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Supporting Information

Chiral Carbon Dot, A Smart Choice for Inhibition of Human Serum Albumin Fibrillation

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Materials and methods

Materials

The chemicals used in the experiment were purchased from the respective company with utmost purity and used without purifications, namely L-tartaric acid and D-tartaric acid was purchased from Merck Ltd, Mumbai, India. Quinine sulphate, Human serum albumin (HSA), Thioflavin T (ThT), dimethyl sulfoxide (DMSO), 3-(4,5-dimethylthiazol-2-yl)- 2,5-diphenyltetrazolium bromide (MTT) and uranyl acetate were purchased from Sigma Aldrich. Phosphate-buffered saline (PBS) was purchased from Gibco Invitrogen, India. Milli Q water was used throughout the experiments.

Synthesis and characterization of LC-Dots

The L-TA-C-Dots were synthesized from L-tartaric acid through pyrolysis without using any passivating agent. The carboxylic acid groups, present in tartaric acid, initiate the dehydration and carbonization process. [50] In a typical synthetic procedure, 10 mL aqueous solution of L-tartaric acid (1 g) was heated in a microwave oven at 800 W for 2 min. The colorless liquid solution changed to brown foamy solid, indicating the formation of C-Dots. Finally, the carbogenic solid was dialyzed for 24 h and carbon dots (named as L-TA-C-Dots) were collected from the supernatant and concentrated through solvent evaporation. The D-TA-C-Dots have been synthesized from D-tartaric acid under the same reaction condition, as discussed above.

The crystalline phase of synthesized L- and D-TA-C-Dots was investigated through X-ray diffraction (XRD) using Cu-K_α radiation over 2θ range of 20°–80° at a scan rate of 3 degrees/min with an applied voltage 40 kV by using Bruker AXS Diffractometer D8 powder XRD (Germany). The presence of functional groups in L- and D-TA-C-Dots surface was confirmed by Fourier transformed infrared (FTIR) spectroscopy using Perkin-Elmer Spectrum RX-II (Model no. 73713, USA) within the scan range of 4000–400 cm^{-1}. The chemical composition of C-Dots was determined by X-ray Photoelectron Spectroscopy (XPS) using Al K_α radiation (hν = 1486.7 eV) by PHI-5000 VersaProbe II Scanning Microprobe (United States). For XPS measurement, the binding energy scale had been calibrated using the standard value of C 1s at 284.5 eV. The particle size and morphology of synthesized L-TA-C-Dots was visualized by transmission electron microscopy (TEM) using TECNAI G^2 20S-TWIN (USA) at an acceleration voltage of 200 kV. The size distribution histogram of L-TA-C-Dots was obtained from TEM image by using ImageJ software (version 1.33; National Institutes of Health, USA). The surface charge potential of the resultant sample at different pH was measured by using Malvern Nano ZS instrument (U.K.). The absorption spectrum of L- and D-TA-C-Dots was obtained by using UV-VIS spectrophotometer (SHIMADZU UV-2450, Japan) and circular dichroism (CD) spectrum was recorded by J-815 Jasco CD spectrometer (United States) at 25 °C. The photoluminescence studies were carried out by using HITACHI F-7000 Fluorescence spectrophotometer (Tokyo, Japan). The Raman spectrum of L- and D-TA-C-Dots was recorded in HORIBA Jobin Yvon T64000 Raman spectrometer (Japan) by using an Ar-Kr laser source of wavelength λ = 514 nm, equipped with a microscope (model BX41 Olympus, Japan).

In vitro cellular uptake studies

MDA-MB-231 cells (approximately 1.5×10^4 per sample) were seeded on each cover slips placed in 60 mm Petri dish and allowed to develop for overnight. Then, the cells were treated with LC-Dots in growth medium and allowed to incubate for varying time intervals of 1, 3 and 6 h. Then, the cells were washed with PBS for two times and fixed with 3.7% paraformaldehyde followed by dehydration steps which were performed in 50, 70, 95 and 100% ethanol for 5 min. Subsequently, the cells were mounted on cover slips using a permanent mounting medium DPX for confocal laser microscopic imaging (CLSM, Olympus FluoView FV1000) [51,52]. Then sample was focused and observed on CLSM by using the imaging software, FV10-ASW2.0 Viewer.

