Supplementary Information

FRET between a Donor and an Acceptor Covalently bound to Human Serum Albumin in Native and Non-Native States

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Table S1: Quantum yield of donor (Φ_D^0, in absence of acceptor at λ_{ex}=405 nm), acceptor (Φ_A^0, in absence of donor at λ_{ex}=470 nm), correction factor (γ) and Förster radius (R_0) in Presence of Different Additives.

System	Φ_D^0	Φ_A^0	γ	R_0(Å)
N	0.63	0.84	1.47	50
U_1	0.61	0.89	1.60	50.5
U_2	0.69	0.92	1.47	51
N′	0.71	0.87	1.35	52
MG-1	0.61	0.9	1.62	51.5
MG-2	0.61	0.92	1.60	48
U_3	0.64	0.87	1.5	49.5
U_4	0.63	0.94	1.66	52

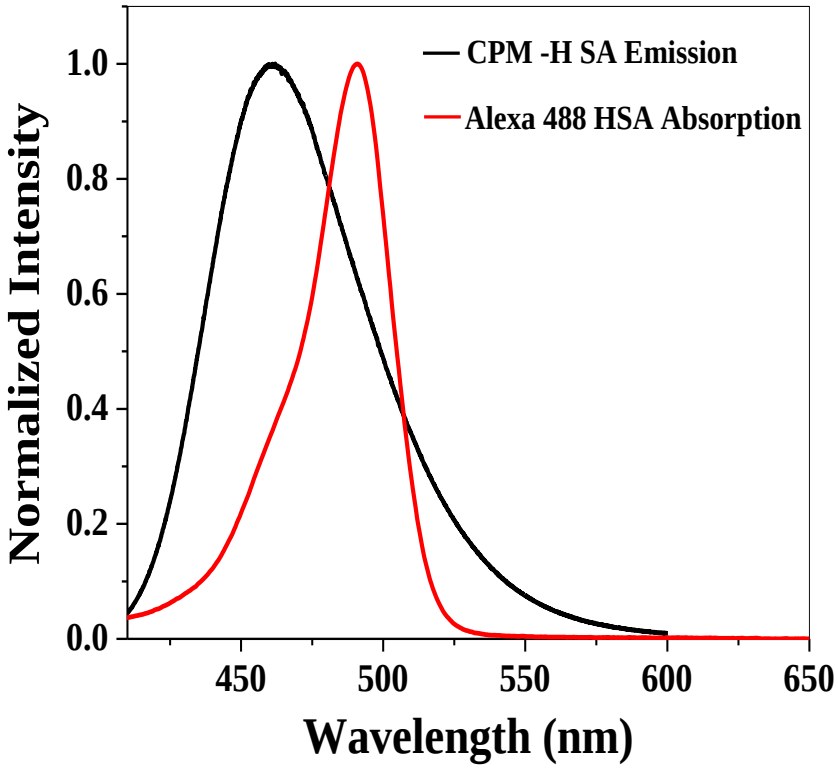

Fig. S1 Overlap between Emission spectra (at λ_{ex} = 405 nm) of donor (CPM–HSA) (black) and absorption spectra of acceptor (alexa 488 HSA) (red) in native state.

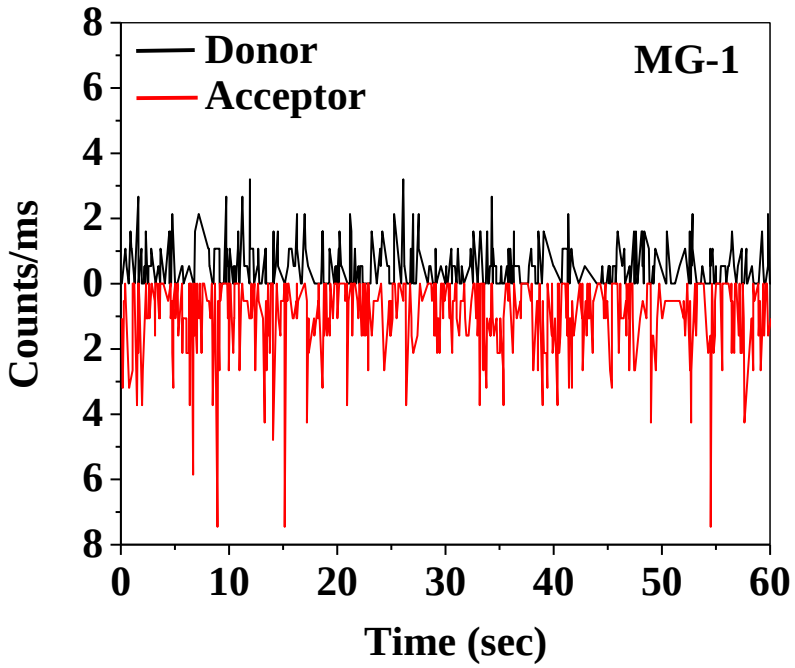

Fig. S2 Photon bursts of donor (black) and acceptor (red) for MG-1. The counts are shown per binning time (1 ms).

