Electronic Supplementary Material (ESI) for Chemical Science.

Electronic Supplementary Information

Cucurbituril Brighten Au Nanoclusters in Water

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1. Experimental Procedures

1.1 Materials and Methods

The peptide N-terminal phenylalanine-glycine-glycine-cysteine (purity > 98%) was purchased from Shanghai Mujin Biological Technology Co., Ltd. All other reagents were purchased from Adamas-beta or TCI Chemicals, and used without further purification. Solvents were purified according to standard laboratory methods. Water was purified using a Millipore Milli-Q system (18.2 MΩ·cm). ^{1}H NMR spectra were recorded on a Brüker AV-400 spectrometer. The experiments were performed at room temperature in deuterated solvent. Chemical shifts were shown in ppm relative to TMS and the solvent residual peak was used as the internal standard. The chemical composition of $Au_{22}(FGGC)_{18}$ clusters was analyzed by using ESI-HRMS spectrometry (Brüker, solanX 70 FT-MS) in positive mode. The sample was prepared in ultrapure water at a concentration of 1 mg/mL and directly injected into the mass spectrometer. UV-Vis absorption spectra were performed on Varian Cray 500 spectrophotometer. Luminescence spectra were recorded on Cary Eclipse Fluorescence Spectrophotometer and HORIBA FluoroMax-4 spectrometer. High-resolution Transmission Electron Microscopy (HRTEM) images were taken on JEOL JEM-2100 electron microscope at an accelerating voltage of 200 kV. TEM samples were prepared by casting a small amount of sample on a perforated copper grid (200 mesh) covered with a carbon film and dried in air. ITC data was recorded on TA Instruments NANO ITC at 298.15 K in pH=7 aqueous solution, the host molecule (CB[7], 0.1 mM and 0.31 mM) was in the sample cell, and guest molecule (FGGC, 1.66 mM) was in the injection syringe.

1.2 Preparation of FGGC-AuNCs

The preparation of $Au_{22}(FGGC)_{18}$ nanoclusters was mainly referred to a literature procedure with some modifications. Aqueous solutions of $HAuCl_4$ (45 μL, 0.5 M) and FGGC (25 mg/mL, 1 mL) were mixed in a 50 mL flask containing 2 mL deionized water, accompanying quickly with a solution color change from flavogreen to colorless. Next, 22 mL deionized water was added to the above mixture. After 1 minute of vigorous stirring, the pH of the reaction solution was adjusted to 11~12 with 1 M NaOH aqueous solution. Further, approximately 250 μL 0.3 mg/mL $NaBH_4$ aqueous solution was added slowly at least for 30 minutes until the solution color changed from colorless to orange-red. Following this, the remaining $NaBH_4$ was quenched by adjusting pH to 2.5 with 1 M HCl with a remarkable color change from orange-red to brown-green. The reaction solution was then kept stirring at 400 rpm for 12 h, forming an orange-red raw AuNCs solution. After pre-purified using Millipore centrifugal filter tubes (4 mL, 10 KD), the solution was further separated by preparative thin-layer chromatography (PTLC, developing solvent: the organic phase of N-butanol/water/acetic acid=4:1:5, R_f=0.3) to obtain orange-red product. The obtained AuNCs were stored at 5 ^{o}C and the absorption and emission spectra nearly unchanged at least one month.

1.3 Photoluminescence Quantum Yield Measurements.

The absolute quantum yields were recorded on Hamamastu absolute PL quantum yield spectrometer C11347 at room temperature. Samples was diluted in aqueous solutions with a pH at 3~4. Pure water was selected as blank sample. The excitation wavelength is at 515 nm and the quantum yields were obtained from emission wavelength from 540 nm to 850 nm. We also testify relative QY of samples by using Rhodamine B in aqueous solution (QY=31% in water when excited at 515 nm) as reference. The difference between absolute and relative QY was less than 0.5% for simple FGGC-AuNCs and 4% for CB[7]/FGGC-AuNCs (200 μL CB[7]).

1.4 Cell Culture and Confocal Laser Scanning Microscopy Measurements.

A549 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS) and 1% antibiotics (50 units/mL penicillin and 50 units/mL streptomycin) at 37 °C in a humidified atmosphere containing 5% CO_2. CLSM was used to evaluate the live cell imaging of CB[7]/FGGC-AuNCs nano-assembly. A549 cells with a density of 1.5 × 10^5 cells per dish were seeded onto the glass-bottomed dish and cultured with DMEM supplemented with 10% FBS for 24 h. Then, the culture medium was replaced by CB[7]/FGGC-AuNCs (5 μg/mL) containing culture medium for 6 h. After being thoroughly washed with PBS solution, the cells were irradiated with an LED lamp (515 nm, 100 mW cm^{-2}) for 0.5 min and incubated with fresh full medium for another 2 h. Cells were fixed with 4% paraformaldehyde in PBS solution for 25 min, then stained with DAPI to mark the nucleus. The CLSM was performed by using a fluorescence microscope (Leica TCS Sp8).

