The 2012 F. M. Becket Summer Research Fellowship — Summary Report

Localized pH Measurements with Scanning Ion-Conductance Microscopy

by Celeste A. Morris

Scanning ion-conductance microscopy (SICM) utilizes a nanopipette to raster-scan a surface with tens-of-nanometer resolution and is best suited for operation in physiological environments.[1-4] The working principle of SICM relies on the ion current through the nanopipette tip. A nanopipette, with a tip tens to hundreds of nanometers in diameter is filled with an electrolyte solution and houses a Ag/AgCl electrode. When the nanopipette tip is submerged in the bath electrolyte of the sample, an ion current is generated due to a potential applied between the pipette electrode and a reference Ag/AgCl electrode. As the nanopipette is approached to the sample surface, the ion current decreases significantly once the probe-surface distance is on the order of the radius of the nanopipette. This ion current is utilized as the feedback signal to control the probe-surface distance of the nanopipette while the pipette scans the surface.

Our specific research goals are to enhance the chemical information recorded by the nanopipette probe—an effort driven by the desire to increase the auxiliary information and analytical applications of SICM. To accomplish this, we fabricated Au electrodes with polyaniline (PANi) films for H^+-sensitive measurements (Fig. 1a).[5] Initially, nanopipettes are fabricated by laser-pulling a quartz capillary to form two nanopipettes. A Au electrode is thermally deposited on the surface of the nanopipette. The nanopipette tip is then isolated in a polydimethylsiloxane mask and the remainder of the nanopipette/electrode is insulated with parylene C. When removed from the polydimethylsiloxane mask, the nanopipette contains a Au electrode localized at the tip.[6,7] For studies of localized pH measurements, polyaniline was electrochemically deposited on the surface of the Au electrode (AuE) (Fig. 1b).

Electropolymerization of PANi films on the Au electrode surface was performed in 0.1 M aniline/ 1.0 M H_2SO_4 with a Ag/AgCl/ 3 M KCl reference electrode and Pt counter electrode (Fig. 2a). To calibrate the EMF response of the PANi film to changes in pH, a differential amplifier was utilized to record the potential difference between the PANi film and a Ag/AgCl/ 3 M KCl reference electrode.[8,9] Near-Nernstian responses of -56.6 and -56.0 mV/pH were obtained for the microscale and commercial pH probes, respectively (Fig. 2b).

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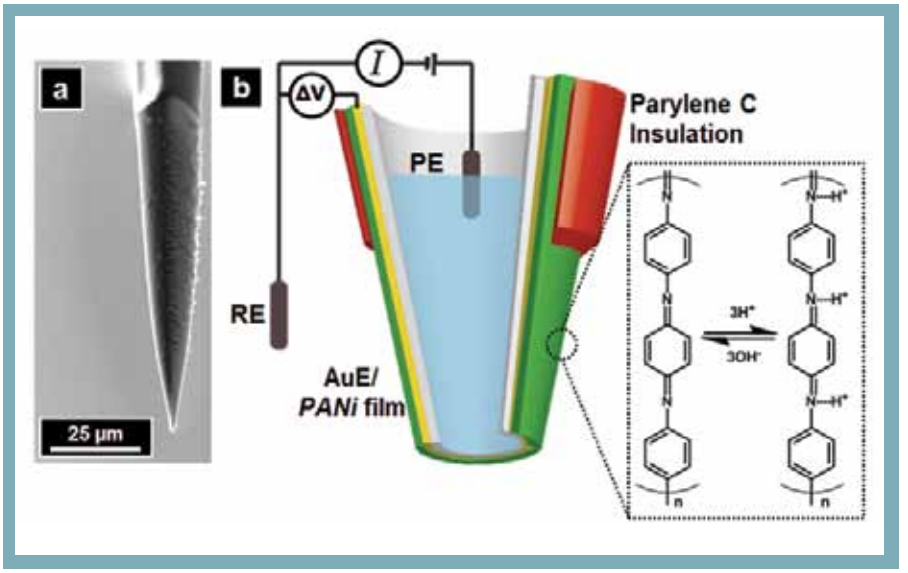

