Supplementary Information

Amplified Luminescence Quenching Effect Upon Binding of Nitrogen Doped Carbon Nanodots to Transition Metal Ions

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1. Experimental section

1.1. Materials

All the reagents were of AR grade unless specified and used as received without further purification. All glass wares were thoroughly cleaned with aqua regia (HNO_3:HCl=1:3), rinsed extremely with water and dried in an air oven prior to use. Millipore water was obtained from a Millipore water purification system (MilliQ, 18.2 MΩ).

EMPLURA® Citricacid anhydrous, EMPARTA® ACS urea for analysis were purchased from Merck Life Science Private Limited., Mumbai, India. EMSURE® ACS, Reag. Ph Eur EMSURE® ACS, Reag. Ph Eur for analysis was from Merck KGaA, Germany. Zinc chloride dry purified, Lead(II) nitrate Purified, Cobalt(II) chloride hexahydrate Purified and Copper(II) chloride dehydrate cryst. Pure were bought from Merck Specialities Private Limited., Mumbai, India. Iron(II) chloride trtrahydrate puriss. p.a.,≥99.0%(RT) was obtained from Sigma-Aldrich, St. Louis, USA. Ruthenium(III) chloride was collected from TCI, Tokyo, Japan. Silver nitrate was acquired from Nice chemicals Pvt. Ltd. Kochi, India. Mercuric chloride was from Fischer Inorganics & Aromatics Ltd. India. Chromium(III) acetate tech was got from CDH, Pvt. Ltd., India. Nickel(II) chloride hexahydrate pure was procured from SRL India. Potassium dichromate puriss AR and Dimethyl sulphoxide were got from Spectrochem Pvt. Ltd., Mumbai, India. Manganese chloride ($MnCl_2.4H_2O$) 97% was purchased from Otto Chemie Pvt Ltd.

1.2. Instruments and Characterisation

HRTEM (High Resolution Transmission Electron Microscopy) analysis of NCD samples was conducted using a JEOL JEM 2100 operating at accelerating voltage of 200 kV. Samples were loaded on to carbon coated copper grids. XPS (X-ray photoelectron spectroscopy) spectra were collected using Thermo Scientific ESCALAB XI$^+$ A1528 XPS instrument. The Fourier transform infrared (FTIR) spectra were obtained with Agilent Technologies Cary 630 FTIR spectrometer with ATR accessory and sample mounting. Spectra were obtained across 650 to 4000 cm^{-1} at 1 cm^{-1} intervals. The surface zeta potential and hydrodynamic diameter distribution of samples were obtained using a HORIBA Scientific nanoparticle analyser SZ-100. The room temperature steady state photoluminescence measurement of all samples were recorded using FP-750 spectrofluorometer of Jasco, Japan. The slit width for excitation and emission were set at 5.0 nm and 5.0 nm respectively. The fluorimeter is operated at low sensitivity for DMSO samples whereas instrument is ran at medium sensitivity for aqueous samples. The time resolved fluorescence (TRF) measurements of samples at room temperature using time correlated single photon counting (TCSPC) technique were obtained with two instruments (Horiba PR 650 provided with excitation at 415 nm & Horiba Fluorolog fluorescence spectrometer with excitation at 330 nm). Delta LED diode lasers (1 MHz frequency, pulse width ± 5 ns) at 415 nm was used as excitation source. Plug and play" interchangeable Nano LED pulsed LEDs (1 MHz frequency, pulse width <1.5 ns) was used at excitation of 330 nm. The prompt measurements were recorded using milk powder dispersion in water as non-fluorescent scatters while detector is set at corresponding excitation wavelength. Weighted average fluorescence lifetimes of NCD and its derivatives, (<τ>), can be obtained by fitting fluorescence decay curve to exponentials.

$$I_t = A_0 + \sum_i A_i e^{-\frac{t}{\tau_i}}$$

$$< \tau > = \frac{\sum_i A_i \tau_i^2}{\sum_i A_i \tau_i}, \qquad i = 1{,}2\ or\ 3$$

The UV-visible absorption spectra of samples were acquired with a PerkinElmer Lambda 365 UV-VIS-NIR spectrophotometer covering range from 200 to 1100 nm. Fluorescence, TCSPC and absorbance spectra were taken in different quartz cuvettes with 10 cm path length. Addition of metal ion quenchers were made by micro-pipette. All spectra were recorded at 25 °C. A POWER SOLO 17D (ONIDA, India) domestic microwave oven provided with 6 power stages was used to prepare NCDs. All pH measurements were done on a Digital pH meter MK VI of Systronics, India. Fluorescence images were taken in an UV cabinet of SAKS Equipments Pvt. Ltd., Cochin, Kerala, India.

1.3. Synthesis of citric acid derived nitrogen doped carbon nanodots

Water soluble NCDs was synthesised following a previously published microwave assisted pyrolysis method.[1] Briefly, 1.5 g of citric acid (7.8 mmol) and 1.5 g of urea (24.9 mmol) were dissolved in water (5 mL) in a 50 mL beaker. The reaction mixture was heated in 700 W microwave oven at 280 W for 2 to 3 minute. This was performed by different steps of heating, each last for 1 minute with 5 second gap between the steps. During this procedure, transparent reaction mixture turn to yellow orange syrupy solid. The syrup kept in same 50 mL beaker was transferred to an air oven and heated at 50°C for 1 hour to remove residual small molecules. The syrup is cooled down naturally to room temperature (~25°C). This orange yellow syrup (~1.92g) was then dispersed in 10 mL of desired solvent (water or DMSO) and centrifuged

(3000 rotation/minute, 2 minute) to remove large or agglomerated particles. The saddle brown (in aqueous medium, see Figure S6 C) or dark sea green (in DMSO medium, see Figure S6 D) supernatant containing NCD were filtered using a syringe driven 0.45 µm pore size Millipore polycarbonate filter. The obtained colloidal solution of NCD is kept as a stock solution (~193 mg/mL) for further use.

1.4. Metal cation stock solution preparation

All stock solutions (mostly 0.1 M except for Ru^{3+} solution at 0.01M) were prepared by adding 5 mL of desired solvent (DMSO or water) to a calculated amount of metal salt powder in a clean 50 mL beaker and stirred with a glass rod until all powder was fully dissolved. Then it is make up to 10 mL.

1.5. Fluorescence quenching experiment

An aliquot (100 µL of aqueous dispersion or 50 µL of DMSO dispersion) of stock NCD was added to desired solvent (3 mL of water or DMSO) in a cuvette. Then certain amount equivalents of metal ion solution was added stepwise to NCD solution by a micro-pipette. As a very small amount of metal ion solution was added, the final volume of the solution was nearly unchanged (3 mL). This mixed solution was shaken for 3 second and then excited at 400 nm. The corresponding emission values during the titrations were recorded.

1.6. Quenching mechanism

Fluorescence quenching process is described by Stern-Völmer equation:

$$I_0/I = K_{SV}[Q]+1$$

Where I and I_0 are the fluorescence intensity with and without quencher (Q), K_{SV} is the Stern-Völmer quenching constant.

In the case of dominant dynamic quenching,

$$K_{SV}=k_q\tau_0.$$

k_q is the bimolecular quenching rate constant (expressed in $M^{-1}s^{-1}$ units) and τ_0 is the excited state lifetime without added quencher.

When static quenching dominates,

$$K_{SV}=K_a$$

K_a is the association constant for the formation of ground-state complex between quencher and fluorescent material.

Deviations -Upward curving Stern-Völmer plot :

(i) Combined Dynamic and Static quenching

$$I_0/I = (K_D[Q]+1)(K_S[Q]+1)$$

This Stern-Völmer equation is second order in [Q] where K_D and K_S are dynamic component and static component of Stern-Völmer quenching constant.

(ii) Amplified quenching or quenching sphere of action

$I_0/I = (K_D[Q]+1)(\exp([Q]VN/1000)$

Where V is the volume of the sphere and N is the Avogadro's number

1.7. J- and H-aggregate concept

J- and H-aggregate concept was originally formulated by Kasha and co-workers to explain photoluminescence behaviour of aggregates of small molecules.[2] In J-aggregates, adjacent fluorophores are oriented in head to tail arrangement.[2,3] The J-aggregates show highly intense fluorescence (superradiance) with a red shifted absorption/excitation band.[2] In contrast, neighbouring fluorophores in a typical H-aggregates are oriented in a more side by side fashion.[2,3] H-aggregates show lower fluorescence with a blue shifted absorption/excitation band.[2]

