Electronic Supplementary Material (ESI) for Environmental Science: Nano.

Supporting Information

Impacts of Broth Chemistry on Silver Ion Release, Surface Chemistry Composition, and Bacterial Cytotoxicity of Silver Nanoparticles

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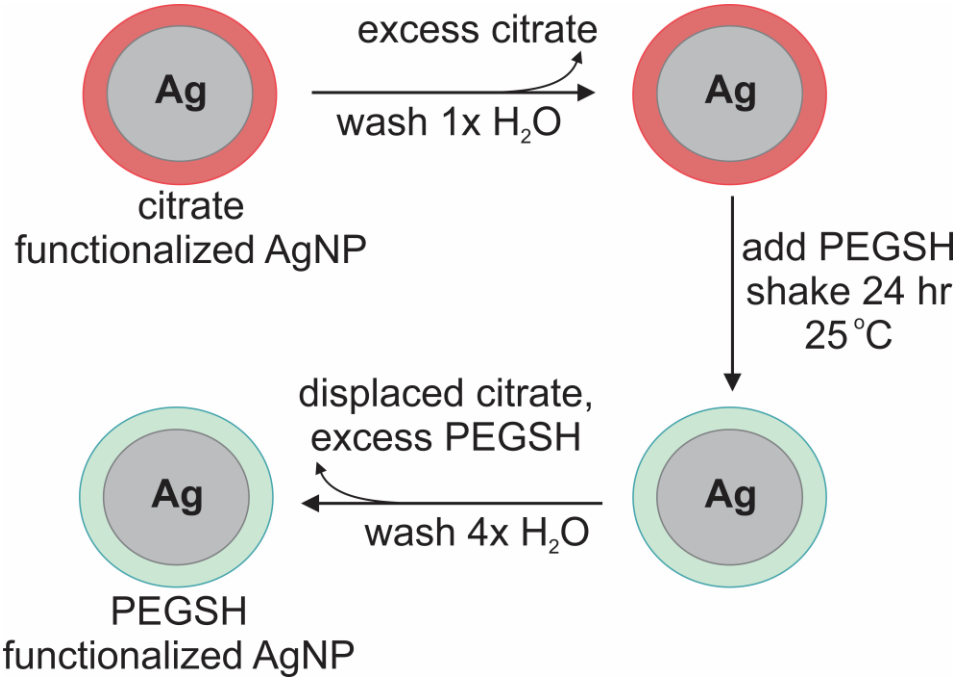

Scheme S1. Ligand exchange procedure for AgNPs originally functionalized with citrate and exchanged with PEGSH.

UV-vis-NIR Absorption Analysis of AgNPs

AgNP solutions (before and after broth incubation for 0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, or 24 hours) were characterized by ultraviolet-visible-near infrared (UV-vis-NIR) absorption spectroscopy using a Cary 5000 spectrophotometer (Agilent, Inc.). Spectra were baseline corrected with respect to the spectrum of the appropriate broth or broth component.

TEM Analysis of AgNPs for Size Determination

An aliquot from each AgNP solution (the “before broth incubation” and “after broth incubation” samples that had been washed 4 times with H_2O) was diluted with H_2O prior to drop casting onto a Formvar-coated copper transmission electron microscopy (TEM) grid (Ted Pella, Inc.). Samples were allowed to slowly air dry and then were dried under vacuum overnight before characterization with a Hitachi H-9500 environmental TEM at 300 kV (NanoScale Fabrication and Characterization Facility, Petersen Institute of NanoScience and Engineering, Pittsburgh, PA). NP size distributions were determined from TEM images of at least 300 NPs from various areas of the grid. ImageJ 1.47d (National Institutes of Health, USA) was used to measure and count all particles.

DLS Analysis of AgNPs for Size Determination

An aliquot of each AgNP solution was placed in a disposable plastic cuvette (Fisherbrand™). Dynamic light scattering (DLS) was used to measure the hydrodynamic diameter and zeta potential (at 25 °C and pH 5.2) of AgNPs (Malvern Zetasizer ZS90). The average hydrodynamic diameters reported here are the Z average size ± the standard deviation obtained from the polydispersity index (PDI) of three runs.

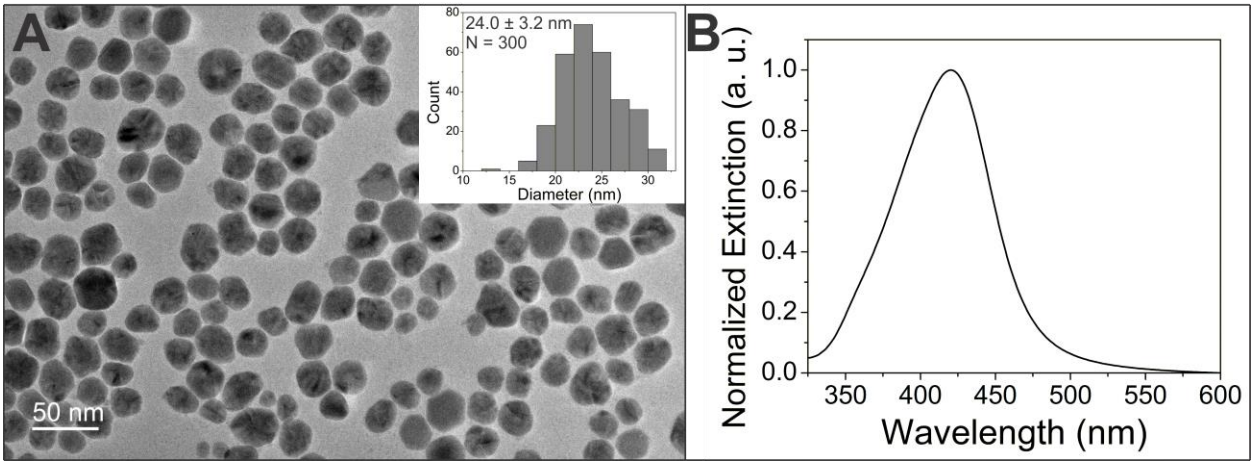

Figure S1. Representative TEM image of AgNPs functionalized with PEGSH, prior to incubation in the various broth components (A), along with the corresponding absorbance spectrum (B). Inset is the diameter distribution histogram generated from sizing at least 300 NPs from this sample.

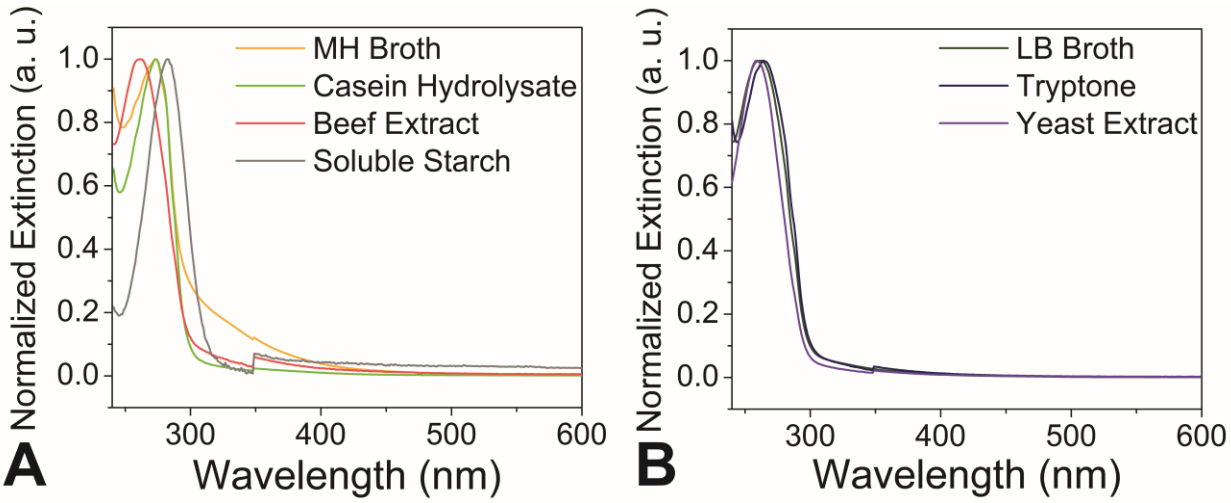

Figure S2. Normalized extinction spectra of MH broth and its components (A) and LB broth and its components (B), with no AgNPs added. The λ_{max} are as follows: MH broth = 273 nm, casein hydrolysate = 273 nm, beef extract = 261 nm, soluble starch = 282 nm, LB broth = 261 nm, tryptone = 264 nm, and yeast extract = 259 nm. In subsequent experiments involving AgNPs, we background correct for each broth component.

Ag(I) Ion Release Units: % Ag(I) Released

We report the amount of Ag(I) ion release as % Ag(I) ion released, which was calculated as shown below. We divided the moles of Ag(I) in the supernatant (measured by ICP-MS after separation of the AgNPs by centrifugation) by the total Ag atom content (in moles) in the entire solution (before centrifugation). To determine the total Ag atom content, we first measured the absorption spectrum of the solution and found the λ_{max}. Then, using a calibration curve (Figure S3) that plots extinction at λ_{max} vs. the concentration of Ag atoms as measured by ICP-MS, we determined the concentration of Ag in the original solution.

$$\%\ Ag^{+}\ released = \frac{mol\ Ag^{+}\ released\ (determined\ by\ ICPMS)}{mol\ Ag\ initial\ (determined\ by\ calibration\ curve)} * 100$$

ICP-MS: $mol\ Ag^{+}\ released = \frac{\mu g\ Ag^{+}}{L} * \frac{1\ mol}{107x10^{6}\ \mu g} * 0.001\ \mathrm{L}$

Calibration curve: $mol\ Ag\ initial = \frac{extinction\ at\ \lambda_{max}}{1.50x10^{-4}} * \frac{1\ mol}{107x10^{6}\ \mu g} * 0.001\ L$

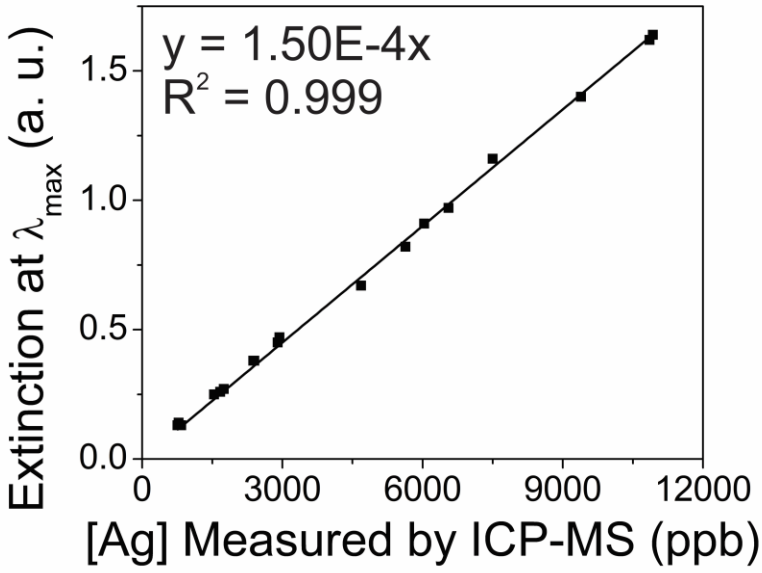

Figure S3. Calibration curve for the determination of Ag atom concentration by UV-vis absorption spectroscopy. Extinction at λ_{max} is plotted vs. [Ag] measured by ICP-MS to determine the Ag concentration in the original AgNP solutions used for the Ag(I) ion release experiments.

