Elastin-Based Stimuli-Responsive Gold Nanoparticles

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Supporting Information

Materials. Dimethylformamide (DMF) was purchased from J.T. Baker and used as received. All other solvents, including solvents for NMR analysis, were purchased from Aldrich and used as received. Column chromatography was performed using 60 H silica purchased from Merck. Deionised water with a typical resistivity of 18.2 MΩ/cm was obtained using a Labconco Water Pro PS purification system. The pH of acidic solutions was adjusted by the addition of hydrochloric acid to deionised water. p-Alkoxybenzyl alcohol "Wang" resin with a loading of 1.14 mmol/g was purchased from Bachem. 9-Fluorenylmethoxy carbamate protected valine (Fmoc Val-OH, >99%), glycine (Fmoc Gly-OH, >99%), and proline (Fmoc Pro-OH, >99%) were purchased from Bachem and were used as received. 1-Hydroxybenzotriazole hydrate (HOBt, g98%) and N,N-diisopropylcarbodiimide (DIPCDI, g98%) were purchased from Fluka and were used as received. Tetraoctylammonium bromide (TOAB, ≥98%) and ethane dithiol (EDT, >99%) were purchased from Merck and were used as received. Hydrogen tetrachloroaurate trihydrate ($HAuCl_4 \cdot 3H_2O$, 99.9+%), sodium borohydride (AF granules, 10-40 mesh, 98%), and 3-mercaptopropionic acid (≥99%) were purchased from Sigma-Aldrich and used as received. Trifluoroacetic acid (TFA, 99%) and 4-(dimethylamino)pyridine (DMAP, 99%) were purchased from Across and used as received. Dialysis was performed using a Spectra/Por molecular porous membrane tubing with a MWCO of 12000-14000 purchased from Spectrum Laboratories.

Instrumentation. ^{1}H NMR and ^{13}C NMR characterizations were performed on a Varian Inova400 instrument working at 400.142 MHz and 100.615 MHz, respectively. Chemical shifts (δ) are reported in parts per million (ppm) relative to tetramethylsilane using the residual solvent peak as a reference standard. Coupling constants (J) are reported in Hertz. UV-vis absorption spectroscopy measurements were performed using a Varian Cary 50 spectrophotometer equipped with a Lauda RM6 - RMS Brinkmann refrigerating circulating bath (-15 °C to 120 °C). Dynamic light scattering (DLS) measurements were performed using a Malvern Instrument Zetasizer Nano-S (ZEN1600) equipped with a He-Ne laser (633 nm, 4 mW) and an Avalanche photodiode detector at an angle of 173 °. All DLS data were processed on a Dispersion Technology Software (Malvern Instruments). CD spectra were measured on a Jasco J-810 spectropolarimeter equipped with a Jasco PFD-425S temperature control unit. LCQ/MS analyses were performed using Thermo Scientific Advantage LCQ Linear-Ion trap Electrospray (ESI-MS). FT-IR spectra were recorded on an ATI Matson Genesis Series FTIR spectrometer with a fitted ATR cell. The vibrations (ν) are given in cm^{-1}. Transmission electron microscopy (TEM) was performed on a JEOL 1010 microscope at an acceleration voltage of 60 kV. Carbon-coated Cu grids (200 mesh) were used for sampling. Thermogravimetric analysis (TGA) was performed on a TA Instruments TGA Q500 V6.7 Build 203. Accurately weighed samples ranging from 1.5 mg to 3 mg were run from 30 °C to 600 °C under nitrogen (60 mL min^{-1}) at a rate of 10 °C min^{-1}.

Synthesis of Fmoc-Glycine-functionalised resin. A suspension of Wang resin (30 g) in 300 mL of DMF was cooled in an ice bath, after which Fmoc Gly-OH (13.5 g, 45 mmol), 9.20 g (60 mmol) of HOBt, and 4.30 g (34.2 mmol) of DIPCDI were added. This mixture was shaken for 6 h. The functionalized resin was filtered and washed repeatedly with dichloromethane (DCM), DMF, and isopropyl alcohol. Unfunctionalised groups on the resin were capped by adding 10.2 mL of benzoyl chloride and 8.4 mL of pyridine to a suspension of the resin in 300 mL of DCM at 0 °C. This mixture was shaken for 30 min, filtered, and washed repeatedly with DCM, DMF, and isopropyl alcohol, and dried.