1.5 Transient Luminescence Spectroscopy Measurements.

The luminescence lifetimes of FGGC-AuNCs and CB[*n*]/FGGC-AuNCs were recorded on FLS 1000 Photoluminescence Spectrometer. Emission wavelength: 640 nm; Excited wavelength: 515 nm. IRF (instrument response function) of all samples in this study were recorded by using LUDOX AS-30 colloidal silica, 30 wt. % suspension in water. Deconvolution and fit procedures of crude data were directly performed on software of spectrometer.

2. Results and Figures

2.1 Detailed Mathematic Operation

The influence of temperature on CB[7]/FGGC assembly is approximately estimated by Van't Hoff equation:

$$\ln\frac{K_2}{K_1} = -\frac{\Delta H}{R}\left(\frac{1}{T_2}-\frac{1}{T_1}\right)$$

Where K_1 and K_2 represent the binding constants at T_1 and T_2. In our system, T_1=278 K, T_2=338 K, R=8.314, ΔH=-29.56 kJ/mol and K=2×10^6 M^{-1} at 298K obtained by ITC. The approximate results are calculated as: K_2=4.6×10^5 M^{-1}; K_1=6.2×10^6 M^{-1}.

Besides,

$$H + G \leftrightharpoons HG$$

$$K_a = \frac{[HG]}{[H][G]};\quad [H] = [H]_0 - [HG];\quad [G] = [G]_0 - [HG]$$

$$[HG] = \frac{1}{2}\left\{\left([H]_0 + [G]_0 + \frac{1}{K_a}\right) + \sqrt{([H]_0 + [G]_0 + \frac{1}{K_a})^2 - 4[H]_0[G]_0}\right\}$$

Where $[H]_0 \approx [G]_0$= 4×10^{-4} M (concentration of FGGC ligand), the term $1/K_a$ could be neglected since $1/K_a \ll [H]_0$ and the $[HG]$ was approximately unchanged ($[HG]\approx[G]_0$=4×10^{-4} M) when temperature elevated from 5°C to 65°C.

2.2 Data processing of PL decay curves

The fitted multi-exponential decay function is written as:

$$\mathrm{R(t)} = B_1 e^{\frac{-t}{t_1}} + B_2 e^{\frac{-t}{t_2}}$$

Where t_1 and t_2 represent the multi-lifetimes of PL decay by time. The value χ^2 reflects the fitting accuracy. The proportion (α) of each lifetime is calculated by B_1, B_2, and B_3. Accordingly, the average lifetime (t_{avg}) is calculated by:

$$t_{avg}=\alpha_1 t_1 + \alpha_2 t_2$$

The details are listed in Table S1.

Table S1. The detailed parameters of fitting PL decay functions.

CB[7] Volume[a]	t_1 (α_1)	t_2 (α_2)	t_{avg}	χ^2
0 μL	0.687 μs (56.72%)	1.780 μs (43.28%)	1.61 μs	1.026
25 μL	1.054 μs (53.53%)	2.726 μs (46.47%)	1.83 μs	1.202
50 μL	1.615 μs (41.85%)	3.676 μs (58.15%)	2.81 μs	1.083
200 μL	1.420 μs (9.23%)	3.719 μs (90.77%)	3.51 μs	1.074

[a] The total volumes and concentrations of all four samples are identical.

