



# Using a glucose meter to quantitatively detect disease biomarkers through a universal nanozyme integrated lateral fluidic sensing platform

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## ABSTRACT

Along with the advance in medical research, more biomarkers emerge as useful indicators for both disease and health index. However, majority of them have no practical or economic testing methods available yet, or rely on high-costing methods such as Enzyme-Linked Immuno-Sorbent Assay (ELISA), High-Performance Liquid Chromatography (HPLC), Mass Spectrum, and immunohistochemistry (IHC). In this article, we develop a universal nanozyme integrated testing platform for biological molecules that incorporates the electrochemical measurement of glucose with lateral flow immunostrip (LFS) for target analytes. This design involves the quantitative conversion of analytes into invertase and then glucose, which can be measured by an extremely affordable meter. The feasibility of this design was validated using 8-hydroxy-2'-deoxyguanosine (8-OHdG) and prostate specific antigen (PSA) as representatives for small molecules and moderate to large proteins respectively. Our approach yields results comparable to commercial diagnostic ELISA kits at a substantially reduced cost and reaction time. Specifically, the design has a detection limit of 0.23 ng mL<sup>-1</sup> for 8-OHdG and 1.26 ng mL<sup>-1</sup> for PSA, and a detection range of 0.1–100 ng mL<sup>-1</sup> for 8-OHdG and 1–100 ng mL<sup>-1</sup> for PSA. By combining the accessibility of well-established glucose testing and LFS, our design can serve as a point of care testing method that can be fully integrated into the personal lifestyle without requiring professional assistance.

## 1. Introduction

Modern clinical tests for most diseases are primarily facilitated at large hospitals or laboratories. Although these locations greatly reduce patient cost by centralizing clinical practice and testing devices, they require both doctors and patients to travel for each appointment and/or laboratory visit. The heavy reliance on travel is especially impractical for many medical conditions requiring chronic and continuous tests. However, the health care industry is shifting care management from periodic testing to individualized continuous health monitoring to prevent and detect diseases at earlier points in time. To facilitate this transition in health care, engineers, researchers, and pharmacists are developing point of care testing (POCT) devices that deliver economic, rapid, on-site diagnostic results without professional assistance (Chan et al., 2013; Hicks et al., 2001; Kost, 2002). POCT has already proven

itself as a health care priority; the POCT market reached USD \$23.16 billion in 2016, at a compound annual growth rate (CAGR) of 9.8% during the forecast period, and will reach USD \$36.96 billion by 2021 ([marketsandmarkets.com](http://marketsandmarkets.com), 2016).

The most successfully marketed over-the-counter POCT device is the personal glucose meter (PGM), widely used by diabetes patients to monitor blood glucose levels at home. Once a blood sample has been obtained by a collection tip, the PGM specifically and reproducibly measures the amount of glucose in the sample through an enzyme catalyzed redox reaction combined with an electronic module. The PGM offers several strengths to users: pocket size, low cost, simple operation, and reliable quantitative results (Heller et al., 2009; Montagnana et al., 2009). Although the properties of blood glucose allow for electrochemical quantification, most biomarkers or vital molecules lack similar electrochemical properties and require more

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sophisticated approaches for measurement. Several methods have been adapted for analyzing such biomarkers, such as Enzyme-Linked Immuno-Sorbent Assay (ELISA), High-Performance Liquid Chromatography (HPLC), Mass Spectrum, and immunohistochemistry (IHC). However, these methods are quite often expensive and require machinery that is difficult to adapt into a point-of-care context.

In order to take advantages of this well-established commercialized POCT technique, effort has been made to establish a relationship between target recognition and glucose generation to quantitatively detect non-glucose target. However, most of them are solution-based tests which still need multiple chemical mixing and handling procedures (Su et al., 2012; Xiang and Lu, 2011, 2012a, 2012b, 2013; Yan et al., 2013). Lateral flow immunostrip (LFS) was incorporated with PGM to address this challenge (Huang et al., 2018; Jingjing Zhang et al., 2016; Zhao et al., 2017; Zhu et al., 2017). LFS, another common category of POCT devices most widely used in qualitative disease diagnostics as well as environmental and food analysis, involves antibodies instead of electrochemistry for detection. In LFS, antibodies specifically recognize the analyte of interest, which is eventually converted to colorimetric density of antibody conjugated nanoparticles. Reagent preloaded LFS minimizes extra solution storage and handling and thus largely simplifies the testing procedure. Integration of the traditional LFS with the PGM to take the advantages of both techniques represents a novel formulation of POCT that can provide quantitative measurement at low cost.

