Electronic Supplementary Material (ESI) for Analyst.
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# Dynamic Sandwich-Type Electrochemical Assay for Protein Quantification and Protein Interaction Analysis

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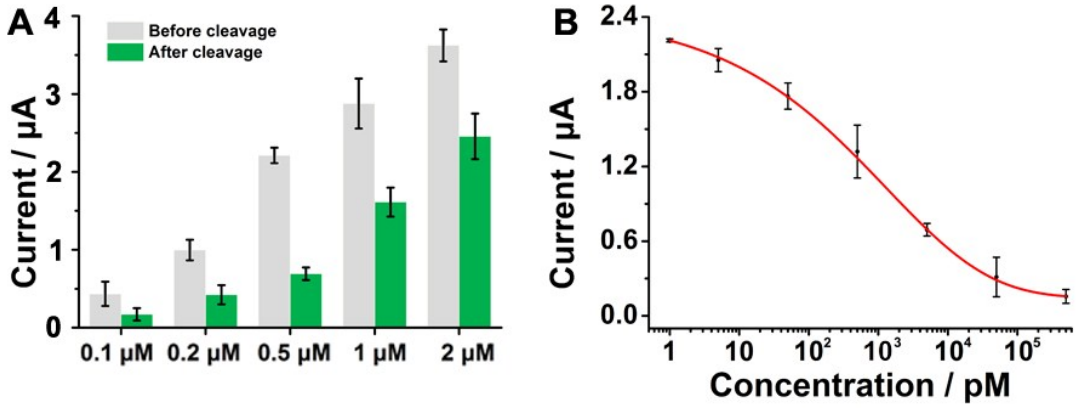


**Figure S1.** Optimization of the substrate DNA density (A) and DNAzyme concentration (B). In order to optimize the sensitivity of dSTEA, we first determined the optimal density of the substrate DNA that is needed to provide both a large electrochemical current and a better signal response after incubation of DNAzyme. (A) To do so, electrodes were incubated in 5 μL of solution at varying concentration of substrate strands for 1 h. Then, DNAzyme with a fixed concentration (10 nM) was introduced to these electrodes to study the rate of cleavage. We find that the final current of our sensors increases with the substrate strand concentration up to a concentration of 2 μM. However, these sensors with higher substrate DNA density also led to worse signal response, probably attributing to the poor DNA hybridization efficiency. In order to obtain the largest signal intension, we therefore performed all dSTEA experiments by functionalizing the electrode using 0.5 μM of substrate DNA ($\Gamma = \sim 4.76\times10^{12}$ molecules/cm$^2$). (B) For the sake of multiple turnover enzymatic condition, the DNAzyme strands in the solution should be less than the substrate DNA (at least a 10-fold substrate excess) on the electrode, while guaranteeing the acceptable electrochemical signal response. We find that 5 nM of DNAzyme in the 5 μL buffer solution ($1.5\times10^{10}$ molecules) can just satisfy this demand.

