Experimental report

18/08/2021

Proposal: 9-13-912 **Council:** 10/2019

Title: Mixed liposomes containing bacterial lipoteichoic-acid (LTA)

Research area: Soft condensed matter

This proposal is a new proposal

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Samples: LTA

Instrument	Requested days	Allocated days	From	To
D11	3	2	26/09/2020	28/09/2020
D22	3	0		

Abstract:

Whilst the biological effect of LTA contributing to bacterial infections is widely reported in microbiological and immunological studies, very little is understood on a physicochemical level; for instance, the amphiphilic nature of LTA would drive its self-assembly into different aggregates such as micelles and liposomes, which would be directly relevant to how it mediates interactions with biological surfaces, biofilm formation, and consequent septic shocks. As part of an H2020 Marie Curie fellowship project, we propose a first SANS study of the mixed liposome structures comprising dipalmitoyl phosphatidylglycerol (DPPG), dipalmitoyl phosphatidylethanolamine (DPPE), cardiolipin (CL) (C16:0) and lipoteichoic acid (LTA) in different molar proportions at pH (1.2, 7 and 12) at 25 and 37 oC. This is expected provide information on the LTA induced structural changes in the mixed liposomes. We also propose measurements on aqueous LTA aggregates at different concentrations (0.2, 2, 5 mg/ml-1) in water at 25 and 37 oC to understand their behaviour as aggregated structures.

Institut Laue-Langevin (ILL), France **Beamline: D11**

Experiment No: 9-13-912

Experimental objectives:

Lipoteichoic acid (LTA, Fig.1), a polymeric amphiphile present in the Gram-positive bacteria membrane, is present in about 0.6-1.4 wt% in the total dry cell mass, while about 5-12 mol% in the lipid membrane. Bacterial such as Bacillus Subtilis can shed LTAs tethered to the cytoplasmic membrane into aqueous biological media, which is identified as a major virulence factor for causing septic shocks and multiorgan failure. Whilst the biological effect of LTA contributing to bacterial infections is widely reported in microbiological and immunological studies, very little is understood on a physicochemical level; for instance, the amphiphilic nature of LTA would drive its self-assembly into different aggregates such as micelles and liposomes, which would be directly relevant to how it mediates interactions with biological surfaces, biofilm formation, and consequent septic shocks. We plan to measure the aqueous LTA micelles (2 and 4 mg mL^{-1}) in D_2O in the presence of Ca^{2+} cations in different concentrations (2, 5 and 10 mM) at 25, 40 and 60 ^oC. This will provide critical experimental results on the self-assembly behavior of LTA, which will help to understand the bioadhesion of these aggregates on the host surfaces.

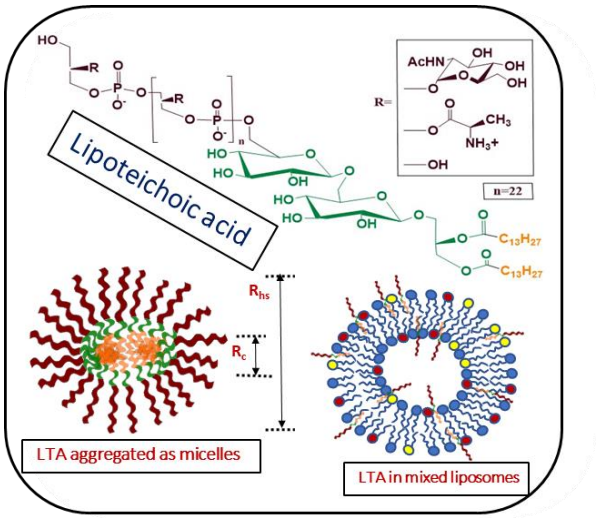

Figure 1. The structure of *Bacillus Subtilis* lipoteichoic acid (LTA) and morphologies formed as micelles and mixed vesicles

Experimental results:

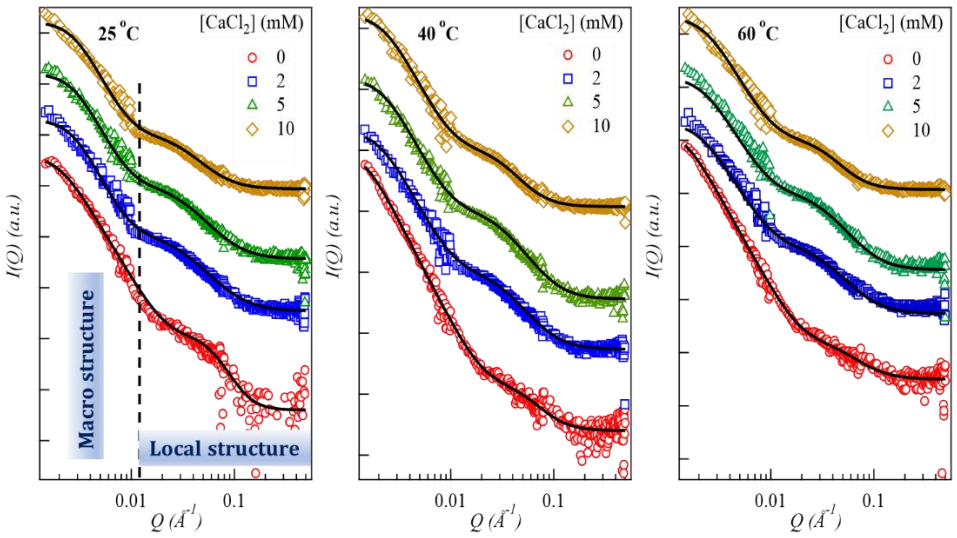

Figure 2. SANS intensity *I* vs *Q* profiles for 2 mg mL^{-1} LTA aqueous solutions containing 0 – 10 mM $CaCl_2$ at 25, 40 and 60 °C. Symbols are the data points and solid lines represent the best fits using the *two Lorentzian* model. For clarity, the SANS profiles are scaled on the *y*-axis.

The scattering intensity decreased with addition of $CaCl_2$ up to 5 mM concentration and remains constant on further increase to 10 mM. The change in the scattering intensity with $CaCl_2$ addition is attributed to the charge neutralization of the negatively charged phosphate groups at the surface of the LTA micelles. We attempted to fit this data using several models e.g. *gel fit, Guinier Porod approximation, correlation length model* etc., but no physically relevant parameters could be achieved. Table 1 demonstrates fitted aggregation parameters using *Two-Lorentzian* model, which calculates an empirical functional form of the SANS data characterized by two Lorentzian type functions. This model calculates two corelations lengths corresponding to the macro structure or clusters (low-*q*) and the local structure (high-*q*). The SANS data was analysed by the *two-Lorentzian model* using the following equation.

$$I(q) = \frac{A}{1 + (Q\xi_1)^n} + \frac{C}{1 + (Q\xi_2)^m} + B \qquad eq.\ (1)$$

where *I(q)* is the scattering intensity, *q* the scattering vector, *A* the Lorentzian scale factor 1, *C* the Lorentzian scale factor 2, ξ_1 and ξ_2 the respective correlation lengths, and *n* and *m* the respective power law components. This model calculates an empirical functional form for the SANS data characterized by two Lorentzian-type functions. The first term (with a subscript 1) describes the low-*q* structures, while the second term (with a subscript 2) the high-*q* structures. A constant (*q*-independent) background B has been introduced to account for the incoherent scattering at high-*q*. The values of correlation lengths describe the variations in the length scales with the concentration or density fluctuations in the system. The exponent *n* characterizes the fractal structure of the long-range inhomogeneities in the low-*q* range. Here, $n > 3$ indicates decrease in the solubility of scatterers and intra-molecular interactions being favoured. The exponent *m* (high-*q*) informs on the extent of water-biopolymer interactions and hence the chain thermodynamics.

Table 1. Fitted SANS parameters for 2 mg. mL^{-1} LTA micelles as a function of $CaCl_2$ concentrations [$CaCl_2$] at 25, 40 and 60 ^oC. Here ξ_1 and ξ_2 are the correlation lengths (Å) corresponding to the low-*q* (clusters) and high-*q* (local structure) region of the SANS data curve. The *n* (low-*q*) and *m* (high-*q*) values are the Lorentz exponents describing the LTA-water interactions, and χ^2 an indication of the goodness of the fit.

