Supporting Information for:

Quantitative, reagentless, single-step electrochemical detection of anti-DNA antibodies directly in blood serum

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Reagents and DNA Sequences

Tris(2-carboxyethyl) phosphine hydrochloride (TCEP), Diethanolamine (DEA), 6-Mercapto-1-hexanol (MCH; Fluka), BSA, potassium phosphate monobasic, dibasic, sodium chloride, 1-naphthyl phosphate, bovine serum albumin (fraction V), polyoxyethylene sorbitan monolaurate Tween 20, fetal calf serum (all from Sigma-Aldrich, St. Louis, MO), and sulfuric acid (Fisher Scientific) were all used as received. Anti-double-stranded DNA monoclonal antibody (dsDNA marker, 100 μg/ml, clone HYB331-01) was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA) aliquoted and stored at 4°C. Anti-single-stranded DNA mouse monoclonal antibody (100 μg/ml, clone F7-26) was purchased from Enzo Life Sciences (Farmingdale, NY, USA) aliquoted and stored at -20°C. Anti-mouse IgG conjugate with alkaline phosphatase (3 mg/ml), was obtained from Vector Laboratories Inc. (Burlingame, CA, USA). Phosphate buffer (potassium phosphate 0.01 M, NaCl 1 M, pH 7.0) was used during sensor preparation. Phosphate buffer at lower salt concentration (potassium phosphate 0.01 M, NaCl 0.05 M, pH 7.4) was used during measurements. 0.97 M DEA buffer (with 1 mM $MgCl_2$, 0.1 M KCl) pH 9.8 was used for electrochemical immunosensor measurements.

The 27-base thiolated, methylene blue-tagged DNA probe we have employed (HPLC purified, Biosearch Technologies Inc., Novato, CA) (sequence: 5'-HS-$(CH_2)_6$-GCAGTAACAAGAATAAAACGCCACTGC-$(CH_2)_7$-MB) and its 22-base complementary strand (Salt free, Mmedial, Italy) (sequence 5'-GCAGTGGCGTTTTATTCTTGTT-3') were used as received.

Electrode Cleaning and Sensor Preparation

A detailed sensor fabrication procedure can be found in the literature [Xiao et al., 2007]. Briefly, gold screen printed electrodes (2mm diameter; EcoBioService, Italy) were prepared by 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 preformed.

Thiolated strand DNA (0.1 mM) was incubated with TCEP (1 μM) for 1 hour to allow reduction of disulfide bonds. This solution was diluted to 100 nM with buffer (0.01 M phosphate, NaCl 1 M, pH 7.0) and electrodes were incubated for 1 hour to allow formation of DNA probe self-assembled monolayer. Electrodes were rinsed with DI water, and incubated in 2 mM MCH in buffer (0.01 M phosphate, NaCl 1 M, pH 7.0) for 1.5 hour to displace nonspecifically adsorbed DNA and passivate the remaining electrode area. To fabricate double-stranded DNA probes these modified electrodes were incubated in a solution containing 200 nM of complementary DNA strand for 1 hour in buffer (0.01 M phosphate, NaCl 0.05 M, pH 7.4). The successful formation of the double-stranded DNA was followed by recording the Square Wave Voltammograms during the hybridization. Decrease of the current signal due to the methylene blue tag demonstrates the formation of the double-stranded DNA on the electrode surface. When the current signal is stable the electrodes were employed for sensing.

Electrochemical Measurements

All electrochemical measurements were performed using an Autolab Instrument (EcoChemie, Utrecht, The Netherlands) or a PalmSens portable instrument (PalmSens, The Netherlands). Square Wave Voltammetry were obtained 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. The current was measured before and after 45 min incubation with the antibody to allow near complete signal saturation. After incubation with the antibody and before current measurement the sensors are shortly washed in pure buffer solution. Results are presented as signal change (difference in peak currents obtained before and after target binding

divided by initial peak current). After antibody testing electrodes are regenerated by immersing them in a solution of urea (8 M) for 2 minutes. Regeneration of double-stranded probes, of course, generates a single-stranded probe, as indicated by the relevant electrochemical signal (Fig. S2).

