Molecules of Life

FEBS3+ Meeting

organized by the Slovenian Biochemical Society,
Croatian Society of Biochemistry and Molecular Biology,
Hungarian Biochemical Society &
Serbian Biochemical Society

and

11th Meeting of the
Slovenian Biochemical Society

September 16-19, 2015
Portorož, Slovenia

Book of Abstracts

Book of Abstracts of the FEBS3+ Meeting "**Molecules of Life**" organized by the Slovenian Biochemical Society, Croatian Society of Biochemistry and Molecular Biology, Hungarian Biochemical Society & Serbian Biochemical Society

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FEBS3+ email: febs3@sbd.si
http://febs3.sbd.si/

VENUE

Grand Hotel Bernardin, Obala 2, 6320 Portorož, Slovenia

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Dear participants of FEBS3+ Meeting "Molecules of Life",

It is a pleasure and honor for us to host you at FEBS3+ Meeting "**Molecules of Life**" in Portorož, Slovenia. The Meeting is organized together by Slovenian Biochemical Society, Croatian Society of Biochemistry and Molecular Biology, Hungarian Biochemical Society and Serbian Biochemical Society under the auspices and support of the Federation of European Biochemical Societies (FEBS)**.** It gives an excellent opportunity to present your scientific achievements at the most of up-to-date topics in the field of molecular life sciences, to enforce your collaboration with academic and other partners, and to establish new acquaintances with scientists from the region and elsewhere. Over 310 participants registrated, excellent plenary and invited lecturers, short oral and poster presentations, participation of numerous colleagues from industry and sponsors' exhibition guarantee exciting gathering and high scientific and professional output.

In addition to dynamic scientific happening we welcome you to enjoy in social events. In the first evening we organize Welcome reception in Hotel Bernardin including the tasting of excellent wines from Lisjak cellar. In Thursday evening you are invited to a guided tour to the city of Piran, whereas in Friday afternoon we organize an excursion for all registered participants to Škocjanske cave, a pearl of Slovenian Carst region. After the excursion the Conference Dinner will take place in Zemono Manor House in the Restaurant "Pri Lojzetu" which offers unique cuisine with flavors and soul of Vipava valley.

We believe that the Bernardin Congress Centre Portorož will satisfy your requirements and that you will be able to use multiple possibilities for different off-program activities. We wish you a pleasant staying in Portorož, fruitful scientific activities and in future, nice memories to our conference!

Sincerely yours,

Janko Kos,
Chair of the Scientific Committee

Nataša Poklar Ulrih,
Chair of the Organizing Committee

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Information

Registration

Registration will take place at the registration desk in the Grand Hotel Bernardin at 12:00 on Wednesday, September 16.
Registration fee for the participant includes: admission to all events (lectures, posters, welcome party, excursion, congress dinner, and coffee breaks), Book of Abstracts, and congress bag.
Registration fee for accompanying persons includes: admission to welcome party, excursion and congress dinner.
The certificate of attendance will be provided at the registration desk.

Language

The official language of the congress is English. There will be no simultaneous translation.

Lectures and oral presentations

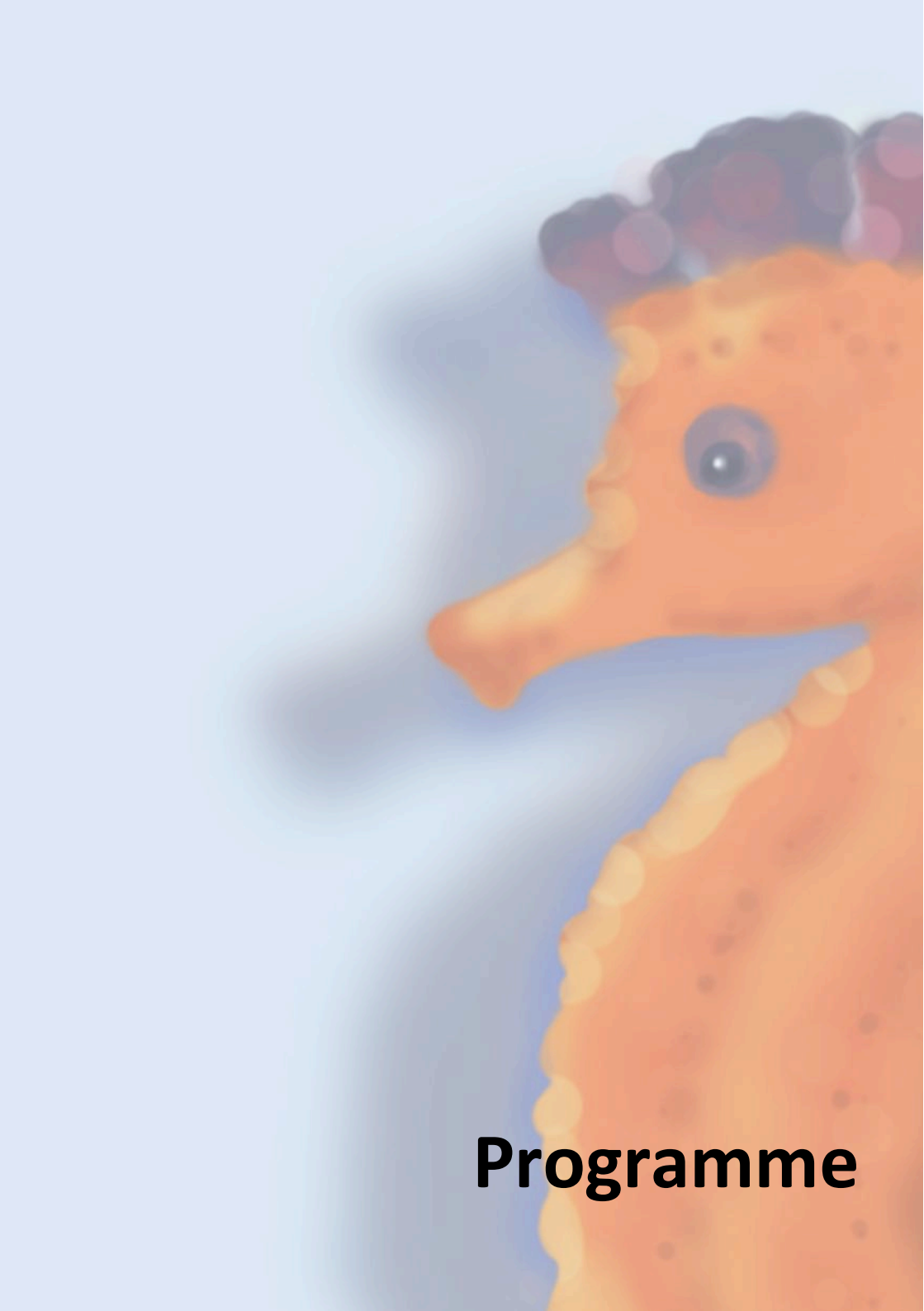
Lectures will be held in the Emerald Room, Aurora and Adria rooms.
Oral presentations should be prepared at MS Power point slides. We will provide laptop for presentations with Windows 7 OS, Office 2010 and Acrobate Reader software. We recommend Apple Macintosh users (without Mini Display port) to convert their presentations into PDF files to avoid compatibility issues.
Presentations need to be tested in advance. Preferably on Wednesday, during the registration, or later during breaks, but not later than one day before the presentation. Presentations should be given on USB memory key.

Poster presentations

Poster should be mounted according to the schedule and to the List of posters in the Book of Abstracts. Poster presentations will be held in the Mediteranea Room.

Social events

Wednesday, September 16	20:00	Welcome Party (Grand Hotel Bernardin)
Thursday, September 17	20:00	Sightseeing walking tour to Piran
Friday, September 18	14:00	Excursion to Škocjan caves
	18:30	Conference dinner at manor house Zemono



Programme

Programme at a glance

Wednesday, September 16, 2015

12:00 – 15:00	Registration	
15:30 – 16:00	**Opening Ceremony**	*Emerald Room*
16:00 – 17:00	**Opening Plenary Lecture** (PL1): Andrej Šali (USA)	*Emerald Room*
17:00 – 18:35	**Structure and Function of Proteins I** L1-L2 & S1-S3	*Emerald Room*
18:35 – 20:00	Dinner break	
20:00 – 22:00	**Welcome Party**	

Thursday, September 17, 2015

8:30 – 9:30	**Plenary Lecture** (PL2): Andras Nagy (Canada)		*Emerald Room*
9:30 – 11:05	**Membrane Structure and Function** L3-L4 & S4-S6 *Emerald Room*	**Synthetic Biology and Biotechnology** L5-L6 & S7-S9 *Aurora Room*	**Stem Cells in Molecular Medicine (HBS)** LH1-LH2 & S10-S12 *Adria Room*
11:05 – 11:35	Coffee break		
11:35 – 13:10	**Molecular Signalling** L7-L8 & S13-S15 *Aurora Room*	**Cell Death and Differentiation** L9-L10 & S16-S18 *Emerald Room*	**Laboratory Reagents & Equipment I** SP1-SP3 *Adria Room*
13:10 – 15:00	Lunch break		
15:00 – 16:35	**Functional Genomics and Gene Maintenance** L11-L12 & S19-S21 *Aurora Room*	**Immunity and Inflammation** L13-L14 & S22-S24 *Emerald Room*	**Lipidomics and Imaging of Mobile Nanodomains (HBS)** LH3-LH4 & S25-S27 *Adria Room*
16:35 – 17:05	Coffee break		
17:05 – 18:15	**Poster Session I** (PI-1 – PI-83)		*Mediteranea Room*
18:15 – 19:05	**Science and Society** (SL1-SL2)		*Emerald Room*
19:00 – 20:00	Dinner break		
20:00 – 21:30	**Assembly of Slovenian Biochemical Society**		*Aurora Room*
	Guided walk to Piran		

Friday, September 18, 2015

8:30 – 9:30	**Plenary Lecture** (PL3): Yosef Yarden (Israel)		*Emerald Room*
9:30 – 11:00	**Structure and Function of Proteins II** L15-L16 & S28-S30 *Emerald Room*	**Molecular Basis of Disease I** L17-L18 & S31-S33 *Aurora Room*	**Laboratory Reagents & Equipment II** SP4-SP5 *Adria Room*
11:05 – 11:35	Coffee break		
11:35 – 13:00	**Poster Session II** (PII-84 – PII-159)		*Mediteranea Room*
13:00 – 14:00	Lunch break		
14:00 – 23:00	**Excursion and Conference Dinner**		

Saturday, September 19, 2015

9:00 – 10:30	**Systems Biology and Bioinformatics** L19-L20 & S34-S36 *Adria Room*	**Molecular Basis of Disease II** L21-L22 & S37-S39 *Emerald Room*
10:35 – 11:05	Coffee break	
11:05 – 12:05	**Closing Plenary Lecture** (PL4): Djordje Miljković (Serbia)	*Emerald Room*
12:05 – 12:45	**Closing Ceremony and Best Poster Awards**	*Emerald Room*

Programme

Wednesday, September 16, 2015

12:00 – 15:30		Registration	
15:30 – 16:00		**Opening Ceremony**	*Emerald Room*
Opening Plenary Lecture			*Emerald Room*
Chairs: Janko Kos, Vito Turk			
16:00	PL1	**Andrej Šali** (San Francisco, United States of America) Integrative modeling of biomolecular assemblies and pathways	
Structure and Function of Proteins I			*Emerald Room*
Chairs: Ivanka Karadjić, László Nyitray			
17:00	L1	**Brigita Lenarčič** (Ljubljana, Slovenia) Novel insights into the biology of tumor marker EpCAM	
17:25	L2	**Mónika Fuxreiter** (Debrecen, Hungary) Fuzziness imparts context dependence on protein interactions	
17:50	S1	**László Nyitray** (Budapest, Hungary) Asymmetric protein-protein interactions in the symmetric homodimeric S100 calcium-binding protein family	
18:05	S2	**Marko Novinec** (Ljubljana, Slovenia) The activity of cysteine cathepsin K is fine-tuned via multiple allosteric mechanisms	
18:20	S3	**Judit Eszter Szabó** (Budapest, Hungary) Characterization of species-specific inhibitory effect of a *Staphylococcal* repressor protein on dUTPases	
18:35 – 20:00		Dinner break	
20:00 – 22:00		**Welcome Party**	

Thursday, September 17, 2015

Plenary Lecture			*Emerald Room*
Chairs: László Fésüs, Beata G. Vertessy			
8:30	PL2	**Andras Nagy** (Toronto, Canada) Pluripotency in the artificial cell space	
Membrane Structure and Function			*Emerald Room*
Chairs: Hrvoje Fulgosi, Gregor Anderluh			*(parallel section)*
9:30	L3	**Gábor Juhász** (Budapest, Hungary) Genetic control of autophagy in *Drosophila*	
9:55	L4	**Hrvoje Fulgosi** (Zagreb, Croatia) Molecular basis of alternative electron partitioning in photosynthesis	
10:20	S4	**Tünde Tóth** (Szeged, Hungary) Carotenoids are essential for the assembly of cyanobacterial photosynthetic complexes	
10:35	S5	**Kristina Sepčić** (Ljubljana, Slovenia) Tracking of cholesterol/ sphingomyelin-rich domains in lipid mono- and bilayers by ostreolysin A-mCherry protein	
10:50	S6	**Marjetka Podobnik** (Ljubljana, Slovenia) Plasticity of listeriolysin O pores and its regulation by pH and unique histidine	

Synthetic Biology and Biotechnology *Aurora Room*

Chairs: Roman Jerala, Imre Boros *(parallel section)*

9:30	L5	**Roman Jerala** (Ljubljana, Slovenia) Designable modularity for engineering cellular logic and new protein folds
9:55	L6	**Marija Gavrović-Jankulović** (Belgrade, Serbia) Recombinant allergens for diagnosis and treatment of allergies
10:20	S7	**Aleš Berlec** (Ljubljana, Slovenia) Engineered probiotics with cytokine/chemokine binding ability
10:35	S8	**Jerica Sabotič** (Ljubljana, Slovenia) Defense proteins from mushrooms offer numerous biotechnological applications
10:50	S9	**Ajasja Ljubetič** (Ljubljana, Slovenia) Folding pathway of a designed protein tetrahedron

Stem Cells in Molecular Medicine (HBS) *Adria Room*

Chair: Balázs Sarkadi *(parallel section)*

9:30	LH1	**Zoltán Ivics** (Langen, Germany) The sleeping beauty transposon system and its applications in molecular medicine
9:55	LH2	**Gregor Majdič** (Ljubljana, Slovenia) Mesenchymal stem cells in veterinary medicine
10:20	S10	**Zsuzsanna Izsvák** (Berlin, Germany) Primate-specific endogenous retrovirus driven transcription defines naïve-like stem cells
10:35	S11	**Ágota Apáti** (Budapest, Hungary) Visualization of calcium signals by genetically engineered calcium indicators; *in vitro* and *in vivo* models
10:50	S12	**Miha Modic** (Cambridge, United Kingdom) TDP-43 safeguards pluripotency by regulation of alternative polyadenylation preventing paraspeckle assembly

11:05 – 11:35 Coffee break

Molecular Signalling *Aurora Room*

Chairs: Mihajlo Spasić, László Buday *(parallel section)*

11:35	L7	**Péter Bai** (Debrecen, Hungary) New ways to revert Warburg-type metabolism
12:00	L8	**Duško Blagojević** (Belgrade, Serbia) Redox regulation: from redox congeners to a systemic molecular physiology approach
12:25	S13	**Robert Vidmar** (Ljubljana, Slovenia) Proteomic identification of legumain physiological substrates
12:40	S14	**Igor Križaj** (Ljubljana, Slovenia) On the role of protein disulfide isomerase in the retrograde cell transport of secreted phospholipases A2
12:55	S15	**Szilvia Krisztina Nagy** (Budapest, Hungary) Autoactivation of AtMPK9 is independent from MAPK cascades

Cell Death and Differentiation *Emerald Room*

Chairs: Boris Turk, Karmen Stankov *(parallel section)*

11:35	L9	**Boris Turk** (Ljubljana, Slovenia) Lysosomal cathepsins in cancer: from cell death to therapy
12:00	L10	**Dušica Vujaklija** (Zagreb, Croatia) Single-stranded DNA binding protein has a key role in chromosome segregation during morphological differentiation of *Streptomyces coelicolor*

12:25	S16	**Endre Karoly Kristof** (Debrecen, Hungary) Functional characterization and gene expression pattern of *ex vivo* differentiated human white, "beige" and brown adipocytes
12:40	S17	**Tomaž Bratkovič** (Ljubljana, Slovenia) Circumstantial evidence for the role of snoRNA SNORD115 in regulation of RNA-editing of serotonin receptor 2c transcript
12:55	S18	**Tvrtko Smital** (Zagreb, Croatia) The role of polyspecific uptake and efflux membrane transport proteins as integral elements of the cellular detoxification and environmental stress response in zebrafish

Laboratory Reagents & Equipment I *Adria Room*

Chairs: Marinka Drobnič Košorok, Marko Goličnik *(parallel section)*

11:35	SP1	**Afif Abdel Nour**, BIO-RAD MIQE Guidelines: Getting a reliable and reproducible qPCR results
12:00	SP2	**Borut Čeh**, Labena, d.o.o. Laboratory grade water - the most important reagent in any analytical laboratory
12:25	SP3	**Torsten Müller**, JPK Instruments AG Nanoscale imaging and quantitative nanomechanical characterization of cells and biomaterials by Correlative Atomic Force and Optical Microscopy

13:10 – 15:00 Lunch break

Functional Genomics and Gene Maintenance *Aurora Room*

Chairs: Damjana Rozman, Beata G. Vertessy *(parallel section)*

15:00	L11	**Ildikó Unk** (Szeged, Hungary) A new way to rescue DNA damage-stalled transcription
15:25	L12	**Lapanje Award Lecture:** **Damjana Rozman** (Ljubljana, Slovenia) A functional genomics approach to understand pathological features of disrupted cholesterol synthesis in the Cyp51 liver knockout mice
15:50	S19	**Ivana Ivančić-Baće** (Zagreb, Croatia) The interaction between HtpG and Cas3 proteins on activity of the *Escherichia coli* type I-E CRISPR-Cas system
16:05	S20	**Rok Košir** (Ljubljana, Slovenia) The influence of genetic background on circadian gene expression patterns in mouse peripheral tissues
16:20	S21	**Ivan Mihaljević** (Zagreb, Croatia) Characterization of Glutathione-S-transferases in zebrafish (*Danio rerio*)

Immunity and Inflammation *Emerald Room*

Chairs: Tihomir Balog, Tanja Ćirković Veličković *(parallel section)*

15:00	L13	**Janoš Terzić** (Split, Croatia) Role of IL-6 in cancer development
15:25	L14	**Tanja Ćirković Veličković** (Belgrade, Serbia) Allegenicity and sensitizing potential of enzymatically cross-linked food allergens
15:50	S22	**Matjaž Deželak** (Maribor, Slovenia) The clinical response to anti-TNF-α drug adalimumab in Crohn's disease patients is associated with genetic polymorphisms in ATG5 gene
16:05	S23	**Milica Perišić Nanut** (Ljubljana, Slovenia) The role of cystatin F in tumour microenvironment
16:20	S24	**Balázs Veres** (Pécs, Hungary) Cyclophilin D-dependent mPT amplifies inflammatory respons in septic shock

Lipidomics and Imaging of Mobile Nanodomains (HBS)			*Adria Room*
Chair: Gábor Balogh			*(parallel section)*
15:00	LH3	**Mario Brameshuber** (Vienna, Austria) Oxidized phospholipids inhibit the formation of mobile cholesterol-dependent plasma membrane nanoplatforms	
15:25	LH4	**Gábor Balogh** (Szeged, Hungary) Towards understanding the role of lipid metabolism in cellular stress response: from yeast models to human diseases	
15:50	S25	**Karoly Liliom** (Budapest, Hungary) Lysophospholipid mediators - players in nanodomain dynamics?	
16:05	S26	**Sergej Pirkmajer** (Ljubljana, Slovenia) Composition of cell culture media modulates basal activity and responsiveness of intracellular signalling pathways	
16:20	S27	**Tamara Popović** (Belgrade, Serbia) Membrane phospholipids fatty acids profiles and lipid peroxidation in aging	
16:35 – 17:05		Coffee break	
17:05 – 18:15		**Poster Session I** (PI-1 – PI-83)	*Mediteranea Room*
Science and Society			*Emerald Room*
Moderator: Radovan Komel			
18:15	SL1	**Karmen Stankov** (Novi Sad, Serbia) Ethical aspects in genomics	
18:35	SL2	**László Fésüs** (Debrecen, Hungary) Science integrity in publishing	
18:55 – 19:05		Discussion	
19:00 – 20:00		Dinner break	
20:00 – 21:30		**Assembly of Slovenian Biochemical Society** (*Aurora Room*) **Guided walk to Piran**	

Friday, September 18, 2015

Plenary Lecture			*Emerald Room*
Chairs: Jerka Dumić, Israel Pecht			
8:30	PL3	**Yosef Yarden** (Rehovot, Israel) EGFR and HER2 in cancer: it takes two to tango	
Structure and Function of Proteins II			*Emerald Room*
Chairs: Zrinka Kovarik, Nataša Poklar Ulrih			*(parallel section)*
9:30	L15	**Ita Gruić Sovulj** (Zagreb, Croatia) Aminoacyl-tRNA synthetase editing preserves the canonical genetic code	
9:55	L16	**Attila Reményi** (Budapest, Hungary) Protein kinases: enzymes working in signaling brigades	
10:20	S28	**Julianna Olah** (Budapest, Hungary) Exploring the catalytic cycle of 3-isopropyl-malate dehydrogenase: A combined experimental and QM/MM modelling study	
10:35	S29	**Davor Obradović** (Ljubljana, Slovenia) The *Aggregatibacter actinomycetemcomitans* cytolethal dystending toxin with a truncated CdtB subunit	
10:50	S30	**Morana Dulić** (Zagreb, Croatia) Specificity of leucyl-tRNA synthetase's editing domain is determined by the chemical step of proofreading	

Molecular Basis of Disease I *Aurora Room*

Chairs: Marija Gavrović-Jankulović, Tamara Lah Turnšek *(parallel section)*

9:30	L17	**Tamara Lah Turnšek** (Ljubljana, Slovenia) Cross-talk of glioblastoma and mesenchymal stem cells modulates signalling pathways in tumour cells
9:55	L18	**Ivan Spasojević** (Belgrade, Serbia) Anticancer effects of vitamin C: The great cell culture swindle?
10:20	S31	**Éva Csősz** (Debrecen, Hungary) Proteomics examination of OSCC-specific salivary biomarkers in a Hungarian population using Luminex-based multiplex assay and SRM-based targeted proteomics method
10:35	S32	**Marko Fonović** (Ljubljana, Slovenia) Cysteine cathepsins affect tumor cell adhesion and intracellular signaling through the shedding of cell adhesion proteins and receptors
10:50	S33	**Ana Mitrović** (Ljubljana, Slovenia) Use of nitroxoline derivatives in regulation of cathepsin B activity

Laboratory Reagents & Equipment II *Adria Room*

Chairs: Sabina Berne, Blaž Cigić *(parallel section)*

9:30	SP4	**Viviane Sternkopf**, RainDance Technology Quantitative and ultra-sensitive, nucleic acid analysis using RainDrop™ Single-Molecule dPCR
9:55	SP5	**Nina Nečimer**, Roche farmacevtska družba d.o.o., Diagnostics Division Roche target enrichment solutions

11:05 – 11:35		Coffee break
11:35 – 13:00		**Poster Session II** (PII-84 – PII-159) *Mediteranea Room*
13:00 – 14:00		Lunch break
14:00 – 23:00		**Excursion and Conference Dinner**

Saturday, September 19, 2015

Systems Biology and Bioinformatics *Adria Room*

Chairs: Aleš Berlec, Sándor Pongor *(parallel section)*

9:00	L19	**Sándor Pongor** (Budapest, Hungary) Microbiome bioinformatics: computational analysis and modeling of signalling in complex bacterial communities
9:25	L20	**Iva Tolić** (Zagreb, Croatia) Forces in the mitotic spindle
9:50	S34	**András Zeke** (Budapest, Hungary) Mapping MAPK interactors: An extensive network with fast-evolving partnerships
10:05	S35	**Željko Popović** (Novi Sad, Serbia) Developing a database on gene and protein expression in animal dormancies
10:20	S36	**Tanja Cvitanović** (Ljubljana, Slovenia) Validation of SteatoNet for prediction the liver network disorders

Molecular Basis of Disease II *Emerald Room*

Chairs: Olgica Nedić, Karmela Barišić *(parallel section)*

9:00	L21	**Boris Rogelj** (Ljubljana, Slovenia) RNA-protein interactions in ALS and FTLD
9:25	L22	**András Váradi** (Budapest, Hungary) 4-Phenylbutyrate reduces calcification in mice expressing human ABCC6 mutants: a preclinical *in vivo* model for allele-specific therapy of PXE and GACI

9:50	S37	**Ana Penezić** (Belgrade, Serbia) Carbonylation of HSA with methylglyoxal affects its copper(II) binding affinity	
10:05	S38	**Anja Pišlar** (Ljubljana, Slovenia) Role of γ-enolase in neuronal development and degeneration: regulation by cysteine protease cathepsin X	
10:20	S39	**Krisztina Kovacs** (Pécs, Hungary) Effect of resveratrol on monocrotalin induced pulmonary hypertension	
10:35 – 11:05		Coffee break	
Closing Plenary Lecture			*Emerald Room*
Chairs: Mihajlo Spasić, Ana Plemenitaš			
11:05	PL4	**Djordje Miljković** (Belgrade, Serbia) From rat to man – an experimental model of multiple sclerosis	
12:05 – 12:45		**Closing Ceremony and Best Poster Awards**	*Emerald Room*

List of Posters

(PI and PII)

Poster Session I

Structure and Function of Proteins (PI-1 – PI-40)

PI-1 **Characterization of the DNA remodeling activity of human HLTF protein**
David Balogh, Yathish Jagadheesh Achar, Dante Neculai, Szilvia Juhasz, Monika Morocz, Himabindu Gali, Sirano Dhe-Paganon, Česlovas Venclovas, Lajos Haracska,

PI-2 **Effect of extracellular S100A4 on cell adhesion and migration of epithelial carcinoma cells**
Beáta Biri, Henrietta Vadászi, Bence Kiss, Zoltán Ligeti, György Csikós, Eszter Lajkó, Orsolya Láng, László Kőhidai, László Nyitray

PI-3 **Structural and functional aspects of Ca2+ loaded S100A4 – what can NMR see?**
Gyula Pálfy, Péter Ecsédi, Bence Kiss, László Nyitray, Andrea Bodor

PI-4 **Investigating the Specificity of Human Elastase 3A (ELA3A) and Elastase 3B (ELA3B) by Phage Display**
Eszter Boros, Andras Szabo, Balint Nemeth, Miklos Sahin-Toth, Gabor Pal

PI-5 **The role of P53 during transcriptional blockage**
Barbara Nikolett Borsos, Ildikó Huliák, Zsuzsanna Újfaludi, Péter Pukler, Tibor Pankotai, Imre Miklós Boros

PI-6 **Interaction of human cholinesterases with bisdimethylcarbamate derivative of albuterol**
Anita Bosak, Anamarija Knežević, Vladimir Vinković, Zrinka Kovarik

PI-7 **The role of the Asp76-Lys41 salt bridge in the amyloid formation of genetic β2m D76N mutant**
Éva Bulyáki, Judit Kun, András Micsonai, Linda Kernya, Zsófia Kele, Yuji Goto, Károly Liliom, József Kardos

PI-8 **Intramolecular interactions and lipid binding of the scaffold protein Tks4**
Anna Cserkaszky, László Radnai, Kitti Koprivanacz, Balázs Merő, Bálint Szeder, László Buday

PI-9 **Hormone-sensitive fluorescent biosensors and *in vitro* assays for steroid receptors and steroid oxidoreductases**
Jovana Plavša, Sofija Bekić, Jovana Krtinić, Andrea Benić, Nevena Kitanović, Edward Petri, Andjelka Ćelić

PI-10 **Tyrosine 24 phosphorylation of annexin A2 regulates isoform-selective S100 protein binding**
Peter Ecsédi, Bence Kiss, Ibolya Leveles, Gergő Gogl, Beáta Vértessy, László Radnai, László Nyitray

PI-11 **Understanding the functional role of segments involved in lipid-mediated regulation of a key lipid biosynthetic enzyme from the malaria parazite**
Hajdu Fanni, Nagy Gergely Nándor, Marton Lívia, Dr. Vértessy G. Beáta

PI-12 **Structural Characterization of an Organophosphate Sequestering Esterase from *Culex pipiens*, a Disease Carrying Mosquito**
Davis Hopkins, Nicholas Fraser, Paul Carr, Colin Jackson

PI-13 **EpCAM oligomerization analysis**
Aljaž Gaber, Miha Pavšič, Brigita Lenarčič

PI-14 **Investigation of potential sterol binding sites in ABCG1 protein**
Zoltan Hegyi, Laszlo Homolya

PI-15 **Using a molecular switch to decipher protein-protein interactions within the living cell: what can we learn about horizontal gene transfer?**
Rita Hirmondó, Szilvia Tarjányi, Kinga Nyíri, Bianka Kőhegyi, Judit Tóth, Beata Vértessy

PI-16 **Characterization of plant dipeptidyl-peptidases III from *Physcomitrella patens* and *Arabidopsis thaliana***
Zrinka Karačić, Marija Abramić

PI-17 **Chloride/formate exchanger (CFEX/Slc26a6) in rat organs; sex-dependent expression in kidneys**
Dean Karaica, Davorka Breljak, Jovica Lončar, Marija Ljubojević, Carol Herak-Kramberger, Vedran Micek, Ivana Vrhovac, Jana Ivković Dupor, Ivan Mihaljević, Petra Marić, Tvrtko Smital, Burckhardt Birgitta Christina, Burckhardt Gerhard, Ivan Sabolić

PI-18 **Pinpointing the interaction domains of plant seryl-tRNA synthetase and metabolic protein BEN1**
Mario Kekez, Vladimir Zanki, Vesna Hodnik, Gregor Anderluh, Anne-Marie Duchêne, Jasmina Rokov Plavec

PI-19 **pH regulated pore-forming activity of listeriolysin O mutant**
Matic Kisovec, Marjetka Podobnik, Gregor Anderluh

PI-20 **Mechanism of Ca2+-dependent membrane bridging by annexin A2 and its regulation by tyrosine phosphorylation and S100 binding**
Bence Kiss, Peter Ecsedi, Gergo Gogl, Gitta Schlosser, Norbert Jeszenoi, Csaba Hetenyi, Laszlo Nyitray

PI-21 **Selection, binding and structural analyses of peptide ligands for the Fc portion of human immunoglobulin G**
Nika Kruljec, Peter Molek, Borut Štrukelj, Tomaž Bratkovič

PI-22 **MD and QM/MM modeling of nitric oxide binding to myoglobin**
Aniko Labas, Julianna Olah, Jeremy Harvey

PI-23 **Insights into NLP activity**
Tea Lenarčič, Isabell Albert, Thorsten Nuernberger, Vesna Hodnik, Marjetka Podobnik, Gregor Anderluh

PI-24 **First characterization of multidrug and toxin extrusion (MATE/SLC47) proteins in zebrafish (*Danio rerio*)**
Jovica Lončar, Marta Popović, Petra Krznar, Roko Zaja, Tvrtko Smital

PI-25 **Compounds with phenanthridine moiety as potential modulators of butyrylcholinesterase activity**
Nikola Maraković, Marijana Radić Stojković, Ivo Piantanida, Stefka Kaloyanova, Goran Šinko

PI-26 **HPLC, ESI qTOF and MALDI TOFTOF MS reveal target sequence and binding stoichiometry of novel Ru(II) complexes to serum albumin**
Marija Nišavić, Romana Masnikosa, Marijana Petković, Mario Cindrić

PI-27 **Secreted aegerolysins and MACPF domain-containing proteins in the filamentous fungus *Aspergillus niger***
Maruša Novak, Nada Kraševec, Urška Čepin, Toshihide Kobayashi, Peter Maček, Gregor Anderluh, Kristina Sepčić

PI-28 **Structural and functional characterisation of a staphylococcal repressor**
Veronika Papp-Kádár, Kinga Nyíri, Judit Eszter Szabó, Ildikó Scheer, Beáta Vertessy G.

PI-29 **Tumor marker Trop2 - a phosphorylation-triggered structural switch at the membrane-cytosol interface**
Miha Pavšič, Gregor Ilc, Tilen Vidmar, Janez Plavec, Brigita Lenarčič

PI-30 **Improved detection of protein-protein interactions using proximity ligation assay**
Mateja Prunk, Milica Perišić Nanut, Jerica Sabotič, Janko Kos

PI-31 **Fourier transform infrared spectroscopy provides an evidence of papain denaturation and aggregation during cold storage**
Brankica Rašković, Natalija Polović

PI-32 **Listeriolysin O membrane interactions and pore formation on giant unilamellar vesicles**
Saša Rezelj, Apolonija Bedina Zavec, Gregor Anderluh

PI-33 **Foreign epitope presentation with chimeric potato virus Y virus-like particle**
Andreja Šink, Nejc Rojko, Marko Šnajder, Magda Tušek Žnidarič, Ion Gutiérrez Aguirre, Nataša Poklar Ulrih, Maja Ravnikar, Gregor Anderluh

PI-34 **Comparison of water and methanol extracts from hyperthermophilic archaeon *Aeropyrum pernix***
Mihaela Skrt, Polona Jamnik, Nataša Poklar Ulrih

PI-35 **Aha1 and cIAP1 isoform-specific interaction with human Hsp90 is mediated by N-terminal and middle domain of Hsp90**
Kamil Synoradzki, Pawel Bieganowski

PI-36 **Alteration of cholinesterase activity in *Daphnia magna* after the exposure to silver nanoparticles**
Goran Šinko, Ivana Vinković Vrček, Lea Ulm, Adela Krivohlavek

PI-37 **Investigation of biochemical, molecular and morphological consequences of the expression of BFSP1 in tumour cells**
Peter Jakus, Kinga Vajda, Fatime Schreck, Ferenc Gallyas, Balazs Sumegi, Antal Tapodi

PI-38 **Characterization of the interaction between metastasis-associated ezrin and S100A4**
Henrietta Vadászi, Beáta Biri, Bence Kiss, László Nyitray

PI-39 **Sodium-glucose cotransporter Sglt1 (Slc5a1) is present in various murine organs; sex-related expression in kidneys**
Ivana Vrhovac, Davorka Breljak, Dean Karaica, Hermann Koepsell, Ivan Sabolic

PI-40 **Evidence for the cytosolic location of NAD(P)H cytochrome b5 oxidoreductase**
Veronika Zámbó, Éva Kereszturi, Mónika Tóth, Gábor Lotz, Miklós Csala

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Membrane Structure and Function (PI-41 – PI-45)

PI-41 **PaNIE binds to plant and yeast membrane lipids**
Vesna Hodnik, Akiko Yamaji, Toshihide Kobayashi, Saša Rezelj, Polona Bedina Zavec, Gregor Anderluh

PI-42 **Absence of cyclophilin D enhances the cholesterol and fat anabolism in mouse liver**
Peter Balazs Jakus, Fruzsina Fónai, Aniko Takatsy, Csenge Antus, Nikoletta Kálmán, Krisztián Erős, Zita Bognár, Balazs Veres

PI-43 **Neuropathy target esterase-related enzyme: a possible role in skeletal muscle energy metabolism**
Maja Katalinić, Katarina Miš, Katarina Gros, Urška Matkovič, Zoran Grubič, Tomaž Marš, Sergej Pirkmajer

PI-44 **Changes in the membrane phospholipid composition in erythrocytes at patients with metabolic syndrome after consumption of pomegranate juice**
Milica Kojadinovic, Aleksandra Arsic, Aleksandra Konic Ristic, Nevena Kardum, Tamara Popovic, Jasmina Debeljak Martacic, Marija Glibetic

PI-45 **α-Synuclein interactions with phospholipid model membranes**
Katja Pirc, Nataša Poklar Ulrih

Synthetic Biology and Biotechnology (PI-46 – PI-59)

PI-46 **Engineering the folding pathway of a designed topological protein**
Igor Drobnak, Ajasja Ljubetič, Karen Butina, Helena Gradišar, Roman Jerala

PI-47 **Synthetic multi-enzymatic intracellular compartments and non-cellular particles**
Rok Gaber, Bojana Stevovic, Tina Fink, Nik Franko, Mojca Benčina, Helena Gradišar, Roman Jerala

PI-48 **Action of propolis in the yeast cell**
Tanja Petelinc, Tomaž Polak, Polona Jamnik

PI-49 **Electrophysiological properties of rhodopsins from selected halotolerant and halophilic fungi**
Tilen Konte, Sabine Panzer, Janja Zajc, Ana Plemenitaš, Ulrich Terpitz

PI-50 **Changes in cytosolic distribution of toxic metals Cd and Pb in liver, gills and intestine of Vardar chub (*Squalius vardarensis*) induced by exposure to mining effluents**
Nesrete Krasnići, Zrinka Dragun, Vlatka Filipović Marijić, Marijana Erk, Sheriban Ramani

PI-51 **Interactions of secondary metabolites from cyanobacteria and invasive tropical algae with the cellular detoxification mechanism in zebrafish (*Danio rerio*)**
Petra Marić, Ivan Mihaljević, Jovica Lončar, Jelena Dragojević, Tvrtko Smital

PI-52 **Designing fluorescent probes by bipartite phage display**
Peter Molek, Mariša Gasparini, Borut Štrukelj, Tomaž Bratkovič

PI-53 **Successful panning of a pre-immune VHH phage display library directly on exosomes**
Milica Popovic, Barbara Toffoletto, Daniela Cesselli, Ario de Marco

PI-54 **Screening Of Endophytic Fungi Isolated From Conifers Needles For Antibacterial Properties**
Matjaž Ravnikar, Matic Terčelj, Damjan Janeš, Borut Štrukelj, Samo Kreft

PI-55 **Codon optimisation is key for pernisine expression in *Escherichia coli***
Marko Šnajder, Nataša Poklar Ulrih, Marko Mihelič, Dušan Turk

PI-56 **Hydrolysis of concentrated raw corn starch with *Bacillus licheniformis* 9945a α-amylase**
Marinela Šokarda Slavić, Zoran Vujčić, Nataša Božić

PI-57 **Oxidative stress biomarkers as predicting factors for boar semen characteristics following short-term liquid storage**
Maja Zakošek Pipan, Janko Mrkun, Marjan Kosec, Alenka Nemec Svete, Petra Zrimšek

PI-58 **Nuclease resistant oligonucleotide receptor for troponin diagnostics**
Zsuzsanna Szeitner, Anna Doleschall, Gergely Lautner, Katalin Keltai, Róbert Gyurcsányi, Tamás Mészáros

PI-59 **Growth parameters and protein production of *E. coli* strains containing different copy number of ribosomal RNA operons**
Gyorfy Zsuzsanna, Draskovits Gabor, Vernyik Viktor, Urmos Kitti, Posfai Gyorgy

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Molecular Signalling (PI-60 – PI-73)

PI-60 **Structural assembly of the signaling competent ERK2–RSK1 complex**
Anita Alexa, Gergő Gógl, Csaba Hetényi, Attila Reményi

PI-61 **Stress triggers mitochondrial biogenesis to preserve steroidogenesis in Leydig cells**
Igor Gak, Sava Radovic, Natasa Stojkov-Mimic, Tatjana Kostic, Silvana Andric

PI-62 **Ubiquitin-dependent phosphorylation of ERKS in a three component signaling complex**
Gábor Glatz

PI-63 **A novel crosstalk of MAPK- and Ca-signaling pathways: comprehensive characterization of S100-MAPKAPK interactions**
Gergo Gogl, Bence Kiss, Anita Alexa, Peter Sok, Attila Remenyi, Laszlo Nyitray

PI-64 **Agrin signalling in primary human myoblasts**
Katarina Gros, Giulia Parato, Urška Matkovič, Zoran Grubič, Tomaž Marš, Paola Lorenzon, Sergej Pirkmajer

PI-65 **PARP-1 inhibitor attenuates mitochondrial ROS production and cell death through PARP-1-ATF4-MKP-1 pathway in oxidative stress**
Enikö Hocsak, Nikoletta Kalman, Ferenc Gallyas Jr., Balazs Sumegi, Boglarka Racz

PI-66 **Melatonin replacement restores the circadian behavior in adult rat Leydig cells after pinealectomy**
Aleksandar Baburski, Srdjan Sokanovic, Sava Radovic, Maja Bjelic, Silvana Andric, Tatjana Kostic

PI-67 **Structural background of the regulation of SH3 domains by tyrosine phosphorylation**
Balázs Merő, László Radnai, Ibolya Leveles, Bálint Szeder, Gréta Kuzma, Anna Fekete, Anna Cserkaszky, Beáta G. Vértessy, László Buday

PI-68 **BEST channels - possible mediators of H2S-induced relaxation of rat uteri?**
Ana Mijuskovic, Nikola Tatalovic, Zorana Orescanin Dusic, Aleksandra Nikolic Kokic, Mihajlo B. Spasic, Dusko Blagojevic

PI-69 **The role of the L5/L11-Mdm2-p53 signaling pathway in response to ribosomal and genotoxic stresses**
Ines Oršolić, Deana Jurada, Siniša Volarević

PI-70 **10 Years of Ibogaine Research in Slovenia**
Roman Paškulin

PI-71 **Myosin phosphatase regulates gene expression via mediating arginine methylation in human hepatocarcinoma cells**
Adrienn Sipos, Judit Iván, Zsuzsanna Darula, Bálint Bécsi, Ferenc Erdődi, Beáta Lontay

PI-72 **Ibogaine relaxes rat arteries: the role of endothelium**
Nikola Tatalović, Mina Bajrica, Ana Mijusković, Zorana Oreščanin Dušić, Aleksandra Nikolić Kokić, Mihajlo Spasić, Duško Blagojević

PI-73 **Fungal lectins: versatile molecular triggers and their potential use in biomedicine**
Simon Žurga, Janko Kos, Jerica Sabotič

Functional Genomics and Gene Maintenance (PI-74 – PI-83)

PI-74 **Correlation between MXR inhibitors in wastewater and zebrafish embryotoxicity**
Sanja Babić, Ruben Strecker, Roberta Sauerborn Klobučar, Rozelindra Čož-Rakovac

PI-75 **Genome stability enzymes are essential for building CRISPR-cas immunity in bacteria**
Ivana Ivančić-Baće, Simon Cass, Wearne Stephen, Edward Bolt

PI-76 **The effect of chronic renal failure on the expression of drug-metabolizing cytochrome P450 enzymes**
Máté Déri, Ádám Kiss, Katalin Tóth, Edit Háfra, Katalin Monostory

PI-77 **An experimental test of the adaptive genome streamlining hypothesis**
Gabor Draskovits, Ildiko Karcagi, Kinga Umenhoffer, Balazs Bogos, Tamas Feher, Zsuzsanna Gyorfy, Frederick R. Blattner, Gyorgy Posfai, Balazs Papp, Csaba Pal

PI-78 **The role of the hepatic cholesterol synthesis in the bile acids synthesis/excretion in mice with Cyp51 liver knockout**
Urša Kovač, Gregor Lorbek, Žiga Urlep, Peter Juvan, Martina Perše, Jera Jeruc, Ingemar Bjorkhem, Claudia Fuchs, Michael Trauner, Damjana Rozman

PI-79 **Examining genome integrity maintenance in human cell lines by site-specific, biallelic gene knock-out technology**
Hajnalka Laura Pálinkás, Gergely Róna, András Horváth, Máté Borsos, Júlia Batki, Beáta Vértessy

PI-80 **Detection of genomic uracil from bacteria to human**
Ildiko Scheer, Gergely Róna, Kinga Nagy, Hajnalka L. Pálinkás, Gergely Tihanyi, Gergely Takács, Beáta G. Vértessy

PI-81 **Clinical relevance of patients' CYP3A-status in clozapine therapy**
Katalin Toth, David Sirok, Gabor Csukly, Judit Bulucz, Istvan Bitter, Pal Szabo, Balazs Magda, Katalin Monostory

PI-82 **VNTR patterns in TPMT promoter region affect TPMT activity via linkage disequilibrium with TPMT*3 alleles**
Dunja Urbančič, Tina Kuhar, David Gurwitz, Andres Metspalu, Irena Mlinarič-Raščan

PI-83 **Variant rs10757278 in 9p21 region is associated with coronary artery disease as comorbidity with carotid atherosclerosis, in males only**
Ivan Zivotic, Ana Djordjevic, Tamara Djuric, Igor Koncar, Dragan Alavantic, Aleksandra Stankovic, Maja Zivkovic

Poster Session II

Stem Cells in Molecular Medicine – HBS (PII-84 – PII-86)

PII-84 **Direct cross-talk between mesenchymal stem cell and glioblastoma cells enhances the expression of the proteolytic enzymes urokinase-type plasminogen activator (uPA) and metalloprotease MMP-9 along with increased invasion of U373 glioblastoma cells**
Barbara Breznik, Miloš Vittori, Helena Motaln, Tamara Lah Turnšek

PII-85 **Kinin Receptor Expression and Activity in Co-cultures of Mesenchymal Stem and Glioblastoma Cells**
Mona N. Oliveira, Michelli M. Pillat, Helena Montaln, Henning Ulrich, Tamara Lah T.

PII-86 **Paracrine effects of mesenchymal stem cells on differentiation of glioblastoma stem-like cells**
Katja Kološa, Helena Motaln, Tamara Lah Turnšek

Lipidomics and Imaging of Mobile Nanodomains – HBS (PII-87)

PII-87 **Nonpolar bases hexylamine and trihexylamine are powerful antioxidants in lipid systems**
Tjaša Prevc, Anja Rečnik, Alenka Levart, Nataša Šegatin, Nataša Poklar Ulrih, Blaž Cigić

Cell Death and Differentiation (PII-88 – PII-103)

PII-88 **Characterization of organic cation transporters in zebrafish (*Danio rerio*)**
Jelena Dragojević, Ivan Mihaljević, Marta Popović, Roko Žaja, Nikola Maraković, Tvrtko Smital

PII-89 **Analysis of changes in neutrophil extracellular trap (NET) proteins profile using proteomic methods**
Bernadett Jakob, Endre Kristóf, Krisztián Csomós, László Fésüs, Éva Csősz

PII-90 **Anticancer potential of steroidal 16,17-seco-16,17a-dinitrile derivatives against triple negative MDA-MB-231 breast cancer cells**
Suzana Jovanović-Šanta, Vesna Kojić, Lidija Aleksić, Gordana Bogdanović, Andrea Nikolić, Marija Sakač, Dimitar Jakomiv

PII-91 **Metformin enhances protective signalling responses but fails to improve survival of cultured myotubes exposed to ischemia and reperfusion**
Matic Kaiser, Katarina Miš, Urška Matkovič, Matej Podbregar, Zoran Grubič, Tomaž Marš, Sergej Pirkmajer

PII-92 **The effect of various antioxidants in cell death-related oxidative stress**
Nežka Kavčič, Katarina Pegan, Peter Vandenabeele, Boris Turk

PII-93 **Orthocaspases: the proteolytically active prokaryotic caspase homologues**
Marina Klemenčič, Marko Novinec, Marko Dolinar

PII-94 **Novel nuclear specific regulatory role of LIN28A protein in pluripotent stem cell lineage acquisition**
Sabina Kolar, Miha Modic, Gregor Rot, Markus Grosch, Tanja Orschmann, Boris Rogelj, Micha Drukker

PII-95 **Converserved functions at the domain level during the uni-multicellular transition**
Jeromos Kun, Illes Farkas

PII-96 **Photosensitizing effect of PARP inhibitor PJ-34 in a UVA irradiated human epidermoid carcinoma cell line model**
Petra Lakatos, László Virág

PII-97 **Altered development of adipose tissue may contribute to the decreased tolerance to cold exposure of tissue transglutaminase knock-out mice**
Andras Madi, Ixchelt Cuaranta Monroy, Kinga Lenart, Timea Veres, Gabor Mocsar, Peter Bai, Laszlo Fesus

PII-98 **Effects of individual and combined treatment of Raloxifene and estrogen on apoptosis in human endometrial stromal ThESC cell line**
Ivana Nikolic, Marina Mitrovic

PII-99 **The TAF10-containing TFIID is necessary for the ecdysone induced larval-pupal transition of *Drosophila melanogaster***
Zoltan Gabor Pahi, Zsuzsanna Kiss, Orbán Komonyi, Barbara Nikolett Borsos, Laszlo Tora, Imre Miklos Boros, Tibor Pankotai

PII-100 **Oleuropein ameliorates cisplatin-induced oxidative damage, inflammation and apoptosis in mice kidneys by modulating CYP2E1, NF-κB and ERK1/2 expression**
Robert Domitrović, Iva Potočnjak, Marko Škoda, Martina Pavletić Peršić

PII-101 **Epimer-specific effects of bile acids on the anti-neoplastic activity of doxorubicin in MCF-7 breast adenocarcinoma cells**
Bojan Stanimirov, Karmen Stankov, Nebojša Pavlović, Maja Đanić, Vesna Kojić, Gordana Bogdanović, Momir Mikov

PII-102 **The role of Hmgb1 in the unique transcriptional mechanism of the matrilin-1 gene and the early step of chondrogenesis**
Tibor Szénási

PII-103 **Role of gamma-enolase in tumor cell survival in starvation and hypoxia: regulation by cysteine protease cathepsin X**
Tjaša Vižin, Anja Pišlar, Janko Kos

Immunity and Inflammation (PII-104 – PII-116)

PII-104 **A dual recognition mechanism of flagellin by cytosolic and membrane sensors**
Vida Forstnerič, Tjaša Plaper, Mojca Benčina, Roman Jerala

PII-105 **Susceptibility of human antimicrobial peptides to the action of aspartic proteases secreted by pathogenic yeast Candida albicans**
Oliwia Bochenska, Maria Rapala-Kozik, Natalia Wolak, Wataru Aoki, Mitsuyoshi Ueda, Andrzej Kozik

PII-106 **Crucial role of cyclophilin D in the pathogenesis of LPS induced acutelung injury**
Fruzsina Fónai, János Krisztián Pribér, Nikoletta Kálmán, Péter Balázs Jakus, Csenge Antus, Balázs Sümegi, Balázs Veres

PII-107 **Impaired autophagy results in increased inflammasome activation in stefin B deficient mice**
Mojca Trstenjak-Prebanda, Katarina Maher, Janja Završnik, Boris Turk, Nataša Kopitar-Jerala

PII-108 **Filamentous phages as immunogenic carriers of Fel d 1 mimotopes for allergen specific immunotherapy**
Jernej Luzar, Manca Ogrič, Mira Šilar, Borut Štrukelj, Peter Korošec, Mojca Lunder

PII-109 **Interaction of perforin monomer with the membrane in micromolar dependence of calcium**
Omar Naneh, Franci Merzel, Mirijam Kozorog, Robert JC Gilbert, Vesna Hodnik, Gregor Anderluh

PII-110 **Examining the role of inflammasome activation and identifying novel diagnostic innate immunity biomarkers in children with hypercholesterolemia**
Tina Tinkara Peternelj, Iva Hafner Bratkovič, Urh Grošelj, Katarina Trebušak Podkrajšek, Nevenka Bratanič, Tadej Battelino, Roman Jerala, Simon Horvat

PII-111 **Minimal oligodeoxynucleotide motifs that are distinctively recognized by human TLR9**
Jelka Pohar, Alenka Kužnik-Krajnik, Miša-Mojca Cajnko, Duško Lainšček, Chikako Yamamoto, Ryutaro Fukui, Kensuke Miyake, Roman Jerala, Mojca Benčina,

PII-112 I**nhibition of the NLRP3 inflammasome formation by the designed peptides**
Petra Sušjan, Roman Jerala, Iva Hafner Bratkovič

PII-113 **Serine proteinase inhibitor ecotin of various pathogen microbes inhibits lectin pathway of the complement system**
Dávid Szakács, Eszter Vígh, Zoltán Attila Nagy, Eszter Boros, Dávid Héja, Péter Gál, Gábor Pál

PII-114 **Colocalization of galectin-1 and osteopontin in mouse uterus during early pregnancy**
Sandra Šućurović, Tamara Nikolić, Biserka Mulac-Jeričević

PII-115 **Identification and function of ghrelin receptor and ghrelin-O-acyltransferase in human keratinocytes**
Miha Vodnik, Eva Knuplež, Patrik Milić, Valentina Kubale Dvojmoč, Mojca Lunder, Borut Štrukelj

PII-116 **An atypical bactericidal, cytotoxic, and modulatory peptide from *Staphylococcus pseudintermedius* exhibits properties of bacteriocin and virulence factor**
Benedykt Wladyka, Marcin Piejko, Monika Bzowska, Piotr Pieta, Ibeth Guevara-Lora, Michal Bukowski, Emilia Bonar, Jacek Miedzobrodzki, Adam Dubin, Pawel Mak

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Molecular Basis of Disease (PII-117 – PII-157)

PII-117 **Biological evaluation of the novel ruthenium(II) complexes**
Maja Anko, Katja Traven, Jakob Kljun, Maša Sinreih, Žiga Ude, Jure Stojan, Iztok Turel, Tea Lanišnik-Rižner

PII-118 **Role of CYP2E1 in female-predominant resistance to hyperoxia**
Željka Mačak Šafranko, Sandra Sobočanec, Ana Šarić, Tihomir Balog

PII-119 **Wound healing process in conditions of CD26 deficiency**
Lara Batičić Pučar, Natalia Jug, Dijana Detel, Jadranka Varljen

PII-120 **Identification and spatio-temporal expression profiling of *Verticillium albo-atrum* effectors in infected hop plants**
Kristina Marton, Vasja Progar, Nataša Štajner, Branka Javornik, Sabina Berne

PII-121 **Molecular background and physiological consequences of altered peripheral 5-HT homeostasis in adult rats perinatally treated with tranylcypromine**
Sofia Blazevic, Dora Persic, Dubravka Hranilovic

PII-122 **Global DNA hypomethylation in white blood cells represents a feature of multiple sclerosis**
Maja Bundalo, Maja Živković, Smiljana Ristić, Nada Starčević Čizmarević, Ivana Babić Božović, Bojana Brajenović Milić , Aleksandra Stanković

PII-123 **LLO related drop in TEER of Caco-2 monolayer is Dependent on Pore Formation with no major role of Ca^{2+} influx**
Miša Mojca Cajnko, Maja Marušić, Matic Kisovec, Nejc Rojko, Mojca Benčina, Simon Caserman, Gregor Anderluh

PII-124 **Finding a new regulatrory function for the proteasome activator PA200 in a cellular model for Huntington's disease**
Rita Czinege, Ciyu Yang, Peter Bay, Erik Lee Snapp, Laszlo Virag, Marion Schmidt, Krisztina Tar

PII-125 **Alterations in ophthalmological parameters and cytokine levels in tears of patients with glaucoma**
Eszter Deák, Éva Csősz, Adrienne Csutak

PII-126 **Relationship between tumor necrosis factor α and insulin sensitivity in rats with impaired glucose tolerance**
Branka Djordjevic, Dusan Sokolovic, Jelena Basic, Andrej Veljkovic, Milena Despotovic, Tatjana Cvetkovic, Tatjana Jevtovic Stoimenov, Davor Djukic, Natasa Zivkovic

PII-127 **Modulation of multiple drug resistance by proprietary potassium ionophores in breast cancer stem cell model**
Katja Ester, Iva Guberović, Marko Marjanović, Lidija Uzelac, Ana-Matea Mikecin, Irena Martin-Kleiner, Tatjana Šumanovac-Ramljak, Kata Mlinarić-Majerski, Marijeta Kralj

PII-128 **Regulative role of Tks4 adaptor protein in the cell differentiation process**
Anna Fekete, Virág Vas, Gyöngyi Kudlik, Metta Dülk, Tamás Kovács, Dalma Csécsy, Krisztián Kvell, Judit Pongrácz, Ferenc Uher, László Buday

PII-129 **Exosomes containing protein Nef are released from microglia and astrocyte cells infected with HIV virus**
Jana Ferdin, Ana Plemenitaš, Matija B. Peterlin, Metka Lenassi

PII-130 **Little babies - Big warriors: The antioxidant response in the umbilical cord of neonates with intrauterine growth restriction**
Agnes Ferencz, Krisztina Nikoletta Dugmonits, Szabolcs Zahoran, Edit Hermesz

PII-131 **Proteomic analysis of gene products that regulate de- and remyelination**
Gabor Szilagyi, Arkadiusz Nawrocki, Janos Schmidt, Zsolt Illes, Ferenc Gallyas

PII-132 **Assessment of toxicity endpoints of selected cytostatic drugs**
Vera Garaj-Vrhovac, Goran Gajski, Marko Gerić, Bojana Žegura, Metka Filipič

PII-133 **Cytogenetic status and activation of BRAF-dependent pathway in patients with thyroid diseases**
Marko Gerić, Renato Janušić, Božena Šarčević, Vera Garaj-Vrhovac

PII-134 **Hepatoprotective effect of cocoa polyphenols (*Theobroma cacao* L.) against carbon tetrachloride-induced hepatic damage in mice**
Jasminka Giacometti, Adriano Pavletić, Damir Muhvić, Ariana Fužinac-Smojver

PII-135 **Polymorphisms in segregation genes contribute to gastric cancer risk**
Petra Hudler, Marija Rogar, Nina Sodja, Aida Zečkanović, Tadej Žlahtič, Radovan Komel

PII-136 **Expression analysis of caveolin-1 in papillary thyroid carcinoma with relation to clinicopathological parameters and BRAF mutation status**
Jelena Janković, Svetlana Paskaš, Ilona Marečko, Tijana Išić Denčić, Svetislav Tatić, Dubravka Cvejić, Svetlana Savin

PII-137 **Alterations in the chemical barrier components in tears of patients with Alzheimer's disease**
Gergő Kalló, Miklós Emri, Zsófia Varga, József Tőzsér, Adrienne Csutak, Éva Csősz

PII-138 **Different experimental approaches for discovery of diagnostic biomarkers of endometriosis**
Tea Lanišnik Rižner, Katja Vouk, Tamara Knific

PII-139 **Non-B DNA structures of C9ORF72 hexanucleotide expanded repeat in ALS and FTLD**
Anja Kovanda, Primož Šket, Matja Zalar, Sabina Vatovec, Jure Pohleven, Maja Štalekar, Vera Župunski, Janez Plavec, Boris Rogelj

PII-140 **Plasma Nef-exosomes: putative biomarkers for active reservoirs in ART treated HIV infected patients**
Jana Ferdin, Pia Pužar Dominkuš, Katja Goričar, Vita Dolžan, Ana Plemenitaš, Steven G. Deeks, Matija B. Peterlin, Metka Lenassi

PII-141 **Development of new peptide drug leads interfering with activity of orexigenic hormone ghrelin**
Mojca Lunder, Miha Vodnik, Eva Knuplež, Valentina Kubale Dvojmoč, Borut Štrukelj

PII-142 **Agrin modulates early stages of skeletal muscle regeneration**
Katarina Gros, Sergej Pirkmajer, Urška Matkovič, Giulia Parato, Katarina Miš, Matej Podbregar, Zoran Grubič, Tomaz Mars, Paola Lorenzon,

PII-143 **Transmembrane protein CD9 and glioma cell stemness**
Helena Motaln, Neža Podergajs, Urška Verbovšek, Miloš Vittori, Christel Herold-Mende, Rolf Bjerkvig, Tamara Lah Turnšek

PII-144 **Loss of a brain-specific tRNA isodecoder affects neuronal function**
Gabor Nagy, Mridu Kapur, Susan Ackerman

PII-145 **Glycogen phosphorylase inhibition has positive effect on metabolism**
Lilla Nikoletta Nagy, Tibor Docsa, Attila Tóth, László Somsák, Tamás Fodor, Mónika Gönczi, Pál Gergely, Péter Bai

PII-146 **Ibogaine effects on uterine smooth muscle contractions: the role of antioxidant enzymes**
Zorana Oreščanin-Dusić, Nikola Tatalović, Aleksandra Nikolić-Kokić, Ana Mijušković, Roman Paskulin, Mihajlo Spasić, Dusko Blagojević

PII-147 **Secreted phospholipase A2 induces the formation of cytosolic lipid droplets enriched with polyunsaturated fatty acids and enables cancer cell survival**
Anja Pucer Janež, Vesna Brglez, Thomas O. Eichmann, Petra Malavašič, Tjaša Blatnik, Jernej Šribar, Igor Križaj, Jože Pungerčar, Robert Zimmermann, Toni Petan

PII-148 ***In vitro* study of a nerve-muscle co-culture after electroporation with FUS, a protein involved in ALS/FTLD**
Sonja Prpar Mihevc, Mojca Pavlin, Simona Darovic, Marko Živin, Tomaž Marš, Boris Rogelj

PII-149 **Localization of dipeptide repeat proteins in human cells**
Anja Pucer Janež, Mirjana Malnar, Anja Kovanda, Sonja Prpar Mihevc, Toni Petan, Boris Rogelj

PII-150 **HIV protein Nef is secreted via exosomes from Nef-transfected human microglia**
Pia Pužar Dominkuš, Anja Kejžar, Ana Plemenitaš, Matija Peterlin, Metka Lenassi

PII-151 **The effect of lipoproteins and western diet on myocardium**
Tadeja Režen, Jera Jeruc, Damjana Rozman, Mauro Giacca

PII-152 **Metabolism of progesterone in endometriotic 12-Z cells**
Maša Sinreih, Sven Zukunft, Jerzy Adamski, Tea Lanišnik Rižner

PII-153 **Effect of Metformin on the Regenerative Capacity of Skeletal Muscle *In Vitro***
Urban Slokar, Nejc Umek, Urška Matkovič, Katarina Miš, Matej Podbregar, Zoran Grubič, Sergej Pirkmajer, Tomaž Marš

PII-154 **Biomarkers of protein and lipid oxidative damage as prognostic factors in ESRD patients**
Sonja Suvakov, Tatijana Pekmezovic, Vesna Coric, Jasmina Ivanisevic, Aleksandra Stefanovic, Zorana Jelic-Ivanovic, Tanja Damjanovic, Nada Dimkovic, Tatjana Simic

PII-155 **γKlotho is a novel marker and cell survival factor in a subset of triple negative breast cancers**
Nuša Trošt, Jurij Stojan, Klementina Fon Tacer, Elisabeth D. Martinez

PII-156 **Proteomic insights in cancer-related extracellular proteolysis with cathepsin K**
Matej Vizovišek, Robert Vidmar, Barbara Sobotič, Lovro Kramer, Veronika Stoka, Boris Turk, Marko Fonović

PII-157 **Phosphorylation of FUS 526Y affects its interaction with TNPO1 and nuclear import**
Simona Darovic, Vera Župunski, Sonja Prpar Mihevc, Maja Štalekar, Youn-Bok Lee, Gregor Gunčar, Christopher E. Shaw, Boris Rogelj

Abstracts of Plenary Lectures

(PL)

PL1

Integrative modeling of biomolecular assemblies and pathways

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The networks and spatial structures of biomolecular interactions provide insights into their function and thus help us to understand the workings of living cells. Detailed structural characterization of large and often dynamic assemblies and their networks is generally impossible by any single existing experimental or computational method. This challenge can be overcome by hybrid approaches that integrate data from diverse biophysical experiments (e.g. X-ray crystallography, NMR spectroscopy, electron microscopy, chemical cross-linking, yeast-two hybrid system, and various chemical genetics and proteomics approaches). We formulate the hybrid approach to structure and/or network determination as an optimization problem, the solution of which requires three main components: the representation of the assembly or network, the scoring function, and the optimization method. The ensemble of solutions to the optimization problem embodies the most accurate characterization given the available information. The key challenges remain translating experimental data into restraints on the structure and/or network, combining these spatial and/or network restraints into a single scoring function, optimizing the scoring function, and analyzing the resulting ensemble of solutions. The approach will be illustrated by several applications to specific biological systems, including the structure determination of the nuclear pore complex and the mapping of the gulonate pathway in *Haemophilus influenzae*.

