Electronic Supplementary Material (ESI) for Nanoscale.

Supporting Information

In-situ nanoscale imaging reveals self-concentrating nanomolar antimicrobial pores

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Figures and Videos

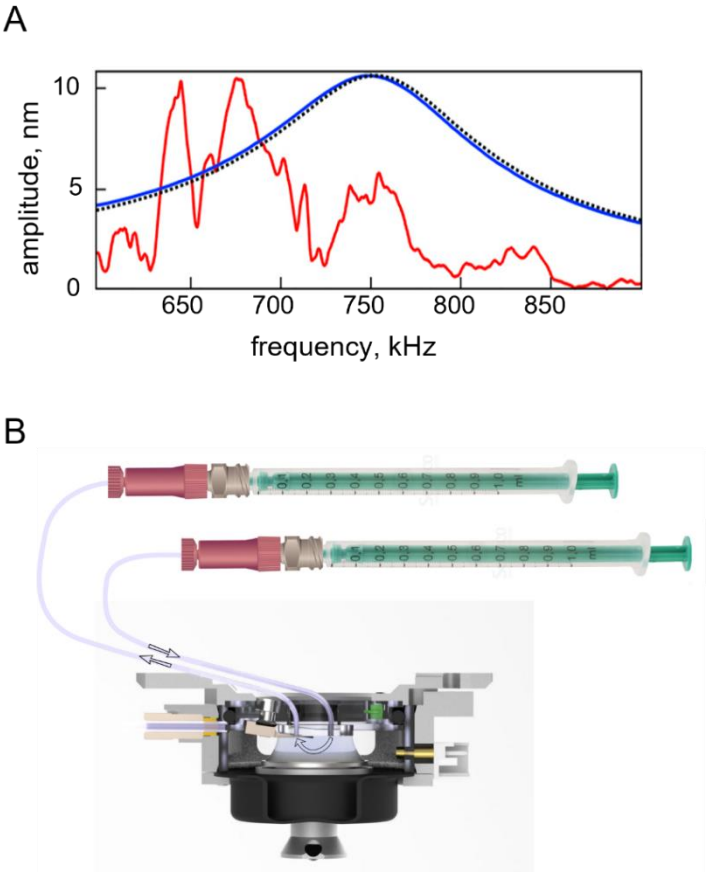

Figure S1. Set up of Cypher ES AFM (Oxford Instruments Asylum Research) equipped with photothermal actuation (blueDrive™). (A) Comparison of cantilever response (amplitude vs frequency) for piezoelectric cantilever excitation (red) and photothermal excitation (blue). Piezoelectric excitation subjects the entire fluid cell to mechanical excitation resulting in multiple peaks. Photothermal methods directly excite the cantilever, resulting in a single peak. Spectra taken in water, reproduced with permission from[24]. (B) Schematic cut away of the sealed measurement cell of the AFM showing connection of tubing to allow the introduction and withdrawal of liquid.

