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Meeting Abstracts

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Part-2 Featured Presentations

Protein Crystal Modeling Using Method of Molecular Dynamics Simulation

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Abstract

It was previously shown practically and theoretically that the Langmuir-Blodgett (LB) crystals have higher stability against radiation damage in comparison with the classical hanging drop (HD) crystals similarly to the space-grown (SG) crystals [1-3]. However, it remains still unclear how and to what extend the shape and micrometer- and nanometer- scale structure of crystals, the methods utilized for the crystal growing and the parameters of an X-ray beam influence the crystal stability and the damage level.

This work is an extension of the previous work [3], and it aims to improve our understanding of the exact organization of crystals, solute and salt orientation around the proteins. MD simulations of crystal lattices containing 16 lysozyme proteins are carried out in different solutions (water and real buffer solution) as well as at different temperatures in order to understand the differences in crystal stability.

The force field is CHARMM36 (full-atom) and MD simulation package is GROMACS.

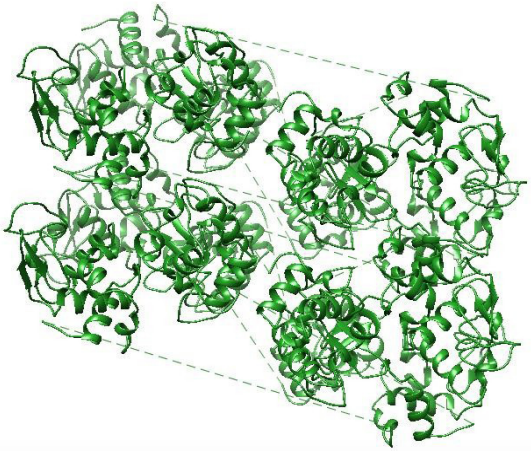

Figure 1: The triclinic supercell containing 16 lysozyme proteins.

The temperature of equilibrium simulation was 293 K. In all simulations periodic boundary conditions were used. The time step is 2 fs, the thermostat is V-rescale (modified Berendsen, with time constant 0.1 ps) and barostat is Nose-Hoover (isotropic pressure, 1 atmosphere for all simulations). Standard structural analysis is provided in order to compare different structures: RMSD and RMSF functions, secondary structure analysis, crystal disorder parameter and analysis of contacts between proteins.

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Beyond Rhodopsin - Cephalopods as a Source of New Interesting Biomaterials for Bioelectronics

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Abstract

The remarkable morphological and behavioral innovations of soft-bodied cephalopods (camera-like eyes, prehensile arms, adaptive colouration system) represent enormous interest for comparative evolutionary, developmental and physiological studies and for various areas of bioinspired engineering. In particular squid and octopus rhodopsins have proved to be a promising starting point for development of optobioelectronoic devices due to unique combination of photochromic and electrooptical properties, in particular, photostability and photoreversibility [1].

Reflectins (RF) and suckerins (SK) are other recently discovered groups of proteins holding significant potential for application in bioelectronics and advanced materials (Figure 1). RFs are found in squid reflective tissues and play key role in cephalopod structural coloration due to their unusual structure and intrinsic ability to undergo reversible changes in condensation and assembly in response to phosphorylation in vivo [2]. Recombinant *E. coli*-made RFs can be manufactured in different types of coatings and films, possessing such properties as wide dynamic tunability of their reflectance, proton conductivity, ability to support stem cell attachment, proliferation, and differentiation [3].

SKs are the sole building blocks of sucker-ring teeth of squids and cuttlefish. These unique silk-like structural proteins self-assemble through hydrogen bond interactions into a robust supramolecular network with mechanical properties that match strong synthetic polymers. Recombinant SKs may assemble into colloids of discrete dimensions in solution, may be fabricated into more complex micro and nanostructures and coated with gold nanoparticles. Due to high Tyr content the elastic properties of SK-based hydrogels and films may be altered by several orders of magnitude by simple modulation of crosslinking conditions to match the elasticity of a wide range of human tissues, from soft liver to stiff bone, thus opening promising avenues in tissue engineering and restorative applications [4].

The ease of recombinant RFs an SKs production in *E. coli* opens new exiting opportunities for altering their properties using protein engineering and chemical modification techniques. Recent genomic and proteomic studies, including the deciphering the octopus genome [5] had revealed wide diversity of RFs and SKs, their close and distant orthologs, other interesting cephalopod proteins that deserve further exploration using recombinant protein expression and nanoscale characterization and manufacturing techniques.

