NANO LETTERS

LETTER

pubs.acs.org/NanoLett

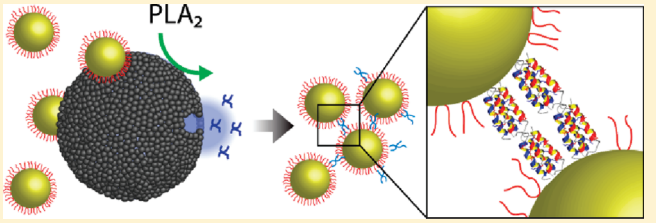
Hybrid Nanoparticle−Liposome Detection of Phospholipase Activity

Daniel Aili,[†] Morgan Mager,[†] David Roche, and Molly M. Stevens*

Department of Materials and Institute of Biomedical Engineering, Imperial College London, SW7 2AZ London, U.K.

Supporting Information

ABSTRACT: A flexible nanoparticle-based phospholipase (PL) assay is demonstrated in which the enzymatic substrate is decoupled from the nanoparticle surface. Liposomes are loaded with a polypeptide that is designed to heteroassociate with a second polypeptide immobilized on gold nanoparticles. Release of this polypeptide from the liposomes, triggered by PL, induces a folding-dependent nanoparticle bridging aggregation. The colorimetric response from this aggregation enables straightforward and continuous detection of PL in the picomolar range. The speed, specificity, and flexibility of this assay make it appropriate for a range of applications, from point of care diagnostics to high-throughput pharmaceutical screening.



KEYWORDS: Phospholipase A_2, gold nanoparticles, liposomes, polypeptides, helix−loop−helix

Assays that enable rapid, high-sensitivity monitoring of biomolecular interactions are of immense importance for point of care diagnostics, drug development, and detection of pathogens.[1] The unique optical and chemical properties of nanoparticles offer the potential to greatly improve the speed, cost, robustness, and sensitivity of current techniques.[2,3] In particular, the colorimetric response of aggregating gold nanoparticles (Au NPs) has received significant research attention in the last 15 years and has been utilized for detection of a wide range of analytes, including enzymes,[4−7] nucleotides,[8] and metal ions.[9,10]

A common approach in Au NP-based enzyme assays is to employ a substrate that induces aggregation or redispersion of the particles as a result of enzymatic activity. Most often, the substrate molecule is immobilized on the nanoparticles. Although such immobilization has proven effective in many systems, there are concerns that it may compromise the interaction with the enzyme or limit the stability of the particles. Further, this functionalization is often unique to the specific particle/molecule pair used. Because of possible variations in molecular surface density, activity and stability, NP-based sensors must be re-evaluated and, frequently, redesigned for each new application. The traditional intimate coupling between enzymatic substrate and the NP transducer can thus limit the versatility and robustness of such systems. These variations are particularly problematic when studying processes affected by the physical as well as chemical state of the biomolecules involved.

One class of enzymatic reaction that is particularly sensitive to substrate presentation is surface-catalyzed reactions such as the degradation of phospholipids by phospholipases. Phospholipases act primarily on aggregated phospholipids organized into lipid bilayers such as the cell membrane and thus, in addition to substrate chemistry, they are also sensitive to the nanoscale intermolecular spatial arrangement of the substrate. Phospholipase activity on free lipid monomers is extremely low as compared to the activity on bilayers, monolayers, and micelles.[11] Studies have even shown that phospholipase activity is sensitive to the phase state[12] and packing density[13] within a bilayer.

Phospholipases are involved in a wide range of physiological processes including digestion, inflammation response, membrane remodeling, and intercellular signaling. One class of phospholipases that have been intensively studied in this context are the phospholipases A_2 (PLA_2), a superfamily of enzymes that degrade phospholipids by cleaving the *sn*-2 acyl ester bond of glycerophospholipids to produce free fatty acids and lysolipids.[14] Dysregulation of PLA_2 is a feature of many pathological conditions including atherosclerosis,[15] pancreatitis,[16] acute sepsis,[17] and some forms of cancer.[18] Because of these diverse biological roles, many assays have been developed to determine PLA_2 concentration and activity. One approach has been the creation of micro- or nanoparticles made of artificial lipid analogues that undergo fluorometric[19,20] or colorometric[21] changes upon hydrolysis. These systems can give rapid, high-resolution readings of phospholipase activity but suffer from the drawback that the enzymatic substrate can never be identical to a natural lipid bilayer. Because of the demonstrated sensitivity of PLA_2 to subtle changes in substrate organization and chemistry, it would be preferable to have an assay based on interactions with unmodified phospholipids. Assays based on PLA_2-mediated release of fluorophores from liposomes can meet these critieria.[22,23] This is a simple and versatile strategy, but it is inappropriate for human diagnostic applications since human serum albumin interferes with the dye used.[24] Such limitations have led to the development of liposome phospholipase assays based on the release of other species such as electroactive[25] or superquenching[26] molecules. Again though, since biological fluids can contain many

