Supporting Information

Highly Stable, Luminescent Gold Nanocluster Assemblies Driven by Depletion and Covalent Linker Interactions

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Experimental Section

Materials: Hydrogen tetrachloroaurate trihydrate ($HAuCl_4.3H_2O$), L-glutathione (GSH), sodium borohydride ($NaBH_4$), Pentaerythritol tetrakis(3-mercaptopropionate) (PTMP) were purchased from Sigma Aldrich. Dimethyl sulfoxide (DMSO), dichloro methane (DCM), acetonitrile was purchased from TCI chemicals.

Methods

Synthesis of $Au_{22}(SG)_{18}$: We adhered to a prescribed protocol with slight modifications.[1, 2] Briefly, a freshly prepared aqueous solution of $HAuCl_4$ (1.5 mL, 20 mM) was added to 27.6 mL of ultra-pure water under stirring, followed by the addition of GSH (0.9 mL, 50 mM). The solution turned from yellow to a turbid white upon the addition of GSH. After 3-4 minutes, the pH was adjusted to 11, causing the turbidity to disappear, and the mixture was stirred at 600 rpm for 20 minutes. 10 mM 75 µL $NaBH_4$ was then added dropwise under a reduced stirring of 200 rpm for 30 min. Subsequently, 1 M HCl was added to lower the pH to 2.53, and the mixture was stirred for 24 hours at 200 rpm. After synthesis, the $Au_{22}(SG)_{18}$ solution was rotary evaporated at 50°C, 73 mbar pressure to near dryness. The product was dissolved in 1.2 mL of H_2O and 1.44 mL of isopropanol. The precipitate was centrifuged and freeze-dried to accomplish further studies.

Synthesis of ca-Au_{22} NCs: After the synthesis of $Au_{22}(SG)_{18}$, depletant PEG 11K at different concentration was added to 0.5 mL of Au_{22}NCs and bath sonicated for 2 min, so that PEG got completely dissolved. 10 µL 2.62 M PTMP was poured over the opaque reaction mixture consisting of Au_{22}NCs and PEG 11K and incubated for 12 hours at room temperature. After prolonged incubation, solution was centrifuged at 12000 rpm, ppt was dissolved in DMSO. Then that DMSO solution was again centrifuged at 12000 rpm and the ppt was dissolved in water. After dialysis, this dispersion was taken for furnishing further analyses.

Synthesis of ua-Au_{22} NCs: 10 µL 2.62 M PTMP was added to 0.5 mL of freshly prepared Au_{22}NCs and incubated for 12 hours at room temperature. After incubation, reaction mixture was centrifuged at 12000 rpm, ppt was dispersed in DMSO. This DMSO solution was again centrifuged at 12000 rpm and finally the ppt was dispersed in water. Solution was dialysed and further characterizations were performed.

Synthesis of AuNCs: We adhered to a prescribed protocol for synthesizing luminescent AuNCs.[3] To prepare a 50 mL stock solution containing 2 mM AuNCs, we initially took 43.5 ml of Milli-Q water in a round bottom flask. Subsequently, 5 mL of freshly prepared 20 mM $HAuCl_4$ solution was added to the flask under stirring conditions at 75°C. Following this, 1.5 mL of 100 mM GSH was introduced into the solution. Notably, the initially yellow-coloured solution turned colourless upon the addition of GSH. The reaction was then left to incubate under stirring conditions for 24 hours, during which the yellow colour gradually reappeared. The resulting orange emitting AuNCs were stored at 4°C for future use.

Synthesis of ca-AuNCs: After the synthesis of AuNCs, depletant PEG 11K at different concentration was added to 0.5 mL of AuNCs and bath sonicated for 2 min, so that PEG got completely dissolved. 10 µL 2.62 M PTMP was poured over the opaque reaction mixture consisting of AuNCs and PEG 11K and incubated for 12 hours at room temperature. After prolonged incubation, solution was centrifuged at 12000 rpm, ppt was dissolved in DMSO. Then that DMSO solution was again centrifuged at 12000 rpm and the ppt was dissolved in water. After dialysis, this dispersion was taken for furnishing further analyses.

