Supplementary Information

Evaporation induced self-assembly approach to prepare polymorphic carbon dots fluorescent nanoprobe for protein labelling

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Experimental section

1.1 Reagents and materials

Anhydrous citric acid (AR), Ethylenediamine (99.5%), Glutaraldehyde (Grade 1, 50% aqueous solution) were purchased from Sigma (St. Louis, MO). Other chemicals were all analytical grade and used as received. Dialysis bag (MWCO 1000 Da) for dialysis was purchased from (Shanghai Biological Engineering Co., Ltd.). Rabbit-derived nano-drug deliver antibody were prepared in our laboratory. Polystyrene 96-well plates or strips (high binding, black or clear, sterilized) were purchased from Greiner (Monroe, NC). Ultrapure water (18.2 $M\Omega \cdot cm^{-1}$) was used throughout the experiments.

1.2 Apparatus

The fluorescence and the absorption spectra were recorded by Hitachi F-4600 fluorescence spectrophotometer and Hitachi UV-2910 spectrophotometer, respectively. Fluorescence intensity measurements were performed on a Microplate Reader (SynergyTM HT; Bio-Tek instruments, Inc. USA). Fourier transform infrared (FT-IR) were measured from a KBr window on a PerkinElmer PE-983 FT-IR spectrophotometer spectroscopy. Characterizations of transmission electron microscopy (TEM) were carried out on a Hitachi HT770. Size distribution were measured by Dynamic light scattering (DLS) (ALV/CGS-8F). X-ray photoelectron spectrum (XPS) (Escalab 250Xi). Energy dispersive X-ray (EDX) spectrum (X Flash 6160). Raman spectrum (inVia Raman microscope). All pH values were measured with a model pHs-3C meter.

1.3 Preparation of the C-Dots

The C-dots were synthesized through a facile hydrothermal method as follows[1]. The citric acid (CA, 0.42 g) and ethylene diamine (EDA, 530 µL) were first dissolved in 10 mL of ultrapure water then mix thoroughly with ultrasound. The solution was then transferred to a 20 mL teflon hydrothermal reactor and heated in an oven at 180 °C for 4-5h. The reaction was slowly cooled to room temperature and then the resulting solution was recovered from the reactor. The crude C-dots solution was dialyzed with ultrapure water for 2-3 days using dialysis bag with MWCO 1000 Da. The concentration of the purified C-dots was determined by weighing the dried samples of C-dots. C-dots solutions were stored under dark conditions at room temperature.

1.4 Preparation of the Carbon nanoclusters

In our studies, at room-temperature one-pot synthesis of carbon nanoclusters in aqueous media, glutaraldehyde mediates the growth of nanoclusters. Glutaraldehyde acts as a bifunctional coupling agent that can cross-link with primary amines on the surface of C-dots. In briefly, the purified C-dots stock solution (1mL, 19 mg/mL) was diluted with 2mL of deionized water. 25% (w/v) glutaraldehyde stock solutions were prepared by diluting the 50% (w/v) glutaraldehyde 5-fold with ultrapure water. Two hundred microliters (200 µL) of this diluted glutaraldehyde was added dropwise into the C-dots solution while stirring at room temperature for 2h. Finally, the mixed solution turns from the initial golden yellow to brown, which means the formation of carbon nanoclusters.[2]

1.5 Preparation of the C-Dots antibody complex

200 µL of 2.7 mg/mL of antibody in phosphate buffered saline (PBS, 100 mM sodium phosphate, 150 mM NaCl, pH 7.4) was added dropwise into the carbon nanoclusters reaction. This conjugation reaction was continuously stirred at room temperature for 1 h. C-dots self-assembly of labeled nano-drug deliver antibody was purified by dialysis use PBS (10 mM PBS) for two days to remove the excess C-Dots and salts with several buffer changes. The C-dots antibody complex solution was diluted to appropriate concentration and stored at 4 °C under dark conditions prior to use.

