

Supporting Information

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Nanopipette Delivery: Influence of Surface Charge

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SI-1. Chemicals and materials

All chemicals were used as received without further purification. (ferrocenylmethyl) trimethylammonium iodide (Strem Chemicals, Newburyport, MA) was converted to hexafluorophosphate salt by metathesis reaction, and was dried and stored at room temperature before use. Potassium ferricyanide (Strem Chemicals, Newburyport, MA) and the hexafluorophosphate salt of (ferrocenemethyl) trimethylammnium were dissolved in supporting electrolyte and the ionic form of those two species, FcTMA^+ and $\text{Fe}(\text{CN})_6^{3-}$ were used as electroactive species in cyclic voltammetry (CV) measurements and amperometric I-T curve measurements. Solutions of potassium chloride (Macron Chemicals, Phillipsburg, NJ) were filtered with 0.22 μm PVDF filter membranes (Millipore Corp., Danvers, MA) and utilized as the supporting electrolyte. (Electrolyte used were: 50 mM KCl, pH = 8.0; 100 mM KCl, pH = 8.0; 100 mM KCl, pH = 5.5; 500 mM KCl, pH = 8.0). Potassium dihydrogen phosphate (J. T. Baker, Phillipsburg, NJ) and potassium dibasic phosphate (J. T. Baker, Phillipsburg, NJ) were used for pH adjustment. Fluorescein sodium salt (Sigma-Aldrich, St. Louis, MO) was used in fluorescence microscopy imaging experiments. Branched polyethylenimine (branched PEI) (Sigma-Aldrich, St. Louis, MO) was used for nanopipette surface modification. Dicholoro-[2.2]-paracyclophane (parylene C) (SCS coating, Indianapolis, IN) was used as the initial dimer for parylene deposition and A-174 silane (SCS Coatings, Indianapolis, IN) was used as the polymer adhesion promoter. All aqueous solutions were prepared from Milli-Q water (resistivity = 18 $\text{M}\Omega\cdot\text{cm}$, Millipore Corp.). In CV measurements and amperometric I-T curve measurements, a Ag/AgCl reference electrode in 3M

KCl and a platinum wire counter electrode were used. In the current-voltage (I-V) measurement, two Ag/AgCl electrodes were used, one was used as a working electrode (WE) and the other one served as a reference electrode (RE).

SI-2. Nanopipette Preparation

Nanopipettes were obtained by pulling quartz capillaries (1.0 mm O.D., 0.7 mm I.D., 7.5 cm length, Sutter Instruments, Novato, CA) with a P-2000 laser-based pipette puller (Sutter Instrument, Novato, CA). Puller program parameters are as follows, heat: 690; filament: 3; velocity: 45; delay: 165, pull: 190.

SI-3. Current-Voltage (I-V) Measurements

A two-electrode system was used to carry out the I-V measurement experiment. A Ag/AgCl electrode was back-inserted into a nanopipette filled with electrolyte solution to complete the working electrode (WE), while another Ag/AgCl electrode was placed in the bulk electrolyte to serve as the reference electrode (RE). Nanopipettes were back filled with fluorophore or redox mediators together with the supporting electrolyte while the bath solution was the supporting electrolyte only. A picoammeter/voltage source (Keithley 6487, Keithley Instruments, Cleveland, OH) was used to record the current-voltage response with respect to the pipette electrode at a step voltage of 0.1 V/s over a range of -1 V to +1 V.

SI-4. Fluorescence Microscopy Imaging

In addition to the electrical characterization, fluorescence microscopy imaging was performed to visualize ion transport in the pipette tip region. A two-electrode system was used and different potentials were applied to the working electrode (a Ag/AgCl electrode inserted into the pipette) by an external power supply with

respect to a second Ag/AgCl reference electrode immersed in the bulk solution. The applied potential was -1 V, 0 V, or +1 V. Images of the enrichment and depletion of an anionic fluorescent probe, fluorescein (200 μ m in different concentrations of supporting electrolyte KCl, 50 mM and 500 mM), were taken with an epi fluorescence microscope (Eclipse E800, Nikon, Inc) equipped with a 40 \times objective, a mercury lamp (HB10101 AF, Nikon, Inc) and a CCD camera. The images were captured with an exposure time of 800 ms and a gain of 10. Image J (NIH, USA) software was used for image analysis.

