Controlling the Protonic Conductivity of Full Colour Emitting Carbon Dots doped Biopolymer: Role of Functional group

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Contents:

1. Material and Method

1.1 Materials

BSA (Bovine Serum Albumin) was purchased from Thermo Chemicals, TFE (Trifluoro ethanol) was purchased from SRL chemical, β-mercapto ethanol, ethylene diamine, and citric acid were purchased from Sigma Aldrich, urea, N, N-Dimethyl formamide, and methanol were purchased from SDFCL. All chemicals were used without any further purification. Synthesis was carried out in HPLC-grade solvent.

1.2 Synthesis

1.2 .1 Synthesis of freestanding BSA thin film:

The BSA film was synthesized following a reported protocol with some modifications. [1] Briefly, the required amount of BSA powder was dissolved in a 1:4 DI water and trifluoroethanol solution and stirred overnight to make a homogenous transparent solution. A small quantity of the β-mercapto ethanol was added and stirred for three hours until the solution became hazy. Finally, 1:1 ethylene diamine and ethanol solution were added to the reaction mixture and stirred for a few minutes. Then, the colourless mixture was dropped cast in a flat glass slide in a fume hood to evaporate the excess reactants and allowed to form the thin film.

1.2 .2. Synthesis of different emitting C-Dots:

The mixture of full-color-emitting CDs was synthesized via solvothermal decomposition of urea and citric acid dissolved in DMF, following the procedure reported in the literature with slight modification.[2] Initially, 1.0 g of citric acid and 1.0 g of urea were dissolved in 15 mL of DMF, and the mixture was sonicated for 10 minutes to ensure complete dissolution. This solution was placed in a Teflon-lined stainless-steel autoclave and heated at 180°C for 12 hours, resulting in a blackish product. The crude product was then purified using simple silica column chromatography, employing a mixture of dichloromethane (DCM) and methanol (MeOH) as the mobile phase. Various emissive pure C-Dots, namely pure B-C-Dots, G-C-Dots, and R-C-Dots, were collected by adding 5%, 15%, and 25% methanol to DCM, respectively.[3,4]

1.2 .3. Synthesis of C-Dot-BSA composite:

A 5 mL stock solution of differently emitting C-Dots was prepared at a concentration of 2 mg/mL in water. A piece of BSA film measuring 1 cm × 1 cm × 0.06 mm (L × W × T) was immersed in the C-Dot solution overnight, allowing the C-Dots to attach to the film via physisorption. Unbound C-Dots were removed by gently washing the films with water. The resulting BSA films doped with various emitting C-Dots were then used for further studies.[5,6]

1.3. Instrumentation and Sample Characterization:

1.3.1. Transmission Electron Microscopy and HRTEM Analysis:

The JEOL JEM 2100F Field Emission Gun-Transmission Electron Microscope (TEM) at 200 kV was utilized to analyze the dimensions and configuration of the produced materials. C-Dots were dissolved in methanol by sonication and drop-cast on the copper grids for capturing TEM images.

1.3.2. Fourier Transform Infrared Spectroscopy:

FTIR spectra were obtained using the FTIR mode on a JASCO FTIR 6600 spectrometer. Potassium bromide (KBr) is dedicated to the IR measurements used for making the pellets. In pellets 1:100 (sample to KBr) ratio was maintained during measurements.

1.3.3. XPS analysis:

XPS measurements were conducted on the Thermo Fisher Scientific K-Alpha X-ray Photoelectron Spectrometer (XPS) System using an Al K micro focus monochromator. The variable operating energy range of 100-4000 eV was utilized to record the spectrum.

1.3.4. UV-Visible Absorbance and Steady State Emission Measurements:

UV-Visible Absorbance Measurement: To investigate the optical properties, we measured absorbance using a Shimadzu UV-2600i UV-visible spectrophotometer. The liquid and solid thin film of C-Dots and BSA-C-Dots were placed different sample holders respectively. Here the stock solution was prepared by dissolving 2 mg of C-Dots in 1 ml water. The absorbance of C-Dots in solution was recorded using water as baseline and the pathlength was 10 mm.

The absorbance of the BSA film and BSA–C-Dot composite films was measured using a thin-film sample holder, with air used as the baseline for solid-state measurements. The free-standing transparent BSA and BSA–C-Dot films with dimension of 3 cm × 1.2 cm × 0.06 mm (L × W × T), were placed perpendicular to the incident light in the sample holder. Thus, the optical path length for the BSA–C-Dot composite film was 0.06 mm.

Steady State Emission Measurements:

The Steady-state fluorescence spectra were acquired using a JASCO spectrofluorometer and a Photon Technology International (PTI) Quanta Master spectrofluorometer. The steady-state PL emission of C-Dots was recorded keeping the optical density of ~1 at 340 nm for all C-Dots.

In the solid state, the PL measurements of free-standing films were conducted using a piece with the dimension of 3 cm x 1.2 cm x 0.06 mm (L x W x T). It was placed in the sample holder at a 45° angle to the incident light, and the excitation and emission slit were fixed at 1 nm, with an integration time kept at 1ms.