PL2

Pluripotency in the artificial cell space

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The discovery of a defined set of transcription factors that can induce reprogramming of somatic cells to pluripotent stem cells (iPSCs) has had an unprecedented impact on our view on future cell transplantation-based tissue repair and restoration of faulty physiological functions. Somatic cell reprogramming is a several weeks long process through which cells reach pluripotency, the developmental state similar to embryonic stem cells. This cascade of events and the driving forces behind the phenomenon are very poorly understood. It is, however, crucial to uncover the fine details of this process in order to comprehend the true properties of iPSCs and so better tailor their future therapeutic use.
We have recently developed a reprogramming method utilizing a transposon-mediated delivery of the reprogramming transgenes. This system has several advantages over the viral delivery-based alternative. Most notably, it allows for a seamless removal of the transgenes once pluripotent stem cells have been generated and they are no longer needed for stem cell self renewal. We also combined the doxycycline inducible transgene expression system with the transposon delivery-based reprogramming and found that these transgenes are very efficiently regulatable by adding or withdrawing doxycycline. In vivo differentiated somatic cells derived from iPSCs can be reprogrammed to “secondary” iPSCs (2ºiPSc) by simply adding doxycycline to the culture medium.
By analyzing the effect of reprogramming factor expression dynamics on the reprogramming process, we have discovered additional classes of pluripotent cells that might have a different spectrum of phenotypes than that of ES cell-like iPS cells. Here, we explore alternative outcomes of somatic reprogramming by fully characterizing reprogrammed cells independently of preconceived definitions of partial or fully reprogrammed iPSC states. These alternative pluripotent states may have different therapeutic values depending on the target disease.

PL3

EGFR and HER2 in cancer: it takes two to tango

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Biological systems robustly integrate metabolic, energy and signaling networks, by maintaining dense webs of control circuits. My lecture will concentrate on systemic defects in signaling networks involved in malignant cell proliferation and migration. As a starting point, I will argue that primordial signaling pathways have been replaced in the course of metazoan evolution by layered signaling networks. Unlike linear pathways, networks can be trained to overcome perturbations, and their control wirings are much more sophisticated. These transitions are relevant to pharmacological attempts to intercept signaling networks, as well as to the excessive reliance of oncogenic networks on 1-2 essential hubs (\'oncogene addiction\'). Using the epidermal growth factor receptor (EGFR) and its kin, a kinase called HER2/ERBB2, I will exemplify defects in system control and feedback regulation, and highlight some of the currently approved drugs that target the EGFR-HER2 axis. Specifically, the lecture will deal with acquired resistance of lung cancer to molecular targeted drugs, such as erlotinib, an EGFR-specific kinase inhibitor, and ways to enhance efficacy of monoclonal anti-HER2 antibodies, such as trastuzumab, an antibody used to treat breast cancer.

PL4

From rat to man – an experimental model of multiple sclerosis

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Multiple sclerosis is a chronic inflammatory demyelinating and neurodegenerative disease of the central nervous system (CNS). A strong autoimmune reaction against the CNS has been attributed the major pathological role in the disease. However, it is still not clear if this autoimmune response is a cause of multiple sclerosis or a consequence of an intrinsic CNS disorder. Generally, there is a considerable knowledge on the disease pathogenesis, but not on the initial steps of the process. Accordingly, numerous therapeutics have been approved for the treatment of multiple sclerosis, yet neither as a definite cure for the disease. Importantly, most of the therapeutics aim at reducing immunity against the CNS and only some of them are simultaneously neuroprotective agents. Our basic research in multiple sclerosis has been performed in a rat model of the disease, i.e. experimental autoimmune encephalomyelitis (EAE). By using this model we have been able to identify novel molecules important for multiple sclerosis pathogenesis and also to test some of novel agents as the therapeutics for the CNS inflammation. My talk will be dedicated to an overview of our studies in rat EAE and its significance for multiple sclerosis research. Particularly, it will present research on the role of a chemokine CXCL12 in rat EAE and multiple sclerosis, as well as on the therapeutic potential of a powerful anti-inflammatory and neuroprotective compound ethyl pyruvate in the CNS inflammatory disorders.

Abstracts of Lectures

(L, SL and LH)

L1

Novel insights into the biology of tumor marker EpCAM

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Integration of structural and functional data is key to both understanding the biology of cells as well as design of effective drugs to battle diseases. In the last decade, one of the molecules that sparked significant interest is the epithelial cell adhesion molecule (EpCAM), a transmembrane tumor marker with cell-cell adhesive and proliferation-enhancing signaling function. To date, numerous EpCAM-targetting binder molecules have been developed with the aim to specifically target carcinoma cells. However, due to complete lack of structural data their development was based on the trial-and-error approach.
To gain insight into the structural features of EpCAM we used a hybrid approach to study the larger extracellular and short transmembrane part of the molecule. The X-ray structure revealed that the extracellular part of EpCAM forms a heart-shaped homodimer which corresponds to a *cis*-dimer at the cell surface. The homodimers further assemble into intercellular *trans*-tetrameric units implicated in cell-cell adhesion. Additional stabilization of the *cis*-dimers is provided by the dimerization of the transmembrane helix as revealed by molecular dynamics simulations. The *trans*-tetramers are believed to assemble into higher order oligomers resembling an intercellular network. The hybrid structural model provides a valuable basis for further basic and applied research, also for design or improvement of new and existing EpCAM-based antitumor approaches.

L2

Fuzziness imparts context dependence on protein interactions

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Binding of intrinsically disordered (ID) proteins to their specific targets is generally assumed to be coupled to folding. Increasing experimental evidence demonstrates however that structural multiplicity or dynamic disorder can be retained in protein complexes and moreover, could be critical to function. Heterogeneous segments in *fuzzy* complexes can perturb conformational equilibrium and/or modulate the flexibility of the binding interface via transient interactions. Bound disordered regions can affect spacing of the globular domains, interaction motif(s), or they could even serve as a competitive partner. In general, *fuzzy* regions are utilized as on/off switches or rheostats to fine-tune binding affinity/specificity by further protein interactions, post-translational modifications or alternative splicing.
Owing to combinatorial motif usage, fuzzy regions often underlie the context-dependence of protein interactions. Molecular mechanisms of *i)* tissue-specificity via rewiring protein-protein interaction networks, and *ii)* evolution of conditional cooperativity of transciption factors will be discussed.

References:
[1] P. Tompa and M Fuxreiter (2008) Fuzzy complexes: polymorphism and structural disorder in protein–protein interactions. Trends in Biochem. Sci 33, 2-8
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[3] M Fuxreiter (2012) Fuzziness: linking regulation to protein dynamics. *Mol Biosystems* 8, 168-177.
[4] M. Buljan, et. al. (2012) Tissue-specific splicing of disordered segments that embed binding motifs rewires protein interaction networks. *Mol Cell* 46, 871-883.

L3

Genetic control of autophagy in *Drosophila*

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The catabolic process of autophagy ensures cellular and organismal homeodynamics *via* lysosomal degradation and recycling of intracellular material. Pioneering genetic screens in yeast identified an evolutionarily conserved set of Atg genes that promote the formation of autophagosomes: double-membrane vesicles transporting cytoplasmic cargo to lysosomes. Recently, several key players mediating autophagosome-lysosome fusion have been also discovered in *Drosophila* and mammalian cells, including a Syntaxin 17-containing SNARE complex and the multisubunit tethering factor HOPS. At the meeting, I will present our latest progress in understanding the molecular mechanisms of autophagosome clearance, and its relevance in lifespan determination, neurodegeneration and survival of environmental stress.

L4

Molecular basis of alternative electron partitioning in photosynthesis

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In vascular plant photosynthesis several electron transport chains have been recognized. Linear electron transport chain (LET) transfers electrons generated by photosystems II and I to a small iron-sulphur protein ferredoxin (Fd). Fd acts simultaneously as a bottleneck and as a hub which distributes high-energy electrons to a multitude of enzymes. The dominant pathway in chloroplasts is, however, the one that produces NADPH, by the activity of ferredoxin-$NADP^+$-oxidoreductase (FNR). FNR is a soluble protein that exists in several isoforms and utilizes two reduced Fds to produce one molecule of NADPH. In order to function efficiently Fd/FNR redox chemistry has to be constantly poised. The question remains how is the partitioning of electrons between various energy-conserving and -dissipating pathways achieved. We have previously shown that thylakoid rhodanase-like protein TROL acts as a *bona fide* membrane attachment point for the FNR [1], although other associations with thylakoid and inner envelope membranes have been described. We have proposed a scheme for the dynamic FNR recruitment to TROL [2]. We posit that TROL-FNR interaction represents branching point between electron-conserving and electron-dissipating pathways. Without the TROL, light-dependent $O_2^{\cdot-}$ generation is reduced, while the generation of other ROS is enhanced. We propose that other Fd-dependent pathways downstream of PSI and different from the linear electron transfer become dominant by the dynamic detachment of FNR form TROL, thus suggesting a novel mechanism of photosynthesis regulation. Alternatively, efficient scavenging of $O_2^{\cdot-}$ by FNR-dependent pathways can be envisaged [3].

References:
[1] Jurić et al. (2009) The Plant Journal
[2] Vojta and Fulgosi (2012) Advances in Photosynthesis - Fundamental Aspects, InTech, Rijeka
[3] Vojta et al. (2015) (Nature) Scientific Reports

L5

Designable modularity for engineering cellular logic and new protein folds

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Modularity is extensively used in engineering to allow rapid and effective construction of complex devices and structures. Biological systems are also composed of versatile structural and functional modules. Construction of complex devices requires large sets of orthogonal elements with similar properties, which may be difficult to obtain from nature. Nucleotide sequence provides a large and easily accessible combinatorial diversity that can underlay programming in biological systems. Knowledge of the recognition code of the sequence-specific DNA binding proteins allows us prepare large number of orthogonal DNA binding proteins. The designable DNA-binding TALE domains can be used to construct genetic logic gates and more complex information processing circuits such as dynamic bistable switches where the nonlinearity is introduced through feedback loops. In terms of the modular construction of structures designable orthogonal coiled-coil dimers provide the basic building blocks based on the specificity of interactions between segments of the polypeptide chain. This principle allows design of new modular protein folds resembling designed DNA nanostructures. The structure and function of designed single chain polypeptide can be encoded by the selected order of coiled-coil forming modules.

L6

Recombinant allergens for diagnosis and treatment of allergies

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Allergic diseases are among the most common chronic disorders worldwide affecting up to 25 % of the population, and therefore require the development of adequate forms of diagnosis and therapy. Allergic manifestations comprise a variety of symptoms ranging from mild forms of hay-fever, severe reactions of bronchial asthma, food allergies, and even fatal outcome in cases of anaphylactic shock. The applicability of natural allergen extracts for diagnosis and allergen-specific immunotherapy (ASIT) is limited by their poor quality, batch-to-batch variations, presence of nonallergenic materials and contaminations.
Nowadays, most of the disadvantages of natural allergen extracts can be overcome because DNA sequences of allergens became available and allowed production of defined recombinant allergens and hypoallergenic allergen derivatives for diagnosis and specific immunotherapy. Unmodified recombinant allergens provide novel opportunities to refine the diagnostic procedures of IgE mediated allergy. They can be combined to form a well-characterized composition containing an optimal amount of relevant allergenic components of a natural extract. In the context of therapeutic treatment of allergy, recombinant allergens enable a more specific diagnosis by determining the sensitization profile before application of specific immunotherapy (SIT). By the employment of genetic engineering, various approaches in molecule design with the modified immunological properties have been created. Hypoallergens that have reduced IgE binding capacity but retained T cell reactivity have also been produced, which have been investigated in clinical trials.
Our present studies are focused on production of recombinant allergens for allergy diagnosis, and design of himeric molecules for modulation of the specific immune response by promoting of specific IgG and reduction of IgE response.

L7

New ways to revert Warburg-type metabolism

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Warburg-type metabolism (named after Otto Warburg, a Nobel laureate) is an adaptation to the metabolic needs of rapid cell division. Warburg metabolism is characterized by decreased mitochondrial oxidation, while the flux of mitochondrial synthetic processes, glycolysis and the pentose-phosphate shunt increases to support the excess need of cells for purine and pyrimidine bases and membrane lipids. Although originally described as a "passive" adaptation of tumor cells to hypoxic environment, it is clear now that switching to Warburg-type metabolism is an active process governed by the intricate web of cellular energy sensors. These energy sensors therefore represent possible pharmacological targets to revert Warburg metabolism and to slow down cellular proliferation.
We aimed to identify pathways that are accessible by pharmacological means and can be used to revert Warburg metabolism. We studied breast cancer, since breast cancer rely on the Warburg-type rearrangement of metabolism, as the presence and the extent of Warburg metabolism affects proliferation, chemosensitivity and clinical outcome. In order to map metabolic changes, we mapped mitochondrial oxidation, the flux of glycolysis and the pentose-phosphate shunt, furthermore, characterized the activity of the energy sensor pathways and changes in cell cycle regulation.
In my talk I will show examples for pharmacological reversion of Warburg metabolism and I will discuss *in vitro* and *in vivo* methods.

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L8

Redox regulation: from redox congeners to a systemic molecular physiology approach

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Redox chemistry lies at the basis of life, providing mechanisms that are directly involved in the generation of cellular energy. Furthermore many redox reactions regulate cellular functions, thus linking ATP production with oxygen demand and mitochondrial functioning. Mitochondrial respiration generates the superoxide anion that precedes different redox congeners, referred to as reactive oxygen species (ROS). Increased concentrations of ROS underlie cellular oxidative stress when the redox balance is shifted towards a prooxidative state. When ROS production is controlled and compartmentalized, the ROS participate in different subtle redox modifications of regulatory molecules. Thus ROS are involved in the regulation of cellular functions. Several cellular active enzymatic mechanisms participate in ROS production, such as NADPH oxidase or cyclooxygenases, which serve as mediators of cellular functions. On the other hand, redox balance is provided by a set of antioxidant enzymes that utilize NADPH and GSH to prevent an uncontrolled shift towards a more oxidative cellular milieu. The sum of these processes is stable but dynamic redox homeostasis. With cellular gases, NO and H_2S, the small redox congeners form a discrete cellular redox-based and reversible network referred to as redox signaling, which is characterized by reversibility and short-lasting activity. The chemical properties of the generated signaling molecules determine the extent and magnitude of their physiological impact. These mediators are ideally suited for short-range effects by virtue of their ability to diffuse through tissue. This is especially important in the physiological regulation and fine-tuning of small vessel vascular tone and smooth muscle contractility. The link between local oxygen demands, the energetic state and physiological regulation is mediated by complex redox interactions in erythrocytes where the redox state can trigger different output signals ranging from NO to ATP. In erythrocytes, phase transitions of redox congeners are partially achieved by superoxide dismutase activity, which has been proposed to represent an enzymatic stabilizer of the cellular feedback mechanism that controls the cell's redox equilibrium, and of the physiological levels of NO.

L9

Lysosomal cathepsins in cancer: from cell death to therapy

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Endolysosomal compartments are responsible for a significant part of intracellular material degradation. However, during lysosomal membrane permeabilization (LMP), the function of endo/lysosomal compartment is compromised and the luminal contents including lysosomal proteases are released into the cytosol to various extents. Such LMP was shown to initiate the lysosomal apoptotic pathway and using the lysosomotropic detergent L-leucine-L-leucine-OMe (LLOMe), we could identify lysosomal cysteine cathepsins as the critical players in the pathway. Following their release into cytosol, they activate Bid and degrade several antiapoptotic members of Bcl2 family leading to subsequent mitochondrial membrane permeabilization, which was found to be a critical downstream event in apoptosis. Especially in cancer, triggering LMP could represent a therapeutic window to kill cancer cells. In addition, even at nonlethal concentrations, such mechanism of lysosomal cysteine cathepsin release itself excludes a protective role of autophagy at the early stage of lysosomal apoptotic pathway, providing an opportunity for the combined therapy with other cytotoxic agents.
However, in cancer cysteine cathepsins are also massively secreted into the tumor microenvironment from various infiltrated cells, especially of immune origin, such as macrophages. Therapeutic inhibition of cathepsins or diminishing their activity by gene ablation was found to have a profound effect on tumor growth in several animal cancer models, thereby demonstrating a therapeutic potential. The two sides of this coin will be discussed

L10

Single-stranded DNA binding protein has a key role in chromosome segregation during morphological differentiation of *Streptomyces coelicolor*

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SSB proteins bind ssDNA with a high affinity and in a sequence independent manner thus protecting transiently formed ssDNA during DNA replication, recombination and repair. In addition, SSBs interact and modulate the activities of various proteins involved in DNA metabolism. Analysis of available bacterial genomes revealed the presence of multiple copies of SSB proteins in many bacteria. The number of *ssb* genes can vary even among closely related species thus indicating that evolution of these proteins in Eubacteria is highly dynamic. However, the role of duplicated SSB proteins is poorly studied. Multicellular bacterium *Streptomyces coelicolor* with two *ssb* genes was selected to study biological role(s) of paralogous SSB proteins. Gene expression analyses suggested that SsbA and SsbB may have different biological roles. In concert with this, the EMSA assays and fluorescent titrations showed that SsbA and SsbB bind to ssDNA with different affinity. In addition, results of the gene disruptions have strongly indicated that *ssbA* is essential for survival while *ssbB* is important during the sporulation process. Crystal structures of these proteins also revealed some structural variations that led us to hypothesize that SsbB binding activity might be regulated during oxidative stress in *S. coelicolor*. To get a better insight into the function of SsbB we examined the impact of disulfide bridges removal on SsbB activity/stability. Cysteine residue (Cys7) in SsbB was substituted by alanine or serine. The gene *ssbB* carrying *cys7* mutation was not able to complement *S. coelicolor* strain lacking *ssbB*. Next, the study of the wild type protein and two SsbB mutants by differential scanning calorimetry showed decrease of thermal stability in the series: SsbBwt>SsbB/Ala>SsbB/Ser. Furthermore, binding of 45dT studied by isothermal titration calorimetry has strongly indicated that the concentration of the active conformation of the protein also declines in that series (SsbBwt>SsbB/Ala>SsbB/Ser). To shed the light on the complex mechanism of cell division in *Streptomyces,* we have constructed double (*ssbBparB; ssbBsmc)* and triple (*ssbBparBsmc*) mutant strains carrying mutations in *ssbB* gene and in the genes previously reported to be important for the chromosome segregation. By fluorescence microscopy we examined the effect of these mutations. The results showed more severe defects in nucleoid segregations during sporulation than previously reported for parental strains.

L11

A new way to rescue DNA damage-stalled transcription

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DNA replication is a high fidelity process that ensures that the genetic information of the daughter cell is identical to the information of the parental cell. However, DNA damage represents a challenge to replication, because the replicative DNA polymerase cannot bypass most DNA lesions. Since stalled replication forks can break leading to genomic rearrangements and cell death, DNA damage tolerance (DDT) mechanisms are activated at stalled forks that can sustain replication on damaged templates without removing the damage. One mechanism of DDT is translesion synthesis (TLS), where specialized DNA polymerases with lowered selectivity and fidelity replace the replicative polymerase and synthesize across the damage. TLS can be error-free contributing to genomic stability, or error-prone leading to genomic instability.
Transcription can also be blocked by DNA damages in the transcribed strand. Transcription blocking lesions can be removed by transcription-coupled repair, during which the lesion is excised from the re-annealed double-stranded DNA, and repair synthesis restores the original sequence. However, *in vivo* experiments suggested the existence of a damage bypass mechanism operating during transcription. So far, translesion RNA synthesis has been thought to be performed by RNAPII itself with the aid of elongation factors. TFIIF, TFIIS, Elongin and CSB purified from HeLa cells were shown to help RNAPII to bypass certain DNA lesions. However, it is still poorly understood how the high variety of transcription blocking lesions can be bypassed, and what other factors influence transcriptional fidelity.
During our work we have identified a new transcription elongation factor and our results show that it can facilitate RNA translesion synthesis.

L12

A functional genomics approach to understand pathological features of disrupted cholesterol synthesis in the *Cyp51* liver knockout mice

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Non Alcoholic (Fatty) Liver Disease (NAFLD) is a progressive liver disease with increasing prevalence due to epidemics of obesity and diabetes. Cholesterol homeostasis is among lipid pathways associated with NAFLD. Our research in *Cre-loxP* mice with deleted *Cyp51* from cholesterol synthesis in hepatocytes (LKO) (Lorbek *et al*., Sci Rep 2015) revealed that a block in cholesterol synthesis leads to steatohepatitis-like features with inflammation and fibrosis but without lipid infiltration (steatosis). Consequently, steatosis and steatohepatitis might represent separate conditions with CYP51 and cholesterol synthesis among the determinants. Gene expression profiling and biochemical analyses indicate that reduced cholesterol esters provoked cell cycle arrest, senescence-associated secretory phenotype and oval cell response, while elevated CYP51 substrates lanosterol and 24,25-dihydrolanosterol promoted the integrated stress response. A female-biased down-regulation of primary metabolism was identified on gene expression level with stronger immune response in LKO males. Interestingly, the most severe *Cyp51* LKO phenotype (runts) was prevalent in males. Mice stopped development prior to adulthood due to severely damaged livers and life threatening jaundice. The expression profiling and pathway enrichment analyses identified additional activation of TGFβ and growth factor (VEGF, PDGF, ErbB) signaling, apoptosis and cancer related pathways, indicating liver regeneration in response to chronic damage. Inflammatory transcription factors (NFkB, c-Jun, Ap-1, Stat3) were enriched while the metabolic (Hnf4a, Fxr, Rxr, Srebp) were damped. Rorc target genes were downregulated in runts further disrupting liver function. This is in accordance with findings that post-lanosterol intermediates play roles as RORgt ligands (Santori *et al*., Cell Metabolism 2015). To simulate the whole body consequences of the *Cyp51* liver conditional knockout we applied the SteatoNet mathematical model (Naik *et al*., PLOS Comput Biol 2014) whose major benefit is the multilevel structure (gene, transcript, enzyme, regulation, etc.) and incorporation of liver interactions with other tissues through the blood.

L13

Role of IL-6 in cancer development

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Immune reaction to malignant tumors has two faces, one that fights cancer and the other, the opposite, which facilitates and supports all aspects of cancer development ranging from tumor initiation to its metastasis. Therefore, inflammation is becoming one of the cancer's hallmark. Understanding both aspects of immune reaction is vital because it opens avenues for manipulating cancer development. Benefits of basic research exploration in this area are becoming visible in the new form of immunotherapy (like CTLA4 and PD-1 inhibitors) as well as use of anti-inflammatory drugs in, for example, colon cancer trerapy. Interleukin-6 (IL-6) was discovered 50 years ago as a lymphocyte B maturation factor and its role as main proinflammatory cytokine is well documented. First hint that IL-6 could be involved in tumor pathogenesis came from studies associating elevated IL-6 serum concentration with increased risk of developing colorectal cancer. Further, the elevated IL-6 serum levels or its expression in tumor tissue is linked to poor prognosis. Importantly, IL-6 is linking two main signaling hubs in cancer-related inflammation, the NF-κB and STAT3 signaling pathway; IL-6 is NF-κB target gene and most of protumorigenic IL-6 signaling is mediated by STAT3. All this positions IL-6 in the center of the cancer - inflammation relationship. Using colorectal cancer mouse models (ayoxymethane and dextran sodium sulphate model), we demonstrated that genetic ablation of IL-6 in mice resulted in reduced number and size of colonic adenomas. Constitutive activation of gp130 protein as well as the addition of recombinant IL-6 protein, led to tumor multiplicity increase and the increase in the tumor size. In contrast, inhibition of IL-6 signaling by soluble gp130 protein or by anti-IL-6 antibody reduced tumor size in the same mouse model. Stimulation of proliferation and inhibition of apoptosis in epithelial cells mediated most of the IL-6 effects on tumor development. Next, bladder cancer (BC) is of particular importance for studying inflammation-cancer relationship since it can be initiated by chronic inflammation caused by *Shistosoma haematobioum* infestation and is successfully treated by acute inflammation induced by BCG infection. Further, urinary bladder is easily accessible organ and is therefore, easily subjected to faster development of appropriate therapy. In addition to data indicating important function of IL-6 in colon cancer development, we will present our preliminary data considering role of IL-6 in bladder cancer development.

L14

Allergenicity and sensitizing potential of enzymatically cross-linked food allergens

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Protein cross-linking in food may occur naturally, during processing, or by exposure to environmental pollutants. Enzymatic cross-linking of proteins that leads to formation of high molecular weight aggregates of proteins (and/or other compounds present in food, such as polysaccharides) is often exploited in the cereal, dairy, meat, and fish processing industry in order to improve mechanical and functional properties of food. Although enzymatic cross-linking of proteins has been carried out extensively, little is known about the health risks of these process-modified food proteins of very high molecular weight. Tailoring of protein structure is necessary for creation of new functional food properties, but brings a risk of creating foods with changed immunogenic potential. Therefore, new proteins and protein derivatives need to be tested before they are released into the food market in order to provide assessment of allergic potential of novel foods regarding prevention of development of *de novo* hypersensitivity and prevention of increase of allergenic potential of allergens already present in foods. IgE-binding of processed and modified foods or proteins is the most common method for examination of how food processing affects allergenicity of food allergens. How processing affects sensitization capacity is generally studied in animal models of food allergy by administration of purified food proteins, food extracts or allergens present in their natural food matrix. There are several reports on the effect of enzymatic cross-linking on human IgE binding to cross-linked food allergens. Most of the published studies confirmed reduction of *in vitro* allergenicity to various enzymatically cross-linked food allergens, but the magnitude of allergenicity reduction was modest in most cases. Several recent studies examined the effects of enzymatic cross-linking on sensitizing potential of food proteins in animal models of food allergy. It has been shown that treatments of peanut proteins with tyrosinase, or milk casein with transglutaminase, did not change protein sensitizing capacity in mouse model of food allergy. However, laccase treatment of beta-lactoglobulin promoted both immunogenicity and allergenicity of this major milk allergen in mice. Cross-linking of beta-lactoglobulin reduced its epithelial uptake, but promoted sampling through Peyer's patches. Similarly to the cross-linking, pasteurization-induced aggregation promoted allergic responses to globular whey proteins, such as beta-lactoglobulin and alpha-lactalbumin, by redirecting their uptake to Peyer's patches. Although IgE-binding of cross-linked food allergens is often reduced due to disruption of conformational IgE-binding epitopes, globular food allergens may show an increased sensitizing potential *in vivo*. Thus, assessment of highly polymerized food proteins is of clinical importance in food allergy.

L15

Aminoacyl-tRNA synthetase editing preserves the canonical genetic code

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Aminoacyl-tRNA synthetases (aaRS) catalyze ATP-dependent covalent coupling of cognate amino acids and tRNAs for ribosomal protein synthesis. The cellular requirements for accurate aminoacylation are high because this reaction defines the genetic code. Yet, some aaRSs are incapable of discriminating against structurally similar amino acids during the synthetic reaction. To keep errors in aminoacyl-tRNA synthesis low these enzymes have evolved additional editing mechanisms. Pre-transfer editing involves hydrolysis of non-cognate aminoacyl-AMP intermediates within the synthetic active site, and can be stimulated by tRNA. Post-transfer editing operates through hydrolysis of misaminoacylated tRNA in a separate dedicated protein domain. The balance between the pre- and post-transfer editing pathways is dictated by kinetic partitioning of aminoacyl-AMP between the aminoacyl transfer step and hydrolysis. The requirement for rapid synthesis of aminoacyl-tRNA within the aaRS synthetic site may have provided an evolutionary driving force for the acquisition of a separate catalytic module committed to proofreading.
This emerging view unveils aaRS proofreading as a fortress of canonical translation. Under various stress conditions nonproteinogenic amino acids may accumulate and threaten the accuracy of protein synthesis. We have shown that the prime target for *Escherichia coli* leucyl-tRNA synthetase (LeuRS) proofreading is norvaline, a nonproteinogenic amino acid that accumulates under microaerobic growth conditions and decreases cell viability when incorporated into the proteome. This contrasts with the generally accepted view in which a key role for LeuRS editing is to prevent the misincorporation of isoleucine at leucine codons. Our detailed kinetic, thermodynamic, structural and *in vivo* analyses have established that LeuRS discriminates well against isoleucine in the synthetic reaction; previous contrasting findings appear to have been based on utilization of impure isoleucine samples contaminated with leucine. Accumulation of norvaline may also jeopardize the accuracy of Ile-tRNA^{Ile} synthesis. Hence, isoleucine/norvaline substitution in proteins is prevented by the editing activity of isoleucyl-tRNA synthetase. This work has uncovered aaRS translational quality control as a novel part of the adaptive response that protects *E. coli* cells in rapidly changing oxygen environments.

L16

Protein kinases: enzymes working in signalling brigades

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Protein kinases control diverse cellular processes by playing a major role in cell signalling as intracellular enzymes. In addition to their upstream activators and downstream substrates, they bind to other signalling proteins. Thus, protein kinases rarely work alone, they are normally part of multi-protein signalling complexes where their impact on a specific biological outcome (e.g. cell division, apoptosis or differentiation) depending on the protein-protein interactions that they form with other dedicated signalling proteins. Using mitogen activated protein kinases (MAPK) as a model system we studied the interactions that govern the assembly of specific MAPK dependent signalling complexes. Our latest results reveal how ERK5, ERK2 and JNK MAPK signalling modules assemble into functional complexes. This set the stage for the discovery of novel compounds that may selectively interfere with specific functions of ubiquitous MAPKs through targeting the interactions rather than the catalytic capacity of the members of a specific “signalling brigade”.

L17

Cross-talk of glioblastoma and mesenchymal stem cells modulates signalling pathways in tumour cells

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The most aggressive subtype of brain tumours is glioma WHO grade IV, the glioblastoma multiformae (GBM), where not only the tumour, but also stromal cells play an important, even crucial role, due to an intensive cross–talk among these cells. The present work focuses on mesenchymal stem cells (MSC), not only as a part of GBM microenvironment but also as potential vectors for cellular therapy, the promising future cancer treatment modality. However, the mechanisms underlying MSC-mediated modulation of tumour behavior *via* paracrine and direct interactions with GBM and their stem cells (*Molina at al., 2014*) is not well understood at the molecular level. Previously, we have confirmed that bone marrow derived MSC (BM-MSC) paracrine effects impaired proliferation, invasion and senescence of several GBM lines (rev. in *Motaln and Lah, 2014*), whereas in contrast enhance aggressiveness of GBMs cells upon direct cellular contacts. By performing transcriptome analyses and cytokine profiling we found striking differences in the two types of interactions, also with respect to the alterations of the respective genes' expression. Focusing on the most relevant activation of invasion facilitating genes, the expression levels of bradykinin receptors B1R and B2R were found increased in GBM cells U87-MG upon direct BM-MSC contacts. The BR1 and BR2s upregulation in the GBM cells were also proven at protein levels, and validated by their activity, *i.e.* Ca^{++} influx and nitric oxide (NO) release. The related intracellular signalling is known to induce migration, associated with invasion and extracellular matrix degradation. We demonstrated that this was accompanied by upregulation of a set of proteases, such as MMP9, urokinase (PA) and cathepsin B. Furthermore, co-culturing of GBM and MSC, not only affected the expression rates of kinin receptors, but also CD73 (5'-ectonucleotidase), a key enzyme of the purinergic system, known to be coupled to kinin receptors. CD73 is metabolizing 5'-AMP into adenosine and thereby determining activity levels of adenosine receptors, affecting GBM cell phenotype. In conclusion, molecular pathways revealed in our studies in the cellular cross-talk concept for the first time, support the notion that MSCs posses an intrinsic ability to alter GBM cells, and the former may be exploited in the design of future cellular therapies targeting glioma.

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L18

Anticancer effects of vitamin C: The great cell culture swindle?

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A renewed interest in the applicability of vitamin C for cancer treatment led to over 20 cell culture studies showing that pharmacological concentrations of vitamin C efficiently kill a variety of cancer cell lines. The mechanisms of action involve iron which, in combination with vitamin C and molecular oxygen, generates hydrogen peroxide. This reactive oxygen species enters cancer cells and inflicts intracellular damage. Most of the examined primary cell types were unaffected by vitamin C, probably because normal cells generally show higher baseline activity of endogenous antioxidative system compared to related cancer cells. However, a drawback in all the *in vitro* studies was their failure to take into account the *in vivo* iron level, which is higher compared to concentration of iron in cell culture media. We showed that the supplementation of media with iron to correct the discrepancy annihilates anticancer effects of vitamin C. At physiological iron concentrations, the balance between hydrogen peroxide production and decomposition is shifted towards the latter, because iron reacts with hydrogen peroxide not allowing its accumulation. It appears that the anticancer effects of vitamin C have been significantly overestimated. This might be true for some other redox-active agents as well, such as (poly)phenols. We urgently need to establish medium formula and culture maintenance settings that are optimal for redox research.

L19

Microbiome bioinformatics: computational analysis and modeling of signaling in complex bacterial communities

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Multispecies microbial communities are a major form of life that occur both in open environments and also as *microbiomes* - symbiotic communities that coexist with other organisms. Their cells compete for space and resources while they also communicate and cooperate via secreted molecules. Signaling systems are used to screen the environment for potential cooperating cells (mostly members of the same strain or species), while cooperative systems use metabolically expensive, secreted molecules that enable the community to solve problems that individual cells can not solve by themselves. The simplest such systems, involved in bacterial *quorum sensing* consist of a signal synthase and a sensor-receptor protein. A computational survey of the available genomes revealed variants of the underlying regulatory circuits [1-2] and also pointed out subgroups of sensor-receptor proteins that specialize on external signals [3]. Computer simulations show that non-communicating mutants can be part of stable communities while non-cooperative ones will collapse the community [4]. Microbiomes can contain many thousands of secreted molecules involved in intercellular interactions within and among species. Sharing of signals seem to stabilize communities [5] while horizontal transfer of resistance genes leads to territorial defense that enables communities to protect themselves with a battery of antimicrobials while preserving the varied metabolic repertoire of the constituent species [6]. Interesting analogies exist between gene spreading and virus integration [7].

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L20

Forces in the mitotic spindle

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At the onset of division, the cell forms a spindle, a precise self-constructed micro-machine based on microtubules and the associated proteins, which divides the chromosomes between the two nascent daughter cells. The attachment of microtubules to chromosomes is mediated by kinetochores, protein complexes on the chromosome. Microtubules generate forces on kinetochores, which are responsible for kinetochore congression to the metaphase plate, silencing of the spindle assembly checkpoint, and segregation of sister chromatids. During metaphase, forces on kinetochores are exerted by k-fibers, bundles of microtubules that end at the kinetochore. Interestingly, non-kinetochore microtubules have been observed between sister kinetochores, but their function is unknown. Here we show by laser-cutting of a k-fiber in HeLa cells that a bundle of non-kinetochore microtubules, which we term ‘bridging fiber', bridges sister k-fibers and balances the tension between sister kinetochores. We found PRC1 and EB3 in the bridging fiber, suggesting that this bundle consists of anti-parallel dynamic microtubules. By using a theoretical model that includes a bridging fiber, we calculate the forces at the spindle pole and at the kinetochore, and show that they depend on the number of microtubules in the bridging fiber. We conclude that the bridging fiber, by linking sister k-fibers, withstands the tension between sister kinetochores and enables the spindle to obtain a curved shape.

L21

RNA-protein interactions in ALS and FTLD

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Amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD) are devastating neurodegenerative diseases that form two ends of a complex disease spectrum. Aggregation of RNA-binding proteins TDP-43 and to a lesser extent FUS is one of the hallmark pathological features of ALS and FTDL, and suggests perturbance of the RNA metabolism in their aetiology. In 95% of all ALS and 60% of FTLD patients the aggregating protein is TDP-43, thus defining the major part of the disease spectrum as TDP-43 proteinopathies. However, only a very small percent of aggregation is caused by TDP-43 mutations. 'FUSopaties' are less common with mutant FUS aggregation associated with ~ 5% ALS and wildtype accumulation in 5-10% of FTLD. Functional and pathognomic similarities between TDP-43 and FUS point to a crucial pathogenic pathway potentially involving RNA processing and transport. The disease importance of RNA related upstream processes has recently been further substantiated with association of the GGGGCC expanded repeat mutation in C9orf72 with ALS/FTLD. The main questions in the field are what makes wild-type or mutated TDP-43 or FUS aggregate in ALS and FTLD and what is the role of perturbed RNA metabolism in these diseases.

L22

4-Phenylbutyrate reduces calcification in mice expressing human ABCC6 mutants: a preclinical *in vivo* model for allele-specific therapy of PXE and GACI

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Mutations in the ABCC6 gene cause ectopic calcification affecting ocular and dermal tissues but also the media of the vasculature in pseudoxanthoma elasticum (PXE) and some generalized arterial calcification of infancy (GACI) cases. ABCC6 facilitates the cellular efflux of ATP from hepatocytes, which is rapidly converted into pyrophosphate (PPi) in the liver vasculature and enters into the circulation. We have found that most missense disease-causing ABCC6 mutations induce conformational changes that prevent normal trafficking to the plasma membrane but often preserve high residual ABCC6 transport activity. We have expressed such mutants *in vivo* in the liver of wt and *Abcc6*-/- mice and demonstrated that these mutants can be "redirected" into the plasma membrane of the hepatocytes upon treatment with a chemical chaperon, 4-phenylbutyrate (4-PBA). Next we addressed whether 4-PBA treatments could rescue the physiological function of ABCC6 mutants *i.e.* calcification inhibition in our humanized mouse model. We used dystrophic cardiac calcification as an ABCC6-specific phenotype in *Abcc6*$^{-/-}$ mice to determine the effect of 4-PBA treatments on transiently expressed disease-associated ABCC6 mutants in the liver. We found that while missense ABCC6 variants expressed in the liver of Abcc6-/- mice do not reduce *in vivo* calcification, the concomitant administration of the chemical chaperon largely attenuates ectopic mineralization (by redirecting the mutants to the plasma membrane), thus restoring the physiological function of the protein.
In summary, we have successfully tested and demonstrated the feasibility of the repurposing use of 4-phenylbutyrate, a drug approved for clinical use by the FDA, for allele-specific therapy to correct the pathophysiological calcification caused by missense ABCC6 mutations associated with both pseudoxanthoma elasticum and generalized arterial calcification of infancy. As 75% of patients are with at least one missense allele, the potential of the described intervention model may be significant. Our results also suggest that plasma PPi is a useful indicator of ABCC6 function.

SL1

Ethical aspects in genomics

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The advances in the field of genomics enabled the discovery of the genes and molecular pathways that play fundamental roles in numerous diseases. The human genome research revolutionized the diagnosis, therapy and prevention of human diseases. This rapid pace of development in genomics is followed by the increased ability to share data, giving a new approach to serious ethical questions of consent, feedback of results, privacy, and the governance of research. For the effective development of human genomics and translation of novel, validated biomarkers into potentially useful clinical applications in personalized medicine, there is a need for clear ethical standards and principles in all phases of clinical research. The magnitude of the medical benefit depends on many technological and analytical factors, but above all on principal bioethical postulates of avoiding any medical or psycho-social harm and on the patient informed consent being a *sine qua non*.
Comprehensive analyses of genomic data sets to advance biomedical research and clinical practice cannot be done without international collaboration, a vast computer infrastructure and advanced software tools. Since genomic projects analyzing thousands of genomes for disease research generate petabytes of data, the researchers are increasingly turning to cloud computing as an approach to store and analyze data. However, although providing several benefits, genomic cloud computing does not come without concerns regarding data security, reliability, data control, confidentiality and transfer.
Strictly ethical and non-discrimination policies such as the European Council protocol on genetic testing for health purposes and Genetic Information Nondiscrimination Act (GINA) in USA, should be applied in each phase of genomic research in medical genomics, during the current period of transition from the investigational to the practical personalized medicine. Patients should be protected from harms of premature translation of research findings, while encouraging the innovative and cost-effective application of novel data into personalized medical care.

SL2

Science integrity in publishing

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Although science publishing has dramatically changed in the past 15 years due to new digital technologies, the rise of interdisciplinary research, appearance of big data science, introduction of alternative peer view systems, novel business models and open access publishing, trust and credibility must remain its most valued core principles. However, increasing number of misconduct cases and retracted publications, serious questions and scrutiny about reproducibility of published scientific results, appearance of unethical practices and predatory journals in the publishing industry have weakened these principles. The healthy reaction of the scientific community and publishers have resulted in several recent initiatives which include the European Code of Conduct for Research Integrity, the San Francisco Declaration on Research Assessment, new data standards, guidelines and recommendations to enhance rigor and further support research that is reproducible, robust, and transparent; these will be reviewed discussing their potential benefits and impacts.

LH1

The sleeping beauty transposon system and its applications in molecular medicine

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The *Sleeping Beauty* (SB) transposon system yields efficient stable gene transfer following gene delivery into primary cell types, including stem cells that are relevant targets for regenerative medicine and gene- and cell-based therapies of complex genetic diseases.
The inherent risks associated with vector insertion in gene therapy need to be carefully assessed. We compared distributions of SB and *piggyBac* (PB) transposon insertions as well as MLV retrovirus and HIV lentivirus insertions in human CD4+ T cells with respect to 40 chromatin states defined by combinations of genomic characteristics specified in 70 different genome-wide datasets. The SB transposon displayed the least deviation from random with respect to genome-wide distribution. We have identified remarkable parallels between integration site distributions of the PB transposon and the MLV retrovirus across all 40 chromatin states. We detect unequal biases across the four systems with respect to targeting genes whose deregulation has been previously linked to serious adverse events in gene therapy clinical trials. Our data highlight the significance of vector choice to minimize the relative chance of insertional oncogenesis in clinical applications.
We applied the SB system for non-viral gene delivery in both mouse and human hematopoietic stem cells. We optimized our protocols by applying minicircle vectors to deliver the transposon and synthetic mRNA to deliver the transposase into this hard-to-transfect cell type. With this optimized protocol in hand, we are in the process of setting up preclinical gene therapy in mouse models of Gaucher disease, an inherited lysosomal storage disorder.
We established iPSC reprogramming of mouse and human fibroblasts by SB transposition. We demonstrate Cre recombinase-mediated exchange allowing simultaneous removal of the reprogramming cassette and targeted knock-in of an expression cassette of interest into the transposon-tagged locus in mouse iPSCs. This strategy would allow correction of a genetic defect by site-specific insertion of a therapeutic gene construct into “safe harbor” sites in the genomes of autologous, patient-derived iPSCs.

LH2

Mesenchymal stem cells in veterinary medicine

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Mesenchymal stem cells are adult stem cells found in different adult tissues such as adipose tissue, bone marrow, and also umbilical blood. Mesenchymal stem cells are capable of differentiation into bone, cartilage and adipose tissue, and several reports also suggest that mesenchymal stem cells might be capable of transdifferentiation into muscle and neural cells. In addition to differentiation potential, mesenchymal stem cells might have other beneficial properties for pathological processes and studies in recent years suggest that mesenchymal stem cells might have immunomodulatory, antiinflamatory and trophic actions, contributing to the healing process in injured/diseased tissues. Osteoarthritis is a chronic, progressive disorder with debilitating effects in both animals and humans. It is particularly common in some dog breeds, but also fairly common in humans. Currently, there is no cure for such conditions, but studies in recent years in both human and veterinary medicine suggest that mesenchymal stem cells might have beneficial effect on chronic osteoarthritis. In our laboratory, we have developed a novel method of treating osteoarthritis using autologous adipose tissue derived mesenchymal stem cells in dogs. Stem cells are collected from patients' adipose tissue, prepared in the laboratory and injected directly into affected joint(s). To prove the efficacy if this method, we have performed blind placebo study in dogs with bilateral osteoarthritis in stifle joints (knee), by treating one joint with stem cells and other with placebo (buffer used for cell delivery). Results of clinical examination revealed beneficial effect of stem cell treatment in osteoarthritic knees and x-ray imaging, and although with important limitations, results do suggest that degenerative processes in the dog stifle joints treated with stem cells were reduced or even reversed by the application of stem cells.

LH3

Oxidized phospholipids inhibit the formation of mobile cholesterol-dependent plasma membrane nanoplatforms

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We have previously developed a single-molecule microscopy method termed TOCCSL ("Thinning out Clusters while Conserving Stoichiometry of Labeling")[1,2], which allows for direct imaging of stable nanoscopic platforms with raft-like properties diffusing in the plasma membrane. As the consensus raft marker we chose monomeric GFP linked via a GPI (glycosylphosphatidylinositol) anchor to the cell membrane; for this probe we previously observed cholesterol-dependent homo-association to nanoplatforms diffusing in the plasma membrane of live CHO cells. I will briefly discuss how the use of the single-molecule FRAP approach termed TOCCSL enabled for the first time the direct imaging of these mobile nanodomains under varying environmental conditions.
In our present study, we report the release of this homo-association upon addition of 1-palmitoyl-2-(5-oxovaleroyl)-sn-glycero-3-phosphocholine (POVPC) or 1-palmitoyl-2-glutaroyl-*sn*-glycero-3-phosphocholine (PGPC), two oxidized phospholipids typically present in oxidatively-modified low density lipoprotein. We found a dose-response relationship of mGFP-GPI nanoplatform disintegration upon addition of POVPC, correlating with the signal of apoptosis marker annexin V-Cy3. Similar concentrations of lysolipid showed no effect, indicating that the observed phenomena were not linked to properties of the lipid bilayer itself. Inhibition of acid sphingomyelinase (aSMase) by NB-19 before addition of POVPC completely abolished nanoplatform disintegration by oxidized phospholipids. In conclusion we could determine the mechanism how oxidized lipid species disrupt mGFP-GPI nanoplatforms in the plasma membrane. Our results favor an indirect mechanism involving aSMase activity and not a direct interaction of oxPL with nanoplatform constituents.

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LH4

Towards understanding the role of lipid metabolism in cellular stress response: from yeast models to human diseases

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Membranes are able to sense environmental changes and, by altering their physical state and microdomain organization, they adjust signals that activate the cellular homeostatic or stress-defence system. The membranes of eukaryotic cells contain thousands of lipid species that are specifically sorted and selected for cellular compartments, involved in membrane trafficking, regulating membrane proteins, and hence determining the biofunctionality. Lipidomics is aiming at quantitative spatial and temporal determination of all lipids to understand how the dynamics of lipid metabolism contributes to the regulation of biological structure and function and how lipid networks and pathways respond to changes in the environment.

To gain insight into interconnection between stress defence system and membrane-lipid metabolic network, yeast models were studied. Mass spectrometric analysis revealed that yeast cells responded to heat stress with profound reorganisation of the whole lipidome. In addition to the bilayer stabilizing compositional shift of membrane lipids, enhanced production of both signal lipids and storage lipids were observed. In the trehalose synthase yeast mutant, where trehalose – that has been shown to stabilize proteins and membranes during heat shock – cannot be formed, the stress-induced membrane-lipid remodelling, the triglyceride (TG) synthesis and the signal lipid generation were more pronounced. Furthermore, the TG-synthesis deficient mutant *S. pombe* cells displayed lipid mediator-related cell cycle arrest upon heat stress.

It has been demonstrated that membrane alterations, signal pathways from membranes to heat shock protein (Hsp) genes and Hsps themselves play fundamental roles in the aetiology of several human diseases, such as type 2 diabetes, neurodegenerative diseases and cancer. Lipidomics provides a powerful tool for the identification of potential lipid biomarker patterns predicting disease risk and uncover novel lipid metabolic pathways related to pathogenesis of the disease.

In respect to therapies, membranes are the typical examples for allo-network interaction sites since lipids are in the neighbourhood of the pharmaceutical target proteins. Molecules developed using the strategy of ‘‘membrane-lipid therapy’’ target the lipid phase of cellular membranes in order to effectively modulate sensory and signalling protein–lipid assemblies allowing the restoration of disease-related malfunctions with negligible side effects.

Abstracts of Short Presentations

(S and SP)

S1

Asymmetric protein-protein interactions in the symmetric homodimeric S100 calcium-binding protein family

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S100 proteins are small, dimeric EF-hand Ca^{2+}-binding proteins that are expressed exclusively in vertebrates and are involved in a number of cellular processes. In the Ca^{2+}-bound form they interact with their protein targets and generate biological effect. Importantly, overexpression of several S100 proteins are associated with serious diseases such as cancer metastasis, chronic inflammations and tissue fibrosis.
S100 binding linear motifs are usually localized in intrinsically disordered domains. Since S100 proteins are homodimers, they could interact symmetrically with two such motifs. However, we have recently determined the atomic structure of an unusual asymmetric complex in which a single non-muscle myosin 2 peptide wraps around the two chains of S100A4 (Kiss et al., PNAS, 2012, 109:6048). This interaction leads to the myosin filaments disassembly and an increase in cell adhesion and migration, ultimately contributing to metastasis.
Here I present evidence that the asymmetric mode of target recognition is more widespread in the S100 family. We solved the crystal structure of the S100A4 in complex with full-length annexin 2A (ANXA2), a non-EF-hand Ca^{2+}- and phospholipid-binding protein involved in diverse cellular, mostly membrane and cytoskeleton related processes and also associated with pathologies especially with cancer progression. The N-terminal disordered domain of ANXA2 interacts with S100A4 as of the myosin peptide, moreover, a second ANXA2 is also bound to one of the canonical hydrophobic target site of the dimer, making the stoichiometry of the complex 2:2. Interestingly, the latter binding site coincides with the binding of a shorter ANXA2 region to S100A10 which forms a symmetric heterotetramer complex. The third asymmetric interaction in the family was found in the complex of S100B (overexpressed in melanomas and gliomas) and Rsk1 (a MAPK-activated protein kinase that signals in diverse targets and implicated in inflammation and cancer). The peculiar feature of this “fuzzy complex” is that the terminal regions of the Rsk1 peptide interacts with the two canonical binding sites, nevertheless the connecting region remains flexible. It is noteworthy that detailed structural studies of these complexes could facilitate development of inhibitors for therapeutic application.

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S2

The activity of cysteine cathepsin K is fine-tuned via multiple allosteric mechanisms

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Allosteric communication between spatially distant sites in proteins is one of the principal modes of biological activity regulation. Targeting allosteric regulatory sites is an emerging strategy for drug design aimed at diverse molecular targets including enzymes and cell surface receptors. Allosteric drugs have the ability to fine-tune protein activity and therefore offer a major advantage over traditional active site-directed drugs.
We have been investigating the mechanisms of allosteric regulation in the family of papain-like cysteine peptidases with the aim of designing allosteric effectors for use *in vitro* and *in vivo*. Our primary model is human cathepsin K, an indispensable enzyme in bone metabolism due to its unique collagenolytic activity and also a promising target for the treatment of osteoporosis. Glycosaminoglycans were the first identified allosteric modifiers of cathepsin K and chondroitin sulfate was characterized as a strong activator of its proteolytic activity towards type I collagen, the major protein constituent of bone. Accumulating structural and functional data now indicate that glycosaminoglycans form diverse interactions with cathepsin K that involve multiple binding modes and can promote or inhibit the activity of cathepsin K on a wide range of substrates.
Using computational and experimental methods, we have identified a novel allosteric site on cathepsin K that binds small ligands and is distinct from the glycosaminoglycan-binding sites. Several hits were obtained from *in silico* library screening that bind at this site. The first characterized compound was NSC13345 which showed increasing inhibitory activity with substrate size, ranging from minimal inhibition with synthetic peptide substrates to full inhibition of type I collagen degradation. The compound acted as an antagonist of chondroitin sulfate and was able to completely abolish its activation effect on cathepsin K. NSC13345 also had good selectivity for cathepsin K over related peptidases qualifying it as a promising candidate for further development.
Several other compounds were characterized recently that bind to the same allosteric site. Interestingly, their activity profiles were different from NSC13345, demonstrating that multiple effects on cathepsin K activity can be triggered by binding to the same allosteric site. At the structural level, the effect of these molecules appears to depend on their mode of interaction with the allosteric site.

S3

Characterization of species-specific inhibitory effect of a staphylococcal repressor protein on dUTPases

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Proteins responsible for genome integrity are preferred targets in drug therapies against various diseases. Accordingly, dUTPase, an enzyme, that is essential for cell viability in many organisms, is subjected as a target of several drug research projects.
It was recently shown that Φ11 phage dUTPase is capable of interacting with a *Staphylococcus aureus* repressor protein called Stl during phage infection. We found that Stl can be a potent inhibitor of not only Φ11 dUTPase(*1*), but other dUTPases, such as the *Mycobacterium tuberculosis* (MTB) dUTPase(*2*). The exploration of species-specific elements of Stl inhibition may serve significant information for designing further species-specific inhibitors. The characterization of the interaction between dUTPases and Stl would also establish the grounds of the *in vivo* inhibition of dUTPases by Stl. Therefore we set out to explore the inhibition mechanism of Stl with the help of kinetic methods. Our results show that Stl inhibition has a slow-tight binding character in case of all investigated dUTPases, however, the extent of the inhibition is differs. For in-depth transient kinetic characterization of the dUTPase – Stl interaction we took advantage of the fluorescence signal changes of an active site tryptophan of dUTPase. By stopped-flow, we found that the Stl is a competitive inhibitor in case of both Φ11 and MTB dUTPases. Further investigation has shown that in case of Φ11 dUTPase three Stl molecules are able to bind to the homotrimeric enzyme with similar affinity, while in case of MTB dUTPase the affinity of the third Stl molecule is somewhat lower, which leads to the observed differences in the extent of inhibition under our assay conditions.
In summary, we have found that the interaction of these dUTPases and Stl is very similar for the individual active sites and the species specific differences in the extent of inhibition are the results of the differences within the quaternary structure of dUTPases.
We have already tried Stl as an in cell inhibitor and gained important results about the effects of dUTPase inhibition in *Mycobacerium smegmatis,* a non-pathogenic model of MTB(*2*).

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S4

Carotenoids are essential for the assembly of cyanobacterial photosynthetic complexes

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Carotenoids (carotenes and the xanthophylls) are pigments with paramount importance in plants and animals, as well. Due to their hydrophobic character, carotenoids are preferentially present inside or in the vicinity of the protein complexes. Occasionally, they can be localised in the lipid membrane environment not bound to any proteins. The carotenoids can influence the membrane dynamics, microviscosity, contribute to protection against (oxidative) stress and might also determine the assembly of some protein complexes e.g. in photosynthetic organisms. In plants and cyanobacteria the conversion of light energy into chemical energy is carried out by the thylakoid membrane associated photosystems amended by light harvesting antennae. Although wealth of information is available on the role of carotenoids in thylakoid membrane of plants, less is known about their impact in cyanobacteria.

We have studied the specific effects of different carotenoid forms on functional organisation of thylakoid membrane under non-stress conditions, which have been hitherto not clarified yet in cyanobacteria. Here we used *Synechocystis sp.* PCC6803 cells mutated at various steps of the carotenoid biosynthesis to obtain sequential elimination of the various carotenoid forms.

