XVI Discussions in Structural Molecular Biology

Annual Meeting of the Czech Society for Structural Biology and the 3rd User Meeting of CIISB

Academic and University Center, Nové Hrady, March 21 - 23, 2019

Organisers:

Jan Dohnálek, Jarmila Dušková, Jan Stránský, Vojtěch Spiwok, Vladimír Sklenář, Kristýna Adámková, Radek Kužel

Thursday, March 21, Session I

L1

ELECTRIC CONDUCTIVITY OF MULTI-HEME PROTEINS

Zdenek Futera[1], Xiuyun Jiang[1], Jan B. D. Elsner[2], Jochen Blumberger[1,3]

[1]Department of Physics and Astronomy, University College London, Gower Street, London WC1E 6BT, United Kingdom
[2]University of Cambridge, Robinson College, Grange Road, Cambridge CB3 9AN, United Kingdom
[3]Institute for Advanced Study, Technische Universität München, Lichtenbergstrasse 2 a, D-85748, Garching, Germany

Multi-heme proteins such as STC or MtrF are membrane proteins facilitating long-range electron transfer (ET) across cell membrane in metal-reducing bacteria [1]. We used classical molecular dynamics (MD) together with electronic-structure calculations based on density functional theory (DFT) to show that in native environment the conducted electrons are transferred by incoherent hopping between the heme cofactors. Kinetics of the ET is significantly enhanced by presence of cysteine linkages [2, 3, 4]. However, recent experimental measurements of current-voltage curves suggested that the ET mechanism changes to coherent electron tunneling in vacuum when the protein is electronically coupled with metal electrodes [5]. Therefore, we performed MD simulations in accurate gold/protein interaction force field [6] to identify adsorption of STC and MtrF between two gold electrodes. By the large-scale DFT calculation on the complete interfacial structure with state-of-the-art band alignment corrections we were able to identify the conduction channels in the STC junction. These are formed predominantly by delocalized heme iron states. Finally, we applied Landauer formalism to compute I-V curves on investigated junctions using the DFT electronic states corrected for band alignment and the ET mechanism is discussed.

1. M. Breuer, K. M. Rosso, J. Blumberger, *Proc. Natl. Acad. Sci. U.S.A.* **2014**, *111*, 611-616.
2. Z. Futera, J. Blumberger, *J. Phys. Chem. C.* **2017**, *121*, 19677-19689.
3. X. Jiang, Z. Futera, E. Md. Ali, F. Gajdos, G. F. von Rudorff, A. Carof, M. Breuer, J. Blumberger, *J. Am. Chem. Soc.* **2017**, *139*, 17237-17240.
4. X. Jiang, B. Burger, F. Gajdos, C. Bortolotti, Z. Futera, M. Breuer, J. Blumberger, *Proc. Natl. Acad. Sci U.S.A.* **2018**, submitted.
5. K. Garg, M. Ghosh, T. Eliash, J. H. van Wonderen, J. N. Butt, L. Shi, X. Jiang, Z. Futera, J. Blumberger, I. Pecht, M. Sheves, D. Cahen, *Chem. Sci.* **2018**, *9*, 7304-7310.
6. Z. Futera, J. Blumberger, *J. Chem. Theory Comput.* **2018**, submitted.

L2

FEMTOSECOND VIBRATION SPECTROSCOPY OF UNPROTONATED RETINAL IN A UV ABSORBING RHODOPSIN BIOLOGICAL PHOTORECEPTOR

Miroslav Kloz[1,3], Yusaku Hontani[1], Matthias Broser[2], Meike Luck[2], Jörn Weißenborn[1], Peter Hegemann[2] and John T.M. Kennis[1]

[1]*Department of Physics and Astronomy, Vrije Universiteit Amsterdam, Amsterdam 1081 HV, De Boelelaan, The Netherlands*
[2]*Institut für Biologie, Experimentelle Biophysik, Humboldt Universität zu Berlin, Invalidenstrasse 42, D-10115 Berlin, Germany*
[3]*ELI-Beamlines, Institute of Physics, Na Slovance 2, 182 21 Praha 8, Czech Republic*