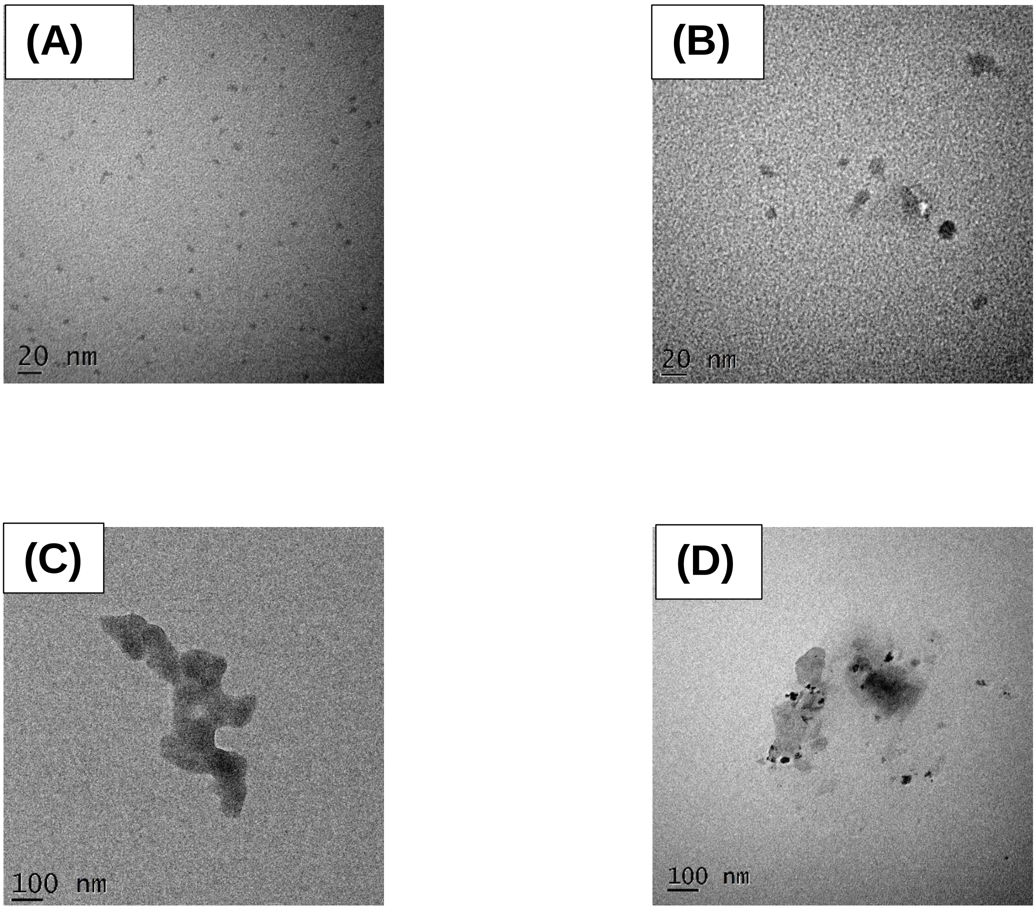

Figure S1. (A) TEM image of aqueous dispersion of NCD revealed a particle size range of 3-5 nm. (B) TEM image of NCD dispersion in DMSO medium shown particle size range of 6-10 nm. (C) TEM image of aqueous NCD dispersion depicted mesoscopic ribbon like- or mono layer sheet like structures at specific location of copper grid. (D) TEM image of NCD dispersion in DMSO illustrated monolayer sheets along with spherical particles.

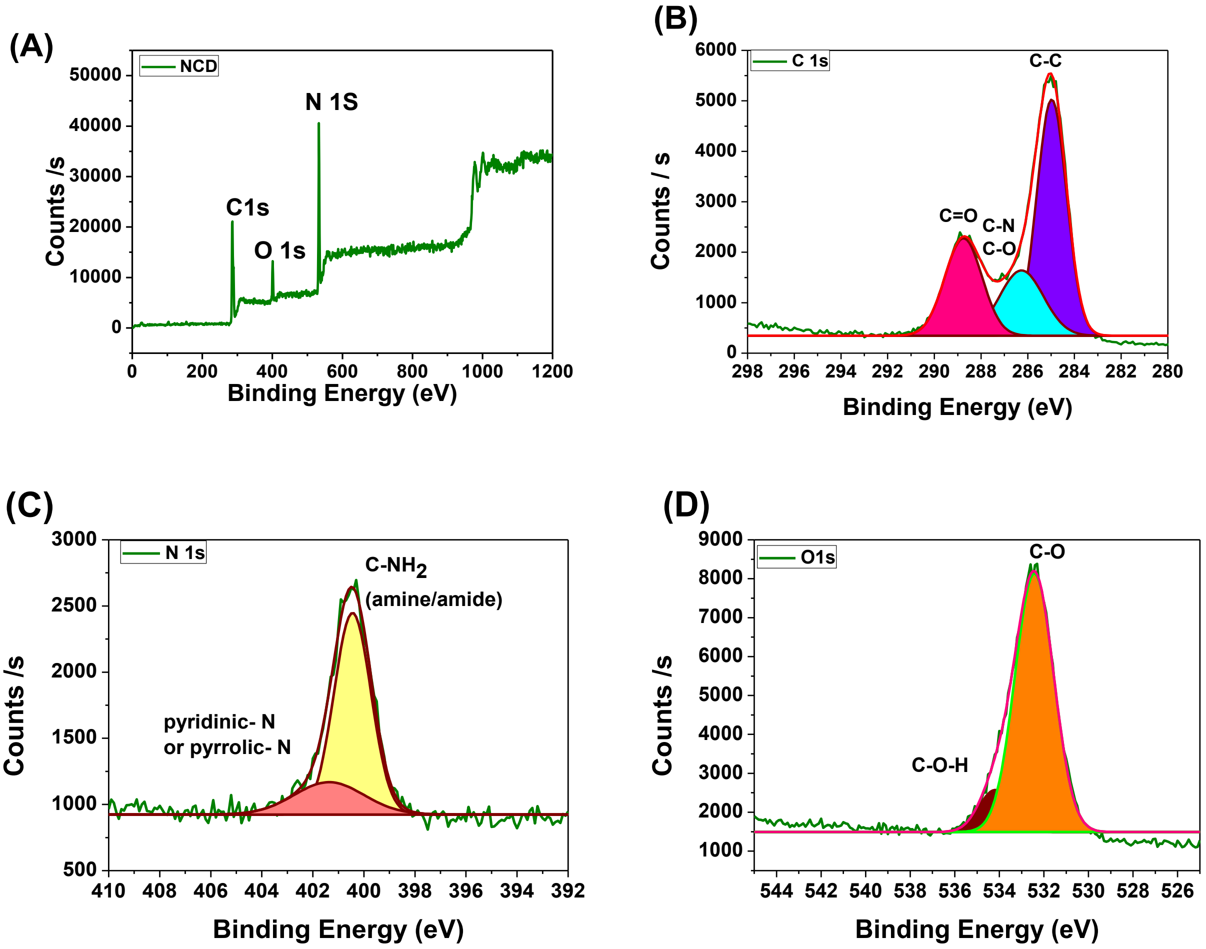

Figure S2. XPS spectra for NCD. (A) The full XPS spectra obtained. (B) The raw data (solid olive line) for (B) C1s (C) N1s and (D) O1s were deconvoluted separately into fitted peaks (coloured area plot). Each corresponding assignment to each fit is presented on each plot, where different colours represent different local bonding.

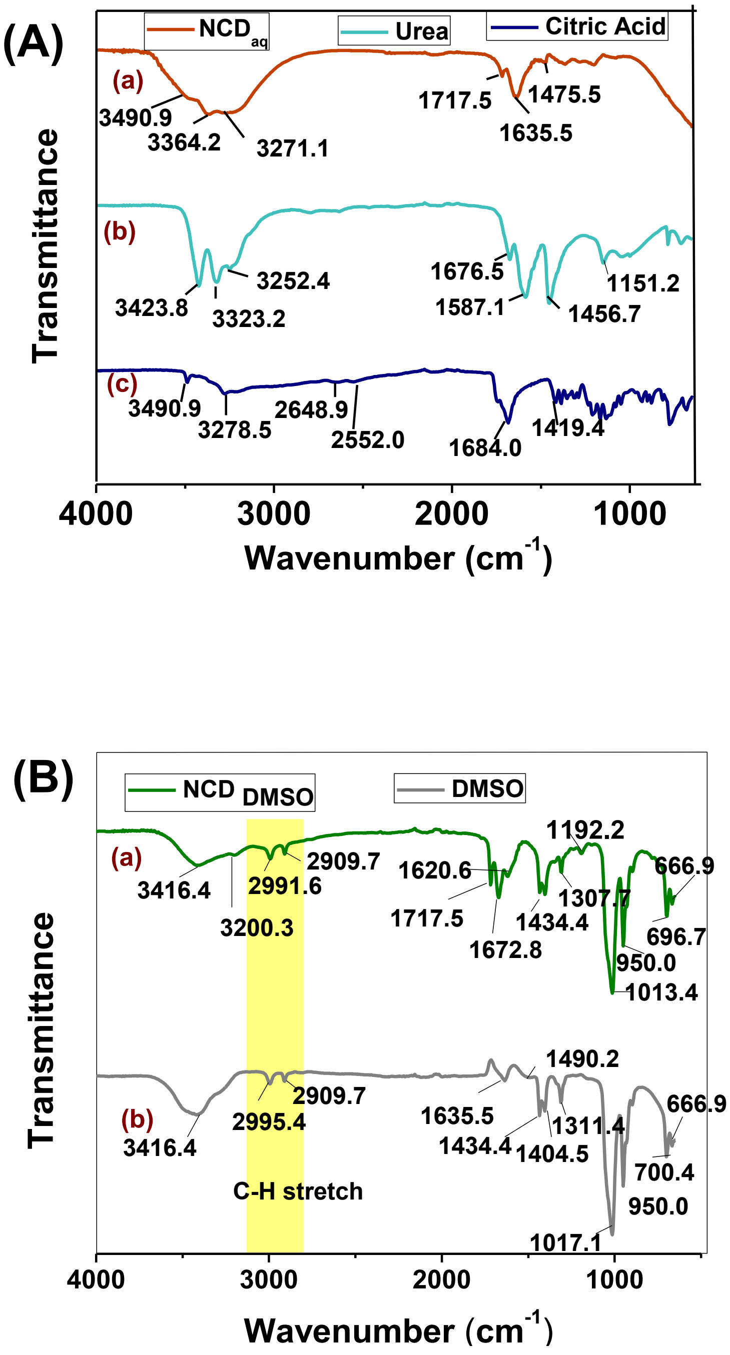

Figure S3. (A) FTIR spectra of (a) aqueous NCD dispersion (brown line), (b) Urea (cyan line) and (c) Citric acid (navy blue line). (B) FTIR spectra of (a) NCD dispersion in DMSO (olive line) and (b) DMSO (grey line). The concentration of NCD dispersion is ~193 mg/mL.

