Improved Methodology for the Preparation of Water-Soluble Maleimide-Functionalized Small Gold Nanoparticles

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Supporting Information
General Materials and Methods:

The following reagents, unless otherwise stated, were used as received. Triethylene glycol monomethylether, tetraethylene glycol, 4-dimethylaminopyridine (DMAP), potassium thioacetate, L-cysteine (97%), deuterated chloroform (CDCl$_3$), tetrachloroauric acid trihydrate, sodium borohydride, $p$-toluenesulfonyl chloride, furan, methylmaleimide, methyamine, and maleimide were purchased from Aldrich. All common solvents, triethyleneamine, magnesium sulfate, dry methanol, hydrochloric acid, sodium hydroxide, and potassium carbonate were purchased from Caledon. Deuterated water (D$_2$O) was purchased from Cambridge Isotope Laboratories. Ethanol was purchased from Commercial Alcohols. Glacial acetic acid (99.7%) was purchased from BDH. Maleic acid was purchased from Eastman Organic Chemicals. Dialysis membranes (MWCO 6000-8000) were purchased from Spectra/Por.

$^1$H and $^{13}$C NMR spectra were recorded on a Inova 400 using CDCl$_3$ or D$_2$O as solvent and reference. Thermogravimetric analysis (TGA) were recorded by loading the sample in a 70 µl ceramic crucible and heating from 25°C to 750°C at rate of 10°C min$^{-1}$. The experiment was run under a nitrogen flow of 70 ml min$^{-1}$ in a Mettler Toledo TGA/SDTA 851 instrument. Transmission electron microscopy (TEM) images were recorded from a JEOL 2010F HRTEM. UV-VIS spectra were collected employing a Varian UV-VIS spectrophotometer model Cary 300 Bio, and dissolving the sample in nanopure water. Zeta Potential measurements were conducted using a Zetasizer Nano-ZS (Malvern Instrument). The cell employed was a polystyrene latex folded capillary cell equipped with electrodes.
Synthesis of compound (1):

9.7 ml of triethylene glycol monomethyl ether (61 mmol), 21.2 ml of triethylamine (0.1525 mol) and 1.74 g of 4-dimethylaminopyridine (DMAP) (14.2 mmol) were dissolved in 400 ml of DCM in a 1 L round bottom flask. The system was cooled to 0°C and 13.2 g of 4-toluenesulfonyl chloride (69 mmol) were added to the solution. After a few minutes the solution darkened and the ice bath was removed. The reaction mixture was stirred for 4 h. The reaction mixture was washed with water, dried over MgSO₄ and the solvent was removed to obtain a yellow oil. The product was purified via liquid chromatography using Ethyl Acetate 3 : DCM 7 as eluent to give 1 with 82% yield. $^1$H NMR (CDCl₃, 400 MHz) δ(ppm): 2.41 (singlet, 3H), 3.34 (singlet, 3H), 3.51 (multiplet, 2H) 3.56 (multiplet, 6H), 3.65 (multiplet, 2H), 4.41 (multiplet, 2H), 7.33 (multiplet, 2H), 7.77 (multiplet, 2H). $^{13}$C NMR (CDCl₃, 400 MHz) δ(ppm): 21.6, 59.0, 68.6, 69.3, 70.5, 70.7, 71.9, 127.9, 129.8, 133.0, 144.7, 175.1. Exact mass (C₁₄H₂₂O₆S) calc: 318.114, found 318.111.

