SUPPORTING INFORMATION

Size-Dependent Molecule-like to Plasmonic Transition in Water-Soluble Glutathione Stabilized Gold Nanomolecules

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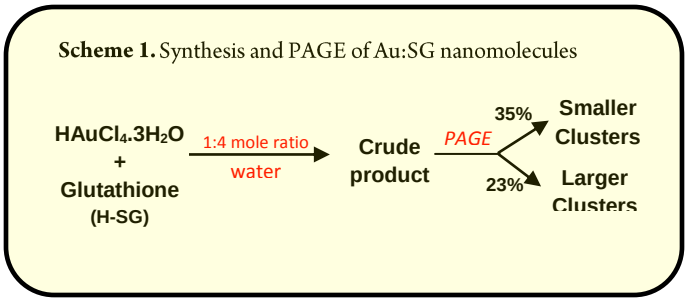
Synthesis of $Au_x(SG)_y$ in water

100 mg of $HAuCl_4$ (0.253 mmol) was dissolved in 50 mL of DI water. Then 307 mg of glutathione (1.00 mmol) was slowly added to the Au solution under slow stirring. A cloudy white suspension was formed after disappearance of the yellow color of the gold salt solution. Next, the solution was cooled in an ice bath. After 30 minutes of cooling, 94.6 mg of $NaBH_4$ (2.5 mmol) dissolved in 12.5 ml of ice cold DI water was added to the mixture all at once while stirring at 1000 rpm. The milky white color of the mixture rapidly turned black after the addition of $NaBH_4$, indicating the formation of nanoparticles. After 1h, the mixture was rotary evaporated until the total volume was reduced to about 5 mL. The temperature of during rotary evaporation was kept below 30 °C. Then 20 mL of methanol was added to the product mixture and centrifuged at 3800 rpm for 3 minutes. The resulted precipitate was washed 3 times with methanol.



Scheme 1. Synthesis and PAGE of Au:SG nanomolecules

Polyacrylamide Gel Electrophoresis (PAGE)

PAGE experiments were carried out using a Bio-Rad protean II xi vertical gel apparatus (dimensions 1.5 x 160 x 160mm) and Thermo Scientific Owl S4S aluminum backed sequencer system (dimensions 1.5 x 200 x 450mm). Gels were made in our laboratory for both systems. The gels made for Protean II used 40% concentration of acrylamide monomer and 29:1 acrylamide to bis-acrylamide ratio.

For optimum separation at *low molecular weight* region, *35% acrylamide* separating gel was used. 10 mg of Au:SG clusters in 450 µl of 5% glycerol/water was loaded. This gels were ran at 200 V for anywhere from 10 to 24 hours at 8 °C. The gels made for the Owl S4S used 30% concentration of acrylamide monomer and 29:1 acrylamide to bis-acrylamide ratio.

For optimum separation at *high molecular weight* region *23% acrylamide* separating gel was used and 20 mg of Au:SG clusters in 450µl of 5% glycerol/water loaded. The gels in Owl S4S ran at 200 V for anywhere from 36 to 48 hours at room temperature.

All separating gels had 0.375M concentration of Tris-HCl and a pH of approximately 8.8. For both systems 4% stacking gels were prepared using 30% concentration of acrylamide monomer and 19:1 acrylamide to bis-acrylamide ratio. Stacking gel has 0.125 M Tris-HCl concentration and separation was enhanced by running samples without lanes across the entire gel. The running buffer consisted of 192 mM glycine and 25 mM Tris-base. After the separation, bands in the gel were cut out individually and crushed and placed in vials with 4 mL of HPLC grade H_2O. The vials were then placed in a refrigerator at 2 ^{o}C for 1 h (or overnight for greater yield) to allow for the clusters to dissolve into the water. Then, any remaining acrylamide gel particles were filtered out using syringe-filter with 0.3 µm pores.

23% vs 35% gels:

The PAGE gels typically consists of acrylamide, bisacrylamide and a buffer with an adjusted pH. A free radical generator and a stabilizer such as ammonium persulfate and TEMED are added to initiate polymerization of acrylamide. The bisacrylamide forms cross links in between polyacrylamide molecules during the polymerization. The pore size of the gels are determined by two factors.

a. The total concentration of acrylamide and bisacrylamide (%T)
b. Amount of cross linker (%C)

When the %T is increased, the pores size inside the gels decreases. Therefore the higher percentage gels are suitable to separate small nanomolecules vice versa. When the %C increases, the pore size decreases. But in this case, we keep the %C constant for all the gels.

Optical absorption spectroscopy

After separation in PAGE, the different sized clusters were extracted and purified. Next absorption spectra of different bands were acquired using a Shimadzu UV-Visible spectrophotometer (UV-1601). Spectra of the bands were used to confirm that the different bands were different size clusters and that the bands were pure, especially for smaller size range.

ESI mass spectrometry

All mass spectra were collected by using Waters Synapt™ High Definition Mass Spectrometer. The materials extracted with bands were washed several times with methanol to remove the smaller gel particles. Then samples were diluted up to 0.25 mg/mL in a solution of 50% methanol in water. Then samples were electrosprayed using stainless steel needle syringe. The instrument parameters are as follows. Mass range, 800 to 3200 Da; Capillary voltage, -2.1 kV; Detector voltage 1650 V; Extraction cone voltage, 5.1 V; Source Temperature 40 °C; Desolvation Temperature 150 °C. The sampling cone voltage was varied between 5 to 50 V in W (high resolution) optics mode. All the mass spectral data processed by using Masslynx 4.1 software (Waters Corp.).

