

Supplemental Information

Variation in LOV Photoreceptor Activation Dynamics Probed by Time Resolved

Infrared Spectroscopy

James N. Iuliano,[†] Agnieszka A. Gil,[†] Sergey P. Laptanok,^{‡,‡} Christopher R. Hall,[‡] Jinnette Tolentino Collado,[†] Andras Lukacs,^{‡,§} Safaa A. Hag Ahmed,[†] Jenna Abyad,[†] Taraneh Daryaei,[†] Gregory M. Greetham,^{||} Igor V. Sazanovich,^{||} Boris Illarionov,[¥] Adelbert Bacher,[#] Markus Fischer,[¥] Michael Towrie,^{||} Jarrod B. French,[†] Stephen R. Meech,^{‡*} and Peter J. Tonge.^{†*}

[†]*Department of Chemistry, Stony Brook University, New York, 11794, USA* [‡]*School of Chemistry, University of East Anglia, Norwich, NR4 7TJ, U.K.* [§]*Department of Biophysics, Medical School, University of Pecs, Szigeti út 12, 7624 Pecs, Hungary.* ^{||}*Central Laser Facility, Research Complex at Harwell, Rutherford Appleton Laboratory, Didcot, OX11 0QX, U.K.,* [#]*Department Chemie, Technische Universität München, D-85747 Garching, Germany,* [¥]*Institut für Biochemie und Lebensmittelchemie, Universität Hamburg, Grindelallee 117, D-20146 Hamburg, Germany*

[‡]Current address (SPL): Biological and Environmental Science and Engineering Division, King Abdullah University of Science and Technology, P.O. Box 4700, Thuwal 23955-6900, Kingdom of Saudi Arabia.

*Authors to whom correspondence should be addressed: Email: s.meech@uea.ac.uk (SRM); peter.tonge@stonybrook.edu (PJT)

Table S1: Assignment of LOV Spectra				
EAS1	AsLOV2	YtvA	LovK	EL222
1FMN*	1375	1379	1382	1378
1FMN*	1413	1418	1424	1419
GS Bleach	1550	1548	1552	1544
GS Bleach	1583	1580	1586	1580
Protein		1615	1612	1615
C2=O	1622	1629	1632	1631
Asn (-)	1669	1663	1662	1652
Gln513	1690	1702	1700	1700
EAS2				
3FMN*	1438	1440	1443	1428
3FMN*	1491	1491	1488	1488
GS Bleach	1550	1548	1552	1544
GS Bleach	1583	1580	1586	1580
Protein		1615	1612	1615
C2=O	1622	1629	1632	1631
Asn (-)	1669	1663	1662	1652
Gln513	1690	1702	1700	1700
EAS3				
Protein (-)	1404	1406	1411	1406
Protein (+)	1417	1417	1424	1418
Protein (-)	1426	1430	1433	1430
Protein (+)	1435	1443	1453	1450
β-sheet N-H		1526		
C4C10a (+)	1541	1541	1547	1553
C4C10a (-)	1553	1550	1553	1541
β-sheet C=O	1625	1619	1633	1631
Jα helix	1634			
β-sheet C=O			1650	1645
FMN/Protein	1665	1667	1669	1665
Gln513	1690	1695	1695	1700
C4=O	1722	1720	1718	

Table S1: Band assignments were based on the effect of ¹³C labelling on the spectrum of AsLOV2.

