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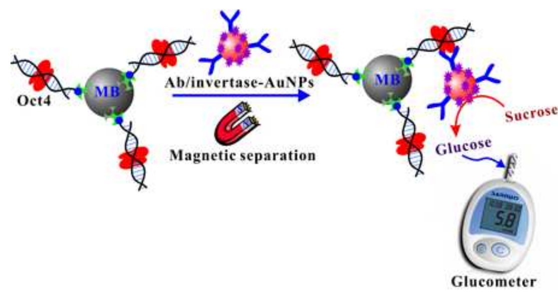
A universal biosensor for portable and quantitative analysis of transcription factors has been constructed using glucometer as the sensing platform.



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Cite this: DOI: 10.1039/c0xx00000x

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ARTICLE TYPE

A universal glucometer-based biosensor for portable and quantitative detection of transcription factors

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Received (in XXX, XXX) Xth XXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX

DOI: 10.1039/b000000x

A universal biosensor for portable and quantitative detection of transcription factors has been constructed using a commercially available glucometer as the sensing platform. With the specific protein-binding DNA and antibody as the recognition elements, invertase as the linker, and glucometer as the transducer, quantitative detection is achieved *via* target-induced capturing of invertase conjugates on magnetic beads, thereby transforming the concentration of the target in the sample into glucose through invertase-catalyzed hydrolysis of sucrose. In comparison with laboratory-based instruments or customized devices, the glucometer-based biosensor has significant advantages of low cost, compact size, wide accessibility, and ease of use, making it as a convenient tool for the public use at home or in the field. As a proof of concept, Oct4, an important transcription factor in regulating the process of embryonic stem cells differentiation, was used as the model target. Using the proposed point-of-care strategy, Oct4 can be quantified in the range from 0.05 to 25 ng/mL with a detection limit of 0.05 ng/mL, which is comparable to the commercial Oct4 test kits. The glucometer-based biosensor is robust and can be used directly to measure the transcription factor activities in crude cell lysate with excellent selectivity. It is expected that this assay principle can be directed toward other DNA-binding transcription factors by simply changing the binding site sequence and the corresponding antibody.

Introduction

Transcription factors are a class of DNA-binding proteins that regulate gene expression programs by binding to specific DNA sequences.[1] They play crucial roles in many cellular processes, such as cell differentiation, proliferation, and apoptosis.[2] Monitoring transcription factor expression levels provides an important assessment of the state of cell populations.[3] Therefore, the development of simple, portable, reliable, and quantitative strategies for detecting transcription factors is urgently needed since these proteins have been recognized as attractive markers or targets for disease diagnosis and drug development.

Traditional methods for the detection of transcription factors include electrophoretic mobility shift assay (EMSA), enzyme-linked immunosorbent assay (ELISA), DNA footprinting, and Western blotting.[4] However, these protocols are usually cumbersome, expensive, and time-consuming, which makes them challenging in routine measurement. Recently, several exquisite sensors have been developed for transcription factor detection using fluorescent,[5a,b] electrochemical,[5c,d] electrochemiluminescent,[5e,f] and Raman scattering[5g] methods. Although these techniques are more convenient and sensitive over traditional approaches, most of them are associated with laboratory-based instruments or customized devices that are not easily accessible to the public for point-of-care (POC) diagnosis or detections. Colorimetric sensors,[6a,b] including lateral flow strips,[6c] have been reported for DNA-binding proteins detection by the naked eye without instrumentation. However, they can only provide qualitative or semi-quantitative results based on color observation that may vary among different people or be affected by the light conditions.

