

Supplementary Information

On-Chip Regeneration of Aptasensors for Monitoring Cell Secretion

S.1 Fabrication of the reconfigurable PDMS microfluidic device

The double-layer reconfigurable device was comprised of a thin (400 μm) fluid layer containing fluidic channels and semi-circular microcups, and a thick (3mm) control layer aiming for pneumatic control. Both Poly(dimethylsiloxane) (PDMS) layers were fabricated employing soft lithography technique. As the first step, SU-8 was spincoated onto a 4'' test-grade silicon wafer and exposed to UV light through a photomask to create the mold for PDMS patterning. The feature height on the mold was 100 μm , which eventually led to 100 μm microstructure height on PDMS. To facilitate the PDMS detachment from photoresist pattern, prior to use, the Si wafer was treated with (Tridecafluoro-1.1.2.2-Tetrahydrooctyl) Trichlorosilane vapor under vacuum for 30 min. PDMS pre-polymers were then poured onto the mold, degassed in a vacuum chamber and baked at 70°C. The ratio of elastomer to curing agent was 20:1 for fluid layer and 5:1 for control layer, respectively. Prior to the baking of the fluid layer, the control layer was placed in oven first and baked for 30 mins in advance. After baking control layer for 1 hour, the control layer was cut out, and holes for the vacuum line were punched using a 16 gauge needle. The control layer was subsequently carefully aligned and placed on top of the half-baked flow layer and heated for an additional 2 hours. Once fully prepared, the construct was cut out and removed from the mold. Fluidic access holes were punched at distal ends of the flow chamber as the final step.

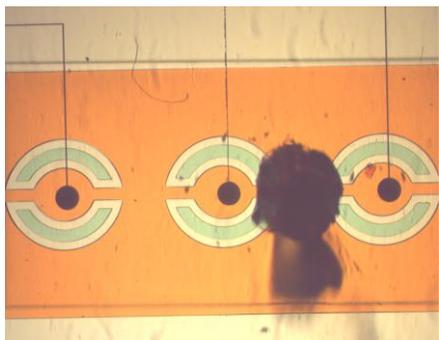


Figure S1. Optical image of the fluid chamber with green dye entrapped within the cups, remaining unmixed with red dye outside in the chamber

Movie S1: Proof-of-concept demonstration of tight cup sealing and mimicking of cytokine diffusion from the inside of cups: Red food dye (representative of cytokines secreted from cells) trapped inside the cups stayed isolated from external solution when the cups were closed; After applying negative pressure to the control chamber, the cups were opened and the dye trapped gradually diffused into the flow chamber.

Movie S2: Proof-of-concept demonstration of urea regeneration in designated area: Red dye (representative of urea buffer) stayed outside the semi-circular cups when they are closed, and the cells inside the cups were well protected.

S.2 Functionality of PDMS cups in cell protection

(i) Demonstration of tight sealing:

Complete isolation of cup inner space in the “closed” mode was confirmed by injecting fluorophore (Alexa 488)-containing solution into the channel after pushing down the cell-protection cups. Fluorescence intensity inside the cups were monitored for up to 3 hours to demonstrate the solution in the main channel couldn't seep into the cups and affect cell function.

