associates with c-SRC in membrane fractions leading to JNK activation via phosphorylation of both c-SRC and p52Shc. The mechanism by which this occurs is not known. The primary objective of this study is to characterize the p52Shc - c-SRC association as a means to determine how c-SRC is activated. In order to study the binding between c-SRC and p52shc, we expressed both proteins in Escherichia coli and purified them for biochemical and biological characterization. We demonstrate that full-length human p52Shc partitions between both membrane and soluble fractions with distinct biochemical, conformational and associative properties. The soluble and membrane associated p52Shc forms have different buffer preferences. Furthermore, the membrane associated form shows two circular dichroism minima at 222 nm and 208 nm while the soluble form shows only one distinct minimum at 210 nm. This is surprising considering the thermal unfolding is the same between both forms. This data would suggest that conformational differences between the two p52Shc forms are important in the binding and activation of c-SRC. Binding studies with both forms and c-SRC are currently underway.

2360-Pos Board B52
Non-Canonical Modular Domain Interactions Dictate PKCz Function

Carter J. Swanson1, Michael Ritt2, William Wang2, Michael Lang2, John J. Tesmer1, Margaret Westfall1, Sivaraj Sivaramakrishnan1.
1Biophysics, University of Michigan, Ann Arbor, MI, USA, 2University of Michigan, Ann Arbor, MI, USA.
The use of modular protein domains has emerged as a prominent feature of increasing phylogenetic complexity. Linking modular domains within a single protein allows complex regulation while conserving the sequence and structure of the individual domains. For instance, spatio-temporal control of signaling proteins is often achieved by stringing together a conserved catalytic domain with one or more regulatory modules. These modules can play multiple roles including masking the catalytic site to inhibit basal activity (auto-inhibition), releasing auto-inhibition through conformational changes triggered by second messenger stimuli, and facilitating translocation to subcellular compartments through binding secondary messengers or scaffolding proteins. Each additional module in a signaling protein provides a combinatorial enhancement to its regulation and cellular function. The protein-context independent structure and cellular function of individual modules have been extensively researched using biophysical approaches such as x-ray crystallography and NMR. Most modular domains have an evolutionarily conserved canonical function. However, coordination of interactions between these domains remains largely unexplored primarily due to the reliance on reductionist structural and biochemical approaches. As a corollary, our current structural understanding of modular signaling proteins does not adequately address the versatility of their cellular function. Using the uniquely persistent ERK x-helix derived from the lever arm of myosin VI combined with genetically encoded fluorophores we have previously developed a methodology termed SPASM to both observe and modulate intra-molecular interactions between domains in multi-domain proteins. Using human protein kinase C α (PKCα) as a model multi-domain signaling protein, we have uncovered intra- and inter-molecular interactions involving each of its modular domains. These interactions contribute to context-dependent spatio-temporal regulation of PKC function in cells. Our findings highlight the importance of intra-molecular interactions in biologically critical multi-domain proteins.

2361-Pos Board B53
The Histidine Button Dictates the Conformation of the pH-Sensitive Region of Troponin I

Sandra E. Pineda-Sanabria1, Ian M. Robertson1–2, Peter C. Holmes1–3, Brian D. Sykes1.
1Department of Biochemistry, University of Alberta, Edmonton, AB, Canada, 2Randall Division of Cell and Molecular Biophysics, King’s College London, London, United Kingdom, 3Department of Biochemistry, University of Oxford, Oxford, United Kingdom.
Along with calcium binding, association of the switch region of troponin I (TnI) to the regulatory N-domain of troponin C (NTnC) is a key step in the regulation of contraction in cardiac and skeletal muscle. The TnI-NTnC interaction has been structurally characterized and the orientation of the switch region of TnI relative to NTnC is known to be very similar for cardiac and skeletal muscle; however, the region of TnI immediately following in the sequence differs substantially and is involved in the differential pH sensitivity of myocytes and cardiomyocyte observed during ischaemia. A single amino acid substitution (A162H, the histidine button) in the acidosis-sensitive cardiac TnI turns the cardiac muscle into the acidosis-resistant phenotype of skeletal muscle. The cause of this improvement is attributed to electrostatic interactions promoted by the histidine button in TnI with NTnC, but little is known about the structural characteristics of this system. In this study, we used NMR spectroscopy to determine the conformation of the pH-sensitive region of cardiac TnI A162H and skeletal TnI when bound to cardiac NTnC at pH 6. The results show that the pH-sensitive regions of both isoforms are in very similar conformations and resemble the conformation of the skeletal TnI when bound to skeletal NTnC. This indicates that the histidine, and not the NTnC isoform or other TnI residues, is the main determinant of the conformation of the pH sensitive region of TnI. The promotion of a conformational change in cardiac TnI in the presence of A162H at low pH then leads to the stabilization of TnI and of the TnI-NTnC interface to continue the contraction mechanism in the face of acidosis.

