# Supporting information

## Benzyl-rich ligand engineering of the photostability of

### atomically precise gold nanoclusters

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#### 1. Materials.

Chloro(dimethylsulfide)gold(I) (97%), were obtained from TCI. Sodium borohydride ( $\geq$  99%), Sodium methoxide solution (0.5 N), were obtained from Sigma Aldrich. 1,3-bis(diphenylphosphino)propane (dppp, 97%) were obtained from J&K. Dichloromethane ( $\geq$ 99.5%, AR), ethanol ( $\geq$ 99.5%, AR), methanol ( $\geq$ 99.5%, AR), ether ( $\geq$ 99.5%, AR), toluene ( $\geq$ 99.5%, AR), petroleum ether (AR) were obtained from Sinopharm Chemical Reagent Co., Ltd.. All the above reagents were used as received. 4-ethynyltriphenylamine (TPA-C=CH), (4-ethynylphenyl)dimesitylborane (TAB-C=CH), alkynyl tetraphenylethylene (TPE-C=CH) were synthesized according to literature methods<sup>1-3</sup>.

#### 2. Measurements

UV-visible absorption spectra was recorded on SHIMADZU UV-2600 spectrometer using a quartz cell with optical path length of 1 cm. Photoluminescence spectra were recorded by Edinburgh FS-5 fluorophotometer using a capped quartz cell with optical path length of 1 cm. The absolute photoluminescene quantum yield was measured by using Edinburgh FLS1000 Fluorescence Spectrometers. Electrospray ionization mass spectra were recorded on an Agilent Technologies ESI-TOF-MS. The single crystal diffraction data was recorded on a Bruker APEXDUO X-ray Diffractometer (Bruker, Germany). Confocal laser scanning microscope images were performed with Leica TCS SP8 using a HC PL APOCS2 63x objective (NA 1.44, oil).

#### 3. Synthesis and Characterizations

[Au<sub>9</sub>(PPh<sub>3</sub>)<sub>8</sub>](NO<sub>3</sub>)<sub>3</sub>: The methanol solution of AgNO<sub>3</sub> added into a dichloromethane solution of Au(PPh<sub>3</sub>)Cl, the mixture was stirred at room temperature in dark for 1h and then filtered. The filtrate was dried in vacuo and got white powder Au(PPh<sub>3</sub>)NO<sub>3</sub>. NaBH<sub>4</sub> in enthanol was added dropwise into Au(PPh<sub>3</sub>)NO<sub>3</sub> in ethanol, the mixture was stirred at room temperature for 2h and then filtered. After filtering and solvent evaporation under vacuum, the residue was dissolved in dichloromethane. The solvent was dried in vacuo, then washed with tetrahydrofuran and hexane and purified by recrystallisation by vapor diffusion of ether into a dichloromethane solution.

[Au<sub>8</sub>(dppp)<sub>4</sub>](NO<sub>3</sub>)<sub>2</sub>: [Au<sub>9</sub>(PPh<sub>3</sub>)<sub>8</sub>](NO<sub>3</sub>)<sub>3</sub> was dissolved in dichloromethane and a

dichloromethane solution of dppp was added, stirred at room temperature for 1h. To this solution toluene was added then got a precipitate, and centrifuged to collect the purple solid. The solid was washed with toluene and ether, then purified by recrystallisation by vapor diffusion of ether into a dichloromethane solution.

 $[Au_8(dppp)_4(R-C=C)_2](NO_3)_2$ : A methanol solution of  $[Au_8(dppp)_4](NO_3)_2$  was added R-C = CH (R = TPA, TAB, TPE) and sodium methoxide. The mixture was stirred at room temperature for 10h, then treated with water and extracted with dichloromethane. The organic phase was dried by MgSO<sub>4</sub>, filtered and evaporated to dryness to give a pinkish solid, purified by recrystallisation by vapor diffusion of ether into a dichloromethane/methanol (~50/50 v/v) solution.

#### Photostability testing of Au nanoclusters

The samples were prepared by dropping 10  $\mu$ L Au<sub>8</sub> nanoclusters (10<sup>-5</sup> mol/L) dispersed in DCM onto confocal dish. They were deposited on the surface of the glass slide when DCM was removed completely after several minutes. Photostability measurements were performed by confocal laser scanning microscope. Au<sub>8</sub> nanoclusters were illuminated with a 514nm laser at 20% intensity for 5 minutes, and detected on 650-750 nm. The laser illumination occurred without intermittence and each measurement was repeated at least eight times using the same settings for all samples. The fluorescence intensity kinetics was analyzed across the entire movie through LAS X software.

