Supporting Information for

Biobar-Coded Gold Nanoparticles and DNAzyme-Based Dual Signal Amplification Strategy for Ultrasensitive Detection of Protein by Electrochemiluminescence

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Table S1. DNA oligonucleotides sequence used in this work.

Name	Sequence (from 5' →3')
Hairpin DNA	CAT CTC TTC TCG AGC CGG TCG AAA TAG TGA GTG GTT GGT GTG GTT GGG AAG AGA TG
Substrate	TGG CAA CTC GTG ACT CAC TAT aG GAA GAG ATG
Capture DNA	GAG AAC TCG ACG AGT ATA GTG AGT CAC GAG TTG CCA-$(CH_2)_6$-SH
cDNA	SH-$(CH_2)_6$-TTTTTTTT-CGT CGA GTT CTC
Signal DNA	SH-$(CH_2)_6$-CTG AGC GTC ATC-$(CH_2)_6$-NH_2

The Melting Temperature (Tm) of hairpin DNA and DNAzyme substrate are 46.5 ^{o}C and 42.2 ^{o}C, respectively. When the hairpin DNA and DNAzyme substrate maintained a stable structure, only one fully complementary sequence between hairpin DNA and DNAzyme substrate termed GAAGAGATG with a Tm of 19.63 ^{o}C (which is much lower than room temperature) formed. This fully complementary sequence is easily to be dissociated. According to the results, the structure of the hairpin DNA and substrate could not be opened by each other without thrombin. On the other hand, the ΔG of TBA-thrombin and hairpin DNA are -35.6 kcal mole^{-1} and -6.04 kcal mole^{-1}, respectively. Thus hairpin DNA could be opened by thrombin easily. After being opened by thrombin, the functional part II of hairpin DNA is free to hybridize with substrate and form a new dsDNA with a Tm of 67.9 ^{o}C, which is very stable. All the Tm and thermodynamic value are calculated by Oligo Analyzer 3.1 under the experimental conditions.

Table S2. Process of feasibility study in Figure 3.

	Capture DNA	Hairpin DNA	Subtrate	Zn^{2+}	thrombin	CdSeTe@ZnS QDs	Bio-bar coded Au NPs- QDs
a	3 μM	-	-	-	-	-	-
b	3 μM	1 μM	3 μM	1 mM	-	-	+
c	3 μM	1 μM	1 nM	1 mM	1 nM	+	-
d	3 μM	1 μM	3 μM	1 mM	1 nM	+	-
e	3 μM	1 μM	1 nM	1 mM	1 nM	-	+
f	3 μM	1 μM	3 μM	1 mM	1 nM	-	+

Gel electrophoresis characterization. The binding process between hairpin DNA, thrombin and substrate strand was further investigated by gel electrophoresis. The results are shown in Figure S1. Lanes 2, 3 and 5 represented the hairpin DNA, substrate strand and thrombin, respectively. Lane 4 represented the mixture of hairpin DNA and substrate in binding buffer. No more bands, except the 2 bands at the same migration position as that in lanes 2 and 3, were observed as no hybridization

happened between them without the addition of Tb. In the presence of thrombin and hairpin DNA (Lane 6, 7 and 8), the bright bands that are higher than 300bp (marked by the red dotted line) indicated the formation of hairpin DNA/thrombin complex with high molecular weight, the bands are trapped in the top of the lanes. However, the band of hairpin DNA and substrate after incubation with thrombin was weaker (lane 7) compared with that of hairpin DNA or substrate only (lanes 2 and 3). This indirectly confirmed the formation of a complex of hairpin DNA, thrombin and substrate. The band of substrate was even weaker in lane 8 as the recycle continued. All the samples for gel electrophoresis were at the same concentration of 1 μM.

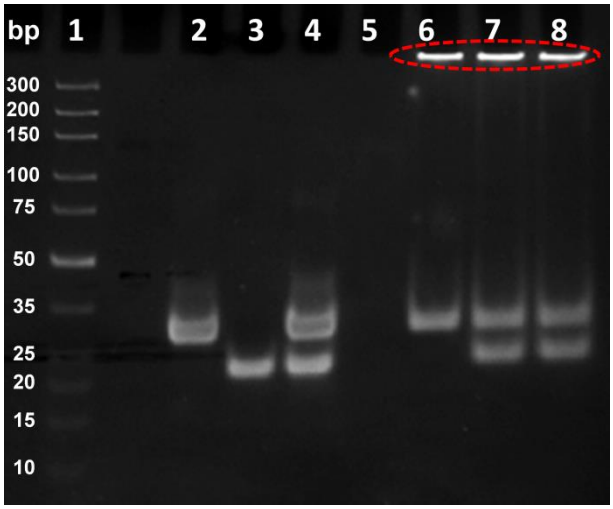

Figure S1. Agarose gel electrophoresis analysis: 1, low molecular weight DNA marker; 2, hairpin DNA; 3, Substrate; 4, Hairpin DNA/substrate; 5, Thrombin; 6, Hairpin DNA/thrombin; 7, Hairpin DNA/thrombin/substrate; and 8, Hairpin DNA/thrombin/substrate with Zn^{2+}.

