INTERDISCIPLINARY FNP CONFERENCE

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WARSAW

ABSTRACT BOOK

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4.	Team of prof. Janusz Bujnicki	Janusz M. Bujnicki
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46.	Team of prof. Andrzej Koliński	Sebastian Kmiecik
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Title: Photoprotection on molecular level: trimer to monomer transition

LHCII is the most abundant membrane pigment-protein complex in the Biosphere and it binds more than half of the chlorophyll pool occurring on the Earth. Under optimal light conditions, LHCII is present as a trimer. This form provides an efficient light absorption in a wide range of the visible area of the spectrum and effective transfer of the excitation energy to the reaction center of the Photosystem II. In light stress conditions the trimer undergoes disintegration into monomers. The subject of our studies were LHCII pigment-protein complexes in trimeric and monomeric form. Oligomers of LHCII were obtained using native green-gel electrophoresis. Samples were analyzed by fluorescence spectroscopy and UV-Vis absorption techniques. Comparison of the excitation and transmission spectra reveal that, in monomers, the excitation energy transfer between chlorophyll b and a is less efficient than in the case of trimeric LHCII. Appearance, of a band at 655 nm in the fluorescence emission spectra of monomer, indicates that part of the energy which was not transferred onto chlorophyll a is emitted through chlorophyll b. Fluorescence emission from chlorophyll b and shortening of fluorescence lifetimes in monomeric LHCII, as well as reversibility of the process of monomerisation, indicate the photoprotective nature of this form of the complex. As revealed by FLIM analysis of a single chloroplast, the process of trimer to monomer transition occurs also in vivo.

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Title: Development of high performance and ecologically friendly supercapacitors for energy management – ECOLCAP project

The quick increase in electric energy consumption requires efficient energy storage (ES) to ensure electric grid quality and to reduce greenhouse gases emissions. Electrochemical devices for ES are attractive due to their high efficiency, low pollution, reliability, etc. Supercapacitors (SCs) are well-suited for several ES applications: stop/start systems, recovering of braking energy, security systems in aircrafts, regulation of electricity production from renewables, etc. The ECOLCAP project is focused on the development of different SC designs: electrical double-layer (EDL), pseudocapacitive and hybrid devices. Exceptionally high cell potentials for EDL capacitors in neutral aqueous electrolytes of up to 1.6 V have been demonstrated with good cycle life. Different strategies for increasing the capacitance of carbon electrodes have been implemented: grafting of carbon surface with quinone-type groups or introducing redox species in the electrolyte (e.g., molybdate and iodide). Studies on protic electrolytes have given beneficial insight into the hydrogen storage processes. Questions have been raised if ionic liquids are the key electrolytes for high performance SCs. Promising results have been obtained in hybrid Li-ion devices with a composite positive electrode of activated carbon and lithium metal oxide, acting as capacitive

electrode and reservoir of Li ions. The realizations of this project by team members hold a high position in the pursuit for high energy/power SCs.

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Title: New materials for organic and hybrid (organic/inorganic) electronics and spintronics

1. New organic semiconductors exhibiting strong electroluminescence and for this reason suitable for fabrication of organic light emitting diodes (OLEDs) were developed, namely: i) solution processable obtained via functionalization of intractable and almost forgotten dyes such as indanthrone and flavanthrone; ii) bithiophene disubstituted thiadiazoles; iii) mono- bi- and terthiophene disubstituted diketopyrrolopyrroles. The latter compounds were known but never studied as electroluminophores. All these compounds were tested as active components of guest/host-type OLEDs with excellent results. 2. Processable organic semiconductors, suitable for air-operating n-channel and ambipolar field effect transistors fabrications were synthesized i.e. a whole series of arylene bisimides differently substituted with triarylamines which showed an excellent performance in test devices. 3. Model high spin molecular compounds consisting of triaryl or carbazole spin-generating units and stiff conjugated spin-coupling units were prepared and their magnetic, structural and electrochemical properties determined. 4. New procedures were elaborated for the preparation of non-stoichiometric quaternary Cu(Ag)-In-Zn-S nanocrystals of tunable band gap and photoluminescence. Hybrids of these nanocrystals with organic semiconducting ligands were also obtained. Pioneering methods of the ligands exchange process investigations by NMR spectroscopy were developed.

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Title: Modeling of RNA and protein-RNA complexes: from sequence to structure to function

Ribonucleic acids (RNAs) are a large class of macromolecules that play a key role in communicating biological information between DNA and proteins. Their functions must be considered in the context of molecular complexes, interaction networks, and metabolic pathways. Our research bridges structural biology with systems biology and theoretical modeling with experimental analyses. It provides a multi-layered understanding of the function of RNA and RNA-protein complexes, which is central to many biological processes. We created an integrated system of databases and bioinformatics methods with the aim to systematize the large body of knowledge about RNA metabolism in model organisms. We also developed tools for modeling and studying RNA 3D structures, RNA-ligand (metal, small molecule) complexes. The key features of these tools are the use of coarse-grained, low-resolution representations,

and utilization of experimental data in the form of distance restraints. We employed a panel of experimental methods for studying RNA and RNP structure that allows for distinguishing fine differences at single nucleotide resolution. The above mentioned tools were applied for modeling of structure and mechanism of action of biologically interesting RNAs and complexes.

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Title: Welcome to the Plant World

The overall aim of the WELCOME 2008/1 project from Foundation for Polish Science was to understand plants cellular and molecular mechanisms, which are involved in electrophysiological, redox and hormonal chloroplast retrograde and nucleus anterograde signaling. Characterization of various Arabidopsis and poplar mutants or transgenic lines generated a new competitive knowledge about novel molecular regulators controlling environmental stress signaling network in plants. This knowledge is already implemented in the development of novel biotechnologies for regulation of plants productivity, in particular in amelioration of mechanisms regulating cellular homeostasis between mitotic cell divisions and cell death. Due to the Welcome 2008/1 project it was possible to build a strong group of young, enthusiastic scientists working in The Laboratory of Physiomics and Crop Design at WULS-SGGW in Warsaw. Results of our experimental work led us to the discovery of novel signaling pathways in plants and were published in top-quality journals (18 publications and 2 patents pending). Four PhD students, 12 masters students and 3 bachelor students successfully obtained their degrees. Cooperation with excellent laboratories in Europe and USA was developed and is continued. Results of the Welcome 2008/1 enabled team members and leaders to receive 7 new projects financed by the Foundation for Polish Science, the National Science Center and the National Centre for Research and Development.

