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Electronic Supporting Information for the Article

A post-labeling strategy based on dye-induced peeling of aptamer off single-walled carbon nanotubes for electrochemical aptasensing

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Experimental section

Materials and apparatus. All electrochemical experiments were conducted on a CHI660C electrochemical workstation (CH Instruments Inc., Austin, TX), and a conventional three-electrode electrolytic cell was used. Au disk electrodes with 3.0-mm diameter (0.07 cm^2 area) served as the working electrode, a KCl-saturated calomel electrode (SCE) as the reference electrode, and a carbon rod as the counter electrode. All potentials here are cited versus SCE. Atomic force microscopy (AFM) images were collected on a PicoPlus atomic force microscope (Molecular Imaging Co., USA). The UV-Vis spectra were recorded on a UV-2450 spectrophotometer (Shimazu, Japan). A computer-interfaced HP4395A impedance analyzer was employed in the QCM experiments, and 9 MHz QCM Au electrodes of a keyhole configuration and 0.29 cm^2 area (Beijing Chenjing Electronic Co., China) were used.

Human α-thrombin was purchased from Sigma. 25-Base apt I (5'-NH_2-$(CH_2)_6$-T_{10}GGTTGGTGTGGTTGG-3'), 45-base apt II (5'-$(TC)_{10}$AGTCCGTGGTAGGGCAGGTTGGGGTGACT-3'), and 25-base thiolated thrombin aptamer (apt III, 5'-SH-$(CH_2)_6$-T_{10}GGTTGGTGTGGTTGG-3') were purchased from Sangon Co., Ltd. (Shanghai, China). SWNTs were purchased from Chengdu Inst. Org. Chem., Chinese Acad. Sci., China. Human blood sera were kindly gifted by the Hospital of Hunan Normal University. 50 mM Tris-HCl buffer containing 100 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, and 1 mM $CaCl_2$ (pH 7.4, denoted below as Tris-HCl buffer for short) was used for binding and rinse, and 0.10 M phosphate buffer solution (PBS, pH 7.4, 0.10 M KH_2PO_4–K_2HPO_4 + 0.10 M

K_2SO_4) was used for electrochemical experiments. All other chemicals were of analytical grade or better quality. Milli-Q ultrapure water (Millipore, ≥ 18 MΩ cm) was used throughout.

Preparation of apt I@MNPs. First, Fe_3O_4 MNPs were prepared using a reported method of chemical codeposition.[1] After rinsed with water, ethanol, and water each three times in an external magnetic field, the yielded MNPs material was dispersed in 0.2 M citric acid, and this suspension was sonicated for 1 h and then stirred for 12 h at room temperature. This procedure can functionalize the MNPs surface with –COOH groups through chemical adsorption of citric acid on iron oxides.[2] Afterward, the MNPs-COOH were rinsed with water three times in an external magnetic field and dispersed in the PBS buffer (0.1 mg mL^{-1} MNPs-COOH), to which 10 mM 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC) and 10 mM *N*-hydroxysuccinimide (NHS) were subsequently added, and this suspension was sonicated for 1 h to activate the -COOH group. 1 μM apt I was then added, and this suspension was stirred for 12 h to ensure the covalent coupling of apt I to MNPs, followed by addition of 1 mM ethanolamine and stirring for another 2 h. After rinsed with PBS in an external magnetic field, the yielded apt I@MNPs (0.9 mg mL^{-1}) were stored at 4 ^{o}C prior to use.

Preparation of apt II@SWNTs. The apt II@SWNTs were prepared according to Zheng's method with minor modification.[3] First, 1 mg mL^{-1} SWNTs were dispersed in water and shortened by 5-h ultrasonication; then 1 mg mL^{-1} apt II and 0.1 M NaCl were added, and this suspension was kept in an ice-water bath and sonicated for 2 h at

a power of ca. 40 W,[4] yielding a homogeneous black suspension. Afterward, the suspension was ultracentrifuged for 40 min at 13,000 *g* to remove SWNTs aggregates, and the supernatant was collected and similarly centrifuged twice. Subsequently, apt II@SWNTs in the supernatant were filtered and collected through a Millipore centrifugal filter (YM-100) to remove excessive apt II not wrapping around the SWNTs. After rinsed with water 5 times, the apt II@SWNTs were re-suspended in ultrapure water (0.3 mg mL^{-1}) and stored at 4 ^{o}C prior to use.

