Supporting Information

Kinetic Defects Induced by Melittin in Model Lipid Membranes: A Solution Atomic Force Microscopy Study

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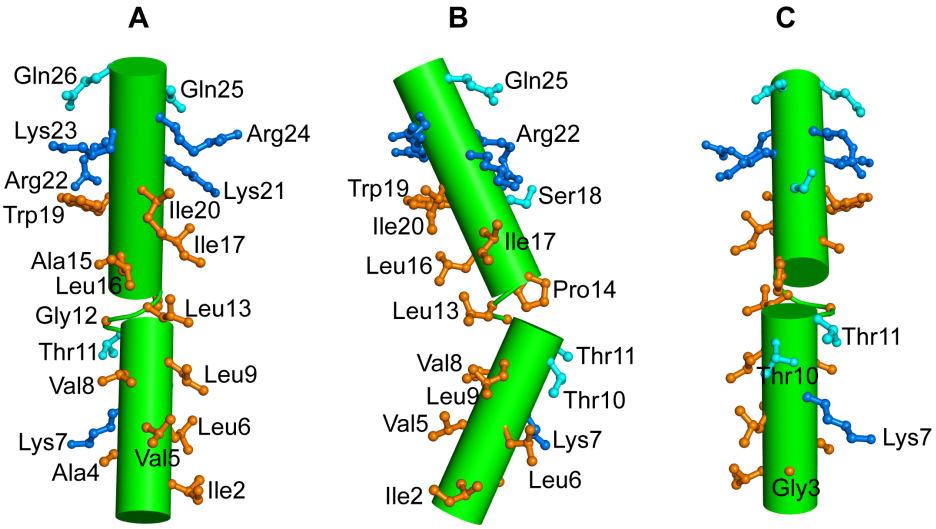

Figure S1 Crystal structure of melittin monomer (PDB: 2MLT). The three images are obtained by successively rotating the monomer by ~90^{o} along the vertical axis. Hydrophobic side chains are colored in orange, polar side chains are colored in cyan, and positively charged side chains are colored in blue. Amphipathic characteristic of the melittin monomer is illustrated by the segregated distribution of hydrophobic and non-hydrophobic side chains.

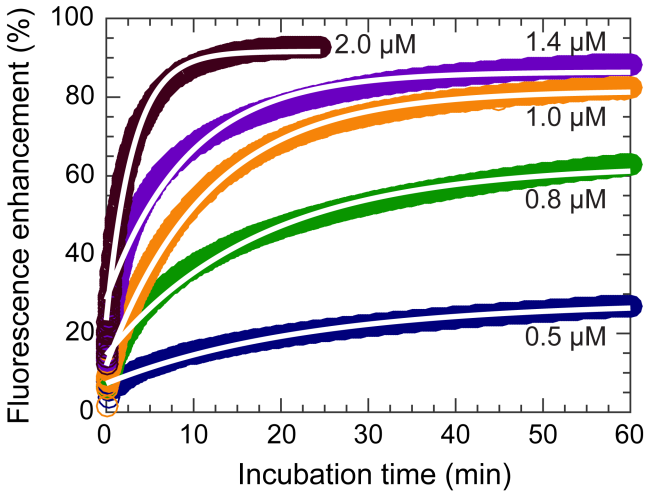

Figure S2 Exponential fitting (white lines) to time-course leakage data induced by melittin. The obtained time constant τ is 23, 18, 12, 9, and 3 min for 0.5, 0.8, 1.0, 1.4, and 2.0 uM of melittin, respectively.

