Searching in the deep.

An Intensive search for H-bond motifs.

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In Short

- Transmembrane helices
- Sequence motifs
- Local Dynamics
- Network Analysis

With this proposal we intend to conduct research in order to address questions about pH-sensoring proteins and water channels through the means of mass analyses of transmembrane ?-helical proteins. We will identify and detect amino acid residues motifs vital for ion transport through channel proteins and pH sensing. pH sensing by proteins is a fundamental reaction that typically involves chemical binding and unbinding of protons at titratable amino acid residue sidechains. The mechanisms by which proteins sense pH are poorly described, particularly whether groups that titrate at physiological pH, such as carboxylate and imidazole groups, function as pH sensors only when found in specific sequence and structure motifs.

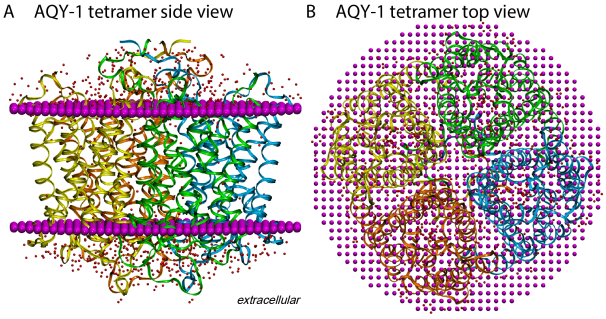

***Figure 1:** The Aquaporin-1 struture. (A) Crystal structure of the Aquaporin-1 tetramer. The protein is shown from a side view. (B) Crystal structure of the Aquaporin-1 tetramer shown from a top view. Protein is depicted in ribbon representation (cyan for chain A, orange for chain B, yellow for chain C, green for chain D). The co-crystallized water molecules are shown as small red spheres. The positions of the headgroups of the lipid bilayer are shown as mauve VDW spheres. Atomic coordinates of the the three dimensional structure were taken from ref1. [1] Molecular graphics were prepared with VMD[4]*

A key motif for proton transfer and protein flexibility and long distance conformational coupling involves the sidechains of Ser or Thr amino acid residues Hydrogen (H)-bonded to side chain of a carboxylate functional group, originating from the sidechain of Aspartate of Glutamate amino acid residues, found in neighboring helices. At the same time the Ser/Thr hydroxyl group can be H-bonded to the carbonyl of the backbone of the amino acid residue located in the i-3, i-4 or i-5 position in the same helix8. Such motifs have been identified in many retinal proteins such as Bacteriorhodopsin (BR)[2], Channelrhodopsin chimera (C1C2)[2], [3], Channelrhodopsin 2 (ChR2) (preliminary data), Anabaena sensory rhodopsin (ASR)[2] and the sodium pump KR2[2]. Rhodopsins are excellent model systems to study the mechanisms of proton and other ion transports. Mutagenesis studies have shown the dramatic changes of the protein function when mutations are introduced, especially in amino acid residues that can H-bond. Thus, the coupling required to move protons or other ions across the cell membrane likely relies on both electrostatic interactions and protein conformational dynamics8. Thus, the question arises, whether groups that titrate at physiological pH, function as pH sensors only when found in specific sequence and structure motifs. Here we address this question by combining data science and molecular dynamics approaches. We established a large hand-curated dataset of three-dimensional structures of membrane proteins, and a subset of representative protein structures solved at high resolution. To identify common motifs of carboxylate and histidine groups, we compute two-dimensional hydrogen-bond maps of all proteins from our dataset, and then extracted from these maps local sub-networks of carboxylate and histidine sidechains. As graphs computed from static protein structures lack information about the local dynamics of the common motifs we found, we will compare interactions in the high-resolution structure of aquaporin with interactions sampled during molecular dynamics.

We will use the HLRN supercomputer cluster to access the local dynamics of the transmembrane helices and the large-scale and long distance communication of the high-complexity H-bond networks and by employing all-atom Molecular Dynamics simulations for the Aquaporin tetramer, embedded in a hydrated lipid membrane environment, resembling high similarity to the native plasma membrane. Additionally, we will investigate the dynamics of the H-bond motifs we have detected in a large database of the Alpha-helical polytopic, containing 130 superfamilies. MD simulations are vital for understanding transmembrane proteins since they can capture the dynamics of the protein/protein - protein/water wa-