2362-Pos Board B54
Purification and Structural Analysis of the Anti-Viral Protein BST-2

Kelly E. Du Pont, Christopher E. Berndsen.
Department of Chemistry and Biochemistry, James Madison University, Harrisonburg, VA, USA.
BST-2 is a human extracellular transmembrane protein that inhibits the release of viruses such as HIV-1 and Ebola from the cell surface. Viruses can evade this inhibition through antagonistic viral protein interactions with BST-2. The BST-2 is a homo-dimer that forms a coiled-coil connected by three disulphide bonds. Recent cellular studies suggest that the extracellular domain of BST-2 is flexible or structurally dynamic. However, x-ray crystallography suggests the coiled-coil structure is rigid. The goal of this study is to understand the relation between the full-length BST-2 structure and function, and the mechanism of viral protein binding. Through limited proteolysis, protein fluorescence, and small-angle x-ray scattering analysis we show that there is a flexible region and a rigid region in the extracellular portion of BST-2. The flexibility of the full-length protein is still unknown. We have purified both the membrane-bound protein of BST-2 and the viral antagonist protein, Vpu for biochemical and structural characterization. We are optimizing conditions for crystallizing the full-length BST-2, Vpu and the BST-2/Vpu complex. This will help us understand how BST-2 functions and the antagonistic interactions with viral proteins.

2363-Pos Board B55
Probing G Protein-Coupled Receptor Dimerisation by FRET and DEER

Patricia M. Dijkman1, Alan D. Goddard2, Oliver K. Castell1, Mark I. Wallace1, Anthony Watts1.
1Biomembrane Structure Unit, Department of Biochemistry, University of Oxford, Oxford, United Kingdom, 2School of Life Sciences, University of Lincoln, Lincoln, United Kingdom, 3Department of Chemistry, University of Oxford, Oxford, United Kingdom.
G protein-coupled receptors (GPCRs) are the largest class of eukaryotic membrane proteins. They trigger intracellular signalling cascades by activation of heterotrimeric G proteins. They are of great pharmaceutical interest, with approximately 40% of medicines being GPCRs. It has been shown that GPCRs can form oligomers in phospholipid bilayers in vivo and in vitro, affecting both ligand binding and G protein coupling [1]. Neurotensin receptor 1 (NTS1) is one of few GPCRs that can be produced in E. coli in an active state, and has been implicated in conditions such as schizophrenia and Parkinson’s and postulated as a biomarker for various cancers [2]. NTS1 has been shown to dimerise in lipid bilayers [3], and though a crystal structure of NTS1 in detergent was recently published [4], there is still no structural data on the receptor and its dimer in a membrane environment. We use a range of biophysical techniques to characterize the structure and function of NTS1 in model membrane systems, including ensemble and single molecule Förster resonance energy transfer (FRET), and double electron-electron resonance (DEER, also known as PELDOR). Fluorescence or nitroxide spin probes are attached to engineered cysteines on the transmembrane helices. By measuring intradimer distances between the probes on each monomer, we are studying dimerisation behaviour of NTS1 to produce a model of its dimeric structure in a more native lipid environment.

References