Electronic Supplementary Information (ESI)

Surface modification of carbon dots via peptide Covalent Conjugation

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Experimental section

Materials

2,3-Dichloro-5,6-dicyano-benzoquinone (DDQ), ortho-xylene, L-phenylalanine, DCC, hydroxy benzotriazole (HOBt), Di-tert-butyl decarbonate (BOC anhydride), NaOH pallets DMF, Ethyl Acetate, formic acid, chloroform, methanol, DMSO, MeCN, pet ether, silica gel (100–200 mesh), were purchased from SRL, India.The water used in all experiments was Millipore Milli-Q grade.

UV-vis spectroscopic analysis

A Cary Varian 50 scan UV-visible optical spectrophotometer equipped with 'Cary Win' UV software was used to investigate the optical properties of C-dots in different solvents.

Fluorescence spectroscopy

Fluorescence studies of CDs in a sealed cuvette were carried out in a PerkinElmer LS55 Fluorescence Spectrometer instrument. All experiments were carried out with an excitation slit width of 5 nm and emission slit width of 5 nm.

FEG-TEM study

TEM studies of C-dots in different solvents were carried out on a JEOL 2100 keV Ultra High-Resolution Field Emission Gun (UHR-FEG) TEM instrument with a voltage of 200 keV using carbon coated copper grids.

X-ray photoelectron spectroscopic (XPS) study

XPS analysis of C-dots was carried out by using the X-ray photoelectron spectroscopy (XPS, Omicron, model: 1712-62-11) method. Measurement was done by using an Al-Kα radiation source under 15 kV voltages and 5 mA current.

Fourier transform infrared (FTIR) study

FT-IR spectra were recorded by using the KBr pellet technique in a Nicolet 380 FT-IR spectrometer (Thermo Scientific).

Time-correlated single-photon counting methodology (TCSPC) study

TCSPC measurements were done by Horiba join Yvon IBH instruments having an MCPPMT Hamamatsu R3809 detector

I–V Measurements

For I–V measurements, the DC currents were measured using a Keithley source meter (model 2410). The dark I–V characteristics were measured after keeping the samples in vacuum for 24 h. For photocurrent transient measurement, a xenon light source (model no. 66902; Newport Corp.) with a power of 1 sun was used for the light illumination.

Preparation of GCD

GCD was synthesized from DDQ by the solvothermal approach. At first, the 50 mg of DDQ were taken in a 100 ml beaker and 50 ml of ortho-xylenewas added followed by a few minutes sonication to make a homogeneous dark red solution. Then the solution was transferred into the Teflon-lined autoclave and heated at 180 ^{0}C for 5 hours in an oven. After that the autoclave was settled to cool down and impure GCD were taken out from the auto clave. Then the solvent was evaporated from the reaction mixture using a vacuum oven at 90^{0}C for 3 hours to get dry carbon dot. These GCDs were dispersed into the milli-Q water and centrifuged for 10 minutes in 5000 rpm. Then the supernatant was dried to get green flourishing carbon dot.

Preparation and Purification of the PCCD

At first 1 mg of GCD is dissolved into the 100 ml ethyl acetate solvent in a 250 ml rotary bottle followed by 10-minute sonication to make a clear dark brown solution. Then 5 milli mole (1.6 mg) of freshly prepared di peptide NH_2-Phe(L)-Phe(L)-OMe (FF) is added into the solution along

with few drops of DMF. The solution is again sonicated for few minutes and then 5 milli mole (0.675mg) of o 1-hydroxy benzotriazole (HOBt) and 5 milli mole (1.03mg) of N, N di cyclohexyl carbodiimide (DCC) are added in consecutive order. Then whole reaction mixture was allowed to stir under the magnetic rotter for 2 days. Then the reaction mixture was diluted by ethyl acetate and filtered to separate N, N- dicyclohexyl urea (DCU). After that the filtrate was washed by the brine water (2×30 ml) and the organic part was dried over sodium sulphate and solvent was evaporated. The formation of the red PCCD was confirmed by the TLC in the MeOH/$CHCl_3$ (fig S15). The crude mixture ware then purified by the silica gel column chromatography using MeOH/$CHCl_3$ to get the pure product. The Schematic illustration of the synthesis rout of the PCCD is provided in (Scheme 1).

Synthesis Procedure of BocNH-Phe-COOH

1.65 g (10 mmol) of L-Phenylalanine (Phe) was taken in a 250 ml round bottom flask. 10 ml 1(N) NaOH and 20 ml dioxane was added to it and cooled to 0°C. 2.20 g (10.1mmol) di-tert-butyl dicarbonate (Boc anhydride) was added to the reaction mixture and stirred for 8 hours at room temperature. Then dioxane was removed by reduced pressure. The resulting mixture was acidified with saturated $KHSO_4$ solution and the aqueous layer was extracted with ethyl acetate (3 x 50 mL). The ethyl acetate extract was dried over anhydrous sodium sulfate and evaporated in vacuum to obtain the colorless sticky product

Yield: 9.1 (mmol) ,91%

Synthesis Procedure of BocNH-Phe-Phe-COOMe

2.38 g (9 mmol) Boc NH-Phe-OHwas dissolved in 10ml dry N, N-dimethyl formamide (DMF) and cooled in an ice bath. H_2N-Phe-OMe (9 mmol) was obtained by neutralization with saturated Na_2CO_3 from its hydrochloride salt and subsequent extraction with ethyl acetate. The ethyl acetate solution was then concentrated to 20 ml and added to the DMF solution followed by 1.35 g (10 mmol) of HOBt and 2.06 g (10 mmol) of N, N cyclohexyl carbodiimide (DCC). The reaction mixture was allowed to come at room temperature and stirred for 24 hr. The reaction mixture was diluted with ethyl acetate and filtered to separate N,N-dicyclohexyl urea (DCU). The ethyl acetate layer was washed with brine (2 × 30 ml). The organic layer was dried over anhydrous sodium sulfate and evaporated to obtain the yellowish product. The product was purified through silica gel column chromatography using ethyl acetate: n-hexane (10-90) as eluent to obtain the pure product.

