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Supporting Information

Versatile Strained-Alkyne Modified Water-Soluble AuNPs for *Interfacial* Strain-Promoted Azide-Alkyne Cycloaddition (*I*-SPAAC)

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Materials and methods

The following reagents, unless otherwise stated, were used as received. Triethylene glycol monomethylether, tetraethylene glycol, 4-dimethylaminopyridine (DMAP), deuterated chloroform ($CDCl_3$), tetrachloroauric acid trihydrate, sodium borohydride, *p-toluenesulfonil* chloride, periodic acid, pyridinium chlorochromate (PCC), N,N-Diisopropylethylamine, 2-propyn-1-amine hydrochloride, 1-ethynylpyrene, copper sulfate, sodium ascorbate and O-Benzotriazole- N,N,N',N'-tetramethyl-uronium-hexafluoro-phosphate (HBTU) were purchased from Aldrich. All common solvents, triethyleneamine, magnesium sulfate, dry methanol, hydrochloric acid, sodium hydroxide, sodium chloride, sodium iodide, and potassium carbonate were purchased from Caledon. Sodium bisulfite was purchased from Fisher Scientific. Thiourea was purchased from J. T. Baker Chemical Company. Deuterated water (D_2O) was purchased from Cambridge Isotope Laboratories. Ethanol was purchased from Commercial Alcohols. Glacial acetic acid (99.7%) was purchased from BDH. Dialysis membranes (MWCO 6000-8000 Da) were purchased from Spectra/Por. Poly(butadiene-b-ethylene oxide) (PBD-PEO) (PDI 1.10) with a composition of 6500 g/mol PBD (> 80 % 1,2 addition) and 3900 g/mol was purchased from Polymer Source (Dorval, Canada).

^{1}H and ^{13}C NMR spectra were recorded on an Inova 400Mhz using $CDCl_3$ or D_2O as solvent and were calibrated against the residual protonated solvent.

Thermogravimetric analysis (TGA) were recorded by loading the sample in a 70 µl ceramic crucible and heating from 25ºC to 750ºC at rate of 10ºC 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 TEM Philips CM10. The TEM grids (Formvar carbon film on 400 mesh copper grids) were purchased from Electron Microscopy Sciences and prepared by dropcasting a drop of nanoparticles solution directly onto the grid surface. The drop was then carefully removed after 30 second with a soft tissue. The vesicle samples were

instead prepared by dropcasting 10 µl of vesicles solution onto the TEM grid. The drop was let to dry completely overnight before recording the TEM images.

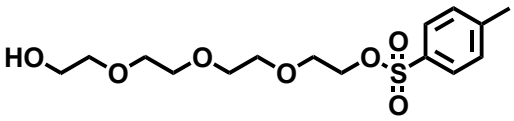
Infrared spectra were recorded using a Bruker Vector33 spectrometer by making a thin film of sample onto a KBr disk.

The XPS analyses were carried out with a Kratos Axis Ultra spectrometer using a monochromatic Al K(alpha) source (15mA, 14kV). XPS can detect all elements except hydrogen and helium, probes the surface of the sample to a depth of 5-7 nanometres, and has detection limits ranging from 0.1 to 0.5 atomic percent depending on the element. The instrument work function was calibrated to give a binding energy (BE) of 83.96 eV for the Au 4f7/2 line for metallic gold and the spectrometer dispersion was adjusted to give a BE of 932.62 eV for the Cu 2p3/2 line of metallic copper. Specimens were mounted on a double sided adhesive tape and the Kratos charge neutralizer system was used on all specimens. Survey scan analyses were carried out with an analysis area of 300 x 700 microns and a pass energy of 160 eV. High resolution analyses were carried out with an analysis area of 300 x 700 microns and a pass energy of 20 eV. Spectra have been charge corrected when needed to the main line of the carbon 1s spectrum set to 285.0 eV for aliphatic carbon. Spectra were analyzed using CasaXPS software (version 2.3.14).

Z-potential measurements were performed using a Zetasizer Nano-ZS (Malvern Instrument). A solution of AuNP in PBS pH 7.5 was prepared with a concentration of 0.5 mg ml^{-1}. 1 ml of this solution was inserted in a latex folded capillary cell equipped with electrodes and the ζ-potential was calculated by employing the Huckel approximation.

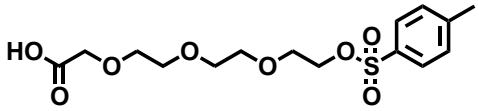
Synthesis of **Compound 1**,

2-(2-(2-(2-hydroxyethoxy)ethoxy)ethoxy)ethyl 4-methylbenzenesulfonate (HO-EG_4-Ts):



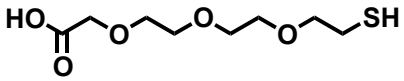
To a 1L round bottom flask containing 400 mL of dichloromethane was added 0.7330 g (6 mmol) of 4-dimethylaminopyridine and 9 mL (65 mmol) of triethylamine. The mixture was set up with vigorous stirring and 10.35 mL (60 mmol) of tetraethylene glycol was added to the flask. The mixture was then cool to 0 °C. After cooling, 3.8130 g (20 mmol) of *p*-toluenesulfonyl chloride was added slowly over 10 minutes. After addition, the mixture was allowed to warm to room temperature and the reaction proceeded for 4 hours. The solvent was removed under vacuum, and the concentrated mixture was then washed with 1M sodium hydroxide, followed by a wash with 1M hydrochloric acid. The product was dried with magnesium sulfate and the solvent was removed under vacuum. A mixture of di-substituted and mono-substituted products was formed, so purification through column chromatography was required. An initial mixture of 4:1 ethyl acetate to acetone was used to elute the product through the column. The di-tosylate product eluted first, and the solvent was then switched to a 2:1 ethyl acetate to acetone mixture to facilitate faster elution of the mono-substituted product. The mono-tosylated product was obtained as a pale yellow oil with a 53% yield. ^{1}H NMR ($CDCl_3$): 7.78 (d, J=8Hz, 2H), 7.33 (d, J=8Hz, 2H), 3.59 (m, 16H), 2.43 (s, 3H). ^{13}C NMR: δ 145.0, 136.1, 72.7, 70.7, 69.5, 68.9, 61.9, 21.91, 21.31. Infrared spectrum (3454cm^{-1}, 2878cm^{-1}, 1583cm^{-1}, 1352cm^{-1}, 1179cm^{-1}). Mass Spectrum (CI) $C_{15}H_{24}O_7S$ $[M+H]^+$: calc: 348.1242, found: 348.1249.

Synthesis of **Compound 2**, 2-(2-(2-(2-(tosyloxy)ethoxy)ethoxy)ethoxy)acetic acid (HOOC-EG$_4$-Ts):



To a clean dry 50 mL 3 neck round bottom flask was added 2.25 g (9.8 mmol) of periodic acid. An addition funnel was placed in one neck of the flask, and 27 mg (2.5% mol) of pyridinium chlorochromate (PCC) was added to it. The system was purged with argon for 15 minutes. To the periodic acid was added 30 mL of dry acetonitrile and 10mL of dry acetonitrile was added to the PCC. The periodic acid mixture was allowed to stir for 15 minute and was then followed by the addition of 1.5 g (4.3 mmol) of **Compound 1**. The reaction flask was then placed in an ice bath to cool the mixture to 0 °C. After cooling, the PCC solution was added dropwise over 15 minutes. The solution became cloudy and opaque with some light orange streaks from the PCC solution. The reaction proceeded for 3 hours before work up. At this point the reaction was still cloudy and opaque but had a greenish tinge to it. The solution was diluted with 60 mL of ethyl acetate and placed in a separatory funnel. The organic layer was washed with 75 mL of saturated sodium bisulphite solution, 3 times with brine, and was finally dried with magnesium sulphate. The product was collected as a yellow oil in 88% yield. ^{1}H NMR ($CDCl_3$): δ 7.80, (d, J=8hz, 2H) 7.35, (d, J=8hz, 2H) 4.17, (s, 2H) 3.59, (m , 12H) 2.45 (s, 3H). ^{13}C NMR ($CDCl_3$): δ 173.0, 144.8, 132.8, 129.8, 127.9, 71.3, 70.53, 70.22, 69.2, 68.70, 68.63 21.6. Infrared spectrum (3266 cm^{-1}, 2906cm^{-1}, 2870cm^{-1} 1755cm^{-1}, 1596cm^{-1}, 1359cm^{-1}, 1172cm^{-1}). Mass Spectrum (CI) $C_{15}H_{22}O_8S$ $[M+H]^+$: calc: 362.1035, found: 362.1107.

