Tackling the Many Ferredoxins Problem in *Chlamydomonas Reinhardtii* with Equilibrium and Enhanced Sampling Molecular Simulations

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Ferredoxins (Fds) are found in all organisms from bacteria, archaea to higher eukaryotes and function to carry electrons between donor and acceptor pairs. Of the nine Fds identified in *Chlamydomonas Reinhardtii*, all feature an iron-sulfur cluster necessary for electron transport, though variations in amino acid sequence may signify unique donor or acceptor specificities. Through molecular simulation, our aim is to rank the different Fds based on their Photosystem I (PSI) binding affinities and binding stability. We use AlphaFold to build Fd models, fill in the structural gaps from the literature and dock these Fds to PSI combining high- and low-resolution information to create bound models for each Fd isoform to the thylakoid-embedded *Chlamydomonas Reinhardtii* PSI. By determining the non-equilibrium work associated with the bound pose, as well as equilibrium simulations, we can identify differences between individual Fd isoforms. Fd1 and Fd2 show smaller deviations from the bound pose during equilibrium simulations, and also had the smallest energetic cost to force the bound pose, suggesting that these isoforms preferentially bind to PSI. By comparison, other Fds have large deviations (> 3Å) from the bound pose in unrestrained simulation, and thus we predict are less favorable to bind to PSI. These qualitative findings from molecular simulations agree with ongoing in-vitro experiments and aid towards future experimental design. To gain insight into the thermodynamics of PSI-Fd binding, we calculate absolute binding free energies using Binding Free Energy Estimator 2 (BFEE2), an enhanced sampling technique. Overall, we demonstrate the reciprocal relationship between molecular simulations and in-vitro experiments, to map the genetic difference between different Fd isoforms to their phenotypic responses during photosynthesis.