Electronic Supplementary Material (ESI) for Chemical Communications

Label-Free Fluorimetric Detection of Histone Using Quaternized Carbon Dot-DNA Nanobiohybrid

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Electronic Supplementary Information (ESI)

Materials:

Betaine, calf thymus DNA (single stranded and double stranded), histone (from calf thymus) and other proteins were purchased from Sigma. Tris, citric acid, glycine and other reagents were procured from spectrochem, India. Milli-Q grade water was used throughout the study. Fluorescence spectra were recorded in Varian Cary Eclipse luminescence spectrometer. ^{1}H NMR spectra were taken in AVANCE 300 MHz (Bruker) spectrometer. UV-visible absorption measured in a Perkin Elmer Lambda 25 spectrophotometer. FTIR spectra were recorded in a Perkin-Elmer Spectrum 100 Spectrometer. Zeta potential was measured in zetasizer Nano-ZS of Malvern instrument limited.

Synthesis of Quaternized Carbon Dot (QCD):

QCD was synthesized by following the reported protocol (Reference 7 in the main manuscript). Briefly, 2 g of betaine hydrochloride was dissolved in 5 mL of water and then to this solution 1.2 g of tris was added (maintaining 1:1 molar ratio) with shaking until complete dissolution. The water soluble organic salt was then extracted with 100 mL of isopropanol and solvent was removed under rotary evaporator. Addition of isopropanol (100 mL) was done three times to extract more sticky mass. It was then dried at 80 ºC for 3 days. This dried

material was heated in a furnace at 250 ºC for 2 h in a porcelain crucible and then cooled to room temperature. Finally, it was extracted with 25 mL water and precipitated after addition of acetone at 1:10 volume ratio. This combined liquid mixture was centrifuged at 14000 rpm for 1 h to precipitate out the quaternized carbon dot. The supernatant was removed and the precipitated brownish black mass was further dried in hot oven at 80 ºC until it became powder in nature.

Synthesis of Anionic Carbon Dot (ACD):

For the synthesis of ACD, initially 1.1 g of glycine (14 mmol) was made to its corresponding carboxylate salt by the addition of equivalent amount of NaOH solution (2 mL). To this, 2 mL of citric acid solution (3 g, 14 mmol) was added by maintaining 1:1 molar ratio. This water-soluble mixture was evaporated to dryness at 100 ºC. The sticky mass was collected and dried in hot oven at 80 ºC for 3 days. The solid was crushed into a fine powder and was heated in a furnace at 300 ºC for 2 h in a porcelain crucible and then cooled to room temperature. The brownish black product was extracted with 25 mL of hot water. The deep brown solution was precipitated after addition of acetone at 1:10 volume ratio and the supernatant was collected. This supernatant was centrifuged at 14000 rpm for 1 h to precipitate out the anionic carbon dot. The supernatant was removed and the precipitated brownish black mass was further dried in hot oven at 80 ºC until it became powder in nature. Zeta potential of ACDs was found to be -23.7 mV.

Quantum yield Calculation:

Quantum yields are generally measured relative to an optically dilute standard fluorophore solution that exhibits a well-known quantum yield (Φ_s). The quantum yields of unknown fluorophore (Φ_u) were determined by using the Parker-Rees method.[S1,S2]

$$\Phi_u = (A_s F_u n_u^2 / A_u F_s n_s^2) \Phi_s$$

Here, A_u = the absorbance of unknown sample at the excitation wavelength, A_s = the absorbance of reference sample at the excitation wavelength, F_u = the total area of integrated fluorescence intensity for the unknown sample when excited at the same excitation wavelength, F_s = the area of integrated fluorescence intensity of the reference sample when excited at the same excitation wavelength. We have used solution having similar absorbance (< 0.1) for the determination of quantum yield. The refractive indices of the solvents in which the unknown and the standard samples are prepared, are given by n_u and n_s respectively. Here, in our study we have chosen quinine sulfate in 0.1 M H_2SO_4 as standard and its quantum yield (Φ_s) is known to be 54%.

Calculation of percentage (%) enhancement in quantum yield after addition of histone:

We have calculated the % enhancement of quantum yield (QY) by following the ratio of enhancement in QY from the maximum quenched state after addition of histone and the net decrease in QY due to addition of ds-DNA in QCD.

% enhancement in QY= (Observed QY at given concentration of histone – lowest QY (maximally quenched state of QCD due to addition of ds-DNA))/Native QY of QCD – lowest QY) × 100.

QCD-ds-DNA nanohybrid formation and histone detection: QCD-ds-DNA nanohybrid was formed by mixing the QCD solution and ds-DNA in aqueous phosphate buffer at different concentration ratio. To this solution, histone was added at required amount and fluorescence spectra were observed after 1 min of the histone addition.

Fluorescence Study: Fluorescence spectra of carbon dots (QCD or ACD) were taken by exciting at 340 nm both in absence and presence of DNA and histone in aqueous phosphate buffer (pH = 7.0, 10 mM). Stern–Volmer constant (K_{SV}) of ds-DNA in presence of QCD was determined using the equation: $F_0/F = 1 + K_{SV}[Q]$, where F_0 and F are the fluorescence

intensities of the QCD in absence and presence of ds-DNA and [Q] signifies the concentration of the quencher ds-DNA. The excitation and emission slits were kept at 20 nm.