NMR Analysis

A typical ^{1}H NMR spectrum of PEGSH is shown in Figure S4. The peaks integrated for quantification of PEGSH ligands are the PEGSH peak labeled “A” and the acetonitrile (ACN) peak at 1.99 ppm. Standard PEGSH solutions of known concentrations (0.01 – 1.00 mM, in D_2O), each with a small amount of ACN added (5 µL of 0.24% v/v solution) were prepared. A calibration curve (Figure S5) was generated by plotting the ratio of the integrated PEGSH:ACN peaks vs. PEGSH concentration. The slope of the line was then used for PEGSH quantification in the unknown samples.

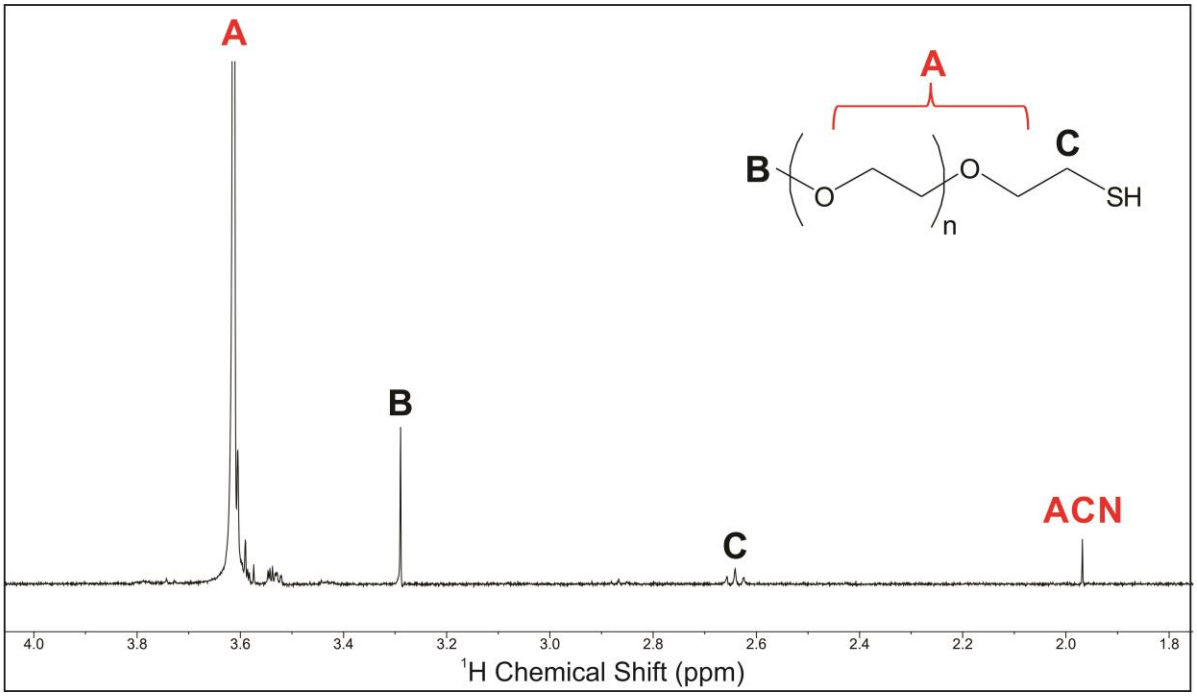

Figure S4. Representative ^{1}H NMR spectrum of PEGSH-exchanged AgNPs in D_2O following particle digestion in nitric acid, with its labeled structure corresponding to plotted ^{1}H NMR peak locations. For the calculation of PEGSH concentration, peak A was integrated and compared to the integrated intensity of the ACN peak at 1.99 ppm.

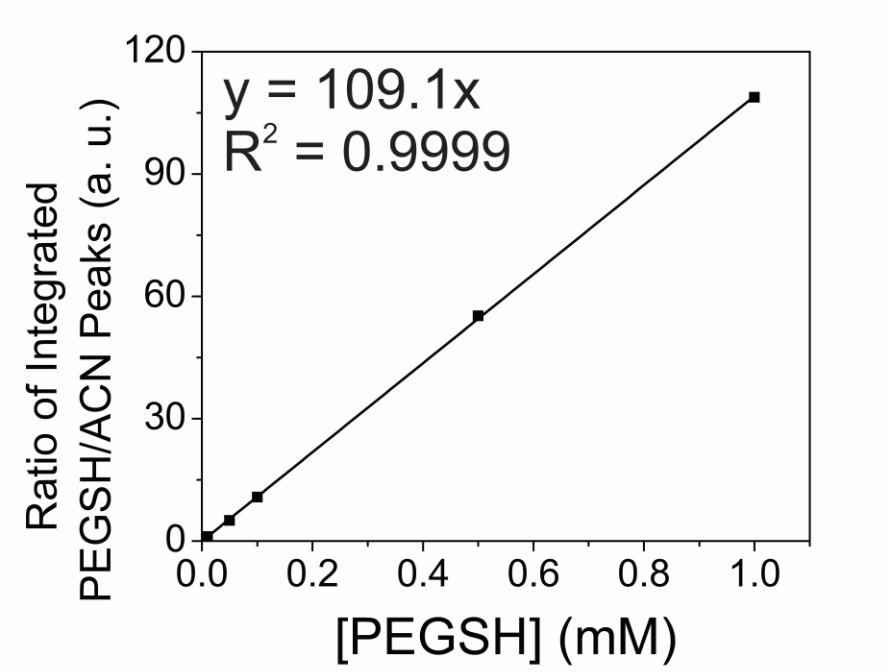

Figure S5. Sample ^{1}H NMR calibration curve obtained for PEGSH by plotting the integrated ratios of PEGSH/ACN vs. the concentration of PEGSH. The equation of the best fit line and R^2 value are displayed.

Additional Information Concerning the Broth Components

Casein hydrolysate: This broth component is the acid digest of casein. The majority of chemical species in this reagent are amino acids, but peptide species are also reported. We attempted to extract additional information about the chemical compositions of this reagent (Figure S4) and found that indeed there are peaks at low molecular weights, consistent with both amino acids and short peptides. However, the sequencing of those peptides and definitive quantification of the amino acid populations were beyond the scope of this report.

Beef extract: This broth component is derived from beef that has been steeped in water (termed, “infusion of beef”) and contains a mixture of peptides, amino acids, oligonucleotides, minerals, and vitamins. The relative proportions of each of these species in the beef extract are not explicitly defined by the manufacturer, however about 80% of the species that make up this beef extract are reported to have molecular weights either less than 250 Da or between 500-2000 Da. We attempted to extract additional information about the chemical compositions of these species (Figure S5), and indeed found peptides consistent with these molecular weight ranges. However, further sequencing of the peptides, and fractionation of the other chemical species present in this component were beyond the scope of the current report.

Soluble starch: The broth component listed as “soluble starch” is a polysaccharide that contains glucose units in two forms: amylose (linear) and amylopectin (branched). Additional details such as molecular weight distributions and synthesis route were not discernible from information provided by the manufacturer.

Tryptone: This broth component is the incomplete pancreatic digest of casein that contains mostly peptides and a few amino acids. About 75% of the species that make up tryptone are reported to have molecular weights either less than 250 Da or between 500-2000 Da.

Yeast extract: This broth component is derived from the autolysis of *Saccharomyces cerevisiae* cells and preserves the naturally occurring B-complex vitamins. It contains peptides, amino acids, carbohydrates, and vitamins as reported by the manufacturer.

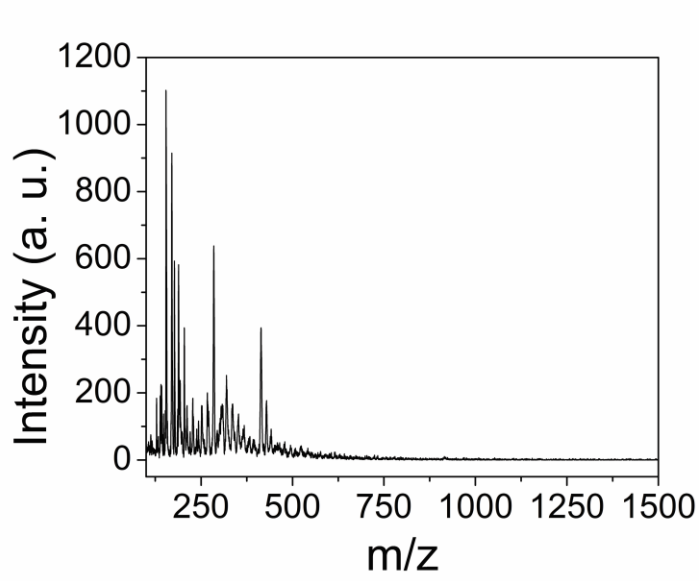

Figure S6. MALDI mass spectrum of casein hydrolysate. The peaks appear at low m/z values, which is consistent with amino acids and short peptides about 2-4 units long.