1.6 Preparation of coating antigen

The method for preparation of immunogen is similar to reference.[3-4] 1 mL PBS (pH=7.4, 0.1 M) containing 1-ethyl-1-3-(3-dimethylamino-propyl)-carbodiimide (EDAC) 0.7 mg and N-hydroxysuccinimide (NHS) 0.1 mg were mixed with 1 mL hydrophilic nanospheres incubating at 37 °C for 30min to activate the PSI_{OAm}-COOH carboxyl groups. The excess EDC and NHS were removed by centrifugation. Followed by 1mL phosphate buffer solution (PBS) containing 1 mg of BSA incubating at 37 °C for 4h and then the BSA-nanocomposite bio-conjugates were collected by centrifugation (11000 rpm, 10 min) and washed with PBS. This purification process was repeated for three times. The obtained BSA conjugated nanocomposites were redisposed in PBS (pH=7.4, 1mL) and stocked at 4 °C for later use.

1.7 Establish direct fluorescent immunoassay measurement procedure

Before fluorescence immunoassay, the 96-well microplates were modified and functionalized as follows: first, 100µL (25µg/mL) of coated antigen (PSI_{OAm}-OVA) were added into wells and incubated at 4 °C overnight. First, the wells were washed

with the wash buffer three times. Second, the wells were blocked with 1% casein at 37 °C for 1 h (the wells were washed with the wash buffer three times). Third, 100μL of diluted C-dots self-assembly of labeled anti-PSI_{OAm} antibody (1μg/mL) in PBS buffer was added. After incubation at 37°C for 90 min, the wells were washed with the wash buffer three times. Fluorescence signal of the direct immunocomplexes were read directly from these dry 96-well plates in end point assay format at λ_{ex}=360 nm, λ_{em}=480 nm.

2. Support picture

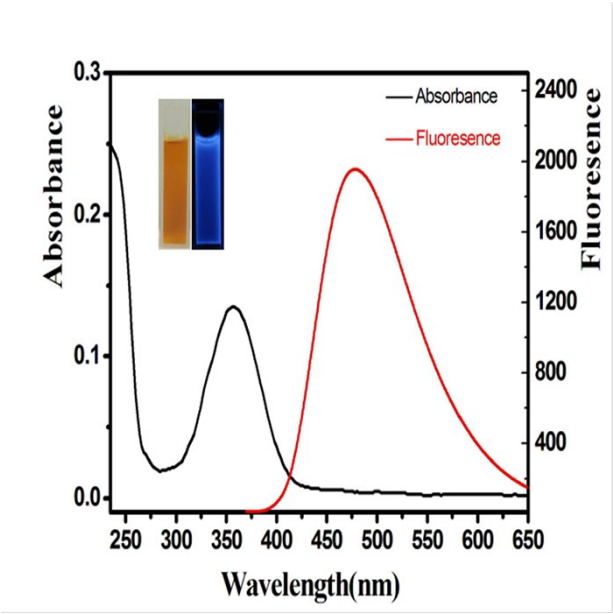

Fig S1. Ultraviolet-visible (UV-vis) absorption spectrum (black line) and fluorescence emission spectrum (red line) of C-dots.

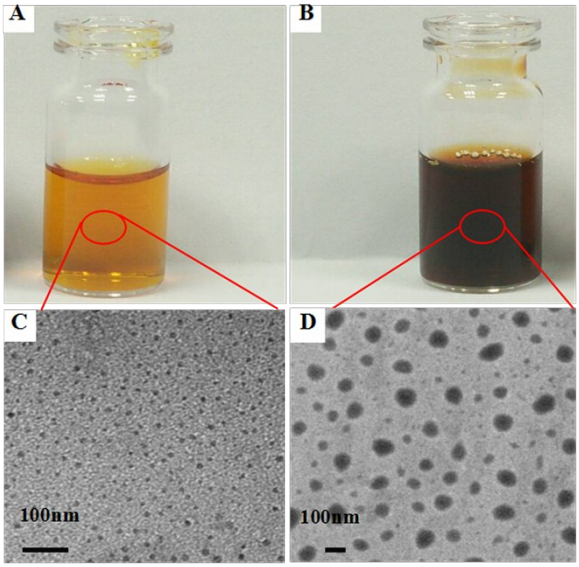

Fig S2. Photographic illustration of the nucleation process of aqueous C-dots precursor before the addition of glutaraldehyde. Before (A) and after (B) maintaining the solution at room temperature for 2 h. The corresponding TEM images are shown in Figure (C) and Figure (D).

