Supporting Information for

Quantum-Size-Effect Accommodation of Gold Clusters with Altered Fluorescence of Dyes

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S1. Chemicals

Tetrachloroauric (III) acid ($HAuCl_4 \cdot 3H_2O$, > 99.99% metals basis) was purchased from Alfa Aesar. L-glutathione (reduced, 99%) and sodium borohydride (99%) were obtained from J&K Scientific Ltd. (Beijing, China). Methanol and ethanol were purchased from Sinopharm Chemical Reagent Co., Ltd. (HPLC, Beijing, China). Ultrapure water (18.2 MΩ) was used in all experiments.

S2. The fluorescence property of gold clusters

Fig. S1 presents the excitation and emission spectra of gold clusters. $Au_{25}(SG)_{18}$ exhibits a maximum emission wavelength at approximately 680 nm when excited at 464 and 523 nm.

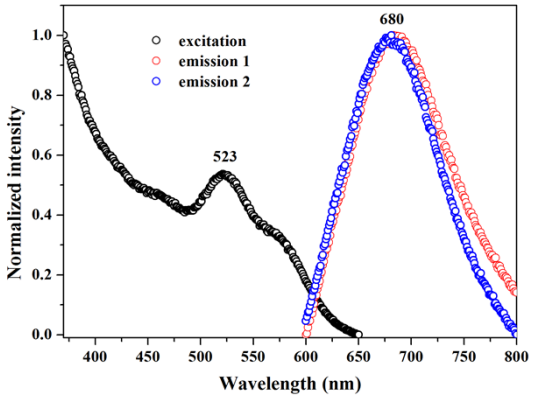

Fig. S1**.** The fluorescence excitation (a) (λ_{em} = 680 nm) and emission (λ_{ex} = 464 and 523 nm) spectra of gold clusters.

S3. The influence of spectral overlap to the fluorescence quenching of dyes

The interactions between gold clusters and several dyes which have different spectral overlap with gold clusters' excitation spectra have been studied. It is noted that the fluorescence of all these dyes can be quenched by the gold clusters (Fig. S2), regardless of their different spectral overlaps with the maximum excitation wavelength of the gold NCs.

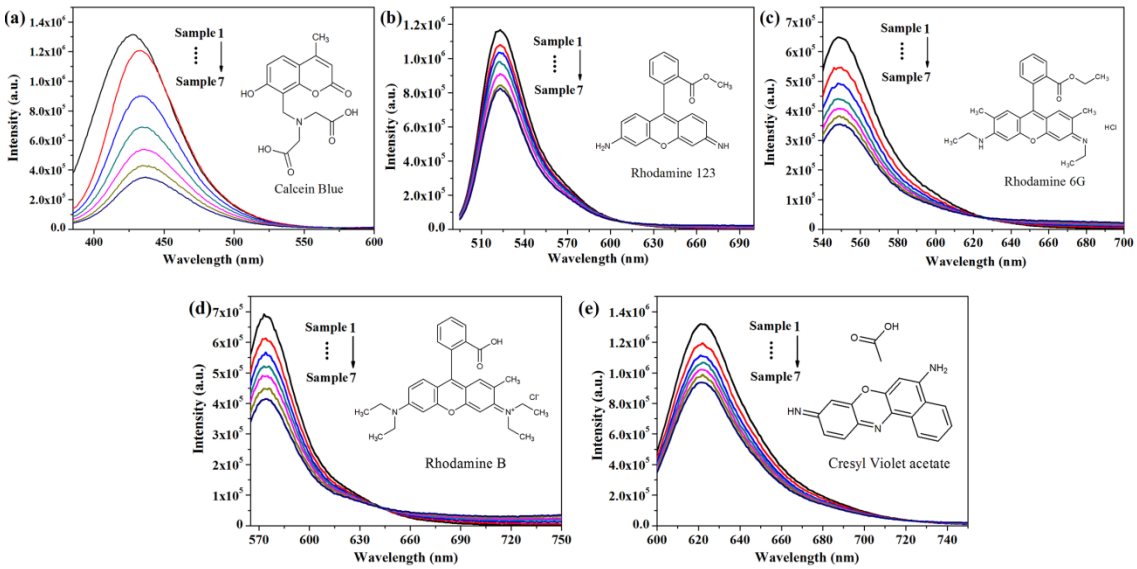

Fig. S2. The fluorescence spectra of Calcein blue (a), Rhodamine 123 (b), Rhodamine 6G (c), Rhodamine B (d) and Cresyl violet acetate (e) with the increasing concentrations of gold NCs: 0 M, 1.6 μM, 3.2 μM, 4.8 μM, 6.4 μM, 8.0 μM and 9.6 μM for samples 1-7 respectively. The concentrations of Calcein blue, Rhodamine 123 and Rhodamine 6G, Rhodamine B and Cresyl violet acetate are 50 nM, 1 nM, 5 nM, 10 nM and 0.5 μM respectively. The insets display the chemical structures of these dyes.

