Supplementary Information

Nanotextured SiNW Forest-based On-Chip Biomolecular Filtering, Separation and Pre-concentration Devices: Nanowires Do it All

Vadim Krevitsky, Lo-Chang Hsiung, Amir Lichtenstein, Boris Brudnik, Raisa Kantaev, Roey Elnathan, Alexander Pevzner, Artium Khatchtourints, and Fernando Patolsky

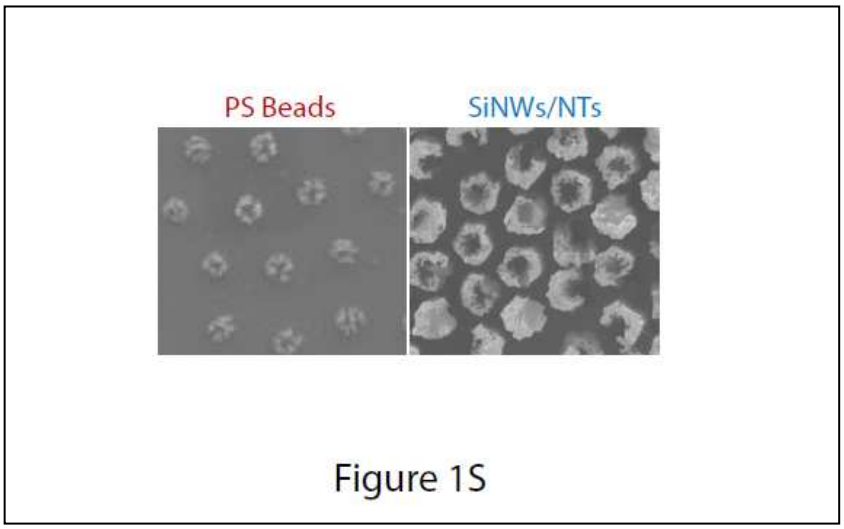

Figure 1S. (left image) Extensively etched holey PS beads elements and (right image) resulting highly rough and porous hollow SiNW elements after the Ag-assisted etching step.

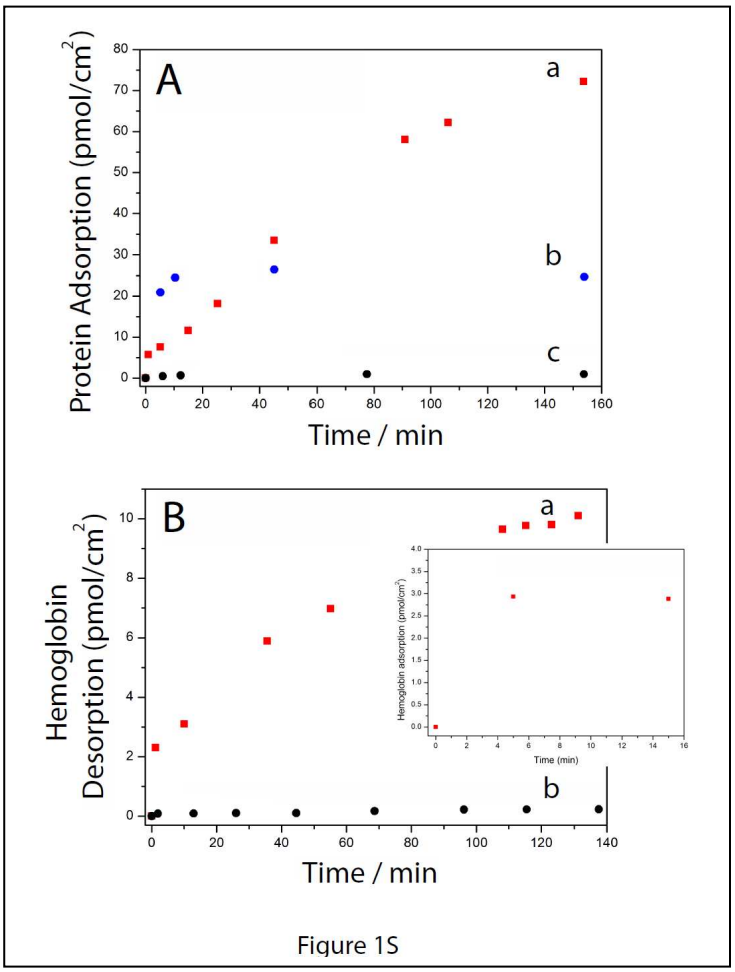

Figure 2S. (A) Results for the simultaneous adsorption kinetics of two proteins on a SiNW-forest capturing device modified with two antibody receptors against eGFP and Hemoglobin from blood samples. Adsorption of Hemoglobin, curve a, and eGFP, curve b. Adsorption of Hemoglobin on a anti-hemoglobin modified flat silicon surface of identical geometrical surface area as in cuves a and b.

