Experimental report

10/01/2022

Proposal: DIR-217 **Council:** 4/2020

Title: Peptide induced lipid flip-flop inasymmetric membrane mimics of gram-negative bacteria

Research area:

This proposal is a new proposal

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Samples: Lipid mixtures of POPE/POPG and antimicrobial peptides

Instrument	Requested days	Allocated days	From	To
D22	2	1	02/02/2021	03/02/2021

Abstract:

REPORT

Peptide induced Lipid Flip-Flop in Asymmetric Membrane Mimics of Gram-Negative Bacteria

Antimicrobial peptides (AMPs) are well-studied compounds with high potential for application against multi-resistant bacterial strains [1]. While the mode of action of AMPs has been studied for decades using lipid-only membranes, the ability to use more realistic mimics of bacterial membranes in form of vesicles with transbilayer asymmetry has become feasible only recently. We used time-resolved SANS to study the response of asymmetric lipid membranes composed of palmitoyl oleoyl phosphatidylethanolamine (POPE) and chain deuterated palmitoyl oleoyl phosphatidylglycerol (POPG-31), to interactions with the frog peptides L18W-PGLa and magainin 2 (MG2a), as well as the lactoferricin derivative LF11-215. In particular we fabricated POPG-d31^{in}/POPE^{out} asymmetric large unilamellar vesicle (aLUVs) using cyclodextrin-mediated lipid exchange [2]. Using POPG-31 then allowed us to generate sufficient contrast between inner and outer leaflets to determine the peptide-induced lipid flip-flop exploiting SANS at D22 (Fig. 1).

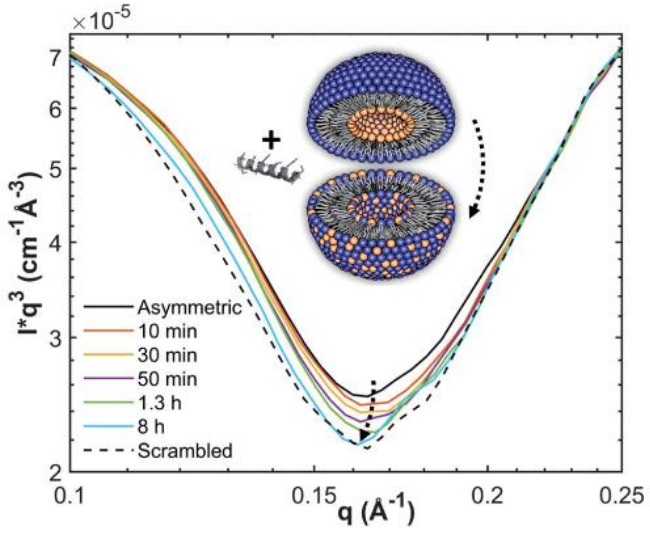

Fig. 1: *Measurement principle and scattering contrast between POPG-d31^{in}/POPE^{out} aLUVs and scrambled LUVS in 100% D2O buffer, as observed by SANS at 37°C. Scattering contrast was additionally enhanced by multiplying the scattered intensities with q^3.*

The change of contrast $\Gamma = \int I\, q^3 dq$ in the q-range shown in Fig. 1 follows

$$\Delta\Gamma = e^{-ik_f t}, \qquad (1)$$

where k_f is the lipid flip-flop rate. Additionally, we fitted all SANS data in the full q-range to make sure that the peptides did not induce any morphological changes, e.g. the formation of multilamellar structures (data not shown).

In the absence of peptides, no significant changes of scattering intensity were observed during the time course of the experiments (i.e. ~24 hours). This signifies that the produced aLUVs are sufficiently stable for all experiments presently reported. Figure 2 shows the contrast changes induced upon the addition of L18W-PGLa and the analysis in terms of Eq (1). Results for all studied peptides are presented in Tab. 1. The addition of L18W-PGLa induced an equilibration of lipid distribution across both leaflets with a rate that strongly increased with peptide concentration. Interestingly, LF11-215 led at an [P]/[L] as high as the the highest L18W-PGLa concentration to no detectable lipid flip-flop (Tab. 1).

