Biomedical Analysis

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Translating Molecular Recognition into a Pressure Signal to enable Rapid, Sensitive, and Portable Biomedical Analysis**

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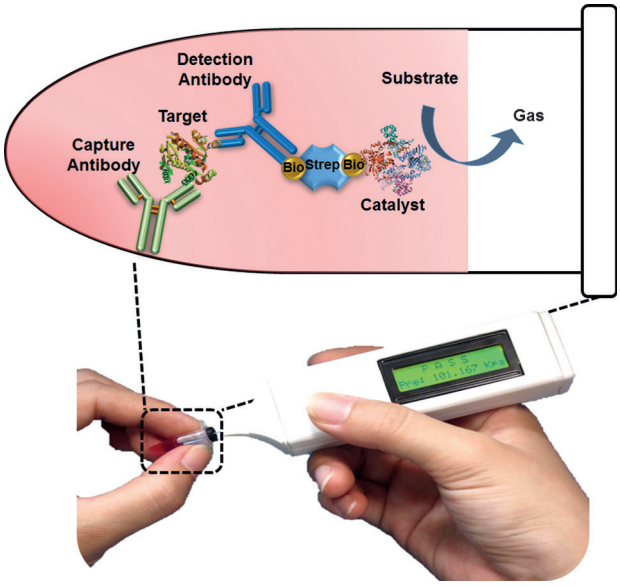
Abstract: *Herein, we demonstrate that a very familiar, yet underutilized, physical parameter—gas pressure—can serve as signal readout for highly sensitive bioanalysis. Integration of a catalyzed gas-generation reaction with a molecular recognition component leads to significant pressure changes, which can be measured with high sensitivity using a low-cost and portable pressure meter. This new signaling strategy opens up a new way for simple, portable, yet highly sensitive biomedical analysis in a variety of settings.*

One of the great challenges in science and engineering today is the development of simple, affordable, yet highly sensitive and specific point-of-care (POC) technologies for biomedical analysis, especially for quantitative detection of disease-specific biomarkers. This capability will streamline healthcare and improve clinical outcomes, substantially improving the standard of living.[1] To meet the growing need for POC testing, developers need to consider both simplification and miniaturization of the devices.[2] To date, optical, magnetic, mass spectrometric, and electrochemical methods for signal readout are still generally regarded as the gold standards in laboratory scenarios. However, for field work or POC testing, they have to overcome certain limitations, such as high cost, environmental interference, or the bulkiness of the instrumentation and the need for experienced operators.[3] For example, optical detection requires light sources, optical filters, photon detectors, and careful optical alignment, which adds complexity and cost for POC miniaturization.[4] To overcome these limitations, alternative approaches based on totally different signaling principles are being pursued. For example, glucose meters have recently been applied for POC testing of non-glucose targets.[5] Some researchers have creatively used some common parameters, such as distance,[6] time,[7] or smell,[8] as the readout for simple and portable POC testing.

Several classical physical principles are widely applied in simple devices, such as thermometers, barometers, hygrometers, for portable, sensitive, and quantitative detection without external bulky instrumentation. For example, pressure measurements, such as for tire maintenance, are performed everyday with simple devices. In fact, pressure measurement has been widely used in industry and other applications to monitor chemical and biochemical processes.[9] Gas-generation reactions, which can produce considerable amounts of gas, can lead to obvious pressure increases in a sealed device. A common example is the decomposition of H_2O_2 to H_2O and O_2. Theoretically, under standard conditions, the decomposition of 1 mmol H_2O_2 (around 100 μL) generates 11.2 mL O_2. If confined to a 100 μL tube, the pressure rises to over 10^7 Pa, which can easily be detected using a pressure meter. If linked with a molecular recognition event, such a gas-generation reaction can translate the molecular-recognition to measurable pressure signal for biomolecule detection with tremendous signal amplification. Based on these simple chemical and physical principles, for the first time we report that the integration of a catalyzed gas-generation reaction with a molecular-recognition component enables pressure measurement as a signaling technique to develop pressure-based bioassays (PASS for short) for the detection of a variety of biomedical targets.

