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of alpha-helix formation in the lipid bilayer approximated as an anisotropic solvent with predefined polarity profiles [1]. All bitopic proteins in the Membranome were classified into 15 functional classes, 660 superfamilies and 1076 families. Interactions between different bitopic proteins are included based on data from ConsensusPathDB and PDB. The database provides linking to amino acid sequences from Uniprot and HGNC, structural domains from Interpro and Pfam, protein-proteins interactions from STRING, experimental structures of water-soluble and transmembrane domains from PDB and OPM databases, and annotations in iHOP. Protein entries are also linked to corresponding biological pathways in KEGG, BioCyc and Reactome databases and small molecule metabolic pathways (from SMPDB). Membranome is intended to provide experimentally validated computational models of homodimers formed by TM alpha-helices of bitopic proteins.
Reference:
[1] I.D. Pogozheva, S. Tristram-Nagle, H.I. Mosberg, A.L. Lomize, Structural adaptations of proteins to different biological membranes, Biochim Biophys Acta, 1828 (2013) 2592-2608.

1263-Pos Board B214
Rosetta-MPDock: A Novel Computational Tool for Protein-Protein Docking within the Membrane Bilayer
Julia Koehler Leman, Rebecca F. Alford, Jeffrey J. Gray.
Chem & Biomol Eng, Johns Hopkins University, Baltimore, MD, USA.
Even though over 50% of drugs target membrane proteins, that are encoded by ~30% of the genome, as little as 1-2% of the structures in the ProteinDataBank belong to them. This discrepancy arises due to major challenges in overexpression, purification, reconstitution into membrane mimetics, and structure determination by various methods. Not surprisingly, interactions between membrane proteins that occur inside the membrane bilayer are even more difficult to study. Here we present a novel method, RosettaMPDock, to predict protein-protein interactions within the membrane. The tool was developed inside the Rosetta software suite, the leading software for biomolecular structure prediction, docking, and design. RosettaMPDock builds upon a new framework for modeling membrane proteins in Rosetta. This framework follows newest standards for object-oriented design and integrates smoothly into Rosetta3's architecture. It further greatly facilitates the development of new protocols such as RosettaMPDock.

1264-Pos Board B215
Super-Resolution Imaging and Reaction Mapping of P450 3A4 and P450 Reductase in Heterogeneous Biomimetics: Starry Night
James A. Brozik, Sara C. Humphreys, Carlo Barnaba, Adam O. Barden, Jeffrey P. Jones.
Chemistry, Washington State University, Pullman, WA, USA.
The dynamics of P450 and P450 reductase (POR) as they move through lipid bilayers, associate with one another, and how they are partitioned within the membrane are all essential elements of how they function. We have undertaken several super resolution fluorescence imaging and single particle tracking studies (1) to understand the nano-scale heterogeneity of a standard P450 lipid membrane composed of DOPC:DLPC:DLPS (1:1:1 wt), (2) to directly observe how P450 and POR are partitioned within and around the lipid ''raft-like'' structures that compose the membrane, and (3) how P450 and POR move and interact with one another in this heterogeneous environment. Moreover, we have conducted super resolution reaction mapping studies using resorufin benzyl ether as a substrate to directly observed single molecule metabolism at individual P450 enzymes. These studies have been used to develop a multiphasic discrete state model(s) for protein-protein interactions and give tantalizing insights into the relationships between these interaction and substrate turnover.

