Supporting Information

Aptamer-based highly sensitive electrochemiluminescent detection of thrombin via nanoparticle layer-by-layer assembled amplification labels

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Experimental Section

Materials: Thrombin, lysozyme, mouse IgG, streptavidin (STV), ethanesulfonic acid (MES), *N*-hydroxysulfosuccinimid sodium salt (NHS), *N*-(3-dimethylamminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC), 3-mercaptopropionic acid (MPA), 6-mercapto-1-hexanol (MCH), Tris-HCl, $HAuCl_4$ and bovine serum albumin (BSA) were purchased from Sigma (St. Louis, MO). Monodispersed carboxyl polystyrene (PS) beads (0.2 μm in diameter) were obtained from BaseLine ChromTech Research Centre (Tianjin, China). Na_2TeO_3 was received from J&K Scientific Ltd. (Guangzhou, China). $NaBH_4$, $CdCl_2$ and trisodium citrate dihydrate were obtained from Kelong Chemical Company (Chengdu, China). Thrombin binding aptamers (TBA_1 and TBA_2), as well as the SH-polyT_{10}-biotin, were all ordered from Shanghai Sangon Biological Engineering Technology and Services Co., Ltd. (Shanghai, China), and the sequences of these oligonucleotides were as follows:

SH-ploy-T_{10}-biotin: 5'-biotin-TTTTTTTTTT-$(CH_2)_6$-SH-3';

TBA_1: 5'-HS-$(CH_2)_6$-GGTTGGTGTGGTTGG – 3';

TBA_2: 5'-biotin-AGTCCGTGGTAGGGCAGGTTGGGGTGACT-3';

All reagents were analytical grade and solutions were prepared using ultrapure water (specific resistance of 18 MΩ-cm).

Apparatus: TEM images and UV-vis spectra were recorded by a Philips TECNAI 10 microscope (Philips Fei Co., OR, USA) and a UV-Vis 8500 spectrometer (Techcomp Ltd., China), respectively. Square wave stripping voltammograms were recorded by a CHI 852C electrochemistry workstation (CH Instruments Inc., Shanghai, China). A conventional three-electrode configuration was used, with a glassy carbon working electrode (GCE, 3 mm in diameter, CH Instruments Inc., Shanghai, China), an Ag/AgCl (3 M KCl) reference electrode, and a platinum wire counter electrode. The square wave voltammetric stripping detection involved in a 1 min pretreatment at +0.6 V, 2 min accumulation at -1.1 V and scanning the potential from -1.0 to -0.3 V with a potential step of 4 mV, a frequency of 25 Hz and an amplitude of 25 mV. Data processing was made using the "linear baseline correction" function of the CHI 852C software. The ECL emission was monitored by a MPI-A electrochemiluminescence analyzer (Xi'an Remax Electronic Science & Technology Co. Ltd., Xi'an, China) in PBS (10 mM, pH 7.4) containing 0.1 M $K_2S_2O_8$ and 0.1 M KCl with the voltage of the photomultiplier tube (PMT) at 800 V in the process of detection.

Preparation of the MPA-capped water soluble CdTe QDs: Water soluble CdTe MPA-capped QDs were prepared according to the previously reported methods.[1-3] In a typical synthesis, $CdCl_2$ (36.89 mg) was first dissolved in water (50 mL), followed by the

addition of trisodium citrate dihydrate (50 mg), Na_2TeO_3 (0.01 M, 1 mL), MPA (33 μl) and $NaBH_4$ (50 mg) with stirring. Afterward, the flask containing the mixture was attached to a condenser and refluxed at 110 °C for 10 h. The resulting CdTe QDs were washed with ethanol, separated by centrifugation, dispersed in water, and stored at 4℃ for further use. The mean size of the CdTe QDs is estimated from the corresponding UV-Vis adsorption peak according to Peng's empirical equation[4] to be 3.1 nm.

