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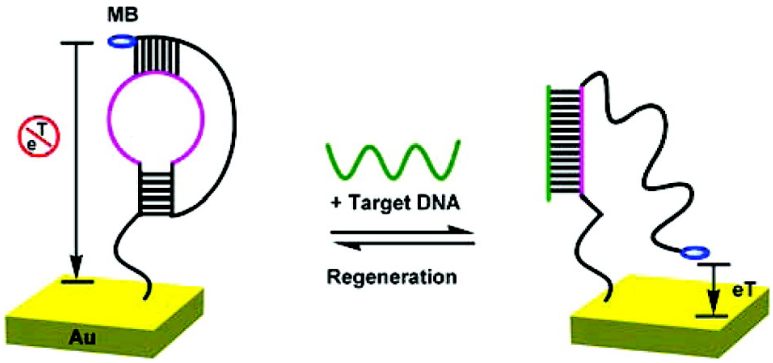

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Label-Free Electrochemical Detection of DNA in Blood Serum via Target-Induced Resolution of an Electrode-Bound DNA Pseudoknot

Yi Xiao,[†,§] Xiaogang Qu,[*,‡] Kevin W. Plaxco,[*,§,¶] and Alan J. Heeger[†]

Department of Physics and Institute for Polymers and Organic Solids, Department of Chemistry and Biochemistry, and Program in BioMolecular Science and Engineering, University of California, Santa Barbara, California 93106 and Division of Biological Inorganic Chemistry, Key Laboratory of Rare Earth Chemistry and Physics, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun, Jilin 130022, China

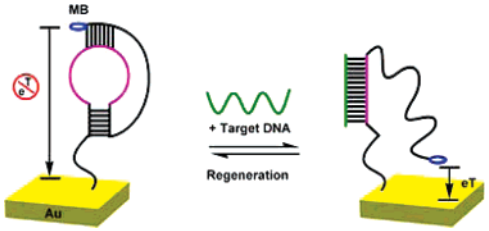
Received June 9, 2007; E-mail: kwp@chem.ucsb.edu; xqu@ciac.jl.cn

Improvements in our ability to detect DNA will translate into improvements to detect and prevent disease,[1,2] an argument that has motivated the development of a large number of optical and electrochemical DNA sensors.[3,4] Recent years, for example, have seen the development of a number of electrochemical DNA (E-DNA) sensors that detect hybridization-induced conformational changes in a redox-modified, electrode-bound probe DNA.[5−9] Broadly, these reagentless, single-step E-DNA sensors fall into two classes. The first class comprises "signal-off" sensors in which target hybridization sequesters the redox moiety from the electrode, reducing the observed Faradaic current.[6−9] In the second class of sensors, in contrast, target hybridization enhances the rate which the redox moiety strikes the electrode, leading to increased current.[5,10]

Signal-on and signal-off E-DNA sensors each have their own strengths and shortcomings. For example, because the signal-off E-DNA architectures reported to date comprise a single probe DNA that is strongly chemi-absorbed to the interrogating electrode,[6,8] they exhibit excellent shelf and operational lifetimes,[11] are stable enough to deploy directly in serum, soil, and other complex sample matrices, and can be readily regenerated via a simple, aqueous wash at room temperature.[9] As signal-off sensors, however, these architectures suffer from false positives when either the probe DNA or the redox tag become degraded and are limited in their signal gain as, at most, the target can suppress only 100% of the original current. Likewise, while the previously reported signal-on architectures avoid these pitfalls, they nevertheless suffer from other significant disadvantages. The first reported signal-on E-DNA architecture, for example, comprises a difficult-to-synthesize DNA−poly(ethylene glycol)−DNA triblock polymer and suffers from high background currents, limiting signal gain.[5] In contrast, the second signal-on architecture, which utilizes a strand-displacement mechanism,[10] exhibits exceptional gain and is comprised of a readily synthesized, double-stranded probe DNA.[10] Unfortunately, however, the double-stranded DNA-based sensor is not reusable—regeneration conditions stringent enough to disrupt target binding cause loss of the signaling strand—and is insufficiently stable for deployment directly in complex samples, such as blood serum.[10]

Motivated by these observations, we report here a novel E-DNA sensor that is based on a target-induced resolution of an electrode-bound, single-stranded DNA pseudoknot. This new E-DNA architecture couples the stability, reusability, and synthetic ease of earlier signal-off designs with the improved signaling characteristics inherent to signal-on architectures. To this end, the new sensor platform is the first signal-on E-DNA sensor that is selective enough to directly employ in blood serum and stable enough to be easily regenerated.

