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Supplementary Materials for

Structure of a Light-Activated LOV Protein Dimer That Regulates Transcription

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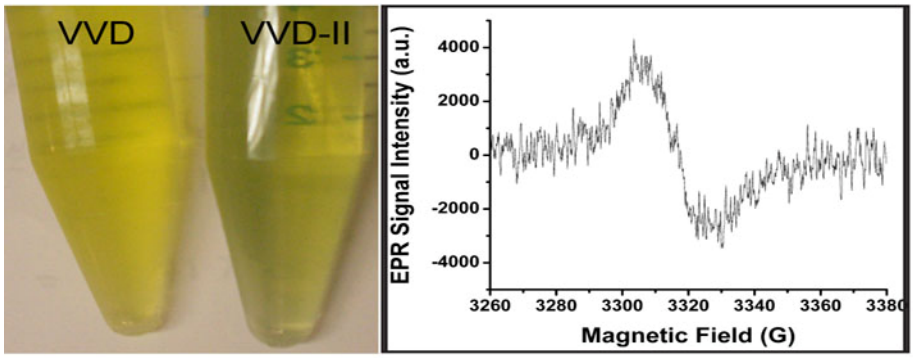

Fig. S1. VVD-II has a high redox potential. VVD-II was purified from *E. coli* in a partial neutral semiquinone state, which was indicated by the green color of the protein compared to the yellow color of the native protein (left panel) and the electron spin resonance (ESR) spectrum of VVD-II as measured with a 9-GHz cw ESR spectrometer (right panel).

