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Label-Free Biosensing with Lipid-Functionalized Gold Nanorods

Edward T. Castellana, Roberto C. Gamez, and David H. Russell*

Department of Chemistry, Texas A&M University, College Station, Texas 77843, United States

Supporting Information

ABSTRACT: The fabrication of a label-free mass spectrometry and optical detection-based biosensor platform for the detection of low-abundance lipophilic analytes in complex mixtures is described. The biosensor consists of a lipid layer partially tethered to the surface of a gold nanorod. The effectiveness of the biosensor is demonstrated for the label-free detection of a lipophilic drug in aqueous solution and a lipopeptide in serum.

Nanomaterials offer versatile platforms for the development of analyte capture and bioanalytical sensor platforms because of their unique optical and physical properties.[1−4] Additionally, the high surface area combined with the capacity to precisely control the surface chemistry gives nanomaterials an inherent advantage when they are employed in capture or separation techniques. Identification of lipophilic analytes is important in a wide range of fields, including pharmacokinetics,[5] food safety,[6] lipidomics,[7,8] and biomarker screening.[9,10] Therefore, a biosensor platform capable of selectively targeting lipophilic analytes in complex solutions for rapid detection would be widely applicable. Here we report a label-free mass spectrometry (MS)-based biosensor platform for the detection of low-abundance lipophilic analytes in complex mixtures (Figure 1).

Gold nanorods (AuNRs) have rapidly become powerful base platforms for the development of optical biosensors,[9,11−14] nanomedicines,[15,16] and gene delivery.[17−19] In previous work, we demonstrated the unique capabilities of AuNRs for IR laser desorption/ionization MS (IR-LDI-MS) analysis of various biomolecules.[20] Recently, several reports have focused on the combination of lipids and nanomaterials for a handful of bioanalytical applications. A limited number of methods for producing lipid-capped AuNRs have been reported to date.[17,21−23] The general procedure involves replacing the cationic surfactant cetrimonium bromide (CTAB), which is necessary for growth of the AuNRs, with lipids. The majority of these methods rely on the interaction of the quaternary amine of the phosphatidylcholine headgroup, which is similar to CTAB, with the surface of the AuNRs. This weak interaction allows for a lipid bilayer to form around the AuNR through a self-assembly process.[21] However, for utility in our experiments, the weak interaction of the chemisorbed lipids is not strong enough to form a stable lipid bilayer that can withstand multiple cycles of centrifugation. That is, once excess lipid is removed from solution, the lipid-capped AuNRs begin to aggregate; this trend also occurs when excess CTAB is removed from solution of CTAB-capped AuNRs. To circumvent this problem, we developed a method of producing

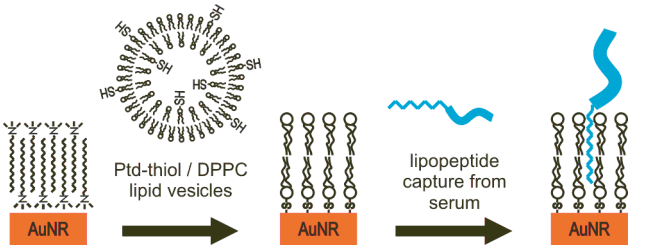

Figure 1. The production of lipid-capped AuNRs was carried out by replacing the CTAB bilayer with a lipid layer using small unilamellar vesicles containing lipids with a thiol headgroup. The lipid-capped AuNRs can be used for the capture of lipophilic analytes from complex solutions for label-free biosensing applications. It should be noted that this figure depicts a lipid bilayer as described by Orendorff et al.[21] As noted by Orendorff, it is difficult to show conclusively that this chemistry is solely due to a bilayer architecture.

stable lipid-capped AuNRs by tethering some of the lipids to the AuNR through a thiol−gold linkage. This method is explained in detail in the Supporting Information.

The lipid-capped AuNRs were characterized by UV−vis spectroscopy (Figure 2a, red trace; also see Figure S1 in the Supporting Information), matrix-assisted LDI-MS (MALDI-MS) (Figure S2), and transmission electron microscopy (TEM) (Figure S3). Analysis of the TEM images provided evidence that the lipid capping layer protects the AuNRs during production of the biosensor and after multiple cycles of centrifugation and resuspension. The TEM images also revealed that the lipids protect the integrity of the AuNRs and prevent aggregation during deployment in analyte capture experiments. Additionally, the longitudinal surface plasmon resonance (SPR) peak (∼850 nm) and the MALDI mass spectra of the lipid-capped AuNRs remained unchanged after several cycles of centrifugation and resuspension (Figure S4). This suggests that the tethered lipid capping not only is stable but also provides ample protection of the AuNRs against aggregation during processing.

