Electronic supplementary information for

Synthesis and Characterization of Mixed Ligand Chiral Nanoclusters

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Experimental

Synthesis of mixed ligand nanocluster

Mixed ligand silver nanoclusters were synthesized by reducing silver nitrate in methanol and water mixture at room temperature (RT). The MilliQ water was used throughout the experiments. First, equal volumes (12.5 ml) of methanol and water were mixed and purged with nitrogen, and the pH of the mixture was adjusted to 11 by the addition of sodium hydroxide solution. We dissolved 4.45 mg (0.036 mmol) of L-cysteine (L-cys) in this solution and then added 5.08 µl (0.036 mmol) of mercaptohexanoic acid. In silver to thiol ligand ratio of 1, 12.5 mg (0.073 mmol) of silver nitrate was used and reduction of silver was carried out by the addition of 13.91 mg (0.36 mmol) sodium borohydride in water. The reaction was kept under vigorous stirring for 6 hours.

Separation in the PAGE

The raw product was separated via PAGE (Cleaver, OmniPAGE mini vertical electrophoresis system) with a separating gel of 30 wt% and a stacking gel of 8 wt% acrylamide monomers (acrylamide/bisacrylamide=93/7), respectively. The size of the gel was 10 cm× 10 cm × 2 mm. A buffer solution of Tris-HCl with pH 8.8 for the separating gel and pH 6.8 for the stacking gel was used. The running electrode buffer was an aqueous mixture of glycine (192 mM) and Tris (25 mM). We took 1 ml of the reaction mixture, precipitated the product in THF, redissolved it in 50 µl water, and added 16% (v/v) of glycerol to this solution. We loaded 5 µl of this solution into the well of the stacking gel and eluted the sample for 3 hours at 4 °C with constant 200 V (Cleaver, CS-300V) to separate the product. To extract silver nanoclusters, the band at each fraction was cut and left in water for a day. Filters with 0.2 µm pore size were utilized to remove remaining lumps of the gel.

Optical characterization of mixed ligand nanoclusters

The absorption spectra of the clusters were recorded with a Shimadzu UV-3150 UV-Vis spectrometer and Jasco J-810 CD spectrometer was used to track chiroptical properties of the clusters. Rectangular quartz cuvettes with a 1 mm path length were utilized throughout the measurements. All the CD data are normalized with respect to relative UV- Vis spectra of the samples.

In addition to different ligand ratios, we also synthesized nanoclusters with different silver to thiol ratios at the same ligand ratio of L-cys:MHA 1:1. UV-Vis spectra show the cluster formation at each reaction condition. Consequently, the normalized CD graph indicates that chirooptical response can be achieved from all batches except the one with 1:0.5 silver to thiol ratio. Although the rest of the reaction conditions result in similar chirooptical responses, the highest amplitude can be seen in nanoclusters with 1:1 silver to thiol ratio (Fig. 1S).

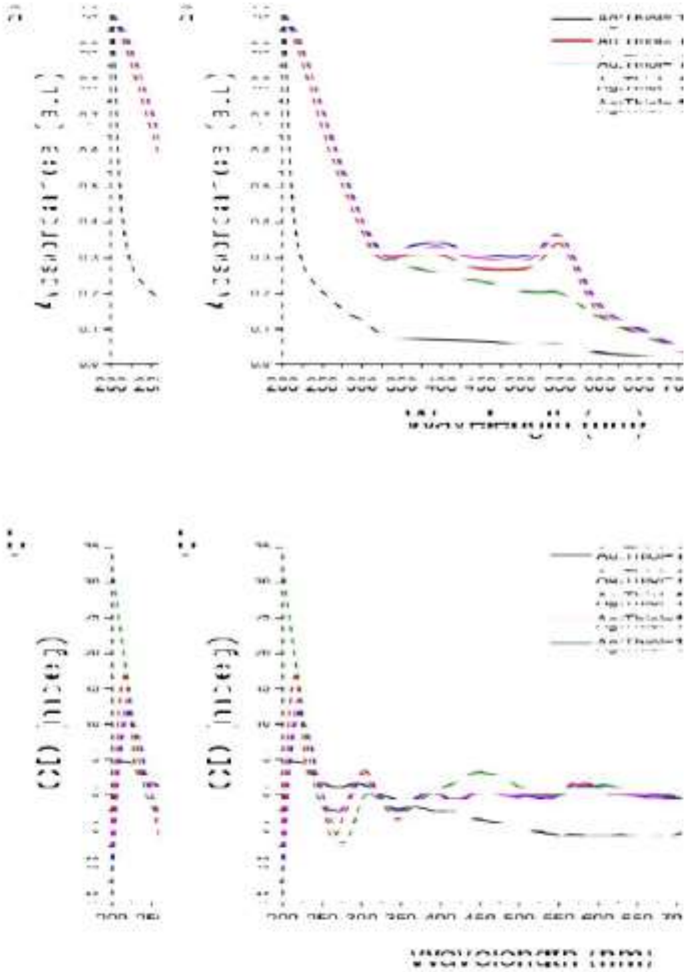


Fig. 1S (a) The UV-Vis spectra of the nanoclusters that are synthesized with different silver to thiol ratios, **(b)** The corresponding CD spectroscopy on the same nanoclusters

We synthesized mixed ligand chiral nanoclusters by combining different enantiomers with MHA. Although we saw that there is a slight difference between yields of clusters in two batches, the amplitudes of chiroptical responses that result from clusters are almost the same (Fig. 2 Sa). While D-cys results in a positive response on the nanoclusters, L-cys leads to a response on the opposite site (Fig. 2Sb).