Gel Electrophoresis: Agarose gel electrophoresis (3 wt %) was performed in 1 × TBE buffer at E = 85 V/cm for 30 min. ds-DNA and the nano-biohybrids of QCD-ds-DNA and ACD-ds-DNA were run on this agarose gel stained with ethidium bromide and photographed upon irradiation with UV light. The band intensities were calculated with quantity one 1-D analysis software (Bio- Rad, USA).

CD Spectra: CD spectroscopic analysis of ds-DNA in aqueous phosphate buffer (pH = 7, 10 mM) in absence and presence of QCD and histone was done in Jasco J-815 using 10 mm path length cell at wavelength 200–350 nm with a scan speed of 50 nm min^{-1}. All the spectra were corrected by subtracting a blank spectrum (without ds-DNA) and accumulated six times. The final concentration of the ds-DNA was kept at 25 μgmL^{-1} in the solution.

FTIR Study: The lyophilized powders of samples were transferred into a mortar-pestle containing IR-grade KBr (ca. 30 mg) and were mixed to prepare the pellet under strictly dry condition to prevent absorption of water vapour. This pellet was further dried by storing in a vacuum desiccator. Spectra of these pellets were recorded and accumulated 512 times at a resolution of 2 cm^{-1} with intervals of 1 cm^{-1}. Each time background correction was performed to eliminate interference from air (or any other parameters).

TEM and AFM study: For TEM, a drop of the carbon dot solution was cast on 300-mesh Cu-coated TEM grid separately and dried under vacuum for 4 h before taking the image. TEM images were taken on a JEOL JEM 2010 and JEM 2100F microscope. The morphology quaternized carbon dot was also studied on a Veeco, model AP0100 atomic force microscope in noncontact mode.

Table S1. Quantum yield value of quaternized carbon dot (QCD) in presence of ds-DNA.

QCD (μgmL^{-1})	ds-DNA ($ngmL^{-1}$)	Quantum yield (%)
1	-	6.3
	50	5.7
	100	5.4
	150	5.2
	200	4.8
	250	4.6
	300	4.5
	400	4.5

Table S2. Zeta potential (ζ) value of QCD in absence and presence of ds-DNA

QCD (μgmL^{-1})	ds-DNA ($ngmL^{-1}$)	ζ value
20	-	23.1
	2.5	20.2
	5	16.2
	10	13.2

Table S3. Quantum yield value of quaternized carbon dot (QCD) in presence of ds-DNA and varying histone concentration.

QCD-ds-DNA (μgmL^{-1}) – ($ngmL^{-1}$)		Histone ($ngmL^{-1}$)	Quantum yield (%)
1	250	-	4.6
		0.1	5.0
		0.2	5.4
		0.5	5.5
		0.8	5.7
		1.0	5.9
		2.0	6.0
		5.0	6.0
		10.0	6.0

Table S4. Quantum yield value of quaternized carbon dot (QCD) in presence of ss-DNA of and histone.

QCD-ss-DNA (μgmL^{-1}) – ($ngmL^{-1}$)		Histone ($ngmL^{-1}$)	Quantum yield (%)
1	-	-	6.3
	50		5.4
	100		4.7
	150		4.4
	200		4.2
	250		4.2
	250	50	4.3
		100	4.3

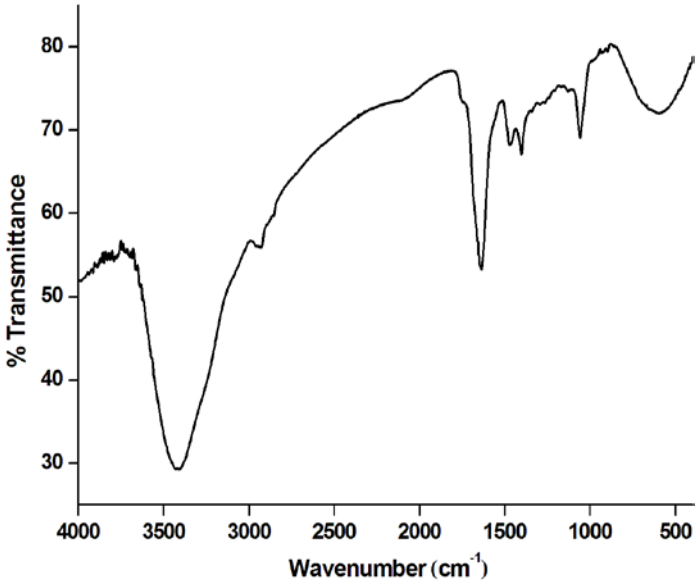

Fig. S1 FTIR spectra of QCD. The presence of peak at 1640 cm^{-1} confirmed the formation of amide (-CONH-) bond. Also, the peaks at 1470 and 1395 cm^{-1} were due to the $–CH_2$ and $–CH_3$ bending peak of the ligand betaine. Simultaneously, peaks around 1120-1060 cm^{-1} and broad peak near 620 cm^{-1} was indicative of C-O-C/C-O stretching of oxygen containing core and graphitic C-H bending or other carbonaceous solids (Reference 7 in the main manuscript).