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Title: Nutrition and ambient temperature during early development can reduce susceptibility to obesity

The beginning of our Welcome project in the summer of 2011 coincided with the discovery that adult humans have significant deposits of brown adipocytes in discrete locations of the body. This has spired hope that brown fat thermogenesis could provide an anti-obesity therapy in humans. But this clinical translation from the mouse to humans is not so simple. A problem to overcome is the fact that most adult humans have very low numbers of brown adipocytes. Here research with the mouse genetic model can make a contribution. Our lab has been investigating 2 aspects of brown fat and the induction of energy expenditure in mice that are common between humans and mice. One is a similar genetic phenotype of both humans and mice that has a large variation in the number of brown adipocytes that can be induced in white fat. A second is the fact that the most effective means to induce brown adipocytes and lower obesity is simply cold exposure; whether these 2 phenotypes are functionally

linked is still uncertain. Our research over the past 3 years has been to establish 1.) the role of ambient temperature on the development of brown fat; 2.) the involvement of the gut microbiota on induction of energy expenditure and resistance to diet-induced obesity via bile acid metabolism; 3.) the characterization of the molecular mechanism controlling temperature dependent involution of brown adipocytes; 4.) mechanisms controlling adipose tissue expansion.

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Title: CpG-specific DNA methyltransferase M.MpeI

Cytosine methylation promotes deamination. In eukaryotes, CpG methylation is thought to account for CpG underrepresentation. Whether scarcity of CpGs in prokaryotic genomes is diagnostic for methylation is not clear. Here, we report that Mycoplasms tend to be CpG depleted and to harbor a family of constitutively expressed or phase variable CpG-specific DNA methyltransferases. The very CpG poor Mycoplasma penetrans and its constitutively active CpG-specific methyltransferase M.MpeI were chosen for further characterization. Genome-wide sequencing of bisulfite-converted DNA indicated that M.MpeI methylated CpG target sites both in vivo and in vitro in a locus-nonselective manner. A crystal structure of M.MpeI with DNA at 2.15-Å resolution showed that the substrate base was flipped and that its place in the DNA stack was taken by a glutamine residue. A phenylalanine residue was intercalated into the “weak” CpG step of the nonsubstrate strand, indicating mechanistic similarities in the recognition of the short CpG target sequence by prokaryotic and eukaryotic DNA methyltransferases.

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Title: Proteomics in characterization of selected protein assemblies

The aim of the project was structural studies of selected biologically important protein assemblies by new method, hydrogen-deuterium exchange mass spectrometry (HDXMS). Over the last two decades, this method has become a valuable technique in the determination of protein structure and dynamics (Iacob, R. E. & Engen, J. R. 2012). Our group has focused on selected protein assemblies that are difficult targets for classic methods like X-ray crystallography or NMR. Based on the results obtained from HDXMS studies, we have defined: 1) oligomerization interface of RAGE receptor (Sitkiewicz, E. et al. 2013), 2) mechanism of pore formation of PFO toxin (Kacprzyk-Stokowiec, A. et al. 2014), 3) dimerization mode of Asc1 protein (Tarnowski, K. et al. 2014), 4) regions involved in filament formation of IF proteins (keratins and vimentins) (Premchandar A. et al., under preparation), 5) binding interface of proteins

important for histone pre-mRNA processing (FLASH, LSM11, NPAT) (Skrajna A. et al., under preparation) 6) DNA binding mode of R.DpnI (Mierzejewska, K. et al. 2014), 7) regions of SnRK2 kinase that are important in stress-response pathway (Tarnowski K. et al., under preparation), 8) interacting regions between NBD1 domain of CFTR and the CB subdomain of Phospholipase A2 (Faure G et al., under revision). These data have provided structural constraints which have allowed the verification of molecular models of many proteins and their assemblies.

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Title: Impaired interaction of a non-catalytic subunit of polymerase ε with the CMG helicase complex affects genome stability in Saccharomyces cerevisiae

DNA replication in eukaryotes depends on large multiprotein complexes - replisomes involving dozens of proteins that act in a coordinated fashion to maintain genome duplication. Cells have to monitor its genome integrity and coordinate these process with cell cycle progression. Main replicative DNA polymerases in yeasts are Polymerase epsilon (Pol ε) and Polymerase delta (Pol δ) that replicate the leading and lagging DNA strands, respectively. It is postulated that targeting of DNA Pol ε to the leading strand depends on the interaction of CMG (Cdc45-MCM-GINS) helicase complex with the non-catalytic Dpb2 subunit of Pol ε. Mutants in DPB2 gene isolated in our laboratory encode Dpb2 variants that exhibit impaired interaction with the GINS complex, and strong mutator phenotype that partially depends on low-fidelity polymerase zeta (Pol ζ). Additionally, inactivation of the proofreading activity of Pol δ results in high increase of mutagenesis in dpb2 mutant. Moreover DPB2 mutants fail to properly activate S-phase checkpoint under replication or genotoxic stress conditions what results in improper cell cycle progression and elevated budding index. Finally, deletion of DDC1 (coding for a subunit of the 9-1-1 complex involved in S-phase checkpoint activation) in the DPB2 mutant gives inviable cells. Therefore, we postulate that perturbed interaction of Pol ε with GINS increases the access of other DNA polymerases to the leading strand and affects S-phase checkpoint activation.

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Title: Molecular mechanisms of insulin resistance and β-cell failure in type 2 diabetes

Obesity-related type 2 diabetes (T2D) develops in individuals with the onset of β-cell dysfunction. Pancreatic islet lipotoxicity is now recognized as a primary reason for the onset and progression of the

disease. Our study showed that the lipotoxic effect on β-cells depends on the type of lipid mediator (e.g., long-chain fatty acids, diacylglycerols, ceramides, phospholipids (PL)), cellular location of its action (e.g., endoplasmic reticulum, mitochondria), and associated-organelle conditions (e.g., membranes, vesicles). It also includes various aspects of lipid action in β-cells, including effects on metabolic pathways, stress responses (e.g., oxidative stress, ER stress, and autophagy), and gene expression. We showed that diminished stearoyl-CoA daseturase (SCD) activity affects the accumulation, composition, and saturation status of cellular membrane PL and neutral lipids. Such an effect was accompanied by aberrant ER stress, mitochondrial injury, and decreases in insulin secretion and cell proliferation. Our data reveal a novel mechanism by which the inhibition of SCD1 activity affects autophagosome-lysosome fusion because of perturbations in cellular membrane integrity, thus leading to an aberrant stress response and β-cell failure. Thus, the beneficial effects of SCD1 inhibition on adiposity may occur at the expense of β-cells, possibly justifying a reconsideration of SCD inhibitors for T2D treatment and allowing the development of alternative therapeutic strategies.

