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Supporting Information

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Assembly of Nanoparticle-Protein Binding Complexes: From Monomer to Ordered Arrays

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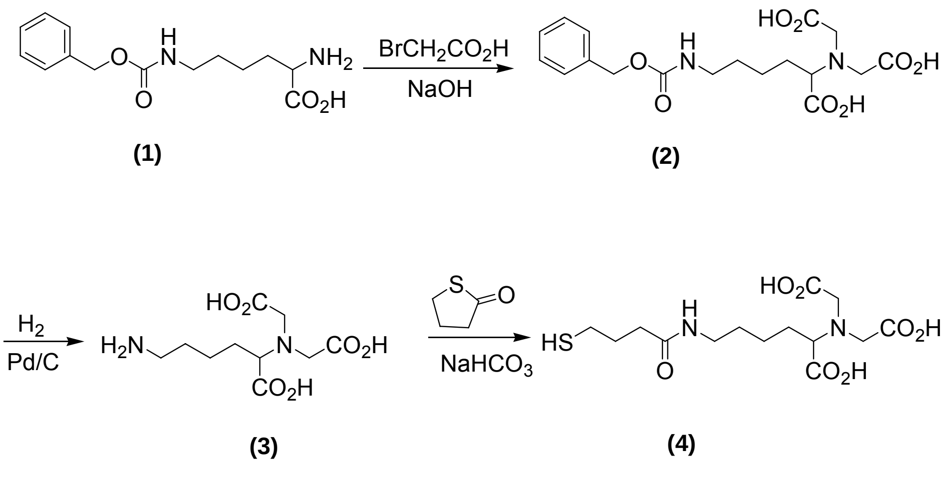
Materials and Methods

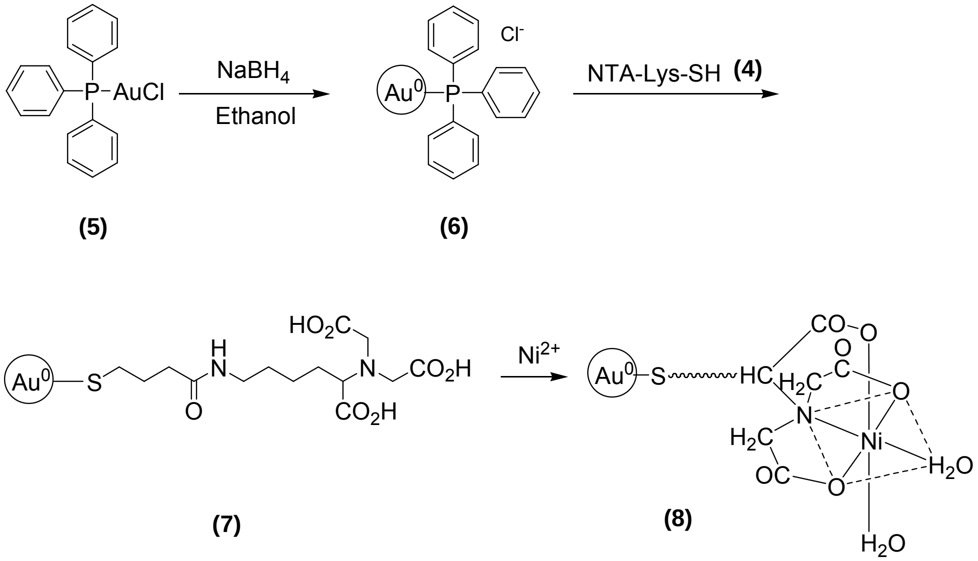
Materials. All chemicals, except where clarified, were purchased from Sigma-Aldrich Inc., and used without further purification.

