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Aptamer-functionalized porous phospholipid nanoshells for direct measurement of Hg²⁺ in urine

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Abstract

A porous phospholipid nanoshell (PPN) sensor functionalized with a specific aptamer sensor agent was prepared for rapid detection of Hg²⁺ in human urine with minimal sample preparation. Aptamer sensors provide an important class of optical transducers that can be readily and reproducibly synthesized. A key limitation of aptamer sensors, and many other optical sensors, is the potential of biofouling or biodegradation when used in complex biological matrices such as serum or urine, particularly when high levels of nucleases are present. We prepared Hg²⁺-responsive, PPN-encapsulated aptamer sensors that overcome these limitations. PPNs provide a protective barrier to encapsulate the aptamer sensor in an aqueous environment free of diffusional restrictions encountered with many polymer nanomaterials. The unique porous properties of the PPN membrane enable ready and rapid transfer of small molecular weight ions and molecules into the sensor interior while minimizing the macromolecular interactions between the transducer and degradants or interferents in the exterior milieu. Using Hg²⁺-responsive, PPN-encapsulated aptamer sensors, we were able to detect sub-100 ppb (chronic threshold limit from urine test) Hg²⁺ in human urine with no sample preparation, whereas free aptamer sensors yielded inaccurate results due to interferences from the matrix. The PPN architecture provides a new platform for construction of aptamer-functionalized sensors that target low molecular weight species in complex matrices, beyond the Hg²⁺ demonstrated here.

Keywords

nanoshell; aptamer; sensor; mercury

Introduction

Heavy metal ions such as mercury are a major concern in the environment and an imminent threat to public health [1]. Mining operations [2], chemical manufacturing [3] and coal-based power plants [4;5] release mercury into the environment. Thus, it is important to monitor the mercury levels in populations with occupational exposure to mercury. Although

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elemental mercury is poorly adsorbed by the skin, inhalation of mercury vapor can lead to an increase of inorganic mercury level in organs and subsequently excreted in urine [6]. According to the WHO, urine mercury levels are considered the best measure of inorganic and elemental mercury for diagnosis since they closely reflect mercury levels in the kidneys [7].

Beyond traditional atomic spectroscopy, there have been numerous reports describing rapid, sensitive and potentially portable approaches for monitoring mercury levels in bodies of water using DNA-based aptamers, synthetic small molecules that can bind mercury and polymeric ion-selective membranes [8–28]. The aforementioned methods do not sufficiently meet the more challenging requirements for monitoring mercury in biological fluids such as urine. For example, non-specific adsorption of proteins to electrode membranes and fluorescent dyes significantly alters the response [29], whereas poor selectivity and low solubility often limit the use of synthetic mercury binding ligands. Although aptamer sensors have excellent selectivity and sensitivity, DNase in human urine can easily degrade the probe if free aptamers are used without protection.

Several approaches have been used to protect the probes from bio-fouling agents in complex biological samples. Kopelman and coworkers developed PEBBLEs (Probes Encapsulated by Biologically Localized Embedding), that typically target small analytes inside living cells [30–32]. PEBBLEs have been constructed using a range of probes that are otherwise not possible, including enzymes and a range of ionophores. Nielsen and colleagues extended this approach to aptamers by encapsulation in polyacrylamide nanoparticles for intracellular sensing [33], where ATP binding increases the fluorescence as a function of ATP concentration. The encapsulated aptamers were resistant to nuclease digestion and bio-fouling, with minimal effect on equilibrium activity [33]. While promising, encapsulation of probes in polymeric matrices can affect dynamic measurements by altering the flux of material into the polymer matrix and thus the sensor response [32].

Our group has previously developed a porous phospholipid nanoshell (PPN) architecture [34] using the synthetic phospholipid bis-sorbylphosphatidylcholine (Bis-SorbPC) [35–37]. PPNs offer a number of key differences compared to traditional polymer nanoparticles including inherent biocompatibility introduced by the phosphatidylcholine (PC) head group of the lipids, an aqueous inner compartment separated from the bulk solution by a thin (ca. 5 nm) membrane, and an interior environment with minimal diffusional impediments. Furthermore, unlike traditional phospholipid liposomes, PPNs present a surface with a size-dependent and charge-independent mass transport across the membrane, functioning similarly to a dialysis membrane [34;38]. The molecular weight cutoff of the PPN is ca. 2,000 Da thus allowing small molecule analytes to readily diffuse across the membrane while at the same time impeding the transport of large molecular weight species, e.g. proteins and enzymes [34;38]. The net result is an environment that facilitates diffusion limited mass transport across the membrane, yet protects the interior cargo from degradation by large molecular weight external components.