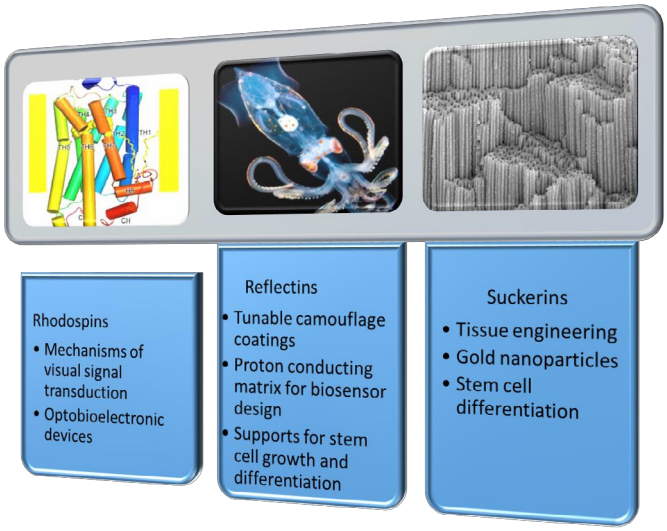

Figure 1: Cephalopod proteins for nanobiotechnology, bioelectronics and advanced materials.

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The Relationship Between Polyamine and Methylation Levels: From Fungi to Cancer Cells

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Abstract

Polyamines (putrescine, spermidine, and spermine) are small, naturally occurring, polycationic alkylamines that are essential for cell growth and development in eukaryotes. Under normal physiological conditions, intracellular polyamine concentrations are tightly regulated through a dynamic network of biosynthetic and catabolic enzymes. The level of polyamines correlates with the rate of cell proliferation. There is also a strong correlation between increased levels of polyamines and different types of cancer. Polyamines are considered fuel to sustain elevated proliferation rates in cancer cells [1]. Quiescent cells have lower polyamine levels than cycling cells. In contrast, in cancer cells the polyamine homeostasis is dysregulated, in many types of cancer the abnormally elevated polyamine levels is observed. Polyamines can be exchanged through gap junction channels between mammalian cells, this process is proposed to play one of key roles in proliferation of tumor cells [1].

Associated with tumor growth polyamine content is regulated by two key enzymes ornithine decarboxylase (ODC) and S-adenosyl methionine decarboxylase (SAMeDC). A number of polyamine metabolism inhibitors (particularly, against ODC and SAMDC) are proposed as drugs for the cancer treatment. From the other side global hypomethylation of genomic DNA is observed in numerous tumor cells and is responsible for overexpression of proto-oncogenes, growth factors and genes which via their protein products are involved in cancer cell proliferation, invasion, and metastases [2]. Since DNA methylation and polyamine metabolism depend on a common substrate, S-adenosylmethionine (SAMe), interaction between both pathways can be expected [2]. Little is known about the relationship between these pathways but studies are available indicating that polyamines and DNA methylation are directly or indirectly interconnected, metabolically as well as physiologically with respect to the regulation of cell growth, differentiation and cancer development [3].

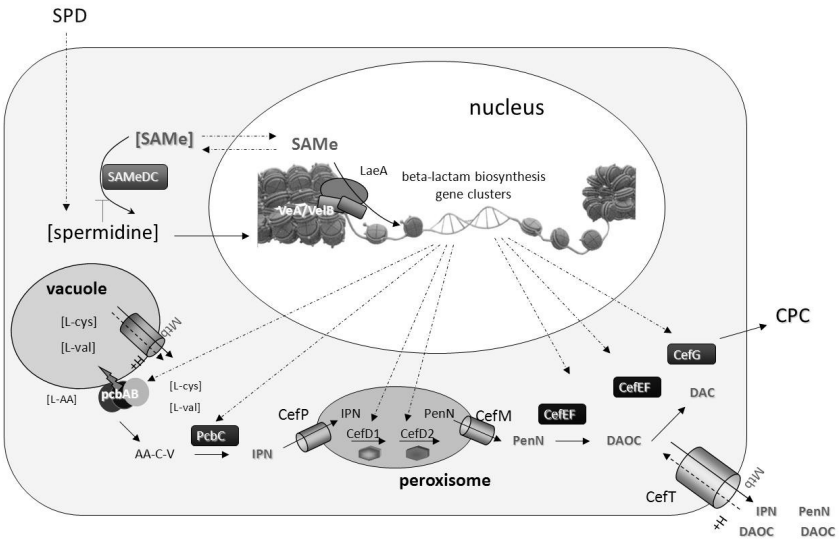

Figure 1: The model of relationship between exogenous polyamines and LaeA- dependent methylation through SAMe in *Acremonium chrysogenum*.

