1 Electronic Supplementary Information

2 Size Specific Emission in Peptide Capped Gold Quantum Clusters with3 Tunable Photoswitching Behavior

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1 **Experimental section**

2 Synthesis of dipeptide H-Leu-Cys-OH:

3 The dipeptide was synthesized by conventional racimization free solution phase peptide synthesis4 method. N terminus was protected with Boc and C terminus with OMe and SH with trityl group.

5 **Trityl protection of Cysteine:** 4 g (33 mmol) of cysteine was dissolved in 60 ml of trifluoroacetic acid 6 (TFA) and to the resulting solution 8.5 g (33 mmol) of Triphenylmethanol (Ph₃COH or TrOH) was added. 7 The reaction mixture was kept for 30 min at room temperature. Then TFA was removed under reduced 8 pressure and diethylether was added to the reddish brown gummy compound. To the ethereal solution, a 9 saturated solution of sodium acetate was added to bring the pH towards neutrality when a thick white 10 precipitate is formed. The precipitate was filtered through suction and washed several times with cold 11 water and small amount of acetone, dried and characterized.

¹H NMR (400 MHz, DMSO-d₆) δ: 2.41-2.45 (1H, m, β-CH₂ of Cys), 2.57-2.61 (1H, m, β-CH₂ of Cys),
2.94-2.97 (1H, m, α-CH of Cys), 7.21-7.36 (18H, m, aromatic Hs of Tr and NH₃⁺). ¹³C NMR (125 MHz,
DMSO-D₆) δ: 33.49, 53.33, 66.00, 126.57, 126.70, 127.45, 127.72, 128.00, 129.09, 144.22, 147.72,
168.14. HRMS (m/z): Calculated for C₂₂H₂₁NO₂S: 363.129, Found: 386.1433 (M+Na)⁺.

16 Synthesis of Boc-Leu-COOH: 1.31 g (10 mmol) of L-Leucine (Leu) was taken in a round bottomed 17 flask. Then 10 mL 1(N) NaOH, 10 mL water and 20 mL 1,4-dioxane were added to it and cooled to 0° C. 18 2.20 g (10.1 mmol) di-tert-butyl dicarbonate (Boc anhydride) was added to the reaction mixture and 19 stirred for 10 h at room temperature. Then volume of the solution was reduced to one third in vacuum. 20 The resulting mixture was acidified with saturated KHSO₄ solution and the aqueous layer was extracted 21 with ethyl acetate (3 × 40 mL). The ethyl acetate extract was dried over anhydrous sodium sulfate and 22 evaporated in vacuum to obtain the white powdered product.

23 Yield: 2.21 g (8.33 mmol, 83.3%).

1 Synthesis of Boc-Leu-Cys(STr)-OMe: 2.21 g (8.33 mmol) of Boc-(L)Leu-OH was dissolved in 12 mL dry N, N-dimethyl formamide (DMF) and it was cooled in an ice bath. H-(L)Cys(STr)-OMe was obtained 2 by neutralization with saturated Na₂CO₃ from its hydrochloride salt and subsequent extraction with ethyl 3 acetate. The ethyl acetate solution was then concentrated to 10 mL and added to the DMF solution 4 5 followed by 1.38 g (9.04 mmol) of HOBt.H₂O and 1.95 g (9.5 mmol) of N, N-dicylohexylcarbodiimide (DCC). The reaction mixture was allowed to come at room temperature and stirred for 24 hours. After 24 6 hrs reaction mixture was diluted with ethyl acetate and filtered to separate N, N-dicyclohexylurea (DCU). 7 The ethyl acetate layer was washed with 1(N) HCl (3×30 mL), brine (2×30 mL), saturated sodium 8 carbonate solution (2 \times 30 mL) and brine (2 \times 30 mL). The organic layer was dried over anhydrous 9 sodium sulfate and evaporated to obtain the yellowish gummy product. The product was purified through 10 silica gel column chromatography using pet ether/ethyl acetate (8:1) as eluent to obtain the pure gummy 11 product. 12

