

# Experimental report

24/10/2024

**Proposal:** CRG-3013

**Council:** 4/2023

**Title:** Effects of binding of polymorphic amyloid fibrils on lipid membrane dynamics

**Research area:** Physics

**This proposal is a new proposal**

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**Samples:** DMPG liposome solution with LP60  
DMPG liposome solution with LP27  
DMPG liposome solution

| Instrument | Requested days | Allocated days | From       | To         |
|------------|----------------|----------------|------------|------------|
| IN13       | 3              | 3              | 28/11/2023 | 01/12/2023 |

**Abstract:**

The abstract is in the scientific case pdf file.

## Effects of binding of polymorphic amyloid fibrils on lipid membrane dynamics

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**Background:** The hallmark of diseases classified as amyloidosis such as Alzheimer's disease is the formation of protein aggregates called amyloid fibrils in various organs. Despite extensive research, the molecular mechanism of amyloidosis has not been elucidated (Iadanza et al., 2018). As an important process underlying the pathogenesis of amyloidosis, toxic effects of fibrils as well as pre-fibrillar oligomers on cells (cytotoxicity) have attracted much attention. The fact that the same protein forms fibrils with different morphology and cytotoxicity depending on solution conditions makes it harder to elucidate the mechanism of cytotoxicity.

As a model protein of amyloid, hen egg white lysozyme (HEWL) has widely been used, partly due to its cytotoxic properties similar to those of human lysozyme, a causative protein of systemic amyloidosis. It is known that HEWL amyloid polymorphs formed at pH 6.0 (hereafter called LP60) show stronger cytotoxicity than those formed at pH 2.7 (LP27) (Fig. 1) (Mocanu et al., 2014). Binding between polymorphs and cell membranes, causing membrane damage, is the first stage of cytotoxicity. Like other amyloids, interactions between HEWL fibrils and membranes depend on the net charge of the membranes. In addition, HEWL has a positive net charge as another amyloidogenic polypeptide IAPP, which causes type-II diabetes.

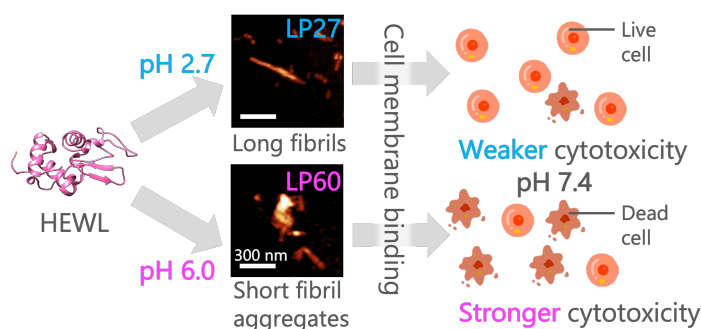


Fig. 1. Lysozyme amyloid polymorphs

Since different interactions between polymorphic fibrils and membranes are related to different levels of cytotoxicity (Bousset et al., 2013), it is essential to understand the molecular mechanism of such interactions.

Since molecular flexibility has large effects on binding and interactions (Forrey et al., 2012) and local motions play an important role in amyloid systems (Schirò et al. 2012; Fujiwara et al., 2013), characterization of the intramolecular motions of polymorphs and membrane lipids both before binding and after binding, is crucial to fully understand the molecular mechanism of cytotoxicity. As a first step toward this goal, we have shown by elastic incoherent neutron scattering (EINS) and quasielastic neutron scattering (QENS) using IN13 that LP60 has enhanced dynamics compared with LP27 in the “before binding” state (Matsuo et al., Front. Mol. Biosci. 2022), which supports a hypothesis on the flexibility of the polymorphs (Araghi & Dee, 2020). The higher flexibility of LP60 implies that it reaches a conformation suitable for membrane binding faster than LP27 and hence membrane binding is promoted. Therefore, as a next step, it is of utmost importance to investigate how the membrane dynamics may be modulated by these different types of amyloid fibrils.

**Aim of the present proposal:** To reveal possible modulation of the internal dynamics of lipid membranes caused by amyloid polymorphs showing different cytotoxicity, we will employ DMPG liposomes and HEWL. DMPG was chosen here as a first step to study the effects of charges of the head group on molecular dynamics since lipid composition depends on organelles and the fibril-membrane interaction is affected by the charges of the head group (Trusova et al., 2019). It is widely accepted that molecular dynamics of bio-macromolecules on the sub-nanosecond timescale and the Ångström length-scale is essential for their functions. Direct measurements on these motions can be achieved by neutron scattering. The aim of this study is to investigate possible differences in dynamics of the DMPG molecules in liposomes caused by binding of LP60 and LP27, which show different levels of cytotoxicity, by EINS. The dynamical features of the lipid obtained by the current proposal would not only give new insights into the molecular mechanism of the fibril-membrane interaction, but also serve as a basis for elucidating molecular mechanisms of amyloidosis.

