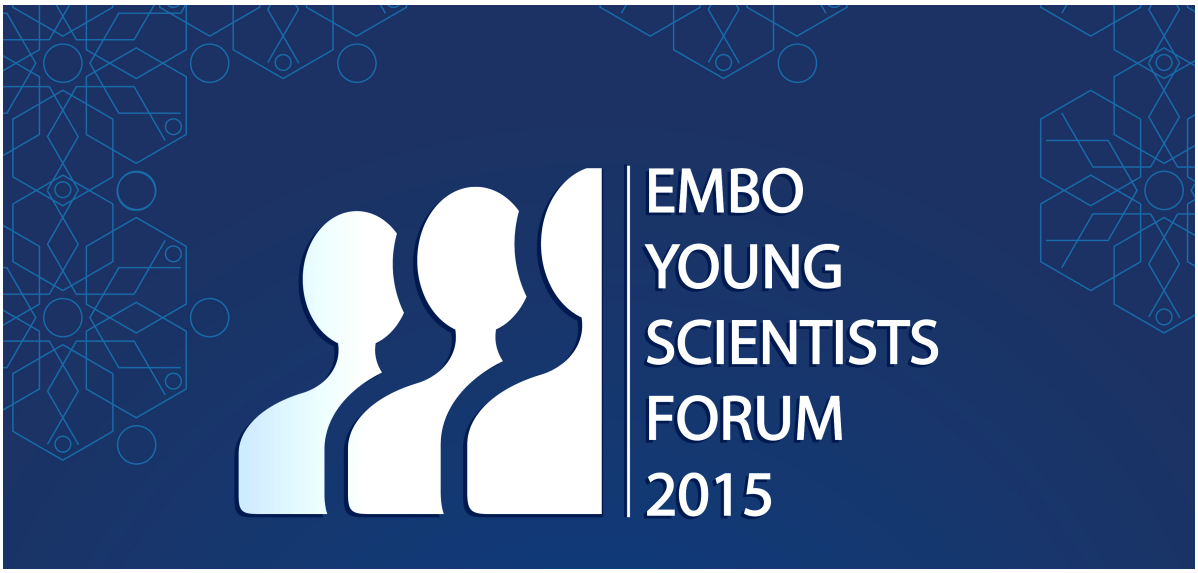
Book of Abstracts



2-3 July 2015

Warsaw, Poland

Contents

General information

1 Organisers

Michał Komorowski Polish Academy of Sciences

Piotr Setny Centre of New Technologies, University of Warsaw

Joanna Sułkowska Centre of New Technologies, University of Warsaw

Tomasz Wilanowski Nencki Institute of Experimental Biology

Dorota Włoga Nencki Institute of Experimental Biology

2 Useful information

Lecture Hall

Lectures will take place in the Conference Hall at the 1st Floor of the Nencki Institute Neurobiology Center.

Dining places

Lunches will be provided in the main hall of the Centre of New Technologies, ul Banacha 2c (see the map attached).
Dinner venue (grill) – yard in front of the Nencki Institute.

Posters' Session

Poster session will take place in the Exhibition Hall of Nencki Institute Neurobiology Center in the Ground Floor.

Participants with their surnames starting with letters from A to K will present their posters on July 2nd (Thursday) from 9:00 am till 5:30 pm. They are kindly asked to be present for discussion between 4:15 pm – 5:30 pm (poster session I).

Participants with their surnames starting with letters from L to Z will present their posters on July 2nd (Thursday evening) from 5:30 pm till July 3rd (early afternoon). They are kindly asked to be present for discussion between 5:30 pm – 6:30 pm (poster session II).

The format of the poster should be A0, portrait orientation. The means to attach posters will be provided.

WiFi

Free WiFi is available both in the Nencki Institute of Experimental Biology and CeNT.
CeNT
network name: **EMBO_YSF**, password: **embo_ysf**
Nencki Institute of Experimental Biology
will be provided

Workshop Office

Registration desk is located next to Conference Hall in the Nencki Institute Neurobiology Center, 1st Floor.

3 Maps

Local map

A - Nencki Institute Neurobiology Center (Conference hall)
B - Centre of New Technologies (lunch)
C - Student Housing

General map

A - Nencki Institute Neurobiology Center

B - Ibis Hotel

C - Hotel Premiere Classe

D - B&B Hotel

E - Student Housing

Food map

A - Nencki Institute Neurobiology Center **B- Centre of New Technologies**

C -Vietnamese bar

D - Canteen at the Faculty of Chemistry

E - Canteen at the Faculty of Biology

F - Vietnamese bar

G - Bistro

H - Canteen at the Faculty of Mathematics, Informatics and Mechanics

I - Kebab

Programme

Thursday, 2nd July (Day 1)

08:00 - 09:00 Registration, putting up posters

09:00 - 09:30 Opening lecture: **Sophie Jackson**
Biological Self Assembly

Session I: Biophysics

09:30 - 10:00 Lecture: **Joanna Trylska**
Functional RNA dynamics: aminoglycoside binding site and thermosensing hairpin

10:00 - 10:30 Lecture: **Martin Zacharias**
Exploring biomolecular dynamics and interactions using advanced sampling methods

10:30 - 10:40 Short talk: **Robert Szoszkiewicz**
Stiffness and internal friction of single protein molecules: AFM study

10:40 - 11:00 Coffee break

11:00 - 11:45 Workshop: **Tomasz Prószyński**
How to stay motivated in science?

11:45 - 12:30 Workshop: EMBO & EMBL - future opportunities
TBA

12:30 - 13:30 Lunch

Session II: Cancer

13:30 - 14:00 Lecture: **Michael Potente**
The link between angiogenesis and endothelium metabolism

14:00 - 14:30 Lecture: **Anna Sablina**
Ubiquitination governs signalling routes of the RAS-like GTPases

14:30 - 14:40 Short talk: **Katarzyna Jastrzębska**
Bioengineered spider silk - an intelligent biomaterial for delivery of anticancer drugs

14:40 - 15:15 Coffee break

15:15 - 15:45 Lecture: **Eric So**
Seed and soil in cancer stem cell biology

15:45 - 16:15 Lecture: **Jesús Gil**
Regulation of the secretome of senescent cells

16:30 - 17:30 Poster session I (first surnames letter A - K)

17:30 - 18:30 Poster session II (first surnames letter L - Z)

18:30 - ... Dinner and party

Friday, 3rd July (Day 2)

Session III: Cytoskeleton

09:00 - 09:30 Lecture: **Gaia Pigino**
3D electron microscopy to reveal structure and function of the cilium

09:30 - 10:00 Lecture: **Frank Schnorrer**
Building muscle - from genes to forces

10:00 - 10:30 Lecture: **Renata Basto**
Investigating the contribution of centrosomes during development and establishment of disease

10:30 - 10:40 Short talk: **Anna Adamiok**
miR-449 controls apical actin network formation during multiciliogenesis through small GTPase

10:40 - 11:00 Coffee break

Session IV: Structural Biology

11:00 - 11:30 Lecture: **Martin Weigt**
Coevolutionary modeling of protein sequences: Inference of 3D structure and protein-protein interactions

11:30 - 12:00 Lecture: **Alex Schug**
RNA: from folding to co-evolutionary structure prediction

12:00 - 12:30 Lecture: **Orsolya Barabas**
How do genes jump: insights from crystal structures and more...

12:30 - 12:40 Short talk: **Jarosław Paszek**
The properties of episode clustering problems for unrooted gene trees

12:40 - 13:00 Special talk: Being a Group Leader at EMBL: Opportunities for talented researchers

13:00 - 14:00 Lunch

14:00 - 14:45 Workshop: **Andrzej Dziembowski**
Writing grant applications

Session V: Systems Biology

14:45 - 15:15 Lecture: **Alfonso Martinez-Arias**
Structure, regulation and function of transcriptional heterogeneities in embryonic stem cell populations

15:15 - 15:45 Lecture: **Richard Moriggl**
Aberrant STAT5 gene dosage and hyperactivation status by cytokine or mutated tyrosine kinase signalling drives disease

15:45 - 15:55 Short talk: **Grzegorz Słodkowicz**
Structural and functional properties of protein sites evolving under positive selection

16:00 - 16:15 **Poster awards** and closing remarks

Invited Speakers

Biological Self Assembly

Sophie Jackson

Department of Chemistry,
University of Cambridge,
Cambridge, UK

Nature has many examples of self-assembly processes, ranging from the folding of nascent polypeptide chains into globular structures, some of which have complex topologies, to the assembly of small molecule natural products such as the aggregation carotenoids into specific highly regular helical complexes. Many of these processes are entirely driven by changes in non-covalent bonding, generally weak in nature, but the assemblies formed are highly specific and functional. Self-assembly processes are associated with many disease states, and include examples where biological species self assemble into structures toxic in nature, and also those examples where a normal biological self-assembly process breaks down. Unwanted self-assembly of biologics is also a significant problem to Biotech and Pharma.

In the talk, I will discuss four different examples of biological self-assembly. This will include

(i) Recent work on the folding of a class of proteins with complex knotted structures.

(ii) The self-assembly of disease-related proteins into amyloid fibrils and the role that molecular chaperones play in controlling this process.

(iii) Unsuccessful attempts to assemble key carotenoid, chlorophyll, protein complexes involved in light harvesting.

(iv) The assembly of carotenoids into large helical assemblies.

Functional RNA dynamics: aminoglycoside binding site and thermosensing hairpin

Joanna Trylska

Centre of New Technologies,
University of Warsaw,
Warsaw, Poland

Internal dynamics of RNA tertiary structure is required for its proper biological function. I will speak about the importance of RNA flexibility in two biologically-relevant RNA motifs. One is the ribosomal decoding site, which is also the binding site for aminoglycoside antibiotics, forming a flexible bulge to accommodate these antibiotics. We investigated the dynamical properties of this bulge in the context of aminoglycoside affinity and stability of the complex. The other one is an RNA hairpin that acts as a thermometer. RNA thermometers are short RNA sequences located in the 5' untranslated regions of mRNA which respond to temperature changes. Local melting of these mRNA fragments exposes the Shine-Dalgarno region for ribosome binding. We have investigated the mechanism of thermal unwinding of a fourU thermometer and repression of heat shock gene expression element (ROSE).

Exploring biomolecular dynamics and interactions using advanced sampling methods

Martin Zacharias

Physik-Department T38,
Technische Universität München,
Garching, Germany

Understanding the dynamics and stability of biomolecules and biomolecular complexes is of critical importance to better understand its biological function. For many applications currently accessible time scales of molecular dynamics simulations are too short to sufficiently sample relevant conformational states. We employ advanced sampling molecular dynamics (MD) simulations based on Hamiltonian replica exchange (H-REMD) to study the dynamics of proteins and nucleic acids. Applications include the study of the fine structure of damaged DNA which can influence the recognition by repair enzymes. A second part will focus on the prediction of the geometry of biomolecular complexes using docking approaches. A coarse-grained docking method has been developed that employs a knowledge-based scoring function for evaluating putative complex structures. The approach also accounts approximately for local and global conformational changes during docking. The application of the methodology to protein-protein and protein-nucleic docking will be presented

The link between angiogenesis and endothelium metabolism

Michael Potente

Angiogenesis & Metabolism Laboratory,
Max Planck Institute for Heart and Lung Research,
Bad Nauheim, Germany

Angiogenesis is a dynamic process that involves the specification of endothelial cells (ECs) into tip and stalk cells, their directed migration and proliferation, as well as the formation of new connections. A primary focus of angiogenesis research has been the understanding of mechanisms that induce such endothelial phenotypes, work that has led to the identification of signaling pathways that control EC migration and proliferation. But the adoption of an angiogenic phenotype has significant consequences for endothelial metabolism as well, a link that has been widely ignored previously. This concept stems from the premise that ECs have to meet the bio-energetic demands of increased proliferation when they switch from quiescence to vessel growth. Indeed, proliferating cells require nutrients, energy, and biosynthetic activity to produce the building blocks for cell duplication. During angiogenesis, ECs, thus, have to rewire their metabolism to generate energy quickly and to facilitate the incorporation of nutrients into biomass. However, the mechanisms that balance endothelial metabolic activity and growth state are not known to date. This presentation will illustrate current concepts of metabolic regulation in the endothelium and will highlight the critical role of FOXO transcription factors in this process.

Ubiquitination governs signalling routes of the RAS-like GTPases

Anna Sablina

Maria Francesca Baietti[*,1,2], *Mikhail Stelkov*[1,2], *Michal Simicek*[1,2], *Layka Abbasi Asbagh*[1,2], *Jonathan Crowther*[1,2], *Enrico Radaelli*[1,2], *Vasily Aushev*[1,2], *Lievens Sam*[3,4], *Mathias Laga*[2,4], *Gevaert Kris*[2,4], *Jan Tavernier*[2,4], *Anna Sablina*[1,2]

[1]Center for the Biology of Disease, VIB, [2]Center for Human Genetics, KU Leuven, Leuven [3]Department of Medical Protein Research, VIB [4]Department of Biochemistry, Gent University, Gent, Belgium

The RAS GTPases are among the most commonly mutated oncoproteins in human cancers. We and others have recently demonstrated a crucial role of reversible ubiquitination in control of the RAS GTPases. We found that NRAS undergoes ubiquitination at multiple sites, and ubiquitination at specific lysines regulates NRAS activity by distinct mechanisms. In particular, ubiquitination at lysines 128 affects its dimerization and subcellular localization, whereas ubiquitination of lysine 5 modulates the ability of RAS to interact with its effector proteins. Using Virotrap approach, we have also identified enzymes that are involved in reversible ubiquitination of NRAS. Strikingly, the identified E3-ubiquitin ligases of RAS behave like typical tumor suppressors, while RAS-specific deubiquitinases have oncogenic properties. This highlights the importance of RAS ubiquitination in cancer development and progression. The results of these studies not only advance our understanding of RAS signaling but also could lead to novel therapeutic strategies for treatment of RAS mutated tumors.

Seed and Soil in Cancer Stem Cell Biology

Eric So

Leukemia and Stem Cell Biology Lab,
King's College London,
London, UK

The recent technological breakthrough in developing high throughout and low cost next generation sequencing technology has facilitated the generation of genetic blueprints of various tumors. While it is clear that DNA mutation is an important and integrated determinant in cancer biology, cancer is more than just DNA mutation but a far more complex biological process. Beyond genetics, epigenetic and transcriptional regulation plays equally important roles in cancer development. Self-renewal is an important feature that distinguishes stem cells from their downstream progenitors. While it becomes evidenced that phenotypically identical cancers carrying same genetic mutations can arise from stem cells or progenitors with different transcriptional programs, it is not clear if cancer stem cells (CSC) originated from distinctive cellular origins will still maintain their transcriptional memories and are capable of utilizing different self-renewal pathways. In this talk, I will put up a seed and soil hypothesis to explain the critical roles of both genetic and epigenetic components in cancer biology. I will show that stem cells-derived, in contrast to progenitors-derived, CSC transformed by MLL fusion can bypass β-catenin requirement for self-renewal, even these cancer cells have the identical genetic driver resulting in phenotypically identical leukemia. On the other hand, the leukemic self-renewal can be however maintained by another key transcriptional memory gene retained in the hematopoietic stem cells (HSC)-derived CSC. We will discuss the significance of these findings and the potential implications to cancer biology and treatment.

Regulation of the secretome of senescent cells

Jesús Gil

MRC Clinical Science Centre,
Imperial College London,
London, UK

Senescent cells secrete a combination of factors collectively referred to as the senescence-associated secretory phenotype (SASP). Although the SASP comprises many pro-inflammatory cytokines, a plethora of other factors including several TGF-β members mediate its actions. The SASP reinforces senescence and activates an immune surveillance response but also display pro-tumorigenic properties and contribute to age-related pathologies. In order to find novel SASP regulators, we have conducted drug and siRNA screens. In the drug screen we uncovered the mTOR inhibitor rapamycin as a potent SASP suppressor. Here we report a mechanism by which mTOR controls the SASP by differentially regulating the translation of the MK2/MAPKAPK2 kinase through 4EBP1. In turn, MAPKAPK2 phosphorylates the RNA binding protein ZFP36L1 during senescence, inhibiting its ability to degrade SASP mRNAs. Consequently, mTOR inhibition or constitutive activation of ZFP36L1 impairs the non-cell-autonomous effects that senescent cells display during tumorigenesis. Altogether, our results place regulation of the SASP as a key mechanism by which mTOR could influence cancer, age-related diseases and immune responses.

3D electron microscopy to reveal structure and function of the cilium

Gaia Pigino

Research Group Leader,
Max Planck Institute of Molecular Cell Biology and Genetics,
Dresden, Germany

Modern biology is increasingly addressing phenomena bridging multiple scales. One such phenomenon is the assembly of large functional machines out of proteins and protein complexes. The cilium is a great example of a sophisticated and large cellular machine that assembles out of many interacting multi-protein complexes. Cilia are highly conserved organelles that are present in nearly all organisms from protists to mammals. Although best known for their role in cellular motility, cilia also serve a multitude of sensory and signaling functions, which are pivotal for organ development, tissue homeostasis and maintenance. All types of cilia share a common microtubule-based structure, the axoneme, which is composed of more than 600 different proteins. Today we do not know the role and often not even the localization of many of those proteins. Also we do not know much about how they assemble into a functioning cilium. However, if the correct assembly of cilia is compromised, cilia-related pathologies (ciliopathies) occur.

In my lab we have a close look at several aspects of axonemal assembly and maintenance processes in Tetrahymena and Chlamydomonas cilia. We use and develop novel methods for cryo-electron tomography and image analysis to show the three-dimensional architecture of axonemal protein complexes. On the basis of our structural and biochemical findings we can finally reason about underlying biological processes.

Building muscle – from genes to forces

Frank Schnorrer

Research Group Muscle Dynamics,
Max Planck Institute of Biochemistry,
Martinsried, Germany

Higher animals built an elaborate muscle-tendon network to perform their daily movements. We use Drosophila flight muscles as a model to dissect the mechanism how developing myotubes stably connect to the skeleton via tendon cells and then assemble their contractile machinery with regularly arranged myofibrils and sarcomeres. Using in vivo imaging we define distinct phases of flight muscle morphogenesis. First, attachment initiation – myotubes extend towards the tendons precursors and the dynamic leading edges of myotubes and tendons extensively interdigitate to initiate attachment. Second, attachment maturation – myotube tips smoothen and stably attach to tendons. Concomitantly, myotubes build up tension and compact in length, while tendons are forced to generate long cellular extensions. Third, myofibrillogenesis – following tension build-up, myofibrillogenesis is initiated simultaneously throughout the entire myofiber. To functionally investigate if force-resistant attachment and tension are a prerequisite for myofibrillogenesis we blocked attachment by removing the transmembrane receptor Kon-tiki (Kon) in muscle. As a consequence flight muscles either round up completely or remain inefficiently attached. In both cases myofibrillogenesis is strongly defective, suggesting that force-resistant attachment and mechanical tension in the muscle are required for the simultaneous assembly of myofibrils. As the sarcomeric architecture of muscles is entirely conserved we predict that a similar tension-dependent mechanism also applies to myofibrillogenesis in vertebrates.

Investigating the contribution of centrosomes during development and establishment of disease

Renata Basto

Biology of Centrosomes and Cilia,
Institut Curie,
Paris, France

The centrosome is the major microtubule organising center (MTOC) of animal cells. It comprises two centrioles surrounded by a mesh of more than one hundred proteins called the pericentriolar material (PCM), which is the site of microtubule (MT) nucleation. Centrosomes contribute to the establishment and maintenance of cell polarity, cytoskeleton organisation and efficient mitotic spindle assembly during cell division. Mutations in centrosome genes are associated with a variety of human diseases such as microcephlay or cancer. In the Basto lab we are interested in understanding the molecular pathways that contribute to centrosome biogenesis and how deviations to these pathways contribute to developmental defects and to the establishment of diasease.

Coevolutionary modeling of protein sequences: Inference of 3D structure and protein-protein interactions

Martin Weigt

Laboratoire de Biologie Computationelle et Quantitative,
Université Pierre et Marie Curie,
Paris, France

Modern-day biology is characterized by an unprecedented wealth of large-scale sequence data. Sophisticated computational approaches are needed to extract information from raw data, and to infer the rules governing complex biological systems from observations. As an example for this general idea, I will discuss the recently developed Direct-Coupling Analysis (DCA), a statistical-inference approach for detecting direct residue coevolution in large multiple-sequence alignments of homologous proteins. Based on sequence information alone, this analysis allows to extract accurate residue-residue contact predictions, which in turn are helpful to predict tertiary and quaternary protein structures, and to reconstruct protein-protein interaction networks.

RNA: From Folding to Co-Evolutionary Structure Prediction

Alex Schug

Multiscale Biomolecular Simulation,
Karlsruhe Institute of Technology,
Eggenstein-Leopoldshafen, Germany

Exploring the interrelationship of structure and function is crucial for the understanding of molecular life. Structured RNAs influence a variety of cellular processes and their structure-function relationship is the topic of vigorous studies. Riboswitches are an important class of RNA-based sensors that regulate gene expression. Despite significant progress of experimental methods, their structural characterization typically preceding any detailed mechanistic exploration of their function remains challenging. In recent years, increasingly ubiquitous availability of sequential information and novel statistical analysis has allowed to trace the co-evolution of residues and predict contact maps based on maximum entropy (e.g. Direct Coupling Analysis, DCA [1]). These contact maps can be exploited for blind structural prediction of proteins [2,3]. We have successfully adapted this method to the specifics of RNA to systematically improve tertiary RNA prediction quality of six representative riboswitches [submitted]. Going beyond structure prediction towards exploring the dynamics and the interconnection of synthesis, folding, and function of riboswitches recent first direct experimental measurement of co-transcriptional folding highlight the underlying challenges [4]. I will present simulations of co-transcriptional riboswitch folding for a range of transcription rates and differentiate scenarios of transcription-rate limited folding and free folding [5]

[1] Weigt M et al., Proc Nat Acad Sci USA (2009) 106, 67-72; F. Morcos et al., Proc Nat Acad Sci (2011) 108, E1293-E1301
[2] Schug A et al., Proc Nat Acad Sci USA (2009) 106, 22124-22129
[3] Dago A et al., Proc Nat Acad Sci USA (2012), 109: E1733-42
[4] Frieda K and Block S, Science 338 (2012): 397-400
[5] Lutz B et al.,: Nucl. Ac. Res., 42 (4), 26872696, 2014

How do genes jump: insights from crystal structures and more...

Orsolya Barabas

Franka Voigt[1], Lisa Wiedemann[2], Irma Querques[1], Cecilia Zuliani[1], Lajos Mátés[3], Zsuzsanna Izsvák[3], Zoltán Ivics[2] and Orsolya Barabas[1]

[1]European Molecular Biology Laboratory, Heidelberg, Germany [2]Paul Ehrlich Institute, Langen, Germany [3]Max Delbrück Center for Molecular Medicine, Berlin, Germany

Transposons are mobile genetic elements with the distinctive ability to autonomously move from one genomic location to another. They constitute a large fraction of modern genomes and their movement (transposition) represents a major force driving genome dynamics and evolution. Moreover, Their ability to autonomously move around in genomes allowed their application as powerful genetic engineering tools. The reconstructed Sleeping Beauty (SB) transposon has revolutionized the functional genomics analysis of vertebrates and found widespread applications in transgenesis including human gene therapy. Nevertheless, the mechanism of SB transposition is poorly understood and no structural data is available, limiting further design of SB based genetic engineering tools.

In this talk, I will present our recent biochemical and structural results that provide long sought insights into SB transposition. Using biochemistry and cell biology approaches, we have described the first steps of SB transposition and show that they follow a distinct pathway, different from homologous transposons. We have also determined the first crystal structure of the SB transposase catalytic domain, which provides important insights into the mechanism of target DNA recognition and integration. In addition, the structure explains hyperactive mutations in the SB transposase and allows us to engineer further improved variants.

Structure, regulation and function of transcriptional heterogeneities in embryonic stem cell populations

Alfonso Martinez-Arias

Department of Genetics,
University of Cambridge,
Cambridge, UK

Embryonic Stem (ES) cells are clonal populations derived from mammalian blastocysts which can be differentiated in culture into all cell types of an organism. Over the last few years, work with mouse ES cells has revealed that they are characterized by dynamic heterogeneities in gene expression. Analysis of these heterogeneities suggests that they are regulated and associated with transient priming for differentiation. I shall discuss the arguments for these suggestions and show some examples of how such heterogeneities are used to build patterns during development.

Aberrant STAT5 Gene Dosage and Hyperactivation Status by Cytokine or Mutated Tyrosine Kinase Signalling Drives Disease

Richard Moriggl

Ludwig Boltzmann Institute for Cancer Research (LBI-CR)
Medical University Vienna, Vienna
University of Veterinary Medicine, Vienna
Institute for Animal Breeding and Genetics

STAT5 signalling is part of a central cancer pathway that can drive other essential core cancer pathways such as survival and cell cycle progression, but it can also promote differentiation and senescence in physiologic context, which opposes cancer formation. Cancer genome landscaping revealed frequent JAK-STAT mutations, but gene dosage questions of essential components lacks behind. The key role of STAT5 in myeloid cancers is well established, other cancer types catch up lately. Particularly, peripheral T cell leukaemia and lymphoma patients (PTCL) were identified with recurrent, somatic STAT5 mutations. Mutations in the JAK-STAT pathway play also a role upon tyrosine kinase drug resistance mechanism. In general, STAT5 transcription factors efficiently dimerize, translocate to the nucleus, change chromatin to regulate gene expression once strongly tyrosine and serine/threonine phosphorylated. Too little or too much hyperactive STAT5 signaling results in hematopoietic failure or neoplasia. Recurrent gain of function STAT5 variants have enhanced tyrosine phosphorylation driving myeloid or lymphoid neoplasia, as seen in PTCL patients associated with poor prognosis due to lack of specific therapeutics. We describe wildtype or gain of function point mutations of STAT5 with different gene dosage in transgenic mice as stringent test systems for cancer development. Upon expression of hyperactive STAT5 from the hematopoietic stem cell stage on PTCL of CD8+ T cell origin developed closely matching gene expression profiles of human PTCL. The consequence is massive T cell expansion, organ failure and death with aggressive, polyclonal disease.

Short talks

Stiffness and internal friction of single protein molecules: AFM study

Presenter: Robert Szoszkiewicz

Warsaw University of Technology

Robert Szoszkiewicz, N. Ploscariu Kansas State University, KS, USA

Using atomic force microscopy (AFM) one can measure physiologically relevant pN forces between an AFM tip and a biomolecule and resolve displacements of less than 1 nm. The last 15 years have witnessed an explosion of interest in single molecule force spectroscopy fueled by: 1) new possibilities to advance in protein folding, 2) possibilities to elucidate molecular mechanisms of various cellular processes, and diseases, and 3) efforts to understand the nanomechanical properties of proteins, polysaccharides and DNAs in order to design biomimetic and/or mechanically functional materials.In this presentation, I will concentrate on our research towards elucidating early folding events in a simple model protein from changes of molecular stiffness and dissipation factors. Using such measurements, we hope in a future to provide basic understanding of early folding events in selected simple proteins.

Bioengineered spider silk – an intelligent biomaterial for delivery of anti-cancer drugs

Presenter: Katarzyna Jastrzebska

NanoBioMedical Centre, Adam Mickiewicz University, Poznan, Poland

Katarzyna Jastrzebska, Katarzyna Jastrzebska, Anna Florczak, Yinnan Lin, Rosalyn Abbott, Andrzej Mackiewicz David L. Kaplan, Hanna Dams-Kozlowska

Spider silk is a durable biopolymer that combines biocompatibility, biodegradability with an ability to self-assemble. Bioengineered spider silk can be designed for various applications, processed into different structural forms and their properties can be controlled by changing their amino-acid sequence. Here, we utilized two bioengineered spider silks: MS1 and MS2. Recombinant proteins were produced in E. coli and purified by thermal extraction. The spherical particles were produced at various conditions to optimize and control particle properties. The spheres made of two bioengineered spider silk proteins showed different morphology and physical properties such as secondary structure content and zeta potential. Both particle types showed no cytotoxic effect and low immunogenity. MS1 particles showed stronger affinity to anticancer drugs than MS2 spheres. The bioengineered spider silk proteins formed stable, biocompatible spherical particles that possess an ability to bind and release anticancer drugs. The bioengineered silk spheres can be modified to target the cancer cells by using a functional domain. These features, will determine the potential of bioengineered spider silk spheres as the drug carriers for cancer therapy.

miR-449 controls apical actin network formation during multiciliogenesis through small GTPase

Presenter: Anna Adamiok

Anna Adamiok, Laurent Kodjabachian, Andrea Pasini

The process of multiple motile cilia formation (multiciliogenesis) is composed of many different steps. Recently, we demonstrated that microRNAs of the miR-449 family control several of these steps. Now, we focused on the role played by miR-449 in two particular aspects of the development of the multiciliated embryonic epithelium of the amphibian Xenopus laevis: the formation of an actin network underneath the apical surface of multiciliated cells and the intercalation of the developing multiciliated cells within the mucous layer of the epidermis. In multiciliated cells, a dense actin network underlying the apical aspect of the cell membrane (actin cap) is required for the anchoring of the multiple basal bodies, and therefore for proper ciliogenesis. Small GTPases play important role in the formation of the actin cap. We identified the small GTPase R-Ras as bona fide targets of miR-449. We demonstrated that apical and subapical actin network reorganization and multiciliogenesis were impaired when R-Ras mRNA was protected from miR-449 binding. Moreover, the actin cap formation and multiciliogenesis were rescued when the translation of protected R-Ras transcripts was prevented.

The properties of episode clustering problems for unrooted gene trees.

Presenter: Jarosław Paszek

Department of Mathematics, Informatics and Mechanics, University of Warsaw, Poland

Jarosław Paszek, Paweł Górecki, Department of Mathematics, Informatics and Mechanics, University of Warsaw, Poland

One of the fundamental issues in evolutionary molecular biology is to discover the locations of gene duplications and multiple gene duplication episodes. We investigate the problem introduced by Guigo et al. in 1996 which is to find a mapping from a collection of rooted, binary gene family trees onto theirs corresponding rooted binary species tree in such a way that the total number of multiple gene duplication episodes is minimized. In the literature there are several approaches presenting models to specify how duplications of genes from gene families can be interpreted as one duplication episode. However, all of them consider only rooted gene trees. This restriction limits the applicability, since unrooted gene family trees are frequently inferred by phylogenetic methods. We would like to propose the extension of the episode clustering problems to the case when the input gene family trees are rooted. Moreover, we would like to present theoretical properties of that problem. In particular, by using theoretical properties of unrooted reconciliation, we show an efficient algorithm that reduces our problem into the episode clustering problems defined for rooted trees.

Structural and functional properties of protein sites evolving under positive selection

Presenter: Grzegorz Slodkowicz

European Bioinformatics Institute / University of Cambridge

Grzegorz Slodkowicz, Nick Goldman, EMBL-European Bioinformatics Institute

Identifying sites under positive selection is a major goal of evolutionary genetics. Statistical methods for detecting positive selection generally assume that selective constraint on each protein site is independent of neighbouring sites. While this is makes computation easier, it is also highly unrealistic: protein structure introduces constraints and interdependencies between sites. With the advent of more sequence data and computational power, it is tempting to relax earlier simplifying assumptions. We investigate how protein structure influences the selective regime experienced by a site. Using a comprehensive set of mammalian alignments and available crystal structures, we estimate the distribution of selective constraint (dN/dS) separately for regions having different secondary structure and different solvent exposure. We also evaluate the evidence for linear and spatial clustering of sites under positive selection. As expected, we find dependence on secondary structure and solvent accessibility as well linear dependencies between sites under positive selection as but surprisingly no statistically significant spatial clustering.

Poster abstracts

Structural determination of 5' UTR RNA motifs

Presenter: Astha Abhu

International Institute of Molecular and Cell Biology, Warsaw

Astha Abhu, Astha1, Grzegorz Chojnowski1, Stanislaw Dunin-Horkawicz1, Elzbieta Purta1, Krzysztof Skowronek1, Radosław Pluta1, Grzegorz Łach1 and Janusz M. Bujnicki1,2 1Laboratory of Bioinformatics and Protein Engineering, International Institute of Molecular and Cell Biology, ul. Ks. Trojdena 4, 02-109 Warsaw, Poland, http://iimcb.genesilico.pl 2Institute of Molecular Biology and Biotechnology, Faculty of Biology, Adam Mickiewicz University, ul. Umultowska 89, 61-614 Poznan, Poland

5' untranslated regions of mRNA contain cis-regulatory elements and play an important role in translation regulation, affecting mRNA's stability and also acting as riboswitches. As impairment of this regulation machinery perturbs cellular metabolism, studying it at a structural level seems to be an important research subject. The aim of my research is to determine the structure of 5' UTR RNA motifs using mainly X-ray crystallography method, in combination with low-resolution structural probing methods and theoretical structure prediction. X-ray crystallography technique employs single crystal X-ray diffraction (SXRD) to unambiguously determine the three dimensional structure at atomic resolution. But as the surface of RNA molecules is dominated by a poor differentiated regular array of negatively charged phosphates, the crystallization of RNAs remains a formidable experimental challenge which makes low-resolution structural probing methods like SHAPE and theoretical structure prediction also important. Structural insight obtain will help in understanding the different mechanisms of translation regulation and impact of 5' UTR structure on gene expression.

The role of DDR proteins in stress-induced premature senescence of cancer cells

Presenter: Olga Alster

Laboratory of Molecular Bases of Aging, Nencki Institute of Experimental Biology PAS, Warsaw, Poland

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Stress-induced senescence (SIPS) is associated with irreversible cell cycle arrest and persistant activation of the DNA-damage response (DDR). The key components of the DDR pathway are: ATM, ATR, CHK2, CHK1 and p53. Since we observed that p53-/- cells can undergo senescence we wanted to verify if downregulation of other members of the DDR pathway would influence the ability of the cells to undergo SIPS. As our experimental model we used HCT116, p53+/+ and p53-/- cell lines which were treated with a cytostatic dose of doxorubicin. Cells with a downregulated level of CHK2 and ATM were able to undergo senescence. In both cases we observed a time-dependent increase in SA-β-Gal activity, a common marker of senescence. To further investigate the role of DDR in SIPS we treated the cells with caffeine, a known inhibitor of DDR kinases ATM, ATR and DNA-PK. Pretreatment with this agent led to a decrease in SA-β-Gal activity. We can conclude that the absence or downregulation of only one or two of the downstream components of the DDR pathway does not prevent the cells from undergoing senescence, while the use of caffeine affected the induction of senescence. This work was supported by National Science Centre, grant 2011/01/M/NZ1/01597.

Interferometric scattering microscopy: a new camera for the nano-world

Presenter: Joanna Andrecka

University of Oxford

Joanna Andrecka, Jaime Ortega Arroyo, University of Oxford, Philipp Kukura, University of Oxford

Recent developments in fluorescence microscopy have enabled routine studies down to the single molecule level and observations beyond the limits defined by diffraction. Despite its many advantages, the fundamental limitation of fluorescence detection is the frequency with which photons can be emitted that defines temporal and spatial resolution. iSCAT (interferometric scattering microscopy) is an alternative approach that relies on light scattering. It is capable of following molecular motion with nanometer precision and microsecond time resolution. As an example, I will first present the results of myosin 5 head tracking which allowed us to reveal structural dynamics of this molecular motor. Second, I will demonstrate that iSCAT can be used for ultra-sensitive imaging of nanoscopic structures without labelling. I will show how a lipid bilayer is formed from nanometer size vesicles in real time. I will also present unlabelled microtubules tracking with sub-nanometer precision that allows resolving 8 nm kinesin steps at 1000 frames per second. Finally, the label free approach can be extended all the way down to the single protein level and I will present a movie of unlabeled myosin 5 moving along an actin filament.

