Supporting Information (SI) for

Time-Resolved Fluctuation during the Photochemical Reaction of a Photoreceptor Protein: Phototropin1LOV2-Linker

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SI-1 Principles of the TG and TrL Methods

(a) The TG Method. The principle of the TG method has been described previously.[1, 2] Briefly, the TG method detects the refractive index change (δn), which is generated by the photo-excitation of chemical species, as the intensity of a diffracted probe beam. There are mainly three contributions to the TG signal: the thermal grating (δn_{th}) due to the thermal energy from photoexcited molecules, the changes in the partial molar volume (δn_{V}: the volume grating) and the changes in the absorption spectrum (δn_{pop}: population grating). The sum of the volume and population grating components are referred to as the species grating component. The TG signal intensity as a function of time is given by:

$$I_{\mathrm{TG}}(t) = \alpha\{\delta n_{\mathrm{th}}(t) + \delta n_{\mathrm{P}}(t) - \delta n_{\mathrm{R}}(t)\}^2, \qquad \text{(S-1)}$$

where α is a constant and the other terms, $\delta n_{\mathrm{P}}(t)$ and $\delta n_{\mathrm{R}}(t)$, are the refractive index changes due to

the product and the reactant, respectively.

The thermal grating component decays single exponentially with a rate constant of $D_{th}q^2$ (D_{th}: thermal diffusivity, q: grating wavenumber).

$$\delta n_{th}(t) = \delta n_{th}\exp(-D_{th}q^2 t) \tag{S-2}$$

The amplitude of the thermal grating is proportional to the thermal energy released from the photo-excited molecules and δn_{th} is expressed by:

$$\delta n_{th} = \frac{dn}{dT}\frac{h\nu\phi_{th}W}{\rho C_p}\Delta N \tag{S-3}$$

where ΔN is the number of photoexcited molecules in a unit volume (mol L^{-1}), ϕ_{th} is the quantum yield of the thermal releasing process, $h\nu$ is the photon energy of the excitation light (J mol^{-1}) and the following are parameters of the solvent water: W is the molecular weight (g mol^{-1}), n is the refractive index, ρ is the density (g L^{-1}), C_p is the heat capacity (J K^{-1} mol^{-1}). The amplitude of the thermal grating of a calorimetric reference sample that releases photon energy promptly as the thermal energy without any reaction is given by Eq. (S-3) with $\phi_{th} = 1$.

The amplitude of the volume grating (δn_V) is given by:

$$\delta n_V = \left(V\frac{dn}{dV}\Phi\Delta N\right)\Delta V \tag{S-4}$$

where Φ is the quantum yield of the reaction, ΔV is the partial molar volume change induced by the photoreaction (cm^3 mol^{-1}), and V is the molar volume of the solution. Here, we assume that V is

given by the molar volume of water. Here, ΔV was calculated by taking a ratio of δn_V to δn_{th} of the calorimetric reference (δn_{th}^{ref}) measured under the same condition. Then ΔV is expressed as:

$$\Delta V = \left(\frac{1}{V}\frac{dV}{dT}\right)\frac{h\nu W}{\rho C_p \Phi}\left(\frac{\delta n_V}{\delta n_{th}^{ref}}\right) = \frac{\alpha_{th} h\nu W}{\rho C_p \Phi}\left(\frac{\delta n_V}{\delta n_{th}^{ref}}\right) \tag{S-5}$$

where α_{th} represents the thermal expansion coefficient of the solvent. The physical parameters of the solvent water (α_{th}, W, ρ, C_p) were obtained from the literature.[3]

The amplitudes of the species grating become weaker as the spatial modulations of the refractive indices become uniform, which are accomplished by the molecular translational diffusion. The temporal profiles of the reactant and the product (including the intermediate) are given by[1]:

$$\delta n_R(t) = \delta n_R \exp(-D_R q^2 t) \tag{S-6a}$$

$$\begin{aligned}\delta n_P(t) = {} & \delta n_I \exp\{-(D_I q^2 + k)t\} \\ & + \delta n_P \frac{k}{(D_P - D_I)q^2 - k}[\exp\{-(D_I q^2 + k)t\} - \exp(-D_P q^2 t)]\end{aligned} \tag{S-6b}$$

where D and δn indicate the diffusion coefficient and the refractive index change, respectively. Furthermore, the subscripts R, I and P denote the reactant, intermediate and the product, respectively. If the reaction completes in a fast time range (faster than the observation time window) and the molecular diffusion coefficient (D) is time-independent, the time profile of the diffusion signal is expressed as:

$$I_{TG}(t) = \alpha\{\delta n_P \exp(-D_P q^2 t) - \delta n_R \exp(-D_R q^2 t)\}^2, \tag{S-7}$$

