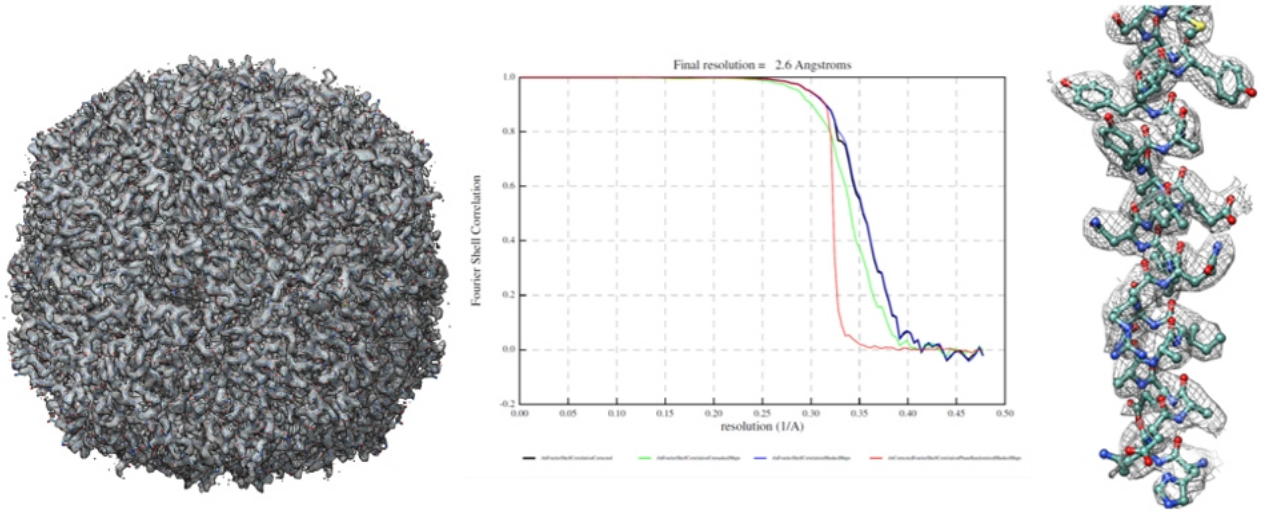

Figure 1. Structure of Apoferritin protein determined at 100 keV. a) 3D reconstruction of apoferritin at 2.6Å resolution, b) Gold-standard FSC plot corresponding to the calculated map, showing the correlation between the phase-randomized (red), unmasked (green) and masked (blue) half-maps, c) Electron density of the 2.6 Å resolution map showing the apoferritin -helix

questions answered. Data was collected with pixel size of 0.75Å, using aberration free image shift (AFIS) technology for less than 4 hours and processed using Relion 3.1 [3]. The data was collected using Thermo Scientific™ EPU™ software with pre-defined settings, were a new functionality in EPU was used – to automatically checks and refines optical alignments and provides system status for high-quality data acquisition.

All these new features that are introduced within the Tundra Cryo-TEM to achieve relevant resolution of their biological samples while keeping an accessible price point. This would make cryo-TEM accessible to many scientists across all life science branches.

1. Michael Eisenstein: The field that came in from the cold, *Nature*, Vol.**13** No.1, January 2016.
2. Mathew J. Peet, Richard Henderson, Christopher J. Russo: The energy dependence of contrast and damage in electron cryomicroscopy of biological molecules, *Ultramicroscopy* **203** (2019) 125–131.
3. J. Zivanov, T. Nakane, B. Forsberg, D. Kimanius, W.J.H. Hagen, E. Lindahl & S.H.W. Scheres "RELION-3: new tools for automated high-resolution cryo-EM structure determination", *eLife* 2018;**7**:e42166.

Friday, March 25, Session V

L23

ON THE IMPORTANCE OF PHYSICALLY CORRECT MODELS FOR PROTEIN-LIGAND BINDING

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Understanding protein-ligand binding in atomistic details is key to success in structure-based drug design. I will discuss two recent approaches: i) effective electronic polarization scheme for classical molecular dynamics (MD) [1] and ii) parametrized semiempirical quantum mechanics (SQM)-based scoring function [2, 3]. The former method includes polarisation in classical additive force fields via charge scaling. We have shown its power in correct structural description of the bridging Ca^{2+} ions in lectin/carbohydrate complex (Fig. 1A, B) [1].

A universal method, which includes also other quantum effects, such as charge transfer or -hole bonding, is quantum mechanics. We have developed an SQM-based scoring function which, due to its chemical generality, outperforms standard academic and commercial packages in sampling, ranking and virtual screening. In summary, developing and applying physically correct models of protein-ligand binding heads toward an unrivalled qualitative and quantitative enhancement of the predictive power of computer-aided drug design.

