P.1.3-027
Fungal bioluminescence system: luciferin, luciferase and luciferin biosynthesis

I. Yampolsky[1,2]
[1]*Institute of Bioorganic Chemistry, Russian Academy of Sciences, Moscow, Russia,* [2]*Institute of Biophysics Siberian Branch of Russian Academy of Sciences, Krasnoyarsk, Russia*

Many living organisms emit light, a phenomenon named bioluminescence. There are estimated to exist ~40 different chemical mechanisms underlying the generation of "cold light". The energy required for light production is generated by the oxidation of a small organic molecule, luciferin, catalyzed by a specific enzyme, luciferase.

More than 100 species of bioluminescent higher fungi are known. The international research group led by the speaker reported elucidation of fungal luciferin in 2015. In 2016 the same team identified and cloned fungal luciferase and the enzymes of luciferin biosynthesis. Discussed will be structure elucidation of fungal luciferin, cloning of fungal bioluminescnce enzymes, light emission mechanism and perspectives of practical applications of fungal bioluminescence.

This work was supported by the Russian Science Foundation grant 16-14-00052.

P.1.3-028
The photosystem II subunit S dynamics under stress

V. Daskalakis, S. Papadatos
Department of Environmental Science and Technology, Cyprus University of Technology, Limassol, Cyprus

The increased spectral range absorption of light exerted by pigments within Light Harvesting Complexes (LHCs) proves an important advantage under low light conditions, in higher plants. However, in the exposure to excess light, oxidative damages and ultimately cell death can occur. It proved, thus, utmost important for the photosynthetic organisms to develop a down-regulatory mechanism called Non-Photochemical Quenching (NPQ). Quantifying this mechanism at the atomic level is still very uncertain. There are several components of the photosynthetic apparatus that are actively involved in NPQ. Apart from the LHCs, and the xanthophyll cycle, the Photosystem II Subunit S (PsbS) is a 22-kDa integral membrane protein that is essential for the response of the photosynthetic apparatus to high-light and it is activated by the protonation of key lumen-exposed glutamate residues. Atomistic details on its involvement in NPQ remain still a mystery. However, It is widely accepted that NPQ (qE) is co-regulated by low lumen pH and ion fluxes (K^+, Ca^{2+}, Mg^{2+}, Cl^-) in Lumen-Stroma areas. It has also been proposed that the activated PsbS may strongly interact with some LHCs enabling quenching by providing an alternative environment for some pigments within these LHCs, or by changing the membrane organization and dynamics. In this study, PsbS (pdb code 4ri2) is embedded in a lipid bilayer model membrane (400–500 POPC lipids, 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine). NPQ conditions are simulated by perturbations in the thylakoid lumen ionic load. Zeaxanthin (Zea) of the xanthophyll cycle that is produced under NPQ is also embedded in the membrane. We employ large-scale Molecular Dynamics simulations to probe the PsbS conformational changes, membrane dynamics, or Zea binding that activate PsbS. We identify two distinct PsbS forms (active-inactive), in response to A) the lumen acidification or ion fluxes, and B) the Zea binding, revealing a PsbS-NPQ relation at the atomic scale.

P.1.3-029
Single-molecule spectroscopy of protein-DNA interactions

G. Rosenblum, F. Wiggers, D. Gruia, D. Liebermann, Y. Harel, H. Hofmann
Weizmann Inst., Rehovot, Israel

Competence in *Bacillus subtilis* is the ability to uptake foreign DNA from the extracellular space. The initiation of this process is a prime example for stochastically driven phenotype switching caused by gene expression noise. The interaction between the transcription factor ComK and its promoter is key for this switch, but the origin of the stochasticity remained elusive. Here, we use single-molecule spectroscopy to study the interaction between the transcription factor ComK and its promoter DNA. Our results show that the structural changes in the promoter during ComK-binding include local rearrangements that are more complex than a simplistic bending model. In contrast to earlier reports, we observe nanomolar affinities (~20 nM) with a cooperativity of binding that varies drastically along the promoter sequence. Our results suggest that at least 10 ComK-molecules bind to its promoter sequence. Together with the estimated *in vivo* copy number of ComK (~100 proteins/cell) this suggests the formation of only a few ComK-DNA complexes per cell. Such a low number of transcription factor-DNA complexes is common for gene expression noise, which may provide an explanation for the stochastic entry into competence.