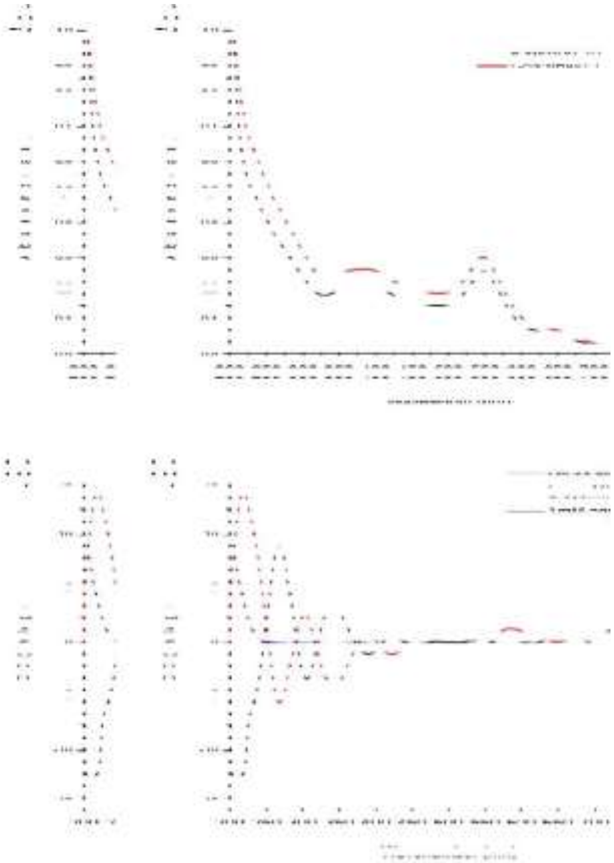


Fig. 2S (a) UV-Vis spectra of the clusters with different enantiomers at cys:MHA ratio of 1:1, **(b)** Corresponding CD spectroscopy of nanoclusters

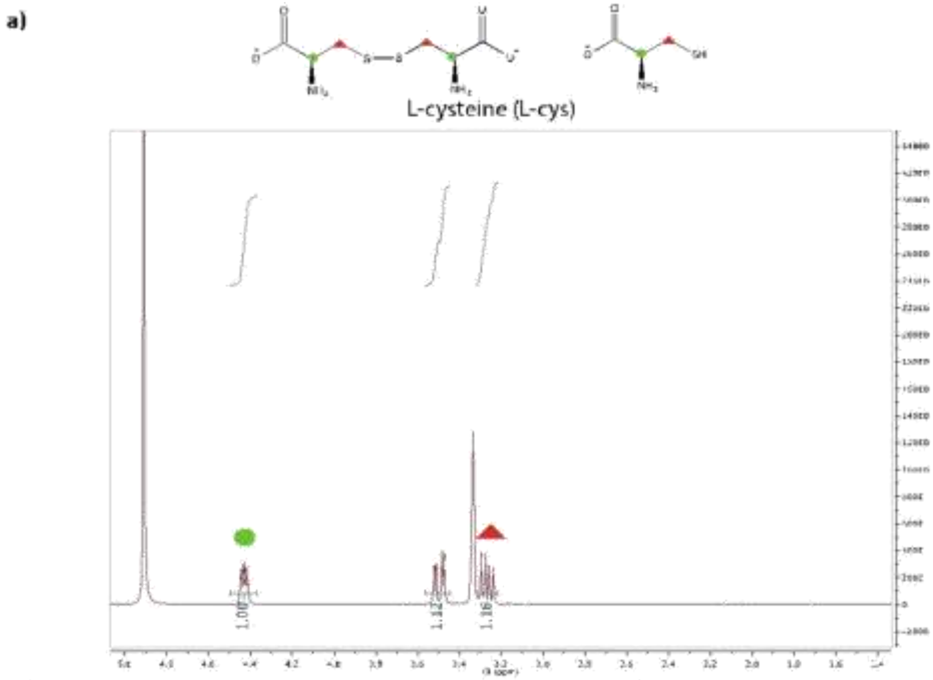
Determination of the relative ratios of ligands on nanoclusters

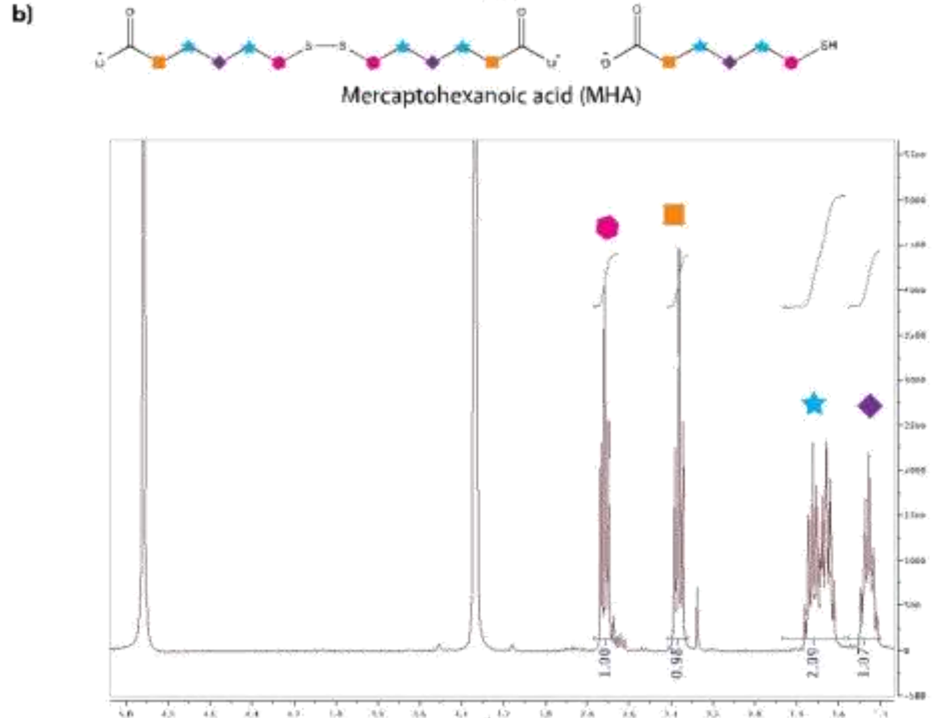
NMR spectroscopy was carried out to determine the ligand composition of ‘Band 4’ of silver nanoclusters with three different feeding ratios (L-cys:MHA of 1:1, 1:2, and 1:3). The products of reactions were separated by PAGE, extracted into water, filtered with 200 nm pore-size membrane (Merck, Millipore), further washed 10 times with water and concentrated by Amicon filters (Merck, Millipore, 3K). Prior to etching, H-NMR in D2O of all three Band 4s provided broad peaks implying the non-existence of free ligands. To find the optimum etching conditions, we tried potassium iodine in methanol-d4 (MeOD), potassium cyanide in D2O and in MeOD. We observed distinguishable peaks in potassium iodine (with concentration of 15 mg/ml) for both ligands, whereas potassium cyanide did not provide distinct peaks for MHA.