To address these limitations, glucometer, the most common commercially available POC diagnostic device, would be an ideal alternative to laboratory-based instruments for portable and quantitative detection of transcription factors at home or in the field. Personal glucometers are compact (pocket size), low-cost (as low as $10), ease-to-use, and widely accessible to the public. Using such digital devices as analytical tools, the time to result is shortened to as little as 25 s and the necessary sample volume is reduced to as small as 5 μL. However, the current glucometer can only detect a single target, glucose. In order to detect and quantify a wide range of non-glucose targets, a link between other targets and the glucose should be established before a glucometer can be used.[7] Invertase, an enzyme that catalyses the hydrolysis of sucrose into glucose, can be used to connect other targets to glucose concentrations because sucrose is completely inert in a glucometer. For example, Lu's group[8] reported an elegant method that combines invertase with a glucometer for DNA, metal ions, toxins, and biomarkers analysis. To explore the new application of a glucometer for other non-glucose analytes quantification, herein we developed a universal POC device for transcription factor detection using specific protein-binding DNA and antibody as the recognition elements, invertase as the linker, and glucometer as the sensing platform. As a proof of concept,

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Oct4, an important transcription factor in regulating the process of embryonic stem cells differentiation, was used as the model target.

Experimental

Chemicals and materials

Invertase from baker's yeast, sucrose, bovine serum albumin (BSA), thrombin, lysozyme, mouse anti-Oct4 monoclonal antibody, and the purified recombinant Oct4 were purchased from Sigma-Aldrich (St. Louis, MO). The purified recombinant TATA-binding protein (TBP) and nuclear factor-kappa B (NF-κB) were purchased from Promega (Madison, WI). Streptavidin-coated magnetic beads (2.8 μM in diameter) were purchased from Life Technologies (Grand Island, NY). Other common chemicals were analytical reagent grade and were used as received. All solution was prepared with ultrapure water (18.2 MΩ/cm) from a Millipore Milli-Q water purification system (Billerica, MA).

Oligonucleotides purified by HPLC were synthesized by Shanghai Sangon Biotechnology Co., Ltd. (Shanghai, China) and listed as follows: (The Oct4-binding site was underlined)

Biotin DNA: 5'-biotin-CATTGTTATGCAAATCAGTC-3'

Complementary DNA: 3'-GTAACAATACGTTTAGTCAG-5'

Procedure for Oct4 detection by a glucometer

Gold nanoparticles (AuNPs) with average diameter 15 ± 3.5 nm were used to prepare the anti-Oct4 antibody (Ab)/invertase-AuNPs conjugates (see the details in ESI†).

1 mL of AuNPs solution was adjusted to pH 8.5 with 0.1 M K_2CO_3. Then, 9 μL of purified mouse anti-Oct4 antibody (10 mg/mL) and 9 μL of invertase (40 mg/mL) were added to the above solution simultaneously. The mixture was incubated at room temperature for 30 min with gentle stirring. A certain volume of 10% BSA was slowly added to the mixture solution to obtain a final concentration of 1% and the stirring was continued for another 30 min. The excess reagents were removed by centrifugation at 12,000 rpm for 30 min. After discarding the supernatant, the red pellets were resuspended in 100 μL buffer containing 10 mM PBS, 5% BSA and 0.05% NaN_3. The resulting Ab/invertase-AuNPs conjugates were stored at 4 °C before further use.

100 μL of 2 mg/mL streptavidin-coated magnetic beads (SA-MBs) were washed three times with the washing buffer (10 mM PBS, 0.02% Tween-20, pH 7.4), and the solid residue after magnetic separation was resuspended in 100 μL of the reaction buffer (10 mM PBS, 0.1 M NaCl, pH 7.4). Biotin-DNA and the complementary DNA were then added to the solution to achieve a final concentration of 1 μM double-stranded DNA (dsDNA), and the mixture was mixed on a shaker for 30 min at room temperature. The resulting SA-MBs-biotin dsDNA complex was washed twice with the washing buffer to remove the excess DNA, and dispersed in 100 μL of the reaction buffer.

Subsequently, the SA-MBs-biotin dsDNA solution was incubated with various concentrations of target Oct4 at room temperature for 30 min. After washing three times using the washing buffer, 100 μL of the SA-MBs-biotin dsDNA-Oct4 solution was further incubated with 10 μL of Ab/invertase-AuNPs conjugates to form the sandwich-type complex (SA-MBs-biotin dsDNA-Oct4-Ab/invertase-AuNPs). After washing the MBs three times using the washing buffer, 20 μL of 0.5 M sucrose in reaction buffer was added to the MBs and incubated at room temperature for 30 min. A portion of 5 μL of the final solution was tested by a commercially available glucometer and the digital reading was obtained after 25 s.

