Fluorescent Carbon Dots as Off-On Nanosensor for Ascorbic Acid

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Synthesis of C-dots

The C-dots were synthesized by rapid one step microwave-assisted hydrothermal method by employing a microwave digestion which can monitor and control the temperature and pressure via fiber optic and pressure transducer in a reference vessel, respectively (XH-800C, xianghu, China). In a typical procedure, 0.3g ascorbic acid was dissolved in 30ml water. Then place it in a microwave digestion container and heated at 180℃ for a period of 5min at 900w power. When cooled down to room temperature, the brown color solution was purified by centrifugation at the speed of 14000rpm for 10min to remove the large particles. C-dots powder was obtained by rotary evaporating the C-dots colloid solution and subsequent drying the concentrated solution in a vacuum oven at 70℃ for 48h.

Characterization of the C-dots

High-resolution transmission electron microscopy (HRTEM) observations were performed on a JEOL-2010 electron microscope operating at 200 kV. Atomic force microscopy (AFM) image was obtained with a Micronano New Spm atomic force microscope (zhuolun, China). Dynamic light scattering experiment of C-dots aqueous solution was conducted by a Nano ZS (Malvern Instrument, UK). X-ray diffraction (XRD) patterns were obtained from a Rigaku D/max-2550VB/PC X-ray diffractometer with Cu Ka radiation (40 kV, 450 MA). The Fourier transform infrared spectroscopy

(FTIR) spectra were measured by an NICOLET iS10 (Thermo) spectrometer with the KBr pellet technique ranging from 500 to 4000 cm⁻¹. UV2450 spectrophotometer (Hitachi, Japan) was used to determine the absorbance of the C-dots. The fluorescence spectra of the CNPs were measured with a F900 fluorescence spectrometer (Edinburgh, UK), with a slit width of 2 nm and 2 nm for excitation and emission, respectively. Cell imaging with C-dots was obtained by the laser scanning confocal microscopy (LSCM, Leica DM6000 CS).
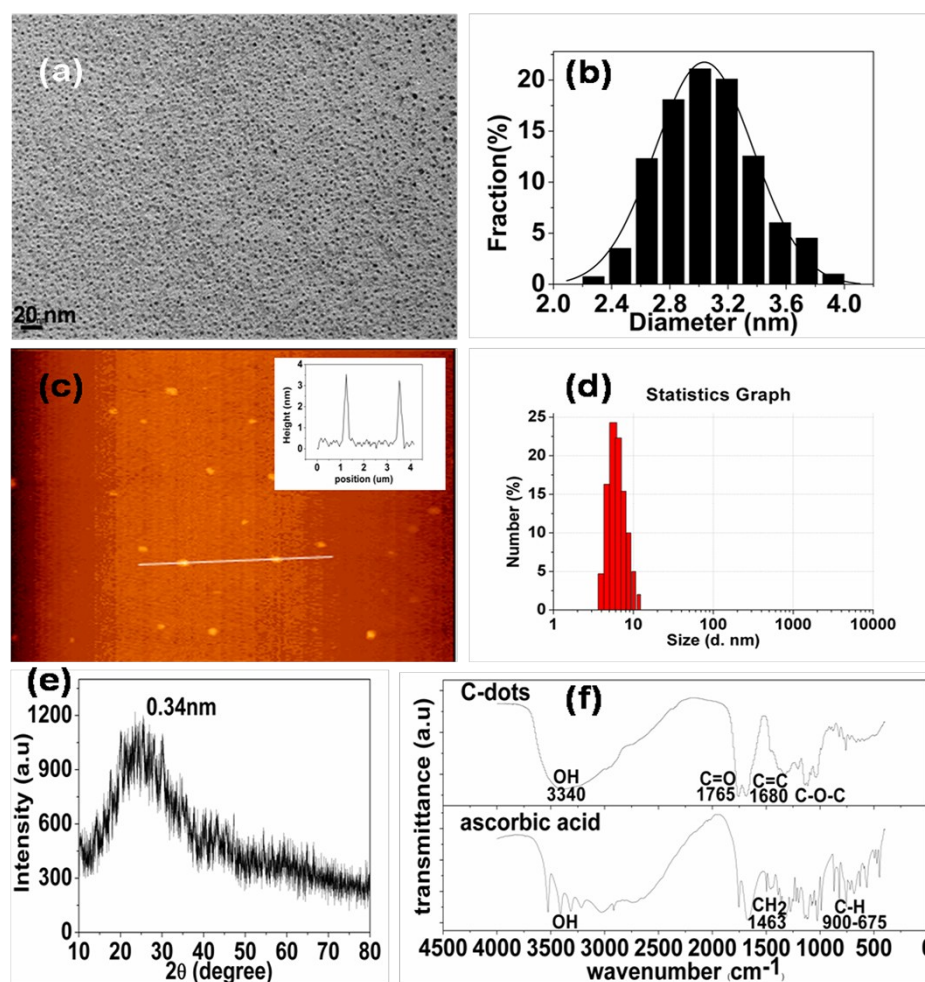

