investigate the significance of coupling information, we simulate a repertoire of artificial WW domain sequences using a physics-based search method called ZAM (Zipping and Assembly method).[2] Our result shows that coupling information has a remarkable influence on the local contacts of N-terminal β-turn of WW domains. This turn would not form correctly if lack of such information. Interestingly, the formation of N-terminal β-turn has been determined as the nucleator and rate-limiting step experimentally.[3] We also identify specific crucial contacts at the beginning of folding process, and accomplish to predict the foldability of a WW sequence, based on its favor of these crucial contacts.

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**3316-Pos**

**Prediction of H Exchange from Perfectly Funneled Structure Based Models**

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Simulations based on perfectly funneled landscapes have been able to capture many of the fundamental aspects of protein folding. When frustration is low enough the topology becomes the main factor determining the folding process. In the most fundamental implementation of the minimal frustration principle only native interactions significantly contribute to the stabililization of the protein structure. Using these ideas and coarse grain models an extensive sampling of the energy landscape could be achieved. We explored the use of such models to interpret subtle dynamic motions near the native state and whether they are able to give a quantitative description of the native protein ensembles. For this aim we developed a method for the quantitative comparison of the local stability of proteins simulated using perfectly funneled structure based models, and detailed experimental measurements of single residue hydrogen/deuterium exchange of backbone amides (HDX) which depends on structural and dynamic properties. The method was applied to ubiquitin, cytochrome-C, HEWL, S6, and IkBalpha70-206. The predicted exchange patterns agree with the experimentally determined HDX protection factors under native conditions. A variety of simulation models with homogeneous, heterogeneous, additive as well as non additive contact potentials were evaluated for their agreement with experiment. We also compare the results obtained using different criteria for structurally defining the open and closed states based on the number of native contacts of each residue, the dynamics of hydrogen bonded residues or a combination of both criteria.

**3317-Pos**

**Computational Prediction of Hotspots in Protein Misfolding for Rational Immunotherapy**

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Physics-based algorithms can predict the misfolding mechanisms of proteins involved in aggregation-related diseases, including SOD1 whose misfolding template-directed conversion is involved in Amyotrophic lateral sclerosis and PrPc, wherein propagation of the misfolded protein is central to the the prion diseases. We have recently developed an algorithm capable of predicting thermodynamically likely regions for misfolding, by employing modeling which involves both atomistic interactions and surface-area based coarse-graining, along with a heterogeneous dielectric function inside the protein. Predictions based upon the algorithm are consistent with recent immunological assays that have uncovered disease-specific epitopes in SOD1 and prion protein, and point to diagnostic and therapeutic applications.
This research was performed in collaboration with Dr. Neil Cashman at the Brain Research Centre, University of British Columbia, and involved joint supervision of M.D./Ph.D. student Will Guest.

**3318-Pos**

**Protonation/deprotonation Effects on the Stability of Trp-cage Miniprotein**

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The effect on the folding/unfolding equilibrium of protonating the aspartic acid on the Trp-cage miniprotein is studied by explicit solvent moleclular dynamics simulations. Replica exchange molecular dynamics (REMD) simulations spanning the temperature range from 280K to 538K were carried out to the micro second scale using the AMBER99SB forcefield in explicit TIP3P water.
The root mean square distance from the backbone of the NMR structure shows two highly populated basins close to the native state with peaks at 0.6 A and 1.6 A which are consistent with previous simulations using the same forcefield. The fraction of folded replicas shows a drastic decrease because of the breaking of the salt bridge. However, significant populations of conformations with the arginine sidechain completely exposed to the solvent, but within the folded basin. This shows the possibility to reach the folded state without formation of the ion pair contrary to the expected.

**3319-Pos**

**Force Field Dependence of Near-Equiilibrium Properties in a Beta Hairpin Peptide**

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All-atom implicit solvent molecular dynamics simulations of the tryptophan zipper trpzip2 were carried out with a fast multiple time stepping integrator and a replica exchange method to improve sampling. Two modifications of the backbone dihedral angle potential energies in the AMBER ff99 parameter set were compared. Individual trajectories were run for over 375 ns, and aggregate simulation times were over 7.5 microseconds. Several measures of folding behavior in simulations begun from both folded and unfolded ensembles showed convergence to near-equilibrium values, allowing thermal phase behavior to be inferred and compared with experiment.

**3320-Pos**

**The Mechanism of Geometrical Frustration in SH3**

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Structure-based protein models build a minimally frustrated energy landscape to focus on the influence of geometrical factors on their dynamics, and they have demonstrated that the native structure is often sufficient to determine the folding mechanism. We customize structure-based models with a flexible interaction potential to investigate this geometrical control of the folding pathway. In the case of SH3 a polarized transition state results from the delayed formation of the N-terminal beta sheet. We isolate the contributions of the native contact map, of chain connectivity and of excluded volume interactions to identify their roles in the creation of this specific mechanism. While the native contacts are a direct expression of the native structure we find that the unspecific repulsion is essential to understand how geometrical frustration guides the folding process.