SI-5. Polyethylenimine (PEI) surface modification

Surface modification of the nanopipette was performed by the strategy described by Shao et al.³⁷ Nanopipettes were backfilled with 0.6% PEI in aqueous solution, followed by 30 min incubation in ambient conditions to allow PEI interact with the pipette surface. Finally, nanopipettes were baked at 120 $^{\circ}$ C for 2 h to remove residual water. To test whether the PEI molecules were successfully adsorbed to the surface, I-V measurements were performed and fluorescence microscopy images were acquired for the PEI-modified nanopipettes.

SI-6. Preparation of adhesion promotion solution

The adhesion promotion solution was made by adding 0.5% (v/v) of A-174 silane solution to an equal volume aliquot of isopropyl alcohol/water mixture. The promotion solution was allowed to sit in air for at least two hours before use.

SI-7. Characterization of the nanopipette-based carbon electrode

Scanning electron microscopy (SEM) was used to determine the diameter of the pipette tip opening and the thickness of the carbon ring electrode. Scanning

transmission electron microscopy (STEM) images were taken to evaluate the thickness of both the conductive PPC layer and the insulation PC layer (**Figure S1, Supporting Information**). Both CV and I-V measurements were performed to further estimate the size of the carbon ring electrode and the pipette tip opening.

Tables

Table S1. Current difference (Δi ; e.g. $|i_{1\text{ V}}| - |i_{0.5\text{ V}}|$) and charge difference (ΔQ ; e.g. $Q_{1\text{ V}} - Q_{0.5\text{ V}}$) at two adjacent delivery potentials.

	Δi (pA)	ΔQ (pC)
i or $Q_{(1\text{ V} - 0.5\text{ V})}$	12.4	68.9
i or $Q_{(1.5\text{ V} - 1\text{ V})}$	7.64	31.1
i or $Q_{(2\text{ V} - 1.5\text{ V})}$	5.07	30.1
i or $Q_{(2.5\text{ V} - 2\text{ V})}$	1.45	2.92