Synthesis of (1S)-N-[5-[(4-Mercaptobutanoyl)amino]-1-carboxypentyl]iminodiacetic Acid (NTA-Lys-SH) (4). **4** was synthesized following a modified literature procedure,[1] as shown in Scheme 1. Briefly, *N*^{6}-carbobenzyloxy-L-lysine (**1**) was reacted with bromoacetic acid under basic conditions to form (1S)-N-(5-carbobenzyloxyamino-1-carboxypentyl)iminodiacetic acid (**2**). **2** was then deprotected by hydrogenolysis in 2:1 THF-water mixture (instead of the reported 20:1 methanol-water mixture)[1b, 1c] under 1 atm of H_2 for 20h to give (1S)-N-(5-amino-1-carboxypentyl)iminodiacetic acid (**3**). Reaction of **3** with γ-thiobutyrolactone gave NTA-Lys-SH (**4**) (1.0 g, 61% yield). ^{1}H NMR (400 MHz, D_2O, 300 K): δ 3.78 (m, 5H), 3.21 (m, 2H), 2.74 (t, *J* = 7.2 Hz, 2H), 2.37 (t, *J* = 7.0 Hz, 2H), 2.00 (q, *J* = 7.2 Hz, 2H), 1.96-1.75 (m, overlapping with the peak at 2.00 ppm, 2H), and 1.62-1.40 (m, 4H) ppm.



Scheme 1. Synthesis of NTA-Lys-SH.

Synthesis of 1.3-nm nickel-nitrilotriacetic acid (Ni-NTA) Au nanoparticles (NPs). Triphenylphosphine gold(I) chloride ($Au(PPh_3)Cl$) in an ethanol solution was reduced with sodium borohydride ($NaBH_4$),[2] then replaced (PPh_3) and chloride ions on surfaces of Au NPs with NTA-Lys-SH via ligand exchange and phase transfer reaction, followed by converting NTA-Lys-SH Au NPs into Ni-NTA-Lys-SH Au NPs (Scheme 2).



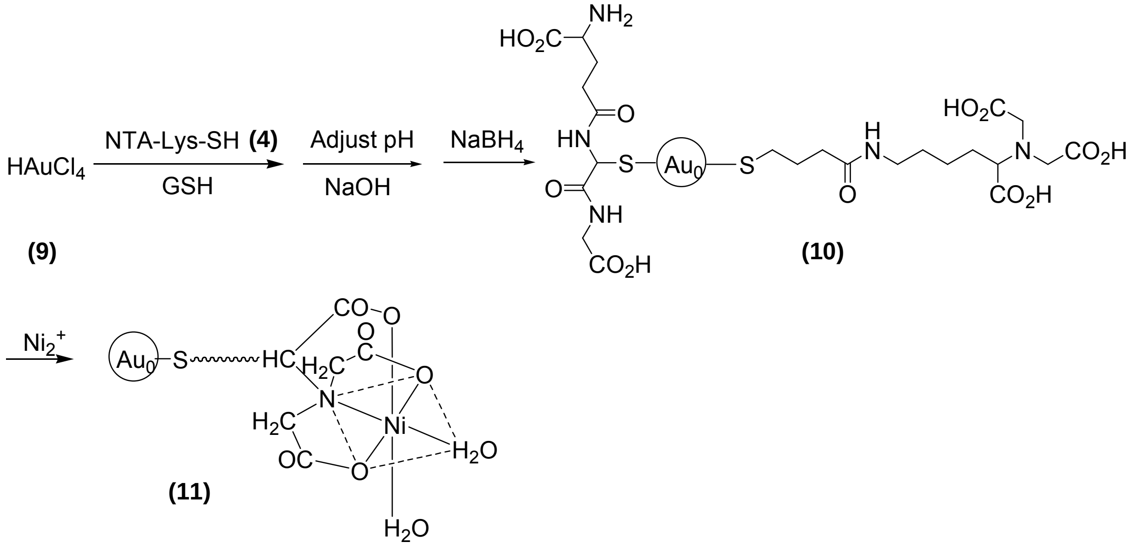
Scheme 2. Synthesis of 1.3-nm Ni-NTA functionalized Au NPs.

