# Supplementary Information

Metal Binding Peptides for Separation and Concentration in Hydrometallurgy

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# Preliminary Experiments

## Au Positive Control

Taking inspiration from Hnilova's unpublished work, a 1:1 ratio of $HAuCl_4$ and sodium citrate was selected as a starting point for establishing a positive control baseline response. Initial experiments with a positive (0.5mM $HAuCl_4$ : 0.5mM Sodium Citrate) and negative (0.5mM $HAuCl_4$ in DI water) control were conducted over a period of two and a half hours in an uncovered 96 well plate measuring the UV-Vis response from 400nm to 1000nm (figure S1).

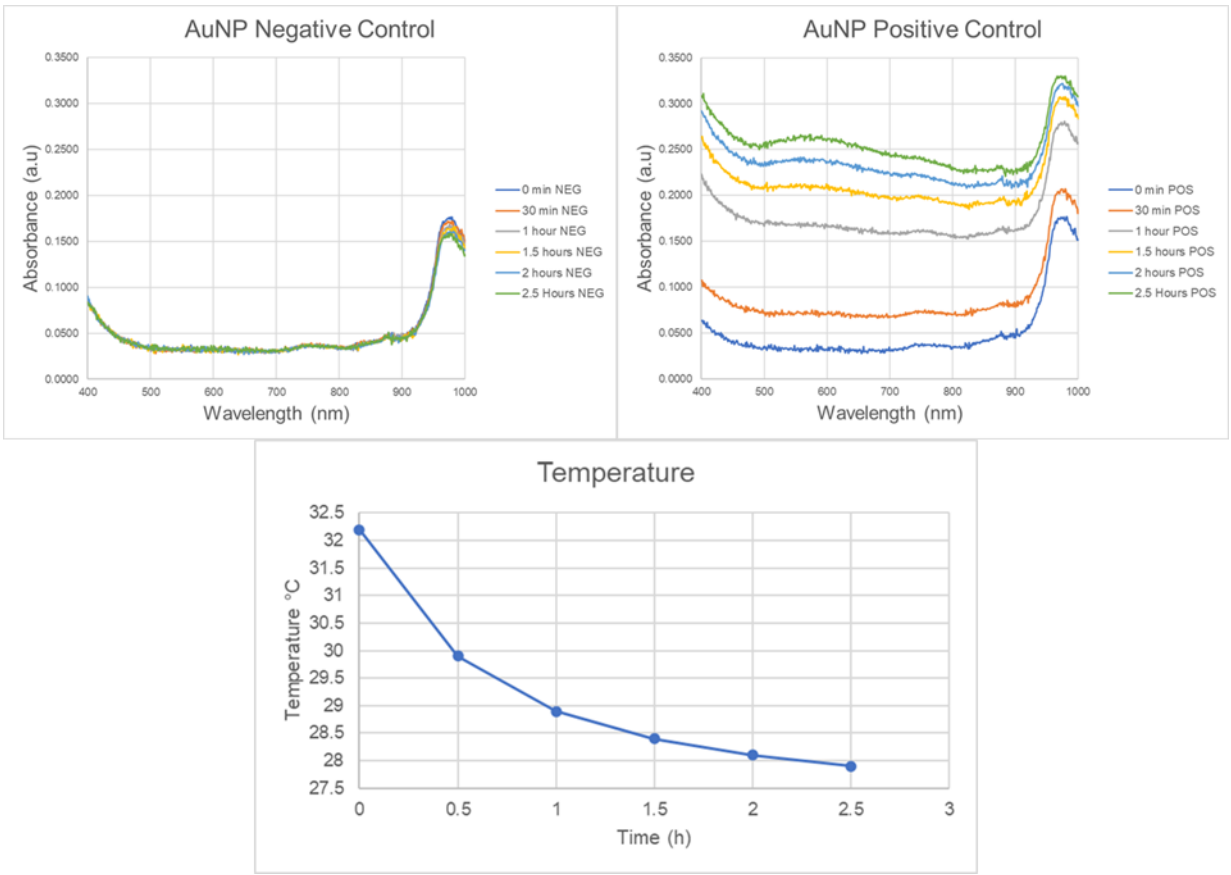


*Figure S1. Initial positive and negative control UV-Vis response.*

The positive control showed broad spectrum increases in absorption with a small peak beginning to form between 500nm and 600nm after one hour. As expected, the negative control showed little change during the measurement. Temperature data logged at each measurement indicated a decrease in temperature from

32°C to 28°C likely due to insufficient time provided for the instrument to reach room temperature following an unrelated experiment at an elevated temperature. Following this initial experiment, several improvements were implemented to produce a more well-defined response and decrease the noise from temperature. These were to increase the measurement to 6 hours and ensure the interior temperature of the spectrophotometer had reached a stable reading.

