Supporting Information

Photoreaction of BlrP1: a role of nonlinear photo-intensity sensor

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SI-1 Primers for gene manipulation

Table.SI-1 Sequence of primers used in this paper.

LIC cloning of BlrP1	**Fwd**	5'-TACTTCCAATCCAATGCAATGATAACCAC-3'
	Rev	5'-TTATCCACTTCCAATGTTATTACAGGTCCA-3'
Replacement of TEV into Precision protease	**insert-Fwd**	5'-AAGTTCTGTTCCAGGGGCCCATGCTAACCACCCTG-3'
	insert-Rev	5'-ATTCCGACAGCATCGCCAGTCACTATGGCGTGCTG-3'
	vector-Fwd	5'-CGATGCTGTCGGAATGGACGATATCCCGCAAGAGG-3'
	vector-Rev	5'-CCTGGAACAGAACTTCCAGCTCGATCCCATTAGTC-3'
LIC cloning of BlrP1-BLUF	**Fwd**	5'-TACTTCCAATCCAATGCAATGATAACCAC-3'
	Rev	5'-TTATCCACTTCCAATGTTATTAGCCGCCGT-3'
T337W mutant	**Fwd**	5'- ATTAGCGGCTGGAAACAGGCGATTGTC-3'
	Rev	5'-CTGTTTCCAGCCGCTAATATGGATATC-3'

For primers of LIC cloning, LIC-tag is underlined.

SI-2 TG signal and SEC profile of a monomer mutant, T337W

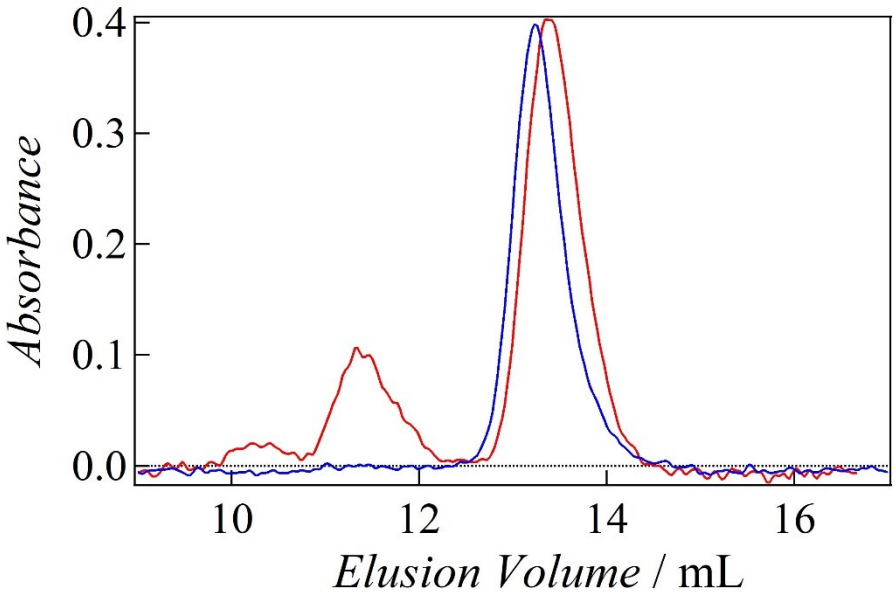

Fig.SI-1

Elution profiles of 200 μM BlrP1 (red) and 190 μM T337W (blue). Peak of larger oligomer disappears for T337W indicating that T337W exists as a monomer.