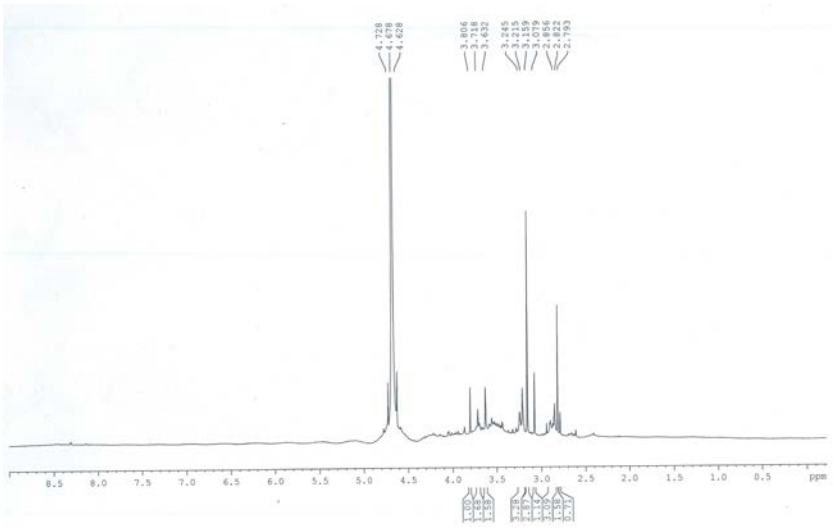

Fig. S2 ^1H NMR spectra of QCD. ^1H NMR analysis of the sample in D_2O also suggests the presence of quaternary ammonium ($+N(CH_3)_3$) and methylene ($-CH_2$) protons with peak at δ 3.2 and 3.8 ppm, respectively (Fig. S2, ESI). Peaks at δ 4.62-4.72 ppm and 2.79-2.85 ppm indicate the presence of graphitic hydrogen (olefinic H and CH_3).