Table S1. The Stern-Volmer equation for the interaction of the dyes with gold NCs.

Dyes	K_{SV} [a] (M^{-1})	R^2 [b]	Intercept [c]
Fluorescein sodium	1.30×10^5	0.99	1.08
Fluorescein isothiocyanate	2.01×10^5	0.97	1.11
Calcein blue	3.57×10^5	0.98	0.36
Rhodamine 123	0.45×10^5	0.97	0.99
Rhodamine 6G	0.79×10^5	0.99	1.07
Rhodamine B	0.64×10^5	0.99	1.02
Cresyl violet acetate	0.36×10^5	0.99	1.06

[a] The Stern-Volmer constant, [b] Correlation coefficient, [c] The intercept of the Stern-Volmer equation.

S4. Experiments of the interactions between the dyes and gold clusters

Fig. S3 presents the fluorescence measurement results of gold NCs at the presence of the dyes. There is almost no change of the fluorescence intensity of the gold NCs, supporting the speculation of no effective FRET at the presence of gold NCs. Also time-resolved fluorescence experiments (Fig. S4) were conducted. Lifetimes of pure Rh 123 and the cluster-Rh123 system are 4.09 ± 0.07 ns and 4.22 ± 0.09 ns respectively, which is consistent with the conclusion based on FDS and FITC.

