Supporting Information

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SI Materials and Methods

Cloning, Expression, and Purification. The DNA fragment encoding *Escherichia coli* TrpR was obtained by colony PCR. A clone of *Avena sativa* phot1 LOV2 was generously provided by Kevin Gardner (University of Texas Southwestern Medical Center, Dallas). The fusion proteins were created from these templates using overlap extension PCR and subcloned into the expression vector pCal-n (Stratagene) so as to be in frame with the amino terminal calmodulin binding peptide. The results were confirmed by DNA sequencing. Rosetta 2(DE3)pLysS *E. coli* (Novagen) carrying these vectors were grown in LB media at 37°C to an $OD_{600} \approx 0.5$ and induced at 20°C by the addition of 0.3 mM IPTG. After ≈16 h, the cells were harvested by centrifugation and resuspended in B-buffer [50 mM Tris (pH 8.0), 150 mM NaCl, 1 mM imidazole, 1 mM $MgCl_2$, 2 mM $CaCl_2$, and 10 mM 2-mercaptoethanol and EDTA-free protease inhibitor mixture (Roche)]. The cells were lysed by the addition of lysozyme and DNase-I. The lysate was cleared by centrifugation and applied to equilibrated calmodulin affinity resin (Stratagene). The resin was washed with W-buffer [50 mM Tris (pH 8.0), 500 mM NaCl, 1 mM imidazole, 1 mM $MgCl_2$, and 2 mM $CaCl_2$) and the protein eluted in 3–5 ml E-buffer [50 mM Tris (pH 8.0), 500 mM NaCl, and 2 mM EGTA). The eluate was applied to a HiPrep 16/60 Sephacryl S-100 HR column (GE Healthcare), wrapped with aluminum foil, and equilibrated with G-buffer [50 mM Tris (pH 8.0), 150 mM NaCl, and 1 mM EDTA) running at 1 ml/min. Fractions corresponding to peaks in A_{447} were collected and analyzed by SDS/PAGE. Concentration was determined by A_{447} using $\varepsilon = 13{,}800\ M^{-1}\cdot cm^{-1}$ for the monomer (1).

Illumination. Blue AlGaInP LEDs (theledlight.com, 20° viewing angle, 8,000 mcd, 468 nm λ_{max} at 3.4 V) were powered by a 3.3-V, 4-A AC adaptor (Mouser). The radiant power delivered to the samples was estimated using a hand-held power meter (New Focus). Irradiance was calculated by using 0.12 cm^2 as the cross-sectional area of the reaction tube.

RsaI Protection Assay (2). The KpnI/RsaI site of plasmid pUC19 was deleted and the duplex oligos 5′-AATCGAACTCGCTAGCGAGTACG-3′ and 5′-ATTCGTACTCGCTAGCGAGTTCG-3′ (IDT) were ligated into the resulting plasmid between the TfiI sites. The deletion and insertion were confirmed by restriction analysis and sequencing. The resulting plasmid, pUC19-KpnIΔ-trpR_s(3A), contains two natural RsaI sites and one site buried in a *trp* operator that binds only one TrpR dimer (3). TrpR activity protects the 1,890-bp fragment from digestion by RsaI to the 1,410- and 480-bp products. A 676-bp internal control results from digestion at unprotected sites. For simplicity, only the 1,890- and 1,410-bp fragments are shown in the figures. Protection assays were performed with 19 nM pUC19-KpnIΔ-trpR_s(3A), 1× NEB Buffer 2 [10 mM Tris·HCl (pH 7.9), 10 mM $MgCl_2$, 50 mM NaCl, 1 mM DTT], 0.1 mg/ml BSA (Promega), and 0.1 mM L-tryptophan (Sigma). Protein in G-buffer, G-buffer supplemented with 350 mM NaCl, or E-buffer was diluted 10-fold into this mixture and incubated for 1 min under ambient conditions. RsaI was diluted to a concentration of 0.5–2 units/μl in dilution buffer (50% vol/vol glycerol, 1× NEB Buffer 2, 0.2 mg/ml BSA) and then diluted 20-fold into the reaction mixture. Reactions were performed in 0.5-ml thin-walled tubes placed in the block of a thermocycler set at 25°C. The reaction was quenched at the desired time points by adding loading buffer (Promega) to 1× and EDTA to 15 mM. The samples were run on a 1.5% agarose gel in 1× TAE, stained with ethidium bromide, and imaged. Digestion was determined as the ratio of the intensity of the product bands (1,410 bp and 480 bp) to that of the product bands plus the reactant band (1,890 bp). The bands were quantitated by using ImageJ (http://rsb.info.nih.gov/ij/) and the results plotted by using Kaleidagraph (Synergy Software).

