It's water after all.

Role of water in membrane protein folding and function

***M. Lazaratos, M. Siemers, A.-N. Bondar**, Department of Physics, Freie Universität Berlin*

In Short

- Retinal Proteins
- Protein Folding
- Water Dynamics couple protein function
- Network Analysis

Membrane proteins perform their biological function in the highly complex environment of the fluid, hydrated lipid membrane, where the protein interacts with the hydrophobic region of the membrane core, with the polar and charged regions of the lipid headgroups, and with bulk water. Moreover, discrete water molecules can be stably bound in the interior of the protein, or visit transiently the protein, where they often have important roles in protein folding and function. Structural water molecules, which occupy largely stable sites inside the protein, can bridge protein groups, thus shaping local conformational dynamics. And, networks of hydrogen-bonded waters can help bridge remote regions of the protein and serve as wires for the conduction of protons.

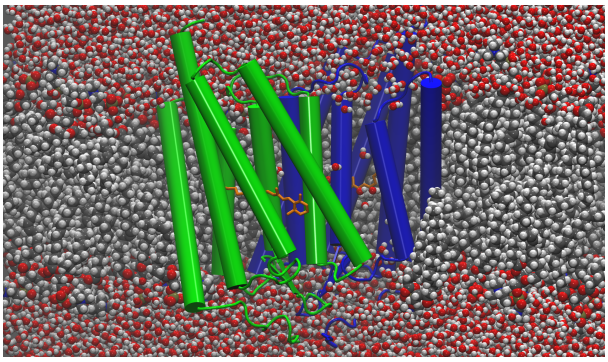

***Figure 1:** Cross section of Channerhodopsin 2 embedded in a hydrated POPC lipid bilayer. Protein is depicted in cartoon representation (green for chain A / blue for chain B). The retinal chromophore is shown as orange sticks. POPC's hydrophobic tails are shown as grey VDW spheres. Molecular graphics were prepared with VMD[4]*

A fundamental distinction between proteins that function as a sensor, a channel or a pump is their coupling to internal water dynamics. In a proton pump, access to water from either side of the membrane must be tightly controlled, such that protons are uptaken from one side of the membrane and released to the other side of the membrane; that is, in a pump continuous water wires that can conduct protons should not stretch from the cytoplasmic to the extracellular side of the protein. Ion channels, by contrast, benefit from continuous water-filled pores that allow ions to pass rapidly, without significant energy costs. No net transfer of ions needs to occur in a sensory protein.

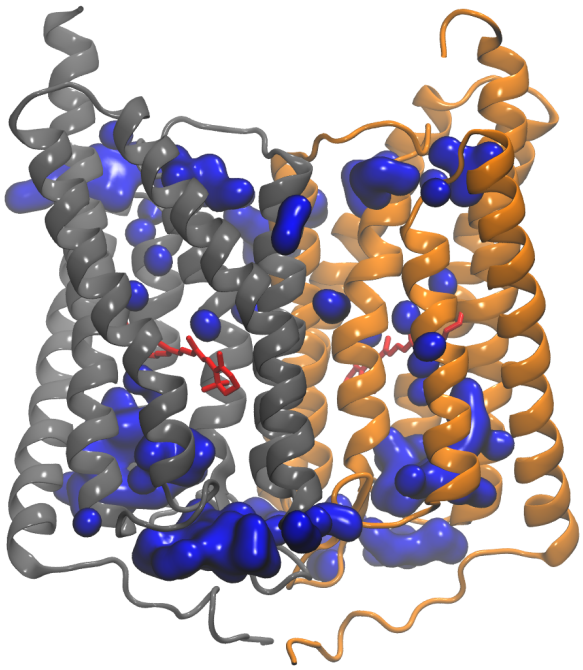

***Figure 2:** Channelrhospin 2 dimer as shown from a snapshot from an MD simulation. For simplicity the lipid bilayer and solvent are not depicted. Protein is represented as ribbons (Grey/Orange and the retinal chromophore as red sticks. Internal water molecules are shown as a surface representation in blue color. We want to emphasize that the protein in water-accessible and the internal water molecules play a significant role in protein function. Molecular graphics were prepared with VMD[4]*

It thus can be concluded that pumps, channels sensors differ in their biological functionality and also their molecular function. A very popular light sensitive non-selective cation channel for example is Channelrhodopsin 2 **(ChR2)**, oroginating from the unicellular alga *Chlamydomonas reinhardtii* Upon illumination with blue light the retinal chromophore isomerizes from an all-trans to a 13-cis state, which triggers a series of conformational changes resulting to the opening of the ion pore allowing cations their passage over the membrane. A very interesting feature of Channelrhodopsins is that they are major tools for optogenetics. Specifically, ChR2 is used as the main tool in numerous laboratories worldwide due to its excellent stability. Wild-type ChR2 is a

retinal protein part of SFB 1078 in Berlin; this is protein for which we have already derived preliminary information by relying on the crystal structure of a chimera protein [1]. Very recently, the crystal structure of wild-type ChR2 was published [2]. For reference simulations of a microbial rhodopsin ion channel, here we will start from this new crystal structure to explore the dynamics of wild-type ChR2.