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Title: A glimpse of the mitochondrial transcriptome of Candida albicans revealed using RNA sequencing

Candida albicans emerges as an attractive model yeast for the study of mitochondrial physiology and genetics. It is a petite-negative facultative anaerobe which, unlike the commonly used model species S. cerevisiae and S. pombe, encodes seven subunits of complex I (NADH:ubiquinone oxidoreductase) in its mtDNA. We used high throughput RNA sequencing to obtain an overview of the mitochondrial transcriptome of common laboratory strains of C. albicans. Our results indicate the presence of 8-9 polycistronic primary transcripts, which are processed using the tRNA punctuation mechanism. The steady-state levels of mature transcripts vary greatly, with tRNAs and rRNAs having the highest expression levels. As the levels of mature RNAs derived from the same primary transcript show very significant differences, RNA stability and degradation has to play a key role in the regulation of mitochondrial gene expression in this species. Open reading frames that are not separated by tRNAs are translated as bicistronic mature transcripts. Our data confirmed the positions of four introns in the COX1 gene and two introns in the COB gene predicted in silico. Two previously unannotated introns were also discovered in the rnl gene encoding the large subunit rRNA. In addition to transcripts corresponding to annotated genes, we also found evidence of transcriptional activity in the inverted repeat regions of the mitochondrial genome, that show no similarity to any known coding sequences.

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Title: Vitamin B12 - beyond the known

Vitamin B12 is an essential nutrient for all mammals and its various forms play a critical role in metabolic processes. However, to date a complete understanding of vitamin B12 reactivity has not yet been achieved. Hence, our goal is to shine a light on vitamin B12 by fully elaborating on its properties. Herein we present our studies on vitamin B12 new reactivity and applications in: A) Drug delivery - due to its specific dietary uptake vitamin B12 is a potential drug carrier for oral delivery. To utilize its uptake properties and to prepare a potential hybrid drug, vitamin B12 was chemically modified and conjugated to selected therapeutics. B) Activation of guanylyl cyclase - vitamin B12 derivative - (CN)2Cbi, can act as NO-independent activator of soluble guanylyl cyclase - an enzyme responsible for smooth muscle relaxation. Synthetic modifications accompanied by biochemical studies demonstrated that the activating properties of (CN)2Cbi are altered with introduction of different peripheral substituents around the corrin macrocycle. C) Catalysis – vitamin B12 is a cofactor in many enzymatic reactions, thus it is a promising, environmentally benign catalyst for organic synthesis. We have confirmed the long-standing hypothesis that the nucleotide loop strongly influences catalytic properties of vitamin B12 and its derivatives.

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Title: Understanding immune dysregulation in cardiovascular disease

While the role of inflammation in cardiovascular risk is relatively well defined, therapeutic targeting of inflammation has not been introduced into clinical practice so far. Recent studies have emphasized the role of perivascular inflammation in cardiovascular disease (CVD). Our lab focuses on the mechanisms of vascular inflammation in cardiovascular diseases in mouse models and human disease. We study the role of the perivascular adipose (pVAT) tissue as a site of inflammation in CVD. Pro-hypertensive and pro-atherosclerotic substance such as angiotensin II increases pVAT quantity, its expression of proinflammatory cytokines and its content of T cells, macrophages and dendritic cells. CVD is associated with increases in pVAT expression of the chemokine RANTES which induces T cell and monocyte chemotaxis. Mice lacking RANTES developed similar degree of hypertension in response to angiotensin II, but were protected against vascular dysfunction, oxidative stress and perivascular inflammation by leukocytes. In a human cohort, a significant inverse correlation was observed between circulating RANTES levels and vascular function. Moreover in patients with aortic abdominal aneurysms, perivascular inflammation is prominent and appears to precede and exceed inflammation in the vessel wall itself. Thus, pVAT represents a unique compartment of inflammation that contributes to vascular dysfunction in hypertension, human aortic abdominal aneurysms and atherosclerosis.

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Title: Investigating the drug-receptor interactions

Prof. Jóźwiak, head of the Biopharmacy Department at Medical University of Lublin, leads the laboratory focused on the investigation of molecular interactions between chemical molecules and their biological targets. The aim of Jóźwiak's team is the design, structure optimization and preliminary validation of new potential drugs. Interdisciplinary approach combines skills and knowledge of the experts in the fields of bioinformatics, medicinal chemistry and molecular biology. Our efforts were acknowledged by grant support from Foundation for Polish Science, National Science Center and National Center for Research and Development. Our recent discoveries enabled us to reveal the mechanism responsible for biased agonism of β2 adrenergic receptor (β2-AR). The emerging paradigm of 'biased agonism' or 'functional selectivity' suggests that binding of one ligand can stabilize receptor conformations preferentially triggering a given signaling pathway, although another ligand stabilizes a receptor state that selectively activates a different signaling cascade. In this manner, ligands can trigger qualitatively distinct signaling events. We concluded that specific interactions between the ligand and the Tyr-308 residue of β2-AR stabilized receptor conformations favoring the receptor-Gs protein coupling (over the coupling to Gi protein) and subsequently resulted in Gs-biased agonism. Here, we will illustrate this and other projects that we are involved in and our main achievements.

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Title: Length-scale Dependent Transport Properties of Colloidal and Protein Solutions

We propose a scaling equation describing transport properties (diffusion and viscosity) in the solutions of colloidal particles. We apply the equation to 23 different systems including colloids and proteins. The particles differ in size (range of diameters: 4 nm to 1 μm), and volume fractions (10^{-3} – 0.56). In solutions under study the colloidal particles interact via steric, hydrodynamic, van der Waals and/or electrostatic interactions. We implement contribution of those interactions into the scaling law. Finally we use our scaling law together with the literature values of the barrier for nucleation to predict crystal nucleation rates of hard-sphere like colloids. Resulting crystal nucleation rates agree with existing experimental data.

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Title: Photonic implementations of quantum enhanced technologies

Recent years have witnessed tremendous progress in experimental capabilities to prepare, control, and detect individual microscopic systems. These advances pave the way for exploiting specifically quantum phenomena, such as wave-particle duality and entanglement, in a broad range of technological applications. Collective states of many photons can improve the precision of interferometric measurements below the standard quantum limit even in realistic scenarios including imperfections. In communication protocols, careful analysis of noise effects reveals the possibility to achieve surprisingly strong levels of cryptographic security despite uncontrolled interactions with the environment. The availability of advanced nonlinear waveguide structures permits the construction of new sources of non-classical light, compatible with requirements of modern fiber optics networks. Manipulating quantum coherence in atomic systems enables the development of high-capacity light-matter interfaces required for massive quantum information processing.

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Title: Novel optical techniques for enhanced structural, functional and dynamical imaging of anterior and posterior segments of the eye

The project consists of two independent parts. The objective of the first part is to broaden knowledge about optical processes in the human retina, especially these based on auto-fluorescence and to develop novel optical imaging modality for visualization of morphology and function of the human retina. The lipofuscin fluorescence imaging of human retina in vivo with highly improved sensitivity is presented. Moreover, we demonstrate the two-photon processes (2ph) in retinal pigments: 2ph-absorption of laser pulses in human photoreceptors responsible for infrared vision phenomenon and 2ph-excited-fluorescence of retinoid compounds produced during visual cycle applied for imaging of mouse eye in vivo. The objective of the second project part is to develop new imaging technology, that will have widespread research and clinical applications, to elucidate changes in morphology and biomechanical properties of the anterior segment of the human eye, and to develop new methods for visualization, or morphometry, which can improve diagnostic sensitivity and the assessment of disease progression. Two aspects of the research related to anterior segment of the human eye are presented. The corneal deformations measured with air-puff swept source Optical Coherence Tomography will be analyzed in details. Additionally novel approach for OCT guided soft contact metrology with its possibilities and limitations are discussed.

