Supplementary Material (ESI) for Chemical Communications

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Supporting Information for

Disruption of protein-protein interactions using nanoparticles: Inhibition of cytochrome *c* peroxidase

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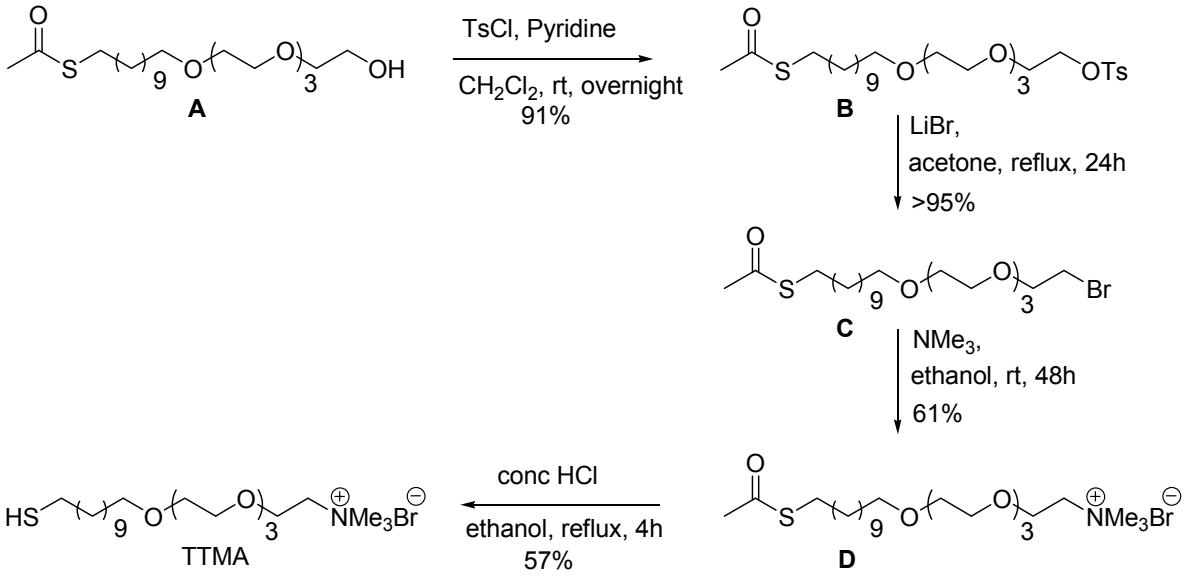
1. Procedure for synthesis of ligands:

A) Ligand TOH (HS-C_{11}-tegOH) and Ligand TCOOH (HS-C_{11}-tegOCH_2COOH) were synthesized according to the literature procedures.[1,2] Ligand TTMA (HS-C_{11}-tegNMe_3^+) was synthesized as follows:



B) Pyridine (24 ml, 300 mmol) was added to an ice-cooled solution of compound **A**[1] (4.22 g, 10 mmol) in dichloromethane. After stirring for 10 min, the reaction mixture was transferred slowly to a solution of Tosyl chloride (2.86 g, 15 mmol) in dcm at 0^0C. The reaction was stirred for overnight at room temperature. Then dcm and pyridine was evaporated. Ice cold water was added to the reaction mixture and extracted with dcm. The organic layer was washed with brine solution, and dried over $MgSO_4$. After evaporating the solvent, the crude product was purified by flash column chromatography using 1:1 EtOAc-Hexane mixture to give 5.24 g (91%) of pure product. **^{1}H NMR** (400 MHz, $CDCl_3$): δ(ppm) 7.74 (d, 2H, J=8.0 Hz), 7.29 (d, 2H, J=8.4 Hz),

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4.11 (t, 2H, J=4.6Hz), 3.64 (t, 2H, J=4.8), 3.61-3.50 (m, 12H), 3.39 (t, 2H, J=6.8), 2.81 (t, 2H, J=7.2), 2.40 (s, 3H), 2.27 (s, 3H), 1.53-1.47 (m, 4H), 1.31-1.19 (m, 14H).

C) LiBr (150 mg, 1.73 mmol) was added to a stirred solution of compound **B** (200 mg, 0.346 mmol) in acetone. The reaction mixture was refluxed for 24h. After removal of acetone, 40 ml water was added to it and the compound was extracted with dcm, and washed with brine. After dried over $MgSO_4$, the solvent was evaporated. A quick filtration was performed using silica to give a quantitative yield (>95%, 160 mg). **^{1}H NMR** (400 MHz, $CDCl_3$): δ (ppm) 3.78 (t, 2H, J=6.32 Hz), 3.64-3.53 (m, 12H), 3.44 (t, 2H, J=6.32), 3.41 (t, 2H J=6.84), 2.82 (t, 2H, J=7.32), 2.29 (s, 3H), 1.57-1.48 (m, 4H), 1.27-1.22 (m, 14H).