Yield: 7.2 (mmol), 80 %

1H NMR (500 MHz, $CDCl_3$), 25 °C:δ 7.33–7.212 (5H, aromatic Hs, m), 7.020-7.002 (1H, NH, br), 6.318-6.303 (1H, NH, br), 4.973 (1H, αH, br), 4.821-4.806 (1H, αH, br), 3.69 (3H, OCH3), 3.129–3.025 (4H, βCH2, m), 1.430 (9H, Boc, s),HRMS (m/z) Calculated : 427, HRMS (m/z) found: 427.18 [M]+.

Synthesis Procedure of NH_2-Phe-Phe-COOMe (FF)

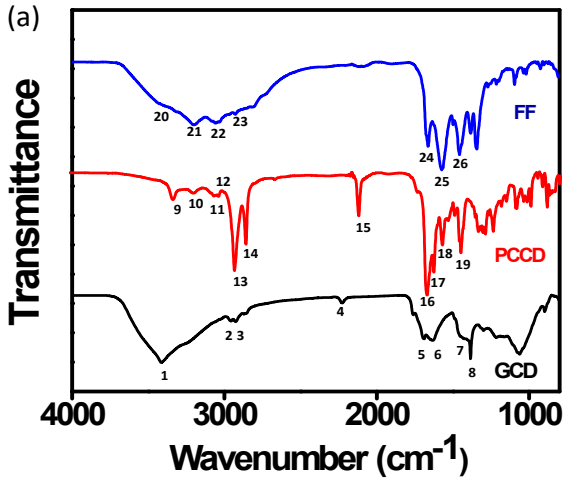
2.9 g (7 mmol) Boc-NH-Phe-Phe-COOMe and 5 ml of 98% formic acid was added and the removal of the Boc group was monitored by TLC. After 7 hours, formic acid was removed under a vacuum. The residue was taken in water (8 ml) and pH of the aqueous solution was then adjusted to 8.0 with 30% aqueous NH_3. The aqueous portion was evaporated in a vacuum. A

white material was obtained, purified using basic alumina in chloroform and methanol (9:1) as eluent.

Yield: 4 (mmol), 57 %

1H NMR (500 MHz, (DMSO-d_6), 25 °C:δ8.4 (1H, NH, br) 7.299-7.191 (5H, aromatic Hs, m), 3.969 (3H, OCH3)3.380 (1H, αH, m),2.825-2.730 (2H, βH, m), 2.249-2.200 (1H, βCH2, m),HRMS (m/z)Calculated 327[M]+HRMS (m/z) found: 327.13 [M]+.

(a)



(b)

Peak Number	Peak Position (cm^{-1})	Assignment
9	3324	N-H stretching (amide)
10	3202	C-N-H bending (overtone)
11	3064	C-N stretching (overtone)
12	2926	C-H stretching
13	2913	CH_2 asymmetric stretching
14	2853	CH_3 asymmetric stretching
15	2124	C≡N stretching
16	1677	C=O stretching
17	1631	C=N stretching
18	1565	CNH bending
19	1447	CH2 bending CH3 asymmetric bending

Fig. S1 (a) the FT-IR spectra of the dipeptide (FF), PCCD and GCD. (b) assignments of peaks of PCCD.

Assignments of GCD

Peak Number	Peak Position (cm^{-1})	Assignment
1	3412	O-H stretching
2	2960	C-H stretching
3	2930	CH_2 Asymmetric stretching
4	2228	C≡N stretching
5	1686	C=O stretching
6	1638	C=C stretching
7	1368	O-H deformation

Assignments of FF

Peak Number	Peak Position (cm^{-1})	Assignment
20	3419	N-H stretching of NH_2
21	3203	N-H stretching of amide
22	3058	C-N stretching
23	2926	CH_2 stretching
24	1662	C=O stretching
25	1571	CNH bending
26	1456	CH_2 bending and CH_3 bending