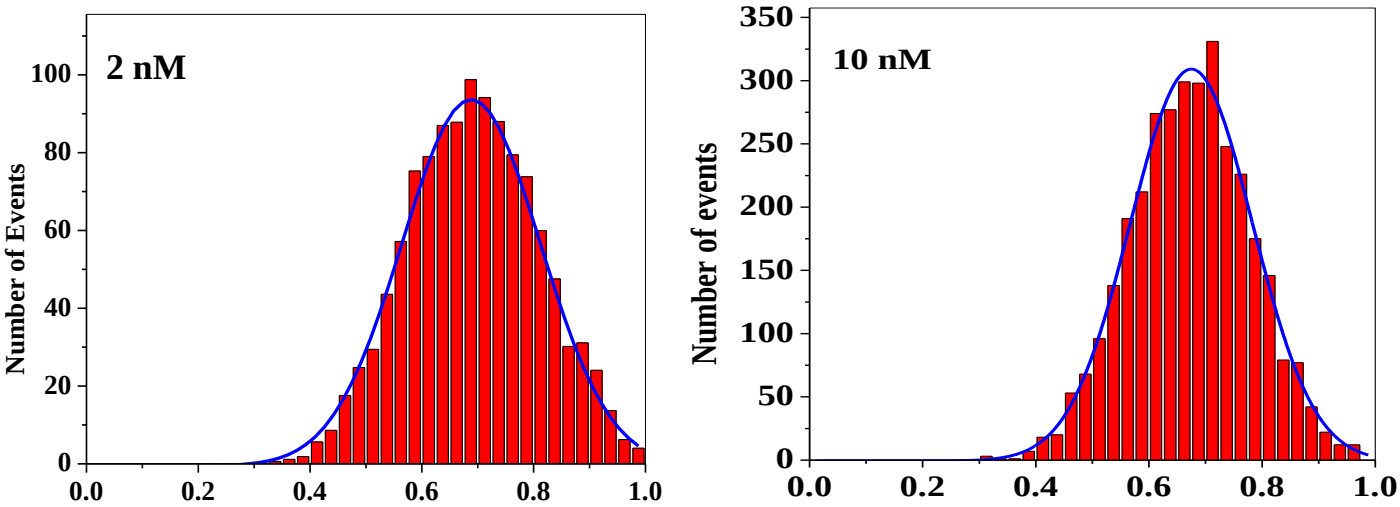

Fig. S3 Efficiency of FRET histograms of doubly labeled HSA in native state for 2 nM and 10 nM concentration of the doubly labeled protein.