Information processing in the JAK-STAT signalling pathway.

Presenter: Katarzyna Andryka

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Katarzyna Andryka, Edyta Głów, Karol Nienałtowski* , Tomasz Jetka* , Michał Komorowski* Institute of Fundamental Technological Research, Polish Academy of Sciences, Warsaw, Poland*

Interferons exhibit their key role of immune modulators through activation of the Jak-Stat signalling pathway. We know substantial amount of molecular details regarding functioning of the pathway. However, to what extend the action of the pathway is dose dependent remains unclear. Specifically it is not know if single cells respond in a digital fashion or their output is analogously dependent on stimulant's concentration. We have used high-throughput confocal imaging combined with an information-theoretic framework in order to provide a thorough, single-cell analysis of the Jak-Stat signalling pathway activated by the type I and type II interferons. We showed that in a baseline state single cells have information hardly sufficient to distinguish between presence or absence of interferons however they can be put in an alert state by an action of the immune system, which allows them to respond more in an analogous fashion. Our results show that the accuracy with which signalling pathways transmit information is not fixed but can be modulated on the contextual basis.

Human population structure and adaptation in the Himalayan region

Presenter: Elena Arciero

Wellcome Trust Sanger Institute

Elena Arciero, Elena Arciero (The Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, United Kingdom), Thirsa Kraaijenbrink (MGC Department of Human and Clinical Genetics, Leiden University Medical Centre, Leiden, the Netherlands), Marc Haber (The Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, United Kingdom), Qasim Ayub (The Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, United Kingdom), Mark Jobling (Department of Genetics, University of Leicester, Leicester, United Kingdom), George van Driem (Himalayan Languages Project, Institut für Sprachwissenschaft, University of Bern, Bern, Switzerland), Yali Xue (The Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, United Kingdom), Peter de Knijff (MGC Department of Human and Clinical Genetics, Leiden University Medical Centre, Leiden, the Netherlands), Chris Tyler-Smith (The Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, United Kingdom)

The Himalayan mountain range has the highest peaks on Earth and has provided a diversity of environments for humans, some of which have required substantial genetic adaptation. There is, however, little understanding of the genetic history of the Himalayans and how culture, geography and genetic selection have shaped Himalayan genomes. In this study, we analyse 700,000 genome-wide SNPs in more than 700 Himalayan individuals from 45 different autochthonous language groups. We find that Himalayan populations have diverged from a shared ancestral population; the development of local fine genetic structure then followed, correlating with language. We detect several admixture events in the history of most Himalayans involving populations related to Central Asians, East Asians and Indians. However, some populations appear to have diverged early in their history from the rest of the Himalayans and remained genetically isolated until today. We find genetic signatures of adaptation associated with variants in the genes EPAS1, VKORC1, and CR1 related to hypoxia, blood clotting, and immune response respectively, showing that the Himalayans have had developed adaptations to survive to one of the most challenging environments on Earth.

Regulation of Crumbs dynamics in morphogenesis

Presenter: Anna Bajur

Max-Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany

Anna Bajur, Elisabeth Knust, Max-Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany

Embryogenesis is a highly dynamic process, for which acquiring and maintaining cell polarity is crucial. Polarised epithelial cells have specialised membrane domains, created by protein complexes and cytoskeleton-induced compartments. The Crumbs (Crb) protein complex, localized to the apical region of the plasma membrane, is critical for apico-basal polarity. Loss or overexpression of Crb affect proper cell morphology, which implies that the levels of Crb must be strictly regulated. This could be achieved by dynamic intracellular traffic control. My project aims at unraveling how the localisation of Crb in the plasma membrane is regulated in the highly dynamic environment of a developing organism by applying following methods: 1.Fluorescence Recovery After Photobleaching assay to analyse the dynamics of Crb within the cell membrane 2.Particle tracking and high-resolution live imaging to identify the trafficking routes responsible for the maintenance of Crb at the membrane. Data obtained thus far suggest that distinct parts of the developing epidermis exhibit different turnover rates of Crb. Further work is aimed to unravel the mechanisms by which the Crb dynamics is regulated.

CCDC113p, a new ciliary protein involved in cilia beating regulation

Presenter: Rafał Bazan

ENCKI INSTITUTE OF EXPERIMENTAL BIOLOGY, POLISH ACADEMY OF SCIENCES

Rafał Bazan, Ewa Wacławek 1, Paulina Urbańska 1, Ewa Joachimiak 1, Dorota Włoga 1, 1-Nencki Institute of Experimental Biology, Polish Academy of Sciences

Cilia are microtubule-based organelles that protrude from the surface of many cell types from protists to mammals, with the exception of fungi and higher plants. These tiny organelles can be divided in two categories, immotile cilia that function as sensors and transducers of the external signals and motile cilia that enable shift of the surrounding fluids or mucus along the cell surface. In humans lack or dysfunction of motile cilia leads to heterogeneous group of human disorders called primary ciliary dyskinesia (PCD). Our research interest is focused on the identification and functional analysis of new proteins that are involved in cilia motility regulation. Recently we identified CCDC113p (coiled-coil domain containing) as a new ciliary protein. N-terminally HA- tagged CCDC113p expressed under the control of its native promoter localizes in cilia of a ciliate Tetrahymena thermophila. Cells with knocked out CCDC113 gene assemble normal-length cilia but with beating defects. The preliminary immunoprecipitation analysis suggests that CCDC113p may interact with cilia dynein arms and thus can alter their function.
Research was supported by the MNiSW Grant (N301706640), NCN “Harmonia 6” grant and EMBO IG No. 2331 to D.W.

Integrative computational model for tissue specific gene regulation

Presenter: Pawel Bednarz

University of Warsaw

Pawel Bednarz, Bartek Wilczynski, University of Warsaw

Understanding the process of cell differentiation, where cells with identical DNA acquire different morphologies requires us to develop accurate models of the mechanisms behind tissue-specific gene expression. There are many factors involved in gene regulation, including chromatin accessibility, nucleosome occupancy and transcription factor binding. To better understand the complex relationships between all these elements, we need computational models integrating available experimental data to make more accurate predictions for any tissue-specific gene. We propose a new model that consists of three layers representing respectively DNA sequential features, nucleosome occupancy and tissue-specific gene expression. To link them together we used machine learning approaches for which the parameters were learned in an expectation-maximization procedure. As a result we obtained a method able to model differences in gene expression between two cell types in adult Drosophila Melanogaster. By further investigating the model's structure we were able to find putative sequential features driving differential gene expression and make predictions regarding the locations of true regulatory elements in the vicinity of tissue-specific genes.

Key role of penetration effects in electrostatic interactions between dimers and in biomolecules.

Presenter: Sławomir Bojarowski

University of Warsaw Faculty of Chemistry

Sławomir Bojarowski, Prashant Kumar University of Warsaw Faculty of Chemistry, Paulina M. Dominiak University of Warsaw Faculty of Chemistry

University at Buffalo Databank (UBDB) is a databank which contains electron density parameters obtained from theoretical structure factors and through avaraging within the certain atom types. It is able to reproduce the electron density just out of atoms coordinates. The main aim of our work was to test the quality of electrostatic properties estimated from UBDB on small compounds from benchmark sets. Values obtained with EPMM method were compared to analogical from GAFF RESP and thereafter to reference values. Results reveal the preponderance of UBDB+EPMM in accuracy of electrostatic interaction energy estimation. This property become overwhelming at smaller distances. Afterwards we examined similar properties on biological systems: interactions between chosen residues from fatty acid amide hydrolase (FAAH) with several ligands. We compared our results from UBDB+EPMM with similar from GAFF. In our method RMSD is about three times lower and correlation coeficient is significantly better. Such tendencies are explained on the theory of so called penetration energy. Multipole expansion aproximation is not sufficient when interacting molecules are close enough, that electron clouds are overlaping themselves.

Acute ethanol exposure disrupts actin cytoskeleton in adult mouse brain slices.

Presenter: Gosia Borczyk

Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw

Gosia Borczyk, Kasia Radwańska, Nencki Institute of Experimental Biology Polish Academy of Sciences, Anna Suska Nencki Institute of Experimental Biology Polish Academy of Sciences

Acute ethanol exposure negatively affects both the adult and developing CNS. It has been shown that ethanol disrupts the actin cytoskeleton but the exact mechanisms of alcohol-induced changes in nerve cells remain unclear. The lack of well-established in vitro models of acute ethanol exposure on the adult brain continues to be an obstacle in investigating the details of its mode of action. Here, we prepared acute brain slices from adult animals and evaluated actin polymerization in the CA1 region of the hippocampus after 30 min of ethanol (50 mM or 100 mM) incubation using phalloidin staining. Both doses of alcohol induced actin depolymerization in this model (effect size about 35%, $p<0.05$). This phenomenon was partially reversed with a common antioxidant ascorbic acid (effect size about 20%, $p<0.05$). We have developed an in vitro model of acute ethanol exposure on adult mice brain. Our results indicate that actin depolymerization by ethanol can be, at least partially, attributed to free radicals as it can be reversed by ascorbic acid. Additionally, the established model can be used for further investigation of the molecular mechanisms of action of ethanol on the adult brain.

C-1748 shows anticancer potential by inducing apoptosis and senescence in pancreatic cancer cells.

Presenter: Barbara Borowa-Mazgaj

Gdańsk University of Technology

Barbara Borowa-Mazgaj, Ewa Augustin, Gdańsk University of Technology, Jerzy Konopa, Gdańsk University of Technology, Zofia Mazerska,Gdańsk University of Technology.

Pancreatic cancer has the highest mortality rate of all major cancers. C-1748 is a DNA binding agent with potent antitumor activity and was selected for phase I clinical trials. Here we investigated the cellular response of Panc-1 and AsPC-1 cells to C-1748 treatment in terms of growth arrest and cell death. Cell cycle analysis following drug treatment showed gradual increase of Panc-1 cells with sub-G1 DNA content (70% after 192 h) considered as apoptotic, on the contrary the fraction of sub-G1 AsPC-1 did not exceed 17%, but starting from 72 h of drug exposure, cells underwent permanent accumulation in G1 phase. The presence of considerable amount of apoptotic Panc-1 cells but only a low amount of apoptotic AsPC-1 cells was confirmed by typical for apoptosis morphological changes and biochemical markers such as: phosphatydylserine externalization, caspase-3 activation, mitochondrial dysfunction. Furthermore, ASPC-1 cells surviving C-1748 treatment were in state of senescence, based on expression of β-galactosidase and increased level of p21 protein. In conclusions, these results highlight the therapeutic potential of C-1748 in pancreatic cancer and support rationale for its further investigation towards this type of malignancy.

Role of focal adhesion anchoring domains in localization and signaling of the adaptor protein CAS

Presenter: Jaroslav Braniš

Charles University in Prague

Jaroslav Braniš, Daniel Rosel, Jan Brábek, Charles University in Prague; Wolfgang Goldmann, Friedrich-Alexander University Erlangen-Nürnberg

The adaptor protein CAS plays a role of the prominent mechanosensing protein in focal adhesions (FAs) where transduces mechanical signals into extension of its substrate domain. During this process substrate domain of CAS is susceptible to be phosphorylated by Src family kinases resulting in activation of downstream signaling. The concept of CAS as a mechanosensor requires two domains which anchor CAS into FAs. The studies concerned with the CAS anchoring into FAs showed N-terminal SH3 and C-terminal CCH domains are essential in FA targeting. For this work we prepared a set of CAS mutants lacking one or both focal adhesion-anchoring domains and mutants where we replaced these domains with the focal adhesion targeting (FAT) domain of FAK or Leu zipper dimerization motif of GCN4 to test the exclusivity of individual focal adhesion-anchoring domain of CAS for mechanosensing properties. We used these mutants to analyze CAS anchoring into FAs; CAS dynamics in FAs and ability of the cells to generate traction forces. Our results suggest that the SH3 domain should be indispensable not only for the CAS stabilization in the FAs but also in the process of the cellular traction forces generation.

Conventional method of liposomes preparation versus novel membrane techniques

Presenter: Anna Bryła

Poznań University of Technology, Institute of Chemical Technology and Engineering, Poland

Anna Bryła, Grażyna Lewandowicz, Poznań University of Life Sciences, Department of Biotechnology and Food Microbiology, Poland

Liposomes are vesicles, composed of phospholipid bilayer, entrapping one or more aqueous compartments. Many methods for liposomes preparation has been described, but there is still need for one-step process and scaling up. The above, can be accomplished with the use of membrane technology. The aim of the work was to compare the suitability of microfiltration systems, working in two different modes and conventional Bangham technique, for liposomes preparation. Elderberry fruit extract was encapsulated into liposomes. Liposomes were prepared by conventional Bangham method, microfiltration trough coated membrane, and ethanol injection. Liposomes suspensions were analysed in terms of size distribution, zeta potential and encapsulation efficiency. It was stated that, elderberry extract can be encapsulated into liposomes using all three methods. However, obtained liposomes suspensions differ significantly in terms of size. Similar values of zeta potential and encapsulation efficiency were evaluated in all cases. However, the different membranes pores size are required for presented membranes techniques. The ethanol injection method revealed the highest potential to continuous work as well as for scaling up.

S-NITROSYLATION OF POSTSYNAPTIC DENSITY PROTEINS IN ALZHEIMER'S DISEASE

Presenter: Katarzyna Bucholc

Institute of Biochemistry and Biophysics PAS

Katarzyna Bucholc, Katarzyna Bucholc, Aleksandra Wysłouch-Cieszyńska, Michał Dadlez, Monika Zaręba-Kozioł (Institute of Biochemistry and Biophysics PAS)

The pathogenesis of Alzheimer's disease (AD) is linked to oxidative/nitrosative stress caused by overproduction of reactive oxygen species and reactive nitrogen species (RNS) and an imbalance in the redox state of the cells. One of the consequences of the RNS interaction with proteins is their S-nitrosylation. This modification is emerging as an important redox signaling mechanism which can regulate a broad range of physiological functions. We have analyzed endogenous S-nitrosylation of postsynaptic density proteins isolated from the brains of APP transgenic mice (models of Alzheimer's disease) and control FVB mice of 3, 6 and 14 months of age. We have used methods enabling us to selectively enrich the PSD fraction in S-nitrosylated proteins and mass spectrometry analysis for their identification. As a result, we have listed numerous S-nitrosylation sites in PSD proteins. Excessive S nitrosylation of these proteins was observed in APP mice from the youngest age. Among the proteins that were differentially S-nitrosylated in young 3-month-old APP mice were cytoskeletal proteins, receptors, signaling proteins and proteins responsible for exo- and endocytosis of synaptic vesicles.

Deamination of methylated and non-methylated cytidines by human AID

Presenter: Lucyna Budzko

Institute of Bioorganic Chemistry Polish Academy of Sciences, Poland

Lucyna Budzko, Lucyna Budzko1, Paulina Jackowiak1, Karol Kamel1, Joanna Sarzyńska1, Anna Philips1, Janusz M. Bujnicki2,3, Marek Figlerowicz1,4 1 Institute of Bioorganic Chemistry Polish Academy of Sciences, Poland 2 International Institute of Molecular and Cell Biology, Laboratory of Bioinformatics and Protein Engineering, Poland 3Institute of Molecular Biology and Biotechnology Faculty of Biology Adam Mickiewicz University, Poland 4Institute of Computing Science Poznan University of Technology, Poland *Corresponding author: marekf@ibch.poznan.pl*

Activation-induced cytidine deaminase (AID) is known for its established function in the antibody diversification. Nevertheless, its ability to deaminate 5-methylcytidine (5mC) suggests that AID can play a role in the genome demethylation. Although an amount of in vivo data confirm AID involvement in the demethylation processes, in vitro studies argue that 5mC is a poor substrate for AID relative to non-methylated cytidine (C). It has been proposed that 5mC is disfavored by the enzyme's active site due to the size restriction against C5 substituent. The data support the opposite hypothesis that the involvement of AID in DNA demethylation is highly unlikely. Basing on studies of mutants and molecular dynamics simulations, we shown that the methyl group impact DNA deamination however not by the previously proposed steric hindrance. We found that AID activity on C and 5mC could be decoupled by the protein mutagenesis and we indicated that these two substrates are differently recognized by the enzyme. Based on our results we conclude that the participation of AID in the demethylation processes by 5mC deamination is feasible in terms of its enzymatic properties.

Translational readthrough of premature termination codons and its use in primary ciliary dyskinesia

Presenter: Zuzanna Bukowy-Bieryllo

Institute of Human Genetics Polish Academy of Sciences, Poznan, Poland

Zuzanna Bukowy-Bieryllo, Bukowy-Bieryllo Zuzanna (1,2), Dabrowski Maciej (1), Zietkiewicz Ewa (1) Affiliations: (1) Institute of Human Genetics PAS, Poznan, (2) International Institute of Molecular and Cell Biology, Warsaw. *Study supported by the National Science Centre grants no. 2011/01/B/NZ4/04840 and 2013/09/NZ4/01692.*

Premature termination codons (PTC) cause premature termination of translation and protein shortening. PTC can be overcame through insertion of a random amino acid in place of PTC(PTC-readthrough, PTC-RT), yielding full-length protein. Stimulation of PTC-RT by aminoglycosides (AAG) is considered an alternative to gene therapy in genetic diseases caused by PTC. Efficiency of PTC-RT depends on many factors: the compound used,PTC type and its surrounding sequence. AAG toxicity also requires finding efficient stimulation conditions, without negative effects on the cell/organism. Our study focused on the use of PTC-RT for primary ciliary dyskinesia (PCD), genetic disorder caused by defects of motile cilia and flagella. PCD causes recurrent airway infections, bronchiectases and lung function decrease, as well as randomization of inner organ symmetry and male infertility. In our study, 14 PTC in 5 PCD-causing genes were used. Oligonucleotides containing PTC and its surrounding sequences were cloned into a reporter vector and translated in vitro with different AAG concentrations. Five most effective PTC were later tested in cell lines. Cyto- and ciliotoxicity of AAG was tested in in vitro differentiated primary airway epithelial cells.

Localization of Nav1.9 ion channel in pyramidal neurons of rat prefrontal cortex

Presenter: Małgorzata Całka

Medical University of Warsaw

Małgorzata Całka,

The main purpose of study was to confirm localization of Nav1.9 ion channel in pyramidal neurons of rats medial prefrontal cortex in two age groups (20 and 60 days old). Method used in experiment was immunofluorescence staining of histological slides of rat brain tissue, results were gathered with confocal laser scanning microscopy. Studies confirmed presence of Nav1.9 channel in prefrontal cortex. Second part of work was to check if there are statistically significant differences in quantity of channel protein in pyramidal cells of V layer of the prefrontal cortex between two age groups. Results show that difference is present in compared groups. This study may be useful for understanding brain development which occur during adolescence.

SK053, an inhibitor of protein disulfide isomerase shows potent anti-leukemic effects in AML cells

Presenter: Justyna Chlebowska

Laboratory of Experimental Medicine, Center of New Technologies, University of Warsaw, Warsaw, Poland

Justyna Chlebowska, Pawel Gaj Department of Immunology Medical University of Warsaw, Michal Lazniewski Laboratory of Functional and Structural Genomics Center of New Technologies University of Warsaw, Malgorzata Firczuk Department of Immunology Medical University of Warsaw, Karolina Furs Department of Immunology Medical University of Warsaw, Radoslaw Sadowski Department of Immunology Medical University of Warsaw, Pawel Leszczynski Department of Immunology Medical University of Warsaw, Piotr Stawinski 5Department of Medical Genetics Medical University of Warsaw, Szymon Klossowski Institute of Organic Chemistry Polish Academy of Sciences, Ryszard Ostaszewski Institute of Organic Chemistry Polish Academy of Sciences, Krzysztof Giannopoulos Department of Experimental Hematooncology Medical University of Lublin, Dariusz Plewczynski Laboratory of Functional and Structural Genomics Center of New Technologies University of Warsaw, Jakub Golab 3Department of Immunology Medical University of Warsaw, Dominika Nowis Laboratory of Experimental Medicine Center of New Technologies University of Warsaw

Introduction of differentiation-inducing agents to the treatment of acute promyelocytic leukemia was a remarkable therapeutic breakthrough. However, there is no such significant progress in the treatment of other acute myeloid leukemia (AML) types. Numerous proteins involved in tumor development have allosteric disulfide bonds amenable to modifications affecting protein structure and function. Targeting allosteric disulfide bonds becomes a novel approach to cancer treatment. Our interest in this field focuses on proteins with thioredoxin fold such as protein disulfide isomerase (PDI). We have developed SK053, a small molecule inhibitor of allosteric disulfide bonds formation. The aim of this project was to evaluate anti-leukemic and differentiation-inducing effects of SK053 and investigation of the potential mechanisms of its activity. We evaluated the biological effects on proliferation and differentiation and the transcriptome changes determined by SK053 in the human myeloid cell lines. Our results show that SK053 reduces cell proliferation evaluated and induces myeloid differentiation.

Cox17 is an auxiliary factor involved in the control of the MICOS complex

Presenter: Magdalena Chojnacka

Międzynarodowy Instytut Biologii Molekularnej i Komórkowej w Warszawie

Magdalena Chojnacka, Agnieszka Gornicka and Agnieszka Chacinska International Institute of Molecular and Cell Biology, Warsaw, Poland

Mitochondria are essential organelles for every eukaryotic cell. Four mitochondrial compartments can be distinguished: outer membrane, inner membrane, intermembrane space and matrix. The mitochondrial inner membrane consists of the inner boundary membrane connected with the cristae membrane by the crista junctions. MICOS, a recently discovered protein complex, is crucial for establishment and maintaining the proper inner membrane architecture. Furthermore, MICOS components were reported to interact with translocase of the outer membrane (TOM) and Mia40 oxidoreductase to facilitate transport of intermembrane space precursor proteins. Therefore, MICOS is also involved in the control of mitochondrial protein biogenesis. It remains to be discovered how the MICOS complex is assembled and regulated. Here, we report a direct link between Cox17, a protein involved in the assembly of cytochrome c oxidase, and the MICOS complex. Cox17 transiently interacts with Mic60, thereby modulating MICOS complex integrity. We propose that Cox17 is a newly identified factor involved in maintaining the architecture of the MICOS complex.

Localized synthesis of proteins at the surface of mitochondria

Presenter: Piotr Chroscicki

Laboratory of Mitochondrial Biogenesis, International Institute of Molecular and Cell Biology, Warsaw, Poland

Piotr Chroscicki, Magdalena Dlugolecka, Agnieszka Chacinska; Laboratory of Mitochondrial Biogenesis, International Institute of Molecular and Cell Biology, Warsaw, Poland

Mitochondria are organelles with their own genome. However, the majority of mitochondrial proteins are encoded in the nucleus. Thus, mitochondria-destined proteins are synthesized in the cytosol and transported into mitochondria as precursor proteins in a post-translational manner. Interestingly, recent studies show that the synthesis of the mitochondrial proteins and their translocation through mitochondrial outer membrane can be coupled. Yet the mechanism of this process is still unknown. We developed a method to efficiently isolate mitochondria with associated cytosolic ribosomes from yeast Saccharomyces cerevisiae. We observed an enrichment of ribosomal markers in the isolated mitochondrial fraction. The association between ribosomes and the Translocase of the Outer Membrane (TOM) was also detected. This method will aid in studying protein-protein interactions between cytosolic ribosomes and mitochondrial surface. Our understanding on the mitochondria-localized protein synthesis coupled to the protein import into mitochondria will be presented.

Crystallization in situ, structural study, physicochemical properties of azetidine cocrystals

Presenter: Grzegorz Cichowicz

Czochralski Laboratory of Advanced Crystal Engineering, Biological and Chemical Research Centre, Department of Chemistry, University of Warsaw, Żwirki i Wigury 101, 02-089 Warsaw, Poland

Grzegorz Cichowicz, Łukasz Dobrzycki, Michał Ksawery Cyrański Czochralski Laboratory of Advanced Crystal Engineering, Biological and Chemical Research Centre, Department of Chemistry, University of Warsaw, Żwirki i Wigury 101, 02-089 Warsaw, Poland, Roland Boese Department of Chemistry, University of Duisburg-Essen, 45117 Essen, Germany

The aim of this study was to cocrystallize azetidine with water and various liquid organic compounds and determine structure of obtained systems. Azetidine is liquid cyclic secondary amine with four membered ring. The crystal structure of the neat amine is known, there is also evidence that azetidine can co-crystallize with water and forms clathrate hydrate. Using IR laser assisted in situ crystallization technique two unknown polymorphs of azetidine and four hydrates were obtained. Amine forms hemi-, monohydrate crystal phases and two hydrates containing seven and twelve water molecules per one amine. Hemihydrate has two transformation polymorphs with phase transition at a temperature below 170 K. In hepta- and dodecahydrates amine molecules interact with water lattice via hydrogen bonds N-H...O. Comparing unit cell parameters, crystal structure of heptahydrate can be considered as a clathrate. Information obtained from the X-ray diffraction experiment shown however that water molecules are forming three distinct cages thus this is new type of hydrate. In addition crystallization of azetidine mixtures with various organic compounds was also performed but the crystals were only obtained in case of cyclobutanole and piperidine.

Lack of S100A10 prevents Ca2+-dependent translocation of TRPV5/6 to plasma membranes of osteoblast

Presenter: Anna Cmoch

Nencki Institute of Experimental Biology

Anna Cmoch, Cmoch A1, Zablocki K1, Groves P2, Pikula S1 1Department of Biochemistry, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland 2Department of Medicinal Chemistry, University of Gdansk, Gdansk, Poland*

The TRPV5/6 channels are involved in the efficient uptake of calcium in osteoblasts, however the molecular mechanisms controlling their distribution and activity remain unclear. Here we provide the first evidence for a novel S100A10-dependent regulatory mechanism in the mineralization of osteoblasts. In the presence of stimulators of mineralization osteoblast-like Saos-2 cells developed a mineralizing phenotype accompanied by an increase in intracellular calcium level, translocation of calcium binding proteins to membranes and matrix vesicles (centres for mineral formation). Interestingly, we confirmed a direct molecular interaction of TRPV5/6 with S100A10 at plasma membrane of mineralizing Saos-2 cells., downregulation of S100A10 in Saos-2 cells showed inhibitory effects on the cellular distribution of TRPV5/6 channels, on the intracellular calcium concentration and on the ability of cells to mineralize extracellular matrix. The work was supported by grant 2012/05/N/NZ3/00330 from the Polish National Science Centre and the statutory grant to the Nencki Institute of Experimental Biology.

Contribution of lipid peroxidation to the aging phenotype of Ercc1 deficient mice

Presenter: Jolanta Czerwińska

Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland

Jolanta Czerwińska, Małgorzata Nowak2, Konrad Kosicki2, Patrycja Wojtczak2, Jarosław Cieśla1, Laura Niedernhofer3, Elżbieta Speina1, Barbara Tudek1,2. 1 Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland; 2 Institute of Genetics and Biotechnology, University of Warsaw, Poland; 3 The Scripps Research Institute, Jupiter, Florida, USA

Ercc1-/- mice are deficient in functional DNA repair endonuclease, ERCC1-XPF. They display untimely many features of aging and are hypersensitive to oxidative stress. One of the consequences of oxidative stress is lipid peroxidation (LPO), that generates reactive aldehydes, e.g. 4-hydroxy-2-nonenal (HNE). The aim of my study was to establish the role of lipid peroxidation in the premature aging phenotype of Ercc1-/- mice. Ercc1-/- murine embryonic fibroblasts (MEFs) were hypersensitive to long chain LPO products, and the most toxic compound was HNE. HNE caused cell death of Ercc1-/- MEFs mainly by inducing necrosis, while of wt MEFs by apoptosis. In Ercc1-/- cells HNE-treatment caused intensive poly(ADP-ribosylation) of proteins, which appears in the presence of DNA single- and double-strand breaks. I found also that HNE markedly inhibited Ercc1-/- cells proliferation and induced senescence to a greater extent of Ercc1-/- than of wt MEFs. Moreover HNE induced transcription of translesion (TLS) DNA polymerases stronger in Ercc1 deficient than in proficient cells. Taken together, these results suggest that LPO may contribute to observed phenotype of Ercc1-/- mice. This work was financed by the grant N N303 819540 from MNiSW, Poland.

Liverworts' antibacterial activity.

Presenter: Magdalena Czołpińska

Adam Mickiewicz University, Faculty of Biology, Insitute of Experimental Biology, Department of Genetics

Magdalena Czołpińska, Katarzyna Buczkowska, Assistant Professor, Adam Mickiewicz University, Faculty of Biology, Insitute of Experimental Biology, Department of Genetics

Many species of plants produce compounds, which exhibit various biological properties. Their chemical structure allows qualify them to terpenoids, phenol derivatives and alkaloids. The evolutionarily oldest land plants – liverworts – has an ability to synthesizing chemical compounds belonging to mentioned earlier groups. So far, published data confirm content, i.a. terpenoids and phenolic compounds in liverworts and give us acknowledgment about their antimicrobial activity. This activity is variable and depends not only on species or region of occurrence but also on method of extraction or distillation or part of plant from which we obtain extracts. Recently we can observe increased interest in use of substances of natural, plant origin in the fight against microorganisms. The using of plant extracts is extremely promising, because so many strains become resistant to commonly used antibiotics. In the present contribution was tested antibacterial activity of extracts from selected liverworts species. Different species of these plants were examined. These results showed that liverworts are source of some chemical compounds which quantity and biological activity should be examined in further researches.

Global topology of proteins with (local) complex lasso motif

Presenter: Pawel Dabrowski-Tumanski

Faculty of Chemistry, University of Warsaw and Centre of New Technologies, University of Warsaw

Pawel Dabrowski-Tumanski, W. Niemyska Institute of Mathematics, University of Silesia, M. Kadlof Faculty of Mathematics, Informatics and Mechanics and Center of New Technologies, University of Warsaw, E. Haglund Center for Theoretical Biological Physics and Departments of Physics and Astronomy, Chemistry and Biochemistry and Cell Biology, Rice University, Houston, USA, P. Sułkowski Faculty of Physics, University of Warsaw and Walter Burke Institute for Theoretical Physics, California Institute of Technology, Pasadena, CA, USA and J. I. Sulkowska Faculty of Chemistry and Center of New Technologies, University of Warsaw

During last two decades great attention has been paid to the importance of topology in protein function. It was found that the complex topology (e.g. in knots) is strongly conserved during the evolution. It is then tempting to conjecture, that topology can influence both function and properties of the proteins (and also other biopolymers). One of the new topological motif besides knots is complex lasso found by us recently [1]. Such structure can arise e.g. in proteins possessing the loop formed by the backbone and closed by cysteine bridge. In such proteins the tail can cross the surface spanned on the loop forming the complex lasso. Proteins with such motif can however possess different number of (potentially pierced) loops, which make them topologically unlike. Here we classify the proteins possessing the complex lasso motif into separate groups depending on the number and mutual relation between the loops. We also seek for the biological importance of such partition. [1] W. Niemyska, P. Dabrowski-Tumanski, M. Kadlof, E. Haglund, P. Sułkowski and J. I. Sulkowska, Complex lasso: new entangled motifs in proteins, sent to PNAS

New genetic markers for human population identification.

Presenter: Patrycja Daca-Roszak

Institute of Human Genetics, Polish Academy of Science

Patrycja Daca-Roszak, P. Daca-Roszak, E. Ziętkiewicz, M. Witt

The goal of our study was to identify new genetic markers, which would allow efficient discrimination between European and East Asian populations. Markers selection was based on the results obtained from the Illumina microarrays-based analysis of human B-lymphocyte cell lines representing European and Chinese/Japanese populations (collaboration with the Institute of Oncology, Gliwice). Twenty genes with different expression profiles between the two populations were selected based on the Illumina transcription microarray results. Thirteen of these genes were validated using TLDA cards, and three represented statistically significant differences in their expression profiles. A set of 24 population-informative genomic SNPs, not associated with the pigmentation genes, was selected based on the Illumina Human OmniExpressExome microarray results.The multiplex minisequencing assay (SNaPshot EurEAs_14_G) for genotyping 14 of these SNPs and a marker allowing gender identification was developed. A set of CpG loci characterized by different levels of methylation in both populations was selected based on the Infinium HumanMethylation450 array results. We are now at the stage of laboratory validation of the selected CpG regions.

Selol - organo-selenium compound affects apoptosis and CAMs expression in prostate cancer cells.

Presenter: Kurpios-Piec Dagmara

Department of Biochemistry, Medical University of Warsaw, Poland

Kurpios-Piec Dagmara, Emilia Grosicka-Maciąg, aDepartment of Biochemistry, Medical University of Warsaw, Emilia Orzechowska, Department of Molecular Biology, Faculty of Biology, University of Warsaw, Ewelina Kiernozek, Department of Immunology, Faculty of Biology, University of Warsaw, Piotr Suchocki, Department of Drug Analysis, Maria Szumiło, Department of Biochemistry, Medical University of Warsaw, Iwonna Rahden-Staroń, Department of Biochemistry, Medical University of Warsaw

Prostate cancer is the second leading cause of male cancer mortality and its metastasis to bones has been observed in 90% of died patients. Thus, one of the main therapeutic problems is the prevention of metastases. Selenium is one of the trace elements which was proved to be a key component of the diet important in the chemoprevention of cancer. Cell adhesion molecules play an important role at each stage of the metastasis. PC3 cells exhibit inhibition of apoptosis, increased ability to migrate through increased activity of MAPK and FAK and increased expression of IKK kinase which stimulates activity of NF-kB, the main regulator of many cell survival proteins functions. Selol is a mixture of selenitriglicerides synthesized from sunflower oil containing selenium (IV) which was carried out at the Department of Drug Analysis at MUW (Pol. PL 176530 (Cl. A61K31/095). To elucidate the effect of organic Se on PC3 cells we carried out an in vitro study aimed at assessing generation of ROS, apoptosis, cellular distribution of NF-κB, and expression of ICAM-1 and ALCAM-1. We have observed that Selol generates ROS, decreases level of apoptosis, and selectively affected an expression of adhesion molecules on the surface of PC3 cells.