Cytotoxicity assay

The cytotoxicity of LC-Dots in MDA-MB-231 breast cancer cells was evaluated using MTT assay. Cells (approximately, 5×10^3 per well) were seeded in 96-well plate and incubated for 24 h at 37 °C. Then the L-TA-C-Dots at varying concentrations (0-250 μg/ml) were treated to the plate and incubated for 48 h. After incubation the suspended carriers, present in medium, were removed and replaced with the 80 μL MTT (1 mg/ml) solutions and again allowed to incubate at 37 °C for 4 h. After incubation, the formazan crystals formed in each well were dissolved in 100 μL DMSO. Then, the absorbance of all the samples was measured at 570 nm using a microplate reader and the cell viability was calculated following the standard procedure.[53,54] The wells with culture medium, L-TA-C-Dots and MTT reagent, without cells were used as blanks.

Preparation of sample for fibrillation study

The HSA stock solution was freshly prepared by dissolving HSA in milli-Q water and its concentration was determined by measuring the absorbance at 280 nm by UV-Visible spectrophotometer (Shimadzu, UV-2450) using molar extinction coefficient value of 35,219 M^{-1} cm^{-1}.[55] HSA fibrils were prepared by incubating HSA solution (50 μM) in 60% (v/v) ethanol at 37 °C for 24 h at pH 7.0 (20 mM phosphate buffer) followed by one day incubation at room temperature. The stock solution of LC-Dots was fixed to 1 mg/mL in milli-Q water. The effect of L- and D-TA-C-Dots towards HSA amyloid fibril formation was performed by varying the amount of LC-Dots solution (50 μL, 100 μL, 150 μL and 200 μL) in HSA (50 μM) before exposing the solution to the fibrillation condition, as discussed above. The samples were diluted by 20 mM phosphate buffer of pH 7.0 for further analysis.

ThT fluorescence

Thioflavin-T (ThT) fluorescence intensity was measured to observe the amyloid aggregation of HSA in absence and presence of various amount of L- and D-TA-C-Dots solution in different aliquots (0-200 μL, 1

mg/mL). Typically, protein and dye concentration were kept at 2 μM and 10 μM respectively. The aliquots were incubated for 5 min at room temperature prior to observing the emission spectra. As the L- and D-TA-C-Dots solutions were itself fluorescent, all the spectra were corrected with respect to the corresponding blank spectra. The fluorescence data were collected by using Horiba JobinYvon Fluoromax-4 spectrofluorimeter at room temperature in a quartz cuvette of an optical path length of 1 cm and scanned using an excitation wavelength of 450 nm. The emission spectra were recorded in the range of 470-600 nm. All the emission spectra were recorded at 5 nm slit width with integration time 0.3 s and scan speed of 100 nm/min. The analysis was performed three times to get precise result.

Circular dichroism (CD) spectroscopy

The far-UV CD spectra of HSA amyloid solutions with and without L- and D-TA-C-Dots of concentration 10 μg/ml were recorded using a J-815 Jasco CD spectrometer at 25 °C in a quartz cuvette with 0.1 cm optical path length. The sample solutions were diluted by 20 mM phosphate buffer (pH 7) to attain final protein concentration of 5 μM. The spectrum was recorded between 190 and 240 nm wavelength at a scan rate of 50 nm/min and corrected with 20 mM phosphate buffer baseline subtraction. As the L- and D-TA-C-Dots solutions were CD-active, each spectrum was subtracted from corresponding blank spectra. The experiments have been performed three times and the error bars in the graph represent the standard error of the mean (±S.E.). In order to get the details of change in the content of percentage of α-helix and β-sheet in the protein, SELCON software was used form online DICHROWEB server.

ThT Fluorescence microscopy

For fluorescence microscopic images aliquots of HSA (10 μL) in absence and presence of L-TA-C-Dots with 5 μL of 1 mM ThT were placed in between glass slide and cover slip, incubated for overnight at room temperature. Samples were visualized using a Leica DM 2500M microscope equipped with a fluorescence attachment and a Leica DFC 310 FX camera. Filter cube no 2 (Leica I3 11513878, BZ: 01) was used for ThT excitation and emission. All images were collected at 10X/0.25 (N PLAN EPI).

