Meso-tetra(4-carboxyphenyl)porphine-Enhanced DNA Methylation Sensing Interface on a Light-Addressable Potentiometric Sensor

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Supporting Information

ABSTRACT: DNA methylation (DNAm) sensors are an emerging branch in the discipline of sensors. It is believed to be able to promote the next generation of epigenetics-based diagnostic technology. Differing from the traditional biochemical sensors that aimed at individual molecules, the challenge in DNAm sensors is how to determine the amount of 5-methylcytosine (5mC) in a continuous nucleotide sequence. Here, we report a comparative study about meso-tetra(4-carboxyphenyl)-porphine (TCPP)-based DNAm sensing interfaces on a light-addressable potentiometric sensor (LAPS), depending on TCPP’s postures that are flat in the π-conjugated TCPP layer on reduced-graphene-oxide-decorated LAPS (#1) and stand-up in the covalently anchored TCPP on glutaraldehyde (GA)-treated LAPS (#2), along with the blank one (only GA-treated LAPS, #3). These DNAm sensing interfaces are also distinct from the traditional biosensing interface on LAPS, that is: it is not functionalized by the sensing indicator (SmC antibody, in this case) but by the target nucleotide sequence. The surface characterization techniques such as Raman spectra, scanning electron microscopy, and X-ray photoelectron spectroscopy are conducted to prove the decorations, as well as the anchored nucleotides. It is found that, though all of them can detect as low as one 5mC in the target sequence, the enhanced DNAm sensitivity is obtained by #2, which is evidenced by the higher output-voltage changing ratio for the SmC site of #2 than those of #1 and #3. Furthermore, the underlying causes for the improved sensitivity in #2 are proposed, according to the conformational and electronic properties of TCPP molecules. Conclusively, TCPP’s synergetic function, including the molecular configuration and the activate (carboxyl) groups on its peripheral substituents, to improve the DNAm sensitivity on LAPS is investigated and demonstrated. This can shed light on a new approach for DNA methylation detection, with the merits of low cost, independence on bisulfite conversion, and polymerase chain reaction.

1. INTRODUCTION

DNA methylation (DNAm) refers to the presence of 5-methylcytosine (5mC) on the gene sequence.¹ As one of the epigenetic mechanisms, it is essential for embryonic development,² genomic imprinting,³ X-chromosome inactivation,⁴ etc. It has been accepted that understanding the DNAm degree in a certain genomic DNA sequence is conducive to the early diagnosis and treatment of disease.⁵ Therefore, an effective technology to profile the landscape of genomic methylation is necessary. Lots of conventional approaches have been extensively developed for the determination of DNAm, such as methylation-specific polymerase chain reaction (PCR),⁶ bisulfite conversion (BC),⁷ fluorescence assay,⁸ high-performance liquid chromatography,⁹ and mass spectroscopy.¹⁰ Recently, attention has been focused on electrochemistry (EC)-based DNAm detections,¹¹ in which the key problem is how to recognize the 5mC and transduce it to the electronic signal. The basic EC-based SmC sensing strategies can be grouped in four categories: (1) the direct detection based on the electro-oxidation peak of SmC;¹² (2) the methylated and demethylated DNA after having undergone endonuclease digestion can be discriminated by the differential pulse voltammogram (DPV) technique;¹³ (3) after suffering BC and PCR, the methylated and unmethylated genomic sequences are in the states of guanine-enrich and adenine-enrich, respectively, which can be measured by EC signals;¹⁴,¹⁵ (4) the anti-SmC monoclonal antibody (anti-SmC)-based DNAm sensing mechanism (as illustrated in Scheme 1A), which can be measured by the stripping peak induced by the anti-SmC-functionalized silver nanoparticles modified on glass carbon electrodes.¹⁶ In brief, it has been confirmed that EC-based DNAm sensors have a promising future to promote the DNAm assay in clinical medicine.

