Characterizing Electrochemical DNA (eDNA) Scaffold Sensors For The Detection of Antibodies in Whole Blood

By Jesse Kasehagen
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http://www.noozhawk.com/schools/article/100910_santa_barbara_middle_school_sees_miracle_in_new_campus_acquisition/
What is a Biosensor?

Pulse Oximeter

Glucose Meter

A blood sample is taken and put on test strip

Strip is put into blood glucose meter

A log book is a helpful aid in keeping track of blood glucose levels
My Focus:

1. What is an eDNA sensor?

2. How specific is this type of biosensor?

3. When will this type of sensor “break” in whole blood?
Materials

- Au Electrodes
- Glass w/ Buffer
- Potentiostat
- Multiplexer
- Timer
- Ag vs AgCl Reference Electrode
- Pt Counter Electrodes
Materials (Cont’d)

Cleaning Solutions

Ethanol + DI Water

Whole Blood

Delbecco’s Phosphate Buffer Solution

3x SSC

6-Mercaptohexanol + DPBS

Blocker: 3x SSC (500mM sodium chloride) + fibrinogen (a protein that aids in blood clotting) + tween-20 (a detergent)
The eDNA Sensor

- Anchoring DNA Strand (with thiol group)
- Methylenblue
- Hybridization
- PNA strand
- SAM
- Carbon electrode
The new sensor platform is sensitive, selective, reusable, and rapid. Both the signal-on and signal-off streptavidin sensors respond sensitively to their target, exhibiting detection limits below 1 nM (Figure 3). Neither architecture responds significantly to nontargeted proteins, such as a mixture of IgG antibodies (Figure S2), and both architectures support the detection of their targets directly in complex sample matrices, such as 50% blood serum, 5% (w/v) soil in buffer, and foodstuffs (Figure 3, bottom and Figure S3). The sensors are also readily regenerable: a short rinse with deionized water to disrupt hybridization and remove the recognition strand before the addition of fresh recognition strand allows reuse for more than five cycles (Figure S4). Finally, both sensors equilibrate

Figure 1.
Here we demonstrate a novel electrochemical sensing architecture that retains the selectivity and convenience of E-DNA sensors while expanding its range to the detection of macromolecules that bind to specific small molecules. The new architecture utilizes a largely double-stranded DNA as a rigid-but-dynamic scaffold to support a small-molecule recognition element. One strand of the scaffold, the “anchoring strand”, is attached to the electrode surface at its thiol-modified 5′ terminus and labeled with a redox tag (here methylene blue) at its 3′ terminus. The second strand, the “recognition strand”, is modified either at its 3′ terminus or, as shown, its 5′ terminus with a small-molecule recognition element. (Left) In the unbound state, the scaffold supports efficient electron transfer between the redox label and the electrode. (Center) The binding of the macromolecular target to this recognition element reduces the transfer efficiency, thus significantly reducing the observed faradaic current. (Right) Shown here are representative square wave voltammograms of the free and target-bound sensor (for the detection of 30 nM anti-digoxigenin antibody in 50% blood serum).

Figure 2.
Sensor response depends on the flexibility of the DNA scaffold. Using a 27 base anchoring strand and 5′ placement of the biotin recognition element (distal to the electrode), we achieve optimal signaling with a recognition strand of 19 bases (centered on the middle of the anchoring strand). In contrast, 3′ placement of the recognition element produces a sharp transition from signal-off behavior at short lengths to signal-on behavior for recognition strands of 21 or more bases. Double-stranded scaffolds 17 bases in length lacking the small molecule recognition element (Ctrl) do not respond to target. All data represent addition of saturating (50 nM) streptavidin target.

Figure 3.
Both the signal-on and signal-off streptavidin sensors achieve subnanomolar detection limits and are able to function in complex samples. Shown on the top are titrations of signal-on (23S17B3) and signal-off (19B5) streptavidin sensors in buffer (the biphasic nature of the curves arises due to the subnanomolar dissociation constant of the streptavidin-biotin interaction). The sensors function comparably in complex samples such as blood serum, soil suspensions, and beer (bottom), yielding similar signals upon addition of saturating (30 nM) streptavidin target. The error bars in this and the following figure represent the standard deviations of measurements conducted using three separately fabricated electrodes. The signal-on construct has large electrode-to-electrode variability in gain, although detection limits are similar for each individual electrode. (Figure S7 presents titration data for individual electrodes.)
The Fabrication Process

Cleaning the Electrodes takes over 1 hour
The Fabrication Process (Cont’d)

- Preparing the scaffold sensor (DNA + Monolayer + Hybridization)
Testing The Sensor
What is it we are Testing?

How specific is the PNA (containing the recognition element/antigen) binding to its target (antibody) in whole blood
Efficacy of Blocker Solution at [25%] Whole Blood

![Bar Chart]

- **Without Blocker**
  - FLAG: 60%
  - 4B3: 30%

- **With Blocker**
  - FLAG: 30%
  - 4B3: 5%
Testing Capability With Blocker
Looking Forward...

We Know...

1. The eDNA Sensor

2. That even in whole blood, this type of sensor can be very specific

3. With the current blocking solution, our sensor works well in 25% whole blood, and even 33% whole blood

What next?

Find a blocking solution to work in 100% whole blood
As a Teacher...

- A renewed interest in the scientific process (perseverance, “big picture”, self checking, and good technique)
- Appreciation for the “nanoscale” world (something exists that you can’t actually see with your eyes)
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