P.1.3-030
Characterization and effects of binding of food-derived bioactive phycocyanobilin to bovine serum albumin

S. Minic[1], M. Radomirovic[1], M. Radibratovic[2], M. Milcic[1], D. Stanic-Vucinic[1], M. Nikolic[1], T. Cirkovic Velickovic[3]
[1]*Faculty of Chemistry, University of Belgrade, Belgrade, Serbia,* [2]*Institute of Chemistry, Technology and Metallurgy, University of Belgrade, Belgrade, Serbia,* [3]*Ghent University Global Campus, Incheon, South Korea*

Microalga *Arthrospira platensis* (Spirulina) is the next big superfood thanks to composition and the numerous health-related benefits. Phycocyanobilin (PCB) is a blue tetrapyrrole chromophore of C-phycocyanin, the main protein of the microalga Spirulina, with numerous proven benefits for human health. Bovine serum albumin (BSA) is blood and food protein capable to bind various bioactive ligands. In this study, we examined and characterized the binding of PCB to BSA and how it affects protein and ligand stability or activity under physiological conditions. Protein fluorescence quenching and microscale thermophoresis results have shown high-affinity binding ($K_a = 2 \times 10^6$/M). Spectroscopic titration experiment with molecular docking analysis revealed two binding sites on BSA for PCB at the inter-domain cleft and at the subdomain IB, while CD spectroscopy indicated the stereo-selective binding of *P* conformer of pigment to protein. In contrast, previous studies have found binding of *M* conformer of PCB to Human Serum Albumin (HSA) at subdomains IB and IIA. Although HSA and BSA have high sequence similarity, subtle differences between the tertiary structures of the two albumins is the most likely explanation for the partial divergence in the binding location of the tetrapyrrole ligand. BSA-PCB complex has increased thermal stability than free protein. Gel electrophoresis data from pepsin digestion study suggest that saturated PCB binding slightly increases digestive stability of protein. Although complex formation partly masked the antioxidant properties of PCB and BSA, a mutually

protective effect against free radical-induced oxidation was found. These results point to subtle differences between PCB binding for bovine *vs.* human serum albumin and that Spirulina health supplements consumption with BSA-containing foods could change bioavailability and bioactivities of participating molecules.

P.1.3-031
Kinetics of aggregation of phosphorylase kinase from rabbit skeletal muscle

N. Chebotareva, S. Roman, T. Eronina, V. Mikhaylova, B. Kurganov
Department of Structural Biochemistry of Proteins, Bach Institute of Biochemistry, Federal State Institution "Federal Research Centre "Fundamentals of Biotechnology" of the Russian Academy of Sciences", 33, bld. 2 Leninsky Ave., Moscow, Russia

Phosphorylase kinase (PhK; EC 2.7.1.38) plays a key role in the regulation of glycogen metabolism in skeletal muscle. PhK with molecular mass of 1320 kDa has a complex molecular organization. The PhK molecule consists of four subunits that form a hexadecamer $(\alpha\beta\gamma\delta)_4$, where the γ-subunit possesses the catalytic activity and other subunits regulate its activity. Ca^{2+} and Mg^{2+} ions stimulate PhK activity by inducing changes in the tertiary and quaternary structure of the molecule and also stimulate association/aggregation of PhK hexadecamer molecules. Ca^{2+}-free PhK and PhK molecules in the presence of Ca^{2+} and Mg2+ ions have different conformations and physicochemical properties. The kinetics of association/aggregation of PhK from rabbit skeletal muscle was studied at 40°C, close to the average physiological temperature of the rabbit, using dynamic light scattering (40 mM Hepes, pH 6.8; 0.1 mM Ca^{2+}, 10 mM Mg^{2+}, 0.1 M NaCl). The initial rate of aggregation was calculated from the kinetic curves describing an increase in the light scattering intensity with time. Based on the analysis of the initial rate of aggregation, depending on the initial concentration of the protein, it has been concluded that the order of aggregation with respect to protein is equal to unity. Thus, the rate-limiting stage of heat-induced aggregation of PhK under used conditions is the stage of unfolding of the protein molecule. Construction of a plot showing the relationship between the light scattering intensity and hydrodynamic radius (R_h) of protein aggregates indicates that the initial stage of PhK aggregation is the stage of formation of the start aggregates. It was shown that the hydrodynamic radius of start aggregates formed at PhK concentration of 0.1 mg/mL is 29 nm and increased to 56 nm at [PhK] = 0.8 mg/mL.