TEM study

The solutions of HSA in absence and presence of 100 μL L-TA-C-Dots were diluted to 10 μM followed by the addition of 1% (w/v) aqueous solution of uranyl acetate for negative staining. Then the samples were applied to carbon coated holey copper grid and dried in air for overnight. The TEM images of the samples were examined with a TECNAI G^2 20S-TWIN transmission electron microscope at an accelerating voltage of 120 kV.

AFM study

The surface topography of HSA incubated with and without L-TA-C-Dots solution was visualized using atomic force microscopy (AFM) (Agilent Technologies USA: AFM 5500). In brief, fibrillar solutions

containing HSA and HSA with 100 μL L-TA-C-Dots solutions were diluted 10 times with 20 mM phosphate buffer (pH 7) and 5 μL of each solution was separately deposited on approximately 1 mm thick glass cover slips (washed with deionized water and ethanol, dried in air) and left 10 min for adsorption. Non-adsorbed portions were washed with water and dried in air. The AFM images were recorded in air using tapping mode method in an area of 1μm × 1μm. The scan rate was in between 1.0 and 1.5 Hz and histogram analysis was achieved using the Pico image basic software.

Interaction study of L-TA-C-Dots with HSA

A stock solution of 50 μM of HSA and 1 mg/mL of L-TA-C-Dots were prepared separately in phosphate buffer (10 μM, pH 7). Typically, protein concentration was kept at 4 μM and L-TA-C-Dots concentration was varied from 0 to 1.66 μM by diluting the samples with phosphate buffer (10 μM, pH 7). The aliquots were incubated for 4 h at three different temperatures (4, 20 and 30 °C) prior to examine the fluorescence spectra within scanning range of 310–450 nm upon excitation at 295 nm wavelength. As the L-TA-C-Dots are itself fluorescent, all the spectra were corrected with respect to the corresponding blank spectra. The fluorescence data were collected by using Horiba JobinYvon Fluoromax-4 spectrofluorimeter. Samples were positioned in a quartz cuvette of an optical path length of 1 cm. All emission spectra were recorded at 5 nm slit width with integration time 0.3 s and scan speed of 100 nm/min.

