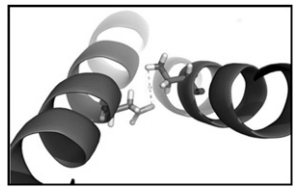
1350-Pos Board B120

Monitoring Dimerization of GpA using FRET

Jennifer K. Binder, Sandip Shinde, Brian Woodrum, Giovanna Ghirlanda, Marcia Levitus.
Arizona State University, Tempe, AZ, USA.

The long-term goal of this project is to design complex membrane proteins that have been functionalized with appropriate cofactors and can serve as components of artificial bioenergetic systems. We seek to achieve this by engineering specific interactions between the helical interfaces of transmembrane proteins. This will allow us to create functional nanostructures that contain the appropriate components in particular orientations.

The association of transmembrane helical proteins to homodimers is driven by a handful of sequence motifs, the most common of which is GXXXG. We used this sequence to introduce additional interactions so that the formation of heterodimers occurred.We designed a buried salt bridge in the transmembrane domain of a well known dimeric membrane protein, GpA. We mutated Thr 87, which is not part of the dimerization interface, to diaminopropionic acid (Dap) on one of the helices and to aspartic acid on the other. Dap and aspartic acid interact electrostatically only in a narrow pH window. We characterized the pH-dependent association of the peptides when incorporated into micelles using fluorescence resonance energy transfer (FRET). This allowed us to establish the pH profile for heterodimer formation and to measure the strength of the interaction.



1351-Pos Board B121

Membrane Protein Secondary Structure Determination via Pulsed EPR ESEEM

You Zhou, Shadi Abu-Baker, Lishan Liu, Indra D. Sahu,
Robert M. McCarrick, Gary A. Lorigan.
Miami University, Oxford, OH, USA.

Membrane proteins are involved in a variety of pertinent roles within the cell such as ion channels, communication, and catalysts for reactions in the membrane. Limited structural information exists for membrane proteins and new approaches need to be developed. A new method has been developed to determine the secondary structure of membrane proteins using pulsed electron paramagnetic resonance (EPR) spectroscopy. The three-pulse electron spin echo envelope modulation (ESEEM) can be used to detect the dipolar coupling between a deuterium-labeled residue and a spin label attached up to 4 residues away (i + 4) and correlate the strength of this coupling with the radial distance between the spin and deuterium labels. In this case, i represents the location of the 2H-labeled residue and 4 represents the location of the spin label 4 residues away. Unique periodic structural difference between an α-helix and a β-sheet can be easily observed in the corresponding ESEEM spectra for i + 2 and i + 3 samples. We have demonstrated these distinct trends with model α-helical (M2δ subunit of the acetylcholine receptor) and β-sheet (first 17-residues of ubiquitin) peptides. Circular Dichorism spectroscopy further confirms the secondary structure of the labeled peptide constructs to ensure the validity of the method. The new ESEEM approach provides site-specific secondary structural information both qualitatively and quantitatively on membrane proteins that otherwise might not have been structurally elucidated.

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Probing the Secondary Structure of Membrane Proteins with the Pulsed EPR ESSEM Technique

Lishan Liu, Dan Mayo, Indra Sahu, Robert McCarrick, Kaylee Troxel, Gary Lorigan.
Miami University, Oxford, OH, USA.

Liu, Lishan; Miami University, Chemistry and Biochemistry; Mayo, Daniel; Miami University, Chemistry and Biochemistry; Sahu, Indra; Miami University, Chemistry and Biochemistry; McCarrick, Robert; Miami University, Chemistry and Biochemistry; Troxel, Kaylee, Miami University, Chemistry and Biochemistry; Lorigan, Gary; Miami University, Chemistry and Biochemistry

A novel approach has been applied to probe secondary structures of membrane proteins and peptides qualitatively utilizing three-pulse Electron Spin Echo Envelope Modulation (ESEEM) spectroscope. The a-helical M2delta subunit of the acetylcholine receptor incorporated into phospholipids bicelles has been used as a model peptide. In order to demonstrate the practicality of this method, a cysteine mutated nitroxide spin label is positioned 1, 2, 3 and 4 residues away from two types of deuterated Leu side chains (denoted *i+1* to *i+4*). The characteristic periodicity of the a-helical structure gives rise to a unique pattern in the ESEEM spectra. By using Leu instead of Val, only i+2 and i+3 become critical to distinguish alpha helical structure. Also, due to the longer side chain of Leu when compared to Val, ^{2}H modulation depth is enhanced. Molecular dynamic simulations provide distance distributions that are consistent with the experimental data. The ESSEM technique can be applied to different deuterated amino acids and provide pertinent qualitative structural information on membrane proteins in a short time with small amounts of sample.