Synthesis of Fmoc-VPGVG-functionalised resin. The Fmoc-VPGVG resin was synthesized by standard solid-phase methods using a Fmoc-glycine-functionalised "Wang" resin.[1, 2] The Fmoc-Gly-functionalized resin (5.0 g, loading 0.64 mmol/g) was swollen in DMF (45 mL) for 20 min and filtered. A DMF solution containing 20% v/v piperidine (45 mL) was then added and the mixture was shaken 20 min to remove the Fmoc group. A positive Kaiser test[3, 4] indicated the presence of free primary amines. The next amino acid was coupled by adding a mixture of Fmoc Val-OH (3.26 g, 9.6 mmol), a 1 M HOBt solution in DMF (11.5 mL, 11.5 mmol) and a 1 M DIPCDI solution in DMF (10.6 mL, 10.6 mmol). The mixture was shaken for 45 min, after which it was washed three times with DMF. A negative Kaiser test indicated the completeness of the reaction. This procedure was repeated with the following three amino acids: Fmoc Gly-OH (2.85 g, 9.6 mmol), Fmoc Pro-OH (3.24 g, 9.6 mmol), and Fmoc Val-OH (3.26 g, 9.6 mmol). The Fmoc-VPGVG resin was then washed repeatedly with DCM, DMF, and isopropyl alcohol, and dried.

Synthesis of thiol-functionalised VPGVG peptide. The Fmoc-VPGVG functionalised Wang resin (2.0 g, loading 0.52 mmol/g) was swollen in DMF (45 mL) for 20 min and filtered. A DMF solution containing 20% v/v piperidine (approx. 45 mL) was then added to remove the Fmoc group. A positive Kaiser test[3, 4] indicated the presence of free primary amines. The thiol end group was coupled by adding a mixture of 3-mercaptopropionic acid (0.26 m, 3.0 mmol), a 1 M HOBt solution in DMF (3.6 mL, 3.6 mmol) and a 1 M DIPCDI solution in DMF (3.3 mL, 3.3 mmol), and diluted to about 45 mL with DMF. The mixture was shaken for 1 hour, after which it was washed three times with DMF. A negative Kaiser test indicated the completeness of the reaction. The resin was then washed repeatedly with DCM, isopropyl alcohol, and diethyl ether, and dried in air. The thiol-functionalised VPGVG was cleaved from the resin using 10 mL of 95% TFA containing 2.5% of water and 2.5% of EDT to reduce any disulfide complexes involving the VPGVG peptide. After cleavage, the resin was removed by filtration and the obtained thiol-functionalised VPGVG peptide was precipitated in diethyl ether. The product was then purified by column chromatography using silica gel and $CHCl_3$/MeOH/water (65:25:4) as eluent. From 2.0 g of Fmoc-VPGVG functionalized Wang resin, 461 mg of peptide were obtained.

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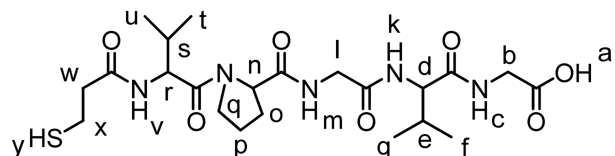
[1] E. Atherton, R. C. Sheppard, *Solid Phase Peptide Synthesis*, IRL Press: Oxford England, 1989.
[2] G. B. Fields, R. L. Noble, *Int. J. Pept. Protein. Res.*, 1990, **35**, 161-214.
[3] E. Kaiser, R. L. Colescott, C. D. Bossinger, P. I. Cook, *Anal. Biochem.*, 1970, **34**, 595-598.
[4] V. K. Sarin, S. B. H. Kent, J. P. Tam and R. B. Merrifield, *Anal. Biochem.*, 1981, **117**, 147-157.