SPD – spermidine; CPC – cephalosporin C; AA-C-V – tripeptide δ-(L-α- aminoadipyl)-L-cysteinyl-D-valine; IPN – isopenicillin N; PenN – penicillin N; DAOC – deacetoxy cephalosporin C; DAC – deacetyl cephalosporin C; Mtb – metabolite;

pcbAB – L-a-aminoadipyl-L-cysteinyl-D-valine-synthetase; pcbC – isopenicillin N-synthase; cefD1 – isopenicillin N-CoA synthetase; cefD2 – isopenicillin N-CoA epimerase; cefM – multidrug efflux pump protein, putative role in penicillin N transport; cefP – multidrug efflux pump protein, putative role in isopenicillin N transport; cefT – multidrug efflux pump protein; CefEF – deacetoxycephalosporin C synthetase/ hydroxylase; cefG – acetylCoA: deacetylcephalosporin C acetyl transferase.

In the experiments with filamentous fungi the addition of exogenous polyamines raised the expression level of SAMe-dependent histone methylase LaeA, the global transcriptional regulator of fungal secondary metabolism [4, 5]. The epigenetic activity of LaeA led in its turn to the increase of beta-lactam antibiotics profile. Apparently the exogenous administration raised the polyamines cell level, reduced their biosynthesis and SAMe consumption which stimulated LaeA- dependent histone methylation and beta-lactam gene expression (Figure 1). This example of the relationship of polyamines and methylation in lower eukaryotes may facilitate the understanding of how the biosynthesis of polyamines and methylation works in healthy and cancerous human cells.

Acknowledgments: Supported by RFBR grant 15-08-03672.

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Investigation of Binding Efficiency of Substituted Polycationic Metallophtalocyanines to Lipopolysaccharides of Gram-Negative Bacteria

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Abstract

Gram-negative bacteria are the causative agents of numerous diseases. Their cell walls with additional outer membrane protect cells from external factors. Lipopolysaccharides (LPSs) of the outer membrane form the first line of bacterial defense. Released from the cell wall, LPS molecules exhibit endotoxic activity. Along with that, these negatively charged molecules serve as potential binding centers for cationic antimicrobials [1]. The presence of positively charged peripheral substituents in polycationic phtalocyanine (Pc) molecules and the ability to create covalently grafted forms allow the use of Pc for photodynamic antimicrobial therapy and disinfection [2, 3]. To study Pc electrostatic binding to bacterial cells, we measured zeta potential neutralization and Pc fluorescence quenching for micelles formed by LPS molecules and LPS molecules embedded into liposomes. The binding capacity of Pc and its photodynamic effectiveness were determined for bacterial strains with various LPS structure and negative cell potential. We showed that the sensitivity of Gram-negative bacteria to photosensitization strongly varied depending on strains and species that possessed different affinity to polycationic dyes.

LPS molecules include three domains: lipid A, inner and outer core oligosaccharides, and O-specific polysaccharide (O-antigen). LPSs' negative charges originate from phosphate, pyrophosphate and carboxyl groups located mainly in the inner core region. Re mutant is a strain without O-antigen chain and with the minimal structure of core part required for bacterial survival [4]. We characterized MALDY mass spectrum of Re mutants and resolved its precise molecular structure (Figure 1). On the base of this Re mutant structure we created molecular dynamic (MD) model of LPS membrane of Gram-negative bacteria. The MD method makes possible calculation of basic features of the model bacterial membrane such as surface area per lipid and electron density. We performed 1200 ns MD simulation. Over the time span of 1200 ns the surface area per lipid holds constant value in the simulation. Electron density study of Re mutant membrane revealed almost symmetric profile, which confirms stability of the model membrane. MD simulation is performed using a software package GROMACS.