We analyzed the peaks of H-NMR spectra of mixed ligand nanoclusters based on the H-NMR spectrum of pure ligands that were subject to the same etching conditions. To calculate the relative ratio of L-cys and MHA, peak between 4.45–4.32 ppm and 1.87–1.57 ppm were chosen for L-cys and MHA, respectively (green circle and blue star in color coding, Fig. 3S). The calculation is carried out based on the integral of the aforementioned peaks. In Fig. 3S, the NMR spectrum that was measured before etching conditions of the clusters display broad peaks indicating the non-existence of the free ligands in the system. We observed approximately 10% (8.5 to 11%) cys on L-cys:MHA feeding ratios of 1:1, 1:2, and 1:3.

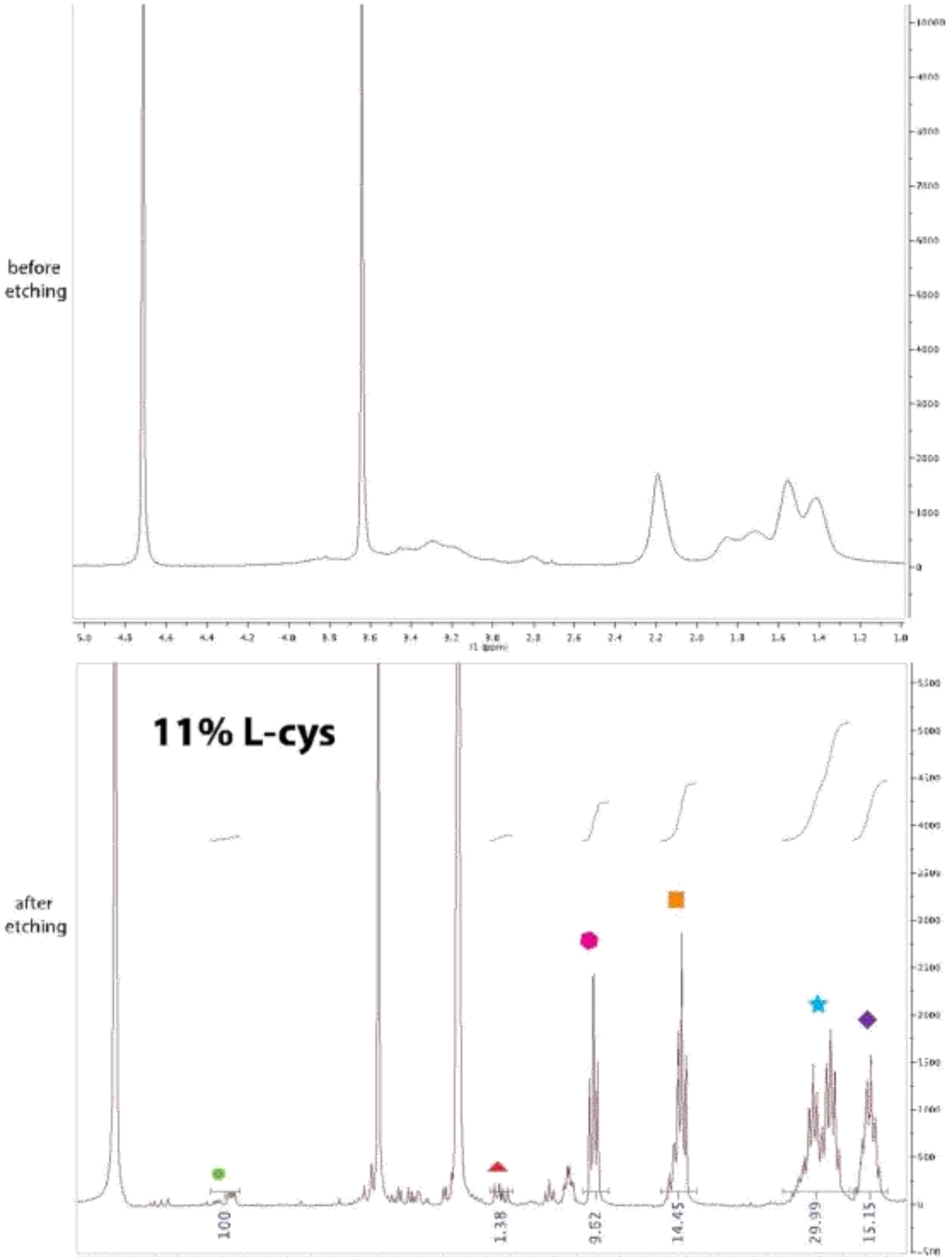
Table 1S. Color and shape coding of H-NMR peaks

Ligand	Coding	Number of H	Peak Values (ppm)
L-cys	Green circle	1H	4.45–4.32
	Red triangle	2H	3.19–3.09
MHA	Pink hexagon	2H	2.77–2.655
	Orange square	2H	2.42–2.26
	Blue star	4H	1.87–1.57
	Purple rhombohedron	2H	1.555–1.4

