Electrochemical Biosensors Employing an Internal Electrode Attachment Site Achieve Reversible, High Gain Detection of Specific Nucleic Acid Sequences

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Running title: Detection of DNA with a High Gain Biosensor

Abbreviations: Phosphate Buffered Saline (PBS), Electrochemical DNA Biosensor (E-DNA), Methylene Blue (MB)

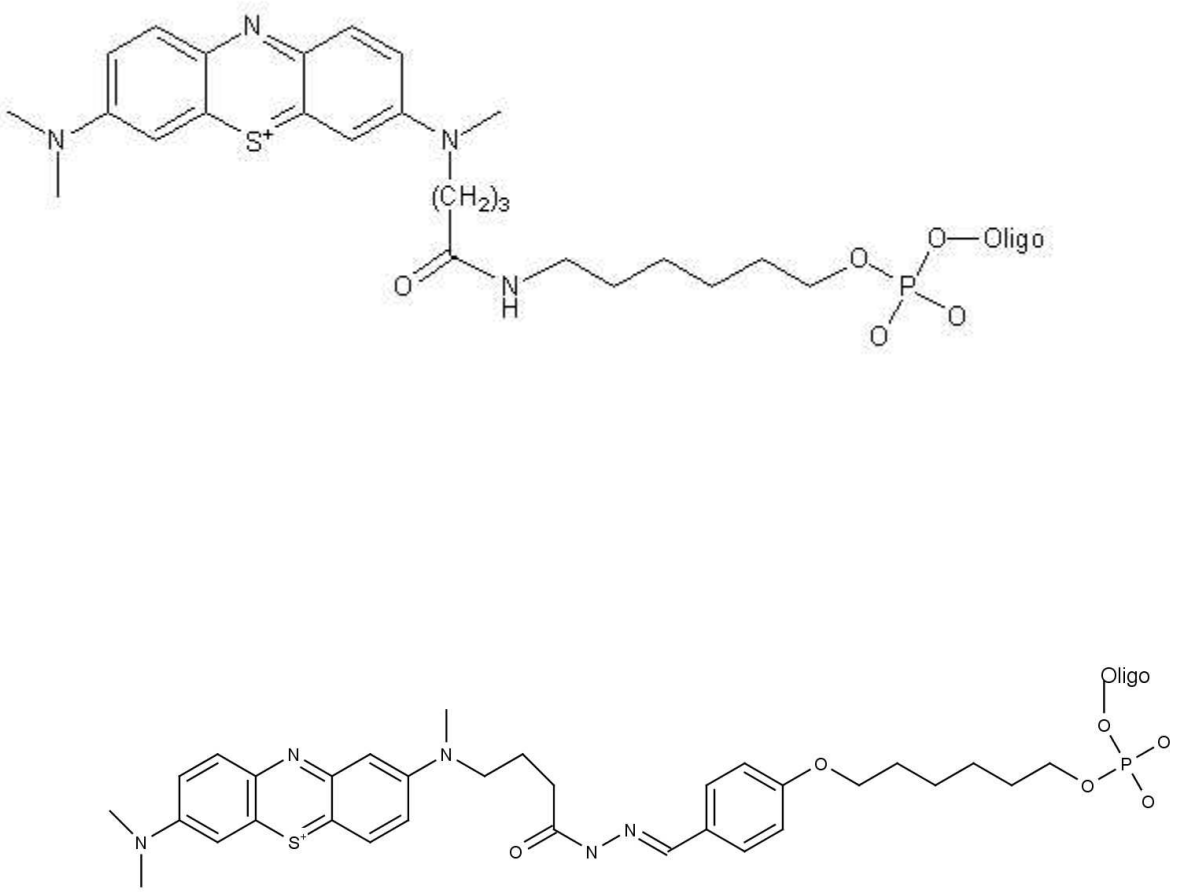

Fig. S1. Two different linker chemistries were used to affix methylene blue to the end of the DNA oligos. The performance of sensors made with these linkers were very similar. Synthesis of the ester linkage, top, is slower and more difficult than construction of the hydrazone linker, bottom.