Structural basis of 5'mRNA cap interaction with Decapping Scavenger enzyme (DcpS)

Presenter: Zbigniew M. Darżynkiewicz

Centre of New Technologies, University of Warsaw

Zbigniew M. Darżynkiewicz, Aleksandra Ferenc-Mrozek, Edward Darżynkiewicz, Janusz Stępiński

DcpS enzyme paritcipates in 3'-5' mRNA degradation pathway, following deadenytalion and exosome-mediated turnover. Human DcpS is a nucleocytoplasmic shuttling protein, its activity may appear in the nucleus and the cytoplasm. DcpS protein is a member of the pyrophosphatases HIT family and use a histidine triad to carry out catalysis in a metal-independent manner, releasing 7-methylguanosine monophosphate(m7GMP) and diphosphate terminated up to 10 nucleotides RNA. Recent data shows that disruption of DcpS decapping activity leads to accumulation of m7GpppN cap structures and is correalted with neurological disorders. It was also found that DcpS is an esssential gene and its deletion is embryonic lethal. Previous structural studies on human scavenger decapping enzyme revealed that DcpS protein is a symmetric homodimer in ligand free form and the asymmetric structure with bound cap analogues. Main goal of this work was to gain insight into the role of first transcribed nucleotide in short mRNA degradation. This nucleotide may play important role in protein-ligand complex formation and stabilization. Biophysical experimental methods, including fluorescence titration and enzyme kinetics were applied to verify this hypothesis.

miRNA transcriptome profiling and functional in vitro study in T-cell acute lymphoblastic leukemia

Presenter: Małgorzata Dawidowska

Department of Molecular and Clinical Genetics, Institute of Human Genetics Polish Academy of Sciences, Poznań, Poland

Małgorzata Dawidowska, Małgorzata Dawidowska1, Bronisława Szarzyńska-Zawadzka1*, Maria Kosmalska1, Łukasz Sędek2, Roman Jaksik3, Anna Lalik3, Katarzyna Derwich4, Anna Pieczonka4, Krzysztof Kałwak5, Marek Ussowicz5, Monika Lejman6, Robert Dębski 7, Jolanta Goździk8, Aleksandra Szczepankiewicz9, Tomasz Szczepański2, Michał Witt1 1 Department of Molecular and Clinical Genetics, Institute of Human Genetics Polish Academy of Sciences, Poznań, Poland 2 Department of Pediatric Hematology and Oncology, Medical University of Silesia, Zabrze, Poland 3 Systems Engineering Group, Institute of Automatic Control, Silesian University of Technology, Gliwice 4 Department of Pediatric Hematology, Oncology and Transplantology, University of Medical Sciences, Poznań, Poland 5 Department and Clinic of Pediatric Oncology, Hematology and Bone Marrow Transplantation, Wroclaw Medical University, Wrocław, Poland 6 Department of Pediatric Hematology, Oncology and Transplantology, Cytogenetic Laboratory, Children's University Hospital, Lublin, Poland 7 Department of Pediatric Hematology and Oncology, Collegium Medicum in Bydgoszcz Mikołaj Kopernik University, Poznań 8 Department of Pediatric Oncology and Hematology, Jagiellonian University Collegium Medicum, Kraków, Poland 9 Laboratory of Molecular and Cell Biology, Department of Pediatric Pulmonology, Allergy and Clinical Immunology, Poznan University of Medical Sciences, Poland *the authors contributed equally*

T-cell acute lymphoblastic leukemia(T-ALL) is rare heterogeneous leukemia with poor prognosis.Based on our preliminary results and data on miRNA expression diversity in cancer vs. normal tissues in various diseases we hypothesise that: miRNA expression profile differs in T-ALL vs. other ALL subtypes/other leukemias and might be key to understanding T-ALL pathomechanisms and heterogeneity. Objectives: Identification of miRNAs specifically expressed in T-ALL and in vitro demonstration of their role in genes' regulation to explore T-ALL pathomechanisms. Correlation of results with T-ALL subtype and treatment outcome to assess miRNAs' diagnostic/prognostic potential. Methods: The project combines miRNA next generation sequencing(miRNA-seq) and in vitro functional experiments on T-ALL cell lines with bioinformatics tools and molecular methods:Dual Luciferase Reporter Assay (miRNA-target 3'UTR interaction); miRNA inhibition/mimicry (in vitro expression changes of miRNAs); miRNA target site blocking (TSB) and degradation of target mRNA (GapmeR) (in vitro modulation of miRNA-mRNA regulatory links);flow cytometric assays,qRT-PCR, Western blotting and integrated transcriptome-proteome analysis to assess the effects of in vitro experiments.

MiRNA expression changes during dedifferentation of Arabidopsis thaliana mesophyll protoplasts

Presenter: Konrad Dełeńko

Department of Cell Biology, Faculty of Biology and Environment Protection, Nicolaus Copernicus University in Toruń

Konrad Dełeńko, Janusz Niedojadło1, Przemysław Nuc2, Katarzyna Niedojadło1, Elżbieta Bednarska-Kozakiewicz1, 1Department of Cell Biology, Faculty of Biology and Environment Protection, Nicolaus Copernicus University in Toruń, 2Institute of Molecular Biology and Biotechnology of Adam Mickiewicz University

After cell wall removal, plant cells becomes totipotent protoplasts which in specific conditions (hormones supplementation of culture medium) are able to proliferation, next differentiation and regeneration of whole plant. Our previous study show that dcl1-9 cells are unable to proliferate and show higher death rate after few days of protoplasting in comparison to wt cells, suggesting that miRNA molecules are involved in dedifferentiation. In this research we have analyzed miRNA transcriptome from A. thaliana wild type cells during first stages of their dedifferentiation. Our analysis showed that transition from differentiated mesophyll cells, through protoplast to proliferating cells is accompanied by expression changes in 94 known miRNA molecules. In protoplast, 15 miRNAs was differentially expressed, 12 miRNAs were down- and 3 upregulated in comparison to mesophyll cells. Potential targets of these 3 upregulated miRNAs are i.a. WRKY transcription factors, methyl-CpG-binding domain (MBD) proteins, heat shock proteins and MYB-HB-like transcription factor. These results show that miRNA molecules could be involved in strong decrease of transcription level in protoplast, which we shown earlier (Dełeńko et al. 2014).

Natural Therapies for Type 2 Diabetes Mellitus

Presenter: Arleta Dołowacka

Department of Medical Biochemistry,Silesian Piasts University of Medicine in Wrocław, Poland

Arleta Dołowacka, Kinga Gostomska, Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wroclaw, Izabela Nawrot - Hadzik, Department of Pharmaceutical Biology and Botany, Silesian Piasts University of Medicine in Wrocław, Poland

We are looking for new natural medicines to use in the treatment of diabetes. Continuous development of pharmacology, which is based on a synthesis of the active chemicals weakened a little interest in herbal medicine. Increasing number of allergic reactions in patients and dangerous interactions this led to a return to phytotherapy. Thus, in recent years interest in herbal medicine in Poland and around the world instantly began to grow. More and more people are convinced to products of natural origin. Thanks to numerous research, it turns out that phytotherapy is an effective method in pharmacovigilance and in the prevention of certain diseases. Most scientific studies that are designed to analyze plant-derived having antidiabetic activity, indicate that about a hundred of plant compounds, for example Vaccinium myrtillus, Lupinus albus or Nigella sativa, may be used in treating and preventing diseases. It has been observed that they can be whole plant and certain parts in the form of dried or fresh. Despite the growing fame of phytotherapy in modern times, weakening its position are as yet in vivo studies in animal models: rats, rabbits with pharmacologically or genetically induced diabetes.

Calculations of PROTEIN HYDRATION FREE ENERGIES FROM EXPLICIT AND IMPLICIT SOLVENT SIMULATIONS

Presenter: Anita Dudek

Centre of New Technologies, Zwirki i Wigury 93, 02-089 Warsaw, Poland

Anita Dudek, Piotr Setny Centre of New Technologies, Zwirki i Wigury 93, 02-089 Warsaw, Poland

The stability of protein conformations is determined by the properties of their free energy landscapes. Although our attention usually focusses just on the details of protein molecular structures, we shouldn't forget that they naturally function in a dense aqueous environment, whose influence may shape the free energy landscape to no lesser degree than internal protein interactions. In order to quantify such solvent effects we developed a protocol allowing for precise calculations of hydration free energy changes accompanying natural protein motions. For explicit solvent molecular dynamics simulations the protocol is based on thermodynamic integration method. Benefitting from the fact that free energy is a function of state and its changes do not depend on particular path between two endpoints, we consider nonphysical transformations between distinct protein conformations. The results of calculations indicate the importance of hydration free energy for protein function. Further, their comparison with estimates obtained with Poisson-Boltzman method allow the assesment of accuracy of this most widely used implicit solvent model.

The mystery of ultra-slow oscillations in subcortical visual areas

Presenter: Katarzyna Dyl

Jagiellonian University

Katarzyna Dyl,

The visual system in the brain consist of neocortical and subcortical structures. Besides perceptual processes, subcortical regions are involved in the regulation of the pupillary light reflex, the sleep-wakefulness cycle and circadian rhythms. Reaserch has shown that majority of these areas contain a population of neurons discharging action potentials in particular activity – ultra-slow oscillations. Oscillatory firing patterns have been found in suprachiasmatic, lateral geniculate and olivary pretectal nuclei. The rhythmic activity is characterized by two subsequent phases: silent and active, with low and high frequency of action potentials. According to recent studies, oscillatory manner is not generated by intrinsic properties of neurons, but is highly dependent on light condition and synchronized activity of retinal cells. The presence of rhythmicity in distant brain areas indicates functional importance, but its role has not been fully recognized so far. It is speculated, that neurons exhibiting oscillatory firing may give rise to oscillations in pupil diameter followed by sleep initiation or underlie the generation of circadian rhythms. However, the significance of ultra-slow oscillations remains an open question.

Deciphering the language of fungal pathogen recognition receptors

Presenter: Witold Dyrka

Wroclaw University of Technology

Witold Dyrka, Witold Dyrka (Inria, IBGC CNRS, Wroclaw University of Technology), Pascal Durrens (LaBRI CNRS), Mathieu Paoletti (IBGC CNRS), Sven J Saupe (IBGC CNRS), David J Sherman (Inria, LaBRI CNRS)

The NLR family of receptors plays a key role in the innate immune system of animals, plants and fungi. In the latter two phyla NLRs adapt quickly to ever-changing pathogen-specific invasion markers thanks to their repeat-based architecture, which can produce diversity of recognition epitopes through unequal crossing-over and mutation. Characterizing computationally the language of these pathogen recognition receptors can provide insight into the molecular mechanisms of immune response and describe the limits of the pathogen targets that can be recognized. In this work, we model generation and selection of the recognition epitopes as a stochastic string rewriting system with constraints, tuned by analysis of observed evolutionary processes and validated with regard to a large dataset of fungal NLRs. Among others, analyzing the feasible set of solutions revealed that the model explained the i/i + 2 periodicity observed in the repeat number distribution of a family of receptors. In addition, in exploring discrepancies between real and simulated data we discovered an overrepresented pattern which potentially has functional importance.

Serine protease evolution in fungi with variable lifestyles

Presenter: Agata Dziedzic

Institute of Biochemistry and Biophysics, Polish Academy of Sciences

Agata Dziedzic, Anna Muszewska, Institute of Biochemistry and Biophysics, Polish Academy of Sciences

Fungi are able to switch between different lifestyles in order to adapt to environmental changes. Their ecological strategy is strongly connected to their secretome. In this study we focus on fungal serine proteases, which distribution is barely described so far. In order to obtain a complete set of fungal proteases, we performed searches against Uniprot database and JGI database. Obtained results suggest that serine proteases are more ubiquitous than expected. From 53 serine protease families described in Merops Peptidase Database, 18 are present in fungi. Interestingly, 17 of them are also present in Metazoa - this suggest that most fungal serine proteases evolved before animals and fungi diverged. This hypothesis is supported by the presence of most serine proteases in ancestral fungal groups, i. e. Chytridiomycota, Microsporidia, Mucorales. Concerning all fungi species together, the contribution of serine protease families varies. The most abundant are S8 proteases (560 species), whereas only 19 species encode proteins from S49 family. Our study shows that S49 is the only one from 18 fungal families not present in Ascomycota. Here, we present a comprehensive evolutionary history of fungal serine protease families.

Optimalisation of StrAlign – an algorithm for structural alignment of proteins

Presenter: Mirosław Falandys

Laboratory of Theory of Biopolymers, Department of Chemistry, University of Warsaw

Mirosław Falandys, Dominik Gront, Laboratory of Theory of Biopolymers, Department of Chemistry, University of Warsaw

StrAlign – a part of Bioshell utility library for bioinformatics - is a program for obtaining structural alignment between pairs of the proteins, using TM-score rotation matrix. Our target was to utilize StrAlign to compute alignments of similar accuracy to TM-align, but at lower CPU cost and further use it to cluster PDB deposits into structurally similar groups (domains). StrAlign was tested on protein domains obtained by cutting PDB deposits into domains. Its results were compared with results from TM-align. CPU time of the two methods was also recorded. Program was modified to improve these parameters. In this work we also used BioShell package to explore the space of structural alignments as calculated by TM-score and StrAlign methods.

DEVELOPMENT OF NOVEL SUBNANOMOLAR P53-MDM2 INHIBITORS

Presenter: Marcin Feder

Adamed Sp. z o.o.

Marcin Feder, Dominika Ujazdowska, Iwona Kalinowska, Joanna Jaszczewska, Ewa Burchard, Wojciech Lewandowski, Urszula Bulkowska

p53 protein responds to a variety of stresses and trigger cell cycle arrest, apoptosis or senescence, thereby protecting against malignant transformation. Almost all of human tumours are believed to harbour a disabled p53, either through mutation of the p53 gene or through aberrant expression of proteins acting as its negative regulators such as Mdm2. Thus designing molecules to block the MDM2- p53 interaction and reactivate the p53 function has been perceived as a promising therapeutic strategy for the treatment of abundant cancers retaining wild-type p53. Herein, I will present an approach that enabled us to identify novel class and Mdm2 inhibitors and to optimise their potency and in vivo efficacy. I will comment on the performance and pitfalls of the chosen computational methods as well as screening assays.

Quantification of ADMA and SDMA in biological samples using LC-ESI-MS/MS.

Presenter: Mariusz Fleszar

Department of Medical Biochemistry, Wroclaw Medical University

Mariusz Fleszar, mgr inż. Mariusz Grzegorz Fleszar, mgr Joanna Piechowicz, dr Jerzy Wiśniewski, prof. dr hab. Andrzej Gamian

Introduction L-arginine and its methylated analogs play an important role in the regulation of nitric oxide (NO) synthesis. Elevated levels of ADMA are associated with reduced NO synthesis and have been found in several diseases of the cardiovascular system. SDMA which is a regioisomer of ADMA, can affect the synthesis of NO by competing with L-arginine for the transport across the cell membranes wherefore is regarded only as a weak intermediate inhibitor of NO synthase. SDMA is considered as an excellent marker of renal function. There is also growing amount of evidence of participation SDMA in inflammation and atherosclerosis, however, they require further examination. Materials and methods The experimental material were fragments of pig myocardium. This method involves simultaneous extraction of the endogenous ADMA and SDMA from myocardium and derivatization of extracted analytes and the internal standard (D7 ADMA). Results and Discussion Our method allows to the simultaneous determination of ADMA, SDMA in myocardium tissues. The calculated concentrations of ADMA and SDMA are within previously reported ranges. For this reasons, our fast and accurate method can be a useful tool in quantitative research in analytical laboratories

Evaluation of probesets to gene mapping approaches for cross-platform microarray data integration

Presenter: Alina Frolova

Institute of Molecular Biology and Genetics of National Academy of Sciences of Ukraine

Alina Frolova, Vladislav Bondarenko, ESC "Institute of Biology" Taras Shevchenko National University of Kyiv, Dr. Maria Obolenska, Institute of Molecular Biology and Genetics of National Academy of Sciences of Ukraine

During last decades idea of gene expression data integration from different studies became a powerful way to access larger samples size. However, microarray chips design is largely different across platforms, which complicates actual integration of different datasets. Important issue, which occurs during datasets merging is incorrect probesets annotation, leading to different kinds of multiple probesets to gene mappings. In our study we analyze impact of several data merging procedures, focusing on Affymetrix and Illumina platforms. We compare three different approaches of microarray processing: using manufacturer probesets definition with random selection of several concurrent probesets, Brainarray custom probesets definition (for Affymetrix) and scoring probesets based on specificity and coverage of a probe sequence. For these purposes, we use publicly available breast cancer datasets (up to 600 samples in total) of three popular platforms - Affymetrix HGU133 Plus 2.0 and HuGene 1.0 ST, and Illumina HumanHT-12 V4.0. In general, we show that approaches based on custom probes summarization and probesets scoring give more consistent results, when merging datasets from different studies and gene-expression chips of different design.

Structural and Mechanistic Analysis of the Slx1-Slx4 Endonuclease

Presenter: Vineet Gaur

International Institute of Molecular and Cell Biology, Warsaw

Vineet Gaur, Vineet Gaur (Laboratory of Protein Structure, International Institute of Molecular and Cell Biology, 4 Księcia Trojdena Street, 02-109 Warsaw, Poland), Haley D.M. Wyatt (London Research Institute, Cancer Research UK, Clare Hall Laboratories, Blanche Lane, South Mimms, Herts EN6 3LD, UK), Weronika Komorowska (Laboratory of Protein Structure, International Institute of Molecular and Cell Biology, 4 Księcia Trojdena Street, 02-109 Warsaw, Poland), Roman H. Szczepanowski (Biophysics Core Facility, International Institute of Molecular and Cell Biology, 4 Księcia Trojdena Street, 02-109 Warsaw, Poland), Daniele de Sanctis (European Synchrotron Radiation Facility (ESRF), 71 Avenue des Martyrs, CS 40220, 38043 Grenoble Cédex 9, France), Karolina M. Gorecka (Laboratory of Protein Structure, International Institute of Molecular and Cell Biology, 4 Księcia Trojdena Street, 02-109 Warsaw, Poland), Stephen C. West (London Research Institute, Cancer Research UK, Clare Hall Laboratories, Blanche Lane, South Mimms, Herts EN6 3LD, UK), Marcin Nowotny (Laboratory of Protein Structure, International Institute of Molecular and Cell Biology, 4 Księcia Trojdena Street, 02-109 Warsaw, Poland)

The SLX1-SLX4 endonuclease required for homologous recombination and DNA repair in eukaryotic cells cleaves a variety of branched DNA structures. The nuclease subunit SLX1 is activated by association with a scaffolding protein SLX4. At the present time, little is known about the structure of SLX1-SLX4 or its mechanism of action. Here, we report the structural insights into SLX1-SLX4 by detailing the crystal structure of Candida glabrata (Cg) Slx1 alone and in combination with the C-terminal region of Slx4. The structure of Slx1 reveals a compact arrangement of the GIY-YIG nuclease and RING domains, which is reinforced by a long α helix. Slx1 forms a stable homodimer that blocks its active site. Slx1-Slx4 interaction is mutually exclusive with Slx1 homodimerization, suggesting a mechanism for Slx1 activation by Slx4.

Characterization and sensitization of glioblastoma cells resistant to Photodynamic Therapy

Presenter: Somayeh Shahmoradi Ghahe

Institute of Genetics and Biotechnology, Faculty of Biology, University of Warsaw, Poland

Somayeh Shahmoradi Ghahe, Somayeh Shahmoradi Ghahe1, Milena Bażlekowa1, Barbara Tudek1,2; 1 Institute of Genetics and Biotechnology, Faculty of Biology, University of Warsaw, Poland; 2 Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland

Photodynamic therapy (PDT) is a minimally invasive cancer treatment that involves two components including light and photosensitizer. PDT leads to generation of cytotoxic oxygen species that damage cell components and cause cell death. In this study glioblastoma cell line (U87) resistant to photodynamic therapy were isolated by applying 5-ALA and appropriate light dose after several cycles of PDT. After isolation of PDT resistant cells, some of their characteristics were studied. PDT resistant glioblastoma cells were bigger and had longer cell doubling time compare to parental cells. Cell cycle analysis showed that G1 phase was elongated in PDT resistant cells. Following PDT, resistant cells were accumulated in G2 phase after 24 hours that refers to activation of DNA damage response. In order to sensitize the resistant cells they were treated with inhibitors of DNA repair enzymes. Best sensitization were observed in response to ATM kinase inhibitor that highlights the role of DNA damage response in PDT resistant glioblastoma cells. Activation of other enzymes of DNA damage response were then studied by western blotting.

Variational formulation of Symmetry Adapted Perturbation Theory

Presenter: Piotr Gniewek

Faculty of Chemistry, University of Warsaw

Piotr Gniewek, Bogumił Jeziorski, Faculty of Chemistry, University of Warsaw, Poland

Non-covalent molecular interactions are very weak, yet they are responsible for wealth of chemical, physical and even biological phenomena. For instance molecular recognition in enzymatic reactions, and base pairing in nucleic acids are caused by weak intermolecular forces. Modern understanding of these interactions is provided by so called Symmetry Adapted Perturbation Theory (SAPT), which makes it possible to rigorously define such physically relevant quantities as electrostatic, induction, dispersion and exchange energy. Conventional formulations of SAPT are based on the modified Bloch equations. Here we present a new variant of SAPT related to the variational principle. Results of calculations for a model system show that the new formulation has exceptionally good convergence properties, which is essential for practical calculations for larger systems.

Analysis of Hsp90 interaction with its putative novel co-chaperone CacyBP/SIP

Presenter: Agnieszka Góral

Nencki Institute of Experimental Biology PAS (Warsaw)

Agnieszka Góral, Wiktor Prus, Nencki Institute of Experimental Biology PAS, Warsaw; Paweł Bieganowski, Mossakowski Medical Research Center PAS, Warsaw; Anna Filipek, Nencki Institute of Experimental Biology PAS, Warsaw

Hsp90 is a molecular chaperone essential for the folding and activation of numerous client proteins involved in important cellular processes and signaling pathways. Hsp90 has the ATPase activity which is tightly regulated by different co-chaperones and post-translational modifications. Some preliminary results have shown that the CacyBP/SIP protein, a homolog of a known Hsp90 co-chaperon, Sgt1, is present in complexes containing Hsp90. The aim of our research was to analyze the interaction between CacyBP/SIP and Hsp90 using purified proteins as well as in cultured cells, and to compare the properties of CacyBP/SIP and Sgt1 in terms of their interaction with Hsp90. Our results have shown that CacyBP/SIP, similarly to Sgt1, directly binds to Hsp90 and exhibits co-chaperone properties. However, there are some differences between Sgt1 and CacyBP/SIP. For instance, Hsp90 inhibitors, radicicol and novobiocin, influence the Hsp90-Sgt1 interaction while do not have any effect on Hsp90-CacyBP/SIP binding. Taken together, our results suggest that CacyBP/SIP might serve as a novel Hsp90 co-chaperone. Currently, more detailed studies are ongoing in order to estimate the role of CacyBP/SIP in the regulation of the Hsp90 chaperone machinery.

Advanced Glycation End-Products (AGE) as a biomarker of diabetes and its complications

Presenter: Kinga Gostomska

L. Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wroclaw, Poland

Kinga Gostomska, Arleta Dołowacka, Department of Medical Biochemistry, Wroclaw Medical University, Wroclaw, Poland

Nowadays, the marker reflecting progression of diabetes is glycated hemoglobin (HbA1c), but it does not reflect fully the actual course of the illness. It seems that the most promising is using for this purpose a stable, long-lived products of advanced glycation (AGE). These products are formed in the non-enzymatic Maillard reaction, which in a cascade of various reactions leads to the formation of highly cross-linked AGE. Glycation occurs between the reducing sugars or low molecular weight aldehydes and amino groups of proteins, lipids or nucleic acids. Created compounds accumulate in the blood vessels or in the walls of organs, causing the disruption of homeostasis. High AGE content is closely related to hyperglycemia and contributes to the emergence of diabetic complications such as nephropathy, retinopathy, angiopathies, neuropathies and cardiomyopathies, which greatly accelerate the progress of disease. Due to the fact that AGE form a very heterogeneous group of compounds, determining their level in the biological material is problematic. Currently the most common methods used for AGE detection are HPLC, mass spectrometry, ELISA and immunohistochemistry.

Identification of co-regulated genes an lncRNAs within chromatin domains.

Presenter: Ilona Ewa Grabowicz

Warsaw University, Mathematics Dept.

Ilona Ewa Grabowicz, Ilona E. Grabowicz, Bartek Z. Wilczyński - Warsaw University

Recently emerging discoveries about DNA chromatin structure have shown that it is organized in topological domains which are delineated by chromatin insulator proteins. Genes located within the same domains were reported to be more co-regulated than expected by chance. Differential gene expression analysis is commonly used to find, for example, tissue-specific genes which change their expression in response to treatment. In our case, we obtained cDNA microarray differential gene expression data from different brain tissues in mice undergoing different diets. As often happens, we are facing very low number of samples and gene expression response can be ambiguous to interpret wherefore it is difficult to find the genes responding to the changes in the diet. For that we have used the knowledge about chromatin topological domain boundaries. We have confirmed very strong gene co-regulation within domains and by using stringent gene exclusion criteria we have selected genes showing consistent response within all samples. Besides, we have found lncRNAs exhibiting strong co-regulation. In this way, we have found not only genes responding to treatment but also gene and lncRNA candidates which potentially interact with or regulate them.

Cytoskeletal changes in senescent vascular smooth muscle cells. The influence of curcumin.

Presenter: Wioleta Grabowska

Laboratory of Molecular Bases of Aging, Nencki Institute of Experimental Biolgy, PAS

Wioleta Grabowska, Wioleta Grabowska1, Emilia Wasiak2, Ewa Sikora1, Tomasz Kobiela2, Anna Bielak-Zmijewska1; 1Laboratory of Molecular Bases of Aging, Nencki Institute of Experimental Biolgy PAS; 2Department of Drag Technology and Biotechnology, Warsaw University of Technology

As a result of cellular senescence, vascular smooth muscle cells (VSMCs) significantly increase their size and become flattened. This feature is considered as one of the senescence markers and can be observed during replicative as well as stress induced senescence. In our laboratory we investigated several models of VSMCs senescence and noticed that depending on the manner of senescence induction (long passaging, curcumin or doxorubicin treatment) the size and shape of the cells differ. Therefore, we analyzed some structural proteins. We focused our interest on filaments (actin) and microtubules (tubulin) as well as proteins involved in focal adhesion (winculin, tallin, alpha-actinin). In curcumin treated senescent VSMCs actin fibers are thicker, while in replicative and doxorubicin induced senescence they are thinner and less prominent. Foci of winculin and tallin are more numerous and bigger after curcumin treatment. Changes in tubulin organization were also observed. Additionally, elasticity of VSMCs was investigated. Summarizing, various inductors of senescence have different effect on the organization of some structural proteins in VSMCs.

The Arabidopsis SWI/SNF complex responds to environmental changes in temperature - dependent manner

Presenter: Dominika Gratkowska

Institute of Biochemistry and Biophysics Polish Academy of Sciences

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The SWI/SNF chromatin remodeling complexes (CRCs) have been shown to play important roles in regulation of gene expression throughout eukaryotes. The Arabidopsis genome encodes four SWI2/SNF2 ATPases, four SWI3, a single SNF5 and two SWP73 subunits. Most of the genes encoding these core components of Arabidopsis SWI/SNF CRCs have critical but not fully overlapping roles during plant growth, including embryo- and sporophyte development. During our study we found that genes encoding the SWI/SNF CRC subunits are ubiquitously expressed and that their expression levels depend on the temperature regime of growth. Furthermore, Arabidopsis mutants impaired in several of these genes growing at lower temperatures show partial alleviation of their phenotypic defects, including reduced fertility, root development, and others. In summary, our data provide novel insight into potential regulatory role of SWI/SNF CRCs activity during plant growth at different temperature ranges.

Prenyl ammonium salts as efficient carriers for gene delivery in mammalian cell transfection

Presenter: Emilia Grecka

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The successful gene therapy requires an effective and safe introduction of therapeutic nucleic acid into the cells. Increasing number of clinical studies concern the use of nonviral gene therapy formulations, which are poorly immunogenic and can be produced on a high scale at a relatively low cost, in comparison to viral specimens. We examined four prenyl ammonium iodides (Amino-Prenols, APs): AP-7, -8, -11 and -15 for their cytotoxicity and ability to transfect genes to the cells. The most efficient cell transfection, comparable to that obtained with commercial agents, was shown for AP-15. The lowest reduction of cell viability and proliferation, considerably lower than that caused by commercial agents, was observed for AP-15. Complexes containing AP-15 and helper lipid dioleoylphosphatidylethanolamine (DOPE) had also high transfection activity and low cytotoxicity. Transfection with AP-15/DOPE complexes affected the expression of only 7 among 44 tested genes associated with lipid metabolism. Finally, complexes containing AP-15 and therapeutic plasmid, deliver the TIMP2 gene to cells efficiently, as found by TIMP2 protein level determination. Obtained results indicate that APs have a great potential for gene therapy applications

How to choose an optimal set of NMR experiments for resonance assignment of IDPs?

Presenter: Katarzyna Grudziąż

University of Warsaw, Faculty of Chemistry, Biological and Chemical Research Centre

Katarzyna Grudziąż, Katarzyna Grudziąż, Wiktor Koźmiński, Anna Zawadzka-Kazimierczuk, University of Warsaw, Faculty of Chemistry, Biological and Chemical Research Centre

Intrinsically disordered proteins (IDPs) are a numerous and functionally important class of proteins. As IDPs lack fixed three-dimensional structure, the primary method to obtain information about those proteins is NMR spectroscopy. However, because of small chemical shift dispersion standard NMR techniques usually do not provide sufficient peak resolution for IDPs studies. A number of high-dimensional techniques have been introduced to overcome this problem, they differ in terms of detected nuclei type, dimensionality and obtained resonances. Choosing an optimal set of experiments can be crucial for the success of the assignment. Here we present a comparison of several sets of high-dimensional experiments with non-uniform sampling. Using chemical shifts data deposited in BMRB we have simulated results of tested experiments, as if they were performed on various IDPs and processed using SMFT algorithm. Peak lists generated in this way were used for automatic resonance assignment using TSAR program.We then investigated how properties of the protein, such as size, number of prolines, number of glycines, presence of repetitive sequences and peak dispersion affect the completeness of the assignment.

Effect of ionotropic receptors on the neuronal SOCE

Presenter: Joanna Gruszczynska-Biegala

International Institute of Molecular and Cell Biology

Joanna Gruszczynska-Biegala, Maria Śladowska1, Jacek Kuźnicki1, 1International Institute of Molecular and Cell Biology, ul. Trojdena 4, 02-109 Warsaw, Poland, Undergraduate student from the Warsaw University of Life Sciences - SGGW, Nowoursynowska 166, 02-787 Warsaw, Poland

Interaction of ER Ca2+ sensors STIM1 and STIM2 with Ca2+ channel-ORAI1 is crucial for store-operated calcium entry (SOCE) in non-excitable cells, but in neurons the molecular mechanism of SOCE remains unclear. Our previous data indicated that both STIMs are involved in Ca2+ homeostasis in neurons, form complexes with endogenous ORAI1, but played a distinct role in SOCE. The aim of this study is to determine, which ionotropic receptors (IR) (NMDAR, AMPAR or kainate receptors - KR), in addition to ORAI channel, react with STIM proteins and are involved in SOCE. In cortical neurons we recorded single-cell Ca2+ levels using Fura-2AM. To investigate the involvement of IR in TG-induced Ca2+ entry, we applied antagonists of these receptors: NS-102 (KR), CNQX (AMPAR/KR), NBQX (AMPAR), MK-801 (NMDAR), memantine (NMDAR), and D-AP5 (NMDAR). We found that SOCE was decreased by CNQX, NBQX, D-AP5, memantine but insignificant changes were observed in the presence of MK801 and NS-102. The results showed that NMDA and AMPA receptors are involved in SOCE pathway. The interaction between endogenous STIM1/STIM2 with IR will be checked by Co-IP. Supported by National Science Centre (2011/01/D/NZ3/02051, JGB).

Enhanced stability and translational efficiency of capped mRNA bearing two phosphorothioate moieties

Presenter: Renata Grzela

Centre of New Technologies

Renata Grzela, R. Grzela1, M. Strenkowska2, M. Lukaszewicz2, M. Majewski2, J. Kowalska2, J. Jemielity1, E. Darzynkiewicz1 1Centre of New Technologies, Warsaw, Poland 2University of Warsaw, Poland

Eukaryotic mRNAs are capped at their 5'-ends by addition of a 7-methylguanosine to the first transcribed nucleotide of the mRNA chain. Considering cap structure crucial for protein synthesis efficiency are two events: cap binding by eIF4E protein and susceptibility of mRNA to 5' to 3' degradation initiated by decapping complex Dcp1/Dcp2. Chemical modification within a cap moiety can influence both factors eIF4E and Dcp2. Engineered mRNA bearing properly modified cap analogs could be a versatile protein delivery molecules in cancer immunotherapies, gene therapies and cells reprogramming. Here we present new cap analogs possessing bis (phosphorothioate) modification in oligophosphate bridge. Their affinities for eukaryotic translation initiation factor 4E (eIF4E) and the stability of mRNAs capped with new analogs were evaluated. Translational potential of bis (phosphorothioate) capped mRNAs was tested in Rabbit Reticulocytes Lysate and human immature dendritic cells (hiDCs). The results revealed that bis (phosphorothioate) modification enhance binding to eIF4E and stabilize cap towards Dcp2 enzyme what subsequently reflects in more efficient mRNA translation in RRL and hiDCs.

Adrenergic control of membrane potential in medial prefrontal cortex (mPFC) pyramidal neurons.

Presenter: Katarzyna Grzelka

Department of Physiology and Pathophysiology, Medical University of Warsaw

Katarzyna Grzelka, Paweł Szulczyk, Department of Physiology and Pathophysiology, Medical University of Warsaw

Impairment of the signal transduction from adrenergic receptors to cellular effectors in prefrontal cortex neurons occurs in many neuropsychiatric disorders. The aim was to clarify the effect of α2-adrenergic receptor activation on membrane potential, determine the cellular effector and the signal transduction pathway responsible for membrane potential changes. Recordings were made in slices isolated from young rats, in layer V mPFC pyramidal neurons in perforated-patch configuration. α2-Adrenergic receptor agonist clonidine evoked membrane hyperpolarisation. The effect was attenuated by the blocker of hyperpolarisation-activated cyclic nucleotide-gated (HCN) channels and by the selective Na+/K+-ATPase inhibitor. It was affected neither by the adenylyl cyclase inhibitor, protein kinase A inhibitor, phospholipase C inhibitor nor the protein kinase C inhibitor but it was attenuated by the G-protein $\beta\gamma$-subunit inhibitor. We conclude that α2-adrenergic receptor activation evokes hyperpolarisation due to HCN channel inhibition and modification of the Na+/K+-ATPase function. The transduction pathway occurs in a membrane-delimited fashion by the G$\beta\gamma$ subunit released from the G-protein. Supported by grants no: NN401584638 and NN301572940.

The impact of coilin mutation on the transcription profile

Presenter: Tomasz Gulanicz

Department of Gene Expression, Adam Mickiewicz University, Poznań

Tomasz Gulanicz, Dariusz Smoliński, Department of Cellular Biology, Nicolaus Copernicus Uiversity

Most genes are transcribed as pre-mRNA containing non-coding sequences called introns which are removed in splicing process. Factors involved into removing introns are snRNP. Maturation of snRNP is Cajal bodies which are a nuclear bodies localized at periphery of nucleolus. Structural protein of CB is coilin and knock-down mutation of this protein leads to the disruption of Cajal bodies. On the other hand over-expression of coilin (PCB-1 mutant) result in increased number of CB. Previously we shown that the effect of coilin over-expression is the accumulation of new transcript localized by the immunolocalization of incorporated bromo-uracil and splicing factor SC35 which do not localize in WT cells. In this studies we determined the localization of active transcriptionally polymerase II phosphorylated at serin 5, 2 and serin 7 in CB under condition of mutation PCB-1 and in WT cells.

Identifying tissue specific enhancers by sequence and histone modifications

Presenter: Julia Herman-Iżycka

University of Warsaw, Institute of Informatics

Julia Herman-Iżycka, Bartek Wilczyński - University of Warsaw - Institute of Informatics

Identifying tissue specific enhancers has been an important field of study in biology for a number of years now. It is necessary for understanding the differentiation of tissues in higher organisms. Many experimental techniques have been used to characterize hundreds of functional enhancers, however given the number of different cell types it is difficult to identify all specific enhancers experimentally. Computational methods usually base on our knowledge of characteristic features of such DNA fragments, including specific sequence motifs or epigenetic markers. We use machine learning approach to predict tissue specific, active human enhancers. In particular, we train random forest classifier on experimentally validated sequences from VISTA database. We combine information about histone modifications occurrence in genome fragments with sequence features. We show that computational identification of regions containing enhancers is possible with good accuracy (AUC between 0.8 and 0.9). We can also distinguish heart and brain enhancers with AUC = 0.83.

