



A graphene oxide-based paper chip integrated with the hybridization chain reaction for peanut and soybean allergen gene detection

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ABSTRACT

Allergen genes of the peanut and soybean were selected as model targets. Four hairpin DNA probes, H1, H2, H3, H4 were designed. Cy3-labeled H1 and H2 were used to detect peanut DNA, while FAM-labeled H3 and H4 were used to detect soybean DNA. Graphene oxide (GO) was used as the adsorption material for capturing the hairpin probes, and as a selective fluorescence quencher to reduce the background signal. To develop an allergen gene detection system with a GO-based paper chip format, we integrated the hybridization chain reaction (HCR) with fluorescence resonance energy transfer (FRET) in our design. The results showed that in the absence of peanut DNA (T_P) and soybean DNA (T_S), the detection probes attached to the GO surface, which quenched their fluorescence. In the presence of T_P or T_S , however, complementary probe binding to the targets initiated HCR, producing long double-stranded DNA products that could not be absorbed onto the GO surface. Hence, a strong red or green fluorescent signal was generated. The detection limit for both peanut and soybean DNA was 1 nM using this method, indicating the high sensitivity of our approach. This method also exhibited good specificity and a single chip could be used to simultaneously detect two different targets.

1. Introduction

Food allergy is a kind of immune response to some foods, which cause serious health problems for allergic people, such as nausea, vomiting, eczema, etc. Allergens commonly exist in foods including milk, eggs, peanuts, soybeans, sesame seeds, walnuts, almonds, wheat [1,2]. Among these, peanut allergens are mainly Ara h 1, Ara h 2, Ara h 3, and Ara h 6 [3], and soybean allergens include Gly m Bd 28 K, Gly m Bd 30 K [4], etc. Enzyme-linked immunosorbent assay (ELISA), western blotting, *et al.* are used for detecting allergenic protein components [5,6]. Many methods have been developed for the detection of low concentrations of DNA including the polymerase chain reaction (PCR) [7], Southern blotting [8], rolling circle amplification (RCA) [9], loop-mediated isothermal amplification (LAMP) [10], strand displacement amplification (SDA) [11], and recombinase polymerase amplification (RPA) [12], *et al.* However, enzymes are indispensable for these DNA detection methods. The operation process of them is very complicated, and harsh conditions of pH, temperature, and ion concentration are required.

The hybridization chain reaction was developed by Dirks and Pierce

in 2004 [13]. This method is not dependent on enzymes and other complex conditions for detecting DNA. Shi and coworkers designed an ultrasensitive photoelectrochemical biosensor to detect HTLV-I DNA using HCR [14]. Ren's group kinetically grafted G-quadruplexes onto DNA nanostructures for detecting DNA using unlabeled HCR [15].

GO is a two-dimensional carbon crystal with one-atom thickness and a unique π - π structure. GO is also an excellent fluorescence quencher and has a higher affinity for single-stranded DNA (ssDNA) than double-stranded DNA (dsDNA) [16]. Accordingly, Zhang and coworkers developed a DNA-stabilized silver nanoclusters/graphene oxide-based platform for the sensitive detection of DNA using HCR [17]. In their study, GO was able to quench the fluorescence of the silver nanoclusters conjugated with partially complementary hairpin probes. However, in the presence of the target DNA, dsDNA was produced by HCR and this DNA detached from the GO, resulting in fluorescence recovery. This platform could, therefore, be used for novel testing strategies.

Paper-based microfluidics or “paper chips” comprise a popular strategy and widespread use for DNA detection [18]. Gong *et al.* analyzed DNA directly by using ion concentration polarization on a paper

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chip [19]. Rosenfeld and Bercovici detected pM concentrations of DNA in a paper-based microfluidic device using electroosmotically balanced isotachopheresis [20]. Li and coworkers tested DNA directly on a paper chip with fluorescence resonance energy transfer (FRET) [21]. We, therefore, decided to pursue the strategy of detecting DNA by integrating HCR with FRET on a GO-based paper chip.

Here, we developed a novel, enzyme-free FRET assay that could simultaneously detect two different DNA targets on a single GO paper chip. We designed hairpin probes to detect allergen genes of the peanut (H1, H2) and soybean (H3, H4). Cy3 was included at the 3' and 5' ends of H1 and H2, respectively, and FAM was included at the 3' and 5' ends of H3 and H4 respectively. In the absence of target DNA, the hairpin probes remained in the hairpin state, and the probe segment labeled with the fluorophore was absorbed onto the GO surface resulting in fluorescence quenching. In the presence of the target DNA, the probes were able to initiate HCR producing long dsDNA products away from the GO surface, resulting in fluorescence recovery. This GO paper chip exhibited high specificity and sensitivity for DNA detection. The target could be clearly distinguished from mismatched sequences or non-target allergen genes of other plant species using this approach.

2. Experimental

2.1. Chemicals and materials

GO was purchased from Sinopharm Chemical Reagent Co., Ltd (China). Glass fiber paper was purchased from Shanghai Kinbio Tech Co., Ltd (China). Reaction buffer solution (50 mM CH₃COONa, 1 M NaCl, pH 7.5) for HCR was prepared in our laboratory [22]. The target DNAs were the peanut gene, Ara h 6 [3] and the soybean gene, Gly m Bd 28 K [23]. All DNA oligonucleotides were synthesized by and purchased from Shanghai Sangon Biotechnology Co., Ltd. (China). The sequences were listed in Table 1 and Table 2. The oligonucleotides were stored at −20 °C and heated to 95 °C for 5 min and gradually cooled to room temperature before use [24]. All other reagents were of analytical reagent grade and used without further purification. All solutions were prepared in ultrapure water.

2.2. Fabrication of paper chips and the container

Paper chips were fabricated from glass fiber. A common paper knife was used to cut glass fiber. Many chips were prepared. Each paper chip was 3 mm in length, 3 mm in width, and 1 mm in thickness (Fig. S1A in the Supplementary Information). The hydrophilic fiberglass chip was put into the well of the hydrophobic styrofoam (container). The container was 10 cm in length, 3 cm in width, and 2 cm in height. Twelve

wells were cut into the styrofoam with a diameter of 4.5 mm and a depth of 2 mm (Fig. S1B in the Supplementary Information). HCR products were added onto paper chips coated with GO (Fig. S2 in the Supplementary Information). The paper chips were then placed onto the container for target detection.

