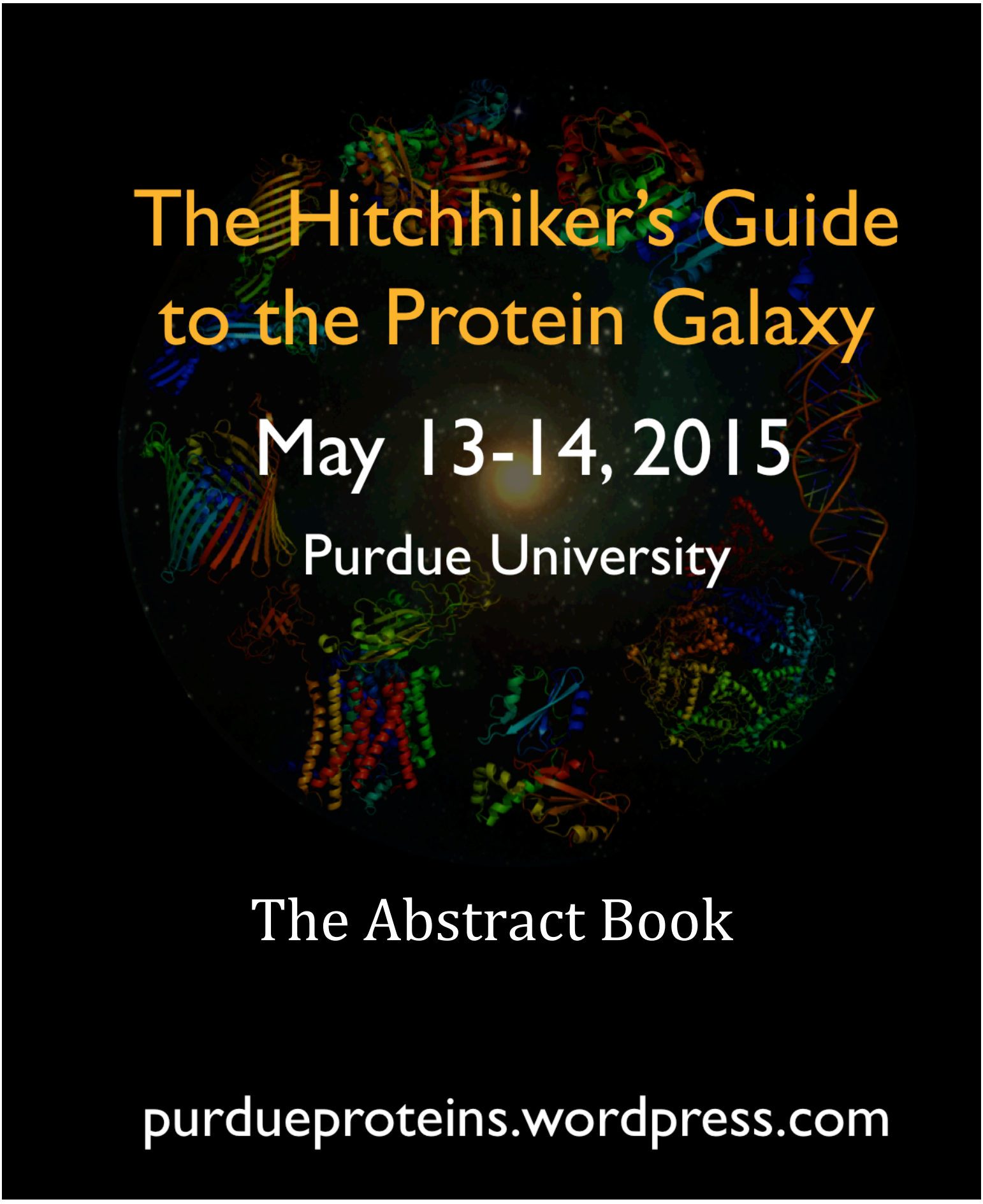

The Abstract Book

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Symposium Abstracts

Targeting the role of tyrosine in Amot protein-lipid binding events

Nawara Abufares and Ann Kimble-Hill (IUPUI)

Amot proteins have been shown to control cell proliferation and differentiation and can selectively bind with high affinity to phosphoinositol containing membranes. This binding event is linked to endocytosis, changes in cellular polarity, and apical membrane sequestration of nuclear transcription factors associated with development of cancer phenotypes. Although the lipid selectivity of the protein has been well characterized, the mechanisms involved in the Amot coiled-coil homology domain (ACCH) binding these membranes are not yet known. The fluorescence properties of the ACCH domain were used to characterize the binding event, however it became clear each of the five native tyrosines proximity to membrane might differ based on fluorescence resonance energy transfer experiments with fluorescently tagged lipids. A variety of short peptides correlating to the amino acid sequence of Amot surrounding these tyrosines were assayed and observed in different membrane mimicking environments to determine if each tyrosine had the ability to bury into the hydrophobic region of the membrane (alcohol study), or simply interacted with the hydrophilic head groups (liposome study). Interactions were characterized by shifts in absorbance, excitation and emission scans peaks. A characterization of these shifts with respect to what is seen with the various tyrosine-phenalanine mutants will further our understanding of whether each tyrosine is buried within the protein or interacts with the membrane.

A proteomic strategy to discover protein complex composition and dynamics in Arabidopsis

Uma K Aryal, Donglai Chen, Jun Xie, Mark C. Hall, and Daniel B. Szymanski (Purdue University)

Knowledge about protein complex composition and dynamics is valuable because it can reveal how cells adapt to different physiological conditions. For example, by forming complexes at the mitochondrial surfaces, glycolytic enzymes can channel metabolites from the cytoplasm to the mitochondria. Sequentially assembled protein complexes precisely execute mechanical tasks like chromosome segregation, vesicle budding, and long distance intracellular transport. A given eukaryotic cell contains hundreds if not thousands of protein complexes. So, there is a strong need to develop new methods to discover and analyze this complexity. To date, affinity purification-mass spectrometry and yeast-two-hybrid are the most widely used methods for high throughput analysis of protein complexes. However, these methods are not suitable for many plant species that are refractory to transformation or genome-wide cloning of open reading frames. To overcome these problems, we recently developed a new method that combines size exclusion chromatography (SEC) with quantitative MS to analyze thousands of proteins leading to the

discovery of hundreds of novel protein complexes. We have recently expanded this technique by developing a new method to predict protein complex composition by combining SEC with an orthogonal ion exchange chromatography (IEX) separation to generate abundance profiles of thousands of proteins using MS1 extracted ion chromatograms (XICs). These abundance profiles were subjected to clustering analysis to identify proteins that co-fractionate, and are putative subunits of native complexes. In our presentation, application of this new technique to study the changes in protein complex composition and dynamics under metabolic stress condition will be discussed.

Yeast Hsp31/DJ-1 is a stress response chaperone that targets a broad range of substrates

Kiran Aslam, Chai-jui Tsai and Tony Hazbun (Purdue University)

A large number of neurodegenerative diseases are associated with protein misfolding and aggregation. Molecular chaperones have been found can prevent aggregation and misfolding of many proteins involved in neurodegenerative disease but there exact mechanism of action and role are still unclear. Saccharomyces cerevisiae Hsp31 is a stress inducible homodimeric protein, which is a member of DJ-1/ThiJ/PfpI superfamily. In this study we characterized the potential role of Hsp31 as molecular chaperone. We showed that Hsp31 abrogates aggregation of a broad array of substrates including α-synuclein (α-Syn) fibrilization, aggregation of citrate synthase and insulin in vitro. We also demonstrated the role of Hsp31 in prevention of in vivo α-Syn foci and a protective effect of Hsp31 against α-Syn cellular toxicity. Furthermore, we established the role of Hsp31 in preventing Sup35 aggregation both in vivo and in vitro. Recently, it is shown that Hsp31 has a methylglyoxalase activity that converts the toxic metabolite methylglyoxal into lactate. We eliminate the possibility that anti-aggregation activity of Hsp31 is due to its methylglyoxalase activity because a catalytically inactive mutant can still prevent α-Syn cellular toxicity. Moreover, we confirmed that Hsp31 expression is induced by H2O2 mediated oxidative stress and further showed an increased expression of Hsp31 under α-Syn mediated proteotoxic stress. Taken together, the results show that Hsp31 is a stress-inducible protein with chaperone and glyoxylase activity and acts on a wide spectrum of misfolded proteins including α-Syn and Sup35. These studies set the stage for further mechanistic insight in the biological roles of the Hsp31/DJ-1 chaperone family.

