

Aptamer probe indirect functionalization enables the reusable detection of cancer protein biomarkers in graphene field-effect transistor

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ABSTRACT: Recently, aptameric graphene field-effect transistors (GFET) have emerged in the field of *in situ* detection of cancer protein biomarkers, thanks to their advantages of fast response, high specificity, and high sensitivity. However, factors such as non-usability have limited their productization process. Here, we propose a novel aptamer indirect functionalization method that enables the reconfigurable modification for aptamer probes on graphene surface, thereby achieving reusable aptameric-GFET detection for protein biomarkers with the calibrated signals. Furthermore, a novel crosslinker, tetrakis(4-carboxyphenyl)porphyrin (TCPP), is exploited for the signal amplification. Results show that our proposed reusable sensor can respond to carcinoembryonic antigen (CEA), a representative cancer protein biomarker, concentration change in 6–8 min with an estimated limit of detection (LOD) of 1.09 ng/mL in 1× phosphate buffered saline (PBS), and the signal is increased by more than half compared to the conventional method. Dissociation occurs at the target temperature and signal degradation is less than 5% after 3 cycles and 20% after 14 days of storage and 10 cycles. Our work provides a novel strategy for measuring CEA quantitatively and sensitively with rapid response, which holds great potential for a wide range of medical application and leads to powerful biomarkers detection devices in healthcare.

KEYWORDS: graphene field-effect transistor, repeatability, aptamer, protein detection, graphene field-effect transistors (GFET) device

1 Introduction

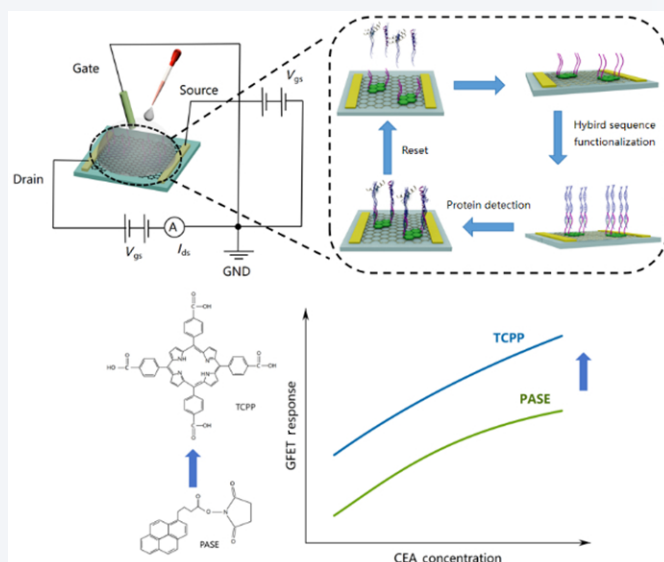
Cancer, such as colorectal, gastric, and lung cancer, has become one of the most prime illness for human death after cardiovascular disease [1]. Due to the fact that early diagnoses and intervention

can enhance survival rates of patients [2, 3], developing detection strategy with high sensitive and accuracy is of great significance for illness prediction and cohort screening. Tumor biomarkers detection [4–7] in human body fluid has become one of the most frequently used diagnose method for its environment convenience with rapid response and assist in cancer diagnoses, distinguishing benign and malignant conditions. Various immunoassay techniques on cancer biomarker detection have been studied over the past decades and enzyme-linked immunosorbent assay (ELISA) has become the most universal method. However, its accuracy in low-level detection and complex operation procedure limit its promotion.

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Stability in electrode materials cannot justify the advantages of the electroluminescence method, such as simple operations and automated analysis [8–12]. Despite the above-mentioned method, photoelectrochemical immunosensor [11], imaging nanoimmunosensor [13], and MXene-based [14] devices are known for their detection convenience but still face challenges such as detection repeatability and low-concentration measurements that limit their practical application.

In recent years, graphene field-effect transistors (GFET) sensing platform has caught the researchers' attraction due to its abundant carrier mobility as well as ease of preparation and surface modification [15–18]. Aptamers are the single strand DNA with strong binding affinity with protein and the advantage of artificial synthetic, and have emerged as promising biorecognition element for high specificity, stability, and reproducibility compared to conventional enzymes and antibodies. Previous studies have validated that aptameric-GFET sensing platform has been widely and extensively applied in biomarker detection and diagnoses among various diseases, including but not limited to ions [19], bacteria [20, 21], reactive species [22–29], and incorporate with clustered regularly interspaced short palindromic repeats (CRISPR) technology [30]. However, in practical scenarios, this sensing platform is still facing limitations on sensitivity and repeatability issues. The non-reconfiguration of the sensor can be attributed to not only the irreversible binding reaction between aptamer and protein but also the conventional immobilization method which utilizes single linker molecule, leading to poor sensitivity performance which is not capable for practical scenario. Previous work has achieved reusable detection on glucose through the reversible binding interaction between PBA and glucose [31], but reusable manner on aptamer sequence has been rarely studied.