1.3.5. Thermal Gravimetric Analysis:

Thermogravimetric analysis (TGA) was performed using a Shimadzu TGA-50 analyser. Approximately 20 mg of BSA and BSA–C-Dot films were heated from room temperature to 900 °C under an inert atmosphere. The data were recorded and analysed using TA-60 software.

1.3.6. Time-Correlated Single Photon Counting Measurement:

Time-resolved fluorescence measurements were conducted using Time-Correlated Single Photon Counting (TCSPC) mode on the Horiba Delta Flex Lifetime System (Horiba Scientific). Samples were excited at two distinct wavelengths, 280 nm, and 340 nm, utilizing a nano LED light source. Emission spectra were collected at 340 nm and 440 nm. For TRES analysis the emission was measured in between 300 to 400 nm after excitation at 280 nm. The instrument response function (IRF) is 1.2 ns both excitations, with preliminary IRF calibration performed using a soap solution in water as a reference standard. C-Dots were uniformly dissolved in water to ensure consistency across measurements.

The lifetime of BSA and BSA-C-Dot composite film was measured by keening films inside the holder at angle nearly 45° in between two quartz slides. Before measurement the films were kept in water for overnight to absorb water. The excess free water present on the surface of BSA film were wiped out to avoid scattering. Data analysis was conducted using Horiba's Data Analyser software, enabling the calculation of average fluorescence lifetimes.

$$\tau_{avg} = \sum \alpha_i \tau_i / \sum \alpha_i \qquad \text{..... S1}$$

The TRES analysis was done by utilizing fitted data following the reported methodology.[7]

The solvation dynamics is calculated by fitting the decay of the solvent correlation function $C(t)$ estimate using the following equation.

$$\boldsymbol{C(t) = \frac{v(t) - v(\infty)}{v(0) - v(\infty)}} \qquad \text{..... S2}$$

Where, $v(0)$, $v(t)$, and $v(\infty)$ are the frequencies at time zero given and infinite, respectively.

1.4. Electrical Study:

1.4.1. Electrochemical Measurements:

To understand proton conduction ability, an electrical study was done by two two-electrode system on the Metrohm Autolab electrochemical workstation. All electrical study was done at a 26 °C temperature and 40% room humidity.

The electrodes were prepared on fluorine tin oxide (FTO) coated glass, which was itched by 1 mm to make separate electrodes. After complete itching, cleaned by sonication in water, ethanol, and acetone for 30 min each, and dried at room temperature. To measure the conductivity of different emitting C-Dots as a control experiment, 2 mg/ml of C-Dots was dissolved in water. Then this solution was drop casted on FTO using a spin coater to check the protonic and electronic conductivity. Similarly, to measure the conductivity of BSA film and BSA-C-Dots composites placed on cleaned electrodes separated by 1 mm. The AC-EIS measurements were conducted in the frequency range of 1 Hz to 1 MHz. The

DC- I-V measurement was carried out in the potential range of -1 to 0.7 V at a scan rate of 100 mVs^{-1} and the applied potential of 1 V.

The complete hydration of the BSA film and BSA-C-Dot composite film was achieved by allowing them to absorb water, keeping the fixed quantity of water overnight for measuring protonic conductivity. Before starting electrical measurements, the absorbed water percentage was determined by weighing and calculating the weight difference between hydrated and dry film. The unbounded water was removed from the surface of the BSA film, carefully wiped with clean tissue paper.

To explore the hydration states, an electrical study was performed using different water-acetonitrile mixtures (10-50 % water in acetonitrile). The EIS measurement was conducted via the addition of 5 µL mixture of water-acetonitrile in the centre of the film and waiting 5 min to complete absorption of the solvent mixture in the BSA film. the acquisition for impedance measurements was started.

Similarly, the kinetic isotopic effect (KIE), measured by determining the ratio of conductivities (KIE = σH / σD) in the presence of 30 % deuterium oxide and 30 % water in acetonitrile. Three different sets were prepared for measuring KIE. The result is reported by taking the average and standard deviation of the calculated KIE in three different sets.

