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Centre of excellence for integrated approaches in chemistry and biology of proteins

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Centre of excellence for integrated approaches in chemistry and biology of proteins

Third Annual Conference 2012

»Immune response and host microbiota in disease development«

15-16 November 2012

HOTEL LEV, Ljubljana

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Ljubljana, 15 November 2012

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Preface

Centre of Excellence for integrated approaches in chemistry and biology of proteins organizes annual conferences with focus on broader topics that are covered within the Centre's research activities. With the third annual conference we are introducing two themes: **Immune response in health and disease** and **Gut Microbiota in health and disease**.

The lectures given by European and Slovenian speakers will cover several different aspects such as characterization of protease degradomes, novel mechanisms of antigen recognition, microbe –host interactions on the examples of three important pathogens: S. aureus, M. tuberculosis and C. difficile, the role of gut microbiota in immune response and other physiological processes and the role of some specific molecules.
Additionally the overview of EU research activities on microbiota will be provided.

The organizing committee would like to welcome you to the conference and we hope that it will stimulate new ideas and new collaborations.

Maja Rupnik, Livija Tušar, Dušan Turk
November 2012

Contents

Program of the third conference "Immune response and host microbiota in disease development"

15 November 2012

13:00-14.15 Registration and lunch

Conference openings

14:15-14:30 Welcome greetings
Dušan Turk, Scientific Director of CIPKEBIP
Mateja Urlep, President of CIPKEBIP Management Council
Maja Rupnik, Principal Investigator of CIPKEBIP and Organizer of the Conference

Section I: Immune response in health and disease

14:30-15:15 **Kris Gevaert**: Positional proteomics shedding light on immune-related proteases
15:15-16:00 **Tom H.M. Ottenhoff:** Identification of Host Signaling Intermediates Regulating *Mycobacterium tuberculosis* Intracellular Survival Using a Library of Novel Kinase Inhibitors
16:00-16:10 **Cornelis J. Korbee**: Identification of Host Signaling Intermediates Regulating *Mycobacterium tuberculosis* Intracellular Survival Using a Library of Novel Kinase Inhibitors Part II
16:10-16:30 Coffee break
16:30-17:15 **Susanne Engelmann**: A proteomics approach to understand host-pathogen interactions
17:15-18:00 **Pavel Kovarik**: TLR-independent recognition of bacteria

Advisory Board Member Lecture

18:00-18:30 **Imre Berger**: The architecture of human general transcription factor TFIID core complex
19:30 Dinner

16 November 2012

08:30 -09.00 Registrations

Section II: Gut Microbiota in health and disease

09:00-09:45	**Alojz Ihan**: The development of gut immune responses
09:45-10:30	**Hervé Blottière**: Metagenomic of the Human Intestinal Tract: advances from the MetaHIT project on our other genome (oriented towards obesity)
10:30-11:00	Coffee break
11:00-11:45	**Maja Rupnik**: Gut microbiota and pathogenic microorganisms: an example of C. difficile infection
11:45-12:20	**Hervé Blottière**: An overview of funded gut microbiota research projects in EU
12:20-12:45	**Saverio Alberti:** Trop molecules in intestinal development, cell growth regulation and cancer Organizing Committee: Closure of the conference

13:00-14:00 Lunch

Program of second meeting of CIPKEBIP Advisory Board

16 November 2012
Hotel Lev, Ljubljana

14:00-14:15	Welcome greeting Urban Krajcar, Ministry of Education, Science, Culture and Sport
	Introduction Dušan Turk, Scientific Director of CIPKEBIP
14:15-14:45	**Maja Rupnik, Mechanisms and pathways of immune response**
	Sašo Džeroski, Analysis of CIPKeBiP data with Machine Learning
14:45-15:15	**Robert Zorec, Intra and Intercellular communication response**
	Marko Fonović, Proteomic identification of extracellular substrates of cysteine cathepsins
	Toni Petan, Secreted Phospholipase A_2 Prolongs the Survival of Invasive Breast Cancer Cells by Modulating Lipid Storage and Metabolism
	Gregor Kosec, Preparation of novel analogues of chelocardin and FK506 using biosynthetic engineering and chemobiosynthesis
	Jernej Iskra, New methods for synthesis of bioactive organic peroxides
	Andraž Stožer, Glucose Stimulated Calcium Dynamics in Islets of Langerhans from Acute Mouse Pancreas Tissue Slices
	Robert Zorec, Vesicle dynamics in astrocytes
15:15-15:45	**Ana Plemenitaš, Adaptation mechanisms of extremophiles to environment response**
	Cene Gostinčar, Sequencing the genomes of halotolerant and halophilic Fungi
	Metka Lenassi, The effect of the Hog1 kinase inhibitor on the HwHog1 activity in fungus *Hortaea werneckii*
	Marko Šnajder, Expression, purification and structural characterization of recombinant pernisine and its catalytic mutant
15:45-16:15	**Dušan Turk, Protein Bank: Proteins, their production, current use and perspectives**

	Aleksandra Usenik, Structural biology of *Clostridium difficile* virulence factors
	Marko Mihelič, "The same" enzymes different activities - comparison of S. aureus Bifunctional Autolysin and SAV2307/Autolysin E activity
16:15-16:30	Coffee Break
16:30-18:00	Discussion with members of CIPKEBIP Advisory Board – CIPKEBIP scientific guidelines for year 2013 and longer-term

Abstracts

Section I: Immune response in health and disease

Positional proteomics shedding light on immune-related proteases

Petra Van Damme[1,2], Kim Plasman[1,2], Francis Impens[1,2] and Kris Gevaert[1,2,3]

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[2]Department of Biochemistry, Ghent University, Ghent, Belgium
[3]Presenting author

Proteases are active in various cellular responses through proteome polishing, generally by activating or inactivating their substrates. Proteomic approaches for studying protease substrates have come to age over the past decade and now allow for detailed characterization of protease degradomes. One of these so-called positional proteomics approaches is N-terminal COFRADIC which has been used amongst others to profile substrates of the inflammatory mouse caspase-1.

During my seminar, I will discuss the latest developments on N-terminal COFRADIC including strategies for more automated protease substrate identification and for distinguishing efficiently processed substrates from inefficiently cleaved ones. In addition, I will zoom in on novel approaches for studying carboxypeptidases, i.e., their substrates and their detailed specificities.

Several of these technologies have been applied to study immune-related proteases such as inflammatory caspases, granzymes and cathepsins, and a selection of results will be discussed. As an example, I will discuss data on a comparative analysis of human granzyme H and murine granzyme C substrates, show that these are probably functional orthologs and that their extended specificity profiles match those of mouse and human granzyme B. Further, by mutagenesis we could demonstrate that granzyme B cleavage susceptibility of a granzyme H/C specific cleavage site can be introduced by altering the P1-residue to aspartate. Our results thus indicate a hitherto generally underappreciated specificity-determining role of extended protease specificity profiles in steering cleavage susceptibility.

Identification of Host Signalling Intermediates Regulating Mycobacterium tuberculosis Intracellular Survival Using a Library of Novel Kinase Inhibitors

Cornelis J Korbee[1], Mariëlle C Haks[1], Nigel D L Savage[1], Jacques Neefjes[2], Hermen Overkleeft[3], Tom H M Ottenhoff[1]

[1] Department of Infectious Diseases, Leiden University Medical Centre, Leiden, The Netherlands; [2] Division of Cell Biology, The Netherlands Cancer Institute Amsterdam, Netherlands, [3] Leiden Institute of Chemistry and Netherlands Proteomics Centre, Gorlaeus Laboratories, Leiden, The Netherlands

The emergence of drug-resistant *Mycobacterium tuberculosis* (*Mtb*) strains poses an increasingly severe global health problem. Despite increased effort to develop novel antibiotics the number of newly approved antibiotic drugs is declining, prompting for complementing therapeutic approaches. Intracellular pathogens such as *Salmonella typhimurium* (*Stm*) and *Mtb* manipulate host signalling networks to induce a niche for survival but knowledge of molecular interactions at the pathogen-host interface is scarce. The *Salmonella* phosphatase SopB blocks vesicle fusion by activating the host kinase PKB/Akt1, which can be prevented by silencing PKB/Akt1 using RNAi. H-89, a chemical inhibitor of PKB/Akt1, efficiently reduces bacterial loads of human cell lines infected with *Stm* but is less potent at clearing *Mtb* infections. This indicates involvement of PKB/Akt1 in regulation of intracellular survival of *Mtb*, but manipulation of additional mechanisms might be required for complete clearance of the pathogen. This prompted us to modify the chemical structure of H-89, generating a library of novel kinase inhibitors with varying inhibitory capacity and specificity for PKB/Akt1. By screening this library on human cell lines infected with *Stm* or *Mtb* inhibitors with different specificities were identified, including inhibitors with bacterium-specific effects and inhibitors that lost PKB/Akt1-inhibiting capacity whilst still decreasing *Mtb* bacterial load, indicating involvement of other regulators in addition to PKB/Akt1.

A proteomics approach to understand host-pathogen interaction

Susanne Engelmann
Institut für Mikrobiologie, Universität Greifswald, Germany

S. aureus is a human pathogen with strong clinical significance due to increasing infections with multi-resistant isolates. To successfully combat the pathogen a better understanding of its physiology and virulence is required. Under infection conditions *S. aureus* is confronted with a multitude of signals

including growth-limiting factors and life-threatening host defense mechanisms and, hence, adaptation of bacterial gene expression is a multi-signal response. Global protein expression profiling is an excellent approach to show the pattern and the level of the proteins expressed under definite conditions. The cytoplasmic proteome of *S. aureus* was analysed in growing cells and in cells exposed to a collection of infection related stress and starvation stimuli. This proteomic view of stress/starvation responses of *S. aureus* promoted follow up-studies aiming at the comprehensive description of single regulons, their signal transduction pathways and their adaptive functions and finally their integration into complex gene expression networks which will be demonstrated for the oxygen starvation response with Rex as a master regulator. A well-studied library of proteomic patterns generated under defined *in-vitro* conditions is an essential basis to decipher the complex adaptational processes in *S. aureus* which are induced by an multi-stimulating environment as it is the case in *in-vivo* experiments and finally in the host. With these tools at hand, it is now possible to move to more complex, infection-relevant experimental models ranging from host cell tissue cultures, to animal infection models and even to the analysis of *S. aureus* isolated from human body fluids and tissue samples.

The virulence of a given *S. aureus* isolate is determined by its ability to express several virulence factors. Extracellular proteins constitute a reservoir of virulence factors and have been shown to play an important role in the pathogenicity of bacteria. The comprehensive analysis of the extracellular proteome of *S. aureus* reveals whether individual virulence genes (i) are expressed at all and secreted, and if yes, (ii) in which quantities, (iii) under which environmental conditions and (iv) whether they are immunogenic. As expected the extracellular protein pattern is very heterogeneous among the different isolates. However, this is not only due to the diversity of the virulence gene repertoire but also to variations in the expression rate of the respective genes as well as to secretion and modification processes which makes the anyhow complex virulence gene repertoire of the different isolates even more complex.

