Supporting Information for:

An Electrochemical Sensor for the Detection of Protein-Small Molecule Interactions Directly in Serum and Other Complex Matrices

Kevin J. Cash[1], Francesco Ricci[2,4], Kevin W. Plaxco[,2,3]*

Department of Chemical Engineering[1], Department of Chemistry and Biochemistry[2], Program in Biomolecular Science and Engineering[3]

University of California, Santa Barbara

*kwp@chem.ucsb.edu

Author Footnote: [4] Current Address: University of Rome Tor Vergata, Rome, Italy

Reagents and DNA Sequences

Tris(2-carboxyethyl) phosphine hydrochloride (TCEP; Invitrogen, Eugene, OR), 6-Mercapto-1-hexanol (MCH; Fluka), sulfuric acid (Fisher Scientific), and bottled Guiness Draught Beer (Dublin, Ireland) were all used as received. Streptavidin (Sigma-Aldrich), monoclonal anti-digoxigenin antibodies (clone 1.71.256; Roche Diagnostics, Indianapolis, IN), and human IgG mixture (Equitech-Bio, Inc., Kerrville, TX) 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. Newborn calf serum (NCS; Sigma-Aldrich) was aliquoted and frozen at -20°C prior to use. Soil samples were taken from the University of California, Santa Barbara campus and suspended in DI water for storage (10% w/v) and diluted with buffer to 5% w/v for use. Saline-sodium citrate buffer (SSC) was diluted from a 20X stock (20XSSC, Sigma Aldrich) to either 6XSSC (for sensor fabrication and buffer experiments) or 12XSSC (for dilution of NCS, beer and soil samples) with DI water.

The thiolated, methylene blue-tagged anchoring strands (HPLC purified, Biosearch Technologies Inc., Novato, CA) were used as received without further purification. Biotin-tagged recognition strands (Integrated DNA Technologies Inc., Coralville, IA) and digoxigenin-tagged recognition strands (Fidelity Systems Inc., Gaithersburg, MD) were used as received. The sequences of the various strands are shown in Table S1.

Table S1: DNA Sequences

Probe DNA Sequence	
Probe	5'-HS-$(CH_2)_6$-GCAGTAACAAGAATAAAACGCCACTGC-$(CH_2)_7$-MB
5' labeled biotin recognition strands (biotin distal to electrode)	
27B5	5'-biotin-GCAGTGGCGTTTTATTCTTGTTACTGC
23B5	5'-biotin-AGTGGCGTTTTATTCTTGTTACT
21B5	5'-biotin-GTGGCGTTTTATTCTTGTTAC
19B5	5'-biotin-TGGCGTTTTATTCTTGTTA
17B5	5'-biotin-GGCGTTTTATTCTTGTT
15B5	5'-biotin-GCGTTTTATTCTTGT
13B5	5'-biotin-CGTTTTATTCTTG
3' labeled biotin recognition strands (biotin proximal to electrode)	
27B3	5'-GCAGTGGCGTTTTATTCTTGTTACTGC-biotin
23B3	5'-AGTGGCGTTTTATTCTTGTTACT-biotin
21B3	5'-GTGGCGTTTTATTCTTGTTAC-biotin
19B3	5'-TGGCGTTTTATTCTTGTTA-biotin
17B3	5'-GGCGTTTTATTCTTGTT-biotin
15B3	5'-GCGTTTTATTCTTGT-biotin
13B3	5'-CGTTTTATTCTTG-biotin
Modified biotin recognition sequences	
23S17B3	5'-GTTTTATTCTTGTTACT-biotin
19S10B5	5'-biotin-TGGCGTTTTA
Digoxigenin recognition sequences	
19D5	5'-digoxigenin-TGGCGTTTTATTCTTGTTA
19D3	5'-TGGCGTTTTATTCTTGTTA-digoxigenin
21D3	5'-GTGGCGTTTTATTCTTGTTAC-digoxigenin
23D3	5'-AGTGGCGTTTTATTCTTGTTACT-digoxigenin
25D3	5'-CAGTGGCGTTTTATTCTTGTTACTG-digoxigenin
Control sequence (no recognition element)	
17NT	5'-GGCGTTTTATTCTTGTT

Electrode cleaning and Sensor Preparation

A detailed sensor fabrication procedure can be found in the literature[25]. 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 preformed.