1. M. Lepsik, et al. Induction of rare conformation of oligosaccharide by binding to calcium-dependent bacterial

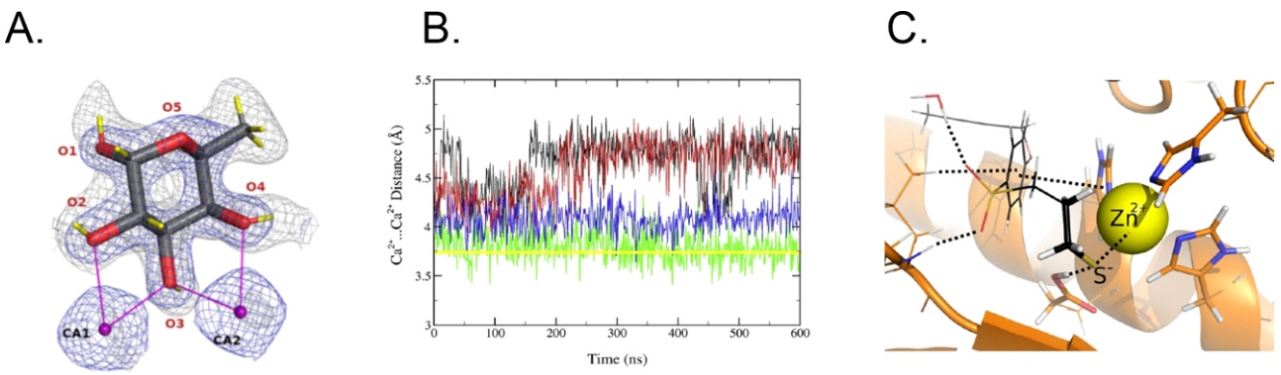

Figure 1. **A.** L-Fucose binding to two calcium ions in LecB lectin (PDB: 7PRG). **B.** $Ca^{2+}\cdots Ca^{2+}$ distances from MD with standard Ca^{2+} parameters (black, red) and scaled-charge parameters (blue, green) compared with the crystal value (yellow). **C.** The active site of metalloprotein (brown ribbon) with Zn^{2+} (yellow) and thiolate (S^-) group of ligand. Noncovalent interactions are shown as dotted lines.

lectin: X-ray crystallography and modelling study. *Eur J Med Chem*., **177**, (2019), 212-220.

2. M. Lepšík, et al. The Semiempirical Quantum Mechanical Scoring Function for In Silico Drug Design. *ChemPlusChem,* **78**, (2013), 921 – 931.
3. A. Pecina, et al., SQM/COSMO Scoring Function: Reliable Quantum-Mechanical Tool for Sampling and Ranking in Structure-Based Drug Design. *ChemPlusChem,* **85**, (2020), 2362 –2371.

ML, PH and JŘ are supported by ChemBioDrug CZ.02.1.01/0.0/0.0/16_019/0000729 project from the European Regional Development Fund (OP RDE). ML acknowledges funding from the Horizon 2020 research programme under the Marie Sklodowska-Curie grant agreement No. 795605, E.U.

L24

"REDNATCO" - MAKING DNATCO MORE USEFUL FOR CRYSTALLOGRAPHERS

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In its current form, the DNATCO web service primarily performs assignment of nucleotide conformers classes (NtCs) to dinucleotides in nucleic acid structures and structure validation. While some functionality for NtC-aware structure modelling and refinement is available, it is currently rather rudimentary and depends on external tools that have to be used separately outside DNATCO. The main goal of the "ReDNATCO" (placeholder name) is to expand the functionality of DNATCO to make it a useful and convenient tool for structural refinement as well.

In this talk we will present an overview of the current and planned features. Most importantly, we hope to get involved with other crystallographers to discuss how they do things and seek collaborations to make sure that the new DNATCO provides the necessary functionality and user convenience.

L25

DESIGNING TRANSMEMBRANE PROTEINS TO ENHANCE TRANSPORT OF PEPTIDES ACROSS CELL MEMBRANES

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Cell membranes are multicomponent semipermeable bilayers providing selective transport of molecules between the cell and its surroundings. Only certain classes of molecules are able to pass directly through the hydrophobic core of the membrane, the transport of other molecules can be catalyzed by transmembrane proteins. One of such proteins are scramblases which facilitate the translocation of lipids between individual membrane leaflets [1].

We hypothesize that scramblases and proteins with similar properties could also enhance membrane translocation of other amphiphilic molecules, such as antimicrobial or cell-penetrating peptides [2, 3].

Using coarse-grained molecular dynamics simulations with free energy calculations, we systematically study and identify properties of transmembrane proteins leading to maximal enhancement of peptide translocation across phospholipid membranes. We show that the optimal translocation-enhancing proteins contain i) hydrophilic residues forming continuous and compact patch, ii) charged residue(s), preferably positioned in the protein center, and iii) large aromatic residues. Furthermore, we reveal that the translocation enhancement originates from i) membrane disruption caused by the protein and ii) stabilizing enthalpic interactions between the protein and the translocating peptide.

Our results demonstrate that naturally occuring scramblases or *de novo* designed proteins or peptides could be used for more efficient transport of amphiphilic peptides into cells. This opens the possibility of developing a drug-delivery system by mixing a peptide-based drug with translocation-enhancer that would integrate into the membrane.

1. H. M. Hankins, R. D. Baldridge, P. Xu, T. R. Graham. *Traffic*, **16**, (2015), 35-47.
2. J. Wang, X. Dou, J. Song, Y. Lyu, X. Zhu, L. Xu, W. Li, A. Shan. *Med Res Rev.*, **39**, (2019), 831-859.

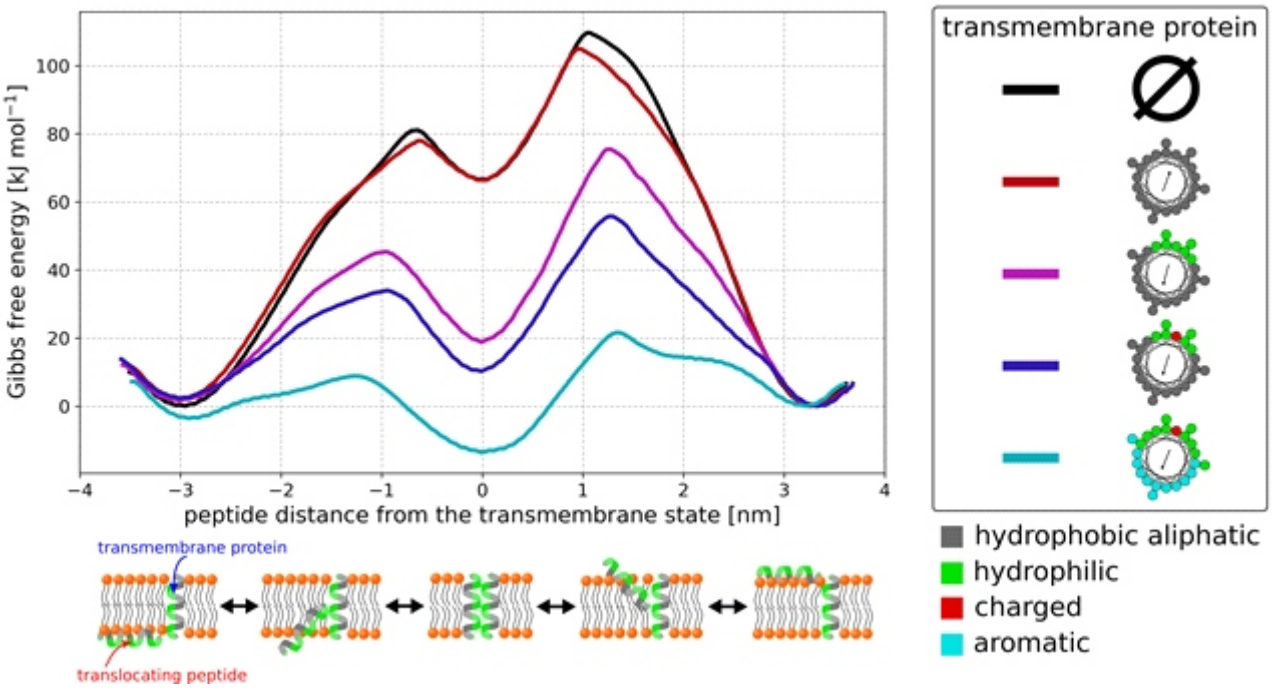

Figure 1. Free energy profiles of a selected amphiphilic peptide translocating through the membrane along various transmembrane proteins. As the translocation-enhancing properties of the transmembrane protein increase, the free energy barrier of peptide translocation decreases. The mechanism of peptide translocation along the transmembrane protein is schematically depicted below the chart.

3. G. Guidotti, L. Brambilla, D. Rossi. *Trends Pharmacol Sci.*, **38**, (2017), 406-424.

This project has received funding from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme (grant agreement No 101001470) and from the Czech Science Foundation (project no. GA20-20152S). Computational resources were provided by the CESNET LM2015042 and the CERIT Scientific Cloud LM2015085 provided under the program Projects of Large Research, Development, and Innovations Infrastructures. Additional computational resources were obtained from IT4 Innovations National Super-computing Center – LM2015070 project supported by MEYS CR from the Large Infrastructures for Research, Experimental Development and Innovations.

L26

MECHANISMS OF CHARGE TRANSFER THROUGH MULTIHEME PROTEIN JUNCTIONS, THEIR DISTANCE AND BAND-ALIGNMENT DEPENDENCIES

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Multiheme cytochromes are redox-active proteins that can efficiently transfer electrons over biological membranes. Electrons flow through such proteins by sequential, thermally activated hops between the heme cofactors and their iron cations, following the Marcus theory of electron transfer. However, experimental measurements on the cytochrome-based junctions between metal contacts in vacuum yielded currents of relatively high magnitudes but practically no temperature dependence [1]. These observations suggested that electrons could coherently tunnel through the protein structures on their way from one electrode to another [2]. Yet, the efficiency of the tunneling mechanism is known to decay exponentially with the distance and cannot explain transport through extraordinary wide junctions.

In the presented theoretical study of stacked small-tetraheme cytochrome (STC) junctions between the gold electrodes, we investigate the distance and band-alignment dependencies of these two mechanisms using DFT calculations and current-voltage curve modeling. We show that the significant potential drop on the protein/metal interface severely hinders the incoherent hopping and supports the off-resonant tunneling as the transport mechanism. However, these two fundamentally different mechanisms begin to be competitive, or their preference is even swapped, as the protein states are brought closer to the electrode Fermi level. Therefore, the specific design of the protein/metal contacts or application of a gate potential controlling the protein electronic levels could drastically affect how electronic charges pass through the redox proteins in nanoelectronic devices.

[1] Garg, K. *et al.*: Direct Evidence for Heme-Assisted Solid-State Electronic Conduction in Multi-Heme c-Type Cytochromes. *Chem. Sci.* **9**, 7304-7310, 2018.

[2] Futera, Z. *et al.*: Coherent Electron Transport across a 3 nm Bioelectronic Junction Made of Multi-Heme Proteins. *J. Phys. Chem. Lett.* **11**, 9766-9774, 2020.

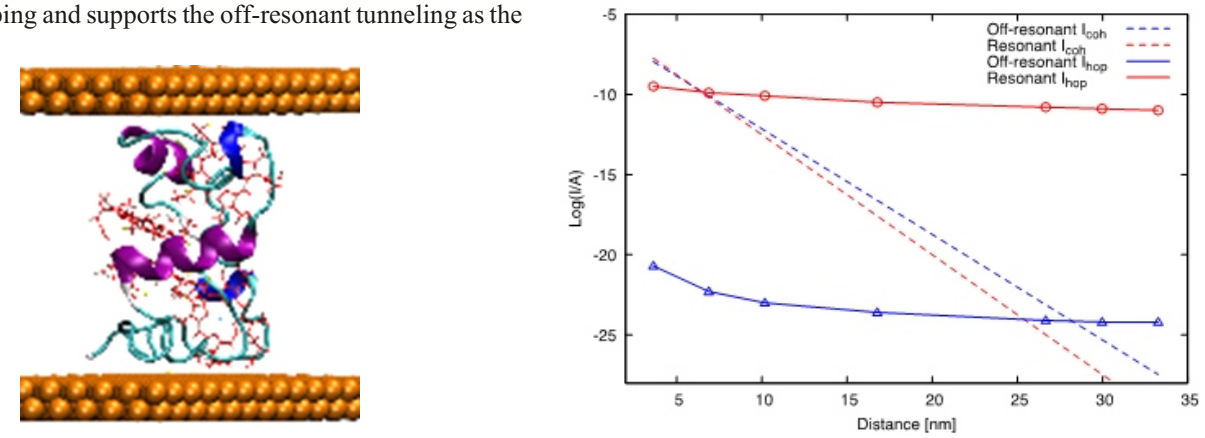

Figure 1. (a) DFT model of STC junction between gold electrodes. (b) Predicted current magnitudes using incoherent hopping and coherent tunneling models under resonant and off-resonant conditions.

L27

EXPLORING REACTION MECHANISMS OF METALLOPROTEINS BY CORRELATING THEORY AND EXPERIMENT

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Among the various essential elements in biocatalysis, metalloproteins play a specific role by catalysing reactions that would not occur under physiological conditions. The presence of metal ions is crucial for the oxidation/reduction processes, electron transfer, spin-forbidden reactions and 'difficult reactions', such as N_2, O_2, C–H bond cleavage (or formation). These processes are intimately involved in the fundamental elements of life, e.g. respiration and photosynthesis. Enormous efforts, both experimental and theoretical, have been exerted to understand the structure and function of metalloproteins. While experiments (e.g., X-ray crystallography, various spectroscopic techniques, electrochemistry) are crucial in initial phases of our understanding to a particular system, theoretical calculations complement these data by providing a unique one-to-one structure-energy mapping. [1] On an example of a multi-copper oxidase, [2-5] and non-heme diiron ^{9}-desaturase, [6,7] I will demonstrate that by correlating experimental and theoretical data, the reaction mechanisms of bioinorganic systems can be fully elucidated. This may eventually lead to a formulation of powerful and qualitative concepts governing catalytic processes involving hydrogen-atom transfer reactions. [8]

1. Rokob, T. A.; Srnec, M.; Rulíšek, L. *Dalton Trans.* **2012**, *41*, 5754-5768.
2. Rulíšek, L.; Solomon, E. I.; Ryde, U. *Inorg. Chem.* **2005**, *44*, 5612-5628.
3. Ryde, U.; Hsiao, Y.-W.; Rulíšek, L., Solomon, E. I. *J. Am. Chem. Soc.* **2007**, *129*, 726-727.
4. Rulíšek, L.*; Ryde, U. *Coord. Chem. Rev.* **2013**, *257*, 445-458.
5. Li, J.–L.; Farrokhnia, M.; Rulíšek, L.; Ryde, U. *J. Phys. Chem. B* **2015**, *119*, 8268–8284.
6. Chalupský, J.; Rokob, T. A.; Kurashige, Y.; Yanai, T.; Solomon, E. I.; Rulíšek, L.; Srnec, M. *J. Am. Chem. Soc.* **2014**, *136*, 15977-15991.
7. Bím, D.; Chalupský, J.; Culka, M.; Solomon, E. I.; Rulíšek, L.; Srnec, M. *J. Am. Chem. Soc.* **2020**, *142*, 10412-10423.
8. Bím, D.; Maldonado-Domínguez, M.; Rulíšek, L.; Srnec, M. *Proc. Natl. Acad. Sci.* **2018**, *115*, E10287-E10294.

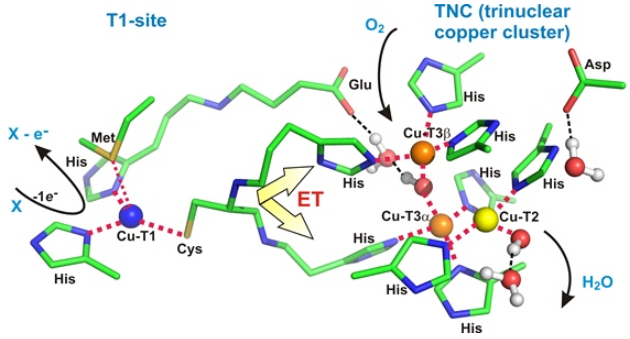

Figure 1. Active Site(s) of Multi-Copper Oxidases.

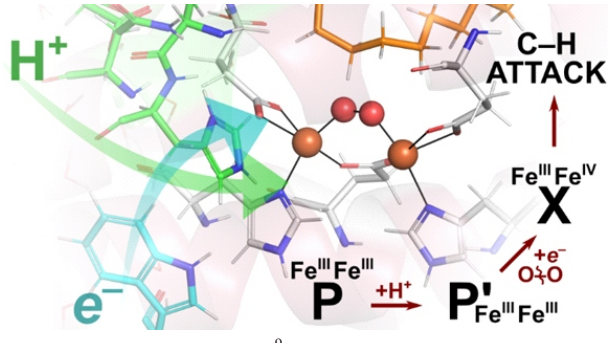

Figure 2. Catalytic Cycle of ^{9}-desaturase.

L28

EMNETIK 24 SYSTEM - DNA CLEANUP FOR GENETIC ENGINEERING

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Beckman Coulter Life Sciences launches the EMnetik System to semi-automate workflows for EMnetik PCR Cleanup Kit and EMnetik Plasmid Purification Kit. These kits use revolutionary magnetic bead-based technology to clean nucleic acids. The small powerful benchtop instrument – Emnetik 24 can run 1 to 24 samples at a time, use electromagnets to mix and separate magnetic beads and navigates user displaying individual steps of the protocol on its screen. Touchpoints to complete PCR cleanup are significantly reduced by up to 80% compared to leading spin-column products. Therefore 24 samples can be proceeded in only 16 minutes.

Figure 1. EMnetik 24 instrument and kits