ELECTRONIC SUPPLEMENTARY INFORMATIONS

Assessing the Impact of Choline Chloride and Benzyltrimethylammonium Chloride-Based Deep Eutectic Solvents on the Structure and Conformational Dynamics of Bovine Serum Albumin: A Combined Steady-State, Time-Resolved Fluorescence and Fluorescence Correlation Spectroscopic Study

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Methods

Synthesis of DESs

Both DESs were synthesized using the typical literature technique.[1-4] Briefly, in a conical flask, ethylene glycol (3 mol) and choline chloride or benzyltrimethylammonium chloride (1 mol) were mixed and heated in an oil bath at 353 K with continual stirring until a transparent homogenous liquid was formed. The liquids were then slowly cooled down to the room temperature (298 K) and dried 323 K for several hours and stored in an inert atmosphere prior to experiments. The purity of the prepared DESs was checked through 1 H NMR spectroscopy in D_2O as solvent.

^{1}H NMR ($\delta_{H, \ ppm}$) of Ethaline: 5.15 brs (1H, O**H**), 4.65 brs (6H, O**H**), 3.55 m (14H, O-C**H₂**), 3.45 m (2H, N-C**H₂**), 3.12 s (9H, N-(C**H₃**)₃). 14H, O-C**H₂**

^{1}H NMR ($\delta_{H, \ ppm}$) of BMEG: 7.4 m (5H, -C₆**H₅**), 4.7 brs (6H, O**H**), 4.35 s (2H, N-C**H₂**), 3.5 m (12H, O-C**H₂**), 2.95 s (9H, N-(C**H₃**)₃).

Sample Preparation: The protein stock solution was made by dissolving the BSA powder in the 10 mM phosphate buffer of pH 7.4. and stirring gently at room temperature. All the samples were prepared fresh and allowed to settle for 15 minutes before being introduced into the instrument. The addition of different quantity of DESs to the protein solution is represented in weight/volume percentage (% w/v) and the amount of DESs in varies from 00 % w/v to 50 % w/v. For the steady-state, time-resolved spectroscopy and CD spectroscopy measurements the concentration of BSA (without dye tagged) was 2μM in experimental buffer. For the CD spectroscopy measurement, the highest concentration of Ethaline and BMEG is 50% and 20 % respectively, so as to keep the HT voltage below 700V. For the studied involving the dye tagged protein, the concentration of BSA-FITC was 4 μM and 4 nM for ensemble average (steady-state and time-resolved spectroscopy) and single molecule measurements respectively.

Following the literature reported method, the dye-to-protein ratio in the labelled protein was calculated using absorbance and molar extinction coefficient values (43824 $M^{-1}cm^{-1}$ for BSA at 280 nm and 68000 $M^{-1}cm^{-1}$ for FITC at 495 nm) and was found to be ~1.2.
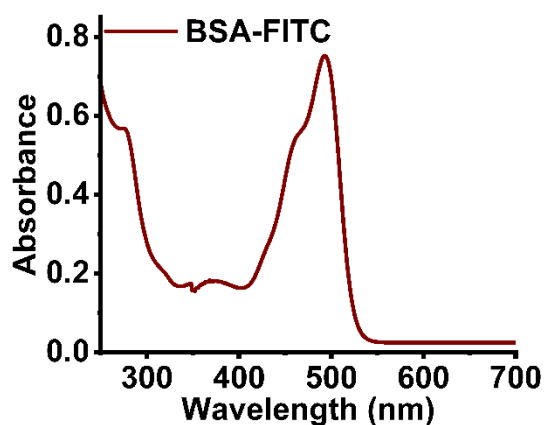