Received: July 10, 2010
Revised: August 18, 2010
Published: August 26, 2010

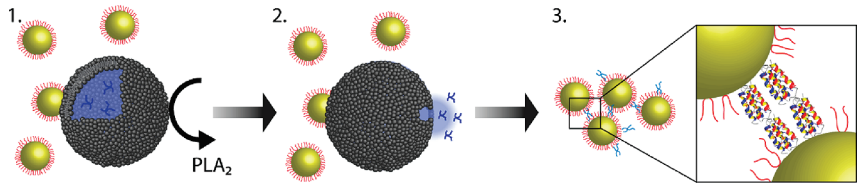


Figure 1. Schematic illustration of the mechanism for colorimetric detection of phospholipase A_2 (PLA_2): (1) Au NPs are coated with a synthetic polypeptide (JR2EC). Liposomes are loaded with a bifunctional complementary polypeptide ($JR2KC_2$) designed to heteroassociate with JR2EC. (2) PLA_2-mediated hydrolysis of the lipids releases the entrapped $JR2KC_2$ from the liposomes. (3) The $JR2KC_2$ associates with the NP-bound JR2EC in a folding-dependent manner to form a four-helix bundle. Because the $JR2KC_2$ is bifunctional, this reaction results in bridging between and aggregation of the NPs.

fluorescently or redox-active compounds, these methods may be susceptible to interference.

In this Letter, we demonstrate that by integrating enzyme-triggered liposome release with controllable NP aggregation, it is possible to realize a sensor system that is both flexible and biologically relevant and which makes use of a natural, non-immobilized lipid substrate. This system comprises two active components that are initially separated: polypeptide-functionalized Au NPs and a complementary polypeptide designed to heteroassociate and fold with the NP-bound polypeptide. The nonimmobilized polypeptide is initially contained within liposomes and is released in the presence of PLA_2. Once released, association and folding of the peptides induce a rapid and extensive particle aggregation. The aggregation in turn alters the resonance condition of the localized surface plasmons, resulting in a significant spectral redshift, clearly visible by the naked eye. A schematic representation of this process for detection of PLA_2 is shown in Figure 1. This assay strategy takes advantage of the tunability of triggered liposome release, in that the biologically relevant enzymatic substrate can be changed without any alteration to the principle of transduction.

The highly specific nature of the NP assembly is governed by the association of two de novo designed polypeptides. The 42-residue polypeptides JR2EC (NAADLEKAIEALEKHLEAK GPCDAAQLEKQLEQAFEAFERAG) and JR2KC (NAADLKK-AIKALKKHLKAKG PCDAAQLKKQLKQAFKAFKRAG) are designed to fold into helix−loop−helix motifs and heterodimerize into four-helix bundles in solution.[27] Both peptides have a cysteine residue located in the loop region. In the case of JR2EC, this cysteine was utilized to immobilize the peptide on the Au NP surface. The cysteine on JR2KC was used to covalently link two of the monomers via a disulfide bridge, forming a bifunctional "cross-linker". In solution, the oxidized peptide ($JR2KC_2$) associates with two of the complementary peptides (JR2EC) to form a hetero-trimeric complex that folds into two disulfide-linked four-helix bundles.[28] When both peptides are oxidized, heteroassociation induces the assembly of peptide fibers composed of disulfide-linked four-helix bundles.[29] Gold nanoparticles functionalized with JR2EC aggregate rapidly and extensively in the presence of nanomolar concentrations of $JR2KC_2$.[28]