Figures

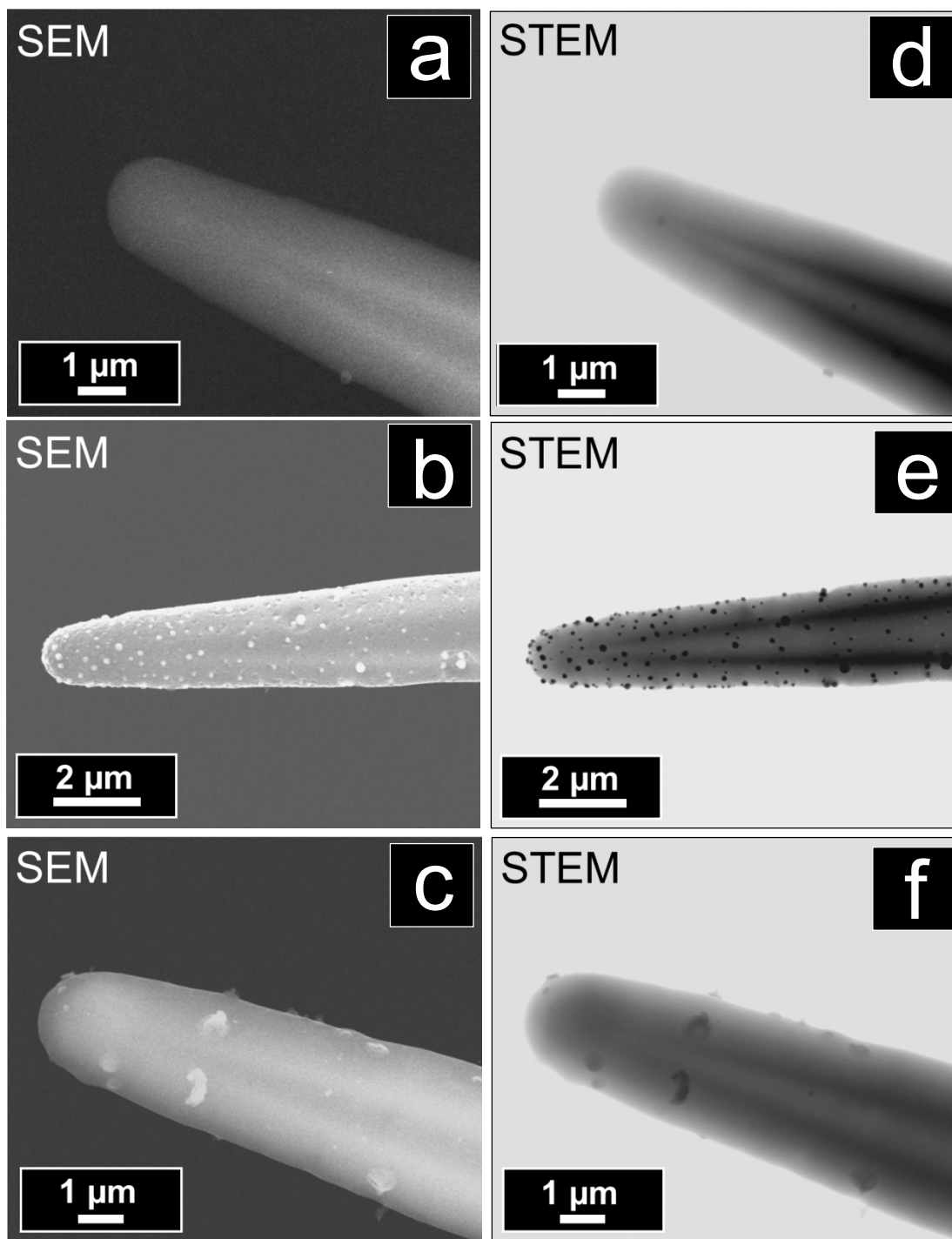


Figure S1. Scanning electron microscopy (SEM) images for (a) PC-coated nanopipette; (b) PPC-coating nanopipette; (c) PPC-coated nanopipette insulated with PC; scanning transmission

microscopy (STEM) images for (d) PC-coated nanopipette; (e) PPC-coating nanopipette; (f) PPC-coated nanopipette insulated with PC.

