

Detection of Sub-fM DNA with Target Recycling and Self-Assembly Amplification on Graphene Field Effect Biosensors

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1 1 Detection of Sub-fM DNA with Target Recycling
2 2 and Self-Assembly Amplification on Graphene
3 3 Field Effect Biosensors

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1 KEYWORDS: graphene field-effect transistor, DNA biosensor, DNA self-assembly
2 amplification, sub-fM limit-of-detection

3 **Abstract**

4 All-electronic DNA biosensors based on graphene field effect transistors (GFETs) offer the
5 prospect of simple and cost-effective diagnostics. For GFET sensors based on complementary
6 probe DNA, the sensitivity is limited by the binding affinity of the target oligonucleotide, in
7 the nM-range for 20-mer targets. We report a $\sim 20,000\times$ improvement in sensitivity through
8 the use of engineered hairpin probe DNA that allows for target recycling and hybridization
9 chain reaction. This enables detection of 21-mer target DNA at sub-fM concentration and
10 provides superior specificity against single-base mismatched oligomers. The work is based on
11 a scalable fabrication process for biosensor arrays that is suitable for multiplexed detection.
12 This approach overcomes the binding affinity dependent sensitivity of nucleic acid biosensors
13 and offers a pathway towards multiplexed and label-free nucleic acid testing with high
14 accuracy and selectivity.

16 **Introduction**

17 Label-free and multiplexed nucleic acid testing is of interest for genetic screening and clinical
18 diagnosis^{1, 2}, and nano-bioelectronics have shown great promise for this application³.
19 Graphene field effect transistors (GFETs) offer advantages of large surface-to-volume ratio,
20 excellent biocompatibility, and high carrier mobility⁴. GFETs can be readily functionalized
21 with single-stranded probe DNA for detection of specific target oligonucleotides with
22 complementary sequences. By detecting the charge of target DNA hybridized with the probe,
23 GFETs typically offer a limit of detection (LOD) ranging from 1 fM to 100 pM⁵⁻⁸. The broad
24 range of sensitivities has been ascribed to the affinity-governed binding kinetics of target and
25 probe DNA, which varies with the length of the complementary sequence^{5, 9}: higher target-

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probe binding affinity and lower LOD are achieved for longer probe and target DNA oligomers. For example, we reported⁵ a LOD of ~100 pM for 22-mer target DNA (as confirmed by others⁸) and 1 fM LOD for 60-mer target DNA due to the stronger target-probe affinity. Achieving a low LOD for oligonucleotides without the constraint of target sequence length, e.g., sub-fM for a ~20-mer, is highly desired for the early diagnosis of various diseases by detecting biomarkers of oligonucleotides, such as cardiovascular disease² and cancer¹⁰. Here we report an approach to overcome the length-dependent sensitivity of GFET nucleic acid sensors based on single-stranded probe DNA. The GFET sensor design was based on a scalable fabrication process⁵. In contrast to traditional GFET DNA biosensors based on a single-strand probe DNA^{5, 6, 8}, our approach included a hairpin-structured probe DNA and a triggered self-assembly pathway to enable target recycling and a hybridization chain reaction to amplify the transduction signal and improve the LOD by a factor of 20,000 or more, depending on the incubation time. The results were in good agreement with a mass action kinetic model. Our tests showed that hairpin probe DNA offered enhanced specificity against non-complementary DNA with a single base mismatch compared to the traditional single-strand probe DNA, and we demonstrated multiplexed detection using the GFET arrays. The work creates the possibility for high sensitivity nucleic acid testing independent of length constraints for the target DNA, which is significant for disease diagnosis in a realistic clinical setting.

Results and Discussion

Figure1a is an optical image of a GFET array fabricated using a scalable photolithography process described in the **Methods Section**. Briefly, large-area graphene (10 cm × 15cm) was synthesized on Cu foil by low-pressure chemical vapor deposition and transferred onto a

Si/SiO₂ substrate with previously fabricated Cr/Au electrodes. GFET channels were defined using an optimized bilayer photolithographic process⁴ and oxygen plasma etching. After processing, the GFET arrays were annealed in a H₂/Ar atmosphere at 225°C to remove resist residues. The GFETs were of high quality as assessed by Raman spectroscopy (Figure S1 of the Supporting Information). As shown in Figure S2, the current-gate voltage characteristics showed good device-to-device uniformity, a narrow distribution of the Dirac point (6.6 ± 1.3 V), and high carrier mobility (2700 ± 700 cm²/V-s).