TLR-independent recognition of bacteria

Pavel Kovarik
Department of Microbiology and Immunobiology, Max F. Perutz Laboratories (MFPL), University of Vienna

Recognition of pathogens by the immune system is fundamentally important for successful host defence against infection. Bacteria are recognized by the immune cells through a number of pathogen recognition receptors (PRR). The receptors of the Toll-like Receptor (TLR) family are the

most frequently employed PRRs. We investigate the mechanisms of the recognition of the Gram-positive *Streptococcus pyogenes* by innate immune system. *S. pyogenes* is a human pathogen that causes a broad range of diseases. The bacterium colonizes the throat and the skin where it can evoke usually mild illness such as strep throat or scarlet fever. Systemic infections are less frequent but can develop into life-threatening diseases such as necrotizing fasciitis and toxic shock syndrome. The immune system launches a usually successful response that is initiated by a so far not understood recognition of this pathogen. These cells produce upon infection a variety of cytokines that orchestrate a full blown protective response.

We excluded the individual role of all well characterized TLRs. The receptor for *S. pyogenes* signals via the adaptor MyD88 since cytokine burst induced upon *S. pyogenes* infection is almost completely abolished in cells lacking MyD88. We demonstrate requirement for Unc93b1, a protein involved in endosomal trafficking. These data suggest that components of *S. pyogenes* are recognized after phagosomal processing of bacteria. Further analyzes revealed critical role of streptococcal RNA in pathogen recognition. We demonstrate that insufficient production of cytokines renders the host more susceptible to infection as mice deficient in type I interferon signaling are more susceptible to infection. Similarly, *S. pyogenes* strain inducing less cytokines is more virulent than a strain inducing strong cytokine burst.

Together, our data suggest that immune cells have evolved ways to recognize bacterial pathogens which escape recognition by the standard repertoire of PRRs.

The architecture of human general transcription factor TFIID core complex

Imre Berger
EMBL, France

Gene transcription is regulated by a plethora of proteins in human cells. General transcription factor TFIID, a 1.6 MDa multiprotein complex, plays a key role in this process. Human TFIID is composed of the TATA box binding protein (TBP) and it's associated factors (TAFs).

A 700 kDa core TFIID module was discovered in vivo, which is of pivotal importance for TFIID integrity and assembly. We have produced fully recombinant human TFIID core complex by using MultiBac, and determined the structure by cryo-electron microscopy and hybrid methods. The structure of core-TFIID, and molecular mechanisms of holo-TFIID assembly, will be presented.

Section II: Gut Microbiota in health and disease

The development of gut immune responses

Alojz Ihan
University of Ljubljana, Medical Faculty, Ljubljana

The infant's immature intestinal immune system develops as it comes into contact with dietary and microbial antigens in the gut. The evolving indigenous intestinal microbiota have a significant impact on the developing immune system and there is accumulating evidence indicating that an intimate interaction between gut microbiota and host defence mechanisms is mandatory for the development and maintenance of a balance between tolerance to innocuous antigens and capability of mounting an inflammatory response towards potential pathogens.

Priming of naive T cells will occur mainly in Peyer's patches (PP), where surface receptors will help to initiate migration of the cells to the submucosa. Various antigen-presenting cells (e.g. dendritic cells, M cells) will present antigen-derived epitopes and initiate either non-responsiveness, or an immune response. The development of specific T helper 1 (Th1)/Th2/Th17 cells is a major branch point in the immune response. It is also an important determinant of whether the response to food will lead to tolerance or hypersensitive reactions.

Metagenomic of the Human Intestinal Tract: advances from the MetaHIT project on our other genome

Hervé M. Blottière
Functionality of the Intestinal Ecosystem – FinE Lab, INRA UMR 1319 Micalis, Jouy en Josas cedex, FRANCE

The human intestinal microbiota is of great complexity. Each individual hosts about one trillion bacteria belonging mainly to three phyla (Firmicutes, Bacteroidetes and Actinobacteria). Dominated by not yet cultivable bacteria, the complexity of the microbiota led to the development of a powerful new approach, Metagenomic, which allowed rapid progress in the characterization of genomic and genetic diversity of human gut microbiota. Thus, the microbial metagenome (microbiome) contains ~ 150 times more genes than the human genome. The microbiota can be considered as an organ of the host in its own right.

To understand the impact of the intestinal microbiota on human health, a thorough knowledge of its genetic potential is essential. The European-funded consortium MetaHIT undertook the sequencing, assembly and characterization of intestinal metagenome. A catalog of 3.3 million non-redundant bacterial genes was obtained from stool samples of 124 European individuals (Qin et al, 2010). The microbiota of each individual in the cohort includes on average 540,000 genes, from which approximately 40% are highly prevalent among individuals, defining conserved core functions, while another part of the genes is rare and often subject-specific. The analysis of the overall metagenomic sequences identified three clusters of individuals characterized by particular microbiota structures called Enterotypes that are observed regardless of the continent or nation of origin (Arumugam et al, 2011). This indicates the existence of a limited and balanced symbiosis between the microbiota and the host. These Enterotypes are defined by the presence of certain bacterial genera and seem not correlated either with age, body mass index, or sex. A comparison of metagenomes from patients with inflammatory bowel disease or obese with controls revealed a state of dysbiosis of the microbiota in patients and allowed the identification of bacterial species associated with these diseases, although it is not yet known if these states of dysbiosis are a cause or a consequence.

During millions of years of coevolution, the host has developed an immune response to control its complex microbial ecosystem. Thus, the gut microbiota contributes to local development and regulation of intestinal homeostasis, maturation of the immune system and maintenance of the ecosystem. Our knowledge of the contributions of the microbiota on health is still in its infancy and the cellular and molecular mechanisms underlying its interaction with intestinal cells remain unclear. To better decipher the mechanisms of interactions between the metagenome and the cells of its host, an innovative approach of functional metagnomics was developed, allowing the identification of bacterial genes involved in the cross-talk with the host (Lakhdari et al, 2010).

The overall work highlights the need to better understand the functions of the gut microbiota and its role in maintaining health. The characterization of our second genome, our metagenome, remains a key step towards a thorough understanding of Human Physiology.

- *Arumugam M, et al. Enterotypes of the human gut microbiome. Nature, 473, 174-80, 2011.*
- *Lakhdari O, Cultrone A, Tap J, Gloux K, Bernard F, Ehrlich SD, Lefevre F, Doré J, Blottière HM. Functional metagenomics: a high throughput screening method to study microbiota-driven cell signaling modulation in the human gut. PLoS One. 5: e13092, 2010.*
- *Qin J. et al. A human gut microbial gene catalogue established by metagenomic sequencing. Nature. 464: 59-65, 2010.*

Gut microbiota and pathogenic microorganisms: an example of *C. difficile* infection

Rupnik Maja[1,2,3], Škraban Jure[3], Livija Tušar[3] , Ženko Bernard[3,4], Džeroski Sašo[3,4,5]
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C. difficile infection (CDI) is a prominent example of the role of intestinal microbiota in protection against pathogens. Although *C. difficile* spores are present in up to 20% of healthy carriers, bacterium can colonize the gut and cause the disease only when gut microbiota is disturbed. The main reason for changes in gut microbiota is oral antibiotic therapy and CDI is well recognized cause of antibiotic associated diarrhoea. The role of commercial probiotics in treatment of CDI is still controversial. However, faecal biotherapy, a method replacing gut microbiota by faecal transplantation, has more than a 95% success rate in patients with multiple recurrences of CDI.

Studies on *C. difficile* colonization and changes in gut microbiota are based on clone libraries, DGGE analyses or on different sequencing approaches. Most of them have studied only the bacterial microbiota and have not included archaea and fungi. We have used a simple molecular method (DHPLC - denaturing high pressure liquid chromatography which separates DNA amplicons based on fragment size and sequence) combined with machine learning analysis methods, to identify different patterns in the composition microbiota in *C. difficile* colonised and uncolonised humans and animals.

The microbes associated with *C. difficile* colonization were found to be different in humans and poultry. In chickens, the absence of *Acidaminococcus intestini* was recognised as the main predictor for the good *C. difficile* growth. In humans, the key predictor associated with *C. difficile* negative samples was *Bifidobacterium longum.* Also, colonization with *C. difficile* ribotype 027 was associated with lower heterogeneity of gut microbiota, suggesting that *C. difficile* could actively influence the microbiota.

In conclusion, this is the first study to show that in addition to bacteria, fungal microbiota is also important in *C. difficile* colonisation and that composition patterns of the gut microbiota and not a single microorganism is predictive for *C. difficile* colonization in humans and poultry.

An overview of funded gut microbiota research projects in EU

Hervé M. Blottière
Functionality of the Intestinal Ecosystem – FinE Lab, INRA UMR 1319 Micalis
& US 1367 MetaGenoPolis Jouy en Josas cedex, FRANCE

The human gut microbiota has been rediscovered over the last decade, thanks to advances in sequencing technologies and bioinformatics. Indeed, it is now possible to investigate the 100 trillion bacteria that harbours a healthy human gut. The first stage in deciphering the symbiotic rapport between intestinal microorganisms and their human host is to characterize the gene composition of a healthy gut microbiome and the differences or dysbiosis associated with diseases. These were the objectives of large consortium such as the European Metagenomics of the Human Intestinal Tract (MetaHIT) and the US Human Microbiome Project. At the initiative of the French National Institute for Agricultural Research (INRA), an international consortium gathering scientist and funding agencies all over the world involved in the analysis of the human microbiome (IHMC) in health and disease agreed to carry out their efforts according to a set of commonly agreed policies. In parallel to MetaHIT, a Marie Curie initial training network named "Cross-Talk" was funded in 2008 with the aim to train early stage scientists towards the emergence of a supra-discipline, the human microbiome metagenomics. Further in 2011, INRA coordinated another European project, the IHMS - International Human Microbiome Standards - was launched to harmonise practices and facilitate the comparison of data between projects of different origins. Another consortium, Tornado acronym for "Molecular Targets Open for Regulation by the gut flora – New Avenues for improved Diet to Optimize European health" headed by scientist from the Karolinska Institute in Stockholm was set up to perform a systemic and comprehensive mechanistic approach to study the health effects of dietary products and the role of gut microbiota. In November 2012, a new Collaborative project METACARDIS, Metagenomics and Integrative Systems Medicine of Cardiometabolic Diseases was launched to study the relationships between gut microbiota, assessed by metagenomics, and host genome expression regulation using systems medicine multilevel approaches. The goal is to improve understanding and innovative care of cardiometabolic diseases (CMD) and their comorbidities.

In the mean time, several national funding agencies supported projects to deeply study the human microbiome and its link with health and disease. Among them, the Irish Eldermet project focussed on the faecal microbiota of elderly volunteers. The purposes were to correlate diversity, composition, and metabolic potential of the faecal microbial metagenome with health, diet and lifestyle indices. Other project focussed on Obesity including the French ANR project, MicroObes, which aimed to qualify the relationship between the gut microbiota and the nutritional and metabolic status of the host. Further, the MetaGenoPolis project was developed in the frame of the French Investment for the Future. MetaGenoPolis has as strategic goal to demonstrate the impact of the human gut microbiota on health and disease, by making available cutting-edge metagenomics technology, quantitative and functional, to the medical, academic and industrial communities.

Human being recognised as a supra-organism, the proliferation of projects taking into account the key role of our second genome will allow a better and thorough understanding of the Human Physiology.