In this paper, we propose a novel aptamer indirect functionalization method that enables the reconfigurable modification for aptamer probes on graphene surface, thereby achieving reusable aptameric-GFET detection for protein biomarkers with the calibrated signals (Fig. 1(b)). By providing multiple functionalization sites, we increased the density of aptamer probes and enhanced sensor sensitivity to meet clinical requirements for protein tumor biomarkers detection. This intermediate method exploit the binding characteristic between the pair of the ssDNA sequence and annealing phenomenon with the increment of temperature to achieve the goal of surface probe reconfiguration. The goal of surface probe reconstruction was achieved due to the annealing and spin separation of ssDNA intermediate chains and nucleic acid aptamers with the increment of temperature (Fig. 1(a)). Furthermore, a tetrakis(4-carboxyphenyl)porphyrin (TCPP) linker was applied to provide abundant functionalization sites for biorecognition element. By activating the 4 carboxyl groups with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC)/N-hydroxysuccinimide (NHS), Schiff-base reaction can be conducted between the amino group of the aptamer and the graphene-immobilized linker. To examine the effectiveness of our approach, carcinoembryonic antigen (CEA), a highly meaningful protein biomarker for clinical diagnoses, was used as a representative. Experimental result shows that this aptameric-GFET sensor, which uses our indirect functionalization approach and multiple sites linkers, can respond to CEA concentration change in 6–8 min with an estimated limit of detection (LOD) of 1.09 ng/mL in 1× phosphate buffered saline (PBS), and signal response was enhanced approximately 1.5 times compare to the conventional linker 1-pyrenebutanoic acid succinimidyl ester (PASE) (Fig. 1(c)). Dissociation occurred at the

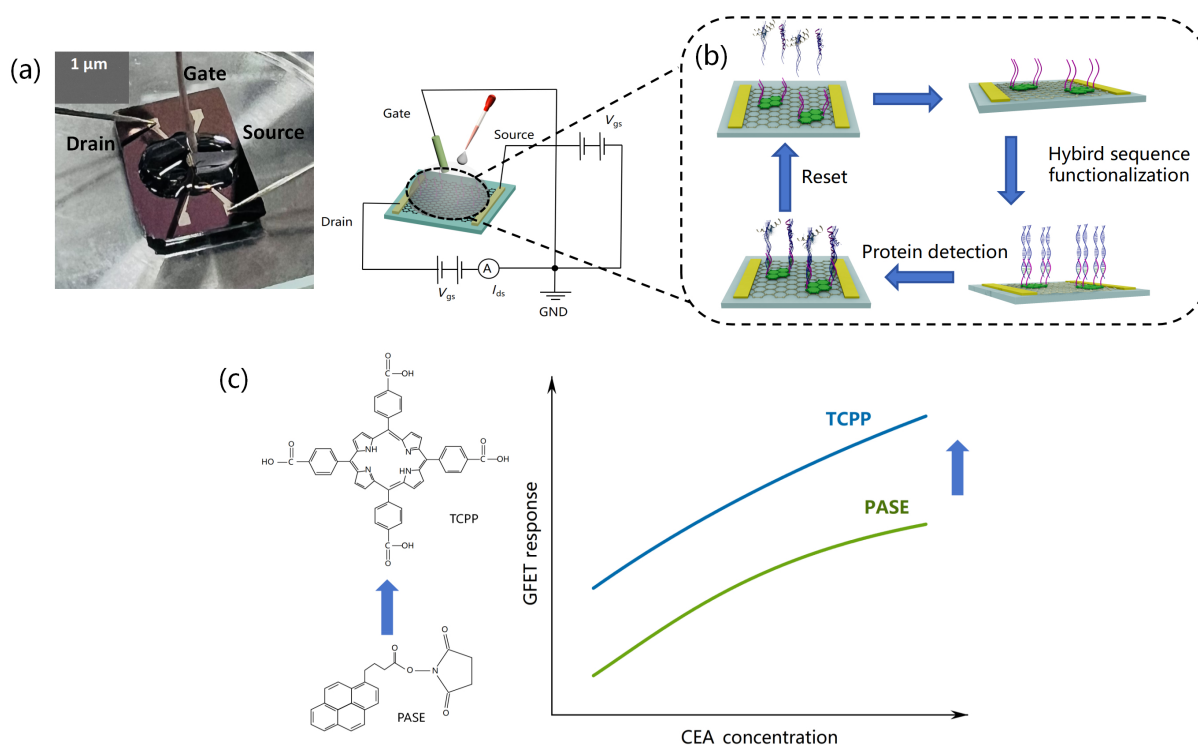


Figure 1 Graphene aptameric nanosensor for CEA detection in a reusable manner. (a) Schematic of the sensor configured as a solution-gated GFET and the liquid handling for nanosensor with the scanning electron micrograph of the single layer graphene in the nanosensor. (b) Sensor fabrication process and detection cycle. (c) A TCPP linker, with 4 carboxyl groups, was used for signal amplification and result indicates that response was enhanced 1.5 times compared to the conventional PASE linker.

target temperature and signal degradation was lower to 95% after 3 cycle and 80% after 14 days storage and 10 cycle, ensuring that our device was capable for practical application.