2.3. HCR/GO on the paper chips

Peanut hairpin probes (H1 and H2) and soybean hairpin probes (H3 and H4) were added to the HCR reaction buffer solution. The final concentrations of the probes were 0.1 μM each. Then, different concentrations of target DNA (1 μL) were added. The final reaction volume was 100 μL. The reaction proceeded at 37 °C for 2 h after which, 6 μL of the reaction products was added onto the paper chip covered with 6 μL of GO (1 mg/mL) [21]. Next they should be kept in the dark for 30 min. An inverted fluorescence microscope (Leica DM14000 B, Germany) was used to detect the fluorescence.

2.4. Agarose gel electrophoresis analysis

H1, H2, H3, and H4 (0.1 μM each) were heated at 95 °C for 5 min and then cooled to room temperature. Before electrophoresis, the target DNA T_p (0.1 μM) was hybridized with H1 and H2 for 2 h, and the target DNA T_s (0.1 μM) was hybridized with H3 and H4 for 2 h. When the reaction was complete, 5 μL of the DNA samples were incubated with 1 μL of 6 × loading buffer. Next, the samples were subjected to 2% agarose gel electrophoresis in 1 × Tris-acetate-EDTA (TAE) buffer at a constant voltage of 110 V. The results were photographed with a gel imaging system (Tanon 3500, Shanghai Tanon Technology Co., Ltd, Shanghai, China).

2.5. Data collection and data processing

Paper chips containing the reaction products were placed onto glass slides. The center of paper chips was selected to take pictures with an inverted fluorescence microscope (Leica DM14000 B, Germany), with a 520-nm laser (40w) as the excitation source for detecting peanut DNA, and a 480-nm laser (40w) as the excitation source for detecting soybean DNA (The detailed parameters of the fluorescence microscope were provided in supplementary material). Then the fluorescence intensity of pictures were calculated with ImageJ software. The intensity of each pixel in a picture was automatically averaged by the software. The final data represent the mean of triplicate experiments.

Table 1

Oligonucleotides for the detection of peanut allergen DNA (Note : The different colors for H1 sequence (5–3') represented for b, c, b*, and a* respectively in Fig. 1. The different colors for H2 sequence (5–3') represented for c*, b*, a, and b, respectively. The underlined letters were mismatched bases.).

DNA name	DNA sequence (5' to 3')
H1	AAACAAAGCCACACCACTGTTCAAGTGGTGTGGCTTTGTTTGCTTCATTCATTGCT-Cy3
H2	Cy3-ACCTGAACAGTGGTGTGGCTTTGTTTAGCAATGAATGAAGCAAACAAAGCCACACCA
Target (T _p)	AGCAATGAATGAAGCAAACAAAGCCACACCA
1-mismatch	AGCAATCAATGAAGCAAACAAAGCCACACCA
2-mismatch	AGCAATCTATGAAGCAAACAAAGCCACACCA
3-mismatch	AGCAATCTATGAAGCAAACAAAGCGACACCA
4-mismatch	AGCAATCTATGAAGCAAACAAAGCGTCACCA
Complete mismatch	CTTGTCAGGTCTAAGTGTTCATCAATGAG

Table 2

Oligonucleotides for the detection of soybean allergen DNA (Note : The different colors for H3 sequence (5–3') represented for b, c, b*, and a* respectively in Fig. 1. The different colors for H4 sequence (5–3') represented for c*, b*, a, and b, respectively. The underlined letters were mismatched bases.).

DNA name	DNA sequence (5' to 3')
H3	CGGAAAGGAGATTGAACTTAGCCGTATTCAATCTCCTTTCCGCTAGTTCATCATCAT-FAM
H4	FAM-TACGGCTAAGTTCAATCTCCTTTCCGATGATGAACTAGCGGAAAGGAGATTGAA
Target (T _S)	ATGATGATGAACTAGCGGAAAGGAGATTGAA
1-mismatch	ATGATGAAGAACTAGCGGAAAGGAGATTGAA
2-mismatch	ATGATGAACAACTAGCGGAAAGGAGATTGAA
3-mismatch	ATGATGAACAACCTAGCGGAAAGGTGATTGAA
4-mismatch	ATGATGAACAACCTAGCGGAAAGGTCATTGAA
Complete mismatch	CCATGCTACTTACCTAAACGCTATCTCCTCG