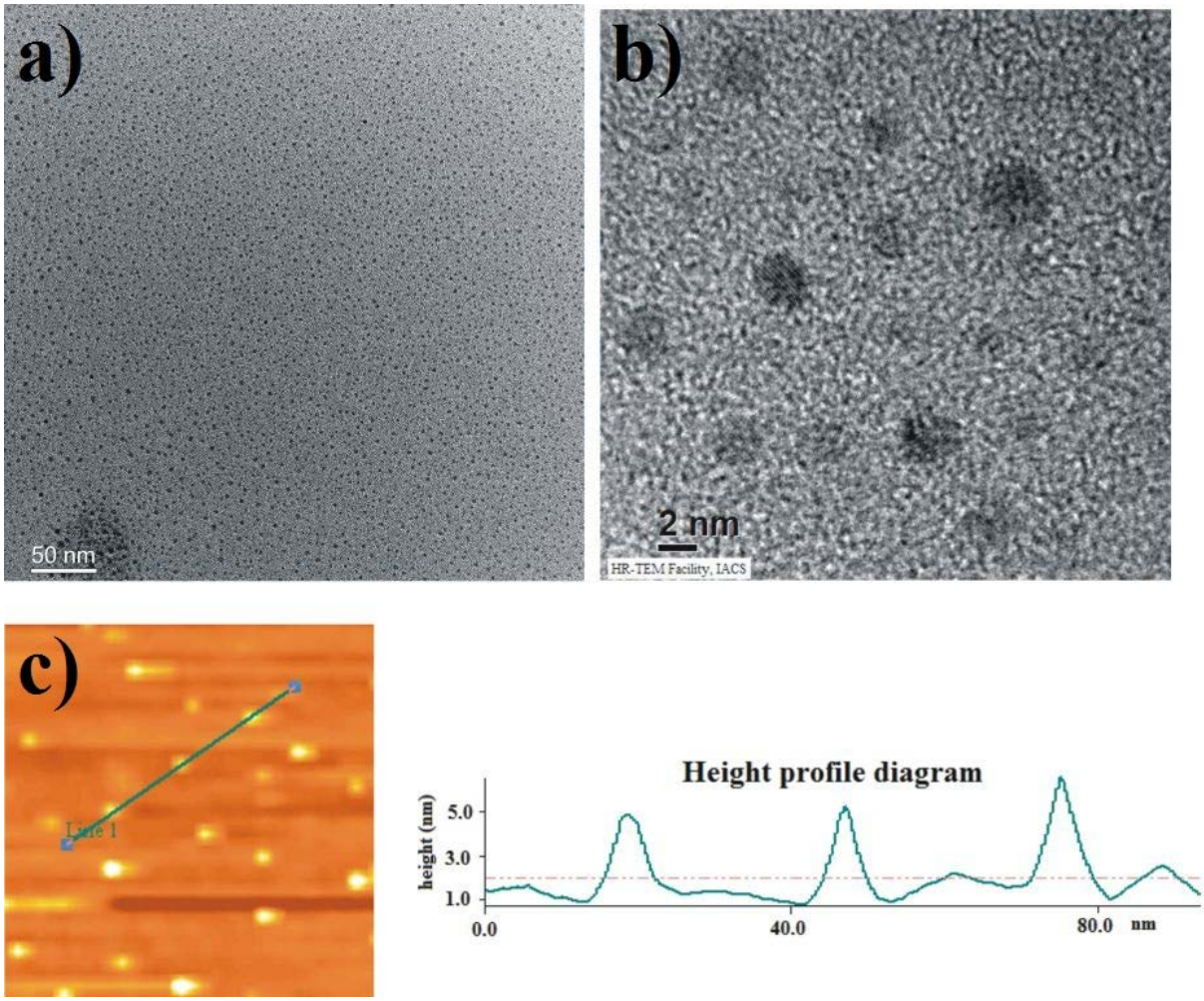

Fig. S3 a,b) TEM, **c)** AFM images of synthesized QCD with different magnification.

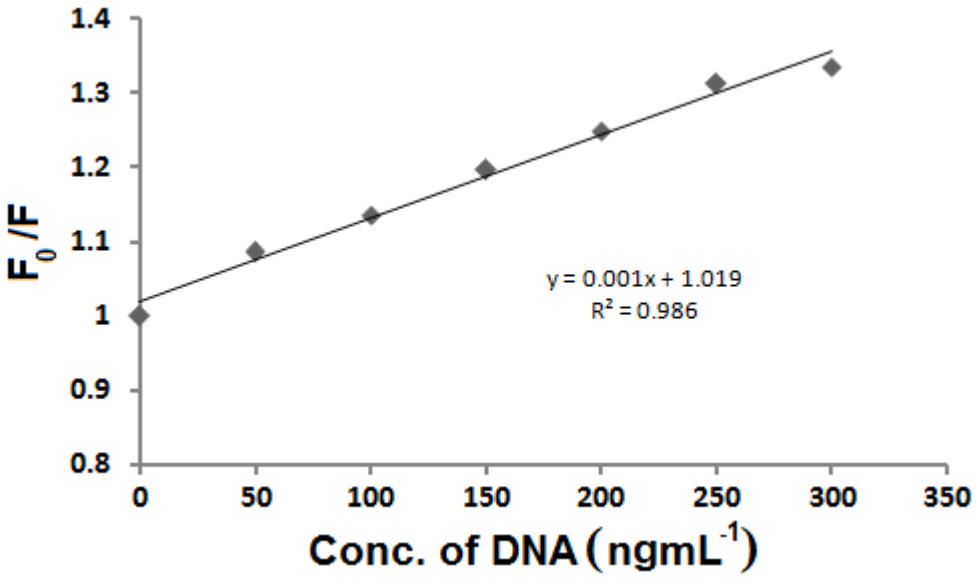

Fig. S4 Stern-Volmer plot of ds-DNA doped in QCD solution ([QCD] = 1 μgmL^{-1}).

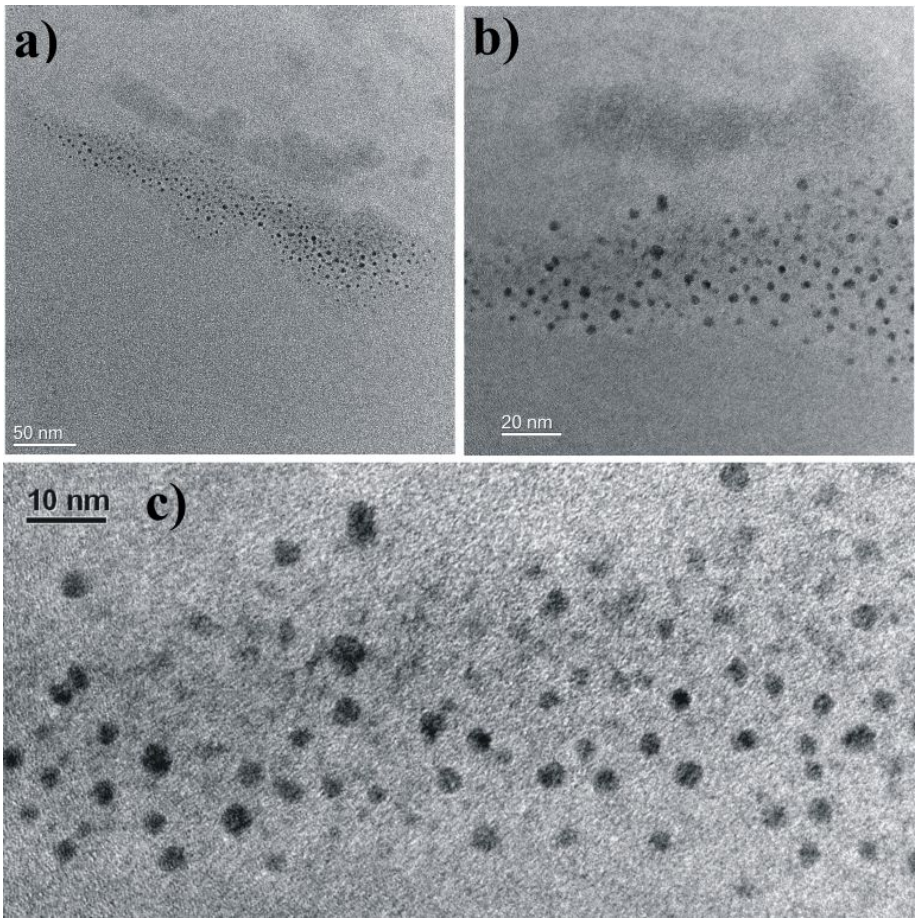

Fig. S5 a-c) TEM imaged of the QCD-ds-DNA nanohybrid with different magnification.