2 Materials and methods

2.1 Materials and reagents

Chemical vapor deposition graphene was grown on an annealed copper (Cu) foil (99.99%, 25 μm). PASE and PBS were purchased from Sigma-Aldrich (St. Louis, MO, USA). TCPP, 2-methoxyethanol, EDC hydrochloride (EDC-HCl), NHS, and polyethylene glycol (PEG) were purchased from Shanghai Yansu Technology Co., Ltd. (Shanghai, China). Human CEA protein, bovine serum, CEA-specific aptamer (sequence 5'-NH₂-ATA CCA GCT TAT TCA ATT-3'), intermediate aptamer (sequence sequence 5'-NH₂-GGG GGG GGT TGG GTT-3'), and hybrid aptamer (sequence 5'-CCC CCA ACC CAA CCC ATA CCA GCT TAT TCA ATT-3') were synthesized and purified by Sangong Biotech (Shanghai, China).

2.2 Fabrication and surface functionalization

Initially, the graphene thin slice was float on the 4% APS solution for copper etching and the graphene was transferred to the deionized (DI) water for washing. Subsequently, the graphene was transferred onto 285 nm SiO₂/Si substrate and kept in a vacuum tube for 12 h. Details can be found in the Electronic Supplementary Material (ESM).

2.2.1 PASE functionalization

The graphene channel was then biochemically functionalized with PASE for its noncovalent π - π interaction. The chip was immersed in the 5 mM solution of PASE in 4 °C environment.

2.2.2 TCPP/EDC/NHS functionalization

Similar to the PASE principle, the chip was incubated with TCPP (50 μM) in 2-methoxyethanol for 2 h at room temperature before rinsed with 1 $\times$ PBS. The carboxyl groups on TCPP were activated via EDC/NHS for 1 h with a mixture of 200 mM of EDC-HCl and 50 mM NHS in DI water.

2.2.3 Probe functionalization

Regardless of the category of linker, the chip was exposed to 40 μL probe (10 μM) overnight at 4 °C in a humidity environment.

2.3 Measurement method

All electrical measurements were performed using two digital source meters (Keithley2450 and Tektronix) with the aid of a commercial software Kickstart.

2.4 Serum solution preparation

Serum solution referred to the 50 $\times$ diluted serum. 500 μL of serum solution was prepared with 10 μL of bovine serum bulk diluted with 490 μL of 1 $\times$ PBS.

2.5 PEG modification procedure

After the probe immobilization, the chip was incubated in 1 μM amino group modified PEG solution for 1 h and followed with ethanolamine (ETH) to form the passivation layer and block the unreacted crosslinker.

3 Results and discussion

3.1 Characterization for surface functionalization and CEA detection

This nanosensor is configured as a graphene-based FET in a liquid-gate format and a multiple linker element was used to enhance the detection sensitivity. Direct binding between the probe and target alters the charge distribution on the graphene-liquid interface, thereby generating equivalent free electrons in the drain source current (I_{ds}) and cause disturbance to the charge carrier in graphene, resulting in detectable changes in the V_{Dirac} , which can be used to determine the CEA level in given solution.

Characterization for TCPP and aptamer functionalization was performed with Raman spectra, X-ray photoelectron spectroscopy (XPS) image, and the shift of the V_{Dirac} . Related details and experiment conducted can be found in the ESM. Porphyrin-based linkers such as TCPP have been shown unsurpassed performance compare to conventional pyrene-based linkers like PASE, with up to three-fold increase in sensor response for exosome detection [32, 33]. Previous studies have primarily utilized TCPP in conjunction with antibodies for biosensing applications [33–36]. Herein, this is the first report of TCPP being integrated with nucleic acid aptamers for cancer protein biomarker detection, marking a significant signal that leverages aptamers' high affinity and stability to overcome antibody limitations, thereby enhancing detection specificity, reproducibility, and reusability in clinical diagnostic scenarios. By activating the 4 carboxyl groups with EDC/NHS, this novel anchor provides 4 times larger functionalization sites than the conventional linker, PASE, for biorecognition element (Fig. 1(c)). Distinct from the conventional functionalization method, this linker requires activation with EDC/NHS before the intermediate sequence immobilization.