While LFS-PGM integration has been exploited for many biomarker detection, practice is limited to proteins of modest to large size (Zhao et al., 2017), or microorganism (Huang et al., 2018). On the other hand testing for small size analyte is largely omitted. Small molecule cannot afford simultaneous binding of multiple antibodies for typical sandwich type immunoreaction posing the biggest challenge for their testing development. Nevertheless development of LFS-PGM with compatibility of small molecule testing is extremely valuable. It not only demonstrates the full range (size) compatibility but also possesses clinical significance as small molecule (metabolic biomarkers, short peptide, nuclear acid) constitutes a large portion of biomarkers for a variety of diseases (Dash et al., 2010; Reyzer and Caprioli, 2007; Shah et al., 2010; Tan et al., 2018).

8-hydroxy-2'-deoxyguanosine (8-OHdG) is one of the predominant forms of free radical-induced lesions of DNA (Pulido and Parrish, 2003; Risom et al., 2005; Valko et al., 2005, 2006) that has been extensively studied as a biomarker for oxidative stress and carcinogenesis (Kasai, 1997; Shigenaga et al., 1989; Valavanidis et al., 2009). We have successfully detected this small molecule in vitro as well as in body fluid by using a gold nanoparticle (AuNPs)-based competitive type LFS (Zhu et al., 2013, 2014). Recently, we also proposed a testing platform that converts the concentration of 8-OHdG to the amount of glucose (Zhu et al., 2017).

It has been shown that serum prostate-specific antigen (PSA) is the most reliable tumor marker to detect prostate cancer (PCa) at the early stage and to monitor recurrence of the disease after treatment (Benson et al., 1992; Bradford et al., 2006; Brawer, 1999; Stephan et al., 2006). Currently, most PSA testings take place at dedicated centralized laboratories using large, automated analyzers, requiring sample transportation, increased waiting time and increased administration and medical costs (Acevedo et al., 2002; Healy et al., 2007). Near-patient or POCT is highly needed to reduce the number of clinic visits, decrease costs to the patient and the healthcare system, increase patient satisfaction and improve clinical outcome.

In this article, we develop a universal nanozyme integrated sensing platform that incorporates the electrochemical measurement of glucose with LFS for target analysis. This design involves the quantitative conversion of analytes into invertase and then glucose, which can be measured by an extremely affordable meter. It is ideally compatible for any type of molecules whose antibodies are available. Performance of this platform was validated using 8-OHdG and PSA as representatives

for small molecules and moderate to large proteins respectively. When compared with commercial testing kit (ELISA), and conclude that our approach is preeminent in terms of remarkably reduced cost, shortened testing time, larger dynamic range, comparable detection limit, and no requirement of advanced analytic devices.

## 2. Material and methods

### 2.1. Materials and reagents

Bovine serum albumin, Invertase from baker's yeast, Sucrose, Gold chloride trihydrate, Sodium borohydride, Sodium periodate, Ethylene Glycol, and Prostate Specific Antigen from human semen were purchased from Sigma-Aldrich (St. Louis, MO). NeutrAvidin Agarose, Streptavidin, EZ-Link Hydrazide-Biotin and Biotin Quantitation Kit were products of Pierce Biotechnology (Rockford, IL, USA). Plastic backing, Nitrocellulose membrane, Absorbing pad and cellulose paper were purchased from Millipore (Billerica, MA). Streptavidin Conjugated Gold Nanoparticles were ordered from Nanocs Inc. (New York, NY). 8-hydroxy-2'-deoxy Guanosine and 8-hydroxy Guanosine were provided by Cayman chemical (Ann Arbor, MI). 8-OHdG ELISA kit (Catalog No. KOG-200S/E) was bought from Genox Corporation (Torrance, CA). Mouse monoclonal antibodies to 8 hydroxyguanosine (biotin conjugated), Mouse monoclonal [PS2] to prostate specific antigen (Biotin), Mouse monoclonal [PS6] to Prostate Specific Antigen and polyclonal Goat anti Mouse IgG were all products of Abcam (Cambridge, MA). PGM and corresponding test strips were manufactured by TRUEtrack and Walgreens respectively.

### 2.2. Preparation of streptavidin coated gold nanoparticles (STV-AuNPs)

The AuNPs were synthesized by using the same protocol as described in previous work (Zhu et al., 2013, 2017, 2014). For Streptavidin labeling, the AuNPs solutions was concentrated 5-fold ( $5 \times$ ) in advance and the pH was adjusted to 6.8–7.2 with  $K_2CO_3$  (0.1 M). Before absorption-based conjugation, the optimal concentration of protein was determined by the same protocol as described in previous work (Zhu et al., 2013, 2017, 2014). Briefly, streptavidin solutions (20  $\mu$ L) of different concentrations (0, 0.1, 0.25, 0.5 and 1 mg mL<sup>-1</sup>) were mixed with gold solution ( $5 \times$ , 100  $\mu$ L, pH 6.8–7.2) and incubated for 15 min at room temperature, and then 10% NaCl solution was added. The color of samples changes from brilliant red to blue as the concentration of Streptavidin decreases. The optimum concentration for labeling was the lowest concentration of mAb that did not change color, which is 0.25 mg mL<sup>-1</sup>.