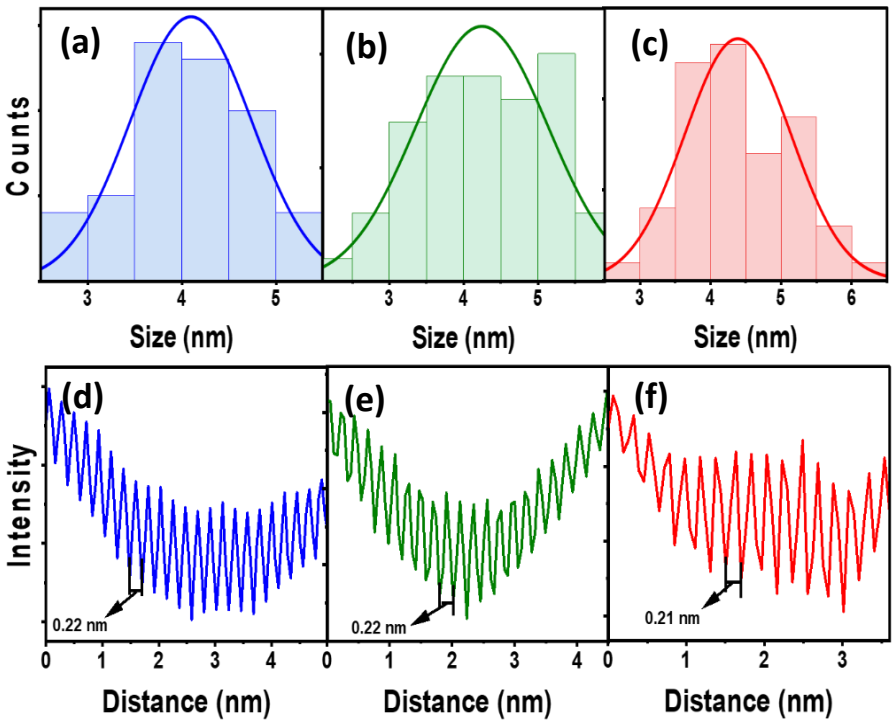

Figure S1: (a-c) HR-TEM interplanar distance measurements, (d-f) Average particle Size distribution analysis for B-C-Dot, G-C-Dot, and R-C-Dot, respectively.

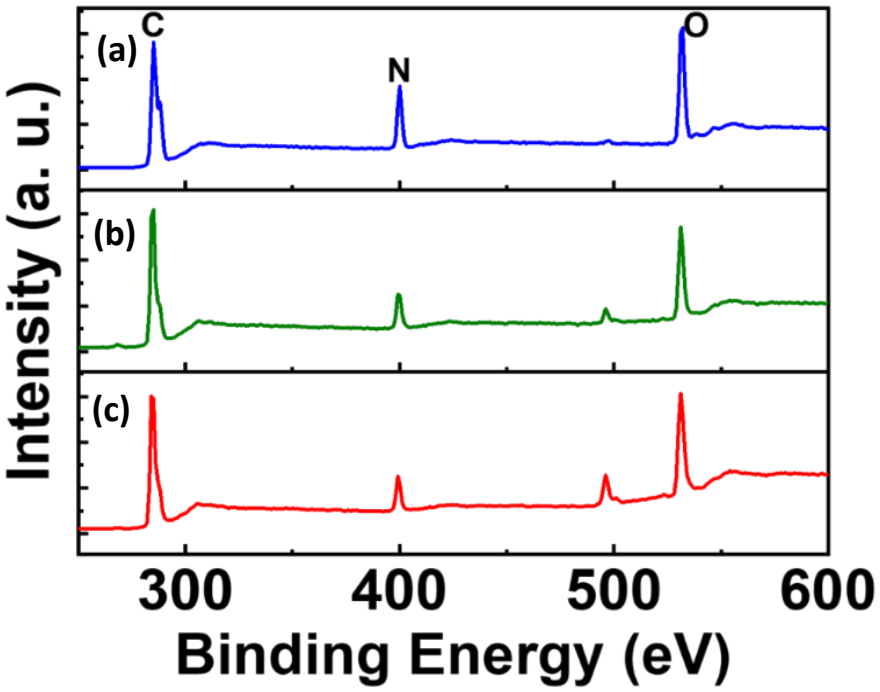

Figure S2: Full survey of elemental analysis of all C-Dots: (a) B-C-Dots, (b) G-C-Dots, and (c) R-C-Dots.

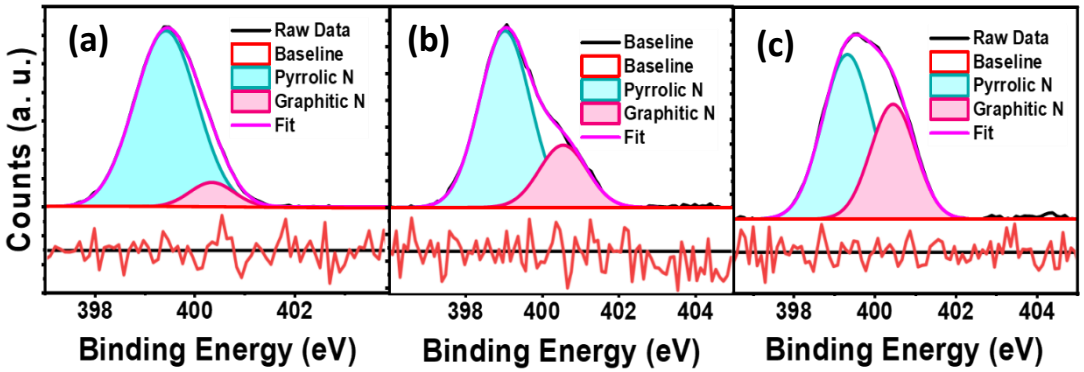

Figure S3: HRXPS of N1s spectra of (a) B-C-Dots, (b) G-C-Dots, and (c) R-C-Dots.