Synthesis of **Compound 3**, 2-(2-(2-(2-mercaptoethoxy)ethoxy)ethoxy)acetic acid (HOOC-EG_4-SH):



To a 100mL 2 neck round bottom flask was added 0.332 g (4.28 mmol) of thiourea, 0.165 g (1.10 mmol) sodium iodide, 0.4 g (1.10 mmol) of **Compound 2**, and 30 mL of water. The solution was purged with argon for 15 minutes and kept under vigorous stirring and then the mixture was heated to reflux at 100 °C for 4 hours. After 4 hours, 6 mL of 2.5M sodium hydroxide was purged under argon in a separate flask for 15 minutes and then added to the reaction via cannula. The reaction was allowed to proceed for another 4 hours under reflux. After 4 hours, the reaction was allowed to cool to room temperature and in the meantime, 18mL of concentrated hydrochloric acid was purged under argon in a separate flask for 15 minutes. After purging, the hydrochloric acid was added to the reaction mixture and the system was allowed to stir for 15 minutes. The mixture was extracted 7 times with chloroform, and the organic layers were collected and dried with magnesium sulfate. The solvent was removed under vacuum to yield a yellow oil in 62% yield. ^{1}H NMR ($CDCl_3$): δ 4.18 (s, 2H), 3.68 (m, 10H), 2.72 (dt J1= 8Hz, J2=8Hz, 2H), 1.60 (t, J=8Hz, 1H). ^{13}C NMR ($CDCl_3$): δ 172.4, 72.8, 71.4, 70.6, 69.9, 68.7, 24.1. Infrared spectrum (3300cm-1, 2938cm^{-1}, 2870cm^{-1}, 2576cm^{-1}, 1741cm^{-1}, 1463cm^{-1}, 1431cm^{-1}, 1355cm^{-1}, 1132cm^{-1}). Mass Spectrum (CI) $C_8H_{16}O_5S$ $[M+H]^+$: Calc: 224.0717, found: 224.0718.

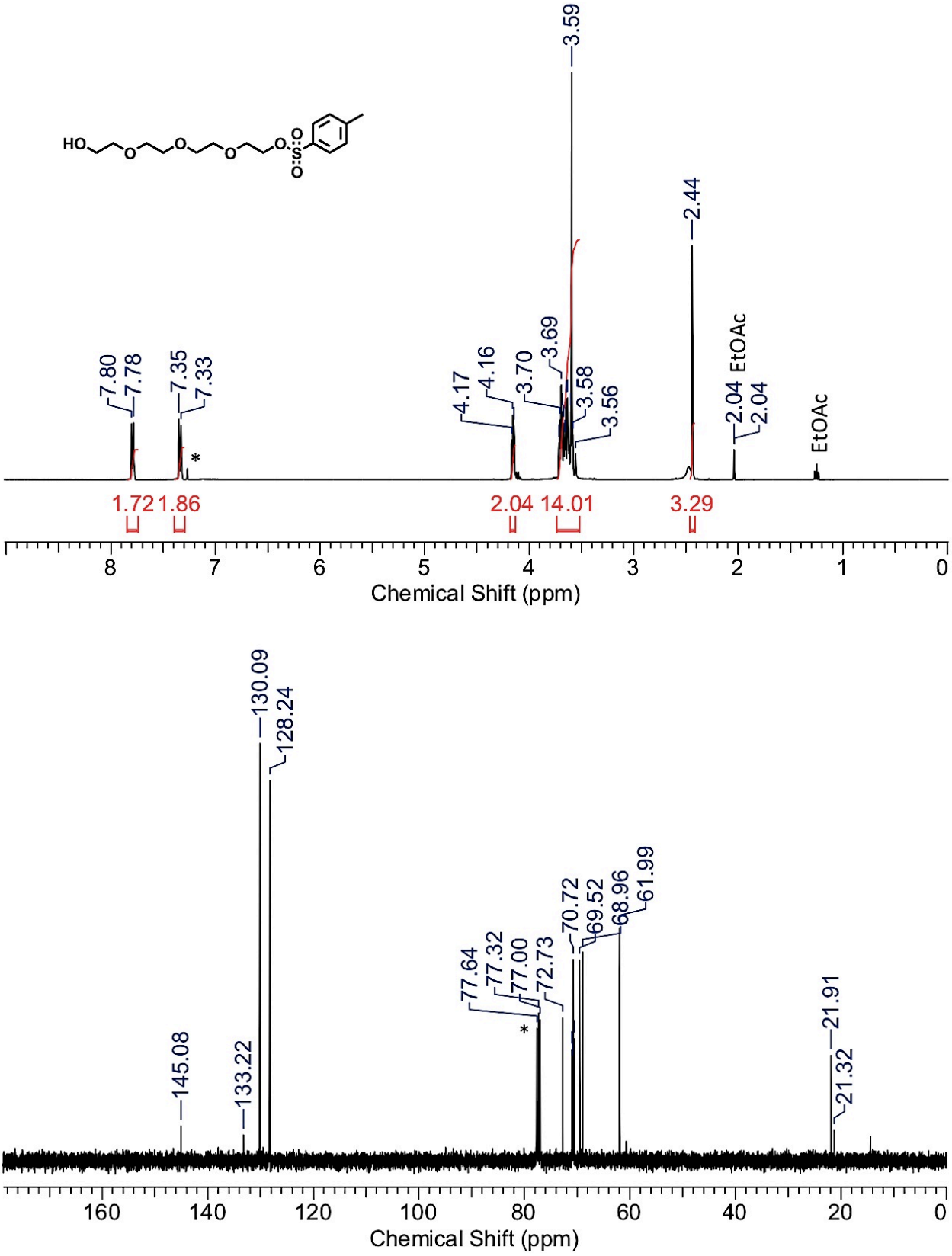


Figure SI1: ^{1}HNMR (top) ^{13}CNMR (bottom) of **Compound 1** HO-EG$_4$-Ts recorded in $CDCl_3$ and calibrated against residual chloroform (*).

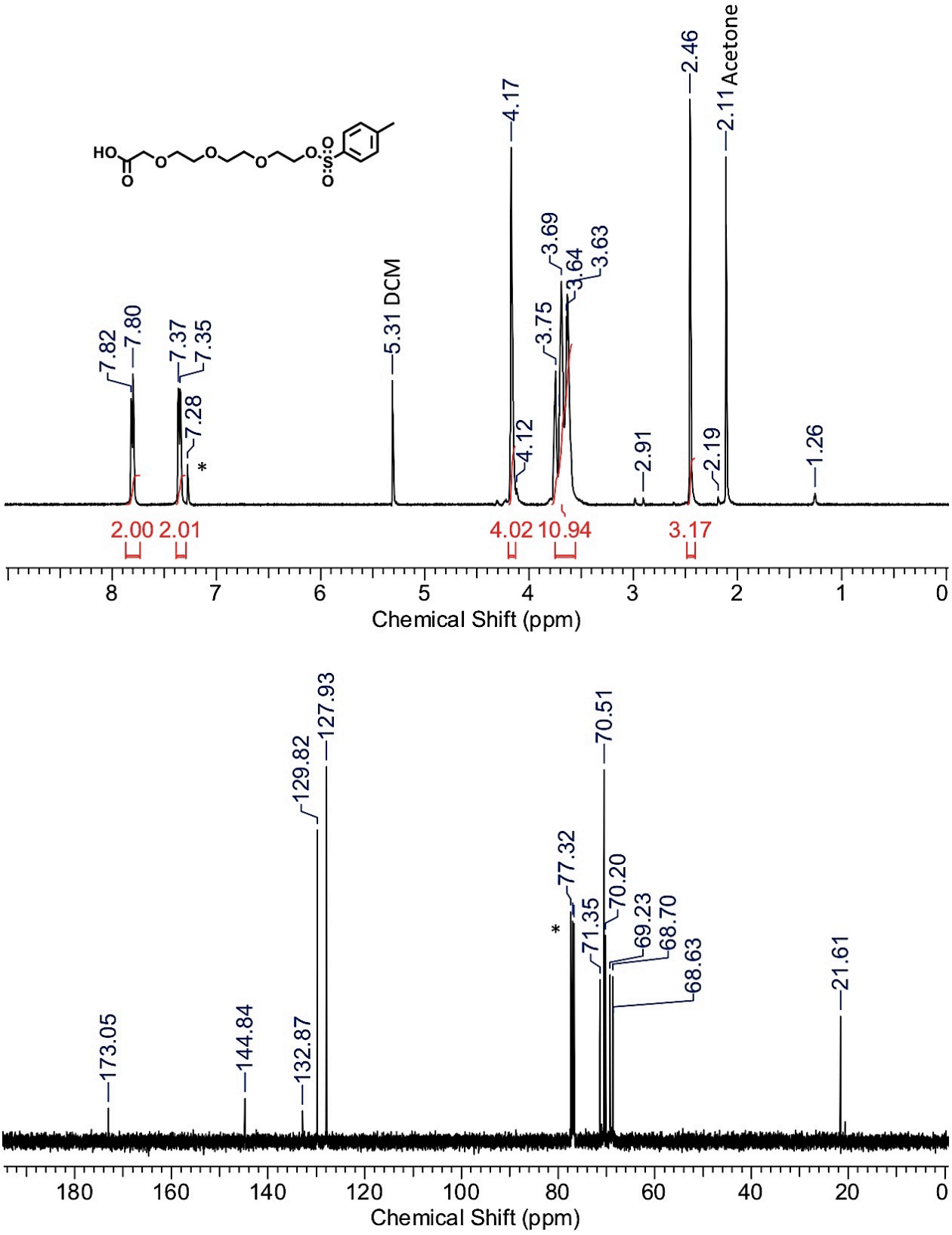


Figure SI2: ^{1}HNMR (top) ^{13}CNMR (bottom) of **Compound 2** HOOC-EG$_4$-Ts recorded in $CDCl_3$ and calibrated against residual chloroform (*).