(b) The TrL Method. The principle of the TrL method has been described previously.[4] In the TrL experiment, a spatially Gaussian shaped laser beam is used for the excitation. The photo-induced refractive index distribution is monitored by the light intensity change at the central part of another probe beam.

$$I_{\mathrm{TrL}}(t) = \{I_{\mathrm{probe}}(t) - I_{\mathrm{probe}}(0)\}/I_{\mathrm{probe}}(0) \qquad \text{(S-8)}$$

where $I_{\mathrm{probe}}(t)$ indicates the probe light intensity at the beam center at a time t and $I_{\mathrm{probe}}(0)$ is that just before the excitation.

The signal intensity ($I_{\mathrm{TrL}}(t)$) is proportional to the photo-induced refractive index change.

$$I_{TrL}(t) = \beta\{\delta n_{\mathrm{th}}(t) + \delta n_{\mathrm{P}}(t) - \delta n_{\mathrm{R}}(t)\} \qquad \text{(S-9)}$$

where β is a constant representing the sensitivity of the system. The origins of the refractive index change are the same as for the TG case; the thermal effect (thermal lens; $\delta n_{\mathrm{th}}(t)$) and a change in chemical species by the reaction (species lens; $\delta n_{\mathrm{P}}(t)$ and $\delta n_{\mathrm{R}}(t)$), which consists of the volume lens and the population lens contributions.

A main difference between the TG and TrL signals is the time dependence of the signal components. Since the excitation beam radius for the TrL method is usually larger than the fringe length of the TG experiment, the thermal and protein diffusion processes in the TrL signal are much slower than those in the TG experiment. Furthermore, the thermal and protein diffusion processes are not expressed by the exponential function, but, for a Gaussian spatial beam, by an expression of $\delta n(t) = \delta n\{1 - 1/(1 + t_{\mathrm{c}}/2t)\}$, where t_{c} is a time constant depending on the beam radius of the excitation beam. Since the temporal profile of the TrL signal is sensitive to the actual beam spatial profile (which could deviate from the Gaussian shape), we used the experimentally observed

temporal profile from the calorimetric reference sample to calculate the TrL signal from the phot1LOV2-linker in the present research.

SI-2. Absorption Spectrum of the Photo1LOV2-linker

UV-Vis absorption spectra of phot1LOV2-linker at various pressures are depicted below.

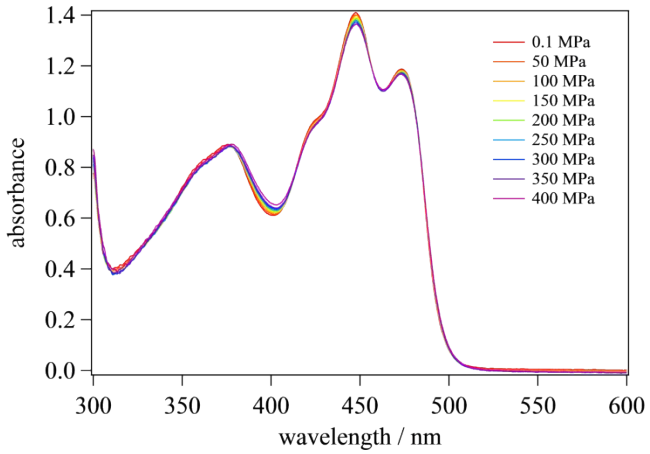

Figure S-1. UV-Vis absorption spectra of the phot1LOV2-linker in a buffer solution at various pressures. The pressures are shown in the legend of the figure. The intensity was corrected for the density change.

