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ARTICLE

Universal DNA detection realized by peptide based carbon nanotube biosensors

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Although DNA recognition has been achieved in numerous biosensors by various sensing probes, the utilization of bio-interaction between DNA and biomolecules was seldom reported in the universal DNA detection. Peptide as a natural molecule owns the unique property to grasp universal DNAs while it has excellent selectivity for DNAs after being functionalized with specific groups. In this work, we report a peptide based carbon nanotube (CNT) thin-film-transistor (TFT) biosensor, which can achieve sensitive sequence-independent DNA detection. In the presence of DNA, a significant increase of ΔI_{on} could be observed within 5 minutes, which was analyzed to the electrostatic adsorption between the opposite Zeta potential of DNA and peptide. With the gradual increase of concentration, the ΔI_{on} signals agrees with the Hill-Langmuir model ($R^2 = 0.98$), indicating a negatively cooperative interaction between peptide and DNA (the Hill coefficient $n < 1$). Compared with the former reported universal DNA bio-detector and nanodrop (a spectrometer from Thermo scientific™), this unique peptide based CNT-DNA sensor demonstrated a broader sensing range from nearly 1.6×10^{-4} to $5 \mu\text{mol/L}$ and much lower detection limit of approximately $0.88 \mu\text{g/L}$. In the quantification of cDNA from T47D cancer cells, this unique peptide based CNT sensor could achieve efficient cDNA detection. To the best of our knowledge, this is the first report on the utilization of peptide as sensing element in the design of CNT based DNA biosensors, which enables highly efficient universal DNA detection.

Introduction

The detection and quantification of nucleic acids are always a desire for clinical diagnosis,¹ forensic investigation² and biological research.³ For instance, in the screening of early-stage cancer, a simple, fast and efficient DNA biosensor is urgently demanded.⁴ To achieve that, various DNA detective biosensors have been developed with high accuracy and efficiency in the past decade, such as: colorimetric^{5,6} fluorescent^{7,8} chemiluminescent^{9,10} and electronic techniques^{11,12}.

Among these methods, the electronic technique could achieve simple and real-time readout of DNA signals, as the transducing elements (such as: nanoparticles,¹³⁻¹⁵ organic conductive materials¹⁶⁻¹⁸ and carbon-based materials¹⁹⁻²¹) could amplify the micro response to a readable signal in seconds. Benefiting from the miniaturization and portability, the

electronic based DNA detectors showed promising potentials for further commercial applications.^{22, 23}

Among the sensor devices, Graphene based transistor was one of the well-developed biosensing platforms with the similar physicochemical properties with CNT-TFT.²⁴⁻²⁶ For instance, Mohanty et al. utilized a modified graphene based transistor to achieve the DNA-hybridization detection.²⁷ However, as graphene has no bandgap, it needs extra chemical and geometric manipulation to control its bandgap, which increases the difficulty of transistor manufacture. Comparatively, the transistor manufacture with the purified carbon nanotubes is relatively simple. Besides, many other FET based DNA sensors could also achieve efficient DNA sensing and detection.²⁸⁻³³

The carbon nanotube thin film transistor (CNT-TFT) was another well-developed biosensing platforms and could capture tiny signals for bio-analysis.³⁴⁻³⁵ The target molecules were recognized and captured on the active CNT channel, resulting in an amplification of tiny response for further detection.³⁶⁻³⁷ For the detection and quantification of DNA molecules, various CNT based biosensors have been developed. For instance, Star et al. immobilized synthetic oligonucleotides to specifically recognize the H63D sequence of HFE gene.³⁸ Maria et al. utilized the polymer NO6 for the immobilization of probe hybridization and achieved efficient DNA detection.²⁰ These works could detect specific DNAs based on sequencing.

