Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Supporting Information

Protein Dynamics of Human Serum Albumin at Hypothermic Temperatures Investigated by Temperature Jump

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1. Deriving the evolutions of the relative fluorescence intensity change

Due to the photobleaching of tryptophan upon long-term ultraviolet exposure, the fluorescence intensity of tryptophan gradually decreased during the measurements. Thus, a blank experiment was performed to eliminate the contribution of photobleaching to tryptophan fluorescence change. A schematic of the procedure is provided in **Fig. S2**. The fluorescence intensity evolutions in the absence (**Fig. S2a**) and presence of 1550 nm IR laser (**Fig. S2b**) were collected, respectively. Then they were converted to their corresponding relative fluorescence intensity change profiles, as shown in **Fig. S2c** and **S2d**, respectively, using the following equation,

$$\frac{\Delta I}{I_0} = \frac{I(t) - I_0}{I_0} \cdot 100\% \qquad \text{(Eq. S1)}$$

where I_0 and $I(t)$ denote the mean fluorescence intensity before the T-jump process and the fluorescence intensity evolution during the T-jump process, respectively. Then the corrected relative fluorescence intensity change evolution with the photobleaching eliminated, as shown in **Fig. S2e**, was derived by subtracting the time trace in **Fig. S2c** from that in **Fig. S2d**. The corrected fluorescence intensity change evolutions for tryptophan ($W(t)$) and HSA ($H(t)$) upon T-jump to different temperatures are shown as blue lines in **Fig. S3**. The corresponding uncorrected relative fluorescence intensity evolutions upon T-jump and fluorescence change evolution attributed to photobleaching are shown in red lines and grey lines in **Fig. S3**, concomitantly.

2. Supplementary figures

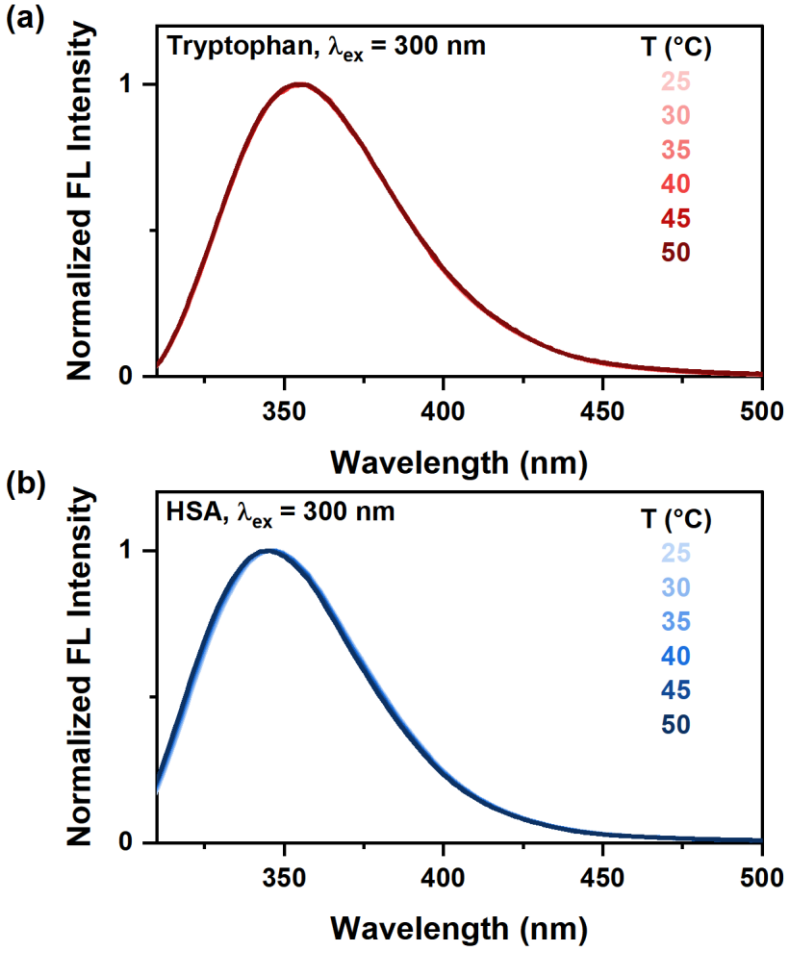

Fig. S1. The normalized fluorescence spectra of (a) tryptophan and (b) HSA at 25 – 50 °C upon 300 nm excitation.

