Experimental report

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Proposal: 8-02-827 **Council:** 4/2018

Title: Location of membrane-active drugs in the lipid bilayer

Research area: Biology

This proposal is a new proposal

Main proposer: Stephan GRAGE

Experimental team: Stephan GRAGE

Local contacts: Bruno DEME

Samples: Lipids
peptides or sterols

Instrument	Requested days	Allocated days	From	To
D16	4	4	20/09/2018	24/09/2018

Abstract:

The activity of many drugs is based on their interaction with lipid bilayers. In this proposal, we envisage to determine how deep such membrane-active drugs penetrate into the membrane in three examples: 1) the helical antimicrobial peptides PGLa and magainin-2, 2) the beta-strand antimicrobial peptide KIGAKI, and 3) the sterol squalamine which prevents amyloid formation in Parkinson disease. Using neutron small angle scattering on oriented bilayer samples, we plan to localize the compounds within the bilayer in order to get clues on their mode of action.

Experimental Report

Location of membrane-active drugs in the lipid bilayer

Proposal: 8-02-827 Instrument: D16 Dates: 20-24.9.2018

Stephan L. Grage
Karlsruhe Institute of Technology, Institute of Biological Interfaces IBG-2

Summary. The activity of many drugs is based on their interaction with lipid bilayers. In our neutron scattering experiments on D16 we studied how deep such membrane-active drugs penetrate into the membrane in three examples: 1) the helical antimicrobial peptides PGLa and magainin-2, 2) the beta-strand antimicrobial peptide $(KL)_5$, and 3) the sterol squalamine which prevents amyloid formation in Parkinson disease. Scattering amplitudes were determined from oriented bilayer samples, which allow to localize the compounds within the bilayer in order to get clues on their mode of action.

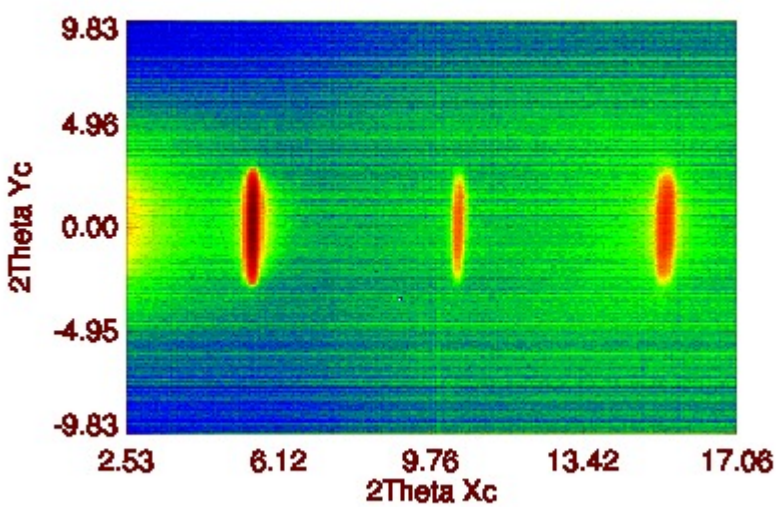
Scientific background. Drugs which target cell membranes have a large potential. For example, bacteria cannot develop resistances against antimicrobial peptides which destroy bacterial membranes as easily as against antibiotics targeting specific enzymes. As another example, squalamine, a sterol from shark, inhibits binding of alpha-synuclein to cell membranes and this way inhibits aggregation of this protein, which is discussed as causing Parkinson disease. In this study, we aimed at characterizing the interaction of such drugs with the lipid bilayer. An important question not yet well understood is the depth of pepnetration of such compounds into the bilayer. Using low resolution neutron diffraction we addressed this point, where we employed oriented samples to determine the scattering length density profile along the membrane normal direction.

