Electronic Supplementary Information

Role of UHPLC in evaluating as-synthesised ligand-protected gold nanoparticles products

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Synthesis of histidine-protected gold nanoparticles (His-AuNPs)

The synthesis of His-AuNPs has been described previously.[1] Briefly, 1.0 mL of 10 mM $HAuCl_4$ aqueous solution was mixed with 3.0 mL of 100 mM histidine aqueous solution at room temperature. The mixture was left for 2 h to allow reaction to proceed. Afterward, the solvent was removed by a freeze-dryer. The His-AuNPs product was kept at 4°C prior to further liquid chromatographic analyses.

Synthesis of *N,N′*-dimethylformamide-protected gold nanoparticles (DMF-AuNPs)

The synthesis of DMF-AuNPs has been described previously.[2] In summary, 150 μL of 0.10 M $HAuCl_4$ aqueous solution was added to 15 mL of DMF at 140°C and refluxed for 6 h under vigorous stirring. Afterward, the solvent was removed under vacuum and the brown residue was further dried by a stream of nitrogen gas and stored in a desiccator.

References

1. Y. Zhang, Q. Hu, M. C. Paau, S. Xie, P. Gao, W. Chan and M. M. F. Choi, *J. Phys. Chem. C*, 2013, **117**, 18697-18708.
2. S. Xie, M. C. Paau, Y. Zhang, S. Shuang, W. Chan and M. M. F. Choi, *Nanoscale*, 2012, **4**, 5325-5332.