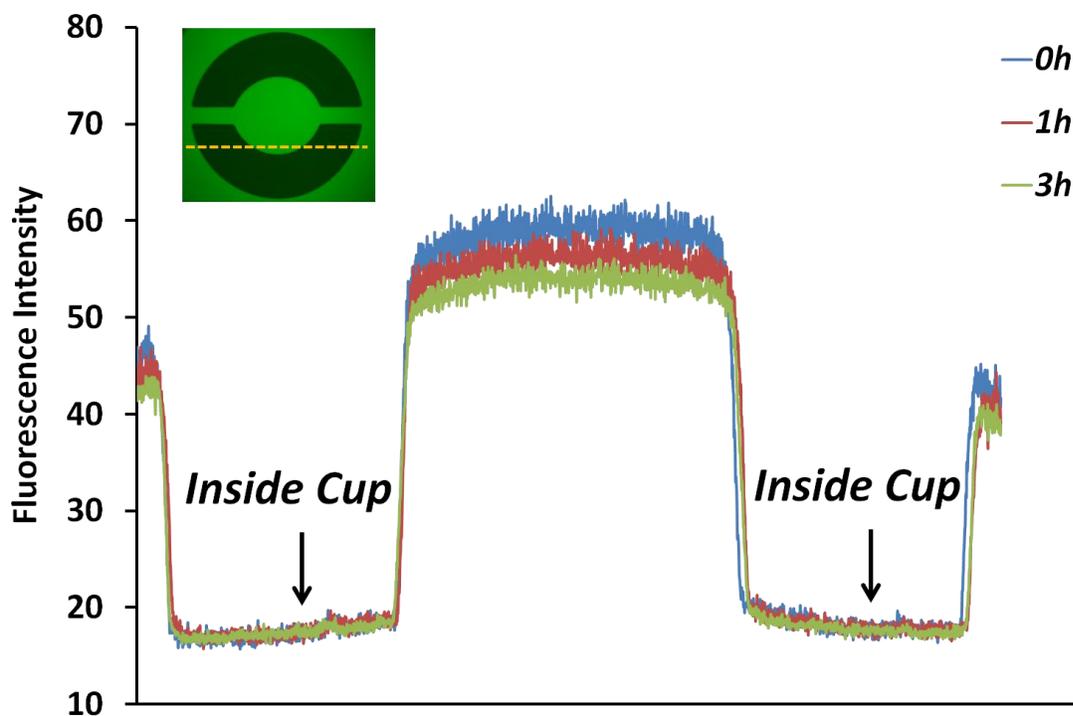


Figure S2. Fluorescence profile of the area indicated by the dashed yellow line: Over the course of three hours, the fluorescence inside the microcups remained low and didn't change over the time course of observation, indicating the effective sealing of the microcups from outside environment.

(ii) Comparison between unprotected and protected cells:

Movie S3: Protection effect of the cups: The cups in this movie were in closed mode. The cells outside the cups got washed away by regeneration buffer while those inside stayed intact.

Movie S4: Control experiment showing that the cells were killed if they were not protected during urea buffer regeneration: The cups in this movie were in open status. Regeneration buffer was injected into the flow chamber. As the movie shows, cells captured inside the cups were killed and vanished gradually from the view.

