RICCEM2023

RUSSIAN INTERNATIONAL
CONFERENCE ON CRYO-ELECTRON
MICROSCOPY
JUNE 4-7

BOOK OF ABSTRACTS

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SECTION I:

STRUCTURE AND FUNCTIONS OF THE TRANSCRIPTION AND TRANSLATION APPARATUS OF THE CELL

Poster abstract
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THE ROLE OF THE RIBOSOMAL PROTEIN UL5 IN THE ASSEMBLY OF THE 50S RIBOSOMAL SUBUNIT IN *ESCHERICHIA COLI*

Baymukhametov T.N.[1], Kravchenko O.V.[2], Afonina Z.A.[2], Korepanov A.P.[2]

[1] National Research Center, "Kurchatov Institute", Moscow, Russia
[2] Institute of Protein Research, Russian Academy of Sciences, Pushchino, Russia

Background: The ribosomal protein uL5 is an essential component of the large ribosomal subunit. In *Escherichia coli*, three proteins, uL5, uL18 and bL25 bind to the 5S rRNA and together form the so-called 5S rRNA-protein complex (5S rRNP), a substantial part of the central protuberance of the 50S ribosomal subunit. Both uL5 and uL18 are required for the attachment of the 5S rRNP to the 23S rRNA and it has long been thought that the 5S rRNP is incorporated during the final steps of the 50S assembly. However, we have shown previously that in the absence of uL5 defective "45S" ribosomal particles are formed in *E. coli*. These particles lacked 5S rRNA and a number of ribosomal proteins belonging to the central protuberance suggesting that uL5 may play an important role early in the 50S assembly. In the present study, we performed cryo-EM analysis and compared the reference 50S ribosomal subunits to the defective "45S" particles formed in *E. coli* cells upon depletion of uL5.
Methods and Results: Quantifoil grids with an additional 2-nm carbon layer were glow-discharged using PELCO easiGlow. Samples were applied to the grids, blotted, and plunge-frozen in liquid ethane using a Vitrobot Mark IV. The datasets were collected using a Titan Krios transmission electron microscope equipped with a spherical-aberration corrector and a Falcon II direct electron detector. Datasets were processed using the following pipeline: Warp – CryoSPARC – RELION (Bayesian polishing) – CryoSPARC – DeepEMhancer. It turned out that the "45S" particles are devoid of the 5S rRNA and ribosomal proteins uL16, uL18, bL25, bL27, bL31, bL33, bL35 and bL36. Electron density was missing for the apical part of the A-site finger (helix 38, domain II of the 23S rRNA) and helices 80, 81, 82, 83, 84, 85, 86, and 87 (domain V). Helices 42, 43, 44 (the GTPase-associated center, domain II); 68, 69, 71 (domain IV); 76, 77, 78 (the L1 protuberance), 74, 89, H90, H91, H92 (adjacent to the peptidyl-transferase ring, domain V) are destabilized.
Conclusion: Our data suggest that uL5 (or, broadly speaking, the 5S rRNP) is required for the assembly of a large portion of the 50S. In the absence of uL5, 23S rRNA regions belonging to different domains are destabilized, including all main functional centers of the 50S subunit.
Key words: ribosome assembly, uL5, *Escherichia coli*, cryo-EM.

This work was supported by the Russian Science Foundation (Grant No. 19-74-20186, https://rscf.ru/en/project/19-74-20186/).

**** Corresponding author:*** *Timur Baymukhametov. E-mail: baymukhametov.timur@gmail.com*

Poster abstract
Abstract category: Structure and functions of the transcription and translation apparatus of the cell

NEW HELICAL CONFORMATION OF THE C-TERMINUS OF THE RIBOSOMAL BINDING FACTOR A FROM *STAPHYLOCOCCUS AUREUS* OBSERVED BY CRYO-EM

Bikmullin A.G.[1], Fatkhullin B.[2], Stetsenko A.[3], GuskovA.[3], Garaeva N.S.[1], Klochkova E.A.[1], Usachev K.S.[1], Yusupov M.M.[2]

[1] Kazan Federal University, Kazan, Russia
[2] Institut de Génétique et de Biologie Moléculaire et Cellulaire, Université de Strasbourg, Illkirch, France
[3] University of Groningen, Groningen, the Netherlands

Background. Despite the structural differences of ribosomes from different domains of life at first glance, the ribosomes are quite similar in general. The biogenesis of ribosomal subunits also has structural similarities. One of example of this point is the ribosomal binding factor A (RbfA), which homologs are found in most eubacteria and archaebacteria, and plant and algae chloroplasts, as well as in the mitochondria of eukaryotes. RbfA is an assembly factor involved in late stages of small subunit maturation. It binds a 3`-end of rRNA and stabilize it before entry to the translation cycle.
Methods. A reconstitution of the 30S–RbfA complex was performed by mixing mature 30S and RbfA (1:30). The mixture was applied on to freshly glow-discharged holey carbon grids (Quantifoil, Au R1.2/1.3 with 2 nm C, 300 mesh), grids were blotted using a Vitrobot Mark IV (FEI Company) and plunge-frozen in liquid ethane. The TEM grids were transferred into a Talos Arctica 200 keV microscope (Thermo Fisher Scientific), equipped with a K2 direct-electron detector (Gatan). The GIF-quantum energy filter was adjusted to a slit width of 20 eV. A nominal magnification of ×130.000 (yielding a pixel size of 1.022 Å) and a defocus range of −0.5 to −2.0 μm were used for image collection. A total of 876 movie images were collected.
Results. The obtained cryo-EM structure of the *S. aureus* 30S–RbfA complex (2.9 Å) revealed an additional helix on C-terminus of RbfA after β3-strand hasn't described before in bacteria. The structure of KH-domain and the manner of RbfA binding with 16S rRNA are similar with *E. coli* complex.
Conclusion. The *S. aureus* RbfA share its KH-domain and C-termius conformations with inward state of mitochondrial homolog RBFA from *M. musculus*. We suggest that these homologs also share its functions and mechanism of action in preventing mRNA binding to immature small ribosomal subunits.

The work is supported by the Russian Foundation for Basic Research (RFBR): grant 20-54-15001.

**** Corresponding author:*** *Aydar G. Bikmullin. E-mail: aydar.bikmullin@gmail.com*

Poster abstract
Abstract category: Structure and functions of the transcription and translation apparatus of the cell

IN VITRO RECONSTITUTION OF THE 50S RIBOSOMAL SUBUNIT FROM *STAPHYLOCOCCUS AUREUS* AND GTP-BINDING PROTEIN YSXC FOR STRUCTURAL STUDIES BY CRYO-ELECTRON MICROSCOPY

Biktimirov A.[1], Garaeva N.[1], Validov Sh.[1], Yusupov M.[2], Usachev K.[1]

[1] Laboratory of Structural Biology, Institute of Fundamental Medicine and Biology, Kazan Federal University, Kazan, Russia
[2] Department of Integrated Structural Biology, Institute of Genetics and Molecular and Cellular Biology, University of Strasbourg, Illkirch, France

Background: Today, about 40% of the world's population is colonized by *Staphylococcus aureus*, the causative agent of a wide range of hospital acquired infections which becomes resistant to current antibiotics. The study of protein interactions with ribosomes makes it possible to identify key proteins that are involved in protein synthesis in bacteria. These proteins can be potential targets for new medicines that can disrupt the normal functioning of the ribosome and thereby stop the growth and reproduction of pathogenic bacteria. One such attractive target is the GTP-binding protein YsxC, whose molecular mechanism of interaction with the ribosome is still unknown.
Methods: We used biochemical purification techniques for proteins and ribosomes for *in vitro* reconstruction of the 50S-YsxC complex. Optimized concentration values for further cryo-EM experiments were determined using negative staining.
Results: Consequently, we successfully determined and validated the optimized conditions for the purification of the YsxC protein and 50S ribosome subunit from Staphylococcus aureus and obtained 50S-YsxC complex for further structural studies cryo-electron microscopy.
Conclusion: Based on the findings of this study, future experiments utilizing cryo-electron microscopy (cryo-EM) hold great potential for further advancing our understanding of protein-ribosome interactions. Building upon the established conditions for complex formation between the protein of interest and the large ribosomal subunit, cryo-EM can be employed to find out the high-resolution structures of the protein-ribosome complex.
Key words: ribosome, *Staphylococcus aureus*, GTPases, Cryo-electron microscopy.

This work was supported by Russian Science Foundation grant 21-74-20034.

**** Corresponding author:*** *Konstantin Usachev. E-mail: k.usachev@kpfu.ru*

Oral abstract
Abstract category: Structure and functions of the transcription and translation apparatus of the cell

STRUCTURAL AND FUNCTIONAL ASPECTS OF EUKARYOTIC RIBOSOME INHIBITION BY QUASSINOIDS

Sorokin I.I.[1#], Pichkur E.B.[2,3#], Garaeva L.A.[2,3], Krasota A.Y.[1,4], Shtam T.A.[2,3], Konevega A.L.[2,3], **Dmitriev S.E.**[1,5]

#These authors contributed equally

[1] Belozersky Institute of Physico-Chemical Biology, Lomonosov Moscow State University, Moscow, Russia
[2] Petersburg Nuclear Physics Institute named by B.P. Konstantinov of NRC Kurchatov Institute, Gatchina, Russia
[3] NRC Kurchatov Institute, Moscow, Russia
[4] Institute of Poliomyelitis, Chumakov Center for Research and Development of Immunobiological Products, RAS, Moscow, Russia
[5] Faculty of Bioengineering and Bioinformatics, Lomonosov Moscow State University, Moscow, Russia

Background: Plants of the Simaroubaceae family are extensively utilized in traditional oriental medicine. Remedies derived from diverse parts of these plants are known for their antitumor and anti-inflammatory properties, and are also used to combat parasites and viral diseases. The major active components of these preparations are quassinoids, small molecules of the triterpene family, such as ailanthone, bruceantin, eurycumanone, and others. Despite the well-documented therapeutic effects of these compounds, their targets in eukaryotic cells and the molecular mechanism of their action remain poorly understood.

Methods and Results: In this study we employed mRNA transfection, *in vitro* translation, and fluorescent toe-printing to demonstrate that quassinoids inhibit protein synthesis in mammalian cells, resulting in translational arrest at the elongation stage. Using high-resolution cryo-electron microscopy, we obtained the structures of the human 80S ribosome in complex with ailanthone and bruceantin, which showed that quassinoids prevent the accommodation of aminoacyl-tRNA in the A-site of the 60S ribosomal subunit. A resolution of 2.1 Å allowed for the detailed examination of the intermolecular contacts of the small molecules with the components of the peptidyl transferase center. We next found that ailanthone inhibited the growth of transformed cell lines and caused cell accumulation in the G0/G1 phase. Though, at lower concentrations, it increased the survival of cultured cells when infected with cytopathic picornaviruses.

Conclusion: We identified protein synthesis as a target for quassinoid activity and elucidated their mechanism of action by obtaining a high-resolution structure of ailanthone and bruceantin on the human ribosome. These findings present promising prospects for the use of quassinoids in clinical applications, specifically for anticancer and antiviral treatments.

The study was supported by the Russian Science Foundation (grant no. 19-74-20146).