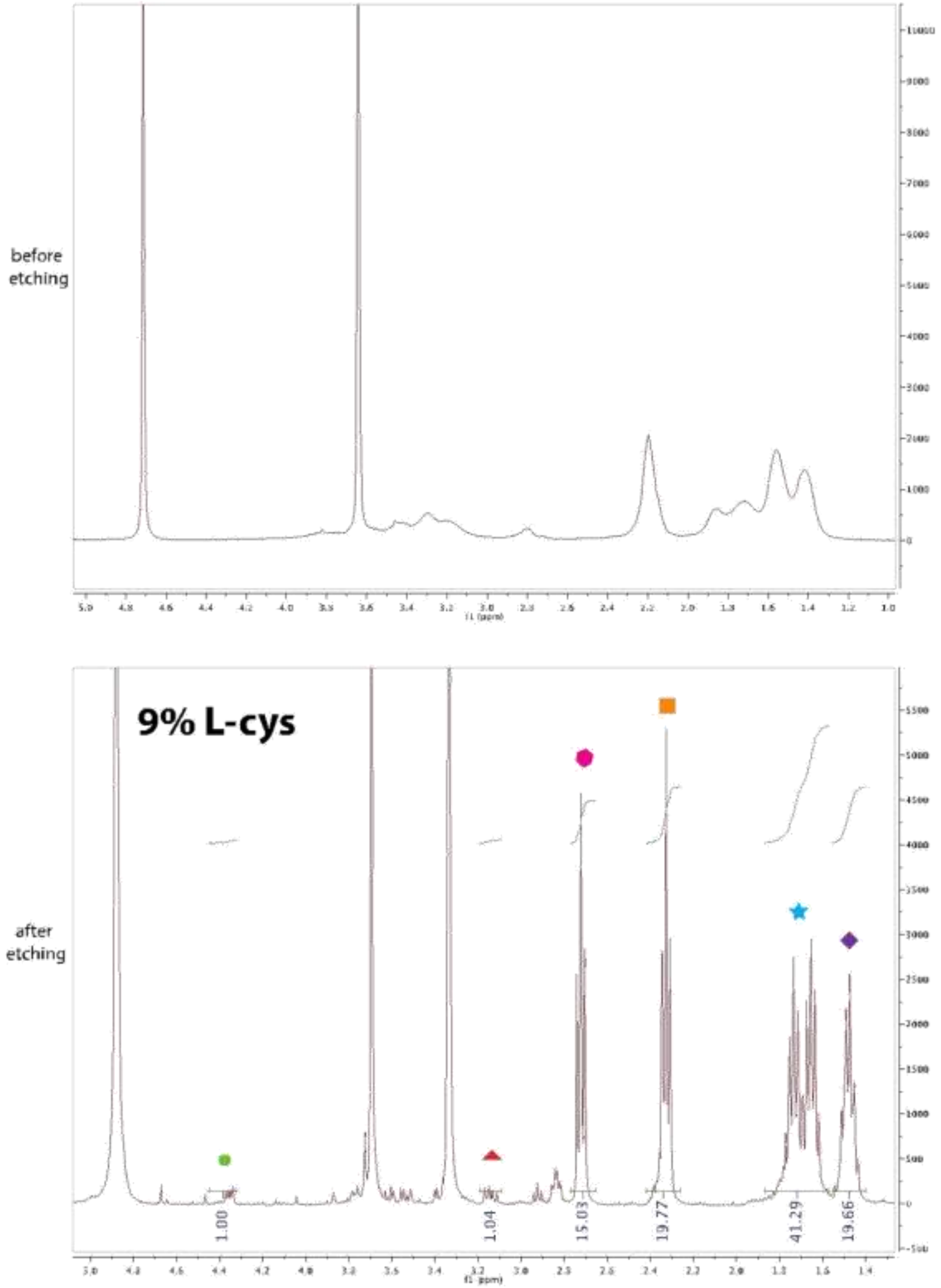

a)
L-cysteine (L-cys)
1.00
1.12
1.16
f1 (ppm)


b)
Mercaptohexanoic acid (MHA)
1.00
0.98
2.00
1.07
f1 (ppm)