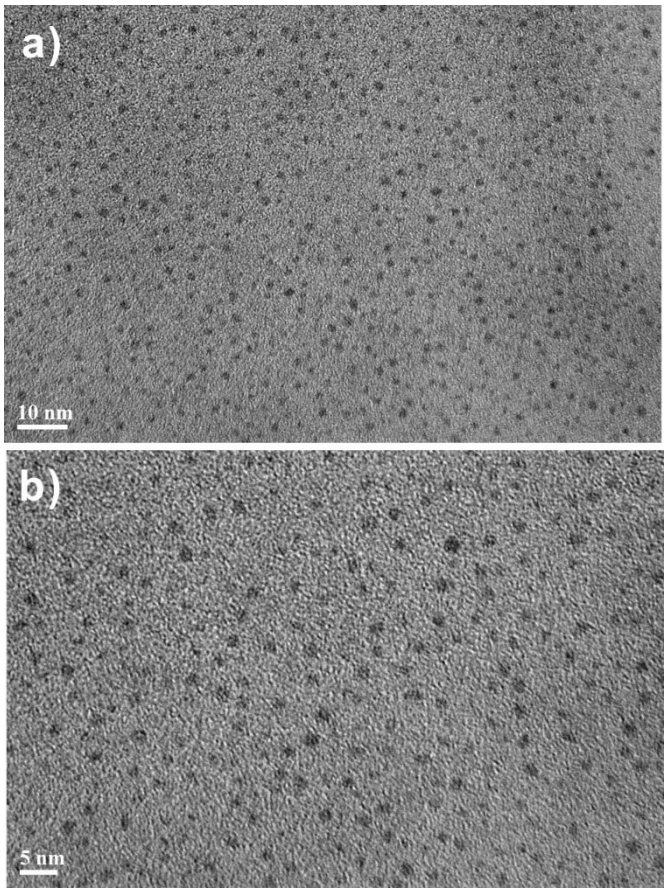

Figure S1. HRTEM images of FGGC-AuNCs at different magnification. (a) Scare bar is 10 nm; (b) Scale bar is 5 nm. The samples were prepared from ~0.1 mM (calculated by AuNCs) aqueous solutions. The average size of nanoclusters referring to TEM photographs is approximately 1.5 nm.

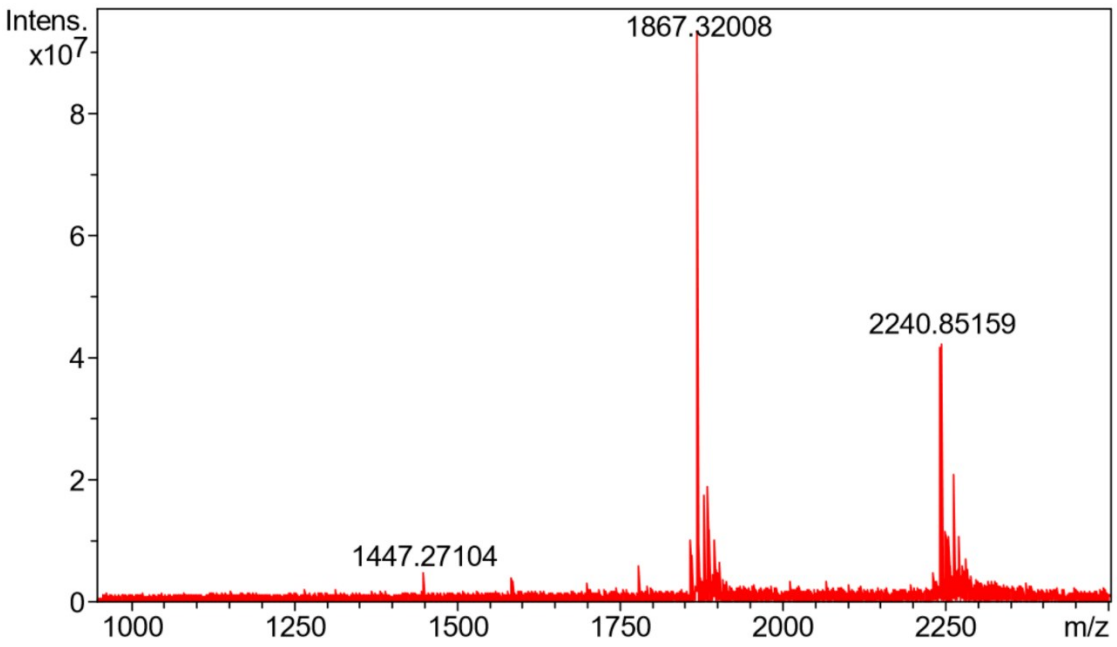

Figure S2. ESI-HRMS spectrum of separated FGGC-AuNCs obtained from the synthesis. The samples were prepared in ultrapure water at a concentration of 1 mg/mL and directly injected into the mass spectrometer. The sample was analysed in polarity-ion positive mode.