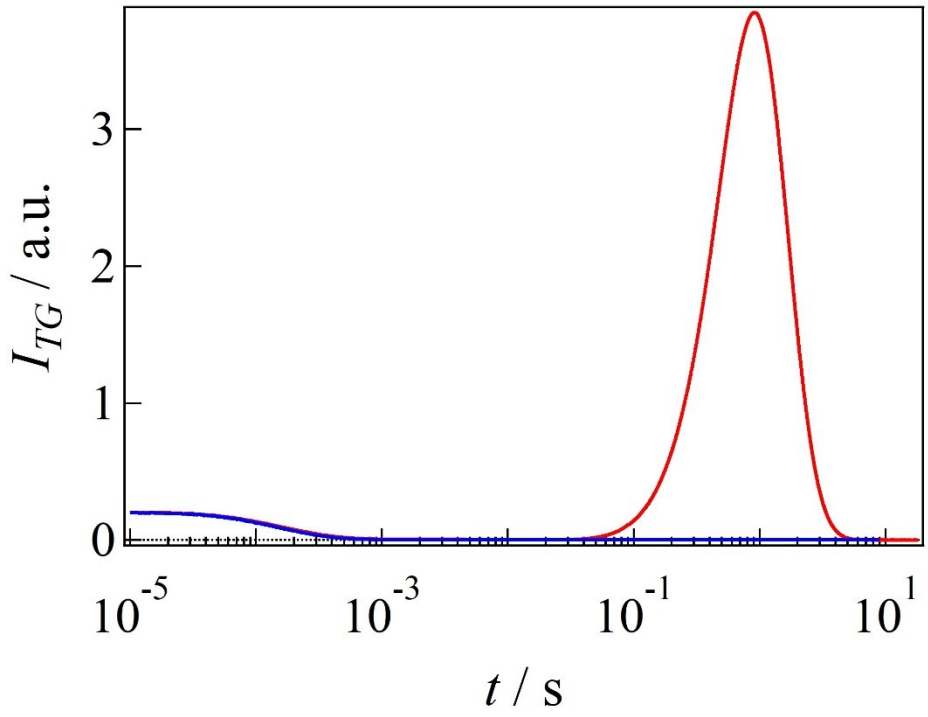

Fig.SI-2

TG signals of 200 μM BlrP1(red) and 200 μM T337W(blue) in 25 mM Trs-HCl, 300 mM NaCl, 5 mM $MgCl_2$, 2 mM EDTA, 2 mM DTT, 5% glycerol, pH 8.0 buffer. Both the signals were normalized at the species grating signal intensity. The diffusion signal of T337W almost disappeared.

SI-3 Excitation light intensity dependence of the diffusion signal

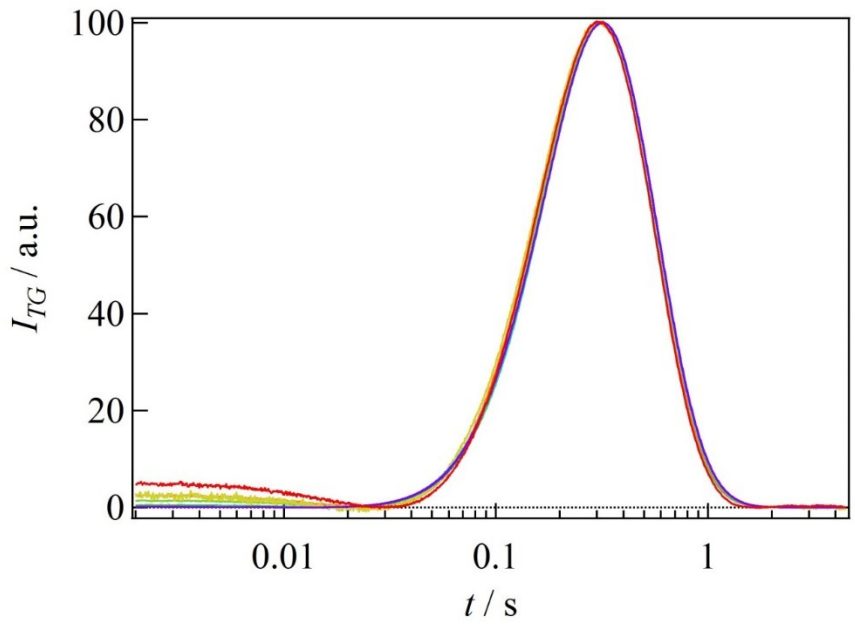

Fig.SI-3

The diffusion signal of 200 μM BlrP1 normalized at peak intensity. Red: lowest laser power, purple: highest laser power. A. $q^2=7.9\times10^{10}$ m^{-2}. The signal does not depend on the excitation laser intensity.

Range of excitation laser intensity is ~5 to ~1000 Jm^{-2} per a pulse.

SI-4 Absorption spectrum change of BlrP1 upon photoexcitation with various light intensities

We measured absorption spectrum of BlrP1 upon photoexcitation with various light intensities to examine a possibile reaction of the chromophore by the photoexcitation of the red-shifted state (Fig.SI-4). If absorption spectrum changes by the photoexcitation of the red-shifted state, the spectrum measured at strong light irradiation should be different from the spectrum measured at weak light irradiation. However, the absorption spectra show isosbestic points indicating that there are two chromophore states (dark and light states).