Scheme 1. Schematic of the Signal-On E-DNA Sensor, Which is Based on the Target-Induced Resolution of an Electrode-Bound, Methylene Blue-Modified, DNA Pseudoknot



The new E-DNA architecture consists of a short, single-stranded DNA pseudoknot[12] that forms two stem-loop structures in which a portion of each loop forms one strand of the stem of the other stem-loop (Scheme 1, left). This pseudoknot DNA (**1**) is modified at its 3′-terminus with a redox-active methylene blue (MB) and covalently attached at its 5′-terminus to a gold electrode via a thiol−gold bond.[13] The surface coverage of the MB-modified DNA probe (**1**) was maintained within the range of 28 ± 2 pmol·cm^{-2} (monitored using cyclic voltammetry[14] and assuming perfect electron transfer efficiency) by controlling the concentration of DNA employed during fabrication; we find that this probe density leads to enhanced signal gain (see Supporting Information (SI), Figure S1). In the absence of target DNA, formation of the pseudoknot structure sequesters the MB tag from the electrode, reducing the Faradaic current (Scheme 1, left). When the sensor is challenged with a 17-base, perfectly matched target (**2**), the observed MB redox current increases significantly. This presumably occurs because target hybridization displaces the seven hybridized bases at the 3′-terminus of the pseudoknot DNA, liberating the flexible, MB-labeled single-stranded element end of the probe that can collide with and transfer electrons to the electrode (Scheme 1, right).

The new E-DNA sensor responds sensitively and reproducibly to its target sequence. In the absence of target, we observe small, reproducible (standard deviation across four electrodes <2%) Faradaic currents (Figure 1A, 0 nM target), presumably arising due to limited, long-range electron transfer from MB to the electrode[15] or due to short-range electron transfer from surface-immobilized probe DNAs not in the pseudoknot conformation. This Faradaic current increases significantly as we titrate the sensor with a short, fully complementary DNA, reaching ∼2 times that of the original current at saturating target concentrations (above ∼100 nM; Figure 1A). The detection limit of the current sensor is 2 nM (defined by a coefficient of variation <1/3), and the useful dynamic range covers from 2 to 100 nM (Figure 1B).

[†] Department of Physics and Institute for Polymers and Organic Solids, University of California, Santa Barbara.
[‡] Division of Biological Inorganic Chemistry, Chinese Academy of Sciences.
[§] Department of Chemistry and Biochemistry, University of California.
[¶] Program in BioMolecular Science and Engineering, University of California.

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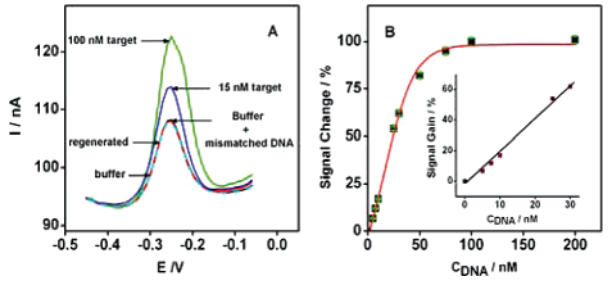

Figure 1. The signal-on E-DNA sensor is sensitive and specific. (A) Shown are alternating current (AC) voltammograms of the E-DNA sensor challenged with either 100 nM of a perfectly matched target or 2 μM of a three-base mismatched target. Our ability to regenerate the sensor is also indicated. (B) The useful range of the sensor spans from 2 to 100 nM. The illustrated error bars represent the standard deviation of three measurements conducted with a single electrode at each concentration; multiple electrodes were used to collect the entire data set. (Relative sensor response (%) is employed because of more reproducible result.)