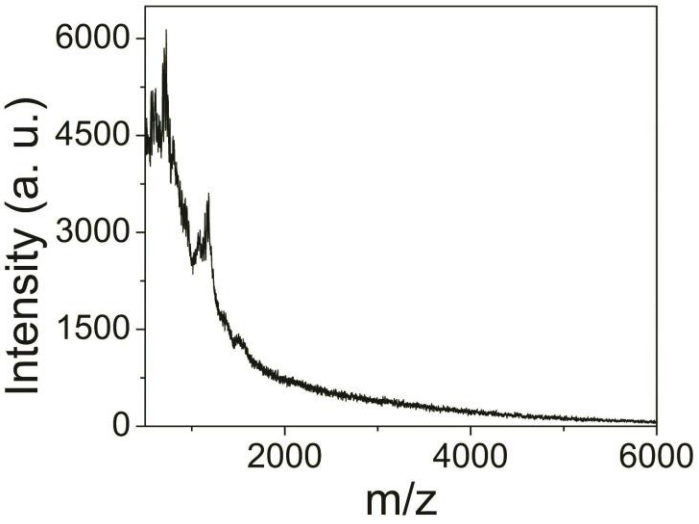

Figure S7. MALDI mass spectrum of beef extract. The peaks appear at low m/z values, which is consistent with peptides between about 5-10 units long.

Table S1. Results of *t* tests[1] for Ag(I) ion release at t = 16 hrs, in the presence of various concentrations of NaCl (0 – 15 mM). The data analyzed here is found in Figure 1. In all cases, the degrees of freedom is equal to 8, and the t_{table} value at the 95% confidence level is 2.306. Green shading indicates that the t_{calc} value is less than t_{table}, and thus the two ion release values are statistically the same. Red shading indicates that the t_{calc} value is greater than t_{table} and thus the two ion release values are statistically different.

	0 mM NaCl t = 16 hrs	**2.5 mM NaCl t = 16 hrs**	**5 mM NaCl t = 16 hrs**	**10 mM NaCl t = 16 hrs**	**15 mM NaCl t = 16 hrs**
0 mM NaCl t = 16 hrs		t_{calc} = 1.45	t_{calc} = 1.53	t_{calc} = 5.57	t_{calc} = 6.56
2.5 mM NaCl t = 16 hrs			t_{calc} = 0.67	t_{calc} = 3.10	t_{calc} = 5.56
5 mM NaCl t = 16 hrs				t_{calc} = 1.28	t_{calc} =4.20
10 mM NaCl t = 16 hrs					t_{calc} = 3.98
15 mM NaCl t = 16 hrs					

Table S2. Results of *t* tests[1] for Ag(I) ion release at t = 24 hrs, in the presence of various concentrations of NaCl (0 – 15 mM). The data analyzed here is found in Figure 1. In all cases, the degrees of freedom is equal to 8, and the t_{table} value at the 95% confidence level is 2.306. Green shading indicates that the t_{calc} value is less than t_{table}, and thus the two ion release values are statistically the same. Red shading indicates that the t_{calc} value is greater than t_{table} and thus the two ion release values are statistically different.

	0 mM NaCl t = 24 hrs	**2.5 mM NaCl t = 24 hrs**	**5 mM NaCl t = 24 hrs**	**10 mM NaCl t = 24 hrs**	**15 mM NaCl t = 24 hrs**
0 mM NaCl t = 24 hrs		t_{calc}=2.56	t_{calc}=2.35	t_{calc}=4.29	t_{calc}=6.56
2.5 mM NaCl t = 24 hrs			t_{calc}=0.95	t_{calc}=1.23	t_{calc}=4.43
5 mM NaCl t = 24 hrs				t_{calc}=0.22	t_{calc}=2.32
10 mM NaCl t = 24 hrs					t_{calc}=3.58
15 mM NaCl t = 24 hrs					

Table S3. Ag speciation in MH broth and LB broth conditions, simulated by Visual MINTEQ (Version 3.1, Jon Petter Gustafsson, KTH, Stockholm, Sweden). The inputs used were: 2.8 μM Ag^+ (approximately equal to the concentration of surface Ag present on the 25 nm AgNPs, which have about 5% of their total atoms on the surface), 10 or 15 mM Cl^- (equal to the concentration of Cl^- present in LB broth or MH broth, respectively), at a temperature of 37 °C at pH 7.

Species	% of Species Present in MH Broth	% of Species Present in LB Broth
Ag^+	1.4	2.0
$AgCl_{(aq)}$	29.7	29.8
$AgCl_2^-$	36.4	24.4
$AgCl_3^{2-}$	0.4	0.2
$AgCl_{(s)}$	32.0	43.6

Powder X-ray Diffraction Analysis

Aliquots of PEGSH-AgNPs were diluted in MH broth or LB broth to a concentration of 1 OD and incubated at 37 °C and 150 rpm for 24 hours. After this incubation time, the AgNPs were washed four times. Samples for powder X-ray diffraction (PXRD) were prepared by drop casting aliquots of this purified AgNP solution on a glass slide (Fisher Scientific). The samples were characterized by PXRD using a Bruker AXS D8 Discover XRD (NanoScale Fabrication and Characterization Facility, Petersen Institute of NanoScience and Engineering, Pittsburgh, PA) at 40 kV, 40 mA for Cu Kα (λ = 1.5406 Å) X-ray source with a scan speed of 3.0 s/step from 10.00-90.00° with a step size of 0.02°.

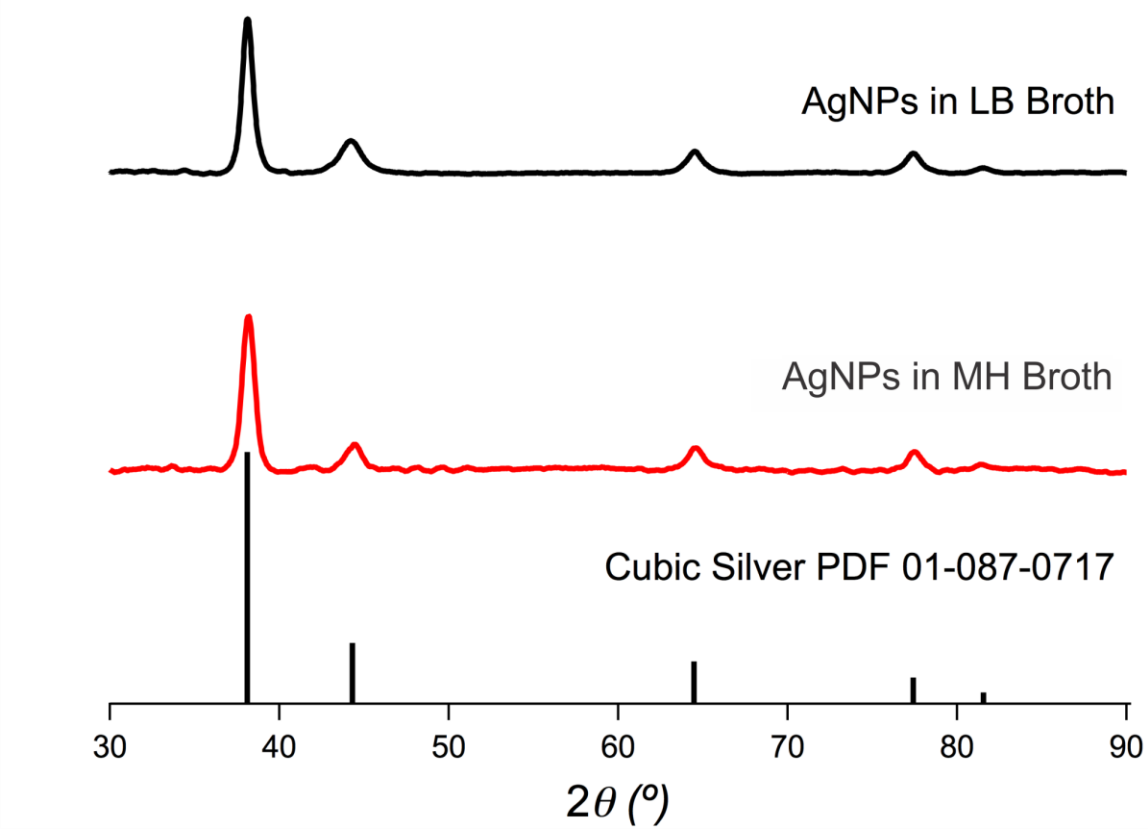

Figure S8. PXRD spectra of AgNPs incubated for 24 hours in either MH broth or LB broth. The spectra do not indicate formation of discrete AgCl NPs.

