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Phytochrome function: structural changes and protonation dynamics

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Phytochromes are red/far-red photochromic biliprotein light sensors that act as master regulators of plant development. They regulate the expression of ~20% of all plant genes, controlling for example germination, pigment production, stem extension and flowering time. Phytochromes are also known in prokaryotes, Cph1 from *Synechocystis*, for example, providing a valuable model for structure/functional studies. De/reprotonation of the chromophore, neighboring histidines and waters is important in both photon absorption and the subsequent photocycle. Following numerous successful X-ray diffraction, solid-state NMR and vibrational spectroscopic studies, we are interested in exploiting neutron diffraction (ND) to investigate protonation dynamics and simultaneously avoid radiation damage in 3D studies of the molecular action mechanism. We have proof-of-principle that phytochrome structure studies can be extended to ND and that Cph1 holoprotein can be deuterated effectively. We hope that it will be possible to generate appropriately deuterated crystals to allow ND at better than 2 Å resolution with low incoherent scattering, and thereby the 3D structure including the positions of functionally-important hydrogens/protons to be solved at near-atomic resolution and with minimal radiation damage. The project will thus provide important, unique information regarding the role of protonation dynamics in phytochrome function in particular and protein function in general.

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