Potentiometric Detection of DNA Hybridization: Supporting Information

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1. Reagents.

TRIS-HCl, 6-mercapto-1-hexanol, potassium dihydrogen phosphate, tris(carboxy-ethyl) phosphine (TCEP), and dipotassium hydrogenphosphate were purchased from Sigma (St. Louis, MO). The nucleic acids were obtained from Integrated DNA Technologies Inc. (Coralville, IA). The following oligonucleotide sequences were used: Probe 1: 5'-HS-GAC CTA GTC CTT CCA ACA GC-3', probe 2: 5'-GGG TTT ATG AAA AAC ACT TTT TTT TT-SH-3', target: 5'-AAA GTG TTT TTC ATA AAC CCA TTA TCC AGG ACT GTT TAT AGC TGT TGG AAG GAC TAG GTC-3', non-complementary: 5'-TTC CTT AGC CCC CCC AGT GTG CAA GGG CAG TGA AGA CTT GAT TGT ACA AAA TAC GTT TTG-3', 2-base mismatch: 5'-AAA GTG TTT TTC ATA AAC CCA TTA TCC AGG ACT GTT TAT AGC TGT TTG AAG GGC TAG GTC-3'.

Chemicals for the synthesis of CdS quantum dots, i.e., sodium bis-(2-ethylhexyl)sulfosuccinate (AOT), $Cd(NO_3)_2$, Na_2S, cystamine, sodium 2-mercaptoethane sulfonate, and the solvents were purchased from Sigma.

The ionophores, *N*,*N*,*N*′,*N*′-tetradodecyl-3,6-dioxaoctanedithioamide (ETH 5435), *N*,*N*-dicyclohexyl-*N*′,*N*′-dioctadecyl-1,3-oxapentanediamide (ETH 5234), sodium tetrakis[3,5-bis(trifluoromethyl)phenyl]borate (NaTFPB), tetradodecylammonium tetrakis(4-chlorophenyl)borate (ETH 500), 2-nitrophenyl octyl ether (*o*-NPOE), poly(vinyl chloride) (PVC), and tetrahydrofuran (THF) were purchased in Selectophore® or puriss. grade from Fluka (Buchs, Switzerland). Methylene chloride and H_2O_2 were obtained from Fisher (Pittsburgh, PA). Poly(3-octylthiophene) (POT) was synthesized as reported[1] and purified according to the patent application.[2] The methyl methacrylate-decyl methacrylate (MMA-DMA) copolymer matrix was obtained as described.[3] All stock and buffer solutions were prepared using autoclave doubly deionized water (18.2 MΩ cm).

2. Preparation of the Oligonucleotide Probe on the Gold Surface.

Immobilization of the oligonucleotide was based on the previously reported protocol.[4] Thiolated nucleotides were received with disulfide protecting groups.

Cleavage of the dithiol protecting group. The disulfide-protected nucleotides (100 μM, 10 μL) were diluted in autoclave water to 100 μL and treated with TCEP (1 mg) for 30 min, followed by purification using a MicroSpin™ G-25 column (Amersham Biosciences, Buckinghamshire, UK).

Gold substrates. The gold substrates were obtained from Denton Vacuum LLC (Moorestown, NJ), machine cut (by Advotech Company Inc., Tempe, AZ) to identical pieces (6 x 3 x 0.2 mm) of uniform thickness.

Preparation of mixed monolayers. Gold substrates were cleaned in *Piranha* solution and rinsed with water prior to use. (*Safety note*: The *Piranha* solution must be handled with extreme caution.) The oligonucleotide monolayer was generated by treating the gold substrates with a 1 μM thiolated oligonucleotide solution (100 μL) in phosphate buffer (0.05 M, pH 7.0) overnight, followed by removal of the solution. The surface of the gold substrates was then blocked by a 10-min treatment with 6-mercapto-1-hexanol (0.1 M, 100 μL), followed by washing with water.

3. Preparation of CdS Quantum Dot Nanocrystals.

Quantum-dot nanoparticles were prepared using a slightly modified procedure reported previously.[5] First, sodium bis-(2-ethylhexyl)sulfosuccinate (AOT) (14.0 g) was dissolved in a mixture of *n*-hexane/water (200 mL/4 mL). The resulting mixture was separated into two sub-volumes of 120 mL and 80 mL. A 0.48 mL aliquot of a 1 M $Cd(NO_3)_2$ solution was added to the 120 mL sub-volume, while 0.32 mL of 1 M Na_2S solution were added to the 80 mL sub-volume. The sub-volumes were stirred for 1 h, then mixed and stirred under nitrogen for an additional hour. The quantum dots were capped by adding cystamine (0.34 mL, 0.32 M) and sodium 2-mercaptoethane sulfonate (0.66 mL, 0.32 M) and mixing under nitrogen for 24 h. Evaporation of hexan *in vacuo* yielded quantum dot nanocrystals, which were washed with pyridine, hexane, and methanol.