(B) Desorption kinetics of hemoglobin from an anti-hemoglobin modified SiNW-forest capturing device, curve a, and desorption of hemoglobin from a similarly modified flat silicon surface of identical geometrical surface area, curve b.

Materials and Methods Section

1. Silicon nanowire forests (SiNWFs) fabrication and antibody immobilization

Wafers cleaning

1. Immerse the wafer in acetone 20 ml, sonicate for 2 min and dry with N2
2. Immerse the wafer in isopropanol 20 ml, sonicate for 2 min and dry with N2
3. Immerse the wafer in fresh piranha solution for 4 min, wash the wafer with DI water and dry with N2.

Preparation of 1% Polystyrene beads suspension

4. Take 0.385ml of 2.6 % Polystyrene beads suspension (0.5μm, Polyscience) and centrifuge it at 4500 rpm for 16 min.
5. Separate the Polystyrene beads carefully with pipette Pasteur from water and add 1ml of methanol and 0.3 mg tween 80 on Polystyrene beads (3% mass percent of tween 80 on Polystyrene beads mass). The solution has: 0.3 mg tween 80, 10 mg Polystyrene beads and 1 ml methanol. Use only fresh.
6. Disperse suspension with “Vortex", do not use ultrasonic disperser, that leads to Polystyrene beads destruction and their agglomeration.

Deposition of the Polystyrene beads

7. Take the needed amount (18-20 μl for wafer 2x2 cm^2, 200 ul for wafer 4x4 cm^2) of a well shacked suspension with pipettor, drip it slowly from a low height in the center of the wafer, and wait for a full spreading of the suspension (1-2 seconds) and start spin coating, adjust the spin rate and time according to wafer dimension (for 2X2 cm^2 1 min at 475 rpm. for 4X4 cm^2 2 minutes at 180 rpm).

Polystyrene beads plasma etching (PECVD)

8. Conditions: O_2=50sccm, P=40mtorr, Bias=15-50w, time=3-15 min (for ø ~200-300nm SiNWs). For a good quality take for one batch 4 wafers only in the center of the chamber.

e-beam Ag-film deposition

9. Thickness 45 nm, deposition rate 1 A/sec

Silicon wafer wet etching

10. Etching solution: HF =4.6M, H_2O_2=0.44M in H_2O. After 9 min etching getting SiNWs with a length of 3 microns.
11. Rinse the wafer with DI water.

Ag-wet etching

12. Drip 2-3 drops of HNO_3 (69%) to wafer, wait for 10 min and rinse it with DI water. Rinse the wafer with IPA and dry at 100°C

Polystyrene beads finishing plasma etching

13. Plasma conditions: P (O_2) = 0.2 torr, t=10min, bias =100w, put the wafer directly on aluminum chamber. (300 nm Polystyrene beads diameter).

Characterization of Silicon Nanowires

The as-synthesized silicon nanowire forests were characterized by scanning electron microscopy (FEI Quanta 200 FEG Environmental SEM) and transmission electron microscopy (TEM, FEG-FEI Tecnai 20).

The surface area was determined using an Autosorb-1 (Quantachrome) volumetric analyzer at 77 K using a liquid nitrogen bath. The samples were degassed overnight before the measurements. Ultra-high purity (99.9999% purity) grade of N_2 and He gases were used for the adsorption experiments. The surface areas were determined by using the data under the relative pressures before the capillary condensation. The pore size distributions were calculated from the adsorption data by the Barret-Joyner-Halenda method.

Antibody immobilization on SSiNW wafer

14. Clean with acetone, DI water and IPA. Dry gently with N_2
15. Plasma 30 min, 100W, 0.200 torr O_2.
16. APDMES modification: 100% APDMES, 50 ^{0}C, 3 hours.
17. Wash with IPA
18. Dehydration: 115 ^{0}C, 30 min.
19. Dialysis: 100 ug of anti-cTnT in 200 ul (500ug/ml) of phosphate buffer (PB) (10 mM, pH=8.5), 5 hours change solution every 20 min, dialysis volume 50 ml.