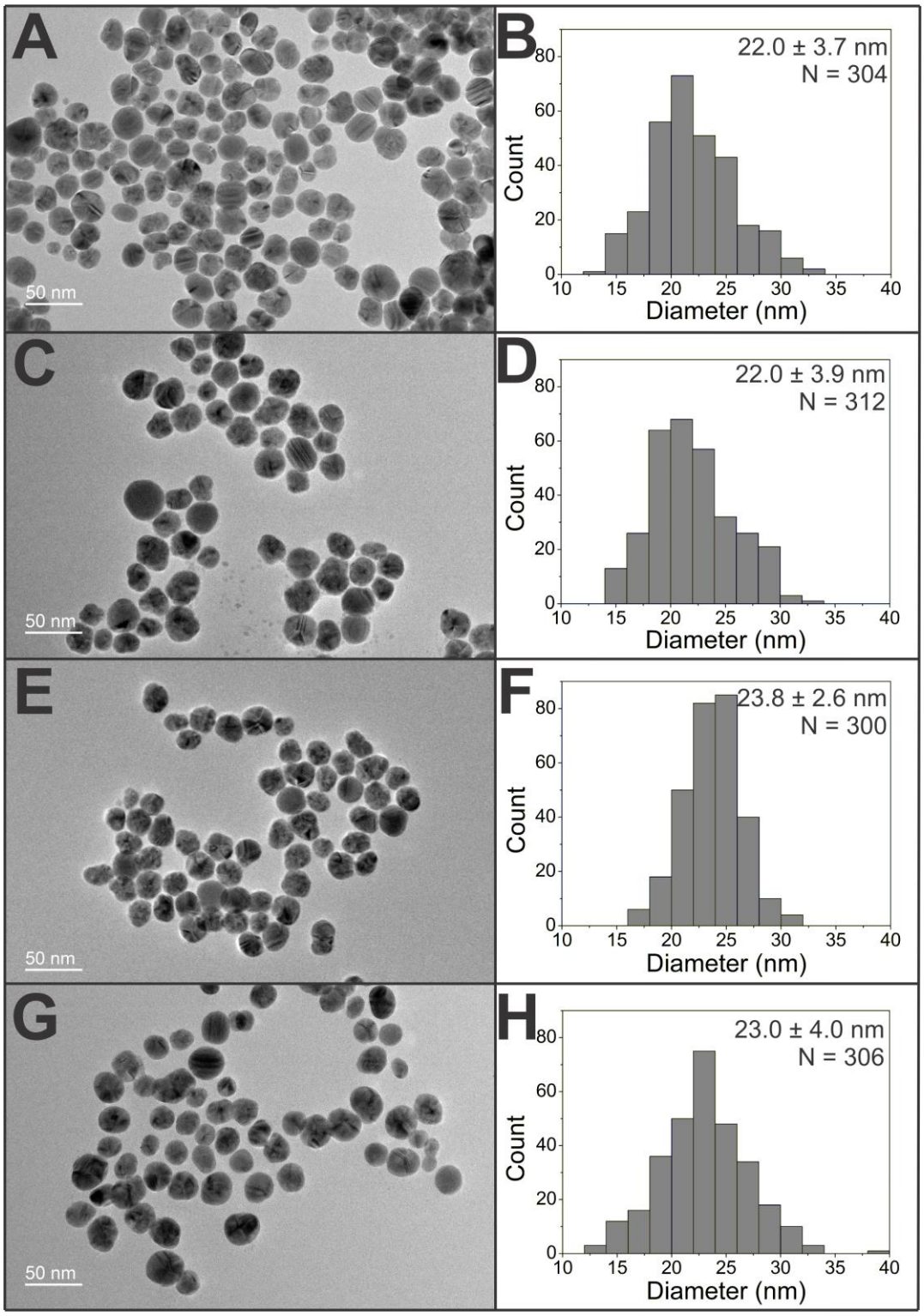

Figure S9. Representative TEM images of AgNPs after 24 hr incubation in MH broth (A), casein hydrolysate (C), beef extract (E), and soluble starch (G). Panels B, D, F, H are the corresponding diameter distribution histograms generated from sizing at least 300 NPs.

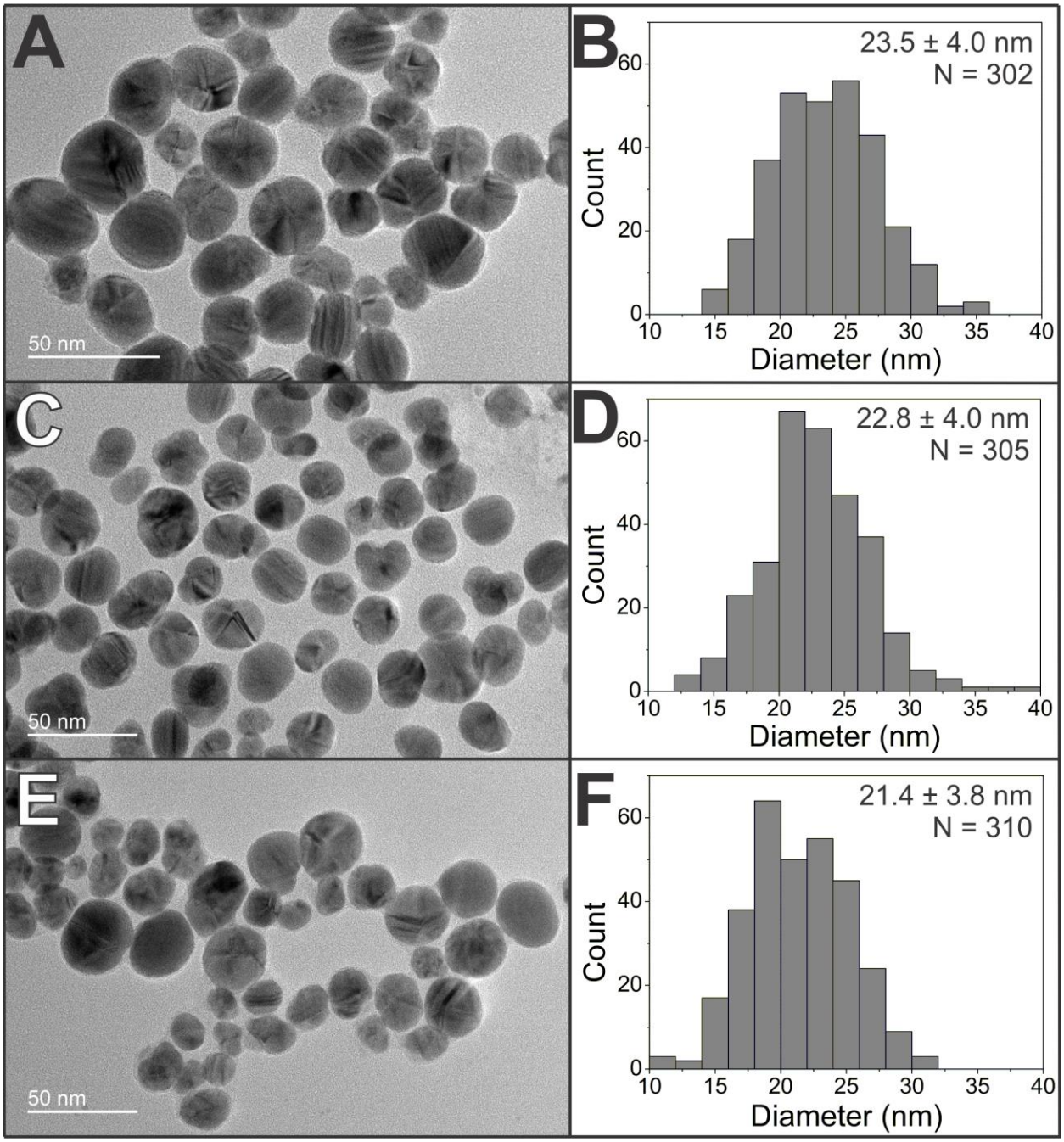

Figure S10. Representative TEM images of AgNPs after 24 hr incubation in LB broth (A), tryptone (C), and yeast extract (E). Parts B, D, F are the corresponding diameter distribution histograms generated from sizing at least 300 particles.

Table S4. Comparison of PEGSH-AgNP diameters before and after incubation in broths or broth components as determined by TEM (sizing at least 300 particles) and DLS.

Sample[a]	Diameter by TEM (nm)	Diameter by DLS (nm)
PEGSH-AgNPs	24.0 ± 3.2	41.5 ± 18.7
MH broth	22.0 ± 3.7	34.6 ± 16.0
Casein hydrolysate	22.0 ± 3.9	33.8 ± 14.2
Beef extract	23.8 ± 2.6	38.4 ± 16.1
Soluble starch	23.0 ± 4.0	39.3 ± 15.2
LB broth	23.5 ± 4.0	39.7 ± 18.3
Tryptone	22.8 ± 4.0	39.3 ± 16.9
Yeast extract	21.4 ± 3.8	43.1 ± 19.1

[a] PEGSH-AgNP diameter is reported immediately after washing steps for the PEGSH ligand exchange ("before broth incubation" samples). All other AgNP diameters are reported after 24 hr incubation in that broth or broth component ("after broth incubation" samples).

X-ray Photoelectron Spectroscopy Analysis

XPS spectra were obtained using an ESCALAB 250XI XPS with a monochromated, microfocused Al Kα X-ray source (spot size = 200 μm). Survey and high-resolution spectra were collected with a pass energy of 150 and 50 eV and a step size of 1.0 eV and 0.1 eV, respectively. Spectra were charge referenced to adventitious carbon (284.8 eV). NPs were drop-cast onto silicon wafers (University Wafer, Boston, MA) that had been cleaned for ultra-high vacuum analysis.

Table S5. Summary of XPS Data

Sample	$Ag3d_{5/2}$ (eV)	$Ag3d_{3/2}$ (eV)	$Cl2p_{3/2}$ (eV)	$Cl2p_{1/2}$ (eV)
NaCl standard	N/A	N/A	199.0	200.4
Ag standard	368.4	374.4	N/A	N/A
AgNPs before incubation	368.1	374.1	N/A	N/A
AgNPs after incubation in H_2O	368.0	374.0	N/A	N/A
AgCl standard	367.4	373.4	197.7	199.4
AgNPs after incubation in MH broth	367.5	373.5	197.6	199.3
AgNPs after incubation in LB broth	367.5	373.5	197.6	199.3

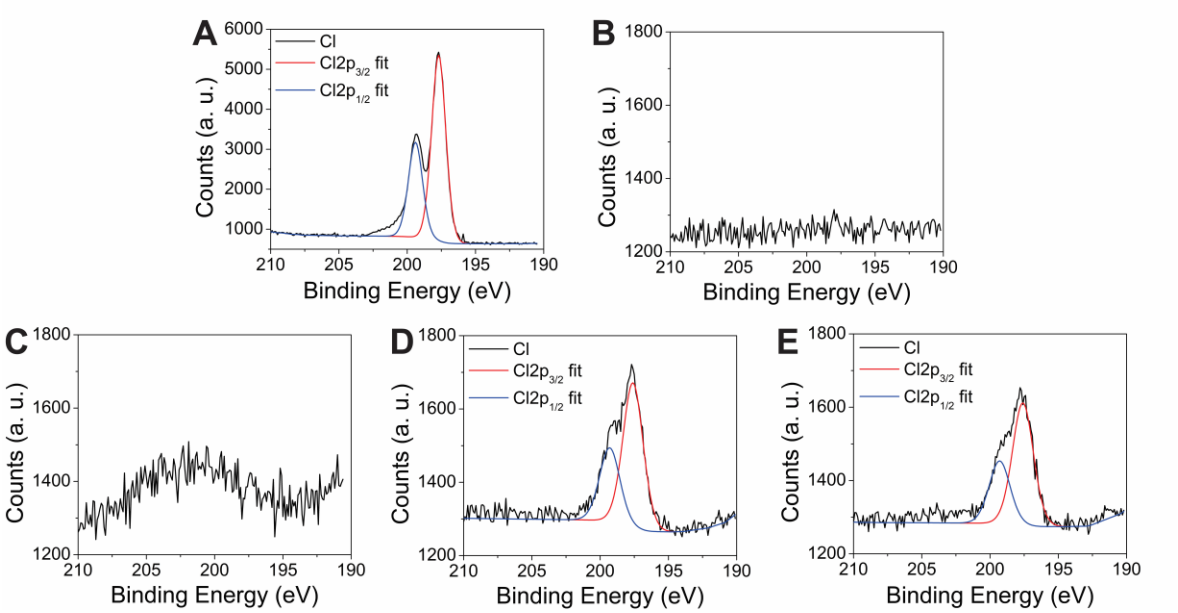

Figure S11. High-resolution Cl 2p XPS spectra for a AgCl standard (A) and PEGSH-AgNPs before broth incubation (B), as well as after incubation in water (C), MH broth (D), or LB broth (E). The spectra indicate that some AgCl may have formed in the MH and LB broth samples based on the binding energy of the Cl 2p peaks.