Although it is generally believed that xanthophylls do not part of the cyanobacterial photosynthetic complexes, our current results demonstrate that this picture has to be modified. We show that xanthophylls stabilise photosystem II (PSII) dimers and influence the structure of PSI trimers. The changes induced by the xanthophyll deficiency can also influence the excitation energy transfer within PSI and result in the decrease of the capability of cells to adapt to modified light conditions. Furthermore, in completely carotenoid-deficient cells the extramembranous light-harvesting complexes, the phycobilisomes are also distorted. This alteration is surprising because carotenoids have never been found as part of the phycobilisomes. In this mutant the number of phycocyanin rods connected to the phycobilisome core is strongly reduced with an increased amount of unattached phycocyanin units. Our results obtained from carotenoid-deficient mutant also cover a new “linker” function of β-carotene in PSI complex. One might speculate that xanthophylls and carotenes are essential ingredients of the assembly and maintenance of the machinery of the photosynthetic complexes in the cyanobacterial cells.

S5

Tracking of cholesterol/ sphingomyelin-rich domains in lipid mono- and bilayers by ostreolysin A-mCherry protein

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Ostreolysin A (OlyA) is a ~15-kDa protein from the mushroom *Pleurotus ostreatus*. It has been shown to specifically bind to membrane domains rich in cholesterol and sphingomyelin that fulfil the criteria of membrane rafts. Its interaction with artificial lipid and biological membranes can be abolished by pre-treatment of these membranes with methyl-β-cyclodextrin or sphingomyelinase.
Recently developed OlyA fused at the C-terminus with fluorescent mCherry protein (OlyA-mCherry) can be used to successfully label cholesterol/ sphingomyelin domains in artificial lipid vesicles, and in plasma membranes of both fixed and living Madin-Darby canine kidney (MDCK) epithelial cells. Double labelling of the MDCK cells with OlyA-mCherry and other toxin-based sphingomyelin- or cholesterol-specific protein markers shows different patterns of binding and distribution of OlyA-mCherry in comparison with these other proteins. Furthermore, OlyA-mCherry can be internalised in living MDCK cells.
Our results indicate that OlyA-mCherry is a promising tool for labelling a distinct pool of cholesterol/ sphingomyelin membrane domains in living and fixed cells. OlyA-mCherry is stable and has a relatively small molecular weight, is not cytotoxic, has optimal fluorescence properties, specifically senses the combination of the two most abundant and important raft-residing lipids, and does not oligomerise in the membrane. However, in contrast to lipid bilayers composed of cholesterol and sphingomyelin, OlyA-mCherry cannot bind lipid monolayers of the same lipid composition. This indicates that in addition to physical characteristics and lipid composition of cholesterol/sphingomyelin membrane nanodomains, specific distribution and availability of the constitutive lipids are essential prerequisites for OlyA-mCherry membrane binding.

S6

Plasticity of listeriolysin O pores and its regulation by pH and unique histidine

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Pore formation of cellular membranes is an ancient mechanism of bacterial pathogenesis that allows efficient damaging of target cells. Several mechanisms have been described, however, relatively little is known about the assembly and properties of pores. Listeriolysin O (LLO) is a pH-regulated cholesterol-dependent cytolysin from the intracellular pathogen *Listeria monocytogenes*, which forms transmembrane β-barrel pores. Here we report that the assembly of LLO pores is rapid and efficient irrespective of pH. While pore diameters at the membrane surface are comparable at either pH 5.5 or 7.4, the distribution of pore conductances is significantly pH-dependent. This is directed by the unique residue H311, which is also important for the conformational stability of the LLO monomer and the rate of pore formation. The functional pores exhibit variations in height profiles and can reconfigure significantly by merging to other full pores or arcs. Our results indicate for the first time a significant plasticity of large β-barrel pores, which is controlled by environmental cues like pH. This is an important step towards better understanding of the mechanism of pore formation by LLO and thus virulence of *Listeria*.

S7

Engineered probiotics with cytokine/chemokine binding ability

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Lactic acid bacteria (LAB) have received considerable attention in recent years, due to their long history of safe usage in food, and their health-promoting effects as probiotics. Recombinant LAB have been engineered for therapy, with most applications aimed at the delivery of antigens or biologicals to the human intestine. Delivery of non-immunoglobulin cytokine/chemokine-binding proteins by recombinant LAB could protect proteins in gastric or intestinal milieu, and neutralize or remove excessive inflammatory mediators. This could result in the alleviation of chronic inflammation, such as in inflammatory bowel disease.
A surface display system for model LAB *Lactococcus lactis* has been engineered by inducibly expressing a protein fusion of secretion signal, binding protein and cell wall anchor domain. Fusion proteins were secreted to the growth medium and enabled non-covalent display of binding proteins on recombinant *Lactococcus lactis*. Additionally, following incubation with used lactococcal growth medium, fusion proteins could also bind to the surface of other non-recombinant species of LAB, thus providing a non-genetically-modified organism alternative. Particularly strong binding was observed on the surface of *Lactobacillus salivarius* ATCC 11741, which has been suggested as an optimal non-recombinant host.
Displayed cytokine-binding proteins included TNFα-binding affibody, IL-17-binding fynomer and IL-23-binding adnectin. Chemokine-binding proteins included evasin-1, evasin-3 and evasin-4 with the ability to bind CXCL-2, CXCL-3, CXCL-8, CCL-3, CCL-4 and CCL-5. Bacterial removal of all cytokines/chemokines was demonstrated *in vitro* by ELISA. Binding efficiency of surface-displayed binding proteins differed. The highest binding was observed with fynomer (85 % of IL-17) and the lowest with adnectin (15 % of IL-23). Heterologous display of multiple binders on *Lactobacillus salivarius* enabled the construction of bacteria with concomitant IL-17-, IL-23- and TNFα-binding ability. Efficacy of engineered probiotics was also demonstrated in a cell model. Lower amount of CXCL-8 was produced by colon epithelial cells after incubation with evasin-3-displaying bacteria.
We have shown that engineered recombinant probiotics, alone or combined, can bind various cytokines/chemokines and interfere with their signalling. This could lead to the amelioration of excessive immune response and could be useful in the treatment of inflammatory bowel disease.

S8

Defense proteins from mushrooms offer numerous biotechnological applications

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Mushrooms are the reproductive structures of higher fungi and despite the short lifespan their protection against parasites and predators is essential. Their defence is mediated by a plethora of secondary metabolites that act as deterrents and as it's been established in the past few years they are also protected by a protein-based defence. Proteins that have been shown to be involved in defence of fungal fruiting bodies against various antagonists include lectins, protease inhibitors, biotin binding proteins, pore-forming toxins and ribotoxins. Many of them are much more abundantly expressed in fruiting bodies compared to the vegetative mycelium. They are small, stable proteins resistant to proteolytic digestion and exposure to extreme pH and temperature conditions and are located in the cytoplasm.
We have been working with two groups of these proteins, lectins and protease inhibitors, and characterized several novel proteins from mushrooms at genetic, biochemical and structural levels. We have characterized two lectins from edible mushrooms that show different degrees of toxicity to the model nematode *Caenorhabditis elegans*. They belong to the family of ricin B-like lectins with β-trefoil fold. CNL from *Clitocybe nebularis* is specific for terminal N,N'-diacetyllactosamine (GalNAcβ1-4GlcNac) and MpL from *Macrolepiota procera* specifically binds repeating units of N-acteyllactosamine (Galβ1-4GlcNac). Furthermore, we have characterized a few protease inhibitors that exhibit entomotoxic activity: two families of cysteine protease inhibitors, clitocypin from *C. nebularis* (Merops family I48), macrocypins from *M. procera* (Merops family I85) and a family of trypsin specific serine protease inhibitors (Merops family I66) with representatives from *C. nebularis* (cnispin) and *Coprinopsis cinerea* (cospin). They all have a beta-trefoil fold.
Versatility and unique features of proteins from mushrooms offer great variety of possibilities for their potential use in the fields of biotechnology, medicine and agriculture. We have shown that protease inhibitors can be used as ligands in affinity chromatography for isolation of specific proteases and in crop protection against insect pests. In addition, lectins have been considered for use in veterinary medicine against parasitic nematodes and as tools in cancer biology, where they could be used as immunomodulators or as tools for targeted delivery and glycoprofiling of cells and malignant transformations.

S9

Folding pathway of a designed protein tetrahedron

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Protein origami is an exciting emerging field, inspired by the success of DNA origami. Proteins however, unlike DNA, do not have simple complementarity rules. This problem can be elegantly solved by using modular components with exactly defined binding partners of which coiled coils are an excellent example[1]. The topology of where the interacting building blocks are located in the sequence determines the final three dimensional shape of such TOPOFOLD proteins. As an example, a topofold tetrahedron has recently been constructed out of six coiled coil pairs[2].

The folding pathway is often critical for the correct structure and function of proteins. In contrast to natural proteins, topofold proteins do not possess a compact hydrophobic core and the folding pathway is therefore determined by the topology of building blocks and the order in which these building blocks assemble.

Here we have examined the folding of the protein tetrahedron using stopped-flow techniques. The obtained chevron plot exhibits peculiar independence on denaturant concentration that is compatible with multistate folding pathways observed in natural proteins. Such folding pathways are typical for proteins displaying long range native contacts[3]. The folding pathway has also been examined via coarse-grained structure-based (Gō) simulations, which help classify the experimentally measured folding pathway. We are using these simulations, along with theoretical calculations of free chain entropy, to test how different arrangements of building blocks in the protein sequence affect its folding pathway. This will allow us to design optimized versions of topofold proteins with folding pathways as smooth and free of energy barriers as possible in order to avoid misfolding and aggregation.

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S10

Primate-specific endogenous retrovirus driven transcription defines naïve-like stem cells

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Naïve embryonic stem cells (ESCs) hold great promise for research and therapeutics as they have broad and robust developmental potential. While such cells are readily derived from mouse blastocysts, to date it has been impossible to easily isolate human equivalents, although human naïve-like cells have been artificially generated (rather than extracted) by coercion of human primed ES cells by modifying culture conditions or through transgenic modification. Here we show that a sub-population within cultures of human ESCs (hESCs) and induced pluripotent stem cells (hiPSCs) manifest key properties of naïve state cells. These naïve-like cells can be genetically tagged, and are associated with elevated transcription of HERVH, a primate-specific endogenous retrovirus (ERV). HERVH elements provide functional binding sites for a combination of naïve pluripotency transcription factors, including LBP9, recently recognized as relevant to naivety in mice. LBP9/HERVH drives hESC-specific alternative and chimeric transcripts, including pluripotency modulating long non-coding RNAs (lncRNAs). Disruption of LBP9, HERVH and HERVH-derived transcripts compromises self-renewal. These observations define HERVH expression as a hallmark of naïve-like hESCs, and establish novel primate-specific transcriptional circuitry regulating pluripotency.

S11

Visualization of calcium signals by genetically engineered calcium indicators; *in vitro* and *in vivo* models

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Calcium signals play a major role in important cellular responses such as proliferation, differentiation or apoptosis, while have a key role in determining pathologic and pharmacologic responses in tissues and organs as well. Human embryonic stem cells (hESC) can serve as a new model to investigate the development and differentiation of various cell types of the human body. First we have studied the intracellular calcium responses to various ligands and the calcium signalling pathways in hESCs and in their differentiated derivatives by using the fluorescent calcium indicator Fluo-4 and confocal microscopy. While this method was informative, several methodological problems, including different dye-loading levels, toxicity of the cleaved dye, etc. hindered this approach. To overcome these difficulties, we have generated human embryonic stem cell lines which express a genetically encoded Ca^{2+} indicator (GCaMP2). We stably expressed GCaMP2 in hES cells by using a transposon-based gene delivery system and tested the effects of various ligands in undifferentiated hES cells. In addition, cardiomyocytes, neural progenitors and neural cells differentiated from hES-GCaMP2 cells have also been characterized by this method. To extend our knowledge towards tissues and organs we have also generated a transgenic rat strain by transposon-based delivery of the GCaMP cDNA into rat zygotes. The resulting transgenic rats contain one copy of the GCaMP2 transgene per allele with a defined insertion pattern, without major genetic or phenotypic alterations. This system provides a new model for studying *in vitro* or *in vivo* cellular calcium signalling and the parallel examination of *in vivo* cellular calcium dynamics and tissue circulation by fluorescent probes opens new possibilities for physiologic and pharmacologic investigations.

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S12

TDP-43 safeguards pluripotency by regulation of alternative polyadenylation preventing paraspeckle assembly

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Alternative polyadenylation (APA) of RNAs is increasingly being recognized as important posttranscriptional mechanism governing gene dosage that is essential for pluripotent stem cell (PSC) differentiation. Over half of mammalian genes harbour multiple polyadenylation sites that regulate translation and RNA localization. APA-pattern is dynamically regulated during early PSC differentiation and reprogramming. However, the factors regulating APA are still poorly understood. We elucidated a novel TDP-43 function in global regulation of APA, role of APA in paraspeckle formation and its connection to the differentiation of PSCs. We show that unique subnuclear structures termed paraspeckles are formed independent of lineage type during exit from the pluripotency and are important for stabilization of the early commitment state. The long nonpolyadenylated isoform of non-coding RNA NEAT1 serves as the scaffold for paraspeckles assembly produced only upon differentiation. Undifferentiated PSCs express only the short NEAT1 isoform that doesn't polymerize paraspeckles. Importantly, we present evidence that TDP-43 maintains production of nucleoplasmic NEAT1 short isoform by directly regulating APA of nascent NEAT1 transcripts in undifferentiated cells, and that TDP-43 itself is sequestered in paraspeckles upon differentiation leading to formation of NEAT1 long isoform and paraspeckle assembly. This indicates the existence of a feed-forward feedback mechanism underlying paraspeckle assembly during onset of differentiation. Furthermore, we analysed how paraspeckle assembly regulates exit from pluripotency; we show that cells lacking NEAT1 and paraspeckle proteins exhibit severe developmental delay. Together, this work implicates developmental regulation of APA in RNA-mediated nuclear restructuring leading to stabilization of the early differentiation state and pluripotency breakdown.

S13

Proteomic identification of legumain physiological substrates

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Legumain, a lysosomal asparaginyl endopeptidase, is evolutionary conserved enzyme that was first identified in germinating bean seeds and later also in animals. Legumain is ubiquitously expressed in mammals with the highest expression in kidney, placenta, spleen, liver and testis. Mice lacking legumain are born viable and fertile but have enlarged spleen and decreased growth rate (Shirahama-Noda et al, 2003). Additionally, legumain knock out mice have impaired kidney function, increased plasma creatinine concentrations and decreased glomerulus filtration rate (Miller et al. 2011).
Past studies on mice lacking legumain suggest important role of this unique enzyme in kidney function and organ homeostasis but lack the molecular explanation for the resulting phenotype. To address this issue we aimed our study to discover legumain proteolytic substrates and subsequently identify signalling pathways involving legumain.
We investigated the changes on a protein level of liver and macrophages in legumain knock out strains compared to the wild type mice. We performed proteomic global analysis and identified and relatively quantified several thousands of proteins. Statistical analysis pointed out a group of lysosomal proteins significantly upregulated in knock out liver. To evaluate if upregulated proteins are in fact legumain substrates we implicated a schematic two-dimensional peptographs that provide a semi quantitative topographical map of proteins in a sample, i.e. PROTOMAP (Dix et al, 2008). With this approach we confirmed that legumain is responsible for the processing of the human single-chain cathepsin L and H into the double-chain form and also successfully identified a set of previously unknown legumain substrates.

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S14

On the role of protein disulfide isomerase in the retrograde cell transport of secreted phospholipases A2

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Despite intensive research on the pathophysiology of secreted phospholipases A_2 (sPLA$_2$s), the molecular basis of many sPLA$_2$-associated phenomena is still not well understood. sPLA$_2$s are enzymes known to act outside cells, however, detection of some types (groups – G) of these molecules inside cells suggests that they can induce certain processes from inside cells also. As mammalian GIB, GIIA and GV sPLA$_2$s, also the structurally closely related neurotoxic snake venom sPLA$_2$s have been detected intracellularly, in the cytosol, synaptic vesicles and mitochondria of nerve cells. In addition, several intracellular binding proteins for these toxins have been described. It is very probable that certain elements of intracellular activity are common to the mammalian and snake venom sPLA$_2$s. Exploring the molecular basis of action of neurotoxic snake venom sPLA$_2$s could thus offer valuable insights into the intracellular pathophysiology of the mammalian sPLA$_2$s that appear to be associated with severe neurodegenerative diseases such as Alzheimer\'s and Parkinson\'s. Experimenting on the rat PC12 cell line, we have studied the passage of a GIIA sPLA$_2$ from the extracellular space, through the plasma membrane, into the cell interior. Ammodytoxin (Atx), one of the most thoroughly studied snake venom neurotoxic sPLA$_2$s, was employed as a model sPLA$_2$. Our results suggest a role for protein disulphide isomerase in the pathophysiology of some snake venom and mammalian sPLA$_2$s by assisting the retrograde transport of these molecules from the cell surface.

S15

Autoactivation of AtMPK9 is independent from MAPK cascades

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Mitogen activated protein kinases (MAPKs) are part of a three-tiered signal transduction modules in eukaryotes, wherein MAPKs are activated by bisphosphorylation in their TXY motif of the T loop by the pertinent dual-specificity MAPK kinases (MAPKKs). Plant MAPKs could be divided into further two subtypes: those possessing the motif TEY belong to the A-C group and those with plant-specific TDY phosphorylation motif to the D group. In contrast to the canonical activation mechanism, atypical MAPKs are elicited in an upstream kinase independent manner. AtMPK9, a representative of unique, plant MAPK family, possesses a TDY phosphoacceptor site, a long C-terminal extension, lacks the common MAPKK binding docking motif, and its activation mechanism awaits for depiction. Our aim was to analyse the regulation of AtMPK9 activity by using *in vitro* translation and protoplast protein overexpressing systems.
We mapped the autophosphorylation sites by mass spectrometry to the TDY activation loop and to the C-terminal regulatory extension. We created phosphoacceptor site mutants, which are confirmed the requirement for bisphorylation at this site to perform maximal kinase activity. Then, we demonstrated that the loss-of-function mutant form of AtMPK9 is not transphosphorylated on the TDY site when mixed with an active AtMPK9 implying intramolecular autocatalytic phosphorylation. Furthermore, we show that *in vivo* AtMPK9 is activated by salt and is regulated by okadaic acid-sensitive phosphatases. We present *in vitro* and *in vivo* data that AtMPK9 is activated independently of any upstream MAPKKs, but it is activated through autophosphorylation, which shows similarities in terms of activation mechanism to mammalian atypical MAPKs, ERK7/8.

S16

Functional characterization and gene expression pattern of *ex vivo* differentiated human white, "beige" and brown adipocytes

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Recent studies verified the presence of active brown adipose tissue (BAT) in healthy adult humans and highlighted the strong negative correlation between obesity and BAT amount. "Deep neck" BAT of humans share many similarities with murine "classical" BAT, while other active, heat producing fat depots have the molecular characteristics of murine "beige" cells rather than "classical brown" adipocytes. Our aim was to isolate and characterize adipose-derived mesenchymal stem cell (ADMSC) populations from supraclavicular "deep neck" and cervical subcutaneous human adipose tissue depots. We intended to clarify whether ADMSCs from these sites are capable of initiating a browning program by using a previously optimized brown adipogenic differentiation protocol.
Human ADMSCs obtained from the aforementioned two sites during thyroid surgery were differentiated into white or brown adipocytes with or without irisin or BMP7 treatment. To complement measurements of gene expression changes from total cell lysates, laser-scanning cytometry based population scale analysis of *ex vivo* brown adipogenic differentiation was performed. It combined texture analysis, which reflects the size of lipid droplets, and detection of Ucp1 and Cidea protein content in single brown adipocytes of mixed cell populations. Functional analysis was carried out using a Seahorse Bioscience XF-96 Analyzer.
To date, there is no evidence whether ADMSCs from various adipose tissue depots have a different potential to develop into white, "classical brown" or "beige" adipocytes. When the brown adipogenic differentiation protocol was applied on ADMSCs from both sources, adipocytes had smaller lipid droplets, higher mitochondrial respiration and contained more Ucp1 and Cidea protein than the differentiated white adipocytes. Adipocytes differentiated from ADMSCs that had been isolated from "deep neck" fat showed higher expression of the brown adipogenic markers and elevated mitochondrial respiration compared to adipocytes of subcutaneous origin. As a next step, we intend to identify molecular markers to characterize those ADMSCs that are capable to implement an effective "classical brown" or "beige" differentiation by analyzing the global gene expression pattern of these cells using RNA sequencing.

S17

Circumstantial evidence for the role of snoRNA SNORD115 in regulation of RNA-editing of serotonin receptor 2c transcript

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Small nucleolar RNAs (snoRNAs) are a class of non-coding RNAs principally involved in nucleotide modification of ubiquitously expressed ribosomal and small nuclear RNAs. As a part of ribonucleoprotein complexes, snoRNAs guide partner enzymes to specific target nucleotides via Watson-Crick base-pairing for either 2'-O ribose methylation or uridine isomerization. Intriguingly, some are expressed exclusively in the brain and do not appear to target canonical RNA substrates. Among these, SNORD115 contains a conserved 18-nucleotide antisense element complementary to a segment of an alternatively spliced exon of serotonin receptor 2c (5-HT2cR) pre-mRNA known to undergo adenosine deamination to inosine (adenosine-to-inosine (A-to-I) editing). Omission of the alternative exon results in a truncated non-functional receptor isoform, whereas A-to-I editing leads to amino acid substitutions (as inosine is read as guanosine by translation machinery) in the protein G-coupling site, affecting receptor's pharmacological properties. Whether SNORD115 affects 5-HT2cR splicing or A-to-I editing (or perhaps both) is still a matter of debate as previous studies came to different conclusions. Using qPCR, we monitored expression levels of SNORD115, 5-HT2cR mRNA and SNORA35, a brain-specific snoRNA harbored in the second intron of 5-HT2cR pre-mRNA, in the murine embryonic carcinoma cell line P19 during retinoic acid-induced neuronal differentiation. All analyzed RNAs were expressed in P19-derived neurons (but not in dopaminergic neurons and to a lesser degree in neuronal progenitor cells derived from the same cell line). Moreover, expression levels progressively increased with differentiation stage. 5-HT2cR alternative splice variants were probed with RT-PCR and qPCR. We detected no significant correlation between SNORD115 expression and the alternative 5-HT2cR exon usage during neural differentiation. In contrast, we found significant differences in A-to-I editing at three out of five adenosine sites known to be prone to deamination, as inferred from direct sequencing of RT-PCR products. The two poorly edited sites encompassed adjacent adenosines of which one is predicted to be targeted by SNORD115 for methylation. Notably, editing at these sites is known to most strongly negatively impact receptor functionality. Thus, our data are consistent with the model where SNORD115 blocks A-to-I editing of 5-HT2cR transcript at crucial nucleotides, thereby preserving receptor signaling.

S18

The role of polyspecific uptake and efflux membrane transport proteins as integral elements of the cellular detoxification and environmental stress response in zebrafish

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During the last decade the mainstream toxicology fully acknowledged an important role of polyspecific membrane transport proteins. A highly complex network of uptake and efflux transporters present in barrier and/or detoxifying tissues of vertebrates transport a diverse group of substances or their metabolites in or out of the cell, enabling proper functioning of the cellular physiology in response to environmental stress. Furthermore, contrary to traditional thought that most lipophilic substances enter cells by passive diffusion, in recent years it has become evident that uptake transport proteins from the SLC (Solute Carrier Class) superfamily play essential role in mediating the entrance of a large number of endo- and xenobiotics into cells. Once inside the cell, however, the activity of ABC (ATP Binding Cassette), or more recently recognized MATE (multidrug and toxic compound extrusion) transport proteins, appears to be critical for their efflux out of the cell. Accordingly, polyspecific membrane transporters appear to be one of critical determinants of the ADME-Tox (absorption, distribution, metabolism, excretion – toxicity) properties of biologically active substances. Nevertheless, despite their physiological importance and role in cellular detoxification well recognized in mammalian toxicology, the knowledge about uptake and efflux transporters in non-mammalian species is modest. The same holds true for zebrafish (*Danio rerio*), making a reliable use of this highly important vertebrate model species and extrapolation of zebrafish data to mammalian models a challenging task. In response to this gap in knowledge our group has been recently doing extensive research on identification, phylogenetic analyses and molecular/functional characterization of (eco)toxicologically relevant polyspecific transporters in zebrafish. Therefore, in this talk our recent data and the actual state of knowledge in this area will be briefly summarized, critical research drawbacks highlighted, and potential biomedical and environmental relevance of this research additionally discussed.

S19

The interaction between HtpG and Cas3 proteins on activity of the *Escherichia coli* type I-E CRISPR-Cas system

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CRISPR (clustered regularly interspaced short palindromic repeats) and its associated Cas proteins provide many bacteria and archaea with a defense mechanism by RNA-mediated targeting of invading genetic elements. *Escherichia coli* type I-E CRISPR-Cas system detects invading DNA by a "Cascade" nucleoprotein surveillance complex that contains CRISPR RNA (crRNA) bound within that recognizes sequences on invader DNA flanked by “Protospacer Adjacent Motifs” (PAMs). This triggers R-loop formation and binding of Cas3 helicase-nuclease that degrades invader DNA. Previous studies showed that cells lacking H-NS have elevated levels of Cascade and crRNA transcripts and are resistant to infection by phage λ*vir* if they contain appropriate anti-lambda spacer. Surprisingly, resistance was strongly dependent on post-infection temperature of incubation: at 30°C *E. coli* Δ*hns* cells containing anti-lambda spacer were fully resistant to phage attack but were sensitive if incubated at 37°C.

In this work we investigated this effect of temperature on CRISPR defence, identifying that although PAM sequences were important for maximal resistance to phage at 30°C, resistance to λ*vir* at 37°C relied on increased expression of Cas3 or HtpG, in Δ*hns* cells. This suggests that levels of active Cas3 are limiting to support efficient resistance to phage at 37°C in Δ*hns* cells. Significantly, we describe the new identification that *cas3* is also under transcriptional control by H-NS but that this is exerted only in stationary phase cells.

S20

The influence of genetic background on circadian gene expression patterns in mouse peripheral tissues

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The biological clock is an important component in body homeostasis. It is however not clear how genetic background could influence circadian expression and regulation. We addressed this question by investigating liver and adrenal gland gene expression patterns of two inbred mouse strains: mixed strain - 129S2/SvPasCrlf × C57BL/6JRj and C57BL6J/OlaHsd in complete darkness (DD) and 12h light 12h dark conditions (LD). Whole exome sequencing was applied to understand how genotype can affect expression of selected core clock and metabolic genes.

24 h gene expression profiles of 25 core clock and metabolic genes were measured using qRT-PCR and analyzed by cosinor analysis. Whole exome sequencing (WES) was performed on Illumina Hiseq2000 to determine genomic differences between strains.

Our study shows a clear influence of genetic background on circadian gene expression patterns. These differences were discovered both in amplitudes and phases of core clock and metabolic genes. The largest effect of genetic background was seen in adrenal glands under LD conditions were phases of 44% and amplitudes of 88% of genes measured were affected. In adrenal glands the time of peak expression of genes occurred earlier in the mixed strain compared to the C57BL6. The adrenal gland has direct neural and humoral connections with the SCN, which could be affected by differences in the genetic makeup of the strains. With the use of whole exome sequencing we discovered 5770 and 85224 single nucleotide variations (SNV's) in C57BL6/OlaHsd and mixed strains respectively compared to the reference C57BL6 strain (NCBI37). The majority of SNVs were found in intron regions but many were also found in exon regions causing nonsynonymous mutations. Importantly, SNVs were also discovered in genes whose expression was seen to be affected by the genetic background of mice.

The differences observed at the level of gene expression between strains can to some extent be explained by the large number of SNVs observed. These SNVs likely influence the pathways which enable the two strains to transmit light information from eyes to the adrenal glands. Such findings have important implications for understanding the genetic bases of the circadian rhythms in human individuals and their susceptibility towards clock-related disorders.

S21

Characterization of glutathione-S-transferases in zebrafish (*Danio rerio*)

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In order to be metabolized and excreted from the organism, xenobiotics need to pass through four phases of the cell metabolism. Detoxification process starts with the phase 0 - initial uptake of xeno- and endobiotics across the cell membrane by polyspecific membrane transporters. After the entrance, the xenobiotics pass through phase I of cell metabolism, which is characterized with enzymatic bioactivation of initial compounds through oxidation-reduction reactions. The phase II encompasses enzymes that mediate conjugation of phase I metabolites or initial compounds to the water soluble moieties, thus increasing the bioavailability of metabolized compounds. Finally, phase III refers to the efflux of parent compounds or metabolites by membrane transporters. Glutathione-S-transferases (GSTs) are one of the key enzymes that mediate phase II of cellular detoxification. The goal of our research was a comprehensive characterization of GSTs in zebrafish (*Danio rerio*) as a valuable vertebrate model species frequently used in various fields of biology and biomedicine. A detailed phylogenetic analysis of the GST superfamily revealed 27 zebrafish *gst* genes: seven classes of cytosolic Gsts, mitochondrial genes within the kappa class, and microsomal genes within the MAPEG class. Further insights into the orthology relationships between human and zebrafish GSTs/Gsts were obtained by the conserved synteny analysis. Expression of *gst* genes in six tissues (liver, kidney, gills, intestine, brain and gonads) of adult male and female zebrafish was determined using qRT-PCR. The results showed ubiquitously high expression of *gstp, gstm* (except in liver), *gstr1*, *mgst3a* and *mgst3b*, high expression of *gsto2* in gills and ovaries*, gsta* in intestine and testes, *gstt1a* in liver, and *gstz1* in liver, kidney and brain. Functional characterization was performed on 9 representatives of cytosolic Gst classes after overexpression in *E. coli* and subsequent protein purification. Enzyme kinetics was measured using GSH as co-substrate and a series of model substrates. The functional characterization revealed specific interactions with model GST substrates and enabled the comparison of kinetic properties with human and fish orthologs. Finally, we conclude that Gstp1, Gstp2, Gstt1a, Gstz1, Gstr1, Mgst3a and Mgst3b have important role in the biotransformation of xenobiotics, while Gst Alpha, Mu, Pi, Zeta and Rho classes are involved in the crucial physiological processes.

S22

The clinical response to anti-TNF-α drug adalimumab in Crohn's disease patients is associated with genetic polymorphisms in ATG5 gene

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In Crohn's disease (CD), tumor necrosis factor-α (TNF) is maybe the most important cytokine, with its elevated concentrations playing role in pathologic inflammation. Controlled trials demonstrated efficacy of adalimumab (ADA), a recombinant human immunoglobulin G1 monoclonal antibody, in treatment of patients with autoimmune and inflammatory diseases, including CD. However, clinical studies revealed that around 30% of patients did not show improvement of disease. Because ADA is relatively expensive and because it may have unpleasant, even fatal side effects, the identification of potential non-responders is of principal importance. Some polymorphisms were associated with response to other anti-TNF drug, infliximab, but pharmacogenetic studies for ADA in CD patients are very rare. Lately, we performed first prospective pharmacogenetic study of ADA in CD patients, which revealed strong association between a gene of autophagy pathway (*ATG16L1*) and response to ADA therapy (Koder *et al*., *Pharmacogenomics* 2015). This seems reasonable since autophagy influences indigenous microbiota and possibly predispose animal to colitis.
To further study involvement of autophagy genes, we focused on genes of other two components of the autophagic protein complex, *ATG5* and *ATG12*. We genotyped three SNPs in *ATG5* and one in *ATG12* in 68 CD patients on ADA therapy 4, 12, 20 and 30 weeks after its beginning. Response was measured according to IBDQ score.
We found no association between response and SNP in *ATG5* but not in *ATG12*. Results for rs9386514 and rs9373839 were expectedly the same since they are in linkage disequilibrium (R^2=0.953). They showed association with response in 12^{th} (p=0.00060; OR=0.115) and 30^{th} (p=0.0406; OR=0.316) week in allelic model where non-responders of 12^{th} week carried 7% of allele C and 40% of allele T. These two SNPs showed association with response also in 12^{th} (p=0.00050; OR=10.909) and 30^{th} (p=0.0479; OR=3.385) week in recessive model where non-responders of 12^{th} week carried 48% of genotype TT and 8% of genotypes TC+CC. rs510432 showed a bit weaker association with response in 12^{th} (p=0.0270; OR=2.442) and 30^{th} (p=0.0939; OR=1.936) week in allelic model where non-responders of 12^{th} week carried 41% of allele A and 22% of allele G. This SNP showed association with response also in 12^{th} (p=0.00149; OR=6.861) and 30^{th} (p=0.0430; OR=3.364) week in dominant model where non-responders of 12^{th} week carried 62% of genotype AA and 19% of genotypes GA+GG.

S23

The role of cystatin F in tumour microenvironment

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Cystatin F is a member of the cystatin type II family of cysteine protease inhibitors expressed mainly in the myeloid cells (macrophages and dendritic cells), natural killer (NK) cells and cytotoxic T lymphocytes (CTLs). As an inactive dimeric protein it is translocated to endosomal/lysosomal compartment, where it is activated. A part of cystatin F is also secreted and can be efficiently taken up and activated by bystander cells. Monomeric N-terminally cleaved form of cystatin F is a potent inhibitor of cathepsins C and H, important activators of progranzymes, serine protease zymogens, which are main effector molecules within secretory lysosomes of NK cells and CTLs. As tumor cells may also express and secrete a sustainable amount of cystatin F, we hypothesize that cystatin F might be a mediator that tumor cells may use to neutralize the cytotoxic effect of NK cells.
In this study we examined the uptake and intracellular trafficking of cystatin F in different tumor cell lines. To that end we made pcDNA3.1 constructs of various cystatin F mutants (N-terminally truncated and mutants lacking specific oligosaccharide chains) and analyzed their subcellular localization upon transfection and staining with markers of biosynthetic/secretory (Golgi and trans-Golgi network) and endosomal/lysosomal compartments. We also analyzed cystatin F uptake from the medium and its subcellular localization in non-transfected cells. In tumor cell lysates overexpressing different forms of cystatin F we analyzed the activity of several target proteases. Finally, we tested the effects of uptake of different forms of cystatin F on cytotoxicity of NK-cell like cell line (NK-92).
Our results show that cystatin F modulates the efficacy of NK cytotoxicity and imply that cystatin F is involved in the mechanisms of tumor-induced immune tolerance.

S24

Cyclophilin D-dependent mPT amplifies inflammatory response in septic shock

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Severe sepsis and septic shock still represent a leading cause of death in intensive care units originating from prolonged production of pro-inflammatory cytokines triggering systemic inflammatory cascades, complement and coagulation systems, reactive oxygen species (ROS) and other factors. Oxidative and nitrosative stress in septic shock trigger opening of mitochondrial permeability transition (mPT) pore which contributes to additional ROS production, mitochondrial swelling, cell death, multiple organ failure and mortality. Cyclophilin D (CypD) is a component of mitochondrial permeability transition pore complex, and disruption of CypD prevents ischemia-reperfusion, calcium overload and ROS-induced mPT and necrotic cell death. No data is available about the role of CypD in septic shock models so far. Here, we show that prevention of CypD-dependent mPT attenuates LPS-induced oxidative/nitrosative damages, DNA breaks, mitochondrial damage, activation of apoptosis (AIF, endonuclease G and cytochrome C release) in the liver, and improves the survival of $CypD^{-/-}$ mice. Positive or negative effects of LPS on gene expressions for ROS, NO, GSH synthesis, death receptor, NRF2, mitochondrial biogenesis, NF-kappaB, acute phase response and complement pathways were significantly weakened in CypD knockout mice, analyzed by whole genome mRNA expression profile. These data show that CypD-dependent mPT is an amplifier of LPS-induced mitochondrial damage, gene expressions, cell death pathways in the liver, and plays a major role in the late phase mortality in LPS-induced septic shock. That is, CypD can be a valuable drug target, and its inhibition may provide a novel possibility to reduce mortality in septic shock.

S25

Lysophospholipid mediators - players in nanodomain dynamics?

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Lysophospholipid species are generated in numerous signaling processes via the activation of effector phospholipases. After cleavage of a hydrocarbon chain and/or headgroup of membrane phospholipids, the remaining lysophospholipid molecule stays in the membrane, serving as a specific surface, new or altered nanodomains locally and temporally. The fate of these lysophospholipids can be metabolic conversion or elimination, as well as recognition by cognate membrane receptors or membrane-associating proteins/protein domains. Lysophospholipid signaling via G protein-coupled receptors is studied in great details in the last two decades, leading to the X-ray structure determination of the two most important receptors, S1P1 for sphingosine-1-phosphate, and LPA1 for lysophosphatidic acid. On the contrary, our knowledge regarding the second messenger-like actions of lysophospholipid mediators is limited to a few clear examples, where the interaction between the lipid and the intracellular protein target is studied in details.
Our objective is to discover connections of the membrane-born lysophospholipids to signaling pathways to find out the mechanisms by which they influence cellular signaling processes. To achieve this aim, we heterologously express and purify several proteins and protein domains we expected to be able to bind lysophopholipids, and study their interactions *in vitro*, applying different binding and functional assays. Here we present new data regarding the recognition of lysophospholipid mediators by several PH, SH3, and SH2 domains. Our results support the hypothesis that locally generated lysophospholipid mediators play roles in fine-tuning the membrane-limited signaling processes via influencing the formation and/or stability of membrane micro-heterogeneities - nanodomains.

S26

Composition of cell culture media modulates basal activity and responsiveness of intracellular signalling pathways

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Cell cultures are widely used to study intracellular signalling pathways. Advantages of using cell cultures include easily controllable experimental conditions and absence of systemic feedback effects that might obscure signalling responses to experimental stimuli. To suppress basal signalling activity, experiments are usually performed in basal media without serum. However, although reduction of signalling activity in serum-starved cells is often accepted as a fact, this supposition has not been rigorously tested. To address this salient issue we studied basal signalling activity in primary human myotubes, rat L6 myotubes and HEK-293 cells during 24-hour serum starvation. We measured phosphorylation of 7 signalling proteins involved in AMP-activated protein kinase (AMPK), the mammalian target of rapamycin (mTOR), and the extracellular signal-regulated kinase (ERK1/2) pathway, across 6 time points and a total of 6 different experimental conditions. Our results show that serum starvation induces rapid, dynamic and time-dependent fluctuations in basal signalling activity. The pattern of signalling responses was dissimilar between different cell types and between different signalling pathways in the same cell type. Unlike serum, whose changeable composition is a major source of variability, basal media have fixed composition and are often thought to guarantee optimal environment for conducting signalling experiments. Nevertheless, basal media are repleted with various nutrients that may impact upon signalling pathways. Notably, some standard media contain high concentrations of nucleosides which could affect the nucleotide-sensitive AMPK pathway. To test this hypothesis, we treated L6 myotubes with AMPK activator AICAR in the presence or absence of nucleosides. We found that nucleoside-free medium augmented AICAR-stimulated AMPK activation in L6 myotubes. Our results suggest that enhanced AICAR action might be due to disinhibition of AICAR uptake in nucleoside-free medium. Alternatively, up-regulation of purine transporters or alterations in purine metabolic pathways may also explain augmented AMPK activation. Collectively, our results show that serum starvation does not induce uniform reduction in basal signalling activity and that choice of basal medium might importantly determine the result of signalling experiments. Physiological extrapolations of results obtained from serum-starved cells grown in pure basal media should be subject to constant scrutiny.

S27

Membrane phospholipids fatty acids profiles and lipid peroxidation in aging

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The phospholipids class, fatty acids (FAs) composition and cholesterol content in cell membranes are basic determinants of their physical properties. They have been shown to influence a wide variety of membrane-dependent functions such as membrane transport, fluidity, enzyme activity and receptor function. The FAs profile in tissues partly reflects not only the dietary fat intake, but also the efficiency of FAs metabolism in the body. Experimental model of aging (young Wistar rats – 3 months, n=10 and aged Wistar rats – 18 months, n=10) in a great extent showed differences in phospholipids fatty acids profiles in liver as well as in lipid peroxidation in hepatocytes.
Overall n-3 are decreased ($p<0.001$) and n-6/n-3 ($p<0.001$) are significantly increased with aging. DHA (22:6, n-3)) is significantly decreased ($p<0.001$) in aged Wistar rats. ETA (20:3, n-6) ($p<0.05$) and linoleic acid (18:2, n-6) ($p<0.001$) are significantly increased compared to young Wistar rats. Stearic acid is significantly increased ($p<0.01$) in aged Wistar rats while palmitic acid is significantly decreased ($p<0.001$) compared to young Wistar rats. Lipid peroxidation (MDA concentration) was significantly increased in aged Wistar rats.
It seems that aging itself is a risk factor and at least in part it leads to higher saturation of FAs in tissues phospholipids. Also n-6/n-3 ratio as a risk factor becomes higher with aging. Lipid peroxidation in aged cells is more pronounced and as a risk factor of oxidative stress more compromised with aging. Dietary FAs composition and aging significantly correlated to cell membrane FAs composition and lipid peroxidation.

S28

Exploring the catalytic cycle of 3-isopropyl-malate dehydrogenase: A combined experimental and QM/MM modelling study

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3-Isopropylmalate dehydrogenase (IPMDH) catalyzes the oxidation and decarboxylation of (2R,3S)-3-isopropylmalate (IPM) to 2-oxo-4-methyl-pentanoate in the presence of NAD^+ and a divalent cation (Mn^{2+} or Mg^{2+}). This reaction is an essential element of the leucine biosynthetic pathway of bacteria, fungi and plants, and as such IPMDH may serve as a possible target for antimicrobial drugs. In our work we combined a variety of experimental (e.g. site-directed mutagenesis, enzyme kinetics, X-ray crystallography) and theoretical (molecular dynamics simulations, combined quantum mechanics molecular mechanics (QM/MM) calculations) methods in order to explore the catalytic cycle of IPMDH and to identify the most important active site residues responsible for catalysis. In addition, we have also unveiled the mysterious origin of the catalytic effect of the K^+ ion on the reaction. In this presentation we will focus on the computational results, and demonstrate how QM/MM calculations can effectively contribute to the interpretation of experimental data. We used the recently determined X-ray structure of the quaternary structure of the enzyme as starting point for the calculations [1]. In the most plausible scenario, the catalytic reaction starts by the deprotonation of the OH-group of IPM by the ε-amino-group of Lys185 via a low-barrier proton-shuttle mechanism via a water molecule, that is followed by the hydride transfer step with an activation energy ΔE* of about 15 kcal/mol [1]. Finally, decarboxylation of the intermediate takes place. According to the X-ray structure of the enzyme K^+ ion interacts with the amide group of the NAD^+. Computational results suggest, in excellent agreement with enzyme kinetic data, that it increases the reaction rate 150 to 1000 fold by increasing the electrophilic character of the nicotine amide ring.[2] Using site-directed mutagenesis Lys185, Asp241, Asp217, and to a lesser extent Asp245 and Tyr139 were identified as the most important residues for the catalytic reaction, and QM/MM calculations were used to rationalize the observed activities of the mutants in the framework of electrostatic catalysis.[3]

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S29

The *Aggregatibacter actinomycetemcomitans* cytolethal dystending toxin with a truncated CdtB subunit

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Aggregatibacter actinomycetemcomitans (*Aa*) is an oral commensal bacterium often found in association with aggressive forms of periodontitis. Fifteen *Aa* strains from patients with generalized advanced chronic periodontitis have been surveyed for virulence factor genes: *ltxA* (leukotoxin), *cdtABC* operon (cytolethal distending toxin, CdtABC), *apaH,* and *flp1* (adherence factors, biofilm formation). Here, we focused on *Aa* CdtABC, an extremely efficient genotoxin that nicks host DNA. We found two *cdtABC*-positive strains harbouring an aberrant *cdtB* gene, coding for a truncated form (deletion of 70-amino acids) of the subunit B of the heterotrimeric CdtABC. This deleted region includes H160, one of the two histidines that are essential for DNase activity of various Cdts. The wild-type and truncated CdtB have been cloned and expressed in yeast or bacterial cells to assess toxicity. Surprisingly, despite missing the catalytic residue H160, the truncated CdtB affected yeast and bacterial growth. Moreover, this truncated protein, overexpressed in *E. coli*, exhibited *in vitro* DNase activity, comparable to the wild-type toxin subunit B. We have managed to confirm that *Aa* bacterium also secretes this truncated *cdtB* gene product in its environment, what further supports its role as potential virulence factor. Further experiments are in progress to characterize enzymatic activity and cytotoxicity of this novel form of cytolethal distending toxins.

S30

Specificity of leucyl-tRNA synthetase's editing domain is determined by the chemical step of proofreading

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Leucyl-tRNA synthetase (LeuRS) covalently attaches leucine to $tRNA^{Leu}$ in an ATP- dependent manner. LeuRS also catalyzes reactions with noncognate norvaline, a noncanonical amino acid that accumulates under microaerobic conditions. To counter this major threat for the accuracy of leucylation, norvaline is eliminated from protein synthesis by deacylation of mischarged tRNA at a separate LeuRS editing domain. To prevent futile ATP consumption, the editing domain efficiently discriminates against cognate Leu-$tRNA^{Leu}$. Although the general perception is that the editing domain operates as a fine sieve that prevents binding of the cognate substrate, the intriguing question remains whether specificity is exercised predominately through the ground state binding or catalysis.

The contribution of ground state binding was examined by measuring the interactions of the isolated LeuRS editing domain from *Escherichia coli* and the non-hydrolysable analogues of the 3' end of aminoacylated tRNA (Nva2AA and Leu2AA) using isothermal titration calorimetry. The dissociation constant for Leu2AA was only 10-fold higher than for Nva2AA, indicating a modest contribution of binding to specificity. Moreover, no significant difference in binding affinity of Nva-$tRNA^{Leu}$ or Leu-$tRNA^{Leu}$ to deacylation-defective LeuRS was observed using microscale thermophoresis. This is likely due to the large interacting surface of the tRNA with LeuRS, which masks the difference in binding of cognate and noncognate amino acid to the editing domain. Next, we compared deacylation of Nva-$tRNA^{Leu}$ and Leu-$tRNA^{Leu}$ by LeuRS under single turnover conditions. We show that the cognate aa-tRNA is discriminated at the chemical step by 10^3-fold. We also confirm the lack of significant differences between the K_ds for Nva-$tRNA^{Leu}$ and Leu-$tRNA^{Leu}$. Our results thus demonstrate that substrate specificity in deacylation is predominately governed by the complementarity established at the transition state.

The T252A substitution was previously shown to undermine the enzyme's specificity by enabling hydrolysis of Leu-$tRNA^{Leu}$. Remarkably, our thermodynamic and kinetic analyses revealed that this substitution does not affect K_d values for Leu2AA or Leu-$tRNA^{Leu}$. Yet, the deacylation rate constant was 500-fold increased as compared to the wild-type enzyme. It thus appears that the major specificity determinant of the editing domain operates by mispositioning of the catalytic residues to prevent hydrolysis of the cognate product.

S31

Proteomics examination of OSCC-specific salivary biomarkers in a Hungarian population using Luminex-based multiplex assay and SRM-based targeted proteomics method

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Oral squamous cell carcinoma (OSCC) is the 6th most common malignancy with increasing incidence and mortality rate accounting for about 90% of malignant oral lesions. Hungarian population occupies the top places of statistics regarding OSCC incidence. The 5-year survival rate is around 50% and one of the possible reasons for the low survival rate may be the delayed detection supporting the need for biomarkers to improve early detection.
14 proteins previously reported in the literature as significantly elevated in saliva of OSCC patients either at gene or protein levels were examined and their amount was measured in the saliva of OSCC patients and age-matched controls. The level of six possible salivary biomarkers (IL-1 alpha, IL-1 beta, IL-6, IL-8, TNF-alpha and VEGF) was examined with Luminex-based multiplex assay and an SRM-based targeted proteomic method was developed for the relative quantification of catalase, thioredoxin, profilin, S100A9, cytokeratin fragment Cyfra 21-1, galectin-3 binding protein, CD44, and CD59. Based on our results protein S100-A9, thioredoxin, IL-6 and TNF-alpha seem to be useful biomarkers for OSCC detection in the Hungarian population.
Using salivary analyses a noninvasive method to detect biomarkers useful for the early diagnosis of OSCC was developed and this seems to be an attractive strategy to decrease morbidity and mortality, to enhance survival rate and to improve quality of life.

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S32

Cysteine cathepsins affect tumor cell adhesion and intracellular signaling through the shedding of cell adhesion proteins and receptors

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Extracellular proteolysis is an important effector of cancer progression, with proteases playing a crucial role in cell surface signaling and extracellular matrix remodeling. Although cysteine cathepsins are primarily known as lysosomal proteases, they can also be translocated to the cell membrane and secreted into the extracellular space. Translocation and secretion are especially important in pathological states such as cancer, where acidic microinvironment of a tumor provides a good milieu for cathepsin activity. Inhibition of extracellular cathepsin activity has been reported to influence cancer-related processes such as cancer cell migration and invasion. The exact mechanism of cathepsin influence remains largely unknown, since very little is known about the identity of their extracellular substrates. Here we report that cathepsins can act as sheddases by cleaving extracellular domains of membrane proteins. Mass spectrometry was used for identification of cleaved ectodomains and cathepsin mediated substrate cleavage was confirmed also on cell based models and on murine pancreatic cancer model with deleted cathepsin genes. Among the identified substrates are CAM adhesion proteins and various receptors (plexins, ephrin receptors and EGFR) which are all known regulators of various stages of cancer progression. Their cleavage could provide a direct mechanistic link between extracellular cathepsin activity and cancer progression.

S33

Use of nitroxoline derivatives in regulation of cathepsin B activity

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Cysteine protease cathepsin B has an important role in variety of physiological processes within lysosomes. In addition, changes in its expression and activity are associated with a number of pathological processes including cancer. In cancer cathepsin B has an important role in degradation of extracellular matrix and tumor invasion and metastasis. The structure of cathepsin B reveals an extra element termed the occluding loop, which determines enzyme's endopeptidase and exopeptidase activity. In addition to endogenous inhibitors, exogenous inhibitors have to be used to regulate its increased activity in pathological processes. To date several groups of exogenous inhibitors have been identified, but due to their low bioavailability and off-target side effects, none of them made its way to clinical practice. Nitroxoline, well-established antimicrobial agent, was recently shown to be potent and reversible non-covalent inhibitor of cathepsin B endopeptidase activity. In order to improve its activity we developed and tested several new derivatives designed on the basis of crystal structure of nitroxoline-cathepsin B complex. For derivatives that potently inhibited cathepsin B endopeptidase or exopeptidase activity we further performed *in vitro* functional tests on cell lines evaluating the effect on tumor cell invasion using real time xCELLigence system and 3D *in vitro* model with implanted multicellular spheroids in Matrigel™. Additionally, we evaluated the impact of the inhibitors on extracellular and intracellular degradation of extracellular matrix.
Novel nitroxoline derivatives showed different potency and selectivity in regulation of cathepsin B endopeptidase and exopeptidase activity. As the best performing inhibitor 2 - {[(8-hydroxy-5-nitroquinoline-7-yl) methyl] amino}-acetonitrile was identified. It selectively and reversibly inhibited cathepsin B endopeptidase activity with much lower constant of inhibition compared to nitroxoline. It also reduced extracellular and intracellular degradation of extracellular matrix and consequently, tumor cell invasion.

S34

Mapping MAPK interactors: An extensive network with fast-evolving partnerships.

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Although mitogen-activated protein kinases (MAPKSs) are one of the best characterized members of intracellular signalling pathways, our knowledge on their substrates is still very limited. However, we do know that the MAPK activating kinases, pathway regulators and most substrates associate with these kinases through auxiliary linear motifs called D-motifs. These were discovered more than 20 years ago, but are still incompletely understood. By applying our structural knowledge, we were able to amend the definition of D-motifs, obtaining a consistent model of MAPK-interacting motifs. This development made computational prediction of human MAPK partners possible. We combined rigorous sequence & structure-based bioinformatic methods with novel, semi-high-throughput validation assays to generate the first relatively reliable interactomes of human MAPKs. Our experiments suggest that specific MAPK-interacting D-motifs are remarkably widespread in the human proteome. These novel partner proteins are involved in a number of intriguing cellular functions, often in a highly tissue-specific manner. Our highly detailed molecular-level maps also help to understand the role of c-Jun N-terminal kinase (JNK) in neuronal development as well as in neurodegeneration or diabetes. The evolutionary analysis of binding motifs also led to a surprising conclusion: Although the MAPKs themselves are an ancient heritage, their substrates and other partnerships evolve much faster than previously anticipated. The evolutionary mechanisms underlying MAPK docking motif emergence are just as varied as the proteins themselves. This study gives the first detailed analysis on how linear motif dependent regulation can develop in disordered protein segments otherwise largely devoid of function.

S35

Developing a database on gene and protein expression in animal dormancies

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Many organisms have evolved the ability to enter into a dormant state in order to survive various environmental adversities. Dormancy is widespread across all biological kingdoms – from the simplest unicellular organisms (bacteria) to the most complex (vertebrates). The type of dormancy is species-specific, occurring at a specific developmental stage and usually once in a life-time. Dormant phase can last from few hours, to several months, years and in extreme cases for decades or centuries. Dormancy is usually defined as a period of temporarily arrested growth, development and physical activity. It engages many behavioural, morphological, physiological, biochemical and molecular alterations that altogether increase the stress tolerance of organisms. Even though dormancy intrigues biologists for centuries, application of "-omic" and other modern technologies was the one that lead to the exponential expansion of scientific data on molecular background of dormancy in the last two decades. Thus, constantly increasing knowledge created a need for organization of produced biological information. In order to meet the demands from the dormancy-based research community, we have started developing a database, named DORMANCYbase, on gene and protein expression during animal dormancy. DORMANCYbase will prove a comprehensive database of functional information on gene and protein expression derived from the scientific literature. Currently the database contains about 800 sequences from approximately 60 different arthropod species. Molecular data from DORMANCYdatabase will enable us to compare gene sets expressed in various resting phases, to search for both conserved and specific molecular processes in different dormancies as well as to start developing functional networks of genes and their products for a given type of dormancy. DORMANCYbase website, which is under development, will be a highly specific resource for sharing information online and supporting researches and studies in the field of animal dormancy. Not only will the database allow scientists to browse for information but they will also be able to submit their own research data. Moreover, DORMANCYbase will be a freely accessible database connected to other relevant resources such as NCBI, UniProt, DDJB and so on.

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S36

Validation of *SteatoNet* for prediction the liver network disorders

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Non-alcoholic fatty liver disease (NAFLD) is a poorly understood disorder that occurs at high frequency in Western populations with unhealthy lifestyle. *SteatoNet* has been developed representing the multi-dimensional nature of NAFLD. *SteatoNet* is a dynamic semi-quantitative model of metabolic and signalling pathways, their interaction with extra-hepatic tissues and hierarchical feedback regulation at the gene expression and signal transduction level to represent the multi-dimensional nature of NAFLD. It is a good *in silico* platform to test biological hypotheses prior to experimental testing (1).
Lanosterol 14α-demethylase (CYP51) is a key regulatory enzyme in the late stage of cholesterol synthesis. Demethylation of lanosterol caused by CYP51 is regarded as a checkpoint in the transformation to cholesterol. Conditional knockout *Cyp51* in the mouse liver (LKO) resulted in hepatomegaly with oval cell proliferation, fibrosis and inflammation, but without steatosis. The key trigger were the reduced cholesterol esters that induced cell cycle arrest, senescence-associated secretory phenotype and oval cell response, while elevated CYP51 substrates promoted the integrated stress response (2).
Applying *SteatoNet* for simulations of *Cyp51* knockout in hepatocytes should predict a network disturbance in adipose tissue, which is a good starting point for further experimental testing. We will measure the effects of liver *Cyp51* knockout on transcriptional level in adipose tissue by evaluating expression of candidate genes suggested by the simulations. On the protein level, western blot analysis will be performed. Adipose tissue will be collected from *Cyp51* LKO mice.
Successful validation and improving the efficacy of *SteatoNet* is an excellent base for better comprehension of the links between the liver and other organs in the sense of understanding the role of cholesterol homeostasis in NAFLD and for adapting the model to other liver-related diseases, like alcoholic liver disease. The improved model opens the door for adaptations to various new applications, including the purposes of personalized medicine.

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S37

Carbonylation of HSA with methylglyoxal affects its copper(II) binding affinity

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Carbonylation of human serum albumin (HSA) with methylglyoxal affects its copper(II) binding affinity

The effects of carbonylation with methylglyoxal on HSA\'s copper(II) binding affinity and copper(II) release from copper-HSA complexes were studied. Carbonylation causes conformational changes in HSA molecule, Cys34-SH content decrease, copper binding affinity decrease and release of copper from copper-HSA complexes. The percent reduction ratio (Cys34-SH group content/HSA bound copper) upon HSA carbonylation and oxidation was the same, indicating that binding/release of copper(II) ions depends mainly on the redox state of Cys34-SH. Observed changes were tested in diabetic group. Cys34-SH and HSA-bound copper(II) ions contents are significantly lower (0.457±0.081 mol SH/mol HSA, 1.07±0.01 µmol/mol HSA, resp.) compared to the control group (0.609±0.027 mol SH/mol HSA, 1.34±0.01 µmol/mol HSA, resp.). Strong correlations between HSA-SH content and HbA1c (R=-0.803), and between HSA-bound copper(II) and the HSA-SH content (R=0.841) were found. Thus, HSA carbonylation lead to decrease of HSA-SH content and to increase of free copper(II) ion level in serum, contributing to further enhancement of oxidative and carbonyl stress in diabetes.

S38

Role of γ-enolase in neuronal development and degeneration: regulation by cysteine protease cathepsin X

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Nervous system depends upon highly specific connections that are formed between neurons during development. In this process, neurotrophic factors are significant and they have important roles in nerve growth and regeneration, cell differentiation and survival. Increasingly, a neurotrophic activity has been identified for the glycolytic enzyme γ-enolase. The C-terminal end of γ-enolase controls neuronal survival and differentiation by activating PI3K/Akt and MAPK/ERK signaling pathways. Recent findings imply the involvement of γ-enolase in neuroprotection of the brain cells during neurotoxic and neurodegenerative processes. γ-Enolase was found to be upregulated in microglial cells surrounding amyloid plaques in Tg2576 transgenic mice and further its neuroprotective role in amyloid-β-related neurodegeneration was demonstrated. The C-terminal end of γ-enolase was able to abolish amyloid-β-induced toxicity through activating signaling pathways leading to neuroprotection. However, γ-enolase neurotrophic activity is regulated by cysteine protease cathepsin X. The latter is abundantly expressed in immune cells and brain cells, with a preference in glial cells and aged neurons and has been suggested to be an important player in degenerative processes. Neurodegenerative action of cathepsin X comprises of the sequential cleavage of the C-terminal dipeptide of γ-enolase, abolishing γ-enolase-triggered neurotrophic and neuroprotective signaling. Additionally, the role of cathepsin X in neurodegenerative processes, which are causative factors in Parkinson's disease, has been shown, where inhibition of cathepsin X resulted in reduced apoptosis of dopaminergic neurons mediated by 6-OHDA. Besides neurons, glial cells are also involved in constant reciprocal signaling both under physiological and neuropathological conditions and the interference of cathepsin X and γ-enolase in the microglial activation process in inflammation-induced neurodegeneration has also been established. Taken together, these findings propose the potential function of neuronal and microglial cathepsin X and its target γ-enolase in neurodevelopment and neurodegeneration. Knowing the involvement of γ-enolase and its regulation by cathepsin X in the processes that are essential for the protection of the injured neurons and their regeneration represent a step towards the development of new molecules for the treatment of neurodegenerative diseases.