Characterization for TCPP and indirect sequence functionalization was performed with Raman spectra, XPS image, and the variation of the V_{Dirac} . The G-band splitting (1590–1621 cm^{-1}) observed in the Raman spectra and the decrease for the ratio between the intensity of 2D and G further confirmed the interaction for graphene and the porphyrin groups on TCPP (Fig. 2(a)). Clear non-overlapping part was discovered in the XPS image, and especially the peak at 133 eV was found after the probe immobilization (Fig. 2(b)). Coupling of TCPP to graphene surface was found to make an increase shift in V_{Dirac} for 130 mV (Fig. 2(c)). And after the aptamer immobilization, a decrease shift of 50 mV in V_{Dirac} was also observed, while the value was 2.5 times higher than the previous reported result (Fig. 2(d)). Thus, it can be concluded that the probe has been successfully immobilized on the graphene surface and its unit density has been increased after using this TCPP linker.

In general, CEA, a broad biomarker among tumor patients [37–39], maintains a comparative low level in human biofluid, for 3–5 ng/mL. Exceeding 10 ng/mL warrants further investigation for potential underlying conditions and higher than 20 ng/mL would consider as tumor patients for elevated concentrations in human serum are associated with an increased risk of cancer [39–41]. Thus, CEA solutions with increasing concentrations varying from 1, 2, 4, 8, 16, and 20 were sequentially introduced to the sensing surface of this nano sensor and measuring capabilities were subsequently tested in PBS buffer (Fig. 2(e) and 2(f)). The binding of CEA to biorecognition element was allowed to reach equilibrium

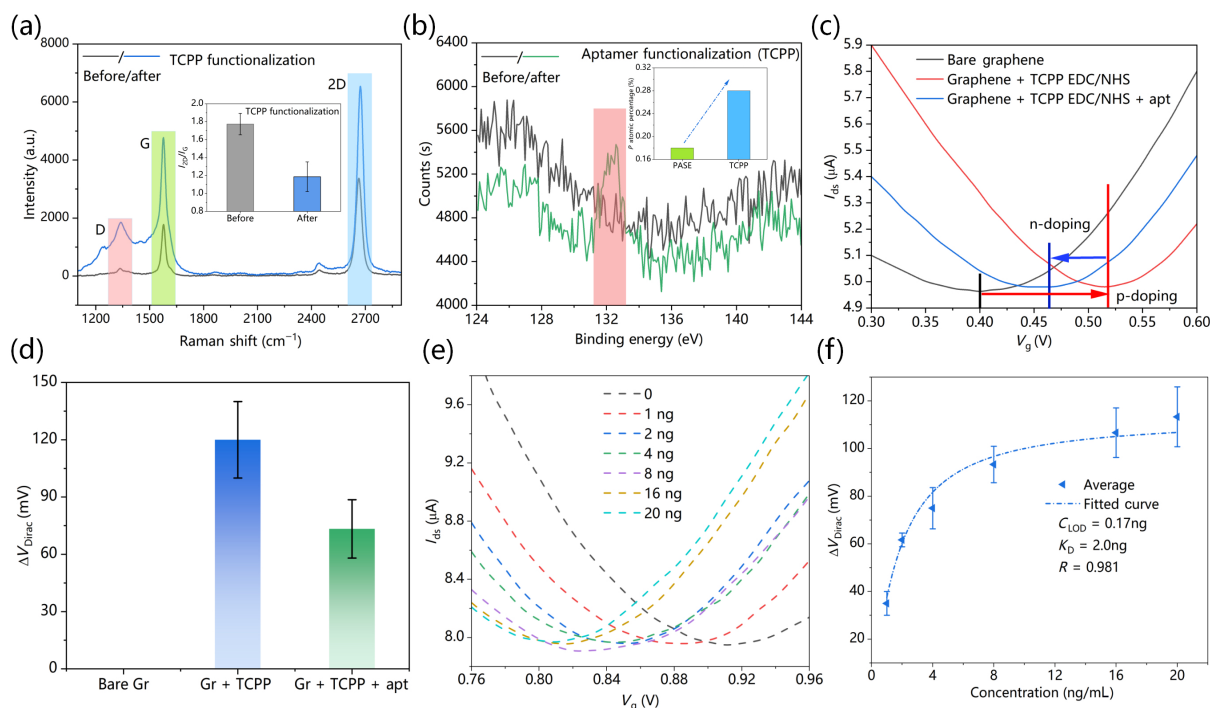


Figure 2 characterization of biofunctionalization and CEA detection performance of this sensor. (a) Distinct peaks and peak intensity characteristics in the Raman spectra of graphene change a lot before and after TCPP functionalization. (b) XPS patterns for P element before and after the aptamer functionalization and obvious non-overlapping part of the peak can be observed after functionalization. Apparent rise in N element percentage was observed after probe immobilization. ((c) and (d)) Variation in V_{Dirac} for pristine graphene, after TCPP functionalization and aptamer functionalization (apt = aptamer). (e) Transfer characteristics measured when the aptamer-functionalized graphene was exposed to CEA solutions (concentration: 1–20 ng). The transfer characteristic curve shifted to the left as a result of the increase of the CEA concentration. (f) Voltage shift (ΔV_{Dirac}) as a function of the insulin concentration. The dashed line is a least-squares fit to the Hill–Langmuir equation, yielding an equilibrium dissociation constant (K_D) of 2 ng and LOD for 0.17 ng/mL.

before replenishment of the sample solution. The measured V_{Dirac} decreased correspondingly with the increment of CEA level, indicating that the binding between CEA to the aptamer generated n-type doping to the graphene surface.