Here, we prepared PPNs that were functionalized with Hg²⁺-binding aptamers and characterized the stability of the encapsulated transducers as well as the capability of the aptamer-PPN for measuring Hg²⁺ in urine samples compared to free aptamer sensors.

Experimental

Reagents and chemicals

Mercury (II) acetate was purchased from Strem Chemicals (Newburyport, MA). Fluorophore/quencher labeled aptamer (5'-FAM-GGT-TGG-TGT-GGT-TGG-DABCYL-3') [8] was purchased from Integrated DNA Technologies, Inc. (Coralville, IA). DNase I (bovine), tris(hydroxymethyl) aminomethane (Tris), and bovine serum albumin (BSA) were purchased from Sigma-Aldrich (St. Louis, MO). 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) was purchased from Avanti Polar Lipids (Alabaster, AL). Bis-SorbPC was synthesized as previously described [39]. All other chemicals were purchased from VWR (Aurora, CO). All chemicals were used as received. Solutions were prepared using deionized H₂O with a measured resistivity of 18 MΩ cm.

Stock Solutions

The aptamer was dissolved to a final concentration of 100 μM in 10 mM Tris-acetate buffer (pH 7.4) that had been filtered through a 200 nm pore size polycarbonate membrane and autoclaved prior to use. Hg(CH₃COO)₂ was initially dissolved at 2.5 mM in 0.1 M HCl, and then diluted with H₂O to yield 5 and 50 μM stock solutions (no precipitates were observed).

Preparation and purification of liposomes

PPNs were prepared by film hydration followed by freeze-thaw and extrusion [40;41]. A 1:1 mass ratio (total weight: 2 mg) mixture of DOPC and bis-Sorb PC in chloroform was equilibrated at room temperature, dried under nitrogen, and then further dried under vacuum overnight to remove residual solvent. A 25 μL aliquot of aptamer stock solution was diluted with 175 μL Tris-acetate buffer, and used to hydrate the dried lipids resulting in encapsulation of the aptamer within the liposome or PPN structure. The resulting suspension was exposed to 11 freeze-thaw cycles (−77° C to 37° C), and extruded through two stacked polycarbonate membranes (nominal pore size = 200 nm) using a mini-extruder (Avanti Polar Lipids) operated at 40° C. Following 21 extrusion cycles, the vesicle suspension was collected and residual, unencapsulated dye was removed by size-exclusion chromatography using Sepharose CL-4B (Sigma-Aldrich) that was packed in a 0.7 × 20 cm column. The column was conditioned with degassed Tris-acetate buffer for 2 hours before use. In the instances where DOPC liposomes were utilized, the liposomes were prepared using 2 mg of DOPC unless otherwise described. PPNs were used within the first two days of preparation, though they were stable in excess of one week.

PPN and liposome size characterization

PPN and liposome size and stability were determined using Quasi-elastic light scattering conducted on a BI8000 auto-correlator (Brookhaven Instruments, Holtsville, NY) with the scattering angle fixed at 90° [42]. The hydrodynamic diameter was calculated using non-negatively constrained least squares fitting [42]. The experiment was performed at 25 (±1) °

C in a refractive index matching solution. Prior works have shown that PPNs retain material with MW larger than the cutoff for indefinite time frames [34;38].

Fluorescence measurements

Fluorescence measurements were performed on a QuantaMaster 40 spectrofluorometer (Photon Technology International, Birmingham, NJ). The excitation wavelength was set at 490 nm. Spectra were corrected for autofluorescence within the individual samples, as well as PPN/liposome scatter. Autofluorescence backgrounds for BSA and urine samples were collected and used for subtraction where indicated. The PPN/liposome scattering background was determined by collecting fluorescence spectra from the aptamer functionalized liposomes before and after surfactant disruption. The resulting difference spectrum was used as the scattering background. All fluorescence measurements were obtained after 10 min of equilibration time following sample mixing.

