Supporting information

Ion channel probes for scanning ion conductance microscopy

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Lipid bilayer characteristic

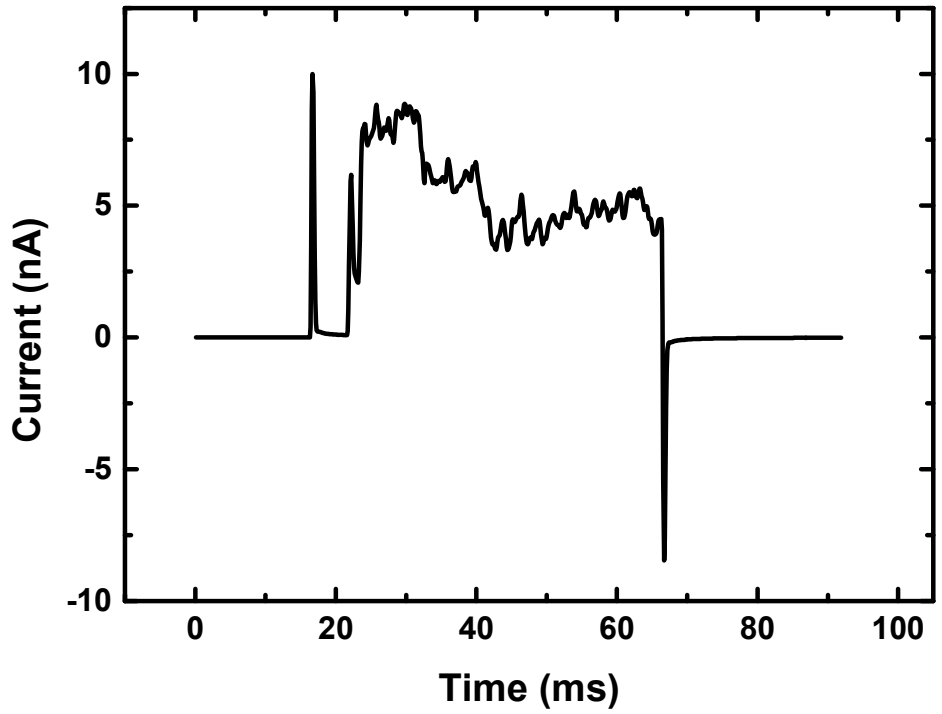

Figure S1. Fluctuations of current due to the formation of transient pores, which happened at a voltage 10 to 20 mV under the breakdown voltage (a complete breakdown). In this BLM, the current fluctuations were observed at 600 mV, prior to complete breakdown.

BLM formation was verified via electrically induced breakdown upon application of increasing potential across the BLM. BLMs that did not break in under 1000 mV were considered clogged and not a true BLM. To monitor breakdown voltage, a holding potential of 0 mV was applied to the pipette for 10 ms and the potential was stepped through 0 mV to 1000 mV in 10 mV increments of 50 ms duration. The pipette was returned to 0 mV for 10 ms between potential protocol steps. Immediately prior to breakdown, slight destabilization of the BLM forms ion conducting transient pores in the

membrane which increase the current. Thus pre-breakdown pore formation serves as an additional indicator of BLM formation across the pipet tip allowing differentiation between BLM, lipid multilayers and clogs.

Current distance characteristic

The distance from the sample surface where a steep current change was observed increased with the number of ion channels reconstituted in the pipet (lower probe resistance, Figure S2.) for ion channel probe (ICP) SICM. The bare pipet approach curves were obtained after applying a sufficiently high potential to irreversibly break the BLMs. The bare pipet approach curves of pipet 1 and pipet 2 are similar, due to similar geometry (pipet 1: I.D. 7.8 μm and O.D. 27.0 μm and pipet 2: I.D. 8.7 μm and O.D. 28.3 μm). Pipet 3 (I.D. 11.0 μm and O.D. 42.6 μm) the surface is detected at greater distances than pipet 1 or 2, which is due to the larger outer diameter and also could have been affected by the angle between the surface and pipet tip.