Table S1. FT-IR assignments of GCD and FF

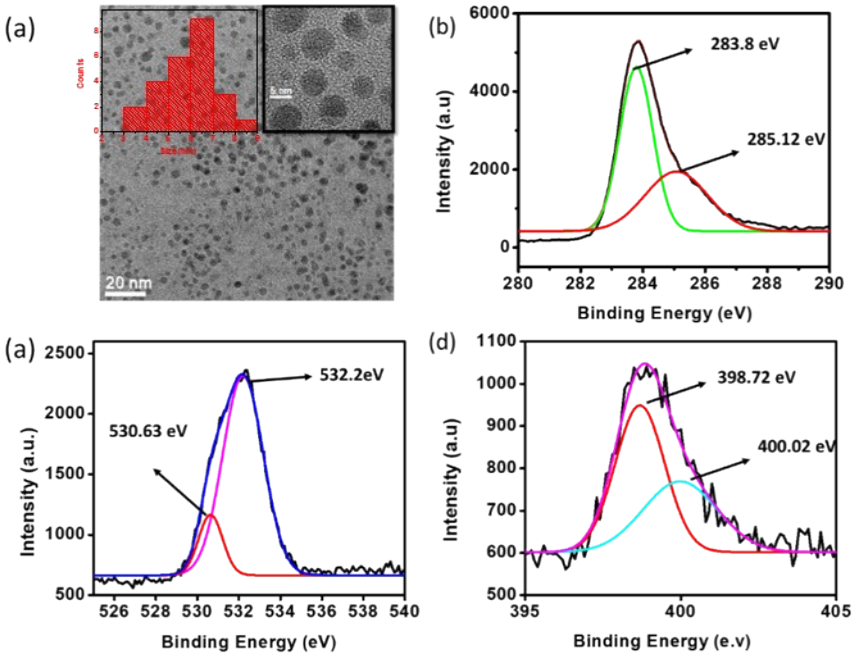


Fig. S2(a) FEG-TEM images of the GCD in water (inset, left- size distribution of the GCD right-close snap of GCD) (b) C1S (b) O1S (c) N1S spectra of the GCD.

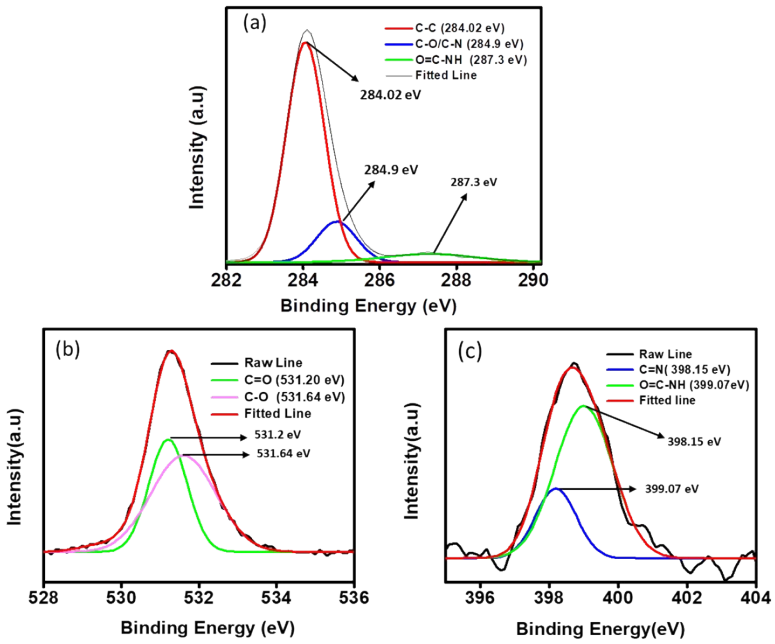


Fig. S3 XPS spectra of the (a) C 1S (b) O 1S (C) N 1S of the PCCD

XPS analysis:

X-ray photoelectron spectroscopic (XPS) analyses of the PCCDs and GCDs were also carried out. The C 1s region of the high-resolution spectrum of the PCCDs (Fig. S3a,) showed C–C (284.02 eV), C–O/C–N (284.9 eV) and O=C–NH (287.3 eV) signals. In the C 1s region of the spectrum of theGCDs, peaks were observed at 283.8 eV and 285.2 eV, which represented the C–C and C–O/C–N bonds, but no peak at 287– 288 eV was found in this spectrum (Fig. S2b,)[1], which confirmed the appearance of the amide bonds in the PCCDs after the surface modification of the GCDs by the FF dipeptide.Furthermore, the O 1s region of the high-resolution spectrum

of PCCDs showed C=O (531.2 eV) and C–O (531.64 eV) signals(Fig. S2b,). The N 1s region showed C=N (398.15 eV) andO=C=NH (399.09 eV) signals,[2] providing further evidencefor the presence of amide bonds in the PCCDs (Fig. S2c,).The XPS spectra of the GCDs are shown in Supporting Information(Fig. S2,).

References:

1. H. Wang, T. Zhang, J. Zhu, Y. Zhai, H. Wang, X. Bai, B. Dong and H. Song, *Nanoscale*, 2017, **9**, 13042–13051.
2. K. Jiang, S. Sun, L. Zhang, Y. Lu, A. Wu, C. Cai and H. Lin, Angew. Chemie- Int. Ed., 2015, 54, 5360–536