Are synthetic (L-Glu)n≤10 peptides apt to form amyloid-like fibrils?

Presenter: Agnieszka Hernik

University of Warsaw

Agnieszka Hernik, Wojciech Puławski(1), Bartłomiej Fedorczyk(1), Dagmara Tymecka(1), Sławomir Filipek(1), Aleksandra Misicka-Kęsik(1), Wojciech Dzwolak(1)
(1) University of Warsaw

Aggregation of misfolded protein molecules into so-called amyloid fibrils has been linked to several neurological disorders such as Alzheimer's disease. Formation of amyloid fibrils is thought to represent a common property of proteins associated with their polymeric nature as polyamides. Essentially the same process can be induced in vitro in synthetic peptides which are not associated with any known diseases what makes poly-L-glutamic acid an interesting biophysical model for amyloidogenesis. Here we are investigating conformational $\alpha \rightarrow \beta$ transition accompanying fibrillation of synthetic (L-Glu)n peptides (n=3,4,5,10...200) using FT-IR spectroscopy and atomic force microscopy (AFM). Under the conditions of this study (L-Glu)n peptides form β–sheet–rich fibrils with "exotic" patterns of bifurcating hydrogen bonds for n≥4. We have discussed seeding ability of pre-formed (L-Glu)n fibrils in the context of pathogenic behavior of amyloid fibrils in vivo.

Cytoplasmic RNP-rich bodies coordinate snRNP assembly during high expression of splicing elements

Presenter: Malwina Hyjek

Nicolaus Copernicus University, Toruń, Poland

Malwina Hyjek, Malwina Hyjek, Department of Cell Biology, Faculty of Biology and Environment Protection, Nicolaus Copernicus University, Toruń, Poland, Natalia Wojciechowska, 3Department of General Botany, Institute of Experimental Biology, Adam Mickiewicz University, Poznań, Poland, Agnieszka Kołowerzo-Lubnau, Department of Cell Biology, Faculty of Biology and Environment Protection, Nicolaus Copernicus University, Toruń, Poland, Dariusz J. Smoliński, Department of Cell Biology, Faculty of Biology and Environment Protection, Nicolaus Copernicus University, Toruń, Poland, Centre For Modern Interdisciplinary Technologies, Nicolaus Copernicus University, Toruń, Poland

Small nuclear ribonucleoproteins (snRNPs) play a crucial role in pre-mRNA splicing in all eukaryotic cells. In contrast to the relatively broad knowledge on snRNPs assembly within the nucleus, the spatial organization of the cytoplasmic stages of their maturation remains poorly understood. Nevertheless, sparse research indicates that similar to the nuclear steps, the crucial processes of cytoplasmic snRNP assembly may also be strictly spatially regulated. In European larch microsporocytes, we determined that the cytoplasmic assembly of snRNPs within a cell might occur in two distinct spatial manners, which depend on the rate of de novo snRNP formation in relation to the steady state of these particles within the nucleus. During periods of moderate expression of splicing elements, the cytoplasmic assembly of snRNPs occurs diffusely throughout the cytoplasm. Increased expression of both Sm proteins and U snRNA triggers the accumulation of these particles within distinct, non-membranous RNP-rich granules, referred to as snRNP-rich cytoplasmic bodies (CsBs). This research was supported by grant NN 303799640.

Evaluation of anti-prion drugs by Saccharomyces cerevisiae expressing artificial prion

Presenter: Takao Ishikawa

Faculty of Biology, University of Warsaw

Takao Ishikawa, Dorota Kruszyńska, Monika Szewczyk

Typically used yeast-based anti-prion drug screening system employ ade1-14 strains accumulating red pigment. However, yeast cells with [PSI+] prion have white colonies due to read-through of stop codons caused by aggregation of the Sup35p translation termination factor. Therefore, if [PSI+] strain is cultivated in the presence of effective anti-prion agent, one can observe formation of red colonies. However, estimation of intensity of red colour, usually done by eye, is the minor point of the system. Here we report the construction of novel yeast-based anti-prion drug screening system. To make the estimation of anti-prion activity easy, we employed Leu2p often used as nutritional marker and constructed artificial [LEU2+] prion. If it is totally aggregated, there is no growth on medium lacking leucine; if artificial prions are partly disaggregated (due to weak anti-prion activity of drug) there is moderate growth of yeast culture; finally, if artificial prions are fully disaggregated (due to strong anti-prion activity of drug) there is vigorous growth of yeast culture. The method presented here allows to quantify the strength of potential anti-prion drugs which makes this method contrasting to systems used until now.

How much is enough?: An optimum number of steps in energy minimization redefined.

Presenter: Rafal Jakubowski

Theoretical Molecular Biophysics Group, Faculty of Physics, Astronomy and Informatics, Nicolaus Copernicus University, Grudziadzka 5, Torun, Poland

Rafal Jakubowski, Jakub Rydzewski, Theoretical Molecular Biophysics Group, Faculty of Physics, Astronomy and Informatics, Wieslaw Nowak, Theoretical Molecular Biophysics Group

Nowadays accessible computer powers allow to perform molecular dynamics simulations (MD) of large biological systems reaching microseconds timescale. Although many life processes occur in a longer time span, a usability of computational methods in solving biological riddles is growing constantly. One of the initial steps in performing MD study is energy minimization, aiming at bringing a biomolecule structure as close to the most stable conformation as possible. Checking the vast literature we have found that this process is rarely analysed and in most cases the minimization is treated upon arbitrary conditions. We present results of our research on the energy minimization procedure performance of a representative set of monomeric proteins. A systematic calculations and analysis gave the surprising conclusion: the energy minimization stage in the majority of currently performed MD simulations leads to biased starting point for further MD steps. We explain why does it happen and give some suggestions how to fix this problem and improve the quality of MD simulations of proteins. This work was supported by "Krok w przyszlosc – stypendia dla doktorantów, V edycja" from the Marshall of Kuyavian-pomeranian voivodeship.

CABS-dock web server for flexible docking of peptides to proteins

Presenter: Michal Jamroz

Faculty of Chemistry, University of Warsaw

Michal Jamroz, Mateusz Kurcinski, Maciej Blaszczyk, Andrzej Kolinski, Sebastian Kmiecik

The CABS-dock web server provides an interface for modeling protein–peptide interactions using a highly efficient protocol for the flexible docking of peptides to proteins. While other docking algorithms require pre-defined localization of the binding site, CABS-dock does not require such knowledge. Given a protein receptor structure and a peptide sequence (and starting from random conformations and positions of the peptide), CABS-dock performs simulation search for the binding site allowing for full flexibility of the peptide and small fluctuations of the receptor backbone. This protocol was extensively tested over the largest dataset of non-redundant protein–peptide interactions available to date (including bound and unbound docking cases). For over 80% of bound and unbound dataset cases, we obtained models with high or medium accuracy (sufficient for practical applications). Server is freely available at http://biocomp.chem.uw.edu.pl/CABSdock

Knot conservation through the DCA lens

Presenter: Aleksandra Jarmolińska

Centre of New Technologies University of Warsaw

Aleksandra Jarmolińska, Joanna Sulkowska, Centre of New Technologies UW

One of the most defining characteristics of proteins, one that enables them to perform their functions, is their structure. That holds especially true for the knotted proteins, since that structure requires a considerable "effort" on part of the protein. Both the evolutionary origin and the folding process of such molecule are still an unknown. Using Direct Coupling Analysis we compare pattern (and contact conservation) within proteins from families with various functions and topologies built around a trefoil (3_1) knot.

Immune system genes expression: discovering interdependencies and providing ancestry specific marks

Presenter: Michal Jerzy

Institute of Computer Science, Polish Academy of Sciences, Poland

Michal Jerzy, Michal Draminski Institute of Computer Science, Polish Academy of Sciences, Poland, Klev Diamanti Department of Cell and Molecular Biology, Uppsala University, Sweden, Jacek Koronacki Institute of Computer Science, Polish Academy of Sciences, Poland, Jan Komorowski Institute of Computer Science, Polish Academy of Sciences, Poland, Department of Cell and Molecular Biology, Uppsala University, Sweden

We aimed to discover interdependencies between immune-related gene expression levels and the origin of a set of individuals. For that purpose we applied classification-based feature selection and rule-based modeling. Interactions among features were modeled as a network of interdependencies, extracted from the classification trees and presented as a directed graph. Then classification rule sets were provided to assign feature values to specific human origin. That allowed us to discover interdependencies in the human immune system responses to various stimuli of CD4+ T-cells depending on the racial background. Biologically, generic-function protein-coding genes (i.e. UTS2) point to function-specific ones (i.e. LYZ). The direction of the significant feature connections followed the time increment of treatment (i.e. genes measured in 4th hour point on measured in 48th). We learnt that gene-responses related to bacteria characterized Afro-Americans; to viruses, Caucasians; and to both characterized Asians. Finally, the refinement to the level of rules showed that the distribution of the attribute values across the classes suggested that African-Americans and Asians were much more homogeneous than Caucasians for the selected genes.

Measuring information transmission from single-cell heterogenous dynamical responses

Presenter: Tomasz Jetka

Institute of Fundamental Technological Research, Polish Academy of Sciences

Tomasz Jetka, Tomasz Winarski, Edyta Głów, Michał Komorowski, Institute of Fundamental Technological Research, Polish Academy of Sciences

All biological organisms need to sense and response to their environment. At the level of single cells, surface receptors convert extracellular cues into activation of transcription factors that control cellular decisions. A considerable unresolved issue is how information about ligand binding is encoded into nuclear activity of the transcription factors. The current challenge is to recognise the features of temporal activity profile that represent information about a given stimulus. A natural strategy to decipher this temporal coding is to scan cellular responses across a range of considered stimuli and identify its most sensitive features. Methods however to quantify sensitivity and information capacity at the single cell level, where stochastic effects play a major role, are virtually missing. We have developed a statistical framework to measure information of cellular outcomes from time-resolved, single cell, heterogeneous responses. We use the method to analyse nuclear translocation of the NF-kB transcription factor upon TNF stimulation. We identified how the information capacity of the system changes with inclusion of time series data and indicate the essential features of the nuclear NF-kB temporal profile.

Multisubtype chimeric influenza VLPs as a potential broader-range vaccine against influenza virus

Presenter: Anna Jurek

Department of Recombinant Vaccines; Intercollegiate Faculty of Biotechnology UG GUMed

Anna Jurek, Karolina Uranowska (Department of Recombinant Vaccines; Intercollegiate Faculty of Biotechnology UG GUMed), Beata Gromadzka (Department of Recombinant Vaccines; Intercollegiate Faculty of Biotechnology UG GUMed), Bogusław Szewczyk (Department of Recombinant Vaccines; Intercollegiate Faculty of Biotechnology UG GUMed)

Influenza virus undergoes high mutation rates and frequent genetic reassortments. Changes in the surface protein, hemagglutinin (HA), gave rise to the new strains that are reason of epidemics affecting millions of people. Currently available seasonal vaccines are strain-specific. Therefore, there is a need for a vaccine that can induce broad immune response. Nowadays, virus like particles (VLPs) are major candidates for vaccines against influenza virus. For that reason we decided to obtain influenza VLPs consisting of M1 (matrix protein 1) and HA from strains: H3N2, H7N9 and H1N1. In case of HA from H1N1 we constructed "headless" protein consisting of stalk domain, which is conserved among influenza species. The plasmids carrying genes: m1, h7, h3 and h1 stalk were constructed and propagated in Sf9 cells using baculovirus expression system. In order to obtain VLPs, insects cells were co-infected with recombinant baculoviruses. We obtained and characterized influenza VLPs consisting of functional hemagglutinin from different influenza strains, morphologically and structurally similar to native virus, which will be futher analized for ability to induce broad immune response.

Lapatinib and ITCs combinations inhibit migration, invasion and viability of breast cancer cells

Presenter: Angelika Kaczyńska

Department of Molecular Biology, University of Gdansk, Poland

Angelika Kaczyńska, Anna Herman-Antosiewicz Department of Molecular Biology, University of Gdansk, Poland

Overproduction of human epidermal growth factor receptor 2 (HER2) is indicated in 25% of all breast cancer cases. Enhanced signal transduction from HER2 via kinases Akt-mTOR-S6K leads to uncontrolled proliferation, migration, neoangiogenesis and evasion of apoptosis. Lapatinib is a small-molecule tyrosine kinase inhibitor, commonly used in breast cancer therapies. However long-term exposure to lapatinib causes elimination of drug-sensitive cells and at the same time increases probability of selection of drug-resistant cells whose percentage rises in the cell population with time and may lead to metastasis. In this work we showed, that combination of lapatinib with one of isothiocyanates (ITCs) sulforaphane, erucin or sulforaphene considerably decreases cell viability, migration, invasion and phosphorylation of proteins crucial in signal transduction from HER2 and more efficiently induces apoptosis as compared to treatment with either agent alone. Our in vitro simulation of drug-dependent selection of resistant cells during lapatinib therapy showed, that combined treatments are efficient even when percentage of resistant cells increases. These results suggest that isothiocyanates may be considered as a promising anti-cancer agents

KnotScore - novel method to assess topology correctness of protein models

Presenter: Michał Kadlof

Faculty of Mathematics, Informatics and Mechanics, Center of New Technologies, University of Warsaw

Michał Kadlof, Recent studies of CASP competition results show that high percentage of submitted structures had wrong or even never observed in proteins topology. Moreover even models with a good GDT score (Global Distance Test - metric used in CASP competition) often had wrong topology. That means that even today, after 20 years of competitive protein modelling, the most fundamental feature of proteins - the topology - remains a non-trivial problem. Here we propose an additional tool for assessing the probability of observing a particular type of knot depending on the secondary structure composition of known knotted proteins. Different stiffness of different types of secondary structures puts additional constrains on allowed topology. This is a statistics based tool dedicated for crystalographers and protein modellers, which could be used by them to further validate their models.

Recent studies of CASP competition results show that high percentage of submitted structures had wrong or even never observed in proteins topology. Moreover even models with a good GDT score (Global Distance Test - metric used in CASP competition) often had wrong topology. That means that even today, after 20 years of competitive protein modelling, the most fundamental feature of proteins - the topology - remains a non-trivial problem. Here we propose an additional tool for assessing the probability of observing a particular type of knot depending on the secondary structure composition of known knotted proteins. Different stiffness of different types of secondary structures puts additional constrains on allowed topology. This is a statistics based tool dedicated for crystalographers and protein modellers, which could be used by them to further validate their models.

Influence of CacyBP/SIP phosphatase on gene expression in colon cancer HCT116 cells

Presenter: Beata Kądziołka

Nencki Institute of Experimental Biology PAS

Beata Kądziołka, Beata Kądziołka, Konrad Dębski, Wiesława Leśniak, Anna Filipek; Nencki Institute of Experimental Biology PAS

CacyBP/SIP was originally discovered in Ehrlich ascites tumor cells and was shown to interact with some proteins from the S100 family as well as with Siah-1, Skp1, tubulin, actin, tropomyosin and (ERK)1/2. In normal tissues eg. stomach or colon CacyBP/SIP is weakly or barely detected whereas in gastric or colon cancer its expression is much higher. Some results suggest association of CacyBP/SIP with tumorigenesis and with multidrug resistance. Thus in this work we assessed functional consequences of the altered level of CacyBP/SIP in highly proliferating cells - colon cancer HCT116. In particular we investigated changes in gene expression in these cells after CacyBP/SIP overexpression or knock-down. Total RNA from cells transfected with plasmid encoding shRNA, which silence CacyBP/SIP expression, or from cells overexpressing CacyBP/SIP was isolated and subjected to microarray analysis. Based on functional analysis we found many genes the products of which are responsible for cell proliferation or involved in immune response. These observations point to CacyBP/SIP as an important player in signaling pathways whose disequilibrium induces relevant changes in cellular "transcriptome".

A novel isoform of HBS1L provides a link between the cytoplasmic exosome and SKI complexes in humans

Presenter: Katarzyna Kalisiak

Institute of Biochemistry and Biophysics, Polish Academy of Sciences; Laboratory of RNA Biology and Functional Genomics

Katarzyna Kalisiak, Katarzyna Kalisiak, Tomasz Kulinski, Rafal Tomecki, Andrzej Dziembowski; 1) Institute of Biochemistry and Biophysics, Polish Academy of Sciences; Laboratory of RNA Biology and Functional Genomics; 2) Department of Genetics and Biotechnology; Faculty of Biology; University of Warsaw; Pawinskiego 5A, 02-106 Warsaw; Poland

Regulation of gene expression relies heavily on RNA metabolism. Many proteins, including nucleases, are involved in RNA decay and quality control in eukaryotes. A multi-subunit exosome complex is one of the major nucleases present in the cytoplasm in human cells. The exosome function in this compartment depends strictly on cooperation with the SKI protein complex, which has RNA helicase activity. Biochemical experiments in yeast proved that the SKI heterotetramer is responsible for delivering RNA substrates directly into the exosome channel and that the exosome/SKI complex cooperation requires the presence of the Ski7 protein, which is a factor linking both complexes. Mechanisms of cooperation between the SKI and exosome complexes in human cells remain unknown, since the human genome does not encode an ortholog of the yeast Ski7 protein. An analysis of cDNA clones suggested that the HBS1L gene expression can give rise to several mRNAs coding for different putative protein variants, however none of the alternative isoforms has been tested so far. Our data indicate that that a short protein variant, which is called HBS1LV3 herein, may be the long-sought "linker" protein – an analog of the yeast Ski7 protein.

Small virus, big problem: why do we still need a drug for flu?

Presenter: Katarzyna Kaminska

International Institute of Molecular and Cell Biology in Warsaw

Katarzyna Kaminska, Katarzyna H. Kaminska, Janusz M. Bujnicki

Influenza virus is a major human and animal patogen with the potential to cause staggering mortality rates. The emergence of the extremely aggressive influenza strains has made the likelihood of the human influenza pandemic and its possible socio-economic impact a major worldwide concern. It has also emphasized the need for the new therapeutic strategies to combat these pathogens. The heterotrimeric influenza virus polymerase, containing the PA, PB1 and PB2 proteins, carries out numerous essential roles in viral replication and pathogenesis. In particular, the polymerase is responsible for 'cap-snatching': that is the cleavage of the capped leaders from the host cell pre-messenger RNA, which are subsequently used to prime transcription of the viral genome. Determination of the crystal structure of the viral polymerase endonuclease domain provided a structural framework for the identification of small molecule compounds that inhibit the nuclease activity of the viral polymerase complex. Using the structure-based virtual screening protocol followed by subsequent experimental verification we have identified the lead compounds that provide the starting point for the rational development of the antiviral medications.

Siderophores and their application in medicine

Presenter: Ewelina Kamińska

Uniwersytet Marii Curie-Skłodowskiej w Lublinie

Ewelina Kamińska,

The majority of organisms need iron as an essential element in many of metabolic and informational cellular pathways. Although iron is required in small amounts, aerobic and partly aerobic microorganisms usually have to face the problem of deficiency of this element. Reduced amount of iron is available to the microbes also in human body. Insufficient concentration of available iron in body fluids and tissues of the host limits bacterial infection development. Under conditions of iron deficiency bacteria produce siderophores which are involved in transport of iron into cells and its storage. Siderophores have potential use in antibiotic therapy of bacterial infections. Scientists created conjugates between siderophores and antimicrobial agents, called sideromycins, to enter drugs into bacterial cells using iron transport ability of siderophores. Such a tactic, known as Trojan Horse Strategy has already been successfully used to carry ampicillin and norfloxacin to pathogenic bacteria like P. aeruginosa. An important benefit of Trojan Horse Strategy is selective drug delivery. This use of siderophores seems to be a promising method of treatment of bacterial infections.

Crystal structure of the 5hmC specific endonuclease PvuRts1I

Presenter: Asgar Abbas Kazrani

International Institute Of Molecular and Cell Biology

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PvuRts1I is a prototype for a larger family of restriction endonucleases that cleave DNA containing 5-hydroxymethylcytosine (5hmC) or 5-glucosylhydroxymethylcytosine (5ghmC), but not 5-methylcytosine (5mC) or cytosine. Here, we report a crystal structure of the enzyme at 2.35 Å resolution. Although the protein has been crystallized in the absence of DNA, the structure is very informative. It shows that PvuRts1I consists of an N-terminal, atypical PD-(D/E)XK catalytic domain and a C-terminal SRA domain that might accommodate a flipped 5hmC or 5ghmC base. Changes to predicted catalytic residues of the PD-(D/E)XK domain or to the putative pocket for a flipped base abolish catalytic activity. Surprisingly, fluorescence changes indicative of base flipping are not observed when PvuRts1I is added to DNA substrates containing pyrrolocytosine in place of 5hmC (5ghmC). Despite this caveat, the structure suggests a model for PvuRts1I activity and presents opportunities for protein engineering to alter the enzyme properties for biotechnological applications.

Biosupercapacitor based on nanocellulose/polypyrrole composite

Presenter: Michał Kizling

Faculty of Chemistry, Warsaw University

Michał Kizling, Krzysztof Stolarczyk, Faculty of Chemistry, Warsaw University; Leif Nyholm, Department of Chemistry, Angström Laboratory, Uppsala University; Renata Bilewicz, Faculty of Chemistry, Warsaw University

The main goal of my present study is preparing of efficient bioelectrodes based on capacitive material for enzymatic biofuel cell (EFC). Progress in nanotechnology has created new direction for the design and development of micro and nanoscale electronic devices suitable for industry, medicine or environmental science. To keep such miniaturized electronic devices operating for long periods of time, low power sources are required. Important features of biofuel cells are the selectivity of processes occurring at the enzymatically modified electrodes and the ability to operate at room temperature and at pH close to neutral. Also, EFCs can be easily miniaturized. Although, present EFCs suffer from insufficient power densities and resistance on high currents load, e.g. during switching powered device on. Therefore, in my researches I focus also on solving this problem by using capacitive matrices based on cellulose and polypyrrole to immobilize enzymes to combine unique capabilities of bioelectrocatalytic systems and supercapacitors. Nextly, device was utilized to power minipotentiostat and amperometric sensing electrode in pulse system for oxygen concentration determination to prove that such system is able to work in real conditions.

Changes of hif-1 α expressions level during neonatal anoxia at different body temperature

Presenter: Hanna Kletkiewicz

Department of Animal Physiology, Faculty of Biology and Environment Protection, Nicolaus Copernicus University

Hanna Kletkiewicz,

Complications after neonatal asphyxia are the most common cause of subsequent neurological disorders. There is a number of evidence that one of the endogenous processes that protect the brain from damage due to perinatal hypoxia is decreasing of body temperature. It is also known, that the transcriptional hypoxia-inducible factor-1α (HIF-1α) plays fundamental role in adaptive process in response to hypoxia. HIF-1α upregulates several genes involved in glycolysis, erythropoiesis and angiogenesis to promote survival. Our experiments aimed at checking the effects of body temperature during simulated perinatal anoxia on the subsequent changes of HIF-1α expression in brain. Two-day-old Wistar rats were divided into 4 temperature groups: hypothermic (31°C), normothermic (33°C), hyperthermic typical to adult rats (37°C) and hyperthermic typical to febrile adults rats (39°C). The temperature was controlled starting 15 min before and continuing during 10 min of anoxia as well as for 2 hours postanoxia. Levels of HIF-1α gene expression were analyzed post mortem: immediately, 3 and 7 days after anoxia using Western blot analysis. The results showed that the body temperature during neonatal anoxia affects the level of HIF-1α expression.

CABS-dock web server for protein-peptide docking without prior knowledge of the binding site

Presenter: Sebastian Kmiecik

Department of Chemistry, University of Warsaw

Sebastian Kmiecik, Mateusz Kurcinski, Michal Jamroz, Maciej Blaszczyk, Andrzej Kolinski and Sebastian Kmiecik

Protein-peptide interactions play a key role in cell functions. Their structural characterization, although very challenging, is important for discovery of new drugs. Based on our methodology for highly efficient simulation of protein dynamics, we developed the CABS-dock web server for protein-peptide molecular docking. While other docking algorithms require pre-defined localization of the binding site, CABS-dock doesn't require such knowledge. Given a protein receptor structure and a peptide sequence (and starting from random conformations and positions of the peptide), CABS-dock performs simulation search for the binding site allowing for full flexibility of the peptide and small fluctuations of the receptor backbone. This protocol was extensively tested over the largest dataset of non-redundant protein-peptide interactions available to date (including bound and unbound docking cases). For over 80% of the dataset cases, we obtained models with high or medium accuracy (sufficient for practical applications). CABS-dock web server is freely available at http://biocomp.chem.uw.edu.pl/CABSdock

Excitation oscillations and hierarchy of feedbacks in MAPK signaling

Presenter: Marek Kochańczyk

Institute of Fundamental Technological Research PAS, Warsaw, Poland

Marek Kochańczyk, et al.

The MAPK pathway involves numerous negative and positive feedbacks. We investigated the functional implications of a positive feedback loop involving SOS and RAS, which links activation of the EGF receptor to activation of the RAF-MEK-ERK cascade. We also considered three negative feedback loops. One, involving ERK-mediated inhibition of SOS, overlaps with the positive feedback loop. The others, involving ERK-mediated inhibition of RAF and MEK, lie downstream of it. Through analysis of a computational model for EGF-stimulated activation of ERK, we find that positive feedback can amplify signals and allow for bistability and hysteretic switch-like responses to signals. Responses can become oscillatory if negative feedback from ERK to SOS is sufficiently strong. Relaxation oscillations, consisting of pulses of ERK activity separated by periods of inactivity, are predicted, which resemble oscillations observed in single cells responding to tonic low doses of EGF.

Aqueous plant extracts can improve treatment of bacterial diarrhea

Presenter: Magdalena Komiazyk

Nencki Institute of Experimental Biology

Magdalena Komiazyk, Palczewska Malgorzata University of Gdansk, Sitkiewicz Izabela National Medicines Institute in Warsaw, Slawomir Pikula Nencki Institute of Experimental Biology, Patrick Groves University of Gdansk

Diarrhea has a large economic impact on society and kills around 2.2 million people each year. The major pathogens causing diarrhea are: Escherichia coli, Vibrio cholerae and Shigella dysenteriae. The main virulence factors produced by these bacteria are AB5 toxins, such as: cholera, shiga and heat-labile enterotoxins, which bound to gagngliosides (GM1 or Gb3) located in the cell membrane. WHO recommends that the main treatment of diarrhea is oral rehydration therapy, which does not directly address the processes involving the toxin. During our project we wanted to improve the diarrhea treatment using a power of medical plants. We studied the anti-enterotoxic activity of selected plant extracts, using few complementary methods: Native Page and Western Blot, modified ELISA with immobilized GM1, flow cytometry and fluorescent microscope assays. These methods gave as a list of fifteen plants, such as: white willow, wild strawberry or star anise, which inhibit the binding of enterotoxin to GM1 and prevent entering toxins into human cell. We hope, the knowledge of antitoxic activity of studied plants can improve treatment of diarrhea.

Interleukin-33 as a factor involved in the regulation of oligodendrocyte precursor cells biology

Presenter: Katarzyna Konarzewska

Nencki Institute of Experimental Biology Polish Academy of Sciences

Katarzyna Konarzewska, Katarzyna Konarzewska, Bartosz Wylot, Beata Kaza, Justyna Ulańska-Poutanen, Małgorzata Zawadzka

Interleukin-33 (IL-33) is a protein which has been recently widely studied because of its an important role in maintaining organism homeostasis by dual activity - it may act as a traditional cytokine or as intracellular nuclear factor. The aim of present study was to characterize the cellular localization of the IL-33 in the rodent central nervous system (CNS). We have shown that IL-33 is present in the nuclei of oligodendrocyte lineage cells in the gray and white matter. Moreover, we demonstrated the changes in the IL-33 protein level associated with response of oligodendrocyte precursor cells, the largest undifferentiated cell population of adult CNS, to the injury. Our in vivo observations where verified by a series of in vitro experiments. Our results suggest that IL-33 may be responsible for maintaining the constant pool of undifferentiated oligodendrocyte progenitors with regenerative potential. The work has been supported by project co-financed by the European Unionunder the European Social Fund

Cap analogs for fluorescent labelling via click chemistry for FRET-based experiments

Presenter: Michal Kopcial

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Michal Kopcial, Blazej Wojtczak, Centre of New Technologies, University of Warsaw, Warsaw, Poland; Joanna Kowalska, Division of Biophysics, University of Warsaw, Warsaw, Poland; Jacek Jemielity, Centre of New Technologies, Universirty of Warsaw, Warsaw, Poland

Cap structure is present at 5' end of eukaryotic mRNAs. It is consisted of 7-methylguanosine bounded via 5',5'-triphosphate bridge to a first nucleoside of a transcript. Cap structure is involved in several steps of gene expression including translation initiation, intracellular transport, pre-mRNA maturation and mRNA stability . For deeper understanding the roles of the cap during a process of gene expression we prepared and characterized novel class of cap analogues with alkyne moiety which enable further modifications. We synthesized series of cap analogs with linkers with a terminal alkyne group or azide group attached to a second nucleobase for functionalization with fluorescent tags in CuAAC reaction. Additionally, cap analogs possess modifications within a phosphate bridge, which modulate cap analog affinity to cap dependent proteins or resistance towards cap-specific pyrophosphatases. We believe that new analogs will serve as a valuable tools for investigation of cap-dependent processes using FRET-based experiments.

Impact of co-purified metal ions on influenza virus endonuclease activity in presence of Mg and Mn

Presenter: Daria Kotlarek

Institute of Physics Polish Academy of Science Warsaw

Daria Kotlarek, Remigiusz Worch Institute of Physics Polish Academy of Science Warsaw

Understanding the contribution of divalent metal ions in the cleavage of nucleic acids constitutes a fundamental stage in designing new inhibitors for medical application. One of the top pharmaceutically relevant enzymes is influenza virus endonuclease (PA-Nter). However, there are contradictory results emerging at the literature concerning the performance of PA-Nter in the presence of Mg and Mn. To explain these discrepancies, we applied agarose and polyacrylamide gel electrophoresis with fluorescence cross-correlation spectroscopy, including various reaction setups. We determined main factors affecting the enzyme performance, which were largely underestimated by other authors. Our study shows significant impact of co-purified metal and concentration regime of ions on enzymatic activity. We report the PA-Nter activity in the presence of both cations and the maximum reaction rate estimated at 0.51 and 0.77 nM/min for Mg and Mn, respectively. For future successful inhibitor design, these findings show the necessity of careful PA-Nter activity examination in the presence of Mg, which is 1000-fold more abundant than Mn in vivo. Supported by National Science Center 2012/07/D/NZ1/04255 and Foundation for Polish Science grants.

The Role of Preformed Template Flexibility in Promoting Aggregation

Presenter: Maksim Kouza

Faculty of Chemistry, University of Warsaw

Maksim Kouza, Maksim Kouza, Phuong H. Nguyen, Andrzej Kolinski, Mai Suan Li

The protein aggregation is a slow and irreversible process. Once the size of the pre-nucleus seed reaches the critical nucleus size, its thermal fluctuations are expected to be small and the resulting nucleus provides a template for sequential accommodation of added monomers. The effect of template fluctuations on fibril formation rates has not been explored either experimentally or theoretically so far. We make the first attempt at solving this problem by two sets of simulations. To mimic template fluctuations, in one set, monomers of the preformed template are kept fixed, while in the other set they are allowed to fluctuate. The kinetics of addition of a new peptide onto the template is explored using all-atom simulations with explicit water and simple lattice models. Our result demonstrates that preformed template fluctuations can modulate protein aggregation rates and pathways. It was shown that template immobility greatly increases the time of incorporating a new peptide into the preformed template compared to the fluctuating template case. This observation has also been confirmed by simulation using lattice models and may be invoked to understand the role of template fluctuations in slowing down fibril elongation in vivo.

Distribution of methylated histone H3 and 5'methylcytosine in male gametophyte cells of hyacinth

Presenter: Marlena Kozlowska

Department of Cell Biology, Faculty of Biology and Enviromental Protection, Nicolaus Copernicus University in Toruń

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Epigenetic mechanisms are one of crucial factors engaged in regulation of transcriptional activity and chromatin structure. Our previous investigations revealed that during maturation of pollen grains, silencing of transcriptional activity and chromatin condensation in vegetative and generative cells were observed. However, in time of pollen tube growing reactivation and differentiation of transcriptional activity took place in those cells (Zienkiewicz et al 2008). The aim of this studies was to localize markers of inactive DNA (5'methylcytosine) and methylated histone variants related to eu- (H3K4me) and heterochomatin (H3K27me) in mature pollen grains and in vitro grown pollen tubes of Hyacinthus orientalis. Results of this experiment show that in mature pollen grain (unhydrated and rehydrated) and growing pollen tubes, in-vegetative and generative nuclei and also after generative cell division in sperm cells nuclei were characterized by relatively high level of DNA methylation. In the vegetative nucleus both variants of histone H3 were localized. Surprisingly, in less active generative nucleus and sperm nuclei only histone H3 variant related to euchromatin (H3K4me) was present.

Profiling of epigenetic enzymes expression in glioma cells reveals downregulation of epigenome

Presenter: Sylwia Katarzyna Król

Laboratory of Molecular Neurobiology, Nencki Institute of Experimental Biology, Warsaw, Poland

Sylwia Katarzyna Król, Marta Maleszewska, Bartosz Wojtas, Bartlomiej Gielniewski, Bozena Kaminska, Laboratory of Molecular Neurobiology, Nencki Institute of Experimental Biology, Warsaw, Poland

Growing evidence indicates that the state of chromatin is crucial for making cell-fate decisions in both normal and malignant cells. Recent studies indicate that epigenetic aberrations have been implicated in the development and progression of brain tumors. Established glioma cell lines are explored to pharmacological and biological studies, however, little is known about epigenetic enzyme expression in GBM cell lines. We performed profiling of epigenetic enzyme expression in 3 established and 2 primary GBM cell lines, and normal human astrocytes by a custom qRT-PCR profiler. The analysis of histone modifications in these cells has been performed using Western blot and immunofluorescence. Our data show that the patterns of epigenetic enzyme expression in primary glioma cell cultures were more similar to astrocytes than established cell lines. Interestingly, the expression of epigenetic enzymes was globally downregulated in all tested glioma cell cultures compared to astrocytes. Histone modifications levels were notably changed in glioma cell cultures compared to non-transformed astrocytes. These results show that epigenetic mechanisms are significantly deregulated in GBM cells and may play an important role in GBM development.

Heterochromatin Protein 1 (HP1β) and PCNA dynamics in replication factories.

Presenter: Maciej Krupa

Nencki Institute of Experimental Biology

Maciej Krupa, Sas-Nowosielska Hanna , Bernas Tytus, Nencki Institute of Experimental Biology

Heterochromatin Protein 1 (HP1), a small non-histone protein, performs diverse roles in cell nucleus. It is involved in establishing of chromatin organization, transcriptional elongation, DNA repair, maintenance of sister chromatid cohesion in centromeres and formation of telomeres. Recently, interaction of HP1 with PCNA has been demonstrated in chromatin replication. Thus, we analyzed the dynamics of HP1β in replication factories. Our data demonstrate the presence of two populations of HP1β inside the replication factories. These populations exhibit transient biding, but differ with respect to their residence time. Moreover, patterns of nuclear distributions of HP1β and PCNA are dynamic and their colocalization changes with S-phase progress.

