metals to an LNR via isothermal titration calorimetry (ITC). In this work, we used a combination of computer modeling and experimental approaches to characterize and compare the Ca^{2+} binding affinities and coordination geometries of various LNR sequences from different proteins. We expect this work to elucidate the basis for Ca^{2+} ion selectivity by the LNRs that is integral for their structural integrity and is required for the proper regulation of the Notch signaling pathway.

2294-Pos Board B264

Regulation of Nuclear PLCβ1 by a Novel Binding Partner called TRAX

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Previous research has led to the discovery that the plasma membrane signaling protein PLCβ1 is sometimes present in the nucleus. Little is known how PLCβ1 is regulated in the nucleus on the plasma membrane. PLCβ1 activity is regulated by G proteins but these have not been found in the nucleus. The focus of this study is to find binding partners for nuclear PLCβ1 and investigate their role in the regulation of its activity in the nucleus. A protein called translin-associated factor-X, TRAX, has been identified as a potential binding partner for nuclear PLCβ1. The work done in this report shows that the two proteins bind in vitro and in living cells. Using a combination of biophysical and biochemical methods, we find the two proteins interact and that TRAX may regulate nuclear PLCβ1 activity.

2295-Pos Board B265

Hybrid Scoring and Classification Using Shape-Based Approaches to Predict Human PXR Activators

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The human pregnane X receptor (PXR) is a transcriptional regulator of many genes involved in xenobiotic metabolism and excretion. Human PXR activators include an extensive range of structurally diverse endogenous molecules, drugs which potentially result in potential drug-drug interactions. Reliable prediction of molecules interacting with this receptor would be valuable for pharmaceutical drug discovery and environmental applications. In the current study, computational models for human PXR activators and PXR non-activators were developed using support vector machine (SVM) algorithms using Shape Signatures and MOE descriptors. The models were validated using separate test sets. The overall test set prediction accuracy for PXR activators with SVM was 72 to 81 % in line with a previous study using VolSurf descriptors and SVM. We have also used the rigorous docking program GOLD and coupled the GoldScore with other scoring functions in an attempt to improve docking results from those previously attained. In this study, the best docking prediction accuracy (61 %) was obtained using 1D Shape Signature descriptors as a weighting factor to the GoldScore. We have also combined the available human PXR data sets into a single larger model (~300 molecules) and described the specific molecular descriptors that we demonstrate can help predict whether a molecule activates PXR. These combined computational approaches could enable us to more confidently identify PXR activators and to further avoid them in various applications.

2296-Pos Board B266

Fluorescence Quenching and Fluorescence Resonance Energy Transfer Studies in the Recombinant N-domain from the Plasma Membrane H(+)-ATPase, Pma1

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Upon substrate binding the isolated plasma membrane H(+)-ATPase (Pma1) from *Kluyveromyces lactis* displays large changes in fluorescence intensity (Sampedro *et al*, 2007 Biochemistry 46:5616-5622). The nucleotide binding domain (N-domain) contains one Trp505 residue, that seems to be responsible for the variations in intrinsic fluorescence. The N-domain was cloned and the protein expressed in *E. coli*. The purified N-domain displayed nucleotide-dependent (ATP and ADP) quenching of fluorescence similar to that observed in the whole Pma1. The dissociation constants (Kd) for ATP and ADP were 100 and 110 uM respectively. Fluorescence resonance energy transfer (FRET) studies were also performed by using mantATP; a fluorescent ATP analog (Ex. 337 nm, Em. 423nm). The absorbance spectra of mantATP overlaps the fluorescence spectra of the N-domain, and thus FRET was observed by exciting at 280 nm. FRET efficiency was 100% indicating a close proximity between Trp505 and the nucleotide. Therefore, in this domain there is a Trp residue located near the substrate binding site which is of high value to determine Kds and molecular distances using fluorescence.

2297-Pos Board B267

NMR Study of the Interaction of Cardiotoxic Drugs with the Extracellular Segment Ile^{583} - Tyr^{597} from the hERG Channel

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Long QT syndrome (LQTS) is a cardiac muscle abnormality caused either by congenital or drug-induced malfunctioning of potassium channels localized in the myocardium cell membranes. LQTS can lead to ventricular arrhythmia and sudden cardiac death. A number of prescription medications inducing long QT have been withdrawn from the market over the past decades, and virtually all cases of drug-induced LQTS are due to the blockade of the heart *human ether-a-go-go-related-gene* (hERG) potassium channel. Evidences show that most of the hERG-channel blockers would exert their activity by binding one or several sites located in the pore region composed of the last two TM helices (S5 and S6) or on the extracellular region connecting S5 and S6 together. In this work, we studied the binding of 4 cardiotoxic drugs (bepridil, cetirizine, diphenhydramine, pentamidine) with a portion of the extracellular segment (Ile^{583} - Tyr^{597}) of the hERG channel and a model membrane. Drug-peptide interactions were studied using ^{1}H liquid-state NMR with pulsed field gradient self-diffusion measurements. According to our CD and ^{1}H NMR results, the peptide appears to be unstructured both in water and membrane mimetic isotropic bicelles. Diffusion measurements suggest that there is no or only weak drug binding to the peptide. However, a strong interaction with the model membrane was evidenced for the bepridil molecule, thus suggesting a potential role of the membrane in the cardiotoxicity of LQTS-active drugs. Our current work, which focuses on drug-membrane interactions and hERG peptide-membrane interactions by ^{31}P and ^{2}H solid-state NMR will also be presented.

2298-Pos Board B268

Protein Selectivity Factors as a Molecular Basis for Metal Toxicity

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Toxic metals are known to displace physiologically-relevant metal ions in proteins, and may activate or deactivate protein function in response to changes in the free metal concentration. To investigate potential relationships between metal/protein complexes and toxicity, an analysis of proteins structural and sequential data was used to establish statistical bases for identifying key selectivity factors associated with Pb^{2+}-protein binding. These data led us to hypothesize that Pb^{2+}, and potentially other toxic metals, may induce opportunistic binding in regions of negative electrostatic potential, thus altering the proteins conformation.

To compare structural/conformational changes, investigate selectivity and affinity, and probe the mechanism of toxic metal-protein interactions, several natural and engineered Ca^{2+}-binding proteins (CaBPs) were analyzed using Fluorescence, CD and 1D and 2D NMR spectroscopy. Engineered proteins were developed based on grafting methods that involved insertion of metal-binding motifs in flexible regions of protein scaffolds to investigate biophysical properties associated with binding reactions in isolated sites. Additionally, the ubiquitous signaling protein calmodulin (CaM) was evaluated extensively to determine changes associated with competitive binding between Ca^{2+} and anthropogenically available toxicants such as Pb^{2+}, Gd^{3+}, La^{3+}, Tb^{3+} and In^{3+}. Results suggest that certain toxic metals may not only displace the biologically-relevant metals in metalloproteins, but support our hypothesis that opportunistic binding occurs in non-sites. This has important implications for the potential binding of toxic metals by non-metalloproteins, as well as providing a basis for understanding the impact of toxicity related to downstream protein-protein interactions.

2299-Pos Board B269

Identification Of The NHERF2 Binding Site For The Chloride/Proton Transporter ClC-5

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The chloride/proton transporter ClC-5 mediates the re-absorption of filtered proteins in the kidney by promoting the formation of the macromolecular endocytic complex and by aiding in endosomal acidification upon complex internalization in proximal tubule cells. Mutations disrupting ClC-5 lead to proteinuria reflecting a severe impairment of renal receptor endocytosis and

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.

2300-Pos Board B270

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.

2301-Pos Board B271

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