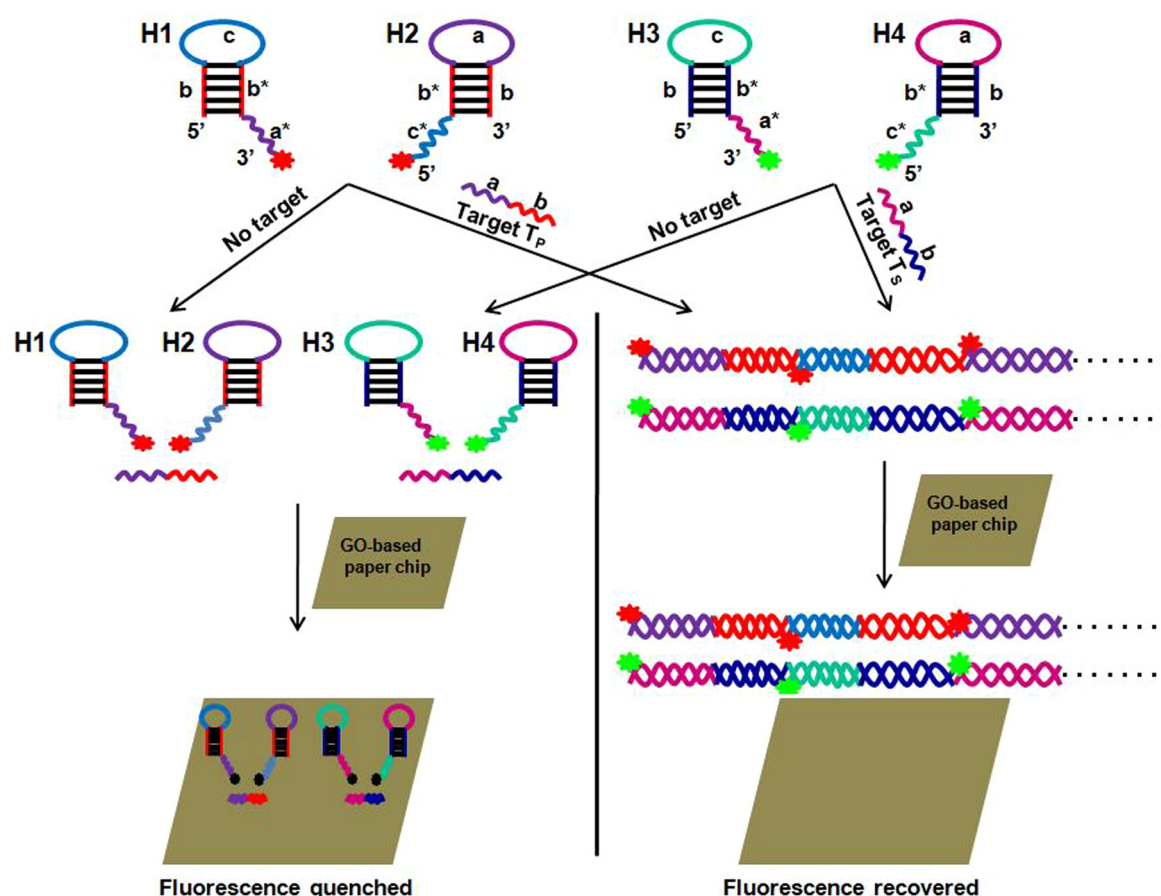


Fig. 1. Schematic of the hybridization chain reaction for the simultaneous detection of the peanut allergen gene (T_P) and the soybean allergen gene (T_S), using the hairpin probe sets H1 and H2, and H3 and H4, respectively. GO, graphene oxide.

3. Results and discussion

3.1. Principle of the HCR/GO chip for the detection of peanut and soybean DNA

In our platform, single-stranded DNA could be detected with HCR using two or more hairpin probes. Special enzymes and high reaction temperatures were not needed, with 37 °C being sufficiently high [24]. GO is known to exhibit a strong fluorescence quenching effect, which was applied here to quench the fluorescence of the fluorophore-labeled ssDNA probes in the absence of the target DNA. During the generation

of dsDNA products from the target DNA, however, the fluorophores were able to move away from the GO surface, allowing fluorescence recovery [25]. Paper chips were used as the reaction carrier because it is inexpensive and very convenient for DNA detection [26]. Using this format, we were able to simultaneously detect peanut and soybean allergen genes on a single paper chip by integrating HCR and GO. The detection process with the HCR/GO chip is depicted in Fig. 1. The detection system included the target T_P (H1 and H2) and T_S (H3 and H4) hairpin probes for the detection of peanut and soybean allergen genes, respectively. These probe sets were each partially complementary to their target DNAs. Cy3 was used to label the 3' end of segment a* of H1

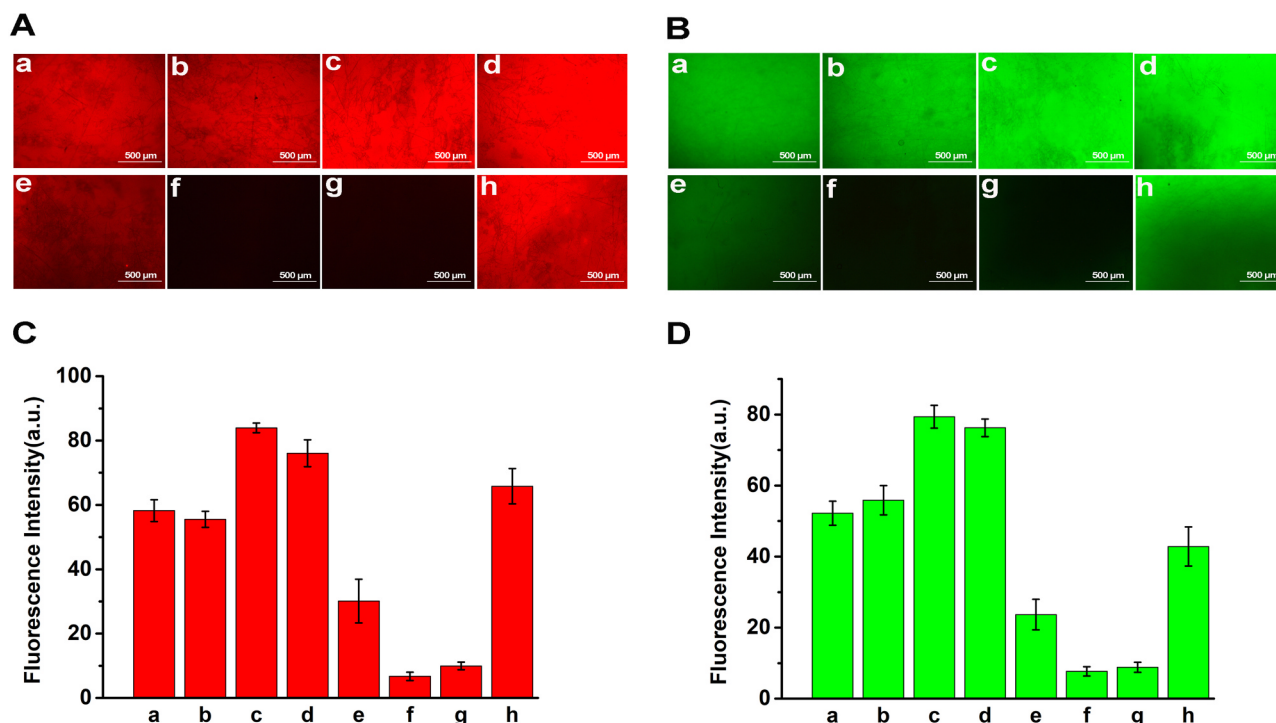


Fig. 2. Fluorescence intensities of different combinations of target DNA and probes tested on the graphene oxide (GO)-coated paper chips. (A, B) Inverted fluorescence microscopy images of the paper chips for the detection of peanut (A) and soybean (B) allergen genes. (C, D) The different reactant combinations for peanut (C) and soybean (D) detection. For peanut, a–h: H1, H2, H1 + H2, H1 + H2 + T_p, T_p + H1 + GO, T_p + H2 + GO, H1 + H2 + GO, and T_p + H1 + H2 + GO, respectively. For soybean, a–h: H3, H4, H3 + H4, H3 + H4 + T_s, T_s + H3 + GO, T_s + H4 + GO, H3 + H4 + GO, and T_s + H3 + H4 + GO, respectively. The concentrations of H1–H4, T_p, and T_s were 0.1 μM each. The concentration of GO was 1 mg/mL. Values represent the mean ± SD, n = 3.