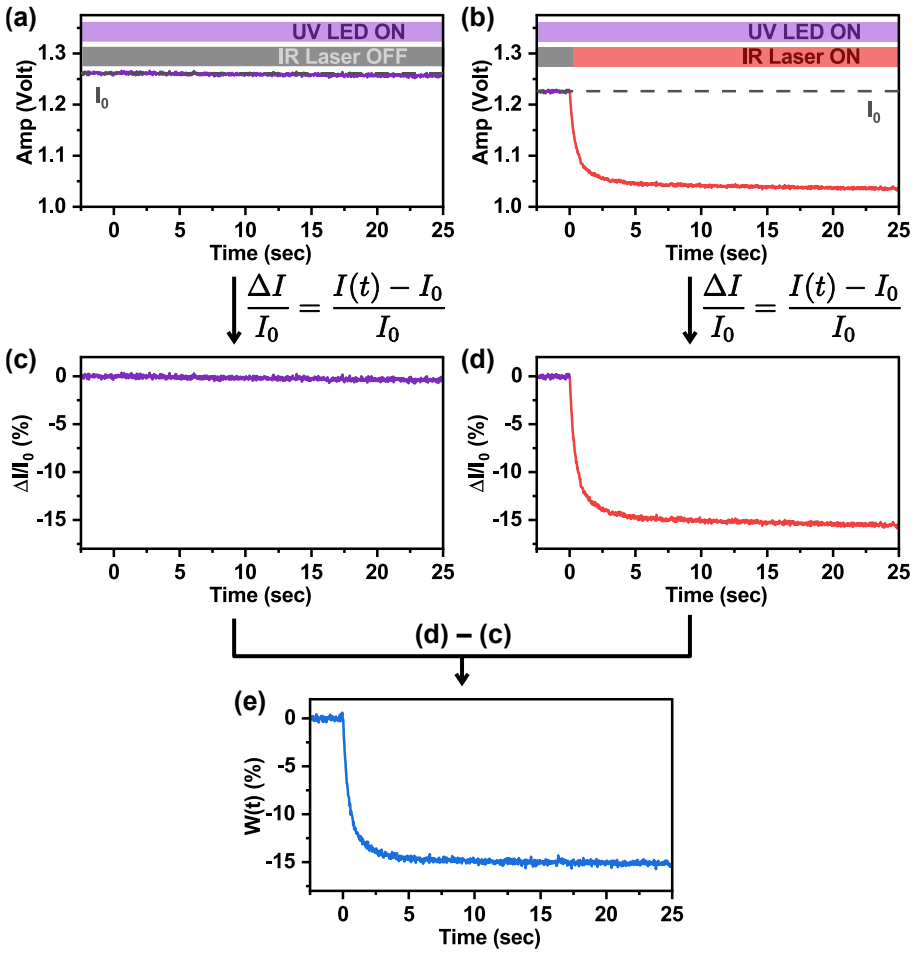

Fig. S2. The schematic procedure to generate the relative fluorescence intensity change evolution for tryptophan (W(t)) upon T-jump by eliminating the photobleaching. Detailed description is provided in **Section 1** in the Supporting Information.

