Experimental report

10/09/2024

Proposal: 8-02-892 **Council:** 10/2019

Title: Interaction of RNA with titratablebilayers

Research area: Other...

This proposal is a new proposal

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Samples: 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC)
O-(Z,Z,Z,Z-heptatriaconta-6,9,26,29-tetraem-19-yl)-4-(N,N-dimethylamino)butanoate (DLin-MC3-DMA)
mRNA
1,2-dioleoyl-3-trimethylammonium-propane (DOTAP)

Instrument	Requested days	Allocated days	From	To
D17	0	2	29/06/2021	01/07/2021
FIGARO	4	0		

Abstract:

Lipid nanoparticles (LNPs) are a growing area of interest as drug delivery vehicles in mRNA therapies. The interactions of these lipids with the cargo drug delivery. Cationic ionisable lipids (CILs) are gaining interest for use in LNPs, as their interactions with their RNA cargo and with anionic lipids in endosomal membranes are theorised to play an important role in LNP formation and endosomal release respectively. Therapeutically relevant LNPs are complex, multicomponent systems with strongly pH dependent structure and interactions. We propose to use a simple system to mimic the RNA interaction with biomembranes with the use of neutron reflectometry to study the interaction. This consists of lipid bilayer containing DOTAP (cationic lipid) or MC3 (CIL) (titratable cationic lipid) and DOPC (phospholipid), which will allow us to reveal the effect of pH and charge density on the adsorption of mRNA.

EXPERIMENT DETAILS

Proposal No: 8-02-892	
Title: Interaction of RNA with titratable bilayers	
Instrument: D17	
Dates scheduled: 29/6/21 – 1/7/21	No. Days allocated: 2

ABSTRACT

Lipid nanoparticles (LNPs) are a growing area of interest as drug delivery vehicles in mRNA therapies(1). Therapeutically relevant LNPs are complex, multicomponent systems with strongly pH dependent structure and interactions. Notably, a formulation showing promise in clinical applications includes the following: an ionisable lipid that is cationic at low pH, distearoylphosphatidylcholine (DSPC, a neutral helper lipid), cholesterol (Chol), and a diffusible polyethylene glycol (PEG)-lipid. (2) Cationic ionisable lipids (CILs) are gaining interest for use in LNPs, as their interactions with their RNA cargo and with anionic lipids in endosomal membranes are theorised to play an important role in LNP formation and endosomal release respectively (3).

Arteta et al. 2018 (4) previously characterised an LNP system of this structure, which includes the CIL O-(Z,Z,Z,Z-heptatriaconta-6,9,26,29-tetraem-19-yl)-4-(N,N-dimethylamino)butanoate (DLin-MC3-DMA or MC3), which has shown therapeutic potential *in vivo*. The complex internal structure of these LNPs is, however, poorly understood, therefore so is the interaction of DLin-MC3-DMA with the mRNA cargo.

To understand the mechanism of drug delivery, it is important to understand the interactions of these lipids with the cargo. In order to understand the more complex system of Arteta et al., we have used a simpler system with the key components that are thought to control the RNA interaction. This consists of a two component lipid bilayer containing DOTAP (cationic lipid) or MC3 (CIL) (titratable cationic lipid) and DOPC (phospholipid), which will allow us to reveal the effect of pH and charge density on the adsorption of mRNA.

It is also important to understand how general these interactions between the lipids and mRNA cargo are. Due to the cost and complexity of working with mRNA, model mRNAs are an attractive alternative to investigate these interactions on a larger scale. It is therefore important to understand whether the interactions, as well as the biophysical properties of these models are representative of full mRNA.

EXPERIMENTAL DETAILS

In our experiment on D17, we investigated the adsorption of erythropoietin (EPO) mRNA and 2 model mRNAs, polyadenylic acid (polyA) and polyuridylic acid (polyU) to lipid layers with 2 different lipid compositions. The lipid layers investigated were 15/85 MC3/DOPC (cationic ionisable lipid, pH6<pKa) and 20/80 DOTAP/DOPC (cationic lipid, pH6 and pH7).

