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

Elucidation of Local Dynamics of Domain-III of Human Serum Albumin over ps-μs Time Regime using a New Fluorescent Label

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Supplementary Information

Figure S1: ^{1}H NMR spectra of *p*-nitrophenyl coumarin ester (NPCE), 400MHz, solvent $CDCl_3$.

Figure S2: Mass analysis of *p*-nitrophenyl coumarin ester (NPCE); inset shows the comparison of experimental and calculated mass.

Figure S3: IR spectra of *p*-nitrophenyl coumarin ester (NPCE).

Figure S4: Spectral overlap between absorption of the acceptor (TMR, pink) and emission spectra of the donor (NPCE, blue).

Figure S5: Emission spectra of only NPCE tagged HSA, only TMR tagged HSA and NPCE-TMR doubly tagged HSA under similar excitation power and similar optical density of the samples at the excitation wavelength.

Figure S6: Fluorescence transients of TMR (acceptor) emission at 572 nm by exciting the NPCE (acceptor) in NPCE-TMR doubly tagged HSA.

Figure S7: Wavelength resolved fluorescence transients of NPCE tagged to HSA in phosphate buffer (pH 7.4, 50 mM). Solid black lines depict the best fit using three exponential function.

Figure S8: Normalized autocorrelation curves of untagged NPCE (cyan) and NPCE tagged in HSA (red) in phosphate buffer (pH 7.4, 50 mM). Solid black lines depict the best fit using single diffusion model for untagged NPCE and one diffusion + one relaxation component fitting model for NPCE tagged HSA.

Figure S9: Laser power dependence of fluorescence autocorrelation of NPCE tagged in HSA in phosphate buffer (pH 7.4, 50 mM). Solid black lines depict the best fit using one diffusion + one relaxation component fitting model.

Figure S1: ^{1}H NMR spectra of *p*-nitrophenyl coumarin ester (NPCE), 400MHz, solvent $CDCl_3$ δ 8.472 (s,1H), 8.276 (d, J=9.16 Hz, 2H), 7.401 (d, J=9.16 Hz, 2H), 6.979 (s, 1H), 3.34-3.39 (m, 4H), 2.890 (t, J=6.4 Hz, 2H), 2.764 (t, J=6.44 Hz, 2H), 1.947-2.010 (m, 4H).