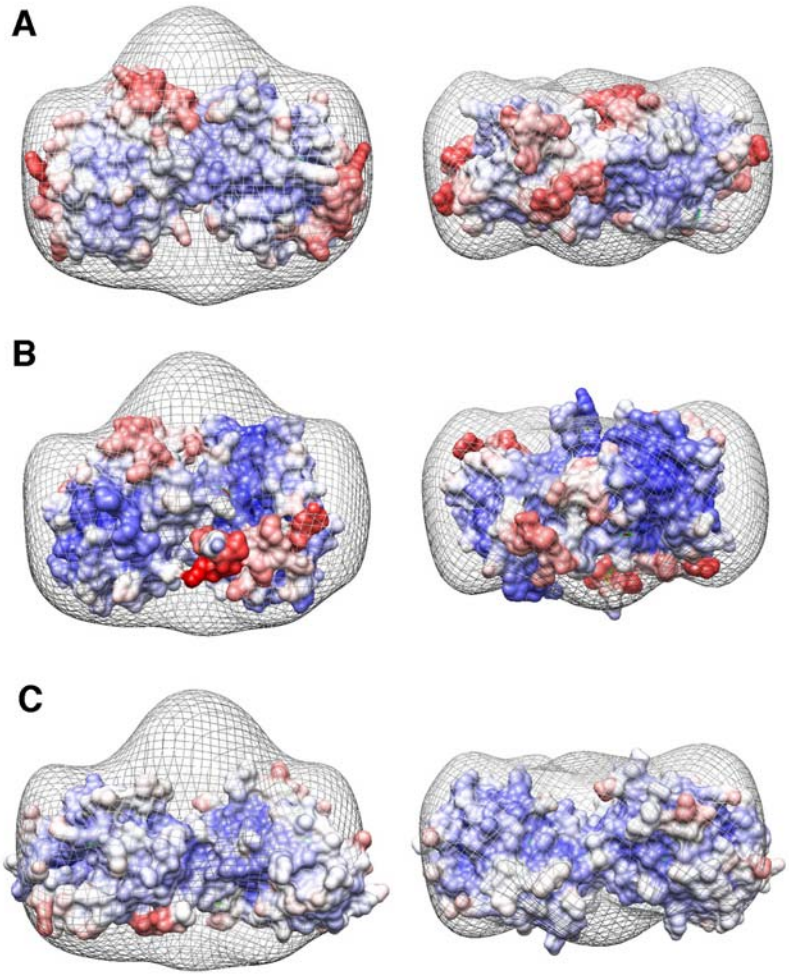

Fig. S2. Comparison of the LSD structure of VVD with time-resolved SAXS data. Superposition of the molecular envelope determined from time-resolved SAXS of light-state VVD and the solvent-accessible surface of several crystallographic dimers of VVD colored by relative thermal B-factor (blue, low; red, high). The views on the right are down from the top as shown on the left. The dimensions of the SAXS envelope generally matched those of the LSD of VVD (**A**), with the exception of a protrusion of SAXS density along the two-fold axis of the dimer; however, this small discrepancy likely reflected dynamics in this part of the molecule. Extended, mobile regions of the VVD structure (denoted by the red surface) pointed toward the two-fold axis of the dimer at the top of the envelope. Note that there is some ambiguity in fitting the structure within the envelope. The procedure that was used to calculate the SAXS envelope (*1*) does not account for conformational heterogeneity; rather, it assumes a constant protein density and a sharp boundary between solvent and protein, which in the more mobile regions of the protein is not a fully representative model. Other crystallographically characterized VVD dimers do not fit the SAXS envelope nearly as well as does the LDS structure. (**B)** The alternative non-physiological dimer present in the LSD crystal lattice. (**C)** The VVD dimer found in dark-state crystals (PDB Code: 2PD7).

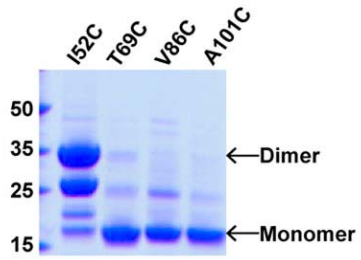

Fig. S3. Targeting the cross-linking ability of the LSD of VVD. The Ile^{52}→Cys variant VVD protein spontaneously cross-linked in the light when purified from *E. coli*, whereas variant proteins from which other positions from the dimer interface (Val^{86} and Ala^{101}) were removed did not cross-link. The band at 25 kD corresponds to proteolytically degraded dimeric VVD. The Thr^{69}→Cys substitution also placed a thiol at the dimer interface, but subunits of this variant protein did not cross-link readily, which may have been a result of the non-ideal orientation of the cysteine thiols to form disulfide bonds.