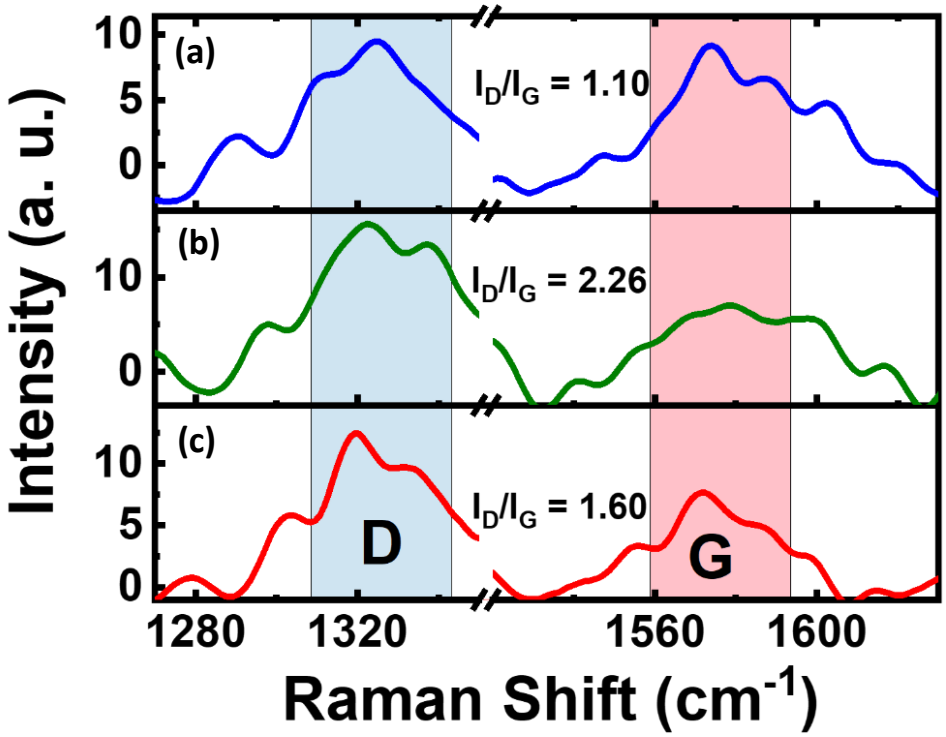

Figure S4: Raman spectrum of emitting C-Dots by exciting with 785 nm laser (a) B-Cots, (b) G-C-Dots, and (c) R-C-Dots.

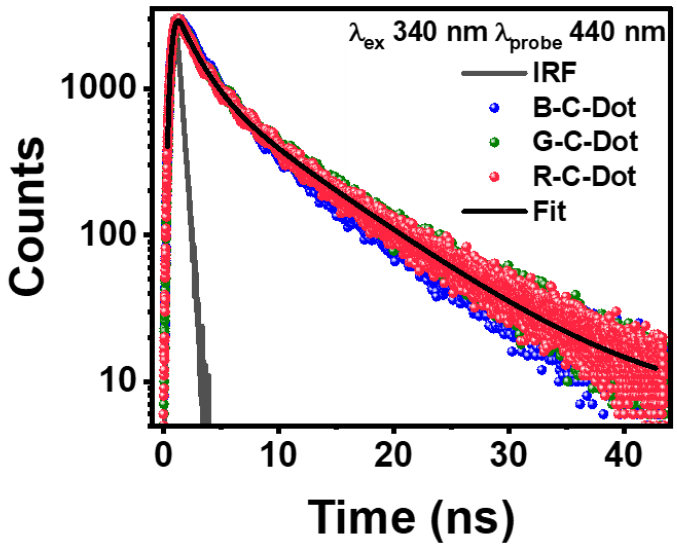

Figure S5: Time resolved fluorescence lifetime measurements of emitting C-Dots after exciting 340 nm and emission monitored at 440 nm.

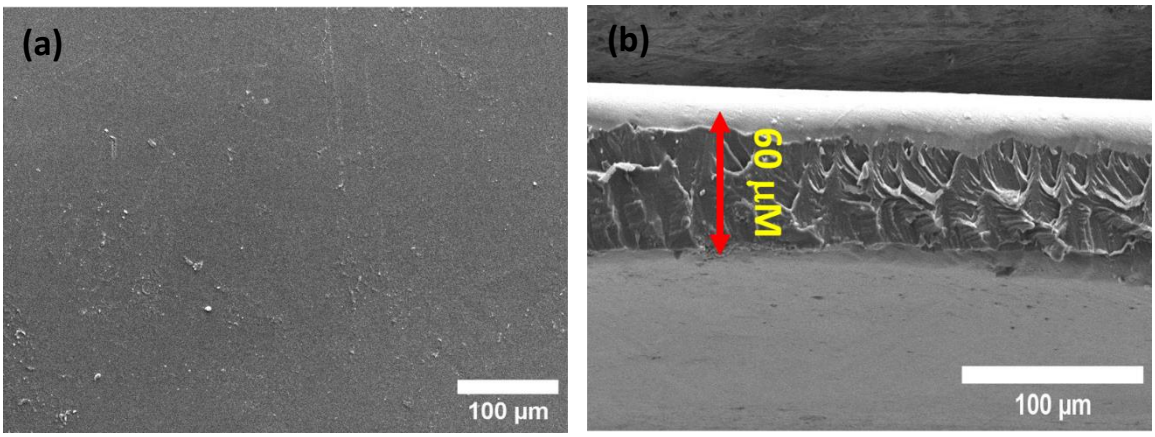

Figure S6: SEM images of BSA film (a) surface and (b) cross section.