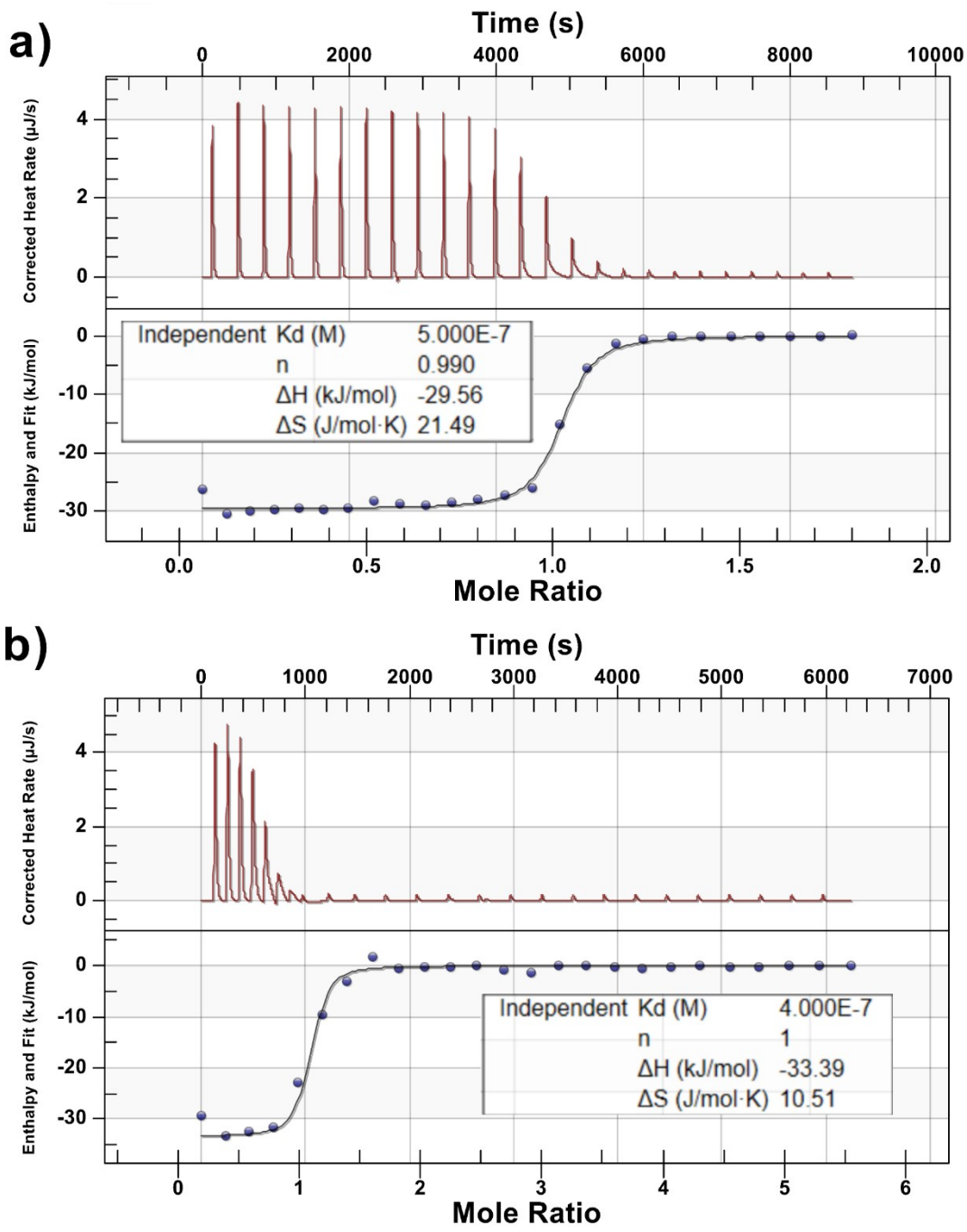

Figure S3. ITC spectra and corresponding thermodynamic values of FGGC peptide titrated with CB[7] in ultrapure water at pH=7.0 and 25 °C. (a) FGGC peptide: 0.31 mM, CB[7]: 1.66 mM. Equilibrium time: 877 s, initial cell volume: 170 µL, injection volume: 1.96 µL; (b) FGGC peptide: 0.1 mM, CB[7]: 1.66 mM. Equilibrium time: 1561 s, initial cell volume: 170 µL, injection volume: 1.96 µL.

