Supplementary Information

Fluorescence Microscopy Apparatus Assembly. A custom apparatus was developed to perform fluorescence microscopy imaging of the electrophoretic transport of analytes in the micro- and nanochannels. The fabrication process flow is presented in Figure S1. After wire bonding, the nDS membrane was placed on a soft silicone rubber film with its Pyrex side facing downward, surrounded by a polyethylene ring (Figure S1-A). An optical UV glue (68, Norland Optical Adhesive) was then used to seal the electrical contacts and to create the source reservoir by gluing the ring to the membrane (Figure S1-B). After curing the glue by UV exposure, the silicone rubber film was peeled off. Next the source reservoir was placed on top of a coverslip spacer (thickness 150 μm), located on top of a glass microscope coverslip. Optical glue (68, Norland Optical Adhesive) was then added and cured, bonding the source reservoir to the bottom coverslip (Figure S1-C). Finally, the spacer was removed and a larger ring was glued around the assembly to create the sink reservoir (Figure S1-D).

![Figure S1. Schematic of the fluorescence microscopy imaging apparatus and process flow of the assembly procedure. In the figure, ir is the ring forming the source reservoir, s is the silicone rubber film, g1 and g2 represent the optical UV-glue, sl is the spacer, cs is the glass microscope coverslip and or is the outer ring forming the sink reservoir.](image)
V-I Characteristics

The characteristic transmembrane I-V behavior (current vs. time at under sequential voltage steps) for the nanochannel membrane in PBS solution (pH 7.4) was measured. Two electrical leads were attached to the membrane electrodes and contact between electrical lead and metallic membrane surface was sealed and insulated with epoxy (Epoxy Technology, H20E parts A and B). After immersion in PBS solution, a series of 0.5 VDC steps was applied to the membrane through a stabilized power supply (Agilent Technologies, E3643A) with a controlled time interval of 60 minutes/voltage step. A 6$rac{1}{2}$ digit multimeter (Agilent Technologies, 34410A) was placed in series with the membrane in order to acquire current measurements. Both instrument control and data acquisition was remotely controlled in real-time by National Instruments LabVIEW to provide for seamless integration of all working parts. The collected data can be seen in Figure S2.

![Current vs. time for a series of voltage steps for the nDS membrane in PBS solution at pH 7.4](image_url)
**Heparin Release Experiment**

In order to perform the release test the nDS membrane was wired and assembled into a testing device made of UV transparent cuvettes. Figure S3 shows the picture of the testing fixture.

![Release testing device](image)

**Figure S3. Release testing device.**

**Wire Bonding.** The nDS2 membranes were wired by using a solids silver-filled conductive epoxy (EPO-TEK® H20E), in a Part A : Part B ratio equal to 1:1. Wires (36 AWG) were bonded to the membrane electrodes by means of small drops of epoxy applied to the stripped extremity of the conductor. During the process it was verified that no conductive epoxy was in touch with the diced sides of the membrane. The epoxy was cured for 15 minutes on a hot plate at 120 °C and for approximately 1 hour in an oven at 110 °C. Finally, the wires were insulated with a thermal epoxy (EPO-TEK® H74) in a Part A : Part B ratio equal to 100:3 and also cured for 15 minutes on a hot plate at 120 °C.

**Testing Device Preparation.** Two UV transparent cuvettes (BrandTech Scientific, Inc, Essex, CT) were cut to 18 mm in height to create the sink and source reservoir. A hole of 4 mm was drilled to one of the non-transparent sides of sink cuvette. The source cuvette was cut in half in the y-plane. Then, the wired nDS membrane was aligned and bonded to the hole of the sink cuvette using a UV epoxy (EPO-TEK® OG116-31). The procedure was carefully performed so that no
epoxy was added on top of the membrane, and any gap was fully insulated. The source cuvette was attached by melting it to the sink cuvette using a soldering iron. Then, it was completely sealed around using the UV epoxy EPO-TEK® OG116-31. One Teflon-coated micro stir bar was placed into the sink reservoir to ensure solution homogeneity by magnetic stirring. During the test, the two cuvettes were capped and sealed to prevent evaporation.

**Wetting Procedure.** The sink reservoir was filled with isopropyl alcohol by piercing the cap with a needle (25 gauge) for the air to escape and using a syringe with a needle for loading. This step prevented air bubbles from remaining at the membrane outlet. After the membrane was wetted, the source reservoir was filled with IPA using the two needle technique described above. IPA was then replaced with Millipore water (exchanged 2 times every 4 hours) which was finally substituted with PBS and left in the sink and source reservoirs overnight.

**Release Test.** A solution of Heparin (Sigma-Aldrich) was prepared in PBS (pH 7.2, GIBCO) at a concentration of 42 mg/mL. 1.328 mL of fresh PBS was loaded into the sink reservoir and 0.450 mL of solution was loaded into the source reservoir. The testing device was placed on a magnetic stirring plate and covered to prevent light from affecting the sample. First, no voltage was applied to the membrane electrodes and concentration-driven release data was collected for approximately 48 hours. Then, 1.8 VDC were applied, followed by a second voltage increase to 3.0 VDC. The absorbance of the solution in the sink reservoir was measured at several time points using a UV-Vis Spectrophotometer (DU 730, Beckman Coulter, Inc) at a peak wavelength of 230 nm. Absorbance data was normalized and the corresponding source concentration determined using an absorbance-concentration standard curve. Figure S4 shows the results of the heparin release.
**Figure S4. Heparin Release Test Results.**

**Transport Schematic within the Channels.** The electrophoretic transport of FITC-BSA occurs simultaneously with an electroosmotic transport of fluid in the opposite as illustrated in Figure S5.

**Figure S5. Schematic illustration of the balance between electroosmosis and electrophoresis for FITC-BSA**