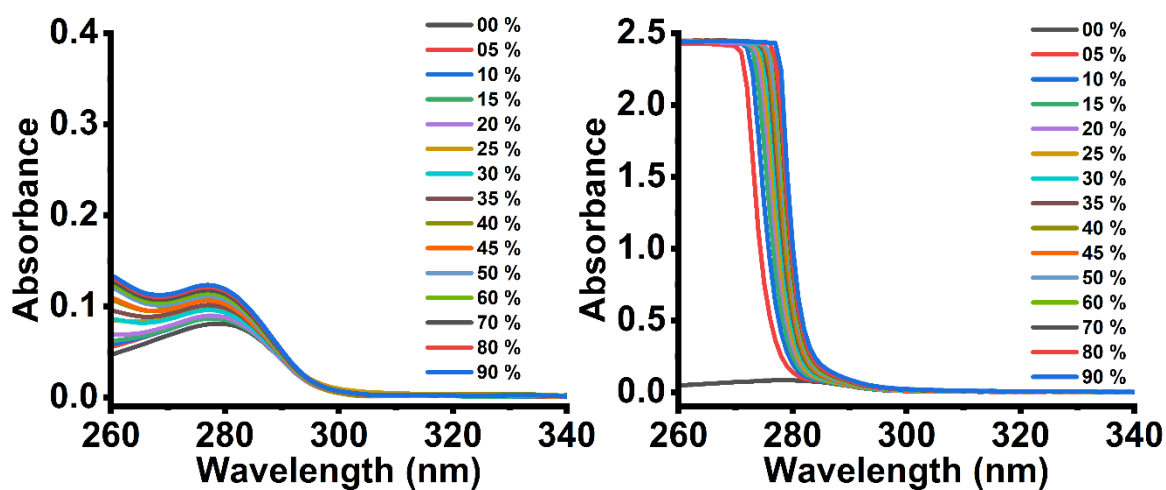**FIG. S1.** Steady-state absorption spectra of BSA-FITC in 10mM phosphate buffer.