Synthesis of (2):

![Chemical structure of 2]
14.0 g of 1 (44 mmol) were dissolved in 500 ml of acetone in 1 L round bottom flask. 6.3 g of potassium thioacetate (55 mmol) were added, the flask was equipped with a condenser and the system was heated to 50ºC. After few minutes a fine white precipitate formed. The reaction was kept under these conditions overnight. Acetone was removed and DCM was added and the solution was washed with water. The collected organic phases were dried over MgSO₄, the DCM was evaporated and a yellow oil was obtained with quantitative yield. ¹H NMR (CDCl₃, 400 MHz) δ(ppm): 2.33 (singlet, 3H), 3.09 (triplet, 2H, J=4Hz), 3.38 (singlet, 3H), 3.55 (multiplet, 2H), 3.60 (triplet, 2H, J=8Hz), 3.63 (multiplet, 6H). ¹³C NMR (CDCl₃, 400 MHz) δ(ppm): 28.1, 30.5, 59.0, 69.7, 70.2, 70.5, 71.7, 77.0, 77.3, 195.5. Exact mass (C₉H₁₈O₄S) calc: 222.093, found 223.099.

**Synthesis of (3):**

For the synthesis of AuNP it is recommended to prepare the thiol right before its use otherwise it could react to form disulfides. 0.51 g of 2 (2.3 mmol) were dissolved in 10 ml of dry MeOH in a 50 ml round bottom flask equipped with a stirrer bar. The flask was sealed with a septum and using a long needle the solution was stirred and purged with Ar for 15 min. To completely avoid the formation of disulfide it is important that the reaction is carried out in complete absence of oxygen, therefore the flask must be kept under a positive pressure of inert gas. In a second 50 ml round bottom flask 2.3 ml of 1 M NaOH solution in EtOH were insert and were purged with Ar for 15 min using a long needle. Using a cannula the 2.3 ml of NaOH solution were transferred to
the MeOH solution. The reaction mixture was stirred for 45 min. In the mean time 6 ml of 1 M HCl in water were purged in a third 50 ml round bottom flask sealed with a septum. After the 45 minutes the HCl solution was transferred to the reaction mixture using a cannula. The solution was then stirred and purged with Ar for 5 minutes. After this time the flask was opened and the thiol was extracted with DCM. The collected organic fractions were dried over MgSO\(_4\), and the solvent was removed to obtain the thiol 3 as a pale yellow oil in quantitative yield without any trace of disulfide. \(^1\)H NMR (CDCl\(_3\), 400 MHz) \(\delta\) (ppm): 1.59 (triplet, 1H, J=8Hz), 2.70 (quartet, 2H, J=8Hz), 3.38 (singlet, 3H), 3.55 (multiplet, 2H), 3.63 (multiplet, 8H). \(^{13}\)C NMR (CDCl\(_3\), 400 MHz) \(\delta\) (ppm): 24.2, 59.0, 70.2, 70.5, 71.9, 72.9, 110.0.

**Synthesis of Triethylene Glycol Monomethyl Ether AuNP (Me-EG\(_3\)-AuNP):**

In a 50 ml round bottom flask 19.3 mg of HAuCl\(_4\)·3H\(_2\)O (49 µmol) were dissolved in 7.5 ml of dry MeOH and 1.25 ml of glacial acetic acid. The solution was yellow. To this mixture 26.3 mg of compound 3 were added (146 µmol). The solution color slightly darkened. The solution was stirred vigorously for 1 hour and the solution color slightly faded. Under vigorous stirring 0.0185 g of NaBH\(_4\) (490 µmol) dissolved in 1.25 ml of nanopure water were added to reaction mixture drop wise. After the first 3-4 drops the solution turned dark brown. The reaction was then stirred overnight. The solution was then concentrated under vacuum, 20 ml of nanopure water were added and the nanoparticles were extracted with toluene. To help the passage of the nanoparticles to the toluene phase, little amounts of sodium chloride were added to the water phase after every extraction. At the end the water phase was colourless. The toluene was then evaporated from the collected organic phases. The Me-EG\(_3\)-AuNP were then dissolved in one
milliliter of toluene and transferred in a clean round bottom flask leaving back any precipitate. The toluene was evaporated and the film of nanoparticles left inside the flask was washed with cyclohexane to remove the excess of thiol. The nanoparticles were then redissolved in nanopure water and purified by dialysis overnight.

