Lipid phase dependence of Equinatoxin II membrane binding and pore formation

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Supplementary Movie S1
EqtII-Cy3B (red) pores diffuse in the L_d phase on a DPhPC:eSM:Chol 4:3:3 bilayer. (a) Functional EqtII-Cy3B pores were visualized by the Ca^{2+} flux through the pore using Fluo-8H fluorescence (green). 17.5 x 17.5 µm image. 100 ms per image acquisition. Playback at 40 ms per image.

Supplementary Movie S2
At low EqtII-Cy3B concentrations (~50 nM) individual molecules can be resolved. 1D diffusion of EqtII-Cy3B at the domain boundaries is evident, as it the presence of EqtII-Cy3B in both phases. Scale bar 10 µm. Images were recorded at 100 ms. Playback at 40 ms per image.

Figure S1

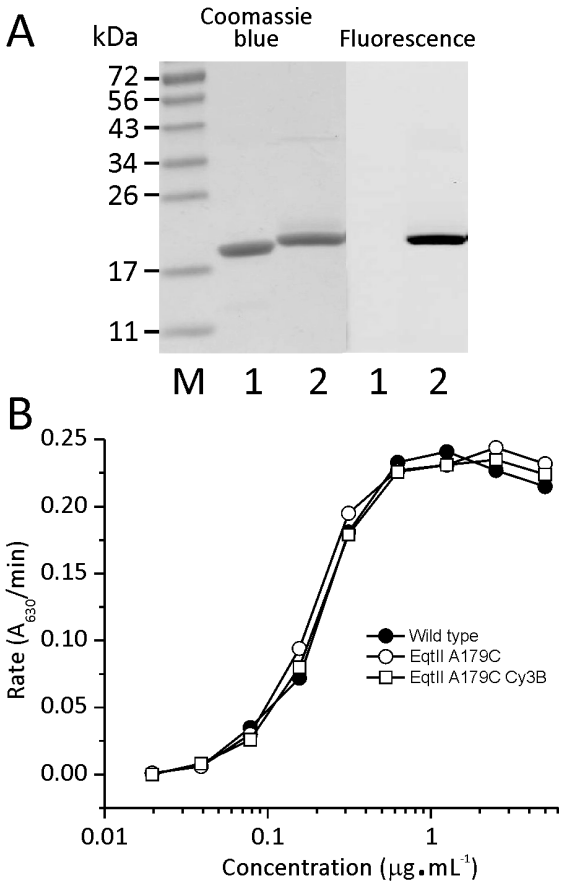

FIGURE S1: Properties of EqtII A179C-Cy3B. (*A*) SDS-PAGE of purified labelled protein (left) and the same gel under UV light prior staining of the gel with Coomassie blue. M, Marker lane; 1, EqtII; 2, EqtII A179C-Cy3B. (B) Hemolytic activity measured using the lysis of bovine red blood cells.

