BOOK OF ABSTRACTS

5th RUSSIAN INTERNATIONAL CONFERENCE ON CRYO-ELECTRON MICROSCOPY 2025

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SECTION I:

STRUCTURE OF MEMBRANE PROTEINS

Poster abstract
Abstract category: Structure of membrane proteins

OLIGOMERIZATION OF PIGMENT EPITHELIUM-DERIVED FACTOR IN THE PRESENCE OF ZINC ALTERS ITS FUNCTIONS

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Background: Zinc is an essential micronutrient involved in numerous neuronal processes, including the facilitation of neurotransmission. Dysregulation of zinc homeostasis can trigger pathological mechanisms leading to irreversible neurodegeneration in the central nervous system and retina. Excess zinc, particularly concentrated in the interneuron space, has been implicated in neurotoxic cascades. Pigment epithelium-derived factor (PEDF) is a multifunctional protein with known anti-angiogenic, anti-tumorigenic, and neurotrophic properties, playing a protective role in retinal and neuronal health.
Methods and Results: We investigated the molecular interaction between zinc ions and PEDF using X-ray diffraction on protein crystals. Our findings revealed that PEDF binds zinc at five distinct high-affinity intermolecular sites. This binding results in a reduction of the protein's overall negative surface charge and promotes reversible oligomerization. Therefore, key functional domains of PEDF involved in neurotrophic and antiangiogenic activity, as well as its interaction with extracellular matrix components such as collagen, become sterically hindered or functionally masked.
Conclusion: These findings suggest that elevated zinc levels in the retinal environment may modulate PEDF activity through direct binding. The resulting structural changes impair PEDF's protective functions, potentially contributing to zinc-induced neurodegeneration. This interaction highlights a novel mechanism through which zinc dysregulation may influence retinal pathology and identifies PEDF as a potential molecular sensor of zinc imbalance in the neuroretina.

This work was supported by Ministry of Science and Higher Education of the Russian Federation (24-15-00171).

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Poster abstract
Abstract category: Structure of membrane proteins

NMR STRUCTURE OF THE TOXIN U_{11}-MYRTX-TB1A FROM THE ANT *TETRAMORIUM BICARINATUM* IN THE MEMBRANE MIMICKING ENVIRONMENT

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In the context of rapidly growing consumer demand, it is necessary to improve the efficiency of the agro-industrial complex. One of the key aspects of increasing the quality of agricultural products is the pest control of cultivated plants. Currently, animal toxins are considered as effective and safe pesticides. The toxin U_{11}-MYRTX-Tb1a (abbreviated U_{11}) from the venom of stinging ants *Tetramorium bicarinatum* exhibits high paralytic activity against blowflies and honeybees and, therefore, holds great promise for the discovery of the novel insecticidal molecules [1]. However, the mechanism underlying neurotoxic activity of U_{11} still remains unclear. Two possible mechanisms have been proposed to explain U_{11}'s neurotoxic effects. One suggests that the peptide modulates voltage-gated potassium channels [1]. Another proposes that U_{11} induces cytolysis by disrupting insect cell membranes [2]. Both mechanisms imply that U_{11} possesses membrane activity. Structural analysis by NMR spectroscopy in aqueous solution unraveled a compact triangular ring helix structure stabilized by a single disulfide bond [1], but the structure and functional behavior in the membrane environment has not been established yet. At the same time, structure-activity relationships for U_{11} in the membrane environment might uncover its potential mode of action.

To elucidate U_{11}'s functional behavior, we performed a comprehensive structural analysis of its interaction with membrane-mimicking environments using NMR spectroscopy. An NMR investigation of U_{11} in DPC micelles revealed a loop conformation containing extended amphipathic α-helix. Upon incorporation in the micelle U_{11} undergoes a significant structural rearrangement. The latter enhances the peptide`s ability to membrane binding, as confirmed by lipid titration experiments, demonstrating moderate membrane activity. As evidenced by the liposome leakage assay, U_{11} binds to the lipid bilayer and causes moderate membrane leakage after six hours of exposure.

The obtained spatial structure and topology of U_{11} in DPC micelles together with the liposome leakage results imply that the toxin likely acts through a «carpet» model of membrane disruption, i.e. covers the membrane surface in a carpet-like manner and disrupts the bilayer upon reaching the threshold concentration [3]. The determination of the exact 3D structure and improved understanding of the toxin mode of action could greatly assist future pharmacological development of next-generation bioinsecticides targeting membrane integrity.

Key words: ant toxins, membrane-active toxins, membrane disruption, NMR spectroscopy.

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Poster abstract
Abstract category: Structure of membrane proteins

OBTAINING STRUCTURES OF NEUROPEPTIDE G PROTEIN-COUPLED RECEPTORS WITH CRYOGENIC ELECTRON MICROSCOPY

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Neuropeptides constitute the most extensive and varied group of neuromediators found in both central and peripheral nervous systems. The majority of neuropeptides function through G protein-coupled receptors (GPCRs), which are the largest family of receptors located in cell membranes. Neuropeptides and their GPCRs are associated with various medical conditions, including obesity, pain, anxiety, mood and sleep disorders [1,2]. Therefore, medications aimed at neuropeptide GPCRs hold significant promise for the creation of new therapeutic agents. Understanding the structure of neuropeptide receptors would greatly simplify the remedy development process by utilizing structure-based drug design (SBDD) approach [3].

The work is focused on obtaining structures of selected neuropeptide GPCRs which involves expression and isolation of a functional and homogeneous receptor sample. In order to increase protein yield and to improve sample monodispersity we employ various strategies, including addition of receptor ligands and introducing stabilizing protein modifications. At the same time approaches like Nano-Luc system, introducing inactivating mutations to the G-proteins and addition of antibody fragments are used to facilitate active and inactive GPCR complexes formation and stabilization.

The proposed strategies significantly improved the quality of the obtained samples, allowing electron microscopy of the complexes with negative contrast. These experiments are essential for selecting optimal conditions for cryo-electron microscopy and obtaining structural data for the complexes

Key words: G protein-coupled receptors, neuropeptide receptors.

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Poster abstract
Abstract category: Structure of membrane proteins

MECHANISM OF MEMBRANE-MEDIATED PROTX-I INTERACTION WITH HUMAN TRPA1 CHANNEL

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Background: TRPA1 is a sensor of various stimuli including temperature and vast diversity of electrophilic ligands and plays major role in nociception. Activation of the channel leads to inflammation eliciting pain or itch, which makes it a potential target for novel analgesics and anti-inflammatory drugs. Protoxin-1 (ProTx-I) is a known antagonist of the human TRPA1 channel.
Methods and Results: In this work we determined a 2.8 Å cryo-EM structure of human TRPA1 in complex with ProTx-I in MSP2N2 lipid nanodiscs. We showed that ProTx-I binds to the VSL (S1-S4) domain of the TRPA1 in a region of S1-S2 and S3-S4 extracellular loops. This binding mode is reminiscent of that in complexes of protoxins with voltage dependent channels. Interactions between Arg23 of the toxin with Glu825 of S3-S4 loop and Glu760 from S1-S2 loop and Arg24 with Gln831 stabilize the complex, while Arg24, His25 and Trp 27 of ProTx-I displace flexible S1-S2 loop. Additionally, toxin interactions with lipids from a membrane surrounding the VSL domain were characterized, suggesting a membrane-mediated mechanism of the toxin-TRPA1 interaction. Probably upon the channel activation, the C-terminal part of the toxin is immersed in the membrane, which creates an additional energy barrier and prevents TRPA1 transition to the open state, explaining the antagonistic action of ProTx-I. This work also characterized the flexibility of the ProTx-I/TRPA1 complex and observed a hydrophobic mismatch between the height of the membrane portion of the channel and the thickness of the lipid bilayer.
Conclusions: Structural insights into ProTx-I interaction with TRPA1 and poposed mechanism of its antagonistic action elucidated it this study will be useful for enhancing understanding of molecular mechanisms of TRPA1 functioning. It also opens a possibility of rational drug design to develop more potent and specific antagonists of TRPA1.

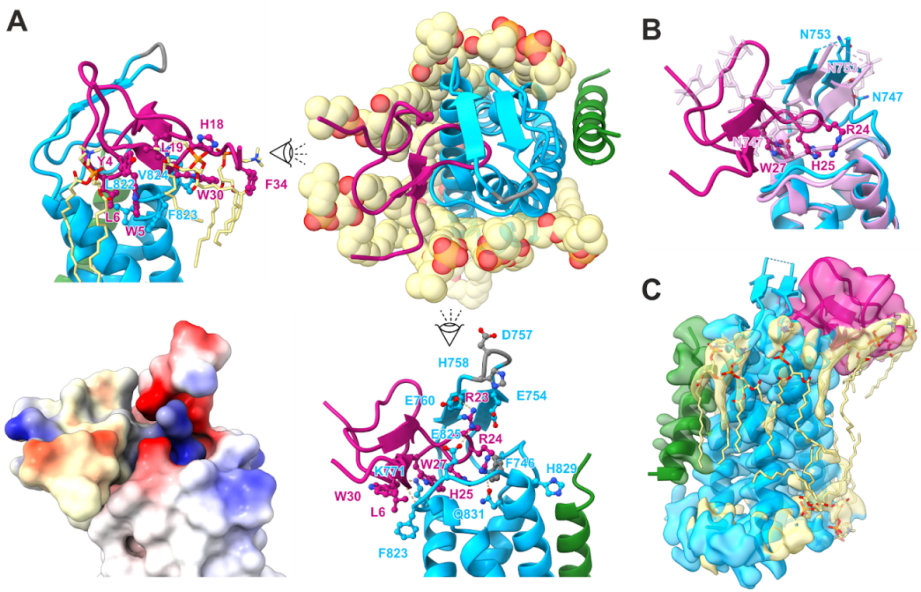

*Fig. 1. – **A.** Interface of ProTx-I binding. **Top right**: Top-down view. **Top left**: view from the bulk of the lipid membrane. Hydrophobic contacts and interaction with lipids are shown. **Bottom right**: View from the side. Residues participating in ionic contacts and h-bonds formation together with some additional charged residues are shown. **Bottom left**: Coulombic potential. **B.** Structural changes of VSLD induced by ProTx-I binding. C. Local refinement Cryo-EM density overview.*

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Poster abstract
Abstract category: Structure of membrane proteins

APPLICATION OF PULSED ELECTRON PARAMAGNETIC RESONANCE TO STUDY CONFORMATIONAL TRANSITIONS IN G PROTEIN-COUPLED RECEPTORS

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Background: G protein-coupled receptors (GPCRs) constitute a large family of membrane proteins (over 800 members) that play a crucial role in organismal physiology [1]. Upon binding specific molecules (ligands), the receptor undergoes a series of conformational changes that lead to the activation of secondary messengers, thereby transmitting extracellular signals into the cell. Dysregulation of these receptors is associated with severe diseases, making GPCRs a major focus of pharmaceutical research. This is evidenced by the fact that approximately 34% of all currently approved drugs target this protein family. Thus, obtaining structural information about GPCRs is a critical and timely objective, both for fundamental and applied science.

Methods and Results: One of the key challenges in understanding receptor mechanisms is investigating their conformational changes in response to ligand binding and interactions with partner proteins. A promising method for studying protein dynamics is electron paramagnetic resonance (EPR) spectroscopy. Pulsed EPR methods significantly expand the range of information that can be obtained from the system under study [2]. For instance, these techniques enable the determination of intramolecular distances within the range of 2–6 nm with angstrom-level precision, allowing researchers to investigate ligand- or protein-induced conformational transitions in proteins. Thus, pulsed EPR approaches can yield essential structural information about protein activation mechanisms that are inaccessible by other means. To apply this methodology to GPCRs, we selected a receptor that plays a key role in neurotransmission, behavioral regulation, and anti-inflammatory responses. Dysfunction of this receptor is linked to several severe disorders, making it an attractive and promising target for studying structural dynamics and activation mechanisms. Potential findings may contribute to the development of novel therapeutics. Notably, due to its high thermostability, this receptor serves as a model system for optimizing techniques that can later be extended to other GPCRs.

Conclusions: In this study, we conducted a series of experiments to optimize site-directed spin labeling protocols, identifying optimal conditions to improve labeling efficiency. Furthermore, we performed extensive pulsed EPR investigations to analyze conformational changes in the target receptor. As a result, valuable insights into the receptor's activation mechanisms, triggered by ligand and G-protein binding, were obtained.

Keywords: GPCR, pulsed EPR, protein conformational transitions.

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Poster abstract
Abstract category: Structure of membrane proteins

CREATION OF A CELLULAR PLATFORM FOR SCREENING LIGANDS OF THE CALCIUM-ACTIVATED POTASSIUM CHANNEL KCa3.1

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Potassium channels play a key role in controlling the cell's membrane potential and managing the transport of potassium ions across membranes. They are essential for the proper functioning of the nervous, immune, and cardiovascular systems. $K_{Ca}3.1$ channel is a calcium-activated potassium channel of intermediate conductance, which facilitates Ca^{2+}-dependent hyperpolarization of the cell membrane and regulates calcium signaling in different cell types. To study interactions of this channel with peptide blockers and search for potent peptide inhibitors, which have considerable therapeutical potential we developed a system based on the cells expressing fluorescently tagged $K_{Ca}3.1$ channel, investigated its cellular distribution and the ability to bind peptide ligands.
We engineered plasmids encoding the α-subunit of the $K_{Ca}3.1$ channel fused with the fluorescent protein mKate2 either at the N- or C-terminus. Fluorescent ligands specific to this channel were produced using a recombinant expression system in *E.coli* cells.
We report on the expression pattern of the $K_{Ca}3.1$channel in Neuro-2a cells, its ability to accumulate in lysosomes, endosomes, mitochondria, endoplasmic reticulum and Golgi apparatus. Mobility of the $K_{Ca}3.1$ channel on the plasma membrane was studied. We characterize the properties of the fluorescent ligands developed, highlighting their potential for imaging of channels in cells. We investigated a number of previously unexplored toxins and discovered several efficient blockers of the $K_{Ca}3.1$ channel.
This work lays the groundwork for advanced cellular assays that facilitate the screening and detailed analysis of ligands targeting the $K_{Ca}3.1$ channel.
Key words: $K_{Ca}3.1$ channel, pore blocker, fluorescence, confocal.

