

## **Supporting Information**

### **Mimicking the Binding and Unbinding of Fe<sup>3+</sup> with Transferrin by Single Biomimetic Nanochannel**

Chao Zhao<sup>a</sup>, Xiaosi Li<sup>a</sup>, Lingyan Li<sup>a</sup>, Xiong Gong<sup>b</sup>, Yung Chang<sup>c</sup>, and Jie Zheng<sup>a\*</sup>

<sup>a</sup> *Department of Chemical and Biomolecular Engineering, The University of Akron, Akron, Ohio 44325, USA. E-mail: [zhengj@uakron.edu](mailto:zhengj@uakron.edu)*

<sup>b</sup> *College of Polymer Science and Polymer Engineering, The University of Akron, Akron, Ohio 44325, USA*

<sup>c</sup> *R&D Center for Membrane Technology and Department of Chemical Engineering, Chung Yuan Christian University, Chung-Li, Taoyuan 320, Taiwan*

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## Experimental Section

**Nanochannel fabrication.** The PET membranes (PET, 12  $\mu\text{m}$  thick) were irradiated with single heavy ion (Au) of energy 11.4 MeV/nucleon at UNILAC linear accelerator (GSI, Darmstadt, Germany). As swift heavy ions pass through the PET film, they deposit the energy along their trajectory to create a cylindrical-like damage track. To produce a conical nanochannel, the PET film with a cylindrical damage track was embedded between the two chambers of a conductivity cell (**Scheme S2**) with a voltage of 1 V applied across the film at 25°C. One chamber was filled with an etching solution (9 M NaOH), while the other chamber was filled with a stopping solution (1 M KCl and 1 M HCOOH) for neutralizing the etchants and slowing down the further etching process once the channel opens. The large opening of the conical nanochannel, named as a base diameter, was determined by scanning electron microscopy (Quanta 200, FEI Corporation, USA). The base diameter was  $\sim 1000$  nm (**Fig. S1**). The small opening of the channel (i.e. tip diameter) was estimated by the conductance method via the following equation<sup>[1]</sup>,  $d_{tip} = 4LI/\pi k(c)UD$ , where  $L$  is the length of the channel,  $I$  and  $U$  is the measured current and the applied voltage, respectively,  $d_{tip}$  is the tip diameter,  $D$  is the base diameter,  $k(c)$  is the special conductivity of the electrolyte. The tip diameter is  $\sim 40$ -60 nm. After the etching process, negatively charged COOH groups were created on the interior surface of the conical channel.

The ionic current flow during the formation of the single conical nanochannel upon chemical etching was monitored. Before the etching process to open the channel ( $\sim 3$  h), no current flow was observed. After 3 h, the nanochannel started to be opened, and the etchants in one chamber were neutralized by the stopping solution in the other chamber, which would slow down and further terminate the etching of the nanochannel, resulting in the current flow to be maintained at  $\sim 1.8$  nA. Then, both chambers in a conductivity cell were filled with dilute etching solution (1 M NaOH) to initiate a slow etching. As a result, the ionic current increased linearly to 20 nA as time for 1.5 h. Use of dilute etching solution (1 M NaOH),

rather than 9 M NaOH as reported in literature<sup>[2]</sup>, is able to easily control not only the size of the nanochannel, but also the smooth interior surface of the channel. The PET film was soaked in MilliQ water (18.2 MΩ) overnight to remove residual salts. Finally, a stable ionic current of ~2.5 nA flowing through the nanochannel was achieved in the presence of PBS buffer (10 mM, 7.4) as electrolyte.

**Nanochannel functionalization.** The terminal carboxylic acid groups of the nanochannel were activated using carbodiimide chemistry prior to apo-Tf immobilization. The COOH-coated nanochannel was incubated in a freshly prepared activation buffer (15mg EDC and 3 mg NHS per milliliter) for 1 hour, followed by 30 min wash steps with DI water. The resulting activated nanochannel was then exposed to an aqueous solution of apo-Tf (2 mg/ml) overnight. After apo-Tf immobilization, the nanochannel was washed by DI water several times before use.

**Current measurement.** Ionic current was measured by a Keithley 6487 picoammeter (Keithley Instruments, Cleveland, OH). A single conical shaped PET membrane was mounted between two chambers of an etching cell (**Scheme S2**). Ag/AgCl electrodes were used to apply a transmembrane potential across the nanochannel film. Forward voltage was applied on the base opening side of the nanochannel. 10 mM PBS buffer was chosen as electrolyte. Scanning voltage was varied from -2 to +2 V with a 40 s period. Each test was repeated 5 times to obtain an average current value at different voltages and 25 °C.

