

Supporting Information

Signal Propagation in LOV-Based Multidomain Proteins: Time-Resolved Infrared Spectroscopy Reveals the Complete Photocycle of YF1 and PAL

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Sequences

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BsLOV ----- 0
YF1 ----- 0
PAL MKVNRPAERASFGSFVLDAGSARFVGSDELALVLFAPGDVLTFAVVLHLHPDDRLEWQAGLQRCLATGRPVVNHLLTAEAEPRPAMTTLTALTEQDRVRAVTGVIDLSDRVRRRA 120

BsLOV -----MHHH 4
YF1 -----MASFQSFQIPGQLE-----VIKK 18
PAL TEAEIRQAVRAAAATRSEIDQAKGIVMAAFDVEDADQAFALKWHSSQSNRKLRLDLATGMIEGLAAANSALPLRRRLSTVFTDMGCPAPSTKGTVPVPTDGLPPTSGLIPTALLPGILTR 240

BsLOV HHHGGSGSVVITDPALEDNPIVYVNOQGFVQMTGYETEELGKNCRFQGGKHTDPAEVDNIRITALQNKPEPTVQIQNYKKDGTMEFWEELNIDPMEIED--KTYFVGIQNDIT----- 113
YF1 ALDHVRVGVVITDPALEDNPIVYVNOQGFVQMTGYETEELGKNCRFQGGKHTDPAEVDNIRITALQNKPEPTVQIQNYKKDGTMEFWEELNIDPMEIED--KTYFVGIQNDITEHQQTQARL 136
PAL AAHDASVAITVADVTPADQPLVYANPAFERLTGYAAAEVLRGNCRFQAESGDPHERSAIRSAIANGDAVTTLIRNFRQDGHAFWNEFHLSFVRNGAGRVTHYIGVQLDVTERVERDQQL 360

BsLOV ----- 113
YF1 QELQSELVHVSRLSAMGEMASALAHENLQPLAAISNYMKGSRLLAGSSDPNTPKVESALDRAAEQALRAGQIIIRRLRDFVARGESEKRVESLSKLIIEAGALGLAGAREQNVQLRFSLD 256
PAL EQLASLEHHHHHH----- 373

BsLOV ----- 113
YF1 PGADLVLADRVQIQVVLVNLFRNALEAMAQSQRRELVTNTPAADDMIIEVEVSDTGSQFQDDVIPNLFQTFFTTKDTGMVGLSISRSIIEAHGGRMWAESNASGGATFRFTLPAADENL 376
PAL ----- 373

BsLOV ----- 113
YF1 EHHHHHHHH 385
PAL ----- 373

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Fig. S1 Sequential alignment of *BsLOV*, *YF1*, and *PAL*. Bold: conserved residues for all three proteins. Purple: β -sheet. Orange: α -helical. Marked in red: F328 (*PAL*) and the corresponding position in *BsLOV* and *YF1*.