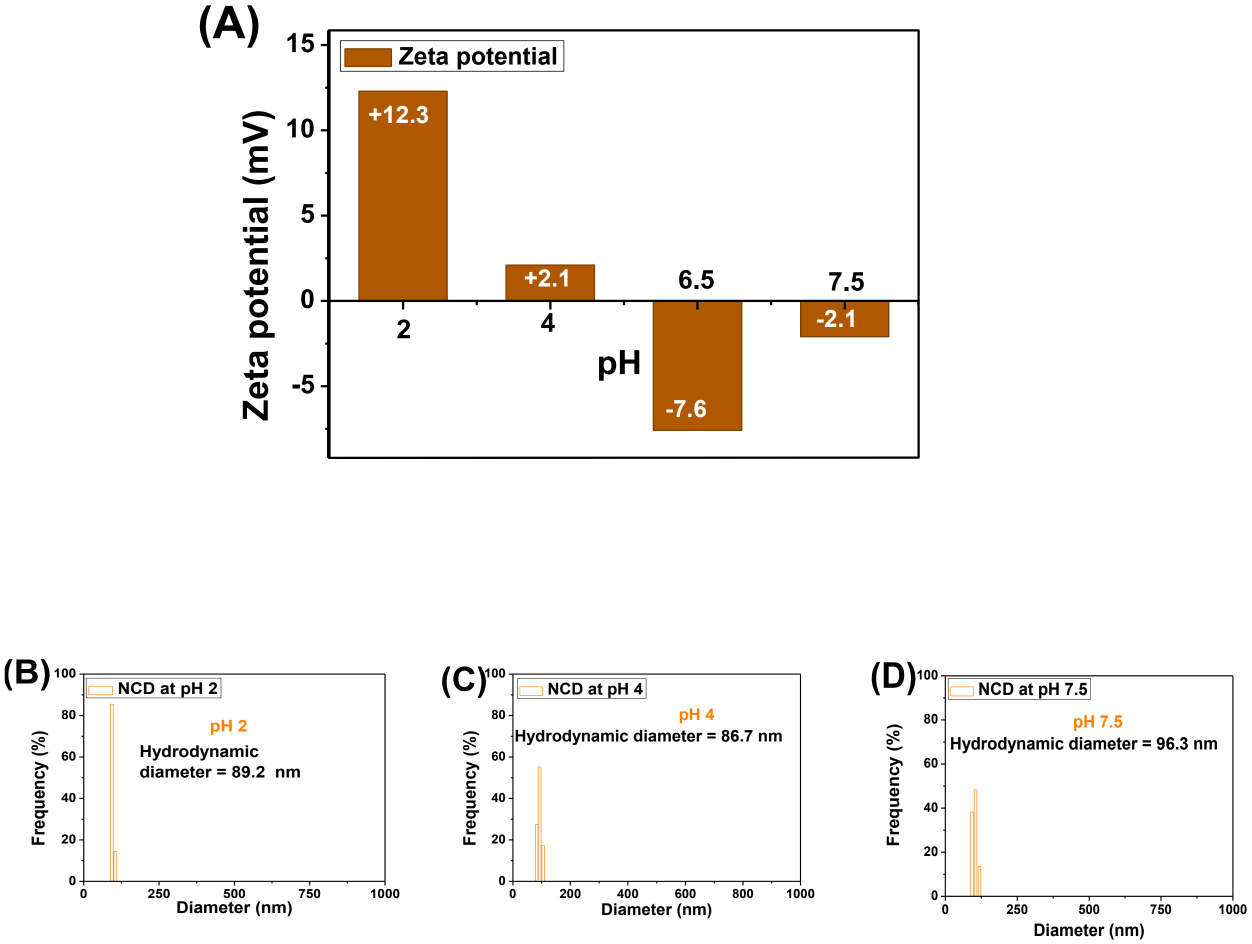

Figure S4 (A) The bar diagram depicting surface charge of aqueous nitrogen doped carbon nanodots (NCD) dispersion as a function of pH. (B-D) The hydrodynamic diameter distribution of NCD dispersion in aqueous medium at different pH measured using Dynamic light scattering (DLS):(B) pH 2, (C) pH 4 and (D) pH 7.5.

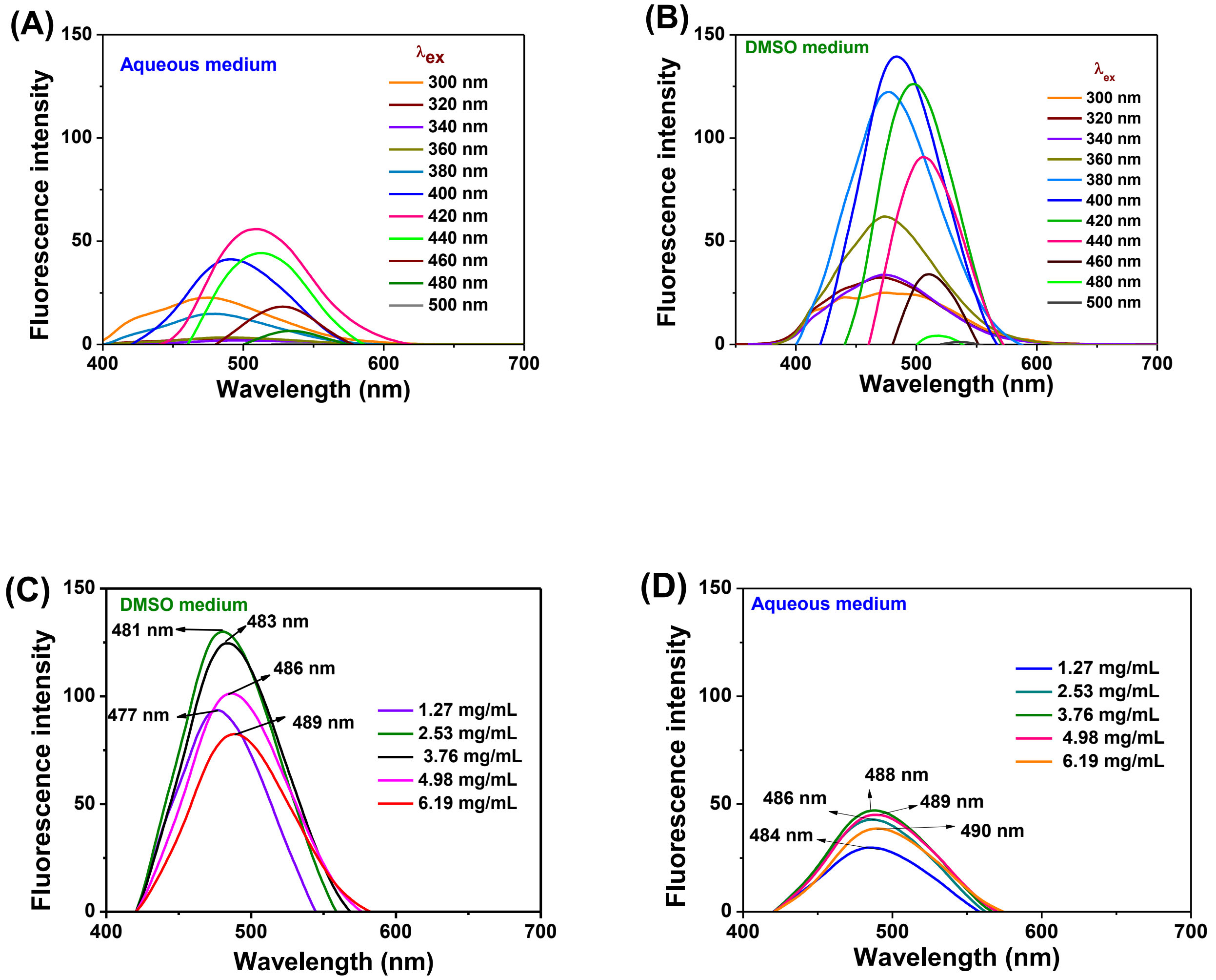

Figure S5. The fluorescence emission spectra of nitrogen doped carbon nanodots (NCD) at different excitation wavelength ranging from 300 to 500 nm with a progression of 20 nm: (A) water medium and (B) DMSO medium. The fluorescence emission spectra of NCD at different concentrations in (C) DMSO medium and (D) water medium.