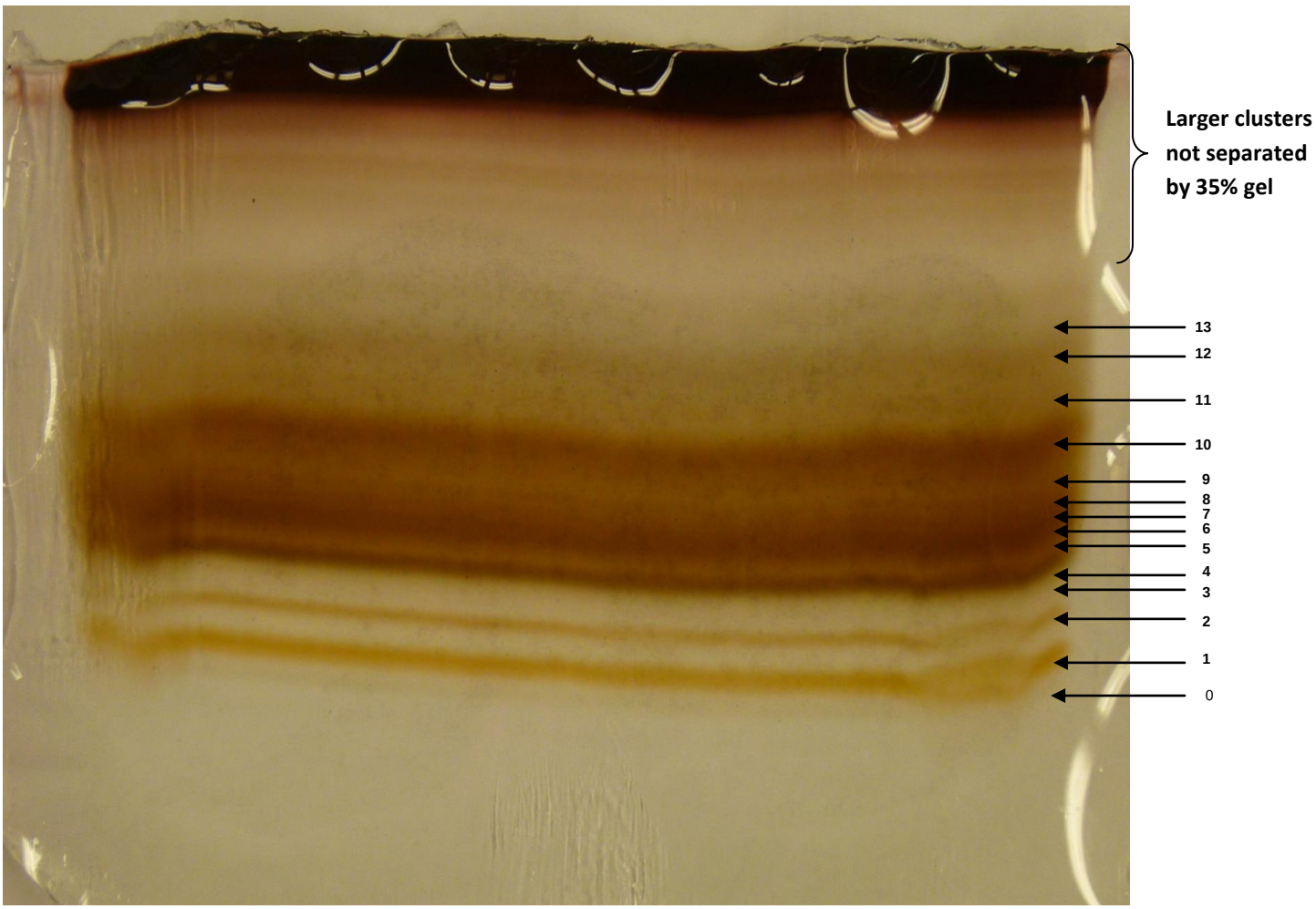


Figure S1: Full Gel picture of 35% gel after separation. Smaller nanomolecules (marked as bands 0 to 13) separated in to bands. Larger nanomolecules, however started to move through the gel up to some level, but were not well separated. The larger plasmonic particles are at the top of the gel.

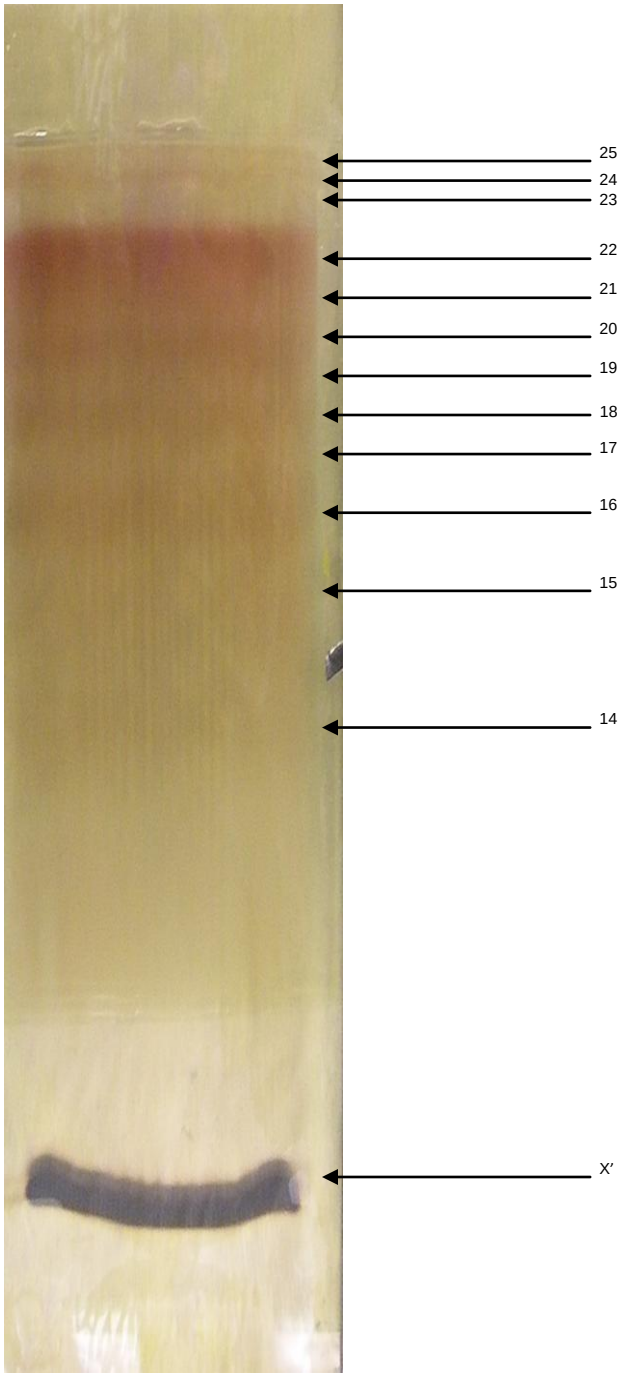


Figure S2: Full Gel picture of 23% gel after separation. Larger nanomolecules (marked as bands 14 to 25) separated as shown above. Smaller nanomolecules, however, were not separated in this 23% gel. These were lumped into one broad black band, down the gel marked as X'.