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Poster abstract
Abstract category: Structure of membrane proteins

STUDY OF THE STRUCTURE OF CARDIOLIPIN, ACYL CHAIN OF CYTOCHROME C WITH CARDIOLIPIN ACTIVATED BY CARDIOLIPIN COUMARINS C_{314} AND C_{525} CHEMILUMINESCENCE UNDER THE ACTION OF HETEROGENEOUS CATALYST

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Background: Modeling of the dimeric structure of cardiolipin, an acyl chain as part of the catalyst surrounded by coumarins showed that the chemiluminescence is 3-4 orders of magnitude higher than the excited ketones themselves, with cardiolipin itself being a proton trap.
Methods: The accuracy of the study is determined by the presence of cardiolipin for pH stabilization, Fe^{2+} quenching and the presence of: natural coumarin dye C_{314}, natural coumarin dye C_{525}. Factors that distort the modeling parameters of the dimeric structure of cardiolipin, acyl chain within the catalyst surrounded by probes: insufficient addition of hydrogen peroxide, excessive amount of nitrogen (II), methanol, protein denaturation, change in the conformation of cytochrome C in the catalyst.
The systems of lipoperoxidase and quasilipoxygenase reactions were analyzed.
Results: The catalyst differs from native cytochrome by the following properties: (1). Possesses fluorescence of tyrosine and tryptophan residues; (2). Loses absorption in the Sore band (405-410 nm) as a result of the breakage of the heme iron-sulfur Met(80) coordination bond in cytochrome C; (3). Possesses enzymatic activity; (4). Catalyzes the formation of lipid radicals in the membrane surrounded by fluorescent coumarin probes. (5). Enzymatic activity depends not only on the concentration of the catalyst but also on the ratio that determines the percentage of the absolute amount of the denatured form; (6). Microviscosity surrounded by coumarins has a different polarization coefficient; (7). At neutral pH, the heme in cytochrome C exists in a low-spin configuration, and the Fe center is hexagonally coordinated by the pyrrolic nitrogen from the porphyrin ring and the axial limands Met(80) and His(18) of the apoprotein; (8) When hydrogen peroxide is formed in a membrane protein, the acyl chain carrying the peroxide group is removed.
Coumarins physical activators of chemiluminescence are oxidized by the catalyst, and the rate of this oxidation is limited only by the concentration of cytochrome C itself, which is also destroyed as part of the catalyst under the action of hydrogen peroxide.
Modeling of the dimeric structure of the enzyme, cardiolipin, which catalyzes the formation of cardiolipin hydrogen peroxide, has shown that its consequence is: (1). Conformational changes in the latter. (2). Relocation of it to the outer mitochondrial membrane. (3). The formation of a pore through which

cytochrome C escapes into the cytosol.
Cardiolipin is a “double” glycerophospholipid. It possesses immune properties.
Conclusions: The results obtained by us can be the basis for the creation of new drugs, which are elements of the cell, respectively, affect cancer cells and are insensitive to synthetic drugs.
Keywords: cardiolipin, chemiluminescence, modeling, proton trap.

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Poster abstract
Abstract category: Structure of membrane proteins

HCIQ2c1: A NOVEL KUNITZ PEPTIDE MODULATING TRPA1 WITH POTENT ANTI-INFLAMMATORY ACTIVITY

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The TRPA1 ion channel is a key mediator of pain and inflammation, representing a valuable target for therapeutic intervention. HCIQ2c1, a Kunitz-type peptide isolated from the sea anemone *Heteractis magnifica*, emerges as a potent modulator of TRPA1 and exhibits both protease inhibitory and anti-inflammatory activities. This study aimed to characterize the spatial structure of HCIQ2c1, investigate binding to the TRPA1 voltage-sensing-like (VSD) domain, and evaluate nociceptive and anti-inflammatory efficacy.

The structure of recombinant HCIQ2c1 was analyzed by NMR spectroscopy, revealing a canonical Kunitz-type fold stabilized by three disulfide bonds, with dynamic glycine-rich loops implicated in functional interactions. Electrophysiological recordings in *Xenopus* oocytes expressing rat TRPA1 demonstrated that HCIQ2c1 binds preferentially to the open state of TRPA1, preventing its transition to closed and hyperactivated states. 15N-HSQC titrations with the VSL-domain TRPA1 in LPPG micelles identified binding interfaces involving the peptide's flexible loops and TRPA1 extracellular loops. Molecular modelling showed that HCIQ2c1 interacts simultaneously with pore and VSL-domain fragments from different TRPA1 subunits, as well as with lipid molecules, stabilizing the channel in an open conformation and restricting hyperactivation.

HCIQ2c1 significantly reduced nociceptive reactions induced by TRPA1 and TRPV1 agonists AITC and capsaicin in mice. Additionally, HCIQ2c1 decreased histamine- and lipopolysaccharide (LPS)-induced intracellular Ca^{2+} release and reactive oxygen species (ROS) production in RAW 264.7 macrophages. It inhibited pro-inflammatory mediators such as TNF-α, inducible NO synthase (iNOS), and 5-lipoxygenase (5-LO) *in vitro* and suppressed gene expression of TNF-α, IL-1β, COX-2, and iNOS in LPS-treated mice. Subplantar administration of HCIQ2c1 reduced carrageenan-induced paw edema by half, comparable to the effect of diclofenac.

HCIQ2c1 is the first Kunitz-type peptide shown to allosterically modulate TRPA1 via VSL-domain interactions, combining protease inhibition with channel stabilization. Its unique structural dynamics and targeted interaction with the VSL-domain underpin potent analgesic and anti-inflammatory effects,

highlighting HCIQ2c1 as a promising lead for development of novel therapeutics targeting TRPA1-mediated pathologies.

Key words: TRPA1 channel, Kunitz peptide, HCIQ2c1, anti-inflammatory, analgetics.

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Oral abstract
Abstract category: Structure of membrane proteins

UNDERSTANDING VIRAL REPLICATION ORGANELLE FORMATION – MOLECULAR ARCHITECTURE OF CORONAVIRUS DOUBLE-MEMBRANE VESICLE PORE COMPLEX

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Positive-stranded RNA viruses substantially remodel intracellular membranes during RNA replication, which is a common feature observed in picornaviruses, flaviviruses, noroviruses and coronaviruses. The replication of coronaviruses, such as MERS-CoV, SARS-CoV-2 and mouse hepatitis virus (MHV), leads to the formation of DMVs in host cells to accommodate viral RNA synthesis and modifications. SARS-CoV-2 non-structural protein (nsp) 3 and 4 are the minimal viral components required to induce DMV formation and to form a double-membrane spanning pore, essential for the transport of newly synthesized viral RNAs. The mechanism of DMV pore complex formation remains unknown. Here we present the molecular architecture of SARS-CoV-2 nsp3-4 pore complex, as resolved locally up to 3.9 Å resolution by cryo-electron tomography and subtomogram averaging within isolated DMVs. Our work establishes a framework for understanding DMV pore formation and viral RNA translocation, providing a structural basis for the development of new antiviral strategies to combat coronavirus infection. In this talk, I will present our recent work about coronavirus DMV pore complex and discuss the application of cryo-ET in virology research.

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Oral abstract
Abstract category: Structure of membrane proteins

OPTOPROTEOMICS AND APPLICATIONS IN NEUROSCIENCE: EXPANDING THE GENETIC CODE TO LINK MOLECULAR KINETICS AND BEHAVIOR VIA CELLULAR MIGRATION

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Understanding how molecular interactions drive neural circuit function and behavior remains a key challenge in neuroscience. This talk introduces optoproteomics, a technology that integrates genetic code expansion, light-based control, and proteomic analysis to study membrane protein dynamics in living systems. By incorporating noncanonical amino acids (ncAAs) such as *p-azido-L-phenylalanine* (*AzF*) at specific protein sites, we achieve light-responsive labeling and crosslinking with single-residue precision. This enables dynamic mapping of protein interactions and signal transduction under physiological conditions. Our prior work on GPCR (rhodopsin) and NMDA receptors revealed novel mechanisms of light-induced phosphorylation and pathway rewiring. These tools were validated in transgenic mice and zebrafish, confirming the feasibility of heritable optoproteomic systems.
Our current research targets – Smoothened (Smo) receptor – to decipher how localized molecular events influence cellular migration, particularly during remyelination and tumor progression. Using cryo-EM, site-specific crosslinking, and functional assays, we aim to correlate receptor activity with outcomes like neuronal cell differentiation and cancer cell metastasis. This presentation outlines a multidisciplinary approach that merges synthetic biology, chemical tools, and structural biology. We highlight how optoproteomics offers unprecedented resolution in linking molecular kinetics to systems-level phenomena. Ultimately, this strategy has the potential to drive innovations in diagnostics and targeted therapies for complex brain disorders.

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Poster abstract
Abstract category: Structure of membrane proteins

CRYO-ELECTRON MICROSCOPY OF MEMBRANE PROTEINS: QUANTITY CHAMPION, QUALITY CHALLENGER?

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Background: Membrane proteins (MPs) play a key role in cellular function and interaction with the environment, acting as transporters, receptors, and more. Determining the structures of membrane proteins is crucial for understanding their functions and mechanisms of interaction with ligands, including pharmaceutical compounds. For a long time, X-ray diffraction (XRD) was the dominant method for obtaining MP structures; however, in recent years, there has been a rapid increase in the number of structures determined by cryo-electron microscopy (cryo-EM). In this study, an analysis was conducted on the distribution of membrane protein structures obtained by cryo-EM, depending on size-related parameters such as molecular weight, protein molecular diameter, and hydrophilic region size. This analysis was performed using software packages for analyzing known MP structures and working with integrated membrane protein databases [1]. A comparative analysis was also carried out between structures obtained by cryo-EM and those obtained by XRD.
Methods and Results: In this study, a software package for the analysis of membrane protein structures was used [1], written in the Python programming language and utilizing libraries such as NumPy, Matplotlib, Pandas, and urllib3. Information from digital membrane protein databases was also used, including mpstruc [2], Orientations of Proteins in Membranes (OPM) [3], PDBTM [4], and SCOP [5]. The results obtained demonstrate the advantages of the cryo-EM method in resolving the structures of large membrane proteins. It was also shown that, on average, the resolution of structures obtained using electron microscopy does not tend to decrease with increasing protein size, in contrast to XRD methods.
Conclusions: In the course of analyzing membrane protein structures obtained by cryo-electron microscopy, it was shown that cryo-EM demonstrates a significant advantage in determining large-sized structures, making it a more favorable method for solving this type of task. However, X-ray diffraction remains the preferred method for obtaining smaller structures, indicating that this approach will continue to be relevant in the near future.
Keywords: membrane protein, protein structure, structural biology, cryo-electron microscopy, database, classification.

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SECTION II:

ADVANCES IN EM TECHNOLOGY, TOMOGRAPHY AND IMAGE PROCESSING

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

OVERCOMING THE «RESOLUTION GAP»: COMBINATION OF SUPER-RESOLUTION MICROSCOPY AND CRYO-ELECTRON TOMOGRAPHY FOR MITOCHONDRIA-VIMENTIN BINDING SITES IDENTIFICATION

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Progress in fundamental research is directly related to the emergence of new research methods that not only expand the established classical concepts, but can provide information that fundamentally changes them. According to our data, vimentin filaments, binding to mitochondria, determine their distribution and mobility in cells (Nekrasova et al., 2011), affect the level of their membrane potential (Chernoivanenko et al., 2015), there is a region responsible for the interaction of vimentin filaments with mitochondria in the N-terminal part of the vimentin molecule (Nekrasova et al., 2011) and similar amino acid sequences are found in other proteins, for example, in desmin. Since direct interaction of vimentin filaments with microtubules (Schaedel et al., 2021) and actin filaments (Esue et al., 2006) has already been shown, these facts together allowed us to assume that the connection of individual cytoskeleton components with each other and with mitochondria is not limited to interaction through cross-linker and motor proteins. Vimentin (and possibly other intermediate filament proteins) can regulate cytoskeletal interactions with mitochondria (Alieva et al., 2024). A revolutionary study performed using cryo-electron tomography and fundamentally changing our understanding of the three-dimensional structure of vimentin filaments (Eibauer et al., 2024) motivated us to use the capabilities of the cryo-electron microscopy method to try to identify the binding sites of vimentin with other cytoskeletal components and mitochondria. To meet this challenge is fundamentally possible if we combine super-resolution microscopy (3D-SIM) and cryo-electron tomography. The task is to identify and to characterize in detail the ultrastructure of the binding sites of vimentin (and possibly other intermediate filaments) to mitochondria - in particular, to test whether the N-terminus of vimentin (desmin) extends through the outer mitochondrial membrane into the intermembrane space, or whether it penetrates through two membranes and enters the mitochondrial matrix Historically, the main problem for addressing appropriate biological questions for fluorescence correlative light and electron microscopy has been the so-called ‘resolution gap’. We hope that 3D-SIM usage instead of conventional fluorescence microscopy in combination with cryo-electron tomography will allow us to overcome the ‘resolution gap’ and solve our problems.
Key words: mitochondria, vimentin, super-resolution microscopy, cryo-electron tomography.

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

CRYO-EM OF THE HUMAN PROTEASOME

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Background: The proteasome is a distinctive multicatalytic proteinase complex that, in conjunction with a ubiquitination system, facilitates the regulated degradation of the majority of intracellular eukaryotic proteins. Given its central role in regulating protein turnover, the proteasome is essential for maintaining cellular homeostasis and responding to stress. Disruption of proteasome-mediated degradation is associated with a range of human diseases, including multiple myeloma, Parkinson's disease, and autoimmune syndromes. The challenge in studying the proteasome stems from the diversity of its intracellular forms, which arise from the modularity of the proteasome assembly process. However, the proteasome is a relatively convenient object for cryo-EM due to a number of features.
Methods: For cryo-EM samples preparation Quantifoil R1.2/1.3 300 mesh grids coated with an additional graphene-oxide film were used. Samples were applied onto the grids, blotted in a range of 1-3 s at 10°C and 100% humidity, and subsequently plunge-frozen in liquid ethane using the Vitrobot Mark IV (TFS). The collection of cryo-EM data was performed using a Titan Krios (TFS) transmission electron microscope, which was equipped with a field emission electron gun X-FEG (TFS), a spherical-aberration corrector (CEOS), a pre- Falcon II (TFS) and a post-energy filter K3 (Gatan) direct electron detector. The initial cryo-EM data were pre-processed in Warp (Tegunov et al., 2019). Subsequent data processing steps were performed using the cryoSPARC (Punjani et al., 2017) and RELION (Zivanov et al., 2019).
Results: A number of cryo-EM structures were obtained at a resolution of 2.0-2.5 Å for 20S and 26S proteasomes in complex with ligands using K3 DED as well as structures of 30S proteasomes at 3.6 Å (Nyquist limit) using Falcon II DED.
Conclusions: Cryo-EM allows both the investigation of the structural features of small molecule interactions with high resolution, as well as visualization of the structural flexibility and conformational variability of proteasome complexes. This poster delineates the technical intricacies and some details of the proteasome cryo-EM data collection and processing pipeline at the NRC "Kurchatov Institute".
Key words: cryo-EM, proteasome.

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

HIGH-RESOLUTION CRYOEM STRUCTURE OF SUB-100KDA PROTEIN: A CASE STUDY OF TRAMTRACK GROUP BTB DOMAINS

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Determining the structures of small proteins using CryoEM remains a challenging task. Particle picking and proper alignment are the main obstacles. Increasing of particle size using Fabs represent a reliable, yet expensive and laborious solution. Here we successfully used the strategy to increase the particle size by fusing target protein with maltose-binding protein (MBP) through an alpha-helical linker with restricted mobility, initially designed for crystallography purposes. Drosophila Tramtrack group BTB domains have unique ability to form hexamers with yet unknown structure. The size of each monomer is about 15 kDa, therefore the size of hexamer is 90 kDa. Whereas the particles are visible, their flat and rounded shape interfere with picking the top views and following particle alignment. Fusing the BTB hexamer to MBP increases the particle size to roughly 350 kDa and substantially improves the reliability of particle picking and subsequent structure solution steps. The fusions were found to be unstable under vitrification conditions, requiring adding detergents to maintain the particle integrity. As a result, we were able to solve the structure of BTB domain from CG6765 protein at 3.3A resolution. The hexamers were found to be consisting of trimer of canonical BTB dimers, yet the symmetry was not perfect, so applying refinements with pre-defined symmetry usually resulted in lower resolution. Using the MBP fusions with flexible linker led to poorer results, obtaining high resolution structures were not possible in these cases. The described strategy can be widely used for structure solution of small multimeric proteins.

