Supporting Information

Detecting Transforming Growth Factor-β Release from Liver Cells Using an Aptasensor Integrated with Microfluidics

Zimple Matharu, Dipali Patel[†], Yandong Gao[†], Amranul Haque, Qing Zhou and Alexander Revzin*

Department of Biomedical Engineering, University of California, Davis, California, United States

Correspondence:

Alexander Revzin Ph.D.
451 Health Sciences Dr.#2519
Davis, CA, 95616
arevzin@ucdavis.edu
†both authors contributed equally to the work

Supporting Information-Table of Content

Surface Plasmon Resonance (SPR) based Characterization of TGF-β1 Aptamer

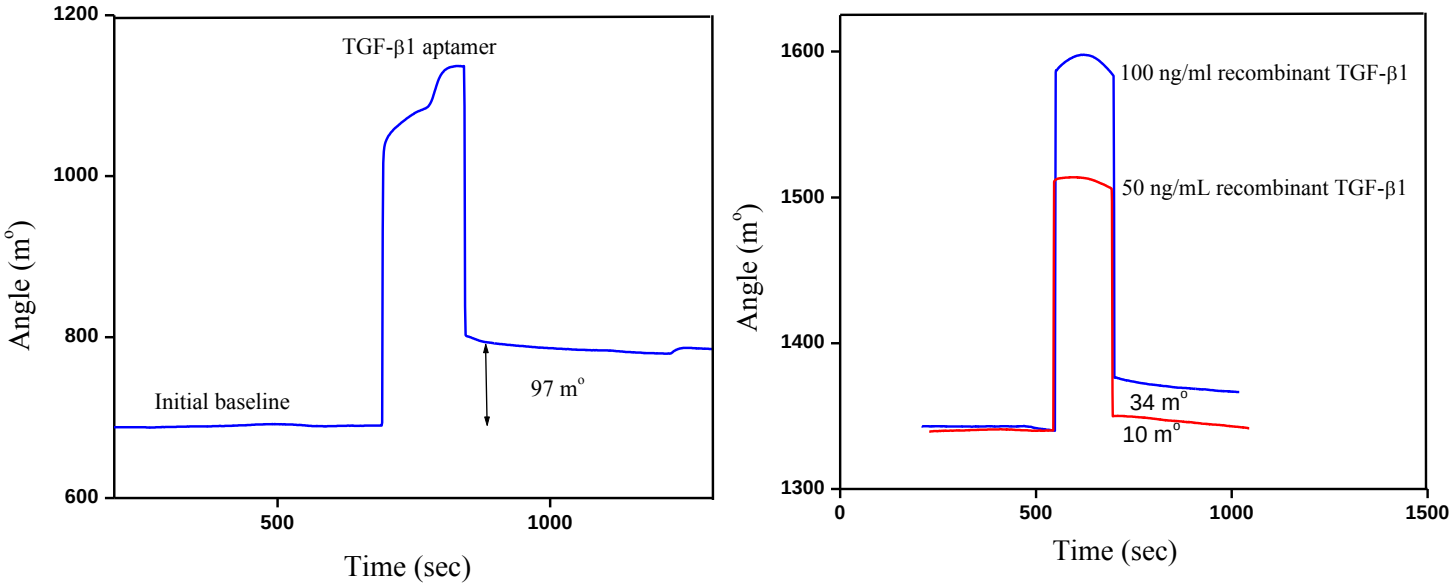

Figure S1. SPR analysis of TGF-β1 aptamer interaction with recombinant TGF- β1. (A) SPR sensogram showing assembly of 1 µM aptamer on SPR Au chip (B) SPR sensogram showing binding of two different concentrations of TGF-β1 on aptamer modified Au chip. HEPES was used as running buffer as well as for making aptamer and recombinant concentrations.