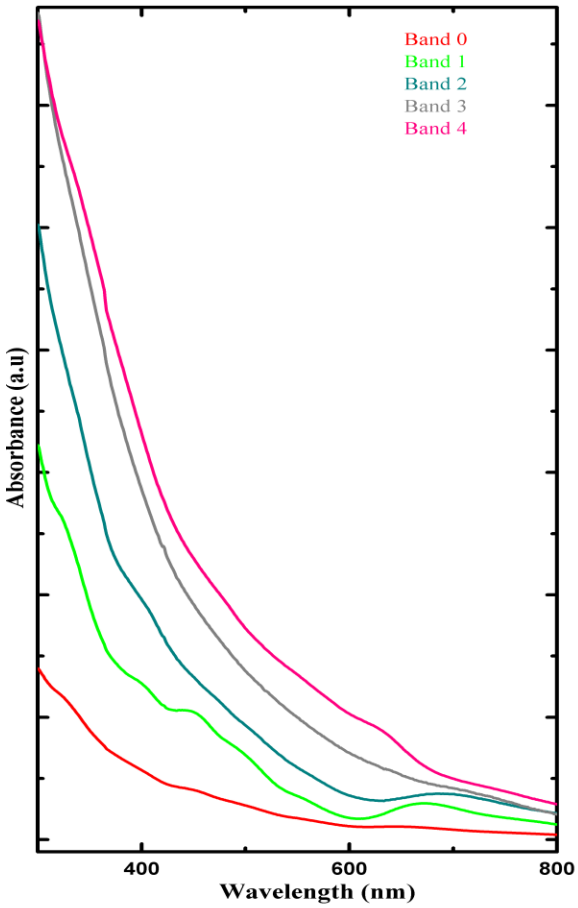


Figure S3: UV-vis spectra of first 5 bands in the 35% gel.

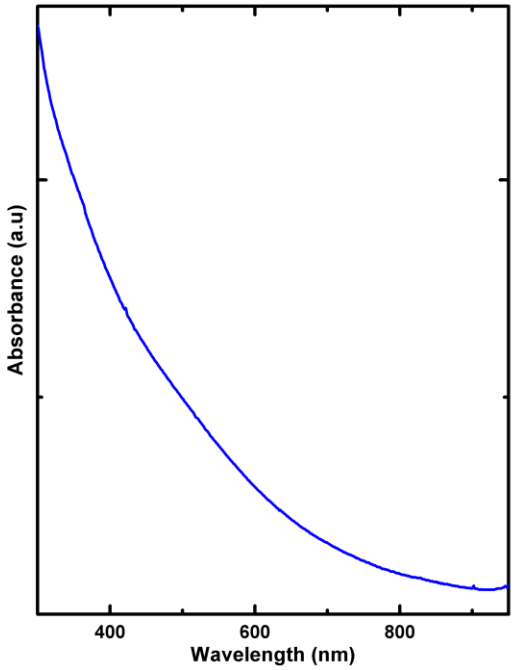


Figure S4: UV-vis spectrum of band X' in 23% gel

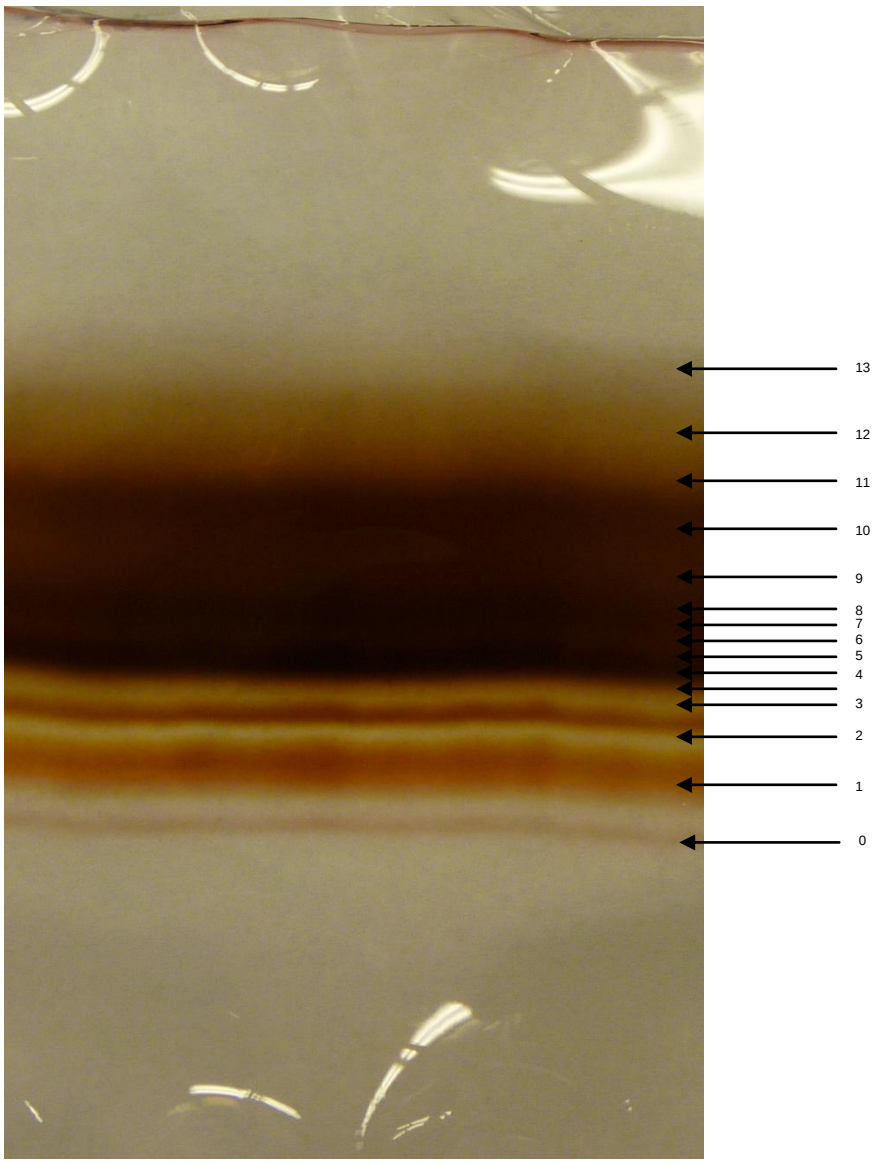


Figure S5: Control experiment. The crude mixture in scheme 1 was subject to solvent fractionation[1] using methanol / water mixture to isolate the smaller nanomolecules. *The soluble fraction* of the product mixture loaded into the same 35% gel, gave the same number of bands as in Figure S1, except for the absence of larger clusters. (Compare with Figure S1, but without the larger plasmonic bands at the top of the gel)