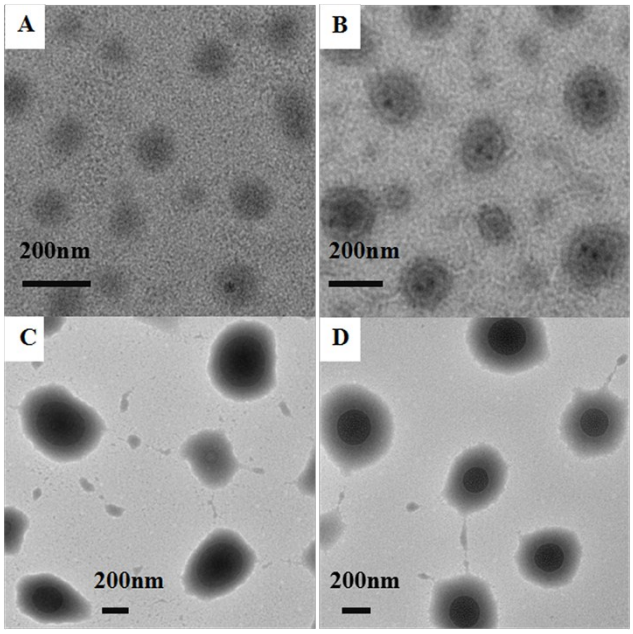

Fig S3. Influence of pH on the evaporation-induced C-dots self-assembly, pH = 5.0 (A), 6.0 (B), 7.0 (C), 7.4 (D). It could be seen that C-dots self-assembly nanocrystalline was synthesized at pH 5.0-7.4. In the present study, a near neutral condition (**pH 7.4**) was employed for the fabrication.

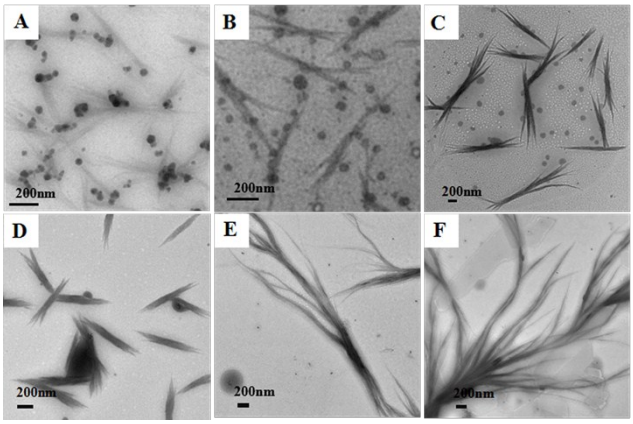

Fig S4. Influence of PBS concentration on the evaporation-induced C-dots self-assembly. A, B, C, D represents adding different volumes of (10 mM, pH=7.4) PBS, E, F represents adding different volumes of (100 mM, pH=7.4) PBS.

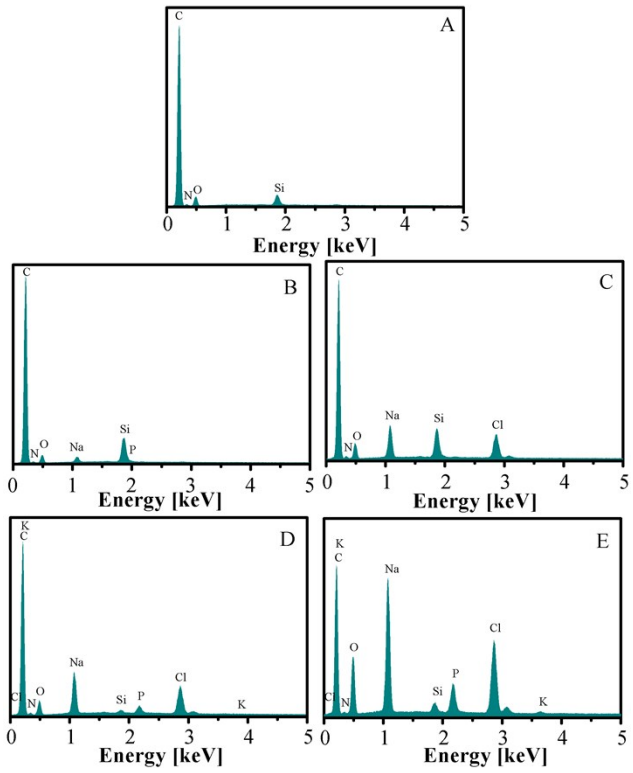

Fig S5. EDX spectra of under different self-assembly conditions, only C-dots (A), (B) pH=7.4, (C) 0.1M NaCl, (D) 0.01M PBS, (E) 0.1M PBS. All EDX test samples are measured on silicon surface.