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However, a CNT based sequence-independent DNA detector is also desired in some applications, like the quantification of total amount of DNA (regardless of sequences) in genomic samples, which was seldom reported. Until recently, Dai et al utilized the instantaneously electrostatic attraction (IEA) between $\text{Ru}(\text{NH}_3)_6^{3+}$ and DNA molecules to design an immobilization free DNA detector.³⁷ The introduction of $\text{Ru}(\text{NH}_3)_6^{3+}$ directly captured the free DNA, resulting in an instantaneous change of electrical signals with further amplification. This unique universal DNA sensing detector displayed its capability for detecting genomic DNAs. In fact, there are various DNA interactive biomolecules that sense DNA and moderate their biofunction in cells. This bio-interaction has the potential to be utilized for the sensing and detection of DNA molecules. However, most of DNA bind biomolecules are difficult to be prepared and may be inactive during the further modification with CNTs³⁹⁻⁴⁰.

Recently, we have found a peptide (sequence with Fmoc-RRMEHRMEWC), which could have special bio-interaction with tiny nucleic acids and brought a significant decrease in Zeta potential.⁴¹⁻⁴² Taking this exciting decrease of Zeta potential into consideration, we envisioned that this change of surface potential (which was resulted from the interaction between peptide and nucleic acids) could be greatly amplified and further detected as a sequence-independent DNA biosensor. Based on that, herein, we report a CNT based DNA biosensor with biological molecules as the receptor to achieve the efficient detection and quantification of nucleic acids. Compared with the former reported universal DNA detection strategy and nanodrop (a spectrometer from Thermo scientific™), this peptide based DNA sensor displays a broader sensing range approximately from nearly 1.6×10^{-4} to $5 \mu\text{mol/L}$ and a lower detection limit of $0.88 \mu\text{g/L}$.³⁷ To the best of our knowledge, it is the first reported CNT based DNA biosensor with peptide as sensing element.

In order to incorporate the peptide to the CNT detector, an appropriate connection between DNA responsive peptide and carbon nanotubes should be guaranteed. As shown in **Fig. 1**, N-(1-pyrene) maleimide was utilized to modify the CNT with active maleimide groups by the hydrophobic interaction between 1-pyrene butyric acid and side wall of CNT. Then, through the bio-orthogonal reaction between -SH and maleimide, the connections between CNTs and decapeptide were realized with the coupling of maleimides on the sidewall surfaces. Subsequently, a significant change of ΔI_{on} could be observed after the incubation of DNA solution for 5 min, indicating this peptide based CNTs could be utilized as DNA biosensor. And the ΔI_{on} signal followed with the Hill-Langmuir equation with the additional concentration of DNA solution ($R^2 = 0.98$).

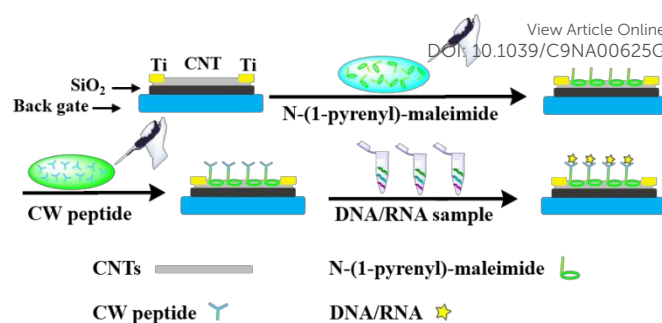


Fig. 1 Schematic presentation of the novel peptide based CNT biosensor.

Furthermore, this unique peptide based DNA sensor could achieve efficient cDNA quantification from T47D cells. These results demonstrated this unique peptide based CNT bio-detector could be utilized for the detection and quantification of DNA.

2. Experimental

2.1 Reagents and materials

The 99% semiconducting purity nanotube powder was purchased from Sigma-Aldrich Company. The 1,2-dichloroethane $\geq 99.9\%$ (GC) solvent was purchased from Aladdin Company. N-(1-pyrene) maleimide and other reactive reagent were purchased from Beijing Bailingwei company. DNA and RNA were purchased from the Shanghai Genepharm company (The sequence of DNA: GAAATGTGGCAACTCGTC; the sequence of RNA: AAGGAAAGCUAGAAGAAAATT).