Light-addressable potentiometric sensors (LAPSs) are a class of surface-charge sensing devices with the structure of electrolyte–insulator–semiconductor (EIS). By constructing a specific biochemical sensing interface, it can be exploited to realize the detection of DNA¹⁷,¹⁸ and bacteria.¹⁹ In this work, a LAPS is developed to be a DNAm sensor (named DNAm-LAPS), for the first time, to our best knowledge. That is, the anti-SmC-based DNAm sensing strategy (illustrated in Scheme 1A) is brought about on the LAPS’ insulator layer (Si₃N₄ in this work, as sketched in the right-upper of Scheme 1B), then LAPS’ surface charges can be altered by this DNAm sensing...
reaction, resulting in a varied output-voltage ($\Delta V_{\text{out}}$) according to LAPS’s working principle, which has been discussed in our previous work and outlined in Figure S1. In this work, the main attention is how to determine the amount of 5mC in the target single-strand DNA (ssDNA), which needs to be fixed on LAPS first, instead of anti-5mC, because anti-5mCs can bind with any 5mC sites no matter they are in one strand, or not. Then, if LAPS is functionalized by anti-5mCs, we cannot be sure. If $\Delta V_{\text{out}}$ is induced by 5mCs from the same strand, then the goal to measure the amount of 5mCs on the concerned ssDNA cannot be realized. Accordingly, how to anchor ssDNAs as much as possible and make them in a suitable posture are of great concern to form an efficient DNAm-LAPS.

In this context, the captivating ideas for constructing DNA—porphyrin adducts are ideal for us to draw upon, which include: (a) intercalating porphyrins into the strand structure to provide insights into the complicated dynamics;\(^{20}\) (b) attaching porphyrins onto the major/minor groove to induce DNA cleavage, forming the G-quadruplex DNA\(^{1,22}\) and they act as indicators for DNA detection;\(^{23}\) (c) stacking them onto the DNA strand by $\pi$-$\pi^*$ conjugation\(^ {21,24}\) or electrostatic interaction;\(^ {25}\) and (d) the covalent binding mod-\(^ {26,27}\)e.\(^ {26,27}\)According to the current understanding of DNA—porphyrin interactions,\(^ {28}\) the covalent approach is utilized to anchor the target nucleotide sequence, for the purpose of exposing the 5mC sites as much as possible. Meanwhile, metalloporphyrins and dsDNA are not used as the prototype in this work because they have a strong tendency to form the $\pi$-stacking interaction with DNA.

Herein, two kinds of mesotetrakis(4-carboxyphenyl)-porphyrin (TCPP)-decorated LAPS interfaces are studied as a model for anchoring the tested ssDNA and the consecutive DNAm detection. In contrast with the blank one, three kinds of ssDNA-anchored LAPS are illustrated in Scheme 1B and named #1, #2, and #3, respectively. The reasons for different modification methods are outlined here. For #1 and #2, TCPP’s morphological features (the shape-persistent macrocycle, the $\pi$-electronic structure, and more carboxyl groups on its peripheral benzene ring) are utilized to form enhanced DNAm sensing interfaces on LAPS, because more ssDNA sequences can be fixed by the covalent bond between TCPP’s carboxyl group and modified amino group on ssDNA’s 5′ terminal, according to the report of TCPP-anchored ssDNA on a graphene field-effect-transistor platform.\(^ {26}\) Meanwhile, the extra layer of reduced graphene oxide (rGO) in #1 is used for

![Scheme 1. Protocol of the DNAm Sensing Interface.](image)

“A (A) the anti-5mC-based methylation recognition strategy; (B) main procedures in forming three DNAm-LAPS, including the reduced graphene oxide (rGO) and TCPP-modified (#1), TCPP-activated (#2), and blank (#3) LAPSs.

Figure 1. Raman spectra of TCPP, rGO, and TCPP on rGO (TCPP@rGO), excited at $\lambda = 532$ nm (A), and SEM photographs of TCPP (B), TCPP on rGO (C), and rGO (D).
the purpose of attaching more TCPP molecules assisted by the \( \pi-\pi^* \) interaction between rGO and TCPP. However, whether higher DNAm sensitivity can be obtained by \#1 than \#2 is still uncertain because the possible configurational discrepancies of TCPP between \#1 and \#2, as diagrammed in Scheme 1B. Finally, the blank one (\#3) is used as a control.

