Experimental report

13/09/2023

Proposal: **DIR-277** **Council:** 10/2022

Title: New approximations for the dynamicstructure factor of vesicles: finite size and membrane viscosity effects

Research area: Soft condensed matter

This proposal is a new proposal

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Samples: SOPC in D2O
CTAB/SOS 3/7 in D2O
CTAT/SDBS 2/8 in D2O/d-glycerol
SOPC in D2O/d-glycerol
CTAT/SDBS 8/2 in D2O
CTAB/SOS 1/9 in D2O
CTAB/SOS 1/9 in D2O/d-glycerol
CTAB/SOS 3/7 in D2O/d-glycerol
CTAT/SDBS 8/2 in D2O/d-glycerol

Instrument	Requested days	Allocated days	From	To
D22	1	0		
IN15	6	5	12/04/2023	17/04/2023

Abstract:

The use of NSE to study membrane dynamics has expanded rapidly in the past twenty years, and more than 15% of papers published in 2020 that mentioned NSE were on membrane dynamics. While interest in these studies continues to grow, there are still major challenges in interpreting NSE data and relating the measured dynamic structure factor (DSF) to fundamental membrane properties of interest. It is currently almost impossible to compare published values of the membrane bending modulus from different NSE studies, and the membrane rigidity values from NSE can be orders of magnitude different from those measured with other common biophysical techniques. Here we propose to test the applicability of newly developed expressions for the DSF that account for the effects of the finite vesicle size and membrane viscosity on NSE data using well-established model systems. These new expressions eliminate the need for exhaustive numerical integrations while still accurately accounting for the finite size effects in NSE data analysis and will enable more robust determination of the membrane rigidity and viscosity.

Preliminary experiment report for DIR-277

The number of neutron spin echo (NSE) papers on membrane dynamics has increased by almost a factor of 30 in the past twenty years. [1] Membrane dynamics also were the focus of almost 40% of proposals submitted for the IN15 Spectrometer at ILL in 2019, and more than 15 % of papers published in 2020 that mentioned NSE. [1] Despite the rapid growth and impact of NSE studies of membrane dynamics, major unresolved challenges in relating the measured dynamic structure factors (DSF) to the the fundamental membrane properties remain.

NSE data from model membrane systems are most often analyzed with the Zilman-Granek (ZG) framework that considers the undulation dynamics of a quasi-planar membrane patch. [2] The ZG framework predicts two important scaling relationships for the DSF. The first is that the DSF decays as a stretched exponential with a stretching exponent of 2/3,

$$S(q,t) \approx S(q)\exp\left[-\left(\Gamma_{ZG,q}t\right)^{2/3}\right] \ , \tag{1}$$

and the second is that the corresponding relaxation rate, $\Gamma_{ZG,q}$, decays as q^3

$$\Gamma_{ZG,q} = \left(\frac{\Gamma[1/3]}{4\pi 4^{2/3}}\right)^{3/2} \frac{(k_B T)^{3/2}}{\eta\kappa^{1/2}} q^3 \ . \tag{2}$$

in which η is the viscosity of the surrounding solvent and κ is the bending modulus of the membrane. While the predicted scaling relationships have been seen in a number of experiments, the extracted values of κ are at least an order of magnitude greater than expected for lipid vesicles. [3] Despite several attempts to rectify this discrepancy, the values of κ remain too high, even when the proposed effects of dissipation within the bilayer are taken into account, [4] or a somewhat arbitrary amplitude is included in the ZG model. [1] These corrections are essentially fudge factors, and it remains difficult to compare absolute values of κ with other experimental methods or even between NSE experiments.

An important difference between the assumptions in the original ZG model and the experiments is the spherical geometry of the vesicle membranes most studied with NSE. As such, the experiments performed in DIR-277 were designed to test new expressions for the DSF that explicitly account for the spherical geometry and finite size effects.

Results

We prepared a series of lipid vesicles composed primarily of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (16:0-18:1 PC, POPC), a well studied model lipid, and doped with 10 mol % charged 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoserine (POPS) to ensure that the vesicles were unilamellar. The solution was extruded through filters with varying pore diameters to form vesicles with radii (R) ranging from $\approx$ 30 nm to 100 nm. Complementary small angle neutron scattering (SANS) measurements on D22 confirmed that the vesicles were unilamellar and relatively monodisperse. The sample properties are summarized in Table 1.

filter pore size (nm)	c_{lipid} **(mg/mL)**	R **(nm) SANS**	**polydispersity**	R_H **(nm) DLS**
400	2	98.3	0.30	99.2
200	4	78.1	0.30	80.3
100	20	48.1	0.30	56.5
50	20	29.7	0.28	40.9

