Supporting Information

Use of thiolated oligonucleotides as anti-fouling diluents in electrochemical peptide-based sensors

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MATERIALS AND METHODS

Materials and Reagents

6-mercapto-1-hexanol (C6-OH), tris-(2-carboxyethyl) phosphine hydrochloride (TCEP), sulfuric acid (H_2SO_4), sodium chloride (NaCl), sodium phosphate monobasic, sodium phosphate dibasic purchased from Sigma-Aldrich (St. Louis, MO) were used as received. All other chemicals were of analytical grade. Synthetic human stimulated parotid saliva was purchased from US Biocontract (San Diego, CA). All solutions were made with deionized water (DI H_2O) purified through a Millipore Synergy system (18.2 MΩ·cm, Millipore, Billerica, MA). The sensors were interrogated either in Phys2 (20 mM Tris, 140 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$ and 1 mM $CaCl_2$, pH 7.4) or 1:9 saliva:Phys2.

A peptide probe (DRY-MB) with the following sequence was purchased from Xaia Custom Peptides (Göteborg, Sweden) (Fig. S1). The probe was modified at the n-terminus with an 11-carbon alkanethiol, and at the c-terminus, a methylene blue (MB) redox label. The MB label was conjugated to the added lysine (K) residue at the c-terminus.

DRY-MB Peptide Probe: (N) HS-$(CH_2)_{11}$- QGPKEPFRDYVDRFYKTLRAE-K-MB (C)

Three types of thiolated DNA diluents with the following sequences were purchased from Biosearch Technologies, Inc. (Novato, California) (Fig. S2).

Thiolated DNA Diluents

T2: 5' HS-$(CH_2)_6$-TT 3'

T4: 5' HS-$(CH_2)_6$-TTTT 3'

T6: 5' HS-$(CH_2)_6$-TTTTTT 3'

The correct target antibody (IgG-Target) was a rabbit monoclonal anti-p24 antibodies purchased from Biomatik Corp (Wilmington, DE). The incorrect target antibodies (IgG-Random) were reagent grade (≥ 95% SDS-PAGE) IgG from human serum purchased from Sigma-Aldrich (St. Louis, MO). The product number is I4506-100MG.

Sensor Fabrication and Target Interrogation

Prior to sensor fabrication, the gold electrodes were polished with a 0.1 μm diamond suspension (Buehler, Lake Bluff, IL), rinsed with DI H_2O, and sonicated in a low power sonicator for approximately 5 min to remove bound particulates. The 2 mm-diameter gold working electrodes (CH Instruments, Austin, TX) were then electrochemically cleaned by a series of oxidation and reduction cycles in 0.5 M H_2SO_4 from -0.3 to 1.5 V. The real area of each electrode was determined from the charge associated with the gold oxide stripping peak obtained after the cleaning process in 0.05 M H_2SO_4.

After electrode cleaning and area determination, 15 μL of 0.5 μM DRY-MB was drop cast onto each electrode for 1 hour, followed by rinsing with DI water for 20 seconds. Next, 15 μL of 25 μM DNA diluent (**T2**, **T4** or **T6**) containing 1.25 mM TCEP was drop cast onto each electrode for 1 hour, followed by the same rinsing procedure. After immobilization of both the peptide probe and DNA diluent, the electrodes were exposed to a 2 mM C6-OH solution for 12-14 hours to displace non-specifically adsorbed molecules. For the **T0** sensor (i.e., sensor without a DNA diluent), the DNA diluent immobilization step was omitted. All immobilization steps were performed at room temperature (RT) and the electrodes were kept away from direct light.