Phosducin-like protein 2A (PHLP2A) is a sperm tail protein

Presenter: Łucja Krzemień-Ojak

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Łucja Krzemień-Ojak, M. Mikosz (Laboratory of Neurobiology, Nencki Institute of Experimental Biology), E. Joachimiak (Laboratory of Cell Movement Physiology, Nencki Institute of Experimental Biology), H. Fabczak (Laboratory of Cell Movement Physiology, Nencki Institute of Experimental Biology)

Mammalian genomes encode two phosducin-like proteins, PHLP2A and PhLP2B, small cytosolic proteins that belong to the highly conserved phosducin-like family. Early studies has shown that PhLP2A is present in most tissues where it modulates the activity of CCT during folding of the cytoskeletal proteins (Stirling et al., 2007). It has been suggested that in germ cells the same role is played by PhLP2B (Willardson, Howlett, 2007). However, using real time PCR we have shown that PhLP2A and PhLP2B are both expressed in rat testes. Moreover, our immunohistochemical analysis revealed that PhLP2A is expressed in both Sertoli cells and spermatids and that the pattern of its localization changes during spermatogenesis. The detailed confocal microscopy studies of single sperm cells revealed that PhLP2A localizes around the flagella axoneme, specifically in the principal piece of sperm tail. To elucidate the function of PhLP2A in rat testes we performed a GST-pull down analysis coupled with mass spectrometry analysis and identified, among other proteins, Rab14 as a PhLP2A potential partner. The interaction between PhLP2A and Rab14 was confirmed by co-localization studies and pull-down analysis.

Impairment of autophagy flux by tacrine-melatonin heterodimer as an alternative anticancer therapy

Presenter: Karolina Kucharewicz

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The most common target of anticancer therapy is the induction of cancer cells death. However, doses of drugs, which kill cancer cells, have also cytotoxic effects for normal cells. For this reason more attention is paid to alternative therapy – induction of cellular senescence or impairment of autophagy, which leads to cell death. In our study we investigated tacrine-melatonin heterodimer C10 activity on breast cancer cells MCF-7. We found that this compound inhibits MCF-7 cells proliferation (IC50 2,5-4μM). Furthermore, we found that C10 treatment for 1-6 h intensified cellular autophagy (increase of autophagy markers such as LC3BII, Atg5, p62 and Beclin), while one-day treatment with C10 caused accumulation of autophagic vesicles in MCF-7 cells. Further investigation, using electron microscopy and transfection of cells with tandem-fluorescence LC3 plasmid, proved that C10 inhibits autophagy at the late stage. We conclude that C10 induces and inhibits autophagy at the late stage. This may be caused by inhibition of autophagy flux or impairment of lysosomes. This multi-action drug may be a new alternative for previous debilitating therapy. The studies were financed by grants: G21804 and 2011/03/B/ST5/01593

Targeting Highly Structured RNA of Coxsackievirus B3 by siRNAs and Helper Antisense Oligomers

Presenter: Jakub Kuczyński

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Jakub Kuczyński, Mariola Dutkiewicz 1, Agata Ojdowska 1, Jakub Kuczyński 1, Vanessa Lindig 2, Heinz Zeichhardt 2, Jens Kurreck 3 and Jerzy Ciesiołka 1. 1 - Department of RNA Biochemistry, Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznan, Poland; 2 - Institute of Virology, Campus Benjamin Franklin, Charite' – University Medicine, Berlin, Germany; 3 - Institute of Biotechnology, Department of Applied Biochemistry, Berlin University of Technology, Berlin, Germany. * - Corresponding author*

Targeting RNA viruses in their poorly accessible, highly structured regions can be advantageous because these regions are often conserved in sequence and thus less prone to viral escape. We developed an experimental strategy to attack highly structured RNA by means of pairs of specifically designed small interfering RNAs and helper antisense oligonucleotides using the 5' untranslated region (5'UTR) of coxsackievirus B3 as a model target. Using criteria for the design of efficient small interfering RNAs (siRNA) and a secondary structure model of the viral 5'UTR, several DNA 19-mers were designed against partly double-stranded RNA regions. Their target sites were located opposite the sites which had been confirmed as accessible for helper oligomers hybridization. Three pairs of oligomers were able to effectively induce RNase H cleavage in vitro. To target 5'UTR in a reporter construct in HeLa cells the DNA 19-mers were replaced by siRNAs. Hybridization of the helper oligomer on the other side of a double-stranded stem substantially improved silencing capacity of the respective siRNA. We assume that the described procedure will generally be useful for designing of nucleic acid-based tools to silence highly structured RNA targets.

How to block bacterial translation by peptide nucleic acid oligomers?

Presenter: Marta Kulik

Centre of New Technologies and Department of Chemistry, University of Warsaw, Poland

Marta Kulik, Agnieszka Markowska-Zagrajek, Centre of New Technologies and Department of Biology, University of Warsaw, Poland; Tomasz Wituła, Centre of New Technologies, University of Warsaw, Poland; Joanna Trylska, Centre of New Technologies, University of Warsaw, Poland

In the process of translation, ribosomes act as protein factories. One of conserved loops of bacterial ribosomal RNA is Helix 69 (H69), which binds aminoglycosides and short peptides that disturb bacterial translation. Another way of targeting bacterial ribosomes is to use antisense techniques: short oligonucleotides designed to hybridize with functional RNA fragments. Since natural oligonucleotides are degraded in cells, we used short oligomers of biostable peptide nucleic acid (PNA). First, we investigated PNA interactions with isolated human and bacterial H69 hairpins, taking into account pseudouridine modifications. We performed melting temperature, isothermal titration calorimetry, circular dichroism and non-denaturing gel electrophoresis assays. Two PNA oligomers were tested: with and without a cell penetrating peptide (KFF)3K. Results show that both PNAs invade the H69 RNA loop and are non-toxic for human ribosomes. We also confirmed the efficiency of translation inhibition of the PNA oligomers in cell-free E. coli extracts. Next, we verified that the PNA-peptide conjugate inhibits E. coli growth. Overall, targeting H69 with PNA is a promising way to inhibit bacterial translation.

Intermolecular interaction between nucleic acid bases in crystalline state.

Presenter: Prashant Kumar

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Prashant Kumar, Paulina M. Dominiak, Biological and Chemical Research Centre, Department of Chemistry, University of Warsaw Żwirki i Wigury 101, 02-089 Warsaw, Poland.

X-ray diffraction experiments not only give atomic position to determine geometry of chemical structure but the exact charge density distribution also be deduced. Accurate electron density studies provide information on chemical bonding that can be used to develop models for the stability and chemical reactivity of molecules and molecular aggregates. Nucleic acids are condensation polymers of nucleotides. To understand the interaction between these nucleotides a subatomic level study is required. For this purpose, high resolution X-ray diffraction data have been collected at 100 K for adenine, guanine and was interpreted in terms of the multipole formalism. The structures were refined to full convergence, first using the fixed model density composed of University of Buffalo pseudoatoms, followed by fit of all parameters including multipole with constrained local symmetries (G.O.F. 1.34, 1.19 respectively). The multipolar refinement and subsequent quantum theory of atom in molecules (QTAIM) gave a comprehensive description of charge density in studies crystals. Further topological studies were performed to analyze the interaction between the molecules, both qualitatively and quantitatively.

Computational tool for multi-chain biopolymer systems modeling.

Presenter: Aleksander Kuriata

University of Warsaw

Aleksander Kuriata, Dominik Gront University of Warsaw, Andrzej Sikorski University of Warsaw,

Mucins are large extracellular, heavy glycosylated proteins that play a role in human immune system by forming a selective molecular barrier and are a target of cancer treatment research. Theoretical studies of mucus properties are therefore necessary to enhance the ongoing effort for mucine-based medical applications. Mucins consist of different but repetitive domains and are capable of polymerizing further by forming disulfide bridges. To better understand the dynamics of mucus net forming and its structural properties it is essential to study a multi-chain system with interactions between different domains evaluated accordingly A coarse grained off-lattice model was introduced due to the size of the examined system. A Monte Carlo sampling algorithm based on the Metropolis was employed with two different simulating methods utilized. Part of this work is aimed to compare the performance and results of the Rouse model and a fixed bond-length model utilizing Verdier-Stockmayer like moves in Monte Carlo simulations. Structural and dynamic behaviour was tested against universal scaling laws for both models.

Structural and energetic landscape of cocrystals of phenylenediboronic acids with aromatic N-oxides

Presenter: Sylwia E. Kutyła

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Phenyleneboronic acids are used in numerous branches of science. Their best known chemical application is the Suzuki coupling reaction. Phenyleneboronic acids have also found applications in biology and medicine, as supramolecular receptors, enzyme inhibitors. Similarly, N-oxides of various heterocyclic compounds are important due to their vast applications as protective groups, ligands in coordination complexes. It is therefore interesting to testify whether it is possible to combine both classes of compounds into solid-state systems of desired properties (e.g. increased biological activity). Such a combination can be achieved by means of crystal engineering via formation of cocrystalline solids. In this respect phenylenediboronic acids are of a particular interest due to their unique abilities to form complex hydrogen-bonded networks as well as to interact via $\pi...\pi$ stacking. Consequently, the purpose of this study was to co-crystallize para- and ortho-phenylenediboronic acids with a series of aromatic N-oxides and explore the structural properties and energetic features of the synthesized cocrystals. The research was founded by the National Science Centre (grant No. NCN 2011/03/B/ST4/02591).

Biomechanical properties of artery after collagen digestion

Presenter: Aleksandra Kuzan

Department of Medical Biochemistry, Medical University of Wroclaw, Poland

Aleksandra Kuzan, Agnieszka Chwiłkowska: Department of Medical Biochemistry, Medical University of Wroclaw, Poland; Marta Kozuń: Wroclaw University of Technology, Faculty of Mechanical Engineering, Department of Biomedical Engineering, Mechatronics and Theory of Mechanisms; Magdalena Kobielarz: Wroclaw University of Technology, Faculty of Mechanical Engineering, Department of Biomedical Engineering, Mechatronics and Theory of Mechanisms, Regional Specialist Hospital, Research and Development Center in Wroclaw.

The biomechanical properties of the artery are mostly determined by the ECM proteins and the layer of myocytes. In this research project was used two collagen-digesting factors to establish the effect of elastin and SMC on strength and elasticity of the tissue. The research material was segments of human aorta. The collagen was digested using Collagenase from Clostridium histolyticum and using formic acid with autoclaving. ELISA, immunohistochemical method and biomechanical properties testing was performed. In the samples digested with collagenase it was found the positive correlation between strength and modulus of elasticity, indicating that after removal of the collagen by collagenase strength of artery increases with its elasticity. In the artery specimens treated with formic acid it was found a negative correlation between modulus of elasticity and the amount of type I collagen and between modulus of elasticity and the amount of hydroxyproline. This publication is part of Project "Wrovasc – Integrated Cardiovascular Centre," co-financed by the European Regional Development Fund within Innovative Economy Operational Program 2007-2013, realized in Regional Specialist Hospital, Research and Development Center in Wroclaw.

5' mRNA cap surveillance by AtDXO1 in Arabidopsis thaliana

Presenter: Aleksandra Kwaśnik

Institute of Genetics and Biotechnology, University of Warsaw

Aleksandra Kwaśnik, Michał Krzysztoń, Agnieszka Gozdek, Joanna Kufel, Institute of Genetics and Biotechnology, University of Warsaw

Co-transcriptional cap addition has long been perceived as a default step of nascent transcript maturation and only recently has it been depicted as an error-prone process that does not always proceed to completion. Accordingly, RAI1/DXO1 protein family members were identified as key components of the cap surveillance machinery, since they proved responsible for the detection and removal of potentially deleterious capping intermediates. Here we provide an insight into the physiological significance and biochemical activity of the RAI1/DXO1 homologue from Arabidopsis thaliana. Substantial function of AtDXO1 in plant RNA metabolism in vivo is exemplified by growth inhibition and sterility, as well as significant transcriptome changes and altered mRNA cap methylation observed for selected T-DNA insertion mutant lines. Series of in vitro assays on uncapped or improperly capped RNA substrates revealed three distinct enzymatic activities of purified AtDXO1, which were severely affected by the presence of N-terminal domain unique for plant RAI1/DXO1 proteins. We envisage that AtDXO1 plays a major role in plant 5' mRNA end surveillance by decapping and subsequent degradation of various aberrant and probably also normal transcripts.

Genome-wide identification of DIS3 targets in human cells and validation of RNAseq data

Presenter: Anna Łabno

Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland; Laboratory of RNA Biology and Functional Genomics

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The human genome is pervasively transcribed but most transcripts originating from unannotated parts of the genome are present at very low levels. hDIS3 is a catalytic subunit of the nuclear exosome complex. In order to identify hDIS3 targets genome-wide we have conducted RNA-seq experiments of samples isolated from HEK293 cells in which wild-type hDIS3 was replaced by its counterparts bearing catalytic mutations. The most prominent effect of DIS3 mutations was observed on pervasive transcription products. Promoter Upstream Transcripts (PROMPTs) accumulated robustly. The second most significant increase was that of snoRNA precursors, suggesting that hDIS3 is probably responsible for pre-snoRNA processing. Interestingly, although general accumulation of excised introns was not the case, hDIS3 mutations led to the increase in the number of the reads mapping to the first intron of numerous genes, but not the second one, indicating that hDIS3 dysfunction is associated with premature transcription termination. In aggregate, our data indicate that hDIS3 is responsible for the main nucleoplasmic exosome activity, which is degradation of pervasive RNA polymerase II transcription products and snoRNA processing.

Accurate high resolution modeling of G protein-coupled receptors complexes.

Presenter: Dorota Latek

Faculty of Chemistry, University of Warsaw

Dorota Latek, Slawomir Filipek, Faculty of Chemistry, University of Warsaw; Marek Bajda, Faculty of Pharmacy, Jagiellonian University

The recent GPCR Dock 2013 competition and the following studies on 5-HT1B, 5-HT2B and SMO targets showed the strengths and the bottlenecks of the currently used computational methods. The test cases of serotonin 5-HT1B and 5-HT2B G protein-coupled receptors proved that both, GPCR structures and ligands binding modes can be predicted with the near-experimental accuracy as long as the target-template sequence similarity is relatively high. Another bottleneck in the current GPCR research, as demonstrated by the 5-HT2B target, is the reliable prediction of global conformational changes induced by the activation of GPCR receptors. In the current work, we report details of our procedure used in GPCR Dock 2013. Our ligand docking and structure prediction procedure which includes our recently published GPCRM web service proved to be successful, especially in case of our model of 5-HT2B-ergotamine complex which was the best prediction in GPCR Dock 2013. Notably, we were also the top score research group in the remaining target categories: 5-HT1B-ergotamine, SMO-LY2940680 and SMO-SANT1 complexes.

TROSPA - an intrinsically disordered protein involved in the tick colonization by Borrelia

Presenter: Dominik Lewandowski

Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poland

Dominik Lewandowski, D. Lewandowski1, A. Urbanowicz1, K. Szpotkowski1, K. Kamel1, M. Jaskólski1,2, M. Figlerowicz1,3; 1Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poland; 2Faculty of Chemistry, Adam Mickiewicz University, Poland; 3Institute of Computing Science, Poznan University of Technology, Poland

Borreliosis caused by bacteria belonging to Borrelia genus is one of the most prevalent tick-borne diseases in North America and Europe. In the latter, Borrelia spirochetes are transmitted by Ixodes ricinus. Earlier it has been demonstrated that a protein produced in tick's epithelial cells called TROSPA is essential for the bacteria to colonize the vector. Bacterial surface protein OspA binds TROSPA and this way enables Borrelia to attach to tick gut. The structure of TROSPA and its basic functions remain unknown. In order to learn more about this protein we produced a recombinant TROSPA_N△44 deletion mutant. It lacked transmembrane domain but still was capable of binding OspA at the similar level to the wild type protein. The structure of TROSPA_N△44 in a solution was examined by using a number of techniques, such as small-angle X-ray scattering, dynamic light scattering , size-exclusion chromatography or circular dichroism spectroscopy. The results coming from our analyses clearly demonstrated that TROSPA shows the features characteristic for intrinsically disordered proteins (IDP). In addition, based on the data collected so far, we generated the first model of three dimensional structure of the TROSPA_N△44-OspA complex.

Association of drug resistance induction and telomerase expression in cancer cells

Presenter: Natalia Lipińska

Department of Clinical Chemistry and Molecular Diagnostics, Poznan University of Medical Sciences

Natalia Lipińska, Natalia Lipińska; Aleksandra Romaniuk; Błażej Rubiś; Department of Clinical Chemistry and Molecular Diagnostics, Poznan University of Medical Sciences

The problem of drug resistance in breast cancer concerns about 25% of new cases and more than 90% of patients with metastasis. Mechanisms of multidrug resistance are complex although in some types of cancer poor response to chemotherapy can be associated with high telomerase activity/expression. Unfortunately, still little is known about the association between telomerase and drug resistance. In this study we developed resistant sublines from the parental human breast cancer cell line MCF7 by stepwise selection in the presence of doxorubicin. We established two sublines of MCF7 up to almost 4-fold higher resistant to doxorubicin. Interestingly, MCF7/DOX cell lines acquired cross-resistance to telomerase inhibitor TMPyP4. The resistant cell lines exhibited characteristic morphological features and expressed high levels of drug transporters ABCC1 and ABCG2 genes, while they did not show altered expression of ABCB1 gene. During the development of drug resistance the expression of hTERT gene was varied. The study indicate that there was a significant correlation between telomerase expression and the development of resistance in breast cancer cells. The study was supported by a grant from the National Science Centre 2014/13/N/NZ7/00307

Study on HAX-1 protein and it's impact on transcriptome and mRNA turnover in the cell

Presenter: Ewelina Macech-Klicka

The Maria Sklodowska-Curie Memorial Cancer Center and Institute of Oncology, Warsaw, Poland

Ewelina Macech-Klicka, Macech-Klicka E.1, Kudła G.2, Helwak A.3, Trębińska A.A.1, Konopiński R.1, Grzybowska E.A.1, 1The Maria Sklodowska-Curie Memorial Cancer Center and Institute of Oncology, Molecular and Translational Oncology, Warsaw, Poland, 2Medical Research Council, Human Genetics Unit, Institute of Genetics and Molecular Medicine, University of Edinburgh Western General Hospital, Chromosomes and Gene Expression, Edinburgh, United Kingdom, 3Wellcome Trust Centre for Cell Biology The University of Edinburgh, Nuclear RNA Processing and Surveillance, Edinburgh, United Kingdom

It is proposed that HAX-1 protein is engaged in apoptosis, cell migration and adhesion. It also binds mRNA and probably plays a role in transcripts localization. We established stable cell lines with silenced expression of HAX-1 gene as well as control cell lines using HeLa, HEK293, MCF-7 and MDA-MB-231 cells - we have the opportunity to study HAX-1 protein function in various types of cells (epithelial and mesenchymal-like). To examine the impact of HAX-1 expression changes on transcriptome, we used microarray-based approach - data were searched against KEGG Pathway Database and Gene Ontology Database. We also conducted qPCR experiments to confirm microarray outcomes. We identify new RNA binding partners of HAX-1 using CRAC, a novel method based on UV-crosslinking of RNA-protein complexes and purifying them. We used stable cell lines with induced overexpression of HAX-1 gene (engineered with adding 5' and 3' sequences, responsible for protein N'- and C'-tagging after the translation). It was confirmed that C–terminal part of HAX-1 molecule is essential for RNA binding. To conclude, we confirmed that HAX-1 protein has an impact on transcriptome and mRNA turnover in selected cell lines.

Computational prediction of posttranscriptional modifications in tRNAs

Presenter: Magdalena Anna Machnicka

Laboratory of Bioinformatics and Protein Engineering, International Institute of Molecular and Cell Biology in Warsaw, , ul. Ks. Trojdena 4, 02-109 Warsaw, Poland

Magdalena Anna Machnicka, Valerie de Crécy-Lagard Department of Microbiology and Cell Science, University Florida, Gainesville, FL, USA , Janusz M. Bujnicki Laboratory of Bioinformatics and Protein Engineering, International Institute of Molecular and Cell Biology in Warsaw, ul. Ks. Trojdena 4, 02-109 Warsaw, Poland,Institute of Molecular Biology and Biotechnology, Adam Mickiewicz University, Umultowska 89, PL-61-614 Poznan, Poland

tRNA molecules contain numerous chemically altered nucleosides, formed by enzymatic modification of the primary transcripts during the tRNA maturation. Unfortunately, commonly used methods for high-throughput sequencing of RNA are unable to identify positions and identity of modified residues, and experimental determination of modified residues in a tRNA repertoire from a given cell is laborious and difficult. The success of computational prediction of modification sites in tRNA has been limited by the difficulty in predicting enzymes responsible for modification. We have developed a prototypical method for predicting modifications in tRNA. It takes as an input a set of unmodified tRNA sequences and a set of protein sequences corresponding to a proteome of a cell, identifies RNA residues that correspond to known modification sites with known enzymes, finds homologs of known tRNA modification enzymes, and maps the predictions onto known pathways of RNA modification, to identify theoretically possible modification reactions for all positions in query tRNAs. The method allows for predicting modification patterns for newly sequenced genomes and for checking tentative modification status of tRNAs expressed in heterological system.

Metabolite-mediated control of transcription in Bacillus subtilis

Presenter: Piotr Machtel

Institute of Bioorganic Chemistry, Polish Academy of Sciences in Poznan

Piotr Machtel, Piotr Machtel1), Kamilla Bąkowska-Żywicka1), Tomasz Twardowski1), Marek Żywicki2)

Riboswitches are specific domains of mRNAs able to directly bind small metabolites and therefore regulate gene expression by their structural rearrangements. Genes controlled by the riboswitches are usually engaged in transport, metabolism or biosynthesis of the ligands which bind to a given riboswitch. This creates usually a negative metabolic feedback loop. The aim of our studies is to describe the transcriptional response of bacteria to the presence or absence of specific metabolites as a function of time. Our efforts focus on amino acid-controlled riboswitches in Bacillus subtilis. The observations indicate differential response to the amino acid absence at the transcriptional level. Moreover, different genes controlled by the same riboswitch present dynamical transcription. In methionine-controlled genes, when Met is eliminated, the genes are expressed in specific order and strength, reflecting the sequence of metabolic reaction leading to SAM synthesis. Similar results were obtained for lysine riboswitches. For metE and samT genes which encode cobalamin-independent or dependent methionine synthase, respectively, additional studies revealed the influence of adenosylcobalamin supplementation on the transcription regulation.

Characteristic of selectivity of binding the inhibitor to methyltransferases with different topology

Presenter: Joanna Macnar

Centre of New Technologies, the College of Inter-Faculty Individual Studies in Mathematics and Natural Sciences, Faculty of Chemistry, University of Warsaw

Joanna Macnar, Agata Perlińska, Centre of New Technologies, Faculty of Mathematics, Informatics and Mechanics, University of Warsaw; Joanna I. Sulkowska, Centre of New Technologies, Faculty of Chemistry, University of Warsaw;

tRNA methyltransferases are a group of enzymes that participate in a process of great biological significance. The methylation of tRNA is needed because a nucleic acid without attached methyl group would block the translation. Both knotted and unknotted topologies can be found in this group of enzymes. Presence of a knot in the binding site forces a bent conformation of the ligand. The enzymes with trivial topology bind cofactor in an open conformation. This trait was used to form the knotted tRNA guanine methyltransferases-selective inhibitor. Knotted structures - TrmD, occur mostly in bacteria, while unknotted - Trm5, mostly in eucaryota. Such designed chemical compound could be used as a new antimicrobial drug for proteins with non-trivial topology. Using numerical simulation, the efficacy of the ligand can be studied theoretically. We performed inhibitor and natural substrate docking to proteins from various methyltransferases' families and organisms with distinct topologies, and on this basis we predicted their selectivity and effectiveness with respect to the knot and Rossmann fold

Presenter: Deepshikha Malik

International Institute of Cell and Molecular Biology, Warsaw

Deepshikha Malik, Marcin Nowotny, International Institute of Cell and Molecular Biology, Warsaw

Rad2 is a member of flap endonuclease family involved in Nucleotide excision repair and falls in the category of structure specific endonuclease. Our lab has discovered the mechanism of DNA binding by Rad2 by solving the crystal structure of the catalytic core of Rad2 in complex with DNA substrate. Rad2 use three structural domains for recognition of double stranded portion of DNA, particularly a Rad2 specific alpha helix for binding the cleaved strand. Now, I am interested in further investigations of the role of this helix by various structural and biochemical studies. I believe that attending the EMBO YSF would give me an opportunity to interact with other scientists involved in myriad aspects of protein biochemistry and broaden my perspective towards the research that I am currently involved in and also help me pave my way for future collaborations given the list of interesting speakers.

Serine protease kinetics from isothermal titration calorimetry.

Presenter: Ksenia Maximova

Ksenia Maximova, Joanna Trylska

Understanding the kinetics of serine proteases catalyzed hydrolyses of peptide bonds could assist in development of inhibitors of these enzymes. Common methods to measure the kinetics are spectrophotometric assays, which require costly and time-consuming labelling of substrates with chromophores. Isothermal titration calorimetry (ITC), which measures the change in the heat of the investigated process, could serve as an alternative technique. Since ITC methodology for kinetics has been scarcely presented in the literature, we have optimized ITC for the measurements of enzymatic activity and inhibition. With ITC we determined kinetics for trypsin- and proteinase 3-catalyzed hydrolyses of various substrates under the influence of inhibitors. In comparison with classical spectrophotometric assay ITC turned out to be an accurate, fast and straightforward method for kinetic and inhibition studies. Supported by the Polish-Norwegian Research Programme (the National Centre for Research and Development under the Norwegian Financial Mechanism 2009-2014, Project Contract No POL-NOR/198939/13/2013). K. Maximova, J. Trylska, Kinetics of trypsin catalyzed hydrolyses determined by isothermal titration calorimetry, Anal. Biochem., under revision

Rapamycin-loaded solid lipid nanoparticles for medical application

Presenter: Jarosław Mazuryk

NanoBioMedical Centre, Faculty of Physics, Adam Mickiewicz University, Umultowska 85, 61-614 Poznań

Jarosław Mazuryk, Stefano Giovagnoli, Department of Pharmaceutical Science, University of Perugia, Alice Polchi, Department of Chemistry, Biology and Biotechnology, University of Perugia, Alessandro Magini, Department of Medical and Biological Sciences, University of Udine, Carla Emiliani, Department of Chemistry, Biology and Biotechnology, University of Perugia, Adam Patkowski, Molecular Biophysics Division, Faculty of Physics, Adam Mickiewicz University

Neurodegenerative disorders belong to most debilitating diseases of affluence that still remain without any efficient, low-cost remedy. Much effort has been paid for inventing brain-targeted drug-carriers to effectively overcome BBB, which regulates the transport of active agents into CNS. At the same time, the rapamycin-mediated inhibition of the mTOR complex, responsible for metabolism and cell proliferation, has been shown to repair the cognitative behavior in an Alzheimer mouse model. Design of well-defined solid lipid nanoparticles carrying rapamycin becomes a powerful strategy to treat the CNS-localized lesions. Rapamycin-loaded SLN, based on Compritol® and stabilized with polysorbate 80, were prepared using cold high-pressure homogenization. Drug content and thermal stability of Rap-SLN were established by UV, FT-IR, Raman spectroscopy and DSC. AFM, EM and DLS revealed colloidal size and spherical shape of the nanoparticles. The internal structure was analyzed by XRD and 1HNMR. Evaluation of Rap-SLN in a SH-SY5Y cell line, an in vitro model for neurodegenerative diseases, by means of MTT, western blotting assays and fluorescence labeling, provided a promising evidence for the release of rapamycin from SLN into the cells.

Another tRNA mystery revealed in Saccharomyces cerevisiae.

Presenter: Anna M. Mleczko

Institute of Bioorganic Chemistry Polish Academy of Sciences, Noskowskiego 12/14, 61-704 Poznań, Poland

Anna M. Mleczko, Marta Kasprzyk, Tomasz Twardowski, Kamilla Bąkowska-Żywicka, Institute of Bioorganic Chemistry Polish Academy of Sciences, Noskowskiego 12/14, 61-704 Poznań, Poland

tRNA is a molecule which main role is formation of the physical link between the nucleotide sequence of mRNA and the amino acid sequence of proteins. In addition to this primary role in protein biosynthesis, tRNA can possess other ex-translational functions. For example, certain stress conditions can induce cleavage of tRNAs in specific sites and forming of stable processing products. Recent findings revealed that tRNA-derived fragments represent a wide functional repertoire, from RNA metabolism through RNAi mechanisms to apoptosis. For further experimental studies of biological functions in RNAi-independent pathways, revealing a complete set of S. cerevisiae tRNA fragments is of special importance. Here, we utilized modification-independent, northern blot-based technology to determine the processing patterns for all tRNAs in Saccharomyces cerevisiae. We have demonstrated the presence of 96 specific fragments derived from all tRNA isoforms in multiple stress conditions. Moreover, we have observed clear isoform-dependent differences in abundance of tRNA fragments. In addition, we provided the first evidence that 3'-part-derived tRNA fragments are as abundant as the 5'-one.

MIA40 interactions in Human Cells

Presenter: Karthik Mohanraj

International Institute of Molecular and Cell Biology

Karthik Mohanraj, Wasilewski M,Sakowska P, Chacińska A Laboratory of Mitochondrial Biogenesis, International Institute of Molecular and Cell Biology, Warsaw, Poland,

Mitochondria play a vital role in various cellular functions like ATP synthesis, apoptosis and signalling in eukaryotic cells. The majority of the mitochondrial proteins are nuclear encoded; hence the precursor proteins should be efficiently imported in to the mitochondria. The import of mitochondrial proteins has been extensively studied in yeast. Among the various import pathways, the mitochondrial intermembrane space assembly machinery (MIA) pathway is responsible for import of intermembrane space (IMS) proteins. The import is facilitated by the redox-active CPC motif (cysteine-proline-cysteine) of MIA40 protein that introduces disulphide bonds in the incoming precursors and thereby trapping the proteins in IMS1. The C- terminal domain of MIA40 that contains the active cysteines is conserved in human but considerably lesser is known about MIA pathway in human cells. We are interested to examine the different interacting partners of MIA40 to understand the various factors governing the import of IMS proteins in human cell lines. We employ pull down assays to identify precursor proteins interacting with Mia40. We use cysteine mutant of MIA40 to understand the role of cysteines in binding to different incoming precursor proteins.

Computational analysis of sequence motifs in different ChIP-exo profiles of related RBF proteins

Presenter: Shamba Sankar Mondal

Laboratory of Molecular Neurobiology and Laboratory of Bioinformatics, Nencki Institute of Experimental Biology

Shamba Sankar Mondal, Yiliang Wei Michigan State University, David N. Arnosti Michigan State University, Bartek Wilczynski Institute of Informatics University of Warsaw

Rbf1 and Rbf2 retinoblastoma corepressor proteins in Drosophila bind DNA in a complex with TFs. Experimental data indicated that Rbf2 targets approximately twice as many genes as Rbf1. We investigated the basis for differential targeting by these two proteins, in four functional groups of genes and in whole genome. By grouping the genes into 4 exclusive classes of bindig: Rbf1 only, Rbf2 only, both or none of the factors, we tested for motif association with ChIP enrichment. Correlation between TFBS affinity scores and ChIP enrichment for the DNA sequences upstream of the TSS was tested, both for individual motifs, and for cooperative interactions between any two TFs. Individual and pair-wise motif enrichment analysis were also done. Although Beaf-32 appeared to be involved in helping Rbf2 bind DNA, a Beaf-32 RNAi experiment demostrated otherwise; but a parallel study showed that the dREAM complex appears to recruit insulator proteins like Beaf-32 to block enhancer activity on divergently transcribed genes. Motif strength analysis identified that Rbf2-specific promoters have different preferred motif affinities for multiple factotrs, suggesting unique targeting mechanisms based on cooperativity of mutiple weakly bound TFs.

Use of tannin rich plants for a supporting treatment of periodontal diseases

Presenter: Izabela Nawrot-Hadzik

Department of Pharmaceutical Biology and Botany, Medical University Wrocław, Poland

Izabela Nawrot-Hadzik, Arleta Dołowacka1, Jakub Hadzik2, 1Department of Pharmaceutical Biology and Botany, Medical University Wrocław, Poland, 2Department of Dental Surgery, Medical University Wrocław, Poland

Periodontal diseases are a very common disease, affecting people worldwide. Studies suggest that natural compounds with a ability of inhibiting the bacterial protease activity or modulating the host inflammatory response, may be useful in the treatment or prevention of the periodontal disease. One of them are tannins because of their antibacterial activity. Couple of studies have shown that tannins have also antibacterial activity for periopathogens, for example proanthocyanidins from cranberry could prevent the formation of P. gingivalis biofilm as well inhibit the attachment of P. Gingivalis to epithelial cells . But not only proantocyanidyn from cranberry have strong inhibitory activity to periopathogenes, also condensed tannins from aplles have shown significantly inhibited proteas activity of P.gingivalis. Green tea contein catechines derivatives, also exhibited inhibition towards Porphyromonas proteas. Recent research provide evidence that tannins can inhibit activity of matrix metaloproteinases. Authors conduct studies of the use tanin rich species: Polygonum cuspidatum, sachalinensis and Sanguisorba officinalis for a possible use in periodontal diseases treatment. Authors present part of the study.

Effect of the Tottori Familial Disease Mutation (D7N) on the Monomers and Dimers of Aβ40 and Aβ42

Presenter: Son Tung Ngo

Institute of Physics, Polish Academy of Sciences

Son Tung Ngo, Son Tung Ngo, Man Hoang Viet, Phuong H. Nguyen, Mai Suan Li, and Philippe Derreumaux

Recent experiments have shown that the mutation Tottori (D7N) alters the toxicity, assembly and rate of fibril formation of the wild type (WT) amyloid beta (Aβ)A β40 and Aβ42 peptides. We used all-atom molecular dynamics simulations in explicit solvent of the monomer and dimer of both alloforms with their WT and D7N sequences. The monomer simulations starting from a random coil and totaling 3 μs show that the D7N mutation changes the fold and the network of salt bridges in both alloforms. The dimer simulations starting from the amyloid fibrillar states and totaling 4.4 μs also reveal noticeable changes in terms of secondary structure, salt bridge, and topology. Overall, this study provides physical insights into the enhanced rate of fibril formation upon D7N mutation and an atomic picture of the D7N-mediated conformational change on Aβ40 and Aβ42 peptides.

A priori identifiability analysis as an approach to experimental design for systems biology models

Presenter: Karol Nienałtowski

Institute of Fundamental Technological Research Polish Academy of Sciences

Karol Nienałtowski, Edyta Głów Institute of Fundamental Technological Research Polish Academy of Sciences, Michał Komorowski Institute of Fundamental Technological Research Polish Academy of Sciences

Dynamical models in quantitive biology are characterised by much more complex structures and substantially larger sets of parameters than models used in physics and engineering. Moreover available experiments usually provide limited set of data, usually corresponding only to fragments of studied systems. In consequence the reliability of used models is limited and our knowledge of studied processes misrepresented. Potential remedy is provided by experimental design techniques. Here we present a method specifically tailored for multi-parameter models of quantitative biology. Our framework enables to determine which parameters of a given model can be identified in a given experiment and predict which experiment should be performed next to maximise the number of identifiable parameters. Our tool is different from methods developed so far as it is focused in verifying identifiability of individual parameters in large dynamical models, which contain even hundreds of parameters. We present applicability of our tool analysing JAK-STAT signalling model. Our method helps to guide experimental design in order to render such parameters identifiable.

