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Electronic Supplementary Information for

FRET-based characterization of surfactant bilayer protected core-shell carbon nanoparticles: Advancement toward carbon nanotechnology

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1. Experimental Section

Materials. Cystine was purchased from SRL, India, sodium hydroxide was procured from Merck, India. The surfactant, cetyltrimethylammonium bromide (CTAB), was procured from Sigma-Aldrich. Phenosafranine and fluorescein dyes (Aldrich) were used as acceptor molecules for the energy transfer study. Triply distilled water was used to make the experimental solutions.

Preparation of fluorescent CNPs. The one step microwave assisted synthetic process yields CNPs that are found to exhibit strong photoluminescence. The CNPs obtained are found to have uniform size distribution. Fluorescent CNPs were prepared from cystine source by microwave treatment following a reported protocol (see manuscript for reference). Briefly, 1g of cystine was dissolved in 10 ml aqueous solution of NaOH and sonicated until a clear solution was obtained. The alkali serves as catalyst for the formation of the CNPs from amino acids. The solution was then put into a microwave oven at 150°C and incubated for 30 seconds. After cooling, a yellow coloured solution was obtained. The solution was centrifuged at 25000 rpm for 20 minutes and the then dialyzed against ultra-pure water through a dialysis membrane (molecular weight cut off = 3.5 kDa, Sigma-Aldrich) for 48 h to remove the excess precursors and resulting small molecules and then stored at 4°C. The concentration of CNP was about 45.4 mg/ml measured by dry weight analysis method. We obtained a moderately high quantum yield of ~17% for the CNPs. Synthesis of fluorescent CNPs from amino acid (cystine) source and in presence of CTAB provides a core-shell structure to the system with a soft-shell comprising of a double layer of cationic surfactant molecules around the CNP core. Formation of the bilayer has been confirmed by monitoring the change in zeta potential of the CNP (Fig. S1). The CTAB coating not only keeps the CNPs well dispersed but also enhances the fluorescence quantum yield to ~22%.

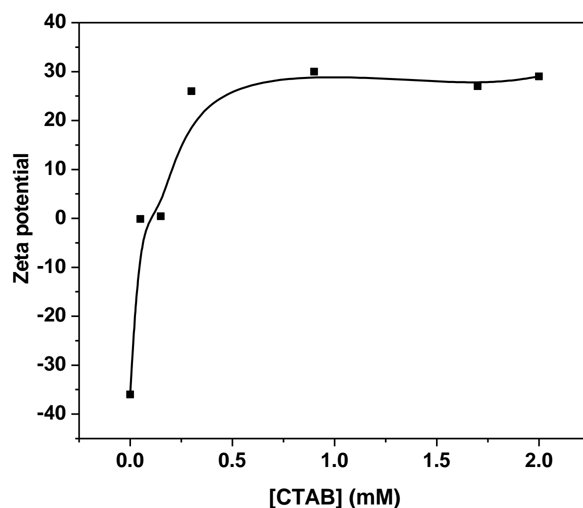

Fig. S1. Change in zeta potential with addition of CTAB to CNP solution in water.

Methods. The absorption spectra were recorded using a Varian Cary 300 Bio UV-vis spectrophotometer. Fluorescence measurements were made using a Fluoromax 3 scanning spectrofluorimeter. The fluorescence lifetimes were measured by the method of time-correlated single-photon counting using a picoseconds spectrofluorimeter from Horiba Jobin Yvon IBH. The instrument was equipped with a FluoroHub single photon counting controller, and a FC-MCP-50SC MCP-PMT detection unit. The excitation wavelength used was 405 nm. The typical detection time of these was 40 ps. To calculate the lifetime, the fluorescence decay curves were analyzed by an iterative fitting program provided by IBH. Size of the CNs were measured by dynamic light scattering (DLS) using a Malvern Zetasizer Nano equipped with a 4.0 mW HeNe laser operating at λ = 633 nm. All samples were measured in an aqueous system at room temperature with a scattering angle of 173°. Size distribution is calculated by Nano x software using a non-negative least square analysis (NNLS). TEM analysis of the CNPs was performed using a Philips CM200 microscope operating at 200 kV equipped with a charge-coupled device (CCD) camera (Gatan).