All electrochemical measurements were performed using alternating current (AC) voltammetry with a CHI 1040A Electrochemical Workstation (CH Instruments, Austin, TX). Gold electrodes served as working electrodes, while platinum wire and Ag/AgCl (3.0 M KCl) served as the counter electrode and reference electrode, respectively (CH Instruments, Austin, TX). The density of DRY-MB on the electrode surface was determined using a method developed by Creager *et al*..[1] AC voltammograms of the sensors were recorded using an amplitude of 25 mV and at a frequency of 10 Hz. Prior to target interrogation, the sensors were allowed to equilibrate in Phys2 until no change in the MB peak current was observed in ACV. Most sensor interrogation experiments were performed in Phys2 at RT. For all four sensors,

sensor specificity was determined by the addition of 70 nM IgG-Random, followed by the addition of 70 nM IgG-Target. Dose-response curves were obtained by sequential addition of different concentrations (1, 5, 10, 25, 40, 65, 80 and 100 nM) of IgG-Target at an interval of 60 min. Mechanical stirring was used in this experiment. In the sensor selectivity experiment, all four sensors were first exposed to 10% saliva, followed by the addition of 70 nM IgG-Target.

Sensor Characterization by X-ray Photoelectron Spectroscopy (XPS)

XPS was used to determine the amount of DNA diluent incorporated into the monolayer. All four sensors were fabricated on glass cover slips coated with 20 Å Cr and 500 Å Au. Prior to sensor fabrication, the gold substrates were placed inside the chamber of a UV ozone cleaner (UVO-Cleaner 42A, Jelight Company, Inc., Irvine, CA) for 1 hour. The protocol used to fabricate sensors on these sputtered gold substrates was the same as that used with the gold disk electrodes.

XPS spectra were acquired using a dual anode X-ray lamp and a hemispherical angle resolved electron analyzer (detector), both elements and the samples were measured inside a ultra-high vacuum (UHV) chamber (~10^{-10} Torr) to prevent impurity scattering events. The X-ray source used the Mg-Kα line at 1253.6 eV, with data taken at normal emission. The XPS data were extracted and analyzed utilizing the CASA software package.

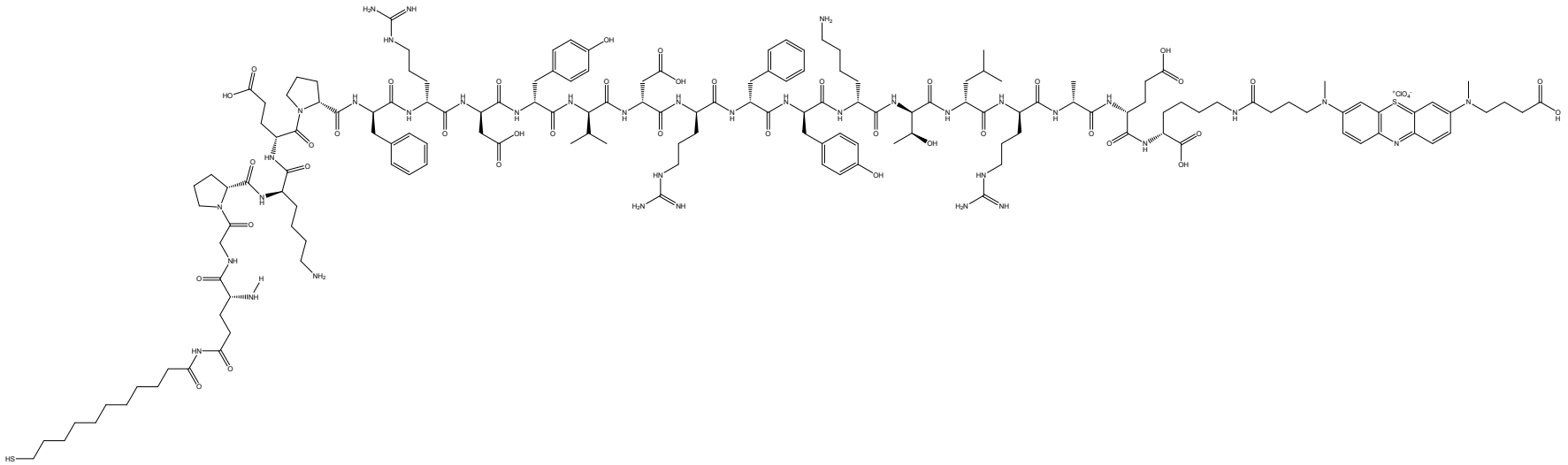

Fig.S1. Structure of the peptide probe (DRY-MB) used in this study.