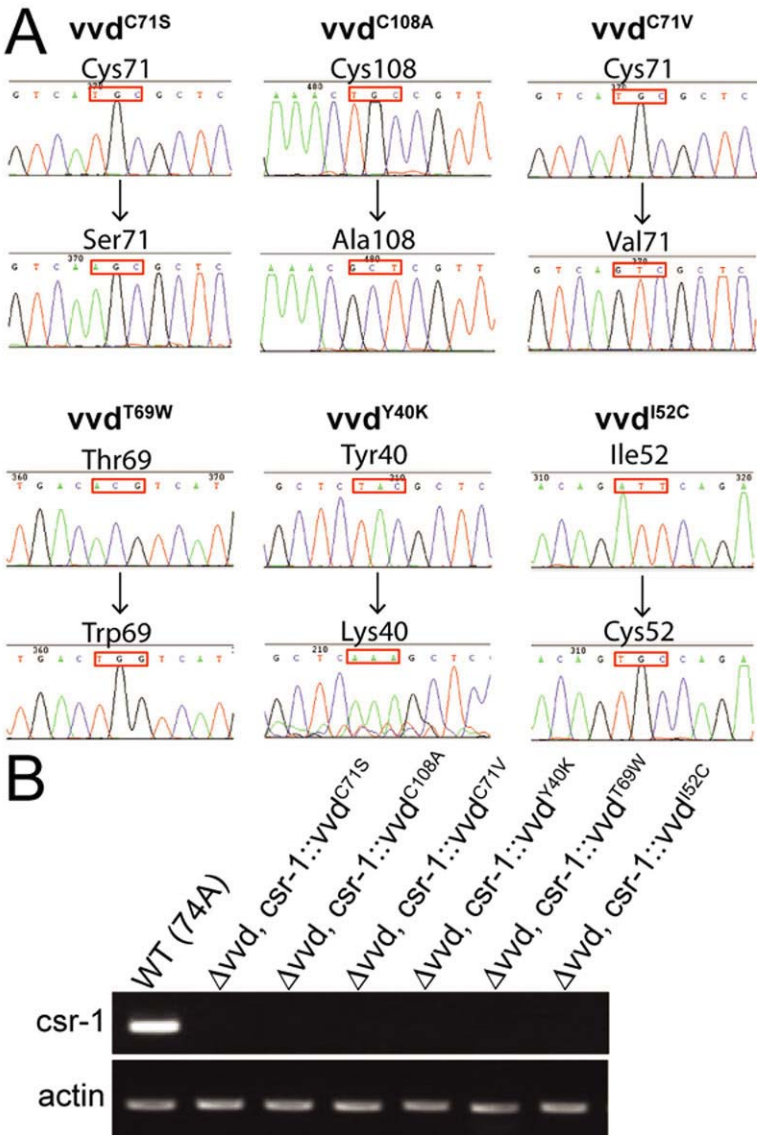

Fig. S4. The *csr-1* knock-in strains used in this study were homokaryons. (**A**) Sequencing data of various *vvd* mutant alleles. (**B**) PCR analysis of the absence of the *csr-1* locus in the genomes of each knock-in strain.