ELISA (enzyme-linked immunosorbent assay) has been used extensively as a diagnostic tool in medicine for protein quantitation. Traditionally, signal readout in ELISA relies heavily on radio- and optical-based measurements. Using ELISA as an example, we demonstrate the applicability of PASS as the signaling technique for protein biomarker detection, (termed PASS-ELISA or PLISA). A schematic representation of the PLISA format is presented in Scheme 1.



Scheme 1. Working principle of PLISA. The sandwich structure is formed between capture antibody, target, and detection antibody. The detection antibody is functionalized with a gas-generation catalyst. Introduction of the substrate initiates a rapid catalytic gas-generation reaction, leading to a significant pressure increase in the reaction chamber allowing a sensitive readout by using a portable pressure meter.

The capture antibodies bound on the solid support will capture target antigens from the sample. The detection antibodies, which are conjugated with catalyst, such as enzymes or catalytic nanoparticles, will then bind to another

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site on the target. Introduction of substrate will initiate rapid catalyzed gas-generation reaction, leading to significant pressure increase in the reaction chamber, which can be sensitively read out by a simple, low cost, and portable pressure meter.

The key step for PASS is the conversion of a biomolecular recognition event into a highly efficient gas-generation reaction for highly sensitive detection. Although there are many textbook examples of gas-generation reactions,[10] the decomposition of H_2O_2 to generate nontoxic O_2 was chosen because the reactant and product are nontoxic and environmentally friendly. Moreover, the rate this reaction can be significantly accelerated by catalase which is capable of converting millions of H_2O_2 molecules to O_2 per second.[11]

To test the feasibility of using the breakdown of H_2O_2 for PASS, catalase with various concentrations was mixed with saturated H_2O_2 solution (9.79 M) to generate O_2 in an airtight tube, and the pressure change in the tube was monitored as a function of time by a portable home-made pressure meter (Figure S1 in the Supporting Information). As shown in Figure 1a, in the presence of catalase, a system pressure change is easily observed within a few minutes, and the pressure change (ΔP), which is defined as the pressure difference between pressure before reaction and the pressure after certain reaction time, increases with increasing catalase concentration, confirming that catalase-accelerated breakdown of H_2O_2 for O_2 generation can lead to a detectable pressure change.

To minimize the destructive effect of the saturated H_2O_2 on the enzyme[12] while still producing O_2 with high efficiency, the concentration of H_2O_2 was further optimized (Figure 1b). Under the optimized H_2O_2 concentration (0.31 M), the pressure change was found to be linearly proportional to the concentration of catalase (Figure S2). The limit of detection (LOD) for catalase was found to be 47.0 pM by 3σ of six measurements of blank samples. The experimental results established the feasibility of combining pressure-based measurements with a gas-generation reaction for highly sensitive biomolecule detection.

After verifying the feasibility of PASS, we designed a sandwich PLISA assay for the detection of prostate specific antigen (PSA) by tagging the detection antibody with catalase. As shown in Figure 1c, the ΔP value was linearly correlated with the concentration of PSA with an LOD of 418.0 pM. The results proved that the pressure change induced by O_2 generation can be used to convert a biomolecular recognition event into a highly sensitive readout for bioanalysis.

Compared with traditional ELSIA using absorbance readout (Figure S3a, LOD for PSA ≈ 8.6 pM), the sensitivity of catalase-PLISA needs improvement. As described above, the limited sensitivity of catalase-PLSIA is attributed to denaturation of the enzyme even in the optimized experimental condition. Alternatively, Pt nanoparticles (PtNPs) were synthesized[13] (Figure S4) and used as catalase mimics because of their good stability and excellent catalytic ability for H_2O_2 decomposition.[10,14] As shown in Figure 2a, the PtNPs exhibited a dramatic increase in catalytic efficiency compared to catalase. With as low as 0.5 pM PtNPs, the catalytic ability is considerably higher than that of 25 nM catalase. Further increase in the concentration of PtNPs to 2.5 pM caused the ΔP to sharply exceed the pressure meter detection range within 8 min. Moreover, in contrast to catalase, PtNPs showed increased catalytic ability with increasing H_2O_2 concentration (Figure 2b), and they could steadily catalyze the generation of O_2 over a long period of time, indicating excellent stability in the H_2O_2 substrate. The inherent catalytic efficiency and longer lifetime of PtNPs resulted in generation of 400-times as much O_2 per second as catalase (Figure 2c). Furthermore, at saturated H_2O_2 concentration, the ΔP value was linearly correlated with the concentration of PtNPs with an LOD of 1.4 fM (Figure 2d), which is more than four orders of magnitude lower than the LOD for catalase (47 pM), demonstrating the excellent sensitivity of PtNPs for quantitative bioanalysis.