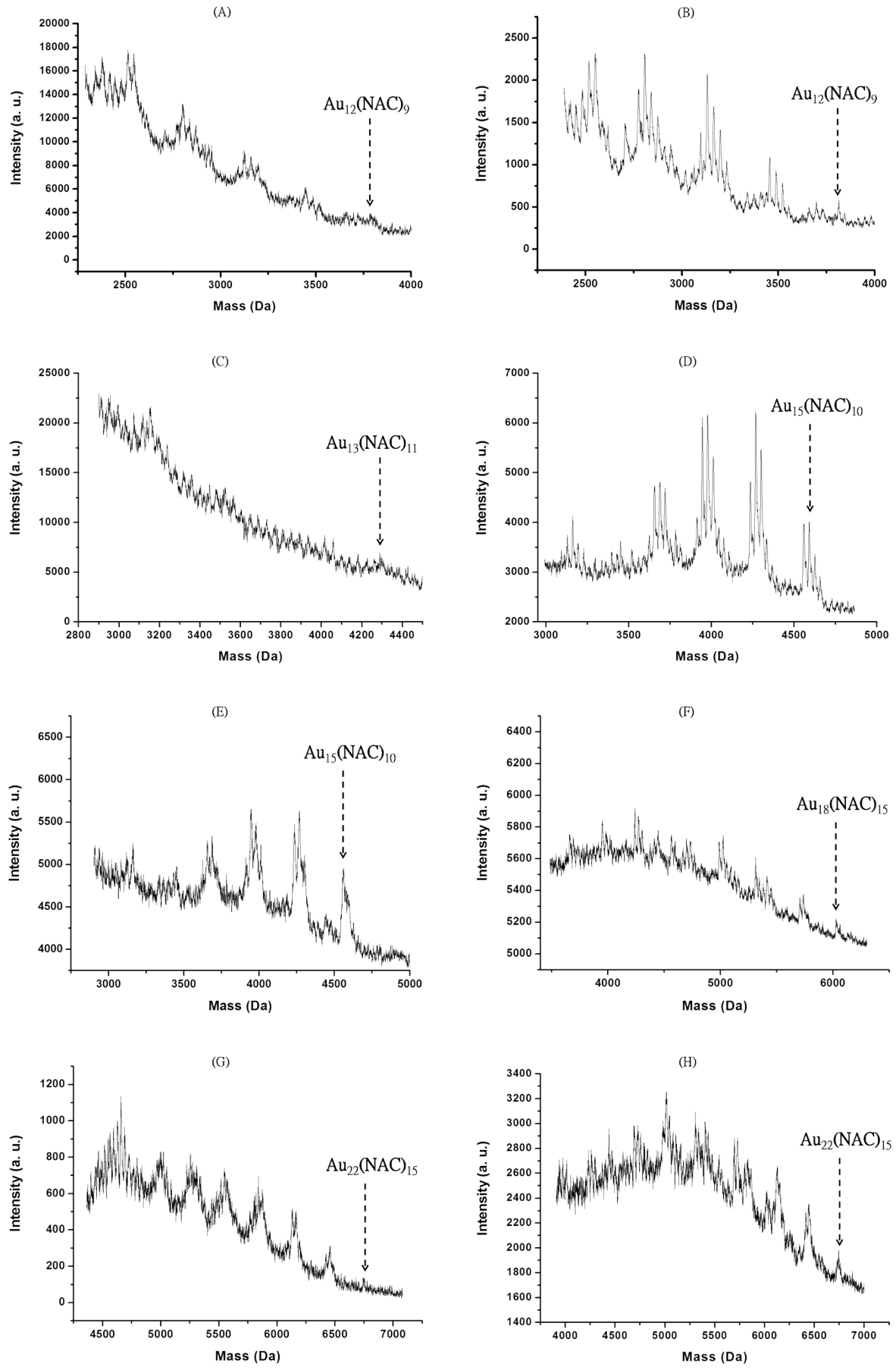

Figure S1. MALDI-TOF mass spectra of Fractions 1–8 (A-H) in Figure 1A(a).

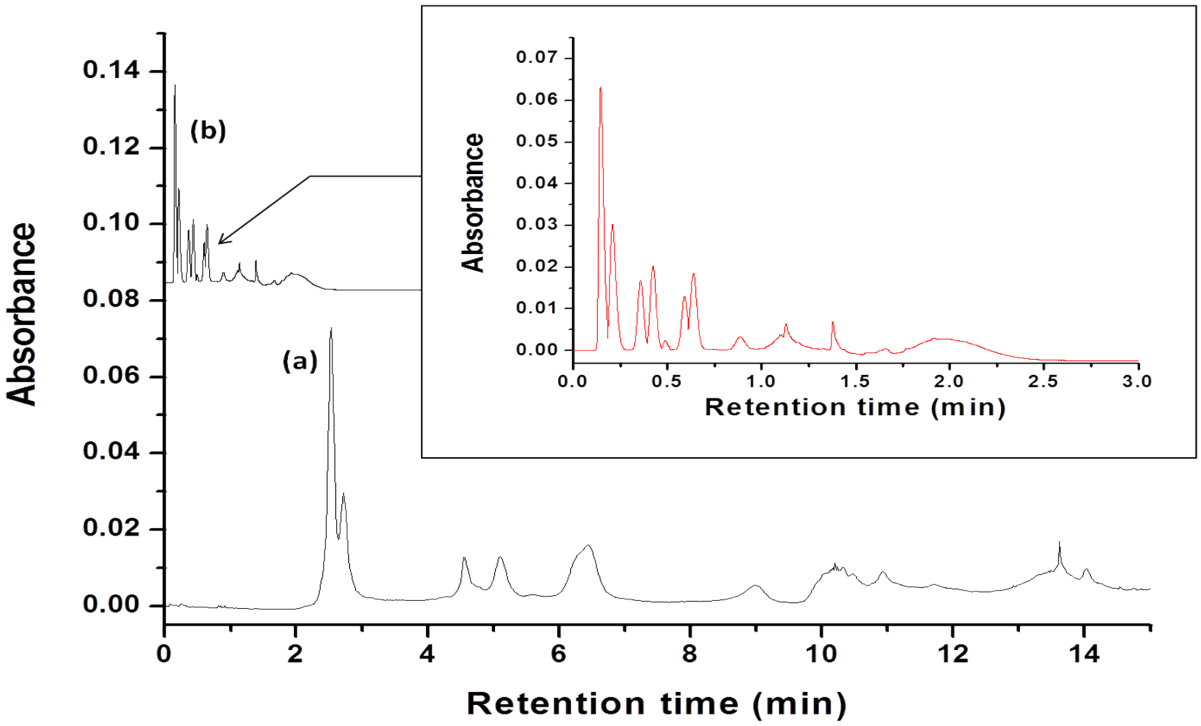

Figure S2. Chromatograms (*a*) and (*b*) are HPLC and UHPLC separations of a 15.00 mg/mL His-AuNPs sample. The inset displays the enlarged view of the UHPLC chromatogram. The mobile phase consisted of 10 mM pH 5.0 ammonium acetate in water (eluent I, pH adjusted by acetic acid) and MeOH (eluent II). For HPLC separation, the injection volume was 20.0 µL. A gradient elution program was used at 0.70 mL/min and as follows: 100% I for 5.5 min, linearly decreased to 90% I from 5.5 to 6.5 min, maintained at 90% I from 6.5 to 9.0 min, linearly decreased to 80% I from 9.0 to 10 min, and kept at 80% I from 10 to 12 min, linearly decreased to 60% I from 12 to 13 min, and kept at 60% I from 13 to 15 min. For UHPLC separation, the injection volume was 2.0 µL. A gradient elution program was used at 0.70 mL/min and as follows: 100% I for 0.33 min, linearly decreased to 90 % I from 0.33 to 0.45 min, maintained at 90 % I from 0.45 to 0.73 min, linearly decreased to 80% I from 0.73 to 0.84 min, and kept at 80% I from 0.84 to 1.07 min, linearly decreased to 60% I from 1.07 to 1.18 min, and kept at 60% I from 1.18 to 3.00 min.The absorbance chromatograms were monitored at 250 nm and offset for clarity.