Many drugs function through interactions with cellular membranes. These interactions include processes such as targeting cell-surface receptors,[24,25] disrupting cell membranes,[26−28] and traversing the cell membrane. Amphotericin B (AmB) is an antifungal drug whose mechanism of action involves formation of pores by insertion into and disruption of cellular membranes, making it an ideal candidate to demonstrate capture of cell-membrane-active drugs in the lipid-capped AuNRs. Additionally, AmB exhibits optical absorption in the UV−vis region because of

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its highly conjugated structure, thus allowing optical confirmation of its capture in the lipid-capped AuNRs.

Capture experiments were performed by incubating lipid-capped AuNRs in 0.5 mL of an aqueous 1 μM AmB solution for 20 min. Following incubation, the AmB−lipid-capped AuNR complex was separated from the solution by centrifugation, washed twice with water, and resuspended. The final AmB−lipid-capped AuNR complex was concentrated to 40 μL prior to analysis by MALDI-MS, and an aliquot was rediluted for UV−vis spectroscopy. Figure 2a shows the optical absorption spectra of the lipid-capped AuNRs (red trace), a 10 μM AmB solution (green trace), and the AmB−lipid-capped AuNR complex (blue trace). For example, the presence of AmB's optical signature was obtained after the capture procedure. Successful capture of the AmB by the lipid-capped AuNRs was confirmed by the absorbance observed in the 300−500 nm region, and the longitudinal SPR band of the rods was also perturbed.

The concentrated AmB−lipid-capped AuNR complex was mixed with the organic matrix 2,5-dihydroxybenzoic acid (DHB) prior to MALDI-MS analysis. As a control, a mixture of the capture solution (10 μM AmB) and lipid-capped AuNRs was mixed together with DHB without incubation or centrifugation. By MALDI-MS, we were able to observe the AmB only after its capture and concentration by the lipid-capped AuNRs (Figure 2b). No AmB signal was observed in the control experiment (Figure 2c). It is also important to note that AmB was not detected by direct MALDI-MS analysis of the AmB capture solution (data not shown). Also observed in both mass spectra (Figure 2b,c) are peaks corresponding to the lipid 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC), the DPPC dimer, and a complex between a DPPC molecule and a CTAB molecule (identified by MS/MS; data not shown).

These results demonstrate for the first time the successful application of a hybrid lipid-capped AuNR biosensor for the capture and label-free detection of membrane-active drugs by MS. Although the unique optical absorption of AmB would have been sufficient to detect its presence, many analytes do not elicit such an obvious change in the absorbance profile. In fact, if the analyte does not strongly absorb in the UV or visible region of the electromagnetic spectrum, then only a small change in the absorbance profile of the lipid-capped AuNRs would be observed. On the other hand, if multiple species are present, any change observed in the optical spectra could not be confidently assigned to an individual analyte. Ultimately, for the analysis of complex samples, where it is desired to capture multiple analytes simultaneously and/or identify unknown analytes, MALDI-MS is one of the best detection methods available. This is particularly important in the development of point-of-care molecular diagnostic assays, where multiplexed high-throughput analysis of body fluids is the key to success.[29]

The applicability of the lipid-capped AuNRs as a label-free biosensing platform was tested by capturing a lipophilic analyte present at low concentration (~10 nM) from a complex biological matrix, calf serum. Capture of the lipopeptide myristoyl-Lys-Arg-Thr-Leu-Arg from fetal bovine serum (0.5 mL, 3 mg/mL) using a method similar to that outlined above for AmB was carried out. The experiments were performed using a 1 μL aliquot of a capture solution containing the lipid-capped AuNRs that was spotted onto a stainless steel MALDI plate, dried under vacuum, and analyzed by MALDI-MS. This process was repeated until 5 μL of solution had been deposited on the sample plate.

