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L-DNA Molecular Beacon: A Safe, Stable, and Accurate Intracellular Nano-thermometer for Temperature Sensing in Living Cells

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Supporting Information

ABSTRACT: Noninvasive and accurate measurement of intracellular temperature is of great significance in biology and medicine. This paper describes a safe, stable, and accurate intracellular nano-thermometer based on an L-DNA molecular beacon (L-MB), a dual-labeled hairpin oligonucleotide built from the optical isomer of naturally occurring D-DNA. Relying on the temperature-responsive hairpin structure and the FRET signaling mechanism of MBs, the fluorescence of L-MBs is quenched below the melting temperature and enhanced with increasing temperature. Because of the excellent reversibility and tunable response range, L-MBs are very suitable for temperature sensing. More importantly, the non-natural L-DNA backbone prevents the L-MBs from binding to cellular nucleic acids and proteins as well as from being digested by nucleases inside the cells, thus ensuring excellent stability and accuracy of the nano-thermometer in a complex cellular environment. The L-MB nano-thermometer was used for the photothermal study of Pd nanosheets in living cells, establishing the nano-thermometer as a useful tool for intracellular temperature measurement.

Measurement of intracellular temperature is of vital significance in biology and medicine. First, as a fundamental physical parameter, temperature affects a wide variety of intracellular chemical reactions and biological processes, including enzymatic reactions, gene expression, and molecular interactions.[1] Second, some pathological cells (e.g., cancer cells) are reported to have higher temperatures than normal cells due to their faster metabolic rates,[2] suggesting intracellular temperature as a potential indicator for related diseases. In addition, accurate monitoring of temperature at the single-cell level is essential for understanding the mechanism of thermal therapy,[3] which utilizes heat to kill cancer cells, as well as evaluation of therapeutic efficiency. Therefore, it is highly important to develop techniques for accurate sensing of intracellular temperature at the single-cell level.

In consideration of the intracellular environment, an ideal intracellular thermometer is expected to have the following features. First, for long-term measurements, the thermometer must be nontoxic to cells. Second, for accurate temperature sensing, the signal of the thermometer is expected to be independent of nonspecific factors in the complex intracellular biochemical environment, such as the presence of biomacromolecules. Third, additional physicochemical properties, such as high spatial resolution, high temperature resolution, excellent temperature-responsive reversibility, and rapid response, are desirable.

Although the traditional thermocouple method[4] is capable of rapid, accurate, and precise measurement of intracellular temperature, it suffers from several limitations, such as cell damage because of insertion through the plasma membrane, low spatial resolution due to its large dimensions (e.g., hundreds of nanometers), and inability to sense temperature at different locations simultaneously. Alternatively, noninvasive fluorescent intracellular thermometers with high spatial resolution and high throughput have been developed using temperature-dependent fluorescent materials,[5] including temperature-sensitive organic dyes,[6] polymers,[7] proteins,[8] quantum dots,[9] upconverting nanoparticles,[10] etc. Unfortunately, current fluorescent sensors suffer from one or more limitations, including cytotoxicity, nonspecific protein adsorption, and relatively large size (tens to hundreds of nanometers). Therefore, a safe, accurate intracellular thermometer with high spatial and temperature resolution is demanded.

Herein, we demonstrate a novel intracellular thermometer employing L-DNA molecular beacons (L-MBs), which combine full biocompatibility and resistance to signal interference with rapid, accurate, and sensitive temperature sensing. Molecular beacons,[11] special hairpin-structured dual-labeled oligonucleotide probes (Figure 1), have been widely applied in areas such as detection of DNA and RNA,[11d−f] monitoring of enzymatic processes,[11g,h] study of protein−DNA interactions,[11k] and investigations of living systems.[11i,j] In the present work, we, for

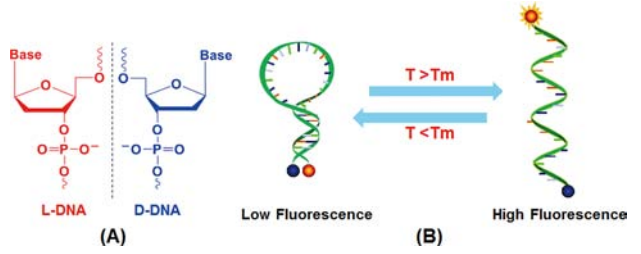

Figure 1. (A) Structure of L-DNA. (B) Principle of the L-MB-based intracellular nano-thermometer.