At the same time, we also measured the zeta potentials of the five mixtures (after 1 h incubation and two times purification). We found that the zeta potential (see Supporting information (SI), Table S1) became less negative when the concentration of streptavidin increased, but there was no obvious change when the concentration is larger than 0.5 mg mL<sup>-1</sup>. Based on the previous two experiments, 0.5 mg mL<sup>-1</sup> was chosen as the optimal concentration for labeling.

### 2.3. Preparation of biotinylated invertase

Biotin was conjugated to sialic acid residues of invertase by using a EZ-Link Hydrazide-Biotin kit by following manufacturer's guide. The efficiency of biotinylation was tested through NeutrAvidin Agarose. Briefly, an aliquot (30  $\mu$ L) of agarose beads were separated and re-suspended in 200  $\mu$ L PBS buffer containing biotin-invertase (0.25 mg mL<sup>-1</sup>), and the mixture was incubated for 1 h which allows fully immobilization of biotin by excess neutravidin. Then the mixture was centrifuged for 2 min and the supernatant was collected. 10  $\mu$ L of the supernatant and biotin-invertase solution (0.25 mg mL<sup>-1</sup>) were mixed with 50  $\mu$ L 0.5 M sucrose solution respectively. After 30 min, the readout of PGM (TRUEtrack Walgreen) was collected. From the result

(see SI, Table S2), we can conclude that the efficiency of the biotinylation of the enzyme was greater than 70%.

#### 2.4. Preparation and characterization of invertase/antibody-AuNPs nanozyme complex

An aliquot (100  $\mu\text{L}$ ) of the streptavidin coated gold nanoparticles (STV-AuNPs) was centrifuged and resuspended with 100  $\mu\text{L}$  PBS buffer containing biotin-invertase (2.5  $\text{mg mL}^{-1}$ ) and biotin-Ab (32.5  $\mu\text{g mL}^{-1}$ ) for 1 h. Then the invertase/Ab-AuNPs nanozyme complex were separated, washed with 1% BSA/PBS and finally resuspended in buffer (50  $\mu\text{L}$ ) for further use.

#### 2.5. Preparation of BSA-8 Hydroxyguanosine conjugates

8-hydroxyguanosine (5 mg) was dissolved in  $\text{NaIO}_4$  (1 mL, 50 mM) and the mixture was incubated for 1 h in the dark. The reaction was stopped by adding Ethylene glycol (2.5  $\mu\text{L}$ ) for 5 min. The mixture was then mixed with BSA (2 mL, 25  $\text{g L}^{-1}$ , pH = 9.5, adjusted by  $\text{K}_2\text{CO}_3$  (50  $\text{g L}^{-1}$ ) under constant stirring dropwise and incubated for another 1 h. After that, Sodium borohydride ( $\text{NaBH}_4$ , 2 mL, 24  $\text{g L}^{-1}$ ) was added and the mixture was incubated in the dark at 4 °C overnight (12–16 h). Finally, the conjugates were dialyzed against 1X PBS and stored at – 20 °C.

#### 2.6. Assembly of the LFS

Details are similar to our previous work (Zhu et al., 2013, 2014) with slight modification. Briefly, the LFS consists of four components: sample loading pad, conjugated pad, nitrocellulose membrane, and absorption pad, all of which were pasted onto a plastic backing plate. The sample pad was made from glass fiber and pretreated with buffer (50 mM borate buffer, 1% BSA and 0.2% Tween-20). And invertase/Ab-AuNPs conjugates (20  $\mu\text{L}$ ) was dispensed by pipette onto the conjugate pad. These pads were dried at room temperature and stored in desiccators at 4 °C before use. The corresponding capture reagents were dispensed by the Linomat 5 dispenser at different locations on a nitrocellulose membrane as the test and control lines respectively. Finally, all four parts were assembled on the adhesive backing layer. Each part overlapped 2 mm to ensure the solution migration through the strip during the assay.