c) Band 4 from Silver Nanoclusters L-cys:MHA 1:1



d) Band 4 from Silver Nanoclusters L-cys:MHA 1:2



e) Band 4 from Silver Nanoclusters L-cys:MHA 1:3

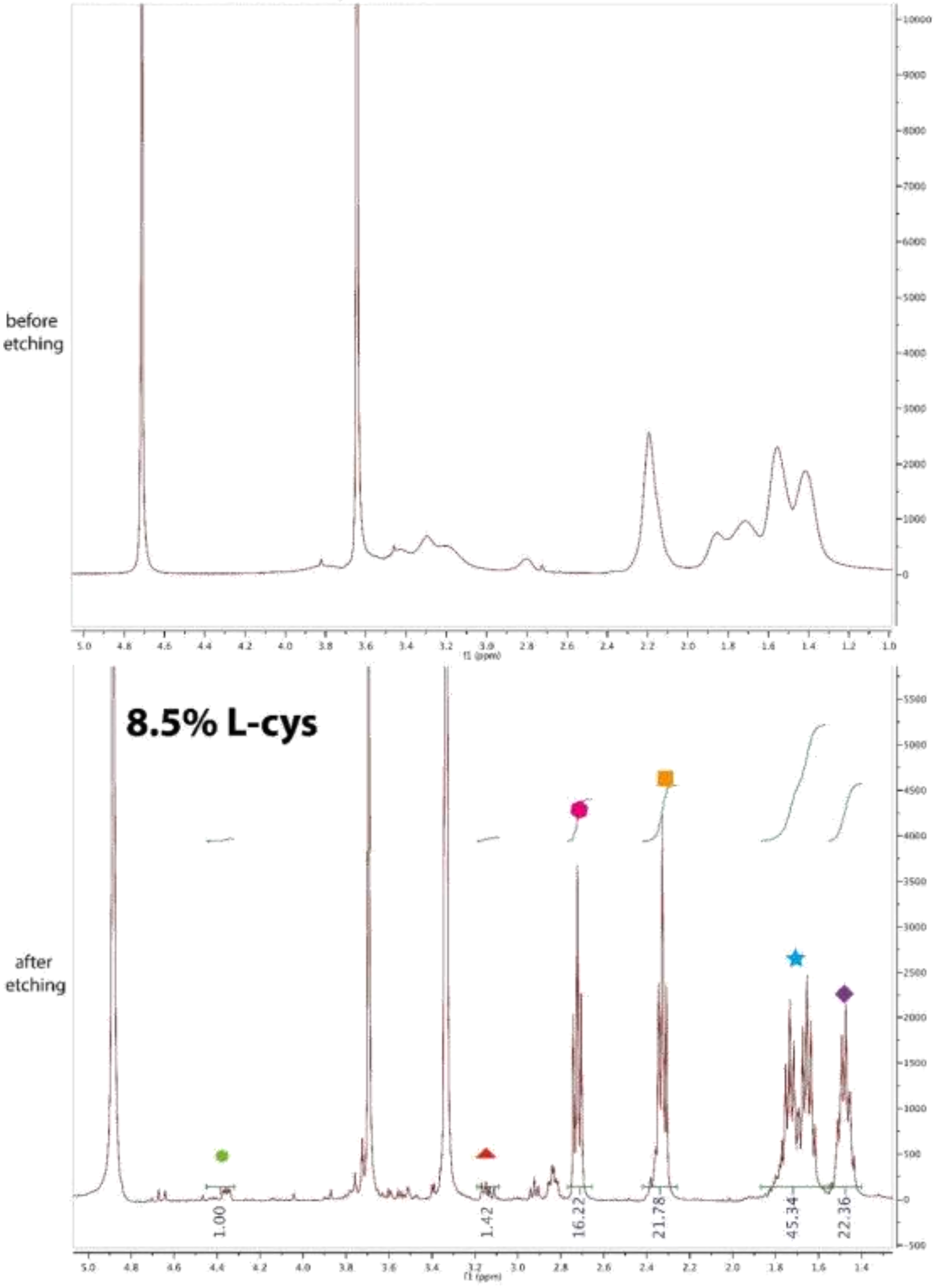


Fig. 3S (a) The chemical derivations of cys that can possibly be formed after etching of potassium iodine, and color coding of hydrogens in these chemicals for relative H-NMR peaks, **(b)** The chemical derivations of MHA that can possibly be formed after etching of potassium iodine, and color coding of hydrogens in these chemicals for relative H-NMR peaks, **(c)** H-NMR spectrum of band 4 of reaction with 1:1 cys:MHA ratio before and after etching, **(d)** H-NMR spectrum of band 4 of reaction with 1:2cys:MHA ratio before and after etching, **(e)** H-NMR spectrum of band 4 of reaction with 1:3 cys:MHA ratio before and after etching

Determination of the size of mixed ligand nanoclusters

i) Polayacrylamide gel electrophoresis (PAGE)

PAGE was run on a Thermo Scientific vertical electrophoresis (P9DS) system with a homemade 5% stacking and 30% resolving gel. As synthesized cluster samples were dissolved directly in 10% aqueous glycerol solution to make a range of concentrations from 50 to 300 mg/mL. Approximately 15–50 µL of these samples were loaded in the gel and ran for 6.5 hours at a constant voltage (200 V) and current (20 mA) using 0 °C coolant.

ii) Sample preparation for mass spectrometry

The dark reddish-brown band 4 of L-cys:MHA nanocluster was excised from the gel and soaked overnight in 2 mL of water at 4 °C. Thus obtained pink solution was centrifuged for 7 minutes at 13,500 rpm to discard any insoluble materials. A thin layer of this solution was deposited on a MALDI target plate without a matrix and allowed to evaporate under ambient conditions. Autoflex speed MALDI-TOF with 1 kHz smartbeam-II laser was used for the data acquisition in linear positive mode.

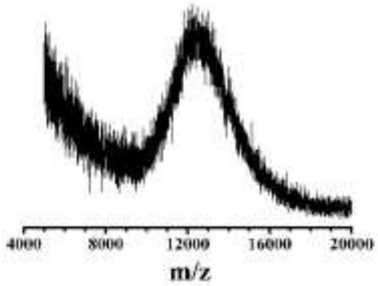


Fig. 4S LDI mass spectrum of band 4 of L-cys:MHA (1:1) nanoclusters.

iii) Determination of partial specific volume (ῡ)

Accurate determination of hydrodynamic parameters such as sedimentation and diffusion coefficients is largely dependent on the correct estimation of the particle density in the form of the partial specific volume ($\bar{\upsilon}$) of the solution species. The average density of the L-cys:MHA (1:1) silver nanoclusters was calculated from its reciprocal relationship with its $\bar{\upsilon}$.

The partial specific volume is the fractional change in the volume of a solution caused by a dissolved solute and can be measured by using the slope of a plot between the concentration and density of L-cys:MHA (1:1) silver nanocluster solutions at 20°C in the Kratky equation.[1] The average partial specific volume of silver L-cys:MHA (1:1) silver nanocluster was calculated to be 0.182 cm^3/g, and thus the particle density, which is essentially the reciprocal of the partial specific volume, is evaluated at 5.49 g/cm^3. This density value is similar to recent estimates for silver nanoclusters in literature.[2]

iv) Determination of sedimentation velocity in the analytical ultracentrifuge

Sedimentation velocity experiments were performed on a Beckman Optima XL-A analytical ultracentrifuge equipped with Scanning Absorption Optics.[3] 12 mm optical path length double sector titanium cells were used in a 4-hole rotor. Experiments were conducted at a speed of 40,000 rpm and at a temperature of 20 °C. Scans were taken every 0.2 minutes and captured by a CCD camera. Sedimentation velocity data were evaluated using Ultrascan-III (The University of Texas Health Science Center at San Antonio - UTHSCA), which discretizes the sedimentation and diffusion coefficients within user-specified limits of sedimentation coefficient and frictional ratio values. Sedimentation coefficients, s (in Svedberg units S, where 1 S = 10^{-13} sec) and diffusion coefficients (in cm^2/s) measured in the solvent (0.01M NaOH) were normalized to standard conditions of the density and viscosity of water at 20 °C by Ultrascan-III to give $S_{20,w}$ and $D_{20,w}$ values.

Sedimentation velocity data of the L-cys:MHA (1:1) silver nanocluster solution to obtain sedimentation coefficient distributions were analyzed with the Ultrascan-III software using the two-dimensional spectrum analysis (2DSA) approach via meniscus optimization.[4] The 2DSA method is capable of resolving heterogeneity in shape and size. Sedimentation coefficients are usually obtained at high resolution and there is a good agreement between the experimental data and the simulation fit achieving typical rmsd values of 0.0031 (Fig.5S). The sedimentation coefficient distributions were obtained following 100 Monte Carlo iterations of the 2DSA simulation fitting routine. Table 2S shows the hydrodynamic parameters from the analysis of the sedimentation velocity data of a solution of L-cys:MHA (1:1) silver nanoclusters. Three major species can be identified in solution with significantly different anisotropies suggesting a heterogeneous solution in terms of solution conformation (particle size and shape distributions). Species 3 has the largest molecular mass and the highest frictional ratio suggesting it is likely to be aggregating solution components as it displays significantly different anisotropy from species 1 and 2.

