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Electronic Supporting Information

Thioalkylated zwitterion: a bioinspired ligand for biocompatible monolayer protected gold nanoparticles in nanomedicine.

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Synthesis of 11-Mercaptoundecylphosphorylcholine (HS-PC)

11-Mercaptoundecylphosphorylcholine (HS-PC) was synthesized as described.[1] A methanol solution (30 mL) containing 11-mercapto-1-undecanol (2.05 g) was titrated by 1 M iodine methanol solution until the solution turned light yellow, and then the reaction was quenched with sodium bisulfite. 11-Hydroxyundecyl disulfide was precipitated from the methanol solution at 0 °C and recrystallized from ethanol with a yield of 1.6 g. 11-Hydroxyundecyl disulfide (0.4 g, 1 mmol) and triethylamine (0.35 mL, 2.5 mmol) were dissolved in 15 mL of chloroform. This solution was slowly added into 2-chloro-1, 3, 2,-dioxaphospholane-2-oxide (0.21 mL, 2.4 mmol) anhydrous THF (40 mL) solution at -18 °C. The mixture was allowed to warm to ambient temperature over 2 h. The reaction mixture was then cooled to 0 °C and filtered. The filtrate was concentrated in vacuo at room temperature without further purification, and the product is a light-yellow powder. The product and trimethylamine (0.5 mL) in anhydrous methane chloride (35 mL) at -15 °C were sealed in a bomb, which was heated to 55 °C and stirred for 24 h. The reaction mixture was cooled and filtered to give a white solid powder (0.33 g). The white powder (0.33 g) was dissolved in ethanol (3 mL), and 1,4-dithio-D,L-threitol (DTT, 0.4 g) was added. The pH of the solution was adjusted to 9 with concentrated $NH_3 \cdot H_2O$. The solution was stirred for half an hour and purified by flash chromatography on silica using a mixed eluent (i.e., chloroform: ethanol: water, 4:8:2). The fraction of HS-PC was concentrated in vacuo by a rotary evaporator at room temperature. Anhydrous ethanol was added to help remove water. The final product is a white solid powder (0.12 g). ^{1}H NMR (CD_3OD, 500 MHz): δ_H: 1.21-1.48 14H, 1.51-1.73 4H, 2.44 2H, 3.15 9H, 3.57 2H, 3.80 2H, 4.18 2H

Synthesis of 16 nm citrate protected gold nanoparticles (Au-NPs)

The synthesis of citrate protected Au-NPs followed the reported procedure.[2] In a 250 mL round-bottom flask equipped with a condenser, 100 mL triply distilled water was added. After boiling, 4.12 mL of 10 mg/mL $HAuCl_4$ was added with vigorous stirring. Rapid addition of 11.57 mL of 38.8 mM sodium citrate to the boiling solution resulted in a color change from pale yellow to burgundy. Boiling was continued for 10 min; the heating mantle was then removed, and stirring was continued for additional 15 min. The UV-Vis spectrum exhibits a characteristic plasmon band at 520 nm. The diameter of the particles, measured from TEM images, was 16 nm ± 2 nm.

Synthesis of 16 nm HS-PC protected Au-NPs

The HS-PC protected Au-NPs were obtained from the citrate coated Au-NPs by exchange of

citrate molecules with HS-PC. This reaction was performed according the literature.[3, 4] A large excess equivalent of thiol ligand was used: 100 monolayers/particle, estimated by assuming that the occupied surface area by a single thiol molecule is ca. 0.20 nm^2.[5] Using this calculation, 1 mL of 0.05 M HS-PC solution (in ethanol) was added into 12 mL of citrate coated Au-NPs solution. The mixture was rapidly stirred at room temperature for 24 h.