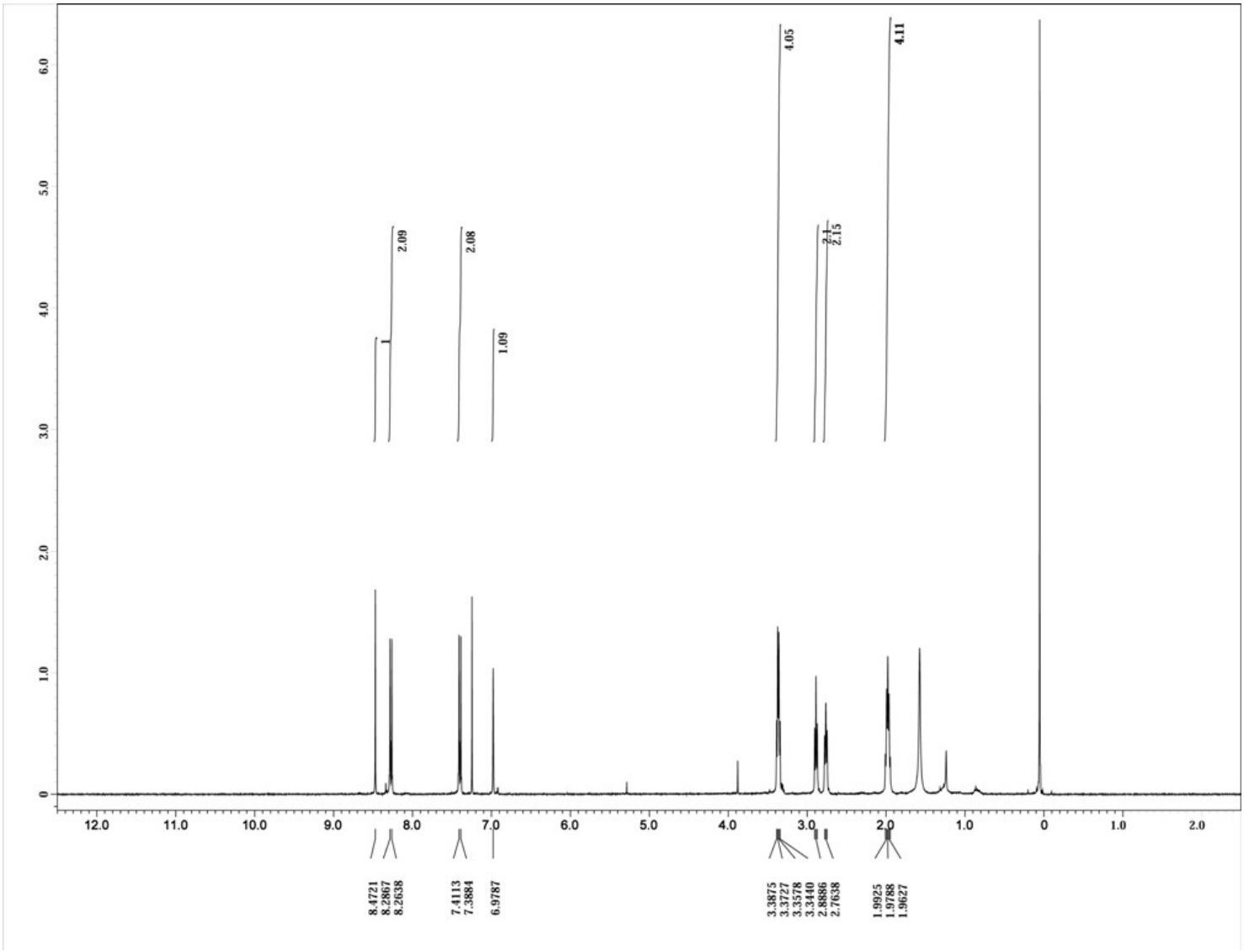

Figure S2: Mass analysis of *p*-nitrophenyl coumarin ester (NPCE); inset shows the comparison of experimental and calculated mass.