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 (or other concentrations for probe packing density experiments) with 6XSSC. Electrodes (thoroughly rinsed with DI water) were incubated in 250 μL of anchoring DNA for 30 minutes. Electrodes were rinsed with DI water, and incubated in 3mM MCH in 6XSSC for 2 hours to displace nonspecifically adsorbed DNA and passivate the remaining electrode area[26]. 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[15]. The probe density used in this research was $2.2 \pm 0.2 \times 10^{11}$ molecules/cm^2 unless otherwise noted. The modified electrodes were then incubated in 100 nM recognition strand solutions for 1 hour to allow hybridization prior to use.

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. With the exception of kinetic measurements, the current was measured after 1 hour incubation to allow near complete signal saturation. 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.

Supporting References

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

[26] Herne, T. M., Tarlov, M. J., *J. Am. Chem. Soc.* **1997**, *119*, 8916-8920.

Supplemental Results

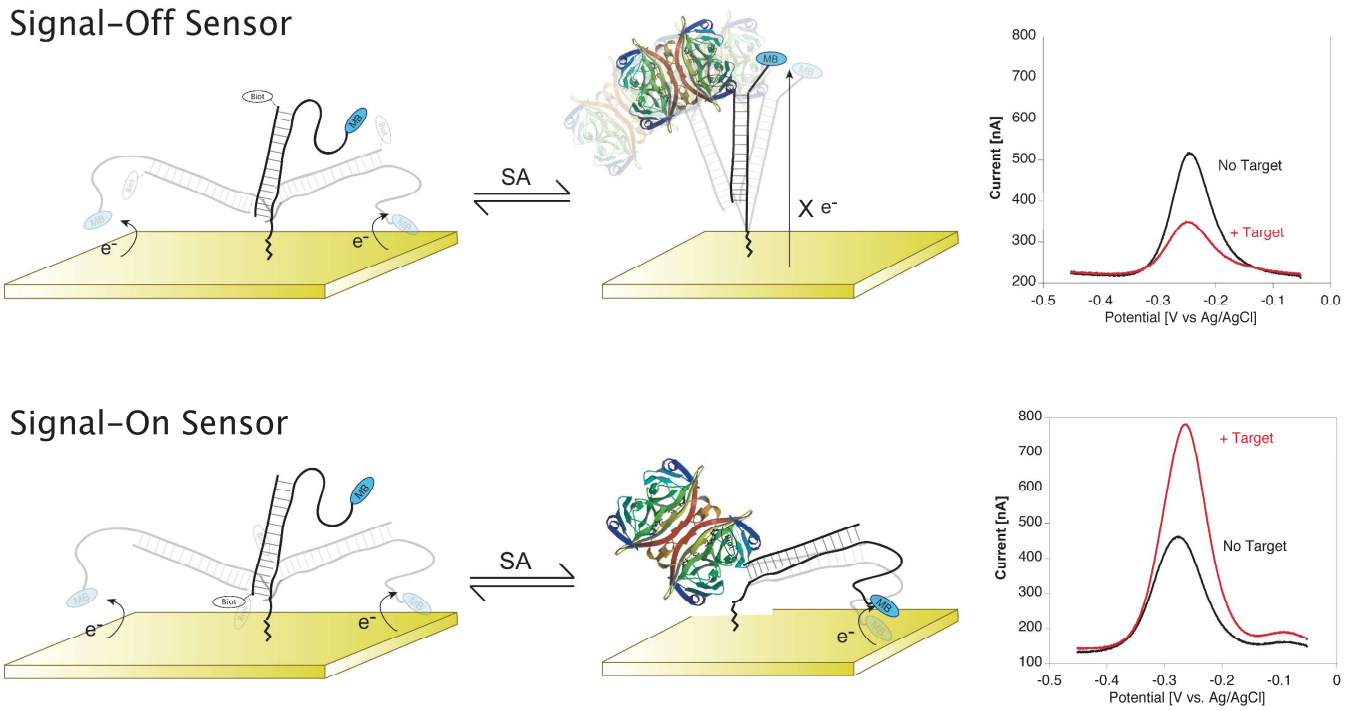