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

STRUCTURAL ANALYSIS OF TUBULIN PROTOFILAMENTS VIA CRYO-ELECTRON MICROSCOPY AND COMPUTATIONAL MODELING: PROGRESS, PITFALLS, AND PERSPECTIVES

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Background: Cryo-electron microscopy and tomography have provided unprecedented insights into cellular architecture, including essential cytoskeletal components such as microtubules. Microtubules are highly dynamic polymers composed of thirteen linear strands of tubulin heterodimers that play critical roles in intracellular transport, maintenance of cellular structure, cell division, and migration. The precise control of microtubule polymerization/depolymerization and the coupling of microtubules with various cellular structures is intimately linked to the nucleotide composition and conformational dynamics of tubulin protofilaments at the dynamic tips of microtubules.
Methods and Results: Here we share our experience and recent observations from integrating cryo-electron microscopy and tomography data with computational approaches, including Brownian and molecular dynamics simulations, to analyze the structure and dynamics of tubulin polymers. We focus on three main topics. First, we review recent findings derived from the application of denoising algorithms, which enhance the signal-to-noise ratio in cryo-electron tomography and enable visualization of the variable configurations of relatively flexible tubulin protofilaments. Second and third, we examine how specific aspects of sample preparation - namely, finite freezing rates and proximity to the water-air interface - may potentially distort the observed structures. We support these analyses with quantitative insights from Brownian dynamics simulations of the process of tubulin protofilament freezing and molecular dynamics simulations of tubulin protofilaments, relaxing from configurations recently reported for single bacterial tubulin protofilaments, assembled *in vitro* and imaged with cryo-electron microscopy.
Conclusions: Our findings be instrumental in guiding future structural studies and refining interpretations of microtubule dynamics and associated cellular processes.

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

CRYO-EM HAS REVOLUTIONIZED STRUCTURAL BIOLOGY AND IS NOW REWRITING THE FOUNDATIONS OF PHYSICS

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The classical Shannon-Nyquist sampling theorem is at the basis of all measurements in the physical sciences, including Cryogenic-Electron-Microscopy (Cryo-EM [1]). Unfortunately, Shannon's proof of the sampling theorem is mathematically incorrect and incomplete. An important aspect of that incompleteness is the lack of symmetry between the Real-space and the Fourier-space sampling. Symmetric-sampling remedies that incompleteness [4], but it also opens a new and wider perspective on sampling in all Physical Sciences. In a series of papers, the measurement problem in Physics is resolved that was believed to be specific to Quantum Mechanics. The fact that the classical Laws of Physics are based on continuous analytical functions implies that they cannot be measured or expressed digitally in a computer with the necessary infinite precision. Strangely enough, the classical sampling theorem itself is a Law of Physics in that sense, a law that does not include any explicit transition from a continuous analytical input signal to an actual discrete physical measurement! This may sound like pedantic remarks of a theoretician, but these problems already worried The QM founding fathers like Erwin Schrödinger, Albert Einstein and Paul Dirac expressed the same worries, a century ago, with respect to the "measurement" ("Born rule") in classical Quantum Mechanics (QM) that they considered "incomplete". The new developed in CryoEM technique of counting arriving electrons, may help remove that 100-years old theoretical conundrum in QM: the measurement postulate. Measurements are primarily the harvesting of information [2,3] under symmetric-sampling rule protocols [4-7], both in Cryo-EM and in QM. The measurement problem in classical Physics and in classical Quantum Mechanics, are due to two sides of the same coin: the prejudice that Physics is Deterministic (quoted from:[5]).

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

EXTENDING THE REACH OF SINGLE-PARTICLE CRYOEM

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Molecular structure determination using electron cryomicroscopy (cryoEM) will surpass X-ray crystallography as the most productive method for experimental determination of new structures during this coming year (2025), at least in terms of PDB depositions. Yet, there are still improvements to be made before we reach the physical limits set by radiation damage and the signal-to-noise ratio in images. I will give an overview of the current status with some examples and try to identify the remaining hurdles to making cryoEM even better.

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

STUDYING COMPOSITION AND STRUCTURE OF IMMUNE COMPLEXES BETWEEN PENTAMERIC C-REACTIVE PROTEIN AND MONOCLONAL IgG ANTIBODIES

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Background: C-reactive protein (CRP) plays a central role in innate immunity and thus is widely controlled in medical diagnostics as a key biomarker of inflammation to estimate severity and dynamics of diseases. The most common form of CRP is pentamer of identical subunits. Therefore, when CRP interacts with bivalent IgG antibodies, complexes of different stoichiometry and spatial structure are formed, which remain unstudied. A detailed characterization of such complexes is important both for understanding both the general patterns of polyvalent immunochemical interactions and for assessing the effects of therapeutic antibodies against CRP. To justify these issues, a cryo-EM reconstruction and structural analysis of CRP-IgG complexes was performed.

Methods and Results: For immune complexes formation, equal volumes of IgG and CRP with concentrations of 2 mg/mL in 20 mM Tris-HCl buffer, pH 7.6, containing 150 mM NaCl, were mixed and incubated. Cryo-electron microscopy was performed using a Titan Krios transmission electron microscope equipped with a Gatan K3 direct electron detector. Cryo-EM data processing was performed using cryoSPARC software.

A total of 2,475 micrographs were subjected to patch motion correction and patch-based estimation. Following several rounds of 2D classification, a set of 170,794 particles was selected. To interpret the data, an atomic model for the CRP monomer and the Fc-fragment of IgG was constructed and subjected to molecular dynamics flexible fitting.

Three-dimensional reconstruction of CRP–IgG complexes was obtained at a resolution of 3.5 Å. CRP is observed in pentameric form, consisting of five identical subunits assembled via non-covalent interactions into a disk-like structure. The CRP-IgG complex consists of two pentamers of CRP bound by 3 to 5 antibodies. The main state, comprising 43% of the dataset, featured five bound antibodies. Each antibody simultaneously attaches to two monomers of CRP from different pentamers.

We have built an atomic model describing the CRP-IgG interactions. Non-covalent interactions occur on the A-face of the CRP molecule. Several residues within this region are capable of forming salt bridges. However, in the observed complex, the antibody interacts with the CRP exclusively through hydrogen bonds.

Conclusions: Extensive knowledge of antigen–antibody recognition is essential for advancing bioengineering aimed at enhancing antibody affinity. We hypothesize that the presence of charged residues within the CRP binding site of specific antibodies suggests the potential for stronger interactions through salt bridges. Engineering such high-affinity antibodies represents a promising direction in the following development of therapeutic preparations.

Key words: C-reactive protein, monoclonal antibody, immune complex, cryo-electron microscopy.

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

ANALYSIS OF CHROMATIN MESOSCALE ARCHITECTURE BY CRYO-ELECTRON TOMOGRAPHY

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Background: Cryo-ET is a powerful tool for studying the structure of biological objects in the native state. However, Cryo-ET is still not widely used to study chromatin structure *in situ*. In our work, we aimed to investigate the possibility of using Cryo-ET to study the spatial organisation of chromatin, with special attention to possible drawbacks. We also focused on comparison with data from the more widely used TEM to study the effects of fixation on the cellular structures.
Methods and Results: 3T3 cells were grown on poly-L-lysine treated Quantifoils for several days. Cells were treated with 10 uM EdU for 10 min and fixed with 1% glutaraldehyde solution in 100 mM sodium cacodylate for 1h, then EdU was visualized by Click-reaction with Cy3-azide. Samples were blotted and plunge-frozen by using Vitrobot Mark IV. To target lamellae to regions of replicating chromatin, we used Leica Cryo CLEM system with 50x 0.9 lens followed by lamella milling in FEI Versa cryoFIB-SEM. Cryotomograms were acquired with Titan Krios at 300 kV. In this way, it was possible to obtain cryotomograms of nuclear regions in the DNA replication phase. There is clearly an increased density of nucleosomes packing on the nuclear periphery, corresponding presumably to peripheral heterochromatin. The rest of the chromatin volume is represented by fiber-like nucleosome assemblies with thickness in 20-30 nm range. We also performed classical electron microscopy on plastic sections to compare the data with Cryo-ET.
Conclusions: We optimized the pipeline for studying chromatin structures in cells using CryoCLEM. Replicative label made it possible to selectively target replicating nuclei, although the manufacture of lamellae is a bottleneck of this approach. We observed a similar morphology of chromatin for samples of plastic sections and cryofrozen samples thus partially ruling out an assumed detrimental effect of dehydration in sample preparation. Further it will be necessary to compare the data obtained with cells frozen without prior fixation. The tested pipeline can be adapted for use with fluorescent proteins to replace fixation and staining without compromising lamellae targeting.
Key words: cryo-electron tomography, correlation microscopy, chromatin.

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

TOMOGRAPHY STUDIES OF THE DENGUE FUSION PROCESS

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During dengue virus (DENV) infection, structural changes in the envelope (E) protein facilitate virus:endosomal membrane fusion. Crystal structures of E protein pre-fusion dimers and post-fusion trimers showed some of the structural information about the start- and end-stages of the fusion process. Here, we used cryo-electron tomography (cryo-ET) and biochemical assays to study the early events of the DENV fusion process. We characterized the interactions of DENV to liposomes at pH conditions that mimic that in the early to late endosomes. We also conducted tomography studies on the structural changes of DENV E proteins and identified an open E trimer fusion intermediate structure. Results offer new insights on the early fusion event of DENV.

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

COMPARATIVE CHARACTERISTICS OF BACTERIAL CELLS OF DIFFERENT SPECIES IN A DIGITAL IMAGE OBTAINED FROM A TRANSMISSION ELECTRON MICROSCOPE

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Background: Hundreds of spots (areas of increased electron density) are visible on digital photographs of bacteria obtained from a transmission electron microscope which reflect certain structures. It is of interest to quantify and compare them in bacteria of different species. It is very difficult to outline the micro-spots and determine their size, contour, and color intensity manually. Computer vision technologies will help speed up and facilitate this process.
Methods and Results: The object of research is cultures of Listeria, Staphylococcus and Proteus.
To obtain digital photographs from ultrathin slices, a JEM 2100 Plus transmission electron microscope (Japan) was used at a magnification of 50,000. A computer program has been developed that makes it possible to isolate individual spots of increased electron density. Briefly, the analysis scheme was as follows: the program "draws" a circle of a certain diameter in the image and determines the number of points on the circle brighter than the central one. This amount is visualized in color. This is how the entire image is processed, except for the edges. First, you need to select the size of this circle and the critical value of the number of points on the circle, less than which the result is considered a local background. The program determines the number of spots, their density, and calculates several parameters for each speck: area, brightness, length, width, and other parameters. The spots selected by the program match well with those selected manually.
The program calculates a set of spots of different area for each image of the cell. The color images obtained as a result of treatment of listeria and staphylococcus clearly show the outer and plasma membranes, while proteus has one outer membrane visible. Hundreds of different spots are detected in the cytoplasm. Detects spots similar to ribosomes and calculates their number. The differences in the sets of spots between cells of the same species were quite significant, which makes it possible to determine their physiological state. Differences in the sets of spots between different species were revealed, especially in the cell membrane.
Conclusions: Using the developed computer program, it is possible to determine various structures on a digital image of bacteria obtained from a transmission electron microscope and quantify them. Both intraspecific and specific differences in the quantitative composition of spots of increased electron density for listeria, staphylococcus and proteus were revealed.
Key words: electron microscopy, computer vision, Listeria, Staphylococcus, Proteus.

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

CRYOSILICO: SILICON-BASED SAMPLE CARRIER FOR CRYO-ELECTRON MICROSCOPY

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Cryo-electron microscopy (CryoEM) has become an essential tool for structural biology and adjacent fields. Despite the advancements in CryoEM, the design of the sample carrier (grid) has remained largely unchanged. However, microfabrication techniques, driven by the semiconductor industry, have made significant progress. In this abstract, we present a silicon-based sample carrier using microfabrication techniques to address the limitations of traditional grids.

Conventional grids are made from soft metals like copper or gold, typically covered with a holey carbon or gold film [1]. These materials are fragile and prone to bending, requiring careful handling. Furthermore, their surfaces are often uneven, making sample preparation challenging. The carbon film is transferred at the individual carrier level, leading to inconsistencies in surface properties even within the same batch. These individual-level processes also increase fabrication time and lead times.

Our silicon-based carriers use nanotechnology and microfabrication techniques to overcome these limitations. These carriers consist of a silicon frame, a holey silicon nitride membrane, and a monolayer graphene layer. Silicon provides a rigid and robust frame, making handling and sample preparation easier. The carriers are fully autoloader compatible, allowing users to seamlessly integrate them with conventional autoloader cartridges and C-clips. The growth of silicon nitride on a silicon substrate results in a highly reproducible, atomically flat surface, and microfabrication allows precise control of material properties, enhancing robustness.

Suspended graphene monolayer produces minimal background noise [2]. In side-by-side comparisons of CryoEM data collected with our silicon-based carriers and traditional gold grids, we observed a significant reduction in beam-induced motion. This increased stability, particularly in the critical first frames, preserves data quality when proteins are least damaged. Additionally, the ultra-flat membranes of our chips resulted in a defocus tilt angle of 0°. This significantly reduces defocus variations between particles, improving contrast transfer function (CTF) estimation. Our ultra-flat membrane, along with the absence of surface topography, also facilitates the formation of a uniform ice layer [3] and better particle adhesion [4,5]. Moreover, the large windows and lack of grid bars simplify patterning and milling during cryoFIB, streamlining lamella preparation.

MEMS-based manufacturing allows customizable design and easy adjustment of layout (e.g. grid square size, number of holes, hole diameter, etc.). It also ensures superior reproducibility across different batches, addressing a common challenge in grid production.

Silicon-based sample carriers represent the next step in CryoEM technology, offering enhanced reproducibility, stability, and flexibility. These carriers open up future possibilities for CryoEM applications, including the integration of nanosensors and nanoactuators.

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SECTION III:

STRUCTURE AND FUNCTIONS OF LARGE CELL MACHINERY

Oral abstract
Abstract category: Structure and functions of large cell machinery

MECHANISMS OF RIBOSOME STALLING BY THE ERMC LEADER PEPTIDE

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One well-characterized class of genes regulated by programmed translational arrest are the *erm* genes that encode ribosomal RNA methyltransferase enzymes conferring resistance to macrolide antibiotics. The *ermC* gene expression is controlled by a short upstream regulatory ORF, the leader peptide ErmCL. In the absence of macrolide antibiotics, the ribosome binding site and start codon of *ermC* are sequestered in a stem-loop structure and are therefore inaccessible to initiating ribosomes. However, in the presence of sub-inhibitory concentrations of the macrolide, ribosomes stall with the isoleucine codon at position 9 (Ile9) of ErmCL in the P site. Despite having been studied for decades, the molecular basis for ribosome stalling on this model leader peptide remains unclear. We present cryo-EM structures of translating ribosomes stalled on the ErmCL leader peptide. The primary stalling site, IFVI/S, is characterized by the unaccommodated A-site seryl-tRNA^{Ser}. The distorted conformation of the nascent ErmCL peptide chain, mediated by the ribosome-bound erythromycin, inactivates the peptidyl transferase center (PTC) of the ribosome by displacing nucleotide U2585, apparently impeding proper docking of the CCA-end of Ser-tRNA into the PTC. Unexpectedly, our cryo-EM analysis uncovers additional ribosome stalling sites along the ErmCL mRNA, illuminating the mechanisms of ErmCL–mediated ribosome stalling that regulate the expression of the ErmC methyltransferase.

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Oral abstract
Abstract category: Structure and functions of large cell machinery

STRUCTURE OF A BUNDLE-SHAPED PHYCOBILISOME

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Phycobilisomes (PBS) are soluble light-harvesting megacomplexes found in cyanobacteria and red algae. While PBS architectures have been extensively characterized in many species, the structural organization in the phylogenetically ancient cyanobacterium *Gloeobacter violaceus* has remained unresolved. In this study, we determined the cryo-EM structure of the *G. violaceus* PBS (GviPBS), a >10-MDa bundle-shaped complex composed of allophycocyanin (AP), phycocyanin (PC), and phycoerythrin (PE) hexamers, along with multiple linker proteins.

