Electronic Supplementary Material (ESI) for RSC Advances.

Supplementary Information

Aconitic acid derived carbon dots as recyclable "on-off-on" fluorescent nanoprobes for sensitive detection of mercury (II) ions, cysteine and cellular imaging

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Fluorescence Quantum Yields and Time-resolved Fluorescence Spectroscopy.

The fluorescence quantum yield (QY) of AA-CDs was determined by absolute method (called absolute QY) using an FLS920 spectrometer (Edinburgh Instruments, U.K.) and the integrating sphere accessory. For the determination of absolute QY, only dilute solutions and the corresponding solvent at the excitation wavelength (λ_{ex} = 360 nm) were used. The absolute QY can be calculated through the following equation (1).

$$\phi_a = \frac{\varepsilon}{\alpha} = \frac{\int L_{emission}}{\int E_{solvent} - \int E_{sample}} \qquad (1)$$

Where ϕ_a is the absolute quantum yield, ε is the emitted photons by the sample, α is the adsorbed photons by the sample, $L_{emission}$ is the luminescence emission spectrum of the sample, E_{sample} is the spectrum of light used to excite the sample, $E_{solvent}$ is the spectrum of light used for excitation with only the solvent in the sphere.

Time-resolved fluorescence spectra were carried out in a time-correlated single photon counting (TCSPC) system from FLS920 spectrometer with λ_{ex} = 360 nm. Curves of the instrument response functions (using a LUDOX scatterer) and sample decays were recorded until they typically reached 1.0×10^4 counts in the peak channel. Obtained curves were fitted as sums of the exponentials, using Gaussian-weighted nonlinear least squares fitting based on Marquardt-Levenberg minimization implemented in the software package of the instrument (equation (2)). Subsequently, the average lifetime ($\bar{\tau}$) can be calculated according to equation (3).

$$Y(t) = A + B_1 \exp(-t/\tau_1) + B_2 \exp(-t/\tau_2) \qquad (2)$$

$$\bar{\tau} = \frac{B_1\tau_1^2 + B_2\tau_2^2}{B_1\tau_1 + B_2\tau_2} \qquad (3)$$

where B_1 and B_2 are the fractional contributions of time-resolved decay lifetime of τ_1 and τ_2. All detailed curve fitting parameters (decay times and pre-exponential factors) were presented in Table S3.

Cytotoxicity test

The cytotoxicity of the prepared AA-CDs to SMMC-7721 cells was evaluated by a standard MTT assay. In brief, 100 μL of SMMC-7721 cells were seeded in 96-well plate with a density of 1×10^5 cells mL^{-1} and allowed to adhere overnight. After an incubation of 24 h at 37 ^oC, the culture media was discarded and cells were cultivated with fresh culture media containing various concentrations of AA-CDs (0-1500 μg mL^{-1}) for another 24 h. At the end of incubation, the culture media were replaced with fresh culture media, following with the addition of 10 μL MTT (5 μg mL^{-1}) into each well. After additional incubation of 4 h, the growth media was removed and 100 μL of DMSO was added to each well to dissolve the formed formazan. After shaking the plates for 5 min, absorbance values of each well were recorded with a microplate reader (RT-6100, Rayto, China) at 490 nm. The cell viability rate (VR) was calculated according the equation: VR = $A/A_0\times100\%$, where A is the absorbance of the experimental group (cells were treated with AA-CDs) and A_0 is the absorbance of control group.