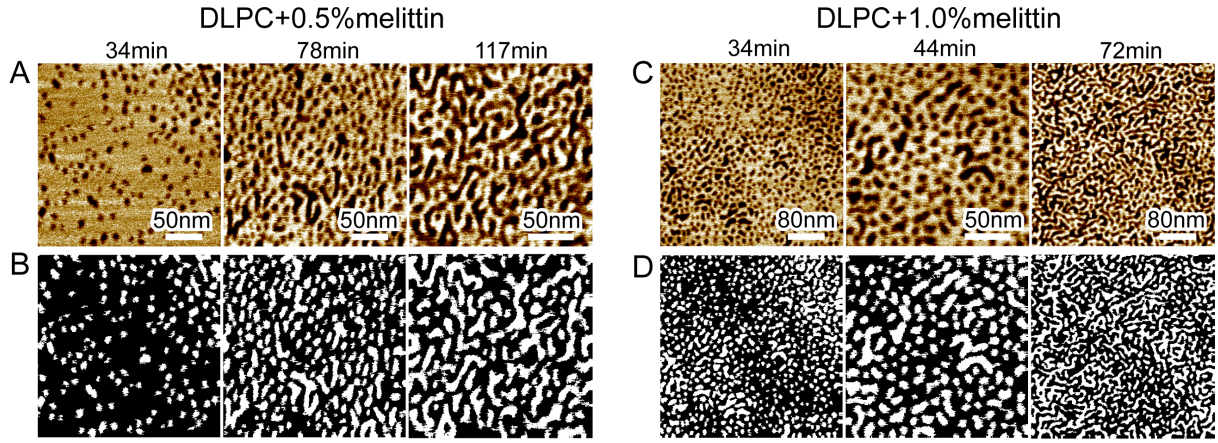

Figure S3 Solution AFM height images of DLPC bilayers doped with 0.5mol% (A) and 1.0mol% (C) melittin (the same as Fig. 2 in the main text). Defects (bright regions) are determined using proper height thresholds (B and D). Defect sizes are calculated by the total number of pixels belong to each defect. Area fraction covered by defects is 0.15, 0.34, and 0.39 for the DLPC+0.5%melittin bilayer at 34, 78, and 117 min, respectively; total area fraction of defects is 0.32, 0.36 and 0.40 for the DLPC+1.0%melittin bilayer at 34, 44, and 72 min, respectively.

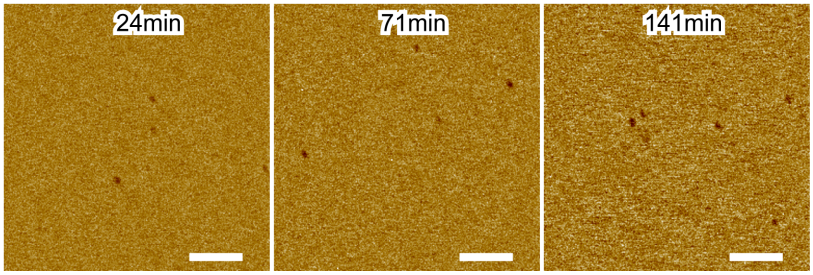

Figure S4 Solution AFM height images of DOPC+1.0mol% melittin bilayer. Scale bars = 100 nm. It is clear that even after incubation of 141 min, very few defects are observed.

