Structural Characterization of Bacterial Red Light Photoreceptors by Atomic Force Microscopy

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Bacteriophytochromes (BphPs) are red-light photoreceptors found in various photosynthetic and non-photosynthetic bacteria. BphPs are composed of a photosensory core module (PCM) that consists of three domains termed PAS, GAF, and PHY along with a signal effector domain, usually a histidine kinase (HK). BphPs utilize covalently attached biliverdin chromophore (BV), a linear tetrapyrrole, to detect light. BV enables photoconversion between red (Pr) and far-red (Pfr) light-absorbing states, which results in global structural changes within the protein. We have Scanning Probe Microscopy (SPM) methods to examine the structure of the PCM and the intact BphP, including HK, in the Pr and Pfr states in a biologically relevant environment; a unique advantage of SPM. Specifically, we have developed a method utilizing atomic force microscopy (AFM) at NEIU and high speed-AFM through a collaboration with The University of Chicago Materials Research Science and Engineering Center to characterize the domain structure of the truncated and intact phytochrome from *S. aurantiaca* (SaBphP2) in its respective Pr and Pfr states. Multiple orientations of BphPs have been observed on the surface. Close comparison of the truncated SaBphP2 to the intact SaBphP2 shows a clear difference in protein length and size. As expected, the intact protein is longer due to the HK that is missing in the PCM construct. Individual dimers of SaBphP2 reveal extensive conformational changes when compared in the Pr and Pfr states. The size, orientation, and structure of SaBphP2 have been further compared to the published cryo-electron microscopy (EM) structures of the related, *D. radiodurans* BphP, which shows similar conformational changes between the Pr and Pfr states.