formation (71VTGVTAVAQKTV82). In the present study, we have investigated the membrane interactions of this 12-mer peptide using 31P solid-state NMR spectroscopy as well as infrared spectroscopy while the peptide structure was studied by infrared spectroscopy. The phospholipids used have different acyl chain lengths and different polar headgroups. The results show that the peptide tends to aggregate and specifically interacts with the negatively charged membranes.

1533-Pos Board B263
Cholesterol Binding Drives Partitioning of the Amyloid Precursor C99 Protein Into Liquid Ordered Membrane Domains
Paul J. Barrett[1], Charles A. Day[2], Yuanli Song[1], Jonathan P. Schlebach[1], **Anne K. Kenworthy**[2], Charles R. Sanders[1].
[1]Biochemistry, Vanderbilt University, Nashville, TN, USA, [2]Molecular Physiology and Biophysics, Vanderbilt University, Nashville, TN, USA.
The association of the 99 residue transmembrane C-terminal domain (C99) of the amyloid precursor protein with cholesterol-rich membrane domains (''lipid rafts'') is thought to promote cleavage of C99 to release the Alzheimer's disease-promoting amyloid-β (Aβ) polypeptide. Here, we test the hypothesis that C99 has an intrinsic affinity for raft-like liquid ordered (Lo) domains and that its domain preference is regulated by cholesterol binding. Fluorescence microscopy shows that C99 undergoes efficient partitioning into Lo domains relative to the co-existing liquid disordered (Ld) phase in giant unilamellar vesicles, establishing C99 as the transmembrane protein with the highest affinity for Lo domains known to date. Lo partitioning is prevented upon mutating residues that are critical for cholesterol binding to C99, indicating that binding of this sterol is a critical determinant of the phase preference of the protein. Addition of coprostanol, a raft-breaking sterol that can compete with cholesterol for binding to C99, shifts partitioning away from the Lo phase. These findings identify cholesterol binding as a novel mechanism that controls the raft targeting of C99 and suggest that pharmacological inhibition of this process could potentially be exploited as a therapeutic approach to avoid or treat Alzheimer's disease.

1534-Pos Board B264
How Do Lipids Localize in Lewy Bodies?
Himanshu Chaudhary[1], Vinod Subramaniam[2,1], Mireille Claessens[1].
[1]Science and Technology, University of Twente, Enschede, Netherlands, [2]Nanoscale Biophysics, FOM Institute AMOLF, Amsterdam, Netherlands.
Lewy bodies are the pathological hallmark of Parkinson's disease (PD). While fibrillar α-synuclein (αS) is the main protein component of Lewy bodies, these structures also contain lipids. To elucidate the presence of lipids in Lewy bodies, we investigated the interaction of lipids with monomeric and fibrillar αS. In vitro, lipid membranes accelerated αS fibril formation under physiological conditions. Moreover lipids and small vesicles co-localized with suprafibrillar structures and individual αS fibrils suggesting that aggregation initiates at the membrane. The presence of lipids in Lewy bodies may therefore be an indication that cell membranes are the major target in aggregation induced neuronal cell death.

1535-Pos Board B265
Interplay Between Amyloid Beta-Peptide and Cholesterol in Bilayer
Durgesh Rai, Divina Anunciado, Hugh O'Neill, Volker Urban, William Heller, Shuo Qian.
Oak Ridge National Laboratory, Oak Ridge, TN, USA.
The interaction between lipid bilayers and amyloid beta-peptide (Aβ) is of great interest in understanding Alzheimer's disease (AD). Accumulating evidence points to a positive association of cholesterol in the membrane and AD, but a molecular-level interaction between cholesterol and Aβ has not been established. As an essential part of the membrane, cholesterol enhances the fluidity of the lipid bilayer, which may reduce membrane permeation caused by Aβ, potentially alleviating its ability to rupture the membrane. On the other hand, cholesterol increases the binding affinity of Aβ to model lipid membranes, as Aβ shows little affinity to cholesterol-free membranes. We studied the Aβ conformations change in a model lipid membrane of DMPC/DMPG/cholesterol by circular dichroism, isothermal titration calorimetry and fluorescence. The structural changes in lipid bilayers caused by Aβ was studied by grazing angle neutron diffraction (GAND) on multilamellar lipid membrane samples in conjunction with solution Small-Angle Neutron Scattering (SANS) on lipid vesicles. The experiments combine to provide new molecular level details about how cholesterol and Aβ interact in the lipid membrane.