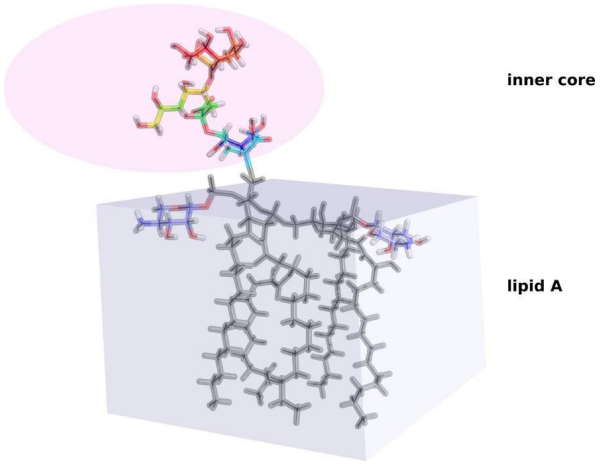

Figure 1: The molecular structure of Re mutant LPS molecule.

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Computer Simulations of Radiation Damage in Protein Crystals

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Abstract

Modern X-ray crystallography is a dominant method for structure determination of large biomacromolecules such as proteins, nucleic acids and their complexes. It allows to reconstruct atomic structure of biomacromolecular specimens with the atomic weight up to millions Daltons and with the resolution down to 1 Å. However, crystal damage due to various interactions of matter with the X-ray radiation (Figure 1A) limits time of the diffraction data deposition and, thus, resolution of the obtained electron density maps, which serve as a starting point for determination of the atomic positions. It remains still unclear how and to what extend the shape and micrometer- and nanometer- scale structure of crystals, the methods utilized for the crystal growing and the parameters of an X-ray beam influence the damage level.

The Monte Carlo approach has gained wide acceptance in multiple fields of natural science and technology as an effective tool for rapid integration in the highly dimensional spaces. It is extensively utilized in nuclear physics, astrophysics, molecular modeling, elementary particle physics, analysis of the diffraction data, etc.

In the present work we have developed a computational routine, which allows one to prepare the molecular geometries corresponding to arbitrary protein crystals (e.g., grown under different conditions or using different techniques) and subsequently simulate the initial processes of radiational damage in them by means of the Monte Carlo approach.

The workflow of the suggested procedure for estimation of the radiation damage is shown in Figure 1B. Firstly, the molecular dynamics approach is used in order to prepare and optimize realistic models of protein crystals including the crystallization buffer. The optimized molecular models of protein crystals are imported then into the Monte Carlo (MC) simulation program code [1] using a corresponding routine written by us using the Python language. Finally, the different observables can be drawn from the MC simulations, such as the total energy deposited in the crystal or number of the happened events of interest (such as photoionization). These analysis is done by our own Python scripts.

In future, we are planning to apply the developed procedure for estimation of the radiation damage in the models of protein crystals of various size and with the different crystallization buffer contents in order to investigate a possible role of the utilized crystallization techniques for the resistance of protein crystals against radiation.

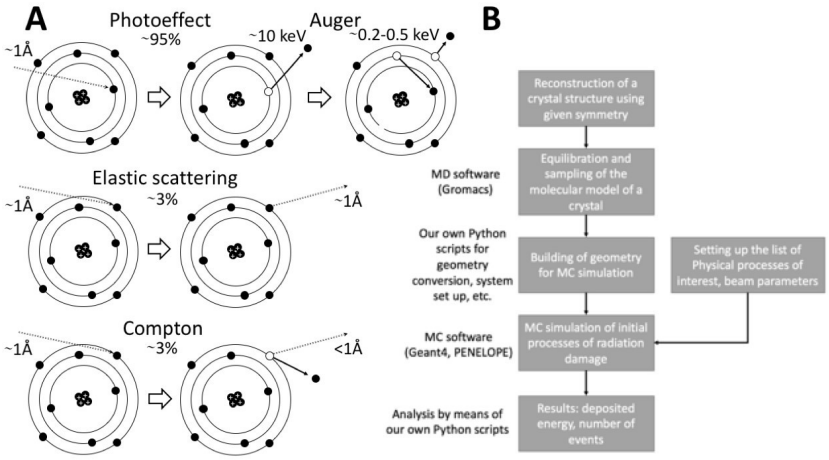

Figure 1: (**A**) Types of interaction of the X-ray photons with matter (electrons are shown as black cycles, electron holes as white cycles, photon tracks as dashed arrows, electron tracks as solid arrows). The four dominant processes are the photoeffect, the Auger effect, the elastic scattering (scattering without the loss of energy, which is responsible for diffraction) and the Compton scattering (scattering with the loss of energy). (**B**) The workflow of a setup of a molecular system representing protein crystal and the subsequent Monte Carlo (MC) simulation. In the left, the programs/tools used in the current procedure are shown.