Figure S2

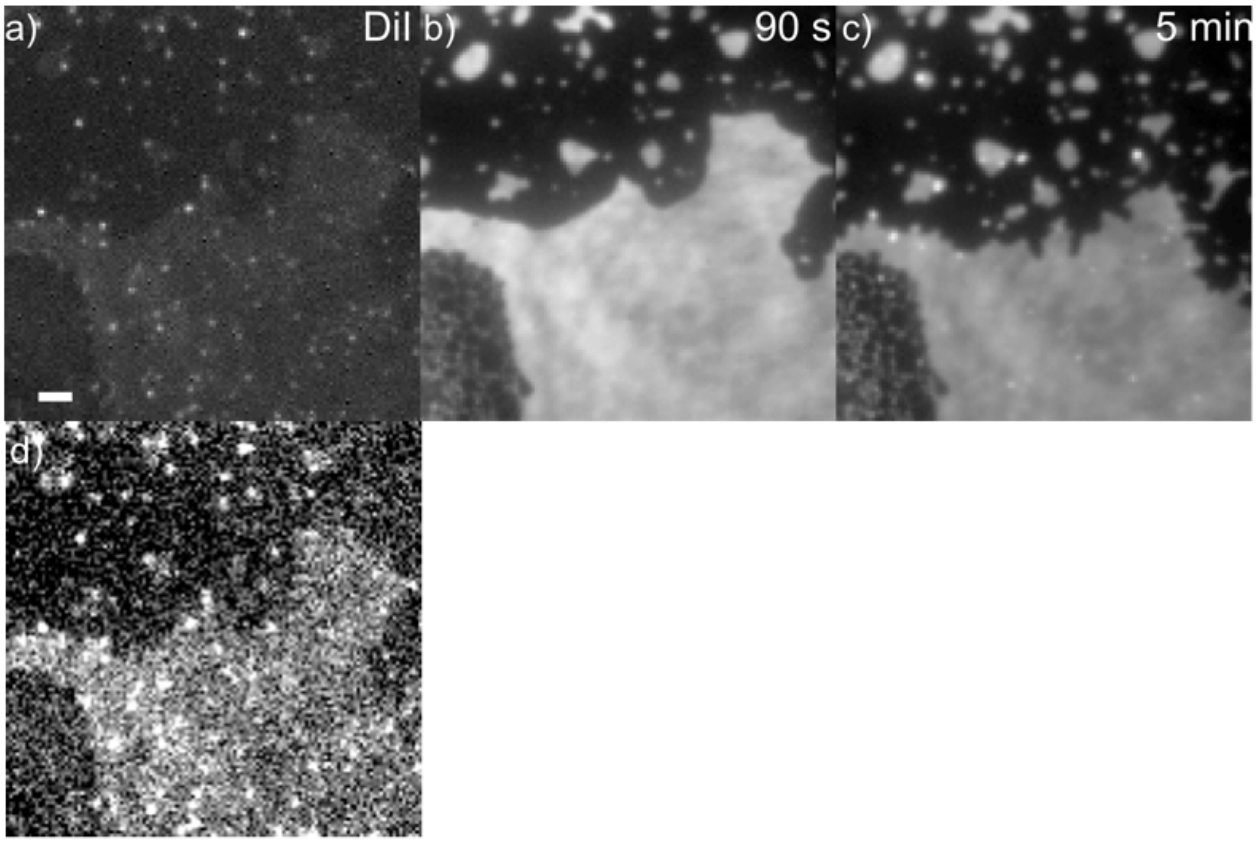

FIGURE S2: Image of a DPhPC:SM:Chol 1:2:2 bilayer containing 0.0001% DiI as a marker for the L_d phase. (a) DiI fluorescence prior to EqtII injection. Contrast is low in this image, in comparison to panels b and c, as reduced laser power is used to limit premature photobleaching. The bright spots are likely due to oil inclusions below the bilayer in which DiI is highly soluble. (b) EqtII-Cy3b (final concentration approximately 700 nM) was injected into the droplet and image was recorded 90 s after injection. (c) 5 min after injection the addition of the toxin has resulted in a reordering of the lipid in the bilayer. (d) Contrast-enhanced duplicate of (a). Scale bar indicates 10 μm.

Figure S3

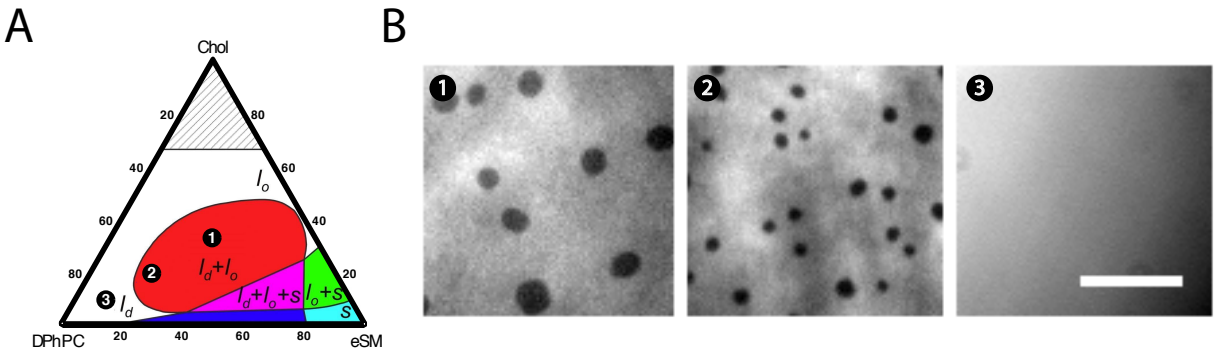

FIGURE S3: Three DPhPC:SM:Chol compositions, expected to correspond to two-phase and single-phase regions of the phase diagram. We are not aware of any published phase diagram for the specific combination of DPhPC:SM:Chol. However in broad terms, phase diagrams for all ternary lipid mixtures of cholesterol with two lipids with different melting temperatures show similarity (1–3). (A) Modified phase diagram from Dimova and co-workers for DOPC:eSM:Chol (4). This is for a different lipid mixture to that used here, but can be used as rough guide to where we might expect phase boundaries to lie. Numbering on this diagram indicates lipid compositions for DPhPC:eSM:Chol chosen to probe the phase diagram (1) 1:1:1, (2) 2:1:1, and (3) 8:1:1. (B) Images of droplet bilayers corresponding to the lipid compositions referred to in (A). Scale bar 10 μm.