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Title: The Araucaria Project: 20 years of measuring precise distances in the Universe

The Araucaria Project is an international astronomical collaboration leaded by a dedicated team created in the Warsaw University Observatory. Our research is focused on precise distance measurements to large sample of celestial objects. Our strategy is to apply wide variety of astrophysical methods in order to determine precise distances in the local Universe. During over 1000 nights and using 16 largest world-class telescopes we collected high-quality data, which allowed us to improve classical methods and invent novel techniques of measuring distances. Among over 50 different distance measurements, the most remarkable achievement is improvement of the accuracy of distance determination to Large Magellanic Cloud, reaching unprecedented 2% precision. Our measurement provides a valuable contribution and establishes important stepping-stone towards reaching much further galaxies. While determining distances to nearby galaxies, we were able to obtain many astrophysical parameters for different types of stars. We also made a surprising discovery of a new class of pulsating stars, enthusiastically accepted by worldwide astronomical society. Our accomplishments give us solid foundations to recalculate and set fine constraints on one of the most important physical parameters describing the Universe - Hubble constant, eventually leading to establishing size and age of the observed Universe.

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Title: Towards new applications of NMR spectroscopy in chemical and biomolecular structural studies

Nuclear Magnetic Resonance (NMR) is nowadays one of the most efficient spectroscopic techniques, providing insight into molecular structure and dynamics. The full exploitation of experimental possibilities of NMR spectroscopy is enabled by acquisition of multidimensional spectra. The main goal of the project was development of new experimental techniques and processing methods aimed at precise determination of spectral parameters from multidimensional NMR spectra. Therefore we have focused on the new methods of data processing, new experimental techniques, and their applications. The project highlights included: compressed sensing and signal separation algorithm in data processing, applications of high- (4-5) dimensional spectra for studies of Intrinsically Disordered Proteins (IDP), four dimensional experiments for structural studies of proteins and RNA, and new diffusiometric experiments. The results of the project enabled significant increase of the scientific outcome and establishing of the widely recognized research group.

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Title: Chemistry of Ag(II): a cornucopia of peculiarities

"Silver is the heavier congener of copper in the Periodic Table, but the chemistry of these two elements is very different. While Cu(II) is the most common cationic form of copper, Ag(II) is rare and its compounds exhibit a broad range of peculiar physico-chemical properties. These include, but are not limited to: (i) uncommon oxidizing properties, (ii) unprecedented large mixing of metal and ligand valence orbitals, (iii) strong spin-polarization of neighbouring ligands, (iv) record large magnetic superexchange constants, (v) ease of thermal decomposition of its salts with O-, N- or C-ligands, as well as (vi) robust Jahn–Teller effect which is preserved even at high pressure. These intriguing features of the compounds of Ag(II) will be discussed here together with (vii) a possibility of electromerism (electronic tautomerism) for a certain class of Ag(II) salts." (W. Grochala and Z. Mazej, Philos. Trans. A. Math. Phys. Eng. Sci., 2015, 373, 20140179)

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Title: Modułowy tomograf holograficzny do badania obiektów biologicznych

Tomografia holograficzna jest techniką umożliwiającą precyzyjny ilościowy pomiar trójwymiarowego rozkładu współczynnika załamania przedmiotów transparentnych o mikroskopowych rozmiarach. W ostatnich latach wzrosło zapotrzebowanie na takie badania w odniesieniu do obiektów biologicznych, a w szczególności żywych komórek i macierzy komórek rakowych. Niniejsza praca prezentuje system modułowego, aktywnego tomografu holograficznego, umożliwiający pomiar w dwóch trybach: (1) z wykorzystaniem obrotu próbki oraz (2) ze zmianą kąta oświetlenia. W pierwszy trybie próbka umieszczana jest w obrotowej kapilarze, co umożliwia zebranie pomiarów w pełnym kącie i dokładną rekonstrukcję. W tej metodzie niepożądane efekty związane z cylindrycznym naczyniem są usuwane numerycznie za pomocą algorytmów korekcji aberracji, bazujących na analizie lokalnego biegu promienia oraz dedykowanego algorytmu rekonstrukcji dla dowolnych warunków oświetlenia. Drugi tryb pracy systemu, opiera się na tomografii w ograniczonym kącie projekcji, w którym statyczna próbka oświetlana jest sekwencyjnie z różnych kierunków. Tryb ten umożliwia szybką tomografię holograficzną obiektów dynamicznych. Co więcej, dzięki zastosowaniu elementów aktywnych (przestrzenny modulator światła), możliwa jest 1) zmiana kierunków oświetlenia badanego obiektu według dowolnego zadanego scenariusza bez wykorzystania elementów ruchomych oraz 2) korekcja frontu falowego wiązki oświetlającej, zwiększająca dokładność pomiaru.

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Title: Breaking Boundaries in Chemistry: from Molecular Precursors to Functional Materials

Design and synthesis of advanced functional materials is a hot issue on the edge of inorganic chemistry and material science. Here we illustrate the diversity of our research interests and bottom-up approach in creating various inorganic-organic materials of desired functionality from well-defined organometallic precursors. The developed mild approaches pay close attention to the relationships between the structure and reactivity, and mechanisms of product formation as well as the in-depth understanding of self-assembly processes of single molecules into complex supramolecular architectures. The acquired fundamental knowledge enables the control over transformations on molecular level as well as in the nanoscale. In particular, the attention is focused on the development of highly-controlled methods of synthesis and functionalization of biocompatible ZnO quantum dots, microporous metal organic frameworks (MOFs) and Bimetallic Single Ion Magnets. Spectacular range of materials and the novelty of synthetic approaches are a great inspiration for further research in these interdisciplinary areas.

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Title: Activation of small molecules in photocatalytic systems

Activation of small molecules belongs to the most fundamental chemical processes. It is also a key step of photocatalyctic reactions. Oxidation processes leading to a complete mineralization of organic pollutants, as well as inactivation of microorganisms, are based on activation of molecular oxygen, water and sometimes hydrogen peroxide. As a result reactive oxygen species are formed. Their generation determines the progress of consecutive redox reactions. In particular, an efficient production of hydroxyl radicals in the presence of TiO2 makes this photocatalyst suitable for oxidation of organic molecules. Our Team managed to clarify several aspects of oxygen and water activation in the presence of TiO2-based, ZnO, Si and other semiconductors. Our research sheds light on singlet oxygen photogeneration and helps understanding the influence of crystal structure and surface morphology on activation of O2, H2O and NO. Beside exoenergetic oxidation processes, photocatalyctic reactions can be used to convert light to chemical energy. These endoenergetic reactions include water splitting, CO2 reduction and some reactions of organic molecules synthesis. Also these processes require activation of small molecules: H2O, CO2 and others. Conditions facilitating these processes usually differ significantly from those necessary for O2 activation. Our systems based on ZnS and few p-type semiconductors open a possibility of a photocatalyctic CO2 reduction.