S39

Effect of resveratrol on monocrotalin induced pulmonary hypertension

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Pulmonary hypertension (PH) is an uncurable progressive disorder where the arterial mid-pressure is over the normal 25 Hgmm resulting in right ventricular failure. In the pathogenesis endothelial dysfunction plays a major role causing plexiform and specific changes in lung arteries and arterioles and also smooth muscle hyperplasia. This leads to hypoxia which is followed by oxidative stress and inflammation.
The resveratrol is a non-flavonoid polyphenol, found in plants. Previous studies have shown it's positive effect on cardiovascular system as well as inflammation. We aimed to study the effect of resveratrol on monocrotalin (MTC) induced pulmonary hypertension.
We induced PH giving MTC once s.c. Animals were randomized into four groups: 1, control (0,1 mg/kg saline, once, s.c); 2, control + resveratrol (0,1 mg/kg saline, once, s.c; 20 mg/kg/day resveratrol, oraly); 3, PH (60 mg/kg MTC, once, s.c); 4, PH + resveratrol (60 mg/kg MTC, once, s.c; 20 mg/kg resveratrol, oraly). We calculated organ-body weight ratio and analyzed the changes in organs with histology and immunhistology; the biochemical changes were detected by using rat citokine array as well as with Western blot.
Resveratrol treatment could diminish the monocrotalin induced changes in lung tissue histology as well as improve remodelling in medicated hearts. The citokine array proved to increase the expression of several inflammatory citokines and chemoattractant proteins as a result of MTC injection. After the resveratrol therapy the activities of these factors were decreased. Our further results showed that the lung/bodyweight and heart/body weight ratios were significantly lower in rats treated with resveratrol, and animals also lived longer than without resveratrol treatment.

SP1

MIQE Guidelines: Getting a reliable and reproducible qPCR results

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Since it is invention (1983) Polymerase Chain Reaction (PCR) was considered a powerful technique in laboratories all over the world. It is not a surprise that more than 800,000 published papers cited the term PCR from 195 countries. The year 2009 was a tipping point for this heavily used technique. The MIQE guideline was published by a group of qPCR experts, with a main objective to point out the pitfalls in experiments. In this talk I will shed the light on how by being compliant to MIQE the qPCR data will be more reliable and easy to publish.

SP2

Laboratory grade water - the most important reagent in any analytical laboratory

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Water. We usually take it for granted due to its abundant presence in the world. But there is more to it than just H20. Water has also its contaminants and particles that have great effect on analytical results. Water is also the most important and widely used regent in every laboratory. Therefore it is important to identify the right contaminants and even more so choose the right water purification techniques to remove those contaminants in order to be able to use laboratory grade or ultra-pure water for the right application you are working on. This lecture will be about which purification technique removes which contaminants from the water in order to fit you application.

SP3

Nanoscale imaging and quantitative nanomechanical characterization of cells and biomaterials by Correlative Atomic Force and Optical Microscopy

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We have developed an imaging mode "Quantitative Imaging" (QI™) which is based on fast force-distance curves to simultaneously obtaining topographic, nanomechanical, and adhesive sample properties. Additionally, even more complex data like contact point, Young´s modulus, or recognition events images can be achieved. To demonstrate the power of QI™, a variety samples have been investigated. Living cells as well as polymer surfaces and single biomolecules have quantitatively been analyzed, and compared with conventional force spectroscopy and traditional AFM imaging modes.
JPK ULTRA Speed AFM combines the tip scanner technologies and compact design allowing fast AFM imaging of approximately 1 frame per second, and can be seamlessly combined with advanced optical methods such as confocal, TIRF, or STED microscopy. The unambiguous correlation between AFM and optical microscopy is achieved by the DirectOverlay™ technique. Thus, individual molecule dynamics can now be studied with AFM. We could gain a high-resolution temporal insight into the dynamics of collagen I fibril formation and its characteristic 67 nm banding hallmark.
The systems newly gained flexibility will also be demonstrated on a study of living fibroblast cells directly imaged in their culture petri dish at 37 degrees C. Here, the dynamics of individual membrane structures is investigated with AFM while simultaneously observing the individual living cell with optical phase contrast.
The inherent drawbacks of traditional AFM imaging modes for fast AFM imaging or for challenging samples can impressively be overcome by NanoWizard® ULTRA Speed and by QI™ mode, respectively.

SP4

Quantitative and ultra-sensitive, nucleic acid analysis using RainDrop™ Single-Molecule dPCR

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There is a need for more accurate and sensitive methods to quantify nucleic acid biomarkers. Next Generation Sequencing (NGS) and Digital PCR (dPCR) technologies have seen recent advances and have facilitated research on both cellular and cell-free material. Single-molecule PCR based methods provide advantages for both NGS and dPCR, enabling multiplexed sample prep for targeted sequencing, or allowing researchers to sensitively quantify a sample's nucleic acids by adding up digital fluorescent counts of single target molecules. This seminar will highlight results from recent studies using the:

- RainDrop Digital PCR System to quantify DNA and RNA in highly sensitive and precise multiplex measurements across a wide dynamic range and spectrum of sample inputs.
- ThunderBolts for Cancer, Myeloid and Custom panels for NGS sample enrichment, enabling simple low-cost Illumina sequencing of low-input samples such as FFPE and circulating tumor DNA.

SP5

Roche target enrichment solutions

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In life science research, understanding the human genome and the diseases associated with genetic mutations are critical. Research has shown the majority of disease causing mutations can occur in the exome or in other disease associated regions. Targeted sequencing enables user specified enrichment to identify and 'capture' only the precise regions of interest that are responsible for disease.
Roche has pioneered targeted enrichment with the development of target enrichment technologies. SeqCap EZ products enable a revolutionary process for the enrichment of selected genomic regions from full complexity human genomic DNA in a single step. Numerous SeqCap EZ Library options are available, depending on organism of choice and genomic regions of interest. Capitalizing on the efficiencies inherent with highly parallel enrichment, researchers can now design economical, high-throughput, and time-saving next-generation sequencing experiments.

Abstracts of Posters

(PI and PII)

PI-1

Characterization of the DNA remodeling activity of human HLTF protein

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The DNA in our cells is continuously damaged by different agents, such as UV irradiation, reactive oxygen species, metabolites and chemicals. These agents are changing the structure of the DNA molecule. To avoid these mutations many DNA repair mechanisms have evolved. These mechanisms are able to set back the original structure of the DNA double helix but some damages get to the S phase of the cell cycle where they can cause the stalling of the replication fork, chromosomal breaks and cell death. To avoid these possibilities the DNA damage bypass pathway has evolved which can protect the stalled replication fork by different ways.
The main step of the pathway is the monoubiquitylation of the PCNA protein, the processivity factor of the polymerases by RAD6/RAD18 complex at the lysine 164 position. After this modification the replicative polymerase can be changed by an alternative polymerase, which is able to synthesize through the lesion. In another error free mechanism the monoubiquitylated PCNA becomes polyubiquitylated by the MMS2/UBC13/HLTF complex through lysine 63 residues, therefore HLTF can reverse the replication fork. On this newly emergent so-called chicken foot structure the stalled strand can be finished using the newly synthesized sister strand as a template. The third possibility is an alternative template switching mechanism.
Our study is focusing on HLTF protein and the better understanding of the function and regulation of the DNA damage bypass pathway. We are analysing the mechanism and function of HLTF DNA remodelling activity. Our ultimate goal is to shed light on the whole molecular mechanism of the damage bypass.

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PI-2

Effect of extracellular S100A4 on cell adhesion and migration of epithelial carcinoma cells

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S100A4 belongs to the Ca^{2+}-binding EF-hand S100 protein family. The most extensively described function of S100A4 is due to its interactions with intracellular partners such as non-muscle myosin IIA, annexin A2 and p53. By binding to myosin IIA S100A4 disrupts myosin filaments, thus increases cell motility. However, S100A4 could also act extracellularly as an angiogenic and motility-inducing factor, though its mode of action, mechanism of internalization and possible receptor(s) are still not well-understood.
Our aim is to study the function of extracellular S100A4 and its mode of internalization. Adhesion of A431 epithelial carcinoma cells was studied by real-time impedance-based assays: xCELLigence system and Electric Cell-substrate Impedance Sensing (ECIS). Cell migration was followed by a similar impedance-based dual-chamber assay system (xCELLigence DP).
According to our results, extracellular S100A4 reduces cell adhesion on fibronectin-coated surface in a concentration-dependent manner. S100A4 induces chemotaxis of cells at a 1 µM concentration optimum. Decrease of cell adhesion could be inhibited by anti-S100A4 antibodies. Analysis of mutants that do not bind to non-muscle myosin IIA and other interacting partners demonstrate that an intact binding interface is required for the effect of S100A4. Interestingly, an oxidized oligomeric S100A4 also failed to reduce cell adhesion. Immunofluorescence studies revealed that S100A4 enters cells shortly (2 min) after adding it to the medium and accumulates in endosomes. The non-functional S100A4 mutants do not enter the cells, indicating that a specific receptor is involved in the process. Work is in progress to identify the S100A4 receptor.
Since extracellular S100A4 is considered a promising therapeutic target protein, our studies could shed more light on the mechanism how S100A4 binds to and has an effect on its target cells. The established impedance-based assays could also be used for testing S100A4 inhibitors in the future.

Supported by the Hungarian Scientific Research Fund (OTKA K108437).

PI-3

Structural and functional aspects of Ca^{2+} loaded S100A4 – what can NMR see?

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S100 proteins are vertebrate specific Ca^{2+} binding proteins of low molecular weight (10-12 kDa) that generally exist as homo- or heterodimers. S100A4 gained increasing attention over the last decade because of its metastasis promoting properties and also its important role in the pathogenesis of rheumatoid arthritis and fibrotic diseases.
Using NMR spectroscopy we extensively investigate the solution characteristics of this protein in interaction with myosin II isoforms (myo2A and myo2B) and the TAD domain of p53. A combination of classical and fast acquisition methods is applied for spectral assignment, relaxation measurements are done to describe backbone dynamics, while solvent exchange and slower motions were characterized by CLEANEX and relaxation dispersion experiments. Translational diffusion studies were performed to follow aggregation and molecular size.
The interaction with a short myo2A fragment was studied from the S100A4 side. The formation of an asymmetric complex was proved and both secondary chemical shift analysis and backbone dynamics revealed the unexpected behavior of helix H1.
Filament disassembly in the myosin system can occur supposedly as a consequence of complex formation between S100A4 and myosin, but the presence of phosphorylated myosin fragments can also induce this phenomena. We characterized a 67-residues long disordered myo2A fragment and a 111-residues long coiled-coil segment in native and phosphorylated form. We proved that phosphorylation does not affect significantly neither the structural propensities, nor backbone dynamics, thus, filament breaking has to be initiated in an intermolecular manner between coiled-coils.
The complete TAD domain of protein p53 (1-63) was investigated in interaction with S100A4. In free form p53 is unstructured with some nascent helical regions corresponding to TAD1 and TAD2 domains. Upon complex formation a disorder-to-order transition is detected and the protein becomes more structured with formation of a real helix, and a short turn motif as proved by secondary chemical shifts and relaxation data.
Our NMR results lead to a better understanding of functional behavior of this pathologically important protein.

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PI-4

Investigating the specificity of human elastase 3A (ELA3A) and elastase 3B (ELA3B) by phage display

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Digestion of food proteins in mammals is carried out by a panel of pancreatic serine proteases that based on primary substrate specificity belong to three major groups, trypsin, chymotrypsin and elastase. While trypsin and chymotrypsin had been classic model enzymes, elastases are less characterized.
Most mammalians have 3 different elastase genes, each coding for an active enzyme: elastase 1, 2 and 3, respectively. Interestingly, unique to humans, the elastase 1 gene is not functional. However, the elastase 3 gene had been duplicated at some point of the human evolution so humans still have three functional elastases. The resulting variants, ELA3A and ELA3B share 93 % sequence identity on the protein level. Both of them are present in the pancreatic juice and have proteolytic activity.
We decided to investigate whether there are any enzymatic differences between these two elastase forms in terms of activity and substrate specificity. Such differences might report on divergent biological functions. For example, one of the two forms might functionally substitute for the missing ELA1 enzyme. In this case the specificity of that ELA3 form should resemble that of ELA1 from other species.
We thoroughly examined the specificity profiles of ELA3A and ELA3B. To do so we applied phage display, a directed protein evolution method. We displayed substrate-like reversible inhibitors on the surface of M13 phage. These inhibitors interact with their cognate enzyme through a surface exposed loop that mimics a substrate. Using combinatorial site directed mutagenesis approach we randomized all non-cysteine protease binding loop positions. Hundreds of millions of variants have been produced and selected separately on ELA3A and ELA3B as target enzymes
By deciphering the identity of large numbers of ELA3A and ELA3B binders through DNA sequencing we generated sequence logos that report on the characteristic positional preferences of the substrate binding sites of these individual proteases. Based on differences between the enzyme-specific logos we produced corresponding inhibitor variants as recombinant proteins and validated the findings obtained by directed evolution in *in vitro* enzyme inhibition kinetic assays.
The results of this comprehensive study will be presented.

PI-5

The role of P53 during transcriptional blockage

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P53 is a well-known tumor suppressor gene, which is affected by inactivating mutations in the majority of the human cancer related diseases. P53 is a sequence-specific transcription factor that preferentially binds to specific sequences of target genes. Under regular conditions, the P53 is present in a low level while following DNA damage, the P53 protein level increases and it blocks the cell cycle prior to S phase allowing repair of the broken DNA. In addition to its several well-characterised functions, P53 has been recently described to affect transcriptional elongation by a so far less well-understood mechanism. It has been shown that the human P53 interacts with RNAPII in *Saccharomyces cerecisiae*. We have also reported recently that P53 localized to actively transcribed gene regions in Drosophila. This observation led us to investigate in human cells whether P53 is present at specific gene regions which are not direct targets of it.

We found that P53 binds to the regulatory regions of genes, which have not been reported earlier as P53 direct targets. We found that P53 and RNAPII co-localized to the examined regions and this localization was ceased upon transcriptional blockage. P53 interacts with the transcriptionally active form of RNAPII. The block of transcription by actinomycin D strengthens the interaction between the two proteins. High concentration of actinomycin D treatment resulted in an increase in P53 protein level, and in a decrease RNAPII level with a concomitant reduction in the S5 and S2 phoshorylation forms of RNAPII. Finally, our data indicate that the transcriptional blockage-induced RNAPII degradation is regulated by the ubiquitin proteasome system.

These observations highlight a mechanism by which the transcriptional blockage could be resolved upon different kinds of DNA damages. The mechanisms we propose here helps to understand how the RNAPII is degraded on a transcribed unit in order to allow access for repair proteins. This is most likely a cell defence mechanism to avoid production of truncated or mutated transcripts of essential genes that would endanger the cell viability.

PI-6

Interaction of human cholinesterases with bisdimethylcarbamate derivative of albuterol

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The selectivity of cholinesterases and their interaction with various compounds, especially drugs, is the subject of many studies in biochemistry and pharmacology, due to their important roles in an organism and involvement in the metabolism of many drugs. One example is bambuterol, a biscarbamate prodrug of terbutaline whose bioconversion in an organism is due to the activity of butyrylcholinesterase (BChE; EC 3.1.1.8), and whose high therapeutic index is associated with an extremely selective BChE inhibition compared to acetylcholinesterase (AChE; EC 3.1.1.7). Albuterol is a short-acting β2-adrenergic receptor agonist used for the relief of bronchospasm in conditions such as asthma. Biscalb, a newly-synthesized bisdimethylcarbamate of albuterol, is structurally related to bambuterol, and we expected it to have a similar BChE inhibition potency and selectivity, as well as stereoselectivity. Inhibition rate can be affected by the BChE gene polymorphism in the human population. The aim of this study was to investigate the inhibition of human BChE (usual, atypical and fluoride resistant) and AChE with biscalb and its (*R*) and (*S*) enantiomers to see if their bioconversion is affected by BChE inhibition the same as with bambuterol. Biscalb proved to be a potent inhibitor of all of the studied cholinesterases with inhibition rate constants within 10^3 $M^{-1}min^{-1}$ range. Biscalb inhibited the fastest usual BChE, while achieving the same effect 40 times slower for atypical BChE. Unfortunately, AChE inhibition was only 10 times slower than that of usual BChE, meaning that this selectivity was very poor compared to the 20,000 faster inhibition of usual BChE as with bambuterol. The studied BChEs did not show any stereoselectivity, while AChE displayed about an 8 times higher preference to (*R*)-biscalb. The obtained results show that the inhibition profile of biscalb is quite different than that of bambuterol, and cannot be taken in consideration for further investigation as a promising lead for prodrug development in analogy with bambuterol.

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PI-7

The role of the Asp76-Lys41 salt bridge in the amyloid formation of genetic β2m D76N mutant

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There are more than 40 proteins that play active role in amyloid disorders, such as Aβ peptides (Alzheimer's disease), α-synuclein (Parkinson's disease), Ig light chain (AL amyloidosis) or β2-microglobulin (DRA). In these diseases, the depositions of amyloid fibrils with cross-β structure can be localized in several organs specifically (as nervous system, heart, etc.) or in the whole body (systemic) and cause serious pathological symptoms. Some amyloidogenic proteins have several mutant variants, e.g., transthyretin (TTR) or Aβ peptide developing hereditary amyloid disorders.
β2-microglobulin (β2m), the light chain of MHC-I, is degraded by the kidney. It has role in several diseases, especially in amyloidoses. The organ-specific dialysis-related amyloidosis (DRA) was reported 30 years ago, while the hereditary systemic β2m amyloidosis was discovered in 2012, in the members of a French family. The first is caused by the wild type, the latest induced by a mutant form (D76N) of β2m. An important question is how this mutation, associated with a charge loss, can influence the molecule and its amyloid formation resulting in completely different amyloidogenic characteristics (e.g., different serum concentration, localization) compared to the wild type protein.
Despite the increasing knowledge on β2m, the mechanism of amyloid formation and the interacting partners/factors which probably act crucial role in the process are not well understood. In our work, we prepared mutants of β2m, affecting the surface charge clusters of the molecule. These clusters play an essential role in stabilizing the native structure of the protein and in interactions with several additives/partners *via* electrostatic forces. We were especially interested in the role of the 76Asp-41Lys ion-pair. We studied the effect of the removal of any of the charges on the stability of the β2m monomers and amyloid fibrils compared to the wild-type protein. Furthermore, we investigated the intermolecular interactions with several partners, such as LPA, SDS, collagen, and heparin.
Our results reveal that the distinct characteristics of the hereditary β2m disorder can be explained by the synergy of three effects, the decreased stability of the mutant monomer, the higher stability of its amyloid fibrils and the altered interaction network of the mutant protein.

PI-8

Intramolecular interactions and lipid binding of the scaffold protein Tks4

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The scaffold protein Tks4 (Tyrosine kinase substrate with four Src homology 3 domains) is a member of the “p47phox-related organizer superfamily”. Tks4 is essential for the formation of actin‑rich membrane protrusions like podosomes and invadopodia that are required for the motility of normal and transformed cells. Therefore, beyond the physiological functions of Tks4 (e.g. motility of macrophages) it plays a role in pathological situations, such as cancer cell invasion and metastasis. Tks4 is regulated by the epidermal growth factor (EGF) signalling pathway and required for EGF induced lamellipodia formation. EGF induces the translocation of Tks4 from the cytoplasm to the plasma membrane, where it associates with the EGF receptor. P47phox, which is another member of the same protein family, share many similarities with Tks4 in the N‑terminal region. Both proteins possess a phosphoinositide-binding *phox* (PX) domain at the N-terminus, which is necessary for membrane translocation, followed by two Src Homology-3 domains (so called “tandem SH3”) and a proline-rich region (PRR). Besides the similar N-terminal region, Tks4 contains two additional SH3 domains and several other PRRs. It has been demonstrated that intramolecular interactions are necessary for an autoinhibited state of p47phox: the tandem SH3 domain binds the PRR while the second SH3 domain interacts with the PX domain, thereby preventing its membrane binding. Based on the conserved structural features of p47phox and Tks4 and the fact that an intramolecular interaction between the third SH3 and the PX domains of Tks4 has also been reported, we have hypothesized that Tks4 is also capable of autoinhibition. In this study we aim to identify all intramolecular interactions within the N-terminal part of the Tks4 protein that are necessary for the presumable autoinhibited conformation. The conserved structural and functional elements, like the PX domain, three SH3 domains and the PRR of this region, or combinations of these are expressed as individual recombinant proteins. Systematic screening for interactions between the obtained protein fragments is in progress. We have already shown that the tandem SH3 domain (SH3-1 and SH3-2) of TKS4 binds the PRR. Moreover, lipid binding assays indicate that the presence of the first two SH3 domains decreases the PX domain\'s affinity to lipids, further supporting our hypothesis.

PI-9

Hormone-sensitive fluorescent biosensors and *in vitro* assays for steroid receptors and steroid oxidoreductases

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Hormone-dependent cancers are a significant social, medical and economic burden. Breast cancer is the most frequent cancer among women and one of the leading causes of mortality in Europe. Hormonal steroids influence the proliferation of hormone-sensitive (e.g. ovarian, prostate, endometrial and breast) and other cancers (e.g. cervical and colorectal). Reduction of circulating sex hormone levels is an important treatment strategy. Steroidal anticancer drugs in clinical use against breast or prostate cancer (e.g. exemestane, formestane, and abiraterone) slow cancer growth by inhibiting steroidogenic cytochrome P450 enzymes (CYP19, CYP17) or steroid oxidoreductases (17b-HSD, AKR1C). Other steroidal drugs (e.g. fulvestrant, megestrol and cyproterone) target hormone receptors, such as estrogen and androgen receptors. Because of the prevalence of cancers resistant to current treatments, screening for compounds with improved anticancer properties remains an active field of research. However, development of new effective drugs is extremely difficult, and rapid, sensitive assays for screening countless newly synthesized and promising compounds are necessary. To identify potential compounds for treatment of hormone-dependent cancers and to investigate mechanisms of enzymes involved in cancer growth, we developed yeast-based hormone-sensitive fluorescent biosensors, *in vitro* assays and computational simulations. Briefly, yeast vectors have been created encoding steroid receptor ligand binding domains fused to yellow fluorescent protein. Cells treated with cognate steroid ligand display dose-dependent increase in fluorescence shown using a fluorescence plate reader and fluorescence microscopy. Combinations of steroid active enzymes and steroid receptors have been cloned for co-expression in yeast for coupled assays. Steroid-active enzymes and oxidoreductases have also been cloned and expressed in *E.coli* for purification, biochemical screening and crystal structure analysis with lead compounds. Molecular docking and computational simulations are utilized to select promising compounds for screening. This combined approach is low-cost, reusable, and non-radioactive and has applications not only for drug screening, but also for detection of environmental chemicals with estrogenic/androgenic properties, and understanding the structural basis of steroid ligand recognition.

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PI-10

Tyrosine 24 phosphorylation of annexin A2 regulates isoform-selective S100 protein binding

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S100A4 is a small, dimeric EF-hand Ca^{2+}-binding protein. Increased concentration of S100A4 promotes metastasis formation through interactions with protein partners like non-muscle myosin-IIA, p53 and annexin A2 (ANXA2). ANXA2 is a non-EF-hand-type Ca^{2+}-binding protein that exhibits Ca^{2+}-dependent phospholipid binding. Its translocation and activation is believed to be regulated by serine and tyrosine phosphorylation that occur in the unstructured N-terminal domain. ANXA2, in complex with different S100 proteins, is involved in diverse cellular processes such as cell motility, membrane aggregation and linkage of membrane associated proteins to the cytoskeleton. It was previously demonstrated that an S100A10 dimer binds symmetrically the two ANXA2 N-termini (Ser^2-Glu^{14}). According to our binding studies, S100A4 interacts with this peptide one order of magnitude weaker than with the peptide Ser^2-Phe^{33} comprising the full unstructured ANXA2 N-terminus, while the extension of the peptide Ser^2-Glu^{14} does not affect the affinity of S100A10. Strikingly, the stoichiometry of the interaction is one S100A4 dimer per one ANXA2. We determined the 2.5 Å crystal structure of the S100A4–ANXA2 complex, which revealed an asymmetric binding mode as the entire ANXA2 N-terminus wraps around the S100A4 dimer. Upon complex formation the N-terminus adopts two helices which bind to the canonical hydrophobic pockets of each S100A4 subunit. Interestingly, the Ser^2-Glu^{14} region of another ANXA2 interacts with one of the S100A4 subunits (as in the case of S100A10–ANXA2 interaction). Liposome aggregation experiments showed that although both S100A4 and S100A10 enhanced ANXA2 mediated membrane bridging, the effect of latter was more pronounced, which is in line with the different affinity and structure of the two complexes. Furthermore, we were able to carry out the selective and quantitative phosphorylation of Tyr^{24} on ANXA2 by EphB1 kinase domain and we solved the crystal structure of ANXA2-Tyr^{24P}. We found that the Tyr^{24} phosphorylation reduces one order of magnitude the affinity of S100A4 to ANXA2 which can be rationalized based on our crystal structures. In conclusion, the novel complex structure of S100A4 implies that the asymmetric binding mode can be more general in the S100 family, additionally we showed that ANXA2 Tyr^{24} phosphorylation could serve as an isoform-selective regulation of S100–ANXA2 interactions.

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PI-11

Understanding the functional role of segments involved in lipid-mediated regulation of a key lipid biosynthetic enzyme from the malaria parazite

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Despite ongoing antimalarial research, malaria is still regarded as one of the most serious infectious diseases, with the vast majority of death cases caused by *Plasmodium falciparum*. Emerging of the multidrug-resistant parasite strains urges the identification of antimalarials with a novel mechanism of action. Accordingly, *de novo* phosphatidylcholine biosynthesis of the parasite was recognized as a validated antimalarial target. Within this pathway, CTP:phosphocholine cytidylyltransferase (*Pf*CCT) was shown to catalyze a rate-limiting step. An interaction with membrane lipid layer essentially regulates the enzyme activity of *Pf*CCT. The understanding of the molecular mechanism of this regulation stands in the focus of our interest. Specifically, we examine two segments of *Pf*CCT that are involved in this action. Firstly, we assess the functional role of different regions within the membrane binding domain of *Pf*CCT by designing relevant truncated constructs for expression in *E coli*. Through analysis of their enzyme activity in presence and absence of lipids as well as biophysical characterization of how membrane interaction affects ligand binding we seek to provide functional explanation for the notable differences between this domain as well as its mammalian CCT counterparts.
Secondly, we characterize catalytic domain constructs of *Pf*CCT with different truncations at its C-terminal αE helix. This region was also indicated to be involved in the lipid-induced regulatory action of the enzyme as ligand binding affects its conformational state. We found that truncation of this segment causes inactivation of the respective *Pf*CCT construct as well as attenuated ligand binding capability. Biophysical characterization of constructs with different truncations at this segment is carried out to reveal the underlying mechanism of the participation of this segment in enzyme action. The prospect of the well-defined role of these key regulatory segments of the enzyme could be further exploited for more specific drug targeting.

PI-12

Structural characterization of an organophosphate sequestering esterase from *Culex pipiens*, a disease carrying mosquito

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Organophosphate (OP) resistance has been reported in multiple insect species with three main modes of resistance: changes in the sensitivity of acetylcholinesterase, hydrolysis of the OP by carboxylesterases (CBEs) and sequestration of the OP by overexpressed CBEs. Resistance to OP insecticides in mosquitoes is generally achieved by CBE gene amplification, resulting in increased CBE expression. Through high affinity binding, the CBEs sequester the OPs and very slowly hydrolyse the compounds. In the mosquito *Culex pipiens,* the two esterases responsible for this are the A and B CBEs which are closely linked on the chromosome. To understand the high affinity of multiple OPs for the mosquito and differences between several reported allozymes, CBE B2 from *C. pipiens* was selected for characterization. Following lysine methylation, the protein was crystallized and the structure solved to 2.6 Å resolution. The structure reveals great similarity (Sequence ID =37%, RMSD = 1.3) to our previously reported CBE structure from the Australian sheep blowfly *Lucilia cuprina* (αE7/E3). However, the *C. pipiens* CBE has a more open active site suggesting the natural substrate is a longer chain fatty acid. The structure of the first CBE from mosquitoes opens up an opportunity to investigate the mechanism of sequestration for OP resistance and to understand the different selection pressures between the two different modes of OP resistance involving CBEs.

PI-13

EpCAM oligomerization analysis

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Epithelial cell adhesion molecule (EpCAM) is a cell surface type I transmembrane glycoprotein, expressed in most epithelial cells and frequently overexpressed in many types of carcinoma. Through its role in cell-cell adhesion and signalling, it affects cell proliferation, migration and metastasis. Due to its localization and frequent overexpression in cancer cells, EpCAM is often used as a diagnostic marker and a drug delivery target.
While it is well established that EpCAM is able to form oligomers, little is known about role of oligomerization with regards to proteins function and ability to interact with other proteins. The current model proposes intracellular *cis*-dimerization and intercellular *trans*-tetramerization of two *cis*-dimers on adjacent cells. Different models proposing even higher oligomeric forms have also been discussed, but their existence is yet to be confirmed.
In our work we characterized and compared oligomerizational properties of different forms of EpCAM using microscale thermophoresis (MST). Our observations explain the role of transmembrane region, glycosylation and N-terminal domain in oligomerization. Transmembrane region decreases dissociation constant (K_d) for both *cis*-dimerization and *trans*-tetramerization. Glycosylation on the other hand appears to increase K_d for *trans*-tetramerization while it doesn't effect *cis*-dimerization. The N-terminal domain contributes to *trans*-tetramerization, but is not involved in *cis*-dimerization. Recent determination of EpCAM *cis*-dimer 3D structure also allowed us to identify several residues on protein surface which could have a crucial role in formation of *trans*-tetramer. The results shed light on the mechanism of EpCAM oligomerization and present the grounds for further investigation of EpCAM oligomeric state, also with regard to interactions with other proteins. Better understanding of EpCAM oligomerization and its role in normal and cancerous cells alike may well represent the base for further optimization and development of novel anti-EpCAM antitumour strategies.

PI-14

Investigation of potential sterol binding sites in ABCG1 protein

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The ABCG1 protein belongs to the ATP binding cassette (ABC) transporter G subfamily. With regards to its function, ABCG1 have been proposed to play a role in HDL-dependent cellular lipid/sterol regulation and can induce apoptosis by an unknown mechanism. Studies investigating the HDL-dependent efflux of different sterol derivatives identified cholesterol, desmosterol, 7-ketocholesterol, etc., as potential substrates of ABCG1. Moreover, accumulation of desmosterol was observed in brain of Abcg1 knock-out mice. However, direct interactions between ABCG1 and these sterols have not been demonstrated yet; neither the regions of ABCG1 interacting with sterol compounds are revealed. Several previous studies identified specific amino acid sequences in proteins involved in cellular sterol homeostasis. The Cholesterol Recognition Amino acid Consensus (CRAC, L/V-$X_{(1-5)}$-Y-$X_{(1-5)}$-R/K) and Sterol Binding Element (SBE, L/M-XX-L-XX-L) motives were examined in several proteins and identified as an important sequence in the interaction with sterol molecules.
In the present work, we mutated the putative CRAC and SBE motives in the ABCG1 protein by replacing the central tyrosine or leucines to alanine by using site-directed mutagenesis. The different ABCG1 variants were expressed in both mammalian cells and *Spodoptera frugiperda* 9 (Sf9) insect cells. The expression and localization of these CRAC and SBE mutants were investigated in Hela and HEK cells by Western blot analysis and confocal microscopy, respectively. The apoptotic effects of the ABCG1 mutants were compared to that of the wild type protein using a High Content Screening instrument.
In addition, the function of the ABCG1 variants was assessed by measuring their ATPase in Sf9 cell membrane preparations. Using this approach the interaction of ABCG1 mutants with different sterol compounds can also be measured. Combining the results obtained with mammalian and Sf9 cells allowed us to map the sterol-sensing regions in the ABCG1 protein, and establish a link between the different functions of the transporter.

PI-15

Using a molecular switch to decipher protein-protein interactions within the living cell: what can we learn about horizontal gene transfer?

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Bacterial genomes may include phage-related mobile genetic elements that encode virulence enhancing factors e.g. toxins termed as pathogenicity islands. There is an intimate relationship between pathogenicity islands and helper phages in *Staphylococcus aureus,* as *S. aureus* pathogenicity islands (SAPIs) are under endogenous repressor control by SAPI specific Stl proteins that are encoded by the pathogenicity island. Helper phages, on the other hand, encode proteins with derepressor functions. After infection, the derepressor protein relieves Stl-exerted repression. A phage-encoded dUTPase was shown to be responsible for derepression of the pathogenicity island SAPIBov1. The mechanism of this derepression is of direct interest for understanding transmission of virulence and pathogenic factors.

Our aim was to construct a switchable model to characterize the Stl driven repression and the dUTPase induced derepression within cellular environments. We have chosen *Mycobacterium smegmatis* as a model to build this system, as we already have various dUTPase mutant strains in this organism. In *Mycobacteria,* dUTP hydrolysis has exclusive biosynthetic role in dTTP biosynthesis, and also serves to eliminate excess dUTP to prevent DNA uracilation. In addition, the inhibition of dUTPase has a well-defined phenotype in *Mycobacteria*. This system is aimed at providing an assay background to check different mutants of dUTPases and Stl proteins for their preserved or diminished functionality in cellular environment.

To characterize the aforementioned interactions, we designed and created a strain, where the following construct was integrated into the genome. Stl binding site was cloned in a promoter element driving expression of a β-galactosidase reporter protein resulting in blue colonies. In contrary, binding of Stl to its recognition site represses gene expression from the reporter gene therefore functional repression results in white colonies. To test functional repression by Stl protein in the cell, the coding region of Stl was randomly mutagenized and nonfunctional Stl variants were sequenced. In addition, we also tested various truncated and point mutant Stl variants in this system and characterized the regions necessary for functional gene repression. We also plan to test the interaction of Stl with various (mutant) dUTPase proteins in this system.

PI-16

Characterization of plant dipeptidyl-peptidases III from *Physcomitrella patens* and *Arabidopsis thaliana*

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Plant dipeptidyl-peptidases III (DPPs III) have been recognized only recently, as data from plant genome sequencing projects have become available. The amino acid sequence showed them to be atypical compared to previously characterized DPPs III, as the signature active site motif of the DPP III family, the hexapeptide HEXXXH, is replaced by a pentapeptide HEXXH motif, common among many other zinc-metallopeptidases[1]. We have cloned, heterologuosly expressed, and purified DPPs III from *Physcomitrella patens* and *Arabidopsis thaliana*. Biochemical characterization by dipeptidyl-2-naphtylamide substrates has revealed significant differences between the two enzymes. Furthermore, we have changed the pentapeptide HECCH motif in *Physcomitrella* DPP III to HECCGH and HECLGH by site-directed mutagenesis and have shown that relatively low peptidase activity of this enzyme is not due to the hexapeptide motif replacement.

The presence of a NUDIX domain on the N-terminus is an additional atypical characteristic, specific to plant DPPs III. This domain is found in proteins belonging to the Nudix hydrolase superfamily, which predominantly catalyze the hydrolysis of small nucleotide substrates composed of a nucleoside diphosphate linked to another moiety X. By bioinformatic analysis, due to its NUDIX domain, *Arabidopsis* DPP III was predicted to be an isopentenyl diphosphate isomerase (IDI)[2]. We have performed a functional colour complementation assay of IDI activity and shown this prediction to be false. In addition, we discuss putative Nudix hydrolase activity of plant DPPs III based on similarity to characterized plant Nudix hydrolases.

Since the biological function of plant DPPs III is currently unknown, our results represent a starting point for understanding of these novel and peculiar proteins.

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PI-17

Chloride/formate exchanger (CFEX/Slc26a6) in rat organs; sex-dependent expression in kidneys

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In mammalian organs, transport of chloride, bicarbonate, oxalate, formate and hydroxyl ion is mediated by the chloride/formate exchanger Slc26a6 (CFEX). Most information about this transporter is known from the studies in mice, whereas its presence in various rat organs has been poorly studied. Here we investigated the presence and possible sex-related expression of CFEX protein and mRNA in various rat organs by qRT-PCR and immunochemical methods, respectively. Specificity of the commercial polyclonal anti-CFEX antibody was confirmed in HEK293 cells transiently transfected with the rat CFEX cDNA; the antibody strongly stained the plasma membrane of CFEX-transfected cells, whereas the mock-transfected cells remained unstained. Thereafter, the CFEX protein was immunolocalized to the brush-border membrane of renal proximal tubules (S1~S2>S3) and intestinal enterocytes (duodenum > jejunum; ileum was negative), canalicular membrane of hepatocytes, and apical domain of pancreatic ducts. By Western blotting of total cell membranes isolated from these organs, the antibody labelled a single protein band of ~120 kDa. The CFEX mRNA expression in most organs followed the protein expression. In the liver, the expression of mRNA was similar in male and female rats. In the gastrointestinal tract, the mRNA expression was highest in duodenum, followed by jejunum and ileum, and sex-independent, and negligible in stomach, caecum and colon. In the kidneys, the CFEX protein and mRNA expression was zone-dependent (cortex < outer stripe), and exhibited sex differences in protein (males > females) but not mRNA (males = females) expression. The renal CFEX protein expression was low and sex-independent in prepubertal rats, whereas in adult animals, it was downregulated by castration and unaffected by ovariectomy. Therefore, CFEX is expressed in various rat organs, whereas in kidneys, the male-dominant protein expression appears after puberty due to androgen stimulation.

PI-18

Pinpointing the interaction domains of plant seryl-tRNA synthetase and metabolic protein BEN1

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Aminoacyl-tRNA synthetases (aaRS) support translational process by attaching appropriate amino acids to cognate tRNAs. Charged tRNA molecules reach ribosome and the amino acid can be transferred onto a growing peptide, according to the genetic code. It is well known that aaRSs can be involved in diverse cellular functions beyond translation independently or by forming protein-protein interactions. Understanding these non-canonical functions opens new perspectives in functional proteomics. However, the studies of plant aaRSs are scarce. Therefore our recent findings on interaction of BEN1, protein involved in metabolism of brassinosteroid hormones, and cytosolic seryl-tRNA synthetase (SerRS) from plant *Arabidopsis thaliana* broadens our knowledge of novel non-translational functions of plant aaRSs. We detected BEN1:SerRS interaction *in vivo* using Y2H screen and confirmed it *in vitro* using SPR method, also determining the dissociation constant of the protein complex. To pinpoint regions responsible for protein-protein interaction truncated variants of both SerRS and BEN1 protein were prepared and analyzed using microscale thermophoresis assay, that also enables K_d determination. K_d for complex containing BEN1 and truncated SerRS variant without basic C-terminal extension was similar to K_d of the SerRS:BEN1 complex indicating that basic C-terminal extension does not participate in interaction. Furthermore, N-terminal tRNA binding domain of SerRS did not form the complex with BEN1. Therefore, we conclude that interaction involves the central part of SerRS that contains globular catalytic domain. Furthermore, using reverse transcription-quantitative polymerase chain reaction we investigated possible correlation of these two proteins on the level of gene expression in wild type plants, but also in transgenic plants overexpressing SerRS. We observed slightly lower expression of BEN1 gene in transgenic plants compared to the wild type. Combining insights from both gene and protein level allowed us to define interaction surfaces and propose functional importance of SerRS:BEN1 assembly.

PI-19

pH regulated pore-forming activity of Listeriolysin O mutant

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Listeriolysin O (LLO) is a Cholesterol-Dependent Cytolysin (CDC). It's expressed as a monomeric protein, able to bind to cholesterol-rich lipid membranes, followed by oligomerization on the surface of the membranes and formation of 25-30 nm wide transmembrane pores. LLO is one of the main virulence factors of *Listeria monocytogenes*, an important food-borne intracellular pathogen that causes listeriosis. Upon infection, LLO is essential for the bacterial escape from the phagolysosome and spread to neighbouring cells. LLO features a peculiar pH-dependent *stability*, being inactivated at temperatures above 30 °C and neutral pH, which is important for its biological role. Our goal was to explore pH sensitive region(s) of LLO in detail by engineering of mutants that would express differential pore-forming activity in different pH environments..
Monomeric LLO has an elongated four-domain structure typical for CDC family of proteins. Domain at the narrow tip of the molecule is the membrane binding domain, responsible for primary interactions of LLO with the membrane, and the other three domains are responsible for oligomerization and pore formation. A number of LLO mutants were designed and prepared by site-directed mutagenesis, and many of them exhibited significant pH dependant pore-forming ability as determined by hemolysis of red blood cells. Y406A mutant was the most interesting since its activity at low pH (5.7) resembled the wild-type activity, while at neutral pH (7.4) it exhibited at least a 100-fold decrease in hemolytic activity. We then set out to explore effects of this mutation on the protein structure. The decrease of the melting temperature of the mutant by about 5 °C indicated a perturbance of structural stability. Molecular dynamics simulations (MD) of the wild-type LLO and Y406A mutant showed significant differences in RMSD and radius of gyration between the two proteins, suggesting the (i) increase of flexibility of the molecule as well as (ii) changes in interdomain contacts upon mutation. Structural changes can result in (long distance) electrostatic effects such as pKa shift of certain amino acid side chains. Changes in pKa could in turn lead to different activity of the LLO Y406A at different pH values.

PI-20

Mechanism of Ca^{2+}-dependent membrane bridging by annexin A2 and its regulation by tyrosine phosphorylation and S100 binding

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Annexin A2 (ANXA2) is a non-EF-hand-type Ca^{2+}-dependent phospholipid-binding protein. It is involved in numerous membrane-related cellular processes such exocytosis, endocytosis and membrane trafficking. Its disordered N-terminal domain (NTD) contains the S100A10 and S100A4 binding site, while the core domain is responsible for Ca^{2+}-dependent binding to acidic phospholipids. It was previously demonstrated that the NTD is not requisite for membrane bridging activity. In contrast, phosphorylation of Tyr24 or mutation of Ser12 and Ser26 to phosphomimetic glutamate interferes with ANXA2 function. Here we investigated the structural and functional consequences of Tyr24 phosphorylation and S100 protein binding by X-ray crystallography and MD simulation. We found that Tyr24 phosphorylation decreases significantly the dynamics of the NTD and increases the thermal stability of ANXA2. Moreover, the NTD is in closer proximity to the core domain in the phosphorylated ANXA2 than in the unphosphorylated protein. Previous results indicated that ANXA2-Tyr^{24P} was not able to aggregate large unilamellar vesicles (LUV) in contrast to ANXA2 and its deletion mutant that lacks the entire NTD. Interestingly, in the presence of either S100A10 or S100A4, ANXA2-Tyr^{24P} exhibited the same membrane bridging activity than the unphosphorylated ANXA2. Furthermore, both S100 proteins lowered 10-20-fold the critical Ca^{2+} concentration of ANXA2-induced LUV aggregation. We compared the affinities of S100A4 and S100A10 to ANXA2 variants in a free or a LUV-bound form. While S100A10 bound to both form of ANXA2 and ANXA2-Tyr^{24P} with similar affinities, S100A4 bound 50-fold stronger to LUV-bound ANXA2. Based on our findings we propose a mechanism for Ca^{2+}-dependent membrane bridging by ANXA2 in which the NTD is a negative regulator of ANXA2 function. Upon association to phospholipids, the NTD detaches from the concave surface of ANXA2 to allow core domain – core domain interactions resulting in membrane bridging. Phosphorylation by $pp60^{c\text{-}src}$ (or PKC) favors a closed conformation, while S100 binding induces an open form of ANXA2 as observed in the crystal structure of the ANXA2–S100A4 complex (see the abstract of P. Ecsedi). These mechanisms might contribute to the fine-tuning of AXNA2-mediated membrane-related processes.

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PI-21

Selection, binding and structural analyses of peptide ligands for the Fc portion of human immunoglobulin G

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Affinity chromatography based on immunoglobulin (Ig)-binding proteins, such as staphylococcal protein A and streptococcal protein G, typically represents the initial step in therapeutic antibody purification process. However, this approach suffers from high cost, poor ligand stability and the requirement for relatively harsh elution conditions that can negatively impact activity and immunogenicity of antibodies. Compared to protein ligands, peptides represent an interesting alternative due to higher stability and easier and less expensive production. Furthermore, the expected lower affinity for immunoglobulins should allow for elution under milder conditions. The aim of our research was to identify a short peptide ligand for the Fc region of human IgGs. We have screened two commercially available phage display libraries of random cyclic and linear heptapeptides for binding to immobilized human Fc region using classical biopanning approach. Five clones (two linear and three cyclic) were shown to specifically interact with the Fc portion of immunoglobulins as verified by a set of ELISA assays. The identified ligands showed partial homology to immunoglobulin-binding protein A, but they did not display a common amino acid motif. Individual phage-displayed peptides were able to recognize specific subclasses of IgG (IgG1, IgG2, IgG3, and IgG4). To determine the minimal binding motif, the highest-affinity phage clone, C11, was subjected to alanine scanning. Experiments to evaluate binding characteristics of clone C11 to Fc region are in progress. The details will be disclosed at the Meeting.

PI-22

MD and QM/MM modeling of nitric oxide binding to myoglobin

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Nitric oxide is a key messenger molecule involved in many biological processes. It is also a notorious inhibitor of many heme-containing enzymes. Despite its importance, the exact mechanism of NO binding to heme-containing enzymes is still unclear. Therefore, we studied the binding of NO to myoglobin, an experimentally well-characterized heme-protein. Our goal was to investigate the diffusion of NO from the bulk phase to the active site using molecular dynamics simulations and to elucidate the reaction mechanism of the spin-forbidden recombination reaction of nitric oxide and myoglobin using QM/MM calculations. Therefore, we carried out long equilibrium molecular dynamics simulations on the system to determine the pathways which can be used by gas molecules to reach the active site of myoglobin and to identify cavities inside myoglobin where NO could be stored. We identified the major residues influencing NO transport into and in the protein. The equilibrium MD simulations were complemented by shorter pulling force MD simulations in order to get a reasonable estimate of the diffusion rate of nitric oxide into myoglobin.
In order to be able to predict the rate of nitric oxide binding to myoglobin we performed QM/MM optimization of snapshots selected from the trajectory of the equilibrium MD simulation. We also performed these QM/MM optimizations to determine the equilibrium structure of heme-NO complexes in various spin states which were followed by the locating the minimum energy crossing points between the various potential energy surfaces. This enabled us to determine the most likely mechanism of the spin-forbidden myoglobin-NO recombination reaction. By studying various snapshots taken from the MD trajectory we were able to get insight into the electrostatic effect of the protein structure on the reaction mechanism.

PI-23

Insights into NLP activity

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Necrosis and ethylene-inducing peptide 1 (Nep1)-like proteins (NLPs) are a group of proteins that are secreted by several phytopathogenic micro-organisms. They trigger leaf necrosis and immunity-associated responses in various dicotyledonous plants. The crystal structure of toxic NLP_{Pya} from *Pythium aphanidermatum* uncovered a central β-sandwich surrounded by α-helices. Tertiary structure-based comparison of the NLP fold revealed structural similarity to actinoporins, a family of cytolytic proteins that act by disrupting the integrity of target cell membrane through pore formation. Fold conservation suggests that the two protein families share a common cytolytic mode of action. Interestingly, HaNLP3 protein, produced by the oomycete *Hyaloperonospora arabidopsidis*, while sharing a high sequence similarity to cytotoxic NLPs, does not induce necrosis. Crystal structure of nontoxic HaNLP3 alongside site-directed mutagenesis of NLP_{Pya} might thus unravel important features for NLP toxicity.
HaNLP3 was expressed and purified from *Pichia pastoris* as a monomeric protein. Circular dichroism (CD) showed a significant proportion of β-structure (> 40 %). In order to define the local environment of tryptophan residues in the native state of HaNLP3, we monitored its intrinsic tryptophan fluorescence as a function of temperature. The results have shown that tryptophan residues are partially buried in the interior and are completely exposed only after denaturation of the protein occurred. These results were confirmed with crystal structure. Although HaNLP3 strongly resembles the overall structure of NLP_{Pya}, it reveals some unique features. It has been shown previously that coordination of a metal ion is crucial for cytolytic activity of NLPs. In HaNLP3, however, the coordination site significantly differs from that of NLP_{Pya}, as well as the conformation of the neighboring three loops. Moreover, an additional nonconserved disulfide bond present in HaNLP3 might contribute to limited conformational flexibility of HaNLP3 and thus potentially the absence of toxicity. Structure-based mutants obtained by site-directed mutagenesis in the region between conserved disulfide bond in NLP_{Pya} showed reduced necrotic activity. Altogether, these data allow a step further towards the understanding of cytolytic activity of NLPs.

PI-24

First characterization of multidrug and toxin extrusion (MATE/SLC47) proteins in zebrafish (*Danio rerio*)

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Multidrug and toxin extrusion (MATE) proteins from the solute carrier superfamily (SLC) are involved in the extrusion of endo- and xenobiotics across the plasma membrane, similar to the ATP-binding cassette (ABC) transporters, but in ATP non-dependent manner since they use proton gradient as the driving force for the efflux. MATEs are conserved from bacteria to mammals with differing number of genes within groups. In humans, three MATEs have been found (MATE1, 2, 2k), whereas in zebrafish (*Danio rerio)* we have found six members annotated as DrMate 3-8. We present first insight into the zebrafish *(Danio rerio)* Mate proteins based on the phylogenetic analysis, tissue expression profiling and functional characterization. Phylogenetic analysis revealed six Mates in teleost fish which form a distinct cluster separated from the tetrapod MATEs. Tissue expression analysis showed high expression of zebrafish Mates in toxicologically important tissues, kidney and liver, as well as in testes.
We have determined transporter kinetic parameters for the uptake of 6 fluorescent dyes out of which 4', 6-diamidino-2-phenylindole (DAPI) was selected as a dye of choice for subsequent testing of more than 20 selected interactors of mammalian MATEs. Inhibition constants (Ki) were determined for more than 10 physiological and xenobiotic compounds including hormones, bile salts, drugs and pesticides.
To summarize, we have characterised zebrafish Mate transporters on the bioinformatical, expressional and functional level giving first insight into fish efflux transporters from SLC superfamily.

PI-25

Compounds with phenanthridine moiety as potential modulators of butyrylcholinesterase activity

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Butyrylcholinesterase (BChE, E.C. 3.1.1.8) is a serine hydrolase closely related to acetylcholinesterase (AChE; EC 3.1.1.7). AChE is a key enzyme in the regulation of cholinergic transmission that catalyzes the hydrolysis of the neurotransmitter acetylcholine (ACh). Since Alzheimer's disease (AD) is characterized by the decline of ACh levels, drugs designed for the treatment of AD are AChE inhibitors that lead to an increase of ACh levels and alleviation of disease symptoms. Although BChE\'s physiological function is yet unclear, it can serve as a co-regulator of cholinergic neurotransmission due to ACh hydrolysis. Moreover, BChE plays an important role in the pathogenesis of AD due to a role in the amyloid beta-peptide aggregation developing into senile plaque deposits. It is reasonable to assume that BChE inhibition will have a beneficial impact on the medical treatment of AD patients. Selective BChE inhibitors could lead to better AD drugs. Crystal structures of AChE-inhibitor complexes have shown that AChE inhibitors usually interact with the active site amino acids *via* arene–arene (π-π) interactions. Even though AChE and BChE active sites have different amino acid compositions with 6 out of 14 aromatic amino acids in the AChE active site corresponding to aliphatic ones in the BChE active sites, BChE can still be considered to have significant potential for π-π interactions with inhibitors. In the search for new BChE inhibitors, we have turned our attention toward small organic molecules with phenanthridine moiety. This moiety is often applied for an ''aryl'' component due to structural features like a permanent or pH-induced positive charge, high polarizability, high electron affinity and highly polar amino groups. The property of induced positive charge may help these compounds to pass the blood-brain barrier and act in the central nervous system. Therefore, we studied the inhibition of BChE with 10-aminophenanthridine and phenanthridines substituted at the position 8 of the phenanthridine ring with biguanide, benzyl carbamate, or urea-based substituents characterized by a combination of binding and steric properties. To demonstrate the significance of positive charge on the stabilization of BChE inhibitors, we also studied the inhibition of BChE with monocationic 2-(cinnolin-4(1*H*)-ylidenemethyl)-4,6-dimethylthiazolo[4,5-b]pyridin-4-ium iodide. All of the compounds reversibly inhibited BChE with inhibition constants (K_i) in the 5–750 micromolar range.

PI-26

HPLC, ESI qTOF and MALDI TOFTOF MS reveal target sequence and binding stoichiometry of novel Ru(II) complexes to serum albumin

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Serum proteins are the first molecules that metallo chemotherapeutics encounter after being administered intravenously. The objective of the present study was to investigate interactions of two novel ruthenium (II) complexes of general formula [Ru(L3)(N-N)X][Y] (where L3 = 4′-chloro-2,2′:6′,2″-terpyridine (Cl-tpy); N-N = 1,2-diaminoethane (en) or 1,2-diaminocyclohexane (dach); X = Cl; Y = Cl, with the major serum transport protein – albumin. The formation of Ru (II) complex-albumin adducts upon binding of the complexes to serum albumin in solution was confirmed by HPLC, using a silica-based SEC column that separated the protein adducts from the free complexes. The Ru (II) complex-albumin adducts were subjected to a tryptic digestion and the resulting peptides were resolved on a reversed-phase HPLC column. The fractions containing the Ru complexes were detected using absorbance at 317 nm. The sequences of the peptides carrying bound Ru complexes were revealed by MALDI TOF TOF MS analysis. The exact number of the complex molecules bound to one albumin molecule was easily assessed using ESI qTOF MS analysis of the intact albumin before and after its incubation with the Ru complexes.
The elution of the albumin adducts from the SEC column could be tracked using an extra peak of absorbance at 317 nm, which originated from the bound Ru complexes. After the digestion of the albumin adducts, the same peak was observed on most of its peptides. As confirmed by the ESI qTOF MS, both Ru (II) complexes showed a high binding affinity toward serum albumin. MALDI TOF TOF MS revealed the existence of multiple Ru binding sites on the albumin, with histidine being the main target for these complexes.
This study demonstrates an easy and reliable method to assess the binding of the Ru (II) complexes to serum albumin. Combining SEC HPLC, ESI MS and MALDI TOF TOF MS analyses, we: i) performed a quick detection of the binding of a transition metal complex to a protein in solution; ii) excluded the possibility that the peptides bearing Ru (II) complexes, which were identified by MALDI TOF TOF MS, were mere artefacts of the binding that occurred in the gas phase of the instrument; and iii) assessed a stoichiometry of the Ru (II) complex-albumin adducts. According to our study, MALDI TOF TOF MS appears to be a suitable method for investigating interactions of metallodrugs with soluble proteins.

PI-27

Secreted aegerolysins and MACPF domain-containing proteins in the filamentous fungus *Aspergillus niger*

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Aegerolysins and membrane attack complex/ perforin (MACPF) domain-containing proteins (Pfam06355 and 01823 protein families, respectively) are found in various kingdoms of life including fungi. In Basidiomycota, proteins of these two families seem to be involved in development of primordia and fruiting bodies, while in filamentous fungi they can also act as virulence factors. Various fungal members of both protein families have been shown to form pores in biological and artificial lipid membranes, either sole or in combination with one another. It appears that the roles of these proteins are pleiotropic and adapted to the fungal life-style.

Aspergillus niger is a saprophytic, filamentous fungus found throughout the world. In its genome, we identified two nucleotide sequences encoding aegerolysins and two nucleotide sequences encoding proteins with MACPF domain. Our aim is to determine the biological role(s) and some characteristics of these proteins in *A. niger* using systematic gene expression studies, gene deletions, protein labelling, studies of protein interactions with lipid vesicles and some toxicological studies. So far, we showed that the increase of the expression of all four target genes coincides with the beginning of conidiation in *A. niger*, and that the prevention of conidiation (either physical or genetic) alters the expression profiles and negatively affects their expression. Deletion of either of the aegerolysin genes did not affect the rate of conidiation or growth on different media. Using the polyclonal antibodies against recombinant aegerolysin (A145), we were able to confirm that aegerolysins in *A. niger* are secreted out of the fungus despite the lack of the corresponding signal peptide. Experiments employing surface plasmon resonance and vesicle sedimentation assay showed that this protein can bind to lipid vesicles composed of ceramide phosphoethanolamine and cholesterol (1:1, mol:mol), but is not able to permeabilize the membrane. Using fluorescently labeled A145 we were also able to stain membranes of cells derived from the insect *Spodoptera frugiperda* that contain considerable amounts of ceramide phosphoethanolamine. Our results suggest that aegerolysins and MACPF domain-containing proteins are produced during conidiation of *A. niger*, but are not actively involved in this process, indicating that their role(s) might be related to some other physiological processes in the fungus, e.g. defence mechanisms, rather than development.

PI-28

Structural and functional characterisation of a staphylococcal repressor

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The *Staphylococcus aureus* pathogenicity islands (SaPI) encode several genes responsible for pathogenicity (e.g. toxins) and have a major role in spreading virulence genes among bacterial populations. The repressor protein Stl obstructs the expression of SaPI transfer initiating proteins in the absence of Φ11 helper phage dUTPase. The presence of dUTPase, however, activates the SaPI transfer via Stl-dUTPase complex formation [1] [2]. Here we aim to provide an in-depth characterization of the Stl repressor with regard to its DNA binding ability and its potentional in vivo function.
At first, we would like to pinpoint the DNA sequence element(s) that constitute a minimal binding site for Stl. We designed a number of oligonucleotides and followed a rational pattern of motif identification, we have used EMSA assays and *in silico* predictions. Our results show that there are at least three binding motifs for Stl located at different regions within the SaPI segment. Each of these binding motifs constitute 23 base pairs, and consist of a 6 nucleotide long palindromic sequence pair, separated by a 5 oligonucleotide long, nonspecific sequence.
Recently, we have also started *in vivo* studies within the staphylococcal cell. Our aim is to complement the *in vitro* analysis with *in vivo* studies. We have initiated expression pattern analysis for several proteins that are key players in uracil DNA metabolism. To verify the effect of these proteins on the potentional synthesis and degradation of uracil-DNA, we plan to determine dNTP pools and the genomic uracil level in Staphylococcus under normal and stress conditions.

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PI-29

Tumor marker Trop2 - a phosphorylation-triggered structural switch at the membrane-cytosol interface

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Human tumor marker and drug delivery target Trop2 is a dimeric bitopic transmembrane glycoprotein with proliferation-enhancing signaling activity. Here, proteolytic cleavage of Trop2 for which triggers are not yet understood results in the release of the short cytoplasmic tail (Trop2IC). This small fragment of 26 amino acid residues is then engaged in a signalling complex and later translocated to the nucleus where it activates/enhances transcription of cell cycle promoting genes. However, an important aspect of Trop2 biology remains unclear - Ser303 phosphorylation within the cytosolic tail. Therefore, we set on to analyze the effect of Ser303 phosphorylation on Trop2IC structure with possible implications in downstream signalling events.
Detailed structural characterization of Trop2IC by NMR showed that phosphorylation triggers salt bridge reshuffling which is accompanied by significant structural changes in the regions flanking the central α-helix of both non-phoshorylated and phosphorylated forms. These changes significantly affect the structure and accessibility of functionally important regions of the cytosolic part - phosphatidylinositol 4,5-bisphosphate-binding site and protein kinase C δ interaction region, which are both believed to be implicated in Trop2 signaling activity. Next, we complemented these results by molecular dynamics simulations of the Trop2 transmembrane region. Here, we demonstrated that cytosolic regions of two Trop2 subunits could be brought into close proximity via transmembrane part dimerization thereby adding another level of complexity to Trop2 signalling mechanism. The observed changes hint that cytosolic tail of Trop2 could function as a phosphorylation-triggered structural switch and provide novel structural insights into regulatory processes at the membrane-cytosol interface.

