Supporting Information

Ligand Symmetry-Equivalence on Thiolate Protected Gold Nanoclusters Determined by NMR Spectroscopy

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Table of Contents

General Methods. All commercially available reagents were used without further purification. Tetrachloroauric (III) acid ($HAuCl_4 \cdot 3H_2O$ 99.99% metal basis, Alfa Aesar), *p*-mercaptobenzoic acid (>95.0%, TCI America), $NaBH_4$ (98-99%, MP Biomedicals), MeOH (99.9%, Fisher Scientific), deuterium oxide (D, 99.9% Cambridge Isotope Laboratories), trifluoroacetic acid (99%, Acros), acetonitrile (HPLC grade, Sigma Aldrich), tetramethylammonium bromide (98%, Sigma Aldrich), sinapinic acid (Bruker Daltonics), 2,5-dihydroxybenzoic acid (Bruker Daltonics) and Prot II (Bruker Daltonics). Nanopure water (resistivity 18.2 MΩ-cm) was produced with a Barnstead NANOpure water system. All NMR samples were dissolved in 0.3 M NaOH D_2O solutions. Simple one pulse and acquire ^{1}H NMR spectra [Au_{102}(*p*MBA)$_{44}$, Au_{144}(*p*MBA)$_{60}$, and *p*MBA] were acquired on a 500 MHz Varian Inova instrument equipped with a Varian 5 mm ^{1}H triple resonance z-pfg probe. Typically, 512 transients were acquired with an 8 kHz spectral window, 2 second acquisition time, 90 degree flip angle and a 5 second delay between transients. The T_2 weighted ^{1}H NMR spectrum of Au_{144}(*p*MBA)$_{60}$ was acquired on a 500 MHz Varian Inova instrument equipped as above by using a standard CPMG-T_2 pulse program. 1,000 transients were acquired with a total recycle time of 7 seconds. The 90º pulse was 5.9 μs. The echo train was 40 ms long with a τ period of 0.5 ms.

Synthesis of Au_{102}(*p*MBA)$_{44}$

$HAuCl_4 \cdot 3H_2O$ was dissolved (0.209 g, 0.50 mmol, a non-metal spatula should be used to weigh out $HAuCl_4 \cdot 3H_2O$) in nanopure H_2O (19.0 mL, 0.028 M based on Au) in a 50 mL conical. In a separate 50 mL conical, *p*-mercaptobenzoic acid (0.292 g, 1.89 mmol) was dissolved in a solution composed of nanopure H_2O (18.43 mL) and 10 M NaOH (0.57 mL, 5.70 mmol). The resulting *p*-mercaptobenzoic acid/NaOH solution was 0.10 M based on *p*-mercaptobenzoic acid, 0.30 M based on NaOH, and the pH was determined to be >9. A 1 L Erlenmeyer flask was equipped with a stir bar and nanopure H_2O was added to it (51.5 mL). In three separate beakers, the following solutions were dispensed: 1) 0.028 M $HAuCl_4$ solution (17.8 mL, 0.5

mmol, 1.0 equiv.), 2) 0.10 M *p*-mercaptobenzoic acid / 0.30 M NaOH (15.5 mL, 1.5 mmol, 3.0 equiv. of *p*-mercaptobenzoic acid and 5.7 mmol, 11.4 equiv. of NaOH) solution, 3) MeOH (75 mL). Under stirring, the $HAuCl_4$ solution was poured into the 1 L Erlenmeyer flask (containing H_2O), this was immediately followed by the addition of the *p*-mercaptobenzoic acid/NaOH solution. The reaction turned from yellow to orange upon the addition of the *p*-mercaptobenzoic acid/NaOH solution. Immediately afterwards the beaker of MeOH was also added to the 1 L flask. The reaction was allowed to stir at room temperature for 1 h. During that time, the reaction turned from dark orange to light orange. After 1 hour, pulverized solid $NaBH_4$ (20.8 mg, 0.55 mmol, 1.1 equiv.) was added to the stirring reaction to reduce the polymer; the reaction continued to stir at room temperature for 17 h. The reaction turned black upon the addition of solid $NaBH_4$. After 17 h MeOH was added to the 1 L flask until the total volume was approximately 800 mL, then 5 M NH_4OAc (40 mL) was also added. The reaction was then split into about twenty 50 mL conicals, which were capped and then centrifuged in a swinging bucket rotor at 4,000 rpm and 4 ºC for 10 min. The supernatant was then decanted and the precipitate was allowed to dry by inverting the conical on a paper towel for about 1 hour. The precipitate in each conical was then dissolved in about 200 μL of nanopure water. The nanoparticle solutions were then combined into 4 conicals. Next, the particles were washed by performing the following: 500 μL of 2 M NH_4OAc was added to each of the four conicals, then MeOH was added until the total volume in each conical was about 45 mL. The conicals were shaken to mix and were centrifuged at 4,000 rpm and 4 ºC for 10 min in a swinging bucket rotor. The resulting supernatant was decanted and the precipitates were dried *in vacuo* at room temperature for at least 2 h. Gel electrophoresis visualization was run on a 20% polyacrylamide gel (19:1, acrylamide : bisacrylamide) at 110 V for 2 h. The nanoparticle bands were visible by eye, thus no staining steps were performed for visualization.