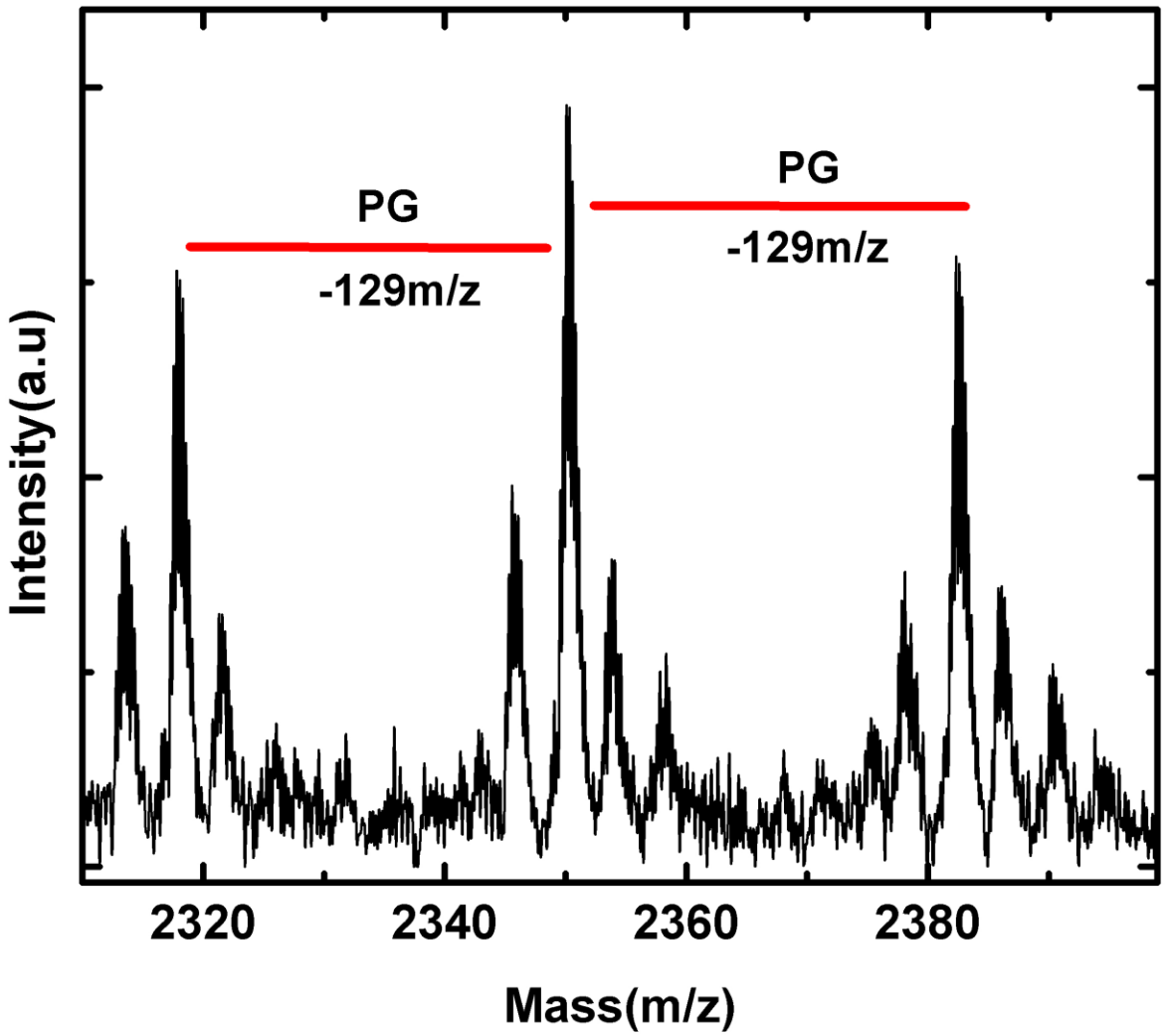


Figure S6: Expansion of ESI mass spectra of band 0. Series of fragmented peaks were produced after the loss of pyroglutamic acid[2] from the molecular ion peak.

