Supporting Information

An electrochemical super-sandwich assay for sensitive and selective DNA detection in complex matrices

Fan Xia,[†, ‡] Ryan J. White,[‡] Xiaolei Zuo,[*, ‡] Adriana Patterson,[‡] Yi Xiao,[‡] Di Kang,[‡]

Xiong Gong,[§] Kevin W. Plaxco[*, ‡] and Alan J. Heeger[*, †]

† Center for Polymers and Organic Solids, University of California, Santa Barbara.

‡ Department of Chemistry and Biochemistry, University of California, Santa Barbara.

§ Department of Physics, University of California, Santa Barbara.

1. The gel-electrophoresis show different base pairs between the traditional structure and the super-sandwich structure.

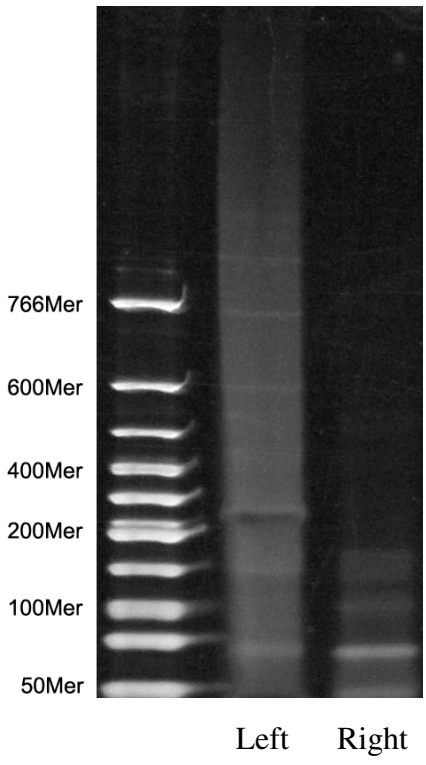

Figure S1. The gel-electrophoresis results confirm the formation of the super-sandwich structure (as we can see, the left lane exhibits a ladder of different length of super-sandwich structure and the maximum length is about 1000 base pairs. Correspondingly, the right lane exhibits traditional-sandwich structure's bands about less than 75 base pairs.).

2. Electron transfer rates

We have used square wave voltammetry to compare the electron transfer kinetics[1] of these two systems and find that the electron transfer kinetics of the super-sandwich assay, 1.0 s^{-1} are quite similar to the 1.2 s^{-1} observed for the traditional assay. Although the super-sandwich contains more methylene blues than the traditional assay, unfortunately, this is difficult to do as the electron transfer from regions of the super-sandwich complex distal from the electrode is likely much less efficient than electron transfer from redox reporters located nearer the electrode.

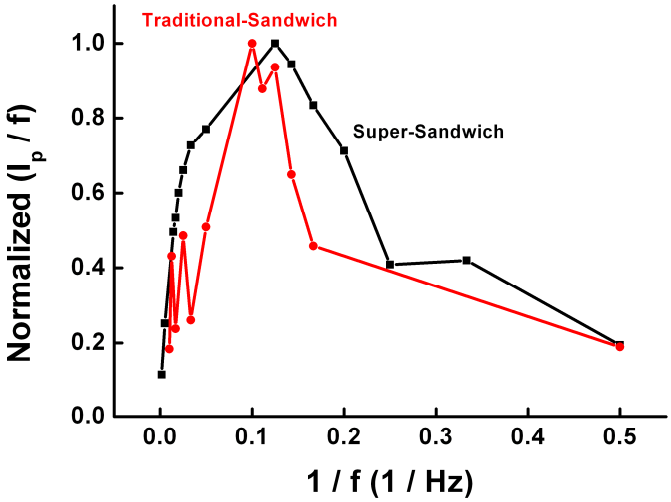

Figure S2. Square wave voltammetry has been used to compare the electron transfer kinetics of these two systems and find that the electron transfer kinetics of the super-sandwich assay, 1.0 s^{-1} are quite similar to the 1.2 s^{-1} observed for the traditional assay.

