Electronic Supplementary Information

Target-induced Molecular-Switch on Triple-helix DNA-functionalized Carbon Nanotube for Simultaneous Visual Detection of Nucleic Acid and Protein

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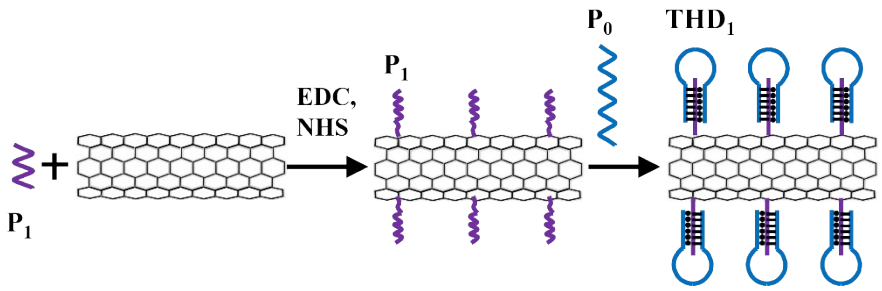
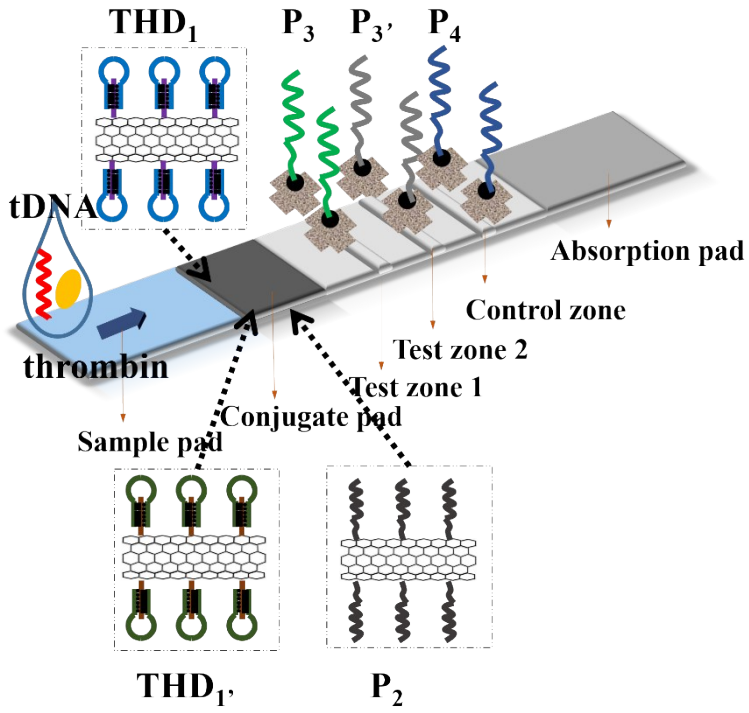


Scheme S1 Schematic illustration of the construction of the triple-helix DNA-functionalized carbon nanotube



Scheme S2 Schematic illustration of lateral flow **nucleic acid** biosensor for visual detection of DNA and thrombin.

Table S1 Sequences of target DNA and different probes used in this work

Name	Sequence 5'—3'
Assistant probe P_0	CTCTCTCATGTGGAAAATCTCTAGCAGTCTCTCTC
Assistant probe P_0'	TCCCTTTGGTTGGTGTGGTTGGTTTCCCT
Amine-modified reporter DNA P_1	Amino / AAGAGAGAG
Amine-modified reporter DNA P_1'	Amino / AGAGGGAAA
Amine-modified reporter DNA P_2	Amino / GTCTGTCAA
Biotinylated capture DNA probe P_3	Biotin / CTCTCTCTT
Biotinylated capture DNA probe P_3'	Biotin / TTTCCCTCT
Biotinylated capture DNA probe P_4	Biotin / TTGACAGAC
Target DNA	ACTGCTAGAGATTTTCCACAT
Single-base mismatch	ACTGCTAGAAATTTTCCACAT
Three-base mismatch	ACTACTAGAAATTTTTCACAT
Irrelevant strand	ATTGAATAAGCTGGTA

