

Electronic Supplementary Information

Fluorescence Fluctuation of Antigen-Antibody Complex: Circular Dichroism, FCS and smFRET of Enhanced GFP and its Antibody

Debmalya Bhunia,^{a#} Rajdeep Chowdhury,^{b#} Kankan Bhattacharyya^{*b} and Surajit Ghosh^{*a}

^aOrganic & Medicinal Chemistry Division, CSIR-Indian Institute of Chemical Biology, Jadavpur, Kolkata-700032, India.

^bDepartment of Physical Chemistry, Indian Association for the Cultivation of Science, Jadavpur, Kolkata-700032, India.

#: Equal Contribution

***E-mail:** sghosh@iicb.res.in, pckb@iacs.res.in.

Table S1. Diffusion time (τ_D), diffusion coefficient (D_t), hydrodynamics radius (r_H) and parameters of proton transfer dynamics for EGFP.

System	[GdnHCl]	τ_D (mS)	D_t (mm^2s^{-1})	r_H (Å)	τ_C [F] (mS)
EGFP	0 M	1000±100	83±8	24.4±2	400±50 [0.6]
	1 M	1400±150	59±6	34.1±2	220±30 [0.5]
	2 M	1700±150	49±6	39.5±2	270±30 [0.69]
	3 M	1900±200	43.5±4	41.3±2	330±30 [0.69]
	4 M	2100±200	39±4	42±2	400±50 [0.72]

Table S2. Diffusion time (τ_D), diffusion coefficient (D_t), hydrodynamics radius (r_H) and parameters of conformational dynamics for alexa 488 labeled anti-EGFP V_HH-His₆.

System	[GdnHCl]	τ_D (\square S)	D_t (\square m ² s ⁻¹)	R_H (\AA)	τ_R (F) (\square S)
Anti-EGFP V _H H-His ₆	0 M	190±20	118±10	18.4±2	83±10 (0.54)
	1 M	212±20	106±10	20.5±2	82±10 (0.6)
	2 M	230±30	97±10	21.1±2	93±10 (0.64)
	3 M	250±30	90±5	21.4±2	100±10 (0.62)
	4 M	275±30	90±5	21.2±2	85±10 (0.6)

Table S3. Diffusion time (τ_D), diffusion coefficient (D_t), hydrodynamics radius (r_H) and parameters of conformational dynamics for EGFP bound to alexa 594 labeled anti-EGFP V_{HH} -His₆.

System	[GdnHCl]	τ_D (mS)	D_t (mm ² s ⁻¹)	R_H (Å)	τ_R [A] (mS)
EGFP: anti EGFP V_{HH} -His ₆	0 M	1680±200	49±5	41±3	240±30 [0.65]
	1 M	1960±200	42±5	48±3	230±30 [0.58]
	2 M	2200±200	38±4	51±3	360 ±40 [0.72]
	3 M	2320±200	36±4	50±3	400 ±50 [0.72]
	4 M	2410±200	34±4	48±3	430 ±50 [0.73]