On the Lipid Dependence of the Stability of the Cytochrome b_6f Complex

S. Bhaduri, S. D. Zakharov, and W. A. Cramer (Purdue University)

Structure-function studies of intra-membrane redox proteins have the particular advantage that function can be readily and quantitatively analyzed. Little is known, however, about the lipid dependence of structure parameters. Crystallization of the eight subunit hetero-oligomeric 270 kDa dimeric cytochrome b_6f complex is dependent on the presence of lipid (1, 2). At least 23 lipid binding sites per monomer are seen in the structure resolved to 2.5 A (3). The function of this lipid, which has been inferred to contribute to the heterogeneous dielectric constant of the complex (4), in the assembly and stabilization of the complex, is

unknown. A dependence of the thermal stability of the secondary structure of the cytochrome complex on state of lipidation is described. From thermal denaturation studies utilizing far-UV circular dichroism analysis, it was found that "melting" (i. e., loss of helical structure) of the b_6f complex starts from T= 45° F for the delipidated complex. The complex is more stable in the presence of anionic lipid, DOPG, for which a significant rate of melting starts at 49°C. The maximum rate of helix melting, determined from the first derivative of the amplitude at 222 nm of the specific molar ellipticity spectrum is 59° F for delipidated complex, while it is 62°F in the presence of DOPG. In the presence of the chemical oxidant, ferricyanide, the melting maximum at approximately 55° and 59°, respectively, for the delipidatedand DOPG-cytochrome b_6f complex. The stability of the complex in the presence and absence of lipid was tested by clear native gel electrophoresis. Both delipidated and lipidated samples were heated, consecutively, to 30°, 40°, 50°, and 60°. In comparison to the lipidated b_6f complex, the rate of monomerization as a function of increasing temperature was enhanced in the delipidated complex.

References: PNAS (2003) **100(9)**, 5160-5163; (2) Science (2003) **302**,1009-1014; (3) Structure (2014) **22**,1008-15;(4) J. Phys. Chem. B (2014) **118**, 6614-25;

Acknowledgement. These studies were supported by NIH GM-038323. We thank Dr. S. Saif Hasan for helpful discussions, and Mr. S. Behmer for initial measurements.

Localization of small-ligand binding to pharmacological targets using cryo-electron microscopy.

Mario J. Borgnia, Soojay Banerjee, Alberto Bartesaghi, Doreen Matthies, Prashant Rao, Alan Merk, Jason Pierson, Jacqueline L Milne and Sriram Subramaniam (National Institutes of Health)

Advances in cryo-electron microscopy are driving the determination of small protein complexes at near-atomic resolution with unprecedented rapidity. For example, computer driven image acquisition together with streamlined image processing pipelines offer the opportunity to localize the binding of ligands with low molecular weight to their target proteins in a relatively automated way. Moreover, improvements in classification algorithms enable the reconstruction of sub-nanometer resolution density maps of distinct conformations from a mixture in single droplet of protein solution.
To test the practicality of this approach, we combined automated procedures and advanced with direct electron detectors to localize ADP (427 Da) and other small ligands on glutamate dehydrogenase (GDH), a clinically significant 365 kDa enzyme that is a relevant pharmaceutical target for cancer, Parkinson's, and diabetes.
Images from single specimens collected in a single session provided enough information to localize nucleotides in a complex at ~3.5 Å resolution. The validity of the results was confirmed by comparison with the crystallographic coordinates of GDH in complex with a variety of ligands. The methods we present provide a streamlined path to rapidly solve the structure of macromolecular complexes and to image the binding target of drug molecules at near atomic resolution.

Effects of Communication on Information Processing in Cells

Tommy Byrd, Andrew Mugler (Purdue University)

Cells can sense and encode information about their environment with remarkable precision. These properties have been studied extensively for single cells, but intercellular communication is also known to be important for both single- and multicellular organisms. Using mathematical models, we investigate how intercellular communication affects information processing in cells. Here, we present preliminary results showing that long-range correlations among cells can emerge from communication, and that single-cell dynamics can be influenced by this communication. We hope to use these insights to build a robust theory explaining the role that intercellular communication has on the sensory properties of cells.

SAXS: A Versatile Biophysical Technique for Studying Structure and Dynamics of Biological Macromolecules.

Srinivas Chakravarthy and Thomas Irving (Argonne National Labs)

While the data collection phase of a typical SAXS experiment is relatively simple, the data quality and interpretability is contingent on the monodispersity of the sample. Sample preparation is therefore often the most crucial and involved part of a successful SAXS experiment. We have commissioned a size-exclusion chromatography setup in-line with the SAXS camera (SEC-SAXS) thus separating the sample from potential contaminants such as aggregates or breakdown products immediately before exposure to x-rays, a technique pioneered at BioCAT. The default data collection strategy for a majority of SAXS experiments at BioCAT is now SEC-SAXS and this has considerably expanded the scope of SAXS at BioCAT as high quality scattering data can be extracted even from samples usually considered too unstable or polydisperse. In addition, SAXS stands alone in its ability to provide global structural information concerning the overall size and shape of macromolecules in the transition states that form within microseconds of the initiation of processes such as folding, ligand binding, allosteric transitions, complex formation and enzymatic reactions. The time-resolved SAXS capabilities at BioCAT include both stopped-flow and continuous flow instruments providing access to time regimes from < 100 microseconds to several seconds. A few examples of projects that have benefited from our versatile SAXS program will be discussed.

Investigating and decoupling the multiple enzymatic activities of the papain-like protease 2 from mouse hepatitis virus to facilitate the development of antiviral therapeutics

Yafang Chen, Anna M. Mielech, Sergey N. Savinov, Susan C. Baker, Andrew D. Mesecar (Purdue University)

Mouse hepatitis virus (MHV) is a positive-sense RNA virus, and serves as a model system for the study of coronaviruses. The papain-like proteases (PLPs) encoded by coronaviruses are responsible for the processing of the N-terminal region of the viral polyprotein, which is essential for virus replication. In addition, several papain-like protease domains from coronaviruses have been shown to have deubiqutinating and deISGylating activities, which may have implications in the viral evasion of the host innate immune response. Our goal is to establish a system to investigate how the deubiquitinating activity and deISGylating activity of MHV PLP2 are involved in immune evasion. To this end, we analyzed the enzymatic activities of recombinant PLP2, and performed X-ray crystallography-gudied protein engineering of PLP2 to selectively disrupt its DUB activity or deISGylating activity, therefore provide a platform to further study the role of PLP2 deubiquitinating and deISGylating activity in immune evasion.