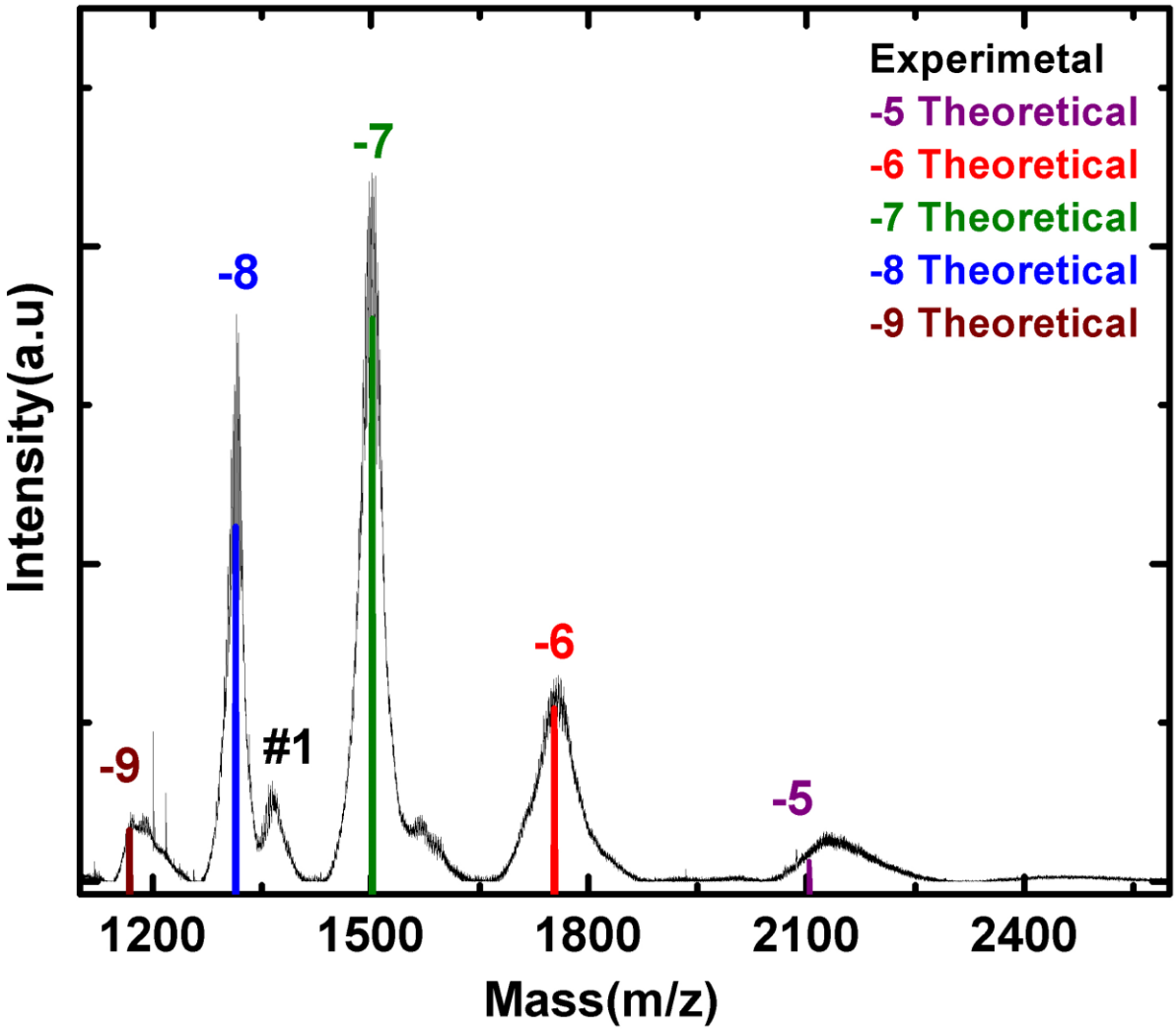


Figure S7: ESI mass spectra of band 1. Molecular Formula of this band is identified as $Au_{25}(SG)_{18}Na_4$ and -5 to -9 charge state are observed. The contamination of $Au_{22}(SG)_{17}$ is labeled as 1#[1].

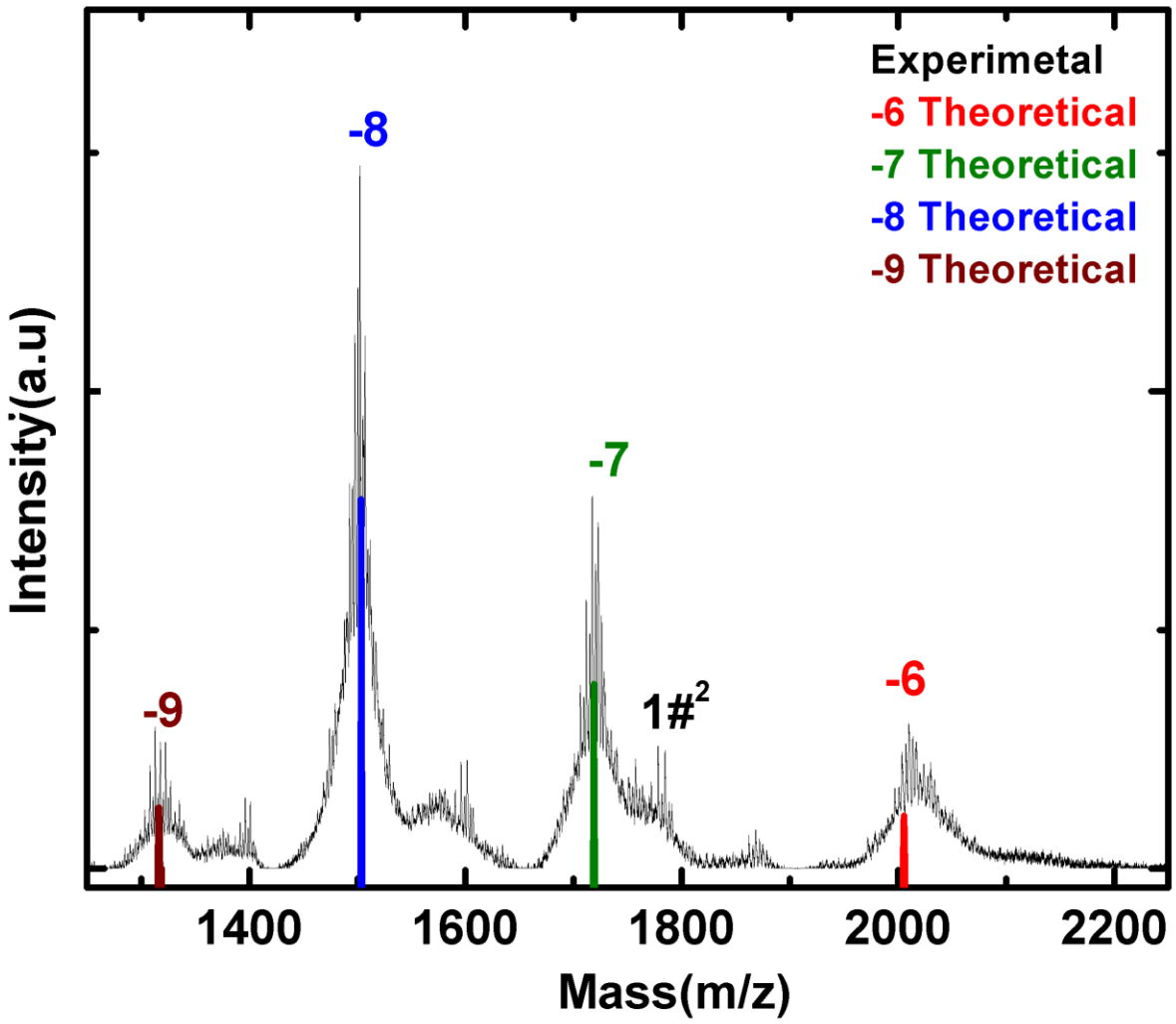


Figure S8: ESI mass spectra of band 2. Molecular Formula of this band identified as $Au_{29}(SG)_{20}Na_8$ and -6 to -9 charge state are observed. The contamination of $Au_{25}(SG)_{18}$ is labeled as $1\#^2$

