Supporting Information

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SI Text

Oriented Circular Dichroism (OCD). OCD is a simple method for detecting the orientation of peptides in membranes, especially for helices (1). It is by now very well established that helical pore-forming peptides adsorb to the membrane interface with helical axes parallel to the lipid bilayers at low peptide concentrations, but as the concentration increases above a threshold, an increasing fraction of the peptides change their helical orientation to become normal to the bilayers (2–5). Neutron in-plane scattering showed the presence of pores in the membranes when, and only when, the normal helical component was detected by OCD (6–8). Fig. S2 shows the OCD of Bax-α5/di18:0(9,10Br)PC mixtures at 1/30 and 1/100 molar ratios in fully hydrated multiple bilayers. Upon dehydration, we found the R phase of the 1/100 sample the same as the R phase of pure di18:0(9,10Br)PC, in the lattice constants as well as in the RH dependence (9), which are quite different from that of the sample 1/30. This implies that Bax-α5 did not form pores in the 1/100 sample. Thus, the spectrum of the 1/100 sample represents the OCD of the helical components of Bax-α5 oriented parallel to the plane of bilayers. Using the OCD of the 1/100 sample and the theoretical OCD for helices normal to the plane of bilayers (1), we analyzed the OCD of the 1/30 sample in Fig. S2 and estimated that ≈30% of the helical components of the peptides in the 1/30 sample were oriented normal to the plane of the bilayers. (If a helix is tilted by an angle θ relative to the normal of the bilayer plane, its normal helical component is $(\cos\theta)^2$ (1).) We applied the same analysis to the CD of Bax-α5 measured in SDS or TFE solutions (10) and found the result consistent with theory (1), i.e., 1/3 of its helical component in the direction of light propagation (normal to the bilayers in OCD).

The OCD of the 1/30 sample at 60%RH, 25°C (not shown) is between the two spectra in Fig. S2. However, this spectrum is not interpretable because we have found that in general not all of the peptides and lipids in the sample convert to the R phase. A detailed analysis of the alamethicin samples (9) showed that the structure of the R phase (as defined by the diffraction lattice and amplitudes) was independent of the peptide–lipid ratio in sample preparation between 1/20 and 1/30. It is as if a free-energy minimum defines a unique (but so far undetermined) peptide–lipid composition for the R phase. Thus, from an arbitrary peptide–lipid mixture, the R phase was formed at the unique peptide–lipid ratio, with extra peptide or lipid (or both) coexisting in a nondiffracting phase (9). It is reasonable to assume that this is a general characteristic of the R phase, including the case of Bax-α5. For example, as described above, the R phase from the 1/100 sample was a pure lipid R phase, implying that the peptides and associated lipid coexisted in a nondiffracting phase.

Electron Density Distribution of the Whole System. Once the phase problem for the Br amplitudes F_2 is solved, so does the phase problem for the whole system amplitudes F_0. This is because the phase relation between F_2 and F_0 was part of the solutions from the anomalous diffraction analysis (Table 1). The electron density distribution of the whole system was constructed the same way as for the Br distribution and shown in Fig. S1*B*. As in the case of alamethicin pore (Fig. S1*A*), only the high-density regions of Br atoms and phosphate groups can be identified (9). Peptides whose average electron density is close to that of water and methylene are not identifiable.

One characteristic of low-resolution electron density distributions is that if there are 2 local density maxima separated by a distance shorter than the resolution limit, the 2 maxima often merge into 1 in between. This appears to be what happens in the center of Fig. S1*B* where there are supposed to be 4 local maxima, a pair from the Br distributions and a pair from the phosphate group distributions. Note that the resolution in the hexagonal plane is worse than the vertical resolution (the highest $q_z >$ the highest q_r in Bragg peaks). This is why the phosphate layers and Br layers are well-separated vertically near the edge of the unit cell but are mixed up near the center mainly because of the poor horizontal resolution. To demonstrate this length dependence, we have reconstructed the stalk structure (11, 12) from lipid di18:0(6,7Br)PC and from di18:0(9,10Br)PC (W.W., L.Y., and H.W.H., unpublished work). Although the Br layers are separated in the stalk of di18:0(6,7Br)PC, they merge and create a maximum at the center in the stalk of di18:0(9,10Br)PC (note that we are talking about the image obtained and not the real structure). The stalk image is clear in di18:0(6,7Br)PC but not in di18:0(9,10Br)PC. The Br layers are clear in Fig. 1, but they are distorted by the low resolution in the distribution of the whole system Fig. S1*B*.

