

Supplementary Information

Diffusion of Organic Dyes in Bovine Serum Albumin Solution Studied by Fluorescence Correlation Spectroscopy

Satyajit Patra, Kotni Santhosh, Ashok Pabbathi and Anunay Samanta*
School of Chemistry, University of Hyderabad, Hyderabad 500046, India

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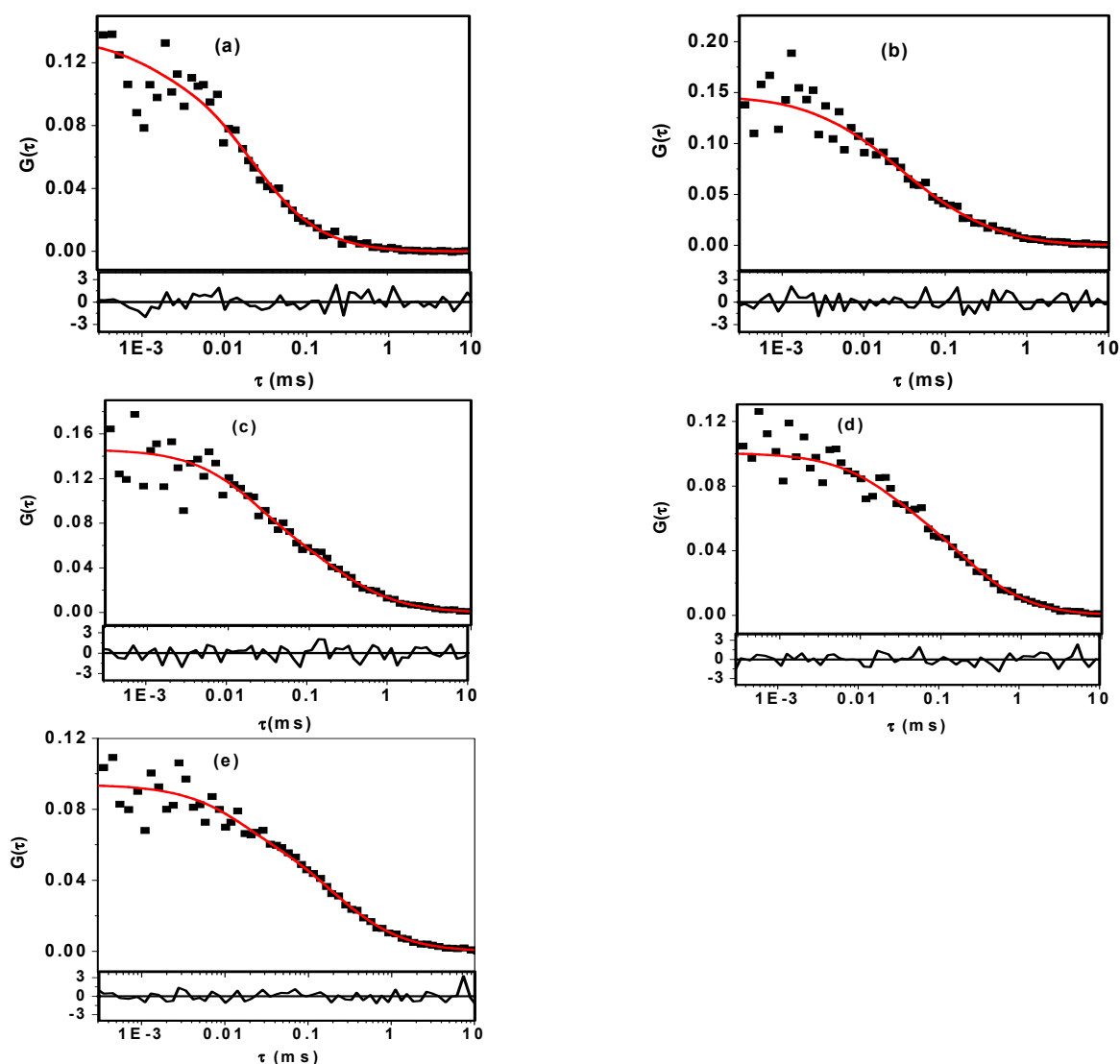


Fig. S1: Correlation curves of C102 for different [BSA]. [BSA] in (a) - (e) are 0, 1, 4, 8, 16 μM respectively. Black squares are the raw data and red lines are the best fit to these data points. Residuals are given at the bottom of each plot. The fit parameters obtained from the fitting of these curves to equation 7 are as follows. (a) $\rho_1=0.1358$, $\tau_1=0.0215$ ms, $T=0.12$, $\tau_{rr}=0.001$ ms, $\langle N \rangle=7.4$ (b) $\rho_1=0.0376$, $\tau_1=0.2111$ ms, $\rho_2=0.1100$, $\tau_2=0.0211$ ms, $T=0.0692$, $\tau_{rr}=0.0023$ ms $\langle N \rangle=7.3$ (c) $\rho_1=0.0903$, $\tau_1=0.21$ ms, $\rho_2=0.051$, $\tau_2=0.025$ ms, $T=0.2095$, $\tau_{rr}=0.02266$ ms, $\langle N \rangle=9$ (d) $\rho_1=0.0983$, $\tau_1=0.20$ ms, $\rho_2=0.005$, $\tau_2=0.029$ ms, $T=0.2512$, $\tau_{rr}=0.01682$ ms, $\langle N \rangle=13$. (e) $\rho_1=0.0890$, $\tau_1=0.190$ ms, $\rho_2=0.005$, $\tau_2=0.029$ ms, $T=0.2299$, $\tau_{rr}=0.01365$ ms, $\langle N \rangle=14$.