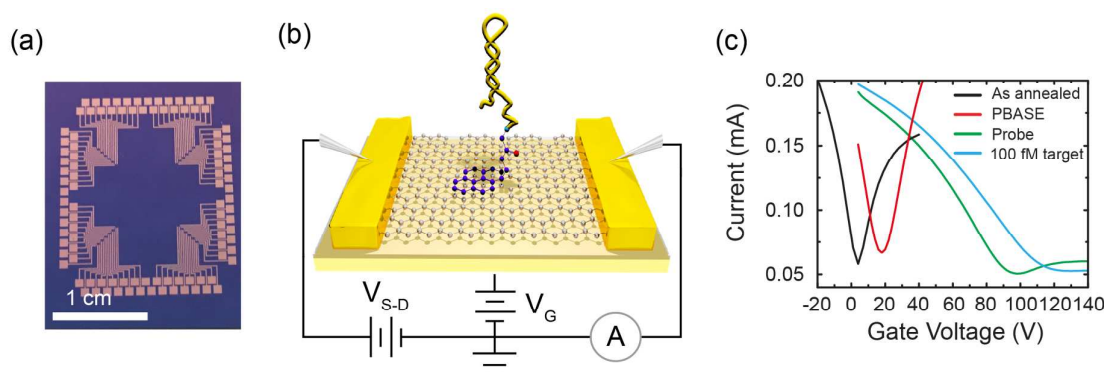


Figure 1. GFET biosensor arrays. (a) Optical image of a graphene field-effect transistor (GFET) array fabricated on 250 nm SiO₂ chip. (b) Schematic of hairpin probe DNA bound to a back-gated GFET using a pyrene linker (purple). (c) Current-gate voltage curve evolution of GFET following different chemical treatment steps.

The GFETs were functionalized by incubation in a solution of 1-pyrenebutyric acid N-hydroxysuccinimide ester (PBASE) in N,N-dimethylformamide (DMF) (see **Methods** for details). The aromatic pyrenyl group of PBASE binds to the graphene basal plane by the π - π stacking interaction^{11, 12}. After functionalization, the GFETs were then incubated in an aqueous solution of aminated hairpin probe DNA (Figure 1b). The probe DNA layer images as a nearly uniform brush in AFM, suggesting a high density of probe DNA immobilized on the GFET surface (Figure S3 of the Supporting Information)⁵. The I-V_g characteristics for a GFET array were measured after each functionalization step. Immobilization of probe DNA

led to an increase in the Dirac voltage ($\Delta V_D = 71.3 \pm 6.0$ V), which was explained quantitatively by assuming chemical gating of 56 elementary charges per probe oligomer, with a probe DNA density of $\sim 1.1 \times 10^3 / \mu\text{m}^2$.

Figure 2 illustrates the operating principle of detection based on target recycling and self-assembly signal amplification. The probe DNA sequence was designed to form a secondary hairpin structure after being annealed by gradually cooling from 95 °C to room temperature. The hairpin probe DNA was metastable and could be specifically opened by target DNA to trigger a self-assembly reaction. A detailed description and DNA designs can be found in the **Methods Section** and Figure S6. The GFET sensor, functionalized with the hairpin probe DNA (H1), was exposed to a mixture of target DNA (T) and three helper DNAs (H2, H3, H4). The target triggered the nucleation between H1 and T via base-pairing, mediating a branch migration that opened hairpin H1 to form a complex H1·T. The protruding segment of H1·T bound to the toehold of hairpin H2 (segment 3*; see Figure 2) to initiate the strand-displacement reaction to form the complex H1·H2 and release T. The dissociated T was recycled to trigger additional self-assembly cycles as described above. Meanwhile, the protruding segment of H1·H2 (segments 4 and 7) nucleated with hairpin H3 and triggered the hybridization chain reaction (HCR) with H4. The presence of T can be circularly used to trigger HCR, leading to long nicked double-stranded polymers for the amplification of DNA products that can be detected by GFET through chemical gating. The effectiveness of the self-assembly amplification was confirmed by electrophoresis analysis (see Supporting Information Figure S4), where a smear band was found due to the formation of H1·H2·H3·H4 complexes with higher molecular weights. The scheme described above is universal for the detection of short DNA or RNA by changing the sequences of segments 1, 2, 3, 1*, 2* and 3*, and H1 and H2 can be correspondingly modified and used with universal H3 and H4 for the amplification. It is noted our scheme is different from a typical competitive assay for

detection of small analytes like small proteins and aptamers, where the labeled ligand is added to the sample to compete with analyte to bind an antibody.¹³ In our approach, the label-free helper DNAs were added to displace target DNA from probe DNA and trigger target recycling hybridization chain reaction, offering the advantages of label-free, amplified signals as well as the capability of detecting short DNAs or RNAs.