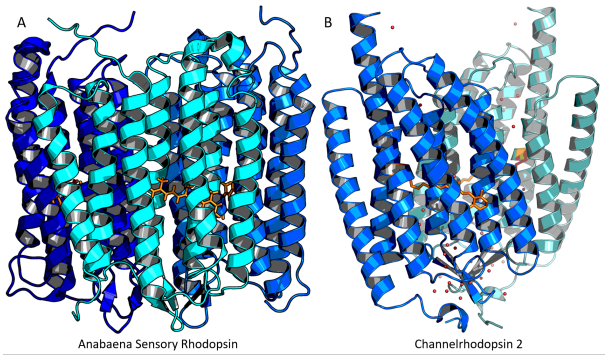

Figure 3: *A) The architecture of the microbial sensory rhosopsin AnbR. B) The cation channel ChR2 originating from Chlamydomonas reinhardtii. Molecular structures are based on coordinates from crystal structures PDBID: 2MG3 [3], 6EID respectively [2]. Helices are depicted with the cartoon representation, (blue/cyan color), internal water molecules are shown as red spheres and the retinal chromophore as orange licorice. Molecular graphics were prepared with PyMOL [5]*

We will use the HLRN supercomputer cluster to access water dynamics by employing all-atom Molecular Dynamics simulations for retinal proteins with low sequence identity in regard to one another, but with similar folding (they follow the 7 transmembrane helical motif), embedded in hydrated lipid membrane environments. MD simulations are vital for understanding retinal proteins since they can capture the dynamics of the protein/protein - protein/water water/water interractions. The important question we aim to adress is the following: How is water dynamics coupled to conformational changes, protein folding protein function?

WWW

http://www.physik.fu-berlin.de/en/einrichtungen/ag/ag-bondar/index.html

More Information

[1] Kato, H.E., Zhang, F., Yizhar, O., Ramakrishnan, C., Nishizawa, T., Hirata, K., Ito, J., Aita, Y., Tsukazaki, T., Hayashi, S. and Hegemann, P., 2012. Crystal structure of the channelrhodopsin light-gated cation channel. *Nature, 482*(7385), p.369. doi: doi:10.1016/0263-7855(96)00018-5

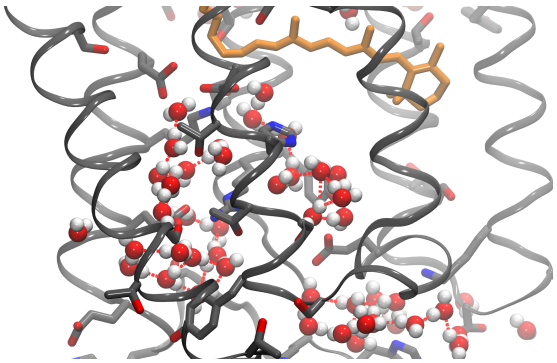

Figure 4: *Close-up view in the Channelrhodospin 2. In the larger vicinity of retinal chromophore numerous water molecules are depicted as red VDW spheres for the oxygen atoms and white VDW spheres for the hydrogen atoms respectively. Red dashed lines show the hydrogen bonds between water molecules, essentially forming chains and internal networks, including protein/water, water/water and mediating protein-protein interractions. The protein's backbone is shown in grey ribbons and essential residues for H-bonding and thus proton transfer are also shown in grey sticks (side chains for simplicity). The retinal chromophore is depicted as orange sticks. Molcular graphics were prepared with VMD[4]*

[2] Volkov, O., Kovalev, K., Polovinkin, V., Borshchevskiy, V., Bamann, C., Astashkin, R., Marin, E., Popov, A., Balandin, T., Willbold, D. and Büldt, G., 2017. Structural insights into ion conduction by channelrhodopsin 2. *Science, 358*(6366), p.eaan8862. doi: doi:10.1126/science.aan8862

[3] Wang, S., Munro, R.A., Shi, L., Kawamura, I., Okitsu, T., Wada, A., Kim, S.Y., Jung, K.H., Brown, L.S. and Ladizhansky, V., 2013. Solid-state NMR spectroscopy structure determination of a lipid-embedded heptahelical membrane protein. *Nature methods, 10*(10), p.1007. doi: doi:10.1038/nmeth.2635

[4] Humphrey, W., Dalke, A. and Schulten, K., 1996. VMD: visual molecular dynamics. *Journal of molecular graphics, 14*(1), pp.33-38. doi: doi:10.1016/0263-7855(96)00018-5

[5] The PyMOL Molecular Graphics System, Version 1.8 Schrödinger, LLC.

Project Partners

L. Brown, University of Guelph, Canada

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