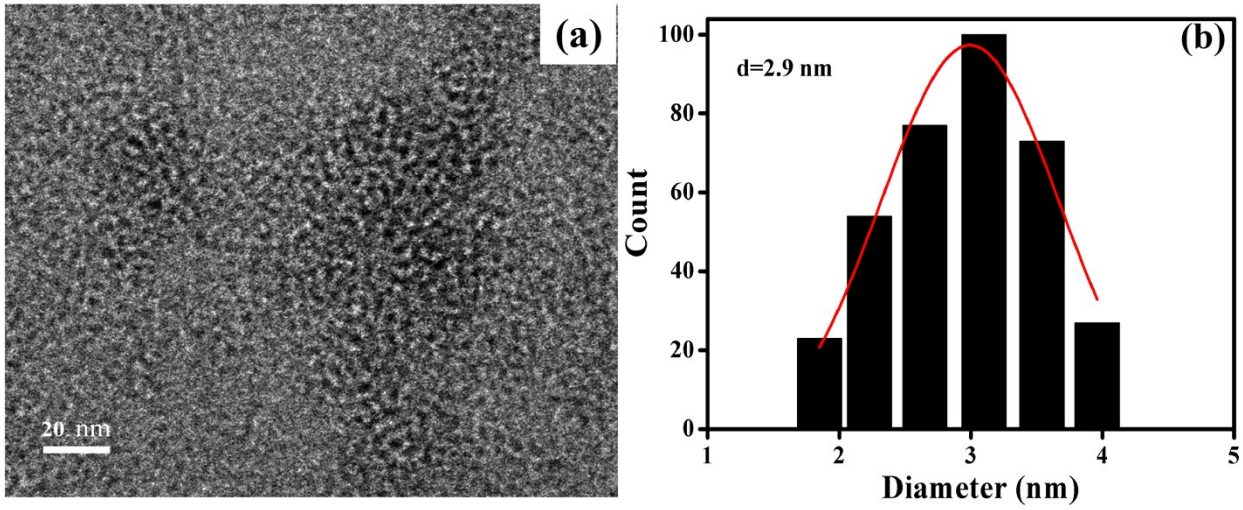

Fig. S1. TEM image (a) and size distribution histogram (b) of AA-CDs.

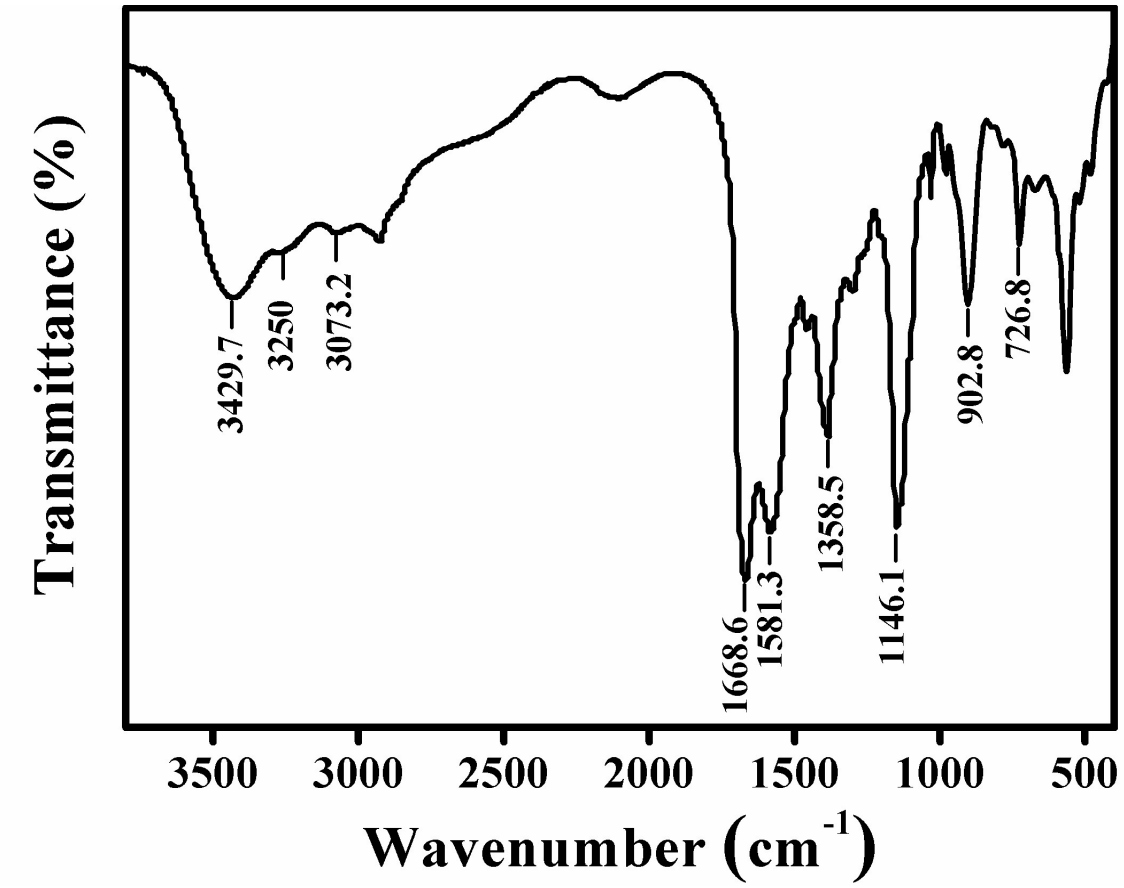

Fig. S2. FTIR spectrum of AA-CDs.