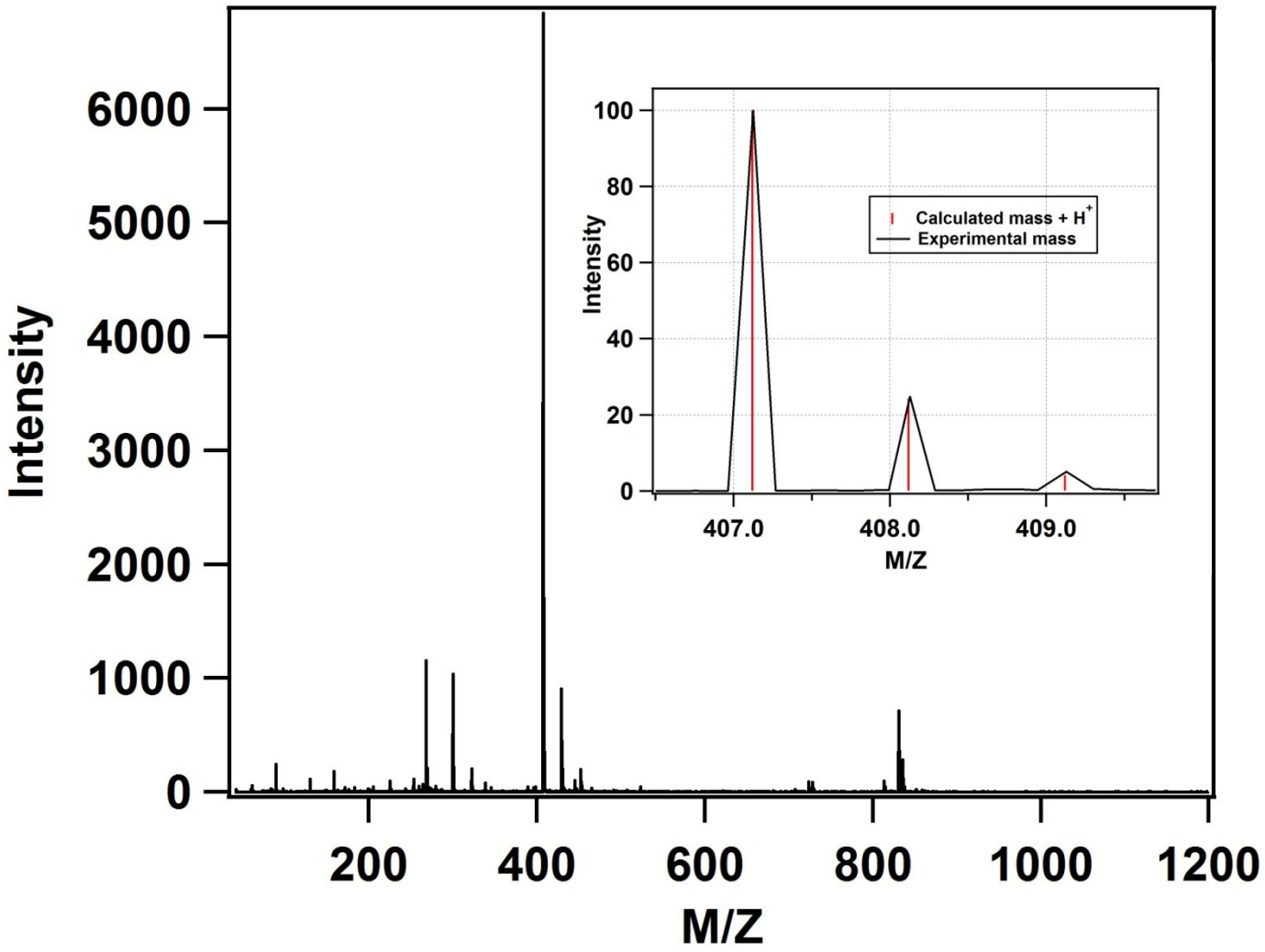

Figure S3: IR spectra of *p*-nitrophenyl coumarin ester (NPCE).

