Supplementary Material (ESI) for Chemical Communications

Electronic Supplementary Information for:

A General Electrochemical Method for the Label-Free Screening of Protein-Small Molecule Interactions

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Reagents and DNA Sequences

Tris(2-carboxyethyl) phosphine hydrochloride (TCEP; Invitrogen, Eugene, OR), 6-Mercapto-1-hexanol (MCH; Fluka), and sulfuric acid (Fisher Scientific) were all used as received. 1000 µg/mL solutions of 2,6-dinitrotoluene and 2,4,6-trinitrotoluene in acetonitrile (Supelco, Bellefonte, PA) were aliquoted and stored at 4°C until use. 2,4-dinitrophenol (DNPOL, Sigma-Aldrich), and 4-(Dimethylamino)-phenylacetic acid (4DMAPA, Sigma-Aldrich) were dissolved in deionized water (DI water; 18 MΩ cm Milli-Q Ultrapure Water Purification, Millipore, Billerica, MA), aliquoted and stored at 4°C for immediate use or -20°C for long term storage. Anti-TNT antibody (Strategic Biosolutions, Newark, DE) was aliquoted and stored at 4°C. Seawater samples were taken from the Pacific Ocean near the University of California, Santa Barbara campus and stored at 4°C. Soil samples were taken from the University of California, Santa Barbara campus and extracted similar to EPA Method 8330B[3]. A 10 g soil sample was ground with morter and pestle, and mixed with 20 mL acetonitrile. The mixture was vortexed for 1 minute, and shaken overnight. After settling for 30 minutes, 8 mL of acetonitrile was removed, and filtered with a 0.45 micron PTFE syringe filter (discarding the first mL). This mixture was then dried, and resuspeded with 4 mL of deionized water and sonicated, then stored at 4°C until use. A similar soil sample was also directly suspended in DI water at a concentration of 10% w/v for storage, and diluted to 5% w/v with buffer before use. Cells used were a kind gift of Patrick Daugherty and Abeer Jabaiah, and were FreeStyle 293-F cells (Invitrogen) grown to 10^6 cells/mL in FreeStyle Expression Medium (Invitrogen), and before use lysed by sonication and diluted to 50% with buffer. Saline-sodium citrate buffer (SSC) was diluted from a 20X stock (20XSSC, Sigma Aldrich) with DI water to either 6XSSC (for sensor fabrication and buffer experiments) or 12XSSC (for dilution of seawater, soil and cell lysate samples to 50%).

The thiolated, methylene blue-tagged anchoring strand as well as the DNP-labeled recognition strands (HPLC purified, Biosearch Technologies Inc., Novato, CA) were used as received without further purification. The sequences of the various strands are shown in Table S1.

Table S1: DNA sequences used in this research

Anchor DNA Sequence	
Anchor	5'-HS-$(CH_2)_6$-GCAGTAACAAGAATAAAACGC CACTGC-$(CH_2)_7$-MB
DNP recognition strands (triethylene glycol linkage between DNA and DNP)	
DNP25	5'-DNP-TEG-CAGTGGCGTTTTATTCTTGTTACTG
DNP23	5'-DNP-TEG-AGTGGCGTTTTATTCTTGTTACT
DNP21	5'-DNP-TEG-GTGGCGTTTTATTCTTGTTAC
DNP19	5'-DNP-TEG-TGGCGTTTTATTCTTGTTA
DNP17	5'-DNP-TEG-GGCGTTTTATTCTTGTT
DNP15	5'-DNP-TEG-GCGTTTTATTCTTGT
Single-stranded DNP Probe Sequences	
TL1	5'-HS-$(CH_2)_6$-AAGGTGGAATGGTT*D*GTC-$(CH_2)_7$-MB
TL2	5'-HS-$(CH_2)_6$-AAGGTGGAATGGTT*T*GTC*D*

D Location of DNP-TEG, *T* Thymine labeled with -$(CH_2)_7$-MB

Electrode cleaning and Sensor Preparation

A detailed sensor fabrication procedure can be found in the literature[20]. Briefly, polycrystalline gold disk electrodes (2mm diameter; BAS, West Lafayette, IN) were prepared by polishing with diamond and alumina (BAS) with sonication in ethanol or water after each step. Following polishing, electrochemical cleaning (a series of oxidation and reduction cycling in 0.5M H_2SO_4, 0.01M KCl/0.1M H_2SO_4, and 0.05M H_2SO_4) and area determination (based on the area of the gold oxide reduction peak in the final cleaning step) were performed.

