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2345-Pos Board B115

Design of Novel Heparin Binding Peptide

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Heparin-binding proteins (HBPs) are responsible for a number of biochemical events that take place in living organisms. The heparin molecule is a glycosaminoglycan, or a polysaccharide made up of linked disaccharide units, with a relatively high level of sulfation. Heparin is an anticoagulant often used to prevent blood clotting. The fibroblast growth factor family is one of the most studied HBPs and consists of twenty-three members of FGF. Important functions of FGF include cell proliferation, cell differentiation and angiogenesis. It has been shown that FGF must bind to heparin before it binds to the fibroblast growth factor receptor (FGFR) on the outside of the cell membrane. Recently, there have been various reports about the presence of some conserved signature sequences (XBBXBX, XBBBXXBX, TXXBXXTBXXXTBB) in various HBPs.
The present study is focused to classify the members of the FGF family based on their affinity for heparin and the presence of each of these HB signature motifs, and also to identify the list of other non-classified HBPs from the Swiss-Prot database.
After building an exclusive database of HBPs with various categories, an exact relationship between the conserved HB signature motifs and affinity to heparin will be ascertained. Based on the analysis, a consensus heparin binding peptide will be designed and its structure and inhibitory role on the cell proliferation activity of FGF will be examined.

2346-Pos Board B116

Biophysical Regulation of 2D Kinetics of Platelet Membrane Receptor GPIB-Alpha and its Ligand VWF

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During the hemostasis and thrombosis process, it has long been recognized that early stage platelet adhesion is primarily mediated by VWF/GPIbα adhesive bond. On the other hand, there is growing evidence that platelet rolling and tethering might be influenced by physical factors that affect binding kinetics of VWF/GPIbα. By monitoring the decrease/resumption in thermal fluctuation of a biomembrane force probe(BFP), we visualized the VWF-GPIbα bond repeating formation and dissociation in real time. Here, we investigated further the effect of varying the physical factors, specifically, receptor-ligand separation distance, BFP spring constant and diffusivity on the kinetics of the GPIbα interaction with the VWF. We found that increasing the gap distance between VWF and GPIbα gradually lowered the on-rate and leveled up the off-rate, but completely abolished the binding at about 80nm that exceeds the total length of the receptor and ligand axis. Moreover, increasing the BFP spring constant or decreasing the diffusivity reduced on-rate but have nothing to do with off-rate. To justify our methods, we measured the kinetics of two VWF A1 mutants, R687E(gain-of-function) and G561S(loss-of-function). Compared with wild-type A1, the former showed both >10-fold higher on-rate and lower off-rate, while the later showed lower on-rate but similar off-rate. These results match well with hypothesis that biophysical regulation of on-rate is governed by transport mechanism(Fokker-Planck type diffusion-reaction equation), while off-rate is by force. The significance of this study is to shed light to quantification of biophysical regulations that allow platelet to sense and response to local changes in their hemodynamic environment.

2347-Pos Board B117

Exploration of the Role of the IκB Pest Sequence in Stripping NFκB from DNA

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Nuclear factor kappa B (NFκB) transcription factors are responsible for the regulation of more than 150 target genes, their expression is induced by many classes of stimuli, and NFκBs play essential roles in the healthy regulation of cellular development and proliferation in inflammatory and immune responses. Diseases such as cancer, heart disease, Alzheimer's disease, and AIDS can be attributed to the wayward regulation of NFκB. The transcriptional activity of NFκB is commanded by its inhibitor—IκB. Two major isoforms of the inhibitor—IκBα and IκBβ—are structurally similar (i.e., crystal structures of the two exhibit a six ankyrin repeat domain followed by a largely unstructured, C-terminal PEST sequence), yet their inhibitory activities markedly differ. It has been shown that IκBα can actively ''strip'' NFκB from DNA *in vitro*, but IκBβ cannot. We are investivating a panel of ''PEST swap'' mutants to probe the role of the PEST sequence in the regulation of NFκB. In addition, the role of individual residues within the IκBα PEST sequence in the ''stripping'' phenomenon is being analyzed.

