Materials and methods

Chemicals

Hen egg-white lysozyme (~70 000) (HEWL), thioflavin T, phosphate buffered saline, glycine, Nile red, fullerene C_{60} (assay 99.5%), single-walled carbon nanotubes (≥80% carbon as SWNT, diameter 0.7–1.4 nm) and diamond nanoparticles suspension (~1% w/w) were purchased from Sigma-Aldrich LTD (Prague, Czech Republic). Dichloromethane, anhydrous citric acid, urea, hydrochloric acid (35%), and ethanol (96%) were purchased from Lach-Ner (Neratovice, Czech Republic).

Preparation of carbon quantum dots (CDs)

Citric acid (6.00 g, 15.6 mmol) and urea (6.00 g, 99.6 mmol) were dissolved in 20 ml water. The solution was heated in microwave oven (600 W) for 4 min. During the reaction, the solution changed from colorless over yellow and then black-brown clustered solid was formed. This solid was heated under vacuum at 60°C for 2 h to remove volatile residuals. This solid was dissolved in 70 ml water and the resulting solution of the CDs was purified in a centrifuge at 830g for 30 min to remove agglomerated particles. Finally, the 2 wt. % solution of CDs was purified on the size-exclusion chromatographic column PD-10® (commercial column pre- packed with Sephadex G-25®). The resulting solution was lyophilized and stored in the fridge. The sample was characterized by elemental analysis, ζ- potential, Small-angle X-ray scattering, UV–Vis absorption, X-ray photoelectron spectroscopy, Infrared spectroscopy (see below). Then the sample was used for experiments with HEWL.

Characterization of carbon nanospecies

Composition: Elemental analyses were done with the Elmer 2400 Series II CHNS/O instrument (Perkin Elmer Inc.).

Absorption and Photoluminescence spectra: UV–Vis absorption spescopy was performed on a Evolution 220 UV–Vis Spectrophotometer (ThermoFisher Scientific). Photoluminescence (PL) spectra were measured by FP-6200 Spectrofluorometer (JASCO).

Measuring ζ- potential: The aqueous solution of CDs at concentration 0.5 wt. % was measured with a Zetasizer Nano ZS (Malvern Instruments) to obtain ζ- potential.

X-ray photoelectron spectroscopy (XPS): Measurements were carried out with a K-Alpha$^+$ spectrometer (ThermoFisher Scientific). The samples were analyzed using a micro-focused, monochromated Al Kα X-ray source (400 μm spot size) at an angle of incidence of 30° (measured from the surface) and an emission angle normal to the surface. Kinetic energy of the electrons was measured using a 180° hemispherical energy analyzer operated in the constant analyzer energy mode (CAE) at 200 eV and 50 eV pass energy for the survey and high resolution spectra respectively. Data acquisition and processing were performed using Thermo Advantage software. The XPS spectra were fitted with Voigt profiles obtained by convolving Lorentzian and Gaussian functions. The analyzer transmission function, Scofield sensitivity factors, and effective attenuation lengths (EALs) for photoelectrons were applied

for quantification. EALs were calculated using the standard TPP-2M formalism. All spectra were referenced to the C1s peak of hydrocarbons at 285.0 eV. The BE scale was controlled by the well-known position of the photoelectron C–C and C–H, C–O and C(=O)–O C1s peaks of polyethylene terephthalate and Cu 2p, Ag 3d, and Au 4f peaks of metallic Cu, Ag and Au, respectively. The BE uncertainty of the reported measurements and analysis is in the range of ± 0.1 eV.

Nuclear magnetic resonance (NMR): ^{1}H NMR and ^{13}C NMR spectra of D_2O solutions were acquired at 295 K with Bruker Avance III 600 spectrometer operating at 600.2 MHz and 150.9 MHz respectively. The width of 90° pulse was 10 μs for ^{1}H NMR, and 8 μs for ^{13}C NMR with relaxation delays 10 s. The acquisition time was 2.18 s with 32 scans and 0.86 s with 31000 scans for ^{1}H NMR, and ^{13}C NMR respectively.