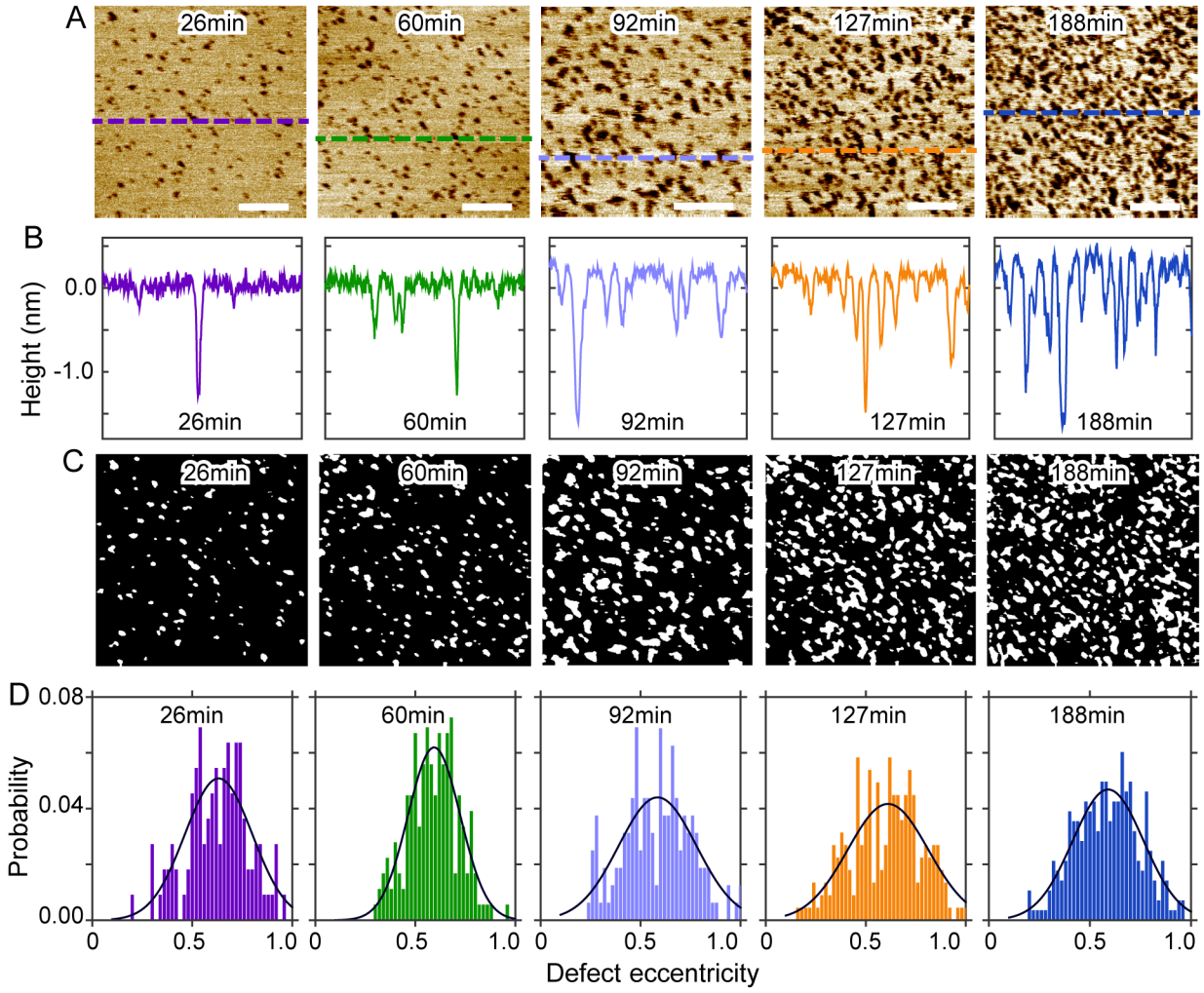

Figure S5 (A) Solution AFM height images of DOPC + 2.0mol% melittin bilayer (the same as Fig. 3 in the main text). Scale bars = 100 nm. (B) Height profiles along dashed lines highlighted in (A). (C) Defects (bright regions) determined using proper height thresholds. Area fraction covered by defects is 0.04, 0.08, 0.17, 0.21, and 0.25 for incubation time of 26, 60, 92, 127, and 188 min, respectively. (D) Normalized probabilities of defect eccentricity. Solid lines are Gaussian fits.

