Supramolecular Tailoring of Protein-Nanoparticle Interactions using Cucurbituril Mediators

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Supporting Information

Experimental section

Preparation of nanoparticles

Gold nanoparticles (AuNPs) protected with 1-pentanethiol (C5) and diaminohexane-terminated thiol ligands (DAH) were prepared following the reported procedure.[1] NPs functionalized with DAH moiety were prepared following the ligand exchange procedure.[2] In a typical reaction, 10 mg of C5 functionalized NP (NP**C5**) was first dissolved in 1 mL of dry dichloromethane (DCM). DAH ligands (10 mg for NP**1** and 40 mg for NP**2**) were dissolved in 5 mL of organic solvent (DCM:MeOH (4:1 v/v)), and then added to the solution of NP**C5**. The reaction mixture was stirred at room temperature for 48 h. After 48 h, the organic solvent was evaporated and NPs were dissolved in MilliQ water. The aqueous solution of the NPs was purified by dialysis.

Cloning and expression of GFP

Genetic engineering manipulation and protein expression were done according to previously reported methods.[3] To construct pQE80-6xHis-GFP plasmid, GFP gene was PCR amplified and cloned from pET21-d EGFP plasmid into *Bam*HI and *Hind* III (downstream of 6xHis tag) restriction sites of pQE80 expression vector. To produce recombinant proteins, plasmid containing the respective gene was transformed into *Escherichia coli* BL21(DE3) strain. A transformed colony was picked up to grow small cultures in 50 mL 2xYT media at 37 °C for overnight. The following day, 15 mL of grown culture was inoculated into one liter 2xYT media and allowed to grow at 37 °C until OD reaches 0.6 by using UV-Vis spectroscopy. At this point, GFP expression was induced by adding isopropyl-b-D-thiogalactopyranoside (IPTG; 1 mM final concentration) at 25 °C. After 16 h of induction, the cells were harvested and the pellets were lysed using microfluidizer. His-tagged GFP were purified from the lysed supernatant using HisPur cobalt columns. The integrity and the expression of native protein were determined by a 12% SDS-PAGE gel and absorbance spectra.

Mass spectrometry instrumentation and conditions

Both laser desorption/ionization mass spectrometry (LDI-MS) and matrix assisted laser desorption/ionization mass spectrometry (MALDI-MS) were performed at positive mode on a Bruker Autoflex III time-of-flight mass spectrometer (Autoflex III). All mass spectra were acquired in reflection

mode. Operating conditions were as follows: ion source 1= 19.00 kV, ion source 2= 16.60 kV, lens voltage= 8.44 kV, reflector voltage= 20.00 kV, and reflector voltage 2= 9.69 kV. For the LDI-MS experiments, 2.5 μL of NP solutions were applied to a metal sample target. After allowing it to dry, the LDI-MS analysis was performed. A saturated α-CHCA stock solution was prepared in 70 % acetonitrile, 30 % H_2O and 0.1 % trifluoroacetic acid for the MALDI-MS analyses. This stock solution was added an equal volume of analyte solutions of the NP-CB[7] complexes. 2.5 μL of this mixture was applied to target and allowed it to dry before the MALDI-MS analysis was performed.

Fluorescence titration assays of GFP-NP and ADA-CB[7] complexation

GFP-NP complexations were carried out in sodium phosphate buffer (5 mM, pH 7.4) using a 96-well plate. GFP (100 nM) was conjugated with various concentrations of NPs in a 96-well plate to produce a range of NP/GFP binding ratio from 0 to 1.6 (final volume for each well was 200 μL). After 30 min of conjugation, the fluorescence endpoint was measured with an excitation wavelength of 475 nm and recorded the fluorescence wavelength of 510 nm using a Molecular Devices SpectraMax M5 microplate reader.

For the ADA-CB[7] complexation, GFP-NP**1**, GFP-NP**1**/50CB[7] and GFP-NP**2**/100CB[7] complexes were conjugated as described previously. After 30 min of conjugation, ADA was added to each well (final concentration of 100 μM) for another 15 min before the fluorescence of GFP was measured.

NPTMA preparation

TMA ligand was synthesized following the procedure reported.[4] The ligand exchange process was similar as the preparation of NP**1**, where 10 mg of NP**C5** and 10 mg of TMA ligand were used in the reactions.