Fractional precipitation of Au_{102}(*p*MBA)$_{44}$

The reaction outcome varies depending on the quality of the solid $NaBH_4$. In the case where many large and/or insoluble products were formed, a simple fractional precipitation removed the majority of the larger products. First, all particles from the reaction were dissolved in nanopure water (9.24 mL) in a 50 mL conical, then 2 M NH_4OAc (0.76 mL, 1.52 mmol, 0.076 M final concentration) was added. The solution was thoroughly mixed followed by the addition of MeOH (10 mL, 50%). The suspension was shaken again to mix and the conical was centrifuged at 4,000 rpm and 4 ºC. The supernatant was decanted into a new 50 mL conical and then re-spun until pellets (larger impurities) no longer formed. The remaining black solution (purified Au_{102}(*p*MBA) $_{44}$) was again transferred to a new 50 mL conical. MeOH was then added to the conical until the total volume was about 45 mL. The conical was shaken to mix and was then centrifuged at 4 ºC for 10 min. The resulting supernatant was decanted and the precipitate (purified Au_{102}(*p*MBA)$_{44}$) was dried *in vacuo* at room temperature for at least 2 h.

Synthesis of Au_{144}(*p*MBA)$_{60}$

A 95.2 mM solution of *para*-mercaptobenzoic acid (*p*MBA, TCI America) was prepared (Day 1) in 0.3 M NaOH and allowed to shake on a vortexer overnight. The following day (Day 2) a 28 mM solution of $HAuCl_4 \cdot 3H_2O$ was prepared in nanopure H_2O. The Au(I)-*p*MBA polymer was then generated in a 50 mL conical using a methanol:water solvent (48 mL total volume, 47% methanol and 53% water in final composition) mixture with a 3:1 molar excess of *p*MBA (0.432 mmol, 9 mM final volume) in relation to $HAuCl_4$ (0.144 mmol, 3 mM final volume) and was immediately shaken by hand, followed by continuous shaking on a vortexer overnight. The polymer should appear clear and faint yellow. The following day (Day 3) a 150 mM solution of $NaBH_4$ was prepared in nanopure H_2O, and 0.5 molar equivalents (0.48 mL) were added (immediately after preparation) to the Au(I)-*p*MBA polymer. The conical was capped and

immediately shaken followed by shaking on a vortexer. Reduction was allowed to proceed for a maximum of two hours. The product was then isolated by centrifugation (without the addition of quenching/precipitation agent) in an Eppendorf® 5810R centrifuge, equipped with a swinging bucket rotor at 4,000 rpm for 5 minutes. The clear supernatant was discarded and the black pellet was fully dissolved in approximately 10 mL of nanopure H_2O to wash the particles. The product was then precipitated with the addition of 1 mL of 2 M NH_4OAc solution and at least 20 mL methanol. The suspension was then centrifuged at 4,000 rpm for 5 minutes and the washing procedure was repeated once more before the particles were finally resuspended in nanopure H_2O or stored as a dried powder prior to resuspension. Product purity was analyzed by polyacrylamide gel electrophoresis (PAGE) on a 15% (19:1) polyacrylamide (with 5% glycerol) gel in 1X TBE run at 110 V for 1 hour. In the event that larger molecular weight products (analyzed by PAGE) were also produced, a fractional precipitation was performed with 80 mM NH_4OAc and 25-30% methanol to remove the larger products. The $Au_{144}(p\text{MBA})_{60}$ MPC could then be isolated with the addition of at least 50% methanol for precipitation. This product was again analyzed by PAGE to ensure removal of any larger contaminants.