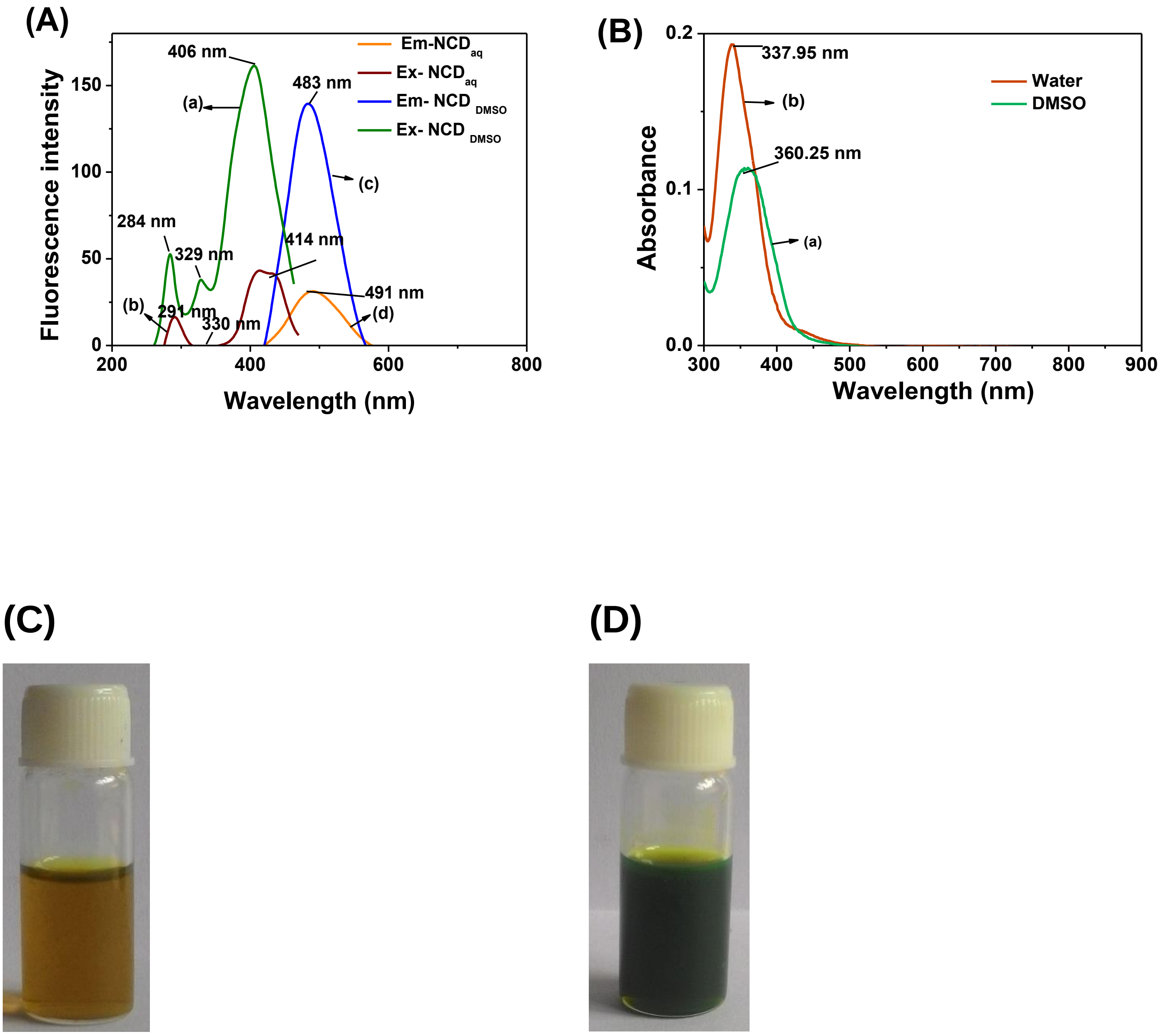

Figure S6. (A) Fluorescence excitation (a,b)(olive line and wine red line) and emission (blue line and orange line) spectra of NCD dispersion in DMSO medium and aqueous medium respectively. (B) Absorption spectra of NCD dispersion in (a) DMSO and (b) aqueous medium. The concentration of NCD in aqueous medium is 6 mg/mL. The concentration of NCD in DMSO medium is 3 mg/mL. Photograph of NCD dispersion (~193 mg/mL) in (C) water and (D) DMSO.

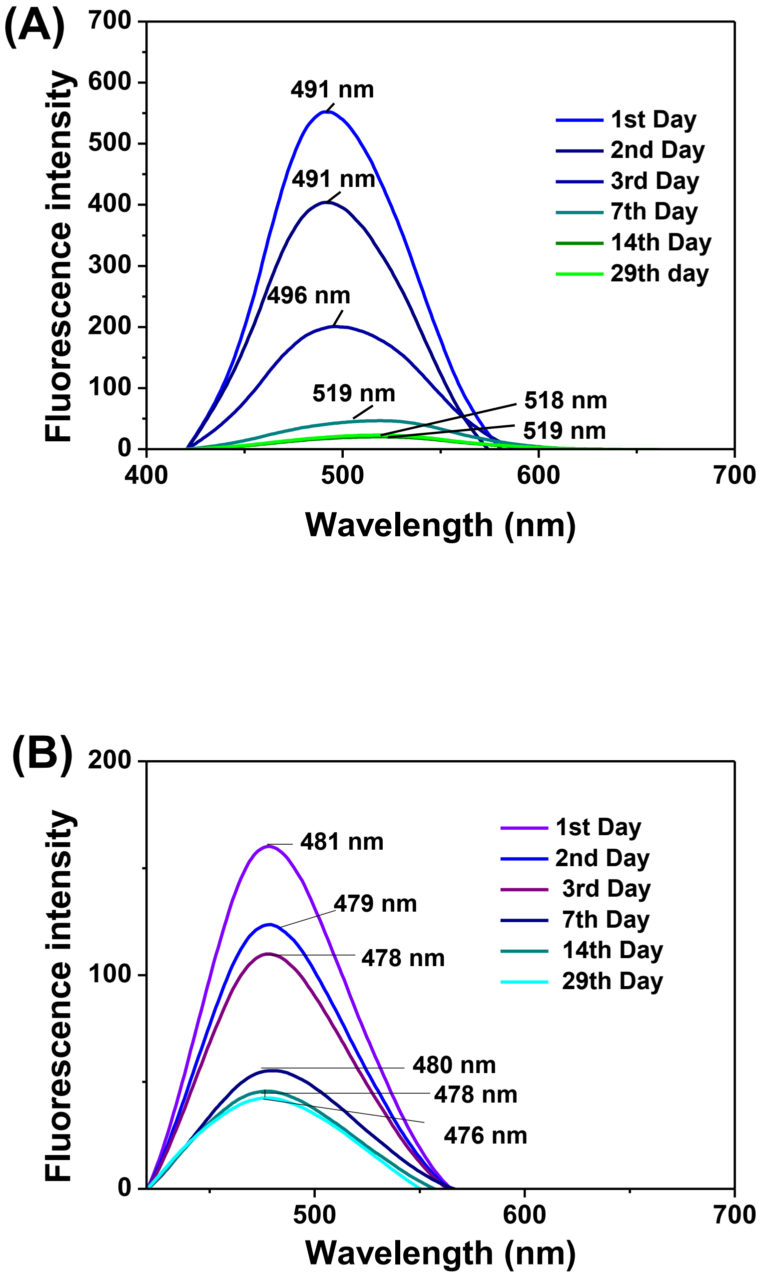

Figure S7 (A) The fluorescence spectra of aqueous NCD dispersion at different storage time. Since NCD exhibit weak fluorescence in aqueous medium, fluorescence spectra is recorded at medium sensitivity. Excitation wavelength: 400 nm. (B) The fluorescence spectra of NCD dispersion in DMSO at different storage time. Since NCD exhibit comparatively stronger fluorescence in DMSO medium, fluorescence spectra is recorded at low sensitivity. Excitation wavelength: 400 nm.

Table S1. The protic polar solvent and aprotic polar solvent used in the study.

Solvent	**Dielectric constant, ε**	**Dipole moment, μ**	**Solvent polarity parameter, $E_T(30)$**	**Kamlet-Taft Parameters**			**Viscosity at 25 °C (cP)**
				Hydrogen bond donation acidity, α	**Hydrogen bond donation basicity, β**	**Polarizability, π***	
Water	80.10	1.82	63.1 kJ/mol	1.17	0.47	1.09	1.00
DMSO	47.24	3.96	45.1 kJ/mol	0.00	0.76	1.00	1.99

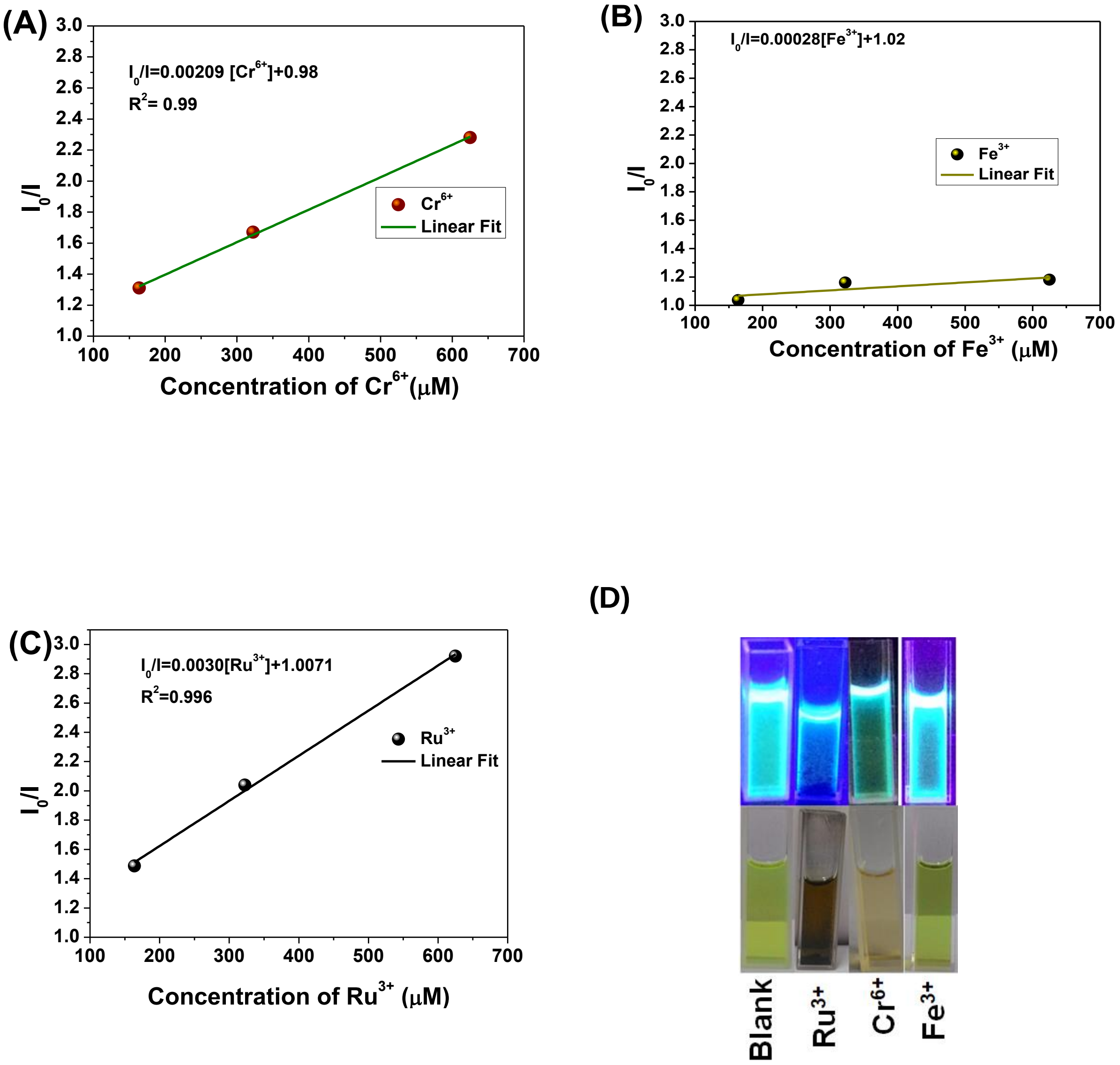

Figure S8. The linear Stern-Völmer plots of NCD fluorescence quenched by lower concentration of transition metal ions in water. Three metal ion quenchers: (A) Cr^{6+}, (B) Fe^{3+} and (C) Ru^{3+}. (D) The photographs of NCD in presence of different metal ions in water at pH 4 taken under UV illumination (first row) and day light (second row). $[Ru^{3+}] = [Cr^{6+}] = [Fe^{3+}]$ =1639.3 μM.