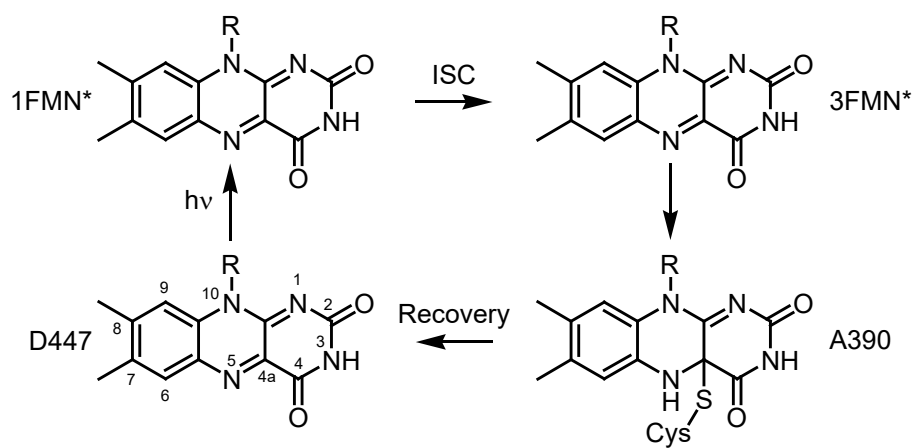


Figure S1: The LOV Photocycle

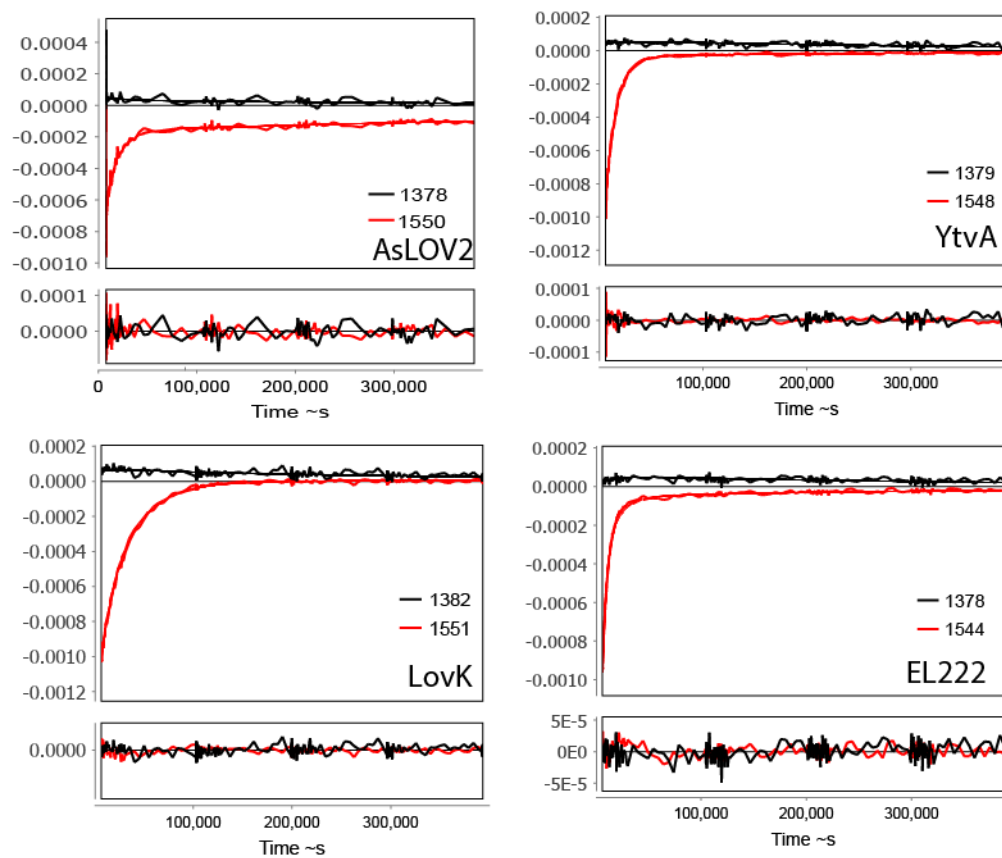


Figure S2: Kinetic traces and residuals showing quality of fit. Excited state decay is shown in black and ground state recovery is shown in red.