3.2 Simulation on intermediate method

To realize the reusable and signal-calibratable capability for this nanosensor, an intermediate method was proposed, which exploited the annealing characterization of the ssDNA sequence. By designing the ssDNA with a specific melting temperature, the sensor could be regenerated through controlled heating and cooling cycles, allowing the dissociation and re-annealing of the probe-target complex. This approach not only enabled reuse of the nanosensor but also provided a consistent signal baseline for calibration, enhancing measurement accuracy and reliability across multiple detection cycles under optimized buffer conditions. Melting temperature (T_m) and annealing ratio are the two key factors among various index of the sequence during design part. T_m is defined as the temperature when 50% of the double-strand DNA was annealed to ssDNA, a key factor indicating the activity and stability. As a biorecognition element, ssDNA should avoid generating secondary or hairpin structure, indicating that T_m should be below 65 °C and higher than 55 °C. Besides, the annealing temperature should be kept under a certain value because the stability of π – π absorption has been experimentally demonstrated by temperature-programmed desorption studies, where aromatic molecules desorb from the graphene surface upon heating [42–44]. To measure the dissociation effect, when the unpair ratio reaches 90%, it will be deemed that annealing has taken place properly.

To realize the best ssDNA sequence, T_m value, pair number, sequence order, and pair ratio are taken into consideration. Initially, pair number and T_m value were concerned because they are the prerequisite factors to determine T_m and pair ratio. At the preliminary stage, the ssDNA sequence exhibits a uniform base composition, with all bases being guanine (G). Considering the aptamer pair number, figures for 12, 13, 14, 15, 18, and 20 were selected to calculate the T_m and simulate the pair proportion. Result shows that T_m and the binding proportion rise whereas the annealing proportion drops with the increase of pair number (Fig. 3(a)). A sharp drop in T_m was observed with the rise of T_{number} , indicating that pair number and T figure should be kept in a reasonable range (Fig. 3(b)). Thereafter, the order of the sequence was taken into account for optimizing the performance. Numerous simulations were conducted for each sequence to find out the best solution with well binding and annealing index. Pair number for 12 and 13 has well performance on binding with its corresponding sequence, whereas majority of the them own a high annealing temperature, resulting in an unstable situation for linker. The simulation results for 12 SE1 confirm the aforementioned trend, namely a high binding affinity at 35 °C, accompanied by comparatively poor annealing performance. Conversely, pair numbers for 18 and 20 exhibit an opposite trend on binding and annealing proportion with high annealing performance and low binding ratio. All the chosen sequence have a low binding trend, surpass 40% and lower than 50%, which is unsuitable for this indirect functionalization method. Simulation outcomes demonstrate this phenomenon for long sequence, low binding affinity, and high annealing performance. Subsequently, because short sequences are lack of annealing ratio