Despite the ambiguity around the center of Fig. S1*B*, the overall features of the phosphate group distribution and the Br atom distribution are still consistent with the toroidal model.

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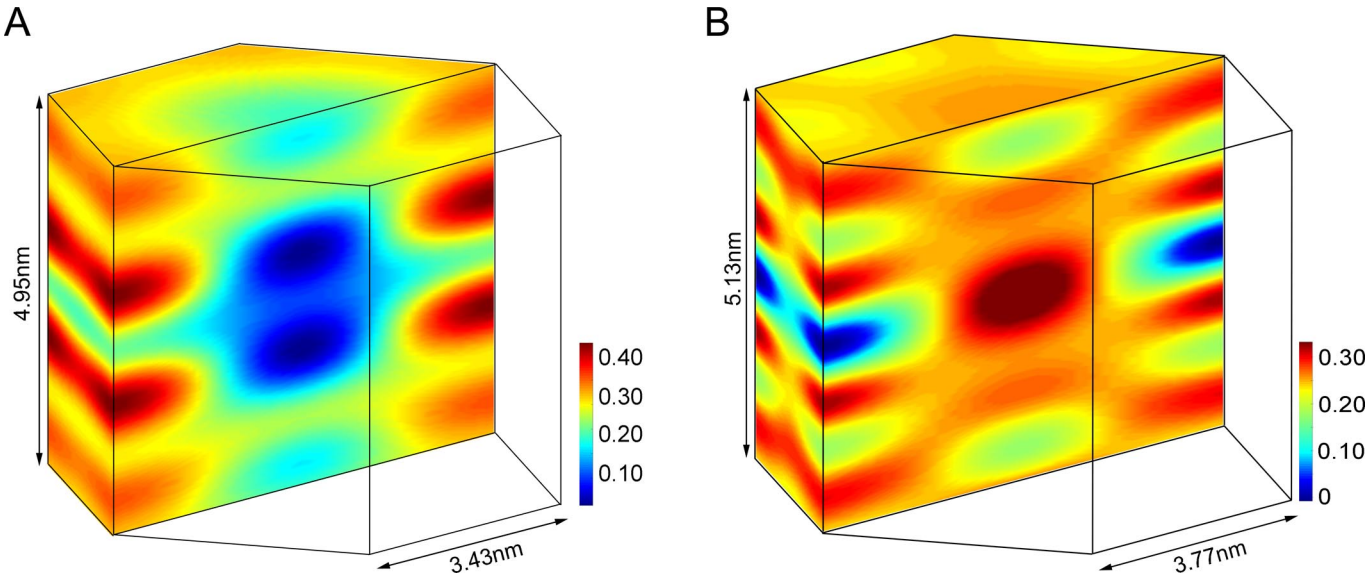

Fig. S1. Normalized electron density distributions of the whole system (peptide, lipid, and water) in a unit cell of the R phase containing alamethicin (*A*) or containing Bax-α5 (*B*). *A* is reproduced from the data of ref. 9 for comparison.