Instruments: UV-vis absorption spectra were recorded using a JASCO V-750 spectrophotometer. PL spectra were collected with a JASCO F-8350 spectrofluorometer. Field emission scanning electron microscopy (FESEM) analysis was performed using a JEOL JSM IT 800 SHL microscope. For FESEM imaging, samples were drop-cast into glass slide, which was then placed in a vacuum oven for 12 h. Following this, a gold sputter coating was applied to the surface of the glass slide under optimized conditions of 30 mA for 20 seconds. High-resolution transmission electron microscopy (HRTEM) images were captured using a JEOL JEM F200 microscope operating at 200 kV. Atomic force microscopy analysis (AFM) images were captured using Bruker Bio-Atomic Force Microscope. Fourier Transform infrared spectroscopy (FTIR) was carried out utilizing a Thermo Scientific Nicolet iS20 smart Itx-Diamond ATR-IR spectrometer. The CLSM images were obtained using an inverted confocal microscope, Leica (Leica TCS SP8). TCSPC experiment was performed in Fluorolog-3 (HORIBA). Relative photoluminescence quantum yield (PLQY) was calculated from the following equation,

$\varphi_U = \varphi_r \times (I_U / I_r) \times (A_r / A_U) \times (n_U^2 / n_r^2)$ ………………….. (1)

where φ_U is the quantum yield of the sample, φ_r is the quantum yield of the reference, I_U and I_r are the integrated emission intensity of sample and reference respectively, A_U and A_r are the absorbance of sample and reference respectively at the excitation wavelength, n_U and n_r are the refractive index of solvent used for sample and reference respectively.

Radiative (K_r) and non-radiative decay constant (K_{nr}) are quantified from the following equations,

$K_r = \varphi/\tau$ …………………………………… (2)

$K_{nr} = (1-\varphi)/\tau$ …………………………… (3), where φ denotes quantum yield and τ is the average lifetime.

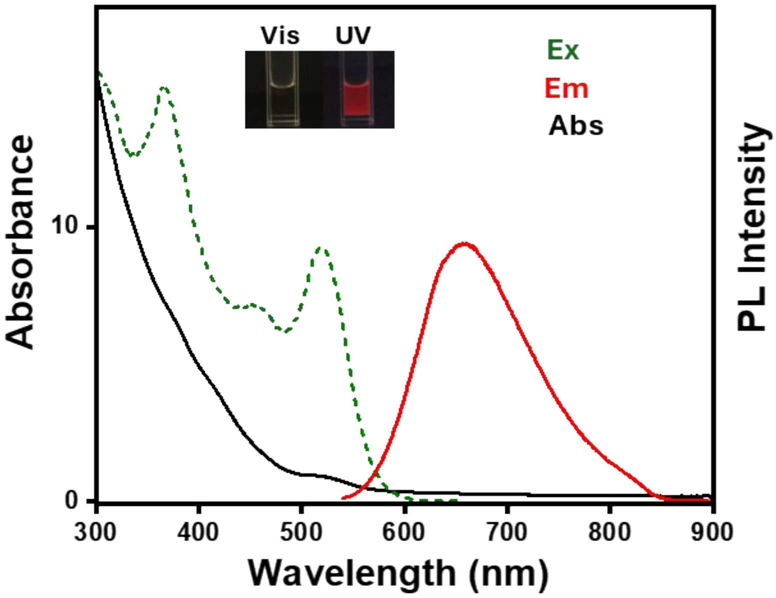


Figure S1. Absorbance (black), emission (red) and excitation (olive green) spectra of $Au_{22}(SG)_{18}$

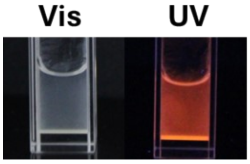


Figure S2. Visual depiction of PTMP mediated assembly of $Au_{22}(SG)_{18}$ taken under visible and UV light respectively.

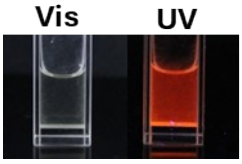


Figure S3. Visual depiction of redispersion of metastable assembly comprising $Au_{22}(SG)_{18}$ and PEG-11K taken under visible and UV light respectively.

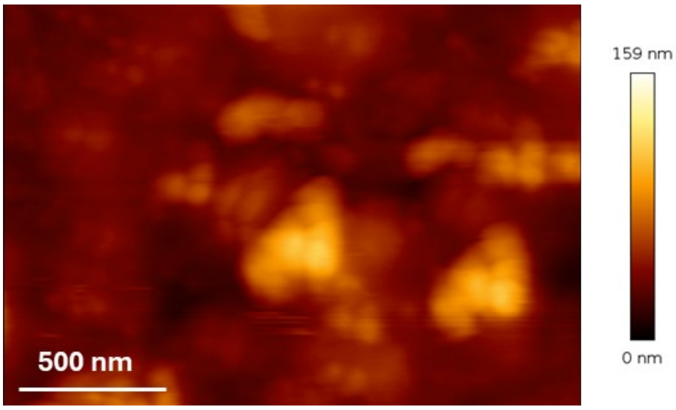


Figure S4. AFM image of ua-Au_{22} NCs.