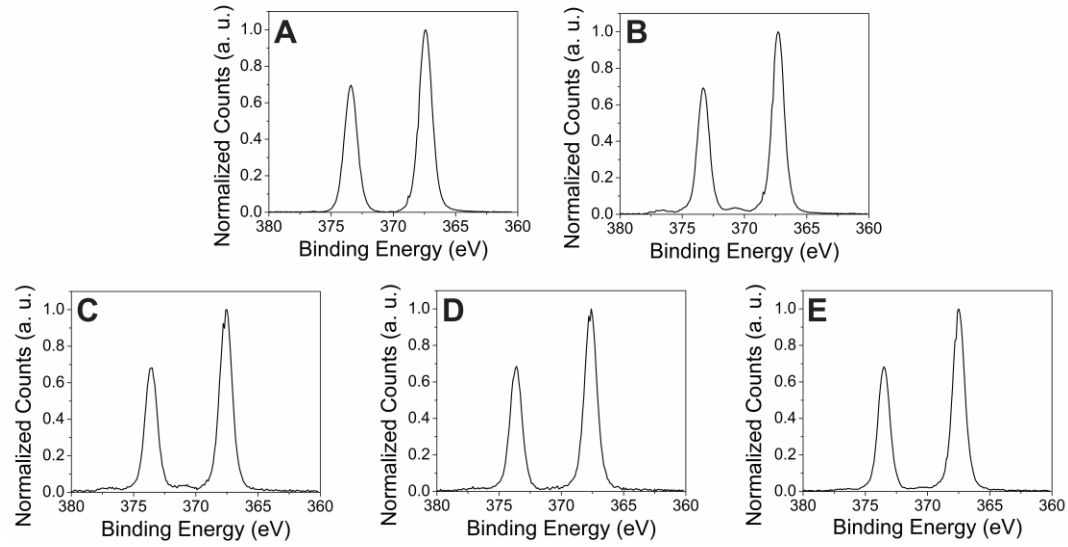

Figure S12. High-resolution Ag 3d XPS spectra for a AgCl standard (A) and PEGSH-AgNPs before broth incubation (B), as well as after incubation in water (C), MH broth (D), and LB broth (E). Loss features (shoulders on the higher energy sides of the main peaks which indicate the presence of metallic Ag) are present for all spectra except for the AgCl standard. However, the binding energies of the peaks indicate that some AgCl also formed.

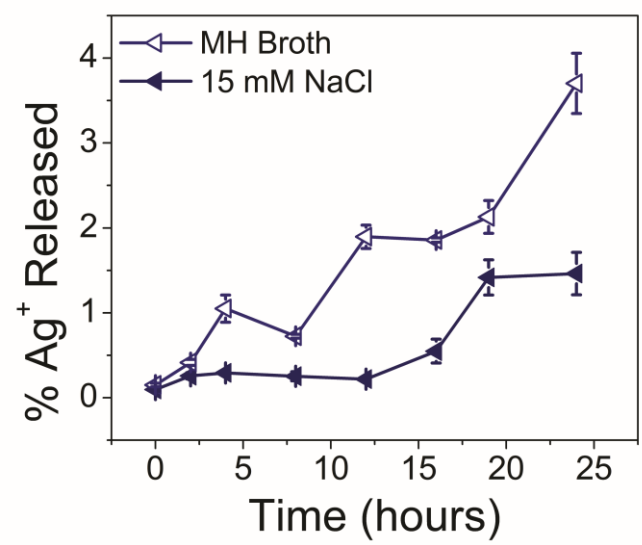

Figure S13. Percent Ag(I) ions released vs. time for AgNPs dispersed in MH broth at 25 °C. The percentage of Ag(I) ions released after incubation of PEGSH-AgNPs in the corresponding concentration of NaCl alone is plotted for comparison. Error bars represent the standard error of at least 5 independent trials.

Table S6. Results of *t* tests[1] for Ag(I) ion release in either MH broth or LB broth, compared to the corresponding concentrations of NaCl. The data analyzed here is found in Figure 2. DF denotes degrees of freedom. Red shading indicates that the t_{calc} value is greater than t_{table} and thus the two ion release values are statistically different.

	0 hr	**4 hr**	**8 hr**	**16 hr**	**24 hr**
MH broth vs. 15 mM NaCl	t_{calc} = 8.46, DF = 15	t_{calc} = 3.99, DF = 18	t_{calc} = 6.20, DF = 17	t_{calc} = 4.78, DF = 10	t_{calc} = 2.27, DF = 12
LB broth vs. 10 mM NaCl	t_{calc} = 2.82, DF = 23	t_{calc} = 3.11, DF = 11	t_{calc} = 48.13, DF = 8	t_{calc} = 16.34, DF = 8	t_{calc} = 2.31, DF = 11

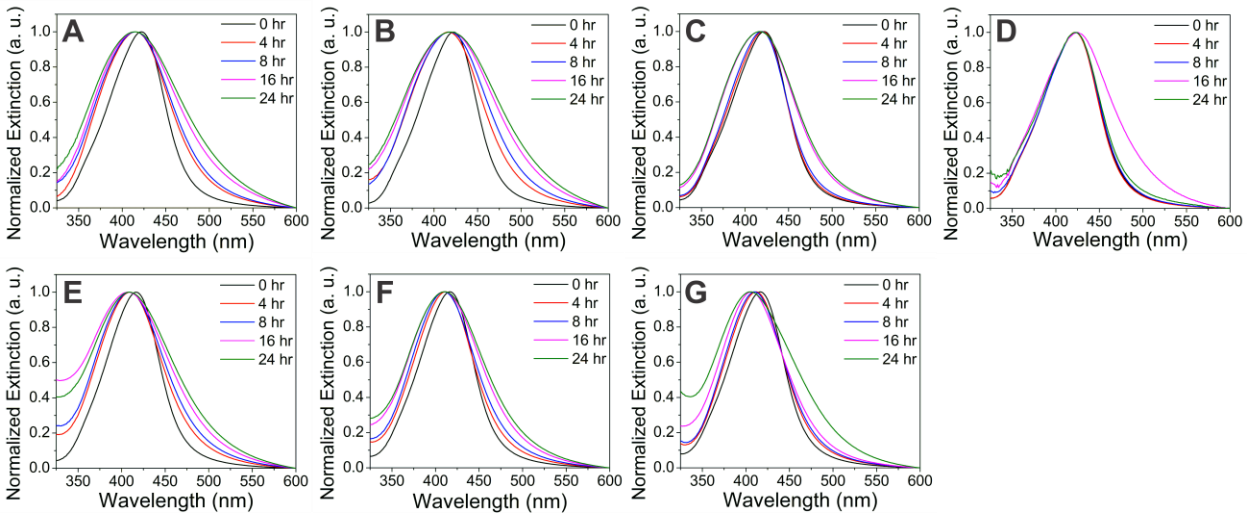

Figure S14. Extinction spectra of AgNPs dispersed in MH broth (A), casein hydrolysate (B), beef extract (C), soluble starch (D), LB broth (E), tryptone (F), or yeast extract (G).

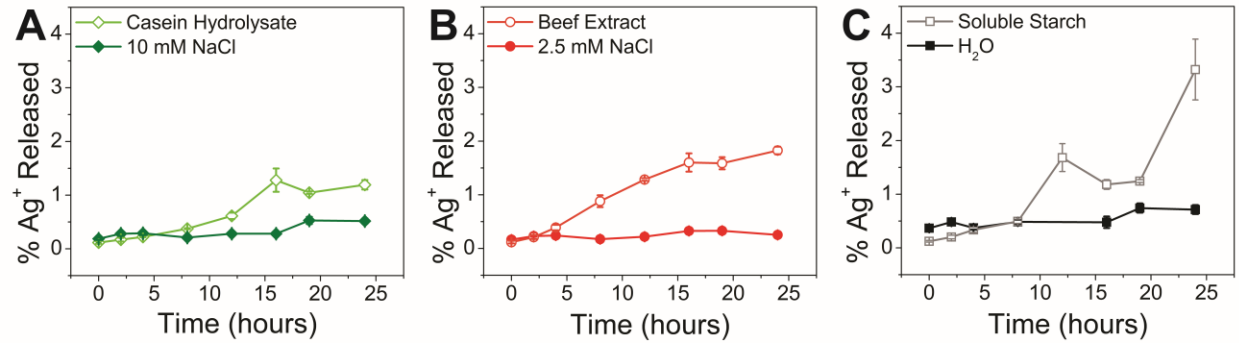

Figure S15. Percent Ag(I) ions released vs. time for AgNPs dispersed in broth components, including casein hydrolysate (A), beef extract (B), and soluble starch (C). In all graphs, the percentage of Ag(I) ions released after incubation of PEGSH-AgNPs in the corresponding concentrations of NaCl alone (or pure water, where NaCl is not present in the component) is plotted for comparison. Error bars represent the standard error of at least five independent trials. All data was collected at 25 °C.