Gli protein activation – from phosphorylation to the primary cilium and beyond

Presenter: Paweł Niewiadomski

Centre of New Technologies, University of Warsaw

Paweł Niewiadomski,

Gli proteins are major transcriptional mediators of the oncogenic hedgehog pathway. Gli2 and Gli3 can both play a role of activators and repressors of transcription of their target genes depending on the state of the upstream components of the pathway. Although the conversion of Gli proteins into transcriptional repressors has been extensively studied, the steps necessary for them to achieve full activating potential have been unclear. Here, we show that multi-site phosphorylation of Gli transcription factors regulates their activity in a graded manner, shedding new light on signal transduction of the hedgehog pathway and opening up new avenues for the treatment of hedgehog-related cancers.

Folding mechanism of tadpole proteins

Presenter: Szymon Niewieczerzał

Centre of New Technologies University of Warsaw

Szymon Niewieczerzał, Joanna I. Sułkowska, Centre of New Technologies University of Warsaw, Faculty of Chemistry University of Warsaw

Disulfide bonds may affect significantly a function of proteins. In some proteins, called tadpole proteins (or lasso proteins), containing one or more disulfide bonds in its structure, their presence leads to an introduction of a nontrivial topology. The existence of disulfide bridges can be regulated by pH value of the solution. We study, by means of structure based models, the folding process of three small tadpole proteins, with one disulfide bond each, under reduced (no SS-bond) and oxidized (SS-bond is present) conditions. We provide thermodynamic and kinetic analysis of the folding process for these proteins and we show an influence of the disulfide bond on this process. We determine free energy landscape and folding pathways. We show, that the presence of the disulfide bridge reduces the energy barrier for the folding process as well as stabilizing the the native state, which is in agreement with previous studies on this subject.

Understanding molecular basis of enantioselective induction of Tau amyloidogenesis.

Presenter: Bartosz Nizynski

College of Inter-Faculty Individual Studies in Mathematics and Natural Sciences, University of Warsaw, Warsaw, Poland

Bartosz Nizynski, Hanna Nieznanska Department of Biochemistry, Nencki Institute of Experimental Biology, Warsaw, Poland; Wojciech Dzwolak Department of Chemistry, Biological and Chemical Research Centre, University of Warsaw, Warsaw, Poland; Krzysztof Nieznanski Department of Biochemistry, Nencki Institute of Experimental Biology, Warsaw, Poland

Amyloidogenesis is a process in which ordered protein aggregates (amyloid fibrils) are formed. Microtubule associated protein Tau, in physiological conditions, stabilizes microtubules in neurons. Amyloidogenesis of Tau leads to the loss of Tau function. On the other hand, Tau can form amyloid fibrils with distinct structure, biological activity and/or toxicity (amyloid strains). Enantioselective induction of Tau aggregation is poorly studied. To understand the importance of the chirality in the context of induction of different Tau amyloid structures we have used enantiomers of polyglutamic acid: poly-L-glutamic acid (PLGA) and poly-D-glutamic acid (PDGA). We have found that kinetics of PLGA-/PDGA-induced Tau aggregation are different which together with distinct morphologies identified by transmission electron microscopy and the content of β-structures examined by Fourier transform infrared spectroscopy imply that obtained Tau amyloid fibrils are polymorphic. We also have observed that these fibrils caused significant toxic effects in rat primary neurons as imaged by confocal microscopy. These results provide a better understanding of the molecular events of the enantioselective induction of Tau amyloidogenesis.

Novel myosin VI-binding partners in neuronal PC12 cells

Presenter: Jolanta Nowak

Laboratory of Molecular Basis of Cell Motility, Department of Biochemistry, Nencki Institute of Experimental Biology, Warsaw, Poland

Jolanta Nowak, 1 Majewski Lukasz, Laboratory of Molecular Basis of Cell Motility, Department of Biochemistry, Nencki Institute of Experimental Biology, Warsaw, Poland 1 Sobczak Magdalena, Laboratory of Molecular Basis of Cell Motility, Department of Biochemistry, Nencki Institute of Experimental Biology, Warsaw, Poland; 2 Lenartowska Marta, Laboratory of Developmental Biology, Faculty of Biology and Environment Protection, Nicolaus Copernicus University, Toruń, Poland; 3 Lenartowski Robert, Laboratory of Isotope and Instrumental Analysis, Faculty of Environment Protection, Nicolaus Copernicus University, Toruń, Poland, Rędowicz Mara Jolanta Laboratory of Molecular Basis of Cell Motility, Department of Biochemistry, Nencki Institute of Experimental Biology, Warsaw, Poland

Myosin VI (MVI), the only known myosin walking towards the minus end of actin filaments, is involved in numerous cellular processes. Remarkably, it was also found within the nuclei of PC12 cells; till now the MVI nuclear function remains, however, poorly understood. Since MVI acts through interactions of C-terminal globular tail (GT) domain with specific partners, we performed a search for MVI partners in PC12 cells with affinity chromatography using GST-tagged MVI-GT as a bait. Using mass spectrometry, we identified several novel potential MVI-binding partners, among them were hnRNP U (heterogeneous nuclear ribounucleoprotein U), ribosomal protein S6 and nucleolar protein nucleolin. These interactions were further confirmed by proximity ligation assay and/or immunocytochemistry. Additionally, we showed that fibrillarin and RNA polymerase I, engaged in ribosome biogenesis, also interact with MVI. Moreover, electron microscopy revealed changes in organization of the endoplasmic reticulum in MVI-depleted cells. In light of these data, we speculate that MVI in PC12 cells could be also engaged in regulation of ribosome biogenesis and ER network organization.

Analysis of cell migration using glioma C6 cellular model

Presenter: Natalia Nowak

Nencki Institute Of Experimental Biology

Natalia Nowak, Natalia Nowak, Laboratory of Imaging Tissue Structure and Function, Nencki Institute Of Experimental Biology; Wanda Kłopocka, Laboratory of Imaging Tissue Structure and Function, Nencki Institute Of Experimental Biology; Paweł Pomorski, Multimodal Laboratory of Cell Adhesion and Motility, NanoBioGeo Consortium, Nencki Institute Of Experimental Biology; Department of Biochemistry, Laboratory Of Molecular Basis of Cell Motility, Nencki Institute Of Experimental Biology;

Numerous cellular functions, including cell motility, are regulated by extracellular nucleotides which acts on specific GPCRs from P2Y group. The P2Y2 receptor may be activated with ATP or UTP and further activate multiple signaling pathways in the cell, such as calcium response and Rac1 and RhoA proteins. This is important signaling pathway that controls glioma tumors invasiveness. Due to the isolation from circulation by the blood-brain barrier, gliomas are characterized by strong dependence of tumor invasion on cell motility. We use glioma C6 cells as the model of tumor cells migration. We present newly developed timelapse techniques to research glioma C6 cells migration and morphological properties which depend on nucleotide signaling from P2Y2 receptor. Chemotactic migration was examined with DIC microscopy with use of mechanized stage. Obtained data was processed with ImageJ plugins to perform time lapse images registration to correct stage inaccuracy which improved the reliability of tracking. We also stitched the fields of view to enable tracking of cells in whole chemotaxis chamber. Metamorph software was used to track cells and then trajectories were analyzed with a Matlab script to calculate migration parameters.

Analysis of functional motions of Decapping Scavenger enzyme using coarse-grained molecular dynamics

Presenter: Anna Nowicka

Departament of Physics, Division of Biophysics, University of Warsaw, Zwirki i Wigury 93, 02-089 Warsaw, Poland

Anna Nowicka, Paweł Dąbrowski-Tumański, Departament of Chemistry, University of Warsaw Pasteura 1, 02-093 Warsaw, Poland, Michał Kadlof, Centre of New Technologies, University of Warsaw Banacha 2c, 02-097 Warsaw, Poland, Jacek Jemielity, Centre of New Technologies, University of Warsaw Banacha 2c, 02-097 Warsaw, Poland, Joanna Ida Sułkowska, Centre of New Technologies, University of Warsaw Banacha 2c, 02-097 Warsaw, Poland

Coarse-grained molecular dynamics simulations can be used to reveal the mechanism of enzyme catalyzed reactions. This approach is essential especially for large molecular complexes which are time consuming. Analysis of such molecular dynamics allows us to observe many cycles of enzyme action, study large functional motions, explore protein conformations during trajectory and analyze them using statistical software. Here, we present analysis of coarse-grained simulations in order to reveal functional movements of the Decapping Scavenger enzyme (DcpS) which appears on the 3'-5' mRNA degradation pathway. Analysis of simulations shows the examined homodimer protein motions to be cooperative. We also detected collective motions using PCA analysis. Obtained data are in agreement with the results from all-atom simulations.

Bid protein fused with TAT domain sensitizes cancer cells to anticancer drugs

Presenter: Emilia Joanna Orzechowska

Department of Molecular Biology, Faculty of Biology, University of Warsaw

Emilia Joanna Orzechowska, Krzysztof Staroń, Joanna Trzcińska-Danielewicz*; * Department of Molecular Biology, Faculty of Biology, University of Warsaw*

Deregulation of apoptotic pathways is a common dysfunction observed in many types of cancer. The main causes of suppression of apoptosis are: lowered expression of proapoptotic proteins; elevated expression of inhibitors of apoptosis or posttranslational modifications. A common result is reduction of the functional dose of protein below the critical level necessary for apoptosis. In our experiments, we tested the effect of increased cellular level of proapoptotic BID protein on sensitivity of different cancer cells to anticancer drugs: camptothecin, doxorubicin and TRAIL. We used a method allowing for controlled delivery of BID into cells as a fusion with a cell penetrating peptide TAT (TAT-BID). We found that delivery of TAT-BID in amounts close to its endogenous level did not induce apoptosis, but sensitized cancer cells to low doses of anticancer drugs, even if cells were previously resistant for their action. Moreover, in PC3 cells BID protein is a critical in apoptotic signaling when apoptosis is induced by doxorubicin, and TAT-BID is involved in mitochondrial pathway of apoptosis. Finally, we observed that phosphorylation of BID protein by CK2 kinase is not a reason of suppression of apoptosis in PC3, A549 and HeLa cells.

Exploring m6A mRNA methylation in neuroblastoma

Presenter: Luigi Pasini

Centre for Integrative Biology - University of Trento - Italy

Luigi Pasini,

Neuroblastoma is a tumor of the developing neural crest and a devastating cause of children death. The embryonic derivation of neuroblastoma progenitor cells may account for a marked involvement of post-transcriptional control of gene expression to maintain the undifferentiated state and promote rapid progression to oncogenesis. In this context, I am focused in investigating N6-methyladenosine (m6A) modification of mRNA. This topic represents a rapidly growing area of interest that starts opening many new questions about post-transcriptional regulation and associated signaling networks, with the potentiality to impact different aspects of mRNA life, and that has already proved to be an indispensable process guiding self-renewal and differentiation of embryonic stem cells. Preliminary results we have collected from neuroblastoma patients and cellular data substantiate the theory that m6A-methyltransferase enzymes may have a role in oncogenic progression. We have found that increased levels of the METTL14 methyltransferase are associated with worse clinical features and poor prognoses, and we are on the way of investigating the cellular function of METTL14-dependent m6A in neuroblastoma cell models and during mouse embryogenesis.

Fragmentation of ribosomal RNA in response to nitrogen starvation

Presenter: Anna Pastucha

Institute of Genetics and Biotechnology, University of Warsaw

Anna Pastucha, Wojtek Tworzynski1, Seweryn Mroczek1,2 and Joanna Kufel1; 1 Institute of Genetics and Biochemistry, University of Warsaw, Poland 2 Institute of Biochemistry and Biophysics PAS, Warsaw, Poland

Nutritional deprivation leads to severe changes in cell homeostasis, ultimately promoting destruction of cellular components and organelles to prevent death. We describe a separate autophagy mechanism, wherein robust fragmentation of mature ribosomal RNA yeast cells is independent of protein degradation. This ribosome recycling mechanism is highly specific, requires a subset of autophagic regulators, Atg proteins, is dependent on the TOR (target of rapamycin) regulatory pathway, but proceeds efficiently in the absence of the Ubp3-Bre5 complex required for the ribophagic degradation of ribosomal proteins. RNA cleavages take place on intact ribosomes in the cytoplasm and are carried out predominantly by the vacuolar ribonuclease Rny1, which is probably released from the vacuole to the cytosol upon activation of autophagy. Endonucleolytic cleavages generate specific and stable RNA fragments, which are further digested by exonucleases, including Nuc1, 5'-3' exonuclease Xrn1 and the 3'-5' exosome complex. The existence of the autonomous rRNA ribophagic pathway substantiates the importance of ribosome breakdown to survive the shortage of nutrients.

Effects of substrate binding on a knotted protein

Presenter: Agata Perlińska

Centre of New Technologies, University of Warsaw

Agata Perlińska, Joanna I. Sulkowska, Centre of New Technologies, Faculty of Chemistry, University of Warsaw

Methyltransferases (MTs), as enzymes with crucial biological function, are widely represented in various organisms – from bacteria to humans. Within this class of proteins different topologies can be found which includes the knot. All of the knotted MTs have the simplest trefoil knot, which is a part of a cross-subunit active site. Interestingly, the quaternary structure of those enzymes is formed by two identical subunits, arranged in such way, that the active sites can interact with each other. Using molecular dynamics simulation we studied three complexes of the TrmD (tRNA methyltransferase D) protein: the apoenzyme (without any substrates), complex with two bound cofactors and the holoenzyme (with two cofactors and a tRNA). Since the active site is within the knot it is expected that the more substrates are bound to the site the more stable it becomes. While it is true for other parts of the active site, it does not appear to be applicable to the knot. Although bound substrates do not affect overall rigidity of the knot, we observe differences between the active sites in the holoenzyme. It is possible that those differences may be the reason why only one tRNA can be bound to this homodimeric protein.

Search for novel proteins with potential for DNA recognition similar to TAL effctors

Presenter: Malgorzata Perycz

Institute of Biochemistry and Biophysics Polish Academy of Sciences

Malgorzata Perycz, Matthias Bochtler, Institute of Biochemistry and Biophysics Polish Academy of Sciences

The aim of this project was identification of proteins that have potential to be developed into genome editing tools. To this end we searched protein database UniRef100 for sequences sharing key features with transcription activator-like effector proteins (TAL effectors, TALEs). TALEs are used by Xanthomonas bacteria to manipulate gene expression in plants they infect. They possess nuclear localization signals and secretion signals and their distinctive feature is presence of c.a. 20 nearly identical 34 residue long repeats, that encode for the protein's specificity towards DNA. TALEs served as a basis for genome editing tools called TALENs (TAL-effector nucleases), which have a nuclease domain fused to a TALE core. To identify proteins of architecture similar to TALEs, UniRef100 database was mined for sequences containing multiple short repeats of 30-43 residue length. This led to identification of over 400 sequences. They were further analyzed for complexity and variability of the repeats, using self-made and online tools. Also, they were checked for presence of nuclear localization signals, secretion signals and known protein domains. Basing on these results we chose the most promising candidates for protein-DNA interactions.

Qualitative screening of amino acids in plasma in patient with sepsi

Presenter: Joanna Piechowicz

Department of Medical Biochemistry, Wroclaw Medical University

Joanna Piechowicz, Mariusz Grzegorz Fleszar, Magdalena Mierzchała-Pasierb, Jerzy Wiśniewski, Andrzej Gamian

Introduction Protein analysis techniques comprise amino acid composition procedures that may have extensive applications. In sepsis the large-scale muscle breakdown influences on level of most of the amino acids in plasma in patient. Better recognition of the dynamic diversity in a set of amino acids is crucial to supply a fundamental for sepsis nutritional care in hospitals. The main aim of our work was a screening of amino acids in plasma. Materials and methods Amino acid analysis was performed on plasma in patients with sepsis. We applied Phenomenex EZ:faastTM amino acid analysis kit that was also used in another studies. The amino acids were separated by liquid chromatography using a nanoAcquity UPLC system. The detection and identification were carried out by using a Xevo G2 Q-TOF. The acquisition and processing of all of the data were accomplished by using MassLynx software from Waters. Results and Discussion The metabolic spectrum of the amino acids altered seriously in patients with sepsis. The outcome is comparable with other evidences presented in the literature. Our assessment of the Phenomenex EZ:faast procedure for amino acid using LC-ESI-MS/MS has indicated the advantages of this method.

Elongin is involved in DOG1 gene regulation in Arabidopsis thaliana

Presenter: Zbigniew Pietras

Institute of Biochemistry and Biophysics Polish Academy of Sciences, Pawinskiego 5a, 02-106 Warsaw, Poland

Zbigniew Pietras, Justyna Kowalczyk, Ruslan Yatusevich, Szymon Świeżewski

Seed dormancy is a phenomenon where mature seeds do not germinate when exposed to favouring conditions until dormancy is released. This phenomenon not only allows for dispersal of seeds and colonisation of new habitats but also is important of many aspects of agriculture for example allowing seed storage. One of the key natural regulators of seed dormancy in Arabidopsis is DOG1. Elongins are a conserved proteins involved in ubiquitination of target proteins that are subsequently degraded by the proteasome. We have observed change in seed dormancy in elongin mutants. This change was correlated with reduced DOG1 expression in those mutants. The reduction of expression was in turn accompanied by increase in RNAPII occupancy on the gene. This suggests that PolII encounters an obstacle on DOG1 gene and that it needs elongin to avoid stopping / pausing. Furthermore we identified mechanism through which elongin aids PolII to overcome the obstacle. Interestingly this pathway is generally associated with DNA damage response and not gene regulation.

Fe(II)- and AlkB-dependent repair of alkylated lesions in DNA

Presenter: Tomasz Pilżys

Institute of Biochemistry and Biophysics, Polish Academy of Sciences

Tomasz Pilżys, Anna Sikora1, Agnieszka M. Maciejewska1, Jarosław Poznański1, Katarzyna Pawlak2 and Elżbieta Grzesiuk1. 1Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland, 2 Department of Analytical Chemistry, Faculty of Chemistry, Warsaw University of Technology, Warsaw, Poland

We showed that Fe(II) in E. coli hemH mutants is double that in hemH+. We recognized the influence of Fe(II) on AlkB-directed repair of alkylated DNA analyzing survival and mutagenesis of irradiation and MMS-treated hemH and alkB mutants. We showed that mutations in alkB+hemH–/pMW1 was 40 and 26% reduced comparing to the alkB+ hemH– and alkB+ hemH+/pMW1 strains. The effect was observed only in bacteria irradiated prior to MMS-treatment. This finding indicates efficient repair of alkylated DNA in photosensibilized cells in the presence of elevated Fe(II) and AlkB levels. Interestingly, 31% decrease in the level of mutations was observed in irradiated and MMS-treated hemH– alkB– comparing to the hemH+ alkB– strain. Also, the level of mutations in the irradiated/MMS treated hemH– alkB– mutant was significantly lower (34%) in comparison to the same strain but MMS-treated only indicating AlkB-independent repair involving Fe(II) and reactive oxygen species that may be caused by non-enzymatic dealkylation of alkylated dNTPs in E. coli cells. In in vitro studies, the absence of AlkB protein in the presence of Fe(II) allowed etheno(ϵ)dATP and ϵdCTP to spontaneously convert to dAMP and dCMP. Funded by UMO-2011/03/B/NZ4/02425

Can inhibition of NADPH oxidases (NOXs) be the way to senescence cancer cells?

Presenter: Katarzyna Piszczatowska

University of Warsaw, Faculty of Biology, 1 Ilji Miecznikowa, 02-096 Warsaw

Katarzyna Piszczatowska, Dorota Przybylska (Nencki Institute of Experimental Biology Polish Academy of Sciences, Department of Biochemistry, Laboratory of Molecular Bases of Aging, 3 Pasteur Street, 02-093 Warsaw), Ewa Sikora (Nencki Institute of Experimental Biology Polish Academy of Sciences, Department of Biochemistry, Laboratory of Molecular Bases of Aging, 3 Pasteur Street, 02-093 Warsaw), Grażyna Mosieniak (Nencki Institute of Experimental Biology Polish Academy of Sciences, Department of Biochemistry, Laboratory of Molecular Bases of Aging, 3 Pasteur Street, 02-093 Warsaw)

Cellular senescence is a permanent growth arrest that results from gradual exhaustion of proliferative potential or is induced by stress factors. It sustain an anticancer barrier activated upon radio-, chemotherapy and restricts tumor growth. Our studies aimed to find out whether permanent growth arrest of cancer cells could be induced by the interfering with signaling pathways important for cell cycle regulation. We treated p53-proficient and p53-deficient human colon cancer HCT116 cells with DPI, which inhibits NADPH oxidases (NOX)-ROS-producing enzymes. It was previously shown that NOXs support mitogenic signaling pathway in cancer cells. Thus, they are postulated to act as an oncogenes. DPI treatment decreased the proliferation potential. Higher concentrations caused cell death, which was more pronounced in p53-/- cells. Inhibition of proliferation was correlated with decreased ROS production. Cells treated with DPI and then cultured in an inhibitor-free media expressed markers of senescence proving that they are growth arrested. Altogether our results show for the first time that inhibition of NOX activity can restrict cancer cells growth in a permanent manner and as such can be considered as an potential anticancer strategy.

The heterogeneity of ventral tegmental area: behavioural response induced by its stimulation

Presenter: Karolina Plucinska

Department of Animal and Human Physiology, University of Gdansk

Karolina Plucinska, Grazyna Jerzemowska, Department of Animal and Human Physiology, University of Gdansk

The ventral tegmental area (VTA) is a key structure of the mesolimbic dopaminergic system involved in motivational function. The aim of this study is to explore the relationship between VTA's stimulated region to specific behavior that the stimulation induced. Animals were implanted with bilateral electrodes aimed at VTA. After recovery from implantation, rats were screened for VTA stimulation-induced behaviour. Trains of square-wave, constant current, 0.1 ms duration were conducted from the stimulator to the electrode by flexible wire leads. The behavioral effect of VTA stimulation in individual animals is unpredictable, may be manifested as an increase in locomotor activity, exploration, food intake, or other. Food intake and feeding-related behavior was observed during stimulation of the ventrolateral part of the rostral and central VTA. Exploratory behavior was obtain from the dorsal part of the rostral and caudal region of the VTA. Stimulation of VTA reflects the heterogeneous nature of this area. The effect of the stimulation may be dependent on the type of activated projections within VTA. Research was financed by the Polish National Science Centre (NCN); decision no: DEC-2013/09/N/NZ4/02195.

Structural studies on DNA nucleases of mobile genetic elements

Presenter: Radoslaw Pluta

International Institute of Molecular and Cell Biology in Warsaw

Radoslaw Pluta, Radoslaw Pluta1, D Roeland Boer1, Alicia Guasch1, Silvia Russi1, José Ruiz-Maso2, Cris Fernandez-Lopez2, Fabian Lorenzo-Diaz2, Maria Lucas3, Gloria del Solar2, Fernando de la Cruz3, Manuel Espinosa2 and Miquel Coll1; 1Institute for Research in Biomedicine (IRB Barcelona), Spain and Institut de Biologia Molecular de Barcelona (CSIC), Spain , 2Centro de Investigaciones Biológicas (CSIC), Madrid, Spain , 3Instituto de Biomedicina y Biotecnologia de Cantabria (IBBTEC), Santander, Spain present address: International Institute of Molecular and Cell Biology (IIMCB), Poland

Plasmids and integrative and conjugative elements (ICEs) are major mobile genetic elements (MGEs) that provide routes for rapid acquisition of new genetic information in bacteria and therefore contribute to the spread of antibiotics resistance. Essential for their action are plasmid/ICE-encoded site- and strand-specific one-metal-ion endonucleases called relaxases. Conjugative relaxases cleave a single strand of the DNA substrate by formation of an intermediate covalent adduct with the scissile phosphate of the DNA nic site. After the ssDNA-relaxase molecule is transferred to the recipient cell, relaxases ensure re-ligation of their DNA cargo. Additionally, plasmids and some ICEs encode for DNA replication relaxases, crucial for their maintenance. Understanding plasmid/ICEs conjugal transfer and replication may aid in combating the spread of antibiotics resistance as well as contribute to the development of new tools for DNA delivery into human cells. Structures of replicative and conjugative relaxases (RepB, MobM and TrwC) that were solved in our lab are compared herein.

Stress-induced signaling in CML promotes release of factors stimulating invasiveness

Presenter: Paulina Podszywalow-Bartnicka

Nencki Institute of Experimental Biology

Paulina Podszywalow-Bartnicka, Paulina Podszywalow-Bartnicka, Anna Cmoch, Agnieszka Wesolowska, Lukasz Bugajski, Marta Tkaczyk, Slawomir Pikula, Michal Dadlez, Tomasz Skorski, Katarzyna Piwocka

We previously showed activation of stress response signaling in CML (Kusio-Kobialka, 2012) thus the next aim was to verify how enhanced status of eukaryotic initiation factor 2 subunit (eIF2) phosphorylation affects leukemia-stroma cells communication and influence cancer progression. We found that higher eIF2-P status increased invasive potential of model cell lines (K562 cells wild type or expressing non-phosphorylable eIF2) and CML primary cells from patients. SILAC mass spectrometry and antibody arrays showed elevated release of enzymes modifying extracellular matrix and other proteins modulating invasiveness. Modification of composition of soluble factors and extracellular vesicles secreted by CML cells influenced invasiveness of bone marrow stroma fibroblasts. This may play a role in the stroma-leukemic cells cross talk and the disease progression. This work was supported by grants from NCN (2011/01/B/NZ3/02145, 2013/10/E/NZ3/00673) and MNiSW (IP2011 043071); P.P-B. is the recipient of EUFP7 POKL.04.03.00-00-060/12-00 fellowship.

The myosin chaperone UNC-45 is organized in tandem modules to support myofilament formation.

Presenter: Wojciech Pokrzywa

Cologne Excellence Cluster on Stress Responses in Ageing-Associated Diseases (CECAD), University of Cologne, Germany

Wojciech Pokrzywa, T. Löwe and T.Hoppe (CECAD, University of Cologne,Germany); L. Gazda, D. Hellerschmeid, T. Clausen (Research Institute of Molecular Pathology, Vienna, Austria); I. Forné, F. Mueller-Planitz (CIPSM, Ludwig Maximilian University, Munich, Germany).

The activity and assembly of various myosin subtypes is coordinated by conserved UCS (UNC-45/CRO1/She4p) domain proteins. One founding member of the UCS family is the Caenorhabditis elegans UNC-45 protein important for the organization of striated muscle filaments. Our recent structural and biochemical results demonstrated that UNC-45 forms a protein chain with defined periodicity of myosin interaction domains. Intriguingly, the UNC-45 chain serves as docking platform for myosin molecules, which promotes ordered spacing and incorporation of myosin into contractile muscle sarcomeres. The physiological relevance of this observation was demonstrated in C. elegans by transgenic expression of UNC-45 chain formation mutants, which provokes defects in muscle structure and size. Collaborating with the molecular chaperones, Hsp70 and Hsp90, chain formation of UNC-45 links myosin folding with myofilament assembly.

Study of polysaccharide-protein interactions via a coarse-grained model

Presenter: Adolfo Poma

IFPAN

Adolfo Poma, Adolfo Poma, M. Chwastyk and Marek Cieplak

Hexaose-Man5B catalytic complexes are characterized in a CG model. We determine contact energies between the carbon (C4) atom and alpha-C (CA) atom in the amino acid residue. These interactions are found to be stronger than the HBs: about 4 times as strong for cellohexaose and 2 times for mannohexaose. Fluctuational dynamics of the CG complexes are found to be compatible with 100 ns all-atom studies.

NADPH oxidase NOX4 is crucial for proliferation of human vascular smooth muscle cells

Presenter: Dorota Przybylska

Nencki Institute of Experimental Biology PAS, Warsaw, Poland

Dorota Przybylska, Dorota Przybylska, Piotr Sunderland, Ewa Sikora, Grazyna Mosieniak, Laboratory of Molecular Bases of Aging, Nencki Institute of Experimental Biology PAS, Warsaw, Poland

Senescence is proposed as one of the mechanisms that drives organismal aging as well as age-related diseases like atherosclerosis. Indeed senescent vascular smooth muscle cells (VSMCs) have been isolated from atherosclerotic plaque. One of the factors that plays a causal role in both atherosclerosis and senescence is oxidative stress which originates from mitochondria or specialized enzymes like NADPH oxidases, NOXs. These ROS-producing enzymes are expressed tissue-specifically and although NOX1, 4 and 5 have been identified in VSMCs, data suggest that NOX4 is the most important isoform. The aim of this study was to investigate the role of NOX4 in the regulation of the cell cycle of VSMCs isolated from the human aorta. We observed that the level of NOX4 decreases during senescence of VSMCs. Downregulation of its level by siRNA in proliferating cells led to a decrease of the level of ROS and induction of senescence. Thus, NOX4 expression is critical for driving ROS-dependent signalling pathways that facilitate cell cycle and this functions could not be taken over by other NOX isoforms. This work was supported by grant 0728/B/P01/2011/40 financed by the Polish Ministry of Science and Higher Education.

Glioma-derived integrin ligands shape tumor microenvironment and immune response in rat glioma model

Presenter: Dominika Pszczółkowska

Nencki Institute of Experimental Biology, Polish Academy of Science

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Malignant gliomas are fast-growing, invasive brain able to attract immune cells and re-program them into tumor-supporting cells. Factors responsible shaping tumor microenvironment in gliomas are poorly known. We identified two proteins: lactadherin (Mfge8) and osteopontin (Spp1) potentially involved in glioma progression. Both Spp1 and Mfge8 are integrin ligands overexpressed in glioma cells, but not in non-transformed astrocytes. C6 glioma cells stably expressing shRNA specific to Mfge8, Spp1 and negative shRNA were implanted into striatum of Wistar rats. Knockdown of Spp1 and Mfge8 resulted in significant reduction of tumor size. Immunochemical analysis revealed similar numbers of infiltrating microglia/macrophages (Iba1 staining), but the reduced number of Arg1+ cells in Mfge8-depleated tumor. Although Mfge8 is known as a protein involved in angiogenesis, Mfge8-depleated tumors do not exhibit reduced blood vessel density. FACS analysis showed that silencing of Spp1 does not affect number of CD11b+ cells, but modulates microenvironment by decreasing percentage of Treg cells infiltrating tumor-bearing hemisphere. Our results suggest that glioma-derived integrin ligands are important factors in glioma progression.

Modeling of protease-substrate complex

Presenter: Wojciech Puławski

Institute of Biochemistry and Biophysics Polish Academy of Sciences; Warsaw University, Chemistry Department

Wojciech Puławski, Sławomir Filipek, Szymon Niewieczerzał

Rhomboid proteases constitute a group of enzymes present in variety of living organisms, and were first recognized in Drosophila, as an important factor for initiating its epidermal growth factor receptor (EGFR) signaling pathway. To this moment, the structural data on substrate-protease complexes are lacking and the mechanism of intramembrane proteolysis is unclear. Here, we modeled the structure of the complex of rhomboid protease with its substrate SPITZ based on molecular modeling and experimental constraints. Moreover, using molecular dynamics we calculated the Potential of Mean Force for the dissociation process of the complex and estimated the influence of mutations on this process.

ECO-HAB - AUTOMATED ASSESSMENT OF SOCIAL IMPAIREMNTS IN MOUSE MODELS OF AUTISM

Presenter: Alicja Puścian

Nencki Institute of Experimental Biology, PAS

Alicja Puścian, Łęski Szymon (a), Winiarski Maciej (a), Boguszewski Paweł (a), Kasprowicz Grzegorz (b,c), Knapska Ewelina (a) (a) Department of Neurophysiology, Nencki Institute of Experimental Biology, Warsaw, Poland; (b) Center for Theoretical Physics, Polish Academy of Sciences, Warsaw, Poland; (c) Warsaw University of Technology, Institute of Electronic Systems, Warsaw, Poland

Impairments of social interactions are a key feature of autism. Existing behavioral tests of social behavior in mice do not allow for longitudinal observations of interactions between littermates. Moreover, their results may be confounded due to the stressors intrinsic to assessment process. In order to alleviate these problems, we designed Eco-HAB - a fully automated tool for assessment of voluntary social interactions in group-housed mice. Using Eco-HAB, we assessed social approach of valproate-treated BALB/c and C57BL/6 mice (in utero exposure to this drug is a pharmacological model of autistic phenotype). We determined that despite previously documented deficits of place learning (BALB/c) and repetitive behaviors (C57BL/6), none of those models displays impairments in social interactions. These data are consistent with results obtained in a three-chambered apparatus test, performed in stress-reducing conditions. We argue that one should focus on particular behavioral impairments of mouse models of autism, rather than try to address a rarely appearing, all-inclusive phenotype. Eco-HAB enables such research and asserts high reliability, thus, we claim it is a valuable tool for assessment of social behavior in-group housed mice.

Cytosine hydroxymethylation in honey bee (Apis mellifera) genome.

Presenter: Dominik Rafalski

International Institute of Molecular and Cell Biology in Warsaw

Dominik Rafalski, Dominik Rafalski 1, Marek Wojciechowski 1, Robert Kucharski 2, Katarzyna Misztal 1, Joanna Maleszka 2, Matthias Bochtler 1, and Ryszard Maleszka 2, 1 - Laboratory of Structural Biology, International Institute of Molecular, Warsaw, Poland, 2 - The Australian National University, Canberra, Australia

Honey bee is an organism whose developmental fate is controlled by epigenetic factors. That makes it an interesting animal model to study DNA modification processes, such as Cytosine methylation and hydroxymethylation. Tet enzymes are responsible for oxidation of 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC). In mammalian genomes the role of these enzymes is known and characterized. However little is known about the function of this Tet in invertebrates. In our work we focused on characterization of the 5hmC in Apis genome as well as characterization of the Tet orthologue, suspected for introduction of this epigenetic mark. We found that 5hmC is present in honey bee genome and its levels differ between castes, tissues and developmental stages. 5hmC is most abundant in testis and ovaries and is established to constitute 7 to 10% of the total level of 5mC, that is comparable to the 5hmC to 5mC proportion in mammalian cells. We also found that Tet protein is responsible for oxidation of 5mC to 5hmC in honey bee genome. Its mRNA levels differ between tissues, castes and life stages. TET mRNA levels are positively correlated with 5hmC levels. Tet is also one of 5% of the most highly expressed genes in honey bee brain.

Cerebral ischemia induced changes in microglial gene expression underlying proinflammatory status

Presenter: Wenson David Rajan

Laboratory of Molecular Neurobiology, Nencki Institute of Experimental Biology, Warsaw

Wenson David Rajan, Bozena Kaminska, Laboratory of Molecular Neurobiology, Nencki Institute of Experimental Biology

Microglia are the resident macrophages of the CNS and the first cells to respond to injury or pathogenesis. Pathological stimuli polarize microglia into two major phenotypes: classical proinflammatory M1phenotype, and alternative anti inflammatory M2 phenotype. Cerebral ischemia activates proinflmmatory microglia neurodegeneration. We used rat transient MCAo intraluminal filament model of focal cerebral ischemia and analyzed gene expression profiles underlying microglial activation. Iba1 and IL1β staining confirmed the presence of activated microglia in the ipsilateral side 24h post 90 minutes transient MCAo. qPCR on CD11b+ cells after 24h I/R upregulated inflammatory genes such as irf7and pim which are STAT dependent as evidenced from our recent finding on LPS stimulated primary microglia. Moreover, the decrease in smad7 gene expression suggests the reduction in neuroprotective TGF-β signaling. Our result shows the induction of the M1 phenotype in microglia and identifies the novel STAT dependent inflammatory genes after transient cerebral ischemia. As a long term goal, it would be interesting to investigate the expression of these inflammatory genes and their possible regulation by STAT and epigenetic modification after MCAo.