**Surface plasmon resonance (SPR) measurement.** An in-house SPR sensor equipped with a four-channel flow cell, temperature control, and a peristaltic pump for sample delivery is built based on the attenuated total reflection method and wavelength modulation. A SPR chip was made of a glass slide coated with an adhesive titanium film (~2 nm) and a gold film (~48 nm) using an electron beam evaporator. The SPR chip was first attached to the base of the prism. Optical contact between two surfaces was realized using a refractive index matching fluid (Cargille). A pre-adsorptive baseline was first established by flowing degassed water through

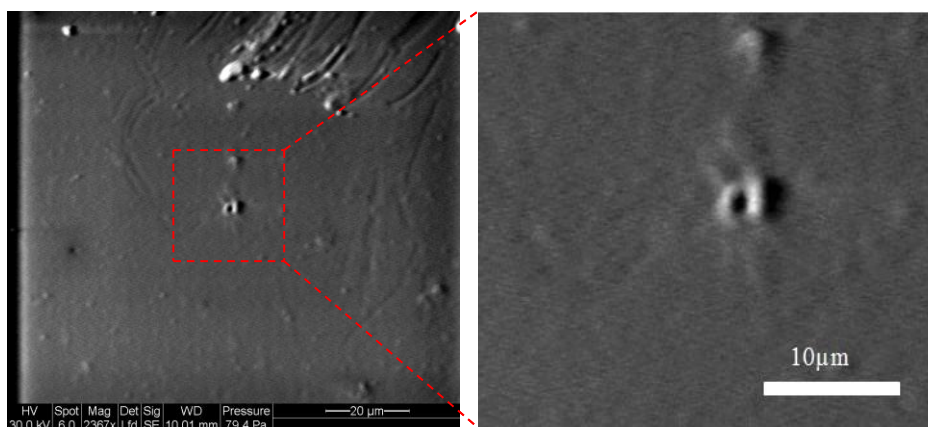
the sensor until the baseline became stable. Sample solutions were then delivered through the channels using a peristaltic pump. A flow rate of 0.05 mL/min was used for all experiments. Tf and Fe<sup>3+</sup> adsorption was finally quantified by measuring wavelength shift between the pre-adsorptive and post-adsorptive baselines.

**Iron-responsive nanochannel.** Ionic current through the apo-Tf functionalized nanochannel was measured for comparison before and after the nanochannel being soaked in 0.75 mM FeCl<sub>3</sub>, 0.75 mM NaCl, and 10 mM PBS (pH 7.4) for 1 h, respectively. Figure S2 showed that as compared to untreated nanochannel, the FeCl<sub>3</sub>-treated nanochannel significantly reduce the ionic current. However, the NaCl-treated or PBS-treated nanochannel remained almost the same ionic current as the untreated nanochannel. These results clearly demonstrate the specific and selective binding of Fe<sup>3+</sup>, not Na<sup>+</sup> and K<sup>+</sup>, to the apo-Tf coated nanochannel.

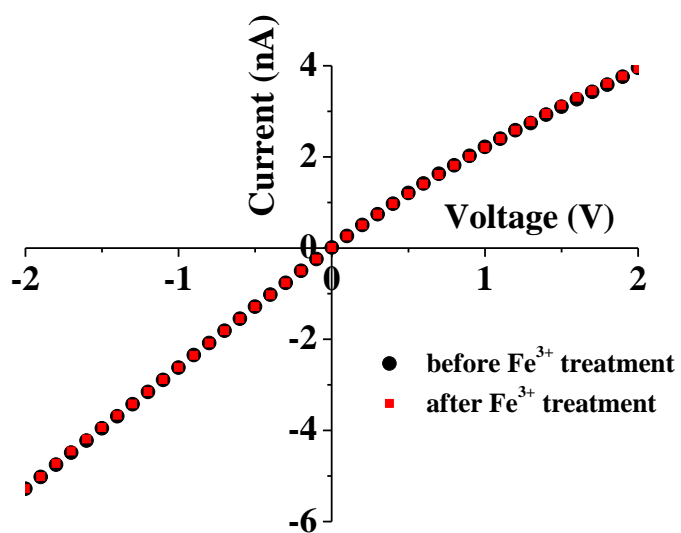
**SAM preparation on SPR chips.** Prior to the formation of 11-mercaptoundecanoic acid (HS(CH<sub>2</sub>)<sub>11</sub>COOH) SAM on gold coated SPR chips, the bare gold substrates were washed with water and ethanol, cleaned with a UV-ozone cleaner for 20 min, washed with water and ethanol again, and then dried under a stream of filtered air. The COOH-SAMs (Sigma-Aldrich Chemical Co.) were then formed by soaking the cleaned gold substrates in a 1 mM ethanol solution of HS(CH<sub>2</sub>)<sub>11</sub>COOH containing 2% (V/V) CF<sub>3</sub>COOH overnight. Then, the COOH-SAM coated chips were rinsed with an ethanol solution of NH<sub>4</sub>OH (10%) and CH<sub>3</sub>COOH (10%), respectively. This step was followed by washing with ethanol and deionized water and drying with compressed air.