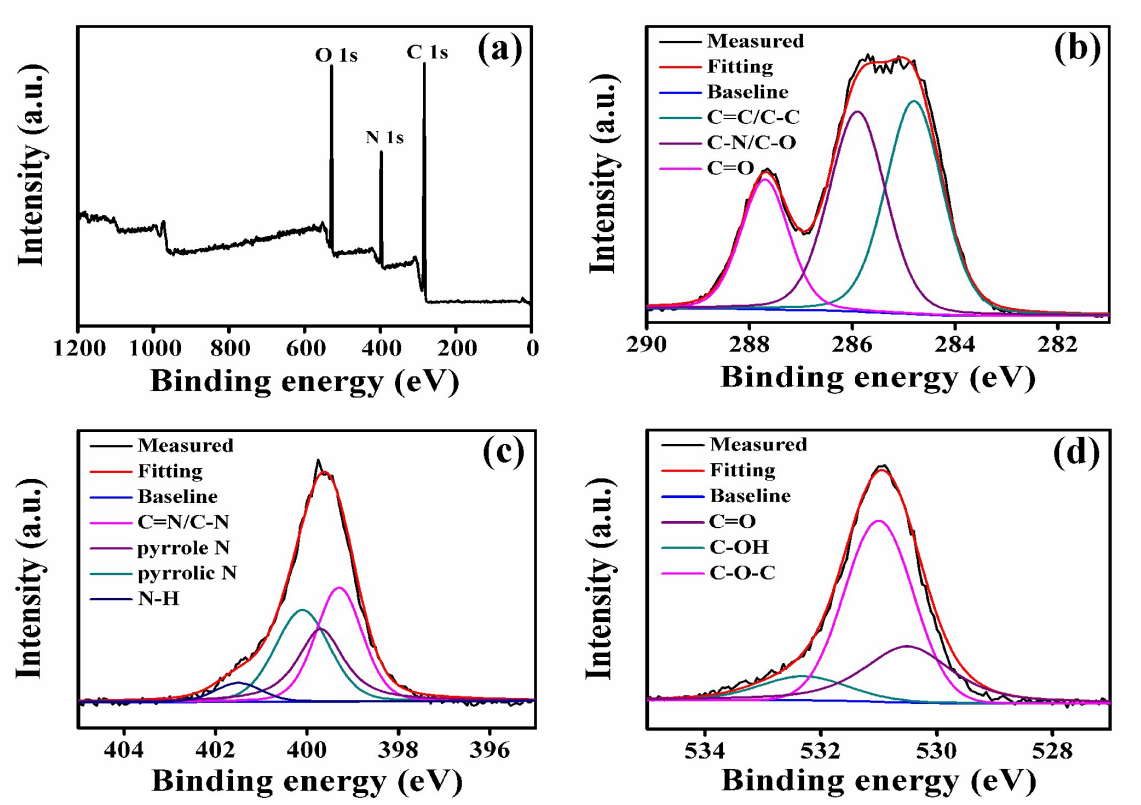

Fig. S3. XPS survey spectrum of AA-CDs (a) and corresponding high resolution spectra for C1s (b), N1s (c) and O1s (d).