**** Corresponding author:*** *Sergey E. Dmitriev. E-mail: sergey.dmitriev@belozersky.msu.ru*

Oral abstract
Abstract category: Structure and functions of the transcription and translation apparatus of the cell

STRUCTURAL STUDIES OF THE RIBOSOME MATURATION FACTOR P (RIMP) IN A COMPLEX WITH THE 30S SUBUNIT FROM *STAPHYLOCOCCUS AUREUS*

Garaeva N.[1,2], Fatkhullin B.[3], Bikmullin A.[2], Kieffer B.[3], Jenner L.[3], Klochkov V.[5], Aganov A.[5], Rogachev A.[6,7], Validov Sh.[1,2], Yusupov M.[3], Usachev K.[1,2]

[1] Laboratory for Structural Analysis of Biomacromolecules, Federal Research Center «Kazan Scientific Center of Russian Academy of Sciences», Kazan, Russia
[2] Laboratory of Structural Biology, Institute of Fundamental Medicine and Biology, Kazan Federal University, Kazan, Russia
[3] Department of Integrated Structural Biology, Institute of Genetics and Molecular and Cellular Biology, University of Strasbourg, Illkirch, France
[4] EPR Lab, Institute of Physics, Kazan Federal University, Kazan, Russia
[5] NMR Laboratory, Medical Physics department, Institute of Physics, Kazan Federal University, Kazan, Russia
[6] Moscow Institute of Physics and Technology, Dolgoprudny, Russia
[7] Joint Institute for Nuclear Research, Dubna, Russia

Background. Ribosome biogenesis is a fundamental and multistage process. It involves the assembly of ribosomal RNA and ribosomal proteins in a highly coordinated way to create functional ribosomes. In this process, even small defects can lead to erroneous RNA conformations, which appear due to degenerate local interactions. Therefore, the assembly process is regulated by a huge number of protein factors known as ribosome maturation factors. Studying the structure of these proteins and their complexes with the ribosome will allow us to understand their mechanism of action and support the development of new inhibitors that stop protein synthesis in the bacterial cell.
Methods. We used an integrated structural biology approach based on the single-particle cryo-EM reconstruction of the 30S-RimP complex together with the solution structure of RimP solved by NMR spectroscopy and uS12-RimP complex analysis by EPR, DEER and SAXS approaches
Results: We obtained the NMR structure of RimP from *S. aureus*, determined the position of the RimP protein relative to the ribosomal protein uS12 by electron paramagnetic resonance (EPR) and double electron-electron resonance (DEER) spectroscopy methods and also by small-angle X-ray scattering (SAXS), and obtained cryo-EM structure of the 30S-RimP complex.
Conclusion: By integrative structural biology approach we show a specificity of RimP binding to the 30S subunit from *S. aureus* and believe that the results obtained in this research contribute to the understanding of the RimP role in the ribosome assembly mechanism.
Key words: ribosome, *Staphylococcus aureus*, ribosome maturation factor P, RimP.

This work was supported by the government assignment for FRC Kazan Scientific Center of RAS [121110200038-7 to K.U.].

**** Corresponding author:*** *Konstantin Usachev. E-mail: k.usachev@kpfu.ru*

Poster abstract
Abstract category: Structure and functions of the transcription and translation apparatus of the cell

COMPARISON OF THE EFFECT OF LINKER HISTONES H1.0 AND H1.5 ON THE STRUCTURE OF THE LINKER REGION OF NUCLEOSOMES

Koshkina D.[1], Maluchenko N.[1], Lyubitelev A.[1], Feofanov A.[1,2], Studitsky V.[1,3]

[1] Faculty of Biology, Lomonosov Moscow State University, Moscow, Russia
[2] Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Moscow, Russia
[3] Fox Chase Cancer Center, Philadelphia, USA

The chromatin of eukaryotic organisms is a complicated, strictly-organised complex of genomic DNA and proteins. The structure and function of chromatin is controlled by a variety of regulatory factors, among which histones proteins play a key role. Histones are divided into two major groups that contain many tissue-, cell-cycle- or organism-specific variants. One group includes histones that form the nucleosome core. These are four histone variants paired together to form a positively charged globule on which DNA is folded in 1.7 turns and efficiently compacted. The diversity of regulatory functions of core histones is mediated by the presence of non-canonical variants and post-translational modifications of histone tails that alter chromatin activity. The second group of histones (linker histones) binds to DNA outside the core and provides for the formation of supranucleosomal levels of chromatin packaging - chromatosomes. This study investigated the effect of linker histones H1.0 and H1.5 on the structure of double-linker mononucleosomes. Both proteins have a similar structure, but histone H1.0 is observed in terminally differentiated cells, whereas H1.5 functions in somatic cells and being cell cycle-dependent is active during S-phase.

To study the structural features of nucleosomes, spFRET microscopy was used to analyse changes in the structure of linker DNA of the nucleosome when forming complexes with different types of H1 histones. In this study, the donor-acceptor pair of Cy3/Cy5 fluorophores was placed 10 base pairs away from the nucleosome entrance and exit, corresponding to the distant arrangement of the DNA strands. From the results, we obtained distributions that correlate with FRET efficiency (E_{PR}) relative to the frequency distribution of nucleosomes. Interaction of nucleosomes with both H1.0 and H1.5 histones transfers the particles into a state with high FRET, consistent with the current studies that H1-type histones bring linker DNA closer together. However, H1.0 brings all nucleosomes in the single state, whereas the same amount of H1.5 only brings part of the nucleosomes into this state. It is possible that such a difference in compaction effect at linker DNA sites is determined by the different biological functions of these histones, since the chromatin state in actively dividing cells should be mobile (H1.5), whereas a denser chromatin conformation is required for terminally differentiated cells (H1.0).

Key words: linker histones, H1.0, H1.5, nucleosome.

The study was funded by a grant from the Russian Science Foundation №19-74-30003.

**** Corresponding author:*** *Daria Koshkina E-mail: koshkina.daria.2015@post.bio.msu.ru*

Poster abstract
Abstract category: Structure and functions of the transcription and translation apparatus of the cell

STUDY OF H2A-H2B DIMER PLASTICITY USING ANALYSIS OF EXPERIMENTAL PDB STRUCTURES AND MD SIMULATIONS

Kniazeva A.S., Armeev G.A., Shaytan A.K.

Faculty of Biology, Lomonosov Moscow State University, Moscow, Russia

The key element of eukaryotic chromatin is the nucleosome. It consists of an octamer of histone proteins (H3, H4, H2A, H2B—two copies of each type) wrapped by a DNA segment of 145-147 bp length. Structural subunits of nucleosome are $(H3\text{-}H4)_2$ tetramer and two H2A-H2B dimers. The nucleosome core is structurally flexible, which is related to DNA dynamics and nucleosome stability. Cryo-EM studies of salt-induced nucleosome reorganization process and structures of nucleosomes with variant histones have shown that the bending of H2A-H2B dimer is observed. Our previous simulations have also presented such a dimer bending in the MD trajectory of a canonical nucleosome, in which dimer bending occurs upon nucleosomal DNA sliding.

To extract evidence of H2A-H2B dimer dynamics from experimental structures, we used three approaches. Firstly, we reconstructed RMSF profiles from reported B-factors using high-resolution X-ray structures of a nucleosome (1KX5) and an H2A-H2B dimer (6K01). Secondly, we used NMR ensembles of structures (2RVQ, 7PJ1) as a possible ensemble of H2A-H2B dimers in solution (unlike crystallographic structures). Lastly, we performed a structural variation analysis of the H2A-H2B dimer within cryo-EM and X-ray structures of nucleosomes and other proteins from PDB. Each approach provided information about H2A-H2B dimer plasticity, which was shown to be consistent with our results from our MD simulations (with some exceptions). The common pattern of dynamics (with different amplitudes) was characterized as H2A-H2B dimer bending, which was observed previously. The highest extent of the bending in structural variation analysis was observed in a group of nucleosomes within complexes with other proteins. We hypothesize that inner dimer plasticity plays a significant role in nucleosome self-dynamics and interactions with other proteins. The results were published in (1).

Key words: MD simulations, cryo-EM structures, nucleosome, histone plasticity.

This research was funded by the Russian Science Foundation grant #18-74-10006 https://rscf.ru/en/project/18-74-10006/. The research was carried out using the equipment of the shared research facilities of HPC computing resources at Lomonosov Moscow State University.

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**** Corresponding author:*** *Anastasiia Kniazeva. E-mail: kniazeva.anastasiia.2015@post.bio.msu.ru*

Oral abstract
Abstract category: Structure and functions of the transcription and translation apparatus of the cell

DNA ARCHITECTURE IN BACTERIA SUBJECTED TO VARIOUS TYPES OF STRESS

Krupyanskii Yu.[1], Loiko N.[2], Kovalenko V.[1], Generalova A.[1], Tereshkin E.[1], Moiseenko A.[3], Tereshkina K.[1], Popov A.[4], Sokolova O.[3]

[1] Semenov Federal Research Center for Chemical Physics RAS, Moscow, Russia
[2] FR Center "Fundamentals of Biotechnology" RAS, Moscow, Russia
[3] Faculty of Biology, Lomonosov Moscow State University, Moscow, Russia
[4] European Synchrotron Radiation Facility, France

Background. The study of DNA condensation in a cell is important for understanding the mechanisms of bacterial survival and for medicine, since ordered DNA condensation ensures the resistance of pathogenic bacteria to the action of antibiotics. DNA is organized in the nucleoid of an actively growing cell hierarchically with three levels of DNA compaction: The lower level (small scale ≥ 1 kb bp) is provided by histone-like NAP proteins. Actively growing cells maintain a dynamic, far from equilibrium order through metabolism. As cells enter a stress of starvation and become dormant (almost complete absence of metabolism), the usual biochemical methods of protecting DNA cease to work, and cells, adapting to new conditions, are forced to use physical mechanisms of DNA protection (dense DNA packing, nanocrystallization of DNA with proteins, etc.). We have studied changes in DNA architecture under the influence of a chemical analogue of the autoinducer of anabiosis 4-hexylresorcinol (4HR) as well. An increase in the 4HR concentration induces the transition of a part of the cells of the population into an anabiotic resting state, and then into a mummified state.
Methods. Electron microscopy and synchrotron radiation diffraction studies were used to reveal distinct forms of DNA condensation in dormant, anabiotic dormant and mummified states of *E. coli* cells.
Results. The study made it possible to find the intracellular nanocrystalline, liquid crystalline, and folded nucleosome-like DNA structures in dormant cells, the DNA architecture in anabiotic dormant states is like those in dormant state, while DNA architecture in mummified state strongly different from those in dormant state
Conclusion. The different types of DNA condensed state found by us in the studied dormant *E. coli* cells (or the heterogeneity of DNA condensation) provide additional arguments in favor of the concept that considers the microbial population as a multicellular organism, while condensed DNA by itself, corresponds to a long-lived intermediate, partially equilibrium state.
Key words: different types of stresses, DNA, condensation, structure.

Research was performed within frameworks of the state tasks for FRC CP RAS of the Ministry of Education and Science of the Russian Federation (FFZE-2022-0011, no. 122040400089-6 and 122040800164-6).