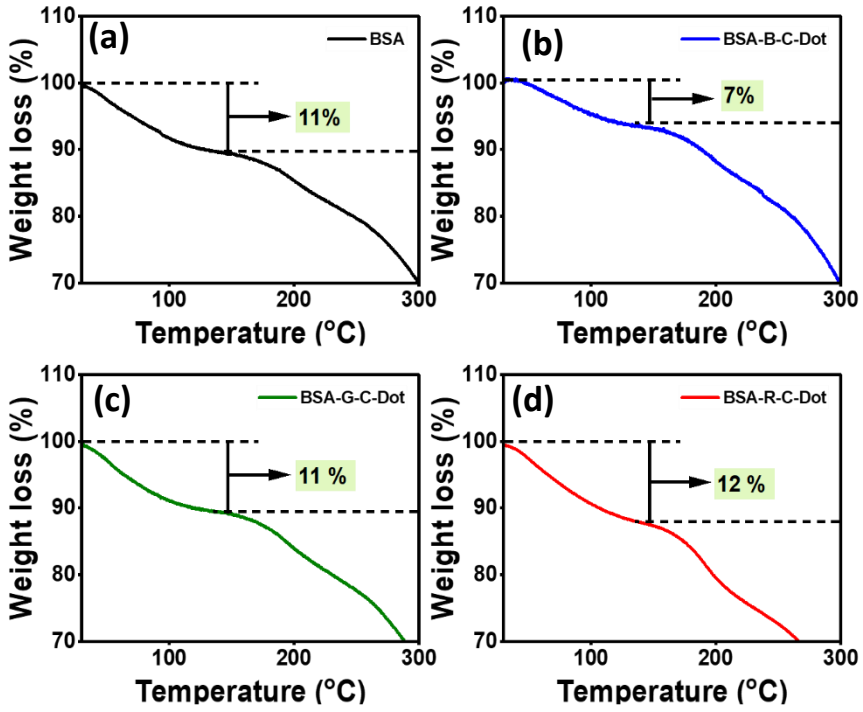

Figure S7: Water content measurements of (a) BSA, (b) BSA-B-C-Dots, (c) BSA-G-C-Dots, and (d) BSA-R-C-Dots films using TGA analysis.

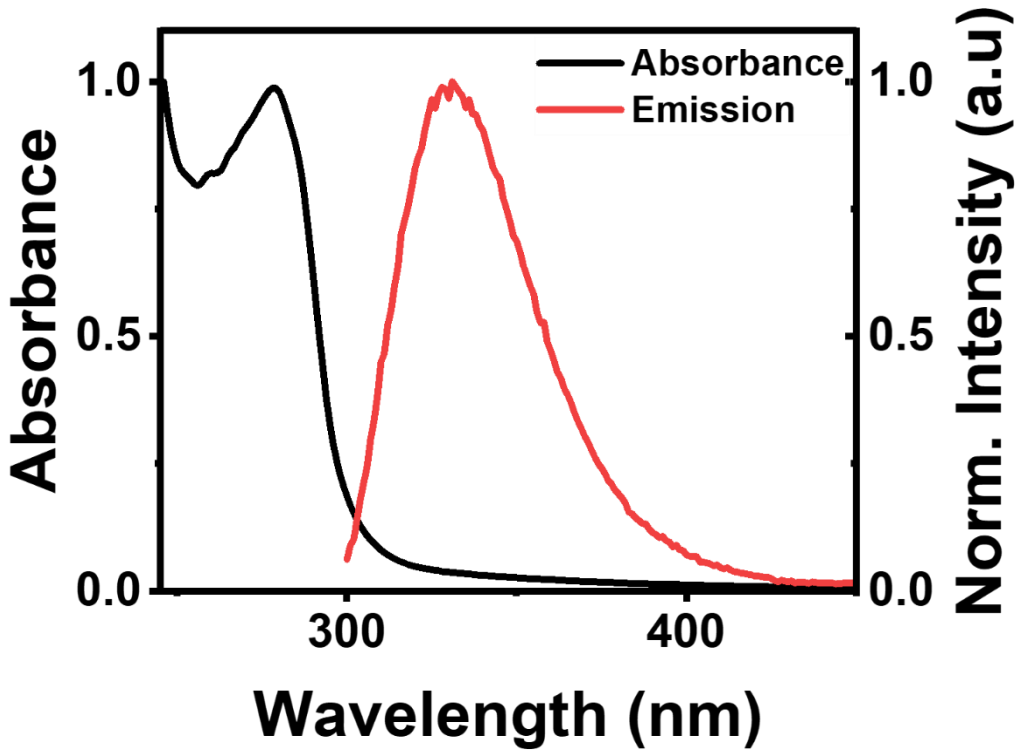

Figure S8: Absorbance and Steady-state emission spectrum of BSA film.