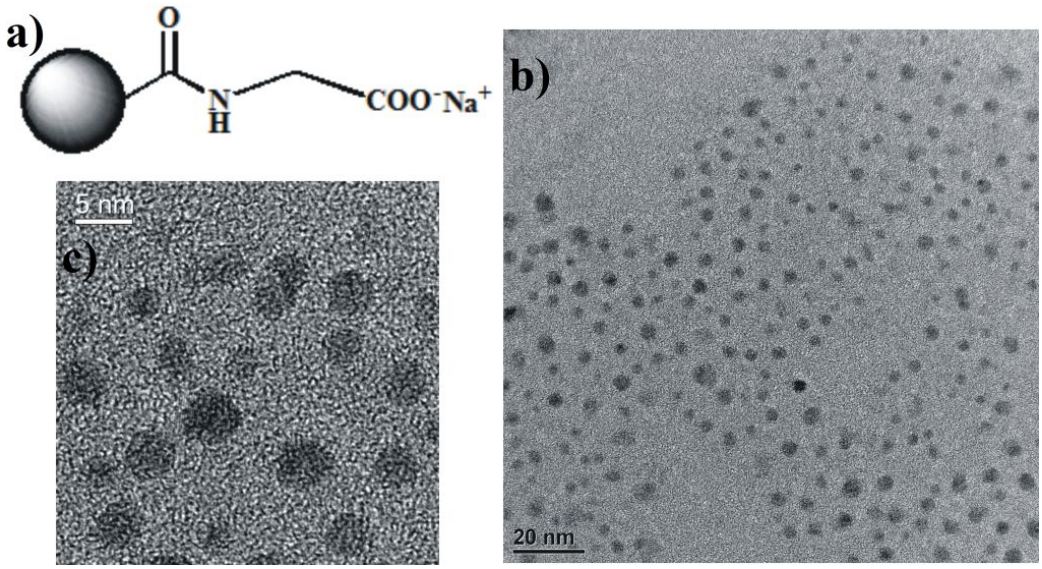

Fig. S6 a) Structure and b,c) TEM images of anionic carbon dot (ACD) with different magnification.

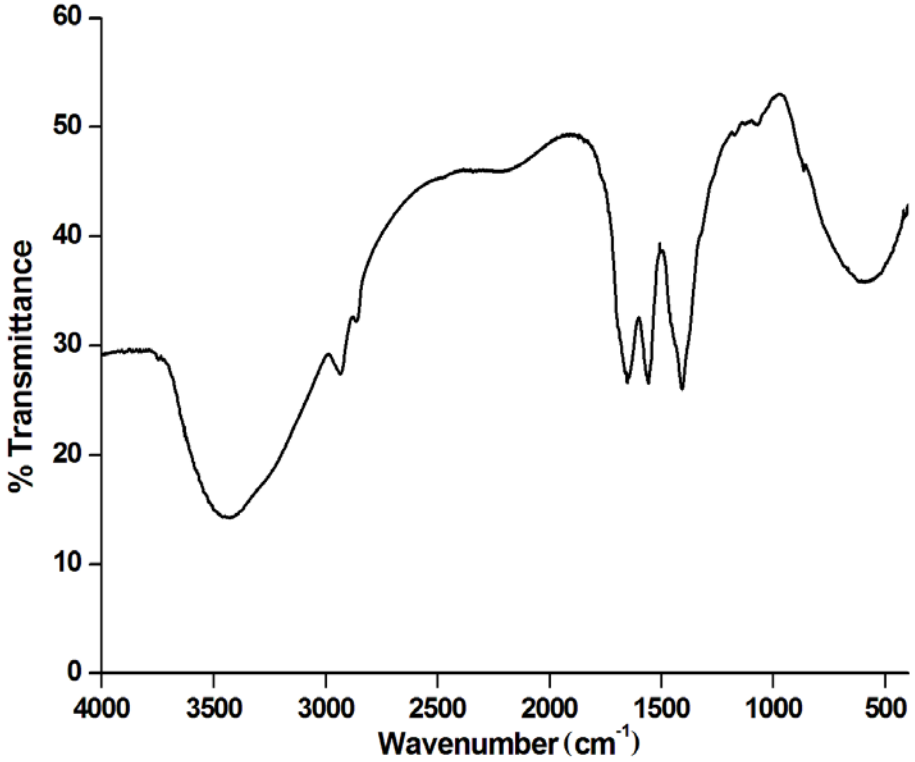

Fig. S7 FTIR spectra of ACD. The peaks at 1652 and 1556 cm^{-1} are the indicative of amide bond and carboxylate moiety, respectively (See Reference 7 in the main manuscript).