4. Preparation of CdS Quantum Dot–Oligonucleotide Conjugate.

The CdS-oligonucleotide conjugate was prepared using a modified protocol.[4,6] A CdS quantum dot suspension (0.2 mg/mL, 500 μL) was exposed to the thiolated oligonucleotide secondary DNA probe (probe 2). The mixture was stirred overnight at room temperature. The quantum dot-DNA conjugate was collected by centrifugation at room temperature at 10,000 rpm for 30 min, removal of supernatant, and resuspension in hybridization buffer (750 mM NaCl, 150 mM Na-citrate).

5. Sandwich DNA Hybridization Assay.

The oligonucleotide-modified gold substrates were incubated for 60 min with the desired amount of target DNA in hybridization buffer (100 μl) followed by washing with washing buffer (50 mM Tris-HCl, 0.1% Tween 20; pH 7.4). Then, the gold substrates were incubated with quantum dot-oligonucleotide secondary probe for 60 min at room temperature. The supernatant was removed, the gold substrates were washed twice with washing buffer (100 μL each), and transferred to new microwells, where were washed 4 times again with the washing buffer (100 μL each) and twice with water.

Dissolution and detection. Hydrogen peroxide was used for the dissolution step since it was observed that it can efficiently oxidize the CdS quantum dots after optimizing concentration and reaction time.[7] Preliminary experiments on dissolving CdS quantum dots with 0.01 M hydrogen peroxide and potentiometric detection of the released Cd^{2+} showed that cadmium was fully oxidized after 15 min. In the final assay, dissolution of CdS was carried out by the addition of 0.01 M H_2O_2 in 10^{-4} M $CaCl_2$ (100 μL) for 1 h to ensure complete oxidation. The detection was performed in microwells (Corning Inc, NY) containing 180 μL of 10^{-4} M $CaCl_2$ and adding 20 μL of sample, using a Ca-ISE as reference and a small magnetic stirring bar. Prior to the measurements, each well was treated with 10% HNO_3 overnight, washed at least 5 times with deionized water, and allowed to dry.

6. Potentiometric Measurements.

Membranes. The membranes for the Cd- and Ca-ISEs were prepared according to the previously described procedure.[8]

EMF measurements. Potentiometric measurements were performed in stirred solutions at room temperature (22 ºC) with a PCI MIO16XE data acquisition board (National Instruments, Austin, TX) connected to a four-channel high Z interface (WPI, Sarasota, FL).

7. ISE Stability and Reproducibility.

The reproducibility of the Cd-ISEs was evaluated in-200 μL solutions by recording three different calibration curves over the concentration range of 10^{-10}–10^{-5} M. After each measurement, the ISE was rinsed for 5 min to eliminate possible memory effects. This washing step was performed in alternating solutions of 10^{-3} M $CaCl_2$, water and 10^{-4} M $CaCl_2$ under continuous stirring for ca. 5 minutes until the readout reached the baseline potential for the background. The standard deviation of the EMF for each concentration was <1.0 mV. During continuous experiments, the Cd-ISEs were found to be capable of measuring more than 45 samples, with good response times and a standard deviation of

<1.5 mV. After recalibration, they can be used for more analyses. After 1 month, the Cd-ISEs showed a loss of detection limit by half an order of magnitude.

8. System Optimization.

The effect of the concentration of the primary DNA probe used in the immobilization was tested with 500, 750, 1000, and 1250 nM solutions in triplicate. The results of binding of 100 nM target DNA with different concentrations of primary DNA probe (probe 1) are shown in Figure 1. Since a stable EMF signal was found with 1 μM primary DNA probe, this level was selected for all subsequent work.

Similarly, the effect of the level of the secondary probe was investigated (in triplicate) by immobilizing 1000 nM primary probe on the gold substrate and using 100 nM target DNA. As seen in Figure 2, the response increased rapidly when increasing the concentration of probe 2 to 750 nM, and more slowly at higher concentrations. In all subsequent work, therefore, a concentration of 1 μM probe 2 was used.

The effect of target hybridization times was studied over the range of 30-75 min. The results from the hybridizations of 100 nM target DNA with the immobilized primary and secondary probes are shown in Figures 3A and 3B, respectively. In both cases, the signal increases upon increasing the hybridization time from 30 to 60 min, and levels off thereafter. Consequently, a 60 min hybridization time was chosen for the two hybridization steps.