and the 5' end of c* of H2. FAM was used to label the same segments in H3 and H4. Segments b and b* of each probe were complementary to each other, allowing the formation of a stable hairpin structure. Segments a* and b* of H1 were complementary to regions (labeled “a” and “b”) on the target DNA, T_p. Similarly, the same segments of H3 were complementary to the target DNA, T_s. In the presence of T_p/T_s, HCR was initiated through complementary binding between these probe segments and the target DNA, resulting in the opening of the hairpin structures of the H1/H3 probes. Segments b and c were thereby exposed, allowing complementary binding between these segments b* and c* of the H2/H4 probes. Then, the hairpin structure of unbound H1/H3 in the solution was opened by complementary binding to the exposed a and b segments of H2/H4, allowing the reaction to repeat. At the end of this process, long dsDNAs comprising T_p–H1–H2 and T_s–H3–H4 were obtained. The long dsDNAs could detach from the GO surface, leading to fluorescence recovery. Red and green fluorescence was thus observed in the presence of T_p and T_s, respectively. In the absence of target DNA, the probes absorbed onto the GO surface through π – π stacking and underwent fluorescence quenching. Using this method, peanut and soybean DNA could be simultaneously detected on a single chip.

3.2. Fluorescence spectra obtained with the HCR/GO chip platform

Using the principle illustrated in Fig. 1, we designed the different combination hairpin probes, applied it to the target DNA on the HCR/GO chip, and measured the fluorescence intensities (Fig. 2). The fluorescence intensities of Fig. 2A–a, b and Fig. 2B–a, b individually on the non-GO-coated surface (55.49 on average) were significantly lower (~1.5-fold) than for Fig. 2A–c and Fig. 2B–c (81.66 on average). This was expected as the absence of GO prevented fluorescence quenching of these probes; thus, the combined probes would be expected to produce higher signals. When each probe set was combined with its respective target DNA (T_p or T_s) on the non-GO-coated surface, the fluorescence

signal remained unchanged.

In contrast, on a GO-coated surface in the presence of the target DNAs T_p or T_s and the individual probes H1 (Fig. 2A–e) or H3 (Fig. 2B–e), respectively, the fluorescence dropped significantly (26.90 on average). This fluorescence signal was the result of partial complementary binding between the target DNA and the individual probes H1 or H3, leading to the opening of the hairpin structures of these probes away from the GO surface and the recovery of the fluorescence signal. When the target DNAs T_p or T_s were combined with the individual probes H2 (Fig. 2A–f) or H4 (Fig. 2B–f), respectively on the GO surface, negligible fluorescence was observed as no complementary binding was possible between these probes and the target DNAs, resulting in fluorescence quenching.

As expected, the presence of the probe sets H1 + H2 (Fig. 2A–g) or H3 + H4 (Fig. 2B–g) alone on the GO surface similarly led to negligible fluorescence signal, because in the absence of their target DNAs, the hairpin probes were absorbed onto the GO surface.

In Fig. 2A–h and Fig. 2B–h, the fluorescence was, however, strongly recovered on the GO-coated surface when the probe sets were combined with their target DNAs (66.82 for T_p and 42.84 for T_s). The hairpin structures of H1/H3 opened following binding to the target DNA, and H1/H3 then opened the hairpin structures of H2/H4. Next, the hairpin structures of unbound H1/H3 in solution were opened by H2/H4. This process occurred repeatedly, resulting in the production of long dsDNA products that could not be absorbed onto the GO surface [27], leading to strong fluorescence recovery. Agarose gel electrophoresis confirmed that HCR had been initiated by the target DNA on the GO-based chips (Fig. S3 in the Supplementary Information). These results all indicate that the method developed in this study was practicable.