This procedure leads to $2.0 \pm 0.3$ nm Me-EG$_3$-AuNP, as showed by UV-VIS spectrum (figure SI 9) and TEM images (figure SI 8). The size of the gold nanoparticles can be easily changed varying the gold:thiol ratio. To a 1:1 ratio correspond $\approx 5$ nm AuNP. When dissolved the solution is ruby. To a ratio 1:0.5 correspond 8 nmAuNP whose solution is purple. The bigger the nanoparticles, the less they are soluble in water. TGA measurement revealed the presence of 2.1 mmol of ligands per milligram of nanoparticles. $^1$H NMR spectroscopy showed the presence of three broad peaks: one at 3.34 ppm and corresponding to the methyl at the nanoparticle interface, one at 3.58 ppm and one at 3.66 ppm related to the protons of the ethylene glycol units.

These Me-EG$_3$-AuNP are well soluble in water, methanol, ethanol, tetrahydrofuran, dichloromethane, chloroform, toluene, ethyl acetate, acetone, dimethylformamide, and acetonitrile.
Figure S1 1: $^1$H and $^{13}$C NMR spectra of compound 1 recorded in CDCl$_3$. 
Figure S1 2: $^1$H and $^{13}$C NMR spectra of compound 2 recorded in CDCl$_3$. 
Figure SI 3: $^1$H and $^{13}$C NMR spectra of compound 3 recorded in CDCl$_3$. 
Synthesis of (4):

4.4 ml of tetraethylene glycol (26 mmol), 9 ml of triethylamine (65 mol) and 0.7418 g of 4-dimethylaminopyridine (DMAP) (6 mmol) were dissolved in 400 ml of DCM in a 1 L round bottom flask. The system was cooled down to 0°C and 11.0 g of 4-toluenesulfonyl chloride (60 mmol) were added to the solution. After few minutes the solution darkened and the ice bath was removed. The reaction mixture was stirred for 4 h. The reaction solution was washed with water, dried over MgSO₄ and the solvent was removed to obtain a dense yellow oil. The product was purified via liquid chromatography using ethyl acetate 3 : hexane 1 as eluent to give 4 with 74% yield. ¹H NMR (CDCl₃, 400 MHz) δ(ppm): 2.45 (singlet, 6H), 3.57 (multiplet, 8H), 3.68 (triplet, 4H, J=8Hz), 4.16 (triplet, 4H, J=8Hz), 7.34 (multiplet, 4H), 7.80 (multiplet, 4H). ¹³C NMR (CDCl₃, 400 MHz) δ(ppm): 21.6, 68.7, 69.2, 70.5, 70.7, 127.9, 129.8, 133.0, 144.8 Exact mass (C₂₂H₃₀O₉S₂) calc: 502.133, found 502.138.

Synthesis of (5):

Compound 5 was synthesized following the previously established procedure.¹ 1.0g of maleimide (10.3 mmol) and 1.05 g of furan (15.5 mmol) were dissolved in 15 mL diethyl ether
in a sealed tube. The system was heated at 100°C for 12 hours. Compound 5 precipitated as a white solid after cooling the mixture to room temperature. The product was then filtered, washed with 3 x 10 mL cold diethyl ether to remove the unreacted maleimide, obtaining the pure product with 62% yield. The NMR spectra indicate that the product is exclusively the exo isomer. $^1$H NMR (CDCl$_3$, 600 MHz) δ(ppm): 8.10 (broad singlet, 1H), 6.52 (doublet, J=0.782Hz, 2H), 5.31 (doublet, J=0.782Hz, 2H), 2.89 (singlet, 2H). $^{13}$C NMR (CDCl$_3$, 600 MHz) δ(ppm): 175.9, 136.6, 80.9, 48.7. Exact mass (C$_8$H$_7$NO$_3$) calc: 165.043, found 165.042.