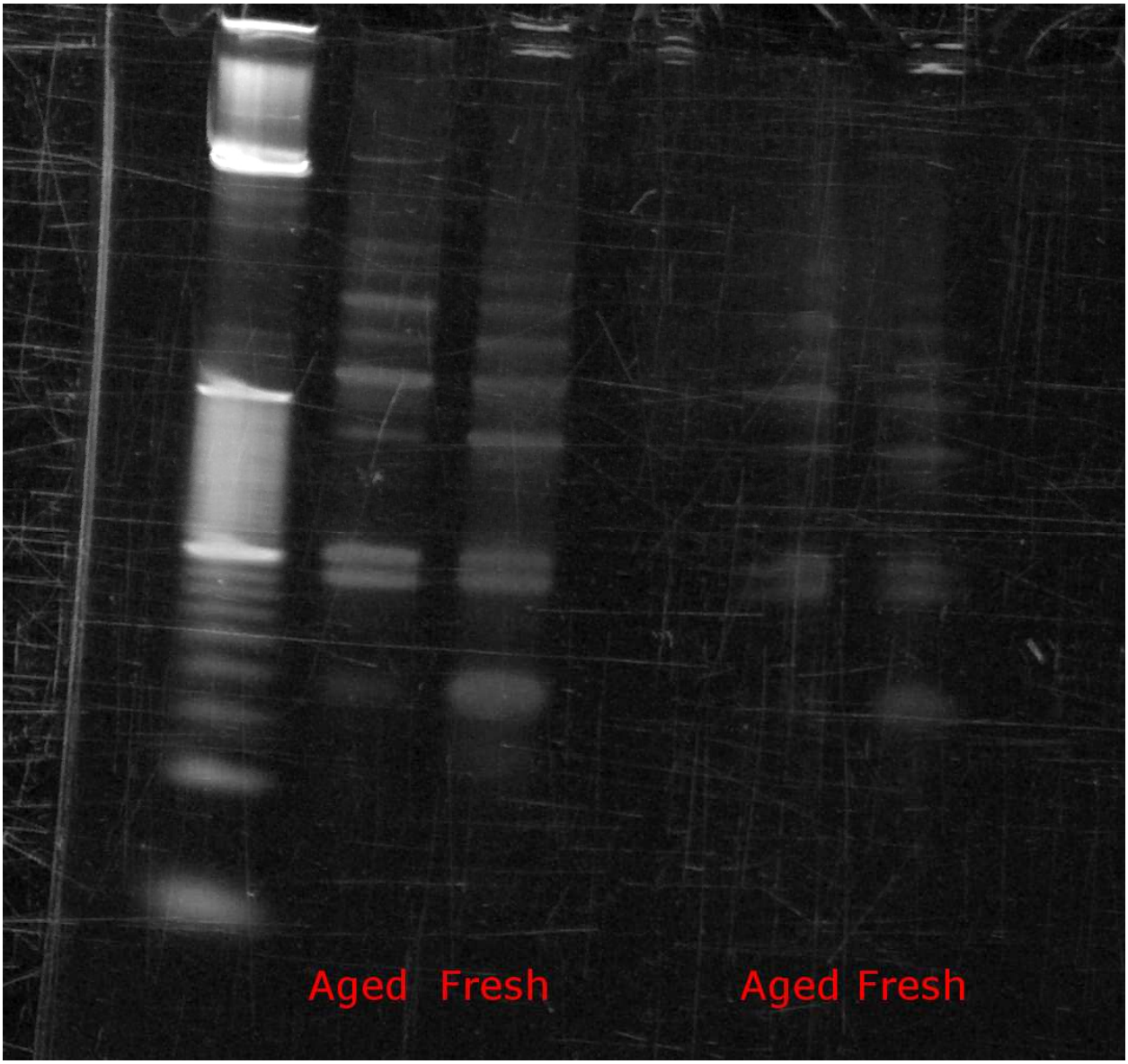

Fig. S2. Gel electrophoresis was performed to better understand the effect of aggregation upon forked DNA probe molecules. Freshly hydrated samples at 2 µM and 500 nM were loaded alongside three week old hydrated samples at the same concentrations in a ten well, pre-cast 4-20% TBE polyacrylamide gel. The gel was run using 1X TBE buffer for 120 minutes at 70V. The gel was then stained with SYBR Gold and imaged using a UV lamp and Kodak Camera-Computer imaging apparatus.