The partial specific volume, ῡ, of the ultra-stable silver nanoparticle, $M_4Ag_{44}(p\text{-}MBA)_{30}$ have previously been determined from x-ray crystallography (0.24 mL/g) and analytical ultracentrifugation (0.27 mL/g).[2] A ῡ of 0.18 mL/g for the L-cys:MHA (1:1) silver nanoclusters suggests that these molecules are comparatively denser than an ultra-stable nanostructure of roughly similar structure and composition. This claim is possible if the L-cys:MHA (1:1) silver nanoclusters has a lower amount of bound surfactants compared to its ultra-stable counterpart or if the nanocluster preparation contains some contaminant species of higher density. However, different solutions of quantum dots have been shown to exhibit heterogeneity in particle density.[2] Interestingly, the 2-DSA 3D plot shows that the solution is heterogeneous (Fig 6S) following Monte Carlo analysis with 50 iterations. In order to verify whether the ῡ estimate from the Kratky method is not an underestimation of the true ῡ of the solution or its components, the sedimentation velocity data were fitted with a constant frictional ratio, f/f_o, of 1.35 (Fig.5S) as well as with a constant ῡ of 0.18 (Table 3S, Fig.8S) and of 0.27 mL/g (Table 4, Fig.9S). The f/f_o value of 1.35 was chosen from the average values of the major species of the latter two fits. The resulting fits show good agreement with experimental data (Fig.8dS and 8eS, Fig.9dS and 9eS). From these analyses, two major species are evident having sedimentation coefficients of 4– 5S and 5–6S with minor components at~ 2S and~ 8S. Contaminants at lower concentrations were also identified with significantly different frictional ratios and ῡ. In addition, the major species were found to have ῡ estimates between 0.15 mL/g and 0.25 mL/g and these values agree with current literature values for these types of molecules.[2] It is, therefore, likely that the solution of L-cys:MHA (1:1) silver nanoclusters contains a mixture of species with densities ranging from 4 to 6.7 g/mL.

Table 2S. 2DSA-Monte Carlo results at a constant partial specific volume, ῡ, of 0.18 mL/g

Parameters	**Species 1**	**Species 2**	**Species 3**
Molecular weight (KDa)	20.4 (17.9, 33.0)	9.8 (7.7, 11.8)	49.1 (41.8, 96.4)
Sedimentation coefficient, $s_{20,W}$(s, × 10^{-13})	4.8 (4.2, 5.5)	6.2 (5.7, 6.7)	8.2 (7.6, 8.7)
Diffusion coefficient, $D_{20,W}$(cm^2/s, × 10^{-7})	7.6 (1.6, 13.7)	18.5 (14.3, 27.3)	5.1 (4.8, 5.8)
Frictional ratio	2.7(1.3, 4.1)	1.3 (1.1, 1.4)	2.9 (1.1, 4.9)
ῡ (mL/g)	0.18	0.18	0.18
Partial conc [OD]	0.17	0.05	0.04
Relative percentage	61.4	17.6	13.1

95% confidence limits are shown in brackets. All values are corrected to standard conditions (equivalent to water at 20 °C).

Table 3S. 2DSA-Monte Carlo results at a constant partial specific volume, ῡ, of 0.27 mL/g

Parameters	**Species 1**	**Species 2**	**Species 3**
Molecular weight (KDa)	12.9 (5.7, 20.1)	18.4 (14.6, 31.7)	14.3 (8.2, 20.5)
Sedimentation coefficient, $s_{20,w}$(s, × 10^{-13})	2.2 (1.8, 2.6)	4.2 (3.7, 4.7)	5.5 (4.8, 6.1)
Diffusion coefficient, $D_{20,W}$(cm^2/s, × 10^{-7})	6.4 (0.3, 12.5)	1.2 (1.0, 3.3)	13.5 (9.3, 17.8)
Frictional ratio	3.4(1.7, 5.2)	2.1 (1.1, 5.4)	1.4 (1.1, 1.8)
ῡ (mL/g)	0.27	0.27	0.27
Partial conc [OD]	0.03	0.08	0.27
Relative percentage	10.3%	27.8%	61.7%

95% confidence limits are shown in brackets. All values are corrected to standard conditions (equivalent to water at 20^{o}C).

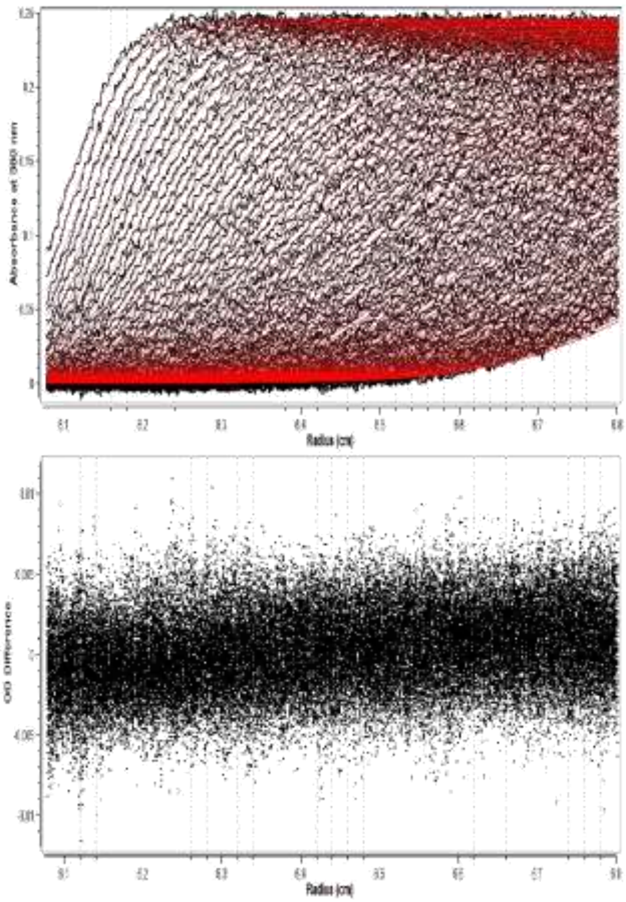


Fig. 5S Plots of **(a)** SV-AUC data (black curves) and 2-dimensional spectrum analysis fit (red curves), **(b)** residuals for L-cys:MHA (1:1) silver nanocluster in a solvent of NaOH/water. Experiment was conducted at 2 °C and 40,000 rpm in a Beckman XLA UltraCentrifuge and data were acquired in intensity mode at 380 nm. Subsequent conversion of s distributions to standard conditions was done using the Ultrascan-III software by BoriesDemeler.