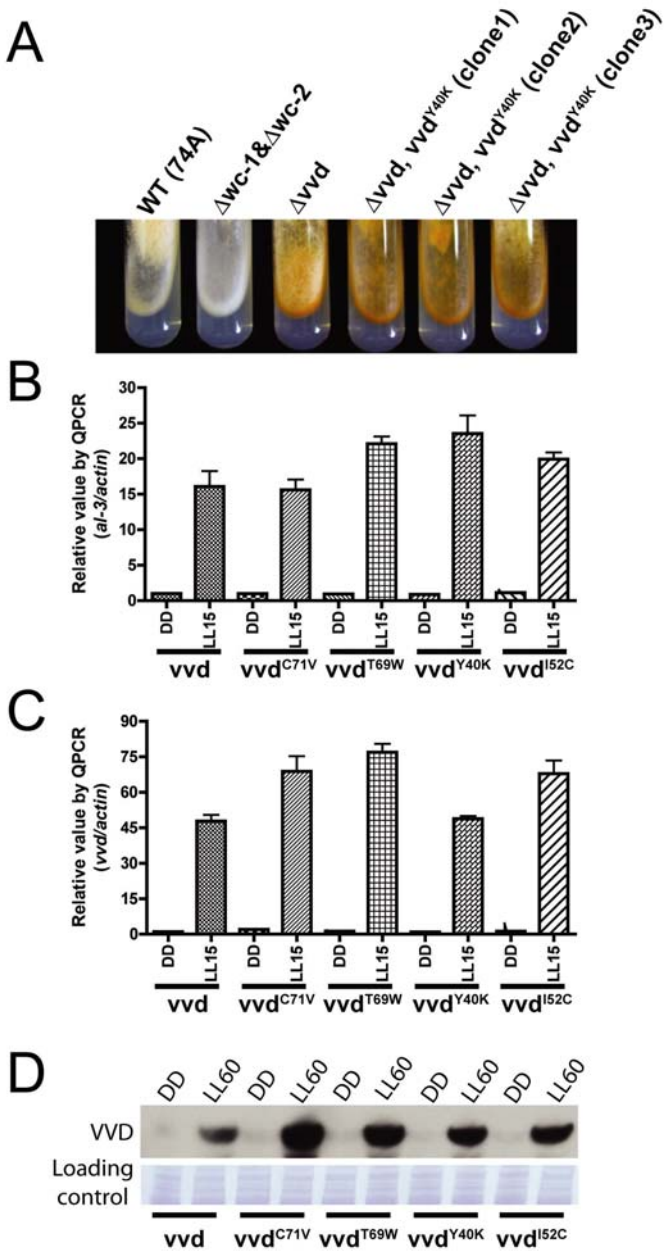

Fig. S5. Light-dependent induction of *vvd* expression and VVD protein production in strains with various *vvd* mutant alleles. (**A**) Independent primary transformants of *vvd*^{Y40K} mutant allele (clones 1, 2, and 3) displayed a consistent phenotype of photoadaptation defects. (**B**) Light-dependent induction of *al-3* and (**C**) *vvd* expression at LL15 in strains with various *vvd* mutant alleles was determined by RT-PCR analysis (n =3 experiments). Data shown are mean values ± standard errors. (**D**) Light-dependent induction of the production of VVD protein at LL60 in strains with various *vvd* mutant alleles. Upper panel shows Western blotting analysis of VVD tagged with a C-terminal V5 epitope with an antibody against V5. The lower panel shows the Coomassie-stained loading control.

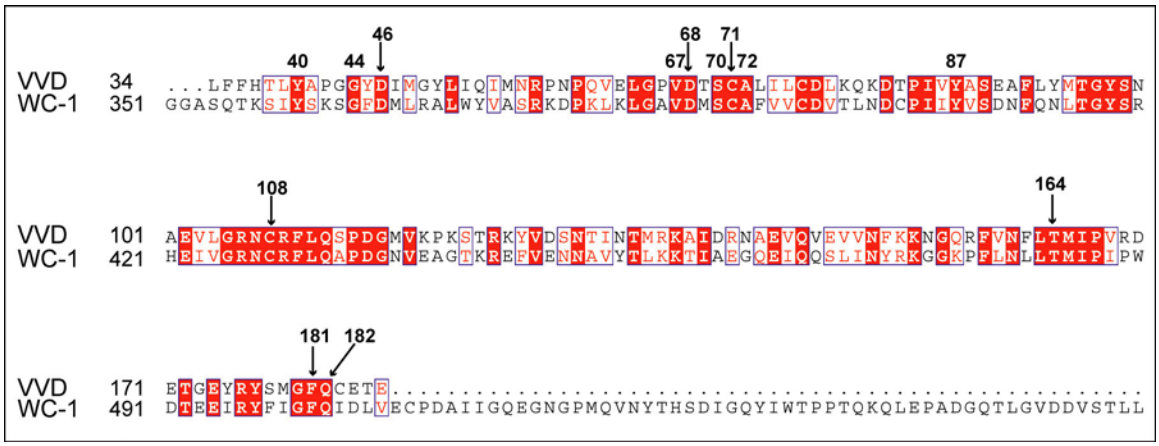

Fig. S6. Sequence similarity between VVD and WC-1. Alignment of the amino acid sequences of VVD and WC-1 proteins shows the conservation of 65% of the residues involved in light-induced signaling and the dimerization of VVD (table S2). The residues involved in the light-sensing, signaling, and dimerization of VVD that are conserved in WC-1 are denoted with VVD numbering.

