Friday, March 24, Session V

L21

ATOMISTIC COMPUTER SIMULATIONS OF THE RIBOSOME

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Atomistic computer simulations have become a valuable tool for understanding the behavior of biological macromolecules at the atomic level. We focus on the ribosome, one of the most complex and essential cellular machines, which is responsible for protein synthesis in all known organisms. The ribosome simulations are especially helpful in addressing questions about conformational heterogeneity and energetics of various ribosome parts. This way the simulations complement other biophysical techniques like cryogenic electron microscopy or fluorescent labeling [1, 2]. Despite the progress in hardware and software development, atomistic simulations of the ribosome remain challenging. The main reason is the ribosome size and complex chemical compositions. Still, using world-class supercomputers, one may gather valuable data in reasonable time.

Over the past few years, we have been using molecular dynamics (MD) simulations to investigate several ribosome's critical sites. Namely, we have studied the exit tunnel through which nascent proteins leave the ribosome [3], the decoding center where correct tRNAs are recognized or a portion of ribosome surface where translation factors bind [4]. In the talk, we will highlight the results of these projects and discuss how effective the computer simulations are in approaching scientific questions about biomolecules.

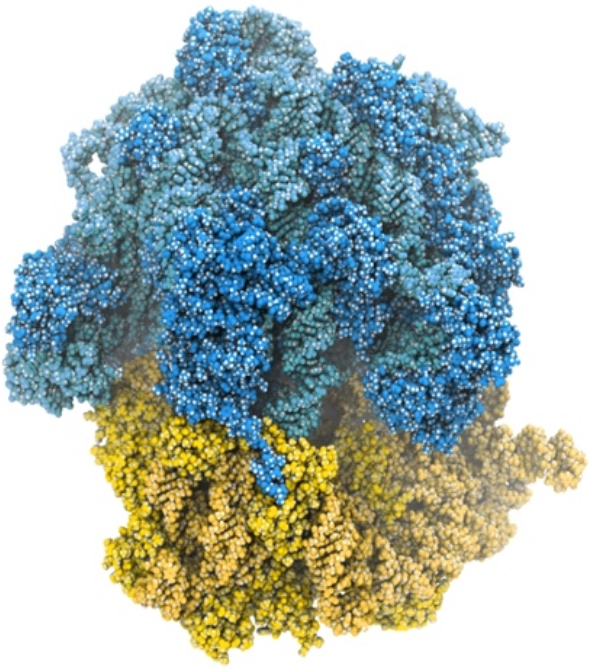

Figure 1: A bacterial ribosome with the small subunit in orange and large subunit in blue. Darker colours represent ribosomal proteins, ligher is the rRNA. Hydrogen in blue. Adopted from Ref. 2.

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2. L. V. Bock, S. Gabrielli, M. H. Kolář, H. Grubmüller, *Ann. Rev. Biophys.*, **52** (2023), 0000.
3. M. H. Kolář, G. Nagy, J. Kunkel, S. A. Vaiana, L. V. Bock, H. Grubmüller, *Nucleic Acids Res.*, **50**, (2022), 2258-2269.
4. H. McGrath, M. Černeková, M. H. Kolář, *Biophys. J.*, **121**, (2022), 4443-4451.

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L22

BINDING OF PEPTIDE DEFORMYLASE TO THE RIBOSOME SURFACE MODULATES EXIT TUNNEL INTERIOR

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Ribosomes are the biomolecular factories in charge of protein synthesis and are thus essential for life as we know it. In order to better understand proteosynthesis it is important to characterize the ways in which it is regulated. The regulatory mechanisms may involve conformational changes of the ribosome induced by external factors possibly transferred over large distances. The principles of this allosteric communication between distant ribosome parts are not fully understood yet. Computer simulations represent a convenient tool to study dynamic complex systems including the ribosome [1]. Here we investigate peptide deformylase, an enzyme that binds to the ribosome surface near the ribosomal protein uL22 during translation and modifies the emerging nascent chain, to understand how conformational motion of the ribosome is affected by external factors [2] (preprint: doi.org/10.1101/2022.04.20.488877).

We have performed all-atom molecular dynamics simulations of the entire ribosome. The simulated system consists of about 2 million atoms so for the microsecond-scale simulations of ours a supercomputing facility involving thousands of CPU cores had to be employed.

We analyzed these simulations using principal component regression, a form of supervised machine learning. The results indicate conformational changes of the ribosomal protein uL22 inside the ribosomal exit tunnel that are correlated with deformylase binding. This suggests a possible effect of the deformylase on the nascent peptide transport through the tunnel.

The research was supported by the Czech Science Foundation (projects 19-06479Y and 23-05557S). MČ acknowledges the support of the Internal Grant Agency of UCT Prague (project 403-88-2091). We also thank the Max Planck Computing and Data Facility.