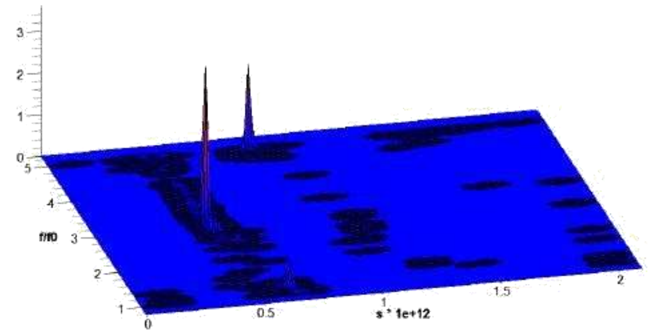


Fig. 6S 2-DSA 3D plots of sedimentation coefficient and frictional ratio against relative concentration for L-cys:MHA (1:1) silver nanoclusters in 0.01M aqueous NaOH solution. Plots were obtained following 50 Monte Carlo iterations. 3 peaks with different frictional ratios are evident. Experimental conditions are the same as in Figure 5S.

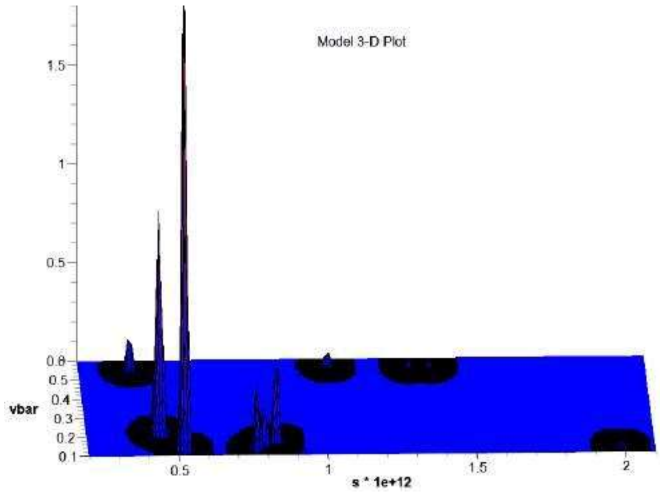


Fig. 7S 3D plots of ῡ against sedimentation coefficient and concentration on the vertical axis

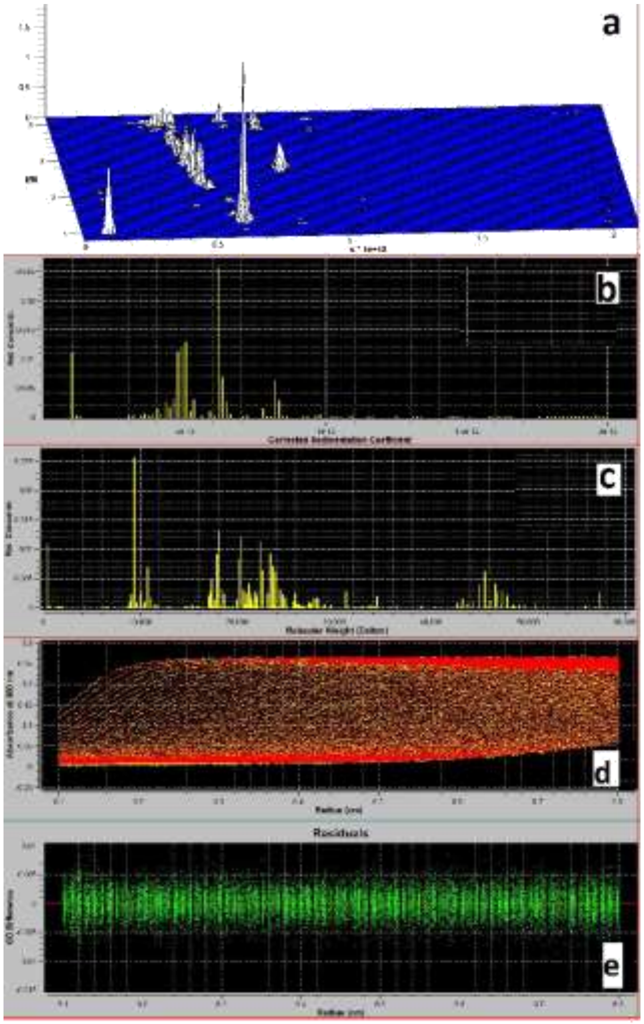


Fig. 8S 2-DSA Analysis of sedimentation velocity data for L-cys:MHA (1:1) silver nanoclusters in a solvent of NaOH aqueous solution. **(a)** 3D plots, **(b)** sedimentation coefficient distribution, **(c)** molecular weight distribution, **(d)** SV-AUC data (yellow curves) and 2-dimensional spectrum analysis fit (red curve**)**, **(e)** residuals A constant ῡ of 0.18 mL/g was used for the fit (green dots)