SI-3. TG Signal of the Phot1LOV2-linker at 0.1 MPa

A typical TG signal of the Phot1LOV2-linker in the wide time region from sub-microseconds to seconds measured at 0.1 MPa is shown below.

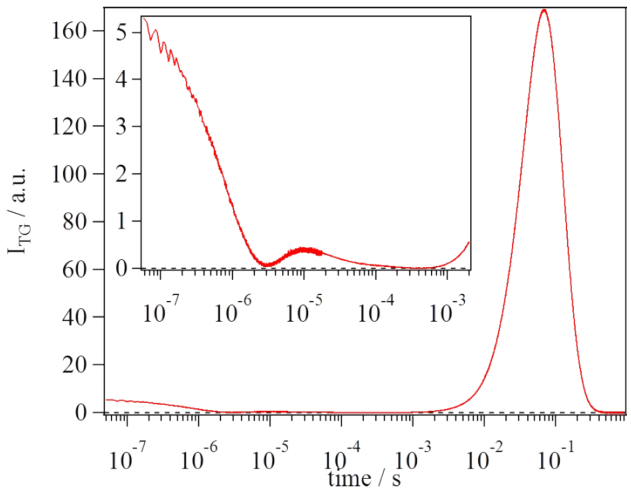

Figure S-2. Typical TG signal of the Phot1LOV2-linker measured at 0.1 MPa with $q^2 = 2.8 \times 10^{11}$ m^{-2}. The inset shows the amplified TG signal in the fast time region. The signal consists of the adduct formation (in a few μs), the thermal grating component (~30 μs), a volume contraction process associated with the transition from the S state to the T state (not clearly seen in the signal with a time constant of ~1 ms), and a peak of the molecular diffusion signal (1 ms–1 s), which represents the change in the diffusion coefficient.

Detailed analysis and features of this TG signal have been described elsewhere.[5] Briefly, this TG signal is expressed by:

$$I_{TG}(t) = \alpha\{\delta n_{ad}\exp(-k_{ad}t) + \delta n_{th}\exp(-D_{th}q^2t) + \delta n_T(t) - \delta n_D(t)\}^2 \quad \text{(S-10)}$$

where δn_{ad} and k_{ad} are the refractive index change and the rate constant of the adduct formation, respectively. Furthermore, the third and the fourth terms, $\delta n_T(t)$ and $\delta n_D(t)$, represent the unfolding reaction of the linker (S species → T species) and the diffusion processes of the T species and the D species. The time dependences of $\delta n_T(t)$ and $\delta n_D(t)$ are given by $\delta n_P(t)$ (Eq. (S-6a)) and $\delta n_R(t)$ (Eq. (S-6b)), respectively.

SI-4. Diffusion Signals at Various Pressures Normalized at the Peak Intensity

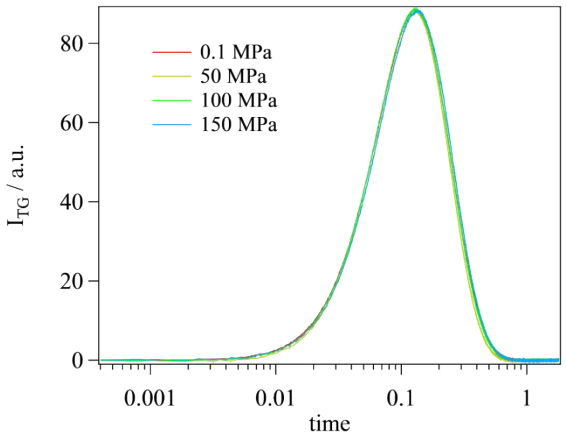

Figure S-3. Diffusion signals of the Phot1LOV2-linker at various pressures normalized at the peak intensity. The pressures are shown in the legend of the figure.

SI-5. Temperature Dependence of the TG Signal at Ambient Pressure

Temperature dependence of the TG signal of the photo1LOV2-linker at ambient pressure is shown in Fig. S-4. The signal intensity decreases slightly with increasing temperature. Since the signal intensity is proportional to the square of the T state formation yield (i.e., reaction yield is given by the root-square of the diffusion signal intensity), the temperature dependence of the fraction of the reactive species is found to be very minor. Furthermore, the diffusion peak intensity does not increase below 283 K, indicating that all molecules exist as reactive species at temperatures below 283 K. These findings indicate that most of the S species is transformed to the T state at an ambient pressure, i.e., $f_0 \sim 1$.