2348-Pos Board B118

The Case for Irreversible Binding of Ice-Binding Proteins to Ice

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Ice-binding proteins (IBPs) include proteins that have the ability to stop ice crystal growth and inhibit ice recrystallization. IBPs do this by adsorbing to the surface of the ice and making the addition of water to the crystal unfavourable. Their surface adsorption causes a thermal hysteresis (TH) between the lowered freezing temperature and slightly elevated melting temperature.The unique interactions of IBPs with ice crystals suggest their potential use in cryopreservation of foods, cells, tissues, and organs. It has been argued that IBP binding must be irreversible because the ice crystals do not grow in the TH gap. However, this seems inconsistent with the observation that TH values are influenced by IBP concentration. We have made three recent experimental demonstrations that IBP binding to ice is indeed irreversible: i) photo-bleaching of GFP-tagged IBP residing on the surface of an ice crystal held in the TH gap shows that there is neither exchange nor overgrowth of the bleached IBP; ii) ice crystals bound by IBPs show a measurable resistance to melting (melting hysteresis) demonstrating that the IBPs remain surface-bound at temperatures above the equilibrium melting point; iii) using a temperature controlled microfluidics apparatus it is possible to entirely replace the IBP solution surrounding an IBP-bound ice crystal in the TH gap with buffer, without losing the bound IBP. According to the anchored clathrate water hypothesis for the mechanism of IBP binding to ice, the ice-binding site of the IBP forms its ligand before merging with it. These results imply that IBP adsorption to the ice surface is irreversible and that TH is a function of the absorbed proteins on the surface and only indirectly a function of the concentration of IBPs in the solution.
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2349-Pos Board B119

Enhancer Interactions with Antifreeze Protein: Mechanistic Insights into the Effect of Antifreeze Activity Enhancement

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Antifreeze proteins (AFPs) found in fish, insects, and many other organisms can lower the freezing point of water in a noncolligative manner and create a difference between the freezing point and the melting point, which is a measure of their antifreeze activity, also referred as to thermal hysteresis (TH). In the presence of certain low molecular weight molecules, the antifreeze activity of AFPs can be further enhanced. Recent studies revealed that interactions between the enhancers and arginine play an important role in the TH enhancement effect of an AFP from the beetle *Dendroides canadensis* (DAFP-1). Several phosphate-containing coenzymes have then been identified as highly efficient enhancers of DAFP-1. This work demonstrates the effect of nicotinamide adenine dinucleotide phosphate (NADP) on the antifreeze activity of DAFP-1 using differential scanning calorimetry (DSC) and provides the first detailed quantitative interaction study between an enahncer and DAFP-1 using isothermal titration calorimetry (ITC). The binding between NADP and DAFP-1 was found to be favored both enthalpically and entropically. The experimental data are analyzed thermodynamically and a simple energetic estimation is given for this enhancement effect. The results imply that the interaction between enhancers and AFPs is a dynamic process involving water molecules, supporting an earlier hypothesis. Possible molecular mechanisms of small molecular weight enhancer action are also discussed.

2350-Pos Board B120

Determination of the Carbohydrate-Binding Specificity of Lectin-Like Domains in *Vibrio Cholerae* Cytolysin

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Vibrio cholerae cytolysin (VCC) is a secreted pore-forming toxin that contains two putative sugar-binding domains: the β-trefoil domain and the β-prism domain. Previous hemagglutinating studies suggest that VCC binds glycoproteins with terminal β1-galactosyl moieties [1]; however, crystallization of monomeric VCC with β-octyl glucoside suggests the β-prism domain may have an affinity for glucosides. We aim to characterize the carbohydrate-binding specificity of the β-trefoil and β-prism domains. The crystal structure of VCC indicates that Asp617 within the β-prism domain forms hydrogen bonds with the bound glucoside moeity. Hemolysis assays demonstrated that the removal of the β-prism domain causes a 1000-fold decrease in lytic activity toward rabbit erythrocytes, while conversion of Asp617 to alanine and lysine decreases the hemolytic activity100-fold, indicating that the sugar-binding activity of VCC may play a role in cell lysis. The β-prism domain was expressed as a separate

construct and isothermal titration calorimetry (ITC) was used to determine the binding affinity for the interaction of the β-prism domain with monosaccharides. The binding of the isolated β-prism domain reflected the binding affinities observed using ITC for full length VCC. The β-prism domain showed an affinity for mannose, but not galactose, indicating that recognition of terminal β1-galactosyl moieties does not occur at the β-prism domain. The carbohydrate-binding activity of the β-prism domain may explain its role in cell lysis.

