Experimental section

Materials

Citric acid (99%), sodium hydroxide, daunomycin hydrochloride (purity ≥ 90%) and calf thymus DNA (ct-DNA) were purchased from Sigma-Aldrich and were used without further purification. The stock solution of ct-DNA was prepared by dissolving 1 mg of ct-DNA in 1 mL of 0.01 M phosphate-buffered saline (PBS). The purity of ct-DNA was verified by monitoring the ratio of the absorbance at 260 and 280 nm, and the ratio of A_{260}/A_{280} was found to be 1.91, indicating that the ct-DNA was sufficiently free from protein contamination. The concentration of ct-DNA solution was spectrophotometrically determined to be 2.03 mM using its known molar absorption coefficient ε at 260 nm = 6600 M^{-1} cm^{-1}. 2.6 mM stock solution of daunomycin hydrochloride was prepared using PBS buffer solution. All the stock solutions were stored at 0–4°C. HPLC grade water was used throughout the experiment.

Synthesis of graphene quantum dots

Graphene quantum dots (GQD) was synthesized using a reported protocol with some modifications.[1] 1g of citric acid was taken in a 50 mL beaker and heated to 200˚C using a heating mantle for 20 mins. After the solid citric acid completely melted to deep orange liquid, 10 mL of 10 mg mL^{-1} NaOH solution was added dropwise under vigorous stirring at room temperature to prepare a solution of pH 7. The obtained GQD solution was centrifuged at 10000g for 30 mins and the supernatant was collected after centrifugation.

Methods

Transmission electron microscopy (TEM). Transmission electron microscopy (TEM) images were recorded with the JEM-2100F, JEOL Field emission gun (FEG) electron microscope using a 200kV electron source at the DST-FIST facility in IISER Kolkata. An aqueous solution of GQDs was drop-casted on a carbon-coated copper grid and dried in air followed by vacuum.

Fourier transform infrared spectroscopy (FTIR). The IR spectrum was recorded on a Perkin Elmer RX1 Spectrophotometer. A KBr pellet was made by mixing 1 drop of GQD stock solution with 100 mg of KBr.

Zeta potential measurement. The ξ-potential measurement was done in a Nanoparticle Analyzer SZ-100 from Horiba Scientific equipped with a He−Ne laser beam operating at 633 nm. An aqueous solution of GQDs was used for this experiment.

Steady state measurements. The absorption spectra were recorded using a Hitachi U-2900 spectrophotometer. The steady state fluorescence spectral measurements were done in a QM 40 spectrofluorimeter from PTI, Inc. Fluorescence quantum yield (QY) of GQD was determined by comparing the wavelength integrated intensity of GQD to that of the standard (quinine sulphate in 0.1 M H_2SO_4, QY= 0.54). Solutions having absorbance (OD) less than 0.05 were considered to avoid concentration related errors. Both GQD and the reference were excited at 360 nm. QY was calculated using the following equation:

$$\Phi = \Phi_R \left(\frac{OD_R}{OD} \right) \left(\frac{I}{I_R} \right) \left(\frac{\eta^2}{\eta_R^2} \right)$$

where, Φ, OD, I, and η stand for quantum yield, absorbance, integrated fluorescence intensity, and refractive index of the solvents, respectively. Subscript R stands for reference (standard dye).

Time resolved measurements. Fluorescence lifetimes in ns-ps time regime were measured by the method of time correlated single photon counting (TCSPC) using a picosecond spectrofluorimeter from Horiba Jobin Yvon IBH equipped with a FluoroHub single photon counting controller, Fluoro3PS precision photomultiplier power supply, and FC-MCP-50SC MCP-PMT detection unit. 375 nm (resolution < 200 ps) and 405 nm (resolution < 70 ps) diode lasers were used as excitation sources. A non-linear least-square iteration procedure using IBH DAS6 (version 2.2) decay analysis software was employed to fit the fluorescence decay curves using a proper exponential decay equation. The goodness of the fit was checked by the χ^2 values.

The average fluorescence lifetime value, $\langle\tau\rangle$, for each decay curve was calculated from the fitted data using the following equation:

$$\langle\tau\rangle = \Sigma_i A_i\tau_i^2 / \Sigma_i A_i\tau_i$$

where, τ_i is the excited-state lifetime component of the decay curve and A_i is the corresponding amplitude of that component.

Steady-state fluorescence anisotropy measurement. Steady-state fluorescence anisotropy measurements were performed in a QM-40 spectrofluorimeter from PTI Inc. using excitation and emission polarizers. Anisotropy (r) was determined using the following equation:

$$r = \frac{I_{VV} - GI_{VH}}{I_{VV} + 2GI_{VH}}$$

where, G is the correction factor ($G = I_{VH}/I_{HH}$) and I is the fluorescence intensity and the suffixes denote the alignment of excitation and emission polarizers, respectively.

Reference

1 X. Wu, F. Tian, W. Wang, J. Chen, M. Wu and J. X. Zhao, *J. Mater. Chem. C*, 2013, **1**, 4676-4684.