Induced Substrate Accommodation in the Mevalonate Diphosphate Decarboxylase from *Enterococcus faecalis* (MDD_{EF})

Chun-Liang Chen and Cynthia Stauffacher (Purdue University)

Mevalonate diphosphate decarboxylases (MDDs) (EC 4.1.1.33) catalyze the ATP-dependent decarboxylation of mevalonate 5-diphosphate (MVAPP) for producing isopentenyl diphosphate (IPP) in the final step of the mevalonate pathway. This pathway has been proved to be essential in *Enterococci, Streptococci and Staphylococci*, and therefore, MDD is an ideal drug target for the treatment of bacterial infections. We have studied the enzyme kinetics and structures of MDD from *Enterococcus faecalis* (MDD_{EF}), which causes clinical enterorococcal infections. In the crystal structure of MDD_{EF} bound with ATP (MDD_{EF}/ATP), the phosphate-binding loop of MDD_{EF} and ATP was defined based on the electron density map and the overall structure is in an open conformation; however, the catalytically unfavored orientation of the phosphate tail of ATP implies the binding of MVAPP would trigger conformational changes of MDD_{EF} for accommodating the binding of ATP. The kinetic data suggests the enzyme mechanism of MDD_{EF} belongs to a compulsory ordered bi-substrate mechanism with MVAPP binding first. An 8-fold decrease of the dissociation constrant (*K*d) value of ATPgS was observed when MDD_{EF} was pre-incubated with MVAPP using isothermal titration calorimetry (ITC). The increased binding affinity of ATPgS suggests the induced binding of ATP to MDD_{EF} can be driven by the prerequisite binding of MVAPP. Indeed, the crystal structure of MDD_{EF} soaked with MVAPP shows the b-10-a-4 loop becomes ordered around the active site. The complex structures of MDD_{EF} bound with ligands indicate a crucial interacting network between ligands and key residues of MDD_{EF}. These crystal structures provide details of conformational changes of MDD_{EF} during the enzyme reaction, and the structural conformers of MDD_{EF} will be utilized for specific drug development.

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Internal Lipid Architecture of the Hetero-Oligomeric Cytochrome b_6f Complex

S. Saif Hasan and William A. Cramer (Purdue University)

The role of lipids in the assembly, structure, and function of hetero-oligomeric membrane protein complexes is poorly understood. The dimeric cytochrome b_6f complex, a 16-mer consisting of eight distinct subunits with 13 trans-membrane helices per monomer, catalyzes trans-membrane proton-coupled electron transfer for energy storage(1). Based on a 2.5 Å crystal structure of the dimeric complex, 23 distinct lipid binding sites per monomer have been identified(2). Annular lipids are proposed to provide a connection for super-complex formation with (i) the photosystem-I reaction center, and (ii) the LHCII kinase enzyme for trans-membrane signaling. Internal lipids mediate (iii) cross-linking to stabilize the domain-swapped iron-sulfur protein subunit, (iv) dielectric heterogeneity within inter-monomer and intra-monomer electron transfer pathways, (v) mediation between the conserved core of the complex and the four peripheral single membrane spanning subunits, and (vi) dimer stabilization through lipid-mediated inter-monomer interactions. This study provides the most complete structure-analysis thus far obtained of lipid-mediated functions in a multi-subunit membrane protein complex, and reveals lipid-sites at positions essential for assembly and function. **References:** (1)Hasan, SS *et al*., 2013. *Proc. Natl. Acad. Sci. U.S.A*., 110:4297-4302, (2)Hasan, SS and Cramer, WA, 2014. *Structure,* 22:1008-1015. Financial support: NIH GM-038323 (WAC) and Purdue University (Graduate Fellowship to SSH).

Targeted Inhibition of Ubiquitin Specific Proteases for Cancer Treatment

Nicole M Davis, Dr. Katie Molland, Dr. Sergey Savinov, and Dr. Andrew Mesecar (Purdue University)

Ubiquitin Specific Proteases (USPs) are deubiquitinating enzymes that are responsible for the removal of ubiquitin and ubiquitin chains from various protein substrates in the cell. Ubiquitination is a widespread post-translational modification that controls numerous cellular processes throughout the body. The most commonly described result of ubiquitination is the proteasomal degradation of the ubiquitinated protein, therefore deubiquitination of the modified protein by USPs stabilizes that protein in the cell, allowing for its continued function. Due to the expansive nature of USP regulation throughout the human body, any inappropriate regulation or mutation of the USPs can lead to many serious diseases, ranging from various types of cancers to Alzheimer's disease. The work described

here is focused on USP7 and USP17, two USPs that, when misregulated, lead to cancer. From a global perspective, we combine insight of the enzyme's kinetic activity with structural insight to aid in the development of small molecule lead inhibitors specific towards the individual USP. Initial hits are identified by high-throughput screening performed at the Bindley Bioscience Center at Purdue University and are developed into lead inhibitors by collaboration with the Computational and Medicinal Chemistry Shared Resource of the Purdue Center for Cancer Research.

Structural Dynamics of a Pentameric Ligand-Gated Ion Channel Assessed by Site-Directed Spin-Labeling (SDSL) and Continuous Wave Electron Paramagnetic Resonance Spectroscopy (CW-EPRs)

Benjamin W. Elberson, D. Marien Cortes, and Luis G. Cuello (Texas Tech University)

Pentameric ligand-gated ion channels (pLGICs) are responsible for producing either an inhibitory or excitatory downstream response – depending on the permeant ion—when a nerve stimulus reaches a synaptic cleft. Prominent examples include the GABA receptor, which is responsible for inhibitory neurotransmission and the nicotinic acetylcholine receptor, which is responsible for propagation of nerve impulses resulting in muscle contraction. Due to the difficulty in expressing and purifying eukaryotic channels, much of our current knowledge came from the work done using prokaryotic analogues. Erwinia chrysanthemi Ligand-gated Ion Channel (ELIC) is one such prokaryotic channel, which is highly amenable to biochemical and structural characterization. Research in this field is currently limited by the lack of reliable structural information concerning different kinetic states as well as the location of the channel gates. An alternative strategy to crystallization is determination of the structural dynamics of these ion channels by tracking a paramagnetic or fluorescent reporter chemical group – attached to cysteine residues systematically introduced along the primary sequence – with CW-EPR and fluorescence spectroscopies. To achieve this goal, we have systematically developed a new and inexpensive protocol for overexpressing, purifying, labeling, and reconstituting milligrams of functional and properly folded ELIC. This methodology will provide a clearer picture of the structural dynamics involved in activation, desensitization, and deactivation.

Insights into the multistate equilibrium thermodynamics of Syk tandem SH2 domains interacting with immunoreceptor tyrosine-based activation motif

Chao Feng, Nina M. Gorenstein, and Carol Beth Post (Purdue University)

The non-receptor spleen tyrosine kinase (Syk) is an important player in signal transduction from immunoreceptors to various downstream targets. It is widely expressed in both haematopoietic and epithelial cells. Syk disorder is closely related with many inflammatory and autoimmune diseases, as well as cancers. Syk associates with immunoreceptors through its tandem SH2 domains (tSH2), which contain two SH2 domains (nSH2 and cSH2) connected by Interdomain A. The association of Syk with immunoreceptors is regulated by the phosphorylation of Y130 in Interdomain A. When Y130 is not phosphorylated, tSH2 can

bind to the doubly phosphorylated immunoreceptor tyrosine-based activation motif (dpITAM) of the cytoplasmic domains of immunoreceptors with very high affinities (nanomolars for Kd). However, when Y130 is phosphorylated, the binding affinities drop drastically (micromolars for Kd). Interestingly, Y130 locates far (>20 Å) from the binding sites and the detailed mechanism of this long-distance regulation is largely unknown. We have carried out a series of experiments trying to understand this long-distance regulatory mechanism by NMR (nuclear magnetic resonance) methods. The results revealed interesting changes in domain-domain coupling and multistate equilibrium thermodynamics of tSH2 interacting with dpITAM. These data suggested a novel regulatory model for Syk release from immunoreceptors. The new knowledge obtained from our work will help to explain the regulatory mechanism of Syk activity, assist design for new drugs to treat Syk related disorders, and promote researches for other kinases containing tandem SH2 domains.

Applications of Spin-labeling with Modern EPR Methods to Study Membrane Proteins Structural and Functional Dynamics

Elka R. Georgieva, Peter P. Borbat, Jack H. Freed (Cornell University)

We show the application of modern Electron Paramagnetic Resonance (EPR) to the study of the structure and functional dynamics of integral and peripheral membrane proteins. We applied pulsed EPR to measure nanometer scale distances in spin-labeled GltPh, a mammalian glutamate transporters homologue representative of this class of proteins. We showed that in solutions and lipid membranes the outward and inward facing conformations of GltPh are almost equally populated, having close energies regardless of the substrate. This conveys a novel view on the energy profile of functional conformations in membrane transporters.