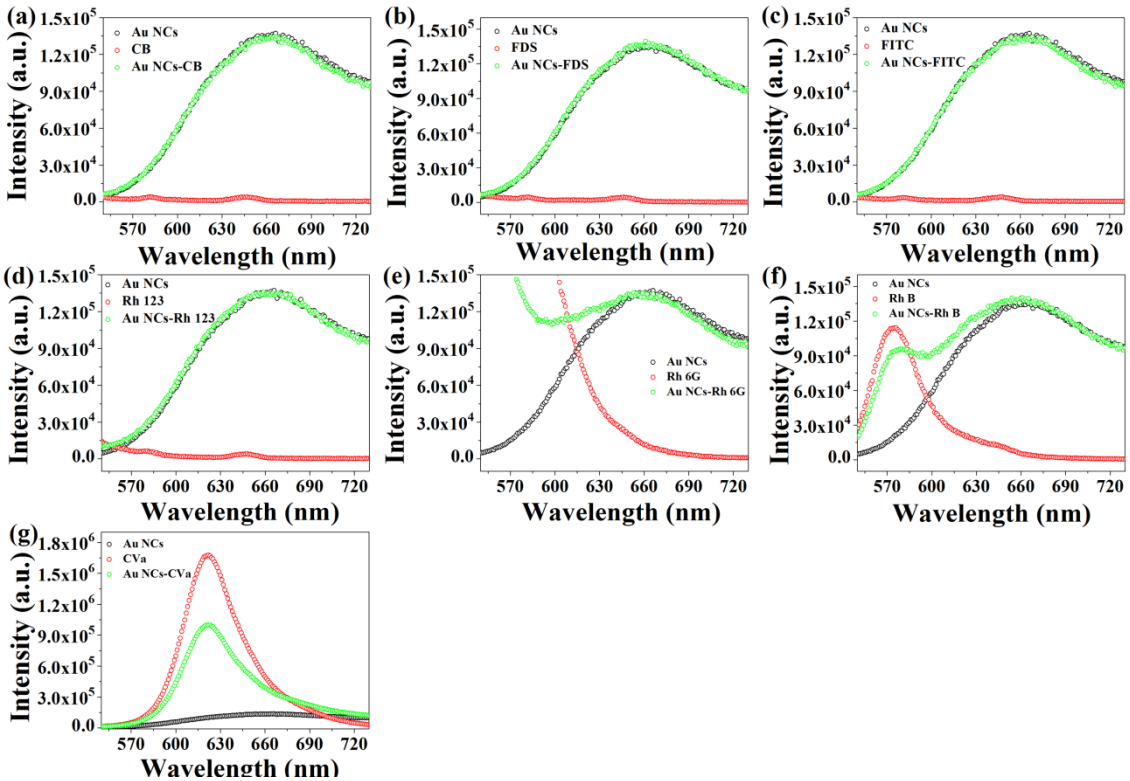

Fig. S3. The fluorescence spectra of the gold NCs (λ_{ex} = 530 nm), comparing with that of the mixtures of dyes and gold NCs, and that of only the dyes: Calcein blue (a), Fluorescein sodium (b), Fluorescein isothiocyanate (c), Rhodamine 123 (d), Rhodamine 6G (e), Rhodamine B (f) and Cresyl violet acetate (g). The concentrations of Calcein blue, Fluorescein sodium, Fluorescein isothiocyanate, Rhodamine 123, Rhodamine 6G, Rhodamine B and Cresyl violet acetate are 50 nM, 2.5 nM, 4 nM, 1 nM, 5 nM, 10 nM and 0.5 μM respectively. The concentration of gold NCs is 9.6 μM for all.

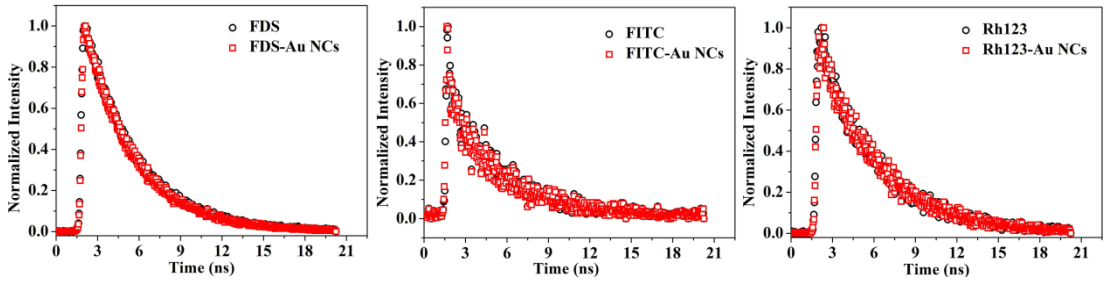

Fig. S4. Time-resolved fluorescence decay curves of FDS, FITC and Rh123 with and without adding gold NCs. The concentrations of FDS, FITC and Rh123 are 2.5 nM, 4 nM and 1 nM respectively. The concentration of gold NCs is 9.6 μM for all.

The magnitude of the fluorescence quenching and the concentration of gold NCs are plotted

according to the Stern-Volmer (SV) equation. The Stern-Volmer quenching constant of Rh123 is 0.45×10^5 M^{-1} which is smaller than FDS and FITC. The gold NCs carry negative charges and Rh123 carries positive charges at neutral environment. Therefore, Rh123 may also readily form nonfluorescent complexes in view of the electrostatic interaction. However, the bond energies of hydrogen bonds may account for a relatively small quenching constant (Table S1), likely due to the weaker bond energy (hence less stable complexes) of N-H···O bond comparing with O-H···O bond for FDS or FITC.