To incorporate PtNPs into the PLISA, PtNPs were conjugated with biotin by a thiol-PEG-biotin hetero-linker (Figure S5), then conjugated with the detection antibody through biotin-streptavidin interaction. The sandwich assay was formed in the same manner as for the catalase-PLISA. Different concentrations of PSA were spiked in buffer and detected by the PtNP-based PLISA (Pt-PLISA). As Figure 3a shows, the ΔP value was directly proportional to the PSA concentration, with an LOD of 870 fM, which is three orders of magnitude lower than that of the catalase-PLISA and one order of magnitude lower than that of the standard ELISA with the same assay time of 6 h for all three methods (Figure S3a).

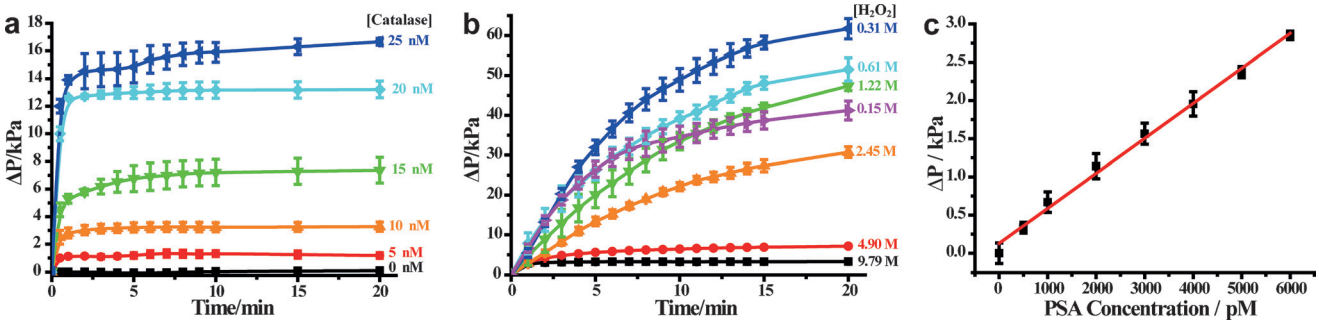

Figure 1. a) Pressure-change profiles of the H_2O_2 decomposition reaction with different concentrations of catalase in saturated H_2O_2 (9.79 M). b) Pressure-change profiles of the H_2O_2 decomposition reaction at different H_2O_2 concentrations ([catalase] = 5 nM). c) Linear response of the pressure change to PSA target concentration ($R^2 = 0.997$).

