A multi-scale computational investigation of protein-biliverdin interactions guiding mutations toward red-shifted fluorescence

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Phytochromes are a large family of photoreceptor proteins containing biliverdin within their chromophore-binding cavity. Biliverdin acts as a chromophore by absorbing light in the red to far-red region of the spectrum. This property enables phytochromes to reversibly switch between photostates with absorption maxima differing by 50-70 nm, depending on the chromophore configuration[1]. Biliverdin can adopt two distinct conformations within the protein binding pocket, known as the extended and compact forms (Figure 1). Understanding the conformational transition between these states and identifying the key amino acids stabilizing each conformation are important for engineering phytochromes with enhanced red-shifted absorption, a desirable feature for deep-tissue imaging applications[2]. However, capturing such large-scale conformational transitions at atomistic resolution is computationally demanding. To address this challenge, we developed a hybrid all-atom/coarse-grained force field combining the ff14SB force field for the chromophore and the binding cavity, with the SIRAH coarse-grained force field for the remaining protein.

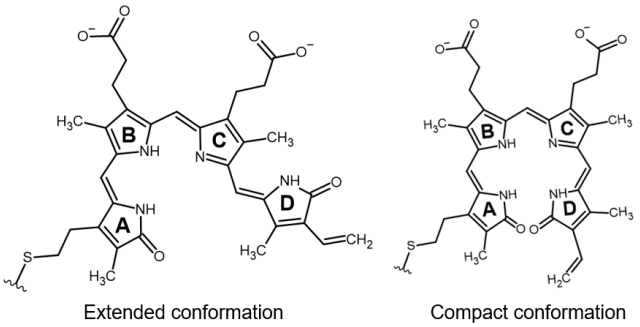

Figure 1. 2D structures of biliverdin extended and compact conformations.

By using umbrella sampling and adaptive steered molecular dynamics, we reconstructed the free energy landscape associated with the extended-to-compact biliverdin transition. These simulations allowed us to identify specific amino acid residues that stabilize each chromophore conformation. Based on these insights, targeted mutations were designed to modulate the chromophore environment and favor red-shifted states. Finally, absorption spectra were computed for the mutated protein structures using QM/MM calculations, enabling direct assessment of the spectral impact of each mutation. This integrative computational strategy led to the identification of candidate phytochrome variants exhibiting absorption in the near-infrared region, highlighting a pathway for the design of optimized photoreceptors for bioimaging applications.

Bibliography:

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