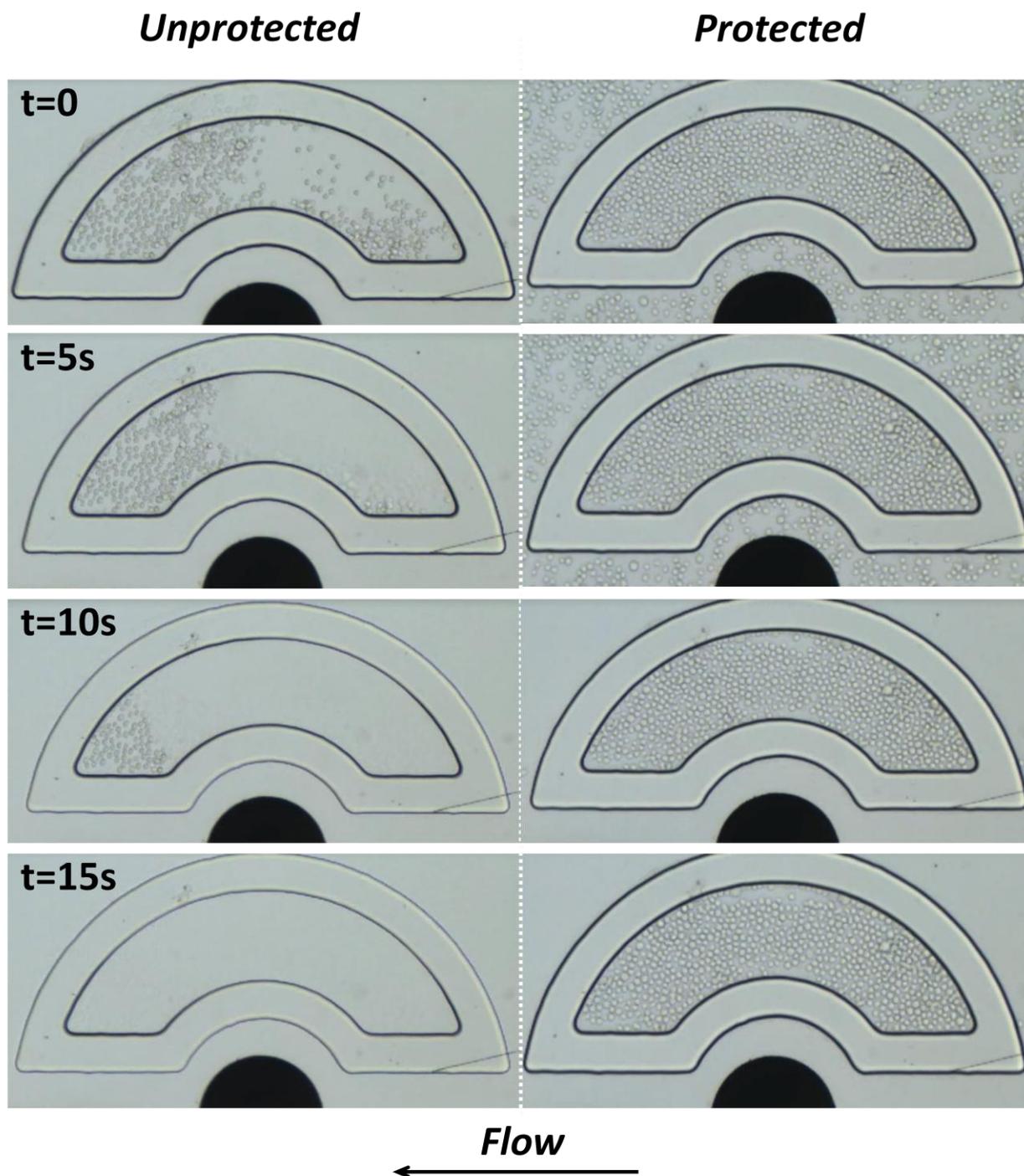
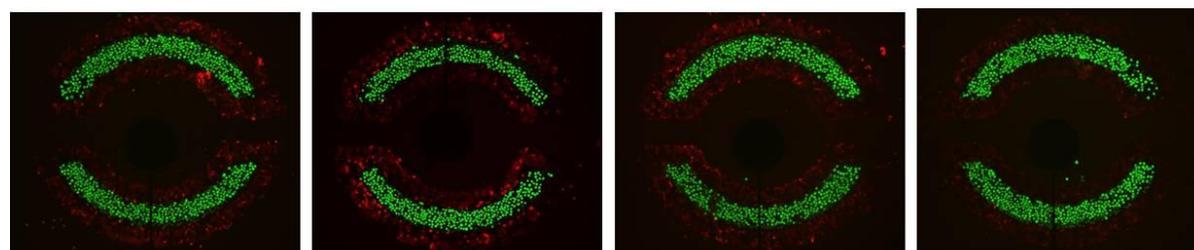


Figure S3. Comparison of unprotected and protected cells while exposed to regeneration buffer: Cells in the unprotected panel were lysed over the course of 15 seconds while the ones protected inside the microcups remain intact.

(iii) Cell viability vs. Regeneration cycles:

LIVE/DEAD staining was used to study the viability of the protected cells after regeneration cycles. The viable cells were stained as green while dead cells were fluoresced in red. The set of four images collected after each of the regeneration cycle demonstrates that cells were unaffected by harsh regenerative buffer present outside the cups in the microfluidic channel. Total cell numbers surrounding each electrode after regeneration cycles were also shown.