Modified RsaI Protection Assay. We modified the above assay to simplify the reaction scheme so that the data are interpretable by using a single exponential model. We digested the plasmid pUC19-KpnIΔ-trpR_s(3A) with PvuI and gel-purified the resulting linear fragment containing only a single RsaI restriction site buried in the *trp* operator. This 1,680-bp reactant is digested to a 375- and a 1,305-bp product by RsaI. Other than the substitution of DNA substrate, the assay was performed essentially as described above. The amount of digestion was determined as the ratio of the intensity of the product bands to that of the product bands plus the reactant band. The bands were quantified by using ImageJ (http://rsb.info.nih.gov/ij/), and the data were analyzed by using IGOR Pro (WaveMetrics). For all fits, the amplitude was constrained to unity and only the rate was allowed to vary. The dissociation constant (K_d) in the lit and dark states was calculated by using the equation

$$K_d = L/(R - 1)$$

where L is the concentration of LovTAP, and R is the ratio of the intrinsic rate of RsaI digestion (k_0) and the rate in the presence of LovTAP (k_{obs}).

Circular Dichroism Spectroscopy. Circular dichroism measurements were performed by using a Jasco J-715 spectropolarimeter equipped with a temperature-controlled cuvette holder. The conditions used were 1–7 μM protein in 150 mM KPO_4 (pH 7.0), 1 mM EDTA at 25°C, 0.1-cm path-length cuvette. Wavelength scans were the average of 10 measurements taken at 1-nm increments with a 2-nm bandwidth, 4-s averaging time, and a speed of 20 nm/min. Data were converted to mean residue ellipticity and plotted by using Kaleidagraph. For kinetic experiments, illumination was from a single blue LED connected to an external switch. The sample was illuminated for 30 s to saturated photoexcitation, and then the LED was switched off and data recorded for 250 s. CD at 222 nm and at 207 nm were recorded in triplicate, averaged, and fit to a single-exponential function by using IGOR Pro.

Size-Exclusion Chromatography. For analysis of the concentration dependence of elution, 0.5 ml of sample in G-buffer was applied to a HiPrep 16/60 Sephacryl S-100 HR column, wrapped with aluminum foil to exclude light, equilibrated with G-buffer and running at 1 ml/min. The elution was monitored by A_{447}.

Small-Angle X-Ray Scattering (SAXS). All SAXS data were collected at the BioCAT beamline at the Advanced Photon Source (Argonne National Laboratory, Argonne, IL). LovTAP samples were prepared to 0.125, 0.25, and 0.5 mg/ml in 50 mM Tris (pH 8.0), 500 mM NaCl, and 1 mM EDTA, and the sample and buffer blanks were filtered to 0.22 μm. Buffer blanks and samples were flowed through a 1.5-mm capillary at a rate of 2 μl/s, and 15 exposures of ≈1-s duration were collected. The exposures were averaged, buffer blanks subtracted, and Guinier analysis per-

formed by using IGOR Pro. I(Q) data were converted to P(r) data by using GNOM (http://www.embl-hamburg.de/ExternalInfo/Research/Sax/gnom.html) (4). Experimental P(r) data were compared to the distribution calculated (5) from our dark-state model plus amino-terminal calmodulin-binding peptide tags modeled as a random walk. Ten *ab initio* reconstructions were generated from the [LovTAP] = 16 μM data by using DAMMIN (http://www.embl-hamburg.de/ExternalInfo/Research/Sax/dammin.html) (6) and averaged by using DAMAVER (http://www.embl-hamburg.de/ExternalInfo/Research/Sax/damaver.html) (7). No assumptions about the final shape were enforced in the reconstruction, except for a 100-Å D_{max} determined in the GNOM P(r) calculation. DAMMIN reconstructions were performed by using data collected at [LovTAP] = 4, 8, and 16 μM, and the shape was found to be independent of concentration.