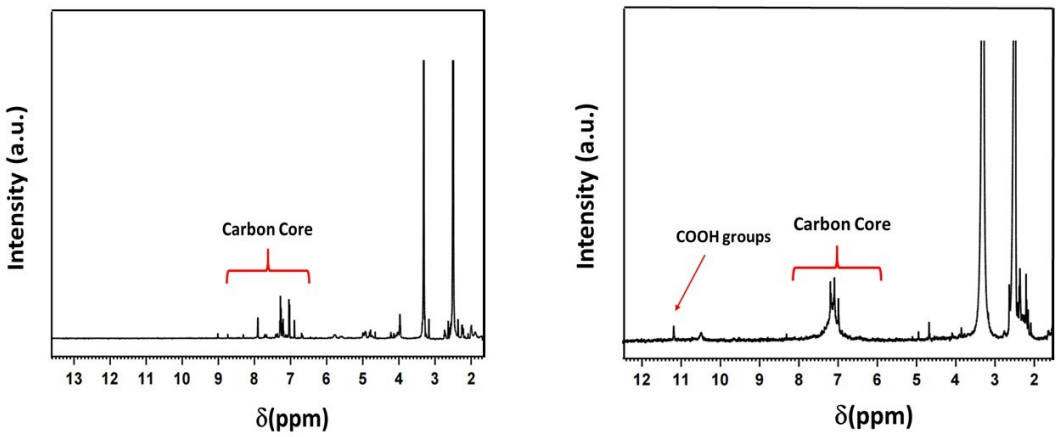


Fig. S4: NMR spectra of the (a) PCCD (b) GCD.

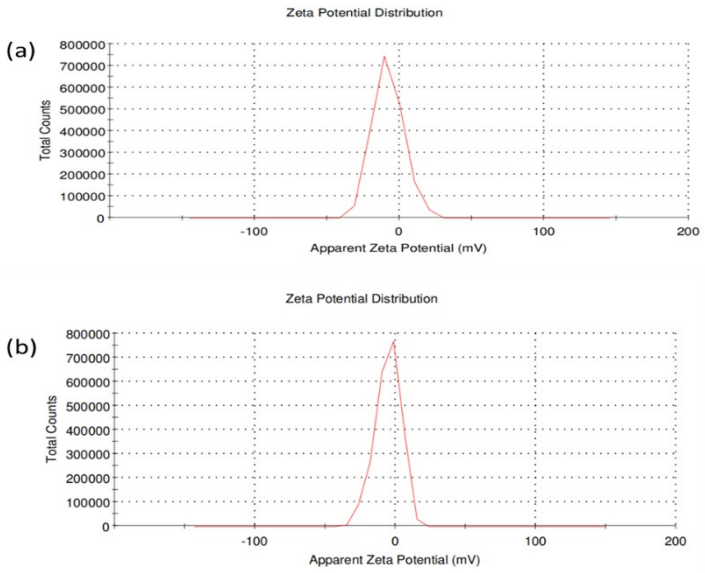


Fig. S5 The DLS analysis of the (a) GCD (b) PCCD

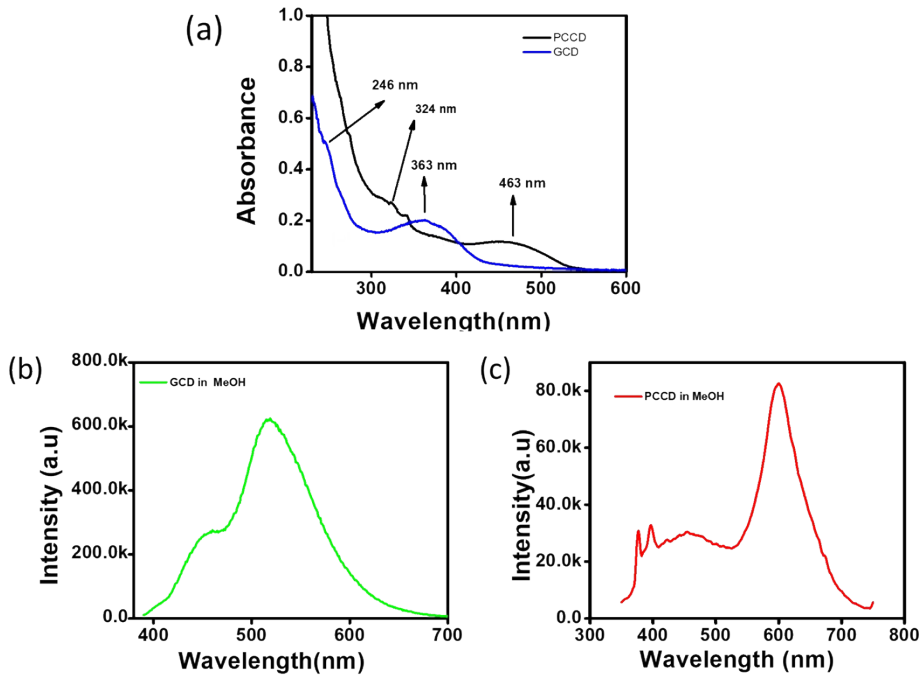


Fig. S6 (a) The UV-visible spectroscopy of GCD and PCCD in methanol. PL (photo luminance) spectra of (b) GCD (c) PCCD in MeOH.