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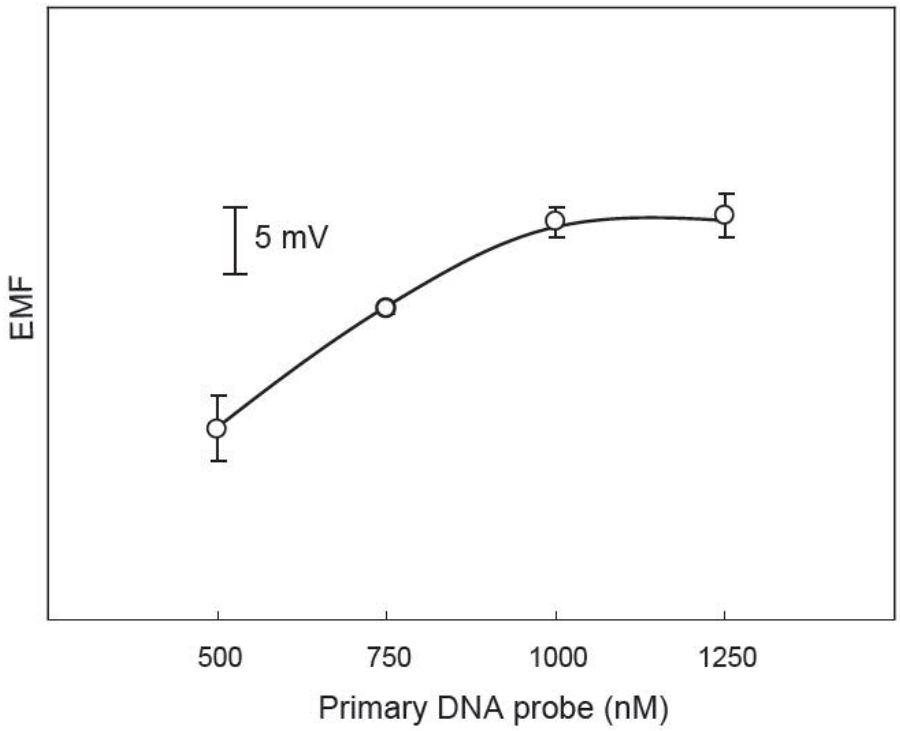

Figure 1. Influence of the concentration of the primary DNA probe, immobilized on the gold substrate, on the response. The immobilized probe was exposed to 100 ppb of target DNA (60 min) upon which 1000 nM secondary DNA probe was added (60 min). The potentiometric measurements were performed in 200-µL samples using 10^{-4} M $CaCl_2$ as background and Ca-ISE as pseudoreference electrode. Error bars: SD, N = 3.

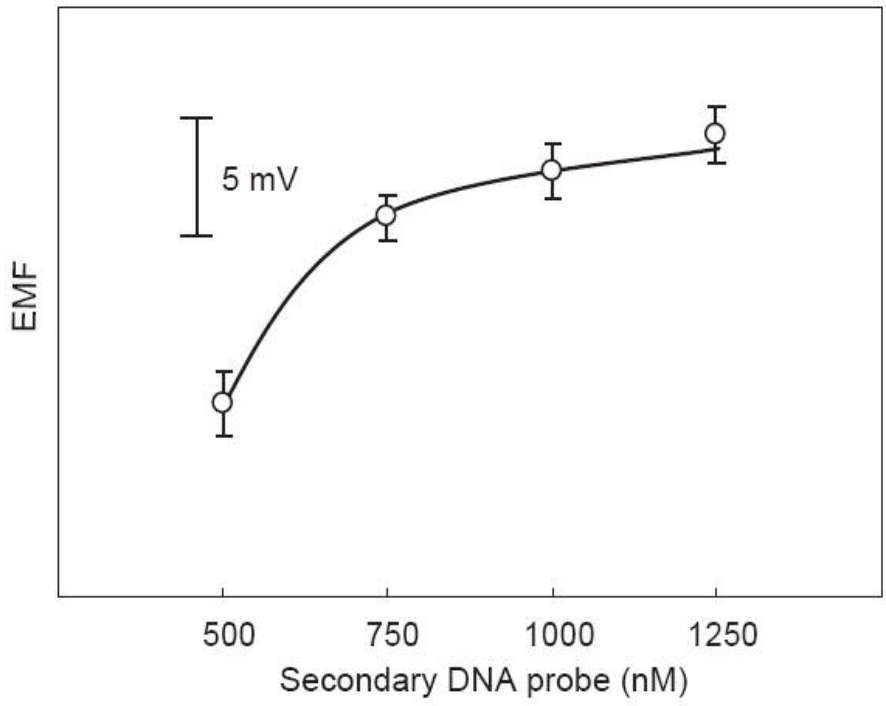

Figure 2. Influence of the concentration of the secondary DNA probe on the response. Other parameters of the assay: 1000 nM primary probe, 100 nM target DNA. Error bars: SD, N = 3. Other conditions as in Figure 1.

