Supplementary Material (ESI) for Chemical Communications

Electronic Supplementary Information for

G-quadruplex signaling probe for highly sensitive DNA detection

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Experimental Section

Chemicals and Materials

Oligonucletides designed in the present study were synthesized by Generay Biotech Co. (Shanghai, China) and had the following sequences:

Capture DNA: 5' HS-$(CH_2)_6$-TTT CTA GTG CGC GGA TTG ACAC 3'

Amino terminal DNA1 (used to prepare the signaling probe1):

5' TT*GGGGG*TTT GTGT CAA TCC GCG C AC TAG-$(CH_2)_3NH_2$ 3'

Amino terminal DNA2 (used to prepare the signaling probe2):

5' TT*TTTTT*TTT GTGT CAA TCC GCG C AC TAG-$(CH_2)_3NH_2$ 3'

Target DNA1: 5' GTGT CAA TCC GCG CAC TAG 3'

Target DNA2: 5' GTGT CAA TCC GCG CAC TA***T*** 3'

Target DNA3: 5' ***T***TGT CAA TCC GCG CAC TAG 3'

Target DNA4: 5' GTGT CAA T***A***C GCG CAC TAG 3'

Target DNA5: 5' TGGA GCT TCA GCC TGA CAC 3' (noncomplementary sequence)

Capture DNA sequences are complementary to amino terminal DNAs as well as target DNA sequences (not including target DNA5). The locations of

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single-nucleotide variations in target sequences are indicated in bold italics. Target DNA5 with the same base composition is designed to be noncomplementary to capture DNA via changing the base sequence. The oligonucleotide stock solutions were prepared with 0.3 M KCl, 10 mM phosphate buffer (pH 7.4) solution (0.3 M PBS) and stored at 4 ℃ until the time of use. The signaling probe was prepared through covalent attachment of ferrocenecarboxylic acid to the amino terminal DNA using standard EDC/NHS (N-ethyl-N'-(dimethylaminopropyl)carbodiimide/N-hydroxysuccinimide) coupling chemistry according to the previous method (1) and dialyzed against 0.3 M PBS. Unless otherwise stated, the signaling probe1 was used throughout.

Other chemicals involved were of analytical grade and used as received without further purification. Milli-Q quality deionized water was used throughout.

Construction of Sensing Interface

A gold electrode (1.0 mm diameter) received from Chenhua Instruments C. (Shanghai, China) was polished to mirror surface with a 0.05-μm alumina powder, followed by sonicating successively in water, absolute alcohol and water for 5 min each. Then, the electrode was cleaned with piranha solution (H_2SO_4:H_2O_2, 7:3 by volume), followed by being electrochemically treated by cycling the potential between -1.0 and +1.55 V in 0.1 M H_2SO_4 until stable voltammograms for gold oxide formation and reduction were observed. The real surface area of the electrode was calculated as about 6.0×10^{-2} cm^2 from the corresponding charge for the reduction of the oxide monolayer. After being washed with water, the gold electrode was dried in a stream of nitrogen.

The construction of electrochemical DNA sensing interface is illustrated in Scheme 1. Prior to exposure to the cleaned gold electrode, capture DNA solution (2.04 μM) was mixed with an equal volume of 3.25 μM signaling probe solution to obtain capture DNA/signaling probe duplexes. A 12-μL droplet of the resulting solution was placed on the surface of gold electrode that was held upside down and kept in a water-saturated atmosphere, and the self-assembly reaction was allowed to proceed for 120 min. Subsequently, the modified gold electrode was exposed to a 1 mM MCH

solution for 20 min. After the signaling probe was removed from the electrode surface by thermal denaturation, the sensing interface for the DNA hybridization detection was accomplished.

Electrochemical Measurements

Alternating current (AC) voltammetry and cyclic voltammetric measurement were performed using CHI 760B electrochemical workstation (Shanghai, China). The reference was a saturated calomel electrode (SCE, saturated KCl). A platinum foil was used as the counter electrode. AC voltammetry was conducted at an amplitude of 25 mV, a frequency of 1 Hz, and a sample period of 1.1 s. All AC voltammograms showed in the present work were the baseline-subtracted currents. Cyclic voltammetric measurements were only carried out when probing the electrochemical characterization of the working electrodes.