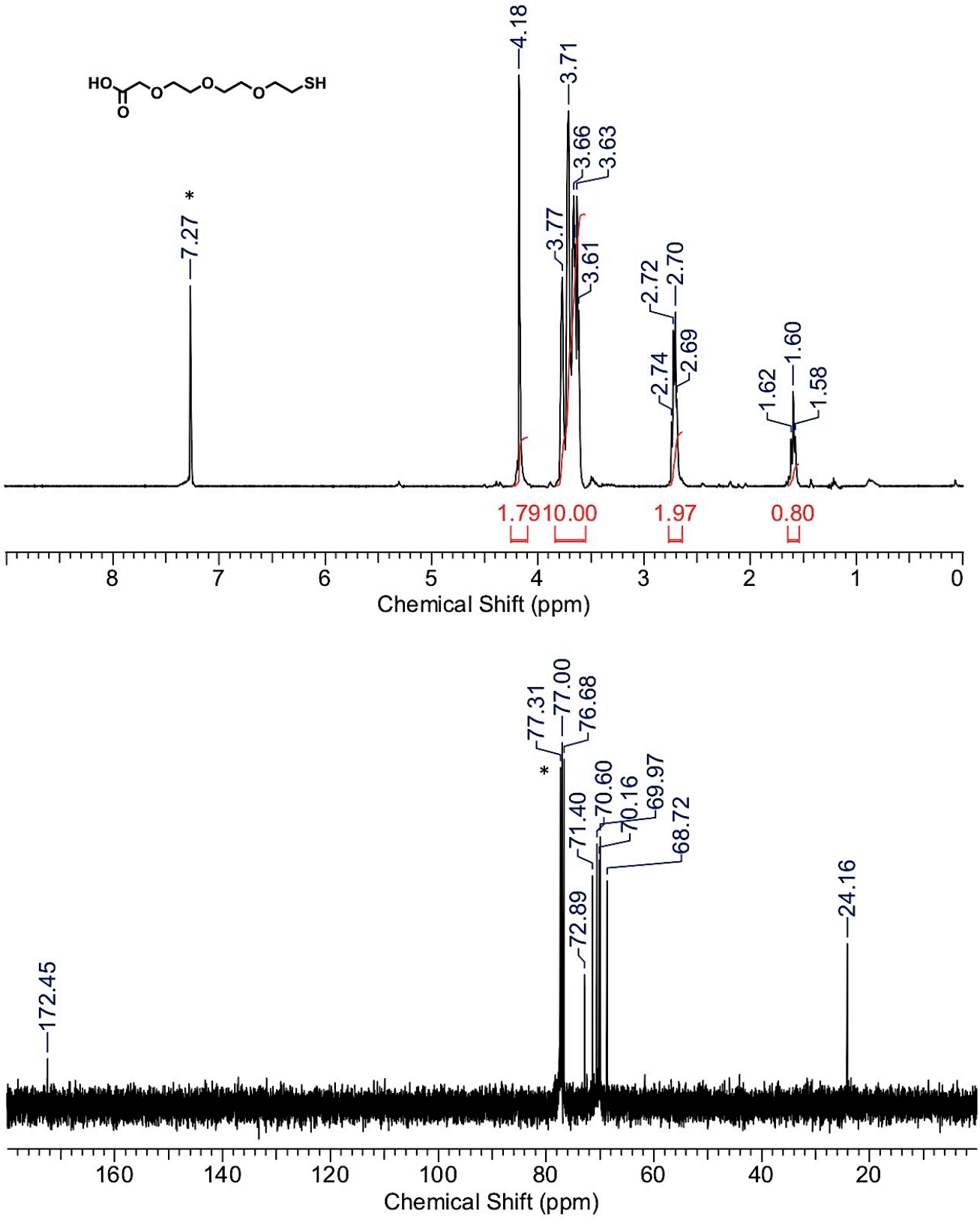


Figure SI3: ^{1}HNMR (top) ^{13}CNMR (bottom) of **Compound 3** HOOC-EG$_4$-SH recorded in $CDCl_3$ and calibrated against residual chloroform (*).

Synthesis of Triethylene Glycol Monomethyl Ether AuNP (Me-EG_3-AuNP)

Me-EG_3-AuNP were synthesized according to our previously established procedure.[1] Briefly, 19.3 mg of HAuCl4·3H2O (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 NaBH4 (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 stirred overnight. The solution was then concentrated at rotavapor, 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 colorless. 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 hexanes to remove the excess of thiol. The nanoparticles were then redissolved in nanopure water and purified by dialysis overnight.

^{1}H NMR 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. The Me-EG_3-AuNPs are well soluble in water, methanol, ethanol, tetrahydrofuran, dichloromethane, chloroform, toluene, ethyl acetate, acetone, dimethylformamide, and acenonitrile.

Synthesis of HOOC-EG$_4$-AuNP

73.9 mg of Me-EG$_3$-AuNP were dissolved in 5 mL of CH_2Cl_2 in a 25 mL round bottom flask. To this mixture was added 10.9 mg of **Compound 3**, HOOC-EG$_4$-SH. The reaction was allowed to proceed for 30 minutes and the solvent was removed under vacuum. The AuNP film was washed with hexanes and isopropyl alcohol and then the HOOC-EG$_4$-AuNP were redissolved in milliQ water. The solvent was removed and the particles were washed and re-dissolved 2 more times. The removal of free thiols was monitored through ^{1}H NMR spectroscopy by the disappearance of sharp peaks.

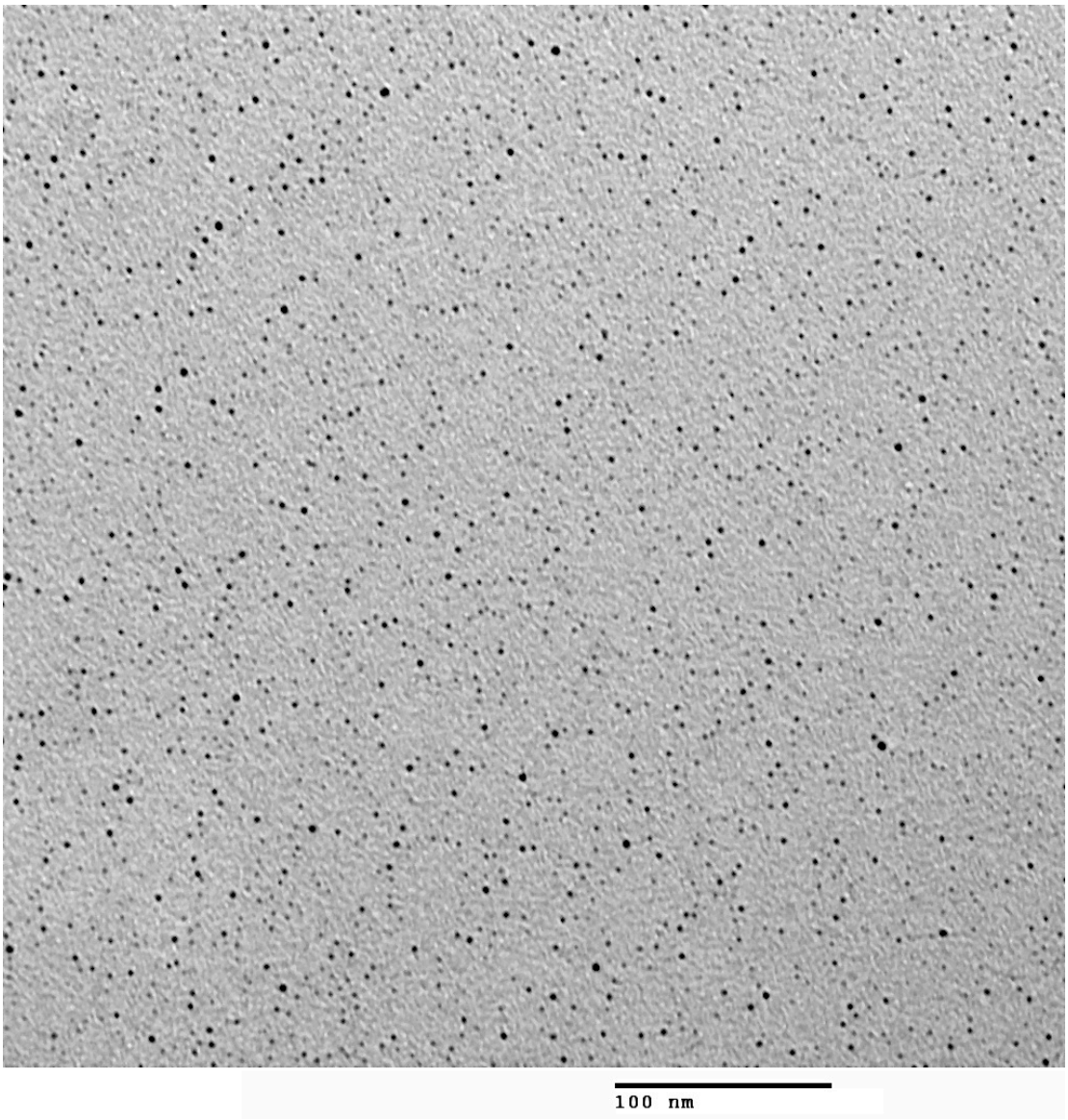


Figure SI4: TEM image of HOOC-EG$_4$-AuNP.