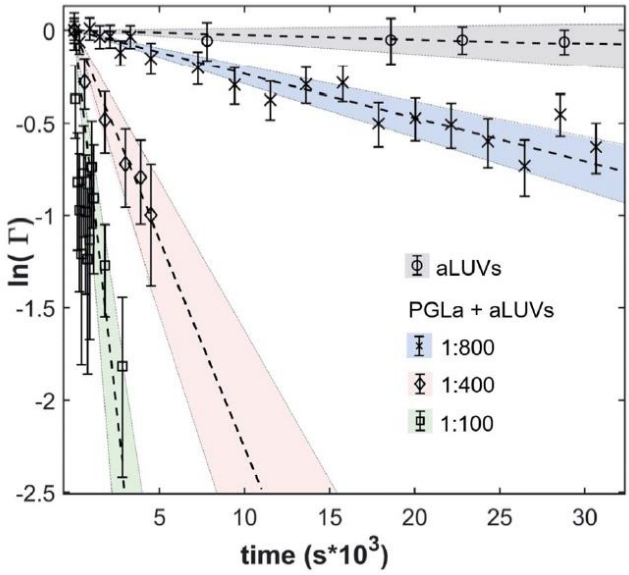

Fig. 2: *Decay of scattering contrast between aLUVs and scrambled LUVs due to L18WPGLa-mediated lipid flip-flop at [L] = 9 mM and different [L]/[P]. As a control, aLUV data in the absence of peptide are also shown.*

Table 1: *Flip-flop rates k_f and flip-flop half-times $t_{1/2}$ for mixtures of asymmetric vesicles with L18W-PGLa, MG2a, their equimolar mixture, and LF11-215 at different [P]/[L] ratios.*

Peptide	[P]/[L]	$k_f \times 10^{-5}$ (s^{-1})	$t_{1/2}$ (min)
L18W-PGLa	1:100	42 ± 13	14 ± 4
	1:400	11 ± 4	52 ± 16
	1:800	1.2 ± 0.4	500 ± 200
MG2a	1:100	1.4 ± 0.5	420 ± 140
	1:200	< 0.6	> 10^3
L18W-PGLa:MG2a	1:800	0.8 ± 0.3	700 ± 300
LF11-215	1:100	< 0.6	> 10^3

Additionally, we studied lipid flip-flop as induced by MG2a and an equimolar mixture of L18W-PGLa and MG2a. MG2a, similar to L18WPGLa, is supposed to remain membrane-surface aligned in the present conditions, while its equimolar mixture is well-known for its synergistic activity [3]. Our flip-flop analysis showed that MG2a is significantly less potent than L18WPGLa in translocating lipids. No detectable lipid flip-flop was found for [P]/[L] = 1 : 200 and rates at doubled MG2a concentration were comparable to L18W-PGLa at [P]/[L] = 1 : 800. Interestingly, the equimolar mixture of L18W-PGLa and MG2a did not exhibit a faster lipid flip-flop at [P]/[L] =1 : 800 than L18W-PGLa alone. However, the equimolar peptide mixture contains only [P]/[L] = 1 : 1600 of either L18W-PGLa and MG2a. Considering that lipid flip-flop will drop significantly for AMP these concentrations, then suggest that the measured half-time for the peptide mixture is indeed a consequence of L18W-PGLa/MG2a-synergism. All results, including complimentary leakage and peptide partitioning data, are reported and discussed in detail in Marx et al. [4]

References

[1] N. Malanovic *et al.*, Biochim Biophys Acta **1862**, 183275 (2020).

[2] M. Doktorova *et al.*, Nat Protoc **13**, 2086 (2018).

[3] a) I. Kabelka *et al.*, Biophys J **118**, 612 (2020); b) M. Pachler *et al.*, Biophys J **117**, 1858 (2019).

[4] L. Marx *et al.*, Faraday Discuss. **232**, 435 (2021).