Synthesis of 50 nm citrate protected Au-NPs

The synthesis of 50 nm citrate protected Au-NPs followed the reported procedure.[6] In a 100 mL round-bottom flask equipped with a condenser, 50 mL triply distilled water was added. After boiling, 0.5 mL of 10 mg/mL $HAuCl_4$ was added with vigorous stirring. 0.5 mL of 10 mg/mL sodium citrate was rapidly added into the boiling solution. Boiling was continued for 15 min. The solution was cooled at room temperature. The diameter of the particles, measured from TEM images, was 50 nm ± 4 nm.

Synthesis of 50 nm HS-PC protected Au-NPs

The synthesis of 50nm HS-PC protected Au-NPs is similar to the synthesis of 16nm HS-PC protected Au-NPs. Briefly, 0.5 mL of 0.05 M HS-PC solution (in ethanol) was added into 20 mL of 50 nm citrate protected Au-NPs solution. The mixture was rapidly stirred at room temperature for 24 h.

Synthesis of 50 nm tetra(ethylene glycol) monolayer protected Au-NPs

The synthesis of 50 nm monohydroxy (1-mercaptoundec-11-yl) tetraethylene glycol ($HS\text{-}EG_4$) protected Au-NPs is similar to the synthesis of 50 nm HS-PC protected Au-NPs. Briefly, 0.5 mL of 0.05 M $HS\text{-}EG_4$ solution (in ethanol) was added into 20 mL of 50 nm citrate protected Au-NPs solution. The mixture was rapidly stirred at room temperature for 24 h.

Synthesis of 2-3 nm HS-PC protected Au-NPs by single-phase reduction method

HS-PC protected Au-NPs in the size range of 3 nm were prepared as described elsewhere[7] with slight modification. In a typical reaction, 25 mL MeOH and 0.5mL acetic acid were mixed in a 50 mL flask. Then 15 mg tetrachloroauric acid and 5 mg HS-PC were added under stirring and the gold salt was reduced by rapid addition of 2.5 mL of a freshly prepared 0.5 M solution of sodium borohydride in methanol. The gold salt solution was immediately turned to dark brown from yellow. After further stirring for 3 h, the particle solution was purified by centrifuging at 60,000 rpm with triply distilled water 4 times and re-dissolved in triply distilled water.

Synthesis of 4-6 nm HS-PC coated Au-NPs by two-phase reduction method

To produce HS-PC protected Au-NPs in the size range of 5 nm, a well established two phase (water/toluene) protocol[8] was followed. An aqueous solution of chloroauric acid (6 mL, 30 mmol/L) was mixed with tetraoctylammonium bromide solution (16 mL, 50 mmol/L in toluene). The resulting two phase mixture was vigorously stirred, allowing complete transfer of tetrachloroaurate into an organic phase. A freshly prepared aqueous solution of sodium borohydride (5 mL, 0.4 mol/L) was added slowly with vigorous stirring and the mixture was

stirred for 3 h. After 3 h, the organic phase was separated and washed with 0.1 M H_2SO_4, NaOH and water.

About 5.5 mg of HS-PC dissolved in 1 mL of 2-propanol was added to Au-NPs in toluene and stirred for 3 h. After 3 h, water (15 mL) was added and the mixture was shaken vigorously. Consequently the organic phase became completely colourless while the colourless aqueous phase became deep wine red indicating that gold clusters have been transferred to aqueous phase. The aqueous phase was separated, washed with diethyl ether (three times) and further purified by centrifugation.

Synthesis of 3 nm tiopronin protected Au-NPs (Au-Tp)

The detailed procedure is described in the literature.[9] In a typical synthesis, 30 mL of methanol and 5 mLof acetic acid were mixed in a 100 mL flask by stirring for 2 min. Then, 40 mg of tetrachloroauric acid and 16.3 mg of tiopronin were added to the above mixed solvents and dissolved by stirring for 5 min, which gave a clear, yellow solution. Next, 60mg NaBH4 was dissolved in 30 mL of triply distilled water. The NaBH4 solution was dropwise added into the above solution with rapid stirring. With the first drop of NaBH4 added, the tetrachloroauric acid solution immediately turned to dark brown from yellow. Rapid stirring was kept for 2 h. The nanoparticle solution was purified by centrifuging at 60,000 rpm with triply distilled water 4 times and re-dissolved in sodium phosphate buffer (50 mM, pH7.4). The average particle size of Au-Tp nanoparticles is 3.2±0.6 nm, as measured by TEM (data not shown).