Native matrix-based in vitro model of alveolar lung tissue

Presenter: Jovile Raudoniute

State Research Institute Centre for Innovative Medicine

Jovile Raudoniute, I. Bruzauskaite, E. Bagdonas, J. Denkovskij, R. Aldonyte; State Research Institute Centre for Innovative Medicine

Diseases of the respiratory system represent an increasing burden on societies. Tools to study respiratory diseases in vitro are limited due to complex architecture and functional properties of the alveolar tissues. Several possibilities exist, including synthetic matrices and native matrix-based models. We plan on employing latter model system to study destruction and regeneration processes in the human alveolar tissue. We have employed pulmonary epithelial cells (line A549) and mesenchymal stem cells of bone marrow origin. Cells were cultured on decellularized cadaver lung matrix for prolonged periods of time and under influence of several pro-regenerative agents. Model proposed represents a relevant platform to study pathologic processes within the alveolar epithelium. Under the influence of alpha-1-antitrypsin, cell proliferation rate rises above control cells. Overall, cell survival in the model is satisfying and provides convenient time period for the assays. Our model may be successfully employed for variety of in vitro studies. Cell proliferation in the model may be regulated. Epithelial and stem cells participate together in the maintenance and regulation of tissue regeneration.

Expression and function of Angiomotin family of proteins in the brain

Presenter: Katarzyna Rojek

Laboratory of Synaptogenesis, Department of Cell Biology, Nencki Institute of Experimental Biology, Warsaw, Poland

Katarzyna Rojek, Katarzyna Rojek Laboratory of Synaptogenesis, Department of Cell Biology, Nencki Institute of Experimental Biology, Warsaw, Poland, Paweł Niewiadomski Laboratory of Synaptogenesis, Department of Cell Biology, Nencki Institute of Experimental Biology, Warsaw, Poland, Krzysztof Bernadzki Laboratory of Synaptogenesis, Department of Cell Biology, Nencki Institute of Experimental Biology, Warsaw, Poland, Hubert Doleżyczek Laboratory of Neurobiology, Department of Molecular and Cellular Neurobiology, Nencki Institute of Experimental Biology, Warsaw, Poland, Marcin Rylski The Medical Center of Postgraduate Education, Warsaw, Poland, Leszek Kaczmarek Laboratory of Neurobiology, Department of Molecular and Cellular Neurobiology, Nencki Institute of Experimental Biology, Warsaw, Poland, Jacek Jaworski International Institute of Molecular and Cell Biology, Warsaw, Poland, Tomasz Prószyński Laboratory of Synaptogenesis, Department of Cell Biology, Nencki Institute of Experimental Biology, Warsaw, Poland

Proper organization of synaptic connections is important for the transmission of information in the central and peripheral nervous systems (CNS and PNS). The molecular mechanisms underlying synaptic plasticity are still poorly understood. We have recently identified the scaffold protein Amotl2 as a potential regulator of neuromuscular junction (NMJ) remodeling. Interestingly, many proteins involved in NMJ remodeling are also implicated in the plasticity of synapses in the brain. Therefore, we investigated in the CNS the expression and function of Amotl2 and closely related proteins Amot and Amotl1, collectively called Angiomotins. We demonstrated that all three angiomotins are widely expressed in the brain with Amotl2 and Amotl1 concentrated at synapses, whereas Amot decorated cytoskeletal structures in neurites. Amot depletion in neurons led to reduced dendritic tree arborization and malfunction of the axon initial segment (AIS). Thus, our experiments identify a novel group of proteins that play a critical role in the organization of neurons and may regulate synaptogenesis both in the CNS and PNS. This research was supported by the NCN grant 2012/05/E/NZ3/00487.

Telomerase inhibition leads to death of breast cancer cells and increases sensitivity to doxorubicin

Presenter: Aleksandra Romaniuk

Department of Clinical Chemistry and Molecular Diagnostics, Poznan University of Medical Sciences, 49 Przybyszewskiego St., 60-355 Poznan, Poland

Aleksandra Romaniuk, Natalia Lipińska, Błażej Rubiś, Department of Clinical Chemistry and Molecular Diagnostics, Poznan University of Medical Sciences, 49 Przybyszewskiego St., 60-355 Poznan, Poland

Telomerase maintains the integrity of chromosomes during cell division. High levels of human telomerase reverse transcriptase (hTERT) are detected in over 85% of cancer cells isolated from primary human tumors. Since enzyme activity is crucial for cancer cells immortality, its inhibition seems to be a promising strategy in cancer therapy. We showed that the knockdown of hTERT effectively inhibited cell viability of human breast cancer cell line MDA-MB-231 and caused cell cycle alterations. We also revealed that telomerase inhibition (lentiviral vector) in combination with chemotherapeutic agent, doxorubicin, provoked a significantly increased cancer cell death rate comparing to the drug used alone. Alternatively, similar combination with rapamycin (drug with a different mechanism of action), did not show such significant differences. Consequently, we also revealed variations in levels of cell-cycle regulatory proteins. These observations suggest that a combined application of telomerase downregulation and doxorubicin might be a very efficient strategy to increase sensitization of cancer cells to therapy and chemotherapeutic agents dosage reduction. The study was supported by National Science Centre Grant No. 2011/03/B/NZ7/00512

Regulation of CREB transcription factor activity by CacyBP/SIP phosphatase in NB2a cells.

Presenter: Sara Rosinska

Nencki Institute of Experimental Biology PAS Warsaw

Sara Rosinska, Wiesława Leśniak, Anna Filipek, (The same affiliation as the author)

CacyBP/SIP is a novel phosphatase directly interacting with and dephosphorylating ERK1/2 kinase. It has two KIM (Kinase Interaction Motive) domains responsible for its phosphatase activity [Topolska-Wos, 2015]. CacyBP/SIP during cell differentiation process translocates to the nucleus where it may influence the Elk-1 transcription factor activity through ERK1/2 [Kilanczyk, 2009; Schneider and Filipek, 2011]. Hence, in the present work we examine influence of this protein on the ERK1/2-CREB-BDNF pathway using mouse neuroblastoma NB2a cells which exhibit a differentiation potential. We have found that in undifferentiated and differentiated NB2a cells, the phosphatase activity of CacyBP/SIP differs. CacyBP/SIP mutants overexpression: K25A/R26A and E217K in undifferentiated cells does not influence ERK1/2 and CREB activity, however in differentiated cells these mutants cause an opposite effect. The level of mRNA BDNF, CREB target gene, changes in the same way as the activity of ERK1/2 and CREB. Two-dimensional electrophoresis has revealed an additional form of CacyBP/SIP in differentiated cells, which may suggests that CacyBP/SIP undergoes some post-translational modifications and might influence its phosphatase activity.

Identification of CD14-associated proteins involved in TLR4 signaling

Presenter: Paula Roszczenko

Nencki Institute of Experimental Biology, Department of Cell Biology, Laboratory of Molecular Membrane Biology

Paula Roszczenko, Dembinska Justyna, Kwiatkowska Katarzyna; Nencki Institute of Experimental Biology, Department of Cell Biology, Laboratory of Molecular Membrane Biology

Lipopolysaccharide (LPS), the component of Gram-negative bacteria, induces production of pro-inflammatory cytokines via TLR4 signaling pathways. LPS initiates this signaling by recruitment and activation of proteins in lipid rafts. CD14, one of raft-associated protein, binds LPS, transfers it to the TLR4/MD-2 complex and controls activation of TLR4-mediated signaling. Thus, we decided to identify proteins associated with CD14 in LPS-stimulated RAW264.7 cells by co-immunoprecipitation. The identification of proteins associated with CD14 was performed by mass spectrometry. This method revealed 29 proteins co-immunoprecipitated with CD14; there were several differences between protein profiles found in non-stimulated and stimulated cells, including the presence of proteins involved in the down-regulation of oxidative stress. Subsequently, to check if the identified proteins might be palmitoylated, the obtained data were compared with the palmitoylation-specific proteomic studies in RAW 264.7 with application of "click chemistry". Some proteins involved in the reduction of oxidative stress are palmitoylated. A further verification of collected results will be the next step in revealing unknown components in TLR4 signaling pathways.

Characterization of novel antimicrobial targets for antisense treatment in Gram-negative bacteria

Presenter: Marcin Równicki

1) College of Inter-Faculty Individual Studies in Mathematics and Natural Sciences, University of Warsaw, Żwirki i Wigury 93, 02-089 Warsaw; 2) Centre of New Technologies, S. Banacha 2c, 02-097 Warsaw

Marcin Równicki, Jakub Czarnecki - Institute of Microbiology, Faculty of Biology, University of Warsaw, Miecznikowa 1, 02-096 Warsaw;. Dariusz Bartosik-Institute of Microbiology, Faculty of Biology, University of Warsaw, Miecznikowa 1, 02-096 Warsaw;. Joanna Trylska - Centre of New Technologies, S. Banacha 2c, 02-097 Warsaw.

Gram-negative bacteria are the cause of serious nosocomial infections with a high incidence of multidrug resistance and mortality. The slow process of production of new antibiotics and the emergence and spread of multi-drug resistant Gram-negative strains limits the therapeutic options, and makes it necessary to develop new methods in the fight against multi-drug resistant bacteria. One promising strategy could be the use of antisense oligonucleotides. In this study peptide nucleic acid oligomers conjugated to a cell penetrating peptide (KFF)3K have been designed to target mRNA transcripts encoding proteins essential for bacterial growth: 1) dnaA and 2) dnaG, both involved in replication process. As a control we also target the transcript of the rfp (red fluorescein protein) reporter gene to evaluate the potential for antisense effects. We constructed a broad-host-range reporter vector expressing rfp – plasmid pBBR(rfp). PNA activities will be tested against different strains of Gram-negative bacteria. The results will verify two new potential targets for antisense technology against Gram-negative bacteria, and indicate if PNAs conjugated to the (KFF)3K peptide are active against Gram-negative strains in vitro.

Interleukin 1 beta gene variability impact on etiology of recurrent aphthous stomatitis

Presenter: Marta Rozmiarek

Dept. of Nucleic Acids Function, Institute of Human Genetics, PAS, Poznan, Poland

Marta Rozmiarek, Zuzanna Ślebioda Dept. of Oral Mucosa Diseases, University of Medical Sciences, Poznan, Poland, Ewa Krawiecka Dept. of Oral Mucosa Diseases, University of Medical Sciences, Poznan, Poland, Elżbieta Szponar Dept. of Oral Mucosa Diseases, University of Medical Sciences, Poznan, Poland , Anna Kowalska Dept. of Nucleic Acids Function, Institute of Human Genetics, PAS, Poznan, Poland

Recurrent aphthous stomatitis (RAS) belongs to the group of inflammatory, ulcerative diseases of the oral mucosa. A variability of human genome may influence the individual susceptibility to RAS. Alterations in the genes of major pro-inflammatory cytokines seem to be very important. The results of the Interleukin-I-β (IL-1β) gene variability analysis in several European and non-European populations are inconsistent. Therefore, we decided to perform genotyping of IL-1β gene in a Polish cohort of patients. The aim of our study is to estimate a distribution of the two following DNA polymorphisms: IL-1β -511 and +3954 among 81 patients with RAS and 52 control individuals (without RAS). The genotyping is carried out with the use of genomic DNA isolated from blood samples and PCR-RFLP approach according to a previous reported method. Results will be evaluated statistically to indicate possible significant differences between the two analyzed groups. As the etiopathogenesis of RAS has not been clearly defined, the treatment is mainly symptomatic and not very effective. Thus, discovering genetic defects of RAS may be helpful in both a risk prediction of the disease and an effective, causative therapy.

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H. pylori mediated immunomodulation of NK cell activity

Presenter: Karolina Rudnicka

Department of Immunology and Infectious Biology, Faculty of Biology and Environmental Protection, University of Lodz, Poland

Rudnicka K., Matusiak A., Miszczyk E., Walencka M., Chmiela M.

The activity of immune cells including NKs infiltrating gastric mucosa during H.pylori infection depends on their ability to effectively react to the bacterial antigens. We evaluated the impact of H.pylori surface glycine acid extract antigens (GE) and the LeXY LPS on the cytotoxic activity, expansion, phenotype and cytokine activity of lymphocytes from H.pylori infected-Hp+ and uninfected donors-Hp-. The cytotoxic activity of peripheral blood lymphocytes was evaluated towards HeLa cells by MTT reduction assay and the granzme B and caspase-8 ELISA. We showed that NK cells of Hp+ individuals have a weaker cytotoxic activity and that this was associated with the domination of CD56bright phenotype as well as higher serum IL-10 concentrations than Hp- donors. The GE antigens stimulated cytotoxic activity and expansion of CD3-CD56+CD25+ cells producing IL-2 and IFN-γ. Weak cytotoxic activity of lymphocytes in the milieu of H.pylori LPS was associated with the lack of CD3-CD56+CD25+ expansion and decrease in IL-2 and IFN-γ synthesis as well as the propagation of IL-10+ NK cells. The domination of H.pylori antigens with downregulating activity towards NK cells may facilitate the persistence of infection.

Accumulation of mRNA in Cajal bodies

Presenter: Magda Rudzka

Nicolaus Copernicus University in Torun, Faculty of Biology and Environment Protection, Department of Cell Biology

Magda Rudzka, Agnieszka Kołowerzo-Lubnau Nicolaus Copernicus University in Torun, Faculty of Biology and Environment Protection, Department of Cell Biology, Dariusz Jan Smoliński Nicolaus Copernicus University in Torun, Faculty of Biology and Environment Protection, Department of Cell Biology

Cajal Body (CB) are evolutionarily conserved structures. Their role is associated with metabolism of various types RNA. However, accumulation of poly(A) RNA in CB, including mRNA encoding protein is phenomenon described only in plant cells. Unique composition CB of larch microsporocytes, it appears that these structures have additional, not yet fully explained functions related to the metabolism mRNA. This process usually occurs after a period of high transcriptional activity precedes the appearance the mRNA in cytoplasm. We performed a detailed analysis of this process concerning the distribution and level of Sm proteins and mRNAs encoding Sm proteins. Initial period is characterized by a high level of transcription and high level of the Sm mRNA in nucleoplasm. Then, after decrease decline the transcriptional activity, the Sm mRNAs accumulate in the CB, and next are transported into cytoplasm where they are translated. During this period in cytoplasm there are many small structures that contain Sm proteins, which often colocalized or are in close proximity to accumulation of mRNAs. Then Sm proteins are transported to nucleus before they are able to perform its splicing function.
This research was supported by grant NN 303799640.

ELECTROSPUN POLY-ϵ-CAPROLACTONE NANOFIBERS AS CELL SCAFFOLDS

Presenter: Marek Rychter

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Extracellular matrix (ECM) is responsible for providing a structural support to the surrounding cells. The nano and microscale fibers produced by electrospinning are in the size range of natural ECM, therefore they can form materials which may be used as cell scaffolds. In this study, poly-ϵ-caprolactone (PCL) nanofibers were electrospun with varying applied voltage and PCL concentration. The diameter distribution analysis based on SEM images indicated the optimal applied voltage in the range of 8 to 10 kV. The nanofibers electrospun from 10% (wt) PCL solutions showed uniform size distribution. No significant differences were observed in the Young modulus of electrospun fibers. The obtained values for nanofibers were in the range characteristic for collagen fibrils in ECM. To visualize nanofiber influence on the fibroblasts (MSU-1.1) growth, nuclei and cytoskeleton were labelled and observed under confocal microscope. The nanofibers revealed significant influence on cell adhesion. Cells incubated with PCL nanofibers were attached and dispersed along the fibers. The PCL nanofibers due to their morphology and mechanical properties can be a promising material for tissue engineering as three-dimensional (3D) scaffolds.

All they need is space: how to snake effectively through protein matrix?

Presenter: Jakub Rydzewski

Institute of Physics, Faculty of Physics, Astronomy and Informatics, Nicolaus Copernicus University, Grudziadzka 5, 87-100 Torun, Poland

Jakub Rydzewski, Wieslaw Nowak, Institute of Physics, Faculty of Physics, Astronomy and Informatics, Nicolaus Copernicus University, Grudziadzka 5, 87-100 Torun, Poland

Ligand travel through a protein interior is a fundamental process governing biological signaling and enzymatic catalysis. At a single molecule level, this process is hard to study experimentally. Moreover, even in standard molecular dynamics simulations, a complex topology of channels in proteins leads often to difficulties in modeling ligand escape pathways. We have developed two novel memetic numerical methods for searching the exit paths and cavity space exploration: Memory Enhanced Random Acceleration (MERA) Molecular Dynamics and Immune Algorithm (IA). In MERA, a pheromone concept is introduced to optimize an expulsion force. In IA, hybrid learning protocols are exploited to predict ligand exit paths. The new algorithms are compared with Random Acceleration Molecular Dynamics on model protein systems with increasingly buried binding sites. The proposed algorithms are general and appropriate in all problems where an accelerated transport of an object through a network of channels is studied.

The Effect of Matrix Metalloproteinases on Presynaptic Vesicle Recycling

Presenter: Ahmad Salamian

Laboratory of Neurobiology, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland

Ahmad Salamian, Martin Heine (Molecular Physiology Group, Leibniz Institute for Neurobiology, Magdeburg, Germany); Leszek Kaczmarek (Laboratory of Neurobiology, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland)

Neuronal synapses are maintained by a complex network of adhesion molecules which form a tight association between pre- and postsynaptic elements. Matrix metalloproteinases (MMPs) are endopeptidases that play an essential role in the dynamic remodeling of extracellular matrix. They are known to be involved in the cleavage of numerous extracellular substrates, such as cell surface receptors and cell adhesion molecules. It was indicated that synaptic activity-dependent proteolytic cleavage of postsynaptic adhesion molecule neuroligin-1 mediated by MMP-9 causes rapid destabilization of its presynaptic partner neurexin-1β. Hence, the hypothesis of our study is whether MMP-9 or other extracellular endopeptidases might have an effect on presynaptic function under synaptic plasticity. The primarily results from uptake of synaptotagmin antibody against its luminal domain indicate significant decrease of activity-dependent vesicle recycling efficiency under treatment with MMPs inhibitors in mature cortical neurons. Additionally, it seems that the efficiency of synaptotagmin uptake is decreased under chemically induced long term potentiation (cLTP) in cells which are pretreated with broad-spectrum MMPs inhibitor GM6001.

4D NMR for RNAs

Presenter: Saurabh Saxena

Biological and Chemical Research Centre, Faculty of Chemistry, University of Warsaw

Saurabh Saxena, Jan Stanek, Wiktor Koźmiński - Biological and Chemical Research Center, Faculty of Chemistry, University of Warsaw, Pasteura1, 02093, Warsaw, Poland;; Mirko Cevec, Janez Plavec - Slovenian NMR Centre, National Institute of Chemistry, Hajdrihova ulica 19, 1000 Ljubljana, Slovenia

With the advent of several new classes of non-coding RNAs (e.g. siRNA, miRNAs) research has been heavily focused on the understanding the role of RNA in cellular processes during normal and diseased states through exploring its structure-function relationship. Over the years, several NMR techniques proved to be highly useful in expanding our knowledge about RNA structure, its basic structural motifs, catalysis and interactions with small molecules/proteins. However, precise structural determination of even moderate size RNAs is still problematic. In addition to low proton density in RNAs, biopolymers made out of only four different nucleotides, chemical-shift dispersion in NMR spectra is also significantly less; which causes severe spectral overlaps even in 3D NMR spectra. To alleviate these difficulties we have developed 4D NMR experiments where chemical shifts are resolved along four dimensions providing hig-resolution and reduced spectral overlap. The performance of the experiments is tested and shown for two RNAs (i) a 34-nt hairpin RNA consisting of two A-RNA form stems, one adenine bulge, an asymmetric internal loop and a GAAA terminal loop; (ii) a 14-nt RNA hairpin capped by cUUCGg tetraloop.

PHOSPHATE2 gene: in the hub of phosphate homeostasis in barley

Presenter: Paweł Sega

Department of Gene Expression, Institute of Molecular Biology and Biotechnology , Adam Mickiewicz University Poznan

Paweł Sega, Katarzyna Kruszka, Zofia Szweykowska-Kulińska, Andrzej Pacak

Phosphate (Pi) level, which is underlying for the structural and metabolic needs of plants, is controlled and maintained by the regulatory network of Pi signaling and homeostasis. The regulation of the phosphate content in plants requires activity of such proteins as PHO1 (PHOSPHATE1), implicated in Pi loading to the xylem, PHO2 (PHOSPHATE2), Pi transporters, several transcription factors and small RNAs, like miRNA399 and miRNA827. The PHO2 gene appears to be an essential player involved in the maintaining of phosphate homeostasis and encodes for a putative ubiquitin-conjugating (UBC) E2 enzyme responsible for the degradation of PHO1 and PHT1 (PHOSPHATE TRANSPORTER 1) proteins. Thereby, the PHO2 protein modulates the acquisition and root-to-shoot translocation of Pi and protects against the phosphate overbalance toxicity. Our present studies show that there are other factors with an impact on the PHO2 expression level and subsequent adapting changes. We found that heat stress downregulates PHO2 expression and increases Pi content in shoots. Further investigations may provide identification of novel phosphate-related regulators, PHO2-interacting proteins and increased understanding of the phosphate homeostasis mechanism.

The molecular and cellular basis of progenitor cell-cell contact formation in zebrafish gastrulation

Presenter: Mateusz Sikora

IST Austria

Mateusz Sikora, M. Sikora (IST Austria, 3400 Klosterneuburg, Austria), J. Slovakova (IST Austria, 3400 Klosterneuburg, Austria), B.A. Truong Quang (Institut de Biologie du Développement de Marseille, 13288 Marseille Cedex 9 – France), M. Cieplak (Institute of Physics, Polish Academy of Sciences, Al Lotnikow 32/46 02-668 Warsaw, Poland) , P.F. Lenne (Institut de Biologie du Développement de Marseille, 13288 Marseille Cedex 9 – France),Carl-Philipp Heisenberg(IST Austria, 3400 Klosterneuburg, Austria)

Gastrulation is the first major morphogenetic process in vertebrate development, where different germ layer progenitor cell types sort from each other and assemble into distinct embryonic germ layers. These progenitor cell types form cell-cell contacts of different size and strength, driving progenitor cell segregation and germ layer formation. Cadherin-mediated cell adhesion functions in progenitor cell-cell contact formation by mechanically coupling the contractile actomyosin cortices of adhering cells at the contact zone via adhesion complex proteins. Mechanosensing properties of adhesion molecules have been suggested, where force-induced changes would enhance their ability to bind Actin depending on cell cortical tension. We show, that adhesion comples proteins change their subcellular distribution from an initial diffuse localization at the cell-cell contact to a distinct ring-like localization in a cortical tension-dependent manner during progenitor cell-cell contact maturation. Our observations also suggest, that α-catenin and Vinculin play a critical role in the processes by which cortical tension controls the mechanical coupling strength of cadherin-mediated adhesion during progenitor cell-cell contact formation.

Biosynthesis of silver nanoparticles by Actinomycetes

Presenter: Marek Składanowski

University of Nicolaus Copernicus, Department of Microbiology

Marek Składanowski, Patrycja Golińska, Hanna Dahm, University of Nicolaus Copernicus, Department of Microbiology

The problem of microbial antibiotic resistance is one of the major challenges of modern medicine. The main reason of spreading the problem is the excessive and inappropriate use of antibiotics, which accelerates the emergence and spread of antibiotic-resistant bacteria. The most important seems to be searching for solutions that will not be temporary but long-lasting and effective. Recent research shows that bacteria witch are resistant to antibiotics aren't resistant to silver nanoparticles. They owe their antimicrobial property due to possession of a large surface area to volume ratio, and unique chemical and physical properties. The nanoparticles act on the bacterial cells of both extra- and intracellularly. Moreover silver nanoparticles synthesized by micro-organisms, in contrast to those from chemical synthesis, are easy to use and environmentally friendly. The aim of this project was to search strains of actinomycetes colonizing so far low explored acidic forest soils, which are able to carry out the synthesis of nanoparticles of silver, and to demonstrate antibacterial properties of these nanoparticles.

Structural studies of human mRNA cap methylation

Presenter: Mirosław Śmietański

International Institute of Molecular and Cell Biology / Laboratory of Protein Structure

Mirosław Śmietański, Maria Werner, International Institute of Molecular and Cell Biology / Laboratory of Bioinformatics and Protein Engineering

The 5' cap of human messenger RNA contains 2'-O-methylation of the first and often second transcribed nucleotides that are important for its processing, translation, and stability. Human enzymes that methylate the first and second nucleotides of the transcript, termed CMTr1 and CMTr2, respectively, have been recently identified. However, the structures of these enzymes and their mechanisms of action remain unknown. In the present study, we solved the crystal structures of the active isolated CMTr1 catalytic domain in complex with a methyl group donor and capped oligoribonucleotide. The structures revealed the mechanism of the specific recognition of capped RNA. This mechanism significantly differs from previously characterized viral enzymes, thus providing a framework for their specific targeting. Based on the crystal structure of CMTr1, a comparative model of the CMTr2 catalytic domain was generated. This model, together with the mutational analysis, led to the identification of residues involved in RNA and methyl group donor binding.

CpG methylation within the Epidermal Differentiation Complex (EDC) during epidermal differentiation

Presenter: Barbara Sobiak

Laboratory of Calcium Binding Proteins, Nencki Institute of Experimental Biology, Polish Academy of Science; 3 Pasteur Street, 02-093 Warsaw, Poland

Barbara Sobiak, Agnieszka Graczyk, Wiesława Leśniak

Considerable gene expression changes occur when transiently amplifying basal keratinocytes differentiate to form the superficial epidermal barrier, separating the organism from environment. Many of genes involved in terminal differentiation of epidermis are located on chr 1q21 and constitute epidermal differentiation complex (EDC). It encodes 4 protein families: the S100, the small proline rich (SPRRs), the late cornified envelope (LCE) and the S100-fused type (SFTPs). To check whether epigenetic factors may govern the EDC gene expression we examined CpG methylation in selected EDC regions during differentiation of primary human epidermal keratinocytes (HEKa cells). The analysis encompassed promoter/coding regions of nine EDC genes and seventeen potential cis-regulatory regions characterized by high density of predicted transcription factor binding sites (TFBS). The methods employed were: bisulfite DNA conversion, conventional sequencing and targeted next-generation sequencing. Only single CpGs revealed substantial changes in CpG methylation and were found to overlap with potential E2F1 TFBS. Other EDC fragments are to be investigated to identify differentially methylated regions (DMRs) of potential regulatory significance.

Study of substrate specificity of Nudt12 enzyme towards dinucleotide analogs of cap structure.

Presenter: Joanna Stelmach

Division of Biophysics, Institute of Experimental Physics, Faculty of Physics, University of Warsaw, Warsaw 02-089, Poland

Joanna Stelmach, Edward Darzynkiewicz Division of Biophysics, Institute of Experimental Physics, Faculty of Physics, University of Warsaw; Megerditch Kiledjian Department of Cell Biology and Neuroscience, Rutgers University; Maciej Lukaszewicz Division of Biophysics, Institute of Experimental Physics, Faculty of Physics, University of Warsaw

Cap is an essential structure involved in synthesis, translation and degradation of eukaryotic mRNA, situated at its 5' end. The structure consists of 7-methylguanosine linked by 5'-5' triphosphate bridge to the first transcribed nucleotide. This bridge is a target for the decapping enzymes, such as Dcp2 and Nudt16, members of NUDIX hydrolase family. Mammalian protein Nudt12 is also a member of the NUDIX family and shows mRNA decapping activity in vitro. The hydrolase properties of Nudt12 were previously shown towards methylated (m7GpppG) and unmethylated (GpppG) dinucleotides (1), the activity specific for DcpS protein – other well studied enzyme involved in cap structure degradation. Here we present the results of our investigation of substrate specificity of Nudt12 towards a library of unmethylated (GpppG, GpppA, ApppA), monomethylated (m7GpppG, m7GpppA) and trimethylated (m32,2,7GpppG, m32,2,7GpppA) dinucleotide cap analogs. We found that Nudt12 preferentially hydrolase substrates consisting of one adenosine and one guanosine, with GpppA being the best one. (1) Song et.al., RNA. 2013 Acknowledgments. This work was supported by National Science Centre grant UMO-2013/08/A/NZ1/00866 (Poland)

The matrix vesicle-mediated mineralization depends on a balance between annexins and fetuin-A

Presenter: Agnieszka Strzelecka-Kiliszek

Department of Biochemistry, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland

Agnieszka Strzelecka-Kiliszek, Lukasz Bożycki, Monika Roszkowska, Slawomir Pikula; Department of Biochemistry, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland

Bone mineralization is initiated by matrix vesicles (MVs), cell-derived structures located within the extracellular matrix (ECM) which are nucleation sites for hydroxyapatite (HA) formation. It is suggested that annexins are mineral-stimulating membrane proteins which exhibit ion channel activity for influx of Ca2+ into MVs. The process is also regulated via enzymatic degradation of inhibitory pyrophosphate by tissue-nonspecific alkaline phosphatase [TNAP]. Another layer of control is exerted by circulating, mineral-inhibiting protein fetuin-A. Human hFOB1.19 osteoblasts and Saos-2 osteosarcoma were used and the mineralization was stimulated by AA/B-GP treatment. We compared vesicularization of cytoplasm, intracellular distribution of proteins and HA formation in control to levamisole- or K-201-treated cells. We isolated MVs from these cells by collagenase digestion and determined TNAP activity. We observed differences in annexins and fetuin-A profile in MVs from resting versus stimulated cell lines. The understanding of annexins and fetuin-A role in MVs function may provide novel insights on mechanisms of physiologic mineralization and may help to create therapeutic strategies to prevent pathologic mineralization.

Is SASP regulated by the proteins involved in senescence induction?

Presenter: Anna Strzeszewska

Nencki Institute of Experimental Biology, PAS

Anna Strzeszewska, Olga Alster Nencki Institute of Experimental Biology, Grażyna Mosieniak Nencki Institute of Experimental Biology, Ewa Sikora Nencki Institute of Experimental Biology

The p53 protein is believed to be crucial for the induction of senescence, which is an irreversible growth arrest caused i.a. by DNA damage. Nevertheless, we observed that HCT116 p53-/- cells undergo senescence upon treatment with genotoxic drug - doxorubicin. One of the hallmarks of senescent cells is so called Senescence-Associated Secretory Phenotype (SASP). We observed SASP (secretion of IL-8 and VEGF) in HCT116 p53-/- cells. Phosphorylation of NFκB p65 protein, which is commonly believed to cause the inflammatory response, was increased upon doxorubicin treatment. Silencing of p65 with siRNA caused a decrease in the level of secreted IL-8, but interestingly it did not affect other markers of senescence, such as SA-β-Galactosidase activity and the level of the cell cycle inhibitor, p21. In contrast, downregulation of CHK2, which is one of the DNA damage response proteins, resulted in increased level of secreted IL-8 and VEGF, however it also did not influence other markers of senescence. These results show that SASP can be regulated independently of other markers of senescence and it does not have a causative role in this process. Supported by grant UMO2011/01/M/NZ1/01597.

Importance of AtUPF1 phosphorylation in plant NMD

Presenter: Aleksandra Sulkowska

University of Warsaw Faculty of Biology Institute of Genetics and Biotechnology

Aleksandra Sulkowska, Paweł J. Sikorski, Izabela Wawer, Joanna Kufel University of Warsaw Faculty of Biology Institute of Genetics and Biotechnology

Nonsense-Mediated mRNA Decay (NMD) is an evolutionary conserved process related to the control of gene expression. This mechanism prevents the production of potentially harmful proteins by eliminating aberrant mRNA carrying premature termination codons. Phosphorylation of the key factor UPF1 is a critical step for NMD in mammals and C. elegans. Phospho-UPF1 is bound by SMG5-7/SMG6 proteins, which trigger mRNA decay and dephosphorylation of UPF1. Despite intense research in recent years, the understanding of NMD mechanisms in plants is still incomplete. We have shown previously that the N- and C-terminal domains of AtUPF1 are phosphorylated, act redundantly during NMD and form the binding platform for AtSMG7. Notably, three of the phosphorylated residues in the N-terminal domain of AtUPF1 were demonstrated to be important for NMD competence. To clarify the role of AtUPF1 phosphorylation in plant NMD, we have analyzed co-localization of specific AtUPF1 and AtSMG7 mutant variants in Arabidopsis protoplasts using confocal microscopy. We have also performed fluorescence resonance energy transfer–fluorescence lifetime imaging assays to investigate the importance of identified AtUPF1 phosphorylation sites for the interaction with AtSMG7.

Dysferlin gene-related dystrophies:genetic, clinical, and biochemical diversity

Presenter: Małgorzata Suszek

Department of Biochemistry, Nencki Institute of Experimental Biology, Warsaw, Poland

Małgorzata Suszek, Jakub Fichna2, Anna Macias3, Cezary Żekanowski2, Anna Kamińska3, Maria Jolanta Rędowicz1; 1Department of Biochemistry, Nencki Institute of Experimental Biology, Warsaw, Poland; 2Department of Neurodegenerative Disorders, Mossakowski Medical Research Centre, Warsaw, Poland; 3Department of Neurology, Medical University of Warsaw, Warsaw, Poland

Limb girdle-muscular dystrophies (LGMD) are very heterogeneous myopathies with autosomal recessive inheritance and progressive weakness and wasting of pelvic and shoulder girdle muscles. LGMD2B subtype is due to mutations in DYSF leading to the absence or decreased level of dysferlin, a transmembrane protein involved in skeletal muscle repair. Besides a transmembrane domain (TM), dysferlin has seven C2 domains (Ca2+-dependent lipid binding), three Fer domains (unknown function) and a Pex24p domain (peroxisomal). DNA from several LGMD patients was sequenced. Causative mutations were found in four patients: 54/01 and 14/05 (siblings) - Q1341E and E1786R (interdomain); 98/10 - I834V (FerB), R1022Q (Pex24p) and R1331L (interdomain), and 107/11 - E1727D (interdomain) and D1876N (last C2). Muscle biopsies were analyzed by immunoblot and immunohistochemistry. Western blot revealed that in patients 54/01, 14/05 and 107/11 there was no detectable amount of dysferlin but in patient 98/10 its level was elevated. These observations were confirmed by immunostaining. The data indicate that severity of dysferlinopathy could depend among others on the number and localization of mutated residues.

Differential expression and coexpression analysis across multiple tissues in twins.

Presenter: Marcin Świstak

Faculty of Mathematics Informatics and Mechanics, University of Warsaw, Warsaw, Poland

Marcin Świstak, Alessia Visconti, Mario Falchi, Veronique Bataille, Tim D. Spector, Department of Twin Research and Genetic Epidemiology, King's College London, London, UK

While most studies on gene expression focus on single tissues and compare data gathered from different subjects, complexity of tissue-specificity still remains elusive. We explore and compare microarray expression data from three tissues: lymphoblastoid cell lines (LCL), skin, and fat. The samples (571 LCL, 478 skin, 569 fat) have been obtained from well-phenotyped healthy female twins (TwinsUK cohort) of the MuTHER resource. It is the first time that the expression data from skin is available. We have analyzed differential expression using limma package finding sets of differentially expressed genes across tissues. Furthermore, we have performed differential coexpression analysis using WGCNA package on each of the datasets. Distinguished modules have been correlated with extensive phenotypic traits showing significant associations. Within the modules functional gene clusters and groups of genes from the same families (eg. keratins) have been found.