[1] Saha, N; Banerjee, K.K. Carbohydrate-mediated Regulation of Interaction of *Vibrio cholerae* Hemolysin with Erythrocyte and Phospholipid Vesicle, J. Biol. Chem. (1997) 272, 162-167.

2351-Pos Board B121

Function of O-Glycans in Leukocyte Rolling on PSGL-1

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In published data from Bernimoulin et al. (2003) it was shown that inclusion of the Core-2 O-glycan branching of PSGL-1 is required for effective L-selectin binding to PSGL-1. Recent data from our lab suggests that a form of PSGL-1 that lacks the Core-2 branching shows significant L-selectin binding to PSGL-1. This result suggests that another carbohydrate branching on PSGL-1 may be capable of providing some of the functionality of the Core-2 branching in the PSGL-1/L-selectin binding interaction. A Core-1 extension is known to exist, as described by Yeh et al. (2001), and when co-expressed with Core-2 in cells with PSGL-1 was shown to provide synergistic improvement of cell rolling over cells expressing Core-2 alone with PSGL-1. Therefore we investigated the possibility that the carbohydrate branching on the modified PSGL-1 might be a Core-1 extension. The transcripts of the cell line were examined for the presence of the Core-1 extension transcript. Cell rolling experiments were performed on E-selectin to confirm full FT7 expression on both Core-2 expressing and Core-2 negative PSGL-1 types. Our data suggests that a carbohydrate branching other than Core-2 on PSGL-1, possibly Core-1 extension, allows for adequate L-selectin binding to PSGL-1 and this form of PSGL-1 may be biologically significant for leukocyte homing and immune response.

2352-Pos Board B122

Mapping the Oligomeric Assembly of Plasma Factor XIII A2B2 by Hydrogen-Deuterium Exchange Coupled with MALDI-TOF Mass Spectrometry

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Factor XIII (FXIII) is responsible for catalyzing the formation of covalent γ-glutamyl-ε-lysyl crosslinks in fibrin chains during blood coagulation. In plasma, unactivated FXIII exists as a heterotetramer consisting of catalytic A2 and regulatory B2 subunits. Each B subunit has 10 tandem repeats called sushi domains. In the presence of thrombin and calcium, the FXIII B2 units are released and the FXIII A2 units are activated. X-ray crystal structures have been solved for FXIII A2, but none are yet available for FXIII A2B2. The current work employed amide proton hydrogen-deuterium exchange coupled with MALDI-TOF mass spectrometry to map sites of contact between the FXIII B2 and A2 subunits. Native gel electrophoresis demonstrated that FXIII A2B2 could be created from mixtures of recombinant A2 and B2 subunits. The high disulfide content of the B2 sushi domains hindered pepsin cleavage of the FXIII B2 dimer and the A2B2 heterotetramer. In the presence of the B2 subunits, sequence coverage for a pepsin digest of FXIII A2 decreased 3-fold further supporting heterotetramer formation. HDX studies then revealed the regions of FXIII A2 that were protected from HDX in the presence of B2. Decreases in percent deuteration were observed for 220-230 (glutamine substrate recognition site), 240-247 (A2 dimer interface), 298-305 (catalytic core domain), 328-338 (catalytic core domain), 607-619 (beta barrel 1), and 632-646 (beta barrel 2). These results support the proposal that the B2 subunits straddle across the A2 subunits protecting the transglutaminase from becoming prematurely activated. Proteolytic or nonproteolytic activation of the A2 units then leads to conformational changes that emphasize the dimer interface, the catalytic core regions, and influences emanating from the calcium binding site. (NIH HL068440)