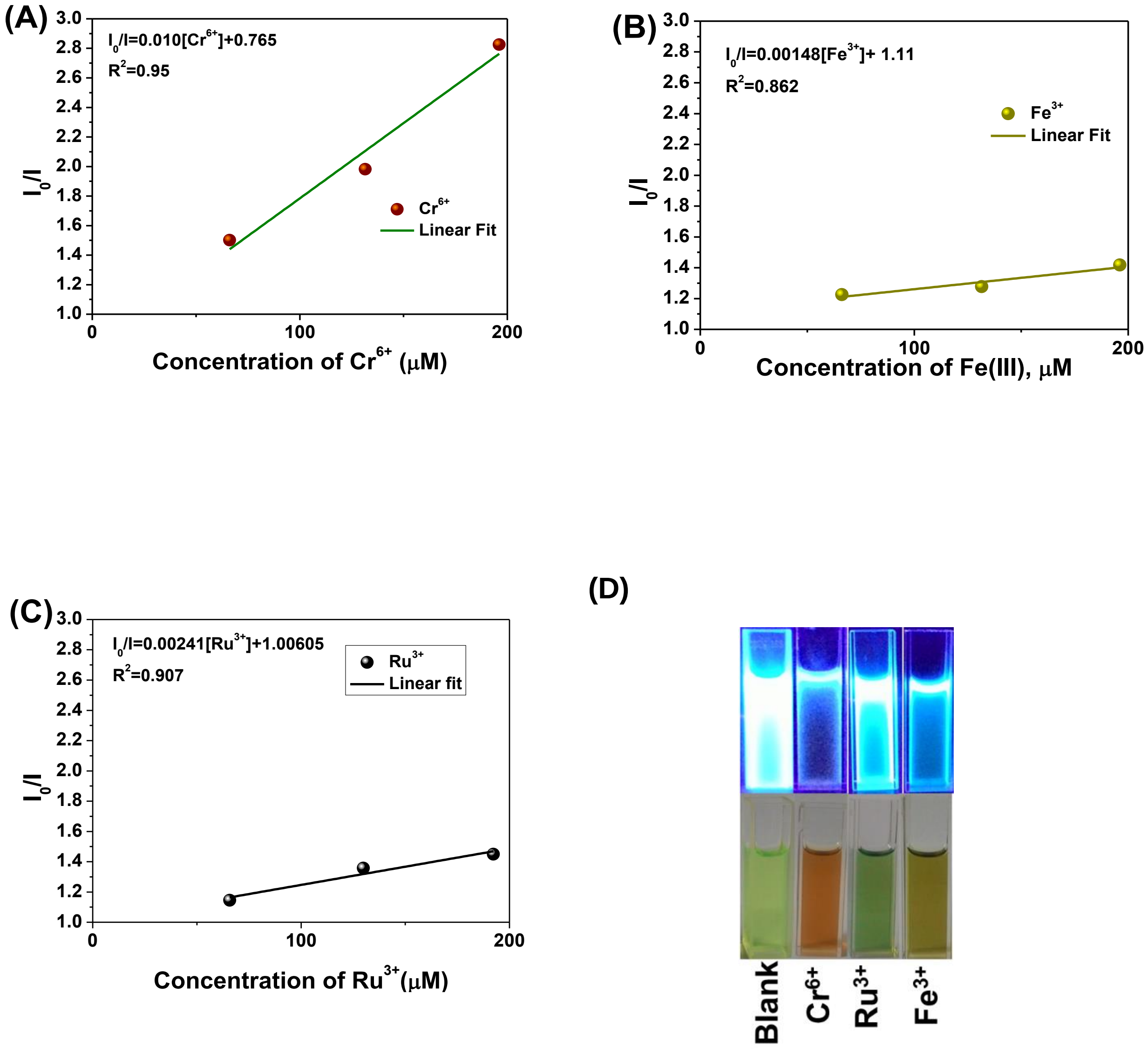

Figure S9. The linear Sten–Völmer plots of NCD fluorescence quenched by lower concentration of transition metal ions in DMSO. Three metal ion quenchers: (A) Cr^{6+}, (B) Fe^{3+} and (C) Ru^{3+}. (D) The photographs of NCD in presence of different metal ions in DMSO taken under UV illumination (first row) and day light (second row). $[Cr^{6+}] = [Fe^{3+}] = 1639.3$ μM. $[Ru^{3+}] = 163.9$ μM.

Table S2. Parameters of amplified quenching in NCD dispersion in water and DMSO.

Metal ion	**Water**		**DMSO**	
	K_{SV} (M^{-1})	**$[Q]_{50}$ (μM)**	**K_{SV} (M^{-1})**	**$[Q]_{50}$ (μM)**
Ru^{3+}	3000	333.3	2410	414.9
Cr^{6+}	2090	478.5	10000	100.0
Fe^{3+}	280	3571.4	1480	675.7

Table S3. Parameters of amplified quenching for previously reported ionic conjugated polyelectrolytes.

System	Parameters	Author and year
Polyphenylene ethynylene based anionic conjugated polyelectrolyte, **PPE-SO$_3^-$** And N,N'-dimethyl-4,4'-bypyridinium [abbreviated as **MV^{2+}**, a cationic quencher]	**K$_{SV}$** 1.4×10^{7} M^{-1} (in Methanol) 2.7×10^{7} M^{-1} (in water)	(Tan *et al.* 2002) [4]
Sulphonate substituted poly(phenylene ethynylene based anionic conjugated polyelectrolyte, **PPESO$_3$** And Cationic cyanine dyes (**HMIDC, DOC, DODC, DOTC, Cy^{3+})**	**K$_{SV}$** 4.6×10^{5} M^{-1} (DOC, in Methanol) 5.8×10^{5} M^{-1} (DODC, in Methanol) 1.3×10^{6} M^{-1} (DOTC, in Methanol) 1.1×10^{6} M^{-1} (HMIDC, in Methanol) 1.0×10^{6} M^{-1} (Cy^{3+}, in Methanol) **[Q]$_{90}$** >5 µM (DOC, in Methanol) 4.8 µM (DODC, in Methanol) 2.2 µM (DOTC, in Methanol) 4.0 µM (HMIDC, in Methanol) 1.4 µM (Cy^{3+}, in Methanol)	(Tan *et al.* 2004)[5]
Metal Organic polymer (**P-Ru and P-Os**) based cationic polymer electrolytes And Anionic electron acceptors [abbreviated as **ND1**]	**K$_{SV}$** 1.6×10^{6} M^{-1} (**P-Ru**, in N,N-Dimethylformamide solution) 0.4×10^{6} M^{-1} (**P-Os**, in N,N-Dimethylformamide solution)	(Liu *et al.* 2002)[6]