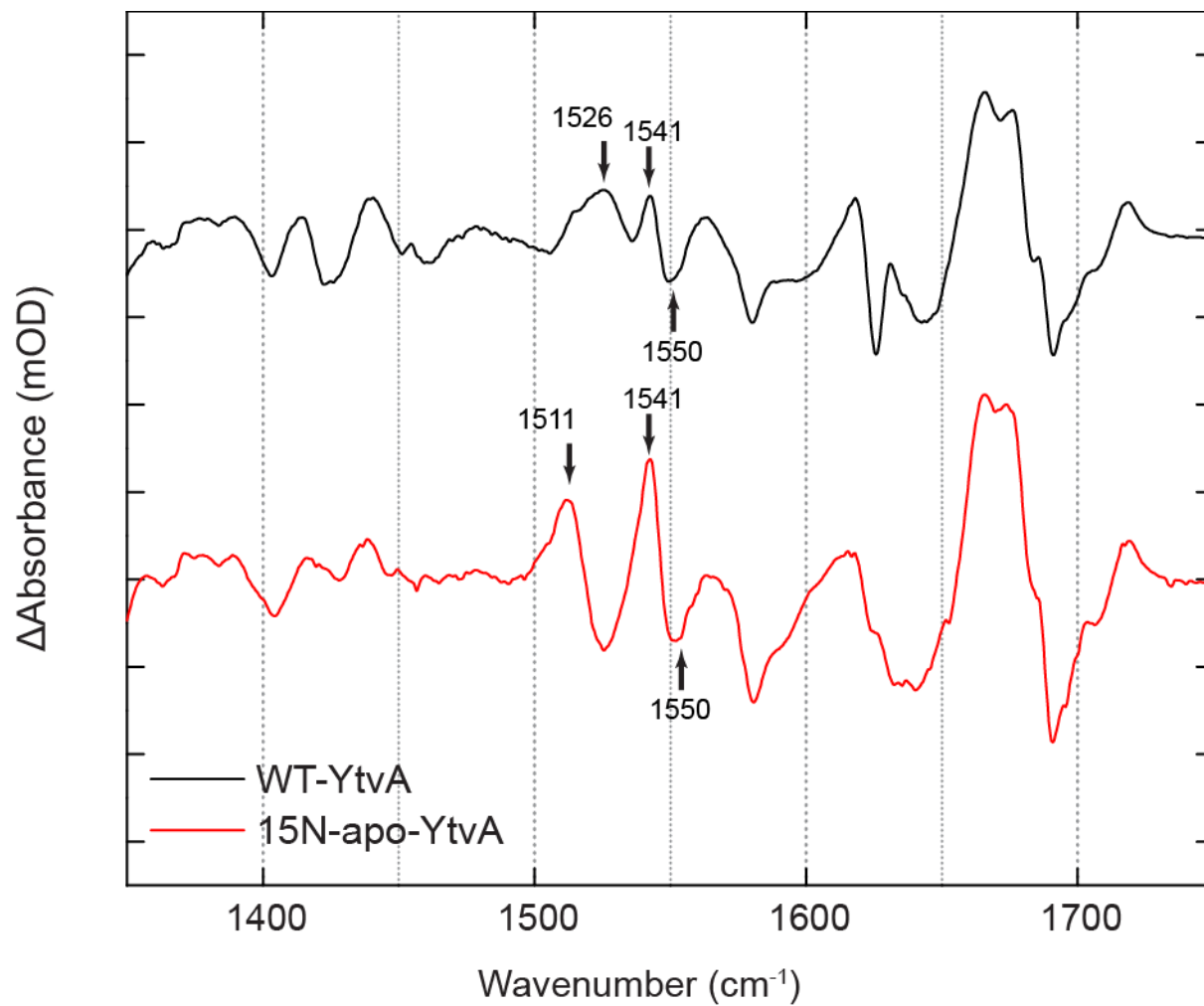


Figure S3: L-D FTIR of [¹⁵N-apoprotein]-YtvA

```

AsLOV2 -----LATTLERIEKNFVITDPRLPDNPIIFA
YtvA MASFQS-----FGIPGQLE-----VIKKALDHVRVGVVITDPALEDNPIVYV
LovK MEDYSESRRAGERLAAGHGVD-----PFAAAISATRMAMIVADATQPDIPPIIFA
EL222 MGQDRP-----IDGSGAPGADDRVEVQPPAQWVLDLIEASPIASVVSDPRLADNPLIAI
          .   :.           :::*      * *::

AsLOV2 SDSFQLTEYSREEILGRNCRFLQGPETDRATVRKIRDAIDNQTEVTVQLINYTKSGKKF
YtvA NQGFVQMTGYETEEILGKNCRFLQ GKHTDPAEVDNIRTALQNKEPVTVQIQNYKKDGTMF
LovK NDAFLRLTGYARDEVI GRNCRFLQ GPDTDPKAIQAVRDALAAGEDVAVDLLNYRKDGSPF
EL222 NQAFDLDLTYSEEECVGRNCRFLA GSGTEPWLTDKIRQGVREHKPVLVEILNYKKDGTPF
  .:. *  : * *  : *  : * : * * * * * * * * :           : *  . :           * * : : * * . * . *

AsLOV2 WNL FHLQPMRDQKGDVQYFIGVQLDGTEHVRDAAER----EGV--MLIKKTAENIDEAAK