M2 is a single-pass transmembrane tetrameric protein, a proton channel, that plays essential roles in Influenza A viral cycle. Our study by double electron-electron resonance spectroscopy (DEER) revealed a cascade mechanism of M2 transmembrane domain assembly in lipid membranes; monomers assemble in tight dimers, which then form dimers-of-dimers i.e. M2 tetramers. Moreover, we developed a DEER-based method to study the assembly of multi-component systems in lipid bilayer.

Misfolding of tau is related to Alzheimer's diseases and other neurodegenerations. Being intrinsically disordered in solution, the microtubule binding domain (MBD) of tau adopts a highly helical conformation when bound to biological membranes. The association with membranes is deemed relevant to tau physiological functions. We found that on membrane surface tau MBD folds into short helices connected by flexible linkers, but no membrane-induced tau aggregation was observed. Thus, the helical structure observed by EPR could be linked to tau physiological functions.

A Spacetime Odyssey: Elucidating Dynamic Protein Interactions at the Centromere/Kinetochore

Kriti Shrestha Thapa and Tony R. Hazbun (Purdue University)

Mitosis is a conserved and essential biological process of all eukaryotic cells and relies on macromolecular protein assemblies such as the kinetochore. The kinetochore is highly dynamic and different components have to assemble/disassemble, attach to the centromere or spindles, sense fidelity signals and communicate with checkpoints to ensure correct chromosome segregation. Recent research efforts have made significant insights into understanding kinetochore structure and function. Several years ago we published a large-scale interaction network of over 600 interactions involving the kinetochore. The regulation of the kinetochore requires a complex network of protein assemblies and protein-protein interactions that are controlled by a myriad series of post-translational modifications that happen at specific points in space and time. We are building on initial system-wide studies to delineate these space/time parameters using biophysical, biochemical, genetic and cellular biological approaches. Our most recent study delineates the exact timing of the interaction between a short sequence motif in the Cnn1 protein and the Ndc80 complex and how it is controlled by phosphorylation via the Mps1 kinase. We hypothesize that many kinetochore interactions are driven by short motifs controlled via phosphorylation. We intend to determine at a high-resolution level the extent of these phosphoregulatory motifs and how they contribute to the kinetochore protein network and dynamically alter the kinetochore throughout the cell cycle.

Adhesive Elastin-Based Proteins as Soft Tissue Surgical Glues

Sydney Hollingshead and Julie Liu (Purdue University)

Over 250 million surgeries occur yearly, and many of them require internal closure of soft tissue wounds. The most common method used to close internal wounds is suturing, but this method can cause damage to soft tissue through infection or punctures of healthy tissue. Surgical adhesives can be used instead to lower the risk of infection and amount of anesthetic used. Effective surgical adhesives must be biocompatible, strongly adhesive in wet environments, and have a mechanical stiffness matching the surrounding tissue. However, there are no commercially available surgical adhesives that fulfill all of these requirements for soft tissue. By combining the adhesive qualities of 3,4-dihydroxyphenylalanine (DOPA) and the elastomeric properties of elastin-like polypeptides (ELPs), we hope to create a flexible, biocompatible adhesive that binds strongly in wet environments and closes internal wounds on soft tissue. To create this adhesive, a recombinant protein containing ELPs and tyrosines was expressed in E. coli. The protein was purified using nickel affinity chromatography with yields of 80 mg per liter of culture. Mammalian cells cultured on the protein show 95% viability, which indicates cytocompatibility. By incorporating DOPA through enzymatic conversion of tyrosines, the protein's adsorption increased over 20-fold on glass, polytetrafluoroethylene, and polyvinyl chloride, compared to unconverted protein. This protein achieved an adhesion strength comparable to Tisseel, a commercially available internal wound sealant.

Preferred Conformations of N-glycan Core Pentasaccharide in Solution and in Glycoproteins

Sunhwan Jo, Yifei Qi, and Wonpil Im (Argonne National Labs)

N-linked glycans are on protein surface and have direct and water/ion-mediated interactions with surrounding amino acids. Such contacts could restrict their conformational freedom of N-linked glycans compared to the same glycans free in solution. In this work, we have examined the conformational freedom of the N-glycan core pentasaccharide moiety in solution using standard molecular dynamics (MD) simulations as well as temperature replica-exchange MD simulations. Both simulations yield the comparable conformational variability of the pentasaccharide in solution, indicating the convergence of both simulations. The glycoprotein crystal structures are analyzed to compare the conformational freedom of the N-glycan on the protein surface with the simulation result. Surprisingly, the pentasaccharide free in solution shows more restricted conformational variability than the N-glycan on the protein surface. The interactions between the carbohydrate and the protein side chain appear to be responsible for the increased conformational diversity of the N-glycan on the protein surface. Finally, the transfer entropy analysis of the simulation trajectory also reveals an unexpected causality relationship between intramolecular hydrogen bonds and the conformational states in that the hydrogen bonds play a role in maintaining the conformational states rather than driving the change in glycosidic torsional states.

Role of heparan sulfate in the entry of RRV-MoMLV pseudotyped virus

Aditi Kesari, David Sanders (Purdue University)

In our lab, we pseudotyped Moloney Murine Leukemia Virus (MoMLV), a retrovirus, with the envelope of Ross River Virus (RRV), an alphavirus, to form such a hybrid virus, that has a core of the MoMLV and the envelope of RRV. To further increase the transduction efficiency of this virus, a potential gene therapy vector, we substituted amino-acid residues of its RRV-envelope glycoprotein with basic amino-acid residues. With cell-culture techniques, we show that this makes the pseudotyped virus utilize heparan sulfate, a ubiquitous molecule present on the surfaces of most cells, as an attachment factor. Attachment to cellular heparan sulfate increases the concentration of virus particles on the cell surface and thus increases their chances of cell entry, thereby increasing their transduction efficiency. Thus, this molecular interaction helps the virus utilizing heparan sulfate to out-compete the virus not utilizing heparan sulfate at the entry level. Although the cellular heparan sulfate facilitates entry of virus, our studies show that it poses a challenge in terms of release of the pseudotyped virus with mutated envelope glycoproteins from the producer cells. Hence, one of our future goals would be to develop a producer cell line that doesn't express heparan sulfate for the generation of this pseudotyped viral gene-therapy vector.

Investigating Syk Tyrosine Kinase and its Critical Involvement in Parasite Egress from Malaria Infected Erythrocytes

Kristina Kesely, Phillip Low (Purdue University)

Despite intense world-wide effort, malaria is still a major cause of morbidity and mortality, especially in third world countries. The intracellular parasite Plasmodium falciparum is the most lethal of the malaria species. Resistance to current anti-malarial therapies is spreading, but can conceivably be circumvented by targeting the host red blood cell (RBC). After invasion, the parasite proliferates during its 48 hour life cycle, eventually culminating in parasite escape and reinvasion of a new red cell. An crucial step in parasite escape includes weakening of the host cell membrane via modification of host membrane proteins, including the integral RBC membrane protein band 3. Over 75% of band 3 is lost by the end of egress, which is critical since the band 3-ankyrin interaction constitutes the major connection linking the cell membrane to the cytoskeleton. Curiously, phosphorylation of band 3 tyrosines by RBC syk kinase also induces release of band 3 from the cytoskeleton, leading to membrane destabilization and fragmentation.
The kinome of the malaria parasite does not encode any tyrosine kinases. Since tyrosine phosphorylation of band 3 is prominent during the later stages of intra-erythrocytic parasite development, we treated infected RBCs with a variety of syk inhibitors to assess their abilities to block parasite egress. Within 48 hours of syk inhibitor addition, parasitemia was found to decline by >95%, with many infected RBCs still containing entrapped parasites. Not surprisingly, syk inhibition also prevented tyrosine phosphorylation of band 3, allowing retention of band 3 in the RBC membrane and preventing membrane fragmentation. To determine the efficacy of syk inhibitors on field isolates, parasitized blood acquired directly from P. falciparum infected patients in both Southeast Asia and East Africa were treated with syk inhibitors. Blood analysis following treatment revealed the same reduction in parasitemia seen with laboratory strains of P. falciparum, demonstrating the efficacy of syk inhibitors in the eradication of malaria.