Anchoring strand DNA (0.1mM) was incubated with TCEP (1 µM) for 1 hour to allow reduction of disulfide bonds. This solution was diluted to 25 nM with 6XSSC. Electrodes (thoroughly rinsed with DI water) were incubated in 250 µL of anchoring DNA for 1 hour. Electrodes were rinsed with DI water, and incubated in 3mM MCH in 6XSSC for 1 hour to displace nonspecifically adsorbed DNA and passivate the remaining electrode area[21]. After thoroughly rinsing with DI water, electrodes were stored in 6XSSC for 30 minutes before use. Probe packing density of DNA was determined using a previously described method[22]. The probe density used in this research was in the range of 2.2 to 3.0 x 10^{11} molecules/cm^2 unless otherwise noted. The modified electrodes were then incubated in 200 nM recognition strand solutions for 1 hour to allow hybridization prior to use. For optimization of antibody concentration, antibody was titrated into buffer containing the electrodes, and allowed to equilibrate for one hour before measurements. For detection of TNT (and other compounds), 4 nM of antibody was added to the solution (buffer or complex sample), and allowed to equilibrate for 3 hours prior to measurements. TNT (and other compounds) in stock solutions were then added to that solution to the desired concentration for measurement, allowing 1 hour incubation to

allow near complete signal saturation for even the lowest concentrations used (with the exception of kinetic measurements).

Electrochemical Measurements

All electrochemical measurements were performed using a CHI630C potentiostat with a CHI684 Multiplexer (CH Instruments, Austin, TX) and a standard 3-electrode cell containing a platinum counter electrode (BAS) and a Ag/AgCl (3M NaCl) reference electrode (BAS). Alternating current voltammograms were obtained in 6XSSC using a 25 mV amplitude signal at 10 Hz from -0.05 to -0.45 V *vs*. Ag/AgCl for the purpose of determining probe packing density of DNA. Experimental data were collected using square wave voltammetry from -0.05 to -0.45V in increments of 0.001V *vs*. Ag/AgCl, with an amplitude of 50 mV and a frequency of 60 Hz. Peak currents were fit using the manual fit mode in the CH Instruments software. Results are presented as signal change (difference in peak currents obtained before and after target binding divided by initial peak current) to allow for better comparison of electrodes differing in surface area. Voltammograms are presented with the current at -0.05V set equal to 0A.

Surface Probe Density Experiments

Surface probe density was controlled through the use of different concentrations of anchoring strand DNA during the immobilization procedure. Before addition of the recognition strand, probe packing density of DNA was determined. Breifly, this method uses electrochemical interrogation with alternating current voltammetry, and relates the peak current to the number of methylene blue redox tags by assuming perfect electron transfer. This method is described more fully in previous research[22].

Sensor Response in Soil Suspension

Of note, detection of TNT was not possible upon addition to soil suspensions (data not shown), presumably from the high adsorption of TNT by particulate organic matter[23]. As a result, selectivity was tested using soil extracted in a method similar to EPA8330B[3].