Preparation of crude cell lysate

The cultured mouse embryonic stem cells (see the details in ESI†) were washed twice with cold PBS and lysed with a non-denaturing cell lysis buffer (20 mM Tris-HCl, pH 8.0, containing 137 mM NaCl, 10% glycerol, 1% NP-40, 2 mM EDTA, 2 mg/mL leupeptin, 2 mg/mL aprotinin, 1 mM DTT, and 1 mM PMSF). Cell debris was removed by centrifugation at 4 °C for 30 min (12,000 rpm). The supernatant was collected as crude cell lysate for analysis. For control experiments, Hela cell lysate was also prepared in a similar procedure. Inactivated cell lysate was obtained by heating at 75 °C for 10 min. All the assay procedures using a glucometer are the same as the foregoing detection in buffer solution.

Results and discussion

Sensing mechanism

Fig. 1 illustrates the sensing mechanism for Oct4 detection using a glucometer. Biotin-dsDNA containing the consensus Oct4-binding site was anchored on SA-MBs via the affinity recognition between SA and biotin. The dsDNA in the SA-MBs-biotin dsDNA can bind Oct4 with high binding capacity and selectivity.[9,10] Anti-Oct4 antibody and invertase were simultaneously immobilized onto AuNPs to form the Ab/invertase-AuNPs conjugates. If the target Oct4 is present in the testing sample, it will bind to the Oct4-binding site in the dsDNA. Upon the addition of the Ab/invertase-AuNPs conjugates, sandwich-type complex (dsDNA-Oct4-Ab/invertase-AuNPs) will be formed on the MBs surface. After magnetic separation, the captured invertase conjugates on MBs can catalyze the hydrolysis of sucrose into glucose, which is monitored by a personal glucometer. The concentration of Oct4 can be calculated from the glucometer readout because of the direct correlation between Oct4 and invertase. Conversely, in the absence of Oct4, the sandwich-type complex cannot be formed on MBs and no invertase could catalyze the sucrose hydrolysis reaction after magnetic separation. As a result, no glucose is generated, and no glucometer reading is observed.

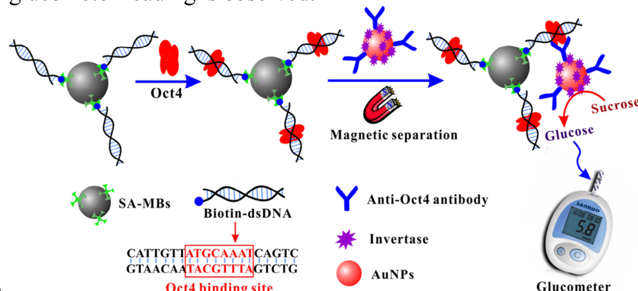

Fig. 1 Schematic illustration of the glucometer-based biosensor for the detection of transcription factor Oct4.

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In our sensing protocol, a commercially available glucometer is used as the transducer. However, the dynamic range of a typical glucometer for glucose monitoring is about 0.6-33 mM,[11] which is much higher than the target protein at pM to nM level in the testing sample. Thus, a highly efficient signal amplification procedure is required if using such device to quantify non-glucose targets. To achieve the goal of signal amplification, we here utilize dsDNA functionalized MBs to concentrate the target Oct4 through Oct4-DNA interaction and magnetic separation. More importantly, AuNPs are employed as carriers to load multiple invertase and anti-Oct4 antibodies, and the formed Ab/invertase-AuNPs conjugates are used as signal amplification labels. So each dsDNA-Oct4-Ab recognition reaction leads to the capture of numerous invertase on MBs. In addition, invertase possesses highly catalytic efficiency for sucrose hydrolysis. Even nM concentration of invertase can convert large amounts of sucrose to mM levels of glucose, resulting a desirable amplification reading. Therefore, based on magnetic beads enrichment, Ab/invertase-AuNPs amplification labels, and the glucometer transducer, we successfully designed a POC protein-detection biosensor for the quantitative electronic readout of Oct4 concentration with high sensitivity.