The Goods, the bad, and the ugly: Charges and their role in lipid phase separation

Merrell A. Johnson, Soenke Seifert, Millicent A. Firestone, Horia I. Petrache, Ann C. Kimble-Hill (IUPUI)

Recent literature has shown that buffers affect the interaction between lipid bilayers through a mechanism that involves van der Waals forces, electrostatics, hydration forces and membrane bending rigidity. This talk will highlight our recent work that shows phase coexistence can be a result of Goods buffer charges on the mixed chain 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) lipid bilayers. Since the two phases must be in osmotic equilibrium with one another, this behavior challenges theoretical models of lipid interactions and introduces new variables to consider for the Gibbs phase rule. This model of lipid charging was then used to explain the mechanisms behind phase separation in lipid mixtures containing charged lipid head groups, particularly phosphatidylinositols. Furthermore, this work is then applied to our understanding of underlying mechanisms involved in membrane protein selective association with phosphoinositols, and later re-

organization of these membranes. This work was supported by NIH NCI K01-CA169078-01 and Indiana University Collaborative Research Grants.

KEYNOTE LECTURE:

Modifying biological function using conformational trapping by customized synthetic antibodies

Anthony A. Kossiakoff (University of Chicago)

Synthetic Antigen Binders (sABs) are a class of customized antibody-based reagents generated using novel phage display libraries and selection strategies. Their attributes provide for the ability to generate sABs that are engineered to: 1) target specific regions on the surface of the protein, 2) recognize specific conformational or oligomeric states, 3) induce conformational changes, and 4) capture and stabilize multi-protein complexes.

As a demonstration of the approach, we have generated a set of sABs that can effectively tune the cross reactivity of cell surface receptors and others that have been tailored to induce conformational changes in F-actin filaments that substantially alter actin cytoskeletal structure by mechanisms involving depolymerizing, severing, bundling and capping of actin filaments. In another example, we show that conformational-specific sABs can selectively capture either the open (apo-) or closed (ligand bound) conformational states of maltose binding protein to dramatically influence the equilibrium of ligand binding.

Strategies to Overcome Drug Resistance Associated with ABC Transporters

Bohn, Kelsey; Lange, Allison; Chmielewski, Jean; and Hrycyna, Christine

ATP-driven transporters recognize hundreds of structurally unrelated compounds and protect the body by effluxing xenobiotics across cell membranes for excretion. The transporters P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP) are of major clinical significance because they transport therapeutics away from the active site, requiring higher concentrations for efficacy.

Our lab devised an approach to inhibit transporter activity by linking two transporter substrates with an ester bond. This bivalent molecule occupies the transporter binding site with higher affinity than the monomer alone, slowing its off-rate and acting as an inhibitor. Intracellullarly the ester bond is cleaved, releasing the original monomer to elicit its therapeutic effect.

This strategy was employed for antipsychotics targeted to the central nervous system, where transporter expression is high. P-gp and BCRP expression in cancer cells contributes to multidrug resistance, so the dimeric inhibitor approach may be useful for reversing resistant phenotypes in malignancies.

EM-tomography investigation to follow and map structural footprints of virus life cycle in host cell

Jason Lanman (Purdue University)

Most positive strand RNA viruses are known to hijack a myriad of host cell membranes to facilitate virus genome replication and assembly within its host cell. The viruses either subvert the membranes in order to construct a safe haven for its replication or it uses the membranes for budding virus particle and virus trafficking. These membrane rearrangements result in extensive large-scale changes in the structure of these cellular membranes. In this work we used EM tomography to dissect the replication complexes and to reconstruct large volumes of the cell to investigate the role these membranes play in virus replication, assembly and egress. From these data we construct spatial models that represent stages of the virus life cycle resident on the Golgi, ER, and mitochondria membranes. This work showcases the power and versatility of using different aspects of EM-tomography in dissecting a virus-host relationship that could aid in more targeted biochemical and therapeutic studies.

Four isoforms of Arabidopsis thaliana 4-coumarate: CoA ligases (4CLs) have over-lapping yet distinct roles in phenylpropanoid metabolism

Yi Li, Jeong Im Kim, Len Pysh, and Clint Chapple (Purdue University)

The biosynthesis of lignin, flavonoids and hydroxy-cinnamoyl esters share the first three enzymatic steps of the phenylpropanoid pathway. The last shared step is catalyzed by 4-coumarate: CoA ligase (4CL), which generates p-coumaryl CoA and caffeoyl CoA from their respective acids. Four isoforms of 4CLs have been identified in Arabidopsis thaliana. Phylogenetic analysis reveals that 4CL1, 4CL2 and 4CL4 are more closely related to each other than to 4CL3, suggesting that the two groups may serve different biological functions. In addition, promoter-GUS analysis shows that 4CL1 and 4CL2 are expressed in lignifying cells whereas 4CL3 is expressed in a broad range of cell types. We report that a 4cl3 single mutant has over-all reduction in flavonoid biosynthesis, suggesting that 4CL3 is the predominant 4CL in flavonoid biosynthesis. Sinapoylmalate, the major hydroxy-cinnamoyl ester found in Arabidopsis is greatly reduced in a 4cl1 4cl3 mutant, showing that both 4CL1 and 4CL3 are important for sinapoylmalate biosynthesis. The 4cl1 4cl2 double mutant and the 4cl1 4cl2 4cl3 triple mutant are both dwarf, and the triple mutant contains less lignin than wild type and 4cl1 4cl2, indicating that 4CL1, 4CL2 and 4CL3 are involved in lignin biosynthesis. We could not find an important role for 4CL4 in any of the organs examined, consistent with its limited expression profile. Together, these data show that the four isoforms of the 4CLs in Arabidopsis have over-lapping yet distinct roles in phenylpropanoid metabolism.

Reprogramming the Substrate Specificity of Natural Product Biosynthetic Enzymes for Combinatorial Biosynthesis

Jeremy Lohman (Purdue University)

Bacterial natural products are one of our best sources of drugs and drug leads. Two clinically important types of complex natural products are polyketides and non-ribosomal peptides. Both are produced by similar biosynthetic strategies, namely tethering the substrate to the biosynthetic enzymes, such that the problem of diffusion is eliminated. This allows upwards of 50 enzymatic reactions to be carried out efficiently and with fidelity. However, the natural products are produced to benefit the producing organism, rather than for clinical purpose. Complimentary to organic synthesis is combinatorial biosynthesis, which is the use of genetics to alter biosynthetic pathways leading to the production of analogs with altered pharmacokinetics. A limiting factor to combinatorial biosynthesis is our understanding of the enzymology of the complex biosynthetic enzymes of PKS and NRPS. My lab is using a crystallography, synthetic organic chemistry and biophysics to characterize enzymology of PKS and NRPS. Using the information gained we will alter the substrate specificities of PKS and NRPS, in order to enable efficient combinatorial biosynthesis.

Molecular Mechanisms of Phospholipase Cβ Regulation

Angeline Lyon (Purdue University)

Phospholipase Cβ (PLCβ) enzymes are key regulators of intracellular calcium levels and are essential for normal cardiovascular function. Dysregulation of PLCβ activity results in profound cellular changes, including cardiac hypertrophy and heart failure. PLCβ enzymes are characterized by very low basal activity and can be potently activated by extracellular signals via direct interactions with the heterotrimeric G proteins Gαq and Gβγ. However, the molecular basis of PLCβ autoinhibition and its activation by Gαq have remained poorly defined. A better understanding of these mechanisms is essential for the development novel therapeutic approaches to treat cardiovascular disease. Recent crystal structures have captured images of PLCβ in its autoinhibited basal state and in complex with Gαq. These structures, along with associated functional studies, define specific regions within the unique PLCβ C-terminal extension that contribute to regulation, Gαq binding, and membrane association. Taken together, these studies reveal an unanticipated allosteric mechanism of PLCβ activation and describe a sophisticated role for the C-terminal coiled-coil domain in dictating both membrane binding and the overall orientation of the signaling complex.

