Experimental report

24/04/2026

Proposal: 9-13-997 **Council:** 4/2021

Title: Unraveling Trans-Membrane CouplingMechanisms in PE-PC-Sphingomyelin containing Asymmetric Lipid Vesicles

Research area: Soft condensed matter

This proposal is a new proposal

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Samples: 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanolamine
1-palmitoyl-2-oleoyl-glycero-3-phosphocholine
Milk Sphingomyelin (CAS No 475662-40-9)

Instrument	Requested days	Allocated days	From	To
D11	0	0		
D33	0	0		
IN15	2	3	28/09/2021	01/10/2021
D22	1	1	01/10/2021	02/10/2021

Abstract:

We have evidence for trans-bilayer coupling mechanisms in asymmetric large unilamellar lipid vesicles, containing either combinations of phosphatidylethanolamine (PE) and phosphatidylcholine (PC), or PC and the highly chain-asymmetric milk sphingomyelin (MSM). The exact mechanisms therefore are however still unclear, as there might be contributions from H-bonding networks and dynamic chain interdigitation. Beamtime on IN15 and D22 would allow us to separate these contributions by the study of trans-membrane structure and bending fluctuations in 3 asymmetric systems: first, PC in the outer leaflet, opposed to the H-bonding PE, a system which is structurally coupled in the gel phase. Second, we use MSM instead of PC, introducing interdigitation stress by the long acyl chains in MSM. Third is an intermediate case, using a 1:1 mixture of PC and MSM in the outer leaflet. We will be thereby able to estimate the contribution strength of either mechanism.

Experimental Report: Anomalous Membrane Stiffening Induced by Transbilayer Aminophospholipid Asymmetry

1. Introduction and Experimental Objectives

Biological membranes are defined by a sophisticated transbilayer lipid asymmetry, which is vital for cellular signaling and mechanical integrity. In mammalian plasma membranes, choline phospholipids such as POPC and sphingomyelin are sequestered in the outer leaflet, while aminophospholipids like POPE and POPS are actively maintained in the inner leaflet by flipases and flopases. While this structural organization is well-documented, the mechanical coupling between these leaflets—specifically the influence of asymmetry on bending rigidity—remains poorly understood.

This study evaluates the mechanical coupling and bending rigidities of asymmetric large unilamellar vesicles (aLUVs) compared to physically scrambled (symmetric) vesicles and cognate leaflet controls. The primary goal is to determine how the specific distribution of lipids across the bilayer dictates mechanical properties beyond simple compositional averaging. Cholesterol was deliberately excluded from the current experimental design. While cholesterol is a fundamental component of biological membranes, it is known to induce nanoscopic lipid domains and heterogeneities. By utilizing pure phospholipid mixtures, this study probes fundamental mechanical interactions without interference from cholesterol-induced domain formation. All results are published in Frewein et al., Biophys. J 2023.

2. Experimental Materials and Methodology

Asymmetric Vesicle Preparation

- aLUVs were engineered using a heavy donor cyclodextrin exchange protocol.
- Acceptor Lipids (Inner Mimics): POPE or POPE/POPS (7:3 mol/mol) mixtures.
- Donor Lipids (Outer Mimics): MSM, ESM, POPC, or equimolar POPC/MSM mixtures.
- Protocol: Acceptor vesicles were incubated with donor lipids complexed with methyl-beta-cyclodextrin. Success was verified by NMR shift reagent experiments and fatty acid analysis.

Technical Specifications of SANS and SAXS

Structural parameters were derived via joint analysis of Small-Angle Neutron Scattering (SANS) and Small-Angle X-ray Scattering (SAXS).

- Instruments: SANS data were collected on the D22 spectrometer (ILL, Grenoble); SAXS data were recorded at the BM29 beamline (ESRF, Grenoble).

- Modeling: A Scattering Density Profile model was utilized to derive bilayer thickness, hydrocarbon thickness, and average area per lipid.

Neutron Spin-Echo Spectroscopy

Mechanical fluctuations and bending rigidities were measured using the IN15 spin-echo spectrometer at ILL.