Figure S1. (a) HRTEM image of C-dots. (b) the diameter distribution according to HRTEM analysis. (c) AFM image of C-dots, the inset shows the height profile analysis along the line (AFM). (d) Statistics graph of DLS analysis of C-dots aqueous solution.

(e) XRD pattern of C-dots. (f) FTIR spectra of C-dots and carbon source ascorbic acid.
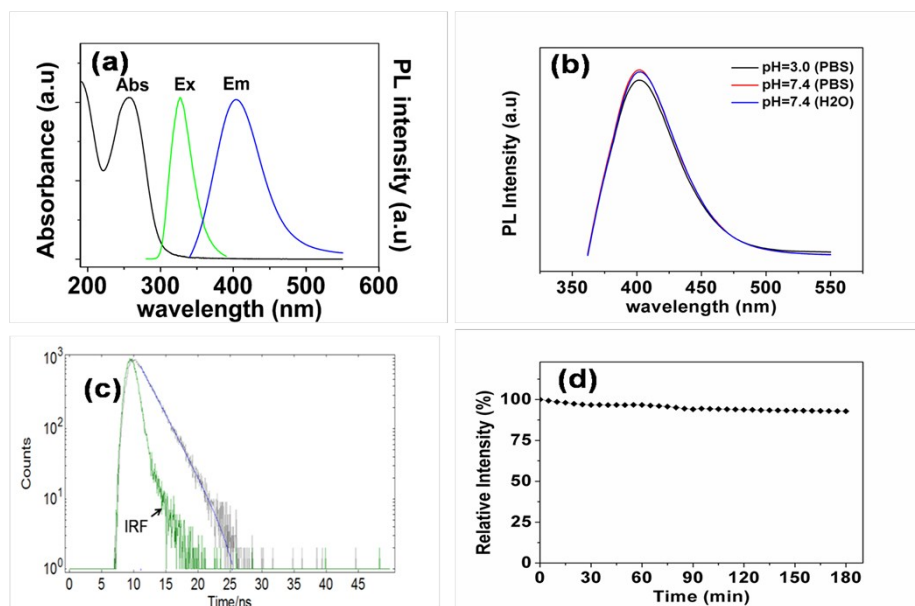

Figure S2. (a) Typical UV/Vis absorption, excitation and emission spectra of C-dots. (b) The effect of pH and buffer on the PL intensity of C-dots aqueous solution. (c) Fluorescence lifetime decay of C-dots aqueous solution at 330nm excitation and 400nm emission. (d) Optical stability of C-dots aqueous solution under irradiation of 330nm.

"Off-on" fluorescence of carbon dots

The "off-on" fluorescent assay was performed in deionized water. In "off" step, 0.1ml C-dots aqueous solution (10mg/ml) was added to 19.9ml Fe^{3+} aqueous solution with different concentrations (0, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000μM). Then in the "on" step, 50uL carbon dots aqueous solution (3mg/ml) and 100uL Fe^{3+} aqueous solution (30mM) was added to 2.85ml ascorbic acid solution with various concentrations(0, 0.2, 1, 2, 5, 10, 20, 40, 50ug/ml). After stirring for 3min, the mixed solution was measured by fluorescence spectrophotometer for PL spectra.

To evaluate the potential interference effect of biomolecules and ions on the PL intensity of C-dots/Fe^{3+} for AA detection, 50uL carbon dots aqueous solution (3mg/ml)

and 100uL Fe^{3+} aqueous solution (30mM) was added to 2.85ml 0.105mM AA with and without 5.25mM citric acid (Cit), glucose (Glu), glycine (Gly), lysine (Lys), bovine serum albumin (BSA), Na^+, K^+, Mg^{2+}, Ca^{2+}. After stirring for 5min, the mixed solution was measured by fluorescence spectrophotometer for PL spectra.

