Experimental report

07/11/2023

Proposal: 9-13-1040 **Council:** 10/2022

Title: Influence of NSAIDs on the Phospholipid Mammalian Neuronal Membrane

Research area: Soft condensed matter

This proposal is a new proposal

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Samples: Ibuprofen
Lipid
Aspirin
Diclofenac

Instrument	Requested days	Allocated days	From	To
IN15	7	5	26/05/2023	31/05/2023
D22	1	1	07/04/2023	08/04/2023
IN5	6	3	11/04/2023	14/04/2023

Abstract:

Phospholipid will be extracted from porcine brain tissue as described Folch et. al. [3].
Phospholipid mixed with phosphate buffer (PBS), pH 7.4, at 0.3 wt/v% and sonicated for ~20-30min and extruded through 100nm polycarbonate membrane, 51 times, to obtain ULV. The appropriate amount of NSAIDs namely aspirin, ibuprofen and diclofenac will be mixed with phospholipid to obtain 10, 20, 30- mol% of NSAIDS. We propose NSE and high resolution TOF techniques to investigate influence of NSAIDS on the dynamics of phospholipid membranes derived from mammalian brain tissues. On one hand NSE can provide the long range membrane fluctuations under the influence of NSAIDs, A-I-D, and IN5 can reveal change in fast phospholipid motions on the molecular length scale. We also propose SANS instrument to investigate shape and structural changes in ULV membrane

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Dates of Experiment and local contact:

Instrument	D22	IN5	IN15
Dates	07-08/04/2023	11-14/04/2023	26-31/05/2023
Local Contact	Lionel Porcar & Olga Matsarskaria	Jacques Ollivier	Czakkel Orsolya

The structure and dynamics of the unilamellalr vesicles (ULVs) prepared from brain phospholipid, extracted from porcine brain tissues, in presence of non-steroidal anti-inflammatory drugs (NSAIDs) aspirin, ibuprofen and diclofenac each at 10, 20, and 30 mol% and neurodegenerative protein amyloid β_{42} ($A\beta_{42}$) studied using different neutron scattering techniques. To investigate the structural changes in the brain phospholipid ULVs in presence of NSAIDs and $A\beta_{42}$ small angle neutron scattering (SANS) experiment was performed. The quasielastic neutron scattering (QENS), and neutron spin echo technique (NSE) experimental techniques were used to investigate the dynamics of brain phospholipid and ULVs membrane fluctuations on pico and nano- second time scale respectively.

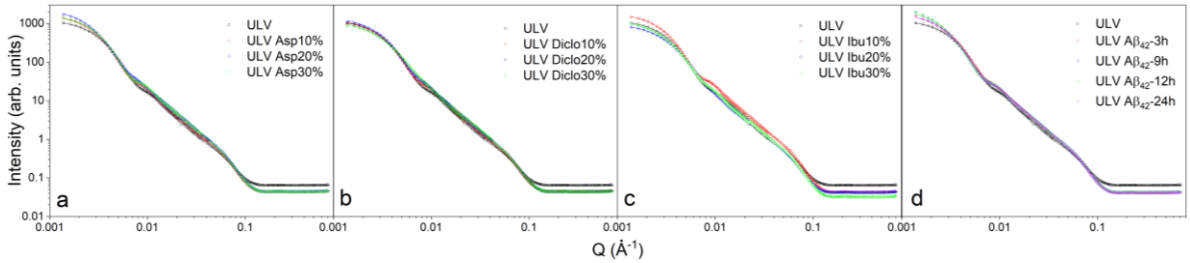

Figure 1: SANS spectra of ULV with and without different mol% of NSAIDs a) Aspirin, b) Diclofenac and c) Ibuprofen. d) ULV with $A\beta_{42}$ equilibrated for different time interval.

Small angle neutron scattering of brain phospholipid ULV with and without 10, 20, and 30 mol% of NSAIDs aspirin, diclofenac, ibuprofen and ULV with 1 mol% of $A\beta_{42}$ equilibrated at 37 C for different time interval were carried out. In all the complexes the SANS data was successfully described by core shell model, as shown in Fig. 1. The radius of ULVs with and without NSAIDs and $A\beta_{42}$ found to be 50-70 nm and bilayer thickness of ~4 nm. The experimental scattering length density of brain phospholipid were found to be 0.313×10^{-6} Å^{-2} which is in good agreement with the theoretical value 0.28×10^{-6} Å^{-2}.

