## Structure and Permeability of Ionchannels by Integrated AFM and Waveguide TIRF Microscopy

Srinivasan Ramachandran<sup>1</sup><sup>\*</sup>, Fernando Teran Arce<sup>1</sup><sup>\*</sup>, Nirav Patel<sup>1</sup>, Arjan. P.Quist<sup>2</sup>, Daniel A. Cohen<sup>3</sup> and Ratnesh Lal<sup>1</sup><sup>\*</sup>

<sup>1</sup> Department of Bioengineering; Department of Mechanical & Aerospace Engineering, University of California San Diego, La Jolla, CA 92093

<sup>2</sup> RC Nano Corporation, 2210 Midwest Road, Oak Brook, IL 60523, USA. Current Address: Innovation and New Ventures Office, Northwestern University, 1800 Sherman Ave., Evanston IL 60201

<sup>3</sup> Department of Materials, University of California Santa Barbara, CA 93106

<sup>\$</sup> Both authors contributed equally to this work

\* Corresponding author

## Supplementary figure S1



Supplementary figure S1. Sequential AFM images following the formation of the DPPC lipid bilayer shown in figure 2c. Approximately 300 ng of DPPC lipid dissolved in chloroform (enough to form 1.5 bilayers on the nanopore chip) were left to dry in vacuum for 45 minutes and form a film on a nanopore chip sample. After mounting this sample on the AFM stage, 100  $\mu$ L of PBS buffer were added to hydrate the lipid film on the sample and form liposomes first, then a supported bilayer by rupture and fusion of liposomes. The sequential images were acquired in contact mode. The times refer to the time elapsed after the lipid-covered chip was exposed to PBS buffer. The arrows are a guide to indicate features common to all three images. The dotted rectangle indicates an area where smaller liposomes ruptured to form a larger patch.

Supplementary figure S2



Supplementary figure S2. Illustration of intensity profile plots. A line segment (dotted blue line in top panel) is drawn across the silicon nitride membrane window over the nanopore region in ImageJ. The function 'plot profile' plots the fluorescent intensity values as a function of pixels position along the line segment (bottom panel). This helps to visualize point-to-point variations in the intensities along the line easily. To avoid overcrowding of image panels the axes were omitted and the plot profile was overlaid on the images directly in figure 4.