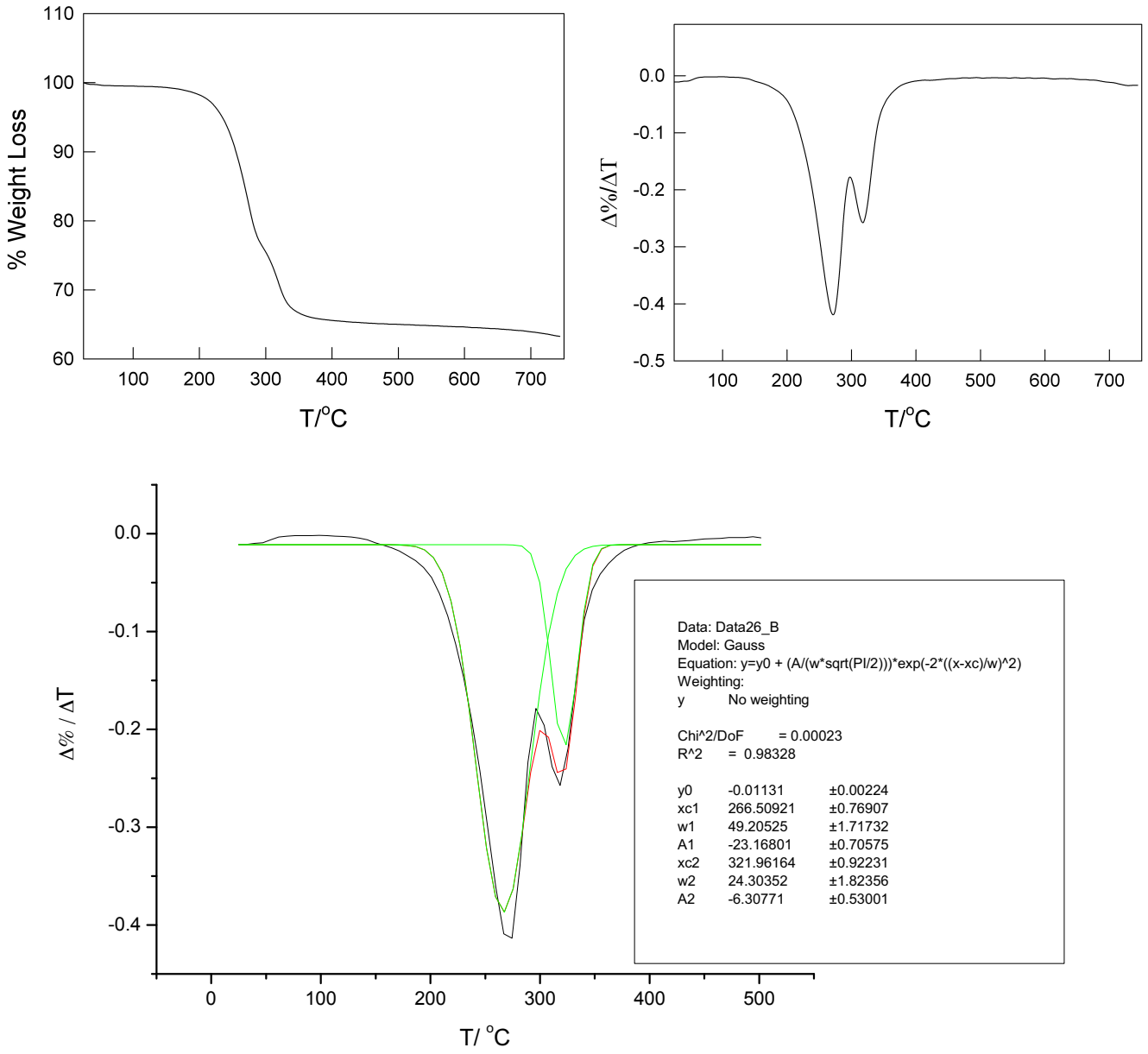


Figure SI5: TGA, TGA derivative, and deconvolution of the TGA derivative curve for the HOOC-EG_4-AuNP.

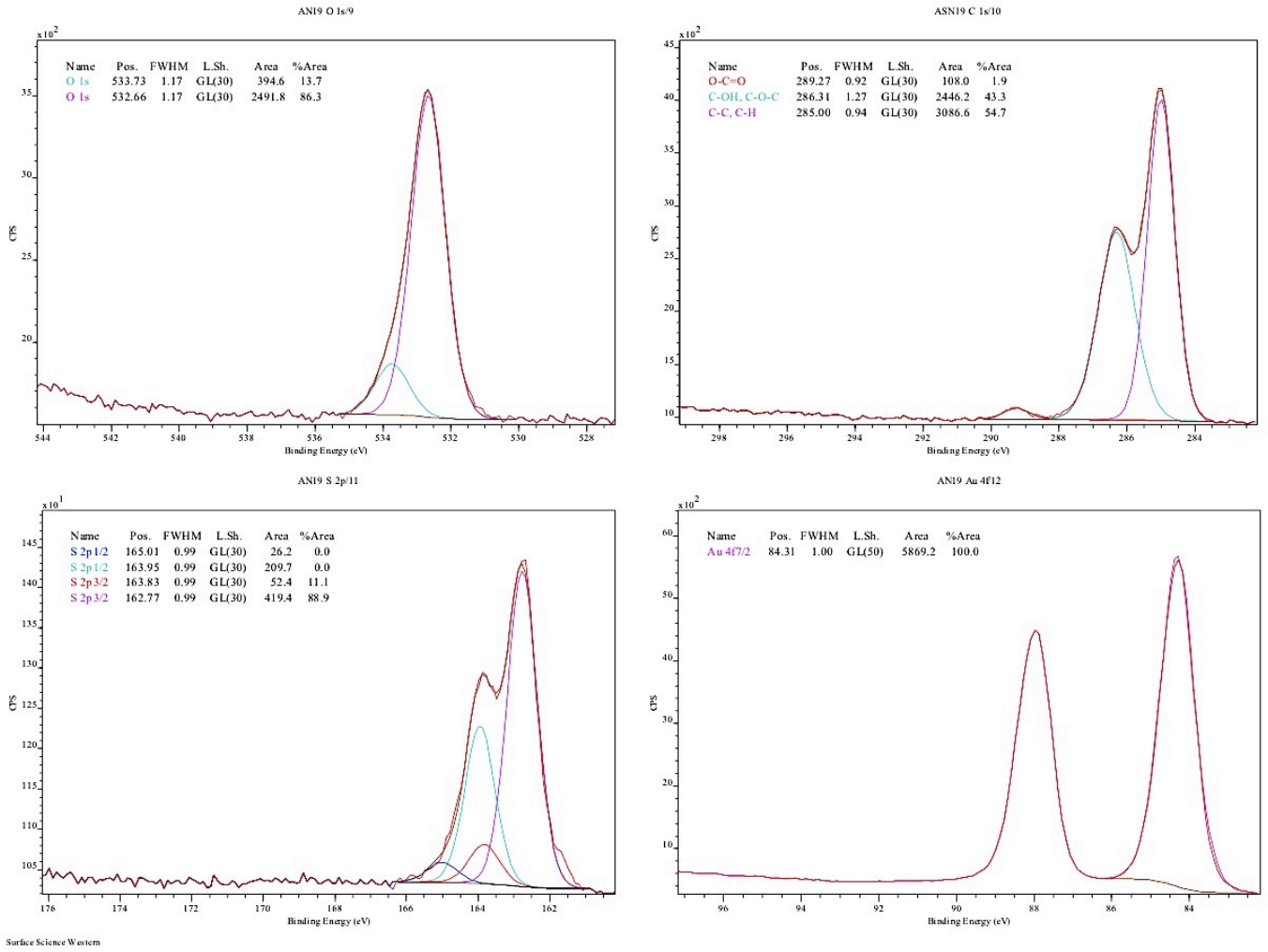


Figure SI6: High-resolution XPS spectra for HOOC-EG$_4$-AuNP.

Composition of the AuNP's corona from XPS data

The percentage of carbon corresponding to the carboxylic group -(C*=O)-OH is 1.9%, while the one corresponding to the C*-O (common to both ligands) is 43.3%. Because of the ligand structures, the percentage of carboxylic functionalities is proportional to the number of carboxy-terminated ligands (HOOC-EG$_4$-S) (eq. 1), while the percentage of C-O is related to the percentage of the two ligands by equation 2.

