Supporting Information

Carbon Quantum Dots as a Macromolecular Crowder

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Materials and Methods:

S1. Hydrophilic Carbon Quantum dots:

**Preparation:** Hydrophilic carbon quantum dots were prepared as described by Bhunia et al.\(^1\) Briefly, to a solution of 14 g of sucrose in 100 ml of water, NaOH was added until the pH reached 12. The reaction mixture was then heated to 90 °C and stirred for 30 min. Following this, the reaction mixture was cooled to room temperature and neutralized by adding HCl. The final product was obtained by dialysis against pure water followed by lyophilization.

S2. Hydrophobic Carbon Quantum dots:

**Preparation:** Hydrophobic carbon Q-dots were prepared by mixing 600 mg of sucrose and 2 g of Octadecyl-amine in 60 ml of octadecene. The reaction mixture was heated to 80°C and stirred for 20 min. Following this, the mixture was cooled to room temperature and mixed with 20 ml of Chloroform and shaken vigorously. The mixture was then washed with 10 ml of water three times and the Octadecyl amine was separated as an insoluble white solid. Chloroform was removed under reduced pressure, resulting in a brown solid. Thus obtained hydrophobic quantum dots were purified with column chromatography, using silicagel as a stationary phase and the Ethyl acetate / Pet-ether as the mobile phase.

S3. Protein Preparation

S3.1. A. Ubiquitin: Ubiquitin containing plasmid, PGLUB was transformed into E. coli BL21 cells. Cells were then grown at 37°C in M9 minimal medium consisting of 1 g/L of \(^{15}\)NH4Cl and 4 g/L of \(^{13}\)C-Glucose. Protein expression was induced at midlog phase (O.D600 ~0.6) by addition of 1.0 mM isopropyl-β-D-1-thiogalactopyranoside (IPTG). Cells were harvested by centrifugation and solubilized then in acetate buffer (5 mM EDTA, 50 mM Na acetate, pH 5). Following sonication, the supernatant containing the protein was loaded on to a pre-equilibrated ion exchange column (SP Sepharose fast flow from GE) and the protein eluted with a salt gradient of 0–0.6 M NaCl. For NMR studies, a sample containing ~2.0 mM of protein in 50 mM Phosphate buffer (95%H2O/5% 2H2O; pH 6.0) was prepared. All NMR experiments on Ubiquitin were performed at 25°C on Bruker 800 MHz spectrometer equipped with a cryogenic probe.

S3.1.B. In Cell Ubiquitin Sample preparation: After 4 hours induction with IPTG, a 50 ml sample of \(^{15}\)N-labeled ubiquitin culture was collected, the cells were centrifuged and washed twice with 50 mM phosphate buffer (pH 6.0) before resuspending very gently in the same buffer with the tip. This sample was then used to assess the HSQC spectra of ubiquitin inside cell with the addition of 5 % D\(_2\)O.\(^2\) After centrifugation, all the supernatant was tested with HSQC spectra to check the leakage of protein and it was not there.
S3.2. L-hIGFBP-2: The gene corresponding to the central flexible (linker) domain of IGFBP-2 [residues 97–190 from the full length protein] was PCR-amplified from the plasmid corresponding to the full length protein and cloned into a pET3a vector with a GST-fusion tag at the N-terminus of the protein resulting in 105 residues (~12 kDa). The constructs were then transformed into BL21 (DE3) cells. The cells were grown at 37°C in M9 minimal medium consisting of 1 g/L of $^{15}$NH$_4$Cl and 4 g/L of $^{13}$C-Glucose and then induced with 0.5 mM IPTG. The protein was purified by binding the cell lysate to GST-beads followed by cleavage and separation of the GST-bound protein by HRV-3C protease using a standard protocol of GST-tag protein purification. The final NMR sample contained ~1.0 mM protein in 50 mM Na-phosphate buffer (pH 6.0) and 50 mM NaCl in 95% H$_2$O/5% D$_2$O.

All NMR experiments on MD-IBP2 were performed at 20°C on Bruker 800 MHz spectrometers equipped with a cryogenic probe.

S3.3. Peptide: The 17 residue peptide (MAGAAAAGAVVGGGY), comprising residues 112-128 of Prion Protein (PrP) was purchased from Custom peptide Synthesis, USV limited, Mumbai, India.

Photographs of (A) Hydrophilic and (B) Hydrophobic CQD; from left to right 100, 50, 25 and 10 mg/ml hydrophilic CQD in water. B shows Hydrophobic CQD in MeOH with 1, 0.1 and 0.01 mg/ml conc. in MeOH from left to the right.
S4. Characterization Techniques:

**Nuclear Magnetic Resonance (NMR):** All the NMR experiments were carried out on Bruker 800.13 MHz spectrometer with Cryogenic probe. For all the HSQCs, we used the standard pulse programme ‘hsqetc3gpsi’ and in each HSQC, the experiment time was 10 min. 7 sec.

**Dynamic Light Scattering (DLS):** DLS measurements were performed using Nanozetasizer machine (Brookhaven Zeta PALS).

**Transmission Electron Microscopy (TEM):** The TEM images were obtained using Technai T-20 machine with an operating voltage 200 kV. A dilute solution was drop cast on carbon coated copper grid and dried overnight at room temperature in a desiccator.

**Scanning Electron microscopy (SEM):** A solution of CQD (1 mg ml-1) 3 µl aliquot was placed on a silicon wafer and allowed to evaporate the solvent under ambient conditions. The resulting residue was desiccated under vacuum for 12 h. All the samples were coated with gold (38 sec) and viewed under an FE-SEM with acceleration voltage of 5 kV.