Trop molecules in intestinal development, cell growth regulation and cancer

Saverio Alberti
Center of Excellence for Research on Aging, Unit of Cancer Pathology,
Foundation University "G. D'Annunzio", Chieti, Italy

Trop-1/EpCam and Trop-2 are monomeric glycoproteins that define a unique family of single-pass type I transmembrane proteins. The TROP genes are highly conserved across species suggesting a strong evolutionary pressure for a conserved functional role. However, Trop-1 is expressed at high level in normal colon and small intestine, whereas Trop-2 is not. To gain insight into the function of Trop-1 in intestinal development, we generated mTrop1/EpCAM knockout mice. Remarkably, mTrop1 knockouts were found to develop congenital tufting enteropathy (CTE) through disregulation of intestinal E-cadherin/β-catenin, and loss of mucosal barrier integrity. mTrop1 knockouts are born alive, but fail to thrive and die soon after birth because of hemorragic diarrhea. The intestine from the mTrop1 knockout mice shows intestinal tufts, villous atrophy and colon crypt hyperplasia as in human CTE. No structural defects were detected in other organs. These results demontrate that TROP1 loss causes CTE. In the affected enteric mucosa E-cadherin and β-catenin were shown to be disregulated, leading to disorganized transition from crypts to villi, with progressive loss of membrane localization and increasing intracellular accumulation, thus unraveling an essential role for Trop-1 in the mantainance of intestinal architecture and functionality.

Consistent with a crucial role during development, Trops were shown to be expressed by progenitor cells of diverse lineages, i.e. epithelial, germinal and hematopoietic, and by cancer stem cells, e.g. Trop-1/ESA in breast cancer, Trop-2 in prostate cancer. Trop-1 and Trop-2 were indeed shown to stimulate cancer cell growth, overexpression above the baseline being sufficient to trigger this growth stimulus. A tightly-interconnected transcription factor regulatory network was found connected to Trop-2 (TP63/TP53L, GRHL1/Get-1,TCF-1/HNF1A, SPI1/PU.1, GLIS2, FOXM1 and FOXP3), with cyclin D1 and NF-kB as major downstream effectors, starting unraveling the major determinants of Trops in cancer growth.

Annual Meeting with the Advisory board members: reporting of workpackage leaders and scientists

Introduction Dušan Turk, CIPKEBIP Scientific Director

By September 15 we have fulfilled the plan for 2012. We managed to spent 80.38% of the total budget, within this 68.39% were spent for the research equipment. Currently 300k EUR are needed to maintain the equipment without the costs for labor.
The main indicators for successful work in 2012 were:
- the number of innovations at least 7 (11 submitted)
- the number of patents at least 1, (1 international patent submitted) (page 42).

CIPKEBIP participated in submission of proposals for calls at national and international levels:
- infrastructure project - national call (CIPKEBIP is coordinator),
- FP7 project (CIPKEBIP as partner),
- first step proposal in ALPE ADRIA IPA call,
- two proposals for early stage researchers – national call,
- 4 large project proposals – national call.

Next year we plan to organize 2-3 workshops with researchers from European countries with the goal to prepare proposals for Horizon 2020 calls mainly addressing biomedicine.

Maja Rupnik, Mechanisms and pathways of immune response

The work package Mechanisms and pathways of immune response is divided into three subtopics, two of which will be covered within other WPs: Generic mechanisms of immune response (overlap with WP 4), Modulation of immune response by small molecules (overlap with WP 2) and Pathogens and their interactions with host. Research on host-pathogen interactions were focused mostly on *C. difficile* epidemiology, potential *S. aureus* and *C. difficile* transmissions between humans and animals, on virulence factors associated with *C. difficile* survival in specific hosts/environments and on microbiota in humans and animals in association with *C. difficile* colonization. The later studies were presented also during the conference programme.

Analysis of CIPKeBiP data with Machine Learning

Saso Džeroski (a,b,c), Dragi Kočev (a), Bernard Ženko (a,c), Marko Fonović (c,d), Boris Turk (c,d), Jure Škraban (c,e), Maja Rupnik (c,e,f)

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c) Centre of Excellence for Integrated Approaches in Chemistry and Biology of Proteins, Jamova cesta 39, Ljubljana, Slovenia
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f) Institute of Public Health Maribor, Prvomajska ulica 1, Maribor

Within CIPKeBiP, the Centre of Excellence for Integrated Approaches in Chemistry and Biology of Proteins, a variety of protein-related data are collected within the different workpackages (WPs). For example, in the WP Intra and Intercellular communication response, proteomic identification is performed of extracellular substrates of cysteine cathepsins. In the WP Mechanisms and pathways of immune response, data are collected on the composition of gut micro-biota in humans and chickens under different conditions (incl. the presence of C. difficile).

We use machine learning methods, and in particular predictive clustering trees, to analyze such data. For the extracellular substrates of cysteine cathepsins, we learn to distinguish between proteins that are enriched and proteins that are not, based on their annotations, which include their functions and the pathways they participate in. For the case of gut micro-biota, we learn to relate the presence of different ribotypes of C. difficile and the composition of the micro-biota.

Enej Kuščer, Robert Zorec, Intra and Intercellular communication response

Work Package 2.2 focuses are studies are signaling pathways both on the level of organelle-mediated signaling as well as protein-mediated:

- the inter and intracellular communications and their mechanisms,
- the link between communication pathway malfunctions and subsequent onset and pathology of the corresponding disease,
- development of novel and existing natural products (small molecules) and their derivatives.

Particularly, we are studying the function of subcellular compartments in astrocytes, their cytoplasmic trafficking and the mechanism of fusion of vesicle membrane with the plasma membrane. We are also studying the role of lysosomes in autophagy and are trying to understand

the role of PKA and Cdk5 in the signaling pathways leading to exocytosis of insulin. On the level of protein-mediated signalling, we are studying protein kinase signalling in diabetes (particularly PKA and Cdk5), protease signalling leading to cancer cell death with focus on lysosomal cathepsins and caspases, and phospholipase signalling in cancer with major focus on the role(s) of sPLA2s.

Better understanding of complex signaling pathways in various biological systems will allow us to develop molecular analogs with improved pharmacological properties, fewer side effects and/or extended area of clinical application in the fields of cancer, autoimmune, cardiovascular, and neurodegenerative diseases. In the scope of this work package we are using a number of existing natural products as well as novel analogs produced by methods of biosynthetic and semisynthetic engineering.

Proteomic identification of extracellular substrates of cysteine cathepsins

Marko Fonović[1,2], Boris Turk[1,2]

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[2]Centre of excellence for integrated approaches in chemistry and biology of proteins, Ljubljana, Slovenia;

Proteases play a crucial role in cell surface signaling pathways and extracellular matrix remodeling. Cell surface proteins can be activated, inactivated or can undergo other changes in their function upon proteolysis. Cysteine cathepsins are a group of papain-like cysteine proteases, which are mainly located within the endosomes/lysosomes. They execute non-specific bulk proteolysis and participate in numerous specific physiological processes such as protein processing, antigen presentation and processing, bone remodeling and apoptosis. In certain pathological states such as cancer, cathepsins can be translocated to the cellular membrane or secreted in the extracellular space, where they are known to promote angiogenesis, proliferation and tumor invasion. Only a few extracellular cathepsin substrates have been identified to date and their exact role in cancerogenesis still remains largely unknown. In the course of our work we have used proteomic approach for identification of cell surface cathepsin substrates, that led to the identification of a novel group of membrane protein substrates for cathepsins L, S and B. We were the first who have shown that cathepsins can act like sheddases and cleave specific protein domains from the cell surface. Among the identified cathepsin substrates are receptors (CD71, plexins, ephrin receptors, neuropilins) and adhesion proteins (CD44, CAM proteins). The majority of identified susbstrates are known to be involved in cancer progression. Our results show, that cathepsin extracellular activity directly promotes cancer cell invasion and migration. Moreover, an analysis of the tumor samples from a pancreatic mouse cancer model demonstrated that these proteins are processed *in vivo* and their levels are reduced in cathepsin S-deficient mice, thus providing a mechanistic link between the cathepsin's activity and cancer progression. Our results have provided a novel insight into the

regulation of cancer development at the molecular level and could open the way toward discovery of novel cancer biomarkers and therapeutical approaches for cancer treatment.

Secreted Phospholipase A_2 Prolongs the Survival of Invasive Breast Cancer Cells by Modulating Lipid Storage and Metabolism

Anja Pucer[1], Vesna Brglez[1], Jože Pungerčar[1], Gérard Lambeau[2], Toni Petan[1]
[1]Department of Molecular and Biomedical Sciences, Jožef Stefan Institute, Ljubljana, Slovenia;
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Tumour cells display progressive changes in metabolism that support their increasing demands for growth and proliferation as well as promote cancer cell survival under conditions of metabolic stress. Increasing evidence suggests that changes in lipid metabolism are an integral part of this metabolic transformation. There are ten structurally distinct secreted phospholipase A_2 ($sPLA_2$) enzymes in humans that are involved in a variety of physiological and pathophysiological processes, ranging from lipid digestion and host defense against pathogens, to lipoprotein remodeling, atherosclerosis, asthma and cancer. The mechanisms of their action in many of these processes are however still unknown, due in a large part to the large variety of metabolic fates and signaling pathways triggered by the products of their hydrolysis, free fatty acids and lysophospholipids. We have found that the expression levels of several human $sPLA_2$s vary greatly in breast cancer cells, mostly due to epigenetic silencing, suggesting distinct functional roles for these enzymes in breast cancer. In line with this idea, the group X $sPLA_2$ displayed disparate effects on the viability, proliferation and survival of breast cancer cell models reflecting different subtypes of the disease. Our latest results reveal that the group X $sPLA_2$ promotes lipid droplet formation through the products of its hydrolysis, which enables prolonged survival of highly invasive breast cancer cells under nutrient and serum starvation induced stress. The stimulation of a lipogenic pro-survival phenotype in cancer cells by the human group X $sPLA_2$ may have important implications for cancer prevention and treatment.

Preparation of novel analogues of chelocardin and FK506 using biosynthetic engineering and chemobiosynthesis

Tadeja Lukežič[1], Ajda Podgoršek[1,5], Gregor Kosec[1,5], Marko Blažič[2], Dušan Goranovič[1], Tomaž Polak[2], Štefan Fujs[1], Jaka Horvat[1], Enej Kuščer[1,5], Branko Jenko[3,5], Stojan Stavber[4,5], Hrvoje Petković[1]
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Polyketides are a large group of structurally versatile but biogenetically related bioactive compounds. In millions of years of evolution their structures have evolved to bind to cellular proteins with very high affinity and thereby specifically affect key metabolic or regulatory (communication) pathways of eukaryotic and prokaryotic cells. Many polyketides are currently used for treatment of disease, based on their antibacterial, antifungal, anticancer, immunosuppressive and other activities. Emerging antibiotic resistance and other unmet needs in other clinical areas, especially cancer, require intensive research in polyketide field in order to further expand their structural diversity and improve their biological activity.

We have taken advantage of increasing availability of DNA sequencing to develop genetic tools and apply biosynthetic engineering approaches to modify two polyketide-producing organisms: *Streptomyces tsukubaensis*, producer of immunosuppressant FK506 and *Amycolatopsis sulphurea*, producer of structurally and functionally unusual tetracycline antibiotic chelocardin.

Using the chemobiosynthetic/mutasynthetic approach on *S. tsukubaensis* we have established that the AT4 domain of FK506 PKS shows broad substrate specificity, allowing us to produce a novel FK506 analogue. After isolation and structure confirmation the biological activity of the compound and its role in cellular communication will be assessed. Furthermore, the compound carries a chemically amenable side chain, thus allowing for further semi-synthetic derivatization and diversification of chemical structure.