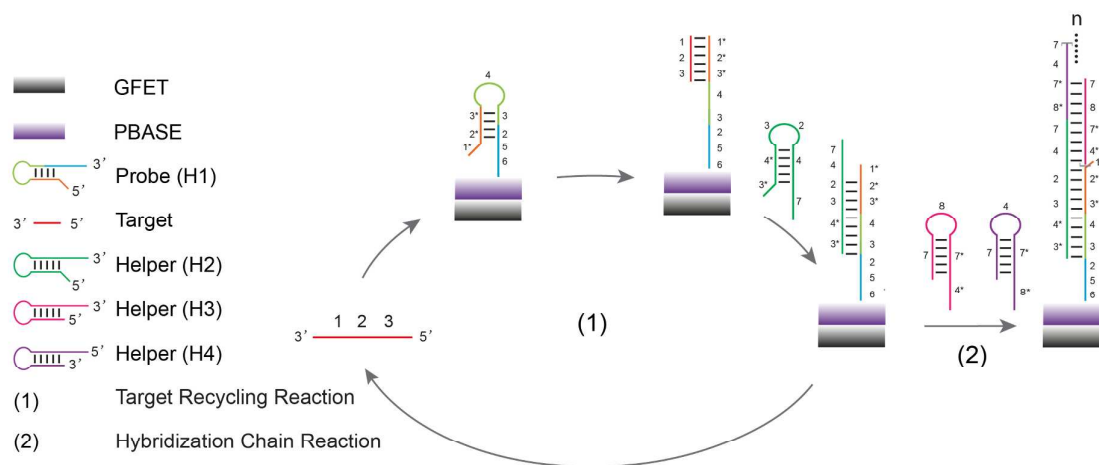


Figure 2. Schematic showing the principle of the triggered self-assembly amplification for DNA detection on GFET. The GFET was functionalized by hairpin probe DNA H1 through the PBASE linker. The target DNA (T) opens the hairpin probe to form the complex H1·T. T is then displaced by helper DNA H2 through the toehold-mediated strand displacement reaction, leading to the formation of the H1·H2 complex and enabling target recycling. Hybridization chain reaction (HCR) was triggered by H1·H2 in the presence of two additional helper DNAs, H3 and H4. Amplified HCR products are then detected through a shift of the GFET Dirac voltage.

In sensing experiments, GFET biosensor arrays were tested against a mixture of a known concentration of the target DNA in the presence of three helper DNA H2, H3, and H4, all at a concentration of 1 μ M, in 5 $\times$ saline-sodium citrate (SSC) hybridization buffer, and the I- V_g

characteristics were measured in the dry state.¹⁴ In all cases a positive shift of the Dirac voltage was observed (Figs 1c and 3a). The p-type doping effect of DNA binding is understood as chemical gating¹⁵ of the GFET by DNA molecules that acquire a negative charge due to deprotonation of phosphate groups in residual water⁵. As done previously⁵, for a given target concentration, the sensor response is reported as ΔV_D^{rel} , the Dirac voltage shift relative to the shift measured upon exposure to the DNA mixtures without target. To minimize the hysteresis effect on sensing^{16, 17}, care was taken to keep the measurement protocol consistent through the entire study (back gate sweep rate: 0.2 V/sec, sweep range: 0 V – 100V), and in all measurements, the forward sweeps were utilized for data analysis. Figure 3a shows the GFET response for 1 hour incubation time. ΔV_D^{rel} varied systematically with the target concentration, which is ascribed to the additional chemical gating of the GFET channel by the negatively charged DNA products by hybridization. It is noted that direct comparison of Dirac voltage shifts between the probe DNA immobilization step (in DI water) and the target DNA binding step (in 5X SSC buffer) is complicated by the very different salt contents of these two solutions. The LOD for the 21-mer target is ~ 5 fM, which is $20,000\times$ lower than earlier reports using single-strand probe DNA^{5, 8}.

To elucidate the dynamics of the self-assembly amplification, we constructed a mathematical model that reflects key biochemical reactions that connect the target DNA oligomer to the initiation of the amplification response mediated by the hairpins H1 and H2. The kinetic model (detailed in **Methods**) is based on the assumption that the HCR reaction with H3 and H4 is non-reversible due to the high concentration of helper DNA species (1 μ M), so the model does not consider reactions involving the helpers H3 and H4. Even in its simplicity, the model recapitulated the measured target DNA dose-response curve (Figure 3c). The model also predicted that the experimental dose-response curve is a function of incubation time: at low target concentration (fM range), the Dirac voltage shift was predicted to grow if

1 the experiments were run for longer periods of time (> 1 hour) because the recycled target
2 DNA would open additional H1 hairpins with time, increasing the number of H1·H2·H3·H4
3 complexes.

