In recent years we have made progress by neutron diffraction in a major structural challenge, namely in detecting and analyzing the structures of peptide assemblies in fluid membranes [1,2]. These experiments were performed with membranes in the form of oriented multilayers. Originally the samples were investigated in full hydration so that the physical properties of the lipid bilayers were close to those at physiological conditions. However, it was soon realized that new phenomena involving peptide-lipid interactions occur when the sample hydration is varied. In general, in full hydration, the peptide organization in each membrane is uncorrelated to the neighboring membranes. As the hydration level decreases, the peptides become correlated between bilayers, even though the membranes are still fluid. In many cases, further dehydration strengthens the correlation such that the peptide organization in the multilayers crystallizes [2]. The crystallization provides the possibility for high-resolution diffractional studies. Investigations along this line might also lead to useful information for crystallization of membrane proteins.

Antimicrobial peptides are inducible innate host defense molecules found in all multicellular organisms, including humans and plants. These peptides have a folded size comparable to the membrane thickness. All evidence indicates that antimicrobial peptides act by permeabilizing the cell membranes of microorganisms. But the molecular mechanisms of their actions are still not clear. We have found that all peptides, when they are bound to lipid bilayers, exhibit two distinct oriented circular dichroism spectra, one at low peptide-to-lipid ratios (P/L) and another at high P/L. This indicates that each peptide has two different physical states of binding to a membrane.

The transition from the low to the high P/L spectrum occurs over a narrow range of P/L as if there is a threshold concentration, called P/L*. At concentrations below P/L*, the peptides are embedded in the headgroup region, as suggested by the peptide orientation and the membrane thinning effect. At concentrations above P/L*, neutron in-plane scattering showed that the peptides form pores in the membranes, while no pores were detected below P/L*. The detection was achieved by exploiting the sensitivity of neutrons to D_2O, which had replaced the water in the membrane pores.

As an example, Fig. 1 shows neutron in-plane scattering taken at Argonne National Laboratory (ANL) of alamethicin in protonated lipid bilayers (Fig. 1a) and in deuterated lipid bilayers (Fig. 1b), with D_2O or H_2O filling the pore channels. The peak in these data is due to the fairly regular pore spacing and is most pronounced when there is a strong contrast, either between D_2O and protonated lipid, or between H_2O and deuterated lipid. All four sets of data are consistent with the model shown in Fig. 2 once the differences in contrast are taken into account (solid curves). Thus we concluded

![Figure 1](image_url)
that alamethicin in DLPC bilayers forms octameric pores in the barrel-stave fashion.

Interestingly, the barrel-stave model is not the only possible pore formation. We have detected another type, called toroidal pores, in which the lipid monolayer bends continuously from one leaflet to another like the inside of a torus [1]. However, while the evidence for the pores is clear by the detection of the water (D$_2$O) channels through the lipid bilayers, the evidence for the pore structures is indirect. Thus the discovery of the crystalline phases is an important new development for the field of antimicrobial peptides.

We developed a method of off-plane scattering [2] to record the diffraction pattern on a SANS instrument that includes both the in-plane and out-of-plane momentum components. Figure 3 exhibits some typical diffraction patterns as recorded on the NG-3 30 m SANS instrument’s detector by this method. The top left panel shows the diffraction pattern of magainin pores in fully hydrated fluid bilayers. When the sample was slightly dehydrated, the pattern changed to the top middle panel. Our analysis [2] showed that the positions of the magainin pores in each bilayer become correlated with the pores in adjacent bilayers, even though the bilayers are still in the fluid phase. The cause of this correlation was hypothesized to be due to the hydration force. Upon further dehydration or cooling, the pore arrangement crystallized into a lattice (the left panel of the middle row) having ABCABC stacking of hexagonally ordered planes.

The other crystalline patterns shown in Fig. 3 were from magainin and other peptides in various lipids at different hydration levels and temperatures. These crystalline phases offer a new way of studying peptide-lipid interactions that will help us to understand the molecular mechanisms of membrane active peptides in cell biology.

REFERENCES