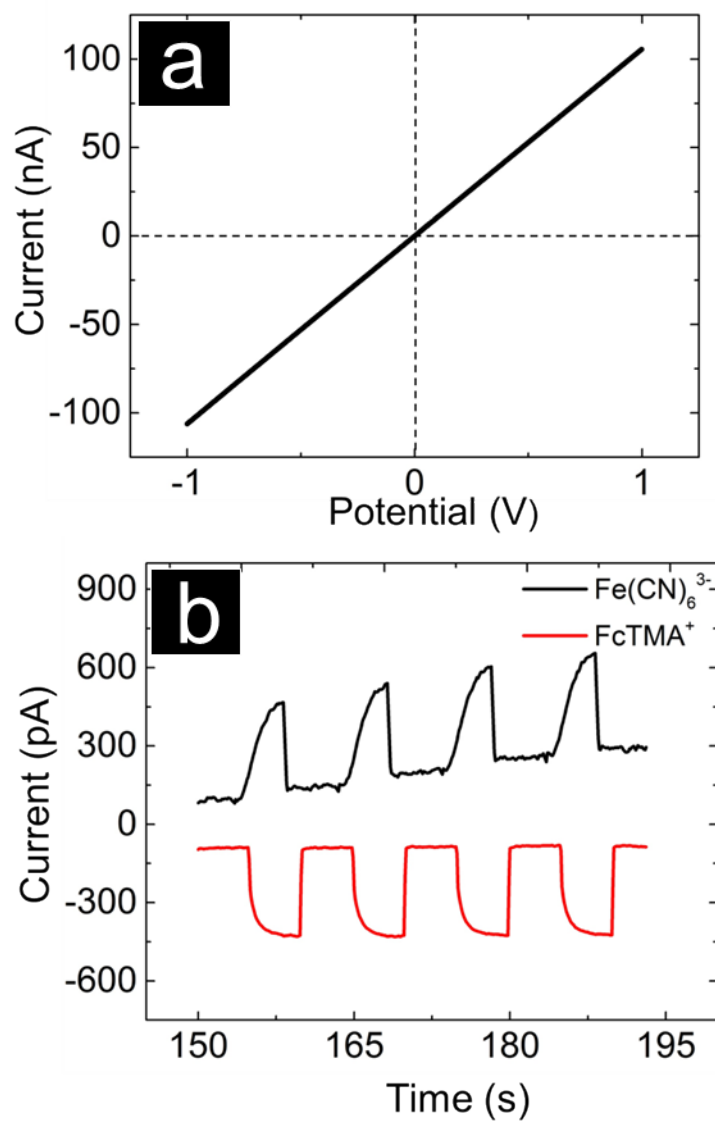


Figure S2. (a) I-V response of the carbon ring/nanopore electrode filled with 100 mM KCl with 2 mM Fe(CN)_6^{3-} and FcTMA^+ , pH = 5.5; (b) Amperometric current-time (I-T) traces collected by the carbon ring electrode for Fe(CN)_6^{3-} and FcTMA^+ .

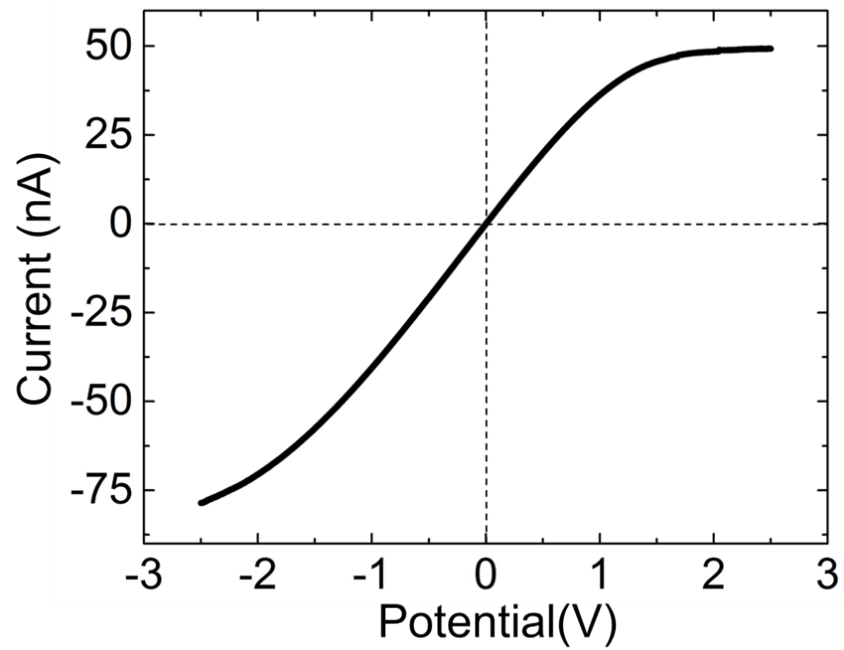


Figure S3. (a) I-V response of the carbon ring/nanopore electrode filled with 100 mM KCl together with 2 mM FcTMA⁺, pH = 8.0.

