

## **Electronic Supplementary Information**

### **A simple and label-free electrochemical biosensor for DNA detection based on the super-sandwich assay**

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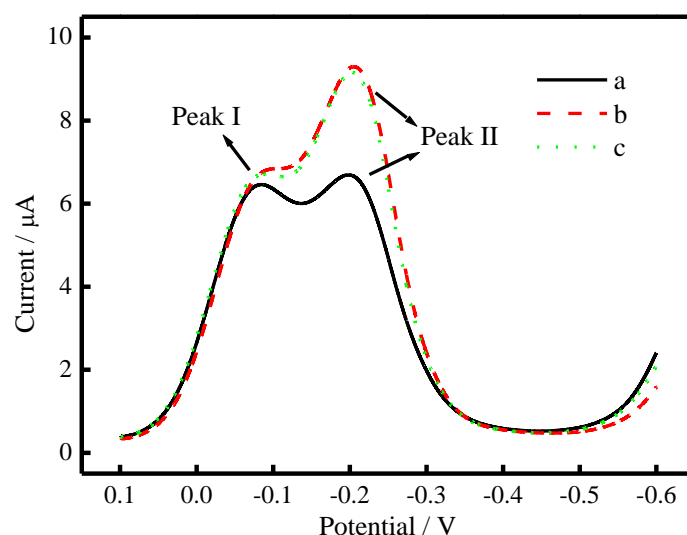
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## Hybridization of the super-sandwich to the electrode

To demonstrate the A2-A3 super-sandwich is easy to approach to the electrode, we did the experiments to prove this and the results are shown in Fig. S1. We hybridized A2 and A3 to the modified electrode by two ways. One way was that the modified electrode was immersed in the solution containing 200 nM A2 and 200 nM A3 for 4 h (curve b). The other was that 100 nM A2 and 200 nM A3 were firstly hybridized for 2 h in the solution to form the super-sandwich assay, and then the modified electrode was immersed in the mixture containing 100 nM A2 and the previous super-sandwich solution for 2 h (curve c). We employed RuHex as the signaling molecule in this DNA sensor. Square wave voltammetry (SWV) was employed to characterize the electrochemistry of RuHex at the gold electrode surfaces with DNA/MCH monolayers.



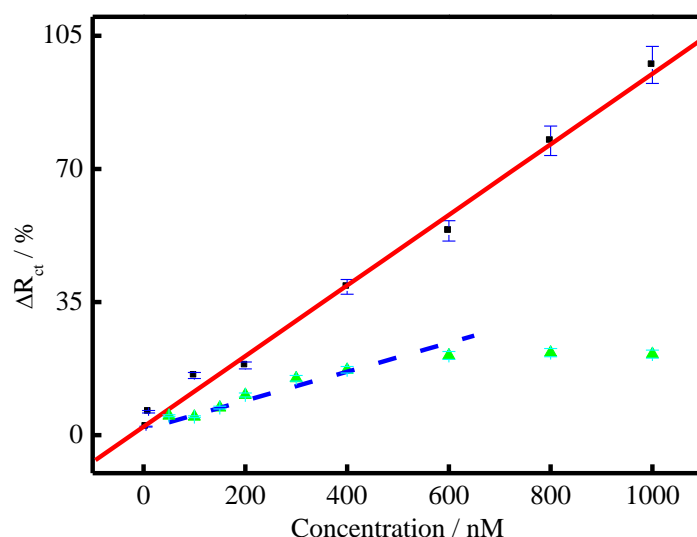
**Fig. S1** SWV responses of the two ways of hybridizing A2 and A3 in 100  $\mu\text{M}$  RuHex; (a) gold electrode modified with A1 and MCH; (b) 200 nM A2 and 200 nM A3 were hybridized simultaneously to the modified electrode for 4 h; (c) the modified electrode was immersed in the mixture containing 100 nM A2 and the previous super-sandwich solution (100 nM A2 and 200 nM A3 were hybridized for 2 h) for 2 h.

Consistent with previous studies<sup>1</sup>, two peaks were observed (Fig. S1): one (peak II) arose due to the redox reaction of RuHex electrostatically bound to the phosphate backbone of DNA, while the other (peak I) was ascribed to RuHex diffused to the MCH portion. Therefore, peak II reflected the amount of DNA strands localized at the electrode surface. Interestingly, in these two ways of hybridizing A2 and A3 to

the modified electrode, similar increases in the peak currents were observed (Fig. S1, curves b and c). These SWV curves and the results, which are consistent with previous study<sup>2, 3</sup>, provided the proof that the A2-A3 super-sandwich is easy to approach to the electrode.

### The linear relationship of the traditional-sandwich assay

To compare the super-sandwich assay with the traditional-sandwich assay, we detected the target A2 by traditional-sandwich assay with the electrochemical impedance spectroscopy to obtain a linear relationship between the resistance change rate ( $\Delta R_{ct}$ ) and the concentration of target A2. The linear range and the detection limit of the traditional-sandwich are 50 – 600 nM and 12.6 nM, respectively, as shown in Fig. S2 (dash). We can observe that the super-sandwich assay is more sensitive than the traditional-sandwich assay.



**Fig. S2** The linear relationships of the super-sandwich (solid) and of the traditional-sandwich (dash).

### Notes and references

- 1 A. B. Steel, T. M. Herne and M. J. Tarlov, *Anal. Chem.* 1998, **70**, 4670–4677.
- 2 T. Yuan, Z. Y. Liu, L. Z. Hu, L. Zhang and G. B. Xu, *Chem. Commun.*, 2011, **47**, 11951–11953.
- 3 X. Chen, Y. H. Lin, J. Li, L. S. Lin, G. N. Chen and H. H. Yang, *Chem. Commun.*, 2011, **47**, 12116–12118.