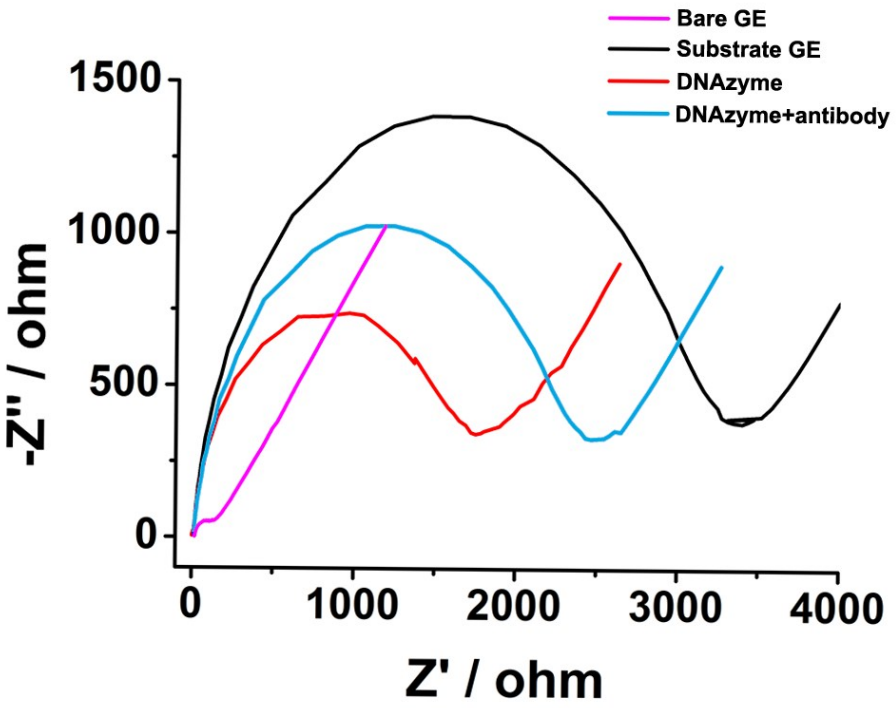


**Figure S2.** EIS results of electrode at different stages. The EIS of bare electrode exhibits an almost straight line (magenta line). After conjugation of substrate oligonucleotides, electron transfer resistance ($R_{et}$) increases significantly because of the formation of negatively charged DNA layer on the electrode surface (black line). Nevertheless, after incubation of the electrode with DNAzyme-containing buffer, the electrochemical impedance dramatically decreases (red line), indicating that the DNAzyme can efficiently cleave surface-tethered DNA and the DNA fragments dissociate from the electrode surface eventually. However, once introduction of antibody-DNAzyme complex, the electrochemical impedance is retained to a certain extent, suggesting the less efficiency of DNA cleavage. Of note, in order to specifically study the change of surface-tethered substrate DNA, all electrodes were incubated with 8 M urea to eliminate interference of DNA hybridization and protein adsorption before final electrochemical detection. Also of note, substrate DNA used here without MB modification.

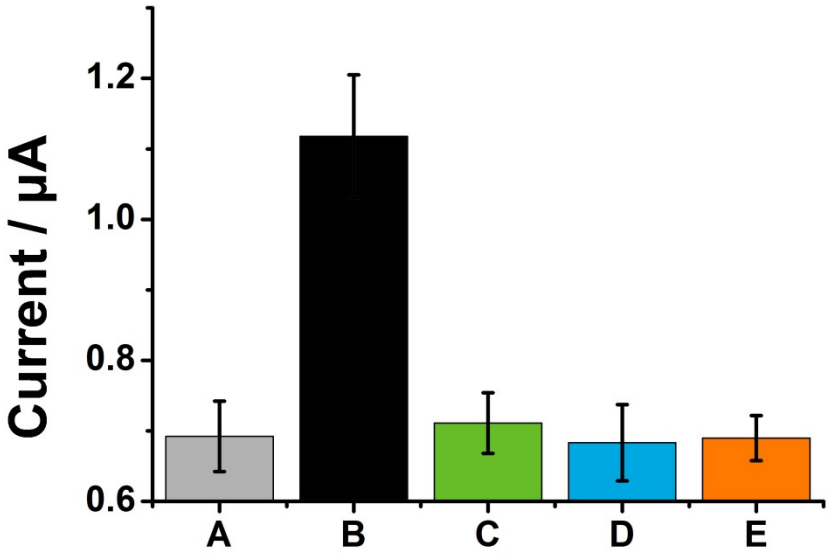


**Figure S3.** Specificity study of dSTEA. (A) without anti-Dig antibody, (B) with anti-Dig antibody, (C) DNAzyme without Dig modification, (D) human IgG, (E) human thrombin. dSTEA's signaling mechanism takes place when the biomolecule specifically binds the ligand on the DNAzyme. To demonstrate this, we designed and tested a series of control groups using the Dig-antibody system. In contrast to the Dig-dSTEA sensor (see Figure 1), the cleavage of control DNAzyme strand or other protein molecules on the electrode surface was not affected.

