versions of SP-C produced in bacteria, by means of ATR-FTIR. This technique is sensitive to the orientation, structure and interactions of SP-C in membranes, which can be correlated with the contribution of SP-C to stabilize compressed cholesterol-containing surfactant films.

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Conformational Differences in Membrane Bound Retroviral Gag Proteins

Marilia C. Do Rego Barros[1], Siddartha A.K. Datta[2], Alan Rein[2], Mathias Losche[1,3], Hirsh Nanda[1,3].
[1]Carnegie Mellon University, Pittsburgh, PA, USA, [2]National Cancer Institute, HIV Drug Resistance Program, Frederick, MD, USA, [3]National Institute of Standards and Technology, NIST Center for Neutron Research, Gaithersburg, MD, USA.

The structural protein termed Gag is an essential component for the assembly of new retroviral particles in infected host cells. Cryo-EM of immaure virions indicated Gag molecules laterally pack on the plasma membrane in long extended conformational states. All Gag proteins contain structured domains that are separated by linker regions. These linkers can be flexible, as in the Human Immunodeficiency Virus (HIV), or rigid, as in he Murine Leukemia Virus (MLV). Previous work by our group showed that HIV-1 Gag can undergo large conformational depending on its biochemical environment. In particular transitioning from a compact to extended conformation required simultaneous binding with both the lipid membrane and single stranded DNA segments[1]. In contrast to HIV-Gag, current evidence from small angel X-ray scattering suggests MLV Gag is constantly extended in solution [2].
The properties of these linkers may indicate different mechanisms for controlling molecular reorganization that leads to proper assembly and membrane budding. However, the structure of MLV Gag on the membrane in intermediate stages of viral assembly is not known. Using reflectivity, we determined the dimensions of MLV Gag bound to the membrane. We compared wild-type MLV Gag to a linker deletion mutant termed dp12. Comparative analysis of the SPR data between these two constructs confirmed similar membrane binding affinity. However, both neutron and x-ray reflectivity measurements showed significant structural differences. As a result we propose a tentative model for the different mechanisms of HIV-Gag and MLV-Gag membrane assembly.
[1]Datta, S.A.K., et al. 2011 Journal Of Molecular Biology, 406(2), 205-214.
[2]Datta, S.A.K., et al. 2011 Journal Of Virology, 85(23), 12733-12741.

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The Effect of Membrane Composition and Protein Lipidation on the Affinity and Structure of the HIV-1 MA Domain

Marilia Barros[1], Siddartha A.K. Datta[2], Alan Rein[2], Mathias Losche[1,3], Hirsh Nanda[1,3].
[1]Carnegie Mellon University, Pittsburgh, PA, USA, [2]National Cancer Institute, HIV Drug Resistance Program, Frederick, MD, USA, [3]National Institute of Standards and Technology, NIST Center for Neutron Research, Gaithersburg, MD, USA.

The retroviral polyprotein Gag is the single essential component required for the formation of new HIV-1 viral particles. Gag proteins are initially expressed in the cellular cytoplasm but eventually target the surface of the plasma membrane (PM) where the assembly occurs. The N-terminal matrix (MA) domain of Gag is the key structural motif that mediates targeting. Several biochemical mechanisms are implicated in MA-membrane binding including electrostatic interactions between a patch of basic residues and anionic lipids, a hydrophobic interaction with a myristolated amino acid group and specific binding to phophatidylinositol 4,5-bisphosphate PI(4,5)P2 found only in the PM. In fact PI(4,5)P2 binding may be a mechanism for directing Gag assembly to specific regions of the PM and potentially induces myristate exposure from a sequestered state within the protein.
To establish the molecular interactions that controls MA-membrane coupling, we conducted surface plasmon resonance(SPR) experiments to determine binding affinities on different membranes composition using both myristolated and non-myristolated MA. Charge density of the lipid membrane had a clear effect on membrane association resulting in high surface coverage. Myristolation significantly increased affinity by more than an order of magnitude. The coupling between binding and orientation of the HIV-1 MA domain on the membrane is investigated by neutron reflectivity. The additional anchoring mechanisms may alter the membrane-binding interface of MA or fix the orientation further. Lipid targeting by the MA domain is a crucial step in viral assembly and may be directed by a hierarchy of biochemical interactions beginning with long-range but weak electrostatic forces and followed by a localized PI(4,5)P2/myristate exposure mechanism for further anchoring. These molecular details may provide a general understanding of how peripheral membrane proteins make reversible and specific interactions with the membrane.