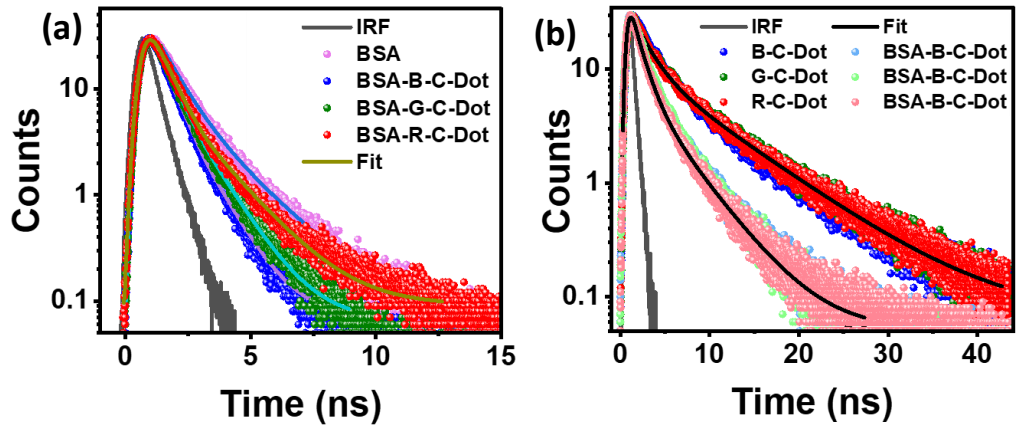

Figure S9: Time-resolved fluorescence lifetime measurements of BSA film and BSA-C-Dots film after exciting at (a) 280 nm and probing at 340 and (b) 340 nm probing at 440 nm respectively.

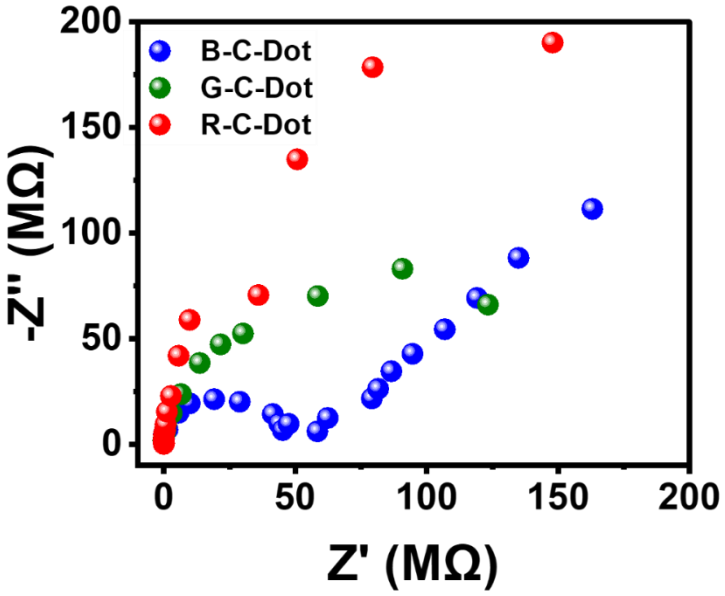

Figure S10: Electrochemical impedance spectra of different emitting C-Dots.

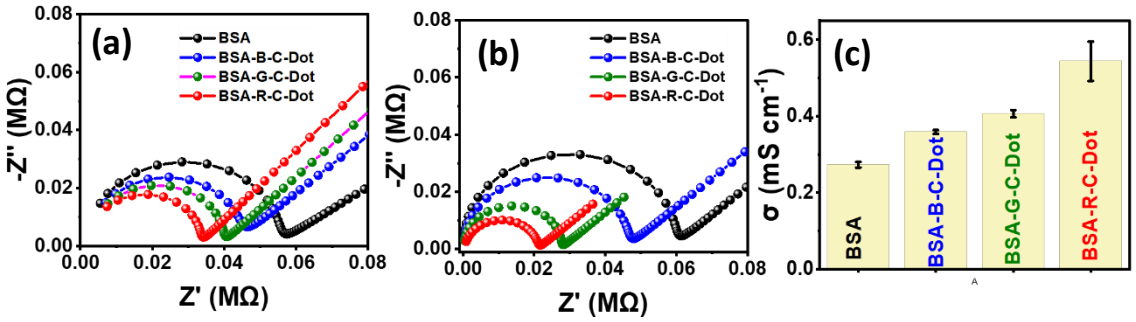

Figure S11: Electrochemical impedance spectra of BSA and BSA-C-Dots films for two sets: (a) Set-II and (b) Set-III, (c) calculated conductivities values of different films with standard deviation.