Global Fitting

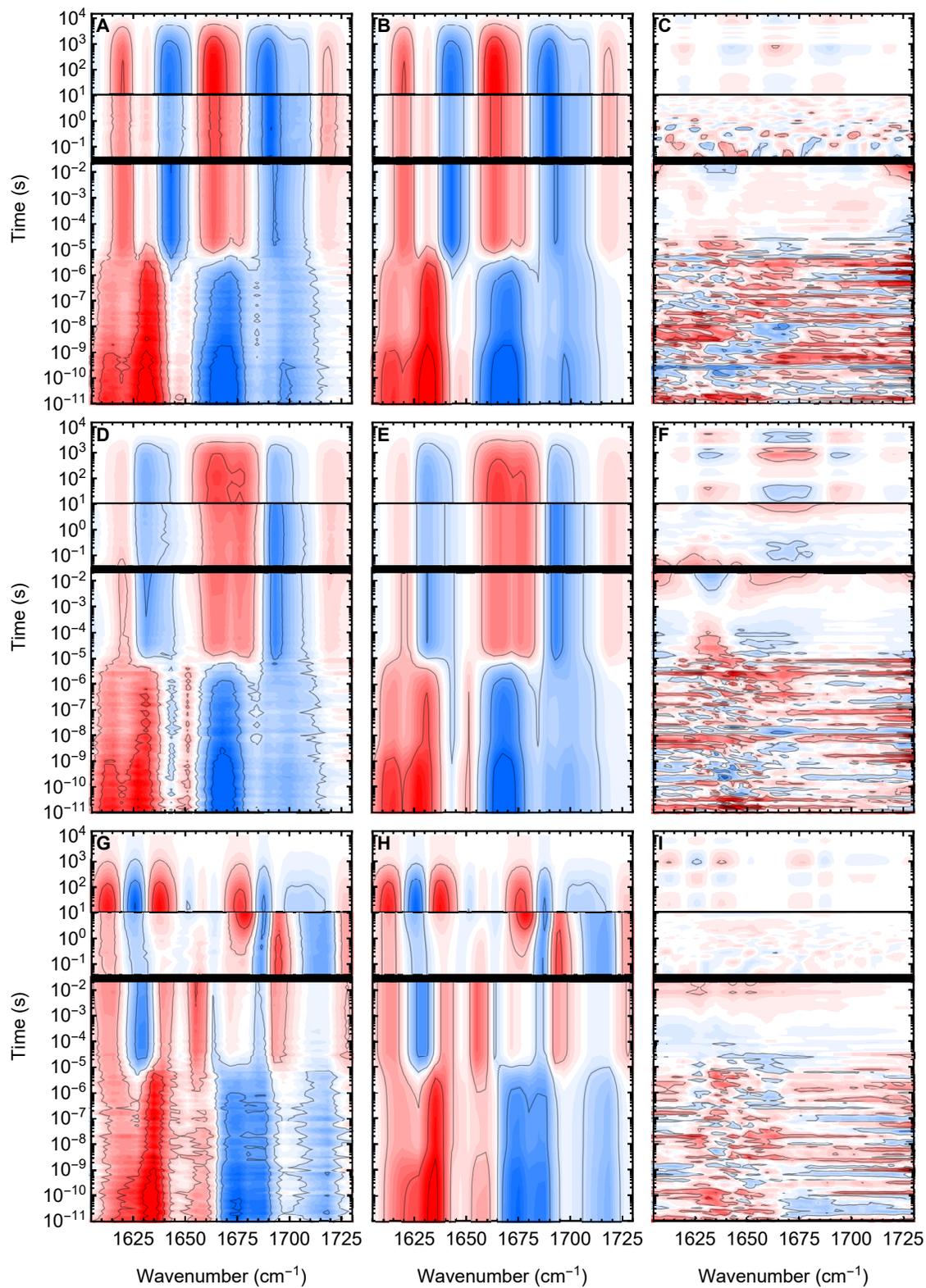


Fig. S2 Contour plots of the experimental TR-IR spectra (A,D,G), the respective global fits (B,E,H), and the fitting residuals (C,F,I) of BsLOV (A-C), YF1 (D-F), and PAL (G-I).

