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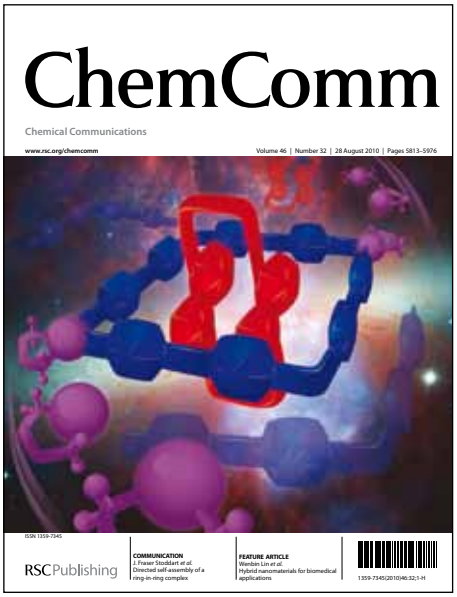

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ARTICLE TYPE

Bioresponsive controlled release of cargo with glucometer readout from mesoporous silica nanocontainer from sensing non-glucose targets†

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A novel sensing platform for monitoring of small molecules without the need of sample separation and washing is developed by using a commercialized personal glucose meter based on bioresponsive controlled release of glucose from aptamer-gated mesoporous silica nanocontainer.

Sensitive, simple, and cost-effective analysis of biomolecules is very important in clinical diagnostics and treatment.[1] A huge effort has been expended in the field of assay development to simplify the assay process while preserving the essential benefits in sensitivity, robustness, broad applicability, and suitability to automation.[2] The assay is generally implemented using certain affinity ligand, *e.g.* antibody or aptamer, which can specifically interact with the protein and thus mediate a target-responsive signal transduction cascade.[3] Despite the high sensitivity of conventional methods including spectroscopy, electrochemistry and chromatography,[4] they have some limitations, such as expensive and complicated instruments, sophisticated operations, professional personnel requirement in laboratories, and complicated washing procedure.

Point-of-care (POC) testing, as a medical testing of patient care, brings the test conveniently and immediately to the patient.[5] Personal glucose meter (PGM) is currently one of the most widely used diagnostic devices in the world because of its portable size, easy operation, low cost and reliable quantitative results.[6] Recently, Lu,[6a,7a,7b,7c] Yang[7d] and Xiang[7e] groups reported a series of methods by coupling functional DNA probes with a PGM for the detection of different target analytes, *e.g.* metal ions, cocaine, virus and disease markers. Unfortunately, these methods usually combined with the hydrolytic enzymes, *e.g.* invertase and amylase, to produce the glucose for glucometer readout. The assays usually required proprietary or specialty enzyme labels. Meanwhile, the sample separation and multi-step washing during the measurement were sometime unavoidable. New signal transduction strategies that provide simple sample pretreatment without the participation of hydrolytic enzymes while still allowing efficient conversion of target recognition events into a cascaded glucose production are thus highly desirable. Herein, we present a simple and general method for quantitative detection of a wide range of non-glucose targets based on target-responsive controlled release of glucose from aptamer–gated mesoporous nanospheres by a portable PGM.

Scheme 1 represents the working principle of target- aptamer-responsive controlled release system. To construct such an assay system, the aminated mesoporous silica nanospheres (MSN) is used as the support, while the adenosine triphosphate (ATP) molecule is demonstrated as the model target analytes. One aminated DNA sequence (DNA1) is initially grafted on the outer surface of the MSN through the glutaraldehyde (designated as DNA1-MSN). Meanwhile, 5-nm gold nanoparticles (AuNP) are functionalized with another thiolated DNA sequence (DNA2) by the Au-S bond (designated DNA2-AuNP). DNA1 and DNA2 are complementary to adjacent areas of ATP aptamer sequence. Detailed DNA sequence, modification, linkage and PGM measurement are described in the Supporting Information (SI). Upon the addition of aptamer, the immobilized DNA1 on the MSN and the conjugated DNA2 on the AuNP hybridize with the aptamer to yield a three-stranded complex (designated as MSN-AuNP), and gated the pores, thus making the glucose molecules entrapped inside. In the presence of ATP, aptamer specifically and preferentially binds ATP to form target-aptamer complex, resulting in the separation of the functional AuNP from the MSN. In this case, the entrapped glucose molecules are released. The released glucose molecules can be quantitatively readout by using a simple, low-cost, user-friendly, and portable PGM without the need of sample separation and washing.