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Complexes of MADS-Box Transcription Factors

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Abstract

Preceding to the divergence of plants and animals, gene duplication lead to the emergence of Type I and Type II MADS-box genes lineages [1]. In plants, MEF2-like Type II MADS-box transcription factors (TFs) family is the one of the most influential regulatory group. The quaternary complexes of MADS-box proteins described by the ABCDE model specify the spatial-temporal flower organs identity and development [2].

Eleven and nine MADS-box cDNAs (CDM and HAM) have been isolated from *Chrysanthemum morifolium* and *Helianthus annuus*, respectively [3]. Functional analysis of these genes was carried out, including structural-phylogenetic specification, expression pattern assay, protein-protein interactions detection in the yeast GAL4-system, the establishing and characterization of model transgenic plants with ectopic expression of CDM and HAM genes (Figure 1(A-C)) [3]. Among them, we revealed the groups of MADS-box genes responsible for the flowering time definition, the identity of flower meristem, perianth, gynoecium and androecium, and the termination of flower meristem. In collaboration with Plant Research International (the Netherlands), the results supporting the model that a C, D, and E-type MADS-box proteins complex controls the stem cell population in the floral meristem were obtained [3]. All our data support the hypothesis about MADS-box genes evolutional origin and diversification through the gene duplication mode, and the conservatism of orthologous MADS-box gene functions and the major developmental mechanisms including MADS-box genes in extant plants. Some revealed features of MADS-box genes activities typical for Asteraceae development indicates the continued plant evolution.

In vertebrates, four MEF2 family members function during embryogenesis, postnatal and adulthood development [4]. In human, MEF2 proteins regulate cardiac, neural, blood and other cell shape, differentiation, proliferation, movement, death and metabolism, while MEF2s with altered activity, being the oncogenes and tumor suppressors, are involved in development of several cancer types. These proteins are considered to be nodes in complex regulatory networks [5].

Human and plant MEF2-like proteins phylogenetic tree rooted with TOPOIIA-A, the putative MADS-TFs ancestor, shows human MEF2 earlier origin compare to plant MADS-box proteins (Figure 1C). The use of genome- and transcriptome scale technologies and comparative interspecies and inter-kingdom genomics may contribute to our understanding of MEF2-

like transcription factors functioning.