4 The model predictions were validated experimentally (Figure 3b). Three target DNA
5 concentrations were used (100 pM, 100 fM, 100 aM) with the same experimental conditions
6 except that incubation time was prolonged to 100 hours instead of 1 hour. Biosensor
7 responses rapidly saturated in less than 1 hour for a concentration of 100 pM. For lower
8 concentrations (100 fM, 100 aM), the response increased more gradually over time. The
9 model gave correct qualitative predictions for the temporal trajectories of the response to the
10 target concentrations tested, although the kinetics of the model were accelerated compared to
11 the experiment (compare the time axes in Figs 4b and 4d). This is most likely the result of not
12 considering the polymerizing reactions involving H3 and H4. As shown in Figure 3b, the
13 LOD for our approach is further decreased by $50\times$ to 100 aM with a sensing time of ~ 15
14 hours. Here, the sensor responses for different incubation times are reported as the Dirac
15 voltage shifts relative to the shifts measured upon exposure to the DNA mixtures without
16 target, incubated for the appropriate sensing time. We observed very little Dirac voltage shift
17 for DNA mixtures without target incubated for up to 3 hours, with the Dirac voltage shift
18 becoming significant (+ 6.1V) for incubation times exceeding 24 hours, presumably due to
19 increased non-specific binding of the helper DNAs. Going beyond the conventional HCR
20 scheme for signal amplification used in other types of biosensors^{18, 19}, our approach includes
21 target recycling for enhanced sensitivity, for all-electronic GFET based DNA detection with
22 the prospect of simple, highly sensitive and label-free diagnostics. Moreover, the reaction
23 kinetics could be accelerated by optimizing the sensing parameters, e.g., H1, H2
24 concentration (see Supporting Information Figure S8) and incubation temperature^{20, 21}, which
25 offers the opportunity to tune the sensing time as needed to enable point-of-care detection.

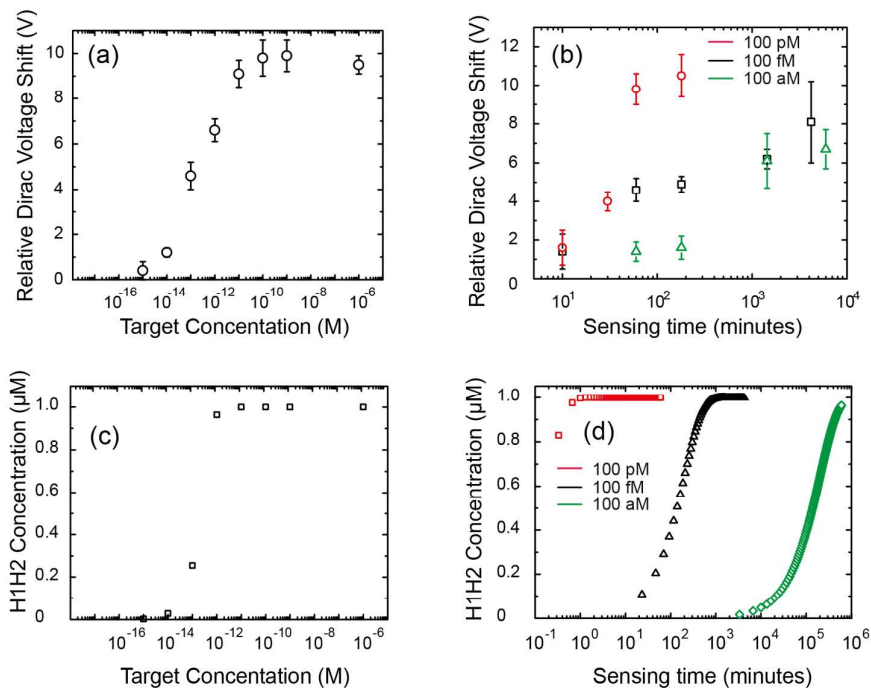
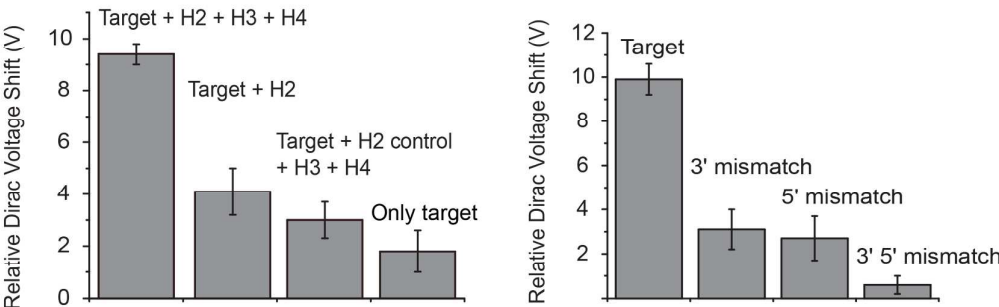


Figure 3. Sensing results and kinetic model. (a) Sensor response as a function of target DNA concentrations. Error bars are standard deviation of the mean. The limit of detection is <10 fM. (b) Sensor response as a function of incubation time for different target concentrations. (c) Simulation results of H1·H2 complex examined for different concentrations of target DNA shows good qualitative agreement with panel a. (d) Simulation results of H1·H2 concentrations as a function of time with different target concentrations, showing trends similar to panel b.