Figure S1. Our sensor approach supports both signal-off and signal-on architectures. The signal off sensor (top left) decreases the population of redox labels near the surface upon binding to target, decreasing electron transfer to the electrode (top right). The signal-on sensor (bottom left) increases the population of redox labels near the electrode, increasing electron transfer (bottom right). The data presented here are for the detection of streptavidin in buffer using the 19B5 (top) and 23S17B3 (bottom) recognition strands.

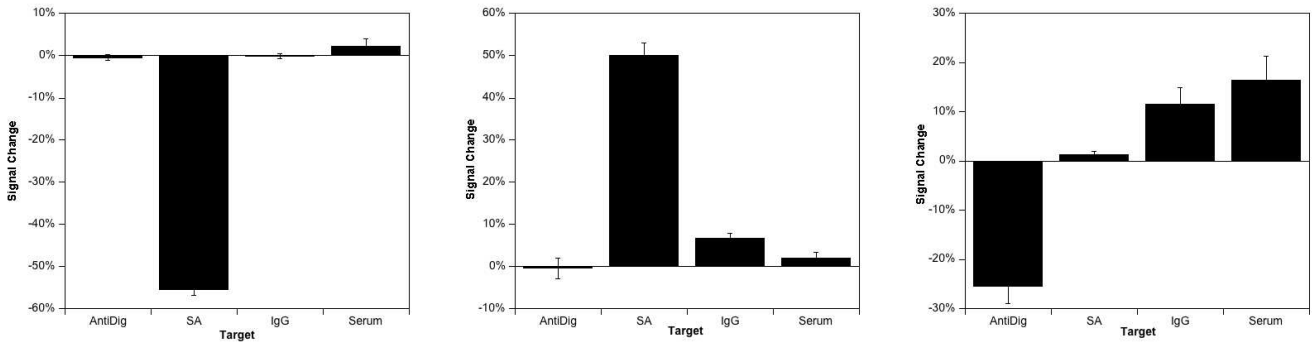

Figure S2. The E-DNA scaffold approach is specific; the signal-off (left, 19B5) and signal-on (center, 23S17B3) streptavidin sensors and digoxigenin sensor (right, 19D5) respond to their correct targets but do not respond significantly to any of the incorrect targets we have investigated. The targets employed here include anti-digoxigenin antibody (AntiDig; 30 nM), streptavidin (SA; 30 nM) a mixture of human IgGs (IgGs; 30 nM) or blood serum (1%). The probe packing densities employed here were 4×10^{11} molecules/cm^2 for 19B5, 8×10^{11} molecules/cm^2 for 23S17B3 and 2×10^{11} molecules/cm^2 for 19D5.