Electrochemical Detection of DNA Hybridization

A 10-μL droplet of target DNA sequence solution at specific concentration was placed on the sensing interface, and the hybridization (the first hybridization) was left to react for 40 min, followed by washing the excess of target sequence with 0.3 M PBS. Then, 10 μL of 3.25 μM signaling probe solution was introduced onto the resulting electrode surface, and kept the hybridization reaction (the second hybridization) for 40 min. After rinsing with 0.3 M PBS, the electrochemical measurement was performed. The first hybridization and the second hybridization took place at 25 ℃. The used gold electrode could be regenerated readily by thermal denaturation.

To valuate the ability of the present assay system to detect single-base mismatches, a single-base mismatched target DNA sequence was used for the first hybridization, and detection experiments were conducted as described above.

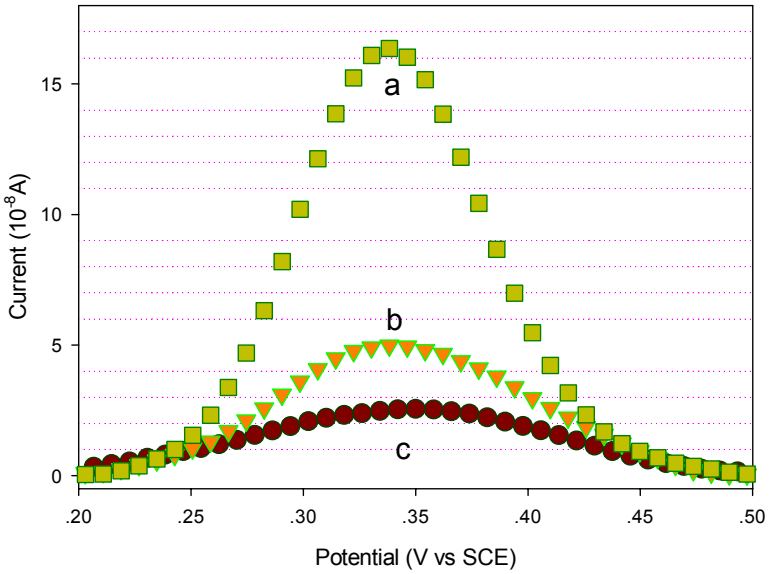

Figure S1 Comparison of the current responses to signaling probe obtained for different sensing interfaces. The line a, b and c indicate the electrochemical responses of the gold electrodes modified with prehybridized capture DNA/signaling probe duplexes using a 120-min assembly, with single-stranded capture DNAs using a 720- and 120-min assembly, respectively.

AC voltammograms for the sensing interfaces prepared using different methods after incubating in signaling probe solution are shown in Figure S1. The preparation of the electrode b and c was carried out under the same conditions as those involved for the electrode a, except that single-stranded capture DNAs were substituted for the prehybridized capture DNA/signaling probe duplexes and a 720-min assembly was used for the electrode b. Under identical conditions, peak current for the sensing interface prepared using double-stranded DNA-based self-assembly strategy (line a) is more than 6-fold higher than that obtained through single-stranded DNA self-assembly (line c). Even though the incubation time for capture DNA immobilization was lengthened from 120 to 720 min, only 30% peak current could be achieved (line b) compared with the current value recorded for the sensing interface prepared using double-stranded DNA-based technique (line a). Possibly, DNA hybrid-based self-assembly could provide sufficient interstitial space for the subsequent target DNA hybridization. Direct comparison of these data indicated that prehybridization of capture DNA with

signaling probe was necessary to successfully prepare the sensing interface.