EDX spectra show that C-dots only appear signals of C, N and O (Fig. S5A); at pH = 7.4 (sodium hydroxide-adjusted PB solution) show signals of C, N, O, Na, and P (Fig. S5B); Signals of C, N, O, Na, and Cl were clearly observed in 0.1M sodium chloride solution(Fig S5C); All shown in signals of C, N, O, K, Na, Cl, P in both 0.01M (Fig. S5D) and 0.01M (Fig. S5E) PBS solutions and the elemental signal in 0.1M PBS was stronger than 0.01M PBS solution. All EDX spectra obtain signals of C, N and O, which indicate that under different self-assembly conditions multi-morphological self-assembled C-dots were synthesized based with a matrix of C-dots.

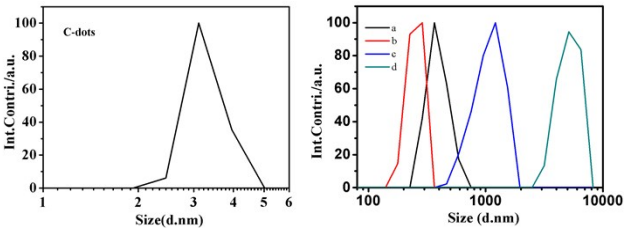

Fig S6. Size distribution of C-dots and a, b, c, d represent under the conditions of pH (7.4), 0.1M NaCl, 0.01M PBS and 0.1M PBS, respectively.