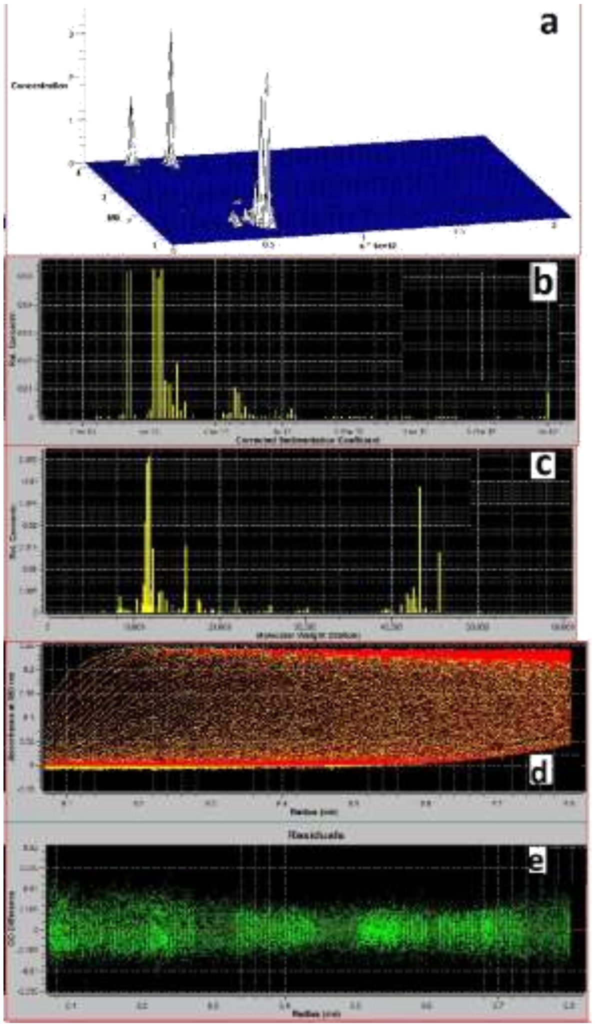


Fig. 9S 2-DSA Analysis of sedimentation velocity data for L-cys:MHA (1:1) silver nanoclusters in NaOH aqueous solution. **(a)** 3D plots, **(b)** sedimentation coefficient distribution, **(c)** Molecular weight distribution, **(d)** SV-AUC data and 2-dimensional spectrum analysis fit **(e)** residuals A constant $\bar{\upsilon}$ of 0.27 mL/g was used for the fit. Legend is as in Figure 8S.

v) Determination of size with scanning transmission electron microscopy (STEM)

Images of nanoclusters were recorded with a FEI TALOS F200X FEG Scanning\Transmission Electron Microscopy, operated at 200 kV. Samples were prepared by drop-casting Ag nanocluster solution on 400 mesh Cu grids (Electron Microscopy Science). Prior to drop-casting, Ag nanoclusters were extracted from PAGE into water, filtered with 200 nm pore-size membrane (Merck, Millipore), further washed 5 times with water and concentrated by Amicon filters (Merck, Millipore, 3kDa). Fig. 10S shows the existence of uniform nanoclusters with a core diameter of 1.33 ± 0.13 nm.

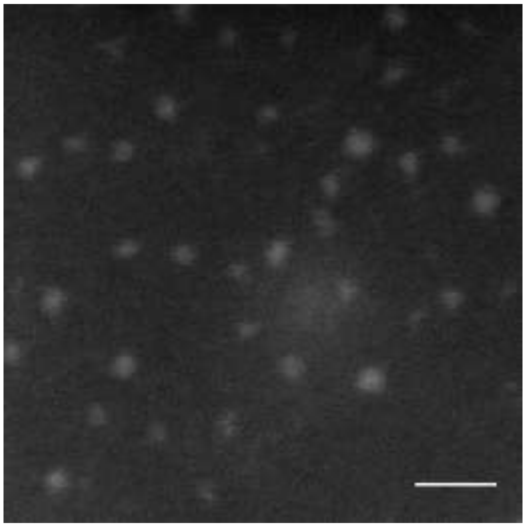


Fig. 10S Bright field scanning transmission electron microscopy image of Ag nanoclusters. The scale bar is 5 nm.

vi) Determination of composition of nanoclusters with energy dispersive x-ray spectroscopy (EDS)

Spectroscopy was conducted in FEI TALOS F200X FEG Scanning\Transmission Electron Microscopy and the samples were prepared by drop-casting of Ag nanoclusters following their extraction from PAGE into water and filtration with 200 nm pore-size membrane (Merck, Millipore). The type of detector was SuperX and the primary energy was 200 keV. The sulphur peak in EDS verified the existence of sulphur coating around the Ag nanoclusters after PAGE extraction.

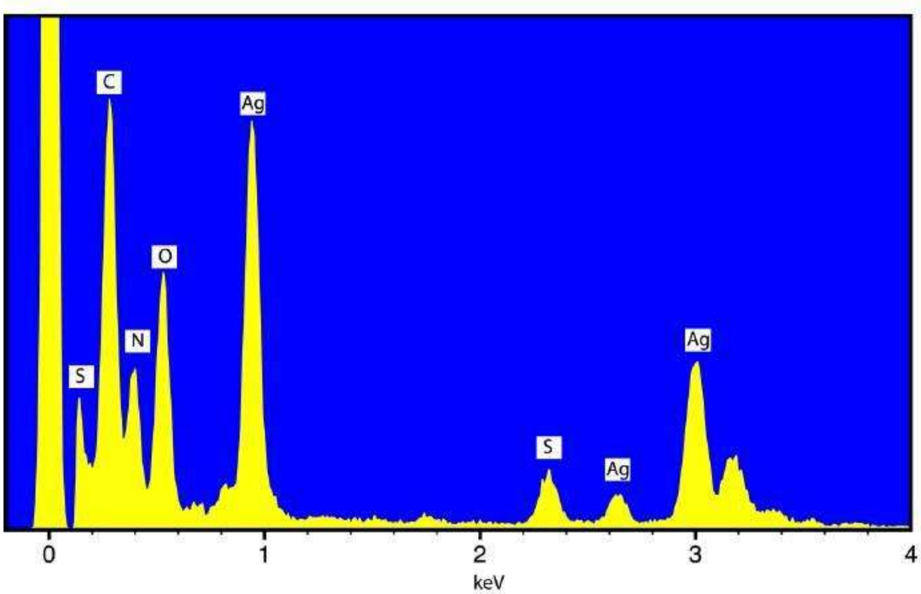


Fig. 11S EDS spectrum of Ag nanoclusters after PAGE extraction

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