^{1}H NMR (DMSO-d_6, 400 MHz): δ 12.50 (bs, 1H, H_a), 8.30 (t, J = 5.8 Hz, 1H, H_c), 8.18 (t, J = 6.0 Hz, 1H, H_m), 8.08 (d, J = 8.5 Hz, 1H, H_v), 7.61 (d, J = 9.0 Hz, 1H, H_k), 4.30 (m, 2H, H_d and H_r), 4.18 (m, 1H, H_n), 3.80-3.40 (m, 6H, H_b, H_l, and H_q), 2.63 (t, J = 7.5 Hz, 2H, H_w), 2.44 (q, J = 7.5 Hz, 2H, H_x), 2.22 (t, J = 7.5 Hz, 1H, H_y), 2.10-1.90 (m, 4H, H_o and H_p), 1.90-1.75 (m, 2H, H_e and H_s), 0.90 (d, J = 6.7 Hz, 3H)*, 0.87 (d, J = 6.7 Hz, 6H)*, 0.82 (d, J = 6.7 Hz, 3H)*.

* Assigned to protons H_f, H_g, H_t, or H_u.

^{13}C NMR (DMSO-d_6, 100 MHz): δ 171.82, 171.16, 170.59, 170.35, 169.87, 168.38, 59.87, 59.22, 55.82, 47.50, 43.24, 42.16, 38.85, 30.86, 30.11, 29.12, 24.52, 20.14, 19.12, 18.90, 18.73, 18.53.

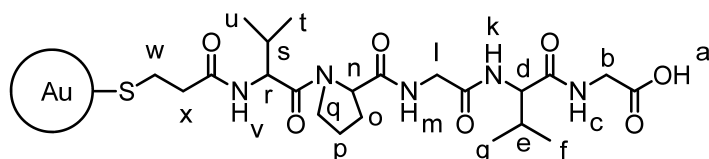
IR (solid): ν 3284 (NH); 3071, 2965, 2924, 2872 (CH); 1622 (C=O amide I); 1530 (amide II); 1443, 1410, 1313, 1233, 1208, 1037 (unassigned).

LC-MS for $C_{22}H_{37}N_5O_7S$ in order of decreasing intensity: 538.5 ($M + Na^+$), 516.4 ($M + H^+$), 554.4 ($M + K^+$), 560.5 ($M - H^+ + 2 Na^+$), 279.2 ($M + 2 Na^+$).

Synthesis of DMAP-stabilised gold nanoparticles. The DMAP-stabilised gold nanoparticles were prepared according to the method proposed by Curasso[5] as described by Lennox.[6] An aqueous solution of hydrogen tetrachloroaurate trihydrate (30 mM, 30 mL) was mixed with a solution of tetraoctylammonium bromide (25 mM, 80 mL (TOAB)) in toluene. The biphasic mixture was vigorously stirred until all the tetrachloroaurate had transferred into the organic phase, giving a deep orange solution. A freshly prepared aqueous solution of sodium borohydride (0.4 M, 25 mL) was added slowly while stirring. The organic phase immediately became deep red. The mixture was stirred for 90 min. The organic phase was then separated and washed with deionised water three times, dried over anhydrous sodium sulphate, and filtered. An aqueous solution of 4-(dimethylamino)pyridine (0.1 M, 80 mL (DMAP)) was then added to the TOAB-stabilised nanoparticles solution. The colour of the aqueous phase progressively became deep ruby as the phase transfer of the particles occurred. The mixture was stirred one hour. The aqueous phase containing the DMAP-stabilised gold nanoparticles was then isolated and diluted with an aqueous solution of DMAP (0.1 M, 97 mL) to obtain a solution of particles with a gold content of 1 mg/mL, as estimated from the mass of gold in the starting material, assuming no loss of gold. The solution was kept at 4 °C for months without apparent degradation.

[5] D. I. Gittins and F. Caruso, *Angew. Chem. Int. Ed.*, 2001, **40**, 3001-3004.
[6] V. J. Gandubert and R. B. Lennox, *Langmuir*, 2005, **21**, 6532-6539.