Fig. 1. *(a) Scanning electron micrograph of the parylene C insulated PANi film/AuE/nanopipette. (b) Schematic of pH detection with SICM. Here, the ion current, utilized as the feedback signal, is measured at the pipette electrode, PE. The EMF, ΔV, is recorded via the PANi film/AuE with a differential amplifier. The PE and PANi/AuE are referenced to the Ag/AgCl/ 3 M KCl reference electrode, RE. The inset demonstrates polyaniline protonation for measurement of ΔEMF.*

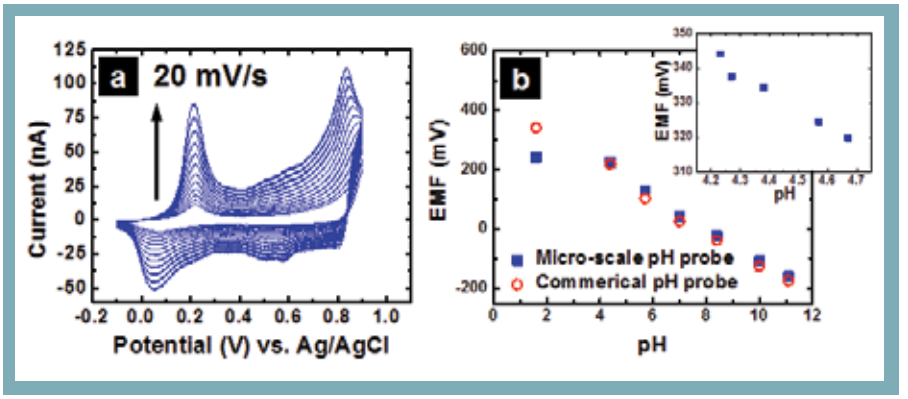

Fig. 2. *(a) Electropolymerization of polyaniline on the AuE in 0.1 M aniline/ 1.0 M H_2SO_4 with a Ag/AgCl/ 3 M KCl reference electrode and Pt counter electrode; 20 mV/s, 15 cycles. (b) Calibration of the EMF response to pH changes (pH 11 – pH 1.5) in 25.0 mM Na_2HPO_4/3.0 mM Na_3BO_3/6.7 mM $Na_3C_6H_5O_7$ buffer for the micro-scale (■) and commercial (○) pH probes which provide a Nernstian response. The inset demonstrates the EMF response to changes in pH of the micro-scale pH probe over a pH range from pH 4.18 to 4.67.*

To study localized differences in pH, we utilized polyimide membranes with 2.5-µm-diameter pores in a diffusion cell. In one experiment, 0.1 M KCl, pH 11.7 was placed in the top chamber while 0.1 M KCl, pH 2.6 was placed in the bottom chamber. As shown in Fig. 3a, localized pH differences were detected by measurement of ΔEMF of the PANi film vs. the reference electrode. When each chamber of the diffusion cell housed 0.1 M KCl, pH 2.6 there was no difference in EMF and thus no difference in pH- as expected (Fig. 3b). The vertical resolution of these probes was studied by repeatedly advancing the probe into pore 1 (Fig. 3a) while recording the ΔEMF. When a pH gradient was present (Fig. 3c) (□), the EMF increased due to the increasing change in pH in the vicinity of the pore and inside the pore. Without a pH gradient (Fig. 3c) (○) a slight decrease in EMF was observed. At present these probes are being applied to make ion-selective measurements of physiological systems.