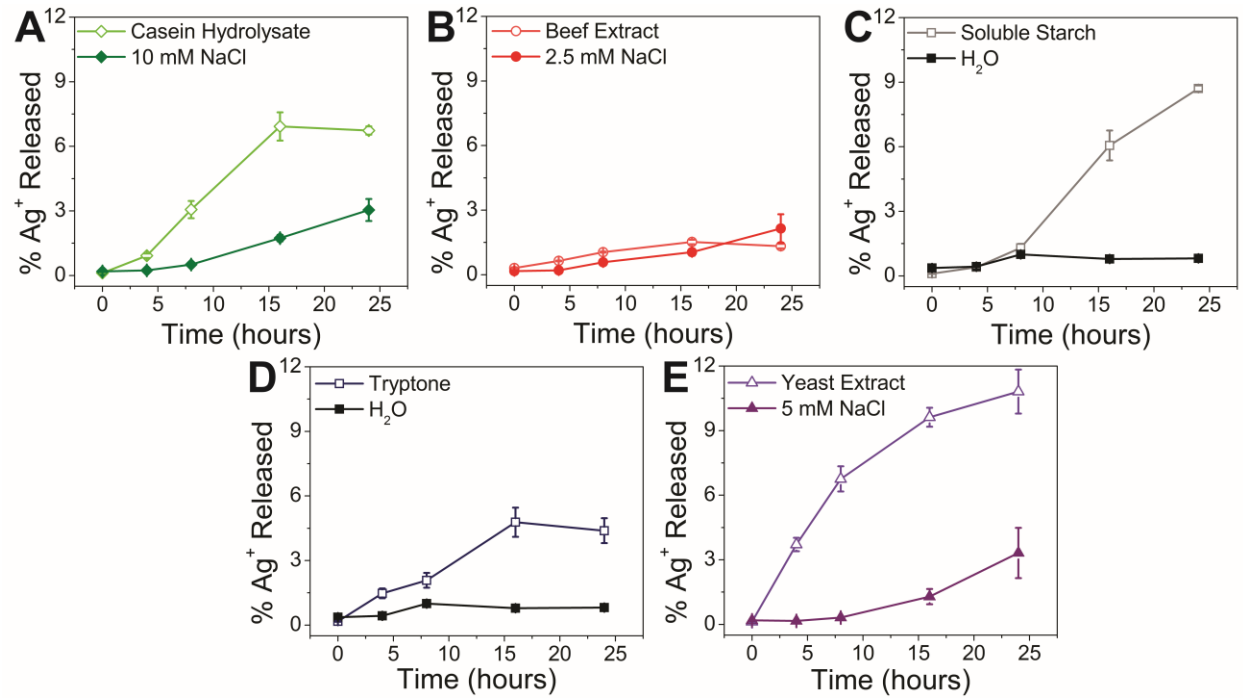

Figure S16. Percent Ag(I) ions released vs. time for AgNPs dispersed in broth components, including casein hydrolysate (A), beef extract (B), soluble starch (C), tryptone (D), and yeast extract (E). In all graphs, the percentage of Ag(I) ions released after incubation of PEGSH-AgNPs in the corresponding concentrations of NaCl alone (or pure water, where NaCl is not present in the component) is plotted for comparison. Error bars represent the standard error of at least five independent trials. All data was collected at 37 °C.

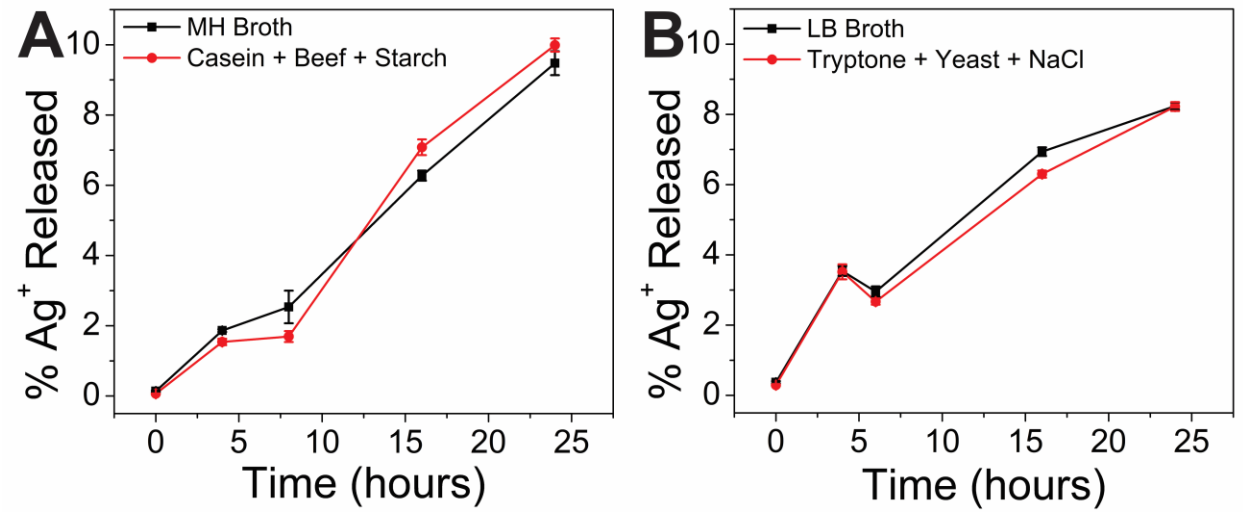

Figure S17. Comparison of Ag(I) ions released over time in the manufacturer's prepared broths (black lines) and the in-house prepared broths (mixture of individual components, red lines).

Table S7. Results of *t* tests[1] for Ag(I) ion release in either MH broth components or LB broth components, compared to the corresponding concentrations of NaCl. The data analyzed here are found in Figure 3 and Figure S16. DF denotes degrees of freedom. Green shading indicates that the t_{calc} value is less than t_{table}, and thus the two ion release values are statistically the same. Red shading indicates that the t_{calc} value is greater than t_{table} and thus the two ion release values are statistically different.

	0 hr	4 hr	8 hr	16 hr	24 hr
Cas. Hyd. vs. 10 mM NaCl	t_{calc} = 1.79, DF = 21	t_{calc} = 4.19, DF = 15	t_{calc} = 3.94, DF = 15	t_{calc} = 4.99, DF = 15	t_{calc} = 7.29, DF = 9
Beef extract vs. 2.5 mM NaCl	t_{calc} = 4.61, DF = 12	t_{calc} = 15.50, DF = 15	t_{calc} = 4.34, DF = 9	t_{calc} = 5.26, DF = 15	t_{calc} = 2.30, DF = 15
Soluble starch vs. H_2O	t_{calc} = 2.48, DF = 37	t_{calc} = 0.74, DF = 9	t_{calc} = 1.37, DF = 9	t_{calc} = 7.56, DF = 8	t_{calc} = 59.10, DF = 8
Tryptone vs. H_2O	t_{calc} = 1.63, DF = 36	t_{calc} = 4.60, DF = 8	t_{calc} = 2.97, DF = 8	t_{calc} = 5.87, DF = 8	t_{calc} = 6.07, DF =
Yeast extract vs. 5 mM NaCl	t_{calc} = 1.22, DF = 11	t_{calc} = 11.30, DF = 8	t_{calc} = 10.98, DF = 8	t_{calc} = 15.37, DF = 8	t_{calc} = 4.82, DF = 8

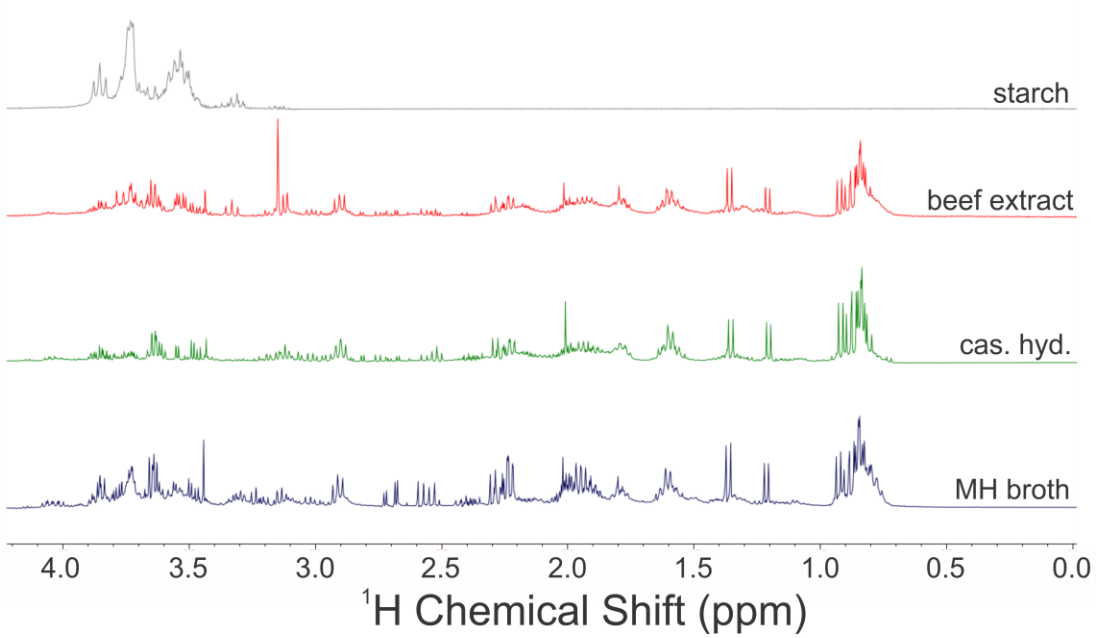

Figure S18. ^{1}H NMR spectra of MH broth and its components, with no AgNPs present.