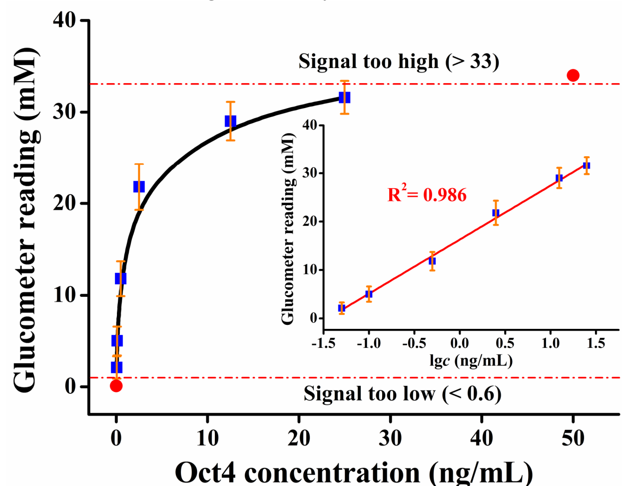

Fig. 2 Detection of different Oct4 concentrations using a glucometer. Inset: the calibration curve of the glucometer readings *vs*. the logarithm of Oct4 concentration. The signals lower than 0.6 mM or higher than 33 mM are beyond the detectable range of a glucometer and those data do not represent the real reading. Reaction time: 30 min; Sucrose concentration: 0.5 M. The error bars represent the standard deviation of three independent measurements.

Sensitivity of the glucometer-based biosensor

The dynamic range and sensitivity of the glucometer-based biosensor were investigated by varying the concentrations of the target Oct4 under optimal experimental conditions. As shown in Fig. 2, the glucometer readings increased with increasing Oct4 concentration. In the absence of Oct4, no readout was observed in a glucometer, which is due to the failure of capturing invertase on MBs. Higher concentrations (> 25 ng/mL) of Oct4 produce signals beyond the upper limit of the glucometer so that they are not included in the calibration curve. The glucometer readout was proportional to the logarithm of Oct4 concentration in the range from 0.05 to 25 ng/mL (inset in Fig. 2). The presence of as low as 0.05 ng/mL Oct4 can yield a detectable signal, indicating that the detection limit of the glucometer-based biosensor for Oct4 detection is 0.05 ng/mL, which is comparable to those commercial Oct4 test kits, such as the E90424 Oct4 ELISA kit (0.057 ng/mL). Importantly, the glucometer-based biosensor is superior to the laboratory-based instruments or customized devices because they are widely available, low cost, portable, and simple to use. Additionally, glucometer-based devices have the potential to become universal tools for POC detection of non-glucose targets using invertase as a signal converter.

Optimization of experimental conditions

The experimental parameters (e.g., the amount of invertase conjugates, the incubation time with sucrose, and the sucrose concentration) that can affect the performance and results of the above analytical system were optimized. In the current study, the glucose was generated through the enzymatic hydrolysis of sucrose by the invertase conjugates. Thus, the sensitivity of the system is strongly related to the amount of invertase conjugates bound to the MBs. As shown in Fig. 3, the glucometer signals increased with the increasing of the volume of invertase conjugates and then leveled off to a saturation value when the volume was higher than of 10 μL. Therefore, 10 μL of invertase conjugates was used in the standard procedure. From Fig. S1 (ESI†), the effective concentration of invertase immobilized on AuNPs can be calculated to be 34 mg/mL. Fig. 4 shows the effect of the incubation time with sucrose on the glucometer response. The amount of glucose produced was proportional to the incubation time with sucrose. To ensure samples with low concentrations of target Oct4 can give a detectable signal, 30 min incubation time with sucrose was used. The response of the glucometer-based biosensor for Oct4 detection is also relevant to the concentration of sucrose. As shown in Fig. 5, 0.5 M sucrose was added to the detection system to achieve the maximum signal in a glucometer.