Supplementary Figures and Experimental Data

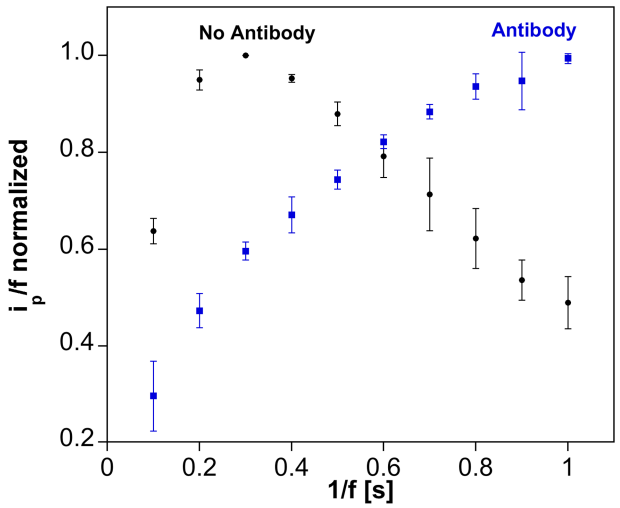

Figure S1: The apparent electron transfer rates are significantly slower in the presence of anti-TNT antibody, supporting the hypothesis that the scaffold dynamics are reduced upon antibody binding. Data represents averaged normalized peak current, for three electrodes in the absence or presence of anti-TNT antibody (70 nM). The frequency with maximal normalized peak current is related to the electron transfer rate constant[24], and can be used to compare the antibody bound and unbound states. Error bars represent standard deviations of the three electrodes.

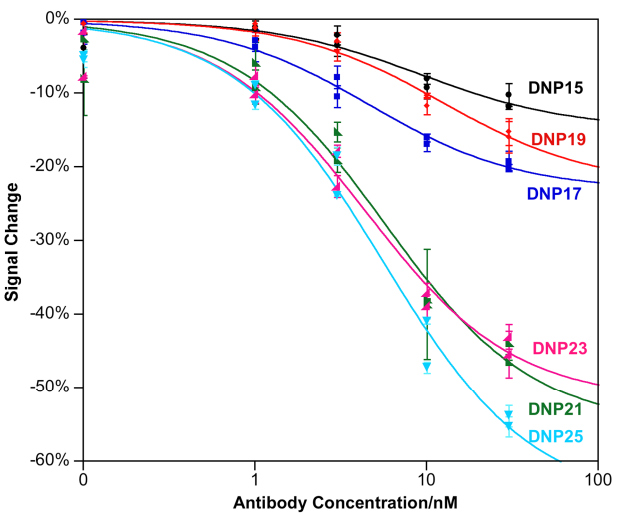

Figure S2: Signal suppression upon antibody binding is a function of recognition strand. While all 6 sequences have comparable affinities (ranging from 4 to 12 nM), maximal signal suppression was obtained with a recognition strand 25 bases in length (using an anchoring strand of 27 bases).

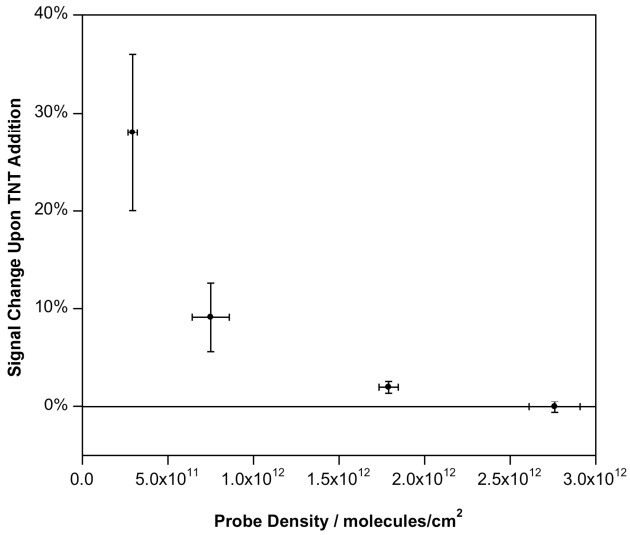

Figure S3: Signal change for TNT detection is highly dependent on probe density on the electrode surface. At high probe densities (obtained with high DNA concentrations used during immobilization), signal change is effectively zero. As probe density decreases, signal gain increases. All data was obtained with addition of 3 µM TNT and use of 4 nM anti-TNT antibody.