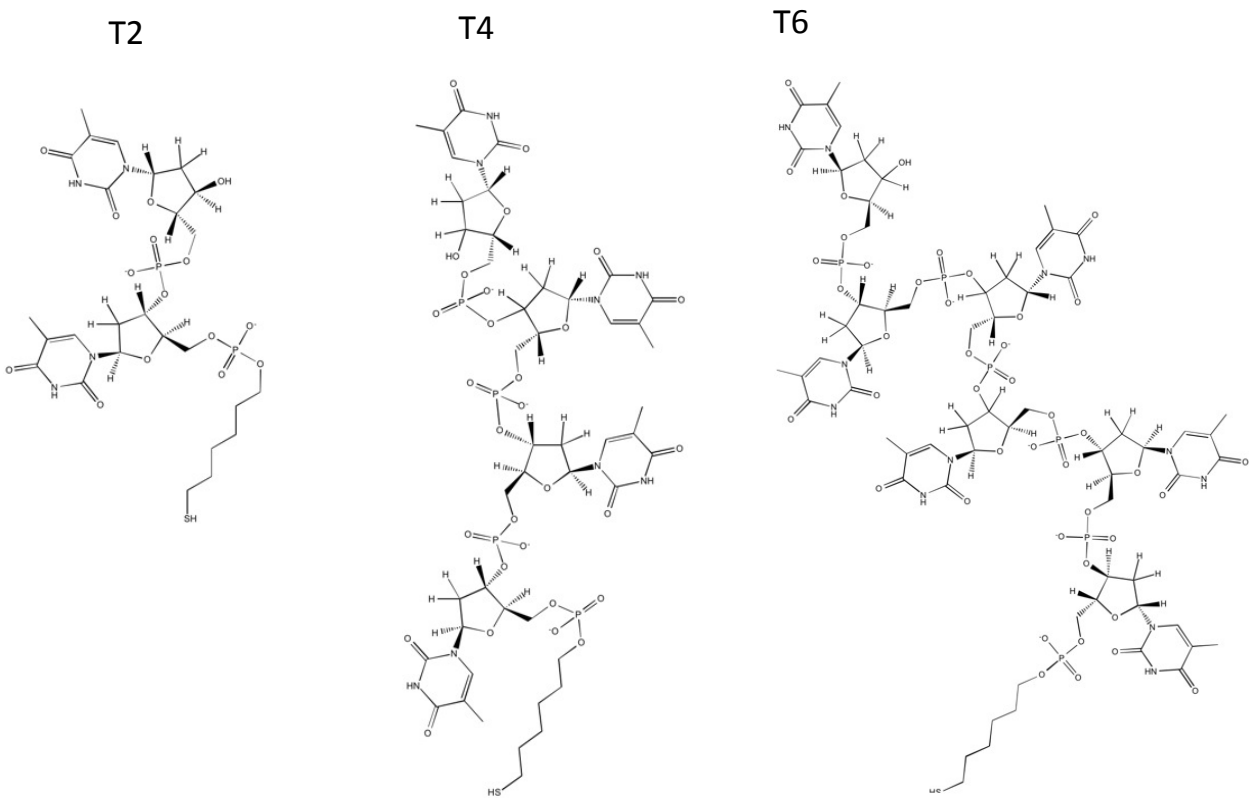

Fig. S2. Structures of the thiolated DNA diluents used in this study.

