several disease-causing mutations in ClC-5 translate into truncations of the carboxy terminus (Ct), highlighting the significance of this region. The Ct interacts with the scaffold protein NHERF2 (Na^+/H^+ exchanger regulatory factor 2) and leads to enhanced endocytic uptake of albumin by kidney cells [Hryciw, D. *et al.* 2006]. This interaction occurs through the second PDZ domain of NHERF2 and an unknown binding site on the Ct of ClC-5 [Hryciw, D. *et al.* 2006]. In the present study NHERF2/ClC-5 Ct interactions were confirmed and a putative internal PDZ binding motif TSII (residues 657-60) was identified in the Ct. Mapping of this motif on the crystal structure of ClC-5 reveals it lies up-stream of a β-turn, a secondary structure element thought to be critical for PDZ domain recognition of an internal motif [Hillier, B. *et al.* 1999]. The strategic S658A mutation completely abolishes NHERF2 binding *in vitro* suggesting the PDZ binding motif was targeted. CD analysis confirmed similar spectra between wildtype and S658A mutant, indicating that this mutation does not lead to gross misfolding of the Ct. The disease mutant R648X which removes this critical S658 abolishes NHERF2 interactions providing further evidence the internal PDZ binding motif was targeted. Future work will focus on identifying proteins interacting with the ClC-5/NHERF2 complex and deciphering the role of this complex in renal endocytosis. This work was supported by the Kidney Foundation of Canada operating gant to C.B. and a NSERC studentship to L.W.

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Structural and Functional Role of Proline Residues in Fibroblast Growth Factor-1

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Fibroblast Growth Factors (FGFs) belong to a large family of β-trefoil polypeptide growth factors and contain no disulfide binds. FGFs are involved in various important biological processes such as development and maintenance of cells. FGFs are about 154 amino acids long and are believed to share a common core of 140 amino acids with 6 invariant proline residues. The secondary structural elements in FGFs include 12 antiparallel beta-sheets arranged in to a beta-barrel motif. Critical analyses of the three-dimensional structure of human acidic fibroblast factpr (hFGF-1) show that proline residues play a critical role in shaping the beta-barrel architecture of the protein. In this context, using a site-directed mutagenesis approach, we examined the effects of substitution of proline on the structure, stability and function of hFGF-1. The stability of of the various proline mutants of FGF-1, were assessed by differential scanning calorimetry, limited proteolytic digestion, and urea-induced equilibrium unfolding. Conformational changes induced in the various proline mutants of hFGF-1 have been studied using 1-anilino-8-napthalene sulfonate binding, far UV circular dichroism, and multidimensional NMR spectroscopy. The heparin and receptor binding affinities of the hFGF-1 mutants were investigated by isothermal titration calorimetry. The results of this study are expected to provide valuable information for a rational design of hFGF-1 with increased stability and enhanced cell proliferation activity.

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Structural Changes in Monomeric HIV-RT Upon Binding the NNRTI Efavirenz

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HIV-1 reverse transcriptase is a complex multi-subunit enzyme that converts single stranded viral RNA into double stranded proviral DNA. This enzyme possesses both DNA polymerase and RNase H activity. Structurally, RT is composed of a 66 kD (p66) subunit and a 51 kD (p51) subunit that lacks an RNase H domain. The homodimers retain DNA polymerase activity but only the p66/p66 homodimer has RNase H activity. In solution, the enzyme exists as an equilibrium mixture of the heterodimer p66/p51, two homodimers p66/p66 and p51/p51 and the two monomers. The nonnucleoside reversible inhibitor (NNRTI) efavirenz enhances dimerization of p66/p51, p66/p66, and p51/p51. The drug binds both monomeric and dimeric RTs. However, structural changes induced by drug binding are unknown. A combination of hydrogen deuterium exchange and mass spectrometry is used to probe the solution structure of RT monomers in the presence and absence of efavirenz. Here we demonstrate that p66 and p51 monomers have similar solution structures, which resemble the p51 subunit in the crystal structure of p66/p51 heterodimer. After drug binding the solution structure of the monomers changes to resemble the p66 subunit in the crystal structure of the heterodimer. We also show that residues in the drug binding pocket of p66/p51-efavirenz complex are affected by drug binding to monomer.