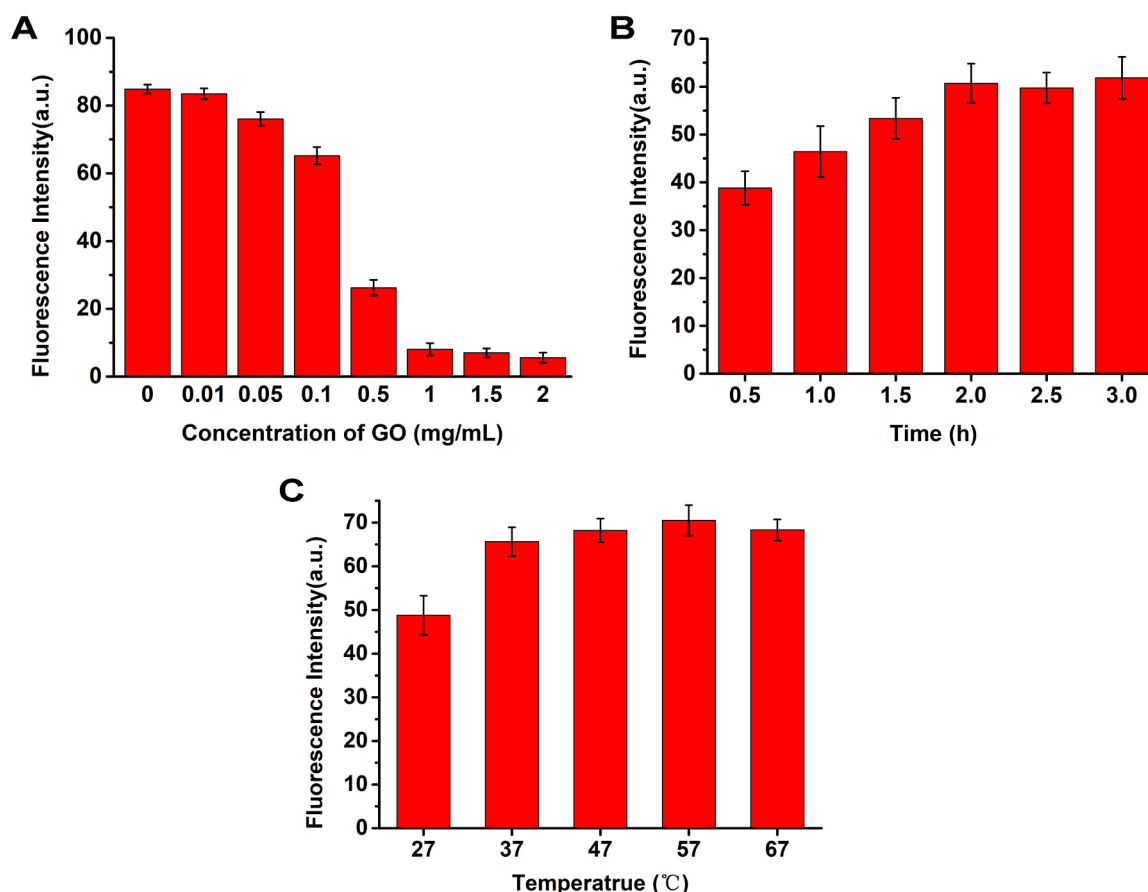


Fig. 3. The effect of graphene oxide (GO) concentration and the hybridization chain reaction time and temperature on the fluorescence intensity of peanut DNA (T_p) detection. (A) The fluorescence intensity of the H1 and H2 probes at different GO concentrations. (B) The fluorescence intensity of H1 and H2 following the detection of T_p with different reaction times. (C) The fluorescence intensity of H1 and H2 following the detection of T_p at different temperatures. The concentrations of H1, H2, and T_p were 0.1 μ M. Values represent the mean $\pm$ SD, $n = 3$.

3.3. Optimization of GO concentration and HCR reaction time and temperature

We found that the fluorescence quenching efficiency was affected by the GO concentration used to coat the chip surface before the addition of the HCR products (Fig. 3A). When the GO concentration was low, the fluorescence of the hairpin probes was not effectively quenched, resulting in a very strong background signal. When the GO concentration was too high, the fluorescence of the hairpin probes was strongly decreased even in the presence of the target DNA, reducing the sensitivity of the assay [28]. When the GO concentration reached 1 mg/mL, ~95% of the fluorescence was quenched to a similar intensity to that of the control, and a higher GO concentration did not produce more effective quenching. We, therefore, selected 1 mg/mL GO to perform all further experiments.

The HCR time and temperature were also found to be important. When investigating the effect of these parameters on peanut DNA detection, we found 2 h and 37 °C to be optimal (Fig. 3B,C). The fluorescence intensity did not significantly improve with longer reaction times or higher temperatures. The same parameters were found to be optimal for the detection of soybean DNA (Fig. S4 in the Supplementary Information).

3.4. Detection limit of the HCR/GO chip for peanut and soybean DNA

Different target concentrations (100, 50, 20, 10, 8, 6, 4, 2, 1, and 0 nM) were prepared and the fluorescence intensity of the products was tested after the HCR ended (Fig. 4). A linear relationship ($R^2 = 0.997$) was observed between the fluorescence intensity and target

concentrations from 0 nM to 10 nM, with the fluorescence intensity gradually increasing with increasing target concentration. The linear regression equations of the insets in Fig. 4A and Fig. 4B were $FL = 6.21C_{T_p} + 4.99$ ($R^2 = 0.997$) and $FL = 3.67C_{T_s} + 2.87$ ($R^2 = 0.997$), respectively. The detection limit for both peanut and soybean DNA was 1 nM, which was close to 1.18 nM detection limit reported by Zhang and coworkers [17]. This results indicated that the detection system could sensitively detect the target peanut and soybean ssDNA.

3.5. The specificity of the HCR/GO chip for peanut and soybean DNA detection

To demonstrate the specificity of the present detection system, the following sample DNAs were selected: peanut, soybean, sesame, pistachio, and walnut (The DNA sequences of sesame, pistachio, and walnut were provide in supplementary material Table S1). Water was used as the negative control. The specificity was verified by testing two sample DNAs per chip (Fig. 5). The fluorescence intensities of non-target DNAs were as low as the background, clearly indicating the specificity of our detection method.

3.6. Analysis of mismatched bases

Mismatched bases occur frequently in organisms and can lead to disease. Detecting mismatched bases is important for discovering single nucleotide polymorphisms (SNP), gene mutations, and DNA damage and so forth [17]. To determine the effect of mismatched bases on target detection with our platform, we designed different mutated variants of the target DNAs. This experiment included completely