Calibration

Small aliquots of stock solution (KCl or $\text{Hg}(\text{CH}_3\text{COO})_2$) were successively added into the aptamer solution (either free aptamer or PPN-encapsulated aptamer) and gently mixed to reach the final concentration at each point. No pH change or precipitation was observed following any additions. The mixed solutions were equilibrated for 10 minutes prior to each measurement. A minimum of three individual samples were prepared at each concentration. The calibration curve was prepared by using the normalized change of fluorescence:

$$((I_0 - I)/I_0)$$

where I_0 is the initial fluorescence and I is the fluorescence intensity at each concentration.

Evaluation of DNase digestion and BSA bio-fouling

A solution containing 3 mM of $\text{Mg}^{2+}/\text{Ca}^{2+}$ in water was mixed with 5 μL DNase and 10 μL free aptamer (1 μM) or 5 μL encapsulated aptamer after SEC purification. The mixture was incubated at 37°C for 1 hour and then diluted to 400 μL (1.0 mL for free aptamers) with tris-acetate buffer for fluorescence measurement. K^+ was then added at a final concentration of 10 mM to evaluate the aptamer activity.

A stock BSA solution (3.5 % w/w) was prepared in tris-acetate buffer. Small fractions of stock BSA solution were successively added to final concentrations of 0.1%, 0.3%, 0.5% BSA.

Preparation of urine samples

Human urine samples were prepared in one of two ways. In the first preparation, samples were centrifugally dialyzed three times at 4000 RPM using a 10 kDa molecular weight cut off spin tube (Vivascience, Littleton, MA) to dilute small molecules by a factor of 320. The reduced volume was compensated with deionized H_2O . The pH of the solution was adjusted with NaOH to between 7.0 and 8.0.

In a second preparation, the urine sample was adjusted to pH 7.0–8.0 with NaOH and no further preparation. “Contaminated” urine samples were prepared by addition of Hg^{2+} to a

final concentration of 100 ppb or 500 nM to monitor the responses of free and PPN-encapsulated aptamers.

Results and Discussion

In this work, we addressed two key goals: a) the design and utilization of a carrier for an aptamer sensor that protects the sensor from interfering biological environments and b) utilization of the resulting protected aptamer sensor to measure Hg^{2+} in complex biological matrices in a stable, selective, rapid, and suitably sensitive manner without the need for substantial sample preparation or expensive and/or complex instrumentation. We have previously demonstrated that PPNs can be polymerized to provide markedly enhanced stability and that these architectures both retain and exclude large molecular weight species suggesting they will protect suitably chosen transducers from external interferents.[43] While a number of fluorescent probes for Hg^{2+} have been developed, we chose to investigate an Hg^{2+} -sensitive DNA aptamer that undergoes a fluorescence intensity change due to collisional quenching upon binding [8]. While this aptamer also responds to Pb^{2+} , the routes of environmental exposure are sufficiently different for Hg^{2+} and Pb^{2+} to suggest minimal interference. Furthermore, this aptamer is well-characterized, allowing this work to focus on the properties of the overall sensor architecture. The use of DNA aptamers presents a number of potential advantages, including the rapid and reproducible synthesis of nucleic acid sequences with suitable fluorescent labels. Additionally, while the primary focus of this work was on detection of Hg^{2+} , aptamers are available for a wide range of biologically important analytes, suggesting a broad applicability for the analytical platforms investigated in this work. Thus, this work also serves as a key proof of concept for developing a myriad of aptamer-based sensors for other analytes.

To prepare Hg^{2+} -responsive, aptamer-functionalized sensors we used a well-characterized aptamer sequence. Chang and coworkers recently demonstrated the utility of the aptamer (5'-FAM-GGT-TGG-TGT-GGT-TGG-DABCYL-3') to detect environmental Hg^{2+} in real world aqueous samples.[8] The sensing mechanism for this probe is depicted in Figure 1. Upon exposure to Hg^{2+} , the aptamer undergoes a conformational change leading to a decrease in the observed fluorescence of the labeled aptamer [8;9;44]. Further investigation revealed that this sequence also responds to mM K^{+} concentrations, though via formation of a G-quadruplex [8]. While the nature of the two conformational changes is different, the net outcome is the same, and thus the sensitivity to Hg^{2+} is likely retained even in the presence of K^{+} due to the substantially lower K_D values for Hg^{2+} binding.