2.2 Preparation of DNA sensing peptide

The preparation of CW peptides (Fmoc-RRMEHRMEWC) was conducted based on the standard Fmoc-based solid phase peptide synthesis (SPPS). The protocol of the SPPS is summarized by literatures and showed below.⁴³ Notably, for the further bio-orthogonal conjugation of -SH and maleimide, cysteine(Cys) was introduced into the original peptide sequence to become CW peptide (Fmoc-RRMEHRMEWC). MBHA resin (loading capacity: 0.37 mmol/g) were swelled with NMP for 30 min. Then the 50% (vol/vol) morpholine in DMF was used to deprotect the -Fmoc group from MBHA resin for 30 min $\times$ 2. After washing with DCM and DMF for 3 times, amino acid coupling mixture (the Fmoc-protected amino acids (5.0 equiv), HCTU (4.9 equiv), DIPEA (10.0 equiv)), which is dissolved in DMF was added for coupling for 2.5 h, followed by washing with DCM and DMF for 3 times. And after that, added 50% (vol/vol) morpholine in DMF to deprotect the Fmoc group for the later amino acid coupling.

After coupled nine amino acids on MBHA resins, the decapeptides were already synthesized to wait for further purification. The resins were treated with a mixture of TFA/H₂O/TIS (95/2.5/2.5) for 2 h and dried by nitrogen blowing. After precipitated with Hexane/Et₂O (1:1 in volume) at 4°C, the mixture was further dissolved by 42% (vol/vol) acetonitrile/



water and purified by HPLC with UV detection at 220 nm or 280 nm and later identified by LC-MS.

2.3 Preparation of CNT TFT for the DNA biosensor

Preparation of carbon nanotube solution: 0.5 mg single walled carbon nanotube (SWCNT) powders with 99% semiconducting purity (an average tube length of 1 μm , Nano Integris Inc.) and the same weight poly m-phenylenevinylene-co-2,5-diethoxy-p-phenylenevinylene (PmPV) dispersant were dissolved into 25 mL 1,2-dichloroethane (DCE) solvent. Meanwhile, ultrasonic treatment was applied to accelerate CNT dispersion. The whole ultrasonication process was conducted with the process temperature strictly guaranteed under 30°C, which assured the dispersion quality. After ultrasonication for 18 hours, a well-dispersed CNT solution was obtained with a concentration of 0.02 mg/mL, which could keep stable and uniform state for several months without CNT aggregation found.

Device preparation: The back-gate CNT TFTs were assumed to ensure the maximum detection area. The high-conductivity p-type silicon substrate (resistivity: 0.001-0.005 $\Omega\cdot\text{cm}$) acts as common back-gate, and 50 nm silicon dioxide growing on silicon by thermal oxidation acts as gate dielectric layer. The silicon dioxide layer was treated by 60 W oxygen plasma for 10 min to make the surface more hydrophilic and promote the contacts between CNTs and the substrate.⁴⁴ Then 200 μL CNT solution (0.02 mg/mL) was spin-coated onto the substrate at the speed of 3000 rpm for 40 seconds to form a homogeneous random-network CNT thin film on the substrate. After that, the CNT film was baked at 400°C for 1 hour to remove impurities and dispersants. The source and drain electrodes were formed by sputter-depositing and patterning a 200 nm thick Titanium(Ti) layer. Secondly, the photoresist was coated and patterned to protect the active region, and the CNTs outside the active region were etched away in an atmosphere of 100 W oxygen plasma for 10 minutes. Then, the photoresist was removed by acetone and alcohol. In the end, the devices were annealed for 1 hour in vacuum at 300°C, which helped to repair the defects in the devices and burned out the impurities introduced during the process.⁴⁵

2.4 Modification and functionization of the CNT active channel

The device was incubated with 6mM N-(1-pyrenyl)-maleimide solution and gently rotated for 4 hours in the shaker at room temperature. After that, DMF and ddH₂O were utilized to wash device several times to remove the residual N-(1-pyrenyl)-maleimide and DMF. The chips were then incubated in ddH₂O containing selective decapeptide and rotated for another 16 hours in the shaker. The entire shaking process was maintained at 25°C and a speed of 60 rev /min, ensuring sufficient reaction and effective retention of CNTs. In order to compare the differences before and after CNT functionization, peptide connection, and DNA capture, the electrical characteristics of the original sensor were measured after

every step. As shown in **Fig. S1-S2**, the amount of CNT film was unwounded after the modification and washing steps. After the CNT functionalization was completed, the devices were washed three times with ddH₂O, and blown dry by N₂, which could remove the unfixed decapeptide and eliminate the effect of water molecules on current.⁴⁶ Finally, the electrical signals are measured as the initial characteristics of the sensor by Agilent B1500A Semiconductor Device Analyzer.