PI-30

Improved detection of protein-protein interactions using proximity ligation assay

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Protein-protein interactions are the driving force for diverse molecular processes. Dysfunctions in these interactions are involved in different pathologies, for example in cancer. Different methods of analysis are employed to study protein-protein interactions. A convenient one is fluorescence microscopy using fluorescently labelled antibodies that bind molecules of interest. If these molecules lie in close proximity, the emission signals overlap indicating the interaction. A limitation of the method is the insufficient resolution of the light microscopy that can lead to misleading or even erroneous conclusions. There are different ways to circumvent this caveat, one of them is proximity ligation assay (PLA). It is based on the use of two antibodies labelled with short DNA strand (PLA probes). If this probes bind in close proximity, short oligonucleotides can hybridize to them and after amplification with a polymerase they are hybridized with fluorescently labeled oligonucleotides. The signal is thus generated only if the probes bind not more than a few tens of nm apart providing means for precise visualization of the interaction. To assess the performance of this novel assay we tested the binding of cathepsins C and H with their endogenous inhibitor cystatin F in different cell lines. Both cathepsins are important cysteine proteases involved in tumour angiogenesis, invasion and growth, in cytotoxic cell-induced apoptosis and many other physiological and pathophysiological processes. Cystatin F is a type II cystatin that is a potent inhibitor of cathepsins C and H in its monomeric form. Our results showed that proximity ligation assay is a specific and sensitive method for detection of protein-protein interactions. Compared to classical colocalization studies it offers higher resolution on a single molecule level and also enables the quantification of the protein-protein interaction.

PI-31

Fourier transform infrared spectroscopy provides an evidence of papain denaturation and aggregation during cold storage

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Papain is a cysteine protease with wide substrate specificity and many applications. Despite its widespread applications, cold stability of papain has never been studied. The main limitation in usage of proteolytic enzymes in many applications is their temperature stability, both at elevated temperatures and during freezing. The main aim of our study was to investigate structural changes of papain upon cold storage and its influence on papain inactivation. We made a hypothesis that secondary structure changes and aggregation cause an increase of inactivation due to cold denaturation to a greater extent than the loss of activity due to autolysis.

We monitored thermal denaturation process, both at low and elevated temperatures. Papain was the most stabile from 45 ºC to 60 ºC with ΔG°_{321} of 13.9 ± 0.3 kJ/mol and T_m value of 84 ± 1 ºC. The beginning of cold denaturation process was captured by differential spectroscopy (15 % reduction of native structure was observed at 5 ºC). After cold storage, papain lost parts of its native secondary structure elements which gave an increase of 40 % of intermolecular β-sheet content (band maximum detected at frequency of 1621 cm^{-1} in Fourier transform infrared (FT-IR) spectrum) indicating the presence of secondary structures necessary for aggregation. The presence of protein aggregates after cold storage was proven by analytical size exclusion chromatography.

Changes in the primary structure of papain induced by autolysis during repeated freeze-thaw cycles was monitored in the assay based on CBB binding, as well as using reverse-phase HPLC. After six freeze-thaw cycles, less than 8 % of protein was lost due to autolysis, which suggests that dramatic inactivation of 65 % cannot be attributed to papain autolysis.

It can be concluded that papain inactivation during cold storage is influenced by secondary structure changes, *i.e.* cold denaturation and aggregation of unfolded protein. Understanding of protein denaturation and aggregation phenomena could lead to better preservation methods which could have the commercial importance for ever growing polypeptide based industry.

PI-32

Listeriolysin O membrane interactions and pore formation on giant unilamellar vesicles

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Listeriolysin O (LLO) is the most important virulence factor of bacteria *Listeria monocytogenes*, which causes the foodborne disease listeriosis. LLO is a member of cholesterol dependent cytolysins (CDCs), which form transmembrane pores in the membranes of the target cells. LLO enables bacteria to escape from the host phagolysosome and to spread into the neighbouring cells. LLO is a unique protein among CDCs, because its activity is pH-dependent. LLO is the most active at acidic pH, because at neutral pH its stability is decreasing at the temperatures over 30 °C. This is the basis of its biologic activity. Giant unilamellar vesicles (GUVs) have been extensively used as a model system for protein-membrane interaction. We also made use of this system to study LLO's mechanism of pore formation. After GUVs preparation by an electroformation method, we observed the GUV's permeability for fluorescent dextrans (FDs) of different sizes upon LLO addition. By analyzing the confocal fluorescence microscopy images with the ImageJ software, we discovered a size and time dependent manner of LLO pore formation. Upon LLO addition, the GUVs made out of POPC and cholesterol were permeable for FDs up to 2000 kDa, in contrast to GUVs made out of DOPC and cholesterol which were permeable only up to 150 kDa FDs. In addition, we showed that the permeability was time dependent, which suggest that LLO's pores are growing in time. Analysis of GUV's population by flow cytometry confirmed that LLO had different effect on GUVs of different membrane compositions. Furthermore, the effects on size and shape of GUVs were dependent on the concentration of LLO.
Since LLO has already found its potential use in medicine as vaccine adjuvant (VPM1002 vaccine for tuberculosis is in phase 3 clinical trials) and carrier molecule for anti-tumor therapies and gene delivery, we believe that our findings can be further utilized in medical and biotechnological applications.

PI-33

Foreign epitope presentation with chimeric potato virus Y virus-like particle

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Potato virus Y is a filamentous virus from Potyviridae family and is among most important plant viruses from scientific and economic point of view. Virus single stranded RNA codes for 10 different proteins, but expressing only recombinant coat protein in bacteria results in virus-like particle (VLP) self-assembly. Traditionally, VLPs are used in vaccine production, because the repetitive antigen presentation on the surface enhances immune response and absence of genetic material removes potential risks of infection in vaccinated organism. PVY VLPs are capable of presenting foreign antigens of up to 71 amino-acid (aa) residues when fused to N-terminal end of coat protein[1], however, larger inserts hamper VLP assembly. Here we studied assembly of PVY coat protein carrying three different polypeptide chains of various lengths: melittin (26 aa), equinatoxin II (eqtII, 179 aa) and yellow fluorescent protein (eYFP, 239 aa). When these proteins fused to virus coat protein are expressed, no VLPs were observed as seen by transmission electron microscopy (TEM). Therefore we used the strategy of expressing native coat protein together with the melittin-, eqtII- or eYFP-coat protein fusion, respectively (chimeric VLP). This strategy results in minimised steric hindrance from extra polypeptide chains fused to coat protein, because VLPs are assembled from both coat protein and coat protein fusion. The assembly was confirmed by TEM and subsequently studied by circular dichroism (CD), differential scanning calorimetry (DSC) and differential scanning fluorimetry (DSF). Results imply thermal denaturation is a two-step process. At low temperature melting point (50-54 °C) VLPs disassemble to individual building blocks and at high temperature melting point (57-61 °C) secondary structure collapses. Moreover, extreme pH values and increasing salt concentration also de-stabilize VLPs. Fluorescent chimeric VLPs assembled from eYFP-coat protein fusion confirm fusion proteins are correctly folded and integrated into VLPs. Because PVY based VLPs are easily prepared, non-infective and capable of carrying large foreign antigens we believe this a promising platform for future vaccine development.

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PI-34

Comparison of water and methanol extracts from hyperthermophilic archaeon *Aeropyrum pernix*

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The aim of our study was to compare the antioxidative activities of extracts obtained from *A. pernix* using two different solvents, methanol and water, and to identify the compounds responsible for the antioxidative activity of these extracts. The extracts were analysed for proteins, for total sulphydryl groups with Ellman's reagent, and antioxidant activity was measured using 1,1-diphennyl-2-picryl-hydrazil. Total phenolics were determinated with Folin-Ciocalteu reagent. The proteins from both of these extracts were analyzed by two-dimensional (2-D) gel electrophoresis, and some of them were identified by mass spectrometry.
The majority of the proteins identified were intracellular proteins, such as proteins involved in the oxidative stress response and in the osmotic stress response, proteins with hydrolase and dehydrogenase activities, proteins common to most organisms. As total phenolic content was higher in methanol extract and antioxidant potential higher in water extract, as well as protein and sulphydryl contents, we suggest that proteins from *Aeropyrum pernix* have more important role in antioxidative mechanisms than secondary metabolites, such as phenolic compounds.

PI-35

Aha1 and cIAP1 isoform-specific interaction with human Hsp90 is mediated by N-terminal and middle domain of Hsp90.

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In the cytosol of human cells, there are two isoforms of Hsp90, named α and β, encoded by distinct genes. Hsp90 is involved in various cellular processes and interacts with over 200 proteins called clients. Only a few clients and one co-chaperone interacting specifically with one isoform of human cytosolic Hsp90 were identified. Hsp90 consists of the N-terminal, middle and C-terminal domains. Each domain plays a distinct function in Hsp90 activity.
We identified the fragments responsible for the isoform-specific functions of Hsp90 α and β using hybrid Hsp90 genes with parts of the Hsp90α sequence swapped with the corresponding sequences of Hsp90β.
We constructed a set of yeast strains with native Hsp90 genes replaced with human Hsp90 isoforms and hybrids to study correlation of the growth rate with the expression of these proteins. The strains that expressed Hsp90α or Hsp90 hybrids containing middle domain of Hsp90α, demonstrated slow growth phenotype at 30 °C and temperature-sensitivity at 37 °C. The yeasts that expressed Hsp90β were insensitive to deletion of AHA1 or HCH1, homologs of human AHA1. However, deletion of AHA1 markedly decreased fitness of the yeasts that expressed Hsp90α. This fact and the results of immunoprecipitation lead to conclusion that, compared to Hsp90β, Hsp90α chaperoning activity is more dependent on stimulation by Aha1.
Tagged Hsp90 hybrid proteins were also expressed in human cells. All the Hsp90 alleles carried mutations that rendered them insensitive to Hsp90 inhibitor 17-AAG. Cells transfected with the hybrids Hsp90 were cultured in the presence of 17-AAG to inactivate native Hsp90. Analysis of the complexes coimmunoprecipitated with the hybrids Hsp90 revealed that Aha1, known as one of the cochaperones that regulate chaperoning activity of Hsp90, strongly associated with Hsp90α. Aha1 bound strongly to the Hsp90α and any Hsp90 hybrid proteins that contained middle domain of Hsp90α.
Depletion of Hsp90β, but not Hsp90α, leads to accelerated degradation of the client proteins cIAP1 and cIAP2. Western-blot analysis of the cIAP1 and cIAP2 level in Hek293 cells that expressed hybrids Hsp90 demonstrated that effective chaperoning of cIAP proteins depends on the Hsp90β N-domain. This observation indicates that N-domain of Hsp90 may play a direct role in chaperoning activity.

PI-36

Alteration of cholinesterase activity in *Daphnia magna* after the exposure to silver nanoparticles

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The environmental impact of nanomaterials is expected to increase in the near future as they are becoming a part of our daily life. Silver nanoparticles (AgNP) are widely used in the form of cosmetics, food packaging, drug delivery systems, therapeutics, antimicrobial agents, biosensors, or labelling agents. Effect of AgNPs on biomolecular level was studied by measuring activity of cholinesterases (ChE) *in vitro*. To asses possible mechanism of silver nanoparticle toxicity on the test organism *Daphnia magna* we measured ChE activity *in vivo*. Due to their sensitivity to toxicants following by rapid response in the aquatic system, *Daphnia magna* neonates are organism used as standardized test species for environmental risk of material with biocidal potency. AgNPs effect *in vivo* and *in vitro* was compared against the effect of ionic Ag^+. Acetylcholinesterase (AChE) and related butyrylcholinesterase (BChE) are chosen because they are present in brain, blood and nervous system of mammals. Acetylcholinesterase (AChE) is a key enzyme in cholinergic neurotransmission and inhibition of AChE may lead to fatal outcome. BChE has important pharmacological and toxicological roles due to its hydrolysis of various xenobiotics, i.e. drugs.
Presence of AgNPs and Ag^+ reduced activity of both AChE and BChE *in vitro*. The major difference was in type of inhibition: AgNPs caused reversible inhibition of enzyme, whereas Ag^+ caused irreversible inactivation of enzyme activity. For *in vivo* experiments, increase in AChE activity in *D. magna* neonates after acute exposure during 48 h to Ag^+ or AgNPs was observed. Such result may be related to a *de novo* synthesis of the enzyme as a response to an initial inhibition as part of *D. magna* compensatory response to toxins. The data indicated also significantly different toxicity of nano and ionic form of Ag in the aquatic system, with Ag^+ being more toxic to *D. magna*.
Since many environmental contaminants exert toxic effects related to change in ChE activity, the more frequent use of various biochemical biomarkers, including ChE, as early indicators of toxic stress to aquatic biota is therefore recommended for evaluation of environmental risk.

PI-37

Investigation of biochemical, molecular and morphological consequences of the expression of BFSP1 in tumour cells

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BFSP1 (beaded filament structural protein one, or Filensin) is an eye lens specific cytoskeletal protein forms intermediate filaments (IFs) with its assembly partner (BFSP2) in the fibre cells of the eye lens. We proved the evidence that splice variants of BFSP1 are expressed in various cancer cell lines. BFSP1 indicated strong membrane binding, which was confirmed by immunohistochemistry of *ex vivo* human breast carcinomas and colon carcinomas. Previously, it was proved that Filensin is a substrate for Caspases, which exposed an internal N-myristoylation site of the Tail-fragment of BFSP1. Filensin is processed by caspases under normal development as well. Our previous work has demonstrated that BFSP1 is a substrate for both human NMTs (huNMT-1, huNMT-2). NMT activity is elevated in various cancers. According to the literature, BFSP1 has been known as a cytoskeletal protein expressing particularly in eye lens so far. The expression of BFSP1 in cancer cells seems unexpectedly and it indicates a new exciting approach in the field of tumour biology. To establish the possible role of a new cytoskeletal protein expressed in tumour cells might have extraordinary significances in the tumour diagnosis.

PI-38

Characterization of the interaction between metastasis-associated ezrin and S100A4

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Ezrin belongs to the ERM (ezrin, radixin, moesin) protein family that has a role in organizing the cortical cytoskeleton by linking filamentous actin to the apical membrane of epithelial cells. In its inactive state, an intramolecular interaction is formed between the N-terminal and C-terminal domains, blocking protein-protein interaction sites. Upon activation the unmasked N- and C-terminal domains bind to the plasma membrane and F-actin, respectively. This activation mechanism is associated with specific cellular functions: cell morphology changes, adhesion and migration. Ezrin is highly expressed in a variety of human cancers and promotes metastasis.
S100P, a member of the Ca^{2+}-binding EF-hand S100 protein family serves as an activator of ezrin: it binds to its N-terminal domain and unmasks the actin-binding site, thus inducing its activation. Here we characterize the interaction of ezrin with another metastasis-associated protein, S100A4. S100A4 is one of the regulators of the motility of tumor cells by inhibiting non-muscle myosin IIA filament assembly. According to our results, S100A4 binds to the N-terminal domain of ezrin with similar affinity as S100P. We demonstrate that full-length ezrin interacts with F-actin in an S100A4-dependent manner. Additionally, competitive binding studies revealed that the ezrin- and myosin-binding sites overlap on S100A4. We validate the interaction by cell culture-based assays, e.g. co-immunoprecipitation and co-localization. As both proteins play an important role in promoting metastasis, the characterization of their interaction could serve as a basis for the design of novel therapeutic agents.
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PI-39

Sodium-glucose cotransporter Sglt1 (Slc5a1) is present in various murine organs; sex-related expression in kidneys

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Diabetes mellitus affects ~400 million people worldwide. Novel antidiabetic drugs have been developed aiming to lower blood glucose by inhibiting the activity of sodium-D-glucose cotransporter 1 (SGLT1 in humans/Sglt1 in rodents) in the intestine and kidney. In rodents, the intestinal Sglt1, localized in the enterocyte brush-border membrane (BBM), is responsible for bulk (>80%) glucose absorption, whereas the renal transporter, localized in the proximal tubule (PT) cell BBM, contributes to minor (~3%) glucose reabsorption. The presence of Sglt1 in other organs is poorly known. As mice are frequently used experimental animals in preclinical studies, knowledge of the Sglt1 distribution in various organs as possible targets of new inhibitors may be of great importance. Here we compared the expression of Sglt1 mRNA and protein in various organs of wild type (WT/Sglt1+/+) and Sglt1 knockout (KO/Sglt1-/-) mice by qRT-PCR and immunocytochemistry (IC). In WT mice, the Sglt1 mRNA expression was highest in small intestine; high in seminal vesicle, kidney and salivary glands; medium in prostate, tongue, eyes and uterus, and small in pancreas, lung and liver. By IC, Sglt1 protein was detected in small intestine (enterocyte BBM), kidney (PT BBM and apical membrane (AM) of thick ascending limb of Henle), liver (bile ducts, AM), pancreas (ducts, AM), salivary glands (serous acini and initial ducts, AM), tongue (taste epithelium), prostate (epithelial cells, AM), bulbourethral gland (ducts, AM), seminal vesicles (intracellular organelles in epithelial and basal cells), and uterus (endometrium, AM). In PT, the staining intensity was 3-fold higher in BBM of S2 segments in the cortex compared to S3 segments in the outer stripe. However, the opposite sex-dependent expression was observed for the renal Sglt1 mRNA (females > males) and its protein (males > females) indicating different transcriptional and post-translational regulations. The presence of Sglt1 in various organs indicates that the potential inhibitors of SGLT1 activity in diabetic humans may target these localizations with unpredictable health consequences.

PI-40

Evidence for the cytosolic location of NAD(P)H cytochrome b5 oxidoreductase

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The recently discovered NADH cytochrome b_5 oxidoreductase (Ncb5or) is a soluble natural fusion protein of unrevealed function. The two redox domains of this soluble enzyme are homologous to Cytochrome b5 (Cyb5) and Cyb5 reductase (Cyb5R) integral membrane proteins of the endoplasmic reticulum (ER). Their concerted action in fatty acid desaturation is well characterized. This structural homology along with the marked alterations in lipid metabolism observed in Ncb5or (-/-) mice strongly suggest the involvement of Ncb5or in fatty acid desaturation. The enzyme likely transfers electrons from NADPH to the desaturase enzyme in the ER membrane. However, controversial data have been published regarding the cytosolic or ER localization of the protein.
In order to clarify whether Ncb5or uses the common cytosolic NADPH or utilizes the separate pyridine nucleotide pool of the ER lumen, we aimed to elucidate the intracellular location of this soluble protein.
Green fluorescent EGFP-Ncb5or fusion protein was expressed in transiently transfected human HEK293T cells and detected by fluorescent microscopy. The location of endogenously expressed Ncb5or was assessed in HEK293T cells by two methods. Cells were harvested and homogenized to separate the subcellular fractions by differential centrifugation. Ncb5or and specific marker proteins of various cellular organelles were detected by Western blotting. In addition, the endogenous protein was also visualized by using *in vitro* immunocytochemistry. Subcellular fractions of rat livers were also isolated and analyzed for the presence of Ncb5or protein.
Purity of the generated nuclear, microsomal, mitochondrial and cytosolic cell fractions were confirmed by immunoblot with characteristic marker proteins of the organelles. Ncb5or could only be detected in the cytosolic fractions of HEK293T cells and rat livers by using Westren blot. EGFP-Ncb5or fusion protein was detected in the cytoplasm of the cells, and it was not co-localized with fluorescent markers labelling either the nucleus or the ER. Similar location of endogenous Ncb5or protein was observed by immunocytochemistry.
Our results clearly prove that Ncb5or is located in the cytoplasm in cultured cells and in liver *in vivo*. Therefore, the utilization of ER luminal reducing equivalents by this enzyme can be ruled out. Further research is needed to confirm the putative role of Ncb5or in the fatty acid desaturation, which in turn will help to understand the contribution of this novel protein to the protection of pancreatic β-cells against lipotoxicity.

PI-41

PaNIE binds to plant and yeast membrane lipids

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Protein PaNIE excreted from *Pythium aphanidermatum* is a member of the NLP (Nep1-like proteins) family. The family consists of effector molecules that are found in taxonomically diverse organisms, including oomycetes, bacteria and fungi. They are known to cause cell death and immune responses in different plants, but the mechanism of their action still needs to be resolved. It is believed that NLPs may associate with the outer surface of the plasma membrane of the dicotyledonous plants. It was shown that the target molecule on the host organism is not of protein origin and it was suspected that the recognition of a specific lipid in the membrane of the host might be important, since structurally similar actinoporins involve recognition of sphingomyelin to begin the pore formation in the membrane.
We demonstrate, with lipid blotting, that PaNIE binds to a specific lipid from the tobacco leaf lipid extract, which could be a sphingolipid according to its separation with thin layer chromatography. In addition we show the binding to yeast sphingolipids using the same approach. We used fluorescently labeled PaNIE to further analyze the protein binding to the membrane of the yeast's spheroplasts. Using confocal microscopy and flow cytometry we show that PaNIE does not bind to intact yeast cells. However, after removing of cell wall with specific enzymes we can see clear binding to the yeast membrane. The most abundant sphingolipid in *Saccharomyces cerevisiae* is mannose-(inositol-P)$_2$-ceramide while sphingolipids in tobacco have the general structure (N-acetyl)glucosamine-glucuronic-inositolphosphoceramide with the addition of additional hexoses and pentoses at either the inositol or glucosamine residues. We used surface plasmon resonance to test the binding of several sugars that build the sphingolipid's core head group in yeast and tobacco.

PI-42

Absence of cyclophilin D enhances the cholesterol and fat anabolism in mouse liver

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Cyclophilin D is a crucial component of the mitochondrial permeability transition pore (PTP). PTP opening causes the collapse of mitochondrial membrane potential leading to cell death and reactive oxygen species formation. The importance of PTP opening has been implicated in various pathological conditions like ischemia-reperfusion injury, however the exact physiological role is still obscure. Inhibition of the PTP, for example by ablation of the CypD gene, favors the intact function of mitochondria; supporting the cell metabolism.
The total mRNA screening of liver from CypD KO mice showed significantly increased expression of genes involved in fat and cholesterol synthesis compared to wild-type. The expression of 20 key genes like HMG-CoA synthase, HMG-CoA reductase, fatty acid synthase, fatty acid binding protein 5, and squalene epoxidase was verified by qPCR. The consequence of diverse lipid metabolism could be observed by histological examination as CypD KO mice exhibited signs of fat deposition. Akhtar Y.N. et. al.[1] has demonstrated that genetic ablation of cyclophilin D improves insulin sensitivity in high-fat fed mice. The published increased insulin sensitivity and our present results together support that the lack of CypD protein could influence the cholesterol and fat anabolism, however the detailed mechanism needs further investigations.

[1] FASEB J. April 2010 24 Meeting Abstract Supplement lb626

PI-43

Neuropathy target esterase-related enzyme: a possible role in skeletal muscle energy metabolism

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Neuropathy target esterase-related enzyme (NRE, PNPLA7), a serine esterase and a member of the family of patatin-like phospholipase domain containing enzymes, is a trans-membrane protein linked to the endoplasmic reticulum and to the lipid droplets in adipocytes and some other cell types and tissues. While NRE has been identified as a lysophospholipase that hydrolyzes sn-1 esters in lysophosphatidylcholine and lysophosphatidic acid, its physiological roles have not been thoroughly examined. The objective of our study was to investigate NRE expression and its functional characteristics in skeletal muscle. Here we found that cultured human skeletal muscle cells express NRE mRNA and protein. Furthermore, by using p-nitrophenyl valerate assay we detected specific NRE activity in these cells. Taken together, our data show that human skeletal muscle cells possess functional NRE. Since skeletal muscles represent approximately 40% of body weight and are one of the key metabolic tissues, we have evaluated a possible role of NRE in skeletal muscle energy metabolism. To investigate whether NRE expression is regulated by substrate availability or by metabolic hormones, we exposed human skeletal muscle cells to different concentrations of glucose, insulin or forskolin, a cell-permeable activator of adenylyl cyclase that mimics effects of insulin antagonists like adrenaline. Insulin treatment tended to suppress NRE expression. Conversely, NRE expression was increased by forskolin, suggesting a role for adrenaline in regulation of NRE expression. Effects of insulin were, in part, dependent upon glucose concentration. Insulin-induced suppression of NRE expression was especially pronounced when skeletal muscle cells were exposed to high (4.5 g/L) glucose concentration. In contrast, normal (1 g/L) glucose concentration blunted suppressive effects of insulin on NRE expression. These results suggest that NRE expression in human skeletal muscle might fluctuate during the starve-feed cycle. Collectively, our results demonstrate that human skeletal muscle cells possess a functional NRE. They indicate that metabolic hormones and glucose concentration regulate expression of NRE in human skeletal muscle cells, therefore implicating a role for NRE in skeletal muscle energy metabolism.

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PI-44

Changes in the membrane phospholipid composition in erythrocytes at patients with metabolic syndrome after consumption of pomegranate juice

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Metabolic syndrome (MS) represents a cluster of cardiovascular risk factors connected to insulin resistance, disturbed glucose metabolism, visceral obesity, arterial hypertension and atherogenic dyslipidemia. In these disorders fluidity and structure of cell membrane play a crucial role and it can be damage.
The aim of this study was to investigate the effects of pomegranate juice (PJ) on lipid peroxidation and phospholipid fatty acids (FA) composition of erythrocytes in patients with MS. Forty women aged from 40 to 60 years, with metabolic syndrome were recruited for this study. They were randomly assigned into two groups. The first group received 300mL of pomegranate juice daily for 6 weeks, and second group did not consume the juice.
Phospholipid fatty acid methyl esters in erythrocytes were analyzed by gas-liquid chromatography on Shimadzu chromatograph GC 2014 (Kyoto, Japan). The levels of thiobarbituric acid-reactive substances (TBARS), as byoproducts of lipid peroxidation in erythrocytes were determined using laboratory kit (Cayman, Ann Arbor, MI 48108, USA). The levels of TBARS were calculated from the calibration curve with malonaldehyde bis (dimethyl acetal) as standard and expressed in MDA equivalents.
Phospholipid fatty acid composition in erythrocytes was not significantly different between intervention and control group, at baseline. There was a significant decrease of relative amounts of DHA and increase ($p<0.05$) of relative amounts of total monounsaturated fatty acid (MUFA) in erythrocytes phospholipids in subjects with metabolic syndrome. There were differences in the percentage of palmitic and arachidonic acid in erythrocytes phospholipids; these differences were not statistically significant. Fatty acid profiles in erythrocytes in control group were not significantly changed during the study. MDA levels in erythrocytes were significantly decreased after 6 weeks of consumption PJ.
FA profile of erythrocytes reflects the FA composition of PL from muscles and other organs and tissues. MUFA affect membrane fluidity, permeability, insulin receptor affinity and up-regulation of glucose transporters. Increasing level of MUFA upon consumption of PJ may have cardio protective effects by reducing the risk of coronary artery disease and be beneficial in subjects with MS. The results suggest that pomegranate juice may reduce the incidence of atherosclerosis and inflammation in patients with MS according to a decrease in lipid peroxidation.

PI-45

α-Synuclein interactions with phospholipid model membranes

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α-Synuclein is a small presynaptic protein that is critically implicated in the onset of Parkinson's disease and other neurodegenerative disorders. It has been assumed that the pathogenesis of α-synuclein is associated with its aggregation, while for its physiological function, binding of α-synuclein to the synaptic vesicle membrane appears to be most important. The present study investigated the mechanism of α-synuclein binding to the lipid membrane. Upon binding to negatively charged small unilamellar vesicles consisting of 1,2-dipalmitoyl-*sn*-glycero-3-phosphoglycerol (DPPG) or 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoglycerol (POPG) in the liquid-crystalline state, α-synuclein undergoes conformational transition from its native unfolded to an α-helical structure. The positively charged N-terminal part of α-synuclein is likely to be involved in the interactions with the negatively charged lipid surface. α-Synuclein did not associate with vesicles consisting of the neutral lipids 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) or 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC). The data obtained by circular dichroism spectroscopy, fluorescence anisotropy measurements, differential scanning calorimetry, and calcein efflux assay indicate that in addition to electrostatic interaction, hydrophobic interactions are important in the association of α-synuclein with membranes. The mechanism of α-synuclein binding to lipid membrane is primarily dependent on the surface charge density of the lipid bilayer and the phase state of the lipids. We propose that α-synuclein has a lipid ordering effect and thermally stabilises vesicles.

PI-46

Engineering the folding pathway of a designed topological protein

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The design of novel proteins to efficiently carry out specific tasks is one of the major long-term goals in biomolecular research. This task is made extremely difficult by the large number of degrees of freedom in protein structures and by the large number of competing interactions that contribute to protein stability. Recent work by our group has demonstrated that the problem can be made more tractable by arranging modular, specifically interacting elements into a sequence that ensures only a specific folded structure will satisfy all available pairwise interactions [1,2]. This concept of topological protein design was used to produce a tetrahedral protein composed of orthogonal coiled coil segments [2]. We have now set out to investigate and optimize its folding pathway - the order in which the individual coiled coil modules form their specific pairwise interactions. Some pathways may allow for smooth folding while others could introduce unnecessary free energy barriers that slow down folding and increase the probability of misfolding and aggregation. We are using theoretical calculations of free chain entropy and Gō model molecular dynamics to determine the preferred order in which native coiled coil pairs are most likely to form. These predictions will be combined with experimental stopped-flow data to determine how different arrangements of the same building blocks affect the kinetics of folding. With this knowledge we will be able to design an optimized version of the tetrahedron, whose folding pathway will be as smooth and free of energy barriers as possible.

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PI-47

Synthetic multi-enzymatic intracellular compartments and non-cellular particles

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Substrate channelling that naturally occurs in some multi-enzymatic biosynthetic pathways is believed to improve overall yield and efficiency of metabolic reactions. Channelling can be mediated by flexible arm that moves attached substrate between individual active sites in multi-enzymatic complex. Alternatively, some enzymes contain structural tunnels that connect several active sites and also charged surface regions that prevent release of unstable intermediates into solution. On the other hand synthetic scaffolds proved to be successful tool for improving biosynthetic pathway efficiency regardless the fact that no channelling mechanism is present in such systems. Computational models and also limited experimental data exist in scientific community, which support hypothesis that enzymatic clustering mimic effect of substrate channelling if size of enzymatic particles is over certain limit. Our aim was to test the activity of large multi-enzymatic complexes where clustering was mediated by polypeptide elements forming ordered structures. Complexes were produced in *Escherichia coli* and tested in small scale fermentation bioprocess. Multi-enzymatic particles were purified in very simple isolation process that includes only cellular lysis, centrifugation and final washing and tested *in vitro* in the production process. Reaction efficiencies of multi-enzymatic particles were compared to reaction systems where soluble enzymes were not linked together or to reaction systems composed of otherwise linked enzymes but not integrated into large subcellular structures.

PI-48

Action of propolis in the yeast cell

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Propolis has wide range of biological activities, including antioxidative. Nevertheless, the exact phenolic compounds and molecular mechanisms behind this activity are still unknown. Here, we fractionated ethanolic extract of propolis (EEP) using solid-phase extraction and evaluated antioxidative activity of particular fractions *in vitro* and in the model organism yeast *Saccharomyces cerevisiae*. No correlation between free radical scavenging activity of the fractions and their effect on yeast intracellular oxidation was found. Furthermore, we determined the most bioactive fraction of EEP, which decreased intracellular oxidation, increased cellular metabolic energy and had no effect on cell viability. Its phenolic compounds were identified: hydroxycinnamic acid esters, prenylated hydroxycinnamic acid and flavonoids . Additionally, we investigated their cellular uptake and it was confirmed only for caffeic acid benezyl ester, caffeic acid phenyethyl and caffeic acid cinnamyl ester. The effect of the most bioactive fraction of EEP was studied also on the yeast subproteome level using differential detergent fractionation and 2-D electrophoresis. There were found changes in proteins involved in carbohydrate and energy metabolism, actin filament dynamics, oxidative stress response, protein folding and other processes. Results gave us better insight into the cellular action of propolis extract rich with phenolic compounds.

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PI-49

Electrophysiological properties of rhodopsins from selected halotolerant and halophilic fungi

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Microbial rhodopsins exhibit a characteristic structure, consisting of seven transmembrane helices forming an interior pocket for the chromophore all-trans-retinal, which is covalently bound via a protonated Schiff-base to a lysine residue. Fungal rhodopsins are usually activated by green light and perform sensory or proton-translocating function. In light-driven fungal rhodopsin pumps, a single proton is translocated per photocycle, which is triggered by light-induced all-trans to 13-cis retinal isomerization. A number of microbial rhodopsins have been used as optogenetic actuators, but the neurophysiologists still seek for faster, more effective proteins. Because halotolerant and halophilic fungi thrive in environments with high salt concentrations their rhodopsins could have evolved the properties, which would draw attention in the field of optogenetics.
In our study, we used the patch-clamp technique to electrophysiologicaly characterize rhodopsins from selected fungi after their expression in mammalian cells. We cloned the cDNAs of fungal rhodopsins from *Wallemia ichthyophaga, Hortaea werneckii,* and *Aureobasidium pullulans* into mammalian expression plasmids allowing for tagging the rhodopsins with fluorescent proteins and for reaching high expression levels by strong viral promoters. We used these plasmids to transfect mammalian cells (HEK293, NG108-15) to explore the physiological role of rhodopsins from selected halotolerant and halophilic fungi as well as to examine their potential for optogenetics.
H. werneckii, and *A. pullulans* contain two rhodopsin genes in their genomes and all four proteins expressed well in mammalian cells. In whole cell patch-clamp experiments they exhibited outward-directed electrical currents between 100 and 800 pA, upon illumination with green light (532 nm solid state laser) under physiological conditions. In contrast, WiOps1 from *W. ichthyophaga* was not expressed well in the mammalian host system and even when we introduced signals for membrane trafficking and ER export to optimize its localization on the plasma membrane we did not observe electrogenic activity. In conclusion, the rhodopsins from *H. werneckii,* and *A. pullulans* are efficient electrogenic pumps with promising biotechnological properties.

PI-50

Changes in cytosolic distribution of toxic metals Cd and Pb in liver, gills and intestine of Vardar chub (*Squalius vardarensis)* induced by exposure to mining effluents

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Mining effluents significantly contribute to metal contamination of freshwaters, presenting an important environmental concern. Aquatic organisms exposed to increased metal levels have various detoxification strategies, such as binding of accumulated metals to specific cytosolic ligands. However, a part of metal accumulated within the cell can still bind to important biological molecules and cause toxic effects. The aim of our study was to establish the changes in Cd and Pb distribution among cytosolic proteins in three organs (gills, liver, intestine) of Vardar chub (*Squalius vardarensis*), which could have occurred due to increased exposure to Cd and Pb in two mining impacted rivers in north-eastern Macedonia (Zletovska and Kriva) in comparison to the reference Bregalnica River.
Size-exclusion high performance liquid chromatography was applied for cytosolic protein separation according to their molecular masses whereas high resolution inductively coupled plasma mass spectrometry was used for metal measurement in obtained fractions, as well as in total cytosolic fractions of three chub organs.
In all three organs, the majority of Cd was eluted with low molecular mass proteins (LMM; 10-30 kDa) with maximum at elution time of metallothioneins (MT; 12.5 kDa). Increase of Cd bioaccumulation resulted mainly with the increase of that MT-peak height, which was most pronounced in chub from mining impacted rivers and corresponded well to cytosolic Cd concentrations. Occurrence of an additional peak due to increase in Cd bioaccumulation was observed only in the gill cytosol, where small Cd portion was found associated with high molecular mass proteins (HMM; >100 kDa).
The best resolved and the highest Pb peaks were found in the chub from the Kriva River, characterized by the highest Pb exposure in the water and the highest Pb bioaccumulation. Unlike Cd, distribution profiles of Pb notably differed between three organs. In liver, two sharp peaks were observed within HMM and LMM regions, where LMM peak coincided with elution time of MTs. In gills, several peaks in HMM and medium molecular mass (MMM; 30-100 kDa) regions were found, but were rather small and not well resolved. In the intestine, Pb was mainly eluted in the MMM protein region in a clear, sharp peak with maximum at 35.9 kDa.
The changes of distribution profiles of Cd and Pb in three organs of Vardar chub clearly reflected the exposure and bioaccumulation levels of these metals.

PI-51

Interactions of secondary metabolites from cyanobacteria and invasive tropical algae with the cellular detoxification mechanism in zebrafish (*Danio rerio*)

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Invasive tropical green algae from the genus Caulerpa and various cyanobacterial species possess diverse and complex composition of secondary metabolites. Some of those are toxic and represent a significant threat to the environment and animal/human health, especially during periods of intensive blooms. Nevertheless, the knowledge on their mechanism(s) of toxicity and interactions with basic cellular defense systems are still scarcely investigated. Therefore, the main goal of this research was to determine interactions of secondary metabolites from C. racemosa, C. taxifolia and selected cyanobacterial strains, including genera Anabaena, Nostoc, Phormidium and Oscillatoria, with four (phases 0, I, II, and III) critical phases of the cellular detoxification in zebrafish (Danio rerio) as a highly relevant vertebrate research model. In addition, we performed a preliminary identification of biologically active substances that cause observed toxic effects using the effects-directed analyses (EDA) approach. Significant toxicity of these complex biological samples towards toxicologically relevant zebrafish uptake transporters DrOatp1d1 and DrOct1 (phase 0), and CYP1A1 detoxification enzymes (phase I) have been determined. Bioactive compounds from Caulerpa species and cyanobacterial strains have been preliminary identified as both polar and lipophilic. Caulerpin (CLP) was determined as the major metabolite in C. racemosa while caulerpenyne (CYN) appeared to be the dominant compound in C. taxifolia. CYN was determined to be the inhibitor of the DrOatp1d1 anion transporter. Aquatic cyanobacterial strains, especially Oscillatoria strain, showed the most significant biological responses to both detoxification phases and are potentially considered to be of high (eco)toxicological relevance.

PI-52

Designing fluorescent probes by bipartite phage display

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Due to the limitations of the fluorescently labelled antibodies, such as expensive production and unpredictable site and extent of labelling, there is a growing interest in alternative biomolecular fluorescent probes. We set out to create (bacterio)phage-based fluorescent probes for detection of specific antigens. Our main goal was to develop a platform enabling efficient concurrent phage display of two different proteins, specifically green fluorescent protein (GFP) reporter and single chain variable fragment (scFv). While presentation of a single protein on the surface of filamentous phage capsid is in most cases a routine task, simultaneous display of two proteins is all but straightforward. By modifying f88-4 phage genome, we have created several variants of type 88 phage vectors harboring the *GFP-gene VIII* (*gVIII*) fusions coupled to distinct signal sequences for translocation of mature proteins to bacterial periplasm. Alternatively, GFP was genetically appended to one of the coiled coils allowing indirect posttranslational attachment of GFP–E-coil fusion to the nascent capsid with incorporated fusion protein of K-coil and major coat protein (p8). Furthermore, to increase the display valency of GFP, the native *gVIII* copy was partially or completely knocked-out. Such GFP-encoding phages were then used as helper phages for phagemid rescue to produce virions displaying two proteins, i.e. the GFP reporter attached to p8 and a scFv fused to minor coat protein p3. Simultaneous display of both proteins was evaluated by ELISA assays, western blot and fluorescence microscopy. N-terminal TorA leader (directing folded proteins to Tat secretory pathway) was found to allow translocation of correctly folded GFP-fusions to periplasm more efficiently compared to the native phage p8 leader (likely directing unfolded proteins to SecB-dependent pathway). Moreover, indirect attachment of GFP onto the phage capsid via the pair of coiled coils improved display efficiency of both proteins. Although antigen-coated agarose beads can be visualized by fluorescent microscopy using our phage probes, display valency of GFP seems to be too low to detect immobilized antigens in fluorescence-linked immunosorbent-like assay. The described platform nevertheless represents the basis for further development of bacteriophage-based probes as well as phage particles of dual functionality for various applications.

PI-53

Successful panning of a pre-immune VHH phage display library directly on exosomes

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Exosomes are cell-derived vesicles present in many biological fluids (blood, urine, cerebrospinal fluid, milk, ascites) that play a key role in the regulation of many physiological processes such as coagulation, intercellular signalling and waste management as well as in many pathological conditions such as inflammation, tumour growth and metastasis [1]. There is an emerging interest in identifying exosome biomarkers to characterize their heterogeneity and potentially apply them in diagnosis and therapy. We evaluated the possibility of panning a pre-immune singe-chain llama antibody (VHH) phage library [2] directly against exosomes and two distinct cell lines were selected as a model: human embryonic kidney (HEK) 293 and human breast adenocarcinoma SKBR3 cells. Intact exosomes were recovered from cell culture supernatant by liquid chromatography on a monolith based chromatography medium and characterized by FACS for exosome specific markers (CD9+). Exosome fractions from HEK293 and SKBR3 cell lines were bound to magnetic beads to simplify the panning procedure. After two rounds of panning, 92 clones were analysed by ELISA and ten strongly positive clones that recognized both cell lines were selected for further validation. Their positivity was confirmed by FACS and sequencing indicated that independent antibodies were selected. The preliminary expression test showed that the selected VHHs express in soluble form in *E.coli.* This is the first report demonstrating the feasibility of panning directly against intact exosomes to obtain recombinant antibody fragments that effectively bind exosome surface markers. This achievement opens the way for applying exosome panning to the discovery of novel markers exposed on the extra-cellular vesicle surfaces that is the preliminary requirement for exosome stratification. Clarifying the exosome heterogeneity could have a great clinical impact because of the possibility to isolate specifically those that are related to a certain pathology or prognosis. We expect that the further optimization of the panning procedure will allow the identification of VHHs able to discriminate between exosome sub-populations.

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PI-54

Screening of endophytic fungi isolated from conifers needles for antibacterial properties

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The problem of drug-resistant pathogens and co-dependent infectious diseases is substantial and still growing. Fungi and in particular endophytes are a promising source of new antimicrobial compounds. We have isolated and cultured endophytic fungi from plant samples, mainly needles of conifers. Extracts of cultured endophytic strains were tested for antimicrobial properties using dilution test. Their activity was compared to antibiotic ampicillin. Samples that exhibited antimicrobial properties were further examined. DNA from 5 active fungal stains was isolated and species-specific DNA regions were amplified and sequenced allowing us to determine samples identity. Active endophytic fungi turned to be two strains of *Lophodermium pinastri*, two strains of *Lophodermium seditiosum* and *Phoma Herbarum*.

PI-55

Codon optimisation is key for pernisine expression in *Escherichia coli*

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Pernisine is an extracellular thermostable serine protease from hyperthermophilic archeon *Aeropyrum pernix* K1. A lower yield from natural host and expression problems in heterologous host inhibits its characterization and potential application in industry.
Challenges of pernisine overexpression in *Escherichia coli* were overcome by codon preference optimization and DNA synthesis *de novo*. Wild type (pernisine^{wt}) and codon-optimized (pernisine^{co}) were cloned into pMCSGx series of vectors and expressed in *E. coli* cells. Fusion tagged pernisine were purified using fast protein liquid chromatography system equipped with Ni^{2+} chelate and gel filtration chromatography columns. The identity was confirmed with N-terminal sequencing, tandem mass spectrometry analysis and immunodetection. Pernisine^{wt} was not expressed and could not be detected even with immunodetection; meanwhile pernisine^{co} was purified as a proform with a yield of around 10 mg per liter of culture. Recombinant pernisine was heat activated at temperature 90 °C for 1 h in buffer 10 mM HEPES pH 8.0 containing 1 mM $CaCl_2$. Proteolytic activity of mature pernisine^{co} was confirmed with zymogramphy at molecular weight 36 kDa. The temperature and the pH optima of the enzymatic activity of the recombinant pernisine, evaluated by azocasein assay, were around 105 °C and pH 7, respectively.
Our findings reveal that codon optimization is crucial for pernisine overexpression in *E. coli*. Recombinant pernisine is activated by autoproteolytical cleavage of its N-terminal proregion consisting of the first 91 aminoacids. Further on, we confirmed that recombinant pernisine retains characteristics of a native one being calcium modulated thermostable serine protease.

PI-56

Hydrolysis of concentrated raw corn starch with *Bacillus licheniformis* 9945a α-amylase

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Starch represents an inexpensive source for the production of glucose, fructose and maltose syrups and for obtaining the products of their fermentation, including biofuels. The importance of the enzymatic liquefaction of granular starch below the temperature of gelatinization has been well recognized, mainly due to energy savings and the effective utilization of biomass, which reduces the overall cost of starch processing. α-Amylase form *Bacillus licheniformis* 9945a (*Bli*Amy) was found to be very efficient in hydrolysis of concentrated raw corn starch at temperatures below the starch gelatinization temperature (60 ˚C). The hydrolysis process of raw corn starch is affected by many variables including solid content, temperature, enzyme loading, pH and time. Response surface methodology (RSM) was used to optimize the reaction parameters of concentrated raw corn starch hydrolysis. This methodology has the advantage of being less expensive and time-consuming than the classical approach. To optimize the hydrolysis reaction, a three-step design consisted of full factorial design (FFD), steepest ascent design (SAD), and central composite design (CCD) was used. FFD was used for identification of the most important factors of the hydrolysis reaction. Three independent variables enzyme loading (X1), solid content (X2) and incubation time (X3) were included in a two level full factorial design. SAD was used to determine the direction toward predicted higher responses (hydrolysis yield). CCD was used to optimize the important factors and maximize the hydrolysis yield.
Regardless of raw starch concentration tested, *Bli*Amy was very effective, achieving almost complete hydrolysis degree on 30% starch suspension after 22 h of hydrolysis. The final hydrolysis rate was dependent on both enzyme amount applied and incubation time.The mode of degradation of native maize starch granules and the changes in the starch structure during the hydrolysis was monitored with TLC, SEM and XRD methods.

PI-57

Oxidative stress biomarkers as predicting factors for boar semen characteristics following short-term liquid storage

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The role of oxidative stress in boar semen has been widely investigated, but there is a paucity of information regarding the use of oxidative stress biomarkers as predictors for semen quality after storage. Therefore the aim of our study was to investigate whether changes in boar semen quality after 3 days of liquid storage can be predicted with superoxide dismutase (SOD), total antioxidant capacity (TAC) and thiobarbituric acid reactive substances (TBARS) in fresh seminal plasma.
Freshly ejaculated boar semen was diluted with Beltsville Thawing Solution at a ratio of 1:2. Semen parameters (motility, progressive motility, viability and morphology) were evaluated and oxidative stress biomarkers (SOD, TAC and TBARS) measured in seminal plasma at 0 and 72 hours after storage at 15-18 °C.
Biomarkers mentioned above that correlated significantly with semen parameters in stored semen were diagnostically evaluated using receiver operating characteristics (ROC) analysis as predictors of semen quality after 3 days of storage. SOD activity in fresh boar seminal plasma showed the strongest correlations with semen parameters in stored semen and was found to be a reliable predictor of the progressive motility (AUC = 0.86; $P < 0.01$) and viability (AUC = 0.85; $P < 0.01$) of stored spermatozoa. We can predict with 88.9 % certainty that fresh semen samples with SOD activity less than 1.22 U/ml will retain more than 25 % progressive motility and, with 100% certainty, that fresh semen samples with activities of SOD less than 1.26 U/ml will retain more than 85 % of viable spermatozoa after storage. Diagnostic evaluation based on fulfilling all quality criteria for satisfactory semen characteristics after storage (viability > 85 %, motility > 70 %, progressive motility > 25 %, normal morphology > 50 %) provided a higher prognostic value of SOD (AUC = 0.97; $P < 0.01$) than similar evaluation based on individual semen parameter. SOD levels of less than 1.05 U/ml lead to the prediction, with 87.5 % accuracy, that the semen will meet the requirements for satisfactory semen characteristics after storage, while semen with SOD levels higher than 1.05 U/ml will not exhibit, with 100 % accuracy, all the required semen parameters after storage.
The results of our study indicate that measurement of SOD in fresh boar seminal plasma can be used as a valuable predictor of semen quality after 3 days of liquid storage.

PI-58

Nuclease resistant oligonucleotide receptor for troponin diagnostics

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Cardiac specific troponins are standard markers of myocardial infarction; thus, various systems have been developed for their fast and sensitive detection. In the recent years, antibodies, the most generally applied receptor molecules in protein detecting devices, have been rivalled by appearance of short single stranded oligonucleotides with highly discriminative molecular recognition and binding capacity. These molecules are superior to antibodies in many ways; they are *in vitro* selected, chemically synthesized, possesses a relatively small size and insensitive to chemical and physical conditions, however their application is hampered due to their susceptibility to enzymatic degradations. Spiegelmers can be seen as biostable version of oligomers because they consist of L-sugar instead of naturally occurring D enantiomer. Considering their advantages, we aimed at producing cardiac troponin I (cTnI) specific Spiegelmers to provide alternative receptors for biosensor development. Our results suggest that protein selective Spiegelmers can be effectively selected by rational identification of protein epitopes and high-throughput screening of isolated candidates. Furthermore, the results of surface plasmon resonance measurements demonstrated that the characterized oligonucleotide bind to cTnI with low nanomolar affinity and discriminate their target even in a complex protein matrix such as blood serum. In order to test applicability of the selected Spiegelmer in sandwich ELISA based assays, we developed an Amplified Luminescent Proximity Homogenous Assay (ALPHA) using our receptor and a commercial cTnI selective antibody. The obtained data corroborated our assumption; the developed assay could differentiate the cTnI positive and negative clinical samples. These results indicate that Spiegelmers could be reasonable alternatives of antibodies in diagnostics.

PI-59

Growth parameters and protein production of *E. coli* strains containing different copy number of ribosomal RNA operons

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The copy number of the ribosomal RNA operons (rrn) is a characteristic trait of bacterial genomes. It influences rRNA availability, which in turn, regulates the amount of ribosomes, key players of cellular physiology and economy. It is thought that the rrn operon number reflects the organism's ecological strategy. Bioinformatic and some experimental data suggest that lower copy numbers are favored in bacteria living in low-nutrient, relatively stable environments, and higher numbers (7 in *E. coli*) seem to be associated with fluctuating, feast and famine conditions. It is not clear, however, what the primary determinant of rrn operon copy number is: the benefit of fast growth, the capability of quick adjustment to favourable conditions, or the economic utilization of nutrients.
We constructed isogenic variants of *E. coli* K-12 with 5 to 10 copies of rrn operons, analysed their growth parameters, measured their RNA and protein contents, and subjected them to pairwise competitions under both fluctuating (serial growth in batch cultures) and stable nutrient influx (growth in a chemostat) conditions. While growth parameters showed only minor changes, competitions revealed a clear pattern: 7-8 copies were optimal under fluctuating conditions, and lower numbers were favored in a stable environment. These patterns persisted at two different growth rates. Interestingly, while the RNA: protein ratio remained constant, an increase in protein and RNA content, accompanied by a slight increase in cell size was observed in the 5 to 8 operon range.
By delineating the effects of nutrient quality and availability, we found that the stability of the environmental conditions is the primary factor determining the optimal number of rrn operons in *E. coli*. Our experiments confirm that the wt rrn copy number of *E. coli* reflects adaptation to fluctuating conditions. However, the results also show that lower rrn copy numbers are beneficial for *E. coli* when environmental conditions are stable. Adjustment of the rrn number to the planned environmental conditions should thus be taken into account when constructing designer bacterial genomes.

PI-60

Structural assembly of the signaling competent ERK2–RSK1 complex

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Mitogen-activated protein kinases (MAPKs) bind and activate their downstream kinase substrates, MAPK-activated protein kinases (MAPKAPKs). Notably, extracellular signal regulated kinase 2 (ERK2) phosphorylates ribosomal S6 kinase 1 (RSK1), which promotes cellular growth. We determined the crystal structure of an RSK1 construct in complex with its activator kinase. The structure captures the kinase–kinase complex in a precatalytic state where the activation loop of the downstream kinase (RSK1) faces the enzyme's (ERK2) catalytic site. Molecular dynamics simulation was used to show how this heterodimer could shift into a signaling-competent state. This structural analysis combined with biochemical and cellular studies on MAPK→MAPKAPK signaling showed that the interaction between the MAPK binding linear motif (residing in a disordered kinase domain extension) and the ERK2 “docking” groove plays the major role in making an encounter complex. This interaction holds kinase domains proximal as they “readjust,” whereas generic kinase domain surface contacts bring them into a catalytically competent state.

PI-61

Stress triggers mitochondrial biogenesis to preserve steroidogenesis in Leydig cells

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Adaptability to stress is fundamental prerequisites for survival. Mitochondria are a key component of the stress response in all cells. For steroid hormones-producing cells, including also Leydig cells of testes, the mitochondria are a key control point for the steroid biosynthesis and regulation. However, the mitochondrial biogenesis in steroidogenic cells has never been explored. Here we show that increased mitochondrial biogenesis is the adaptive response of testosterone-producing Leydig cells from stressed rats. All markers of mitochondrial biogenesis together with transcription factors and related kinases are up-regulated in Leydig cells from rats exposed to repeated psychophysical stress. This is followed with increased mitochondrial mass. The expression of PGC1, master regulator of mitochondrial biogenesis and integrator of environmental signals, is stimulated by cAMP-PRKA, cGMP and ß-adrenergic receptors. Accordingly, stress-triggered mitochondrial biogenesis represents an adaptive mechanism and does not only correlate-with but also is an essential for testosterone production, being both events depend on the same regulators. Here we propose that all events induced by acute stress, the most common stress in human society, provoke adaptive response of testosterone-producing Leydig cells and activate PGC1, a protein required to make new mitochondria but also protector against the oxidative damage. Giving the importance of mitochondria for steroid hormones production and stress response, as well as the role of steroid hormones in stress response and metabolic syndrome, we anticipate our result to be a starting point for more investigations since stress is a constant factor in life and has become one of the most significant health problems in modern societies.

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PI-62

Ubiquitin-dependent phosphorylation of ERKS in a three component signaling complex

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Mitogen-activated protein kinases (MAPKs) are well-conserved elements of human signal transduction. Mammals possess four classical MAPK pathways that play critical roles in many biological processes, e.g. proliferation, differentiation, stress-induced signaling and apoptosis. ERK5 and c-jun N-terminal kinase (JNK) pathways have paralogues protein elements and share a common upstream avtivator (MEKK3). Structural and biochemical studies of MEKK3-MKK5-ERK5 interactions can give a mechanistic insight on how signaling cascades using common components and can achieve functionally distinct and specific outcomes.
Pull-down and fluorescence polarization (FP) based assays showed that minimally two MKK5 interacting regions are required to bind ERK5. A Phox and Bem1 (PB1) domain and a linear motif (D-motif) from MKK5 cooperate to mediate high affinity binding to ERK5. MAPK activation depends on a linear binding motif found in all MAPK kinases (MKK). I present the crystal structure of ERK5 in complex with an MKK5 construct comprised of the PB1 domain and the linear binding motif. Structural and biochemical characterization revealed that the MKK5 PB1 domain cooperates with the MAPK binding linear motif to achieve substrate specific binding. In addition this domain also enables co-recruitment of the upstream activating enzyme and the downstream substrate into one signaling competent complex. The upstream activator kinase for MKK5 is MEKK2/3, which also activates MKK7 that in turn activates JNK signaling. ERK5 and JNK signaling is functionally distinct and I demonstrate that ERK5 pathway activity is diminished upon MEKK2/3 ubiquitination by XIAP (X-linked Inhibitor of Apoptosis Protein). Interestingly, JNK activation is not inhibited by ubiquitination of this shared upstream activator kinase.

PI-63

A novel crosstalk of MAPK- and Ca-signaling pathways: comprehensive characterization of S100-MAPKAPK interactions

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Mitogen-activated protein kinase activated protein kinases (MAPKAPK) are the terminal kinases in MAPK signaling pathways. Their conserved core contains a CaMK type protein kinase domain and a C-terminal MAPK binding linear motif. A recent publication showed that overexpression of S100B, a small CaM like Ca^{2+} activated dimeric protein can directly inhibit ERK2 specific MAPKAPK (RSK1) phoshorylation in melanoma cells. We are studying the biochemical and structural properties of this interaction.
We confirmed the presence of this interaction *in vitro* and also validated the inhibition of ERK2 phosphorylation. The binding interface was mapped and found that the whole C-terminal part of RSK1 is involved in S100B binding. In CaMK type kinases a C-terminal inhibitor region blocks the substrate binding groove which is generally released upon CaM binding. Because this autoinhibitory region is also important in S100B binding along with the MAPK binding linear motif there is a strong possibility that S100B binding not only protects RSK1 from ERK2 phosphorylation but can be an important step in the RSK1 activation mechanism. Interestingly we found that a single RSK1 peptide fragment binds to an S100B dimer. The knowledge on similar asymmetric S100 interactions are very limited and was only described in case of S100A4 and S100A10. We also solved a crystal structure of the S100B-RSK1 protein-peptide complex which shows a rather fuzzy structure between the two partners. In this binding mode RSK1 anchors itself only to the main hydrophobic pockets of each S100B subunit with its N- and C-termini while the intervening region remains highly flexible. We also found that not only RSK1 but also MK2, the p38 specific MAPKAPK is an S100B binding partner and we also expand our studies to other S100 proteins.
These *in vitro* results suggest a wide interaction network between the S100 and MAPKAPK family and therefore an additional, so far unrecognized crosstalk between the MAPK and Ca-signaling pathways.

PI-64

Agrin signalling in primary human myoblasts

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Neural agrin is a heparan-sulphate proteoglycan that plays a major role in formation and maintenance of the neuromuscular junction. Recent evidence suggests that neural agrin could be used as a pharmacological agent to promote stabilization of the neuromuscular junction in ageing skeletal muscle, which could prevent age-related denervation of skeletal muscle fibres and the attendant muscle wasting. Neural agrin promotes stabilization of the neuromuscular junction by activating the muscle-specific kinase (MuSK) *via* LDL receptor-related protein 4 (Lrp4). During skeletal muscle regeneration Lrp4/MuSK pathway is up-regulated upon differentiation of myoblasts into myotubes. Thus, myoblasts are usually assumed to be unresponsive to neural agrin. However, this assumption has not been thoroughly examined.

Here we examined whether primary human myoblasts are responsive to neural agrin. We found that myoblasts express Lrp4 and MuSK, which form the canonical agrin receptor in myotubes and mature muscle fibres. Gene silencing of Lrp4 and MuSK markedly reduced myoblast proliferation, indicating a functional role for Lrp4/MuSK receptor in myoblasts. To further examine its role in myoblasts, we assessed the Abl-CrkII and MEK1/2-ERK1/2 signalling pathways, which are activated by binding of neural agrin to the Lrp4/MuSK receptor. In myoblasts treated with neural agrin phosphorylation of Abl and its downstream target CrkII remained unaltered, while ERK1/2 was transiently dephosphorylated, suggesting neural agrin did not activate Lrp4/MuSK. Notably, we found that myoblasts express agrin mRNA. Treatment of myoblasts with agrin-Z0, a muscle-specific agrin isoform that does not activate Lrp4/MuSK, induced dephosphorylation of ERK1/2, while the activity of Abl-CrkII pathway remained unaltered. This shows that Lrp4/MuSK activation is not required for agrin signal transduction in human myoblasts.

