of the type of interactions governing the homodimerization and antobody complexation phenomena and the role that the membrane plays on that.

3375-Pos Board B103
Investigating Influenza a M2-Cholesterol Interactions by Oriented Sample Magnetic Resonance
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The influenza A M2 protein is a homotetrameric membrane protein that is vital to the viral life cycle. M2 has been shown to interact with cholesterol to induce cell membrane curvature and scission in the production of new virus particles. Previous work from our group used site-directed spin labeling electron paramagnetic resonance spectroscopy (SDSL-EPR) to probe the conformation and dynamics of M2 constructs consisting of the transmembrane and C-terminal domains, revealing multiple conformational states in a cholesterol-modulated equilibrium. The observed conformational changes were proposed to arise from both a direct M2-cholesterol interaction through an amino acid consensus region in the C-terminal domain and indirectly through cholesterol-induced changes to the bilayer environment.
We present work using lipid bicelles, which spontaneously orient in a magnetic field, as a membrane alternative to detergent micelles and bilayered vesicles. Nuclear magnetic resonance (NMR) and EPR were used to obtain new information describing the specific M2-cholesterol interaction.

3376-Pos Board B104
New Insights into the Mechanism of Action of the Antimicrobial Peptide Aurein 1.2. Isothermal Titration Calorimetry and Confocal Microscopy Studies
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Aurein 1.2 is a short (13 residue) cationic antimicrobial peptide (AMP) from the glandular skin secretions of *Australian anurans* of the *Litoria genus*. It has been suggested that this peptide disrupts membranes in a detergent-like manner. This peptide is considered too short to span the membrane and may possibly lie on the membrane surface without pore formation. Here, we use isothermal titration calorimetric (ITC) and phase-contrast microscopy to study the interaction of AU with membrane mimetic composed of SOPC/SOPG (95:5) and SOPC/SOPG (50:50). The hypothesis is that different membrane composition may lead to different peptide membrane interaction and to different mechanisms of action. ITC data showed an exothermic event at low lipid/peptide ratio, which is stronger for large unilamellar vesicles (LUVs) composed of SOPC/SOPG (50:50). These results showed that the affinity of AU is higher for the most charged vesicles. Phase contrast microscopy studies showed that AU solubilizes giant unilamellar vesicles (GUVs) containing SOPC/SOPG (50:50), but form pores on SOPC/SOPG (95:5) GUVs. These results showed that the mechanism of action of AU dependent on the membrane composition, making pores on low charged membranes but solubilizing the highest. It is according with the proposed in literature that the detergent mechanism can be considered and extreme of the toroidal pore mechanism.

3377-Pos Board B105
How Pegylation Stabilizes a Protein
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Protein PEGylation has been widely applied by the pharmaceutical industry for making protein drugs with lower immunogenicity, longer circulatory half-life and better bioactivity. However, the efficacy of PEGylation depends on parameters such as attachment site and chain length. Characterizing the relationship between PEGylation pattern and protein stability would therefore be beneficial to designing improved protein PEGylation. Although recent studies have shown that site-specific PEGylation has impact on protein conformation stability and folding rate, there is no clear consensus on how PEGylation stabilizes a protein on the atomistic scale.
Through all-atom molecular dynamics simulation, we explored the interaction between the attached PEG oligomer and a β-sheet protein WW domain. While experiments showed clues that stabilizing effect could be attributed to specific interactions between PEG and protein surface residues, a direct correlation cannot be drawn from simulation results. The attached PEG oligomer was flexible and undergoes rapid transitions between extending into the solvent and collapsing onto the protein surface, on a 10 ns time scale. A mechanism including water mediated interaction is therefore suggested.

3378-Pos Board B106
Understanding the Structural Determinants of the Extreme Thermal Stability of Rubredoxin
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Rubredoxins are a class of redox-active, iron-sulfur proteins found in strict anaerobes. These 6-7 kDa proteins remain stable at very high temperatures. Rd functions as part of the oxygen detoxification system with a melting temperature near to 200°C. Rd structure consists of a three-stranded β-sheet, a hydrophobic core, a middle loop, and a hydrophilic tail. The β-strands are joined by two iron-coordinating loops each containing two Cys residues, which are liganded to an iron ion to form a tetrahedral iron-sulfur complex. In the present study, Rd gene from Pyrococcus furiosus was successfully cloned into pET22b, E.coli expression vector and the recombinant Rd was successfully purified to homogeneity. While structural information about Rd is known, this study aims to gain a more comprehensive understanding of the structural stability through various biochemical and biophysical studies under different experimental conditions such as change in pH, metal and ionic strength. The details of these results will be discussed in depth.

3379-Pos Board B107
Stabilization Effects of Disulfide Bonds and Dimerization on Cxcl7
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Chemokines are a family of small signaling proteins that are responsible for an array of biological processes ranging from the chemotaxis of leukocytes to more general homeostatic activities. The oligomerization of chemokines within the human biological system may lead to the biological activity different from that of the chemokine monomer. Here, we use a combination of computational (Anisotropic Network Model (ANM), molecular dynamics (MD) simulations, minimal Distance Constraint Model (mDCM) and experimental (Circular Dichroism (CD) and Nuclear Magnetic Resonance (NMR) spectroscopy) methods to assess the dynamics and stability of CXCL7 monomer and dimer, one the most abundant platelet chemokines. Using this combined approach, we analyzed effects of oligomerization and disulfide bonds on the stability/flexibility and mechanical coupling of different secondary structure elements within the CXCL7. The data is discussed in terms of the biological function of chemokines. Through these models we have shown a large variation in how disulfide bonds affect the stability of CXCL7. The ANM model suggests that the disulfide bonds Cys5-Cys31 shows large fluctuations in protein motion while Cys7-Cys47 shows much lower fluctuations due to the location of Cys47 within the beta sheet structure. The mDCM has shown that the heat capacity of the dimer form is more than twice that of the monomer forms, suggesting a higher degree of cooperativity of folded-unfolding transition in the dimer. Likewise the higher energy barrier suggests that dimerization has a stabilizing effect on the protein. Surprisingly we also saw that the removal of Cys7-Cys47 disulfide bond does not increase the flexibility at Cys31 or Cys47 however we do see global flexibility changes suggesting long-range constraint network changes due to the existence of these disulfide bonds.

3380-Pos Board B108
Effects of Tryptophan Mutation on the Thermal Stability and Catalytic Activity of Cold-Adapted Esterase At Ambient Temperatures
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The cold-adapted enzymes display high catalytic activity at low temperatures due to their flexible structure. But, they are susceptible to denaturation by heat at ambient temperatures compared with their mesophilic or thermophilic counterparts. The extracellular esterase, EstK, from cold-adapted *Pseudomonas mandelii* contains 5Trp residues, whereas its thermophilic homologs EstE1 and AFEST have 2 Trp residues. In this study, the effects of Trp to Phe mutation on the thermal stability and catalytic activity of EstK were investigated. Among 5 Trp residues, W^{208} contributed to maintaining the thermal stability and an intact conformation of the enzyme. The intramolecular interactions surrounding W^{208} were also important to maintain the stability and activity of the enzyme. Three additional mutants, $W^{208}A$, $W^{208}K$, and $W^{208}Y$, were generated, and, interestingly, the mutant $W^{208}Y$ showed an 18-fold increase in activity at 20°C and a 12-fold increase at 40°C, compared with the wild-type enzyme. Furthermore, $W^{208}Y$ was resistant to heat treatment at the catalytic site and had enhanced α-helical contents at 40°C, suggesting that the catalytic site of $W^{208}Y$ was stabilized upon substrate binding, while the other parts