

Saturday, March 25, Session VII

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REORGANIZATION FREE ENERGY FOR AZURIN OXIDATION AT GOLD INTERFACES EVALUATED BY PERTURBED MATRIX METHOD**O. V. Kontkanen^{1,2}, D. Biriukov², Z. Futera¹**¹*Faculty of Science, University of South Bohemia, Branisovska 1760, 370 05 Ceske Budejovice, Czech Republic*²*Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Flemingovo nam. 2, 16610 Prague 6, CR
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Charge transfer reactions of various complexities naturally occur in redox proteins and their complexes. On the other hand, these proteins could be incorporated into nanobio-electronic devices as a charge-carrier components, which could benefit in new electronic properties. However, the charge transfer reactions have to be fully understood in various environments such as in vacuum, which provides the challenge to investigate them accurately. From the theoretical point of view, a lot of computational resources (tools, time) are required to capture the details of charge transfer, e.g. by computing the reorganization free energy, in a reasonable accuracy. Traditionally, QM/MM is used to investigate large systems, namely proteins, but it can grow demanding as the size of the system grows. Alternatively, Perturbed Matrix Method (PMM) [1,2] could be applied, as a faster approach, which can handle large number of molecular-dynamics (MD) samples and provide thus statistically-converged data.

We previously investigated azurin and its reorganization free energy in vacuum and adsorbed on the gold surface through QM/MM [3]. Our study suggests that azurin gains structural flexibility near redox center in vacuum, which gives similar reorganization free energies as in solution. Now we apply PMM for the same azurin systems in vacuum and show that a) PMM is applicable outside from the aqueous solution, b) it can re-produce the results within a reasonable accuracy when compared to QM/MM ($\Delta = 0.1-0.2$ eV), and c) to go beyond, we compute the reorganization free energy of azurin junction, 0.73 eV, in vacuum (**Fig. 1**). The reorganization free energy is similar to the azurin in solution, $\sim 0.6 - 0.7$ eV, which indicates that the flexibility gained from vacuum is restrained in the junction structure. In conclusion, PMM is able to replicate the reorganization free energies in vacuum and on bio/metal sur-

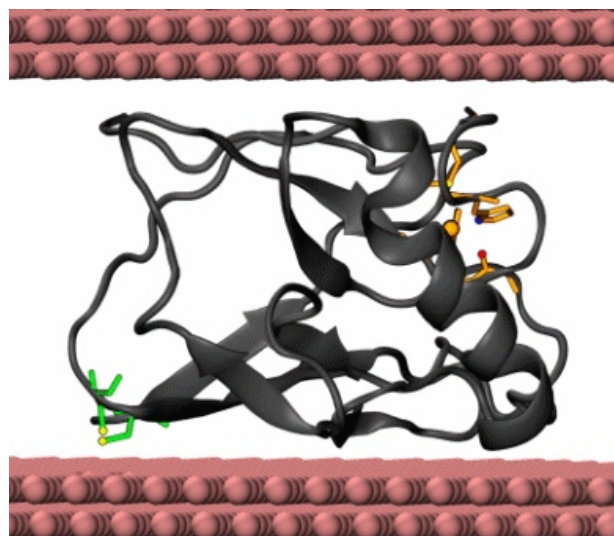


Figure 1. Azurin adsorbed through both disulfide bridge and Cu-site on gold in vacuum.

faces within the same accuracy as QM/MM with an appropriate description of the the redox center.

1. Aschi, M., Spezia, R., Di Nola, A. and Amadei, A., 2001. A first-principles method to model perturbed electronic wavefunctions: the effect of an external homogeneous electric field. *Chemical physics letters*, 344(3-4), 374.
2. Spezia, R., Aschi, M., Di Nola, A. and Amadei, A., 2002. Extension of the perturbed matrix method: application to a water molecule. *Chemical physics letters*, 365(5-6), 450.
3. Kontkanen, O. V., Biriukov, D., & Futera, Z. (2022). Reorganization free energy of copper proteins in solution, in vacuum, and on metal surfaces. *The Journal of Chemical Physics*, 156(17), 175101.



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SEQUENTIAL ALL-TRANS TO 13-CIS TO 11-CIS PHOTOISOMERIZATION IN BESTRHODOPSIN, AN UNUSUAL RED-ABSORBING MICROBIAL LIGHT-GATED ANION CHANNEL AS REVEALED BY FEMTOSECOND STIMULATED RAMAN SPECTROSCOPY

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Bestrhodopsins are a newly discovered class of light-regulated ion channels that consist of two rhodopsins in tandem fused with a bestrophin ion channel domain, together forming a giant pentameric ion channel structure. Bestrhodopsin of the marina alga *Phaeocystis antarctica* covalently bind all-trans retinal as Schiff-base (RSB) chromophores uniquely absorbing in the red at 660 nm, which conveys an excellent potential for optogenetic applications. Upon red light absorption, the rhodopsins switch to a metastable state absorbing green light at 540 nm denoted as P540. The P540 state corresponds to an unusual 11-cis