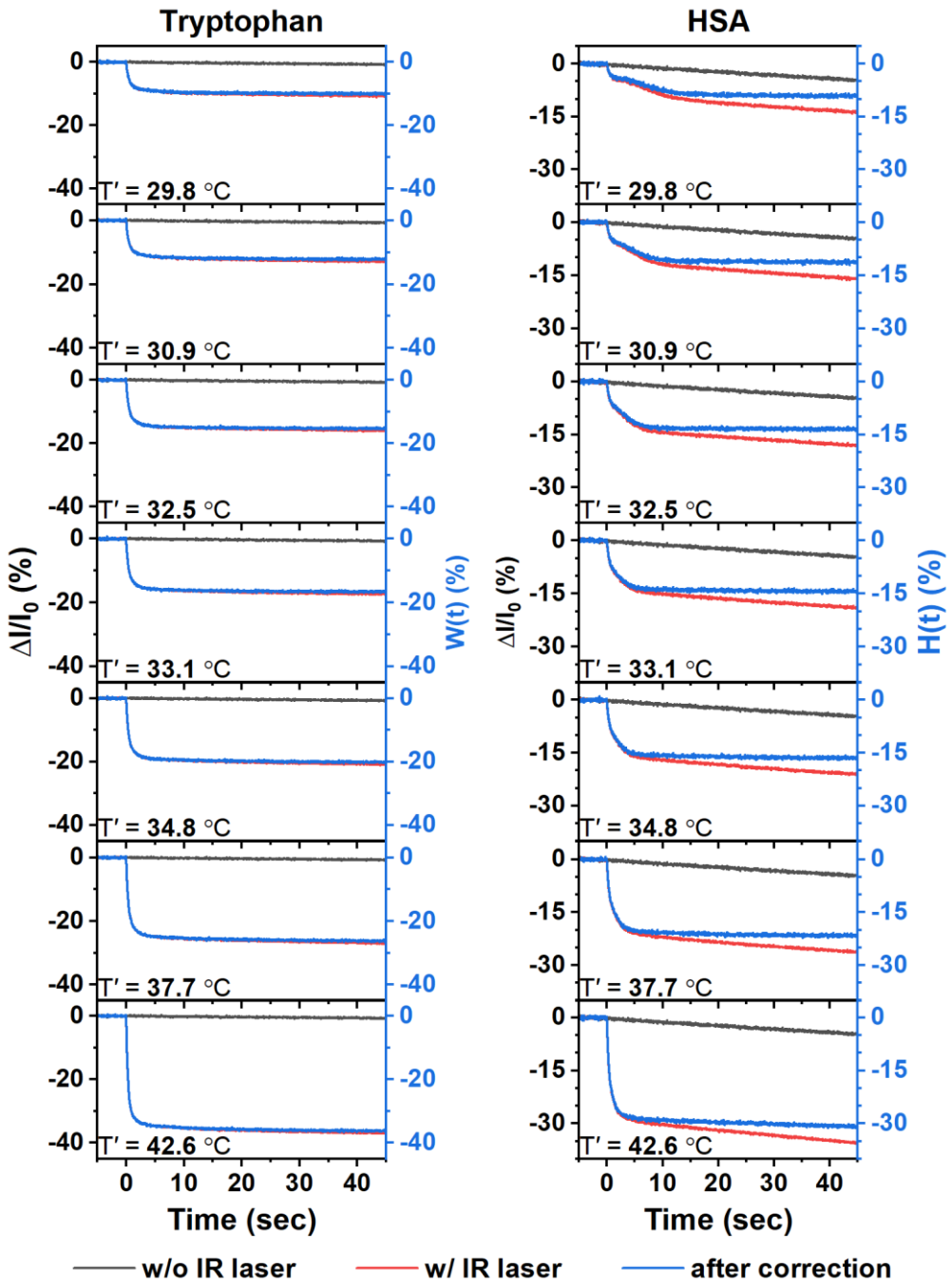

Fig. S3. Raw data of the evolutions of the fluorescence change of dissolved tryptophan (left-hand frame) and HSA (right-hand frame) without infrared laser heating (grey) and with infrared laser heating (red). The traces were averaged from triplicate experiments. The corrected profiles (blue) described in **Section 1** in Supporting Information were summarized in **Fig. 3a**. The starting temperature was 25.0 °C. The final temperatures in each experiment were determined by W(t) at stationary state within ca. 20–30 s.

3. Supplementary tables

Table S1. Laser powers of the continuous-wave 1550 nm laser for temperature jump experiments from initial temperature of 25.0 °C.

Power of 1550 nm CW Laser (Watt cm^{-2})	Jumped Temperature (T'/ °C)
14.3	29.8
17.9	30.9
24.7	32.5
28.3	33.1
32.9	34.8
47.8	37.7
78.9	42.6

Table S2. A_1, A_2, y_0, k_1, k_2, ϕ_B/ϕ_A, and ϕ_C/ϕ_A at different temperatures.

T′ (°C)	A_1	A_2	y_0*	k_1	k_2	R^2	ϕ_B/ϕ_A	ϕ_C/ϕ_A*
29.8	-0.09 ± 0.01	0.09 ± 0.01	1.01	0.70 ± 0.04	0.16 ± 0.01	0.908	1.07 ± 0.01	1.01
30.9	-0.13 ± 0.01	0.12 ± 0.01	1.01	0.87 ± 0.06	0.33 ± 0.02	0.915	1.09 ± 0.01	1.01
32.5	-0.23 ± 0.04	0.21 ± 0.04	1.02	0.98 ± 0.08	0.50 ± 0.03	0.919	1.12 ± 0.02	1.02
33.1	-0.18 ± 0.05	0.15 ± 0.05	1.03	1.26 ± 0.14	0.65 ± 0.06	0.823	1.10 ± 0.03	1.03
34.8	-0.27 ± 0.12	0.22 ± 0.12	1.05	1.44 ± 0.18	0.86 ± 0.11	0.815	1.14 ± 0.05	1.05
37.7	-0.26 ± 0.10	0.20 ± 0.10	1.06	1.79 ± 0.24	1.01 ± 0.13	0.750	1.15 ± 0.05	1.06
42.6	-0.32 ± 0.15	0.23 ± 0.16	1.09	2.46 ± 0.39	1.42 ± 0.25	0.706	1.19 ± 0.07	1.09

* uncertainty < 1×10^{-3}