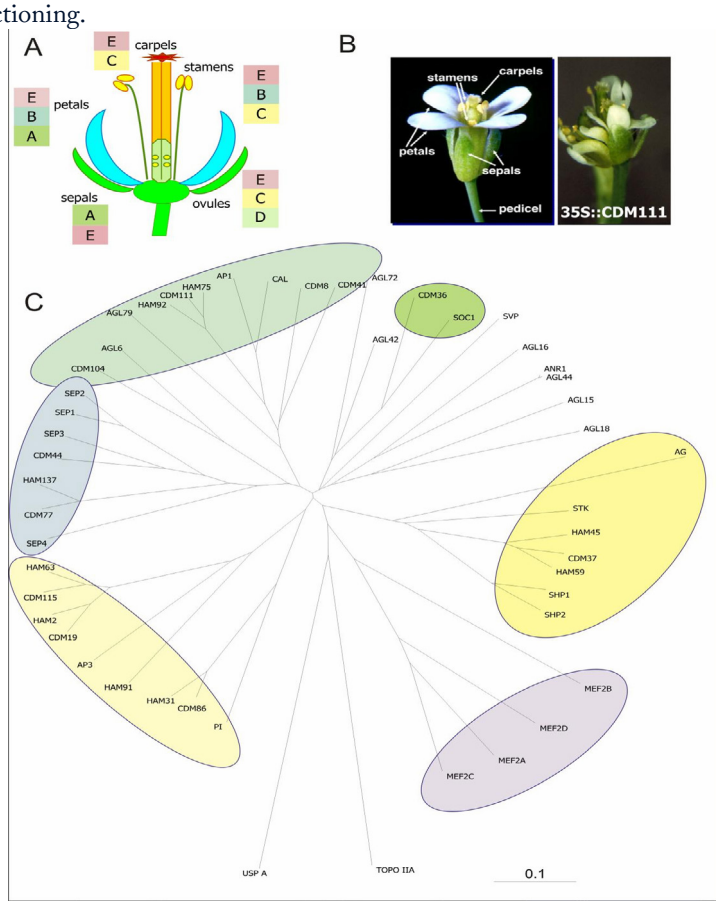

Figure 1: MADS-box transcription factors in plant development. (**A**) Genetic ABCDE model of flower: organ differentiation. (**B**) Transgenic A. thaliana flower over-expressing chrysanthemum MADS-box gene CDM111. (**C**) Phylogeny of *C. morifolium* and *H. annuus* MADS-box genes. The tree was rooted with human MEF2 transcription factors.

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Modular NanoTransporters for Cancer Therapy. Novel Constructions and Their Interactions

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Abstract

In the field of anticancer drug discovery, a great effort is done to reduce normal cell damage by the drug, that can be achieved by using locally acting agents, especially Auger electron emitters (AE). As they undergo decay, high ionising density electrons are emitted. It has been shown that AE do not possess cytotoxicity when being outside cells and should be transported to the cellular nucleus in order to become toxic [1].

With the purpose of targeted drug delivery to the nucleus, Modular NanoTransporters (MNTs) have been invented in our lab. MNT is a chimeric protein molecule which consists of several modules, each responsible for a specific stage of delivery. Its ligand module binds to over-expressed internalizable receptors on the target cell, MNT undergoes receptor-mediated endocytosis and turns out into acidifying endosomes. Then endosomolytic module activates, and MNTs form pores in endosomal

membranes. Due to this module MNT molecule undergo endosome escape. In the cytoplasm nuclear import module interacts with importin dimer, and MNT molecule (with the tagged AE) undergoes nuclear import [2].

It has been shown, that 111Indium cytotoxicity is enhanced by 270 times when this AE is tagged to the MNT [3]. Now we see two ways of further MNT improvement. The first one is increase cytotoxicity of MNT by putting the AE in a close proximity to DNA. To do this, one additional module, derived from eukariotic p21 protein. has been added. p21 interacts with PCNA (a sliding clamp on DNA) via its C-terminal domain [4]. Ka for p21-MNT – PCNA interaction measured by surface plasmon resonance method was 2.75 ·107 1/M which is consistent with the literature data for p21 [4]. Another way to improve MNT is to extend its plasma half-life by adding an Albumin Binding Domain (ABD) of Streptococcus Protein G [5]. Kd for interaction ABD-MNT – BSA, measured by microscale thermophoresis method, was 23 ± 9 nM.

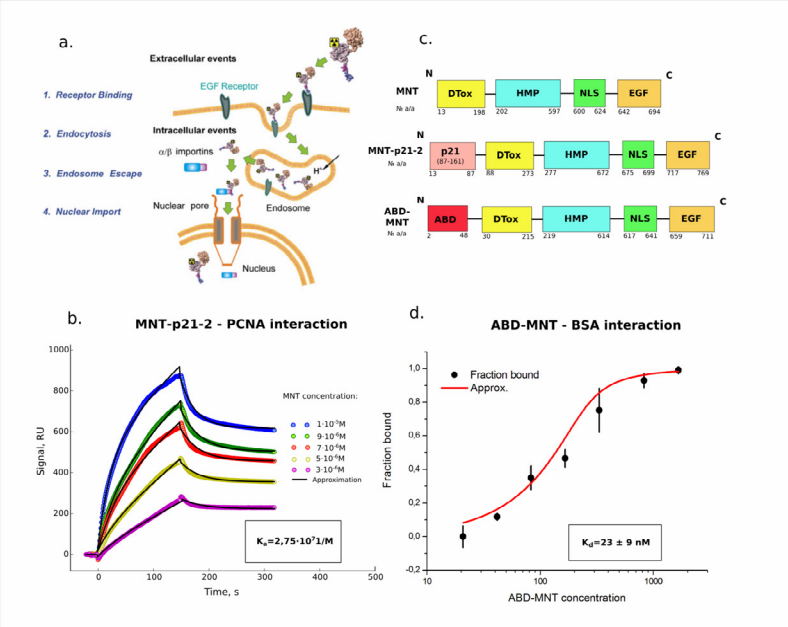

Figure 1: (**a**) Steps of targeting MNT from the cell surface to the nucleus [2], (**b**) SPR curves for interaction p21-MNT – PCNA, approximated with a two-state interaction model, (**c**) schematic representation of sequence of modules in different MNTs: DTox – translocation domain of a diphteria toxin, HMP – hemoglobin-like protein, NLS – nuclear localization signal, EGF – epidermal growth factor, p21 – C-terminal fragment of p21 protein, ABD – albumin binding domain, (**d**) interaction curve for ABD-MNT – BSA, measured by a microscale thermophoresis method.