MALDI-MS Data Collection

MALDI-MS data was collected on a Bruker UltraFlex MALDI TOF/TOF instrument in positive ion, reflector mode using an accelerating voltage of 25 kV. The laser power was varied between 75−100%. All matrixes were prepared prior to spotting at 10 mg/mL in a solution of 50% acetonitrile (ACN), 0.1 % trifluoroacetic acid (TFA). External calibration was performed using Prot II (a mixture of 4 to 6 peptides), which was spotted with the sinapinic acid (SA) matrix adjacent to the sample. For each sample, 1 μL of sample was spotted, followed by 1 μL of dihydroxybenzoic acid (DHB). Data was processed and analyzed using mMass software.

Characterization of Purified Au_{102}(*p*MBA) $_{44}$ and Au_{144}(*p*MBA)$_{60}$.

Dispersity of the synthesized and purified compounds was characterized by PAGE and Transmission Electron Microscopy, with each (qualitative) technique suggesting the particles are prepared with near-atomic dispersity and appropriate relative size. Formula assignments of the clusters were made using MALDI-Mass Spectrometry. Additionally, product crystallization in known mother liquors yielded single crystals with similar sizes, morphologies and diffraction properties consistent with crystals of these compounds.[1,2] UV-vis spectroscopic measurements of these MPCs were also consistent with previous reports.

MALDI Mass Spectrometry of MPCs typically results in mass spectra that contain many fragments of the parent compound, even when ESI mass spectra show the presence of a single discrete compound. We were unable to acquire ESI data on *p*MBA-protected compounds, despite several attempts. The fragmentation pattern of the MALDI spectrum of Au_{102}(*p*MBA)$_{44}$ contains peaks that correspond to exact fragments of the cluster, as shown in Fig. 1 in the main text. This mass spectrum, which was smoothed with a 50 m/z Savitzky-Golay operation in the mMass mass spectroscopy processing package to suppress noise, shows two dominant peaks which we assign as a 'mostly bare' Au core peak and a second peak that corresponds approximately to the mass of intact Au_{102}(*p*MBA)$_{44}$, possibly with some counterions contributing to the mass. To our knowledge this is the first observation of an intact mass spectrum of the Au_{102}(*p*MBA)$_{44}$ nanocluster. Notably, we could assign the fragments near the bare gold peak to exact masses of Au_{102}(*p*MBA)$_{44}$ fragments.

The mass spectrum of Au_{144}(*p*MBA)$_{60}$ shows a broad peak without identifiable fragments, centered at 30.5 kDa. This spectrum is annotated to show the locations for exact molecular masses of Au_{144}, $Au_{144}S_{60}$ and Au_{144}(*p*MBA)$_{60}$. Several other groups have reported MALDI mass spectra for $Au_{144}(SR)_{60}$ compounds with similar resolution.[3-5]