[$CaCl_2$] (mM)	ξ_1 (Å)	ξ_2 (Å)	*n*	*m*	χ^2
25 ^{o}C					
0	516.8 ± 13.7	16.8 ± 0.6	3.4 ± 0.02	4.2 ± 0.04	1.9
2	387.8 ± 11.9	34.7 ± 0.9	4.0 ± 0.07	2.6 ± 0.05	1.5
5	380.9 ± 13.7	33.9 ± 0.9	3.9 ± 0.08	2.7 ± 0.05	1.2
10	377.2 ± 12.7	33.1 ± 0.8	3.9 ± 0.07	2.8 ± 0.05	1.3
40 ^{o}C					
0	773.0 ± 34.3	17.9 ± 1.2	3.30 ± 0.02	3.1 ± 0.04	1.4
2	505.6 ± 27.0	32.0 ± 0.9	3.30 ± 0.07	2.7 ± 0.06	1.4
5	471.0 ± 27.5	32.4 ± 0.9	3.34 ± 0.08	2.8 ± 0.06	1.3
10	453.2 ± 21.8	31.5 ± 0.8	3.35 ± 0.07	3.0 ± 0.06	1.3
60 ^{o}C					
0	763.5 ± 24.8	19.7 ± 1.6	3.5 ± 0.03	2.7 ± 0.03	1.2
2	456.9 ± 18.9	32.8 ± 0.9	3.5 ± 0.07	2.7 ± 0.07	1.5
5	453.2 ± 20.8	30.3 ± 0.7	3.5 ± 0.07	3.1 ± 0.06	1.3
10	452.5 ± 20.8	30.0 ± 0.7	3.4 ± 0.06	3.2 ± 0.07	1.3

The experiments were also conducted at 4 mg. mL^{-1} LTA concentrations and the obtained SANS data is shown in the below figure. The analysis of these scattering profile is under progress.

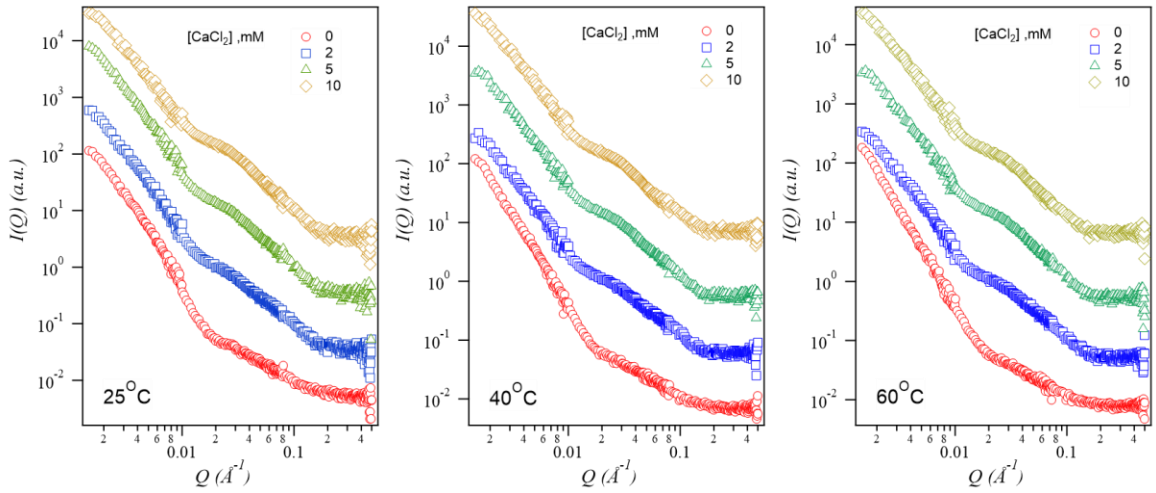

Figure 3. SANS intensity *I* vs *Q* profiles for 4 mg mL^{-1} LTA aqueous solutions containing 0 – 10 mM $CaCl_2$ at 25, 40 and 60 ^oC. Symbols are the data points and solid lines represent the best fits using the *two Lorentzian* model. For clarity, the SANS profiles are scaled on the *y*-axis.