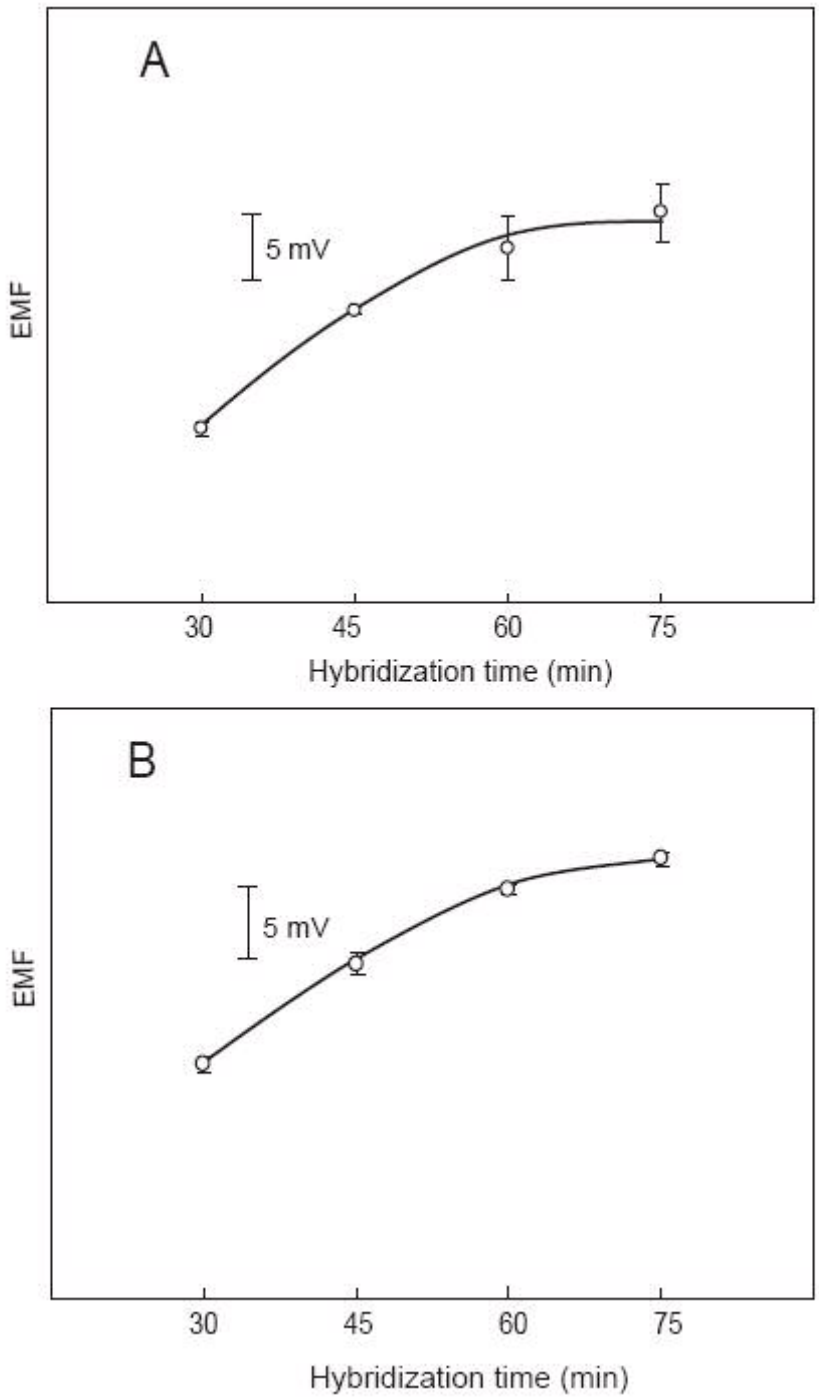

Figure 3. Effect of time of the primary (A) and the secondary (B) DNA hybridization on the potentiometric response to 100 nM target DNA (error bars: SD, N = 3). Other conditions as in Figure 1.