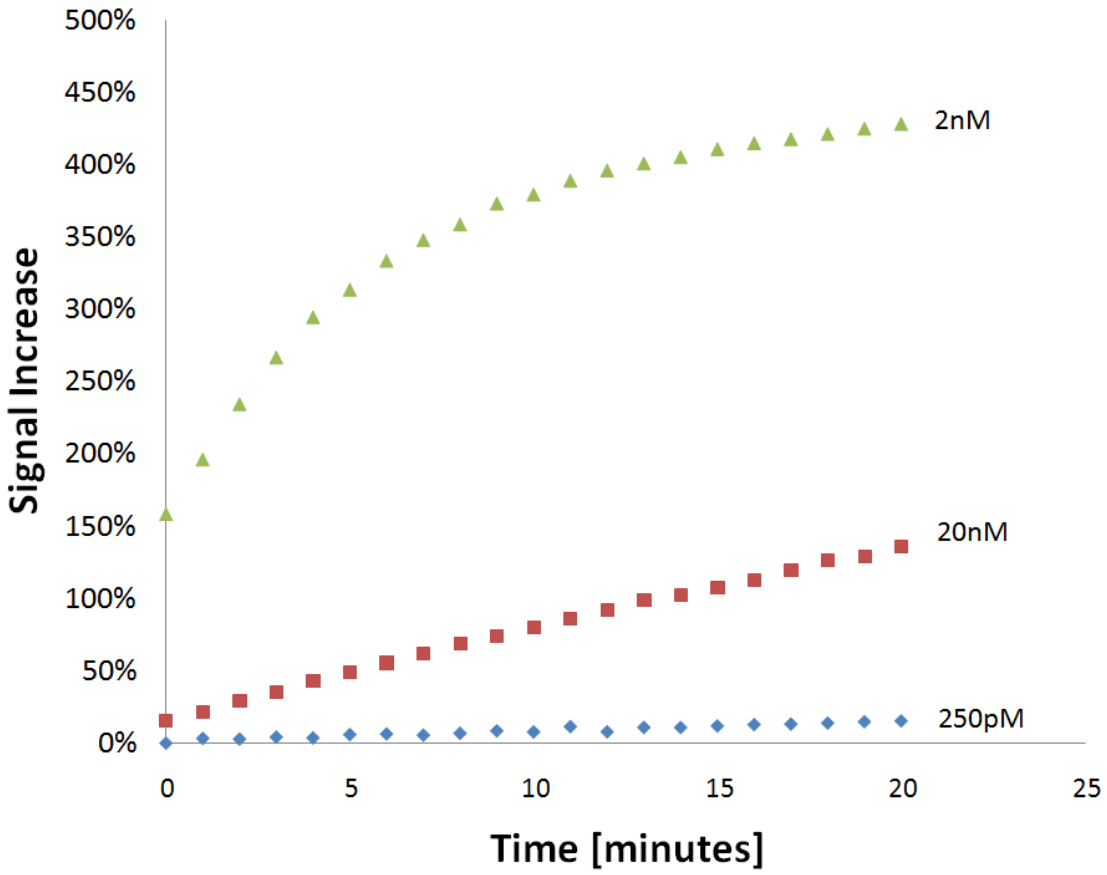

Fig S3. Three gold disk electrodes were immersed in 750 mL of phosphate buffered saline enriched with 0.2 mM MgCl2. Synthetic target DNA was added after the first measurement, every 20 minutes thereafter. The mixture was stirred continuously with a magnetic bar throughout the measurement period. For these experiments, the sensors had a particularly low surface coverage of probe DNA molecules. During the preparation procedure, the electrodes were immersed in a 100 nM probe solution for only five minutes. Since we have employed large gold disk electrodes in this study, and the surface area of the biosensors on those electrodes is quite high, and thus a large absolute amount of target DNA was necessary to achieve a significant signal gain. In theory, the same results could be obtained by using electrodes with a much lower surface area, but this would require potentiostats that can make high quality square wave voltammetry measurements in the sub-picoamp range.