1.9 = [-(C*=O)-OH] = [HOOC-EG$_4$-S] (% of carboxy-terminated ligands) (eq.1)

$43.3 = [C^*\text{-}O] = 6[Me\text{-}EG_3\text{-}S] + 6[HOOC\text{-}EG_4\text{-}S]$ (6 = number of C*-O per ligand) (eq.2)

$[Me\text{-}EG_3\text{-}S] = (43.3 - 11.4) / 6 = 5.3$ (% of $Me\text{-}EG_3$-S ligands)

The relative % of the two ligands and the composition of the corona can now be easily calculated:

$(1.9 * 100)/ (5.3+1.9) = 26.4$ (% of $HOOC\text{-}EG_4$-S on the corona)

$100-26.4 = 73.6$ (% of $Me\text{-}EG_3$-S on the corona)

Synthesis of DBCO-AuNP

To a 25 mL round bottom flask was added 65 mg HOOC-AuNP. The flask was purged under argon for 15 minutes and then 9 mL of dry DMF was added. After addition of the DMF, 16 µL (90 µmol) of N-N-Diisopropylethylamine was added to the reaction mixture and the reaction was cooled down to 0°C in an ice bath. In a separate flask, 23 mg (60 µmol) of HBTU (O-Benzotriazole- N,N,N',N'-tetramethyl-uronium-hexafluoro-phosphate) was placed under argon followed by addition of 5mL of dry DMF. The HBTU solution was purged for 15 minutes and was then added to the reaction mixture and allowed to stir for 15 minutes. In another flask, 17 mg (60 µmol) of DBCO-amine was placed under argon and dissolved in 3 mL of dry DMF. After 15 minutes of purging, the DBCO-amine was added to the reaction flask and the ice bath was removed. The reaction was allowed to proceed overnight under an inert atmosphere. The next day the reaction mixture was insert into a dialysis bag (MWCO 6-8 KDa) and dialyzed against DMF to remove the reaction byproducts. The DMF was changed every 2 hours for two times. Finally the sample was dialyzed against water overnight.

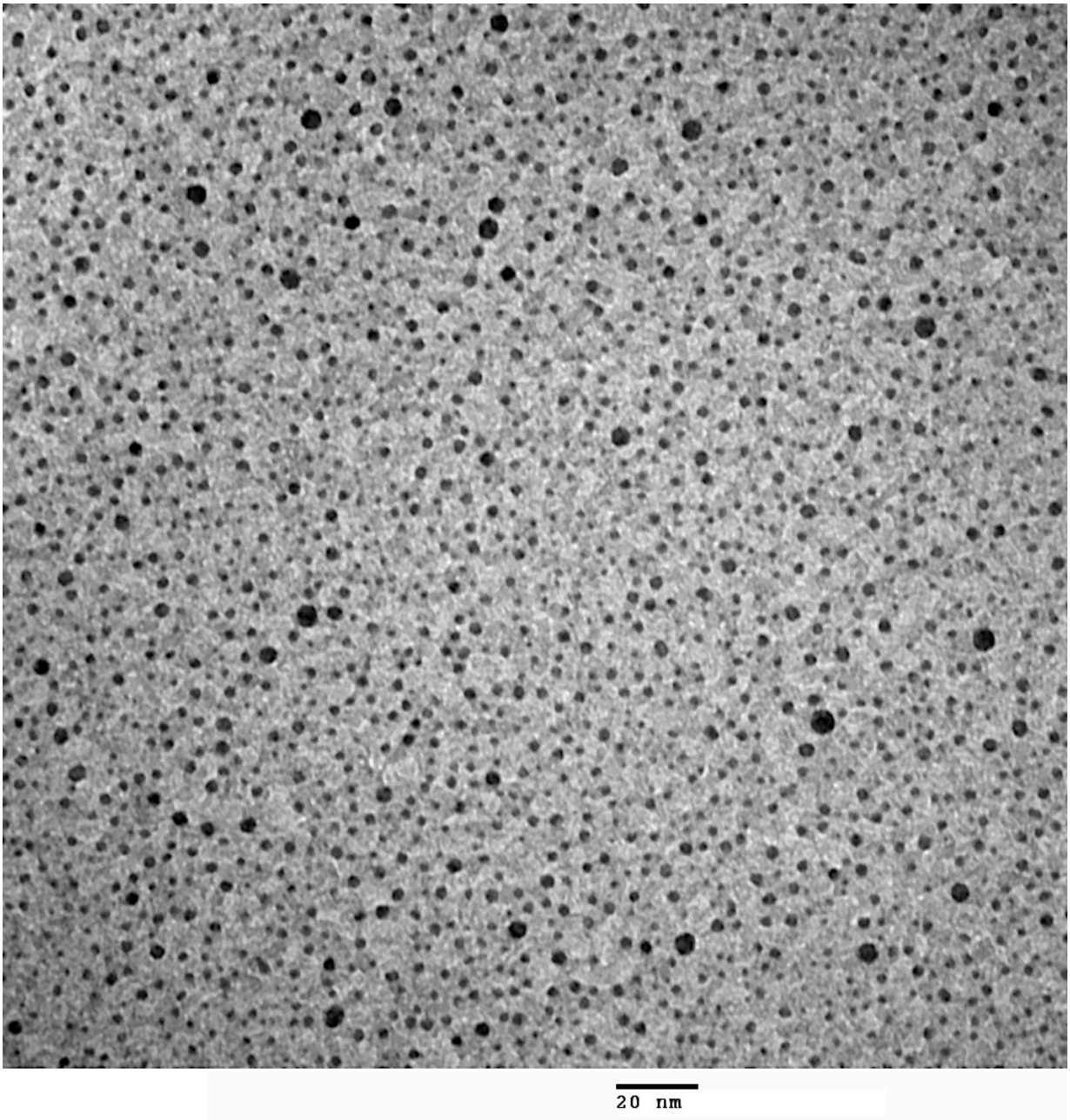


Figure SI7: TEM image of DBCO-AuNP.

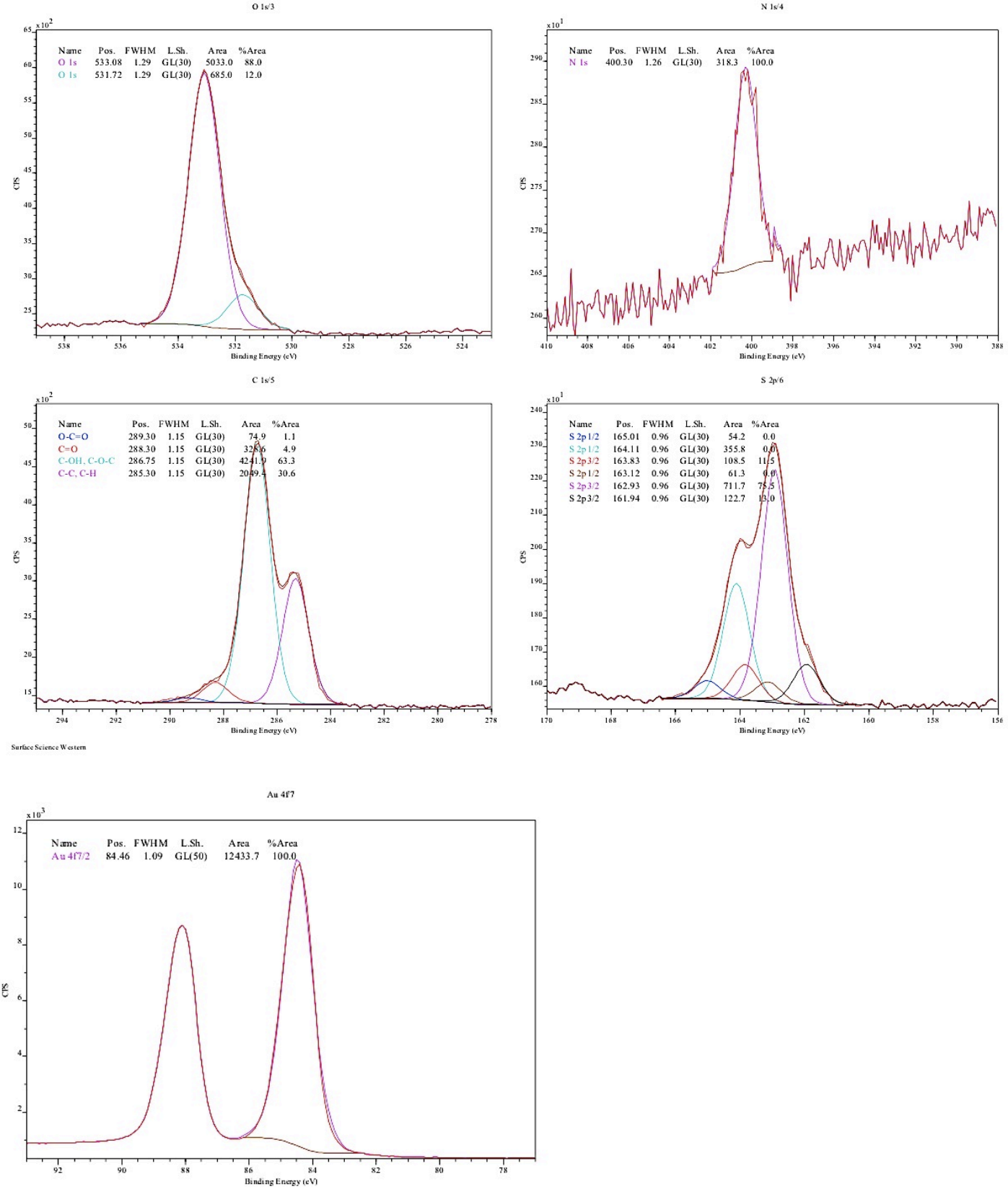


Figure SI8: High-resolution XPS spectra for DBCO-AuNP.

