Magnetic Graphene Field-Effect Transistor Biosensor for Single-Strand DNA Detection

Jinjin Sun¹,², Xiaohui Xie¹,², Ke Xie¹,², Shicai Xu³, Shouzhen Jiang¹,², Junfeng Ren¹,², Yuefeng Zhao¹,², Huaqiang Xu¹,², Jingjing Wang¹,²* and Weiwei Yue¹,²*

Abstract
Herein, a magnetic graphene field-effect transistor biosensor was prepared through the transfer of a chemical vapor deposition graphene film onto a glass substrate to produce a sensing film and conductive channel. By fixing 1-pyrenebutanoic acid succinimidyl ester onto graphene film as an anchor, a probe aptamer was immobilized on the graphene film in order to capture magnetically labeled complementary single-stranded DNA. Our experiments showed that, within a periodic magnetic field, the biosensor impedance exhibited a periodic oscillation, the amplitude of which was correlated to the complementary DNA concentration. Based on this principle, the magnetic graphene field-effect transistor was utilized to detect single-stranded DNA with detection limit of 1 pM. The results were rationalized using a model wherein the magnetic force causes the DNA strand to bend, thereby resulting in magnetic nanobeads/DNA modulation of the double conductive layer of graphene transistors. Furthermore, since a periodic magnetic field could be introduced to produce a periodic impedance changes of MGFETs, sampling integration could be used to improve the signal-to-noise ratio efficiently by increasing the number of periods of the external magnetic field. Therefore, a novel biosensor for DNA detection with high sensitivity has been presented in this work. Based on the detection principle, this system may also be a potential tool for detecting other bio-molecules, cells, etc.

Keywords: Magnetic, Graphene, Field-effect transistor, Biosensor, Magnetic nanobeads, DNA

Introduction
The detection of DNA is of great significance for the study of molecular biology and the diagnosis of genetic diseases [1–3]. To date, various biosensors for DNA detection have been developed, including fluorescent biosensors [4, 5], electrochemical biosensors [6–9], and field-effect transistor (FET) biosensors [10–13], with the latter having attracted widespread attention due to their high sensitivity and specificity. Kaisti et al. [12] developed a FET biosensor to detect unlabeled single-stranded DNA using peptide nucleic acid DNA hybridization. Kim et al. [13] fabricated a FET-type DNA charge sensor based on standard complementary metal oxide semiconductor technology.

Due to its high specific surface area, high electrical conductivity, and excellent electron mobility, graphene has been heralded an ideal material for the fabrication of FET biosensors [14–16]. Cai et al. [15] developed a graphene FET (GFET) biosensor for ultrasensitive detection of DNA via peptide nucleic acid DNA hybridization. Our group has also proposed a multi-channel GFET biosensor to determine the binding kinetics and affinity of DNA hybridization and single-base mismatched [16].

In a conventional GFET, an external gate electrode electric field generates a double conductive layer at the interface between the graphene film and the solution electrolyte [17–19]. Based on a captive model of GFETs [16], the gate electrode charges and discharges the double conductive layer through the electrolyte, thereby modulating the GFET conductivity. Therefore, the conductivity of a GFET is related to the intensity of the external electric field and the ion concentration in electrolyte.

During the research, it was found that the research on the sensitivity of GFETs has reached the fM level. For example, Ping et al. [20] and Zheng et al. [21] have reported conventional GFET biosensors with detection limit in fM level. However, the above literature achieves extremely high sensitivity by semiconductor analyzer...
A Raman microscopic system (SPEX-1403, SPEX) was used to characterize the quality of graphene as well as to verify the functionalization of MGFETs. A fluorescence photometer (LS55, PerkinElmer) was used to characterize the coupling of magnetic nanoparticles to cDNA. A lab-made data acquisition system was used to record the impedance of MGFETs in real time.

