Friday, March 21, Session V

L17

AMINO ACIDS IN THE CONTEXT OF PROTEIN STRUCTURE: WHAT WE CAN LEARN FROM PROTEIN LARGE LANGUAGE MODELS

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Proteins are extremely diverse in structures and functions, despite the fact that they are composed only from 20 amino acids. Any of these amino acids can play a different structural and functional role depending on the context of the sequence and structure. For example, histidine can be a charged residue on the protein surface, a specialized residue in "catalytic triad", a metal-chelating residue, etc. Analysis of this context was recently enabled by advanced machine learning methods originally developed for the analysis of natural language. It is possible to analyze the amino acid sequence of a protein as a "sentence" composed of amino acid residues as "words". We analyzed sequences of all human proteins using a large language model ESM-2 (Evolutionary Scale Modeling). Each amino acid residue was converted into its profile that reflects its structural role in the context of the overall sequence and structure of the protein. These profiles can be visualized as a 2D map. We plan to annotate this map to assign a 3D structure to each cluster of this map. We will illustrate how this map can help us to understand the structure and function of different regions of any protein.

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L18

GATING OF THE RIBOSOME EXIT TUNNEL

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Ribosomes are complex biomolecular machines essential for proteosynthesis and thus for life as we know it. As the nascent peptide is assembled one amino acid residue at a time, the growing chain escapes through the ribosomal tunnel. The ribosomal tunnel is mostly made up of rRNA. However, there is a narrowing of the tunnel, called the constriction site (CS), formed by the extended loops of two ribosomal proteins: uL4 and uL22. This CS is where the first protein--protein contact of the nascent chain takes place. The tips of uL4 and uL22 include charged amino acid residues that come into close contact with the nascent chain and may be of special importance in the early stages of translation.

To understand the conformational variability of the CS in various chemical contexts, we explored 222 experimental *E. coli* ribosome structures from the Protein Data Bank

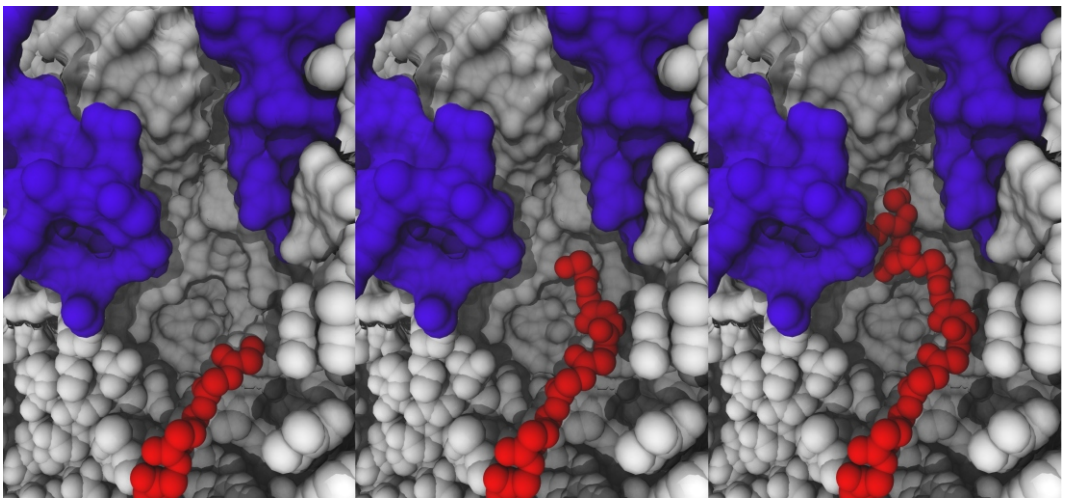

Figure 1. Varying lengths of polyglycine (red) in proximity to the constriction site (blue) in the ribosomal tunnel.

and their distribution of distances of the tips of uL4 and uL22. These ribosomes contained various cofactors, mutations, or ligands. Our analysis reveals that the CS is flexible and the uL4 and uL22 tip distance -- hence the tunnel width -- varies in different chemical contexts.

In our ongoing research, we are exploring the CS dynamics and the interaction of a nascent peptide with the CS using molecular dynamics simulations. We study four systems with varying lengths of polyglycine (11, 8 or 5 glycines or no peptide at all) in the ribosomal tunnel. In the talk, I will present our preliminary results that suggest that the peptides affect not only the dynamics of the CS but also more distant parts of the ribosome.

L19

LONG-RANGE ELECTRON TRANSFER IN PROTEIN-METAL JUNCTIONS

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Cytochrome b_{562} (Cyt b_{562}) is a small redox-active heme protein that has served as a key model system for understanding biological electron transfer processes. Electron transport in such proteins plays a crucial role in various biochemical functions, including respiration and enzymatic catalysis. Investigating its transport properties in protein-metal junctions provides valuable insights into charge transfer mechanisms relevant to bio-electronic interfaces. Recent experimental studies have demonstrated the conductive properties of Cyt b_{562} on gold surfaces [1], but a deeper theoretical understanding of its charge transport mechanism is necessary. This study presents a comprehensive theoretical analysis of electron transport in Cyt b_{562}-based junctions using a multiscale computational approach, examining both coherent and incoherent transport processes.