Construction of the aptasensor and detection of thrombin. Bare Au electrodes were cleaned according to the reported protocol.[5] Briefly, the Au electrodes were carefully polished in 0.5- and 0.05-μm alumina suspensions in sequence. After thoroughly rinsed with water, the polished electrodes were ultrasonically treated sequentially in water, ethanol, and water each for 5 min to remove residual alumina powder, respectively. Then, the Au electrodes were treated with Piranha solution (H_2SO_4:H_2O_2, v/v 3:1, highly oxidizing and corrosive, treat with great care) for 15 s. Afterward, the Au electrodes were subjected to electrochemical rinse in 0.50 M H_2SO_4 to thoroughly remove impurities, namely, potentiostatic treatments at 2 V for 5 s and at −0.35 V for 10 s, and potential cycling for 6 cycles from −0.35 to 1.55 V at 4 V s^{-1}. The cleaned Au electrode was finally characterized by cyclic voltammetry from -0.35 to 1.55 V in 0.50 M H_2SO_4, which showed a single sharp reduction peak at ca. 0.9 V and multiple overlapping oxidation peaks at potentials ranging from 1.2 to 1.5 V. The cleaned Au electrodes were immediately immersed into 10 mM ethanethiol (ET)/ethanol solution and kept for 10 h to ensure the full self-assembly of ET and thus

obtain a hydrophobic surface (ET/Au) which could efficiently capture carbon nanotubes through the van de Waals and hydrophobic interactions.[6]

To 12 μL Tris-HCl buffer containing thrombin at various concentrations, 1 μL 1 mg mL^{-1} BSA, 2 μL apt I@MNPs, and 5 μL apt II@SWNTs were orderly added, this mixture was incubated for 30 min at 37 ^{o}C to yield an apt I@MNPs–thrombin–apt II@SWNTs sandwich complex, and the nonspecific sites were blocked by largely excess BSA.[7] After rinsed with Tris-HCl buffer three times in a magnetic field, the yielded sandwich complex was magnetically transferred to the surface of the ET/Au electrode. This sandwich complex modified electrode was then immersed in PBS containing 1 mM MB for 30 min at room temperature to ensure replacement of apt II with MB and thus release of SWNTs from apt I@MNPs–thrombin–apt II complex. After immersed in PBS four times each for 5 min to remove un-adsorbed MB, the modified electrode was faced to an external magnetic field to remove the isolated apt I@MNPs–thrombin–apt II complex to improve the conductivity of electrode surface,[6] remaining MB-adsorbed SWNTs on the surface. Finally, DPV curves were recorded to detect MB on the electrode surface.

References

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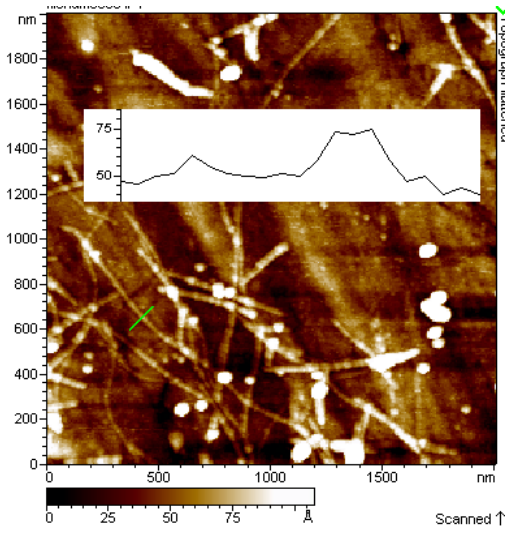

Fig. S1. AFM images of apt II@SWNTs with diameters of ca. 2.5 nm.

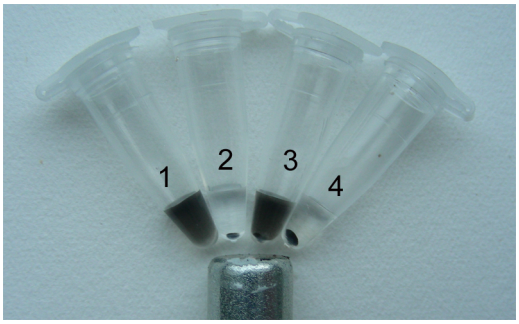

Fig. S2. Digital picture of Tris-HCl buffer containing apt II@SWNTs + thrombin (1), apt I@MNPs + thrombin (2), apt I@MNPs + apt II@SWNTs (3), or apt I@MNPs + thrombin + apt II@SWNTs (4) in a magnetic field. The concentrations of apt II@SWNTs, thrombin, and apt I@MNPs were 0.13 mg mL^{-1}, 100 nM, and 0.33 mg mL^{-1}, respectively.

