Experimental report

06/11/2023

Proposal: 9-13-542 **Council:** 4/2019

Title: Understanding a macromolecule translocation & reservoir attachment mechanism: slow drug release to membranes

Research area: Soft condensed matter

This proposal is a continuation of 9-13-485

Main proposer: Richard CAMPBELL

Experimental team: Javier SOTRES
Erik WATKINS
Marite CARDENAS

Local contacts: Richard CAMPBELL

Samples: D2O
1-palmitoyl-2-oleoyl-phosphatidyl-choline
1-palmitoyl-2-oleoyl-phosphatidyl-glycerol
deuterated 1,2-dihexanoyl-glycero-3-phospho-choline
deuterated 1,2-dihexanoyl-glycero-3-phospho-glycerol
poly(amido amine) dendrimers - generation 6

Instrument	Requested days	Allocated days	From	To
FIGARO User-supplied	6	6	05/12/2014	09/12/2014
			04/05/2015	06/05/2020

Abstract:

This proposal follows on from our recent FIGARO publication in ACS Macro Letters: "Key Factors Regulating the Mass Delivery of Macromolecules to Model Cell Membranes: Gravity and Electrostatics". The aim of the project is to tune the attachment of reservoirs of macromolecules in the form of lamellar aggregates to supported lipid bilayers for continuous diffusion and slow release. We have shown already that the orientation of the membrane is of great importance and a macromolecule translocation + aggregate attachment mechanism only occurs on membranes of sufficient charge. There are 3 aims of this experiment. [1] To determine the underlying nature of the mechanism in terms of kinetic vs. thermodynamic barriers. [2] To resolve the translocation mechanism in terms of clustering of charged lipid first in the outer leaflet as the macromolecule binds then in the inner leaflet after translocation. [3] To determine the factors which affect surface multilayer formation in this complex biophysical system. With these results we aim to write one more research paper of relevance to the drug delivery community and one technical paper of interest to the neutron community before the end of 2014.

FINAL EXPERIMENTAL REPORT: #9-13-542

Understanding a macromolecule translocation & reservoir attachment mechanism: slow drug release to membranes

Richard Campbell, Erik Watkins and Marité Cardenás

Abstract

This proposal follows on from our recent FIGARO publication in ACS Macro Letters: "Key Factors Regulating the Mass Delivery of Macromolecules to Model Cell Membranes: Gravity and Electrostatics". The aim of the project is to tune the attachment of reservoirs of macromolecules in the form of lamellar aggregates to supported lipid bilayers for continuous diffusion and slow release. We have shown already that the orientation of the membrane is of great importance and a macromolecule translocation + aggregate attachment mechanism only occurs on membranes of sufficient charge. There are 3 aims of this experiment. [1] To determine the underlying nature of the mechanism in terms of kinetic vs. thermodynamic barriers. [2] To resolve the translocation mechanism in terms of clustering of charged lipid first in the outer leaflet as the macromolecule binds then in the inner leaflet after translocation. [3] To determine the factors which affect surface multilayer formation in this complex biophysical system. With these results we aim to write one more research paper of relevance to the drug delivery community and one technical paper of interest to the neutron community before the end of 2014.

Comments

The experiment was successful in part, but complexities with the data analysis as a result of (A) isotope-specific effects, and (B) features in the data that were not possible to analyse using either a model that averaged scattering length densities or one that averaged volume fractions, due to inhomogeneities across length scales that varied according to the coherence length dependence on Qz meant that to date a publishable analysed dataset has not been achieved. The experimental team is not averse to investing more time in the data analysis, but the key expertise in the analysis is now working in a different area and current resources mean that the data remain unexploited until funding can be found to invest in a consider endeavour. The proposers have a strong track record in publications resulting from ILL data, and the disappointing outcome of this experiment is very much an exception to the rule. Nevertheless, this report states openly and honestly that the outcome of this investment of beam time looks not to be as positive as the previous FIGARO experiment in the same project, which resulted in a high impact publication in ACS Macro Letters (2014, 1, 121–125):

ABSTRACT: We show that both gravity and electrostatics are key factors regulating interactions between model cell membranes and self-assembled liquid crystalline aggregates of dendrimers and phospholipids. The system is a proxy for the trafficking of reservoirs of therapeutic drugs to cell membranes for slow diffusion and continuous delivery. Neutron reflectometry measurements were carried out on supported lipid bilayers of varying charge and on hydrophilic silica surfaces. Translocation of the macromolecule across the membrane and adsorption of the lamellar aggregates occur only when the membrane (1) is located above the bulk liquid and (2) has sufficient negative charge. The impact of such dramatic directionality effects due to bulk phase separation and gravity is emphasized for future biochemical investigations. Further, the potential to switch on the interaction mechanism through tuning the charge of the aggregates to activate endocytosis pathways on specific cell types is discussed in the context of targeted drug delivery applications.