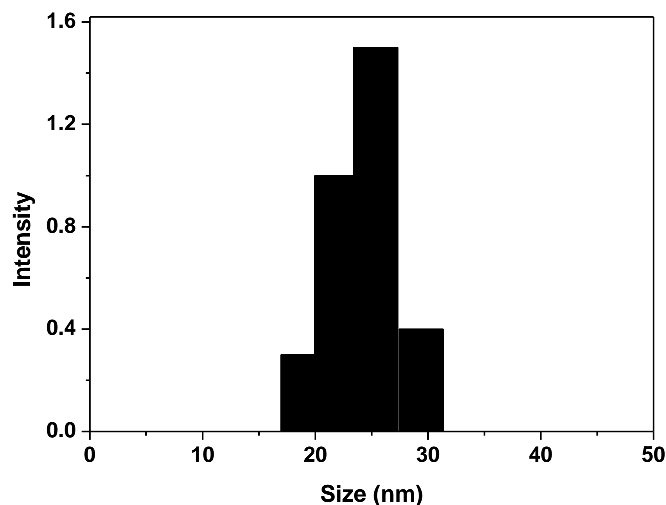

Fig. S2 Dynamic light scattering analysis of the CNPs.

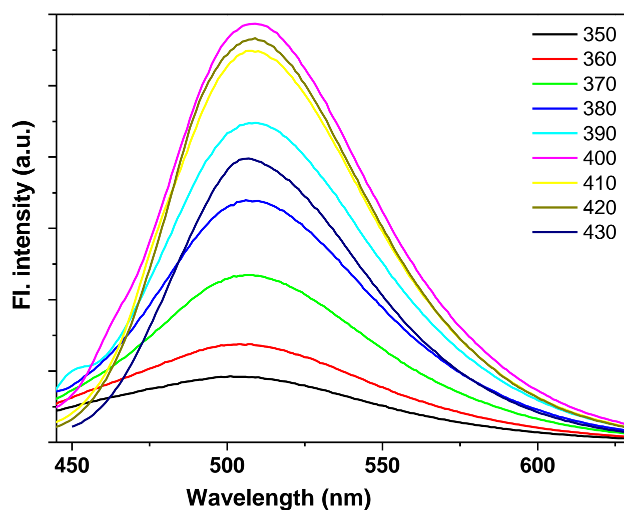

Fig. S3 Fluorescence emission spectra of CNP on excitation at different excitation wavelengths.

Calculation of Förster distance for the donor-acceptor pairs

The equation used to calculate the overlap integrals for the donor-acceptor pairs is

$J(\lambda)=\int_0^\infty F_D(\lambda)\varepsilon_A(\lambda)\lambda^4 d\lambda$, where $F_D(\lambda)$ is the normalised fluorescence intensity of donor at a particular wavelength range, $\varepsilon_A(\lambda)$ is the molar extinction coefficient of acceptor at the same wavelength range.

The equation used to calculate the Förster distance (R_0) between donor and acceptor is

$R_0 = 0.211[\kappa^2 n^{-4} J(\lambda)]^{1/6}$, where κ^2 is the orientation factor and n is refractive index of the medium.

Thus, for CNP+PSF pair: $R_0 = 0.211\ [(2/3) \times (1.4)^{-4} \times 0.17 \times 3.022 \times 10^{17}]^{1/6} = 93.72$ Å and for CNP-CTAB+PSF pair: $R_0 = 0.211\ [(2/3) \times (1.4)^{-4} \times 0.22 \times 2.79 \times 10^{17}]^{1/6} = 96.52$ Å.