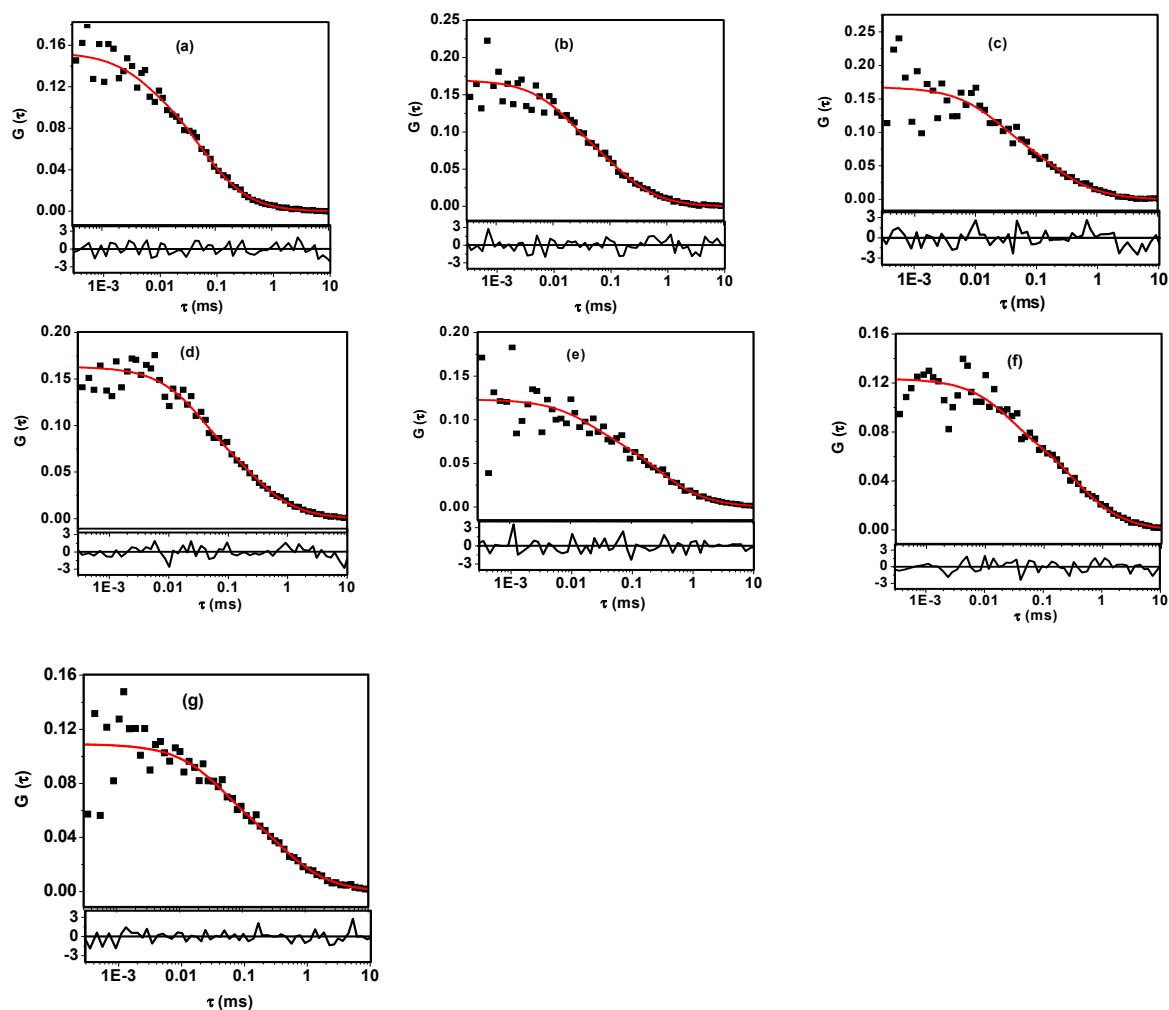


Fig.S2: Correlation curves of FL in (a) 0 μM , (b) 8 μM , (c) 16 μM , (d) 25 μM , (e) 40 μM , (f) 60 μM and (g) 70 μM concentrations of BSA. The black squares are the data points and red lines are the best fits to them. Residuals are given in the lower part of the each Residuals are given at the bottom of each plot. All the curves are fitted to equation 7. The fit parameters are as follows. (a) $\rho_1 = 0.1525$, $\tau_1 = 0.0532$ ms, $\langle N \rangle = 8$, $\tau_{rr} = 0.00492$, $T = 0.1580$ (b) $\rho_1 = 0.1989$, $\tau_1 = 0.0982$ ms, $\langle N \rangle = 7.6$, $\tau_{rr} = 0.00684$ ms, $T = 0.3398$ (c) $\rho_1 = 0.1517$, $\tau_1 = 0.1489$ ms, $\langle N \rangle = 8$, $\tau_{rr} = 0.01516$ ms, $T = 0.2154$. (d) $\rho_1 = 0.1632$, $\tau_1 = 0.192$ ms, $\langle N \rangle = 9$, $\tau_{rr} = 0.02911$ ms, $T = 0.3150$ (e) $\rho_1 = 0.1235$, $\tau_1 = 0.2432$ ms, $\langle N \rangle = 11$, $\tau_{rr} = 0.01919$ ms, $T = 0.2558$ (f) $\rho_1 = 0.1236$, $\tau_{rr} = 0.02721$ ms, $\langle N \rangle = 12$, $\tau_{rr} = 0.02298$ ms, $T = 0.2968$ and (g) $\rho_1 = 0.1091$, $\tau_1 = 0.02860$ ms, $\langle N \rangle = 13$, $\tau_{rr} = 0.03993$ ms, $T = 0.3095$.

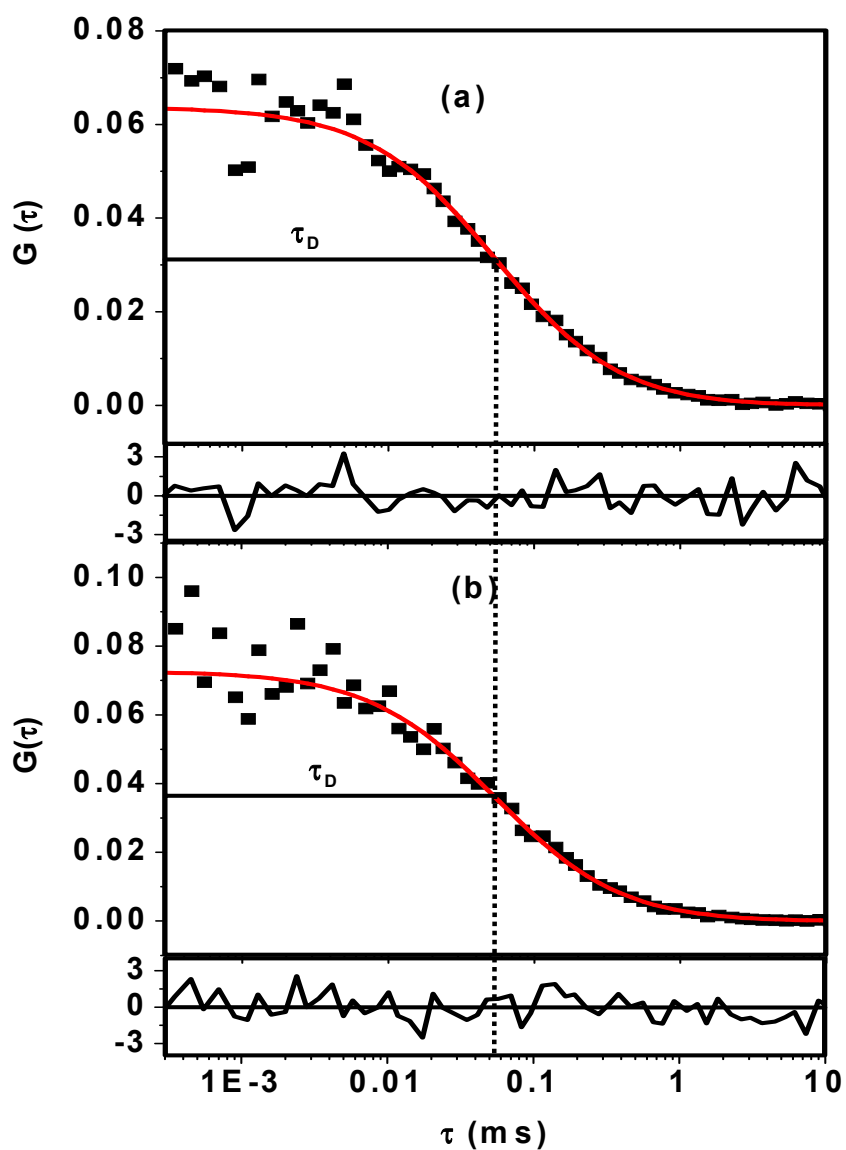


Fig. S3: Correlation curves of FL measured in phosphate buffered solution (pH = 7.0) (a) in absence (top panel) and (b) in presence of 4 M urea (bottom panel). Red lines are the fit to the raw data points. Residuals are given at the bottom of each plot. τ_D represents the diffusion time. There is no change in the diffusion time in absence and presence of 4 M urea as indicated by the dotted line.

