

Structural Basis of Photoactivation of a Bacterial DNA-binding Protein

The ability of organisms to sense and respond to light availability is of great interest to those wishing to exploit these mechanisms in the areas of biotechnology and agriculture. A particular light sensing domain, termed the light-oxygen-voltage (LOV) domain, is found in plants, animals, and bacteria to control a wide variety of biological processes¹⁻⁵. LOV domains oftentimes signal to a variety of attached effector domains including kinases, F-boxes, and DNA-binding domains. This leads to the questions: How does light stimulation of the LOV domain cause activation to such a wide variety of effector domains? And is there a shared mechanism? Understanding the molecular mechanism of how organisms use LOV domains might enable engineered light-induced control of pathways.

E222 is a two-domain 24-kDa protein consisting of a light-sensing LOV domain and a LuxR-like DNA binding domain. The LOV domain has the traditional PAS fold of $\beta_2\alpha_4\beta_3$ surrounding a central flavin (FMN) chromophore. Upon blue-light stimulation at 450 nm, electrons in the FMN isoalloxazine ring delocalize and a covalent bond is formed between the FMN C4a carbon and the conserved Cys75 in the *E222* LOV domain.

Diffraction data were collected at SSRL Beam Line 9-1. Since structures of isolated LOV and HTH domains from other organisms were known, we used molecular replacement to solve the structure of the first naturally occurring sensor/effector pair. The J α linker helix was not used in the initial search but subsequently built by hand into positive difference density that illustrated a connection between the two domains. The structure was iteratively refined to a resolution of 2.1 Å.

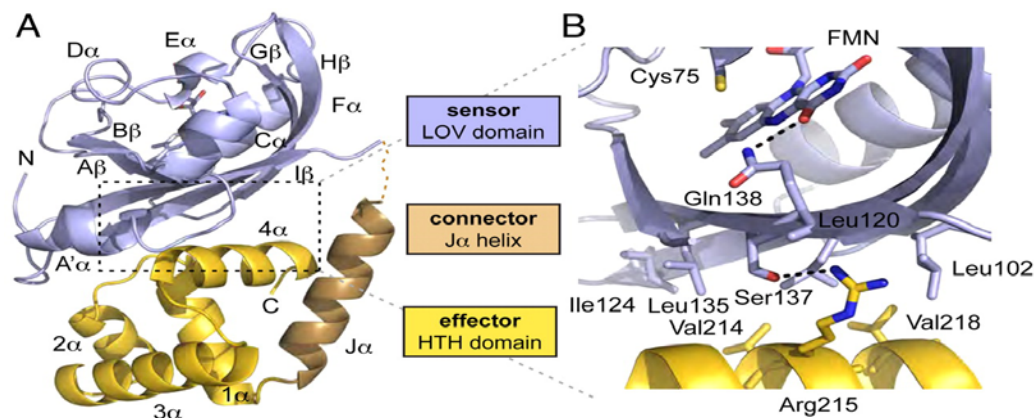


Fig. 1: The dark-state crystal structure of *E222* reveals extensive LOV-HTH interactions predicted to inhibit HTH DNA-binding activity. (A) Overview of *E222* structure, highlighting locations of the LOV (blue) and HTH (gold) domains and the J α -helix (bronze) connecting the two. The LOV domain binds to the HTH domain using the LOV β -sheet surface. (B) Expansion of the LOV/HTH interface as observed in chain A of the *E222* structure, as indicated by the boxed region in A. To bind the LOV domain, the HTH domain presents the 1 α -2 α linker and 4 α -helix, the latter of which typically provides a dimerization interface for DNA-bound HTH domains. Thus sequestered, the 4 α -helix is unable to participate in HTH/HTH interactions observed in many DNA-bound HTH complexes.

LuxR-like HTH domains dimerize via their 4 α helices and subsequently bind to the major groove of DNA via their DNA recognition helix 3 α . In *E222*, the HTH domain is unable to dimerize in the dark state because it interacts with the LOV domain β -sheet (Fig. 1A). The interface between the two domains is quite hydrophobic with one hydrogen bond between Ser137 and Arg215 (Fig. 1B). In elucidating how light stimulation of the LOV domain can traverse the LOV domain to affect the HTH domain, we found a hydrogen bond network connecting the LOV domain to the HTH domain. In the dark state, the FMN carbonyl is hydrogen bonding with Gln138. The adjacent residue, Ser137, hydrogen bonds to Arg215 on the HTH domain. Thus it is plausible that the light-induced Cys75-FMN adduct propagates steric and electronic changes through the β -sheet to modulate LOV domain interaction with the HTH domain.

In order to confirm the FMN β sheet signaling mechanism for other LOV domains, we analyzed currently deposited LOV domain-containing structures for interactions with the LOV core. All external LOV domain interactions of at least 5 Å were highlighted on the LOV core (Fig. 2). In all cases external portions interact with the LOV domain β sheet in a similar fashion.

Furthermore, based on our crystal structure, we proposed a site-directed single amino acid mutation at the β sheet interface that we predicted would disrupt the interaction between the LOV domain β sheet and the HTH domain 4 α helix. Unlike the wild type, which requires light for DNA binding, this engineered construct shows DNA binding even in the dark and is this constitutively active.

This work has implications for having a light-controlled expression system. Using light-controlled proteins to modulate cell activity is part of an emerging field called optogenetics. In addition to the obvious research uses, the concept is that light-based therapy might one day supplement or completely replace pills.

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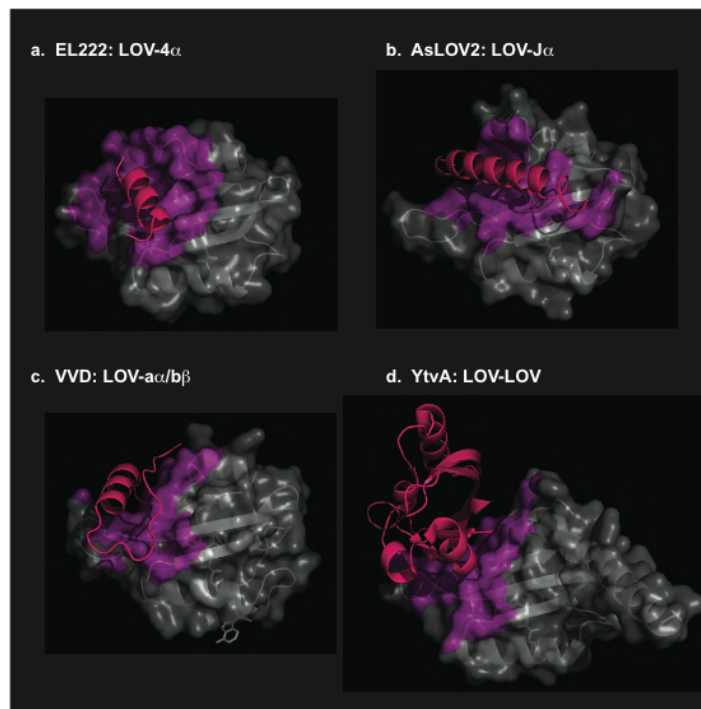


Fig. 2: Interactions of various LOV domain β sheets with α helices (a-c) or in a dimer with a second LOV domain β sheet (d).

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