Histidine kinase rhodopsin 1 (HKR1) is a unique UV-absorbing microbial rhodopsin featuring unprotonated retinal Schiff-base. It serves as a model system of UV-absorbing animal rhodopsins, including those of *H. sapiens*. Unlike the situation for canonical rhodopsins which bind a protonated retinal Schiff base, the photoreaction dynamics of unprotonated retinals have remained essentially unknown. We report the photoisomerization and protonation dynamics of HKR1 probed by transient absorption (TA) and femtosecond stimulated Raman spectroscopy (FSRS) from the femto- to submillisecond timescales. We demonstrate that energy level ordering is inverted with respect to canonical rhodopsins, i.e. that photoexcitation occurs from the S_0 to the S_2 state, and that the lowest-lying S_1 state is optically forbidden. We observe that photoexcitation elicits a double isomerization reaction on distinct potential energy surfaces: optical excitation to the S_2 state results in C13=C14 *trans-cis* isomerization on the S_2 - S_1 evolution in 40 fs, followed by C15=N16 *anti-syn* isomerization on the S_1 - S_0 evolution in 4.8 ps. This results in two deprotonated ground-state photoproducts, all-*trans*/15-*anti* and 13-*cis*/15-*syn*. Protonation of the former occurs in 58 microseconds, whereas the latter is protonated in ~3 ms, resulting in a stable blue-absorbing form of HKR1 comprising two distinct protonated retinal Schiff base conformers. We thus demonstrate the complete excited-state and ground-state dynamics of protein-bound unprotonated retinal Schiff base, which constitutes a benchmark of the photochemistry of UV-absorbing rhodopsins of all kingdoms of life.

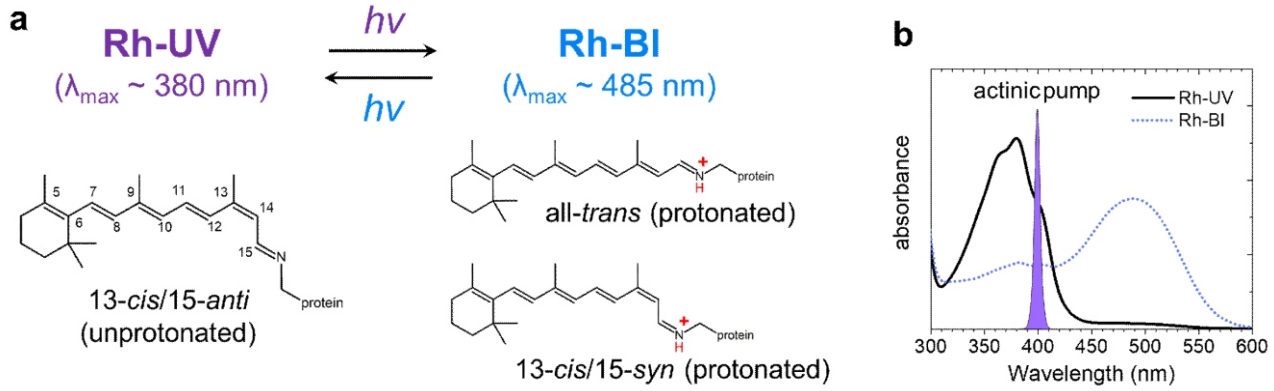

Figure 1. Retinal conformers in HKR1. (**a**) UV-absorbing (Rh-UV1) and blue-absorbing (Rh-Bl) states of HKR1. (**b**) Steady-state absorption spectra of Rh-UV1 (black solid line) and Rh-Bl (blue dotted line) states.