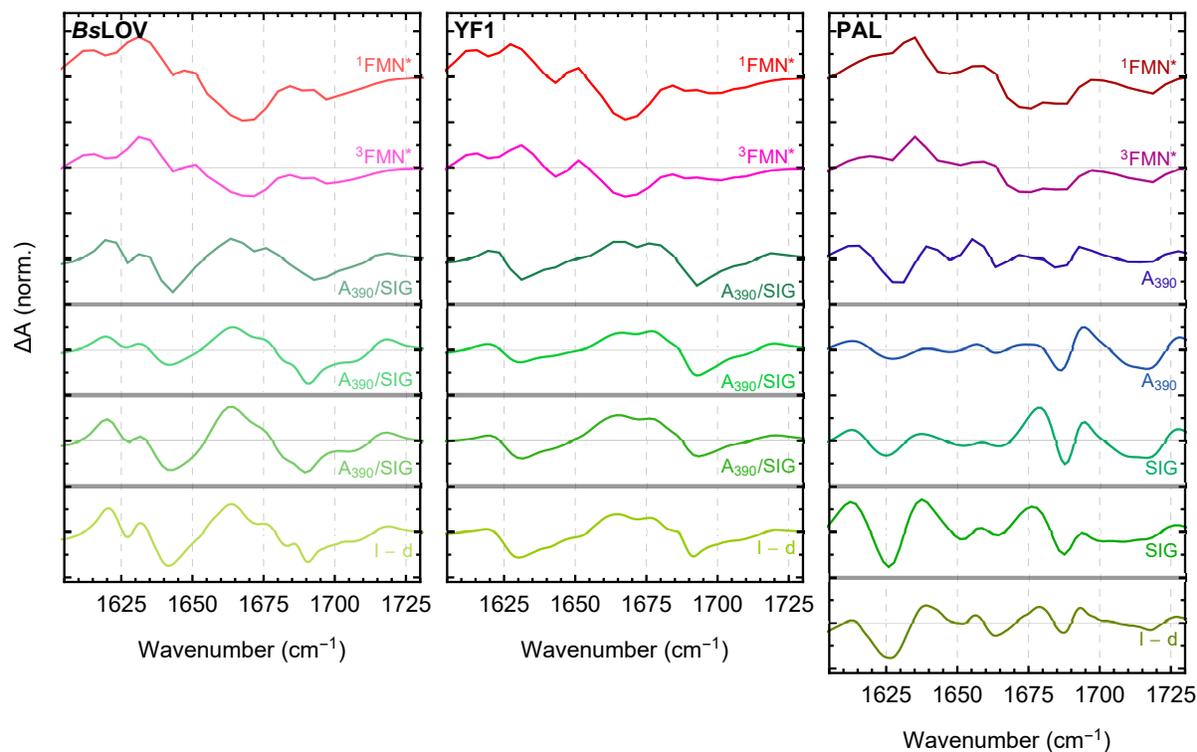


Fig. S3 EADS obtained from global fits to the TR-IR data. Thick gray lines represent the cut between the individually recorded and fitted time-windows. The EADS directly before and after each gray line represent an EADS associated with the same photophysical state.

Setup

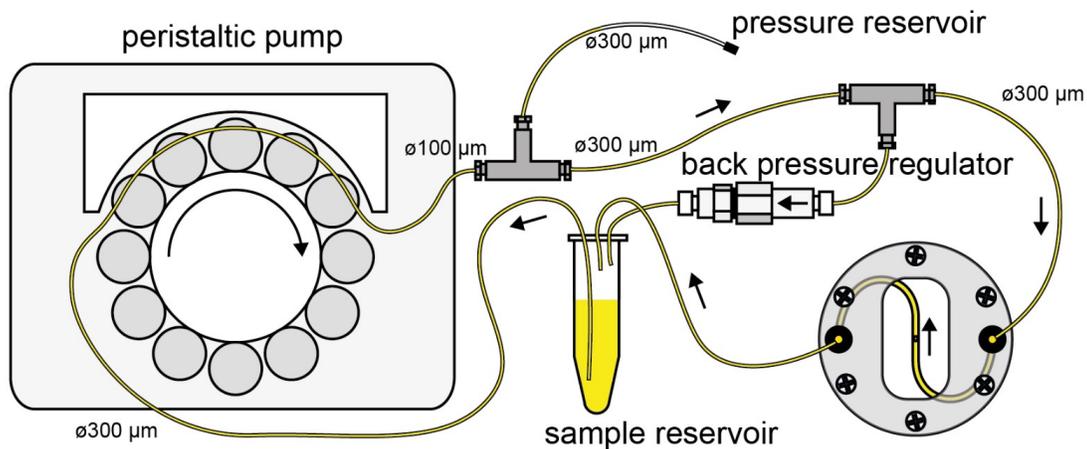


Fig. S4 Schematic illustration of the stop-flow sample-delivery system as adapted from Ref 1 with the additional flowpath through a back-pressure regulator.

