

# Supporting Information

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## SI Text

**Transient Grating Signal of TePixD at 0.1 MPa.** The typical transient grating (TG) signal of TePixD in the wide time region from submicroseconds to seconds was measured at 0.1 MPa (Fig. S1). Detailed analysis and features of this TG signal have been described elsewhere (1).

**Absorption Spectrum of TePixD.** UV-Vis absorption spectra of TePixD (Fig. S2), transient absorption difference spectra and temporal profile after the photoexcitation at various pressures (Figs. S3 and S4) are depicted below.

**Principles.** Detailed principles of the TG method have been reported previously (2, 3). Here, we briefly introduce only the basic concepts and principles that are necessary to understand this study. In the TG method, the refractive index change ( $\delta n$ ), which is generated by the photo-excitation of chemical species, is detected as the intensity of a diffracted probe beam. This diffracted beam is the TG signal. Under a weak diffraction condition and a condition where the probe beam is not absorbed, the intensity of the TG signal [ $I_{TG}(t)$ ] is proportional to the square of  $\delta n$ . The refractive index change arises from two factors owing to the temperature increase of the solution by the heat release (thermal grating;  $\delta n_{th}$ ) and photo-chemical reactions of the excited species (species grating;  $\delta n_{spe}$ ), both of which are generally time dependent. Therefore

$$I_{TG}(t) = \alpha [\delta n_{th}(t) + \delta n_{spe}(t)]^2, \quad [S1]$$

where  $\alpha$  is an experimental constant reflecting the sensitivity of the setup.  $\alpha$  has been validated to be constant even under the application of pressure (4).

The amplitude of the thermal grating is proportional to the thermal energy released from the photo-excited molecules, and  $\delta n_{th}$  is expressed as

$$\delta n_{th} = \frac{dn}{dT} \frac{h\nu\phi W}{\rho C_p} \Delta N, \quad [S2]$$

where  $\Delta N$  is the number of reaction molecules in a unit volume ( $\text{mol}\cdot\text{L}^{-1}$ ),  $\phi$  is the quantum yield of the thermal releasing process,  $h\nu$  is the photon energy of the excitation light ( $\text{J}\cdot\text{mol}^{-1}$ ), and the following are parameters of the solvent water:  $W$  is the molecular weight ( $\text{g}\cdot\text{mol}^{-1}$ ),  $n$  is the refractive index,  $\rho$  is the density ( $\text{g}\cdot\text{L}^{-1}$ ), and  $C_p$  is the heat capacity ( $\text{J}\cdot\text{K}^{-1}\cdot\text{mol}^{-1}$ ). For a calorimetric reference sample that releases photon energy promptly as thermal energy without any reaction,  $\phi$  is unity.

The species grating  $\delta n_{spe}$  consists of two contributions: the changes in the partial molar volume ( $\delta n_V$ : the volume grating) and the changes in the absorption spectrum ( $\delta n_{pop}$ : population grating). The volume grating ( $\delta n_V$ ) is given by

$$\delta n_V = \left( V \frac{dn}{dV} \Phi \Delta N \right) \Delta \bar{V}, \quad [S3]$$

where  $\Phi$  is the quantum yield of the reaction,  $\Delta \bar{V}$  is the partial molar volume change induced by the photoreaction ( $\text{cm}^3\cdot\text{mol}^{-1}$ ), and  $V$  is the molar volume of the solution. Here we approximate it by the molar volume of water.  $\Delta \bar{V}$  was calculated by taking a ratio of  $\delta n_V$  to  $\delta n_{th}$  of the calorimetric reference ( $\delta n_{th}^{ref}$ ; i.e., di-

viding Eq. S3 by Eq. S2) measured under the same condition as the sample. Then  $\Delta \bar{V}$  is expressed as

$$\Delta \bar{V} = \left( \frac{1}{V} \frac{dV}{dT} \right) \frac{h\nu W}{\rho C_p \Phi} \left( \frac{\delta n_V}{\delta n_{th}^{ref}} \right) = \frac{\alpha_{sol} h\nu W}{\rho C_p \Phi} \left( \frac{\delta n_V}{\delta n_{th}^{ref}} \right), \quad [S4]$$

where  $\alpha_{sol}$  represents the thermal expansion coefficient of the solvent, and  $\Phi$  is the quantum yield of the photoreaction of TePixD, which has been reported to be 0.29 (5). The physical parameters of the solvent water ( $\alpha_{sol}$ ,  $W$ ,  $\rho$ , and  $C_p$ ) were obtained from the literature. With only the experimental values in the last parentheses,  $\Delta \bar{V}$  was determined.