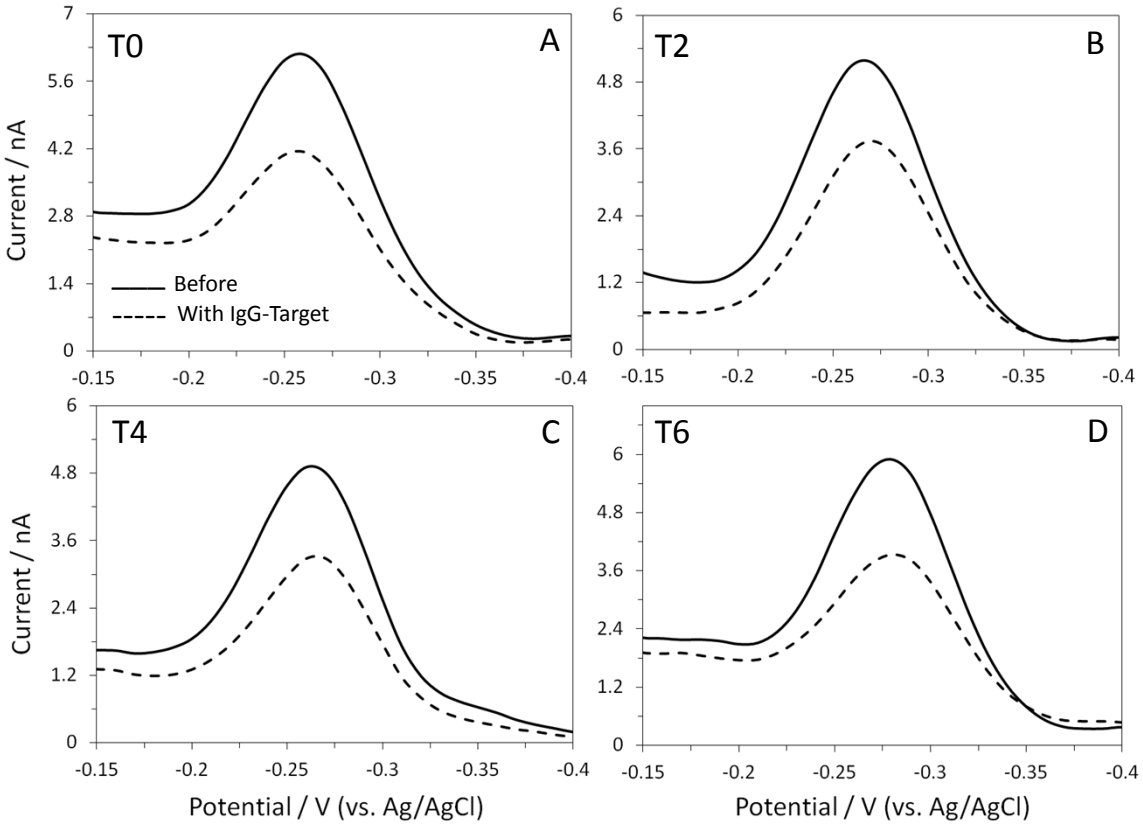

Fig. S3. Representative AC voltammograms of the **T0** (A), **T2** (B), **T4** (C), and **T6** (D) sensors in the absence and presence of 65 nM IgG-Target. All scans were collected at 10 Hz in Phys2.