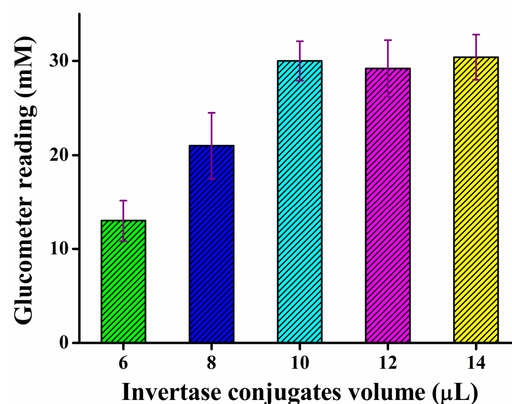

Fig. 3 Effect of the volume of the invertase conjugates on the response of the glucometer-based biosensor for Oct4 detection. Oct4 concentration: 25 ng/mL. Sucrose concentration: 0.5 M. Incubation time with sucrose: 30 min. All experiments were performed at room temperature. The error bars represent the standard deviation of three independent measurements.

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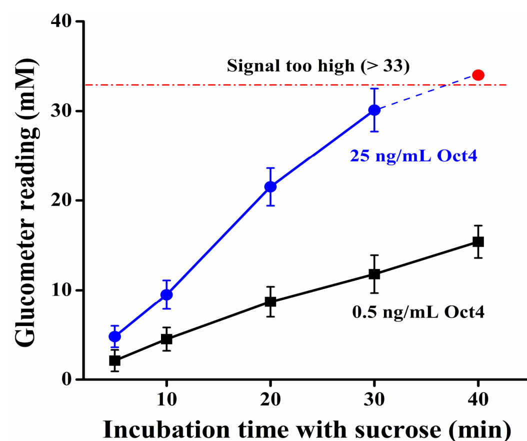

Fig. 4 Effect of the incubation time with sucrose on the response of the glucometer-based biosensor for Oct4 detection. The signals higher than 33 mM are beyond the detectable range of a glucometer and they do not represent the real reading (marked in red). The error bars represent the standard deviation of three independent measurements. Sucrose concentration: 0.5 M. All experiments were performed at room temperature.

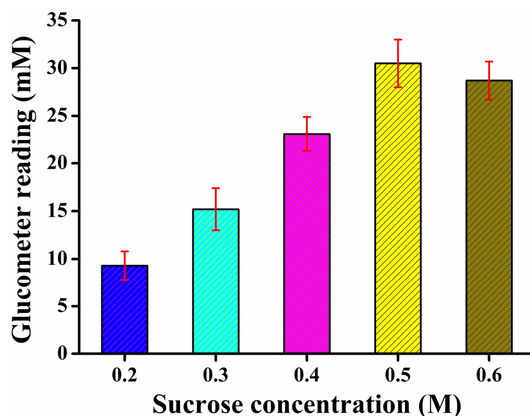

Fig. 5 Effect of the sucrose concentration on the response of the glucometer-based biosensor for Oct4 detection. Oct4 concentration: 25 ng/mL. Incubation time with sucrose: 30 min. All experiments were performed at room temperature. The error bars represent the standard deviation of three independent measurements.

Selectivity of the glucometer-based biosensor

To evaluate the selectivity of the glucometer-based biosensor for Oct4 detection, four other proteins: thrombin (Thro), lysozyme (Lyso), TATA-binding protein (TBP), and nuclear factor-kappa B (NF-κB) were tested as the negative controls under the same conditions. As shown in Fig. 6, 0.5 ng/mL Oct4 could produce a high glucometer reading, while other proteins even at concentrations of 50 ng/mL did not give any signal. These results demonstrate that our constructed POC strategy exhibits an excellent selectivity to Oct4 over other non-target proteins. In addition, a series of eight repetitive measurements of 10 ng/mL Oct4 produced a relative standard deviation (RSD) of 5.8%, indicating that the reproducibility of the proposed method was acceptable.