Synthesis of VPGVG-capped gold nanoparticles. An aqueous solution of DMAP-stabilised gold nanoparticles (1 mg/mL gold content, 10.8 mL, 0.054 mmol of Au) was added to the thiol-functionalised VPGVG peptide (15.5 mg, 0.03 mmol) in a small round-bottomed flask equipped with a magnetic stirring bar. The solution was stirred overnight, after which time it was transferred to a dialysis bag and dialysed against deionised water for 24 hours. The VPGVG-capped gold nanoparticles were then freeze-dried to obtain a dark purple solid. The size distribution of the gold core was determined from TEM pictures using ImageJ 1.41o image analysis software. A mean core diameter of 3.2 nm with a standard deviation of 1.0 nm was obtained from various images containing a data set of at least 200 particles.



^{1}H NMR (DMSO-d_6, 400 MHz)[7, 8]: δ 8.30 (t, J = 5.8 Hz, 1H, H_c), 8.18 (t, J = 6.0 Hz, 1H, H_m), 8.08 (d, J = 8.5 Hz, 1H, H_v), 7.61 (d, J = 9.0 Hz, 1H, H_k), 4.30 (m, 2H, H_d and H_r), 4.18 (m, 1H, H_n), 3.80-3.40 (m, 6H, H_b, H_l, and H_q), 2.10-1.90 (m, 4H, H_o and H_p), 1.90-1.75 (m, 2H, H_e and H_s), 0.90 (d, J = 6.7 Hz, 3H)*, 0.87 (d, J = 6.7 Hz, 6H)*, 0.82 (d, J = 6.7 Hz, 3H)*.

* Assigned to protons H_f, H_g, H_t, or H_u.

IR (solid): ν 3284 (NH); 3071, 2964, 2924, 2872 (CH); 1621 (C=O amide I); 1529 (amide II); 1442, 1394, 1311, 1237, 1200, 1033 (unassigned).

TGA (on 1.727 mg and 2.525 mg samples): weight loss of 30 % between 250 °C and 500 °C. The number of thiol-VPGVG ligands on the surface of each Au NP is evaluated to be approximately 211, assuming the presence of 1289 gold atoms in the core of a nanoparticle with a diameter 3.2 nm.[9]

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[7] A minimal amount of TFA was added to fully solubilise the particles.
[8] The signals for protons H_x and H_w are probably too broad to be observed.
[9] M. J. Hostetler, J. E. Wingate, C.-J. Zhong, J. E. Harris, R. W. Vachet, M. R. Clark, J. D. Londono, S. J. Green, J. J. Stokes, G. D. Wignall, G. L. Glish, M. D. Porter, N. D. Evans and R. W. Murray, *Langmuir*, 1998, **14**, 17-30.

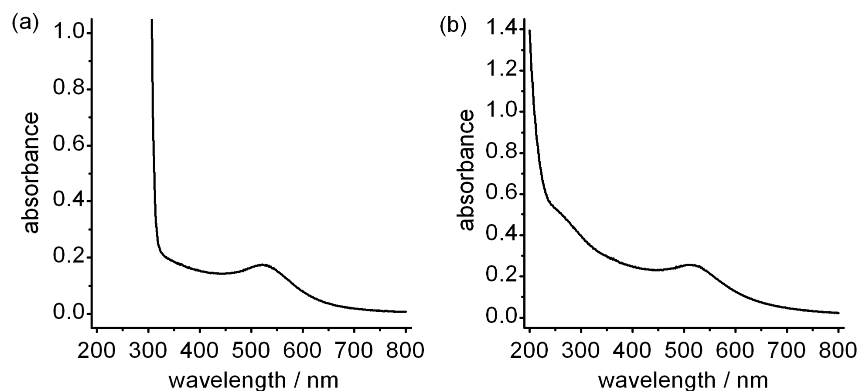

Fig. S1 UV-visible spectra of (a) DMAP-stabilised Au NPs and (b) VPGVG-stabilised Au NPs in deionised water showing that the plasmon band does not shift after the ligand-exchange reaction, suggesting that the integrity of the particles size and shape was maintained.