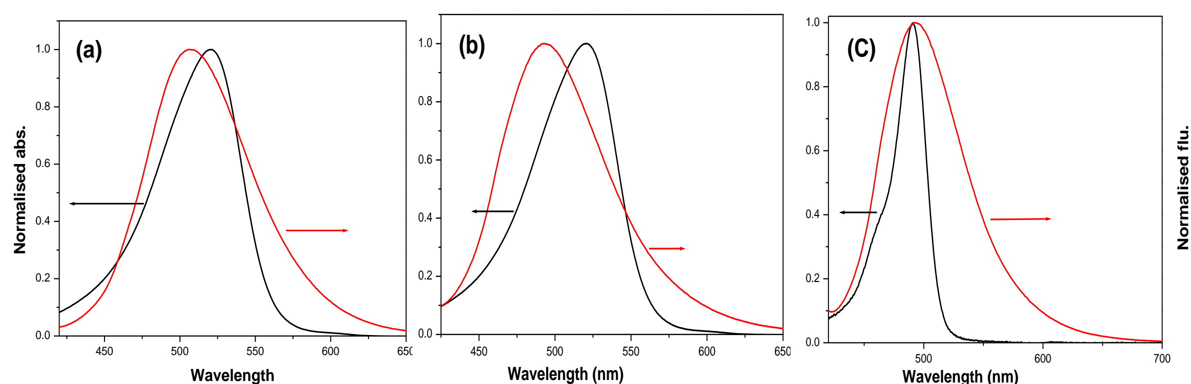

Fig. S4 Spectral overlap of donor (CNP) emission and acceptor (organic dyes, PSF and FL) absorption. Red lines represent emission spectra of donors and black lines indicate absorption spectra of respective acceptors. Excitation wavelength for CNP is 400 nm.

To further clarify our proposed FRET model and distribution of PSF in CNP and CNP-CTAB conjugate, we intend to state that prbability of existence of PSF adsorbed on the CNP surface in presence of CTAB is minimal. This can be proved by plotting the FRET efficiency against PSF concentration. If PSF stickes to CNP surface in presence of CTAB, then the FRET efficiency must be same (at least up to some extent) in both CNP-CTAB + PSF and CNP-PSF pair with changing the acceptor concentration. However, this is not the case in the

present study as can be seen in the following plot of variation of FRET efficiency against acceptor concentration.

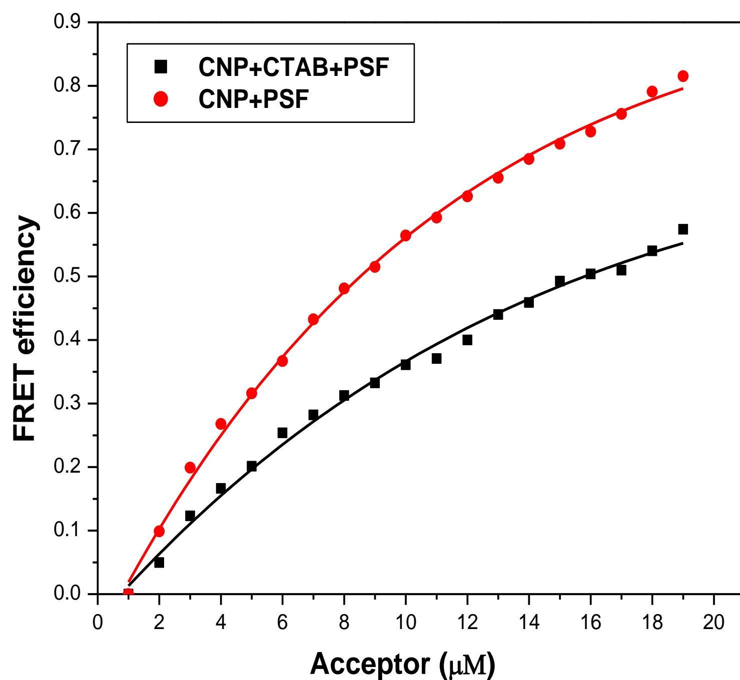

Fig. S5 Plot of FRET efficiency of CNP-PSF and CNP-CTAB-PSF against concentration of acceptor (PSF).

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Table S1: Time-resolved fluorescence data for the different donor-acceptor systems. The excitation wavelength was 405 nm and the emission wavelengths are shown beside the respective donors. The digits beside the acceptors indicate their concentrations in solution in μM. The figures in parentheses in the lifetime (τ) columns indicate the contributions to the existing components in the decay profile. The negative decay indicates a growth component, which is typical for FRET. The χ^2 values indicate the goodness of the fits to the raw data.

Donor	Acceptor (μM)	τ_1 (a1) (ps)	τ_2 (a2) (ns)	τ_3 (a3) (ps)	χ^2
CNP @580 nm	PSF 0	2.56 (38.9)	6.38 (61.1)	-	1.27
	PSF 16	847 (83.9)	5.32 (20.7)	41 (-4.6)	1.22
	PSF 26	852 (91.6)	5.62 (14.1)	49 (-5.7)	1.26
CNP+CTAB @570 nm	PSF 0	2.01(23.9)	8.16 (76.1)	-	1.18
	PSF 50	803 (85.1)	5.83 (16.3)	77 (-1.4)	1.04
	PSF 200	819 (98.6)	6.54 (7.9)	48 (-6.5)	1.16
CNP+CTAB @515 nm	Flu 0	1.58 (30.0)	6.11(70.0)	-	1.28
	Flu 50	4.04 (48.0)	6.20 (53.4)	676 (-1.4)	1.05
	Flu 200	4.46 (52.3)	6.34 (49.6)	520 (-2.0)	1.10