Determination of entrapment efficiency of ascorbic acid liposome

Liposome was prepared by membrane evaporation method. In detail, 2mg/ml egg phosphatidylcholine was dissolved in a mixture of chloroform and methanol (V/V=2). Then the mixed solution was evaporated at 43℃ by rotary evaporation. The obtained lipid membrane was then dissolved in 2mg/ml ascorbic acid aqueous solution and stirred for 1h at room temperature. To remove the unencapsulated ascorbic acid, ascorbic acid-liposome mixed solution (1ml) was put into a dialysis bag (MWCO: 8000-14000) and dialysis against deionized water at temperature for 24h. The dialysate was collected and diluted to certain volume for further concentration determination.

The entrapment efficiency of ascorbic acid in liposome was calculated as the following equation:

EE %=$(m_{AA} - m_{dialysate})/m_{AA}$*100%= $(1-C*V_{\text{dialysate}}/m_{AA})$*100%.

where $m_{AA}, m_{dialysate}$, C and $V_{dialysate}$ are total mass of AA added in liposome, mass of AA in the dialysate, AA concentration in dialysate and dialysate volume, respectively.

For HPLC assay, samples were analyzed by HPLC on an Agilent 1200 instrument mainly consisting of an Agilent Eclipse XBD-C18 colume and UV detector (Agilent Technologies 1200 series, Beijing, China). Ultrapure water was prepared by a DZG

303A ultrapure water system (Liding, Shanghai, China). The dialysate was diluted to 1000ml and the diluted solution was filtered by syringe filter with 0.22μm pore size before HPLC analysis. The mobile phase was acetonitrile : water (40:60, V/V) with the flow rate at 1ml/min. The colume oven was set at 25℃ and the UV detector was set at 265 nm. The concentration of ascorbic acid(C, μM) in the dialysate was determined using a standard curve of integrated peak area vs. concentration (Fig. S3). The entrapment efficiency of ascorbic acid is determined by the equation: 1-0.088C.

For off-on fluorescent assay, the dialysate was diluted to 200ml and the diluted solution was measured at by fluorescent spectrophotometer at excitation wavelength of 330nm. The concentration of ascorbic acid in the dialysate was determined using a standard curve of PL intensity vs. concentration as showed in Fig 2c. The entrapment efficiency of ascorbic acid is determined by the equation: 1-0.0176C.
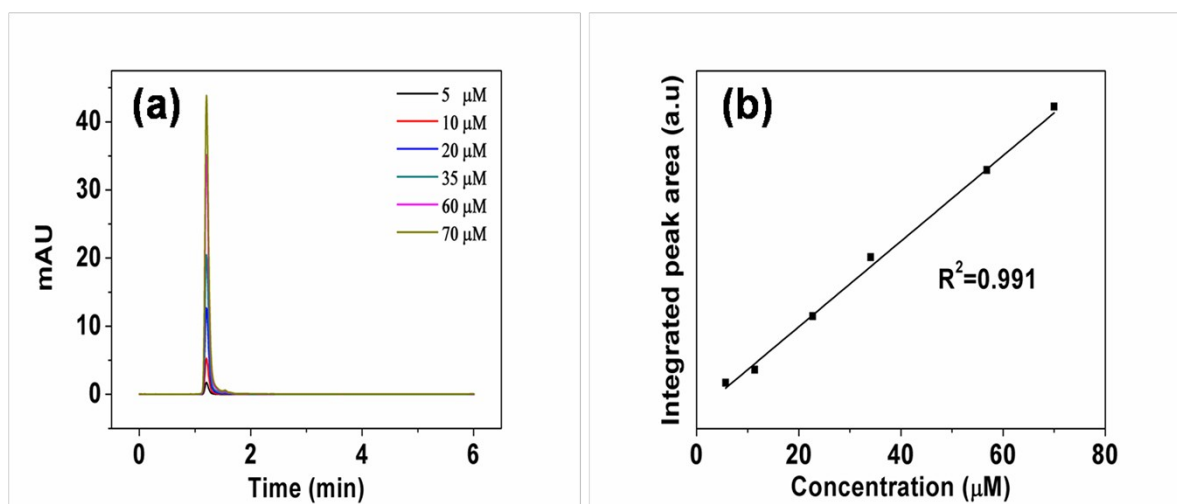

Fig S3 (a) the HPLC chromatogram of AA aqueous solution and (b) the standard linear curve for the integrated peak area in HPLC *vs.* the concentration of AA

Table S1 Determination of entrapment efficiency of liposome

Assay	Detection Range (μM)	Concentration (μM)	Entrapment Efficiency (%)	R.S.D (%, n=3)
HPLC	5-70	5.4	52.5	3.12
Off-On	0.2-280	26.7	53.0	2.87