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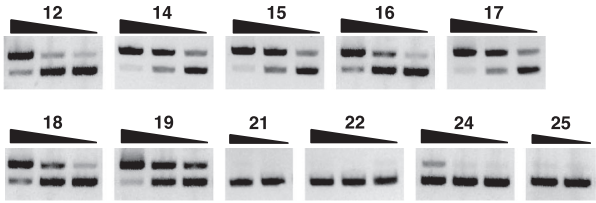

Fig. S1. DNA-binding activity of LOV2-TrpR constructs. Numbering indicates the first included TrpR residue at the point of fusion. Concentrations are 320, 100, and 30 nM monomer (100 and 30 nM for constructs 21 and 25). Specific DNA binding to the *trp* operator protects the large fragment from digestion to the small fragment by RsaI.

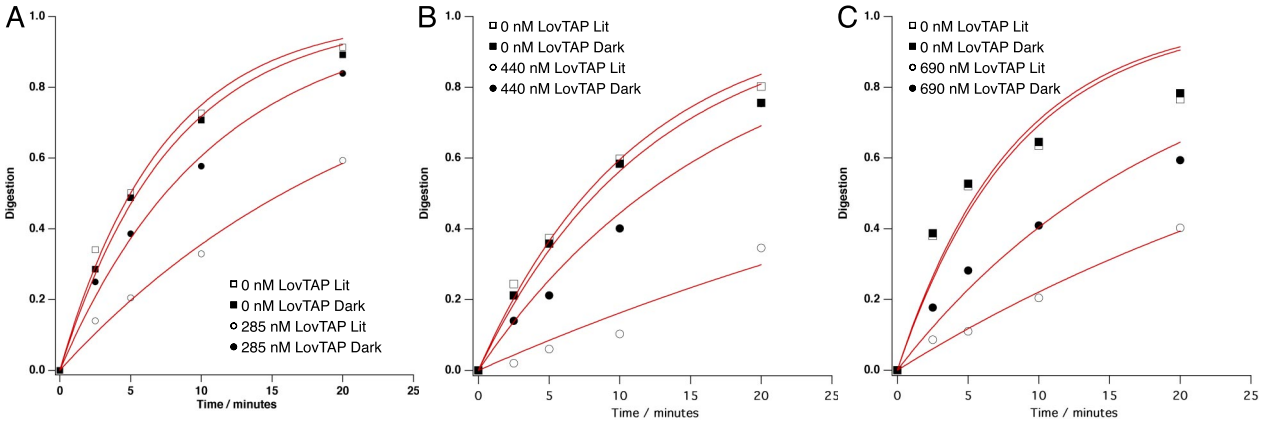

Fig. S2. Kinetic analysis of DNA protection by LovTAP. Cleavage in the absence (squares) and presence (circles) of LovTAP at three dimer concentrations; in the dark (solid symbols) or light (open symbols). Red lines are single-exponential fits to the data.

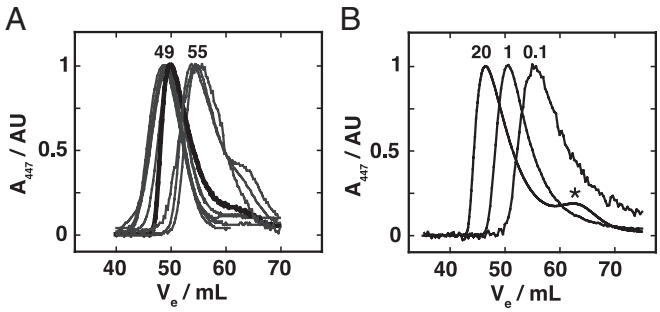

Fig. S3. Analysis of oligomeric state by size-exclusion chromatography. Columns were run in the dark. (*A*) Comparison of LOV2-TrpR constructs. LovTAP is in black. Nonallosteric constructs are in gray. Of the nonallosteric constructs, all with Trp-19 intact are in the group eluting at ≈49 ml, whereas all with Trp-19 substituted are in the group eluting at ≈55 ml. At high concentration, LovTAP elutes at ≈49 ml. (*B*) Concentration dependence of LovTAP elution. Concentrations shown (in μM) indicate the peak monomer concentration at elution. The 46-ml (20 μM) and 55-ml (0.1 μM) peaks represent 60- and 30-kDa proteins, respectively, corresponding to dimeric and monomeric species. The 50-ml peak (1 μM) represents a mixture in equilibrium on the time scale of the experiment. The 63-ml peak (asterisk) is a stable proteolytic fragment of the LOV domain.