To identify TCPP molecules, as well as the immobilized ssDNA on three interfaces (\#1, \#2, and \#3), surface characterization techniques such as Raman spectra, scanning electron microscopy (SEM), and X-ray photoelectron spectroscopy (XPS) are performed first. Then, electronic examinations of DNAm-LAPS are conducted step by step, to evaluate the TCPP-enhanced DNAm sensing interface on LAPS. Furthermore, the capability of TCPP to enhance DNAm sensitivity is evaluated by the relation of LAPS’ changing ratios of \( \Delta V_{\text{out}} (V_{\text{out}}/V_{\text{out},0}) \) to the amount of 5mC sites on the target sequences by comparison of three DNAm sensing interfaces. Finally, the theoretical deductions are provided for the measured results, based on the amount of anchored ssDNAs which are evidenced by XPS characterization, as well as ssDNA morphological variations on each of the interfaces. It is believed that the improved DNAm sensing interface on LAPS by vertically anchored TCPP molecules is an effective and promising candidate for determining the methylation degree of the anchored sequences, with the merits of independence on the extra processes like bisulphite conversion and PCR.

2. RESULTS AND DISCUSSION

2.1. Raman and SEM. In Figure 1A, by using TCPP (green) as a control, the TCPP molecule in TCPP@rGO (red) can be identified by its characteristic peaks, which are contributed by the vibrations of \( C_\alpha-C_\alpha \) (1004 and 1552 cm\(^{-1}\)), \( C_\alpha-N \) (1242 cm\(^{-1}\)), \( C_\alpha-C_\beta + C_\beta-H \) (1330 and 1457 cm\(^{-1}\)), and the weak peak at 1497 cm\(^{-1}\).29 The positions of carbon \( \alpha, \beta \), and m are marked in the inset of Figure 1A. The superimposed peaks of rGO and TCPP can be distinguished in TCPP@rGO (red) by using rGO (gray) as a control, which are the D band (\(~1346\) cm\(^{-1}\)), G band (\(~1598\) cm\(^{-1}\)), and two-dimensional (2D) band (\(~2672\) cm\(^{-1}\)). The confirmation about a reduced GO layer is made by the existence of a 2D peak; meanwhile, its full width at half-maximum (about 140 cm\(^{-1}\)) indicates that the sp\(^2\) carbon network is distorted. What’s more, the intensity ratio of the D peak to G peak (\( I_D/I_G \)) is about 1.28, which means that there are still structural defects in the rGO layer. By comparing “TCPP@rGO” and “rGO”, the diminished 2D band is deduced to be caused by the \( \pi-\pi^* \) conjugation of TCPP flakes.

The SEM image in Figure 1B indicates that the directly coated TCPP flakes on GA-treated LAPS are not flat, which is similar to the reported morphology of porphyrin films.30 The cross-stand TCPP flakes agree with the binding pattern of TCPP as illustrated in Scheme 1B#2. In contrast, the TCPP flakes on rGO (Figure 1C) can be identified by the flat strips on rGO’s nanosheets, which can be identified from the pure rGO SEM image (Figure 1D). This overlapped TCPP and rGO shape corresponds to the \( \pi-\pi^* \) conjugation, as mentioned above and illustrated in Scheme 1B#1.

2.2. XPS Characterization. It is found from the wide spectra (Figure 2A) that the atoms of C, N, and O exist in all samples, while P, Na, and Cl are only found in the ssDNA-immobilized ones, also proved by component analysis (Figure S2). In Figure 2B, the resolved curves of P 2p core spectra (SP_\( i \), \( i = 1, 2, 3 \)) can be classified into two groups, which are close to 134.0 \( \pm \) 0.1 and 133.0 \( \pm \) 0.1 eV, and they agree with P atoms in the phosphate backbone of unpaired ssDNA.31–33 By comparing the N 1s core spectra (Figure 2C) of TCPP-decorated ones (#1 and #2) with #3 (blank), the existence of TCPP can be identified by the peaks at about 397.6 and 399.9 eV, which are contributed by the N atoms in the porphyrin ring. Moreover, the peak fitting analyses of N 1s and C 1s core spectra (Figures S3 and S4) can provide more proofs of the porphyrin ring and carboxyl in TCPP.34,35 Finally, the