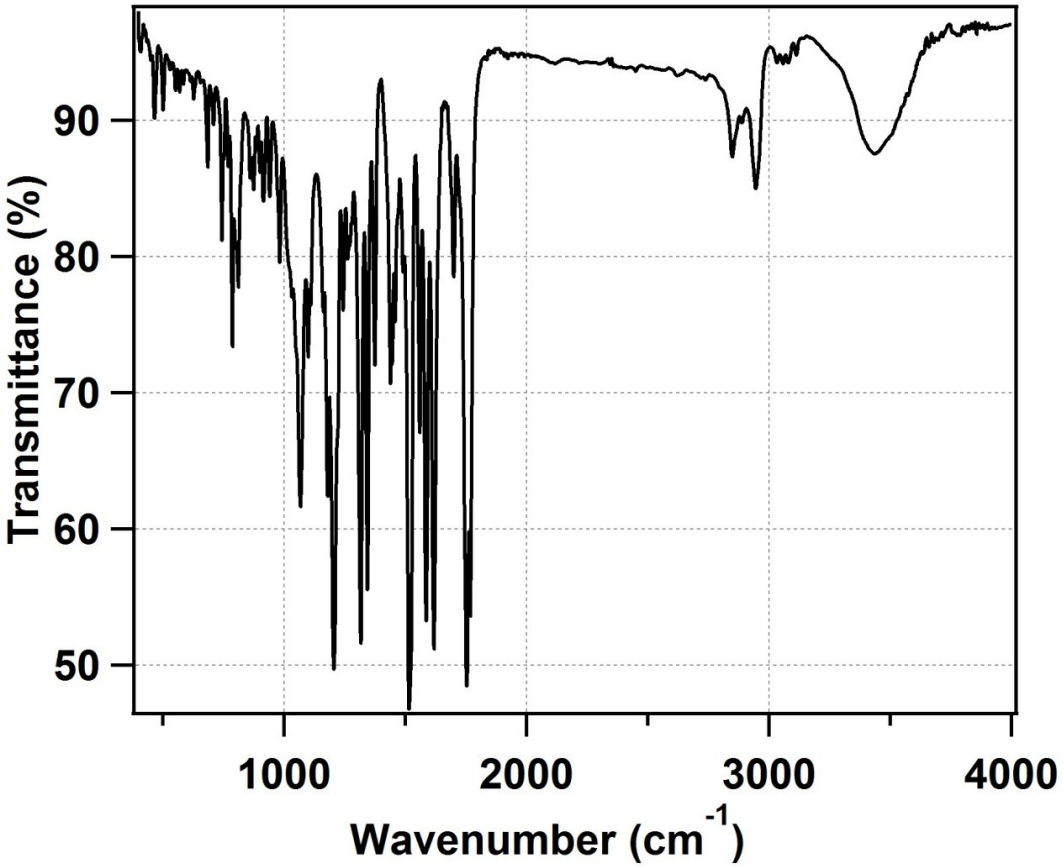

Figure S4: Spectral overlap between absorption of the acceptor (TMR, pink) and emission spectra of the donor (NPCE, blue).

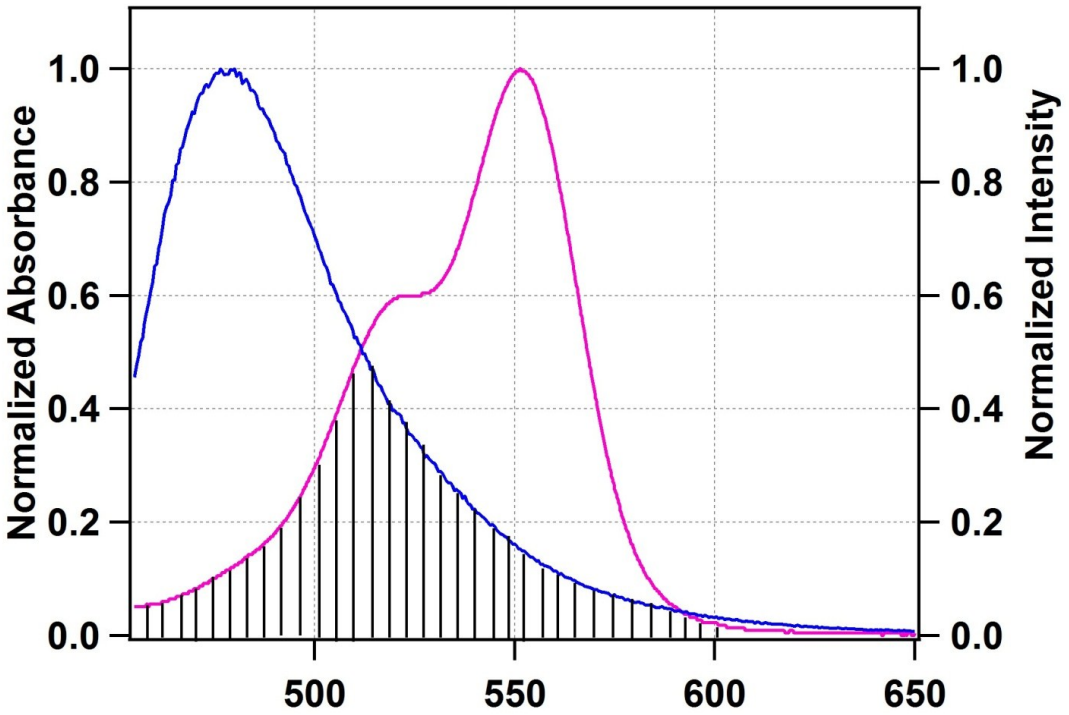

Figure S5: Emission spectra of only NPCE tagged HSA, only TMR tagged HSA and NPCE-TMR doubly tagged HSA under similar excitation power and similar optical density of the samples at the excitation wavelength. (excitation wavelength 446 nm)