2.5 Electrical measurement of nucleic acid

Nucleic acids (DNA or RNA) were dissolved in ddH₂O or DEPC water and diluted to different gradient concentrations. A little drop of nucleic acid was added to the devices, and incubated for 4 minutes, then washed off and dried with deionized water and nitrogen for three times. Finally, the responding electrical signals were detected by Agilent B1500A Semiconductor Device Analyzer. For the detection of standard curves, all the electrical signals were conducted three times. The error bars represent the standard error of mean square root values from two independent experiments.

2.6 cDNA quantification from T47D cells

T47D cells were cultured in 1640 medium with 10% serum and 1% PS. After washed with PBS for three times, TRIzol reagent (Invitrogen) was used to split T47D cells for 5 min at room temperature. After mixed with 100 μL chloroform and rest for 5 min, the sample was centrifuged at 4°C with 12000 rpm for 15 min. Then carefully collected the supernatant liquid to a clear tube without Rnase and mixed with isopropanol of the same volume. After incubated on the ice for 10 min, the sample was centrifuged at 4°C with 12000 rpm for 15 min. Then, discarded the supernatant and washed the sediment with 75% ethyl alcohol for two times. After that, discarded the supernatant and put it rested on ice for 10 min to remove the remianing ethanol. Finally, 20 μL DEPC water was added to dissolve the sediment (RNA). After that, took 2 μL extracted RNA from T47D cells, and added 1 μL Oligo-dT(Invitrogen). After incubating the mixture at 65°C for 5 min, put the mixture on the ice immediately.⁴⁷⁻⁴⁸ mixed the solution with Rnase Inhibitor, dNTP, reverse transcriptase, and its buffer, and use PCR reaction for reverse transcription amplification. Finally, the PCR product cDNA from T47D cells were collected and stored at -20°C, waiting for dilution and further detection by the peptide based CNT biosensor.

3. Results and discussion

3.1 Design of peptide based DNA biosensor

As shown in **Fig. 2a**, Ti electrodes were designed at both ends with CNT channel positioned at the middle. Besides, scanning electron microscope (SEM) image of the middle active channel region clearly shows that the CNTs formed uniform random network with a density about 10 tubes/ μm (**Fig. 2b**). As shown



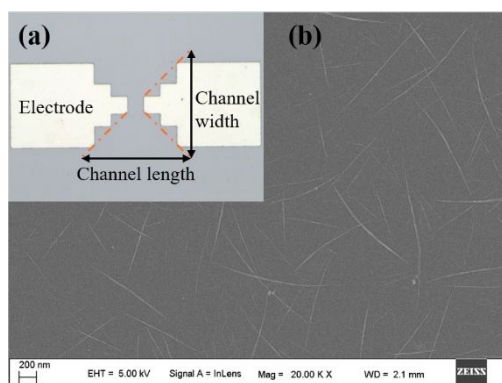


Fig. 2 (a) the optical microscope image of CNTTFT with Ti electrodes and CNT.; (b) the SEM image of the uniform CNT network.

in **Fig. S1a** and **Fig. S1a**, amounts of carbon-nanotubes were placed on the CNT with relative uniform morphology, which enable the further functionalization.

Then N-(1-pyrene)maleimide incubation was applied on the CNT transistor for the further connection of DNA sensitive peptide. And this functionalization process could be monitored by the change of the transfer characteristic curves (I_{ds} - V_{gs}). As shown in **Fig. 3a**, the initial signal (black line) exhibited representative p-type characteristics at drain voltage V_{ds} of -0.1 V with width and length of both 50 μm . After the incubation of N-(1-pyrene)maleimide, the value of I_{ds} (red line) showed a significant decrease at $V_{gs}=-10$ V compared with initial curve (black line), indicating the formation of π -stacking interaction of N-(1-pyrene)maleimide and the sidewalls of CNTs.⁴⁹ Subsequently, the incubation of the decapeptide (Fmoc-RRMEHRMEW) further resulted in a clear reduction of the I_{ds} signal, indicating the formation of connection between peptide and CNTs. These results demonstrated that the decapeptide could be successfully modified on the CNTs through the linkage of -SH and maleimide. And these changes on electrical characteristics verified the effectiveness of the design, which displayed a promising potential for further DNA detection.