Figure 2. XPS wide spectra (A), P 2p core spectra (B), and N 1s core spectra before (C) and after ssDNA immobilization (D). Split curves (SP_\( i \), \( i = 1, 2, 3 \)) in (B) are decomposed by CasaXPS.
existence of ssDNA can also be evidenced by the peaks at about 401.5 eV in Figure 2D and their peak fitting analyses in Figure S3. Based on these XPS characterization results, it is inferred that the amounts of anchored ssDNA on three kinds of interfaces can be ordered as #2 > #1 > #3. This deduction can be explained from two points: (1) the carboxyl groups in TCPP-decorated ones (#1 and #2), after being treated by EDC and NHS, can provide more covalent binding sites for the amino-modified ssDNA sequences.26 (2) the less-anchored ssDNA on #1 than #2 can be illustrated by the morphological differences of TCPP molecules. Although there are more free carboxyl groups on #1 (10.59%) than #2 (3.99%) (Figure S4), the flat posture of TCPP on #1 (evidenced by Raman and SEM characterizations) makes the anchored ssDNA chains consume more surface area. By contrast, the erected TCPP molecules on #2 is more helpful to reduce the steric hindrance, so more ssDNA sequences are anchored on #2 than #1.

2.3. Electronic Measurements. First, the basic working curve of blank LAPS is examined. The gradually growing $V_{\text{out}}$ with the changing of $V_{\text{REF}}$ from left to right (Figure 3A) agrees with LAPS' theory for the p-type Si substrate.31 The measured $V_{\text{out}}$ is the out-express of the inner electric field ($E_{1}$) from SI to the bulk of p-Si, which can be altered by the interface state at SI and insulator SiO$_2$/Si$_3$N$_4$ (SI), as illustrated in Figure 3A-1, A-2. (1) Exhausted state. It is induced by positive $V_{\text{REF}}$ or charges on the insulator. In this state, $E_{1}$ can be easily altered by $V_{\text{REF}}$, resulting in $V_{\text{out}}$ changing; (2) Accumulated state, which is induced by the negative $V_{\text{REF}}$ or charges. In this state, $E_{1}$ cannot be easily altered because of the homologous carriers (hole) in the SI region and bulk of p-Si. The steep curve at around $V_{\text{REF}}$ 0.4 V indicates that LAPS' $V_{\text{out}}$ is sensitive to the altered surface potential. Therefore, in the following experiments, $V_{\text{REF}}$ is controlled at 0.4 V.

From Figure 3B, it is found that changing ratio ($\Delta V_{\text{out}}/V_{\text{out,0}}$) is decreased, after LAPSSs (#1 and #2) are incubated with higher concentrations of TCPP solutions (C_TCPP). $V_{\text{out,0}}$ is the data of $V_{\text{out}}$ when C_TCPP is zero. It is deduced to be caused by the electronegativity of TCPP’s carboxyl groups,32 which can introduce negative charges on the LAPS surface, then more holes may be induced at the SI interface and weaken the exhausted state, which can subsequently reduce $V_{\text{out}}$. Meanwhile, the discrepancy between #1 and #2 is explained from two sides: (1) the oxygen-containing groups and the resistance of in rGO coating (#1),36 which can increase the negative charges and lower $V_{\text{REF}}$, respectively. (2) morphological differences of TCPP at two interfaces which are the evenly $\pi-\pi$ conjugated TCPP on rGO (#1) and the vertically fixed TCPP on GA-treated LAPS (#2). Then, there will be more free electronegative carboxyl groups on #1 than on #2, as evidenced by C 1s core spectra analyses (Figure S4), so $V_{\text{out}}$ of #1 is reduced more than that of #2.

2.4. Responding to ssDNA Immobilization. After incubation with different concentrations of ssDNA (C_DNA, by using DNA m1 as an example), three kinds of LAPS (#1–#3) are measured in similar working conditions. Their data of $V_{\text{out}}$ and $\Delta V_{\text{out}}/V_{\text{out,0}}$ ($V_{\text{out,0}}$ is $V_{\text{out}}$ when C_DNA is zero) are presented in the bilongitudinal coordinates (Figure 3C). Their main trends are falling down with the increase of C_DNA (0–100 nM), and it is inferred to be caused by the negative charges on the anchored phosphate backbone, which can make the SI interface state changed from A-1 to A-2 (Figure 3A). According to this common feature in three kinds LAPSSs (#1–#3), 100 nM ssDNA solutions are used in the subsequent experiments. Meanwhile, two divergences are also obvious, which are the upshift $V_{\text{out}}$ curves (#1–#2) and the weakly varied $\Delta V_{\text{out}}/V_{\text{out,0}}$ curve (#1).