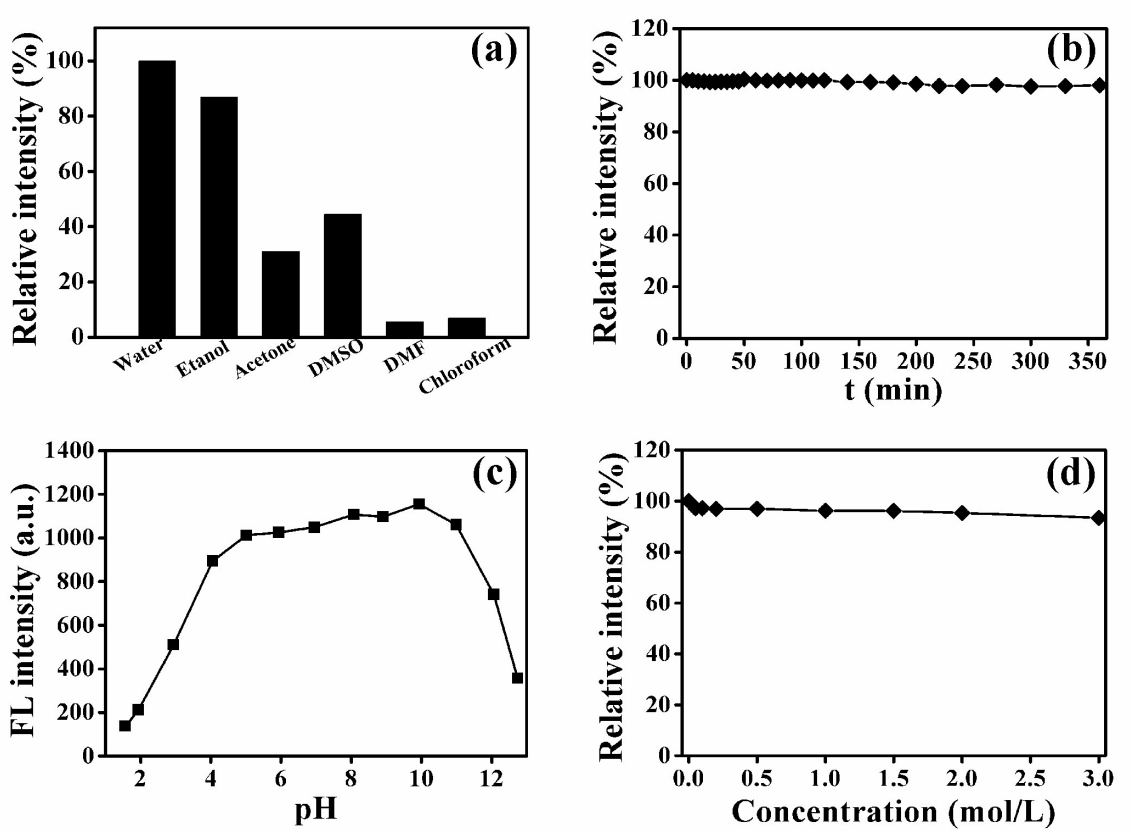

Fig. S4. The fluorescence responses of AA-CDs toward ambient environments. (a) The fluorescence intensities for AA-CDs in different solvents. (b) The fluorescence responses of AA-CDs under light illumination. (c) The fluorescence responses of AA-CDs at different pH. (d) The fluorescence intensities of AA-CDs in various concentrations of NaCl solutions.

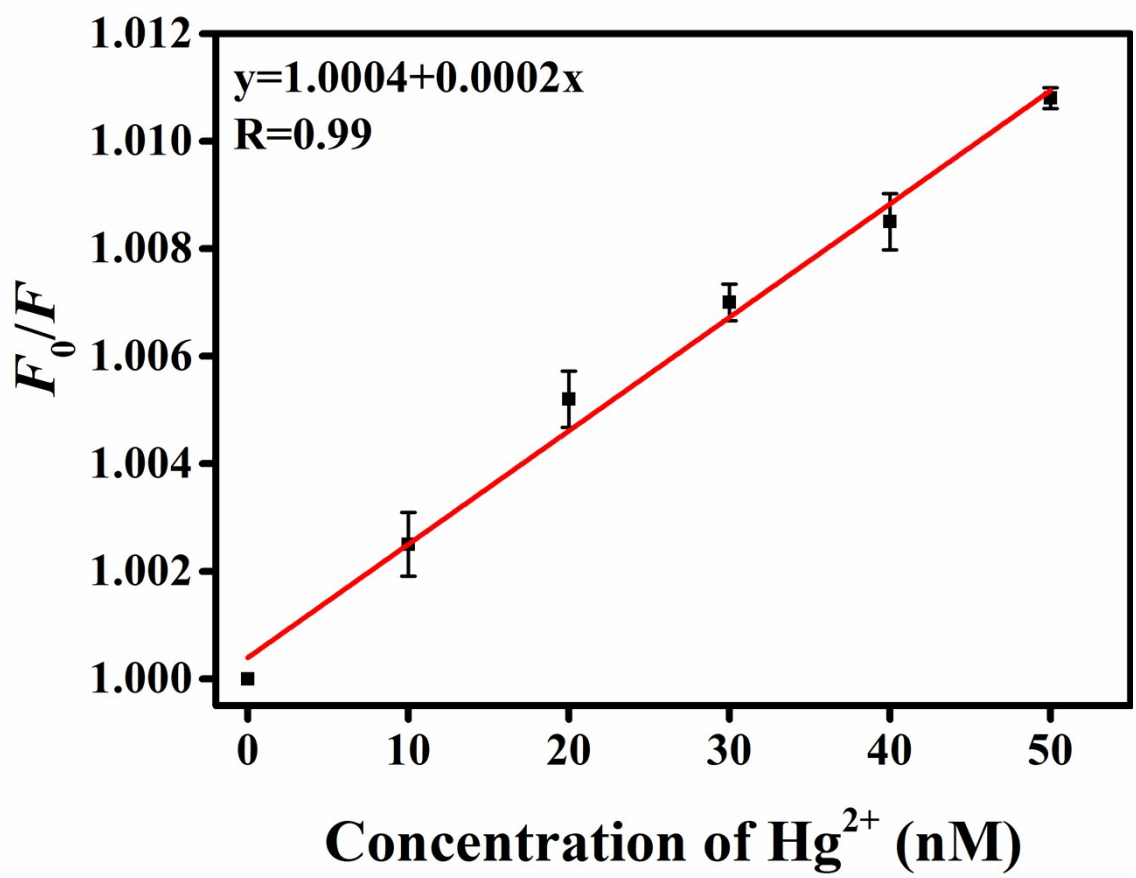

Fig. S5. The dependence of the fluorescence intensity ratio (F_0/F) of AA-CDs at 450 nm (λ_{ex} = 360 nm) versus the concentrations of Hg^{2+} ions (F_0 and F correspond to the fluorescence intensities of AA-CDs in the absence and presence of Hg^{2+} ions, respectively).