13 Yield: 4.5 g (7.6 mmol, 91.2%).

¹H NMR (500 MHz, CDCl₃) δ: 0.93-0.95 (6H, m, δ-CH₃ of Leu), 1.43 (10H, s, γ-CH of Leu of Boc-CH₃),
1.61-1.70 (2H, m, β-CH₂ of Leu), 2.60-2.67 (2H, m, β-CH₂ of Cys), 3.69 (3H, s, OMe), 4.11 (1H, brs, αCH of Leu), 4.49-4.53 (1H, m, α-CH of Cys), 4.88 (1H, brs, NH), 6.51-6.52 (1H, d, J=7 Hz, NH), 7.207.39 (15H, m, aromatic H of STr). ¹³C NMR (125 MHz, CDCl₃) δ: 22.13, 23.08, 24.81, 28.44, 33.83,
41.62, 51.34, 52.68, 67.00, 127.01, 128.02, 128.12, 129.63, 144.42, 170.70, 172.30. HRMS (m/z):
Calculated for C₃₄H₄₂N₂O₅S: 590.281, Found: 613.1810 (M+Na)⁺, 629.1703 (M+K)⁺.

Synthesis of Boc-Leu-Cys(STr)-OH: 4.5 g (7.6 mmol) of Boc-Leu-Cys(STr)-OMe was taken in a round bottomed flask and dissolved in 50 mL of methanol. 16 mL of 1(N) NaOH was added to it and kept under stirring condition for 6 hours. The progress of hydrolysis was monitored by thin layer chromatography (TLC). After the completion of the reaction, as indicated by TLC, the methanol was removed in vacuum. The aqueous part was then taken in 50 mL water and washed with diethyl ether (2 × 30 mL). The 1 remaining solution was acidified with 1(N) HCl and extracted with ethyl acetate (3 × 40 mL). The ethyl 2 acetate extract was dried over anhydrous sodium sulfate and evaporated in vacuum to obtain a white 3 powdered product.

4 Yield: 4.23 g (7.5 mmol, 98.2%).

¹H NMR (400 MHz, DMSO-d₆) δ: 0.85-0.86 (6H, m, δ-CH₃ of Leu), 1.24 (9H, s, Boc), 1.60-1.62 (3H, m,
β-CH₂ and γ-CH of Leu), 2.39-2.51 (2H, m, β-CH₂ of Cys), 3.88-4.51 (2H, α-CH of Leu and Cys), 6.86
(1H, brs, NH), 7.13-8.03 (15H, m, aromatic-H of Tr), 8.11 (1H, brs, NH), 12.79 (1H, brs, COOH). ¹³C
NMR (100 MHz, DMSO-D₆) δ: 21.54, 22.84, 24.11, 28.11, 33.04, 33.28, 50.85, 51.05, 52.66, 65.90,
65.97, 126.16, 126.54, 126.71, 127.43, 127.70, 127.97, 128.26, 128.96, 129.50, 144.13, 147.70, 155.13,
171.38, 172.30. HRMS (m/z): Calculated for C₃₃H₄₀N₂O₅S: 576.226, Found: 599.0681 (M+Na)⁺.

11 Synthesis of H-Leu-Cys(STr)-OH: 4.2 g (7.5 mmol) of Boc-Leu-Cys(STr)-OH was dissolved in 12 minimum volume of formic acid then the reaction mixture was stirred for seven hours and progress of the 13 reaction was monitored by thin layer chromatography. Then excess formic acid was removed under 14 vacuum, dissolved in water and neutralized with ammonium hydroxide solution. The solution was then 15 removed under vacuum to get the solid product.

16 Yield: 3.5 gm (7.4 mmol, 98%)

¹H NMR (400 MHz, DMSO-d₆) δ: 0.84-0.87 (6H, m, δ-CH₃ Leu), 1.31-1.76 (3H, β-H and γ-CH of Leu),
2.36-2.44 (β-CH₂ of Cys), 3.49-3.52 (1H, m, α-CH of Leu), 3.75-3.78 (1H, m, α-CH of Cys), 7.11-7.33
(19H, aromatic Hs, NH₃⁺, NH). ¹³C NMR (100 MHz, DMSO-d₆) δ: 21.57, 22.88, 24.15, 24.21, 33.06,
33.30, 50.88, 51.08, 52.64, 55.85, 65.93, 66.00, 126.21, 126.59, 126.75, 127.47, 127.73, 128.01, 128.30,
129.00, 129.54, 144.16, 171.42, 172.39. HRMS (m/z): Calculated for C₂₈H₃₂N₂O₃S: 476.213, Found:
499.1309 (M+Na)⁺.