For the first one, it is deduced to be caused by the positive (Na$^+$) charges that are generated by the EDC and NHS.
treatments for #1—#2, as illustrated by the inset in Figure 3C and evidenced by the XPS spectrum of EDC and NHS modified chip in Figure S5; they can turn the EIS interface state to the “exhausted state” (as shown in Figure 3A-1) and increase $V_{\text{out}}$ data at zero. Subsequently, accompanied by ssDNA immobilization, these positively charged groups are replaced by negatively charged ssDNA sequences, so the decreased $V_{\text{out}}$ can be measured.

For the latter one, $\Delta V_{\text{out}}/V_{\text{out},0}$ data of #1 (the blue dash curve) are only varied around $-10$ to $-30\%$, whereas for #2—#3, their data are decreased gradually in the range of 0–100 nM. This coincides with the morphological discrepancy of ssDNA on them. For #1, there are two ways for fixing ssDNA: (1) covalently anchored on the peripheral carboxyl groups of the lateral-orientated TCPP; (2) $\pi$-conjugated on TCPP’s macrocycle or residual rGO. Both of them can make ssDNA lie on LAPS, induce a large amount of negative charges on the insulator layer, and turn the SI interface state into the “accumulated state” (Figure 3A-2) quickly, even when C_DNA is as low as 10 pM. However, for #2—#3, the covalently anchored nucleotide strands tend to stand on LAPS, owing to the rigid macrocycle of erected TCPP (#2) and the short length of the tested ssDNA (#3). In this case, the interface resister at SI, which can be increased with increasing C_DNA, is the main issue to lower $V_{\text{out}}$ according to our previous work. As a consequence, the curves of #2—#3 are similar, and they gradually fall from $-5$ to $-60\%$ and from $-25$ to $-80\%$ (C_DNA = 10 pM to 100 nM).

2.5. Responding of DNAm-LAPS to Anti-5mC. Based on the optimized experimental conditions, three kinds of DNAm-LAPS (#1—#3) are examined in the reactions with anti-5mC, after they are functionalized by different DNAmi ($i = 0, 1, 2, 3, 4$) with similar concentrations (100 nM). When DNAmi ($i = 0, 1, 2, 3, 4$) is different, the DNAm-LAPS responding to the concentration of anti-5mC (C_anti-5mC) is shown in Figure 4.

First, there are negatively increased data of $\Delta V_{\text{out}}/V_{\text{out},0}$ in the curves of DNAmi ($i = 1, 2, 3$, and 4); $V_{\text{out},0}$ is the measured $V_{\text{out}}$ when C_anti-5mC is zero.). It is in agreement with the increased anti-5mC molecules captured by 5mC sites, and they can increase the interface impedance, depress the exhausted state (Figure 3A-1), and decrease $V_{\text{out}}$ consequently. In contrast, almost no C_anti-5mC-related LAPS responding could be found in the curves of DNAm0-LAPS (black curves in #1—#3). The comparison indicates the methylation information about the amount of 5mC on the anchored DNAmi can be transduced to the changed $V_{\text{out}}$.

Secondly, TCPP-decorated DNAm-LAPS (#1—#2) show a wider responding range for C_anti-5mC than the DNAm-LAPS without TCPP (#3) that demonstrates once more, more DNA sequences are anchored on TCPP-decorated LAPS. Besides, it is also found in Figure 4, when C_anti-5mC < 1 $\mu$g/mL, the data of $\Delta V_{\text{out}}/V_{\text{out},0}$ vs C_anti-5mC cannot be ordered regularly from $i = 0$ to 4, although the trends of these curves are downward. It is surmised to be caused by the randomly captured anti-5mC molecules. So, it is necessary to provide an enough methylation indicator (anti-5mC) for DNAm detection, and its concentration is optimized as 1 $\mu$g/mL.

2.6. Methylation Determination. Methylation determination by three kinds of DNAm-LAPS (#1, #2 and #3) is
compared in Figure S5A, after they are incubated with similar anti-5mC solutions (1 μg/mL). Generally, the data of $-\Delta V_{\text{out}}/V_{\text{out,0}}$ grow up when the amount of 5mC is varied from 0 to 4, which is coincident with the increased anti-5mC molecules captured by 5mC sites. It is found that the DNAm changing ratios of TCPP-decorated DNAm-LAPS are higher than that of the blank one, due to the increased ssDNA by TCPP’s peripheral carboxyl groups, which is evidenced by the XPS analyses.