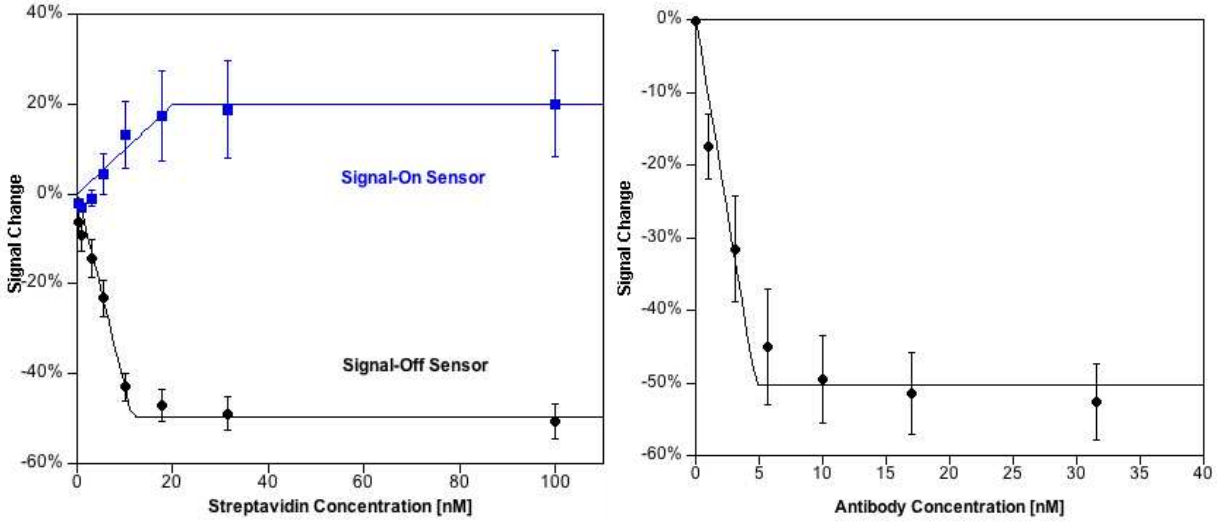

Figure S3. Our sensors perform well even when challenged directly in blood serum and other complex matrices. Shown are titration curves for streptavidin binding (left) and anti-digoxigenin binding (right) performed directly in 50% blood serum. Results are similar to the titration curves obtained in buffer (Fig. 3 and Fig. 4), with the relatively small differences likely resulting from different probe packing densities employed (2.3×10^{12} molecules/cm^2).

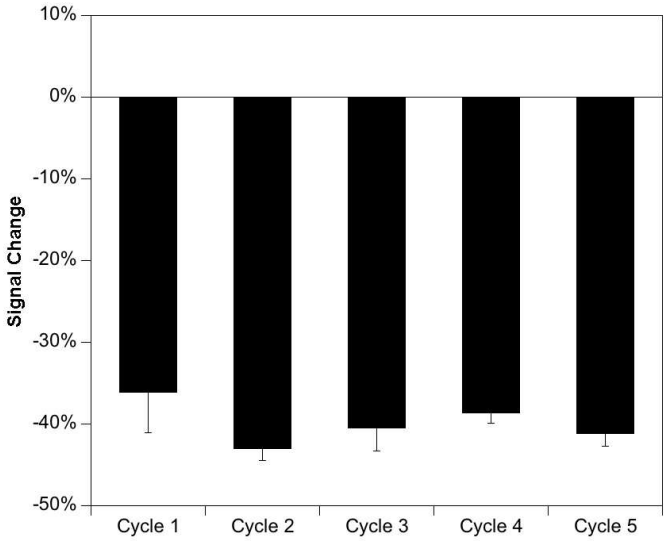

Figure S4. The E-DNA scaffold system is readily reusable via a 20 s deionized water wash (which removes the recognition strand/target complex) followed by the re-introduction of fresh recognition strand. Shown here is the signal obtained when a sensor (19B5) is repeatedly challenged with 10 nM streptavidin, washed, regenerated and reused.