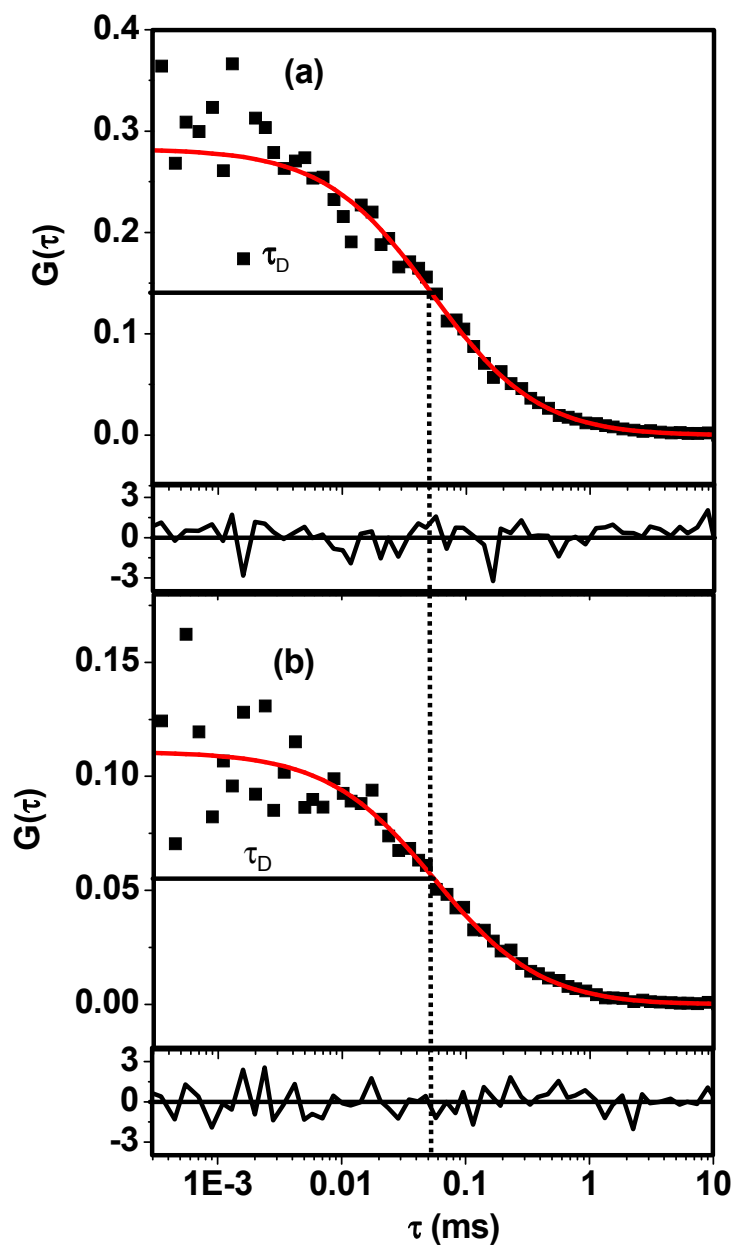


Fig. S4: Effect of 4 M urea on the correlation curves of R6G in phosphate buffered solution (pH = 7.0). (a) Top panel in absence of urea (b) bottom panel in presence of 4 M urea. Diffusion time (τ_D) remains almost same in both the case. Here red line is the fit to the raw data points. Residuals are given at the bottom of each plot.

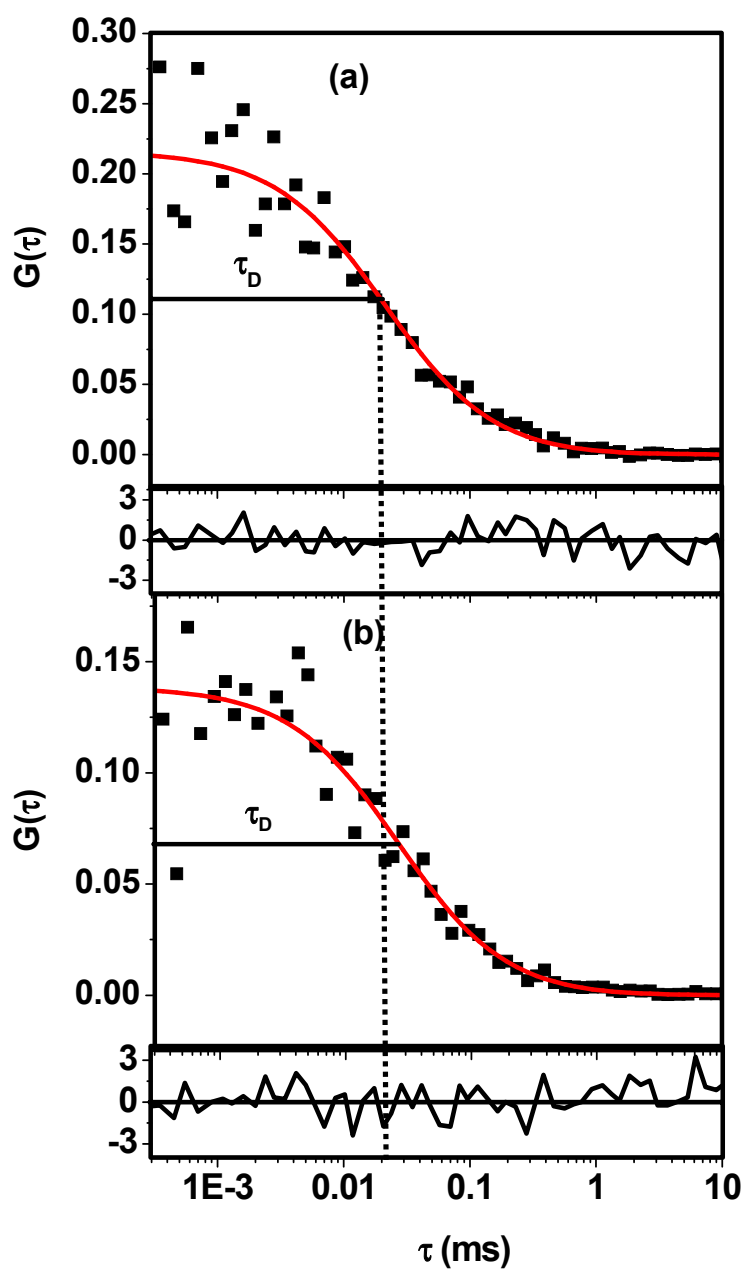


Fig. S5: Correlation curves of C102 in phosphate buffered solution (pH = 7.0) (a) in absence and (b) in presence of 4 M urea. Here red line is the fit to the raw data points (black squares). Residuals are given at the bottom of each plot. Diffusion time slightly shift to longer time scale in presence of 4 M urea as shown by the dotted line.

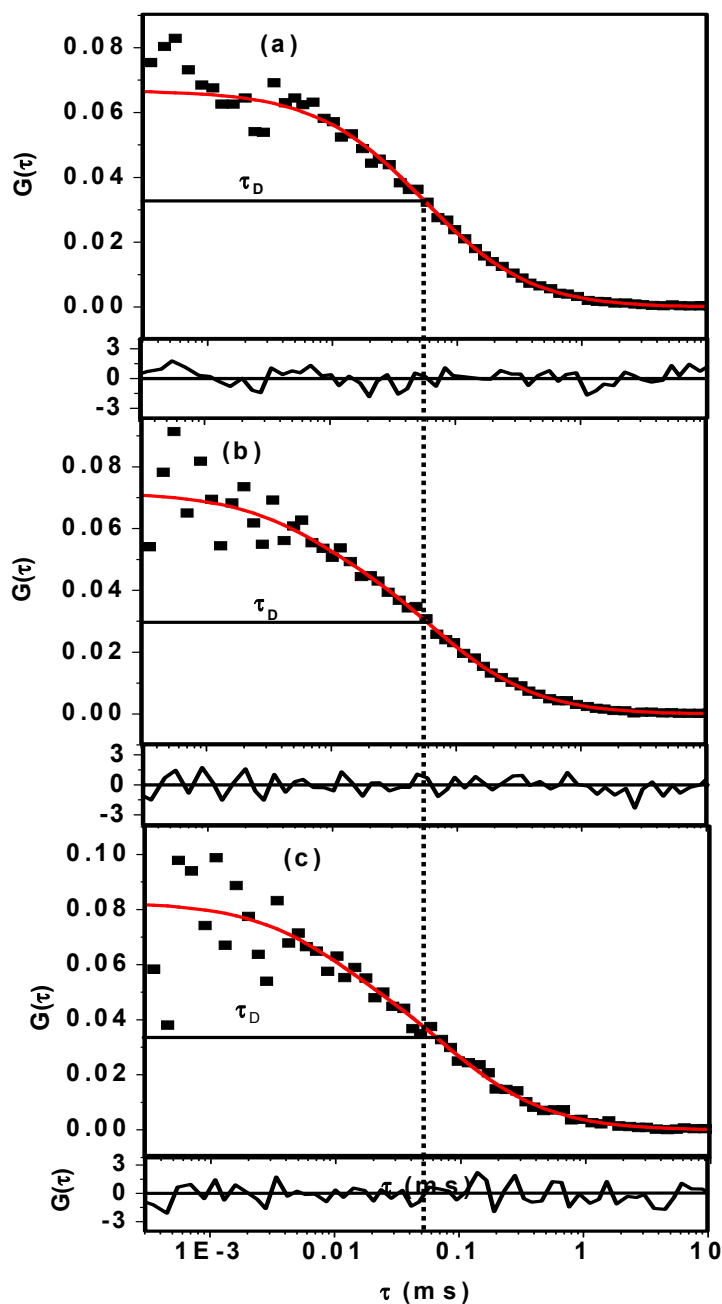


Fig. S6: Effect of NaCl on the correlation curves of FL in phosphate buffered solution (pH = 7.0). The concentration of NaCl are (a) 0 M (b) 1.5 M (c) 3 M respectively. Here the black squares are the data points and red lines are the best fits to them. Residuals are given at the bottom of each plot. There is slight increase in the diffusion time with increasing concentrations of NaCl as shown by the dotted line.