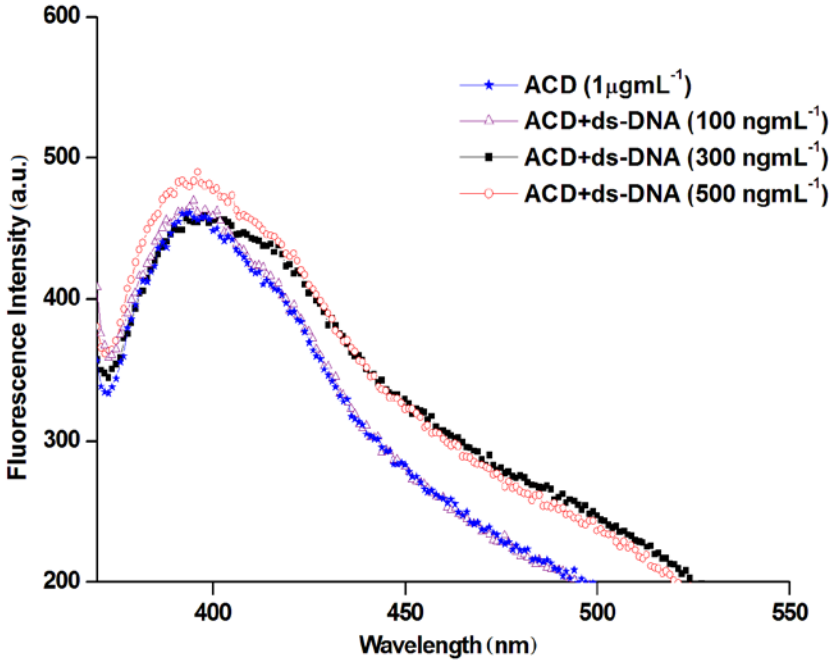

Fig. S8 Fluorescence spectra of ACD in absence and presence of varying DNA concentration (excitation wavelength = 340 nm).

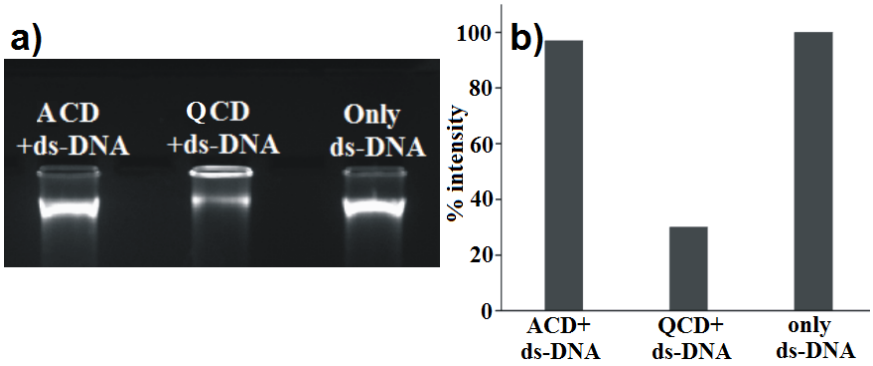

Fig. S9 a) Agarose gel electrophoresis of ds-DNA in absence and presence of ACD and QCD ([DNA] = 0.1 mgmL^{-1}; [ACD] and [QCD] = 0.2 mgmL^{-1}; b) Densitometric analysis of luminescent domain of gel.

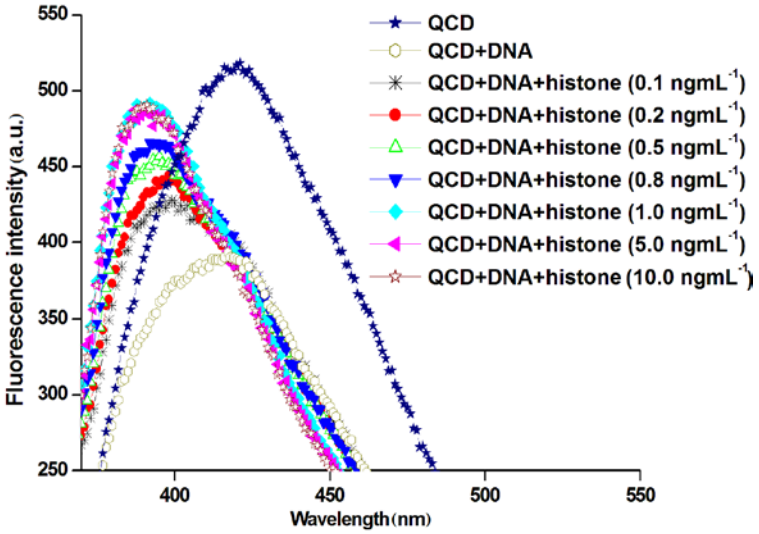

Fig. S10 Fluorescence spectra of QCD in absence and presence of ds-DNA and histone (excitation wavelength= 340 nm). [QCD] = 1 µgmL^{-1}, [ds-DNA] = 250 ngmL^{-1}, [histone] = 10 ngmL^{-1}.