The following samples were measured:

1. 15/85 MC3/DOPC, pH6 with polyA
2. 15/85 MC3/DOPC, pH6 with polyU
3. 20/80 DOTAP/DOPC, pH7 with EPO mRNA
4. 20/80 DOTAP/DOPC, pH6 with EPO mRNA

The 4 Si blocks were initially characterised in D2O, then cell 1 was also characterised in H2O. The lipid layers were prepared by vesicle deposition (0.5mg/mL vesicles in PBS), then rinsed with Milli Q to ensure complete rupture followed by characterisation in 50 mM sodium phosphate buffer in 2 contrasts (H_2O, D_2O). Cells 1 and 2 were additionally characterised in buffer contrast matched to Si (CMSi). The relevant (model) mRNA in 50 mM sodium phosphate buffer in D_2O was manually injected and incubated for 45 mins – 1 hr, then the cell was rinsed with 50 mM sodium phosphate buffer in D_2O. For all cells, the lipid layers + (model) mRNA were characterised in 3 solvent contrasts (H_2O, contrast matched Si (CMSi), D_2O) and cells 1 and 2 (containing MC3) were additionally characterised in buffer contrast matched to mRNA (CMpolyA/CMpolyU).

RESULTS

Along with the data collected here, QCM-D measurements for 15/85 MC3/DOPC lipids layers (the composition in cells 1 and 2) with polyA and polyU were performed, in which the observed frequency and dissipation changes after addition of polyA and polyU are different at this %MC3 and pH, indicating different adsorption behaviours. In combination with fitting of the reflectivity profiles collected here (Figure 1), these adsorption behaviours can be interpreted as adsorption to the surface of the layers for polyA and penetration into and rearrangement of the layer for polyU. This data complements data from a previous neutron reflectometry beamtime comparing the adsorption of EPO mRNA to lipid layers composed of varying MC3/DOPC ratios in pH6 and pH7 buffer. For EPO mRNA, a combination of these behaviours was observed in these conditions (15% MC3, pH6) but to a much greater extent