Preparation of the STV-CdTe and biotin-CdTe bioconjugates: To prepare the STV-CdTe bioconjugates, the CdTe QDs (30 μL) were mixed with EDC (80 mM) and NHS (20 mM) for 30 min in MES buffer (100 μL, 0.1 M, pH 5.9). This is followed by centrifugation at 5000 rpm for 3 min and removal of the supernatant. Next, STV (20 μL, 2 mg mL^{-1}) was mixed with the above QDs for 1 h. Finally, the STV-CdTe bioconjugates were collected by centrifugation and re-suspended in PBS for further use. The biotin-CdTe bioconjugates were prepared by mixing the SH-polyT_{10}-biotin (12 nmol) with a CdTe QD suspension (1.2 nmol) in Tris buffer (10 mM Tris-HCl, 100 mM NaCl, pH 7.6) overnight at room temperature, followed by centrifugation at 15000 rpm for 10 min, removal of supernatant, washing with PBS, and re-suspension in PBS.

Preparation of the STV-PS beads: An aliquot (25 μL) of the PS beads was transferred into a centrifuge vial and washed twice with PBS. The beads were then re-suspended in MES buffer containing EDC (400 mM) and NHS (100 mM) and mixed for 1 h, followed by centrifugation at 5000 rpm and washing with PBS. Next, STV (200 μL, 0.2 mg mL^{-1} in PBS) was added and incubated with the beads for 1h. After washing twice with PBS, the resulting STV-PS were re-suspended in PBS (50 μL) for further use.

Preparation of the PS-$(CdTe)_n$ and TBA_2-conjugated PS-$(CdTe)_2$ assemblies: In brief, the STV-PS beads (2.5 μL) was transferred into a centrifuge vial and the CdTe-biotin (12.5 μL) as well as PBS-BSA (50 μL, 0.15% BSA, pH 7.4) were added to the vial and incubated for 30 min. Subsequently, the PS-biotin-CdTe assemblies were centrifuged at 8000 rpm for 5 min to remove the supernatant. These beads were washed three times with PBS-T (0.1% Tween 20, pH 7.4) to remove any non-specifically adsorbed CdTe-biotin conjugates. Next, the STV-CdTe conjugates in PBS-BSA were incubated with the beads for 30 min, followed by centrifugation at 8000 rpm for 5 min to remove the supernatant and washed with PBS-T. Additional CdTe layer could be attached by using the above procedure consecutively to obtain the PS-$(CdTe)_n$ assemblies. After the attachment of the STV-CdTe layer (n=2), the TBA_2 (2 μM) was incubated with the PS-$(CdTe)_2$ for 30 min, followed by centrifugation and washing with PBS. The TBA_2-conjugated PS-$(CdTe)_2$ assemblies were then re-suspended in PBS.

Thrombin sensing protocol: A bare GCE was polished with 0.3 and 0.05 μm alumina slurries, respectively, and sonicated sequentially in water, ethanol and water for 5 min each. A layer of gold nanoparticles (AuNPs) was electrochemically deposited on the electrode surface in 1% $HAuCl_4$ by controlling the deposition potential at -0.2 V for 30 s. The electrode was then dried with N_2, followed by incubation with TBA_1 (20 μL, 5 μM) overnight at room temperature in humidity. Next, the electrode surface was blocked with 1 mM MCH for 2 h and washed with PBS. The modified electrode was incubated with thrombin at various concentrations for 1 h, followed by washing with PBS-T. Afterward, the TBA_2-conjugated PS-$(CdTe)_2$ assemblies (20 μL) were incubated with the electrode for 1 h. After washing with PBS-T, ECL measurement was performed.

ECL mechanism:

In a typical ECL measurement involving QDs (CdTe) and the $S_2O_8^{2-}$ co-reactant, a cathodic potential scan is applied to the modified working electrode. During the scan, the electrons transfer from the electrode surface to the out layer of the CdTe QDs labels. Since CdTe QDs are semiconductor nanoparticles and electrons can migrate between these QDs. This leads to the reduction of CdTe QDs to $CdTe^{-}\bullet$. Meanwhile, the cathodic potential scan also reduces the $S_2O_8^{2-}$ co-reactant to $SO_4^{-}\bullet$. The species $SO_4^{-}\bullet$ is a strong oxidant and further reacts with $CdTe^{-}\bullet$ to generate the excited state CdTe* in the aqueous solution. In addition, the dissolved oxygen can also participate in assisting the formation of CdTe*. When CdTe* falls from the excited state to the ground state, light is emitted and detected. The mechanism can be expressed as follows:[5-9]