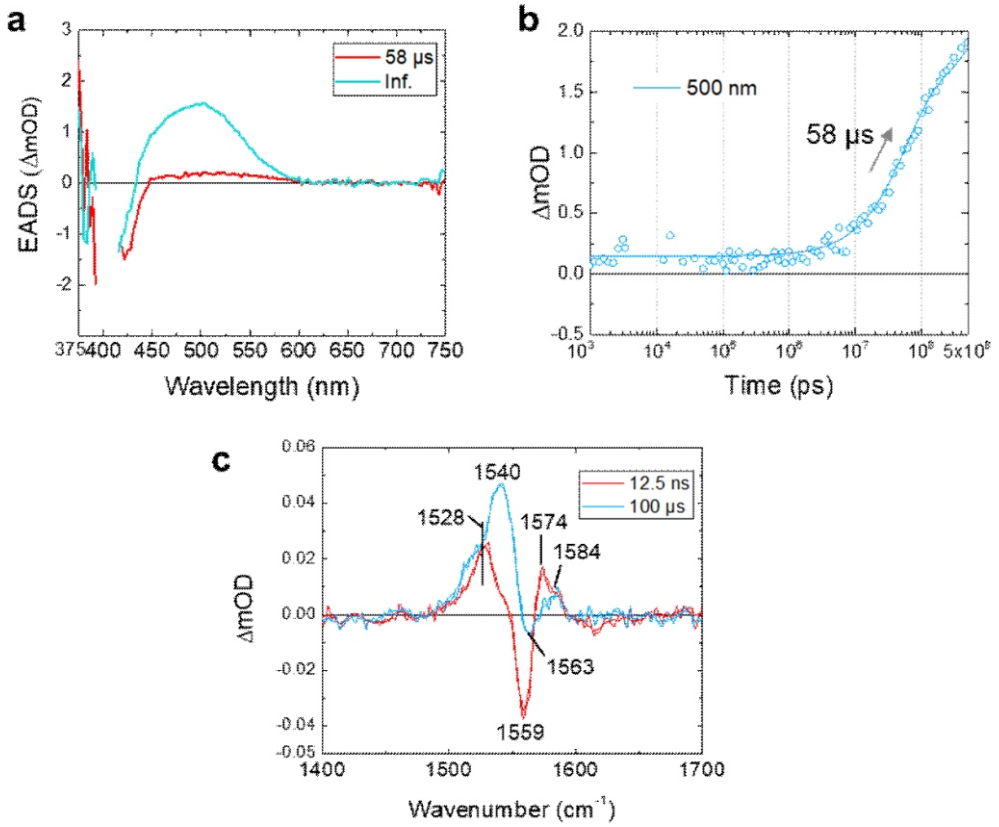

Figure 2. Evolution associated Decay spectra (EADS) of the 58 μs and infinite components of the transient absorption experiments. (a) EADS of the transient absorption signals. (b) A ns-μs time trace at 500 nm of the transient absorption experiments. The open dots and the solid line show raw data and a fitting curve, respectively. (c) FSRS spectra taken at 12.5 ns and 100 s time delay.

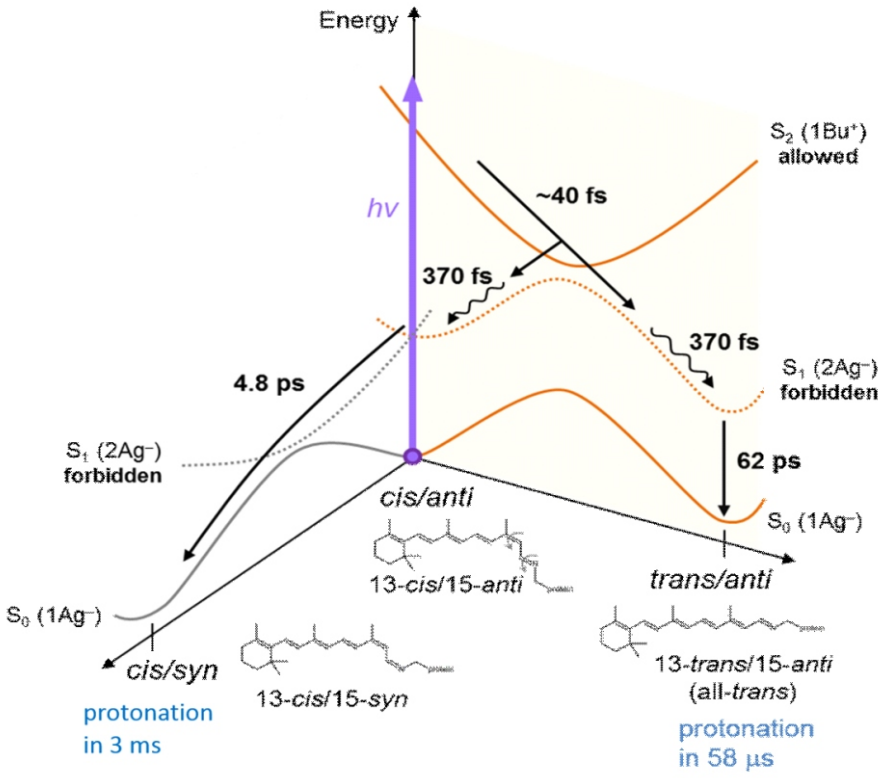

Figure 3. Excited-state reaction model of Rh-UV1 HKR1.