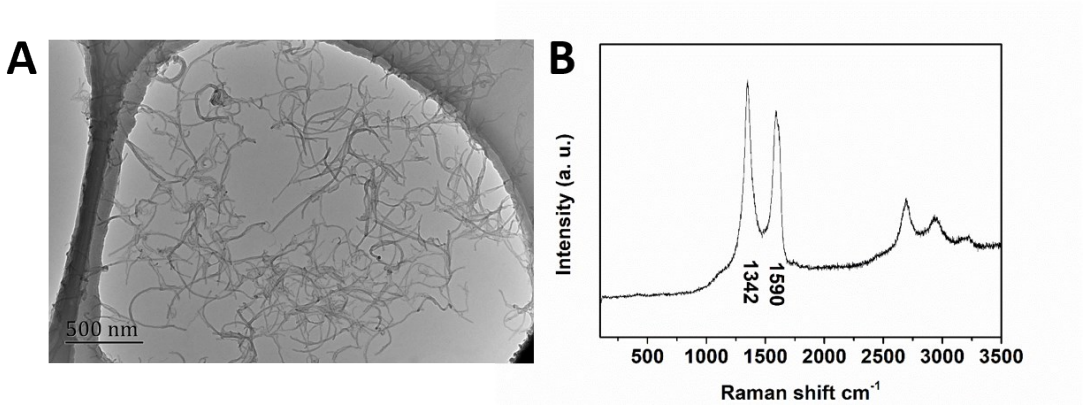


Figure S1 (A) TEM image and (B) Raman spectrum of shortened multi-wall CNTs.

The morphologies of the shortened MWCNTs were characterized by transmission electron microscope (TEM). One can see the shortened CNTs have a length of 0.5 to 3 μm (Figure S1A). Figure S1B presents the Raman spectrum of the shortened CNT. The dominant peak at ~1342 cm^{-1} of CNT can be attributed to the D band of CNTs, while another intense peak at ~1590 cm^{-1} can be ascribed to the G band of CNTs.

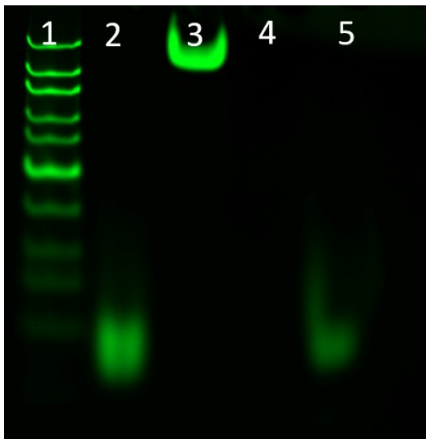


Figure S2 Polyacrylamide gel electrophoresis. Lane-1: Ladder (10 – 300 bp); Lane-2: Detection probe + complementary DNA; Lane-3: CNT-DNA conjugate + complementary DNA; Lane-4: CNTs; Lane-5: CNT + detection DNA probe +complementary DNA.

Figure S2 presents the typical polyacrylamide gel electrophoresis image. One can see that the formed duplex DNA in lane 2 (without CNT) had greater mobility than that in lane 3, in which the duplex DNA were formed on the CNT surface. Such mobility difference was caused by the formation of CNT-duplex DNA complexes, which had larger mass than the free duplex DNA and slow down its movement in the polyacrylamide gel. Control experiments were performed by adding pure CNTs (without conjugation with the detection DNA probe) in lane 4 and the mixture of CNTs, detection DNA probe and complementary DNA in lane 5. It can be seen that there is no band observed in lane 4, and a bright band at the same position as in lane 2 is shown in lane 5. The above results confirmed that the detection DNA probes were conjugated with CNTs successfully.

