This article can be cited before page numbers have been issued, to do this please use: J. Zhang, H. Xing and Y. Lu, Chem. Sci., 2018, DOI: 10.1039/C7SC05325H.

This is an Accepted Manuscript, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. We will replace this Accepted Manuscript with the edited and formatted Advance Article as soon as it is available.

You can find more information about Accepted Manuscripts in the author guidelines.

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal’s standard Terms & Conditions and the ethical guidelines, outlined in our author and reviewer resource centre, still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this Accepted Manuscript or any consequences arising from the use of any information it contains.
Translating Molecular Detections into a Simple Temperature Test Using Target-Responsive Smart Thermometer

Jingjing Zhang, a Hang Xing a and Yi Lu a

While it has been well recognized that affordable and pocket-size devices play a major role in environmental monitoring, food safety and medical diagnostics, 1-5 it is often necessary to quantify a broad range of targets at home or in the field. Devices such as a fluorimeter are often required, and, given the wide availability of smartphones, devices that can interface with smartphones are also preferred. 6-7 Despite progress made so far, most portable devices still require a fair amount of resources to develop and manufacture, making the devices unaffordable to most users at home. In addition, most of these devices are not small enough to be placed in pockets for convenient and frequent use. A major challenge in using a thermometer to measure a broad range of targets beyond temperatures is to find a method that can link the binding of any targets with change of temperature so that one can use any thermometer to detect the target and measure its concentrations. Conventional calorimetric biosensors utilize enzymatic reactions to generate heat and correlate it to the concentration of the substrate. 23-28

 Thermometer is arguably the most widely available, smallest and cheapest quantitative device for measurements. The determination of the temperature is one of the most important analytical methods in environmental monitoring, food safety and medical diagnostics. 7-17 As a result, various thermometers were developed for scientific research, 18, 19 environmental monitoring, 20, 21 and personal healthcare. 22 However, most of the commercialized thermometers are still limited in that it can detect only a single “target”, temperature.

A major challenge in using a thermometer to measure a broad range of targets beyond temperatures is to find a method that can link the binding of any targets with change of temperature so that one can use any thermometer to detect the target and measure its concentrations. Conventional calorimetric biosensors utilize enzymatic reactions to generate heat and correlate it to the concentration of the substrate. 23-28 Most of the methods, however, are either time-consuming, labor-intensive, high cost, or require highly technical expertise and sophisticated instrumentation, which limits their application for field work or POC testing. 29, 30 To overcome the limitation, integrated sensors based on photo-thermal effect of hemoglobin have been described, but their sensitivity is low, due to relatively low thermal generation efficiency of hemoglobin. 31-33 While the exothermic chip sensor, based on an aptamer-modified hydrogel to modulate the capillary fluid flow rate to control the heat produced by the exothermic reagent (e.g. NaOH) dissolution, can provide high sensitivity, 34 its performance is often affected by the endogenous fluid viscosity that is not suitable for analysis of biological samples (e.g. blood or serum). Recently,
nanoparticle-mediated photothermal bioassay using a thermometer as the signal reader has been developed for detecting biomolecules (e.g. PSA) using antibodies. However, antibodies don’t have good sensitivity and selectivity when applied for detecting small molecular targets (e.g. metal ions and small molecular drugs). Therefore, it is still a challenge to integrate thermometers with a convenient and highly sensitive sensing system for quantitative bioanalysis in a wide range of targets.

In this paper, we take advantage of excellent photo-thermal property of indocyanine green (ICG) that is encapsulated in liposome and target-responsive functional DNA (aptamer or DNAzyme) conjugated to phospholipases A2 (PLA2) to provide quantitative link between the target and temperature readout by a thermometer for on-site and real-time detections. Our sensor system include several key features. First, a biotin–DNA immobilized onto magnetic beads (MBs) by means of streptavidin–biotin binding, and the DNA-PLA2 conjugate synthesized by the maleimide-thiol reaction, both of which hybridize with the target-responsive functional DNAs (e.g. structure-switching aptamer or RNA-cleaving DNAzyme) to form a DNA sandwich structure; this design can provide a general platform for the recognition of a wide range of targets. Second, ICG is the only near-infrared (NIR) photosensitizer dye approved by the United States Food and Drug Administration (FDA) for clinical use and has a wide range of applications in imaging and photo-thermal therapy because it can effectively convert NIR laser light into heat. Third, liposomes are spherical lipid vesicles consisting of a phospholipid bilayered membrane structure, which has been widely used as carriers of different signaling tracers with high carrying capacity. Finally, the encapsulated ICG can be easily released by PLA2, which is a superfamily of enzymes that degrade phospholipids by cleaving the sn-2 acyl ester bond of glycerol phospholipids to produce free fatty acids and lysolipids. In this case, the PLA2 could provide a link between the target concentration and the temperature readout.