L3

CRITICAL DEFECTS IN CRYOPRESERVED CELL NUCLEI: DNA STRUCTURE CHANGES

Martin Falk[1], Iva Falková[1], Eva Pagáčová[1], Olga Kopečná[1], Alena Bačíková[1], Daniel Šimek[2], Martin Golan[2,4], Stanislav Kozubek[1], Michaela Pekarová[1], Shelby E. Follett[3], Bořivoj Klejdus[5,6], K. Wade Elliott[7], Krisztina Varga[7], Olga Teplá[8,9] and Irena Kratochvílová[2]

[1]The Czech Academy of Sciences, Institute of Biophysics, Královopolská 135, CZ-612 65 Brno, Czech Rep.
[2]The Czech Academy of Sciences, Institute of Physics, Na Slovance 2, CZ-182 21, Prague 8, Czech Rep.
[3]Department of Chemistry, University of Wyoming, 1000 E. University Ave Laramie, WY 82071, USA
[4]Faculty of Mathematics and Physics, Charles University in Prague, Ke Karlovu 5, CZ-121 16 Prague 2, Czech Republic
[5]Institute of Chemistry and Biochemistry, Faculty of Agronomy, Mendel University in Brno, Zemědělská 1, CZ-613 00
[6]CEITEC-Central European Institute of Technology, Mendel University in Brno, Zemědělská 1, CZ-613 00 Brno, Czech Republic
[7]Department of Molecular, Cellular, and Biomedical Sciences, University of New Hampshire, 46 College Road, Durham, NH 03824, USA
[8]ISCARE IVF a.s. Jankovcova 1692, CZ-160 00 Praha 6
[9]VFN Gynekologicko-porodnická klinika, Apolinářská 18, CZ-120 00, Czech Republic

In this work, we shed new light on the highly debated issue of chromatin fragmentation in cryopreserved cells. Moreover, for the first time, we describe replicating cell-specific DNA damage and higher-order chromatin alterations after freezing and thawing. We identified DNA structural changes associated with the freeze-thaw process and correlated them with the viability of frozen and thawed cells.

We simultaneously evaluated DNA defects and the higher-order chromatin structure of frozen and thawed cells with and without cryoprotectant treatment. We found that in replicating (S phase) cells, DNA was preferentially damaged by replication fork collapse, potentially leading to DNA double strand breaks (DSBs), which represent an important source of both genome instability and defects in epigenome maintenance. This induction of DNA defects by the freeze-thaw process was not prevented by any cryoprotectant studied. Both in replicating and non-replicating cells, freezing and thawing altered the chromatin structure in a cryoprotectant-dependent manner. Interestingly, cells with condensed chromatin, which was strongly stimulated by dimethyl sulfoxide (DMSO) prior to freezing had the highest rate of survival after thawing. Our results will facilitate the design of compounds and procedures to decrease injury to cryopreserved cells [1].

1. M. Falk, I. Falková, E. Pagáčová, O. Kopečná, A. Bačíková, D. Šimek, M. Golan, S. Kozubek, M. Pekarová, S. E. Follett, B. Klejdus, K. W. Elliott, K. Varga, O. Teplá, I. Kratochvílová, *Scientific Reports*, **8**/14694, 1-18.

L4

FLEXIBILITY OF DNA MISMATCHES AND ITS IMPLICATION FOR MISMATCH RECOGNITION

T. Bouchal[1,2], I. Durník[1,2], P. Kulhánek[1,2]

[1]CEITEC – Central European Institute of Technology, Masaryk University, 625 00 Brno, Czech Republic
[2]National Centre for Biomolecular Research, Faculty of Science, Masaryk University, 625 00 Brno, Czech Republic
kulhanek@chemi.muni.cz