Moreover, it is also found that TCPP directly decorated DNAm-LAPS (#2, red line) exhibits higher methylation sensitivity than #1 (blue). Why rGO coating in #1 (blue) cannot further increase DNAm-LAPS sensitivity? The reasons may be as follows: (1) the amount of the anchored ssDNA sequences on #1 is lower than that on #2, which has been evidenced and explained in the section of XPS characterization; (2) the configuration of ssDNA on #1 is lying, and the attachment of ssDNA on the substrate may overlap the 5mC sites. Therefore, the directly decorated TCPP method is demonstrated to be more effective than the methods of no TCPP (#3) and with an extra rGO layer (#1).

Finally, the selectivity of TCPP directly decorated LAPS (#2) to the 5mC site is examined, as presented in Figure S5B. It is found that the corresponding data ($-\Delta V_{\text{out}}/V_{\text{out,0}}$) for the mixture of DNAm1 and DNAm0 are almost halved. The results are in agreement with the anchored sequences and the amount of 5mC on each of them, that is: (1) for the analyte of only DNAm1 ($i = 1, 2, 3, 4$), the growing columns agree with the increased 5mC sites on the anchored sequences; (2) for the analyte of the mixture “DNAm1 + DNAm0” with V/V ratio of 1:1, and there are amino groups on all of them, the chances of the methylated and unmethylated nucleic acids should be 50–50, then, when the other conditions are same, the captured anti-5mC molecules should be halved. However, because of the complexity and randomness in the binding process, the decreased data ($-\Delta V_{\text{out}}/V_{\text{out,0}}$) are not exactly half, as shown in Figure S5B. Nevertheless, the reduced columns’ height by mixing DNAm0 can also be observed. Moreover, their shifting trend from left to right is in a similar way to “DNAm1”, that means, the methylated sites on the anchored sequences can be selectively detected.

3. CONCLUSIONS

In this work, for the particular DNAm sensing application that is aimed to evaluate the amount of 5mC sites along the concerned nucleotide sequence, an enhanced sensing interface is proposed and discussed on the versatile sensing platform LAPS, according to the TCPP’s distinct structural property, the highly π-conjugated macrocycle, as well as the functional groups on its external substituents. It is found that by the LAPS electronic measurements and the XPS evidence, the initial idea of using TCPP decoration to enlarge the number of the anchored strands is accomplished; then under the action of the immunoonidicator (anti-5mC), the 5mC sites on them can be recognized and the increased sensitivity for the amount of methylated sites is achieved by the TCPP-decorated LAPS (#1→#2). However, the idea of using rGO in #1 fails to further enhance the amount of the anchored ssDNA and the DNAm sensitivity, due to the planar oriented molecules of the π-conjugated TCPP on rGO. In conclusion, the enhanced DNAm sensing interface is achieved by the erectly coated TCPP, and the succeeded DNAm-LAPS by this method may be nominated as an efficient device for epigenetic assay.

4. EXPERIMENTAL SECTION

4.1. Materials. The tested ssDNA sequences are synthesized by Sangon Biotech Co. Ltd. (China). They have the same sequence, which is 5′-TTG CCG GCC GTG CGT CTC TTG AAC TTC-3′ and is modified by −NH2 at the 5′ terminal, but differ from the amount of 5mC, as illustrated in Scheme 1A with the name of DNAmi ($i = 0, 1, 2, 3, 4$). TCPP (95%) is from Hangzhou Expo biotech Co., Ltd., China. The main chemicals are (1) APTES and GA from Sigma-Aldrich, (2) anti-5mC from Aviva Systems Biology, China, (3) N,N-dimethylformamide (DMF) from J&K Scientific, (4) 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide (EDC) and N-hydroxysulfosuccinimide (NHS) from Thermo. Others like H2SO4 (98.0%), hydrogen peroxide (H2O2, 30%), acetone, etc. are of analytical grade from Tianjin Chemical Reagent wholesale company (China). Deionized water (DIW) was used during the experiments.