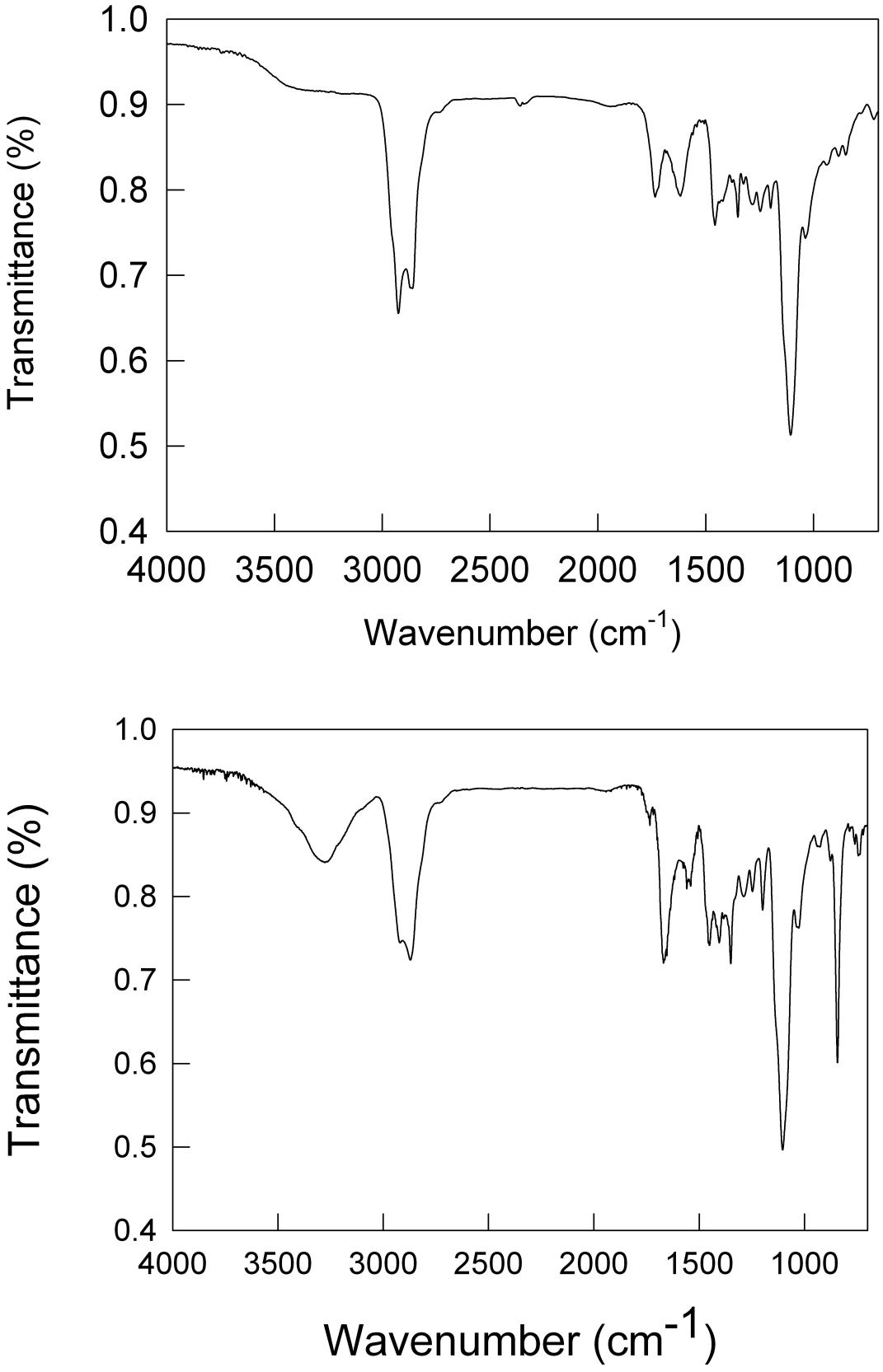


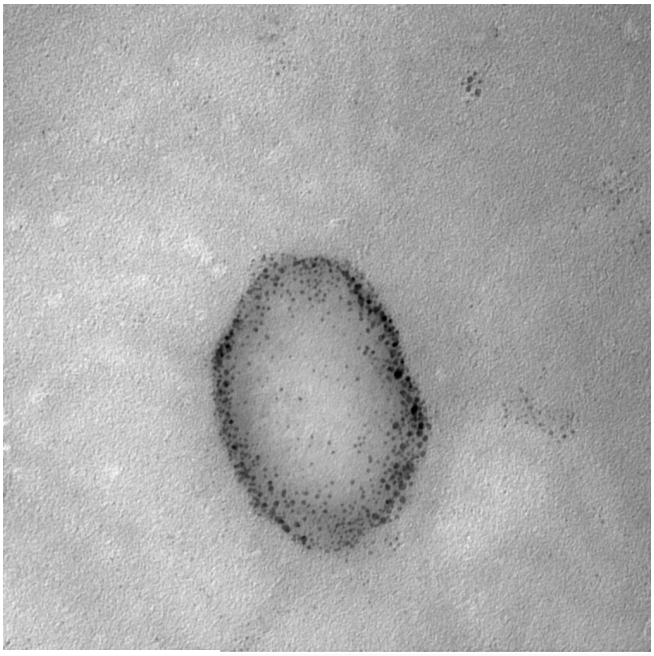
Figure SI9: IR spectra of the HOOC-EG_4-AuNP (top) and DBCO-AuNP (bottom).

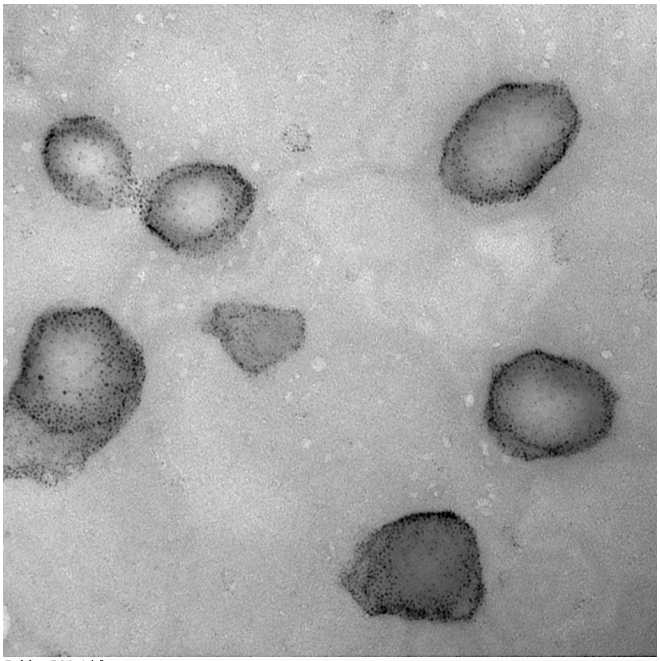
Synthesis of PBD-PEO-N_3:

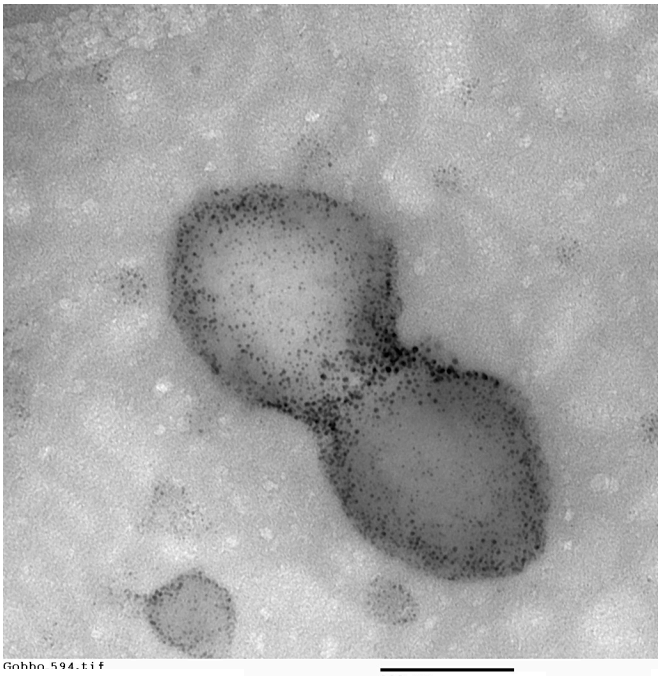
PBD-PEO (50 mg, 4.8 μmol, 1.0 equiv.) was dissolved in dry CH_2Cl_2 (0.5 mL). Azido acetic acid (9.7 mg, 96 μmol, 20 equiv), dicyclohexylcarbodiimide (DCC) (20 mg, 96 μmol, 20 equiv), 4,4-dimethylaminopyridine (DMAP) (2.9 mg, 24 μmol, 5.0 equiv) and 4,4-dimethylaminopyridinium p-toluenesulfonate (DPTS) (7.1 mg, 24 μmol, 5.0 equiv) were added, then the reaction mixture was stirred overnight under nitrogen at room temperature. The reaction mixture was diluted with CH_2Cl_2, then the dicyclohexylurea (DCU) byproduct was filtered off using a cotton plug and washed with a small amount of CH_2Cl_2. The solvent was evaporated and the resulting residue was taken up in EtOAc. Residual DCU was removed by filtering the resulting suspension. The filtrate was dialysed against 200 mL of DMF for 24 h using a 25K MWCO membrane (Spectra/Por, regenerated cellulose). The DMF was evaporated to provide polymer 3 (46 mg, 91 %). ^{1}H NMR (400MHz, $CDCl_3$): δ 5.60-5.21 (m, 152H), 5.00-4.84 174H), 1.38-0.95 (m, 264H). IR (thin film from $CHCl_3$): 2108, 1830, 1733,1640.

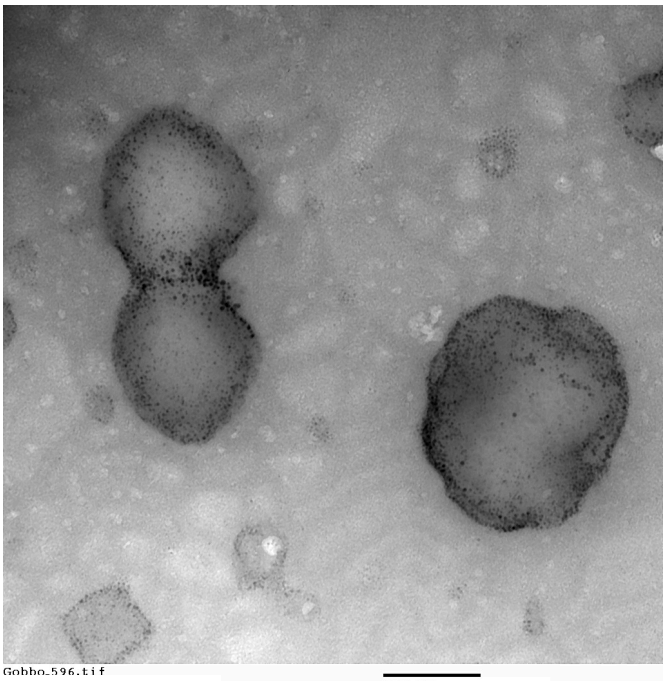
Synthesis of Azide-Functionalized Polymersomes

The azide-functionalized polymersomes were prepared as previously reported by the Gillies group.[2] Briefly, PBD-PEO-N3 (5 mg) were dissolved in CH_2Cl_2 (0.5 mL) in a 5 mL round bottom flask. The solvent was removed under a stream of air to produce a film of the polymer on the flask. Deionized (DI) water (1 mL) was added and the resulting sample was stirred for 0.5 h at 45 °C. The sample was then sonicated for 0.5 h and finally stirred for 24 h at 45 °C followed by an additional 24 h at room temperature. The resulting polymersomes were extruded 2 times through each of 1000 nm, 400 nm, 200 nm and 100 nm polycarbonate membranes (Whatman, Nuclepore) at 45 °C using a pressure driven Lipex Thermobarrel Extruder (1.5 mL capacity, Northern Lipids).









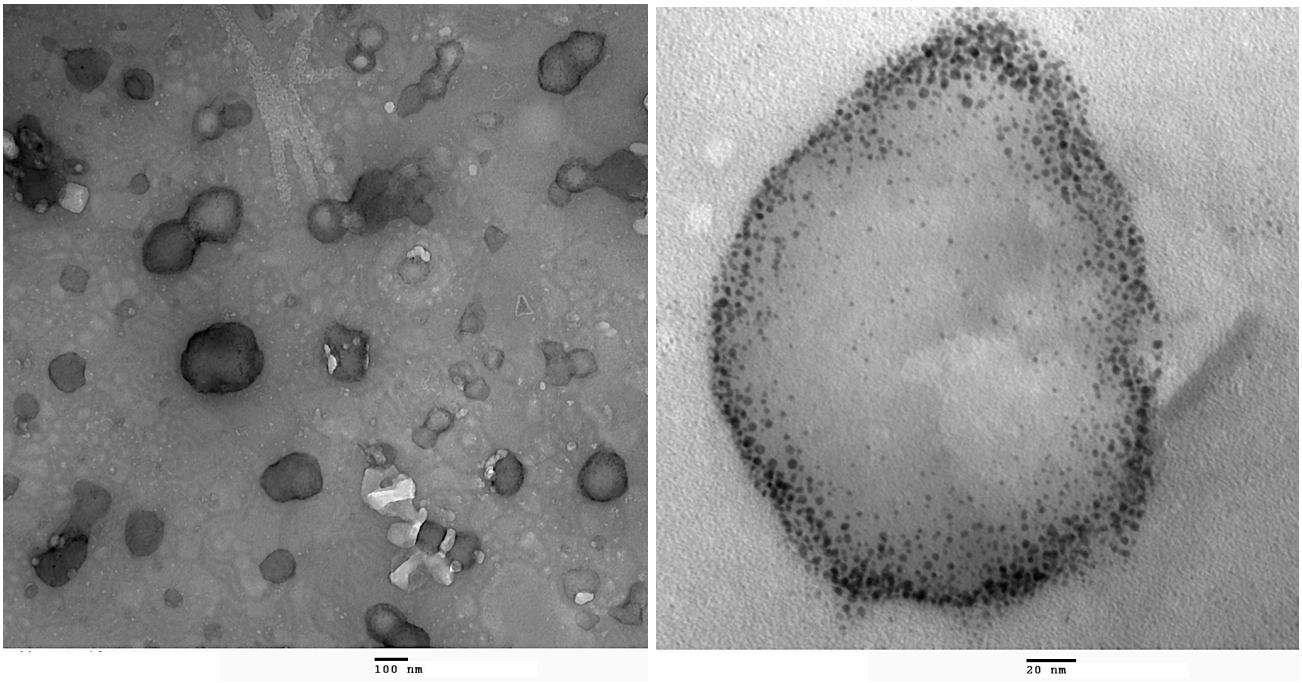


Figure SI10: TEM images of polysomers functionalized with DBCO-AuNP.