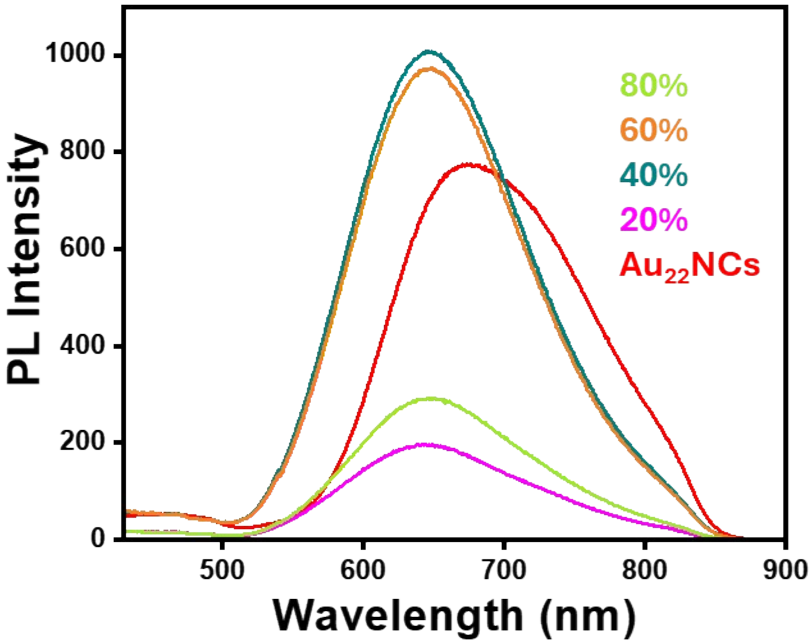


Figure S5. PL spectra of assemblies comprising of PEG 11K at varying concentrations, ranging from 20% (w/v) to 80% (w/v) and Au_{22} NCs. (λ_{ex}: 375 nm)

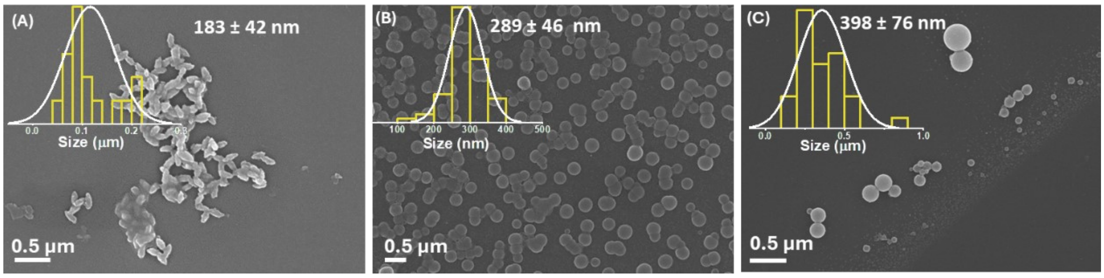


Figure S6. FESEM image of ca-Au_{22} NCs comprising $Au_{22}(SG)_{18}$ and (A) 20% PEG-11K, (B) 40% PEG-11K and (C) 60% PEG-11K. Histograms in inset signify size distribution of corresponding assemblies.

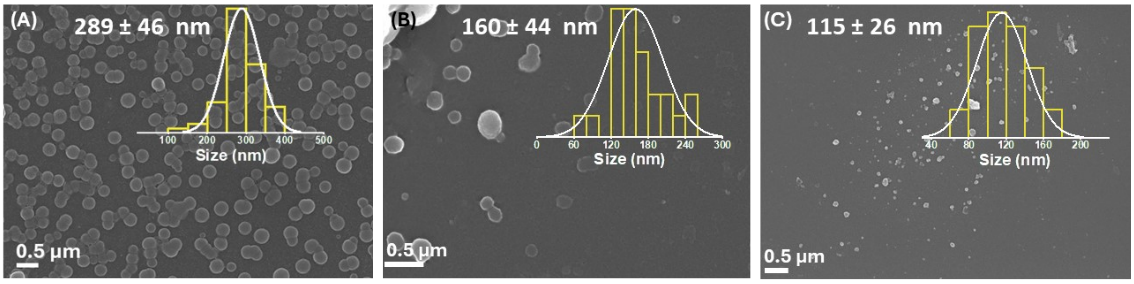


Figure S7. FESEM image of ca-Au_{22} NCs at an incubation of (A) 12 hours, (B) 24 hours and (C) 48 hours. Histograms in inset signify size distribution of corresponding assemblies.