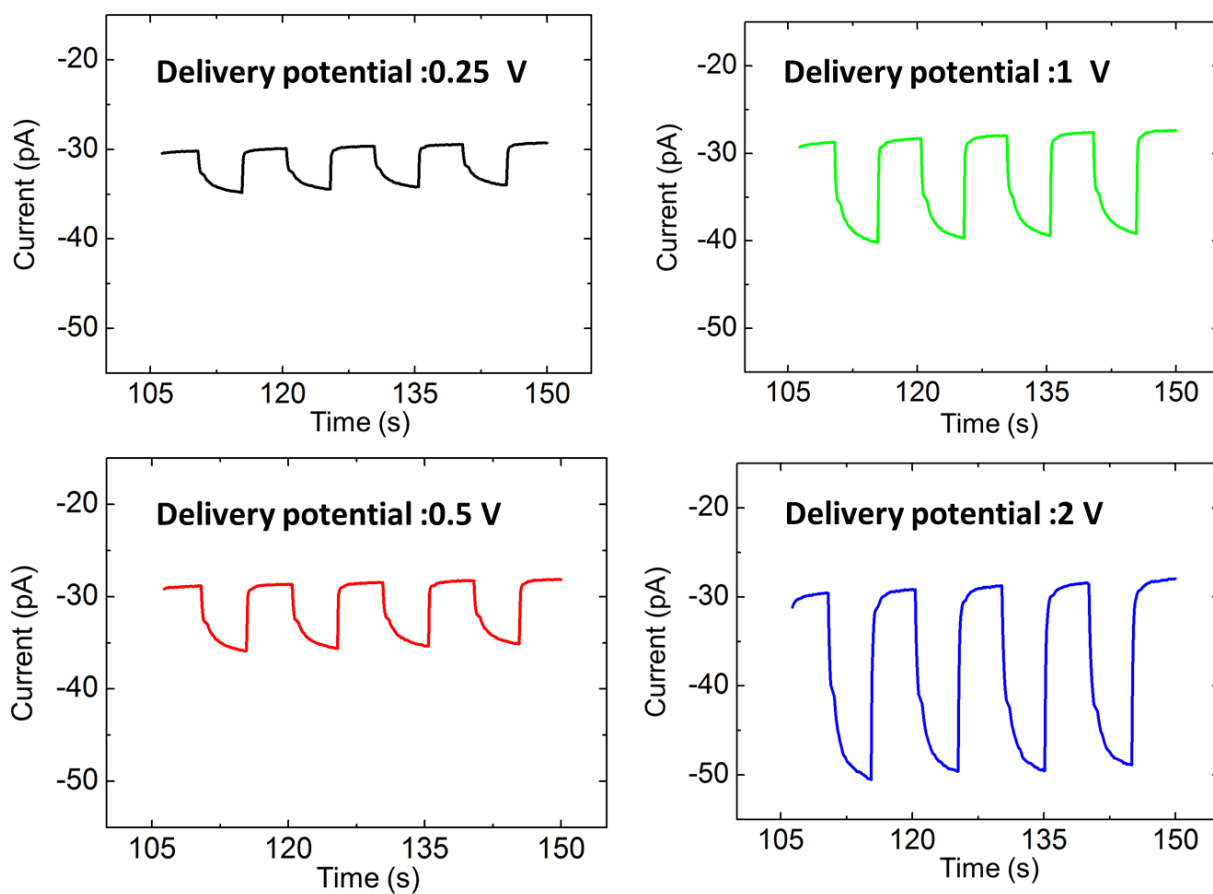


Figure S4. Amperometric I-T curves collected by the carbon ring for FcMeOH. The frequency of the square wave is kept at 0.1 Hz, the retaining potential is kept constant, $V_{\text{retaining}} = -0.5$ V, the delivery potentials used were: 0.25 V, 0.5 V, 1 V and 2 V.

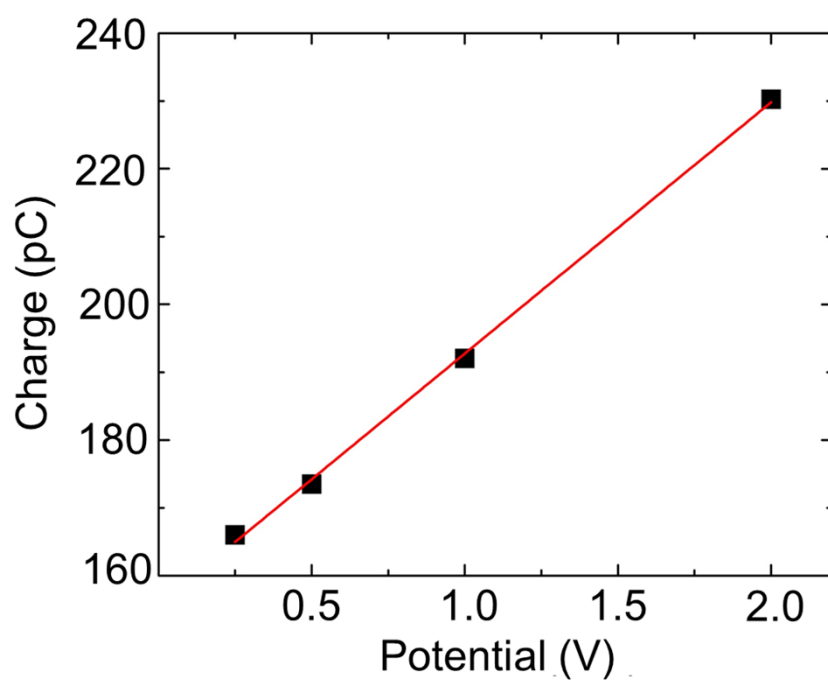


Figure S5. Amount of charge detected by the carbon ring electrode for FcMeOH at different delivery potentials.