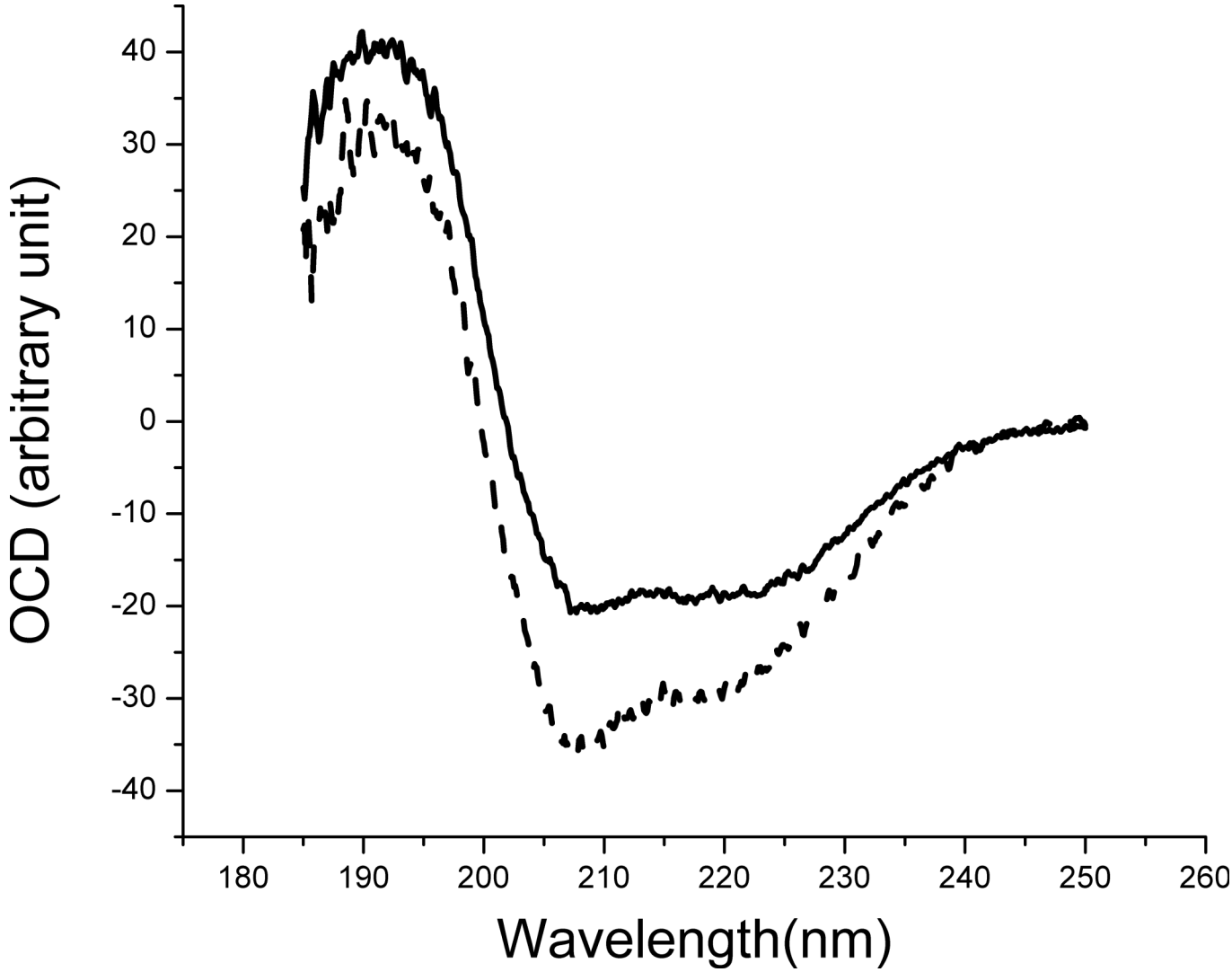

Fig. S2. OCD of Bax-α5/di18:0(9,10Br)PC mixtures at 1/30 (solid line) and 1/100 (dashed line) molar ratios in fully hydrated multiple bilayers. The lipid background has been removed, and the 2 spectra are normalized relative to each other. The spectra $<$195 nm were suppressed by strong UV absorption and are therefore not reliable.