RSB isomer, rather than the canonical 13-cis isomer found in nearly all other microbial rhodopsin photoreactions. To assess the reaction and isomerization dynamics of bestrhodopsin, we performed femtosecond – submillisecond transient absorption (TA) and femtosecond stimulated Raman spectroscopy (FSRS). Steady-state stimulated Raman spectroscopy of the bestrhodopsin dark state (D661) showed that it is very similar to the bacteriorhodopsin O intermediate, with a highly distorted all-trans RSB and protonated counterion, partly explaining the unusual red absorption of bestrhodopsin.

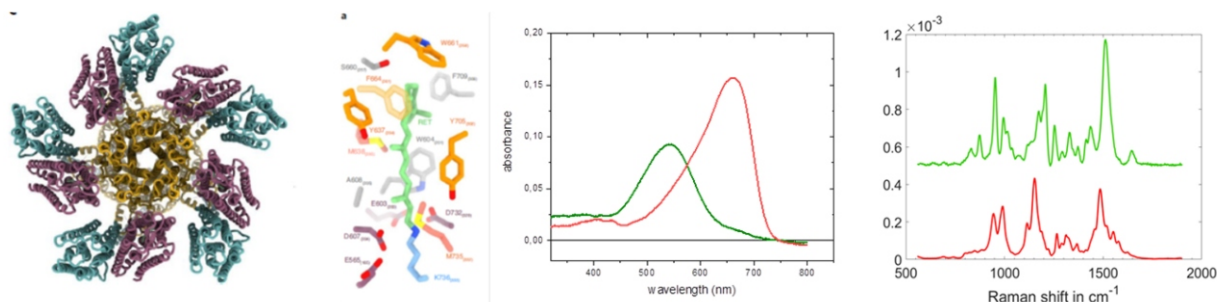


Figure 1. Structure of bestrhodopsin oligomer, configuration of retinal in the binding domain and electronic absorption and Raman vibration spectra of the Red and Green state.

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THE ELIBIO ODYSSEY: A 6-YEAR RECAP

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The ELIBIO (Structural dynamics of biomolecular systems) project explores new frontiers in light and optics to create breakthrough science in biology, chemistry and physics. The research team of ELIBIO is formed at the interface between two complementary research centres, ELI Beamlines oriented to photon science, and the Institute of Biotechnology (IBT) oriented to biomedical, protein engineering and structural biology research. The milestones include the creation of biologically oriented research facilities (Biolab), and the strengthening of research infrastructure at the IBT. We have developed new instrumentation and methods, in particular, genetic code expansion technology, and a dual transient visible absorption and femtosecond-stimulated Raman spectroscopy (FSRS) set-up. The unique combination of genetically encoded non-canonical amino acids carrying vibrational probes, and ultrafast time-resolved optical spectroscopy (UV/Visible, infrared and Raman) down to the femtosecond time scale has allowed us to gain new insights in signal propaga-

tion pathways. We have applied these novel methodologies to study photosensory receptors (EL222, CarH, and KR2) and non-photoactive proteins (myoglobin). In addition, quantum chemistry calculations have permitted comprehensive assignments of the experimentally-determined spectral bands, and have made testable predictions. We have also complemented our results with other techniques, like nuclear magnetic resonance spectroscopy, X-ray crystallography, and small-angle scattering. We conclude that the joint use of unnatural amino acids and time-resolved methods can reveal non-equilibrium protein structural dynamics residue-by-residue.

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PLASTICITY OF SARS-CoV-2 SPIKE'S RBD DOMAIN AND ACE2 INTERACTION AND ITS IMPLICATIONS

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Viruses rapidly evolve to stay a step ahead of their hosts. Several new variants emerged during the course of pandemics, with Omicron sublineages BQ.1.1 and XBB being the most recent. These variants displayed higher fitness taking over a significant part of the virus population. Although each variant revealed mutations across the whole genome, a significant portion of the phenotype can be attributed to mutations in the Spike protein and its mutational hotspot Receptor Binding Domain (RBD). We study the ongoing evolution and adaptation of SARS-CoV-2 to humans, focusing on the interaction that dictates its immuno-

logical properties. Apart from the structural similarity, the interface of SARS-CoV-2 – ACE2 is composed of different amino acids from that of the RBD of SARS-CoV. While both RBDs bind the same location, mostly helix one on the ACE2, they differ in 11 interface residues and the new variants are similarly different. Parallel solutions for the same interaction suggest high interface plasticity. In my talk, I will describe how current *in vitro* evolution techniques can help explain this observed plasticity and what we have learned about protein interface evolution in general.