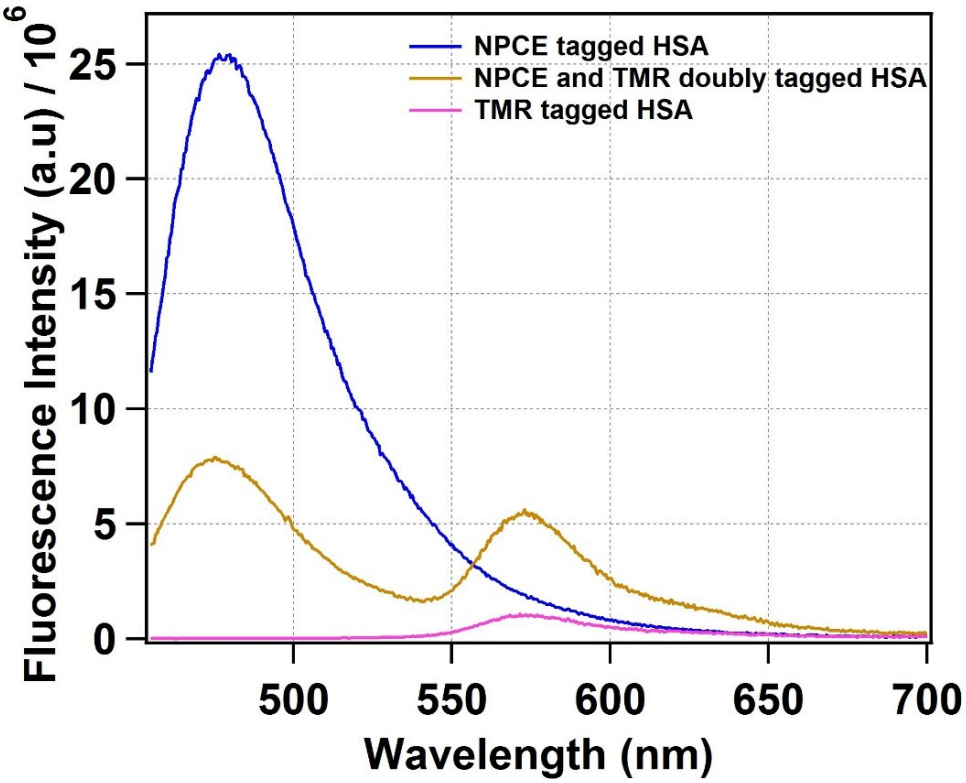

Figure S6: Fluorescence transients of TMR (acceptor) emission at 572 nm by exciting the NPCE (acceptor) in NPCE-TMR doubly tagged HSA.