**Measurements :** Following samples were prepared using the Chemical Lab in the Sciece Building.

- 1) DMPG liposome solution
- 2) DMPG liposome solution with LP60 (HEWL:DMPG = 1:86)
- 3) DMPG liposome solution with LP27 (HEWL:DMPG = 1:86)

EINS measurements were carried out on IN13 at  $\approx 8 \mu\text{eV}$  energy resolution between 280–320 K in temperature, which covers the phase transition temperature of DMPG ( $\sim 296 \text{ K}$ ). In addition to the above three samples, the corresponding  $\text{D}_2\text{O}$  buffer, the empty cell, and the vanadium slab were measured. At the molar ratio of HEWL:DMPG = 1:86, the scattering contribution of DMPG is 89% of the total scattering, suggesting that scattering contribution of amyloid fibrils to the measured EINS curves is negligible.

**Results :** Data reduction was carried out using the software LAMP. The subsequent analysis has been conducted using the software IGOR PRO. The measured EINS curves were analysed by fitting using the Gaussian approximation to extract the mean-square displacement (MSD) of atomic motions of DMPG molecules. Fig. 1 shows the results of the fitting at all the temperatures employed. The data of DMPG molecules in the absence of amyloid fibrils is labelled « DMPG », that in the presence of LP27 is labelled « DMPG+LP27 », and that in the presence of LP60 is labelled « DMPG+LP60 ».

Fig. 2 shows the temperature dependences of the MSD of atomic motions of DMPG molecules in all the three samples. It is found that the MSD of DMPG increased abruptly between 294 K and 300 K, which is most likely to be due to the phase transition from the gel to the liquid crystalline phase. On the other hand, this feature is not seen for other two samples, implying that the dynamical features of DMPG molecules are modulated by interaction with amyloid polymorphic fibrils.

Upon binding of LP27 (DMPG+LP27), the MSD decreased largely. Upon binding of LP60 (DMPG+LP60), the MSD also tended to decrease compared with the pure DMPG vesicles, but the magnitude of decrease is much smaller for DMPG+LP60 than for DMPG+LP27, suggesting that the atoms of DMPG molecules are still able to fluctuate more freely in the presence of LP60 than in the presence of LP27. This result is consistent with our previous observation using Laurdan fluorescence measurements that LP60 causes more structural disorder in the DMPG membranes than LP27 (Matsuo et al., 2024). The detailed analysis is currently under progress.

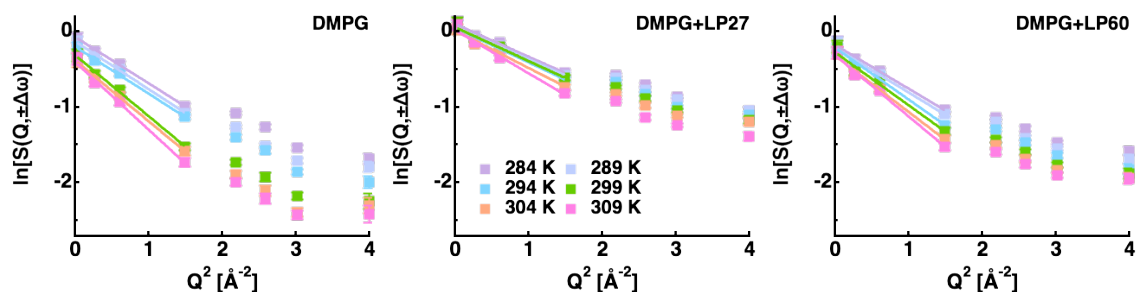


Fig. 1. Fitting results of the measured EINS curves by the Gaussian approximation.

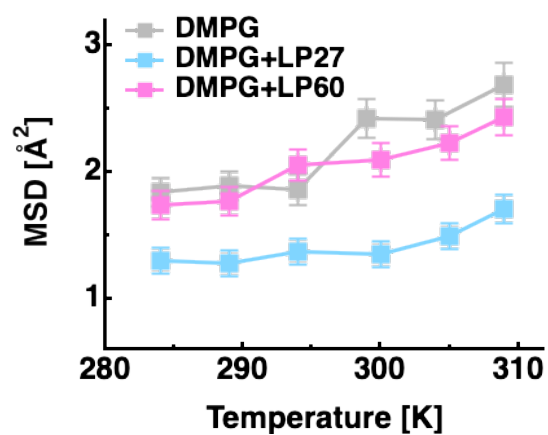


Fig. 2. Temperature dependence of the MSD obtained by the Gaussian approximation.