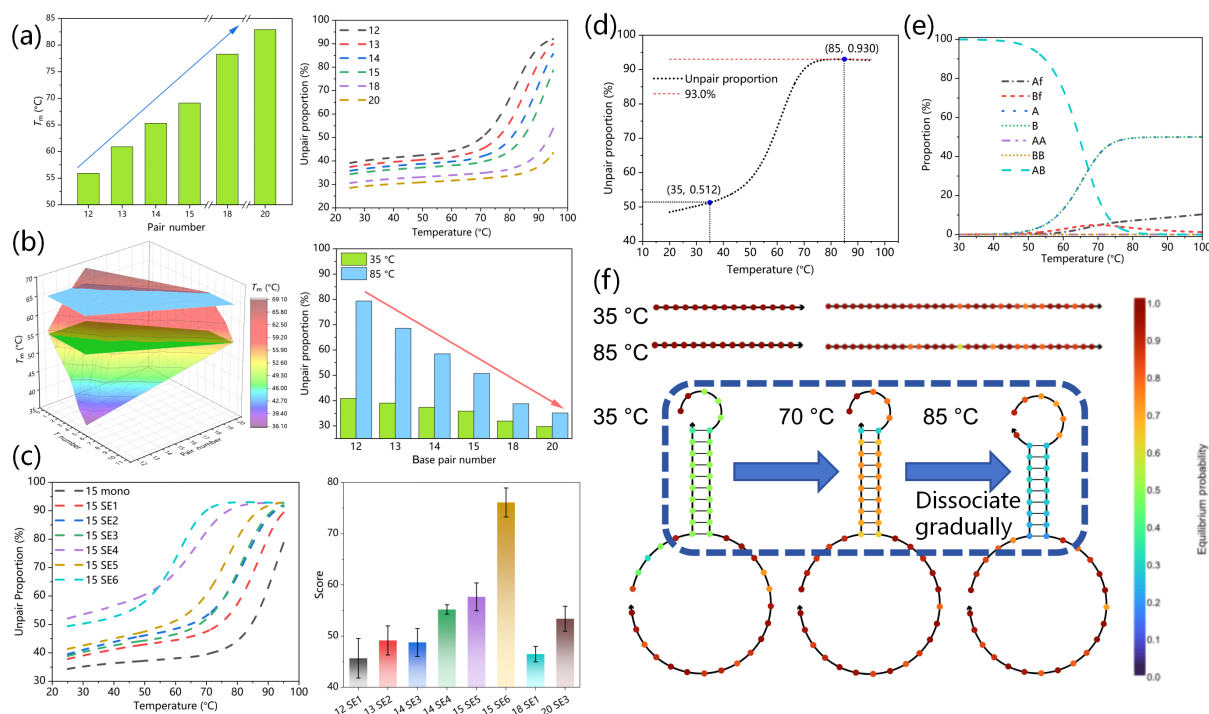


Figure 3 Simulation of annealing and binding performance of the chosen sequence. (a) Melting temperature and annealing curve vary with the increment of pair number. (b) Annealing proportion dropping sharply with the rise of pair number. (c) A evaluation system was established for the best choice of each group to assess the annealing and binding index of each sequence. Scores for annealing and binding index were calculated and the aggregate result indicates that 15 SE6 was the best choice after 3 ablation study. Simulation for 15 SE6 on self intersection, unpair proportion, and element ratio. (d) Unpair proportion curve. (e) The component proportion varies with increasing temperature from 35 to 85 °C. All the simulation data were collected from Mfold web server. (f) Self intersection test and temperature influence on equilibrium probability for intermediate sequence and hybrid sequence (35, 70, and 85 °C, respectively). Simulation conducted with NUPACK.

and long sequence owns comparative low binding proportion, numbers for 14 and 15 with different order were further investigated. With the increment of T number, group 14 exhibits a trend for binding proportion decreases in a comparatively low value and annealing ratio rises dramatically from 60% to nearly 90%. Group 15 exhibits the same “rising” trend with group 14 and all the sequences with rearrangement order show a better pair and untie performance compared to the mono pattern ones. Among all the selected sequence, 15 SE6 shows a general pair ratio and excellent annealing performance for simulation result, indicating that this one has a broad “annealing field”, 90% annealing proportion at 70 °C, and kept in high untie ratio till the temperature rise to 95 °C, compared to other sequences. Untie at such a comparatively low temperature indicates that the linker, which is highly sensitive to the increasing temperature, can be kept in a stable situation and this sequence has been highly focused attributed to this unique character. Details can be found in the ESM.

Finally, two optimal solutions from groups 12–15 and 18–20 were selected for the final comparison. A evaluation system has been established to assess the annealing and binding performance of each sequence (Fig. 3(c)). Score calculation was divided into two parts, annealing and binding index. The annealing part can be divided into two core parts, annealing field and proportion. Annealing field refers to the distance between 95 °C and the boundary temperature, which refers to the first temperature at which annealing proportion reaches 90%. Annealing and binding proportions refer to the pair ratios at 35 and 85 °C, respectively. All the results were calculated with a percentage system and added together with a certain weight. Since this paper focuses on the dissociation behavior of the ssDNA, the weight assigned to the

annealing part is greater than that assigned to the pairing part. The aggregate outcome indicates that 15 SE6 scored the highest after 3 ablation studies, demonstrating that it can be the best choice for the indirect functionalization ssDNA sequence. Details can be found in ESM.

Furthermore, self intersection test, unpair proportion, and element ratio simulation were conducted (Fig. 3(d)). This nanosensor exploits this best solution, 15 SE6, for indirect functionalization sequence and annealing temperature chooses 70 and 85 °C to achieve the detection repeatability. Annealing curve for 15 SE6 exhibits a general pair proportion for 51.2% at 35 °C and a long dissociation field with high untie ratio, ranging from 70 to 85 °C, and the annealing proportion is as high as 93% (Fig. 3(e)). Each element in the solution was simulated and the result validates the previous proposals. Besides, simulation for self intersection for intermediate and its corresponding sequence and equilibrium probability graph with the increment of temperature, indicating that the dissociation phenomenon occurred [45].

3.3 Experimental validation for the indirect functionalization method

This indirect method requires intermediate sequence functionalization at first and followed by a hybrid sequence added into the solution. The hybrid sequence refers to the direct linking between the corresponding sequence of the intermediate sequence

and the CEA probe sequence. Protein detection was conducted after the hybrid sequence was introduced into the solution and subjected to the annealing procedure, by adding a small drop of PBS buffer at the target temperature into the testing solution. Experimental results show that the measured Dirac curve shifted left correspondingly with the increment of CEA concentration and returns to the beginning stage after annealing, representing that the dissociation phenomenon occurs properly at both 70 and 85 °C (Fig. 4(a)). Experiment conducted with PASE can be found in the ESM.

