

## 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  $\mu$ M) and untagged (5.03  $\mu$ M) human serum albumin in buffer. (b) tagged human serum albumin (HSA) in buffer (3.74  $\mu$ M) and in water-pool of AOT reverse micelle  $W_0=20$  (overall concentration of HSA is 1.65  $\mu$ M).

**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.

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

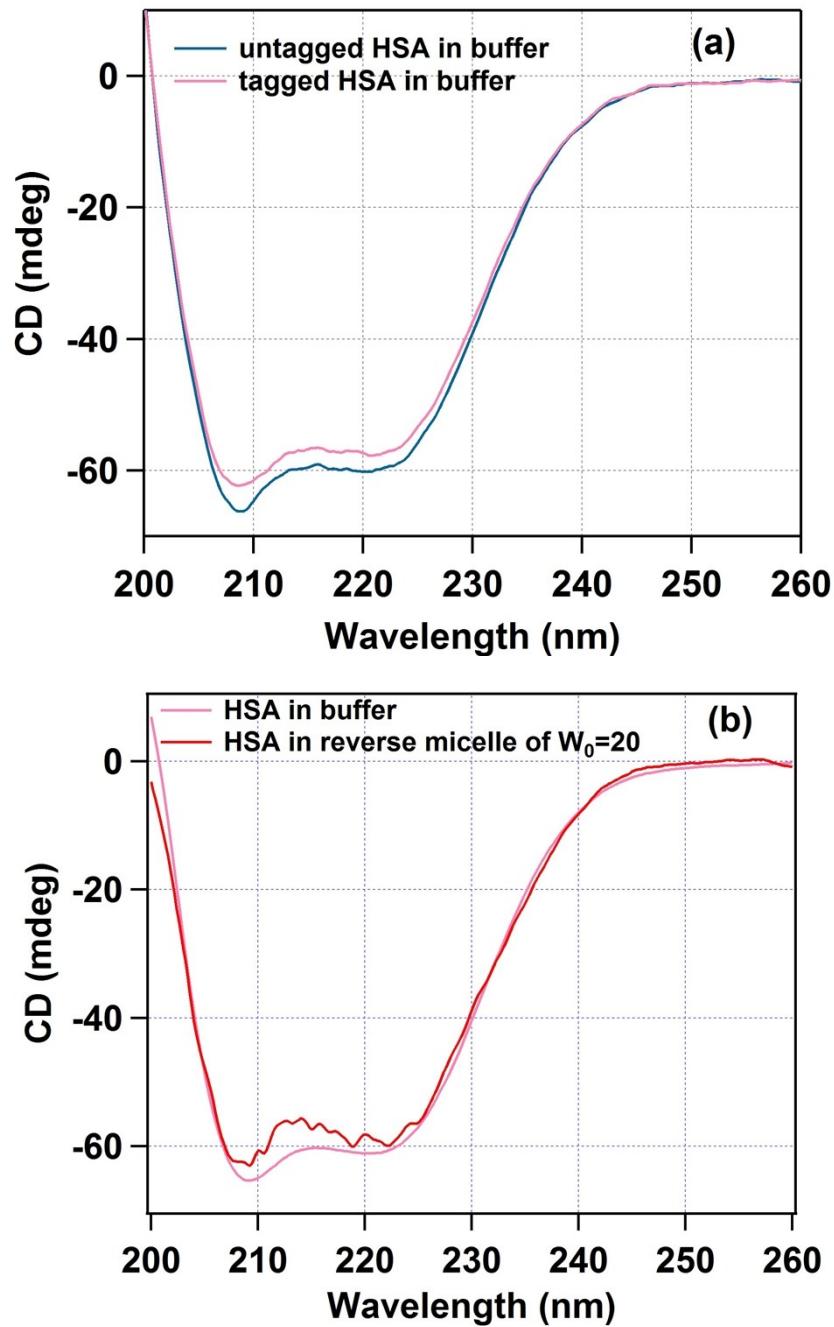
**Figure S5:** Normalized fits of autocorrelation curves of TMR tagged HSA in  $W_0=20$  AOT reverse micelle at different temperatures.