*Synthesis of Au NPs capped with triphenylphosphine chloride (**6**).* **6** were synthesized following a modified literature procedure.[3] $NaBH_4$ (38 mg, 1.01 mmol) was slowly added to a stirred solution of $Au(PPh_3)Cl$ (**5**) (500 mg, 1.01 mmol) in ethanol (27.5 mL) over a period of 15 min. After 2 h, the solution was poured into hexane (500 mL), and allowed to crystallize overnight at 4 °C. The brown solids were collected, and then washed successively with hexane, 1:1 CH_2Cl_2-hexane (4×10 mL), and 3:1 CH_2Cl_2-hexane (5 mL). The residue was redissolved in CH_2Cl_2, and further purified by column chromatography (silica; 9:1 CH_2Cl_2-methanol, then 4:1 CH_2Cl_2-methanol). The first brown fraction was collected and crystallized from CH_2Cl_2-hexane to give **6** (85 mg, 21% yield).

*Synthesis of 1.3 nm NTA-Lys-SH Au NPs (**7**).* A solution of NTA-Lys-SH (**4**) (33.5 mg, 92 μmol) in water (30 mL) was added to a solution of **6** (20 mg, 4.6 μmol) in $CHCl_3$ (30 mL). After the mixture was stirred vigorously at 55 °C for 16 h, the aqueous layer turned from colorless to brown due to the phase transfer reaction. The aqueous layer was collected and washed with $CHCl_3$ (3×15 mL). The crude product was concentrated by rotary evaporation, and purified by gel filtration chromatography (GH-25, water) to give **7** (10 mg). NMR analysis indicated the ligand exchange was complete and no $Au(PPh_3)Cl$ residues remained on Au NP surfaces. NMR analysis indicated the ligand exchange was complete. ^{1}H NMR (400 MHz, D_2O, 300 K): δ 3.78 (m, 5H), 3.19 (m, 2H), 2.72 (m, 2H), 2.35 (m, 2H), 2.05-1.75 (m, 4H), 1.65-1.35 (m, 4H) ppm. TEM analysis showed these Au NPs have an average size of 1.3 nm with a standard deviation of 0.5 nm (Figure S1).

*Synthesis of 1.3 nm Ni-NTA-Lys-SH Au NPs (**8**).* A cation exchanger CM-Sepharose (Pharmacia) was washed with water, then suspended in 0.05 M $NiCl_2$ aqueous solution, and then washed with water again. Finally, it was packed in a 5×100 mm column. An aqueous solution of **7** was passed through the nickel cation exchange column, collected, and used for the protein assembly experiments.

Synthesis of 4.4-nm Ni-NTA Au NPs. Hydrogen tetrachloroaurate ($HAuCl_4$) was reduced with $NaBH_4$ under the protection of mixed ligands of L-glutathione (γ-glu-cys-gly, GSH) and synthesized (1S)-N-[5-[(4-Mercaptobutanoyl)amino]-1-carboxypentyl]iminodiacetic (NTA-Lys-SH), followed by converting GSH/NTA-Lys-SH Au NPs into GSH/Ni-NTA-Lys-SH Au NPs (Scheme 3).



Scheme 3. Synthesis of 4.4-nm Ni-NTA functionalized Au NPs, and their binding to 6×His-tagged proteins.