**Synthesis of (6):**

2.25 g of compound 4 (4.5 mmol) were dissolved in 100 ml of dry acetonitrile in a 250ml two necks round bottom flask. To this solution 0.3692 g of compound 5 (2.6 mmol) were added and the mixture was stirred for few minutes until everything was dissolved. At this point 0.2399 g of K$_2$CO$_3$ (1.7 mmol) were added, the system was equipped with a condenser and the reaction mixture was heated up to 50°C. The reaction took 40 h to reach completion; it could be monitored through TLC using ethyl acetate as eluent. The best way to see the product spot on the TLC is to use a potassium permanganate based stain. After the reaction reached completion the acetonitrile was substituted with ethyl acetate and the latter was washed with water. The orange
organic phase was dried over MgSO$_4$ and the solvent was evaporated to obtain a viscous orange oil. The crude product was purified via liquid chromatography using ethyl acetate as eluent. Compound 6 was obtained as a viscous yellow oil with 56% yield. $^1$H NMR (CDCl$_3$, 400 MHz) δ(ppm): 2.45 (singlet, 3H), 2.87 (singlet, 2H), 3.57 (multiplet, 12H), 3.69 (multiplet, 4H), 4.16 (triplet, 2H, J=4), 5.27 (singlet, 2H), 6.52 (singlet, 2H), 7.34 (multiplet, 2H), 7.79 (multiplet, 2H). $^{13}$C NMR (CDCl$_3$, 400 MHz) δ(ppm): 21.6, 38.1, 47.4, 67.1, 68.62, 69.23, 70.03, 70.48, 70.52, 70.67, 80.85, 127.93, 129.8, 136.6, 144.7, 176.1.

**Synthesis of (7):**

![Chemical Structure](attachment:image.png)

0.966 g of 6 (2 mmol) were dissolved in 50 ml of acetone in a 250 ml round bottom flask. 0.289 g of potassium thioacetate (2.6 mmol) were added, the flask was equipped with a condenser and the system was heated to 50ºC. After few minutes a fine white precipitate was formed. The reaction was kept under these conditions overnight. Acetone was removed and substituted with ethyl acetate and the solution was washed with brine. The organic phase was dried over MgSO$_4$, the DCM was evaporated and a pale yellow dense oil was obtained with quantitative yield. $^1$H NMR (CDCl$_3$, 400 MHz) δ(ppm): 2.33 (singlet, 3H), 2.86 (singlet, 2H), 3.08 (triplet, 2H, J=4Hz), 3.62 (multiplet, 14H), 5.26 (singlet, 2H), 6.51 (singlet, 2H). $^{13}$C NMR (CDCl$_3$, 400 MHz) δ(ppm): 28.8, 30.5, 38.2, 47.4, 67.1, 69.70, 70.06, 70.27, 70.48, 70.56, 80.9, 136.5, 176.1.