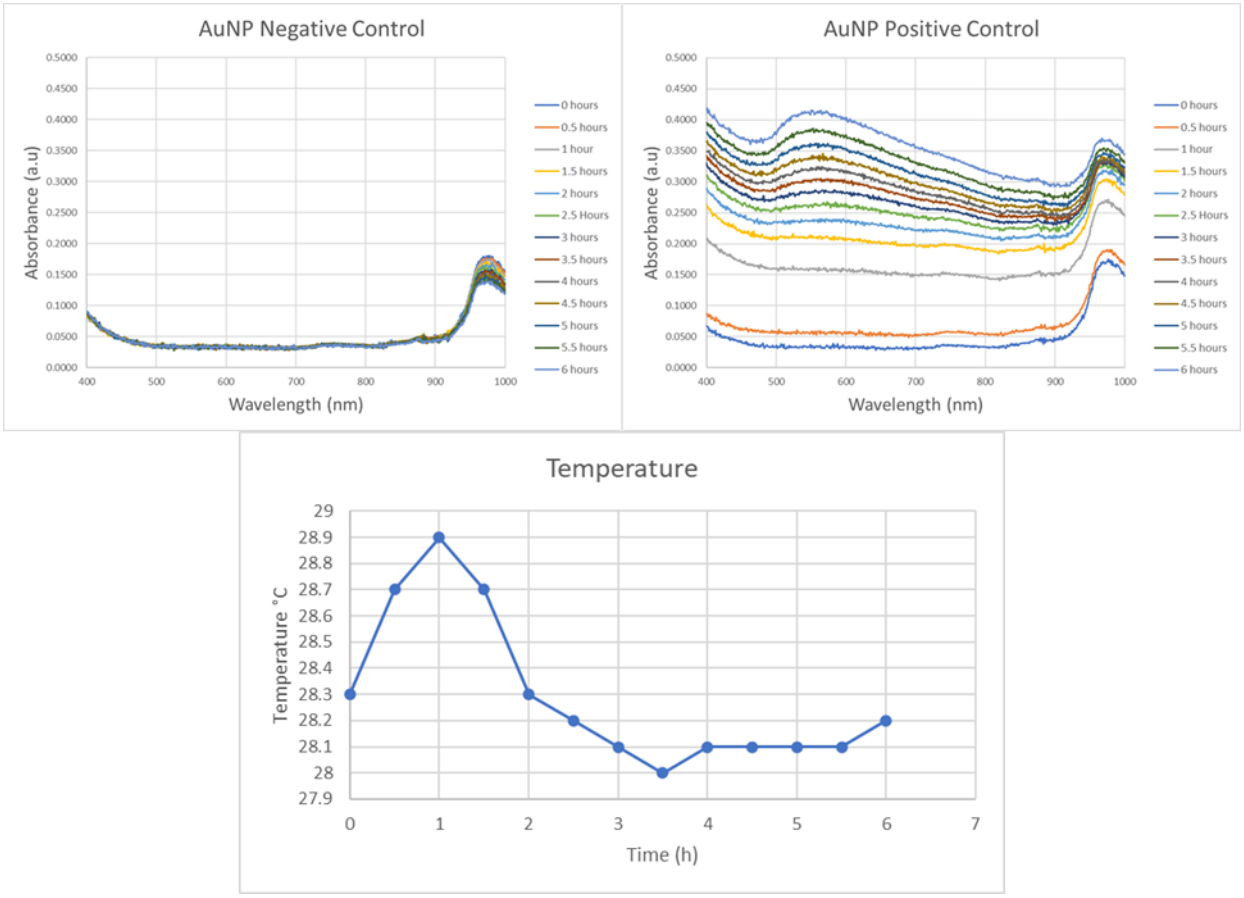


*Figure S2. First improvement to Au positive control*

The same 1:1 ratio was used in the following experiment with a longer measurement and more stable temperature, the results of which can be seen in figure S2. The spectra show an increased response near 550nm compared to the first experiment and temperature fluctuations stayed between 28°C and 28.9°C. However, the UV-vis response from this experiment indicates a high degree of asymmetry which is not consistent with the expected response (Haiss et al., 2007). Upon review of solution preparation, a

calculation error likely produced a sodium citrate solution in excess 0.5mM during this experiment which may have contributed to the observed asymmetry. Further changes were implemented to produce more consistent UV-vis spectra closer to expected results. This included extending the measurement time to 13 hours and correcting the solution preparation error. A 13-hour trial with freshly prepared reagents produced a far more characteristic response with increased symmetry and sharper peak around 550nm (Figure S3) (*UV-Vis Spectroscopy Open Source Reference Data Library for Nanoparticles*, 2021). A negative control was not measured for this experiment to conserve reagents. A longer test was suggested to observe whether the reaction had reached an asymptotic response.

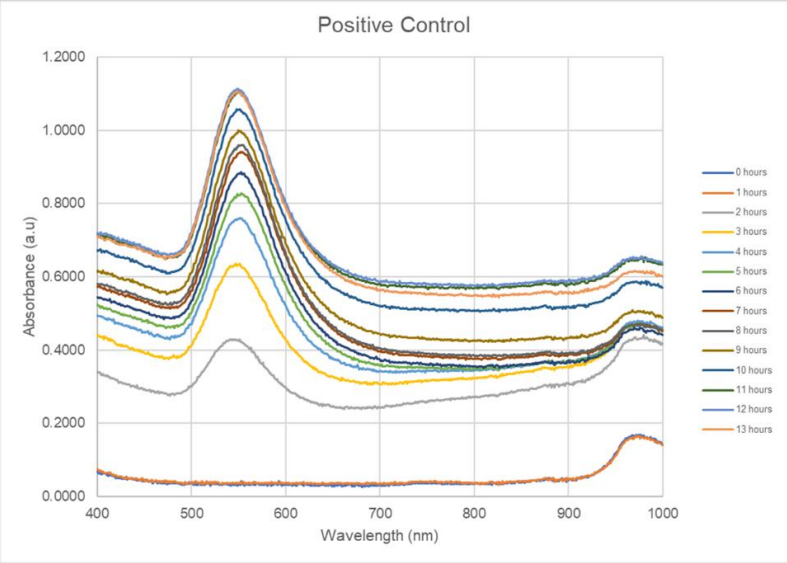


*Figure S3. Second improvement to Au positive control (13 Hours)*

A 20-hour test was conducted under the same reaction conditions revealing a trend reversal. After 16 hours the optical absorbance began to decrease (Figure S4). Visual observation of the sample indicated precipitates had settled out of solution and significant evaporation had taken place during the experiment. This prompted further improvements to prevent settling of precipitates and evaporation. High and low concentration experiments: (0.5mM $HAuCl_4$ : 0.5mM Sodium Citrate) and (0.05mM $HAuCl_4$ : 0.05mM Sodium Citrate) respectively were carried out in triplicate on an uncovered 96 well plate (Figure S5) and concurrently in 1.5mL Eppendorf tubes (Figure S6) (the reaction volume in the tubes was doubled). Tubes

were placed in a dark environment with a low 10 rpm orbital mixing overnight and spectra for tubes was measured once after 22 hours. Triplicates on the plate were prepared using the same stock solutions pipetted into three separate wells. With the uncovered plate, the UV-vis response was not consistent for the high concentration across each of the trials while the low concentration showed diminished, but consistent responses. As before, significant evaporation had taken place in the wells during the measurement. The reactions carried out in the tubes, to eliminate evaporation, showed diminished responses compared to the plate because the effective concentration did not increase as in the case of the open well plate. For the high concentration trial, precipitates had settled out of solution and were mixed prior to measuring the UV-vis spectra. The high concentration synthesis showed red shifting of the peak compared to previous experiments (Figure S3) and the low concentration provided a miniscule response.

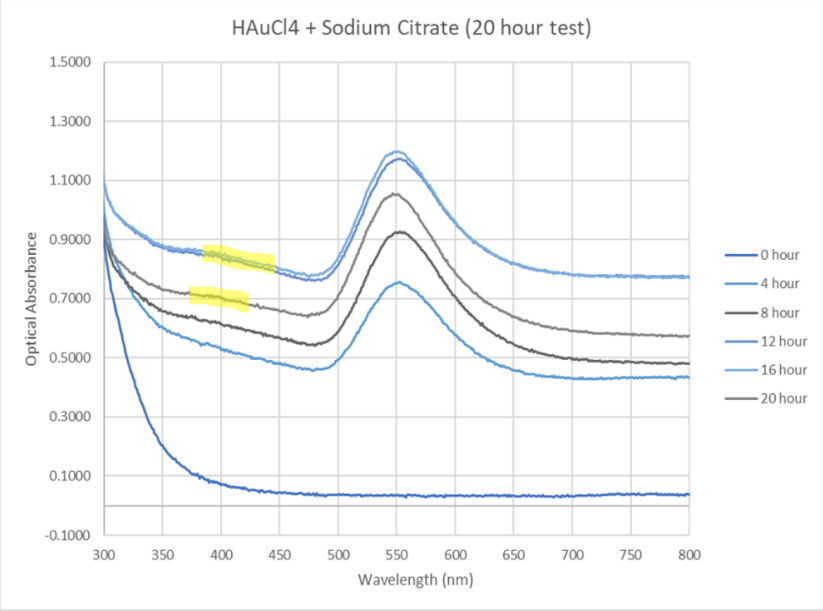


*Figure S4. Third Improvement to Au Positive Control (20 Hours). Decrease in absorbance after 16 hours highlighted in yellow.*