**** Corresponding author:*** *Yurii Krupyanskii. E-mail: yuriifkru@gmail.com*

Oral abstract
Abstract category: Structure and functions of the transcription and translation apparatus of the cell

X-RAY CRYSTALLOGRAPHY AND SINGLE-PARTICLE CRYO-EM ANALYSIS OF DRUG INTERACTIONS WITH THE *CANDIDA ALBICANS* RIBOSOME

Usachev K.[1], Kolosova O.[2], Zgadzay Y.[2], Stetsenko A.[3], Atamas A.[3], Validov Sh.[1], Rogachev A.[4], Jenner L.[2], Dmitriev S.[5], Guskov A.[3], Yusupov M.[2]

[1] Laboratory of Structural Biology, Institute of Fundamental Medicine and Biology, Kazan Federal University, Kazan, Russia
[2] Department of Integrated Structural Biology, Institute of Genetics and Molecular and Cellular Biology, University of Strasbourg, Illkirch, France
[3] Groningen Biomolecular Sciences and Biotechnology Institute (GBB), University of Groningen, Groningen, the Netherlands
[4] Moscow Institute of Physics and Technology, Dolgoprudny, Russia
[5] Belozersky Institute of Physico-Chemical Biology, Lomonosov Moscow State University, Moscow, Russia

Background. *Candida albicans* (*C. albicans*) is a fungal pathogen causing superficial and hematogenous infections in immunocompromised patients or individuals with imbalanced microflora. Since the clinical candidiasis rate increases yearly, efficient drugs against *C. albicans* are on demand. The design of the drugs with high selectivity and low toxicity requires unique targets in vitally important macromolecules of *C. albicans.* Our study aimed at finding a high-resolution structure of the *C. albicans* ribosome and identifying its structural determinants. Our findings elucidate the mechanism of action of these inhibitors in eukaryotes, which is critical for creating novel inhibitors to treat human disorders.
Methods. We used an integrated structural biology approach based on the single-particle cryo-EM and macromolecular X-ray crystallography to establish the details of drug interactions with the *C. albicans* ribosome.
Results. We have developed a novel crystallization protocol that does not involve the use of osmium, thereby enabling efficient screening of a large number of compounds through X-ray crystallography analysis. Initially, this method resulted in significant improvements in the resolution of *C. albicans* ribosome crystals, achieving a resolution of 3 Å. Our primary focus was to investigate potential eukaryotic inhibitors with unknown mechanisms of action, specifically ailanthone (ALT), which belongs to quassinoid family. In our study, we determined the crystal structure of the 80S ribosome from *C. albicans* in complex with ALT, revealing its specific binding to the peptidyl transferase center (PTC) in the A site. Furtherly, the absence of heavy atoms in the decoding center (DC) also provided us with an opportunity to study other inhibitors, such as hygromycin B, which binds in close vicinity to the DC.
Conclusion. In the future, X -ray crystallography and cryo-EM can be combined for screening and obtaining structural information on inhibitor binding, as well as for the development of novel inhibitors.
Key words: ribosome, *Candida albicans*, X-ray crystallography, Cryo-electron microscopy.

This work was supported by Russian Science Foundation grant 20-65-47031.

**** Corresponding author:*** *Konstantin Usachev. E-mail: k.usachev@kpfu.ru*

Poster abstract
Abstract category: Structure and functions of the transcription and translation apparatus of the cell

STRUCTURAL FEATURES OF SUBNUCLEOSOME REORGANIZATION BY YEAST CHAPERONE FACT

Volokh O.[1], Nazarova A.[1], Afonin D.[1], Studitsky V.M.[1,2], Sokolova O.S.[1]

[1] Faculty of Biology, Lomonosov Moscow State University, Moscow, Russia
[2] Fox Chase Cancer Center, Philadelphia, USA

Background: The DNA transcription regulation in chromatin, which is required for controlled genes expression, occurs with the participation of various regulatory factors, in particular, the FACT chaperone. It has been previously shown that yeast FACT (yFACT) is able to ATP-independently and reversibly unfold nucleosomes in the presence of Nhp6 protein, reorganizing nucleosomes in nearly linear structures. However, the detailed mechanism of this process remains unclear.
Methods: Here we studied the artificially prepared subnucleosomes (SNs) missing one H2A/H2B dimer and reorganized by yFACT in presence of Nhp6. Images were taken using JEOL 2100 TEM. Micrographs were captured with 2.31 Å pixel size. EM images pre-processing and single particles analysis were performed in CryoSPARC [1]. Total number of single particles used for the final analysis was ~490 000.
Results: Based on 2D-data analysis we found structural intermediates, emphasizing the gradual unfolding of SNs by FACT, which were similar to intact nucleosomes (Ns) reorganized by yFACT/Nhp6 [2]. The distribution of 2D averages and their characteristic views are presented on Figure 1(a, b).
Comparison of the linear dimensions demonstrated similar “beads-on-a-thread” architecture of unfolded SNs and Ns intermediates. Structural analysis of yFACT (Figure 2a,b) and previously described ”joint”-like role of dimerization domains of Spt16/Pob3 propose the potential localization of yFACT domains mapped on Figure 2c.
Conclusions: Based on our EM data we propose that the mechanism of subnucleosome reorganization follows the same steps as in intact nucleosomes.

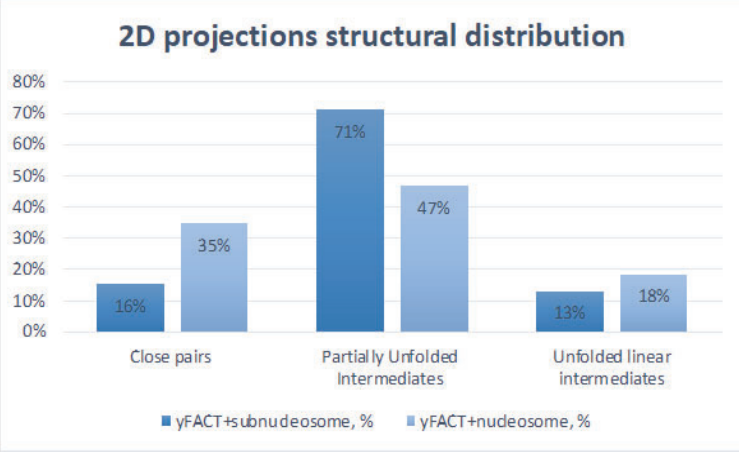

Fig. 1.A. – Single particles structural distribution between the states.

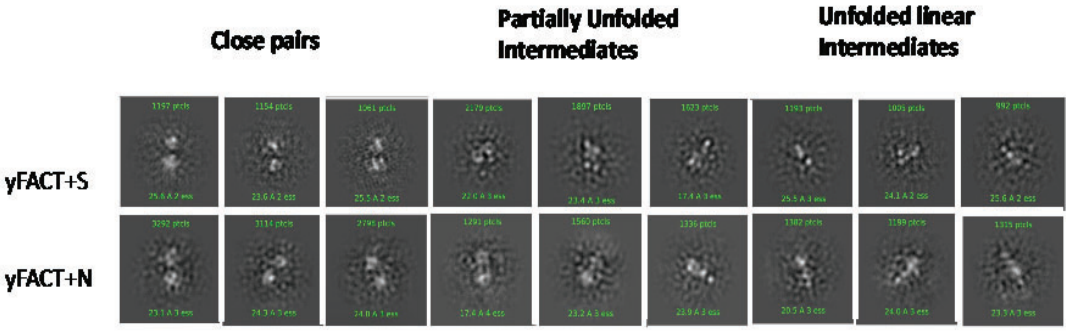

Fig. 1.B. – 2D classes, S – subnucleosome, N – nucleosome.

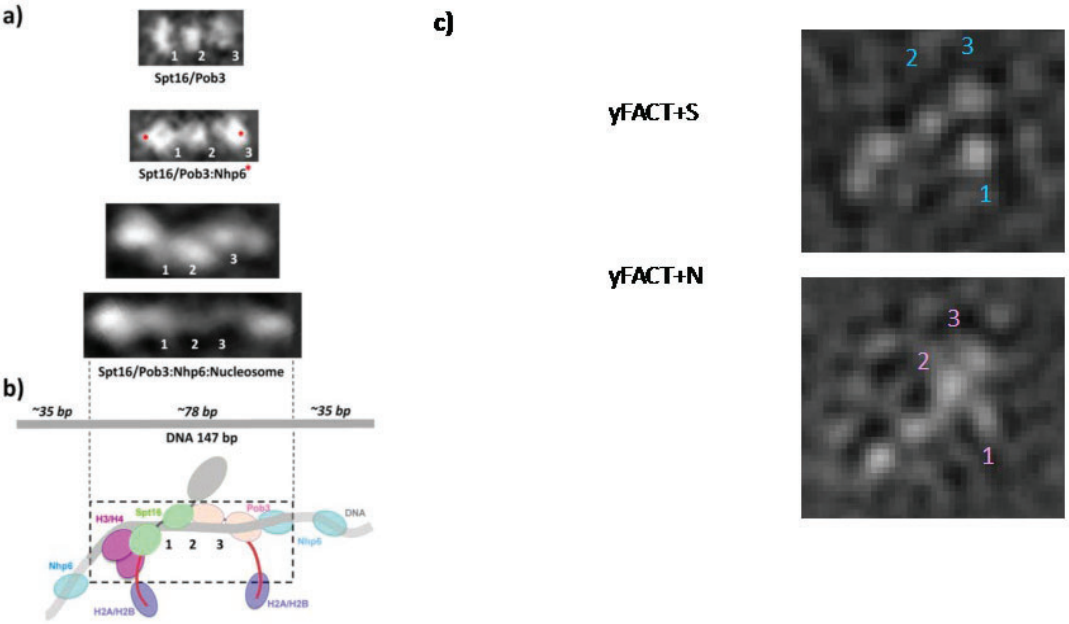

Fig. 2. – a), b) - model of nucleosome reorganization by yFACT w/Nhp6 from Sivkina et al., 2022; c)1 - Spt16-MD, 2 - Spt16-DD/Pob3-NTD-DD, 3 - Pob3-MD.

This work was supported by the Russian State Foundation (Grant 19-74-30003). Electron microscopy was performed on Unique scientific installation '3D-EMC'.

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**** Corresponding authors:*** *Vasily Studitsky. E-mail: Vasily.Studitsky@fccc.edu*
Olga Sokolova. E-mail: sokolova184@gmail.com

SECTION II:

ADVANCES IN EM TECHNOLOGY, TOMOGRAPHY AND IMAGE PROCESSING

Poster abstract
Abstract category: Advances in EM technology, tomography and image processing

ANALYZING DNA-PROTEIN COMPLEXES' STABILITY USING FLUORESCENCE POLARIZATION TO FACILITATE SAMPLE PREPARATION FOR ELECTRON MICROSCOPY

Feskin P.G., Mamaeva N.U., Shaytan A.K.

Faculty of Biology, Lomonosov Moscow State University, Moscow, Russia

Background. DNA-protein interactions play crucial role in functioning of living systems, including genomes of all living organisms. Electron microscopy is a popular technique that is often used to study structures of DNA-protein complexes. To efficiently prepare samples of these complexes for EM one requires some prior knowledge of the binding strength between DNA and protein. To date a lot of methods for detecting protein-DNA interactions have been developed. One approach relies on fluorescently labeled molecules: upon interaction of the labeled binders the changes in the fluorescence intensity or polarization may be detected. Fluorescence-based methods allow for a quantitative estimate of binding constants directly in the bulk solution. In this work we describe an experimental protocol based of fluorescence polarization measurements and present a data processing algorithm (developed by us using Python programming language), which allows to estimate binding constants from the experimental data and accounts properly for the background signal and measurement uncertainties.

Methods and Results. We carried out preliminary measurements of the parallel and perpendicular components of the fluorescence of the FAM-labeled DNA and the free fluorophore. We estimated the signal-to-noise ratio, blank signal, the dependence of measurement accuracy on the concentration of the fluorophore and sample volume. A Python program was written for the analysis. We developed a program for the numerical simulations of the accuracy of the binding constant estimation depending on the concentration of the labeled DNA and signal-to-noise ratio of the measurements at different experimental parameters (sample volume, buffer content, PMT gains, etc).

Conclusions. It was found that the most optimal solvent for measurements is the Tris buffer (HCl, Mg, Tris-HCl, pH 7.5). The fluorescence of the used label – fluorescein is most intense in this solvent; therefore, the resulting deviations will be smaller compared to the measured value. It was found that fluctuations in the sample volume in different wells of the microplate have a negligible effect on the resulting polarization value, because measurements equally affect both perpendicular and parallel component of the fluorescence intensity. It was shown that fluorescence can be detected and binding constant estimated at low nanomolar concentrations with a 1-2% error.