3.2 Response to the DNA molecule

To further investigate the electronic response of decapeptide modified CNTs, 10 μM DNA solution was incubated for 5 min (**Fig. S3**). Excitingly, an unambiguous increase of I_{ds} could be observed in I_{ds} - V_{gs} curve (blue line), as shown in **Fig. 3a**. Compared with various electrical parameters extracted from multiple experiments, I_{on} was identified with the most significant changes, considering its high sensitivity and stability simultaneously. Thus, ΔI_{on} was selected to evaluate the response of each stage in every former step. As shown in **Fig. 3b**, after connected with N-(1-pyrene)maleimide and decapeptide, I_{on} of the CNT TFT markedly decreased by approximately 40% and 60%, respectively. While, the DNA incubation resulted in an increase of I_{on} about 30%. These changes of electrical characteristics demonstrated the functionalization of CNTs could be successfully achieved by the incubation of

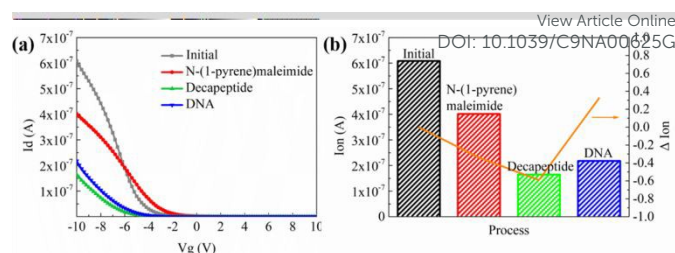


Fig. 3 (a) I_{ds} - V_{gs} characteristics at $V_{ds}=-0.1$ V with $W,L=50$ μm and (b) their significant changes of I_{on} at subsequent stages of functionalization and detection. The characteristics of initial stage (black) and incubation stages with N-(1-pyrene)maleimide (red), decapeptide (green), and DNA (blue) were measured by Agilent B1500A Semiconductor Device Analyzer. I_{on} was defined as the drain current at $V_{gs}=-10$ V and $\Delta I_{on} = (I_{on}' - I_{on}^0)/I_{on}^0$ represents the relative responses of I_{on} compared with prior stages.

N-(1-pyrene)maleimide and DNA response peptide. And the decapeptide modified CNTs could further serve as DNA biosensor.

Two control biosensors with/without peptide functionalization were also fabricated to further verify that the increase of ΔI_{on} was contributed by the peptide based responsive elements. As shown in **Fig. 4**, No obvious increase of I_{on} signal could be observed in the CNT TFT sample without-peptide, indicating that the free DNA could not be detected if no sensing element of peptide connected on CNTs. While, with the presence of the peptide, a significant improvement of I_{on} signal could be observed after the incubation of DNA solution. These results demonstrate that the sensitive response of I_{on} is contributed to the existence of the DNA responsive peptide, and the sensor platform with the peptide shows obvious response when detecting DNAs.

Besides, different length of single DNA were detected by this peptide-CNT biosensor. And their ΔI_{on} signals displayed the similar and consistent electronic response, indicating the sensor really can detect different length of DNA with stable ΔI_{on} signal responses (**Fig. S4**). Furthermore, DNA with different GC contents and double strand DNA were also investigated by this peptide based CNT biosensor. As shown in **Fig. S5** and **Fig. S6**, both the DNA with high GC content and double strand DNA could be detected and result in a similar ΔI_{on} response. These results demonstrated that this peptide based CNT biosensor could achieve universal DNA detection with stable ΔI_{on} response.

3.3 Analysis for detection range, limitation and detection mechanism

Furthermore, the detections of DNA solutions with different concentrations from 5×10^{-5} to 5 $\mu\text{mol/L}$ were carried out to explore the detection range of this novel peptide based biosensor. As shown in **Fig. 5**, the responses of I_{on} signal were gradually improved with the increase of DNA concentration. The fitted curve shows a Hill-Langmuir relationship with the degree of fitting correlation coefficient (R^2) reaching 0.98. These results further demonstrates that this decapeptide



modified CNT TFTs can be utilized for the quantification of DNA, potentially.