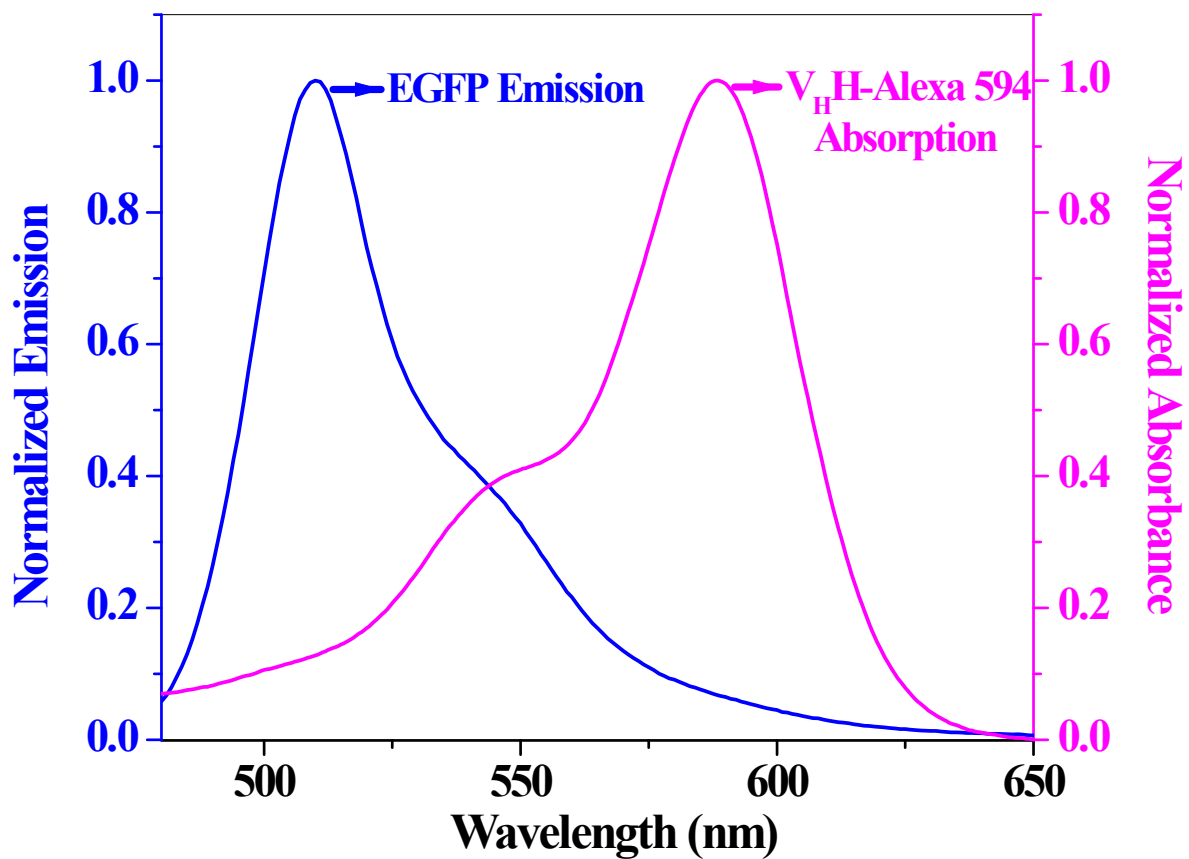


Figure S1. Spectral overlap between EGFP (donor) emission and absorbance of alexa 594 labeled anti-EGFP V_HH-His₆ (acceptor).

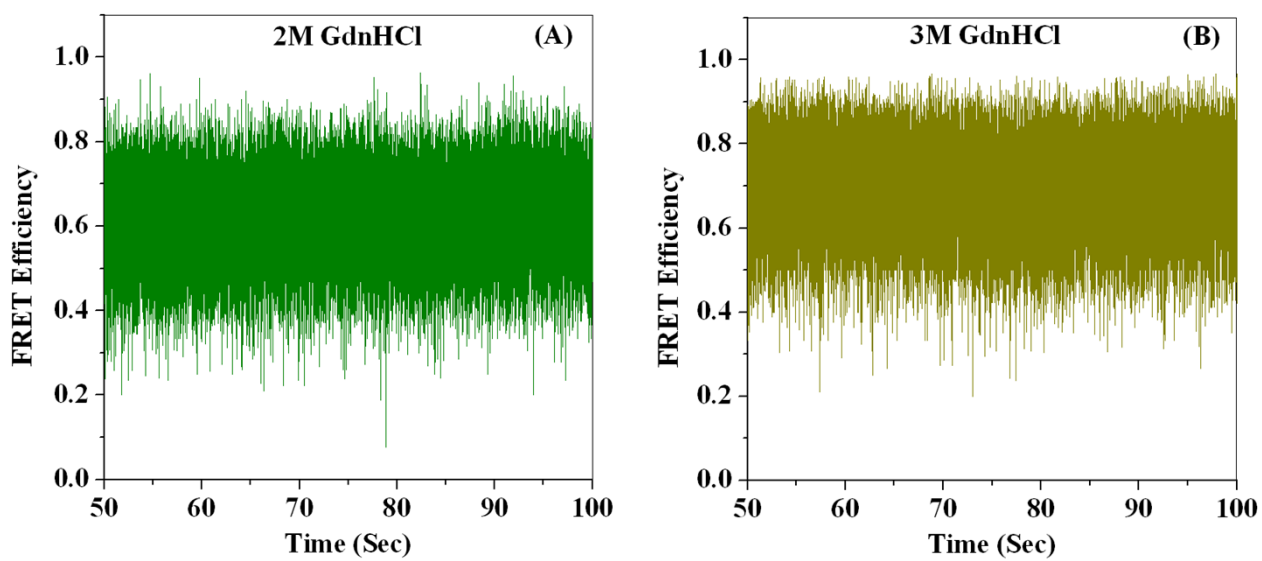


Figure S2. Time trajectory of FRET efficiency for EGFP: anti-EGFP V_HH-His₆ complex in presence of (A) 2M and (B) 3M GdnHCl.