D) Compound **C** (160 mg, 0.33 mmol) was taken in ethanol. To this solution, 33% by weight solution of NMe_3 (600mg, 3.3 mmol) in ethanol was added. The reaction mixture was stirred at room temperature for 2 days. Then ethanol was removed by *rotavap*. The product (112 mg, 61%) was collected by precipitation in diethyl ether. **^{1}H NMR** (300 MHz, $CDCl_3$): δ (ppm) 3.98 (br, 4H), 3.69-3.53 (m,12H), 3.47 (s,9H), 3.42 (t, 2H, J= 6.8), 2.85 (t, 2H, J=7.1), 2.31 (s, 3H), 1.59-1.49 (m, 4H), 1.42-1.35 (m, 14H).

TTMA) 1 ml conc HCl was added to a stirred solution of compound **D** (112 mg, 0.21mmol) in EtOH, and refluxed for 4h. Then ethanol was removed. The product was precipitated out by washing with diethyl ether (60 mg, 57%). **^{1}H NMR** (400 MHz, $CDCl_3$): δ (ppm) 3.99 (br, 2H), 3.89(br, 2H), 3.71-3.55 (m,12H), 3.44 (t, 2H, J= 7.0), 3.42 (s,9H), 2.54-2.49 (m, 2H), 1.63-1.52 (m, 4H), 1.38-1.26 (m, 14H).

Procedure for preparation of nanoparticles:

Au-TOH and Au-TCOOH

Particles (water soluble) were prepared according to the reported procedures.[3,4]

Au-TTMA

Octanethiol covered 2 nm gold nanoparticles (Au-C_8) were prepared by following the Brust method in a toluene-water two-phase procedure.[5] 20 mg of Au-C_8 was dissolved in 10ml of dichloromethane, and the ligand 10ml dichloromethane was then added after both solutions were sparged with Ar for one hour. The solution was stirred at room temperature for 2 days, during which point the particle precipitated. The precipitate was washed with dichoromethane (5 x 20 ml) and dried under vacuum.

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(2) Hong, R.; Fischer N. O.; Verma, A.; Goodman, C. M.; Emrick T.; Rotello, V. M. *J. Am. Chem. Soc.* **2004**, *126*, 739-743.

(3) Kanaras, A. G.; Kamounah, F. S.; Schaumburg, K.; Kiely, C.J.; Brust, M. *Chem. Commun.* **2002**, 2294-2295.

(4) Hong, R.; Emrick, T.; Rotello V.M. *J. Am. Chem. Soc.* **2004**, *126*, 13572-13573.

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2. DLS Analysis:

Samples were prepared using 10 mM Tris-HCl buffer (pH 7.4) filtered with 0.2 μm filter (Fisher Sci.). The CcP and Cyt c was prepared according to previously described and allowed to incubate with the nanoparticles for 5 minutes. The sizes were measured on a MALVERN Zetasizer Nano ZS instrument. The average of at least nine measurements was reported.

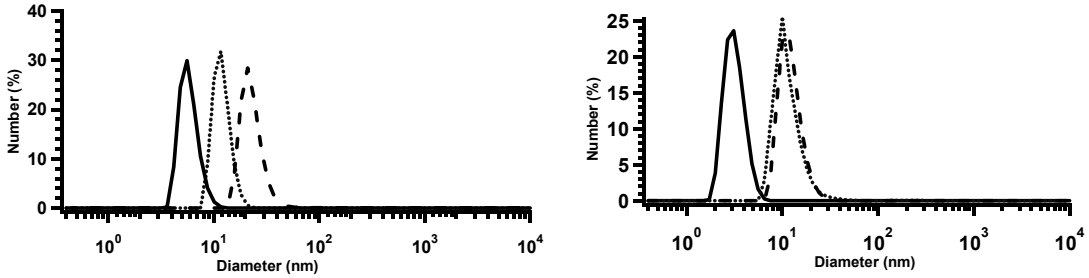

Figure S1. (A) 2.0 μM of CCP (solid line), 0.9 μM of Au-TTMA (dotted line), and the mixture of CcP and Au-TTMA (dashed line). (B) 20.0 μM of Cyt c (solid line), 1.2 μM Au-TCOOH (dotted line), and a mixture (dashed line).

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3. Gel shift assay for binding ratio:

Agarose gels were prepared in 10 mM Tris-HCl buffer at pH 7.4 with 0.7% final agarose concentration. Appropriately sized wells (40μL) were formed by placing a comb in the center of the gel. After the protein and particle was mixed, 4μL of 80% glycerol was added to ensure proper well loading and a constant voltage (100 V) was applied for 20 min for sufficient separation. Gels were placed in staining solution (0.5% Coomassie blue, 40% methanol, 10% acetic acid aqueous solution) for 1 h, followed by extensive destaining (40% methanol, 10% acetic acid aqueous solution) until protein bands were clear.