**Cell imaging and confocal co-localization.** HeLa cell were seeded and cultured at 37  $^{\circ}$ C. After incubation with 100µM Au nanoclusters for 6 h, the cells were washed with PBS three times and subjected to imaging analysis using a laser scanning confocal microscope. For Au<sub>8</sub> nanoclusters, the excitation filter was 514 nm and the emission filter was 530-750 nm. For the co-staining assay, HeLa cells were incubated with commercial dyes tracker red and DAPI for 20 min, the cells were washed with PBS three times and subjected to imaging analysis, respectively. For DAPI, the excitation filter was 488 nm, the emission filter was 505-573 nm; for lyso tracker red, the excitation filter was 633 nm and the emission filter was 650-700 nm.

**Photostability testing in cells.** The cells stained with  $Au_8$  nanoclusters were excited at 514 nm for 20% laser power. The first scan of  $Au_8$  nanoclusters was set to 100%, followed

by which the pixel intensity values were averaged and plotted against the scan number. The resulting curve represents the bleaching rate. The scanning speed was 3 s per scan, and the repeated image scans were taken 100 times. The data was analyzed through Leica analysis software.



Fig. S1 Synthesis and characterization of benzyl-rich ligands modified Au<sub>8</sub> NCs. (a) Reaction of divalent Au<sub>8</sub> nanocluster cations ( $[Au_8(dppp)_4]^{2+}$ ) with terminal alkynes (Ar-C=CH) and the structure of benzyl-rich ligands employed in this study featuring a variety of benzene rings. Mass spectrometric characterization of (b) Au<sub>8</sub>-TPA, (c) Au<sub>8</sub>-TAB (d) Au<sub>8</sub>-TPE, along with the structure obtained by X-ray crystallography, yellow = Au, green = N, orange red = B, gray = C, H atoms omitted for clarity. Inset, observed versus calculated isotope pattern.



Fig. S2. ESI-MS spectrum and the crystal structure of Au<sub>8</sub>-Ph dissolved in DCM, yellow = Au, gray = C, H atoms omitted for clarity. Inset, observed versus calculated isotope pattern.  $[Au_8(dppp)_4(Ph-C=C)_2]^{2+}$  at m/z = 1713.22. The cif file obtained from the reported work<sup>4</sup>.



Fig. S3. Absorption spectra of  $Au_8$  nanoclusters solved in DCM (10<sup>-5</sup> mol/L).



Figure S4. Absorption spectra of  $Au_8$  nanoclusters against degradation in DCM. (a)  $Au_8$ -Ph, (b)  $Au_8$ -TPA, (c)  $Au_8$ -TAB, (d)  $Au_8$ -TPE.



Figure S5. X-ray crystal structures of Au8 nanoclusters: partial structures of Au8 nanoclusters. (a) Au8-TPA, (b) Au8-TAB, (c) Au8-TPE. Yellow = Au, green = N, red = B, gray = C, H atoms omitted for clarity. The navy dashed lines correspond to the measured distances.

	Au <sub>8</sub> -Ph	Au <sub>8</sub> -TPA	Au <sub>8</sub> -TAB	Au <sub>8</sub> -TPE
d(Au <sub>edge</sub> -Au <sub>exo</sub> )	2.93, 3.02 Å	3.05, 3.10 Å	3.08, 3.14 Å	3.02, 3.12 Å
$d(Au_{edge}-C_{\alpha})$	3.41, 3.53 Å	3.22, 3.45 Å	3.25, 3.33 Å	3.28, 3.49 Å
$d(Au_{exo}-C_{\alpha})$	2.02 Å	2.02 Å	1.96 Å	2.04 Å
$\alpha(Au_{edge}-Au_{exo}-C_{\alpha})$	84.8, 86.7°	83.2, 74.5°	75.0, 79.0°	78.2, 82.4°
Tilt angle	2.0°	13.2°	13.1°	10.3°

Table S1. Selected structural parameters of Au<sub>8</sub>-Ph, Au<sub>8</sub>-TPA, Au<sub>8</sub>-TAB and Au<sub>8</sub>-TPE



**Fig. S6** PL spectra of (a)  $Au_8$ -Ph, (b)  $Au_8$ -TPA, (c)  $Au_8$ -TAB, (d)  $Au_8$ -TPE in solution, aggregate and solid states, excited at 510 nm. Inset: photograph of  $Au_8$  NCs taken under 365 nm UV irradiation in different states. (e-h) Plots of I/I<sub>0</sub>-1 versus hexane fractions of  $Au_8$  NCs respectively, where I<sub>0</sub> is the PL intensity in pure DCM solution.