Ligand exchange reaction of HS-PC protected Au-NPs

An additional advantage of the Au-NPs system is the ease of surface modification by ligand exchange reactions. Ligand exchange reactions on Au-NPs have been extensively used to create new functionalized Au-NPs. Biomolecules can also be introduced onto Au-NPs surface by ligand exchange reactions. To show this, an ethanolic solution (10 mL) containing 10 mg of HS-PC protected Au-NPs and 15 mg of hydrophobic 1-dodecanethiol was stirred overnight. 1-dodecanethiol protected Au-NPs formed by this process precipitated quantitatively and they would readily re-dissolve in chloroform. (Fig. S4)

Stability Test

The stability HS-PC and HS-EG_4 protected Au-NPs at various situations was tested by UV-vis spectra collected within a range of 400-800 nm. The Au-NPs solution was first dialyzed against freshly prepared triply distilled water prior to test. During this procedure, water outside the tubing was refreshed several times, until the two systems reached the same level of conductivity. To determine nanoparticle stability with respect to various NaCl concentration, the nanoparticles were redispersed in 1.5 mL of 10 mM phosphate buffered solution (PBS) at pH 7.4. The desired NaCl concentrations were achieved by adding aliquots of 2.5 M NaCl in PBS (pH 7.4) to 1 mL of the buffered nanoparticle solution. To determine nanoparticle stability with respect to pH, the nanoparticles were redispersed in 1.5 mL of triply distilled water. A 100 μL aliquot of the concentrated nanoparticle solution was diluted in 900 μL of different PBS (10 mM) solutions at pH 1-13. In a 1 mL quartz cuvette, 600 μL solution was used. The spectra was recorded 10 min after the pH and NaCl concentration of the nanoparticle solution was adjusted. In experiments

studying the reversible dispersion-agglomeration of HS-PC protected Au-NPs at pH=5, 600 μL Au-NPs solution at pH=5 was kept for 30 min, the UV-vis spectra was recorded. Then, 100 μL PBS (10mM, pH=13) was added. The UV-vis spectra was recorded at once. To determine nanoparticle stability in physiological phosphate buffer solution (8 g NaCl, 0.2 g KCl, 2.9 g $Na_2HPO_4 \cdot 12H_2O$, 0.2 g KH_2PO_4 in 1000 mL triply distilled water), the nanoparticles were redispersed in 1 mL of physiological phosphate buffer solution. To determine nanoparticle stability in plasma, platelet-poor plasma was used. Platelet-poor plasma was collected as follows: Fresh blood collected from a healthy human was immediately mixed with 3.8 wt% sodium citrate solution at a dilution ratio of 9:1. It was then centrifuged at 3000 rpm and 8 °C, for 20 min to obtain the platelet-poor plasma. A 100 μL aliquot of nanoparticle solution was added into 900 μL platelet-poor plasma. After 10 min, The UV-vis spectra was recorded.

Flocculation parameter characterization

The "flocculation parameter" was firstly defined as the integrated area between 600 and 800 nm in the optical absorption by Whitesides et al.[10] Flocculation of individual spheres will result in red shifting and broadening in the visible spectrum. So the flocculation parameter will increase. The 50 nm HS-PC and HS-EG_4 protected Au-NPs were used to determine the flocculation parameter. To the HS-PC protected Au-NPs, 50 μL of 0.05 M HS-PC solution (in ethanol) was added into 2 mL of 50 nm citrate protected Au-NPs solution. The UV-vis spectra was recorded once HS-PC solution was added. The UV-vis spectra was followed every 5 min. The flocculation parameter of HS-PC protected Au-NPs was obtained by the integrated area between 600 and 800 nm in the UV-vis spectra. The flocculation parameter of HS- EG_4 protected Au-NPs was determined similar to the HS-PC protected Au-NPs, except that HS- EG_4 was used.