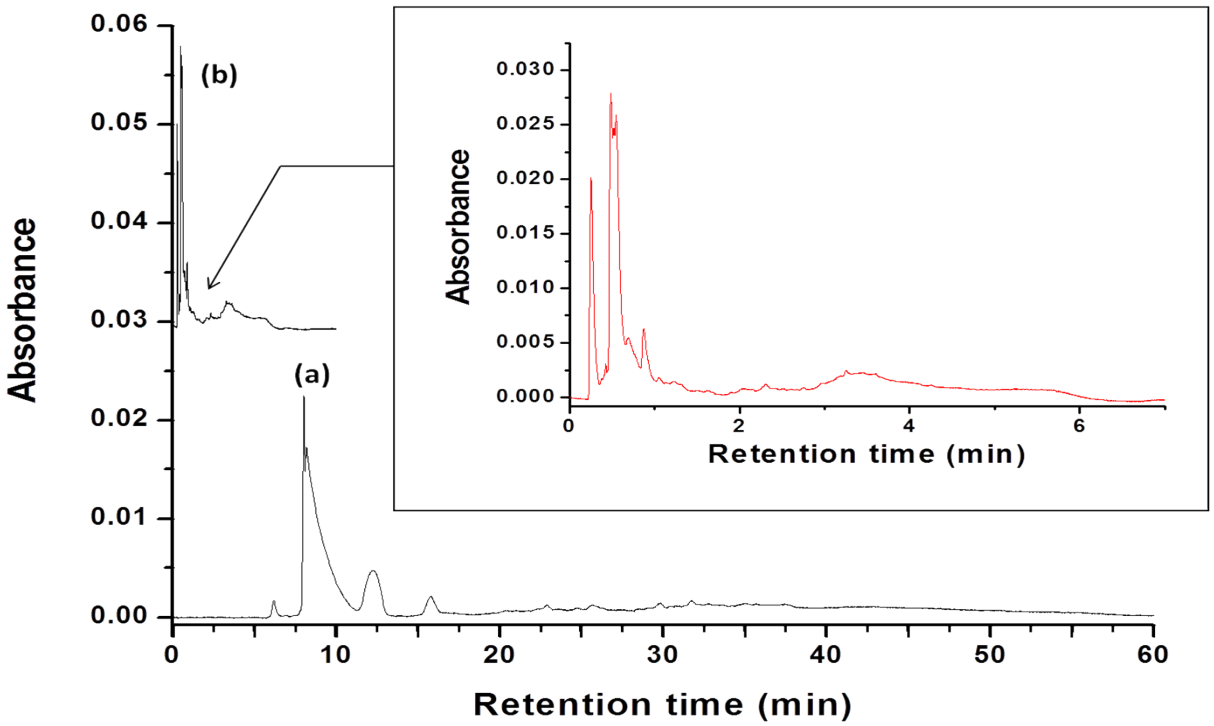

Figure S3. Chromatograms (*a*) and (*b*) are HPLC and UHPLC separations of a 1.00 mg/mL DMF-AuNPs sample. The inset displays the enlarged view of the UHPLC chromatogram. The mobile phase consisted of water (eluent I) and MeOH (eluent II). For HPLC separations, the injection volume was 10.0 μL. A gradient elution program was used at 0.40 mL/min and as follows: 97% I for 11 min, linearly decreased to 90 % I from 11 to 15 min, maintained at 90 % I from 15 to 20 min, linearly decreased to 80% I from 20 to 25 min, and kept at 80% I from 25 to 60 min. For UHPLC separation, the injection volume was 1.0 μL. A gradient elution program was used at 0.40 mL/min and as follows: 97% I for 0.74 min, linearly decreased to 90% I from 0.74 to 1.19 min, maintained at 90% I from 1.19 to 1.76 min, linearly decreased to 80% I from 1.76 to 2.32 min, and kept at 80% I from 2.32 to 7.00 min.The absorbance chromatograms were monitored at 250 nm and offset for clarity.