Coarse-Grained Molecular Dynamics Simulations
Oligomerization of Amphipathic Peptides in a Membrane Studied by
2369-Pos
Field Laboratory, Tallahassee, FL, USA.

Clues for the Mechanism of SEVI HIV Enhancement from Structural
Studies of the SEVI Precursor Peptide PAP248-286 in a Membrane Environment
by NMR
Ravi Prakash Reddy Nanga, Jeffrey R. Brendler, Vivekanandan Subramanian, Natašija Popovych, Ramamoorthy Ayyalusamy. University of Michigan, Ann Arbor, MI, USA. Despite the rapid progress of the AIDS pandemic, the HIV virus is a surprisingly weak pathogen in vitro. The large difference between in vitro and in vivo infection rates suggests that cofactors absent in vitro but essential for the natural transmission of the virus may be responsible for this discrepancy. A recently identified peptide in human semen, PAP248-286, has emerged as a clear candidate for the missing cofactor as it dramatically enhances the infectivity of HIV by up to five orders of magnitude. PAP248-286 appears to enhance HIV infection by forming amyloid fibers known as SEVI, which are believed to enhance the attachment of the virus by bridging interactions between virion and host-cell membranes. To understand the unique ability of SEVI to enhance HIV infection, we have solved the atomic-level resolution structure of the SEVI precursor peptide, PAP248-286, using NMR microscopy in SDS micelles. In contrast to other toxic amyloid peptides that generally penetrate into the core of the membrane, non-toxic PAP248-286 binds superficially to the surface of the micelle. Unlike most amyloid peptides that bind to the membrane in an α-helical state, PAP248-286 is mostly disordered when bound to the surface of the micelle. The highly disordered nature of the SEVI peptide may explain the high ability of SEVI to enhance HIV infection, as partially disordered amyloid fibers will have a greater capture radius for the virus than more compact amyloid fibers. Two regions of nascent structure match the prediction of highly amyloidogenic sequences and may serve as nuclei for aggregation and amyloid fibril formation. NMR studies of the binding of PAP248-286 to the anti-amyloid agent ECGC will also be presented.

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Formation of Toroidal Pores by Amyloid Proteins: Evidence of Lipid Transbilayer Exchange Induced by Islet Amyloid Polypeptide
Daniel W. Youngstrom, Jeffrey R. Brendler, Pieter E.S. Smith, Kevin Hartman, Ayyalusamy Ramamoorthy. University of Michigan, Ann Arbor, MI, USA. Recent computer simulations have indicated that amyloid peptides disrupt membranes by the formation of lipid-toroidal pores caused by excess membrane curvature, but experimental evidence for this mechanism has largely been lacking. A directly measurable consequence of this phenomenon is the significantly accelerated transbilayer exchange of lipids, which is a feature of the toroidal pore mechanism but not of other mechanisms of membrane disruption. Using vesicles asymmetrically labeled on the outer leaflet by pyrene-labeled lipids, we show that toxic versions of islet amyloid polypeptide, an amyloid peptide implicated in the pathogenesis of type II diabetes, induce rapid lipid flip-flop between bilayers. This manner is consistent with antimicrobial peptides known to disrupt membranes by the toroidal pore mechanism. We further demonstrate that a clear difference between toxic and non-toxic versions of IAPP can be observed in their binding to bicelles containing DMPC and the detergent DHPC, in which DHPC forms highly curved regions resembling toroidal pores. Using this model of a pre-structured toroidal pore we show that toxic IAPP1-19 binds in the highly curved, pore-like DHPC enriched region while non-toxic IAPP1-37 binds to the flat lamellar DMPC enriched region away from the pore. Similarly, DSC indicates that toxic versions of the IAPP peptide strongly favor the formation of negative curvature membranes, while the non-toxic rIAPP1-37 peptide does not. Further results on other amyloid peptides (including Aβ, calcitonin, and insulin) will also be presented as well as results from antimicrobial peptides.

2368-Pos
Structural Characterization of Amyloids Comprised of Anchorless Prion Proteins
Jan Steoerh. David Colby, Kurt Giles, Stanley B. Prusiner, Holger Wille. UCSF, San Francisco, CA, USA. Prion diseases are fatal, neurodegenerative diseases that afflict sheep, cows, and humans. The key event in prion diseases is the conversion of the α-helical, cellular isoform of the prion protein (PrPα) to an insoluble, β-sheet-rich, infectious isoform (PrPβ). Host-encoded PrPα is anchored to the cell membrane by a glycosylphosphatidylinositol (GPI) anchor and converts to GPI-anchored PrPβ during prion infection. Here we describe prion infection in transgenic mice (Tg8015) that lack the signal sequence for attachment of the GPI anchor and therefore express non-anchored, free-floating, PrPβ (ΔGPI-PrPβ). The lack of the GPI anchor leads to incomplete modification of PrP, resulting in a unglycosylated molecule. Therefore, Tg8015 mice produce unglycosylated GPI-PrPSc and macroscopic amyloid de...