The pentacylindrical core of GviPBS is assembled from AP hexamers stabilized by the core linkers ApcE and ApcC. GviPBS exhibits a unique structural adaptation: two laterally positioned PC hexamers (Rx/Rx′) extend from the core, likely enabling the formation of higher-order PBS arrays. Furthermore, we identified two *Gloeobacter*-specific multidomain linker proteins, Glr1262 and Glr2806, critical for maintaining the bundle-shaped architecture. Glr1262 harbors three PBS-binding domains and adopts a triangular arrangement at the base of the rod-associated PC hexamers, while Glr2806 facilitates the attachment of the Rx/Rx′ hexamers to the core.

The GviPBS rod bundles consist of stacked PE and PC hexamers, extending from the pentacylindrical core, which is encircled by auxiliary PC hexamers. This arrangement introduces significant conformational flexibility, distinguishing GviPBS from canonical PBS structures. The bundle-like architecture of GviPBS expands the known structural diversity of PBS and provides insights into the evolutionary adaptation of light-harvesting systems in early-branching cyanobacteria.

Key words: phycobiliproteins, light-harvesting complexes, evolution.

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Poster abstract
Abstract category: Structure and functions of large cell machinery

H3-H4 TETRASOME PLASTICITY AND ITS FUNCTIONAL IMPLICATIONS

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Chromatin is a dynamic complex that packs the eukaryotic genome within the cell nucleus. The basic unit of chromatin is the nucleosome, a complex consisting of a histone octamer – formed by two copies each of the H2A, H2B, H3 and H4 histones – and DNA. Histone sequences and posttranslational modifications are a source of epigenetic variability and gene regulation. Histone-DNA complexes can assemble and disassemble naturally and dynamically. Complexes of DNA with only four out of eight histones (two copies each of H3 and H4) can also be found in chromatin. For instance, after transcription, H2A-H2B dimers are exchanged, yet the $(H3\text{-}H4)_2$ tetramer remains bound to the DNA. In this study, we examine the dynamics of the tetrasome, a complex consisting of the $(H3\text{-}H4)_2$ tetramer and DNA [1].

We performed molecular dynamics (MD) simulations and enhanced MD simulations, as well as analysing experimental X-ray data from the Protein Data Bank. In our equilibrium MD simulations (1–2 μs trajectories using Gromacs, Amber ff14SB+bsc1+CUFIX and the TIP3P water model), we observed that the H3 and H4 histones were more dynamic in the tetrasome than in the nucleosome. Based on the X-ray structure B-factors, we obtained RMSF profiles that were consistent with the MD-derived histone plasticity. Using principal component analysis, we extracted two major modes of $(H3\text{-}H4)_2$ tetramer dynamics: tetramer opening (pacman-like mode) and torsion of the tetramer along the superhelix axis. Tetrasome plasticity was systematically estimated in enhanced MD simulations (Methodynamics simulations), for which two modes of tetramer plasticity were used as collective variables. Two-dimensional free energy profiles showed that tetrasome dynamics couple both modes, with low free energy barriers observed for left-to-right handedness transitions of the DNA superhelix. Furthermore, feather metadynamics modelling disrupted the interface between two H3-H4 dimers, but not the histone-DNA contacts.

Key words: epigenetics, histones, DNA, molecular dynamics.

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Poster abstract
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STRUCTURAL STUDIES OF THE RIBOSOME FUNCTIONAL COMPLEX FROM *ACHOLEPLASMA LAIDLAWII*

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Background: *Acholeplasma laidlawii* is a small bacterium of the *Mollicutes* class that lacks a cell wall. Like other *Mollicutes*, these organisms are parasitic in eukaryotic cells due to their inability to synthesize many essential enzymes. *A. laidlawii* is a widespread saprophyte and commensal organism inhabiting mucous membranes of animals and birds, capable of evading immune responses and suppressing host immunity. The bacterium produces toxic lipoglycans that mimic host antigens and exhibits resistance to numerous antibiotics. Due to the absence of a cell wall, only protein biosynthesis-inhibiting drugs are effective against this bacterium. However, some *Mollicutes* have already developed resistance to these drugs, and the mechanisms responsible for the development of this resistance remain an important research challenge.
Methods: We used the single-particle cryo-EM reconstruction of the functional 70S ribosome complex with mRNA and tRNAs.
Results: In this work, we developed a protocol for the purification of 70S ribosomes from *A. laidlawii* and obtained high resolution cryo-EM data of 70S ribosomes in complex with mRNA and tRNAs.
Conclusion: Cryo-EM data reveals the presence of multiple classes of ribosomes, including a vacant 70S, a complex with mRNA and tRNA in the P-site, and a complex with mRNA and tRNA in the P- and A-sites.
Key words: ribosome, *Acholeplasma laidlawii*, cryo-electron microscopy.

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Poster abstract
Abstract category: Structure and functions of large cell machinery

ANALYSIS OF PROTEIN-PROTEIN INTERACTIONS OF HUMAN PIONEER TRANSCRIPTION FACTORS BASED ON PREDICTIONS OF THE STRUCTURES OF THEIR COMPLEXES

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Pioneer transcription factors (PTFs) such as SOX2, OCT4, and KLF4 play a critical role in cellular reprogramming by binding to heterochromatin and recruiting chromatin-modifying proteins. However, the molecular mechanisms of their protein-protein interactions (PPIs) remain uncovered. This study aims to predict and analyze the structures of PTF complexes with nuclear proteins to elucidate their functional roles in chromatin remodeling.

We employed AlphaFold-Multimer to predict binary complexes of SOX2, OCT4, and KLF4 with 3,557 human nuclear proteins. Structural and functional annotations were integrated from databases such as Pfam, InterPro, and TFRegDB. Quality metrics (ipTM, pDockQ, ipSAE) were used to filter predictions, and functional enrichment analysis was performed using Gene Ontology and the SimChrom classification.

We identified 1,453 high- and medium-confidence complexes, revealing novel interactions between PTFs and chromatin-associated proteins. Key findings include:

- KLF4 interacts with nuclear hormone receptors via a non-canonical motif (VAELL), resembling co-activator binding.
- SOX2 and OCT4 form complexes with methyltransferases (e.g., EEF2KMT, METTL23), suggesting roles in epigenetic regulation.
- A recurrent oncogenic mutation in KLF4 (K409Q/N) slightly disrupts interactions with transcriptional regulators, implicating its role in cancer.
- Transition regions from disordered to ordered states were mapped, highlighting the dynamic nature of PTF interactions.

Our study provides a comprehensive structural and functional map of PTF interactions, uncovering novel mechanisms in chromatin regulation and cellular reprogramming. These predictions offer a foundation for experimental validation and further exploration of PTF roles in development and disease.

Keywords: pioneer transcription factors, protein-protein interactions, AlphaFold2, chromatin remodeling, structural bioinformatics.

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Poster abstract
Abstract category: Structure and functions of large cell machinery

STRUCTURAL STUDIES OF THE IMMATURE 30S RIBOSOMAL SUBUNIT IN COMPLEX WITH THE ERA GTPASE ASSEMBLY FACTOR OF *STAPHYLOCOCCUS AUREUS*

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Background: Antibiotic resistance of such common microorganisms as *Staphylococcus aureus* leads to a constant search for new targets of action in the cells of these pathogens. The study of maturation and assembly factors of *Staphylococcus aureus* ribosome opens up possibilities for influencing the process of protein biosynthesis, which is essential for the vital activity of the cell. GTPase Era plays an important role in the maturation of the 30S ribosomal subunit at late assembly stages and its activity depends on the GTP level in the cell. An unusual mutual orientation of domains was showed in *Staphylococcus aureus* Era GTPase, which was not observed for Era GTPases from other microorganisms. In this regard, the location of the Era protein in the structure of the immature 30S subunit of the *Staphylococcus aureus* ribosome is of particular interest.
Methods: We used single-particle cryo-EM reconstruction of the complex 30S-Era from *Staphylococcus aureus*.
Results: In this work we obtained a cryo-EM map of 30S ribosomal subunits of *Staphylococcus aureus* in complex with the Era GTPase.
Conclusion: The 30S-Era cryo-EM map shows an additional density in the neck region of immature 30S subunits corresponding to the Era GTPase.
Key words: Era GTPase, ribosome, *Staphylococcus aureus*, 30S subunit, cryo-electron microscopy.

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Poster abstract
Abstract category: Structure and functions of large cell machinery

COOPERATIVE MODULATION OF NUCLEOSOME STRUCTURE BY P53 AND PARP1

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Poly(ADP-ribose) polymerase 1 (PARP1) and p53 are well-recognized for their central roles in regulating a wide array of biological pathways, including DNA repair, cellular differentiation, metabolism, and aging. Although both proteins have been extensively studied individually and are known to interact, the precise nature of their combined influence on chromatin structure remains to be fully elucidated. The present study seeks to clarify how PARP-1 and p53 together affect nucleosome organization. For this purpose, mononucleosomes labeled with fluorescent dyes were reconstituted on DNA templates containing the high-affinity 603 Widom nucleosome positioning sequence, flanked by two 20-base pair linker DNA segments. These templates were generated by PCR using fluorescently tagged oligonucleotides, with Cy3 and Cy5 fluorophores incorporated at positions 10 base pairs from the start of the 603 sequence within the linker regions. Protein-nucleosome complexes were assembled by incubating PARP-1 and the DNA-binding domain of p53 (p53DBD) with the nucleosomes in a buffer containing potassium at a physiologically relevant concentration (150 mM K^+). The formation and composition of these complexes were subsequently analyzed by native polyacrylamide gel electrophoresis (EMSA). The experimental design included two complementary approaches: in the first, increasing concentrations of p53 (25–200 nM) were added to pre-formed nucleosome–PARP-1 complexes (50 nM); in the second, PARP-1 (50 nM) was introduced to nucleosome–p53 complexes that had been pre-incubated with varying amounts of p53 (25–200 nM). In both experimental series, multiple complexes with different stoichiometries were detected. Interestingly, p53 displayed enhanced binding activity when introduced to nucleosomes already associated with PARP-1, while PARP1 showed increased interaction when added to nucleosome–p53 complexes. These results suggest that the sequence in which the proteins are added modulates the assembly and stability of the resulting nucleoprotein complexes. Overall, our findings support the notion that PARP-1 and p53 can exhibit both cooperative and independent binding behaviors when interacting with nucleosomes, with the potential for either synergistic action or mutual displacement depending on the context. This work advances our understanding of the dynamic interplay between PARP-1 and p53 in chromatin remodeling and highlights their potential roles in orchestrating cellular stress responses at the level of chromatin structure.
Key words: chromatin, p53, PARP-1, nucleosome, epigenetics.

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Oral abstract
Abstract category: Structure and functions of large cell machinery

CONDENSED DNA STRUCTURE IN BACTERIA SUBJECTED TO VARIOUS TYPES OF STRESS

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Background: The knowledge of the changes in the structural organization of DNA in bacteria in response to stress will allow us to understand the mechanisms of bacterial survival and to approach the solution of the most important problem of modern medicine: overcoming the resistance of pathogenic and other microorganisms to drugs (including antibiotics). DNA is organized in the nucleoid of an actively growing cell hierarchically with three levels of DNA compaction. 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.). The investigation aims to identify changes in condensed DNA structure when bacteria are exposed to various types of stress such as starvation stress, the influence of a chemical analogue of the autoinducer of anabiosis (4HR); the action of the antibiotic ciprofloxacin (CIP); the cells of Dps null strain were studied at stress of starvation
Methods: Electron microscopy and small angle synchrotron radiation scattering (SAXS) were used to reveal distinct forms of DNA condensation in dormant cells, dormant cells of Dps null strain, anabiotic dormant, mummified and antibiotic stressed 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. The effect of the antibiotic ciprofloxacin is visually like the effect of 4HR and leads to the appearance of a Bragg maximum at a characteristic distance of 45 Å. Dormant cells of Dps null strain were studied. The study showed that the absence of the Dps protein does not prevent the cells from forming ordered DNA structures, 45 Å maximum appears as well.
Conclusions: The different types of DNA condensed structure found by us in the dormant *E. coli* cells additionally prove the heterogeneity of DNA condensation, while condensed DNA by itself corresponds to a long-lived intermediate, partially equilibrium state.
Key words: different types of stress, DNA, condensation, structure.

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Poster abstract
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REAL-TIME VISUALIZATION OF PARP INHIBITOR MECHANISMS USING CONFOCAL FLUORESCENCE MICROSCOPY

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Background: Confocal microscopy enables detailed study of fluorescently labeled molecules, providing insights into molecular interactions. We propose its application as a novel method to elucidate the mechanisms of action of PARP (poly(ADP-ribose) polymerase) inhibitors. PARP enzymes play a critical role in DNA repair processes, and their inhibition has emerged as a promising therapeutic strategy in cancer treatment, particularly in tumors with defective DNA repair pathways. There are different types of PARP inhibitors classified based on their mechanisms of action. Some enhance the DNA-binding activity of PARP and promote the retention of PARP2 on DNA, thereby suppressing its catalytic activity. Others weaken the DNA-binding activity, which leads to the release of DNA from PARP. Understanding the precise mechanisms by which PARP inhibitors exert their effects is essential for optimizing their clinical use and developing more effective therapeutic strategies.

Methods and Results: Using PARP2 as a model protein, we demonstrated the feasibility of directly visualizing the impact of various inhibitors on the DNA-binding activity of PARP2, as well as the trapping effect associated with PARP inhibition. Our experimental approach involved the use of agarose beads (diameter ≈100μM) functionalized with Ni-NTA to immobilize His-tagged PARP2. Upon introducing fluorescently labeled nucleosomes (Cy5) into the solution containing the PARP2-bound beads, we observed bright fluorescence around the beads in confocal fluorescence mode, indicating the interaction between PARP2 and nucleosomes, which reflects its DNA-binding activity. Furthermore, the addition of NAD+ resulted in a gradual decrease in fluorescence signal over a 40-minute incubation period, corresponding to the poly(ADP-ribosyl)ation reaction, leading to the dissociation of PARP2-nucleosome complexes and subsequent diffusion of fluorescent nucleosomes into the solution. The addition of clinical inhibitors: talazoparib (promotes the retention of PARP2 on DNA), and veliparib (weakens the DNA-binding activity of PARP), into the reaction mixture (PARP2-bound beads + nucleosomes + NAD^+) allowed us to visualize the inhibitors' effects on both the DNA-binding and catalytic activities of PARP2 through changes in confocal fluorescence intensity.

Conclusion: Our study highlights the potential of confocal fluorescence microscopy as a powerful tool for investigating the mechanisms of action of PARP inhibitors. By enabling real-time visualization of protein-nucleosome interactions and the effects of inhibitors, this method provides valuable insights into the dynamics of PARP activity and its modulation by therapeutic agents. This approach not only enhances our understanding of PARP biology but also paves the way for the development of more effective PARP-targeted therapies in oncology.

Key words: PARP inhibitors, confocal fluorescence microscopy, nucleosome.

