Signaling Transduction Pathway of AsLOV2 Revealed by Time-Resolved Vibrational Spectroscopy

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Transient vibrational spectroscopy was used to investigate the photoactivation mechanism of LOV2 from *Avena sativa* (AsLOV2). Ultrafast relaxation reveals singlet to triplet conversion of the flavin chromophore. Slower microsecond components represent formation of the cysteinyl-flavin adduct and unfolding of the Jα helix.

The Light, Oxygen, and Voltage sensor domains (LOV) compose a major class of biological photoreceptors in plants responsible for phototropism, cellular growth regulation, and chloroplast motility[1]. LOV2, in particular, is known to undergo largescale conformational changes following blue-light excitation of the central Flavin mononucleotide chromophore (FMN). The prevalent mechanism implicates transduction of ultrafast sidechain movements near the chomophore, through a mediating ß-sheet, to the Jα helix domain docked on the protein surface[2]. To date, no experimental effort has clearly captured the complete transient light-induced response of AsLOV2 and a unified signal transduction mechanism is still under debate. We report femto-to microsecond transient vibrational spectra that reveal dynamic components of 2 ns, 10 and 243 µs, and asymptote that does not evolve on the experimental time window (Figure 1). We assign these to singlet to triplet conversion of the FMN chromophore, formation of the Cys-FMN adduct, and unfolding of the Jα helix. A strong match between the asymptotic component and the steady state difference spectra indicates our results fully capture the light-induced signaling response of AsLOV2. Transient 2DIR experiments are currently underway to identify possible coupling between participating vibrational modes.

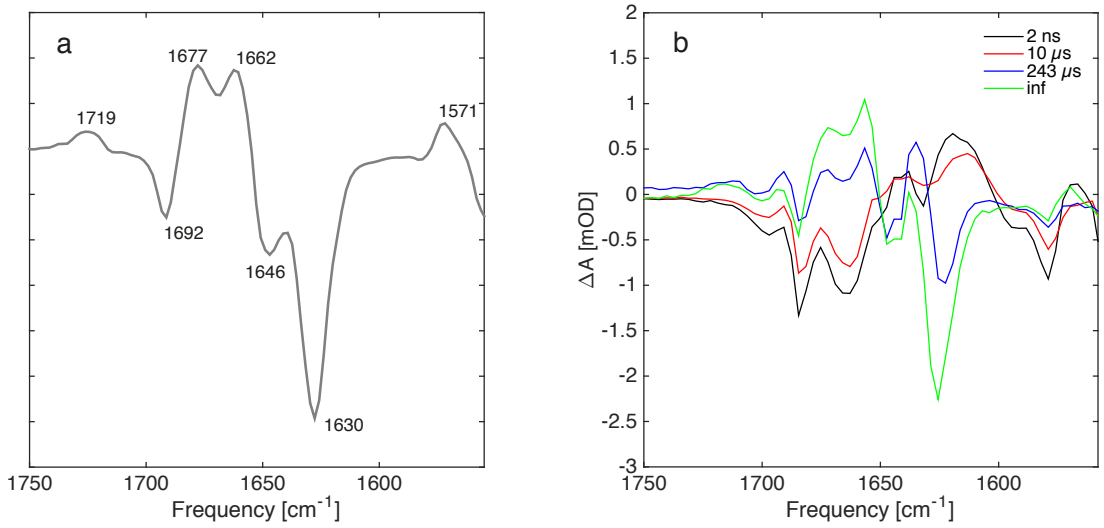

Figure 1. (a) Steady state light minus dark FTIR spectra of AsLOV in D2O. The strongly negative band at 1630 cm^{-1} is assigned to loss of the Jα helix. (b) EADS determined from global analysis of transient vibrational spectra.

[1]Christie, J. M. 2007. Phototropin Blue-Light Receptors. Annu. Rev. Plant Biol. 58:21–45
[2]Harper, S. M., L. C. Neil, and K. H. Gardner. 2003. Structural basis of a phototropin light switch. Science. 301:1541–1544.