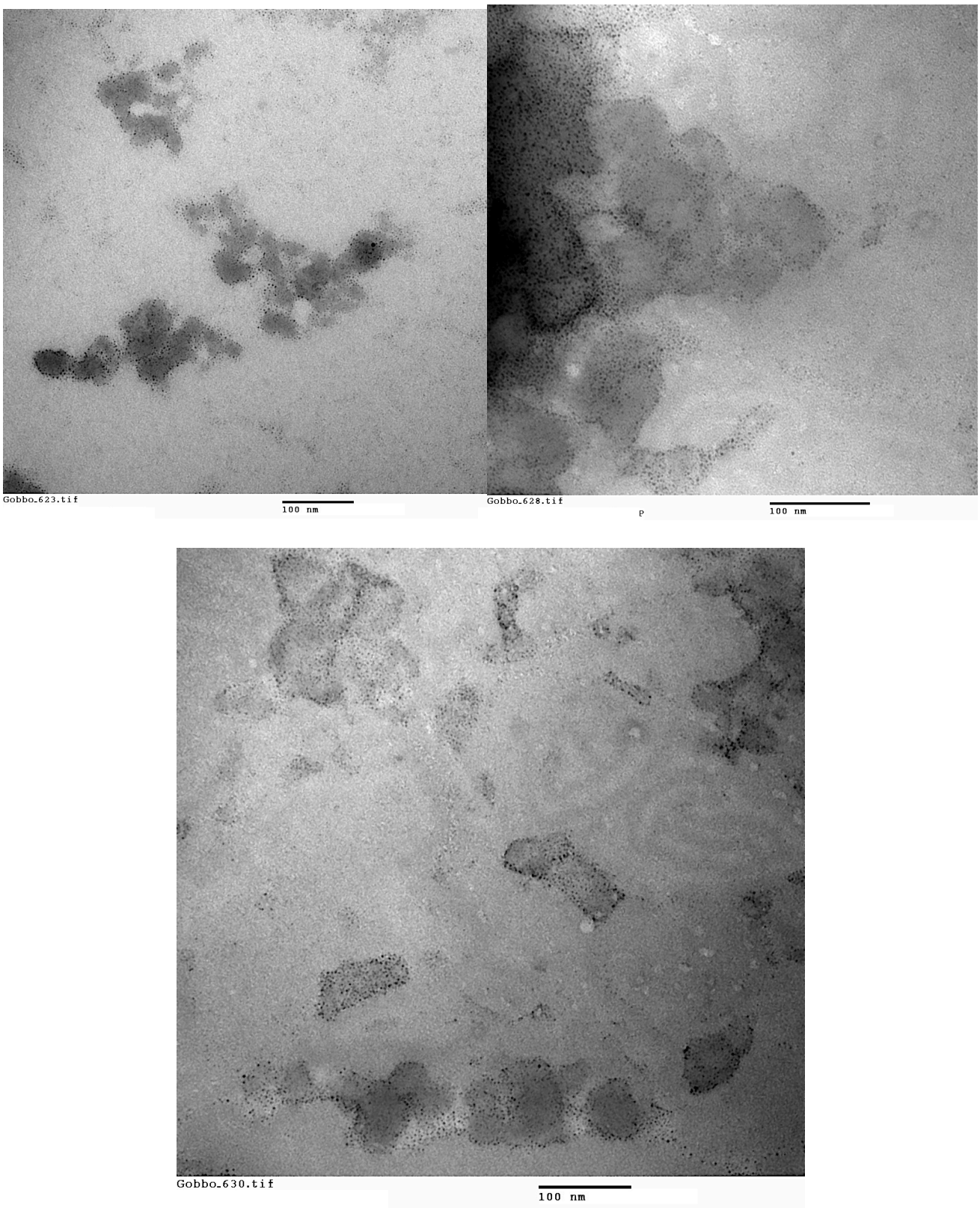


Figure SI11: TEM images of the control experiment: polysomers + Me-EG_3-AuNP.

Synthesis of Azide-AuNP

Azide-AuNP were synthesized according to our previously established procedure.[3] Briefly, 50.0 mg of Me-EG_3-AuNP were mixed in acetone for 20 minutes in presence of 10.0 mg (42.5 μmol) of N_3-EG_4-SH. After 20 minutes the solvent was removed and the resulting Azide-AuNP film was washed with hexanes and isopropanol for three times. The nanoparticles were readily redissolvable in H_2O, acetone, acetonitrile, methanol, ethanol, DMF, DMSO and DCM with little to no aggregation. ^{1}HNMR (CD_3CN, 400 MHz): δ_H (ppm): 3.60, 3.49, 3.39, 3.31. ^{1}HNMR (D_2O, 400 MHz): δ_H (ppm): 3.66, 3.57, 3.43, 3.32. IR (KBr disk, cm^{-1}): 2921, 2871, 2101, 1443, 1349, 1292, 1244, 1198, 1119, 1033.

Cu-catalyzed Huisgen Cycloaddition on Azide-AuNP

To test the copper-catalyzed Huisgen cycloaddition at the Azide-AuNP interface, Azide-AuNP were reacted with 2-propyn-1-amine hydrochloride or 1-ethynylpyrene in presence of copper sulfate and sodium ascorbate. In a typical experiment 10 mg of Azide-AuNP (10.6 μmol of azide) were mixed with 106 μmol of alkyne (using 2 ml of H_2O as the solvent for 2-propyn-1-amine hydrochloride; using 2 ml of a mixture 3:2 of acetonitrile and isopropanol as the solvent for 1-ethynylpyrene). To this mixture 1 ml of a freshly prepared water solution containing 2 μmol of $CuSO_4$ and 4 μmol of sodium ascorbate was added. The reaction mixture was mixed for 48 hours. After 48 hours, a black precipitate was observed inside the flask indicating AuNP aggregation/degradation. Attempts have been carried out using shorter reaction times (24 hours and 14 hours). In these cases AuNP did not precipitated entirely. The reaction mixture was transferred into a clean flask leaving back the AuNP aggregates and the solvent was removed. The AuNP film was washed with hexanes and isopropanol, and finally the AuNP were dialyzed overnight against water. ^{1}H NMR spectroscopy was carried out on the remaining fraction of purified AuNP and showed the starting material only.

***I*-SPAAC reaction on Azide-AuNP**

In a typical experiment 10 mg of Azide-AuNP (10.6 μmol of azide) were mixed with 29.3 mg of DBCO-amine (160 μmol) in acetonitrile for 1 hour. After 1 hour the acetonitrile was evaporated, and the AuNP were washed away with acetone where the DCBO-amine is not soluble. This washing procedure was repeated three times. No trace of AuNP aggregation was observed. ^{1}H NMR spectroscopy showed the appearance of cycloaddition product (see figure SI12). From the integration of the aromatic protons and those of the reference peak at 3.28 ppm, corresponding to the Me-EG_3-S

ligands, and knowing that the 35% of the ligands has an azide functionality, it was possible to estimate 60% yield for the I-SPAAC reaction between Azide-AuNP and DBCO-amine.

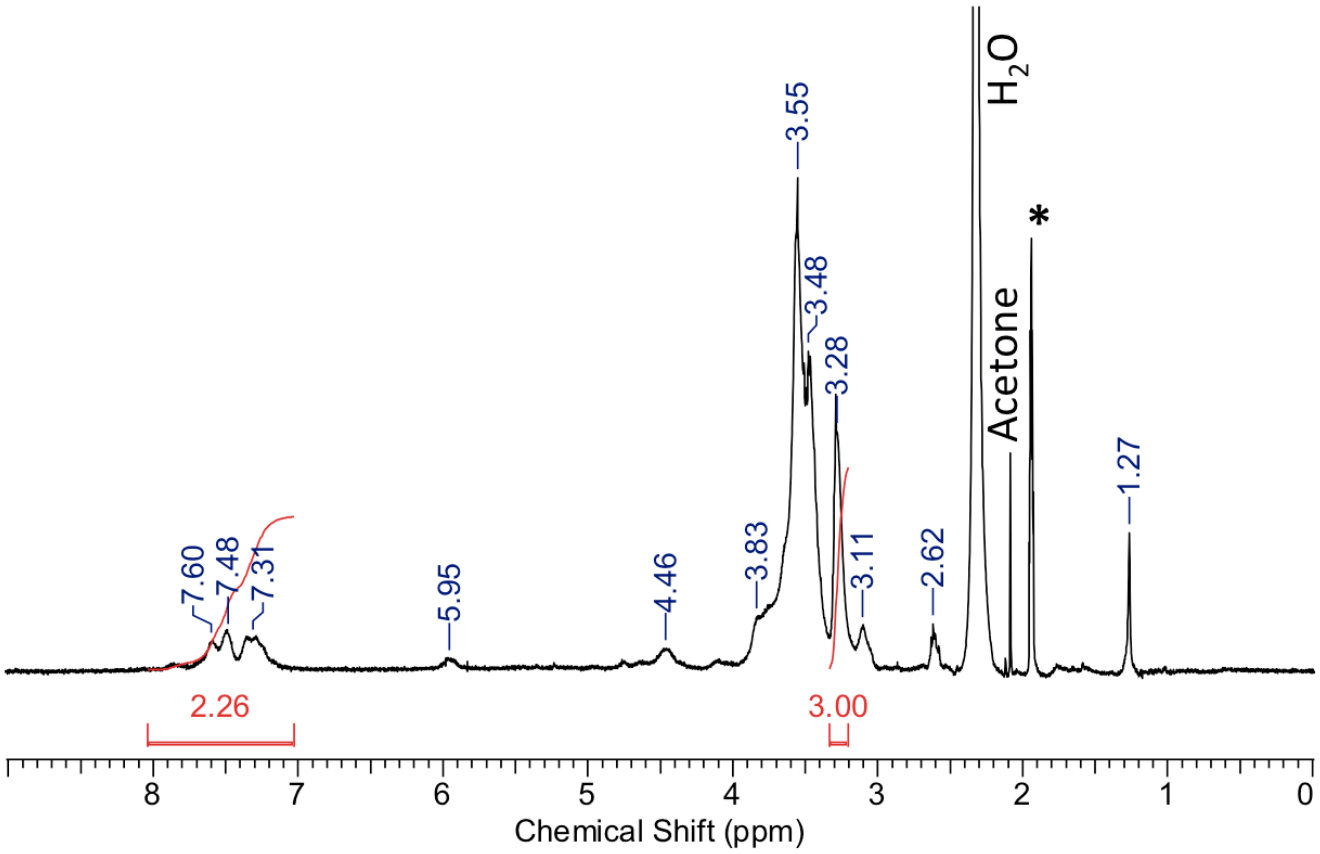


Figure SI12: ^{1}HNMR spectrum of amine-DBCO-N_3-EG_4-AuNP recorded in CD_3CN and calibrated against residual acetonitrile (*).

References:

1. P. Gobbo and M. S. Workentin, *Langmuir*, 2012, **28**, 12357-12363.
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3. P. Gobbo, S. Novoa, M. C. Biesinger and M. S. Workentin, *Chem Commun*, 2013, **49**, 3982-3984.