2369-Pos
Oligomerization of Amphipathic Peptides in a Membrane Studied by Coarse-Grained Molecular Dynamics Simulations
Myunghi Yi1,2, Huan-Xiang Zhou1. 1Florida State University, Tallahassee, FL, USA, 2National High Magnetic Field Laboratory, Tallahassee, FL, USA. To gain insight into the aggregation and oligomerization of antimicrobial or amyloidogenic peptides, we carried out molecular dynamics simulations of a 26-residue amphipathic peptide at different concentrations (8, 16, and 32 copies) in a fully hydrated bilayer composed of 1600 POPC lipid molecules. With a coarse-grained representation of the molecules, >28 microseconds of simulations were accumulated for each system. Oligomers of various orders were observed to form. The system with 32 copies of the peptide was finally comprised of a 4-, 7-, 8-, and 13-mer. The final compositions of the systems with

16 and 8 copies of the peptide were 3-, 4-, and 9-mer for one and 3- and 5-mer for the other. Higher oligomers were formed by addition of monomers and by association of preformed lower oligomers (see Figure). Dissociation was not observed. In the lower oligomers (up to 4-mer) only the hydrophilic side of each copy was buried, but in the higher oligomers the hydrophilic sides of some copies were also buried. These simulations provide molecular insight into oligomerization of peptides inside membranes.

2367-Pos
Detecting the Aggregation of Amyloid Peptides by Spin Label EPR
Maryam Hashemi Shabestari1, Irina Sepkhanova1, Malte Drescher1, Nico J. Meeuwenoord1, Dimitri V. Filipov2, Ronald Limpens3, Roman L. Konings1, Martina Huber4. 1Leiden University, Leiden, Netherlands, 2Department of Chemistry, Leiden University, Leiden, Netherlands, 3Leiden University Medical Center, Leiden, Netherlands. Plaques containing aggregated β-Amyloid (Aβ) peptide in the brain are the main indication of Alzheimers disease. These plaques consist of Aβ fibrils. Oligomers of Aβ have been implicated as infective agents in the disease and may also be intermediates of fibril formation. Therefore, methods to study oligomers on the timescale of aggregation are sought. We show that by EPR the dynamics of spin-labeled Aβ in solutions in which fibrils are formed can be determined. The EPR experiments were performed on solutions of the Aβ peptide with 42 residues (1-42 Aβ) containing an N-terminal cysteine, which was spin labeled with the MTSL spin label (1-oxyl-2,2,5,5-tetramethyl-3-pyrroline-3-methyl]methanethiosulfonate) (SL-Aβ). For diamagnetic dilution, SL-Aβ was mixed with unlabeled Aβ. Fibril-formation in these solutions is shown by Congo-red binding and electron microscopy. Continuous wave, 9 GHz EPR reveals three fractions of different spin-label mobility, a fast one attributed to monomeric Aβ, one with a mobility that corresponds to a multimer of eight to 15 monomers, and a slow one due to larger aggregates or fibrils. The approach, in principle, allows detection of oligomers on the timescale of aggregation.

2370-Pos
Clues for the Mechanism of SEVI HIV Enhancement by NMR
Ravi Prakash Reddy Nanga, Jeffrey R. Brendler, Vivekanandan Subramanian, Natašija Popovych, Ramamoorthy Ayyalusamy. University of Michigan, Ann Arbor, MI, USA. Despite the rapid progress of the AIDS pandemic, the HIV virus is a surprisingly weak pathogen in vitro. The large difference between in vitro and in vivo infection rates suggests that cofactors absent in vitro but essential for the natural transmission of the virus may be responsible for this discrepancy. A recently identified peptide in human semen, PAP248-286, has emerged as a clear candidate for the missing cofactor as it dramatically enhances the infectivity of HIV by up to five orders of magnitude. PAP248-286 appears to enhance HIV infection by forming amyloid fibers known as SEVI, which are believed to enhance the attachment of the virus by bridging interactions between virion and host-cell membranes. To understand the unique ability of SEVI to enhance HIV infection, we have solved the atomic-level resolution structure of the SEVI precursor peptide, PAP248-266, using NMR microscopy in SDS micelles. In contrast to other toxic amyloid peptides that generally penetrate into the core of the membrane, non-toxic PAP248-286 binds superficially to the surface of the micelle. Unlike most amyloid peptides that bind to the membrane in an α-helical state, PAP248-286 is mostly disordered when bound to the surface of the micelle. The highly disordered nature of the SEVI peptide may explain the high ability of SEVI to enhance HIV infection, as partially disordered amyloid fibers will have a greater capture radius for the virus than more compact amyloid fibers. Two regions of nascent structure match the prediction of highly amyloidogenic sequences and may serve as nuclei for aggregation and amyloid fibril formation. NMR studies of the binding of PAP248-286 to the anti-amyloid agent ECGC will also be presented.