Prognostic significance of genetic factors in pediatric T-cell acute lymphoblastic leukemia

Presenter: Bronisława Szarzyńska-Zawadzka

Department of Molecular and Clinical Genetics, Institute of Human Genetics PAS

Bronisława Szarzyńska-Zawadzka, Małgorzata Dawidowska1, Maria Kosmalska1, Łukasz Sędek2, Tomasz Szczepański2, Michał Witt1,3 and members of the Polish Pediatric Leukemia Lymphoma Study Group (PPLLSG) 1)Department of Molecular and Clinical Genetics, Institute of Human Genetics, Polish Academy of Sciences, Poznań, Poland 2) Department of Pediatric Hematology and Oncology, Medical University of Silesia, Zabrze, Poland 3) International Institute of Molecular and Cell Biology, Warsaw, Poland.

T-cell acute lymphoblastic leukemia (T-ALL) - a rare subtype of ALL, remains a challenge in pediatric oncology because risk stratification relies solely on the response to treatment and patients with relapsed T-ALL face a dismal prognosis. Thus, special emphasis is placed on the identification of prognostic factors that allow to determine the individual risk of recurrence and adjust treatment regimens. The project aim is to evaluate prognostic significance of gene mutations in pediatric T-ALL. High Resolution Melting Analysis (HRMA) and Sanger sequencing are used to assess mutation frequency of NOTCH1 (exons 26, 27, 28, 34), FBXW7 (exons 9, 10), FLT3 (exons 11,12), PTEN (exons 5, 6, 7, 8), WT1 (exons 7, 9), IL7R (exon 6), RUNX1 (exons 3, 4, 5, 8), and DNMT3A (exons 8-23). Currently the research is in progress. After collecting all molecular data mutational status will be correlated with the minimal residual disease (MRD) and clinical data. The project will contribute to a more precise molecular definition of T-ALL subgroups and to the identification of the prognostic relevance of genetic changes. The results may facilitate risk stratification and impact on the intensity of conventional chemotherapy. NCN (2013/11/N/NZ5/03730)

AGGRESCAN3D (A3D): server for prediction of aggregation properties of protein structures

Presenter: Agata Szczasiuk

University of Warsaw, Faculty of Chemistry, Pasteura 1, Warsaw, Poland

Agata Szczasiuk, Rafael Zambrano1; Michal Jamroz2; Agata Szczasiuk2; Jordi Pujols1; Sebastian Kmiecik2; and Salvador Ventura1; 1 - Institut de Biotecnologia i Biomedicina and Departament de Bioquímica i Biologia Molecular, Universitat Autònoma de Barcelona, Bellaterra, 08193, Spain; 2 - University of Warsaw, Faculty of Chemistry, Pasteura 1, Warsaw, Poland

Protein aggregation underlies an increasing number of disorders and constitutes a major bottleneck in the development of therapeutic proteins. There are many predictive algorithms to identify aggregation-prone sites. A majority of these methods rely only on sequence. Therefore, they find difficulties to predict the aggregation properties of folded globular proteins, where aggregation-prone sites are often not contiguous in sequence or buried inside the native structure. The AGGRESCAN3D (A3D) server overcomes these limitations by taking into account the protein structure and the experimental aggregation propensity scale from the well-established AGGRESCAN method. Using the A3D server, the identified aggregation-prone residues can be virtually mutated to design variants with increased solubility, or to test the impact of pathogenic mutations. Additionally, A3D server enables to take into account the dynamic fluctuations of protein structure in solution, which may influence aggregation propensity. This is possible in A3D Dynamic Mode that exploits the CABS-flex approach for the fast simulations of flexibility of globular proteins. The A3D server can be accessed at http://biocomp.chem.uw.edu.pl/A3D/

Discovering the role of non-coding RNAs in regulation of bacterial virulence.

Presenter: Krzysztof Szczepaniak

International Institute of Molecular and Cell Biology in Warsaw, Laboratory of Bioinformatics and Protein Engineering, 4 Ks. Trojdena Street, 02-109 Warsaw, Poland

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Pathogenic bacteria developed many mechanisms facilitating their survival in the host environment. Many of those mechanisms involve regulation of virulence genes expression. Recent studies indicated that small non-coding RNAs (ncRNAs) can serve as such regulators. This study aimed at better understanding of mechanisms of this process. First, we collected data regarding proteins involved in bacterial pathogenesis and predicted operons encompassing them. 5'UTRs of all these operons were scanned for the presence of putative ncRNA motifs. All motifs were collected into a publicly available database. Then, we searched our database for novel ncRNA motifs that were not annotated by the existing Rfam families, but were characterized by secondary structure conservation and were present in similar genomic context across the operons. Finally, ten candidates were selected for further studies. We are running biochemical experiments to confirm secondary structure and to discover potentially interacting proteins. With this data we hope to formulate hypothesis about the role of those motifs in the bacterial virulence regulation.

Ncd:EB1:microtubule complex during EB1-dependent microtubule plus-end tracking by kinesin-14 Ncd

Presenter: Ewa Szczęsna

Nencki Institute of Experimental Biology

Ewa Szczęsna, Ewa Szczęsna, Andrzej A. Kasprzak

Ncd is a kinesin-14 which crosslinks and slides microtubules (MTs) during mitotic spindle formation. Ncd is located at the plus ends of growing spindle MTs due to the interaction with EB1, a protein which specifically binds and follows the ends of polymerizing MTs in a process called "plus-end tracking". In order to elucidate the mechanism of MT plus-end tracking by Ncd, we measured Ncd-EB1 affinity in the solution: Kd was 9 μM. We also reconstituted MT dynamics using purified and fluorescently labeled proteins and observed it under the TIRF microscope. We analyzed the behavior of Ncd and EB1 on dynamic MTs. We constructed 2 Ncd mutants with lowered affinity to MTs and one which did not bind to MT. Next, we measured the efficiency of the tracking for all the mutants as a function of increasing EB1 concentration. We observed an inverse correlation between the affinity of the Ncd mutant to MT and the concentration of EB1 required to obtain high tracking efficiency. The dwell time of single Ncd molecules at the MT end was much longer than that for EB1. The dwell time of EB1 molecules also increased in the presence of Ncd tail. These results suggest that Ncd, EB1 and MT form a ternary complex crucial for the plus-end tracking.

Lipid peroxidation products in etiology of Fanconi anemia

Presenter: Ewelina Maria Szmajda

Institute of Biochemistry and Biophysics PAS

Ewelina Maria Szmajda, Konrad Kosicki1,2, Barbara Tudek1,2, Elżbieta Speina1; 1 Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland, 2 Institute of Genetics and Biotechnology, University of Warsaw, Warsaw, Poland

Fanconi anemia (FA) is a genetic disorder characterized by bone marrow failure. Cells derived from patients are genetically instable and hypersensitive to DNA cross-linking agents. Disease arises through mutations in FA pathway genes which cooperate in genome stability maintenance. FA proteins are involved in removal of DNA cross-links (ICLs) but it is still unknown if ICLs are generated endogenously. The aim of the study was to determine the role of lipid peroxidation (LPO) products in etiology of the FA phenotype. Our investigation showed that fibroblasts derived from FA patient (FANCD2-/-) were hypersensitive to three major LPO end aldehydes, 4-hydroksynonenal (HNE), croton aldehyde (CRO) and acrolein (ACR). Next, we examined the transduction of the DNA damage response signals. We observed higher amount of double strand breaks (DSB) in mutant cells which was accompanied by increased phosphorylation of repair kinases such as ATM, ATR and Chk2. Cell cycle analysis revealed accumulation of cells in G2 phase. To research DNA replication, we measured lengths of DNA tracks. Results demonstrated decreased velocity of replication and increased number of stalled replication forks in both cell lines after treatment with HNE, CRO and ACR.

Cytotoxicity of N-isopropylacrylamide nanoparticles; potential application as a bioscaffold.

Presenter: Tobiasz

NanoBioMedicalCenter, AdamMickiewicz University

Tobiasz, Alicja Warowicka NanoBioMedical Center, Adam Mickiewicz University; Anna Woźniak NanoBioMedical Center, Adam Mickiewicz University; Mikołaj Grzeszkowiak NanoBioMedical Center, Adam Mickiewicz University; Division of Macromolecular Physics, Faculty of Physics, Adam Mickiewicz University Maciej Jarzębski NanoBioMedical Center, Adam Mickiewicz University; Molecular Biophysics Division, Adam Mickiewicz University, Faculty of Physics Magdalena Bednarowicz NanoBioMedical Center, Adam Mickiewicz University Adam Patkowski NanoBioMedical Center, Adam Mickiewicz University; Molecular Biophysics Division, Adam Mickiewicz University, Faculty of Physics Ryszard Słomski NanoBioMedical Center, Adam Mickiewicz University; Department of Biochemistry and Biotechnology, Poznan University of Life Sciences

Polymeric nanoparticles based on poly-N-isopropylacrylamide (pNiPAM NPs) and their biomedical applications have been widely investigated in recent years. These tunable nanoparticles are considered to be great candidates for drug delivery systems, biosensors and bioanalytical devices. Thus, the biocompatibility and toxicity of these nanoparticles is clearly a crucial issue. In this work, the cytotoxicity of thermo-responsive pNiPAM nanoparticles was studied, followed by a detailed analysis of the NPs morphology in growing cell cultures and their 3D structure. Cytotoxic examination was conducted for two cell cultures - HeLa (cervical cancer cell line) and HeK293 (human embryonic kidney cell line), employing MTT (3-4, 5-dimethylthiazol-2-yl-2, 5-diphenyltetrazolium bromide) assay and viability tests. We used Cryo-SEM (scanning electron microscopy) and fluorescence microscopy (IN Cell Analyzer) in order to investigate the morphological structure of the polymer network. We show that pNiPAM nanoparticles do not exhibit any cytotoxicity effects on the investigated cell lines. Additionally, we report that the pNiPAM nanoparticle based scaffold promotes cell growth.

Ligand- and mutation-induced conformational selection in the CCR5 chemokine GPCR

Presenter: Bartosz Trzaskowski

Centre of New Technologies, University of Warsaw

Bartosz Trzaskowski, Ravinder Abrol (Cedars-Sinal Medical Center), William A. Goddard (Caltech)

The C-C chemokine receptor type 5 (CCR5) G protein-coupled receptor (GPCR) is a prime target for preventing HIV invasion. A major difficulty in developing effective therapeutics is that the CCR5 exhibits an ensemble of ~10–20 distinct low-energy conformations, each of which might favor binding to different ligands and/or lead to different downstream functions. X-ray structures generally provide only one of these conformations. We applied the GEnSeMBLE methodology to predict this ensemble, and we designed and carried out 11 experiments to validate the ability of this ensemble to predict binding of an HIV therapeutic to CCR5. We found that each of the mutations changes the binding site. The predicted effects of mutations on binding are in excellent agreement with experiments, providing CCR5 structures for designing new ligands.

NPDock – a web server for protein-nucleic acid docking

Presenter: Irina Tuszyńska

Institute of Informatics, Univeristy of Warsaw

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Protein-RNA and protein-DNA interactions play fundamental roles in many biological processes. A detailed understanding of these interactions requires knowledge about protein-nucleic acid complex structures. Because the experimental determination of these complexes is time-consuming and perhaps futile in some instances, we have focused on computational docking methods starting from the separate structures. Docking methods are widely employed to study protein-protein interactions; however, only a few methods have been made available to model protein-nucleic acid complexes. Here, we describe NPDock; a novel web server for predicting complexes of protein-nucleic acid structures that implements a computational workflow that includes docking, scoring of poses, clustering of the best-scored models, and refinement of the most promising solutions. The NPDock server provides a user-friendly interface and 3D visualization of the results. The smallest set of input data consists of a protein structure and a DNA or RNA structure in PDB format. Advanced options are available to control specific details of the docking process and obtain intermediate results. The web server is available at http://genesilico.pl/NPDock.

Dicer-like gene family in Medicago truncatula, a model legume plant

Presenter: Aleksander Tworak

Institute of Bioorganic Chemistry, PAS

Aleksander Tworak, Aleksander Tworak, Anna Urbanowicz, Jan Podkowiński, Anna Kurzyńska-Kokorniak, Natalia Koralewska, Marek Figlerowicz, Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznań, Poland

Four functional types of Dicer-like ribonucleases (DCL1-4) are encoded in plant genomes. DCL1-type enzymes mainly produce 21 nt miRNAs. The products generated by DCL2-, DCL3-, and DCL4-type ribonucleases belong to various classes of siRNAs that are 22, 24 and 21 nt in length, respectively. By screening the most recent Medicago genome assembly, we identified three new DCL genes in addition to the MtDCL1, 2 and 3 genes characterized in previous studies; the new genes included MtDCL4 and two new MtDCL2 homologs. All six MtDCL genes are constitutively expressed in plant cells and significantly upregulated in root nodules. The first of the newly identified MtDCL2 paralogs encodes for a truncated version of the DCL2 protein, while the second undergoes substantial upregulation in the root nodule. Additionally, we identified an alternative splicing variant of MtDCL1 mRNA, similar to the one identified in Arabidopsis. Our results indicate involvement of DCL genes in the nodule development and functional specialization of DCL2 homologs. In addition, we hypothesize that the alternative splicing variant of MtDCL1 mRNA may be involved in tissue-specific regulation of DCL1 enzyme abundance in various plant species.

Co-crystallization of cyclohexylamine with water and alcohols. Structural and spectroscopic study

Presenter: Piotr Tylkowski

Czochralski Laboratory of Advanced Crystal Engineering, Biological and Chemical Research Centre, Department of Chemistry, University of Warsaw, Żwirki i Wigury 101, 02-089 Warsaw, Poland

Piotr Tylkowski, Łukasz Dobrzycki, Michał Ksawery Cyrański Czochralski Laboratory of Advanced Crystal Engineering, Biological and Chemical Research Centre, Department of Chemistry, University of Warsaw, Żwirki i Wigury 101, 02-089 Warsaw, Poland, Roland Boese Department of Chemistry, University of Duisburg-Essen, 45117 Essen, Germany

Cyclohexylamine is an aliphatic primary amine miscible with water. The aim of my work was to investigate possibility of the amine to crystallize with water to form hydrates and/or hydrate clathrates as well as to check the capability of cyclohexylamine to co-crystallize with various alcohols. At ambient conditions the amine and its mixtures with water are liquids. To obtain crystals suitable for single crystal diffraction, I used in situ crystallization technique. I analyzed 9 co-crystals containing alcohols from methanol to butanol including all isomers. In these co-crystals both the amine and alcohol molecules are engaged in hydrogen bonds forming columns. With water cyclohexylamine forms two hydrates containing 0.5 and approximately 9.4 H2O molecules per one amine. The latter structure is similar to $9\frac{3}{4}$ hydrate of tert-butylamine, however some part of the amine molecules substitute water entities in the H2O framework thus leading to non-stoichiometric composition of the crystal. I established crystal structures of all obtained systems using single crystal diffraction technique. In addition all the crystals were investigated by in situ Raman spectroscopy.

Insights into radial spokes heterogeneity.

Presenter: Paulina Urbanska

Nencki Institute of Experimental Biology PAS

Paulina Urbanska, Urbanska P1, Song K2, Joachimiak E1,5, Krzemien-Ojak L1, Koprowski P1, Hennessey T3, Jerka-Dziadosz M1, Fabczak H1, Gaertig J4, Nicastro D2, Wloga D1. 1) Laboratory of Cell Movement Physiology, Nencki Institute of Experimental Biology PAS, Pasteur 3, 02-093 Warsaw, Poland 2) Department of Biology and Rosenstiel Basic Medical Sciences Research Center, MS029, Brandeis University, 415 South Street, Waltham, MA 02454, USA. 3) Department of Biological Sciences, University at Buffalo, 109 Cooke Hall, Buffalo, NY 14260, USA 4) Department of Cellular Biology, University of Georgia, Athens, GA 30602 5) Department of Animal Physiology, Faculty of Biology, University of Warsaw, Miecznikowa 1, 02-096 Warsaw, Poland

Motile cilia function not only as locomotory organelles in sperm cells and some protist but also enable transport of the extracellular fluids or mucus along the surface of the ciliated epithelia in multicellular organisms. In humans lack or dysfunction of motile cilia results in primary ciliary dyskinesia (PCD). The skeleton of motile cilia is composed of 9 peripheral microtubular doublets, a pair of central microtubules and macro-complexes that are attached to the microtubule surface such as outer and inner dynein arms or radial spokes (RS). Cilia beating is regulated by the mechanochemical signals that are transmitted from central microtubules through the radial spokes to dynein arms. Radial spokes are structurally heterogeneous and appear in triplets of RS1, RS2 and RS3. Structural differences suggest also variation in their protein composition and function. We have shown that in Tetrahymena cells the CSC complex (calmodulin and spokes associated complex) composed of FAP91, FAP251, FAP61 and calmodulin is a specific RS3 docking complex. Moreover, we identified proteins that are likely building blocks of RS3, demonstrating the differences in protein composition between radial spokes.

Overexpression of katanin regulatory subunit leads to inhibition of cytokinesis in Tetrahymena

Presenter: Ewa Wacławek

Nencki Institute of Experimental Biology, Polish Academy of Sciences

Ewa Wacławek, Ewa Joachimiak1,2, Małgorzata Hall3, Dorota Włoga1 1 Laboratory of Cell Movement Physiology, Nencki Institute of Experimental Biology, Polish Academy of Sciences, 3 Pasteur Street, 02-093 Warsaw, Poland 2 Department of Animal Physiology, Faculty of Biology, University of Warsaw, 1, Miecznikowa Street, Warsaw

Katanin, the microtubule severing enzyme generates internal breaks in microtubule lattice and splits it into short fragments. Katanin functions as a heterodimer composed of p60 catalytic subunit and p80 regulatory subunit. The HA-tagged p80 accumulates in the region of basal bodies, contractile vacuoles pores, division furrow and weakly in cilia. Katanin complex is indispensible to complete Tetrahymena cell division. Knockout of either p60 or p80 leads to the formation of multinucleated cells blocked in cytokinesis. Surprisingly, prolonged overexpression of p80 inhibits cell division and phenocopies p60 knockout. Similar phenotype is observed in cells overexpressing N-terminal domain of p80 containing WD40 motifs, indicating that N-terminal fragment of p80 may negatively regulate p60 katanin activity. The inhibitory effect of the prolonged overexpression of p80 on Tetrahymena cell division is enhanced in cells treated with proteasome inhibitor, that maintains the higher level of p80 protein. This suggests that p80 is degraded via proteasomal pathway. Support: financial resources of the MNiSW for science within years 2011-2015 for the implementation of the co-financed project, Maria Curie IRG (277122) and EMBO IG No. 2331.

Asymmetric dimethylarginine as a potential risk factor for ischemic stroke

Presenter: Paulina Werner

1. Laboratory of Physical Biochemistry, Intercollegiate Faculty of Biotechnology UG-MUG

Paulina Werner, Paulina Werner, Grzegorz Gawron, Leszek Kadziński, Bogdan Banecki

Strokes are one of the major causes of death or permanent disability in the world. They are divided into hemorrhagic and ischemic, of which the latter account for the vast majority of all cases. The optimal strategy to reduce the incidence of stroke is to determine the risk factors of its occurrence. The proper functioning of the blood vessels is required the presence of nitric oxide (NO), which has a spasmolytic action on smooth muscle. An important role in the control of NO in the human body play NO synthase inhibitors - iNOS. One of the primary inhibitors appears to be asymmetric dimethylarginine - ADMA. The level of ADMA and its derivatives in plasma is used as a marker of ischemic stroke. The aim of the project is to determine the role of nitric oxide synthase inhibitor (iNOS) - ADMA as a factor in the pathogenesis of ischemic stroke. The project employed a standard technique for determining the level of ADMA in the plasma which is based on high performance liquid chromatography using a fluorescent label. Gas chromatography was used as a comparative method to analyze the contents of ADMA and its derivatives. The data obtained have demonstrated a correlation between the level of ADMA and selected risk factors for stroke.

Hirshfeld atom refinement HAR and wave function fitting XCW – new quality in X-ray crystallography

Presenter: Magdalena Woińska

Chemistry Department, University of Warsaw

Magdalena Woińska, Simon Grabowsky - Fachbereich 2 – Biologie/Chemie, Universität Bremen, Leobener Str. NW2, D-28359 Bremen, Germany, Paulina M. Dominiak - Chemistry Department, University of Warsaw, ul. Pasteura 1, 02-093 Warsaw, Poland, Krzysztof Woźniak - Chemistry Department, University of Warsaw, ul. Pasteura 1, 02-093 Warsaw, Poland, Dylan Jayatilaka - School of Chemistry and Biochemistry, University of Western Australia, 35 Stirling Highway, Crawley WA 6009, Australia

HAR and XCW are novel methods of crystallographic X-ray data refinement based on Hirshfeld partition of electron density. HAR yields improved positional parameters of atoms and XCW allows to include experimental contribution to electron density. This constitutes progress in comparison with the most popular in crystallography Independent Atom Model (IAM) or the multipole model (MM). Describing positions of H atoms is believed to be achievable with the desired precision and accuracy only with the use of neutron diffraction experiments. Basing on HAR carried out for 81 high resolution X-ray data sets for organic compounds, it can be concluded that X-H bond lengths averaged within certain types differ from the neutron ones only by 1-2 X-ray standard deviations and also have comparable precision. Moreover, anisotropic refinement of H thermal motions can be achieved. The second problem considered is QTAIM properties based evaluation of electron density reconstructed from crystallographic data refined with MM, HAR and XCW. The results are checked against the benchmark values from periodic DFT calculations. In the overall view HAR provides the best agreement with the theoretical properties while the other methods diverge slightly more.

Programmed Cell Death (PCD) as a key process of seasonal dying of ephemeral organs in plants.

Presenter: Natalia Wojciechowska

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PCD is an important process that occurs during plant ontogeny and their stress tolerance mechanisms. A specific example of PCD is the senescence of ephemeral plant organs. It is an active process usually connected with the clearance of cytoplasm followed by vacuole rupture and final cell degradation. First indications of PCD can be characterised by a number of morphological, cytological and physiological symptoms, including chromatin condensation, DNA fragmentation, as well as carbon and nitrogen compounds translocation from senescent organs. The majority of researches on this topic is based on leaves and petals. However, very little is known about this pathway in ephemeral absorptive roots, known as fine or fibrous. The aim of our work was to assess if fine roots of black cottonwood (Populus trichocarpa) undergo seasonal senescence and dying with PCD symptoms featured. The results of anatomical and cytological analyses show that death of fine roots correlates or even ahead of the leaf senescence time. We suggest that PCD might play a crucial role in this process, both in leaves and roots. This work was supported by the grant no. 2012/07/E/NZ9/00194 from the National Science Center.

Interactions of peptide nucleic acids with ribosomal RNA helix 18

Presenter: Monika Wojciechowska

Centre of New Technologies, University of Warsaw

Monika Wojciechowska, Monika Wojciechowska, Joanna Trylska

Helix 18 is a conserved fragment of 16S ribosomal RNA (rRNA) formed by nucleotides 500 to 545 (according to Escherichia coli numbering) [1]. Its secondary structure contains an internal loop and nucleotides 505-507 and 524-526 form a three-dimensional pseudo-knot. Targeting helix 18 in a sequence-specific manner, especially the nucleotides involving the pseudo-knot, should be an effective strategy to inhibit translation [2]. We designed a peptide nucleic acid (PNA) oligomer complementary to rRNA in helix 18 and with different experimental techniques assessed the formation of helix 18/PNA complexes. We analyzed the difference in hybridization of PNA to helix 18 between free PNA and PNA covalently linked with the bacterial cell-penetrating peptide (KFF)3K [3]. Acknowledgements: This work was supported by National Science Centre (DEC-2012/05/B/NZ1/00035). Bibliography: [1] T. Powers, H.F. Noller, A functional pseudoknot in 16S ribosomal RNA., EMBO J. 10 (1991) 2203–14. [2] B. Llano-Sotelo, D. Klepacki, A.S. Mankin, Selection of small peptides, inhibitors of translation., J. Mol. Biol. 391 (2009) 813–9. doi:10.1016/j.jmb.2009.06.069. [3] P.E. Nielsen, M. Egholm, An Introduction to Peptide Nucleic Acid, 1 (1999) 89–104.

Contact maps of proteins.

Presenter: Karol Wołek

Institute of Physics PAS

Karol Wołek, Àngel Gómez-Sicilia - Instituto Cajal CSIC, Instituto Madrile˜no de Estudios Avanzados en Nanociencia; Marek Cieplak - Institute of Physics PAS

Folding and stretching properties of proteins studied within structure-based coarse-grained models depend primarily on the selection of the contact map. Sometimes the presence or absence of a small group of contacts may have a profound impact. It is thus important to develop appropriate methods to determine valid contact maps. Here, we focus on two algorithms to generate contact-maps: the overlap-based (OV) and one based on a modified CSU that includes repulsion and ionic bridges (rCSU). Both contacts maps overlap considerably but are distinct and the simple combination of them can provide improvements to the prediction of physical properties. The case of lack of known native structure, such as multi-domain proteins where structures of only single domains are known, present a bigger challenge. Here we propose to use statistical contact maps for inter-domain contacts: Based on the analysis of rCSU contact maps of large a set of protein structures, we determine what are the most probable contacts and their parameters.

Deregulation of signal transduction pathways in Niemann-Pick type C disease

Presenter: Marcin Woś

Nencki Institute of Experimental Biology PAS

Marcin Woś, Joanna Bandorowicz-Pikuła, Laboratory of Cell Metabolism, Department of Biochemistry, Nencki Institute of Experimental Biology, Warsaw, Poland

Up to now the Niemann Pick type C (NPC) storage disorder, caused by mutation in the NPC1 and NPC2 genes, was linked to the disruption of the cholesterol transport. The recently obtained data revealed that the molecular background of this disease is much more complex. Accumulation of cholesterol in NPC cells may affect mitochondrial membrane composition and, therefore, mitochondrial functions. Indeed, the analysis of NPC cells revealed significant changes in mitochondria biogenesis, oxygen consumption rate and ATP synthesis (Woś et al. 2015). Moreover, in NPC cell lines, dysfunction of mitochondria coexists with the changed signal transduction pathways (changes in phosphorylation of kinases such as Akt, PDK, GSK3-β and JNK) that can modulate mitochondrial functions and are associated with impaired metabolism. These differences in signal transduction in NPC cells may suggest complex molecular mechanisms, involving the cellular energy metabolism and its regulation that lead to perturbed lipid distribution in NPC cells. This work was supported by the National Science Center grant NN401642740, by statutory funds from the Nencki Institute of Experimental Biology and by scholarship for PhD student from the Mazovia Region.

Enhancing the effectiveness of chemotherapy by electroporation applied on pancreatic cancer cells.

Presenter: Olga Wysocka

Olga Wysocka, Olga Wysocka (1), Julita Kulbacka (1), Katarzyna Bieżuńska-Kusiak (1), Paweł Surowiak (2), Jolanta Saczko(1); (1) Department of Medical Biochemistry, Wroclaw Medical University; (2) Department of Histology and Embryology, Wroclaw Medical University

Pancreatic cancer is one of the most aggressive malignancies. Currently applied chemotherapy (CT) requires the usage of high drug doses, often resulting in the serious side effects occurrence. Electroporation (EP) is an innovative technique increasing cells permeability. Electrochemotherapy (ECT) represents a revolutionary approach for pancreatic cancer treatment. The aim of the study was to evaluate the influence of ECT with bleomycin and compared to standard CT. The experiment has been carried out on two cell lines of human pancreatic carcinoma: EPP85 -181P and EPP85 -181RDB, subjected to an electric field action of strength 800 V/cm and 1200 V/cm in buffer containing 30 nM and 75nM of bleomycin, respectively. Cellular viability was measured using the SRB assay after 24 and 72 h of incubation. Additionally, the activity of GSTπ has been evaluated. A significant decrease in cells viability and a slight increase in the expression of GSTπ has been observed as a result of ECT application. The highest level of cytotoxicity was observed after 72 h with combination of 75nM bleomycin and 1200 V/cm EP. The obtained results indicate a great potential of combining EP with CT in order to decrease the drug dose and reduce the side effects.

Genes co-localization in topologically associating domains indicates higher co-expression.

Presenter: Rafał Zaborowski

Institute of Informatics, University of Warsaw

Rafał Zaborowski, Torgeir Hvidsten, Department of Chemistry, Biotechnology and Food Sciences, Norwegian University of Life Sciences; Bartosz Wilczyński, Institute of Informatics, University of Warsaw

Hi-C is the technique to study genome architecture by analysis of higher order chromatin interactions. Recent studies exploiting Hi-C revealed hierarchical structure of chromatin. In particular a phenomenon of so-called topologically associating domains (TADs) emerged as strongly self-interacting regions of a genome found universally across different species. There are indications that TADs may be responsible for separating different functional loci by influencing the frequency of long range contacts between different parts of chromosomes. It was also reported that TADs are conserved between mice and humans. In this study we investigate whether genes spatial co-localization is preserved across evolution. In our analysis we map genes of two evolutionary related organisms, mouse and human to corresponding TADs. We then calculate average co-expression for each domain based on publicly available microarray experiments data. Finally we look for co-expression patterns depending on whether domain structure were maintained or changed during evolution.

Multielectrode biosensor system to study ion fluxes across the monolayer of epithelial cells.

Presenter: Mirosław Zając

Warsaw University of Life Sciences

Mirosław Zając,

Epithelial cells form a barrier for water and ion transport in lungs, pancreatic ducts and sweat glands. There are different ion channels, transporters and pump molecules present in epithelial cell membranes. The defect in single anion channel CFTR is responsible for the most common fatal human genetic disorder – cystic fibrosis. The defect in the CFTR ion channel changes passive osmotic water transport across epithelium, which leads to dense mucus formation prone to opportunistic bacterial infections. Multiple ions are transported across the epithelial cell monolayer i.e. sodium, potassium, chloride, hydrogen and bicarbonate. Measurement of all these ions is the key to understand the mechanism of cystic fibrosis. In our laboratory we built and successfully tested the small volume multielectrode biosensor system which can measure transport of all these ions but bicarbonate. Recent studies show that ion transport across 16HBE-14σ is very complex phenomenon. For example introduction of chloride gradient across the cell monolayer causes not only transport of chloride ions but also transport of potassium ions and pH change. Blocking of one particular ion channel species affects transport of all the ions across the cell layer.

Steroid hormones as regulators of the O6-methylguanine-DNA methyltransferase (MGMT) transcription

Presenter: Nidoieva Zarina

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O6-methylguanine-DNA methyltransferase (MGMT) is the DNA repair enzyme that prevents mutations and cell death. On the other hand, MGMT can provide the cancer cell resistance to alkylating agents. These drugs are often used in combination with the hormone therapy. However, there is a lack of data about effect of hormones used in clinic on the MGMT transcription and the chemotherapy efficacy. Only glucocorticoids are known to up-regulate this gene expression. Thus, the aim of the project is to study the regulation of the MGMT transcription by steroid hormones. We predicted several novel cis-elements that bind with steroid hormone receptors and thyroid hormone receptor-like factors within the human MGMT gene promoter by in silico analysis using different programs. According to our preliminary data, there is the fluctuation in the MGMT expression at mRNA and protein levels in MCF7 and 293 cells after hormones treatment. Estradiol increased the gene expression at concentrations corresponding to such in women blood before ovulation and during pregnancy. Other tested concentrations tend to down-regulate MGMT. Similarly, progesterone activated MGMT transcription at the concentrations corresponding to such in blood of pregnant women.

Abnormalities of HTERT gene and telomere length in pituitary adenomas.

Presenter: Joanna Zbijewska

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Pituitary adenomas (PAs) are among the most frequent brain tumors in humans. Abnormal telomerase activity and telomere legthening are features of tumor cells. They may result from mutations in promoter region or amplification of HTERT, that encode catalytical component of telomerase. Such changes were found in vairety of tumors including those of brain. The incidence of HTERT genetic abnormalities was not assesed in PAs. Aim of the study was to evaluate the frequency of HTERT promoter mutations and amplification and to assess their role in tlomerese lengthening. Study involved 102 piatient with PA including both nonfunctioning and functioning subtypes DNA was isolated from fresh-frosen tumor tissue and normal blood samples from healthy donors with commercial kit. Promoter mutations were assesed using Sanger sequencing whereas HTERT copy number variation was assesed using quantitative PCR (qPCR). We observed a missense mutatation in one patient only and qPCR results indicated increased HTERT copy number in 10,8% of patients. Variable telomere length was observed in patients, however no raltionship with HTERT abnormalities was found. The results indicate low incidence and limited role of HTERT abnnormalities in PA pathogenesis.

The unfolding pathway of knotted protein inside chaperone

Presenter: Yani Zhao

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It's well known that chaperonin is essential to avoid protein aggregation in vivo, but it is still unclear how chaperonin can assist protein folding mechanisms. In our work, we try to understand how chaperonin can unfold a knotted protein based on numerical simulations. Additionally, we are investigating the mechanism of knot untying. The knotted protein under consideration is the S-adenosyl-L-methionine (AdoMet)-dependent α/β-knot superfamily of SPOUT methyltransferases (MTases), with a high structural homology to YbeA (PDB code: 1VH0). The results show there are at least two consecutive intermediate states in the untying of the protein. First, the protein unfolds but still contains a knot. Then, the protein unties to form a linear chain with trivial topology. Our results also show that the knot can stay in the native position long after the N-terminal domain of the protein has unfolded itself. Surprisingly, the protein is able to unfold and refold back several times inside the chaperonin. In the untying process, protein unties a knot in 90% by the C-terminal.

Tetraphosphate cap analogues modified in polyphosphate bridge are inhibitors of Dcp1/2 complex

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Dcp1/2 is the major eukaryotic RNA decapping complex, which release m7GDP molecule from m7G capped transcripts. Its activity is crucial for RNA quality control and turnover hence deregulation of those processes may be detrimental. Since m7GDP is bound by the Dcp1/2 catalytic subunit, Dcp2 with only milimolar affinity a small library of synthetic m7G nucleotides was screened, bearing modifications in their oligophosphate chain, in order to find better Dcp1/2 binders. Using a radioactivity-based decapping assay compounds binding Dcp2 much tigher than m7GDP was identified. All these nucleotides are mRNA cap analogues based on m7Gppppm7G structure and contain either boranophosphate or phosphorothioate moiety in the phosphate chain. The most potent inhibitor, m7GpSpppSm7G is about 20-times more potent than m7GDP. NMR binding experiments revealed that both regulatory and catalytic domains of Dcp2 recognise that compound with submilimolar affinities. Single-turnover kinetics inhibition assay showed that mentioned compound is a mixed inhibitor with higher affinity for apo enzyme than for E-S complex. It is the first report of a small molecule inhibitor of Dcp2 and it may be beneficial for further studies on RNA decapping.

Length of 5'UTR and structural context of AUG1 codon influence translation initiation of p53 mRNA

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There is evidence that translation initiation of p53 mRNA may be regulated by the structure and length of its 5'UTR. Previously, we proposed the secondary structure model of 5'-terminal regions of p53 mRNA, which revealed two characteristic hairpin motifs: G56-C169 and U180-A218. Here, we answer the question how length and structure of 5'UTR influence the ribosome scanning and translation initiation of p53 mRNA. We performed in vitro translation analysis in rabbit reticulocyte lysate, using mRNA constructs with variable length 5'UTRs and encoding Renilla luciferase reporter protein, monitoring continuously protein synthesis in situ. Moreover, four constructs of p53 mRNA were prepared: two with altered structural context of AUG1 codon, and two with shortened G56-C169 or removed U180-A218 hairpin motifs. The bulge structure of AUG1 codon facilitates the initiation of translation and accelerates the scanning, whereas the codon embedded in double-stranded RNA reduces the translation efficiency. Surprisingly, reduction of G56-C169 hairpin does not accelerate the initiation, while removal of U180-A218 hairpin causes delay of translation initiation. This work was supported by the Polish National Science Centre grant 2013/09/B/NZ1/01884.

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