(a) *Reg 1* *Reg 2* *Reg 3* *Reg 4*

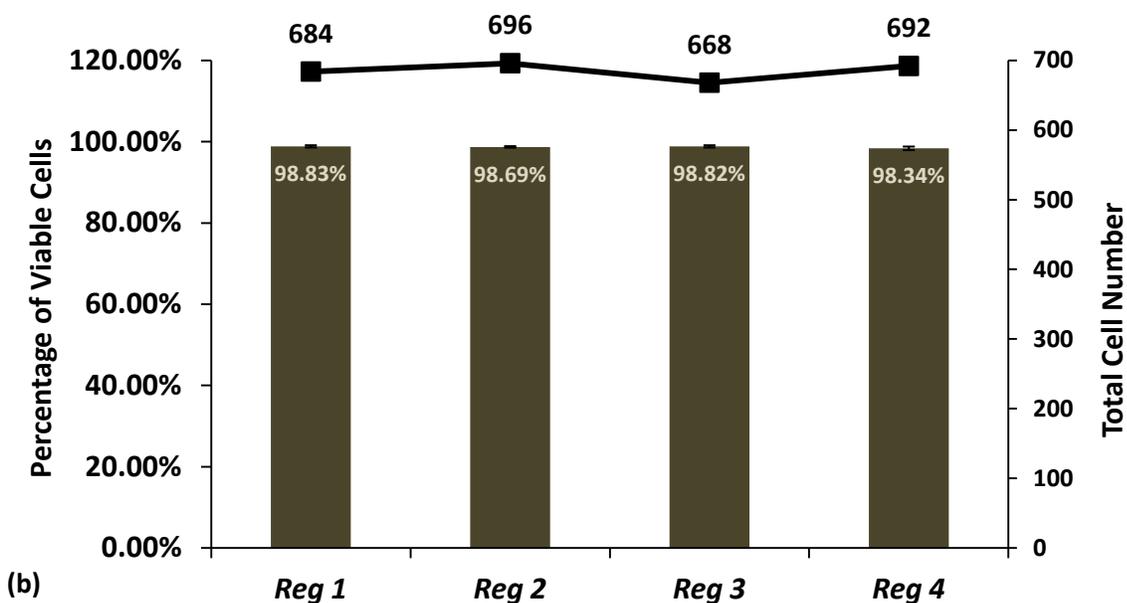


Figure S4. (a) Images of cell viability staining after various number of regeneration cycles (b) Percentage of viable cells and total cell numbers surrounding each electrode plotted against number of regeneration cycles

S.3 Sensing principle of IFN- γ aptasensor

As reported in our previous publication¹, the IFN- γ aptamer was engineered to contain a hairpin structure, with thiol group on the 3'-end to allow for self-assembly on gold electrode, and redox reporter covalently attached at the 5'-end for electrochemical sensing. As shown in Figure S5a, at rest status, the aptamer was naturally folded, placing MB group in close proximity to the electrode. The electron transfer efficiency is thus high upon application of the redox potential, quantified via square wave voltammetry (SWV). In the presence of IFN- γ , the binding event unfolds the hairpin structure of the aptamer, increasing the distance between MB and gold electrode, which eventually leads to suppressed electron transfer efficiency and a lower current peak. The decrease in signal is reported as "signal suppression" and used to interpret the concentration of IFN- γ . The "signal suppression" is defined as the ratio of SWV peak current loss at a given IFN- γ concentration to SWV peak current in the absence of analyte.

Aptamer hairpin sequence: 5'-NH₂-C₆-GGGGTTGGTTGTGTTGGGTGTTGTGTCCAACCC3'-SH-3'

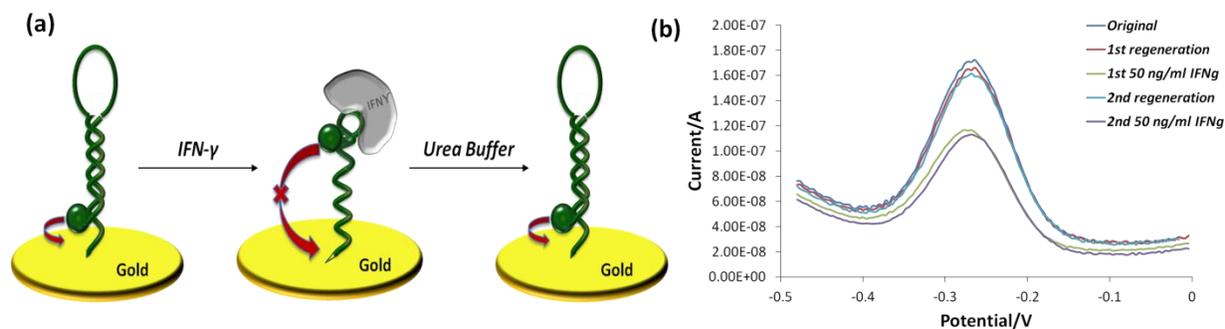


Figure S5. (a) Scheme showing the sensing/regeneration principle of the IFN- γ aptasensor; (b) Corresponding signals from SWV voltammetry. In the presence of IFN- γ , the peak current decreases; subsequent rinsing with regeneration buffer restores the original signal

