with cytosolic proteins viz., calmodulin and modulate cytoskeletal binding to the oligodendrocyte plasma membrane.
(Supported by CIHR, NSERC and MS Society of Canada)

1208-Pos
A Study of the Conformation of HIV Nef Bound to Lipid Membranes by Neutron Reflectivity
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Nef is one of six HIV-1 accessory proteins and directly contributes to AIDS progression. Nef associates with membranes and may require a transition from a solution conformation to a membrane-associated conformation. It has been hypothesized that a transition from a ''closed'' conformational form to an ''open'' form enables interaction of Nef with cellular proteins. Despite its obvious disease importance, there is little or no direct information about the conformation of membrane-bound Nef. In this work we used neutron reflection to reveal details of the conformation of membrane-bound Nef. Nef was bound through an N-terminal His tag to Langmuir monolayers of DPPC mixed with a synthetic metal-chelating lipid. Several methods were found to achieve a dense monomolecular layer of membrane-bound Nef, despite its tendency to form oligomers at high concentration. At the conditions of this initial study (65% DSIDA 35 mol% DPPC, 20 mM Tris, 20 C, pH 8.2), for the large majority of the bound population the core domain of membrane-bound Nef lies within a few Å of the lipid headgroups. The N-terminal arm is directly against the lipid headgroups with a small portion inserted. The results also indicate that the disordered loop extends from the core domain into the solution. The data also suggest that for a very small fraction of the bound population the N-terminal arm extends normal to the membrane and the core domain is displaced ~50 Å from the membrane. Some ramifications of these results for the activity of Nef are discussed.

1209-Pos
Site-Directed Spin Labeling Electron Paramagnetic Resonance Studies of Flap Conformations and Flexibility in Multiple HIV-1 Protease Variants
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Human immunodeficiency virus type 1 protease (HIV-1PR) is a 99 amino acid homodimeric aspartic protease that plays an essential role in the maturation and life cycle of the retrovirus HIV-1, as it functions in regulating post-translational processing of viral polyproteins gag and gag-pol. Accessibility of substrate to the active site is mediated by the conformational changes within two β-hairpins, or flaps. Site-directed spin labeling (SDSL) in conjunction with continuous wave and pulsed electron paramagnetic resonance spectroscopy was used to monitor the conformations of the flaps in HIV-1PR. Six inactive (D25N) HIV-1PR constructs were purified and spin labels were incorporated into the flaps at the aqueous exposed sites K55 and K55'. Constructs included Subtypes B, C, F, circulating recombinant form CRF01_A/E, and patient isolates V6 and MDR 769. For all constructs, two naturally occurring cysteine residues (C67 and C95) were substituted to alanine residues to allow for site-specific labeling as well as to avoid intramolecular disulfide cross-linking. B, F, C, and CRF01_A/E constructs contained three stabilizing mutations that provide protection from autocatalytic cleavage: Q7K, L33I, and L63I. Pulsed EPR results have shown that sequence variations within the subtypes of HIV-1 protease alter the average flap conformations within the apo-enzymes. With detailed data and error analysis, these altered distance profiles can be understood as shifts in the conformational sampling of four distinct HIV-1PR conformations, with some states having enhanced flexibility or structural instability, which may play an important role in viral fitness and drug-resistance. Additionally, DEER data was collected on each construct in the presence of nine different FDA-approved protease inhibitors and a non-hydrolysable substrate mimic called Ca-P2. Continuous wave EPR was used to monitor the autoproteolysis of active (D25) Subtype F and CRF01_A/E constructs.

