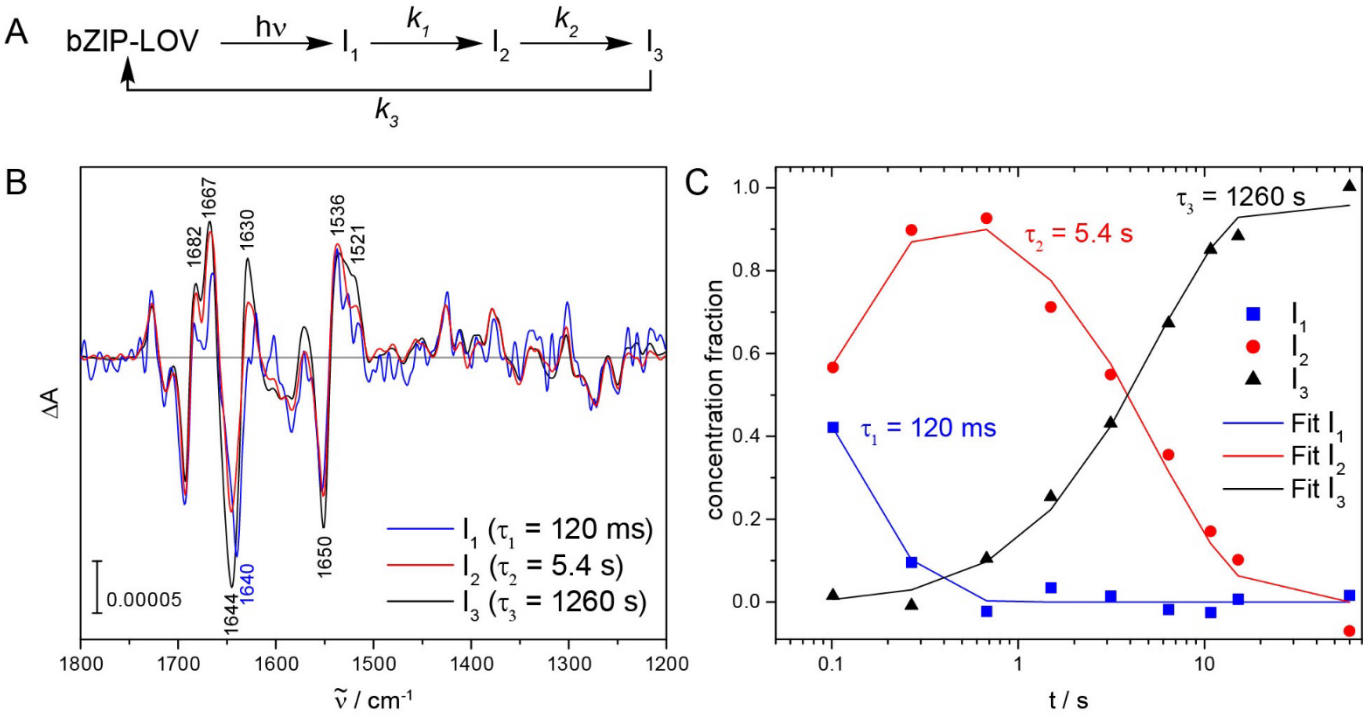
Supplementary Material

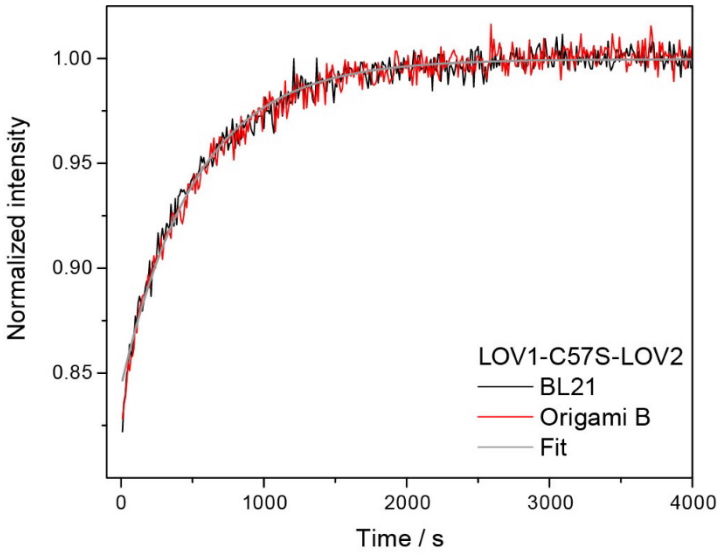
Time-resolved Infrared Difference Spectroscopy in Cells: Response of the Basic Region Leucine Zipper of Aureochrome