Fig. S7. PL spectra of (a) Au8-Ph, (b) Au<sub>8</sub>-TPA, (c) Au<sub>8</sub>-TAB, (d) Au<sub>8</sub>-TPE in DCM-Hexane mixtures (10µM) with different hexane fractions ( $f_{n-Hex}$ ), excited at 510 nm. Inset: photos of Au<sub>8</sub> NCs in DCM/Hexane mixtures ( $f_{n-Hex}$  = 0 and 90%, 70%), taken under 365 nm excitation.

	$\lambda_{ m abs}$ a	ε×10 <sup>4</sup>	λ	em	C	QY	
cluster	(nm)	(L ⋅mol <sup>-1</sup> ⋅ cm <sup>-1</sup> )	(n	ım)	('	%)	
			Soln. <sup>a</sup>	Aggre. <sup>b</sup>	Soln. <sup>a</sup>	Aggre. <sup>b</sup>	$\alpha_{\text{AIE}}{}^{\text{c}}$
Au <sub>8</sub> -Ph	509	1.87	574	692	0.48	3.37	7.02
Au <sub>8</sub> -TPA	511	3.52	578	689	0.42	16.34	38.9
Au <sub>8</sub> -TAB	509	2.95	584	696	0.66	5.73	8.68
Au <sub>8</sub> -TPE	510	2.06	585	696	0.04	0.25	6.25

Table S2 Optical properties of Au<sub>8</sub> nanoclusters

<sup>a</sup> In DCM solution (10<sup>-5</sup> M). <sup>b</sup> Maximum PL intensity of Au<sub>8</sub> clusters in aggregation state, respectively. <sup>c</sup> Values of AIE effect factor, calculated by QY (Aggre.) / QY (soln.).



Figure S8. Absorption spectra of  $Au_8$ -Ph against sulfur etching in 6 hours. (a) NaHS (1  $\mu$ M), (b) cysteine (200  $\mu$ M), (c) Glutathione (10 mM).



Figure S9. Absorption spectra of Au<sub>8</sub>-TPA against sulfur etching in 6 hours. (a) NaHS (1  $\mu$ M), (b) cysteine (200  $\mu$ M), (c) Glutathione (10 mM).



Figure S10. Absorption spectra of Au<sub>8</sub>-TAB against sulfur etching in 6 hours. (a) NaHS (1  $\mu$ M), (b) cysteine (200  $\mu$ M), (c) Glutathione (10 mM).



Figure S11. Absorption spectra of Au<sub>8</sub>-TPE against sulfur etching in 6 hours. (a) NaHS (1  $\mu$ M), (b) cysteine (200  $\mu$ M), (c) Glutathione (10 mM).



Fig. S12. Chemical stability of Au<sub>8</sub> nanoclusters with different ligands against sulfur etching, relative absorbance intensity of peaks in 6 hours. (a) NaHS (1  $\mu$ M), (b) cysteine (200  $\mu$ M), (c) Glutathione (10 mM). In a biological system, a normal cell has only a few micromoles of NaHS, but Cys and GSH levels up to 200  $\mu$ M, 10 mM respectively<sup>5</sup>.



Fig. S13. CLSM images of HeLa cells incubated with Au<sub>8</sub> NCs for 6 h, DAPI and Iyso tracker for 20 min. The images from left to right are the bright-field, fluorescence and merged microscopy images, respectively. Scale bars represent 20  $\mu$ m in all images.