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Poster abstract
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ARTIFICIAL POLYNUCLEOSOMES AS A MODEL OF INTERPHASE CHROMATIN

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Background: DNA eukaryotic nuclei is compacted and organized by formaton of hierarchically organized DNA-protein complex termed as chromatin. Chromatin DNA is organized into nucleosomes – octamers of histone proteins wrapped by ~150 bp of DNA. Structure and dynamics of nucleosomes have been well-characterized during the last few decades, whilst higher levels of chromatin organization remain relatively understudied. Higher-order chromatin structure is known to play an important role in transcription and epigenetic regulation. Development of new chromatin models and imaging approaches may help broaden our understanding of these processes.
Methods: Polynucleosome (PN) samples were prepared using *X. laevis* recombinant histone octamers and circular plasmid DNA containing 13 high affinity S601 nucleosome positioning sequences. Formation of nucleosomes was confirmed by *AluI* digestion followed by primer extension assay. The PNs were analyzed using atomic-force microscopy (AFM) and transmission electron microscopy (TEM). For both imaging methods, they were fixed with 1% glutaraldehyde and deposited onto a substrate in the presence of Mg^{2+} ions.AFM imaging was carried out using an INTEGRA microscope, TEM imaging was carried out using JEM-1400 and JEM-2100 microscopes at 120 kV and 200 kV, correspondingly.
Results: The images of the PNs are shown in Figure 1. When imaged using AFM, they appeared as beads on a string, and the typical height of an individual nucleosome was 2.7±0.7 nm (mean±SD). When imaged using TEM, the PNs appeared as "bunches of grapes", because the DNA was not distinguishable between the individual nucleosomes. Both imaging methods indicated that the nucleosomes were bound not only at the positioning sequences, but to the non-specific sites as well.
Conclusion: The PNs described in the current work can be useful as a model system to assess the functions of the DNA-binding proteins which participate in chromatin function regulation.
Key words: chromatin.

The TEM measurements were carried out at the User Facilities Center "Electron microscopy in life science". The work was supported by Russian Science Foundation (Grant 21-64-00001).

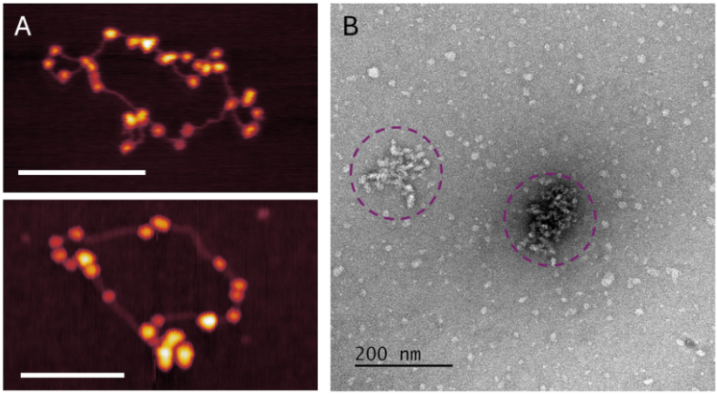

Fig. 1. – The images of PNs obtained using AFM (A) and TEM (B). The scale bar is 200 nm in all the images.

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Poster abstract
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SAMPLE PREPARATION OF NUCLEOSOME CORE PARTICLES BASED ON THE WIDOM 603 DNA SEQUENCE FOR THEIR STUDY BY CRYOELECTRON MICROSCOPY AND SMALL-ANGLE X-RAY SCATTERING

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Background: The nucleosome is an elementary structural unit of chromatin and is a complex consisting of a histone octamer and ~147 bp DNA. Nucleosomes with the Widom 603 DNA sequence are suitable systems for studying transcription *in vitro*. We have obtained a sample of such nucleosomes for their study by structural biology methods.
Methods and Results: We obtained nucleosome samples with the DNA sequence Widom 603 (147 bp) and human canonical histones. Quality control was performed at each step of nucleosome sample preparation, including nucleosomal DNA purification, histone production and purification, histone octamer purification and nucleosome particle assembly. The obtained nucleosome samples were subjected to quality control by native gel electrophoresis, after which, including for the purpose of additional quality control of the samples, the samples were examined using a low-resolution cryo-EM JEM-2100 (JEOL, Japan) TEM with a side-entry holder, a reconstruction of the nucleosome structure (a resolution of ~10 Å) was obtained. SAXS experiments were performed on these samples using the BioMUR beam line at the Kurchatov synchrotron radiation source, Moscow. The obtained experimental data together with the modeling data were subsequently used to characterize the unwrapping of nucleosomal DNA, the results were published in [1].
Conclusion: Nucleosome samples with the Widom 603 DNA sequence were obtained, suitable for study by SAXS and cryo-EM methods, which made it possible to study the structure and dynamics of these nucleosomes.
Key words: nucleosome, cryo-EM, SAXS, sample preparation.

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Poster abstract
Abstract category: Structure and functions of large cell machinery

STRUCTURAL STUDIES OF THE *GALLUS GALLUS* RIBOSOME

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Background: Ribosome hibernation is a conserved regulatory mechanism that modulates translational activity across diverse species, serving as a critical adaptive response to environmental and physiological stress. During studies on the impact of hypothermic conditions on chicken microtubules in the 1970s, highly ordered bidimensional layers of ribosomes were discovered [1]. Furtherly, extensive analysis of chicken embryos subjected to cold stress, characterized by significantly elevated translation rates, the formation of aggregates was widespread and independent of cell type. The first studies on the avian ribosomes were never completed due to methodological limitations [2].
Methods and Results: The presence of two-dimensional aggregates in chicken embryo tissues was discovered through negative staining experiments. Subsequently, cryo-EM grids were prepared, and cryo-EM data were collected for these two-dimensional aggregates and 80S *Gallus gallus* ribosomes. During data processing, software-related issues were encountered, which prevented us from achieving high-resolution reconstructions for the two-dimensional aggregates of the *G. gallus*. We decided to start from obtaining the high-resolution structure of the *G. gallus* 80S ribosome derived from cold-treated chicken embryos. The 80S ribosomes containing elongation factor eEF2 with GDP (eEF2), SERPINE1 mRNA binding protein 1 (SERBP1), and tRNA in the P/E position is commonly referred to as translationally inactive. This class of the ribosomes shows common features with complexes already studied in mammals. Detailed analysis of the expansion segments within the 28S ribosomal RNA of *G. gallus* has revealed unique features distinct from those of mammalian ribosomes.
Conclusion: These structural differences may underpin species-specific regulatory mechanisms and provide clues to the evolutionary pressures shaping ribosome architecture in birds versus mammals and forms a solid molecular background for future structural and functional studies of ribosomal aggregates occurring under the stress. This could bring new insights into the fundamental mechanisms of hibernation in higher eukaryotes.
Key words: ribosome, hibernation, structure, *Gallus gallus*.

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Oral abstract
Abstract category: Structure and functions of large cell machinery

LIGHT AT THE END OF THE TUNNEL: MOLECULAR MECHANISM OF ACTION OF RUMICIDINS

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Background: Antimicrobial resistance along with the difficulties of antibacterial drugs finding dictate the need to develop new antibiotics. Animal antimicrobial peptides can be considered a promising class of antibiotics, since they bypass resistance mechanisms, are non-toxic and are easily synthesized. Here, we determine the structural and functional aspects of the molecular mechanism of action of full-length and truncated variants of representatives of a new class of proline-rich peptides of ruminants – rumicidins.
Methods: Bacterial *in vitro* translation system reconstructed from individual purified *E. coli* components with fluorescent and radioactive labels for characterization of partial reactions of the translation elongation cycle by methods of pre-steady-state kinetics (stopped flow, quenched flow), peptide analysis, study of thermodynamic characteristics of reactions, as well as the method of cryogenic electron microscopy for determination of structural features of inhibition.
Results: Oncocin-like proline-rich peptides in general and rumicidins in particular inhibit bacterial translation by binding to the ribosome exit tunnel in reverse orientation and forming multiple contacts (Panteleev P.V. et al., Nature Communications (2024) 15:8925). The N-terminal part of the peptide is located in the A-site cleft, disrupting the accommodation of aminoacyl-tRNA, stimulating its dissociation and blocking the peptidyl transferase reaction. Peptides with a truncated N-terminus also protrude into the A-site region in the case of a vacant A-site, but do not lead to inhibition of A-site reactions and allow peptidyl transfer. We found out, that simultaneous location of aminoacyl-tRNA and N-truncated proline-rich peptide on the ribosome leads to disruption of the coordination of the central part of the peptide within the exit tunnel, without affecting the key contacts of the conservative Trp23-Phe24 dyad of rumicidins with the constriction point of the exit tunnel. Thus, the conservative Trp23-Phe24 dyad, which is a unique feature of rumicidins, has a predominant role in fixation of the peptide in the ribosome exit tunnel and can be used to optimize the sequence of peptide inhibitors.
Conclusion: Rumicidins are promising molecular scaffolds for development of ribosome-targeting antibiotics.
Key words: ribosome, antibiotics, cryo-EM, proline-rich peptide.

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Poster abstract
Abstract category: Structure and functions of large cell machinery

STRUCTURAL STUDIES OF NOVEL MATURATION STATES OF THE 30S SUBUNIT OF THE *STAPHYLOCOCCUS AUREUS* RIBOSOME

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Background: One of the most effective strategies for combating pathogenic microorganisms involves developing selective inhibitors that specifically target the protein synthesis machinery - the ribosome. These inhibitors can act through several distinct mechanisms: either by blocking functional ribosome activity or by inhibiting ribosomal subunit maturation. Ribosomal subunit assembly represents a complex, multi-stage process requiring numerous protein factors. Any errors in this process may lead to local energy minima corresponding to aberrant ribosome conformations. Therefore, investigating ribosome maturation pathways remains a critical research priority in modern science.
Methods: We used the single-particle cryo-EM reconstruction of the immature 30S ribosome subunit of *Staphylococcus aureus*.
Results: We obtained cryo-EM map of immature 30S ribosome with missing density of helix h44 and unstable position of the ribosomal head.
Conclusion: Based on these data, we probably observe a new step in the maturation process of the 30S ribosomal subunit.
Key words: ribosome, *Staphylococcus aureus*, 30S subunit, Cryo-electron microscopy.

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Poster abstract
Abstract category: Structure and functions of large cell machinery

INVOLVEMENT OF THE WGR DOMAIN OF PARP2 IN ZINC BINDING AND ZINC-DEPENDENT NUCLEOSOME REORGANIZATION

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Background: Poly(ADP-ribose)Polymerase 2 (PARP2) is an enzyme that plays an important role in the regulation of DNA repair, transcription, apoptosis, necrosis, cell division, and many other processes. PARP2 binds to the DNA damage and performs poly(ADP-ribosylation) - posttranslational modification of proteins by a homopolymer chain of ADP-ribose. In PARP2, the WGR domain is responsible for binding to DNA. The WGR domain of PARP2 is also an important link in the regulation of poly(ADP-ribosylation). It was previously shown that PARP2 acquires the ability to change the structure of nucleosomes in the presence of zinc ions, making the nucleosome structure less dense. That is interesting, because PARP2 does not have any obvious Zn^{2+}-binding sites. Considering that WGR is an important domain for the DNA-binding and enzymatic activity of PARP2, it was suggested that the WGR domain may contain Zn^{2+}-binding sites and also be responsible for changing the structure of nucleosomes.
Methods and Results: To study the role of the WGR domain in the chromatin reorganizing activity of PARP2, a recombinant WGR domain of human PARP2 (hPARP2) was obtained. The WGR hPARP2 gene was obtained by PCR using the hPARP2 gene. The WGR hPARP2 gene was cloned into the *E. coli* pET-15b-TEV plasmid, and the plasmids were transformed into electrocompetent *E. coli* Rosetta 2(DE3)pLysS cells. The protein was induced by the addition of IPTG, after which it was purified in several stages: 1) metallaffinity chromatography (Ni^{2+}-sepharose); 2) affinity chromatography (heparin column); 3) final purification of WGR PARP2 by gel filtration (cefacril). The obtained WGR domain of PARP2 was studied by tryptophan fluorescense spectroscopy. The results showed that the presence of Zn^{2+} ions increases tryptophan fluorescense in WGR. The effects of the WGR domain of PARP2 on the structure of nucleosomes were studied by single particle FRET microscopy using fluorescently labeled nucleosomes. The results showed that in the presence of Zn^{2+} ions WGR domain of PARP2 has a similar effect on the structure of nucleosomes as in full-length PARP2.
Conclusion: The results confirm that WGR domain is capable of binding zinc ions and responsible for the Zn^{2+}-dependent change in the structure of nucleosomes.

The study was carried out with the financial support of the Russian Science Foundation (project No. 19-74-30003).

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Poster abstract
Abstract category: Structure and functions of large cell machinery

SPECIFICS OF STRUCTURAL INTERACTIONS AT THE PROTEIN-PIGMENT INTERFACE OF CAROTENOPROTEINS

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Carotenoproteins are protein complexes with a stoichiometrically bound carotenoid component, thereby acquiring important biological functions. Despite the non-covalent nature of the bond, a strict specificity of interaction between carotenoids and specific protein regions is observed. The mechanisms of this specificity remain insufficiently studied, which is relevant in light of the development of new biotechnological tools based on carotenoproteins and the demand for their targeted modification.
The aim of this work was to identify the features of protein-carotenoid interaction through a detailed analysis of the molecular structures of carotenoprotein holo-forms from the PDB database. Particular attention was paid to structures obtained using cryo-electron microscopy (cryo-EM), which provides data on the native distribution of conformations in an ensemble of specific structures.
The enrichment or depletion of the protein-pigment interface with specific types of residues relative to their background occurrence in proteins deposited in the Swiss-Prot database was analyzed. A comparative analysis was performed both for the environment of the entire polyisoprenoid chain of the carotenoid and separately for the environment of the ionone rings. Special attention was given to the geometric parameters of interactions between the ionone rings of carotenoids and the aromatic amino acids of the protein. To identify ligand-specific patterns in the amino acid environment and visualize structural differences, the UMAP dimensionality reduction method was applied.
The analysis showed that the carotenoid binding interface is significantly enriched in hydrophobic amino acids and, conversely, strongly depleted in polar and charged residues, which is a key property for specific interaction. Tryptophan and phenylalanine achieve the highest frequency of occurrence relative to the background (4 times more frequent), while lysine achieves the lowest (128 times less frequent).
Geometric analysis of interactions between ionone rings and aromatic amino acids showed a predominance of perpendicular (T-shaped) orientations, especially for phenylalanine. Tryptophan demonstrated the ability to form both T-shaped and parallel (stacking) interactions, while tyrosine showed geometric non-specificity. Furthermore, ligand-specific differences in the amino acid composition of binding sites were found, which is confirmed by UMAP data clustering.
Thus, the analysis succeeded in identifying specific organizational features of carotenoid-binding sites.

The obtained results will be applied to the further research of rational design of new biotechnological tools based on carotenoproteins. This may include the development of new drug delivery methods, the creation of biosensors, and the development of new approaches to protect plants from stress and damage.

Key words: carotenoproteins, protein-ligand interactions, binding sites, structural bioinformatics.

