Engineering Light-Responsive Protein Nanomachines: Characterization of a NIR Bacteriophytochrome

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Área temática: E. Nanobiointerfases y procesos biológicos

Genetic and protein engineering have transformed the pharmaceutical industry by enabling the precise design and large-scale production of safer, more effective, and highly targeted therapies. Proteins can be viewed as responsive nanoparticles, given their nanometric dimensions and ability to undergo structural changes in response to external stimuli. In this framework, optogenetics has emerged as a powerful branch of protein engineering, enabling precise spatiotemporal control of cellular processes using light-sensitive proteins operating as biological on/off switches. Most current tools rely on blue (370–500 nm) or green (~560 nm) light, limited by poor tissue penetration and phototoxic effects. Achieving deep-tissue control requires photoreceptors responsive to near-infrared (NIR, 650–900 nm) light. Bacteriophytochromes (BphPs) are particularly promising, operating within the NIR window using biliverdin as a chromophore. They undergo reversible photoconversion between a red-absorbing Pr state ($\lambda_{max} \approx$ 700–720 nm) and a far-red-absorbing Pfr state ($\lambda_{max} \approx$ 740–760 nm). This photoconversion is driven by nanoscale structural dynamics: a single photon induces biliverdin isomerization at the ångström scale, propagating conformational changes across domains spanning several nanometers and ultimately triggering a biological response. In bathy-type BphPs, the Pfr state is thermodynamically favored, an advantageous feature for optogenetic applications. However, limited high-resolution structural data have hindered their rational engineering. Here, we present the characterization of *Rhizobium leguminosarum* BphP (*Rle*BphP) as a novel NIR optogenetic candidate. Both the full-length protein and its photosensory module (PSM) were analyzed combining photochemical, biophysical, and structural methods. UV–Vis spectroscopy confirmed efficient chromophore incorporation, reversible photoconversion, and bathy-type dark reversion. Static and dynamic light scattering and mass photometry revealed a concentration-dependent monomer–dimer equilibrium. Cryo-EM and X-ray diffraction studies are underway, with preliminary results supporting suitability for high-resolution analysis. These findings establish *Rle*BphP as a well-characterized and structurally tractable BphP governed by nanoscale conformational dynamics, providing a foundation for its engineering as a next-generation NIR optogenetic tool with broad biotechnological and biomedical applications.