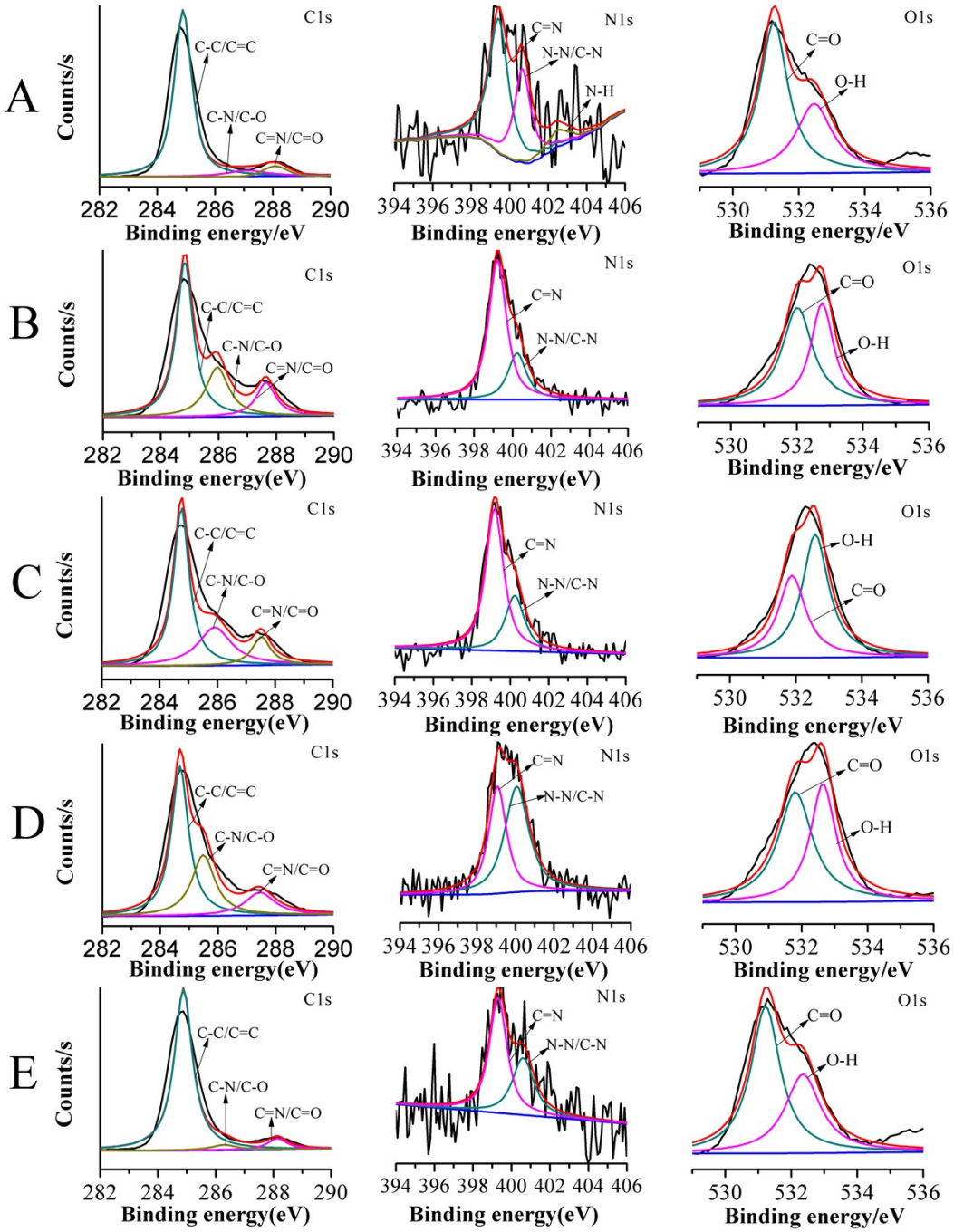

Fig S7. X-ray photoelectron spectroscopy (XPS) of under different self-assembly conditions, only C-dots (A), (B) pH=7.4, (C) 0.1M NaCl, (D) 0.01M PBS, E) 0.1M PBS.

The XPS analysis in Fig S7 indication that different C-, N-, and O-containing units upon the C-dots and multi-morphological self-assembled C-dots surface. In the XPS spectra of C-dots (Fig S7A), High-resolution C1s spectrum indicate that there exist three types of C including sp^2 C carbon atoms (C=C, peaks at 284.7 eV), sp^3 C carbon atoms (C-C, C-N, C-O, peaks at 286.6 eV) and (C=N/C=O, peaks at 288. 6 eV), respectively. The N 1s spectrum of C-dots showed two peaks at 399.25 eV and 400.49 eV, which were attributed to aromatic N and surface NH_2 groups, respectively.[5] As show in the Fig S7(B, C, D, E), all X-ray photoelectron spectra obtain signals of C, N and O. These indicate that under different self-assembly conditions multi-morphological self-assembled C-dots were assembled based on C-dots instead of residue salt in CDs solution after evaporation.

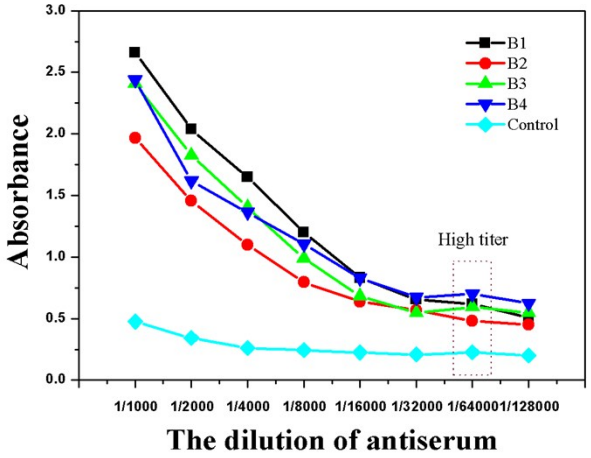

Fig S8. PSIOAm-BSA as an immunogen, and determination of antibody titer produced by immunizing New Zealand white rabbits. The result shows that the high titer reached at 1:64000.