Collectively, our results demonstrate that primary human myoblasts are responsive to neural as well as to the muscle-specific agrin. Although myoblasts possess the canonical agrin receptor Lrp4/MuSK, both agrin isoforms likely signal *via* an alternative agrin receptor. Finally, our results suggest that myoblast-derived agrin might be involved in autocrine and paracrine signalling in skeletal muscle. Agrin signalling in myoblasts may have important implications for the development of agrin-based therapeutic strategies for muscle wasting disorders.

PI-65

PARP-1 inhibitor attenuates mitochondrial ROS production and cell death through PARP-1-ATF4-MKP-1 pathway in oxidative stress

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Several studies showed that poly(ADP-ribose) polymerase-1 (PARP-1) inhibitors reduce oxidative stress-induced JNK and p38 MAP kinase activation, which significantly contribute to cell death and disease progression. Molecular link between PARP and the MAPKs have not yet well defined, although we have raised the role of MKP-1 expression in PARP inhibitor induced JNK and p38 MAPK inactivation. Here, we search for the transcription factor (TF) which involved in PARP-inhibitor mediated MKP-1 expression and for its role in oxidative stress. Analyzing several TFs we found that suppression of ATF4/Creb2 prevented PARP inhibitor, or PARP-1 suppression, induced MKP-1 expression. In ATF4 suppressed cells MAP kinases activities were much higher than in normal cells, and PARP inhibitor can not suppress JNK and p38 MAPK activation in oxidative stress. Active PARP-1 poly-ADP-ribosylated ATF4, and so inhibited its specific binding to CRE sequence. PARP-1 binds to the CRE sequence to specifically, and its activation augments its binding. PARP inhibitor protected cells, suppressed mitochondrial ROS production and protected mitochondrial membrane potential in oxidative stress but suppression of ATF4, or MKP-1 diminished its protective effects. That is, PARP-1 inhibitor protects mitochondrial through ATF4-MKP-1-JNK/p38 MAPK retrograde pathway which could be important in oxidative stress-related diseases. JNK activation plays an important role in the tumor-initiating capacity of cancer stem cells, therefore data showing that PARP inhibitor reduces JNK activities in A-549 and T24/83 cancer cells raising the significance of the PARP inhibitors mediated retrograde pathway in cancer therapy.

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PI-66

Melatonin replacement restores the circadian behavior in adult rat Leydig cells after pinealectomy

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Melatonin actions on oscillators in reproductive organs are poorly understood. Here we analyzed melatonin effects on rhythmic expression of clock and steroidogenic-related genes in adult rat Leydig cells (LCs). The effect of melatonin was tested both *in vivo* using pinealectomized and melatonin-substituted rats and *in vitro* on isolated LCs. Data revealed 24-h-rhythmic expression of clock genes (*Bmal1, Per1,2,3, Rev-erba,b, Rorb*), steroidogenic genes (*Star, Cyp11a1, Cyp17a1*), and genes of steroidogenic regulators (positive-*Nur77*, negative-*Arr19*). Pinealectomy increased 24-h-oscillations of serum testosterone and LC`s cAMP levels, expression of *Insl3, Per1*, *Star*/StAR, *Hsd3b1/2*, *Nur77*, decreased *Arr19* and canceled *Per2* oscillatory expression pattern. At hypothalamic-pituitary level, pinealectomy increased mesor of *Gnrh, Lhb* and rhythm robustness of *Mntr1a* expression. All parameters disturbed were restored by melatonin-replacement. However *in vitro* studies did not confirm direct melatonin effects on neither clock nor steroidogenic genes. Results suggested melatonin influence 24-h-rhythmic LC-function likely through hypothalamic-pituitary axis and consequently cAMP-signaling in LCs.

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PI-67

Structural background of the regulation of SH3 domains by tyrosine phosphorylation

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Src homology 3 (SH3) domains are protein-protein interaction domains in eukaryotes involved in various intracellular signalization pathways. These domains bind short, proline rich sequences within intrinsically unstructured regions of partner proteins. More and more evidence have been accumulated in the recent years showing that phosphorylation on different conserved tyrosine residues of SH3 domains is a common regulatory strategy. Most of these tyrosines are part of the ligand binding groove, and the result of this post-translational modification was the inhibition of partner binding *in vivo* in the majority of reported cases. However, *in vitro* studies demonstrating the effects of tyrosine phosphorylation on individual tyrosine residues (e.g. partner binding assays) has not been published yet. Atomic resolution structures would also be essential to fully understand the role and function of the introduced phosphate groups. In this study we successfully overcome all technical limitations, and investigate the structural and functional consequences of phosphorylation *in vitro*. The SH3 domains of the Abl1 and Abl2 tyrosine kinases have been chosen as model systems for our experiments. We successfully phosphorylated the SH3 domains on two physiologically relevant tyrosine residues by the recombinant kinase domain of Ephrin B1 receptor. According to our preliminary results, the SH3 domain of Abl1 kinase phosphorylated on Tyr70 and Tyr115 show reduced affinity to a peptide ligand corresponding to the binding motif of the 3BP-1 protein (residues: 616-625). We have already obtained well diffracting crystals of both phosphorylated SH3 domains and ongoing X-ray diffraction experiments are expected to reveal the structural background of this common regulatory mechanism.

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PI-68

BEST channels - possible mediators of H_2S-induced relaxation of rat uteri?

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Hydrogen sulphide (H_2S) reduces uterine contractility and appears to be an important signaling molecule in rat uterus. BEST proteins were shown to recapitulate the properties of native CaCCs - the major anionic conductance in myometrium. To the best of our knowledge there were no studies showing expression of bestrophin channels in rat uterus in estrus. The aim of this study was to explore the mechanism of sodium sulphide (Na_2S) - induced relaxation of non-pregnant rat uterus in estrus, investigating importance of redox effects and ion channel-mediated mechanisms, as well as interactions between these two mechanisms. Pharmacological assessment of sodium sulphide (Na_2S, H_2S/HS^- "donor") effects on uterine strips were performed by isolated organ bath studies (uteri were allowed to contract sponatneosly or contractions were induced by external calcium). Antioxidative enzyme activities were measured in homogenates of treated uteri. Expression studies of the bestrophin channel 1 (BEST-1) were performed by Western blotting and RT-PCR. In this study, we demonstrated that relaxation is DIDS sensitive which was recently found to be highly selective for BEST-1 channels. Relaxation was not affected by other CaCC modulators since T16Ainh-AO1, tannic acid and NFA failed to inhibit Na_2S induced relaxation or by the absence of extracellular HCO_3^-. Although, it has been considered that K_{ATP} channels are the main reasponsible for H_2S effects, our data shows limited importance of K_{ATP} channels. We showed that BEST-1 is expressed at the mRNA level in rat myometrium. Additionally, BEST-1 channels are expressed at protein level in rat uterus in estrus, suggesting a role for BEST-1 in the control of uterine contractility. L-type calcium channel activator S-Bay K failed to affect Na_2S-induced relaxation. BEST-1 appears to function as a regulator of voltage-dependent L-type Ca^{2+} channels. Antioxidative enzyme activities from tissues pretreated with DIDS, followed by Na_2S exposure were not different from activities measured in uteri treated only with Na_2S. This implies that there is no feedback from DIDS-sensitive Cl^--channel to antioxidative system activity. However, the inhibition of K_{ATP} channels did affect antioxidative system. This study demonstrates the significance of DIDS-sensitive Cl^- - pathway in Na_2S relaxatory effects which appear to be redox and Ca^{2+} dependent.

PI-79

The role of the L5/L11-Mdm2-p53 signaling pathway in response to ribosomal and genotoxic stresses

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The exposure of cells to various DNA-damaging stressors activates p53 to preserve cellular and genetic stability, preventing tumor development in mice and humans. The critical role of p53 in tumor suppression is supported by the observation that approximately 50% of all human cancers have mutations within this gene. Although it was largely accepted that common to all p53-activating stresses is DNA damage, research over the last decade has shown that disruption of ribosome biogenesis promotes binding of several distinct ribosomal proteins (RP) to Mdm2 resulting in inhibition of its E3 ubiquitin ligase activity towards p53. As a result, p53 accumulates within the cell and transcriptionally activates genes that regulate apoptosis, cell cycle checkpoints, metabolism and senescence. We have recently shown that ribosomal proteins (RP) L5 and L11 play a major role in p53 activation upon ribosomal biogenesis as well as DNA damaging stresses. Given the importance of RPL5 and RPL11 in p53 activation, we initiated a project to understand their role in the development of malignant tumors. Our efforts led to the identification of the first somatic cancer-associated missense mutations in the RPL5 and RPL11 genes in humans. Our current research focuses on understanding the molecular mechanisms by which RPL5 and RPL11 participate in p53 activation in response to ribosomal or genotoxic stress as well as providing insights into the functional significance of these cancer-associated mutations in *RPL5* and *RPL11* genes in p53 regulation and tumorigenesis.

PI-70

10 Years of ibogaine research in Slovenia

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The root bark of iboga plant - *Tabernanthe iboga* has been used traditionally in Central Africa as a psychoactive substance in religious rituals, while in smaller doses it is appreciated due to its stimulant properties. The iboga root bark, iboga extract or pure ibogaine are being recognized in the West as an anti-addiction remedy and their use is increasing. The project aims to disclose the common mechanism of action at these seemingly diverse indications for iboga use, to predict eventual adverse effects and to build the grounds for its safe and beneficial utilization.
Our results showed that ibogaine triggers adaptation of house keeping metabolism. Under the initial energy load this results in a stabile shift in epigenetic landscape that improves cellular energetic state and can be considered as nootropic. While healthy organism profits from improved fitness and mental performance and can withstand higher stress without risking a disease, due to the same principles ibogaine provides beneficial support at the recovery after diseases including addiction syndrome.

PI-71

Myosin phosphatase regulates gene expression *via* mediating arginine methylation in human hepatocarcinoma cells

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The myosin phosphatase (PP1M) holoenzyme is a serine/threonine specific protein phosphatase. It is involved in the regulation of cell contractility *via* dephosphorylation of the 20 kDa light chain of myosin. The fact that several novel non-cytoskeletal protein substrates of PP1M have been identified indicating the complex function of PP1M in different cellular processes. In PP1M protein phosphatase-1 catalytic subunit is associated with myosin phosphatase target subunit 1 (MYPT1). MYPT1 was found to be localized not only in the cytosol and cytoskeleton but in the nuclei of *aortic smooth muscle*, primary neuronal as well as of human hepatocarcinoma (HepG2) cells. Our goal was to investigate the nuclear functions of PP1M by determining the subnuclear localization and the interacting proteins of MYPT1. The dominant nuclear protein phosphatase was found to be the PP1 in HepG2 cells. Numerous nuclear MYPT1-interacting proteins were identified such as histone 1, splicing factor proteins as well as the members of the methylosome complex, i. e. protein arginine methyltransferase 5 (PRMT5). In addition PRMT5 was found to be phosphorylated at Thr80 by Rho-associated protein kinase *in vitro,* whereas PP1M diminished the phosphorylation level of this site. Silencing of MYPT1 significantly induced the general symmetric dimethylation (PRMT5 specific methylation) level on arginine3 residues of histone H2A and histone H4 and caused a global change in gene expression. Our data suggests novel physiological roles of PP1M in the nuclear dephosphorylation processes related to the regulation of transcription, RNA splicing and the functions of the methylosome complex.

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PI-72

Ibogaine relaxes rat arteries: the role of endothelium

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Ibogaine is a naturally occurring alkaloid isolated from the bark of the roots of the West African *Tabernanthe iboga* plant. It is well known for its anti-addictive effects. On the other hand, its pharmacology is quite complex, affecting many different neurotransmitter systems simultaneously. Ibogaine binds to several types of receptors: 5-Hydroxytryptamine (5-HT), opioid, nicotinic and N-methyl-D-aspartate (NMDA) receptors, dopaminergic and 5-HT transporters and monoamine oxidase enzyme (MAO). Based on our previous study showing ibogaine effects on ATP liberation (127 pM) from erythrocytes *in vitro*, we wanted to investigate direct pharmacological ibogaine effects on aorta and mesenteric artery and to compare them with effects of ATP. Its effects were tested by isolated organ bath studies using aorta and mesenteric artery rings (with and without endothelium) isolated from Wistar rats. Aortic and mesenteric artery rings were precontracted with phenylephrine (10 μM). Ibogaine (64.4 mM) produced a relaxation in the aortic as well as in mesenteric artery rings, in a similar way. Realaxation effects were followed in time (5, 10, 20, 30, and 60) and it was shown that ibogaine effects are time-dependent. In addition, ibogaine effects are also endothelium dependent since presence of endothelium facilitated relaxation. ATP (127 pM) induced relaxation in the aortic as well as in mesenteric artery rings, and this effect is completely endothelium-dependent. Taken together these findings suggest that ibogaine affect smooth muscles directly. Additionally, relaxation is endothelium dependent (possibly is mediated *via* nitric oxide) and ATP-mediated.

PI-73

Fungal lectins: versatile molecular triggers and their potential use in biomedicine

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Lectins are ubiquitous carbohydrate-binding proteins. They have many important biological functions in living organisms. Their location inside the cytoplasm, different organelles, membranes or extracellular compartment is crucial in the transport of proteins, and cell interactions. Binding of exogenous lectins to cell surface glycosylated moieties results in activation of receptors, crosslinking receptors into clusters destined for endocytosis, and in changed membrane permeability. Lectins can have either mitogenic, antiproliferative or toxic effects on cells. Many newly discovered fungal lectins have been thoroughly studied and are known to possess considerable diversity of glycan recognition and are toxic to various organisms. Recent studies are focusing on the use of these lectins in biomedical research.

Our group focuses on two β-trefoil lectins CNL and MpL from mushrooms *Clitocybe nebularis* and *Macrolepiota procera* [1, 2], respectively. MpL binds best to one of the most common glycosylation motifs *N*-acetyllactosamine (Galβ1-4GlcNac) and CNL to a less pronounced *N,N'*-diacetyllactosamine (GalNAcβ1-4GlcNac). Selectivity of these lectins results in the fact that MpL is not toxic to human cell lines, whereas CNL exerts toxicity only toward human leukemic T cells (Jurkat and Mo-T). Moreover, CNL lectin binds to the cell membrane and presumably acts in a cytokine-like pathway of activating receptors by crosslinking. MpL binds to cell surface targets and is internalized *via* clathrin/receptor-mediated endocytosis in several minutes upon addition to the cells. Once located inside early endosomes it is transported to Golgi network *via* retrograde transport. Mechanisms of endocytosis triggering by single domain lectin MpL are speculative and remain to be determined.

Activities of MpL and CNL toward cell lines can be useful for treatment of leukemic diseases, immunomodulation, glycoprofiling and for targeted delivery of biomedicines to cellular organelles.

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PI-74

Correlation between MXR inhibitors in wastewater and zebrafish embryotoxicity

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Waste water treatment plants (WWTPs) are important point sources of a broad range of xenobiotics in the aquatic environment. Once released from WWTPs, xenobiotics represent a potential risk to environment, but also to human health. Since it is very hard to predict their possible impact on different levels of biological organization, it is necessary to use "early warning" biomarkers that can provide information on potential environmental impact. Zebrafish embryos are frequently used organisms in ecotoxicology studies due to their short embryonic ontogenesis and transparent chorion. The fish embryo test (FET), as a substitute for acute toxicity with adult zebrafish, is a great biomarker in toxicity testing of pollutants that are residues from incomplete elimination in WWTPs. Screening sub-lethal and lethal endpoints after exposure to wastewater included coagulation of embryos, presence of somites, tail detachment, heart functioning, blood circulation, pigmentation, formation of edema and developmental retardation. Zebrafish embryos also possess a multixenobiotic resistance mechanism (MXR) mediated by ABC transport proteins that acts as a protective barrier against the cell uptake and accumulation of potentially toxic compounds dissolved in the water. MXR mechanism can be modified by the presence of a wide range of xenobiotics frequently present in wastewaters (pharmaceuticals, chemicals used in industry and agriculture, personal care products). Concentration of model fluorescent substrate in the embryo after exposure to environmental samples, gives us an insight into the degree of inhibition and accumulation of xenobiotics from wastewater in the whole embryo. The integration of embryotoxicity and MXR creates a sensitive tool for the evaluation of early developmental responses of zebrafish to xenobiotics present in waste waters on whole organism level, but also on the cellular level. These responses help us to better understand possible toxic effects of wastewater effluents on aquatic organisms.

PI-75

Genome stability enzymes are essential for building CRISPR-cas immunity in bacteria

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CRISPR-cas is a prokaryotic immune system built from capture and integration of invader DNA into CRISPR loci by Cas1 and Cas2 proteins, termed 'Adaptation'. In *E. coli*, Cascade-Cas3 degrades invader DNA to enact immunity, termed 'Interference'. Adaptation can be stimulated by interference ('primed'), or can be independent of interference ('naive'). We identified that host genome stability enzymes are required for adaptation; primed adaptation requires RecG and PriA, naïve adaptation requires RecB and both types require DNA polymerase I (PolA). Analysis of *recG* and *priA* phenotypes indicates interplay between primed adaptation, blocked replication forks and R-loop processing. We further show that Cas1 and Cas2 proteins specifically target substrates mimicking blocked forks. A model is proposed for DNA capture enabled by RecG, PriA and Cas3, or by RecB, according to different types of compromised DNA replication. PolA is proposed to synthesize DNA to fill single strand gaps during capture or integration.

PI-76

The effect of chronic renal failure on the expression of drug-metabolizing cytochrome P450 enzymes

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The drug metabolizing capacity of kidney transplant recipients largely determines the outcome of postoperative drug therapy. In order to reduce the risk of drug failure and side effects, it is recommended to obtain information on patients' current drug metabolizing potential. The members of cytochrome P450 (CYP) enzyme family play a great clinical role in the metabolism of drugs applied in during the post-transplant therapy.
The hepatic drug metabolism mainly depends on the expression and the activity of CYP enzymes. Information about CYP activities in the liver can be obtained from CYP expressions in white blood cells, since we have displayed a strong correlation between the hepatic CYP enzyme activities and the amount of CYP mRNA in leukocytes.
Due to high sensitivity, the quantitative RT-PCR method is suitable for detection of low mRNA levels; however, optimization of sample preparation and the measurement steps are always required. The main aim of our work was to improve the quantification of CYP expressions by optimizing RNA isolation, reverse transcription and by designing novel, more specific primers and probes.
The expression of main drug-metabolizing isoenzymes, CYP1A2, CYP2C9, CYP2C19 and CYP3A4, has been determined in 106 patients with chronic renal failure, immediately prior to kidney transplantation. Amongst the evaluated patients, particularly high proportion (50-75%) of low CYP expressers were found, which can be attributed to the hepatotoxic effects of increased levels of parathyroid hormone, various cytokines and uremic toxins caused by uremia.

PI-77

An experimental test of the adaptive genome streamlining hypothesis

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Genome reduction is a prominent evolutionary process that pervades nearly all major bacterial lineages. Two main hypotheses have been suggested about why some prokaryotic genomes are especially compact and contain reduced gene sets (Giovannoni *et al*. 2014 and Mira *et al*., 2001). The non-adaptive loss scenario suggests that relaxed purifying selection together with a strong mutational bias towards deletions in bacterial lineages account for genome reduction. In sharp contrast, the streamlining hypothesis posits that selection acts to reduce genome size to improve cellular economics via two mechanisms. It minimizes the metabolic burden of replication, and it allows concomitant reduction in cell volume. In this work, we explicitly tested assumptions of the genome streamlining hypothesis. By extending a previous series of deletions of genomic segments, we reduced the *Escherichia coli* genome by up to 20.3%. The resulting 69 multiple-deletion series (MDS) strains provided an unprecedented opportunity to study the phenotypic consequences of genome reduction, not least because the deleted segments harbor genes that have been repeatedly lost in *E. coli* relatives. The following major conclusions were drawn. Eradication of large genomic segments 1) had no beneficial effects on growth rates, 2) reduced metabolic yield under nutrient starvation, and 3) caused a major perturbation of genome-wide gene expression. 4) A systematic experimental survey revealed that multiple-deletion strains exhibit severe defects in nutrient utilization. Last, 5) there was no significant association between genome size and cell size. In sum, we failed to find systematic evidence for beneficial effects of genome reduction in most cellular traits investigated.

PI-78

The role of the hepatic cholesterol synthesis in the bile acids synthesis/excretion in mice with *Cyp51* liver knockout

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In the liver, bile acid transporters play a critical role in maintaining the enterohepatic circulation and bile acid homeostasis. Hepatocyte is a polarized cell with a basolateral (sinusoidal) and an apical (canalicular) plasma membrane. The two fundamental parts of bile acid enterohepatic circulation are secretion from the liver and absorption from the intestine. Transporters on the hepatocyte basolateral (sinusoidal) membrane initiate the transport of bile acids. Once inside the hepatocyte, bile acids are transferred *via* various mechanisms to the apical (canalicular) pole for their transport to the bile. To understand the role of hepatic cholesterol synthesis in the bile acid (BA) synthesis and excretion we applied the *Cyp51* liver conditional knockout with defect in the hepatic cholesterol synthesis. The aim was to investigate the impact of defected cholesterol pathway on expression of the bile acid transporters in *Cyp51* liver knockouts (LKOs) with a block of cholesterol synthesis in the post-lanosterol path. We measured the expression of selected genes from bile acid transport pathway in livers of females and males on a standard laboratory diet (LFnC). RT-qPCR with Sybr Green was applied.

We took a closer look at the expression of selected genes from bile acid transport pathway to determine the difference between LKOs and LWTs. As expected, some of bile acid transporters were up-regulated and some of them were down-regulated in LKOs compared to LWTs. We observed a consistent trend of higher gene expression in LKOs encoding the transporters localized on the apical (canalicular) side of the cell and being responsible for the excretion of bile acids. We also noticed a higher expression of genes in LKOs encoding the transporters from the basolateral side, which are responsible for bile acids efflux, while genes that encode transporters responsible for bile acids influx are down-regulated in LKOs compared to LWTs.

With our further study we aim to explain the potential sexual dimorphism and expand the study by incorporating other extrahepatic tissues which play a role in maintaining the cholesterol and bile acid homeostasis.

PI-79

Examining genome integrity maintenance in human cell lines by site-specific, biallelic gene knock-out technology

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Numerous physiological pathways with multiple protein factors play diverse roles in the faithful maintenance of genomic integrity which is indispensable for life. The object of our studies, dUTPase, catalyzes the hydrolysis of dUTP into pyrophosphate and dUMP, supporting low cellular dUTP/dTTP ratio, thus immunity against genomic uracil accumulation. It has been shown that deficiency in dUTPase function leads to cell death in several organisms e.g. in *Mycobacterium smegmatis* and in *Drosophila melanogaster*.
The molecular mechanism of thymine-less cell death, induced by the lack of dUTPase, is poorly understood although several routinely applied chemotherapeutic drugs in the clinic (fluoropyrimidines, methotrexate and its derivatives) interfere with the *de novo* thymidylate biosynthetic pathway. Overexpression of dUTPase causes partial resistance against fluoropyrimidines, while its deficiency sensitizes the cells. Therefore a better knowledge of dUTPase role and function is particularly important in medicine. Until now, dUTPase function was only investigated with gene silencing but gene knock-out strategy has not been applied. Our aim is to investigate the potential mechanism of cell death caused by dUTPase knock-out in human cancer cell lines, thus gaining a better understanding of how cancer cells get resistant to chemotherapeutic agents. We applied a state-of-the-art method, the zinc finger nuclease (ZFN) technology, which allows site-specific, poliallelic genomic manipulations. Considering the fact that we are knocking-out a putative essential gene, we first had to provide the cells with conditional exogenous source of the protein, otherwise a viable knock-out cell line could not be established. Therefore an exogenous floxed dUTPase source was also integrated to another well-established point of the genome, which expresses both the nuclear and the mitochondrial isoforms. Expression could be terminated at any desired time point by the help of a ligand-inducible Cre recombinase.
With the inducible dUTPase knock-out cell line we will be able to clarify the role of dUTPase in genome integrity maintenance and their involvement in clinical therapy. The applied combined knock-out/integration strategy could also be used for other potentially essential genes which could be of wide interest.

PI-80

Detection of genomic uracil from bacteria to human

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Uracil can incorporate into DNA via two mechanisms: instead of thymine, through DNA polymerases or via cytosine deamination. Base-excision repair and regulation of nucleotide pools are responsible for the prevention of uracil accumulation in DNA. The two key enzymes in this process are uracil-DNA glycosylase and dUTPase. However, uracil also appears under normal physiological conditions, such as in some heavily uracilated phages, and in the genome of HIV. Uracil in DNA is a normal intermediate in acquired immunity in human B lymphocytes, and emerges in elevated levels in friutfly larvae, pupae and imagoes. However, in most organisms genomic uracil level is low.
There are some genomic uracil quantification methods with varying specificity, sensitivity and price. MS-based method [Galashkaya *et al*., DNA Repair 2013] is sensitive, but requires a quite expensive instrument and specialized knowledge. Aldehyde reactive probe assay measures AP-sites done by UNG. A quantitative qPCR based method [Horváth and Vértessy, Nucleic Acids Research 2010] is suitable for relative quantification.
All current absolute quantification methods excise uracil, or scarf the DNA, and do not rely on *in situ* detection. Our new method uses a catalytically inactive UNG, capable of binding but not excising uracil. Our uracil bound UNG sensor was designed in a way that it could be detected with conventional antibodies in dot-blot and ELISA applications along with *in situ* detection using an immunocytochemical approach. We validated our uracil sensor in all three approaches, using samples derived from CJ236 *dut-, ung- E. coli*, BL21 (DE3) *ung*-151 *E. coli*, holometabolas and also mouse embryonic fibroblast cells with altered base excision repair background, in combination with chemotherapeutic drugs. Our method has high sensitivity, with as low as 1 uracil / million bases. It is a versatile tool for quantification of genomic uracil of any kind of organism, while also giving the possibility to gain position and sequence specific information on genomic uracil content. Using knowledge of uracil content of DNA of several species, interesting biological questions can be answered.

PI-81

Clinical relevance of patients' CYP3A-status in clozapine therapy

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The prevalence of schizophrenia is approximately 1% of the total population in Hungary. The clinical protocol for the management of schizophrenia dictates mandatory use of antipsychotics as part of the treatment. First generation antipsychotic drugs, such as chlorpromazine, though proving to be effective in reducing the positive symptoms, had serious side effects (extrapyramidal symptoms, EP). The second generation of antipsychotics (e.g. clozapine, quetiapine, olanzapine) are characterized by largely reduced EP symptoms. Up to one third of patients are resistant to most commonly used antipsychotics. Clozapine is proved to be more effective for these non-responder patients. However, monitoring of serum levels is recommended because of the serious rare side effects such as agranulocytosis and hepatotoxicity.
The genetic polymorphisms of cytochrome P450 (CYP) enzymes involved in clozapine metabolism can significantly influence the serum levels of clozapine. The association between CYP genotypes and occurrence of side effects (or ineffectiveness of treatment) is at best modest. A possible explanation is the fact that CYP expression is the factor that largely determines the drug metabolizing capacity of patients. Determining the expression of CYP genes and identifying defective CYP genes can provide a more accurate picture of the patients' drug metabolizing capacity and facilitate a more personalized treatment.
According to previous publications, CYP1A2, CYP3A4 and CYP2D6 to some degree are postulated to be involved in clozapine metabolism. CYP-status (CYP genotype and CYP expression) of patients (n=74) treated with clozapine was determined by qPCR. The expression of the relevant CYP genes in leukocytes was also determined by qPCR. Serum levels of clozapine were detected by HPLC-MS/MS. We found strong association between CYP3A4 expression and normalized clozapine serum levels, which enables us to predict the effective therapeutic dosage of clozapine based on CYP3A4 phenotype.

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PI-82

VNTR patterns in TPMT promoter region affect TPMT activity via linkage disequilibrium with TPMT*3 alleles

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Thiopurine-S-methyltransferase (TPMT) is one of the finest examples in translation of genetic research to clinical practice. Gene's ORF-based SNPs strongly correlate with lower TPMT enzymatic activity and, consequently, higher toxicity of thiopurine drugs. Besides, its GC-rich promoter region contains variable number of tandem repeats (VNTR). Population studies and research on transfected cells tried to link the VNTR patterns with several phenotypic characteristics. However, due to poor qualitative determination of motive arrangements, these analysis left inconclusive results.
In order to clarify the influence of VNTR patterns on TPMT activity and TPMT gene expression, we applied an "*in vitro*" model – EBV-transformed lymphoblastoid cell lines (LCL) obtained from 38 healthy individuals. TPMT activity was assessed in LCL lysates through standard HPLC procedure. Determination of TPMT SNPs and TPMT expression were assayed by means of TaqMan technology. Expression of housekeeping genes, GUSB and TBP, was measured using Sybr dye qPCR. VNTR alleles were qualitatively resolved by sequencing of promoter region.
Since the population of LCLs was selected to study TPMT-deficient cells, the frequencies of variant alleles were higher than in randomly selected population: 0.03 (*3C) and 0.17 (*3A). Expectedly, the SNP genotype correlated with TPMT activity (Mann-Whitney test; t-unpaired test, in both $p<0.001$). LCLs containing four VNTR alleles, namely AB2C, AB4C, AB5C or A6BC, had significantly lower TPMT activity (Mann-Whitney test; t-unpaired test, $p\leq0.02$) compared to LCLs with other VNTR alleles. The assumption that ABC combinations influence TPMT activity via gene expression regulation was statistically declined. We observed that certain VNTR patterns appeared only in the LCLs with TPMT *3 allele. The calculation of linkage disequilibrium (LD) revealed that AB2C was in LD with *3C allele ($D'=1$, $r^2=1$), meanwhile AB4C and AB5C presence correlated with *3A allele ($D'=1$, $r^2=0.674$ and $r^2=0.250$, respectively).
Our recent findings suggest that particular VNTR alleles in TPMT gene correlate with lower enzymatic activity via LD with two most common *3 alleles. Based on VNTR evolutionary model, we can assume that the *3C variant appeared close to AB2C modification and that the *3B variation occurred jointly with further accumulation of B motives. Additional larger scale population studies are needed to prove the stated phenomenon.

PI-83

Variant rs10757278 in 9p21 region is associated with coronary artery disease as comorbidity with carotid atherosclerosis, in males only

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Genome-wide association studies have recognized the 9p21 region as a significant independent risk region for coronary artery disease (CAD). The single nucleotide polymorphism (SNP) rs10757278, which was designated as the lead SNP in the haplotype block in the 9p21 region, was significantly associated with both CAD and carotid atherosclerosis, in separate studies. Here we aimed to explore the possible association of variant rs10757278 with CAD as comorbidity with advanced carotid atherosclerosis (CA) among patients from the population of Serbia. The study group included 387 (230 males) patients with CA, consecutively admitted for carotid endarterectomy, of which 143 (85 males) had CAD as comorbid disease. Those with previous myocardial infarction or with stable angina pectoris evaluated by selective coronarography that confirmed or revealed coronary artery disease were characterized to have CAD. The rs10757278 was genotyped using TaqMan® technology on 7500 ABI Real-Time PCR. We have found significant association of rs10757278 with CAD in the G allele in a dose-dependent manner, but only among males. The odds ratio for CAD was 1.64 for a single G allele carriers (95% CI 1.02 - 2.63, p = 0.039) and 2.68 (95% CI 1.04 – 6.95, p = 0.039) for GG genotype, after correction for type 2 diabetes, age, hypertensive status, smoking status, alcohol consumption, HDL and TG. In females we didn't find significant association of SNP with CAD (p = 0.7). Preliminary results in this study suggest that variant rs10757278 is associated with CAD as comorbid disease with advanced carotid atherosclerosis in a gender-specific manner. Further, larger studies are needed to resolve the genetic architecture of atheroclerosis and its complex expressions in humans strongly taking into account traditional risk factors.

PII-84

Direct cross-talk between mesenchymal stem cell and glioblastoma cells enhances the expression of the proteolytic enzymes urokinase-type plasminogen activator (uPA) and metalloprotease MMP-9 along with increased invasion of U373 glioblastoma cells

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Glioblastoma multiforme (GBM), the most aggressive brain tumour, is characterized by single GBM cell invasion into the healthy brain tissue. This process is assisted by tumour microenvironment where mesenchymal stem cells (MSCs) have not yet well understood role. MSCs become part of the tumour microenvironment as they are recruited from bone marrow via blood or from tissue niches within brain by tumour secreted signalling molecules (Motaln et al., 2015). Direct cross-talk between MSCs and GBM cells in vitro caused inhibition of GBM cells proliferation and enhanced GBM cells invasion (Schichor et al., 2012). Here, we aimed to confirm the effect of MSCs on GBM invasion *in vitro* as well as *in vivo*. Furthermore, we aimed to reveal if and which proteolytic enzymes, being crucial for GBM cells invasion (Lah et al., 2006), are up-regulated upon direct contact with bone marrow derived MSCs (BM-MSCs). The gene expression level of proteases was measured by qRT-PCR and the protein levels by flow cytometry, immunocytochemistry and Western blot. The invasion of BM-MSCs and GBM cells in direct co-cultures was analysed by 3D-spheroid-assay, using collagen I, laminin and Matrigel as an embedding substrate. To investigate the effect of MSCs on GBM invasion *in vivo*, MSCs and GBM cells were co-injected into the brain of zebrafish embryos. Our results implicate on crucial role of urokinase-type plasminogen activator (uPA) in the direct cross-talk between MSCs and GBM cells. uPA was found to be up-regulated in MSCs-GBM direct co-cultures both at gene and proteins level, compared to GBM cells grown alone. Beside the uPA, the expression of matrix metalloprotease MMP-9 was up-regulated in GBM cells. When U373 eGFP cells were directly co-cultured with MSCs, the invasion of GBM cells was significantly enhanced *in vitro* and also *in vivo*. In contrast, the invasion of directly co-cultured U87 dsRED was impaired *in vitro*. Since qRT-PCR analysis revealed that U87 cells express significantly higher levels of MMPs and uPA receptor than U373 cells, we may conclude that MSCs increase invasion only in poorly aggressive GBM cells. To conclude, our findings suggest that direct contact between MSCs and GBM cells promotes the invasion of some types of GBM cells, via activation of protease signalling cascade involved in degradation of the extracellular matrix proteins. The signalling in direct cross-talk between MSCs and different GBM cell subtypes needs to be elucidated in our future research.

References: Motaln et al., Protein Pept. Lett. 22(4):322-31, 2015; Schichor et al., Exp Neurol. 234(1):208-19, 2012.; Lah et al., Expert Opin Biol Ther. 6(3):257-79, 2006.

PII-85

Kinin receptor expression and activity in co-cultures of mesenchymal stem and glioblastoma cells

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Glioblastoma (GBM), the WHO grade IV glioma is highly proliferative and the most invasive tumour of CNS. Infiltrating mesenchymal stem cells (MSCs) are attracted by GBM cells; however it remains unclear, if MSCs support or suppress GBM progression, as result of cellular cross-talk mediated by chemokines in a paracrine manner or direct contact. Kinin or bradykinin receptors (BR) are expressed by the bone marrow (BM)-MSC and U87-MG GBM cells, however, the mechanism of the observed expression upregulation and activation of BR1 and BR2 in GBM during tumor progression is poorly understood. The present work aims to elucidate the activity, expression and the role of both BRs as a result of GBM:BM-MSC cell interaction

The human GBM cell line U87-MG was stably transfected with dsRed protein, single cell cloned, expanded and co-cultured with human BM-MSCs cultures at equal cell number ratio for 1-9 days. Validation of mRNA expression by qRT-PCR, flow cytometry, immunocytochemistry and calcium imaging were used for expression and activity studies of BRs. Scepter cells counter and flow cytometry analysis were employed for proliferation. Cell viability was determined with the MTT assay at increasing BR agonist and antagonist concentrations.

We demonstrated that the direct cellular interactions significantly increased expression and activity of B1R as confirmed by quantitative assessment using flow cytometry and immunocytochemistry. Direct cell complexes also differentially provoked $[Ca^{2+}]i$ oscillations, indicative of increased des-Arg-bradykinin binding to B1R and bradykinin binding B2R and its downstream signalling in U87-MG cells. Treatment with antagonist provoked toxicity at about 30 μM. Interestingly, in the co-cultures with MSC, the cells were more resistant against the toxic effects of BR antagonist. Direct cellular interactions significantly increased proliferation of U87-MG after 3-5 day in 3D spheroids.

Taken together, this data corroborate the hypothesis that MSCs in direct contact with the tumor, would increase GBM cell stability and previously reported migration possibly *via* kinin- B1 and B2 receptors signalling, both being activated in GBM cells. Further research is needed to elucidate the mechanisms of BRs upon GBM:MSC interactions, possibly leading to a more informed and safe cell therapy in glioma in the future.

PII-86

Paracrine effects of mesenchymal stem cells on differentiation of glioblastoma stem-like cells

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Glioblastoma multiforme (GBM) remains an incurable disease due to often tumour recurrence and resistance to conventional chemo/radiotherapy. One of the reasons may be the hypothetical chemoresistance of a subpopulation of highly tumorigenic GBM stem-like cells (GSLCs), residing within a bulk tumour mass. Mesenchymal stem cells (MSC) exhibit an intrinsic ability to migrate towards tumours and secrete numerous chemokines and cytokines to modulate tumour characteristics.

Thus we aimed to evaluate the impact of MSC's paracrine factors on proliferation and differentiation of GSLCs, either exposed to the MSC conditioned media or indirectly co-cultured with MSCs. Here, we exposed the two GSLC lines (NCH421k, NCH644) and two primary GBM cell lines enriched for GSLCs (NIB-26 and NIB-50) to the umbilical cord (UC)- and bone marrow (BM)-derived MSCs conditioned media for 72h. Additionally, the NCH421k cells were indirectly co-cultured with BM and UC-MSCs.

We demonstrated a decrease of proliferation, associated with simultaneous down-regulation of cyclin D1 gene expression in all GSLC lines after exposure to BM-MSC and UC-MSC conditioned media. Moreover, a significant increase of β-galactosidase activity in GSLCs was detected upon exposure to both types of MSCs conditioned media and activation of senescence was further confirmed by p21 and p16 gene up-regulation. In all GSLC lines exposed to conditioned media of BM and UC-MSCs or when the cells were indirectly co-cultured with BM and UC-MSCs, a change in expression profile of stemness cell markers was evidenced, i.e. down-regulation of marker Sox-2 and up-regulation of the differentiated astrocytes cell markers, vimentin and GFAP.

Taken together, our data demonstrate the MSC's potential to induce senescence and affect the GSLCs stemness by shifting them towards more differentiated and less malignant phenotype. These findings support the notion that MSCs possess a favorable antitumor ability to modulate the GSLC characteristics and represent a promising strategy for glioblastoma treatment.

Reference: Kološa et al., Cell Transplant, 2015.

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PII-87

Nonpolar bases hexylamine and trihexylamine are powerful antioxidants in lipid systems

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Products of oxidation of unsaturated fatty acids are highly reactive molecules that display marked biological effects. They can be formed *in vivo* or in various food matrices. Both dietary and endogenously formed lipid peroxides and their degradation products are toxic compounds. We have analyzed the influence of nonpolar bases hexylamine (HA) and trihexylamine (THA) on the formation of hydroperoxides in vegetable oils and model lipid systems. HA and THA in millimolar range attenuated formation of peroxides and improved stability of tocopherols in vegetable oils at 60°C. Experiments in model systems revealed that THA is an efficient antioxidant even in the absence of tocopherols. Both, HA and THA acted synergistically with γ-tocopherol, whereas synergism was not observed in the presence of α-tocopherol. The synergistic effect could be attributed to the increase in proticity of nonpolar solvents in the presence of THA and HA that results in higher rate of reaction of γ-tocopherol with DPPH radical.

PII-88

Characterization of organic cation transporters in zebrafish (*Danio rerio*)

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Uptake transporters represent integral elements in the ADME (Absorption, Distribution, Metabolism, Excretion) processes and toxicological response. They play crucial role in the initial phase of cellular metabolism of xeno- and endobiotics by mediating the uptake of compounds across the cell membrane. The majority of uptake transporters belong to the SLC (Solute Carrier) protein superfamily which is one of the largest known protein superfamilies. Organic cation transporters (OCTs) are members of SLC22A family. There are three human OCT members, OCT1-3. OCT1 and OCT2 are dominantly expressed in liver and kidney, respectively. OCTs are localized in basolateral membranes of blood-tissue barrier cells and they are responsible for polyspecific transport of numerous organic cations. Using phylogenetic and synteny analysis of previously identified two members of zebrafish Octs, we determined orthological relationship with human and other vertebrates Octs. We also determined dominant expression of Oct1 in zebrafish kidney and liver, whereas Oct2 showed lower but ubiquitous expression. Functional characterization was performed using newly identified fluorescent Oct1 substrates, which were used as a tool for determination of interactions with various environmentally and physiologically relevant compounds. Initial interaction screen with ASP+ as fluorescent substrate and numerous tested compounds revealed steroid hormones as potent interactors of Oct1, with the highest inhibition constants for progesterone (K_i = 2 μM), androstenedione (5.3 μM) and testosterone (13.2 μM). Among environmental contaminants Oct1 showed high interaction with organotin compounds, especially with dibutyltin and tributyltin chloride with the lowest K_i values of 0.4 and 3.9 μM, respectively. We also modeled tertiary structure of Oct1 and performed molecular docking analysis with model substrates and determined interactors, revealing crucial amino acid residues within the Oct binding region. In conclusion, this study represents the first functional characterization of organic cation transporters from Slc22 family in fish. The obtained knowledge provides new insight into possible roles of Octs in crucial physiological, defensive and pathological processes in zebrafish, enabling a more reliable use of zebrafish in understanding of OCT/Oct functions in higher vertebrates and human.

PII-89

Analysis of changes in neutrophil extracellular trap (NET) proteins profile using proteomic methods

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Neutrophils are the most abundant leukocytes in plasma. Mature neutrophils act as the first line of the innate immune system migrating to the site of infection in response to the microbial invasion. They are able to attack pathogens directly in three different ways: phagocytosis, release of antimicrobial peptides and neutrophil extracellular trap (NET) generation. During activation, neutrophils produce reactive oxygen species (ROS) through NADPH oxidase required for neutrophil extracellular trap formation. NET is the result of a unique form of cell death in which neutrophils eject their mixture of nucleoplasm and cytoplasm components into the extracellular space forming a web-like structure. Hereby the invaded pathogens are trapped, neutralized and therefore their dissemination is inhibited. A number of antimicrobial proteins (such as azurocidin, lactoferrin) and proteases (such as neutrophil elastase, myeloperoxidase and cathepsin G) are essential constituents of the NET contributing not only to the direct microbial activity but also participating in the proteolytic process, therefore the viability of pathogens can be diminished.
Our aim was to characterize the protein crosslink profile alterations during NET generation upon the effect of different treatments. One of our hypotheses was that polyamines are able to undergo chlorination in the presence of hypochloric acid generated by myeloperoxidase in activated neutrophils. These reactive polyamines might be able to react with proteins resulting in crosslinked peptides. Another hypothesis was that the crosslink formation among NET proteins might be catalyzed by transglutaminase. NET proteins were identified by LC-MS/MS based mass spectrometry analysis and using the MS/MS data, the site and the type of crosslinks were identified using the StavroX protein cross-link examination software. The results indicate that most probably both mechanisms might contribute to the generation of protein crosslinks during NET formation.

PII-90

Anticancer potential of steroidal 16,17-seco-16,17a-dinitrile derivatives against triple negative MDA-MB-231 breast cancer cells

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Breast cancer is among the most widespread diseases with a fatal outcome. About 10–20% of invasive breast cancers belong to triple-negative breast cancers (TNBC). These cancers demonstrate the absence of estrogen (ER) and progesterone (PR) receptors, as well as human epidermal growth factor receptor 2 (HER2). This aggressive subgroup of breast cancer is resistant to existing hormonal therapy, due to deficiency of the appropriate hormone receptors. Consequently, TNBC are managed with standard treatment; however, such treatment leaves them associated with a high rate of local and systemic relapse. Defects in the apoptotic pathways are recognized as crucial in many pathological processes, including cancer. Targeting apoptotic signaling pathways in treatment of different cancer types is one therapeutic strategy, while others are targeting the cell cycle or selective induction of necrosis. Searching for new effective anticancer drugs includes a broad spectrum of research. A new drug candidate, except antiproliferative effect, should also have good selectivity and low system toxicity. Some steroidal compounds, originated from natural sources or synthetic ones, seem to be promising anticancer drugs. Secosteroids are an important group of modified steroids. Studies on secosteroids showed that modification of the rigid steroidal carbon skeleton by cleavage of the internal C-C bond provides more flexible compounds with new biological properties. Having in mind all of these facts, antiproliferative and pro-apoptotic potential of selected steroidal 16,17-seco-16,17a-dinitrile derivatives was studied. All tested compounds decreased proliferation of MDA-MB-231 breast cancer cells. They also affected the cell cycle distribution and induced apoptosis and /or necrosis in the same cell line. Almost all compounds increased expression of proapoptotic Bax protein and decreased expression of antiapoptotic Bcl-2 protein. Cleavage of PARP protein confirmed the completion of apoptotic process in all treated samples after 72 h of exposure. Caspase 3 activation was found in some samples, suggesting induction of apoptosis in a caspase-dependent manner. Results showed that tested secosteroids have a substantial biomedical potential and could be candidates for anticancer drug development, especially having in mind lack of the influence on the healthy cells.

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PII-91

Metformin enhances protective signalling responses but fails to improve survival of cultured myotubes exposed to ischemia and reperfusion

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Ischemia-reperfusion injury, a common complication of surgical procedures, can lead to muscle fiber necrosis, prolonged postoperative recovery, and life-threatening complications like the crush syndrome. The extent of injury might be mitigated by preconditioning skeletal muscle with pharmacological agents that target energy metabolism. The AMP-activated protein kinase (AMPK), an energy sensor that protects against energy depletion, is a particularly attractive target to develop novel strategies for limitation of ischemia-reperfusion injury in skeletal muscle. We explored whether metformin, the most widely used drug for type 2 diabetes and an AMPK activator, might protect skeletal muscle against ischemia-reperfusion injury.

To test effects of metformin, we used cultured myotubes obtained from rat L6 skeletal muscle cells. Since metformin activates AMPK by targeting mitochondria, we first assessed mitochondrial function of L6 myotubes. FCCP, an uncoupler of oxidative phosphorylation, increased phosphorylation of AMPK and phosphorylation of its downstream target acetyl-CoA carboxylase. FCCP-induced AMPK activation indirectly demonstrates that L6 myotubes have functional mitochondria. We also determined that L6 myotubes express metformin uptake transporter OCT1 and that acute metformin exposure induces dose-dependent AMPK activation, which demonstrates that L6 myotubes are responsive to metformin. To explore whether metformin protects against ischemia-reperfusion injury, L6 myotubes were pretreated with metformin for 4 days and then exposed to simulated ischemia and reperfusion. Metformin pretreatment suppressed lactate production and promoted AMPK activation. Furthermore, up-regulation of hypoxia-inducible factor-1α, a master regulator of cellular oxygen homeostasis, was markedly enhanced in ischemic myotubes. These results suggest that metformin promotes protective signalling responses. However, metformin failed to suppress ischemia-induced release of lactate dehydrogenase, an indicator of cellular necrosis, indicating that enhanced signalling responses did not translate into improved survival of L6 myotubes.

In sum, we show that metformin enhances signalling responses that protect against energy and oxygen depletion, but fails to improve survival of cultured myotubes. Collectively, our results suggest that pharmacological preconditioning with metformin would not be an effective strategy for limitation of ischemia-reperfusion injury in skeletal muscle.

PII-92

The effect of various antioxidants in cell death-related oxidative stress

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The pool of reactive oxygen species (ROS) are normally mitochondria and peroxisomes. When the ROS production is superfluous and cellular antioxidant defence system is disrupted, oxidative stress occurs, leading to energy metabolism impairment and cell death. Effective cellular antioxidant systems can be complemented by exogenous antioxidants during therapy.
In our study we wanted to clarify the role of ROS produced during four different types of cell death:

- necrosis, induced by menadione (MD),
- intrinsic apoptosis, triggered by staurosporine (STS),
- extrinsic apoptosis, induced by tumour necrosis factor-α in combination with cycloheximide (TNF-α CHX), and
- necroptosis, triggered by co-treatment with tumour necrosis factor-α, cycloheximide and caspase inhibitor (TNF-α zVAD).

Moreover, we tested the capability of antioxidants to affect induced oxidative stress in different cells and to resolve the exact effect of antioxidants that might be beneficial in some therapeutic approaches. Therefore, cells´ media was enriched by the selected antioxidants: NAC, α-tocopherol, MnTBAP and tempol.
According to our results we can conclude that MD, STS and TNF-α treatment caused oxidative stress, followed by cell death. All tested antioxidants showed scavenging capacity independently of the trigger. However, cell viability was not always restored by antioxidant usage. NAC seemed to be more efficient antioxidant in comparison to α-tocopherol and MnTBAP. On the other hand, tempol usage is tricky due to its low antioxidant potential and detected contrary action. With this study we would like to put forward that tempol is often used carelessly and its effect should be re-evaluated. Nevertheless, tempol appears to have a great potential for being used in co-treatment with other chemotherapeutics.

PII-93

Orthocaspases: the proteolytically active prokaryotic caspase homologues

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Caspases are a family of cysteine-dependent proteases known to be involved in the process of programmed cell death in metazoans. Two homologous families belonging to caspase-like superfamily of proteases have been identified so far and were termed metacaspases (found in plants, yeasts and protozoa) and paracaspases (found in metazoa and slime molds). All share the characteristic p20 caspase subunit, which contains the catalytic His/Cys dyad. Recently, bacteria were also found to contain caspase-like homologs, but their existence has only been identified *in silico* up to now. Performing a sequence homology search against *Microcystis aeruginosa* PCC 7806 protein sequence database we have identified six putative caspase-like genes (*MaOC1* to *MaOC6*). We have expressed the *MaOC1* gene in *E. coli* and shown that it encodes for a 78 kDa protein. Kinetic characterization has shown that MaOC1 is an endopeptidase with a preference for arginine in the P1 position and a pH optimum of 7.5. MaOC1 exhibited high catalytic rates with the k_{cat}/K_M value for Z-RR-AMC substrate of the order of 10^6 $M^{-1} \cdot s^{-1}$. In contrast to metacaspases, whose activity is calcium-dependent, or paracaspases that need dimerization for activation, MaOC1 was activated by autocatalytic processing after residue Arg219, which separated the small p20-like domain and the remaining 55 kDa subunit. The Arg219Ala mutant was resistant to autoprocessing and exhibited no proteolytic activity, confirming that processing of MaOC1 is a prerequisite for its activity. Due to their structural and functional differences to other known caspase-like proteins we suggest to name these evolutionary primitive proteins orthocaspases.

PII-94

Novel nuclear specific regulatory role of LIN28A protein in pluripotent stem cell lineage acquisition

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Over recent years, the highly conserved LIN28A RNA-binding protein has emerged as a factor that defines stemness in several tissue lineages and is even one of four factors that convert human fibroblasts into induced pluripotent stem cells. Although originally identified as a cytoplasmic protein, LIN28A protein has been observed to shuttle between the nucleus and the cytoplasm. However, its localization still remains unclear. Recently, methyl-transferase SETD7 has been identified as a post-translational modulator of LIN28A protein, resulting in increased protein stability and nuclear retention with preferential localisation in the nucleoli. Importantly, little remains known of nuclear function of LIN28A.
Using CRISPR/Cas technology, we generated a stable human pluripotent stem cell (PSC) line expressing fusion protein LIN28A-eGFP. This reporter line remained equally competent at producing differentiation progenitors and did not significantly alter protein stability. Modification was easily detected using western blotting, flow cytometry and microscopy, enabling us to quantify LIN28A-eGFP protein level during early lineage specific stem cell differentiation. Although exposure of PSCs to differentiation conditions does not lead to reduction of LIN28A protein level, we observed nucleolar translocation of LIN28A upon early mesodermal differentiation following exit of pluripotency. We performed total RNA-sequencing for analyzing the changes in the amount of transcripts in immediate-early differentiation time points and observed that SETD7 methyltransferase is not expressed in pluripotent stem cells and gets upregulated first upon exit of pluripotency. To elucidate the nuclear role of LIN28A we performed co-immunoprecipitation and iCLIP (individual resolution Crosslinking and Immunoprecipitation) experiments, independently on nuclear and cytoplasmic subcellular fractions. These revealed distinct cytoplasmic vs. nuclear protein interaction network and novel RNA targets of LIN28A, demonstrating that upon differentiation, LIN28A obtains an important nuclear specific regulatory role governing early pluripotent stem cell transition.

PII-95

Conserved functions at the domain level during the uni-multicellular transition

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Multicellularity arose from unicellular ancestors at least 10-15 times. Systematic large-scale sequence-based comparative studies of this transition focus on changes. For example, Pincus *et al.* (PNAS 2008) measured the emergence (during the uni-multicellular transition) of a group of three signaling domains that read, write and delete a molecular signal. As a contrast, here we focus on constant functions throughout the transition. We investigate with PFAM and GO all protein domains in 9 organisms that are close to the base of the Metazoa. Many of these organisms have both uni- and multicellular lifestyles. We find a group of 48 PFAM domains to be present in all 9 organisms and a smaller group (10 domains) to be most frequently co-appearing pairwise in the proteins of all 9 organisms. The statistically most significant shared Gene Ontology (GO) functions of these 48 protein domains are related to metabolism, its control, and the binding of DNA, GTP and ATP. Interestingly, signaling, which is key to cell-cell communication is underrepresented in our results. We are now performing further analyses to identify the reason for this result.

PII-96

Photosensitizing effect of PARP inhibitor PJ-34 in a UVA irradiated human epidermoid carcinoma cell line model

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Photodynamic therapy (PDT) is a popular treatment that selectively destroys tumor or other target cells. The process involves a photosensitizing agent that is activated by light to produce reactive oxygen species (ROS). One type of PDT is the PUVA therapy which is the combination of a psoralen and UVA irradiation and is used in psoriasis treatment for example. Poly(ADP-ribosyl)ation is a protein modification carried out by members of the PARP [poly(ADP-ribose) polymerase] enzyme family. One of the most important functions of PARP-1 is to assist in DNA repair in case of single strand DNA breaks. Photosensitizer reactions are known to alter cellular function via generation of ROS, which can induce DNA damage. We set out to investigate the role of PARylation in PUVA-induced cell death of epidermoid carcinoma cells. We found that PARP-1 silencing or pharmacological inhibition of the enzyme activity with a potent PARP inhibitor Veliparib had no effect on viability of A431 (human epidermoid carcinoma cell line) treated with 8-methoxypsoralen (8MOP) and UVA (2.5 J/cm^2) irradiation, indicating that PARP-1 is not a major player in PUVA-induced cell death. Interestingly another commonly used PARP inhibitor PJ-34 increased the toxic effect of 8MOP+UVA and it had a photosensitizing effect with equal potency as 8MOP. Characterization of this phototoxicity showed that PJ-34 triggers overproduction of ROS, induces single and double strand breaks of DNA, increases the activity of caspase-8 and caspase-3 and causes excessive DNA fragmentation. PJ-34+UVA induced cell death could not be prevented by antioxidants (ascorbic acid and Trolox) in our model, but could be significantly suppressed by caspase-8 and caspase-3 inhibitors. In conclusion, PARP inhibitor PJ-34 acts as a photosensitizer and induces caspase-mediated apoptosis which is at least partially independent of its PARP-1 inhibitory activity.

PII-97

Altered development of adipose tissue may contribute to the decreased tolerance to cold exposure of tissue transglutaminase knock-out mice

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Adipose tissue is a specialized connective tissue that functions as the major storage site for fat in the form of triglycerides. It is present in mammals in two different forms: white adipose tissue (WAT) and brown adipose tissue (BAT), with WAT being more abundant. In rodents, prolonged cold exposure can provoke the appearance of "beige" adipocytes with brown fat-like characteristics within WAT. These inducible cells support cold tolerance of these animals by heat generation.
As our knowledge on adipose tissue is continuously growing, new insights into normal physiological regulation as well as new strategies for treatment of obesity and its related disorders may be expected. Tissue transglutaminase (TGM2) can be a notable target for investigation on adipose tissue development as the expression pattern for TGM2 suggests that it promotes differentiation of clinically important cell types. To substantiate a potential role of TGM2 in adipogenesis, we have studied its effects on *in vivo* adipose tissue formation in mouse models. Although wild type and TGM2 knockout (KO) mice produce the same amount of adipose tissue, we have found that the expression of uncoupling protein-1 (UCP-1) is significantly lower in gonadal WAT of KO animals. The brown and "beige" marker UCP-1 catalyzes a proton leak across the inner mitochondrial membrane, thus "uncoupling" fuel oxidation from ATP synthesis and generating heat. In addition, we have detected significantly different expression of two important marker genes (decreased CIDEA and increased PRMD16) in BAT of KO mice. Altered development of adipose tissue is also reflected in the significantly lower tolerance to cold exposure of KO animals compared to wild-type mice. We also investigate tissue characteristics of animals such as size and density of both adipocytes and blood vessels in WAT and BAT.

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PII-98

Effects of individual and combined treatment of Raloxifene and estrogen on apoptosis in human endometrial stromal ThESC cell line

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Stimulation of cells proliferation and differentiation by steroid hormones is one of the crucial regulatory points in the development of life and diseases as well. In physiological milieu, estrogen stimulates growth and inhibits apoptosis through estrogen receptor-mediated mechanisms in estrogen dependent tissues. The key regulation between life and disease is coordinated via apoptotic processes. Local high level of estrogen, in the absence of progesterone, results in the development of osteoporosis, breast tumors and endometrial hyperplasia. There are strong evidences suggesting that estrogen switches from being a proliferative agent to paradoxically cell growth inhibitor and inducer of apoptosis in various cancer cells. Selective estrogen receptor modulators, such as Raloxifene, are drugs commonly used in the treatment of endometrial hyperplasia. Because of its antagonistic effect on uterus, Raloxifene inhibits the growth of cell lines derived from the uterus. There are no experimental data demonstrating the direct apoptotic effect of both Raloxifene and estrogen on endometrial stromal ThESC cell line. The aim of this work was to investigate both cytotoxic and apoptotic mechanisms of Raloxifene and estrogen induced death in ThESC cell line. In order to determine their cytotoxic and apoptotic effects, various doses and treatments of both Raloxifene and estrogen were applied on the ThESC cell line and their effect investigated by MTT assay, FACS analysis and immunofluoroscence methods. Our results demonstrated that combined application of investigated substances induced strong cytotoxic effect and induction of apoptosis compared to their single effects in ThESC cell line. Apoptotic effects of investigated substances were carried out by activating the mitochondrial apoptotic pathway through decreasing Bcl-2 expression and activation of BAX and caspase 3.