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Title: Secure Lottery based on Bitcoin

Bitcoin, introduced in 2008, is an IT system that works as a digital currency running in the Internet. Just as a standard monetary or banking system it enables accumulating currency - bitcoins - on accounts, money transfers and exchanging bitcoins for standard currencies like dollars or euros. What makes it unique is its decentralization. No one (no bank, institution or a country) is controlling the currency or transactions, no one can create new bitcoins, steal them from users accounts or close the system. Bitcoin correctness and security is based only on mathematics and cryptographic protocols. Besides the decentralization, the main features of Bitcoin are: partial anonymity, very fast transactions and low or even zero fees. Additionally Bitcoin syntax allows more complex transactions than simply transferring the money. Because of that Bitcoin recently gained noticeable popularity - its current capitalization is around 4 billions dollars. In our work we construct protocols for secure lotteries using the Bitcoin currency, without relying on a trusted authority (e.g. casino). Users can use them to run a lottery over the internet with unknown, untrustworthy participants. Our protocols guarantee fairness for the honest parties no matter how the loser behaves, e.g. if one party interrupts the protocol then her money is transferred to the honest participants. Our protocols are practical and in principle could be used in real life as a replacement for the online gambling sites.

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Title: Biogenesis and turnover of mitochondrial intermembrane space proteins

Mitochondria play an important role in metabolism and regulatory processes in the cell. Thus, the formation of mitochondria is essential for cellular function in the entire eukaryotic kingdom, from unicellular organisms to mammals. Mitochondria comprise 1000-1500 cellular proteins, which are synthesized outside of the mitochondria in the cytosol. The biogenesis of mitochondria relies on the efficient import, sorting, and maturation of proteins governed by conserved protein translocases and other complex machineries. We aim to understand the complex and dynamic processes involved in the formation of functional mitochondria, the maintenance of mitochondrial protein homeostasis, and their failure that results in pathology. Our major interests are also directed to redox-dependent processes involved in mitochondrial protein biogenesis. We concentrate on the following issues: • Redox-related protein biogenesis events driven by MIA in yeast and higher eukaryotes. • Cross-talk between mitochondrial architecture and dynamic events that are involved in mitochondrial protein biogenesis. • Impact of protein transport pathways on mitochondrial and cellular protein homeostasis

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Title: Driving properties of magnetic nanostructures

In the last decade much attention was paid to magnetic nanostructures due to their interesting properties and potential applications in spintronics and magnetic logic devices. The most interesting effects are the appearance of perpendicular magnetic anisotropy (PMA) when layer thickness is decreased and discovery of the giant magneto-resistance effect (GMR), awarded in 2007 by the Noble prize. Patterned nanostructures with PMA can be used for e.g. mass memories together with magnetic ratchet memories, magnetic field sensors, spin waves applications or controllable transport of magnetic beads. Post-growth modifications of nanostructures using ion or light irradiation will be discussed, focusing on the recently discovered phenomena: creation of new PMA phases, increase of magnetooptical effects, changes of coercivity field. We will show a possibility of tuning these parameters by changing irradiation fluence in a broad range of values, up to ones enabling material sputtering. The presented methods of modifications of nanostructures are possible to be applied on a submicrometer scale, to create new metamaterials such as magnonic and magnetophotonic crystals.

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Title: Polish Registry of Pediatric and Adolescent Diabetes - Nationwide Genetic Screening for Monogenic Diabetes

The project entitled „Polish Registry of Pediatric and Adolescent Diabetes - Nationwide Genetic Screening for Monogenic Diabetes" was focused on the molecular background of a rare subtype of diabetes. Monogenic diabetes is a heterogenous group of ailments caused by single gene mutations that lead ultimately to the dysfunction of insulin-producing pancreatic islet beta cells. Until recently, monogenic diabetes was assumed to be a marginal problem, affecting less than 1% of patients. Epidemiological reports, performed by our group on a nationwide scale showed the actual prevalence to be much higher, with current estimates placing the figure at 5-10%. Throughout the project, the research team moved from diagnostics of neonatal diabetes, through epidemiological estimates of monogenic diabetes in childhood into the final stage – translational medicine applied through pharmacogenetic interventions. Notable achievements of the team in that field include substitution of insulin by orally-ingested sulphonylurea derivatives among carriers of Kir6.2/SUR1 activating mutation, insulin discontinuation in patients with mutations of the glucokinase gene and recently undertaken attempts to stall the progression of Wolfram syndrome (WFS1 gene defect) – the most severe form of monogenic diabetes which includes neurodegeneration symptoms. Finalization of the TEAM project enabled the Department to follow through with several spin-off projects focused on biomarkers, clinical features of monogenic

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Title: Biomimetic asymmetric carbon-carbon bond formation - catalysts design and application

TEAM Project Biomimetic asymmetric carbon-carbon bond formation - catalysts design and application coordinated by Jacek Młynarski was realised at Jagiellonian University in years 2010-2014.
Since the beginning of organic synthesis, chemists have dreamed of preparing complex molecules in an elegance and efficiency similar to that of Nature way. In doing so, chemists have designed many biomimetic catalytic processes and natural product syntheses. Presented TEAM project was composed around this important concept of biomimetic transformations. Planed research in broad terms focus on organic synthesis and the discovery of new reaction methodology, in particular stereoselective and catalytic asymmetric reactions which contain following topics: - development of new efficient catalysts for asymmetric aldol reaction, - asymmetric synthesis of carbohydrates, - application of pyruvates in asymmetric synthesis, - biomimetic synthesis of natural products and bioactive compounds. Program contains 24 fellowships which include 5 post-doc positions, 5 PhD students and 14 undergraduate students. As a result of realised research included in TEAM programme, fifteen articles were published in prestigious journals with high IF (average IF: 7,579). Obtained results were also presented at national and international conferences as poster and oral communications which won several awards.

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Title: High-resolution force and mass metrology using actuated MEMS/NEMS devices – FoMaMet

The micro- and nano-electro-mechanical systems (MEMS and NEMS) can be used to investigate the mass adsorption and force at the nanoscale. The experiments in this field started with invention of the scanning tunneling microscopy (STM) and atomic force microscopy (AFM) in late 1980s. This technology which, in the general form, applied modified AFM spring cantilevers, was utilized to observe forces between functionalized microtips and surfaces. One of the possible measurement configurations can lead to biochemical diagnostics and nanoscale recognition of chemical nature of the chemical bindings between the microprobe and surfaces. Unfortunately, the described technology enables only qualitative investigations (which means detection) of forces in the range of several piconewtons. Another application of huge potential of the MEMS/NEMS technology is the detection of changes of the mass adsorbed on the cantilever surface. The sensitivity of these investigations reaches attogram range, but in the same way as in the force experiments any reliable method enabling calibration of the applied methods is well established. Therefore, the scientific goal of the project High-resolution force and mass metrology using actuated MEMS/NEMS devices – FoMaMet is to develop metrological methods and techniques for scaling, calibration of high-resolution novel MEMS- and NEMS-based devices for precise investigations of force and mass changes in the femtonewton and femtogram range.