We also used biosynthetic engineering in order to obtain novel chelocardin analogues with potentially superior activity and pharmacological properties. Two strains of *A. sulphurea* obtained by targeted mutagenesis in chelocardin biosynthetic cluster showed a particularly high productivity of 2 novel analogues and additional 2 mutations lead to novel analogues in lower yields. The two former strains were cultivated in larger volume and the compounds purified from fermentation broths using solid phase extraction and/or preparative HPLC methods. The structure of one of the compounds, where polyketide synthase starter unit has been modified, was confirmed using LC-MS and NMR and is currently being tested for antibacterial activity.

New methods for synthesis of bioactive organic peroxides

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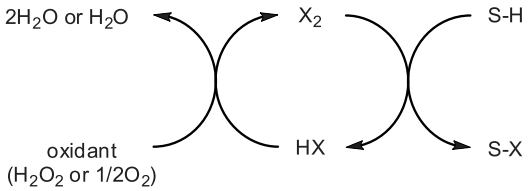
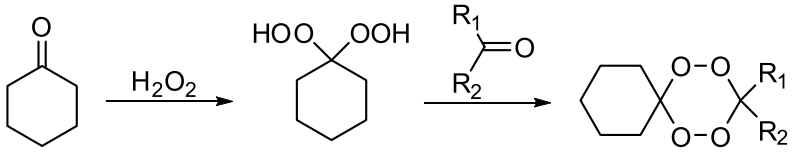
Oxidation is one of the basic chemical trasformation as well as the main process in biological systems.

Our study is connected with the investigation of new methods in oxidation reactions with emphasis on "green" oxidants – hydrogen peroxide and oxygen. In our research on halogenations we are studying oxidative halogenation reaction as an interesting method that can replace a use of molecular halogens that are corrosive, toxic and hazardous chemicals. The main challenge is in activation of oxygen or hydrogen peroxide to act as oxidant for in situ generation of active oxygen species.[1,2]



Another aspect of hydrogen peroxide chemistry is connected with organic peroxides. We are studying peroxidation of carbonyl compounds (ketones, aldehydes) into bioactive peroxides. We have found out, that hydrogen peroxide is activated in situ by solvent evaporation leading to fast, simple and efficient synthesis of *gem*-dihydroperoxides. This molecules were further transformed into 1,2,4,5-tetraoxanes, a potent bioactive molecules acting as antimalarials, anti-helmintic, anti-proliferative agents etc. Our results show that different hydroperoxidic products can be formed from ketones leading to the preparation of different types of cyclic and acyclic peroxides. As a consequence the structural diversity of bioactive cyclic peroxides is importantly enlarged.[3,4]



1. A. Podgoršek, M. Zupan, J. Iskra, ***Angew. Chem. Int. Ed***. **2009**, 48, 8424 – 8450.
2. J. Iskra (ed.) *Halogenated Heterocycles: Synthesis, Application and Environment*. In series: Topics in Heterocyclic Chemistry, Springer Verlag, **2012**.
3. J. Iskra, Antimalarial peroxides : from artemisinin to synthetic peroxides. ***J. malar. res.***, **2011**, vol. 1, no. 1/2, str. 1-40.
4. K. Žmitek, S. Stavber, M. Zupan, D. Bonnet-Delpon, S. Charneau, P. Grellier, J. Iskra, Synthesis and antimalarial activities of novel 3,3,6,6-tetraalkyl-1,2,4,5-tetraoxanes. ***Bioorg. med. chem***., **2006**, vol. 14, str. 7790-7795.

Glucose-Stimulated Calcium Dynamics in Islets of Langerhans from Acute Mouse Pancreas Tissue Slices

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In endocrine cells within islets of Langerhans cell stimulation is coupled to hormone secretion by calcium ions. Since the advent of modern fluorimetry, numerous *in vitro* studies employing almost exclusively isolated mouse islets have investigated the effects of glucose, the physiologically most important secretagogue on cytoplasmic calcium, predominantly in insulin-secreting beta cells. Due to technical shortcomings, insights of these studies were inherently limited to a rather small subpopulation of outermost cells. The results of these studies also depended on various factors, like culture conditions and duration, and were not always easily reconcilable with findings *in vivo*. The main controversies regard the types of calcium oscillations, presence of calcium waves, and the level of synchronized activity. We set out to combine the *in situ* acute mouse pancreas tissue slice preparation with noninvasive fluorescent calcium labeling and subsequent confocal laser scanning microscopy to shed new light on the existing controversies utilizing an innovative approach enabling the characterization of responses in many cells from all layers of islets. Our experiments reproducibly showed stable fast calcium oscillations on a sustained plateau rather than slow oscillations as the predominant type of response in acute tissue slices. Furthermore, we found evidence that calcium waves are the mechanistic substrate for synchronization of oscillations. We also found indirect evidence that even a large amplitude calcium signal was not sufficient and that metabolic activation was necessary to ensure cell synchronization upon stimulation with glucose. This way, employing a novel method we resolved existing controversies and showed that our experimental approach has the potential to help answer important physiological questions, making it one of the methods of choice for the foreseeable future.

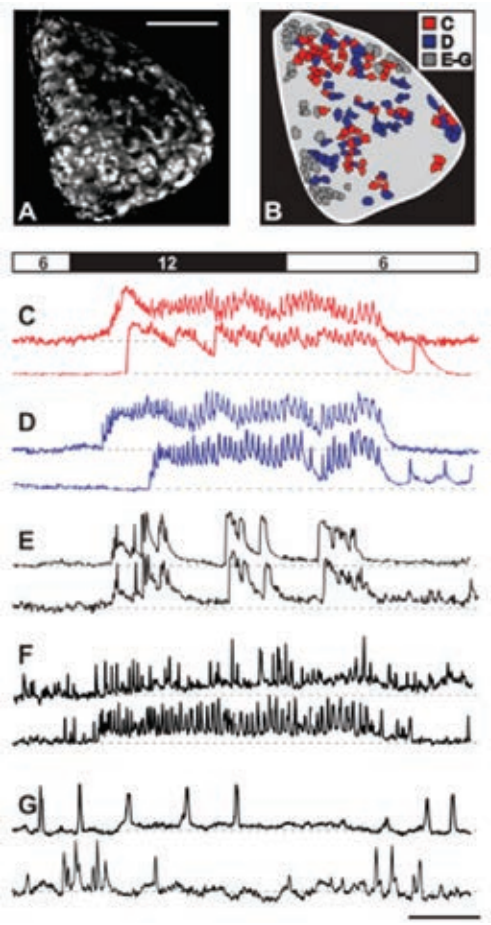

Figure 1. Spatiotemporal $[Ca^{2+}]_i$ patterns in a representative islet of Langerhans upon stimulation with 12 mM glucose. A A high resolution image, used as a reference to choose regions of interest corresponding to individual cells and to assess motion artefacts, showing that Oregon Green® 488 BAPTA-1 effectively labeled most of the cells within the focal plane. **B** A schematic color-coded representation of the position of cells in A that responded to stimulation with 12 mM glucose as shown in C-E (N = 177 cells). The grey area indicates unlabelled or unresponsive cells. We detected six different types of responses to glucose. The types of responses presented in C and D were predominant and are characteristic of beta cells. **C** Slow transient response followed by oscillations superimposed on a sustained plateau (type 1, N = 63 cells). Note the difference in time required for activation between the upper and lower trace, the synchronicity of Ca^{2+} oscillations superimposed on the sustained plateau and of deactivation, as well as the presence of a transient increase in Ca^{2+} in the lower trace after the sustained plateau has subsided. **D** Response as in C but without a clear transient phase (type 2, N = 61 cells). **E** Responses representative of non typical beta cells. **F** Responses representative of delta cells. **G** Responses representative of alpha cells. Scale bar in A indicates 100 microns. Scale bar in G indicates 200 seconds. In Y axes signals were normalized to individual maxima.

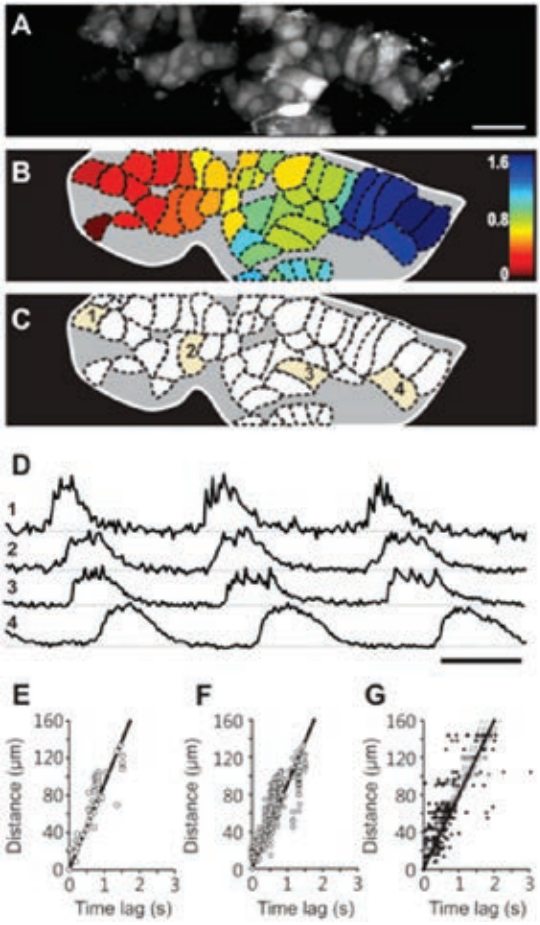

Figure 2. Spatiotemporal characterization of $[Ca^{2+}]_i$ oscillations superimposed on the sustained plateau. A High resolution image (1024x256 pixels) that served as a reference to select regions of interest indicated in B and to assess possible motion artefacts. Scale bar indicates 20 microns. **B** Color-coded time delays for every cell demonstrating the average direction of spreading of $[Ca^{2+}]_i$ waves for 6 consecutive $[Ca^{2+}]_i$ oscillations. **C** 45 individual cells whose signals were included in the analyses. Temporal traces of highlighted cells indicated with 1-4 are plotted in D. **D** 3 consecutive $[Ca^{2+}]_i$ oscillations in 4 cells indicated in C. Fluorescence is presented as F/F_0, with maximum equaled for the presented cells. Scale bar indicates 2 seconds. **E** Time delays between the beginning of a $[Ca^{2+}]_i$ oscillation in the cell in which the wave originated and the beginning of the $[Ca^{2+}]_i$ oscillation in any given cell as a function of the Euclidean distance between the cell of wave origin and the respective cell, for a single $[Ca^{2+}]_i$ oscillation in 45 cells from the islet shown in A-C. The regression line gives an average velocity of 92 µm s^{-1} (R^2=0.76, p<0.001). **F** After taking into account 6 consecutive spikes in the same set of cells, the same average speed was obtained (R^2=0.73, p<0.001). **G** For 4 different islets, with 6 consecutive $[Ca^{2+}]_i$ oscillations in 10 cells from each islet, the average speed was 80 µm s^{-1} (R^2=0.62, p<0.001). The respective values obtained in 4 islets were 98 µm s^{-1} (R^2=0.83, p<0.001), 88 µm s^{-1} (R^2=0.34, p<0.001), 80 µm s^{-1} (R^2=0.40, p<0.001), and 74 µm s^{-1} (R^2=0.46, p<0.001). In Figures E-G, x and y axes are chosen as to enable representation of speed by the slope of the regression lines. However, velocities were calculated with distances representing the independent and time lags being the dependent variable.