FigureS1 (A,B) shows SPR results for aptamer assembly and TGF-β1 binding. The SPR system was purged with running buffer solution (HEPES) to set and stabilize an initial baseline as shown in Figure S1(A). Subsequently, 200 µL of 1 µM TGF-β1 aptamer solution was infused at the flow rate of 40 µL/min in the SPR flow system and allowed to interact with the Au surface. At the completion of binding, the surface was washed with running buffer and 1mM MCH was injected to block the unmodified Au regions. A change of 97 m^o in the SPR angle (Figure S1(A))

corresponds to the change in refractive index due bound TGF-β1 aptamer. Figure S1(B) shows the binding of TGF-β1 aptamer with two different concentrations of recombinant human TGF-β1 injected at the flow rate of 40 µL/min. The SPR binding curves were used to calculate the binding constants of aptamer and target using kinetic analysis software (Scrubber). This analysis revealed equilibrium dissociation constant (K_d) as 1.07 nM.

Electrochemical Characterization and Specificty Studies of TGF-β1 Aptamer. Electrochemical characterization of the TGF-β1 apatmer was done in batch-mode based electrochemical cell as shown in Figure S2. This electrochemical set up utilizes Pt wire as counter and Ag/AgCl as reference electrode. A piece of Au-coated silicon wafer was used as a working electrode. To complete an electrochemical cell the Au surface was sandwiched between the two parts of batch mode of which one contained a hole of circular area 1.13 cm^2. A rubber O-ring was placed at the Au surface for sealing the working area. In this way we get Au working electrode inside the set up. 1µM of MB-tagged TGF-β1 aptamer was immobilized on the working electrode inside the electrochemical cell. To take the electrochemical measurements 1 mL of HEPES buffer was put inside the cell containing reference and counter electrodes and set up is connected to the CHI instrument.

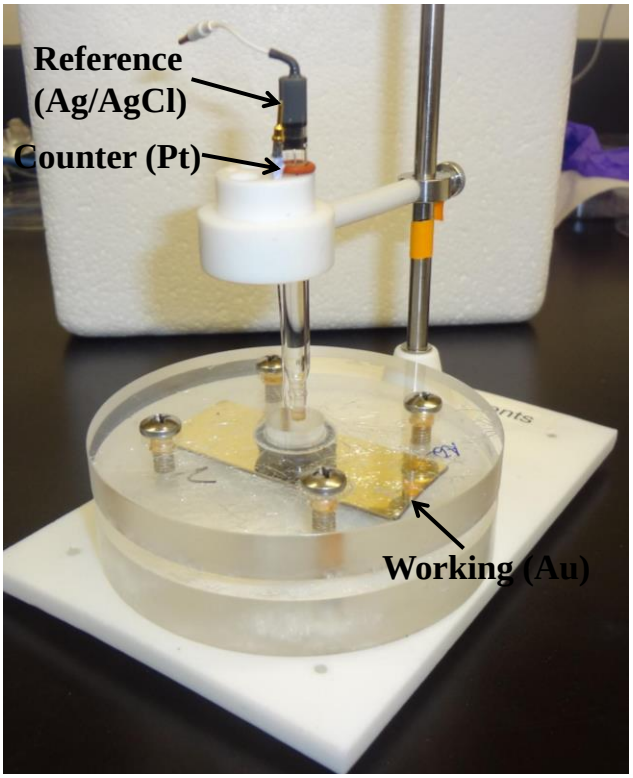

Figure S2. Picture of electrochemical batch mode set up for characterization of aptasensor in HEPES buffer.

The MB-tagged TGF-β1 apatmer was immobilized on Au surface. SWV measurements were taken after incubating the aptamer modified surface with different concentrations of recombinant TGF-β1 in HEPES for 30 min. This time was found to be optimal for complete binding of target molecule with the aptamer in buffer. Figure S2(A) shows the SWV measurements taken in HEPES buffer after binding of different TGF-β1 concentrations to the aptamer-modified surface. The MB current progressively decreased. Figure S2(B) illustrates change in the MB current as a function of different concentrations of recombinant TGF-β1 revealing sensitivity of 44.9 nA $ng^{-1}mL$.