S5. More Details of the Altered Fluorescence of the Dyes

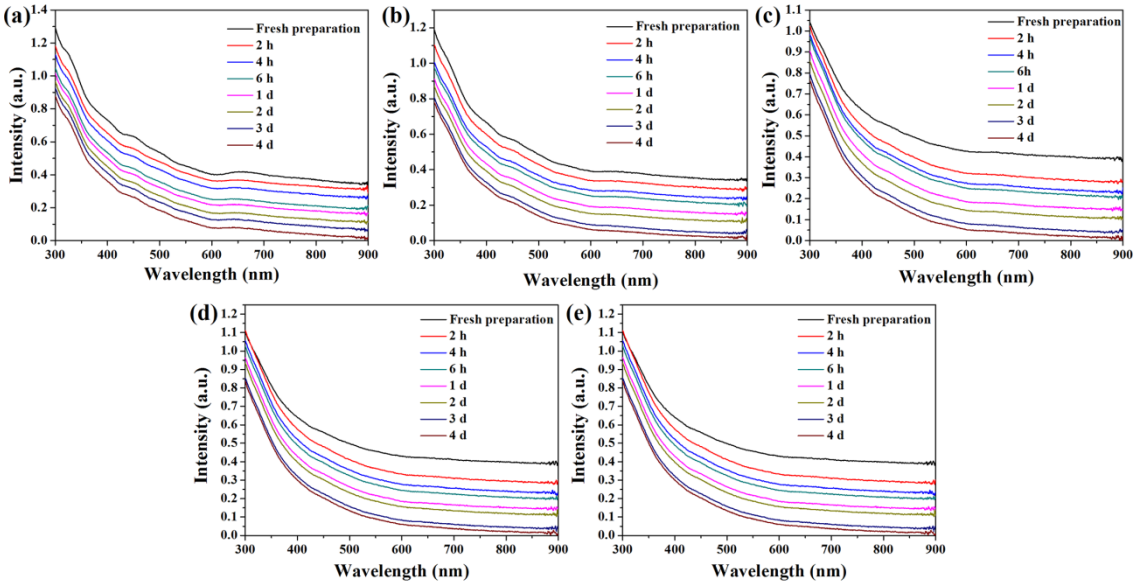

Fig. S5. The changes of absorption spectra of gold nanoclusters and nanoparticles over time at different irradiating time: (a) 0 min, (b) 1 min, (c) 3 min, (d) 6 min and (e) 10 min.