Hybrid QM/MM Simulations

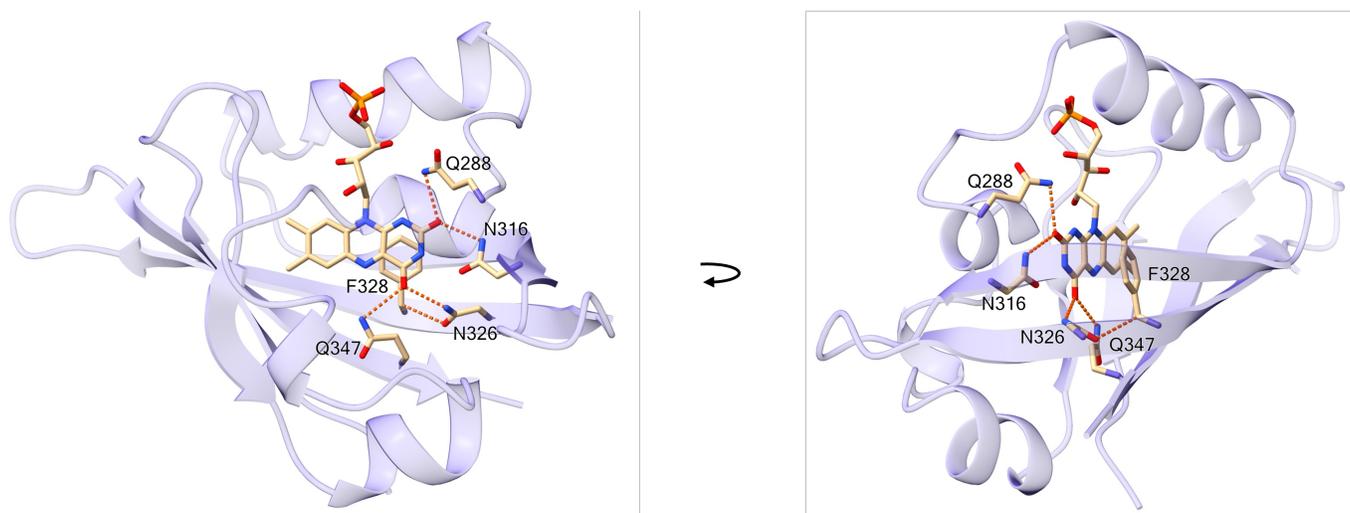


Fig. S5 The QM-MM partitioning chosen for *NmLOV* in two different views. The carbon atoms in the QM region are marked in beige, while the carbon atoms outside the QM region are marked in purple.

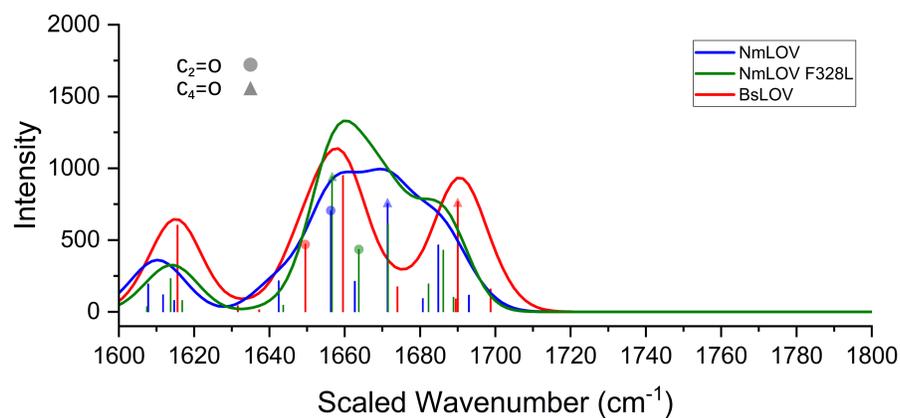


Fig. S6 The calculated QM/MM IR spectra of *NmLOV*, its F328L mutant and *BsLOV*, computed at B3LYP-D3BJ/6-311G* level of theory. The purpose of the mutation was to test the influence of the F328's sidechain on the C₂=O and C₄=O vibrations. The vibrations of C₂=O and C₄=O are highlighted by circles and triangles, respectively, on top of the sticks.

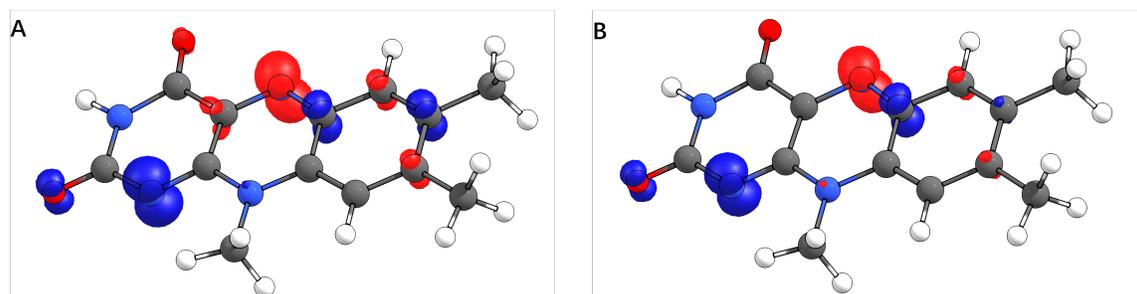


Fig. S7 Electron density difference maps of lumiflavin. (A) $S_1 - S_0$; (B) $T_1 - S_0$. Calculations were performed at the S_0 -optimized geometry at the CAM-B3LYP/6-311G* level of theory. Regions of electron density depletion and accumulation are indicated in blue and red, respectively.

References

- 1 D. Buhrke, J. Ruf, P. Heckmeier and P. Hamm, *Rev. Sci. Instrum.*, 2021, 92, 123001.