To model electron transport, molecular dynamics (MD) simulations were employed to generate junction geometries under both vacuum-dried and solvated conditions, where the protein was covalently bound to gold contacts in various configurations [2]. Charge transport was analyzed through two mechanisms: coherent tunneling, studied using the Landauer-Büttiker formalism within the Density Functional Theory (DFT) framework, and incoherent hopping, modeled using the semi-classical Marcus theory.[3]

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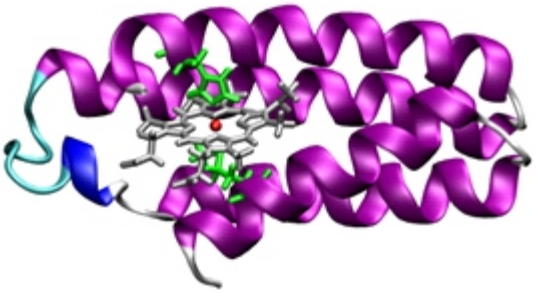

Figure 1: Crystal structure of Cyt b_{562}

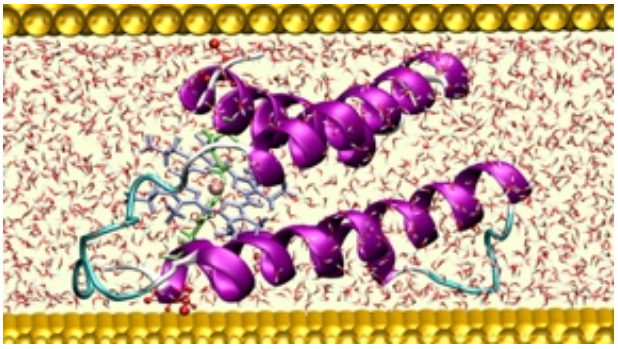

Figure 2: Solvated lying junction of Cyt b_{562}

L20

HIGH-RESOLUTION LIQUID CELLS FOR TIME-RESOLVED CRYO-EM

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A large portion of protein dynamics occurs on micro- and millisecond time scales, including large-scale domain motions and protein folding processes [1]. Insight into the dynamic states of these processes is necessary for understanding how proteins function.

Microsecond time-resolved cryo-electron microscopy (Cryo-EM) has recently emerged as a promising method for capturing intermediate states reached after tens of microseconds of dynamics [2, 3]. A cryo specimen is locally flash melted using a laser beam *in situ* of a transmission electron microscope. As the laser is switched off, the specimen revitrifies, trapping the particles in their transient states. The time window for the dynamics (time-in-liquid) is tuned by the duration of the laser pulse and usually sits at 30 μs with the temporal resolution of the method is better than 5 μs [4]. Moreover, the near-atomic resolution capabilities of standard single-particle Cryo-EM are preserved even after the flash melting process [5]. However, exceeding time-in-liquid beyond 30 μs has proven to be difficult using this setup.

The inherent limitation of the above-described method stems from evaporation of the sample in the liquid phase. This limits the maximum achievable time-in-liquid to the tens of microseconds, as well as the maximum reachable temperature, which plateaus at approximately room temperature due to evaporative cooling [4, 6].

To overcome this limitation, we developed a method that prevents evaporation by depositing a sealing membrane onto the cryo sample prior to laser flash melting. This is achieved using physical vapor deposition of silicon dioxide in an in-house built setup. The thickness of the deposited sealing layer is precisely controlled and can be as thin as 1.5 nm on each side of the sample. This thickness is sufficient to retain the sample even after 300 μs spent in the liquid, extending the observational time-window for protein dynamics from tens to hundreds of microseconds and. Moreover, due to the absence of evaporative cooling of the specimen, temperature jump experiments can also be performed. Importantly, the resolution loss resulting from the sealing layers is negligible, as demonstrated by sub-1.8 A resolution reconstruction of apoferritin.

Experiments prove that time windows up to milliseconds can be achieved with a sufficiently thick membrane (e.g., 10 nm on each side), opening new possibilities not only in time-resolved Cryo-EM, but also in liquid cell electron microscopy.

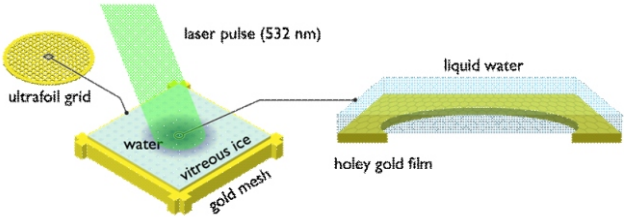

Figure 1. Schematic principle of laser melting. A cryo sample on a gold grid is first illuminated by a laser beam, causing heating of the gold film and subsequent local melting of the vitreous ice. Surrounding areas on the grid square, as well as remaining grid squares, remain unaffected by melting.