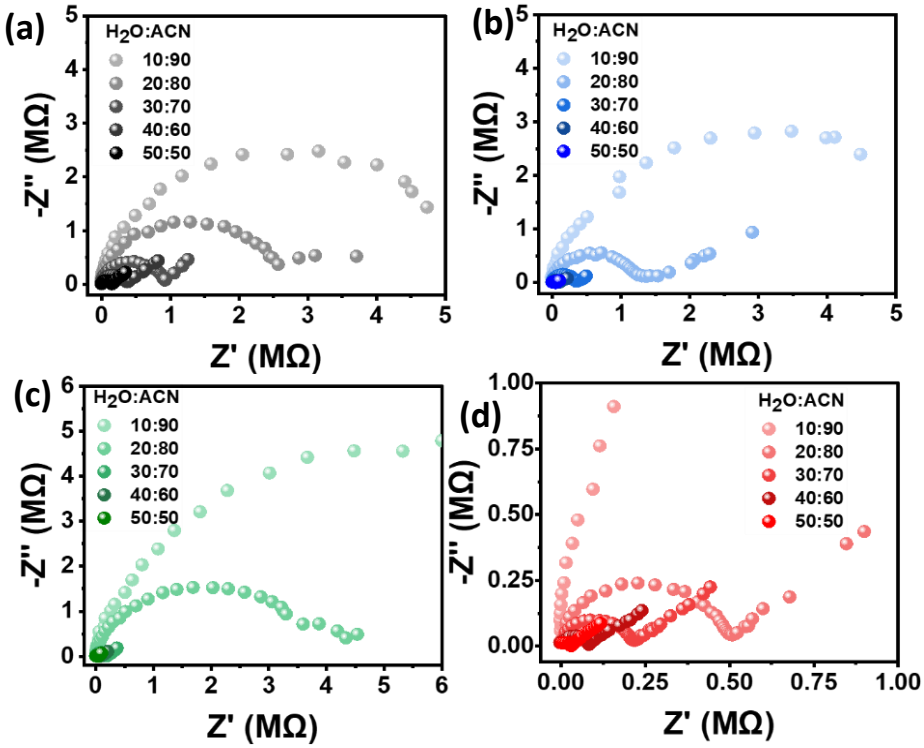

Figure S12: Electrochemical impedance spectra of (a) BSA (b) BSA-B-C-Dots, (c) BSA-G-C-Dots, and (d) BSA-R-C-Dots films at different hydration states.

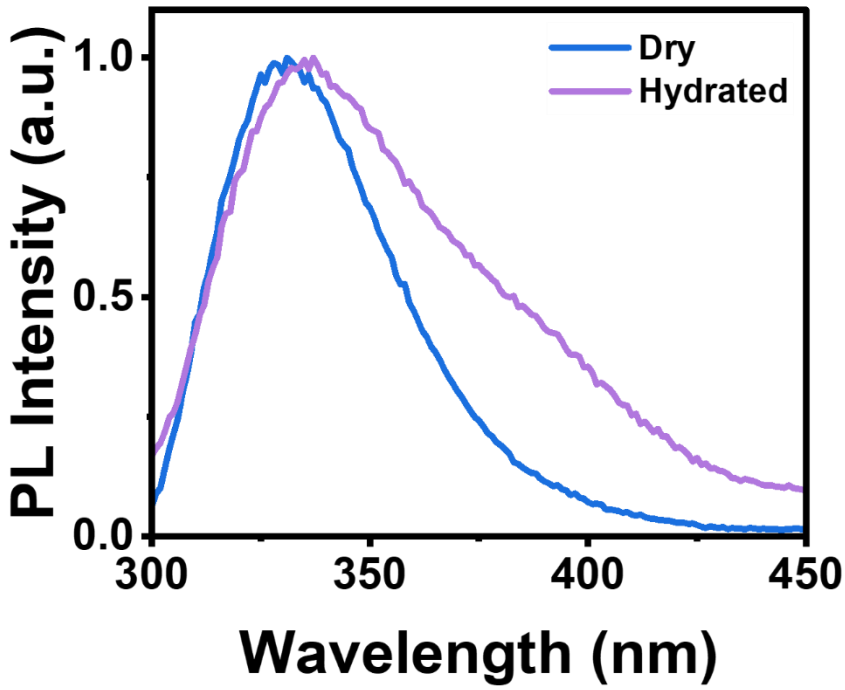

Figure S13: Steady-state fluorescence emission of BSA film on exciting at 280 nm in the dry and hydrated state.