Vesicle dynamics in astrocytes

Marko Kreft, Jernej Jorgačevski, Priyanka Singh, Saša Trkov, Maja Potokar, Matjaž Stenovec, Valentina Lacovich, Ajda Flašker, Alenka Guček, Boštjan Rituper, Nina Vardjan, Helena Chowdhury, Robert Zorec

University of Ljubljana, Medical Faculty

Vesicles are subcellular organelles playing a number of important functions in eukaryotic cells, including a role in cell-to-cell communication. To visualize theses structures in astrocytes, the most abundant glial cells in the central nervous system, super-resolution optical methods are being employed. In the last period the STED microscope has been successfully tested for the first optical channel. In the second half of April 2012 Dr. Jernej Jorgačevski in Dr. Marko Kreft have been involved in the assembly and testing of this platform at Laser Laboratorium Goettingen with Dr. A. Egner and Dr. C. Geisler. During this period the system for fluorophore depletion was established, with the adjustments of excitation and emission optical lines. In June, 2012 the Fianium laser was sent for calibration and upgrades to the manufacturer to readjust the synchronisation of laser pusles at different outputs. To achieve time synchronicity the optical fibres were adjusted also for polarization. Dr. Jernej Jorgačevski was again in Goettingen at the end of September and most of October, 2012, where the first prepartions made in Ljubljana were imaged. The Figure shows and example of confocal and STED images of vesicles.

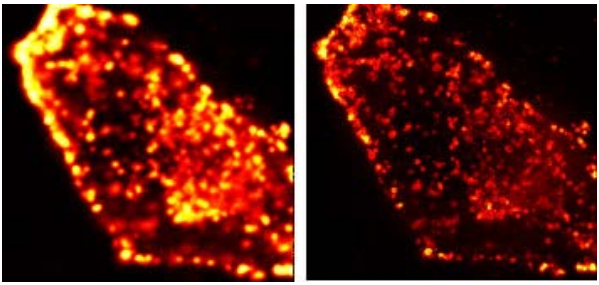

Figure: Left panel shows a confocal micrograpf of a lactotroph, labelled by the vesicular marker prolactin. The same cell is imaged on th eright by the STED microscope. Modified form Jorgačevski et al., (2011) *J. Neurosci.* 31:9055.

During the last period we have also been using the system for super-resolution SIM (Elyra), where the sub-vesicular architecture of the synaptobervin 2, a vesicular protein playing a role in memnrane fusion, was studied. Yellow synaptophluorin (spH), cosntruvted by the Dr. Parpura's lab (University of Alabama, Birmingham, USA) specially for these experiments was employed. In addition to transfecting this construct into astrocytes, we also used immunocytochemistry to label cytoplasmic domains of synaptobrevin 2/VAMP. In thi syear several papers have been published in relation to vesicle dynamics in our labs: Vardjan et al. (2012) *J. of Neuroinflammation* 9:144; Potokar et al. (2012). *Glia* 60(4):594; Trkov et al., (2012) *Glia*; 60(9):1406.

Nina Gunde-Cimerman, Ana Plemenitaš, Adaptation mechanisms of extremophiles to environment response

Sequencing the genomes of halotolerant and halophilic Fungi

Cene Gostinčar[1,2], Metka Lenassi[2,3], Martina Turk[1], Nina Gunde-Cimerman[1,2], Ana Plemenitaš[2,3]

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[2] CIPKeBiP Centre of excellence for integrated approaches in chemistry and biology of proteins, Ljubljana, Slovenia
[3] Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

Hypersaline environments, in which high concentrations of salt prevent growth or even survival of the majority of microorganisms, include food, soil and water habitats. Despite the hostility of these environments, microbial life can exist over the whole range of salt concentrations. Not only specialized Archaea and Bacteria, some Eukaryotes are also able to withstand the loss of water and the sodium toxicity. Among them, Fungi are especially successful and adaptive. The extremely halotolerant black yeast *H. werneckii* for example has been found in nature across the entire salinity range – in contrast to halophilic Archaea, which are killed by the non-saline medium.

Research on halotolerant and halophilic organisms has been made worthwhile due to their large biotechnological potential. They are a rich source of industrially important enzymes, they are important for bioremediation of hypersaline environments and represent a valuable (but still largely unused) genetic resource. Although most studies are focused on prokaryotic halophiles, for some applications their fungal counterparts are even more promising. Good examples of this are the efforts to increase halotolerance (and drought tolerance) of baker's yeast *Saccharomyces cerevisiae* (important for the production of bioethanol fuel) and various crops (soil salinization is a major agricultural problem worldwide).

Our research on *H. werneckii* and ecologically similar fungi has already led to several discoveries of halotolerance enabling mechanisms. Until recently, studies have been hindered by the fact that the genome sequences of these species were not available. For this reason we have acquired the whole genome sequence of *H. werneckii* in collaboration with the Canada's Michael Smith Genome Sciences Centre. These data are expected to open new possibilities of studying eukaryotic halotolerance, especially when they will be combined with knowledge gained by sequencing and analysing the genomes of other halotolerant/halophilic fungi. Two of these have already been sequenced in collaboration of the researchers from University of Ljubljana with institutions abroad.

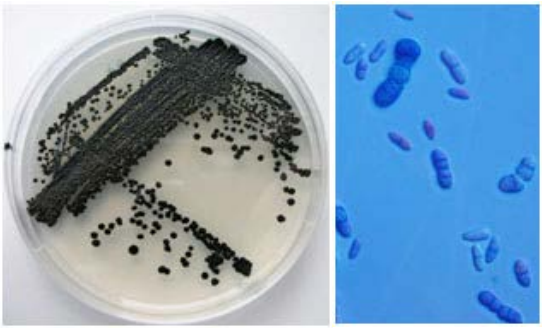

Figure: *Hortaea werneckii* on YNB medium (left) and under the microscope (right).

The effect of the Hog1 kinase inhibitor on the HwHog1 activity in fungus *Hortaea werneckii*

Anja Kejžar[1], M. Grötli[2], M. Tamás[3], Ana Plemenitaš[1], Metka Lenassi[1,4]

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[2] Medicinal Chemistry, Department of Chemistry, University of Gothenburg, Göteborg, Sweden
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Despite several previous studies on osmotolerance of the extremely halotolerant *Hortaea werneckii*, the key role of the MAP kinase HwHog1 in the process remains unsolved. HwHog1 activation is regulated by the HOG signaling pathway, through which the signal is transferred from the membrane osmosensors to the MAP kinase cascade inside the cell. This triggers HwHog1 phosphorylation, which leads to its activation and regulation of expression of target genes and activity of target proteins. To resolve this question, we employed a chemical-genetic approach using a novel inhibitor of the native Hog1 kinase (PD447), a *Saccharomyces cerevisiae* homologue of the HwHog1 kinase.

First, we constructed a yeast plasmid with inserted intron-less *HwHOG1* gene, transformed it into the *S. cerevisiae* strain with deleted *HOG1* gene *(Δhog)* and confirmed with western blot analysis that HwHog1 was expressed. The described system enabled us to study the effect of the inhibitor in the same genetic background as used for the Hog1 inhibitor testing. Functional complementation assays and western blotting using antibodies against phospho(p38) showed, that HwHog1 complemented the MAP kinase Hog1 function in *Δhog* mutant and rescued its osmosensitive phenotype. Next, the inhibitor plate assays and growth curve measurements in 96-well microtiter plates showed that

PD447 efficiently inhibits the HwHog1 kinase when the kinase is expressed in *Δhog* mutant. The PD447 dependent growth restriction was less pronounced when observing the effect of inhibitor in liquid media.
Importantly, inhibitor plate assays and growth curve measurements showed effect on the osmotolerance also in *H. werneckii*. We observed partial growth inhibition at osmolarities higher than 3.5 M NaCl , which is consistent with full activation phenotype of HwHog1 only at 3,5 M salt concentration or higher. To check if the retained osmotolerance of *H. werneckii* is the consequence of the effective export of PD447 from *H. werneckii* cells by the Pdr5 pleiotropic drug resistance efflux pumps, we inactivated the pumps by inhibitor FK506. The inhibition assays surprisingly demonstrated partial growth inhibition of *H. werneckii* even in cultures with only FK506 added, and only small additional inhibition when PD447 and FK506 were present. It seems that PD447 is retained in the *H. werneckii* cells and that the remaining growth is the consequence of other mechanisms involved in osmotolerance (like adaptations in cell wall structure, membrane fluidity and function of different pumps and transporters). Considering the impaired growth of *H. werneckii* in the presence of salt and FK506 inhibitor, a pathway likely involved in osmotolerance is the calcineurin-mediated Ca^{2+} signaling pathway.

Expression, purification and structural characterization of recombinant pernisine and its catalytic mutant

Šnajder Marko[1], Mihelič Marko[2,3], Turk Dušan[2,3], Poklar Ulrih Nataša[1,2]
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[3] Institute Jožef Stefan, Ljubljana, Slovenia

Pernisine is a serine protease secreted from hyperthermophilic archaeon *Aeropyrum pernix* K1 that grows optimally at temperatures higher than 90°C. Thermostable proteases are potentially biotechnologically interested for food industry, decontamination processes of waste or leather industry.

Protein expression with non-optimized nucleotide sequence did not produce pernisine at detectable level. We constructed expression vectors with codon-optimized synthetic sequence adjusted for *E. coli*. Expression of recombinant pernisine and its proposed catalytic mutant (S355A) was performed in *E. coli* using autoinduction media. Proteins were purified with automated Aktaxpress™ chromatography system using Ni^{2+} Sepharose and Superdex S200 column. The recombinant pernisine was identified with tandem mass spectrometry.

Pernisine activity was confirmed by zymogramphy. The mutation of S355A in pernisine caused completely lost of its activity.

The secondary structure elements of pernisine were estimated from circular dihroism (CD) spectra. The pernisine at pH 8.0 and 25°C had 30% α-helix, 46% β-sheet, 10% β-turn and 14% random structure, while at 90°C the amount of α-helix, β-sheet, and β-turn decreased. Predicted secondary structure of pernisine obtained by NPS program contained 13% α-helix, 30% β-structure and 56% random structure. Near-UV CD-spectra of pernisine indicated at 90°C the complete lack of the tertiary structure. From the UV-melting experiments, we determined the temperature of denaturation at 61°C.

Homology based 3D model structure of pernisine was made using GENO3D program.

Figure 1: 3D model structure of pernisine using Geno3D program

Basic crystallization screening tests of pernisine and its catalytic mutant resulted in the appearance of new crystals that will be further used for 3D structure determination.