Results and discussion

The target-responsive smart thermometer (TRESTM) system or sensor reported in this work is comprised of three components (Scheme 1): a functional DNA conjugated to the PLA2 enzyme at 5’ end and immobilized onto magnetic beads (MBs) at 3’ end, which establishes the relationship between target concentration and enzyme concentration based on the target-induced release of DNA–PLA2 conjugate; a liposome-encapsulated ICG, which acts as a signal amplifier; and a NIR-laser integrated thermometer, which is used as a downstream transducer for temperature signal output. The binding of a target by the functional DNA can result in the release of PLA2 from the functional DNA–PLA2 conjugates that are immobilized on the MBs, and the released PLA2 then catalyzes the hydrolysis of liposome to release the ICG from its core. Upon NIR-laser light irradiation in the photo-thermal chamber (Scheme 1b), the released ICG can convert the light excitation energy into heat, producing a temperature increase in solution, which is detectable using a super-fast thermapen (Scheme 1c and d). Since the output temperature signal is related to the presence and concentration of the released ICG in solution, which in turn depends on the presence and concentration of the released PLA2 by the target, the presence and concentration of the target can be determined by monitoring the temperature change in the system. Furthermore, by taking advantage of the enormous amplification afforded by liposome-encapsulated ICG, the system can greatly enhance the high sensitivity of the novel thermometer transducer system, and hence lead to an ultrasensitive thermosensor.

Figure S1a illustrates the synthetic approach to prepare DNA–PLA2 conjugate using the maleimide-thiol reaction. The UV-vis absorption spectrum of the purified DNA-PLA2 conjugate overlays well with the sum of the spectra of its two components, DNA and PLA2 (Figure S1b), suggesting successful conjugation. The successful conjugation has also been confirmed by SDS–PAGE, followed by fluorescence and photographic imaging. In this experiment, the thiolated DNA was modified with fluorescein so that the free DNA and DNA–PLA2 conjugate could be fluorescently imaged (Figure S1c), while the free PLA2 was stained by Coomassie Brilliant Blue (Figure S1d). Another important component of our thermometer system is the liposome that contains ICG, which can be prepared by the film hydration and extrusion method.
to sequester a large amount of the ICG dye for signal amplification.\textsuperscript{5-8} The UV-vis absorption spectrum of liposome-ICG shows a significant red shift (by 22 nm) from that of free ICG (Figure 1a), suggesting that the ICG has been integrated into the lipid bilayers of liposome after encapsulation, as reported previously.\textsuperscript{\text{49}} Dynamic light scattering (DLS) spectrum suggests an average hydration diameter \( \sim 155 \) nm with a uniform distribution (Figure S2). After incubating the liposome-ICG with PLA2 for 1 h, the average hydration diameter increased to about 221 nm, indicating the hydrolysis of liposome-ICG by PLA2. The enzymatic hydrolysis reaction was further analyzed by monitoring the released ICG in the supernatant using UV absorption spectra (Figure 1b), demonstrating even subtle changes in PLA2 concentration (e.g. 5 nM) could release a large amount of the ICG dye, which can be subsequent transduced into an enhanced temperature signal.

To test the ability of the above TRESTM to detect a broad range of targets, we first explored quantitative detection of cocaine using a cocaine aptamer. Cocaine is an addictive drug, and serves as a representative model target for testing new aptamer-based analytical techniques due to unmet diagnostic needs.\textsuperscript{50,51} As shown in Figure 2a, a DNA sandwich structure was assembled on MBs by connecting the DNA–PLA2 conjugate to a biotinylated capture DNA (biotin–DNA) through simultaneously hybridization with the cocaine aptamer. In the presence of cocaine, a target-specific structure switching of the aptamer could cause the disassembly of DNA sandwich structure.\textsuperscript{52} This disassembly process was verified by fluorescence assays using FAM-labeled DNA–PLA2 conjugate (Figure 2b). The fluorescence intensity increased as a function of increasing cocaine concentrations, establishing a quantitative relationship between the concentration of the target cocaine and the amount of DNA–PLA2 released to the solution. The released DNA–PLA2 conjugates were allowed to catalyze the hydrolysis of liposome to release the ICG from its core, which converted the light excitation energy into heat, producing a temperature increase in solution that can be measured by a thermometer. To release as much ICG as possible for maximal signal output, the concentration of liposome-ICG, determined using a calibration curve of UV absorbance versus ICG concentrations (Figure S3), was chosen as 0.25 mg/mL in subsequent experiments. In addition, to obtain high analytical performance, some important detection parameters, including the time of enzymatic hydrolysis and NIR irradiation, were also optimized (Figure S4).