$CdTe + e^{-} \rightarrow CdTe^{-}\bullet$

$S_2O_8^{2-} + e^{-} \rightarrow SO_4^{-}\bullet + SO_4^{2-}$

$CdTe^{-}\bullet + SO_4^{-}\bullet \rightarrow CdTe^{*} + SO_4^{2-}$

$O_2 + H_2O + 2e^{-} \rightarrow OOH^{-} + OH^{-}$

$2\ CdTe^{-}\bullet + OOH^{-} + H_2O \rightarrow 2CdTe^{*} + 3OH^{-}$

$CdTe^{*} \rightarrow CdTe + h\upsilon$

UV-Vis and ECL characterizations of the prepared CdTe QDs:

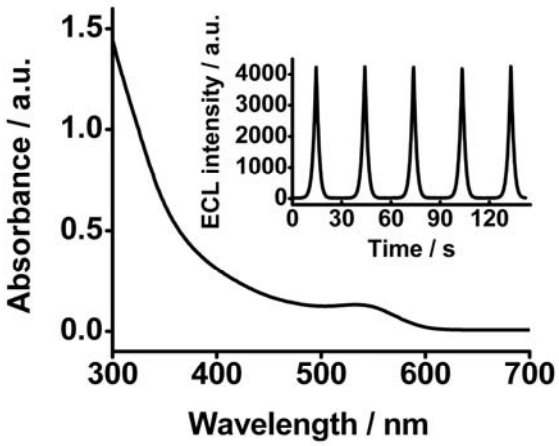

Fig. SI1 UV-Vis spectrum of the MPA capped CdTe quantum dots in ultrapure water. Inset: ECL emission curves of the MPA capped CdTe quantum dots in PBS containing 0.1 M $K_2S_2O_8$ and 0.1 M KCl on a GCE with 5 continuous potential scanning cycles at a scan rate of 100 mV s^{-1}.

Electrochemical impedance spectroscopic characterization of the sensing electrode:

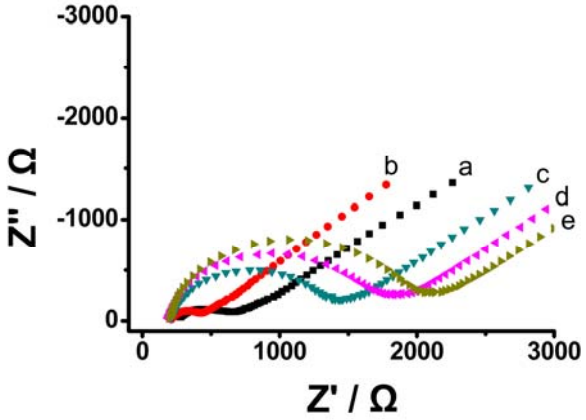

Fig. SI2 Electrochemical impedance spectra corresponding to (a) bare GCE, (b) GCE/AuNPs, (c) GCE/AuNPs/TBA_1/MCH, (d) GCE/AuNPs/TBA_1/MCH/thrombin (150 pM), and (e) GCE/AuNPs/TBA_1/MCH/thrombin/TBA_2-PS-$(CdTe)_2$. The impedance spectra were recorded in 0.1 M KCl solution containing 5 mM (1:1) $[Fe(CN)_6]^{3-/4-}$ with the range from 10 kHz to 50 MHz and an alternate voltage of 5 mV.

In a typical electrochemical impedance spectrum, the semicircle portion observed at higher frequencies corresponds to the electron-transfer limited process. The increase of the diameter of the semicircle reflects the increase of the interfacial charge-transfer resistance (R_{et}). From Fig. SI2, we can see that the electrodeposition of AuNPs on the GCE surface leads to a decrease in R_{et} (from curve a to b) due to the excellent conductivity of the Au NPs. After formation of the mixed self-assembly monolayer of TBA_1 and MCH on the AuNPs, a dramatic increase in R_{et} (from curve b to c) is observed. Subsequent incubation of the electrode with 150 pM thrombin results in an increase in R_{et} (from curve c to d). The addition of the TBA_2-PS-$(CdTe)_2$ leads to a further increase in R_{et} (from curve d to e).

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