Thermodynamic stabilities of base pairs composed of 36 unique combinations of A, G, T, and C nucleobases in both *anti* and *syn* conformations in the central part of 13-nt long palindromic DNA were characterized by biased molecular dynamics simulations. The bias was introduced through two base pair parameters, opening and shear, which cover all possible arrangements of nucleobases in the base pair plane. In total, we identified 107 different free energy minima. We found excellent agreement between calculated free energy minima and experimental structures of mismatches in both free DNA and DNA complexed with the MutS enzyme. The latter suggests that MutS evolved in such a way that the mismatch recognition is achieved by probing a mismatch towards the minor groove, where mismatches exhibit stable albeit energetically less favourable structures already in the free form while the canonical base pairs do not. We also found that opening of mismatch towards minor groove provides better discrimination from the canonical base pairs than previously suggested bending of DNA. This finding can be helpful in better understanding of sequence-dependent mutability or designing chemical substances targeting damaged DNA.

This research was carried out under the project CEITEC 2020 (LQ1601) with financial support from the Ministry of Education, Youth and Sports of the Czech Republic under the National Sustainability Programme II and the Grant Agency of the Czech Republic (GA16-11619S/2016). Computational resources were provided by the CESNET LM2015042, the CERIT Scientific Cloud LM2015085, and the IT4Innovations National Supercomputing Center LM2015070 provided under the program "Large Infrastructures for Research, Experimental Development and Innovations" by the Ministry of Education, Youth and Sports.

Thursday, March 21, Invited lecture

L5

STRUCTURAL BIOLOGY AT THE DIFFRACTION LIMITED SYNCHROTRON SOURCE MAX IV

Marjolein Thunnissen

MAX IV Laboratory Lund University, Lund, Sweden
marjolein.thunnissen@maxiv.lu.se

The MAX IV Laboratory is a new synchrotron in Lund, Sweden which operates two storage rings and a short-pulse facility. The MAX IV 3 GeV storage ring is the first synchrotron ring with a multi-bend achromat design providing higher photon beam brilliance and coherence.

A number of beamlines interesting for Structural Biologists is in operation or under development at this facility. Amongst these beamlines are the protein crystallography beamline BioMAX and MicroMAX, the small angle scattering beamline CoSAXS and the X-ray absorption spectroscopy beamline Balder. There are also opportunities for using imaging techniques such as m-XRF and soft-Xray STXM. The beamlines will be presented with a slight emphasis on the protein diffraction beamlines.

Friday, March 22, Session II

L6

COMPARATIVE STRUCTURAL ANALYSIS OF LECTIN FAMILY FROM *PHOTORHABDUS SPP*

Josef Houser[1,2], Eva Fujdiarová[2], Gita Jančaříková[1,2], Filip Melicher[1], Michaela Wimmerová[1,2]

[1]CEITEC-Central European Institute of Technology, Masaryk University, Kamenice 5, 625 00 Brno, Czech Republic
[2]National Center for Biomolecular Research, Faculty of Science, Masaryk University, Kotlářská 2, 611 37 Brno, Czech Republic
[3]Department of Animal Physiology and Immunology, Institute of Experimental Biology, Masaryk University, Kamenice 5, 625 00 Brno, Czech Republic

Photorhabdus is a genus of gram-negative bioluminescent bacteria living in a symbiosis with *Heterorhabditis* nematodes forming a highly entomopathogenic complex. Such a complex is used in agriculture as a nature-based insecticide. However, some members of this genus are human pathogens as well. Understanding the mechanisms that determine interaction between *Photorhabdus* and its symbionts/hosts could be highly beneficial not only in biotechnologies but also in clinical reasearch and drug development.

Cell-cell interactions are frequently mediated by sugar-binding proteins – lectins. Based on the genome analysis, there has been identified potential lectins in number of *Photorhabdus* species. Recently, we examind two of them in further details, namely PLL from *P. laumondii* (formerly *P. luminescens*) [1] and PHL from *P. asymbiotica* [2, 3]. Both lectins share the basic structural features, e.g. -barrel fold with seven blades or presence of multiple binding sites per monomer. However, despite rather high sequence similarity, some non-marginal differences were detected: oligomeric state, binding site preferences and organization. This lead us to investigate this lectin family in further details.

We analyzed several homologues of proteins PLL and PHL from *Photorhabdus spp.* We managed to prepare some of them in recombinant form and perform basic analysis, as well as solve structure of these proteins in free form and in complexes with naturally occuring saccharide lig-