Transient compressibility of reaction intermediates can be obtained from the pressure dependence of the species grating ( $\delta n_{spe}$ ). Of the two components of the species grating ( $\delta n_{pop}$  and  $\delta n_V$ ),  $\delta n_{pop}$  can be regarded as pressure independent, because the transient absorption of TePixD was almost independent of pressure. Therefore, provided that  $\Delta$  represents any change caused by pressure, we obtain

$$\Delta \delta n_{spe} = \Delta (\delta n_{pop} + \delta n_V) \approx \Delta \delta n_V. \quad [S5]$$

Here  $\delta n_V$  is related to the partial molar volume change given in Eq. S3. Regarding the term in the parentheses in Eq. S3, we confirmed that  $\Phi \Delta N$  was almost constant under high pressures by the transient absorption method.  $dn/dV$  can be also regarded as constant because the pressure dependences of both the refractive index ( $n$ ) and the partial molar volume ( $V$ ) of the water are negligibly small. Hence, the proportional constant between  $\Delta \bar{V}$  and  $\Delta \delta n_V$  in Eq. S3 can be regarded as constant. Then, we obtain from Eq. S5

$$\Delta \delta n_{spe} \approx \Delta \delta n_V \approx \left( V \frac{dn}{dV} \Phi \Delta N \right) \Delta \Delta \bar{V}_{g \rightarrow e}, \quad [S6]$$

where  $\Delta \bar{V}_{g \rightarrow e}$  means the volume change from the ground state to the excited state. If we know the volume change in Eq. S3 at 0.1 MPa, we can determine  $\Delta \Delta \bar{V}_{g \rightarrow e}$  at any pressure by comparing  $\Delta \delta n_{spe}$  with  $\delta n_V$  of 0.1 MPa. With the pressure dependence of  $\Delta \bar{V}_{g \rightarrow e}$ , the compressibility change between the ground and excited states ( $\Delta \bar{K}_{g \rightarrow e}$ ) was finally determined by Eq. S7

$$-\frac{d\Delta \bar{V}_{g \rightarrow e}}{dP} = \Delta \bar{K}_{g \rightarrow e}. \quad [S7]$$

Using the fitting parameters of Eq. 1, the species grating ( $\delta n_{spe}$ ) for  $I_1$  and  $I_2$  states of TePixD can be expressed as  $\delta n(I_1) = \delta n_V + A$  and  $\delta n(I_2) = A$ , where  $A$  is defined by Eq. S9. Here, we determined the volume change ( $\Delta \bar{V}$ ) corresponding to  $\delta n_V$  as  $\Delta \bar{V} = 4 \pm 0.5 \text{ cm}^3$  per 1 mol of TePixD monomer by taking the ratio of  $\delta n_V$  to the thermal grating ( $\delta n_{th}$ ) of the calorimetric reference. Therefore, an increase (or decrease) in  $\delta n_{spe}$  provides the pressure-induced change in  $\delta n_V$  (i.e.,  $\Delta \delta n_V$ ), and it further provides the changes in  $\Delta \bar{V}_{g \rightarrow e}$  with changing pressure.

**Derivation of Eq. 1.** The molecular diffusion signal of TePixD is generally expressed by the below two-state model equation, which assumes the reaction scheme that the reactant ( $R$ ) is converted to the intermediate ( $I$ ) immediately by photo-excitation, and the product ( $P$ ) is produced from  $I$  at the reaction rate of  $k$

(i.e.,  $R \xrightarrow{h\nu} I \xrightarrow{k} P$ ). The mathematical expression of the two-state model is as follows (2):

$$I_{TG}(t) = \alpha \left[ \delta n_I \exp[-(D_I q^2 + k)t] + \delta n_P \frac{k}{(D_P - D_I)q^2 - k} \times \{ \exp[-(D_I q^2 + k)t] - \exp(-D_P q^2 t) \} - \delta n_R \exp(-D_R q^2 t) \right]^2 \quad [\text{S8}]$$