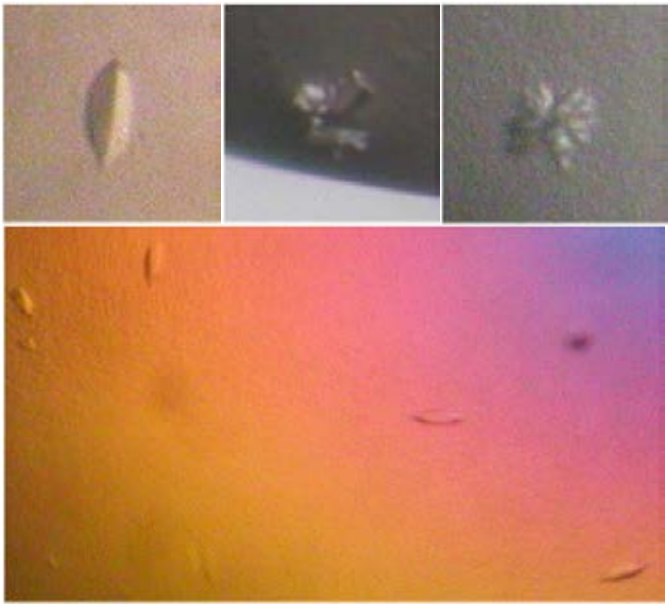

Figure 2: Crystals of recombinant pernisine

Dušan Turk, Protein Bank: protein production, use and perspectives

High throughput protocols for *E.coli* and insect and mammalian cell lines are established. The protein production platform setup is almost complete (missing are the static light scattering analyzer and mammalian cell line facility). Crystallization platform (automatic crystal growth imaging) is in the process of being purchased.

Through the high throughput protein production and the medium throughput structure determination we have reached the low throughput stage of functional analysis of targeted proteins and their chemistry.

The following groups of proteins are being tackled:

- lysosomal proteins / hydrolases (Marko Mihelič, Andreja Doberšek, Miha Renko) The crystal structures of two human hydrolases Alpha fucosidase I and di-N-acetilhitobiase were determined. Characterization of the active site residues by mutagenesis and cocrystallization with substrates is in progress. Expression of MHC class II complexes was published in FEBS Lett. (Majera et al., 2012)
- surface proteins from human pathogens
  - *S. aureus* (Marko Mihelič, Andreja Doberšek, Miha Renko) From final 27 targeted proteins on the surface of the bacteria understanding of involvement of autolysins in biofilm formation is most advanced (report below M. Mihelic), while 4 additional ones are in the structure determination process/
  - *C. difficile* (Aleksandra Usenik, Miha Renko, Gregor Pretnar, Maja Rupnik) From 12 targeted surface proteins 3 are now in crystal structure phasing step. (report by S. Usenik)
- proteins from extremophiles
  - *H. Wernecki* (Marko Mihelič, Metka Lenasi, Miha Renko, Ana Plemenitaš) Crystal structures of Hal2a and Hal2b have been determined, yet did not provide insight into the role of the specific peptide, which is assumed to be crucial for the adaption to the environment of high salt concentration.
  - *Aeropyrum pernix* (Marko Šneider, Marko Mihelič, Naraša Poklar Urlih, Hrvoje Petković) Detailed report is given by Marko Šnajder.

Structural biology of *Clostridium difficile* virulence factors

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Clostridium difficile is a spore-forming, anaerobic bacillus and a major cause of antibiotic-associated diarrhea that can lead to potentially lethal pseudomembranous colitis. In the past ten years substantial increase in incidence and severity of nosocomial as well as communal *C. difficile* infection worldwide has led to discovery of new virulence factors, nevertheless the effects of these virulence factors variants on strain pathogenicity and human immune response have yet to be elucidated. Moreover, out of the two categories of *C. difficile* virulence factors only a few crystal structures of parts of toxins (TcdA, TcdB, CdtA) and cell surface proteins (SlpA) have been determined so far. Therefore a set of proteins has been investigated: several cell surface proteins out of the 17 open reading frames (CD2782,..., CD2799) in the surface protein cluster, toxins (TcdA, TcdB) and regulatory proteins involved in expression and secretion of toxins (PaLoc proteins TcdR, TcdC, TcdE and CdtLoc protein CdtR). In addition to the C terminal functional domain crystals of spontaneously truncated protein CD2786, crystals of the intact proteins CD2784 and CD2799 were also obtained. Native X ray diffraction data sets to resolutions 2 Å, 2.2 Å and 2.5 Å were collected, respectively. In order to solve the phase problem, derivative crystals are in preparation. Trypsinisation of other proteins (CD2791, CD2795 and CD2796) was successfully implemented as crystallization rescue strategy to gain crystals of their stable fragments (functional domains). Optimization of crystallization conditions and crystal testing are in progress. Out of all the investigated proteins at least three structures are expected to be determined and along with biochemical characterization to provide background for the rational drug design and/or vaccine discovery.

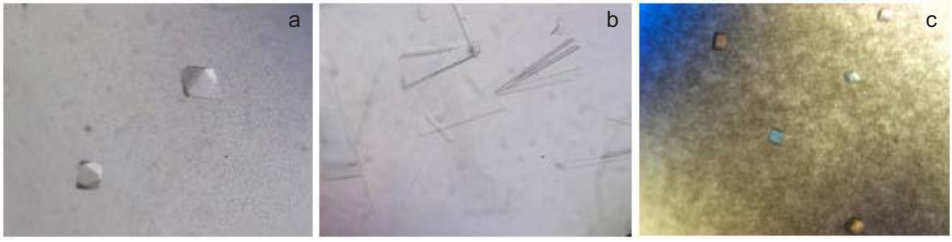

Figure 1: Crystals of CD2786 C terminal domain (a), CD2799 (b) and CD2784 (c)

"The same" enzymes different activities - comparison of S. aureus Bifunctional Autolysin and SAV2307/Autolysin E activity

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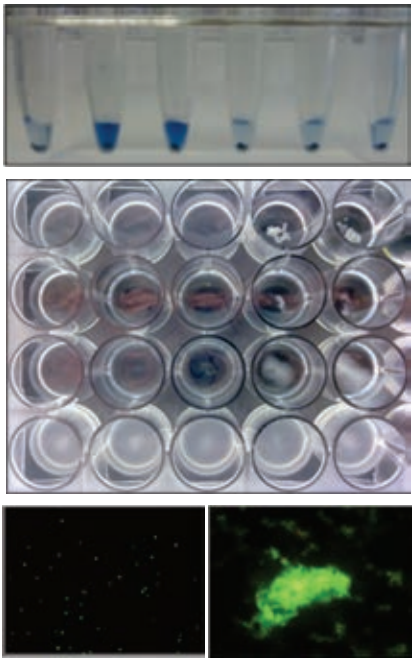

Autolysins are group of the bacterial cell-wall modifying enzymes that play an essential role in a number of different processes including cell growth and division. On the basis of their specificity they can be divided in three broad groups: amidases, glucosaminidases and peptidases. Since they participate in the key processes of bacterial survival, they are considered as primary candidates for development of novel strategies in combating the spread of bacterial infection.

Staphylococus aureus MRSA strains are one of the most common sources of hospital-acquired infections, which are associated with increased mortality of infected patients. Therefore S. aureus is considered as key model for studying bacterial pathogenesis. Several different peptidolycan hydrolases (Autolysins) from S. aureus have already been characterized including Bifunctional Autolysin (BFAtl). It is believed that BFAtl is the predominant staphylococcal Autolysin. It consists of two enzymatic domains, one with the amidase and the other one with the glucosaminidase activity. The silencing of the BFAtl gene causes severe defects in the cell growth and division. Sequence analysis of various S. aureus strains, however, showed presence of at least 4 additional genes with the high degree of sequence homology (50 %) to glucosaminidase domain of BFAtl . The activity and function of these sequences, which are present as single domain proteins or as part of multidomain proteins, is still unclear.

To examine the activity of S. aureus glucosaminidases, we have developed a system for production of recombinant glucosaminidase domains and adopted protocols for assessment of their activity. Comparison of the activity of BFAtl glucosaminidase domain and SAV2307/Autolysin E showed striking differences - not only at the level of protein stability and activity, but also on the ability of the living S. aureus cells to form biofilms. Our data suggest that these high-sequence gene homologues do not play redundant roles, but are more likely involved in specialized cellular processes.

Poster Session

Dihaloiodates(I): Synthesis with Hydrogen Peroxide and Halogenating Activity

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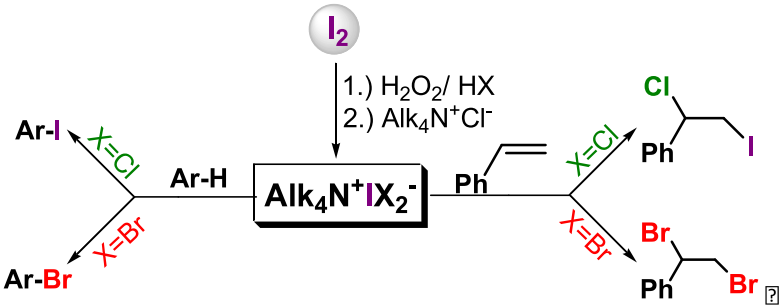
In organoiodine molecules iodine can be found in different oxidation states and forms. Most common are iodine (I) compounds, however, iodine is also capable to form stable hypervalent compounds, usually as iodine(III) and iodine(V) species. Iodine(I) compounds differ in chemistry to iodine(III) and (V) compounds and serve mainly as electrophylic iodinating reagents (NIS, IPy_2BF_4, ICl).

Dichloroiodates(I) are less known analogues, although they were used for iodination of aromatics, alkenes, enaminones and flavones. Structural analogues of dichloroiodates(I) are dibromoiodates(I) and difluoroiodates(I). There is little known on chemistry of dihaloiodates(I). According to the literature, dichloroiodates(I) are prepared using strong and dangerous oxidants *i.e.* ICl, Cl_2 or hypochlorite. Due to our interest on the use of "green" oxidants for oxidative halogenation,[3] we will present a study on the use of hydrogen peroxide for oxidation of iodine into iodine(I) compounds (IX_2^- salts) with an emphasis on a solvent-free method for the preparation of dihaloiodates(I) using different forms of hydrogen peroxide for oxidation of iodine in the presence of halide ions. In addition, a study on the activity of tetraalkylammonium and pyridinium dichloroiodates(I) ($Alk_4N^+ICl_2^-$) and dibromoiodates(I) ($Alk_4N^+IBr_2^-$) as halogenating reagents will be presented.



1. Zhdankin, V. V.; Stang, P. J., *Che.l Rev.* **2008,** 108, 5299.
2. Filimonov, V. D.; Semenischeva, N. I.; Krasnokutskaya, E. A.; Ho, Y. H.; Chi, K. W., *Synthesis* **2008**, 401. Zefirov, N. S.; Sereda, G. A.; Sosonuk, S. E.; Zyk, N. V.; Likhomanova, T. I., *Synthesis* **1995**, 1359. Hajipour, A. R.; Arbabian, M.; Ruoho, A. E., *J. Org. Chem.* **2002,** 67, 8622-.
3. Podgorsek, A.; Zupan, M.; Iskra, J., *Angew. Chem. Int. Edit.* **2009,** 48, 8424.

Epigenetic Silencing Of Secreted Phospholipases A_2 In Human Breast Cancer Cell Lines

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Cancer cell survival depends on aberrant epigenetic modifications that enable tumourigenesis and cancer progression. Secreted phospholipases A_2 ($sPLA_2$s) are lipolytic enzymes that liberate fatty acids and lysophospholipids from membranes and have recently been associated with the pathology of colorectal, gastric and prostate cancers. The aim of this study was to determine the involvement of epigenetic mechanisms in the regulation of $sPLA_2$ expression in a panel of cell line models of breast cancer.