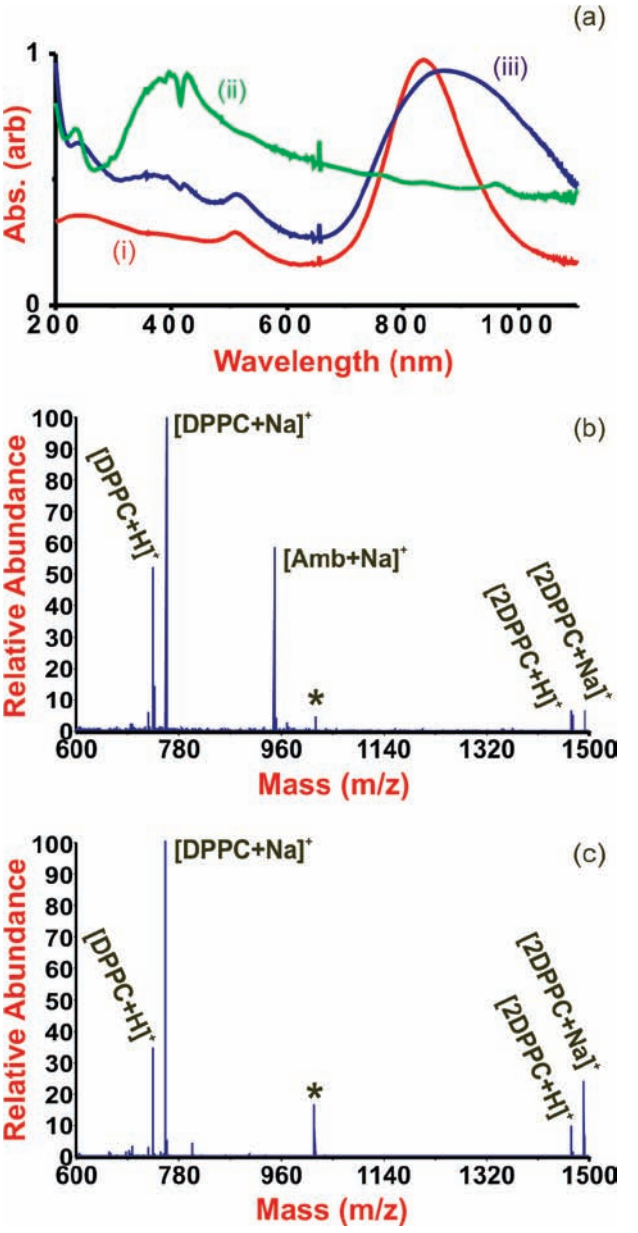

Figure 2. Capture of amphotericin B (AmB) with lipid-capped AuNRs followed by label-free detection using UV−vis spectroscopy and MALDI-MS. (a) UV−vis spectra of (i) lipid-capped AuNRs, (ii) a 10 μM solution of AmB, and (iii) a solution of the AmB−AuNR complex. (b) MALDI-MS mass spectrum of an AmB−AuNR complex after AmB was captured from a 1 μM AmB solution. (c) MALDI-MS mass spectrum of lipid-capped AuNRs. In (b) and (c), the peak labeled with an asterisk (*) was identified as a CTAB−DPPC complex by MS/MS.

Finally, 1 μL of a 1 mg/mL methanol solution of α-cyano-4-hydroxycinnamic acid (CHCA) was spotted over the dried sample spot. The deposited sample was then analyzed by MALDI-MS (Figure 3a). The dominant signal in the spectra for the capture experiment corresponds to the lipopeptide $[M + H]^+$ ion at m/z 883.6. The spectrum also contains signals corresponding to $[DPPC + H]^+$ and $[DPPC + Na]^+$. Additional peaks in the spectra labeled with numbers represent unidentified species present the serum. As a control experiment, an identical sample preparation procedure was repeated for the lipopeptide/serum solution without the addition of lipid-capped

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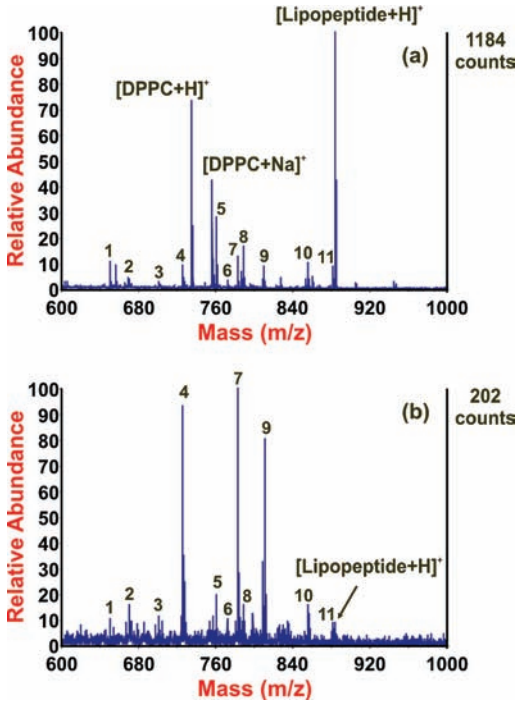