Key words: fluorescence polarization, DNA-protein interactions, binding affinity.

This work was supported by the Russian Science Foundation (grant no. 19-74-30003).

**** Corresponding author:*** *Pavel Feskin. E-mail: p.feskin@intbio.org*

Poster abstract
Abstract category: Advances in EM technology, tomography and image processing

"CRYOCONVEYOR" PROTOCOLS FOR CRYOCLEMS AND DIFFERENT MULTI-SCALE COMBINED OPTICAL AND ELECTRON CRYOMICROSCOPY TECHNIQUES

Gradov O.V.

N.N. Semenov Federal Research Center for Chemical Physics Russian Academy of Sciences, Moscow, Russia

When analyzing biosamples by any cryomicroscopy methods, it is necessary to maintain the same (and spatially equal) temperature at all stages of the study. If the temperature is not sufficiently stable, artifacts may get into the result of the study, and the sample may be damaged to structural degradation. The basic focus of research in modern structural biology is shifted towards Cryo-TEM – at the level of single macromolecules and their ensembles, but this fact does not detract from the importance of other methods of cryomicroscopy in the life sciences (*sensu lato*). Therefore, it is necessary to ensure the stability of maintaining the temperature in these methods or series of methods associated in individual cryomicroscopy protocols (even in cases where the protocols do not reach super-resolution cryoelectron microscopy, due to the problem being limited by larger-scale structural objects, indicating cellular changes on the ultrastructural or microstructural level that was elementary achieved >30 years ago). Both CryoSEM electron microscopy methods (including CryoESEM / CryoASEM) and different optical cryomicroscopy methods can be considered such methods. As examples of the wide distribution and high integrability of optical cryomicroscopy methods, one can cite: Raman cryomicro(spectro)scopy and confocal Raman cryomicroscopy; integrated FTIR and optical / confocal cryomicroscopy; multi-photon cryomicroscopy; synchrotron-assisted cryoprotocols; non-optical spectral range cryomicroscopy methods – including UV / VUV based ones; cryomicroscopy integrated with DSC and TDA; etc. Considering the worldwide distribution of optical microscopy methods and their integrability with Cryo-SEM (both in the form of Cryo-CLEM and in sequential implementations), in 2014-2015 the "Cryoconveyor" system was proposed and tested, in which one sample in a special cuvette ("chip") is consistently passed through the serial protocol of optical cryomicroscopy methods (with different geometry of cooling elements and different physical principles of image registration: from lensless cryomicroscopy to cryo-SEM). Subsequently, this approach was diversified, because in the absence of the Cryo-SEM / Cryo-CLEM stage, a wider range of chip-cuvette geometries and materials can be used, including capillary cryo-microscopy methods (Cryo-SPIM or Cryo-LSFM, flow interference cryo-microscopy, etc.). In particular, we have developed methods and instrumental schemes for differential interference contrast cryomicroscopy and polarization interference cryomicroscopy, phase-contrast and/or anoptral cryomicroscopy, multi-angle / holographic lensless optical cryomicroscopy and cryotomography on a chip, etc.. The remains of this technique are still at our disposal, however, there are currently no experimental tasks. The scheme of the serial protocol "Cryoconveyor" is shown in Fig. 1.

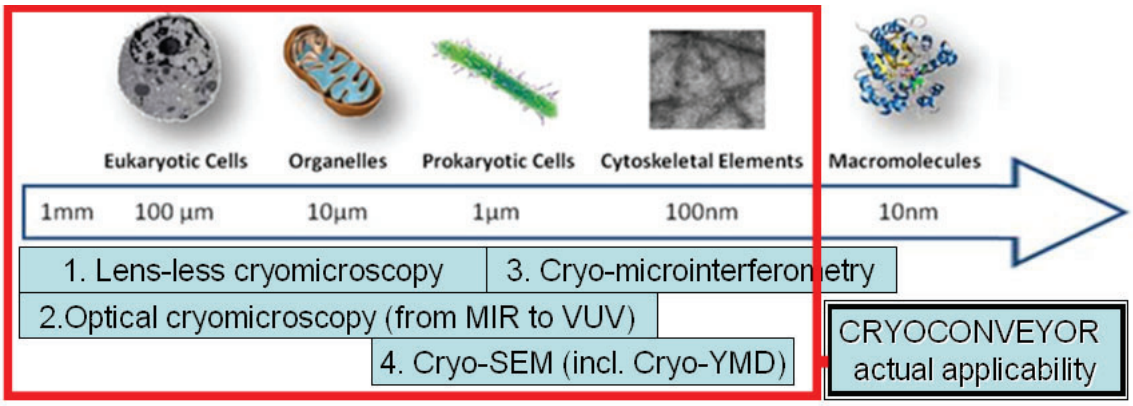

Fig. 1: Applicability of different spatial scale microscopy unit operations in the sequential CRYOCONVEYOR protocol, integrable with unified laboratory information system (LIS).

**** Corresponding author:*** *Oleg Gradov. E-mail: o.v.gradov@gmail.com*

Oral abstract
Abstract category: Advances in EM technology, tomography and image processing

THE COMPUTER PROGRAM FOR QUANTITATIVE ANALYSIS OF THE IMAGE OF THE DIFFERENT STRUCTURES OF BACTERIA IN MICROGRAPH RECEIVED FROM ELECTRON MICROSCOPES

Navolnev S.O., Shevlyagina N.V., Zhukhovitsky V.G.

Gamaleya National Research Center for Epidemiology and Microbiology, Moscow, Russia

Background: A transmission electron microscope image of a bacterium shows hundreds of spots. This is of interest for quantitative characterization of different bacterial cell structures. It is very difficult to isolate spots manually, to determine the size, contour, coloration features. Computer vision can help speed up this process.
Methods and Result: The object of our research was the *Hilicobacter pylori* culture. Samples were fixed in glutaraldehyde and subjected to the standard procedure of dehydration, contrasting, casting and cutting. A JEM-2100 Plus (Japan) transmission electron microscope was used for analysis of ultrathin sections. A computer program was developed to determine morphological and brightness parameters of digital images of bacteria obtained with a transmission electron microscopeThe photographs manually delineate the area of the cell (outer membrane, plasma membrane, protoplasm, vesicles) that will be analyzed by the program. The program calculates many parameters in a spot with increased electron density: area, brightness, length, width, etc. The spot area extracted by the program corresponds well to the area extracted visually. The program is written on Visual Basic 6 and can work on IBM compatible computers with Windows XP and above.
Conclusion: Using the computer program developed by us, it is possible to evaluate various morphological and luminance parameters of different bacterial structures in digital images obtained from transmission electron microscope. Parameter values are given in quantitative form. What usually takes a person a pretty long time, the computer allocates it in a couple of seconds. The use of such parameters makes it possible to establish more quickly and accurately the physiological features of the bacterial cells under study.
Key words: electron microscopy, computer vision, *Hilicobacter pylori*.

**** Corresponding author:*** *Sergei Navolnev. E-mail: navoln@mail.ru*

Poster abstract
Abstract category: Advances in EM technology, tomography and image processing

ALPHAFOLD2 AND DISORDERED PROTEINS: CAN AN INTERACTION PREDICTION BY AI IMPROVE Cryo-EM DATA?

Podruzhko E.A.[1], Korshunova A.V.[2,3]

[1] Moscow Institute of Physics and Technology, Dolgoprudny, Russia
[2] Center for Theoretical Problems of Physicochemical Pharmacology, Russian Academy of Sciences, Moscow, Russia;
[3] Physics Department, Lomonosov Moscow State University, Moscow, Russia

AAA Intrinsically Disordered Proteins (IDPs) and Regions (IDR) of proteins play an important role in protein-protein interaction. Many IDPs belong to transcription factors and use as interaction hubs in metabolic pathways. But they are difficult in studying of their interaction interface and contacts, since modern experimental approaches to obtaining the 3D structure of proteins are severely limited in the ability to determine the 3D of such regions and proteins. Often these parts are simply deleted in X-ray experiments, because IDPs can interfere with the crystallization of proteins, or do not have clear electron densities in Cryo-EM.

The new AI-based algorithm AlphaFold2 (AF2), which is capable to predict protein structure from the primary amino acids sequence, is currently the most effective tool not only in the *de novo* 3D structure prediction, but has proven to be able to calculate protein-protein contacts and build multimeric protein complexes. It was noted that if the protein was a dimer, a heterodimer etc., an accuracy of its predicted 3D protein structure in multimers was higher than when predicting process was for a monomer only. This observation was described for globular proteins. However, nothing is known about IDPs and they remain an unexplored area. On the one hand, AF2 was not trained on structural data about IDP due to their absence in the database, but on the other hand, it turned out that AF2 somehow learned to determine protein-protein interactions despite the fact of absence any training for this purpose. If AF2 can determine protein-protein interaction in case of IDP, it will make AF2 the most useful tool for IDP studying.

We conducted a study to determine the ability of AF2 to predict the interactions of various cases with IDPs. In this work, we considered 4 cases of protein interaction with IDP: when an unstructured protein acquires a structure as a result of interaction with a globular partner (*disorder-to-order*), interaction with a globular partner (*disorder-to-disorder*), long proteins with IDR as flexible linkers (*coiled-coils with linkers*), and interaction with disordered partner (*disorder+disorder*).

Based on the analysis of the obtained data, it was shown that in most cases the predicted by AF2 3D model of a single unstructured protein has a high similarity with its structure in the composition of the protein complex (for cases of disordered-to-ordered). It was found that when calculating the 3D structure of an unstructured protein in the presence of a partner protein, the AlphaFold2 algorithm correctly identifies the bonds for this protein complex. In addition, the AlphaFold2 algorithm suggests new bonds in such protein complexes that need further experimental verification. . For coiled-coils with linkers proteins we observed that the presence of a natural interaction partner significantly improved

tertiary structure prediction. Only in the case of disorder+disorder, the study showed that AF2 was unable to detect any protein-protein interaction.
Thus, we have shown that AF2 is able to determine protein-protein interactions in a number of cases with IDP, and can be used as an additional tool for structural data.

**** Corresponding author:*** *Ekaterina Podruzhko. E-mail: podruzhko99@gmail.com*

SECTION III:

STRUCTURE OF MEMBRANE PROTEINS

Poster abstract
Abstract category: Structure of membrane proteins

CONFORMATIONAL CHANGES OF GPCRS CAN BE MONITORED USING DYES BASED ON GFP-CORE

Belousov A.[1], Maslov I.[1], Orekhov P.[2], Khorn P.[1], Mishin A.[3], Baranov M.[3], Zernii E.[4], Permyakov S.[5], Mishin A.[1], Borshchevskiy V.[1]

[1] Moscow Institute of Physics and Technology (National Research University), Dolgoprudny, Russia
[2] Faculty of Biology, Shenzhen MSU-BIT University, Shenzhen, China
[3] Institute of Bioorganic Chemistry, Russian Academy of Sciences, Moscow, Russia
[4] Belozersky Institute of Physico-Chemical Biology, Moscow, Russia
[5] Institute of Biology, Russian Academy of Sciences, Pushchino, Russia

From *in vivo* imaging to single-molecule tracking, the green fluorescent protein (GFP) has become an indispensable tool for many biological studies [1]. Structurally modified synthetic analogues of the GFP chromophore represent a class of benzylidene imidazolones that found many applications as versatile labels due to their exceptional fluorescent properties, small size, and easy synthesis [2].
GPCRs constitute the largest class of membrane proteins in humans that regulate critical physiological processes, e.g. vision, taste, neurotransmission, and inflammation. More than one third of drugs approved by FDA have GPCRs as their primary targets [3]. The ligand binding causes structural changes propagating across the receptor towards the intracellular side. Orthosteric ligands can directly control GPCR activity: agonists increase the basal signaling, antagonists occupy the ligand-binding site but do not affect the receptor's activity, and inverse agonists decrease the basal signaling.
Here we assessed four GFP-inspired fluorophores for their potential to serve as environmentally sensitive labels to report on conformational changes in proteins. For this we attached a maleimide group to them for cysteine labeling and studied their spectral properties in solvents with varying polarity and viscosity. The best of them were then employed to label two proteins: bovine recoverin and A_{2A}AR. Recoverin is a convenient model protein to evaluate the performance of environmentally sensitive labels. This is attributed to the presence of a cysteine residue and the considerable conformational alterations upon activation by Ca^{2+}. Using the best performing dye attached to A_{2A}AR, we investigated effects of various ligands on its fluorescence and observed reliable and distinctive changes. Finally, we conducted molecular dynamic simulations of the A_{2A}AR complexes with the dyes to obtain structural insights into the observed changes.