Single-Particle Electron Microscopy Analysis of Keap1 reveals a novel domain organization and conformational variability

Julia P. Luciano, Aimee Eggler, Sergey Savinov, Andrew Mesecar (Purdue University)

Ubiquitination of cellular proteins is a major regulatory mechanism of cellular protein concentration, which is essential to highly ordered eukaryotic cells. Changes in the

ubiquitination patterns of cellular proteins often leads to detrimental results such as the development of cancer, obesity, and neurodegenerative disease. The Cullin3-Keap1 ubiquitin E3 ligase complex (Cul3-Keap1) ubiquitinates Nrf2, a major transcription factor known to increase levels of cytoprotective proteins. Keap1 is the adaptor and substrate recognition protein that presents Nrf2 to Cullin 3 for ubiquitinatioin. Nrf2 concentrations are normally kept low in the cytoplasm through proper positioning and targeted degradation via ubiquitination by the Cul3-Keap1 ligase complex. Dietary compounds react with Keap1 and stop ubiquitination of Nrf2 leading induction of cytoprotective genes. To characterize the structural components of this complex, we are utilizing different single-particle electron microscopy (EM) strategies, combined with molecular dynamics (MD) simulations and small angle x-ray scattering (SAXS). Our EM data of the Keap1 homodimer reveals conformational heterogeneity within the Keap1 dimer. 2D classifications show that the Kelch domains of each protomer of Keap1 exist in different conformations. Initial models from 3D EM reconstruction elucidate a novel domain arrangement of Keap1 and support the conformational variability and flexibility hypothesis. Chimeric homology models and MD simulations corroborate our findings and illustrate a range of possible Keap1 conformations. SAXS models were also generated and taken together, our data indicate to a new mechanical interaction within Keap1 and target molecules.

Tuning the temperature of single-cells: a new tool to investigate protein dynamics and temperature sensing

Kaho Long, Hairong Ma (Drexel University)

Temperature stress is a common environmental cue that profoundly alters many essential cellular activities. Recent study shows that single cells employ the actin and actin binding proteins (ABP) as both a sensor and mediator of the thermal response. One critical question is how the dynamics of these temperature sensitive proteins are associated with the temperature perturbation. The effort is stymied by the lack of appropriate tools that can induce a wide range of temperature perturbations in live cells with adequate time resolution, so that the protein dynamics can be tracked in real time. Toward this goal we have developed a novel platform by combining infrared laser induced temperature-jump (T-jump) with live-cell imaging, whereupon we can tune the temperature of single cells or cell populations up to 60 degrees Celsius with millisecond time resolution.

Multimeric Protein Fitting by Cryo-EM Map Manipulation

Lyman Monroe, Daisuke Kihara (Purdue University)

Cryo-electron microscopy (cryEM) aided Protein structure determination has improved significantly in the past years. However, there is a lack of effective computational methods for the elucidation of high resolution multi-protein complexes from electron microscopy data [1]. Resolution of electron microscopy images has improved drastically in recent years; however, methods should be able to handle low-resolution (around 8 Å or lower) images as well, as low resolution EM maps are still regularly determined [2]. We propose a

novel computational protocol for the refinement of multiple, rigidly fitted structures to electron microscopy maps. In our method, we iteratively subtract portion of the density map so that individual chains can be independently fitted to their respective regions. Each regions are determined by the positions of all other chains in the fitting, such that the region that corresponds to chain i is produced by removing the areas of the map that are occupied by all chains j where j≠i. This iterative map manipulation allows for the independent flexible fitting of each chain in such a way that protein-protein interactions do not need to be calculated, and only the internal energy of the chain and the density values of the target map are used to calculate the fit.

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From molecules to cells to tissue: how cells use molecular communication to sense collectively

Andrew Mugler (Purdue University)

Single cells have evolved to sense their environment with remarkable precision. In recent years the evidence has accumulated that in a wide variety of biological domains, this precision even approaches the limits of what is physically possible. In this talk, I will describe examples of optimal sensing by bacteria, amoebae, and fruit flies. I will present the physical arguments that set bounds on their abilities, as well as the experimental techniques used to probe them. Then I will describe my own work in mammalian tissue, where cells can beat these bounds by exploiting cell-to-cell communication. These examples reflect the power of simple modeling to make quantitative sense of complex biological phenomena.

Coarse-Grained Molecular Simulations of Allosteric Cooperativity

Prithviraj Nandigrami and John J. Portman

We develop a mixed Monte Carlo-Molecular Dynamics scheme to simulate the classic Monod-Wyman-Changeux (MWC) model of allostery at the molecular level. Ligand binding in this model is cooperative due to the coupling between the binding sites provided by the conformational transition of the protein. We present results for calcium binding to the two binding loops within each domain of Calmodulin (CaM). We find that relative binding free energies of an individual loop is determined by the conformational compatibility of the binding site in the bound conformation, as well as the conformational flexibility of the binding site in the unbound conformation. This simple coarse-grained model captures the qualitative differences for calcium binding to the isolated domains of CaM such as overall

affinity and the relative binding cooperativity. A simple two state MWC model provides an accurate description of the simulated population for the ligation states of each domain as a function of concentration.

The Role of BamA in the Biogenesis of Beta-barrel Membrane Proteins

Nicholas Noinaj (Purdue University)

Beta-barrel membrane proteins are essential for nutrient import, signaling, motility, and survival. In Gram-negative bacteria, the beta-barrel assembly machinery (BAM) complex is responsible for the biogenesis of beta-barrel outer membrane proteins (OMPs), with homologous complexes found in mitochondria and chloroplasts. Despite their essential roles, exactly how these OMPs are formed remains unknown. The BAM complex consists of a central and essential component called BamA (an OMP itself) and four lipoproteins called BamB-E. While the structure of the lipoproteins have been reported, the structure of full length BamA has been elusive. Recently though, we described the structure of BamA from two species of bacteria: Neisseria gonorrhoeae and Haemophilus ducreyi. BamA consists of a large periplasmic domain attached to a 16-strand transmembrane beta-barrel domain. Together, our crystal structures and molecule dynamics (MD) simulations revealed several structural features which gave clues to the mechanism by which BamA catalyzes beta-barrel assembly. The first is that the interior cavity is accessible in one BamA structure and conformationally closed in the other. Second, an exterior rim of the beta-barrel has a distinctly narrowed hydrophobic surface, locally destabilizing the outer membrane. Third, the beta-barrel can undergo lateral opening, suggesting a route from the interior cavity in BamA into the outer membrane. And fourth, a surface exposed exit pore positioned above the lateral opening site which may play a role in the biogenesis of extracellular loops. In this presentation, the crystal structures and MD simulations of BamA will be presented along with our work looking at the role of these four structural features in the role of BamA within the BAM complex.

Selective Stabilization of a Partially Unfolded Protein by a Metabolite

Chiwook Park (Purdue University)

When proteins fold *in vivo*, the intermediates that exist transiently on their folding pathways are exposed to the potential interactions with a plethora of metabolites within the cell. However, these potential interactions are commonly ignored. Here, we report a case in which a ubiquitous metabolite interacts selectively with a non-native conformation of a protein and facilitates protein folding and unfolding process. From our previous proteomics study, we have discovered that *Escherichia coli* glyceraldehyde-3-phosphate dehydrogenase (GAPDH), which is not known to bind ATP under native conditions, is apparently destabilized in the presence of a physiological concentration of ATP.[1] To decipher the origin of this surprising effect, we investigated the thermodynamics and kinetics of folding and unfolding of GAPDH in the presence of ATP. Equilibrium unfolding of the protein in urea showed that a partially unfolded equilibrium intermediate accumulates in the presence of ATP. This intermediate has a quaternary structure distinct from the native protein. Also, ATP significantly accelerates the unfolding of GAPDH by selectively stabilizing a transition

state that is distinct from the native state of the protein. Moreover, ATP also significantly accelerates the folding of GAPDH. These results demonstrate that ATP interacts specifically with a partially unfolded form of GAPDH and affects the kinetics of folding and unfolding of this protein.[2] This unusual effect of ATP on the folding of GAPDH implies that endogenous metabolites may facilitate protein folding *in vivo* by interacting with partially unfolded intermediates.