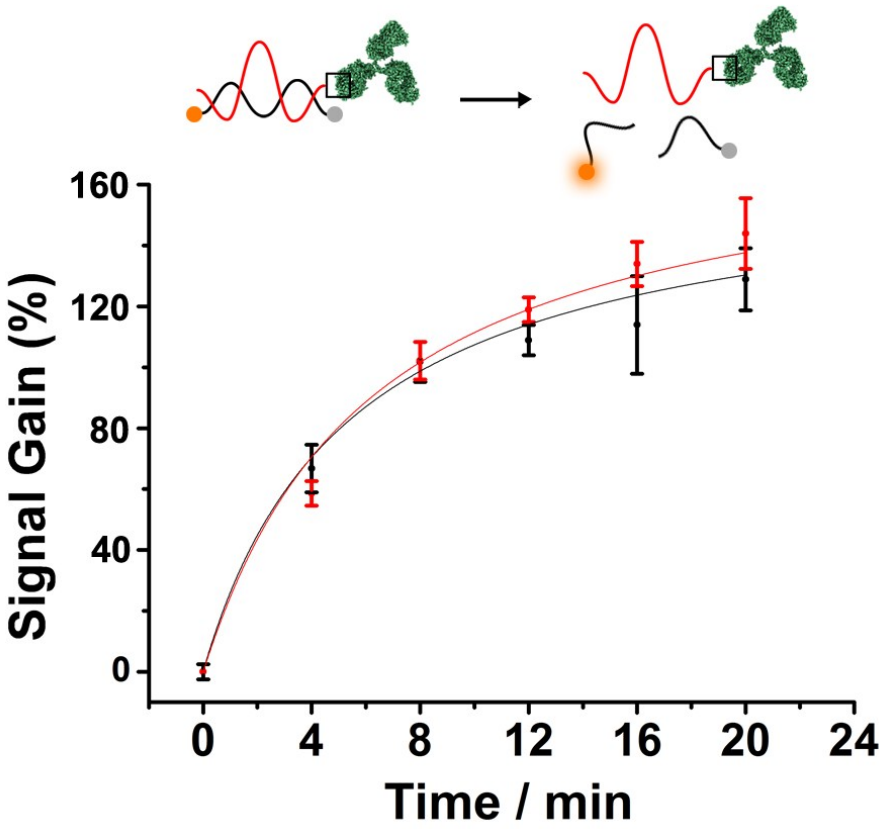


**Figure S4.** dSTEA fails to work in a homogeneous protocol since the attachment of protein to the DNAzyme will not affect the substrate DNA hybridization and cleavage in the solution. Black line: without antibody, Red line: with antibody.