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Title: Ultra-precise spectroscopy of atoms and molecules

Development of ultra-narrow and stable laser systems, laser cooling and trapping methods as well as highly-sensitive spectroscopy are crucial for optical control and metrology of quantum systems at the Hz-level of precision. Extremely narrow sub-Hz transitions in alkaline earth metals are very interesting from the point of view of experiments aimed at precise measurements of the second, testing

fundamental theories or temporal variation of physical constants. In this matter we present our results of 27-Hz-level spectroscopy of neutral strontium atoms confined in the 1D optical lattice. On the other hand, high-resolution spectroscopy of high-finesse cavity modes shapes gives a new perspective for development of an alternative, ultra-sensitive and accurate methods for molecular systems metrology. In this context, we present our cavity mode-width and cavity mode-dispersion spectroscopy techniques based on absorptive broadening and dispersive shift of cavity modes, respectively. Both methods are compared to the well-known and commonly used cavity ring-down spectroscopy. The sub-Hz precision achieved in the cavity mode width and position determination is demonstrated as well.

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Title: Polymeric inhibitors of human coronavirus HCoV-NL63

Human coronavirus HCoV-NL63 is generally classified as a common cold virus which may cause upper and lower respiratory tract diseases, including severe ones. Thus, there is an interest in the development of effective prevention and treatment procedures for these infections. The aim of this study was to synthesise and explore anticoronaviral activity of polymer-based compounds. Four polycations, i.e. N-(2-hydroxypropyl)-3-trimethylammonium chitosan chloride (HTCC), hydrophobically-modified HTCC (HM-HTCC), O-(2-hydroxypropyl)-3-trimethylammonium poly(vinyl alcohol) chloride (HTPVA) and poly(allylamine hydrochloride) (PAH) were obtained and studied. In order to evaluate the inhibitory activity of the tested polymers the in vitro studies using Macaca mulatta rhesus kidney cells (LLC-MK2 cells) and the ex vivo studies using human airway epithelium (HAE) cultures were performed. The viral cytopathic effect (CPE) was correlated with a quantitative RT PCR based assay. The polymer cytotoxicity was examined by an XTT assay and a Neutral Red assay. The results showed that selected cationically-modified polymers were effective inhibitors of HCoV-NL63 replication. The cationically-modified chitosan derivative (HTCC) was found to be the best inhibitor of HCoV-NL63 replication at nontoxic concentrations. The reference experiments carried out with polymers before modification (chitosan, polyvinyl alcohol) have shown that they have no anticoronaviral activity.

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Title: Translation regulation by bacterial noncoding RNAs binding to the coding sequence of mRNAs

Small non-coding RNAs (sRNAs) are involved in the bacterial cell adaptation, maintenance of cellular homeostasis, and virulence. They regulate translation by binding to complementary regions in target mRNAs. This regulation is dependent on the Sm-like, ring-shaped Hfq protein, which contains three independent RNA binding sites, which allow it to enter into complex interactions with RNA molecules. The majority of sRNAs interfere with translation by binding to the 5'-untranslated region. However, recent Hfq profiling data in a pathogenic E.coli strain showed that the coding sequence could also be an important target of this regulation. Indeed, Salmonella ompD mRNA is regulated by four sRNAs binding to its coding sequence, which accelerate its decay involving RNase E. Although Hfq binds to ompD mRNA and affects its expression, it remains enigmatic how it contributes to the interactions of sRNAs with this mRNA. Here, the kinetics of association of RybB and MicC sRNAs to the 5'-terminal ompD mRNA fragment was monitored using a gelshift assay. The results showed that Hfq markedly increased the rates of association of both sRNAs to this mRNA. To further analyze these interactions, the effects of Hfq and sRNA binding on the secondary structure of ompD mRNA were investigated. Moreover, in vitro translation assays in bacterial cell lysates were carried out to better elucidate the molecular mechanism of the interplay between sRNAs and Hfq in the control of ompD mRNA translation.

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Title: The quest for new physics: from the Universe to the microcosmos

In our group we try to tackle some of the most pressing puzzles in contemporary particle physics: – elusive nature of new physics beyond the Standard Model. – theories behind the Higgs boson discovered at the LHC. – the nature of the dark matter of the Universe. – the early moments of the Universe after the Big Bang We address questions like: Is nature supersymmetric? Why no new theory has been observed yet? Where could it be hiding? Have we explored all possible venues or could we missing something? Investigations must be carried out on different fronts, analysis involves great amount of data: – new physics searches at the Large Hadron Collider at CERN – underground experiments to detect dark matter in the Universe – Galactic and extra galactic signals of dark matter One important result of our research: the Higgs boson discovered at the LHC implies a specific dark matter particle with mass close to 1000 x proton mass. Our results are tested by experimenters at the Large Hadron Collider and in dark matter searches. We use the most advance techniques: – highly developed theoretical scenarios – modern advanced statistical techniques – highly efficient computational tools The tools and techniques we develop can be used in other branches of physics and science in general.

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Title: Novel hybrid materials for photonics manufactured by crystal growth methods

Two novel bottom-up manufacturing methods of nanoplasmonic materials and metamaterials will be presented utilizing the crystal growth techniques: (i) directional solidification of eutectic composites, and (ii) direct doping of dielectric matrices with plasmonic nanoparticles (NanoParticles Direct Doping - NPDD). Eutectics are simultaneously monolithic and multiphase materials forming self-organized micro/nanostructures, which enable: (i) the use of various component materials including oxides, semiconductors, metals, (ii) the generation of a gallery of geometrical motifs and (iii) control of the size of the structuring, often from the micro- to nanoregimes. On the other hand, the novel method of direct doping of dielectric matrices with nanoparticles utilizing directional solidification provides three-dimensional nanoplasmonic materials enabling doping with nanoparticles of various chemical composition, various size and shape, as well as co-doping with other chemical agents. Materials with plasmonic resonances at visible and IR wavelengths, as well as materials with enhanced photoluminescence and with anomalous refraction will be presented. Our new approach may lead to novel manufacturing solutions for photonic applications in areas such as metamaterials, plasmonics, as well as photovoltaics.