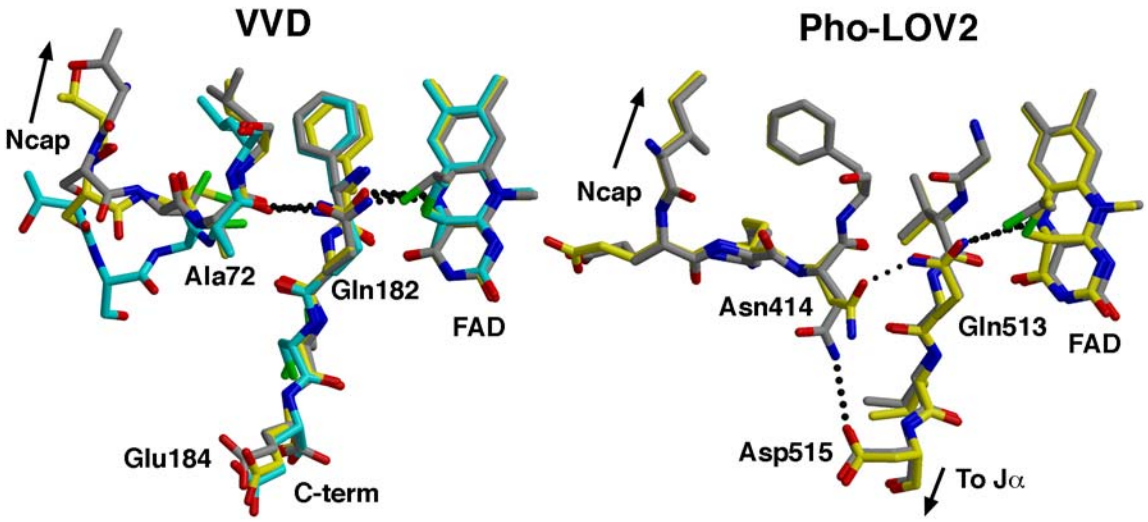

Fig. S7. Comparison of the light-induced changes in hydrogen bonding found for VVD and the *A. sativa* phototropin LOV2 domain. In both VVD (left panel) and the *A. sativa* phototropin LOV2 domain (right panel), formation of cysteinyl-flavin adducts caused similar changes to hydrogen bonding between the first and last β-strands of their respective PAS domains. In VVD, protonation of the N5 of flavin flipped Gln^{182} in the light state (yellow and cyan for the two superimposed subunits), which resulted in the formation of a new hydrogen bond to the carbonyl group of Ala^{72}. In LOV2, the analogous glutamine residue formed a similar hydrogen bond with the first residue of the first β-strand in the light state (yellow), but in this case, the acceptor was the side chain of Asn^{414}, which no longer formed hydrogen bonds with Asp^{515}, as it does in the dark state (gray). In the VVD LSD, these changes resulted in restructuring of the linker loop leading to the N-cap. The changes in the cyan subunit were larger than those in the yellow subunit. No such large-scale changes were seen in the LOV2 Ncap; however; potential rearrangements were probably limited by the crystal lattice. *A. sativa* LOV2 domains are shown for PDB codes 2V1A (dark state) and 2V1B (light state).

Table S1. Data collection and phasing statistics. Data for outermost resolution shell are given in parenthesis.

Parameter	***Value***
Wavelength (Å)	0.97918
Space group	$P2_1$
Cell Parameters	a = 37.59, b = 77.55, c = 54.78, β= 99.73
Resolution (Å)	30 - 2.75 (2.85 - 2.75)
No. of observations	31885
No. of unique reflections	8227
Completeness (%)	98.6 (97.9)
R_{sym}[a]	0.134 (0.485)
I/σ(I)	10.3 (2.0)
Refinement statistics	
Resolution range	30-2.75 (2.85 - 2.75 Å)
R factor, %	22.33 (34.67)
R_{free}, %	28.76 (39.32)
Wilson B factor (Å^2)	42.1
Molecules / Asym unit	2
Residues	2 X 149
Atoms	
Protein	2346
Solvent	207
Cofactor (FAD)	106
Mean B-values (Å^2)	
Overall	41.7 Å^2
Main chain	40.8 Å^2
Side chain	42.7 Å^2
Solvent	33.0 Å^2
Rmsd from ideal geometry	
Bonds	0.017 Å
Angles	2.14°
Ramachandran plot, %	
Most favored	83.6
Additionally allowed	15.6
Generously allowed	0.8
Disallowed	0.0
Missing residues	None

[a] $R_{sym} = \Sigma\Sigma_j |I_j - \langle I \rangle| / \Sigma\Sigma_j I_j$

Table S2. Conservation of light-dependent signaling and dimerization in the fungal LOV domains The output of 170 protein sequences from Pipealign was classified into 10 groups (left panel). *Ascomycota*, *Basidiomycota*, and *Zygomycota* are three phlya of the kingdom Fungi. UC, uncharacterized proteins. The amino acid residues (in single-letter code) involved in light-sensing (*sense*), conformational signaling (*signal*), and dimerization (*dimer*) in VVD and the corresponding residues across the ten groups (right panel) are denoted as: ✓, the residue is conserved in that group; □, the corresponding residue is not conserved in the group; -, the corresponding residue is not present in the group; X/Y, the residues X and Y are conserved in the group instead of that found in VVD. Residues in green are a part of the N-terminal latch; residues in blue are found in aα, those in magenta are in the hinge of VVD, and those in red are conserved in all the groups.