For electrochemical immunosensor experiment the single stranded DNA probe was incubated 30 min in a solution of buffer (0.01 M phosphate, NaCl 0.05 M, pH 7.4) with 2% of BSA to block the sensor surface and avoid non specific absorption. The sensors were rinsed with Tween-containing buffer (0.05% Tween in 0.01 M phosphate, NaCl 0.05 M, pH 7.4) and incubated in a solution of anti-dsDNA antibody for 45 minutes. Of note, without the use of the blocking step and of the Tween as washing reagent the signals of the blank sample obtained with electrochemical immunosensors after the whole immunological chain were almost indistinguishable from those obtained in the presence of anti-dsDNA antibodies. After incubation with the anti-dsDNA antibody the sensors were rinsed with Tween in buffer and incubated with the secondary antibody conjugated with alkaline phosphatase (diluted 1:100) for 15 min. After a new wash with Tween in buffer the sensors are immersed in a solution of DEA buffer containing 1-naphthyl phosphate (5 mg/ml) and the signal due to the enzymatically produced 1-naphtol was measured with DPV after 2 minutes. Secondary antibody concentration and incubation time were optimized to obtain the best sensitivity.

Supporting References

Xiao, Y., Lai, R. Y., Plaxco, K. W., *Nat. Protoc.* **2007**, *2*, 2875-2880.

Supplemental Results

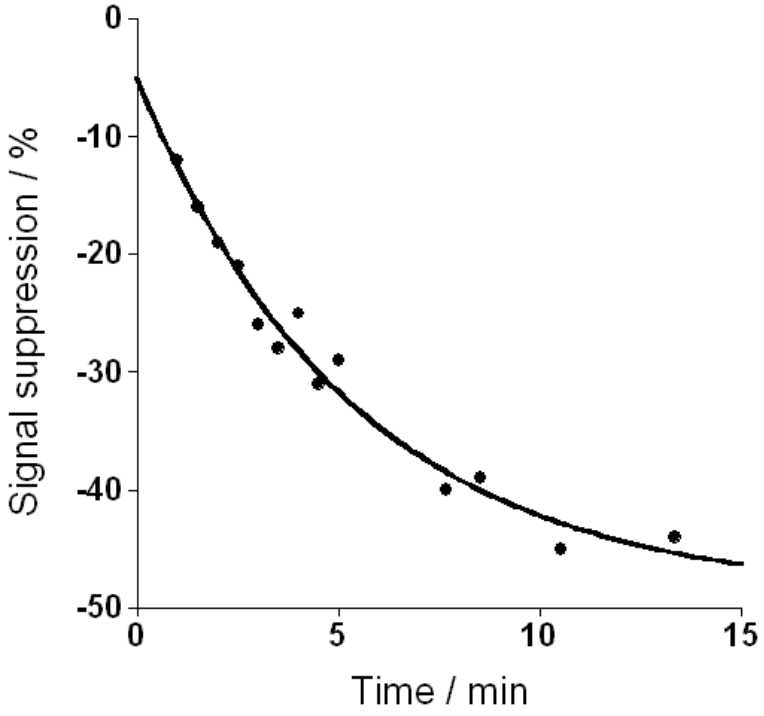

Figure S1. The detection of anti-DNA antibodies is rapid. Shown is the sensor response upon the addition of 50 nM anti-dsDNA antibody. The sensor equilibrates with a single-exponential kinetics (solid line) with a time constant of ~ 3 minutes.

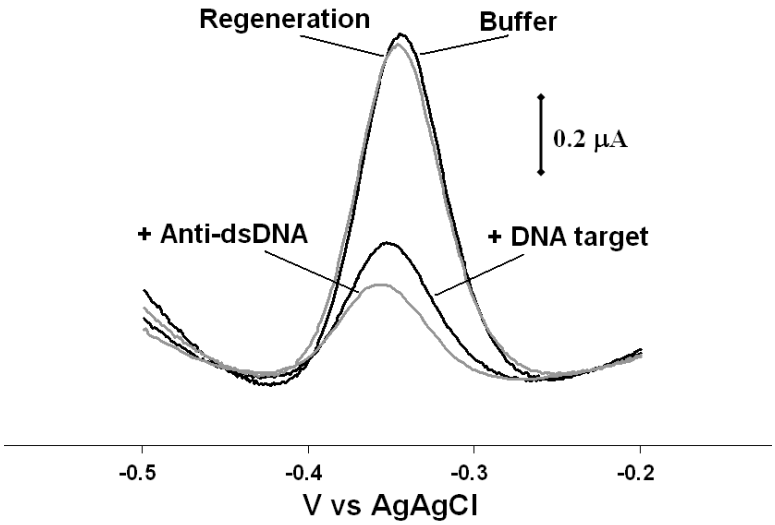

Figure S2. The sensors are readily regenerable: a 30 s wash in 8 M urea disrupt antibody-DNA complex and remove the second DNA strand thus regenerating the original signal. The addition of fresh complementary strand regenerates the original sensor signal allowing for re-use.