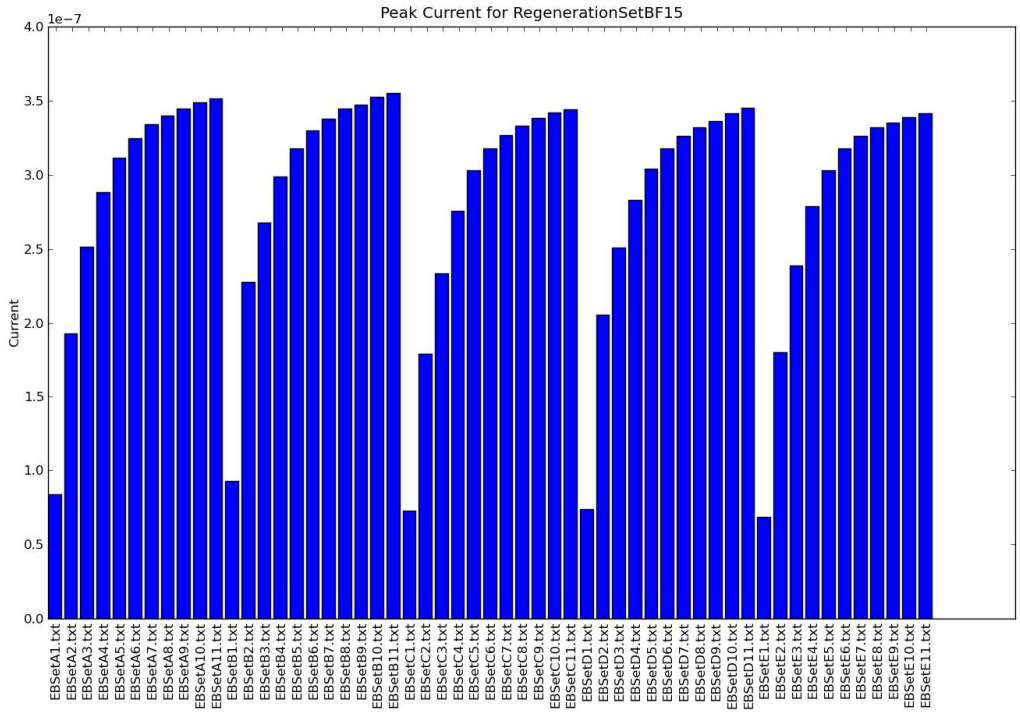

Fig. S4. Five regeneration cycles of the F17 forked DNA sensor. In each cycle, the sensor was immersed in 15mL buffer and scanned once per minute for 10 minutes. After the first scan, target DNA was introduced. The solution was mixed, bringing the concentration up to 200nM. After each cycle, the sensor was rinsed with a spray of DMSO for 60 seconds, rinsed with water, scanned once, and then sprayed with DMSO for another 60 seconds, and rinsed again with water.