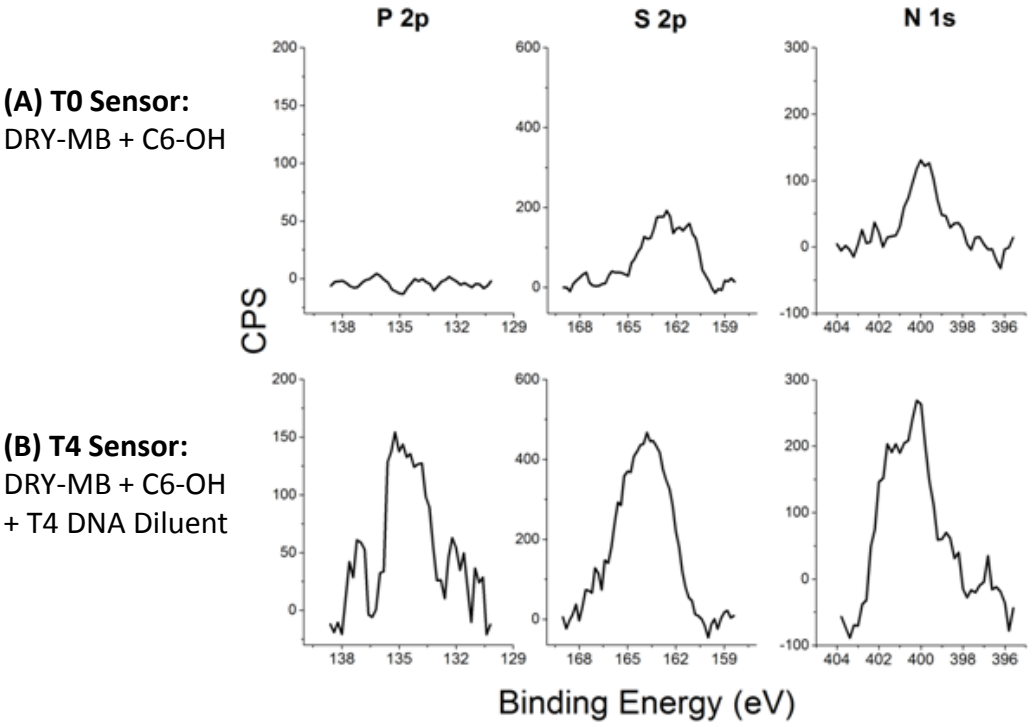

Fig. S4. XPS spectra of phosphorous (P), sulfur (S), and nitrogen (N) for the **T0** (A) and **T4** (B) sensors. The presence of the phosphorous peak confirms successful incorporation of DNA diluents into the monolayer of the **T4** sensor. Similar spectra were obtained for the **T2** and **T6** sensors (data not shown).

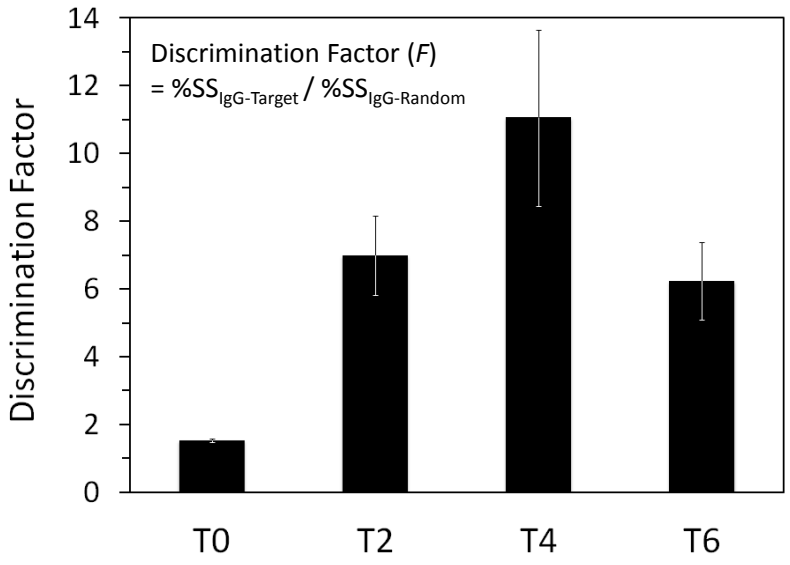

Fig. S5. Discrimination factors (*F*) determined for the four sensors used in this study.

Sensor	*P/S*[1]	*Coverage (%)*[2]	*Molecules/cm^2 (x 10^{13})*
T0	0	0	0
T2	0.42	21±2.5	9.5±1.1
T4	0.55	14±2.5	6.3±1.1
T6	0.35	6.0±3.0	2.7±1.4

[1] Corrected for cross-section and analyzer transmission.

[2] Corrected for electron attenuation and DNA chain length.

Table S1. Phosphorous to sulfur ratio and calculated DNA diluent coverage for all four sensors based on the XPS data. The DNA diluent coverage was calculated with the assumption that the maximum surface density of thiolates in a self-assembled monolayer on gold is 4.5 x 10^{14} molecules/cm^2.[2]

References:

1 [a] S. D. O'Connor, G. T. Olsen, S. E. Creager, *J. Electroanal. Chem.*, 1999, **466**, 197. [b] J. J. Sumner, K. S. Weber, L. A. Hockett, S. E. Creager, *J. Phys. Chem. B*, 2000, **104**, 7449.

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