*Synthesis of 4.4-nm (GSH/NTA-Lys-SH)-Au NPs (**10**).* A solution of **9** (8 mg, 0.022 mmol) in water (1.3 mL) was mixed with a solution of GSH (20 mg, 0.066 mmol) in water (3.4 mL). The mixed solution was added to a solution of $HAuCl_4$ (5 mg, 0.015 mmol) (Strem Chemical) in water (0.5 mL), followed by adjusting the pH to 6.0-6.5 with 1 N NaOH. Finally, a solution of $NaBH_4$ (5.6 mg, 0.15 mmol) in water (2.8 mL) was added to the above solution, and was vigorous stirred for 10 min at rt., then stayed overnight. The NTA Au NPs were purified by using GH-25 gel column chromatography, followed by centrifugal concentration with Amico Ultra-4 centrifugal filter (Millipore). TEM analysis shows these Au NPs have an average size of 4.4 nm with a standard deviation of 1.0 nm (Figure S2). Their size distribution can be narrowed down to 0.7 nm by further centrifugation, giving Au NPs with an average size of 3.7 nm (Figure S3). These NPs are stable in aqueous solutions at 4 ºC for 3 months.

*Synthesis of 4.4-nm (GSH/Ni-NTA-Lys-SH)-Au NPs (**11**).* A solution of **10** was passed through an ion exchange column treated with $NiCl_2$ solution, following the same procedure as that of modifying 1.3-nm NTA Au NPs, to give **11**, and used for the protein assembly experiments.

Scheme 4. Binding of Ni-NTA Au NPs to 6×His-tagged proteins.

Assembly of Ad12 knob and Mtb 20S proteasome proteins. For Ad12 knob proteins, 14 μl (1 mg/mL, 0.24 nmol) protein solution in 150 mM NaCl and 10 mM PBS at pH 7.5 was added to 40 μl (1 μM, 0.04 nmol) aqueous solution of 1.3-nm or 4.4-nm Ni-NTA Au NPs. The mixed solution was kept at rt. for 3 min, and used for transmission electron microscopy (TEM) analysis. The binding complexes of 1.3-nm Ni-NTA Au NPs and Ad12 knob proteins were further separated by using size exclusion high performance liquid chromatography, and the first two components were collected, and analyzed by using scanning transmission electron microscopy. For Mtb 20S proteasome proteins, 40 μl (3 mg/mL, 0.16 nmol) protein solution in 150 mM NaCl and 10 mM phosphate buffered saline (PBS) at pH 7.5 was added to 40 μl (1 μM, 0.04 nmol) aqueous solution of 3.7-nm Ni-NTA Au NPs. Additional PBS and NaCl were added to keep their concentrations the same as before in the protein solution. Finally, the solution was incubated at 4 ºC for 2-4 days, followed by TEM analysis.

Chromatography. Size exclusion high performance liquid chromatography (SE-HPLC) was performed for the binding complexes of 1.3-nm Ni-NTA Au NPs and Ad12 knob proteins. The chromatographic system consisted of a Superdex 200 column (Amersham Biosciences, 10 × 300 mm, 13 μm) and an HPLC system (HP 1040A). The sample solution was analyzed at rt. using a solution (150 mM NaCl, 0.01 M PBS, pH 7.4) at a flow rate of 0.5 mL/min at a pressure of 1.2 MPa. Absorbance at 280 nm was monitored. Two major fractions at 32.0 and 37.4 min were collected, respectively (Figure S4), and used for scanning transmission electron microscopy analysis.

Electron Microscopy. Negative stain (nano-W, Nanoprobes Inc.) images of proteins were recorded using a JEM 1200CX (JEOL) operating at 120 kV at a low electron dose (< 10 e/Å^2) with either a 1k×1k CCD camera (Gatan) or electron image films (Kodak SO-163). TEM specimens were prepared by evaporating sample solutions on plasma-cleaned Cu mesh grids covered with carbon thin films. Dark field images of unstained, freeze-dried proteins were recorded using a scanning transmission electron microscopy (STEM facility at the Brookhaven National Laboratory) operating at 40 kV. The sizes of Au NPs were analyzed with TEM images using ImageJ software (NIH). Cryo-TEM images were recorded using a JEM 2010F (JEOL) operating at 200 kV at a low electron dose. Cryo-TEM specimens were prepared using a glow-discharged quanti-foil grid (Ted Pella) and a Vitrobot (FEI). Images were recorded on a Kodak SO-163 negative film at a magnification of 50,000, and digitized in a high resolution scanner (Nikon Coolscan 8000) with a step size of 2000 dots/inch (2.54 Å/pixel).