2302-Pos Board B272

Stabilizing Interactions In TNF Ligand-receptor Binding

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High-affinity binding of extracellular protein ligands belonging to the tumor necrosis factor (TNF) superfamily to their conjugate transmembrane cell surface TNF receptors initiates the extrinsic signaling cascade that results in apoptotic cell death. Intriguingly, despite a high-degree of structural homology among the family of ligands and receptors, the ligand binding is both high-affinity and has high-specificity. As yet, it is poorly understood what confers this specificity. Starting from the crystal structures for the TRAIL-death receptor 5 and LTα-TNF receptor 1 complexes, we used all-atom molecular dynamics simulations to investigate the stabilizing interactions between these ligand-receptor pairs. Additionally, we simulated both complexes with destabilizing point mutations. The simulation results yield insight into published experimental data as well as the underlying mechanism of high affinity ligand-receptor binding.

2303-Pos Board B273

Understanding the Mechanism of Autoregulation of FGF Signaling

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Fibroblast growth factors (FGFs) are heparin binding proteins that help regulate key cellular processes such as wound healing and differentiation, cell proliferation, cell migration, morphogenesis, and angiogenesis. The FGF signaling is generated by the binding of the ligand (FGF) to the extracellular domain of the FGFR, this binding induces dimerization of FGFR, which is an essential step in FGF signaling. Fibroblast growth factor receptor (FGFR) extracellular domain consists of three Ig domains D1, D2, and D3. Between the Domains D1 and D2 is a short span of acidic residues called the ''acid box''. The D1-D2 linker is thought to play a role in regulation of FGF interaction with FGFR. Many of the FGF binding sites can be found on the extracellular D2 domain of the receptor. It is believed that ''acid box'' can regulate FGF binding to FGFR. The ''acid box'' can mimic heparin like compounds and bind at the heparin binding sites located on the surface of the D2 region of FGFR. In the present study, we synthesized a twenty-eight amino acid box region peptide and studied its interaction with D2 domain of FGFR using various biophysical techniques including multidimensional NMR spectroscopy. Equilibrium unfolding experiment monitored steady state fluorescence, far-UV circular dichrosim and proteolytic digestion experiments reveal that acid box binds to D2 domain very weakly. Two-dimensional nuclear magnetic resonance ^{1}H-^{15}N HSQC experiments show that the acid box binds to the FGF-1 and heparin binding sites in the N-terminal end of the D2 domain of FGFR. Our results clearly show that the acid box peptide binds to the ligand binding domain of the fibroblast growth factor receptor.

2304-Pos Board B274

Interactions Defined between S100A13 and Annexin Peptides: Insight into Non-Classical Secrection

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S100A13 is a calcium binding chaperone protein, known to be involved in the non-classical export of signal peptide less proteins such as fibroblast growth factor (FGF-1) and interleukin-1α across the cell membrane. It has also been shown that the interaction of S100A13 with Annexin II, which exhibits an inducible flip-flop mechanism across the cell bilayer, helps the multiprotein release complex to traverse the membrane bilayer. The interaction of S100A13 and annexin II has been characterized using various biophysical techniques including multidimensional NMR spectroscopy. Results of the Isothermal titration calorimetry (ITC) experiments show that holo-S100A13 exhibits preferential binding to annexin II with high affinity in the micro molar rang compared to apo-S100A13. Equilibrium guanidine hydrochloride denaturation monitored by steady-state fluorescence and limited trypsin digestion analysis reveals holo-S100A13 is significantly stabilized upon binding to annexin II peptide compared to apo-S100A13. ANS (8-anilino-1-napthalene sulfonate) binding experiments indicates that the presence of annexin II peptide does not increase the solvent availability of hydrophobic residues in holo-S100A13. Availability of the solvent-exposed hydrophobic surface(s) in apoS10013 does not facilitate its interaction with the annexin II peptide, which is unique characteristic of S100A13. 1H-15N- HSQC NMR experiments reveal that the binding site of annexin II peptide on holo-S100A13 is modestly different from other S100/Annexin interactions. S100 proteins typically interact with more than one Annexin protein. In order to define the specificity of S100A13 for Annexin 2, the interaction of S100A13 with other Annexin peptides was characterized and data