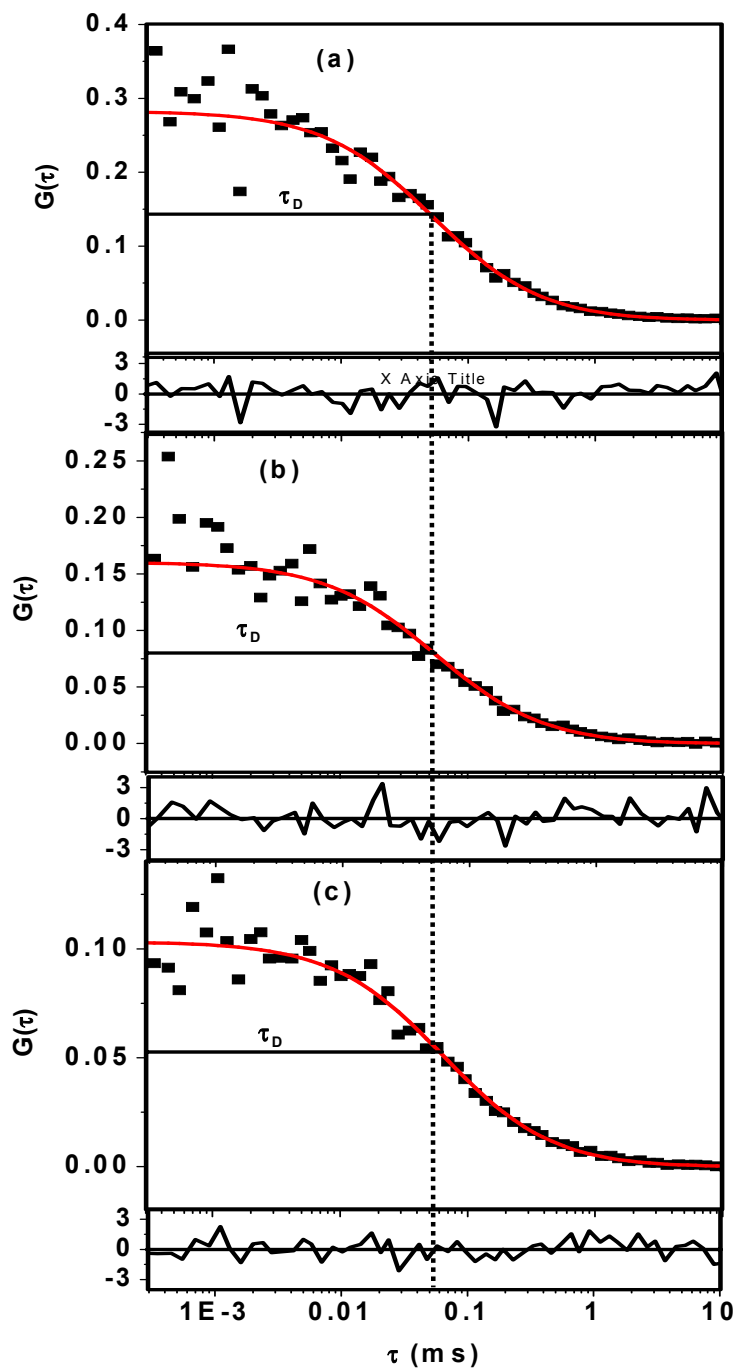


Fig. S7: Fluorescence correlation data points along with the best fits (solid lines) of R6G in phosphate buffered solution (pH = 7.0) in presence of (a) 0 M (b) 1.5 M (c) 3 M concentrations of NaCl. Residuals are given at the bottom of each plot. There is slight increase in diffusion time (τ_D) on addition of NaCl.

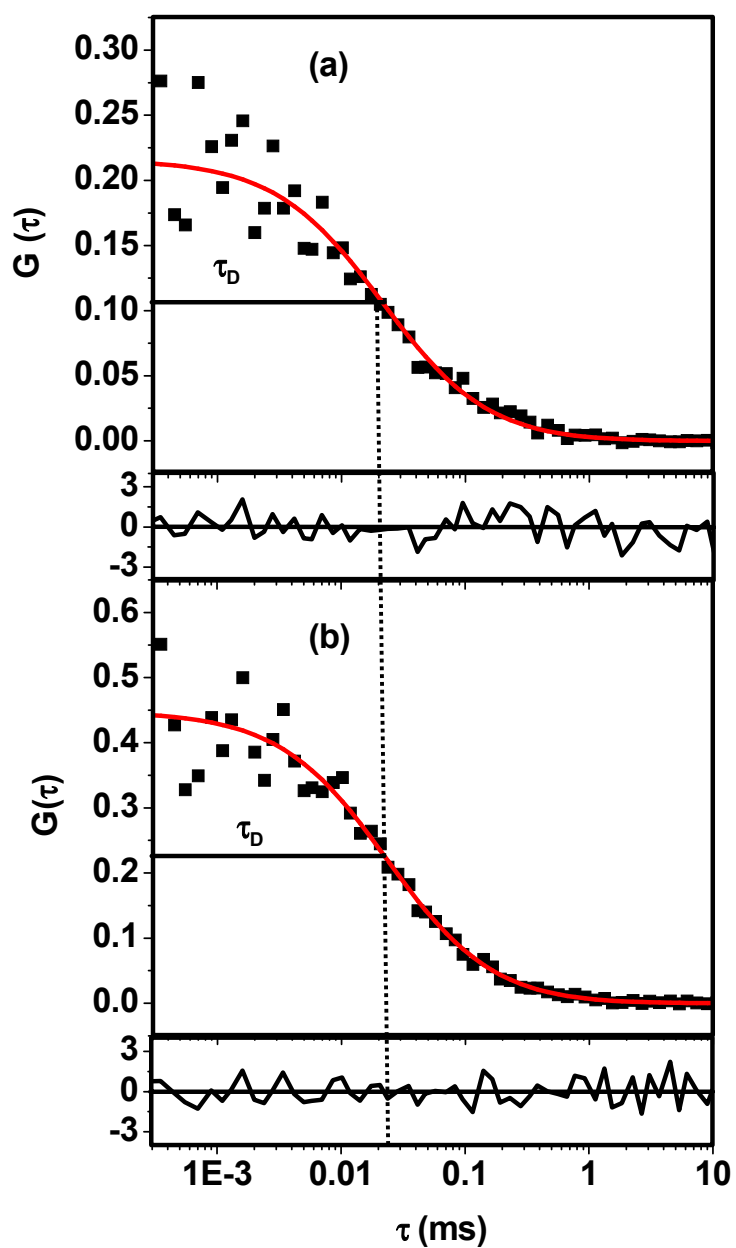


Fig. S8: Correlation curves of C102 in phosphate buffered solution (pH = 7.0) (a) in absence and (b) in presence of 1.5 M NaCl. Residuals are given at the bottom of each plot. Red line is the best fit to the raw data points (Black Square).