Protein adsorption test

Protein binding reactions were monitored by gel electrophoresis. The experiments were done by mixing 10 μL of ~ 0.1 mM Au-NPs with 1.0 μL of 10 mg/mL protein in phosphate buffer solution (50 mM, pH 7.4). After incubation at room temperature for 10 min, the entire reaction mixture was loaded on a 1% agarose gel. Gel electrophoresis was run in 1X TBE buffer (Tris-borate-EDTA) at 90V constant voltage for 20 min. Gel pictures were taken by directly scanning the gel on a HP ScanJet 2400.

Instrumentation and Measurements

FT-IR Measurements. FT-IR spectra of solid NPs samples pressed into a KBr plate were recorded from 4000 to 750cm^{-1} with a Bruker Vector2 spectrometer. For the citrate coated Au-NPs, 1M NaCl was added into the citrate coated Au-NPs solution. The addition resulted in a color change from red to black. Solid precipitates could be collected after centrifugation. Then, the precipitates were centrifuged for several times to remove any residual salt and then dried in vacuum at 60 ºC. For the HS-PC protectted Au-NPs, the solution was first dialyzed against freshly prepared triply distilled water. During this procedure, water outside the tubing was refreshed several times, until the two systems reached the same level of conductivity. Then, the HS-PC coated Au-NPs solution was centrifuged to remove water and then dried in vacuum at 60 ºC.

UV-vis spectra studies. UV-vis spectra were carried out with a UV-vis Shimadzu UV-2505

spectrometer using 1-cm-path length quartz cuvettes. Spectra were collected within a range of 400-800 nm.

TEM Measurements. Transmission Electron Microscopy (TEM) analysis was performed on a JEM-1200EX TEM operating at 200 kV in bright field mode. Au NP samples were prepared by placing a drop of the colloidal solutions on a 400-mesh carbon-coated copper grid and air-drying the grid at 25 °C. For the determination of particle size, over 100 particles were counted in Multiple pictures from different areas of the TEM grid.

^{1}H NMR Measurements. HS-PC powders were dissolved in MeOD, and the ^{1}H NMR spectra was recorded on a Bruker DMX500 instrument and scanned in the range 0-15 ppm.

Zeta potential Measurements. Zeta potential measurements were carried out using a Malvern Zetasizer3000HSA instrument. The zeta potential (ζ) was calculated from the electrophoretic mobility (u) using the Smoluchowsky relationship, $\zeta = \eta u/\varepsilon$, assuming that $ka >> 1$ (where η is the solution viscosity, ε is the dielectric constant of the medium, and k and a are the Debye-Hückel parameters and the particle radius, respectively). A 300 μL aliquot of the concentrated Au-NPs solution was diluted in 3 mL of different PBS (10 mM) solutions at pH 2-9. The diluted Au-NPs solutions (pH 2-9) were used to determine the zeta potential.

