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# Integrative time-resolved structural biology of the light-regulated transcription factor EL222

## Content

The bacterial LOV (light-oxygen-voltage) domain-containing protein EL222 is a natively photocontrolled transcription factor that has found practical use in optogenetic applications. Excitation of the embedded flavin mononucleotide (FMN) cofactor triggers conformational changes that ultimately lead to EL222 association with DNA and gene expression. However, our knowledge of the light-adapted state(s) and the molecular mechanism underlying the transition between dark and lit states remains incomplete. Here we employ a solution-state integrative structural biology approach combining infrared spectroscopy, nuclear magnetic resonance (NMR) spectroscopy, small-angle neutron scattering (SANS), and quantum mechanics (QM) simulations, to unveil the structural evolution of EL222 on timescales ranging from femtoseconds to hours upon blue-light absorption. Transient mid-infrared data and QM cluster calculations including the FMN chromophore and key nearby residues resolved intermediate species up to millisecond delays. NMR experiments under continuous illumination identified two distinct monomeric conformations of EL222 with slow conversion kinetics. Finally, time-resolved SANS measurements revealed the reversible assembly of EL222 into large oligomers in a concentration- and irradiance-dependent manner. Taken together, our results illustrate the utility of complementary methods to disentangle complex photoinduced structural dynamics of proteins across multiple length and time scales.

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