Synthesis of H-Leu-Cys-OH: 3.4 g (7.1 mmol) of H-Leu-Cys(STr)-OH was dissolved in 60 mL of dry dichloromethane. Triethylsilane (25 mg, 21.3 mmol) was added to the solution and stirred for 10 min. 50 mL trifluoroacetic acid was then added and the reaction mixture was stirred for 1 hour. The solution was then concentrated under reduced pressure and diethyl ether was added. The precipitate formed in the reaction was then dried and dissolved in minimum volume of methanol and re-precipitated by adding diethylether, this process was repeated three times and the solid was dried in high vacuum pump to get the final product as white solid.^{S1}

8 Yield: 1.1 gm (5 mmol, 70.4%)

¹H NMR (400 MHz, DMSO-d₆) (Fig. S1) δ: 0.84-0.87 (6H, m, 2 δ-CH₃ of Leu), 1.6-1.62 (3H, m, 2 β-H
and 1 γ-H of Leu), 2.38-2.40 (2 β-H of Cys), 3.98 (1H, m, α-H of Leu), 4.17-4.18 (1H, m, α-H of Leu),
8.03 (1H, m, NH₃⁺). ¹³C NMR (100 MHz, DMSO-d₆) (Fig. S2) δ: 21.88, 22.87, 23.55, 25.75, 40.64,
59.20, 64.51, 169.53, 171.10. HRMS (m/z) (Fig. S3): Calculated for C₉H₁₈N₂O₃S: 234.104, Found:
235.0579 (M+H)⁺.

14 Synthesis of the gold quantum clusters

15 The quantum clusters, QC1, QC2 and QC3 were synthesized following the procedures similar to the 16 methods reported previously.^{S2}

Synthesis of QC1: 10 mg of the dipeptide Leu-Cys was taken in a 50 mL round bottomed flask and it 17 was dissolved in 4 mL of 50 mM phosphate buffer of pH 7.46. 2 mg of HAuCl₄ (tetra chloro auric acid) 18 in 500 μ L MilliQ water was added to that solution. The round bottomed flask was then fitted with a bulb 19 condenser and the reaction environment was made inert by using nitrogen gas balloon fitted to a 20 condenser. The total set up was put on an oil bath with vigorous stirring having bath temperature 140 °C. 21 22 2 mg of sodium borohydride (NaBH₄) in 500 µL MilliQ water was then injected / added to the hot reaction mixture slowly. The progress of reaction was monitored using fluorescence spectroscopy. After 23 16 hrs of reaction time, the total solution became very light yellow in colour. This solution showed blue 24

colour fluorescence behaviour on irradiation at 365 nm with an UV torch (Fig. 1 in the main text).
 Methanol was added to this aqueous solution to make 1:1 ratio of the water-methanol mixture. This was
 centrifuged for 20 minutes at 13,000 rpm to remove any excess dipeptide ligand and bulk gold. Methanol
 was then removed from the supernatant by evaporation and the rest was lyophilized to obtain powdered
 dry sample. This QC1 was redissolved in water and used for all experiments.

6 Synthesis of QC2: The same procedure was followed for QC2 synthesis as for the synthesis of QC1. 7 However, the amount of HAuCl₄ was 2 mg in this case and 2 mg of trisodium citrate was used as the 8 reducing agent. The amount of the ligand, Leu-Cys was also increased to 16 mg for this synthesis and the 9 bath temperature was kept at 160 °C. After 16 hrs of reaction time, the total solution became yellow in 10 colour. This solution showed green colour fluorescence behaviour on irradiation at 365 nm with an UV 11 torch (Fig. 1 in the main text). Methanol was added and centrifuged as before. This QC2 was redissolved 12 in water and used for all experiments.

Synthesis of QC3: QC3 was also synthesized according to the procedures used for QC1 and QC2. Here, 14 10 mg of Leu-Cys, 2 mg of HAuCl₄ and 4 mg of trisodium citrate were taken to successfully synthesize 15 QC3. After 16 hrs of reaction time at 140 °C bath temperature, the total solution became yellow in colour. 16 This solution showed red colour fluorescence behaviour on irradiation at 365 nm with an UV torch (Fig. 1 17 in the main text). Methanol was added and centrifuged as before. This QC2 was redissolved in water and 18 used for all experiments.