β-galactosidase (β-gal) activity assay studies

The β-galactosidase (β-gal) was purchased from Sigma-Aldrich and the colorgenic substrate chlorophenol-red-β-D-galactopyranside (CPRG) was purchased from Roche Applied Science. In this study, β-gal, nanoparticle and CPRG solutions were prepared in sodium phosphate buffer solution (5 mM, pH 7.4). In the activity assay studies, β-gal (1 nM) was conjugated with various concentrations of NPs in a 96-well plate, and the final volume for each well was 200 μL. After 30 min conjugation, CPRG was added for another 10 min (final concentration of CPRG was 1.5 mM). The enzymatic activity was followed by monitoring product formation at absorbance wavelength of 595 nm using a Molecular Devices SpectraMax M5 microplate reader.

Different chemical agents were used to interfere with the major interactions in the complexes

GFP-NP and GFP-NP/CB[7] complexes were conjugated in a 96-wells plate for 30 min before the adding of different chemical reagents with different final concentrations (i.e., NaCl (400 mM), Tween-20 (1%) and urea (400 mM)). After incubation with the chemical agents for another 15 min, the fluorescence of GFP was measured and analyzed.

Different proteins were used to investigate the selective interactions between particles and proteins

GFP-NP**1** and GFP-NP**1**/50CB[7] complexes (GFP:NP = 1:1, 200 μL) were conjugated in a 96-wells plate for 30 min before the addition of protein solutions (10 μL of bovine serum albumin (BSA), lysozyme (Lys) or β-galactosidase (β-gal)), where the final concentration of the protein was 100 nM. After 30 min incubation with protein, the fluorescence of GFP was measured and analyzed.

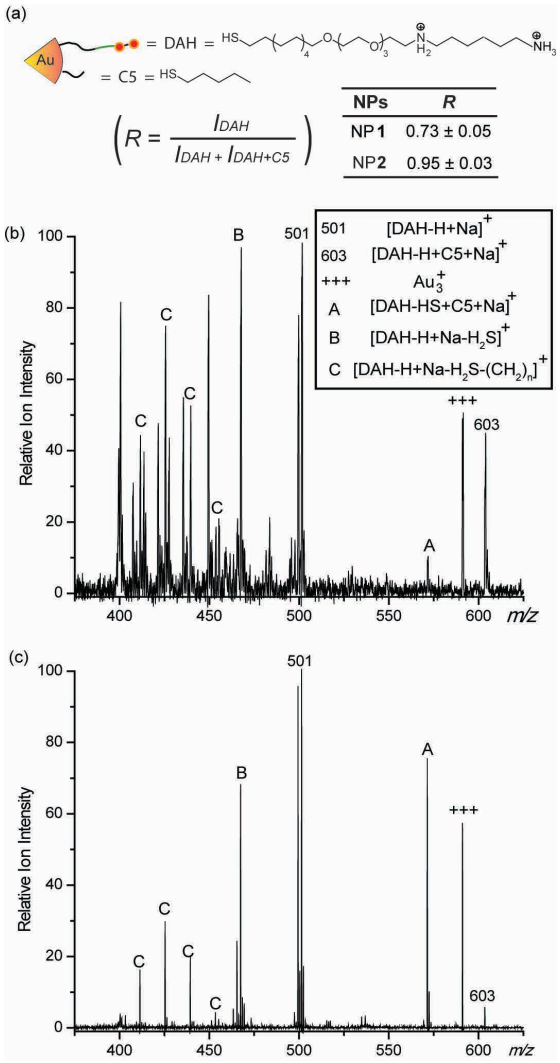

Fig. S1 (a) Structure of a mixed monolayer-protected AuNP featuring pentanethiol (C5) and diaminohexane (DAH) terminated thiol ligand. (b) NP**1** and (c) NP**2** presented different coverages of DAH ligand based on the LDI mass spectra. In the LDI mass spectra, *R* value was defined as the peak intensity ratio of DAH ($\boldsymbol{I}_{DAH}$, where M_{DAH} was *m/z* 501, sodium adduct ion)) to the sum of DAH ($\boldsymbol{I}_{DAH}$) and disulfide species DAH+C5 ($\boldsymbol{I}_{DAH+C5}$, where M_{DAH+C5} was *m/z* 603, sodium adduct ion). The spectral analysis showed that the coverage of DAH ligand on NP**2** (R_{NP2}= 0.95 ± 0.03) was higher than that on NP**1** (R_{NP1}= 0.73 ± 0.05).