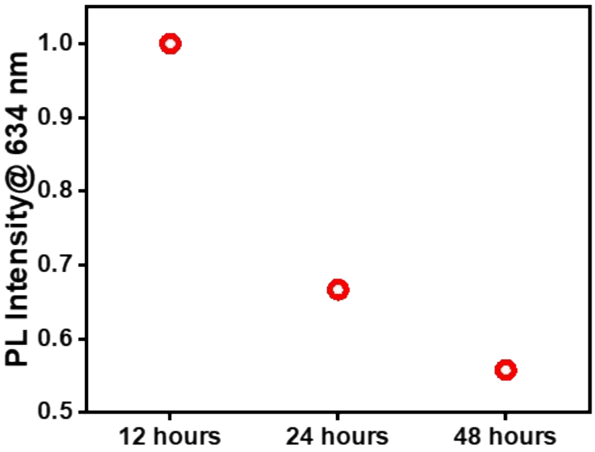


Figure S8. Time dependent PL spectra of ca-Au_{22} NCs.

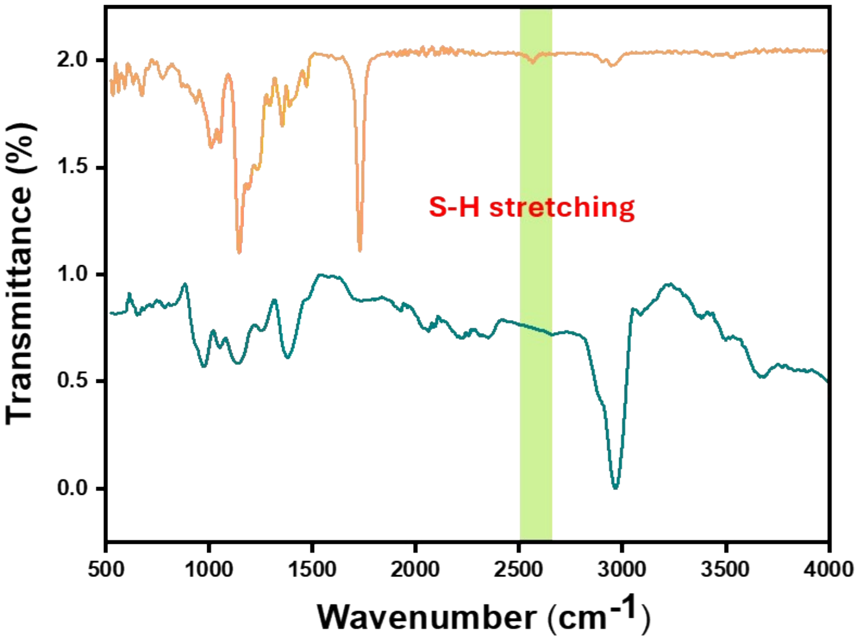


Figure S9. FTIR spectra of PTMP and ca-Au_{22} NCs.