PII-99

The TAF10-containing TFIID is necessary for the ecdysone induced larval-pupal transition of *Drosophila melanogaster*

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In eukaryotes, transcription is a tightly controlled process consisting of several regulatory steps. Protein complexes with versatile functions participate at different steps in transcription: the TFIID complex is required in preinitiation complex assembly for positioning the RNA polymerase II around the transcription start sites of different core promoters. On the other hand histone acetyltransferase complexes like SAGA and ATAC, modulate transcription at several steps through modifications of specific core histone residues.
In this study we investigated the function of *Drosophila melanogaster* (d)TAF10 and TAF10b proteins which are subunits of dTFIID and dSAGA, respectively. We generated a mutation which eliminated the production of both *Drosophila* TAF10 orthologues. The simultaneous deletion of the two d*Taf10* genes impairs the recruitment of a dTFIID subunit dTAF5 to polytene chromosomes, while binding of another TFIID subunit, dTAF1 and RNAPII seems not to be affected. This suggests that in the absence of dTAF10 proteins, functional dTFIID complex can be formed. The lack of both dTAF10 proteins results in failures in the larval-pupal transition during metamorphosis and in the transcriptional reprogramming at this developmental stage. Surprisingly, unlike dSAGA mutations, mutations of a dATAC subunit result in very similar changes in the steady state mRNA levels on approximately 5000 genes than does the ablation of both d*Taf10* genes, indicating that dTAF10- and/or dTAF10b-containing complexes and dATAC affect similar pathways.
Importantly, the phenotype resulting from d*Taf10+dTaf10b* mutation can be rescued by ectopically added ecdysone, suggesting that dTAF10- and/or dTAF10b-containing complexes are involved in the expression of ecdysone biosynthetic genes. Indeed, in d*Taf10+*d*Taf10b* mutant flies the cytochrome genes (*spookier, phantom, disembodied,* and *shadow*) involved in steroid conversion in the ring gland are down-regulated, while *shade*, the product of enzyme, which converts ecdysone into its active form (20-OH ecdysone) outside of the ring gland is up-regulated. Our data supports the idea that the presence of dTAF10 proteins in dTFIID and/or in dSAGA is required only at specific developmental steps. We propose that distinct forms of dTFIID and/or dSAGA exist during *Drosophila* metamorphosis, wherein the different TAF compositions could serve in targeting the RNAPII at different developmental stages and tissues.

PII-100

Oleuropein ameliorates cisplatin-induced oxidative damage, inflammation and apoptosis in mice kidneys by modulating CYP2E1, NF-κB and ERK1/2 expression

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Oleuropein, a phenolic compound isolated from the leaves and fruits of the olive tree (*Olea europea* L.), has been shown to possess numerous pharmacological activities. The aim of the current study was to investigate the renoprotective effects of oleuropein against cisplatin (CP)-induced kidney injury. Male BALB/cN mice were gavaged daily with 5, 10 or 20 mg oleuropein/kg body weight for two days, 48 h after intraperitoneal injection of CP (13 mg/kg). Four days after CP administration, serum creatinine and blood urea nitrogen (BUN) levels were significantly elevated, with histopathological changes in renal tissue. Oxidative stress in the kidneys was evidenced by overexpression of 3-nitrotyrosine (3-NT), 4-hydroxynonenal (4-HNE), cytochrome P450 E1 (CYP2E1) and heme oxygenase-1 (HO-1). The expression of nuclear factor-kappaB (NF-κB) p65, tumor necrosis factor-alpha (TNF-α) and cyclooxygenase-2 (COX-2) in the kidneys increased upon CP treatment, suggesting renal inflammation. CP intoxication increased expression of p53, Bax and active caspase-3, indicating apoptotic cell death, which was further confirmed by the terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay. CP administration resulted in enhanced phosphorylation of extracellular signal-regulated kinases 1 and 2 (ERK1/2), involved in CP-induced renal cell apoptosis, but not c-Jun N-terminal kinases 1 and 2 (JNK1/2) and p38 mitogen activated protein kinase (MAPK). All these effects were dose-dependently diminished by oleuropein treatment. The results of the current study suggest that oleuropein attenuates CP-induced oxidative stress, inflammation and apoptosis at least in part through the suppression of CYP2E1 and NF-κB overexpression and the reduction of ERK1/2 phosphorylation.

PII-101

Epimer-specific effects of bile acids on the anti-neoplastic activity of doxorubicin in MCF-7 breast adenocarcinoma cells

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Bile acids (BAs) are endogenously produced amphiphatic molecules. In addition to transport-facilitating properties of various xenobiotics across biological membranes, BAs emerged as signalling molecules that regulate cell homeostasis and death. These properties of BAs are determined by their hydrophobicity, number and stereo-orientation of hydroxyl groups on the steroid nucleus. The aim of our study was to characterize the differential effects of two BA epimers, chenodeoxycholic (CDCA) and ursodeoxycholic acid (UDCA), on modulation of doxorubicin (DOX) induced cytotoxic activity and apoptosis signalling in MCF-7 breast adenocarcinoma cell line.
Cytotoxic effects determined using the colorimetric MTT assay revealed that treatment with CDCA and UDCA alone exerted the inhibition of cell growth in a dose dependent manner, with IC_{50} values 22.5 μM and 321.0 μM, respectively. The co-incubation of cells with 0.25 μM of DOX and non-toxic (IC_{20}) concentrations of BAs over 48h resulted in statistically significant increase of cytotoxicity: co-treatment with UDCA and CDCA increased the cytotoxic effect up to 39.6% ($p<0.05$), and 67.9% ($p<0.01$), respectively, compared to DOX treatment alone, which inhibited growth in 33.1% of cells. Quantitative analysis of dose-effect relationship using CompuSyn software by determining the combination index (CI) value indicated that combination treatment with UDCA resulted in weak antagonistic effect (CI=1.2), whereas with CDCA in strong synergistic activity (CI=0.18) with DOX. In accordance to this, we observed the significant changes in the expression of mitochondrial pro-apoptotic Bax and anti-apoptotic Bcl-2 mRNA levels and the increase in Bax/Bcl-2 ratio in both groups co-treated with BAs, compared to DOX alone. However, more pronounced mitochondrial apoptosis-promoting effects were associated with CDCA co-treatment.
These results indicate that both BAs modulate the cytotoxic activity of DOX and increase the susceptibility of MCF-7 cells to undergo a mitochondrial-initiated apoptosis, however, on an epimer-specific pattern. Given that CDCA synergistically acts with DOX, CDCA may be further investigated as a novel chemo-sensitizing agent with the primary aim to improve the therapeutic response to DOX-containing chemotherapy regimens, while potentially decreasing its harmful effects.
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PII-102

The role of Hmgb1 in the unique transcriptional mechanism of the matrilin-1 gene and the early step of chondrogenesis

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The architectural high mobility group box 1 (Hmgb1) protein acts as both a nuclear and an extracellular regulator of various biological processes including skeletogenesis. We reported its contribution to the evolutionarily conserved, distinctive regulation of the matrilin-1 gene expression in amniotes. We previously demonstrated that uniquely assembled proximal promoter elements restrict Matn1 expression to specific growth plate cartilage zones by allowing varying doses of L-Sox5/Sox6 and Nfi proteins to fine-tune their Sox9-mediated transactivation. To shed more light on its role in chondrogenesis, we studied the effect of Hmgb1 on the Sox-mediated cartilage-specific regulation using the Matn1 control region as a model system.
In early steps of chondrogenesis, declining Hmgb1 expression overlaps with the onset of Sox9 expression. Unlike repression in late steps, Hmgb1 overexpression in early chondrogenesis increases Matn1 promoter activation by the Sox trio, and forced Hmgb1 expression in COS-7 cells facilitates induction of Matn1 expression by the Sox trio. The conserved Matn1 control elements bind Hmgb1 and SOX9 with opposite efficiency in vitro. They show higher HMGB1 than SOX trio occupancy in established chondrogenic cell lines, and HMGB1 silencing greatly increases MATN1 and COL2A1 expression. Together, these data thus suggest a model whereby Hmgb1 helps recruit the Sox trio to the Matn1 promoter and thereby facilitates activation of the gene in early chondrogenesis. We anticipate that Hmgb1 may similarly affect transcription of other cartilage-specific genes. We suggest a model for contribution of Hmgb1to the activation of the gene at the onset of chondrogenesis in amniotes.

These results constituted the major part of my PhD thesis supervised by Dr. Ibolya Kiss at the Institute of Genetics, BRC, Hungarian Academy of Sciences, Szeged.

PII-103

Role of gamma-enolase in tumor cell survival in starvation and hypoxia: regulation by cysteine protease cathepsin X

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Gamma-enolase is an enzyme of the glycolytic pathway, catalyzing conversion of 2-phosphoglycerate to phosphoenolpyruvate, a process which is enhanced in tumor cells. In neuronal cells it was shown also to promote cell survival by activating specific signaling pathways. This function is manifested through an additional active site, localized in the C-terminal part of the molecule. Cathepsin X, a cysteine carboxypeptidase, was shown to sequentially cleave amino acids from the C-terminal end of gamma-enolase and to abolish its function. Both molecules are abundantly present in tumor cells and therefore, this interaction could affect their survival in stressful conditions within the tumor microenvironment.

To prove that, we cultured different cancer cells lines in serum starvation media and in media with hypoxia mimetic desferrioxamine and evaluated the levels of C-terminally uncleaved gamma-enolase in cell lysates by western blotting. In all cell lines, starvation or hypoxia mimicking conditions significantly upregulated the levels of uncleaved gamma-enolase. The up-regulation was even more evident in the presence of the cathepsin X specific inhibitor AMS-36 or in cells silenced for cathepsin X by siRNA, which both significantly increased cell survival. The co-localization of the molecules within the cells was observed also by confocal microscopy.

Our results demonstrate that C-terminally uncleaved gamma-enolase promotes survival and adaptation of cancer cells to stressful conditions and that cysteine protease cathepsin X regulates its function.

PII-104

A dual recognition mechanism of flagellin by cytosolic and membrane sensors

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Flagellin, the main structural protein of bacterial flagella and a virulence factor of several human pathogens, is recognized by the innate immune system via two separate pathways. The cytosolic NAIP5-NLRC4 inflammasome evokes an immune response to the 35 C-terminal amino acids of flagellin triggering secretion of inflammatory cytokines, while the membrane-bound Toll-like receptor 5 (TLR5) has been shown by crystal structure studies to interact with flagelllin through distinct interaction surfaces within the conserved D1 domain of flagellin and evoke an immune response via the NF-κB pathway. However, the identified interactions between flagellin and TLR5 are not sufficient for receptor activation, as deletion of the D0 domain completely abrogates signaling. To investigate the role of the D0 domain of flagellin in TLR5 activation we used deletion constructs to identify the C-terminal region of D0 as crucial for TLR5 activation, while the N-terminal D0 seems to have a disposable role. Further, by the use of alanine scanning mutagenesis we have identified several conserved amino acid residues in the CD0 domain to play a crucial role in TLR5 activation, including a terminal hydrophobic region previously reported to be essential for the assembly and activation of the NLRC4 inflammasome. Our results show, in contrary to previous belief, that while TLR5 recognition is targeted to a larger segment of flagellin, the same region crucial for inflammasome assembly is in fact also necessary for the activation of the membrane receptor TLR5, pointing to a dual recognition mechanism of this highly conserved region of flagellin by the host immune system.

PII-105

Susceptibility of human antimicrobial peptides to the action of aspartic proteases secreted by pathogenic yeast *Candida albicans*

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Candida albicans belongs to the most common fungal pathogens of humans. The versatility of the pathogenic potential of this fungus results from its ability to colonize different niches in the host organism and to cope with the host immune defense. Among numerous virulence factors used by *C. albicans* to infect the human host, major roles are attributed to a family of ten secreted aspartic proteases (Saps) that exhibit a broad substrate specificity and are active in both neutral and acidic environment.
During the infections, reactions of the human innate immune system are activated, one of the earliest events being the secretion of an array of antimicrobial peptides (AMPs). Taking into consideration a constantly growing attention of researchers to the contribution of AMPs to the host defense against fungal infections, our current study was undertaken to verify a hypothesis that the diverse group of ten Saps can neutralize the antifungal activity of the major human AMPs, representing all main classes of the mechanism of antimicrobial activity and differing in the mode and place of their synthesis.
We found that major groups of human AMPs significantly differed in the susceptibility to Sap action over a broad range of pH, reflecting different niches of the host that are colonized by *C. albicans*. Among all investigated classes of AMPs, only α-defensins (HNP-2) were resistant to the proteolytic action of Saps, while an α-helical, cationic peptide LL-37 and an anionic peptide dermicidin (DCD-1) were effectively cleaved by Sap1-4, Sap8 and Sap9, whereas a histidine-rich peptide histatin-5 was degraded by Sap1-4 and Sap7-9. Of degradable AMPs, the lowest susceptibility to Sap action was observed for DCD-1 while LL-37 was cleaved most rapidly. On long-time scale, the peptides were completely degraded and lost their antimicrobial potential but after short times of Sap treatment, the peptides were released that still possessed fungicidal properties.
Our results provide a new insight into the mechanisms that fungal pathogens exploit to modulate or evade the innate immunity of the host.

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PII-106

Crucial role of cyclophilin D in the pathogenesis of LPS-induced acute lung injury

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Gram negative sepsis caused by lipopolysaccharide (LPS) component of bacteria is characterized by an intense systemic inflammatory response with high mortality rate. This systemic process also affects the lungs causing acute respiratory distress syndrome (ARDS). The role of mitochondrial dysfunction and consequential reactive oxygen (ROS) and nitrogen species (RNS) production have already been proposed in the pathogenesis of sepsis, however the exact molecular mechanism is poorly understood. Mitochondrial permeability transition pore (MPTP) is a multicomponent transmembrane channel that opens upon stimuli like ROS or cytosolic Ca^{2+} overload, and causes free diffusion of solutes under 1.5 kDa between the mitochondrial matrix and the cytosol. Consequential dissipation of the mitochondrial membrane potential enhances mitochondrial ROS production contributing to the pathogenesis of acute lung injury. The importance of MPTP opening has been studied in several pathological conditions like cardiovascular and neurodegenerative diseases, however its role in the pathogenesis of LPS-induced lung injury has not been evaluated yet.
In our present study we investigated LPS-induced lung injury in mice lacking cyclophilin D (CypD), an indispensable component of MPTP. Acute lung injury was induced by intraperitoneal LPS injection. 24 hours after LPS challenge CypD-deficient (KO) mice exhibited less severe histological damage of the lungs. Immunohistochemistry revealed significantly attenuated nitrotyrosine and 4-hydroxy-2-noneal Michael adduct formation in KO mice after LPS treatment, both important markers of oxidative damage. As a result of the lower oxidative milieu redox sensitive cellular pathways were also downregulated. Western blot showed inhibited phosphorylation, thus activation of MAP kinases, Akt, NF-κB and I-κB. Decreased expression of NF-κB mediated proinflammatory genes in the lungs of LPS-treated KO mice was confirmed by quantitative PCR. The lack of CypD reduced the release of proinflammatory cytokines TNFα and IL-1ß determined by ELISA.
We demonstrated that the lack of CypD significantly reduces the severity of LPS-induced lung injury and has clear impact on the LPS-induced signaling cascade and proinflammatory gene expression involved in the pathogenesis of septic sequel. These effects are based on the decreased ROS and RNS production in KO mice leading to further downregulation of redox sensitive MAPKs and of the inflammatory master regulator NF-κB.

PII-107

Impaired autophagy results in increased inflammasome activation in stefin B-deficient mice

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Objective of this work is the elucidation of the mechanism how does the lack of cysteine proteinase inhibitor, stefin B (cystatin B) influence autophagy, mitochondrial reactive oxygen species (ROS) generation and consequently caspase-11 expression and Nlrp3 inflammasome activation.
Bone marrow–derived macrophages (BMDMs) were prepared from wild-type and stefin B-deficient (KO) mice, cells were primed with lipopolysaccharide (LPS) and then stimulated with ATP to activate inflammasomes. Caspase-11 expression was determined with qPCR and inflammasome activation with Western blots. Mitochondrial ROS were determined with flow cytometry, cytokines in serum and supernatant were determined by multiplexing flow cytometry and ELISA.
Upon stimulation with LPS plus ATP, stefin B KO BMDMs secreted significantly more IL-1β and IL-18, as well as active, cleaved caspase-1 and IL-1β, than did wild-type BMDMs. Consistent with the *in vitro* results, serum concentrations of IL-1β and IL-18 were significantly higher in endotoxemic stefin B KO mice than in their wild-type control animals and stefin B KO mice were more susceptible to LPS-induced mortality than were wild-type mice due to increased caspase -11 expression. Upon inflammasome activation, the amount of mitochondrial ROS in stefin B KO BMDMs was greater than that in wild-type BMDMs. Treatment with LPS plus ATP induced autophagy in wild-type BMDMs, but less in stefin B KO BMDMs. Stefin B deficient BMDMs also had less p62 protein upon LPS stimulation than did wild-type BMDMs.
The loss of stefin B resulted in impaired autophagy and the production of excessive mitochondrial ROS and consequently in increased caspase-11 expression and hyperactivation of the Nlrp3 inflammasome.

PII-108

Filamentous phages as immunogenic carriers of Fel d 1 mimotopes for allergen-specific immunotherapy

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Cat allergy is one of the most prevalent allergies worldwide. The major cat allergen Fel d 1 is a well-defined protein, which is produced in the salivary glands and in the skin of cats. In contrast, its epitopes are still not clearly defined. Identification of epitopes or mimotopes could have an important role in development of allergen-specific immunotherapy. However, mimotopes, which are short peptides, need to be administered on an immunogenic carrier to enhance immunogenicity. Due to their particle properties and ability to accomplish a focused antibody response against synthetic peptides, phages are considered as suitable immunogenic carriers.

We identified and characterized five mimotopes of Fel d 1 allergen. Identification of mimotopes was done by phage display technique. Furthermore, mimotopes were characterized by ELISA assays and immunoblotting. To develop the immunotherapeutic agent, mimotopes were expressed on the surface of an immunogenic carrier – a filamentous phage. Recombinant phages expressed around 150 copies of selected mimotopes. Basophil activation tests and lymphocyte activation tests were used for characterization of phages as immunogenic carriers.

Mimotopes showed IgE reactivity by binding specific IgE from cat allergic patients' sera. However, *ex vivo* cellular tests showed that mimotopes were not able to activate basophils, due to the absence of allergenic activity of short peptides. In comparison with Fel d 1 allergen, which activated basophils of cat allergic patients, mimotopes seem to be too short to cause IgE cross-linking. Nevertheless, phages were able to activate T lymphocytes independently of displaying mimotopes or not. Furthermore, T helper cells as well as cytotoxic T cells were activated. Activation of T cells has proven that phages are able to elicit specific immune response. Given this, phages showed the potential to be used as immunogenic carriers.

Phages displaying IgE mimotopes should be considered as one of potential therapeutic agents in allergen-specific immunotherapy, due to their low allergenic activity, and abundant immunogenicity and T cell activity.

PII-109

Interaction of perforin monomer with the membrane in micromolar dependence of calcium

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Perforin (PFN), a pore forming protein, plays a fundamental role in both adaptive and innate immune systems. To eliminate malignant and infected cells, cytotoxic lymphocytes T and natural killer cells release PFN monomers to kill their targets. Once released, PFN binds to target membranes, oligomerizes into giant beta-barrel structures and forms arch- or ring-like channels - pores. Pores induce lysis of cells and/or allow entry of granzymes, serine proteases, into the targets. Binding of PFN and pore formation are strongly associated with calcium concentration and pH of the environment. The prominence of PFN's role becomes apparent in severe immune disorders when its function or processing is impaired – e.g. in familial hemophagic lymphohistiocytosis, juvenile arthritis or lupus. Despite its significance, resolving PFN's nature remained a challenge – PFN is membrane protein, toxic for recombinant systems, highly glycosylated and contains a number of cysteine bridges. Due to the low-yield production of the native form and difficult production of the functional recombinant form, a significant part of PFN's nature remains unsolved. Using optimized baculovirus expression system and a novel, one-step purification technique, we isolated pure and active recombinant mouse PFN. The product is cytotoxic and forms pores on model membranes only in the presence of calcium. In addition, we established novel biochemical techniques to determine its interactions with ions and membranes. Using surface plasmon resonance, we evaluated affinity of native human and recombinant mouse PFN for calcium. In addition we confirmed micromolar affinity of PFN for calcium ions using microscale thermophoresis and fluorescently labelled nanodiscs, membrane-protein discoidal complexes. The results are in good agreement with PFNs calcium-dependent hemolytic activity. All-atom molecular simulations of PFN in the solution confirm that calcium ions stabilize its structure. In addition, ions induce exposure of hydrophobic residues that are required for PFN's interaction with the membrane. To investigate interactions of PFN monomer with the membrane, we performed cryo-electron microscopy and single-particle reconstruction of nanodisc-PFN complexes. The results are new insights in biochemical properties of PFN and indicate further possibilities for investigation of interaction of proteins with membranes.

PII-110

Examining the role of inflammasome activation and identifying novel diagnostic innate immunity biomarkers in children with hypercholesterolemia

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Hypercholesterolemia in children has been recognized as an important risk factor associated with cardiovascular dysfunction at a young age and later in adulthood. It has been suggested that inflammation and perturbed innate immunity play critical part in early development of diseases such as atherosclerosis. In animal models, cholesterol crystals have been shown to activate NLRP3 inflammasome, an intracellular multiprotein complex responsible for inflammatory processes also involved in atherosclerosis.
The main objective of this study is to examine the role of inflammasome activation in children with hypercholesterolemia, as a possible preceding condition to development of cardiovascular disease. Isolated peripheral blood mononuclear cells (PBMCs) were exposed to a series of priming (first) signals (Toll-like receptor agonists, e.g. LPS) and the second signals (e.g. ATP, nigericin, silica, cholesterol, LDL, oxidised LDL). In PBMCs from healthy adults we optimized experimental protocols and identified optimal read-outs. Silica and nigericin demonstrated the most potent inflammasome activation and the cytokines IL-1 , IL-6 and TNF- were determined as the most informative output assays. In a smaller-scale preliminary study, we found higher concentration of these cytokines in plasma of hypercholesterolemic children and differential inflammasome responses of their PBMCs. Ongoing are comparative studies of inflammasome activation between hypercholesterolemic and healthy children, as well as comparisons before and after the therapy. As the second objective, we aim to identify early immunity-specific biomarkers in hypercholesterolemia. We compared 35 samples of plasma from hypercholesterolemic children with 34 healthy controls for 17 key biomarkers of inflammation using a multiplex Bio-Plex cytokine assays. ANOVA analysis correcting for total cholesterol, sex and age revealed several significant differences in inflammatory cytokines. Larger-scale screens are planned to confirm these results and examine their functional importance.
Improved understanding of key innate immunity processes in hypercholesterolemia, such as inflammasome function, can lead to novel mechanistic insights to early atherosclerosis development. Apart from such studies of basic research interest, our search for robust non-invasive plasma inflammatory biomarkers could lead to improved diagnosis of hypercholesterolemia and cardiovascular diseases in early asymptomatic stages.

PII-111

Minimal oligodeoxynucleotide motifs that are distinctively recognized by human TLR9

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Oligodeoxynucleotides (ODNs) containing unmethylated deoxycytidylyl-deoxyguanosine dinucleotide (CpG) motifs recapitulate activation of Toll-like receptor 9 (TLR9) by microbial DNA. Activation of TLR9 is dependent on the nucleotide sequence, backbone modifications, length, and oligomerization properties of ODNs. However, the key sequence determinants that govern activation of TLR9 by ODNs have not been well defined.
The minimal ODN that activate human TLR9 comprises two CpG dinucleotides separated by six to ten nucleotides, where the first CpG motif is preceded by the 5'-deoxythymidine and elongated by the poly-deoxythymidine tail at the 3' end. The distance between the stimulatory CpG motifs within the ODN as well as their length fine-tunes activation of B-cells. The minimal ODN that specifically activates murine but not human TLR9 comprises one CpG motif separated by one to three nucleotides from the 5'-TCC motif. The position of the CpG motif four to six nucleotides from the 5'-end and ODN's length fine-tune activation of murine macrophages.
The minimal ODNs were further used to provide an insight into the molecular mechanism of TLR9 ligand recognition to minimise the effects of other CpG motives that are present in commonly used ODNs.

PII-112

Inhibition of the NLRP3 inflammasome formation by the designed peptides

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NLRP3 inflammasome is a multiprotein complex which forms within cells in response to various triggers (ATP, microbial components, crystals, amyloid plaques etc.) The complex consists of a sensor protein NLRP3, adaptor protein ASC and procaspase-1, which self-activates and converts cytokine pro-forms into their mature forms. Mutations in the gene encoding NLRP3 lead to cryopyrinopathies, autoinflammatory diseases that are treated by therapy targeting preferentially IL-1R signaling. NLRP3 inflammasome was also shown to be involved in a variety of common diseases, such as diabetes type II and neurodegenerative diseases. Despite increasing knowledge on the involvement of NLRP3 in a variety of pathologies, the mechanism of inflammasome activation is not understood and inhibitors targeting early steps of the inflammasome assembly are lacking.
Based on the available structures of the proteins that comprise the inflammasome and on the NLRP3 regions carrying pathological mutations, we designed two groups of putative inhibitory peptides which were most likely to disrupt the formation of the inflammasome. Using immunoenzymatic tests and Western blotting we showed that peptides inhibit the activation of caspase-1 and the release of IL-1β and IL-18 from myeloid cells in a concentration-dependent manner. Peptides from the protein interaction sites exhibited inhibitory effect independently of the NLRP3 inflammasome trigger. On the contrary, inhibition with the peptides from the NLRP3 regions with pathological mutations was dependent on the type of trigger. Furthermore, we found that some of the peptides specifically inhibited the NLRP3 inflammasome but not other inflammasomes.
Designed peptides provide an insight into the mechanism of NLRP3 inflammasome assembly as well as the basis for the development of novel inflammasome inhibitors.

PII-113

Serine proteinase inhibitor ecotin of various pathogen microbes inhibits lectin pathway of the complement system

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The complement system is a network of about 35 plasma and cell surface proteins and it is an essential part of the innate immune system. It can be activated through three different routes: the classical, the lectin and the alternative pathway. The lectin pathway recognizes ancient molecular patterns on the invading pathogens via its recognition molecules mannan binding lectin, ficolins and other collectins (CL-L1, CL-K1). These molecules circulate in complex with zymogens of **m**annan binding lectin **a**ssociated **s**erine **p**roteinases MASP1 and MASP2. After recognition of the pathogen, MASP1 autoactivates and activates MASP2. The activated enzymes initiate complement activation by cleaving downstream complement proteins yielding a C3 convertase. This results in complement activation leading to opsonization and lysis of the pathogens, recruitment of immune cells and triggering inflammation. In this process the alternative pathway provides a positive feedback loop for the other two pathways.
Ecotin is a periplasmic homodimeric serine proteinase inhibitor protein first isolated from *E. coli*. Ecotin inhibits a large number of serine proteinases (e.g. trypsin, chymotrypsin, elastase, urokinase and fXa) with K_I values of 10^{-9} – 10^{-13} M. Potent inhibition of so many proteases having different specificities is a unique feature of ecotin in the family of canonical, substrate-like serine proteinase inhibitors. Orthologs of ecotin are expressed in some pathogenic bacteria like *Yersinia pestis* and *Pseudomonas aeruginosa* and in the eukaryotic parasite *Leishmania major*.
Inhibiting the activation of the lectin and the alternative pathway through the panspecific serine protease inhibitor, ecotin could provide at least partial protection for the pathogen against the immune system and therefore it might represent an evolutionary advantage.
Using purified recombinant human MASPs and ecotins from various pathogenic microbes we conducted enzyme inhibition experiments. Moreover, we also tested the effects of the various ecotins on complement activation using human serum assays. We show that ecotins are potent inhibitors of MASP2 and inhibit lectin pathway activity efficiently in human serum.
The results suggest that lectin pathway inhibition through ecotin could provide an important advantage to the pathogen during the infection process.

PII-114

Colocalization of galectin-1 and osteopontin in mouse uterus during early pregnancy

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Pregnancy is highly regulated event in which endocrine, immune and metabolic systems co-operate in order to sustain life. Galectin-1 (Gal-1) is a glycoprotein found in endometrium during pregnancy. Important role of this glycoprotein in early pregnancy was established in study with Gal-1 knockout mice; those mice exhibit high rate of fetal loss. Further, detail analyses of Gal-1 function during pregnancy have shown its role in angiogenic modulation, immune response and placental development. Placental development is also under control of specific population of natural killer (NK) cells known as uterine NK cells (uNK). As it known, some of uNK cells specifically recruit to placental attachment sites where intensive formation of new maternal blood vessels takes place. Subpopulation of uNK cells express osteopontin (OPN). Osteopontin is an Arg-Gly-Asp (RGD) containing integrin-binding glycoprotein postulated to regulate cell adhesion and invasion in many normal and pathological conditions.
During peri-implantation period endometrium becomes a structurally and functionally segregated tissue divided to mesometrial and antimesometrial pole. Our analysis has shown that during decidualization Gal-1 is localized in the stromal cells at the antimesometrial and mesometrial side. Additionally, OPN positive cells are present at mesometrial side of uterus during decidualization.
This study was undertaken to analyze the spatial relationship between OPN and Gal-1 expression in mouse uterus during early pregnancy. Analysis of the cell type expressing Gal-1 will be presented.

PII-115

Identification and function of ghrelin receptor and ghrelin-O-acyltransferase in human keratinocytes

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Obesity increases the risk for the development and severity of psoriasis. The main underlying cause is the low-grade inflammation resulting from the pro-inflammatory cytokines produced by the adipose tissue of obese individuals. Other endocrine factors, like reduced levels of anti-inflammatory orexigenic hormone ghrelin, might also influence the pathogenesis of psoriasis. We investigated the presence of ghrelin receptor and ghrelin-O-acyltransferase in human keratinocytes with an aim to elucidate the potential role of ghrelin in the development of skin disorders.
RNA from human keratinocyte cell line HaCaT was isolated and reverse transcribed to cDNA. The expression of ghrelin, receptor GHS-R1a and enzyme ghrelin-O-acyltransferase was investigated with RT-PCR. The response of HaCaT cells to ghrelin stimulation was studied with intracellular calcium mobilization assay. TNF-α stimulated HaCaT cells were used to study the effect of ghrelin on inflammatory cytokine expression with quantitative PCR.
Ghrelin receptor GHS-R1a and ghrelin-O-acyltransferase, but not ghrelin, were expressed in HaCaT cell line. Measurements of intracellular calcium mobilization revealed that the receptor is functional and the cells respond to ghrelin stimulation. Stimulation with TNF-α increased the expression of IL-1β, IL-6, IL-23, TNF-α and ICAM-1 in HaCaT cells, but did not influence the expression of GHS-R1a and ghrelin-O-acyltransferase. Ghrelin (100 ng/mL) downregulated the expression of ICAM-1 in activated keratinocytes.
We have shown that keratinocytes HaCaT express the functional ghrelin receptor and respond to ghrelin stimulation. Ghrelin is able to suppress expression of adhesion molecule ICAM-1 which is important for migration of leukocytes into the psoriatic lesions. Reduced ghrelin concentration might contribute to the more severe form of psoriasis clinically observed in obese patients. The biological function of ghrelin-O-acyltransferase in these cells remains to be identified.

PII-116

An atypical bactericidal, cytotoxic, and modulatory peptide from *Staphylococcus pseudintermedius* exhibits properties of bacteriocin and virulence factor

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Staphylococcus pseudintermedius is a commensal bacterium colonizing the skin and mucosal surfaces of household animals, especially dogs. However, it also emerges as a dangerous opportunistic pathogen, comparable to *S. aureus* for humans. The epidemiological situation is further complicated by the increasing number of methicillin-resistant *S. pseudintermedius* infections and evidences of transmission of genes driving antibiotic resistance between staphylococci colonizing human and zoonotic hosts. Here, we describe isolation and characterization of a unique peptide BacSp222, which possesses features characteristic for both bacteriocins and virulence factors. The peptide is plasmid-encoded and excreted in high quantities by *S. pseudintermedius* strain 222 isolated from dog skin lesions. BacSp222 is a 50 amino-acid long linear and predominantly alpha-helical peptide, formylated at the N-terminus. The peptide is rich in tryptophan, lysine, and arginine but its sequence does not exhibit significant similarities to any other known peptides or proteins. BacSp222 kills Gram-positive bacteria at doses ranging from 0.1 to several micromoles but is inactive toward Gram-negative bacteria and fungi. Importantly however, regardless of the bactericidal properties, at micromolar concentrations BacSp222 demonstrates also significant cytotoxic activities toward eukaryotic cells, including keratinocytes and fibroblasts. However, at nanomolar concentrations, it also possesses modulatory properties, efficiently enhancing interferon gamma-induced nitric oxide release in murine macrophage-like cell lines. BacSp222 may be considered as the first example of a multifunctional peptide which breaks the convention of splitting virulence factors and bacteriocins into two unrelated groups.

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PII-117

Biological evaluation of the novel ruthenium(II) complexes

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Platinum-based drugs are clinically used in anticancer chemotherapy for the last 30 years, nevertheless they suffer from side effects, sensitivity and resistant mechanisms. Ruthenium complexes proved to be promising alternatives, as two compounds already entered clinical trials: NAMI-A and KP1019. Human aldo-keto reductases from the AKR1C subfamily, especially AKR1C1 and AKR1C3 are related to resistance to a variety of anticancer drugs including the platinum-based cisplatin and carboplatin. Although the mechanism of resistance has not been defined in detail, the AKR1C enzymes have been reported to regulate the generation of cisplatin-associated reactive oxygen species, and to disrupt apoptotic pathways. The increased expression of AKR1Cs in the cisplatin- and carboplatin-resistant cancers thus calls for drugs that can overcome this resistance by either not affecting the AKR1C levels or by decreasing the AKR1C enzymatic activities. Our group already published the study that has examined the interactions between the AKR1C enzymes and selected ruthenium complexes.
The aim of this study was to evaluate 1) the interaction between novel ruthenium complexes and recombinant AKR1C enzymes and 2) the effect of ruthenium complexes on cell proliferation. Four ruthenium(II), complexes of two types and two ligands were included in the study: the first type of complexes with a general formula of [Ru([9]aneS$_3$)(dmso)(*pyO-S or pyO-O*)](PF$_6$) (compound 1 and 2) and the second with the general formula of [(η^6-*p*-cymene)RuCl(pyO-S or py O-O)] (compound 3 and 4). To evaluate the potential inhibitory actions of these compounds on the AKR1C enzymes, we followed an enzymatically catalyzed oxidation of the substrate 1-acenaphthenol by NAD^+ in the absence and presence of the individual compounds. WST-1 assay was performed to evaluate the impact of ruthenium complexes on proliferation of a breast cancer cell line MCF-7.
The obtained data demonstrate that the compound 3 is the most effective inhibitor of recombinant AKR1C enzymes as well as a very effective inhibitor of the cell proliferation. Compound 3 and 4 inhibited all three types of AKR1C enzyme whereas compound 2 inhibited only AKR1C1 and compound 1 was not effective towards AKR1C enzymes. Compounds with *O,S*-donor ligand effectively inhibit cell proliferation in contrast to compounds with *O,O*-donor ligand that have the opposite effect.

PII-118

Role of CYP2E1 in female-predominant resistance to hyperoxia

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Hyperoxia is one of the oldest oxidative stressors used to assess defense response to oxidative stress. It reduces lifespan, induces same level of oxidative damage and similar gene expression patterns as ageing. Thus, it serves as a good model for studying ageing. The resistance to oxidative stress appears to be sex-related, with females being more resistant to oxidative damage. Females are found to have lower levels of ROS, better antioxidant defense, higher mitochondrial activity, overall lower oxidative damage, which takes part in their longer lifespan. CYP2E1 is responsible for metabolism of ketone bodies, glycerol and fatty acids. It participates in lipid metabolism homeostasis and depletion of lipid peroxidation (LPO) products. Protective role of CYP2E1 in development of lung injury and survival rate after hyperoxia treatment in mice has been shown previously in CYP2E1 null mice. We have previously found that 4 months old male mice are more sensitive to hyperoxia, compared to females. Our next aim is to investigate role of CYP2E1 on sex-related differences in susceptibility to hyperoxia. CBA/H mice of both sexes, 4 and 12 months old, were exposed to 95% O_2 for 18 hours. After hyperoxia, animals were sacrificed. LPO and the expression of CYP2E1 were analyzed in supernatant of their lungs and liver. Expression of CYP2E1 was also analyzed in $ER\alpha^+$ and $ER\alpha^-$ breast cancer cells exposed to hyperoxia. In 4 months old CBA mice, differences in expression of CYP2E1 mRNA were sex-related in liver and lungs after hyperoxia. Expression was increased only in females, and was accompanied with absence of change in LPO. In 12 months old mice sex-related difference in expression after hyperoxia was diminished. *In vitro* hyperoxia exposure lead to altered expression of CYP2E1 in ERα positive and negative breast cancer cells. These results implicate important contribution of CYP2E1 in oxidative stress resistance that can take part in managing the aging process.

PII-119

Wound healing process in conditions of CD26 deficiency

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Dipeptidyl peptidase IV (DPP IV/CD26) is a ubiquitous multifunctional membrane-bound glycoprotein, acting as a proteolytic molecule, receptor and binding protein expressed on the surface of various cell types. Its soluble form, resulting most probably by proteolytic cleavage of the membrane-bound form, is found in different biological fluids. DPP IV/CD26 plays an important role in different physiological as well as pathological processes, including cell adhesion, migration, apoptosis and extracellular matrix degradation, which are all key processes in wound healing. Therefore, we hypothesized that DPP IV/CD26 influences wound healing processes, especially proliferation of basal layer cells of epidermis and neovascularization. In order to investigate the role of DPP IV/CD26 in wound healing, experimental wounds were induced in CD26 deficient ($CD26^{-/-}$) and wild type (C57BL/6) mice which were sacrificed on days 2, 4, 7, 10 and 15 following wounding. Histomorphometrical, patohistological and immunohystochemical analyses were done and revealed different wound healing dynamics between $CD26^{-/-}$ and wild type mice. In conditions of CD26 deficiency, the epithelization process was more intense. The formation of new capillaries started promptly in $CD26^{-/-}$ mice, on day 2 of wound healing, and their number was considerably higher than that of C57BL/6 mice in all analyzed days. Likewise, the proliferation of basal layer cells of epidermis was more pronounced in $CD26^{-/-}$ mice on days 4 and 7 of wound healing compared to wild type mice. On day 10 of wound healing a restoration of normal structure of the skin was noticed in both mice strains, but the re-establishment of different layers of epidermis was noticed only in $CD26^{-/-}$ mice. The resulting scar tissue in $CD26^{-/-}$ mice was better vascularized and having more solid structure compared to wild type mice. The activity of DPP IV/CD26 was also measured in wild type mice and showed a significant reduction on day 4 of wound healing. Obtained results indicate an important role of DPP IV/CD26 in wound healing processes, which was found to be more efficient in conditions of CD26 deficiency. These findings contribute in insights of wound healing mechanisms and could give a contribution in finding new therapeutic approaches for wound healing and tissue regeneration.

PII-120

Identification and spatio-temporal expression profiling of *Verticillium albo-atrum* effectors in infected hop plants

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Hop wilt caused by *Verticillium albo-atrum* (Vaa) is a fungal disease ravaging hop gardens worldwide. Successful colonization and proliferation inside xylem vessels requires bypassing several plant immune responses. To achieve that, the fungus secrets various toxins, cell wall degrading enzymes and effector molecules. Effector genes are expressed early in infection, encode small cysteine-rich secreted proteins and lack recognizable protein domains or homologues in other fungi.
The objectives of our research were i) *in-silico* identification of candidate Vaa effector genes, ii) determination of their spatio-temporal expression and iii) confirmation of their biological role in *Verticillium* wilt pathogenesis.
Using an in-house generated bioinformatic pipeline and comprehensive biological datasets available from our previous genomic, RNAseq and proteomic studies, we first determined *in silico* the secretome of Vaa comprising 962 putatively secreted proteins. After removing proteins with predicted enzymatic activity and dismissing proteins with NLS and ER signals, we obtained 369 putative Vaa effectors. We ranked them by several criteria (expression *in planta*, small Cys-rich proteins lacking functional domains, unique sequences, similarity to sequences in PHI database, etc.) and selected 10 candidate effector genes for expression profiling studies with qPCR. As best reference genes, topoisomerase and splicing factor 3a2 were used and the expression of effector genes was further normalized to the fungal mass in infected hop. Pathogenicity tests of the corresponding effector gene deletion mutants are ongoing to confirm that the effector gene is required for virulence of *V. albo-atrum*.
The most important part of studying effector biology is identification of host protein targets (interactors). Knowing what signalling or metabolic pathways are modulated by a certain effector may provide candidate host proteins that could be exploited in engineering resistance.

PII-121

Molecular background and physiological consequences of altered peripheral 5-HT homeostasis in adult rats perinatally treated with tranylcypromine

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Developmental exposure to 5-HT-enhancing agents has been reported to induce long-lasting changes in the brain, but there is hardly any data on the long-term effects of perinatal 5-HT enhancement on 5-HT balance and function in the peripheral compartment. Perinatal treatment of rats with monoamine oxidase (MAO) inhibitor tranylcypromine (TCP), leads to persistent imbalance in central (increased 5-HT degradation and decreased 5-HT concentrations in the brain) and peripheral (increased platelet and decreased plasma 5-HT concentrations) 5-HT homeostasis. In this study, we explored the molecular background of peripheral serotonin imbalance, and its possible consequences on bone remodelling and haematopoiesis. Jejunum, liver and blood samples were collected from TCP- and saline-treated rats on post-natal day 70. Relative mRNA levels for tryptophan hydroxylase 1 (TPH1) and MAO A were analysed using quantitative RT-PCR, femoral trabecular bone parameters were measured using micro-computed tomography, while peripheral blood cell number was determined by cell counter. TCP-treated rats displayed significant decrease in expression of *Tph1*, and significant increase in percentage of bone volume, trabecular number, connectivity density, and leukocyte number. Significant negative correlation was observed between relative concentrations of TPH1 mRNA and trabecular bone parameters. Significant long-lasting decrease in the intestinal *Tph1* expression, along with accumulation of 5-HT into platelets, may represent the main mechanism of peripheral compensation for 5-HT excess during treatment, presumably leading to increase in trabecular bone structure (supporting the negative influence of peripheral 5-HT on bone accrual) and leukocyte number (indicating negative *in vivo* effect of 5-HT in regulation of leukocyte development and/or sustainment).

PII-122

Global DNA hypomethylation in white blood cells represents a feature of multiple sclerosis

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Multiple sclerosis (MS) is a chronic, inflammatory disease of a central nervous system. It is thought that both hereditary and environmental factors contribute to the elevated risk for the disease occurrence. Environmental factors that are associated with epigenome modifications, such as vitamin D level, smoking, Epstein-Barr virus (EBV) infection and diet were shown to impact the MS risk. DNA methylation, a process that involves addition of methyl group to the C5 position of cytosine in C-G dinucleotides, is the most studied epigenetic mechanism. The majority of CpG dinucleotides are found in repeated DNA sequences such as Long Interspersed Nucleotide Element (LINE) family. The majority of them are truncated but those designated as LINE-1, which comprises nearly 17% of human genome, can be transcribed. LINE-1 methylation correlates with global DNA methylation. The aim of this study was to determine whether there were differences in the global DNA methylation in white blood cells between MS patients and healthy controls. We collected blood samples from 36 Croatian patients with MS and 100 healthy controls. After DNA isolation we performed the sodium bisulphite modification. Global DNA methylation was assessed by quantifying the methylation of LINE-1 elements using the real-time methylation specific PCR (MethyLight). We used Alu-based real-time PCR control reaction in order to normalize DNA input. The MethyLight data specific for methylated repetitive elements were expressed as percent of methylated reference (PMR) values, while the levels of unmethylated repetitive elements were expressed as percent of unmethylated reference (PUR) values. Our results showed that the methylation level of LINE-1 elements was significantly reduced in MS patients compared to the healthy controls (M-W U test; $p<0.05$). Neither gender nor age was found to be associated with the LINE-1 methylation level in patients or controls. Based on our findings, we can conclude that patients with MS display global DNA hypomethylation in white blood cells. Since peripheral blood is easily accessible, measurement of LINE-1 methylation in white blood cells could be used in future for the development of new prospective biomarkers for MS. Still, it remains unknown weather this hypomethylation is the cause or the consequence of the disease and this will be the subject of our future research.

PII-123

LLO related drop in TEER of Caco-2 monolayer is dependent on pore formation with no major role of Ca2+ influx

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Listeria monocytogenes is a pathogenic bacteria present in soil and contaminated foods. It secretes a pore-forming toxin listeriolysin O (LLO) as its major virulence factor. Intestine is the primary site of the bacterial invasion; therefore we tested the effects of LLO on an intestinal epithelial cell line Caco-2 and compared them to effects of an unrelated pore-forming toxin equinatoxin II (EqtII). Apical application of both toxins resulted in rapid drop in transepithelial electrical resistance (TEER) but with different kinetics. For the same change in TEER higher LLO concentrations or prolonged exposure was needed than with EqtII. The drop in TEER was found to be dependent on pore formation and coincided with rearrangement of claudin-1 within cellular tight junctions and associated actin cytoskeleton. However, no significant increase in Caco-2 monolayer permeability to fluorescein or 3 kDa FITC-dextran could be measured. Both toxins exhibit similar effects on Caco-2 epithelium morphology and physiology. Taken together, LLO action upon the Caco-2 cell membrane is much slower than EqtII but results in compromised epithelium for a longer period of time at lower concentrations than EqtII. We assume this could favour listerial invasion in hosts resistant to E-cadherin related pathway of infection.

PII-124

Finding a new regulatory function for the proteasome activator PA200 in a cellular model for Huntington's disease

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The conserved Blm10/PA200 activators bind to the proteasome core particle gate and facilitate peptide and protein turnover *in vitro*. We provided evidence for an additional regulatory function of the proteasome in mitochondrial homeostasis. We demonstrated that the mitochondrial fission protein Dnm1 is degraded by the proteasome and that proteasome-mediated Dnm1 turnover involves the proteasome activator Blm10. We have also shown that loss of BLM10/PA200 upon expression of mutant huntingtin protein (mHtt) leads to a severe growth defect, increased aggregate formation of mHtt and excessive mitochondrial fragmentation in yeast and HEK293 cells. Assuming that the impact of Blm10 on Dnm1 turnover is conserved in mammals, we propose that upregulation of its mammalian ortholog PA200 might provide a neuroprotective function through the regulated degradation of Drp1and is involved in the altered mitochondrial dynamics and function during the development of Huntington's disease (HD).We generated PA200 knock-down SH SY5Y human neuroblastoma cell lines (shPA200) using shRNA lentiviral technology. We looked at Drp1 turnover by cycloheximide (CHX) chase assay and have shown that PA200 is required for the correct degradation of Drp1 by the proteasome. Furthermore, we have observed that loss of PA200 leads to impaired mitochondrial morphology including increased mitochondrial fragmentation and decreased mitochondrial length.We demonstrated in yeast that mitochondrial respiratory capacity and aconitase activity are altered in *blm10* deleted cells. Therefore we checked mitochondrial function by measuring oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) in shPA200 and control cells using Seahorse XF Analyzer. Our results show that loss of PA200 leads to mitochondrial dysfunction.Moreover, Drp1 is stabilized in shPA200 cells upon expressing different length of mHtt while in its respective control Drp1 level is dramatically decreased. A previous study showed that mutant Htt binds to Drp1, changes its structure and increases its enzymatic activity. The mechanistic details are unknown. Our results lead to speculate that PA200 has a regulatory function while HD progresses through its impact on Drp1 turnover.
Our results may provide novel information on the role of Drp1 turnover in the progression of Huntington's disease and on the neuroprotective role of PA200 and later might point toward Drp1 as a new target for Huntington's disease therapy.

PII-125

Alterations in ophthalmological parameters and cytokine levels in tears of patients with glaucoma

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Glaucoma is a progressive, chronic neurodegenerative disease with a multifactorial pathophysiology that affects the optic nerve and may cause loss of function up to the blindness. This disease has several types, but generally categorized by the anterior chamber (iridocorneal) angle. The open-angle glaucoma (OAG) and the angle-closure glaucoma (ACG) can be divided into primary and secondary forms and also can be categorized as acute or chronic. Actually, the elevated intraocular pressure (IOP) is the only risk factor that can be influenced with therapy such as medications or glaucoma surgery. In routine clinical use, different classification systems are used to evaluate and quantify the anterior chamber angle. One of them is based on Scheimpflug imaging, like the Oculus Pentacam. This imaging technology provides quantitative data regarding of anterior chamber characteristics and may serve as a useful adjunct for glaucoma diagnosis and monitoring reducing chances of this error bias. One of the advantages of the Pentacam over other noncontact instruments such as optical pachymetry is that the anterior chamber volume (ACV), anterior chamber depth (ACD) and anterior chamber angle (ACA), as well as the corneal thickness and corneal topography can be obtained at the same time.
The corneal tear film contains numerous proteins the qualitative and quantitative pattern of which can be pathognomonic in various ophthalmic conditions. The tear can be collected *via* non-invasive way, so it is an appropriate sample type for biomarker studies. Results from previous research showed that there is a link between the inflammatory cytokine level and the different type of glaucoma eye structural changing.
Our aim was to identify specific correlations between these parametric imaging changes and examine how to correlate with the proteome changes with cytokine profiles in glaucoma. The cytokine profile in tear samples of 10 OAG, 10 ACG patients and 10 age and sex-matched healthy volunteers was analyzed by multiplex cytokine assay.

PII-126

Relationship between tumor necrosis factor α and insulin sensitivity in rats with impaired glucose tolerance

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Introduction: Impaired glucose tolerance is a pre-diabetic state of hyperglycemia associated with reduced sensitivity to the action of insulin that may precede type 2 diabetes mellitus by many years. Tumor necrosis factor α (TNF-α) is a cytokine produced by adipocytes that has been implicated in reducing sensitivity to insulin.
Objective: We aimed to find out the association of serum TNF-α levels with insulin sensitivity in the rodent model of impaired glucose tolerance.
Methods: Twenty 10-week-old male Wistar rats were divided into two groups, A (control) and B (impaired glucose tolerance). Impaired glucose tolerance was induced by intraperitoneal streptozotocin injection following nicotinamide injection (group B), while control group received citrate buffer and saline respectively. All groups received standard diet for 6 weeks. After 6 weeks animals were sacrificed in deep ketamine anesthesia. Blood samples were taken and glucose, insulin and TNF-α were determined. The quantitative insulin sensitivity check index (QUICKI) was calculated.
Results: Fasting serum glucose levels were significantly higher in group B when compared to the group A (group A, 4.81 ±0.18 mmol/L *vs.* group B, 6.51 ± 0.32 mmol/L; $p < 0.001$). No significant difference in insulin levels between healthy rats and rats with impaired glucose tolerance was found (group A, 1.24±0.10 *vs.* group B, 1.09±0.09 ng/ml, NS). Serum TNF-α was higher in animals with impaired glucose tolerance when compared to control group (group A, 11.27 ± 0.48 pg/ml *vs.* group B, 13.21 ±0.41 pg/ml, $p< 0.001$). Furthermore, moderate negative correlation between TNF-α and QUICKI index was obseved ($r= -515$, $p=0.071$) as well as strong positive correlation between TNF-α and blood glucose ($r= -846$, $p<0.001$).
Conclusion: These results suggest that an increase in circulating TNF-α concentration is associated with with reduced sensitivity to insulin and increased plasma glucose in animals with impaired glucose tolerance.

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PII-127

Modulation of multiple drug resistance by proprietary potassium ionophores in breast cancer stem cell model

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Cancer stem cells (CSCs) represent a subpopulation of cancer cells responsible for tumor formation, relapse and metastasis. These cells share properties of embryonic stem cells like pluripotency and self-renewal. CSCs exhibit resistance to a whole range of drugs with different cellular targets, via phenomenon called multiple drug resistance (MDR). Mechanisms of MDR include enhanced survival pathways and increased activation of drug efflux pumps, P-gp and ABCG2. Also, CSC resistance to therapy is related to enhanced reactive oxygen species (ROS) defense capability.
Recently, breast CSC model established by Weinberg group enabled screening of a large library of compounds for selectivity against CSCs. The most selective compound identified was salinomycin, a natural potassium ionophore. Salinomycin can act as a P-gp inhibitor and this feature has been attributed to its selectivity towards CSC.
Based on the above mentioned studies and anticancer activity of crown-ethers that act as K^+ ionophores (previously published by our group), we hypothesized that these compounds could show selectivity towards breast CSCs by modulation of MDR. Therefore, the aim of this study was to identify CSC selective drugs and to elucidate their mechanisms of action, with the focus on their strategies to overcome MDR: P-gp inhibition as well as abrogation of ROS defense.
We used the breast CSC model, which consists of two isogenic epithelial breast cell lines $HMLE^{shGFP}$ and $HMLE^{shEcad}$, with latter showing markers of CSC. In addition, we used breast cancer cell lines with different degree of differentiation/invasiveness, SUM159 and MCF7, and a multidrug resistance cell model, A2780 and A2780/Adr. The use of these cell lines enabled us to examine effects of compounds on cell viability and P-gp activity. Abrogation of ROS defense was evaluated by compounds' ability to induce ROS levels in CSCs, which are known to possess stable low levels of basal ROS.
The compounds' toxicity evaluated by MTT and xCELLigence system showed toxicity of several compounds on $HMLE^{shEcad}$ and SUM159 cells. The most promising compounds exhibited inhibition of P-gp activity evaluated by Rhodamine 123 efflux assay. In addition, ROS levels upon treatment with crown ethers, measured by both flow cytometry and fluorometry, showed an increase over time in several cell lines.
To conclude, proprietary crown ether compounds modulate MDR in breast CSCs via inhibition of P-gp and induction of ROS.

PII-128

Regulative role of Tks4 adaptor protein in the cell differentiation process

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Scaffold proteins modulate cellular signalling in cells by bringing in close proximity regulatory proteins, enzymes or actin structures. The Tks molecules are recently described large scaffold proteins that received their name based on the observation that they serve as tyrosine kinase substrates of Src kinase. One particular member of Tks family, the Tks4 scaffold protein has already described central role in EGFR signalling, in the formation of podosomes and cell movements, production of ROS, and in the adipogenic cell-commitment. The null mutation of Tks4 gene in humans cause the congenital abnormality called Frank-ter Haar Syndrome (FTHS). Although the Tks4 function in cells is well-documented, the exact nature how the mutant versions of Tks4 cause the abnormally differentiated tissue-phenotype in FTHS symptoms is yet not known.
The Tks4 mutant mice present many features of FTHS phenotype and are available in our hand. We studied the Tks4 null mice with the aim to provide explanation for the impaired tissue differentiation observed in FTHS.
As the affected tissues (bone, cartilage, fat) in FTHS patients have mesenchymal origin, we tried to study mesenchymal stem cells (MSCs). According to our experiments, the Tks4 KO MSCs have impaired potential in adipocyte and osteocyte differentiation. We tracked the MSCs differentiation processes with appropriate molecular markers (PPARγ, adiponectin, runx2, osteocalcin, etc.). To understand the involvement of Tks4 in the adipogenic differentiation, we performed quantitative analyses of the expression of 41 lipid-related genes in the Tks4 KO visceral tissue fat cells. The experiment showed that the overall gene expression profile of the Tks4 KO fat tissue is dramatically changed compared to that of wild type.
We expect to describe a new regulative role of Tks4 in differentiation processes and to gain knowledge about novel function of Tks4 through the characterization of the abnormal metabolic status of the KO mice.

PII-129

Exosomes containing protein Nef are released from microglia and astrocyte cells infected with HIV virus

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While combination anti-retroviral therapy has improved and saved the lives of millions of people living with HIV, the prevalence of HIV-associated neurocognitive disorder is increasing. Previous studies support the involvement of Nef in neuropathology of HIV, but the exact mechanism is unexplored. We hypothesize that Nef is exported from HIV infected microglia and astrocytes *via* exosomes, which then target and affect surrounding cells similar to what we have previously shown for HIV infected T-cells.
To test this hypothesis, VSV-G pseudotyped HIV viruses (NL4-3, YU2 and NL4-3 Δnef) were produced in 293T cells and tested for infectivity using luciferase assay in TZM-bl cells, while Western blot was used to check their composition. After infection of microglia and astrocytes with these viruses, culture media were filtered and exosomes/viruses pelleted by ultracentrifugation and later separated on Optiprep gradient. Samples before and after gradient separation were analysed for AChE activity using enzyme assay, concentration of HIV p24 was determined by ELISA and the presence of p24, Nef, flotilin and AChE by western blot analysis.
In microglia, secretion of proteins Nef and p24 was detected 3 days post infection with HIV virus. Furthermore, the level of Nef in microglia decreased after 5 days post infection, while p24 stayed constant. In astrocytes viral infection resulted in secretion of p24 within 3 days and Nef after 5 days. According to these results samples used for further analysis were collected 5 days post infection for microglia cells and 7 days for astrocytes. Later Optiprep gradient fractions efficiently separated exosomes and viruses from infected cells, as AChE activity was present in upper 6 fractions, whereas p24 was present in lower 5 fractions. In microglia cells Nef protein was detected in medium fractions of Optiprep gradient, while in astrocytes it was detected in most of the fractions.
We showed that infected microglia and astrocytes besides viruses secrete exosomes containing protein Nef. In further studies we will focus on the functional role of Nef-exosomes on the other cells of the central nervous system.

PII-130

Little babies - big warriors: the antioxidant response in the umbilical cord of neonates with intrauterine growth restriction

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Intrauterine growth restriction (IUGR) is one of the major complications of pregnancy and accounts for significant neonatal mortality and morbidity. An IUGR newborn weighs less than 90% of all other newborns of the same gestational age.
The pregnancy is a physiological state associated with an enhanced metabolism and an increased demand for oxygen. The increasing production of reactive oxygen species (ROS) may cause cells damage as lose their pro-oxidant/antioxidant balance. The premature and/or the low birth weight neonates are more vulnerable, because their antioxidant defense systems are impaired.
Antioxidant enzymes; the superoxide dismutases (SOD) and the catalase (CAT) take part in the direct elimination of ROS; such as superoxide anion and hydrogen peroxide. The heme oxygenases (HOs) play roles in heme degradation, yielding equimolar quantities of biliverdin, carbon monoxide (CO) and free Fe ions. The CO and the nitric oxide (NO) produced by endothelial nitric oxide synthase (eNOS) are considered the main vasodilator agents in foetoplacental circulation lack innervations of umbilical cord vessels.
Our aim was to characterize the antioxidant status of neonates born with IUGR compared to the appropriate-for-gestational-age neonates. The umbilical cord blood and arteries were used in classical biochemical (enzyme activity assays, determinations of ROS) and molecular biological methods (qPCR and immunohistochemistry) to specify the oxidative status of neonates.
Reduced expressions of *sod*, *cat* and *ho*s were detected in the umbilical cord blood and arteries of IUGR group. The lowered levels of gene expressions may refer to the inadequate antioxidant protection of neonates with IUGR.
Elevated production of peroxynitrite, a harmful oxidant molecule, was detected in the umbilical blood of a part of IUGR neonates, which reduces the level of available NO and indicates an increased $O2^{\bullet -}$ production.

PII-131

Proteomic analysis of gene products that regulate de- and remyelination.

