Supporting Information: Label-Free Detection of the Antigen-Specific T-Cell Immune Response

Eric Stern, Erin R. Steenblock, Mark A. Reed, and Tarek M. Fahmy

Materials and Methods

Sensor fabrication. Sensors were fabricated as described previously.[1] Briefly, starting with commercially available ultra-thin silicon-on-insulator wafers, the active silicon layer was thinned to 40 nm by subsequent thermal oxidation and buffered oxide etching steps. The NWs were then defined lithographically and realized using tetramethylammonium hydroxide, an anisotropic silicon etchant. (The majority of the device processing occurred at the Cornell Nanofabrication Facility.) The resulting NWs have electronic transport properties similar to bulk silicon, due to the smooth, pristine sidewalls produced by the wet chemical etch.[1] Devices used in this study were patterned with a final photoresist layer that opened vias to the die only over active devices (Clariant AZ 5218-E inverting resist; hardbaked for 1 hr at 140ºC).[2] This prevented solution-induced device failure by eliminating leakage paths to the backgate; devices on protected chips are stable in buffered solutions for >1 hr.

Mice. All animals were routinely used at 6-8 weeks of age and were maintained under specific pathogen free conditions and routinely checked by the Yale University Animal Resource Center. OT-1 transgenic breeder mice were a gift from (Ruslan Medzhitov,

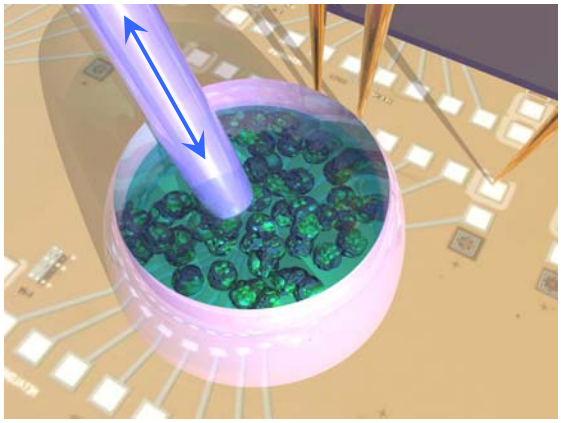
Yale University) and 2C TCR animals were gift from (Herman Eisen, Massachusetts Institute of Technology). C57B/6 (B6) mice were obtained from Jackson Laboratories (Bar Harbor, ME). All transgenic mice were maintained as heterozygous by breading on a C57BL6 background in our animal facility. Phenotypes were tested with the clonotypic 1B2 antibody (for 2C mice) and Valpha and CD8 for OT-1.

Cells. All cells used were obtained from homogenized naive mouse spleens after depletion of RBC by hypotonic lysis (Acros Organics). Splenocytes were used without further purification. For experiments involving inhibition of cellular signaling, 1 mL of 1 mg/mL genistein (Quality Biological) was added to splenocytes at 1×10^7 cells/mL followed by incubation for 1 hr at 4°C. Cell viability was assessed with trypan blue before and after genistein treatment.

Sensing measurements. The low-buffered solution was created by diluting 1X PBS tenfold and adding sodium chloride to a final concentration of 150 mM. Cells were resuspended in this solution immediately prior to sensing measurements at a concentration of 1×10^7 cells/mL. 7 μL of this solution was initially present in the sensor reservoir for all cellular measurements, thus $\sim 7 \times 10^4$ total cells were present. 2 μL of stimulant (anti-CD3 or peptide/MHC dimer, provided by Jonathan Schneck, Johns Hopkins School of Medicine) was added at a concentration of 0.5 mg/mL for all cellular measurements. Mixing was induced throughout the measurement. The control sensing measurement in Fig. 2A was performed by adding dilute sulfuric acid (at *time* = 0) to the same buffer used for cellular sensing measurement (pH 7.4) to achieve a final pH of 6.9. The pre- and post-titration pH values indicated in the figure were measured using an Orion 720A pH meter.

System Design

Supp. Fig. 1 is a schematic (not to scale) of the device design superimposed on an optical micrograph of the wafer. The reservoir (light pink) is defined by epoxying a section of thin-walled polypropylene tubing to the wafer. Probetips (gold) are used to contact the device (source and drain) and the backgate. The solution containing cells (cells are shown as spheres in the aqua interior of the chamber) is added to the reservoir directly prior to commencing the sensing measurement. After the establishment of an initial baseline current, the stimulant is manually injected with a micropipette, represented by the light blue tube, and subsequently mixed, as indicated by the double-arrow.



Supp. Fig. 1

References

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2. Stern, E.; Wagner, R.; Sigworth, F. J.; Breaker, R.; Fahmy, T. M.; Reed, M. A. *Nano Lett.* **2007**, 7, 3405-3409.