**Transmembrane proton pumps: Characterizing the protonation microstates in proton loading sites and transfer paths**

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The MCCE program generates a Boltzmann distribution of protonation and tautomer states for all residues within a protein. MCCE also keeps side chain rotamers at equilibrium with the protonation states. The main constraint is that the backbone is rigid.

Average protonation states are routinely used to obtain residue $pK_a$s by MCCE and other similar programs that use MC sampling. New functionality within MCCE allows analysis of the millions of accepted microstates in a converged simulation. The distribution of (1) the total protein charge, (2) proton distribution amongst the protonatable residues in microstates with the same change (tautomer) and the (3) system enthalpy and (4) correlation of protonation states of groups of residues will be described for lysozyme (for a small protein) and Complex I (a large proton pump) and cytochrome c oxidase (a smaller proton pump).

We find that there is a large enthalpy distribution amongst the accepted microstates so that often the proton distribution with the lowest enthalpy is not the one that is most often found. This poses interesting questions about the best protonation states to be used to seed MD trajectories. The movement of protons with a given charge state will be shown to play a functional role in proton transport processes.