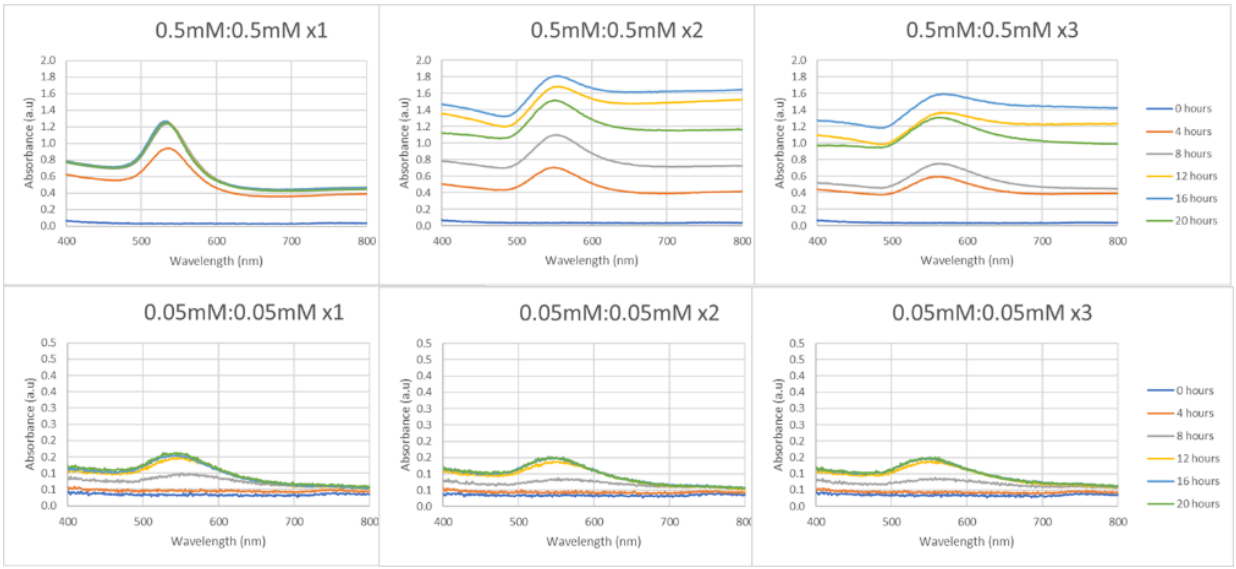


*Figure S5. High and low concentration experiments ($HAuCl_4$:Sodium Citrate) in triplicate*

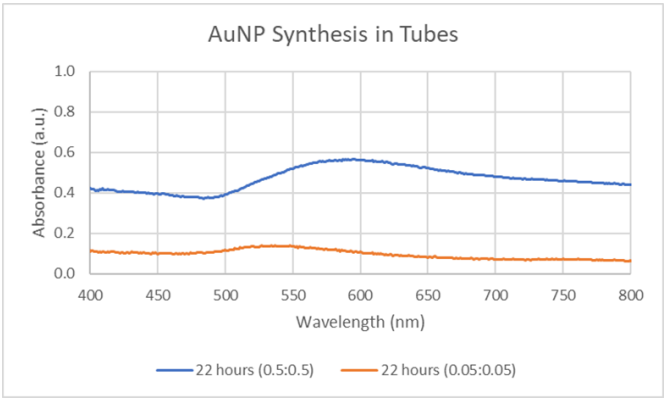


*Figure S6. $HAuCl_4$ and sodium citrate reaction spectra after 22 hours in Eppendorf tubes for high and low concentrations.*

A new 96 well plate with a lid was procured to provide more a consistent reaction environment. Because the response of the 0.05mM:0.05mM trials were very low, the low concentration condition was increased to 0.25mM $HAuCl_4$ :0.25mM Sodium Citrate. The new round of experiments in the covered well plate produced more consistent, symmetric, and asymptotic responses in triplicate runs (Figure S7). These data provide a baseline response for Au precipitation under positive control conditions. Temperature stayed between 24.4°C and 26.3°C.

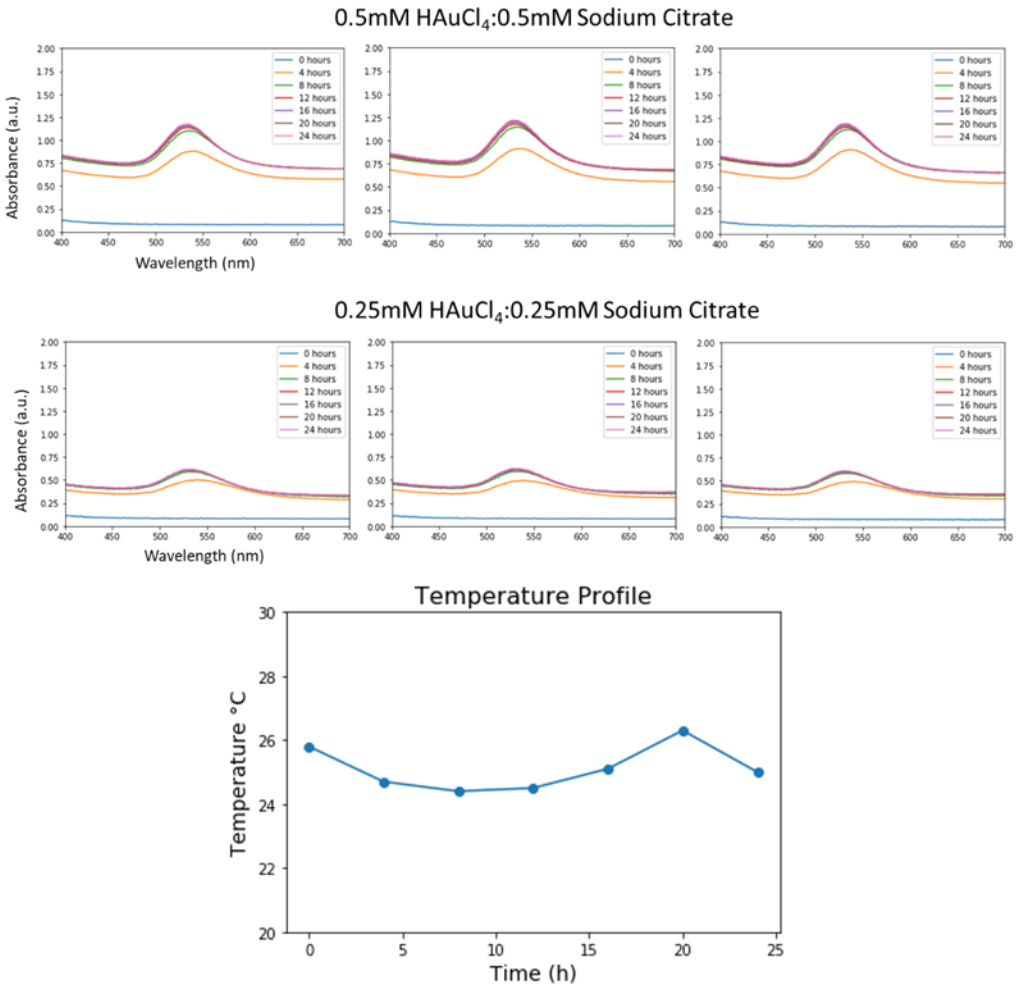


*Figure S7. Improved Au Positive Control Baseline Spectra*

Scanning electron microscopy was carried out on a (0.5mM HAuCl:0.5mM Sodium Citrate) sample synthesized in an Eppendorf tube over the course of 24 hours under mild stirring and dark conditions (Figure S8). The imaging reveals several distinct features. First are small agglomerations of what appear to be semi-spherical particles roughly 100nm in diameter. These small particles likely formed first during a broad nucleation event where citrate reduces Au (III) to Au (0) forming an Au nanoparticle capped by citrate molecules. These Au nanoparticles (AuNP) agglomerated likely to reduce surface energy. Triangular morphologies of gold particles seemed to have also formed likely at a later stage and over a longer period when the activity of sodium citrate was lower. The lower activity of sodium citrate later in the reaction

seems to have allowed gold particles to grow larger. Facet selective absorption of sodium citrate may have also led to the formation of triangular shape preserving growth morphologies.