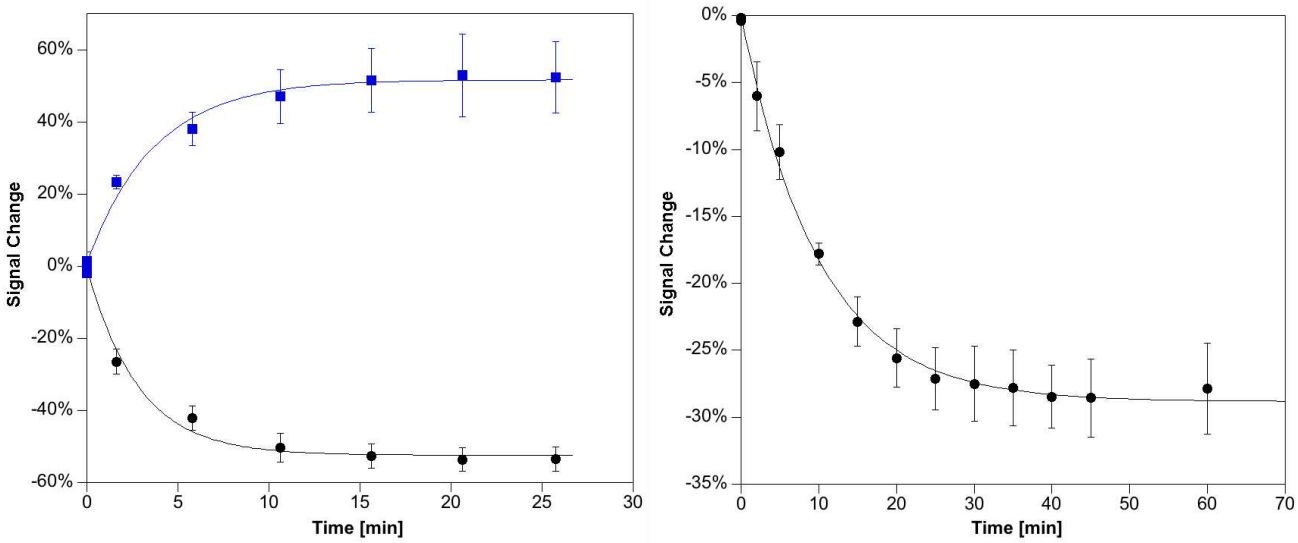

Figure S5. The new sensor architecture responds rapidly, exhibiting exponential equilibration kinetics with time constants of 2.8 minutes for streptavidin binding to the 19B5 recognition strand, 3.6 minutes for streptavidin binding to the 23B3 recognition strand (left) and 9.9 minutes for antibody binding to the 19D5 recognition strand (right).

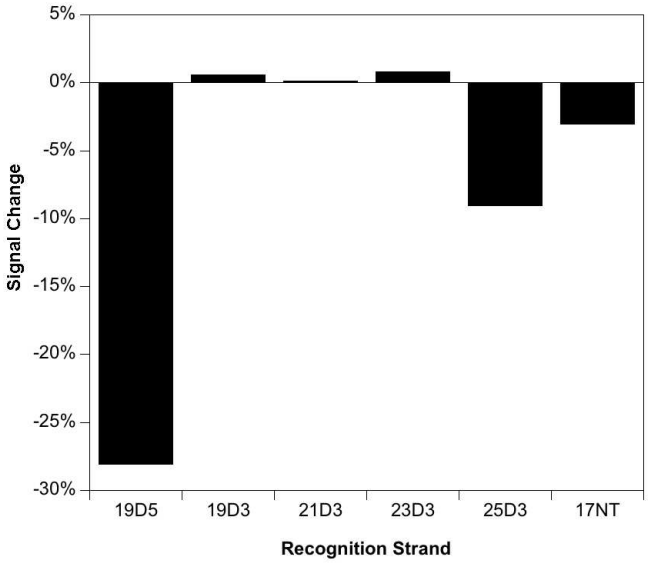

Figure S6. In contrast to sensors directed against streptavidin, no signal-on response was observed for sensors directed against anti-digoxigenin antibodies. This may indicate a lack of generality for the signal-on approach, or simply the need for further optimization of probe geometry, flexibility and probe packing density. Shown are the responses upon addition of 40 nM anti-digoxigenin antibody of a single 5' labeled recognition strand (19D5), four different length 3' labeled strands (19D3, 21D3, 23D3, and 25D3), as well as an unlabeled control strand (17NT).