YtvA WNE LNIDPMEIE--DKTYFVGIQNDITKQKEYEKL-----LED SLTEITALS-
LovK WNA LNMSPVRNDAGQLVYFFGSQVDVTDKKVVELRARDHSDGLQOMVEERTRE-----
EL222 RNA VLVAPIYDDDDDELLYFLGSQVEVDDDDQPNMGMARRERAA--EM-----
          * . : * : : : * * . * * : ..

AsLOV2 EL
YtvA --
LovK --
EL222 --

```

Figure S4: Sequence alignment of the AsLOV2, YtvA, LovK, and EL222 LOV domains. Conserved LOV core motif is highlighted in yellow and shows the Gln to Ala replacement in EL222 and the prediction of Gln for LovK. F494 in AsLOV2 and corresponding residues in YtvA, LovK, and EL222 are highlighted in green.

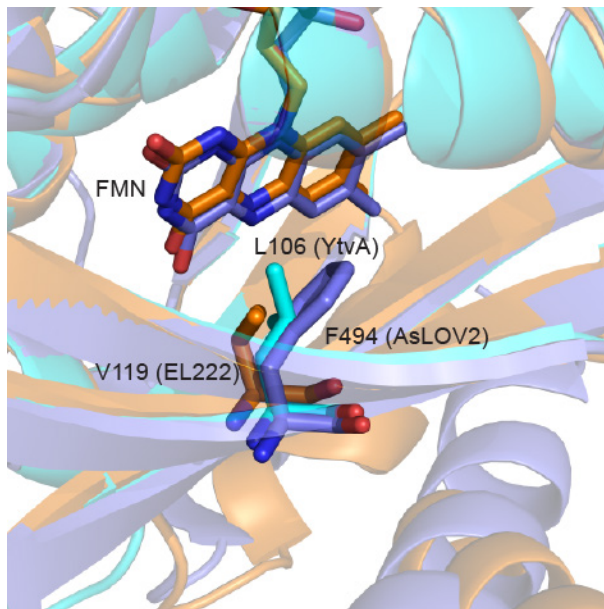


Figure S5: F494 forms a π -stacking interaction with the isoalloxazine ring of FMN.

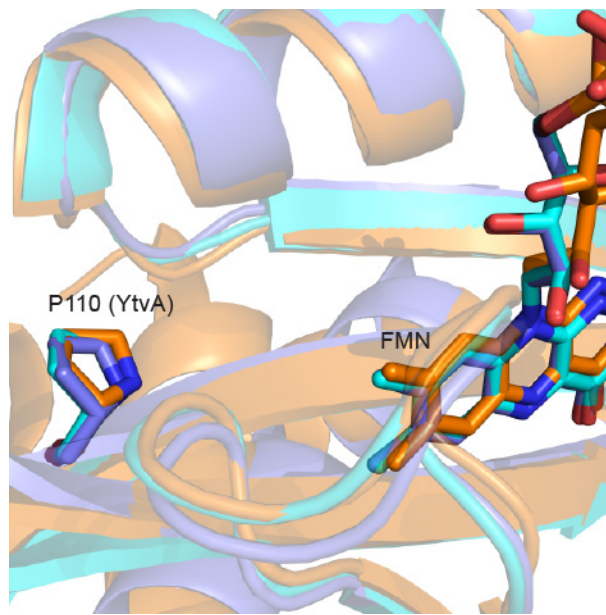


Figure S6: Proline residues on the LOV β -sheet.