## Heme Proteins

**3321-Pos**

**A Biophysical-Biochemical Comparison of Hemoglobins from Mammoth, Asian Elephant, and Human**

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This study is aimed at investigating the molecular basis of environmental adaptation of hemoglobin from tropical climate to low temperature in the Arctic region. We have carried out a biochemical-biophysical characterization of the structural and functional properties of hemoglobins from woolly mammoth (Hb WM) and Asian elephant (Hb AE) and compared those to human hemoglobins (Hb A and Hb A2) in 0.1 M phosphate buffer. Hb A consists of two α and two β subunits. Hb AE was found to contain two α subunits and two β/δ fusion subunits. Hb WM was expressed by inserting Asian elephant α-like and β/δ-like cDNA into our E. coli Hb plasmid (pHE2), and then introducing the mammoth-specific residue differences (αK5N, β/δT12A, β/δA86S, and β/δG101Q) into the Asian elephant plasmid. Since Hb AE and Hb WM contain β/δ fusion chains, we have also compared them to Hb A2, which contains δ chains instead of the β subunits present in Hb A. Oxygen affinity, Bohr effect, and cooperativity of oxygenation were measured at different temperatures and pH and 1H-NMR spectra were obtained for structural comparisons for each Hb. Our results show: (i) Hb AE has the higher O2 affinity as compared to Hb WM, Hb A2, and Hb A; (ii) the effect of an allosteric effector, inositol hexaphosphate (IHP), is the most prominent on Hb A2 as compared to Hb A, Hb AE, and Hb WM. 1H NMR results indicates that the α1β/δ1 and α1β/δ2 interfaces are perturbed in both Hb AE and Hb WM, whereas only the α1δ1 interface is perturbed in Hb A2 compared to Hb A. Hb AE and Hb WM have structural

features that are very different from that of Hb A2 and Hb A, consistent with the altered functional properties.

**3322-Pos**

**The Use of Glassy Films and Sol-Gel Matrices to Probe Nitrite Mediated Reactions of Met Hemoglobin**

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There have been growing indications that under certain conditions hemoglobin (Hb) can undergo nitrite mediated reactions that result in the formation of bioactive forms of nitric oxide (NO) capable of reversing vasoconstriction due to NO scavenging. This process is especially relevant for the design of Hb based blood substitutes that typically cause vasoconstriction when administered. In this presented work the use of both trehalose-derived glassy films and silane derived sol-gel matrices are used to isolate both reactive intermediates and key steps in nitrite-mediated reactions of met Hb. The glassy films allow for controlled production NO within the glass and controlled access of the NO into the distal heme pocket of the met nitrite derivative of Hb. The use of the sol-gel allows for trapping either the T or R state forms of Hb and for facile separation of products (e.g. nitrosothiols such as GSNO) from the Hb containing sol-gel phase. The contributions of added NO and small thiol containing molecules (L-cysteine and glutathione) are exposed. The results are consistent with the formation of a relatively stable intermediate capable of forming S-nitrosothiols such as GSNO. The intermediate has properties consistent with one proposed by Gladwin, Kim-Shapiro[1] and coworkers which has the potent nitrosating agent $N_2O_3$ coordinated to a ferrous heme.

[1.] Basu, S., *et al*, *Nature Chemical Biology*, (2007) vol. 3, p.785.

**3323-Pos**

**General Mechanisms for the Folding and Assembly of Myoglobins and Hemoglobins**

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Mammalian myoglobin has served as the archetype globin for understanding the folding properties of single domain globins with the 3 on 3 helical fold. After removal of heme, the resultant apo-Mb shows a loss of structure in the proximal F helix and adjacent loops, and during acid or GdmCl-induced denaturation, apo-Mb populates at least one intermediate. In contrast, unfolding of holo-Mb appears to be a simple two-state process with little protein concentration dependence but the underlying mechanism is much more complex. The lack of protein concentration dependence implies that heme either interacts with the unfolded polypeptide, self-associates, or both. The observed steepness of the unfolding curves for holo met-Mb requires that the affinity of hemin for the intermediate and completely unfolded states must be at least be 1000 fold weaker than that for the native apo-state, and as a result, unfolding of holo met-Mb is governed primarily by the affinity of the folded native apo-state for hemin. The generality of this conclusion for holo-Mb has been tested in several other monomeric hemoglobins, including the miniglobin from *Cerebratulus lacteus* and the thermoglobin from *Aquifex aeolicus*.

Human hemoglobin unfolding is even more complex due to association of the $\alpha$ and $\beta$ subunits into dimers and tetramers. Removal of hemin leads to formation of an apo-$\alpha_1\beta_1$ dimer and its unfolding appears to involve an intermediate whose stability is dependent on protein concentration. This dependence suggests the formation of a dimer intermediate with partially folded subunits still attached to each other through the $\alpha_1\beta_1$ interface. Folding and assembly of holo-Hb is even more complex because there are significant differences in hemin affinity between the $\alpha$ and $\beta$ subunits, and between tetramers, dimers and monomers.