This work was supported by Ministry of Science and Higher Education of the Russian Federation (075-03-2023-106, project FSMG-2020-0003).

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**** Corresponding author:*** *Anatoliy Belousov. E-mail: Belousov.as@phystech.edu*

Poster abstract
Abstract category: Structure of membrane proteins

DETERMINATION OF DISSOCIATION CONSTANTS OF GPCR-LIGAND COMPLEXES USING THERMAL SHIFT ASSAY

Dashevskii D., Luginina A., Khorn P., Maslov I., Borshchevskiy V., Mishin A.

Moscow Institute of Physics and Technology, Moscow, Russia

G-protein coupled receptors (GPCR) are both the most common human membrane proteins and the most popular drug targets, since these receptors are involved in signaling in most physiological processes of the body. One of the most important tasks in the field of GPCR research is the search and description of the interaction of new ligands, the characterization of the effect of allosteric modulators [1]. Thermal shift assay (TSA) [2] can become an easily accessible alternative to the currently used methods for analyzing the interaction of GPCR and ligands, such as radioligand assay and cellular functional tests. The speed of measurement, low consumption of the receptor and ligand, the accuracy of the obtained values, the possibility of carrying out measurements with some allosteric modulators represent the main advantages of the TSA.

TSA is based on the measurement of the melting curve, which displays the dependence of the proportion of molten protein on the temperature of the solution. The amount of protein in the molten state is determined either by the fluorescence of its own tryptophans in the case of nanoscale scanning fluorometry (nanoDSF), or by the fluorescence of a dye reacting to protein denaturation. The dissociation constant (K_d) of the protein-ligand complex is calculated from the magnitude of the shift of the melting curve to the region of higher temperatures, which occurs when the ligand is added to the solution and the protein is stabilized, due to the formation of the complex. During work experimental conditions were selected for TSA method in the presence of the dye 7-diethylamino-3-(4'-maleimadylphenyl)-4-methylcoumarin (CPM). A mathematical model of data processing was formed and tested for the TSA/CPM approach. As a result, K_d for several orthosteric and allosteric ligands of the adenosine receptor of the second type ($A_{2A}AR$) were obtained by the TSA method.

The obtained values of constants for orthosteric ligands are close to those known from the literature measured by radioligand assay, which confirms the possibility of using TSA as an alternative method.

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**** Corresponding author:*** *Dmitrii Dashevskii. E-mail: dashevskij.de@phystech.edu*

Poster abstract
Abstract category: Structure of membrane proteins

OPTIMIZATION OF PROTOCOLS FOR EXPRESSION, ISOLATION AND PURIFICATION OF G-PROTEIN COUPLED RECEPTORS FOR EXPERIMENTS ON THE STUDY OF ITS CONFORMATIONAL DYNAMICS

Kapranov I., Khorn P., Maslov I., Luginina A., Mishin A., Borshchevsky V.

Moscow Institute of Physics and Technology, Moscow, Russia

The class of G-protein coupled receptors (GPCRs) is the ubiquitous family of membrane proteins which can specifically bind different signal molecules regulating by this biochemical and physiological processes in cells. Disorders related to GPCRs can cause serious diseases that's because this group of receptors is the biggest class of targets for drugs (more than 34% of all medicines on the pharmacological marketspace), and its structural and functional research are crucial not only for fundamental sciences but also for applied disciplines [1]. One on the most important task to understand the receptor activity is investigation its conformational dynamics – special changes in protein structure in time in response to the ligand binding. Not all modern methods of structural biology allow watching structural movements of protein domains in real time. That's because one of the most perspective techniques of investigation the protein conformational dynamics is the single-molecule FRET-microscopy (smFRET) [2]. This approach is based on the phenomenon of resonance energy transfer emitted by the fluorescent label-donor to the label-acceptor what leads to the appearance of the fluorescence signal from the second. It has place only if the absorbance spectrum of the label-acceptor overlaps the emission spectrum of the label-donor and the efficiency of the transfer depends on the range between fluorescent dyes. Thus, by disposing two labels on the mobile parts of the protein of interest we can track its conformational changes in response to some ligands by the appearance or disappearance of fluorescent signal from the laber-acceptor.
To apply smFRET to GPCR we chose the receptor playing a vital role in physiology of whole organism. In this work the wide range of work has been carried out to create and optimize the methods of GPCR expression, isolation, purification and fluorescent labelling. Obtained protocols allowed us to constitute the stable extraction of functional wild type protein. Also, several constructions for smFRET have been designed and created by the methods of gene engineering. They allowed to test methods of fluorescent labeling of the receptor by two dyes and to check the efficiency of lipid nanodisks assembly. Thus, the protocol for protein samples preparation for smFRET experiments were created, optimized and used in practice.

We express our gratitude to the Ministry of Science and Higher Education of the Russian Federation for supporting the work (075-03-2023-106, project FSMG-2020-0003).

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**** Corresponding author:*** *Ivan Kapranov. E-mail: kapranov.ia@phystech.edu*

Poster abstract
Abstract category: Structure of membrane proteins

PURIFICATION OF MUTANT KV11.1 ION CHANNEL EXPRESSED IN EUKARYOTIC CELL LINES

Kravchuk E.V.[1], Glukhov G.S.[1,2], Sokolova O.S.[1,2]

[1] Faculty of Biology, Lomonosov Moscow State University, Moscow, Russia
[2] Faculty of Biology, MSU-BIT Shenzhen University, Shenzhen, China

The object of this study is the human potential dependent channel hERG (Kv11.1). In 2017 R. McKinnon and colleagues obtained a structure of hERG in an open conformation with a resolution close to atomic using cryoelectron microscopy. So far, there is no study on its structure in a closed conformation. It was shown that when cysteines are introduced into the S4-S5 linker and the C-terminus of S6 under oxidizing conditions, disulfide bonds are formed that fix the contact. Thus, for the D540C-L666C mutant hERG variant, it was shown that in the presence of tbHO2 it does not conduct current when voltage steps are applied by O.A. Malak et al. in 2017. In this work, the D540C-L666C hERG mutant variant was used.

Gibson assembly was used to clone the mutant channel sequence into PIRES2-EGFP with the 1D4-tag for purification. The channel was expressed in CHO cell line and its expression was confirmed using immunocytochemistry. Then the mutant channel was expressed in HEK293, but the level of expression was not satisfactory for purification and EM analysis. We decided to obtain a construction with two extended deletions as in R. McKinnon's study (hERGΔ140-351Δ870-1006 of hERGt). We performed immunocytochemistry and western blotting and showed that the expression increased. Electrophysiological experiments confirm that the current remains in D540C-L666C mutant hERG variant and in hERGt. We transfected HEK293 cell line with hERGt, solubilized in 2.5 % DDM and purified the protein using NHS activated sepharose conjugated with monoclonal anti-Rho-1D4 antibodies (Fig. 1A). The obtained protein was studied by electron microscopy with negative staining (Fig. 1B). We analyzed the data. Particles were collected using crYOLO. Totally 85884 particles were collected. After CTF-correction of images, the resulting set of particles was imported into RELION3.0, where two-dimensional classification was carried out (fig. 1C). After two-dimensional classification, a part of the classes was selected, which included 45873 particles.

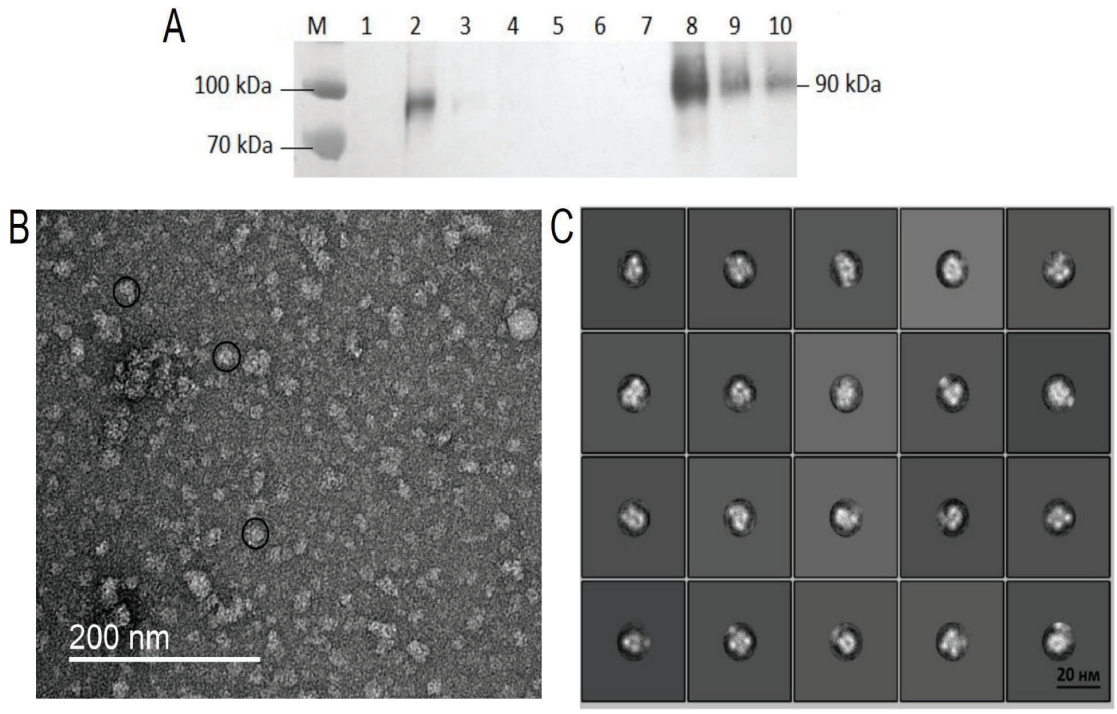

Fig. 1. A – hERGt purification results, western blotting. M - molecular weight marker, 1- control HEK293, 2 - precipitate after solubilization in 2.5% DDM, 3 - supernatant after solubilization in 2.5% DDM, 4 – unbinded fraction, 5-7 – washes, 8-10- elutions. B - electron micrograph of the mutant Kv11.1 channel, negative staining, magnification 40,000 times. C - examples of selected class sums.

These results will form the basis for further biochemical and structural studies of hERGt in a closed conformation.