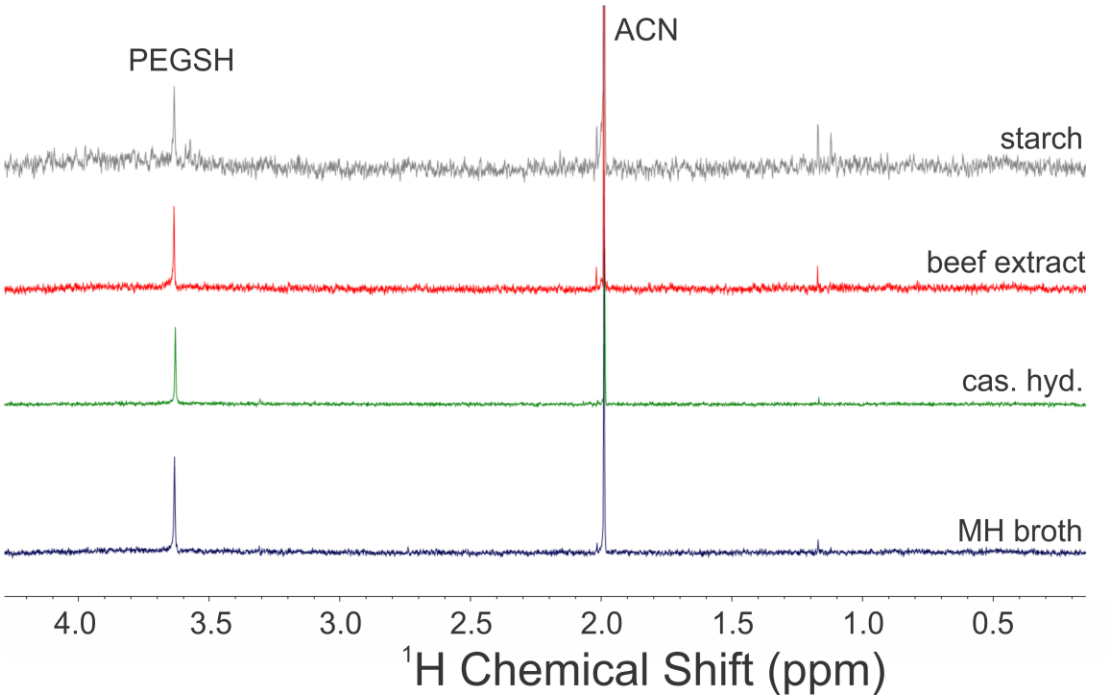

Figure S19. ^{1}H NMR spectra of purified, digested PEGSH-AgNPs after 24 hour incubation in MH broth or its components. Absence of peaks corresponding to broth components indicates that there is no significant component adsorption to the AgNPs.

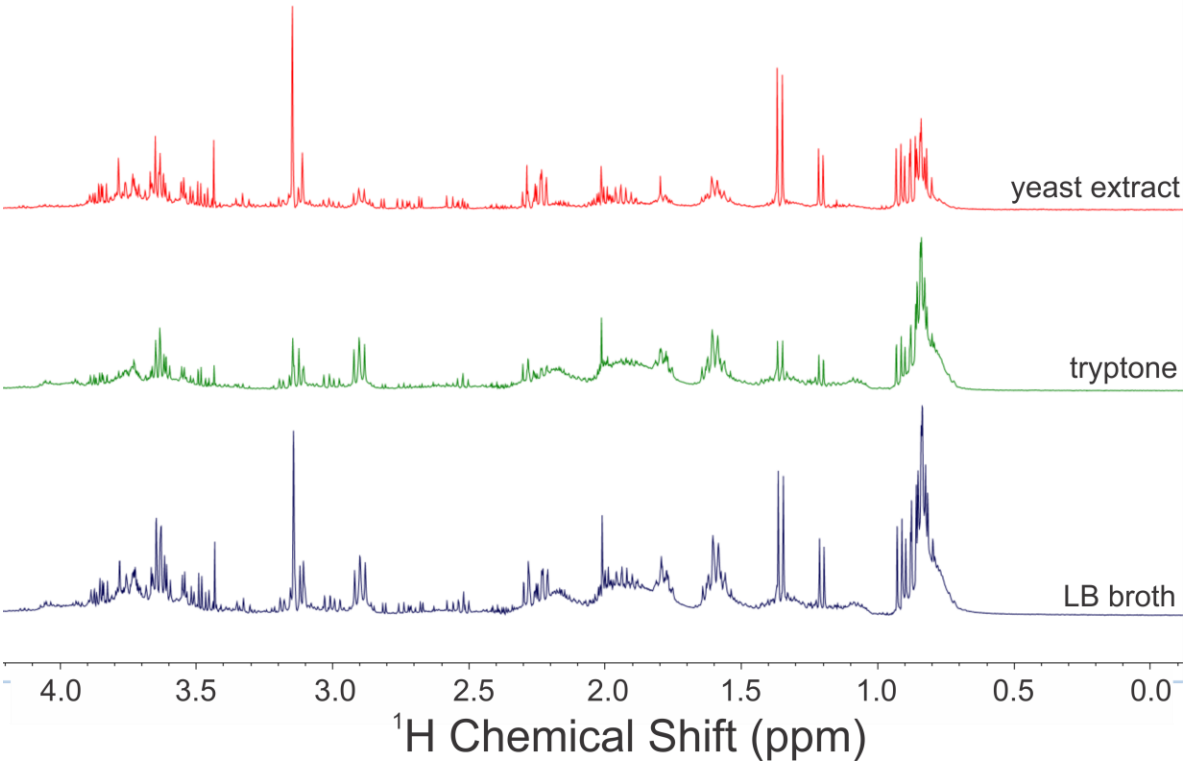

Figure S20. ^{1}H NMR spectra of LB broth and its components, with no AgNPs present.

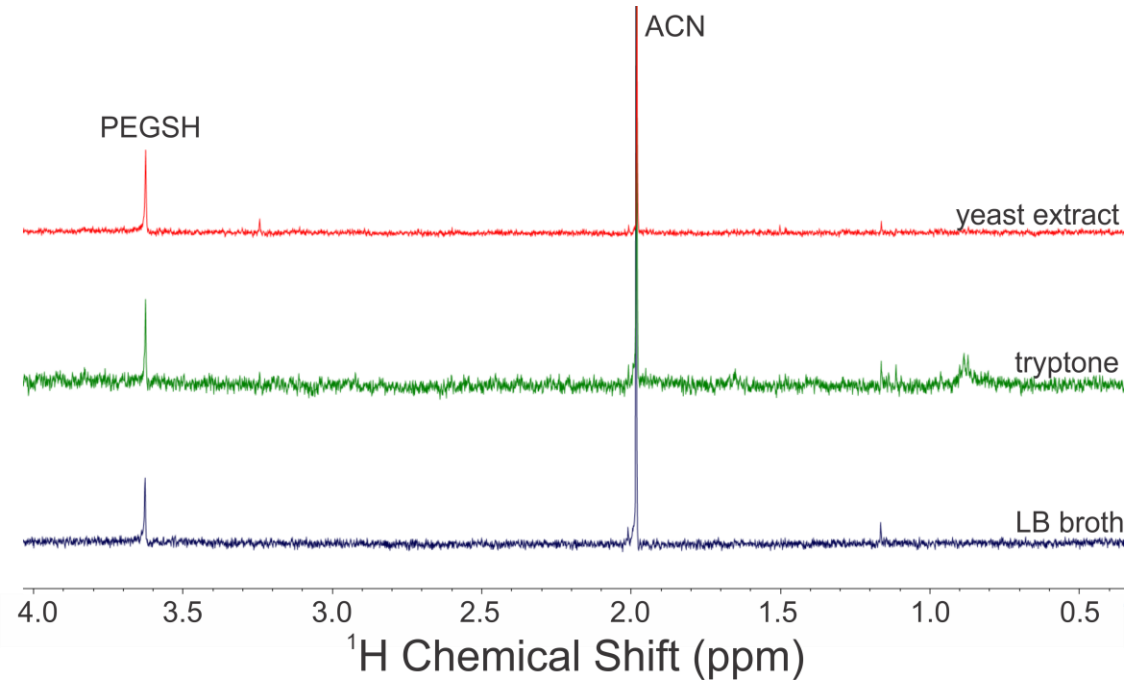

Figure S21. ^{1}H NMR spectra of purified, digested PEGSH-AgNPs after 24 hour incubation in LB broth or its components. Absence of peaks corresponding to broth components indicates that there is no significant component adsorption to the AgNPs.

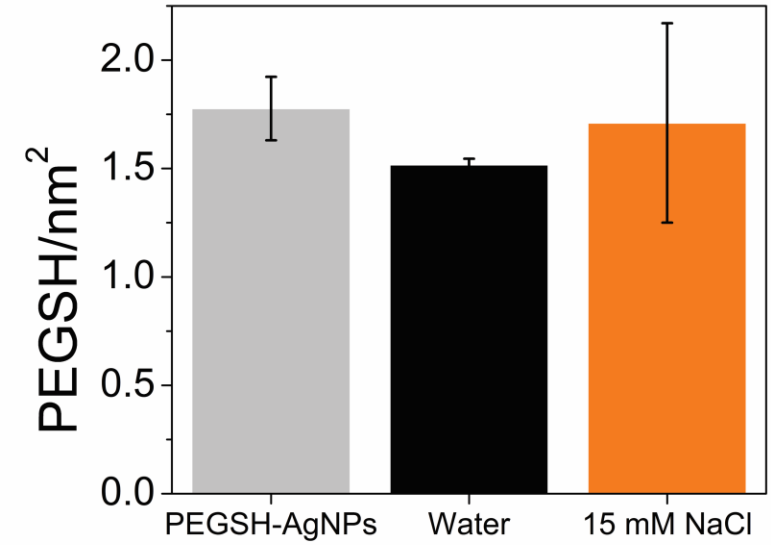

Figure S22. PEGSH density for the original PEGSH-AgNPs (gray bar) and after 24 hr incubation at 37 °C in water (black bar) and 15 mM NaCl (orange bar). There is no statistical difference between these PEGSH densities.