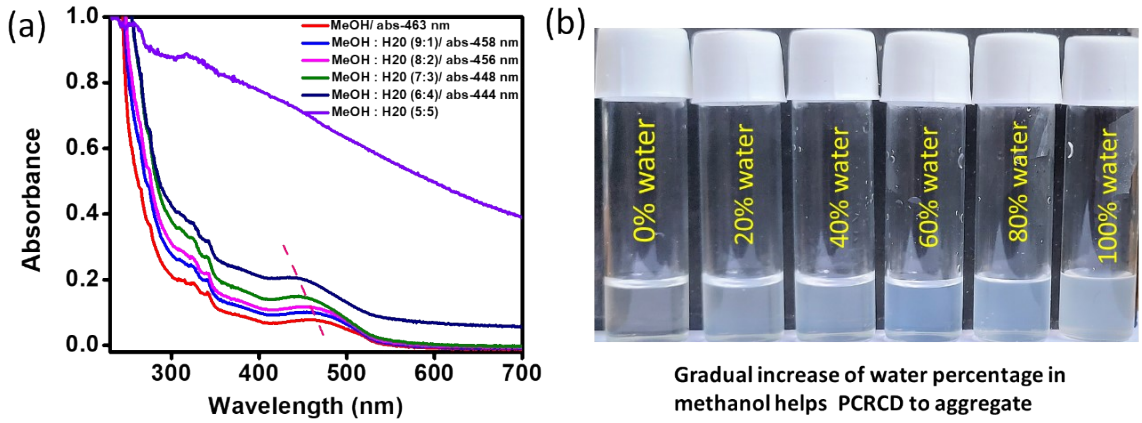


Fig. S7 (a) The blue shift of UV- vis spectra of the PCRDC in the different ratio between MeOH and water, represents H type aggregation (b) Change of color from clear to viscous upon increasing of aggregation solvent (water) under day light.

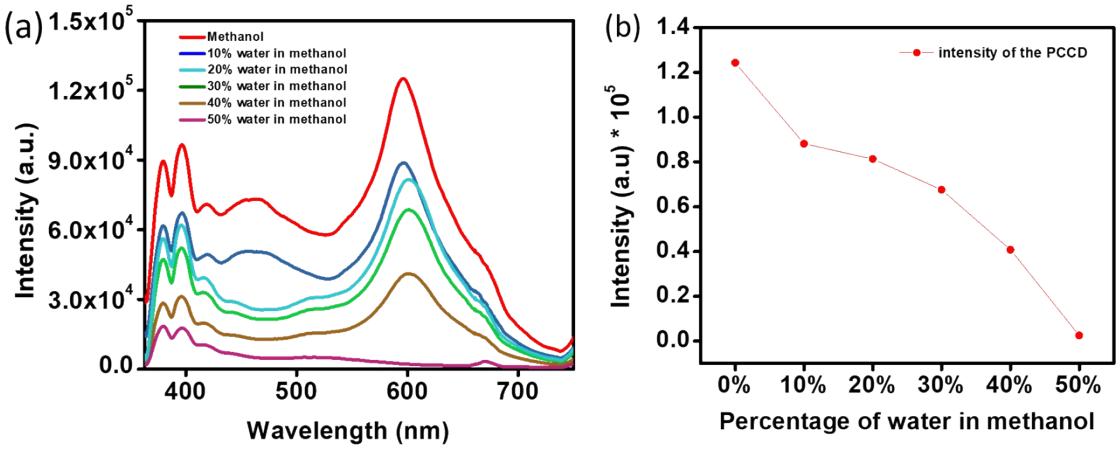
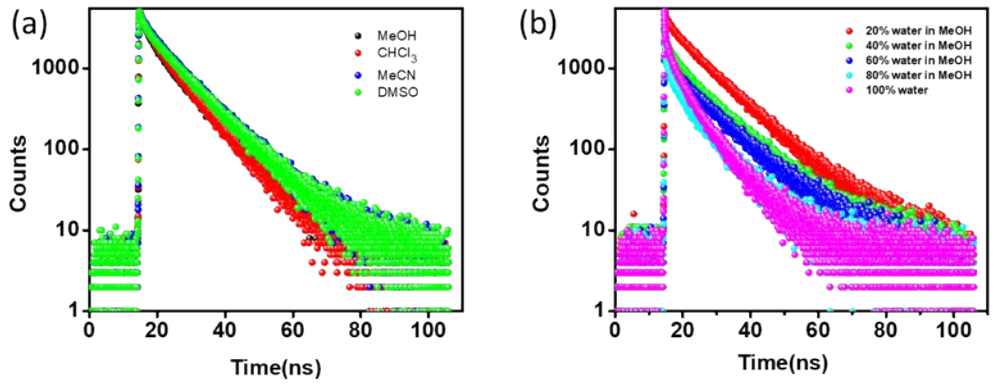


Fig. S8(a) The steady quenching of fluorescence of PCCD with the increasing of water percentage in MeOH. (b) Decay plot of intensity of fluorescence of PCCD with water percentile in MeOH (inset: the aggregation caused quenching photograph.



(c)

% of Water	τ_1(ns)(A_1%)	τ_2(ns)(A_2%)	τ_3(ns)(A_3%)	Average Life Time (ns)
20%	4.03(11.36)	11.59(86.40)	0.36(2.24)	6.07
40%	3.22(13.28)	11.26(74.95)	0.17(11.76)	1.25
60%	3.01(7.9)	11.13(79.72)	0.13(12.37)	0.964
80%	2.49(17.12)	9.39(61.49)	0.13(21.39)	0.566
100%	2.58(26.68)	7.80(56.58)	0.18(16.74)	0.922

Fig. S9 TCSPC curve of the PCCD (a) in different organic solvent (b) in methanol increasing water percentile with the gradual increasing order (c) the change of the average life time of PCCD with the increasing water in MeOH.

solvent	τ_1(ns)(A_1%)	τ_2(ns)(A_2%)	Average Life Time (ns)
MeOH	2.55(25.5)	10.14(74.5)	5.77
$CHCl_3$	2.38(17.72)	8.75(82.28)	5.9
MeCN	2.14(9.64)	9.93(90.36)	7.46
DMSO	1.14(7.89)	9.72(92.11)	6.64

Table S2. The life time PCCD in different organic solvents.

