Structures of the archaerhodopsin-3 transporter reveal that disordering of internal water networks underpins receptor sensitization

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Like other photoreceptor proteins, the archaerhodopsin-3 (AR3) protein has a desensitized, inactive state which is formed in the prolonged absence of light. This dark-adapted (DA) state must be converted to the light-adapted (LA) or resting state, before the protein can generate a proton motive force. In general, receptor desensitization is commonly achieved through reversible covalent or non-covalent modifications, which typically modulate intramolecular bonding networks to stabilize a conformation that is distinct from the active resting or ground state.

Here, we present high-resolution crystal structures of the LA and DA states of AR3, solved to 1.1 Å and 1.3 Å resolution respectively [1]. We observe significant differences between the two states in the dynamics of internal water molecules that are coupled via H-bonds to the retinal Schiff base. These changes modulate the polarity of the environment surrounding the chromophore, influence the relative stability of 13-cis and all-trans retinal isomers and facilitate the conversion between the two forms. These crystal structures also allow us to gain a better understanding of the extent to which the conformation of the chromophore is coupled to the networks of internal water molecules, see Fig. 1. They highlight how minimal displacements of charged and hydrophilic groups within the low dielectric environment of the membrane can induce changes in ligand conformation and vice versa. Finally, these structures also provide high-resolution structural information that increases our understanding of the mechanism of H+ translocation by AR3, and will facilitate the design of further, more efficient AR3 mutants for applications in optogenetics.

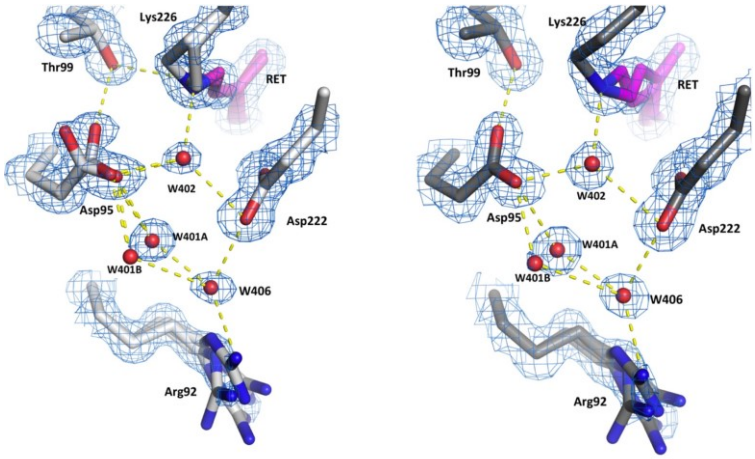

Figure 1. Structures of the region between the retinal SB and Arg92 for the light-adapted (Left) and dark-adapted (Right) AR3. The mF_{obs}-DF_{calc} electron density map (blue mesh) is contoured at ±2.3σ. Water molecules are shown as red spheres and retinal chromophore is colored pink. Predicted hydrogen bonds are shown as dashed yellow lines.

[1] Bada Juarez, J.F., Judge, P.J., Adam, S. et al. (2021). *Nat. Commun*. **12**, 629

Keywords: Membrane Proteins, Photoreceptor, Receptor sensitization, Microbial rhodopsins