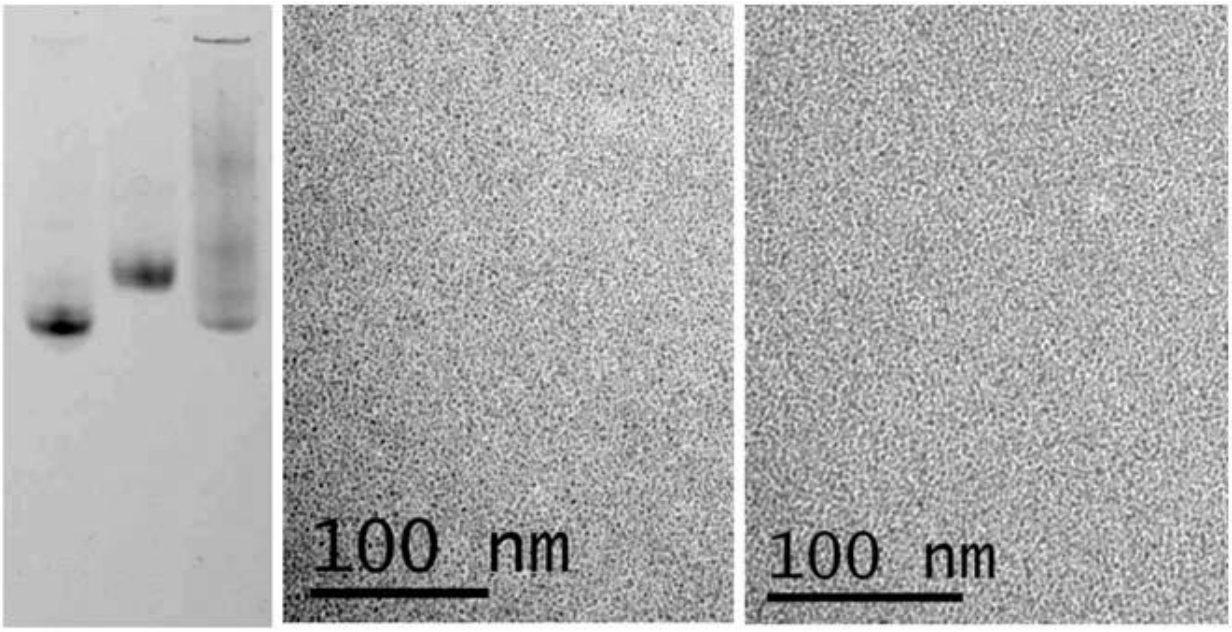

Fig. S1 Qualitative dispersity characterization of Au_{102}(*p*MBA)$_{44}$ and Au_{144}(*p*MBA)$_{60}$. Left panel shows a 20% (19:1) polyacrylamide gel. The lanes from left to right show Au_{102}(*p*MBA)$_{44}$, Au_{144}(*p*MBA)$_{60}$ and an example of a polydisperse synthesis containing Au_{102}(*p*MBA)$_{44}$. The center and right panels show TEM images of Au_{102}(*p*MBA)$_{44}$ and Au_{144}(*p*MBA)$_{60}$ respectively.