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Combinatorial fragment docking for sequence-based structure prediction of disordered protein interactions

Lenna X. Peterson, Daisuke Kihara

Proteins perform innumerable vital biological tasks, including catalysis, signaling, and maintenance of cellular structures. Many essential protein functions involve interactions with other proteins. Thus, understanding protein-protein interactions (PPIs) is central to understanding both normal and pathological functions of living organisms. Classical PPIs combine two well-folded protein partners. However, an increasing number of PPIs involve one well-folded protein and one intrinsically disordered protein, which lacks a stable structure when not involved in a PPI. These disordered protein interactions occur in important signaling and regulatory pathways. While classical PPIs can be predicted using protein-protein docking methods, no computational method is widely available for prediction of disordered protein interactions. Classical docking methods require a priori knowledge of the structure of the disordered partner. In contrast, protein-peptide docking methods have short peptide length limits. We have developed a method to apply an established protein docking method to the prediction of disordered protein interactions. Given the structure of the ordered partner and the sequence of the disordered partner, the method predicts both the structure and the docking location of the disordered partner. These predictions will allow improved understanding of biologically relevant disordered protein interactions.

A History of Crystallization Techniques

Edward E. Pryor (Anatrace)

Since the determination of Myoglobin in 1958 by Kendrew and Perutz, the crystallographic community has contributed over 100,000 structures into the Protein Data Bank, the majority of which have been determined by X-ray crystallography. During this time, we have witnessed the technological evolution of many crystallization techniques – including batch crystallization, vapor diffusion, free-interface diffusion, and even microgravity

experiments performed on the International Space Station. This talk will provide an overview of many crystallization techniques, with a particular focus on how capillaries have been used in the crystallization of many of the first protein structures. The focus on capillaries serves as an introduction to the Microlytic Crystal Former, a high-throughput, capillary-based plate for free interface diffusion crystallization experiments. We'll conclude with an exciting review of customer crystallization successes of both membrane and soluble proteins using the Crystal Former. Following this presentation, there will be a hands-on workshop for anyone interested in setting up and/or visualizing crystallization conditions in the Microlytic Crystal Formers.

The Effect of Macromolecular Crowding on the Structure of Superoxide Dismutase

Ajith Rajapaksha

Macromolecular crowding can alter the structure and function of biological macromolecules. We used small angle scattering (SAS) to measure the effects of macromolecular crowding on the size of a protein complex, superoxide dismutase (SOD). Crowding was induced using 400 MW polyethylene glycol (PEG), triethylene glycol (TEG), methyl-alpha-glucoside (alpha-MG) and trimethylamine N-oxide (TMAO). Parallel small angle neutron scattering (SANS) and small angle x-ray scattering (SAXS) allowed us to unambiguously attribute apparent changes in radius of gyration to changes in the structure of SOD. For a 40% PEG solution, we find that the volume of SOD was reduced by 9%. Considering the osmotic pressure due to PEG, this deformation corresponds to a highly compressible structure. SAXS done in the presence of TEG suggests that for further deformation---beyond a 9% decrease in volume---the resistance to deformation may increase dramatically.

ROLE OF FIC-MEDIATED ADENYLYLATION IN THE UNFOLDED PROTEIN RESPONSE PATHWAY

Anwesha Sanyal, Andy J. Chen, Ernesto S. Nakayasu, Cheri S. Lazar, Erica A. Zbornik, Carolyn A. Worby, Antonius Koller and Seema Mattoo (Purdue University)

Maintenance of endoplasmic reticulum (ER) homeostasis is a critical aspect of determining cell fate and requires a properly functioning unfolded protein response (UPR) cascade. We have discovered a hitherto unknown role of a novel post-translational modification, termed adenylylation, in regulating signal transduction events during UPR. Adenylylation entails the covalent attachment of an adenosine monophosphate (AMP) to the protein substrate. This adenylylation reaction is catalyzed by the Fic (filamentation induced by cAMP) family of enzymes, which are evolutionarily conserved from bacteria to humans. Fic proteins are defined by an HXFD/E)(G/A)N(G/K)RXXR motif, where the His residue is essential for catalytic activity. Despite the existence of over 8000 Fic proteins in nature, the human genome encodes only one Fic protein, HYPE (Huntingtin yeast interacting protein E). We previously demonstrated that HYPE possesses adenylyltransferase activity against mammalian RhoGTPases in vitro. However, HYPE's physiological substrates and cellular

function had remained elusive. Here we demonstrate that HYPE localizes to the ER lumen where it modifies the ER-chaperone, BiP. BiP serves as a sentinel in the mammalian UPR pathway and determines cell fate by regulating ER homeostasis. We find that adenylylation enhances BiP's ATPase activity, which is required for refolding misfolded proteins while coping with ER stress. Accordingly, HYPE expression levels increase upon stress. Further, siRNA-mediated knockdown of HYPE prevents the induction of an unfolded protein response. Thus, HYPE represents a new player in UPR signaling and reveals an exciting, novel network between Fic-mediated adenylylation and ER homeostasis.

PL-PatchSurfer2.0: A fast virtual screening program using surface patch based on 3D Zernike descriptors

Woong-Hee Shin, Daisuke Kihara (Purdue University)

Computer-aided drug discovery (CADD) has been widely used to reduce human efforts in early stage of drug discovery. CADD methods can be classified into two categories based on the target molecule type: receptor-based virtual screening (RBVS) and ligand-based virtual screening (LBVS). RBVS method requires the receptor structure as the name indicates. Protein-ligand docking is one of the popular RBVS methods. However, as the size of chemical database increases, required computation time for molecular docking is also increases largely because it optimizes the binding pose and affinity of ligand within binding pocket. Therefore, there is a need for a fast virtual screening technique. On the other hand, LBVS method compares the small molecule database with the known drug. If the ligand is similar to the target molecule, then it is considered as a lead compound or drug candidate. Because LBVS method just compares the chemical properties and/or molecular shapes, it is a relatively fast method and used when the receptor structure is not available. However, LBVS method should require the known ligand for target disease. In addition, it has a tendency to find the molecules biased to the drugs. Therefore, LBVS method is not suitable for finding novel compound.Last year, our group proposed a novel molecular local surface-based RBVS method, called PL-PatchSurfer (Hu, Zhu, Bures, & Kihara, Int. J. Mol. Sci, 2014). It segments the surface of binding pocket and ligand. After partitioning the surface into a number of patches, it converts the surface and electrostatic potential on the surface patches into three-dimensional Zernike descriptors (3DZD). Then it compares patch 3DZDs of protein with those of ligand to determine the ligand is likely to bind the target receptor or not. Because PL-PatchSurfer searches interaction of protein and ligand based on patch Zernike descriptors, not based on atomic pairwise scoring function in traditional RBVS, our program showed a much faster speed (~40 times faster than AutoDock4), showing comparable performance with other RBVS methods. Although the former version of PL-PatchSurfer presented a good performance, only shape and electrostatic potential are used as a physicochemical feature, so sometimes it could not catch other properties such as hydrogen bonding. In this presentation, a new version of PL-PatchSurfer will be proposed. It adds two properties: hydrogen bonding and hydrophobicity, which are also important for protein-ligand interaction. A benchmark performance of this new program will be also introduced in this presentation.