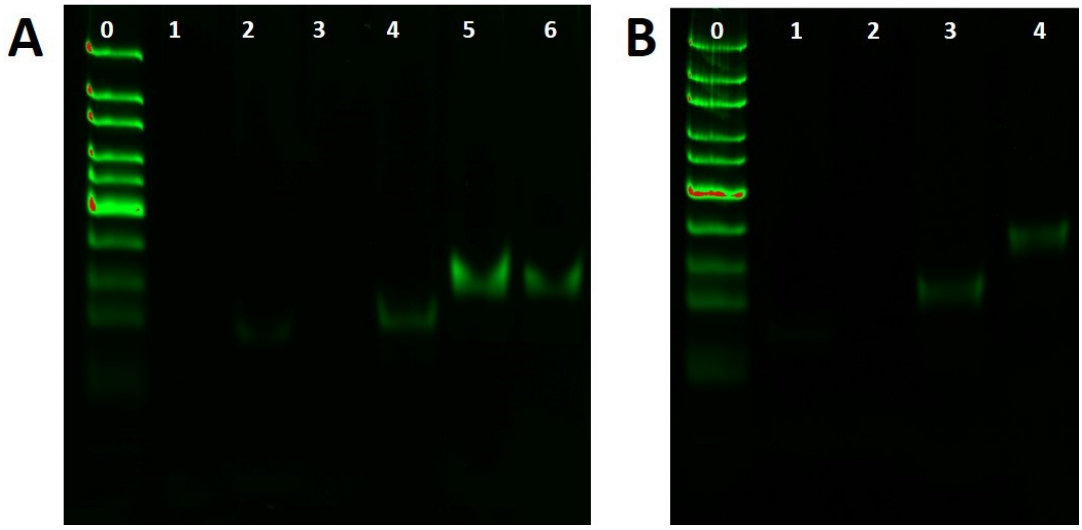


Figure S3 Polyacrylamide gel electrophoresis analysis of (A) 2 pmoles of DNA marker (lane 0), NH2-DNA (P1, lane 1), P0 (lane 2), single strand target DNA (lane 3), P0+P1 (Triplex DNA, lane 4), P0+P1+Target DNA (lane 5), P0+Target (lane 6); (B) 2 pmoles of DNA marker (lane 0), P0' (lane 1), NH2-DNA (P1', lane 2), P0'+ P1' (Triplex DNA, lane 3), P0'+ P1' + thrombin (Triplex DNA, lane 3).

In order to further verify the process, polyacrylamide gel electrophoresis was performed to analyze the DNA composition of different condition. In Figure S2A, amine-modified DNA P_1, P_0 probe and target DNA was put in lane 1, lane 2 and lane 3, respectively. The mixture solution after reaction of P_0, P_1, without/with target were put in lane 4 and lane 6, respectively. P_0 and target mixture was put in lane 6. No bands were observed in lane 1 and lane 3 because the two DNA sequence is too short to be dyed. A relatively light green band in lane 2 indicating the existence of P_0. One can see a bright band in lane 4 that moved slower than lane 2, which proved the formation of the THD. The bright bands in lane 5 and lane 6 with worse mobility than that in lane 4 were the duplex DNAs formed by P_0 and target, thus P_1 would be released from the triplex DNA and worked as a signal probe. In Figure S2B, P_0', P_1', P_0'+ P_1', P_0'+ P_1'+thrombin was put in lane 1, lane 2, lane 3 and lane 4, respectively. Also a light green band in lane 2 indicating P_0'. A bright slower band in lane 3 represents the THD probe. Upon the addition of thrombin to THD probe, a conformational change happened and aptamer/target conjugate was formed, leading to the corresponding band shifted to a higher molecular weight position compared to THD band.

Experimental Section

Apparatus and Reagents

A high-resolution FEI F30 transmission electron microscope (TEM; Tokyo, Japan) was used for images taking of the CNTs. Raman spectrums were measured using an inVia-Reflex confocal Raman microscope (Renishaw, England). The XYZ3010 dispenser, and the Guillotine cutting module CM 3010 purchased from Jiening Biotech. Co., Ltd. (Shanghai, China) were used to prepare LFNABs. A portable strip reader DT1030 (Shanghai Goldbio Tech. Co.) was used for signal recording. Canon 650D camera (Canon, Japan) was used to take the photo images of lateral flow strip biosensors.

Human α-thrombin, N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC), N-hydroxysulfosuccinimide (sulfo-NHS), 2-(4-Morpholino) ethanesulfonic acid (MES), sucrose, Tween 20, bovine serum albumin (BSA) and phosphate buffer saline (PBS, 0.01 M, pH 7.4) were purchased from Sigma-Aldrich (St. Louis, MO). Fusion 5 fiber, glass fibers 8965, cellulose fiber H-1, and PVC laminated cards were purchased from Jiening Biotech. Co., Ltd. (Shanghai, China). Nitrocellulose membranes (HFB18004) were purchased from Millipore (Billerica, MA). Streptavidin, Rabbit IgG and SYBR Green I dye were purchased from Sangon Biological Engineering Technology & Co. Ltd. (Shanghai, China). Alpha fetoprotein (AFP) was purchased from Fitzgerald (Acton, MA, USA). Carbohydrate Antigen 125 (CA 125) recombinant protein were purchased from MyBioSource (San Diego, CA, USA). The oligonucleotides were purchased from Sangon Biological Engineering Technology & Co. Ltd. (Shanghai, China) and purified using high-performance liquid chromatography. The sequences were shown in Table 1.

All the chemicals used in this study were analytical reagent grade. Solutions were prepared with ultrapure (Z18 MΩ) water from Millipore Milli-Q water purification system (Billerica, MA, http://www.merckmillipore.com).