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Comparative Proteome Profiling of *Acremonium chrysogenum* Strains After Fermentation with 1, 3-diaminopropane and Spermidine

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Abstract

Polyamines play multiple roles in cell growth, survival, proliferation and are associated from one side with longevity [1] and from the other - with diseases and cancer [2]. A number of contributions for understanding the polyamine functions in cancer

have been made due to research on microorganisms [3].

In last decade it was shown that biogenic polyamines - 1,3-diaminopropane (1,3-DAP) and spermidine (SPD) - can increase the expression of beta-lactam biosynthetic genes in filamentous fungus *A. chrysogenum* [4]. These effects are mediated by LaeA - a global transcriptional regulator of filamentous fungi, controlling epigenetically expression of fungal secondary metabolism and differentiation genes by heterochromatin reorganization. We performed comparative analysis of the influence of exogenous polyamines on cephalosporin C (CPC) production and protein expression in industrial and laboratory stains of *A. chrysogenum* ATCC 11550 (wild-type) and *A. chrysogenum* RNCM F-4081D (HP, high-producer), differing more than hundred-fold in the rate of CPC biosynthesis.

We applied the proteomic profiling by the tandem mass spectrometry method to reveal the differences after the addition of 5mM of 1,3-DAP and 5 mM SPD to the fermentation medium. CPC biosynthesis was stimulated by 1,3-DAP and SPD in both strains. Differentially expressed proteins were identified and analyzed by using gene ontology (GO) classification and various bioinformatics tools (Figure 1).

The results were correlated with previous data of comparative expression for genes, involved in primary and secondary metabolisms [5], as well as, reveal a number of other proteins that are differentially expressed after the addition of exogenous polyamines. Enriched gene ontology analysis indicates on possible balance between polyamine, beta-lactam and sulfur-dependent biosynthetic levels and may explain the so-called "methionine puzzle" – known for decades but still unclear positive regulation of exogenous methionine on the production of CPC in *A. chrysogenum*.

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Figure 1: (**a**) The comparative analysis of proteomes of *A. chrysogenum* ATCC 11550 and ⊠⊠ F-4081D strains after 120 h of fermentation in GO terms; (**b**) Venn diagrams from the comparative proteome analysis of *A. chrysogenum* ATCC 11550 and

F-4081D strains after 120h of fermentation with 5 mM 1,3-DAP and 5 mM SPD.

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Marine Actinobacteria Clade Rhodopsin (MACR) Crystallization

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Abstract

As membrane proteins perform signal, transport, enzyme and other vital cellular functions, studying of their structure could help us to understand cellular processes more deeply. Protein structure can be solved by using several methods in structural biology such as NMR, SAXS, Cryo-EM, etc. However, X-ray diffraction analysis is still the critical method in the field of structural biology. X-ray crystallography tool demands proteins in form of crystals for diffraction measurements. Crystal preparation is a weak point in protein structural analysis especially for membrane proteins and their complexes. The lipid cubic phase (LCP) crystallization is one of the most promising modern methods. LCP is a type of lipid phase, it consists of a single bicontinuous lipid layer, so protein molecules can diffuse thought all lipid layer, while this process is embarrassed in other lipid phase types. There are more than 200 structures in protein data bank to date, related to LCP crystallization, it makes the method very promising to obtain new structures. [1]. However, an additional investigation of this method is necessary and it demands model proteins.

Marine Actinobacteria clade rhodopsin (MACR) is a member of light-sensitive proteins family which has a number of applications today [2]. Similar to other rhodopsins MACR is an integral membrane protein with seven transmembrane -helices and loops between them. The unique feature of MACR which makes preparation of crystals easier is that it is smaller in comparison with other rhodopsins and, even more important, MACR has shorter loops which are the most flexible parts in a protein and may prevent crystallization process. Recombinant MACR can be expressed in E. coli and purified by standard protocol [3].