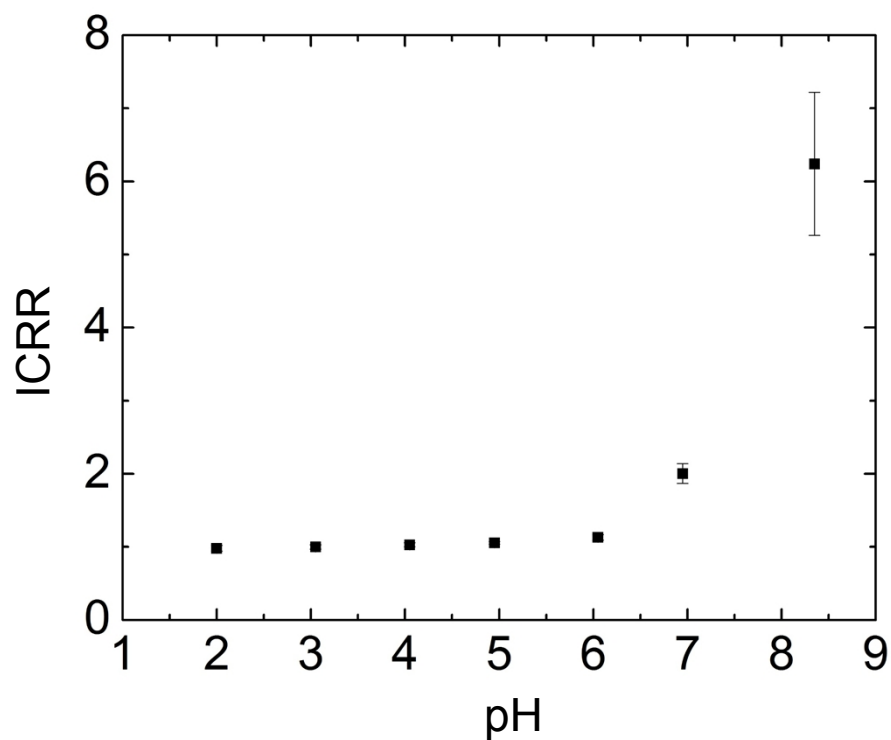


Figure S6.(a) Ion current rectification ratio (ICRR, $\text{ICRR} = \frac{i_{-1V}}{i_{+1V}}$) at different electrolyte pH. Electrolyte used was 100 mM KCl buffered with 2 mM KH_2PO_4 or K_2HPO_4 . pH = 2, 3, 4, 5, 6, 7, 8.4. ICRR \approx 1 when the electrolyte pH is lower than 6.

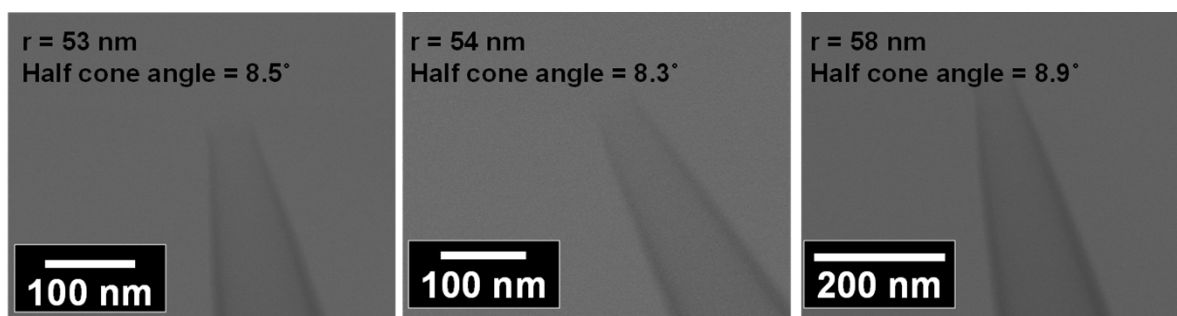


Figure S7. Representative STEM images of nanopipettes used in the pH dependent ion current rectification study.

Reference

37. S. Liu, Y. Dong, W. Zhao, X. Xie, T. Ji, X. Yin, Y. Liu, Z. Liang, D. Momotenko, D. Liang, H. H. Girault and Y. Shao, *Anal. Chem.*, 2012, **84**, 5565-5573.