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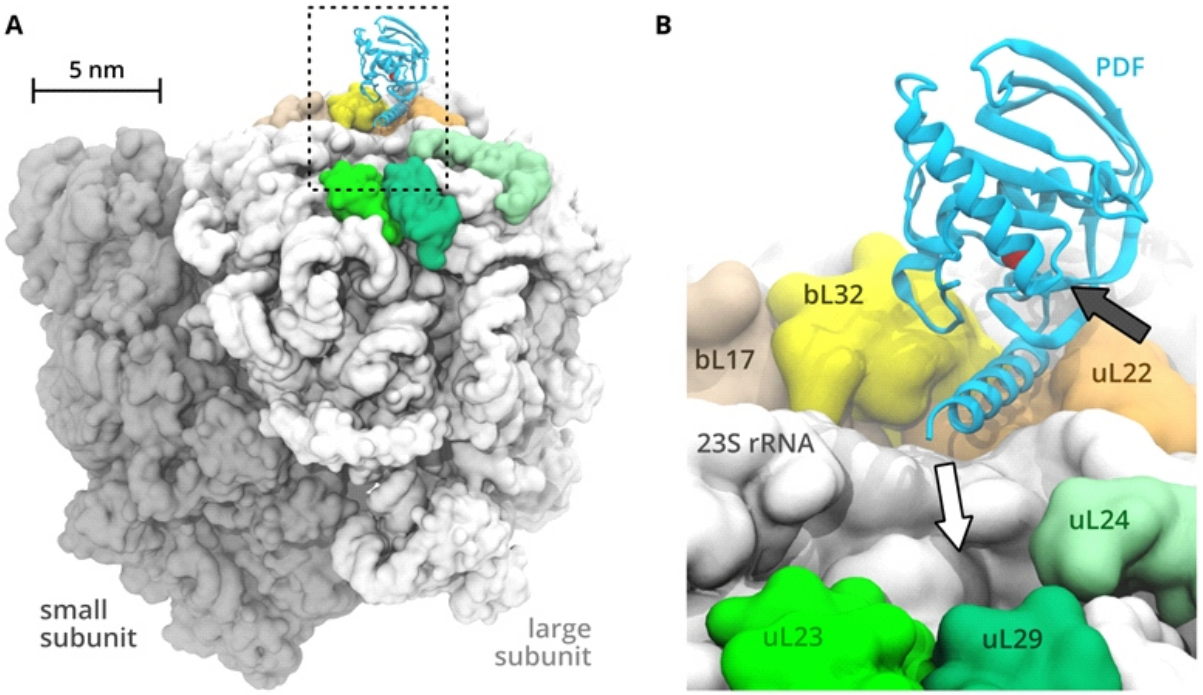

Figure 1.: **A:** The ribosome with peptide deformylase bound to the surface. **B:** Detail of bound peptide deformylase.

L23

CHARGE TRANSPORT PROPERTIES OF CYTOCHROME b_{562} IN METAL JUNCTIONS

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Life sustaining process, including respiration, photosynthesis, and various enzymatic catalytic activities, rely on electron transfer reactions mediated by redox proteins. One such protein, cytochrome b_{562} (cyt b_{562}) found in *Escherichia coli,* contains a redox active heme ($Fe^{2+/3+}$) cofactor bonded to the protein matrix, coordinated by axial histidine (His102) and methionine (Met7) ligands (Fig. 1.). The conductive properties of single cyt b_{562} adsorbed on gold surfaces were recently investigated using Electrochemical Scanning Tunneling Microscopy (EC-STM) [1], and we further examine the related adsorption structures by using computational techniques to elucidate the charge transport properties and mechanism.

We use classical molecular dynamics (MD) to study the structure and configuration of cytochrome b562 on gold surfaces, and a quantum mechanical approach based on Density Functional Theory (DFT) to investigate its electronic states at the protein/metal interfaces and junctions [2,3]. We simulate adsorption of mutated cyt b_{562} on the flat gold surface and use the obtained structures for the preparation of the cytochrome junction between gold contacts. We use the DFT+ approach to predict electronic state alignment, followed by interfacial coupling calculations using Project Operator-Biased Diabatization (POD) method to evaluate tunneling current with Landauer formalism [3,4]. Incoherent electronic flux through redox site was also computed and these findings have implications for the development of bioelectronic devices and materials.

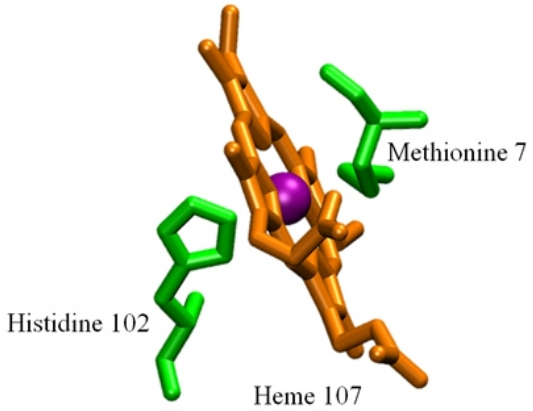

Figure 1. Details of the redox-active heme site.