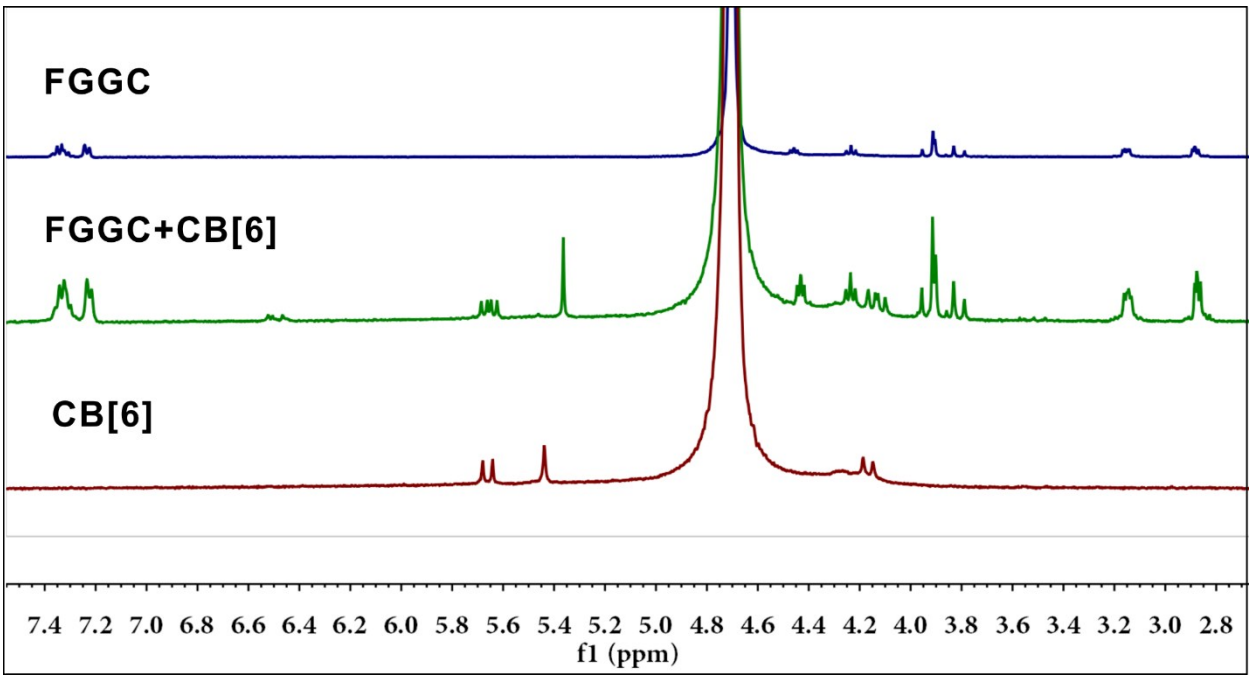

Figure S4. ^{1}H-NMR spectra of FGGC, CB[6], and FGGC/CB[6] mixture in D_2O at 298K. Despite of the existence of slight shift of CB[6] proton signals, ^{1}H-NMR signals of FGGC peptide are hardly changed upon adding CB[6], indicating the formation of the proximity in spatial rather than the host-guest encapsulation between FGGC and CB[6].

