Supporting Information

Modulation of Gold Nanorod Growth via Proteolysis of Dithiol Peptides for Enzymatic Biomarker Detection

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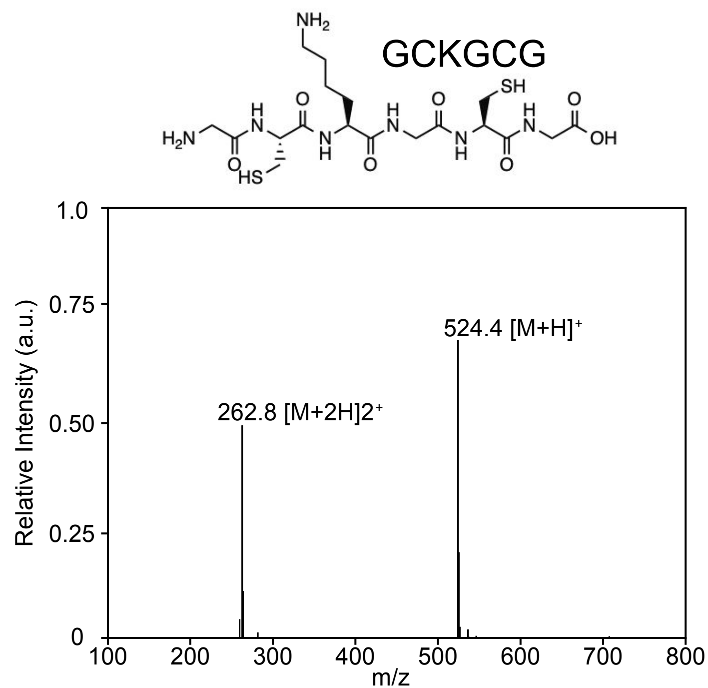
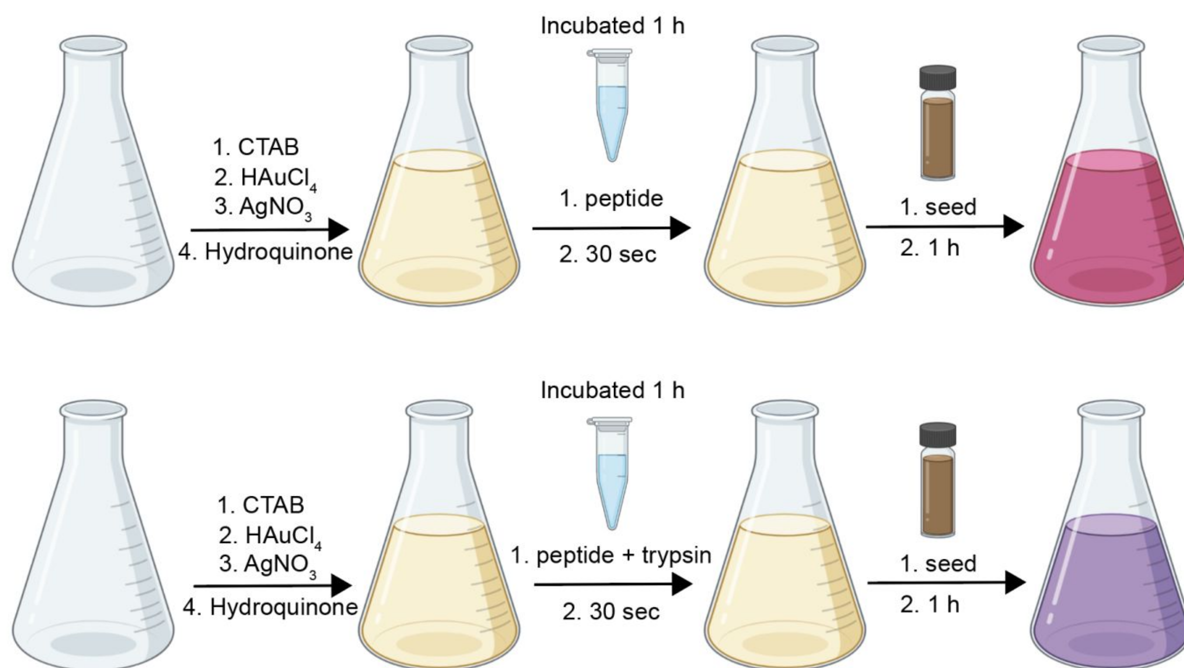


Figure S1. Chemical structure and MS-ESI spectrum of GCKGCG.



Scheme S1. Detection of trypsin using a control synthesis (top, peptide only) and sample synthesis (bottom, peptide and trypsin).

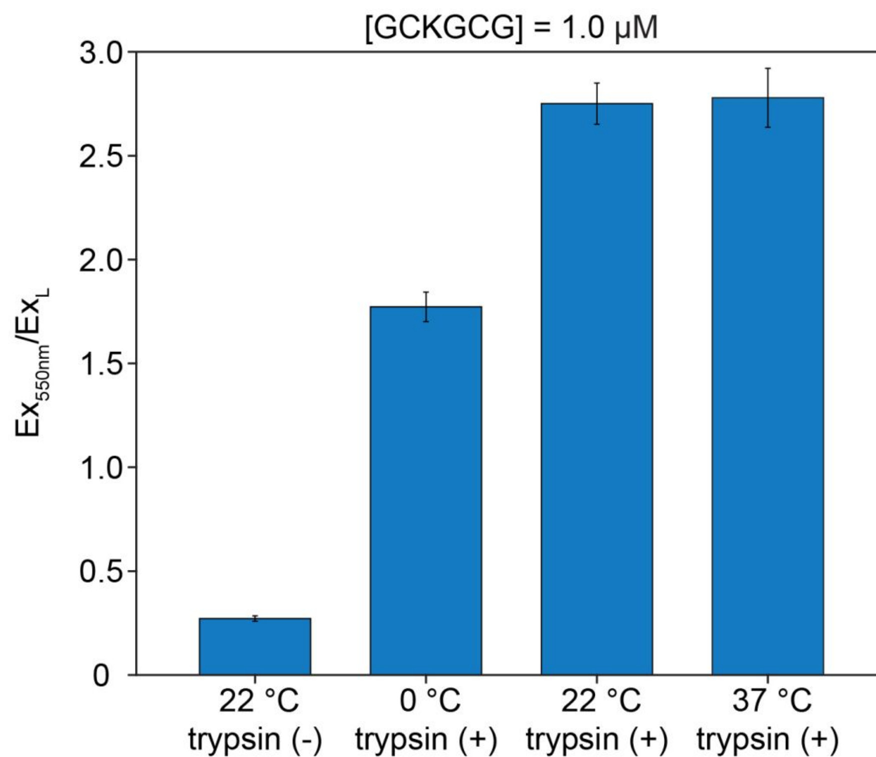


Figure S2. Sensing 500 pM of trypsin at different incubation temperatures. Triplicate experiments (n = 3) were performed in an ice-bath, at room temperature, and in an incubator.

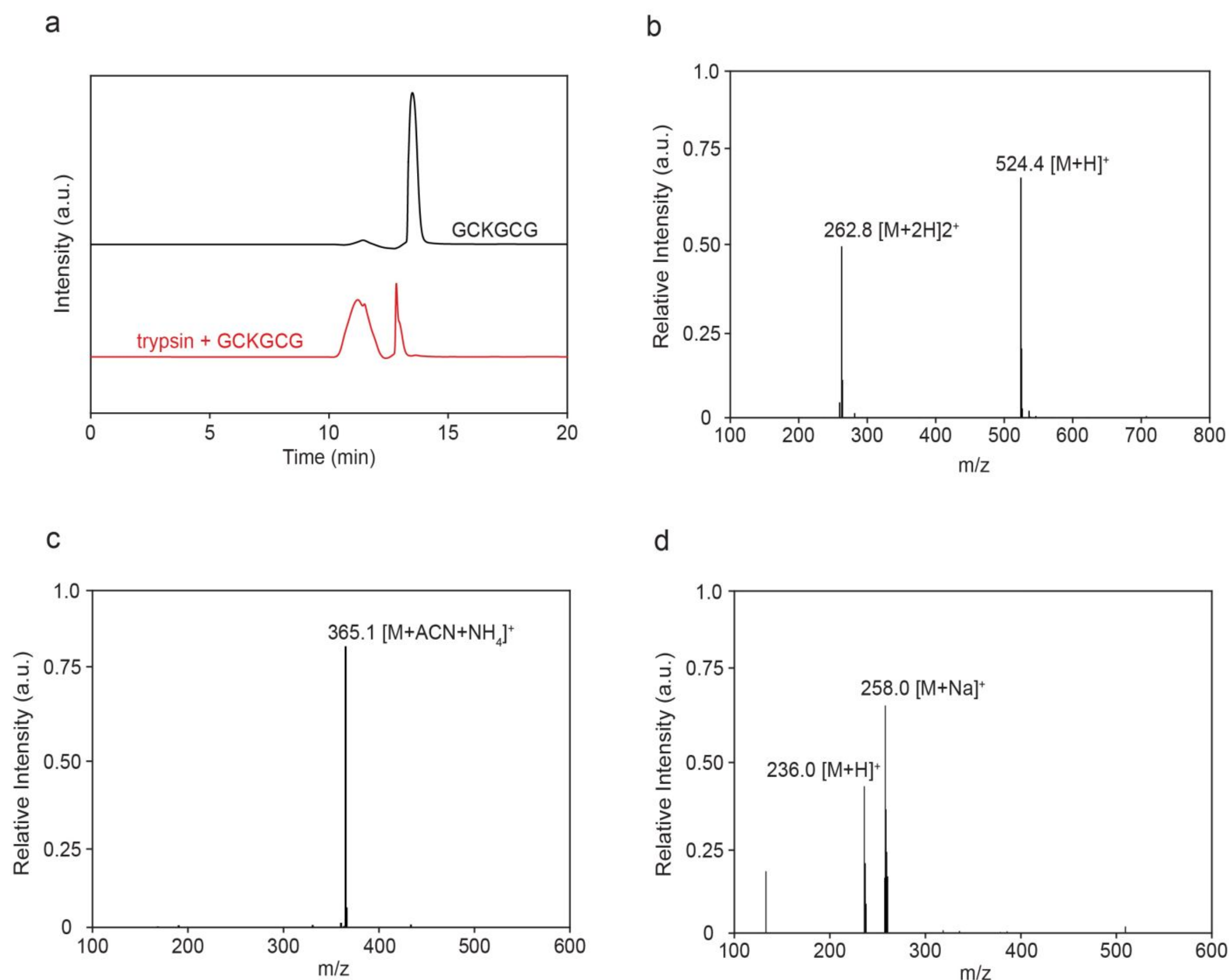


Figure S3. Cleavage of GCKGCG after the lysine residue by trypsin. (a) HPLC chromatogram of GCKGCG (0.5 mg/mL) prepared without and with trypsin (0.04 mg/mL) incubation. (b) MS-ESI of GCKGCG. (c,d) MS-ESI of GCKGCG incubated with trypsin (red chromatogram): the peak eluted at ~11 min (GCK) and the peak eluted at ~13 min (GCG).

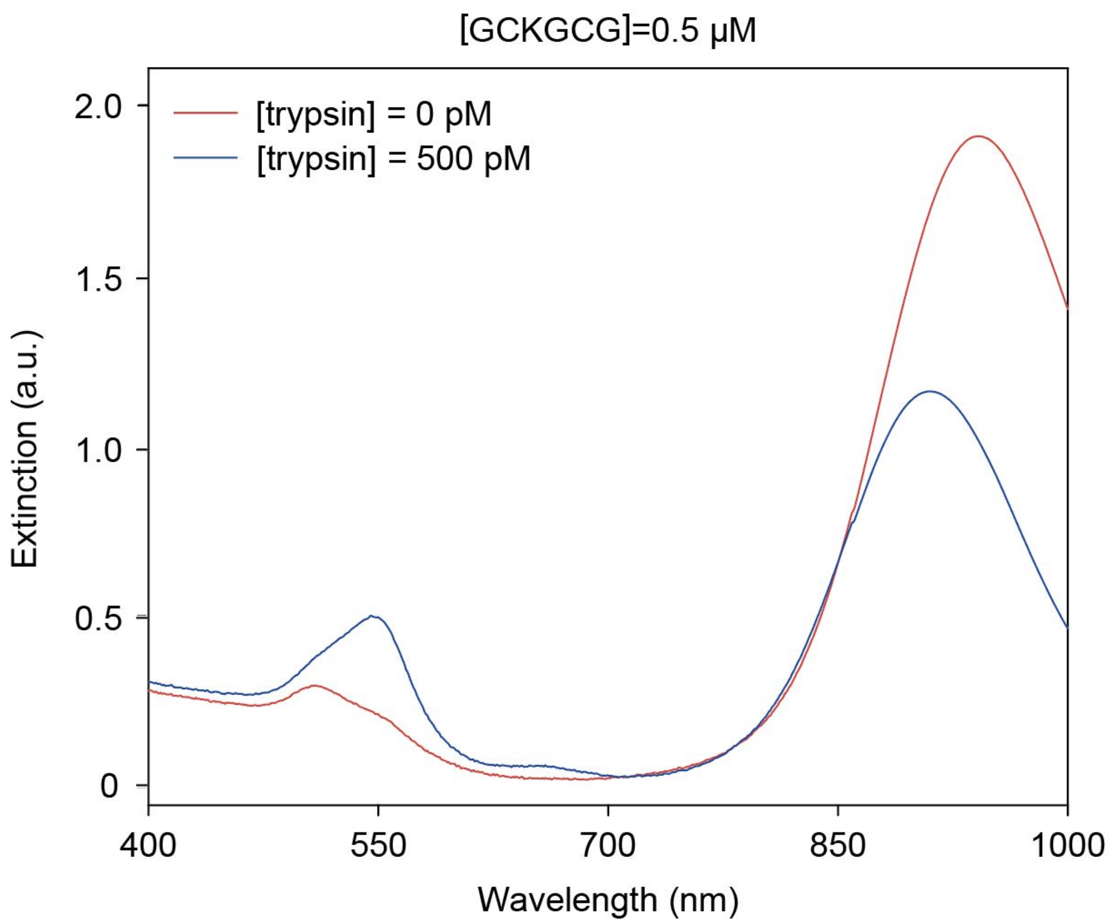


Figure S4. UV-Vis spectra showing the LSPR shift of AuNRs synthesized without and with trypsin. [GCKGCG] = 0.5 μM for both spectra.

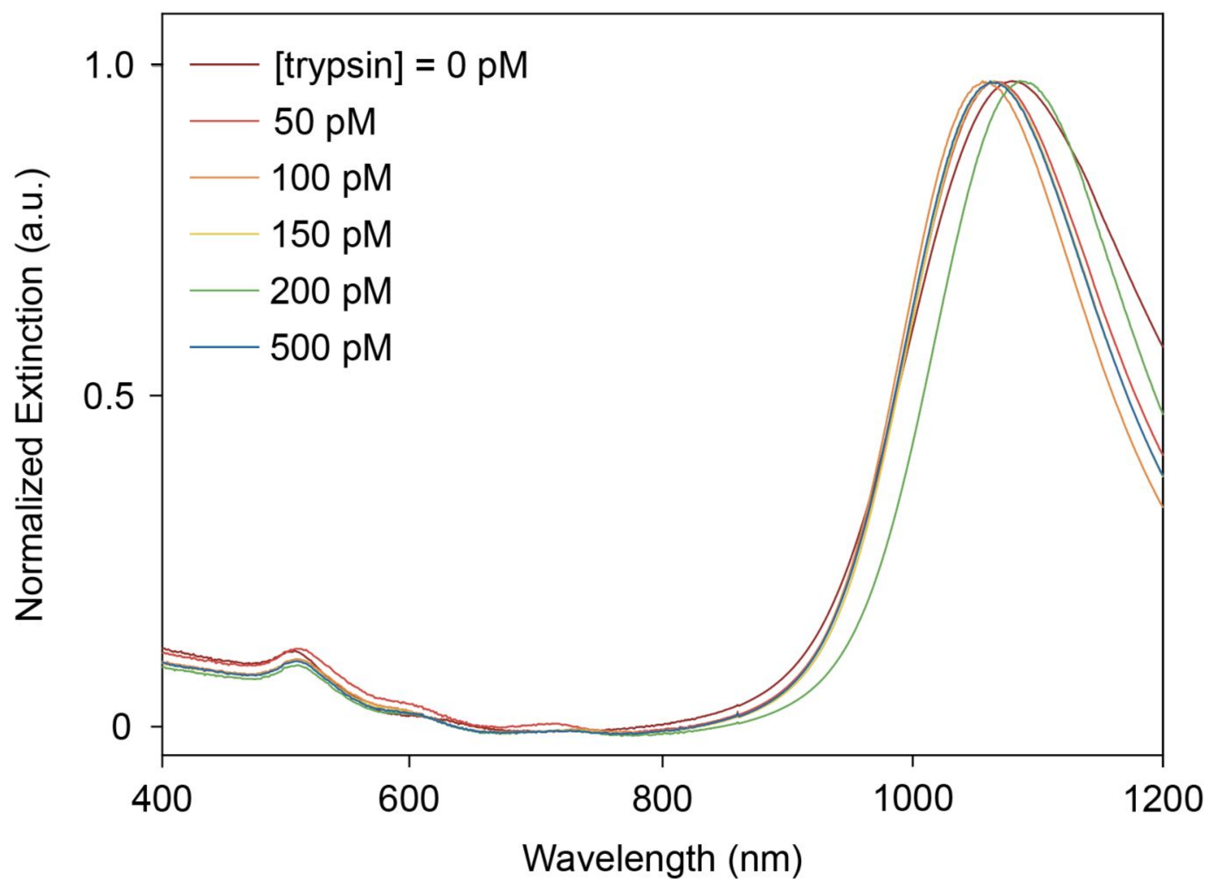


Figure S5. UV-Vis spectra showing no correlation between trypsin concentration and the LSPR of synthesized AuNRs.

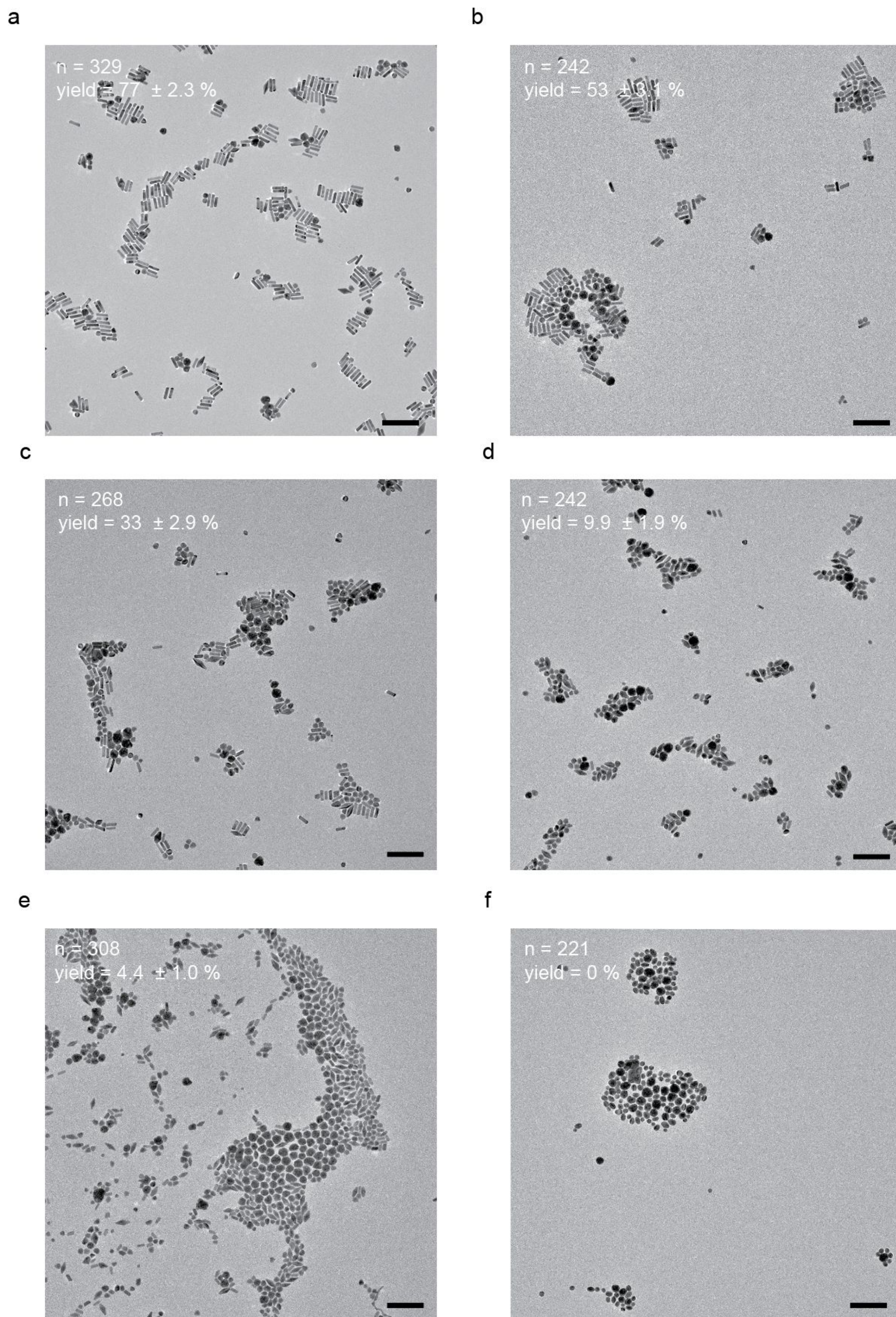


Figure S6. AuNRs prepared with GCKGCG (1.0 μM), increasing trypsin, and constant incubation time (1 h) of the peptide protease mixture before injection into the growth solution (a-f) 0, 50, 100, 150, 200, and 500 pM trypsin. Scale bars, 200 nm.

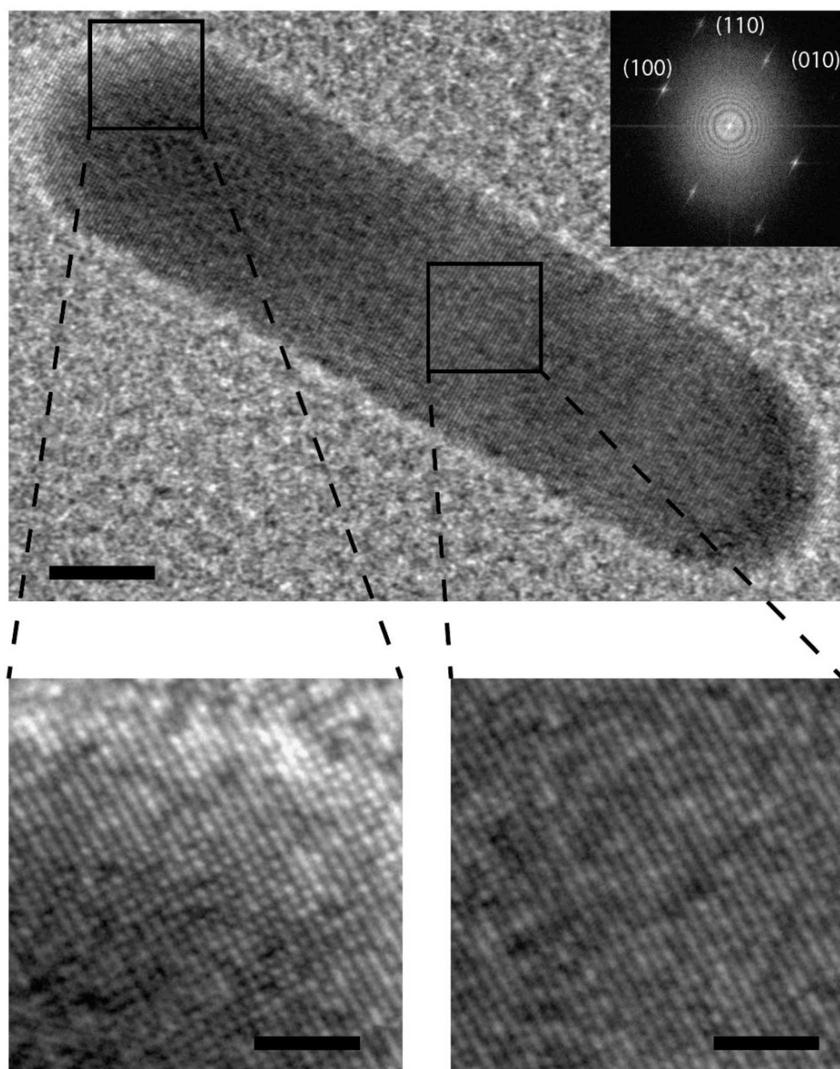


Figure S7. HR-TEM image and the FFT pattern showing the single crystalline structure of AuNRs synthesized with GCKGCG (1.0 μM) and trypsin (0 pM). Scale bars 5 nm, 1 nm (insets).

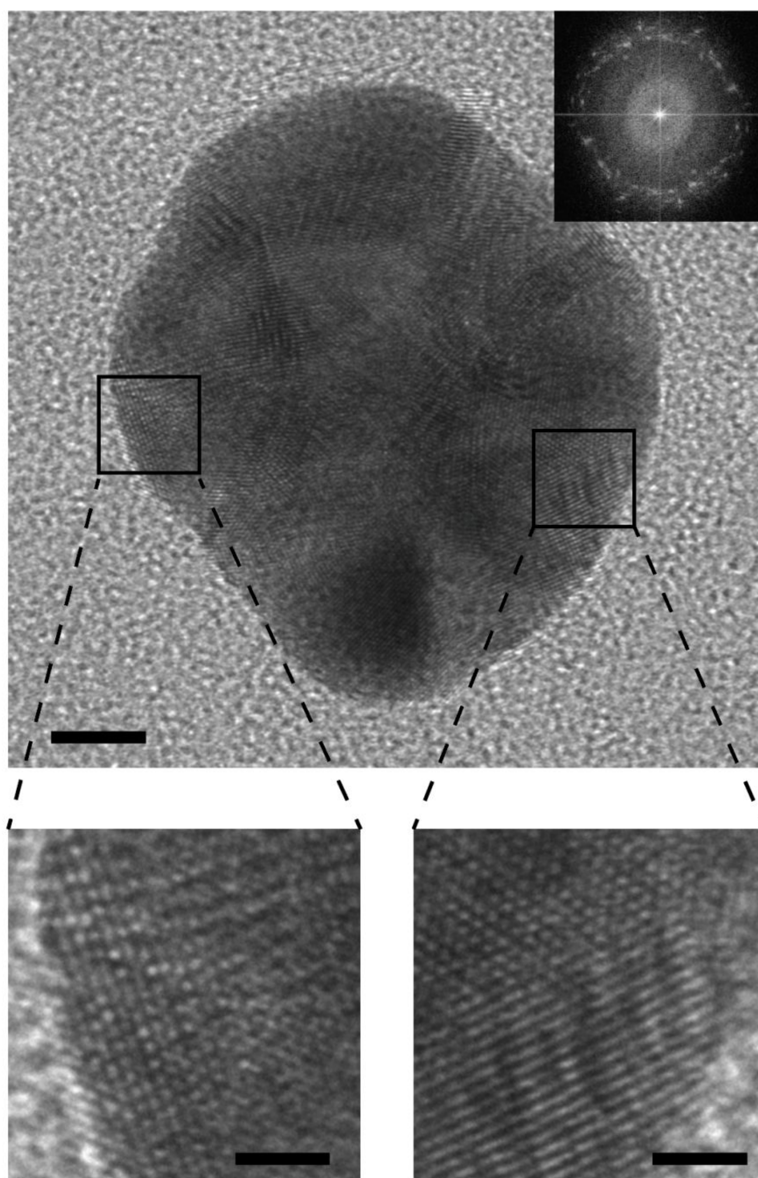


Figure S8. HR-TEM image and the FFT pattern showing the polycrystalline structure of the spherical impurities synthesized with GCKGCG (1.0 μM) and trypsin (500 pM). Scale bars 5 nm, 1 nm (insets).

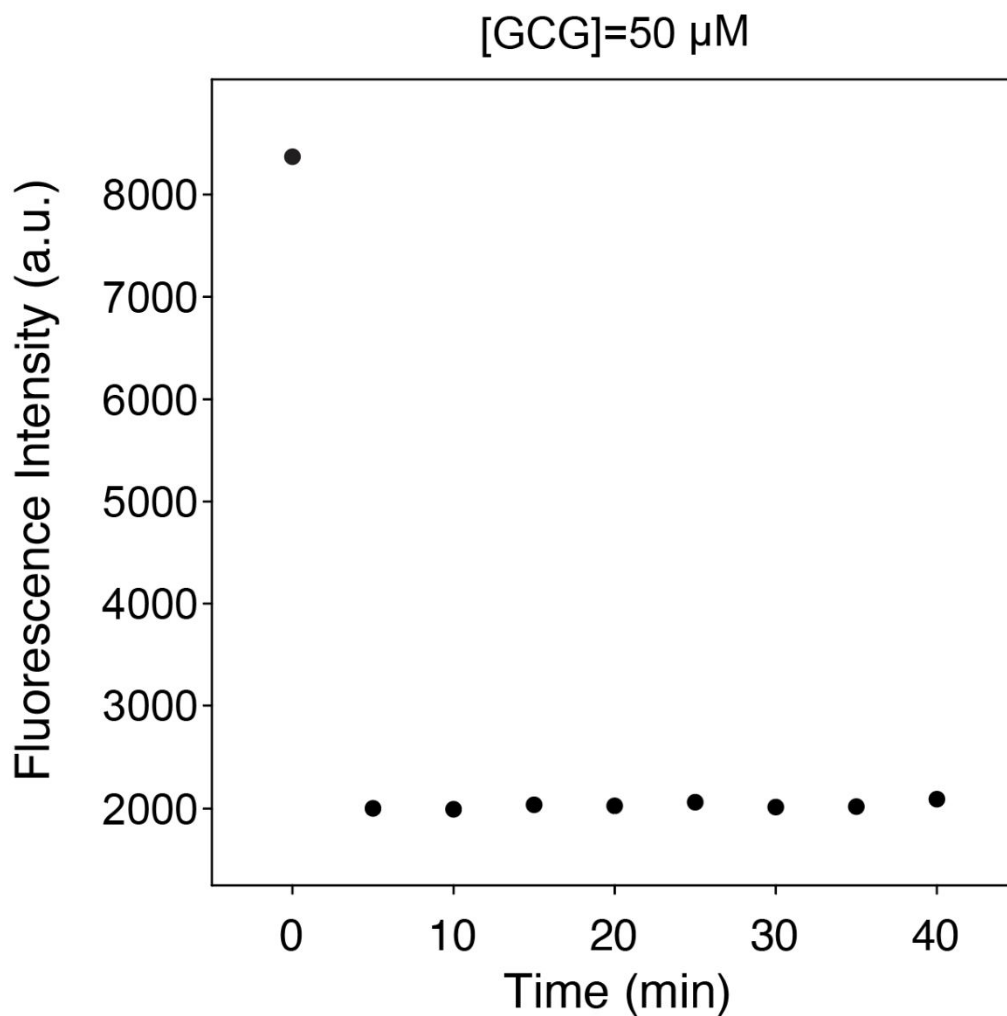


Figure S9. Au—S binding kinetics using GCG and gold nanorods. Experiment was performed by adding GCG to a gold nanorod solution, pelleting down the particles using centrifugation, and then measuring the thiol concentration in the supernatant using a thiol-sensitive fluorescent probe.

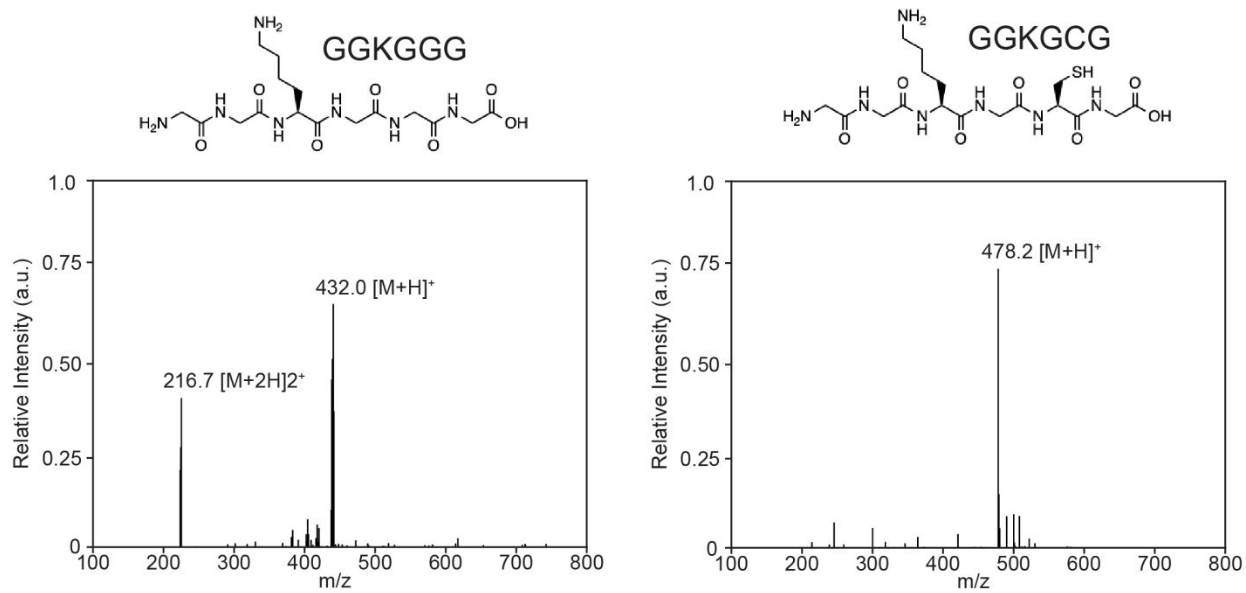


Figure S10. Chemical structure and MS-ESI spectra of the purified control probes, GGKGGG and GGKGCG.

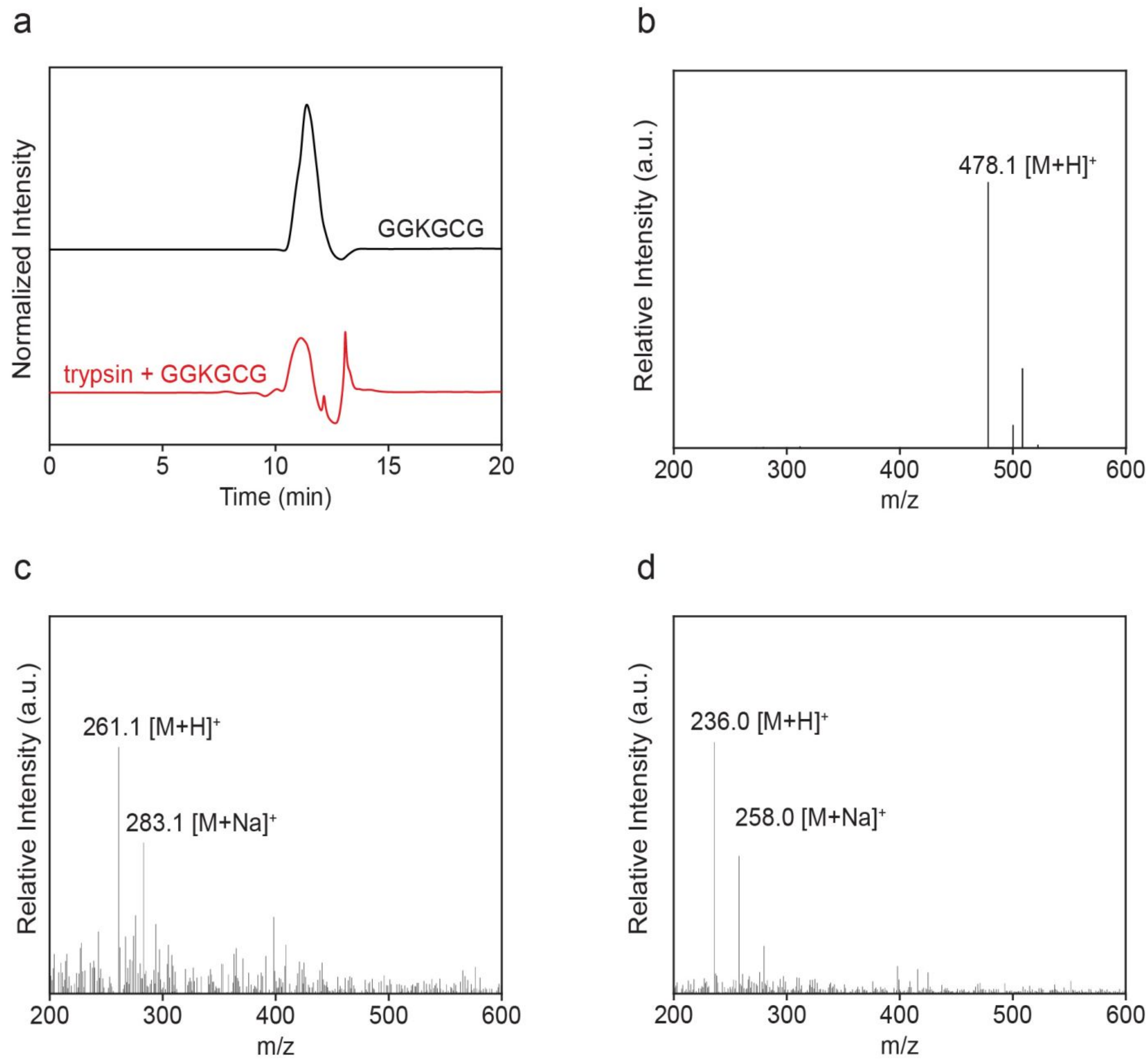


Figure S11. Cleavage of GGKGCG after the lysine residue by trypsin. (a) HPLC chromatogram of GGKGCG (0.5 mg/mL) prepared without (black) and with (red) trypsin (0.04 mg/mL) incubation. The black curve was collected using a pump rate of 1 mL/min with a 30 min gradient from 10% to 30% acetonitrile. The red curve was collected using a pump rate of 1 mL/min using constant 16% acetonitrile. (b) MS-ESI of GGKGCG. (c,d) MS-ESI of GGKGCG incubated with trypsin: the peak eluted at ~11 min (GGK) and the peak eluted at ~13 min (GCG).

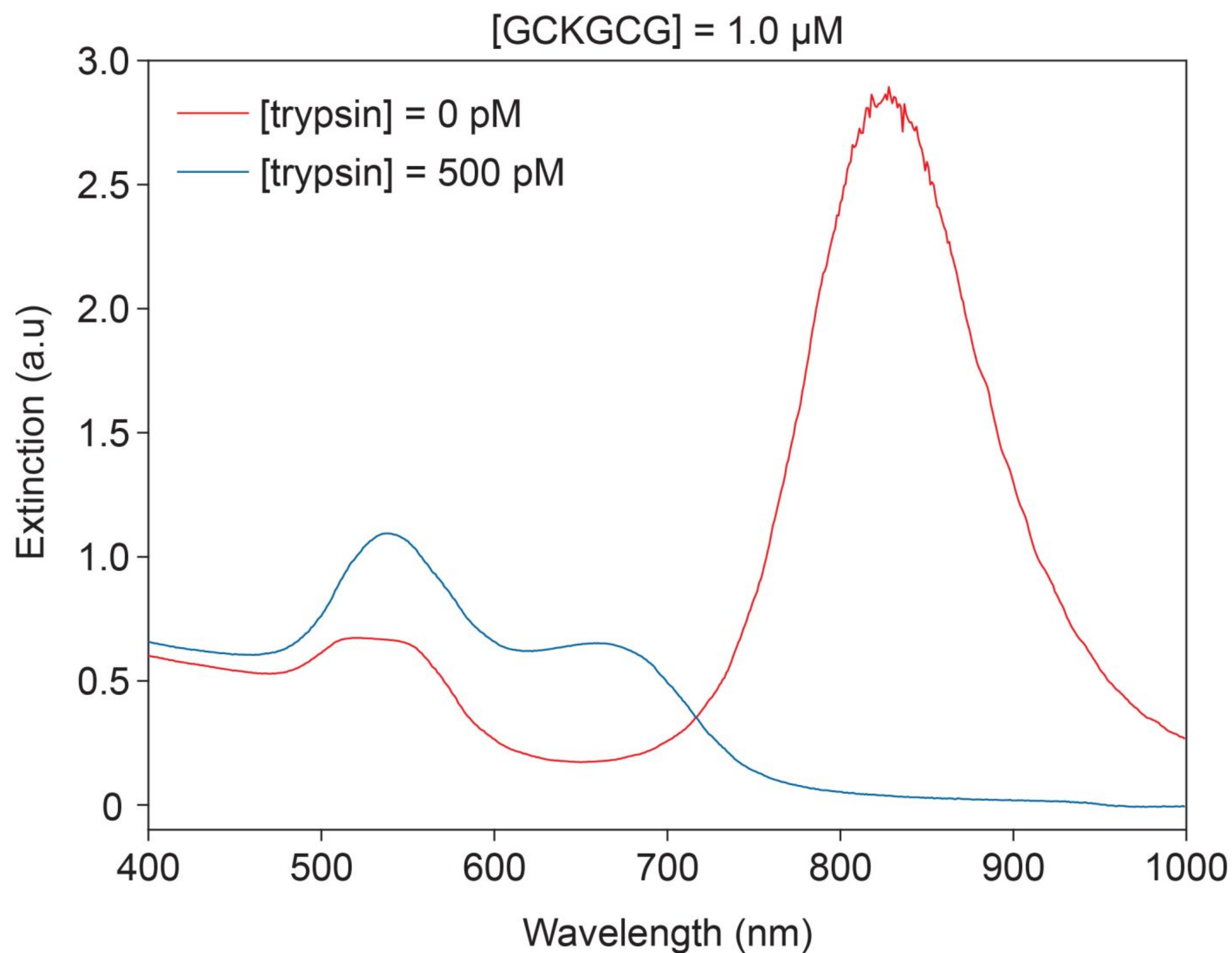


Figure S12. UV-Vis spectra of nanoparticles taken 18 hours after seed injection. Samples incubated without trypsin (red curve) and with trypsin (blue curve). Both samples contained 1.0 μM of GCKGCG.

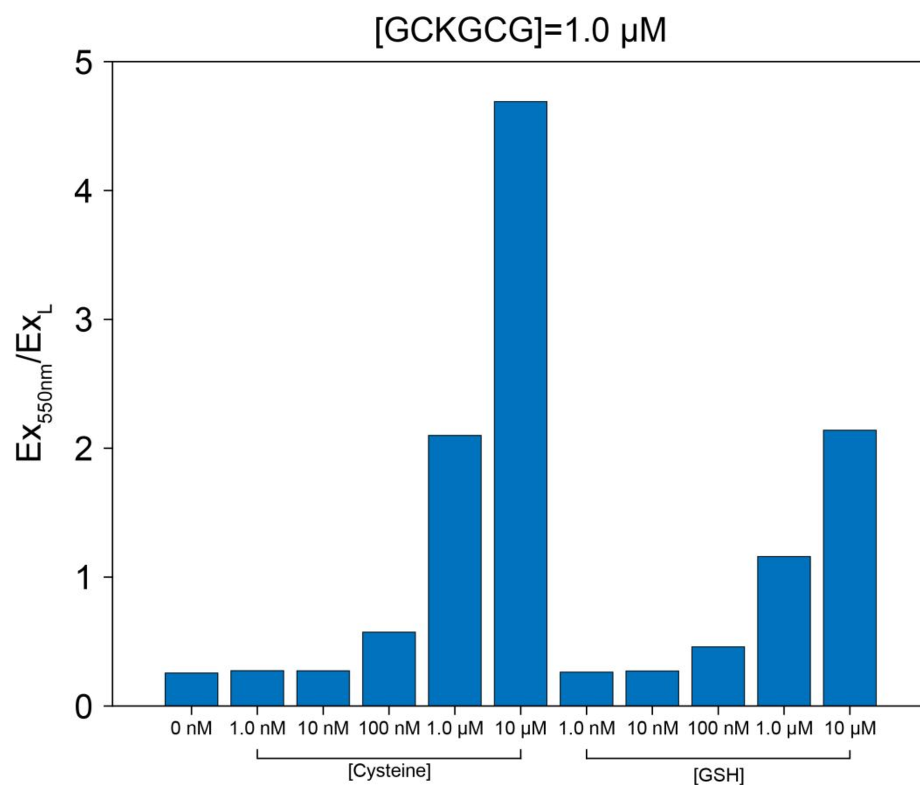


Figure S13. Interference experiment where AuNRs were prepared with a constant enzyme substrate concentration ([GCKGCG]=1.0 μM) and increasing amounts of thiolated biomolecules.

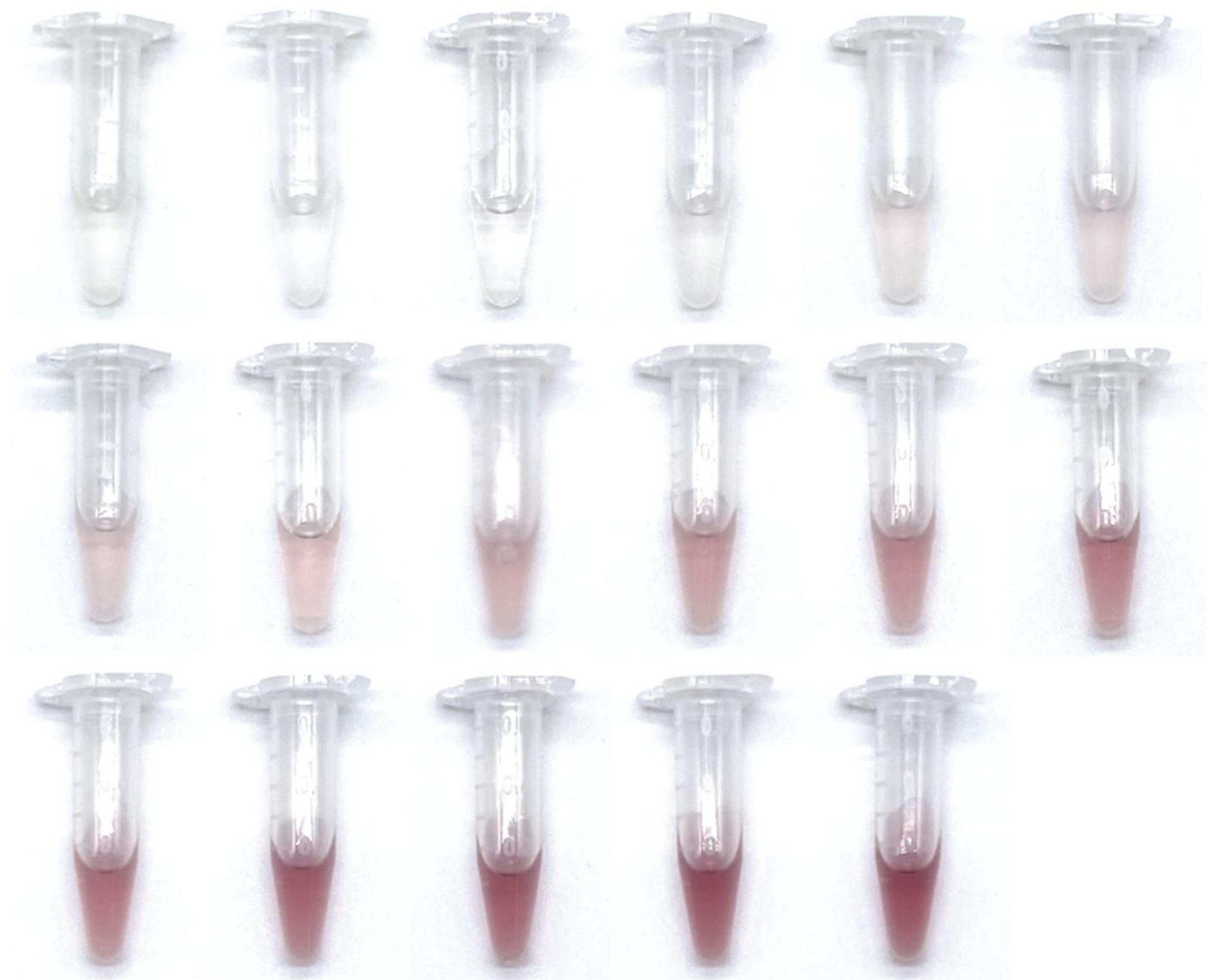
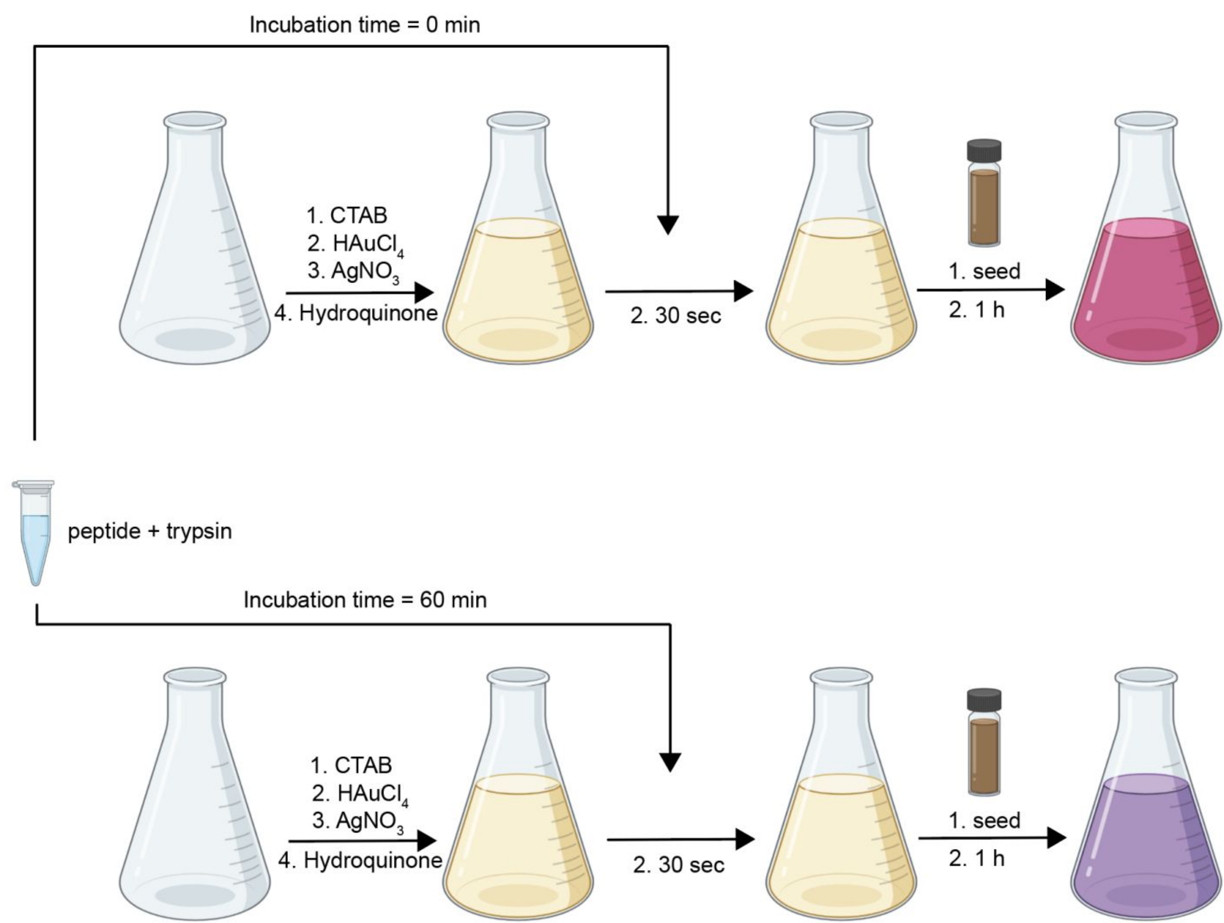


Figure S14. Images showing the growth of nanoparticles in GCKGCG (1.0 μM) with no trypsin incubation prior to seed injection. 200 μL aliquots were removed from the growth solution every 15 minutes after initiation.

Figure S15. Images showing the growth of nanoparticles in GCKGCG (1.0 μM) and trypsin (500 pM) incubation prior to seed injection. 200 μL aliquots were removed from the growth solution every 15 minutes after initiation.



Scheme S2. Incubation time dependent detection of trypsin at constant peptide and trypsin concentration.

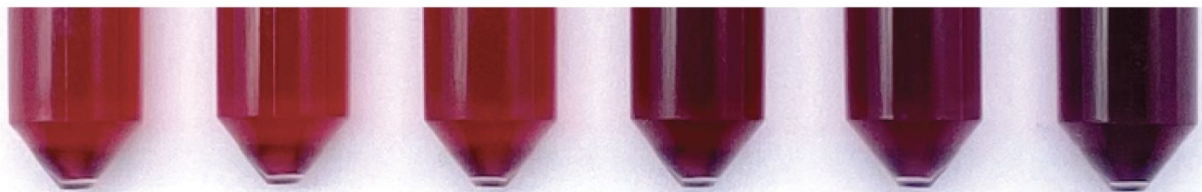


Figure S16. Images of nanoparticles prepared with [GCKGCG] = 1.0 μM, [trypsin] = 500 pM, and varying incubation time before injection into the growth solution. Samples represent incubation times from left to right of 0, 5, 10, 20, 40, and 60 min.

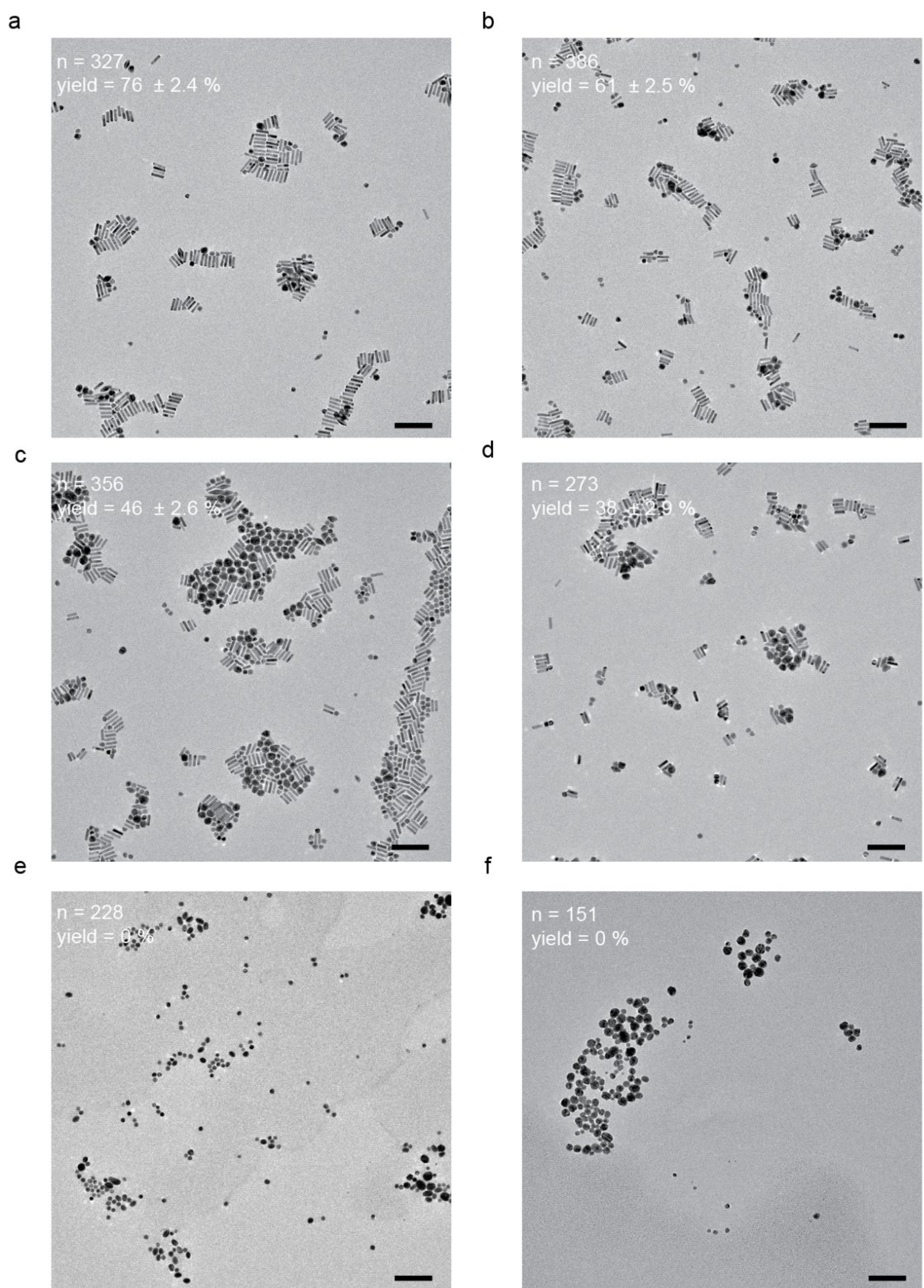


Figure S17. AuNRs prepared with GCKGCG (1.0 μM), constant trypsin (500 pM), and increasing incubation time of the peptide and protease mixture before injection into the growth solution. (a-f) 0, 5, 10, 20, 40, and 60 min. Scale bars, 200 nm.

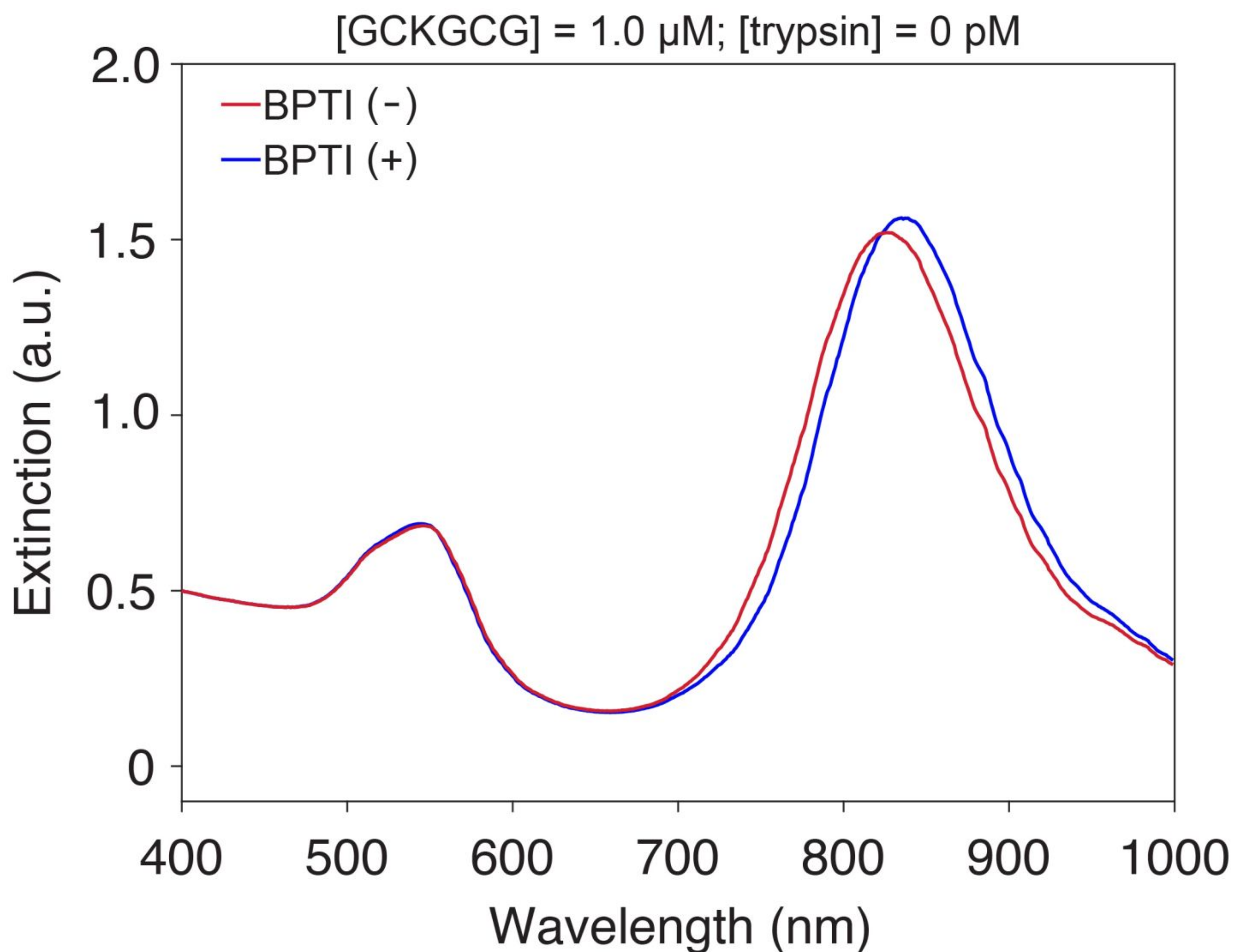


Figure S18. Experiment performed in triplicate (n = 3). AuNRs prepared with GCKGCG (1.0 μM), no trypsin (0 pM), and 0 nM (-) and 5 nM (+) of BPTI. BPTI does not alter AuNR growth in the absence of trypsin.

Limit of Detection Calculation

The limit of detection (LOD) was calculated using the limit of blank (LOB). The LOB is defined as the highest signal generated from a sample that contains no analyte. It is calculated by taking replicates of a blank sample and finding the mean and standard deviation.

$$LOB = mean_{blank} + 1.645(SD_{blank})$$

The LOB encompasses 95% of observed blank values while the remaining 5% contains a response that could have been generated from a low analyte concentration. The LOD is defined as the minimum analyte concentration that can be reliably distinguished from the LOB.

$$LOD = LOB + 1.645(SD_{low\ concentration})$$

The LOD represents an analyte concentration in which 95% of measured samples are distinguishable from the LOB while the remaining 5% erroneously appear to contain no analyte. In our experiments, the LOB and LOD were calculated from Figure 2C using 0 pM and 50 pM of trypsin, respectively.