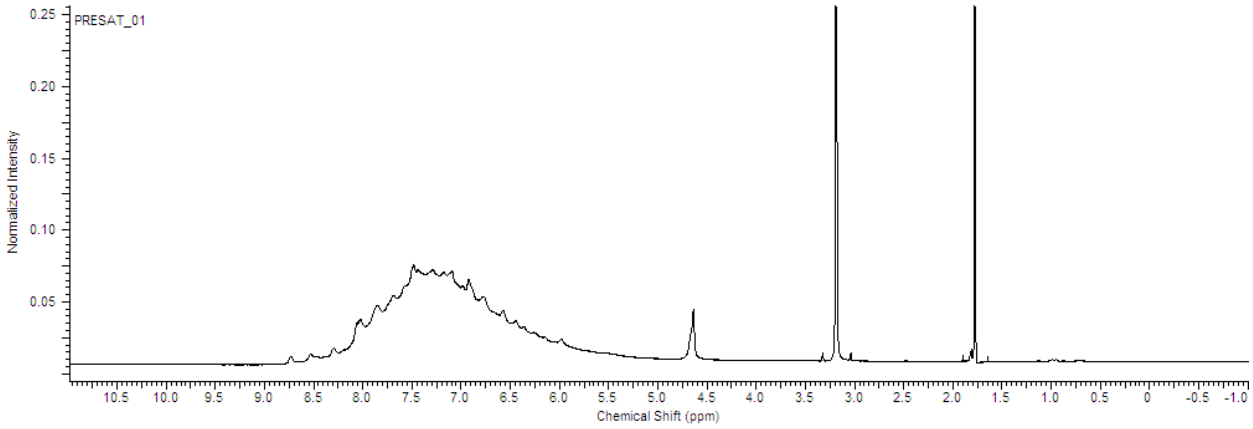

Fig. S2 Full ^{1}H NMR Spectrum of Au_{102}*p*MBA$_{44}$

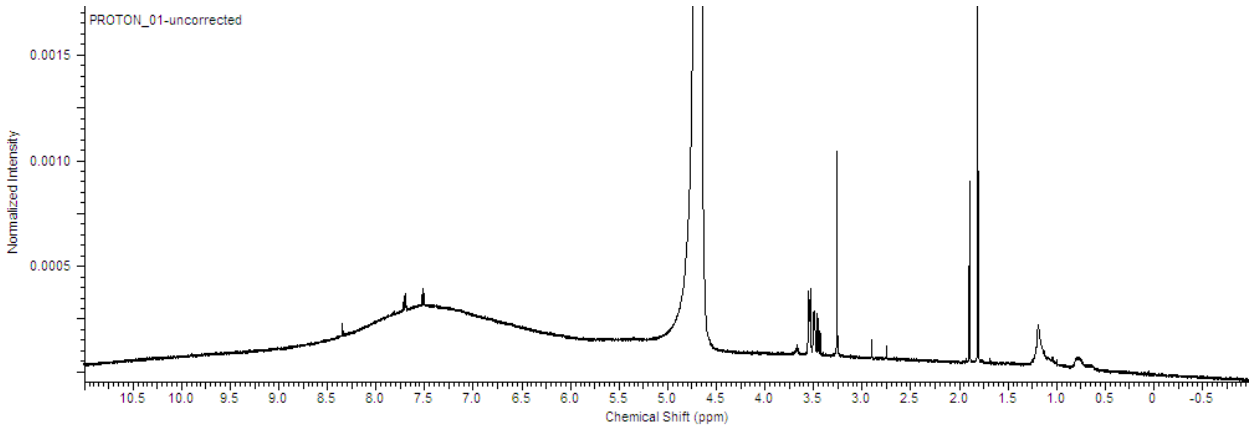

Fig. S3 Full ^{1}H NMR Spectrum of Au_{144}*p*MBA_{60}

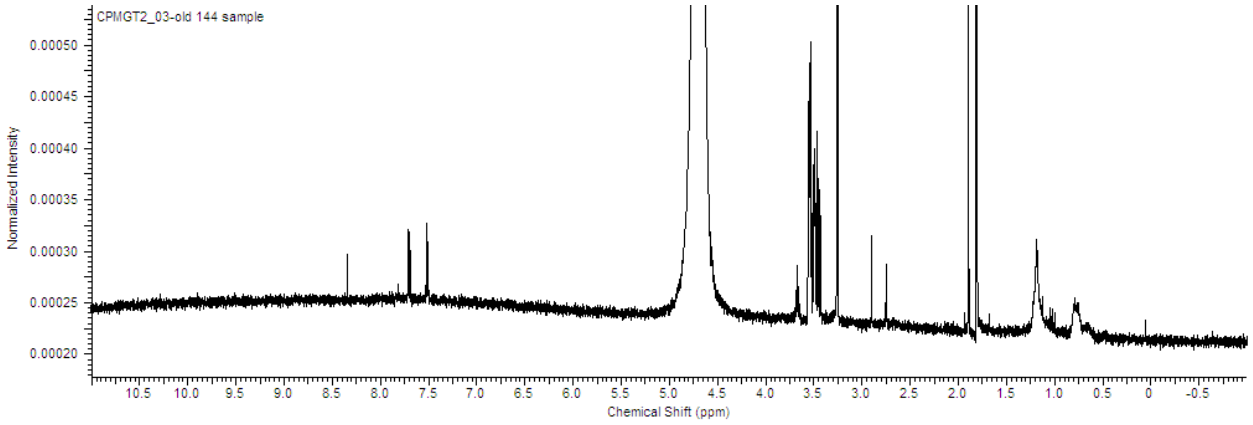

Fig. S4 Full ^{1}H NMR Spectrum of Au_{144}*p*MBA_{60} (t_2 cpmg)

For the aromatic region: 500 MHz, 0.3 M NaOH in D_2O, δ 8.34 (s), 7.71 (d, *J* = 5.0 Hz), 7.52 (d, *J* = 5.0 Hz)