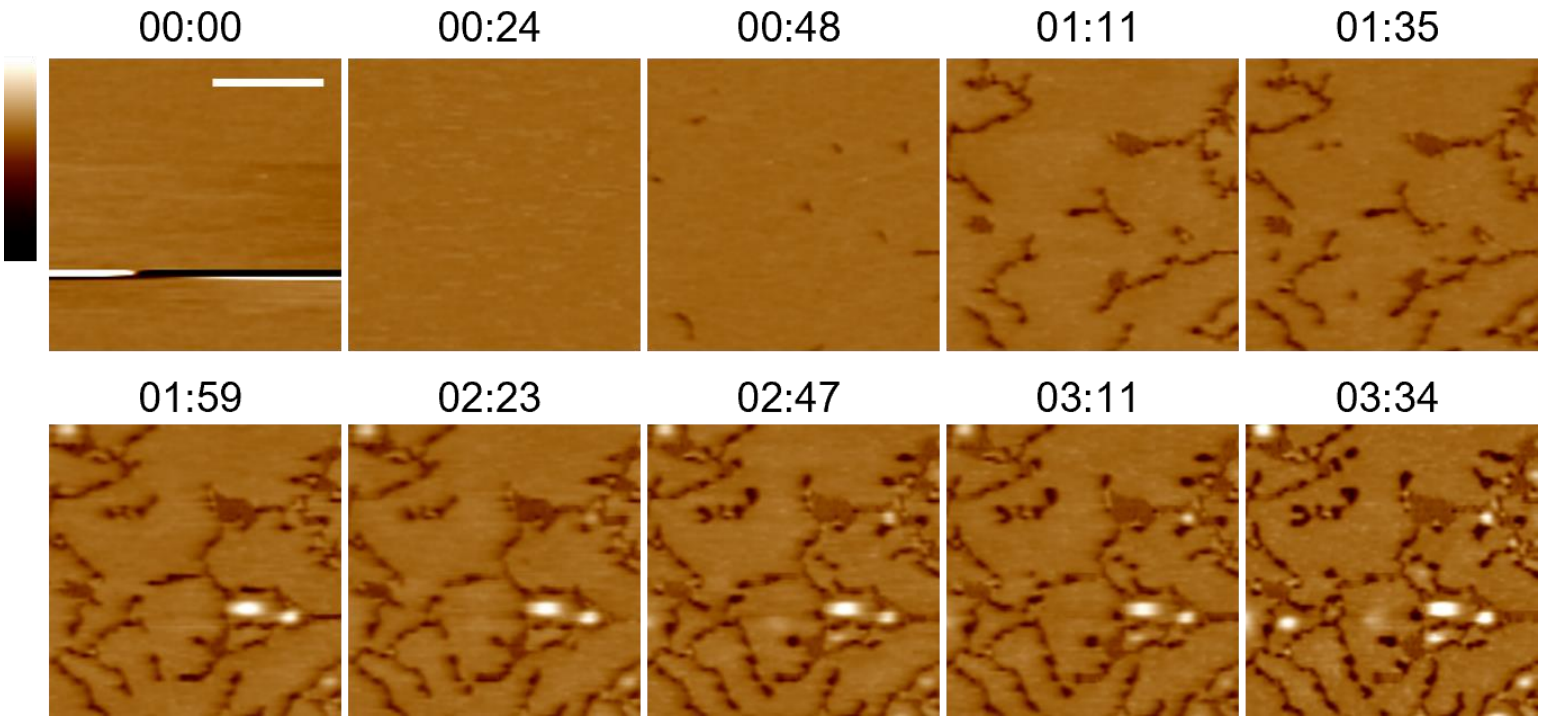

Figure S2. Time-resolved imaging of membrane disruption by NI01. (A) Representative AFM topography images of anionic POPC/POPG (3:1, molar ratio) SLBs following injection of NI01 (750 nM) at 00:00 min. The images are digital zooms of selected frames from Video S2. Scale and colour (height) bars are 500 nm and 15 nm, respectively.