**Figure S6:** Normalized fits of autocorrelation curves of TMR tagged HSA in  $W_0=25$  AOT reverse micelle at different temperatures.

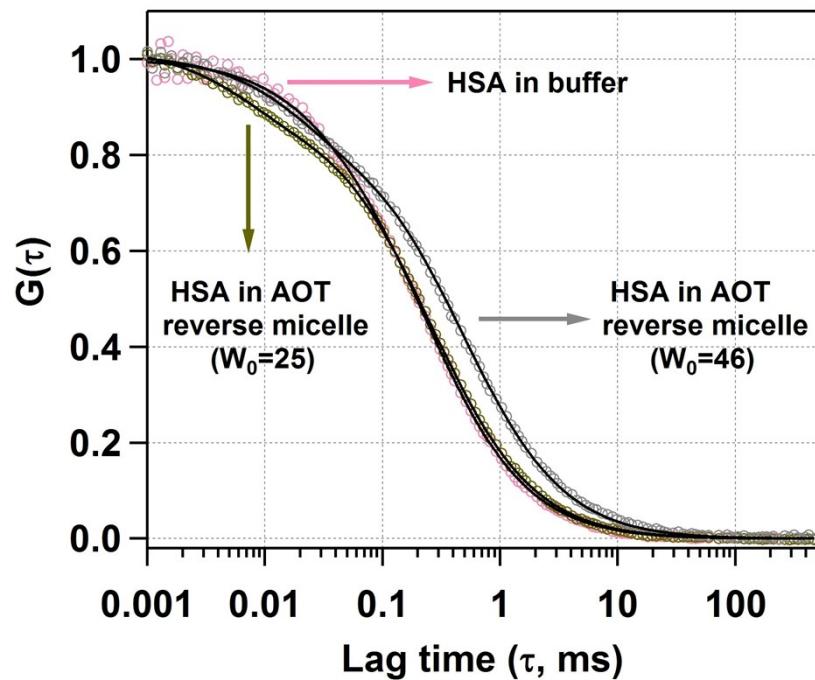
**Figure S7:** Normalized fits of autocorrelation curves of TMR tagged HSA in  $W_0=30$  AOT reverse micelle at different temperatures.

**Figure S8:** A comparison of the fluorescence autocorrelation curve of TMR tagged HSA in the water-pool of AOT reverse micelle ( $W_0=25$ ) and untagged HSA along with Rhodamine 6G in the water-pool of AOT reverse micelle of same dimension at different temperatures.