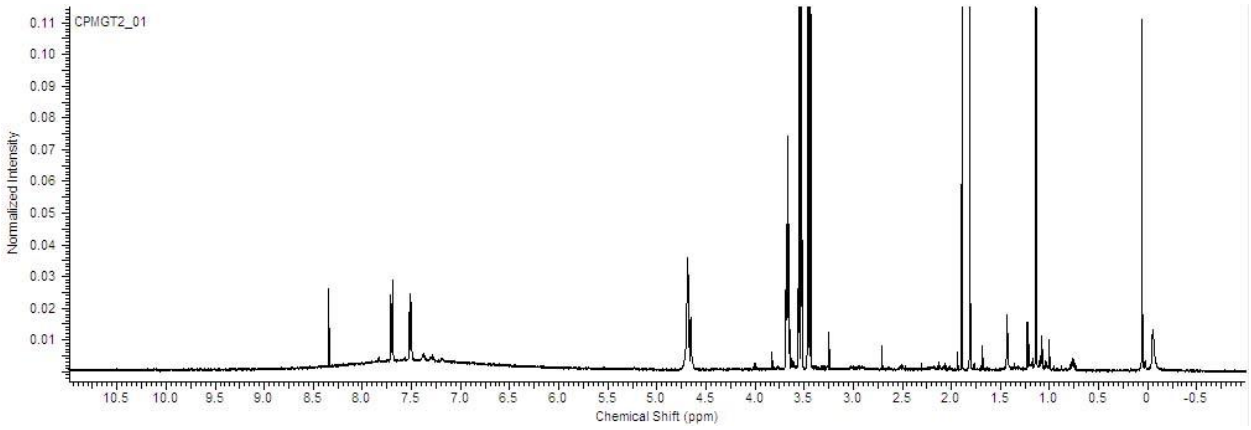

Fig. S5 Full ^{1}H NMR Spectrum of Unknown AuNP (t_2 cpmg)

For the aromatic region: 500 MHz, 0.3 M NaOH in D_2O, δ 8.34 (s), 7.84 (d, *J* =5.0 Hz), 7.70 (d, *J* = 10.0 Hz), 7.57 (d, *J* = 10.0 Hz), 7.51 (d, *J* = 10.0 Hz), 7.38 (d, *J* = 10.0 Hz), 7.29 (t, *J* = 10.0 Hz), 7.18 (t, *J* = 10.0 Hz)

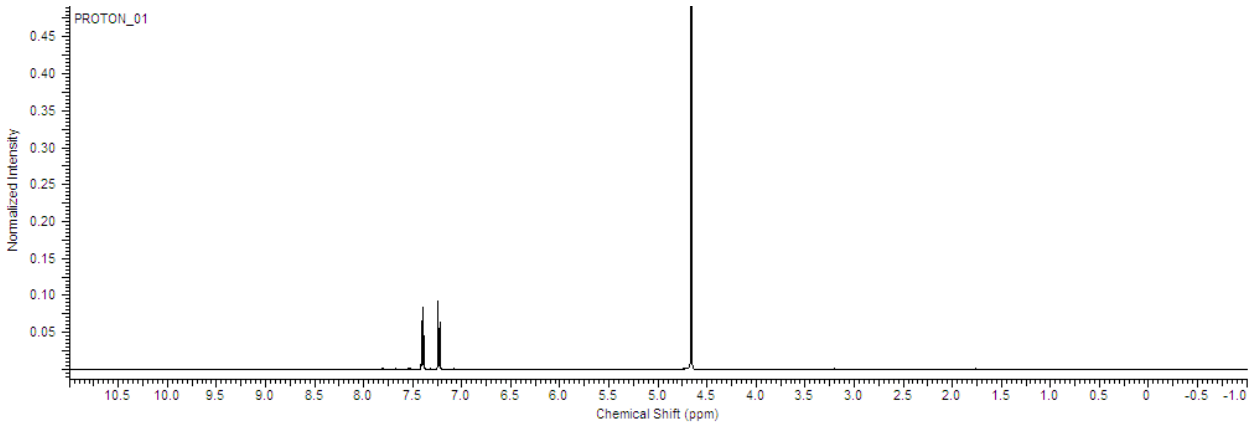

Fig. S6 Full ^{1}H NMR Spectrum of *p*MBA

For the aromatic region: 500 MHz, 0.3 M NaOH in D_2O, δ 7.40 (d, *J* =10.0 Hz), 7.23 (d, *J* = 10.0 Hz)