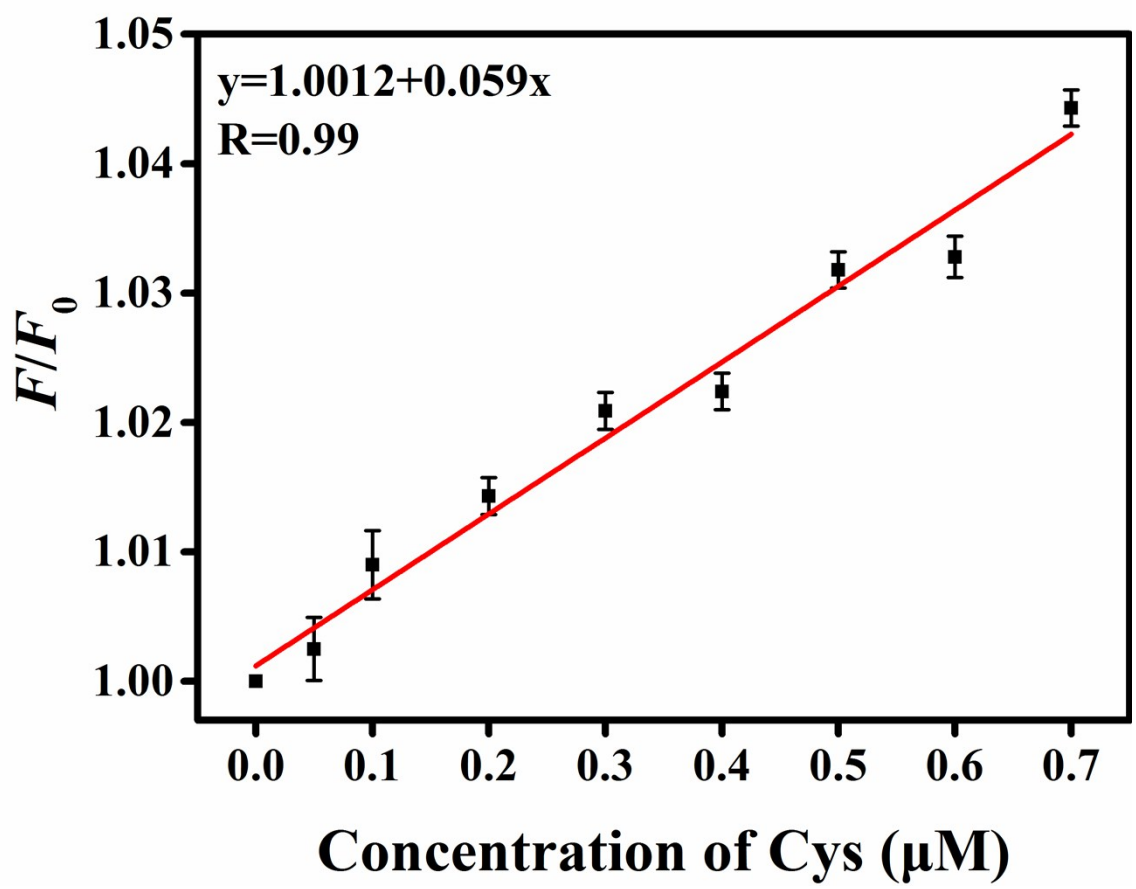

Fig. S6. The dependence of the fluorescence intensity ratio (F/F_0) of AA-CDs-Hg^{2+} at 450 nm (λ_{ex} = 360 nm) versus the concentrations of Cys (F and F_0 correspond to the fluorescence intensities of AA-CDs-Hg^{2+} in the presence and absence of Cys, respectively).