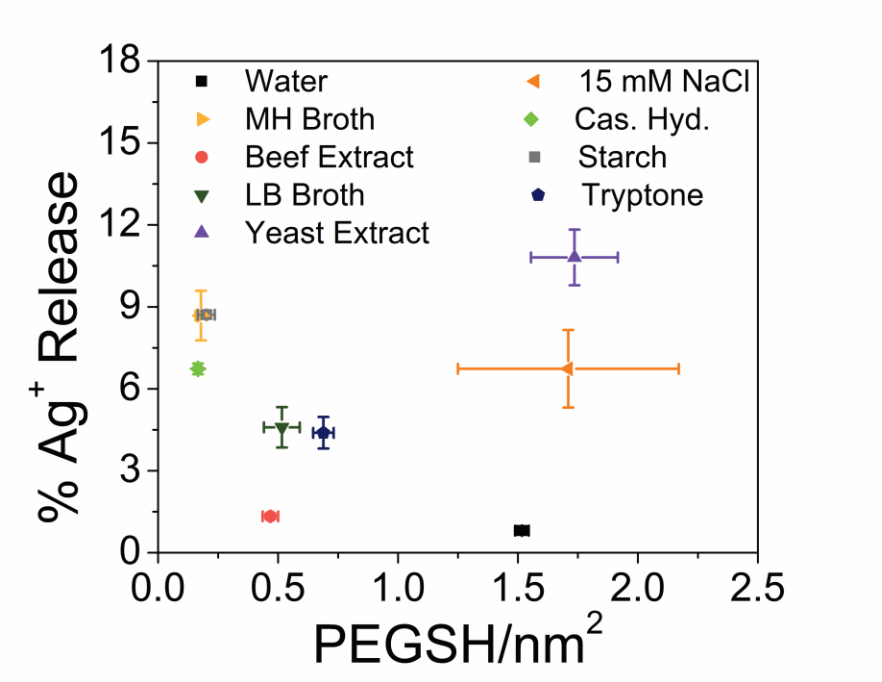

Figure S23. PEGSH/nm^2 vs. percent Ag(I) ion released for AgNPs incubated in the broths or broth components for 24 hours at 37 °C.

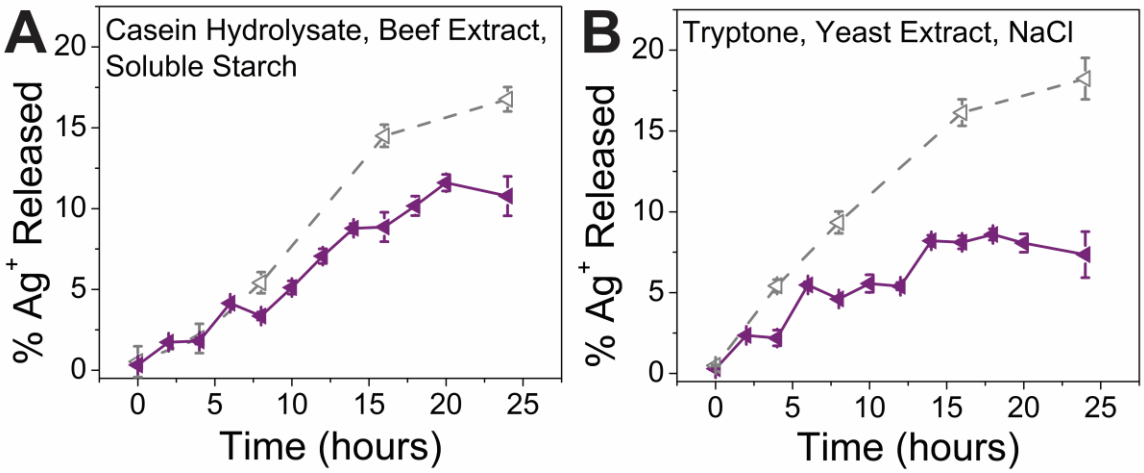

Figure S24. Percent Ag(I) ion released vs. time for the three MH broth components (A) and the three LB broth components (B). The purple solid line is data for experiments where the three broth components were together in the same solution (same data as Figure 2, replotted here). The gray dashed line is the sum of data for experiments where the three broth components were studied in separate experiments (same data as Figure 3, replotted here for comparison). All data was obtained at 37 °C.

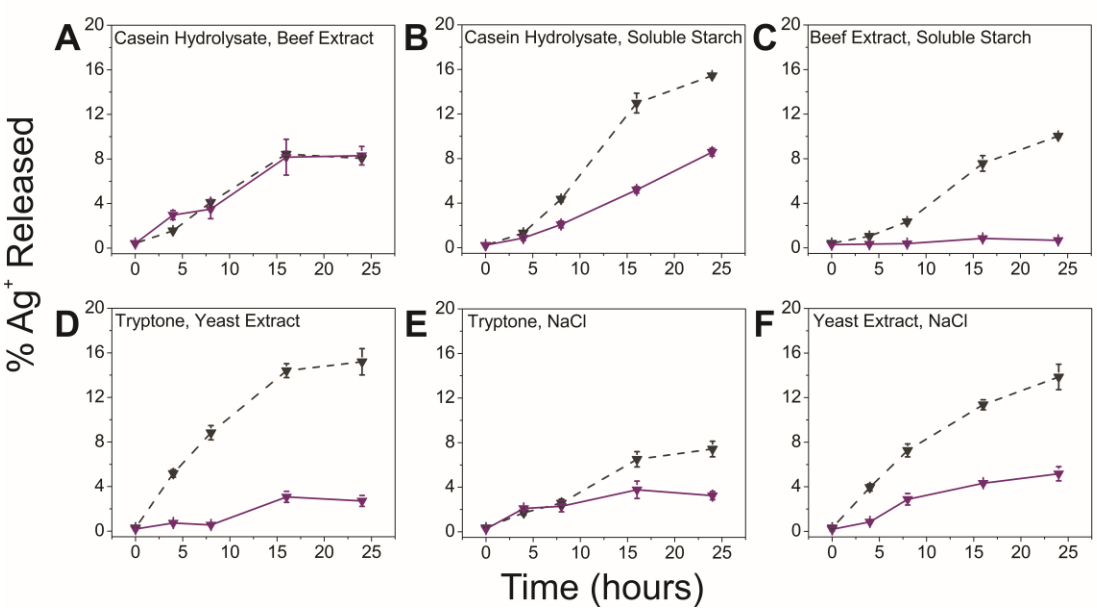

Figure S25. Percent Ag(I) ion released vs. time for combinations of two MH broth components (A-C) and combinations of two LB components (D-F). The purple solid line is the data for experiments where the two broth components were present together in the same solution. The gray dashed line is the sum of the data for experiments where the two broth components were studied in separate experiments. (*N. B.* gray dashed lines represent the sum of the same data as presented in Figure 3, shown here in order to facilitate comparisons between experiments. Error bars represent the standard error of at least 5 independent trials. All data was obtained at 37 °C.

Table S8. Results of *t* tests[1] for Ag(I) ion release in combinations of two broth components, either dispersed in the same solution or in separate solutions. The data analyzed here is found in Figure 5 and Figure S25. DF denotes degrees of freedom. Green shading indicates that the t_{calc} value is less than t_{table}, and thus the two ion release values are statistically the same. Red shading indicates that the t_{calc} value is greater than t_{table} and thus the two ion release values are statistically different.

	0 hr	4 hr	8 hr	16 hr	24 hr
Casein+Beef separate vs. together	t_{calc} = 0.03, DF = 15	t_{calc} = 7.90, DF = 27	t_{calc} = 1.34, DF = 21	t_{calc} = 0.33, DF = 27	t_{calc} = 0.48, DF = 21
Casein+Starch separate vs. together	t_{calc} = 0.59, DF = 15	t_{calc} = 3.48, DF = 21	t_{calc} = 4.50, DF = 21	t_{calc} = 4.68, DF = 20	t_{calc} = 22.75, DF = 14
Beef+Starch separate vs. together	t_{calc} = 3.08, DF = 15	t_{calc} = 10.41, DF = 20	t_{calc} = 6.77, DF = 15	t_{calc} = 5.12, DF = 20	t_{calc} = 37.54, DF = 20
Tryptone+Yeast separate vs. together	t_{calc} = 2.53, DF = 13	t_{calc} = 9.09, DF = 13	t_{calc} = 8.95, DF = 13	t_{calc} = 11.81, DF = 13	t_{calc} = 7.21, DF = 13
Tryptone+NaCl separate vs. together	t_{calc} = 3.28, DF = 13	t_{calc} = 1.14, DF = 13	t_{calc} = 0.48, DF = 13	t_{calc} = 2.45, DF = 13	t_{calc} = 4.02, DF = 13
Yeast+NaCl separate vs. together	t_{calc} = 1.11, DF = 13	t_{calc} = 6.84, DF = 13	t_{calc} = 4.79, DF = 13	t_{calc} = 10.81, DF = 13	t_{calc} = 5.09, DF = 13

Citrate-capped AuNP Synthesis and Ligand Exchange with PEGSH

AuNPs were synthesized using a similar procedure as Smith et al.[2] Here, the molar ratio of Au:citrate used was 1:2.2 for 25 nm AuNPs. Prior to use, AuNPs were filtered using a poly(vinylidene fluoride) (PVDF) filter membrane with a pore size of 0.45 µm (25 mm GD/XP disposable filters, Whatman, Inc.). Immediately after filtration, the citrate-capped AuNPs were concentrated by centrifuging 1.50 mL aliquots in 1.5 mL centrifuge tubes (40 total tubes) at 20,000 rcf for 6 min. The supernatants were removed, and the particles were resuspended in another 1.50 mL aliquot of AuNPs and centrifuged again. The supernatants were removed, and the particles were resuspended in 1.00 mL of H_2O. The particles were then washed by centrifuging once more. The supernatants were again removed, and the particles were resuspended in 1.00 mL of H_2O. Then, 1.1 µL of PEGSH (12.9 mM) was added to each tube. These mixtures were vortexed and then placed on a temperature-controlled mixer for 24 hr. Immediately after this mixing time, the particles were washed four times with H_2O. After the last wash cycle, the supernatant was removed to yield the concentrated pellet of AuNPs.

References

1. Harris, D. C. *Quantitative Chemical Analysis*; W. H. Freeman and Company: New York, 2007.
2. Smith, A. M.; Johnston, K. A.; Marbella, L. E.; Hartmann, M. J.; Crawford, S. E.; Kozycz, L. M.; Seferos, D. S.; Millstone, J. E. Quantitative Analysis of Thiolated Ligand Exchange on Gold Nanoparticles Monitored by ^{1}H NMR Spectroscopy. *Anal. Chem.* **2015**, *87*, 2771-2778.