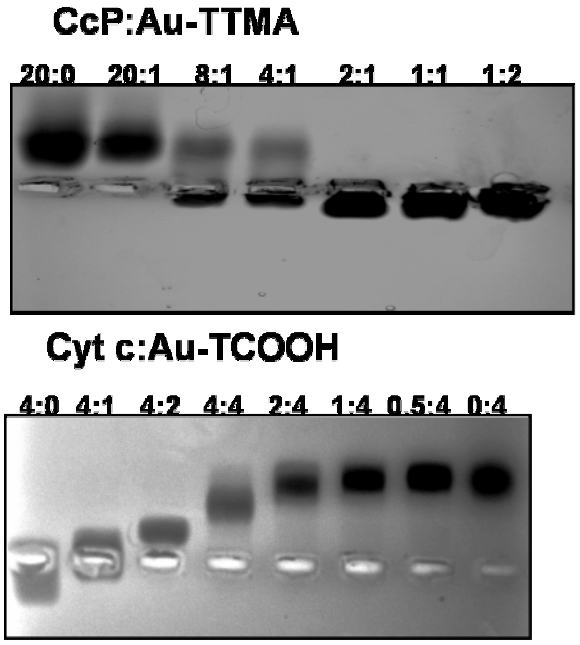

Figure S2. (A) CCP (20 μM) was mixed with varied concentration of Au-TTMA (from 0 μM to 40 μM). (B) Cyt c (10 μM) was mixed with varied concentration of Au-TCOOH (0 μM to 80 μM).

4. CD titration and estimation of the binding constants and ratios

The adduct formation of protein (P) with a binding site (site) on the Au-MMPC could be expressed by equation S1:

$$P{:}Site \rightleftharpoons P + Site \quad \text{(S1)}$$

Where K_D denotes the dissociation constant of the bound protein. Defining $X = \frac{[P:Site]}{[P]+[P:Site]}$, K_D can be defined as:

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$$K_D = \frac{[P]\times[Site]}{[P:Site]} = \frac{(1-X)\times(Site_o - P_o \times X)}{X}$$

(S2)

Where $Site_o$ and P_o denote the initial concentrations of binding site and protein respectively. The relationship between the concentration of binding site and Au-MMPC is denoted by $[Site_o]$ = $n\times[Au_o]$ where n indicates the number of identical and independent binding sites on Au-MMPC. After a few manipulations of equation S2 is solved for X to give equation S3.

$$X = \frac{I - I_o}{I_f - I_o} = \frac{([P]_o + n\times[Au]_o + K_D) - \sqrt{([P]_o + n\times[Au]_o + K_D) - 4\times n\times[P]_o\times[Au]_o}}{2\times[[P]_o}$$

(S3)

Where P_o and $Site_o$ is replaced by $[P]_o$ and $n\times[Au]_o$ respectively. On the basis of equation S3, the binding constant and binding ratio were determined by using nonlinear least-squares curve fitting analysis with IGOR version 4.01 (Wavemetrics Inc., Oregon, USA).

5. CD Measurements

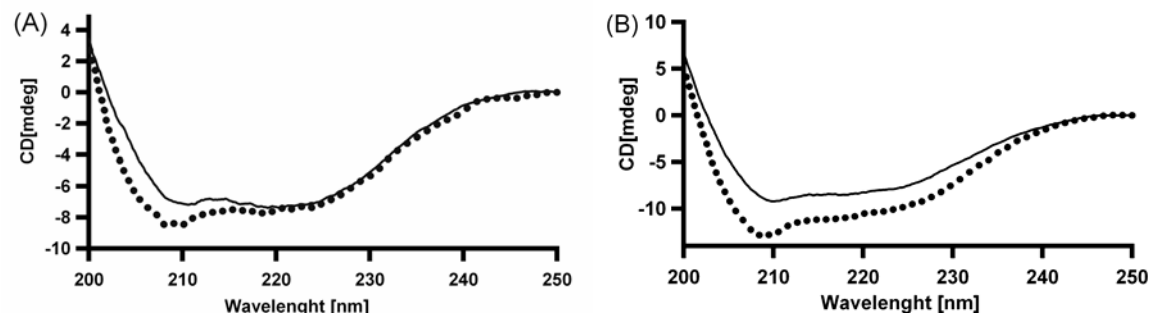

Figure S3. (A) 2.0 μM of Cyt c (straight line) was mixed with of 2.1 μM of Au-TCOOH (dotted line). (B) 3.9 μM of CCP (straight line) was mixed with of 3.2 μM of Au-TTMA (dotted line).