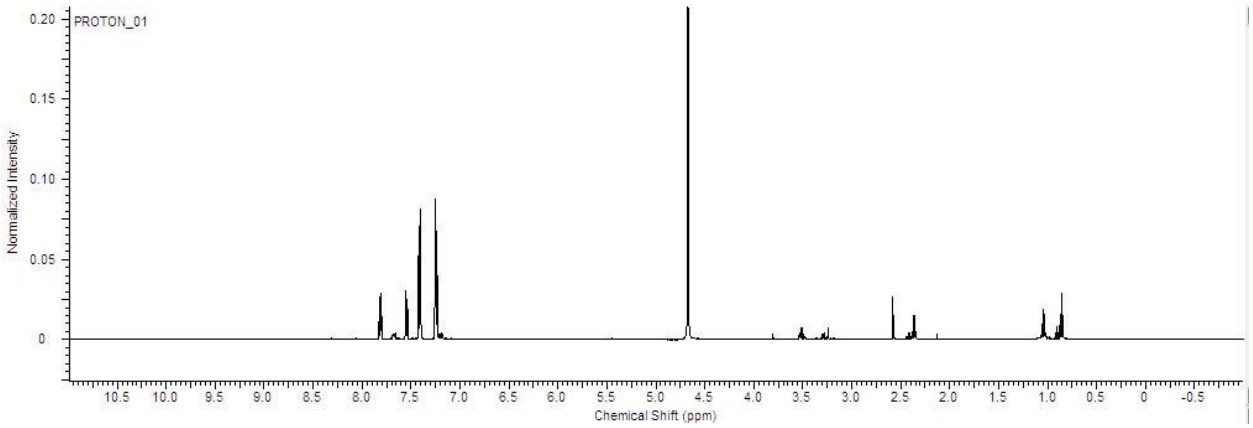

Fig. S7 Full ^{1}H NMR Spectrum of a mixture of disulfide of *p*MBA and *p*MBA

For the aromatic region: 500 MHz, 0.3 M NaOH in D_2O, for disulfide of *p*MBA δ 7.82 (d, *J* = 5.0 Hz), 7.55 (d, *J* = 5.0 Hz), for *p*MBA δ 7.41 (d, *J* = 10.0 Hz), 7.23 (d, *J* = 10.0 Hz)