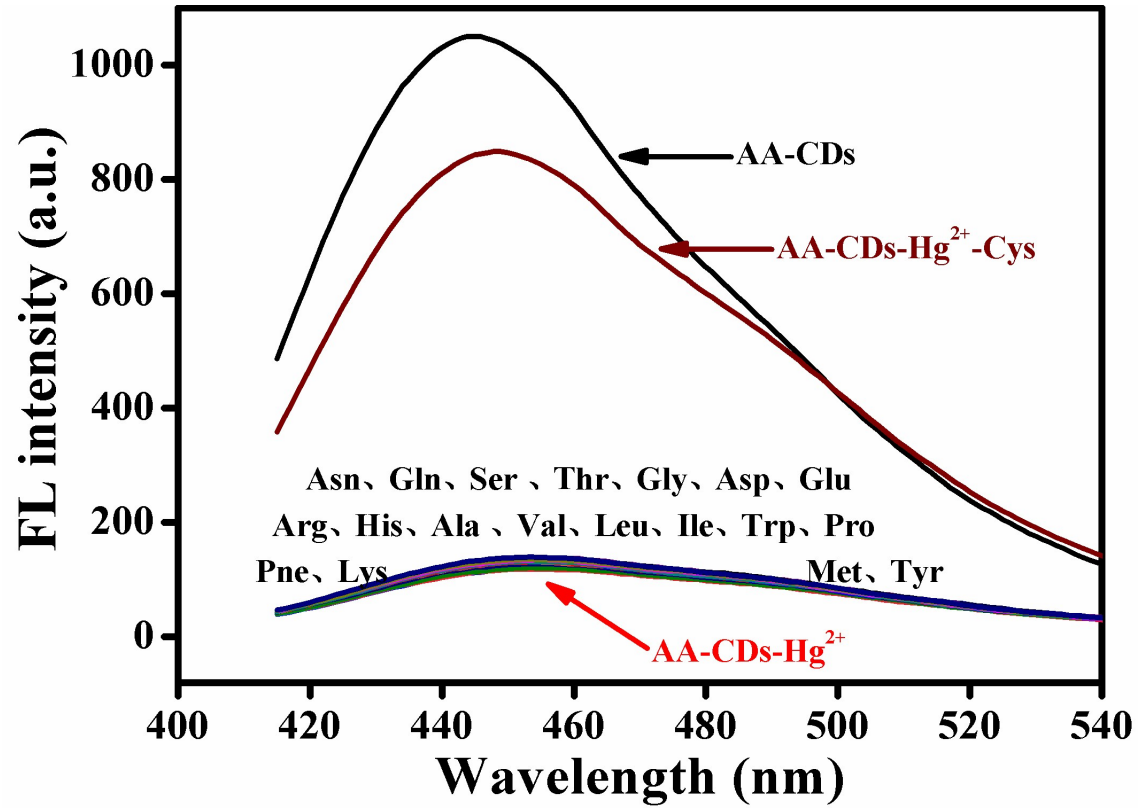

Fig. S7. Fluorescent emission spectra of AA-CDs, AA-CDs-Hg^{2+} and AA-CDs-Hg^{2+} by adding different amino acids (the concentration for all amino acids is 63 μM).