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Despite of substantial basic and clinical research, two major questions are still elusive in the pathogenesis of multiple sclerosis; (i) why susceptibility of oligodendrocyte subsets to death stimuli varies, and (ii) why oligodendrocyte precursor cells in affected areas fail to replace eliminated oligodendrocytes. To answer these questions, we performed proteomic analysis in a cuprizone-induced demyelination model.
Eight week-old male C57BL/6 mice were fed by standard rodent chow containing 0.2% of cuprizone for 4 weeks to induce demyelination. The mice were sacrificed after termination of the treatment (CPZ), and 2 (2DR) or 14 (2WR) days later. Dissected *corpora callosa* were homogenised in buffered protease and phosphatase inhibitor containing 2% sodium dodecylsulphate solution by sonication. Proteins were collected by 20000 xg centrifugation after precipitating them by -20°C acetone and trichloroacetic acid 8:1, solubilised, alkylated by iodoacetamide and digested by lysyl endopeptidase. Peptides were purified on a C8 then a C18 reverse solide phase extraction columns before isobaric tags for relative and absolute quantitation labelling was performed on them, and were were analysed with EASY-nLC 1000 liquid chromatography coupled to nano-electronspray ionisation mass spectrometry MS system (Thermo Orbitrap Veloso). The peptides were separated on a 3 µm C18 analytical column (75 µm x 150 mm) using 400 nl/min gradient elution of (A) aqueous (0.1%) and (B) acetonitrilic formic acid solution (0.1%). The initial 3% B was increased to 10% over 10 min, then increased to 40% in 80 min than was re-equilibrated to 3% B in 30 min. The scanning range of mass spectrometry was m/z=100–3,000 in positive mode. Each intensive peptide was fragmented and the completed data was processed through the Thermo Proteome Discoverer software. Identified proteins were subjected to bio-informatics analysis.
We found substantial differences between the groups among proteins involved in apoptosis inducing factor- and caspase-mediated apoptotic processes, and negative regulation of nuclear factor kappa B.
Significant gene-expressional differences are associated with cell death and axonal guidance processes during de- and remyelination.

PII-132

Assessment of toxicity endpoints of selected cytostatic drugs

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The use of cytostatic drugs is increasing due to constant increase in cancer prevalence worldwide. According to GLOBOCAN, approximately 32.6 million people have diagnosed cancer. The use of cytostatic drugs can pose threat on several different levels. They are toxic to patients and therefore optimisation of therapies is done; then to the environment through their release by patients' excretion, improper disposal, and during production. Finally, it poses threat to medical staff that is preparing chemotherapy.
In this study, three cytostatic drugs [5-fluorouracil (5-FU), cisplatin (CDDP), and etoposide (ET)] were selected based on their different mode of action and tested for their cyto- and genotoxic potential in concentrations that are relevant for environmental, therapeutic, but also for occupational exposure (0-50 μg/mL). Human peripheral blood lymphocytes were selected as an experimental model, since they are sensitive biomarkers of human exposure. Time- and dose- response was observed in cytotoxicity testing, where IC_{50} values were estimated. For 5-FU IC_{50} values were >50 for 4, 24, and 72 h exposure, and 21.81 μg/mL for 48 h exposure. On the other hand, CDDP showed IC_{50} >50, 15.81, 8.11, and 4.16 μg/mL for 4, 24, 48, and 72 h exposure, respectively. Similarly, ET showed IC_{50} >50, 38.93, 7.23, 26.47 μg/mL for 4, 24, 48, and 72 h exposure, respectively.
As for genotoxicity endpoints concentrations up to 10 μg/mL were selected. The comet assay showed significant increase in genome damage only for ET at 10 μg/mL after 48 h exposure. The micronucleus (MN) test showed significant induction of MNi for CDDP at 10 μg/mL after 24 and 48 h exposure and for ET at concentrations 1 and 10 μg/mL after 24 and 48 h exposure, but also at 0.1 μg/mL after 48 h exposure.
The presented results imply that occupational exposure might pose risk for increase in genome damage of medical staff. Further analyses are necessary to establish more sensitive biomarkers of cytostatic exposure. It is also important to maintain constant biomonitoring of exposed staff so that potential health risks can be minimised.

PII-133

Cytogenetic status and activation of BRAF-dependent pathway in patients with thyroid diseases

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Thyroid cancer is one of the fastest growing types of cancer in the world. Its molecular pathogenesis and mechanisms are closely related to changes in the genome what makes it a good model for such study. The aim of this study was to assess cytogenetic status of patients with thyroid diseases and to determine the rate of mutated proteins in thyroid tissues.
The study population consisted of 80 volunteer untreated patients (67 female: 13 male, average age 51.56±12.61 years, 21 smokers) diagnosed with follicular adenoma (33), papillary cancer (26), goitre (18) and thyroiditis (3). The analysis of DNA damage in peripheral blood lymphocytes for this group using Comet assay resulted in: tail length (TL) 10.28±2.52 μm, tail intensity (TI) 4.04±2.12% and tail moment (TM) 0.21±0.32. When compared to matched control population that consisted of 80 healthy volunteers (67 female: 13 male, average age 51.15±13.22 years, 21 smokers), significantly ($p<0.05$) lower average TL (9.34±1.28), TI (2.19±0.76), and TM (0.09±0.04) were observed.
The expression of Raf-B and Ret variant proteins was also evaluated where out of 80 tissue samples, 82.50% was positive for Raf-B and 21.25% for Ret. In addition, only 2 samples were negative for both proteins whereas 5 were positive for both of them.
As both mutations are consequences of changes in genome, cytogenetic methods could be used for biomonitoring of human population in order to detect which individuals are more prone to this type of disease and therefore improve its prevention, although, the quest for ideal biomarker for thyroid diseases diagnosis and prediction is still on.

PII-134

Hepatoprotective effect of cocoa polyphenols (*Theobroma cacao* L.) against carbon tetrachloride-induced hepatic damage in mice

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Cocoa is a food rich in polyphenols, especially flavonoids, which possesses antioxidant and anti-inflammatory activity. Their potential health effects include general antioxidant effects protecting against reactive oxygen species (ROS) that are involved in the etiology of many degenerative diseases. But, little is known about their impact on liver diseases.
In this study, we have investigated the hepatoprotective activity of cocoa polyphenols (CP) and epicatechin (EPI), as the major antioxidant in cocoa extract, in carbon tetrachloride (CCl_4)-induced liver injury in female BALB/cN mice.
Cocoa extract and epicatechin in doses of 3.37 mg/kg and 2.24 mg/kg were administrated after previously CCl_4-induced liver damage (in dose 1 mg/kg). Differences between the groups (control, CCl_4, EPI and CP groups) assessed by a nonparametric Kruskal–Wallis test and Mann-Whitney test with $p<0.05$ were considered to be statistically significant.
CCl_4 intoxication resulted in hepatic necrosis and increased plasma transaminases (ALT, AST, and the ALP). Blood glucose and lactate concentration were also, significantly increased. Liver injury was associated with a significant decrease in activity of mitochondrial superoxide dismutase (SOD) activity and cytosolic glutathione peroxidase (GPx) and catalase (CAT) activity as well as total protein thiols. Serum TNF-alpha and IFN-gamma were significantly increased.
EPI and especially CP increased cytosolic, plasma membrane and mitochondrial total antioxidant capacity (TAC). SOD, GPx and CAT were elevated markedly after EPI and CP treatments. Serum TNF-alpha and IFN-gamma were significantly decreased compared to CCl_4 group.
The results of the current study suggest that cocoa and epicatechin exhibit a significant hepatoprotective activity. Cocoa polyphenols also provided better hepatoprotection when compared to epicatechin. The present study demonstrates antioxidant and antiinflammatory activity of cocoa polyphenols. Due to these health impacts, it seems that cocoa belongs to “super foods”.

PII-135

Polymorphisms in segregation genes contribute to gastric cancer risk

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Gastric cancer is in decline in most developed countries; however, it still accounts for a notable fraction of global mortality and morbidity related to cancer. It is the third leading cause of cancer-related death in both sexes worldwide. Despite a vast amount of data on molecular aberrations found in gastric adenocarcinomas, the exact molecular mechanisms of its development are unclear. Advances in genomics are showing an important role of chromosomal instability (CIN) in gastric carcinogenesis. CIN is likely caused by aberrations in genes, involved in the maintenance of genome integrity.
The primary aim of this study was to determine whether selected polymorphisms in mitotic kinase *BUB1B* and kinetochore proteins contribute to gastric cancer risk.
A group of 520 patients diagnosed with gastric cancer and control subjects were included in the study. Genotyping was carried out using real-time PCR and sequencing. The results were statistically evaluated with the X^2 test and odds ratios (OR). The influences of genotypes and histopathological characteristics on survival were evaluated using Kaplan-Meier estimations and Cox proportional hazard model.
AA genotype of BUB1B rs1801389 was marginally more represented in gastric cancer patients compared to control group (F=6.36, p=0.036). A significantly higher proportion of men with gastric cancer had TT genotype of CASC5 rs11855334 compared with control subjects (OR=1.723, 95% CI (1.163-2.555); p=0.009). Additionally, CT genotype was under-represented in the group of patients with undifferentiated tumours. Survival analysis showed that the survival of patients was associated with perineural invasion and lymph node involvement.
The study revealed significant associations of polymorphisms in genes that are regulating chromatid segregation with gastric cancer risk and certain histopathological features. These observations suggest a potential role of polymorphic alleles to predict more aggressive types of gastric cancer and indicate risk of developing gastric cancer in male population.

PII-136

Expression analysis of caveolin-1 in papillary thyroid carcinoma with relation to clinicopathological parameters and BRAF mutation status

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Papillary thyroid cancer (PTC) is a well differentiated cancer, which generally has a favorable prognosis, but it may become invasive and develop regional and distant metastases. Therefore, ways of recognition of the more aggressive PTCs are being explored. Most studies implicate BRAF V600E mutation as a marker of aggressive PTC, since it activates pathways that lead to tumor spread. Caveolin-1 (cav-1), a ubiquitously expressed protein of membrane caveolae, was reported to play a role in tumor cell migration and invasion, however its loss from the tumor stroma correlates with a worse prognosis in some malignancies.
With the aim to investigate the role of cav-1 in PTC pathogenesis, we evaluated the expression of cav-1 in PTC histotypes by WB and compared the findings with immunohistochemical (IHC) expression of cav-1 in both epithelial and stromal compartments. The results were related to clinicopathological features and BRAF V600E mutation status of PTCs.
Caveolin-1 expression was found in malignant thyroid epithelium and more abundantly in tumor stroma, but varied in both compartments within and between PTC subtypes. Correlation analysis revealed positive association between epithelial cav-1 and lymph node metastasis, yet a significant negative correlation between total cav-1 and BRAF status. Interestingly, stromal but not epithelial IHC expression of cav-1 was inversely correlated with BRAF status as well as with the depth of tumor infiltration.
The pathogenesis of PTC might be influenced by the altered expression of cav-1 in the thyroid epithelial and stromal compartments. The possible role of stromal cav-1 in the progression of thyroid cancer and its potential relation to BRAF mutation should be further investigated.

PII-137

Alterations in the chemical barrier components in tears of patients with Alzheimer's disease

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Alzheimer's disease (AD) is the most common age-related dementia affecting millions of people worldwide. The number of people affected by this disorder is expected to increase in the coming years, particularly in the developed and in the developing countries. Several neuropathological and biochemical studies revealed a link between inflammation and AD development. Inflammation can alter the level of the components in the chemical barrier of the first line host defense, such as the levels of antimicrobial an immunmodulatory proteins/peptides. Tear is a protein rich body fluid containing hundreds of proteins and as far as it can be collected by non-invasive way it is a possible new source for biomarker studies. A considerable part of the tear proteome is made up of diverse antimicrobial proteins forming the chemical barrier of the eye. In order to examine the changes of the antimicrobial and immunmodulatory protein levels caused by AD, we have developed an SRM-based targeted proteomic approach for the analysis of tear samples of 15 patients with Alzheimer's disease and 10 age and sex-matched volunteers without AD. The multiplex feature of the SRM-based approach is a powerful advantage of this technique; multiple analytes can be monitored from one sample, even if the sample volume is limited. The developed SRM method was optimized using stable isotope labeled synthetic peptides and the level of 12 proteins was examined in tears. Regarding the results, the levels of lipocalin−1, lysozyme-C, lactotransferrin, prolactin-inducible protein and extracellular glycoprotein lacritin was found to be reduced, while the level of dermcidin was found to be elevated in the tears of AD. The results show that the inflammatory condition present in AD could alter the composition of the chemical barrier in the eye.

PII-138

Different experimental approaches for discovery of diagnostic biomarkers of endometriosis

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Endometriosis is an estrogen-dependent inflammatory disease which is characterized by the presence of endometrial tissue outside the uterine cavity. There are three types: ovarian, peritoneal and deep infiltrating endometriosis with different aetiologies and pathogenesis. The gold standard for diagnostic of endometriosis is invasive laparoscopy that is combined with histological analysis. Consequently, it takes seven years on average before a correct diagnosis can be obtained. Due to this invasive procedure reliable biological markers for non-invasive diagnostic have been searched for in peripheral blood and urine samples and although more than 100 potential biomarkers have been investigated, none of these has proven useful in clinical practice.

Our research team has identified several panels of biomarkers in blood samples by global non-targeted and targeted approaches. Based on genome wide and low density array expression analyses we identified 25 genes encoding secretory proteins that were differentially expressed in endometriotic *versus* control tissue. Some of these genes have been further evaluated in blood samples of endometriosis patients and controls. With targeted proteomic approach we searched for serum biomarkers among cytokines and other secretory proteins and found models including concentrations of leptin, ficolin 2 and glycodelin-A. Our metabolomics approach in plasma samples showed elevated levels of sphingomyelins and phosphatidylcholines in patients *versus* controls. Based on a stepwise logistic regression we selected the best biomarkers and built a model with a sensitivity of 90% and specificity of 84.3% and thus a high diagnostic potential.

Further studies are now needed for a selection and validation of biomarkers based on serum/plasma analysis of larger groups of patients with endometriosis and subjects with endometriosis-like symptoms. One approach is the use of protein chip analysis which would enable us to analyse hundreds of native proteins simultaneously on a single slide. Alternative method would be the use of magnetic bead-based multiplex assay. The greatest advantage is the small sample volume needed and the ability to simultaneously detect multiple different proteins in one sample.

This kind of approach would enable us to define a panel of biomarkers that could be clinically useful and might thus allow a non-invasive diagnosis of endometriosis.

PII-139

Non-B DNA structures of C9ORF72 hexanucleotide expanded repeat in ALS and FTLD

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The G_4C_2 hexanucleotide repeat expansion, located in the first intron of the *C9ORF72* gene is the most common genetic feature of fatal neurological disorders amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD) in patients of European origin. Several pathogenic mechanisms involving the expanded repeats have been proposed: haploinsufficiency of C9ORF72, formation of toxic RNA transcripts and RNA foci that inactivate RNA binding proteins, and accumulation of toxic dipeptide repeat proteins (DPRs) that are translated from the expanded repeat RNA *via* repeat associated non-ATG (RAN) translation (1). Due to their high GC content, the expanded repeats enable the formation of several non-B DNA structures, such as G-quadruplexes and hairpins. Using nuclear magnetic resonance and circular dichroism spectroscopy we show that the orientation (parallel/anti-parallel) and molecularity (intra- or inter-molecular) of G-quadruplexes is dependent on the length of the repeats (2). This structural heterogeneity may have important implications in disease pathogenesis as it likely affects DNA duplication, transcription as well protein binding affinity of the repeats.

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PII-140

Plasma Nef-exosomes: putative biomarkers for active reservoirs in ART treated HIV infected patients

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HIV remains a major global public health issue. Although HIV blood levels can be efficiently controlled by antiretroviral therapy (ART), there is no cure and HIV still persists in different tissue and cell reservoirs, causing HIV-associated neurocognitive disorders (HAND) and other health issues in long term. Currently, HIV reservoirs are routinely determined by measuring cell-associated unspliced RNA, but this does not tell us anything about the possible expression of HIV viral proteins. Nef is one of the early HIV transcripts which does not require viral protein Rev for nuclear export and translation in the cytosol. Significantly, Nef was detected in the blood of HIV infected ART treated or untreated patients.
To evaluate the importance of Nef as a biomarker for active reservoir size, we have collected 150 plasma samples from well-characterized HIV-infected patients from the SCOPE cohort (SFGH), stratified them into four groups (HIV-uninfected, non-controllers, ART supressed, and elite controllers), and measured Nef concentration with Nef-ELISA assay. We have shown that in many patients with undetectable plasma HIV RNA levels (ART supressed and elite controllers), plasma Nef levels were similar to levels observed in untreated HIV infected patients. We hypothesise that plasma Nef is released from infected cells with exosomes (vesicles 40 - 100 nm in diameter), as we and others have already shown for HIV-infected CD4+ T cells. Similarly Nef-exosomes could also be released from other HIV reservoirs like plasma and peripheral blood mononuclear cells and macrophages, and also from the central nervous system, as exosomes were shown to cross the blood-brain barrier. Indeed, we have shown that Nef-GFP transfected microglial cells release Nef with extracellular vesicles with cup-shaped morphology and protein markers (like flotillin, Hsc70 and Tsg101) typical for exosomes. Additionaly, vesicle quantification with NTA and by total exosome protein content showed that Nef-expressing microglia release 4-times or 8-times more exosomes compared to control, respectively. Importantly, Nef-exosomes were also released from HIV-1 infected (isolates NL4-3 or YU2) microglia and astrocytes, in addition to produced viruses.
We conclude that plasma Nef-exosomes are interesting putative biomarkers for active reservoirs in ART treated HIV infected patients. Further studies are needed to address their role in HAND in HIV infected patients over the long term.

PII-141

Development of new peptide drug leads interfering with activity of orexigenic hormone ghrelin

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Peripheral orexigenic peptide hormone ghrelin has become one of the most interesting therapeutic targets for appetite regulation in pharmacological treatment of obesity and diseases accompanied by cachexia. Our objective was to find peptide drug leads modulating the ghrelin signal transduction by targeting the growth hormone secretagogue receptor.
Various linear peptide motifs resembling the ghrelin's primary structure were selected from random 7-mer and 12-mer peptide phage libraries. Two anti-ghrelin polyclonal antibodies mapping to C-terminal and N-terminal part of ghrelin were used as targets. Since they recognize an epitope on the ligand crucial for receptor interaction, selection resulted in peptides mimicking the receptor ligand. Four peptides, two resembling N terminal (P1 and P2) and two central part of ghrelin (P3 and P4) were synthesized and their agonistic and antagonistic activity was tested by measuring the release of intracellular calcium in HEK-293 cell line stably expressing ghrelin receptor GHS-R1a.
Peptide P1, mimetic of octanoylated N-terminal part of ghrelin exhibited antagonistic activity in nanomolar range (IC_{50}=1.3 nm). Partial antagonistic activity was revealed also for peptide P2 and P4. None of the tested peptides showed agonistic activity.
We have identified new peptide antagonists of ghrelin. They represent much in demand leads in development of small molecular and peptidomimetic drugs for pharmacological support during weight loss and other medical conditions associated with increased body weight and appetite.

PII-142

Agrin modulates early stages of skeletal muscle regeneration

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Skeletal muscle regeneration is a multistep process important for maintenance of skeletal muscle mass and function throughout the life span. Satellite cells, key cells involved in regeneration, are activated to form proliferating myoblasts which later form new muscle fibers and thereby repair damaged muscle tissue. Agrin, trophic factor released from motor neurons plays a major role in development and maintenance of the neuromuscular junction. Accelerated agrin degradation has recently been linked to degeneration of the neuromuscular junction and pathogenesis of the ageing-related sarcopenia. Pharmacological strategies to enhance agrin action in sarcopenic muscle may therefore alleviate muscle wasting in the elderly.
We explored whether agrin modulates the early stages of muscle regeneration. Using cultured human myoblasts we found that proliferation of myoblasts from young donors remained unaltered upon acute or chronic agrin exposure. Conversely, agrin enhanced proliferation of myoblasts from aged donors. Notably, myoblast fusion and maturation of the excitation-contraction coupling in myotubes remained unaltered in the presence of agrin, indicating increased proliferation was not linked to impaired myogenic differentiation. While myoblasts expressed agrin receptor Lrp4/MuSK, agrin exposure did not stimulate phosphorylation of downstream kinases ERK1/2 and Abl. In addition, pharmacological activation of Abl markedly suppressed myoblast proliferation. These data suggest that agrin does not stimulate myoblast proliferation *via* the canonical agrin receptor Lrp4/MuSK.
Taken together, our results demonstrate that agrin effects muscle regeneration. Furthermore, we show that agrin stimulates proliferation of myoblasts from old donors, which suggests that agrin could promote the regenerative capacity of sarcopenic muscle. Collectively, our findings support the notion that agrin-based pharmacological approaches may lead to novel treatments for sarcopenia.

PII-143

Transmembrane protein CD9 and glioma cell stemness

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Glioblastoma (GBM) is the most common primary brain tumour, as well as the most aggressive of primary gliomas. Despite modern treatments following diagnosis, the overall median survival remains only about 15 months. The hypothesis that a tumour contains tumour cells acting as stem cells, which are responsible for tumour development, was proposed more than a decade ago, and is still under investigation (1). Yet GBM stem-like cells (GSC) were confirmed to exhibit greater resistance to chemotherapy (2). New approaches for GBM treatment are thus aimed at selective targeting GSC. For this, the selective transmembrane protein markers appear most suitable, due to their accessibility and ease of detection, as compared to intracellular proteins.
By bioinformatics analysis of our own and publicly available omic datasets we searched for genes that encode plasma-membrane proteins, in particular cell surface receptors associated with kinase signalling, which are often overexpressed in GBM (3). The candidate gene CD9 encoding transmembrane protein tetraspanin met these criteria. We showed that CD silencing in GSC lines leads to decreased proliferation, survival, invasion and self-renewal ability, accompanied by altered expression of stem-cell markers CD133, nestin and SOX2. In addition our subsequent *in-silico* investigations within the REMBRANDT database for brain tumours confirmed the prognostic value of CD9, whereby its more than two fold up-regulation correlates with shorter patients' survival. Moreover, orthotopic xenotransplantation of CD9-silenced GSCs exhibiting altered activation patterns of Akt, MapK and Stat3 signal transducers, into nude rats promoted prolonged survival.
We thus confirmed the contribution of CD9 to the malignancy of gliomas and GSCs, and propose CD9 to be investigated in future as a therapeutic target for glioblastoma treatment.
Acknowledgements: This work was supported by Slovenian Research Agency (Programme P1-0245) and INTERREG – SLO -ITA - GLIOMA project 2007-2013.

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PII-144

Loss of a brain-specific tRNA isodecoder affects neuronal function

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The nuclear genomes of higher eukaryotes contain hundreds of transfer RNA (tRNA) genes. Thus, most codons/anticodons are represented by multiple tRNA genes, which led to the assumption that isodecoder tRNAs, that is, tRNAs that share the same anticodon and differ only in their body sequence, are functionally redundant, and compensate for any mutation affecting a single tRNA. This may be true with certain tRNA families, as no disease-linked mutation in a nuclear-encoded tRNA gene has been reported. However, growing evidence suggests that isodecoder tRNAs may have different functional efficiencies, and might not fully compensate for the loss of an isodecoder. Our lab recently identified the first tissue-specific mammalian tRNA gene, *n-Tr20*. *n-Tr20* is one of 5 isodecoders in the nuclear-encoded $tRNA^{Arg}_{UCU}$ family in the mouse genome, and, in contrast to its isodecoders, is exclusively expressed in the central nervous system. We also reported a mutation in *n-Tr20* that impairs its maturation and that loss of *n-Tr20* dramatically reduces the total $tRNA^{Arg}_{UCU}$ pool in the brain. Here, we examined how this loss affects mouse behavior. We found that *n-Tr20* expression modulates seizure susceptibility and neuronal excitability, suggesting a scenario where isodecoders are not fully redundant.

PII-145

Glycogen phosphorylase inhibition has positive effect on metabolism

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Glycogen metabolism regulated by function of glycogen phosphorylase (GP) and glycogen synthase has major role in hepatic glucose production (HGP). Therefore GP inhibition is a potential target in type II diabetes to modulate glucose levels.
We investigated the metabolic effects of glucose-based GP inhibitors KB228 (N-(3,5-dimethyl-benzoyl)-N'-(β-D-glucopyranosyl)urea; K_i = 937 nM), BeVa335 (3-β-D-glucopyranosyl-5-(2-naphtyl)-1,2,4-triazole; K_i= 0.411 nM) by laboratory of László Somsák and CP-316819 (Ingliforib, IC_{50} = 40 nM).
We fed mice with chow or high fat diet (diabetic, obese C57/Bl6J mice on 60% fat hypercaloric diet for 3 months) and investigated the effects of KB228 treatment. KB228 enhanced oxygen consumption and RQ and in the liver led to the overexpression of uncoupling protein-2 (UCP2) in animals and in HepG2 cells. In addition, KB 228 induced mammalian target of rapamycin complex 2 (mTORC2) which may take an active part in increased glycogen deposition and contribute to metabolic adaptation in the cells.
Furthermore, our data demonstrate that KB228 does not only reduce glucose levels but enhance glucose-induced insulin release in mice, therefore we investigated these effects on the cellular model of pancreatic β-cells, MIN6 cells. KB228, BEVA335, SzA37 and CP-316819 improved mitochondrial function, insulin production and insulin secretion in MIN6 -cells in a time-dependent manner. KB228 and BeVa335 treatment intensified MIN6 and beta cell proliferation in mice.
Our data suggest that GP inhibitors not only reduce HGP but induce beneficial metabolic rearrangements too.

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PII-146

Ibogaine effects on uterine smooth muscle contractions: the role of antioxidant enzymes

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The drug ibogaine, which is used for the treatment of substance abuse, is prepared from the root of the shrub *Tabernanthe iboga*. Its biological effects are the result of its interactions with different types of neural transmitter receptors. However, ibogaine also influences cellular energy, redox state and antioxidant capacity in a dose- and time-dependent manner. It has been reported that ibogaine application leads to a decrease in cellular ATP level and an increase in CO_2 production in the first hour of exposure, followed by increased cellular respiration and the production of reactive oxygen species (ROS) which changed redox homeostasis. It has been proposed that the systemic effect of ibogaine is a combination of receptor- and redox-mediated cellular events. Our results show that a single dose of ibogaine (10 mg/L) intensified spontaneous uterine contractility, followed by decreased Cu and Zn containing superoxide dismutase (CuZnSOD) activity after 2 h, and elevated glutathione peroxidase (GSH-Px) activity after 4 h. In Ca^{2+} activated uteri, the same dose was less effective and led to slight inhibition of contractile activity after 4 h, however, it also decreased MnSOD and CuZnSOD activities and elevated GSH-Px and catalase (CAT) activities at the same time point. This points to a changed uterine cellular redox milieu. Pretreatment of active uteri with glibenclamide, a selective ATP-dependent K^+ channel inhibitor, had no effect on the ibogaine-induced changes in contractility, neither in spontaneous or Ca^{2+}-induced uteri during 4 h. However, pretreatment with glibenclamide prevented the decrease in MnSOD activity in Ca^{2+}- activated uteri, and the decrease in CuZnSOD activity in both types of active uteri after 2h. Glibenclamide also prevented the ibogaine-induced increases in GSH-Px and CAT activities. Propranolol, a non-selective beta-adrenoceptor antagonist, also antagonized the ibogaine-induced decreases in MnSOD and CuZn SOD activities after 2h, as well as the ibogaine-induced increases in CAT and GSP-Px activities. Our results suggest that SOD, aside from having an antioxidant role, is also involved in receptor-coupled signaling pathways, at least during ibogaine action. On the other hand, the elevated CAT and GSH-Px activities strongly suggest that hydrogen peroxide is involved in the cellular actions of ibogaine.

PII-147

Secreted phospholipase A2 induces the formation of cytosolic lipid droplets enriched with polyunsaturated fatty acids and enables cancer cell survival

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Cells store neutral lipids within cytosolic lipid droplets (LDs). These newly recognized organelles are composed of a core of neutral lipids (triacylglycerols (TAGs) and cholesterol esters), and are covered with a phospholipid monolayer and LD-associated proteins. Recent evidence suggests that LDs are not passive repositories of energy, but act as platforms integrating cell signalling and metabolism with important implications for metabolic diseases and cancer. Secreted phospholipases A2 (sPLA2s) are lipolytic enzymes that hydrolyse membrane phospholipids to liberate free fatty acids (FAs) and lysophospholipids. They act in the extracellular space on cell membranes and lipoproteins and are involved in atherosclerosis, inflammation and cancer. We have recently described a novel metabolic role of sPLA2s in cancer cells, which includes a stimulation of LD formation and global changes in lipid metabolism leading to prevention of cell death during starvation. We show here that the activity of sPLA2 on cancer cell membranes leads to the release of a mixture of mono- and polyunsaturated FAs (PUFAs). We found that sPLA2-released FAs are taken up by the cell and are incorporated into TAGs of growing LDs. Importantly, lipidomic analysis of LDs isolated from cancer cells treated with sPLA2 revealed a specific enrichment of TAG with PUFAs. Cells treated with PUFAs and sPLA2 show a similar pattern of changes in lipid metabolism, cell survival and activation of AMP-activated protein kinase (AMPK), but differ from those induced by oleic acid, the most abundant product of sPLA2 activity. To the best of our knowledge, this is the first example of PUFA enrichment in mammalian LDs and its (patho)physiological relevance is not yet known. We thus identify sPLA2 as a novel modulator of lipid metabolism that promotes the growth and survival of invasive cancer cells by altering both the composition and amount of cytosolic LDs. Our results also suggest that LDs function as integrators of PUFA metabolism and cell signalling, which may be crucial for the understanding of the various biological roles of sPLA2s and other lipid-modifying enzymes.

PII-148

In vitro study of a nerve-muscle co-culture after electroporation with FUS, a protein involved in ALS/FTLD

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In familial amyotrophic lateral sclerosis (ALS) with fused in sarcoma (FUS) gene mutations, cytoplasmic inclusions positive for the FUS protein accumulate in the neurons and glial cells. Until recently pathological and physiological studies have focused mainly on neuronal abnormalities in neurodegenerative disorders, but it is becoming increasingly evident that astrocytes, oligodendrocytes microglia and skeletal muscle cells also play an important role in neurodegeneration. These cells are responsible for many functions, including maintenance of the extracellular environment, stabilization of cell–cell communications, maintenance of synaptic function, and facilitation of immune response during inflammation, all of which is important in the maintenance of the neuronal environment and the progression of the disease. In this study we employed a complex *in vitro* model of neuro-muscular junction (NMJ) formation using rat embryonic spinal cord explants co-cultured with primary human myoblasts which might give valuable insight into abnormal accumulation and mislocalisation of proteins involved in ALS. Differences in neurite outgrowth and NMJ formation were observed after electroporating spinal cord explants with wild type (pEGFP: FUSwt) and mutant FUS-EGFP (pEGFP: FUS Y526X, pEGFP: FUS Y526F, pEGFP: FUS Y526A, and pEGFP: FUS Y526E). Cell type specific incorporation of FUS was detected by imunocytochemical stains followed by confocal microscopy. All cell types present in the spinal cord were electrotransfected, namely EGFP signal was detected in neurons, astrocytes, oligodendrocytes and Schwann cells. This model might represent a platform to study the role of different ALS/FTLD related proteins in triggering and/or worsening the pathology in a complex system.

PII-149

Localization of dipeptide repeat proteins in human cells

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Amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD) are severe neurodegenerative diseases that represent two ends of a complex disease spectrum. Aggregation of RNA binding proteins is one of the hallmark pathological features of ALS and FTLD, defining them as proteinopathies. Mutations in more than 10 different genes were linked to familial ALS/FTLD, including genes coding for TDP-43 (TARDBP), fused in sarcoma (FUS), and a mutation in the gene C9ORF72. Disease-associated expansions of the intronic hexanucleotide repeat GGGGCC in the C9ORF72 gene can undergo repeat-associated non-ATG (RAN) translation which results in the accumulation of aggregates of pathogenic dipeptide repeat (DPR) proteins: poly(GA), poly(GR), poly(PR), poly(PA) and poly(GP). Poly(GA), poly(GP) and poly(PA) are highly hydrophobic. We will present our study of molecular interactions between ALS/FTLD-associated aggregating hydrophobic DPR proteins and cellular lipid droplets. Constructs for mammalian expression of proteins containing 125 repeats of the dipeptides GA, GP, PA, PR, GR were generated and transiently transfected into HeLa and SH-SY5Y model cell lines. Analysis of apoptotic cells by flow cytometry shows that the proteins $(GA)_{125}$, $(PA)_{125}$ and $(GP)_{125}$ result to be neurotoxic in the model cell lines. Immunofluorescent staining followed by confocal microscopy revealed that the overexpressed proteins $(GA)_{125}$ and $(GP)_{125}$ are localized mainly in the cell nucleus, while $(PA)_{125}$ proteins form filamentous cytoplasmic structures. These results provide an insight into the cellular localization and toxicity of DPRs that are important for our further studies of the interactions between DPRs and lipid droplets in a model of ALS/FTLD.

PII-150

HIV protein Nef is secreted *via* exosomes from Nef-transfected human microglia

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The main targets of HIV in the brain are microglia and astrocytes. Microglia are a type of glial cells that are involved in brain defence and immune system and constitute 10-15% of the brain cells. HIV protein Nef is one of the first and most abundant viral proteins and it was previously detected in autopsied brains of HIV patients. We and others have shown that Nef stimulates its own export *via* exosomes, which affect viability of neighbouring cells. The aim of this study was to examine the effects of Nef-GFP expression on exosome release from human microglia.

hTERT immortalised human microglia, transfected with Nef-GFP plasmid were grown in DMEM supplemented with exosome-depleted FBS. Exosomes were isolated from cell culture media after 1 to 4 days of incubation using one of the following methods: (i) pelleting by ultracentrifugation, washing with PBS and subsequent separation on sucrose gradient; (ii) filtering through 0.22 μm filter, purification on 20% sucrose cushion and washing with PBS; (iii) filtering through 0.22 μm filter and pelleting by ultracentrifugation. Exosomal proteins were extracted with RIPA buffer or TCA precipitation and analysed by western blot for different protein markers. Concentration of exosomes was determined by NTA analysis or by total exosome protein content.

Nef-GFP transfected microglial cells efficiently expressed Nef protein with maximum expression on the second day. Sucrose gradient analysis showed that Nef-containing vesicles separate to fractions that correspond to exosomal fractions. The exosomal character of vesicles was additionally confirmed with electron microscopy. Kinetic studies showed that most of the Nef-containing exosomes are released on the third day after transfection, so we selected this day for all further analyses. Exosome samples contained specific exosomal protein markers like flotillin, Hsc70 and Tsg101. Furthermore, we were interested whether Nef increases the quantity of released exosomes. Quantification with NTA showed that Nef-expressing microglia release 4 times as many exosomes, while the total exosome protein content predicts 5 times as many exosomes, compared to control.

We conclude that Nef increases the release of Nef-containing exosomes. In the future, we will study the function of these exosomes.

PII-151

The effect of lipoproteins and western diet on myocardium

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Elevated blood cholesterol level is a risk factor for development of cardiovascular diseases. The effect of lipoproteins on macrophages and endothelium and their role in the development of atherosclerotic plaque is well studied. However, recent studies indicate that elevated lipoprotein levels also affect the heart itself. In particular, animal models on high-fat diet indicate that the diet induces pathological changes in the myocardium. Even short-term diet aggravates damage after myocardial infarction. Aim of this project is to study the effect of cholesterol on cardiac function, through feeding mice western diet and treating cardiomyocytes with different lipoproteins also in conditions of oxidative stress.
Feeding wild type and heterozygous *Cyp51* knockout mice [1] with western diet consisting of 20% of lipids and 1.25% of cholesterol (versus normal diet with 10% of lipids) leads to adaptive changes in expression of key genes in lipid/cholesterol homeostasis. In left ventricle we measured down-regulation of lipoprotein lipase and up-regulation of cholesterol transporters *Abca1* and *Abcg1*. We observed no pathological changes in myocardial histology (cell area and interstitial connective tissue). Treatment of the mouse HL-1 cell line (atrial cardiomyocytes) with lipoproteins and free cholesterol induced changes in lipid droplet size and intracellular cholesterol level. They affected expression of genes involved in cholesterol homeostasis, endoplasmic reticulum stress and selected miRNA. Lipoproteins affect survival of cells in low serum conditions and oxidative stress induced by H_2O_2. Gene expression studies indicated that two regulatory pathways, the SREBF and LXR/PPAR signalling pathways, are responding to lipoproteins and oxidative stress. VLDL receptor is known to be involved in lipid infiltration in different tissues and its regulation by lipoproteins, cell cholesterol level and oxidative stress will be presented.
The myocardium/cardiomyocytes respond to changes in cholesterol/lipoproteins in their environment (blood, cell medium) by modulating expression of genes involved in cholesterol homeostasis. Lipoproteins affect HL-1 survival under a variety of stress inducing factors.

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PII-152

Metabolism of progesterone in endometriotic 12-Z cells

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Endometriosis is a chronic gynecological disease diagnosed in 30% to 50% of patients with fertility problems. It is an estrogen-dependent disease where the protective actions of progesterone are reduced. This might be the result of 1) decreased expression of the nuclear progesterone receptor or 2) increased metabolism of progesterone in the endometriotic tissue. The aim of our study was to explore metabolism of progesterone at the gene expression and metabolite levels in the model cell line of peritoneal endometriosis 12-Z and in the cell line of normal proliferative endometrium HIEEC. Our results revealed increased expression of *SRD5A1* and decreased expression *AKR1C* in 12-Z cells compared to HIEEC cells, which can lead to increased formation of 5α-pregnanes and lower formation of 4-pregnenes. The gene expression data were confirmed by identification of progesterone metabolites in the 12-Z and HIEEC cells by employing a recently established combined liquid chromatography-tandem mass spectrometry method. These cell lines differed both in metabolic profiles and in the rate of metabolism, which was higher in HIEEC cells. In the 12-Z cells mostly 5α-pregnanes were formed, progesterone was reduced to 5α-pregnane-3,20-dione, 3β-hydroxy-5α-pregnane-20-one, and 3α-hydroxy-5α-pregnane-20-one. In the HIEEC cells progesterone was reduced to 20α-hydroxy-pregn-4-ene-3-one, 5α-pregnane-3,20-dione, 20α-hydroxy-5α-pregnane-3-one, 5α-pregnane-3β,20α-diol, and 5α-pregnane-3α,20α-diol. The si-RNA mediated gene silencing confirmed that in the 12-Z cells, 5α-reduction was catalyzed by SRD5A1, while 3-keto reduction was catalyzed by the AKR1C enzymes. The silencing of *SRD5A1* in the 12-Z cells decreased the rate of progesterone metabolism and reduced the formation of 5α-pregnanes. Our data show that SRD5A1 represents a potential target for treatment of endometriosis.

PII-153

Effect of metformin on the regenerative capacity of skeletal muscle *in vitro*

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Metformin, the most commonly used drug for type 2 diabetes, is in focus of interest due to its antiproliferative effects in cancer. While underlying mechanisms are not fully understood, antiproliferative effects are most likely conveyed through the AMP-activated protein kinase (AMPK). However, proliferation is also an essential step in skeletal muscle regeneration, which importantly determines the mass of regenerated muscle tissue after injury. Thus, while metformin-induced AMPK activation and suppression of proliferation may protect against cancer, they may concomitantly impair the regenerative capacity of skeletal muscle. To explore this possibility, we examined effects of metformin on proliferation of cultured myoblasts and their subsequent fusion into myotubes.

Here we determined that acute exposure to metformin (3-10 mM) markedly suppresses BrdU incorporation in rat L6 myoblasts. Conversely, 10 mM metformin only slightly suppressed BrdU incorporation in human myoblasts, indicating L6 myoblasts are more sensitive to metformin. Low concentrations of metformin (30 and 100 μM), which are similar to those obtained *in vivo* during treatment of type 2 diabetes, did not alter BrdU incorporation neither in L6 nor in human myoblasts. Chronic treatment with 3 mM metformin reduced formation of L6 myotubes by 70% and formation of human myotubes by 30-40%, which again suggests L6 and human myoblasts differ in their sensitivity to metformin. To examine whether lower sensitivity of human myoblasts may be due to the absence of metformin transporter OCT1, we measured expression of OCT1 mRNA. We found that OCT1 mRNA is robustly expressed in L6 and human myoblasts, indicating that differences in metformin uptake likely do not underpin differential metformin sensitivity in these cells. Furthermore, metformin-induced phosphorylation of AMPK and phosphorylation of its downstream target acetyl-CoA carboxylase did not differ between L6 and human myoblasts. Taken together, these results suggest that proliferation of human myoblasts is less sensitive to activation of AMPK than proliferation of L6 myoblasts.

In sum, our results demonstrate that high metformin concentrations may impinge on regenerative capacity of skeletal muscle. However, we also show that human myoblasts are less sensitive to metformin than L6 myoblasts. Collectively, our data indicate that metformin, in doses used for treatment of type 2 diabetes, likely does not impair skeletal muscle regeneration in humans.

PII-154

Biomarkers of protein and lipid oxidative damage as prognostic factors in ESRD patients

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Increased oxidative stress is a hallmark of end-stage renal disease (ESRD) contributing to poor cardiovascular (CV) and overall outcome. It has been shown that susceptibility to oxidative stress in ESRD is influenced by the genetic polymorphism in antioxidant and detoxifying enzymes glutathione transferases (GST). We evaluated the prognostic significance of GST polymorphisms and markers of oxidative stress.
A total of 199 ESRD patients on hemodialysis and 199 age- and gender matched controls were included in the study. GSTA1, GSTM1, GSTP1 and GSTT1 genotypes were determined by PCR and RFLP-PCR method. Markers of oxidative protein (advanced oxidative protein products, AOPP ; carbonyl groups) and lipid (malondialdehyde, MDA; MDA adducts) damage were determined spectrophotometrically and by ELISA. Prooxidant-antioxidant balance (PAB) has also been measured. A 5-year all-cause mortality and cardiovascular mortality were prospectively registered.
Elevated protein and lipid markers of oxidative damage are significantly associated with investigated GST-null/low activity genotypes, predominantly with GSTM1-null genotype (carbonyl groups: p=0.005; AOPP: p=0.001, MDA adducts: p=0.001). The level of oxidative stress is even more pronounced in the patients with all null or low activity GST genotypes. Cox regression analysis demonstrated that stratified values of MDA (HR=1.5, p=0.049, 95%CI=1.00-2.28) and PAB (HR=2.22, p=0.001, 95%CI=1.39-3.52) were independent all-cause mortality predictors. AOPP has shown to be cardiovascular mortality predictor (HR=2.32, p=0.006, 95%CI=1.27-4.24) together with stratified values of MDA (HR=1.89, p=0.021, 95%CI=1.1.-3.25) and PAB (HR=2.5, p=0.003, 95%CI=1.36-4.47). Regarding GST genotype, only GSTM1-null genotype was independent predictor of all-cause mortality (HR=1.79, p=0.009, 95%CI=1.15-2.77).
It may be concluded that null/low GST genotypes are associated with enhanced susceptibility to oxidative stress in ESRD patients. Moreover, GSTM1-null genotype might be considered as a genetic marker of overall death risk. These results also suggest that AOPP, MDA and PAB could contribute to risk prediction of overall and cardiovascular death over known indicators which could improve attempts towards individualization in antioxidant therapy.

PII-155

γKlotho is a novel marker and cell survival factor in a subset of triple negative breast cancers

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Klotho proteins are recently uncovered coreceptors for endocrine FGFs. The family consists of three evolutionary conserved proteins, Klotho, βKlotho, and γKlotho. Klotho is required for the endocrine function of FGF-23 in phosphate homeostasis. Additionally, in an FGF-independent manner, it regulates the function of ion channels, suppresses oxidative stress in cells and inhibits signaling of growth factors. βKlotho is required for the metabolic function of FGF-19 and FGF-21 that control bile acid and energy metabolism. Interestingly, Klothos as modulators of FGF signaling are often epigenetically silenced in several cancers and are generally accepted as tumor-suppressors. The physiological function of the third member, γKlotho is still not known. Here, we investigated the role of role γKlotho in breast cancer. Our results showed that γKlotho is significantly up-regulated in breast cancer, specifically in triple-negative tumors (TNBC), where Klotho and βKlotho are down-regulated. Further, we found that γKlotho is expressed in a subset of TNBC cell lines and when depleted provoked cell cycle arrest and apoptosis. In addition, γKlotho overexpression in MDA-MB-231 cells promoted clonogenic cell growth suggesting γKlotho may represent a potential oncogene for TNBC. To understand the molecular basis of γKlotho oncogenic activity, we performed Illumina microarray gene expression analysis of γKlotho positive HCC1395 cells after treatment with γKlotho siRNA and the corresponding control. Results revealed 205 differentially expressed genes of which 64 are known to be involved in cancer pathogenesis. Interestingly, genes participating in reactive oxygen species (ROS) homeostasis presented one of the prevalent altered categories. Oxidative-stress responsive genes were extensively induced and genes conferring protection against ROS were mostly down-regulated after γKlotho knockdown. Further, we observed persistent high activation of ERK in γKlotho depleted cells, implying increased ROS levels in these cells what we confirmed by flow cytometry analysis. Altogether, our results suggest that γKlotho may be involved in protection of cancer cells against increased oxidative stress and thus facilitating their rapid growth. Thus, γKlotho might represent a marker for patients that would benefit from oxidative cancer therapy and even a novel drug target for TNBC.

PII-156

Proteomic insights in cancer-related extracellular proteolysis with cathepsin K

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Cathepsin K is a lysosomal cysteine protease with collagenolytic activity secreted mainly from osteoclasts with physiological role in bone development. Beside the role in osteoarthritis, recent research has shown that cancer cells can have significant amounts of cathepsin K and that the selective inhibitors can reduce the cancer-related osteolysis and bone metastasis burden (1). To investigate the role of extracellular cathepsin K in the tumor microenvironment we treated different cancer cells with recombinant cathepsin K. This way we identified extracellular proteins released (shed) in the supernatant during the cathepsin treatment. We divided them into two major groups: cell adhesion molecules (e.g. ALCAM, CD44) and cell membrane receptors (e.g. EGFR, TFR-1). The majority of identified targets are known to have important roles in different processes, such as cell migration and invasion. We also identified cathepsin K cleavage sites within collagens, especially in the triple-helical polyproline region. This finding is in good agreement with our recent proteomic profiling of protease specificity, where cathepsin K displayed preference for Pro at P2 position, thus explaining the cleavages in polyproline regions of collagens (2). The group of extracellular cathepsin K substrates is conserved over different cancer cell lines, suggesting its importance in cancer development and progression.

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PII-157

Phosphorylation of FUS 526Y affects its interaction with TNPO1 and nuclear import

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Frontotemporal lobar degeneration (FTLD) and amyotrophic lateral sclerosis (ALS) are neurodegenerative disorders with clinical, genetic, and neuropathological overlap. Aberrant cytoplasmic aggregation of fused in sarcoma (FUS) is associated with 3 % of familial ALS and 10 % of all FTLD cases (FTLD-FUS). FUS is a nuclear RNA/DNA binding protein with PY type nuclear localization signal present at its C-terminus which enables interaction with transportin-1 (TNPO1) and its transport into the nucleus. ALS patients with FUS positive cytoplasmic inclusions contain mutations in gene encoding FUS. The majority of these mutations fall within the nuclear localization signal which disables its transport to nucleus. On the other hand, patients with FTLD-FUS do not have FUS mutations but FUS still accumulates in cytoplasmic inclusions, suggesting a different mechanism of inclusion formation in ALS and FTLD. Our aim is to elucidate if the nuclear localization signal of FUS is subjected to posttranslational modifications that have impact on its localization. We have identified a novel posttranslational modification on the C-terminal tyrosine of FUS. This modification significantly reduces interaction with TNPO1 and consequently affects transport of FUS into the nucleus. We have also identified the tyrosine kinases that lead to the phosphorylation. Our study implicates the 526Y phosphorylation as one of the mechanisms by which nuclear transport of FUS is regulated and potentially perturbed in ALS and FTLD.

PII-158

Drug metabolism and circadian clock interplay in mouse liver

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Circadian rhythms are processes that display endogenous oscillations of about 24 hours. Circadian transcriptome studies show that different organs feature different sets of rhythmic genes with different peak phase distributions. Over 3000 genes of the mouse liver are expressed in a circadian manner, including drug metabolism genes. Circadian clocks are endogenous transcription-translation feedback loop oscillators driving daily rhythmic gene expression. The core clock system (with E-box, D-box, and ROR-element promoter regulation) drives expression of clock-controlled genes; although the core clock system is well studied, details about other clock-controlled processes are still vague.
According to literature, ROR-elements and the REV-ERB/ROR systems (regulators of ROR-elements) could represent a link between the circadian clock and drug metabolism, and are inter-connected to BMAL1 regulation. REV-ERBs have heme as their natural ligand, and cholesterol and other oxidized sterols bind to the activation modulator ROR. In contrast to lipid metabolism, the link of the ROR/REV-ERB system to drug metabolism is not well understood.
Analysis of our core clock mathematical model, which is based on clock gene expression data in mouse liver, shows that most of the phase variability from transcriptome data can indeed be traced back to E-box and ROR-element regulation. Based on the theoretical analysis, we evaluated phase I, phase II and drug transporter genes for their circadian rhythmicity and potential regulation by REV-ERB and BMAL1 in mouse liver.
We measured gene expression of clock and drug metabolism genes in liver by qPCR in C57BL/6 and 129/Sv x C57BL/6 mice after circadian entrainment in 12 h light: 12 h dark conditions. Rhythmically expressed genes from phase I, phase II and drug transporter genes show group-specific phase profiles. Additionally, some genes are only circadian in one mouse genotype. Potential regulation by REV-ERB and BMAL1 was examined through analysis of publicly available ChIP-seq data. As expected, few genes are potential targets of BMAL1, and more of REV-ERB. Model simulations of phase distributions of ROR-element regulated genes support the conclusion that REV-ERB directly regulates some drug metabolism components.

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Analysis of communication networks of glioblastoma stem cell markers

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Gliomas are malignant tumors of glia, the brain connective tissue. Glioblastoma multiforme (GBM) is the most dangerous type of this malignancy. The median survival time of GBM patients after diagnosis is only 15 months despite surgical treatment combined with aggressive chemotherapy. The fatal outcome has been attributed to glioblastoma stem cells (GSCs), which cannot be totally removed by tumor resection because of their location in perivascular niches on tumor edges. After a resection left-over GSCs represent a focal point of cancer relapse. Targeted destruction of GSCs is a promising approach to GBM therapy since it would prevent tumor recurrence. To enable such therapy, GSCs markers need to be discovered and a search is ongoing for new marker candidates.

We have focused on five proteins recently proposed as GSCs markers, namely CD9, FTL, S100A9 [1], β-actin and TRIM28 [2]. There are substantial differences among the five proposed candidates in their cellular locations, functions, connections with other malignancies and experimental methods by which these proteins have been linked to GSCs. Our aims are to analyze cell communication networks of each of the five proposed candidates, to find possible connections between the networks, to clarify the role of the candidates in GBM carcinogenesis and possibly to discover new, better GSCs marker candidates.

By using system biology approaches, we intend to create, for each candidate respectively, cell communication networks for various malignancies in which a particular candidate is involved and where its role has already been explained. These networks will be compared to find similarities and create, by analogy, network models that explain the roles of the candidates in GBM carcinogenesis. The models will be compared to see if they have common points indicating connections among the marker candidates. Such common points may represent or lead to new marker candidates which would be screened with the same approach as the original five. After the screening, the most promising candidates will be experimentally validated.

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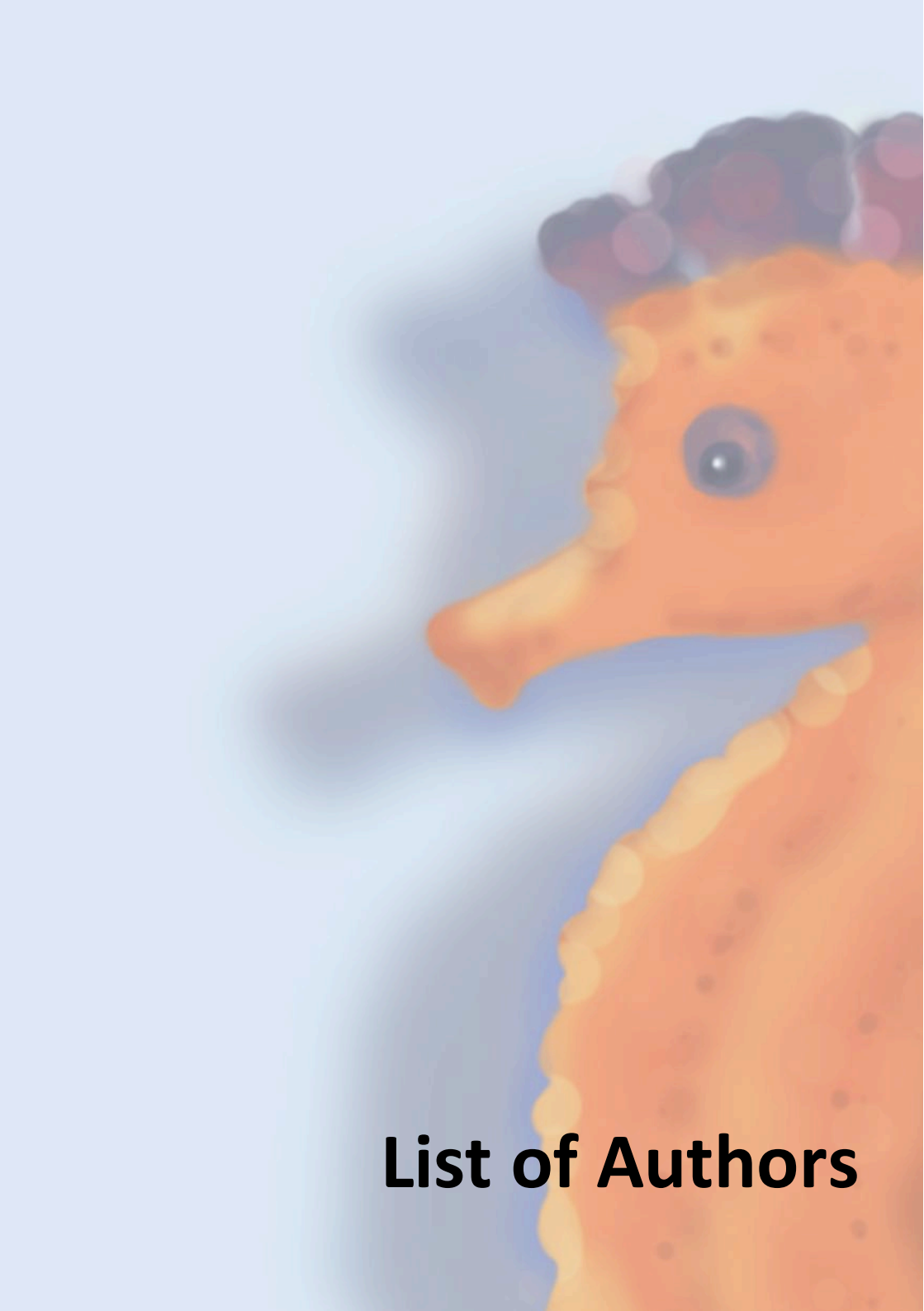

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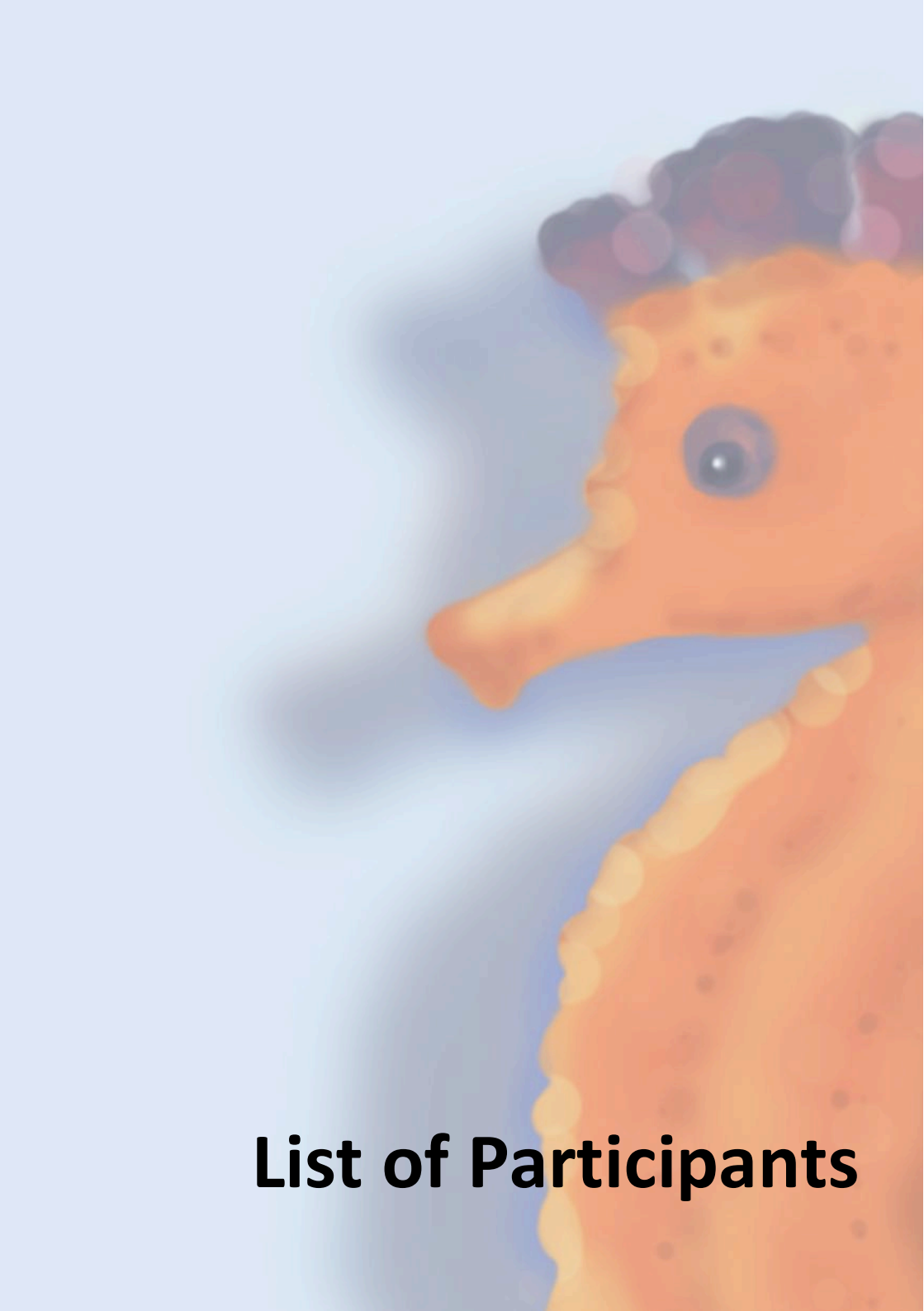

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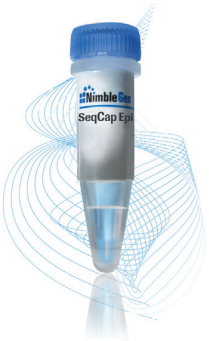

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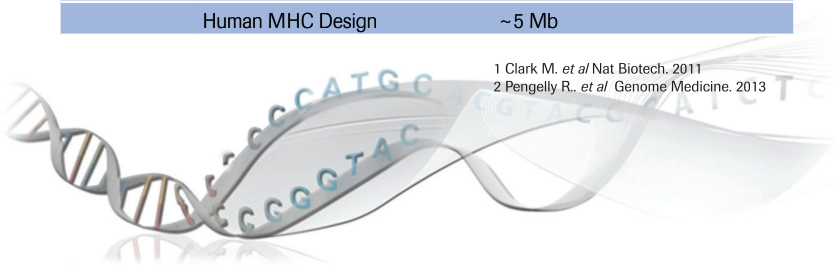

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