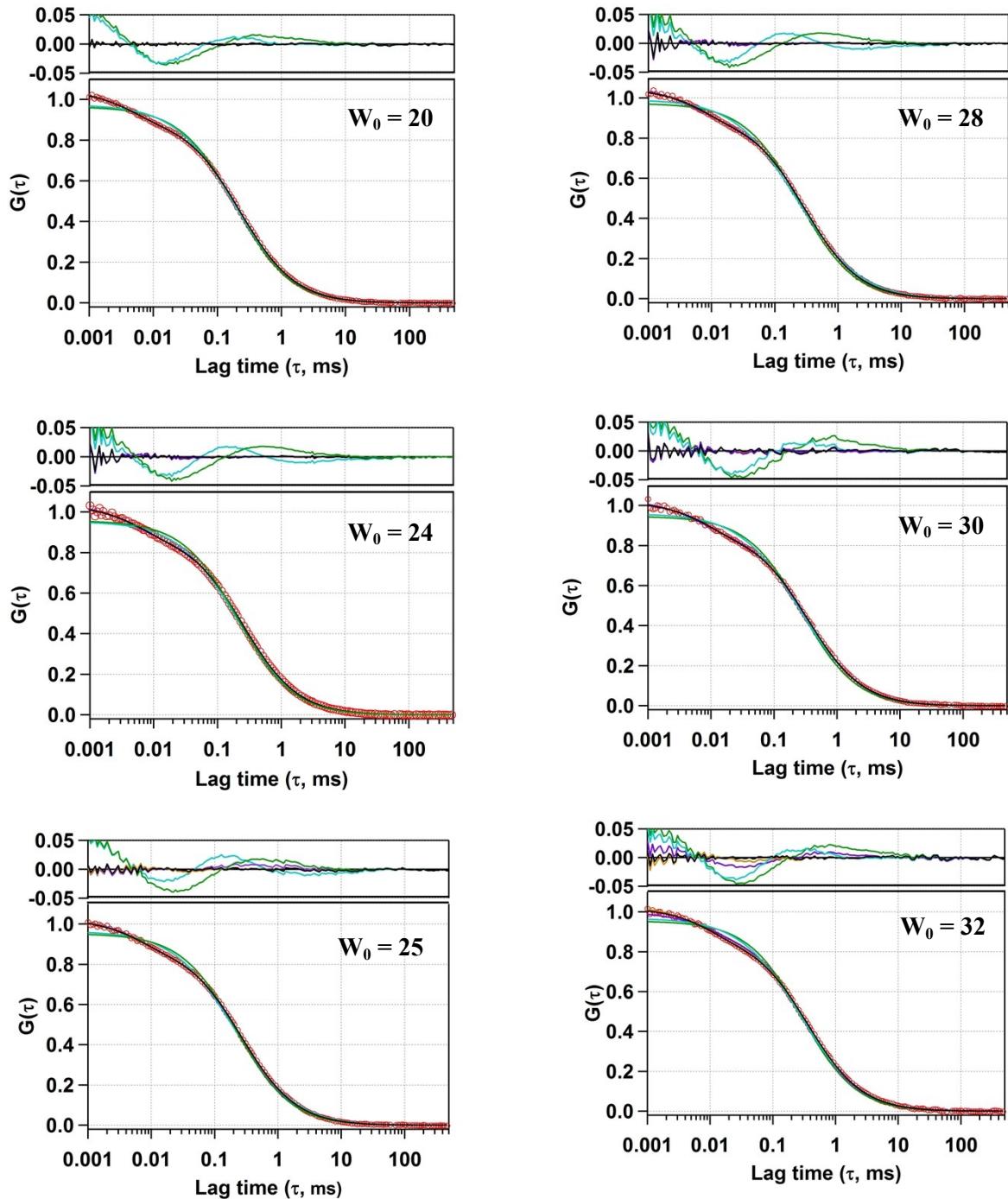
**Figure S1:** Circular dichroism spectra of (a) TMR-tagged and untagged human serum albumin in buffer. (b) human serum albumin in buffer and in water-pool of AOT reverse micelle  $W_0=20$  (overall concentration of HSA is maintained at 5  $\mu\text{M}$ ).

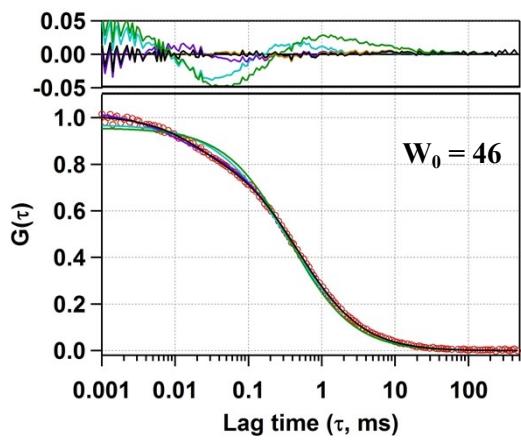
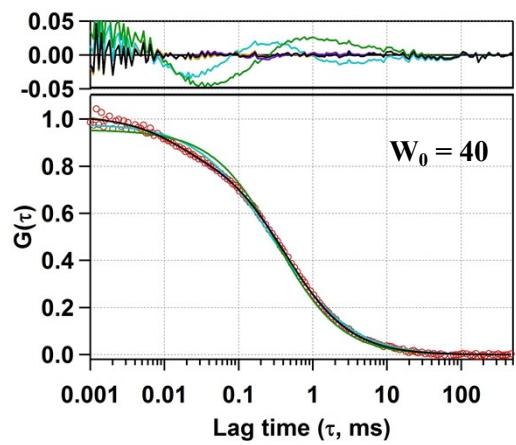
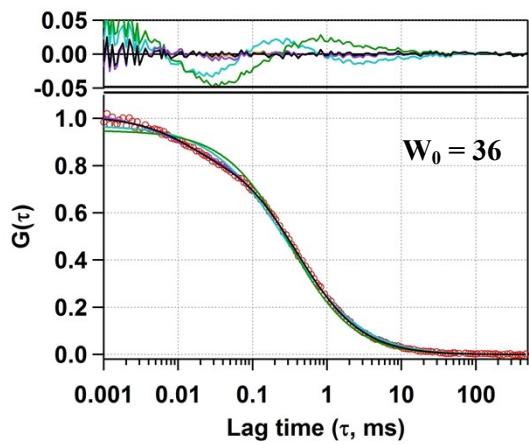


