Supporting Information For:

The power of fluorescence excitation-emission matrix (EEM) spectroscopy in the identification and characterization of complex mixtures of fluorescent silver clusters

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Fig S1. Absorbance spectra of AgNCs prepared using different ratios of Ag:GSH:NaBH₄, whereby the concentration of reducing agent NaBH₄ was changed in each instance from 1:1:1 to 1:1:16.



Fig S2a. Diode array chromatogram representing the sum of absorbances from 250 – 600 nm.



Fig S2b. High performance liquid chromatogram plotted using absorbance at 450 nm, demonstrating the separation of 3 strongly absorbing AgNCs: $Ag_A - 6.5$ minutes, $Ag_B - 13.0$ minutes, $Ag_C - 19.0$ minutes.

AgNC's separated on a Waters H-Class UPLC using an amide column (Waters BEH Amide; 2.1mm x 100mm; 1.7 μ m particle size) via isocratic mobile phase gradient of 51:49 acetonitrile: 10 mM ammonium acetate at a flow rate of 0.500 mL/min for 30 minutes. AgNC's detected by diode array absorbance scanning from 250 nm to 600 nm with a resolution of 4.8 nm.









Figure S3. A) Crude AgNC mass spectrum, B) close-up of high mass region, C) annotation demonstrating loss of S, AgS, and Ag_2S to form common fragments of AgNCs, D) isotope simulation of $Ag_9(C_5H_6NO_3S)$, a common glutathione fragment.¹





Figure S4. Mass spectra of A) Ag_A , B) Ag_B and C) Ag_C ; all AgNCs present the same fragmentation patterns with this mass range (<4500 m/z). See absorbance spectra of Ag_A , Ag_B and Ag_C in Figure 3.



Figure S5. TGA of Ag_A, Ag_B and Ag_C obtained from RP-HPLC and L-glutathione ligand (GSH); run with 5°C/minute ramp up to 900°C under argon gas. The three AgNC species show four steps in mass loss upon heating from 25°C to 900°C, whereas GSH destructs in three steps. As seen in previous studies, the first drop observed in the mass loss of the silver clusters can be attributed to the evaporation of adsorbed water molecules, as the ligand curve shows no mass loss until 180°C.² At 800°C the destruction of GSH is complete, and only the silver cores present in these clusters would be expected to remain after heating the samples to 900°C. This allows for the comparison of the mass of ligands present in each sample (destructed from 180°C to 800°C) relative to the amount of silver (remaining mass at 800°C). Based on this calculation, the percent composition for each fraction is: $Ag_A 1$ 57.8% GSH: 42.2% Ag, Ag_B 55.7% GSH: 44.3% Ag and Ag_C 53.4% GSH: 46.6% Ag.





Figure S6. a) Absorbance spectrum of glutathione (GSH) prepared at a 5mM concentration in water, b) EEM spectrum of aqueous 5mM GSH that has not been corrected for Raman or inelastic scattering, which results in the extremely weak emission shown. This showcases that there is no emission from free GSH ligands.

References

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- 2 M. Farrag, M. Thämer, M. Tschurl, T. Bürgi and U. Heiz, J. Phys. Chem. C, 2012, **116**, 8034-8043.