3. Specificity of the super-sandwich assy.

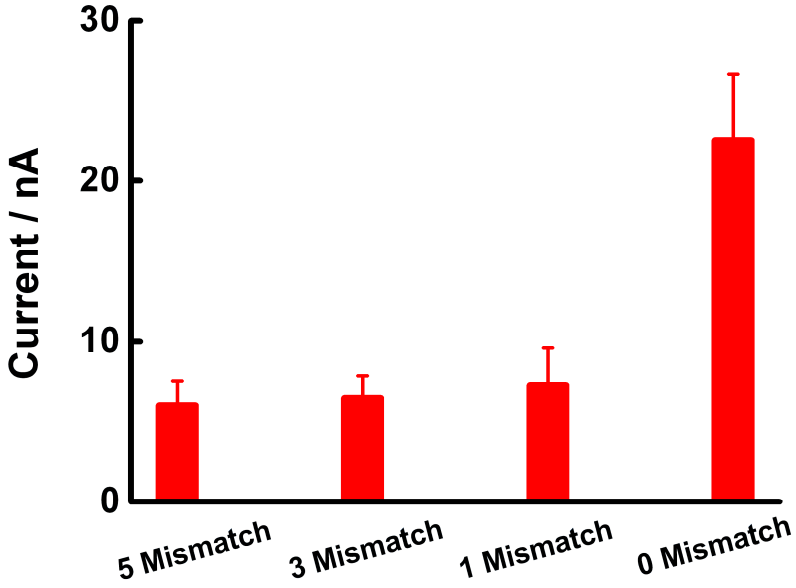

Figure S3. The super-sandwich assay is also specific. In order to evaluate this, we challenged our assay using one-base, three-base and five-base mismatched targets and found that it readily discriminates between these mismatched targets.

4. Materials and methods.

Materials.

Labeled oligonucleotides were synthesized by Biosearch Technologies, Inc. (Navato,CA), purified by C18 HPLC, confirmed by mass spectrometry and used as received. The sequences of these oligomers are:

Capture probe:

5'-SH-$(CH_2)_6$-CGGC ACC TGG GGG AGT ATT GCG GAG GAA GGT GCCG-3'

Signal probe (Super-sandwich):

5'-CGGC ACC TGG GGG AGT ATT GCG GAG GAA GGT GCCG-Methylene Blue-3'

Target:

5'-T ACTCCCCCAGGT GCCG A CGGC ACCTTCCTCCGC A-3'

Signal probe (Traditional sandwich):

5'-CGGC ACC TGG GGG AGT AT-Methylene Blue-3'

1-Mismatch Target:

5'-T ACTCCCCCAGGT GCGG A CGGC ACCTTCCTCCGC A-3'

3-Mismatch Target:

5'-T ACTCCCCCAGGT CGGG A CGGC ACCTTCCTCCGC A-3'

5-Mismatch Target:

5'-T ACTCCCCCAGGA CGGC A CGGC ACCTTCCTCCGC A-3'

Super-sandwich Assay Sequences:

5'-(SH)-CGGCACCTGGGGGAGTATTGCGGAGGAAGGTGCCG-3' **(Capture Probe)**

3'-ACGCCTCCTTCCACGGCAGCCGTGGACCCCCTCAT-5' **(Target)**

5'-CGGCACCTGGGGGAGTATTGCGGAGGAAGGTGCCG(MB)3' **(Signal Probe)**

3'-ACGCCTCCTTCCACGGCAGCCGTGGACCCCCTCAT-5' **(Target)**

(Signal Probe) 5'-CGGCACCTGGGGGAGTATTGCGGAGGAAGGTGCCG(MB)3'

.....

.....

Traditional Assay Sequences:

5'-(SH)-CGGCACCTGGGGGAGTATTGCGGAGGAAGGTGCCG-3' **(Capture Probe)**

3'-ACGCCTCCTTCCACGGCAGCCGTGGACCCCCTCAT-5' **(Target)**

5'-CGGCACCTGGGGGAGTAT(MB)3' **(Signal Probe)**

5. Fabrication of Sensors:

Polycrystalline gold disk electrodes (2mm diameter, CH Instruments, Austin, TX) were prepared by polishing with 1 μm diamond and 0.5 μm alumina (Buehler, Lake Bluff, IL), sonicating in water, and electrochemically cleaning (a series of oxidation and reduction cycles in 0.5 M H_2SO_4, 0.01 M KCl/0.1 M H_2SO_4, and 0.05 M H_2SO_4) before modification with probe DNA by incubating the clean electrode in 0.6 μM DNA / 5 μM TCEP (tris(2-carboxyethyl) phosphine hydrochloride) in Tris buffer (pH 7.4) for 30 min. The surface was then rinsed with water and subsequently passivated with 2 mM 6-mercaptohexanol in Tris buffer, for 2 hours. Prior to use, electrodes were rinsed with deionized water.

Electrochemical measurements were conducted using square wave voltammetry (SWV) with a CHI 650b potentiostat (CH instruments, Austin, TX). SWV was performed using a potential window -0.15 to -0.4 V (versus Ag/AgCl), a potential step of 0.001 V, an amplitude of 0.05 V and a frequency of 60 Hz. The electrolyte and hybridization buffer used in this study was 10 mM Tris buffer (pH 7.4) with 500 mM NaCl and 1 mM $MgCl_2$. The hybridization time is 1 hour.

The data points and error bars represent the average and standard deviations from at least three independently fabricated electrodes and are dominated by sensor-to-sensor variation arising from the fabrication process (relative sensor response (%), is employed because this is more reproducible from electrode-to-electrode than the absolute current change).

Reference

1. White, R. J.; Plaxco, K. W. *Anal. Chem*. **2010**, *82*, 73-6.