The QENS experiment were carried out at two different wavelength 5 Å and 8 Å corresponding to instrumental resolution of 76μeV and 20μeV respectively to investigate the slow and fast dynamics of the brain phospholipids. Significant high quasielastic broadening observed in pure brain phospholipid ULV and with NSAIDs and $A\beta_{42}$ over the instrumental resolution. At 30mol% of NSAIDs, highest quasielastic broadening is observed in presence of aspirin compared to the ibuprofen and diclofenac. This indicates that aspirin induces much faster dynamics in the brain phospholipid compared to ibuprofen and diclofenac, as shown in Fig. 2 (a). In the presence of $A\beta_{42}$, equilibrated at 37C for different time, brain phospholipid ULVs show very high QE broadening compared to the pure brain phospholipid ULV's, as shown in Fig. 2 (b). It is observed that ULV- $A\beta_{42}$ equilibrated for 1h show slight higher quasielastic broadening compared to the ULV- $A\beta_{42}$ equilibrated for 6h. This indicates that

depending upon incubation time of neurodegenerative peptides has ability to perturb the membrane mimetic system. From preliminary analysis, it is found that the QENS spectra can be described by motion on two different time scale which can be attributed to the slow long range translational diffusion of phospholipids and fast localized internal motion of phospholipids. The QENS spectra corresponding to all the complexes are being analyzed.

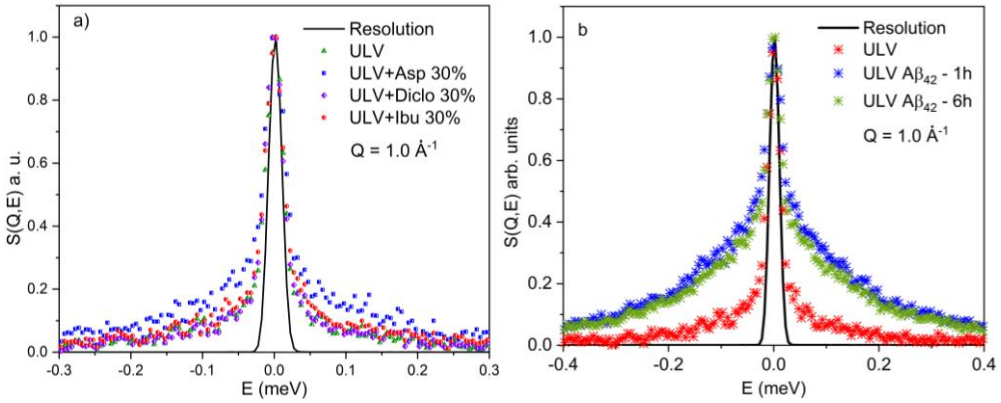

Figure 2: Normalized QENS spectra of ULV with and without 30 mol% of NSAIDs, aspirin, diclofenac and ibuprofen. b) Normalized QENS spectra of ULV with $A\beta_{42}$ equilibrated for different time interval.

The NSE measurement was carried out a 3 different configurations with wavelength (λ) 12, 10, and 8 Å at detector angle of 6°, 7.5°, and 8° respectively.

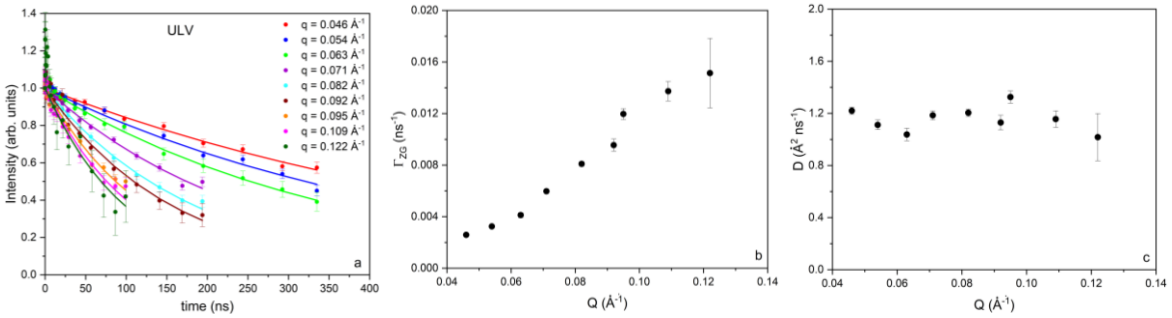

Figure 3: a) Intermediate scattering function corresponding to pure unilamellar vesicles, solid line represents Zilmann Granek model. b) Variation of relaxation rate Γ_{ZG} and c) diffusion coefficient (D) with Q.

It is found that the NSE spectra corresponding to pure ULVs can be well described by Zilman-Granek (ZG) model $S(Q,\tau) = exp\left(-\left(\Gamma_Q \tau\right)^{2/3}\right)$ where $\Gamma_{ZG} = 0.025\gamma\sqrt{\frac{kT}{\kappa}}\frac{kT}{\eta}Q^3$, K is bending modulus and, η viscosity. The ZG fits to NSE data corresponding to membrane fluctuations of pure brain phospholipid ULV are shown in Fig. 3 (a). The variation of relaxation rates Γ_{ZG} and diffusion coefficient (D) of brain phospholipid ULVs membrane are shown in Fig. 3 (b) and (c) respectively. The NSE spectra corresponding to different complexes are being analyzed by similar approach.