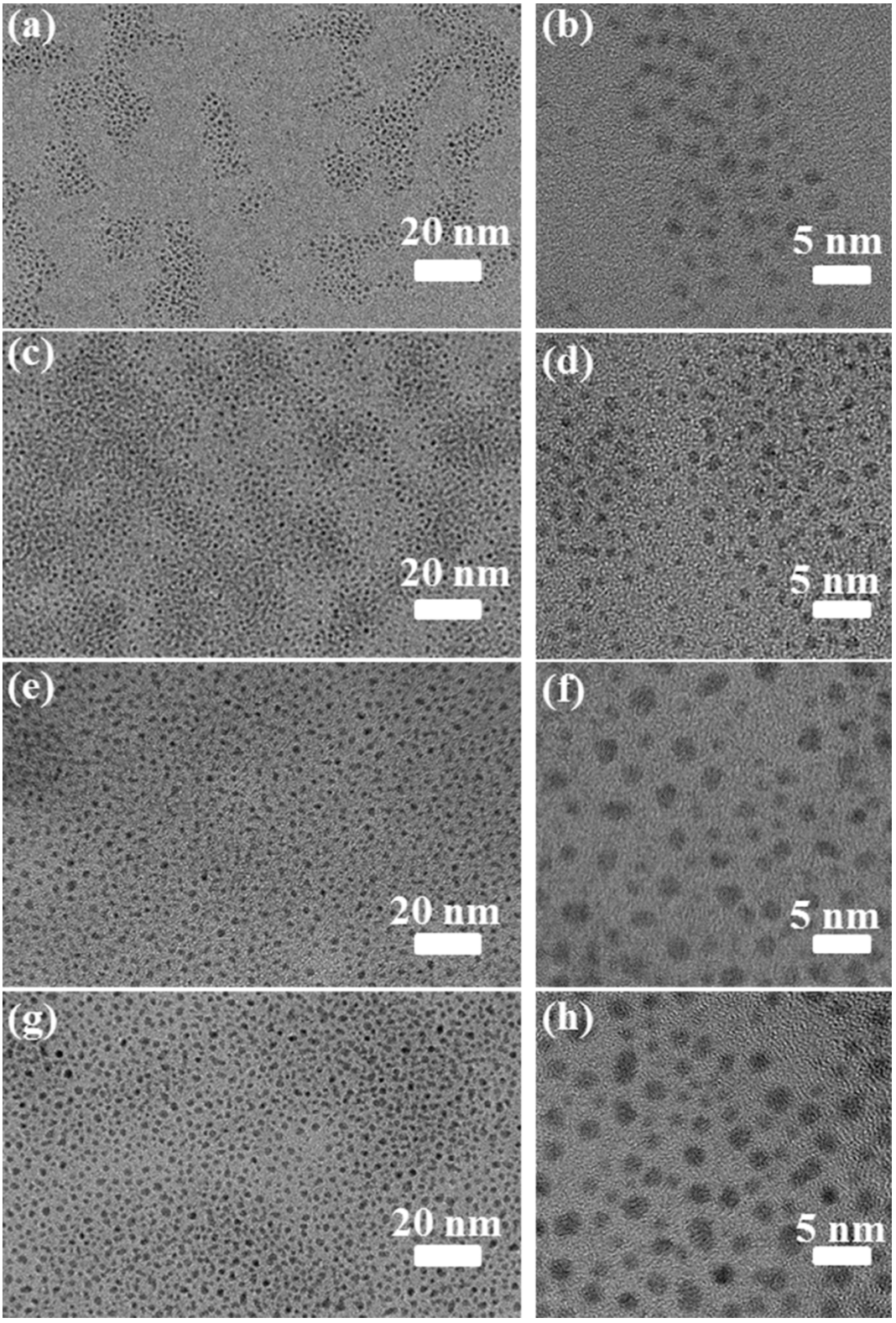

Fig. S6. TEM images and the size distributioins of the samples four days after preparation at different irradiating time: 1 min (a, b,), 3 min (c, d), 6 min (e, f) and 10 min (g, h).

In order to exclude the influence of ligand glutathione to the fluorescence enhancement, with the same irradiating conditions, glutathione instead of the ligand-stabilized gold NCs was irradiated

independently. However, monotonous fluorescence quenching to the dyes under various irradiating conditions were observed, as shown in Fig. S7. Therefore, it is believed that the emission enhancements of the dyes are caused by the built-up gold NPs.

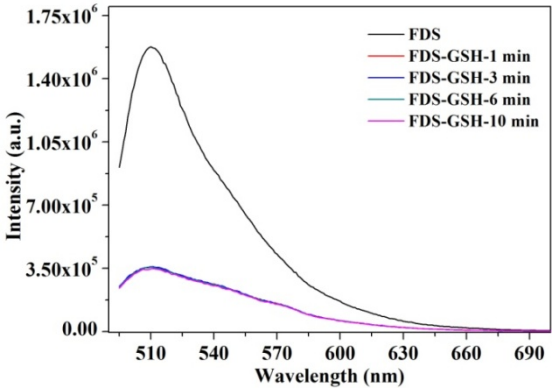

Fig. S7. The fluorescence spectra of FDS (λ_{ex} = 480 nm) without and with the irradiated samples of glutathione at different irradiating time. The concentration of FDS is 2.5×10^{-8} M.

It is still worthy of mentioning that, recently glutathione protected 2-nm and 4-nm gold NPs were found to both display fluorescence quenching for dyes intercalated into DNA (*J. Phys. Chem. C* **2011**, *115*, 20105), but this is not contradictory with our finding in this work as the surfaces of the built-up gold NPs (derived from irradiation of gold NCs) are protected with less glutathione molecules. Also different from biological molecular systems such as DNA where the distances from NPs are tunable simply by choosing different DNA lengths, the distances of gold NPs/NCs with dye molecules are difficult to quantitatively control but may alter with their relative concentrations. At low concentrations (e.g. < 6 ug/ml), gold NPs tend to exhibit a statistical regularity of enhanced fluorescence due to its strong localized surface plasmon resonance (LSPR) effect and adequately dispersive molecules within the LSPR region (*Adv. Exp. Med. Biol.* **2010**, *662*, 407), as depicted in Fig. S8a. Simply for free gold NPs and fluorophores, when the dye concentration is fixed, the distances between NPs and the molecule are determined by the NPs concentration. At a higher NP concentration (Fig. S8b), the distances between NPs and molecule are shorter allowing more dye molecule near the surface of NPs, resulting in fluorescence quenching. When the concentration of analyte is ultralow, the average distance between dyes and NPs is beyond the range of LSPR, hence unaffected. However, for gold NCs (Fig. S8c), the weak bonding and shielding media (i.e., protection ligand of the NCs) dominate the interactions of dyes with gold NCs pertaining to a static quenching mechanism. For such a system, whatever the distances are controlled, no fluorescence enhancement will be gained owing to the non-plasmonic property of gold NCs.

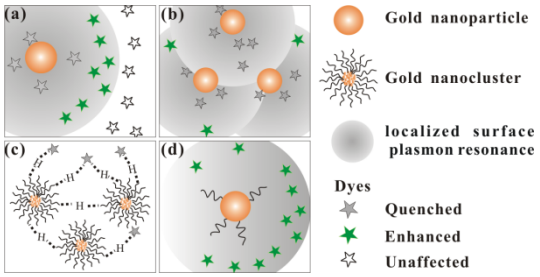

Fig. S8. A sketch showing the fluorescence quenching/enhancing mechanism for dye molecules around gold NPs/NCs. The gray region sketches the localized field of surface plasmon resonance.