Small-angle X-ray scattering (SAXS): SAXS experiments were performed using a pinhole camera (modified Molecular Metrology System, Rigaku, Japan) attached to a microfocused X-ray beam generator (Rigaku MicroMax 003) operating at 50 kV and 0.6 mA (30 W). The camera was equipped with a vacuum version of Pilatus 300K detector. Two experimental setups were used to cover the q range of 0.005–1.1 $Å^{-1}$. Scattering vector, q, is defined as: $q=(4\pi/\lambda)\sin\theta$, where λ is the wavelength and 2θ is the scattering angle. Typical exposure time was 4 hours. Calibration of primary beam position and sample-to-detector distances was performed using AgBehenate sample. Water was used as absolute intensity calibrant.

Infrared spectroscopy: Fourier transform infrared (FTIR) spectra were recorded in the range of 650–4000 cm^{-1} at 256 scans per spectrum at 4 cm^{-1} resolution using a fully computerized Nicolet NEXUS 870 FTIR Spectrometer (ThermoFisher Scientific). Attenuated total reflection (ATR) method was applied using a single reflection Golden GateTM Diamond ATR crystal. The spectra were corrected for the water vapor and carbon dioxide in the optical path.

Raman spectroscopy: Raman spectra excited with an Ar-ion 514 nm laser were collected on a Renishaw inVia Reflex Raman spectroscope. A research-grade Leica DM LM microscope with an objective magnification 50× was used to focus the laser beam. The scattered light was analyzed by the spectrograph with a holographic grating 2400 lines mm^{-1}. A Peltier-effect cooled CCD detector (576 × 384 pixels) registered the dispersed light.

Transmission electron microscopy (TEM): Two microliters of nanospecies suspensions was placed on carbon-coated copper grid and dried. The morphology of all carbon nanospecies was observed with Transmission Electron Microscopy Tecnai G2 Spirit (FEI). Voltage was 120 kV.

Dynamic light scattering (DLS): For characterization of size of nanospecies in solution, DLS was performed on a Zetasizer Nano ZS (Malvern Instruments). DLS analyzes the velocity distribution of particle movement by measuring dynamic fluctuations of light scattering intensity caused by the Brownian motion of the particle. This technique yields a hydrodynamic radius, or diameter, to be calculated via the Stokes-Einstein equation from the aforementioned measurements.

Preparation and characterization of hen egg white lysozyme (HEWL) amyloid fibrils

In order to generate amyloid fibrils the stock solution of protein was prepared at concentration 2 mg/ml in 10 mM glycine-HCl buffer, pH 2.0. The solutions were filtered through-out a 0.22 μm filter. The stock solution was divided into glass vials to which were added various concentrations of nanospecies. Depending on the experiment the final protein concentration in all vials was even up adding Milli-Q water or filtered dichloromethane. The solutions were incubated at the 57°C. Samples (10 μl) were taken at different time and were detected by thioflavin T, Nile red, Transmission electron microscopy (see below).

Thioflavin T (THT) fluorescence assay: Solution of phosphate buffered saline (PBS) was prepared from tables. The solution was used for dissolution ThT at a final concentration 10 μM. HEWL samples (10 μl) taken at different times were added to 240 μl of the ThT solution into one well in 96-well plate. Before measuring fluorescence 96-well plate was briefly mixed on plate reader. ThT fluorescence intensity measurements were performed by exciting samples at 440 nm and recording emission intensities at 485 nm using with Synergy H1 Hybrid plate-reader (Biotek).

Nile red (NR) fluorescence assay: Nile red has the poor solubility in aqueous solution therefore a stock solution of NR at concentration 2.5 mM was prepared in pure ethanol and was kept at 4°C. The working solution was prepared by diluting the stock solution into 25 mM HCl (pH 1.6) at a final concentration of NR 10 μM. HEWL samples (10 μl) taken at different times were added to 240 μl of NR working solution into one well in 96-well plate. The mixed samples kept 1 h at room temperature before measuring fluorescence. Samples were excited at 550 nm and emission was recorded at 640 nm using with Synergy H1 Hybrid plate-reader (Biotek).

TEM: Two μl of sample was placed on carbon-coated copper grid and dried with paper. The sample was stained with 1 wt. % uranyl acetate for 30 s. Images were obtained with Tecnai G2 Spirit (FEI) at an accelerating voltage of 120 kV.

Statistical analysis: Experimental data were analyzed with Q-test and one–way ANOVA and the significance level was set at $\alpha<0.05$ for all experiments.