### 3. Results and discussion

#### 3.1. Design and detection mechanism

We have successfully demonstrated a concept that converts 8-OHdG amount to the amount of glucose for the first time through a two conjugation pad testing platform (Zhu et al., 2017), though apparent limitations do exist. 1. This configuration is only compatible with small molecule sensing; 2. More sensing components (two conjugation pads) on strips are associated with increased difficulty in fabrication and decreased reproductivity and stability. Therefore, we intend to develop a new universal nanozyme integrated sensing platform here to eliminate these limitations. In theory, the new platform can be used for any type of biomolecules (small, moderate and large) detection through a single nanozyme based conjugation pad with the signal readout that is more straightforward.

We designed a universal testing platform for the diagnostics of various biomarkers as shown in Fig. 1a. The test strip is composed of four components which were preloaded with different reagents and pasted onto a plastic backing plate. The sample pad pretreated with buffer (50 mM borate buffer, 1% BSA and 0.2% Tween-20) is where the strip contacts with the liquid sample. Conjugation pad, which is composed of glass-fiber material, contains invertase/antibody (Ab)-conjugated AuNPs. Next to the conjugation pad is a flow membrane, which

is made of nitrocellulose. There are two reaction zones on membrane, the test line and control line. BSA/8 hydroxyguanosine conjugates (1  $\text{mg mL}^{-1}$ ) or Mouse anti-PSA [PS6] antibody (1  $\text{mg mL}^{-1}$ ) was used as the test line capture reagent, while Goat anti-Mouse IgG Ab (1  $\text{mg mL}^{-1}$ ) was used as the control line capture reagent. Sample moves along the strip due to capillary action and finally gets collected by last component known as the absorption pad (Fig. 1a).

To realize non-glucose target detection using a PGM, the relationship between target recognition and glucose generation must be established. In our design, as illustrated in Fig. 1b and c, the detection is based on two steps: the first step is that of competitive-type (for 8-OHdG, Fig. 1b (A)(B)(C)(D)) or sandwich-type (for PSA, Fig. 1c (A)(B)(C)(D)) immunoreactions in the LFS; the second step (Fig. 1b (E)(F)(G) or Fig. 1c (E)(F)(G)) contains an enzymatic reaction which converts sucrose into detectable glucose for measurement by a PGM.

Upon target introduction (Fig. 1b(A) and c (A)), the target moves along the strip due to capillary force and rehydrate the conjugation pad, where the immunoreactions take place (Fig. 1b(B) and c(B)). Subsequently, the invertase/Ab-AuNPs can be captured separately by the corresponding reaction zone based on the format of the Ab (Ab with free antigen-binding site will be captured by the BSA/8 hydroxyguanosine conjugates on the test line Fig. 1b (C)); Ab saturated with 8-OHdG from samples will be captured by the Goat anti-Mouse IgG on the control line (Fig. 1b (D)); Ab bounded with PSA will be captured by anti-PSA Ab2 on the test line (Fig. 1c (C)); the rest Ab will be fixed on the control line (Fig. 1c (D)). Competitive-type immunoreactions make the target (sample) compete with the antigen (test line) for the antibody from conjugation pad. As a result, the color intensity of the test line as well as the amount of invertase captured on the test line are inversely proportional to the target concentration in the sample. Instead, the amount of invertase captured on the test line is proportional to concentration of PSA in the sandwich type assay.

Followed by the first step (< 10 min), as a conceptual and validation study, the test (2 mm) and control (2 mm) lines are cut by scissors and placed on a hydrophobic plate (Fig. 1b (E) and c (E)). Then, 20  $\mu\text{L}$  0.5 M sucrose solutions are added on top of each zone (Fig. 1b (F) and c (F)). After a specific period of time, the enzyme invertase fixed on each zone catalyzes the hydrolysis of sucrose to produce a specific amount of glucose for quantitative readout by the PGM (Fig. 1b (G) and c (G)). Sophisticated engineering to have generation of invertase on testing lines and the production of glucose integrated will be needed following the current study for ultimate mass production.

#### 3.2. Check the properties of bifunctional nanozyme complex

The bifunctional Nanozyme complex which includes enzyme (invertase) and antibody on AuNPs is one of the most critical components in our design. The enzymatic product of invertase (glucose) can be monitored by a PGM. And the invertase/Ab-AuNPs are also used as signal amplifier, which converts limited event of antibody-antigen recognition to considerable changes in the amount invertase on test lines and subsequent glucose product, when given a properly designed complex. As a result, target-induced competitive or sandwich immunoreactions can lead to the capture of numerous invertases and generate a measurable amount of glucose that falls in the dynamic range of the PGM (0.6–33.3 mM or 10–600  $\text{mg dL}^{-1}$ ). Therefore, two aspects of the functions of the complex were examined before being used for the conjugation pad: enzymatic activity (invertase part) for glucose production and immunoreactivity (antibody part) for competitive or sandwich immunoreactions.