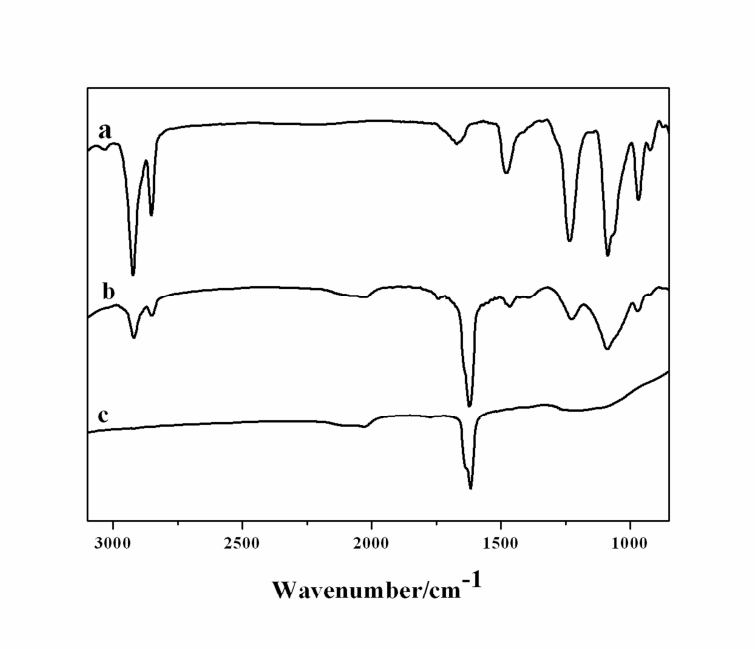


Figure S1. FTIR spectra of HS-PC (a), HS-PC protected Au-NPs (b), citrate protected Au-NPs precipitated by 1 M NaCl (c).

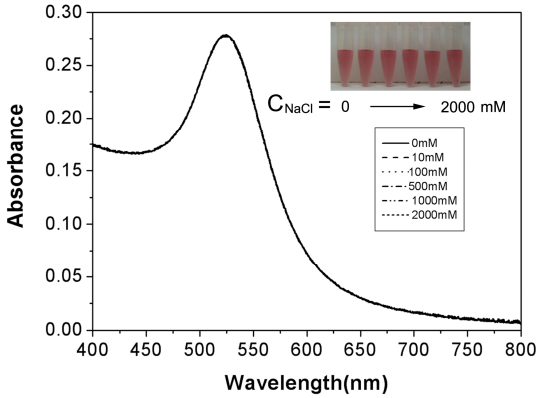
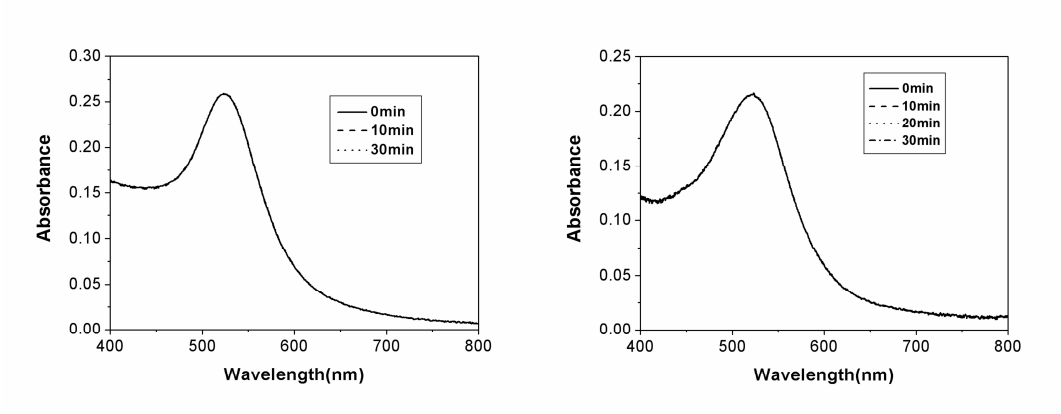


Figure S2. HS-PC protected Au-NPs stability test against various NaCl concentrations (0-2000 mM).



(a) (b)

Figure S3. HS-PC protected Au-NPs stability test in (a) physphysiological PBS solution (b) plasma.

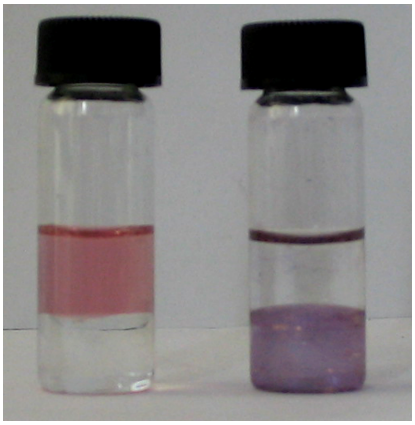


Figure S4. Optical images Au-NPs upon ligand exchange from HS-PC (in water, upper) to 1-dodecanethiol (in chloroform, underside).

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