Figure 3. Capture of the lipopeptide myristoyl-Lys-Arg-Thr-Leu-Arg from calf serum with lipid-capped AuNRs and label-free detection using MALDI-MS. (a) MALDI-MS mass spectrum of the lipopeptide−lipid-capped AuNR complex after the lipopeptide was captured from an 11 nM solution of lipopeptide in a 3 mg/mL serum solution. (b) MALDI-MS mass spectrum of the serum containing 11 nM lipopeptide. The peaks labeled with numbers correspond to unidentified species present in the calf serum.

AuNRs (Figure 3b). Here the most abundant peaks in the spectra correspond to serum components (peaks labeled 4, 7, and 9). More importantly, the lipopeptide is barely discernible in the spectrum [signal/noise (s/n) ratio < 3]. MALDI-MS analysis of the solution following the selective capture and concentration of the lipopeptide by the lipid-capped AuNRs revealed a drastic improvement in our ability to detect the lipopeptide. In fact, the s/n ratio of the lipopeptide $[M + H]^+$ peak increased to ~550 and became the most abundant signal (Figure 3a).

To further verify that selective capture of the lipopeptide occurred because of the presence of the lipid capping the AuNRs, control experiments employing non-lipophilic capping agents (mPEG-thiol or 4-aminothiophenol) were performed. In order to eliminate any effect or possible interference from components of the serum, these control experiments were carried out with aqueous lipopeptide solutions and were performed using a capture protocol and sample preparation parameters identical to those for the lipid-capped AuNRs. An additional control experiment utilizing CTAB-capped AuNRs was also performed. However, as previously noted, the CTAB-functionalized AuNRs were unable to withstand multiple washing steps, and no analyte signals were observed by MALDI-MS because of AuNR aggregation. The MALDI-MS spectra for the capture experiments utilizing mPEG-thiol-capped AuNRs (Figure S5) and 4-aminothiophenol-capped AuNRs[30] (Figure S6) contained no discernible lipopeptide signals above the background. It is important to note that incubation was essential for analyte capture. For example, when the lipid-capped AuNRs were mixed with the serum solution and analyzed immediately, there was no significant increase in the lipopeptide signal (Figure S7) in comparison with the capture experiment (Figure 3a).

The data presented here illustrate the utility and applicability of lipid-capped AuNRs as a label-free biosensor platform for both optical and MS detection. Previous studies have addressed the importance of lipid bilayers for stabilization of AuNRs, and tethering of lipid bilayers to the surface of the AuNRs also provides an effective medium for the capture of lipophilic analytes from solution.[31,32] The AmB and lipopeptide capture data clearly illustrate the utility of the lipid-capped AuNR biosensor for the selective capture, detection, and identification of lipophilic analytes.

This work underscores the potential of lipid-capped AuNRs for biosensor applications; however, lipid-capped AuNRs may provide avenues for developing powerful multiplexed label-free sensor platforms. For example, tailoring the chemical and physical properties by altering the composition of the lipids or by attaching targeting molecules to the headgroups of the lipids would allow for the incorporation of additional degrees of selectivity into the biosensor platform. Such approaches would significantly enhance the selectivity of the lipid-capped AuNR platform, thereby extending the capability of the biosensor to target different types of analytes. For example, it should be possible to develop a multiplexing system that incorporates an array of lipid-capped AuNR biosensors, which would serve to simultaneously target a variety of analytes in solution. Mass spectrometry then would become an essential component of the label-free detection of the multiple targeted analytes because of the low specificity of optical detection methods.

■ ASSOCIATED CONTENT

Supporting Information. Detailed experimental section, MALDI-MS data for the lipid-capped AuNRs, MALDI-MS data for the capture control experiments described, and complete ref 7. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author
russell@chem.tamu.edu

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