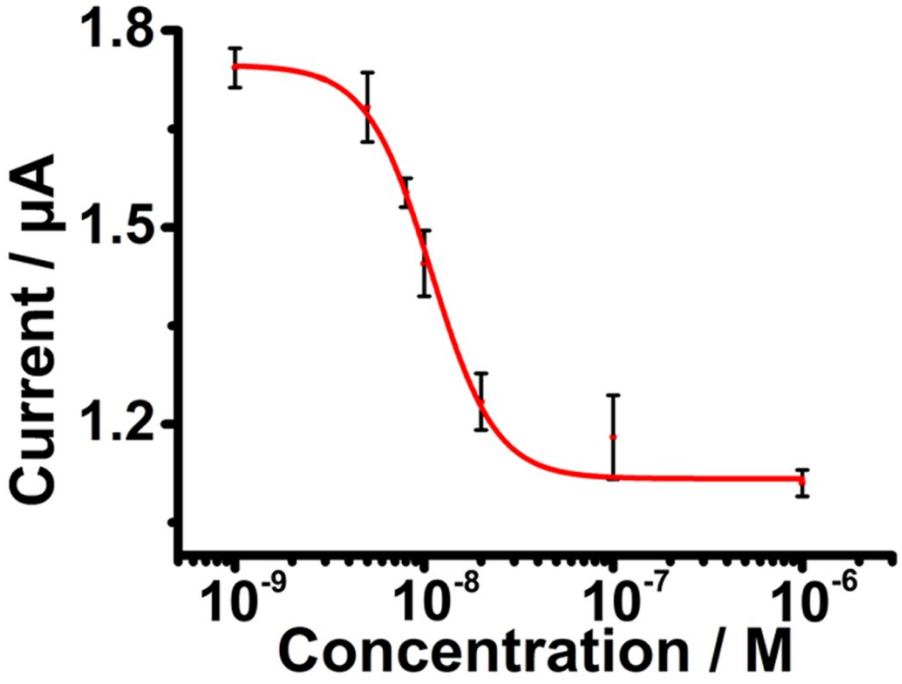


**Figure S5.** Dose-response curves of cAMP in the presence of intact PKA (50 nM).

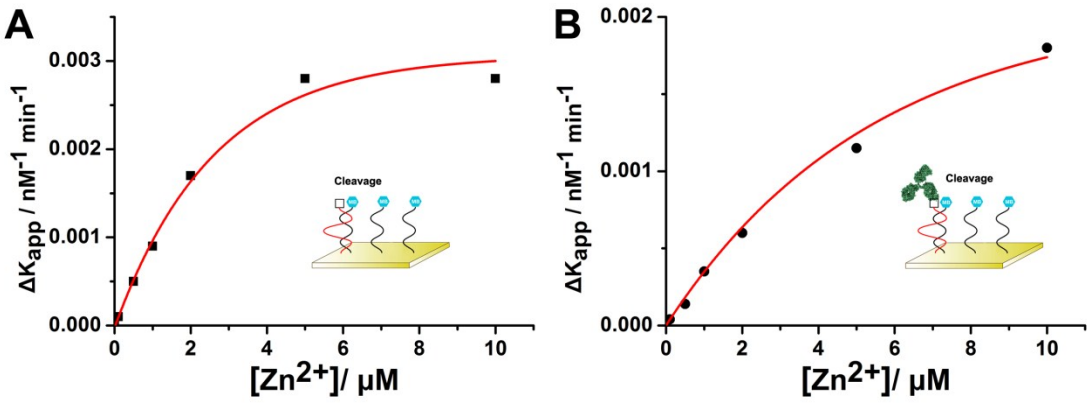


**Figure S6.** The relative apparent cleavage rate ($\Delta k_{app}$) of the 8–17 DNAzyme in the absence of binding protein (A) and in the presence of binding protein (B).

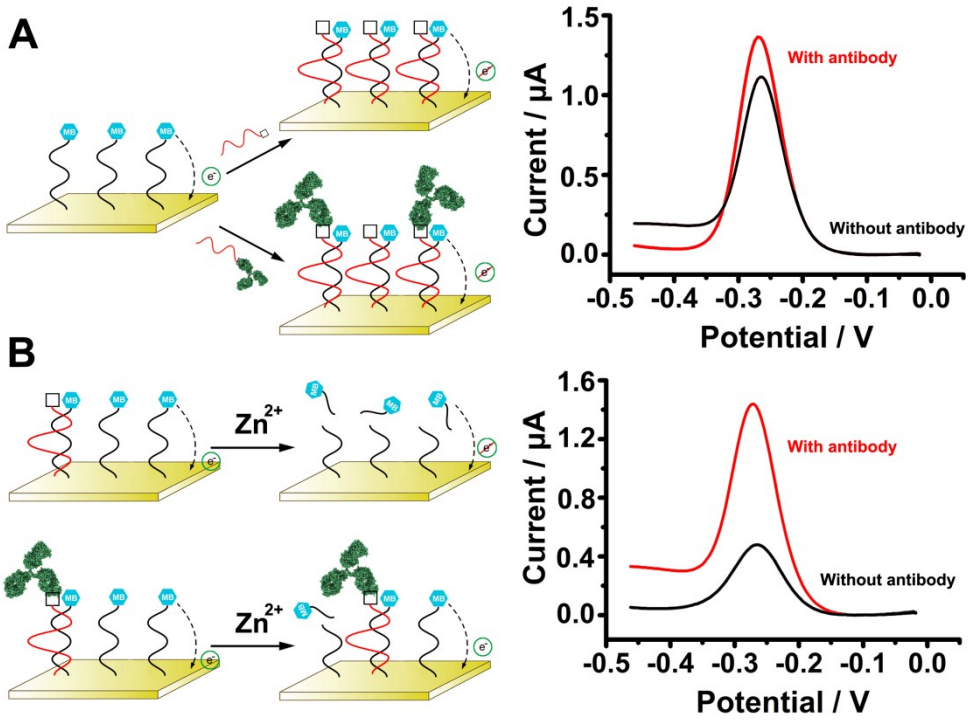


**Figure S7.** Signal contribution study of dSTEA. (A) SWV-response difference between direct incubation of substrate DNA modified electrode with excess DNAzyme or DNAzyme-antibody complex in absence of $Zn^{2+}$. (B) SWV-response difference of substrate DNA cleavage after addition of $Zn^{2+}$-containing buffer with pre-incubation of substrate DNA modified electrode with DNAzyme or DNAzyme-antibody complex. In order to deeper understand the principle of dSTEA, the whole assay is divided into two independent parts: the diffusion of DNAzyme to the electrode surface and the migration of DNAzyme on the electrode surface. Firstly, we have performed anti-Dig antibody sensor without addition of $Zn^{2+}$ that is necessary for the activity of DNAzyme, thus the signal response only stems from the difference of diffusion rate of DNAzyme-antibody complex. In the absence of DNAzyme, the soft single-stranded substrate DNA is free to collide with the electrode surface, thus producing a large faradaic current for MB. Upon DNAzyme hybridization, this current is reduced because the rigid duplex reduces the efficiency with which the redox tag collides with the electrode surface. Secondly, after sufficient incubation of electrode with DNAzyme and then Ab1, another $Zn^{2+}$-containing buffer is added into the electrochemical pool to activate the DNAzyme, thus the signal response only stems from the difference of migration rate of DNAzyme-antibody complex