**Fluorescent measurement.** Absorption fluorescence spectra were recorded by a LS55 spectrofluorimeter (PerkinElmer, USA) in a 2 mm path length quartz cell at room temperature. The fluorescence spectra of the apo-Tf (0.05 mg/ml) in water, NaCl solution (0.75 mM), PBS buffer, and FeCl<sub>3</sub> solution (0.075 mM, 0.15 mM, and 0.75 mM) were measured, respectively. Intrinsic fluorescence spectra were recorded between 310 and 400 nm with excitation wavelength of 295 nm to avoid the contribution from tyrosine residues.

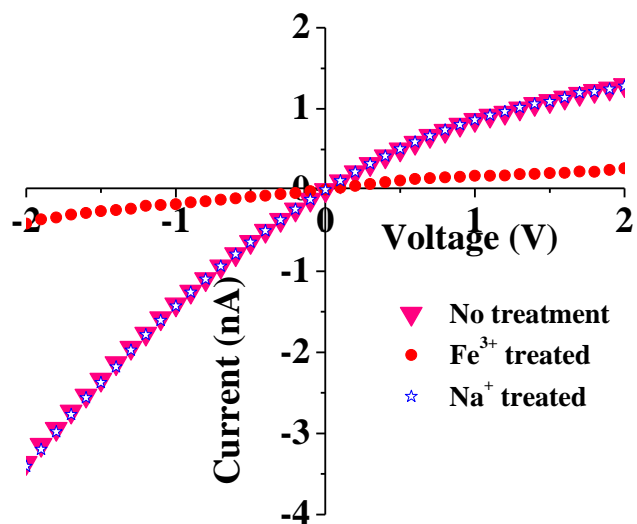
**Contact angle (CA) measurement.** Contact angle was measured using a sessile drop technique on a Rame-Hartgoniometer (Model 100-00, Mountain Lake, NJ) at ambient temperature. Before the CA test, the sample was dried by N<sub>2</sub>. Then, DI water drop of 2 μL was carefully dispensed onto the sample surface. For each sample, CA values were averaged by three readings from each of five different positions of the same sample.



