# Molecular dynamics simulation of a putative $H^+$ transport pathway in Photosystem II – insights from comparisons of *in silico* results and *in vivo* data

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Given the tightly packed protein matrix of Photosystem II (PSII), specific channels and pathways for the transport of substrate water and products ($H^+$ and $O_2$) to and from the active site $CaMn_4$ cluster have been proposed.[1] To move beyond a static view of the PSII structure, and take into account the all-important dynamic movements of the protein,[2] molecular dynamics (MD) calculations with explicit solvation have been performed on PSII. Such considerations are particularly important for the study of $H^+$ pathways, as $H^+$ do not diffuse as "naked" ions through the protein, but are transported through chains of hydrogen-bonded water molecules and suitable amino acid residues (Grotthuss mechanism). Therefore, investigation of the dynamic interactions between water molecules and the protein are crucial for identifying and understanding the $H^+$ transport pathway within PSII.

A putative $H^+$ exit pathway in PSII was studied through MD simulations. This pathway consists of residues surrounding a dynamically stable water chain leading from the $CaMn_4$ cluster towards the lumen. A number of these residues have previously been shown by *in vivo* mutagenesis to be important for $H^+$ transport, and they are found to directly interact with this water chain, consistent with the Grotthuss mechanism. Where there is a break in the water chain due to steric constraints, the involvement of a suitable residue ensured a continued $H^+$ path. Analysis of the MD trajectories allowed the study of *e.g.* bridging water molecules connecting important residues, the stability of the water chain, and the evolution of the chain and its interactions with the surrounding residues over time. For instance, the well-known D1-D61 and -E65 residues were found to be part of this pathway, and they were bridged by one or more hydrogen-bonded water molecules in stable and high occupancy positions over 95% of the time. This is in agreement with their proposed roles in $H^+$ transport.

Furthermore, a number of residues not previously regarded as being involved in $H^+$ transport have been identified as being of significant structural and perhaps electrostatic importance. In particular, the residue D1-R334 was found to be critical in the maintenance of the well-ordered water chain, as well as an efficient connection between the D61 and E65 residues. This was exemplified by further MD simulations performed on PSII mutated *in silico* at this and other positions. Very significant changes in the stability of the water chain and the conformations of the residues involved in the $H^+$ pathway were observed in these mutants. These results could be correlated with available studies *in vivo* mutants, and reconcile data that have until now seemed anomalous.[3] The connection between the $H^+$ pathway with a proposed group of residues acting as a localised buffer for receiving $H^+$ released during water oxidation[4] is also discussed. Overall, comparisons of our MD results of *in silico* mutants with *in vivo* mutagenesis data have revealed some unifying themes in the molecular basis of the roles played by different residues involved in $H^+$ transport.

Keywords: Photosystem II, molecular dynamics, proton pathway, channels, mutagenesis.

[1] Ho & Styring (2008) *Biochim. Biophys. Acta*, **1777**, 140-153.
[2] Ho (2008) *Photosynth. Res.*, **98**, 503-522.
[3] Li & Burnap (2002) *Photosynth. Res.*, **72**, 191-202.
[4] Shutova *et al.* (2007) *Biochim. Biophys. Acta*, **1767**, 434-440.