**Synthesis of (8):**
For the synthesis of Maleimide-AuNP the thiol must be prepared right before its use otherwise it reacts to form disulfide. 0.2658 g of 7 (2.3 mmol) were dissolved in 10 ml of dry MeOH in a 50 ml round bottom flask equipped with a stirrer bar. The flask was sealed with a septum and using a long needle the solution was stirred and purged with Ar for 15 min. To completely avoid the formation of disulfide it is important that the reaction is carried out in complete absence of oxygen, therefore the flask must be kept under high pressure of inert gas. 2.3 ml of 1 M NaOH solution in EtOH was insert an a second 50 ml round bottom flask and was purged with Ar for 15 min using a long needle. Using a cannula the 2.3 ml of NaOH solution were transferred to the MeOH solution. The reaction mixture was stirred for 45 min. In the mean time 5 ml of 1 M HCl in water were purged in a third 50 ml round bottom flask sealed with a septum. After the 20 minutes the HCl solution was transferred to the reaction mixture using a cannula. The solution was then stirred and purged with Ar for 5 minutes. After this time the flask was opened and the thiol was extracted with DCM. The collected organic fractions were dried over MgSO₄, and the solvent was removed to obtain the thiol 8 as a pale yellow and viscous oil in quantitative yield without any trace of disulfide. ¹H NMR (CDCl₃, 400 MHz) δ(ppm): 1.60 (triplet, 1H, J=8Hz), 2.70 (quartet, 2H, J=8Hz), 2.87 (singlet, 2H), 3.63 (multiplet, 14H), 5.27 (singlet, 2H), 6.52 (singlet, 2H). ¹³C NMR (CDCl₃, 400 MHz) δ(ppm): 24.3, 47.5, 67.1, 70.10, 70.21, 70.52, 70.59, 72.6, 80.9, 136.5. Exact mass (C₁₆H₂₃NO₆S) calc: 357.124, found 357.123.
Synthesis of Protected Maleimide AuNP (PtMaleimide-EG₄-AuNP):
The protected maleimide tetraethylene glycol ligands (PtMaleimide-EG₄-SH) were inserted in the nanoparticles shell through a place exchange reaction. In a typical synthesis we used 1.0 mg of PtMaleimide-EG₄-SH per 2.7 mg of Me-EG₃-AuNP. The freshly prepared thiol was dissolved in a mixture of dry methanol and acetone (dry methanol : acetone, 8:1). When the thiol was totally dissolved, the nanoparticles were added and the reaction mixture was stirred vigorously for 15 min. The solvent was evaporated and the nanoparticle film was washed with cyclohexane to remove the most of unreacted or exchanged ligands. PtMaleimide-EG₄-AuNP were then purified by dialysis overnight.

Synthesis of Deprotected Maleimide AuNP (DptMaleimide-EG₄-AuNP):
The deprotection of the maleimide was carried out using the retro Diels-Alder reaction similar to that one reported previously for organic soluble maleimide AuNP.¹ The PtMaleimide-EG₄-AuNP were dissolved in toluene in a round bottom flask equipped with a condenser. The system was then heated at 100°C overnight. Toluene was evaporated under vacuum and the resulting nanoparticle film inside the flask was washed with cyclohexane.
Figure SI 4: $^1$H and $^{13}$C NMR spectra of compound 4 recorded in CDCl₃.
Figure S1 5: $^1$H and $^{13}$C NMR spectra of compound 6 recorded in CDCl$_3$. 
Figure S1 6: $^1\text{H}$ and $^{13}\text{C}$ NMR spectra of compound 7 recorded in CDCl$_3$. 
Figure SI 7: $^1$H and $^{13}$C NMR spectra of compound 8 recorded in CDCl$_3$. 
Figure SI 8: TEM images of A) Me-EG$_3$-AuNP (2.0 ± 0.3 nm), B) PtMaleimide-EG$_4$-AuNP, (2.0 ± 0.3 nm), C) Maleimide-EG$_4$-AuNP, (2.5 ± 0.3 nm) and D) Hydrolysis product of Maleimide-EG$_4$-AuNP. Note: on prolonged heating in water there is some aggregation due to hydrolysis products. This leads to larger apparent AuNP.
Figure SI 9: UV-VIS spectra (A) and their derivative (B) of Me-EG₃-AuNP (black line), PtMaleimide-EG₄-AuNP (blue line), and Maleimide-EG₃-AuNP (red line). Spectra recorded in nanopure water.
Figure SI 10: Derivative of the TGA carried out on Me-EG$_3$-AuNP (solid line), PtMaleimide-EG$_4$-AuNP (dashed line), and Maleimide-EG$_4$-AuNP (dotted line).
Figure SI 11: Kaiser test carried out on the hydrolysis product of N-methylmaleimide after heating dissolved in water (left vial) and on a solution of N-methylmaleimide in water prior to heating (right vial). Blue color indicates presence of a primary amine.
Figure SI 12: $^1$H NMR spectra recorded in D$_2$O of i) hydrolysis products of N-methylmaleimide, ii) hydrolysis products of N-methylmaleimide plus addition of methylamine, iii) hydrolysis products of N-methylmaleimide plus addition of methylamine and maleic acid.
Figure SI 13: $^{13}$C NMR spectra recorded in D$_2$O of i) hydrolysis products of N-methylmaleimide, ii) hydrolysis products of N-methylmaleimide plus addition of methylamine, iii) hydrolysis products of N-methylmaleimide plus addition of methylamine and maleic acid.
Figure S1 14: $^1$HMR spectra recorded in D$_2$O showing the progression of the hydrolysis of the maleimide functionality of the model ligand. In addition to the formation of furan (indicated by arrows), note the growth of proton G (maleimide), which under prolonged heating in water disappears, and the appearance of H (and I). Also the hydrolysis of the thioacetate functionality to acetic acid takes place under the same conditions, as indicated by the growth of the peak L due to acetic acid.
**Figure SI 15:** $^1$HMR spectra recorded in D$_2$O showing the progression of products from deprotection of PtMaleimide-AuNP by heating in water. In addition to the formation of furan note the growth of proton F (maleimide) which disappears and the appearance of H (and G).
**Figure SI 16:** $^1$H NMR spectrum recorded in D$_2$O of the product resulting from heating Maleimide-EG$_4$-AuNP in water and then adding authentic maleic acid (peak at 6.37 ppm). The signal originally at 6.31 ppm is slightly shifted downfield to 6.37 ppm because of the increased acidity of the solution after adding maleic acid. The superimposition of the signal of the authentic maleic acid with that of the signal on the AuNP supports the conclusion that on heating in water the Maleimide-EG$_4$-AuNP is hydrolysed to maleic acid/maleate and an amine (ammonium)-terminated AuNP. (i.e., supports the formation of maleic acid on heating N-methylmaleimide in water).
Figure SI 17: $^1$H NMR spectrum recorded in D$_2$O of Maleimide-EG$_4$-AuNP after Michael addition reaction with L-cysteine. Note loss of signal at 6.85 ppm due to maleimide and new signals at 3-3.5 ppm due to protons of the adduct.
**Zeta Potential Measurements:**