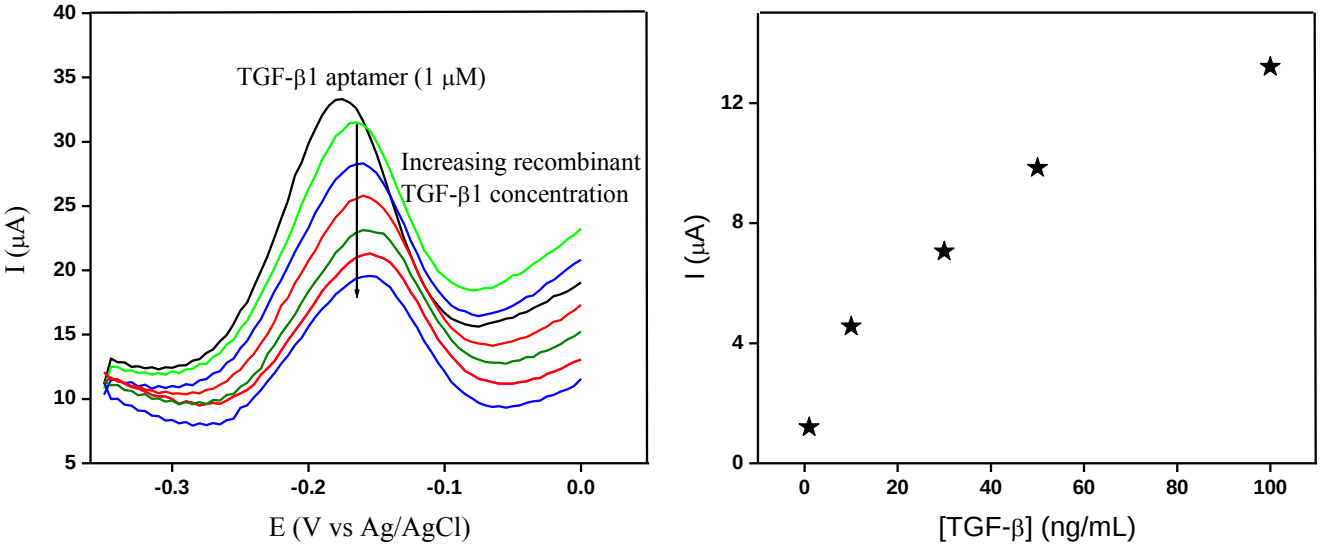

Figure S3. (A) SWV plots taken in HEPES buffer showing gradual decrease in MB current after challenging MB-tagged TGF-β1 aptamer (1 μM) modified Au surface with increasing recombinant TGF-β1 concentrations. The decrease in MB current is attributed to conformation change in the aptamer structure while target binding that pushes away the MB from the electrode surface (B) Plot showing change in MB current as a function of different concentrations of recombinant TGF-β1.

Computational Model for Determining Secretion Rates.

To predict the production rate of TGF-β1 from stellate cells, we developed a diffusion-reaction model that accounts for the transport of cytokines (TGF-β1) in the solution and the binding of those cytokines to the aptamers immobilized on electrode surface. The transport of cytokines was only due to diffusion which is governed by the following equation;

$$\frac{\partial C}{\partial t} = D\nabla^2 C \qquad \text{Eq. (S1)}$$

Where C is the concentration of cytokines in the media and D is the diffusion coefficient of the cytokine. The cytokine-aptamer binding on the electrode surface was modeled by the first-order reaction kinetics,

$$\frac{dB}{dt} = k_{on} C A_o - B - k_{off} B \qquad \text{Eq. (S2)}$$

In the above equation, B is cytokine-aptamer conjugation on the sensor's surface; A_0 is the initial surface concentration of TGF-β1 aptamer; k_{on} and k_{off} are the association and dissociation constants, respectively. The binding process depends on the concentration of available binding sites, A_o–B and the cytokine concentration C at the aptasensor surface whereas the unbinding process depends on concentration of bound cytokines only. The surface reaction and bulk diffusion are coupled together through a boundary condition on the sensor surface

$$D\frac{\partial C}{\partial n} = k_{on} C A_o - B - k_{off} B \qquad \text{Eq. (S3)}$$