The chemicals in experiments are prepared as follows: (1) APTES solution is in DIW with the volume ratio (v/v) of 1:10 and adjusted to pH = 7.4. (2) The concentration of GA dilution solution is 2.5% (v/v) in DIW. (3) The phosphate buffer solution (PBS) is adjusted at about 7.4. (4) The concentrations of TCPP solutions are 0.001, 0.01, 0.1, 0.25, 0.5, and 1 mM in DMF. (5) The concentrations of DNAmi solutions are 10 mM, 100 mM, 1 mM, 10 mM, and 100 mM in PBS. (6) The concentrations of anti-5mC solutions are 0.01, 0.05, 0.1, 0.5, and 1 μg/mL in PBS.

4.2. Synthesis of rGO. The production for rGO is based on the modified Hummers method and the hydrazine hydrate reduction method.30 The procedures are outlined as follows: (1) GO powder is prepared by the modified Hummers method; (2) thoroughly rinsed and dried GO flakes are suspended in DIW solution (3 mg/mL), then subjected to bath sonication and centrifugation to obtain “GO suspension”; (3) hydrazine hydrate and ammonia are added to the mixture and adjusted to pH = 10; (4) the water bath at 95 °C is executed in the mixture of (3) for 1 h.

4.3. DNAm Sensing Interface on LAPS. The surface of LAPS is activated by three methods as sketched in Scheme 1B. The main steps executed on the LAPS surface are outlined here: (1) all of the LAPS chips are incubated with APTES solution (50 °C, 2 h) and GA solution (at room temperature, 1 h), successively; (2) the first part of APTES and GA-treated LAPS (named #1) is coated by rGO at room temperature, incubated with TCPP solutions (37 °C, 3 h), and after being treated by EDC (2 mM) and NHS (5 mM), they are incubated with different DNAmi solutions (37 °C, 4 h); (3) the second part of LAPS (named #2) is directly treated by solutions of TCPP, EDC, NHS, and DNAmi at the same conditions in step (2); (4) the others are named #3, and they are directly treated by DNAmi solutions at the same conditions in step (2).

4.4. Experimental Procedures. The data $V_{\text{out}}$ of blank LAPS (#3) are first measured in PBS, at different $V_{\text{REF}}$ (−0.8 to 0.8 V) and the controlled illumination modulation frequency (1 kHz). Second, TCPP-decorated devices (#1–#2) that are incubated with TCPP solutions are examined in similar PBS, respectively, at the controlled illumination background and optimized $V_{\text{REF}}$. Third, after the treatments of EDC (2 mM) and NHS (5 mM) on #1→#2, $V_{\text{out}}$ data of three kinds of LAPS that are incubated with DNAmi solutions (0–100 nM) are measured at similar conditions to those in the second step. Fourth, DNAmi-LAPS ($i = 0, 1, 2, 3, 4$) are
prepared after incubating three kinds of LAPS (#1, #2, #3) in the optimized DNAmi solutions, respectively. After they have reacted with anti-SmC solutions (0–1 μg/mL) and rinsed with PBS, the data of $V_{out}$ are measured by the same method as in the third step.

4.5. Methylation Detection. DNAmi-LAPS ($i = 0, 1, 2, 3, 4$) based on three kinds of sensing surfaces (#1, #2, and #3) are prepared by incubating them with DNAmi solutions (the optimized concentration). After being incubated with anti-SmC solution with the optimized concentration and rinsed with PBS, the methylation detections are conducted on each of them and their $V_{out}$ data are measured by the same method as in the fourth step in Experimental Procedures. Finally, the selectivity experiments are executed by mixing unmethylated nucleic acid (DNAm0, 100 nM) into the methylated ones DNAmi ($i = 1, 2, 3, 4$) with a similar concentration and $V/V$ ratio of 1:1 and incubating them onto the optimized DNAmi-LAPS. The methylation detections are conducted following the method in the fourth step of Experimental Procedures.

4.6. Apparatus. SEM examinations are executed by SEM S-3500N (Hitachi, Japan). XPS measurements are conducted by AXIS Ultra DLD (Kratos Analytical Ltd., U.K.). Raman spectra are measured by RTS-HiR-AM (Titian Electro-Optics Co. LTD). The home-made LAPS detection system that has been used previously is utilized to perform the electronic measurements, in which the reference voltage ($V_{REF}$) and the illumination’s modulation frequency are controlled as optimized values.

ASSOCIATED CONTENT

Supporting Information
The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.9b00980.

Analyses of XPS characterization, including atomic concentrations, and the peak fitting of N 1s and C 1s core spectra (PDF)

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