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the first time, utilized MBs for intracellular temperature sensing due to their inherent features. First, MBs consist of nucleic acids and thus are nontoxic to cells. Second, the stability of loop-stem structure and fluorescence intensity of MBs are temperature-dependent.[12] The thermal stability of MBs is reflected in the melting temperature (T_m), defined as the temperature at which half of hairpin structure is dissociated to single-stranded DNA (ssDNA). As shown in Figure 1B, below T_m, MB form a loop-stem structure with fluorophore and quencher in close proximity, leading to low fluorescence; with the temperature increasing toward or beyond T_m, the MB gradually "melts", so that the fluorophore moves away from the quencher, and the fluorescence is restored (Figure 1B). In consideration of the complex cellular environment, instead of using natural D-DNA bases, our MBs consist of non-natural L-DNA bases, a mirror-image isomer of D-DNA (Figure 1A),[13] which cannot hybridize with any D-DNA strands or bind with natural proteins. As artificial nucleic acids, L-DNAs are also resistant to enzymatic degradation.[13a−c] These properties of L-DNA allow L-MBs to function in complex cell environment with excellent resistance to interferences and biological degradation.

To confirm the feasibility of our thermometer, we first designed an L-MB with four base pairs in the stem (named L-MB4; the sequence is shown in Table S1) and studied its response to temperature. As shown in Figure 2A, the

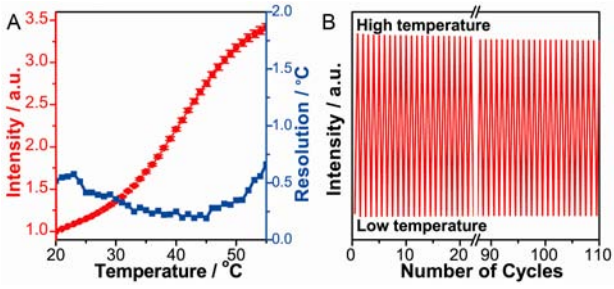

Figure 2. L-MBs show (A) temperature-dependent fluorescence intensity (red line, left axis; fluorescence intensity was normalized to the value at 20 °C) and good temperature resolution (blue line, right axis), as well as (B) excellent reversibility of fluorescence change at different temperatures (20, 50 °C) in PBS buffer (10 mM phosphate, 140 mM NaCl, pH 7.4, 500 nM L-MB4).

fluorescence intensity of L-MB4 increased as the temperature rose from 20 to 55 °C, due to the dissociation of hairpin structure at high temperature and the consequent separation of fluorophore from quencher. Moreover, the temperature resolution, evaluated as the product of the inverse of the slope of intensity versus temperature and the standard deviation of the averaged fluorescent intensity,[7c] was determined to be smaller than 0.7 °C, which is comparable to or better than the resolution of current fluorescent thermometers.[7b,c,9b,10b] Figure 2B shows that the fluorescence change between low temperature (20 °C) and high temperature (50 °C) is reversible even after 110 cycles, indicating that our novel thermometer with excellent reusability can be used for long-term monitoring or repeated temperature cycling. In addition, the temperature response range of MBs is tunable by changing of the number of base pairs in the stem (Figure S1 and Table S2). With an increasing number of base pairs, the T_m of MB increases and the response range changes from low temperature (20−55 °C) to high temperature (48−79 °C). Meanwhile, a broader response range (25−75 °C) can be achieved by mixing different MBs in certain concentration ratios (Figure S1 and Table S2).

