Linear, redox modified DNA probes as electrochemical DNA sensors†

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We show here that hybridization-linked changes in the dynamics of a redox-modified, electrode-bound linear (as opposed to stem-loop) probe DNA produce large changes in Faradaic current, allowing for the ready detection of target oligonucleotides.

E-DNA sensors, which consist of a redox-tagged stem-loop DNA covalently attached to an interrogating electrode, are the electrochemical equivalents of optical molecular beacons.[1–9] We show here, however, that unlike molecular beacons, which rely on a rigid, binding–induced conformational change (to segregate a fluorophore–quencher pair),[10–12] E-DNA signaling arises due to binding-induced changes in the dynamics of the probe DNA. We do so by demonstrating that hybridization-linked changes in the dynamics of an electrode-bound linear (as opposed to stem-loop) probe DNA efficiently support E-DNA signaling. That is, whereas a large Faradaic current is observed from a redox-modified, single-stranded DNA probe, this current is reduced upon hybridization to the appropriate target DNA sequence due to changes in the rate with which the terminal redox label collides with the electrode surface (Fig. 1).

We have fabricated E-DNA sensors using a 27-base linear probe sequence that, in order to facilitate direct comparison with earlier studies, is directly analogous to a previously characterized stem-loop E-DNA sensor[9,13] save that the five base sequences at the two termini are identical and thus do not form a double stranded stem. In the absence of target, the sensor gives rise to a sharp, well-defined AC voltammetry peak consistent with the ~ -0.26 V (*vs.* Ag/AgCl) formal potential of the methylene blue redox moiety employed (Fig. 2). Upon hybridization to a fully complementary, 17-base target this current is significantly reduced. Furthermore, because the observed signal change arises due to a hybridization-specific change in DNA dynamics (as opposed to the simple adsorption of mass or charge to the sensor surface), we can readily observe this change even when the sensor is challenged with complex, multi-component sample matrices, such as target-doped blood serum (Fig. 2, right). Finally, like the original stem-loop E-DNA architecture, the linear-probe E-DNA sensor is label-free and reusable: a 30 sec wash in room temperature distilled water or (after deployment in blood serum) room temperature detergent solution is enough to regenerate >97% original sensor current (Fig. 2).

The signaling characteristics of linear probe E-DNA sensors are improved relative to those of the equivalent stem-loop sensor. Whereas a linear probe E-DNA sensor exhibits an 85% signal reduction at a given target concentration (Fig. 2), the equivalent stem loop sensor exhibits only 71% signal suppression at this target concentration.[9] We presume this difference arises because, in

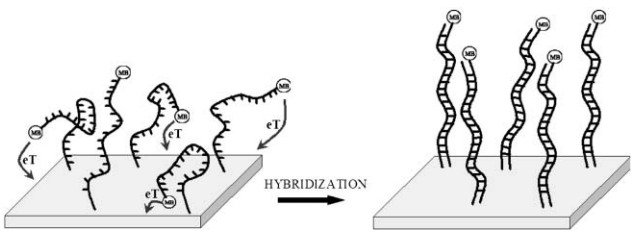

Fig. 1 E-DNA signaling arises due to hybridization-induced changes in probe dynamics (rather than to a conformational change *per se*) and thus redox-modified linear probe DNAs serve as effective E-DNA sensors. The Faradaic current arising from such a linear probe DNA is significantly reduced in the presence of a complementary target sequence because, as demonstrated here, hybridization reduces the rate with which the terminal redox tag collides with the electrode surface and transfers electrons.

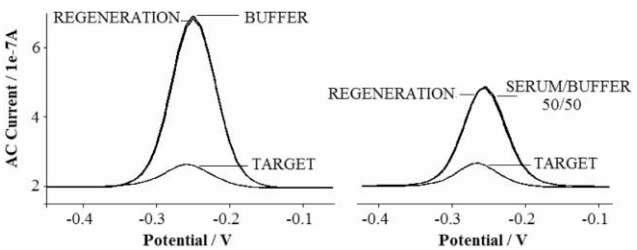

Fig. 2 Linear-probe E-DNA sensors respond well in either (left) buffer or (right) 50% blood serum. And while the currents observed in serum are lower, the signal suppression observed in serum (77%) is quite similar to that obtained in buffer (85%) and equivalent sensor regeneration (>97%) is observed under both conditions. The reduced currents observed in serum may be due to the greater viscosity and/or the reduced ionic strength of this medium, which would reduce collision rates and electron transfer efficiency; no similar drop-off is observed for stem-loop sensors,[3] presumably because the stem structure fixes the MB near the surface, rendering its electron transfer rate relatively independent of these effects. The very similar gains observed in serum and in buffer suggest that none of the hundreds of proteins that have been identified in serum to date affect sensor performance. These experiments were conducted after $\sim$30 min. incubation with 200 nM of a 17-base, fully complementary target. Regeneration is achieved with a 30 sec, room temperature wash in (left) distilled water or (right) 10% sodium dodecyl sulfate.