Empirical formula	C148 H132 Au8 N2 P8	
Formula weight	3762.04	
Temperature	120 K	
Wavelength	0.71073	
Crystal system	monoclinic	
Space group	P 2 <sub>1</sub> /c	
Unit cell dimensions	a = 18.9535(19) Å α= 90°	
	b = 19.0583(17) Å $\beta$ = 108.192(3)°	
	$c = 24.088(2) \text{ Å} \qquad \gamma = 90^{\circ}$	
Volume	8266.2(13) Å <sup>3</sup>	
Z	2	
Density (calculated)	1.511 g/cm3	
Absorption coefficient	7.188mm <sup>-1</sup>	
F(000)	3572.0	
Crystal size	0.12 x 0.08 x 0.06 mm <sup>3</sup>	
Theta range for data collection	3.92 to 55.908	
Index ranges	-24<=h<=24, -25<=k<=25, -31<=l<=28	
Reflections collected	132508	
Independent reflections	19806 [R(int) = 0.1170]	
Completeness to theta = 55.908°	99.7 %	
Max. and min. transmission	0.300 and 0.746	
Refinement method	Full-matrix least-squares on F2	
Data / restraints / parameters	19806 / 1209 / 616	
Goodness-of-fit on F2	1.013	
Final R indices [I>2sigma(I)]	R1 = 0.0539, wR2 = 0.1247	
R indices (all data)	R1 = 0.1033, wR2 = 0.1539	
Largest diff. peak and hole	3.78 and -2.70 e.Å <sup>-3</sup>	

**Table S3.** Crystal data and structure refinement for  $[Au_8(dppp)_4(TPA-C\equiv C)_2]^{2+}$ .

Empirical formula	C160 H156 Au8 B2 P8	
Formula weight	3923.95	
Temperature	120.0 K	
Wavelength	0.71073 Å	
Crystal system	monoclinic	
Space group	P 2 <sub>1</sub> /c	
Unit cell dimensions	a = 21.632(2) Å α= 90°	
	b = 19.298(2)) Å β= 113.934(3)°	
	c = 24.055(2) Å γ= 90°	
Volume	9178.3(17) Å <sup>3</sup>	
Z	2	
Density (calculated)	1.420 g/cm <sup>3</sup>	
Absorption coefficient	6.477 mm <sup>-1</sup>	
F(000)	3756.0	
Crystal size	0.14 x 0.1 x 0.07 mm <sup>3</sup>	
Theta range for data collection	4.61 to 55.566°	
Index ranges	-28<=h<=28, -25<=k<=24, -31<=l<=26	
Reflections collected	136144	
Independent reflections	21265 [R(int) = 0.1911]	
Completeness to theta = 55.566°	98 %	
Absorption correction	MULTI-SCAN	
Max. and min. transmission	0.746 and 0.425	
Refinement method	Full-matrix least-squares on F2	
Data / restraints / parameters	21265 / 1290 / 675	
Goodness-of-fit on F2	1.035	
Final R indices [I>2sigma(I)]	R1 = 0.1270, wR2 = 0.2950	
R indices (all data)	R1 = 0.2594, wR2 = 0.3929	
Largest diff. peak and hole	6.30 and -2.76 e.Å <sup>-3</sup>	

Table S4. Crystal data and structure refinement for  $[Au_8(dppp)_4(TBA-C\equiv C)_2]^{2+}$ .

Empirical formula	C164 H142 Au8 P8	
Formula weight	3936.26	
Temperature	173 K	
Wavelength	1.34138 Å	
Crystal system	monoclinic	
Space group	P 2 <sub>1</sub> /n	
Unit cell dimensions	a = 24.4379(19 Å α= 90°	
	b =17.5485(14)Å β= 102.922(2)°	
	c = 39.122(3)Å γ= 90°	
Volume	16352.53(200)Å <sup>3</sup>	
Z	4	
Density (calculated)	1.59876 g/cm <sup>3</sup>	
Absorption coefficient	9.740 mm <sup>-1</sup>	
F(000)	7512.0	
Crystal size	0.12 x 0.14 x 0.21 mm <sup>3</sup>	
Theta range for data collection	5.548 to 117.998°	
Index ranges	-31<=h<=31 -22<=k<=22, -49<=l<=49	
Reflections collected	157524	
Independent reflections	35702 [R(int) = 0.0424]	
Completeness to theta = 117.998°	99.9 %	
Absorption correction	MULTI-SCAN	
Max. and min. transmission	0.752 and 0.467	
Refinement method	Full-matrix least-squares on F2	
Data / restraints / parameters	35702 / 279 / 1748	
Goodness-of-fit on F2	1.010	
Final R indices [I>2sigma(I)]	R1 = 0.0521, wR2 = 0.1619	
R indices (all data)	R1 = 0.0581, wR2 = 0.1669	
Extinction coefficient	n/a	
Largest diff. peak and hole	4.47 and -3.29 e Å <sup>-3</sup>	

**Table S5.** Crystal data and structure refinement for  $[Au_8(dppp)_4(TPE-C\equiv C)_2]^{2+}$ .

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