characterizing these interactions will be presented. Finally, defining the interaction between S100A13 and Annexin 2 peptide will give much needed insight into the non-classical release of the signal peptide-less protein Fibroblast Growth Factor-1

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Redefining The Interaction Domain Of Cx43CT With RXP-E And Cx43CL

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Connexins are integral membrane proteins that oligomerize to form intercellular gap junction channels. These channels allow the passage of ions, small molecules, and second messengers that are essential for the coordination of cellular function. Many factors have been identified to regulate the channel gating of the major cardiac connexin, connexin43 (Cx43) and our laboratory has focused on pH-regulation, which is a cause of ischemia-induced arrhythmias. Our previous studies have suggested that regulation of Cx43 channels results from the association of the carboxyl-terminal domain (Cx43CT), acting as a gating particle, and the cytoplasmic loop domain (Cx43CL), acting as a receptor for the gating particle. Recently, we have identified a synthetic peptide, RXP-E (30-mer peptide containing a RXP sequence specifically recognized by Cx43CT, where X represents any amino acid, and R and P correspond to arginine and proline) that interacts with the Cx43CT and can prevent closure channel. The question remains as to whether this peptide is involved in the disruption of the Cx43CT/Cx43CL interaction. Using NMR, we investigated the changes in the structure of Cx43CT with RXP-E and identified the binding site in the first half of the Cx43CT. The identification of this new specific binding domain was furthermore confirmed by mutagenesis. Additionally, we studied the interaction of Cx43CT with Cx43CL and identified a similar binding region as RXPE, confirming the potentiality of our peptide to act as a ''scaffold'', thereby holding the channels in its open state and reducing life threatening arrhythmias.

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Amyloid Fibrillation of Bovine Alpha Lactalbumin

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In recent years, scientific community has paid great attention to characterize the protein folding and unfolding processes. Interestingly they can exhibit wide range of structures like oligomers and aggregates. In the present work, acidic fibrillation of bovine alpha-lactalbumin (α-LA) was studied in presence and absence of natural small nontoxic compounds at 37°C in 10mMTris/10mM NaCl buffer, pH 2.2.

Various techniques including, ThT intensity, Congo Red absorbance, far- and near-UV, circular dichroism (CD) and intrinsic fluorescence spectroscopy, ANS intensity, Gel filtration, TEM microscopy, were applied to characterize the inhibitory effect of them.

Congo Red clearly demonstrated the inhibitory effect of one of these compounds in amyloidogenesis pathway. The formation of beta structures was evidently demonstrated by CD and the instability of α-LA by intrinsic fluorescence and ANS intensity through analysis of nucleation step is confirmed. To give more assurance, one of these compounds was added at different times including 0, 12, 18, 24 and 48 hours. Our data indicated that it does not play an important role in the stability of native α-LA. However its addition after 18 hours inhibited amyloid fibrillation.