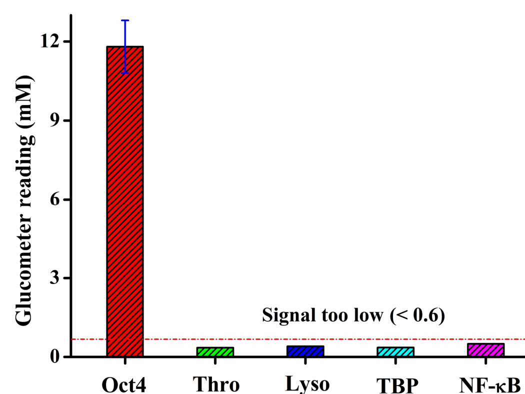

Fig. 6 Selectivity of the glucometer-based biosensor for Oct4 against other non-target proteins. The concentration was 0.5 ng/mL for Oct4 and 50 ng/mL for other proteins. The signals lower than 0.6 mM is beyond the detectable range of a glucometer and those data do not represent the real reading.

Real sample analysis

Transcription factor Oct4 can serve as the biomarker for the identification of stem cells.[3] Thus, the capability of the glucometer-based biosensor for practical applications is demonstrated by applying it to measuring Oct4 activity in mouse embryonic stem cells. Crude cell lysate containing different amounts of Oct4 was analyzed directly using the glucometer-based biosensor and a commercial ELISA kit (Uscn Life Science Inc). As shown in Table 1, there are no significant differences between the results obtained by the two methods. By heating the cell lysate at 75 °C for 10 min to inactivate Oct4, no signal was observed, indicating that the proposed methods can distinguish active DNA-binding proteins from inactive ones. There was also no detectable response in Hela cell lysate, demonstrating that other cellular components do not affect the performance of the assay. The glucometer sensing approach thus provides a convenient and robust platform for the routine assessment of transcription factor activities in cultured cell populations.

Table 1 Determination of Oct4 (ng/mL) in cell lysate using commercial ELISA kit and the glucometer-based biosensor

Sample	ELISA kit[b]	Glucometer[b]	Relative error (%)[c]
Stem cell 1	4.3 ± 0.4	4.6 ± 0.5	6.5
Stem cell 2	10.6 ± 1.5	10.2 ± 1.8	-3.9
Stem cell 3	18.8 ± 2.1	20.4 ± 2.5	7.8
Stem cell 3[a]	–	–	–
Hela cell	–	–	–

[a]Heat inactivated for 10 min at 75 °C.
[b]Average of five determinations ± standard deviation.
[c]Glucometer-based biosensor *vs*. commercial ELISA kit.
"–" represents no signal.

Conclusions

In summary, we have successfully developed a general methodology for portable, digital, and quantitative detection of transcription factor using a commercially available glucometer as the sensing platform. The quantification is based on target-induced capturing of Ab/invertase-AuNPs conjugates on MBs,

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thereby transforming the concentration of Oct4 into glucose *via* invertase-catalyzed hydrolysis of sucrose. In comparison with laboratory-based instruments or customized devices, the glucometer-based biosensor has significant advantages of simple operation, low cost, compact size, and wide accessibility, making it as a convenient tool for the public use at home or in the field. The proposed POC strategy possesses high sensitivity for the reliable monitoring of Oct4, with a detection limit as low as 0.05 ng/mL, which is comparable to the commercial Oct4 ELISA kit. In addition, the glucometer-based biosensor is robust and can be used directly to measure the transcription factor activities in crude cell lysate with excellent selectivity. Thus, personal glucometer can serve as an ideal quantitative tool for the routine assessment of the state of cell populations. It is expected that this assay principle can be directed toward other DNA-binding transcription factors by simply changing the binding site sequence and the corresponding antibody.

Acknowledgments

Financial support was provided by the Ministry of Science and Technology 973 Project (2013CB967100) and the Special Key Project of Science and Technology Bureau of Guangzhou (2010U1-E00611).

Notes and references

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† Electronic Supplementary Information (ESI) available. See DOI: 10.1039/b000000x/
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