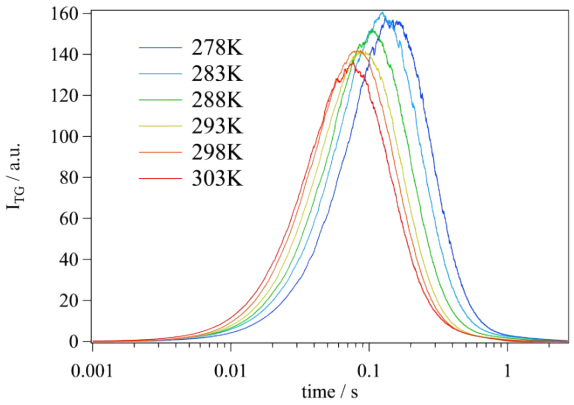

Figure S-4. Temperature dependence of the TG signal of the photo1LOV2-linker at ambient pressure measured at $q^2 = 2.5\times10^{11}$ m^{-2}.

SI-6. Calculation of the Volume Change from the Signal

The refractive index change detected by the TG and TrL methods is related to the volume change and the thermal contribution, if the absorption spectrum does not change during the reaction. Since the absorption spectrum of the S and T states are identical for the phot1LOV2-linker, the refractive index change during the S → T transformation ($\delta n_T - \delta n_S$) is decomposed into the thermal ((δn_T –

$\delta n_S)_{th}$) and volume ($(\delta n_T - \delta n_S)_V$) contributions.

The thermal contribution comes from the enthalpy change associated with the S → T transition (ΔH(S-T)). Hence, $(\delta n_T - \delta n_S)_{th}$ normalized by the thermal contribution of the calorimetric reference sample is given by:[6, 7]

$$\frac{(\delta n_T - \delta n_S)_{th}}{\delta n_{th}^{ref}} = -f(P)\frac{\Phi \Delta H(S-T)}{h\nu} \tag{S-11}$$

where f(P) is the fraction of reactive molecules at a pressure of P MPa.

Using Eq.(S-5), the volume grating term ($(\delta n_T - \delta n_S)_V$) can be written as:[6-8]

$$\frac{(\delta n_T - \delta n_S)_V}{\delta n_{th}^{ref}} = f(P)\frac{\rho C_p \Phi}{\alpha_{th} h\nu W}\Delta V(S-T) \tag{S-12}$$

where ΔV(S-T) is the volume change associated with the S → T transition. Therefore, the total refractive index change ($\delta n_T - \delta n_S$) is given by:

$$\frac{\delta n_T - \delta n_S}{\delta n_{th}^{ref}} = \frac{\rho C_p \Phi}{\alpha_{th} h\nu W} f(P)\left\{\Delta V(S-T) - \frac{\alpha_{th} W}{\rho C_P}\Delta H(S-T)\right\} \tag{S-13}$$

From the signal intensity $\frac{\delta n_T - \delta n_S}{\delta n_{th}^{ref}}$ and the physical parameters in Eq. (S-13), the volume change can be calculated and the enthalpy change corrected by the pressure dependent fraction f(P) using a relation of:

$$f_0\{\Delta V(S-T) - \frac{\alpha_{th}W}{\rho C_P}\Delta H(S-T)\} = \frac{\delta n_T - \delta n_S}{\delta n_{th}^{ref}}\left(\frac{h\nu\alpha_{th}W}{\Phi\rho C_P}\right)\left(\frac{f_0}{f(P)}\right) \quad \text{(S-14)}$$

This equation was used for the analysis of the TrL experiment in the main text (Section 3.2). If the thermal contribution can be ignored in the signal due to a fast thermal diffusion (accomplished, e.g., under the TG condition), the volume change can be calculated by:

$$f_0\Delta V(S-T) = \frac{\delta n_T - \delta n_S}{\delta n_{th}^{ref}}\left(\frac{h\nu\alpha_{th}W}{\Phi\rho C_P}\right)\left(\frac{f_0}{f(P)}\right) \quad \text{(S-15)}$$

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