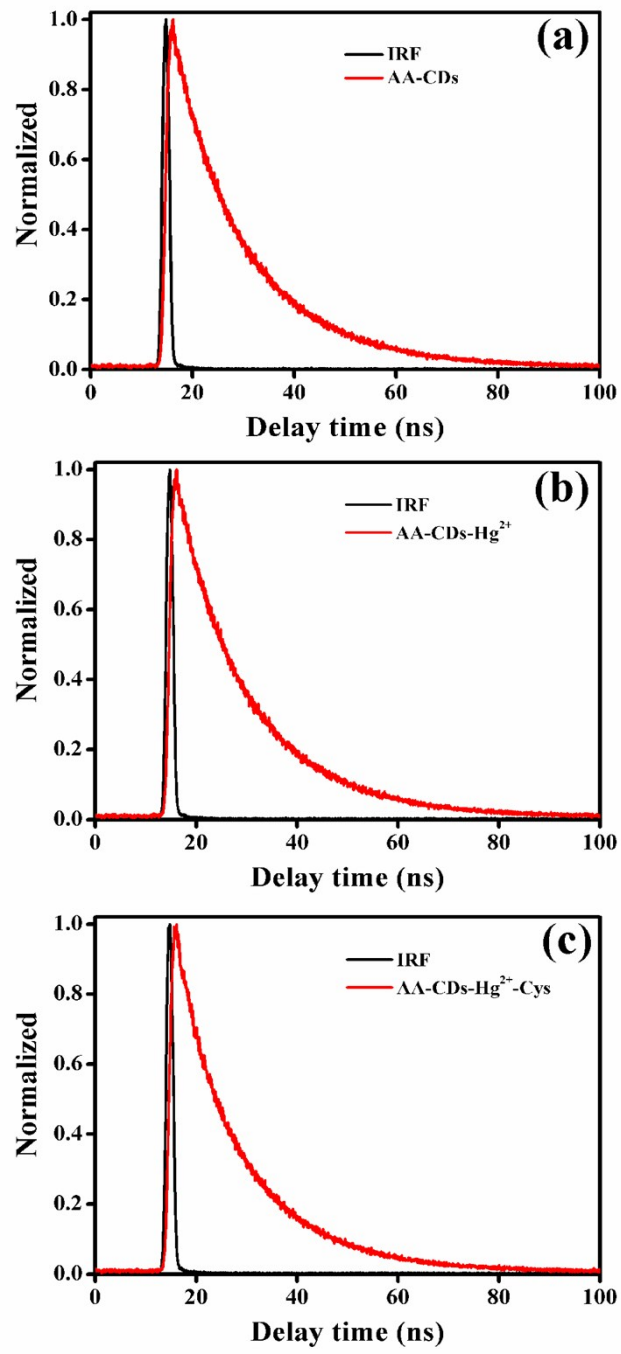

Fig. S8. Fluorescence decay curve of AA-CDs (a), AA-CDs-Hg^{2+} (b), and AA-CDs-Hg^{2+}-Cys (c), λ_{ex} = 360 nm and λ_{em} = 450 nm.

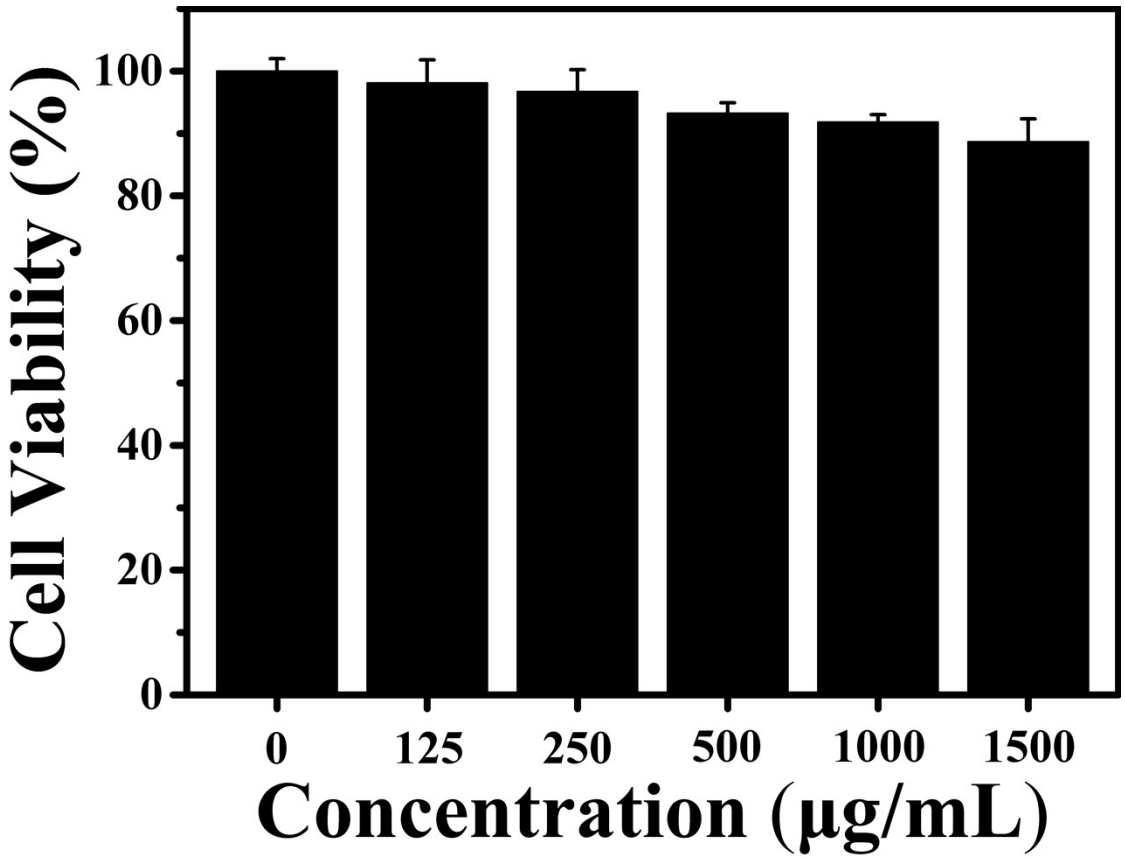

Fig. S9. The viability of SMMC-7721 cells treated with AA-CDs for 24 h.

Table S1 Comparison of different CDs-based fluorescence sensors for Hg^{2+} ions detection.