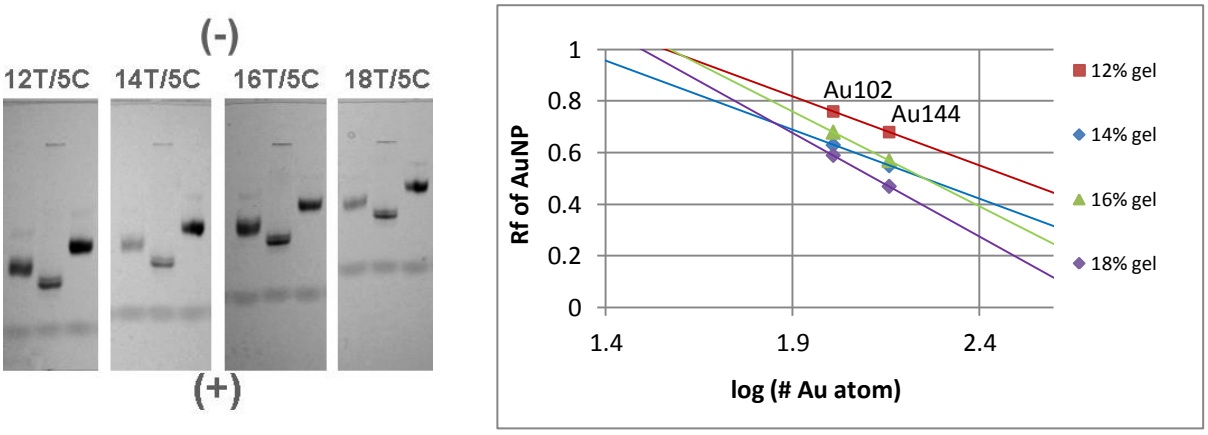

Fig. S8 Size Determination with PAGE of Unknown AuNP. Left panel shows the acrylamide gels that are used for size determination of unknown AuNP. Samples from left to right of each gel: Au_{144}(*p*MBA)$_{60}$, Au_{102}(*p*MBA)$_{44}$, unknown AuNP, and orange G is defined as R_f = 1. From left to right: 12% polyacrylamide (19:1 / 5% crosslinker) ($R_{f\text{-}144}$=0.68, $R_{f\text{-}102}$=0.76, $R_{f\text{-}unknown}$=0.56), 14% ($R_{f\text{-}144}$=0.55, $R_{f\text{-}102}$=0.63, $R_{f\text{-}unknown}$=0.45), 16% ($R_{f\text{-}144}$=0.57, $R_{f\text{-}102}$=0.68, $R_{f\text{-}unknown}$=0.41), and 18% ($R_{f\text{-}144}$=0.47, $R_{f\text{-}102}$=0.59, $R_{f\text{-}unknown}$=0.37) acrylamide. Right panel: graph of log (# Au atoms) vs. R_f. The number of Au atoms and number of *p*MBA ligands were

calculated independently using the formula of the trend lines. The particle weight 59 kDa is the average of the resulting unknown formula (see Table S1 below).

Gel%	# Au	# *p*MBA	Mass (Da)
10T/5C	263	75	63136
12T/5C	283	103	71304
12T/5C	218	100	58046
14T/5C	204	80	52268
14T/5C	228	92	58808
16T/5C	203	81	52222
16T/5C	249	92	62945
18T/5C	194	77	49845
AVERAGE	**230**	**88**	**58572**
Standard Dev	32	11	7158

Table S1: Tentative assignment of unknown particle on the basis of comparative R_f values relative to $Au_{102}(pMBA)_{44}$ and $Au_{144}(pMBA)_{60}$. While the band in the gels appears molecular in dispersity, the electrophoresis method for assigning a formula leaves some uncertainty.

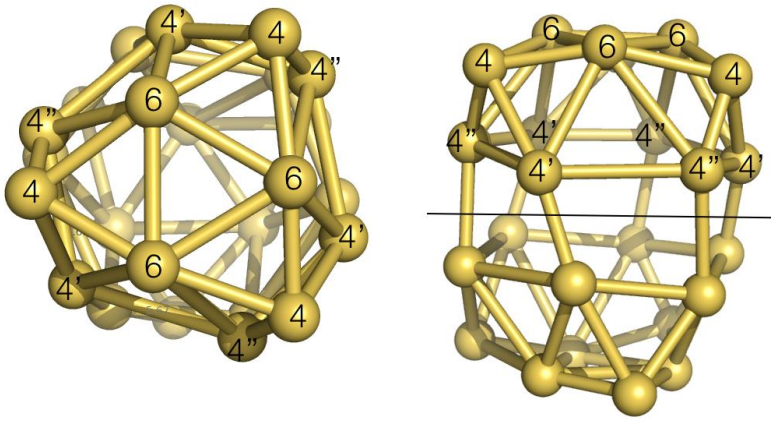

Fig. S9 Symmetry environment analysis of $Au_{38}(SR)_{24}$. Polyhedra are formed by edges connecting all sulfur atoms (vertices) within 5.7 Å. The $Au_{38}SR_{24}$ nanocluster contains a fusion plane, denoted with a line in the right panel, meaning that the symmetry environments of the top 12 sulfur atoms are the same as the symmetry environments of the bottom 12 sulfur

environments. The sulfur vertices on one side of the fusion plane are labeled according to how many edges are attached. For the 3 vertices labeled 4, all of the edges share an edge-edge angle of approximately 72°. The edges attached to the vertices labeled 4' and 4'' are at dramatically different angles, distinguishing them from the vertices labeled 4. The verticies labeled 4' and 4'' differ from each other in that they are approximately mirror images. The vertices labeled 6, 4, 4' and 4'' comprise 4 distinct symmetry environments.

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