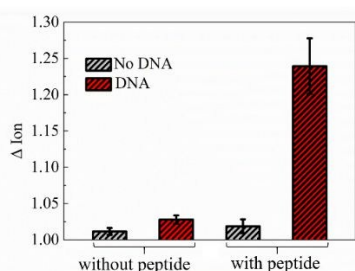


Fig. 4 Comparison of responses to DNA for biosensors with and without peptide. The current increase only occurred in the sensors with peptide and the response signal of this biosensor in blank control buffer was around 0.018. The error bars represent the standard error of mean square root values from two independent experiments.

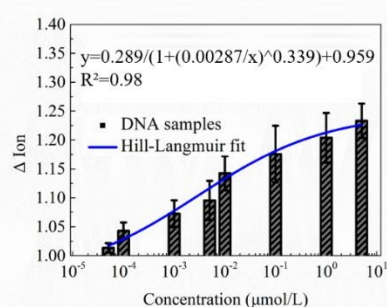


Fig. 5 The ΔI_{on} signal responses to DNA concentrations from 5×10^{-5} to $5 \mu\text{mol/L}$. The relationship between ΔI_{on} and DNA concentration could be fitted with the Hill-Langmuir model with the fitting correlation coefficient R^2 approximately to 0.98. The error bars represent the standard error of mean square root values from two independent experiments

According to the electrostatic adsorption mechanism, the curve was fitted with the Hill-Langmuir model, which was used to describe the degree of cooperativity of the ligand binding to the receptor.⁵⁰

$$\Delta I_{on} = A / (1 + (K_A / c)^n) + Z$$

Where A is the response signal at saturation when all decapeptide are occupied, Z is an overall response offset in blank test, K_A is known as the dissociation constant describing the concentration at which half receptors are occupied, and n is the Hill coefficient describing cooperativity of binding. The fitting curve shown in the figure shows a maximum response $A = 0.28942 \pm 0.01678$, response offset $Z = 0.9585 \pm 0.00617$, dissociation constant $K_A = 0.00287 \pm 0.00085$, Hill coefficient $n = 0.33861 \pm 0.02975$, and the degree of fitting correlation coefficient $R^2 = 0.97694$. The value of $n = 0.33861 \pm 0.02975$ indicates the negatively cooperative interaction of decapeptide and DNA. Compared with the former reported universal DNA detection strategy, this peptide based DNA sensor displayed a broader sensing range approximately from nearly 1.6×10^{-4} to $5 \mu\text{mol/L}$ and a lower detection limit of $0.88 \mu\text{g/L}$.³⁷

Besides, benefiting from the sensitive response to nucleic acids, this unique peptide based detector could also be utilized

for the quantification of RNAs. As shown in **Fig. S7**, this peptide based biosensor displayed significant I_{on} response in the presence of RNA solutions with different concentrations from 1 – $0.0001 \mu\text{mol/L}$. With the addition of RNA concentration, the I_{on} response was also fitted with Hill-Langmuir relationship with the R^2 about 0.99. These results demonstrated that this unique peptide based CNTs biosensor could be used for the universal nucleic acid detection.

Furthermore, Zeta potential assay was conducted to investigate the potential mechanism of this unique ΔI_{on} signal increase in peptide based CNT biosensor. As shown in **Fig. 6a**, in the absence of DNA, the Zeta potential of peptide-DNA complex displayed a significant decrease. Notably, the forming complex had the same negative ZP with DNA, indicating that once the DNA molecule incorporated with decapeptide, the complex may display a reduced absorption towards DNA. These results were consistent with the Hill-Langmuir model, especially with the negatively cooperative interaction situation ($n < 1$).