- Conditions: Experiments were conducted at 50°C to ensure all lipids remained in the fluid lamellar phase.
- Fourier Time Range: Data were collected from 0.01 to 300 ns to capture the undulatory motion of the bilayers.

3. Theoretical Framework

Dynamics are captured via the intermediate scattering function. According to the model, the decay of fluctuations follows a characteristic power-law relationship. The decay constant is linked to the effective bending rigidity and depends on solvent viscosity and thermal energy. To convert effective rigidity to absolute bending rigidity, the model incorporates internal dissipation within the bilayer.

4. Results: Structural and Compositional Analysis

Compositional analysis confirmed varying degrees of exchange, with POPC systems achieving higher asymmetry than those containing sphingolipids. Structural data demonstrate that asymmetric vesicles are generally more laterally condensed compared to scrambled analogs, with one noted exception.

5. Discussion: Anomalous Membrane Stiffening

The data reveal a clear departure from standard additive mechanical models. For most systems, asymmetry significantly increases bending rigidity. Some asymmetric vesicles exhibit anomalous stiffening, where rigidity exceeds that of both symmetric reference systems. MSM contains highly asymmetric hydrocarbon chains that allow for transbilayer interdigitation, which appears to alleviate the stiffening effect. The asymmetric distribution of hydrogen-bonding and charged lipids likely induces strong intraleaflet coupling, increasing contributions from short-wavelength fluctuations.

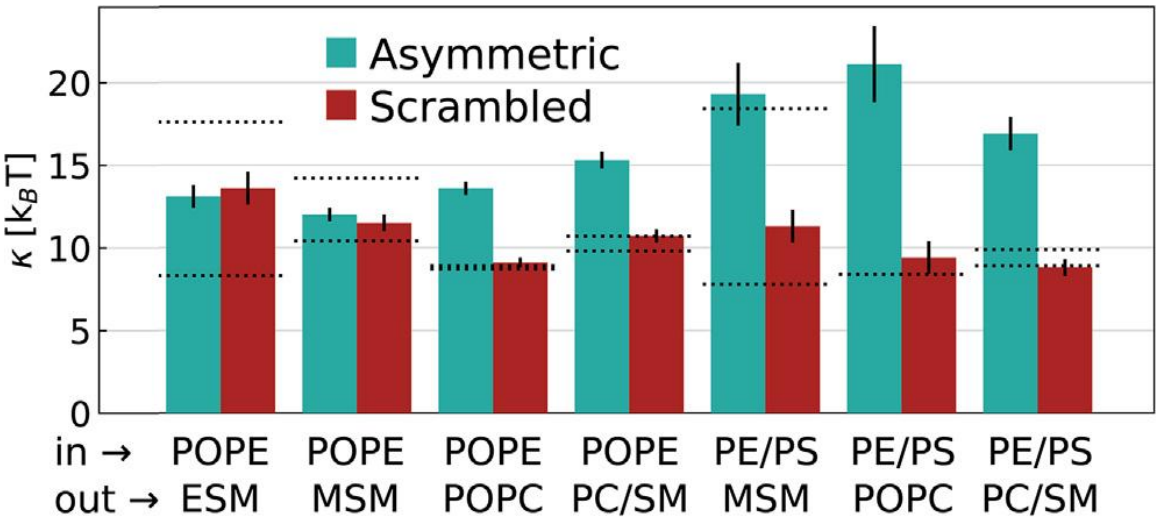

Figure 1: Bending rigidities transitions across lipid systems. Most asymmetric systems show higher rigidity. Some systems show more than double the rigidity compared to scrambled counterparts.

6. Conclusion

This research demonstrates that transbilayer aminophospholipid asymmetry induces a mechanical stiffening transition that cannot be explained by membrane thickness or differential stress. The stiffening exceeds that of either leaflet alone, suggesting cells may use lipid asymmetry to regulate membrane mechanics.

7. References

Frewein, M. P. K., et al. (2023). Distributing aminophospholipids asymmetrically across leaflets causes anomalous membrane stiffening. Biophysical Journal, 122, 1-12 doi: 10.1016/j.bpj.2023.04.025