**** Corresponding author:*** *Ekaterina Kravchuk. E-mail: kravchuk_ekaterina01@mail.ru*

Poster abstract
Abstract category: Structure of membrane proteins

STUDY OF THE MICROVISCOSITY OF A MEMBRANE SURROUNDED BY A PROBE ACTIVATED BY COUMARIN C-525 CHEMILUMINESCENCE UNDER THE ACTION OF A CYTOCHROME C COMPLEX WITH CARDIOLIPIN

Levchenko I.N.[1], Vladimirov G.K.[2], Volodyaev I.V.[3]

[1] N.I. Pirogov Russian National Research Medical University, Moscow, Russia
[2] I.M. Sechenov First Moscow State Medical University, Moscow, Russia
[3] Faculty of Biology, Moscow State University, Moscow, Russia

Background. The use of physical activators enhances the intensity of the glow by 2-3 orders of magnitude without affecting the chemical processes taking place in the process. The physical activator coumarin C-525 intercepts the excitation of triplet-excited ketones formed during recombination of peroxide radicals by the Russell mechanism and is a fluorescent probe. When the microviscosity of the membrane is found in the environment of the probe, chemiluminescence is 3-4 orders of magnitude higher than the excited ketones themselves.
Materials and methods. The accuracy of the solution is determined by the presence of cardiolipin for pH stabilization, quenching of Fe2+ and the presence of coumarin C-525. Factors that distorted the value of the microviscosity of the membrane in the environment of the coumarin C-525 probe: insufficient addition of hydrogen peroxide, excessive amount of nitrogen (II), methanol, protein denaturation, a change in the conformation of cytochrome C in the cytochrome and cardiolipin complex. The systems of lipoperoxidase and quasi-lipoxygenase reactions are analyzed.
Results. The cytochrome C and cardiolipin complex differs from the native cytochrome in properties: (1) has the fluorescence of tyrosine and tryptophan residues; (2) loses absorption in the Cope band (405-410 nm); (3) has peroxidase activity and catalyzes the formation of lipid radicals in the membrane surrounded by the fluorescent probe of the dye C-525.
Conclusion. (a) knowing the polarization coefficient of coumarin C-525, the microviscosity of the membrane in the probe environment was determined. (b) peroxidase activity depends not only on the concentration of cytochrome C and cardiolipin, but also on the ratio determining the percentage of the absolute amount of the denatured form.
Key words: microviscosity, membrane, coumarin C-525, cytochrome C, cardiolipin.

**** Corresponding author:*** *Irina Levchenko. E-mail: irnlevchenko@yandex.ru*

Poster abstract
Abstract category: Structure of membrane proteins

DETERGENT-FREE SOLUBILIZATION METHOD FOR STRUCTURAL STUDY OF THE SARS-CoV-2 SPIKE PROTEIN

Mamaeva N.Y.[1], Glukhov G.S.[1,2], Sokolova O.S.[1,2]

[1] Faculty of Biology, Lomonosov Moscow State University, Moscow, Russia
[2] Faculty of Biology, MSU-BIT Shenzhen University, Shenzhen, China

The study of integral membrane proteins is one of the main objectives of modern research in structural biology. In native conditions, membrane proteins are embedded in the lipid bilayer, which is involved in maintaining the stability of their structure and is necessary for their proper functioning. However, for detailed structural and functional studies, membrane proteins need to be isolated from the lipid environment while maintaining their stability and activity. Therefore, purification of membrane proteins is more challenging than purification of soluble proteins. Currently, there are many ways to solubilize membrane proteins. Various approaches include using detergents to solubilize – into micelles and then replacing them with more stabilizing agents such as amphipols. In addition, membrane proteins can be incorporated into lipid bilayer-forming media such as bicelles, lipid vesicles, or lipodiscs. The current approach is to use a copolymer of styrene and maleic acid. In contrast to detergents, which typically remove most or all lipids in close proximity to the membrane protein, styrene and maleic acid copolymer isolates proteins directly from the membrane, preserving part of the membrane environment. The object of the study in this work is a full-length spike protein of wild-type coronavirus SARS-CoV-2 (Wuhan-Hu-1), which is the causative agent of the COVID-19 epidemic.

For structure studies, a full-length S-protein fused to 1D4-tag was expressed in the HEK293 cell line. During the protein purification, solubilization was performed using 2.5% SMA (styrene-maleic acid). The purified protein samples were analyzed by electron microscopy using negative staining on a JEM-2100 microscope (JEOL) at 200 kV. Reconstruction was performed using CryoSPARC image processing suites. The result was 20 representative classes that included 7,300 particles for which further refinement was conducted. The final resolution was 17.67Å.

We have developed a protocol for the direct extraction of the S-protein SARS-CoV-2. The data we obtained show the possibility of using SMA as a stabilizing agent for the membrane domain of the protein, which is important for obtaining structural data of the full-length S-protein. Further studies of the S-protein structure, using cryo-electron microscopy in combination with detergent-free purification, may provide information on the unresolved regions of the protein.

Key words: styrene-maleic acid, S-protein, electron microscopy, membrane protein purification.

**** Corresponding author:*** *Naida Mamaeva. E-mail: n.mamaeva@intbio.org*

Oral abstract
Abstract category: Structure of membrane proteins

MEMBRANE-ACTIVE SPIDER TOXINS RECOGNIZING CONSERVED STRUCTURAL ELEMENTS IN P-LOOP CHANNELS

Shenkarev Z.O.[1,2,3], Mironov P.A.[2,4], Chernaya E.M.[2,4], Zaigraev M.M.[2,3], Kulbatskii D.S.[2], Shulepko M.A.[5], Paramonov A.S.[2], Krumkacheva O.A.[1], Lyukmanova E.N.[2,4,5]

[1] International Tomography Center SB RAS, Novosibirsk, Russia
[2] Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, RAS, Moscow, Russia
[3] Phystech School of Biological and Medical Physics, Moscow Institute of Physics and Technology, Russia
[4] Faculty of Biology, Lomonosov Moscow State University, Moscow, Russia
[5] Shenzhen MSU-BIT University, China

The superfamily of P-loop channels includes voltage-gated Na^+, K^+, and Ca^{2+} channels, as well as TRP channels. These channels are modular and include a central pore domain and four peripherally located S1–S4 gating domains. The S1–S4 domains are embedded in the membrane and usually have short extracellular loops. Therefore, spider toxins that affect the gating of P-loop channels exhibit affinity for lipid membranes and attack the extracellular loops of the S1–S4 domains from the membrane surface. Phα1β (PnTx3-6) is a neurotoxin from the spider *Phoneutria nigriventer* venom, originally identified as an antagonist of two ion channels involved in nociception: N-type voltage-gated calcium channel ($Ca_V2.2$) and TRPA1. In animal models, Phα1β administration reduces both acute and chronic pain.
Here we report the spatial structure and dynamics of Phα1β determined using NMR spectroscopy and a model of the Phα1β/TRPA1 complex built using a combined docking/molecular dynamics approach. N-terminal domain of the toxin (Ala1–Ala40) contains the inhibitor cystine knot (ICK) motif, which is common to spider neurotoxins. The C-terminal α-helix (Asn41–Cys52) stapled to ICK by two disulfides exhibits the μs–ms time-scale fluctuations. The Phα1β structure with the disulfide bond patterns Cys1–5, Cys2–7, Cys3–12, Cys4–10, Cys6–11, Cys8–9 is the first spider knottin with six disulfide bridges in one ICK domain, and is a good reference to other toxins from the ctenitoxin family. Phα1β has a large hydrophobic region on its surface and demonstrates a moderate affinity for partially anionic lipid vesicles. Surprisingly, 10 μM Phα1β significantly increases the amplitude of diclofenac-evoked currents through the rat TRPA1 channel expressed in *Xenopus oocytes*. Computer modeling revealed Phα1β binding to the S1-S2 loop of S1-S4 domain from a membrane bound state.
Targeting two unrelated ion channels ($Ca_V2.2$ and TRPA1), membrane binding, modulation of TRPA1 channel activity, and binding to the S1-S2 loop allow Phα1β to be considered as a typical membrane-active gating modifier toxin.

This work was supported by the Ministry of Science and Education of RF (Contract No. 075-15-2021-580) and by the Russian Science Foundation (project no. 22-14-00326).

**** Corresponding author:*** *Zakhar Shenkarev. E-mail: zakhar-shenkarev@yandex.ru*

Oral abstract
Abstract category: Structure of membrane proteins

MONITORING PROTEIN NANOPARTICLE SELF-ASSEMBLY USING SAXS AND EM

Sudarev V.V.[1], Gette M.S.[1], Kurkin T.S.[1], Vlasova A.D.[1], Bazhenov S.V.[1,3], Ryzhykau Yu.L.[1,2], Gushchin I.Y.[1], Bondarev N.A.[1], Manukhov I.V.[1,3], Kuklin A.I.[1,2], **Vlasov A.V.**[1,2]

[1] Research Center for Molecular Mechanisms of Aging and Age-Related Diseases, Moscow Institute of Physics and Technology, 141701 Dolgoprudny, Russia
[2] Joint Institute for Nuclear Research, 141980 Dubna, Russia
[3] National Research University Higher School of Economics, 101000, Moscow, Russia

Ferritin is one of the most widespread iron-containing proteins. It consists of 24 identical subunits, forming a hollow globular protein shell. The process of its self-assembly is very stable, making ferritin a perspective tool for biotechnological applications (1). Engineering of vaccines based on ferritins is a promising approach, rapidly developing for the last decade (2, 3). Control of the self-assembly process of globular structure is an important step in engineering of ferritin-based vaccines.
In the present work, we studied self-assembling chimeric ferritin-based constructs by small-angle X-ray scattering (SAXS) and negative stain electron microscopy (EM). Namely, we designed several chimeric protein constructs based on ferritin from *H. pylori*. We studied the constructs with and without addition of SUMO protein tag or the receptor binding domain (RBD) of the SARS-CoV-2 S protein.
The conditions for optimal negative staining EM data collection for ferritin particles were obtained. The micrographs were recorded at a magnification of 10,000–40,000 in Tecnai Polara G2 (FEI) transmission electron microscope (FEG cathode source operated at 300 eV of accelerating voltage) with the Gatan Orius 4k × 2.67k digital camera.
SAXS experiments were conducted using the in-house instrument Rigaku equipped with an X-ray source Micromax-007HF (4), the emission wavelength λ = 1.54 Å. Scattered beam light was recorded by an ASM DTR Triton 200 position-sensitive detector.
Obtained data were used to characterize the oligomeric state of protein complexes. We demonstrate the complete self-assembly into a 24-mer of the apoferritin from *H. pylori* (Fer), Fer fused with the SUMO protein (Fer-SUMO), and Fer fused with the RBD of S protein from SARS-CoV-2 and the SUMO protein (Fer-RBD-SUMO). The intermediate self-assembly into an 8-mer was observed for Fer fused with the RBD (Fer-RBD). These results might have a promising application for further immunological studies.

The study was supported from the Ministry of Science and Higher Education of the Russian Federation (agreement 075-03-2023-106, project FSMG-2021-0002).

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**** Corresponding authors:*** *Vsevolod Sudarev. E-mail: sudarev.vv@phystech.edu*
Aleksei.Vlasov. E-mail: aleksei.vlasov@phystech.edu

Oral abstract
Abstract category: Structure of membrane proteins

STRUCTURAL INSIGHTS TO LIGAND BINDING AND SIGNALING MECHANISM USING EXAMPLES OF MELATONIN RECEPTORS AND GPR88

Zheng Liu

Kobilka Institute of Innovative Drug Discovery, School of Medicine, Chinese University of Hong Kong, Shenzhen, Guangdong, China

G-protein-coupled receptors (GPCRs) are the largest and most diverse group of membrane receptors in eukaryotes. These cell surface receptors act like an inbox for messages in the form of light energy, peptides, lipids, sugars, and proteins. Employing the advanced methods of cryo-EM, we determined high resolution structures of a number of the human GPCRs in complex with auxiliary subunits, toxins, and drugs, which reveal the mode of action of some GPCRs. Based on the structural discovery, we discovered some novel ligand binding, activation and signaling mechanism on melatonin receptors and GPR88. These new insights will facilitate the innovative drug discovery using structural based on rational design.