**Methods**

**Materials and Instrument**

A glass substrate with ITO electrodes was purchased from Hua Nan Xiang Cheng Ltd. (China). The probe aptamer, cDNA, and mismatched DNA were purchased from Sangon Biotech Inc. (Shanghai, China). The sequence of the probe aptamer was 5′-NH₂-TGG ACC CCC TCA TAA CGC CTC CTT TTC-FAM-3′, sequence of the complementary DNA was 5′-NH₂-GAA AAG GAG GCG TTA TGA GGG GGT CCA-3′, sequence of the completely mismatched DNA was 5′-NH₂-TCC CCT TCT TAT GGC CTG TTT TTC AAC-3′, and sequence of the single-base mismatched DNA was 5′-NH₂-GAA AAG GAG TCG TTA TGA GGG GGT CCA-3′. PBASE and dimethyl sulfoxide (DMSO) were obtained from Sigma-Aldrich (Shanghai, China). Magnetic nanobeads (MBs) modified with carboxyl groups (10 mg/mL) were obtained from Xianfeng Nano Material Technology Co., Ltd. (Nanjing, China). 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (2 mg/mL), N-hydroxysuccinimide (2 mg/mL), 200 μL of 1-pyrenebutanoic acid succinimidyl ester (PBASE), and sodium dodecyl sulfate phosphate-buffered saline (PBS, pH 7.4) were purchased from Sigma-Aldrich (Shanghai, China). Sodium dodecyl sulfate phosphate-buffered saline (PBS, P5368-10PAK; pH 7.4) were purchased from Sigma-Aldrich (Shanghai, China). Magnetic nanobeads (MBs) modified with carboxyl groups (10 mg/mL) were obtained from Xianfeng Nano Material Technology Co., Ltd. (Nanjing, China). Magnetic nanobeads/DNA (MB/cDNA) conjugates were washed three times with PBS and dispersed in PBS for future use.

**Fabrication of MGFETs**

The preparation of MGFETs is described in detail below. Firstly, a CVD graphene film was transferred onto a glass plate as the conductive channel between the two ITO electrodes (Fig. 1a), as described previously [18, 19]. Secondly, PBASE (10 mM) dissolved in DMSO was injected into the MGFETs for 12 h at room temperature and allowed to react completely with graphene through π–π stacking (Fig. 1b). The MGFETs were then washed successively with DMSO and PBS to remove any unreacted PBASE. Thirdly, 2 μM of the probe aptamer was introduced into the MGFETs and incubated with PBASE for 4 h at room temperature, allowing the probe aptamer to react sufficiently with PBASE (Fig. 1c). The MGFETs were then respectively washed with 0.2% SDS three times to remove any unbound probe aptamer.

**Results and Discussion**

**Characterization of MGFETs**

Graphene film produced by the CVD method was transferred onto a glass substrate as a conductive channel between two ITO electrodes (Fig. 1a). The transferred graphene film was characterized with Raman spectrum (Fig. 2). The appearance of the three characteristic peaks of the graphene demonstrated the successful transfer of the graphene film onto the glass substrate [24, 25]. The intensity ratio between the 2D band and the G band (I₂D/I,G) indicated that the transferred graphene was a multilayer film [26]. Further, the intensity ratio between the D band and the G band (I_D/I_G) was small, indicating a very low defect density.
Due to the lack of functional groups, the aptamer chains were difficult to modify on the CVD graphene film. Therefore, based on its aromatic pyrenyl group, PBASE was modified on the graphene films via π–π stacking as a linker. On the other end of PBASE, the succinimide portion of PBASE could be coupled to the 5′-NH$_2$-labeled probe aptamer based on the N-hydroxysuccinimide (NHS) crosslinking reaction (Fig. 1c). In order to assess the binding of the probe aptamer on graphene film, the 3′-end of the probe aptamer was labeled using the FAM fluorophore (sequence: 5′-NH$_2$-TGG ACC CCC TCA TAA CGC CTC CTT TTC-FAM-3′). Immediately following aptamer introduction, the fluorescence intensity was obviously enhanced, indicating its successful modification on the graphene surface (Fig. 3). Increasing the probe aptamer concentration led to an increase in fluorescence intensity, reaching a constant value, and therefore indicating probe aptamer saturation on the MGFETs, at approximately 2 μM. Therefore, subsequent experiments were performed at a probe aptamer concentration of 2 μM.