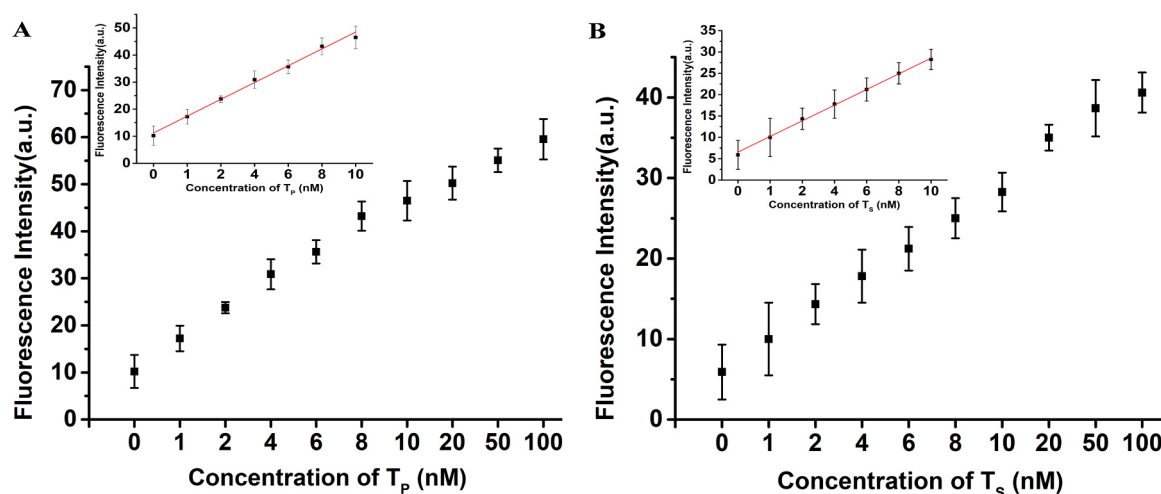


Fig. 4. The detection limit for peanut and soybean target DNA was determined to be 1 nM each. The fluorescence intensities resulting from different concentrations of peanut DNA (A) and soybean DNA (B) were measured. The concentrations of H1–H4 were 0.1 μ M. The insets indicate the linear detection range (0–10 nM) of the assays. Values represent the mean $\pm$ SD, $n = 3$.

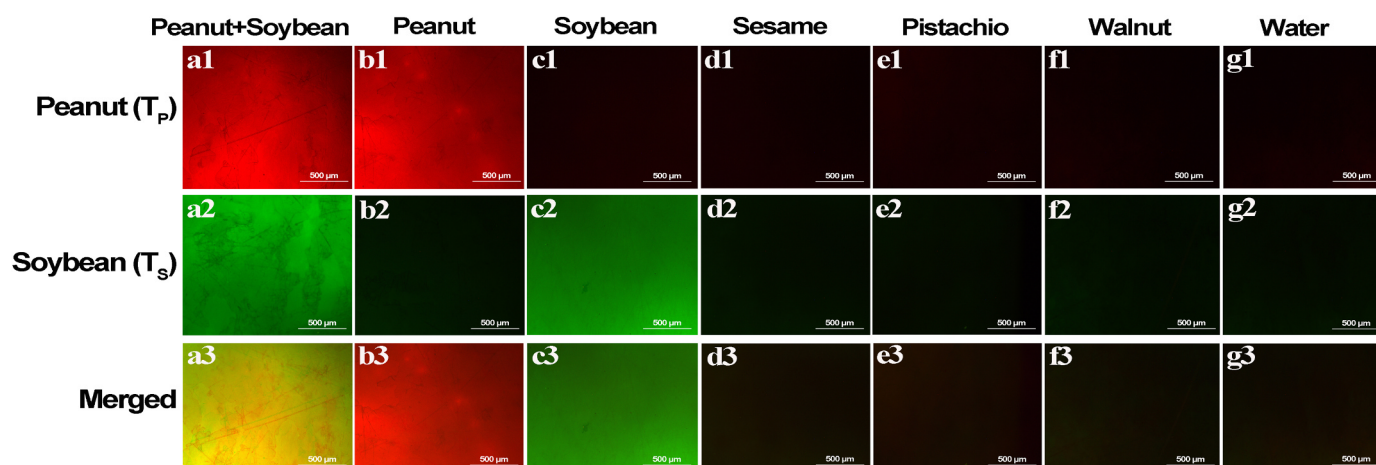


Fig. 5. The specificity of peanut and soybean target DNA detection on the graphene-coated chips. Inverted fluorescence microscopy was performed with excitation at 520 nm (top panel) for the detection of reaction products from the peanut probes H1 and H2, and with excitation at 480 nm (middle panel) for the detection of reaction products from the soybean probes H3 and H4. The respective merged images are also shown (bottom panel). The samples tested were: a, soybean and peanut allergen genes; b, peanut only; c, soybean only; d–f, sesame, pistachio, and walnut allergen genes, respectively; and g, water (negative control). The concentrations of H1–H4, T_p , T_s , and the other sample DNAs were 0.1 μ M. The graphene oxide concentration used to coat the chips was 1 mg/mL.

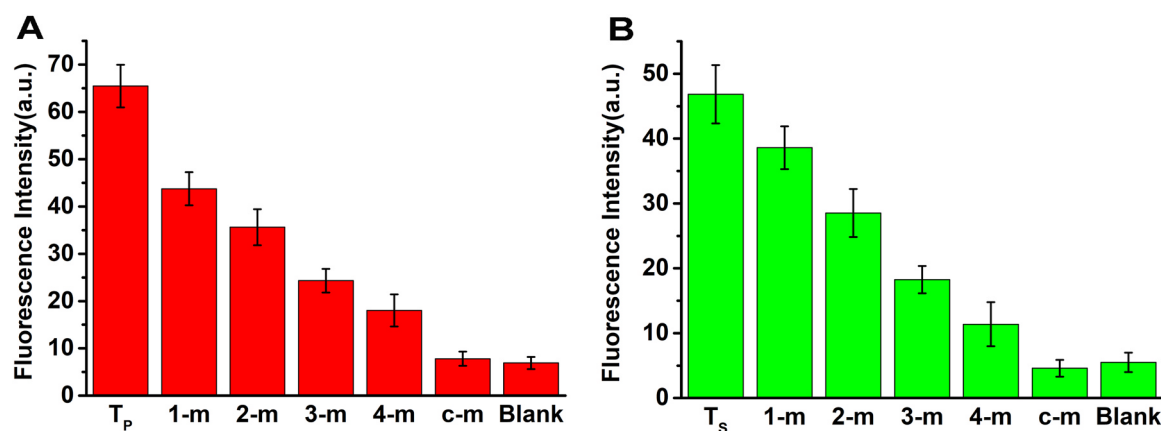


Fig. 6. The effect of mismatched bases on target DNA detection. A series of mismatched variants of the peanut target DNA (A) and soybean target DNA (B) were designed and the resulting fluorescence intensities were detected: T_p , completely matched peanut target DNA; T_s , completely matched soybean target DNA; 1-m to 4-m, single-base, two-base, three-base, and four-base mismatched target DNAs; c-m, completely mismatched target DNAs; Blank, water. The concentrations of H1–H4, T_p , T_s , and the other target DNAs were 0.1 μ M. The graphene oxide concentration used to coat the chips was 1 mg/mL. Values represent the mean $\pm$ SD, $n = 3$.

matched target DNA (T_p and T_s), single-base mismatched DNA (1-m), two-base mismatched DNA (2-m), three-base mismatched DNA (3-m), four-base mismatched DNA (4-m), completely mismatched DNA (c-m), and the blank. The fluorescence intensities of mismatched DNA targets were 66.81%, 54.40%, 37.14%, 27.50%, 11.93% of T_p (Fig. 6A), and 82.41%, 60.91%, 39.98%, 24.30%, 9.80% of T_s (Fig. 6B). The results indicated that the target DNA could be clearly distinguished from mismatched DNA using our method, which would be beneficial for the analysis of SNPs [29].