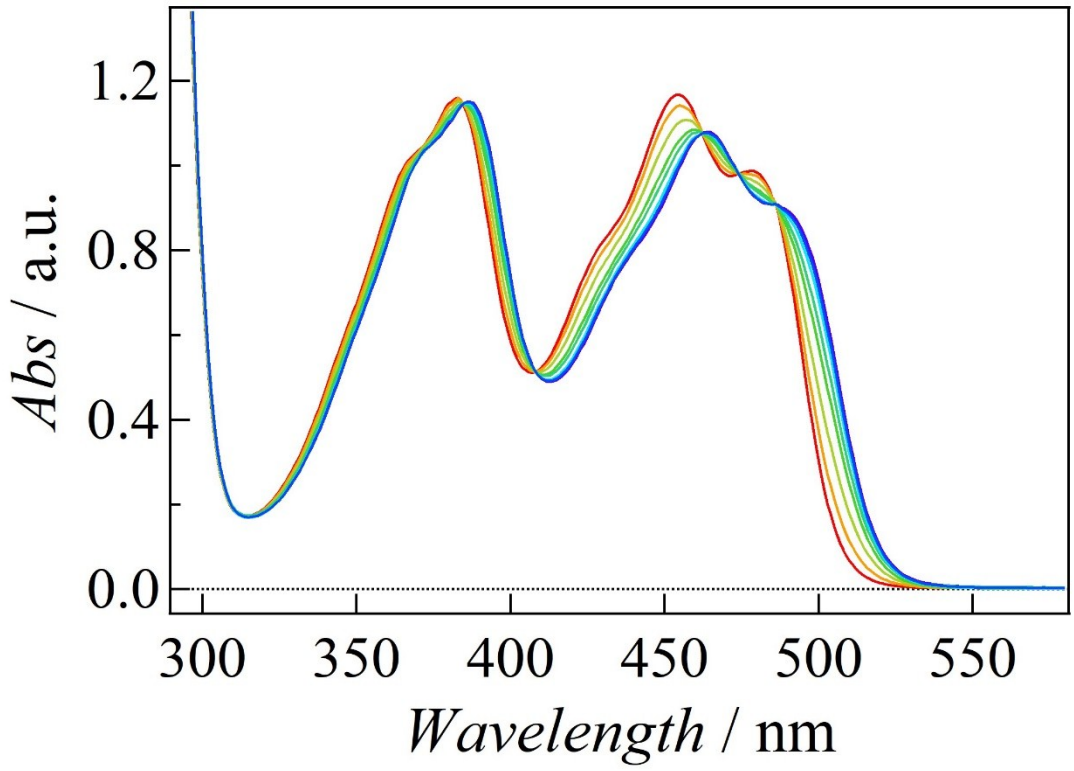

Fig. SI-4

Absorption spectra of BlrP1 measured during light irradiation with various light intensities. The light intensities were varied in a range of 30 mW/cm^2 (blue line) -0.1 mW/cm^2 (orange line) and the red line represent the spectrum of the dark state.

SI-5 Analysis of the light intensity dependence of the diffusion signal

In this section, we describe a method to analyze the light intensity dependence of the diffusion signal of BlrP1.

The peak intensity of the diffusion signal, which represents the amount of species that exhibits the conformation change, is given by

$$I_{TG}(peak)= \alpha\{\delta n_P \exp(-D_P q^2 t_{peak}) - \delta n_R \exp(-D_R q^2 t_{peak})\}^2 \quad \text{(SI-1)}$$

where t_{peak} is the peak time of the diffusion signal. Since the diffusion peak is a sum of contributions of the one-monomer-unit excited dimer ($\delta n_{1(}t_{peak})$) and the two-monomer-unit excited dimer ($\delta n_2(t_{peak})$), it is written as

$$I_{TG}(peak)=\alpha\{\delta n_{1(}t_{peak}) + \delta n_2(t_{peak})\}^2 \quad \text{(SI-2)}$$

The diffusion signal was normalized by the amount of the red-shifted state ($I_{TG}(R)$), which is given by

$$I_{TG}(R)= \alpha(\delta n_I - \delta n_R)^2 \quad \text{(SI-3)}$$

for eq.(3) in main text. Hence, we analyzed the light intensity dependence of

$$I_{TG}(peak)/ I_{TG}(R)= \{\delta n_{1(}t_{peak}) + \delta n_2(t_{peak})\}/ (\delta n_I - \delta n_R)\}^2. \quad \text{(SI-4)}$$

The saturation effect of light absorption is usually written as[1]

$$\alpha_n=\alpha_0/(1+I_L/I_S)$$

where α_n is the measured non-linear absorption coefficient, α_0 is the absorption coefficient at low light intensity, I_L is the laser intensity, and I_S is the saturation intensity. Therefore, the total concentration of the photoexcited monomer units (N_{ex}) is proportional to α_n times I_L.

$$N_{ex} \propto I_L\alpha_n \propto \alpha_0 I_L/(1+I_L/I_S) \propto \alpha_0 I_S I_L/(I_L+I_S)$$

The fraction of the photoexcited protein f_{ex}, which is given by N_{ex} divided by the total protein concentration, is expressed by

$$f_{ex} = I_L/(I_L+I_S) \qquad \text{(SI-5)}$$

Since the two-monomer-unit excitation of a dimer is a two-photon process, the fraction of two-monomer-unit excitation of a dimer (f_2) is proportional to the square of f_{ex};

$$f_2 = f_{ex}{}^2 = \{I_L/(I_L+I_S)\}^2 \qquad \text{(SI-6)}$$

The fraction of the one monomer excitation f_1 is given by $f_1 = f_{ex}-f_2$. The laser power dependence of f_{ex}, f_2 and f_1 are calculated and shown in Fig.SI-5.