Table S1: Diffusion coefficients of the probes in buffer in absence and presence of NaCl and urea.

| Fluorophores | D_t ($\mu\text{m}^2/\text{s}$) | | | |
|--------------|------------------------------------|----------|------------|----------|
| | In buffer | 4 M urea | 1.5 M NaCl | 3 M NaCl |
| FL | 420 ± 10 | 400 ± 15 | 390 ± 25 | 370 ± 20 |
| R6G | 440 ± 15 | 415 ± 15 | 390 ± 30 | 350 ± 15 |
| C102 | 640 ± 60 | 520 ± 30 | 550 ± 15 | |

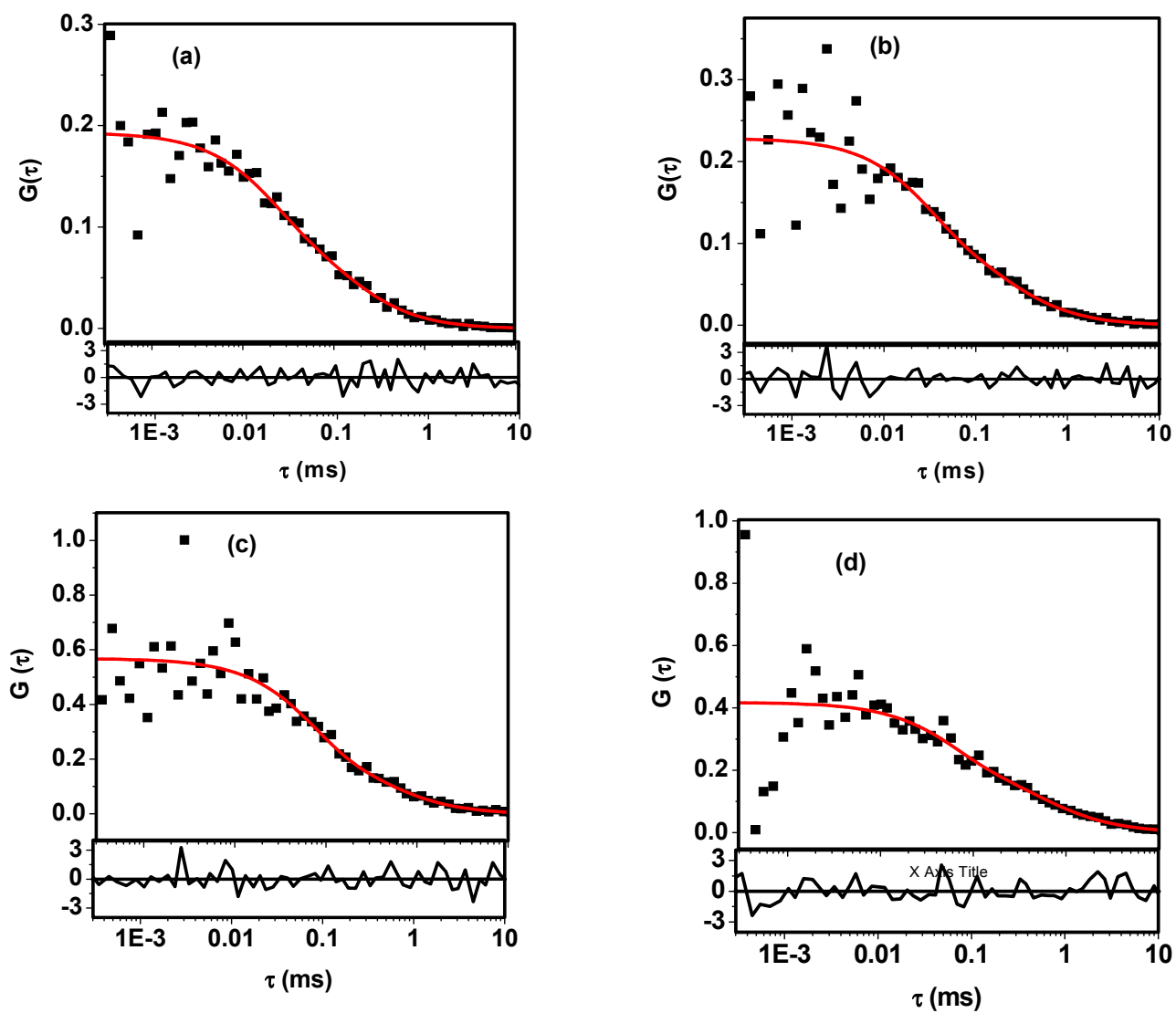


Fig.S9: Effect of addition of NaCl on the correlation curves (data points along with the best fit) of R6G in 8 μ M BSA for different concentrations of NaCl. [NaCl] in (a) to (d) are 0 M, 0.5 M, 1.5 M and 3 M respectively. Residuals are shown in the lower part of the each plot.

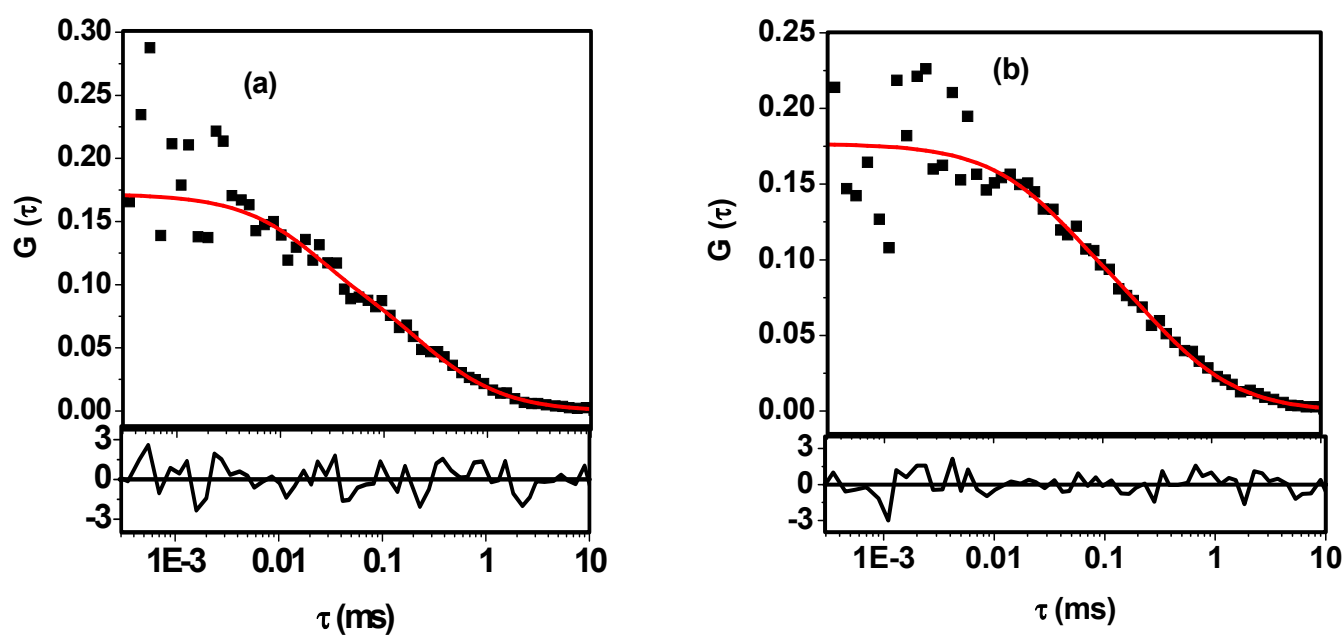


Fig.S10: Correlation curve of C102 in 8 μ M BSA (a) 0 M NaCl and (b) 1.5 M NaCl. Residuals are given in the lower part of the figures.