**3324-Pos**

**Time Resolved Thermodynamic Studies of Ligand Binding/release to Sol-Gel Encapsulated Horse Heart Myoglobin**

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The successful confinement of proteins in solid state materials allows for a multitude of applications in the study of protein dynamics, as well as, advances in biotechnologies. The importance of bulk solvent and restricted space on the conformational dynamics of proteins can be identified by encapsulating the biomolecules (peptides, proteins, enzymezes, etc) in environments where both can be regulated. In addition, the affect of encapsulation on ligand binding and preservation of reactivity once encapsulated are of equal importance. With the use of Photoacoustic calorimetry (PAC) and Photothermal beam deflection (PBD) methods along with picosecond-millisecond transient absorption and fluorescence techniques we are investigating enthalpy, molar volume changes, and kinetics associated with with CO dissociation and rebinding to horse heart myoglobin encapsulated in sol-gels. Preliminary PAC data suggest that the dissociation of CO from HHMb results in four kinetic phases: <7ns, ~125ns, ~260ns, and ~600ns. We will discuss the differences in molar volume and enthalpy changes associated with the conformational dynamics of these events in sol-gels relative to native protein in aqueous solution.

**3325-Pos**

**The Role of the Internal Disulfide Bond in the Conformational Dynamics of Neuroglobin**

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Neuroglobin (Ngb) is a member of the globin family expressed mainly in brain tissue of mammals and other vertebrates that plays a role in the neuronal response to hypoxia and ischemia. Human Ngb has two cysteine residues (Cys46 and Cys55) within the CD loop and the D helix of the protein that were shown to form an internal disulfide bond, however these two residues are replaced by Gly in rat Ngb and the internal disulphide bond is missing. Therefore, to investigate the impact of the internal disulfide bond on the dynamics and energetics in Ngb we used photoacoustic calorimetry and transient absorption spectroscopy and determined the time-resolved volume and enthalpy changes associated with CO rebinding to human Ngb, rat Ngb and a rat Ngb mutant with and engineered internal disulfide bond (Cys46rNgb). The relaxation of the protein structure associated with the ligand photo-release is fast ( $< 50$ ns) and involves a decrease in the volume of the protein matrix. The enthalpy change associated to CO photo-dissociation for all samples studied was 19 kcal $mol^{-1}$, whereas the reaction volume changes for human Ngb and Cys46rNgb were roughly two times higher than for rat Ngb. The reaction volume changes obtained for human and Cys46rNgb were $13.4 \pm 0.9$ mL $mol^{-1}$ and $10.3 \pm 0.6$ mL $mol^{-1}$, respectively, and $4.6 \pm 0.3$ mL $mol^{-1}$ for rat Ngb. These results indicate that the presence of the engineered internal disulphide bond in Cys46rNgb leads to a structural volume change that is similar to that found for human Ngb indicating that the internal disulphide bond control, to some extend, conformational dynamics associated with the ligand binding to deoxy Ngb.

**3326-Pos**

**Design and Characterization of an Enzymatically Active Amphiphilic Maquette Protein**

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Many questions still exist about how quinone molecules act as substrates for membrane oxidoreductase enzymes, as well as how quinones can act as a catalyst in energy conversion mechanisms. We apply our knowledge of electron tunneling and protein design towards defining the basic engineering requirements for quinone reactivity in natural membranes and heme proteins. We have synthesized and characterized a transmembrane, amphiphilic maquette protein, AP6, which extracts the basic structural components from Complex III necessary to perform transmembrane proton-coupled electron transfer. We have shown that our AP6 peptide assembles as a four-helix bundle protein and can potentially bind up to six bis-histidine ligated hemes tightly across a membrane interface. Given its sequence and heme binding capabilities, our AP6 design could accomplish a variety of potential functions, including: transmembrane electron transfer, electron transfer with aqueous proteins, proton-coupled electron transfer, or combining these, quinol-cytochrome *c* oxidoreductase activity. Through standard Complex III activity assays, we have demonstrated that AP6 has quinol-cytochrome *c* oxidoreductase activity in detergent micelles that is within two orders of magnitude of the activity of natural Complex III purified from *R. capsulatus*. This activity can be generated with a variety of reduced quinone substrates, and is dependent on the concentration of cytochrome *c* present. With no obvious quinone-binding site included in our protein design, AP6 provides clear evidence that a specific quinone-binding site within a membrane protein is not essential for generating significant quinol-cytochrome *c* oxidoreductase enzymatic activity from a heme protein.

**3327-Pos**

**The Effect of Non-Coordinated Water in the Heme Pocket on the Ligand Binding Dynamics of Heme Proteins**

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Water molecules in internal protein cavities play fundamental roles in satisfying the H-bonding potentials of main chain atoms in turns, coils, and loops,