Preparation of carbon nanotubes (CNTs)

Ten milligrams of multiwalled CNTs were treated with 4.8 mL H_2SO_4 and 1.6 mL HNO_3 under vigorous ultra-sonication for 3 h. The shortened CNTs was centrifuged 20 min at 6000 rpm min^{-1}, washed with water several times until the solution was neutral. During the washing steps, the shortened CNTs suspension was centrifuged 40 min at 14500 rpm min^{-1}. The collected pellet in the final washing step was suspended in 10 mL water for further use.

Preparation of CNT–DNA conjugates

The CNT-DNA were prepared according to the reported methods with slight modifications.[1] The shortened carboxylated MWCNTs (0.5 mL) was spun down. The supernatant was discarded and the precipitation was mixed with 9.6 mg EDC and 5.43 mg sulfo-NHS in 1.0 mL MES buffer (0.1 M, pH 4.7). After shaking at room temperature for 30 min, the mixture was washed by centrifuging at 14,500 rpm for 20 min. Discard the supernatant and resuspend the pellet in PBS buffer. Repeat the above operations/treatments three times to remove the excess reagents. The amine-modified DNA

detection probe P_1 or P_1' or P_2 was then added to the activated MWCNT solution with a final concentration at 0.1 OD mL^{-1} and the solution was incubated overnight at room temperature. This mixture was centrifuged at 14500 rpm for 20 min. The supernatant was discarded and the pellet was resuspended in PBST for further processing.

Preparation of CNT–Triple helix DNA conjugates

CNT–Triple helix DNA conjugates were prepared as follows using the procedure reported by Zheng et al[2]. one The THD_1 or THD_2 conjugates was obtained by reacting an equal volume of the above CNT–DNA conjugate (CNT-P_1 or CNT-P_1') and 1 μM single-stranded DNA P_0 or P_0' in 20 mM PBS (2.5 mM $MgCl_2$, 0.1 M NaCl, pH 6.0) for 90 min. The supernatant was discarded and the pellet was resuspended in PBST. After repeating the above step 3 times, the pellet was resuspended in 1 mL eluent buffer containing 20 mM $Na_3PO_4 \cdot 12\ H_2O$, 5% BSA, 10% sucrose, and 0.25% Tween-20. The CNT–DNA conjugate solution was stored at 4 °C before further use.

Preparation of streptavidin-biotin-DNA conjugates

One hundred nanomoles of biotinylated DNA probe (capture DNA probe P_3 or P_3' or P_4) was mixed with 2.5 mg mL^{-1} streptavidin and incubated at room temperature for 1 h. After adding 500 μL PBS to the mixture, the new solution was centrifuged using a centrifugal filter (molecular retention 30000) for 20 min at 6000 rpm at 4 ℃ to remove the unbound DNA. The above step was repeated for 3 times. The remaining solution in filter was diluted with PBS and used for dispensing.

Preparation of lateral flow nucleic acid biosensor (LFNAB)

A lateral flow nucleic acid biosensor (LFNAB) consists of the following components: sample application pad, conjugate pad, nitro-cellulose membrane, and absorbent pad. The sample application pad (17 mm × 30 cm) was made from glass fibers 8965. 1.5 μL of the CNT–THD_1 solution, 1.5 μL of the CNT–THD_1' solution and 1.5 μL of CNT-P_2 conjugates solution were dispensed on the glass fiber conjugate pad with an Airjet dispenser. Streptavidin-biotin-capture DNA probe P_3, streptavidin-biotin-capture DNA probe P_3' and streptavidin-biotin-capture capture DNA probe P_4 solutions were dispensed on the nitrocellulose membrane by the XYZ3010 dispenser to form the test and control zones, respectively. After dispensing, the conjugate pad and nitrocellulose membrane were dried at 37 °C for 1 h before assembly. Finally, all the components were assembled onto a plastic adhesive backing (60 mm × 30 cm) using a clamshell laminator. Each part overlapped 2 mm to ensure the solution could migrate through the strip during the assay. Strips with 3 mm width were cut by the Guillotin cutting module CM 3010.

Sample assay procedure

Sample solutions with di□erent concentrations of target DNA and proteins were prepared in the running bu□er (5×SSC + 4% BSA). In a typical assay, 100 μL of sample solution was applied onto the sample pad, then the solution migrated toward absorption pad. During the assay process, the solution migrated up by capillary force. 10 minutes later, another 70 μL of running buffer was applied to the sample pad to wash the strip and facilitate the flow of the conjugate. The test zones and control zone were evaluated visually within 20 min. For quantitative measurements, the

intensities of the test line and control line were recorded by a portable strip reader.

References

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(2) Zheng, J.; Hu, Y.; Bai, J.; Ma, C.; Li, J.; Li, Y.; Shi, M.; Tan, W.; Yang, R. *Anal Chem* **2014**, *86*, 2205.