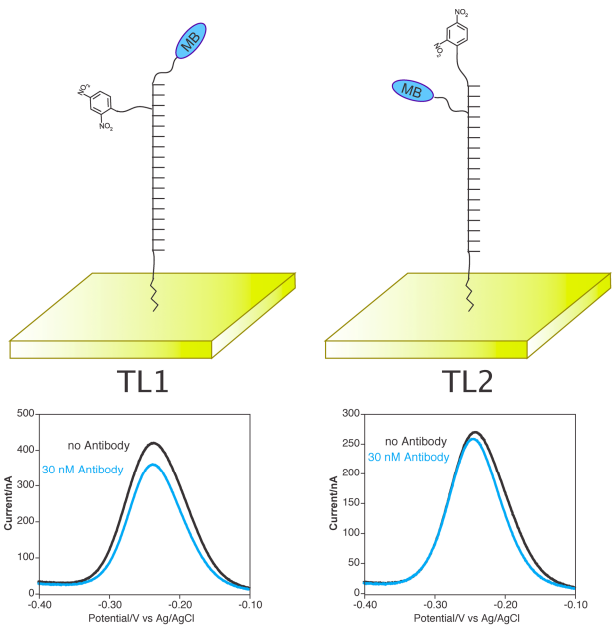

Figure S4: Shown are the structures (top) and representative square wave voltammograms (bottom) for the single stranded constructs. Structure TL1 had a signal suppression of 14%, and structure TL2 showed a suppression of 4% upon addition of 30 nM anti-TNT antibody.

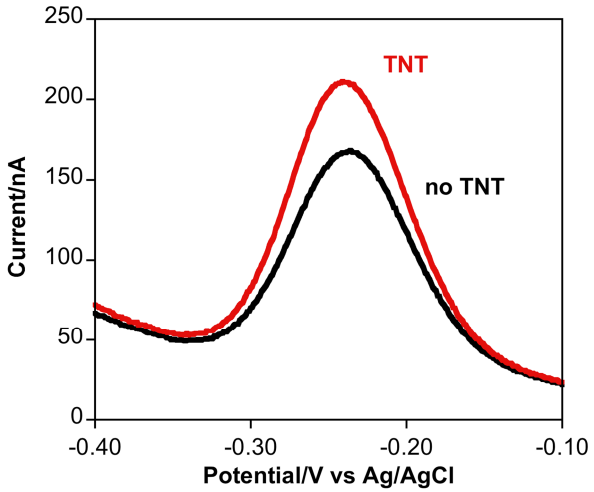

Figure S5 TNT detection is also possible in cell suspensions. Shown is a representative square wave voltammogram for addition of 100 nM TNT to the sensor in the presence of 5 x 10^5 cells/mL partially lysed human kidney cells (Cell line 293 F) in growth medium diluted to 50% with 12X SSC buffer. For three electrodes the average gain was 26 ± 12%.

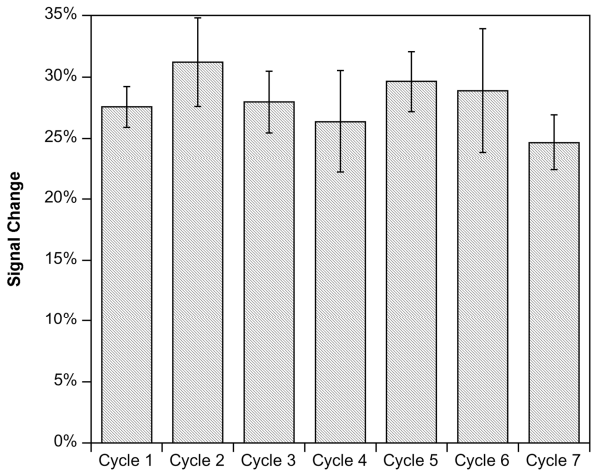

Figure S6: This sensor is readily regenerated and reused. Addition of saturating TNT, followed by a rinse with buffer and addition of fresh antibody is able to regenerate the sensor for reuse at least 7 times without significant difference in results obtained.

Notes and additional references

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