**** Corresponding author:*** *Zheng Liu. E-mail: liuzheng@cuhk.edu.cn*

SECTION IV:

STRUCTURE OF VIRUSES AND CHAPERONINS

Oral abstract
Abstract category: Structure of viruses and chaperonins

STRUCTURE AND DYNAMICS OF REGN10987 FAB INTERACTION WITH S-PROTEINS OF DELTA AND OMICRON SARS-CoV-2

Lyukmanova E.[1], Pichkur E.B.[2], Nolde D.E.[3], Kocharovskaya M.V.[3,4], Kirpichnikov M.P.[3,5], Shenkarev Z.[3]

[1] Shenzhen MSU-BIT University, China
[2] National Research Center "Kurchatov Institute", Russia
[3] Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Russia
[4] Phystech School of Biological and Medical Physics, Moscow Institute of Physics and Technology (National Research University), Russia
[5] Interdisciplinary Scientific and Educational School of Moscow University «Molecular Technologies of the Living Systems and Synthetic Biology», Faculty of Biology, Lomonosov Moscow State University, Moscow, Russia

Background. Knowledge how neutralizing antibodies recognize various SARS-CoV-2 variants is critical for design of vaccines and antibody-based therapeutics.
Methods and Results. Here, we reported 2.3 Å cryo-EM structure of full-length trimeric S-protein of the SARS-CoV-2 Delta variant in a complex with recombinant analogue of REGN10987 antibody's Fab. Receptor-binding domain (RBD)/Fab regions were locally refined to 3.2-3.4 Å. Two RBDs were in the 'down' state, while third RBD adopted the 'up' conformation. Fab interacted with RBDs in both conformations occupying a fragment of the receptor-binding motif and blocking ACE2 recognition. 3D variability analysis revealed high mobility of the RBD/Fab regions. Molecular dynamics simulations explained the differences observed in the Fab interaction with Delta and Omicron RBDs and revealed amino acid residues of Omicron S-protein responsible for virus evasion of REGN10987 antibody.
Conclusion. Our study provides a structural insight into the role of RBD mutations of the Omicron in the REGN10987 evasion. Data obtained will be useful for development of new therapeutic antibodies.
Key words: coronavirus; neutralizing antibodies; RBD; cryo-EM.

**** Corresponding author:*** *Ekaterina Lyukmanova. E-mail: lyukmanova_ekaterina@smbu.edu.cn*

Poster abstract
Abstract category: Structure of viruses and chaperonins

MORPHOLOGY AND INNER STRUCTURE OF INFLUENZA AND SARS-CoV-2 VIRIONS DETERMINED BY CRYO-ELECTRON MICROSCOPY

Kordyukova L.[1], Moiseenko A.[2], Timofeeva T.[3], Shanko A.[4]

[1] Belozersky Institite of Physico-Chemucal Biology, Lomonosov Moscow State University, Moscow, Russia
[2] Faculty of Biology, Lomonosov Moscow State University, Moscow, Russia
[3] D.I. Ivanovsky Institute of Virology, FSBI N. F. Gamaleya NRCEM, Ministry of Health of the Russian Federation, Moscow, Russia
[4] R&D Department, FORT LLC, Moscow, Russia

Background: Influenza viruses belong to *Orthomyxoviridae* family of enveloped viruses with segmented negative RNA genome; SARS-CoV-2 belongs to *Coronaviridae* family with positive RNA genome. The interaction of glycoproteins incorporated into the lipid envelope with matrix layer underneath, and the matrix protein with the nucleocapsid are poorly understood. We aimed to compare the morphology and overall inner structure of virions of two Influenza virus types (A and B) and SARS-CoV-2 using negative staining transmission electron microscopy (TEM) and cryo-EM.

Methods and Results: The Influenza viruses A/Puerto Rico/8/34 (H1N1) and B/Phuket/3073/13 were grown in the allantoic cavity of embryonated chicken eggs and purified via ultracentrifugation through 20% sucrose cushion. The SARS-CoV-2 hCoV-19/Wuhan/WIV04/2019 strain was passaged in Vero cells up to 1 x 10^8 $TCID_{50}$/ml titre, inactivated using β-propiolactone (1:2000, 36h) or UV-radiation, concentrated using Amicon Ultra-15 100 kDa units, and kept at -80°C. For negative staining 2% phosphotungstic acid (pH 7.0) was used. For cryo-EM, the thawed virus was concentrated by ultracentrifugation. EM was performed with a JEM-2100 200kV LaB6 transmission electron microscope (JEOL, Japan) equipped with a Direct Electron DE20 direct detector and Gatan Elsa cryotransfer holder. TEM studies were carried out at the Shared Research Facility “Electron microscopy in life sciences” at Moscow State University (Unique Equipment “Three-dimensional electron microscopy and spectroscopy”, ID RFMEFI61919X0014).

The cryo-EM of A/Puerto Rico/8/34 (H1N1) and B/Phuket/3073/13 virus strains revealed mostly spherical virions. In most virions a clear matrix layer is visible adjacent to an outer lipid monolayer, while the inner lipid monolayer is poorly resolved. In some virions there are “gaps” found in the matrix layer, or the matrix layer is absent. Interestingly, in the latter case, there is often no high electronic density visible inside the virion implying a crucial role of matrix M1 protein in the incorporation of the nucleocapsid during virions buddying. While examining SARS-CoV-2 virions using negative staining, we found many particles entirely decorated with a typical S spike “crown”, some particles partly spike-decorated or entirely spikeless. The ultracentrifugation of thawed SARS-CoV-2 led to great losses, thus only few particles were examined using cryo-EM.

Conclusion: Cryo-EM helped to visualize a natural morphology of Influenza virus virions, matrix layer and nucleocapsid inside the virions. Partial integration of M1 protein into the lipid bilayer may be

hypothesized. Compared to a coat of tightly positioned hemagglutinin/ neuraminidase spikes of Flu virions, the coronavirus S spikes are longer, rarely located and much more fragile.

Key words: Influenza A and B viruses, SARS-CoV-2, cryo-EM, negative staining.

This work was supported by the Russian Foundation for Basic Research (grant 20-54-14006).

**** Corresponding author:*** *Larisa Kordyukova. E-mail: kord@belozersky.msu.ru*

Oral abstract
Abstract category: Structure of viruses and chaperonins

OVERVIEW OF THE PIPELINE FOR CHARACTERIZATION OF INACTIVATED TBEV SAMPLES FOR SINGLE PARTICLE STRUCTURE DETERMINATION

Samygina V.R.[1,2], Vorovitch M.F.[3,4], Pichkur E.[1], Konarev P.V.[1,2], Peters G.[1], Osolodkin D.I.[3,4], Ishmukhametov A.A.[3,4], Egorov A.M.[3,5]

[1] NRC «Kurchatov Institute», Moscow, Russia
[2] FSRC "Crystallography and Photonics", Moscow, Russia
[3] FSASI "Chumakov FSC R&D IBP RAS" (Institute of Poliomyelitis), Moscow, Russia
[4] Institute of Translational Medicine and Biotechnology, Sechenov First Moscow State Medical University, Moscow, Russia
[5] Department of Chemistry, Lomonosov Moscow State University, Moscow, Russia

Background: Determining the structure and function of biological nanomachines is a key goal of molecular biology. Single-particle techniques offer unique opportunity to understand the role of structural variability in biological function. For enveloped viruses, such as flaviviruses, cryo-EM or single particle imaging (SPI) at XFEL are the only suitable ways to resolve the structure at the atomic level. Quality of the sample is a crucial milestone of any structural method. High homogeneity, relatively high concentration, absence of aggregates is necessary for both methods. Additionally, large volume of sample is required for SPI at XFEL. Such requirements are suppose reliable quality control. Tick-borne encephalitis virus (TBEV) from flavivirus family is an important human pathogen. Single particle structural studies can provide new insight into TBEV structure to further improve the vaccine design.
Methods and Results: An approach for the sample quality control combines negative staining TEM, cryoEM, AFM microscopy, Dynamic Light Scattering and small-angle X-ray scattering. Differential mobility analysis measurements were carried out in order to measure the particle size distribution after the sample was transferred into an aerosol. The obtained sample was successfully delivered to the X-ray interaction region point at the SPB/SFX instrument of European XFEL, and diffraction patterns were obtained from the individual iTBEV particles, thus providing a proof-of-concept for possibility of iTBEV single-particle imaging experiments. Cryo-EM data for high cryo-EM structure determination were collected using cryo-TEM Krios (Thermo-Fisher, USA) at 300kV using DED Falcon II. Dataset was preprocessed using Warp. Further processing was performed in Relion 3.1 and CisTEM. The quality control pipeline allowed us to prepare a concentrated, homogenous, non-aggregated TBEV sample. Moreover, we were able to solve the high-resolution iTBEV structure with the best resolution to date (3.02Å).
Conclusion: We demonstrated a diffraction pattern obtained with a single X-ray pulse from inactivated TBEV particle for the first time – using SPB/SFX instrument of the EuXFEL. Our quality assessment workflow may be useful for the preparation of other challenging virus samples for single particle imaging at different FELs. The high resolution cryo-EM structure further confirming the high quality of the sample obtained using quality control pipeline.

Key words: tick-borne encephalitis virus, virus characterization, single particle imaging, high resolution cryoEM.

This work was supported by the RFBR (Grant No. 18-02-40026) in part of quality control pipeline development and Russian Ministry of Science and Education (Agreement No. 075-15-2021-1355 (12 October 2021)) in part of high resolution cryo-EM structure determination.

**** Corresponding author:*** *Valeriya R Samygina. E-mail: lera@crys.ras.ru*

Poster abstract
Abstract category: Structure of viruses and chaperonins

THREE-DIMENSIONAL CRYO-ELECTRON MICROSCOPY RECONSTRUCTION OF THE TAIL OF GIANT BACTERIOPHAGE AR9

Sirotkin I.[1], Moiseenko A.[1,2], Sokolova O.[1,3]

[1] Faculty of Biology, Lomonosov Moscow State University, Moscow, Russia
[2] N.N.Semenov Federal Research Center for Chemical Physics, Russian Academy of Sciences, Moscow, Russia
[3] Department of Biology, Shenzhen MSU-BIT University, Shenzhen, Guangdong, China

There has recently been reported an increased attention to giant bacteriophages, which comprise a special group of bacterial viruses characterized by a large genome of over 200 kbp. This group raises many questions concerning their genome composition and structural features, which are reflected in new infection strategies and increased resistance to the host defense systems. The object of this study is the *Bacillus subtilis* phiKZ-like giant bacteriophage AR9 encoding their own multisubunit RNA polymerase. No high-resolution phage structures have been obtained for members of this group, which complicates our understanding of the organization of these biological objects.
Cryo-electron microscopy of AR9 phage samples was performed and single particle analysis was used to obtain three-dimensional reconstructions. As a result, the three-dimensional structure of the phage tail with a resolution of 2.7 Å was obtained. The AlphaFold2 neural network was used to build atomic models of phage tail proteins. The complete atomic model of the phage tail was obtained and successfully validated with *MolProbity*, attesting to the quality of the model.
The low conformational heterogeneity of AR9 phage tail allows to reveal its structure with near-atomic resolution. According to the reconstructed atomic models, sheath protein of the contractile tail has the structure of the inner and middle domains similar to typical members of *Myoviridae* (for example, phage T4). The structure of the outer domain of the sheath protein is new, not previously found among giant bacteriophages. The sheath proteins are bound to each other by a single β-sheet formed by β-strands of the inner domain of the underlying and the terminal domains of the two overlying proteins.