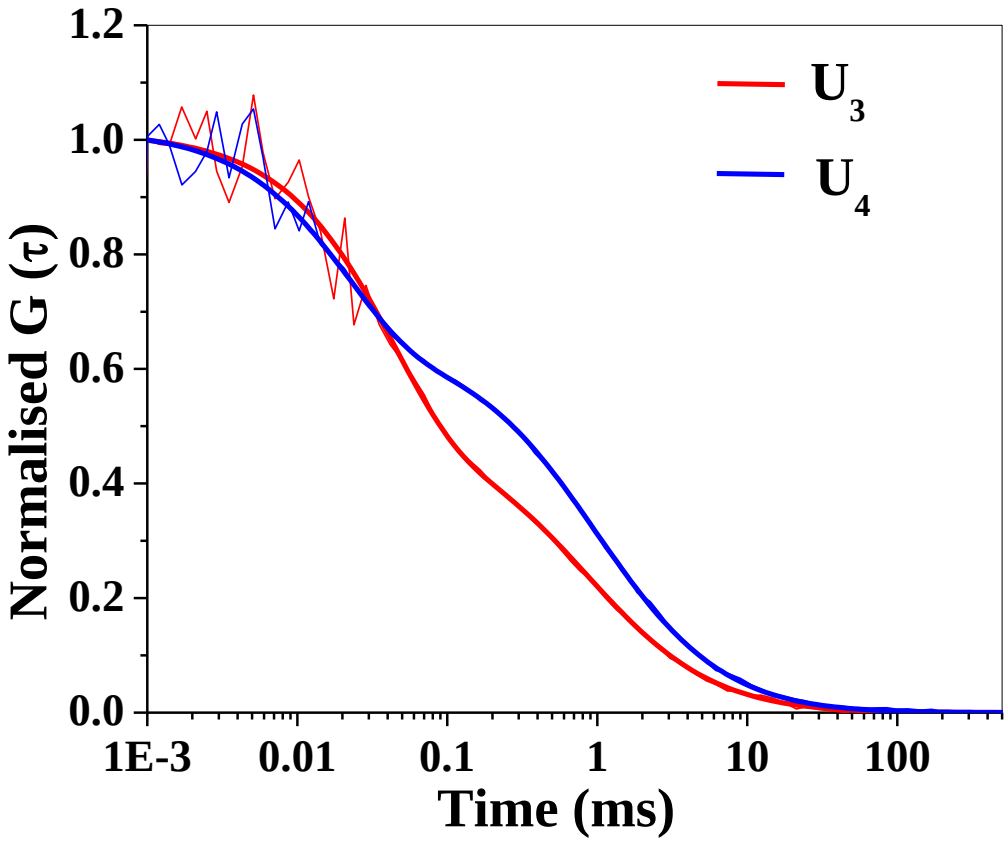

Fig. S4 FCCS traces of doubly labeled HSA at pH 8.5 (U_3, red) and pH 10 (U_4, blue) along with their fit.

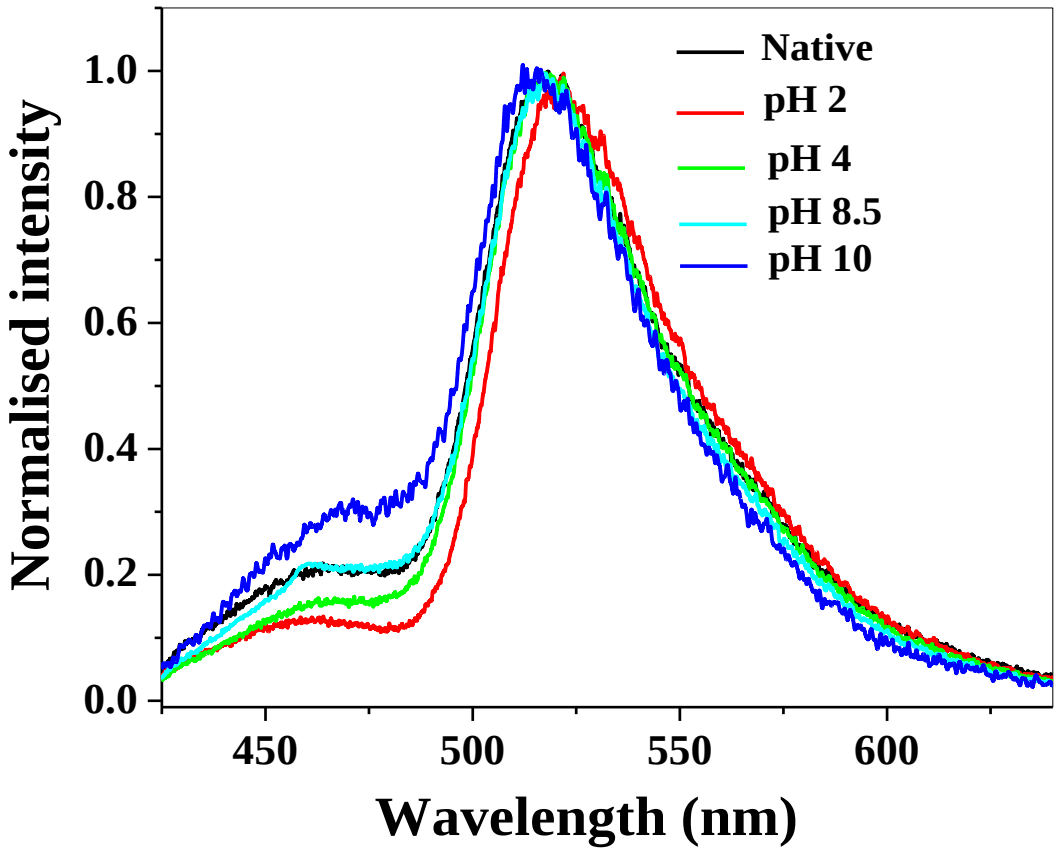

Fig. S5 Steady state emission spectra of doubly labeled HSA in native state (black), at pH 2 (red), at pH 4 (green), at pH 8.5 (cyan) and at pH 10 (blue). The background signal (buffer of various pH) has been corrected.