$JR2KC_2$ loaded liposomes composed of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) were prepared by extrusion through a polycarbonate membrane with 100 nm pores (Nucleopore). Untrapped peptides were removed from the liposome suspension by gel filtration through a Sephadex G-100 column. The resulting liposomes had a narrow size distribution with an average hydrodynamic radius (R_H) of 51 ± 6 nm and a zeta potential (ζ) of −7.6 ± 1.2 mV. The hydrodynamic radius and zeta-potential of POPC liposomes

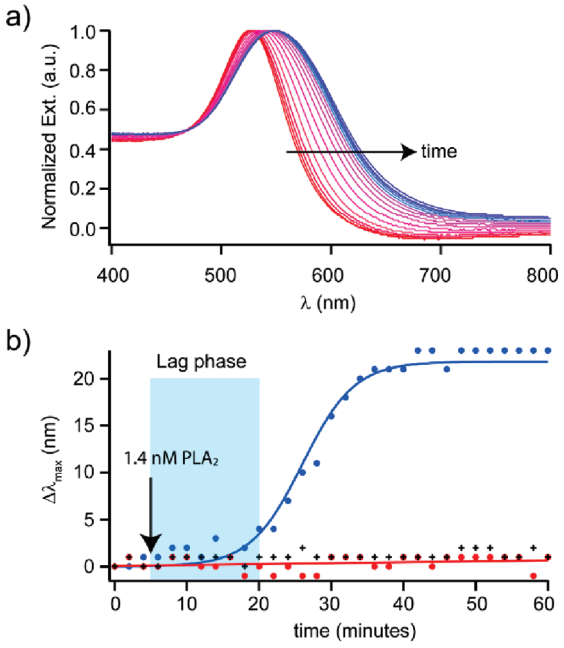


Figure 2. (a) The PLA_2-triggered release (1.4 nM PLA_2) of $JR2KC_2$ resulted in extensive particle aggregation and a large redshift of the LSPR-band. (b) The release of $JR2KC_2$ was preceded by a concentration-dependent lag phase (blue). No particle aggregation was observed in the absence of Ca^{2+} (red) or if the particles were modified with a peptide unable to fold (+).

without $JR2KC_2$ was not significantly different (R_H = 49 ± 2 nm and ζ = −6.6 ± 0.4 mV) indicating that the peptides were not extensively associated with the outer leaflet of the lipid membrane. The slightly negative zeta potential of these zwitterionic POPC liposomes is in good agreement with previously reported data.[30]

The $JR2KC_2$ loaded liposomes were diluted in PBS buffer pH 7.4 containing 0.5 mM Ca^{2+}, to a total lipid concentration of 0.1 mg/mL. The concentration of $JR2KC_2$ loaded into the liposomes was 0.25 mM, which would yield a concentration of about 0.1 μM of $JR2KC_2$ in the event of 100% release. At this concentration of $JR2KC_2$ an extensive aggregation of the JR2EC modified particles is induced.[28] When exposed to PLA_2, the degradation of the liposomes resulted in a release of the entrapped peptides that induced a significant redshift of the localized surface plasmon resonance (LSPR) band of the Au NPs (Figure 2a). This rapid transduction and large optical shift enabled real-time monitoring of the enzymatic activity. The peptide loaded liposomes exhibited a remarkable stability; after

dx.doi.org/10.1021/nl1024062 |*Nano Lett.* 2011, 11, 1401–1405

4 months of storage at 4 °C there was no detectable background leakage but the liposomes maintained full responsiveness toward PLA_2 (Figure S1a in the Supporting Information).

Because the catalytic activity of the PLA_2 used here (*naja mossambica mossambica*) is highly dependent on the presence of millimolar concentrations of Ca^{2+},[14] repeating the experiments in the absence of Ca^{2+} is a useful negative control. As expected, no particle aggregation was observed in the absence of Ca^{2+} upon addition of PLA_2 (Figure 2b). This observation confirms that the aggregation of the particles is dependent on the PLA_2-mediated hydrolysis of the lipids, rather than simply the presence of the enzyme. Moreover, the assembly of the particles was highly specific and dependent on the ability of the peptides to heteroassociate and fold. When replacing all L-alanines in JR2EC with D-alanines, the folding of the polypeptides is completely abolished and the heterotrimeric complex cannot be formed.[28] Particles coated with this modified peptide did not aggregate upon PLA_2-mediated release of $JR2KC_2$ (Figure 2b).