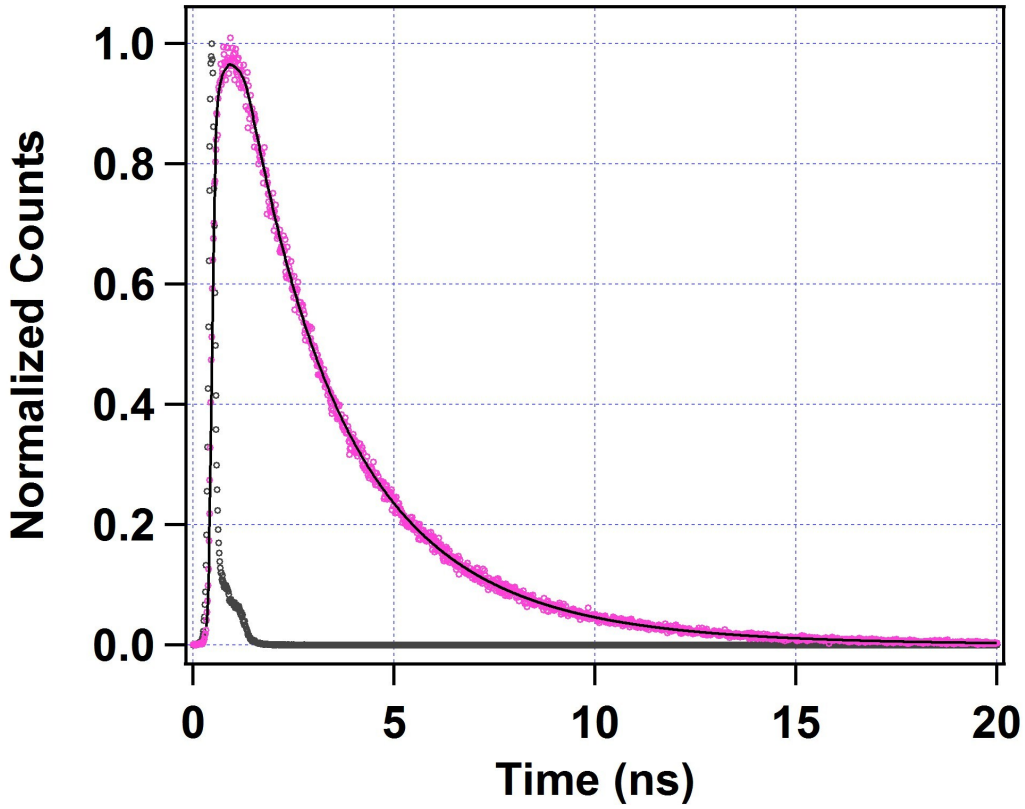

Figure S7: Wavelength resolved fluorescence transients of NPCE tagged to HSA in phosphate buffer (pH 7.4, 50 mM). Solid black lines depict the best fit using three exponential function.