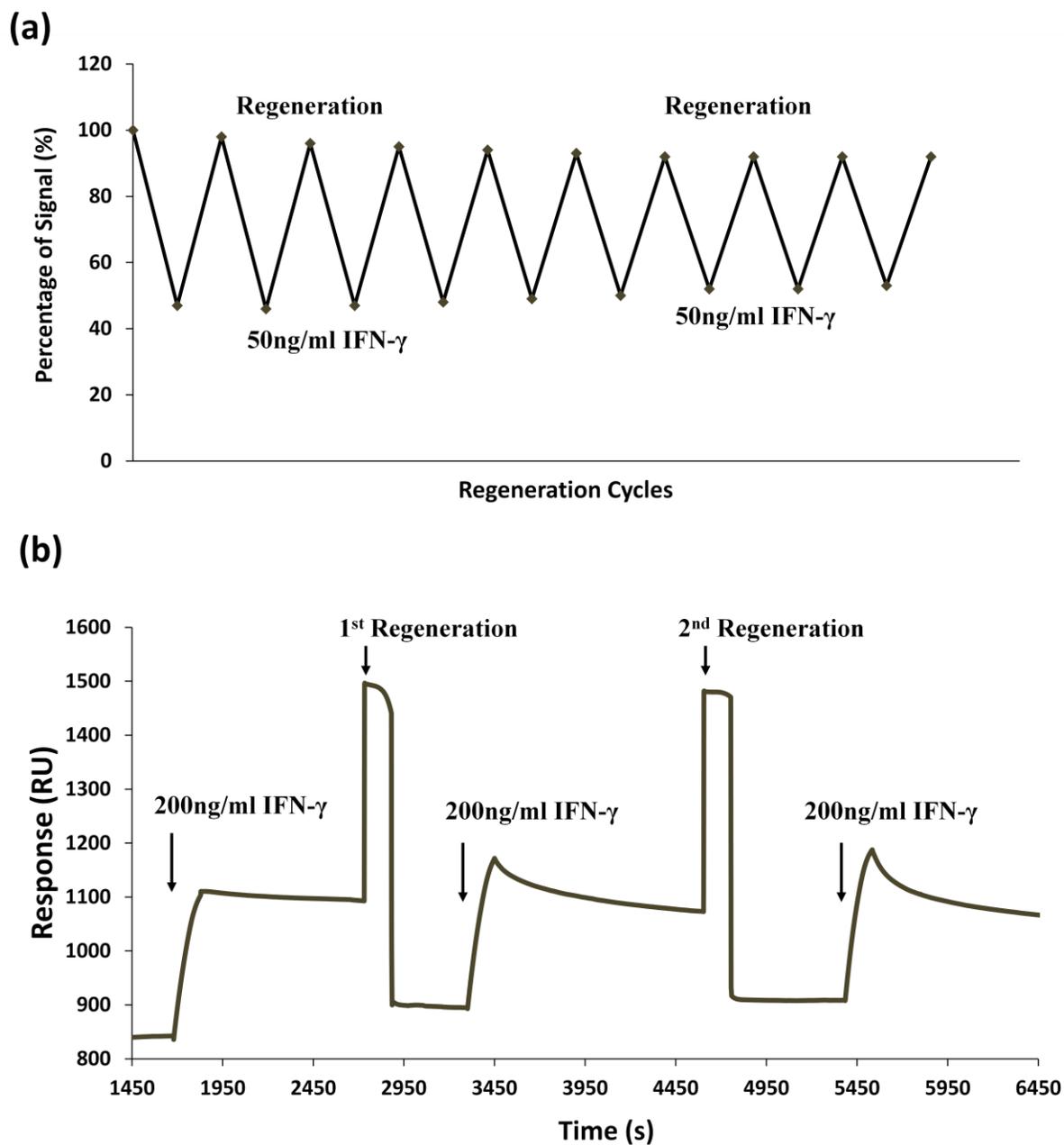


Figure S6. (a) Electrochemical sensor response within 10 cycles of regeneration (b) Sensorgram of repetitive IFN- γ challenging (200ng/ml) and aptamer regeneration using Surface Plasmon Resonance (SPR)