Treatment of cells with a DNA-methyltransferase inhibitor (DNMTi) led to a significant increase in the expression of human group IIA, III and X (hGIIA, hGIII and hGX, respectively) $sPLA_2$s, indicating that DNA hypermethylation is responsible for $sPLA_2$ silencing in breast cancer. Bisulphite sequencing of hGIIA, hGIII and hGX $sPLA_2$ promoter regions and treatment of cells with transcription factor inhibitors suggested that Sp1, estrogen receptor α, retinoic acid receptor α and SREBP transcription factors are crucial for $sPLA_2$ silencing by hypermethylation.

Additionally, the expression of hGIIA, hGIII and hGX $sPLA_2$s was restored in cells treated with a histone deacetylase inhibitor (HDACi), especially in the most tumourigenic cell line, and it was even further augmented upon treatment with both DNMTi and HDACi. This indicates that both DNA hypermethylation and histone acetylation are involved in $sPLA_2$ silencing in breast cancer, particularly in highly tumourigenic and invasive cells.

Our results confirm the importance of epigenetic factors in downregulation of hGIIA, hGIII and hGX $sPLA_2$ expression in breast cancer, suggesting a functional importance of these enzymes in development and progression of the disease.

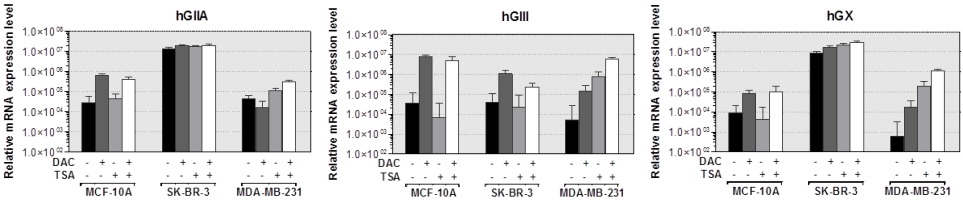

Fig. 1: Relative mRNA expression levels of hGIIA, hGIII and hGX $sPLA_2$s in different breast cancer cell lines upon treatment with DAC or/and TSA.

Group X Secreted Phospholipase A_2 Stimulates Growth And Survival Of Invasive Breast Cancer Cells by Releasing Fatty Acids And Modulating Lipid Metabolism

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Recent studies have implicated several secreted phospholipase A_2 ($sPLA_2$s) enzymes in the pathology of cancer, with roles in either tumour promotion or inhibition, depending on the tissue and biochemical microenvironment of the tumour involved. A pro-tumourigenic role has been suggested for the group X $sPLA_2$ ($sPLA_2$-X) in colon cancer, however, its role in breast cancer has not been studied yet. The aim of our study was to elucidate the influence of $sPLA_2$-X on cell growth and survival of model mammary cell lines with different tumorigenicity. Low nanomolar concentrations of exogenously added recombinant $sPLA_2$-X increased cell survival and proliferation of the highly invasive cell line MDA-MB-231, but decreased the viability of weakly and moderately tumorigenic cells. Ectopic expression of $sPLA_2$-X promoted cell proliferation of MDA-MB-231 as well. The proliferative effect was strictly dependent on $sPLA_2$ enzymatic activity, as it was completely prevented by the $sPLA_2$ inhibitor varespladib and was not observed in cells treated with the enzymatically inactive active-site H48Q mutant of $sPLA_2$-X or in those forcibly expressing the same mutant. Most importantly, exogenously added as well as ectopically expressed $sPLA_2$-X prevented serum-withdrawal induced cell death of MDA-MB-231 cells most probably by inducing pro-survival and anti-apoptotic signalling associated with changes in lipid storage and fatty acid metabolism. The effects of $sPLA_2$-X on the growth and survival of breast cancer cells suggest distinct and possibly contrasting roles for the enzyme at different levels of progression of the disease and reveal previously unknown connections between $sPLA_2$-mediated fatty acid release and alterations of lipid metabolism in cancer.

AEROBIC OXIDATIVE IODINANTION OF ORGANIC COMPOUNDS CATALYSED WITH NITRIC(V) ACID

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An investigation concerning an environmentally friendly method for oxidative iodination of organic compounds using molecular iodine, air oxygen as the oxidant and catalytic amounts of HNO_3 was carried out. In accordance with the principles of green chemistry an efficient and selective transition-metal free system I_2/air/$HNO_{3(cat.)}$/$H_2SO_{4(cat.)}$ for introducing iodine atom into organic molecules with 100 % iodine atom economy was developed. The effect of reaction conditions on the aerobic oxidative iodination of acetophenone was studied and an efficient and selective α-iodination of different aryl-alkyl ketones was achieved. In the case of 2,4-dimethoxyacetophenone influence of solvent on regioselectivity of iodination was observed: in MeCN corresponding α-iodo ketone was exclusively formed, while in AcOH iodination of aromatic ring was preferential. Furthermore, iodofunctionalization of 1,3-diones and benzene derivatives was studied. The catalytic system I_2/air/$HNO_{3(cat.)}$/$H_2SO_{4(cat.)}$ in AcOH proved to be very efficient for iodination of methoxy and methyl substituted benzenes but less suitable in the case of 1,3-diones, since iodinated compounds seem to be very unstable in acidic media. While activated benzene derivatives required only short reaction times and mild reaction conditions, successful iodination of benzene and bromobenzene was achieved in TFA as solvent and with longer reaction time.

BINDING OF α-SYNUCLEIN TO MODEL LIPID MEMBRANES

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Alpha-synuclein is 140-amino acid protein that is widely distributed in the brain. Although the native function of α-synuclein remains unknown, it is proposed that the protein is involved in the maintenance of the synaptic vesicle reserve pool of the brain. Alpha-synuclein is remarkable for its structural variability. It exists as almost unstructured monomer in aqueous solution, while its aggregation leads to oligomeric and fibrillar β-structures which are thought to be involved in the patoghenesis of Parkinson's disease and related synucleinopathies. After binding to particular

membranes protein forms α-helical structure. It is assumed that binding depends on electrostatic interaction and size of lipid structures.

We investigated the SUV binding and the coil-helix transition of human wild-type (**WT**) α-synuclein and its mutants (**Y39A**, **Y125A** and Y(125,133,136)A - **3X**) by using circular dichroism (CD) and differential scanning calorimetry (DSC).

After binding to negatively charged small unilamellar vesicles á-synuclein and its mutants undergo a transition from unordered into á-helical structure. Neutral lipid vesicles do not impact protein secondary structure. The highest increase in the formation of α-helix was observed at temperature of gel-to-liquid phase transition, suggesting a key role of co-existance of different lipid phases for α-synuclein insertion into the membranes. Comparison of the effect of specific Tyr → Ala mutations shows the importance of C-terminal part for interactions with lipid vesicles. 3X mutation decreases α-helix formation in presence of DPPG vesicles. DSC and fluorescence polarization measurements support the results obtained by CD, suggesting that in addition to electrostatic interaction, hydrophobic interaction is also important in the association of α-synuclein with membranes.

Green approach to halogenation of organic compounds: Aerobic oxidative halogenation of ketones catalyzed by nitrate anion

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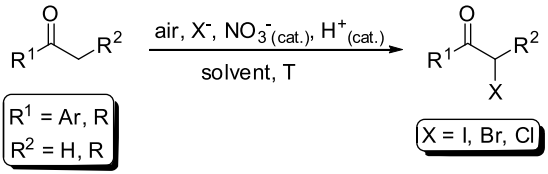
Carbonyl compounds bearing halogen atom at the alpha position (α-halo carbonyl compounds) serve as valuable bioactive molecules in numerous applications in medicine and agriculture, as well as versatile building blocks, precursors or intermediates in various organic transformations resulting in a synthesis of more complex organic molecules, including natural products.1

Synthetic methods for the preparation of α-halo carbonyl compounds are numerous, but only a few of them fulfill some of the principles of green chemistry. Reactions using elemental halogens exhibit low halogen atom economy, with iodine requiring some form of activation, while other molecular halogens are too reactive (F_2, Cl_2) or at least hazardous chemicals (Br_2) demanding special manipulation techniques. Transferring halogen atoms into carbonyl compounds through reagents bearing reactive heteroatom-halogen bond, such as N-X compounds (N-halo succinimides, N-halo sacharines, N-F reagents: Selectfluor™, NFSi...; etc) is experimentally easier, usually with higher halogen atom economy and low overall atom economy, but since production of these reagents

includes the use of molecular halogens and after halogenations, their ligand parts represent a reaction waste, overall green characteristics of these processes are at least disputable. In nature enzymatically supported oxidative halogenations processes are co-catalyzed by high valence metal species and use oxygen or hydrogen peroxide as terminal oxidants. In these processes natural organohalogen compounds, including α-halo carbonyl substituted molecules, are formed. Following this, (or in parallel) in classical organic synthesis, a green approach to haloorgano compounds is supposed to be oxidative halogenation methodology using these two oxidants as the cheapest and environmentally friendly reagents.[2,3]

We report the discovery and the development of the method for metal-free aerobic oxidative halogenations (iodination, bromination, chlorination) of carbonyl compounds, catalyzed by nitrate anion. After optimization of reaction parameters a variety of structural types of ketones have been halogenated, regioselectively and efficiently resulting in the formation of α-halo-substituted carbonyl compounds in high yields.



References

1. Erian, A. W.; Sherif, S. M.; Gaber, H. M., The chemistry of alpha-haloketones and their utility in heterocyclic synthesis. Molecules 2003, 8 (11), 793-865.2. Podgorsek, A.; Zupan, M.; Iskra, J., Oxidative Halogenation with "Green" Oxidants: Oxygen and Hydrogen Peroxide. Angewandte Chemie-International Edition 2009, 48 (45), 8424-8450; 3. Stavber, G.; Iskra, J.; Zupan, M.; Stavber, S., Aerobic Oxidative Iodination of Organic Compounds with Iodide Catalyzed by Sodium Nitrite. Adv Synth Catal 2008, 350 (18), 2921-2929.

Publications in 2011/2012

Scientific papers

Georgy Mikhaylov (IJS), Ursa Mikac (IJS, EN-FIST), Anna A. Magaeva, Volya I. Itin, Evgeniy P. Naiden, Ivan Psakhye, Liane Babes, Thomas Reinheckel, Christoph Peters, Robert Zeiser, Matthew Bogyo, Vito Turk (IJS, CIPKEBIP), Sergey G. Psakhye, Boris Turk*(IJS, UL FKKT, CIPKEBIP, CO NANO), Olga Vasiljeva* (IJS). **Ferri-liposomes as an MRI-visible drug delivery system for targeting tumours and their microenvironment**. 2011, Nature nanotechnology, vol. 6, September 2011.

Janezic S, Strumbelj I, Rupnik M. **Use of modified PCR ribotyping for detection of Clostridium difficile ribotypes directly in stool samples**. J Clin Microbiol. 2011, 49(8):3024-5.

Zidaric V, Kotnik Kevorkijan B, Oresic N, Janezic S, Rupnik M. **Comparison of two commercial molecular tests for the detection of Clostridium difficile in the routine diagnostic laboratory**. J Med Microbiol. 2011, 60: 1131-1136.

Maša Skelin, Dr. Marjan Rupnik, **cAMP increases the sensitivity of exocytosis to Ca2+ primarily through protein kinase A in mouse pancreatic beta cells**, Cell calcium 2011;49(2):89-99.