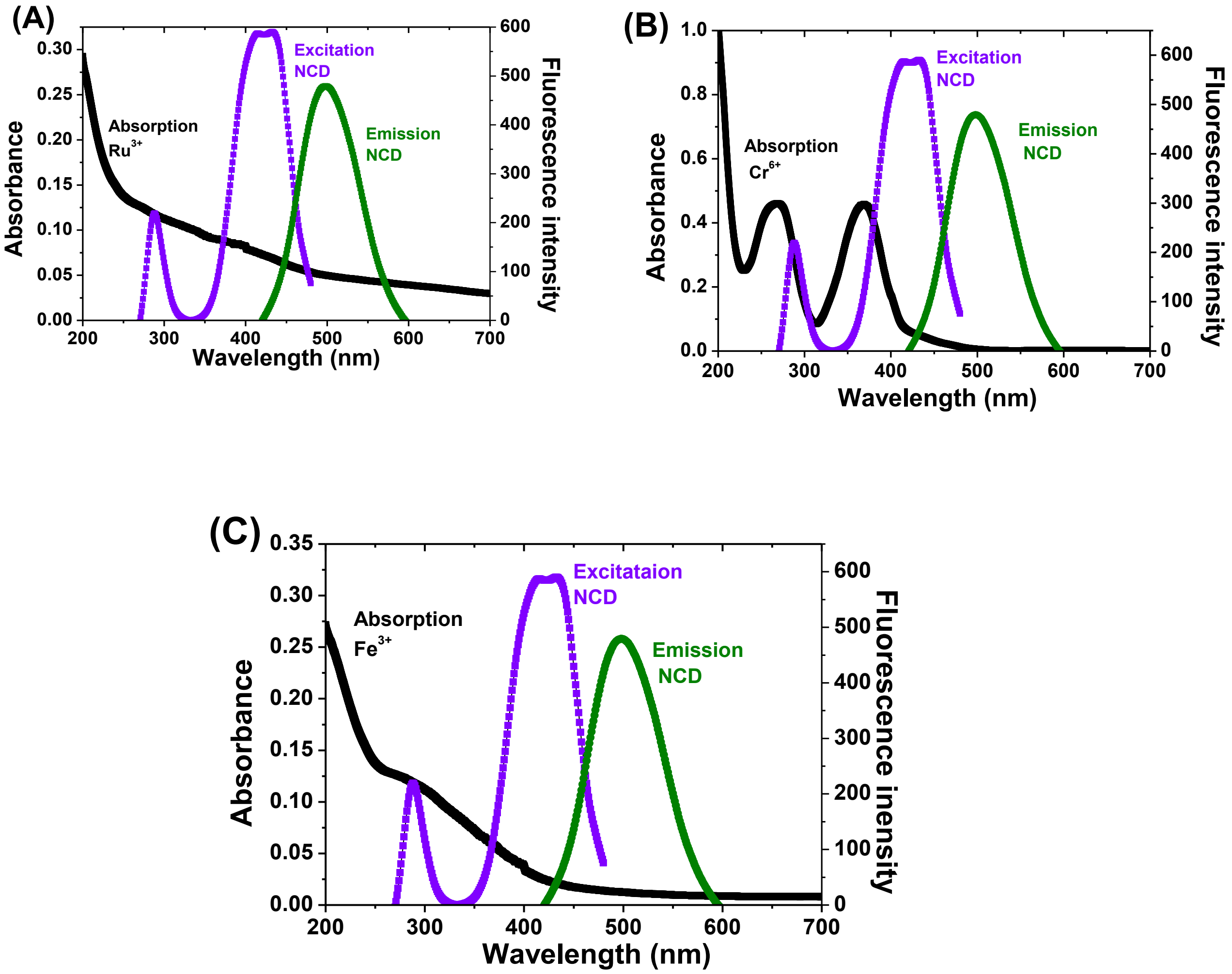

Figure S10. The spectral overlap of UV-visible absorption spectra (black scatter) of metal ion quenchers and excitation (violet scatter)/emission (olive scatter) of NCD in aqueous solution. The metal ion quenchers are: (A) Ru^{3+}, (B) Cr^{6+}, (C) Fe^{3+}.

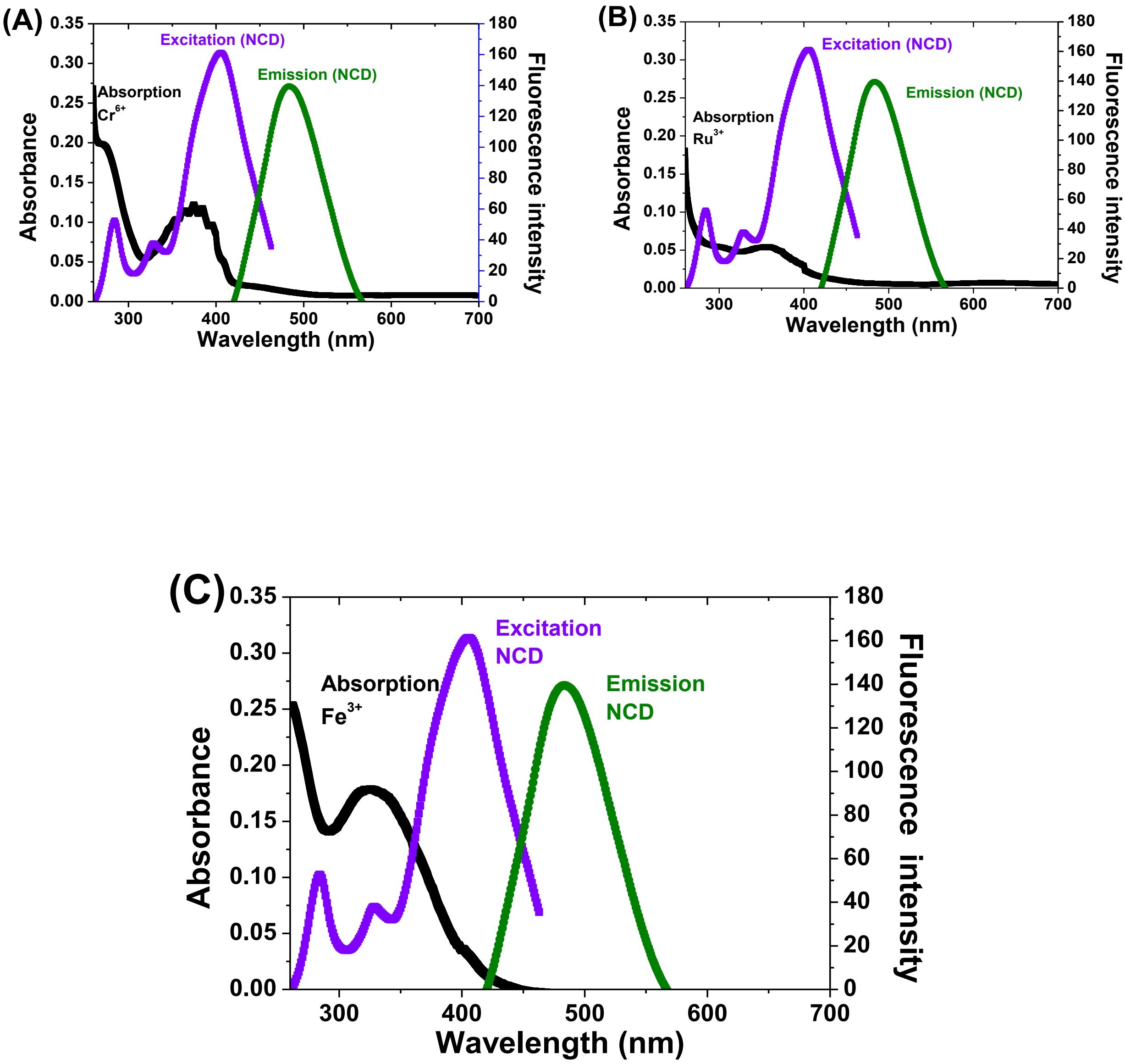

Figure S11. The spectral overlap of UV-visible absorption spectra (black scatter) of metal ion quenchers and excitation (violet scatter)/emission (olive scatter) of NCD in DMSO solution. The metal ion quenchers are: (A) Cr^{6+}, (B) Ru^{3+}, (C) Fe^{3+}.

Table S4. Time resolved fluorescence data (*f* ~ fractional contribution) of nitrogen doped carbon nanodots (NCDs) before and after the addition of transition metal ion solution at ambient temperature in (A) highly viscous protic solvent-water and (B) aprotic solvent having low viscosity-DMSO. Excitation wavelength = 415 nm. The concentration of Cr^{6+}, Fe^{3+}, Ag^{+} and Hg^{2+}solution were kept at 1639.3 µM. The concentration of Ru^{3+} was 163.9 µM.

(A)

Sample	**JH aggregate**		**H-aggregate**		**J-aggregate**		***$\langle\tau\rangle$ (ns)***	**χ^2**	***k_{ET} (ns^{-1})***
	f_1 (%)	***τ_1(ns)***	***f_2 (%)***	***τ_2 (ns)***	***f_3 (%)***	***τ_3 (ns)***			
NCD$_{aq}$	21.42	3.03	76.97	8.1	1.61	0.07	7.62	1.21	0.00
NCD$_{aq}$/Cr^{6+}	20.14	2.68	78.00	6.27	1.85	0.09	5.91	1.24	0.04
NCD$_{aq}$/Fe^{3+}	24.97	3.16	73.42	7.56	1.61	0.07	7.01	1.16	0.01
NCD$_{aq}$/Ru^{3+}	21.95	3.08	76.52	8.01	1.53	0.09	7.52	1.17	0.00
NCD$_{aq}$/Ag^{+}	37.00	2.90	60.81	6.49	2.20	0.08	5.72	1.43	0.04
NCD$_{aq}$/Hg^{2+}	36.76	2.92	61.05	6.73	2.19	0.10	5.94	1.35	0.04

(B)

Sample	**JH-aggregate**		**H-aggregate**		**J-aggregate**		***$\langle\tau\rangle$ (ns)***	**χ^2**	***k_{ET} (ns^{-1})***
	f_1 (%)	***τ_1(ns)***	***f_2 (%)***	***τ_2 (ns)***	***f_3 (%)***				
NCD$_{DMSO}$	22.33	5.23	75.56	12.97	2.11	0.30	12.14	1.36	0.00
NCD$_{DMSO}$/Cr^{6+}	22.32	4.99	74.92	13.20	2.76	0.35	12.36	1.38	0.00
NCD$_{DMSO}$/Fe^{3+}	17.93	4.57	79.65	13.16	2.42	0.31	12.53	1.35	0.00
NCD$_{DMSO}$/Ru^{3+}	20.41	4.81	76.94	12.93	2.65	0.34	12.91	1.40	-0.01
NCD$_{DMSO}$/Hg^{2+}	16.62	3.91	79.72	13.54	3.66	0.22	12.98	1.6	-0.01