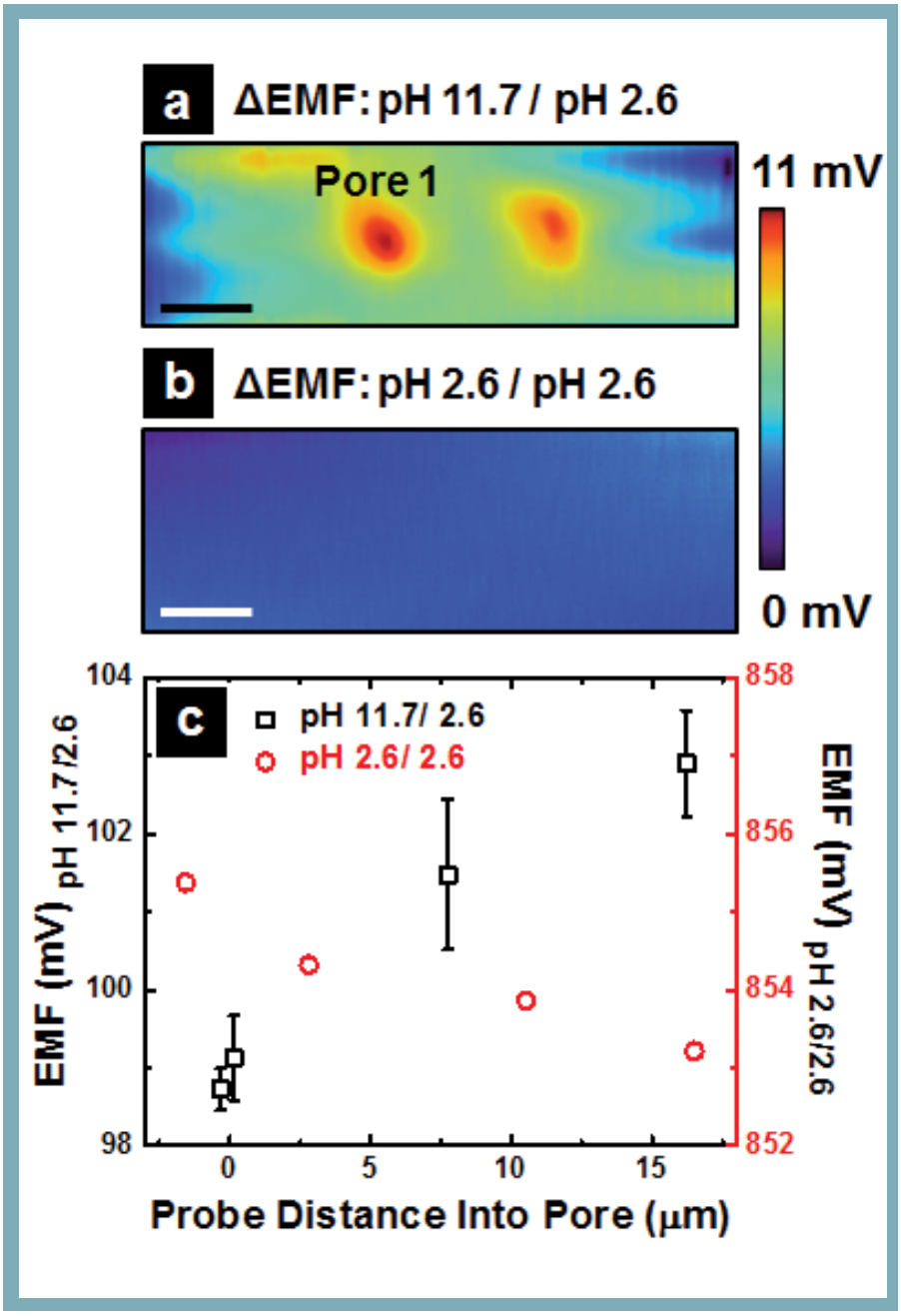

Fig. 3. *(a) EMF image obtained from the differential amplifier which demonstrates local pH differences above two pores in a polyimide membrane with the pH gradient of 0.1 M KCl pH 11.7 / 2.6 top and bottom chambers, respectively. (b) No significant EMF difference is obtained without the pH gradient (0.1 M KCl pH 2.6 in both chambers). Scale bars equal 2 µm. (c) EMF response of a pipette as it moved into pore 1 (Fig. 3a) with a pH gradient (□), 0.1 M KCl, pH 11.7 (top chamber)/ pH 2.6 (bottom chamber) and without a pH gradient (○), 0.1 M KCl, pH 2.6 in both chambers of the perfusion cell. Error associated with EMF response without a pH gradient is smaller than the legend utilized.*

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