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Title: Different activities of MBNL1, MBNL2 and MBNL3 proteins on RNA metabolism

Muscleblind-like (MBNL) are conserved RNA-binding proteins that regulate cytoplasmic and nuclear RNA metabolism. There are three MBNL paralogs in human which contain few partially self-regulated alternative exons that might modulate its cellular localization, affinity to RNA targets, splicing activity and protein-protein interaction capacity. Each MBNL might exist in numerous protein isoforms at different expression level across tissues and developmental stages. The understanding of differences between MBNL1, 2 and 3 and their splicing isoforms might shed more light on their impact on RNA metabolism in normal and pathological stages. We compared the activity of three paralogs and their splicing isoforms on alternative splicing activity of several endogenously expressed pre-mRNAs and few exogenous constructs. We noticed that MBNL paralogs regulate the same splicing events but they differ significantly in the strength of splicing activity. We tested whether these differences relied on various affinity to RNA targets or subcellular distribution of MBNLs. We also compared the ability of MBNL paralogs and their isoforms to interact with toxic transcript containing expanded CUG repeats in nuclear foci. Using microscopic technics we characterized the impact of MBNLs on foci formation and the mobility of trapped MBNL proteins. Our results suggest that all isoforms have similar impact on foci formation, but their mobility in these structures are significantly different.

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Title: Multi-Scale Local and Non-Local Approaches for Elastic Wave Propagation

The ultimate objective of the project was to develop the local simulation platform for efficient, and reliable multi-scale wave propagation modelling tool in complex, nonlinear media with added temperature field and sharp interfaces. This new modelling tool is suitable for numerous applications in physics, medicine, material science and other areas of engineering. Examples related to wave propagation modelling in engineering and medicine are provided.

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Title: MMP-9 contribution to physiological and pathological synaptic plasticity

Matrix metalloprotease 9 (MMP-9) is an extracellularly operating protease playing key role in the morphological reorganization of dendritic spines harboring excitatory synapses as well as learning and memory. Herein we investigated the role of MMP-9 in functional and structural synaptic plasticity. Mice in the IntelliCages were trained to acquire alcohol addiction. MMP-9 KO mice displayed lower motivation towards ethanol as compared to the wild type controls (WT). Moreover, chronic alcohol drinking

produced alterations in dendritic spine shape of both WT and KO. To test the functional relevance of altered structural plasticity we performed electrophysiological analyses of glutamatergic synapses. We have discovered that increased MMP-9 activity elevates the number of silent synapses – immature synapses considered as substrates for increased plasticity. We then investigated the formation and fate of these synapses in our model of ethanol addiction and discovered that ethanol consumption elevates the number of silent synapses, especially in mice displaying extremely high motivation for reward. Taken together these data suggest that MMP-9 is involved in synaptic plasticity associated with alcohol addiction. The change of spine morphology together with elevated silent synapse number might represent an ongoing circuitry reorganization that primes neurons for enhanced learning, which then might lead to the out-of-control ethanol use.

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Title: Prospects of X-ray photoemission electron microscopy at the first beamline of the Polish synchrotron facility "Solaris"

The synchrotron radiation facility "Solaris" is currently being built in Krakow, Poland. The first experimental beamline at Solaris will use bending magnet radiation and two exchangeable end stations: a spectroscopic X-ray photoemission electron microscope and a soft X-ray absorption spectroscopy chamber. We present the beamline specifications and exemplary results obtained with our end station microscope, which (in statu nascendi of Solaris) has been operated at the NanoXAS beamline in the Swiss Light Source. The end stations should be available for a broad user community at Solaris at the end of 2015.

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Title: A novel gene expression-targeted therapy for lysosomal diseases

Lysosomal storage diseases (LSDs) are a group of inherited metabolic disorders caused by dysfunction of one of lysosomal proteins. They are characterized by accumulation of particular compounds that cannot be degraded in lysosomes. Currently, the only available treatments for some LSDs are bone marrow or hematopoietic stem cell transplantation and enzyme replacement therapy (ERT). Although these treatments can be effective in management of somatic symptoms, they are not able to correct neuropathy, occurring in most of LSDs, because enzymes, either produced by transplanted cells or administered intravenously, cannot cross the blood-brain-barrier. Thus, we focused on development of

an alternative therapy that might be effective in treatment of lysosomal metabolic brain diseases. We found that some natural flavonoids , as well as synthetic derivatives of genistein, can impair the synthesis of glycosaminoglycans and modulate metabolism of sphingolipids through regulating expression of particular gene. Moreover, genistein was found to stimulate lysosomal biogenesis due to positive regulation of the transcription factor EB. Because of its mode of action, a LSD therapy based on the use of flavonoids was named gene expression-targeted therapy (GETT). Experiments with mice indicated that genistein can correct behavior in Sanfilippo disease. Finally, it appears that a combination of GETT and ERT can be considered as a more effective treatment than each of these therapies used alone.

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Title: Technologies for Information Transfer and Processing Based on Phenomena of a Strictly Quantum Nature

Information transfer and processing are the essential task in the digital world. Quantum mechanics promises an improvement in realizing some of information processing tasks by using the principle of superposition. This advantage may be in terms of enhanced security, lower gate query number, or reduced cost of communication. We review some of the results obtained within FNP TEAM project. We report advances on new all-versus-nothing (GHZ-type) paradoxes for collections of higher-dimensional systems. We also present new results on quantum steering, a quantum protocol, in which a state belonging to a chosen basis remotely, by a proper measurement on another particle. We also study the Hardy paradox and its use in solving the Byzantine agreement problem, as well as randomness extraction, in which quantum correlations are used to obtain truly random strings of bits. In addition, we show how two copies of the Einstein-Podolsky-Rosen state lead to a refutation of their original ideas. The misleading concept of the elements of reality can be put to a test in a feasible experiment. Finally, we argue that quantum entanglement can bring advantage to a pair of players in the game of bridge.

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Title: Application of NMR Spectroscopy for the Development of Inhibitors of the p53-MDM2 interaction

Protein-protein interactions (PPI) play pivotal roles in virtually every cellular process. Frequently such interactions are weak and transient. NMR spectroscopy has the unique capability to provide information about such interactions. We will present a brief overview of NMR methods for the characterization of protein-protein interactions. We will also describe the application of NMR in the identification and characterization of small molecules for inhibition of protein function - a primary objective of rational drug design. Application of NMR for studying PPIs will be illustrated by studies of the Mdm2-p53 interaction.The tumour suppressor p53 protein, "the guardian of the genome", has an overarching role in protecting the organism from cancer. In order to escape the safeguard system mediated by p53, nearly all human cancers have compromised the effectiveness of the p53 pathway. The restoration of the impaired function of the single gene, p53, by disrupting the Mdm2-p53 interaction, offers a fundamentally new avenue for anticancer therapy across a broad spectrum of cancers.

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Title: Calculation of hydrodynamic properties of bead models of macromolecules with different-sized overlapping spheres

The Rotne-Prager-Yamakawa (RPY) approximation is commonly used to model the hydrodynamic interactions between small spherical particles suspended in a viscous fluid at low Reynolds number. It is used for investigation of various processes on different scale: from single particle dynamics, through particle-particle interactions to calculation of macroscopic properties like solution viscosity. However, when the particles overlap, the RPY tensors lose their positive definiteness. Here we show, how to generalize the RPY approach to the case of overlapping spherical particles of different radii. So that, the new class of molecules can be described. We also show an example of application of our results to modelling complex macromolecules using bead models.