To validate the effectiveness of detection based on target recycling and self-assembly amplification, the GFET arrays were tested against several positive controls. As shown in Figure 4a, the mixture of target DNA (1 μ M T) and helper DNAs (1 μ M H2, 1 μ M H3, 1 μ M H4) gave the highest response of 9.5 ± 0.4 V. For the positive control with target recycling but no HCR (only mixture of T + H2), the signal dropped to 4.2 ± 0.9 V, consistent with the expectation that HCR leads to increased formation of DNA products, consistent with the electrophoresis test (Figure S4 of the Supporting Information). The sensor response further

1 decreased to 3.1 ± 0.7 V by using a random sequenced H2 to suppress the target recycling
2 reaction, which is similar to the response for the control sample with T only (1.9 ± 0.7 V).
3 The GFET arrays were also tested against various negative controls, all at a concentration of
4 10 nM, to confirm that the response reflected specific binding of the complementary target
5 DNA. As seen from Figure 3a, this 10 nM test concentration corresponded to a fully saturated
6 sensor response for the T strand. As shown in Figure 4b, the target DNA give the largest
7 signal (9.9 ± 0.7 V), while for target DNA with a single base mismatch at the 3' or 5' end, the
8 responses were significantly smaller, 3.1 ± 0.9 V and 2.7 ± 1.0 V, respectively, which would
9 correspond to a T concentration of 100 fM for the target DNA. This very strong rejection of
10 binding for the single-base mismatch at the 3' or 5' end was greatly superior to our earlier
11 report for GFET DNA sensors based upon single-stranded probe DNA.⁵ The higher
12 specificity is attributed to the stem-and-loop structure of the hairpin probe DNA.^{22, 23} The
13 hybridization to the target DNA disrupts the hairpin structure of the probe DNA and is less
14 thermodynamically favorable than the hybridization of target DNA to the single-stranded
15 probe DNA.^{23, 24} The control oligomer with two mismatches at the 3' and 5' ends had a
16 relative Dirac voltage shift of 0.6 ± 0.4 V, essentially identical to the Dirac voltage shift for
17 buffer.
18 Finally, we demonstrated multiplexed detection of target DNAs T and T' with a GFET sensor
19 array, through the use of site-specific functionalization using two different hairpin probe
20 DNAs. The second set of probe DNA (H1') and helper DNA (H2') were redesigned
21 according to the base sequence of the second target DNA (T') to trigger the self-assembly
22 reaction in the presence of H3 and H4. As shown in Figure S5, exposure of the DNA mixture
23 (100 nM T, 100 aM T', 1 μ M H2, 1 μ M H3 and 1 μ M H4 in 5 $\times$ SSC buffer) leads to
24 distinguishable response for the site-specifically functionalized GFET sensors (10.4 ± 1.9 V
25 for H1 site, versus 0.6 ± 1.2 V for the H1' site). The multiplexed sensor array is therefore

1 capable of quantifying concentrations of T and T' simultaneously in a single measurement,
2 which is potentially valuable for use in a clinical setting.



3
4 **Figure 4.** Biosensor response to positive and negative controls. (a) Relative Dirac voltage
5 shifts for various positive control experiments based upon concentrations of 1 μ M for the
6 target DNA and the specified helper DNAs. (b) Relative Dirac voltage shifts for 10 nM
7 target DNA and negative controls with base mismatches at the ends; detailed DNA sequence
8 are listed in Table S1. Error bars are standard deviation of the mean. Helper DNAs were of 1
9 μ M.

11 **Conclusions**

12 We developed manufacturable GFET nucleic acid sensors based on hairpin probe DNA
13 designed to enable signal amplification by target recycling and a hybridization chain reaction.
14 Based upon a 1 hour detection time, the limit of detection was $\sim$ 5 fM for a 21-mer, an
15 improvement of 20,000x over earlier reports based on complementary probe DNA. The limit
16 of detection could be lowered to below 1 fM by extending the time, in agreement with a
17 simplified kinetic model we developed. The approach showed excellent specificity against
18 single base mismatches at the 3' or 5' end, as expected due to the design characteristics of the
19 hairpin probe, and we also demonstrated simultaneous detection of multiple targets. Our
20 approach offers a platform for DNA detection at a low concentration even for short DNA

1 targets. The scalability and sensitivity of the GFET devices make them potentially applicable
2 for disease diagnosis and label-free genetic diagnosis.

3 **Materials and Methods**

4 **Graphene synthesis:** Graphene was synthesized in a low-pressure chemical vapor deposition
5 system (OTF-1200X-4-C4-SL-UL, MTI Corp.). Cu foils (Alfa Aesar Item #46365) were
6 cleaned with 5.4% HNO₃ for 40 seconds and two DI water baths for 2 min, and then
7 thoroughly blown dry using N₂ gas. The reaction chamber was pumped to a base pressure of
8 50 mTorr. The Cu growth substrate was annealed at 1020 °C for 30 minutes with a gas flow
9 of 500 sccm Ar and 80 sccm H₂. Monolayer graphene was then grown using methane as a
10 carbon source at a flow rate of 5 sccm for 5 mins and then 10 sccm for 15 mins with 80 sccm
11 H₂. The reactor was rapidly cooled to room temperature under a flow of 80 sccm H₂ and 10
12 sccm CH₄.