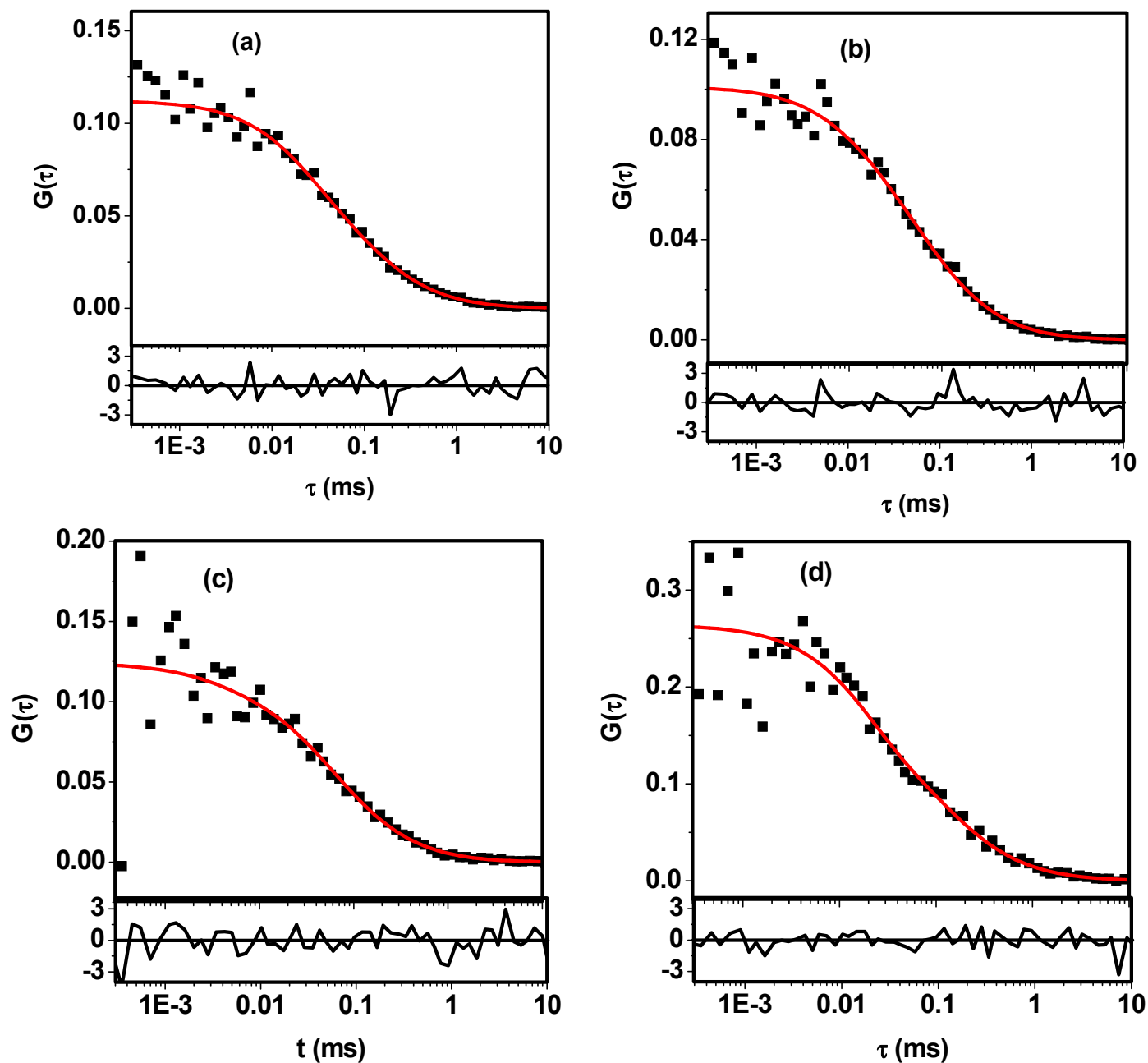


Fig.S11: Correlation curves of FL in 8 μM BSA in presence of (a) 0. M (b) 0.5 (c) 1.5 M (d) 3 M NaCl. Red lines are the best fit to the raw data points. Residuals are shown in the lower part of each figure.

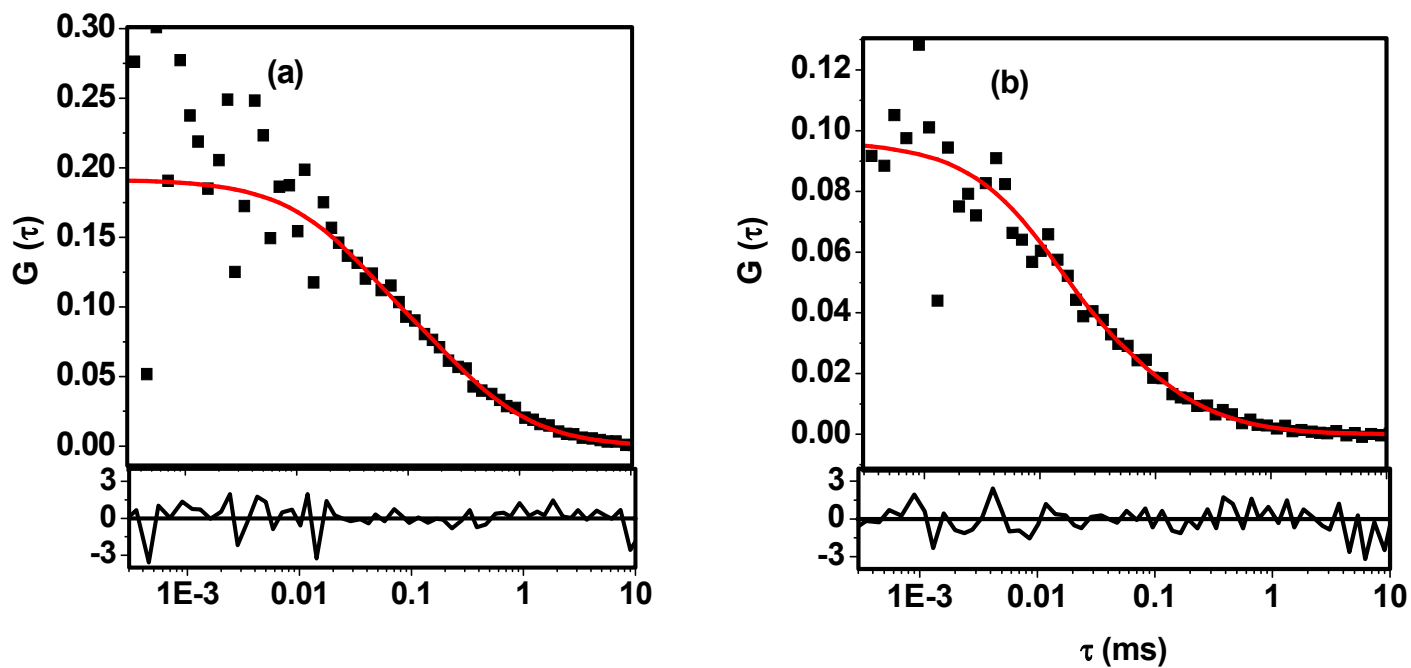


Fig.S12: Effect of addition of 4 M urea on the correlation curves of C102 in the presence of constant amount of BSA ($8 \mu\text{M}$) (a) 0 M urea (b) 4 M urea. Black squares represent the raw data and solid lines are the best fits to these data points. Residuals are shown in the lower part of the figures.