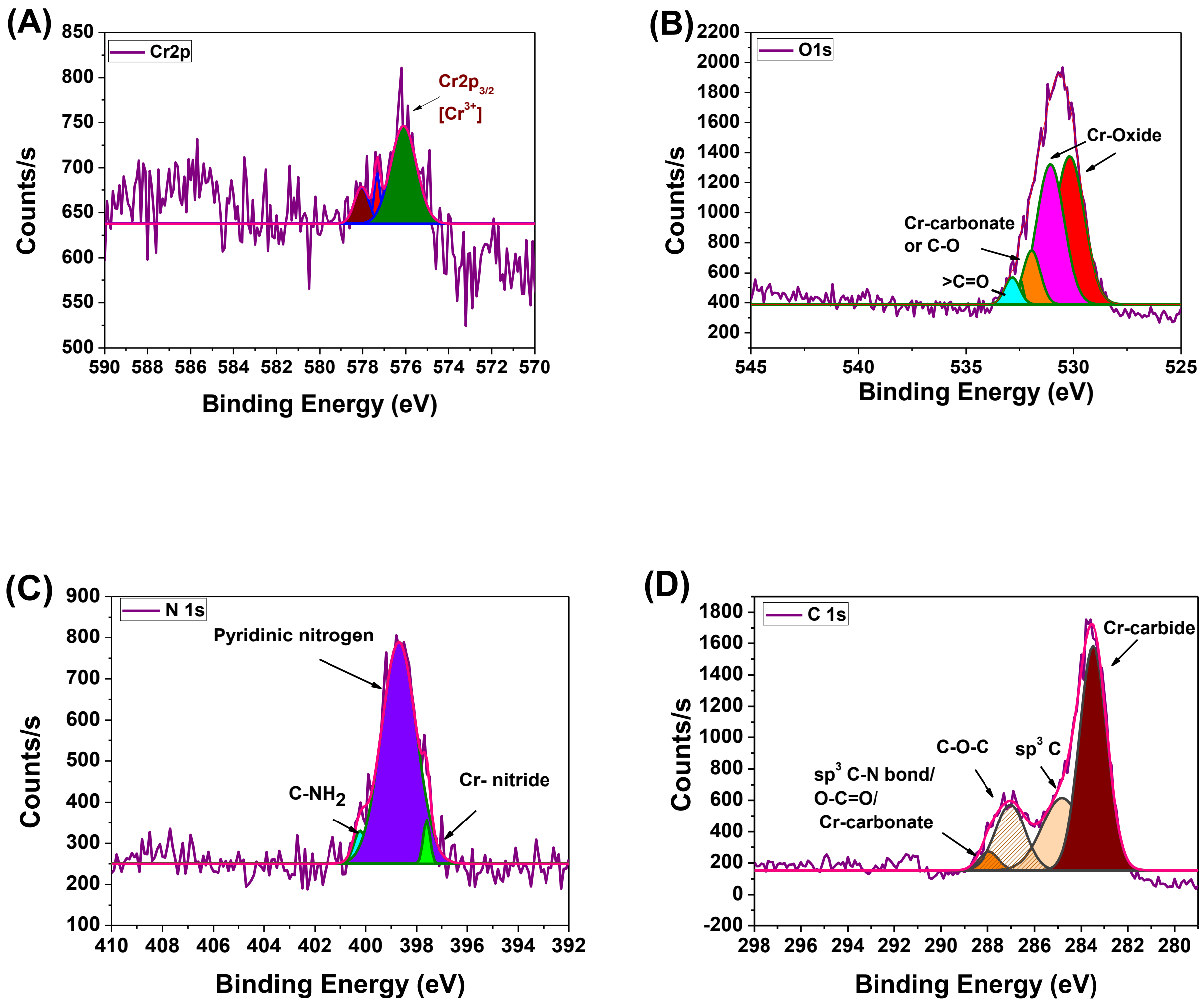

Figure S12. XPS spectra for Cr^{6+} quenched NCD. The raw data (solid purple line) for (A) Cr2p, (B) O1s, (C) N1s and (D) C1s were deconvoluted separately into fitted peaks (coloured area plot). Each corresponding assignment to each fit is presented on each plot, where different colours represent different local bonding.

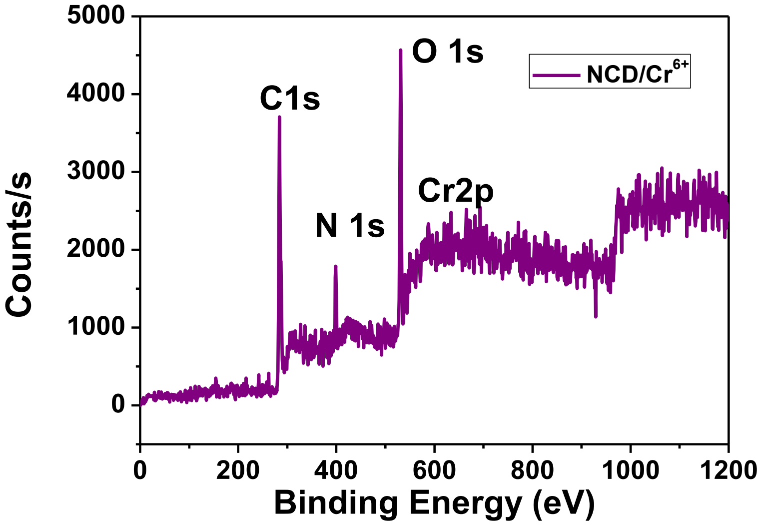

Figure S13. The full XPS spectra obtained for Cr^{6+} quenched NCD.

Table S5. Time resolved fluorescence data (f_i ~ fractional contribution) of nitrogen doped carbon nanodots (NCDs) before and after the addition of transition metal ion solution at ambient temperature in (A) highly viscous protic solvent-water and (b) aprotic solvent having low viscosity–DMSO. Excitation wavelength = 330 nm. The concentration of Cr^{6+}, Fe^{3+}, Ag^{+} and Hg^{2+}solution were kept at 1639.3 µM. The concentration of Ru^{3+} was 163.9 µM.

(A)

Sample	**JH aggregate**		**H-aggregate**		**J-aggregate**		**<τ> (ns)**	χ^2	k_{ET}
	f_1 (%)	τ_1(ns)	f_2 (%)	τ_2 (ns)	f_3 (%)	τ_3 (ns)			
NCD_{aq}	9.20	4.66	77.83	10.28	12.97	1.07	9.85	1.22	0.00
NCD_{aq}/Cr^{6+}	10.75	4.89	80.42	8.999	8.83	1.09	8.63	1.19	0.01
NCD_{aq}/Fe^{3+}	2.73	3.46	84.70	9.798	12.57	0.94	9.60	1.27	0.00
NCD_{aq}/Ru^{3+}	8.82	4.56	78.54	10.19	12.64	1.02	9.79	1.18	0.00
NCD_{aq}/Ag^{+}	10.01	5.01	77.00	10.31	12.99	1.05	9.85	1.18	0.00
NCD_{aq}/Hg^{2+}	5.59	3.22	82.60	10.18	11.81	0.93	9.92	1.22	0.00

(B)

Sample	**JH-aggregate**		**H-aggregate**		**J-aggregate**		**<τ> (ns)**	χ^2	k_{ET}
	f_1 (%)	τ_1(ns)	f_2 (%)	τ_2 (ns)	f_3 (%)	τ_3 (ns)			
NCD_{DMSO}	19.91	7.62	77.29	14.42	2.81	0.94	13.58	1.13	0.00
NCD_{DMSO}/Cr^{6+}	22.25	6.89	74.79	13.34	2.96	0.81	12.46	1.09	0.01
NCD_{DMSO}/Fe^{3+}	12.98	6.63	83.87	14.21	3.15	0.78	13.67	1.20	0.00
NCD_{DMSO}/Ru^{3+}	19.99	7.37	76.96	14.05	3.05	1.00	13.22	1.07	0.00
NCD_{DMSO}/Hg^{2+}	16.47	6.24	77.91	14.12	5.62	0.90	13.39	1.1	0.00

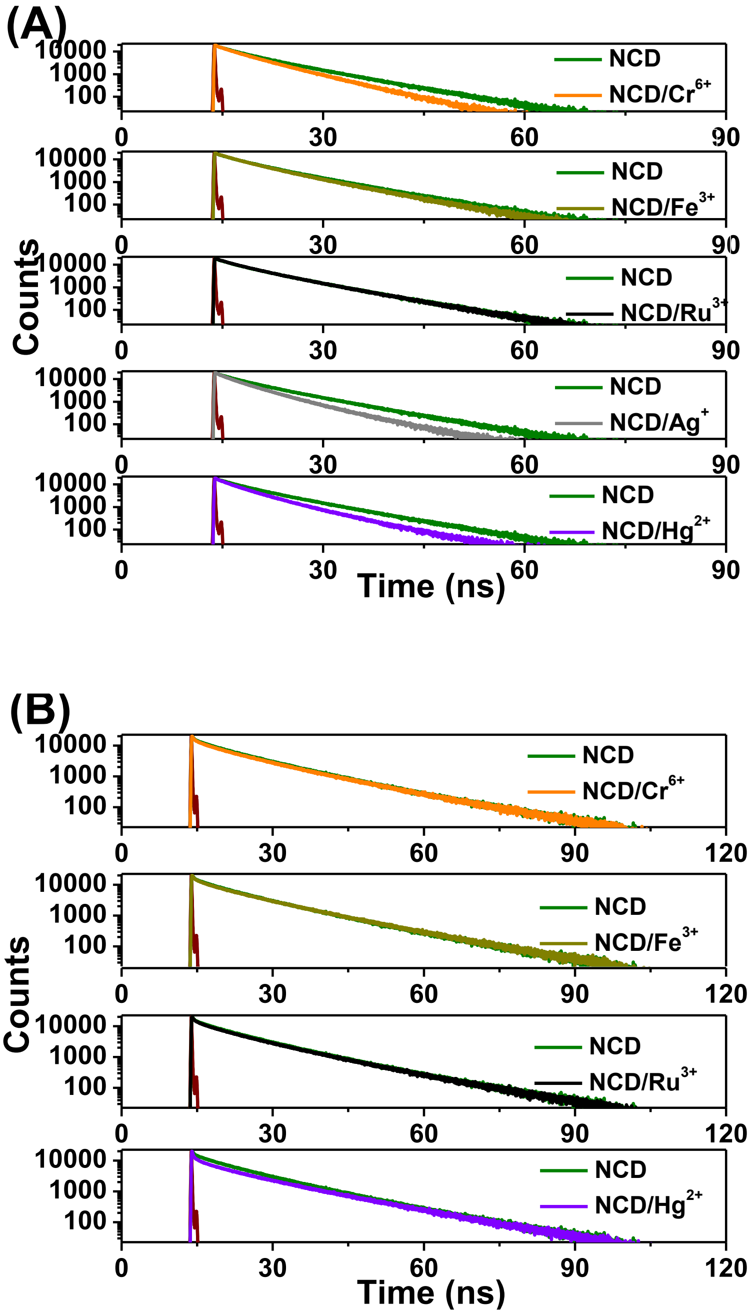

Figure S14. (A) Fluorescence decay traces of aqueous dispersion of NCD (6 mg/mL, pH 4) before and after addition of metal ion quenchers when monitored at 415 nm excitation. The metal ion quenchers are: (a) Cr^{6+}, (b Fe^{3+}, (c) Ru^{3+}, (d) Ag^{+}, and (e) Hg^{2+}. (B) Fluorescence decay traces of NCD dispersion in DMSO medium (3 mg/mL) before and after addition of metal ion quenchers when monitored at 415 nm excitation. The metal ion quenchers are: (a) Cr^{6+}, (b Fe^{3+}, (c) Ru^{3+}, and (d) Hg^{2+}. The concentration of Fe^{3+}, Cr^{6+}, Ag^{+}, and $Hg^{2+}$is 1639.3 µM. The Ru^{3+} is added at 819.65 µM.

