SUPPORTING INFORMATION

TITLE: Flavin Radical Formation in the Light-Oxygen-Voltage-sensing Domain of the Photozipper Blue-light Sensor Protein

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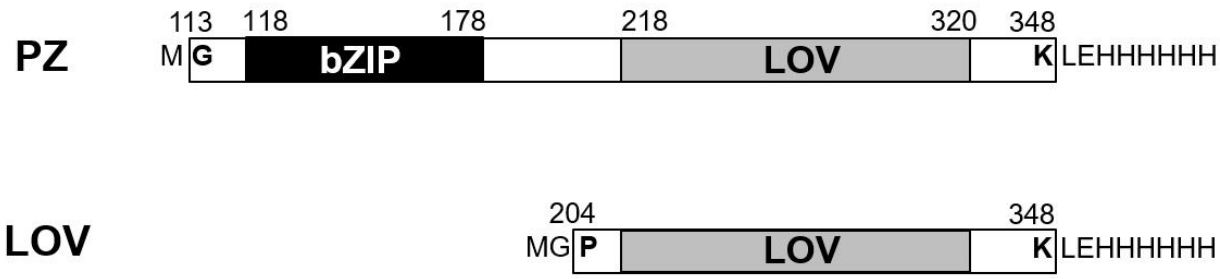

Figure S1. Schematic drawings of the PZ and LOV domain.

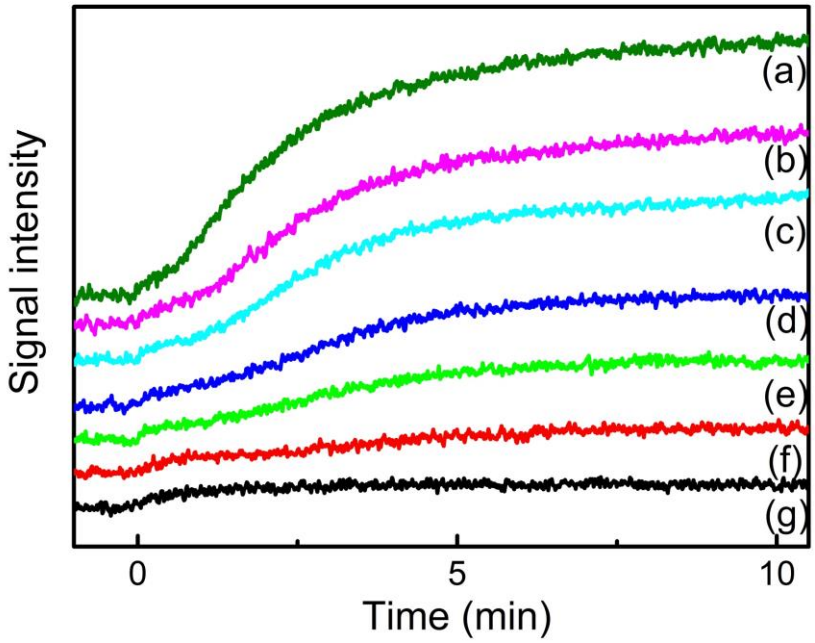

Figure S2. Time course of CW EPR of the LOV-C254S in the presence of DTT. The illumination was started at 0 min. Laser power was 30 mW. The sample concentration was (a) 70 μM, (b) 60 μM, (c) 50 μM, (d) 40 μM, (e) 30 μM, (f) 20 μM and (g) 10 μM, respectively. Experimental conditions: microwave frequency, 9.62 GHz; modulation frequency, 100 kHz; modulation amplitude, 15 G; time constant, 655 ms.

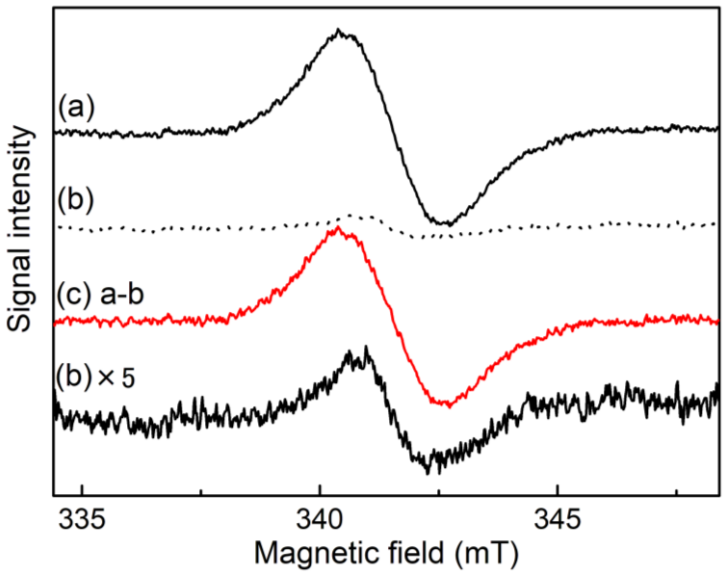

Figure S3: Field-swept EPR spectra of LOV-C254S in the presence of dithiothreitol (DTT). The samples were illuminated with diode laser for 20 min. The sample concentration was (a) 40 μM and (b) 10 μM, respectively. Trace c is the subtraction of trace b from trace a. Experimental conditions: microwave frequency, 9.62 GHz; modulation frequency, 100 kHz; modulation amplitude, 15 G; time constant, 655 ms.