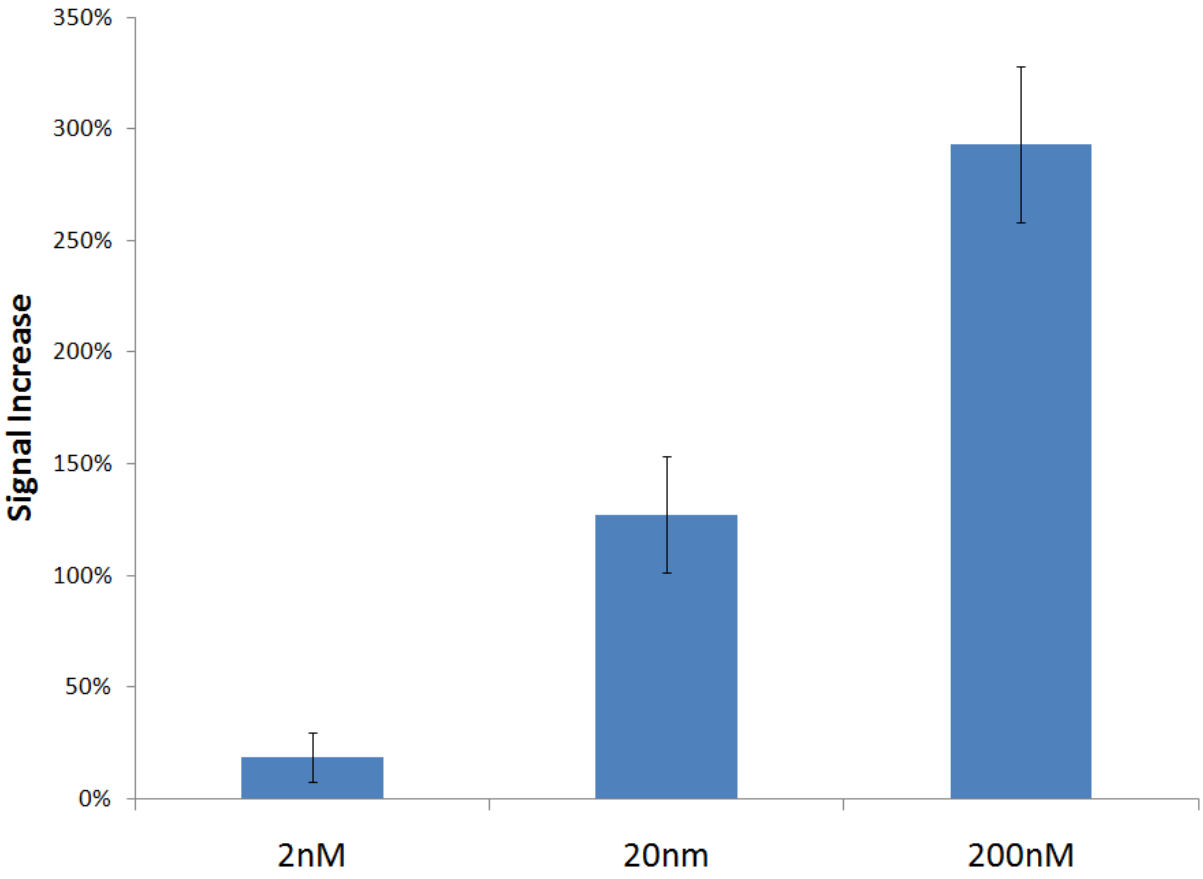

Fig S5. The sensor is functional in BioRad Supermix iQ PCR mix. In each test, the sensors were immersed in 2mL of PCR mix and scanned once per minute for ten minutes. After the first scan, target DNA was added. Error bars represent the standard deviation from three separate sensors.

Tips for Reproducing This Work

- Have the forked DNA probe shipped in a large number of separate vials. Do not add water to the sensor DNA until you are ready to prepare a batch of sensors.
- Use a very low coverage of DNA on the sensor surface. For instance, incubate the electrodes in a 100nM solution of the probe DNA for only 5 minutes. Rinse with water, dry them off, and then store in a 6mM mercaptohexanol solution.
- To re-use the sensor, rinse it with a steady stream of DMSO several times for at least 30 seconds. Also rinse with water.
- Be careful not to touch the surfaces of the sensors.
- Take a look at our *JOvE* video, which details our sensor preparation methods.
- We have recently observed that these sensors perform well when fabricated on GeneFluidics gold electrode arrays. These sensors do not work well on DropSens screen printed electrodes. For all of the work presented in this paper, we used standard gold disk electrodes.
- Once you have fabricated the sensors, we recommend storing them in 6mM mercaptohexanol solution or in DMSO.
- When preparing a TCEP solution to reduce the thiols in the probe DNA, don't do it in a PBS buffer. TCEP is not stable in PBS.
- Dithiols such as 1,6-hexanedithiol can be combined with the probe molecule during immobilization to create a ternary monolayer like the ones described by the Joe Wang group.
- If you want to design a new probe sequence, be sure to use mFold or the Vienna RNA folding prediction tool to check its secondary structure. Probe DNA molecules with complicated secondary structures may not function properly.