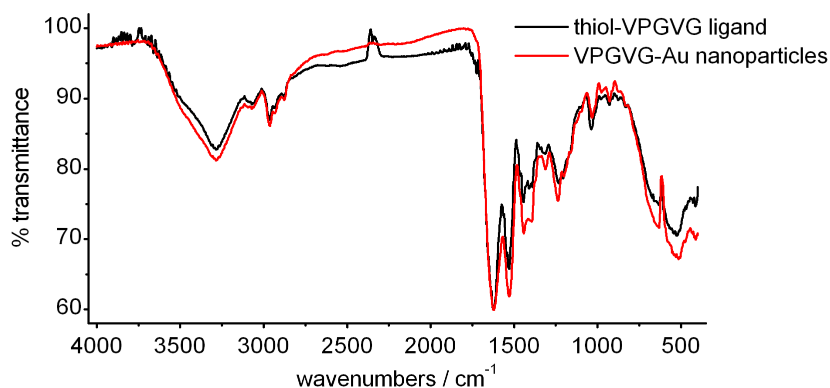

Fig. S2 Infrared spectra of the thiol-VPGVG ligand (black) and VPGVG-Au NPs (red).

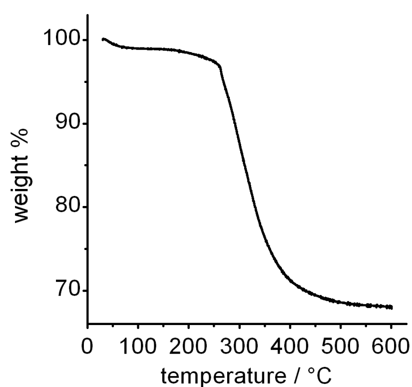

Fig. S3 Thermogravimetric analysis of freeze-dried VPGVG-stabilised Au NPs.

Analysis of the LCST Behaviour

Typical solution preparation. A sample of previously freeze-dried VPGVG-Au NPs (0.52 mg) was weighed in a vial, to which acidic water was added (3 mL, prepared by the addition of a 0.2 M HCl solution to deionised water). The pH of the resulting solution was evaluated using a pH meter. The solution was then immediately placed in a refrigerator at 4 °C and was left to equilibrate for at least 30 min before any variable temperature analysis.

Typical variable temperature UV-visible spectroscopy experiment. The internal temperature of the UV-visible spectrometer was adjusted to the lowest temperature of analysis (5 °C, 10 °C, or 20 °C). The VPGVG-Au NP solution was transferred to a 1 cm path length quartz cuvette and placed in the spectrometer. Spectra were acquired periodically until no recordable changes were observed before increasing the temperature in increments of 2.5 °C or 5 °C.

Cyclic variable temperature UV-visible spectroscopy experiment. The internal temperature of the UV-visible spectrometer was adjusted to 10 °C. A VPGVG-Au NP solution (0.18 mg/mL, pH 2.8) was transferred into a 1 cm path length quartz cuvette and placed in the spectrometer. The solution was left to equilibrate for 30 min before the start of the analysis. The spectra were acquired at 10 °C and 50 °C for 5 cycles. After each temperature change, spectra were acquired periodically until no recordable changes were observed. The temperature was changed directly between 10 °C and 50 °C.

Typical variable temperature CD spectroscopy experiment. The internal temperature of the CD spectrometer was adjusted to 5 °C. The VPGVG-Au NP solution was transferred into a 1 mm path length quartz cuvette and placed in the spectrometer. The solution was left to equilibrate 30 min before the start of the analysis. CD spectra were acquired in the 5 °C – 50 °C range with 5 °C intervals (band width: 1 nm, response: 2 s, sensitivity: high, data pitch: 0.5 nm, mode: continuous, scan speed: 100 nm/min, accumulations: 3).