Recently, glucose aqueous solutions confined in MCM-41 silica pores have been studied by molecular dynamic simulations and quasielastic neutron scattering.[8] Molecular dynamic simulations revealed a strong preferential interaction of glucose molecules with the silica walls, which induced significant concentration gradients within the pore. In this work, we initially synthesized the MCM-41-type mesoporous silica nanospheres consulting to the literatures.[8,9] To realize our design, TEM was initially used to investigate the interaction between DNA1-MSN and DNA2-AuNP (Figure 1). Figure 1a shows typical TEM image of the aminated MSN, and the average size of the as-synthesized MSN was 120 nm. Meanwhile, we also clearly observed that there were a large number of pores on the silica nanospheres. The pore size distribution curve shows a narrow size distribution of the pores with a BJH pore diameter of 3.2 nm. The aminated MSN has a BET surface area of 956 m^2 g^{-1} and a pore volume of 0.42 cm^3 g^{-1} (Figures S1). As seen from Figure 1c, a large number of DNA2-AuNP were attached on the MSN after mixing with DNA1-MSN, DNA2-AuNP and the aptamers, indicating that DNA2-AuNP could be adsorbed onto the surface of DNA1-MSN through the aptamers. In contrast, when target ATP (0.1 mM) was introduced in the system, partial DNA2-

AuNPs were dissociated from the DNA1-MSN, and dispersed into the solution (Figure 1d). The results revealed that DNA2-AuNP could be readily capped and uncapped from the DNA1-MSN by the aptamer and a specific target-aptamer interaction, respectively.

To investigate the aptamer-target-responsive release behaviour of the developed system, glucose was loaded as the guest by soaking the DNA1-MSN into a PGM buffer (pH 7.3). The loading efficiency of glucose was determined as described in the supporting information (Figure S3). On this basis, several control tests were investigated by using the synthesized DNA1-MSN and DNA2-AuNP (Figure 2). Initially, we examined the capping efficiency of DNA1-MSN toward glucose by aptamer and DNA2-AuNP. The DNA1-MSNs loading with glucose were dispersed in pH 7.3 PGM buffer without target ATP, the suspension (column '*b*') was almost the same as the blank sample (column '*a*'), indicating no obvious leakage of the glucose, whereas the uncapped glucose/DNA1-MSN by DNA2-AuNP exhibited a strong PGM signal (column '*c*'). The results revealed that the capping strategy based on the aptamers was successful with good efficiency. For comparison, the trigger release of glucose was also monitored by addition of 0.1 mM ATP to the MSN-AuNP system. As shown from column '*d*', the PGM signal was obviously higher than those of columns '*a*-*b*'. The reason might be most likely a consequence of the fact that the three-stranded complexes were dissociated by target ATP, and DNA2-AuNP was uncapped from the DNA1-MSN, thus resulting in the release of glucose from the pores. The results suggested that the PGM-based system was sensitive for monitoring target ATP.

Next, the developed system was applied for quantifying ATP standards with various concentration based on target-responsive controlled release of glucose from the MSN-AuNP. Reactions were carried out in a 200-μL PCR tubes containing 10 μL of MSN-AuNP suspension and various-concentration ATP. The tubes were shaken occasionally during the reaction at RT. A 3-μL aliquot of the supernatant was removed for glucose measurement by using a commercialized Roche PGM. Target ATP concentrations of 0 – 0.8 mM were assayed with three measurements each in parallel. As seen from Figure 3a, samples containing ATP were ability to produce PGM signals, confirming that the MSN-AuNP could response to ATP to trigger glucose generation for PGM readout. The maximum release was observed at 0.8 mM ATP, indicating the quantitative detection capability of the PGM-based sensing platform. Moreover, the PGM signals increased with the increasing target ATP concentrations. A linear dependence between the PGM signals and ATP concentrations was obtained in the range from 0.01 μM to 0.8 μM. The linear regression equation was $y = 24.59x + 1.26$ (μM, $R^2 = 0.9848$, $n = 30$). The detection limit (LOD) was 8 μM at the $3S_{blank}$ level.