Firstly, the enzymatic activity of the complex was tested by mixing 10  $\mu\text{L}$  Nanozyme solution with 50  $\mu\text{L}$  0.5 M sucrose solutions. The readout of PGM was 436  $\text{mg dL}^{-1}$  ( $n = 3$ ) after 10 min, indicating that complex possess a high enzymatic activity. Secondly, the immunoreactivity of the complex was tested through colorimetric antibody-antigen immunoreactions. Fig. S1 depicts the reduction of redness

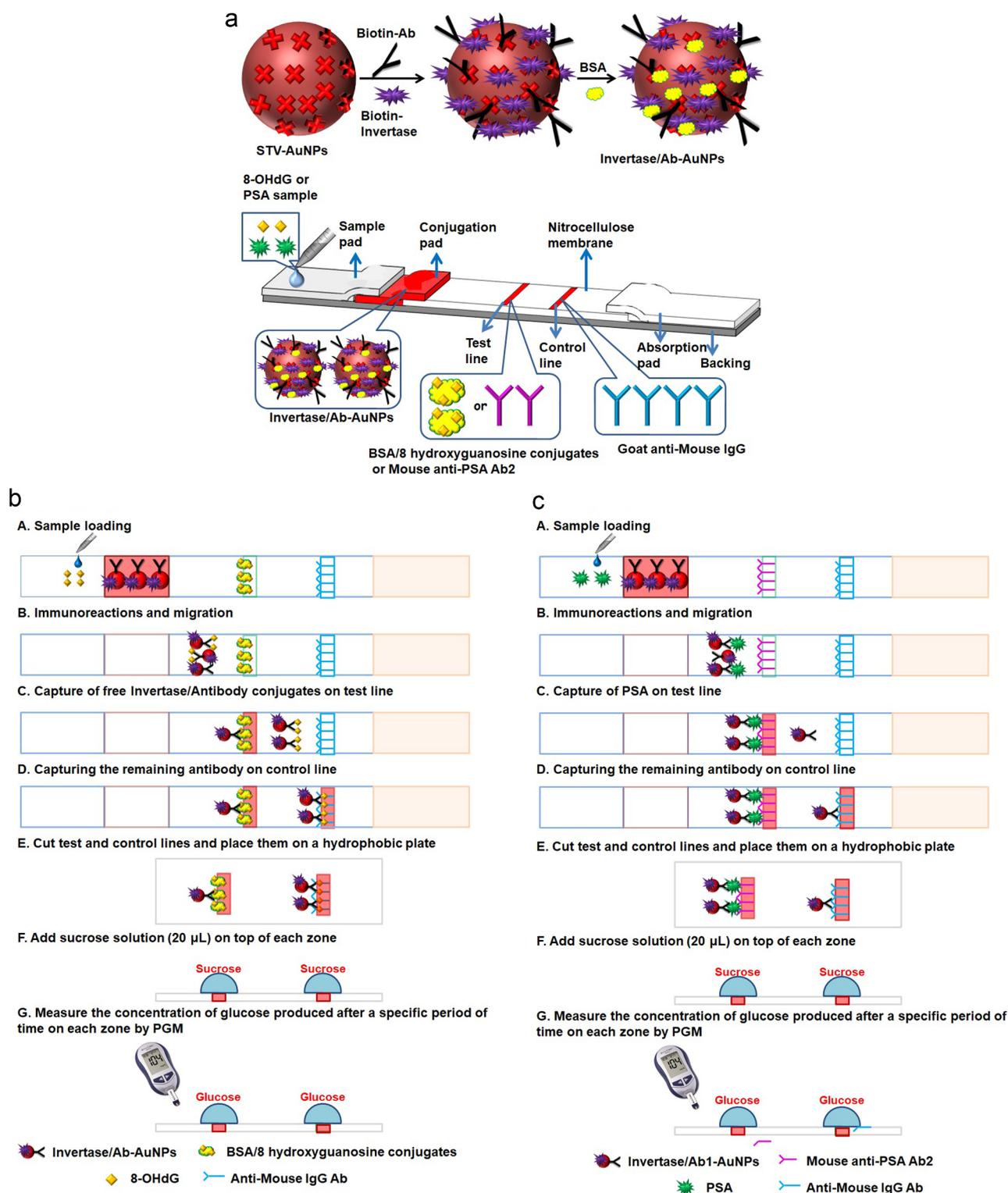


Fig. 1. (a) Design of test strips for quantitative detection of 8-OHdG or PSA; (b) Mechanism for 8-OHdG detection by a PGM; (c) Mechanism for PSA detection by a PGM.