20. glutaraldehyde modification: 8.3% glutaraldehyde containing 12 mM sodium cyanoborohydride, 3 hours.
21. wash with: DI, acetone, IPA and DI again.
22. Ab modification: Anti-cTnT (from dialysis) in PB (100 ug), containing 12 mM sodium cyanoborohydride, at 4 ^{0}C, for overnight on SSiNW wafer.
23. Blocking: ethanolamine (100 mM) containing 12 mM sodium cyanoborohydride in PB (pH8.67), for 3 hours under shaking ~ 30 RPM
24. Wash with PB.

2. Nanowire FET Fabrication

SiNW-FET devices were fabricated by photolithography. Briefly, source and drain electrodes were deposited with the use of a multilayer photoresist structure consisting of 500 nm LOR5A (Microchem) and 500 nm 1805 (Shipley). After exposure and development of the electrode patterns, the contacts were metallized by e-beam and thermal evaporation of Ni (60 nm) respectively, and were then passivated with an insulating layer of 20 nm alumina (ALD deposition using a Cambridge Nanotech Savannah 200 system) and a layer of Si_3N_4 (60 nm-thick) deposited by plasma-enhanced chemical vapor deposition at 80C^o (ICP-PECVD, Axic Inc.). The separation between the source and drain electrodes for each FET was 2 μm.

3. Fluid-Delivery System

The fluid-delivery system was fabricated from flexible polydimethylsiloxane (PDMS) elastomer mixed in a 10:1 ratio with base as curing agent. The PDMS was cured overnight in an oven at 60ºC and then cut into rectangular pieces. The dimensions of the PDMS were 10 × 10 × 5 mm.

4. Data Acquisition, Electrical Setup and Sensing

The basic electrical properties of the SiNW devices on the sensor chip were first characterized in air as this provides means for quality control before completion of the sensor structure. Then, the sensor device chip was integrated with a custom-made PDMS microfluidic channel and wire bonded to the outside conductive pads for the electrical measurements. The conductance of the SiNW-FET was measured by a DC custom-made electronic board of 64 measuring channels. The

drain current was amplified and filtered. The action of injecting the solution might introduce some noise into the electrical read-out signal. All studies were carried out at room temperature.

5. Nanowire-FET device surface modification

1. Wash the chip with acetone, DI water and IPA.
2. Plasma 30 min, 100W, 0.200 torr O_2.
3. APDMES modification: 100% APDMES, 50 ^{0}C, 30 min.
4. Wash with IPA.
5. Dehydration: 115 ^{0}C, 25 min.
6. Dialysis: 40 ug of anti-cTnT in 200ul (200ug/ml) in PB (10 mM, pH=8.5)
7. glutaraldehyde modification: 8.3% glutaraldehyde containing 12 mM sodium cyanoborohydride, reaction time 30 min, under shaking 30 rpm, cover from light during the reaction.
8. Chip wire-bonding.
9. Assemble the PDMS channel.
10. Ab modification: Anti-cTnT in PB (16 μg/ml), containing 12 mM sodium cyanoborohydride, at 4 ^{0}C, flow at 2~2.4 μl/min for overnight.
11. Blocking: ethanolamine (100 mM) containing 12 mM sodium cyanoborohydride (pH8.67), flow at 5 μl/min for 3 hours.
12. Flow sensing buffer (SB) (10 μM phosphate buffer pH=8.00) at 5 μl/min for 30 min to wash the channel, then do transconductance test.

6. Protein capturing and sensing

1. Incubation: incubate 400 fmol cTnT in 200 μl blood (i.e. 2 nM) the NWs forest capturing wafer, immobilized with anti-cTnT for a period of 5 minutes.
2. Washing: 30 seconds with PBS via squeeze bottle (~30 ml) (flow of 500μl/min), wash the capturing forest with flow of 10 seconds SB (desalting step).
3. Elution: incubate NW forest in circulating flow of 400 μl Sensing Buffer (150μM PB).
4. Sensing: flow the eluted solution from the wafer into sensing system. V_g=-0.1 v, V_{ds}=0.1v, flow rate=5μl/min.