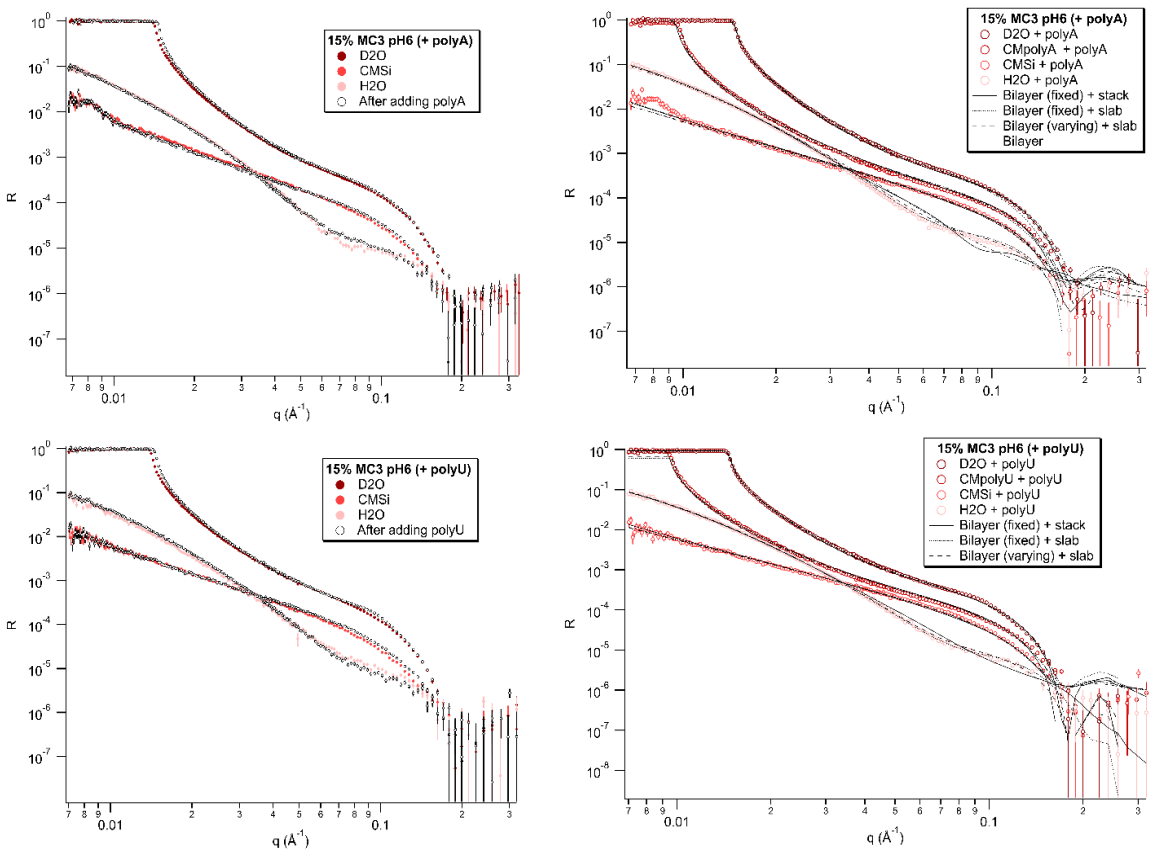

Figure 1: The reflectivity profiles and the corresponding model fit comparison for the 15% MC3 lipid layers before and after incubation with polyA (top row) and polyU (bottom row) are shown.

(i.e. larger adsorbed mass on the surface and a greater extent of rearrangement of the lipid layer).

The results from this section are described in the following publication: Gilbert, J., Ermilova, I., Fornasier, M., Skoda, M., Fragneto, G., Swenson, J., & Nylander, T. (2024). On the interactions between RNA and titrateable lipid layers: implications for RNA delivery with lipid nanoparticles. *Nanoscale*, *16*(2), 777–794. https://doi.org/10.1039/D3NR03308B

It was also of interest to us to compare the effect of including a cationic lipid (DOTAP) or a cationic ionisable lipid (MC3) on the lipid layer structure and cargo interaction. This data complements both data from the beamtime mentioned above and data from another neutron reflectometry beamtime comparing the adsorption of polyA and polyU to lipid layers composed of varying DOTAP/DOPC ratios. Interestingly the adsorption behaviour of EPO mRNA to this lipid layer in both pHs is very different to that observed for MC3/DOPC layers at the same pHs; here, minimal changes observed for both pHs after incubation with mRNA, whereas multilayer formation was observed at both pHs with the MC3/DOPC layers, as shown in Figure 2 below for pH7.

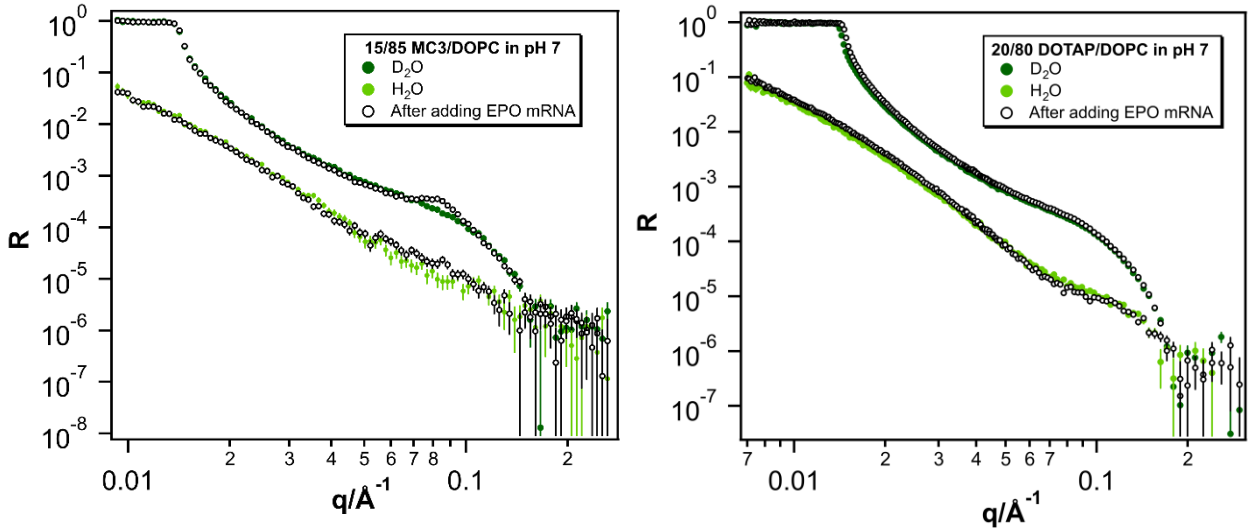

Figure 2: Comparison between adsorption of EPO mRNA to lipid layers containing either a cationic ionisable lipid, MC3 (left) or cationic lipid, DOTAP (right).

REFERENCES

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