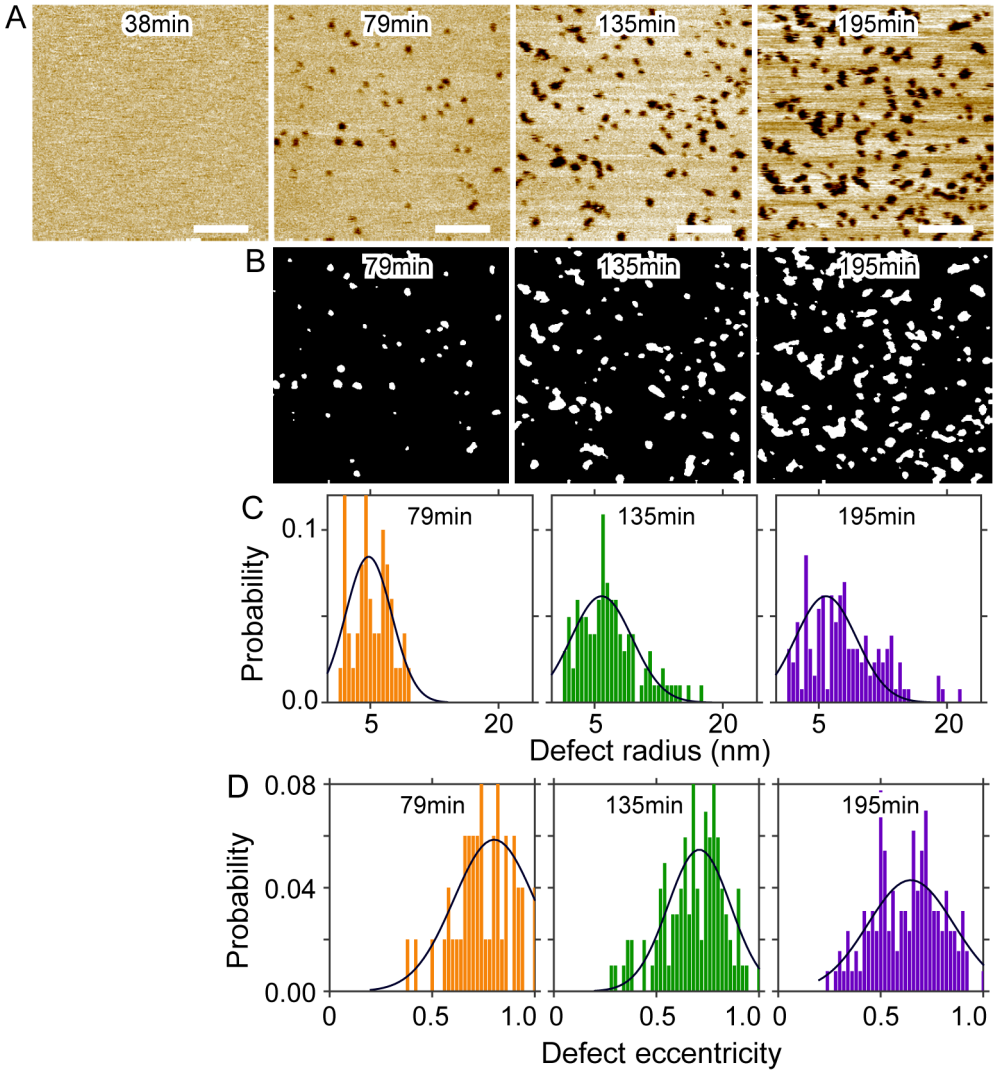

Figure S6 (A) Solution AFM height images of the DOPC bilayer exposed to 1.0 μM melittin. Scale bars = 100 nm. (B) Defects determined using proper height thresholds. Area fraction covered by defects is 0.02, 0.08 and 0.14 for incubation time of 79, 135 and 195 min, respectively. (C) Normalized probabilities of defect radius. (D) Normalized probabilities of defect eccentricity. Solid lines (C and D) are Gaussian fits.