Zeta potential measurements of Maleimide-EG$_4$-AuNP and hydrolyzed Maleimide-EG$_4$-AuNP were carried out by measuring the nanoparticles electrophoretic mobility in nanopure water. A solution of Maleimide-EG$_4$-AuNP in nanopure water was prepared with a concentration of 0.5mg/ml. The solution’s pH was found to be 6. 1ml of this solution was inserted in a folded capillary cell equipped with electrodes and the zeta potential was by calculated employing the Smoluchowski method. The zeta potential was found to be $-36.9 \pm 4.2$ mV (see Figure SI 18), indicating a good stability of the Maleimide-EG$_4$-AuNP in water. The mother solution was then warmed up overnight at 100ºC to hydrolyze the maleimide functionalities of the nanoparticles. Water was then evaporated and a solution with a concentration of 0.5mg/ml in nanopure water was newly made. The pH of this solution was found to be 6. Zeta potential was then calculated by using the same conditions as before and the same method. The zeta potential for the hydrolyzed Maleimide-EG$_4$-AuNP was found to be $-24.6 \pm 3.3$ mV (see Figure SI 18). The more positive value found for the hydrolyzed nanoparticles can be ascribed to the presence of the ammonium functionalities. However, the difference is not so marked because the counterion, the maleate, is strongly electrostatically bound to the surface, being that the nanoparticle basically a polycation. Noteworthy is that after the hydrolysis the zeta potential dropped into the region of nanoparticles instability ($-30$ mV up to $+30$mV), indicating that the hydrolyzed Maleimide-EG$_4$-AuNP slowly tend to flocculate because the coulombic repulsion is no longer strong enough to keep them far apart.
Figure SI 18: Zeta potential measurements. Top: the Maleimide-EG₄-AuNP and Bottom: the hydrolyzed Maleimide-EG₄-AuNP.

References: