Supplementary Material (ESI) for New Journal of Chemistry


# Supporting Information

# Novel Trimethyl Lock Based Enzyme Switch for the Self-Assembly and Disassembly of Gold Nanoparticles

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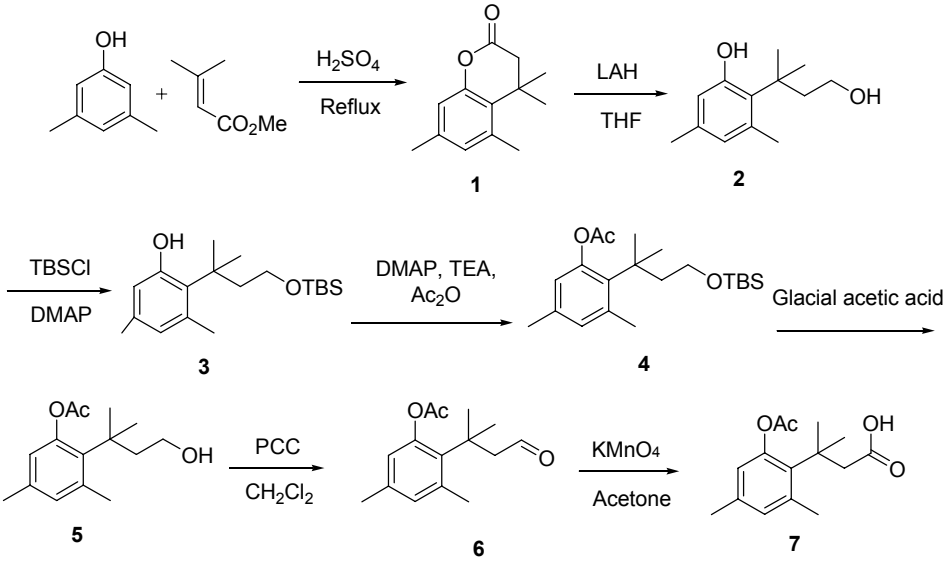
**Materials and Chemicals:** The purified esterase from hog liver (131 units/mg protein) was purchased from Fluka. Thermolysin from *Bacillus thermoproteolyticus rokko* (lyophilized powder, 68 units/mg protein) was purchased from Sigma. Fmoc-amino acids and rink amide resins were obtained from Sigma-Aldrich. 10 nm gold nanoparticles were purchased from Sigma. Their average diameter measured by TEM (100 particles) was found to be 8.8 ± 1.0 nm. Dipotassium bis (*p*-sulfonatophenyl) phenylphosphine dihydrate was purchased from Aldrich. All the other commercially available reagents and chemicals were obtained from Sigma or Aldrich and used without further purification unless noted. Milli-Q water (18.2 MΩ) was used, obtained from an ultrapure water system (Millipore) with 0.22 μm filter. The solvents were analytical grade or better and dried over according to regular protocols. HPLC grade acetonitrile and methanol were used for peptide purification.

**Instruments for Characterization and Purification:** $^1$H NMR was taken on Bruker Advance 300 MHz. When deuterated chloroform with TMS was used as a locking agent, TMS $^1$H (0 ppm) peaks were used as a reference. ESI-MS spectrometric analyses were performed at the Thermo Finnigan LCQ Deca XP Max. Analytical reverse-phase high performance liquid chromatography (HPLC) was performed on Alltima C-18 column (250 × 3.0 mm) at a flow rate of 1.0 mL/min and semi-preparative HPLC was performed on the similar C-18 column (250 × 10 mm) at a flow rate of 3 mL/min. UV-Vis absorption spectra were recorded on Beckman Coulter DU 800 UV-Vis spectrophotometer using quartz cuvettes. Background adjustments were made using deionized water. Solid-phase peptide was synthesized on WS180$^o$ Shaker.

# Synthesis of Acetylated "Trimethyl lock".




**Scheme S1.**

**Preparation of compound 1:** 3, 5-dimethylphenol (2.1 g, 17.3 mmol) and 3, 3-dimethylacrylate (2.15 mL) were dissolved in 15 mL benzene solution. Then 1.2 mL concentrated sulfuric acid was added forming a dark yellow solution. The mixture was stirred for 4 hours under reflux. After removal of solvent from the rotatory evaporator, the reaction mixture was washed with ethyl acetate (50 mL), water (20 mL), 1.0 M sodium hydroxide (10 mL), brine (20 mL x 3) and finally dried over anhydrous magnesium sulfate. The solvent was removed under vacuum affording 2.512 g (71.6 %) yellow oil compound. $^1$H NMR (300 MHz, $CDCl_3$): δ6.77 (s, 1H), δ6.57 (s, 1H), δ2.61 (s, 2H), δ2.50 (s, 3H), δ2.28 (s, 3H), δ1.46 (s, 6H). ESI-MS: found ($M+H^+$) 205.44, calculated 204.3.

**Preparation of compound 2**: Compound 1 (2.512 g, 12.3 mmol) was suspended in 30 mL anhydrous THF under ice bath with subsequent addition of lithium aluminum hydride (467.8 mg, 12.3 mmol). The mixture was stirred for 1 hr under nitrogen. Then the reaction was quenched with 1.0 M HCl (10 mL) and filtrated. After the solution was concentrated under reduced pressure, it was washed with ethyl acetate (30 mL), water (15 mL), brine (15 mL x 3) and dried over anhydrous magnesium sulfate. The residue was purified with column chromatography (silica gel) with eluent hexane/ethyl acetate (3:2) to afford 2.203 g white powder product (87.7 %). $^1$H NMR (300 MHz, $CDCl_3$): δ6.51 (s, 1H), δ6.36 (s, 1H), δ3.64 (t, $J$ =

7.26, 2H,), δ2.50 (s, 3H), δ2.28 (t, *J* = 7.26, 2H), δ2.19 (s, 3H), δ1.58 (s, 6H). ESI-MS: found [M+H$^+$] 209.18, calculated 208.3.

**Preparation of compound 3**: To a solution of compound 2 (2.203 g, 10.6 mmol) and 4-dimethylaminopyridine (2.025 g, 16.6 mmol) in anhydrous THF (20 mL) was added *tert*-butyldimethylsilyl chloride (1.8 g, 12.0 mmol). The reaction was stirred under an ice bath for 14 hours. Then the mixture was extracted with ethyl acetate (20 mL), water (10 mL), brine (10 mL x 3) and dried over anhydrous magnesium sulfate. The residue was then concentrated under reduced pressure and purified with flash chromatography with eluent hexane/ethyl acetate (3:2) to afford 3.2g (93.9%) white solid. $^1$H NMR (400 MHz, $CDCl_3$) δ6.54 (s, 1H), δ6.44 (s, 1H), δ3.69 (t, *J* =7.35, 2H), δ2.54 (s, 3H), δ2.28 (t, *J* =7.35, 2H), δ2.24 (s, 3H), δ1.63 (s, 6H), δ0.97 (s, 9H), δ0.13 (s, 6H). ESI-MS: found [M+Na$^+$] 345.06, calculated 322.5.

**Preparation of compound 4:** The compound from 3 (3.2 g, 9.94 mmol) was further reacted with anhydrous acetic anhydride (1.74 mL, 18.5 mmol) with the addition of triethylamine (2.79 mL, 20 mmol) and 4-dimethylaminopyridine (313.7 mg, 2.57 mmol). The mixture was stirred for 2 hours under nitrogen at room temperature. Then the solution was quenched and purified by column chromatography (silica gel) with eluent hexane/ethyl acetate (3:2) which later gave 3.402g (94%) yellow oil title product. $^1$H NMR (300 MHz, $CDCl_3$): δ6.88 (s, 1H), δ6.66 (s, 1H), δ3.61 (t, *J* =7.35, 2H), δ2.61 (s, 3H), δ2.31 (s, 3H), δ2.30 (s, 3H), δ2.15 (t, *J* =7.35, 2H), δ1.59 (s, 6H), δ0.97 (s, 9H), δ0.09 (s, 6H). ESI-MS: found [M+H$^+$] 365.21, calculated 364.6.

**Preparation of compound 5:** To 3.402 g (9.34 mmol) of compound 4 was added 20 mL of anhydrous THF and 10 mL of deionized water. Glacial acetic acid (20 mL) was added into above solution and stirred overnight under nitrogen in an ice bath. The mixture was concentrated under reduced pressure and extracted with ethyl acetate (30 mL), water (10 mL), 10 % sodium bicarbonate (50 mL) and brine (10 mL x 3). Then the residue was dried over anhydrous magnesium sulfate and purified with silica gel with eluent hexane/ethyl acetate (1:1) to afford title compound (2.013 g, 85.8 %). $^1$H NMR (300 MHz, $CDCl_3$)

δ6.80 (s, 1H), δ6.56 (s, 1H), δ3.40 (t, $J$ = 7.65, 2H), δ3.06 (br, 1H), δ2.49 (s, 3H), δ2.22 (s, 3H), δ2.20 (s, 3H), δ2.00 (m, 2H), δ1.46 (s, 6H). ESI-MS: found [M+$H^+$] 251.11, calculated 250.2.

**Preparation of compound 6:** The 2.013 g (8.05 mmol) of compound 5 was dissolved in 10 mL of dichloromethane. Then pyridine chlorochromate (3.45 g, 16 mmol) was added into above solution forming a black suspension. The mixture was stirred for 1 hour under room temperature and was filtered to collect the eluent. After evaporation of solvent, the residue was purified with column chromatography with eluent hexane/ethyl acetate (2:3) to obtain 1.5 g (75.0 %) of the title product. $^1$H NMR (300 MHz, $CDCl_3$) δ9.53 (t, $J$ = 2.58,1H), δ6.84 (s, 1H), δ6.62 (s, 1H),δ2.81 (d, $J$ = 2.55, 2H,), δ2.53 (s, 3H), δ2.26 (s, 3H), δ2.22 (s, 3H), δ1.56 (s, 6H). ESI-MS: found [M+$H^+$] 248.31, calculated 247.3.

**Preparation of compound 7:** Compound **6** (1.5 g, 6.04 mmol) was dissolved in 10 mL of acetone and 10 mL of deionized water. Then potassium permanganate (VII) (955 mg, 6.04 mmol) dissolved in 5 mL of deionized water and 5 mL of acetone was added dropwise to the above solution. The reaction mixture was stirred for 17 hours under ambient temperature. After evaporation of solvent, the product was extracted with dichloromethane (30 mL), washed by water (5 mL), brine (5 mL x 3) and finally dried over anhydrous magnesium sulfate. The mixture was then concentrated under reduced pressure and purified with flash chromatography with eluent hexane/ethyl acetate (1:4) to obtain solid powder of trimethyl lock product (942 mg, 66.2 %). $^1$H NMR (300 MHz, $CDCl_3$) δ6.88 (s, 1H), δ6.66 (s, 1H), δ2.90 (s, 2H), δ2.60 (s, 3H), δ2.34 (s, 3H), δ2.29 (s, 3H), δ1.64 (s, 6H). ESI-MS: found [M+$H^+$] 265.33, calculated 264.3.

**Synthesis and purification of polypeptide:** The polypeptide sequence, SH-Ph-$CH_2$-Gly-Gly-Gly-Phe-Gly-Gly-Lys ($NH_2$)-$CONH_2$, which could be cleaved by thermolysin, was synthesized on Fmoc-Rink amide polystyrene resin by standard Fmoc-Solid Phase Peptide Synthesis strategies.[1] Typically, each amino acid was coupled for two hours with TBTU/HOBT as coupling reagent in anhydrous DMF. Finally, 4-thiol phenyl acetic acid was coupled to the N-terminus of above polypeptide, which contains amide at the C-terminus, in anhydrous DMF following the solid phase coupling approach. After cleavage and deprotection from resin in strong acid solution, the above crude

polypeptide was obtained and dried in vacuum. Then, the polypeptide was purified by reverse-phase semi-preparative HPLC using 20 % - 80% water/acetonitrile gradient eluting system containing 0.1 % TFA, which was monitored by UV-Visible absorbance at 280 nm. After frozen and lyophilized, 8.6 mg white powder of pure peptide was obtained. Figure S1 shows the analytical HPLC graph for purified polypeptide. ESI-MS Found [$M+H^+$]: 728.3; calculated 727.3.

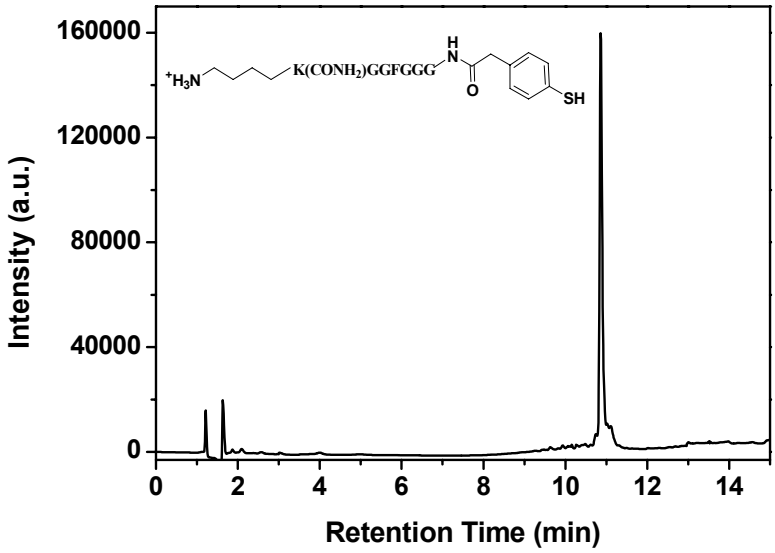



**Figure S1.** RP-HPLC chromatogram of purified polypeptide.

**Preparation of "trimethyl lock" conjugated polypeptide substrate.** To a stirred suspension of "trimethyl lock" compound **7** (15.6 mg, 0.06 mmol) in 200 µL anhydrous dichloromethane, thionyl chloride (43 µL, 0.59 mmol) was added dropwise at cooled temperature (ice bath). The reaction mixture was warmed slowly to room temperature and stirred 18 h. The solvent was evaporated in vacuum to give the oil product without purification. Then, the oil product was reacted with above purified polypeptide (8.6 mg, 0.012 mmol) in 150 µL anhydrous DMF followed by addition of triethylamine (13 µL, 0.093 mmol) under nitrogen atmosphere for 12 hours. After removing DMF, the crude product was purified by reversed-phase semi-preparative HPLC with 20 % - 80 % water/acetonitrile gradient system containing 0.1 % TFA to give the final "trimethyl lock" peptide substrate product 7.4 mg (53.1%). As shown in

Figure S2, the purified substrate was analyzed by analytical HPLC. ESI-MS Found [M+H$^+$]: 1220.63, [M+Na$^+$]: 1242.85; calculated 1219.6.

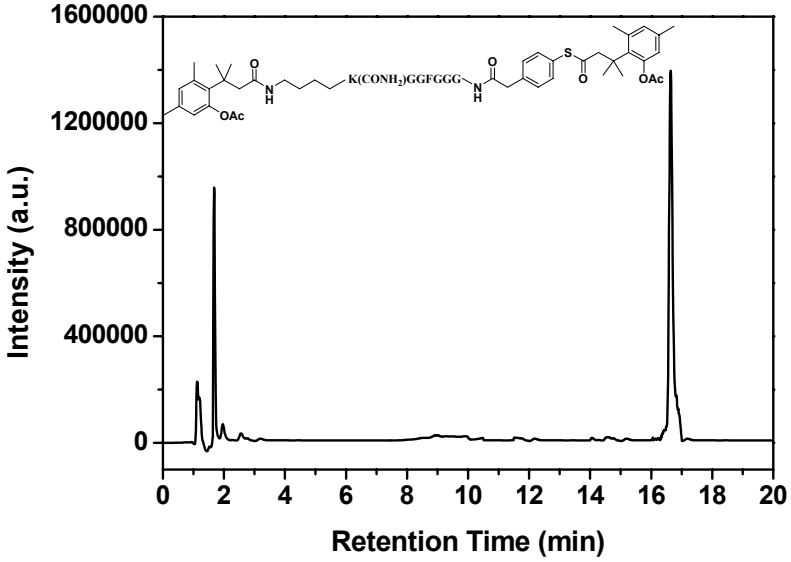



**Figure S2.** RP-HPLC chromatogram of "trimethyl lock" peptide substrate.

**Cleavage of "trimethyl lock" conjugated peptide substrate with esterase/thermolysin.** The "trimethyl lock" conjugated peptide substrate (85 μM) was firstly incubated with esterase (125 μg/mL) in PBS buffer (pH 7.4) for 2 hrs. Separately, another part of "trimethyl lock" peptide substrate (85 μM) and thermolysin (600 μg/mL) were mixed in PBS buffer (pH 7.4) for 4 hrs. Both of the reactions were monitored by RP-HPLC (20 % - 80 % water/acetonitrile containing 0.1% TFA linear gradient system).

Upon the esterase treatment, a peak with an elution time of 10.7 min was identified (Figure S3b) which corresponded to the polypeptide fragment $NH_2$-K($CONH_2$)GGFGGG-$CH_2$-Ph-SH as determined by ESI-MS: Found [M+H$^+$]: 727.6, calculated 727.3. Similarly, in the thermolysin proteolysis product, a peak at t = 14.6 min (Figure S3c) was observed which were corresponding to the fragment acetyl trimethyl lock-SH-Ph-$CH_2$-GGG as determined by ESI-MS: Found [M+H$^+$]: 585.9, calculated 585.6. RP-HPLC analysis of peptide substrate solution exposed to esterase/thermolysin confirmed that substrate has been quantitatively cleaved.

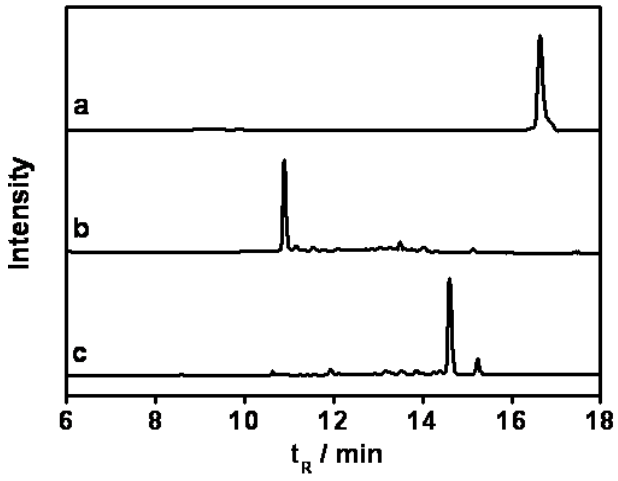



**Figure S3.** RP-HPLC profiles of cleavage experiments. a) RP-HPLC chromatogram of intact "trimethyl lock" peptide substrate; b) "trimethyl lock" peptide substrate treated with esterase; c) "trimethyl lock" peptide substrate hydrolyzed by thermolysin. The peaks at 10.7 min (trace b) and 14.6 min (trace c) correspond to the fragment $NH_2$-K($CONH_2$)GGFGGG-$CH_2$-Ph-SH and acetyl trimethyl lock-SH-Ph-$CH_2$-GGG, respectively.

**Stabilization of gold nanoparticles.** Purchased gold nanoparticles were stabilized by co-dissolving with freshly prepared dipotassium bis (*p*-sulfonatophenyl) phenylphosphine dihydrate solution (0.5 mM) for more than 10 h.[2] After centrifuging and harvesting the gold nanoparticles, PBS buffer solution was used to redissolve gold nanoparticles with the concentration of 8.2 nM.[3] The stabilized gold nanoparticles were stable in PBS buffer at room temperature over a period of days.

**Colorimetric assay for enzyme hydrolysis of "trimethyl lock" substrate:** To the solution of 0.4 mL of monodispersed dipotassium bis (*p*-sulfonatophenyl) phenylphosphane stabilized AuNPs (10 nm), the peptide substrate and esterase in 0.05 mL of PBS buffer (pH 7.4) were added to afford their final concentrations of 8.5 μM and 1.25 μg/mL, respectively. The mixture was incubated for 2 hrs at 37°C for enzyme hydrolysis. Gold nanoparticles were induced to highly aggregated clusters which displayed purple-blue color and surface plasmon resonance shifts from 520 nm to 600 nm. Both the decreased absorbance at 520 nm and the increased absorbance at 600 nm were monitored by UV-vis spectrum as time increased (Figure S4). The aggregated gold nanoparticles were further incubated with thermolysin

(60 μg/ml) for 4 hrs, the aggregated gold clusters were driven disassembly, which displayed red color and surface plasmon resonance shifts back from 600 nm to 520 nm again.

The different concentration of trimethyl lock substrate was found to influence the process of self-assembly and disassembly of gold nanoparticles. The different concentration of enzyme treated peptide substrate was incubated with AuNPs solution and the self-assembly and disassembly process were monitored by naked eyes and UV-Vis spectrotrophometer. As shown in Figure S5, the higher concentration of substrate induced the aggregation of gold nanoparticles in shorter time. While, in the process of the disassembly of the aggregated AuNPs, the higher concentration of trimethyl lock peptide substrate displays longer dispersion time upon thermolysin (60 μg/ml) cleavage (Figure S5b).

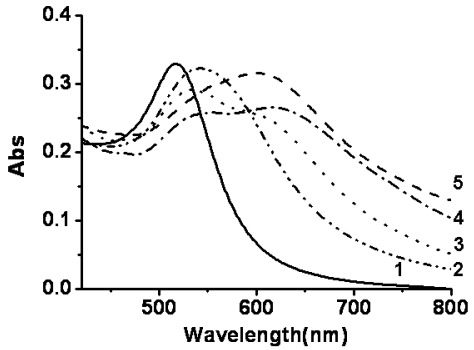



**Figure S4.** Uv-vis spectra of gold nanoparticles with "trimethyl lock" peptide substrate (8.5μM) treated with esterase (12.5μg/mL) as a function of time. The time interval is 0.5 hr from state 1 to state 5.

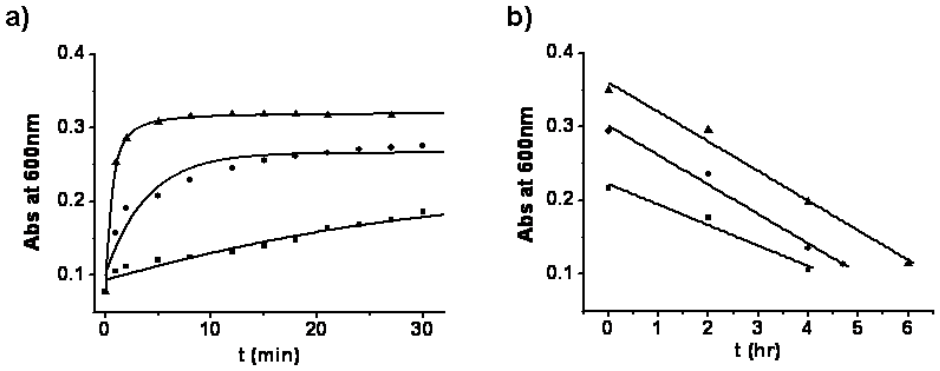



**Figure S5.** Absorbance change of gold nanoparticles at 600nm as a function of time with "trimethyl lock" peptide substrate 4.3 μM (square), 8.5 μM (circle) and 12.9 μM (triangle) and the corresponding concentration ratio of substrate/gold nanoparticles is 524 (square), 1036 (circle), 1573 (triangle),

respectively.[3] (a) "Trimethyl lock" peptide conjugate treated with esterase and gold nanoparticles. (b) Self-assembled gold nanoparticles treated with thermolysin for disassembly.

**Transmission electron microscopy (TEM) for particle size analysis.** All the TEM photographs of gold nanoparticles were taken on JEOL 2000 EX transmission electron microscope at 200 kV. The gold nanoparticles solution was dropped onto the carbon-coated copper grids (200 mesh) which had been pre-treated by UV-light to reduce static electricity. Then the nanoparticles solution was allowed to settle on grids for 5 mins before the excess solution was wicked away with filter paper.

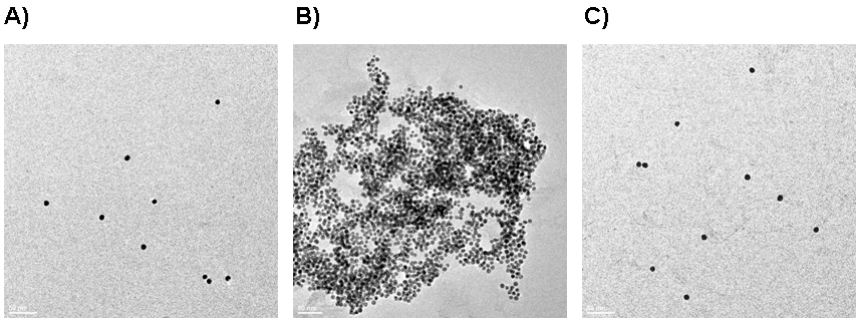



**Figure S6.** Transmission electron microscopy images of GNPs. A), GNPs with "trimethyl lock" peptide conjugate (8.5 μM); B), GNPs with esterase (1.25 μg/mL) treated "trimethyl lock" peptide conjugate (8.5 μM); C), Aggregated GNPs with protease thermolysin treatment (60 μg/mL). Scale bar: 50nm.

## References:

(1) Chan, Weng C; White, Peter D. *Fmoc solid phase peptide synthesis; a practical approach*. Oxford University Press: New York, 2000.

(2) C. J. Loweth, W. B. Caldwell, X. Peng, A. P. Alivisatos, P. G. Schultz, *Angew. Chem. Int. Ed.* 1999, **38**, 1808.

(3) X. Liu, M. Atwater, J. Wang, Q. Huo, *Colloids Surf. B Biointerfaces,* 2007, **58**, 3.