XFEL structures of the influenza M2 proton channel at 1.4 Å: room temperature water networks and insights into proton conduction

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The M2 proton channel of influenza A is a drug target that is essential for the reproduction of the flu virus. It is also a model system for the study of selective, unidirectional proton transport across a membrane. The channel is a homotetramer whose gate consists of four histidines and four tryptophans. When the channel is at near-neutral pH, the gating His tetrad has a charge of +2 [1]. Proton transport is thought to occur as the channel cycles from a +2 to a +3 charge state on its His gate. Water wires inside the channel pore have been proposed to play a role in both the conduction of protons to the gating His tetrad and the stabilization of multiple positive charges within the channel. In previous studies[2], lipidic cubic phase crystallization techniques were used to solve high resolution (1.10 Å) structures of M2 under cryogenic data collection conditions at a synchrotron source; in these structures, continuous water wires span the channel pore from the N-terminus of the channel to the gating His residues. Room temperature data collection techniques at a synchrotron source revealed a water network that was more mobile. However, it was not clear if this loss of solvent ordering at room temperature was a result of the increased amount of radiation damage during data collection. Room temperature data were collected to a resolution of 1.4 Å using an X-ray free electron laser (XFEL) to visualize the solvent in the pore of the channel while minimizing the effects of radiation damage. Three different pH conditions were examined: pH 8.0, pH 6.5, and pH 5.5. At pH 5.5, which is the pH condition at which the M2 channel has maximal proton conductance[3], the solvent network within the pore of the channel has a hydrogen bonding network that continuously spans the vertical length of the channel. This is consistent with a Grotthuss mechanism for proton transport. An increasing volume of ordered solvent density, and correspondingly a larger number of pore waters, is observed with decreasing pH. This ordered solvent could act to stabilize the multiple positive charges that build up on the gating His tetrad during the proton transport cycle.

References:
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