**Characterization of MB/cDNA**

The morphology of the MBs and MB/cDNA conjugates was characterized by transmission electron microscopy (TEM) (Fig. 4a, b). The particle size distribution of MBs showed an average particle size of approximately 7 nm (Fig. 4c). In order to ensure sensitivity and accuracy in the biosensing for cDNA, MBs should be excessive for

---

**Fig. 1** Functionalization and detection principle of the MGFETs. a Graphene film grown by chemical vapor deposition. b Functionalization of graphene by PBASE. c Immobilization of probe aptamer via PBASE. d Hybridization of the probe aptamer with cDNA. e Photograph of the detection device

**Fig. 2** Raman spectrum

**Fig. 3** Characterization of MGFETs modification by probe aptamer. Error bar represents the standard deviation of 5 independent analysis.
cDNA in order to capture cDNA completely. MBs at a concentration of 4 mg/mL were activated to ensure binding to the cDNA samples use herein. Through labeling of cDNA by FAM, the fluorescence intensity was exploited to characterize the coupling efficiency and optimize the cDNA concentration (Fig. 4d). Indeed, the fluorescence intensity of the supernatant decreased obviously following the introduction of MBs into the cDNA solutions, indicating that cDNA was captured and enriched by the MBs. The successful capturing of cDNA by MBs was confirmed by the observation that, at a cDNA concentration of 10 nM, the fluorescence intensity of the supernatant was equivalent to that of PBS, indicating that all the cDNA was captured and enriched by MBs (Fig. 4d).

Analysis of Magnetic Field Intensity
MB/cDNA conjugates were added into the MGFETs for 10 min to allow complete cDNA hybridization with the probe aptamer. Since the probe aptamer could not couple with MBs without the modified amino groups, the excess MBs could be removed through washing of the MGFETs three times with PBS. Therefore, only the MB/cDNA conjugates were left on the MGFETs (Fig. 1d). A permanent magnet was mounted onto a rotating motor to apply a periodic magnetic field to the MGFETs (Fig. 1e). A lab-made detection device was used to record the impedance fluctuation of the MGFETs.

Since impedance of MGFETs was modulated by a magnetic field as the back gate, the correlation between magnetic field intensity and impedance of MGFETs was investigated to optimize the magnetic field intensity parameters (Fig. 5). It is generally believed that the double conductive layer formed between the graphene and the electrolyte is modulated by the external electric field, thereby modulating the conductivity of GFETs [19, 27, 28]. In MGFETs, through the magnetic force between the MBs and the magnetic field, the distance between MB/cDNA conjugates and the graphene film was controlled mechanically, thereby modulating the double conductive layer of MGFETs [29, 30]. MGFET biosensors impedance varied with the increasing magnetic field intensity in three stages which could be explained through taking the MB/cDNA chain as an elastic thin rod [31]. The first stage occurred at a magnetic field intensity of less than 100 mT in this work. Based on the elastic thin rod model of DNA chains, because the magnetic field force is less than the radial support force of...
the DNA strand, the magnetic field force is difficult to cause the DNA strand to bend; therefore, the MGFETs is not sensitive to the magnetic field. In the second stage with the magnetic field strength from 100 to 200 mT, the magnetic field strength is sufficient to overcome the radial support force of the DNA elastic thin rod, resulting in a rapid bending of the MB/cDNA and then a sensitive response of the MGFETs to the magnetic field. Finally, in the third stage with magnetic field intensity above 220 mT, the bending of the DNA elastic rod reaches its limit; therefore, the MGFETs will not respond to the change of the magnetic field, resulting in a stable impedance of the MGFETs as shown in Fig. 5b.

Detection of cDNA

The changes in MGFET impedance with varying MB/cDNA conjugate concentrations were measured under a fixed magnetic field strength of 240 mT to determine the feasibility and sensitivity for cDNA detection.