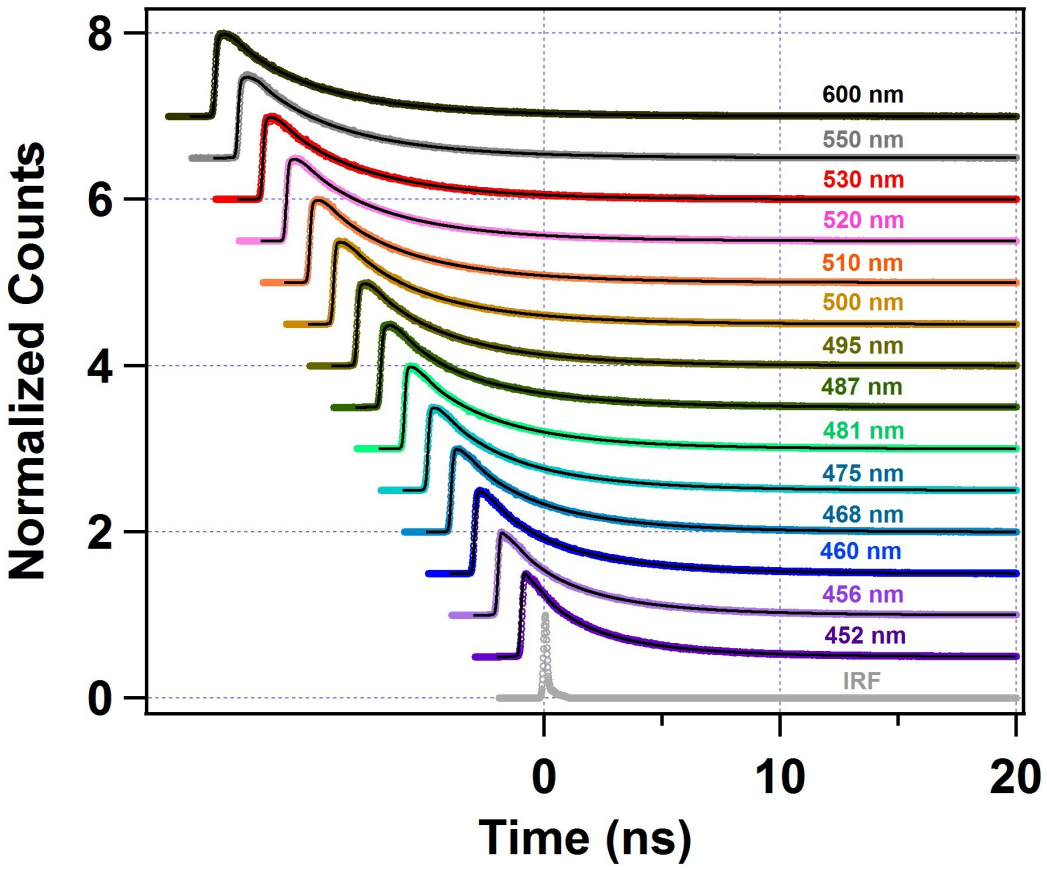

Figure S8: Normalized autocorrelation curves of untagged NPCE (cyan) and NPCE tagged in HSA (red) in phosphate buffer (pH 7.4, 50 mM). Solid black lines depict the best fit using single diffusion model for untagged NPCE and one diffusion + one relaxation component fitting model for NPCE tagged HSA.

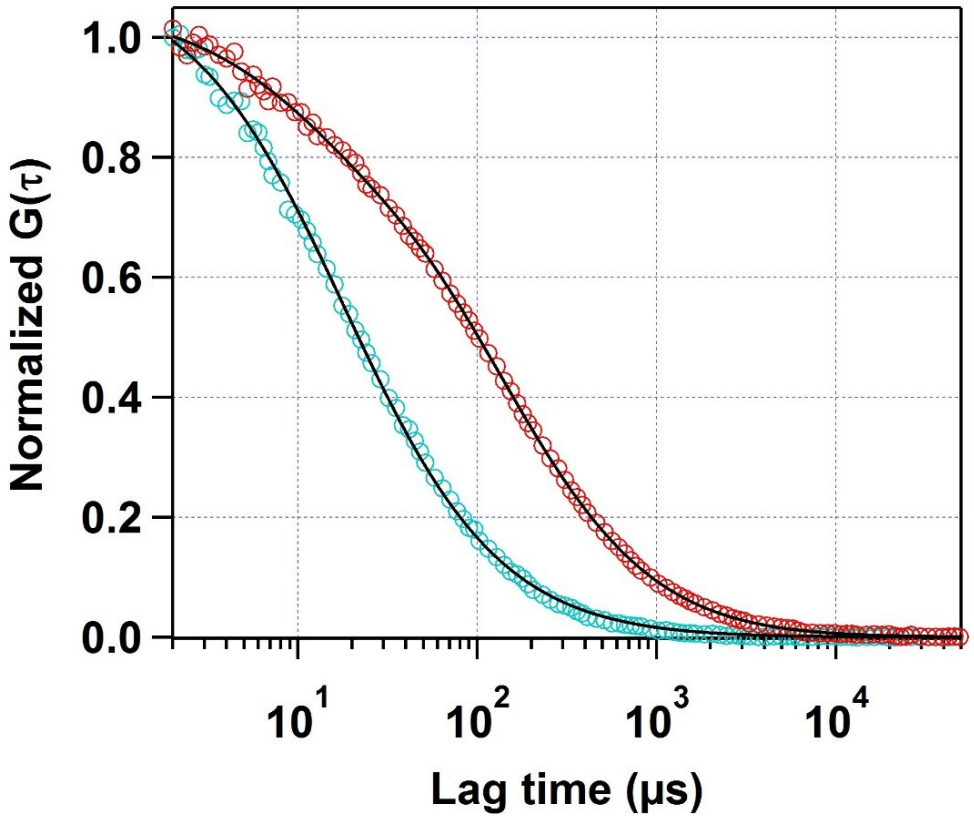

Figure S9: Laser power dependence of fluorescence autocorrelation of NPCE tagged in HSA in phosphate buffer (pH 7.4, 50 mM). Solid black lines depict the best fit using one diffusion + one relaxation component fitting model.