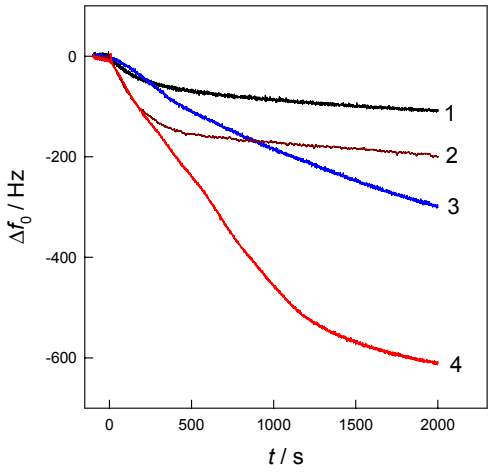

Fig. S3. QCM monitoring of thrombin binding at apt III/QCM (1) and at apt I@MNPs/QCM (2) as well as subsequent binding of apt II@SWNTs at thromin/apt III/QCM (3) and at thromin/apt I@MNPs/QCM (4). 100 nM thrombin or 0.03 mg mL^{-1} apt II@SWNTs (final concentration) was added into stirred Tris-HCl buffer at time zero. The apt I@MNPs/QCM electrode was prepared via cast-dry method, which was then exposed to 1 mg mL^{-1} BSA to block the nonspecific sites. The apt III modified QCM Au electrode was prepared via self-assembly of apt III for 12 h, and then exposed to 2-mercaptoethanol to block the unoccupied Au surface sites. We comparatively examined the binding of thrombin to thiolated apt I (apt III) or apt II modified QCM Au electrode and found comparable binding rates, the obviously slower kinetics for the binding of large-sized apt II@SWNTs to surface-captured thrombin is thus ascribed to the spacial limitation at the electrode|solution interface.

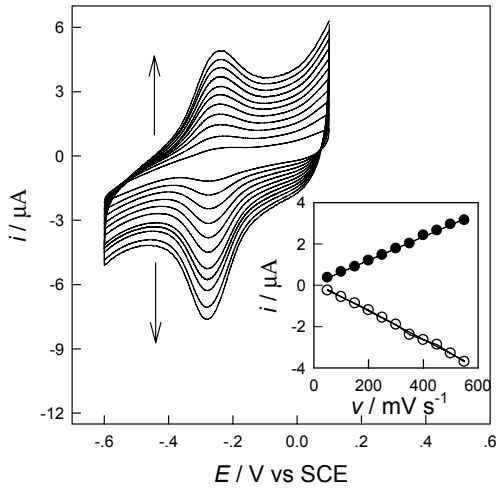

Fig. S4. Cyclic voltammetric curves of a MB-adsorbed SWNTs modified Au electrode at various potential scan rates in 0.1 M PBS. Insert: anodic and cathodic peak currents vs scan rate.

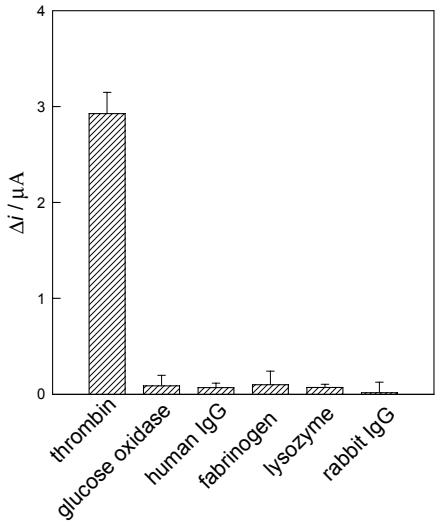

Fig. S5. The responses of the prepared aptasensor to 5 nM thrombin and several other proteins each at 0.3 μM.

Table S1. Detection of thrombin in the human blood serum substrate (n = 3) using the developed aptasensor.

Added /nM	Measured /nM	RSD /%	Recovery /%
1.00	0.96	8.3	96
2.00	2.02	7.4	101
3.00	2.71	6.9	90
4.00	3.77	9.3	94
5.00	4.81	8.5	96