**Figure S1.** SEM image of the base side of the nanochannel after chemical etching by NaOH (9M)

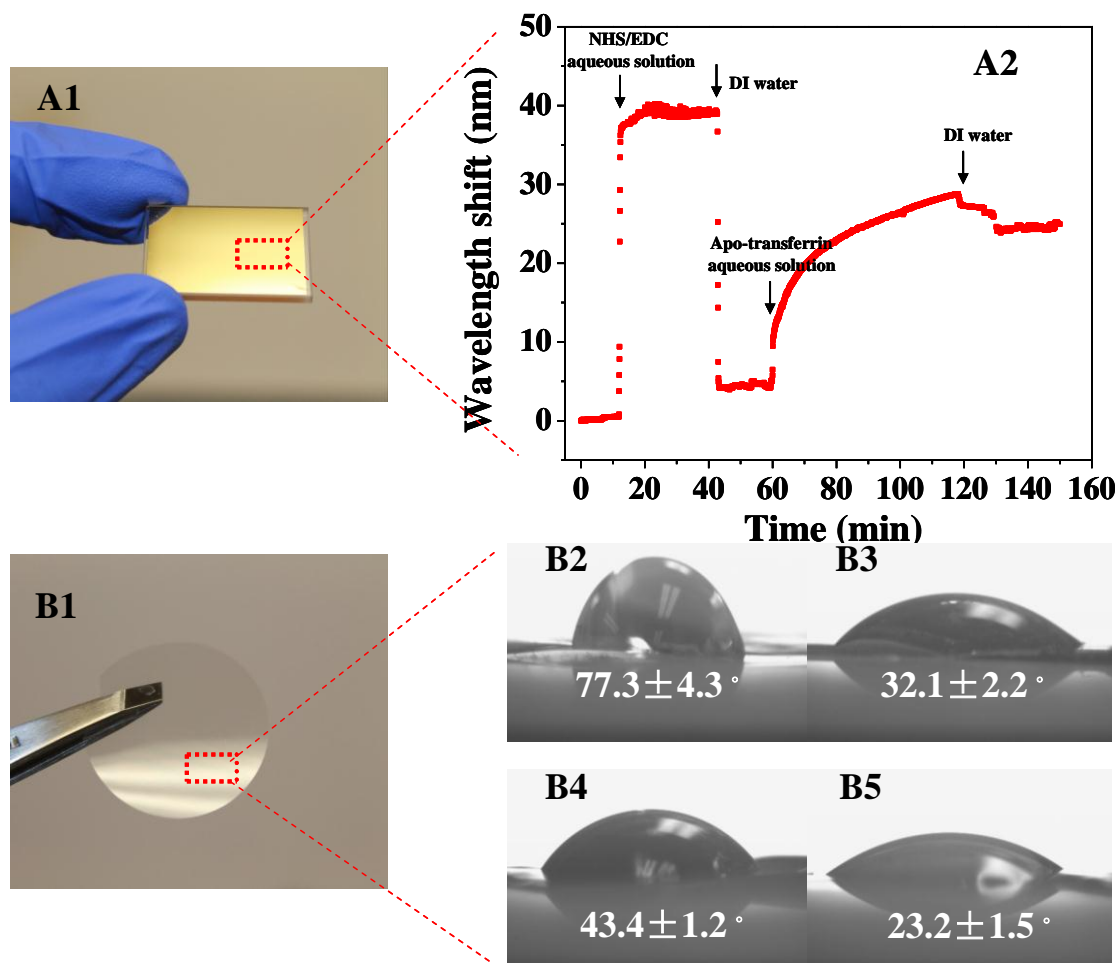


**Figure S2.** Comparison of current-voltage (I-V) curves between the single Nanochannel-I before (black) and after 0.75 mM FeCl<sub>3</sub> (red) treatment. The two well-overlapped current curves indicate that (i) no current signal changes before and after Fe<sup>3+</sup> treatment of Nanochannel-I and (ii) Fe<sup>3+</sup> ions do not bind to the COOH-coated Nanochannel-I.

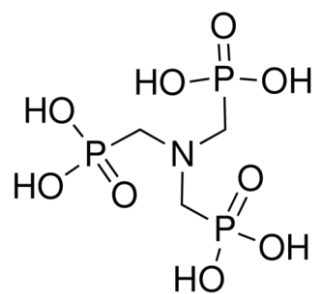


**Figure S3.** Current-voltage (I-V) properties of the single apo-Tf functionalized nanochannels (Nanochannel-II) before (pink) and after 0.75 mM FeCl<sub>3</sub> (red) or 0.75 mM NaCl (blue) treatment.

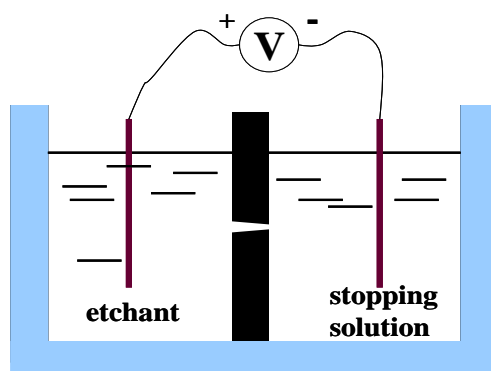




**Figure S4.** (A1) A SPR chip and (A2) SPR sensorgrams comprising NHS/EDC activation of COOH-SAM, DI water washout, covalent attachment of apo-Tf onto the activated COOH-SAM surface, and DI water washout. (B1) A PET film and contact angle of (B2) original PET film, and PET film (B3) etched by NaOH (9 mM) solution, (B4) followed by NHS/EDC activation, and (B5) incubation in apo-Tf (2 mg/mL) solution.



**Scheme S1.** Molecular structure of nitrilotri(methylphosphonic acid) (NTP) used as an exchange ligand to remove  $\text{Fe}^{3+}$  from Tf.



**Scheme S2.** Schematic representation of an electrochemical cell used for the etching process and current measurements.

**References:**

- [1] X. Hou, W. Guo, F. Xia, F.-Q. Nie, H. Dong, Y. Tian, L. Wen, L. Wang, L. Cao, Y. Yang, J. Xue, Y. Song, Y. Wang, D. Liu, L. Jiang, *Journal of the American Chemical Society* 2009, 131, 7800.
- [2] X. Hou, H. Dong, D. Zhu, L. Jiang, *Small* 2010, 6, 361.