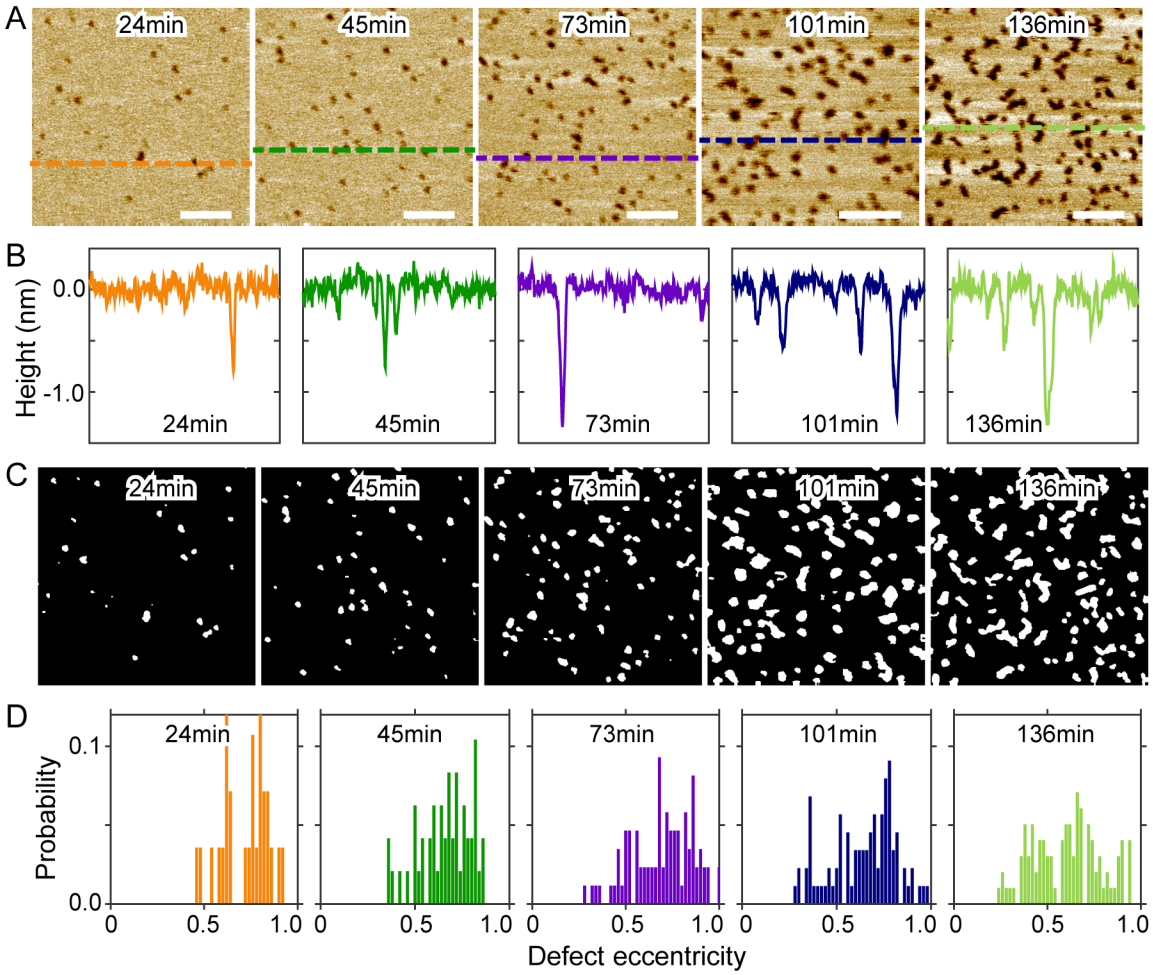

Figure S7 (A) Solution AFM height images of the DOPC + 2.0mol% melittin bilayer doped with 30mol% Chol (the same as Fig. 4 in the main text). Scale bars = 100 nm. (B) Height profiles along dashed lines highlighted in (A). (C) Defects determined using proper height thresholds. Area fraction covered by defects is 0.01, 0.03, 0.07, 0.16, and 0.17 for incubation time of 24, 45, 73, 101, and 136 min, respectively. (D) Normalized probabilities of defect eccentricity.