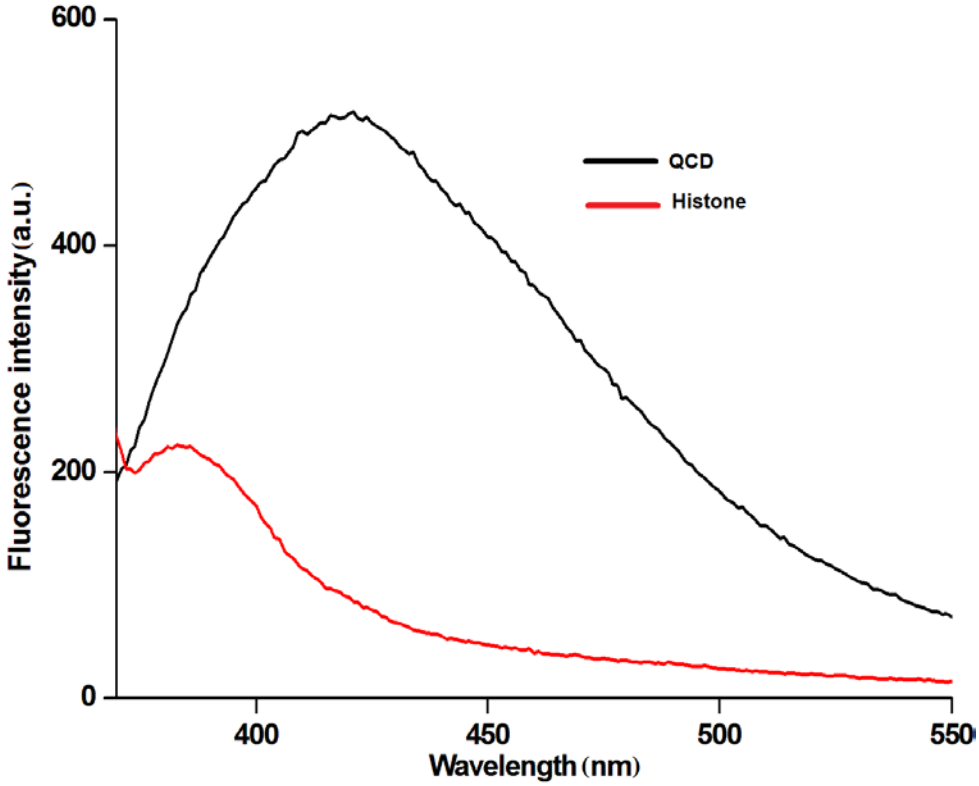

Fig. S11 Fluorescence spectra of QCD and histone (excitation wavelength= 340 nm). [QCD] = 1 μgmL^{-1}, [histone] = 50 $ngmL^{-1}$.

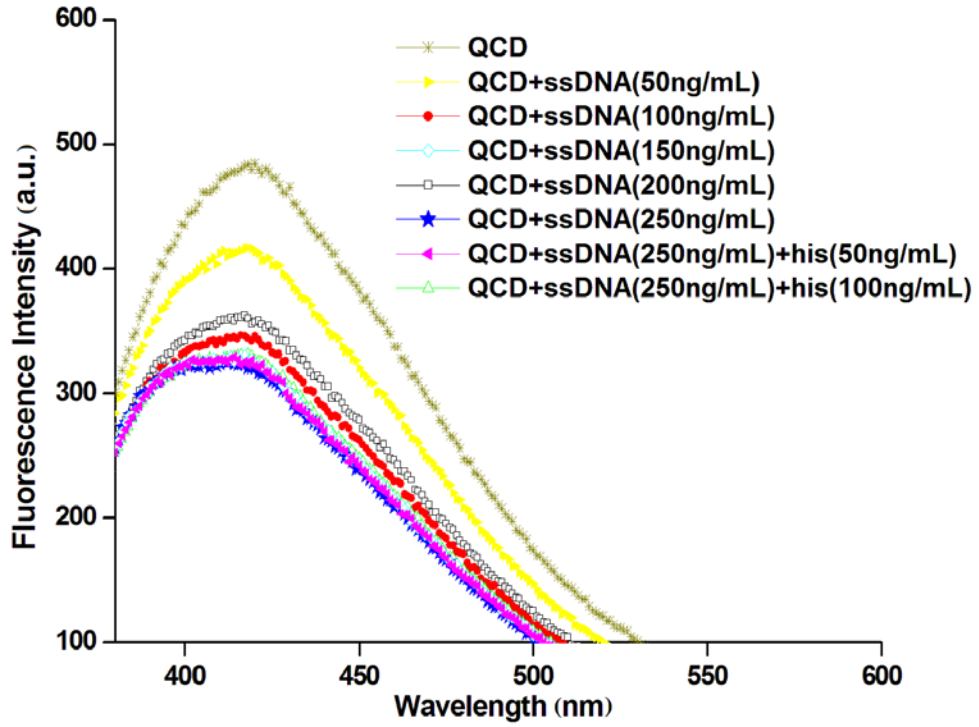

Fig. S12 Fluorescence spectra of QCD in presence of different concentration of ss-DNA and after addition of histone ((excitation wavelength= 340 nm).