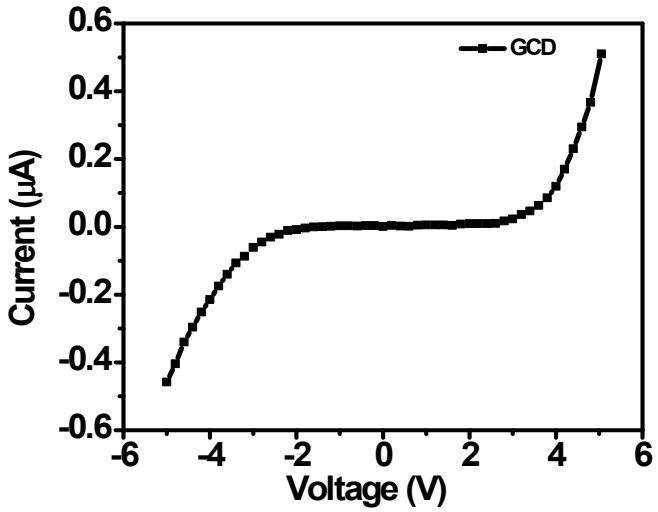


Fig. S10I-V measurements of the GCD

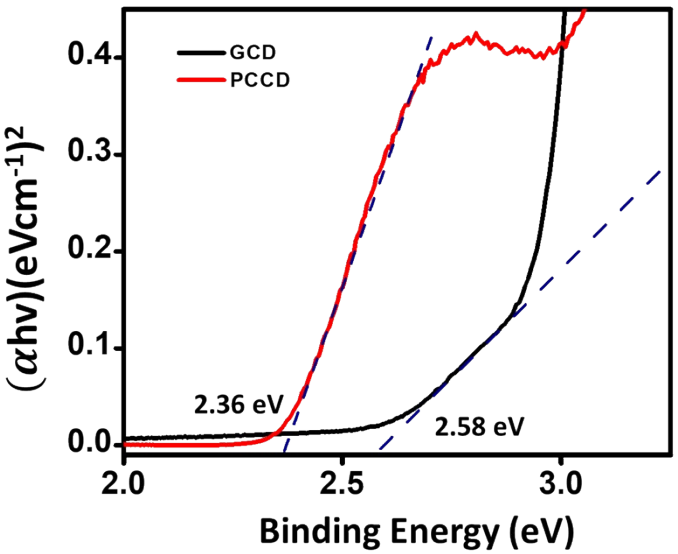


Fig. S11 Tauc's plot of the GCD and the PCCD

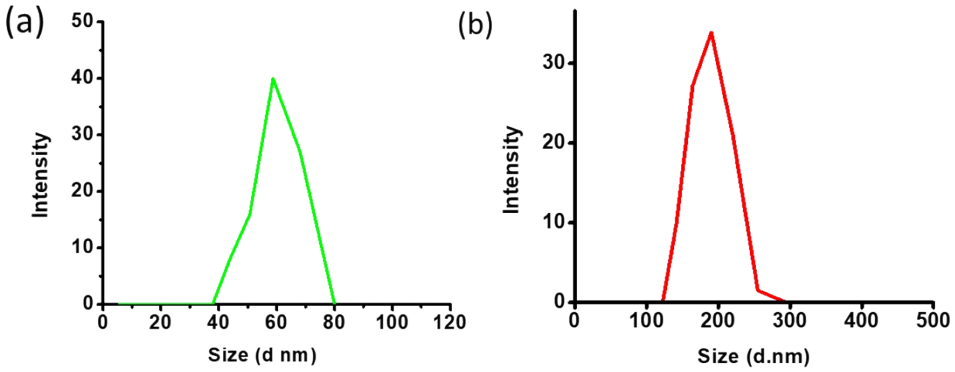


Fig. S12 Hydrodynamic diameters of the GCD and PCCD

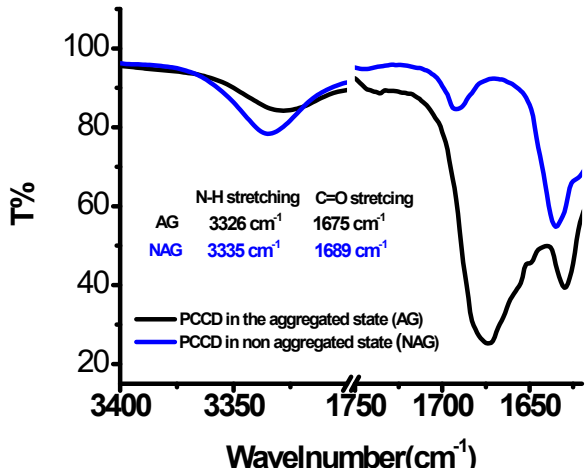
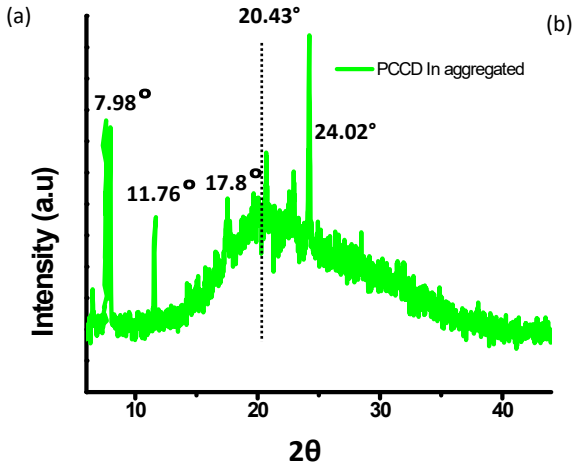