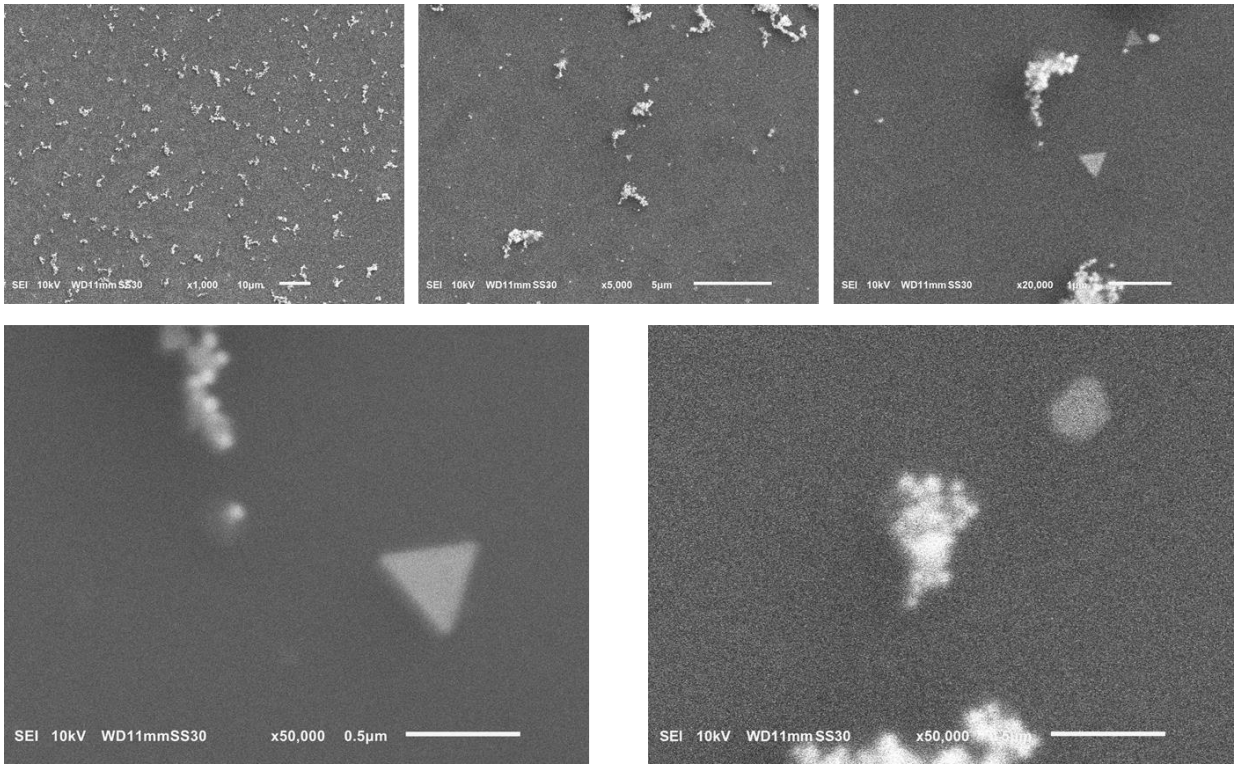


*Figure S8. SEM Images of sodium citrate reduced Au Particles (24 Hours) [Taken by Hanson Fong]*

## Ag Positive Control

A naïve replication of Au positive control mineralization protocols replacing $HAuCl_4$ with $AgNO_3$ was carried out to determine whether the previously optimized conditions would work for creating silver particles in ambient conditions. A peak in the spectra between 400nm and 450nm is expected upon the presence of silver nanoparticles (AgNP) (*UV-Vis Spectroscopy Open Source Reference Data Library for Nanoparticles*, 2021). However, the UV-vis spectra in Figure S9 does not seem to indicate the presence of AgNP at either high or low concentration of $AgNO_3$. The recorded temperature increased from a low of 25.4°C to a high of 28.9°C. This result may be explained by the different redox potentials of silver and gold respectively. $Ag^+ + e^- \rightleftharpoons Ag(s)$ has a redox potential of 0.7996V while $AuCl_4^- + 3e^- \rightleftharpoons Au_{(s)} + 4Cl^-$ has a reduction potential of 1.002V (P1, 2013). The higher reduction potential of Au (III) means that Au (III)

more easily accepts electrons compared to Ag (I). A higher ratio of sodium citrate to $AgNO_3$ may be needed to overcome this difference in redox potential to form AgNPs by increasing the concentration of available donor electrons.

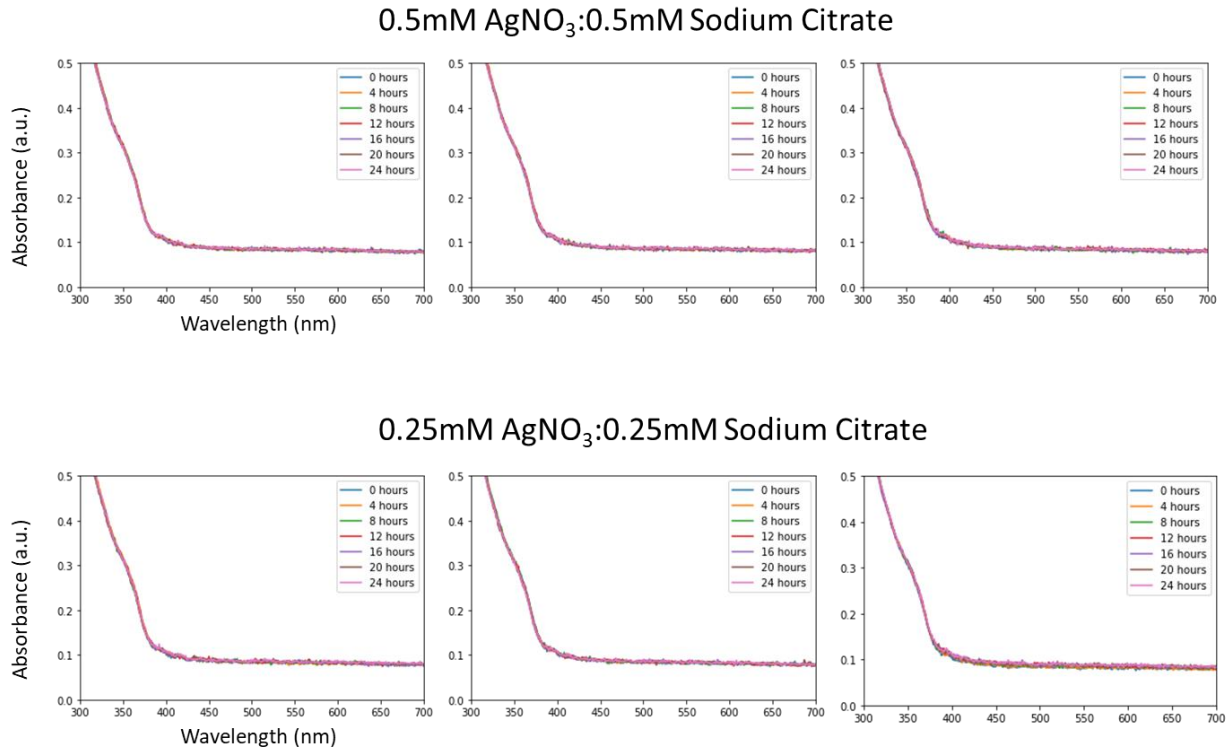


*Figure S9. Initial tests of silver nitrate and sodium citrate*

## Au + Ag Mixture Test

An oversight of "one of the most famous reactions in chemistry" (Silver Chloride, 2021) presents an obstacle for easily preparing solutions of mixed Ag and Au ions from $HAuCl_4$ with $AgNO_3$. The UV-vis spectra of a reaction of $HAuCl_4$ with $AgNO_3$ in Figure S10 shows a broad spectrum increase in optical absorption compared to $AgNO_3$ and sodium citrate. This is explained by the quick formation of insoluble AgCl precipitates observed visually as a white cloudiness in the solution. A potential approach to mitigating this obstacle could be the preparation of a gold and silver nitrate solution by reacting gold chloride with excess silver nitrate and filtering out the solid AgCl precipitate. However, further experiments to establish

new baseline mineralization spectra would be required for contextualizing spectra from peptide-based mineralization activity.

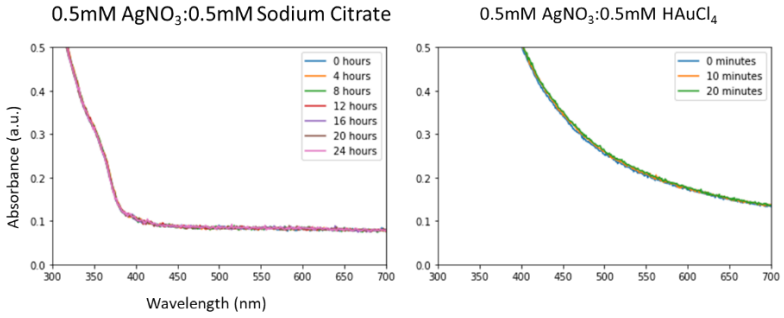


*Figure S10. Comparison of Ag with sodium citrate and Ag with HAuCl4*

# Initial Exploratory Round and Sources of Error

| | | | 1:1 Peptide to Ion | 1:10 Peptide to Ion | 1:100 Peptide to Ion |
|---|---|---|---|---|---|
| | | | **1** | **2** | **3** |
| AuBP1 | Au | **A** | 125uL 1mM AuBP1 + 125uL 1mM HAuCl4 | 125uL 0.1mM AuBP1 + 125uL 1mM HAuCl4 | 125uL 0.01mM AuBP1 + 125uL 1mM HAuCl4 |
| AuBP1 | Ag | **B** | 125uL 1mM AuBP1 + 125uL 1mM AgNO3 | 125uL 0.1mM AuBP1 + 125uL 1mM AgNO3 | 125uL 0.01mM AuBP1 + 125uL 1mM AgNO3 |
| AgBP1 | Au | **C** | 125uL 1mM AgBP1 + 125uL 1mM HAuCl4 | 125uL 0.1mM AgBP1 + 125uL 1mM HAuCl4 | 125uL 0.01mM AgBP1 + 125uL 1mM HAuCl4 |
| AgBP1 | Ag | **D** | 125uL 1mM AgBP1 + 125uL 1mM AgNO3 | 125uL 0.1mM AgBP1 + 125uL 1mM AgNO3 | 125uL 0.01mM AgBP1 + 125uL 1mM AgNO3 |
| | AuBP1 | **E** | 125uL 1mM AuBP1 + 125uL DI | 125uL 0.1mM AuBP1 + 125uL DI | 125uL 0.01mM AuBP1 + 125uL DI |
| | AgBP1 | **F** | 125uL 1mM AgBP1 + 125uL DI | 125uL 0.1mM AgBP1 + 125uL DI | 125uL 0.01mM AgBP1 + 125uL DI |
| | Au | **G** | 125uL 1mM HAuCl4 + 125uL DI | 125uL 1mM HAuCl4 + 125uL DI | 125uL 1mM HAuCl4 + 125uL DI |
| | Ag | **H** | 125uL 1mM AgNO3 + 125uL DI | 125uL 1mM AgNO3 + 125uL DI | 125uL 1mM AgNO3 + 125uL DI |

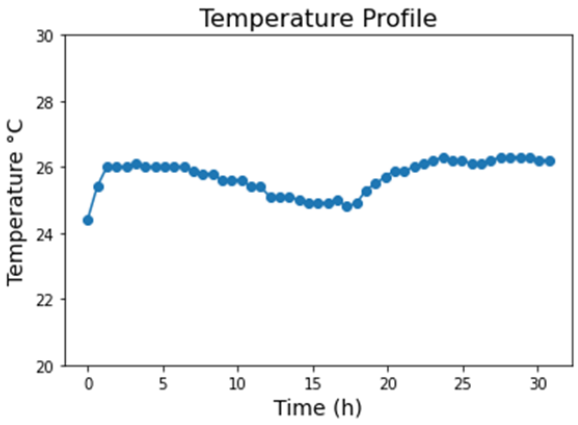


*Figure S11 Initial exploratory experimental matrix and temperature profile*

The experimental matrix in figure S11 was designed to test the mineralizing activity of both l-AuBP1 and l-AgBP1 in both gold and silver precursor solutions (A1:D1). Ratios of 1:1, 1:10, and 1:100 peptide to Au and Ag precursor concentration indicate how mineralization activity changes as less peptide is available (A2:D3). Control solutions for observing background UV-Vis spectra are included (E1:H3). All solutions were prepared using DI water and pH control was avoided to reduce side reactions between precursors and buffering agent. Following measurement of UV-Vis spectra over the course of 30 hours, solution pH was recorded for each well using a spear tip probe (figure S12). All reactions took place concurrently at room temperature between 24.4 and 26.3°C with mean temperature of 25.7°C measured from inside the plate reader.

| | | | 1:1 Peptide to Ion | 1:10 Peptide to Ion | 1:100 Peptide to Ion |
|---|---|---|---|---|---|
| | | | 1 | 2 | 3 |
| AuBP1 | Au | A | 3.19 | 3.38 | 3.48 |
| AuBP1 | Ag | B | 3.46 | 4.09 | 4.60 |
| AgBP1 | Au | C | 3.23 | 3.38 | 3.47 |
| AgBP1 | Ag | D | 3.64 | 4.37 | 5.28 |
| | AuBP1 | E | 3.56 | 4.23 | 4.89 |
| | AgBP1 | F | 3.63 | 4.40 | 4.99 |
| | Au | G | 3.51 | 3.52 | 3.51 |
| | Ag | H | 4.89 | 4.68 | 4.72 |

*Figure S12 pH measurements of initial exploratory round*

UV-Vis Measurement: The optical absorbance was recorded over a range of 230nm to 1000nm with a step size of 1nm. The number of flashes was reduced from 10 (default) to 4 to reduce the measurement time gap between adjacent wells in the plate to 96 seconds. The absorbance spectra of all 24 wells were collected for 49 cycles in intervals of 38 minutes over the course of approximately 31 hours. A time delay of 4 minutes and 14 seconds occurred between the start of the reaction in well A1 and the beginning of absorbance measurements. Absorbance was measured in a serpentine pattern following the sequence A1, A2, A3, B3, B2, B1, C1, …, G3, H3, H2, and H1 allowing for the absorbances of primary reactions to take place before those of background signals. Absorbance spectra of 24 individual wells was collected over the course of 31 hours with sampling intervals of 38 minutes (minimum time needed to collect absorbance on 24 wells).