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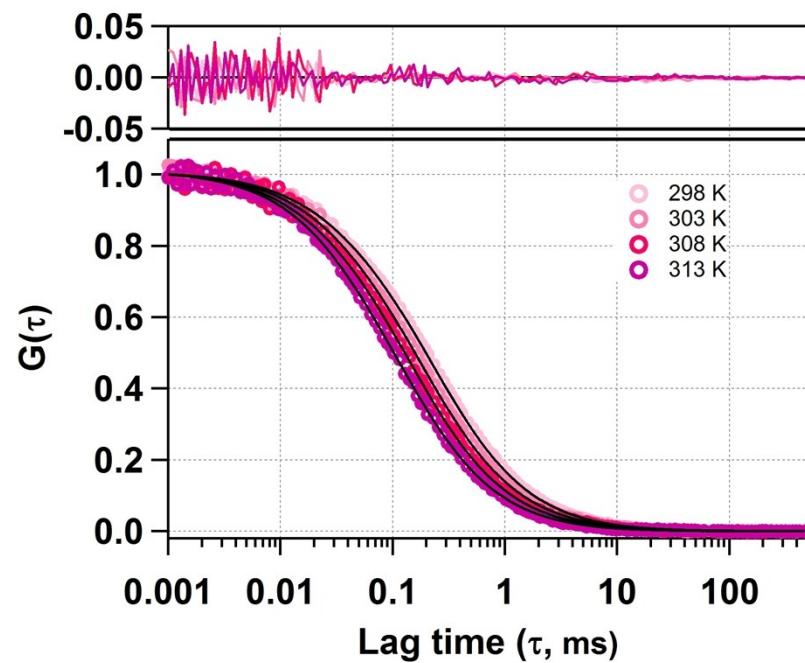


**Figure S3.** Experimental fluorescence autocorrelation curve of TMR tagged HSA in the water-pool of AOT reverse micelle of different  $W_0$  values at 298 K and its fitting with (i) 1 diffusion model (green line), (ii) 1 diffusion + 1 conformational relaxation component model (violet line), (iii) Gaussian 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.

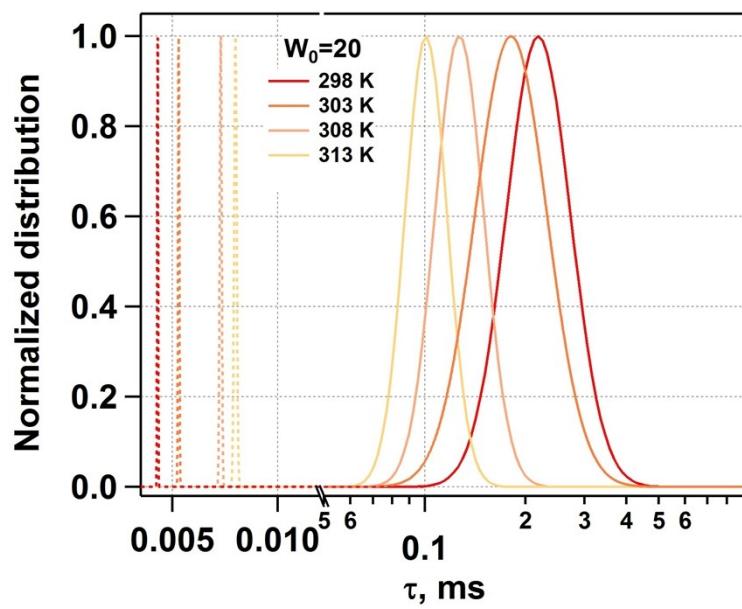
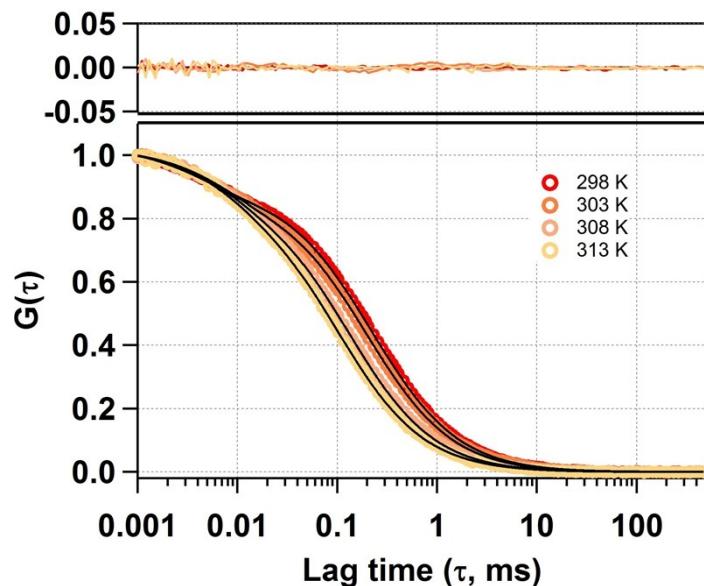