As mentioned above, two other factors are extremely important for an intracellular thermometer: resistance from interference and stability in the complex cellular environment. For this purpose, non-natural L-DNA bases rather than natural D-DNA bases were employed for MB construction because of their inherent properties. In our experiment, both L-DNA- and D-DNA-based MBs (L-MB4 and D-MB4) were synthesized, and their binding properties with complementary natural nucleic acids or proteins, as well as their stability in the presence of nuclease or in a complex biological environment, were evaluated. In Figure 3A, fluorescence enhancement was observed for D-MBs

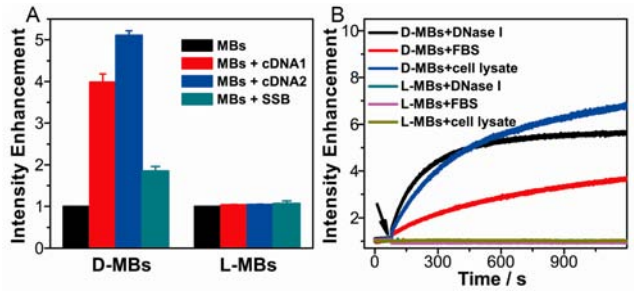

Figure 3. L-MBs show (A) little or no interference in fluorescence intensity in the presence of complementary DNA (cDNA1, complementary to loop; cDNA2, complementary to loop and stem) or SSB proteins and (B) resistance to enzyme digestion in different environments (DNase I, 10% FBS, and cell lysate). The black arrow indicates the time of addition.

in the presence of complementary DNA (cDNA1, complementary to loop; or cDNA2, complementary to loop and stem) or DNA-binding proteins (single-stranded DNA-binding protein, SSB protein), since they could be recognized by natural nucleic acids or D-DNA binding proteins. On the contrary, L-MBs showed little or no fluorescence increase, indicating that our L-MBs can efficiently avoid signal interference from D-DNA-binding proteins and naturally occurring nucleic acids.

In addition, as shown in Figure 3B, D-MBs suffered severely from enzymatic digestion, evidenced by the significant increase in D-MBs fluorescence upon the addition of DNase I, fetal bovine serum (FBS), or cell lysate. In contrast, L-MBs showed the desirable property of resistance to enzyme digestion in different biological environments (DNase I, FBS, and cell lysate). These results demonstrate the excellent interference resistance and stability properties of L-MBs, thereby avoiding false-positive results, as required for real biological applications. Furthermore, the melting temperatures of L-MBs were found to change very little across intracellular variation range of ionic strength (150−200 mM)[14] and pH (6.8−7.4)[15] (Figures S2 and S3), suggesting their usability inside the cells.

To test the performance and evaluate the cytotoxicity of our thermometer, L-MB4 was introduced into live HeLa cells via liposome transfection. Figure 4 shows confocal microscopy images of HeLa cells with L-MBs at different temperatures ranging from 20 to 37 °C. The fluorescence becomes more intense with increasing temperature. Through Image J analysis, the cellular temperature−fluorescence intensity curve was obtained (Figure 4C), which was similar to the response curve of L-MBs in PBS buffer. In contrast, the fluorescence intensity of cells transfected with D-MB4 remained unchanged when the temperature increased from 20 to 37 °C (Figures 4C and S4). It

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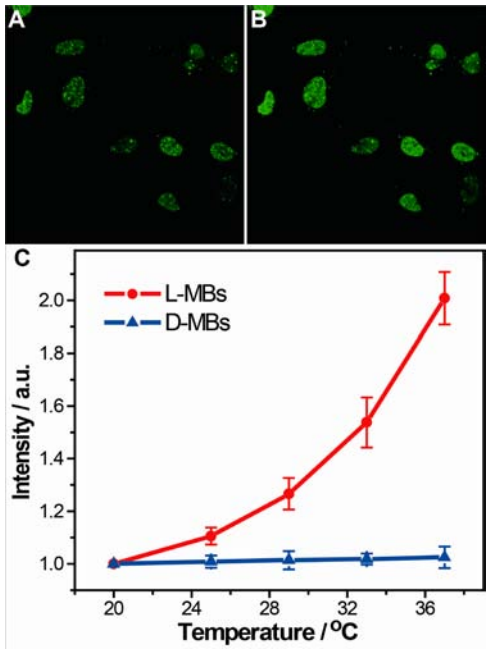

