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

2305-Pos Board B275

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

Gaelle Spagnol[1], Wanda Coombs[2], Steven M. Taffet[2], Bjarne Due Larsen[3], Mario Delmar[4], Paul L. Sorgen[1].
[1]UNMC, Omaha, NE, USA, [2]SUNY Upstate Medical University, Syracuse, NY, USA, [3]Zealand Pharma A/S, Glostrup, Denmark, [4]University of Michigan Health System, Ann Arbor, MI, USA.

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.

2306-Pos Board B276

Amyloid Fibrillation of Bovine Alpha Lactalbumin

Mehriar Amininasab, Mohamad-Bagher Ebrahim-Habibi, Azadeh Ebrahim-Habibi, Mohsen Nemat-Gorgani.
University of Tehran, Tehran, Iran, Islamic Republic of.

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

Christian Sieben[1], Ilona Papp[2], Anna Wozniak[3], Kai Ludwig[2], Christoph Böttcher[2], Thomas Korte[1], Andreas Herrmann[1].
[1]Humboldt-University Berlin, Berlin, Germany, [2]Freie Universität Berlin, Berlin, Germany, [3]JPK Instruments AG, Berlin, Germany.

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

Rajintha M. Bandaranayake[1], Madhavi Kolli[1], Moses Prabu-Jeyabalan[1], Madhavi Nalam[1], Christina Ng[1], Junko Kakizawa[2], Annie Heroux[3], Nancy M. King[1], Wataru Sugiura[2], Celia A. Schiffer[1].
[1]University of Massachusetts Medical School, Worcester, MA, USA, [2]National Institute of Infectious Diseases, Tokyo, Japan, [3]Brookhaven National Laboratory, Upton, NY, USA.

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.

2309-Pos Board B279

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

Lorry M. Grady.
Wesleyan University, Middletown, CT, USA.

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.