A primary disadvantage of aptamer sequences in biological matrices is the sensitivity of nucleic acid sequences to nucleases that are present in many biological fluids, including urine and serum. Thus the capability to stabilize analyte-specific aptamers in such a manner that retains access to the analyte would provide a key advancement in the development of aptamer-based optical sensors. To enhance the stability of the aptamer sensor in biological fluids, we encapsulated the aptamer within a PPN. PPNs are water-filled nanoshells that have an inherent porosity within the nanoshell membrane [34;38]. Prior work has shown that PPNs retain compounds greater than 3 kDa for an indefinite time frame, yet allow unimpeded access of compounds less than 1.5 kDa, irrespective of charge [34;38]. Thus,

nucleases which would otherwise degrade the aptamer sensor and proteins which may yield non-specific signals upon interacting with fluorophores, should not affect the aptamer function, though Hg^{2+} will readily cross the membrane to interact with the aptamer.

To determine if aptamer encapsulation within PPNs would affect aptamer sensor response or activity, responses to Hg^{2+} and K^+ were monitored as a function of concentration for aptamer sensors in free solution as well as aptamers sensors encapsulated within PPNs. As a control, liposomes prepared solely from DOPC that do not form porous membranes and thus inhibit transport of the analyte to the encapsulated aptamer sensor were used. Aptamer sensors that were encapsulated in PPNs show similar responses to Hg^{2+} compared to that of the free aptamer sensors (Figure 2), suggesting unimpeded access of the aptamer sensor to the analyte via the pores in the nanoshell membrane. Furthermore, these results mirror those reported by Liu et. al who observed a linear range from 10–200 nM Hg^{2+} and very similar fluorescence ratios at higher Hg^{2+} concentrations.[8] Interestingly, when DOPC liposomes were utilized, the response for Hg^{2+} was lower than the free or PPN-encapsulated aptamer but still much higher than anticipated when the concentration exceeded 100 nM. This finding agrees well with a previous investigation into the ability of inorganic Hg to increase the permeability of phosphatidylcholine lipid membranes [45], a unique property of inorganic Hg that significantly increases the toxicity.

While it is clear that the PPN supports Hg^{2+} detection and aptamer function, we sought to further confirm that the unexpected permeability observed in Figure 2A was confined to the inherent lipid membrane permeability of inorganic Hg using an ion that should be non-permeant in DOPC membranes. As mentioned, the aptamer sensor utilized in this work also responded to high concentrations of K^+ . Thus, we compared the response of aptamer sensors encapsulated within PPNs and DOPC liposomes to free aptamer sensors as a function of K^+ concentration (Figure 2B). The response of aptamer sensors encapsulated within PPNs to K^+ was found to be identical, within experimental error, to the response of free aptamers, whereas the response of DOPC-encapsulated aptamer sensors was less than 2% of the maximal value, due to the low permeability of the DOPC membrane to K^+ . To confirm that the aptamer sensor encapsulated within DOPC liposomes was still active, Triton X-100 was used to disrupt the DOPC liposome membranes. No response was observed after spiking 10 mM K^+ into DOPC liposomes into which aptamer sensor had been encapsulated, though upon introduction of Triton X-100, a fluorescence intensity decrease was observed (Figure 2C) with a magnitude comparable to that of free aptamer sensor supporting the continued functionality of the aptamer sensor. Overall, these results confirm that encapsulation within the PPN does not exhibit a deleterious response of the aptamer sensor and suggest that the PPN exhibits unique transport properties for analytes that are otherwise impermeant to lipid membranes.

A commonly encountered problem with biosensors in general, and aptamer sensors in particular, is the loss of sensor response due to biodegradation and/or biofouling [43]. To elucidate the protective capabilities of PPNs for preparing aptamer sensors, PPN-encapsulated and free aptamer sensors were incubated in DNase solution for 1 hour at 37° C. Surprisingly, after digestion, free aptamers still showed moderate fluorescence response to Hg^{2+} in the solution (see Electronic Supplementary Material (ESM), Fig. S1), possibly

due to the fact that DNase used for the experiment yields a tetra-nucleotide as the smallest average fragment. One hypothesis for this observation is that after digestion, Hg^{2+} still chelates the individual fragments of the resulting 5'-FAM-GGT and TGG-DABCYL-3' to yield a decreased fluorescence intensity, though other factors may also influence this binding. In all cases, the magnitude of the fluorescence intensity change is significantly less for the free aptamer sensor following digestion by DNase.