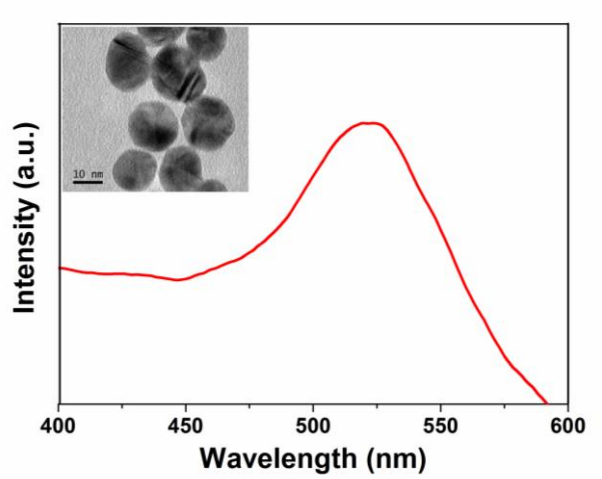

Figure S2. Typical TEM image and UV-vis absorption spectrum of the prepared Au NPs.

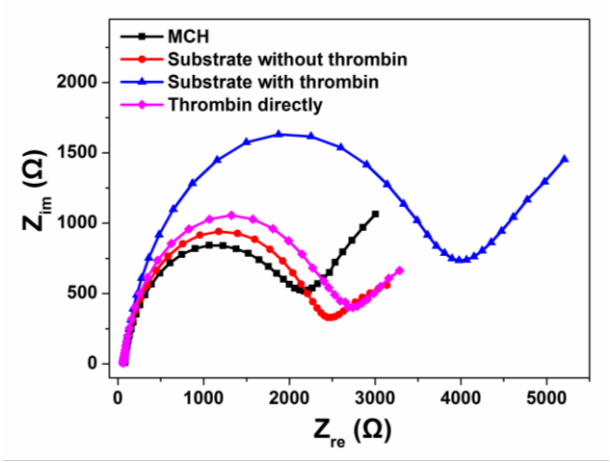

Figure S3. EIS of substrate modified Au electrode with or without thrombin. The electrolyte for EIS detection: 0.1 M KCl + 2 mM $[Fe(CN)_6)]^{3-}$/$[Fe(CN)_6]^{4-}$.

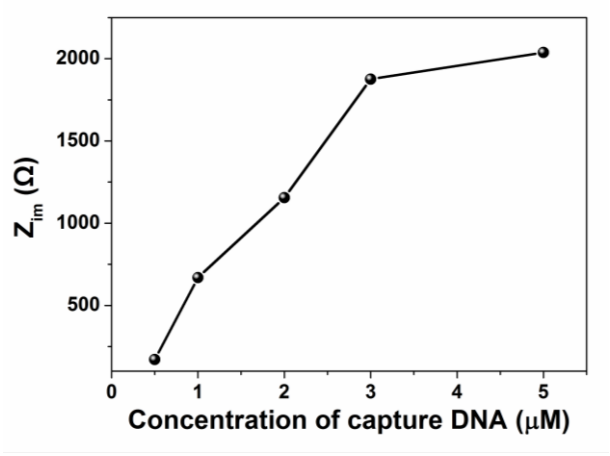

Figure S4. EIS of different concentration of capture DNA on Au electrode.

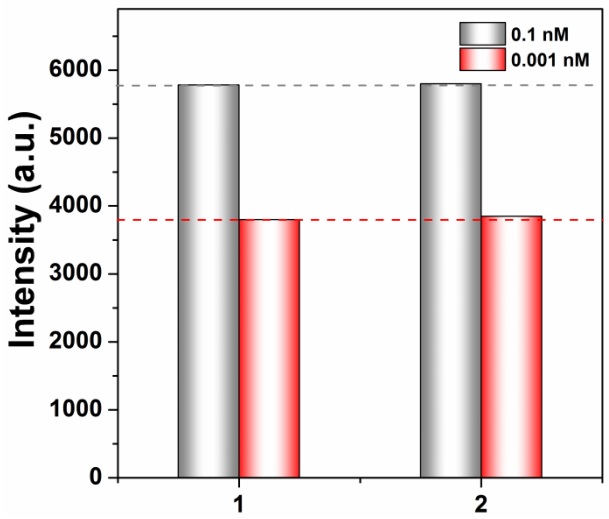

Figure S5. ECL signals of batch-to-batch variations for preparing the biobar-coded Au NPs-QDs, 1 and 2 stand for two batches.