Figure 4. Confocal microscopy images of HeLa cells transfected with L-MBs at different temperatures: (A) 20 °C and (B) 37 °C. (C) Temperature–fluorescence intensity curve of cells transfected with L-MBs or D-MBs. The fluorescence intensity of the cells at different temperatures was normalized by taking the fluorescence intensity at 20 °C as 1.0.

has been reported that unmodified phosphodiester oligonucleotides possess a half-life as short as 15–20 min in living cells.[16] As a result, the life span of DNA MB inside cells is less than 30 min, after which it becomes nonfunctional inside the cell.[17] During the 4 h transfection process, D-MBs are believed to be completely digested by intracellular nuclease, leading to their inability to respond to temperature changes. Meanwhile, the working performance of L-MBs after long-term incubation inside cell was also investigated. As shown in Figure S4, within 12 h after transfection, the cells with L-MBs produced similar temperature response curves. In addition, L-MBs were demonstrated to function well in different types of cells (Figure S5). These results indicate that L-MBs can be used for long-term (as long as 12 h) intracellular temperature monitoring in different types of cells. Furthermore, the sub-10 nm size of L-MBs, which is smaller than most current thermometers and comparable to the smallest one (8.9 nm in hydrodynamic diameter for a fluorescent polymeric thermometer[11]), offers higher spatial resolution, another important feature for study of intracellular temperature distribution. Moreover, because they are composed of nucleic acids, L-MBs are friendly to cells, as proven by the *in vitro* cell viability experiment (Figure S6).

After obtaining the calibration curve inside HeLa cells, we applied our nano-thermometer to investigate the photothermal effect on live cells. Recently, thermal therapy for cancer, which makes use of significant temperature increases in organisms to kill cancer cells, has attracted attention because of the low side effects, excellent temporal and spatial resolution, and improved therapeutic efficiency compared to radiation therapy and chemotherapy.[3,18] However, the lack of an effective, noninvasive method for real-time monitoring of the temperature changes in organisms, especially at the single-cell level, has been an obstacle to in-depth understanding of this promising technique. To employ L-MBs for monitoring the temperature change during a photothermal process, ultrathin hexagonal Pd nanosheets (side length 41 nm, thickness 1.8 nm, Figure 5A) were synthesized