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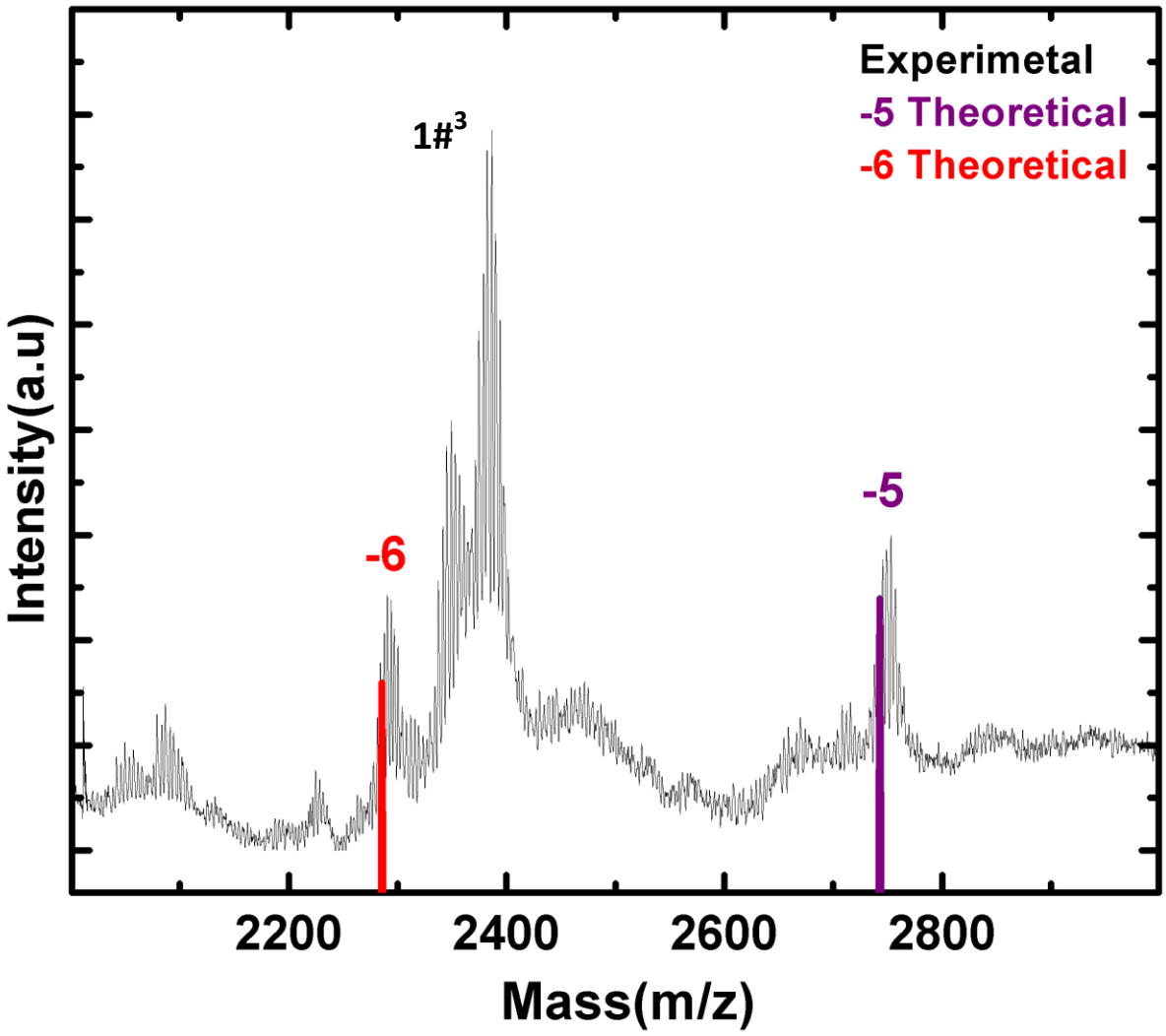

Figure S9: ESI mass spectra of band 3. Molecular Formula of this band is identified as $Au_{35}(SG)_{22}Na_4$ and -5 to -6 charge state are observed. The contamination of $Au_{29}(SG)_{20}$ is labeled as $1\#^3$

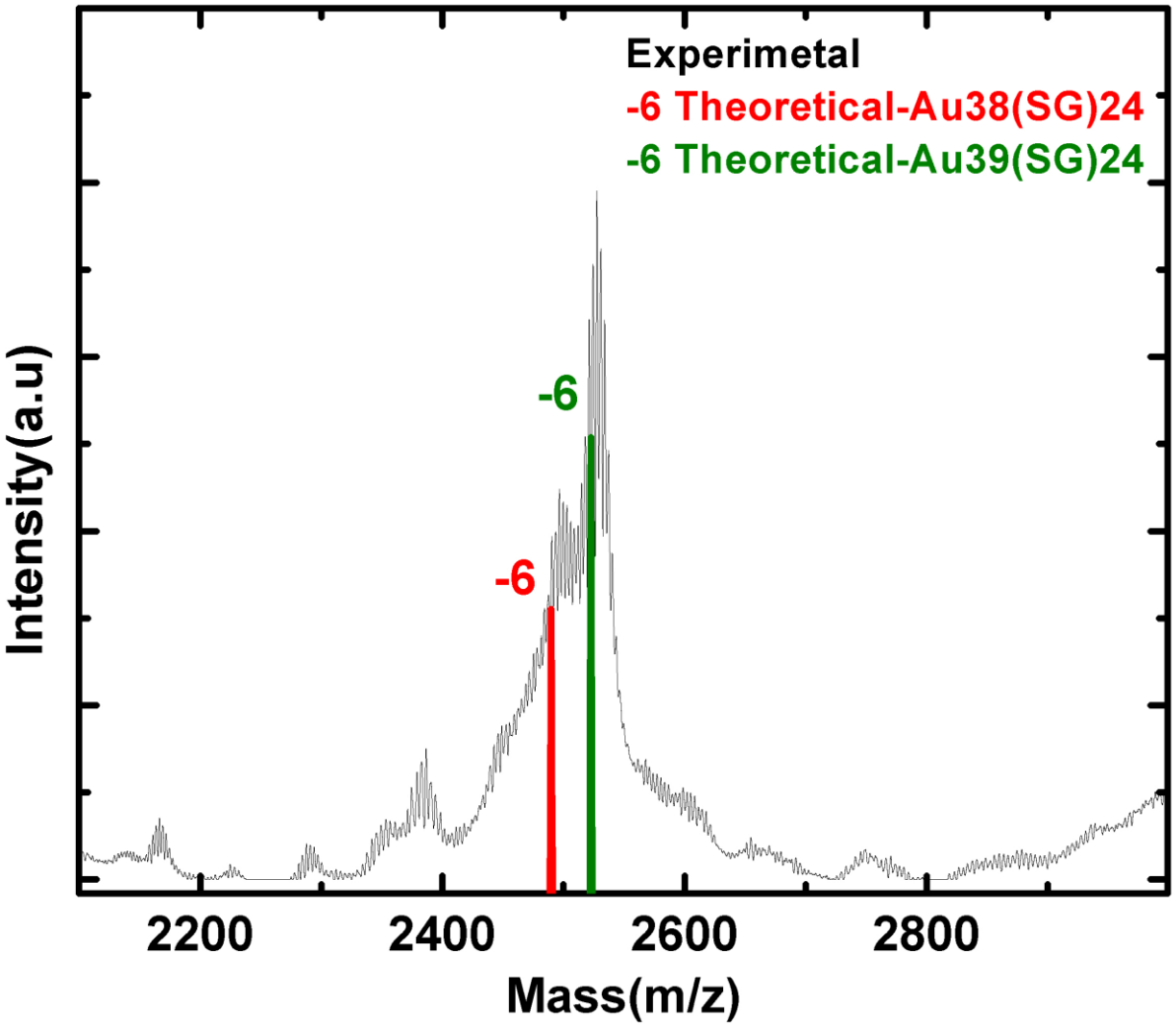

Figure S10: ESI mass spectra of band 4. Two clusters present in this band. Molecular Formula of nanomolecules in this band identified as $Au_{38}(SG)_{24}Na_4$ and $Au_{38}(SG)_{24}Na_4$.