This work was funded by the Russian Science Foundation (grant 16-14-10055).

P.1.3-032
Negative charge and membrane tethered viral 3B cooperate to recruit viral RNA dependent RNA polymerase 3Dpol

A. Dubankova, J. Humpolickova, J. Silhan, A. Baumlova, D. Chalupska, M. Klima, E. Boura
Institute of Organic Chemistry and Biochemistry of the CAS, Prague, Czech Republic

RNA dependent RNA polymerases (RdRp) are key enzymes for +RNA viruses. These enzymes are not present in human genome which makes viral RdRp an ideal target for drug design. Therefore, RdRps are highly studied enzymes, however, their regulation is not fully understood. Most +RNA viruses replicate at replication organelles (ROs); membranous structures derived from the host intracellular membranes (Golgi or ER most often). These ROs provide microenvironment needed for efficient viral replication and also provide shelter from innate intracellular immunity while serving as a platform for viral replication. The lipid hallmark of the RO membrane is phosphatidylinositol 4-phosphate (PI4P). However, +RNA viruses do not possess any phosphatidylinositol 4-kinase (PI4K), instead they hijack the human enzyme PI4K. Many picornaviruses use the Golgi resident acyl-CoA-binding domain-containing protein-3 (ACBD3) to hijack the lipid kinase PI4KB. Using pure recombinant proteins and biomimetic model membranes we show that the nonstructural viral 3A protein is sufficient to induce membrane hyperphosphorylation given the proper intracellular cofactors PI4KB and ACBD3. However, our bio-mimetic *in vitro* reconstitution revealed that not PI4P but rather the negative charge is responsible for the recruitment of RdRp enzyme to the viral replication sites. Additionally, we show that membrane tethered 3B protein cooperates with the negative charge to increase the efficiency of RdRp membrane recruitment.

P.1.3-033
Interaction of the N-terminal extension of myosin essential light chain-1 with F-actin studied by fluorescence resonance energy transfer

D. S. Logvinova[1,2], A. M. Matyushenko[1,2], O. P. Nikolaeva[3], D. I. Levitsky[1]
[1]A.N. Bach Institute of Biochemistry, Research Center of Biotechnology of the Russian Academy of Sciences, Moscow, Russia, [2]Department of Biochemistry, School of Biology, Moscow State University, Moscow, Russia, [3]A.N. Belozersky Institute of Physico-Chemical Biology, Moscow State University, Moscow, Russia

Rabbit fast skeletal muscle myosin has two isoforms of the essential light chain (ELC), called LC1 and LC3. The LC1 differs from LC3 by the presence of N-terminal extension of 41 residues containing seven pairs of Ala-Pro repeats, which form an elongated structure, and two pairs of Lys residues near the N-terminus. When isolated myosin head (myosin subfragment 1, S1) binds to F-actin, these Lys residues may interact with the C-terminus of actin thus forming an additional actin-binding site on S1. Here we applied fluorescence resonance energy transfer to measure for the first time the distances between Cys374 on actin and different sites on the N-terminal extension of LC1 associated with S1. Cys374 of actin was labeled with 1,5-IAEDANS as a donor, and S1 was reconstituted with various recombinant LC1 mutants which were fluorescently labeled with 5-IAF (acceptor) at different positions in their N-terminal extension and then introduced into the S1 regulatory domain. At physiological ionic strength (120–150 mM NaCl) and S1:actin molar ration equal to 1:3 (i.e. under conditions when the LC1 N-terminal extension interacts with actin), the following distances were calculated between Cys374 on actin and different sites on the N-terminal extension of LC1: >6 nm to Cys41, 4–5 nm to Cys15 located among Ala-Pro repeats, and 3–4 nm to Cys residues located near Lys residues at the N-terminus. At higher ionic strength (above 300 mM NaCl) and S1:actin molar ration equal to 1:1 (i.e. under conditions preventing interaction of the LC1 N-terminus with actin) all these distances significantly increased. These results are consistent with the previously proposed concepts and also reveal new interesting details of the molecular mechanism of the interaction of LC1 N-terminal extension with actin, which may play an important role in actin-myosin interaction during muscle contraction.

This work was supported by RFBR (grant 15-04-03037).