4. Conclusions

Based on the HCR principle and the quenching efficiency of GO, we developed an assay for the simultaneous detection of peanut and soybean allergen genes on a single chip with the use of a combination of red and green fluorescence-labeled probes. This method could distinguish peanut and soybean DNA from other non-target DNA (sesame, pistachio, and walnut). Furthermore, mismatched DNA could be clearly distinguished. Our assay could, therefore, be beneficial for the detection of single nucleotide polymorphisms, gene mutations, and DNA damage. The detection limit of our assay was 1 nM for both peanut and soybean DNA. This assay is simple to perform and the HCR reaction could be carried out at 37 °C without enzymes and harsh reaction conditions. The paper chips and container were easy to fabricate and could, therefore, be rapidly manufactured. The chips and substrates used were very cheap, making the assay very inexpensive to perform. However, it should be noted that this method was performed in well controlled conditions and the real-world application could be hard. The method can only be applied to the detection of single-stranded DNA but not double-stranded DNA. Therefore, our future studies will focus on developing double-stranded DNA detection with HCR and applying the method to the real-world conditions.

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Conflict of interest

The authors declare that there is no conflict of interest.

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.talanta.2018.12.036.

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Supplementary Information

A Graphene Oxide-Based Paper Chip Integrated with the Hybridization Chain
Reaction for Peanut and Soybean Allergen Gene Detection

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32 **1. Imaging analysis of paper chips**

33 The center of paper chips was selected to take pictures with an inverted
34 fluorescence microscope (Leica DM14000 B, Germany). The parameters were set as
35 follows. The objective lens magnification was 5×. The shutter time was 399 ms. The
36 gain was 4.9×. The saturation was 1.95. The gamma was 1.15. Then the fluorescence
37 intensity of pictures was calculated with ImageJ software. The intensity of each pixel
38 in a picture was automatically averaged by the software.

39 **2. The DNA sequences of sesame, pistachio, and walnut**

40 **Table S1. The DNA sequences of sesame, pistachio, and walnut**

DNA name	DNA sequence (5' to 3')
Sesame	TGAGGAACGTGGACGAGAGGTGCCGCTGTGA
Pistachio	CAAAAGCAGTTCGAGGAGCAGCAAAAATTCA
Walnut	CAGATTCAACGCCAGCAAAACCTCAACCACT

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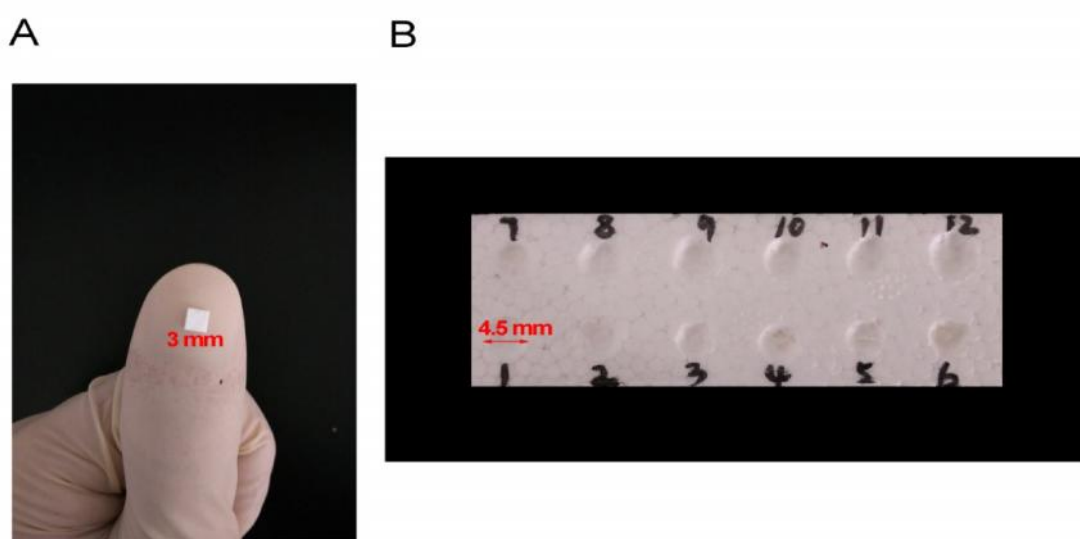


Fig. S1. Paper chip (A) and reaction substrate (B).