19 Conductivity and Photoresponse studies

To study the current vs. voltage nature of the red and green quantum clusters and their phororesponse nature device was fabricated with indium tin oxide (ITO) coated glass slides. For this, two same sized (approx. 2.5 cm \times 1.2 cm) ITO coated glass slides were taken and at the middle, an area of 0.9 cm \times 1.2 cm was etched with Zn dust/HCl to make a non-conducting area. Both the sides of this non conducting area (0.8 cm \times 1.2 cm) remain ITO coated which can serve as electrodes. Then a device was fabricated by

1 drop-casting a solution of the clusters (15 mg of lyophilized quantum cluster in 500 μ L water) on the etched non conducting area. Then the sample containing ITO coated glass slide was dried under high 2 vacuum for two days to obtain a thin layer of the sample. Two copper wires were then connected with the 3 two ITO electrodes with the help of conducting silver paste to complete the device. The current-voltage 4 5 (I-V) measurement between two electrodes was carried out in the voltage range 0-5 Volt by using Keithley Electrometer 6517A. The photocurrent was measured under illumination of white light 6 irradiance of 0.65 Wcm⁻² which was obtained by using Kratos universal Arc Lamp, Model 1152/1144 7 (150XE/200HG-XE). The time-resolved photocurrent measurement was carried out by illuminating the 8 light for 30 s and then switching off the light for another 30 s periodically under an applied bias of 5 V. In 9 all the experiments films were kept in a vacuum chamber with a transparent window. 10

11 Capacitance Measurements

12 Frequency-dependent dielectric constant measurements of the devices (made of green and red emitting13 gold clusters) were performed with an impedance analyzer of Agilent 4192A.

14 Instrumentation

15 UV-Vis spectroscopic analysis

We used a Cary Varian 50 scan UV-Vis optical spectrometer equipped with 'Cary Win' UV software toelucidate the optical properties of gold quantum clusters.

18 Fluorescence spectroscopy

Fluorescence studies of gold quantum clusters in a sealed cuvette were carried out in a Perkin Elmer LS55
Fluorescence Spectrometer instrument. All the experiments were carried out with the excitation slit width
5 nm and emission slit width 5 nm.

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1 Time-Correlated Single Photon Counting (TCSPC) study

2 TCSPC measurements were performed by means of Horiba Jobin Yvon IBH having MCP PMT
3 Hamamatsu R3809 detector instrument and all data were fitted using Data Station v2.3. We have used
4 NANO-LED source for excitation of samples at 340 nm and LASER source for excitation of samples at
5 440 nm.

6 MALDI-TOF MS study

7 The MALDI-TOF MS analyses were done using Bruker Daltonics flex Analysis mass spectrometer.

8 TEM study

9 TEM study of the red clusters was carried out in a JEOL 2100 KeV Ultra High Resolution Field Emission
10 Gun (UHR FEG) TEM with voltage 200 KeV.

11 FTIR study

12 The FT-IR spectra were taken by using Shimadzu (Japan) model FT-IR spectrophotometer. In the solid13 state FT-IR studies, powdered were mixed with KBr for preparing thin films.

14 X-ray Photoelectron Spectroscopic (XPS) Study

15 XPS analysis of dried NIR quantum cluster was carried out by using an X–ray photoelectron
16 spectroscopic (XPS, Omicron, model: 1712–62–11) method. Measurement was done by using an Al-Kα
17 radiation source under 15 kV voltages and 5 mA current.

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1 Figures:



9 Fig. S1. ¹H NMR spectrum of Leu-Cys in DMSO-d₆.



17 Fig. S2. ¹³C NMR spectrum of Leu-Cys in DMSO-d₆.



9 Fig. S3. HRMS spectrum of Leu-Cys.



15 Fig. S4. Time dependent fluorescence spectra showing the formation kinetics of blue (QC1), green (QC2)16 and red (QC3) emitting gold clusters.