The MGFET impedance at each cDNA concentration was recorded in real time (Fig. 6a). When a permanent magnet was loaded onto the back of the MGFETs, the impedance increased rapidly. Conversely, when a periodic magnetic field was applied, a periodic change in impedance was observed. Based on this impedance periodicity, a sample integration algorithm (SIA) was used to increase the signal-to-noise ratio of the MGFETs. Given the period
without applying magnetic field was $T_0$ and the period with applying magnetic field was $T_M$ (Fig. 6a), the SIA could be described with the following steps: (1) during $T_0$, all the data points, produced by noise, was normalized to zero, (2) the data points obtained during each $T_M$ period were sampled and averaged in order. After SIA processing over four cycles, the periodic impedance change in MGFETs was obtained as shown in Fig. 6b. In theory, the signal-to-noise ratio of the MGFETs could be effectively improved using sufficiently long sampling times.

The impedance changes in MGFETs had a positive correlation with the cDNA concentration (Fig. 6b). The correlation between the impedance change of MGFETs and concentration of cDNA was assessed (Fig. 7). The high sensitivity of the MGFET biosensors in this work is mainly based on the following two aspects: firstly, the mechanical movement of MB/cDNA conjugates could enhance the modulation effect on the double conductive layer compared to the case of DNA alone, and secondly, since a periodic magnetic field could be applied to produce a periodic impedance changes of MGFETs, based on the sampling integration principle, only the MGFET impedance with the magnetic field was sampled and integrated to reduce the noise. Therefore, the system signal-to-noise ratio could be greatly optimized by increasing the number of periods of the external magnetic field.

**Selectivity of the MGFETs**

The specificity of the MGFETs was evaluated by detecting two different target DNA sequences, including completely mismatched DNA chains and single-base mismatched DNA chains. Similar to the procedure described above, a completely mismatched DNA (sequence: 5′-NH$_2$-TCC CCT TCT TAT GGC CTG TTT TTC AAC-3′) and single-base mismatched DNA (sequence: 5′-NH$_2$-GAA AAG GAG TCG TTA TGA GGG GGT CCA-3′) were coupled to MBs respectively. The mismatched MB/DNA dissolved in PBS solution was added into the MGFET biosensors for 10 min to react with the aptamer sufficiently. The MGFETs was washed with PBS for three times to remove the mismatched DNA. For completely mismatched DNA chains, due to the conjugate of MB/DNA could not hybridize with aptamer, almost all the MB/DNA conjugates were removed. Therefore, the addition of completely mismatched MB/DNA has almost no effect on the conductivity of graphene as shown in Fig. 8, which indicates a high selectivity of the biosensor. Furthermore, we have also investigated the selectivity of the biosensors through single-base mismatched DNA chains as shown in Fig. 7. It can be found that the MGFET impedance change with single-base mismatched chains was slightly lower than the complementary strands and higher than the non-complementary target strand on each certain concentration. Therefore, the single-base mismatched strand could be detectable in this work. Although the aptamer and the complementary DNA chains are all commercial products which mainly determined the selectivity of the biosensors, the MGFETs and its detection system have also provided contribution to the high sensitivity for DNA detection.

**Conclusions**

Herein, a MGFET biosensor based on graphene and magnetic nanoparticles was presented to detect cDNA.
In the MGFTs, magnetic nanoparticles were modified onto the end of the cDNA sequence. Through the magnetic force between the MBs and the magnetic field, the distance between the MB/cDNA conjugates and the graphene film was mechanically controlled, thereby modulating the double conductive layer of the MGFTs. Furthermore, we can also conclude that, for a particular DNA strand, the impedance of the MGFTs will reflect the stress of the DNA strand, which in turn reflects the bending of the DNA strand (inset, Fig. 5b). Thus, the present MGFTs have the potential to be used in the study of the mechanical parameters of DNA chains. Therefore, the MGFTs may not only function as a biosensor for cDNA detection but may also potentially detect the mechanical parameters of DNA chains.