Image Processing. Image averaging was done using imaging processing packages of SPIDER[3] and EMAN[4] through a procedure of micrograph screening, defocus value estimation, picking particles, phase contrast correction, and averaging on a Linux workstation.

Supporting Figures

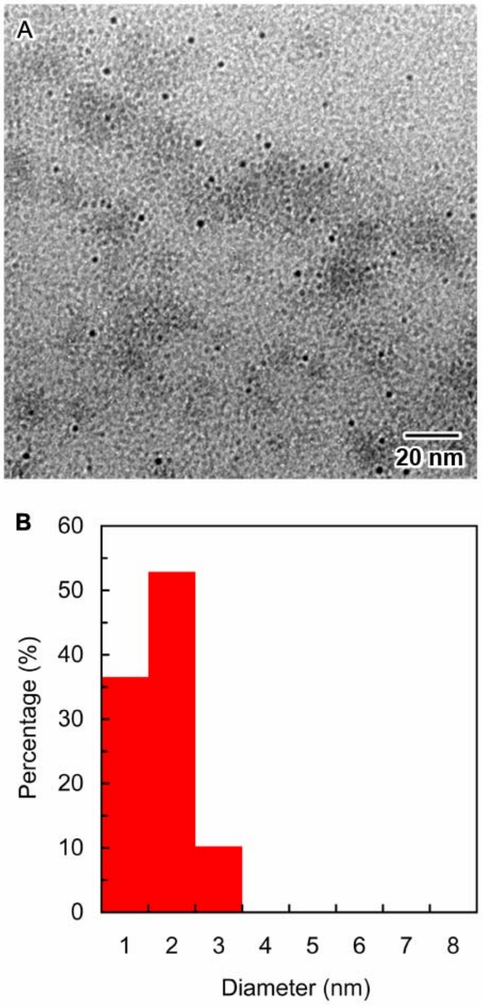


Figure S1. As-synthesized 1.3-nm GSH/NTA-Lys-SH Au NPs. (a) TEM image of well-dispersed Au NPs. (b) Size distribution showing an average size of 1.3 nm with a standard deviation of 0.5 nm.

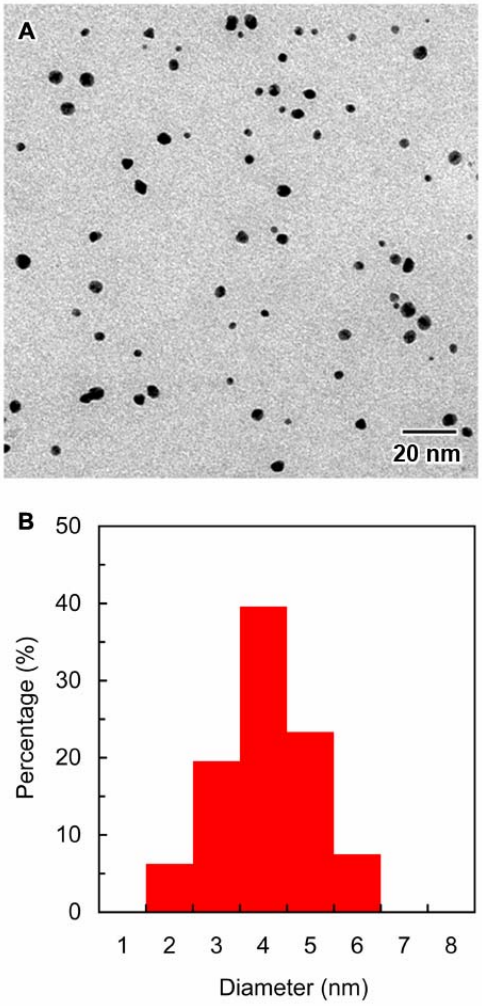


Figure S2. As-synthesized 4.4-nm GSH/NTA-Lys-SH Au NPs. (a) TEM image of well-dispersed Au NPs. (b) Size distribution showing an average size of 4.4 nm with a standard deviation of 1.0 nm.