on test line upon increasing 8-OHdG being loaded onto the strips. 20  $\mu$ L of the Nanozyme complex was applied onto the conjugation pad, which was subsequently assembled into a strip. 100  $\mu$ L of 8-OHdG samples with different concentrations were applied to the sample pad. After 10 min, photographs were taken by using digital camera. Fig. S1 shows the typical responses of the strips to 8-OHdG with seven concentrations (0, 1, 2, 5, 10, 20, 100  $\text{ng mL}^{-1}$ ). The color intensity of the test line decreased when the sample concentration increased, which was

consistent with the theory of detection of competitive format and validates the function of antibody in the complex. Color changes starts to be visually discriminated at a concentration of  $\sim 2 \text{ ng mL}^{-1}$  of 8-OHdG, an excellent starting point for following applications.

### 3.3. Quantitative analysis of 8-OHdG by integrating PGM with LFS

To demonstrate the feasibility of our method for 8-OHdG detection,

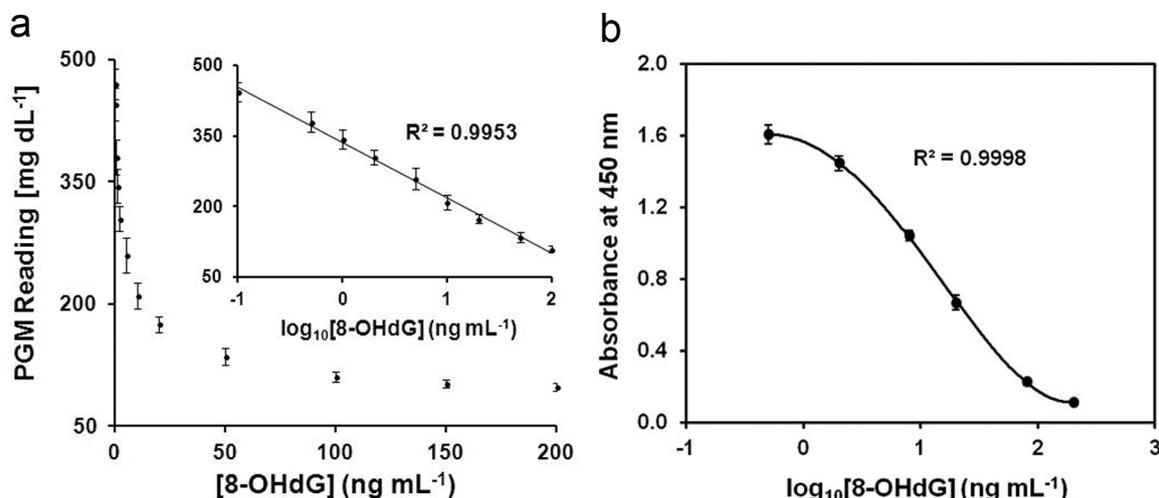


Fig. 2. (a) left: detection of 8-OHdG in buffer based on the PGM. The concentration of 8-OHdG was varied from 0 to 200 ng mL<sup>-1</sup>. Error bars: SD, n = 6; (b) right: conventional colorimetric ELISA for various concentrations of 8-OHdG. The absorbance spectra were recorded at 450 nm. Error bars: SD, n = 3.

a series of 8-OHdG solutions with concentrations of 0, 0.1, 0.5, 1, 2, 5, 10, 20, 50, 100, 150 and 200 ng mL<sup>-1</sup> in 1X PBS buffer were prepared and applied to the strips. After 10 min, the test (2 mm) and control (2 mm) lines were cut out by scissors and placed on a hydrophobic plate. 20  $\mu$ L of 0.5 M sucrose solution was put on top of each zone, and PGM was used for readout after 45 min. Here only the PGM readings of the test line were used for generating the correlation with 8-OHdG concentration, since signal from the control line is composed of a high noise resulting from incomplete capture of 8-OHdG-free antibody at the test line due to affinity limit of anti-8-OHdG antibody.

As shown in Fig. 2a (left), PGM readings (test line) decreased when the 8-OHdG concentration increased, which is consistent with the theory of the competitive format of detection. When the concentration is larger than 100 ng mL<sup>-1</sup>, there was no more obvious change in PGM readings. And 0.1 ng mL<sup>-1</sup> is the minimum concentration we tested which can lead to a readable PGM reading in a fixed time range (45 min). Nevertheless, a dynamic response from 0.1 to 100 ng mL<sup>-1</sup> is obtained, and as low as 0.23 ng mL<sup>-1</sup> of 8-OHdG can be detected by the definition of  $3\sigma_b/\text{slope}$  ( $\sigma_b$ , standard deviation of the blank samples), a significant improvement from stand-alone LFS testing. In addition, we also test the stability of the sensing platform through a three-week course. Fig. S2(A) demonstrate that the sensing platform is quite stable over three weeks, and there are only slight decreases in the average PGM readings comparing the result got from 3rd week to that from the original.