13 **GFET sensor array fabrication:** Photolithographic processing was used to define an
14 electrode array for 100 back-gated graphene FETs on a highly p-doped Si wafer with a 250
15 nm thermal oxide layer. The contact metallization was 5 nm Cr / 40 nm Au, deposited by
16 thermal evaporation. Graphene was then transferred onto the metallized SiO₂/Si chip using
17 the PMMA assisted “bubbling” transfer method.⁴ Briefly, PMMA-coated graphene/Cu was
18 slowly immersed into a 50 mM NaOH aqueous solution with a 20 V potential difference
19 applied between the copper foil and the solution. PMMA-supported graphene was separated
20 from the Cu foil by hydrogen gas bubbles formed at the Cu surface. After three deionized
21 water baths (resistivity of 18.2 MΩ-cm), the PMMA/graphene film was transferred onto the
22 metallized SiO₂/Si chip, followed by air drying and baking at 150 °C for 3 minutes. After
23 removal of PMMA by immersion in acetone overnight, the chips were spin coated with a
24 photoresist bilayer of PMGI (MicroChem Corp.) and S1813 (Shipley). Graphene channels
25 were defined using photolithography and oxygen plasma etching (Pressure: 1.25 Torr, Power:

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1 50 W, Duration: 35 seconds). The photoresist residue on graphene channels was removed by
2 a N-Methyl Pyrrolidinone (NMP) based stripper (NANOTM Remover PG, MicroChem
3 Corp.), acetone and IPA to obtain the array of 100 FETs. Finally, the array was annealed in
4 H₂/Ar forming gas at 225 °C to reduce photoresist residues.

5 **AFM Characterization:** An atomic force microscope (AFM, Icon Bruker) equipped with a
6 probe with a tip radius of <10 nm (TAP300Al-G, Budgetsensors) was used to evaluate the
7 height increase for the PBASE functionalization and probe DNA immobilization.

8 **1-pyrenebutyric acid N-hydroxysuccinimide ester (PBASE) functionalization with**
9 **hairpin probe DNA immobilization and testing against target or control solutions:**

10 GFET sensors were soaked in a 0.2 mM PBASE (Sigma-Aldrich) in DMF for 20 h and then
11 washed thoroughly with DMF, IPA, and DI water for 3 min each. Care was taken to blow dry
12 the GFET gently with N₂ gas at a pressure of 10 psi for ~ 1 min. The hairpin structure of the
13 probe DNA was formed by heating the probe DNA at 95 °C for 5 minutes, allowed by
14 gradual cooling to room temperature before use. GFET sensors were then incubated in 1 μM
15 aqueous solution of hairpin probe DNA (sequence is listed in Table S1) in DI water for 3
16 hours in a humid atmosphere to suppress the evaporation of the DNA solution. This was
17 followed by washing with two DI water baths (2 min each) and drying with N₂ gas gently
18 with a pressure of 10 psi for ~ 1 min. After I-V_g measurement, the probe DNA-immobilized
19 GFET devices were immersed in 200 μL of DNA mixture with known concentrations of
20 target and helper DNA (1 μM for H2, H3 and H4, respectively) for 1 hour to allow for DNA
21 hybridization. While incubating in the target DNA solution, GFET sensors were placed in a
22 container with a hot water bath to create a humid atmosphere and suppress evaporation of the
23 DNA solution. The hot water was replaced every few hours; no significant decrease in the
24 volume of the DNA solution was observed. The devices were washed with two DI water

baths (2 min each), followed by drying with N₂ gas gently with a pressure of 10 psi for ~ 1 min before measurement of the electrical properties.

The GFETs I-V_g characteristics were measured after each functionalization step. Self-assembly of the PBASE layer led to an increased Dirac voltage ($\Delta V_D = 18.8 \pm 1.5$ V), which is explained by assuming that NHS groups are hydrolyzed into carboxyl groups, which deprotonate and acquire a negative charge^{15, 25}. Functionalization with PBASE also led to decreased hole carrier mobility to $\mu = 1070 \pm 200$ cm²/V-s. Immobilization of probe DNA led to a further increase in the Dirac voltage ($\Delta V_D = 71.3 \pm 6.0$ V), corresponding to a hole carrier density of $\sim 6 \times 10^{12}$ cm⁻² induced by the hairpin probe DNA. This is explained quantitatively by assuming chemical gating of 56 negative charges for each probe oligomer, so the observed Dirac voltage increase corresponds to a probe DNA density of $\sim 1.1 \times 10^3$ / μm^2 . This high density of immobilized probe DNA is consistent with the AFM images in Figure S3, where individual probe DNA molecules are not resolved.