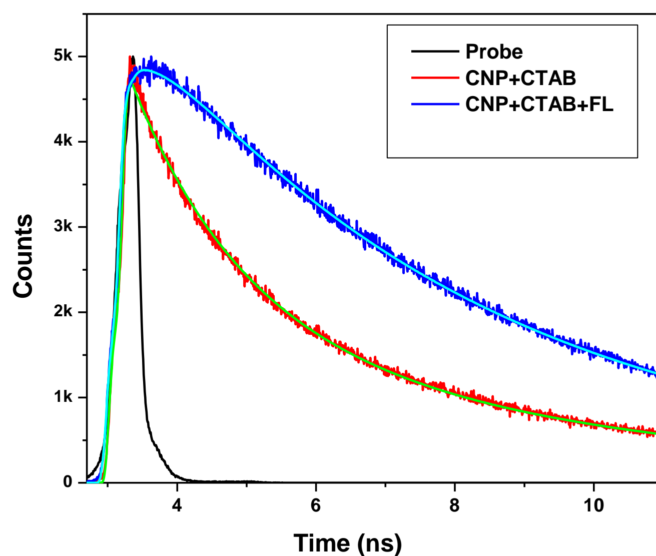

Fig. S6 Time-resolved fluorescence data for CNP-CTAB and CNP-CTAB conjugate and FL. The donor was excited at 405 nm and the emission was monitored at 515 nm.

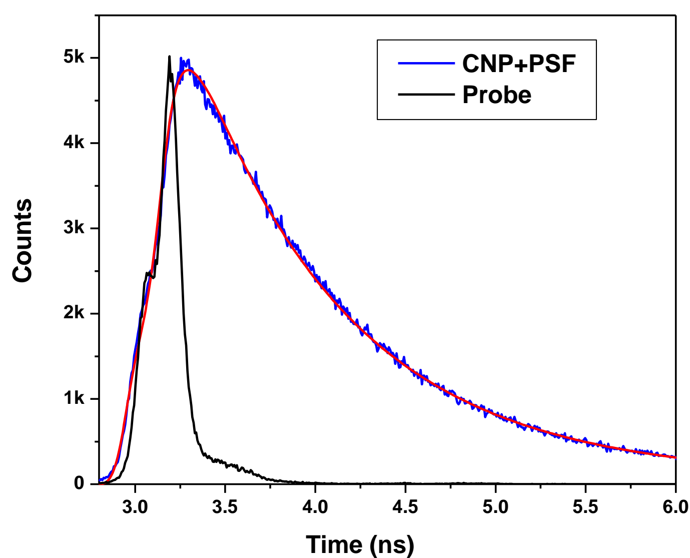

Fig. S7 Time-resolved fluorescence data for CNP and PSF. The donor was excited at 405 nm and the emission was monitored at 580 nm.

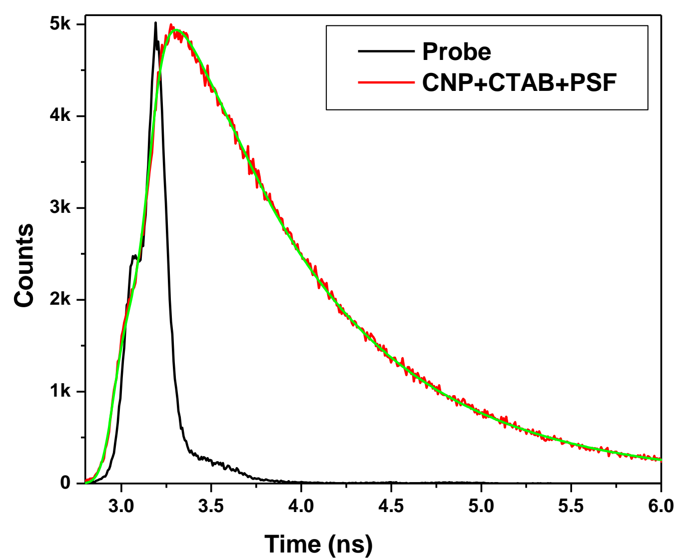

Fig. S8 Time-resolved fluorescence data for CNP-CTAB conjugate and PSF. The donor was excited at 405 nm and the emission was monitored at 570 nm.