Where $D\frac{\partial C}{\partial n}$ is the molar flux of C normal to the sensor surface. We assumed that the rate of TGF-β1 production was a constant value during the entire detection process, which is described by a boundary condition in the model,

$$D\frac{\partial C}{\partial n} = \sigma \frac{N_c}{A_c} \qquad \text{Eq. (S4)}$$

Where σ is the cell secretion rate, N_c is the total number of stellate cells captured in the cell capture region A_c. The measured signal S(t) is the integration of the fraction of occupied binding sites over the whole sensor surface Ω,

$$St = \frac{B(t)}{A_o} d\Omega \qquad \text{Eq. (S5)}$$

The above equations were solved with a finite element method using COMSOL Multiphysics (COMSOL Inc., Burlington, MA). A cylindrical coordinate system where the axis is at the center of the electrode was used to reduce the calculation burden. The finite element model was set up in COMSOL taking into account the device geometry.

Inhibition of TGF-β Detection Using Anti-TGF Antibodies.

1μM TGF-β1 aptamer was first functionalized on Au microelectrodes. The aptasensors were protected via PDMS cups and stellate cells were seeded inside the chambers. PDGF (20 ng/mL), TGF-β1 capture antibody (100 μg/mL, obtained from ELISA kit) and TGF-β1 detection antibody (13 μg/mL, obtained from ELISA kit) were prepared in DMEM supplemented with 0.5% FBS and 1% PS and infused into the channels. The liquid flow inside the channels was stopped and PDMS cups protecting aptasensor were raised. SWV signal was recorded over the course of time. Interestingly no significant change in SWV signals was observed (Figure S4). This suggests that the TGF-β1 antibodies present inside the chamber binds with the secreted TGF-β1 molecules and prevents binding of the target analyte with the aptamer.

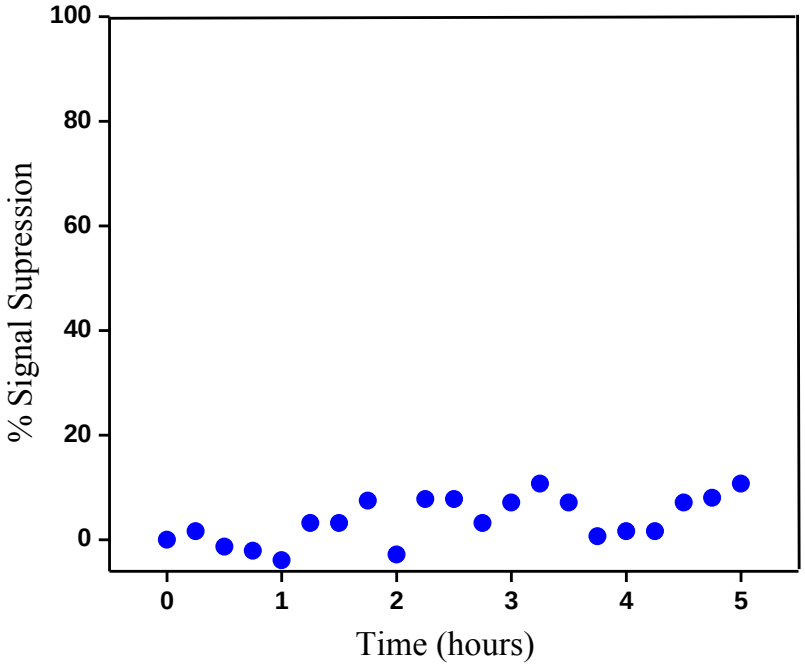

Figure S4. Signal suppression obtained from aptasensor having TGF-β1 blocking antibodies present inside the cell chamber. No significant signal suppression indicates potential blocking of TGF-β1 molecules secreted by stimulated stellate cells.