Experimental report

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Proposal: 8-02-778 **Council:** 4/2016

Title: Membrane interactions of a viral scission protein: A combined neutronreflectometry and SAXS study.

Research area: Biology

This proposal is a new proposal

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Samples: Lipid bilayers (POPC:POPG:Cholesterol)
Synthetic peptides

Instrument	Requested days	Allocated days	From	To
FIGARO Langmuir trough	4	3	05/09/2016	08/09/2016

Abstract:

Influenza virus is an enveloped virus of significant medical importance. One of the least understood aspects of influenza virus biology is the complicated process of virus particle assembly and budding. Budding is a key step in viral replication that relies on the essential activities of multiple proteins. Recently, we identified a novel role for the amphipathic helix (AH) domain in the influenza virus M2 protein: the mediation of membrane scission. The M2 AH was found to bind cholesterol and alter membrane curvature in a cholesterol-dependent manner, causing the release of budding virions. However, we still do not fully understand how the M2 AH affects the membrane bilayer nor how membrane cholesterol modifies this activity. Through a combination of neutron reflectometry and small angle X-ray scattering we will investigate the cholesterol-dependent effects of M2 AH on bilayer structure. These results will further our understanding of the molecular mechanisms of influenza virus budding, specifically as mediated by the M2 protein.

Report on: Membrane interactions of a viral scission protein:
A combined neutron reflectometry and SAXS study

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Proposed experiment:

It is known that the M2 AH interacts with lipid bilayers and causes membrane scission. However, it is not known what structural effects the M2 AH has on lipid bilayers that results in sufficient alterations of membrane curvature to cause scission, nor is it know how membrane cholesterol content affects curvature generation. Neutron reflectometry (NR) and small angle X-ray scattering (SAXS) represent two key tools for examining structural alterations of lipid membranes. By using isotopic contrast variations in NR it is possible to study model bilayer structures and determine the interaction with the M2 AH peptide, showing the effect of peptide insertion on bilayer thickness and roughness. Using SAXS we can then examine the interaction of peptide with lipid vesicles and the generation of the Ia3d cubic phase, allowing for estimation of peptide induction of negative Gaussian curvature. These experiments will be combined with ongoing NMR experiments examining the structure of the M2 AH bound to different lipid environments.

Peptide effects on bilayers of increasing cholesterol concentrations will be examined using NR at the solid-lipid and lipid/solution interfaces. A Langmuir-Schaefer bilayer will be applied to a silicon surface through using Langmuir-Blodgett followed by Langmuir-Schaeffer deposition. Lipid bilayers will then be examined using D_20, H_2O and silicon matched 38% D_20 buffered solutions in the presence or absence of the M2 AH peptide or an inactive control peptide, providing the full range of isotopic contrasts required to elucidate the membrane bilayer structure and to determine the subsequent effect of the M2 AH peptide. To perform these experiments we were awarded 3 days of time on FIGARO and one shift on the SAXS beamline BM29.

Objectives:

1) Create DOPC/DOPG Langmuir-Schaefer lipid bilayers with varying amounts of membrane cholesterol and matched DOPC/DOPG/Cholesterol Small Unilamellar Vesicles (SUVs).
2) Evaluate bilayer structure using D20, H2O and silicon matched 38% D20 buffered solutions on the FIGARO beamline.
3) Add M2 AH peptide to bilayers and evaluate the impact on the structure of the different cholesterol-containing bilayers using D20, H2O and silicon matched 38% D20 buffered solutions on the FIGARO beamline.
4) Add M2 AH peptide to SUVs and determine the induction of the Ia3d cubic phase by SAXS on BM29.

Results:

Upon arriving, our team prepared several lipid bilayers containing different lipid compositions. Using a Langmuir trough we created lipid bilayer on a silicone substrate sample block using Langmuir-Blodgett deposition followed by Langmuir-Schaeffer deposition. The DOPC:DOPG bilayers were successfully created with 0, 15 and 30 molar % cholesterol.

We then imaged the structure of each bilayer in D20, H2O and silicon matched 38% D20 Hepes buffered solutions. Following data collection of each contrast, a 10uM solution of the M2 AH scission peptide in D2O was flowed on to the lipid bilayer during data collection. After equilibrium,

data collection would begin again with the three contrast solutions. All of these buffer/contrast changes were automated, allowing for rapid and continuous data collection. This procedure was then repeated for each of the three different lipid bilayers. Most of the data collection proceeded smoothly, however, there was a pump error during the imaging setting that caused a significant delay and the loss of some usable data. However, enough data was collected to process for determination of the effect of the M2 AH peptide on the bilayer structure.

Initial data analysis shows good bilayer formation for all lipid compositions. Analysis of peptide binding shows that the 10uM solution of peptide came to equilibrium during equilibration run time. At present the data show some effect of the M2 AH peptide on the structure and organization of the lipid tails in the bilayer; however, further analysis is necessary to fully model the effect of the peptide on the bilayer.

During FIGARO imaging, SUVs of 30nm in diameter were prepared, containing DOPC:DOPG with 0, 15 and 30 molar % cholesterol, matching the above bilayers. The SUVs were mixed with increasing concentrations of the M2 AH peptide and imaged on the BM29 SAXS beamline using a 96 well plate and the BioSAXS robot. Control SUVs, imaged in the absence of the M2 AH peptide showed a typical scattering profile of small lipid vesicles. Addition of the M2 AH peptide showed a small effect on the scattering profile, we observed peaks indicative of the presence of a small amount of the Ia3D cubic lipid phase. However, the addition of the peptide only produced a small amount of the Ia3D phase, so additional experiments will be necessary in order to further optimize the system.

Together these experiments have provided valuable data that will be used to more precisely define the effect of the M2 AH peptide on the lipid bilayer and to determine how this activity is modulated by increasing amounts of membrane cholesterol. Eventually these results will be included in a forthcoming publication showing how membrane cholesterol levels regulate M2-mediated membrane scission and the release of budding influenza viruses.