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Oral abstract
Abstract category: Structure and functions of large cell machinery

Structural aspects of *Staphylococcus aureus* ribosome biogenesis

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Background: One promising approach to directed regulation of pathogen translation is to impact protein factors that control ribosome operation. This could involve factors that influence ribosome maturation or assembly, or hibernation factors that ensure survival under stressful conditions. Ribosome biogenesis is a highly regulated, multistep process involving protein assembly factors. An integrated structural biology approach using XRD, SAXS, NMR and cryoEM techniques was employed to determine the structural features and mechanisms of action of the RbfA, RsfS, RimM, RimP, Era, EttA and YsxC factors from *Staphylococcus aureus*.
Methods: The XRD data were collected using a Cu K-alpha radiation from a PhotonJet-S microfocus sealed tube X-ray generator (Rigaku XtaLAB Synergy-S); ID30B beamline at the European Synchrotron Radiation Facility (ESRF, France); LNLS SIRIUS MANACÁ beamline at the Brazilian Synchrotron Light Laboratory (LNLS-Sirius; Brazil); BL 14.1 beamline at the Berlin Electron Storage Ring Society for Synchrotron Radiation (BESSY, Berlin, Germany). The X-ray scattering data were collected using the BL19U2 station of the SSRF synchrotron (Shanghai, China). 2D and 3D NMR spectra were collected on a Bruker Avance III HD 700 MHz spectrometer equipped with a QCI cryoprobe. Cryo-EM single particle approach was used for structure determination. Data collection was performed on a Titan Krios (FEI Company) at 300 kV and Glacios (FEI Company) at 200 kV electron microscopes.
Results: The protein structures were established using NMR, XRD and SAXS methods and were then analyzed in relation to their complexes with ribosomal subunits. This process resulted in the visualization of different states of the maturing ribosome and the identification of differences in the mechanisms of action of the proteins in S. aureus.
Conclusion: Inhibiting *Staphylococcus aureus*-specific elements of the protein synthesis apparatus will enable the development of new, more selective anti-staphylococcal drugs.
Key words: ribosome, translation, protein biosynthesis.

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Poster abstract
Abstract category: Structure and functions of large cell machinery

STRUCTURE OF THE TRANSLATION PRE-INITIATION COMPLEX FROM A PLANT EXTRACT

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Background: Translation pre-initiation complexes are typical components of the cytoplasm of eukaryotic cells, consisting of 40S ribosomal subunits bound to initiation factors. Actively studied in mammals and protozoa, these complexes are involved in the initiation of mRNA translation. We studied the abundance and structure of *plant* pre-initiation complexes using single particle cryo-EM analysis of wheat germ extract preparations.
Methods and Results: A total of ~250,000 images of particles containing the 40S ribosomal subunit were collected. 3D classification revealed that about 25% of free 40S subunits form a complex with the initiation factor eIF3. By using local refinement, we were able to reconstruct the body of the complex with a resolution better than 3 Å and the core of factor 3 with a resolution of 3.5 Å. It turned out that, unlike the 40S/eIF3 complexes isolated from human cell lysates, which always contain the initiation factor eIF1, the plant complexes only contain the initiation factor eIF1A. We constructed the atomic model of the iIF3 core (eIF3a, eIF3c, eIF3e, eIF3k, eIF3l, and eIF3m subunits), as well as the distal subunits eIF3b, eIF3d, and eIF3c-β, which reside on the 40S surface apart from the factor core. The models of the wheat 18S rRNA and SSU proteins that we published previously were corrected to account for the structural changes caused by the complex formation. Interestingly, structural analysis revealed that the distal eIF3b subunit and rRNA expansion segment ES6a have a tight interaction, which was not observed in the animal preinitiation complexes. This may clarify the role of the eukaryote-specific ES6 segment, which still remains unclear.
Conclusion: We obtained the first structural data on the plant pre-initiation complex which reveal notable differences from those of mammalian cells.
Key words: eukaryotic translation, plant ribosome, pre-initiation complex.

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Poster abstract
Abstract category: Structure and functions of large cell machinery

SUBSTRATE RECOGNITION BY PARP3 GOVERNS ITS INVOLVEMENT IN DNA REPAIR PATHWAYS

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Poly(ADP-ribose)polymerase (PARP) family proteins play a key role in the recognition and repair of DNA damage, as well as in the regulation of transcription, apoptosis, inflammation, and other cellular processes [1], [2], [3], [4]. While the functions of PARP1 and PARP2 are well characterized, the role of PARP3 remains insufficiently understood. Nevertheless, according to several studies, PARP3 may promote classical non-homologous end joining (c-NHEJ) while suppressing alternative DNA repair pathways, such as homologous recombination (HR) and alt-EJ [5], [6]. One of the factors that may determine PARP3 function in the context of DNA repair pathway choice is its substrate specificity; however, the molecular basis of this specificity and the mechanisms regulating its catalytic activity remain unclear.

For this study, recombinant human PARP3 was expressed in *E. coli* and purified using a three-step chromatographic procedure including immobilized metal affinity chromatography (IMAC) on Ni^{2+}-sepharose, heparin affinity chromatography, and size-exclusion chromatography. Optimization of expression and purification conditions allowed us to increase the protein yield more than tenfold compared to previously published protocols [7], enabling large-scale biochemical studies.

According to published data, the catalytic activity of PARP3 is enhanced in the presence of nicked DNA substrates. Using gel electrophoresis, we additionally demonstrated that such damage also increases the affinity of PARP3 for DNA, indicating its pronounced substrate specificity. The use of model oligonucleotide substrates revealed that PARP3 exhibits higher affinity for nicked DNA compared to intact DNA of the same length.

The obtained results expand our understanding of the role of PARP3 in the mechanisms of DNA repair pathway choice. The established substrate specificity of PARP3 toward nicked DNA substrates suggests that the protein may act as a sensor of specific DNA damage, directing the cell toward preferential use of c-NHEJ. This functional orientation is particularly important in the context of a rapid response to DNA stress, such as exposure to ionizing radiation or chemotherapeutic agents. In this regard, PARP3 is of interest not only as an object of basic research, but also as a potential therapeutic target and prognostic marker of tumor cell sensitivity to genotoxic stress.

Key words: PARP3, protein expression, nicked substrate, DNA repair pathways.

This work was supported by the Russian Science Foundation (19-74-30003).

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SECTION IV:

STRUCTURE OF VIRUSES AND PHAGES

Oral abstract
Abstract category: Structure of viruses and phages

NEAR-ATOMIC RESOLUTION STRUCTURE OF THE BACTERIOPHAGE T4 VIRION

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Background: Tailed double-stranded DNA bacteriophages are the most abundant biological entities on Earth, with an estimated population of 10^{31}. Among tailed phages, members of the Myoviridae family have the most sophisticated virion architecture, which includes a long contractile tail providing very high infection efficiency. During infection, the tail contracts and penetrates the bacterial envelope, creating a conduit for injection of the phage DNA. T4 is the most well-characterized myophage that infects *E. coli* and has a 170 kbp genome. The T4 virion comprises a 1200-Å-long and 860-Å-wide prolate capsid (or head), and a 1200-Å-long tail, that consists of a multiprotein baseplate, a tube, and a contractile sheath, surrounding the tube. The tail and the capsid assemble via separate pathways before joining to form the infectious virion. The mature T4 virion is composed of ~40 different proteins and thousands of polypeptide chains.

The T4 capsid assembles via formation a proteinaceous (DNA-free) intermediate called prohead, which is then filled with dsDNA by an ATP-driven packaging motor through the portal protein located at a special capsid vertex. Once the capsid is full of DNA, the motor terminates packaging and departs from the portal vertex. During the virion assembly, the prohead expands, increasing its volume by ~70%.

Methods and Results: We determined near-atomic-resolution structures of T4 prohead in the unexpanded and expanded states and described conformational changes occurring in the capsid shell during expansion. In addition, we determined the structure of the capsid from the mature T4 virion full of DNA and unexpectedly found significant conformational changes in the portal vertex. In the expanded empty capsid, the portal protein dodecamer has the shape of a flying saucer, whereas in the full capsid, it has a mushroom-like shape. Moreover, in the full capsid, the portal dodecamer is pushed down by ~10 Å relatively to the capsid shell, presumably by the pressure of the highly condensed genome. These conformational changes of the portal probably transmit the "head-full" signal, triggering the packaging termination. After departure of the packaging motor, the portal vertex is temporarily sealed by the head completion proteins gp13 and gp14.

Recently we determined a near-atomic-resolution cryo-EM structure of T4 tail from the native virion. The structure suggests mechanisms of the contractile sheath assembly and stabilization by β-sheet augmentation, with N- and C-terminal β-strands of sheath subunits extending β-sheets of adjacent subunits.

T4 virion contains the hexameric tail terminator protein, gp15, that binds to the top of the tail, stops the sheath's polymerization, and creates an interface for attachment of an independently assembled

capsid. Our structure shows that the head-tail interface between gp14 and gp15 is reinforced by electrostatic interactions between negatively charged gp14 and positively charged gp15 residues.
Conclusion: Our cryo-EM reconstructions of the capsid and tail allowed building the atomic model of the entire T4 virion, providing new insights into the assembly of the tailed dsDNA phages.
Key words: Myoviridae phage assembly, Capsid maturation, Contractile tail, Portal protein.

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Poster abstract
Abstract category: Structure of viruses and phages

STRUCTURAL STUDIES ON BACTERIOPHAGE T5 TAIL FRAGMENTS

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Representatives of the large Siphoviridae family of bacteriophages, which belong to the double-stranded DNA (dsDNA) viruses and are characterized by a long, flexible, non-contractile tail, are capable of infecting a wide range of Gram-negative bacteria. Despite the significant representation of this phage family, the currently available information on the structure and assembly of the tail cannot be considered comprehensive. The tail appendage ensures specific interaction with bacterial cells, perforation of the cell wall, and the injection of viral DNA from the capsid into the cytoplasm of the infected bacterium.

Although a number of recent studies have focused on the structure and function of the tail region of one of the Siphoviridae representatives – bacteriophage T5 – the structure of some of its components remains unknown.

We isolated tail appendages of bacteriophage T5 and collected cryo-EM data from it. Data processing allowed us to obtain high-resolution cryo-EM density maps of several regions of the tail appendage of bacteriophage T5. The main part of the T5 phage tail consists of 40 rings of trimers of the main tail protein pb6. The structure of pb6 within the phage tail appendage was determined at a resolution of 2.45 Å. The tail tip region, which includes proteins p140, p132, pb9, and pb3, as well as several adjacent pb6 rings, was resolved at a resolution of 3.06 Å.

The major findings are the newly described structures of fragments of the pb1 protein (long tail-fiber, Ltf) within the T5 phage tail, which were resolved at resolutions ranging from 3.31 Å to 4 Å, as these elements are described for the first time in this work.

Key words: bacteriophages, T5 family, cryo-EM, long tail-fiber (Ltf).

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Poster abstract
Abstract category: Structure of viruses and phages

TEM, CRYO-EM AND AFM CHARACTERIZATION OF COLD-ADAPTED ATTENUATED SARS-COV-2 MUTANTS

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Background: SARS-CoV-2 belonging to Coronaviridae family evoked a devastating pandemic in 2019-2022. Currently, several sublineages of Omicron variant continue to circulate in the human population. Thus, immunogenic and morphological features of prospective vaccine candidates based on cold-adapted (*ca*) temperature sensitive (*ts*) mutants of Omicron-like virus is reasonable to investigate. For this, transmission electron microscopy (TEM), cryoelectron microscopy (cryo-EM), and atomic force microscopy (AFM) were applied.

Methods and Results: To obtain a *ts* mutant, laboratory SARS-CoV-2 strain FEB2 (Omicron BA.5.2, GenBank ID OP920753.1) isolated from COVID-19 patients and genetically characterized in I. Mechnikov Research Institute of Vaccine and Sera was used possessing high reproductive activity *in vero* CCL-81 cell line (ATCC, USA). The parent FEB2 strain was grown at 37°C, while the *ts* mutant F-F3 was adapted to grow at 23-24°C but did not reproduce at 37-39°C. Inactivation of the virus containing fluid was carried out by treating with ultraviolet light (λ = 253.7 nm) using a bactericidal irradiator "TUV 30W/G30 T8" (Philips). The viruses were concentrated on Amicon centrifuge units (100 kDa), aliquoted and stored at -80°C. The virus titers were 0.5-1 x 109 TCID50/ml. To explore the immunogenic properties of the viruses, they were incubated with immune rabbit anti-Omicron virus sera.

For negative staining, 2% water solution of phosphotungstic acid (pH 7.0) was used. The EM 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) using JEM-2100 200kV LaB6 transmission electron microscope (JEOL, Japan) equipped with a Direct Electron DE20 direct detector and Gatan Elsa cryotransfer holder. AFM experiments were carried out using FemtoScan microscope and FemtoScan Online software.

Most SARS-CoV-2 virus particles were of 80-120 nm in diameter and entirely decorated with a typical S spike “crown”. All analyzed spikes were of flail-like shape indicating their pre-fusion conformation. Both the parent FEB2 strain and its *ts* mutant F-F3 formed immune complexes after incubation of viruses with rabbit immune sera against Omicron strain, detected as large aggregates of virions. No morphological differences between the parent and *ts* mutant viruses were found. We also tried to study virus samples using cryo-EM and AFM. Unfortunately, the high concentration of impurity protein (from the cell culture medium) prevented the acquisition of good-quality images. Nevertheless, we were able to obtain several images of the FEB2 sample, in which the concentration of virions was about five times

higher.

Conclusion: TEM of negatively contrasted virions, cryo-EM and AFM helped to visualize the natural morphology of virions of Omicron-like SARS-CoV-2 *ts* mutants and their spikes and revealed no structural differences from the original parent strain. It has been shown that the *ts* mutant F-F3 retains its antigenic properties.

Key words: SARS-CoV-2, Omicron, cold-adapted ts mutant, negative staining, cryo-EM.

The study was conducted under the state assignment of Lomonosov Moscow State University.

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Poster abstract
Abstract category: Structure of viruses and phages

HIGH-AFFINITY RECOMBINANT MONOCLONAL ANTIBODY RSV-MAB WITH ENHANCED NEUTRALIZING ACTIVITY AGAINST RESPIRATORY SYNCYTIAL VIRUS

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Background: Respiratory syncytial virus (RSV) accounts for an estimated 3–4 million hospitalizations and over 100 000 deaths annually in children under five worldwide, with a second peak of severe infection in adults over 65. RSV pathology is characterized by epithelial cell fusion and syncytia formation, driving bronchiolitis and pneumonia. Despite decades of research, the only approved prophylactic monoclonal antibody, palivizumab, demonstrates suboptimal potency, requires monthly dosing due to limited half-life, and is cost-prohibitive for large-scale public health programs. Recent structural studies reveal conformational plasticity of the RSV F protein, suggesting opportunities to design next-generation antibodies targeting conserved epitopes.

Methods and Results: We engineered a full-length human IgG1 antibody, RSV-mAb, by optimizing F10/H7 germ-line variable domains and transiently expressed it in CHO-K1 cells. At 24 h post-transfection the culture was supplemented with EmACF CHO 203 Feed at 8 % (day 2) and the temperature was shifted from 37 °C to 33 °C. A second feed (5 % v/v) was added on day 5. Under these conditions, mean harvest titres reached ≈ 19.5 mg L^{-1} for the F10 lineage and ≈ 9.1 mg L^{-1} for H7. The protein was purified using Protein A affinity chromatography, concentrated by ultrafiltration, and validated through SDS-PAGE, size-exclusion chromatography (SEC), and native agarose electrophoresis. These analyses confirmed a purity exceeding 95% and the absence of aggregates.

Neutralizing activity was measured at the Smorodintsev Research Institute of Influenza of the Ministry of Health of Russia in a microneutralization assay on Vero cells against reference RSV strains A2 and B 9320. The mean IC50 values, determined from three independent mAb samples, ranged from 6.03 to 6.46 ng/mL for RSV-B 9320 and from 14.23 to 18.77 ng/mL for RSV-A2. These values were approximately 15-fold lower than those observed for palivizumab, which exhibited IC50 values of 99.2 ng/mL and 294.1 ng/mL for RSV-B 9320 and RSV-A2, respectively (fig. 1).

Conclusions: The RSV-mAb developed in this study combines enhanced affinity, broad neutralization of both RSV subtypes, and suitability for scalable CHO production, making it a promising candidate for RSV prophylaxis.