Experimental results show that the transfer curve measured during the detection kept shifting left correspondingly after the rising concentration CEA solution was introduced into this sensor and returned to the beginning status when the temperature rose to 70 °C. Signal response was then calculated and apparent rise in response for each concentration was found compared to the conventional method with an estimated LOD for 1.09 ng/mL (Figs. 4(b) and 4(c)). This result suggested that the proposed TCPP linker is reasonable for CEA detection and the high response for this proposal can be attributed to the increase in the unit probe density, validating the rationality for this proposal.

A decrease in signal response and concentration range was observed compared to the conventional probe detection method. The reason for this problem can be attributed to two major factors: First, the annealing curve for this ideal sequence shows a pair ratio of approximately 50% in room temperature, indicating a gap for the unit density probe between this indirect method and the conventional ones. Second, previous work [19, 46] has validated that the shift of the Dirac point is attributed to the change in carrier density in graphene induced by the interaction between target and the compact secondary structure. Compared with conventional

probes, this hybrid probe is longer, meaning fewer target molecules are brought into the electrical double layer. Consequently, fewer electrons are generated for the same number of recognition events, ultimately leading to signal loss.

Subsequently, alpha-fetoprotein (AFP) was employed as a comparison protein due to its nature as a glycoprotein biomarker. Similar to CEA, AFP contains glycan moieties that influence its interfacial behavior and molecular recognition properties. Although AFP has a lower molecular weight and a reduced degree of glycosylation compared to CEA, it still shares key physicochemical characteristics of glycoproteins. Control experiments to examine the sensor specificity, using cardiac troponin I (cTnI), creatine kinase-MB (CKMB), interleukin-6 (IL-6), and AFP at 4 ng/mL, were conducted, respectively (Fig. 4(d)). Result shows that the sensor only shows normal response to target analyte CEA, whereas abnormal or negligible signal can be observed when other interference protein was introduced into the sensor. Moreover, the proposed biosensor could be extended for the analysis of other targets by changing the specific aptamer for recognition of different target analytes.

Furthermore, long-term stability detection and reproducibility of this sensor were further investigated after 14 days of storage and 10 cycles of aptamer immobilization reproduction. The chip was prepared at the first day and CEA measurement was conducted at the same time for reference after 3 cycles of aptamer immobilization reproduction was conducted at the same time for reference. Afterwards, the sensor was stored in 1× PBS at 4 °C for 14 days and subsequently experienced 10 cycles of signal reproduction before the final test. The experimental result shows that this device functions properly after multiple cycles of reproduction. The sensor signal for 6 ng CEA was around