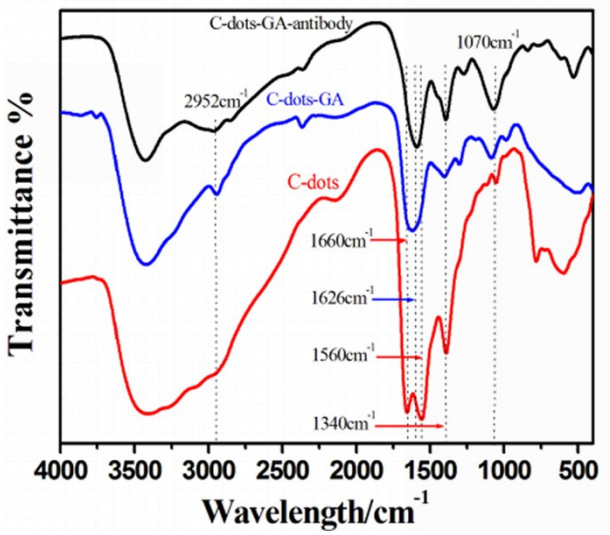

Fig S9. FT-IR spectra of C-dots (read line), C-dots GA complex (blue line) and C-dots marked antibody (black line). Exhibiting obvious absorption peaks at 400-4000 cm^{-1}.

FT-IR spectra show 1560 cm^{-1} corresponded to the stretching vibration of acyl group (CONR). The absorption bands between 3500-2940 cm^{-1} were attributed to O-H, N-H and C–H stretching vibrations. The characteristic absorption peak of C–H stretching vibration was observed at 2952 cm^{-1}.[6] The characteristic stretching vibration of C=C (1633 cm^{-1}) and C=O/C=N (1660 cm^{-1}) were also observed. The narrow and strong peak (1340 cm^{-1}) corresponded to the stretching vibration of C-N. Typically, C-dots contain many groups, such as the -OH, -C=O, and -NH-,-NH_2 groups moieties at their surface. After adding the protein, it can be clearly seen that the peaks at 1560 cm^{-1} and 1660 cm^{-1} disappeared and new peaks appeared at 1626 cm^{-1} and 1070 cm^{-1}, which means that the C-dots nanoclusters reacted with the protein.

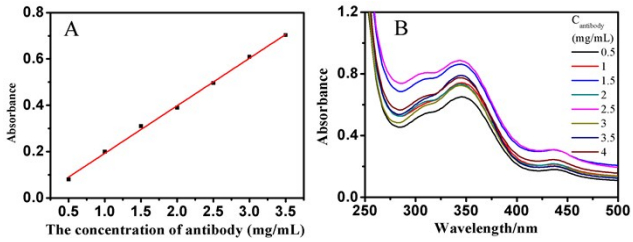

Fig S10. The calibration curves of antibody (A), and optimized adsorption capacity of antibody (B). The concentration of antibody added was from 0.5mg/mL to 4mg/mL, increment is 0.5mg/mL. Optimized adsorption capacity of antibody is 2.5mg/mL.

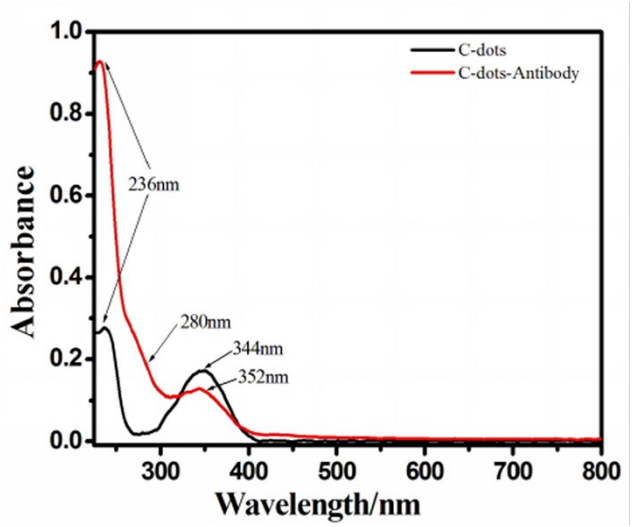

Fig S11. UV-Vis spectra of C-dots (black line) and C-dots labeled antibody (red line). UV-Vis spectra show presence of a sharp peak at 236nm followed by a hump at 344nm (black line), which arise due to $\pi \rightarrow \pi^*$, In comparison to red line (352nm), there was blue shift of 8nm in UV-Vis spectrum (red line), in addition, a new absorption peak appears at 280nm can be seen (red line). [7]

3. Notes and references

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