Design of hairpin structures: The principle of kinetically trapped nucleic acid hairpin assembly and the mechanism of the hybridization chain reaction (HCR) were demonstrated by Pierce and co-workers.^{24, 26, 27} We designed the target triggered self-assembly amplification circuit based on the ingenious combination of four-hairpin DNA. Figure S3 depicts the design of hairpin structures. The sequence of hairpin structures is described in terms of numbered domains, each of which represents a short fragment (5-15 nt) of DNA sequence. Numbered domains marked with * are complementary to the corresponding unmarked numbered domains. The length of toehold, stem, loop and target regions were chosen based on kinetic and thermodynamic considerations. By using the UNAFold web server (<http://unafold.rna.albany.edu/>), we found this target triggered amplification is sensitive to short DNA with an optimum sequence length of 20-32 nt. We also set out to engineer hairpins that maximize the free energy benefit per polymerization step while

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3 1 retaining hairpin metastability in these conditions. Hairpin DNAs have identical secondary
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5 2 structures, with stems of length 15 bp, toehold of length 6-8 nt and loop of length 8-15 nt.
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7 3 The four hairpin structures do not initially interact with each other.
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10 4 **Triggered self-assembly HCR amplification:** After immobilization of hairpin probe DNA
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12 5 H1 on GFETs, the target DNA with a known concentration (100 aM to 1 μ M) and three
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14 6 helper DNAs (H2, H3, H4) at a concentration of 1 μ M were introduced in an aqueous
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16 7 solution mixture with 5 $\times$ SSC buffer. The four hairpin species, H1 (Probe), H2 (Helper), H3
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18 8 (Helper) and H4 (Helper), coexist in metastable state in the absence of T (target). Exposure of
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20 9 target triggers the nucleation of H1 and T via base-pairing to the single-stranded toehold “1*”
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22 10 of T (Figure 2), mediating a branch migration that opens the hairpin H1 to form a complex
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24 11 H1·T and release a single-stranded segment “3 and 4” (Figure 2). H1·T complex then
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26 12 nucleates with hairpin H₂ by means of base-pairing to toehold “3*”, mediating a branch
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28 13 migration that opens the hairpin to form the complex H1·T·H₂, which is inherently unstable.
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30 14 T then dissociates from the complex H1·T·H₂, leaving a stable H1·H₂ duplex with a
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32 15 protruding segment “4 and 7”. The dissociated T is recycled into the DNA mixture, nucleates
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34 16 with a new H1 and triggers the same circuit as described above.
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36 17 The protruding segment of H1·H₂ nucleates with hairpin H₃ via base-pairing to single-
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38 18 stranded toehold “4*”, mediating a branch migration that opens the hairpin H₃ and forms a
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40 19 complex H1·H₂·H₃ releasing of single-stranded segment “8 and 7”. This complex nucleates
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42 20 with hairpin H₄ by means of base-pairing to toehold “8*”, mediating a branch migration that
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44 21 opens the hairpin to form complex H1·H₂·H₃·H₄ releasing of single-stranded segment “4 and
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46 22 7”. Thus, the initiator sequence is regenerated, providing the basis for a hybridization chain
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48 23 reaction of alternating H₃ and H₄ polymerization steps. In this amplification system, target
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50 24 DNA can be circularly used to trigger HCR amplification, which results in the generation of
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52 25 numerous long nicked double-stranded amplification polymers. Due to the target recycling
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and self-assembly amplification nature, the presence of low concentration of target is expected to generate a large number of annealed H1·H2·H3·H4 complexes, which can potentially result in a significant change of Dirac voltage for GFET detection of trace amount of target DNAs and RNAs.

Electrical measurement and evaluation: Electrical measurements were performed under ambient conditions in a probe station equipped with a probe card that is capable of measuring the 100 devices simultaneously. Current-gate voltage (I - V_g) measurements were carried out using a Keithley 2400 sourcemeter, with a bias voltage of 0.1 V. The gate voltage was applied using a Keithley 6487 voltage source. In order to avoid the readout errors induced by extracting the Dirac voltage from the Dirac peak directly, the Dirac point voltage and hole carrier mobility were extracted by fitting the hole branch of the σ - V_g curve to the equation²⁸⁻³⁰:

$$\sigma^{-1}(V_g) = [\mu c_g (V_D - V_g)]^{-1} + \sigma_s^{-1}$$

where c_g is the gate capacitance per unit area for the 250 nm thick SiO_2 (11.5 nF/cm²), μ is the hole carrier mobility, V_D is the Dirac voltage, and σ_s is the saturation conductivity as $V_g \rightarrow -\infty$.