Fig. S13 the change of FTIR after the aggregation state



(b)

Values	Significance
17.8°(d=4.85Å)	β sheets
24.02°(d=3.6Å)	π-π stacking of phenylalanine
20.43°(d=4.22Å)	Characteristics amorphous graphitic core of the carbon dot

Fig S14. (a) XRD spectra of the aggregated PCCD (b) significance of peaks

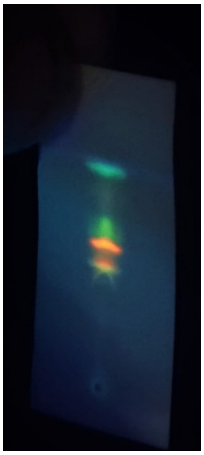


Fig. S15TLC of PCCD in the 3% MeOH/$CHCl_3$.

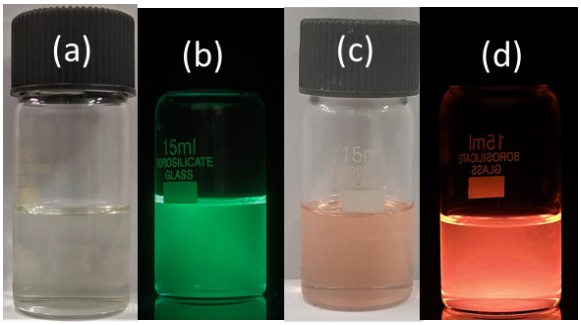
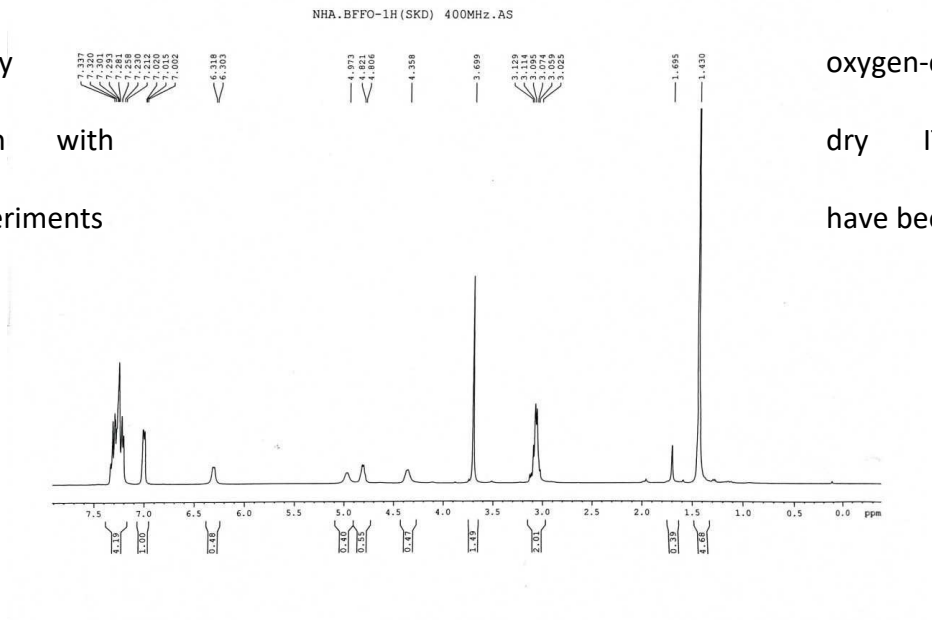


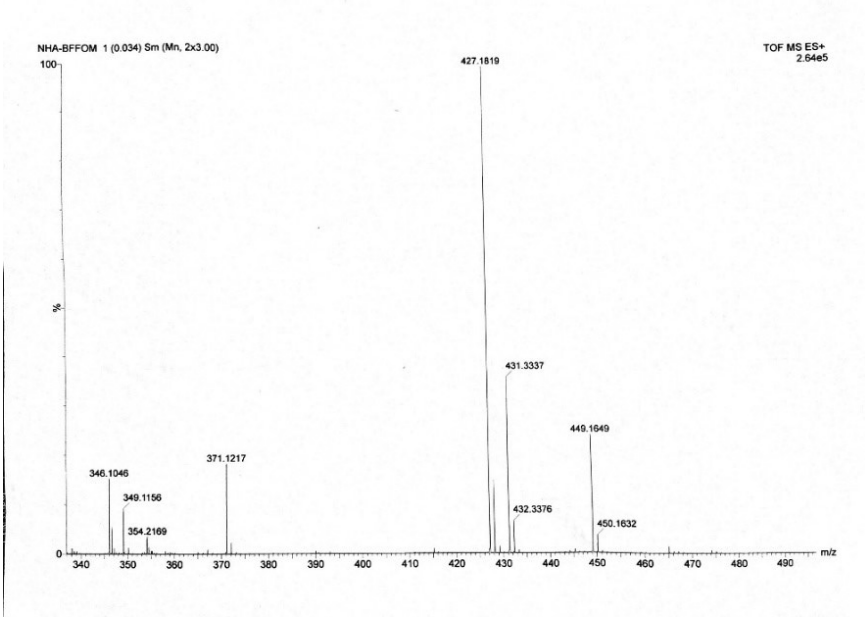
Fig. S16 (a and b) Photographs of GCDs in methanol (a) in daylight and (b)under UV light. (c and d) Photographs of PCCDs in methanol (c) in daylightand (d) under UV light.

