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**Supporting Material**

**Peptide-induced domain formation in supported lipid bilayers: Direct evidence by combined atomic force and polarized total internal reflection fluorescence microscopy**

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# **Peptide-induced domain formation in supported lipid bilayers: Direct evidence by combined atomic force and polarized total internal reflection fluorescence microscopy**

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## **Supplementary Files**

Figure S1: A 1x1  $\mu\text{m}$  AFM image of a 3:1 POPE/TOCL SPB before addition of PR-9. The image demonstrates that even at smaller scan windows, phase separated lipid domains were not observed in this type of membrane. Scale bar = 200 nm.

Movie S2: Time-lapse pTIRFM image sequence of PR-9 addition (concentration (I'), 13  $\mu\text{g}/\text{mL}$ ) to a 3:1 POPE/TOCL SPB fluorescently labeled with NAO. Image acquisition was carried out as described in the methods section of the main text. The injection event of the peptide is symbolized by the appearance of the "\*" symbol in the upper right corner of the ROI. Each frame is time-stamped with the number of milliseconds that have elapsed since the acquisition was initiated. Scale bar = 5  $\mu\text{m}$ . Movie playback is sped up to 30 frames per second.

Movie S3: Time-lapse pTIRFM image sequence of PR-9 addition (concentration (II'), 6.7  $\mu\text{g}/\text{mL}$ ) to a 3:1 POPE/TOCL SPB fluorescently labeled with NAO. Image acquisition was carried out as described in the methods section of the main text. The injection event of the peptide is symbolized by the appearance of the "\*" symbol in the upper right corner of the ROI. Each frame is time-stamped with the number of milliseconds that have elapsed since the acquisition was initiated. Scale bar = 5  $\mu\text{m}$ . Movie playback is sped up to 30 frames per second.

Movie S4: Time-lapse pTIRFM image sequence of PR-9 addition (concentration (III'), 2.0  $\mu\text{g}/\text{mL}$ ) to a 3:1 POPE/TOCL SPB fluorescently labeled with NAO. Image acquisition was carried out as described in the methods section of the main text. The injection event of the peptide is symbolized by the appearance of the "\*" symbol in the upper right corner of the ROI. Each frame is time-stamped with the number of milliseconds that have elapsed since the acquisition was initiated. Scale bar = 5  $\mu\text{m}$ . Movie playback is sped up to 30 frames per second.

Figure S5: Combined pTIRFM fluorescence (A) and AFM topography images (B-D) of a 4:1 POPE/cardiolipin (extracted from bovine heart) SPB fluorescently labeled with NAO. This type of supported bilayer exhibits phase separation at small length scales. (A) and (B) are 20x20  $\mu\text{m}$  images. AFM image (C) is a 5x5  $\mu\text{m}$  scan of the boxed region in (B) and AFM image (D) is a 1x1 scan of the boxed region in (C). The black star symbol in (A) and (B) help guide the reader's eye to identical xy spatial locations in the images. Scale bar = 5  $\mu\text{m}$ .

Figure S6: Combined pTIRFM fluorescence (A) and AFM topography images (B-D) of a 4:1 POPE/cardiolipin (extracted from bovine heart) SPB fluorescently labeled with NAO 45 minutes after exposure to PR-9 at a concentration of 2.0  $\mu\text{g}/\text{mL}$ . (A) and (B) are 20x20  $\mu\text{m}$  images. AFM image (C) is a 5x5  $\mu\text{m}$  scan of the boxed region in (B) and AFM image (D) is a 1x1 scan of the boxed region in (C). The black star symbol in (A) and (B) help guide the reader's eye to identical xy spatial locations in the images. Scale bar = 5  $\mu\text{m}$ .

POPE/TOCL (3:1)