The diffusion coefficient ( $D$ ) of each species and reaction rate  $k$  have been reported as  $D_R = 4.9 \times 10^{-11} \text{ m}^2 \cdot \text{s}^{-1}$ ,  $D_I = 4.4 \times 10^{-11} \text{ m}^2 \cdot \text{s}^{-1}$ ,  $D_P = 3.2 \times 10^{-11} \text{ m}^2 \cdot \text{s}^{-1}$ , and  $k = 250 \text{ s}^{-1}$ . In the observation time here ( $t < 2 \times 10^{-4} \text{ s}$ ) and the grating wave number ( $q^2 = 3.5 \times 10^{12} \text{ m}^{-2}$ ), each term in the exponential function  $[(D_I q^2 + k)t, D_P q^2 t, D_R q^2 t]$  becomes smaller than 0.1. Then, each exponential term can be well approximated as  $e^{\alpha x} \approx 1 + \alpha x$ , and we obtain a much simpler expression

$$I_{TG}(t) = \alpha(A + Bt)^2 [A = \delta n_I - \delta n_R B = (\delta n_P - \delta n_I)k + (\delta n_R D_R - \delta n_I D_I)q^2]. \quad [\text{S9}]$$

Eq. S9 is a simplified expression to fit the diffusion signal in a fast time range. Taking the other two components (thermal diffusion signal and volume change signal) into account, we obtain Eq. S10 (which is the same as Eq. 1)

$$I_{TG}(t) = \alpha [\delta n_{th} \exp(-D_{th} q^2 t) + \delta n_V \exp(-k_V t) + A + Bt]^2, \quad [\text{S10}]$$

where the first term represents the thermal diffusion process ( $\delta n_{th}$ : thermal grating,  $D_{th}$ : diffusion coefficient of the heat), the middle term represents the volume expansion process ( $\delta n_V$ : volume grating,  $k_V$ : rate constant of the volume change from  $I_1$  to  $I_2$ ), the last term ( $A + Bt$ ) represents the contribution of the molecular diffusion signal, and  $\alpha$  is a proportional constant.

**TG Signal of Photo-Inactive Mutant of TePixD (Q50A).** For a negative control experiment, we measured the TG signal of a photo-inactive mutant of TePixD (Q50A). The key glutamine residue for the blue light using FAD (BLUF) photoreaction was replaced by alanine in this mutant, and it has been shown that this mutant does not create the spectral red shift species (i.e., this is a photo-inactive mutant) (7). Since this mutant does not react, we have expected that there is no volume change and the dissociation reaction and we should not observe any TG signal except the thermal grating. We prepared the Q50A TePixD mutant as described elsewhere (7, 8). Site-directed mutagenesis to gen-

erate the Q50A TePixD mutant was performed using PCR-based QuikChange site-directed mutagenesis kit (Stratagene) with primers (sense, 5'-GGCATGTTTCTGGCAACCCTTGAGGGC-3' and antisense, 5'-GCCCTCAAGGGTTGCCAGAAACATGCC-3'). The plasmid carrying the desired substitution was confirmed using nucleotide sequencing with the BigDye terminator fluorescence detection method (Applied Biosystems) and a capillary sequencer (PRISM 310 Genetic Analyzer; Applied Biosystems). The expression and purification were performed in the same way as that of WT TePixD.

As expected, the TG signal after photo-excitation of this mutant consisted of only the thermal grating signal. Fig. S5 shows the comparison of the TG signal of the WT representing the volume change and the Q50A mutant in the same time range under the same conditions (at 500  $\mu\text{M}$ ,  $q^2 = 4.68 \times 10^{12} \text{ m}^{-2}$ , laser intensity of 13  $\text{mJ} \cdot \text{cm}^{-2}$ ). This result confirms that the experimental conditions in our experiment (even at the high laser intensity and high concentration) did not cause any artifact in the signal.

**Derivation of the Expression of  $f_n$ .**  $f_n$  can be written using the equation of Poisson distribution ( $P_n$ ) as follows. When  $\lambda$  monomers in the oligomer are excited on average, the probability that  $n$  monomers in an oligomer are excited is described as

$$P_n = \frac{\exp(-\lambda) \times \lambda^n}{n!} \quad \text{where} \quad \lambda = \frac{cI}{1 + I/I_s}, \quad [\text{S11}]$$

$I$  is the laser intensity ( $\text{mJ} \cdot \text{cm}^{-2}$ ),  $c$  is a constant, and  $I_s$  is the saturation intensity.