To evaluate the selective delivery process, we challenged the system with several ATP analogues: cytosine triphosphate (CTP), guanidine triphosphate (GTP) and uridine triphosphate (UTP). As shown from Figure 3b, these ATP analogues did not induce the characteristic release similar to the release for ATP.

The reproducibility of determinations was investigated by repeatedly assaying 3 ATP various standards, using identical batches of MSN-AuNP throughout. Results indicated that the coefficients of variation (CVs) of the intra-assay between 6 runs were 3.6%, 3.1% and 4.4% for 0.1 μM, 10 μM, and 0.5 mM ATP, respectively, whereas the CVs of the inter-assay with various batches were 5.3%, 7.8% and 4.9% for ATP towards the mentioned-above targets. The low CVs indicated the possibility of MSN-AuNP batch preparation.

To verify the generality, the synthesized MSN and AuNP were applied for the detection of small molecules (cocaine used as an example) by using the corresponding DNA1, DNA2 and aptamer. The PGM-based method allowed the quantitative detection of cocaine with alinear range from 0 to 1 mM (Figure 4a). Meanwhile, the control experiments with cocaine metabolites, such as benzoly ecgonine and ecgonine methyl ester, did not produce enhanced PGM signals, indicating good selectivity for the PGM assay system (Figure 4b). Hence, the stratgey is a generic approach that can be modifed with different aptamer sequences for portable and quantitative detection of a wide range of non-glucose targets.

Logically, another concern arises as to whether the PGM-based assay system could be applied for determination of the sample containing endogenous glucose. Due to the presence of endogenous glucose, *e.g.* in human serum, it might interfere with the final assay results. To tackle this problem, the glucose concentration in an unknown sample must be monitored using the PGM prior to target detection. When the background signal plus the signal generated by the target exceeds the upper limit of the PGM readout, however, an appreciate dilution should be preferable. In this case, the background signal can be subtracted from the signal obtained in the subsequent actual test. As indicated from Figure S5, the results of cocaine detection in the samples containing different-level glucose after pretreatments were comparable to those in the glucose-free samples. Therefore, the pretreatment for glucose-containing samples was reliable for successfully eliminating glucose interference.

In summary, we for the first time demonstrate the ability of target-responsive controlled release of glucose conjugates without the participation of glucose hydrolytic enzymes for the detection of non-glucose targets. The controlled release profile completely depends on the high affinity and specificity between the aptamer and target. Compared with conventional PGM-based assay protocols,[7] the system is inexpensive, rapid, portable and user-friendly without the need of sample separation and washing. Just as simple operation, low cost, and wide availability of the PGM, the methodology demonstrated in this work can be utilized by the public for quantitative detection of proteins and small molecules by controlling the used DNA sequences, thereby representing a versatile detection method. Nevertheless, only one disadvantage of the developed strategy is the long-time incubation time for the formation of MSN-AuNP. To fulfil the potential application for point-of-care (POC) testing, future work should focus on the improvement of interaction time.

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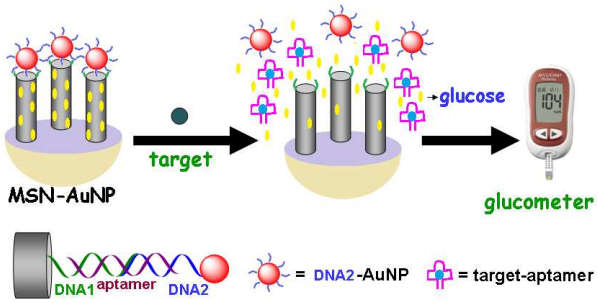

Scheme 1 Schematic illustration of the PGM-based assay method.