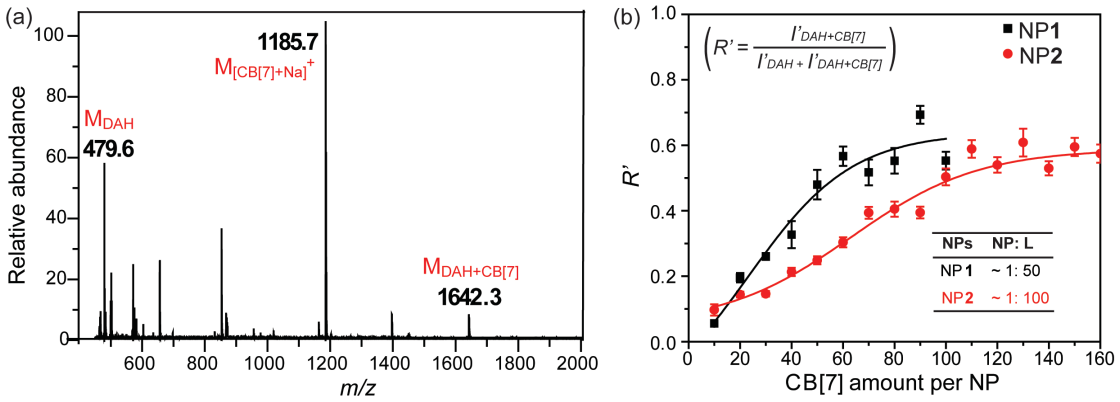

Fig. S2 (a) A representative MALDI mass spectrum used for the calculation of CB[7] binding amounts on NP surface. The characteristic ion peaks of DAH ligand (M_{DAH} *m/z* 479) and DAH/CB[7] ligand ($M_{DAH+CB[7]}$ *m/z* 1642) on NP surface are shown. (b) Mass spectrometric titration experiments were performed by adding different amounts of CB[7] to a fixed amount of NPs (final concentration of 2 μM) to calculate the saturated CB[7] amount on each NP. The peak intensity ratio (*R'*) of DAH+CB[7] ($\boldsymbol{I'}_{DAH+CB[7]}$) to the sum of DAH ($\boldsymbol{I'}_{DAH}$) and DAH+CB[7] ($\boldsymbol{I'}_{DAH+CB[7]}$) gradually increased upon addition of CB[7], indicating the continuous attachment of CB[7] to the terminal DAH ligands on NP surface in the titration process. The *R'* value reached a saturation at a CB[7] to NP ratio of ~50 and ~100 for NP**1** and NP**2**, respectively.

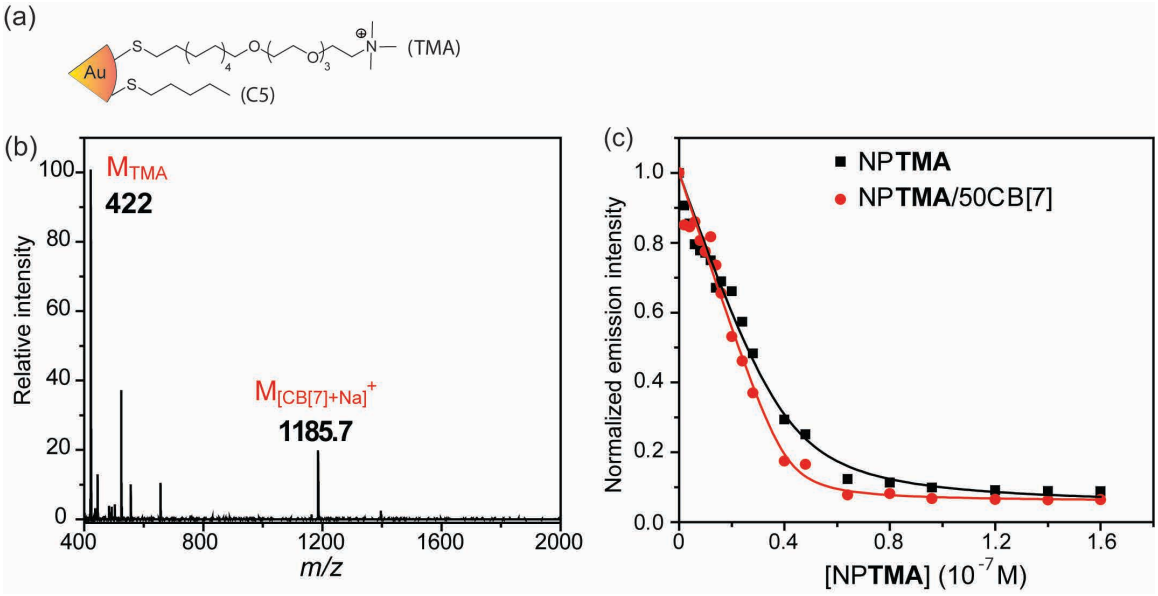

Fig. S3 (a) Structure of NP**TMA** featuring mixed monolayers of pentanethiol (C5) and trimethylamine (TMA) terminated thiol ligand. (b) Mass spectra of NP**TMA**/50CB[7]. (c) GFP and NP**TMA** complexations in the absence and presence of CB[7].

References

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