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**

**Caroline Whitaker**, David Ader, Michael Lawrence.
University of Virginia, Charlottesville, VA, USA.

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**

**Muriel Maurer**, Ricky Woofter.
University of Louisville, Louisville, KY, USA.

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**

**Nils Carlsson**, Catherine C. Kitts, Bjorn Akerman.
Chalmers University of Technology, Gothenburg, Sweden.

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**

Eloise Mastrangelo[1], Margherita Pezzullo[2], Delia Tarantino[2], Roberto Petazzi[2], Francesca Germani[2], Dorothea Kramer[3], Jacques Rohayem[3], Martino Bolognesi[2], **Mario Milani**[1].
[1]National Research Council, Milano, Italy, [2]University of Milano, Milano, Italy, [3]University of Technology, Dresden, Germany.

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**

**Saumya S. Pandey**, Shailendra Asthana, Paolo Lacola, Attillio Vargiu, Paolo Ruggerone.
University of Cagliari, Italy, Cagliari, Italy.

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**

**Bence Kiss**[1], Anette Duelli[2], Laszlo Radnai[1], Katalin A. Kekesi[1], Gergely Katona[2], Laszlo Nyitray[1].
[1]Eotvos Lorand University, Budapest, Hungary, [2]University of Gothenburg, Gothenburg, Sweden.

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