1265-Pos Board B216
Application of Luminescence Resonance Energy Transfer (LRET) for Study of Substrate Peptide Binding to PglB from N-Linked Glycosylation Pathway in C. Jejuni and C. Lari
Monika Musial-Siwek[1], Barbara Imperiali[2].
[1]Department of Biology, Massachusetts Institute of Technology, Cambridge, MA, USA, [2]Department of Biology and Department of Chemistry, Massachusetts Institute of Technology, Cambridge, MA, USA.
PglB, a monomeric membrane-bound protein, is the oligosacchryltransferase (OTase) of the N-linked glycosylation system from the gram-negative bacterium *Campylobacter jejuni* (*C. jejuni*). PglB is a homologue of the Stt3p subunit of the multimeric eukaryotic OTase and represents a simplified model of this highly conserved enzymatic process. OTases transfer glycans from polyisoprenyl diphosphate-linked carriers to selected Asn residues of target proteins. A recently published 3.4Å-resolution crystal structure of PglB from *C. lari* (Lizak C. et al., 2011 *Nature* 474, 350–355) provides important insight into the binding determinants of the peptide, although some of the critical periplasmic loops are not well-ordered and it has not yet been possible to experimentally determine the glycan substrate binding site. To gain insight into the potential conformational changes involved in PglB substrate binding and catalysis we have established a Luminescence Resonance Energy Transfer (LRET) approach for studying the system. The strategy devised includes expression of PglB with an N-terminal genetically encoded lanthanide-binding tag (LBT). The LBT is an encodable peptide sequence comprising 24 amino acids that binds and sensitizes Tb^{3+} as a luminescence donor in LRET experiments. The LBT enables intermolecular LRET measurements between the LBT-Tb^{3+} complex and a Bodipy-TMR maleimide acceptor attached to a cysteine-containing peptide substrates. Here, we report the establishment of a system to study structural changes associated with substrate peptide binding to PglB from *C. jejuni* and *C. lari* using LRET technique. LRET is measured through the span of the membrane with Å accuracy, since the Tb^{3+} donor emission is unpolarized. Additionally, the long-lived luminescence lifetime of the bound Tb^{3+} is exploited by including a short (50 μs) time delay to eliminate background emission of the acceptor.

1266-Pos Board B217
Probing Enzyme-Substrate Complex Structural Dynamics during Intramembrane Proteolysis
Jason W. Cooley, Mia Brown, Renee D. JiJi.
Chemistry, University of MIssouri, Columbia, MO, USA.
Understanding how intramembrane proteolysis is carried out and controlled is important for a variety of reasons, not the least of which is its implied importance in Alzheimer's disease onset/progression. One of the most challenging questions in this field lies in identifying how substrates, single transmembrane helical domains (or type-I,II,III membrane proteins), are recognized by the intramembrane-cleaving protease (I-CLiP) complexes in the absence of discernible sequence motifs. Until this is understood, how cleavage sites are defined by the substrate-enzyme interaction will remain a mystery. Of course, this is the big question, as diseases like Alzheimer's are referred to be resultant from ''mis-cleavage'', which is manifested by promiscuity of cleavage site choice by the enzyme. One hypothesis for substrate recognition/cleavage site choice that has gained broader acceptance is that specific substrate structural perturbations during enzyme-substrate interactions differentiate cleaved from non-cleaved single span helical domains. Here we present our findings demonstrating the structural dependence of substrate sequences to potentially helix breaking residue content during their interactions with various I-CLiPs. Interestingly, using a combination of isotopic labeling/exchange coupled to UV-resonance Raman we are able to characterize subtle structural perturbations during the protein-protein interactions within a membrane as well as define the types and extent of non α-helical structures explored by the bound substrate.

1267-Pos Board B218
Tracing Lipids and their Association with Keratin in the Adhesive Gecko Setae by NMR Spectroscopy
Dharamdeep Jain, Alyssa Stark, Peter Niewiarowski, Toshikazu Miyoshi, Ali Dhinojwala.
University of Akron, Akron, OH, USA.
The gecko toe is one of the intriguing examples of adhesion in nature. Based on a complex structure of hairy features known as 'setae', the toe is composed primarily of keratinous material. Recently however, lipids have been found to be associated with the structure of the adhesive setae. However, the organization and the molecular-level behavior of lipids and keratin in the setae is still not known. Here, we demonstrate the use of Solid and Solution State NMR spectroscopy to detect lipids and understand their association with keratin in the molts termed as 'sheds' from the toe pad and the non-adhesive regions of the epidermal skin. Our results show a distribution of similar lipids in both the skin and toe shed but with different dynamics at a molecular level. The study has implications in understanding the organization of materials in nature and provides insight to improve fabrication for synthetic adhesives.

1268-Pos Board B219
Investigating the Structural Dynamics Transitions of Human Adipocyte Fatty Acid Binding Protein by NMR Spectroscopy
Kim N. Ha[1], Youlin Xia[2], Yenchi Tran[1], Adedolapo Ojoawo[1], Gianluigi Veglia[3], David A. Bernlohr[4].
[1]Chemistry and Biochemistry, St. Catherine University, St. Paul, MN, USA, [2]Minnesota NMR Center, University of Minnesota, Minneapolis, MN, USA, [3]Chemistry and Biochemistry, Molecular Biology, and Biophysics, University of Minnesota, Minneapolis, MN, USA, [4]Biochemistry, Molecular Biology, and Biophysics, University of Minnesota, Minneapolis, MN, USA.