Precursors	Preparation methods	λex/λem (nm)	QY (%)	LOD	Refs
Pomelo peel	Hydrothermal (200 °C, 3 h)	365/444	6.90[a]	10 nM	[20]
Sodium citrate, NH_4HCO_3	Hydrothermal (180 °C, 4 h)	340/435	68.22[b]	10 nM	[21]
Flour	Microwave (180 °C, 20 min)	365/442	5.40[a]	0.5 nM	[22]
Melamine, trisodium	Microwave-assisted hydrothermal	350/437, 575	-[c]	45 nM	[23]
CA, EDA	Hydrothermal (200 °C, 5 h)	360/438	65.50[a]	226 nM	[24]
AEAPMS, CA	Heat (240 °C)	360/452	55.40[a]	845 nM	
Glutathione	Solid-phase thermal (260 °C, 1 h)	371/442	39.90[a]	0.05 nM	[25]
CA, thiourea	200 °C, 2 h	369/444	14.50[a]	0.37 nM	[26]
CA, dithiooxamide	Microwave (800 W, 2.5 min)	360/448	17.60[a]	0.18 μM	[27]
CA, Urea, Cys	Microwave (5 min)	353/450	25.20[a]	2 μM	[28]
Urea, sodium citrate	Electro chemical	351/433	11.90[a]	3.3 nM	[29]
CA, EDA	Hydrothermal (165 °C, 150 min)	350/448	8.90[a]	0.57 nM 0.55 nM	[30]
Chitosan gel, PEG	Microwave (3 min)	280/412	13.4	6.8 nM	[31]
Sodium citrate, Glutathione	Hydrothermal (200 °C, 4 h)	355/450	21.03[a]	25 nM	[32]
Citric acid, DETA	Reflux	360/440	82.40	0.201 μM	[33]
PAMAM, APTES	Hydrothermal (185 °C, 3.5 h)	354/442	52.6[a]	87 fM	[34]
Citric acid, Biuret	Microwave (600W, 12 min)	350/430	15.3[a]	0.017 μM	[40]
Citric acid, $Mg(OH)_2$, EDA	Hydrothermal (200 °C, 3 h)	348/440	58.8[a]	0.02 μM	[41]
Aconitic acid, Urea	Microwave (700 W, 6 min)	360/450	45[d]	5.5 nM	This work

[a] The quantum yield (QY) was measured by relative method using quinine sulfate as a standard.

[b] The quantum yield (QY) was measured by relative method using Rhodamine 6G in ethanol as a standard.

[c] The result was not provided.

[d] The QY of AA-CDs (this work) was measured by absolute method.

Table S2 Comparison of different CDs-based fluorescence sensors for Cys detection.

Precursors	Preparation methods	$\lambda_{ex}/\lambda_{em}$ (nm)	QY (%)	LOD	Refs
EDTA	Prolysis	-[a]/410	11.00[c]	4.9 nM	[35]
Triammonium citrate, Na_2HPO_4	Sonication (2 min)/750W, 140 s	350/440	14.30[d]	0.05 μM	[36]
CA, NaOH	Oil bath (200 °C)	365/450	15.40[d]	4.5 nM	[37]
Melamine/trisodium•$2H_2O$	Microwave/180 °C, 12 h	350/437	-[a]	-[a]	[38]
Chitosan gel, PEG, DTT	Microwave (3 min)	280/412	9.50[e]	72 nM	[39]
Citric acid, Biuret	Microwave (600W, 12 min)	350/430	15.3[d]	0.29 μM	[40]
Citric acid, Mg(OH)2, EDA	Hydrothermal (200 °C, 3 h)	348/440	58.8[d]	-[a]	[41]
Aconitic acid, Urea	Microwave (700 W, 6 min)	360/450	45[f]	30 nM	This work

[a] The result was not provided.

[b] The quantum yield (QY) was measured by relative method using tryptophan aqueous solution as a standard.

[c] The quantum yield (QY) was measured by relative method using Rhodamine B as a standard.

[d] The quantum yield (QY) was measured by relative method using quinine sulfate as a standard.

[e] The standard was unmentioned.

[f] The QY of AA-CDs (this work) was measured by absolute method.

Table S3 Results for the lifetime of AA-CDs, AA-CDs-Hg^{2+}, AA-CDs-Hg^{2+}-Cys systems.

Samples	Param.	Value/ns	Rel./%	τ/ns	χ^2
AA-CDs	t_1	5.21	6.87	14.92	0.976
	t_2	15.64	93.13		
AA-CDs-Hg^{2+}	t_1	4.13	34.13	9.93	1.106
	t_2	12.94	65.87		
AA-CDs-Hg^{2+}-Cys	t_1	3.82	5.47	14.03	1.014
	t_2	14.66	94.26		