FIG. S2. Steady-state absorption spectra of 2 µM of BSA in the presence of Ethaline (left panel) and BMEG (right panel).
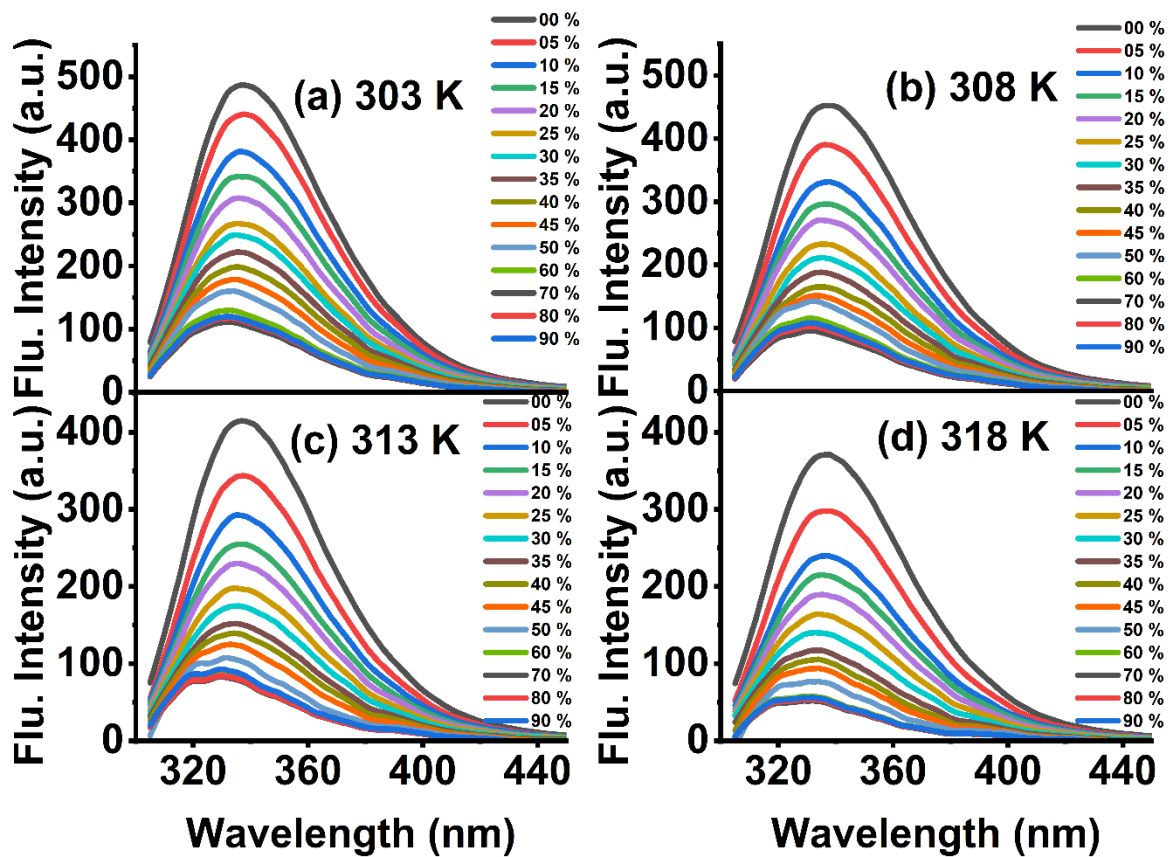
FIG. S3. Steady-state fluorescence spectra of BSA (2µM) in presence of different quantity of BMEG at 303 to 318K.
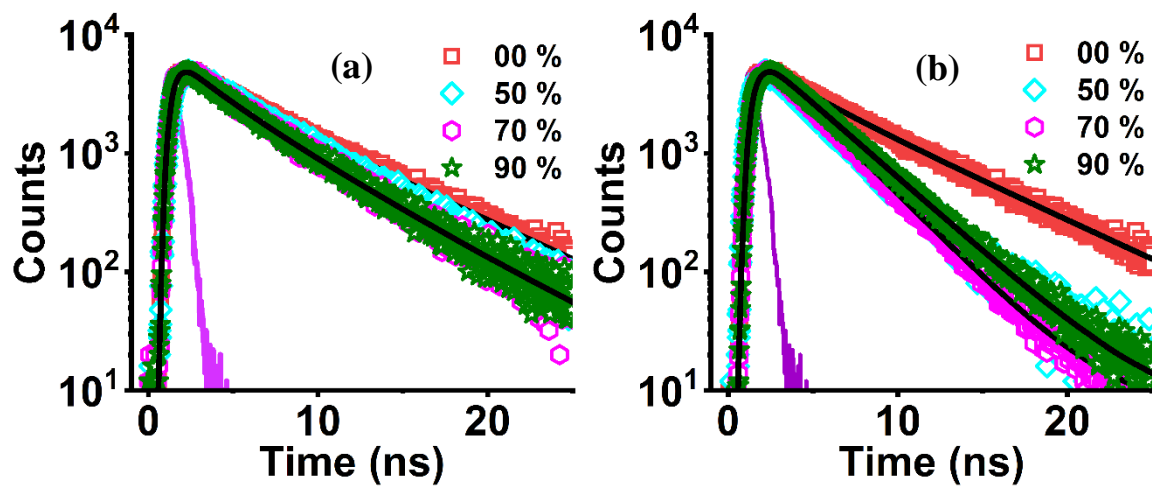