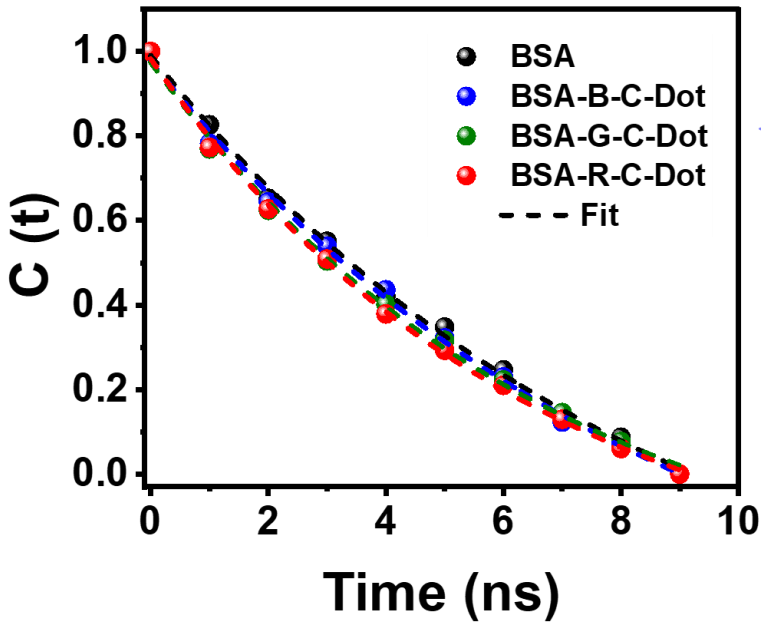

Figure S14: Solvation correlation function analysis for BSA-C-Dot film.

Table S1: HRXPS Fitting constants of N1s signal of C-Dots.

C-Dots	Functional Group	BE (eV)	Area under the curve	Composition (%)	χ^2
B-C-Dot	Pyrrolic N	399.3	89324	87.03	0.999
	Graphitic N	400.4	13301	12.96	
G-C-Dot	Pyrrolic N	398.9	54761	75.12	0.996
	Graphitic N	400.2	18134	24.87	
R-C-Dot	Pyrrolic N	399.3	50778	61.46	0.999
	Graphitic N	400.5	31841	38.53	

Table S2: HRXPS Fitting constants of C1s signal of C-Dots.

C-Dots	Functional Group	BE (eV)	Area under the curve	Composition (%)	χ^2
B-C-Dot	C=C	284	66745	24.48	0.999
	C-O/C-N	284.9	144166	52.88	
	C=O	287.6	61670	22.62	
G-C-Dot	C=C	284.2	218901	57.38	0.998
	C-O/C-N	285.7	59900	15.70	
	C=O	287.2	102676	26.91	
R-C-Dot	C=C	284.1	176231	35.75	0.999
	C-O/C-N	284.8	120766	24.50	
	C=O	286.8	195865	39.74	

Table S3: HRXPS Fitting constants of O1s signal of C-Dots.

C-Dots	Functional Group	BE (eV)	Area under the curve	Composition (%)	χ^2
B-C-Dot	C=O	531.1	185710	64.58	0.999
	C-O-C/C-OH	532.2	101835	35.41	
G-C-Dot	C=O	530.5	91882	60.11	0.998
	C-O-C/C-OH	531.5	60955	39.88	
R-C-Dot	C=O	530.4	95743	45.75	0.999
	C-O-C/C-OH	531.4	113487	54.24	

Table S4: Time constants of time-resolved fluorescence lifetime measurements of C-Dots.

	C-Dots	τ_1 (ns)	B_1 (%)	τ_2 (ns)	B_2 (%)	$\langle\tau\rangle$ (ns)	χ^2
λ_{ex} = 340 nm **λ_{em}= 440 nm**	**B-C-Dot**	2.36	44.58	7.91	55.42	5.44	1.28
	G-C-Dot	1.92	33.48	7.99	66.52	5.96	1.25
	R-C-Dot	1.93	34.65	7.95	65.35	5.86	1.27

Table S5: Time constants of time-resolved fluorescence lifetime measurements of BSA-C-Dots.

	C-Dots	τ_1 (ns)	B_1 (%)	τ_2 (ns)	B_2 (%)	$\langle\tau\rangle$ (ns)	χ^2
λ_{ex}= 280 nm λ_{em}= 340 nm	**BSA**	<1	61.25	2.13	38.75	1.33	1.29
	BSA-B-C-Dot	<1	57.49	1.01	42.51	0.54	1.10
	BSA-G-C-Dot	<1	51.84	1.14	48.16	0.67	1.13
	BSA-R-C-Dot	<1	56.53	1.57	43.47	0.87	1.35
λ_{ex}= 340 nm λ_{em}= 440 nm	**BSA-B-C-Dot**	<1	52.03	4.22	47.97	2.68	1.24
	BSA-G-C-Dot	<1	49.8	3.99	50.2	2.58	1.35
	BSA-R-C-Dot	<1	55.48	4.16	44.52	2.51	1.22

Table S6: Fitting constants for electrochemical circuit of in impedance analysis.

Film	Rs	Rp	CPE	W	χ^2
BSA	2044.1	50119	4.3091E-12	1.9495E-06	0.33535
BSA-B-C-Dot	1267.3	38690	6.5858E-12	1.6671E-06	0.25023
BSA-G-C-Dot	566.01	31485	1.1105E-11	1.9821E-06	0.22317
BSA-R-C-Dot	1116.8	23429	3.7943E-11	2.4875E-06	0.20403

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