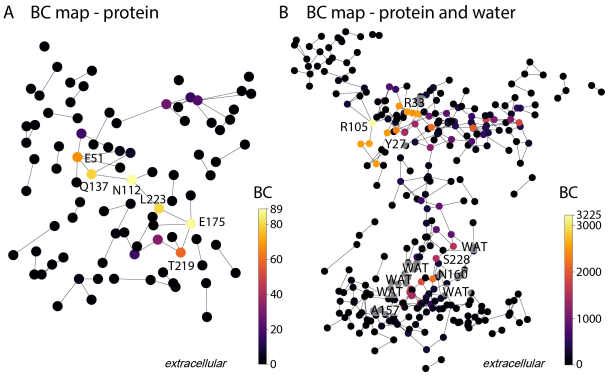

Figure 2: *Betweenness centrality maps for the Aquaporin monomer. (A) BC map for the Aquaporin monomer, for protein-protein interactions only. (B) BC map for the Aquaporin monomer, for protein-protein and protein-water interactions.*

ter/water interractions. The important question we aim to adress is the following: How is water dynamics coupled to conformational changes and protein function?

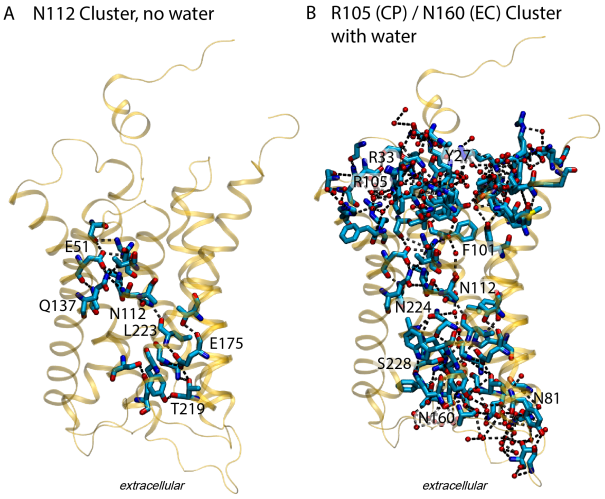

Figure 3: *Atomistic depiction of high-complexity H-bond clusters in the Aquaporin monomer. (A) Molecular graphics for the high BC H-bond cluster when considering protein-protein interactions. (D) Molecular graphics for the high BC H-bond clusters when considering protein-protein and protein-water interactions, found in the cytoplasmic side (top) and the extracellular side (bottom).*

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

More Information

[1] Eriksson, U.K., Fischer, G., Friemann, R., Enkavi, G., Tajkhorshid, E. and Neutze, R., 2013. Subangstrom resolution X-ray structure details aquaporin-water interactions. *Science, 340*(6138), pp.1346-1349. doi: doi:10.1126/science.1234306

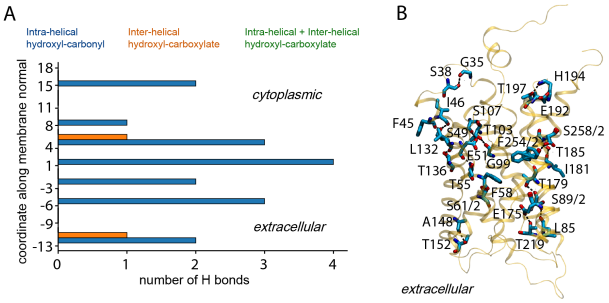

Figure 4: *Distribution of the number of H-bond motifs as a function of their coordinate along the membrane normal for the aquaporin monomer (PDB ID: 3ZOJ[1]). The bars are colored according to the H-bond motif class. Intra-helical hydroxyl-carbonyl H-bonds are blue, Inter-helical hydroxyl-carboxylate H-bonds are orange and Intra-helical combined with Inter-helical hydroxyl-carboxylate H-bonds are green. (B) Molecular graphics for the H-bond motif distribution in the aquaporin monomer.*

[2] Bondar, A.-N.; Smith, J. C., Protonation-state coupled conformational dynamics in reaction mechanisms of channel and pump rhodopsins. 2017. *Photochem. Photobiol., 93*(10), p.1336-1344. doi: doi:10.1111/php.12790

[3] Siemers, M.; Lazaratos, M.; Karathanou, K.; Guerra, F.; Brown, L. S.; Bondar, A. N., Bridge: A Graph-Based Algorithm to Analyze Dynamic H-Bond Networks in Membrane Proteins. 2019, *J Chem Theory Comput , 15*, p.6781-6798. doi: doi:10.1021/acs.jctc.9b00697

[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

Project Partners

L. Brown, University of Guelph, Canada

Funding

DFG Sonderforschungsbereich (SFB) 1078