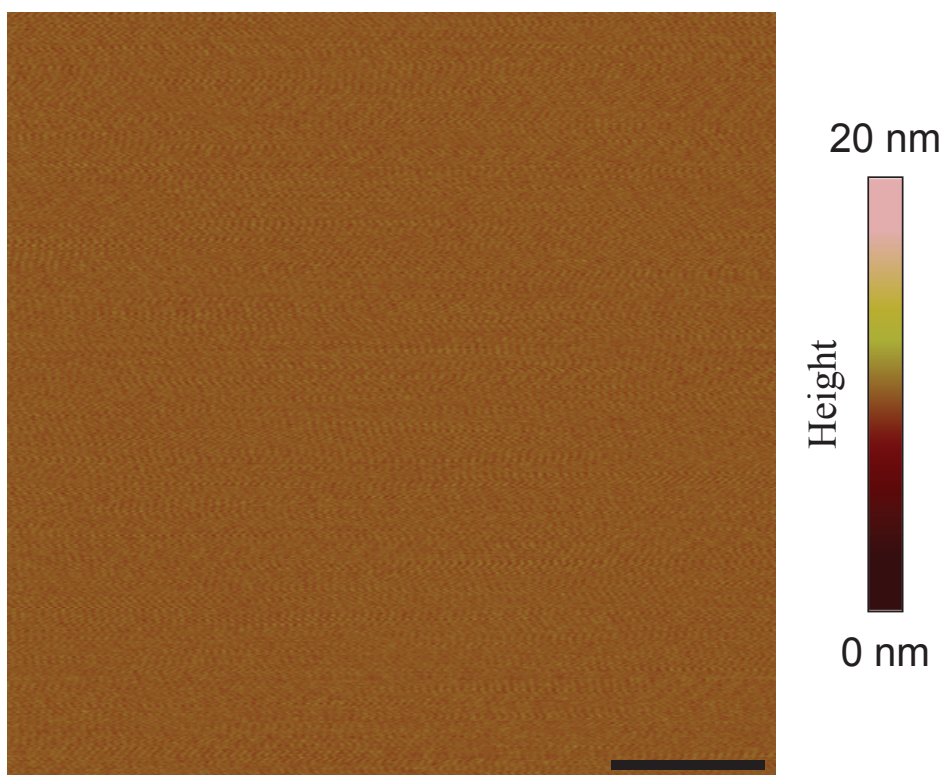


Figure S1

POPE/CL bovine heart (4:1)

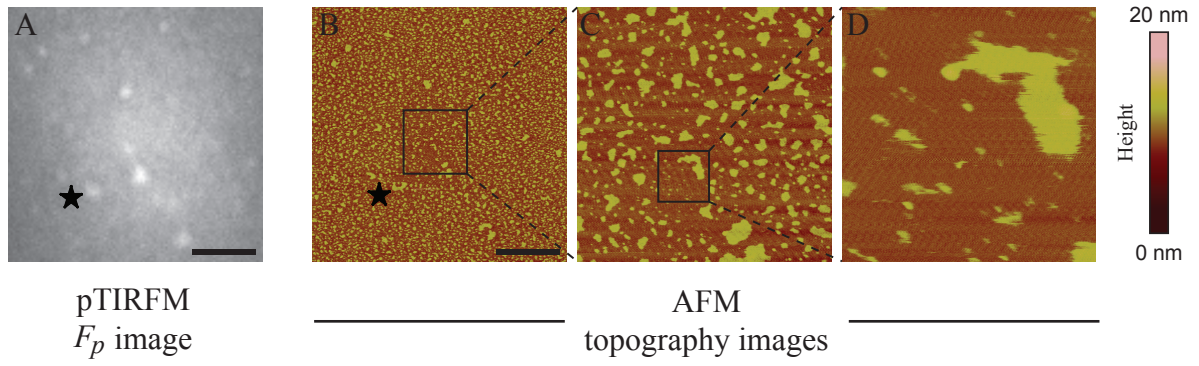


Figure S5

POPE/CL bovine heart (4:1) + PR-9 (2.0  $\mu\text{g}/\text{mL}$ ,  $t=45$  min)

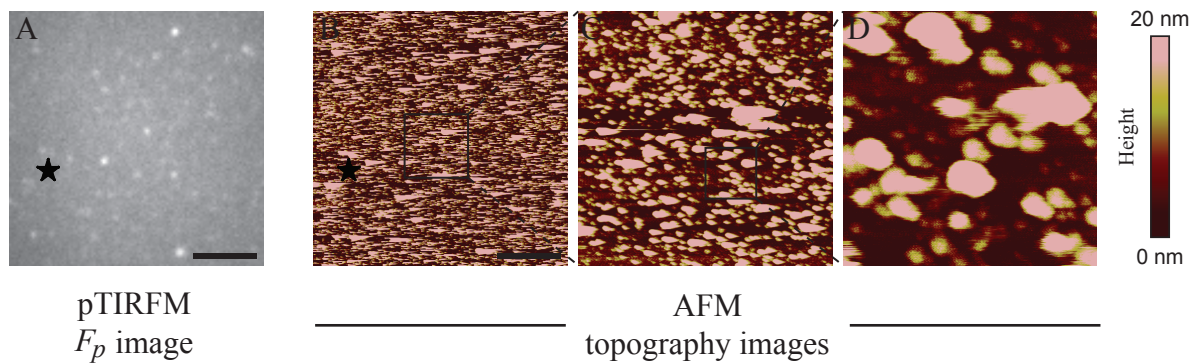


Figure S6