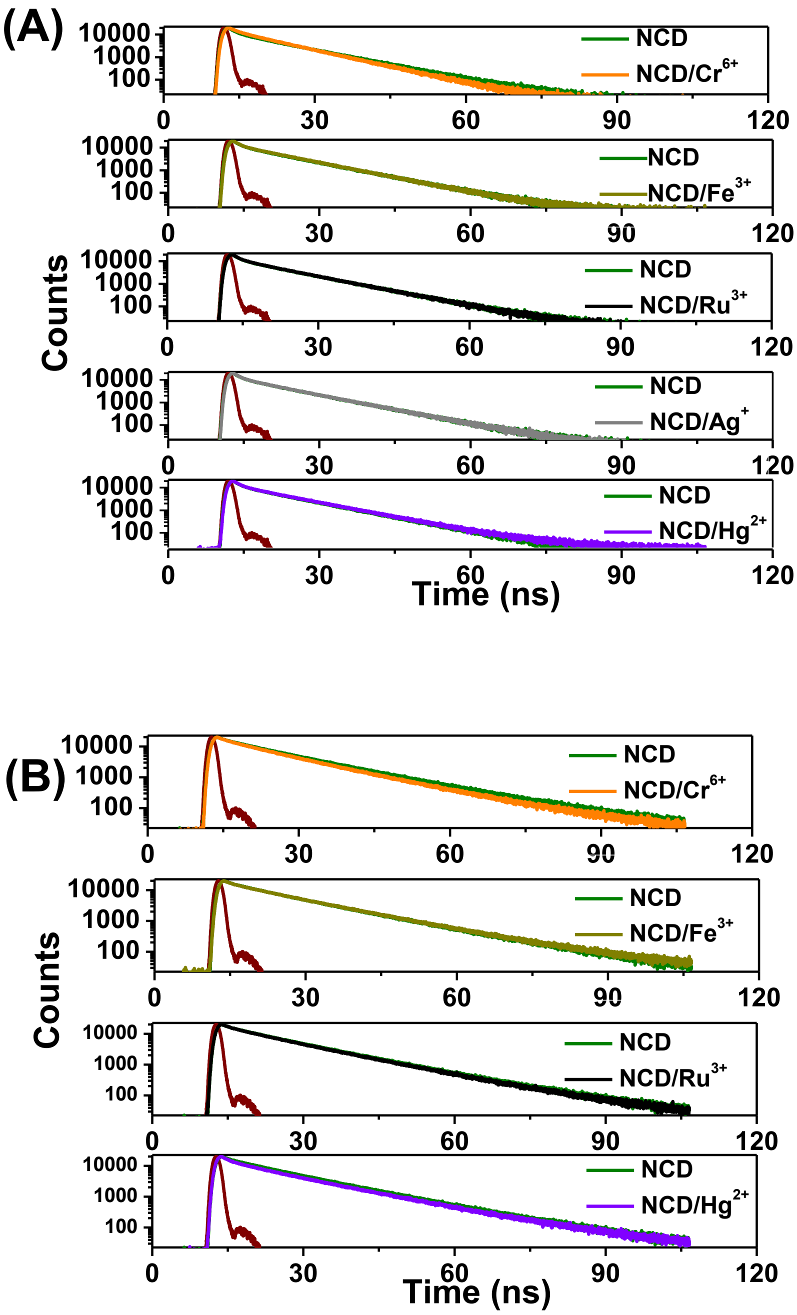

Figure S15. (A) Fluorescence decay traces of aqueous dispersion of NCD (6 mg/mL, pH 4) before and after addition of metal ion quenchers when monitored at 330 nm excitation. The metal ion quenchers are: (a) Cr^{6+}, (b Fe^{3+}, (c) Ru^{3+}, (d) Ag$^{+=,}$ and (e) Hg^{2+}. (B) Fluorescence decay traces of NCD dispersion in DMSO medium (3mg/mL) before and after addition of metal ion quenchers when monitored at 330 nm excitation. The metal ion quenchers are: (a) Cr^{6+}, (b Fe^{3+}, (c) Ru^{3+} and (d) Hg^{2+}. The concentration of Fe^{3+}, Cr^{6+}, Ag^{+}, and Hg^{2+}is 1639.3 µM. The Ru^{3+} is added at 819.65 µM.

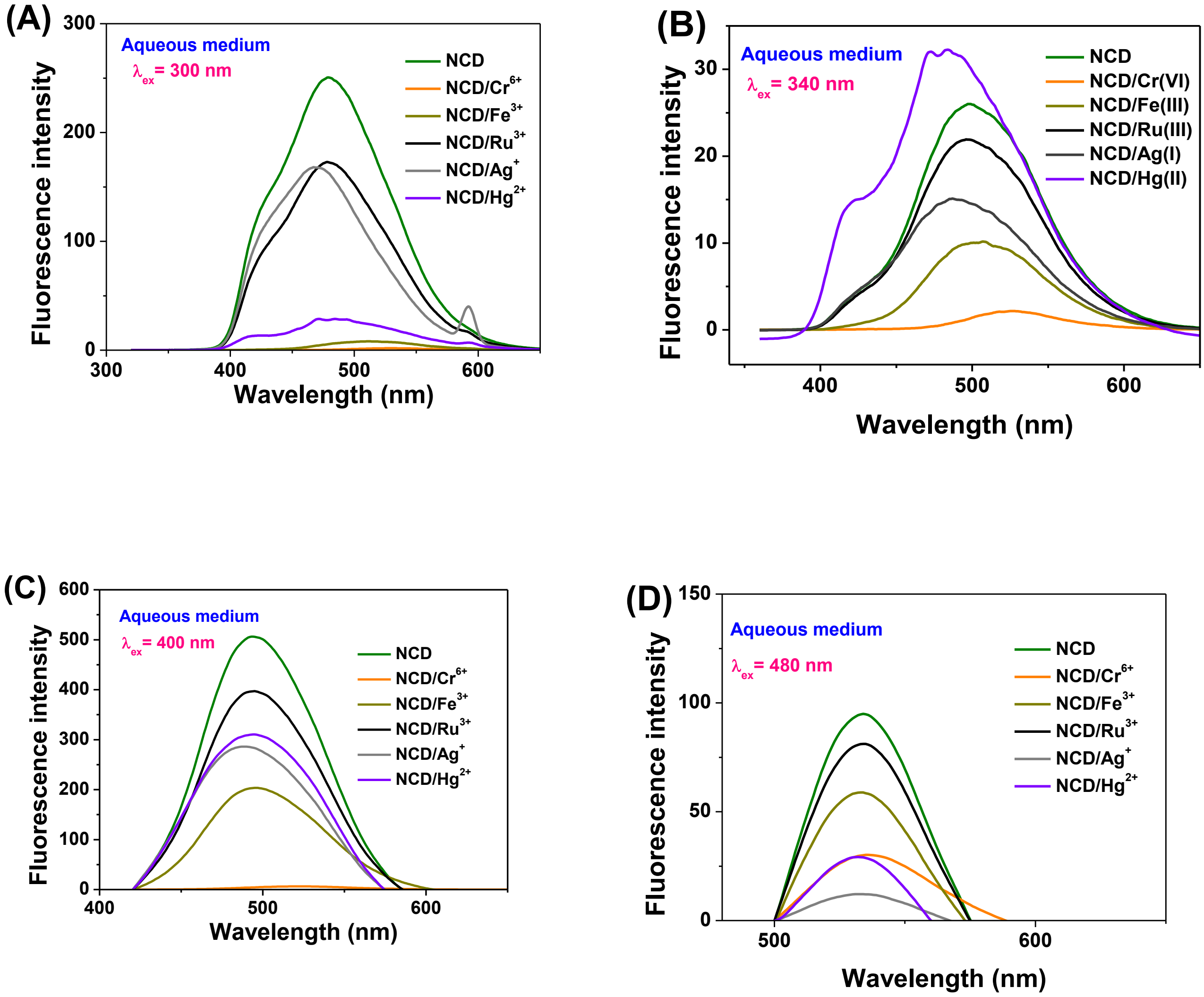

Figure S16. The fluorescence response of aqueous NCD towards metal ions [Cr^{6+}, Fe^{3+}, Ru^{3+}, Ag^{+}, Hg^{2+}] in aqueous solution at different excitation wavelength. (A) 300 nm, (B) 340 nm, (C) 400 nm and (D) 480 nm. The concentration of all metal ions except Ru^{3+} is 1639.3 µM. The concentration of Ru^{3+} is 163.9 µM.

References

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