Previously, it was reported that  $\delta n_R$  determined from the molecular diffusion signal of TePixD is proportional to  $P_1$  (6). In this study,  $\delta n_R$  at 0.1 MPa was determined at various laser powers ( $I$ ) (Fig. S6).  $\delta n_R$  corresponds to the relative number of decamers that produced the final product. This laser power dependence was well fitted by  $aP_1$  ( $a$ : proportional constant), and we determined the parameters as  $a = 2.88 \pm 0.08$ ,  $I_s = 37.2 \pm 10 \text{ mJ} \cdot \text{cm}^{-2}$ , and  $c = 0.215 \pm 0.016$ .

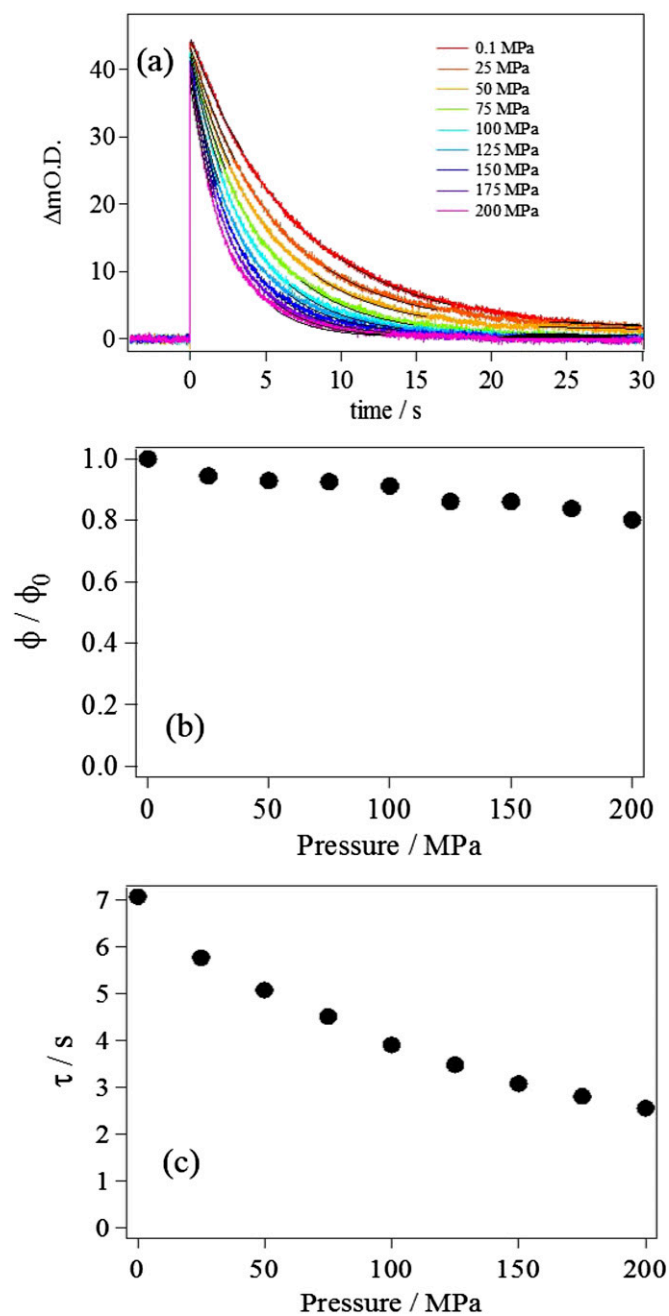
The probability that an oligomer absorbs at least one photon is expressed as  $1 - P_0$ . Hence,  $f_n$  should be expressed as

$$f_n = \frac{P_n}{1 - P_0} = \frac{\exp(-\lambda)}{1 - \exp(-\lambda)} \frac{\lambda^n}{n!}. \quad [\text{S12}]$$

Using the determined parameters in  $\lambda$  (i.e.,  $c$  and  $I_s$ ), the calculated  $f_n$  (from  $n = 1$  to  $n = 5$ ) at each excitation laser power is shown in Fig. S7. From this calculation, we estimated that the fraction of triple excited species ( $f_3$ ) is smaller than 15% of the total excited decamers below 8  $\text{mJ} \cdot \text{cm}^{-2}$ .