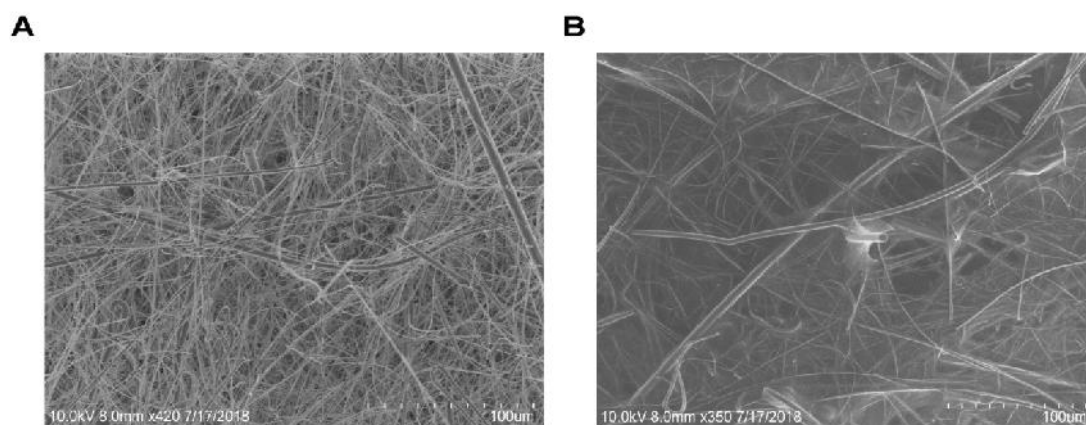
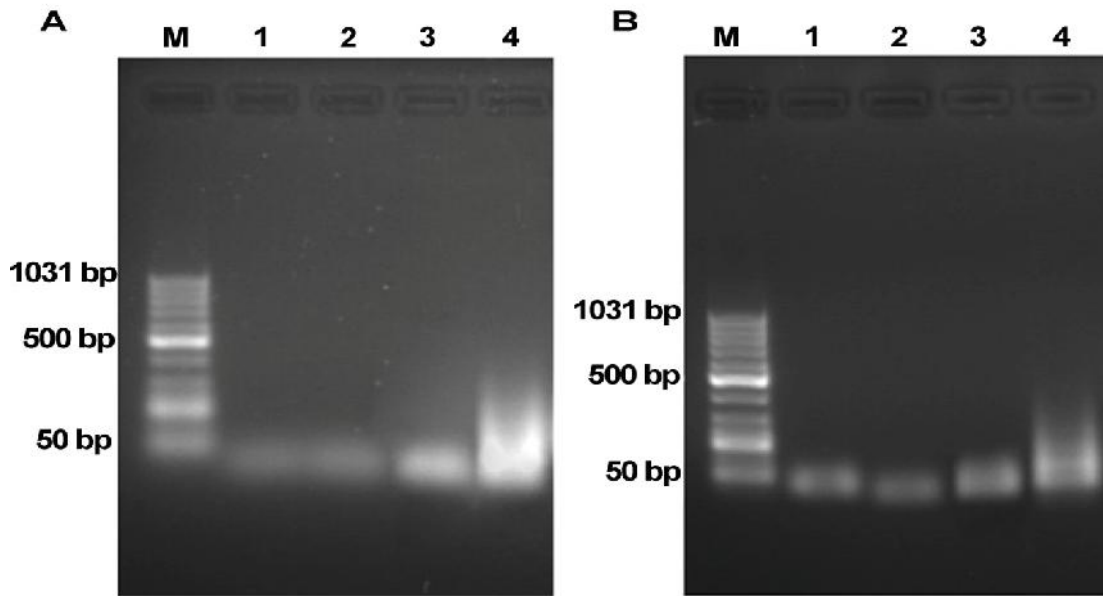


Fig. S2. Scanning electron microscope (SEM) characterization of the paper chip (A) and the GO-coated paper chip (B).

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69 **Fig. S3.** Agarose gel electrophoresis validation of the hybridization chain reaction

70 (HCR) products. (A) HCR products from the detection of peanut target DNA. M,

71 DNA marker; lanes 1–4, H1, H2, H1+H2, and H1+H2+T_p. (B) HCR products from

72 the detection of soybean target DNA. M, DNA marker; lanes 1–4, H3, H4, H3+H4,

73 and H3+H4+T_s. All reactant concentrations were set at 1 μ M.

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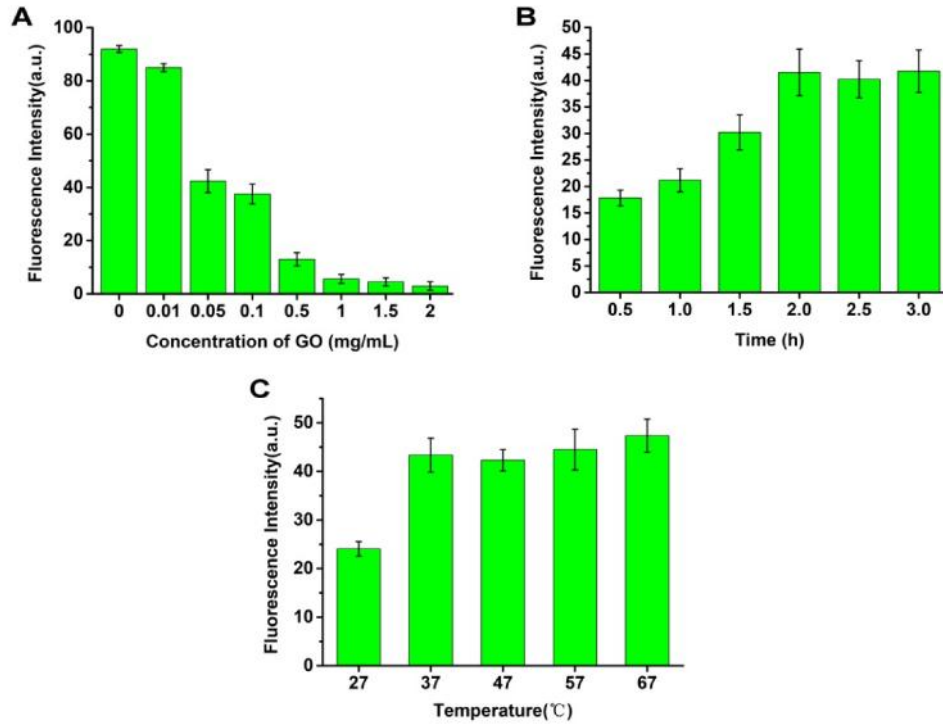


Fig. S4. The effect of graphene oxide (GO) concentration and the hybridization chain reaction time and temperature on the fluorescence intensity of soybean (T_S) detection. (A) The fluorescence intensity of the H3 and H4 probes at different GO concentrations. (B) The fluorescence intensity of H3 and H4 following the detection of T_S with different reaction times. (C) The fluorescence intensity of H3 and H4 following the detection of T_S at different reaction temperatures. The concentrations of H3, H4, and T_S were 0.1 μ M. Values represent the mean $\pm$ SD, $n=3$.