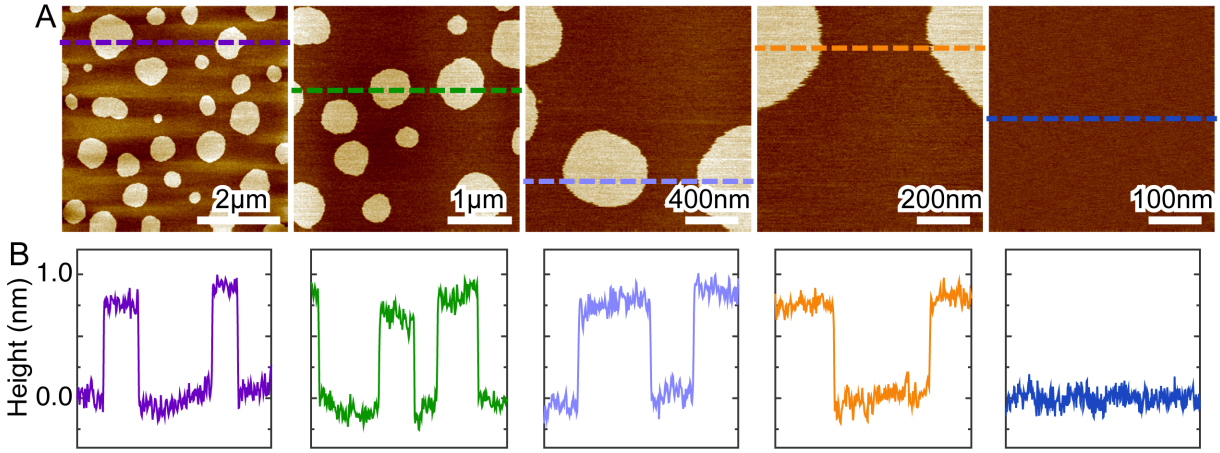

Figure S8 (A) Solution AFM height images of the DOPC/eSM/Chol 0.48/0.32/0.20 bilayer obtained with different magnifications. (B) Height profiles along dashed lines highlighted in (A).

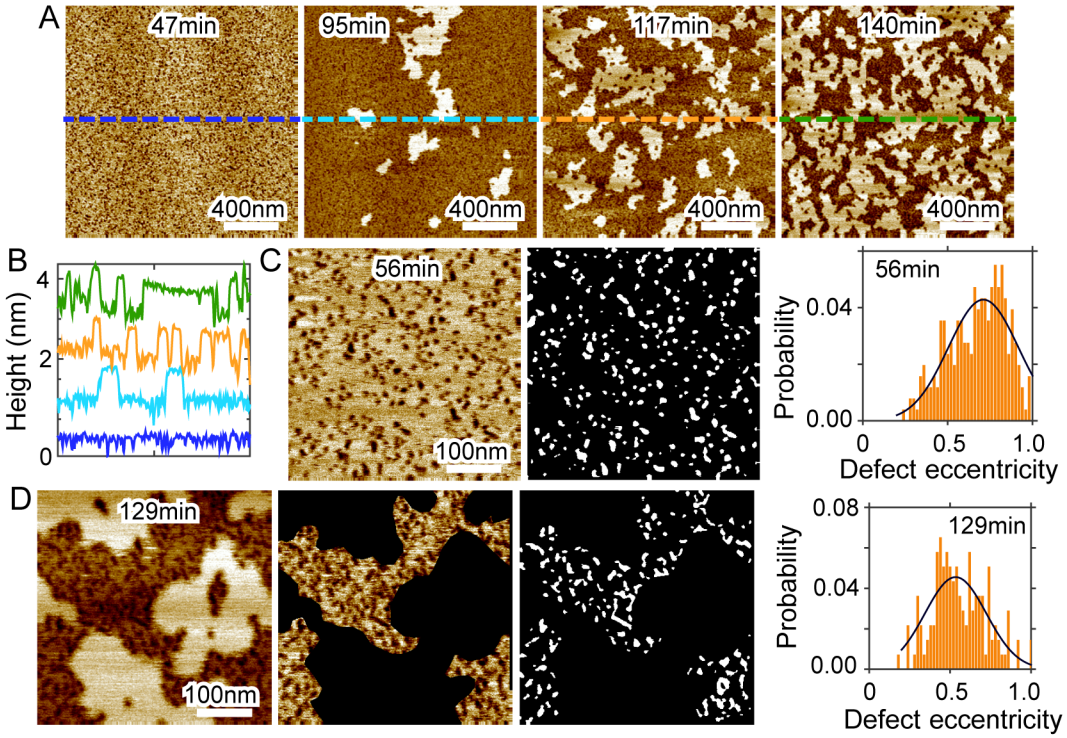

Figure S9 (A) Solution AFM height images of the DOPC/eSM/Chol 0.48/0.32/0.20 bilayer doped with 2.0mol% melittin (the same as Fig. 6 in the main text). (B) Height profiles along dashed lines highlighted in (A). (C) Defects in the Ld-phase bilayer. (D) Defects in the Ld-phase region of the Ld+Lo phase coexisting bilayer. Solid lines (C and D) are Gaussian fits.