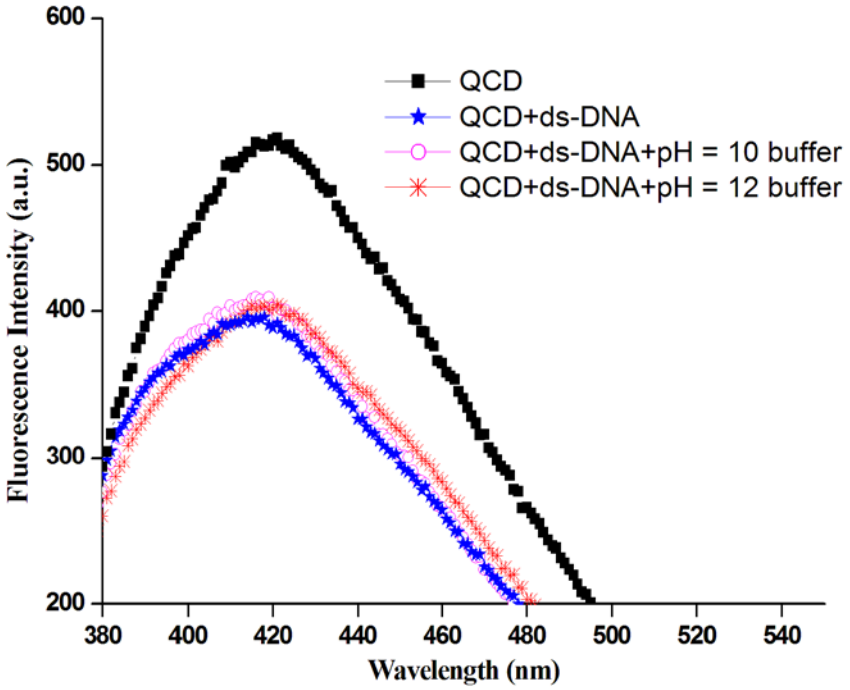

Fig. S13 Fluorescence spectra of QCD in presence of ds-DNA and after addition of pH = 10 buffer (carbonate-bicarbonate, 50 mM) and pH =12 buffer (phosphate-NaOH, 50 mM) (excitation wavelength= 340 nm).

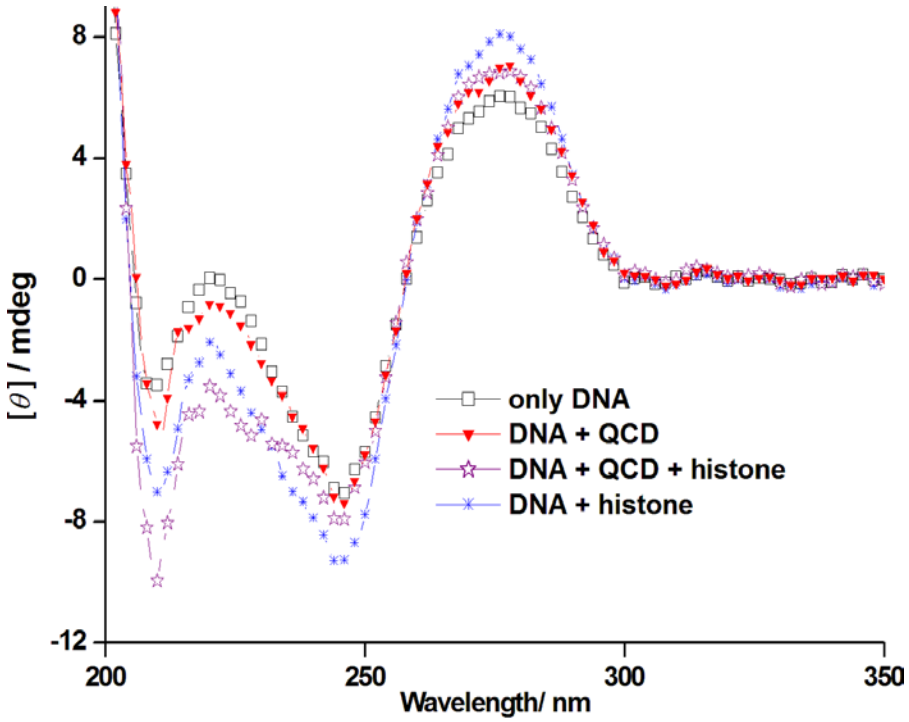

Fig. S14 CD spectra of DNA in absence and presence of QCD and histone in aqueous phosphate buffer (pH 7, 10 mM). ([DNA] = 25 μgmL^{-1}; [QCD] = 50 μgmL^{-1}; [histone] = 5 μgmL^{-1}).

References:

S1. J. N. Dames and G. A. Crosby, *J. Phys. Chem.* 1971, **75**, 991.
S2. C. A. Parker and W. T. Rees, *Analyst* 1962, **87**, 83.