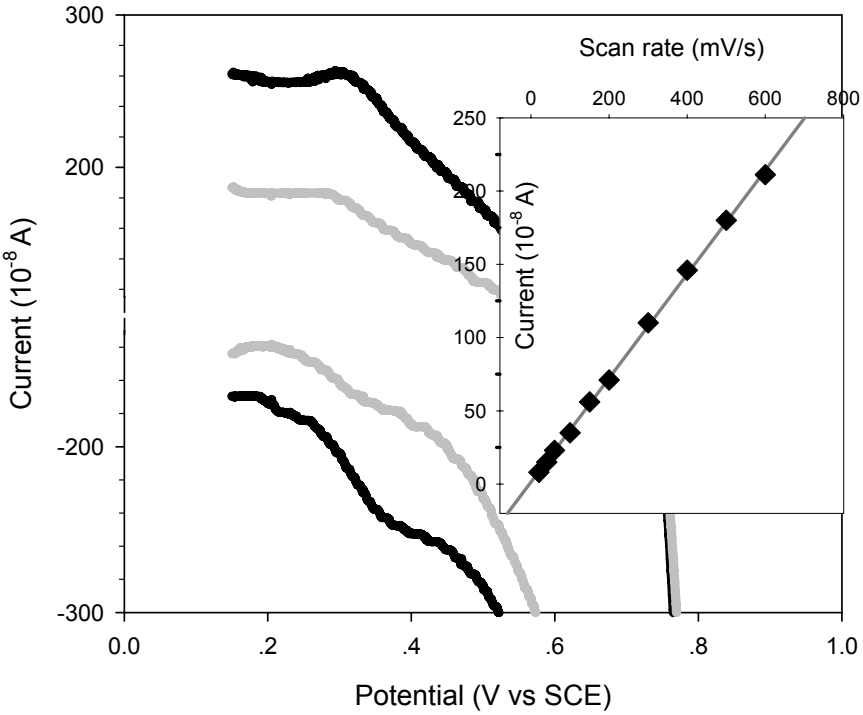

Figure S2 Cyclic voltammograms of the capture DNA/signaling probe duplexes on the sensing interface in the presence (light gray) or absence (dark gray) of target DNA sequences at a scan rate of 100 mV s^{-1}. The inset shows the variation of the current intensity with the scan rate in the range of 20-600 mV s^{-1}. The modified electrode used was that without target DNA.

Figure S2 shows cyclic voltammograms of the sensing interface collected after hybridization with the signaling probe in the absence and presence of 3.4×10^{-10} M complementary target DNA. A marked decrease of redox peak current is clearly seen due to the introduction of the target sequence to the gold electrode. Such a result provided strong evidence that the sensing platform prepared using the present scheme can be employed for the detection of target DNA sequences. According to the method reported in the literature (2), a modified electrode without target DNA after exposure to signaling probe solution was used to investigate the dependence of peak current on the scan rates. The baseline-corrected current intensity is plotted versus the scan rate as shown in the inset of Figure S2. Clearly, the peak current was linearly proportional to the potential scan rate in the range from 20 to 600 mV s^{-1}, indicating a

surface-confined redox reaction.