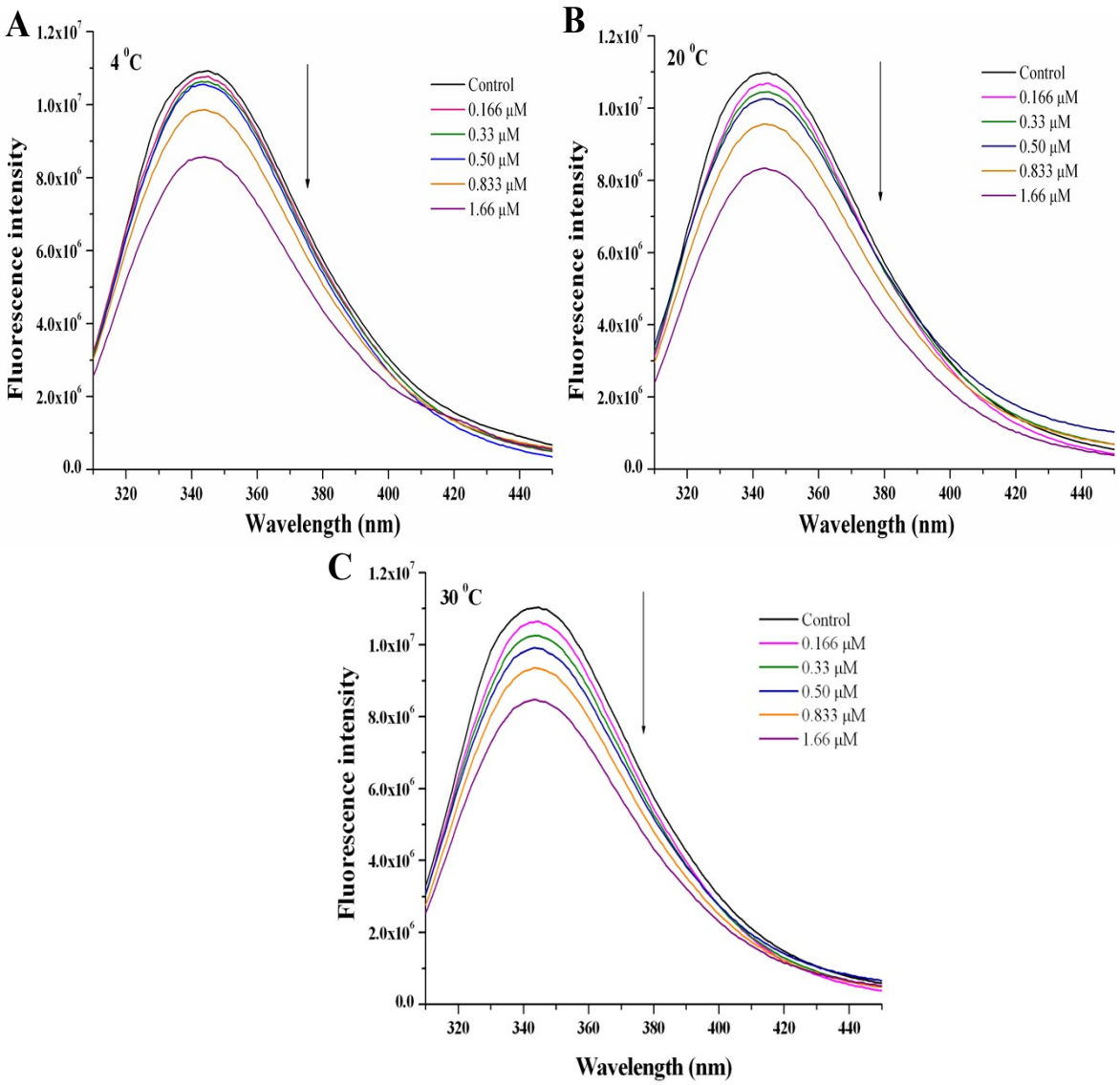

Fig. S1: Interaction study in between HSA molecules and L-TA-C-Dots at three different temperatures (A at 4 °C, B at 20 °C and C at 30 °C).

Characterization of CDs derived from D-Tartaric acid (DC-Dots):