FIG. S4. Fluorescence decay traces (λ_{ex}= 295nm) of BSA in absence and presence of different quantity of (a) Ethaline and (b) BMEG DESs at 298 K
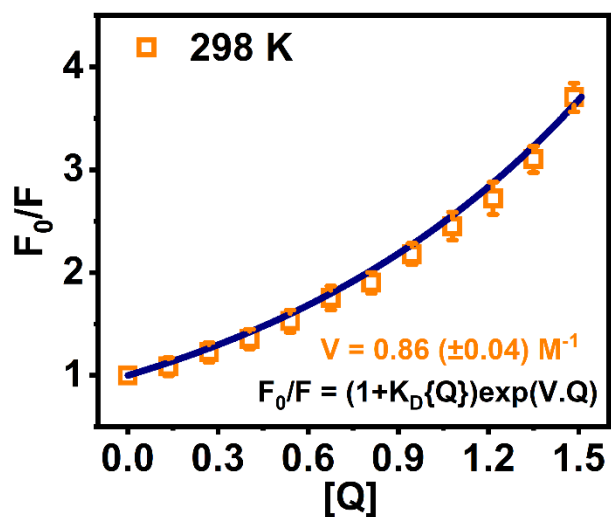
FIG. S5. Stern-Volmer plots for quenching of intrinsic BSA fluorescence by BMEG at 298 K. The solid line represents the simulated curve obtained by using equation 6.
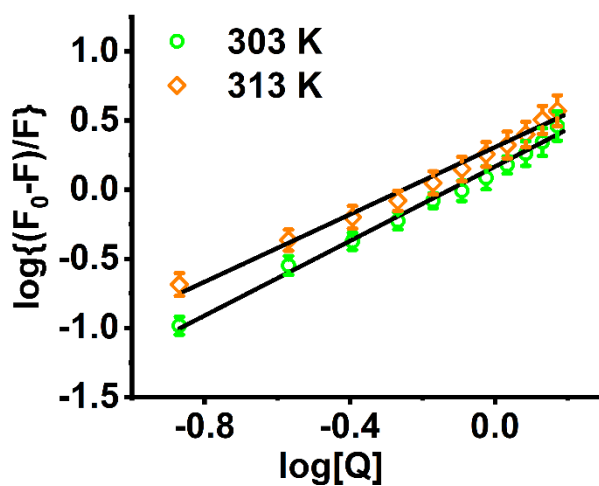

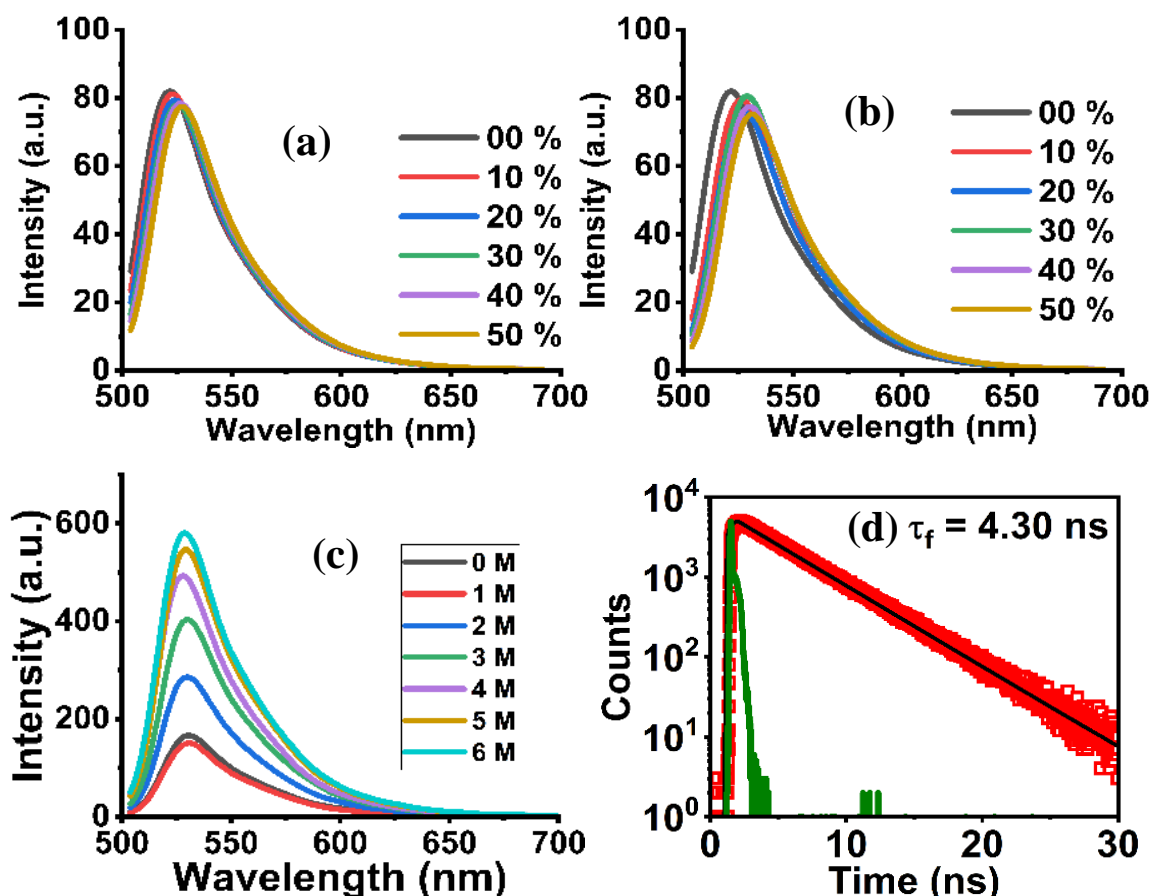**FIG. S6.** Double logarithmic plot at 303K and 313K for BSA-BMEG