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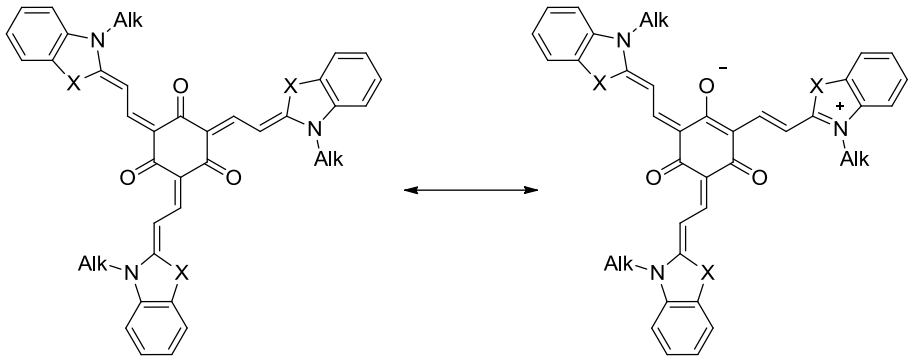
Title: Novel nonlinear octupolar merocyanine chromophores

Design of novel chromophores possessing nonlinear optical (NLO) properties such as two-photon absorption (2PA) has been attracting considerable attention in recent years owing to the prospect of using them in a wide range of applications including two-photon excited fluorescence (TPEF) microscopy of biological objects, optical limiting, 3D microfabrication etc.

From the structural point of view, the majority of 2PA chromophores belong to the family of push-pull polyenes with electron donor and acceptor at the edges of the conjugated system. Merocyanine dyes are similar to polyenes, have electrically neutral chromophore, but in contrast to the latter they are

characterized by more efficient intramolecular charge transfer (ICT) between electron-donor and electron-withdrawing groups, which potentially results in higher two-photon cross-section at the shorter conjugated chain. Our research addresses to synthesis and investigation of novel octupolar conjugated merocyanine dyes as a combination of three dipolar chromophores in one system.[1]



X = $C(CH_3)_2$; S, -CH=CH-

Changing electron-donor properties of the end heterocyclic nuclei makes it possible to change the contribution of polyene and zwitterionic mesomeric forms. This results in changing ICT and dipole moment within the dipolar arms of the octupolar chromophore, and hence allows to control 2PA.

Dyes obtained are characterized by the intense long-wavelength absorption bands within the range λ_{max} = 560-660 nm (ε = 140000-220000) and short-wavelength bands at 418 – 530 nm. Merocyanine dyes of this series possess fluorescent properties and values of fluorescent quantum yield reaches 0.17. Synthesized dyes are also characterized by the intense nonlinear response, values of 2PA cross-section (σ) for such octupolar chromophores are in the range 1200-2000 GM.

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Title: Electrostatics and hydration in aminoglycoside-RNA complexes

Aminoglycoside antibiotics are mainly used to cure hospital-acquired infections caused by aerobic Gram-negative and selected Gram-positive bacteria. They are usually injected because their oral absorption is poor. Applying higher doses of aminoglycosides and their prolonged use often results in their ototoxicity and nephrotoxicity. Another problem is the development of bacterial resistance.

[1] Y. M. Poronik, V. Hugues, M. Blanchard-Desce, D. T. Gryko, *Chem. Eur. J.* **2012**, *18*, 9258–9266.

Therefore, finding new modifications of existing natural and semi-synthetic aminoglycosides could help overcome the resistance and toxicity.

Aminoglycoside antibiotics bind to bacterial ribosomal RNA. Electrostatic interactions are believed to be the main driving force in aminoglycoside recognition by RNA. Based on a computational study of electrostatics and hydration patterns in many aminoglycoside-RNA complexes, we selected aminoglycoside groups that could be modified to achieve better affinity of these antibiotics to the bacterial RNA target.

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Title: Molecular modeling web servers for protein-peptide docking, simulations of protein flexibility, prediction of protein structure and aggregation propensities

Recently, we developed a series of molecular modeling tools dedicated for: protein-peptide docking (CABS-dock) [1], simulations of protein flexibility (CABS-flex) [2], prediction of protein structure (CABS-fold) [3] and aggregation propensities (Aggrescan3D) [4]. These tools have been made available as web servers and can be easily managed via web browser interface. The web servers are freely available from the laboratory website: http://biocomp.chem.uw.edu.pl

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Title: Pet imaging of receptor for advanced glycation end-products in murine model of hindlimb ischemia

Introduction. The endothelial expression of the receptor for advanced glycation end-products (RAGE) was found to be enhanced in patients with a range of peripheral occlusive vascular diseases. In this study we evaluate a novel 64Cu-labeled RAGE-targeted probe as PET imaging agent and report the applicability to non-invasively assess spatial and temporal changes of RAGE expression in murine model of hindlimb ischemia.

Methods. RAGE-targeted PET probe was synthesized by functionalization of PAMAM dendrimer (G4) with Nε-carboxy-methyl-lysine (CML) modified albumine. Control non-targeted probe contained unmodified albumine (HSA). Radiolabeling was performed by chelation of 64Cu with bifunctional p-SCN-Bn-NOTA (Macrocyclics, Inc.). Surgical right femoral artery ligation (HLI) was performed on C57BL/6 male mice (n=12). At 1 week (n=4) and 2 weeks (n=8) post HLI, ~100μCi of 64Cu-G4-CML or 64Cu-G4-HSA were injected via jugular vein and 60 min later in vivo microPET-CT imaging performed. After imaging, mice were euthanized and selected organs and hindlimb muscle tissues were excised for gamma well counting (GWC) of 64Cu activity. Raw counts were corrected for background, decay and weight, and converted to μCi/g using previously determined calibration curve using 64Cu aliquots. For image analysis, cylindrical VOIs were drawn on microPET images to segment ischemic and non-ischemic regions. Counts derived from GWC and image analysis were converted to both ischemic-to-nonischemic ratios (I/N) and percent-injected dose (%ID/g).

Results. Biodistribution studies demonstrated similar retention of both targeted and non-targeted radiotracers. Image analysis correlated well with the GWC results (R2=0.63). Both GWC and microPET imaging permitted quantitative analysis and demonstrated a focal uptake of both radiotracers in ischemic hindlimb. However, the 64Cu-G4-CML uptake was 343% higher than that for the non-targeted probe at 1 wk post HLI, which returned to basal levels at 2 wks post HLI.

Conclusions. In the setting of hindlimb ischemia, augmented RAGE expression/activation can be monitored non-invasively with targeted RAGE imaging. This targeted microPET imaging strategy may allow for serial in vivo studies of RAGE/AGE pathophysiology and has a great potential for translation to clinical studies in patients with peripheral arterial disease (PAD).