Startling Temperature Effect on Proteins when Confined: Single Molecular Level Behaviour of Human Serum Albumin in Reverse Micelle

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Supplementary Information

Figure S1: Circular dichroism spectra of (a) tagged (4.96 µM) and untagged (5.03 µM) human serum albumin in buffer. (b) tagged human serum albumin (HSA) in buffer (3.74 µM) and in water-pool of AOT reverse micelle W₀=20 (overall concentration of HSA is 1.65 µM).

Figure S2: Fluorescence autocorrelation spectra of TMR tagged human serum albumin (HSA) in buffer and in AOT reverse micelle (W₀=46). Solid black lines indicate best fits of the corresponding data.

Figure S3: Experimental fluorescence autocorrelation curve of TMR tagged HSA in the water-pool of AOT reverse micelle of different W₀ values at 298 K and its fitting with (i) 1 diffusion model (green line), (ii) 1 diffusion + 1 conformational relaxation component model (violet line), (iii) Gaussian distribution on diffusion model (cyan line), (iv) Gaussian distribution on diffusion + 1 conformational relaxation component model (brown line), and (v) Gaussian distribution on diffusion + Gaussian distribution on conformational relaxation component model (black line). Upper panel shows the residuals of the corresponding fits.

Figure S4: Normalized fits of autocorrelation curves of TMR tagged HSA in buffer at different temperatures.

Figure S5: Normalized fits of autocorrelation curves of TMR tagged HSA in W₀=20 AOT reverse micelle at different temperatures.

Figure S6: Normalized fits of autocorrelation curves of TMR tagged HSA in W₀=25 AOT reverse micelle at different temperatures.

Figure S7: Normalized fits of autocorrelation curves of TMR tagged HSA in W₀=30 AOT reverse micelle at different temperatures.

Figure S8: A comparison of the fluorescence autocorrelation curve of TMR tagged HSA in the water-pool of AOT reverse micelle (W₀=25) and untagged HSA along with Rhodamine 6G in the water-pool of AOT reverse micelle of same dimension at different temperatures.
**Figure S1:** Circular dichroism spectra of (a) TMR-tagged and untagged human serum albumin in buffer. (b) human serum albumin in buffer and in water-pool of AOT reverse micelle $W_0=20$ (overall concentration of HSA is maintained at 5 µM).
**Figure S2:** Fluorescence autocorrelation spectra of TMR tagged human serum albumin (HSA) in buffer and in AOT reverse micelle ($W_0=46$). Solid black lines indicate best fits of the corresponding data.
Figure S3. Experimental fluorescence autocorrelation curve of TMR tagged HSA in the water-pool of AOT reverse micelle of different $W_0$ values at 298 K and its fitting with (i) 1 diffusion model (green line), (ii) 1 diffusion + 1 conformational relaxation component model (violet line), (iii) Gaussian distribution on diffusion model (cyan line), (iv) Gaussian distribution on diffusion + 1 conformational relaxation component model (brown line), and (v) Gaussian distribution on diffusion + Gaussian distribution on conformational relaxation component model (black line). Upper panel shows the residuals of the corresponding fits.

$W_0 = 20$

$W_0 = 24$

$W_0 = 28$

$W_0 = 30$

$W_0 = 25$

$W_0 = 32$
Figure S4: Normalized fits of autocorrelation curves of TMR tagged HSA in buffer at different temperatures.
Figure S5: Normalized fits of autocorrelation curves of TMR tagged HSA in $W_0=20$ AOT reverse micelle at different temperatures.
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