Keywords: Respiratory Syncytial Virus, monoclonal antibody, neutralization assay, CHO expression.

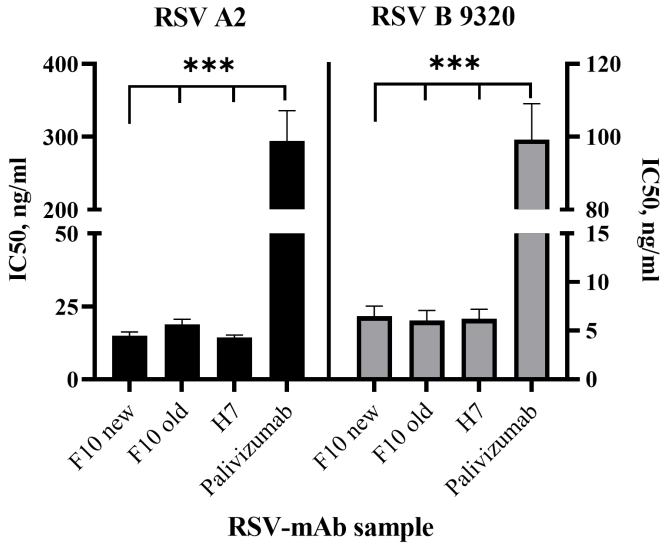

Fig. 1. – Neutralizing potency (IC50) of independent RSV-mAb samples (F10 new, H7, F10 old) versus Synagis® (palivizumab) against RSV strains A2 (left) and B 9320 (right). mean ± SD, p<0.01 (one-way ANOVA with Tukey's test).

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Oral abstract
Abstract category: Structure of viruses and phages

PORTRAIT OF A DANGEROUS PHAGE: STX-CONVERTING BACTERIOPHAGE Phi24b WHOLE VIRION STRUCTURE

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Background: Temperate bacteriophages may contribute significantly to pathogenic potential of the lysogenic strains. Stx-converting phages of *Eescherichia coli* may turn moderately pathogenic strain into Shiga-toxin producing *E. coli* (STEC) strains causing life-threatening foodborne infections. Phage Phi24b exemplifies the most prevalent type of Stx-bacteriophages. The infection and/or lysogenization of commensal *E. coli* by Phi24b-like viruses released from the pathogen may contribute to in-patient toxin production and give raise to new STEC variants. Therefore, the virion structure and host recognition by Phi24b are of significant importance for understanding its ecology and epidemiology.
Methods and Results: Using Phi24b:Cat lysogens obtained from environmental *E. coli* isolate 4s we produced highly purified phage stock suitable for Cryo-EM and proteomics. The proteomics identified 15 proteins including gp47 and gp84 previously believed to be non-structural proteins. The conserved esterase gp84 was found in proteolytically processed form in old phage stocks and non-processed form in freshly prepared samples.
The cryo-EM reconstruction revealed the T=9 capsid which is unusual for lambdoid phages. The capsid is stabilized by the capsid-cementing protein gp67. In the central cavity of central hexamers of the facets contain the hexamers of gp84 N-terminal 54 a.a. domain. No density of the gp84 central domain hexamer (ca. 200 KDa per hexamer) is detectable. This result indicates that gp84 has two sites of self-processing instead of one previously reported.
The Phi24b tail is built of two adaptor dodecameric rings instead of one in most podoviruses and nozzle protein hexamer. The nozzle accomodates the central fiber (needle) that could not be resolved because of the symmetry shift. Six tail fibers made of gp61 trimers attached at adaptor B – nozzle interface. Only N-terimnal coiled-coil fragments of the fibers could be resolved. These fragments are predicted by AlphaFold3 to be followed by long flexible collagen-like filament capped with a small globular domain.
The overall phage structure except the fibers and gp84 decoration protein is highly similar to recently described *Ralstonia solanacearum* phage GP4 (Zheng et al. JMB, 2023) despite very low sequence similarity of the structural protein. The genomic data and low-resolution TEM microscopy suggest that *Erwinia amylovora* phage Stean may also have similar virion structure although the protein sequence homology to Phi24b is almost non-detectable.

Conclusion: The structure of Phi24b virion indicates that it belongs to very diverse but rare type of T=9 temperate podoviruses. The model obtained provides some hints to understanding physiology of the host cell recognition by Phi24b-related viruses.

This work was supported by the Russian Science Foundation (Grant No. 25-24-00110).

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Poster abstract
Abstract category: Structure of viruses and phages

CRYO-EM RECONSTRUCTION OF THE PHIK601 JUMBO PHAGE

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Jumbo phages have attracted attention for their potential to use against antibiotic-resistant bacteria [1, 3]. Their large capsids can package genomes over 200 kbp, a size approaching the smallest bacteria, allowing for diverse infection strategies and enhanced evasion of bacterial defense systems. However, large particle size and complex protein composition pose challenges for structural determination. Recent advances in cryo-EM have enabled high-resolution reconstructions of large viral particles, yet only two jumbo phage structures have so far been resolved at high resolution [2, 5].

This study presents the high-resolution reconstruction of the capsid of jumbo phage phiK601, which infects *Pseudomonas aeruginosa* and was isolated from Powai Lake, Mumbai, India. The phage was purified using CsCl gradient ultracentrifugation and dialyzed in SM buffer. Cryo-EM data were collected using a 300 kV Titan Krios G4 microscope with a Gatan K3 detector (super-resolution pixel size: 1.05 Å, total dose: 14 $e^-/Å^2$, defocus: 1 µm). Data were processed in CryoSPARC. An initial 3D reconstruction based on 11,502 single particles with icosahedral symmetry achieved a resolution of 7.0 Å. Symmetry expansion and local refinements were performed to improve resolution, focusing on capsid vertices, face centers, and edge centers.

The resulting three reconstructions reached 3.8 Å resolution (FSC 0.143). The phiK601 capsid shows isometric icosahedral symmetry with a triangulation number T = 27 and a diameter of ~1300 Å, making it smaller than related jumbo phages phiKZ and phiKP24 [2, 5]. Like phiKZ, phiK601 contains an inner body and internal minor decorating proteins, which are absent in phiKP24 [4]. These reconstructions expand structural knowledge of jumbo phages and provide a foundation for atomic-level modeling of capsid proteins and host interaction studies.

Key words: jumbo phage, Cryo-EM, 3D reconstruction.

The work was funded with RSF 24-44-02003.

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Oral abstract
Abstract category: Structure of viruses and phages

ADVANCES IN STRUCTURAL STUDIES OF BACTERIOPHAGES USING CRYOEM

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Structural studies of biocomplexes using single-particle cryo-electron microscopy (cryoEM) is a well-established technique nowadays in structural biology and outdoing X-ray crystallography. The development of digital registration methods at recording images in EM and algorithms for the fast subsequent analysis facilitated the determination of structures at near-atomic resolution. The current studies demonstrate structures of protein complexes at 2-4 Å resolution for an extremely broad range of sizes from ~200 kDa up to hundreds of megadaltons. Bacteriophages represent huge bio complexes that comprise many different components where their flexibility is essential for their bioactivity in nature. Here I will describe a general workflow and the logistics of image processing in croEM through the basic steps required for reconstructions of bacteriophages and their refinement to nearly atomic resolution.

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Oral abstract
Abstract category: Structure of viruses and phages

CRYO-EM STUDIES OF INACTIVATED FLAVIVIRUSES

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Background: Flaviviruses are among the most important emerging viruses known to man being transmitted by arthropod vectors. Established flavivirus threats continue to cause over 400 million infections annually and are significant global health and economic burdens. There are no specific antiviral drugs for treatment; vaccination is the only effective method of preventing and controlling the disease. The development of improved vaccines would benefit from the atomic resolution structure of the antigen. Here we report first single-particle cryo-electron microscopy (cryo-EM) structure of the inactivated mature flaviviruses: tick-borne encephalitis (Sofjin–Chumakov strain) and yellow fever virus (17D strain).
Methods and Results: Cryo-EM data from tick-borne encephalitis (iTBEV) and yellow fever virus (iYFV) inactivated by formaldehyde 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 CryoSPARC. Model building was performed using program Phenix, Coot and Isolde programs. iTBEV structure was solved with the best resolution to date (3.02Å) among the different strains of TBEV, first high resolution YFV structure was solved at 4.1Å. The structure of these two viruses is similar to other known flavivirus structures, with 180 copies of protein E arranged in a herringbone pattern that makes up the icosahedral shell. Disorder in the transmembrane region was discovered for both structures. High-resolution structure of the inactivated virion provides insight into specific features of E protein ectodomains. We have superposed our iTBEV and iYFV structures to the previously published structures of TBEV or YFV (cryo-EM or X-ray of E protein) complexed with fragment of neutralizing antibodies. The superposition of the epitopes did not reveal substantial differences.
Conclusion: The inactivation of the high pathogenic orthoflaviviruses is a way for structure studies at low level biosafety facilities. Our structures confirm directly formaldehyde inactivation is soft enough and suitable for the flavivirus structure studies in general. Single particle structural studies of inactivated flaviviruses can provide new insight into structure to further improve the vaccine design.
Keywords: flaviviruses, virus inactivation, single particle analysis, high resolution cryo-EM.

This work was performed within the framework of the State assignment to the National Research Centre "Kurchatov Institute" and Federal Scientific and Technical Program for the Development of Synchrotron and Neutron Research and Research Infrastructure for 2019-2027).

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Oral abstract
Abstract category: Structure of viruses and phages

STRUCTURAL STUDIES OF THE EARLIER STAGES OF PHIKZ INFECTION

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During infection, the giant phiKZ phage forms a specialized structure at the center of the host cell called the phage nucleus. This structure is crucial for safeguarding viral DNA against bacterial nucleases and for segregating the transcriptional activities of late genes. Here, we describe a morphological entity, the early phage infection (EPI) vesicle, which appears to be responsible for earlier gene segregation at the beginning of the infection process. Using cryo-electron microscopy, electron tomography (ET), and fluorescence microscopy with membrane-specific dyes, we demonstrated that the EPI vesicle is enclosed in a lipid bilayer originating, apparently, from the inner membrane of the bacterial cell. The phiKZ EPI vesicle contains both viral DNA and viral RNA polymerase. EPI vesicle further migrates from the cell pole to the center of the bacterial cell together with ChmA, the primary protein of the phage nucleus. Thus, EPI vesicle acts as a membrane transport agent, efficiently delivering phage DNA to the phage nucleus while protecting it from the nucleases of the bacterium.

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Poster abstract
Abstract category: Structure of viruses and phages

MOLECULAR MODELING OF THE METHYLENE BLUE INTERACTION WITH THE SARS-COV-2 CORONAVIRUS VIROPORIN

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Background: Viroporins are small pentameric membrane proteins that play a critical role in the life cycle of enveloped viruses, including SARS-CoV-2, influenza virus, and HIV. These proteins form ion channels in infected cells, facilitating virion release and pathogenesis. In this regard, viroporins are a promising target for antiviral therapy. Methylene blue is a well-known antiseptic and antiviral compound that exhibits activity against a wide range of viruses. However, the molecular mechanisms of its interaction with viroporins remain poorly understood.

Methods and Results: In this work, the interaction of methylene blue with the viroporin channel of the SARS-CoV-2 was studied using the molecular dynamics method using Umbrella Sampling technique. Calculations were performed in a system containing a lipid bilayer simulating the membrane of the endoplasmic reticulum-Golgi intermediate compartment (ERGIC). Analysis of MD trajectories revealed that methylene blue preferentially binds to the central hydrophobic region of the channel, forming stable stacking interactions with aromatic phenylalanine residues. The calculation of the potential of mean force by the weighted histogram analysis method (WHAM) showed a global energy minimum value of -13.56 kJ/mol, indicating a high affinity of the compound for the channel.

Conclusions: Using computer modeling methods, we show that, following the initial electrostatic contact of methylene blue with the negatively charged glutamic acid residues of the N-terminus of the E proteins of SARS-CoV-2, upon further immersion into the viroporin channel, methylene blue molecules enter multiple π-interactions with sufficiently high binding energy. This indicates the prospect of further experimental studies of methylene blue as a viroporin inhibitor.

Keywords: viroporins, methylene blue, molecular dynamics, SARS-CoV-2.

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Poster abstract
Abstract category: Structure of viruses and phages

STRUCTURES OF BACTERIOPHAGE RB43 REVEALED UNDER CRYO-EM

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Pseudo-T-even bacteriophages generally have wide host ranges and are present in commercial phage therapy cocktails. Bacteriophage RB43 was recognized as a type strain within the branch of pseudo-T-even phages, with a high structural similarity and low sequence similarity compared with phage T4. However, no structural analysis has been conducted on RB43 previously.
Phages were propagated using *E. coli* BL21 as host in LB medium and infected at the multiplicity of 10^{-2} pfu/cfu. Lysed phages were pelleted at 75,000 × g (1 h, 20 oC), resuspended in SM buffer and loaded on top of a sucrose step gradient and centrifuged at 75,000 × g (1h, 20 oC). 4 µL aliquots of sample were applied to R 2/1 Quantifoil grids, blotted for 8 s, and plunged into liquid ethane-propane with Vitrobot Mark IV. Images were collected on a Titan Krios TEM at 300 kV and collected at a total electron dose of 65 electrons/Å^2 and defocus ranging between -0.7 and -2.8 µm. All image processing operations were performed with cryoSPARC based on corresponding symmetry. Proteins were predicted with AlphaFold 2 and AlphaFold 3, refined with Coot and ISOLDE, and validated with Phenix.
As a result, the phage has been refined separately. The capsid was refined with D5 symmetry at 4.50 Å resolution. The portal vertex of pre-contraction phage was refined with C12 symmetry at 3.03 Å, and 2.97 Å for contracted phage portal. The entire neck interface was refined with C6 symmetry at 3.42 Å for pre-contraction phage, and 3.78 Å for contracted phage portal. The pre-contraction tail was refined with helical symmetry at 2.84 Å resolution and 3.56 Å for contracted tail. The pre-contraction baseplate was refined with C3 symmetry at 3.51 Å and C6 symmetry at 3.62 Å, and C6 symmetry at 3.61 Å for contracted baseplate. Based on the reconstruction, proteins of the phage have been modelled and refined. The structure of proteins may provide insights on the conformational change mechanism of the phage, contributing to improving the understanding on similar Myophages.

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SECTION V:

MEDICAL APPLICATIONS OF CRYO-EM AND TOMOGRAPHY

Oral abstract
Abstract category: Medical applications of cryo-EM and tomography

NANOSCALE STRUCTURES ON THE ERYTHROCYTE MEMBRANE IN PATHOLOGY

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Background: According to the urgent need to use extracellular vesicles (EV) in the development of "targeted drug delivery", the aim of the work was a number of test methods causing the release of EV from erythrocytes *in vitro*, statistical data of EV in terms of their size, biogenesis and hemoglobin content, as well as identifying the spread of EV on the surface of erythrocytes during oncology.
Methods and Results: Using scanning electron microscopy (SEM, JEOL JSM-7800F) and the use of nanostructured graphene substrates, as well as using SEM - KYKY-EM6200 and TESCAN VEGA 3 SBH) it was found that when the state of erythrocytes is renewed (A23187-Ca^{2+}, SDS, LPA and heat), extracellular vesicles (from 322 nm to 505 nm) are formed on the cell surface, in which hemoglobin can be preserved, and in oncological diseases, vesicles (from 30 to 300 nm), in which DNA fragments are presented.
Conclusions: It is assumed that when EV structures appear on the surface of the plasma membrane, they are caused by changes in both the state of the cell itself and the adsorption of tumor exosomes circulating in the blood.
Key words: extracellular vesicles, scanning electron microscopy, erythrocyte, exosome, oncology.