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The Conformation of Membrane-Associated HIV Nef and its Interactions with Hck

Michael S. Kent[1], Bulent Akgun[2], Sushil Satija[2], Hirsh Nanda[2], Briana C. Vernon[1], Xiaomeng Shi[3], Gregory F. Pirrone[3], John R. Engen[3].
[1]Sandia National Labs, Albuquerque, NM, USA, [2]National Institutes of Standards and Technology, Gaithersburg, MD, USA, [3]Northeastern University, Boston, MA, USA.

Nef is one of several HIV-1 accessory proteins and directly contributes to AIDS progression. Nef has no catalytic activity but instead realizes its functions by interacting with cellular proteins. Nef is myristoylated on the N-terminus, associates with membranes, and undergoes a transition from a solution conformation to a membrane-associated conformation. It has been hypothesized that conformational rearrangement enables membrane-associated Nef to interact with cellular proteins. Despite its obvious disease importance, there is little or no direct information about the conformation of membrane-bound Nef. In this work we used neutron reflection to reveal details of the conformation of membrane-bound Nef. The conformation of myristoylated Nef was studied upon binding to Langmuir monolayers of negatively-charged lipids. By adjusting the surface pressure, the extent of insertion of the myristate group could be controlled. At sufficiently high surface pressure such that the myristate group did not insert, adsorbed Nef was in a condensed state with the core domain directly against the lipid headgroups. At lower surface pressure such that the myristate group inserted into the membrane, adsorbed Nef was found to be in an extended state with the core domain displaced ~ 70 Å from the lipid headgroups. Thus, insertion of the myristate group triggers a conformational transition to an open configuration. This may have important ramifications for the ability of Nef to interact with host proteins. Prior work has shown that Nef interaction with the SH3 domains of Src-family kinases is a common early event in HIV-infected cells that generates important downstream signals essential for viral pathogenesis. We will report on the ability of membrane-bound Nef in each conformation to interact with the SH3 domain of Hck.

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Ion Channel Water Pockets Examined by Neutron Diffraction: The M2 Peptide Channel of H1N1 Influenza Virus

David Worcester[1,2], Mihaela Mihailescu[3,4], Stephen H. White[5].
[1]University of California-Irvine, Gaithersburg, MD, USA, [2]University of Missouri, Columbia, MO, USA, [3]Inst. Bioscience/Biotechnology Research, Rockville, MD, USA, [4]NIST Center Neutron Research, Gaithersburg, MD, USA, [5]University of California-Irvine, Irvine, CA, USA.

Many ion channels have remarkably large water-containing spaces (pockets) within the membrane-spanning region of the channel protein. These are often adjacent to a selectivity filter. The pockets are unusual since they are often formed by rather hydrophobic protein surfaces. In some cases, "de-wetting" transitions have been suggested as having roles in channel gating. Evaluation of the amounts and trans-membrane distribution of water within ion channels can be determined by neutron diffraction from multilayers using D2O/H2O exchange. The main difficulty is the high protein/lipid molar ratios needed in multilayers if water in the channels is to be a accurately measured. We used the M2 peptide of H1N1 influenza virus to form multilayers of high protein content. This peptide forms tetrameric proton channels by self-assembly in lipid bilayers. Protein to lipid molar ratios as high as 1/3 formed good multilayers for neutron diffraction. We obtained trans-membrane water distributions using the AND/R instrument at the NIST Center for Neutron Research. Neutron diffraction data for three lamellar orders were obtained and Fourier D2O/H2O difference profiles show considerable water penetration into the membrane, consistent with water approaching the selectivity filter. The M2 channel water profile is strikingly different from that of the lipid-only membrane, which has a profile that is flat in the hydrocarbon region. For further comparison, the water profile obtained for purple membranes, which are of similar high protein content, is also presented and shows little water in the hydrocarbon region. Aspects of water in hydrophobic pockets are discussed.

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Exploring Water in the Hydrophobic Cavity of the Bacterial Potassium Ion Channel KcsA

Joseph Blasic[1], David Woecester[2], Ella Mihailescu[3].
[1]NIST, Rockville, MD, USA, [2]University of California, Irvine, Irvine, CA, USA, [3]Institute for Bioscience and Biotechnology Research, Rockville, MD, USA.