Group (Number of sequences)	Proteins Representative primary accession numbers Classification of the sequences (number of sequences)
1 (7)	**VVD and its variants from *Neurospora crassa*** Q9C3Y6, Q1K5Y8 VVD (1) + VVD variants (4) + Predicted (2)
2 (22)	**GATA factors and predicted proteins from Ascomycota** Q7ZA36, A1D8C9, C8VHD4 GATA factor (11) + Predicted (8) + WC-1 (2)
3 (9)	**Putative WC-1 and WC-1 from Zygomycota** B5M9P6, Q14SS7, B8YIE4 WC-1 (2) + Predicted (7)
4 (15)	**Putative WC-1, VVD and ENVOY from Basidiomycota** A4RDB4, Q6DMM2, B8P2A7 WC-1 (3) + VVD (1) + ENVOY (1) + Predicted (7) + UC (3)
5 (21)	**WC-1 from Ascomycota** B2VXI8, C6K2I9, D2Y4Q8 WC-1 (19) + Predicted (1) + UC (1)
6 (16)	**Putative WC-1 and WC-1 from Ascomycota** A7F3V7, Q5PXG9, D1Z8I0 WC-1 (4) + Predicted (7) + UC (5)
7 (20)	**Phototropin and uncharacterized proteins from plants** C5YK68, Q5DW42, A7LI54 Phototropins (12) + Predicted (2) + UC (6)
8 (11)	**Phototropins from plants** B9T0R3, D7LMS0, Q8H934 Phototropins (7) + Predicted (3) + UC (1)
9 (12)	**Putative phototropins from plants** C5YPQ1, B8BLR3, O48547 Phototropins (4) + Predicted (5) + UC (3)
10 (37)	**Histidine kinase and sensor proteins** D5RKE3, A5EAE7, B7KW67 Histidine kinase (18) + Predicted (15) + UC (1)

	Group →	1	2	3	4	5	6	7	8	9	10
Dimer	Y40	✓	✓	✓	✓	✓	✓	E	D/E	E	
Dimer	A41	✓	S	S/A	S	S	S	R	R/S	R	
Signal	G44	✓	✓	✓	✓	✓	✓	S	S/D/E	S	
Dimer	D46	✓	✓	✓	✓	✓	✓	D/E	✓	E/D	
Dimer	M48	✓	M/A	I/L/V	L	L	L		K	E	
Dimer	I52	✓	A	S		R	Y/L/R	R	K/R	K	
Signal Dimer	P66	✓	A/P	✓	✓	S	A	R	R	R	V
Signal	V67	✓	✓	I/V	I	✓	✓	I	I	I	E/R
Signal Dimer	D68	✓	✓	✓	✓	✓	✓	E	E	E	T/A
Signal Dimer	T69	✓	L/M	L/M		L	M	K	K	K	✓
Signal	S70	✓	✓	✓	✓	✓	✓	N	N	N	R
Signal Dimer	C71	✓	✓	✓	C/V	✓	✓	-	-	-	M
Signal	A72	✓	✓	S	S/A	✓	✓	-	-	-	P/A
Dimer	V86	✓	V/I	V/I	V/I	✓	I	I	I	I	I/V
	Y87	✓	✓	✓	✓	✓	✓	F	F	F	F/Y
Dimer Sense	C108	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Dimer	F162	✓	L	L	L	L	L	L	L	L	A
Dimer	T164	✓	✓	✓	✓	✓	✓	H	H	H	F/Y
Dimer	F181	✓	✓	L	✓	✓	✓	V	V	V	S
Signal	Q182	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

Reference

1. J. S. Lamb, B. D. Zoltowski, S. A. Pabit, L. Li, B. R. Crane, L. Pollack, Illuminating solution responses of a LOV domain protein with photocoupled small-angle x-ray scattering. *J. Mol. Biol.* **393**, 909–919 (2009).