# Experimental report

04/02/2021

**Proposal:** 8-02-871 **Council:** 4/2019

**Title:** Influence of spontaneous curvatureof membrane-active drugs on their insertion into lipid bilayers

**Research area:** Biology

**This proposal is a new proposal**


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**Experimental team:** Stephan GRAGE

**Local contacts:** Bruno DEME


**Samples:** Water
peptides
LIPIDS

| Instrument | Requested days | Allocated days | From | To |
|---|---|---|---|---|
| D16 | 5 | 5 | 06/02/2020 | 11/02/2020 |


## Abstract:

The insertion into lipid bilayers plays an important role for many drugs. The spontaneous curvature of lipids has been discussed as having a pronounced influence on how far such guest molecules penetrate into a lipid bilayer. Vice versa, also the spontaneous curvature of amphiphilic guest molecules themselves is expected to direct guest molecules either towards the interior of the membrane or the water/bilayer interface. In this proposal we plan to use a series of amphipathic alpha-helical peptides with different spontaneous curvatures to evaluate the role of this parameter on the depth of insertion into a lipid bilayer. The set of peptides is designed to possess different cross section shapes leading to different spontaneous curvatures. We envisage to compare the insertion depth in bilayers with different lateral pressure profiles, to monitor the interplay of peptide and membrane spontaneous curvature. Following a previous study at ILL (proposal 8-02-827, see corresponding experimental report), we propose to use neutron small angle diffraction on oriented bilayer samples to determine the scattering length density profile across the lipid bilayer.

# Experimental Report

## Influence of spontaneous curvature of membrane-active drugs on their insertion into lipid bilayers

Proposal: 8-02-871 Instrument: D16 Dates: 6-11.2.2020

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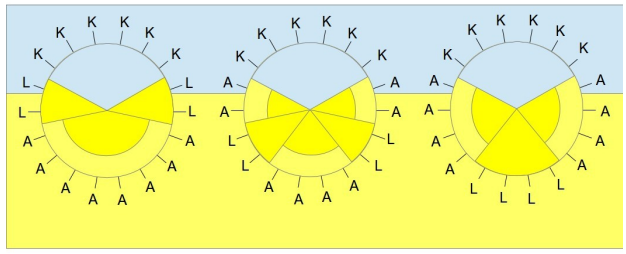
**Summary**. The insertion into lipid bilayers plays an important role for many drugs. The spontaneous curvature of lipids has been discussed as having a pronounced influence on how far such guest molecules penetrate into a lipid bilayer. Vice versa, also the spontaneous curvature of amphiphilic guest molecules themselves is expected to direct guest molecules either towards the interior of the membrane or the water/bilayer interface. In this project, a series of amphipathic alpha-helical peptides with different spontaneous curvatures was studied using small angle neutron diffraction, to evaluate the role of this parameter on the depth of insertion into a lipid bilayer.
The peptides were reconstituted into oriented lipid bilayers. The scattering length density profile across the lipid bilayer was determined by measuring the neutron scattering for a series of different sample orientations with respect to the incident beam. Up to the 6th order Bragg reflection could be detected. As the Bragg peaks vary in intensity, we are confident that the data can be analyzed in terms of a scattering length density profile across the lipid membrane, leading to conclusions of the position of the peptides within the bilayer. Furthermore, a membrane thinning induced by the peptides could be observed from the positions of the reflections.

**Scientific background.** Many drugs interact with cell membranes, as they need to cross membranes to reach their target, or even target the membrane of pathogens directly. Depending on how such molecules interact with membranes, they may possess a cell penetrating activity or be able to destroy membranes of pathogens. A decisive parameter for the mode of membrane-activity is the depth of insertion into the bilayer. Here, the physical properties of the lipid bilayer, in particular the spontaneous curvature, have been discussed as being important for the insertion of, for example, antimicrobial peptides into membranes. The insertion of molecules in the bilayer is namely influenced by the shape of the lipid molecules, i.e. whether they possess a larger headgroup or extended tail region. Similarly, also membrane proteins have been attributed a cross sectional shape, and conformational changes of the protein can be explained by an adaptation of this shape profile to the lateral pressure profile of the lipid bilayer.
Also in the case of smaller amphiphilic peptides, the distribution of large and small sidechains leads to a shape profile and spontaneous curvature of the peptide. In this study we aimed at elucidating the influence of this shape profile of the peptide on its insertion into the membrane. Three different alpha-helical, amphiphilic peptides will be prepared which possess a different distribution of large leucine residues, giving the peptides a different shapes. From the helical wheel plots (see Figure), we expected a different depth of penetration in membranes with different lateral pressure profile. To modulate the lateral pressure profile in the lipid bilayer, we added lysolipids or DOPE to DMPC bilayers.

**Samples**. Three peptides with different cross section profiles were compared:
L45: KAAKLALKAAKLALKAAK
L67: KALKAAAKLLKAAAKLAK
L89: KLAKALAKAAKALAKALK



All peptides were characterized in lipid bilayers of chain-deuterated dimyristoyl-phosphatidyl-choline (DMPC-d54). To obtain membranes with different lateral pressure profiles, 20 mol% lyso-dimyristoylphosphatidylcholine (lysoMPC) or 10 mol % dioleoylphosphatidylethanolamine (DOPE) was added to DMPCd54. The membranes with lysoMPC or DOPE were compared with membranes of just DMPCd54. A peptide:lipid ratio of 1:40 was used.

**Sample preparation.** The peptides and lipids were dissolved in methanol for stock solutions. Aliquots were mixed to obtain the various sample mixtures containing each 0.7mg peptide and 10mg lipid. The solutions (~400µl) were spread onto quartz slides (2" x 1" x 1mm, Precision Optics), and dried on air and under vacuum to obtain a lipid/peptide film. Water was added before drying in some samples if the film was not smooth.

**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 contrasts were used: 30 vol% $D_2O$, 70 vol% $D_2O$, 100 vol% $D_2O$. 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 = 29°, respectively. At Gamma = 12°, Omega was scanned in the range from -1.0° to 12° in steps of 0.05°, and at Gamma = 29°, 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 10s or 20s at each Omega position for Gamma=12° or 29°, respectively. The detector-sample distance was 955 mm and the detector width was 20°. Collimation slits were set to 4.95 mm (slit 1) and 4.64 mm (slit 2). The wavelength was 4.477 Å.

**Results.** Aim of the study was to determine how deep peptides with different cross sectional shapes immerse into lipid bilayers, which possess different lateral pressure profiles. To answer this question, neutron diffraction data was acquired to obtain the depth of insertion from the lateral pressure profiles. Using uniformly aligned bilayers allowed to measure the intensities of reflections resulting from the resulting multilayer liquid crystal structures. As seen in the representative scattering data of the peptides in DMPCd54 membranes (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. Furthermore, the reflections shift to larger scattering angles in the samples with peptides as compared to the control sample (bottom of the figure). This reflects a membrane thinning induced by the peptides, which has been described as a typical interaction of amphiphilic peptides with lipid bilayers.