FIG. S7. Steady-state fluorescence spectra of FITC dye only in presence of (b) Ethaline and (c) BMEG, respectively. (c) Steady-state fluorescence spectra of BSA-FITC in presence of GdHCl, (d) Time-resolved fluorescence spectra of FITC dye only in buffer.
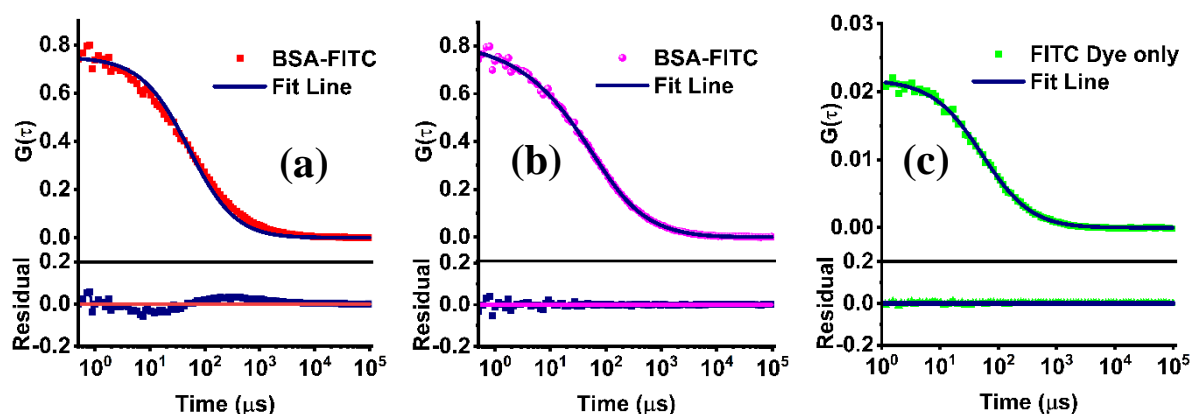