9 Fig. S5. Comparison of the emission of the as-synthesized Au_5 , Au_{13} and Au_{23} with the emission 10 predicted by the jellium model.





Fig. S6. (a) The emission peak at 490 nm for QC2 (black line) remains unmoved on changing excitation wavelength. (b) No alternation of the peak at 628 nm for QC3 with change in excitation (black line).

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1 Table S1. Quantum yields and excited state lifetime details of the clusters from TCSPC measurements.

Clusters (Emitting Colour)	Quantum Yield (%)	τ ₁ (%) ps	τ ₂ (%) ns	τ ₃ (%) ns	τ ₄ (%) μs	τ_{av} ns
Au ₅ L ₃ (blue)	2.31	320 (87.1)	1.7 (9.4)	6.8 (3.5)	-	0.68
Au ₁₃ L ₇ (green)	0.42	180 (81.8)	1.2 (12.2)	5.0 (6.0)	-	0.59
Au ₂₃ L ₁₇ (red)	1.69	2399 (83.5)	27.3 (6.6)	287.9 (4.5)	1.96 (5.4)	122.86

3 Table S2. Reaction conditions, FL, FLE values and cluster sizes and calculated band gap from FLE.

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Amount of reagents in synthesis	Reducing agent used	Temperatu re (°C)	Emittin g Colour	Fluorescen ce excitation (FLE) (nm)	Fluorescen ce emission (FL) (nm)	Calculate d Band gap (eV) from FLE	Size of the clusters from MALDI -TOF MS analysis
10 mg LC + 2 mg HAuCl ₄ + 2 mg Na- borohydrid e	Sodium Borohydrid e	140	Blue	FLE: 333	FL: 418 nm	3.73	Au ₅ L ₃ + Au ₈ L ₂ (small)
$\begin{array}{c} 16 \text{ mg LC} \\ + 2 \text{ mg} \\ HAuCl_4 + \\ 2 \text{ mg Na-} \\ \text{citrate} \end{array}$	Sodium Citrate	160	Green	FLE: 376 (major), 346, 410	FL: 490	3.30	Au ₁₃ L ₇
10 mg LC + 2 mg HAuCl ₄ + 4 mg Na- citrate	Sodium Citrate	140	Red	FLE: 410, 376	FL: 628 (major) and 492 (less intense)	3.02	Au ₂₃ L ₁₇

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8 **Fig. S7.** FT-IR of Leu-Cys (black curve) and Leu-Cys capped QC1 (blue), QC2 (green) and QC3 9 (red). (a) Leu-Cys exhibiting small band at 2576 cm⁻¹ for –SH which is absent for the blue 10 (QC1), green (QC2) and red (QC3) emitting gold clusters. (b) Enlarged portion of the area within 11 the dashed box in (a). I₁ and I₂ indicate peaks at 1680 cm⁻¹ and 1636 cm⁻¹ respectively. (c) Raman 12 spectral analysis with blue cluster QC1, showing a peak at 237 cm⁻¹ for the Au-S metal thiolate 13 bond.



Fig. S8. XPS spectra of the red emitting QC3 gold cluster. The Au $4f_{7/2}$ binding energy black line) could be deconvoluted into two components (the fitted result is shown as the red dashed line), that gives peaks at 84.23 eV (blue line) and 85.08 eV (dark yellow line). These two components could be assigned to Au(0) and Au(I) respectively.



2 Fig. S9. (a) Photoresponse curve of green emitting QC2 over more than 10 cycles. (b) Bi-exponential
3 fitting of the photo and dark current values.



Fig. S10. Capacitance nature of the thin films formed from (a) green and (b) red emitting quantum 15 clusters.

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1 References

2 (S1) Barman, A. K.; Verma, S. Sunlight mediated disruption of peptide-based soft structures decorated 3 with gold nanoparticles. *Chem. Commun.* **2010**, *46*, 6992-6994.

4 (S2) Roy, S.; Baral, A.; Bhattacharjee, R.; Jana, B.; Datta, A.; Ghosh, S.; Banerjee, A. Preparation of

5 multi-coloured different sized fluorescent gold clusters from blue to NIR, structural analysis of the blue

6 emitting Au₇ cluster, and cell-imaging by the NIR gold cluster. *Nanoscale*, **2015**, *7*, 1912–1920.