### Abbreviations
- cDNA: Complementary magnetically labeled single-stranded DNA
- CVD: Chemical vapor deposition
- DMDO: Dimethyl sulfoxide
- FET: Field-effect transistor
- GFET: Graphene field-effect transistor
- MBs: Magnetic nanobeads
- MGFET: Magnetic graphene field-effect transistor
- NHS: N-Hydroxysuccinimide
- PBASE: 1-Pyrenebutanoic acid succinimidyl ester
- PBS: Sodium dodecyl sulfate phosphate-buffered saline
- SDS: Sodium dodecylbenzenesulfonate
- TEM: Transmission electron microscopy
- ITO: Indium tin oxide

### Acknowledgements
This research project was jointly supported by the Shandong Natural Science Fund Project (Grant No.ZR2019MF025) and the National Natural Science Foundation of China (Grant No. 61401258, 11674199, 11674197 and 21303096).

### Authors’ Contributions
JJS conducted the experiments and wrote the papers. WWY and JJW designed this work and supervised the overall test process. XHX, KX, and SZJ guided the transfer of graphene. SCX provided graphene grown by CVD. JFR designed this work and supervised the overall test process. XHX, KX, and SZJ conducted the experiments and wrote the papers. WWY and JJW wrote the papers.

### Availability of Data and Materials
All data generated or analyzed during this study are included within the paper. All authors read and approved the final manuscript.

### Competing Interests
The authors declare that they have no competing interests.

### Author details
1Shandong Province Key Laboratory of Medical Physics and Image Processing Technology, School of Physics and Electronics, Shandong Normal University, Jinan 250358, People’s Republic of China.
2Institute of Materials and Clean Energy, Shandong Normal University, Jinan 250358, People’s Republic of China.
3College of Physics and Electronics, Dezhou University, Dezhou 253000, People’s Republic of China.