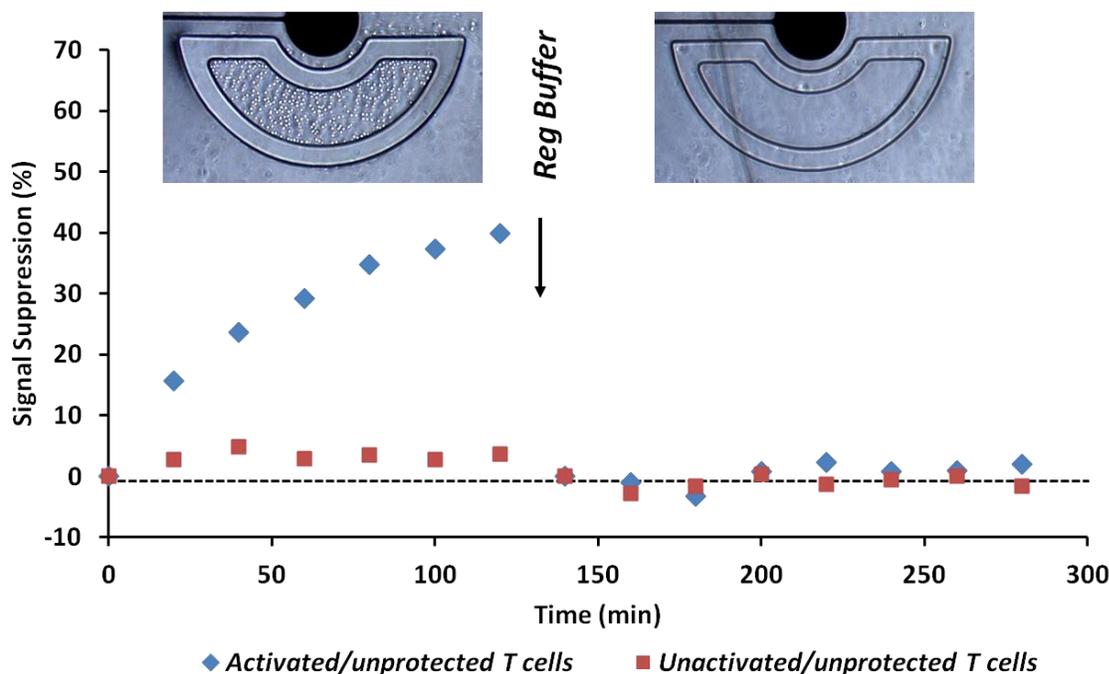


Figure S7. Sensor response from the activated/unactivated cells if they are not protected: The cell-protection cups remained in open status upon regeneration buffer infusion, and the sensor signal before/after flushing was recorded.

S.4 Electrode arrays and data collection:

The electrodes were fabricated using standard photolithography and metal etching processes. Glass slides (75mm×25mm) coated with 15nm Cr adhesion layer and 100nm Au layer, and then patterned to contain eight 300µm diameter electrodes on each half-slide. The electrode arrays were connected to the CHI 824B potentiostat (CH Instruments, TX) and controlled by a home-made multiplexing setup capable of collecting data automatically. We connected the ER-16 electromechanical relay (National Instruments, TX) to a computer through NI PCI-6503 (National Instruments, TX). To Interface the accompanying NI software and the CHI software, a program written in Sikuli controlled the switching between different working/counter/reference electrodes, took an SWV scan, and saved the data automatically for analysis.

S.5 Description of the Computational Model

To determine cellular production rate of IFN- γ , we used COMSOL to create a diffusion-reaction model that incorporates diffusion of cytokines in the solution in addition to the binding of cytokines to the aptamer-functionalized electrode surface. We assumed the transport of cytokines was purely by diffusion,

$$\frac{\partial C}{\partial t} = D\nabla^2 C \quad (1)$$

with C being the concentration of IFN- γ in the media and D is the diffusion coefficient. The cytokine-aptamer binding was modeled by the first-order reaction kinetics²,

$$\frac{dB}{dt} = k_{on}C(A_0 - B) - k_{off}B \quad (2)$$

Here, B represents the concentration of cytokine-aptamer complexes on the sensor's surface; A_0 is the initial surface concentration of aptamer; k_{on} and k_{off} are the association and disassociation constants, respectively. Eqns. (1) and (2) can be coupled through a boundary condition on the sensor surface, yielding

$$D \frac{\partial C}{\partial n} = k_{on}C(A_0 - B) - k_{off}B \quad (3)$$

where $D \frac{\partial C}{\partial n}$ is the molar flux of C normal to the sensor surface. We assumed a constant rate of cytokine production during each sensing cycle (i.e. we acquired unique secretion rates post-cycle), and this is modeled by the following boundary condition:

$$D \frac{\partial C}{\partial n} = \dot{\sigma} \frac{N_c}{A_c} \quad (4)$$

where $\dot{\sigma}$ is the cytokine secretion rate, and N_c is the total number of cells captured in the cell capture region A_c . The measured signal $S(t)$ is the integration of the fraction of surface binding over the whole sensor surface Ω ,

$$S(t) = \int_0^t \frac{B(t)}{A_0} d\Omega \quad (5)$$

The above equations were solved with a finite element method using COMSOL Multiphysics (COMSOL Inc., Burlington, MA). The finite element model was set up in COMSOL taking into account the device geometry as described in the main text. The values of parameters used in simulations are listed in Table S1.