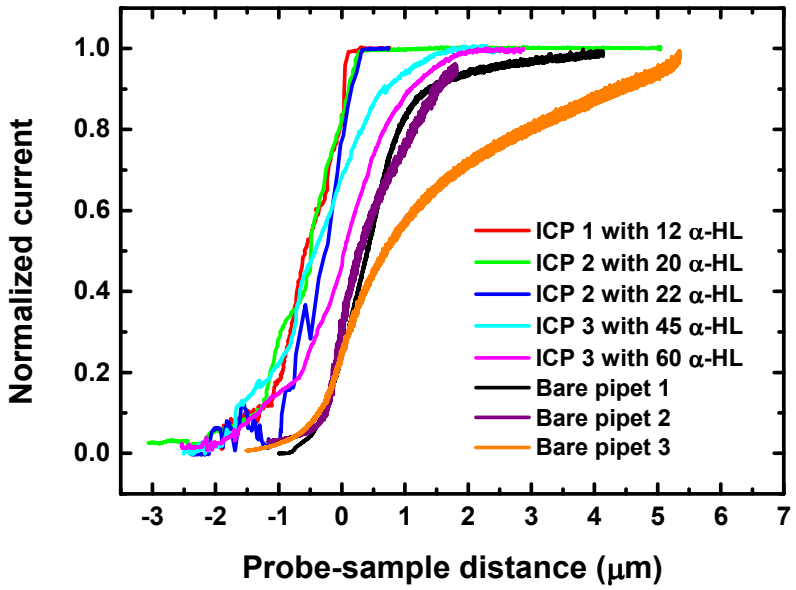

Figure S2. Approach curves of ICPs functionalized with different numbers of ion channels and the associated bare pipets.

Line scan

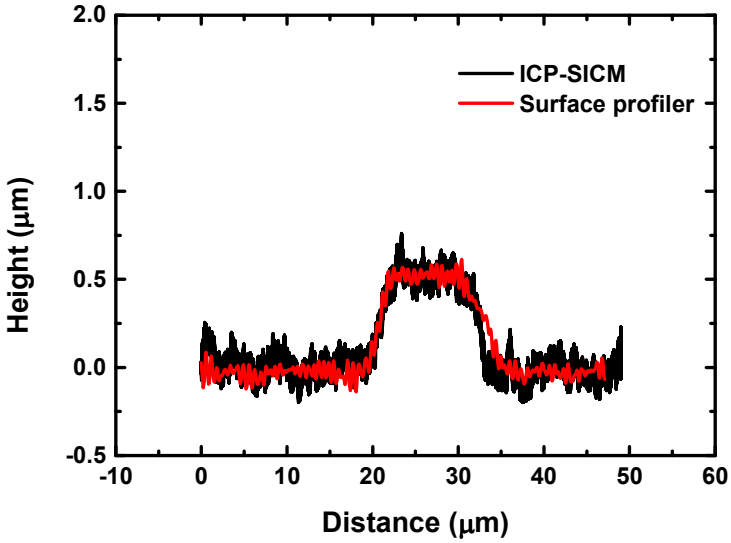

Figure S3. Line scans of the sample feature measured by ICP with SICM and a surface profiler.

Figure S3 shows a second experiment with additional comparison between the line scans obtained on a PDMS bar feature with the ICP in SICM and surface profiler (Dektak 6 M, Veeco, Plainview, NY). The specific probe used here exhibited a current of 1.72 nA (48 α-HL channels) and was controlled approximately 1 μm above the sample surface. Vertical resolution of the surface profiler is 1 nm. Thus the similarity between the surface profiles in the two line scans supports a high vertical resolution for the ICP with SICM. Further, the stylus used in the vertical profiler had a radius of 12.5 μm, comparable to the ICP, providing equivalent lateral resolution.