1 Time-resolved FTIR spectroscopy on bZIP-LOV in vitro



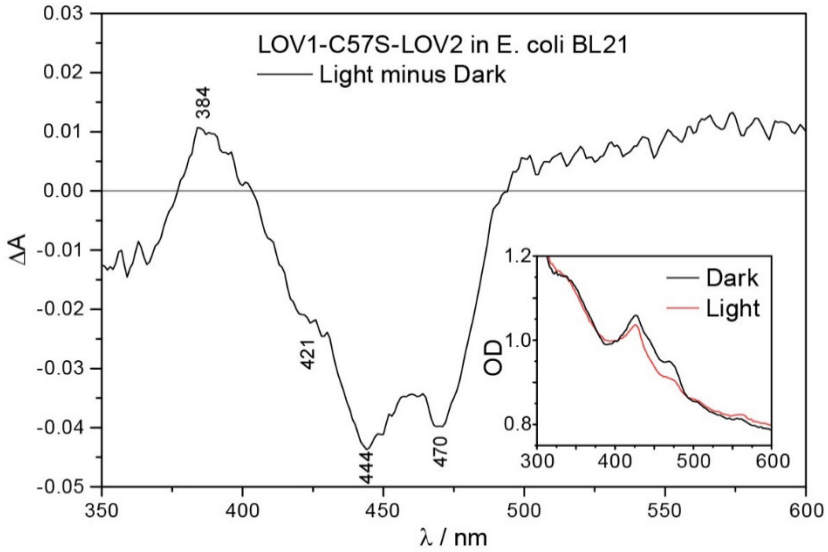
Supplementary Figure S1. Species associated difference spectra (SADS) and concentration profiles of the intermediates from the global fit of time-resolved infrared difference spectra of bZIP-LOV *in vitro*. (A) The kinetic model of sequential first order reactions used for global fit analysis contains three intermediates. (B) Intermediate I_1 exhibits fingerprint of LOV photoconversion including a signal at 1640 (-) cm^{-1} associated to the Jα helix unfolding (Herman et al., 2013) but lacks any signal at 1630 (+) cm^{-1}. Intermediate I_2 shows an increase in contributions at 1682 (+), 1667 (+) and 1630 (+) cm^{-1} compared to I_1. We assign these changes to the signal progression within the LOV core including some reorganization of the β-sheet. Intermediate I_3 shows a fully evolved signal at 1630 (+) cm^{-1}, which has been assigned to the formation of the light dimer of LOV (Herman and Kottke, 2015). Moreover, the additional negative contribution at 1644 (-) cm^{-1} compared to I_2, can be assigned to the partial unfolding of the bZIP (Banerjee et al., 2016). (C) Concentration profiles of the intermediates show mixed concentration fractions of I_1 and I_2 at early time points. Resulting time constants of reactions from I_1 to I_2 and I_2 to I_3 are τ_1 = 120 ms and τ_2= 5.4 s, respectively. The dark state recovery τ_3 is much slower than the time window of the measurements of 60 s, which is in agreement with the time constant of τ = 1560 s found in literature (Banerjee et al., 2016).