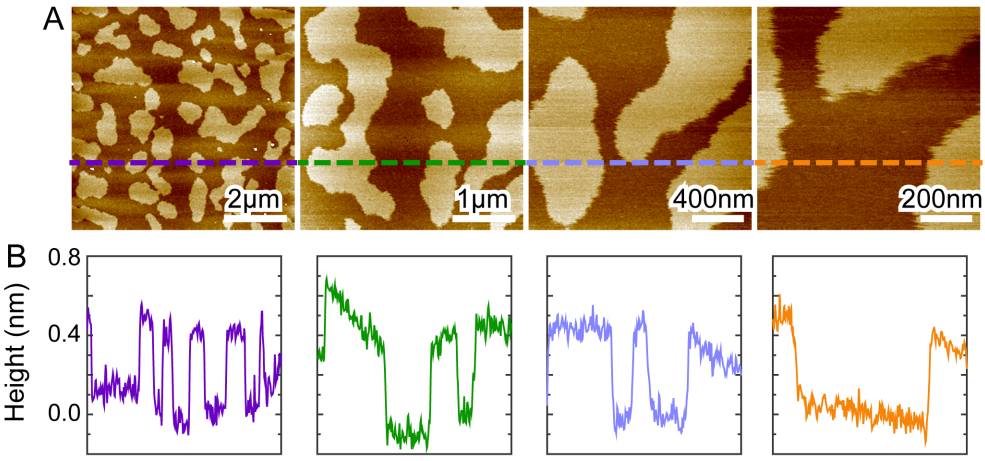

Figure S10 (A) Solution AFM height images of the DOPC/eSM/Chol 0.41/0.27/0.32 bilayer obtained with different magnifications. (B) Height profiles along dashed lines highlighted in (A).

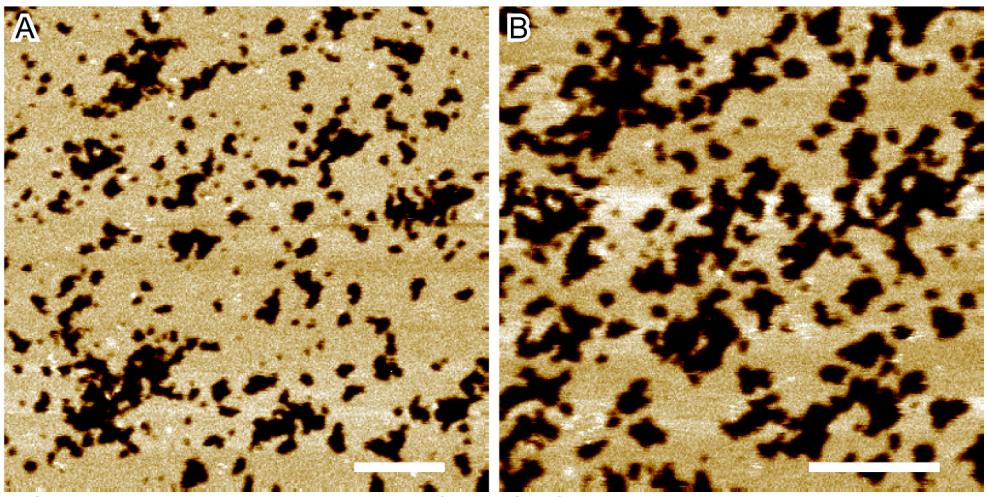

Figure S11 Solution AFM height images of a DOPC bilayer incubated with 0.5 mM nonionic detergent Triton X-100. Scale bars = 200 nm. Lipid dissolution yields transmembrane defects with large heterogeneity in terms of defect size and shape.

Table S1 Area fraction for melittin induced defects as a function incubation time.

Lipid composition	Melittin	Defect area fraction	Incubation time
DLPC	0.5mol%	0.15	34 min
		0.34	78 min
		0.40	117 min
DLPC	1.0mol%	0.32	34 min
		0.36	44 min
		0.40	72 min
DOPC	2.0mol%	0.04	26 min
		0.08	60 min
		0.17	92 min
		0.21	127 min
		0.25	188 min
DOPC	1.0 uM	0.02	79 min
		0.08	135 min
		0.14	195 min
DOPC+cholesterol[a]	2.0mol%[b]	0.01	24 min
		0.03	45 min
		0.07	73 min
		0.16	101 min
		0.17	136 min

[a] Cholesterol mole fraction is 0.3 of the total mixture DOPC/cholesterol/melittin
[b] DOPC to melittin ratio is 98:2 mol/mol