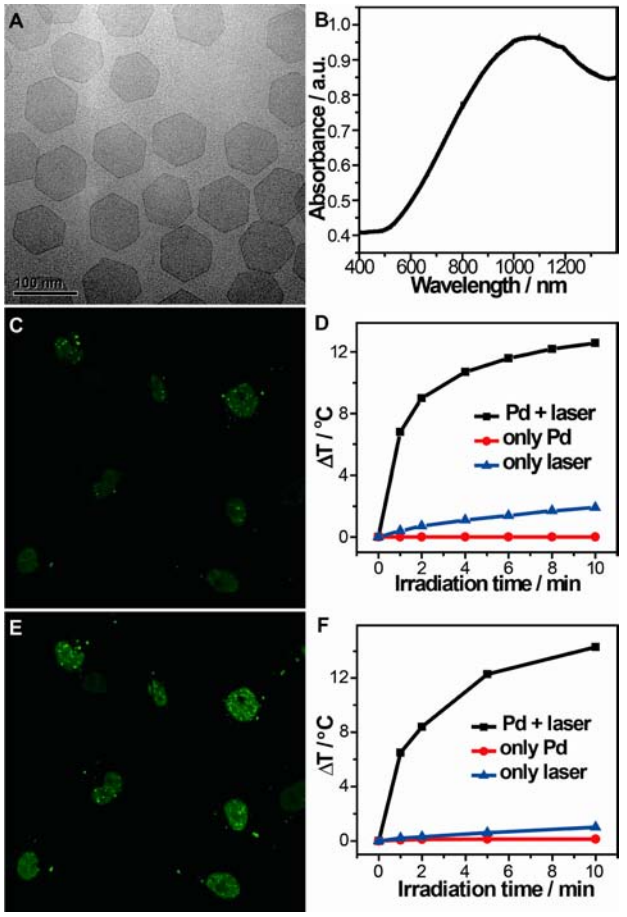

Figure 5. Photothermal study using Pd nanosheets for living HeLa cells: TEM (A), absorption spectra (B), and photothermal effect in DMEM medium (D) of Pd nanosheets; confocal images of HeLa cells with Pd nanosheets before (C) and after (E) irradiation by 808 nm laser for 10 min; and the photothermal effect of Pd nanosheets on living cells (F).

according to the reported method[19] and used for photothermal study in live cells. The Pd nanosheets showed strong absorption (extinction coefficient, 4.1×10^9 M^{-1} cm^{-1} at 1045 nm) in the NIR region, ranging from 800 to 1400 nm (Figure 5B). The photothermal effect induced by NIR surface plasmon resonance absorption was confirmed by monitoring the temperature of 1 mL of cell culture medium DMEM containing 5 ppm of the Pd nanosheets irradiated by an NIR laser (808 nm, 2 W). As shown in Figure 5D, the temperature of the DMEM solution increased about 12.5 °C after 10 min of irradiation, while no significant temperature change was observed from control samples without Pd nanosheets or laser irradiation (Figure 5D). These results demonstrated the excellent photothermal property of the Pd nanosheets.[19] After treatment with L-MB4, HeLa cells with or without the addition of Pd nanosheets in the DMEM medium were irradiated with or without the 808 nm laser. The results showed that the fluorescence intensity of cells with Pd nanosheets significantly increased after 10 min of 808 nm laser irradiation (Figure 5C,E), while for the cells without Pd nanosheets or without laser treatment, little change in fluorescence intensity was observed (Figure S8). After

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conversion of fluorescence intensity to temperature on the basis of the calibration curve in Figure 4C, the real-time temperature changes are shown in Figure 5F. The temperature of cells with Pd nanosheets rose gradually from 20.0 to 34.3 °C after 10 min laser irradiation, while no obvious temperature change occurred in the control experiments. The temperature change profile of living cells (Figure 5F) agrees well with that of DMEM (Figure 5D), although there is a small difference in overall temperature increase after laser treatment (14.3 °C for cell solutions vs 12.5 °C for DMEM buffer), possibly due to a difference in insulation of the containers (Petri dish vs cuvette). These results established that our L-MBs thermometer is a promising tool for measuring the temperature during the course of thermal therapy for cancer.

In conclusion, we have developed a novel nanoscale thermometer with L-MBs for intracellular temperature measurement. The L-MB thermometer can sense temperature with high resolution and rapid response. Compared to other fluorescent intracellular thermometers, our L-MB nano-thermometer has several advantages. First, its composition of nucleic acids makes it fully biocompatible with cells, which is essential for long-term temperature monitoring. Second, the signal of L-MBs is undisturbed by binding of nucleic acids and proteins, or by the presence of nucleases ubiquitously existing in cell environments, ensuring the accurate measurement of temperature. Finally, additional physicochemical features, such as small size (less than 10 nm), excellent response reversibility, and tunable respond range, make L-MBs suitable for potential applications in a variety of situations. The L-MB nano-thermometer was successfully utilized for a photothermal study in living cells, establishing the nano-thermometer as a useful tool for intracellular temperature measurement.

■ ASSOCIATED CONTENT

Supporting Information

Experimental details and additional characterization data. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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