In the absence of cocaine, the temperature increase (\( \Delta T \)), defined as the temperature change of the supernatant solution before and after the NIR laser irradiation, was \( \sim 2 \) \(^\circ\)C, which may be due to the nonspecific release of DNA–PLA2 conjugate or ICG leakage during centrifugation. In contrast, under the same incubation condition, the \( \Delta T \) was increased with the increasing input of cocaine and reached a plateau at 300 \( \mu\)M (Figure 2c). This observation is in agreement with the fluorescence results, which indicated all the releasable DNA–PLA2 conjugates were released at this high concentration of cocaine. This TRESTM exhibited a wide dynamic range from 0 to 500 \( \mu\)M and showed a limit of detection of 3.8 \( \mu\)M. This sensitivity is comparable to the commercial cocaine test kits, and other portable meter-based cocaine sensors.\textsuperscript{10}

To demonstrate the selectivity of the TRESTM, we further carried out a control experiment with two common biological compounds (adenosine and glucose). Continuous addition of up to 0.5 mM adenosine or glucose showed a negligible signal change. In contrast, the temperature response of cocaine at 100 \( \mu\)M was much higher (Figure 2d). These results indicate that the TRESTM retained the aptamer selectivity with a highly specific response to the target, cocaine.

To test the feasibility of applying the TRESTM sensor in real samples, we explored detection of cocaine in human serum, one of the most challenging media that contain a variety of proteins and other interfering molecules.\textsuperscript{53} By testing 18 serum samples spiked with different concentrations of cocaine with our TRESTM sensor, we found that the calibration curve in the presence of 20 % serum (Figure S5) was similar to that in the PBS buffer. The detection limit was 15.1 \( \mu\)M, which was comparable with that obtained in buffer, suggesting that the other components of the serum did not interfere significantly.
with sensor performance. The results revealed that the proposed TRESTM system showed promising applicability in complex body fluids.

Another key issue that needs to be addressed, especially for in-home or in-field diagnostics, is the acceptable shelf life of the TRESTM system. Previous research has shown that aqueous-instability of ICG limits its application as a fluorescence agent for imaging and sensing purposes. To address the question of whether such an instability affects the temperature signal in target detection, we monitored the output temperature signal to assess the stability of liposome-ICG as a signal reporter species by using 250 µM cocaine as input. As shown in Figure S6, after refrigerated storage for one month, our TRESTM system performed more than 80% of the initial sensitivity, demonstrating that the liposome-ICG are stable and have a suitable shelf life for practical applications.

To demonstrate the generality of our TRESTM sensor, we extended our methodology from aptamers to DNAzymes for the on-site detection of uranyl (UO$_{2}^{2+}$), which is a radioactive and potentially carcinogenic species. To achieve the detection, the DNA–PLA$_2$ conjugates were connected to biotin-DNA immobilized MBs by the UO$_{2}^{2+}$-specific DNAzyme (39E) and its substrate (39S) via 12-base-pair hybridization (Figure 3a). Upon addition of UO$_{2}^{2+}$, the DNAzymes catalyzed the cleavage of its substrate at the 3’-phosphoester bond of the ribonucleotide A (rA) and subsequent release of the DNA–PLA$_2$ conjugate, which was verified by fluorescence assays (Figure 3b). A dose-response fluorescence curve for UO$_{2}^{2+}$ detection was demonstrated in Figure 3b inset, indicating the possibility to further connect the UO$_{2}^{2+}$ detection with the TRESTM system. As expected, our TRESTM sensor allowed the quantitative analysis of UO$_{2}^{2+}$ with a dynamic range from 0 to 1 mM (Figure 3c). The detection limit was calculated to be 25.7 nM (6.8 µg l$^{-1}$), which is comparable to our previous PGM-based detection system (9.1 nM) and much lower than the US Environmental Protection Agency (EPA) regulated level (30 µg l$^{-1}$ or 113 nM) for drinking water. In addition, the sensor maintained excellent selectivity for UO$_{2}^{2+}$ over other various biologically relevant metal ions at physiologically relevant concentrations (Figure 3d).

Conclusions

In summary, we have demonstrated a new sensor system in which the presence and concentration of a target can be transformed to a detectable temperature changes using a portable thermometer. The sensor system is based on the target-induced release of DNA–PLA$_2$ conjugate from a functional DNA duplex immobilized on MBs, and subsequent hydrolysis of liposome-ICG and NIR-laser irradiation to produce a temperature signal. Using this system, cocaine and uranium have been detected quantitatively through a direct temperature reading. As more functional DNAs are available to bind a broad range of targets, our TRESTM will provide a generic approach for quantitative detection of a wide range of targets at home or in the field using a portable thermometer, which is among the cheapest and most widely used analytical device. Although the sensing system was not fully integrated into a single device, as a proof-of-concept, the sensing platform holds considerable promise toward portability by taking advantage of well-developed microfluidic or lateral flow technology in the future.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

We wish to thank the U.S. National Institutes of Health (ES16865) for financial supports.

Notes and references

A novel target-responsive smart thermometer for translating molecular detection into a temperature test has been developed.