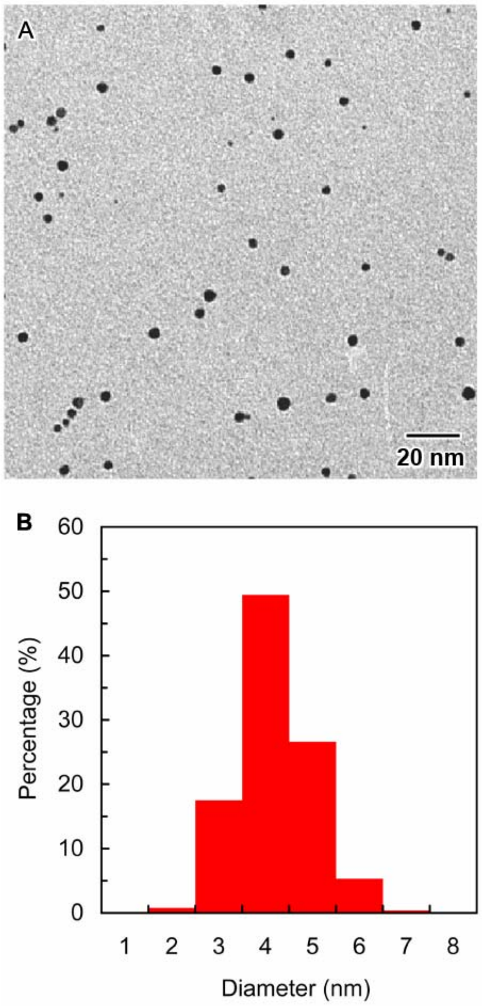


Figure S3. Size-selected 3.7-nm GSH/NTA-Lys-SH Au NPs by centrifugation from as-synthesized 4.4-nm Au NPs. (a) TEM image of well-dispersed Au NPs. (b) Size distribution showing an average size of 3.7 nm with a standard deviation of 0.7 nm.

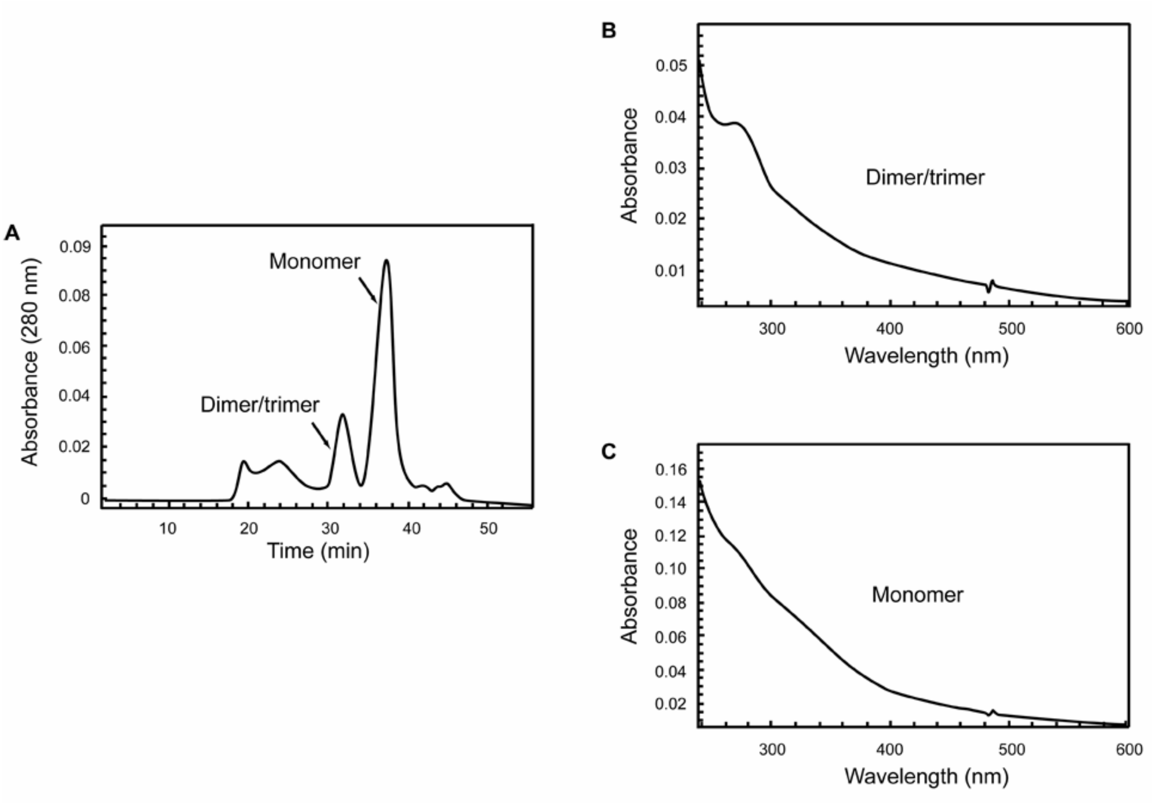
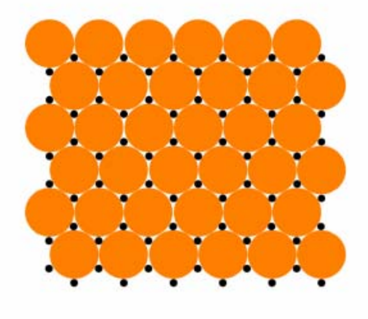
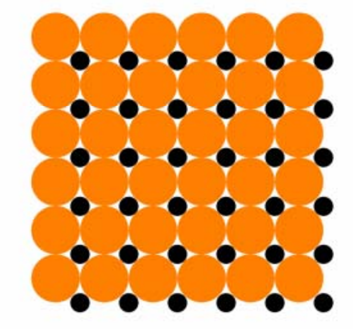
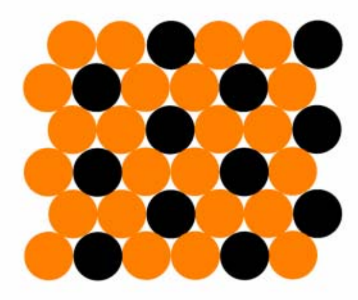


Figure S4. Separation of monomer and dimer (occasional trimer) of binding complexes of 6×His-tagged Ad12 knob proteins and 1.4-nm Ni-NTA Au NPs. (a) SE-HPLC profile of binding complexes. UV-Vis spectra of two fractions collected at (b) 32.0 and (c) 37.4 min, both of which show the absorption characteristics of 1.3-nm Au NPs (continuously from 200 to 500 nm) and proteins (280 nm). The fraction between 18 and 28 min was found to be disordered aggregates of Au NPs and proteins.

Table S1. Simple packing constraint in 2D assembly of binary systems. The black particle presents the NP, and the orange particle presents the protein, B. The size ratio of 3.7-nm Au NPs to 11.5-nm proteasome proteins was estimated to be between 0.32 (without a Ni-NTA layer) and 0.60 (with a fully extended 1.6-nm Ni-NTA layer), which agrees with the steric hindrance in tetragonal packing (0.41-1.00).

Size ratio r_A/r_B	Stoichiometry A:B	Architecture	Illustration
0 – 0.155	1:1	Linear	
0.155 – 0.414	2:1	Hexagonal	
0.414 – 1.000	1:1	Tetragonal	
> 1.000	1:2	Hexagonal	

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