In order to evaluate the analytical performance of the proposed approach for 8-OHdG detection, a conventional ELISA approach was also performed for comparison. In the ELISA detection format, Horse Reddish Peroxidase (HRP) catalyzes the chromogenic substrate, which produce a strong colorimetric signal that can be measured with assistance of a multi-well plate reader. After the formation of the 8-OHdG-Ab1-Ab2/HRP in the ELISA plate wells, the 3,3',5,5'-tetramethylbenzidine (TMB) substrate for HRP was added and the absorption at 450 nm was recorded after acidification with phosphoric acid. As displayed in Fig. 2b (right), the standard ELISA method for 8-OHdG possesses a detection range from 0.5 to 200 ng mL<sup>-1</sup> (indicated by the manufacturer) and the detection limit is about 0.64 ng mL<sup>-1</sup>, by the definition of  $3\sigma_b/\text{slope}$  ( $\sigma_b$ , standard deviation of the blank samples). We performed a comparison between our method and ELISA on the detection of 8-OHdG in buffer with known concentrations, which is summarized in Table 1. From this table, we can see that the results obtained from our method are close to real concentration indicated in the first column. Moreover, Table 2 illustrates that our PGM-based method can provide a simple, rapid, on-site and cost-effective platform with acceptable detection limit and range in comparison with ELISA.

Table 1

Comparison between PGM-based method and ELISA on 8-OHdG detection in buffer samples (n = 6).

8-OHdG in buffer (ng mL <sup>-1</sup> )	PGM-based method (ng mL <sup>-1</sup> )	ELISA kit (ng mL <sup>-1</sup> )
1	0.90 (0.31)	0.70 (0.14)
10	12.38 (2.48)	9.86 (0.62)
20	24.33 (3.45)	21.60 (1.86)
50	53.62 (6.34)	45.30 (6.23)

Evaluate the PGM-based strip for 8-OHdG detection in human urine samples by comparing it with a conventional ELISA testing kit. Urinary 8-OHdG has been measured most frequently to indicate the extent of oxidative damage because of its noninvasiveness and less involvement of sample processing. To evaluate the PGM-based strategy for the detection of 8-OHdG in real biomedical applications, urine samples from four individuals with varying 8-OHdG concentrations were tested. These samples were also assayed with a commercial 8-OHdG ELISA kit as reference. As shown in Table 3, the 8-OHdG reading and orders obtained by the PGM-based method (8-OHdG concentration: #4 > #1 > #3 > #2) are generally in agreement with those obtained by the ELISA approach (8-OHdG concentration: #4 > #1 > #3 > #2) for the investigated urine samples, which suggests that the developed PGM-based method could be applied to clinical samples. However, transition from standard solution to urine samples poses potential challenges in the testing: (1) residual trace of electroactive interferants on the test line from human urine might alter PGM reading, and eventually an inaccurate analyte concentration; (2) immune-suppressive conditions of urine may reduce the antigen-antibody reaction on the test line; (3) high viscosity of urine causes an overall reduction of matter mobility on strip which also leads to reduced invertase accumulation on the test line.

#### 3.4. Quantitative analysis of PSA by integrating PGM with LFS

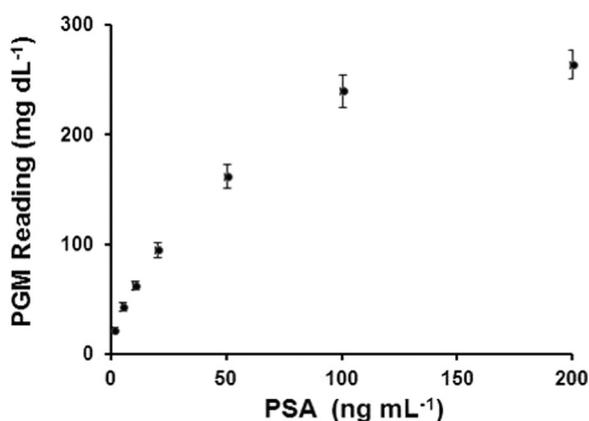
Another major category of biomarkers is proteins of larger molecular mass. This type of molecules are good analytes for the sandwich type of immunoassays. The current platform was also used to resolve PSA as a representative for protein biomarkers. Since 1986, PSA test has been utilized to screen for potential prostate cancer patient with a guided safe value of 4 ng mL<sup>-1</sup> in blood. Though more recent studies have shown that some men with PSA levels below 4.0 ng mL<sup>-1</sup> have prostate cancer and that many men with higher levels do not have prostate cancer (Thompson et al., 2004), PSA screening is still widely