Addition of PLA_2 in the concentration range from 700 pM to 7 nM did not induce an immediate particle aggregation but rather showed a typical lag-burst behavior (Figure 2b). The origin of the lag phase has been suggested to be due to an accumulation of hydrolysis products in the bilayer, especially fatty acids. Fatty acid molecules have a tendency to form segregated domains in lipid bilayers to which PLA_2 binds with a higher affinity. The sudden burst at the end of the lag period may be related to a relief of local product inhibition caused by the accumulation of hydrolysis products close to the surface bound enzyme.[31] An increase in the amount of membrane bound PLA_2 has also been observed to coincide with the onset of the rapid hydrolysis at the end of the lag phase.[32] A lag-burst behavior has also been described for other types of phospholipases.[33]

The duration of the lag phase is an important kinetic parameter that depends on the concentration and activity of phospholipase in the sample.[34] With this in mind, a systematic investigation was performed to determine whether the lag time could be used as an appropriate assay metric. The length of the lag phase was found to be clearly dependent on the concentration of PLA_2, and spanned from 5 to 40 min for concentrations ranging from 7 nM to 700 pM (Figure 3) in the presence of 0.5 mM Ca^{2+} at 37 °C. The large number of parameters affecting the length of the lag phase further offers numerous possibilities to tune the dynamic range of the assay by, for example, varying the relative concentrations of the assay components. As shown in Figure 4a, temperature also had a significant influence on the length of the lag phase. At a concentration of 7 nM PLA_2, the lag period was about 20 min longer at room temperature than it was at 37 °C.

As a first demonstration of tunability, the release rate was increased by addition of human serum albumin (HSA) after incubation of the liposomes with PLA_2. HSA is a ubiquitous protein in serum that, among other roles, transports fatty acids to the liver. HSA has seven binding sites capable of hosting one fatty acid each.[35] Addition of 4 mg/mL of HSA to the peptide-loaded liposomes 5 min after addition of PLA_2 led to an almost immediate release of the peptides and extensive particle aggregation (Figure 4b). HSA alone did not cause any particle aggregation. Similarly, addition of HSA after PLA_2 in the absence of Ca^{2+}, or in the presence of Ca^{2+} when using liposomes not loaded with $JR2KC_2$, failed to induce aggregation as shown in Figure 4c. We therefore hypothesize that HSA binds to the liposome surface and removes the fatty acids that have

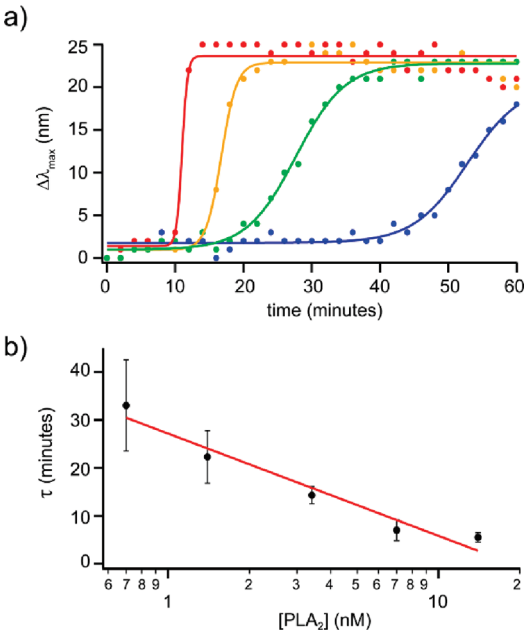


Figure 3. (a) Response for 7 nM (red), 3.5 nM (orange), 1.4 nM (green), and 700 pM (blue) PLA_2 at 37 °C. Lines are drawn as a guide for the eye. (b) Length of the lag time (τ) as a function of PLA_2 concentration ($R^2 = 0.97$). Error bars are standard error of the mean, $n \geq 3$.

accumulated in the membrane, destabilizing the liposomes and resulting in a more rapid lysis. This progression is in keeping with previously reported results.[36] The shift of the plasmon peak is also larger in the presence of HSA, indicating that the presence of a high concentration of fatty acids may interfere with the association and folding of the polypeptides.