Received: 16 January 2019 Accepted: 12 June 2019

Published online: 24 July 2019

### References
1. Samanta A, Medintz IL (2016) Nanoparticles and DNA - a powerful and growing functional combination in biotechnology. Nanoscale 8(17):9037
2. Speit G, Hartmann A (2006) The comet assay: a sensitive genotoxicity test for the detection of DNA damage and repair. Methods Mol Biol 314:275
3. Shen L, Zhang X, Jin W (2012) Signal amplification based on DNA hybridization-dehybridization reaction on the surface of magnet submicrowave for ultrasensitive DNA detection. Analyst 137(20):4849–4854
4. Frommer WB, Davidson MW, Campbell RE (2009) Cheminform abstract: genetically encoded biosensors based on engineered fluorescent proteins. Cheminform 38(10):2833–2841
5. Lorimier RMD, Smith JJ, Dwyer MA et al (2002) Construction of a fluorescent biosensor family. Protein Sci 11(11):2655–2675
6. Pan LH, Kuo SH, Lin TY et al (2017) An electrochemical biosensor to simultaneously detect VEGF and PSA for early prostate cancer diagnosis based on graphene oxide/sDNA/PPLA nanoparticles. Biosens Bioelectron 89(Pt 1):598–605
7. Luo LQ, Zhang Z, Ding YP et al (2013) Label-free electrochemical impedance biosensor based on 1-aminonaphtalene/graphene hybrids. Nanoscale 5(13):5893–5896
8. Mascini M, Palchetti I, Marraza G (2001) DNA electrochemical biosensors. Fresenius Journal of Analytical Chemistry 369(1):15–22
9. Xu W, Deng W, Lei S et al (2015) A sensitive quenched electrochemiluminescent DNA sensor based on the catalytic activity of gold nanoparticle functionalized MoS2. New J Chem 39(10):8100–8107
10. Hung SC, Cheng NJ, Yang CF et al (2014) Investigation of extended-gate field-effect transistor pH sensors based on different-temperature-annealed bi-layer MWCNTs-In 2 O 3 films. Nanoscale Res Lett 9(1):502–502
11. Marchenko SV, Soldatkin O, Kasp 80 et al (2016) Creatinine deiminase adsorption onto Silicolate-modified pH-FET for creation of new creatinine-sensitive biosensor. Nanoscale Res Lett 11(1):173
12. Kastri M, Kerko A, Aarikka E et al (2017) Real-time wash-free detection of unlabeled PNA-DNA hybridization using discrete FET sensor. Sci Rep 7(1):15734
13. Kim DS, Jeong YT, Park HJ et al (2004) An FET-type charge sensor for highly sensitive detection of DNA sequence. Biosens Bioelectron 20(1):69–74
14. Kiani M, Ahmadi M, Hediyeh KF et al (2013) Analytical modelling of monolayer graphene-based ion-sensitive FET to pH changes. Nanoscale Res Lett 8(1):173
15. Cai B, Wang S, Huang L et al (2014) Ultrasonic label-free detection of DNA-PNA hybridization by reduced graphene oxide field-effect transistor biosensor. ACS Nano 8(3):2623–2638
16. Xu S, Zhan J, Man B et al (2017) Real-time reliable determination of binding kinetics of DNA hybridization using a multi-channel graphene biosensor. Nat Commun 8:14002
17. Ohno Y, Maehashi K, Yamashiro Y et al (2009) Electrolyte-gated graphene field-effect transistors for detecting pH and protein adsorption. Nano Lett 9(9):3318–3322
18. Yue W, Jiang S, Xu S et al (2014) Fabrication of integrated field-effect transistors and detecting system based on CVD grown graphene. Sensors Actuators B Chemical 195:467–472
19. Yue W, Wang C, Wang C et al (2017) An electricity-fluorescence double-checking biosensor based on graphene for detection of binding kinetics of DNA hybridization. RSC Adv 7(70):44559–44567
20. Ping J, Vishubhota R, Vrudhula A et al (2016) Scalable production of high-sensitivity, label-free DNA biosensors based on back-gated graphene field effect transistors. ACS Nano 10(9):8700–8704
21. Zheng C, Huang L, Zhang H et al (2015) Fabrication of ultrasensitive field-effect transistor DNA biosensors by a directional transfer technique based on CVD-grown graphene. ACS Appl Mater Interfaces 7(31):15073016002004
22. Hua X, Zhou Z, Yuan L et al (2013) Selective collection and detection of MCF-7 breast cancer cells using aptamer-functionalized magnetic beads and quantum dots based nano-bio-probes. Anal Chim Acta 778(14):135–140
23. Vanlincck ID, Henighan T, Loomholt MTV et al (2011) Highly parallel magnetic tweezers by targeted DNA tethering. Nano Lett 11(12):5489
24. Tang B, Guo X, Gao H (2010) Raman spectroscopic characterization of graphene. Appl Spectrosc Rev 45(5):369–407
25. Cong C, Yu T, Sato K et al (2011) Raman characterization of ABA- and ABC-stacked trilayer graphene. ACS Nano 5(1):8760
26. Lenski DR, Fuhrer MS (2011) Raman and optical characterization of multilayer turbostratic graphene grown via chemical vapor deposition. J Appl Phys 110(1):289
27. Hailer H, Chatoor S, Manekin J et al (2010) Influence of electrolyte composition on liquid-gated carbon nanotube and graphene transistors. J Am Chem Soc 132(48):17149–17156
28. Fu W, Abbassi ME, Hasler T et al (2014) Electrolyte gate dependent high-frequency measurement of graphene field-effect transistor for sensing applications. Appl Phys Lett 104(16):666–669
29. Chou FC, Lipfert J, Das R (2014) Blind predictions of DNA and RNA tweezers experiments with force and torque. PLoS Comput Biol 10(8): e1003756
30. Mosconi F, Allemand JF, Bensimon D et al (2009) Measurement of the torque on a single stretched and twisted DNA using magnetic tweezers. Phys Rev Lett 102(7):078301
31. Xiao Y, Huang Z, Qiang L et al (2015) Elastic response of DNA molecules under the action of interfacial traction and stretching: an elastic thin rod model. Modern Physics Letters B 29(31):1550193

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.