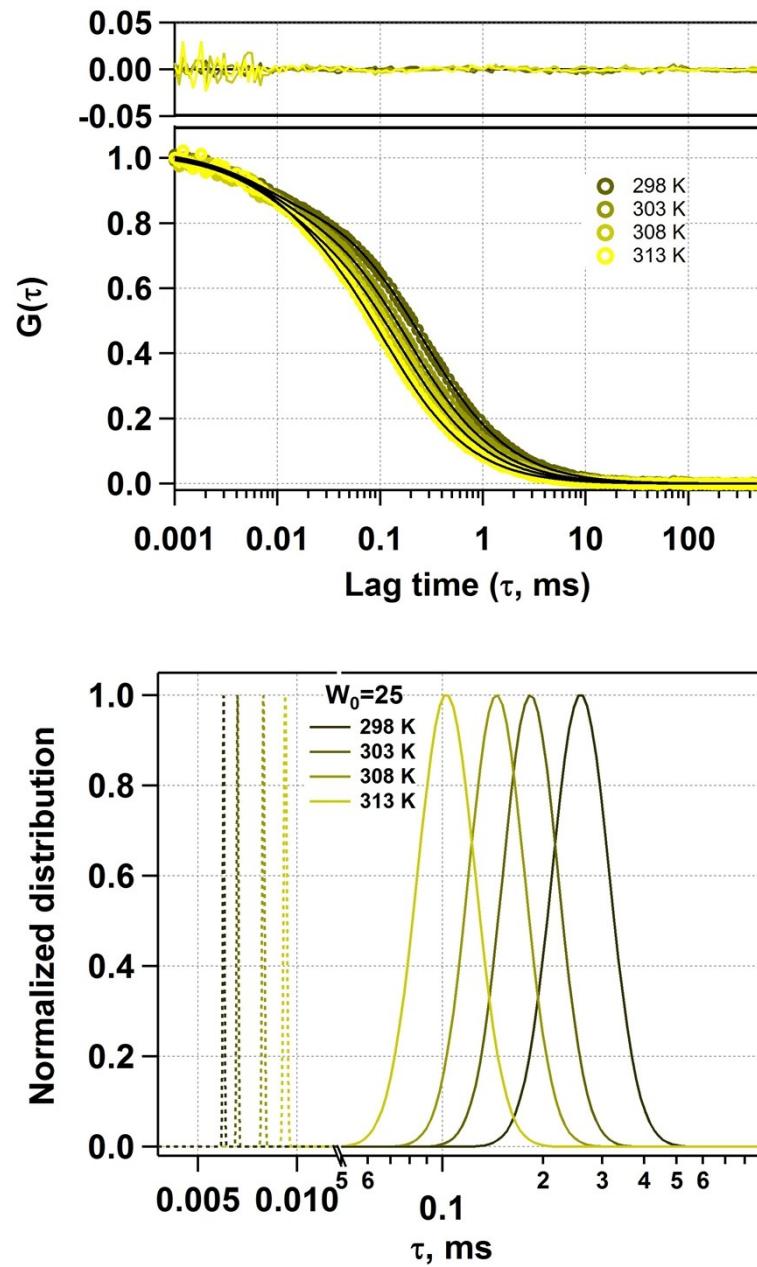
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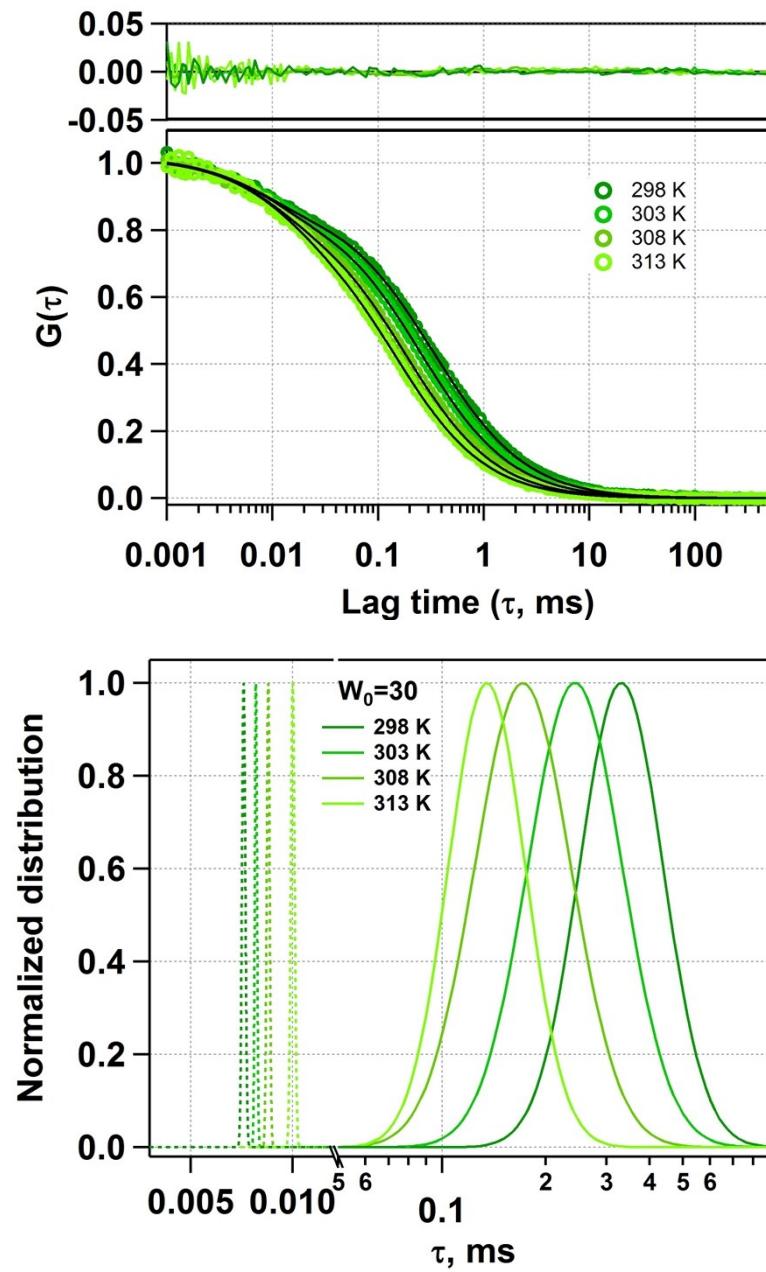
**Figure S5:** Normalized fits of autocorrelation curves of TMR tagged HSA in  $W_0=20$  AOT reverse micelle at different temperatures.



**Figure S6:** Normalized fits of autocorrelation curves of TMR tagged HSA in  $W_0=25$  AOT reverse micelle at different temperatures.



**Figure S7:** Normalized fits of autocorrelation curves of TMR tagged HSA in  $W_0=30$  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 ( $W_0=25$ ) and untagged HSA along with Rhodamine 6G in the water-pool of AOT reverse micelle of same dimension at different temperatures.