The response of the assay depends primarily on the quantity of active PLA_2 present in the sample and the amount of time for which this enzyme is incubated with the liposomes. Using HSA, it was possible to detect less than 10 nM PLA_2 after only 5 min of enzymatic action at room temperature. The results for a range of enzyme levels are given in Figure 4d and show a sigmoidal response with concentration. In order to compare the nanoparticle-based assay with a more traditional fluorescence based assay, liposomes were loaded with 50 mM of the dye carboxyfluorescein (CF). CF is self-quenched at millimolar concentrations. Release (and therefore dilution) of the dye consequently results in a large increase in fluorescence intensity. Addition of PLA_2 to the CF-loaded liposomes resulted in a dramatic increase in the rate of CF release (Figure S2 in the Supporting Information). The overall sensitivity and range of the traditional CF-based assay were similar to our system though, as discussed above, adaptability of that system is much more limited. Stability of the CF system was also much lower, with more than 10% of the CF passively leaking out after 24 h of storage at 4 °C. The remarkable stability of the peptide system in comparison to the CF assay is presumably due to the large size and polar nature of the $JR2KC_2$ species, which hinders nonspecific leakage.

A further advantage of the present approach is the flexibility afforded by the fact that the substrate (liposomes) and transducer (NPs/peptides) are not chemically linked. Because of this decoupling, the composition of lipids in the liposomes can easily be varied without affecting the assembly of the particles. The inclusion of lipids modified with poly(ethylene glycol) (PEG) is a common modification for drug delivery applications as it

dx.doi.org/10.1021/nl1024062 |*Nano Lett.* 2011, 11, 1401–1405

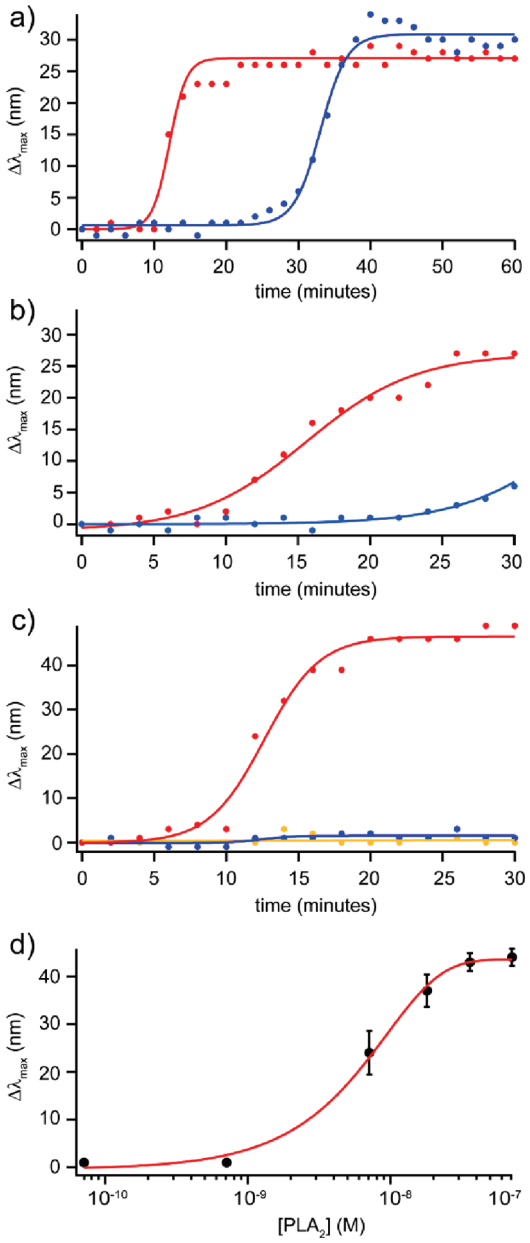


Figure 4. (a) Given the same concentration of PLA_2 (7 nM), the lag phase was considerably longer at room temperature (RT) (blue) than at 37 °C (red). (b) At RT with 7 nM PLA_2, the assay response was significantly enhanced by the presence of 4 mg/mL HSA (red) as compared to the same conditions without HSA (blue). (c) HSA alone does not induce a response without enzymatic activity and specific, peptide-mediated aggregation. With 4 mg/mL HSA and 70 nM PLA_2 at RT (red), the system gives a robust response. Removal of Ca^{2+} (orange) or $JR2KC_2$ (blue) under otherwise identical conditions completely abolishes this response. (d) Response as a function of PLA_2 concentrations 10 min after addition of 4 mg/mL HSA. Error bars are standard error of the mean, $n \geq 3$. Lines drawn as a guide for the eye.