Table 1: Summary of sample properties

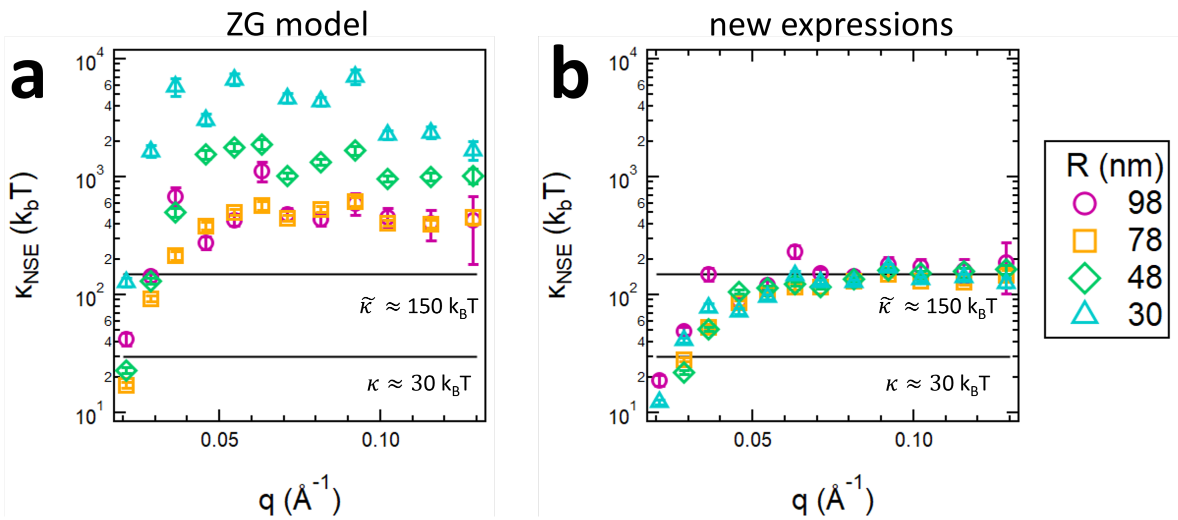

Figure 1: (a) Extracted bending moduli from NSE measurements of 90%POPC + 10%POPS vesicles of different sizes using the conventional stretched exponential expressions [2] and (b) using our new expressions that take into account the spherical geometry and finite size effects. The solid lines indicate the expected values for κ and $\tilde{\kappa}$ as indicated on the plots.

NSE data were then collected on these samples on the IN15 instrument using neutron wavelengths (λ) of 13.5 Å, 12 Å, 10 Å and 8 Å at scattering angles Θ of 3.5°, 6°, 7.5°, and 8.5°, respectively, covering a combined q-range from 0.02 Å^{-1} to 0.18 Å^{-1} and Fourier times up to 300 ns. As seen in Fig. 1a, fitting the measured DSF with the traditional ZG-framework (Eq. 1 and 2) gave values of the bending modulus (κ_{NSE}) that strongly depended on R and were orders of magnitude larger than those extracted from other experimental techniques or predicted theoretically.

Quite promisingly, fitting the data with our new DSF expressions with the values of R and $polydispersity$ from the SANS data gave consistent values of κ_{NSE} for all sizes (Fig. 1b). What is more, the extracted $\kappa_{NSE} \approx 140$ k$_B$T are in excellent agreement with predicted values of the effective bending modulus, $\tilde{\kappa} \approx 150$ k$_B$T, that accounts for the extra source of dissipation at the nanoscale. [5, 6] We are currently testing the sensitivity of the analysis as well as comparing the extracted κ_{NSE} values with different theoretical predictions for membrane undulation dynamics. We expect to submit a manuscript on the results by the end of the year. [7]

References

[1] I. Hoffmann. *Frontiers in Physics* **8**, 620082 (2021).
[2] A. Zilman and R. Granek. *PRL* **77**, 4788 (1996).
[3] Z. Yi, M. Nagao, and D. P. Bossev. *Journal of Physics: Condensed Matter* **21**, 155104 (2009).
[4] E. G. Kelley, E. E. Blick, V. M. Prabhu, *et al.* *Frontiers in Physics* 288 (2022).
[5] U. Seifert and S. A. Langer. *Europhysics Letters* **23**, 71 (1993).
[6] M. C. Watson and F. H. Brown. *Biophysical Journal* **98**, L9 (2010).
[7] R. Granek, I. Hoffmann, E. Kelley, *et al.* *to submitted in 2023.*