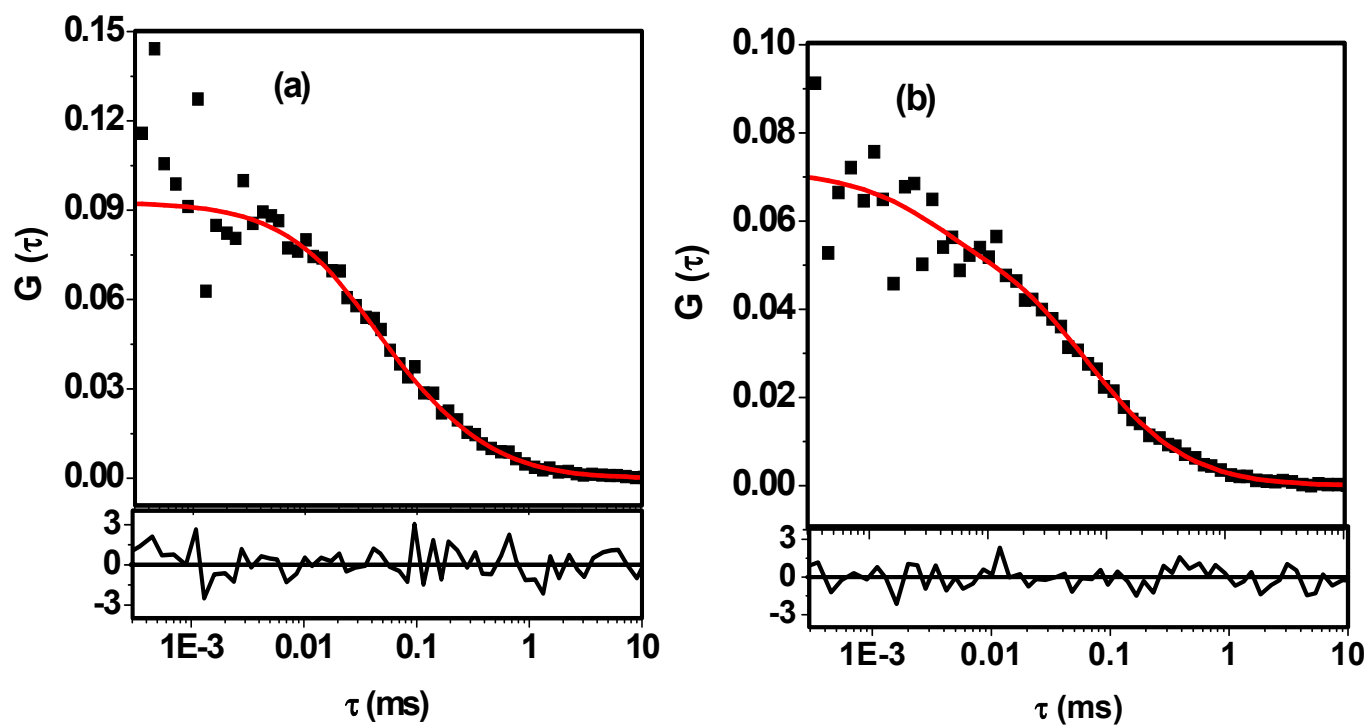


Fig.S13: Raw data points (black squares) along with the best fits (solid lines) of FL in 8 μ M BSA (a) in absence and (b) in presence of 4 M urea. Residual is shown in the lower part of the each figure.

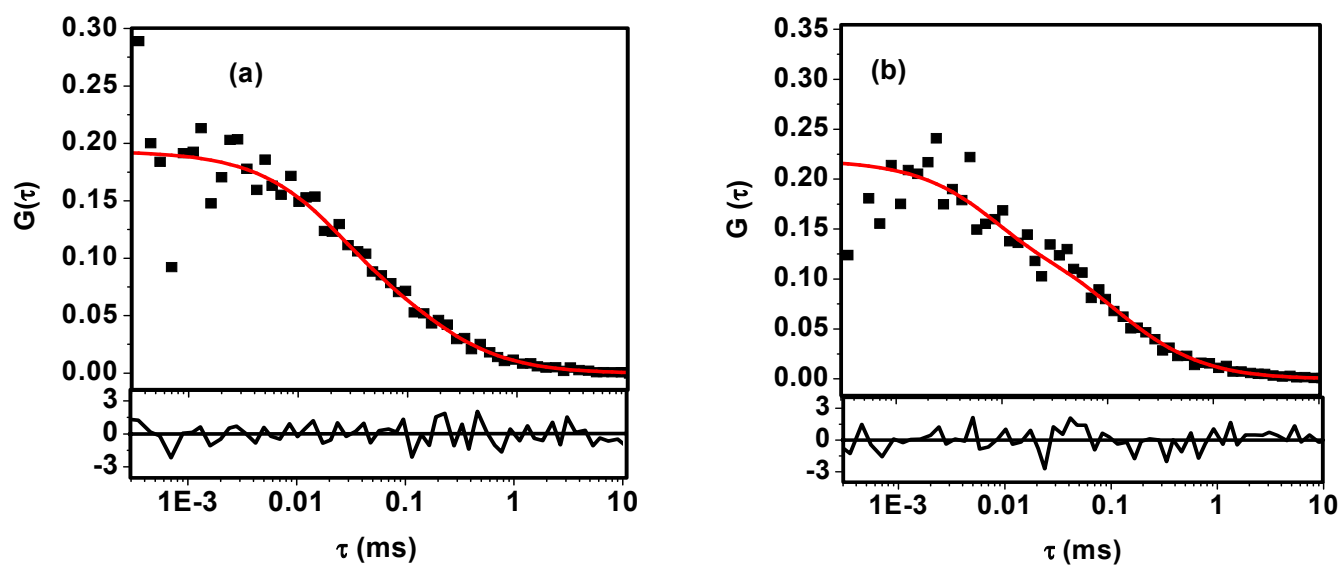


Fig.S14: Correlation curve of R6G in 8 μ M BSA (a) in absence and (b) in presence of 4 M urea. Red line (solid) is fit to the raw data (black squares). Residuals are shown in the lower part of the corresponding figures.

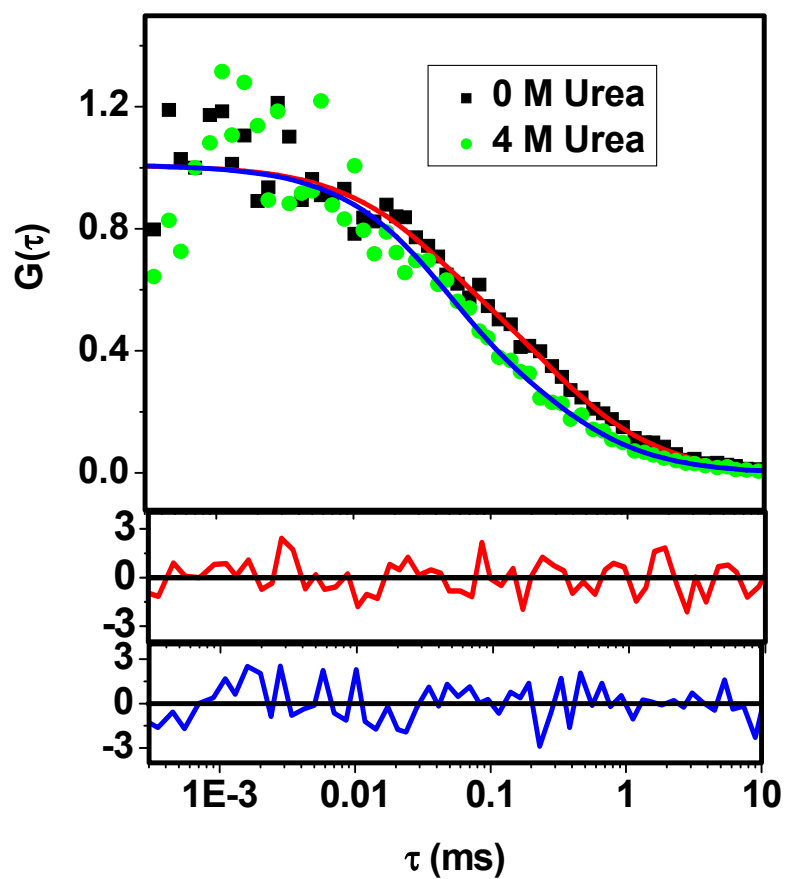


Fig.S15: Normalized correlation curves of FL in 60 μ M BSA in 0 M (black squares) and 4 M (green circle) urea. Residuals are shown in the lower part of the figure with the corresponding color of the curves. Here the solid lines are fits to the raw data.