7. Hemoglobin experiment

Specific adsorption

1. Block the surface sites that non-specifically adsorbed proteins on the wafer with 0.06 g/ml milk powder in PBS buffer pH=7.45 for 1 hour at room temperature.
2. Washing: 30 sec with PBS via squeeze bottle (~30 ml), wash the wafer with 30 ml PBS in petri dish 10 min on shaking plate (30 rpm) and wash 20 sec with PBS via squeeze bottle.
3. Measure the amount of Hemoglobin adsorption by the wafer via spectrophotometer (absorption in 406 nm) from a blood like solution: PBS 155 [mM] pH 7.45, BSA 85 [mg/ml] and the initial Hemoglobin concentration in the experiment 0.063 [mg/ml] in 0.5 [ml] on
4. 4 cm^2 SSiNW surface on the wafer.

Non-specific adsorption

5. Block the surface sites that un-specifically adsorbed proteins on the wafer with 0.06 g/ml milk powder in PBS buffer pH=7.45 for 1 hour at room temperature.
6. Washing: 30 sec with PBS via squeeze bottle (~30 ml), wash the wafer with 30 ml PBS in petri dish 10 min on shaking plate (30 rpm) and wash 20 sec with PBS via squeeze bottle.
7. Measure the amount of Hemoglobin adsorption by the wafer via spectrophotometer (absorption in 406 nm) from blood like solution: PBS 155 [mM] pH 7.45, BSA 85 [mg/ml] and the initial Hemoglobin concentration in the experiment 0.074 [mg/ml] in 3.5 [ml] on 4 cm^2 SSiNW surface on the wafer.

Desorption

8. Block the surface sites that non-specifically adsorbed proteins on the wafer with 0.06 g/ml milk powder in PBS buffer pH=7.45 for 1 hour at room temperature.
9. Washing: 30 sec with PBS via squeeze bottle (~30 ml), wash the wafer with 30 ml PBS in petri dish 10 min on shaking plate (30 rpm) and wash 20 sec with PBS via squeeze bottle.
10. 2.5 hours of Hemoglobin adsorption from blood like solution: PBS 155 [mM] pH 7.45, BSA 85 [mg/ml], 0.063 [mg/ml] human Hemoglobin in 3.5 [ml] on 4 cm^2 wafer.
11. Washing: 30 sec with PBS via squeeze bottle (~30 ml), wash the wafer with 30 ml PBS in

petri dish 10 min on shaking plate (30 rpm) and wash 20 sec with PBS via squeeze bottle.

12. Slots elimination: Putting the wafer in DI solution for 3 sec.
13. Hemoglobin elution from silicon wafer into 3.5 ml of DI water on the wafer.
14. Measure the amount of Hemoglobin desorption by the wafer via spectrophotometer (absorption in 406 nm) to DI water on the wafer.

8. eGFP experiment

Specific adsorption

1. Block the surface sites that non-specifically adsorbed proteins on the wafer with 0.06 g/ml milk powder in PBS buffer pH=7.45 for 1 hour at room temperature.
2. Washing: 30 sec with PBS via squeeze bottle (~30 ml), wash the wafer with 30 ml PBS in petri dish 10 min on shaking (30 rpm) plate and wash 20 sec with PBS via squeeze bottle.
3. Measure the amount of Hemoglobin adsorption by the wafer via spectrophotometer (absorption in 488nm) from blood like solution: PBS 155 [mM] pH 7.45, BSA 85 [mg/ml] and the initial eGFP concentration in the experiment 0.017 [mg/ml] in 0.5 [ml] on 4cm^2 SSiNW surface on the wafer.
4.

Desorption

5. Block the surface sites that non-specifically adsorbed proteins on the wafer with 0.06 g/ml milk powder in PBS buffer pH=7.45 for 1 hour at room temperature.
6. Washing: 30 sec with PBS via squeeze bottle (~30 ml), wash the wafer with 30 ml PBS in petri dish 10 min on shaking plate (30 rpm) and wash 20 sec with PBS via squeeze bottle.
7. 30 min of eGFP adsorption from: blood like solution:PBS 155 [mM] pH 7.45, BSA 85 [mg/ml], 0.017 [mg/ml] eGFP in 3.5 [ml] on 4cm^2 wafer. 5.3.2. Blood with Trisodium citrate [concentration], 1 ml, 0.06 eGFP [mg/ml].
8. Washing: 30 sec with PBS via squeeze bottle (~30 ml), wash the wafer with 30 ml PBS in petri dish 10 min on shaking plate (30 rpm) and wash 20 sec with PBS via squeeze bottle.
9. Slots elimination: Putting the wafer in DI solution for 3 sec.
10. eGFP elution from silicon wafer into 3.5 ml of DI water on the wafer.