decreases nonspecific interactions between the liposomes and proteins present in solution.[37] Interestingly, despite this diminished nonspecific interaction, the specific hydrolysis of lipids by phospholipases is actually increased in liposomes containing a small fraction of PEG-terminated lipids. This effect is most likely mediated by the negative net charge carried by the PEG−lipids.[38] In liposomes with 2% 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine-*N*-[methoxy(polyethylene glycol)] a significantly faster release of the peptides was observed at room temperature (Figure S1b in the Supporting Information). The lag time decreased from about 25 min in POPC liposomes to 10 min with the incorporation of PEG. The total response was also slightly higher as compared to POPC liposomes.

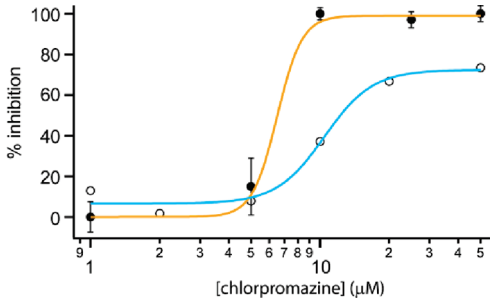


Figure 5. Inhibition of PLA_2 by chlorpromazine as determined using the NP−peptide assay (orange) and a traditional CF-based fluorescence assay (blue). Error bars are standard error of the mean, $n \geq 3$. The IC_{50} was estimated by fitting to the monophasic Hill equation $y = \min + (\max - \min)/[1 + (x/IC_{50})^{Hillslope}]$. For the NP-based and CF-based assays, the IC_{50} had a value of 6.5 and 10 μM, respectively.

One application in which it is crucial to measure the activity of an enzyme rather than just its concentration is high-throughput screening for inhibitors. Chlorpromazine is a prototypical, water-soluble small molecule inhibitor of PLA_2 and is therefore a good test case for the utility of this assay in such screening. The assay response was recorded after incubating various concentrations of the inhibitor with PLA_2 (7 nM), functionalized Au NPs (0.1 nM), and liposomes (1.4 nM) for 10 min at 37 °C. These experiments yielded an IC_{50} for chlorpromazine of 6.5 μM (Figure 5). This value was obtained by fitting the data to the monophasic Hill equation

$$y = \min + \frac{(\max - \min)}{1 + \left(\frac{x}{IC_{50}}\right)^{n}}$$

where min is the fully inhibited value, max is the uninhibited value, x is the concentration of inhibitor and n is the Hill coefficient. The lag time increased from about 5 min without the inhibitor to approximately 15 min in the presence of 10 μM of chlorpromazine (Figure S3 in the Supporting Information). This lag time indicates that the concentration of active non-inhibited PLA_2 was about half of the actual PLA_2 concentration present in the sample (Figure 3b). The inhibition by chlorpromazine was confirmed using CF loaded liposomes, which gave an IC_{50} value of 10 μM (Figure 5). Slightly higher values have been previously reported for chlorpromazine (IC_{50} = 10−100 μM),[39] indicating that the assay method used may affect the magnitude of the obtained IC_{50} value. This discrepancy could be due to the relative time scales involved. Since many traditional assays (e.g., radiometric assays) are very time-consuming and do not allow for continuous monitoring of enzymatic activity,[40] quantitation may occur after the lag phase has completed and the burst phase has begun. Accordingly, a relatively small amount of remaining active enzyme could result in a large response and, therefore, a larger concentration of inhibitor might be needed to obtain the same apparent inhibition as in a faster assay. If this discrepancy proves consistent with other inhibitor systems, the advantage of natural substrate presentation in addition to the real time measurements

dx.doi.org/10.1021/nl1024062 |*Nano Lett.* 2011, 11, 1401–1405

offered by this assay may prove critical in the future development of drug candidates. Moreover, unlike with electroactive or fluorometric systems, the present system is less likely to be perturbed by charged or highly conjugated inhibitors.