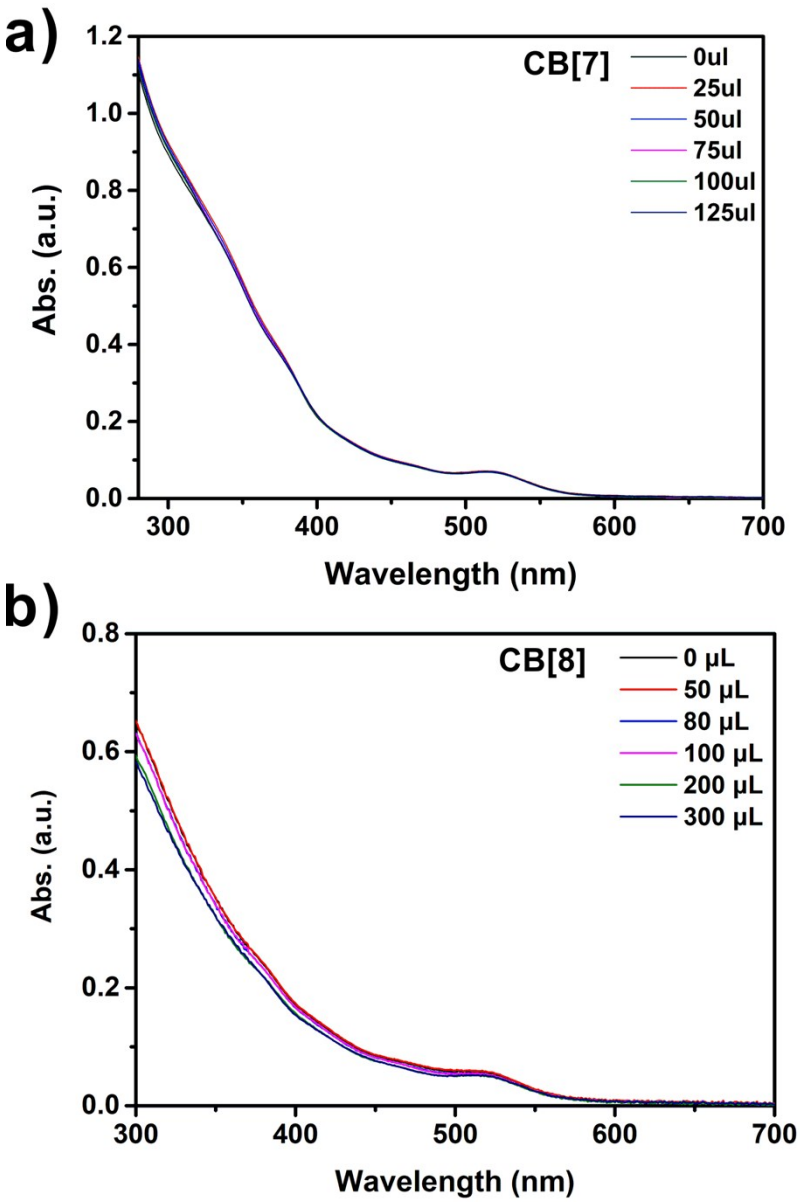

Figure S5. Absorption spectra of FGGC-AuNCs (2.5 mL, ~5 μM) with increasing amounts of CB[7] (a) and CB[8] (b) in aqueous solutions. The adding volume is increasing from 0 to 125 μL for CB[7] (1 mM) and 0 to 300 μL for CB[8] (0.2 mM), respectively. There exist nearly no changes in the absorption spectra of FGGC-AuNCs with adding CB[7] and slightly decline of absorbance in case of CB[8]. Overall, no obvious wavelength changes are presented in absorption spectra.