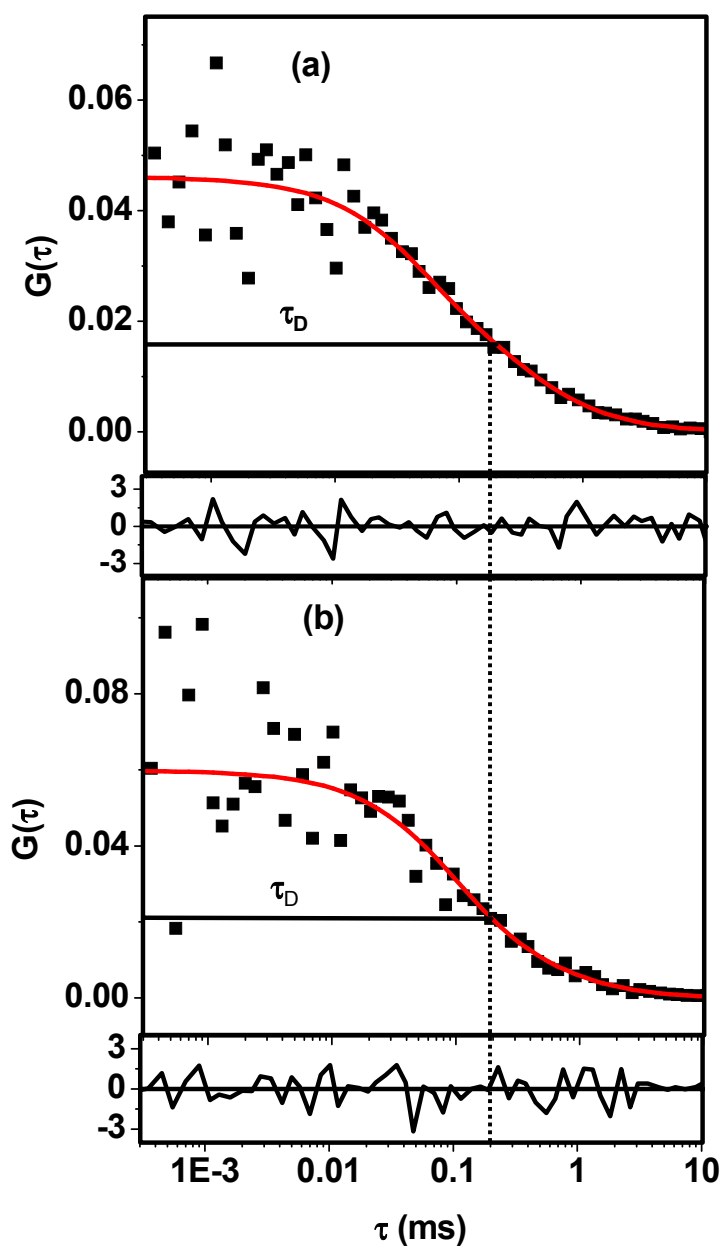


Fig.S16: Correlation curves (data points along with best fits) of R6G in 60 μ M BSA (a) in absence (0 M urea) and (b) in presence of 4 M urea. Residuals are shown in the lower part of each figure.

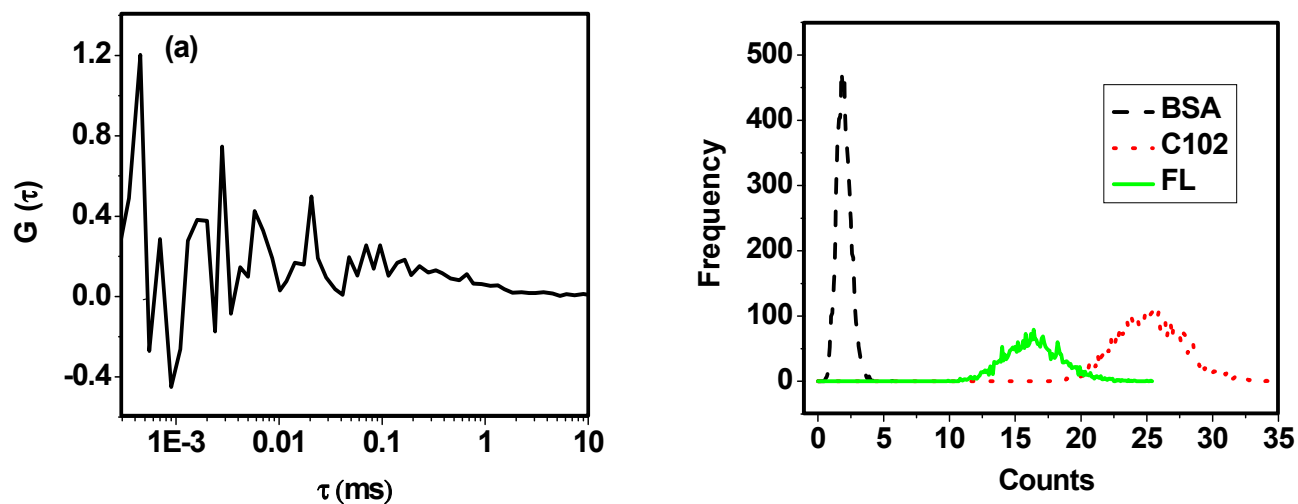


Fig S17: (a) Correlation trace of 25 μM BSA in pH 7 phosphate buffer. (b) Count rate histograms of 25 μM BSA (---), C102 (···) and FL (—) in 25 μM BSA.

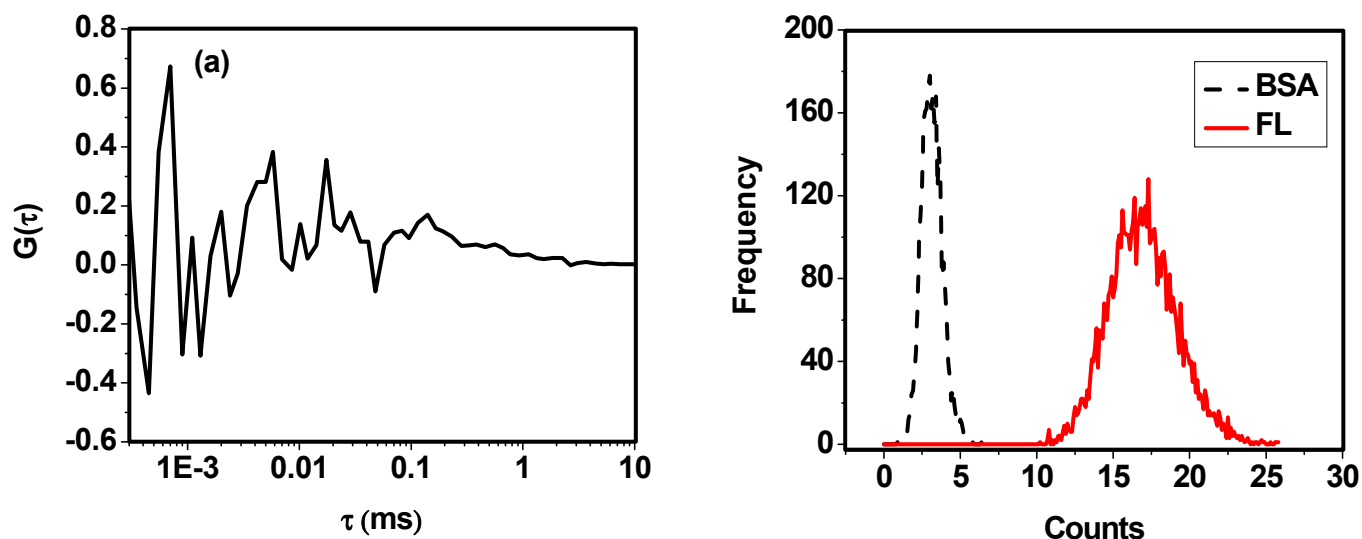


Fig S18: (a) Correlation trace of 60 μM BSA in pH 7 phosphate buffer. (b) Count rate histograms of 60 μM BSA (---) and FL in 60 μM BSA (—).