Supramolecular Tailoring of Protein-Nanoparticle Interactions using Cucurbituril Mediators

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Abstract

Supramolecular modification of nanoparticle surfaces through threading of cucurbit[7]uril (CB[7]) onto surface ligands is used to regulate protein-nanoparticle interactions.

Nanoparticles (NPs) provide tailorable surface chemistries that allow facile modulation of protein-NP interactions. [1] These interactions are useful tools for modulating enzymatic activity,[2] delivering proteins [3] and sensing cancerous cells. [4] Furthermore, regulating protein-NP assemblies offers an effective route to construct novel hybrid materials and devices.[5]

Supramolecular host-guest chemistry provides an alternative to covalent approaches[6] to modulating NP surface functionality.[7] The properties of monolayers on NP surface can be efficiently tailored through the complexation with guest molecules, where the physicochemical properties (e.g., hydrophobicity and charge) of the guest molecules are imparted to the NP surface. In reported studies, tailored surface charge, [8] hydrophilicity/phobicity [9] and redox potential [10] of NPs have been achieved through the reversible threading/dethreading of the guest molecules on the NP surface. This supramolecular tailoring approach provides an important "post-synthetic" strategy for surface modification of NPs to regulate molecular recognition and binding strength of the target molecules.[8]

We report here the use of supramolecular host-guest chemistry to modulate protein-NP interactions through control of the hydrophilicity/phobicity of the NP surface. Gold nanoparticles (AuNPs) functionalized with a diaminohexane motif were modified using cucurbit[7]uril (CB[7])[11] to form pseudorotaxane structures (Scheme 1a). Binding of CB[7] to the NP surface modulated the surface properties of NPs, with concomitant regulation of protein-NP interactions. The complexation of the CB[7]-diaminohexane motifs on NP surface was quantified by matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) and the protein-NP interactions were studied through fluorescence titrations.

Increased binding constants (K_s) and binding stoichiometries (n) of the protein-NP complexes were observed with increasing amounts of CB[7] bound on NP surface (Scheme 1b), demonstrating that the CB[7] molecules act as non-covalent regulators for controlling protein-NP interactions in a specific and reversible fashion.

Cationic AuNPs featuring diaminohexane (DAH) functional groups were prepared following the reported procedure.[12] Briefly, pentanethiol (C5) capped AuNPs (~2 nm core diameter) were synthesized through Brust-Schiffrin two-phase method[13] and used for ligand exchange reactions.[14] Different amounts of DAH ligand were used in the ligand exchange process to generate two AuNPs with different ligand coverages (NP**1** and NP**2**) (See ESI[†]). These two NPs allowed us to investigate the role of ligand coverage in the protein-NP interactions. The coverage of DAH ligand on each NP was determined by laser desorption/ionization mass spectrometry (LDI-MS)[15] (Fig. S1, ESI[†]). It was observed that the coverage ratio of DAH ligand on NP**1** was 0.73 and that of NP**2** was 0.95.

The DAH moiety on the NP surface serves as a recognition unit for complexation with cucurbit[7]uril (CB[7]) (association constant $\sim 1 \times 10^8$ M^{-1}).[16] Matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) was used to quantify the number of CB[7] units around a single NP by titrating CB[7] into NP solution (Fig. S2, ESI[†]). The CB[7] binding amounts per NP were ~ 50 and ~ 100 for NP**1** and NP**2**, respectively.

The effect of supramolecularly modified NPs on protein-NP interactions was investigated by studying the binding with a model protein, green fluorescent protein (GFP). In this study, anionic GFP (pI 6.0 at pH 7.4) [17] efficiently bound with positively charged AuNPs, with concomitant fluorescence quenching by AuNPs.[4] From the fluorescence titrations, it was clear that the fluorescence intensity of GFP decreased upon the addition of NPs, where NP**2** displayed more efficient quenching ability (Fig. 1a). The higher quenching efficacy of NP**2** was expected based on the higher number of cationic ligands available to electrostatically bind to GFP surface. These results were consistent with the reported studies demonstrating that the particles with higher cationic ligand coverage can interact more strongly with proteins.[18]

To investigate the effect of CB[7] on the GFP-NP complexations, CB[7]-threaded NPs (NP/CB[7]) were prepared with varying CB[7] to NP ratios. When the NP/CB[7] complexes were titrated with GFP, the particles quenched the fluorescence of GFP in a CB[7] concentration-dependent manner (Fig. 1b and 1c). The binding parameters such as binding constant (K_s) and binding stoichiometry (n) of the GFP-NP conjugates were determined through nonlinear least-squares curve-fitting of the titration data.[19] The correlation plots of K_s and n of GFP-NP and the corresponding GFP-NP/CB[7] complexes were shown in Fig. 2. Both the K_s and n values of GFP-NP/CB[7] complexes were higher compared to that of GFP-NP complexes, indicating that the NPs/CB[7] presented an increased protein binding affinity as well as the amount of protein bound. This CB[7]-responsive binding behavior of GFP-NP complexes were further confirmed by the higher fluorescence quenching with increasing CB[7] amounts at a fixed GFP:NP**1** ratio (4:1) (Fig. 1d). Taken together, the NP/CB[7] complexes exhibited a greater GFP binding efficiency than the NPs only. In addition, controlling the amount of CB[7] enabled the tuning of protein-NP interactions.

A significant difference in the GFP-NP interactions was reflected in the larger change in the slope of the titration curve for NP**1** than that of NP**2** at the same amount of CB[7]. At a certain amount of CB[7] the change in K_s for NP**1** was much greater than that of NP**2**, such as the change of K_s for NP**1** was ~ 20-fold compared to that for NP**2** (~ 8-fold) at the CB[7] to NP ratio of 50. These results demonstrated that a greater impact of CB[7] on regulating the GFP-NP complexations was observed for NP**1**, indicating that the NPs with lower cationic ligand coverage possessed a broader modulation window. Therefore, the cationic ligand coverage on NPs not only influenced the GFP-NP interactions but also determined the impact of CB[7] on regulating protein-NP binding.

The CB[7] moiety is a good synthetic receptor for amino acids (e.g., tryptophan and phenylalanine), peptides and proteins.[20] Thus, it can potentially affect GFP-NP interactions by binding to GFP. To test the effect of CB[7] on the present GFP-NP/CB[7] binding, we used trimethylamine-functionalized NP (NP**TMA**) as a negative control, wherein CB[7] did not bind to trimethylamine terminal group (Fig. S3a and S3b, ESI[†]). A minor change in the titration curve of the GFP-NP**TMA** complexes was observed in the presence of free CB[7] in solution (Fig. S3c, ESI[†]). From these control studies it can be inferred that CB[7] molecules did not have significant impact on the GFP-NP interactions through binding to surface functionality of GFP.

Reversibility of host-guest binding using CB[7] chemistry provides an important tool to modulate surface properties of the supramolecular complexes. We utilized the competitive disruption of the NP/CB[7] complexes by 1-adamantylamine (ADA) to tune the surface properties of the NPs. ADA was used as a competitive guest molecule to dethread CB[7] from NP surface to form more favorable ADA-CB[7] complexes (association constant ~1.7 × 10^{12} M^{-1}).[12,16] After adding ADA to the solution of GFP-NP**1**/50CB[7] complexes, the fluorescence titration curve of GFP-NP**1**/50CB[7] was very similar to that of GFP-NP**1** (Fig. 3a). A similar result was observed for GFP-NP**2**/50CB[7] complexes (Fig. 3b), demonstrating the utility of this mode of protein binding regulation.

We utilized GFP fluorescence to probe the major non-covalent interactions involved in protein-NP and protein-NP/CB[7] complexation, The effect of different chemical additives on the interactions in the GFP-NP and GFP-NP/CB[7] complexes was explored: NaCl (electrostatic attractions), Tween-20 (hydrophobic interactions) and urea (hydrogen bonding). In the presence of these chemical reagents, the fluorescence recovery of GFP was observed when the complexes were treated with NaCl and Tween-20, while the negligible change was shown in the presence of urea (Fig. 4). These results indicated that both electrostatic and hydrophobic interactions contributed in GFP-NP and GFP-NP/CB[7] complexation. It should be noted that NP/CB[7] still presented a stronger GFP affinity compared to the NP in the presence of Tween-20 that disrupted the hydrophobic interactions (Fig. 4b and 4e), suggesting stronger electrostatic interactions involved in GFP-NP/CB[7] complexes than that in GFP-NP complexes.

NPs with hydrophobic and charged surface tend to adsorb more proteins due to higher protein binding affinity or the presence of more protein binding sites. [21] CB[n] possesses a hydrophilic exterior and a hydrophobic cavity.[11] However, the "equatorial" region of the

outer surface of CB[n] is somewhat positively charged as observed in its electrostatic surface potentials[22] and is hence able to interact with negatively charged molecules.[23] According to our findings, NP/CB[7] presented stronger electrostatic interactions with GFP, implying that the NPs possessed higher cationic surface charge after CB[7] complexation. Therefore, the threading of CB[7] on NP surface may enhance the surface charge of NPs and subsequently increase the protein binding affinity and the amounts of protein binding.

Building on our GFP studies, we next investigated the effect of these supramolecularly tailored NPs on regulating enzyme activity. β-galactosidase (β-gal), a negatively charged protein (pI 4.6 at pH 7.4), can be inhibited by cationic AuNPs through electrostatic complex formation.[24] The inhibition of β-gal was studied with NP**1**, NP**1**/50CB[7], NP**2** and NP**2**/100CB[7]. The activity of β-gal was monitored by the hydrolysis of the chromogenic substrate chlorophenol red β-D-galactopyranoside (CPRG), where the active β-gal converted yellow substrate into red product to provide a colorimetric readout. As shown in Fig. 5a, the extent of inhibition increased with increasing of NP concentration. It was observed that NP**1**/50CB[7] and NP**2**/100CB[7] produced a greater enzyme inhibition than the uncomplexed NP**1** and NP**2**, respectively. As observed with GFP, NP**1** exhibited a larger change in the slope of the inhibition curve than that of NP**2**. NP**1** threaded with different amount of CB[7] was further used to inhibit the activity of β-gal at a fixed β-gal:NP ratio (1:15) (Fig. 5b). The β-gal activity was inhibited by NP**1** in a CB[7] concentration-dependent manner, indicating the amount of CB[7] threaded on NP**1** enabling the fine-tuning of β-gal activity.

We also investigated the selective protein binding ability of NPs in the absence and presence of CB[7]. Three proteins (i.e. bovine serum albumin (BSA), lysozyme (Lys) and β-gal were added into the GFP-NP**1** and GFP-NP**1**/50CB[7] complex solutions, with different fluorescence responses were generated due to the competitive binding of proteins on NP surface (Fig. 6). The increased fluorescence indicated that GFP was released from NP surface by BSA and β-gal, while the addition of Lys did not show obvious fluorescence change. These results demonstrated that different proteins presented different binding affinities to NP**1** and NP**1**/CB[7]. It also indicated the use of CB[7] can potentially make NPs selectively bind to a target protein, which is a promising property of NPs in the design of biosensors.[25]

In this study we used NPs with 2 nm core size to demonstrate the tunable surface properties of NPs by adding different amounts of CB[7]. This general strategy can be applied to NPs with different sizes to modulate the NP surface properties as well as the protein-NP interactions. However, the curvature of the NP determines the organization of the self-assembled monolayers (SAMs)[26] and protein adsorption[27] on NP surface. The addition of CB[7] to the NPs with different curvature may affect the SAMs and regulate the protein-NP interactions. The role of NP curvature along with CB[7] molecules on the SAMs and protein adsorption could be further investigated using computational stimulation[28] and analytical tools.[29]

In summary, we have demonstrated that protein-NP interactions can be efficiently tailored by threading different amounts of CB[7] on NP surface. We showed that supramolecular

host-guest chemistry provided a simple and straightforward method to modulate the surface properties of NPs and concomitantly regulate the protein-NP interactions. Taken together, this supramolecular tailoring approach can open new possibilities to effectively control protein stabilization for biotechnology and delivery applications.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Notes and references

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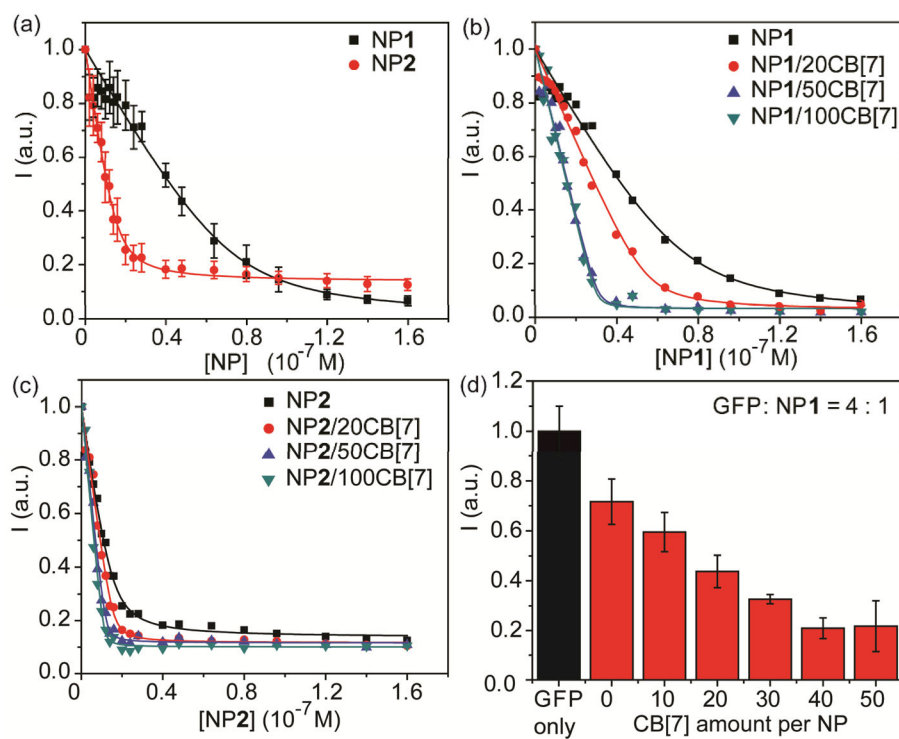**Fig. 1.**
(a) Fluorescence titration of GFP with NP**1** and NP**2**, where the abbreviation "I" represents the fluorescence intensity of GFP. (b) NP**1** and (c) NP**2** in the presence of different CB[7] amounts on NP surface were titrated with GFP. (d) Fluorescence intensity of the GFP-NP**1** complexes in the presence of different CB[7] amounts on NP**1** surface at a fixed GFP to NP**1** ratio of 4. Each titration experiment was performed in three replicates.
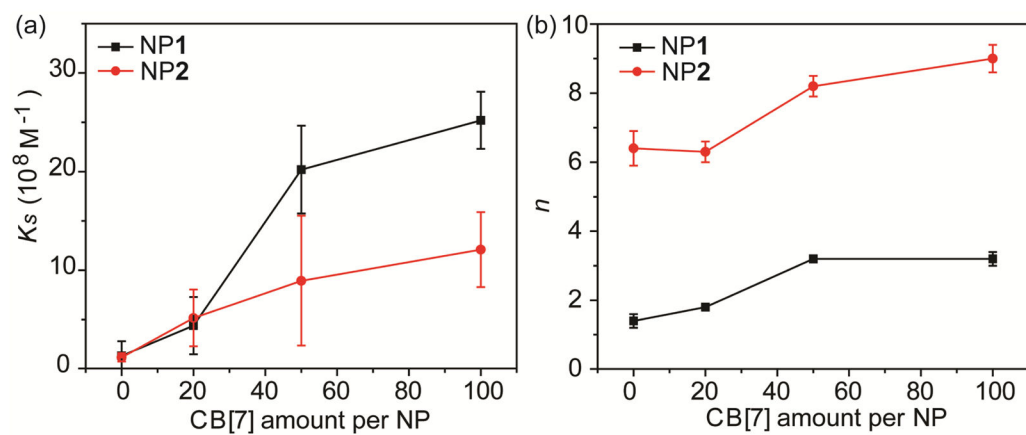
Fig. 2.

Correlation between (a) K_s and (b) n values of the GFP-NP complexes in the presence of different CB[7] amounts on NP surface
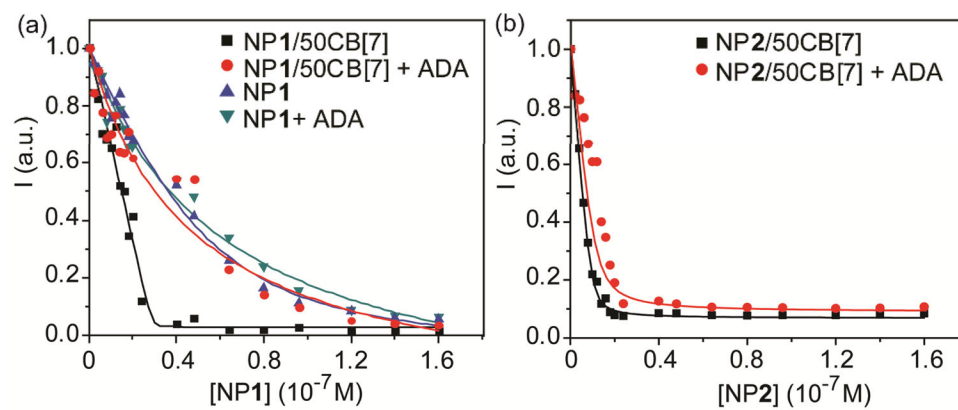
Fig. 3.
Fluorescence titration curve of (a) GFP-NP**1**, GFP-NP**1**/50CB[7] and (b) GFP-NP**2**/50CB[7] complexes and the responses after the addition of ADA.
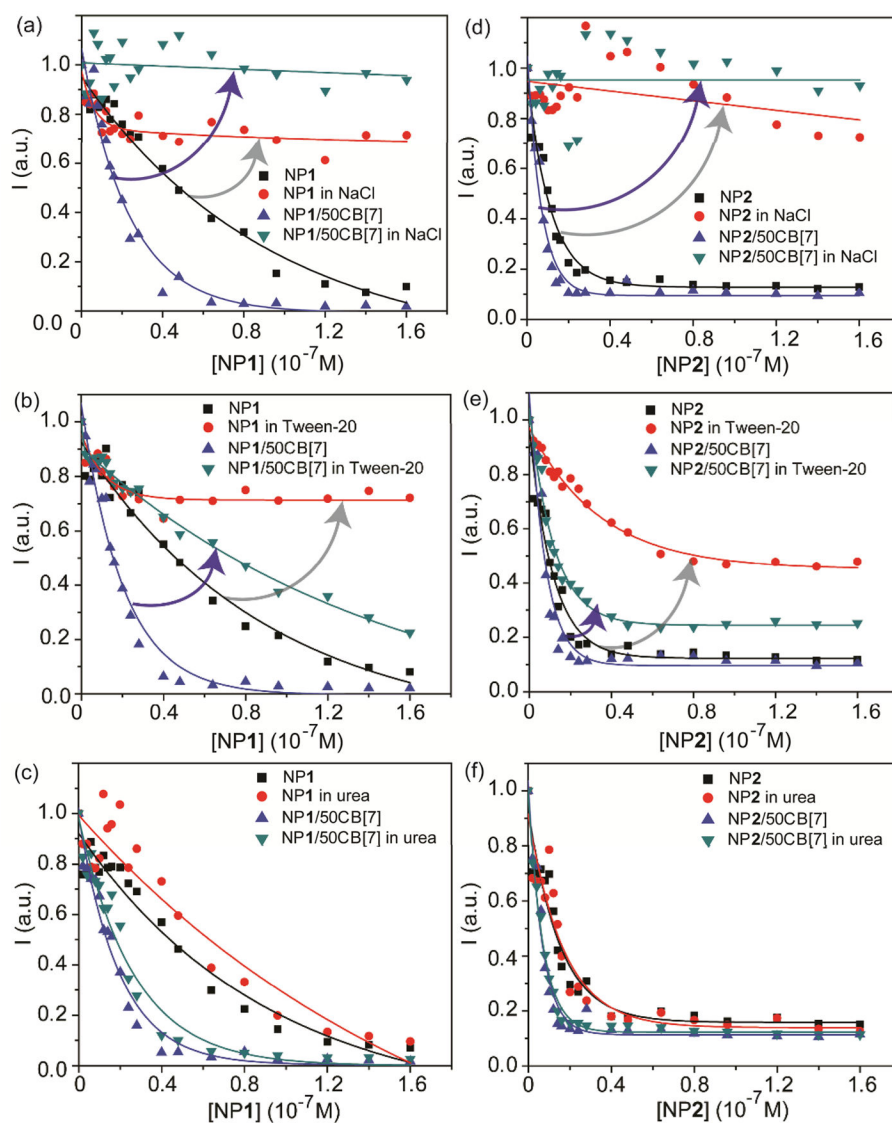
Fig. 4.
Different chemical agents were used to interfere with the major interactions in the complexes: (a–c) GFP-NP**1** and GFP-NP**1**/50CB[7] complexes; (d–f) GFP-NP**2** and GFP-NP**2**/50CB[7] complexes.
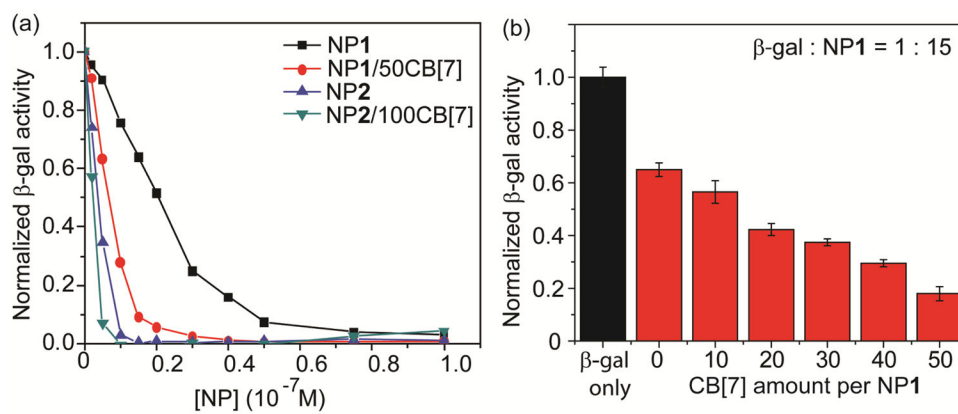
Fig. 5.
(a) Inhibition of β-gal activity upon addition of different NPs. (b) Inhibition studies of β-gal-NP1 complexes in the presence of different CB[7] amounts on NP1 surface at a fixed NP1 to β-gal ratio of 15. Each titration experiment was performed in three replicates.
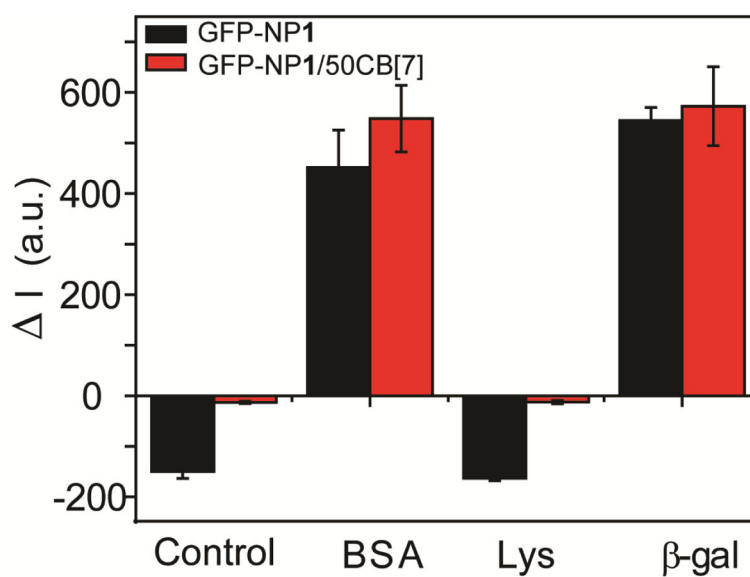
Fig. 6.
Fluorescence response (I) of GFP-NP**1** and GFP-NP**1**/50CB[7] conjugates after the incubation with different proteins at a 100 nM concentration. These GFP-NP conjugates were prepared in a fixed GFP concentration (100 nM) and GFP to NP ratio (1:1). Each titration experiment was performed in three replicates. Protein information: BSA: 66.3 kDa, pI 4.8; Lys: 14.4 kDa, pI 11.0; β-gal: 540 kDa, pI 4.6.
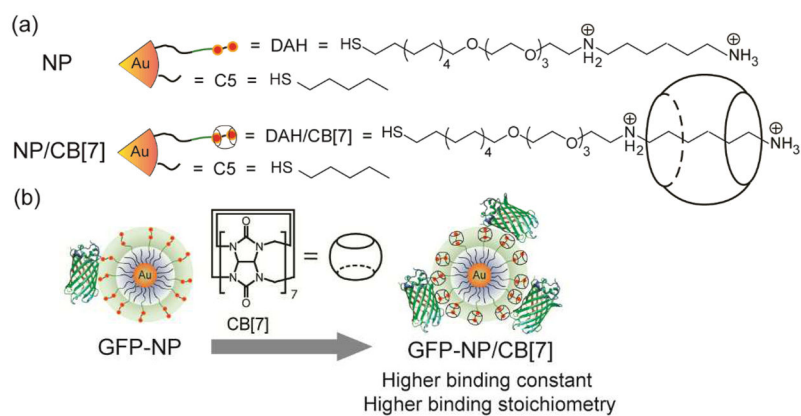
Scheme 1.
(a) Structure of a mixed monolayer-protected AuNP featuring pentanethiol (C5) and diaminohexane (DAH) terminated thiol ligand. The DAH moiety on the NP surface is a recognition unit for complexation with CB[7]. (b) Schematic of the protein-NP interactions in the absence and presence of the guest molecules CB[7].