2307-Pos Board B277

Inhibition of Influenza Virus Activity by Sialic Acid Conjugated Multivalent Particles

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Influenza virus binds via its envelope protein hemagglutinin (HA) sialic acid (SA) residues of glycoproteins on the plasma membrane of the host cell. The affinity of a single HA-SA pair is low (10^3 M^{-1}) compared to the overall affinity between virus and cell surface caused by multiple simultaneous interactions. For example, the affinity between virus and erythrocytes - serving as a model for a host cell - is about 10^{13} M^{-1}. Multivalency is ubiquitous in biology and can dramatically enhance affinities. We use synthetic polymers with multiple sialic acid moieties to investigate their inhibitory effect on virus binding and fusion. The aim of this study is to gain insights into the molecular mechanism and the effect principles of multivalent inhibitors. Furthermore we aim to measure the accurate affinity between a virus particle and the target erythrocyte membrane by using a new developed optical tweezers system. With this approach we are able to explore the action of multivalent inhibitors on the single molecule level. Furthermore, we study binding of labelled virus particles to human erythrocytes using fluorescence activated cell sorting (FACS). The fusion activity is examined by detection of fluorescence de-quenching of R 18 labeled viruses attached to human erythrocyte ghosts.

2308-Pos Board B278

Structural Explanations to Altered Drug Resistance Pathways in HIV-1 Non-Clade B Proteases

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The majority of human immunodeficiency virus-1 (HIV-1) infections across the world result from non-B clades. CRF01_AE is predominantly seen in Southeast Asia and the protease differs by ~10% when compared to the clade B protease. Polymorphisms in CRF01_AE are often associated with drug resistance in the clade B protease. CRF01_AE protease has been observed to develop the unique N88S mutation in response to nelfinavir (NFV) therapy which is not commonly seen in clade B protease. We present here, structural and binding thermodynamic data on CRF01_AE protease in order to explain how sequence polymorphisms within CRF01_AE protease might affect its activity as well as to explain the altered NFV resistance pathway observed in CRF01_AE. The crystal structure of HIV-1 CRF01_AE N88S protease in complex with Darunavir (DRV) was determined to a resolution of 1.76 Å and was compared with the clade B protease in complex with DRV. The CRF01_AE structure shows a significant change in the flap hinge region of the protease when compared to the clade B structure. The Ser88 side chain in the CRF01_AE structure is involved with a novel network of hydrogen bonds and interacts with the side chain of Asp30. This likely disrupts a critical hydrogen bond required for NFV binding. Binding constants and thermodynamic parameters for CRF01_AE and clade B protease were determined by isothermal titration calorimetry (ITC). Calorimetric data indicate that wild type CRF01_AE protease has ten fold and two fold weaker to NFV and DRV respectively when compared to clade B protease. This weakened affinity may permit the alternative pathway for NFV resistance via N88S, which is a mutation outside the active site.

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The *In Vivo* Analysis Of *Escherichia coli's* SecA Membrane Topology In The Nucleotide Binding Domain I

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In Eubacteria, the ATPase SecA transports secretory proteins across the inner membrane using the membrane embedded channel, SecYEG. SecA drives the preprotein through the channel in an ATP dependant manner that promotes a series of conformational changes associated with the insertion and deinsertion of a region of SecA during the translocation cycle. To gain additional insight into the interactions between SecA and SecYEG, an *in vivo* sulfhydryl labeling technique was developed to probe monocysteine SecA mutants through the channel during protein translocation using N-(3-maleimidylpropionyl)biocytin (MPB). Our lab demonstrated multiple residues of SecA that are exposed to the trans side of the membrane through the SecYEG channel. These residues, which were located throughout most domains of SecA, resided on a single face within the *Bacillus subtilis* SecA crystal structure. This provided some insight into the regions of SecA that are in fluid contact with the channel. Interestingly, two residues located on the opposite side of the labeled face of SecA in the nucleotide-binding domain I (NBD-I) also labeled. In the present study, the NBDI domain was further investigated to determine if there were other residues in fluid contact with the SecYEG channel. Additional monocysteine SecA mutants were created in the NBDI domain and analyzed using the same methodology. After looking at the crystal structure with the newly labeled residues, the labeled face of SecA seemed less apparent. These results emphasize the dynamic nature of SecA and identified regions of SecA that may interact with the channel. The NBDI mutants will be further analyzed using an *in vivo* photocrosslinking technique.