Electronic Supplementary Information

Surface Initiated DNA Self-Assembly as an Enzyme-Free and Nanoparticle-Free Strategy toward Signal Amplification of an Electrochemical DNA Sensor

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Experiment details

1. DNA sequences (5’-3’):
   Capture: thiol-TTTTTTTTTGCGCGAACCTATA
   Reporter: TCTATCCTACGCTTTTT
   ACTGGAACTAGTTGATGAAGCTG
   (underline bases correspond to an initiator sequence)
   Target: AGCGTAGGATAGATATACG
   TTTCGCGC (underlined is a G-to-T mutation)
   L1: GTGTGCGTATTATGTCTCCTCCTCTCAGCTTCATCAACTAGTCTCCAGT
   C1: CTAGTTGATGAAGCTGGACATAATACGCACAC
   L2: AGGAGGAGACATAATACGCACACACTGGAACTAGTTGATGAAGCTG
   C2: CAGCTTCATCAAACGTGGGTGCMTATGTC
   R1: TGCGTATATGTCCAGCTT
   R2: GCACACCTAGTTGGATGAAG
   All oligonucleotides were purchased from Sangon Inc. (Shanghai, China) and purified by denaturing (8 M urea) polyacrylamide gel electrophoresis (PAGE).

2. Assaying process:

   Electrode pretreatment:
   A 2 mm diameter gold disk electrode sealed in a cylindrical Kel-F tube (Shanghai Chenhua Co., China) was polished with 0.05 μm gamma alumina slurry followed by sequential soninations in doubly distilled water (ddH₂O), ethanol, and ddH₂O for 10 min each. The electrode was then scanned in a 0.1 M H₂SO₄ solution at 0.1 V s⁻¹ from -0.8 V to 0.9 V (v.s. Pt quasi-reference) until a stable background was reached. The electrode was then sonicated in ddH₂O for another 10 min, blown dry with compressed high-purity nitrogen.

   Immobilization of capture DNA:
   A 16 μL solution droplet containing 30 nM thiolated capture DNA (dissolved in a buffer containing 0.5 M NaCl, 20 mM Tris-HCl, 1 mM EDTA, 1 mM TCEP) was pipetted onto an inverted gold electrode and allowed to stay on the gold surface for 2 hours. During the incubation, the electrode was capped with a plastic microcentrifuge tube to avoid liquid evaporation. The electrode surface was then washed with a copious amount of ddH₂O, and blown dry with nitrogen. The resulting electrode was backfilled with 1 mM 6-mercaptohexanol for 1 h to minimize nonspecific DNA adsorption.

   The 2 mm diameter well-polished gold electrode modified with the capture DNA and MCH was then measured for the surface density of the capture DNA. Chronocoulometry (CC) in a low ionic strength buffer (10 mM Tris-HCl) was employed for such a determination. Trivalent [Ru(NH₃)₆]³⁺ (RuHex) (50 μM) was used as a cationic redox indicator, which quantitatively (in a ratio of 1:3) displaced the monovalent counterions of DNA phosphate backbones. The Faradaic charge related to the reduction of surface adsorbed RuHex (related to the amount of surface DNA), together with the accumulated double layer charge, was associated with the intercept on a $Q \sim t^{1/2}$ plot linearly
extrapolated to \( t = 0 \). After subtracting the double layer background (pre-measured by CC in the absence of RuHex) from the intercept charge, the surface coverage of DNA strands could be calculated following a literature method (A. B. Steel, T. M. Herne, M. J. Tarlov, *Anal. chem.*, 1998, **70**, 4670-4677). Typical surface density for the 24-base long capture DNA was \( 1.5 \times 10^{12} \) molecules/cm\(^2\) or around in our experiments.

**Target hybridization:**
The electrode modified with the capture DNA was covered by a solution containing target and reporter DNAs (10 nM reporter, target concentration varied, 0.5 M NaCl, 20 mM Tris-HCl, 1 mM EDTA) for 12 hours at 23°C. The electrode was then rinsed with a washing buffer (0.1 M NaCl, 20 mM Tris-HCl) before any further experiments.

**Polymerization:**
On the above electrode was then spotted with a polymerization cocktail (0.25 μM M1, 0.25 μM M2, 0.75 μM R1, 0.75 μM R2, 100 mM NaCl, 5 mM MgCl\(_2\), 20 mM Tris-HCl, 1 mM EDTA, at pH 8.0). The polymerization went on at 23°C for 22 hr. After polymerization, the electrode was rinsed with a washing buffer (0.1 M NaCl, 20 mM Tris-HCl) before electrochemical measurements.

**Electrochemical measurement:**
Cyclic voltammograms were acquired on a CHI 400A electrochemical analyzer (Shanghai Chenhua Co., Ltd., China). A conventional three electrode system was employed, containing the above obtained working electrode, an Ag/AgCl (3 M KCl) reference and a platinum wire auxiliary electrode. Electrolyte solution was composed of 10 mM Tris-HCl and 5 μM [Ru(NH\(_3\))\(_6\)]\(^{3+}\). Potential was scanned between -0.05V and -0.4V at a scan rate of 0.3 V/s.

**3. Gel electrophoresis**
Samples were prepared as mentioned above and incubated for two hours at room temperature before being combined with 2 μl of 75% glycerol and run on a 15% non-denaturing polyacrylamide gel in 1×TAE buffer (40 mM Tris.base, 20 mM Acetic acid, 2 mM EDTA) for 1 h at 32 V/cm.