Figure S4

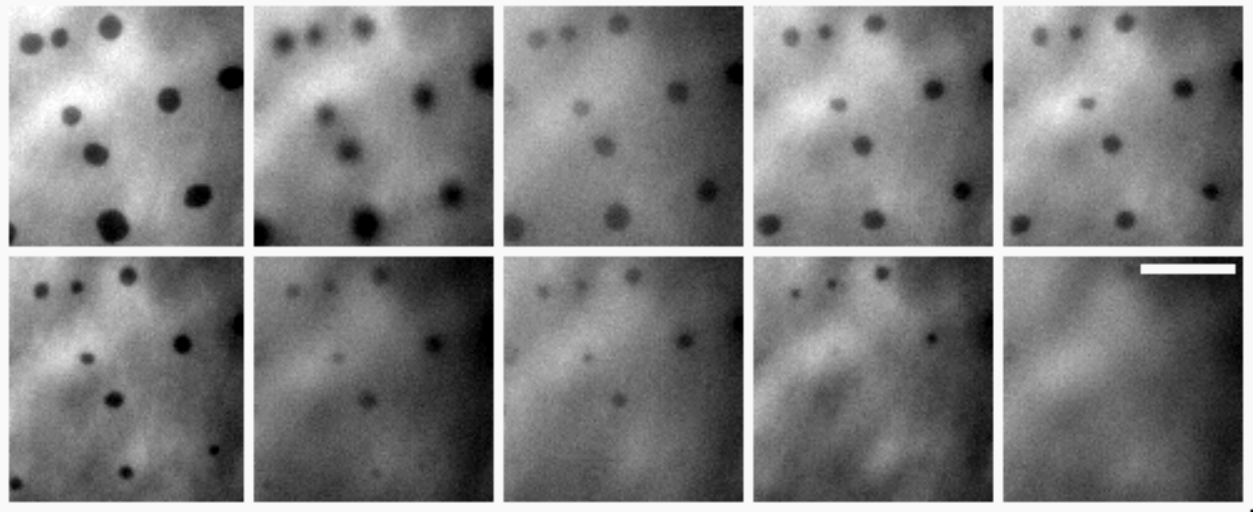

FIGURE S4: Image sequence following heating of a DIB formed from a 1:1:1 DPhPC:SM:Chol mixture with 0.5% *N*-(6-tetramethylrhodaminethiocarbamoyl)-1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine (TRITC-DHPE) used as marker for the L_d phase (5). Heating from 22 (top left) to 30°C (bottom right) over a period of 5 minutes results in shrinkage of the area fraction of the putative L_o phase. Scale bar 10 μm.

Figure S5

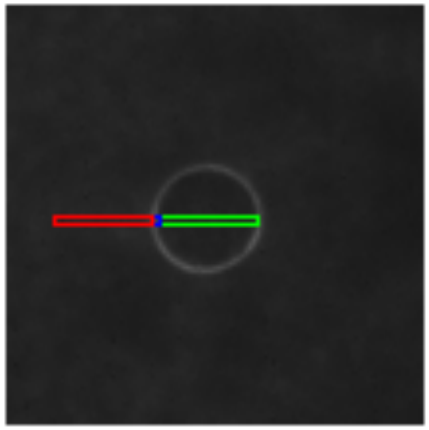

FIGURE S5: Image from Fig 3B depicting the regions of interest used to compute the time-dependent changes in fluorescence intensity seen in Fig. 3D. As per the colour coding of Fig. 3D, red is outside the domain, blue is at the domain boundary, and green is inside the domain boundary.

Figure S6

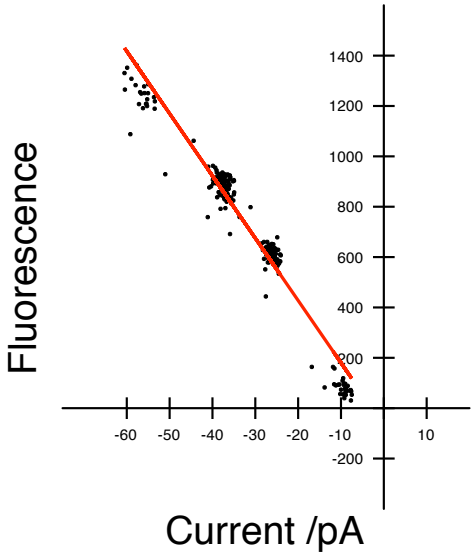

FIGURE S6: (B) Fluorescence-current plot of the data in Fig. 2C is linear.

Supplementary References

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