**X-Ray Photo Electron spectroscopy (XPS):** XPS chemical analysis was performed using Axis Ultra DLD (Kratos Analytical Ltd., Manchester). High resolution XPS spectra’s were deconvoluted using Origin software.

**Fourier Transformed Infrared Spectroscopy (FT-IR):** The FT-IR spectra were recorded using FT-IR spectrometer (Perkin-Elmer Spectrum one model) at room temperature using KBr pellet method. Dried powder samples were used for making the KBr pellet.

**Viscosity measurement:** Viscosity measurement were done using MCR 301 rheometer. The shear rate was varied smoothly from 0 to 500 S⁻¹. The temperature was kept constant at 298 K.

**Fluorescence Microscope:** Fluorescence Microscope measurement was done in Olympus instrument (Model-IX 71) using a 19V 100W Mercury lamp housing source and DP 71 camera with the ND25 Neutral density filter and U-DICT analyzer. The software is Image-pro Express 6.0 and the software for hot stage temp controller is Linksys 32 being used for this purpose.
Figure S1: SEM and Fluorescence Microscope Image:

Figure S1: SEM image of hydrophilic CQD showing the self-aggregation in A; fluorescence microscope images of hydrophilic CQD in water (A1), hydrophobic CQD in methanol (B1) and hydrophobic CQD with 0.1mM peptide in methanol (B2) has been shown respectively. The scale bar of 100 µm is given on the right-bottom side on each of the A1, B1 and B2.
Figure S2: XPS characterization of Hydrophilic and Hydrophobic Carbon Quantum Dot

The high-resolution C1s spectra was deconvoluted into four peaks. The peaks at 284.3 eV, 285.7 eV, 287.1 and 288.3 eV are assigned to carbon atoms involved in C-C (sp2 & sp3) bonds, C-O bonds, C-O-C bonds and C=O bonds, respectively.

The high-resolution C1s spectra was deconvoluted into three peaks. The peaks at 284.4 eV, 286.0 eV and 288.3 eV are assigned to carbon atoms involved in C-C (sp2 & sp3) bonds, C-O bonds and C=O bonds, respectively.
Figure S3: FTIR spectra of (a) Hydrophilic and (b) Hydrophobic CQD shows peak at ~3300 cm\(^{-1}\), ~1650 cm\(^{-1}\) and 1470 cm\(^{-1}\) which corresponds to COOH/NH\(_2/\)OH, C=O, C-O (ether) stretching frequencies, respectively.
Figure S4a: Dynamic Light Scattering (DLS) Study to show the self-aggregation

Figure S4a: DLS spectra of Hydrophilic CQD with 5 mg/ml (A) and 50 mg/ml (B) conc. in water, Hydrophobic CQD with diluted 10 µg/ml (C) and 100 µg/ml (D) conc. in methanol, Ficoll with 50 mg/ml (E) and 400 mg/ml (F) conc. in water shows the increase in particle size as a result of self-aggregation with the conc. increment in each cases. 

\[\text{Mean Diameter} = 635 \text{ nm} \]
\[\text{Mean Diameter} = 4725 \text{ nm} \]
\[\text{Mean Diameter} = 369 \text{ nm} \]
\[\text{Mean Diameter} = 3599 \text{ nm} \]
\[\text{Mean Diameter} = 795 \text{ nm} \]
\[\text{Mean Diameter} = 9411 \text{ nm} \]
Figure S4b: Dynamic Light Scattering (DLS) Study to illustrate that self-aggregation property of Hydrophobic CQD is unaffected in presence of 1 mM Peptide (PrP) in Methanol
Figure S5: Viscosity vs. shear stress plot for different conc. of Ficoll in (A) and for different conc. of CQD in (B). There is not much change in the viscosity for hydrophobic CQD in MeOH, and hence not shown. The same concentrations of Ficoll and CQD for crowding study of both the protein (Ubiquitin and MD) has been chosen for viscosity measurement as shown above.
Figure S6a: Comparison of NMR spectra of ubiquitin observed in-cell with that in presence of hydrophilic CQD: An overlay of NMR spectra of Ubiquitin in intact cells (blue) with ubiquitin in 25 mg/ml hydrophilic CQD (pink). Both spectra were recorded at 298K on a Bruker 800 MHz NMR spectrometer.
Figure S6b: Comparison of NMR spectra of L-hIGFBP2 observed in-cell with that in presence of hydrophilic CQD: An overlay of NMR spectra of L-hIGFBP2 in intact cells (blue) with L-hIGFBP2 in 25 mg/ml hydrophilic CQD (red). Few extra blue peaks are appearing from the other proteins present inside the cell under in-cell condition of L-hIGFBP2. Both the spectra were recorded at 293 K on a Bruker 800 MHz NMR spectrometer.
Figure S7: Comparison of molecular crowding by CQD with Ficoll using NMR spectroscopy

Figure S7: (A) An overlay of 2D $^{15}$N-$^1$H HSQC spectra of ubiquitin in presence of 100 mg/ml Ficoll with free ubiquitin and (B) in presence of 25 mg/ml CQD overlaid. (C) and (D): The similar comparison for L-hIGFBP2.
Figure S8: CD spectra
Figure S9a: Dynamic Light Scattering (DLS) Study to illustrate that self-aggregation of Hydrophilic CQD in methanol remains unaffected in (A) presence of 1 mM Peptide (PrP) and (B) in absence of the peptide similar to Hydrophobic CQD

Figure S9b: Fluorescence Microscope Image of Hydrophilic CQD with peptide in Methanol

Figure S9b: Fluorescence microscope images of hydrophilic CQD in methanol (A), only peptide in methanol (B) and hydrophilic CQD with the peptide in methanol (C) has been shown respectively. The scale bar of 100 µm is given on the right-bottom side on each of the A, B and C.
References:

