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Mass Spectrometric Detection of Nanoparticle Host–Guest Interactions in Cells

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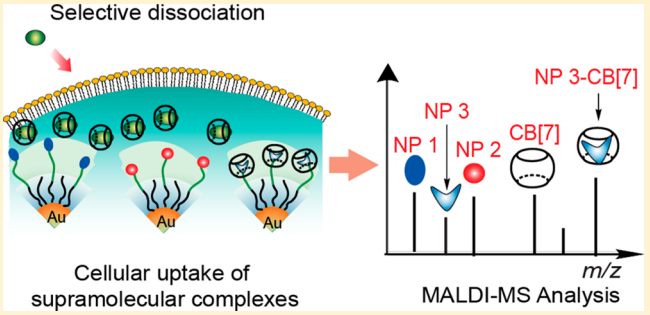
Supporting Information

ABSTRACT: Synthetic host–guest chemistry is a versatile tool for biomedical applications. Characterization and detection of host–guest complexes in biological systems, however, is challenging due to the complexity of the biological milieu. Here, we describe and apply a mass spectrometric method to monitor the association and dissociation of nanoparticle (NP)-based host–guest interactions that integrates NP-assisted laser desorption/ionization (LDI) and matrix assisted laser desoption/ionization (MALDI) mass spectrometry. This LDI/MALDI approach reveals how NP surface functionality affects host–guest interactions in cells, information difficult to achieve using other techniques.



Host–guest chemistry using engineered molecular systems provides controllable platforms for biomedical applications such as cell targeting,[1,2] biosensing,[3] imaging,[4] drug delivery,[5−7] and cancer therapeutics.[8] The reversibility of the association/dissociation process plays a vital role in these applications, allowing host systems to regulate the release of drug guests.[9,10] Multifunctional nanomaterials provide particularly versatile scaffolds for these host–guest systems due to their biocompatibility and functional versatility.[8,9−11] For example, the cytotoxicity of gold nanoparticles (AuNPs) can be triggered in cancer cells using competitive host–guest binding molecules, providing a new strategy for potential therapeutic applications.[8]

Effective use of nanomaterial-based supramolecular chemistry in biomedical applications requires the ability to monitor the association and dissociation of the noncovalent conjugates inside cells.[12] Characterization of host–guest interactions is traditionally performed in simple solutions using techniques such as NMR[13,14] and isothermal titration calorimetry (ITC).[15,16] These methods, however, cannot be used to analyze host–guest interactions in biological systems due to the complex environments in cells and tissues. Fluorescence spectroscopy is an alternate strategy to detect host–guest complexes in complicated biological samples.[17] The use of florescent probes, especially when additional labeling steps are required, can affect the biological behavior of original host–guest complexes due to the alteration of surface properties by the dye.[18−20] Moreover, it is challenging for this method to simultaneously probe multiple host–guest complexes.

Mass spectrometry (MS) is an effective tool for characterizing host–guest interactions in solution.[12,21−24] For example, electrospray ionization (ESI) MS[25−27] and matrix assisted laser desoption/ionization (MALDI) MS[28,29] have been utilized for the detection of host–guest complexes. However, to the best of our knowledge, detecting host–guest interactions inside cells using MS has not been reported, due in large part to the large number of interfering ions generated from biological samples.

We report here a direct method to monitor the association and dissociation of multiple NP-based host–guest complexes inside cells (Figure 1a) using a standard MALDI mass spectrometer. Supramolecular complexes formed by the surface ligands of AuNPs and cucurbit[7]uril (CB[7]) serve as "mass barcodes" to indicate the presence of AuNP-CB[7] complexes inside cells. This method integrates NP-mediated laser desorption/ionization (LDI-MS)[30−34] with MALDI using an organic matrix and acts to selectively desorb/ionize supramolecular complexes of the ligands, allowing observation of these species in the presence of other cellular materials. Using this method, the intracellular association and dissociation of AuNP-CB[7] complexes were monitored, as well as competitive dissociation of these complexes using 1-adamantylamine (ADA) (Figure 1b).

EXPERIMENTAL SECTION

Cell Culture Experiments. 60k HeLa cells per well were plated into a 24 well plate 24 h before the experiment. Cells were incubated with AuNP-CB[7] complexes (250 nM, 500 μL) for 24 h in DEMEM media containing 10% FBS and 1% antibiotics and then washed 3 times with phosphate-buffered saline (PBS) (500 μL for each washing). Beta Gal lysis buffer

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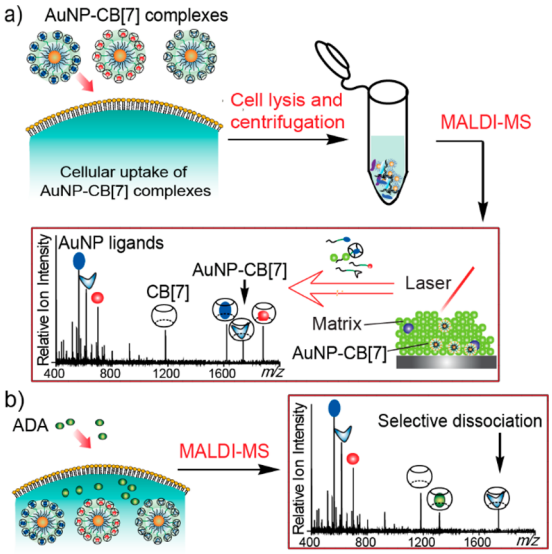

Figure 1. (a) Schematic illustration of the MALDI-MS detection process of supramolecular complexes in cells. AuNP-CB[7] complexes are measured as complex ions between CB[7] and AuNP surface ligands, and these ions appear at m/z values above 1600. (b) Monitoring the selective dissociation of the supramolecular complexes after adding the competitive binding molecule ADA. The addition of ADA dissociates some AuNP-CB[7] complexes and also leads to a new ADA-CB[7] complex ion at m/z 1314.

(250 μL per well, 5 times diluted) was used to lyse the cell, with the cell culture plate kept at room temperature on a vibrator for 30 min.

ADA Treatment. 60k HeLa cells were treated with a single type of NP-CB[7] complex or a mixture of three NP-CB[7] complexes for 24 h. Then, they were washed 3 times with PBS (500 μL) and treated with ADA at a concentration of 1.8 and 3.6 μM for 1 h (total ADA amount: 0.9 and 1.8 nmol, respectively). After that, cells were washed 3 times with PBS and lysed with Beta Gal lysis buffer.

Cell Sample Preparation for MALDI-MS. The cell lysate samples were transferred from the 24-well cell culture plate to 1.5 mL centrifuge tubes. Then, they were centrifuged at 14 000 rpm for 30 min. After removal of the supernatant containing the lysis buffer, the pellets were transferred to the stainless steel MALDI-MS sample carrier. A saturated solution of the matrix α-cyano-4-hydroxycinnamic acid (α-CHCA) solution was prepared in 70% acetonitrile and 30% water for the MALDI-MS analysis. 2.5 μL of the matrix solution was applied on top of each pellet. The samples were air-dried before MALDI-MS analysis.

MALDI-MS Instrumentation. MALDI-MS experiments were carried out on a Bruker Autoflex III MALDI-TOF mass spectrometer (Bruker Daltonics, Bremen, Germany), equipped with a Smartbeam 2 Nd:YAG laser. MALDI-MS 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, reflector voltage 2 = 9.69 kV, and positive reflectron mode in a mass range of m/z 400–3000. A total of 200 laser shots was fired per measurement. The laser energy was optimized to ~40 μJ/pulse. Data processing was performed using the Bruker flexAnalysis (version 3.3) software.

RESULTS AND DISCUSSION

We chose the cucurbituril (CB) supramolecular family for our studies. These host–guest complexes are particularly promising for biomedical applications due to their solubility in aqueous media, high affinity, and nontoxicity.[35−39] We probed intracellular CB interactions using AuNPs with three types of surface functionalities (Figure 2a). The AuNP-CB[7] complexes were formed by mixing AuNPs with excess CB[7] (molar ratio of AuNP/CB[7] = 1:200). The initial LDI/MALDI-MS detection of the AuNP-CB[7] host–guest complexes was first in simple aqueous solutions (Figure 3).

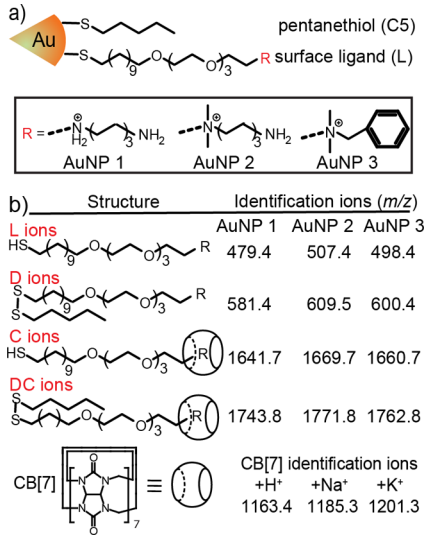

Figure 2. (a) Structures of the surface functionalities on the AuNPs used in this work. (b) The mass-to-charge (m/z) ratios of ligands and their corresponding supramolecular complexes monitored by MALDI-MS. Letter code key: molecular ions of the surface ligands (L ions), disulfide ions (D ions) formed by surface ligands and pentanethiol ligands, supramolecular complex ions (C ions) formed by surface ligand and CB[7], and disulfide ions formed by CB[7] and D ions (DC ions).

The molecular ion of the surface ligand (L1) of AuNP **1**, which has a diaminohexyl ending group, was readily detected using LDI/MALDI-MS (Figure 3a). The disulfide ion (D1), previously reported in LDI/MALDI-MS analysis of self-assembled monolayer surfaces,[40−43] and the molecular ion (L1) confirmed the presence of AuNP **1**. The supramolecular complex ions C1 and DC1 (formed by the D1 ion and CB[7]) indicate the detection of the host–guest complexes (Figure 3a, see the inset for the enlarged region between m/z 1600 and 1800; ion identities are shown in Figure 2b). This MALDI-MS method for monitoring the AuNP-CB[7] host–guest interactions is able to detect the intact supramolecular complexes without generating fragments of the gold clusters[44,45] or ionizing the intact AuNPs[46,47] and thus provides information on the ligand-CB[7] interaction. We applied this method to supramolecular complexes formed by CB[7] and AuNPs with different surface functionalities, and analogous mass spectra were acquired using AuNP **2**-CB[7] and AuNP **3**-CB[7] containing solutions (Figure 3b,c). The LDI/MALDI-MS characterization of the AuNP-CB[7] supramolecular structures can also be applied to NPs with a wide range of surface functionalities (Supporting Information, Figures S-1 and S-2).

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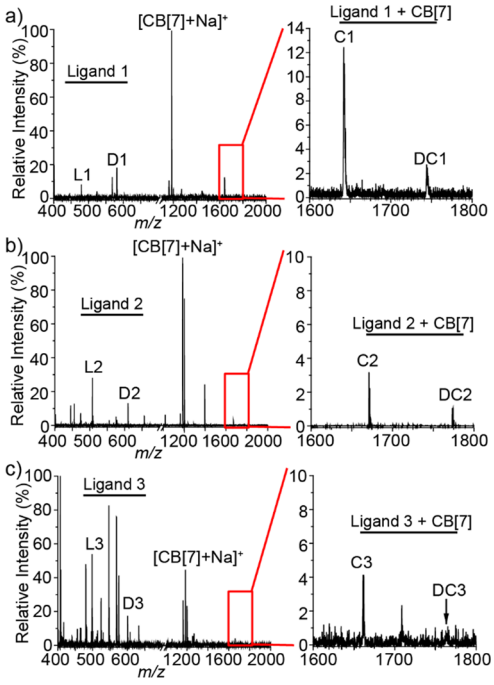

Figure 3. Monitoring AuNP-CB[7] interaction in solution using MALDI-MS. (a) AuNP **1**-CB[7]. (b) AuNP **2**-CB[7]. (c) AuNP **3**-CB[7]. [AuNP] = 2 μM, [CB[7]] = 400 μM. See Figure 2 caption for the identities of the L, D, C, and DC ions.

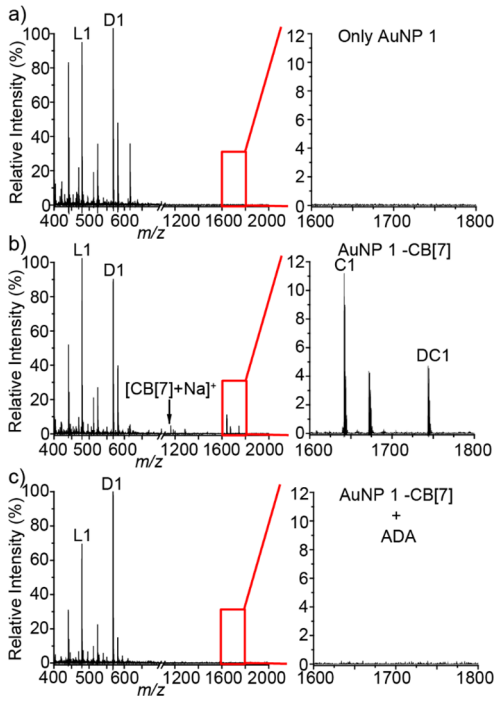

Figure 4. Monitoring AuNP-CB[7] interactions using MALDI-MS. (a) Detection of AuNP **1** in cells after incubation with 250 nM AuNP. (b) Detection of AuNP **1**-CB[7] ([AuNP] = 250 nM, [CB[7]] = 50 μM) taken up by the cells. (c) The dissociation of the host–guest complex by adding ADA (4 μM) to the cells containing AuNP **1**-CB[7].

Building on the solution phase experiments, we next explored the ability of this method to selectively ionize and detect NP host–guest complexes in cells. HeLa cells were incubated with uncomplexed ligands (250 nM) and complexed ligands (250 nM AuNP, 200 equiv of CB[7]) and washed with PBS three times to remove the AuNPs and AuNP-CB[7] complexes that were not taken up by the cells. After the cells were lysed, the resulting samples were transferred to centrifuge tubes, and the pellets containing AuNPs or AuNP-CB[7] complexes were collected after the centrifugation (Figure 1a). The high density of AuNPs relative to the biomolecules in the cells allows one to concentrate the AuNPs and AuNP-CB[7] complexes to some extent, minimizing interferences from biological molecules in the cell lysate. We then transferred the pellets to the MALDI-MS sample carrier and applied a thin layer of matrix on top of the pellets (Figure 1a). Figure 4a,b shows typical LDI/MALDI mass spectra that are obtained. The surface ligand ions (L1 and D1) are observed, indicating the existence of AuNPs in the pellets (Figure 4a). The supramolecular ions (C1 and DC1) are also readily observed, showing successful detection of host–guest complexes inside cells (Figure 4b). The gold cores of the AuNPs and the added matrix seem to work together to enable the selective ionization of the surface ligands and complexes that are attached to the AuNPs. Interestingly, the relative intensities of the complexed and uncomplexed ligands in cells (Figure 4b) are different than those in solution (Figure 3a); we are investigating the origins of this disparity.

We next used LDI/MALDI to monitor the dissociation of host–guest complexes using ADA, a strong binding competitor for CB[7]. Since a similar amount of particle was taken up with each of the ligands (Supporting Information, Figure S-3), the same amount of ADA was added for each particle. The host–guest complex "mass barcodes," both C1 and DC1 ions, disappear after the cells containing AuNP-CB[7] complexes are treated with ADA (Figure 4c), indicating the dissociation of supramolecular complexes. Comparing the results in Figure 4a,c, ADA treatment of cells incubated previously with AuNP **1**-CB[7] complexes leads to very similar mass spectra as the cells treated with only AuNP **1**. Figure 4 demonstrates the successful tracking of the association and dissociation of AuNP-CB[7] supramolecular complexes in cells by LDI/MALDI-MS.

Multiple supramolecular complexes can be followed simultaneously using MALDI-MS.[30] This multiplexed detection could provide direct ratiometric measurements, significantly reducing the variability introduced from studying different supramolecular complexes in separate cell populations. Cells were incubated with three AuNP-CB[7] complexes (AuNP **1**-CB[7], AuNP **2**-CB[7], and AuNP **3**-CB[7]) to demonstrate this multiplexing capability. Ions corresponding to the surface ligands (L1, L2, and L3) of three AuNPs and the host–guest complexes (C1, C2, and C3) are readily detected (Figure 5a); however, the intensities of the complex ions detected by MALDI-MS vary due to the different amounts and different ionization efficiencies of the supramolecular complexes.

As above, ADA was used to trigger the dissociation of the AuNP-CB[7] complexes inside the cells. In this study, 1.8 and 3.6 μM of ADA (total ADA amount: 0.9 and 1.8 nmol, respectively) were added to the cells containing AuNP-CB[7] complexes. We used the intensity ratios of all the supramolecular complex ions (C and DC ions) and all the ligand related ions (L, D, C, and DC ions) to evaluate the ADA-triggered dissociation of the complexes. All the ion intensity ratios were then normalized (Figure 5b) relative to cells without ADA treatment. The decrease in the normalized ion intensity ratios show the dissociation of these three supramolecular complexes is different. A more detailed examination

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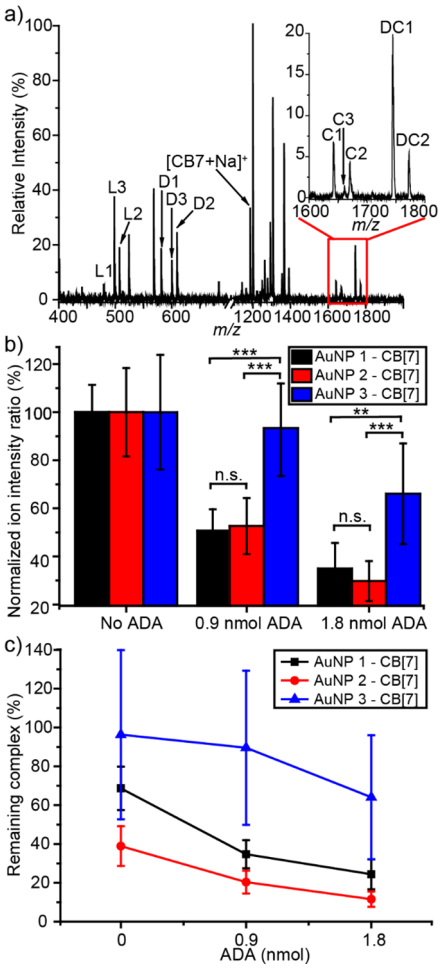

Figure 5. Monitoring the dissociation of three AuNP-CB[7] complexes in cells. (a) Typical mass spectrum of cell samples incubated with a mixture of three AuNP-CB[7] complexes. (b) Normalized ion intensity ratios indicating the relative amount of the remaining supramolecular complexes after ADA treatments. n.s., no significant difference. **, $0.001 < p \leq 0.01$; ***, $p \leq 0.001$ through one-way ANOVA ($n = 9$). See detailed p values in the Supporting Information, Table S-1. (c) The residual complexes in the cell lysates after ADA treatment based on relative ionization efficiencies (Supporting Information, Table S-2).

using one-way ANOVA reveals that AuNP **3**-CB[7] complexes are much more stable to ADA treatment than the other two ligands. This observation of selectivity illustrates the utility of the of the LDI/MALDI-MS method to screen multiple host–guest interactions in cells.

CONCLUSIONS

In summary, we have demonstrated the use of LDI/MALDI-MS to detect AuNP-CB[7] complexes in cells, confirming that both formation and dissociation of host–guest interactions inside cells can be monitored. We predict that this method is adaptable for monitoring other host–guest systems with various types of NPs,[30] with the inherent multiplex capabilities of the mass barcode approach facilitating high-throughput screening.

ASSOCIATED CONTENT

Supporting Information

AuNP synthesis, ligand synthesis, ligand characterization, complexation of AuNP with CB[7], LDI/MALDI-MS analysis of additional AuNP-CB7 complexes, ICPMS analysis of the cellular uptake amounts of AuNP-CB7 complexes, additional experimental details and results. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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