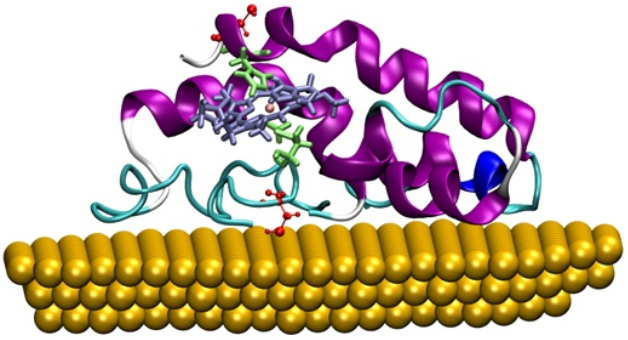

Figure 2. Mutated cyt b542 adsorbed onto a gold surface.

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L24

ELUCIDATING THE MOLECULAR BASIS OF VOLTAGE- AND LYSOPHOSPHOLIPID-INDUCED TRPC5 CHANNEL GATING

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TRPC5 channel has recently emerged as a novel potential target for the treatment of peripheral and visceral pain, and its abnormal functioning has been linked to pathophysiologically increased levels of lysophosphatidylcholine (LPC) [1, 2]. Therefore, a deep understanding of the underlying molecular mechanism of TRPC5 regulation by LPC has become urgent for the design of new effective analgesics. Like several other members of the large family

of Transient Receptor Potential (TRP) channels, TRPC5 can be also tightly regulated by membrane voltage that dampens its activity at negative potentials and enhances it at positive potentials. The structural bases for these modes of regulation are unclear. We measured whole-cell membrane currents from recombinant human TRPC5 channels expressed in HEK293T cells in response to LPC 18:1 at various membrane potentials ranging from -100 to +200 mV. Using molecular docking and molecular dynamics analysis, we identified a pocket within the outer pore region (dubbed lipid/xanthine binding domain) as a potential site from which the activity of the channel can be effectively up-regulated by LPC 18:1 and through which strongly depolarizing voltage may act to promote the transition of the pore loop toward the open channel conformation. Replacing the highly conserved tryptophan residue 577 from the pore helix by alanine (W577A) rendered TRPC5 completely insensitive to depolarizing voltage. Surprisingly, LPC activated this mutant to a similar extent as the wild-type channel but only at highly depolarizing potentials. Substitution of conserved glycine 606, located in the sixth transmembrane segment directly opposite W577, with tryptophan (G606W) enhanced activation by voltage and LPC but abolished activation by xanthine. The W577A/G606W double mutation partially rescued the voltage-dependent activation of the channel, which was further enhanced by LPC. These results may help identify sites of effective pharmacological targeting of TRPC5 and, given the considerable degree of homology between TRP proteins and the similar role of lipids in their regulation, may provide insights in the search for a general mechanism of TRP voltage and lipid activation.

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L25

PUSHING THE BOUNDARIES IN BIOMOLECULAR INTERACTION ANALYSIS WITH GCI AND waveRAPID

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The WAVEsystem with the Grating-coupled interferometry (GCI) is the novel surface-based, label-free biosensing technology to measure binding kinetics and affinity, with superior resolution in signal and time compared to SPR. The WAVEsystem allows researchers to quickly and accurately measure label-free binding kinetic rates, determine affinity constants, and monitor the concentrations of even low abundance interacting analytes in crude samples such as biofluids. Paired with no-clog WAVEchips®, a wide range of molecules can be immobilized using various chemistries or capturing techniques, while remaining compatible with crude samples or complex matrices. The Creoptix® WAVEsystem combines the enhanced sensitivity of GCI technology with the high throughput method waveRAPID® to reduce screening time and complexity.

GCI is our cutting-edge biophysical characterization method commercially available since 2015 in the WAVE family of laboratory devices. Its evanescent field penetrates less deep into the sample and extends the light-to-sample interaction length for improved signal-to-noise ratios (< 0.01 pg/mm^2). Standard immobilization techniques such as amine-coupling, Ni-NTA capture and streptavidin-biotin capture are available for the Creoptix WAVEsystem, as well as alternative lipidic interactions or Protein A/G capture. It is suitable for Fragments, Small Molecules, Peptides, Proteins, Viruses, Cell Culture Supernatants, Serums, Cell lysates etc.

The above statements will be supported by application data.

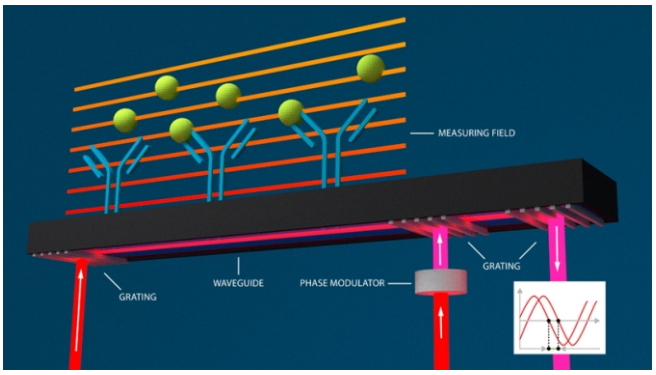

Figure 1. Measurement principle of Grating-Coupled Interferometry used in WAVE systems.