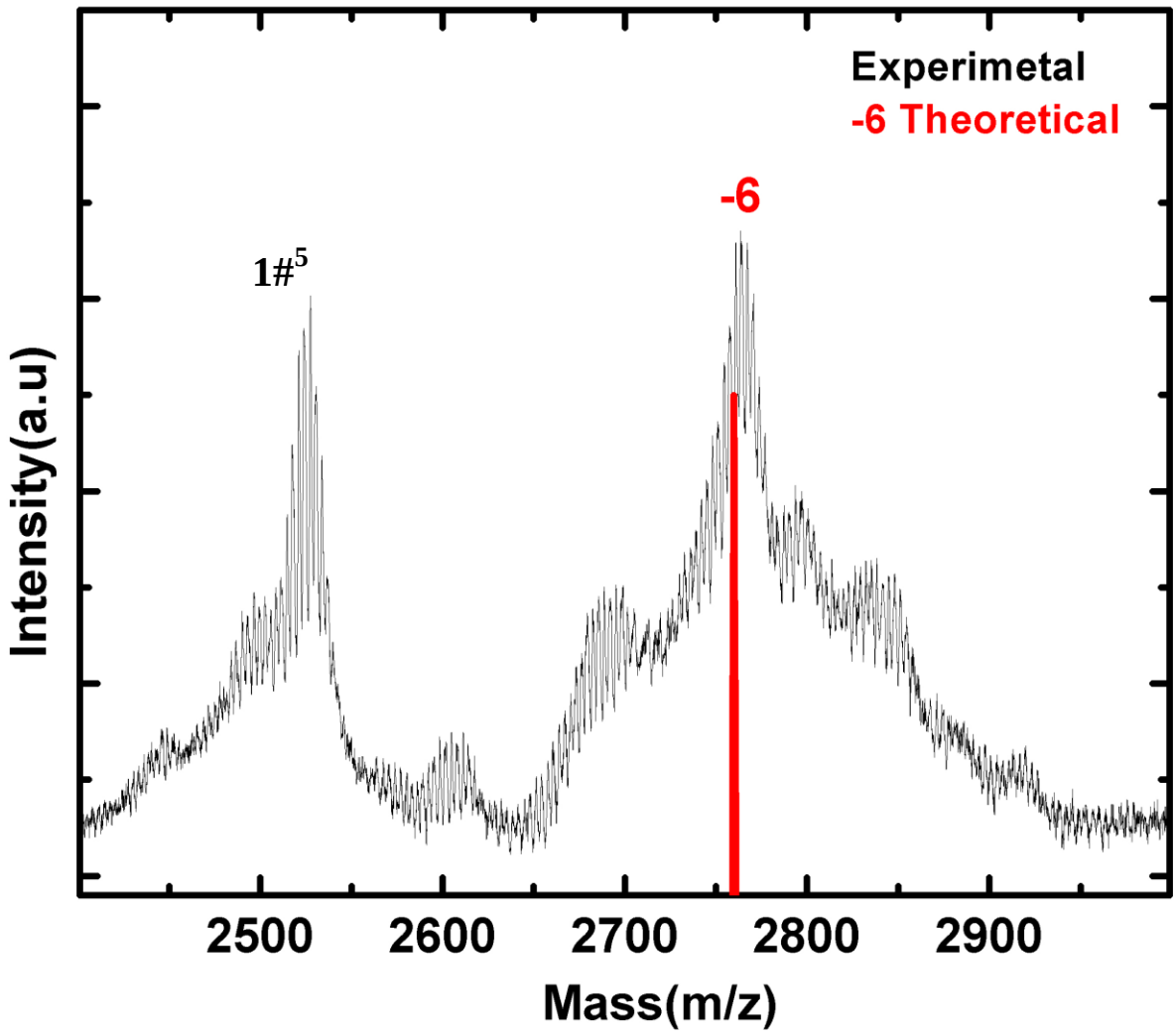

Figure S11: ESI mass spectrum of band 5. Molecular Formula of this band is identified as $Au_{43}(SG)_{26}Na_6$. The contamination with $Au_{38}(SG)_{24}Na_4$ and $Au_{39}(SG)_{24}Na_4$ is labeled as $1\#^5$.

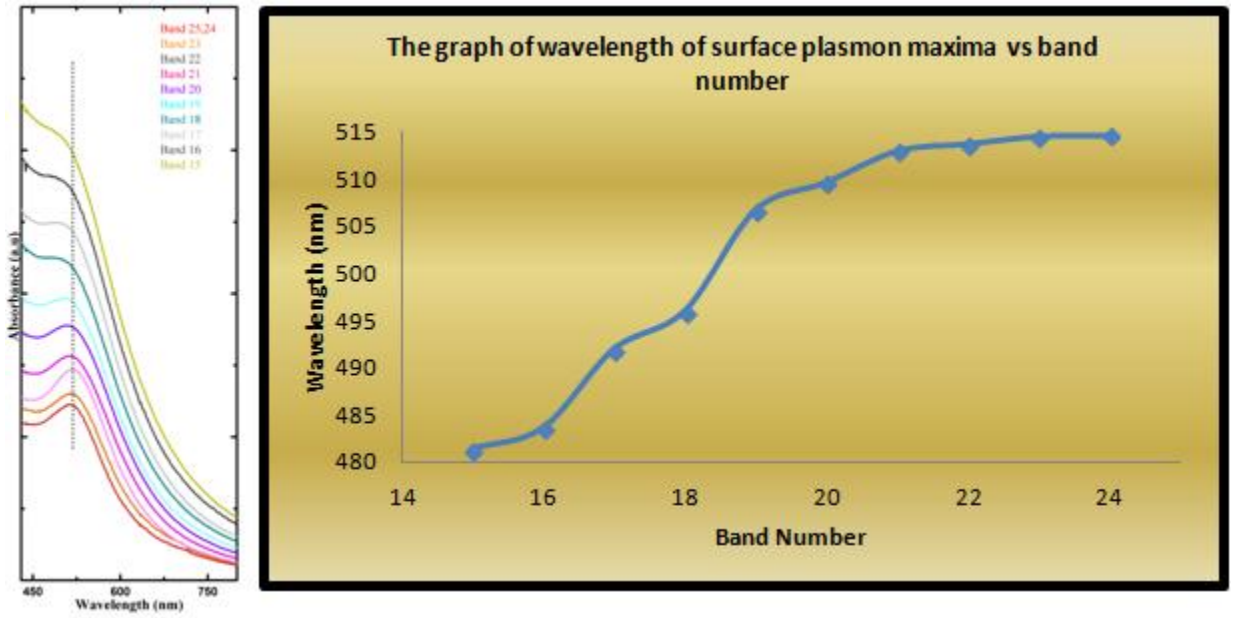

Figure S12: The plot showing surface plasmon peak maxima shifting to higher wavelength with an increase in the size of the nanomolecule