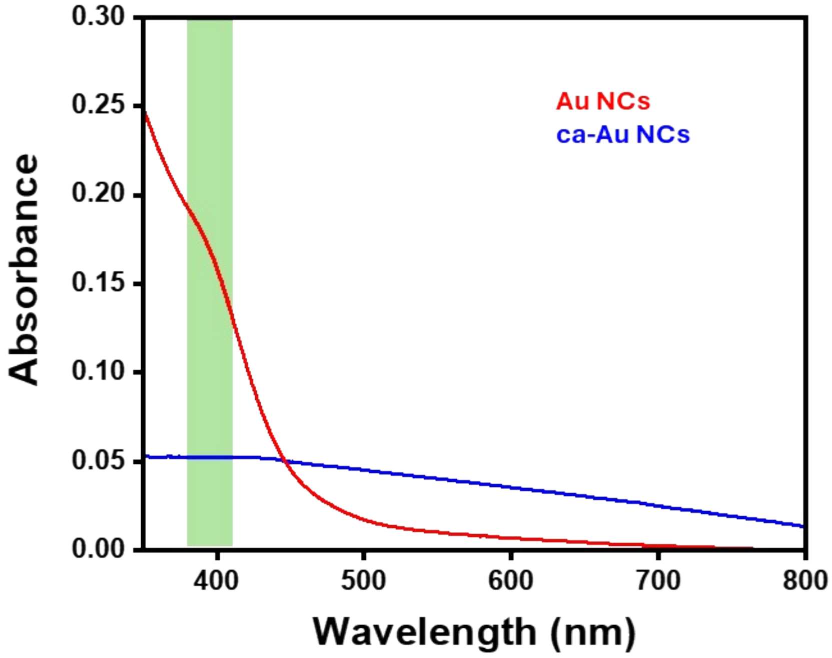
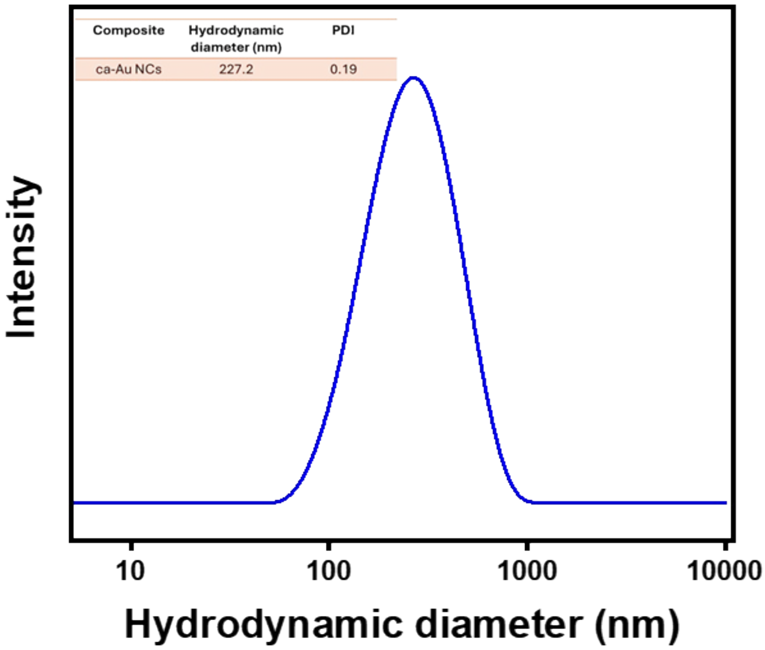


Figure S10. Absorbance spectra of Au NCs and ca-Au NCs.



Composite	Hydrodynamic diameter (nm)	PDI
ca-Au NCs	227.2	0.19

Figure S11. DLS spectra of ca-Au NCs, inset table denotes hydrodynamic diameter and PDI of this assembly.

Table S1. Hydrodynamic diameter and PDI of ca-Au_{22} NCs and ua-Au_{22} NCs.

Composite	Hydrodynamic diameter (nm)	PDI
ca-Au_{22} NCs	**325**	**0.23**
ua-Au_{22} NCs	**648**	**0.38**

Table S2. Summary of the lifetime values of Au_{22} NCs and ca-Au_{22} NCs.

Composite	A_1	τ_1 (ns)	A_2	τ_2 (ns)	τ_{avg} (ns)	K_r (10^7 S^{-1})	K_{nr} (10^7 S^{-1})
$Au_{22}(SG)_{18}$	**1536.71**	**0.91**	**808.44**	**21.81**	**8.11**	**0.171**	**12.16**
ca-Au_{22} NCs	**1361.38**	**1.36**	**1224.05**	**20.83**	**10.58**	**0.397**	**9.05**

Table S3. Summary of the quantum yield of Au_{22} NCs and ca-Au_{22} NCs.

Composite	Slope	PLQY (φ) (%)
Rhodamine 6G	**532494.98**	**95 (Reported)**
$Au_{22}(SG)_{18}$	**14430.29**	**1.39 (Calculated)**
ca-Au_{22} NCs	**44410.34**	**4.2 (Calculated)**

References

1. H. Deng, K. Huang, L. Xiu, W. Sun, Q. Yao, X. Fang, X. Huang, H. A. A. Noreldeen, H. Peng, J. Xie and W. Chen, *Nat. Commun.*, 2022, **13**, 3381.
2. K. Pyo, V. D. Thanthirige, K. Kwak, P. Pandurangan, G. Ramakrishna and D. Lee, *J. Am. Chem. Soc.*, 2015, **137**, 8244-8250.
3. Z. Luo, X. Yuan, Y. Yu, Q. Zhang, D. T. Leong, J. Y. Lee and J. Xie, *J. Am. Chem. Soc.*, 2012, **134**, 16662-16670.