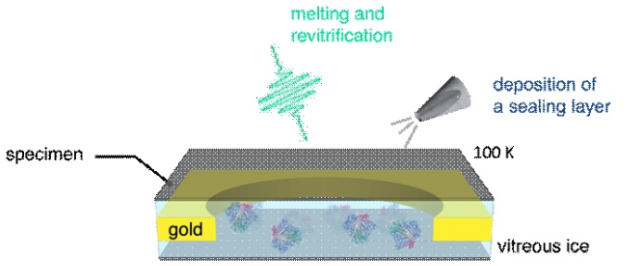

Figure 2. Sealing and revitrification of a cryo sample. First, silicon dioxide membranes are deposited from both sides onto the vitreous ice. Subsequently, sample is flash melted with a microsecond laser pulse, allowing for the dynamics to occur. After the laser is switched off, specimen revitrifies and traps the particles in their transient state. Thanks to the SiO2 membranes, the specimen evaporation is limited.

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L21

STRUCTURAL BASIS OF THE 14-3-3/CYCLIN Y-MEDIATED REGULATION OF CDK16

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CDK16, also known as PCTAIRE1, is a member of the PCTAIRE family of cyclin-dependent kinases (CDKs). CDK16 plays a crucial role in various physiological processes, such as neurite outgrowth, vesicle trafficking, spermatogenesis, glucose homeostasis, and muscle differentiation. [1] Unlike other CDKs, CDK16 activation is mediated through interaction with cyclin Y, phosphorylated at residues Ser100 and Ser326, in complex with 14-3-3, as depicted in Figure 1. CDK16 has a distinct unstructured N-terminal extension that includes two residues, Ser119 and S153, these residues can undergo PKA phosphorylation, resulting in the formation of 14-3-3 consensus binding motifs. When phosphorylated, these residues are reported to decrease CDK16 activity [2]. CDK16's activity is associated with the progression of various cancers, including breast cancer, lung cancer, endometrial cancer, melanoma, and others. In this context, the phosphorylation of specific substrates promotes cell proliferation [3]. Research has demonstrated that inhibiting CDK16 activity leads to a reduction in cancer cell growth [4]. Given CDK16's crucial role in regulating cell proliferation in cancer, it is essential to understand its activation mechanism at the molecular level. Herein, we solved the structure of the complexes involved in CDK16 activation using Cryo-EM, showing the role of cyclin Y and 14-3-3 in CDK16 activation.

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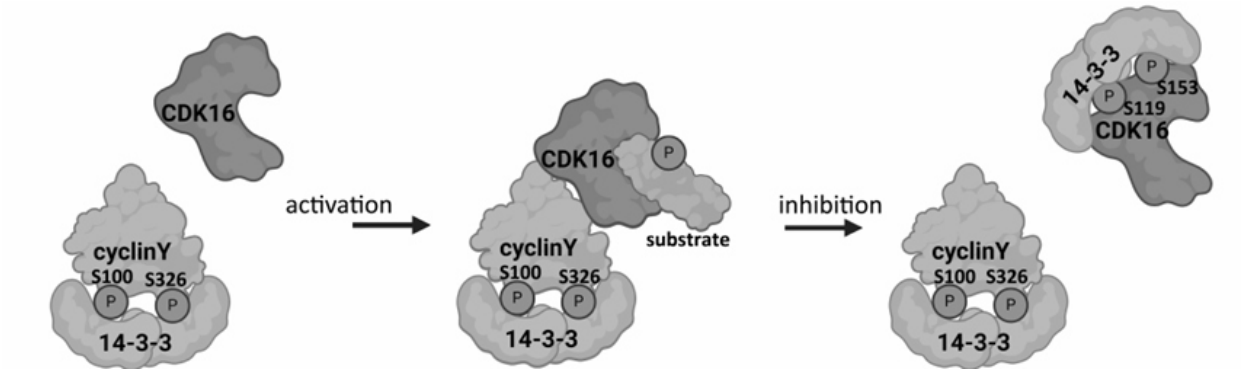

Figure 1. Graphical scheme of CDK16 regulation via cyclin Y and 14-3-3. Created with BioRender.com

L22

SEEING IS BELIEVING: PROXIMITY LIGATION ASSAY

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The Duolink proximity ligation assay (PLA), an exclusive product of Merck, represents a powerful technique for studying protein interactions in situ at endogenous protein levels, enabling researchers to visualize and quantify interactions at the cellular level. This simple yet innovative method employs a unique combination of proximity ligation and fluorescence microscopy, providing high sensitivity and specificity in detecting and localizing biomolecular interactions. The assay utilizes two primary antibodies that bind to the target proteins, which are then linked by DNA oligonucleotides. When the antibodies are in close proximity, a ligation reaction followed by DNA amplification with fluorescent probes occurs, generating a detectable signal.

The benefits of Duolink PLA include its ability to provide spatial information about protein interactions within their native cellular context, facilitating the study of complex biological processes. Furthermore, the assay's compatibility with various sample types and its potential for multiplexing allow for comprehensive analyses of protein networks. Merck offers full application support to ensure optimal use of the Duolink PLA, assisting researchers in maximizing their experimental outcomes. This presentation will delve into the technical aspects of the Duolink PLA, highlight its advantages over traditional methods, and discuss its typical applications in the field of life sciences.

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