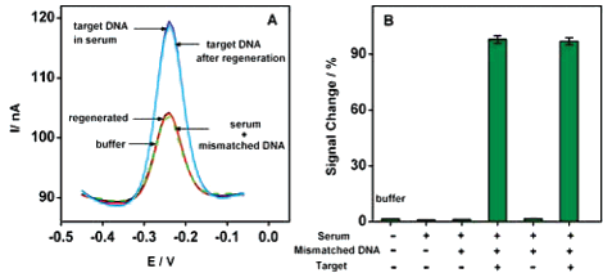

Figure 2. The E-DNA sensor readily discriminates between 100 nM of a perfectly matched target (**2**) and 2 μM of a three-base mismatched DNA (**3**) even in a complex, contaminant-ridden sample such as blood serum. Shown are the original AC voltammograms (A) and a histogram representation of the same data (B). Illustrated on the histogram (from left to right) are the signal changes observed from target-free buffer, target-free 50% fetal calf serum, serum doped with 2 μM mismatched DNA (**3**), serum doped with 2 μM mismatched DNA (**3**), and 100 nM of the target DNA (**2**), after regeneration and, finally, upon challenging the regenerated electrode a second time with target-doped serum.

The specificity of the new E-DNA architecture is improved over that of the original E-DNA sensor.[6] Control experiments reveal that a 17-base target with three mismatches (**3**) or five mismatches (**4**) produces less than a 2% signal increase at concentrations as high as 2 μM (Figure 1A), which is 20 times the concentration at which the signal from the perfectly matched target saturates. As such, the specificity of the pseudoknot-based sensor significantly outpaces that of the original E-DNA sensor,[6] presumably because the free energy of hybridization of the mismatched targets cannot compete with the opening of two 7-base pair stems. The equilibration time of the E-DNA sensor is also reasonably rapid; 75% of the final response is observed within 30 min at 100 nM target (see SI, Figure S2).

Because signaling is based on a target hybridization-induced change (rather than the absorption of mass or charge to the sensor surface) and because the probe DNA is fully covalent, the new sensor architecture is relatively insensitive to the nonspecific binding of contaminants and is stable in complex media. Because of this, we can readily detect perfectly matched target (**2**) doped in 50% serum (diluted with buffered saline to control the pH and ionic strength) (Figure 2). Mismatched targets (**3** or **4**), in contrast, do not produce any significant signal change (<2%) under these conditions (Figure 2), indicating that the sensor's specificity is not reduced even in complex clinical materials. Likewise, under the same conditions, the sensor's response to its target (**2**) is unaffected by the presence of a 20-fold greater concentration of mismatched DNA (Figure 2).

Sensor regeneration is critical in order to ascertain that a signal is arising due to specific interactions with the target and is not simply due to some other nonspecific modification of the probe or sensor head. Fortunately, because the MB-tagged DNA pseudoknot is a single-stranded element strongly chemi-adsorbed to the electrode surface, the sensor is stable enough to allow for ready regeneration: a brief, low ionic strength wash (30 s in room temperature distilled water) is sufficient to recover >96 ± 2% of the original sensor signal even for sensors that have been challenged directly in 50% blood serum (Figure 2B), and we have reused individual sensors more than a half-dozen times before significant degradation is observed (see SI, Figure S3).

We and others have previously shown that E-DNA-like sensing is supported by the binding-induced disruption of a DNA stem-loop,[6,9] the binding-induced folding of DNA aptamers,[16] the hybridization of single-stranded probe DNA,[5,8] and via a strand-invasion mechanism.[10] Here we have further extended the range of E-DNA sensing architectures by demonstrating an E-DNA sensor based on the target-induced resolution of a DNA pseudoknot. This new, fully covalent, signal-on sensor is easily fabricated, exhibits good specificity, is selective enough to deploy in directly blood serum, and is stable to be readily regenerated. It thus appears among the more promising E-DNA architectures reported to date. Moreover, when taken with the observation that any single-stranded DNA or RNA sequence, including aptamer sequences, can be modified to form pseudoknots, and given that the target-binding-induced folding of an aptamer should also disrupt pseudoknot formation, this sensor architecture may also prove suitable for the detection of proteins, small molecules, and other non-nucleic acid targets even in complex clinical samples.

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Supporting Information Available: The preparation of the E-DNA sensor, time-course and regeneration experimental results are available. This material is available free of charge via the Internet at http://pubs.acs.org.

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