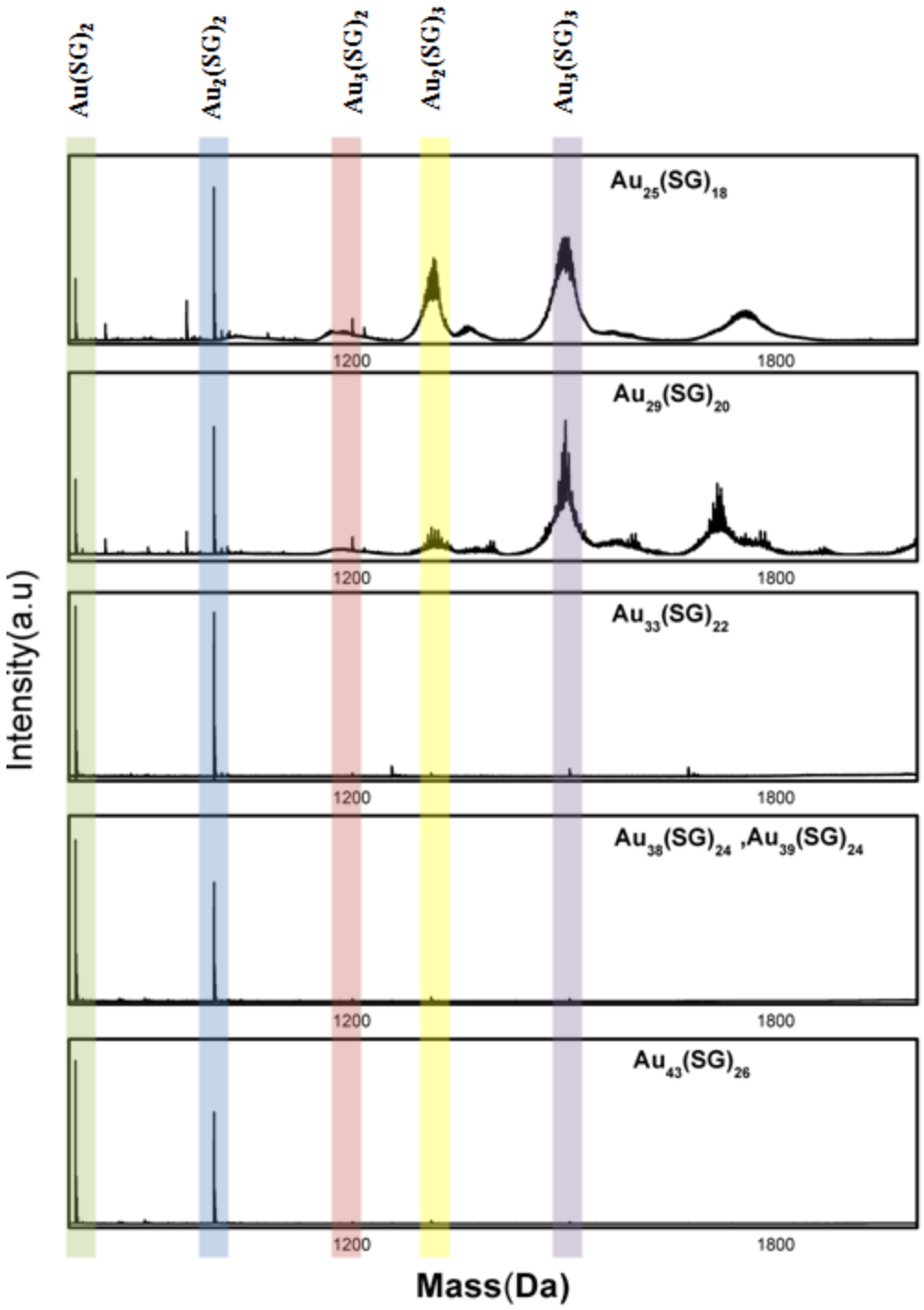

Figure S13: The fragmentation pattern of ESI mass spectra of nanoparticles at first 1-6 bands. The observed fragments are $Au(SG)_2$, $Au_2(SG)_2$, $Au_3(SG)_2$, $Au_2(SG)_3$ and $Au_3(SG)_3$

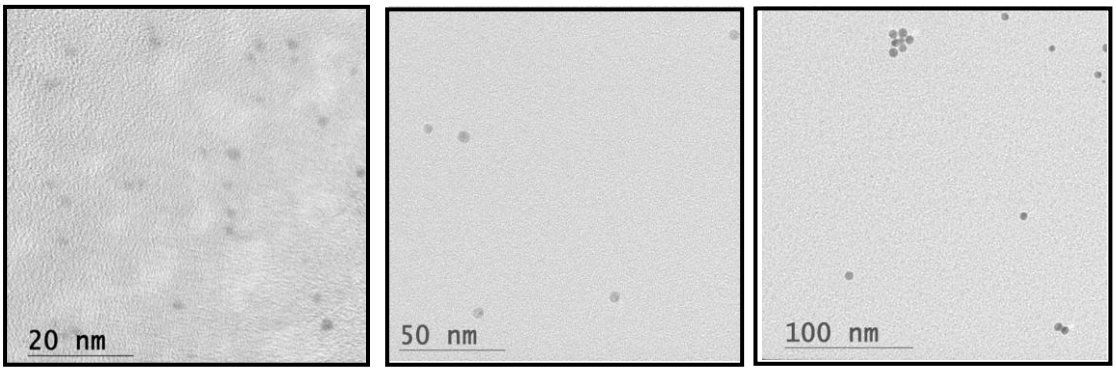

Figure S14: The TEM images of nanoparticles in band 10 (~1.8 nm), 14 (~3.0 nm) and 22 (~6.0 nm). Average particle size measured gien in the brackets.

References:

(1) Wu, Z.; Chen, J.; Jin, R. *Adv. Funct. Mater.* 2011, *21*, 177-183.

(2) Guo, J.; Kumar, S.; Bolan, M.; Desireddy, A.; Bigioni, T. P.; Griffith, W. P. *Anal. Chem.* 2012, *84*, 5304-5308.