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**Fig. S4.** (A) Temporal profiles of the transient absorption of TePixD after excitation at 462 nm recorded at every 25 MPa from 0.1 to 200 MPa. Pressures are shown in the legend in the figure. The best-fit curves by a single-exponential function are shown by broken lines. (B) Pressure dependence of the relative quantum yield ( $\phi_0$  is the quantum yield at 0.1 MPa) of the photoreaction of TePixD, obtained from each amplitude of the time profiles of A. (C) Pressure dependence of the time constant for the dark recovery of TePixD.

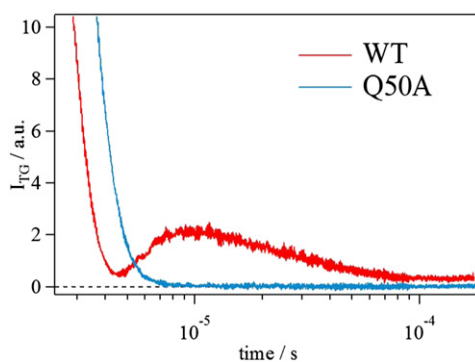


Fig. S5. The TG signals of the WT (red line) and the Q50A mutant (blue line) of TePixD measured under the same condition at  $13 \text{ mJ/cm}^2$ . The signals decaying in microseconds time range for both samples are the thermal grating component.

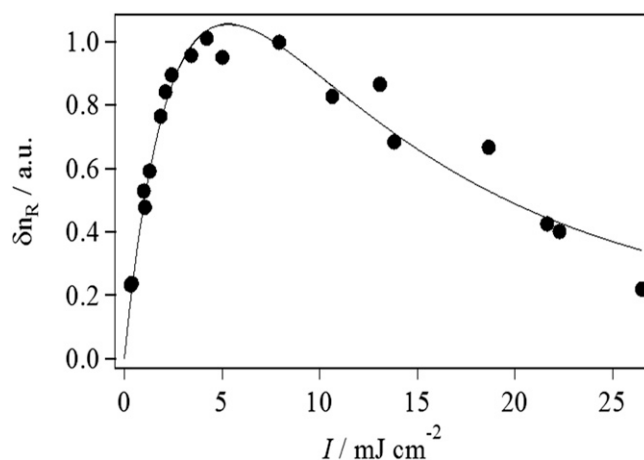


Fig. S6. Laser power dependence of  $\delta n_R$  determined from the intensity of the molecular diffusion peak of the TG signal.  $\delta n_R$  corresponds to the relative number of decamer molecules that underwent the diffusion coefficient change. The solid curve is the best fit line using the equation of Poisson distribution ( $n = 1$  in Eq. S11).

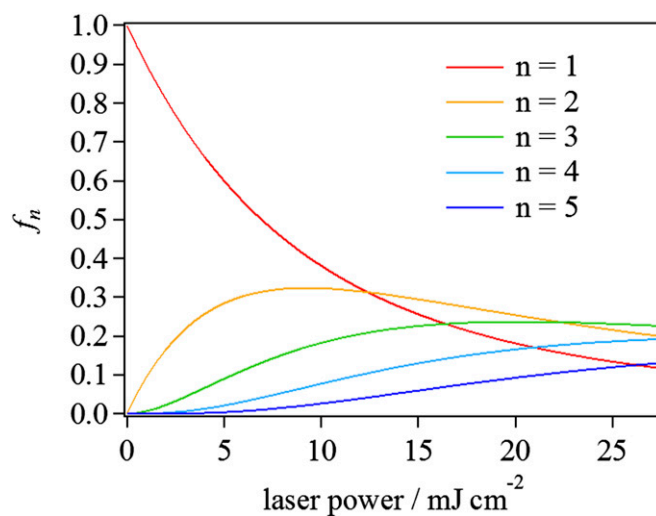


Fig. S7. Laser power dependence of the fraction of  $n$ -excited species (from  $n = 1$  to  $n = 5$ ) calculated by Eq. S12. The number  $n$  is shown by the legend in the figure.