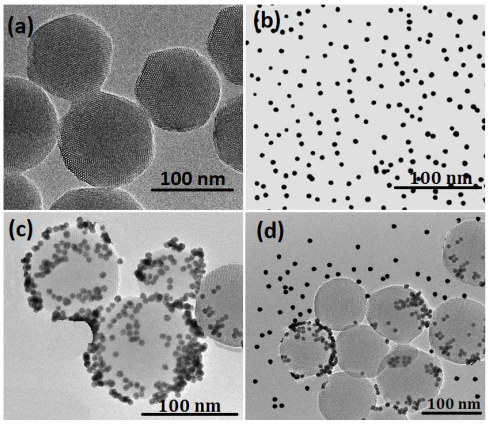

Fig. 1 TEM images of (a) the aminated MSN, (b) AuNP, (c) DNA2-AuNP-capped DNA1-MSN by the aptamer (MSN-AuNP), and (d) MSN-AuNP in the presence of 0.1 mM ATP.

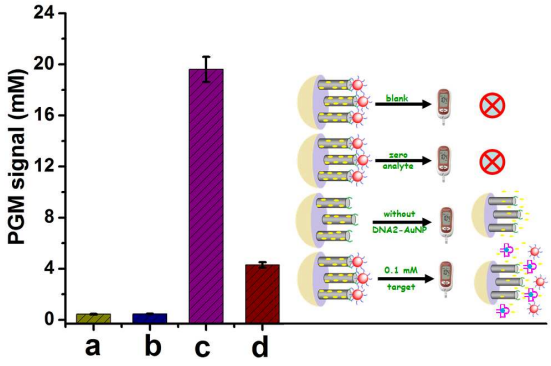

Fig. 2 Study of the entrapping capacity of DNA1-MSN toward cargo (glucose) molecules into the mesoporous nanocontainer by the aptamer and DNA2-AuNP: (a) MSN-AuNP loading with glucose in the blank PGM buffer, (b) MSN-AuNP loading with glucose toward zero analyte, (c) DNA1-MSN toward glucose without DNA2-AuNP, and (d) MSN-AuNP loading with glucose in the presence of 0.1 mM ATP. Incubation time: 6 h.

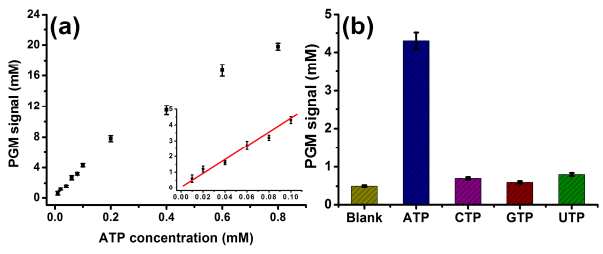

Fig. 3 (a) Calibration curve of the PGM-based assay system toward various-concentration ATP standards. A linear standard curve from 0 to 0.8 mM was obtained with a detection limit of 50 nM. (b) Selectivity release profiles for aptamer-target-reponsive controlled-delivery system trigged by ATP, CTP, GTP, and UTP, respectively (0.1 mM in this case, measured after 6 h).

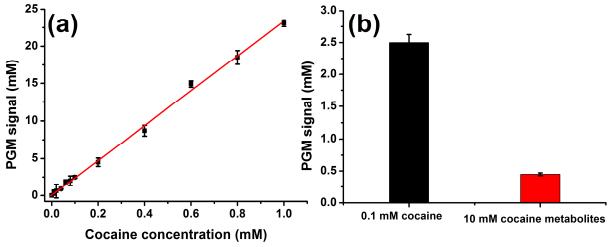

Fig. 4 (a) Detection of Cocaine with the PGM-based assay system, and b) responses of the system to 0.1 mM cocaine and 10 mM benzyol ecgonine and ecgonine methyl ester in pH 7.3 PGM buffer.

Notes and references

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† Electronic supplementary information (ESI) available: Experimental procedures. See DOI: 10.1039/xxxxxxx.

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