Sample preparation for IV and photocurrent measurements:

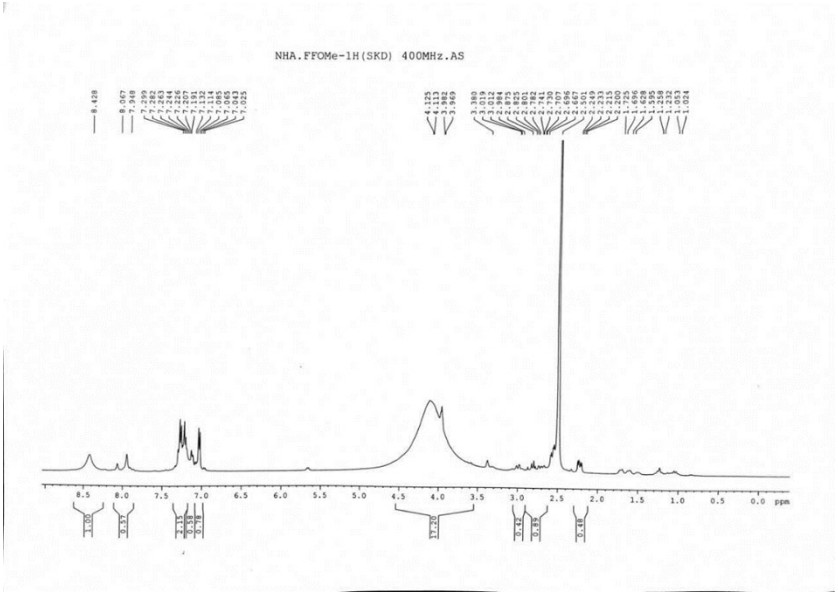
To perform thecurrent–voltage (I–V) and photocurrent studies in both states (aggregated and non-aggregated), we dissolved thePCCDs in methanol and dispersed the resulting solution in awater medium. In the methanol and water, the PCCDsremained in the non-aggregated and aggregated states, respectively,so we drop-cast PCCDs from those solutions onto twoseparate washed and cleaned ITO glasses. After the dropcasting,the two ITO glasses were sandwiched with each otherand kept together using a crocodile clip. Then they were keptunder a vacuum atmosphere for 24 hours to mitigate any effectsof moisture as even small amounts of moisture can inhibitachieving the desirable conductivity by creating defect sites asCD surfaces contain many oxygen-containing groups. Then with dry ITOs the final experiments have been performed.



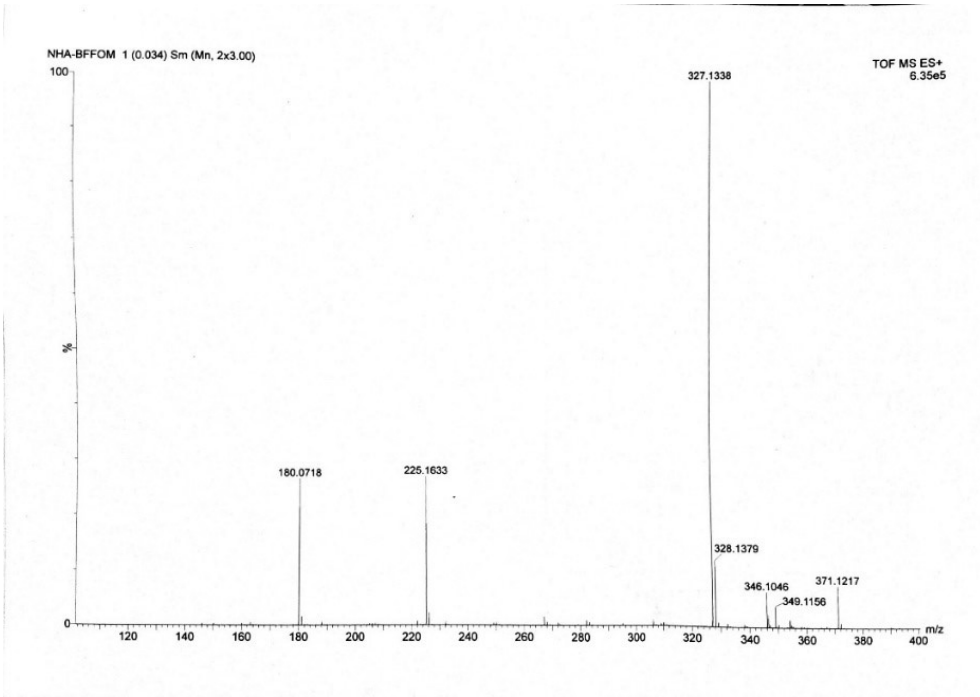
NMR spectrum of Boc-NH-Phe-Phe-OMe



HRMS spectrum of Boc-NH-Phe-Phe-OMe



NMR spectrum of NH_2-Phe-Phe-OMe



HRMS spectrum of NH_2-Phe-Phe-OMe