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Reaction dynamics of a UV photosensor UVR8

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S-1. Principles

Detailed principles of the TG method have been reported previously (1, 2). Here we briefly introduce only the basic concepts and principles which are necessary in this study. In the TG method, the refractive index change (δn), 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 δn. The refractive index change arises from two factors; due to the temperature increase of the solution by the heat release (thermal grating; δn_{th}) and photo-chemical reactions of the excited species (species grating; δn_{spe}). Therefore, the TG signal intensity is given by

$$I_{TG}(t) = \alpha[\delta n_{th}(t) + \delta n_{spe}(t)]^2 \qquad \text{(S-1)}$$

where α is an experimental constant reflecting the sensitivity of the set up. The thermal grating decays single exponentially with a time constant of $D_{th}q^2$, where D_{th} is the thermal diffusivity, and q is the grating wavenumber. In this study, information on the reaction dynamics comes from the time profile of the species grating signal.

If the chemical reaction is simple as

Scheme S-1 $R \xrightarrow{h\nu} P$

the TG signal profile is given by a sum of protein diffusion of the reactant and the product;

$$I_{TG}(t) = \alpha\left[\delta n_{th}\exp(-D_{th}q^2t) - \delta n_R\exp(-D_Rq^2t) + \delta n_P\exp(-D_Pq^2t)\right] \quad \text{(S-2)}$$

where δn_P and δn_R are, respectively, the refractive index changes due to the product and reactant, and D_P and D_R are the diffusion coefficients of the the product and reactant, respectively.

If 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*, the reaction scheme is written as

Scheme S-2 $R \xrightarrow{h\nu} I \xrightarrow{k} P$

In this case, the time dependence of the refractive index of the reactant (δn_R), intermediate (δn_I), and the product (δn_P) are given by

$$\delta n_R(t) = -\delta n_R\exp(-D_Rq^2t) \quad \text{(S-3 a)}$$

$$\delta n_I(t) = \delta n_I\exp\{-(D_Iq^2+k)t\} \quad \text{(S-3 b)}$$

$$\delta n_P(t) = \frac{k}{(D_I-D_P)q^2+k}\delta n_P\left[\exp(-D_Pq^2t) - \exp\{-(D_Iq^2+k)t\}\right] \quad \text{(S-3 c)}$$

Hence, the total TG signal is given by,

$$I_{TG}(t) = \alpha\left[\delta n_{th}\exp(-D_{th}q^2t) + \left(\delta n_I - \frac{k}{(D_I-D_P)q^2+k}\delta n_P\right)\exp\{-(D_Iq^2+k)t\}\right.$$
$$\left. - \delta n_R\exp(-D_Rq^2t) + \frac{k}{(D_I-D_P)q^2+k}\delta n_P\exp(-D_Pq^2t)\right]^2 \quad \text{(S-4)}$$

When there are two intermediates (I_1 and I_2), the reaction scheme may be written as

Scheme S-3 $R \xrightarrow{h\nu} I_1 \xrightarrow{k_1} I_2 \xrightarrow{k_2} P$

where k_1 and k_2 are the decay rate constants of the first and the second intermediates, respectively. In this case,, the time profile of the TG signal is given by,

$$\begin{aligned} I_{TG}(t) = \alpha \Big\langle & \delta n_{th} \exp(-D_{th} q^2 t) - \delta n_R \exp(-D_R q^2 t) \\ & + \left[\delta n_{I_1} + \frac{k_1}{(D_{I_1} - D_{I_2}) q^2 + k_1 - k_2} \left(-\delta n_{I_2} + \frac{k_2 \delta n_P}{(D_{I_1} - D_P) q^2 + k_1} \right) \right] \exp\{-(D_{I_1} q^2 + k_1) t\} \\ & + \left[\frac{k_1}{(D_{I_1} - D_{I_2}) q^2 + k_1 - k_2} \left(\delta n_{I_2} - \frac{k_2 \delta n_P}{(D_{I_2} - D_P) q^2 + k_2} \right) \right] \exp\{-(D_{I_2} q^2 + k_2) t\} \\ & + \frac{k_1 k_2 \delta n_P}{(D_{I_1} - D_{I_2}) q^2 + k_1 - k_2} \left(\frac{1}{(D_{I_2} - D_P) q^2 + k_2} - \frac{1}{(D_{I_1} - D_P) q^2 + k_1} \right) \exp(-D_P q^2 t) \Big\rangle^2 \end{aligned} \tag{S-5}$$

S-2. SDS- PAGE measurement of C132T

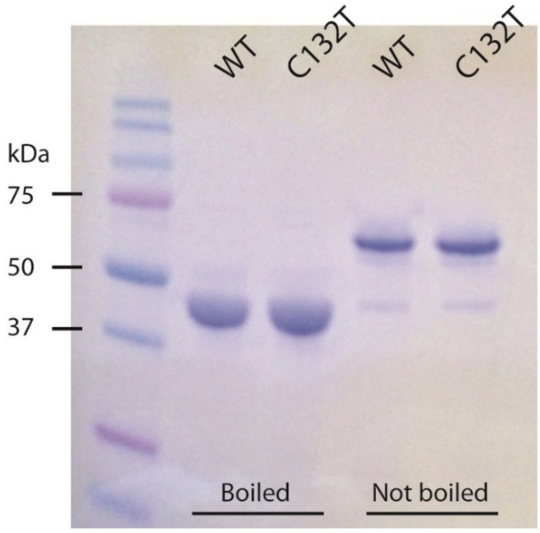

Fig.S1

SDS- PAGE of C132T showing that C132T exists as a dimer. "Boiled" means heating of the sample solution to dissociate the dimer. Both WT and C132T without heating show the dimer form.

Reference

1. Terazima M (2006) Diffusion coefficients as a monitor of reaction kinetics of biological molecules.

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2. Terazima M (2002) Molecular volume and enthalpy changes associated with irreversible photo-reactions. *Journal of Photochemistry and Photobiology C: Photochemistry Reviews* 3(2):81-108.