In conclusion, we have demonstrated a highly sensitive enzymatic assay utilizing Au NPs and liposomes. Using this assay, we were able to detect enzyme concentrations as low as 700 pM in real time. Higher sensitivity could be reached with an extended incubation time. In a rapid testing format, we were able to detect enzyme concentrations as low as 10 nM within 5 min by addition of HSA. The dynamic range shown here was about 3 orders of magnitude, covering the biologically relevant concentration range for phospholipases. In addition to determining phospholipase levels, we have also shown that this assay can be used to identify and study PLA_2 inhibitors. These results highlight the necessity and benefit of this hybrid approach, utilizing two very different nanomaterials. The use of liposomes allows investigation of enzymes acting on a biologically relevant substrate, while the use of polypeptide folding to induce aggregation of Au NPs offers a highly specific and robust transduction mechanism. Further, by decoupling the substrate from the readout material, we have created a flexible system in which each aspect can be independently tuned to the needs of a particular experiment. As a demonstration of this flexibility, we have modified the liposomes with antifouling PEG lipids while simultaneously increasing the system sensitivity. We expect that this system will have applications in both point of care diagnostic testing and research-level high throughput pharmaceutical screening of potential drug candidates.

■ ASSOCIATED CONTENT

Supporting Information. Additional data and detailed experimental procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: m.stevens@imperial.ac.uk.

Author Contributions

†These authors contributed equally to this work.

■ ACKNOWLEDGMENT

Financial support from the Knut and Alice Wallenberg Foundation is gratefully acknowledged (DA). M.M.S. thanks the EPSRC, ERC grant "Naturale" and the World Gold Council for funding. The authors thank Professor Lars Baltzer, Dr. Johan Rydberg, and Dr Karin Enander for introducing us to the field of helix−loop−helix polypeptides.