The refractive index change after the thermal grating component and before the diffusion signal, $(\delta n_I - \delta n_R)$, is proportional to the concentration of photo-created red-shifted state, which is proportional to f_{ex}. Therefore, $(\delta n_I - \delta n_R)$ is given by

$$(\delta n_I - \delta n_R) = a\, I_L/(I_L+I_S) \qquad \text{(SI-7)}$$

where a is a proportional constant. Since the diffusion signal intensity due to the two excited monomers should be proportional to f_2, it is given by

$$\delta n_{2(}t_{peak}) = ab_2\{I_L/(I_L+I_S)\}^2 \qquad \text{(SI-8)}$$

where b_2 is a proportional constant representing the relative amplitude of the diffusion peak to $(\delta n_I - \delta n_R)$ due to the two excited monomers. The diffusion signal intensity due to the one excited monomer is given by

$$\delta n_{1(}t_{peak}) = ab_1\{I_L/(I_L+I_S) - (I_L/(I_L+I_S))^2\} \qquad \text{(SI-9)}$$

where b_1 is a proportional constant representing the relative amplitude of the diffusion peak to $(\delta n_I - \delta n_R)$ due to the one excited monomers.

Using these equations, the excitation light intensity dependence of the peak intensity of the diffusion signal relative to the $I_{TG}(R)$ (eq.(SI-4)) is expressed by,

$$\frac{I_{TG}(peak)}{I_{TG}(R)} = \left(b_1 + c\frac{I_L}{I_L+I_S}\right)^2 \qquad \text{(SI-10)}$$

where $c = (b_2 - b_1)$. The laser intensity dependence of the diffusion signal intensity was well reproduced by this equation (Fig.7) with the parameters of b_1=1.3±3.2, b_2=581±18, and I_s=86±14 J/m^2. The very small b_1 compared with b_2 (b_1: b_2 =1:447) indicates that the diffusion signal of the one excited monomer is very weak compared with that of the two excited monomers.

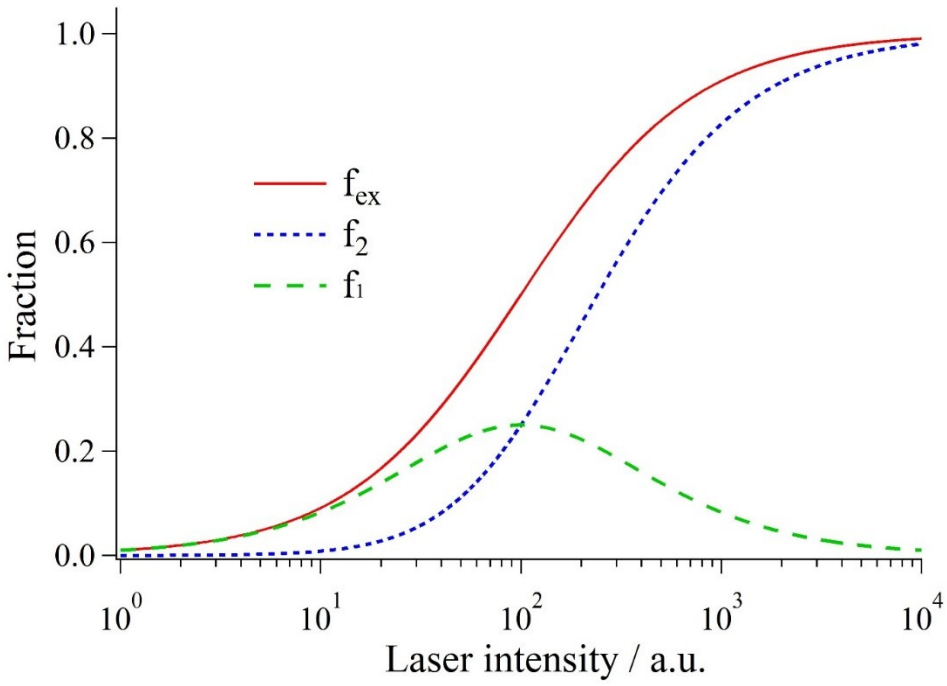

Fig.SI-5 Calculated laser intensity dependence of fraction of the excited species to total concentration, f_{ex}, $f_{2,}$ and f_1. For this calculation, I_S =100 was used.

Reference

1. Demtroeder, W. (2008) Laser Spectroscopy: Vol. 2: Experimental Techniques, fourth ed, Springer-Verlag, Berlin, Heidelberg.