## Results of Initial Exploration

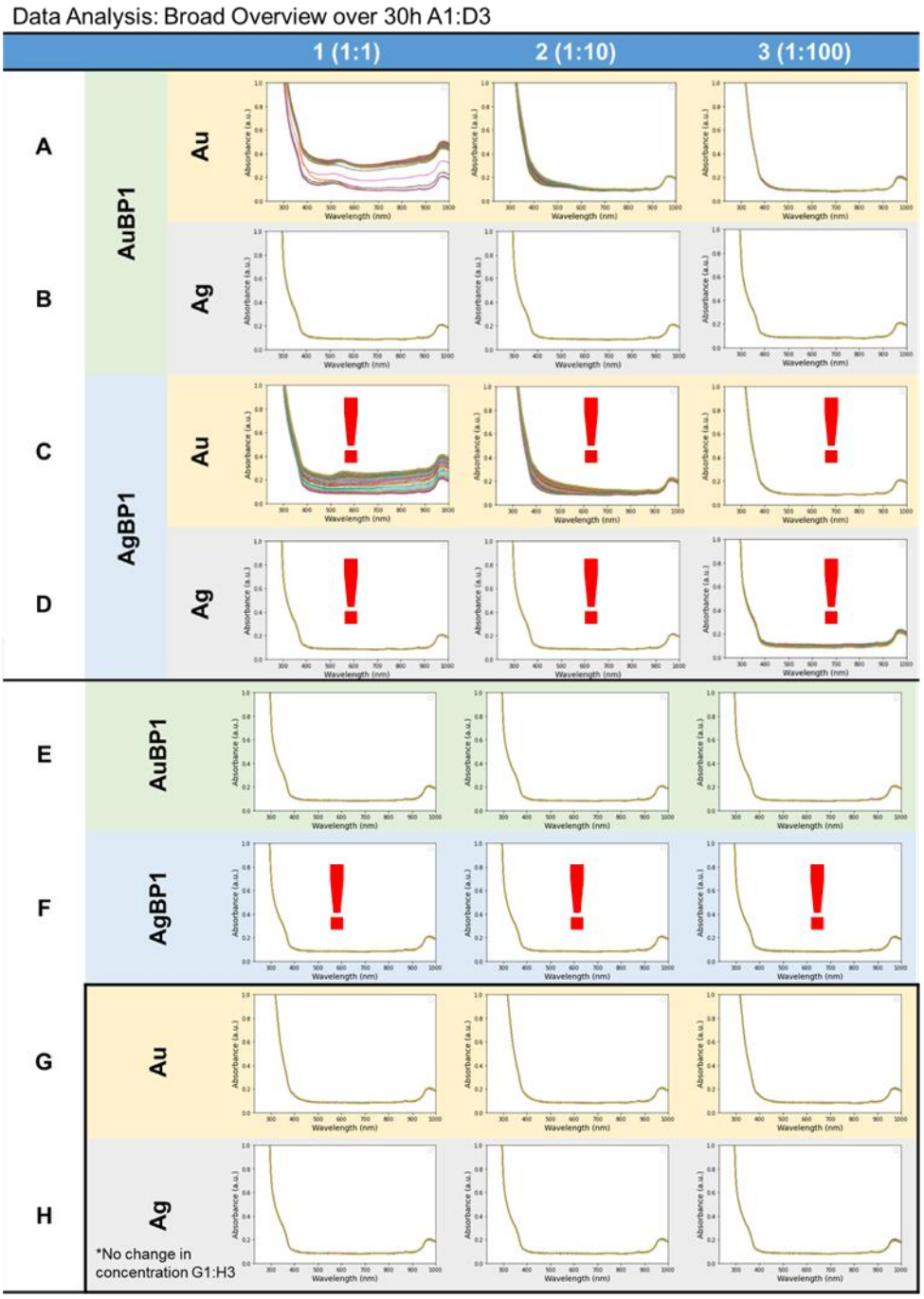


*Figure S13 Initial exploratory round UV-Vis spectra*

The results show that both peptides seem to induce precipitation of $HAuCl_4$, however this result was not replicated in subsequent triplicate experiments indicating an erroneous result for l-AgBP1 in figure S13.

Comparison of this result with the primary experimental results indicate that both peptides in this experiment were likely l-AuBP1. Interestingly, a small increase in absorption was found in a 1:100 well containing $AgNO_3$ with a pH of 5.28. While there are observed differences in the kinetics between the peptides measured in this study (figure S14) this is likely due to differences in stock concentration. The protocol followed for this experiment used the weight printed on the vial of lyophilized peptide (4.0mg). The protocol in the primary experimental matrix remedied this by measuring the mass of the lyophilized peptide and adding an appropriate amount of diluent to reach a desired stock concentration. During execution of the primary experimental matrix, mass of peptide in the vials was found to vary between 3.0 and 5.1mg.

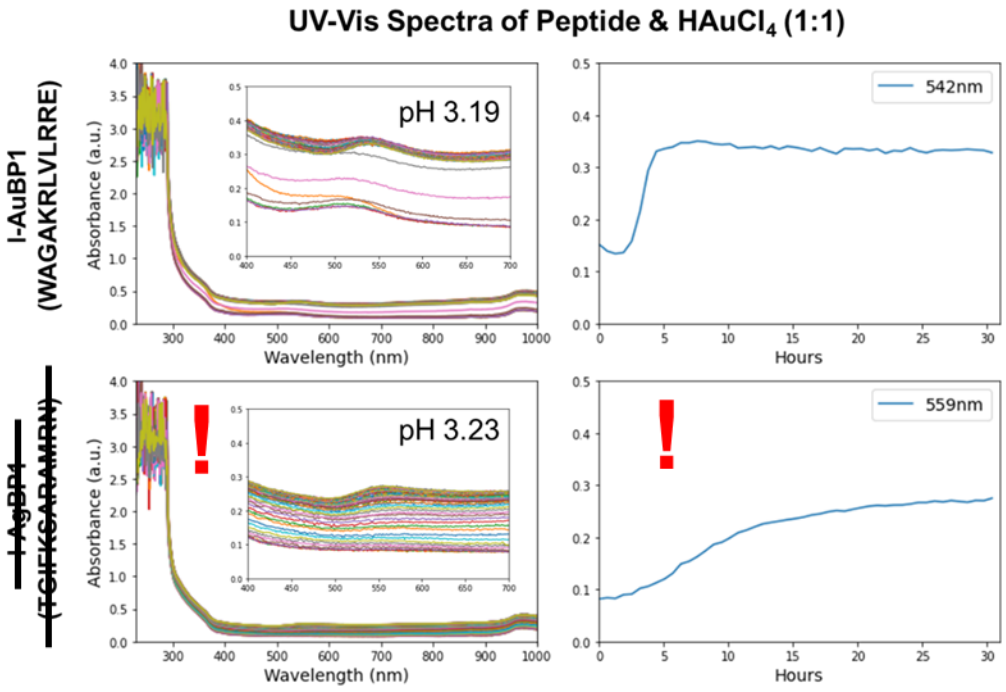


*Figure S14 Comparison of peptides with $HAuCl_4$ precursor (1:1)*

In the two solutions containing $HAuCl_4$ precursor and peptide in 1:1 ratio, both peptides led to a broad increase in absorption with a characteristic peak forming around 542nm and 559nm for both each peptide respectively (calculated as the mean lambda max between 500-700nm during the last 15 cycles or approximately 10 hours of measurement). A side-by-side comparison of the two spectra is shown in figure

S14. Isolating the absorption trend of lambda max between 500-700nm over the course of measurement reveals two different trends. With l-AuBP1, lambda max shows nearly step wise jump in absorbance while ~~l-AgBP1~~ (likely l-AuBP1 at a lower concentration) shows a more elongated sigmoidal curve taking longer to reach a maximum absorbance.

## Shortcomings

The exploratory round was not conducted in a statistically robust manor and its results could be spurious due to uncontrolled pH that varied between 3.19 and 5.28. Another source of error in the concentrations of peptide stock solutions was found following the experiment where a packaging label of 4.0mg was used as if accurate, but, ranged between 3.0 and 5.1mg. Control of pH using acetate buffer and improved reagent preparations were implemented for the next round of experimentation. Triplicates of the (1:1) done in the primary experimental matrix did not replicate the observed results and demonstrate how an error made in the initial stages of the experiment could propagate unnoticed without independent trials.

# 48 Hour Follow Up

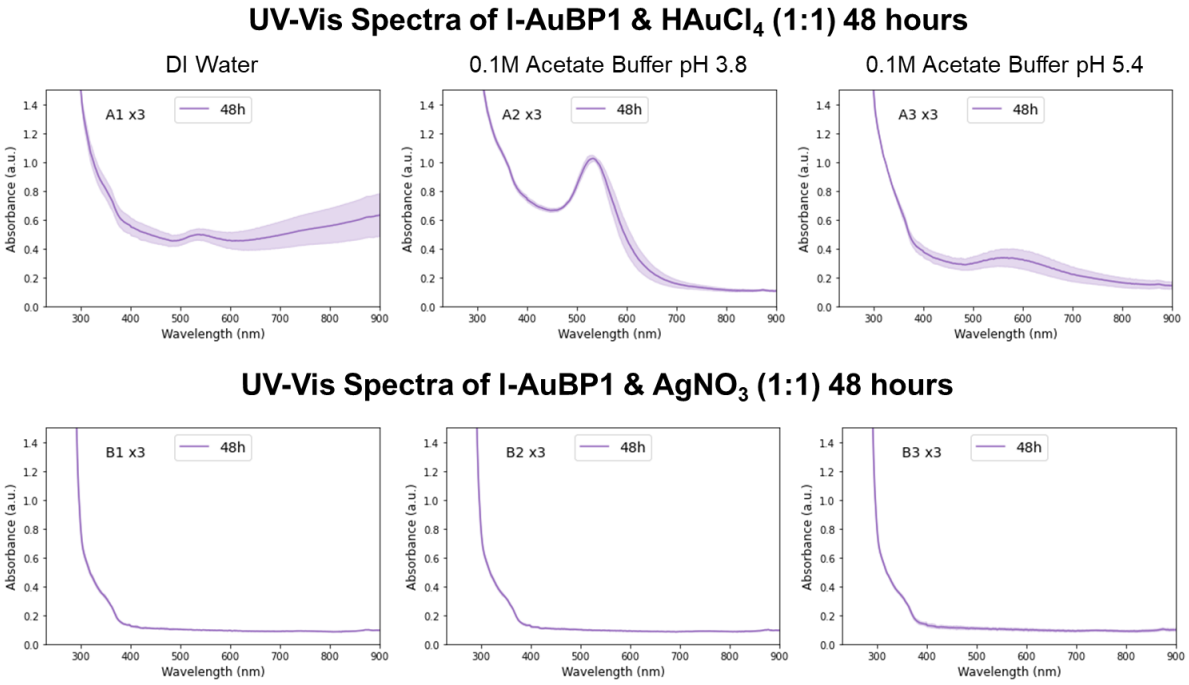


*Figure S15 48h follow up of l-AuBP1 and $HAuCl_4$ and $AgNO_3$ (triplicate study)*

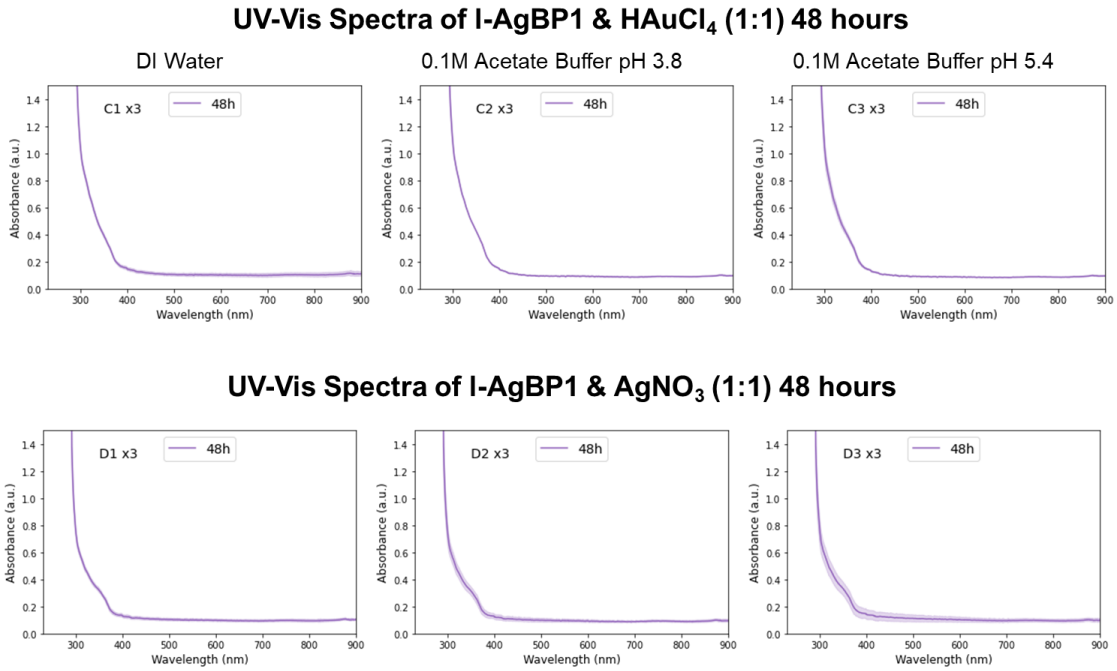


*Figure S16 48h follow up of l-AgBP1 and $HAuCl_4$ and $AgNO_3$ (triplicate study). Note slight increase in absorption for l-AgBP1 and $AgNO_3$ as pH increases.*

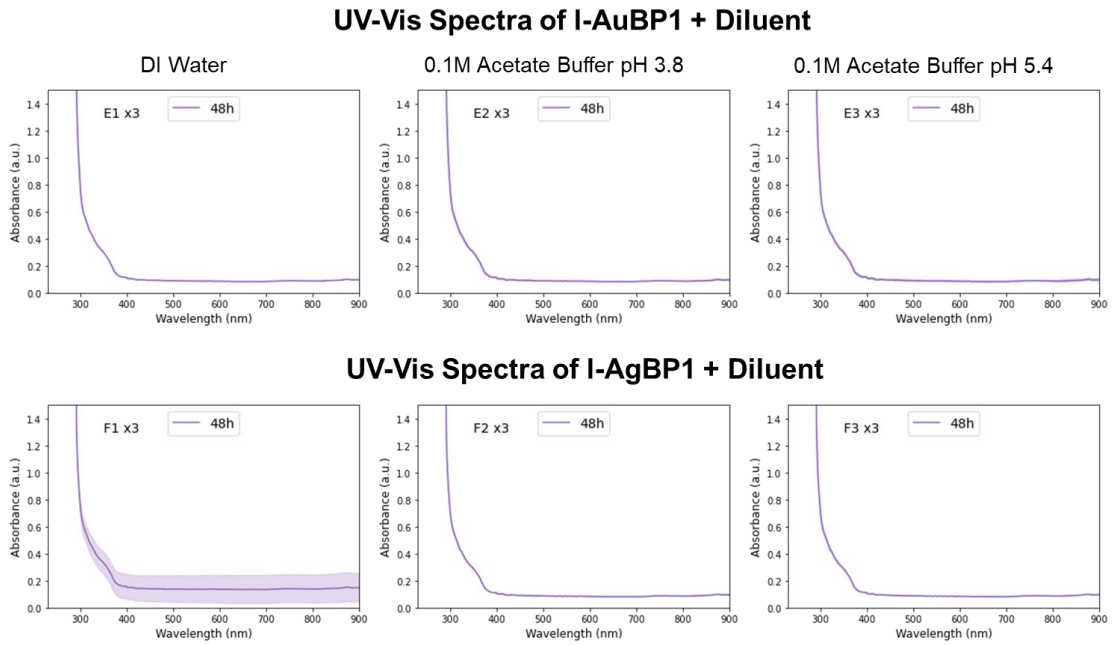


*Figure S17 48h follow up of l-AuBP1 and l-AgBP1 in diluent*

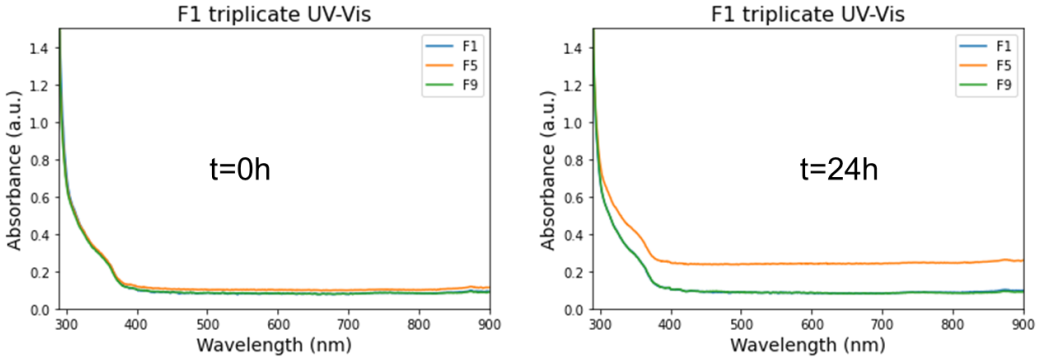


*Figure S18 Identification of F1 outlier trial*

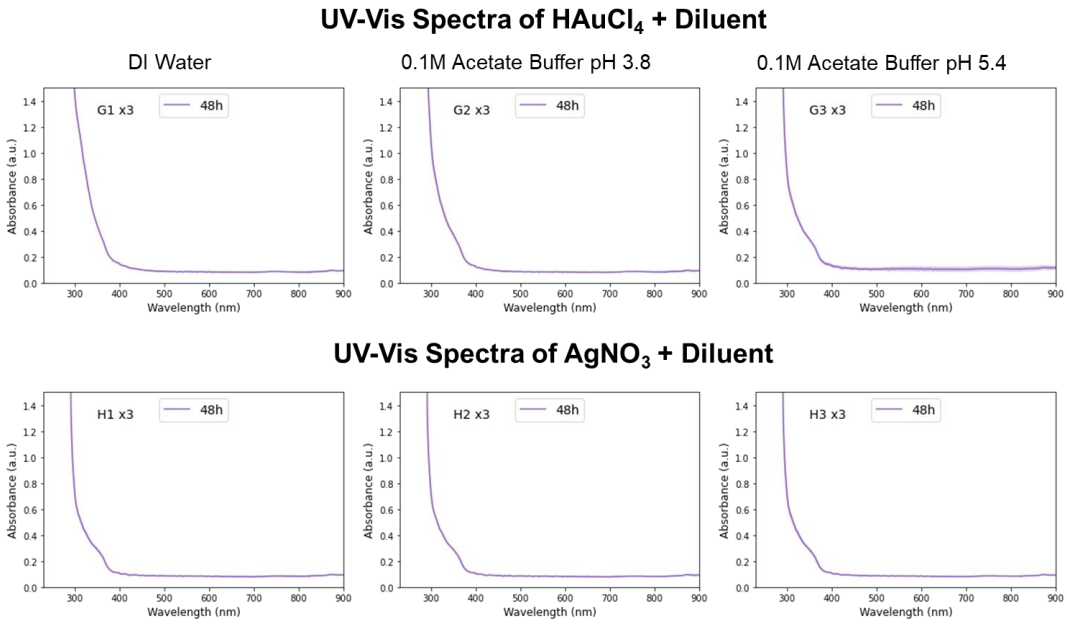


*Figure S19 48h follow up of precursor solutions in diluent*

# Temperature Plot for Primary Experiment

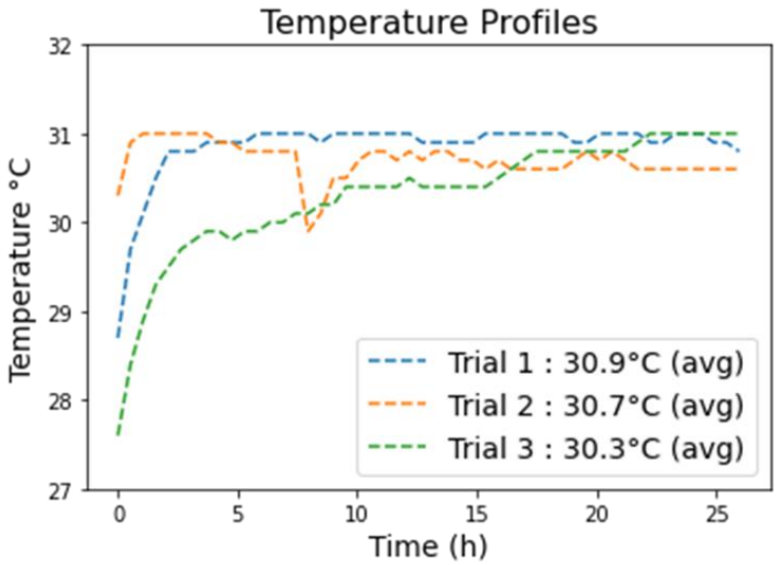


*Figure S20 Temperature profiles for each trial in the primary experimental matrix*

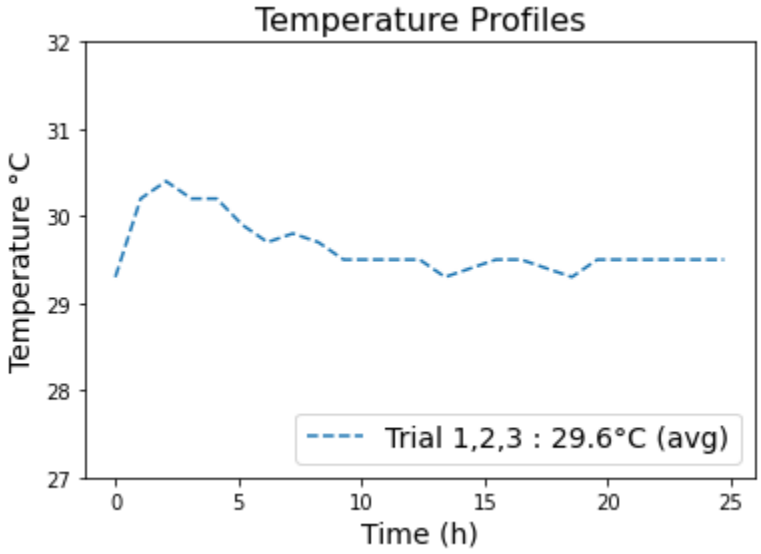


*Figure S21 Temperature profile for follow up experiment with l-AuBP1 at different concentrations*

# References

Haiss, W., Thanh, N. T. K., Aveyard, J., & Fernig, D. G. (2007). Determination of Size and Concentration of Gold Nanoparticles from UV−Vis Spectra. Analytical Chemistry, 79(11), 4215–4221. https://doi.org/10.1021/ac0702084

UV-Vis Spectroscopy Open Source Reference Data Library for Nanoparticles. (2021, April 23). InstaNANO. https://instanano.com/characterization/reference/uv-vis-spectroscopy/

P1: Standard Reduction Potentials by Element. (2013, December 2). Chemistry LibreTexts. https://chem.libretexts.org/Ancillary_Materials/Reference/Reference_Tables/Electrochemistry_Tables/P1%3A_Standard_Reduction_Potentials_by_Element

Silver chloride. (2021). In Wikipedia. https://en.wikipedia.org/w/index.php?title=Silver_chloride&oldid=997648805