

From old find new: Unravelling phytochrome structural dynamics from single-particle cryo-EM using cryoSPHERE

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Light-driven processes are widely recognized as the foundation to signalling cascades essential for sustaining life. Light-transducing proteins like phytochromes are the start point of this mechanism and thus bare a highly complex mechanism propagating small conformational changes via the whole structure activating the following reaction interface. Over the last decades, many experimental studies have tried to decipher the mechanism behind light-activation, but all have a common conundrum: limited investigation of structural dynamics of big proteins and protein complexes. While crystallography results in static structures, NMR is limited by size, which in the case of phytochromes demands for small constructs omitting the bigger picture and cryo-EM was previously only looking at average structures. To overcome this obstacle, cryoSPHERE has been developed to determine structural heterogeneity from single cryo-EM images.

Here, we provide a thorough investigation of the conformational ensemble of phytochromes reconstructed in cryoSPHERE. This enables us to highlight the highly dynamic behaviour of these proteins while rationalizing the previously published cryo-EM averaged conformations. Additionally, cryoSPHERE also facilitates the study of full length phytochromes, which is challenging in averaged cryo-EM maps due to the high flexibility of the output domain and thus low resolution. This enables for the first time an in-depth analysis of the output module in direct interaction with the photosensory module. Thus, cryoSPHERE allows for a more detailed understanding of phytochromes, their conformational mechanisms and structural features providing new insights into the light transduction phenomenon.