As discussed, the conformational change for K^+ binding requires a higher level of structural integrity. Upon digestion with DNase, the free aptamer sensor response to K^+ was lost (see ESM, Fig. S2). Thus, we used K^+ to more clearly elucidate whether the PPN is capable of protecting the aptamer sensor from DNase degradation. Following digestion with DNase, free and PPN-encapsulated aptamer sensor was exposed to a saturating K^+ concentration. Whereas, the signal was almost totally abolished for free aptamer sensor upon DNase digestion, the PPN-encapsulated aptamer sensor was comparable to the undigested free aptamer (Figure 3).

To evaluate protection from bio-fouling via PPNs, the fluorescence signal of free and PPN- or DOPC-encapsulated aptamer sensors was monitored as a function of added BSA. BSA provides an excellent model for proteins that may be present in biological samples and is actively involved in biofouling in a wide range of sensors. BSA increases the fluorescence intensity of fluorophores and fluorescent sensors in solution, in an analyte-independent manner [32;33]. Upon addition of BSA, a large increase in the normalized fluorescence intensity was observed for free aptamer sensor, even at BSA concentrations as low as 0.1% (w/w). In contrast, aptamer sensors encapsulated in either PPNs or DOPC liposomes yielded changes of normalized fluorescence of < 5 – 20 % upon increasing BSA concentrations (Figure 3B) compared to > 100% for free aptamer. Interestingly, these changes were similar for both PPNs and DOPC liposomes, suggesting that interactions between BSA and the lipid membrane may occur at a low level, a factor that would be minimized upon polymerization of the PPN [34].

Following validation of aptamer sensor function when encapsulated in PPN matrices, coupled with the reduced effects of biofouling and biodegradation afforded by the PPN membrane, we sought to use the PPN-encapsulated Hg^{2+} -responsive aptamer sensor to measure Hg^{2+} directly in urine with minimal sample preparation. Urine is a difficult sample matrix that contains DNase as well as other potential interferents. Furthermore, mercury is nephrotoxic [46;47] and microalbuminuria and macroalbuminuria are commonly observed in kidney dysfunction [48] suggesting the need for enhanced stability and minimized interferences in Hg^{2+} measurements within urine.

To evaluate the performance of PPN-encapsulated aptamer sensors compared to free aptamer sensors, human urine was collected and centrifugally dialyzed with a 10 kDa molecular weight cut off spin tube to remove low molecular weight species but to retain high molecular weight species, e.g. DNase (MW = 30 kDa) that may potentially biofoul or degrade the sensor response. The aptamer sensor samples were suspended in varying solution compositions of urine ranging from 0 – 50 % dialyzed urine and subsequently spiked with saturating concentrations of Hg^{2+} (1.2 μM) or K^+ (10 mM) (Figure 4). For each

analyte, the PPN-encapsulated aptamer sensor was minimally affected by the presence of urine components in the sample, whereas the free aptamer sensor yielded unreliable responses with errors exceeding 50%. These results agree well with the observations from model systems in Figure 3 supporting the further utilization of PPN-encapsulated aptamer sensors for quantitative measurements in complex biological matrices.

We next investigated the capability to detect Hg^{2+} in human urine with minimal sample preparation using free aptamer sensor and PPN-encapsulated aptamer sensor. For these experiments, human urine samples were used and the only sample preparation involved the adjustment of the pH using NaOH and pH paper and dilution. “Contaminated” urine samples were prepared via addition of mercury (II) acetate to a final concentration of 500 nM, a concentration chosen based on the urine mercury threshold of 100 $\mu\text{g/L}$ (~500 nM) [7]. Unlike the data shown in Figure 4, no dialysis, sterilization or filtration was performed on the urine sample for these measurements. The contaminated urine sample was mixed directly with PPN-encapsulated aptamer sensor or free aptamer sensor. A 1:10 (v/v) dilution was performed on the sample prior to final analysis to achieve a concentration 10-fold below the action threshold. H_2O spiked with 50 nM Hg^{2+} and urine lacking the addition of Hg^{2+} (denoted as “clean” urine) were used as controls. Similar to the results shown in Figures 3 and 4, utilization of free aptamer sensors showed high variation in both “contaminated” and “clean” urine compared to the signal obtained for free aptamer sensor in H_2O with 50 nM Hg^{2+} (Figure 5). Based on calibration values, the normalized intensities measured in clean and contaminated urine samples correspond to 150–200 nM Hg^{2+} , irrespective of the actual Hg^{2+} concentration, whereas the response observed for the free aptamer in response to 1.2 mM Hg^{2+} in 50% urine in Figure 4 corresponded to an artificially low concentration of 50 nM Hg^{2+} . These results support the argument that non-specific biofouling and/or biodegradation lead to unreliable responses for free aptamer sensors in complex biological media. In contrast, the PPN-encapsulated aptamer sensor provided more accurate measurements. The normalized intensity obtained for the contaminated H_2O matrix agreed within 4% of the calibration value shown in Figure 2. Furthermore, there were no statistical differences between the values obtained for the contaminated urine and H_2O samples, supporting the utilization of this approach for measuring Hg^{2+} in unprocessed urine samples. The small response observed in clean urine corresponded to an 8% signal change and may result from small molecule interferents present within the unprocessed urine or from K^+ background. Importantly, these interferences minimally affect the ability to obtain accurate measurements at the 50 nM and higher level as seen in Figure 5. Additionally, as previously described the aptamer sensor employed in this work responds to K^+ in the mM concentration range. Interestingly, if the signal observed from “clean” urine arises only from K^+ , then the signal corresponds to a urinary K^+ concentration that is less than 0.3 mM for these samples, well below the normal urinary output in humans. This observation would suggest that K^+ does not significantly interfere with PPN-encapsulated aptamer sensor and that other small molecule interferents may play a more dominant role in false signals.