■ REFERENCES

(1) Giljohann, D. A.; Mirkin, C. A. *Nature* **2009**, *462*, 461–464.
(2) Rosi, N. L.; Mirkin, C. A. *Chem. Rev.* **2005**, *105*, 1547–1562.
(3) Aili, D.; Stevens, M. M. *Chem. Soc. Rev.* **2010**, *39*, 3358–3370.
(4) Laromaine, A.; Koh, L. L.; Murugesan, M.; Ulijn, R. V.; Stevens, M. M. *J. Am. Chem. Soc.* **2007**, *129*, 4156–4157.
(5) Aili, D.; Selegård, R.; Baltzer, L.; Enander, K.; Liedberg, B. *Small* **2009**, *5*, 2445–2452.
(6) Gupta, S.; Andresen, H.; Ghadiali, J. E.; Stevens, M. M. Kinase-Actuated Immunoaggregation of Peptide-Conjugated Gold Nanoparticles. *Small* **2010**, *6* (14), 1509–1513.
(7) Ghadiali, J. E.; Stevens, M. M. Enzyme-Responsive Nanoparticle Systems. *Advanced Materials* **2008**, *20* (22), 4359–4363.
(8) Elghanian, R.; Storhoff, J. J.; Mucic, R. C.; Letsinger, R. L.; Mirkin, C. A. *Science* **1997**, *277*, 1078–1081.
(9) Aili, D.; Enander, K.; Rydberg, J.; Nesterenko, I.; Björefors, F.; Baltzer, L.; Liedberg, B. *J. Am. Chem. Soc.* **2008**, *130*, 5780–5788.
(10) Lee, J. S.; Han, M. S.; Mirkin, C. A. *Angew. Chem., Int. Ed.* **2007**, *46*, 4093–4096.
(11) Sarda, L.; Desnuelle, P. *Biochim. Biophys. Acta* **1958**, 30.
(12) Burack, W. R.; Dibble, A. R. G.; Allietta, M. M.; Biltonen, R. L. *Biochemistry* **1997**, *36*, 10551–10557.
(13) Lehtonen, J. Y. A.; Kinnunen, P. K. J. *Biophys. J.* **1995**, *68*, 1888–1894.
(14) Dennis, E. A. *J. Biol. Chem.* **1994**, *269*, 13057–13060.
(15) Kugiyama, K.; Ota, Y.; Takazoe, K.; Moriyama, Y.; Kawano, H.; Miyao, Y.; Sakamoto, T.; Soejima, H.; Ogawa, H.; Doi, H.; Sugiyama, S.; Yasue, H. *Circulation* **1999**, *100*, 1280–1284.
(16) Agarwal, N.; Pitchumoni, C. S. *Am. J. Gastroenterol.* **1991**, *86*, 1385–1391.
(17) Green, J. A.; Smith, G. M.; Buchta, R.; Lee, R.; Ho, K. Y.; Rajkovic, I. A.; Scott, K. F. *Inflammation* **1991**, *15*, 355–367.
(18) Abe, T.; Sakamoto, K.; Kamohara, H.; Hirano, Y.; Kuwahara, N.; Ogawa, M.; Agarwal, N.; Pitchumoni, C. S. *Int. J. Cancer* **1997**, *74*, 245–250.
(19) Rose, T. M.; Prestwich, G. D. *ACS Chem. Biol.* **2006**, *1*, 83–92.
(20) Chemburu, S.; Ji, E.; Casana, Y.; Wu, Y.; Buranda, T.; Schanze, K. S.; Lopez, G. P.; Whitten, D. G. *J. Phys. Chem. B* **2008**, *112*, 14492–14499.
(21) Okada, S. Y.; Jelinek, R.; Charych, D. *Angew. Chem., Int. Ed.* **1999**, *38*, 655–659.
(22) Rex, S.; Schwarz, G. *Biochemistry* **1998**, *37*, 2336–2345.
(23) Fugman, D. A.; Shirai, K.; Jackson, R. L.; Johnson, J. D. *Biochim. Biophys. Acta* **1984**, *795*, 191–195.
(24) Lelkes, P. I.; Tandeter, H. B. *Biochim. Biophys. Acta* **1982**, *716*, 410–419.
(25) Kim, H. J.; Bennetto, H. P.; Halablab, M. A.; Choi, C. H.; Yoon, S. *Sens. Actuators, B* **2006**, *119*, 143–149.
(26) Zeineldin, R.; Piyasena, M. E.; Sklar, L. A.; Whitten, D.; Lopez, G. P. *Langmuir* **2008**, *24*, 4125–4131.
(27) Enander, K.; Aili, D.; Baltzer, L.; Lundström, I.; Liedberg, B. *Langmuir* **2005**, *21*, 2480–2487.
(28) Aili, D.; Enander, K.; Baltzer, L.; Liedberg, B. *Nano Lett.* **2008**, *8*, 2473–2478.
(29) Aili, D.; Tai, F.-T.; Enander, K.; Baltzer, L.; Liedberg, B. *Angew. Chem., Int. Ed.* **2008**, *47*, 5554–5556.
(30) Kunze, A.; Svedhem, S.; Kasemo, B. *Langmuir* **2009**, *25*, 5146–5158.
(31) Burack, W. R.; Biltonen, R. L. *Chem. Phys. Lipids* **1994**, *73*, 209–222.
(32) Wacklin, H. P.; Tiberg, F.; Fragneto, G.; Thomas, R. K. *Biochim. Biophys. Acta, Biomembr.* **2007**, *1768*, 1036–1049.
(33) Majd, S.; Yusko, E. C.; MacBriar, A. D.; Yang, J.; Mayer, M. *J. Am. Chem. Soc.* **2009**, *131*, 16119–16126.
(34) Honger, T.; Jorgensen, K.; Stokes, D.; Biltonen, R. L.; Mouritsen, O. G. *Methods Enzymol.* **1997**, *286*, 168–190.
(35) Bhattacharya, A. A.; Grüne, T.; Curry, S. *J. Mol. Biol.* **2000**, *303*, 721–732.
(36) Wilschut, J. C.; Regts, J.; Scherphof, G. *FEBS Lett.* **1979**, *98*, 181–186.
(37) Blume, G.; Cevc, G. *Biochim. Biophys. Acta* **1990**, *1029*, 91–97.
(38) Vermehren, C.; Kiebler, T.; Hylander, I.; Callisen, T. H.; Jørgensen, K. *Biochim. Biophys. Acta* **1998**, *1373*, 27–36.
(39) Lindahl, M.; Tagesson, C. *Inflammation* **1993**, *17*, 573–582.
(40) Reynolds, L. J.; Washburn, W. N.; Deems, R. A.; Dennis, E. A. *Methods Enzymol.* **1991**, *197*, 3–23.