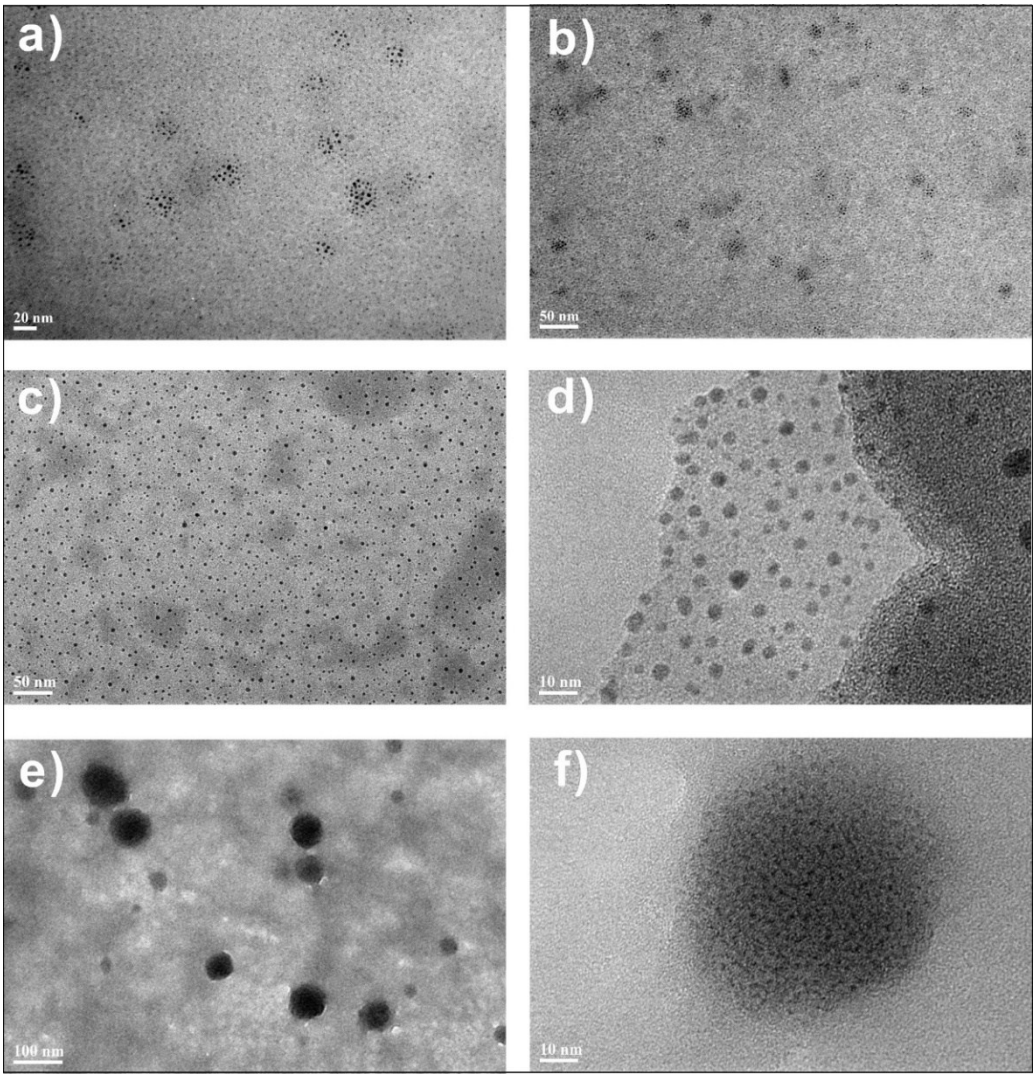

Figure S6. HRTEM photographs of FGGC-AuNCs (2 mL, the absorbance at 515 nm is 0.05) with different amounts of CB[8] (0.1 mM) in aqueous solutions. The images show TEM morphologies of FGGC-AuNCs with adding 80 μL (a, b), 200 μL (c, d), and 300 μL (e, f) CB[8] aqueous solutions at different scales.

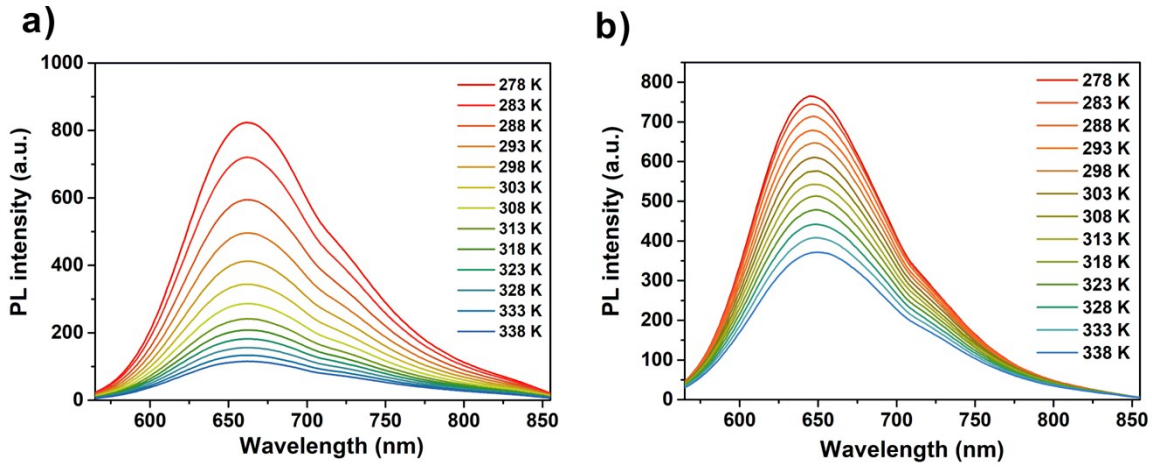

Figure S7. Photoluminescence of FGGC-AuNCs (a) and CB[7]-FGGC-AuNCs (b) in aqueous solutions at different temperatures. Excitation wavelength is 515 nm.

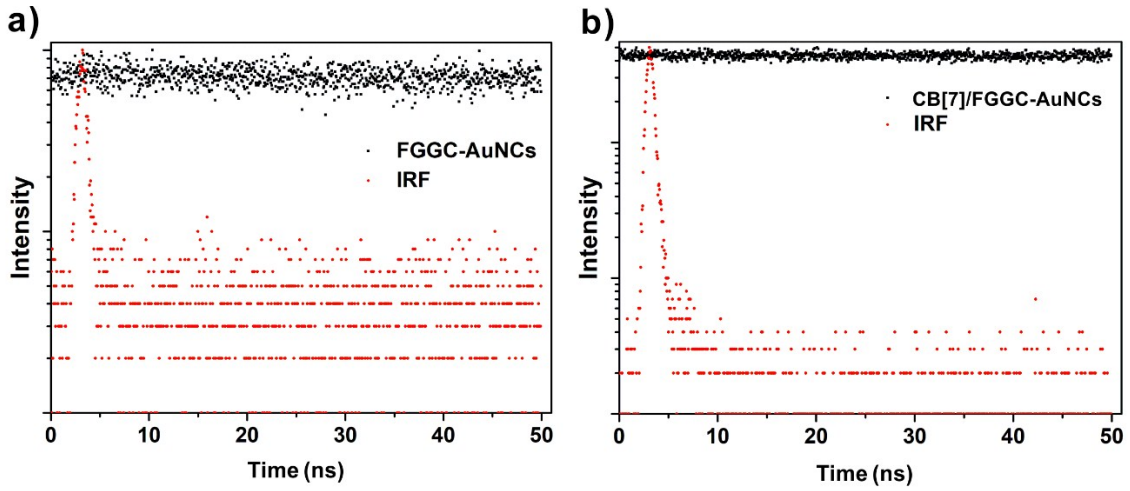

Figure S8. PL decay traces of (a) FGGC-AuNCs and (b) CB[7]/FGGC-AuNCs within 50 ns in aqueous solutions. The red traces are decay signals of colloidal silica, 30 wt. % suspension in water which represent the instrument response function (IRF) curves of spectrometer.