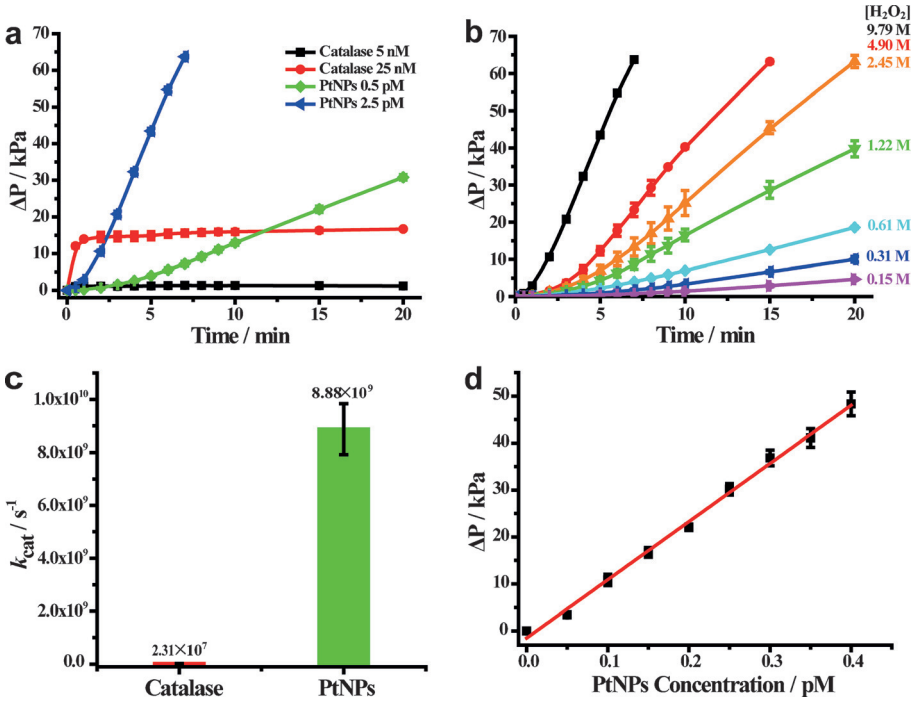

Figure 2. a) Pressure-change profiles of the H_2O_2 decomposition reaction catalyzed by different concentrations of catalase or PtNPs in saturated H_2O_2. b) Pressure-change profiles of the H_2O_2 decomposition reaction catalyzed by PtNPs using different H_2O_2 concentrations. c) The apparent turnover number (k_{cat}) of catalase and PtNPs. d) Linear relationship of pressure change and PtNP concentration in saturated H_2O_2 ($R^2 = 0.991$).

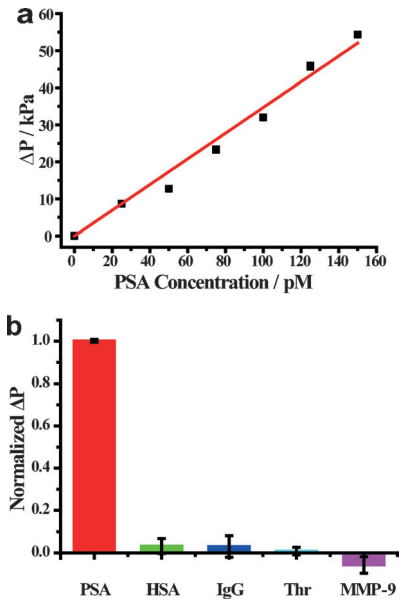

Figure 3. a) Linear response of pressure change to the concentration of PSA ($R^2 = 0.995$). b) The selectivity of Pt-PLISA for PSA detection, 100 pM for each protein.

The specificity of Pt-PLISA for PSA detection was then investigated with some common proteins found in serum as the negative controls, including human serum albumin (HSA), immunoglobulin G (IgG), matrix metalloproteinases-9 (MMP-9) and thrombin (Thr). In Figure 3b, with the same concentrations of proteins tested (100 pM), PSA exhibited significant pressure change, while the control proteins gave only negligible signals. Therefore, these experiments unequivocally demonstrate that the pressure change observed in the presence of PSA is not generated by non-specific interactions with the substrate, but is in fact related to selective molecular recognition by antibody.

We further investigated the versatility of Pt-PLISA by detecting Influenza A/H5N1 virus (H5N1)[15] with Pt-PLISA in buffer, and an LOD of 160 fM was obtained (Figure S6a), almost five-times lower than 790 fM by standard ELISA (Figure S3b). The selectivity was investigated by control proteins, including IgG, HSA, H3N2, and SARS (Figure S6b), and the ΔP was negligible for the controls. We also performed the detection of H5N1 in spiked serum sample with Pt-PLISA and a standard ELISA method. Results from both methods matched very well with a slope of 0.98 ($R = 0.998$, Figure S7). These results clearly demonstrated that the PASS method is highly applicable with a sandwich-based platform for the sensitive and accurate detection of a wide variety of proteins.

To demonstrate portable detection capability of the method and reduce the assay time, we used magnetic beads as support for immobilization of the capture antibodies, and used Pt-PLISA for the detection of PSA in spiked serum. As shown in Figure 4a, the ΔP increased linearly with PSA concentration in serum, realizing an LOD of 1.42 pM. It is noteworthy that detection was achieved with the portable device within 2 h, including multiple incubation and washing steps, and final signal readout. Therefore, our pressure-change detection platform is well suited for highly sensitive protein detection in clinical and POC settings. Furthermore, to demonstrate the user-friendly nature of the method, three users unfamiliar with the technique were asked to perform the measurement of a same unknown sample. The concentration results were obtained with very small deviation by all three users (Figure S8).