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The Allosteric Modulation of Syntaxin 1A by Munc18: Characterization of the Open and Closed Conformations of Syntaxin

Damian Dawidowski, David S. Cafiso.
University of Virginia, Charlottesville, VA, USA.

Syntaxin 1A is a plasma membrane SNARE that contains a SNARE motif (H3 domain) and a regulatory Habc domain. It is generally believed that syntaxin can exist in both an open and closed conformation, where the H3 domain is either free or associated with the Habc domain. Munc18, a regulatory SM protein was discovered on the basis of its high affinity to syntaxin. It is believed to arrest membrane fusion by locking syntaxin 1A into a closed state so that it cannot assemble into a SNARE complex with SNAP25 and synaptobrevin. In the present work, we use continuous wave and pulse EPR to characterize the open and closed states of syntaxin 1A. The data indicate that the H3 motif is always closely associated with the Habc domain, irrespective of the presence of munc18. Segments of the H3 motif are in conformational exchange between ordered and disordered states and munc18 acts to shift this conformational equilibrium to the ordered state. The results indicate that munc18 functions as an allosteric regulator of syntaxin 1A, and we postulate that this regulation functions to control the dynamics of those segments of syntaxin that participate in SNARE recognition and assembly.

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Control of Transverse Position of the Notch Transmembrane Helix by Amino Acid Sequence: Effect on γ-Secretase Mediated Cleavage and Activity of Notch

Diya Bhattacharjee, Erwin London.
Stony Brook University, Stony Brook, NY, USA.

Notch is a single-pass, transmembrane (TM) receptor that mediates intercellular signaling. In metazoa, the Notch signaling cascade regulates cell fates, cell proliferation and cell death during development as well as in self-renewing adult tissues. Notch signaling deregulation is associated with several pathological conditions, including cancer. Notch signaling is initiated by a receptor-ligand interaction. This is followed by a series of proteolytic cleavages. A crucial cleavage mediated by the γ-secretase complex occurs within the TM segment. This leads to the release of the Notch Intracellular Domain (NICD), which migrates to the nucleus and regulates gene expression. There has been evidence that the γ-secretase mediated cleavage of Notch does not occur at a strictly specific scissile peptide bond within the Notch TM segment. Instead, the cleavage site is variable. Little is known about the mechanisms that regulate the selection of site of the scissile peptide bond that is cleaved. Here, we study the effect of mutations that induce shifts in the transverse position of TM helices (established previously via fluorescence studies upon synthetic peptides incorporated into model membranes) upon the cleavage of the Notch TM segment, and the activity of the signaling pathways it controls. Our studies indicate that substitutions with strongly polar and charged residues that induce large transverse shifts that move C-terminal TM residues towards the bilayer interior, correlate with lower Notch stability and activity in mammalian cells. In contrast, mildly polar, uncharged substitutions which induce little or no transverse shifts, exhibit Notch stability and activity comparable to the wild type. These results suggest that different transverse positioning of the Notch TM helix alters the identity of the scissile peptide bond exposed to the active site of γ-Secretase.

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The Oncogenic Jaagsiekte Sheep Retrovirus Cytoplasmic Tail Adopts a Unique Conformation on a Phosphocholine Surface

Ali Alhoshani, Naoyoshi Maeda, Jessica Schulz, Saskia Milton, Charles Glabe, Hung Fan, Melanie Cocco.
University of California, Irvine, Irvine, CA, USA.

Jaagsiekte sheep retrovirus (JSRV) is the etiologic agent of a transmissible lung cancer in sheep, ovine pulmonary adenocarcinoma (OPA). OPA resembles bronchiole-alveolar carcinoma in humans, and it is an excellent animal model for this disease. A unique feature of JSRV is that the viral envelope (Env) protein also functions as an oncogene, in that expression JSRV Env protein causes morphological transformation of fibroblast and epithelial cell lines, and