We have shown that MACR can be crystallized in LCP in a wide range of PEGs and salt conditions. Different buffers and pH can be used for crystallization. So far, we have received three forms of MACR crystals: two with plate-like shape and one with hexagonal shape (Figure 1). Here we show that MACR can be readily crystallized in LCP in various crystal forms. It can be used in diverse perspective directions such as studies of LCP crystallization mechanism, X-ray radiation damage and other crystallographic studies.

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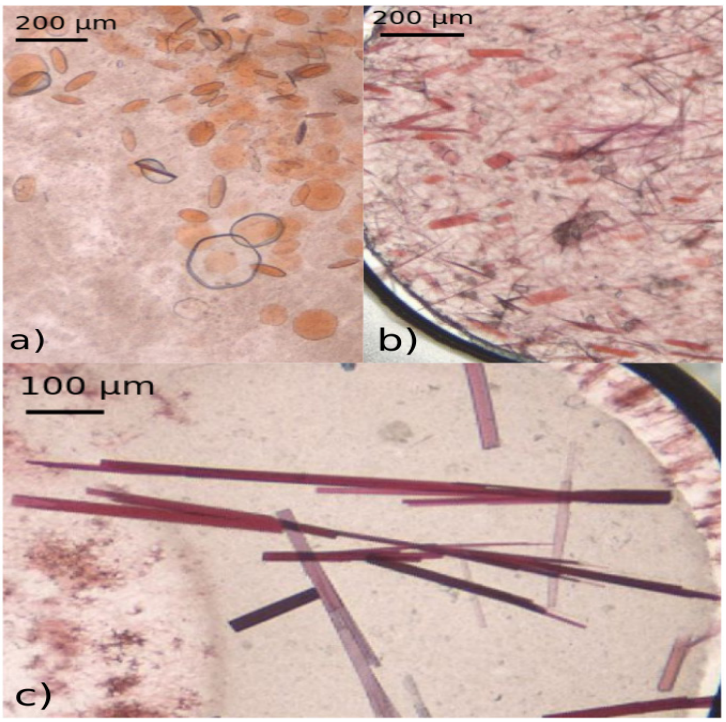

Figure 1: Three forms of MACR crystals. (**a**) Hexagonal shape, (**b**, **c**) plate-like shape.

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Quasi-Smiles as an Alternative of Smiles for Building Up Predictive Models of Nanomaterials Endpoints

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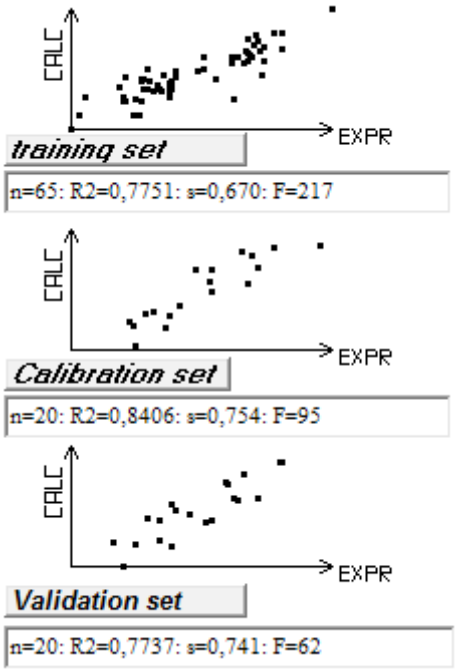
Abstract

Traditional quantitative structure – property / activity relationships (QSPRs/QSARs) are a tool to predict the physicochemical and or biochemical behavior of organic compounds. This prediction is based on structural descriptors which are representing of architecture of their molecules. Molecular architecture can be represented by simplified molecular input-line entry systems (SMILES), i.e. line of characters encoded the structure. Attractive alternative of the SMILES are quasi-SMILES, which encoded physicochemical or biochemical conditions of acting of nanomaterials. Thus, quasi-SMILES give possibility to use paradigm "endpoint is a mathematical function of conditions" instead of paradigm "endpoint is a mathematical function of molecular structure". In praxis, quasi-SMILES are lines of characters, which reflect impact of different doses (concentrations), presence or absence of irradiation or heating, and so on. In addition, the quasi-SMILES give possibility to predict impact of mixtures of substances in general and nanomaterials in particular. This approach has been studied in recent works [1-5].



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