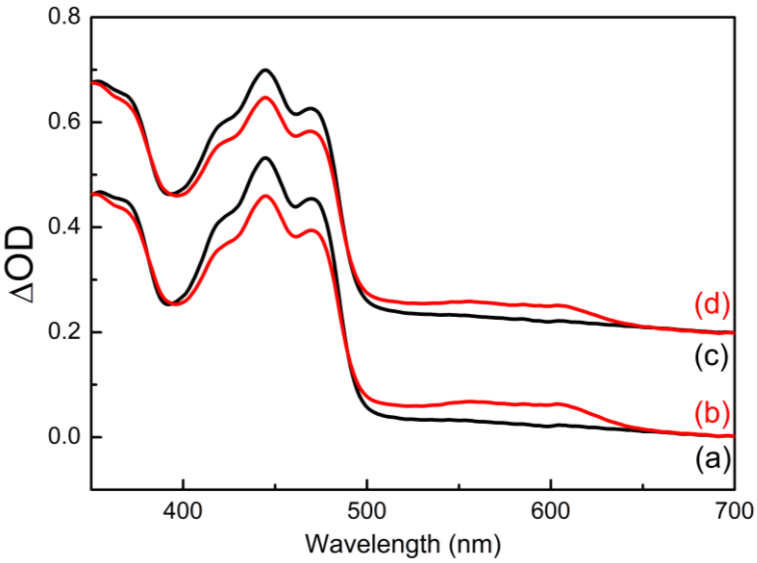

Figure S4: Absorption spectra of LOV-C254S in (a) initial state, (b) after illumination for 4 min, (c) dark adapted for 15 min after illumination and (d) re-illumination for 1 min in the presence of 1 mM DTT. Laser power was 30 mW. Sample concentration was 40 μM with the volume of 100 μl. Hitachi U-3300 spectrometer was used.

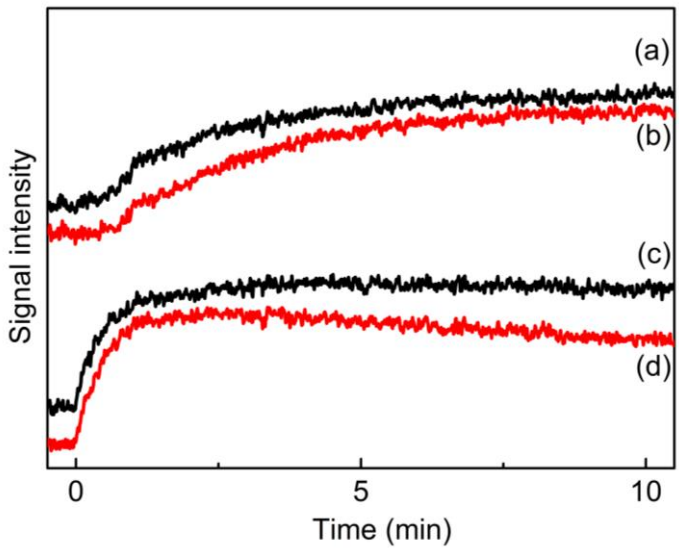

Figure S5: Time course of CW EPR signals of the (a, c) untreated and (b, d) O_2 degassed PZ-C254S domain in the presence of DTT. The samples were illuminated (a, b) in the initial states and (c, d) dark adaptation for 30 min after initial illumination for 30 min. The illumination was started at 0 min. Laser power was 40 mW. The sample concentration was 40 μM. The trace acquired at 10 μM was used for subtraction. Experimental conditions: microwave frequency, 9.62 GHz; modulation frequency, 100 kHz; modulation amplitude, 15 G; time constant, 655 ms.

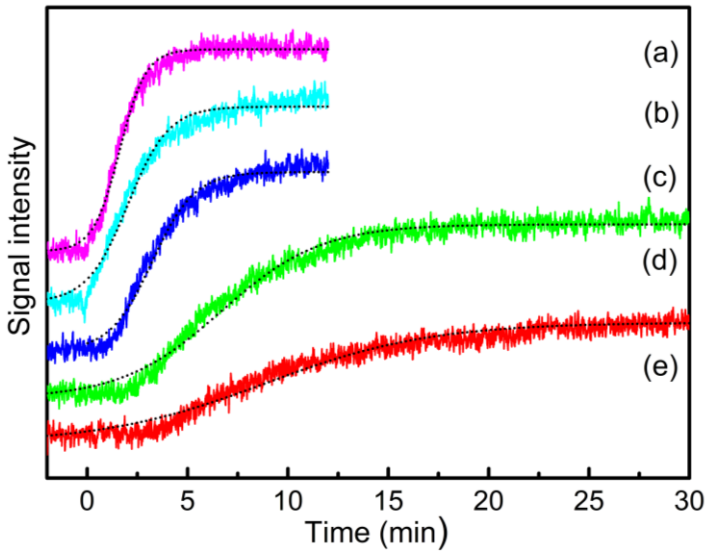

Figure S6: Laser power dependence of the time courses for LOV-C254S. The sample concentration was 40 μM. Laser power was (a) 50, (b) 40, (c) 30, (d) 20 and (e) 10 mW, respectively. The dotted lines are curves of best fit, where *k* was (a) 1.53, (b) 0.99, (c) 0.94, (d) 0.40 and (e) 0.24, respectively. The trace acquired at 10 μM was used for subtraction. Experimental conditions are the same as in fig. S2.