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Poster abstract
Abstract category: Medical applications of cryo-EM and tomography

EVOLUTION OF NETWORKS OF WORMLIKE MICELLES INTO PERFORATED VESICLES

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Background: The formation of micellar aggregates and changes in their morphology are crucial for numerous practical applications of surfactants. Nevertheless, the correct structural characterization of complex micellar nanostructures remains a challenge. This work demonstrates the achievements of cryo-electron tomography (cryo-ET) in identifying the structural characteristics accompanying the evolution of aggregates of surfactants.
Methods: Using cryo-ET in combination with cryo-electron microscopy (cryo-EM), small-angle neutron scattering and rheometry, studies of a model system consisting of zwitterionic and nonionic surfactants were carried out. In this system, the molecular packing parameter increased gradually due to an increase in the molar fraction of the nonionic surfactant.
Results: Structural transformations were observed: linear wormlike micelles → branched wormlike micelles → saturated network → perforated vesicles (stomatosomes). Transformations occur due to an increase in the number of branches due to cylindrical sub-chains and hemispherical end caps number decrease. 3D structure of multiconnected saturated networks has been obtained and described. Stomatosomes were formed when the length of the subchains of saturated networks became much shorter than the persistence length, as a result of which the three-dimensional (3D) structure transformed into a two-dimensional (2D) membrane.
Conclusions: Thus, a mechanism of structural changes from wormlike micelles networks into perforated vesicles has been identified, which can later be used in the design of various self-assemblies of surfactants. Perforated vesicles are suitable for controlled delivery applications because of their nanometer-sized pores.
Key words: perforated vesicles, wormlike micelles, surfactants, cryo-electron tomography.

The study was conducted under the state assignment of Lomonosov Moscow State University.

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Poster abstract
Abstract category: Medical applications of cryo-EM and tomography

A COMPUTER PROGRAM VERONICA FOR MARKUP OF SERIAL CRYO-IMAGES OF SMALL EXTRACELLULAR VESICLES

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The aim of the study was to develop a new computer program to markup membrane vesicles on serial cryo-images, to store the results accurately and reliably and to analyze them. The program/environment uses the MongoDB service as the result storage service. This is a universal database storing data in JSON format, a popular tool in the environment of open source projects. To work with the program in Linux, it is recommended to create and use a virtual environment.

In the context of marking up images with vesicles, veronica makes possible to markup the following graphical elements:

- vesicle: closed spline curve, drawn in blue;
- vesicle segment: unclosed spline curve, drawn in purple;
- interference line: unclosed spline curve, drawn in light blue;
- blot: closed spline curve, limited area is drawn with light blue shading;
- dirt: a closed broken line, the bounded area is drawn with a blue hatch.

It is recommended to mark only vesicle types and vesicle segment and not to mark vesicles if they extend beyond the image boundaries, while having a regular shape.

For elements of the “closed spline curve” type (vesicle/blot) the minimum configuration consists of two segments - 6 control points. At the initial definition of such an element two points are defined: a spline curve close to a circle is drawn and resting on these points as on the (almost) diameter. During further editing, the element can be shaped as a very complex curve: both by moving the control points and by inserting additional segments (with the Shift key). When defining a complex shape, it is recommended (but not obligatory) to start the marking by specifying the two farthest from each other “opposite” points.

Application of abovementioned algorithm allowed successful image labeling for two sets of cryoEM images of small extracellular vesicles, purified from gastric juice of a healthy volunteer and a patient with gastric cancer and obtained on JEOL2100 electron microscope (as part of Unique scientific facility “3D-EMC” of MSU).

The Veronica computer program allows automatic calculation of metrics characterizing the samples of interest: vesicle diameter and nestedness. The average calculated vesicle diameter from the control sample was 218.9 px and from the patient sample was 225.6 px. Given a scale of 2.15 px/nm, this corresponds to sizes of 102 and 105 nm, respectively. Thus, the diameters of the vesicles in the control

and patient samples did not differ.
On the other hand, the samples differed significantly in the number of vesicles nested within each other. We applied the term “branching” to describe the number of nested vesicles. If no nested vesicles are observed in a vesicle, branching is 0. The number of vesicles with nested vesicles for the healthy volunteer control sample was 49.23% of the total number, while for the patient sample only 9.66%. This confirms that the parameter of vesicle attachment can be used for sample identification.

This research has been supported by the Interdisciplinary Scientific and Educational School of Moscow Lomonosov University «Molecular Technologies of the Living Systems and Synthetic Biology» (#24-III04-14).

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Poster abstract
Abstract category: Medical applications of cryo-EM and tomography

EVALUATION OF THE BACTERIOPHAGES ACTIVITY AGAINST BIOFILMS OF ANTIBIOTIC-RESISTANT *PSEUDOMONAS AERUGINOSA* CLINICAL ISOLATES

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Background: *Pseudomonas aeruginosa* is a common pathogen with the ability of forming robust biofilms, which confer extreme tolerance to antibiotics. This study investigates the anti-biofilm activity of 2 species phages: phiKZ (Phikzvirus), phi10/2 and phi14/1 (Pbunavirus) against a clinical *P. aeruginosa* strain Ur1 and Ur14 isolated from a urinary tract infection. All the phages shown good lysis ability on corresponding host in plaque assay. These 2 strains exhibit resistance to almost all the common antibiotics of treating *P. aeruginosa*, shown the urgency to explore phage-based alternatives.
Methods and Results: Mature Ur1 and Ur14 biofilms (24 h incubation) were treated with each kind of phages at MOI=0.01 for 24 h. Biofilm architecture was analyzed via scanning electron microscopy (SEM) to assess structural integrity, including extracellular polymeric substance (EPS) matrix preservation. Biofilms from 2 strains shown large different physical property. Ur1 biofilm displays high viscosity with low elastic recovery, it is thicker but results in deformation under minimal external forces (e.g., pipette tip contact), and the cells are loosely arranged with large gaps. While Ur14 biofilm exhibits low apparent viscosity with thinner biofilm, with a densely packed cellular arrangement which form a rather rigid structure resistant to mechanical disruption. Ur1 biofilm exhibited significantly higher sensitivity to the effects of different phages species than strain Ur14, with slightly less EPS fibers than Ur14 samples, although both strains shown high EPS density after phage exposure from SEM images. This paradox highlights the incompletely correlation between structural integrity and functional biofilm eradication. Additionally, both biofilms retained EPS architecture post-treatment, suggesting that residual eDNA-polysaccharide complexes maintain structural cohesion even after bacterial lysis.
Conclusions: This research underscore that biofilm clearance efficiency depends not only on EPS disruption but on multi-factors such as biofilm physical properties and phage lytic enzymes specificity. These findings also demonstrate the necessity of developing personalized phage therapeutic protocols.
Key words: *P. aeruginosa* biofilm, phage therapy, scanning electron microscopy.

This work was supported by RSF (24-44-02003).

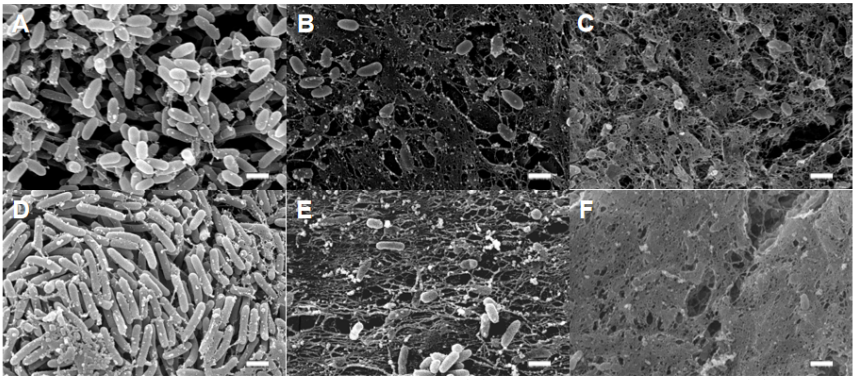

Fig. 1. – SEM images of biofilms after overnight culture (A) Ur1 without phage treatment, (B) Ur1 after phi14/1 treatment, (C) Ur1 after phiKZ treatment. (D) Ur14 without phage treatment, (E) Ur14 after phi10/2 treatment, (F) Ur14 after phiKZ treatment. Size bar-1um.

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Oral abstract
Abstract category: Medical applications of cryo-EM and tomography

MORPHOLOGICAL AND STRUCTURAL INVESTIGATION OF ENVELOPED VIRUSES USING TRANSMISSION ELECTRON AND CRYO-ELECTRON MICROSCOPY: INFLUENZA VIRUS AND SARS-COV-2

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Background: Enveloped viruses include serious pathogens of humans and animals. Influenza virus and SARS-CoV-2 belonging to *Orthomyxoviridae* and *Coronaviridae* families, respectively, evoked two devastating pandemics in the XXI century and continue to circulate in the human population. To develop efficient and safe cold-adapted (*ca*) attenuated or inactivated split vaccines against those infections one should visualize the shape and resolve the structural details of virus antigens located at the virions' surface. High resolution cryo-electron microscopy (cryo-EM) is by far the most powerful and advanced tool for this. Yet, this method requires a lot of time and effort and requires a large amount of material without protein and cellular impurities.

Methods and Results: To compare the morphology and surface antigen structures of Influenza viruses and SARS-CoV-2 and assess the quality of commercial and candidate antiviral vaccines, we applied a simplified method of cryo-EM based on the upgraded JEM-2100 transmission electron microscope (TEM) equipped with a Direct Electron DE20 direct detector and Gatan Elsa cryotransfer holder available at the Shared Research Facility "Electron microscopy in life sciences" at Moscow State University. A classical approach of studying negatively stained virions using TEM was also applied. We visualized the hemagglutinin spikes, matrix layer and inner structure of Influenza A and B viruses and characterized Influenza inactivated split vaccines. The SARS-CoV-2 Wuhan- and Omicron-like strains and the prospective vaccine candidates based on cold-adapted (*ca*) temperature sensitive (*ts*) mutants of Wuhan- and Omicron-like viruses were investigated. The viruses were inactivated using β-propiolactone or UV-radiation. Depending on the inactivation method, we revealed either the natural pre-fusion (flail-like) or abnormal post-fusion (needle-like) shape of S-spikes. No post-fusion spikes were found in the UV inactivated *ca* attenuated Wuhan- and Omicron-like mutants.

Compared to a coat of tightly positioned hemagglutinin/ neuraminidase spikes of Influenza virions, the coronavirus S-spikes are longer, rarely located and much more fragile. A clear matrix layer adjacent to an outer lipid monolayer was resolved within the Influenza A and B virions, while the inner lipid monolayer is poorly resolved. A partial integration of M1 protein into the lipid bilayer of Influenza virus virions may be hypothesized.

Conclusion: The inactivation method should be taken into account when developing new inactivated vaccines against SARS-CoV-2. It should be noted that the presence of surface antigens in the non-native conformation can lead to the production of non-neutralizing antibodies, which always carries the risk of antibody-dependent enhancement (ADE) of respiratory disease, as previously shown for many enveloped viruses, including MERS-CoV/SARS-CoV.
Key words: SARS-CoV-2, Influenza virus, negative staining, cryo-EM.

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Poster abstract
Abstract category: Medical applications of cryo-EM and tomography

DIVERGENT CONTRIBUTION OF CYTOPLASMIC ACTINS TO NUCLEAR STRUCTURE OF LUNG CANCER CELLS

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Background: In both normal and transformed epithelial cells, the actin cytoskeleton is composed of two non-muscle cytoplasmic actin isoforms: β-cytoplasmic actin and γ-cytoplasmic actin. Our previous studies have demonstrated that the relative balance between these actin isoforms has a profound impact on cell morphology and behaviour. In this study, we further show that the chromatin texture and composition of the nuclear lamina are modulated by the predominant expression of specific non-muscle actin isoforms.

Methods and Results: Using shRNA interference in combination with Western blot analysis, immunofluorescence, and laser scanning microscopy, we have shown that the two non-muscle actin isoforms exert distinct regulatory effects on the nuclear structures of human lung adenocarcinoma cells.

Selective downregulation of β-actin, accompanied by a compensatory increase in γ-actin expression, resulted in an expanded nuclear projection area, a decrease in A-type lamins, and an increase in lamin B2 levels. This was accompanied by a redistribution and concentration of lamins within the nuclear envelope zone. In contrast, suppression of γ-actin, along with a corresponding rise in β-actin levels, led to an increased lamin A/B ratio, primarily due to elevated expression of A-type lamins.

We observed that suppression of β-actin reduced the nuclear DAPI staining, indicating chromatin decondensation. Conversely, the predominance of β-actin resulted in increased DAPI staining with prominent chromocenters, suggesting elevated heterochromatin levels. Our findings also revealed that histone expression profiles were modulated in accordance with the relative abundance of specific actin isoforms.

Histone H3 post-translational modifications were also influenced by the balance of actin isoforms. In A549 cells with β-actin downregulation, the level of dimethylated H3K9me2 decreased while acetylated H3K9ac increased. In contrast, downregulation of γ-actin expression resulted in a decrease in H3K9ac. Preliminary RNA sequencing data revealed that the demethylase KDM3A, which specifically demethylates lysine 9 of histone H3, was significantly upregulated in A549 cells expressing shRNA targeting β-actin.

Conclusion: Our study of human lung adenocarcinoma cells revealed that the balance between actin isoforms influences nuclear lamina composition and chromatin compaction, which subsequently affects nuclear stiffness and cellular behaviour. The dominance of β-actin promoted chromatin compaction and deactivation, whereas the prevalence of non-muscle γ-actin led to chromatin decondensation.

Keywords: β-actin; γ-actin; lamin; histone; nucleus.

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Poster abstract
Abstract category: Medical applications of cryo-EM and tomography

STRUCTURAL STUDY OF THE COMPLEX OF HEAVY CHAIN ANTIBODY G2.3 WITH H1N1 INFLUENZA VIRUS HEMAGGLUTININ

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Influenza remains one of the most common and contagious respiratory infections, causing over 1 billion infections and approximately 500,000 deaths annually. The main problem in influenza prevention and therapy is the extremely high variability of its surface viral proteins (hemagglutinin and neuraminidase), which leads to the rapid emergence of resistance to existing antiviral drugs and vaccines. The surface trimeric viral protein hemagglutinin (HA) is one of the main components of the viral envelope, and use of antibodies to its conserved regions in the "stem" domain could be an effective antiviral therapy strategy. Of particular interest in this regard are single-domain antibodies found in representatives of the Camelidae family, which lack light chains and have a more compact antigen-binding site compared to canonical vertebrate antibodies.

Previously, we obtained a series of single-domain antibodies to the "stem" domain, among which clone G2.3 turned out to be one of the most affinity with a dissociation constant of 0.55 nM. To map the epitope and study the details of the interaction of G2.3 with HA, the complex of this antibody with HA of the H1N1 influenza virus was studied by cryoEM technique. Based on the obtained data with a resolution of about 3Å, a reconstruction of the structure was performed. For each trimeric HA molecule there are three G2.3 molecules bound to the "stem" domain. The obtained data allowed to identify residues involved in the formation of the antigen-antibody interface. The mechanism of the significant influence of HA glycosylation at position 304 on binding to the antibody was revealed, which is consistent with the experiments on obtaining escape mutants, as well as with the results according to which G2.3 competes with the previously developed SD38 antibody, which epitope partially overlaps with the epitope of G2.3.

Keywords: influenza, hemagglutinin, single-domain antibodies, immunotherapy, cryoEM.

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