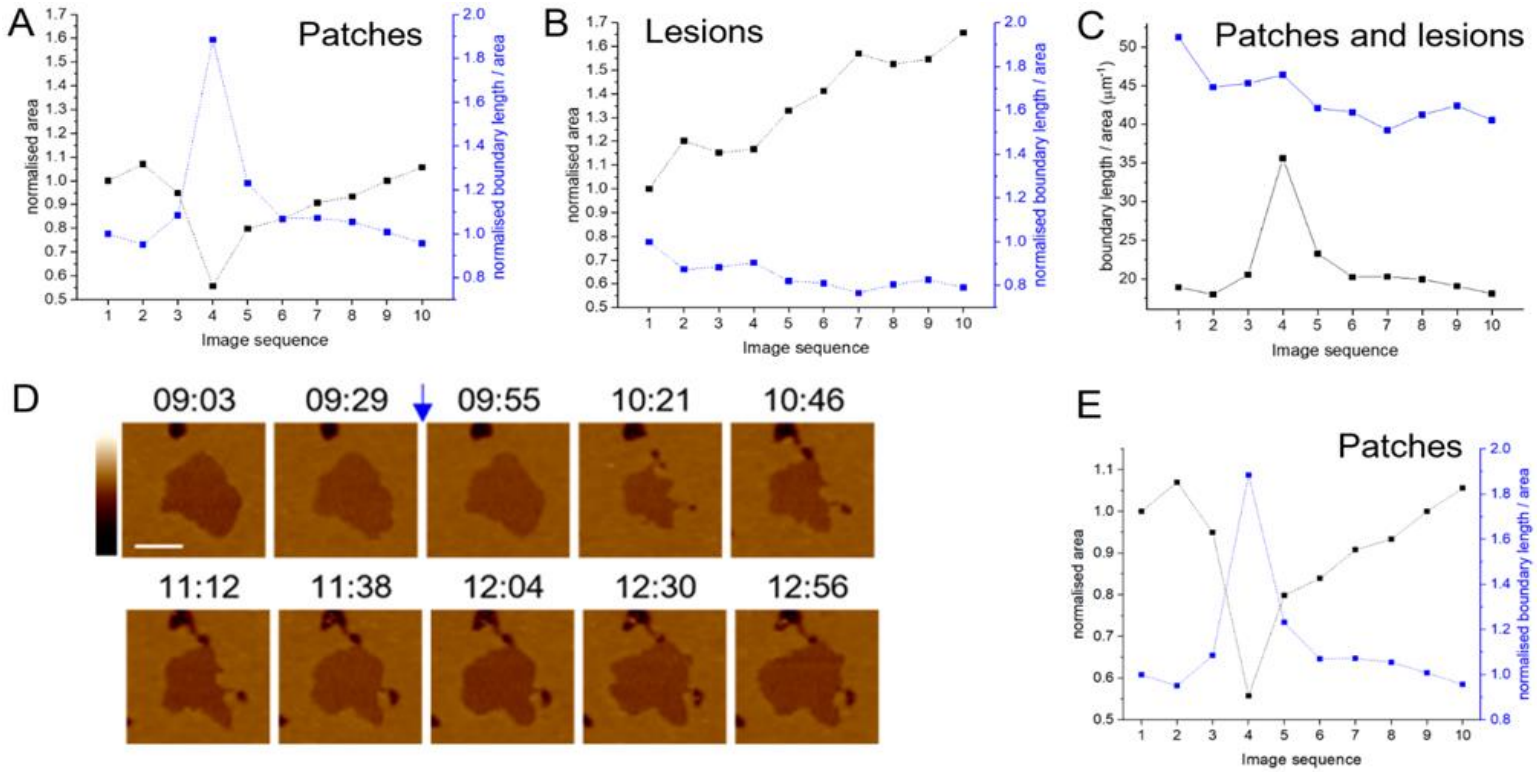

Figure S3. Response of membrane thinning patches to additional injections of NI01. (A, B) Plots of the surface area (black) and boundary length per unit area (blue) for the thinned membrane patches (A) from topography images in Fig 3C. Image sequence numbers refer to the topography images labelled from left to right, and the point of injection occurs after image 2, as highlighted by the blue arrow. Values are normalised to the value in image 1. (C) Comparison of the absolute value for boundary length per unit area between transmembrane channels (blue) and patches (black) in Fig 3C. (D -E) topography images (D) and corresponding plots of the surface area (black) and boundary length per unit area (blue) for a thinned membrane patch (E) . Image sequence numbers refer to the topography images in (D), labelled from left to right and the point of injection is highlighted by the blue arrow. Values are normalised to the value in image 1. Images in (D) are digital zooms of selected frames from continuous imaging (Video S1). Colour (height) and scale bars are 15 nm and 180 nm, respectively.

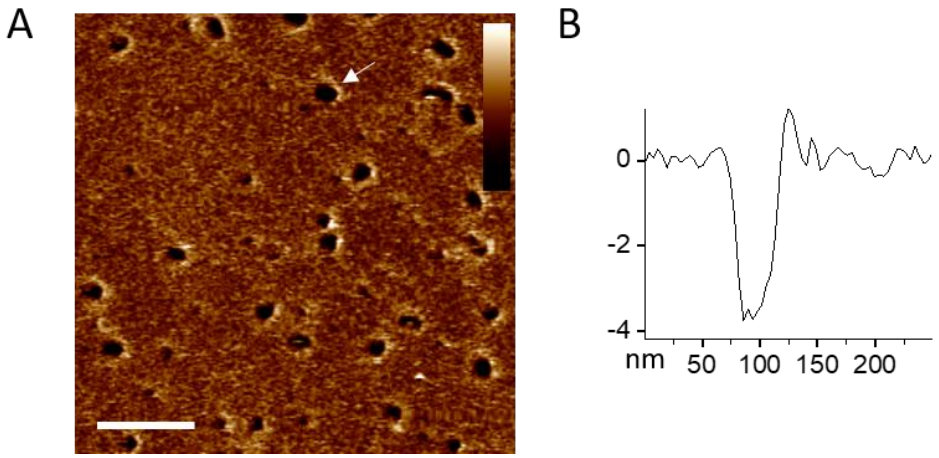

Figure S4. Membrane disruption with higher concentrations of magainin 2. AFM topography image of anionic POPC/POPG (3:1, molar ratio) SLB treated with magainin 2 (200 nM, 20 min incubation). Transmembrane pores with a heterogenous diameter of 5 – 40 nm form across the surface. Scale and colour bars are 200 nm and 5 nm, respectively.

Video S1. Time-resolved scanning of membrane disruption by NI01. The video shows consecutive scans from 3 min prior to peptide injection, to 30 min after peptide injection, for anionic POPC/POPG (3:1, molar ratio) SLBs treated with NI01 (375 nM). The video is recorded in a Cypher ES, with an AC10 cantilever, in tapping mode, at a line rate of 20 Hz and 25.8 seconds per frame. Scan size is 4 µm.

Video S2. Time-resolved scanning of membrane disruption at higher NI01 concentrations. The video shows consecutive scans from 1 min prior to peptide injection, to 6 min after peptide injection, for anionic POPC/POPG (3:1, molar ratio) SLBs treated with NI01 (750 nM). The video is recorded in a Cypher ES, with an AC10 cantilever, in tapping mode, at a line rate of 20 Hz and 23.8 seconds per frame. Scan size is 4 µm.