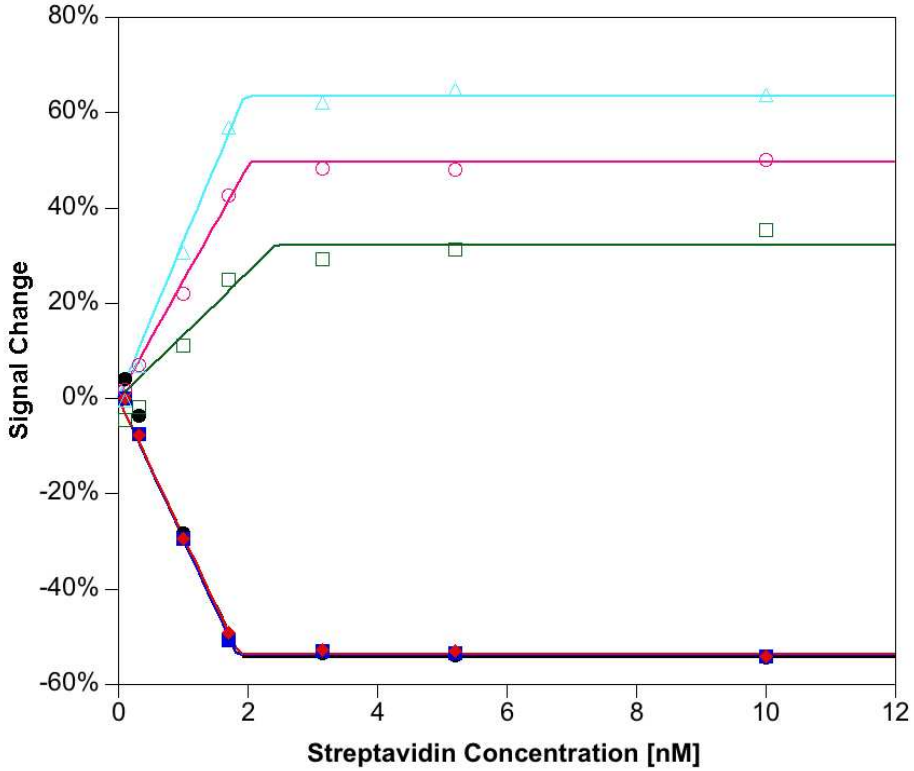

Figure S7. By plotting the responses of various, individual sensors we see that most of the variance in the signaling of the signal-on sensors (shown are data for the 23S17B3 recognition strand) arises due to sensor-to-sensor variability in gain. In contrast, the sensor-to-sensor reproducibility of the signal-off architecture (shown is the 19B5 recognition strand) is exceptional.

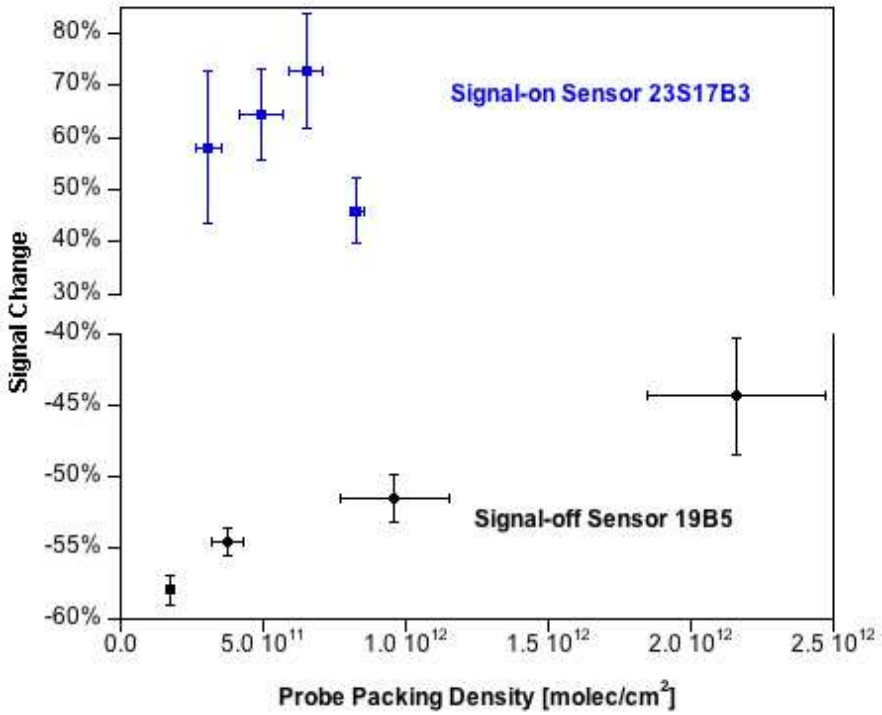

Figure S8. The probe packing density of the DNA scaffold, which can be controlled by varying the concentration of signal strand used during sensor fabrication, effects signaling. The dependence of signal change upon probe density is linear for 19B5 recognition strands (bottom) but is nonlinear and sensitive for 23S17B3 recognition strands (top).