The authors express their deep gratitude to Maria Yakunina and Daria Antonova (Peter the Great St. Petersburg Polytechnic University, St. Petersburg, Russia) for the provided purified and concentrated phage samples for cryo-EM. The authors are also grateful to Fuxing Wang and Zheng Liu (Kobilka Institute of Innovative Drug Discovery, School of Medicine, Chinese University of Hong Kong, Shenzhen, Guangdong, China) for conducting cryo-electron microscopy experiments. This work was funded by RSF (21-44-07002).

**** Corresponding author:*** *Ilia Sirotkin. E-mail: sirotkinil5@gmail.com*

SECTION V:

EM RESEARCH RELATED TO MEDICINE

Poster abstract
Abstract category: EM research related to medicine

SPATIAL ORGANIZATION OF AMYLOID FIBRILS, FORMED BY YEAST PROTEIN SUP35, STUDIED BY CRYOELECTRON MICROSCOPY

Burtseva A.D.[1,2], Baymukhametov T.N.[3], Moiseenko A.V.[4], Dergalev A.A.[1], Kushnirov V.V.[1], Boyko K.M.[1]

[1] Bach Institute of Biochemistry, Research Center of Biotechnology RAS, Moscow, Russia
[2] Moscow Institute of Physics and Technology (NRU), Moscow, Russia
[3] "Kurchatov Institute" National research center, Moscow, Russia
[4] Faculty of Biology, Lomonosov Moscow State University, Moscow, Russia

Prions represent an infectious variety of amyloids - fibers with a common cross-β structure. Amyloid formation is of particular importance in the context of a range of medical disorders that include Alzheimer's and Parkinson's diseases and type 2 diabetes. These disorders are becoming increasingly common in the modern world and now affect some 500 million people worldwide [1]. All known prion diseases in mammals are caused by the endogenous protein PrP [2], which has similar properties to the prion proteins of the yeast *Saccharomyces cerevisiae* [3]. Sup35, the best studied yeast prion, , is a convenient research model for determination of amyloid structure and properties. The conformational diversity of amyloid fibrils underlies the existence of structural variants of the Sup35 prion [4]. According to protease mapping data, these variants can be divided into two types: strong (S) and weak (W) [3,4].

The Sup35 amyloid structure has not been established yet and the proposed models are somewhat contradictory [3]. Furthermore, cryoelectron microscopy (cryo-EM) is the only method that allows study the structure of such objects with a high resolution.

This work is devoted to the study of the spatial structure of the prion protein Sup35 isolated *ex vivo* from the yeast *S. cerevisiae* by cryo-EM. Negative staining of the W variant of the Sup35 was performed and the low-resolution 3D-model of the fibril was obtained. The model demonstrates the characteristic features of the helical structure and has ~100 Å diameter. However, cryoEM data showed the presence of a dense "coat" around fibrils. The "coat" seems to be formed by GFP molecules, covalently attached to Sup35 to simplify the isolation procedure. 2D-classification revealed that the presence of GFP prevents high-resolution reconstruction of the structure. Currently, for further research GFP-less construction of Sup35 is being obtained.

This work was supported by Russian Science Foundation grant № 23-74-00062.

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**** Corresponding author:*** *Anna Burtseva. E-mail: a.burtseva@fbras.ru*

Poster abstract
Abstract category: EM research related to medicine

MOVABLE NETWORK OF POLYSACCHARIDE AND NATURAL NANOTUBES

Molchanov V.S., Glukhova S.A., Philippova O.E.

Physics Department of Moscow State University, Moscow, Russia

Background: Recently, additive technologies have become extremely popular. They make it possible to obtain products by layer-by-layer addition of material (3D printing) and are able to save resources and ensure waste-free production, which is important not only from an economic, but also from an environmental point of view. From an environmental point of view, it is also important to develop 3D printing of natural biodegradable polymers, which, at the end of use, will not pollute the environment. Such polymers include, in particular, polysaccharide gels. One of the important directions in the development of this technology has become 3D bioprinting for use in medicine. The materials currently used for recovery and regeneration are predominantly based on materials of natural origin. The advantage of natural polymers for 3D bioprinting and other tissue engineering applications is their similarity to the human extracellular matrix and their inherent biological activity.
It is shown that the most promising method of 3D printing for gels is layer-by-layer extrusion due to its simplicity, low cost and high quality printing. However, it was found that products obtained from traditional hydrogels are characterized by low mechanical strength. The problems can be solved by introducing inorganic nanomaterials into the polymer matrix.
Methods: Rheometry and cryo-transmission electron microscopy were combined to investigate the structure and properties of the hydrogels.
Results: To create grids used in the form of ink for 3D printing, only natural components were used: sodium alginate polysaccharide and aluminosilicate nanotubes of halloysite clay. To improve the mechanical properties of the gel, natural nanotubes of halloysite clay. At a low concentration of such nanotubes, "ordinary" nanocomposite hydrogels were obtained, and at a concentration of nanotubes exceeding the concentration of their overlap, double network structures were obtained, in which the polymer network is combined with a network of inorganic nanoparticles.
Conclusions: Thus, nanocomposite hydrogels based on natural components have been developed and the conditions corresponding to the fastest fixation of the structure, as well as the greatest mechanical strength of the resulting gels, have been determined. It is shown that the most durable gel is formed in the case of double network structures, in which the polymer network is combined with a network of inorganic nanoparticles.
Key words: polysaccharide, clay nanotubes, 3D printing.

This work was supported by the Russian Science Foundation (project № 23-13-00177).

**** Corresponding author:*** *Vyacheslav Molchanov. E-mail: molchan@polly.phys.msu.ru*

Poster abstract
Abstract category: EM research related to medicine

STUDY OF THE MORPHOLOGY OF SMALL EXTRACELLULAR VESICLES OF THE H358 CELL LINE BY CRYOELECTRON MICROSCOPY

Senkovenko A.M.[1], Moiseenko A.V.[1], Skryabin G.O.[2], Tchevkina E.M.[2], Bagrov D.V.[1]

[1] Faculty of Biology, Lomonosov Moscow State University, Moscow, Russia
[2] Institute of Carcinogenesis, N. N. Blokhin National Medical Research Center of Oncology, Moscow, Russia

Background. Small extracellular vesicles (sEV) are particles with a diameter of 30 nm to 150 nm, surrounded by a lipid bilayer, and secreted by cells into the extracellular space. These objects are found in large quantities in biological fluids or culture media of any cells. EVs are a promising object for research since their physical parameters and chemical contents serve as markers for the development of various diseases.

Transmission electron microscopy (TEM) is the most popular method for visualizing EVs. However, the field of view may be contaminated with proteins from the solution or crystals of the staining agent. The cryo-EM method does not imply adsorption of the sample onto the substrate and preserves the morphology of objects by instantly freezing them. Thus, cryo-EM will provide a more accurate assessment of the size and morphology of particles than imaging using TEM.

Methods and results. An experiment on the study of EVs using cryo-EM was carried out at Moscow State University for the first time. For the experiment, we used a sEV sample isolated from the cultivation medium of the H358 cell line. Most of the studied particles had a regular shape and one membrane. However, approximately 20% of the sEV in the experiment had a different morphology. For example, particles with two or more membranes were observed, particles of a non-spherical shape, as well as multilayer objects with a complex structure that had one or more other vesicles inside. The scatter in size is the largest in sEVs with a complex structure (83 ± 40 nm) since their morphology is diverse. The mean sizes of vesicles measured by TEM with negative staining (79 ± 36 nm) and cryo-EM (47 ± 26 nm) turned out to have a significant difference, which can be explained by the deformation of particles upon adsorption onto a substrate and a size decrease upon drying. The results of nanoparticle tracking analysis also show an increase in the mean size of particles (112 ± 4 nm) due to the non-specific registration of vesicle aggregates.

Conclusion. According to the results of the experiment, it can be judged that sample preparation for TEM significantly affects the size of EVs. As a result, there is a difference in the diameters of objects measured by cryo-EM and TEM. Cryo-EM images show the morphological features of individual vesicles, which usually change during sample preparation for TEM imaging with negative staining.

Key words: Cryo-EM, TEM, extracellular vesicles, morphology.

**** Corresponding author:*** *Alexey Senkovenko. E-mail: senkovenkoam@my.msu.ru*

Poster abstract
Abstract category: EM research related to medicine

CRYO-ELECTRON MICROSCOPY OF EXTRACELLULAR VESICLES OF ADIPOSE TISSUE IN OBESITY AND TYPE 2 DIABETES

Shtam T.A.[1,3], Dracheva K.V.[1,2], Yastremsky E.V.[3], Garaeva L.A.[1,3], Pobozheva I.A.[1,2,3], Anisimova K.A.[2], Balandov S.G.[2], Hamid Z.M.[2], Vasilevsky D.I.[2], PchelinaS.N.[1,2,3], Konevega A.L.[1,3], Miroshnikova V.V.[1,2]

[1] Petersburg Nuclear Physics Institute named by B.P. Konstantinov of NRC Kurchatov Institute, Gatchina, Russia
[2] Pavlov First Saint Petersburg State Medical University, St.-Petersburg, Russia
[3] NRC Kurchatov Institute, Moscow, Russia

Background: Extracellular vesicles (EVs) are membrane-bound vesicles of cellular origin that play an important role in cell-to-cell communication and physiology. EVs deliver biological information from producer cells to recipient cells by transporting various cargoes such as proteins, RNA molecules, and lipids. Adipose tissue EVs can regulate metabolic and inflammatory interactions within the adipose tissue depot, as well as in distal tissues. Thus, it is suggested that adipose tissue EVs are involved in the pathologies associated with obesity, especially insulin resistance and type 2 diabetes mellitus (TDM2). In this study, for the first time, we characterized EVs secreted by visceral (VAT) and subcutaneous adipose tissue (SAT) of obese patients with type 2 diabetes mellitus using standard methods, and analyzed their morphology using cryo-electron microscopy.
Methods and Results: Cryo-electron microscopy allowed us to visualize heterogeneous population of EVs of various size and morphology including single EVs and EVs with internal membrane structures. Single vesicles prevailed (up to 85% for SAT, up to 75% for VAT) and their mean size was smaller for SAT when compared with VAT in obese patients with/without T2DM. The total proportion of EVs with internal membrane structures was higher in the samples of VAT EVs compared to the SAT. Decreased size of: single and double SAT EVs, large proportion of multilayered VAT EVs and all EVs with internal membrane structures distinguishes obese with/without T2DM from the control group. These findings could support the idea of modified biogenesis of EVs during obesity and type 2 diabetes mellitus.
Conclusion: The study describes for the first time the high degree of morphological variability of EVs in adipose tissue. A feature of VAT is a large proportion of vesicles with an internal membrane structure, including multilayered explosives, compared to SAT. A decrease in the single and double size of EVs in the SAT, as well as a large proportion of multilayer EVs and EVs with an internal membrane structure characteristic of VAT, distinguish obese patients with/without T2DM from the control group. Morphologically different subpopulations of EVs in different forms of obesity may have certain functions, the definition of which requires further research.
Key words: cryo-EM; extracellular vesicles; exosomes; obesity; 2 diabetes mellitus; adipose tissue.

The study was supported by the Russian Science Foundation (grant no. 19-74-20146).

**** Corresponding author:*** *Tatiana A. Shtam. E-mail: shtam_ta@pnpi.nrcki.ru*