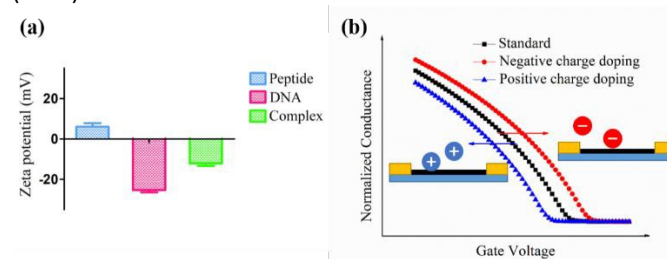


Fig. 6 (a) Zeta potential values of CW peptide, DNA, and their complex. The electrostatic adsorption of peptide and DNA occurred because of their opposite ZP types and forming complex had the same type ZP with DNA, which hindered the continuous adsorption with DNA. (b) Illustration of current change sensitive to charge doping. The negative charge doping induced a right shift of characteristic curve, resulting in a current enhance, and vice versa.

As for the peptide based CNT biosensor, the DNA molecule with negative surface potential could serve as the centralized carrier.⁵¹ Thus, attributed to the selectivity of peptide, DNA molecule could be captured, further leading to a significant change in Zeta potential, which could be amplified and detected with the ΔI_{on} signals. The following simulation of charges effect on p-type transistor supported this hypothesis. As shown in **Fig. 6b**, the increase of negative charges results in the reduction of current; while the enhancement of positive charges led to the increase of current, which is consistent with the experimental observations.

3.4 cDNA quantification from T47D cells

As this unique peptide based DNA biosensor could achieve sequence-independent DNA detection, we further applied this novel peptide based CNT detector to the cDNA quantification of T47D cells. As shown in **Fig. 7**, the detected concentration of cDNA from T47D cells was $7.32 \text{ ng}/\mu\text{L}$ by peptide based CNT, which was consistent with the results by nanodrop of 10.36



ng/ μ l. These results demonstrated that this novel peptide based DNA biosensor could also be utilized for the quantification of

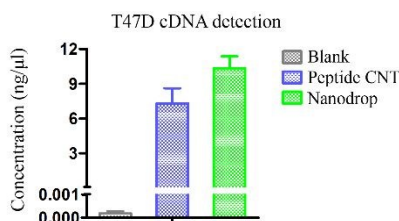


Fig. 7 Detection of cDNA from T47D cells by peptide based CNT biosensor, with ddH₂O as blank and nanodrop as control. The concentration of cDNA detected by peptide CNT was 7.32 ng/ μ l. The concentration of cDNA detected by nanodrop was 10.36 ng/ μ l. (The concentration of cDNA was quantified by Hill-Langmuir equation: $y = 0.289 / (1 + 0.01586/x)^{0.339 + 0.959}$, where the unit of concentration was converted to ng/ μ l.) The error bars represent the standard error of mean square root values from two independent experiments.

cDNA from T47D cancer cell lines, regardless of sequence. Furthermore, as our peptide based CNT biosensor displayed a broader sensing range and low detection limit to 8.8×10^{-4} ng/ μ l, it could be potentially utilized for tracing the amount of DNA in the future.

Conclusions

Although numerous DNA detection strategies have been developed with various sensing elements for DNA capture, the utilization of biomolecule for the universal sequence-independent DNA quantification was seldom reported. In this work, we have realized a universal DNA detection platform by incorporating the DNA sensitive CW peptide into the CNT TFT platform to amplify and monitor the sensitive electronic signals. In the presence of DNA, a significant increase of ΔI_{on} could be observed in the peptide based CNT bio-detector. With the gradual increase of the DNA concentration, the ΔI_{on} signal followed with the Hill-Langmuir relationship with the R^2 about 0.989 ($n < 1$). Compared with the former reported sequence-independent DNA detection strategy and nanodrop, the sensing range of this peptide based biosensor was more than 12-time broader (from nearly 1.6×10^{-4} to 5 μ mol/L) and the detection limit is 4300-time lower to only 0.88 μ g/L.³⁷ To our best knowledge, it is the first reported CNT TFT based DNA biosensor with peptide as sensing element to realize universal detection. We believe, with the wider sensing range and better detection limit, the proposed sensor could greatly save the amount of subject nucleic acid sample and be applied for detecting the DNA isolated from PB (peripheral blood) or tissues in the future.

Conflicts of interest

There are no conflicts to declare.

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Note

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