Kinetic model: We constructed a computational model that simulates the amplification response that enables the detection of a DNA oligomer. Specifically, the model reflects key biochemical reactions that connect the target DNA oligomer sequence to initiation of the amplification response mediated by the Hairpin H1 and H2. The model is based on mass action kinetics and comprises 5 coupled ordinary differential equations (ODEs), each of which expresses the rate of change in the concentration of a biochemical species:

$$\frac{dH1}{dt} = -k_{1on} \times H1 \times T + k_{1off} \times H1T \quad (1)$$

$$\frac{dT}{dt} = -k_{1on} \times H1 \times T + k_{1off} \times H1T + k_{2on} \times H1T \times H2 - k_{2off} \times H1H2 \quad (2)$$

$$\frac{dH1T}{dt} = k_{1on} \times H1 \times T - k_{1off} \times H1T - k_{2on} \times H1T \times H2 + k_{2off} \times H1H2 \quad (3)$$

$$\frac{dH2}{dt} = -k_{2on} \times H1T \times H2 + k_{2off} \times H1H2 \quad (4)$$

$$\frac{dH1H2}{dt} = k_{2on} \times H1T \times H2 - k_{2off} \times H1H2 \quad (5)$$

The model's input is the target DNA oligomer concentration and its output is the concentration time course (i.e., kinetic trajectory) for each biochemical species considered. The initial concentrations of the Hairpin H1 and H2 are set to 4 μ M and 1 μ M, respectively. The model reactions and parameters representing the rates of different molecular and cellular processes, such as target DNA oligomer-Helper association/dissociation, are defined in Table S2. All computational analyses were performed in the software suite MATLAB R2017a (MathWorks, Natick, MA), and the ODEs were solved using the ODE15S solver with an absolute tolerance of 10^{-15} μ M and a relative tolerance of 10^{-12} .

Agarose gel electrophoresis: The target triggered self-assembly amplification was confirmed on a step-by-step basis using agarose gel electrophoresis (Figure S1). Hairpin H1 reacts with target and leads to a band corresponding to the product of H1·T, which migrates slower than H1 in agarose gel. Complex of H1·T then reacts with hairpin H2 to produce a band that corresponds to the product of H1·H2, which migrates similarly as the H1·T in agarose gel. Displacement reaction between H2 and H1·T releases T which is recycled to react with another H1 to produce H1·T until the supply of H1 is exhausted. H1·H2 reacts with hairpin H3 and produces a band that correspond to products of H1·H2·H3, which migrates similarly with H1·H2 in agarose gel. In 2 hours, there remains a band of H3, which does not react with H1·H2. H1·H2·H3 reacts with hairpin H4, which causes a chain reaction of alternating kinetic escapes by H3 and H4 species corresponding to polymerization into a

nicked double helix. Amplification continues until the supply of H3 and H4 are exhausted. As shown in Figure S1, H1·H2·H3·H4 complex with larger molecular weight exhibits a smear band.

ASSOCIATED CONTENT

Supporting Information

DNA sequences employed in this work. Parameters of the computational model. Optical image and Raman spectrum of a GFET after the photolithographic processing. Electronic characterization of 100 devices in a GFET array. AFM images and line scan profiles for an as-prepared GFET, GFET functionalized with PBASE and GFET immobilized with aminated hairpin probe DNA. Agarose gel electrophoresis results for the self-assembly amplification. Secondary structures and sequences design of DNAs for self-assembly amplification.

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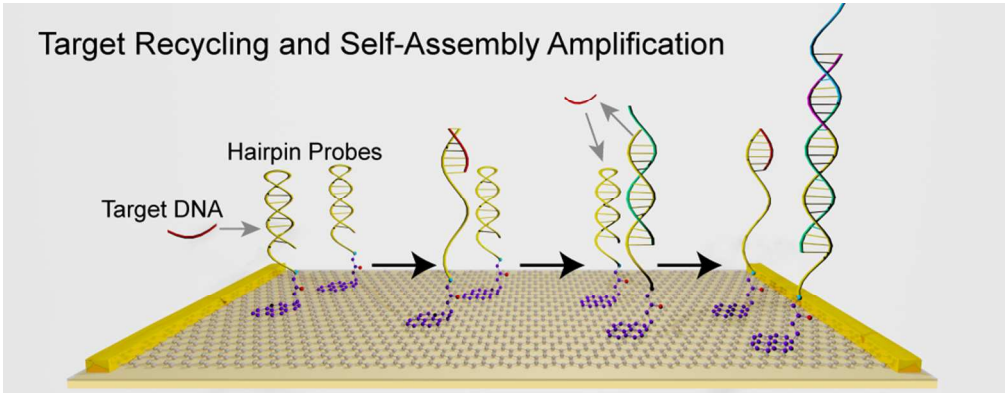
3 **Notes**

4 The authors declare no competing financial interest.

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ToC image

90x35mm (300 x 300 DPI)