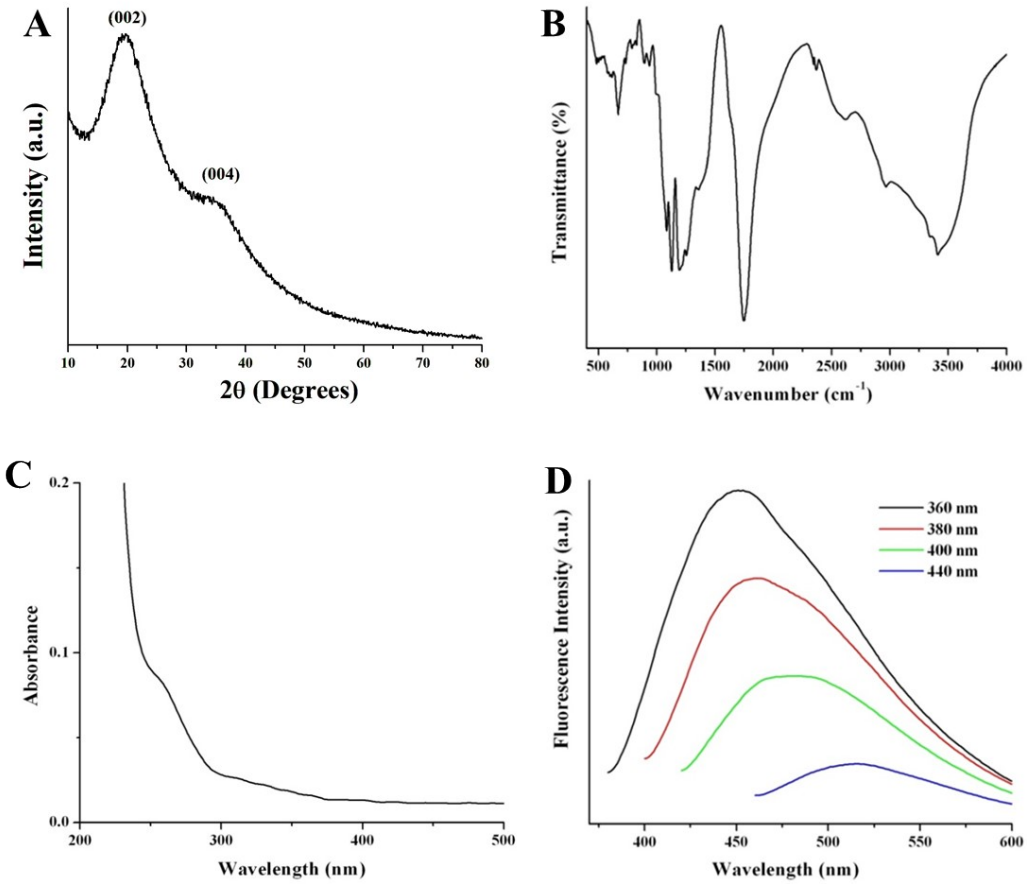

Fig. S2: (A) XRD pattern, (B) FTIR spectra, (C) UV-Visible spectra, and (D) excitation wavelength dependent fluorescence spectra of D-TA-C-Dots.

The FTIR spectrum of D-TA-C-Dots depicted in fig S2B The peaks at around 1085, 1132 and 1750 cm^{-1} are assigned to C–O–C, C–OH and C=O stretching mode of vibration of carboxylic acids confirming the presence of carboxylic carbonyl group assurface functional group. Similar to the LC-Dots, the UV-Visible absorption spectrum of DC-Dots (Figure S2C) depicts two broad shoulders at around 310 nm and 260 nm due to n → π* transition of C=O bond and π → π* transition of C=C bond respectively. The fluorescence emission spectra (Figure S2D) predominant blue emissive nature of the carbon dots.

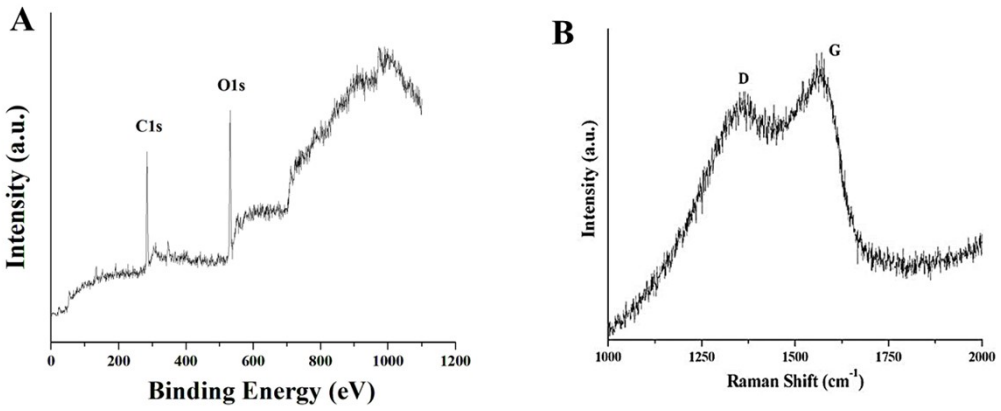

Fig. S3: (A) XPS survey spectrum, and (B) Raman spectrum of D-TA-C-Dots

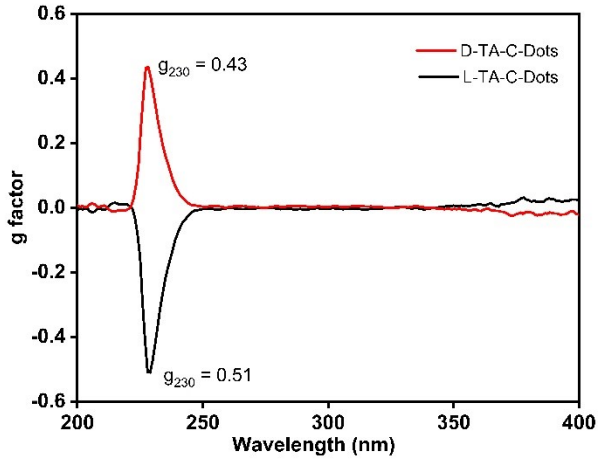

Fig. S4: Anisotropy spectra of L-TA-C-Dots (Black) and D-TA-C-Dots (Red).