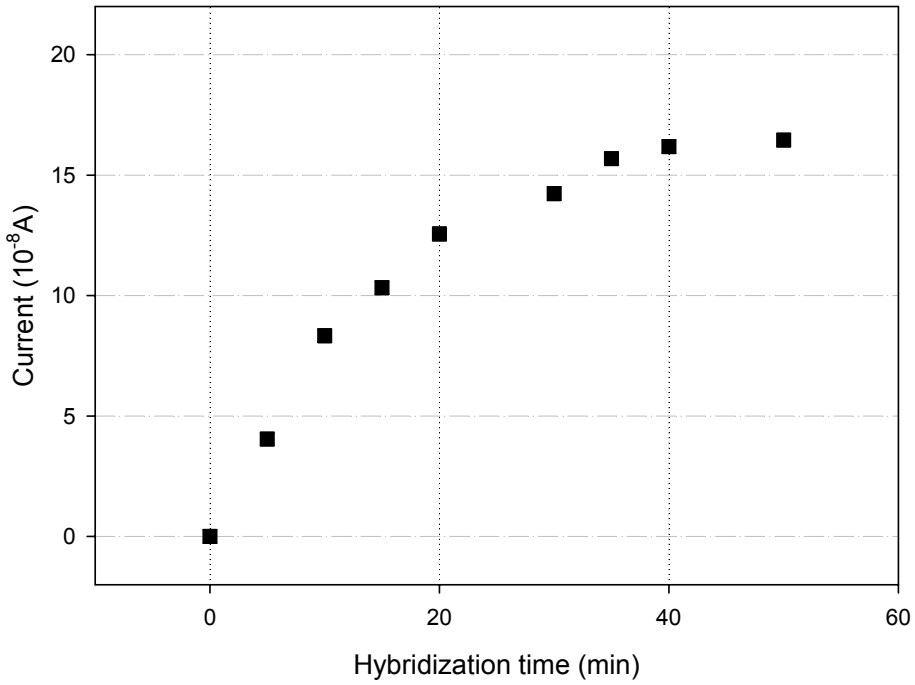

Figure S3 Hybridization kinetics on the sensing interface for signaling probe.

The incubation time for hybridization between the surface-confined capture DNAs and signaling probes was investigated to improve the hybridization efficiency and the subsequent response to target DNA sequence. Figure S3 shows peak currents of AC voltammograms for the working electrode obtained by incubating sensing interface in signaling probe solution for different periods of time. Peak current increased rapidly with the hybridization time up to 40 min, after which it varied slightly. Taking into account that some prehybridized target DNA sequences on the surface of working electrode might be possibly replaced by signaling probes due to competitive hybridization, an incubation time of 40 min rather than a longer time was set as the hybridization time in subsequent experiments.

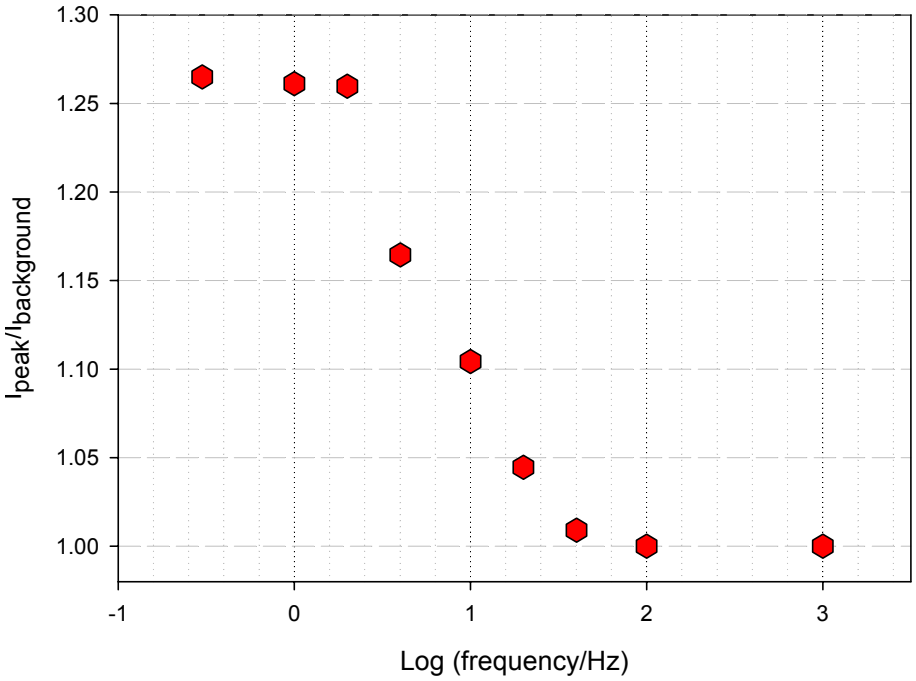

Figure S4 Plot of the peak ratio ($I_{peak}/I_{baseline}$) against log of working frequency prepared using the AC voltammetric data. The AC voltammograms are collected at different working frequency for signaling probe/capture DNA duplex-modified gold electrode surface. $I_{peak}/I_{baseline}$ is plotted as a function of the logarithm of the frequency, where I_{peak} is the absolute peak current while $I_{baseline}$ is the baseline current.