Conclusion

Porous phospholipid nanoshells provide a unique sensor platform that enables the utilization of analyte-responsive aptamer sensors. The PPN provides a protective environment to

minimize the effects of biofouling and biodegradation on aptamer sensors, a common problem when using aptamers in complex biological samples. The porous membrane of the PPN provides easy access of small molecule and ionic analytes, including Hg^{2+} and K^+ , to the sensor transducer in the interior of the PPN with minimal perturbation of the signal. Using the PPN-encapsulated aptamer sensor, Hg^{2+} levels below the action level were detected in unprocessed human urine. When utilized in complex matrices such as urine, PPN-encapsulation markedly improves the performance of aptamer sensors and enables rapid measurements with minimal sample preparation. Such measurements may translate well into lower resource environments lacking expensive instrumentation such as ICP-MS and ICP-OES. Furthermore, the rapidly expanding catalog of aptamer sensors that target small molecules are directly amenable to integration into the PPN platform. Though unpolymerized PPNs were used in this work, the capability to polymerize the PPN sensor geometry provides additional potential advantages that will be investigated in the future.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Schematic of aptamer-PPN sensor platform. **a** Upon ion binding, conformational changes in the aptamer lead to a change in FRET signal. Hg^{2+} induces a fold within the aptamer by forming T-Hg-T complex (top) whereas K^{+} induces a G-quadruplex (bottom). In both cases, donor fluorescence is decreased. **b** Analyte-selective aptamer sensor is encapsulated within a PPN. Low molecular weight analytes readily penetrate the membrane through ca. 2 nm pores generating an optical signal. The aptamer is shielded from large molecular weight interferences in the external matrix such as nucleases and non-specific protein binding.

Figure 2.

Encapsulated aptamer sensor response to analyte ions. Responses to **a** Hg^{2+} and **b** K^+ were measured for free aptamer sensor (black squares), PPN-encapsulated aptamer sensors (blue triangles) and DOPC-encapsulated aptamer sensors (red circles). **c** Response to 10 mM K^+ for aptamer sensors encapsulated within DOPC liposomes before (–) and after (+) vesicle rupture with Triton X-100.

Figure 3.

Investigation of biodegradation and biofouling in PPN-encapsulated aptamer sensors. **a** Response of free and PPN-encapsulated aptamer sensors to DNase degradation. Sensors were exposed to 10 mM K⁺ before (–) and after (+) exposure to DNase to evaluate the effect of degradation on aptamer function. **b** Fractional fluorescence increase over background of free (blue), PPN-encapsulated (red) and DOPC liposome-encapsulated (gray) aptamer sensors to increasing levels of BSA.

Figure 4. Comparison of free (blue) and PPN-encapsulated (red) aptamer sensors in dialyzed urine samples. **a** Aptamer sensor response to 10 mM K^+ . **b** Aptamer sensor response to 1.2 μM Hg^{2+} .

Figure 5.

Response of free (blue) and PPN-encapsulated (red) aptamer sensor in the presence (+) and absence (–) of 50 nM Hg^{2+} in minimally treated samples. In all cases, the only sample treatment was adjustment of pH to 7.0–8.0 using NaOH.