FIG. S8. Fitting of the FCS trace of BSA-FITC in 10 mM phosphate buffer through (a) single component diffusion model (equation), and (b) single component diffusion model along with stretched relaxation component (equation). (c) Fitting of FITC dye only in buffer through single component diffusion model. The corresponding residual shows the goodness of the fit.
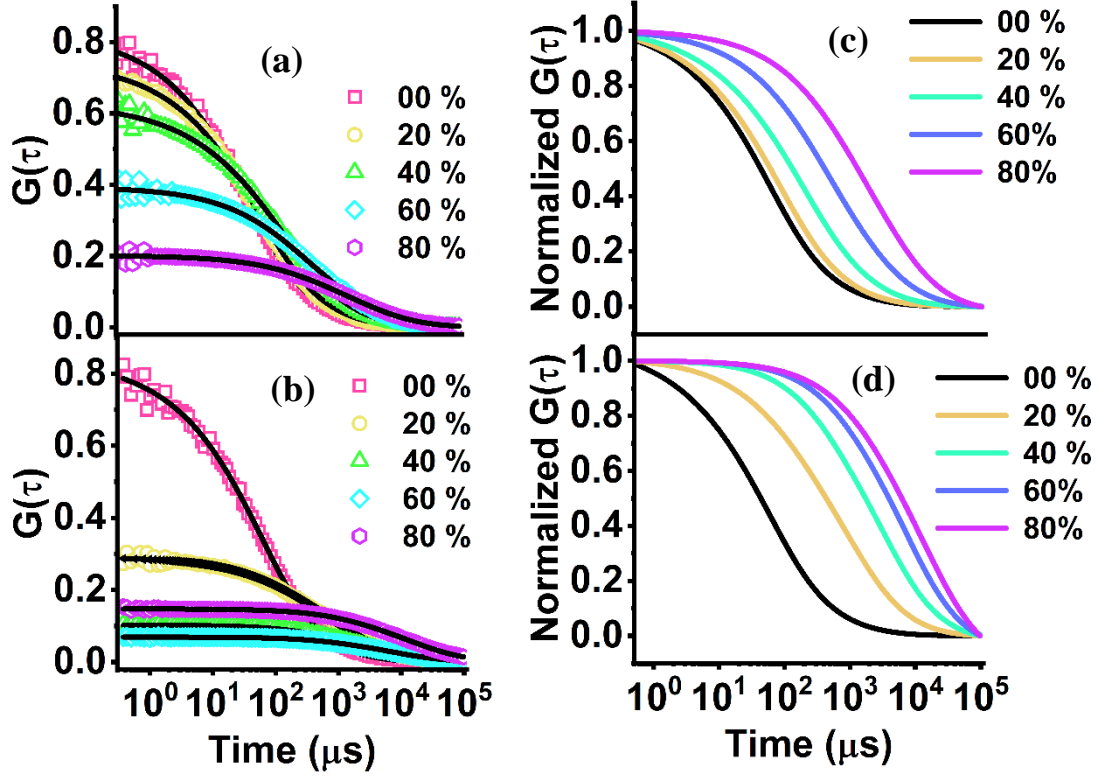
FIG. S9. FCS traces of BSA-FITC in presence of (a) Ethaline and (b) BMEG. (b) Normalized fitted FCS traces of BSA-FITC in (a) Ethaline and (b) BMEG.

Table S1. Estimated fitted parameters of FCS data of BSA-FITC in presence of DESs