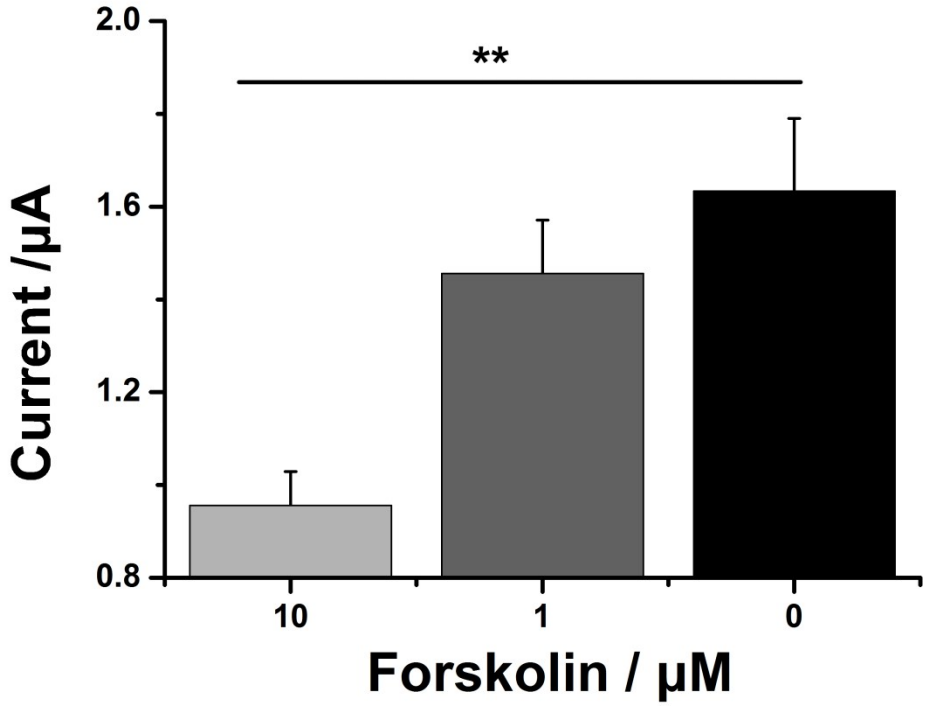


**Figure S8.** Electrochemical response of PKA in the HEK 293 cell extract treated with forskolin (Student's test: **P < 0.05).

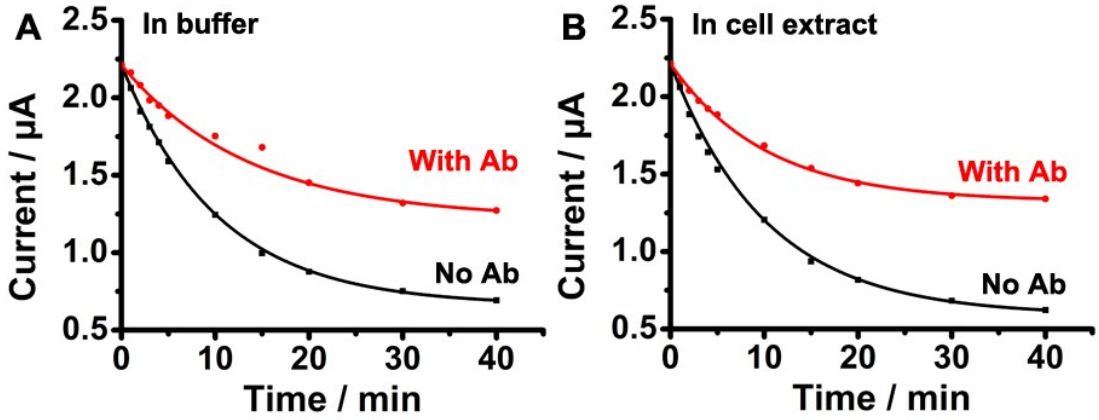


**Figure S9.** dSTEA performs equally well in buffer and cell extract (5 nM of anti-Dig antibodies).

**Table S1. The LOD comparisons between the proposed method and various developed methods for antibody detection.**

| Assay | LOD | Ref. |
|---|---|---|
| Electrochemistry | 10 nM | 1 |
| Electrochemistry | 1 nM | 2 |
| Electrochemistry | below 1 nM | 3 |
| Colorimetry | 39 pM | 4 |
| Fluorescence | Below 1 nM | 5 |
| Surface plasmon resonance | 70 fM | 6 |
| Electrochemistry | 100 fM | This work |

## References


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