Cells, biomembranes and membrane proteins: IR and X-ray techniques come at high spatial and temporal resolution

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Proteins are flexible nanomachines. Biological pumps that use retinal isomerization to move protons across a membrane have been studied extensively [1] but the mechanisms involved in moving sodium and chloride ions, which have both a different charge and different coordination requirements, are less understood. In a collaboration with Nogly´s group, we combined time-resolved X-ray crystallography by applying free electron laser sources, molecular spectroscopy, and QM/MM simulations to provide direct molecular insight into the dynamics of active ion transport across biological membranes. By these means, molecular movies were generated of ion transport through two microbial rhodopsins: The sodium pump rhodopsin 2 from *Krokinobacter eikastus* (*KR*2) [2] and the chloride-pumping halorhodopsin from *Nonlabens marinus* (*Nm*HR) [3]. Tracing these structural alterations over the entire chemical time range from femtoseconds to seconds provides an atomic-level understanding of how ions can be pumped against a concentration gradient. It is also demonstrated how spectroscopy and simulations can provide essential information not accessible to X-ray crystallography alone.

In the second part of the presentation, I will showcase the new scattering-type scanning near-field optical microscopy (sSNOM) and Fourier-transform infrared spectroscopy (nanoFTIR) with a spatial resolution far beyond the diffraction limit. Herewith, nanoscale surface and volumetric chemical imaging are performed using the intrinsic contrast generated by the characteristic absorption of mid-infrared radiation by covalent bonds. sSNOM is applied to study the subcellular structures of eukaryotic (*Chlamydomonas reinhardtii*) and prokaryotic (*Escherichia coli*) species, revealing chemically distinct regions within each cell, such as the microtubular structure of the flagellum [4]. Serial 100 nm-thick cellular cross-sections were compiled into a tomogram yielding a three-dimensional infrared image of subcellular structure distribution at 20 nm spatial resolution. The presented methodology can image biological samples complementing current fluorescence nanoscopy but with less interference due to the low energy of infrared radiation and the absence of labeling.

References:

[1] Heberle, J. *Proton transfer reactions across bacteriorhodopsin and along the membrane.* Biochim. Biophys. Acta – Bioenergetics 1458 (2000), 135-147.

[2] Skopintsev, P., Ehrenberg, D., Weinert, T., …, Nogly, P., Schapiro, I., Milne,C., Heberle, J., Standfuss, J. *Femtosecond to millisecond structural changes in a light-driven sodium pump.* Nature 583 (2020), 314–318.

[3] Mous S, Gotthard G, Ehrenberg D, Sen S, Weinert T, …, Milne C, Standfuss J, Schapiro I, Heberle J, Nogly P. *Dynamics and mechanism of a light-driven chloride pump.* Science 375 (2022), 845-851.

[4] K. Kanevche, D.J. Burr, D.J. Nürnberg, P.K. Hass, A. Elsässer, J. Heberle. *Infrared Nanoscopy and Tomography of Intracellular Structures*. Commun. Biol. 4: 1341. doi.org/10.1038/s42003-021-02876-7 (2021).