**Table 2**  
PGM-based method vs. ELISA on 8-OHdG detection.

	PGM-based method	Commercial ELISA kit
Detection limit (ng mL <sup>-1</sup> )	0.23	0.64
Detection range (ng mL <sup>-1</sup> )	0.1–100	0.5–200
Time (h)	< 1	3.5–4
Cost (US dollars)	5/strip	700–800/test kit
Equipment required	PGM (\$20)	Microplate reader, Incubator, and others

**Table 3**  
Comparison between PGM-based method and ELISA on detection of 8-OHdG in human urine samples (n = 6).

Urine sample #	PGM based method (ng mL <sup>-1</sup> )	Commercial ELISA kit (ng mL <sup>-1</sup> )
1	22.70 (1.25)	17.72 (0.16)
2	17.07 (0.97)	13.70 (0.55)
3	20.01 (1.83)	15.24 (1.10)
4	25.24 (2.12)	18.81 (1.14)



**Fig. 3.** Detection of PSA in buffer based on the PGM. The concentration of PSA was varied from 0 to 200 ng mL<sup>-1</sup>. Error bars: SD, n = 6.

employed for elderly over 50 years old.

A series of PSA solutions with concentrations of 1, 5, 10, 20, 50, 100 and 200 ng mL<sup>-1</sup> in 1X PBS buffer were prepared and applied to the strips. After 10 min, the test lines (2 mm) were cut by scissors and placed on a hydrophobic plate. 20  $\mu$ L of 0.5 M sucrose solution was put on top of each zone, and the PGM was used for readout after 30 min. As shown in Fig. 3, PGM readings increased when the concentration of applied PSA sample increased, which is consistent with the theory of the sandwich format of detection. When the concentration is larger than 100 ng mL<sup>-1</sup>, there was no more obvious change in PGM readings. 1 ng mL<sup>-1</sup> is the minimum concentration we tested which can lead to a readable PGM reading in a fixed time range (30 min). A dynamic range of 1–100 ng mL<sup>-1</sup> can be obtained, and as low as 1.26 ng mL<sup>-1</sup> of PSA can be detected, by the definition of  $3\sigma_b/\text{slope}$  ( $\sigma_b$ , standard deviation of the blank samples). This finding suggests our PSA detection approach applicable to clinical setting. Stability of the sensing platform for PSA were also tested, and the results are demonstrated in Fig. S2(B). The stability is acceptable over three weeks, and there are only slight decreases in the average PGM readings comparing the result got from 3rd week to that from the original.

We also compared our PGM-based method with a commercialized ELISA kit (see SI, Table S3) on the performance on PSA detection in aspects of detection limit, detection range, assay time, assay cost as well as portability. It demonstrates that our PGM-based method can provide a simple, rapid, low cost platform with acceptable detection limit and detection range.

#### 4. Conclusions

In summary, we reported a universal nanozyme integrated lateral fluidic sensing platform linked to a PGM for sensitive and portable detection of targeted disease biomarkers. The concept was demonstrated by using a small molecule (8-OHdG) and a large protein molecule (PSA). The device is able to detect 8-OHdG in both PBS and urine samples with comparable detection limit and dynamic range with that of commercial ELISA kit in less than 1 h. Feasibility of the approach for protein type biomarkers is validated with PSA whereas as low as 1.26 ng mL<sup>-1</sup> PSA in buffer can also be detected. The advantages of this approach rely on (1) establishment of a proportional relationship between disease biomarkers and the amount of glucose in one-step configuration, therefore taking advantages of commercial PGM for quantification of a variety of non-glucose biomarkers; (2) using LFS which is easy to fabricate and convenient to be adopted by different biomarkers through using suitable antibodies; (3) the combination of these two techniques that realizes the low cost and portability, providing great potential for POCT development. In the near future, the sensing platform is expected to be further optimized into a widespread method applicable for the detection of other disease biomarkers (both small and large molecules). And how to connect LFS and PGM seamlessly, as current formulation still requires manual intervention at the point of test line excision and mixing with substrate solution will be explored. Additionally, more samples need to be tested and compared in the assay to ensure the reproducibility and accuracy. Last but not least, telemedicine is a new perspective for POCT development. Therefore, a wireless-enabled monitoring system could be incorporated for instant biomarker detection, data collection and transmission.

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#### Declarations of interest

None.

#### Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.bios.2018.11.033](https://doi.org/10.1016/j.bios.2018.11.033).

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