1210-Pos
Correlated Force-Optical Spectroscopy of Single Human Nucleolar Phosphoprotein-140 Immobilized on Nano-Array Patterns
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Human nucleolar phosphoprotein-140 (hNopp140), an intrinsically unstructured protein (IUP), is known for its regulatory behavior of uncontrollable cell growth like in cancer and its unusual structural characteristics of a high percentage of flexible regions or extended loops. It is also one of the most highly phosphorylated mammalian proteins, of which interactions with ligands and other proteins depend strongly on the degree of phosphorylation. In this work, we employed a correlated force-fluorescence imaging and spectroscopic technique in conjunction with a nano-array patterning technique, to investigate the intrinsic shape of hNopp140 and its structural changes by phosphorylation, ligand binding and interactions with other proteins. For this, we prepared nano-array patterned surfaces containing hexagonally well-separated spots (~300 nm in diameter, ~ 2 um in distance), functionalized the spots with specific covalent linkers for targeting the cys residue of hNopp140, and immobilized single hNopp140 covalently on each spots. The presence of single hNopp140 on each spot was confirmed by single molecule fluorescence behavior of fluorescently labeled hNopp140 in TIRF mode and by subsequent high resolution AFM imaging. We finally performed single molecule force-fluorescence spectroscopy of hNopp140 itself and its complexes with ligands such as doxorubicin, mitoxantrone, and proteins such as CK2 under the precisely controlled nano-environments, and attempted to reveal the structural nature of hNopp140 and its intra- and inter-molecular interactions at single molecular level.

1211-Pos
Allosteric Inhibition of Thermus Thermophilus Phosphofructokinase
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Thermus thermophilus phosphofructokinase (TtPFK) comes from an extreme thermophile and exhibits entropically-driven inhibition by phosphoenolpyruvate (PEP). Interestingly, a PFK from the moderate thermophile Bacillus stearothermophilus also exhibits entropically-driven inhibition, while enthalphically-driven inhibition is observed for PFK from mesophilic E. coli. Although the thermodynamics of inhibition are similar for TtPFK and BsPFK, TtPFK exhibits a much weaker coupling between the inhibitor and substrate ($\Delta Gay = 1.60 \pm 0.04$ kcal/mol) when compared to that of BsPFK ($\Delta Gay = 5.0 \pm 0.9$ kcal/mol). Sequence alignment and crystal structures of BsPFK suggest that there is a network of interacting residues leading from the allosteric binding site to the active site. In the apo form, H215 forms a hydrogen bond with T158 and T156 interacts with D12 across the interface. In the inhibitor-bound form, T158 forms a hydrogen bond with D12. In TtPFK these interactions are missing due to substitutions at positions 215 (Ser) and 158 (Ala). Changing the amino acid residues at these positions to the corresponding amino acids in BsPFK resulted in an increase in coupling free energy to $\Delta Gay = 2.47 \pm 0.02$ kcal/mol for S215H and $\Delta Gay = 2.45 \pm 0.04$ kcal/mol for A158T. Currently the double mutant A158T/S215H is being investigated to see if the coupling can be augmented to the level of BsPFK coupling. Supported by NIH grant GM33216 and Welch Foundation grant A1548.

1212-Pos
Nucleotide Binding Induces Conformational Changes in Full-Length ClC-5
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Mutations within the Cl^-/H^+ transporter ClC-5 lead to Dent's disease, a kidney condition characterized by proteinuria. Numerous disease-causing mutations in ClC-5 translate into truncations of the carboxy terminus (Ct), a region binding adenine nucleotides and mediating ATP-dependent regulation of ClC-5 activity [Meyer, S. *et al.,* 2007; Zifarelli, G. & Pusch, M., 2009]. As the mechanism underlying allosteric regulation of ClC-5 activity is unknown the major goal of this work was to capture dynamic nucleotide-dependent conformational changes in ClC-5 Ct. Sedimentation velocity on purified Ct revealed a more compact peptide with ATP bound evident by an increase in its sedimentation coefficient (1.18 to 1.23 $s_{20,\omega}$) and a decrease in its frictional coefficient ratio (1.55 to 1.46) with ATP present. Photo-affinity labelling of ClC-5 in crude membranes with the analog γ^{32}P-ATP-γbenzophenone ensured full-length protein directly bound ATP. As the intracellular Ct is tethered to the membrane domain through the R helix, conformational changes in response to ATP binding could evoke global structural changes in ClC-5. In support of this hypothesis, ATP binding mediated global conformational changes in membrane bound ClC-5 as revealed by ATP-dependent increases in the accessibility of endogenous cysteine residues (2.04 ± 0.34 times the control). Importantly, changes in accessibility were not observed in D727A ClC-5, the mutant unable to specifically bind ATP in photo-labelling experiments. Future work will focus on uncovering the physiological significance of nucleotide-dependent regulation of ClC-5 activity in endocytic uptake and acidification in the kidney. This work was funded by a Kidney Foundation of Canada operating grant to CB and a studentship from NSERC to LW.