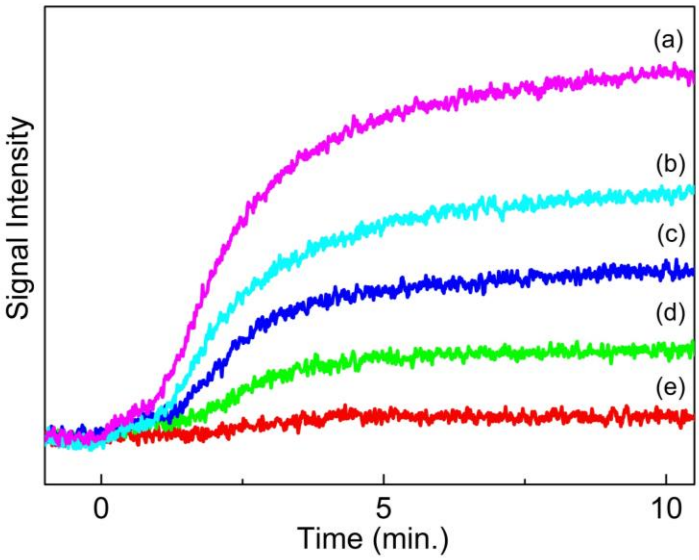

Figure S7: Time course of CW EPR of the PZ-C254S in the presence of DTT. The illumination was started at 0 min. Laser power was 30 mW. The sample concentration was (a) 100 μM, (b) 80 μM, (c) 60 μM, (d) 40 μM and (e) 20 μM, respectively. Experimental conditions: microwave frequency, 9.62 GHz; modulation frequency, 100 kHz; modulation amplitude, 15 G; time constant, 655 ms.

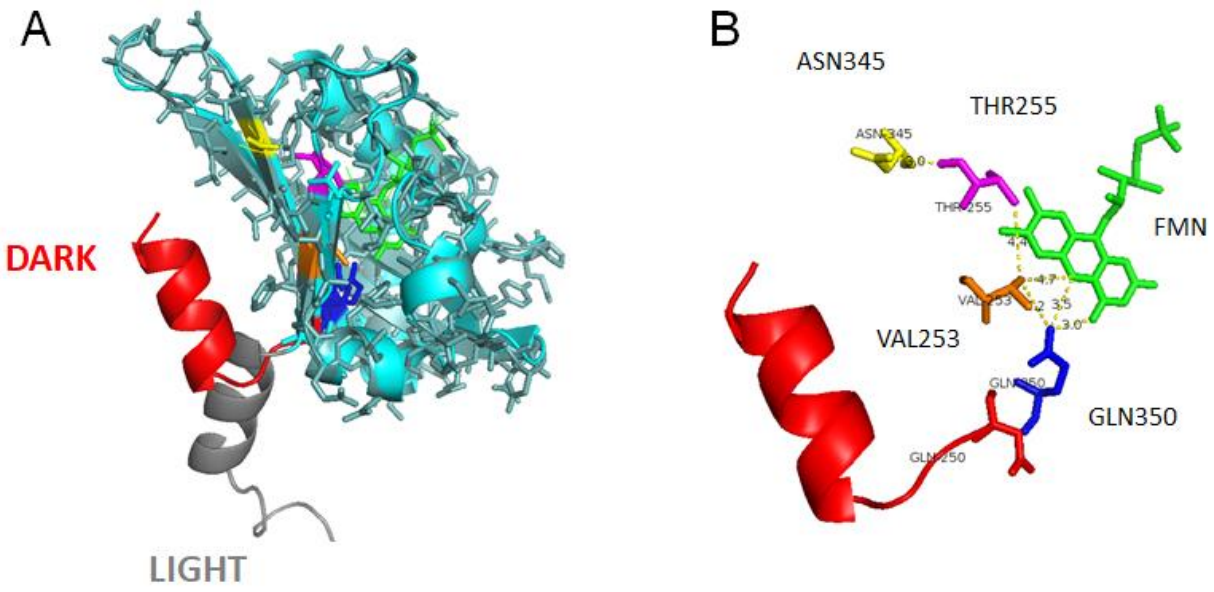

Figure S8: The illustration taken from the crystal structure of aureochrome. Panel A was overlaid model of the monomers in the dark structure (PDB:5dkk) and light structure (PDB:5dkl). Panel B: VAL253, Gln350, THR255, ASN345 and FMN were extracted from the dark structure (PDB:5dkk).