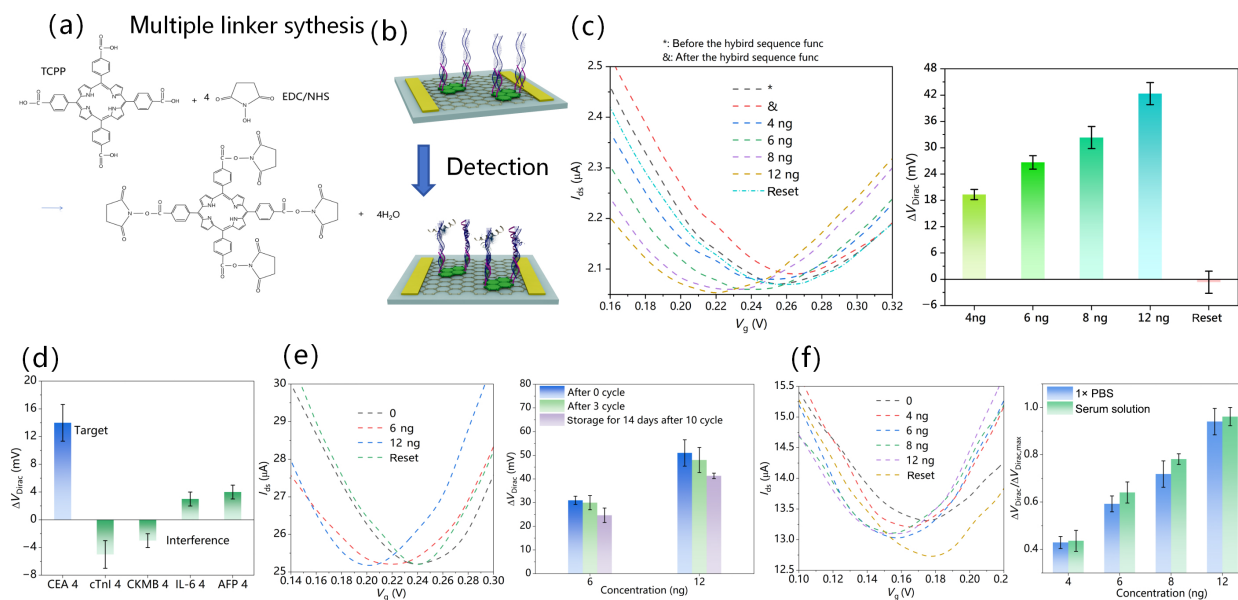


Figure 4 Experimental validation of the intermediate method. ((a) and (b)) Schematic illustration for TCPP activation and detection of the intermediate method. A hybrid sequence was introduced into the liquid after the intermediate sequence was immobilized on the surface of the graphene and the binding was dissociated with the rise of temperature. (c) After annealing at 70 °C, transfer characteristics were measured while sensor exposed 4, 6, 8, and 12 ng/mL CEA. (d) Control experiments were performed using cTnI, CKMB, IL-6, and AFP at 4 ng/mL for comparison. The sensor only responds to the target analyte CEA whereas abnormal or negligible response signal can be observed while exposure to other interference protein. (e) The transfer characteristic curves and response signals of the sensor toward 6 and 12 ng/mL CEA before and after multiple cycles of reset. (f) Detection of CEA in serum solution incorporating with the ssDNA intermediate functionalization method. The left figure shows the transfer curve measured with the sensor exposure to 4–12 ng/mL CEA solutions and one cycle reset (annealing occurred at 70 °C). Right figure shows the sensor signal response to various concentrations of CEA solutions in 1× PBS and serum solution. To eradicate device-to-device non-uniformity, the signal was normalized, and the result indicates a negligible difference from the detection results obtained in 1× PBS.

29–31 mV and dropped to around 24–28 mV after 14 days of storage and 10 cycles of reproduction. The same trend can be observed for 12 ng CEA detection, with a decrease from 46–55 to 40–45 mV in the signal. Compared to the previous research result [47], the signal degradation range is acceptable. The reason for this signal degradation probably can be attributed to the susceptible of the π - π stacking between PASE and graphene, which would have influence on the aptamer immobilization density (Fig. 4(e)).

As for practical scenario, the performance of this reusable sensor was tested with PEG with molecular weight of 5kDa (PEG5k) modified sensing platform (Fig. 4(f)). Similar to the previous procedure, after probe immobilization, the chip was incubated with 1 μ M amino-PEG5k to form the isolating layer and block the unreacted crosslinker. Previous work has validated that PEG can suppress non-specific adsorption effectively while performing detection of biomarkers in biofluid using graphene biosensor [48]. The detection was carried out in serum solution. The results demonstrate that the sensor functions well in biofluid media, and the transfer curve shifts correspondingly when a higher-concentration solution is introduced into the testing zone. Annealing occurred at the target temperature and a signal degeneration compared to the detection in 1 $\times$ PBS was observed during the test, which can be attributed to the formation of isolating layer. In addition, PEG has been exploited in various surroundings for suppressing non-specific adsorption, and future effort can focus on target biofluid separation [49].

4 Conclusions

Herein, we proposed an aptameric-GFET nanosensor in a reusable manner, incorporated with an amplification method, to achieve ultra-sensitive, rapid, and specific detection of a broad cancer-related biomarker, CEA, enabling a detection range of 4 to 12 ng/mL with an estimated LOD of 1.09 ng/mL. Our work exploits a novel crosslinker incorporated with an aptamer and an indirect functionalization method to achieve signal reconfiguration, thereby providing a reliable strategy for quantitative and ultra-sensitive detection of CEA in a reusable manner, which holds great potential to be extended to a wide range of applications for detecting other disease markers. GFET biosensors and their detection platforms are expected to achieve nucleic acid amplification-free detection and miniaturized portable devices, with great potential for applications in point-of-care technology.

Electronic Supplementary Material: Supplementary material (fabrication procedure, cycle validation, and evaluation system demonstration) is available in the online version of this article at <https://doi.org/10.26599/NR.2026.94908804>.

Data availability

All data needed to support the conclusions in the paper are presented in the manuscript and the Electronic Supplementary Material. Additional data related to this paper may be requested from the corresponding author upon request.

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Declaration of competing interest

The other contributing authors report no conflict of interests in this work.

Author contribution statement

M. L. R.: Conceptualization, data curation, formal analysis, investigation, methodology, validation, visualization, writing – original draft, writing – review & editing. J. H. L.: Formal analysis, investigation. M. L. Q.: Investigation, methodology. X. H. X.: Methodology, resources. T. T. Z.: Formal analysis, investigation. Q. T. Z.: Funding acquisition, resources. J. Z. D.: Funding acquisition, resources. Z. H.: Conceptualization, methodology, project administration, supervision, writing – review & editing, funding acquisition, resources. All the authors have approved the final manuscript.

Use of AI statement

None.

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