2353-Pos Board B123

Coomassie Blue Binding to Amyloid Fibrils and Native Proteins

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Coomassie brilliant blue G-250 (CB) is the dye used in the common Bradford assay for protein concentration determination. In this work we investigated the binding of CB to lysozyme and insulin in the native and amyloid fibril states by several optical spectroscopic techniques. We found that Coomassie blue binds both to the native proteins and to amyloid fibrils, but give distinctly different spectral responses. In addition, we investigated how the solvent polarity and viscosity affect the CB absorption and fluorescence spectra, and applied this understanding to the protein observations. The absorption and fluorescence spectra of CB indicated that the binding sites in the fibrils are less polar and holds the CB dye more rigidly than in the native forms. The spectral comparison of CB bound to the two different fibrils showed that the binding sites are different. This was most likely due to differences in secondary structure, which was monitored by circular dichroism. Linear dichroism was used to show that the fibril-bound CB is oriented preferentially parallel to the insulin amyloid fibril axis.

2354-Pos Board B124

Structure Based Inhibition of the Calicivirus RNA-Dependent RNA-Polymerase

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Caliciviridae are RNA viruses with single stranded positive-oriented genome causing a broad spectrum of diseases such as acute gastroenteritis in humans. The structures of the RNA-dependent RNA-polymerase (RdRp) of several Caliciviruses have been reported. The RdRp is predicted to play a key role in genome replication, as well as in the synthesis and amplification of subgenomic RNA.
Starting from crystal structures of human (hNV) and Murine Norwalk virus (MNV) RdRp, we performed an in silico docking search to identify commercially available compounds with predicted high affinity for the enzyme active site. The best candidates were tested in vitro to assay their effective inhibition of MNV and hNV RdRp. The results of such combined computational and experimental screening approach led to the identification of two high-potency inhibitors: EM01 and EM02. The crystal structure of MNV in the presence of the two inhibitors showed a common binding site close to the protein active site where the addition of new nucleotides to the nascent RNA occurs. From such structures we could identify the main residues involved in inhibitor binding. We inserted a point mutation in one of these key residues showing the reduction of inhibitory potency in both MNV and hNV RdRps. Finally, we identified a common moiety present in the two inhibitors likely carrying most of the inhibitory power. Such fragment can be elongated/modified to enhance its druglikeness in order to develop novel anti-viral drugs.

2355-Pos Board B125

Mechanism of Inhibition By, and of Drug Resistance to, a Benzimidazole Inhibitor of the RNA-Dependent RNA Polymerase of Bovine Viral Diarrhoea Virus

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Bovine viral diarrhoea virus (BVDV), a major pathogen in cattle and other ruminants, has been used as a surrogate in vitro model for the development of HCV inhibitors. Recently, we have identified a very potent and selective benzimidazole derivative (227G) that: i) targets the RNA-dependent RNA polymerase (RdRp) of both BVDV and HCV, ii) allows the selection of BVDV resistant mutants characterized by the point mutation I261M located in the *finger* domain of the enzyme.
This work deals with the effects of 227G and I261M on the function of the wt and mutated BVDV RdRp, respectively, investigated by using non-conventional approaches that include molecular dynamics, cluster analysis, flexible docking and metadynamics. Briefly, binding of 227G to the wt RdRp induces conformational changes that prevent entrance of the RNA primer into the enzyme's polymerization cavity. Viceversa, the I261M mutation results in structural changes that allow access of the primer to the polymerization cavity and binding of 227G into a site different from that occupied by the inhibitor in the wt enzyme.

2356-Pos Board B126

Hugging Interaction: Asymmetric Binding of Metastasis Associated Protein S100A4 to Non-Muscle Myosin 2A Tail

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S100A4 is a member of the S100 family of calcium-binding proteins that is directly involved in tumor metastasis. In the cytoplasm it binds to the heavy chain of non-muscle myosin 2A (NM2A) near the assembly competence domain (ACD) promoting filament disassembly which could be associated with