Constant	Value	Units
Molecular Weight ³	17145.6	g/mol
Diffusion Coefficient ⁴	1.457×10^{-6}	cm ² /s
Association Constant ⁵	1.38×10^5	dm ⁵ /mol/s
Dissociation Constant ⁵	6.339×10^{-5}	1/s
Aptamer Surface Density ⁵	1.6×10^{-13}	mol/cm ²

Table S1. Values for the constants used in the simulation.

Expected signal profiles were calculated given a constant $\dot{\sigma}$, and comparisons of the modeled signals to the observed signals yielded R^2 values to determine the secretion rates for each cycle, which were 0.00144pg/cell/hour, 0.00048pg/cell/hour, and 0.00040pg/cell/hour. The fraction of cytokines captured ranged from 7.7%, 9.9%, and 10.2% for each cycle.

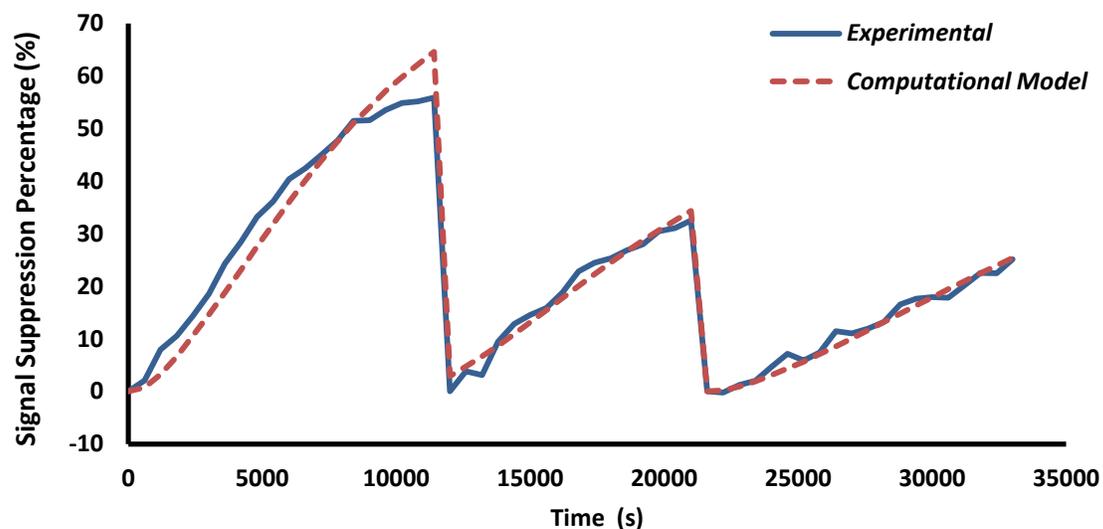


Figure S8. Modeled signal profiles assuming a constant secretion rate for each regeneration cycle. Each cycle is linked to a different secretion rate: 0.00114pg/cell/hour, 0.00048pg/cell/hour, and 0.00040pg/cell/hour for each cycle respectively.

References

1. Y. Liu, J. Yan, M. C. Howland, T. Kwa and A. Revzin, *Analytical chemistry*, 2011, **83**, 8286-8292.
2. T. M. Squires, R. J. Messinger and S. R. Manalis, *Nat Biotechnol*, 2008, **26**, 417-426.
3. M. A. Palladino, F. R. Bahjat, E. A. Theodorakis and L. L. Moldawer, *Nat Rev Drug Discov*, 2003, **2**, 736-746.
4. M. E. Young, P. A. Carroad and R. L. Bell, *Biotechnology and Bioengineering*, 1980, **22**, 947-955.
5. Y. Liu, Q. Zhou and A. Revzin, *The Analyst*, 2013, **138**, 4321-4326.