Jurij Dolenšek, Maša Skelin, Marjan Rupnik, **Calcium dependencies of regulated exocytosis in different endocrine cells**, Physiological Research (2011).

Pasquale V, Romano V, Rupnik M, Capuano F, Bove D, Aliberti F, Krovacek K, Dumontet S. **Occurrence of toxigenic Clostridium difficile in edible bivalve molluscs**. Food Microbiol. 2012 Sep;31(2):309-12. Epub 2012 Mar 12. PubMed PMID: 22608238.

Janezic S, Ocepek M, Zidaric V, Rupnik M. **Clostridium difficile genotypes other than ribotype 078 that are prevalent among human, animal and environmental isolates**. BMC Microbiol. 2012 Mar 27;12:48. PubMed PMID: 22452857; PubMed Central, PMCID: PMC3353227.

Pasquale V, Romano VJ, Rupnik M, Dumontet S, Cižnár I, Aliberti F, Mauri F, Saggiomo V, Krovacek K. **Isolation and characterization of Clostridium difficile from shellfish and marine environments**. Folia Microbiol (Praha). 2011, Sep;56(5):431-7. Epub 2011 Sep 8. PubMed PMID: 21901293.

Cene Gostinčar, Martin Grube, Nina Gunde Cimerman: **Evolution of Fungal Pathogens in Domestic Environments?** *Fungal biology*, 2011, vol. 115, issue 10, str. 959-970.

Metka Lenassi, Janja Zajc, Cene Gostinčar, Alenka Gorjan, Nina Gunde-Cimerman, Ana Plemenitaš. **Adaptation of the glycerol-3-phosphate dehydrogenase Gpd1 to high salinities in the extremely**

halotolerant *Hortaea werneckii* and halophilic *Wallemia ichthyophaga*. *Fungal biology*, 2011, vol. 115, no. 10, str. 1008-1018.

Nina Gunde-Cimerman, Martin Grube and Sybren de Hoog. **The emerging potential of melanized fungi: Black yeast between Beauty and the Beast.** *Fungal Biology*, 2011 Oct;115(10):935-6.

Turk M., Plemenitaš, A., Gunde-Cimerman N. **Plasma-membrane fluidity as determinant of stress tolerance.** *Fungal biology*, 2011, vol. 115, no. 10, str. 950-958.

Zalar P., Novak M., de Hoog G.S., Gunde-Cimerman N. **Dishwashers – a man-made ecological niche accommodating human opportunistic fungal pathogens.** *Fungal biology*, 2011, vol. 115, no. 10, str. 997-1007.

Cene Gostinčar, Metka Lenassi, Nina Gunde-Cimerman, Ana Plemenitaš (MF, Inštitut za biokemijo). **Fungal adaptation to extremely high salt concentrations.** *Advances in applied microbiol.*, 2011, vol. 77, str. 71-96.

GMAJNER, Dejan, AHLIN GRABNAR, Pegi, TUŠEK-ŽNIDARIČ, Magda, ŠTRUS, Jasna, ŠENTJURC, Marjeta, POKLAR ULRIH, Nataša. **Structural characterization of liposomes made of diether archaeal lipids and dipalmitoyl-L-[alpha]-phosphatidylcholine. *Biophysical chemistry***. [Print ed.], 2011, vol. 158, str. 150-156.

GMAJNER, Dejan, POKLAR ULRIH, Nataša. **Thermotropic phase behaviour of mixed liposomes consist of archaeal diether and conventional diester lipids**. *J. therm. anal. calorim.*, 2011, vol. 106, str. 255-260.

Cene Gostinčar (CIPKEBIP, BF), Martina Turk (BF), Nina Gunde Cimerman (BF): **Genetic Resources of Extremotolerant Fungi: A Method for Identification of Genes Conferring Stress-Tolerance.** *Bioresour. technol.*. [Print ed.], 2012, vol. 111, str. 360-367.

B.Mirković, M.Renko, S.Turk, I.Sosič, Z.Jevnikar, N.Obermajer, D.Turk, S.Gobec, J-Kos: **Novel mechanisem of cathepsin B inhibition by antibiotic nitroxoline and related compounds**, ChemMedChem, 2011, Vol.6, iss.8, str. 1351-1356, IF 3.306.

C.Jelinska, P.J.Davis, M.Kenig,E.Žerovnik, S.Kokalj-Jenko, G.Gunčar, D.Turk, V.Turk, D:T.Clarke, J.P.Waltho, R.A. Staniforth: **Modulation of contact order effects in the two-state folding of stefins A and B**, Biophysical journal, 2011, vol. 100, no.9, str. 2268-2274, IF 4.218.

Majera D, Kristan KČ, Neefjes J, Turk D, Mihelič M., **Expression, purification and assembly of soluble multimeric MHC class II-invariant chain complexes**. FEBS Lett. 2012 586(9):1318-24.

Mattiazzi, M., Sun, Y., Wolinski, H., Bavdek, A., Petan, T., Anderluh, G., Kohlwein, S.D., Drubin, D., Križaj, I. and Petrovič, U. (2012a): **A neurotoxic phospholipase A2 impairs yeast amphiphysin activity and reduces endocytosis**. *PLoS ONE* 7(7), e40931.

Mattiazzi, M., Petrovič, U. and Križaj, I. (2012b): **Yeast as a model eukaryote in toxinology: a functional genomics approach to the studies of the molecular basis of action of pharmacologically active molecules**. *Toxicon* 60, 558-571.

Kurtović, T., Leonardi, A., Lang Balija, M., Brgles, M., Habjanec, L., Križaj, I. and Halassy, B. (2012): **The standard mouse assay of antivenom quality does not measure antibodies neutralising the haemorrhagic activity of the *Vipera ammodytes* venom**. *Toxicon* 59, 709–717.

Križaj, I. (2012): **Characterising and cloning the venom components of the nose-horned viper that affect the human haemostatic system**. *Circulation* 126(1); f5-f6.

Leonardi, A., Biass, D., Kordiš, D., Stöcklin, R., Favreau, P. and Križaj, I. (2012): ***Conus consors* snail venom proteomics unveils functions, pathways and novel families involved in its venomic system**. *J. Proteome Res.*, doi: 10.1021/pr3006155.

Violette, A., Leonardi, A., Piquemal, D., Terrat, Y., Biass, D., Dutertre, S., Noguier, F., Ducancel, F., Stöcklin, R., Križaj, I. and Favreau, P. (2012): **Recruitment of glycosyl hydrolase proteins in a cone snail venomous arsenal: further insights into biomolecular features of *Conus* venoms**. *Mar. Drugs* 10, 258–280.

ŠNAJDER, Marko, VILFAN, Tanja, ČERNILEC, Maja, RUPREHT, Ruth, POPOVIĆ, Mara, JUNTES, Polona, ČURIN-ŠERBEC, Vladka, POKLAR ULRIH, Nataša. **Enzymatic degradation of PrPSc by a protease secreted from Aeropyrum pernix K1**. *PloS one*, 2012, vol. 7, no. 6, 1-12.

OTA, Ajda, GMAJNER, Dejan, ŠENTJURC, Marjeta, POKLAR ULRIH, Nataša. **Effect of growth medium pH of Aeropyrum pernix on structural properties and fluidity of archaeosomes** : *Archaea*. [Online ed.], 2012, vol. 2012, str. [1-9], no. ID=285152.

TERPINC, Petra, POLAK, Tomaž, MAKUC, Damjan, POKLAR ULRIH, Nataša, ABRAMOVIČ, Helena. **The occurrence and characterisation of phenolic compounds in Camelina sativa seed, cake and oil**. *Food chem.*. [Print ed.], 2012, vol. 131, issue 2, str. 580-589.

MOŽE BORNŠEK, Špela, ŽIBERNA, Lovro, POLAK, Tomaž, VANZO, Andreja, POKLAR ULRIH, Nataša, ABRAM, Veronika, TRAMER, Federica, PASSAMONTI, Sabina**. Bilberry and blueberry anthocyanins act as powerful intracellular antioxidants in mammalian cells**. *Food chem.*. [Print ed.], 2012, vol. 134, issue 4, str. 1878-1884.

SKRT, Mihaela, BENEDIK, Evgen, PODLIPNIK, Črtomir, POKLAR ULRIH, Nataša. **Interactions of different polyphenols with bovine serum albumin using fluorescence quenching and molecular docking**. *Food chem.*. [Print ed.], 2012, vol. 135, str. 2418-2424.

TERPINC, Petra, ČEH, Barbara, POKLAR ULRIH, Nataša, ABRAMOVIČ, Helena. **Studies of the correlation between antioxidant properties and the total phenolic content of different oil cake extracts.** *Ind. crops prod.*, 2012, vol. 39, str. 210-217.

WECHTERSBACH, Luka, POKLAR ULRIH, Nataša, CIGIĆ, Blaž. **Liposomal stabilization of ascorbic acid in model systems and in food matrices**. *Lebensm.-Wiss. + Technol.*, 2012, vol. 45, issue 1, str. 43-49.

POGAČNIK, Lea, POKLAR ULRIH, Nataša. **Application of optimized chemiluminescence assay for determination of the antioxidant capacity of herbal extracts.** *Luminescence (Online)*, 2012, str. [1-6, v tisku].

ŠUŠTAR, Vid, ZELKO, Jasna, LOPALCO, Patrizia, LOBASSO, Simona, OTA, Ajda, POKLAR ULRIH, Nataša, CORCELLI, Angela, KRALJ-IGLIČ, Veronika. **Morphology, biophysical properties and protein-mediated fusion of archaeosomes**. *PloS one*, 2012, vol. 7, no. 7, str. 1-15.

PIRC, Katja, POKLAR ULRIH, Nataša. Alpha-synuclein interactions with membranes. V: QAYYUM RANA, Abdul (ur.). **Etiology and pathophysiology of Parkinson's disease.** Rijeka: InTech, 2011, str. 87-110.

Invited lectures

Rupnik M., What can we learn from microbes? Out of the box conference on innovative ways to improve the culture of living, Maribor, Slovenija, 15-17. May 2012

GOSTINČAR, Cene. Stress-tolerant fungi : adaptations, evolution, applications, dangers. *[Lecture at Uniformed Services University of the Health Sciences, School of Medicine, Bethesda, USA, May 30 2012]*. 2012.

Nina Gunde-Cimerman, Polona Zalar, Monika Novak, Sybren de Hoog. Polyextremotolerant human opportunistic black yeasts inhabit dishwashers around the world. The 14th International Symposium on Microbial Ecology, ISME14, Copenhagen, Denmark, 19-24 August 2012.

Guest Editors

FEMS Microbiology Ecology: Special Thematic Issue on Polar and Alpine Microbiology
Editors: **Nina Gunde-Cimerman**, Dirk Wagner and Max M. Häggblom

Patent

Authors: Robert Zorec, Matjaž Stenovec, Saša Trkov, Nina Vardjan, Maja Potokar, Marko Kreft, Mateja Gabrijel, Jernej Jorgačevski
Patent application no. PCT/EP 2012/001759, Title: Screening methods based on vesicle mobility
Involved institutions:

1. University of Ljubljana, Kongresni trg 12, Ljubljana
2. Celica d.o.o., Technology park 24, Ljubljana
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Short explanation:
The present invention relates to methods and systems for screening for pharmaceutically active substances. The invention also relates to methods, systems and a reference compound for studying subcellular organelle traffic and related disease states.

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