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**

**Omoz Aisiku**[1], Mario Rebecchi[1], Loren Runnels[2], Suzanne Scarlata[1].
[1]Stony Brook University, Stony Brook, NY, USA, [2]UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ, USA.

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**

**Sandhya Kortagere**[1], Dmitriy Chekmarev[2], William J. Welsh[2], Sean Ekins[2].
[1]Drexel University College of Medicine, Philadelphia, PA, USA, [2]University of Medicine and Dentistry of New Jersey, Robert Wood Johnson Medical School, Piscataway, NJ, USA.

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**

**Yadira G. Ruiz-Granados**[1], Nayelli Cabrera[1], Salvador Uribe[1], José G. Sampedro[2].
[1]Instituto de Fisiologia Celular, UNAM, Mexico, Mexico, [2]University of Michigan, Ann Arbor, MI, USA.

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**

**Etienne Chartrand**, Alexandre A. Arnold, Séverine Convenant, Sarah Jenna, Isabelle Marcotte.
University of Quebec in Montreal, Montreal, QC, Canada.

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**

**Michael Kirberger**, Jin Zou, Jie Jiang, Jenny Yang.
Georgia State University, Atlanta, GA, USA.

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**

**Leigh Wellhauser**[1,2], Himabindu Penmatsa[3], Anjaparavanda P. Naren[3], Christine E. Bear[1,2].
[1]Department of Biochemistry, University of Toronto, Toronto, ON, Canada, [2]Department of Molecular Structure & Function, Hospital for Sick Children, Toronto, ON, Canada, [3]Department of Physiology and Biophysics, University of Tennessee, Memphis, TN, USA.

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