Computational Prediction and Experimental Validation of pKa Shifts in the Q57D Mutant of *Lepidium virginicum*WSCP: Implications for Tailored Chlorophyll Protein Design

Nicolas de Cordoba[1], Mohamed A. Elrefaiy[2], Andres S Urbina[1],
Gehan A. Ranepura[3], M. R. Gunner[3], Lyudmila Slipchenko[1], Doran Raccah[2] and Mike Reppert[1]

[1] Department of Chemistry, Purdue University. West Lafayette, IN 47907, USA.
[2]Department of Chemistry, The University of Texas at Austin, Austin, TX 78712, USA3.
[3]Department of Physics, City College of New York, New York, NY 10031, USA.

Engineering chlorophyll-binding proteins with customized optical properties is essential for advancing photobiology and renewable energy applications. Achieving this goal requires a thorough understanding of how the chlorophyll's local environment within protein complexes influences its spectral characteristics. A previous study demonstrated that a simple electrostatic model accurately predicted mutation-induced frequency shifts for eight out of nine single-point mutants of the Water-Soluble Chlorophyll Protein from *Lepidium virginicum* (LvWSCP). However, the Q57D mutant exhibited a small red shift typical of neutral amino acids instead of the anticipated large blue shift expected from negatively charged residues.

To elucidate the anomalous behavior of the Q57D mutant, we integrated computational predictions with experimental validations. Using Multi-Conformation Continuum Electrostatics (MCCE), we predicted the pKa values of ionizable residues and employed a simple electrostatic model using the Charge Density Coupling (CDC) method to calculate the expected absorption peak shifts. Our computational models predicted that all four Q57D residues in the LvWSCP tetramer have shifted pKa values, leading to their protonation at physiological pH (~7). We experimentally confirmed the protonation of all four Q57D residues at physiological pH through site-directed mutagenesis and spectroscopic analyses, including absorption and fluorescence spectroscopy.

Our findings underscore the critical importance of considering local pH environments and pKa shifts in engineering light harvesting proteins. By demonstrating the effectiveness of a combined computational and experimental approach, our study paves the way for the rational design of chlorophyll-binding proteins with tailored optical properties.