Cyclic variable temperature CD spectroscopy experiment. The internal temperature of the CD spectrometer was adjusted to 5 °C. A VPGVG-Au NP solution (0.18 mg/mL, pH 2.8) was transferred into a 1 mm path length quartz cuvette and placed in the spectrometer. The solution was left to equilibrate 30 min before the start of the analysis. CD spectra were acquired at 5 °C and 50 °C for 8 cycles (band width: 1 nm, response: 2 s, sensitivity: high, data pitch: 0.5 nm, mode: continuous, scan speed: 100 nm/min, accumulations: 3). After each temperature change, the sample was allowed to equilibrate for 5 min.

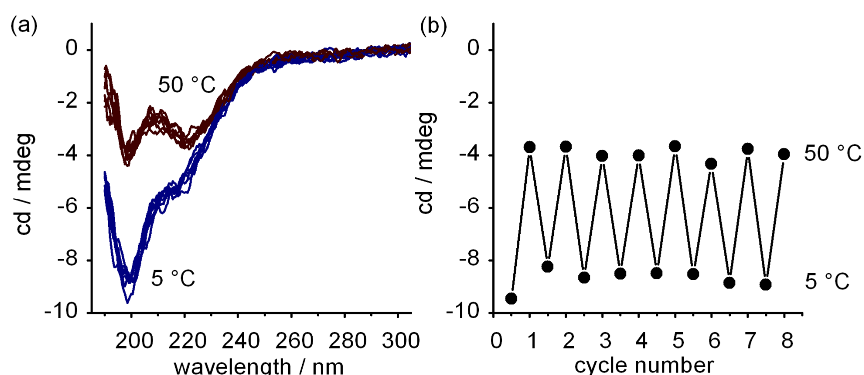

Fig. S4 The changes in (a) the CD spectra and (b) the CD signal at 198 nm of a VPGVG-Au NPs solution (0.18 mg/mL, pH 2.8) when alternating the temperature 8 times between 5 °C and 50 °C.

Variable temperature DLS experiment. The internal temperature of the particle size analyser was adjusted to 5 °C. A VPGVG-Au NP solution (0.18 mg/mL, pH 2.8) was transferred into a 40 µL polystyrene cuvette and placed in the instrument. The solution was left to equilibrate 30 min before the start of the analysis. The data were acquired from 5 °C to 50 °C with 5 °C intervals and 20 min equilibration times. The derived count rate values were averaged over 10 acquisitions. The viscosity and refractive index of the solvent (water) was automatically adjusted by the software at each temperature. Once the temperature of 50 °C was reached, the temperature was kept constant for a few hours and data were acquired periodically.

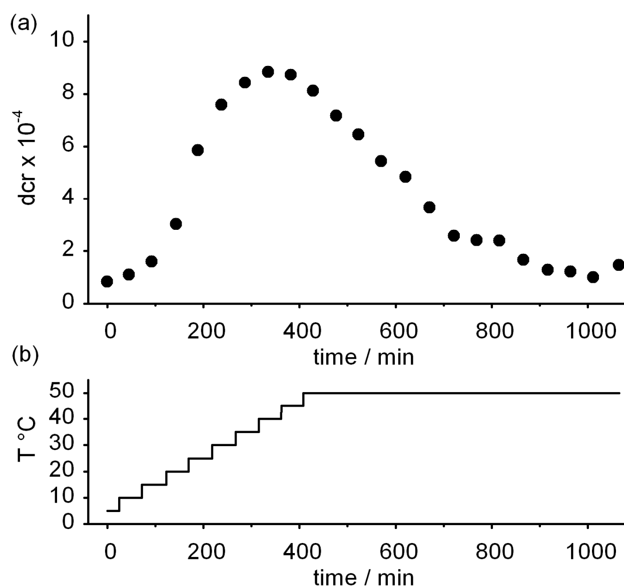

Fig. S5 (a) The changes in the derived count rate (dcr) of the scattered light over time for a VPGVG-Au NPs solution (0.18 mg/mL, pH 2.8) as the temperature in increased from 5 °C to 50 °C following the temperature profile shown in (b).