2 Kinetics of Recovery of LOV1-C57S-LOV2 by In-Cell Fluorescence Spectroscopy



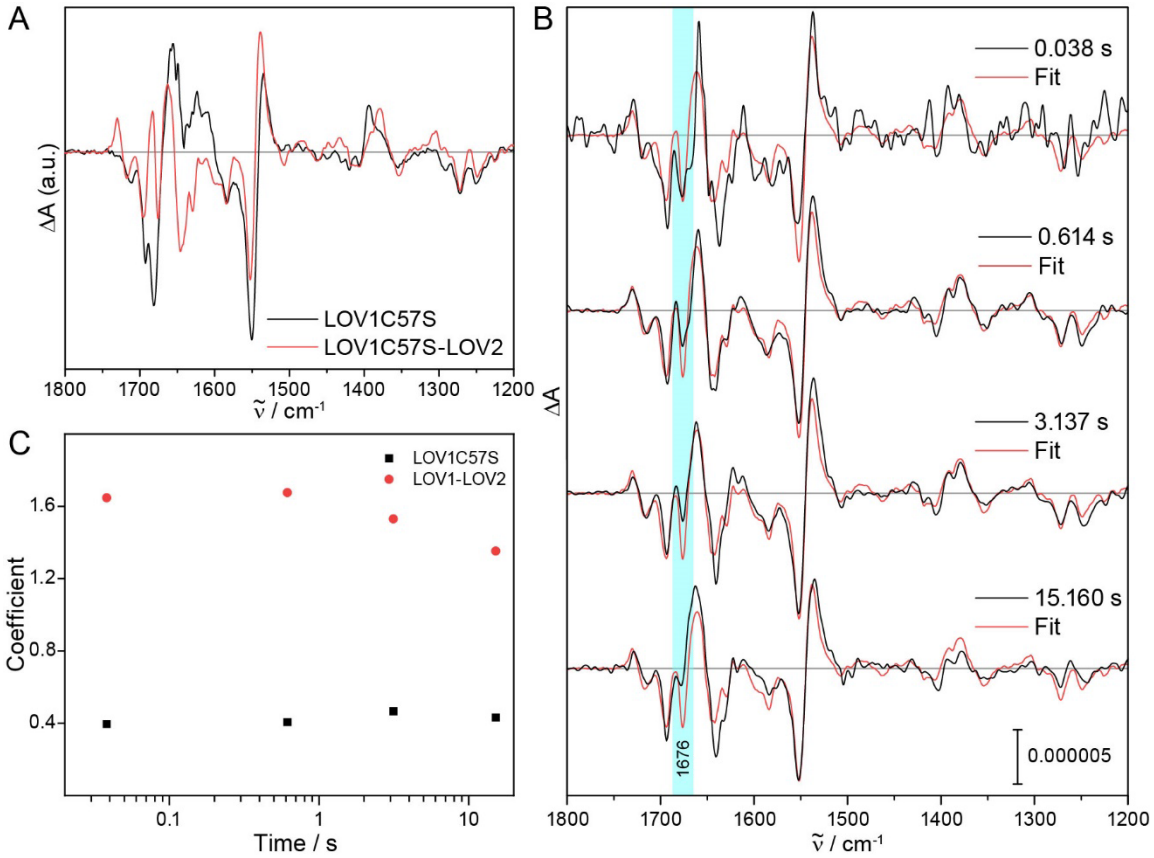
Supplementary Figure S2. Fluorescence recovery kinetics of LOV1-C57S-LOV2 in *E. coli* BL21 and *E. coli* Origami B cells. A monoexponential fit reveals time constants of recovery of 473 (±72) s and 486 (±60) s in *E. coli* BL21 and *E. coli* Origami B cells, respectively. Full recovery of the sample was estimated from the time constant to be reached at about 1500 s. The less-reducing intracellular redox potential of the *E. coli* strain Origami B (Prinz et al., 1997) did not affect the fluorescence recovery kinetics so that we proceeded with BL21 cells. E. *coli* BL21 (DE3) or Origami B (DE3) cells expressing LOV1-C57S-LOV2 were prepared for in-cell fluorescence spectroscopy and fluorescence recovery kinetics were recorded as described previously (Goett-Zink et al., 2020). The fluorescence recovery kinetics were recorded with excitation at 448 nm for 1 s every 10 s at 495 nm and at 20 °C after 100 ms blue-light illumination of the cells expressing LOV1-C57S-LOV2. A linear time-dependent fluorescence decay of LOV1-C57S-LOV2 in Origami B cells was corrected by fitting and subtraction.

3 In-Cell UV/vis Spectroscopy on LOV1-C57S-LOV2



Supplementary Figure S3. In-cell UV-vis spectra of LOV1-C57S-LOV2 in *E. coli* BL21 cells before and after illumination. The difference spectrum was obtained by subtracting dark and light spectra of the optical density before and after illumination with blue-light for 2 s (inset). Signals of the FMN photoreaction to the adduct are detected at 384 (+) nm, 444 (-) nm and 470 (-) nm. The broad signal between 500 nm and 600 nm is attributed to the formation of the flavin neutral radical in the LOV1-C57S domain (Kottke et al., 2003). Cells expressing LOV1-C57S-LOV2 were prepared as described previously (Goett-Zink et al., 2020). A cell suspension in 50 mM phosphate buffer, pH 8, 300 mM NaCl with an OD_{600} of 40-60 was placed in a fluorescence cuvette with a magnetic stirrer bar rotating. Spectra were recorded with a UV-vis spectrometer (UV-2450, Shimadzu) equipped with an Ulbricht sphere to reduce scattering before and after 2 s illumination with an LED (455 nm, 14 $mW \cdot cm^{-2}$, Phillips Lumileds).

4 Time-Resolved In-Cell FTIR spectroscopy on LOV1-C57S-LOV2



Supplementary Figure S4. Data analysis of time-resolved ICIRD spectra of LOV1-C57S-LOV2 in *E. coli* cells. (A) Difference spectra of LOV1-C57S-LOV2-Jα were simulated via a linear combination of two steady-state spectra of LOV1-C57S and LOV1-C57S-LOV2-Jα representing LOV1 radical formation and LOV2 blue-light response, respectively. Reference spectra were obtained from (Pfeifer et al., 2010). (B) Simulations agree well with recorded spectra with only slight deviations in the millisecond time range from 40 ms to 614 ms. From 3 s to 15 s, the signal at 1676 (-) cm^{-1} deviates between the simulations and recorded spectra indicating an influence of the intracellular environment on the blue-light response of LOV1-C57S-LOV2. (C) Coefficients of the linear combination of spectra show that deviations cannot be explained by the LOV1 radical decay since fractions of radical remain constant. The decay of the photo adduct of LOV2 is observed already after 3 s.

5 Supplementary References

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