Samples. The insertion depth was studied for several membrane-active compounds: 1) two alpha-helical antimicrobial peptides, 2) two beta-strand forming peptide sequences, and 3) the sterol squalamine.
1) The two alpha-helical peptides PGLa and magainin-2 originate from frog skin, and have been found in different orientations in the membrane previously. We also studied the mixture of PGLa and magainin-2 (1:1), as for this combination of peptides a synergistic enhnacement of activity and a transmembrane insertion of PGLa has been observed.
2) As an example for a beta-strand forming peptide we prepared also samples with $(KL)_5$, both without (KL10) and with (KL10meth) additional N-methylation of the leucine residues. (The originally envisaged KIGAKI peptide sequence was replaced by the KL repeat sequence the latter shows a high antimicrobial activity.)
3) In addition, we monitored potential changes in the membrane penetration depth of the KL sequences induced by squalamine.
All peptides were reconstituted into chain deuterated lipid bilayers consisting of DMPC-d54 and DMPG-d54 (4:1). The peptide:lipid ratio and squalamine:lipid ratios were 1:40 (mol:mol) and 1:10 (mol:mol), respectively. An extra sample with squalamine:lipid = 1:30 was prepared on-site. The bilayers were oriented by deposition on a glas slide, where 10mg of lipids and ~0.4 mg peptide and ~1mg squalamine was used per sample. Three different contrasts were achieved by hydrating the samples with 30%, 70% or 100% D_2O (vol%).

Sample preparation. Peptides, squalamine and lipids were co-dissolved in methanol/chloroform/water mixtures, and some water was added to achieve better spreading in the next step (final volumes of methanol/chloroform/water: PGLa: 0.6ml/0.1ml/0.1ml, magainin-2: 0.7ml/0.2ml/0.1ml, PGLa+magainin-2: 0.7ml/0.1ml/0.1ml, KL10: 0.4ml/0.2ml/0.2ml, KL10meth: 0.4ml/0.2ml/0.2ml, squalamine: 0.4ml/0/0.1ml, KL10+squalamine: 0.5ml/0/0.1ml). The solution for each sample was spread on a quartz glass slide (2in x 1in, 1mm thick, PLANO), dried under vaccuum, and stored at room temperature until measurement.

Sample environment. The glass slides with the dry lipid film were mounted vertically inside a sample chamber, which allowed precise temperature and humidity control (development Bruno Demé, D16 at ILL). After alignment of the beam in the actual position of measurement, the sample was incubated inside the closed chamber for at least 2-3h before measurement, and then measured in the same sample cell. The contrast was adjusted by using the desired H_2O/D_2O mixture as water reservoir in the sample chamber. Three sample chambers were used to allow the incubation of samples in advance. All samples were measured at 100% humidity and at 35°C.

Instrument setup. Experiments were performed on D16. The sample glass slide was mounted vertically, and rotated about the vertical axis (Omega angle) in the course of an acquisition. Such Omega-scans were conducted for two detector positions, where the detector was turned out of the direction of the incident beam by Gamma = 12° or Gamma = 27°, respectively. At Gamma = 12°, Omega was scanned in the range from -1.0° to 12° in steps of 0.05°, and at Gamma = 27°, Omega was scanned from 10° to 20° in steps of 0.05°. An additional scan was performed with inserted attenuator (attn 5) for Gamma = 12° in the Omega-range of 1.5° to 3.5°. Exposure time was 15s at each Omega position. The detector-sample distance was 920 mm and the detector width was 20°. Collimation slits were set to 4.0 mm (slit 1) and 4.9 mm (slit 2). The wavelength was 4.52 Å.



Results. Aim of the study was to determine the interaction of a representative set of antimicrobial peptides and squalamine with lipid bilayers, and, in particular, to determine how deep they insert into the lipid bilayer. Using uniformly aligned bilayers allowed to measure the intensities of the reflections resulting from this multilayer liquid crystal structure. As seen in the representative detector image of PGLa in DMPCd54/DMPGd54 (see Figure), indeed several reflections could be measured, in some samples up to the 6th order reflection. The position of the first Bragg reflection was observed in the range of 2 Theta = 5.1° to 5.5°, which results into bilayer repeat distances between 46 Å and 52 Å. These values are in good agreement with literature values reported for DMPC bilayers, and indicate full hydration. The intensities of the reflections encode the position of the guest molecules in the bilayer matrix, their analysis is in progress. However, we noted already variations in the intensity patterns of different samples, indicating different positions of the guest molecules in the bilayer.