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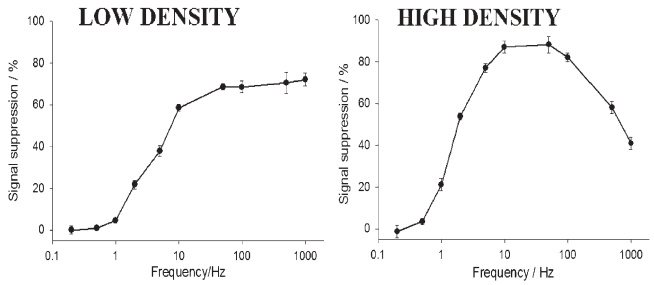

Fig. 3 Sensor response is a strong function of the ACV frequency employed to probe electron transfer, supporting the collisional mechanism proposed here. The signal suppression observed with lower-density sensors (left) rises rapidly above ~0.5 Hz before saturating at ~10 Hz. This presumably occurs because, at lower AC frequencies, the MB moiety on both single-stranded and more rigid, double-stranded probes have sufficient time to collide with the electrode and transfer electrons (and thus the *difference* in the current arising from free and target-bound probes is reduced). In contrast, the signal change associated with higher-density sensors (right) rises at lower frequencies and then decreases again above ~50 Hz. We presume the latter fall-off occurs because, under these conditions, the collision dynamics of even single-stranded probes do not support efficient electron transfer (of note, no similar drop-off is observed for stem-loop sensors of any density,[9] presumably because the stem structure fixes the MB near the surface, ensuring an extremely rapid collision rate). Shown are the signal ratios between the current obtained in absence and presence of 200 nM of a 17-base, fully complementary target after 30 min equilibration. The illustrated error bars represent the standard deviation of measurements conducted with three independently fabricated sensors.

contrast to the stem-loop probe, the target binding to the linear probe is not coupled to a competing equilibrium (stem formation) and is thus favored. The response time and specificity of the E-DNA sensor are, in contrast, not significantly influenced by the geometry of the probe DNA. For example, we observe the same ratio of suppression obtained with the fully complementary target to that obtained with a three base mismatched target for both stem-loop and linear probe sensors (*e.g.*, ~1.17 at a probe density of 1.6×10^{12} molecules cm^{-2}—Table SI1† and ref. 9), and the equilibration times of both classes of sensors are quite rapid (near complete equilibration is achieved in <5 min. for lower density sensors—Fig. SI1† and ref. 9). As with the original E-DNA architecture,[9] the length and structure of the target affect linear-probe E-DNA signaling, with longer and/or bulkier targets producing greater signal suppression (Table SI2†). Finally, as with its stem-loop predecessor, the signals observed from the linear-probe sensor are quite reproducible in both the absence and presence of the 17-base fully complementary target (RSD < 10%, and 3% respectively, $n = 3$) (Table SI1†) and the sensor is relatively stable (24–48 h in buffer at room temperature, data not shown).

E-DNA signal gain is a function of the density of the DNA probes on the sensor surface, an effect that provides insights into the E-DNA sensing mechanism. By varying the probe DNA concentrations employed during sensor fabrication (over the range 0.005 to 2 μM) we can control this important variable and can readily and reproducibly fabricate linear-probe sensors of probe densities from 1.2×10^{10} to 1.6×10^{12} molecules cm^{-2} (assuming perfect electron transfer efficiency) (see Fig. SI2†). This range of probe densities corresponds to mean probe-to-probe separations of ~97 to ~8 nm. (Given, however, that perfect transfer efficiency is unlikely, these numbers presumably overestimate the actual probe-to-probe distance.) A dramatic rise in signal suppression is observed as the mean probe separation drops (Fig. SI3†), presumably because, with increased crowding, the dynamics of target-probe duplexes are reduced preferentially, increasing the binding-induced change in electron transfer efficiency.

The ACV frequency dependence of the response of linear-probe sensors (Fig. 3) provides further evidence for this collisional model of E-DNA signaling. At low ACV frequencies no significant signal suppression is observed, presumably because the collision rates of both unhybridized and hybridized probes are rapid enough to support efficient electron transfer under these conditions. The target-induced signal suppression then increases as the ACV frequency rises until, for lower-density sensors, it plateaus at ~10 Hz. At still higher frequencies the suppression observed for higher-density sensors once again falls. We presume this occurs because, at higher probe densities, the collision rate of the single-stranded probe is slow enough that electron transfer from unbound probes is also inhibited under these conditions. In support of this collision-limited signaling mechanism, we find that the rate of electron transfer slows by approximately an order of magnitude upon target binding (see Fig. SI4†).

All of the groups responsible for the initial development of E-DNA sensors employed stem-loop DNA probes,[1–9,14,15] presumably due to the misconception,[1–3,9,14] shared by us, that, by analogy to molecular beacons, a specific conformational (*i.e.*, geometric) change is required in order to support robust signaling. We have shown here, however, that binding-induced changes in DNA dynamics are sufficient to support E-DNA signaling. Indeed, although the stem-loop probe provides a more controlled and predictable structure (which in turn appears to minimize the effects of changing viscosity and/or ionic strength on the absolute signal current),[3] the linear-probe sensor exhibits improved signal gain over its stem-loop counterpart. Moreover, like these counterparts, linear-probe E-DNA sensors are label free, reusable, sequence specific and selective enough to employ directly in complex sample matrices such as blood serum, thus rendering them well suited for clinical applications.

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