1536-Pos Board B266
Mimicking Lysosomal Degradation of α-Synuclein
Jennifer C. Lee, Ryan P. McGlinchey, Mercedes Vargas.
Laboratory of Molecular Biophysics, National Heart, Lung and Blood Institute, Bethesda, MD, USA.
Pathological accumulation of α-synuclein (α-syn) is a feature of Parkinson's disease. This accumulation may be counteracted by mechanisms of protein degradation that involve the proteasome and lysosome. Specifically, the lysosomal protease, Cathepsin D (CatD), has been suggested to be the main enzyme involved in the degradation of α-syn in vivo. In vitro, only C-terminal truncated species are generated, arguing that other mechanisms are needed to fully explain α-syn degradation. Here, we show that N-terminally acetylated α-syn also generates C-terminal as well as N-terminal truncated variants in the presence of CatD. These species are shown to be more aggregation prone. Since α-syn associates with membranes, we have investigated the effects of various glycosphingolipids such as glucosylceramide (GlcCer), on CatD degradation of α-syn. It is known that GlcCer buildup is a hallmark of the lysosomal storage disorder Gaucher disease and that these patients have an increased risk of developing PD. Our data clearly shows that in the presence of GlcCer and CatD, α-syn is completely proteolyzed. These data offer new mechanistic insight into α-syn degradation in the lysosome.

1537-Pos Board B267
Small Angle Scattering of Fibrinogen Polymerization Kinetics and of Alpha1 Antitrypsin Interactions with Lipid Membranes
Luis A. Palacio[1], Christopher B. Stanley[2], Soenke Seifert[3], Horia I. Petrache[1].
[1]Physics, Indiana University Purdue University Indianapolis, Indianapolis, IN, USA, [2]Biology and Soft Matter Division, Oak Ridge National Laboratory, Oak Ridge, TN, USA, [3]Chemical and Materials Science X-ray Science Division, Argonne National Laboratory, Argonne, IL, USA.
Fibrinogen and Alpha 1-Antitrypsin (A1AT) are plasma glycoproteins with different, but specific functions. A1AT has been shown to have protective roles of lung cells against emphysema, a disease characterized by lung tissue destruction [1], while fibrinogen is a major factor in the blood clotting process. Most known glycoproteins have been shown to play a role in cellular interactions but the exact role of the glycan chains is still under investigation. Previous electrophysiological measurements show that A1AT has a strong affinity to lipid bilayers perturbing the function of ion channels present in the membrane. This study was designed to investigate how protein-membrane and protein-protein interactions affect the native conformation of the protein and membrane in question. Two different glycoproteins were used for comparison purposes. For A1AT, we performed contrast-matching small-angle neutron scattering (SANS) and small angle x-ray scattering (SAXS) experiments to study the structural changes of the glycosylated form of A1AT in the presence of three different lipid membranes: POPC, POPS and DLPC. For fibrinogen, we performed dynamic light scattering (DLS) measurements to find a suitable protein concentration that would yield the reaction rate needed for a time resolved SANS study of the structural evolution of fibrinogen polymerization in solution. Guinier fits were used as a first approximation to obtain the radius of gyration (Rg) of A1AT, fibrinogen and fibrin. Pair distribution functions were used to monitor the shifts in structural parameters and Bragg peaks were used to study the structural changes of lipid vesicles. We observed that the A1AT interacts with unilamellar vesicles and that fibrin structure is affected by its polymerization rate. [1] Petrusca, et al., JBC 2010.

1538-Pos Board B268
Novel Properties of the Smurf1 C2 Domain in Cellular Lipid Binding
Jordan L. Scott[1], Robert V. Stahelin[2,1].
[1]Chemistry and Biochemistry, University of Notre Dame, Notre Dame, IN, USA, [2]Biochemistry and Molecular Biology, Indiana University School of Medicine, South Bend, IN, USA.
Ubiquitin ligases are essential regulators of cellular homeostasis and have been shown to contribute to cancer metastasis and new virus formation. The Nedd4 family of E3 ubiquitin ligases target a number of cellular substrates including regulators involved in TGFβ growth signaling and cellular motility. This 9-member family of ubiquitin ligases has WW domains for target recognition and C-terminal HECT catalytic domains for the covalent linkage of ubiquitin to substrates. In addition, these proteins have N-terminal C2 domains that bind lipids and enable them to localize to membranes.
We have discovered new properties of the C2 domain of the Nedd4 family member Smurf1. The Smurf1 C2 domain acts as both a specific phospholipid-binding protein and as an anionic charge sensor in vitro and in