| Systems	A	τ_R (µs)	β	τ_D (ms)	r_H (Å)
00 %	0.75 ± 0.04	46.1 ± 2.4	0.50 ± 0.03	0.31 ± 0.02	34.7 ± 1.4
20 % ETH	0.69 ± 0.04	50.5 ± 2.8	0.51 ± 0.03	0.39 ± 0.03	36.5 ± 1.6
40 % ETH	0.63 ± 0.02	56.6 ± 3.4	0.52 ± 0.04	0.79 ± 0.06	46.5 ± 1.7
60 % ETH	0.52 ± 0.04	62.3 ± 3.1	0.57 ± 0.04	1.74 ± 0.13	59.6 ± 2.1
80 % ETH	0.40 ± 0.02	67.2 ± 2.6	0.59 ± 0.05	4.02 ± 0.24	66.7 ± 2.6
20 % BMEG	0.36 ± 0.03	60.5 ± 4.2	0.61 ± 0.05	1.1 ± 0.10	68.8 ± 3.1
40 % BMEG	0.25 ± 0.02	79.3 ± 4.6	0.80 ± 0.04	3.45 ± 0.23	78.2 ± 3.4
60 % BMEG	0.21 ± 0.02	82.1 ± 4.4	0.79 ± 0.05	7.91 ± 0.64	86.2 ± 3.9
80 % BMEG	0.34 ± 0.03	98.7 ± 4.3	0.80 ± 0.04	17.2 ± 0.96	119.2 ± 4.3
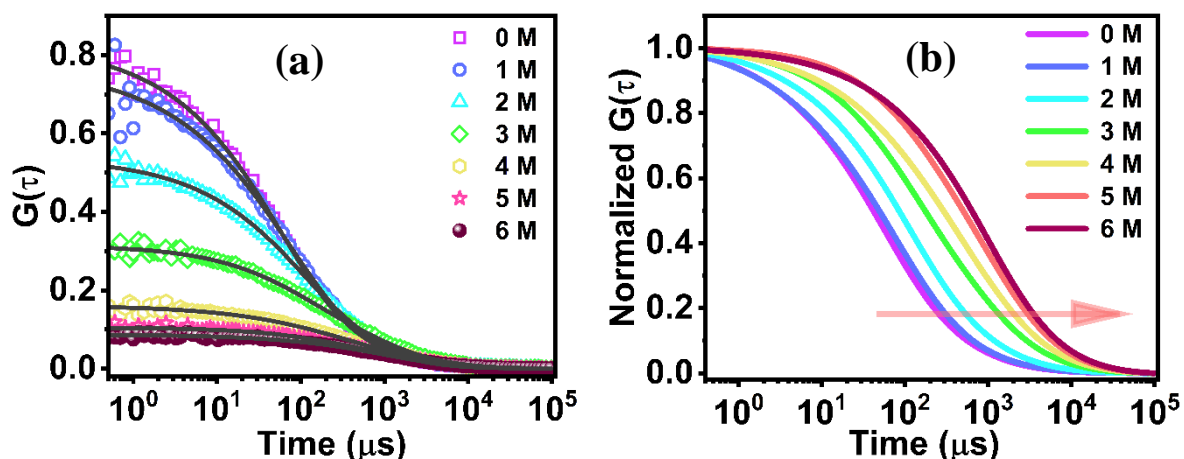
FIG. S10. (a) FCS traces of BSA-FITC in presence of GdHCl. (b) Normalized fitted FCS traces of BSA-FITC in GdHCl.

Table S2. Estimated fitted parameters of FCS data of BSA-FITC in presence of GdHCl

Systems	A	τ_R (µs)	β	τ_D (ms)	r_H (Å)
00 %	0.75 ± 0.04	46.1 ± 2.4	0.50 ± 0.03	0.31 ± 0.02	34.8 ± 1.4
1 M GdHCl	0.73 ± 0.03	45.2 ± 2.2	0.56 ± 0.04	0.37 ± 0.03	35.7 ± 1.5
2 M GdHCl	0.65 ± 0.03	57.9 ± 3.1	0.59 ± 0.04	0.51 ± 0.05	46.2 ± 1.8
3 M GdHCl	0.49 ± 0.03	62.1 ± 3.6	0.64 ± 0.06	0.78 ± 0.05	65.6 ± 2.4
4 M GdHCl	0.42 ± 0.02	69.5 ± 2.9	0.67 ± 0.05	0.99 ± 0.08	76.7 ± 2.9
5 M GdHCl	0.29 ± 0.02	77.6 ± 3.9	0.76 ± 0.05	1.13 ± 0.09	81.2 ± 2.8
6 M GdHCl	0.27 ± 0.03	78.9 ± 3.7	0.73 ± 0.04	1.29 ± 0.07	80.8 ± 2.7

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