AC voltammetry has proven to be very useful for studying redox kinetics in monolayers on electrodes (3), particularly when the amount of the surface-confined redox-active molecules is small. Thus, AC voltammetry was utilized for the quantitative detection of target DNA sequence. As indicated in the previous work (4), the plot of the ratio of the peak current to background (current ratio) against the logarithm of AC frequency shows the sigmoidal shape. Therefore, it is necessary to optimize the AC frequency. Figure S4 presents the dependence of AC voltammogram on the frequency of the applied AC voltage. It is very clear that there are two data plateaus in the plot of the ratio of peak current to baseline current versus log (frequency): at low frequency, the ratio is almost constant; at high frequency, the ratio

approaches one as the ferrocene oxidation-reduction reaction can no longer occur rapidly enough to keep pace with the fluctuating potential (5). Between these two plateaus is a transition region where the current ratio decreases sharply with the frequency. The experimental results were consistent with those reported in literature (6). The AC voltammetric experiments in this study were carried out at a low frequency of 1 Hz for the applied AC potential as it was in the middle of the plateau value of the current ratio.

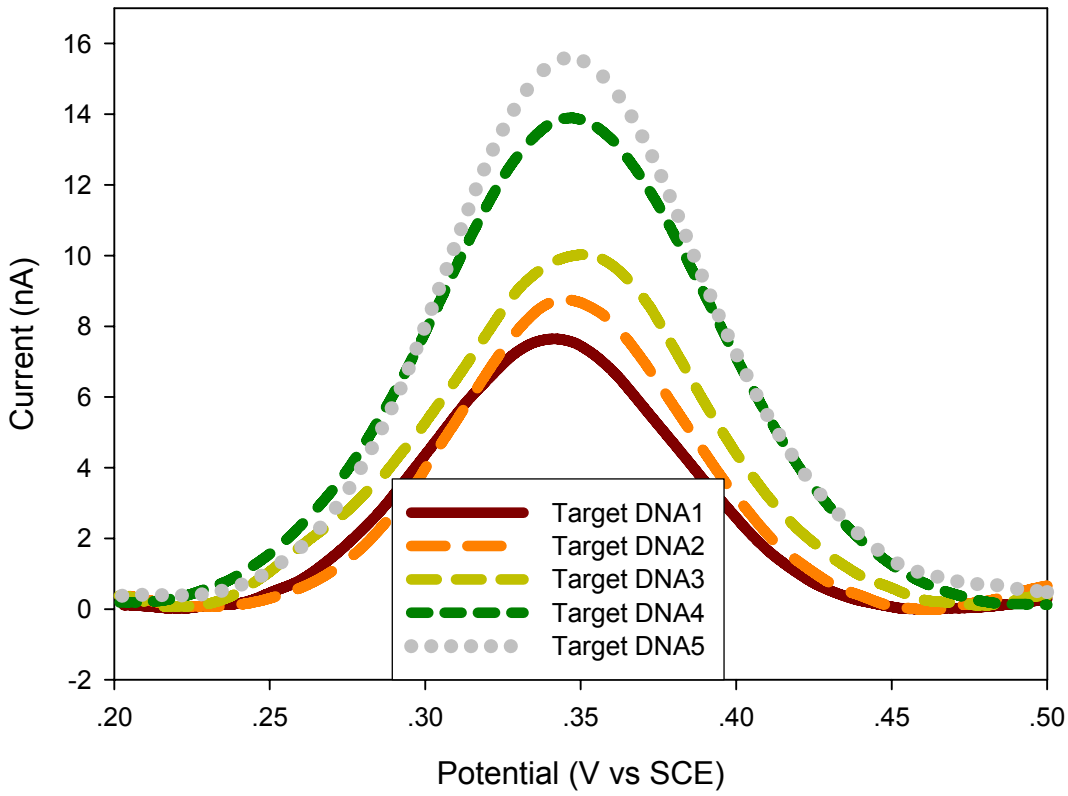

Figure S5 The selectivity of the electronic DNA assay system. AC voltammograms of the modified electrodes are recorded after incubation in signaling probe solution following hybridization with full-matched, mismatched or noncomplementary target sequences at 9.92×10^{-10} M. These data are corrected by the subtraction of the baseline electrochemical current.

References

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