To confirm the accuracy of the Pt-PLISA for PSA detection in real patient samples, we tested 17 serum samples and compared the results with current benchmark for PSA detection in hospitals, that is, chemiluminescence microparticle immuno assay (CMIA). As shown in Figure 4b and Figure S9, the two methods correlate well with a slope of 0.99 ($R^2 = 0.998$). Thus, the accuracy of the portable Pt-PLISA is at least as good as that of the standard ELISA method for real-sample analysis, indicating the potential use in monitoring

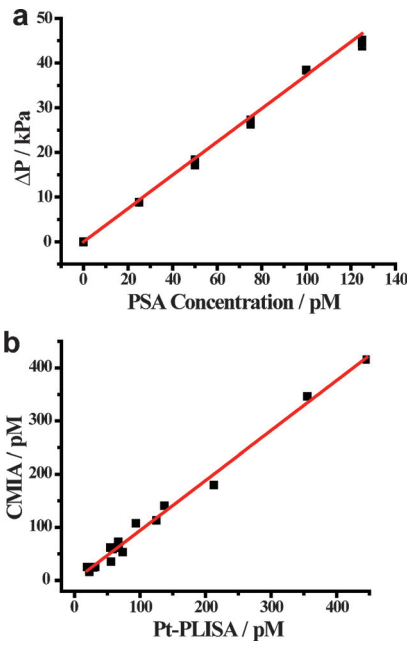

Figure 4. a) Linear standard curve of PSA detection in serum by Pt-PLISA with magnetic beads ($R^2 = 0.998$). b) Correlation of Pt-PLISA analysis results with standard immunoassay CMIA results for PSA detection in 17 serum samples ($R^2 = 0.995$).

disease progress in prostate cancer patients in resource-constrained settings.

In conclusion, we demonstrated for the first time that gas-pressure increase induced by the catalyzed gas-generation reaction can be used as signal readout for highly sensitive bioanalysis. There are several advantages offered by the pressure-based signaling strategy for bioanalysis. First of all, PASS affords highly sensitive detection resulting from two important signal-amplification factors. When gas is generated from a reaction and released from the solution, its volume expands by 2–3 orders of magnitude, leading to significant pressure increase in a sealed device. More importantly, with the excellent catalytic properties of catalase/PtNP, one molecular recognition event leads to the generation of more than one million O_2 molecules per second. This combination of liquid to gas volume expansion and extremely high turnover number could theoretically result in a signal enhancement factor of more than 10^{10} with a few minutes of reaction. Compared to absorbance- or chemiluminescence-based ELISA, PLISA is one order of magnitude higher in sensitivity even with a handheld pressure meter. Second, the pressure-based measurement is free from optical or magnetic field noise. This unique property simplifies instrumental design and further contributes to the excellent sensitivity and accuracy of the method. Third, the substrate used is H_2O_2, while the reaction products are H_2O and O_2. The reactant and products are all environmental friendly, which is an attractive factor for POC applications, where safe use by inexperienced personnel is an important concern. Finally, unlike optical detection devices that require light sources, optical filters and photon detectors, pressure based measurement can be easily achieved using a very simple pressure sensor, such as pressure meter, which is lightweight, small, inexpensive, and portable. On the other hand, it should be noted that pressure reading is sensitive to temperature and elevation changes. Like most of analysis methods, a calibration curve should be obtained under the same experimental condition to remove these effects. Using the PASS technology, we have demonstrated that pressure-based bioanalysis can be achieved for highly sensitive detection of important protein biomarkers, including cancer biomarker PSA and virus biomarker H5N1. With the advantages of simplicity of both design and operation, low cost, portability, and excellent sensitivity, the pressure-based signaling strategy opens up a new way for simple, sensitive, affordable biomedical assays in clinical and point-of-care settings, as well as routine analyses of a variety of analytes.

Keywords: gas generation · hydrogen peroxide · immunoassay · point-of-care testing · pressure measurement

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