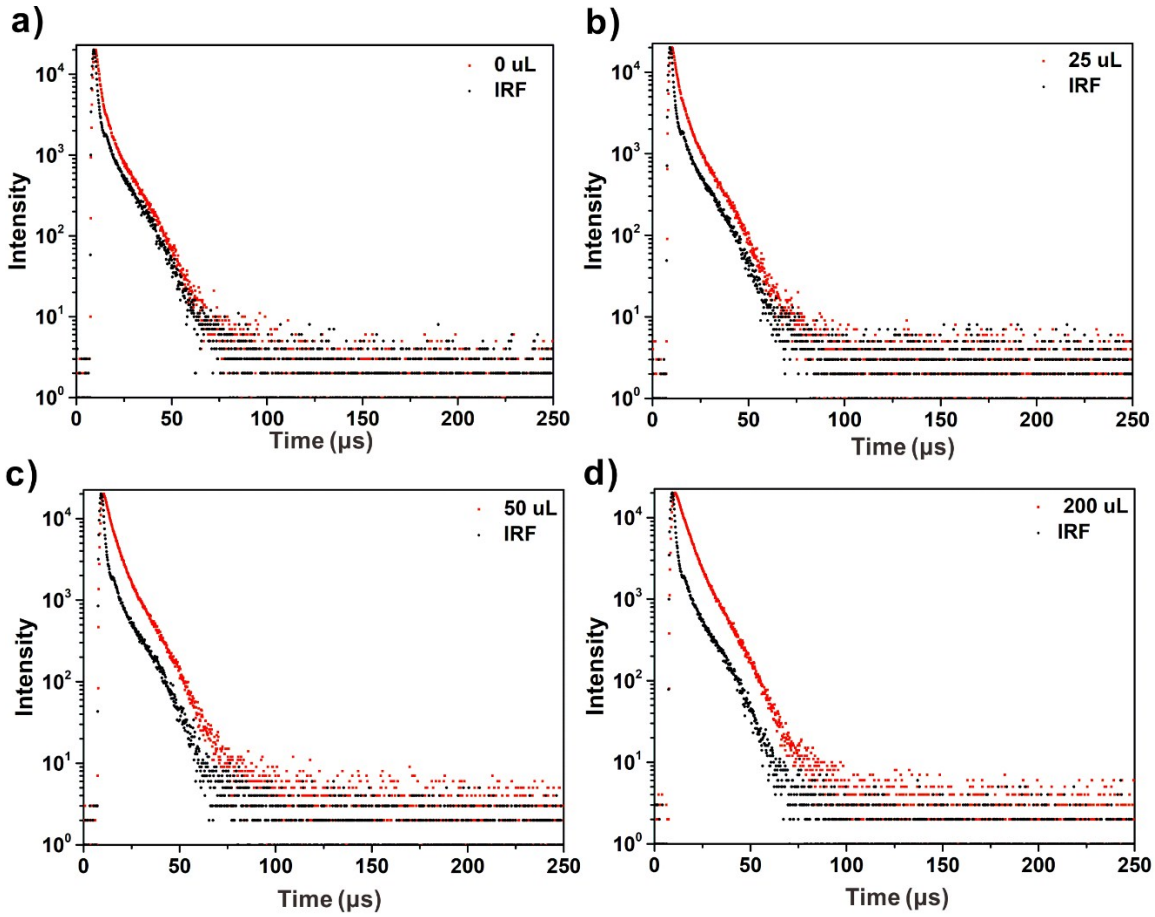

Figure S9. PL decay traces of FGGC-AuNCs with adding 0 μL (a), 25 L (b), 50 uL (c), and 200 uL (d) CB[7] (1mM) in aqueous solutions. The concentrations of FGGC-AuNCs in all samples are identical (absorbance at 515 nm is 0.03). The red traces are decay signals of colloidal silica, 30 wt. % suspension in water which represent the instrument response function (IRF) curves of spectrometer.

Table S2. Optical parameters of FGGC-AuNCs with different volumes of CB[7].

CB[7] volume (μL)	Φ(%)	τ (μs)	k_r (s^{-1},$\times 10^5$)	k_{nr} (s^{-1},$\times 10^5$)
0	7.5	1.61	0.64	7.97
25	11.3	1.83	0.62	4.84
50	24.8	2.81	0.88	2.68
200	51.0	3.51	1.45	1.40