Function of the Cytochrome b6f Complex in Trans-Membrane Signalling

S.K. Singh, S. Saif Hasan, and W. A. Cramer (Purdue University)

The hetero-oligomeric eight subunit 270 kDa dimeric cytochrome b6f complex, currently resolved at 2.5 Å (1), catalyzes proton-coupled redox reactions of the lipid soluble substrate plastoquinone/plastoquinol (PQ/PQH2), to generate a trans-membrane electrochemical proton gradient utilized for ATP synthesis. The quinol site on the electrochemically positive (p) side of the complex, proposed to function as a "redox-sensor", at which quinol binding is inferred to signal for activation of a kinase ("Stt7") (2-3) functioning on the n-side that phosphorylates the light-harvesting pigment-proteins, thus facilitating their light-dependent re-distribution (4, 5). The activation and catalytic sites of the kinase, inferred to be located on the p and n-sides, are separated by a transmembrane distance of ≈ 30 Å. This passage of information across the membrane is caused by a presently unknown signalling mechanism and resultant conformational change. Activation of the enzyme is proposed to be dependent on the bonded status of the conserved cysteine pair and on mediation by thioredoxin (6). Based on p-side superoxide (O2-.) formation (7), it is inferred that O2- dismutates to yield hydrogen peroxide in the presence of the organelle superoxide dismutase, the peroxide reacts with thioredoxin, leading to its oxidation, cysteine oxidation, and kinase disulfide bond formation, thus activating the kinase.
[NIH GM-038323]. (1) Structure (2014) 22, 1008-; (2) PNAS (1997) 94, 1585-; (3) EMBO J. (1999) 18, 2961-; (4) Science (2003) 299, 1572-; (5) Ann. Rev. Plant Biol. (2014) 65, 287-; (6) J. Expt. Botany (2005) 56, 1439-; (7) Biochemistry (2013) 52, 8975-.
We thank J.-D. Rochaix and G. Fucile for helpful discussions.

Time-Resolved Circular Dichroism for Photosynthesis Research

Valentyn Stadnytskyi, Sergei Savikhin

Fenna-Matthews-Olson (FMO) complex is a photosynthetic light harvesting protein that channels electronic excitations from chlorosomal antenna to reaction center in green sulfur bacteria. Each subunit of this trimeric complex contains 8 bacteriochlorophyll a (BChl a) molecules enclosed into a beta-sheet of a proteins. Close spacing leads to strong interaction between pigments and causes excitonic delocalization of electronic excited states. Excitonic nature of the excited states gives a rise to coherences and quantum beats between different states and it has been suggested that such quantum interference can enhance the efficiency of energy transfer(Fleming, Shulten). This phenomenon relies on a particular realization of the excitonic excited state structure within FMO, defined by the energies of individual noninteracting pigments and interactions between the pigments. Since the individual pigment energies are not directly observable, conclusions about their values are made using exciton modeling of available experimental data.
In this work we develop and apply time-resolved circular dichroism (CD) nanosecond spectroscopy to study exciton structure of the FMO (via triplet-state dynamics) and refine the exciton model parameters. Unlike unpolarized optical spectra, excitonic CD is extremely sensitive to mutual orientation of interacting pigments, which will allow to greatly enhance the sensitivity of the model to model parameters. With modifications, this new technique will allow us to measure changes in CD spectrum of ΔAR-L∼10-6, which should be sufficient to measure a change in CD absorption of the FMO protein.

Building a Better Antibiotic

Nic Steussy, Mark Lipton, Mohamed Saleem, Tim Schmidt, Matt Hostetler, Daneli Lopez, Cynthia Stauffacher (Purdue University)

Multi-drug resistant bacterial infections are a significant and growing clinical problem. Since the 70s the incidence of both hospital and community acquired resistant bacterial infections has ballooned, while drug company research into new antibiotics has dwindled (1). We were excited to discover that the bacterial mevalonate pathway, which the laboratory had long studied, was present and essential in pathogenic gram positive bacteria (2). Since then we have done extensive *in vitro* screening against HMG-CoA reductase, the rate limiting enzyme of this pathway, from *Enterococcus faecalis.* That effort discovered a lead compound kinetically effective in the low micromolar range. This lead has been refined and improved using x-ray crystallography, *in vitro* kinetics and *in vivo* anti-bacterial experiments. It was found to be effective *in vivo* against both Vancomycin Resistant *Enterococcus faecalis* (VRE) and Methicillin Resistant *Staphylococcus aureus* (MRSA). In addition to our compound's effectiveness against these pathogens the biology of our target provides a novel aspect to its deployment; it is specific to the low g+c gram positive cocci that utilize the mevalonate pathway. This implies that the commensal bacterial community will be unaffected by treatment, thus avoiding the morbidity and mortality associated with broad spectrum antibiotic use. We are in the process of refining our inhibitor structure to increase its potency and specificity

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Extracting Diffusion Models from FCS Studies of Transcription Factor in the Nucleus

K.Tsekouras, A.P. Siegel, R.N. Day and S. Presse (IUPUI)

Fluorescence correlation spectroscopy (FCS) is a noninvasive technique that probes the diffusion dynamics of proteins down to single molecule sensitivity in living cells. Critical mechanistic insight is often drawn from FCS experiments by fitting the resulting time-intensity correlation function, G(t), to known diffusion models. When simple models fail, the complex diffusion dynamics of proteins within heterogeneous cellular environments can be fit to anomalous diffusion models with adjustable anomalous exponents. Here we take a different approach. We use the maximum entropy method to show – first using synthetic data – that a model for proteins diffusing while stochastically binding/unbinding to various affin- ity sites in living cells gives rise to a G(t) that could otherwise be equally well fit using anomalous diffusion models. We explain the mechanistic insight derived from our method. In particular, using real FCS data, we describe how the effects of cell crowding and binding to affinity sites manifest themselves in the behavior of G(t). Our focus is on the diffusive

behavior of an engineered protein in i) the heterochromatin region of the cell's nucleus as well as ii) in the cell's cytoplasm and iii) in solution. The protein consists of the basic region-leucine zipper (BZip) domain of the CCAAT/enhancer-binding protein (C/EBP) fused to fluorescent proteins (FPs).

Visualization of subnanometer-resolution native structures and conformational dynamics of viral particles captured directly from cell culture

Frank Vago

Collective Cell Sensing and Cellular Migration

Julien Varennes

Cells use inter-cellular communication to coordinate behavior. Our goal is to understand the interplay between the epithelial-mesenchymal transition (EMT), collective sensing and cell migration. We are using tools from statistical physics to explore the fundamental limits to communication-aided sensing. Collectives of cells in the hybrid E/M state are both mobile and best at sensing a gradient — therefore, does an optimally sensing cellular unit of a particular size emerge computationally?
I plan on reporting my findings on single cell and grouped cell diffusion. In the future I want to explore how introducing a bias in the cell's diffusion can give insights as to the advantages of grouped cellular migration.

Exploring the FcRn-Albumin interaction: Effect of glycation and fluorescent tag addition

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Serum albumin is the most abundant carrier protein in blood and interacts with both small and large molecules. The focus of this study was to address specific albumin modifications and their impact on albumin-FcRn binding and cellular handling. Since FcRn binds albumin strongly at acidic pH and releases it following transcytosis at physiological pH, binding at pH 6.0 and 7.4 was performed. KD was measured using Microscale Thermophoresis. Some studies have shown that glycated albumin is excreted in the urine at a higher rate than unmodified albumin. In our studies glucose and methylgloxal modified albumin (21day) all had reduced affinity to FcRn at pH 6.0 suggesting that these albumins would not be returned to the circulation via the transcytotic pathway. To address why modified albumin has reduced affinity we analyzed the structure of the modified albumins using SAXS. This analysis showed significant structural changes occurring to albumin with glycation that could explain reduced affinity. Understanding how modified albumins interact with FcRn and other receptors i.e. Megalin/Cubilin, RAGE is critical to developing a complete picture of how the kidney handles this important protein.

Probing the TolC Trans-Periplasmic Pathway of the Cytotoxin, Colicin E1

Karen S. Jakes, Stanislav D. Zakharov, Xin S. Wang, Ilya Seleznev, and William A. Cramer (Purdue University)

The pathway of cellular entry of colicins and viral DNA is a fundamental structure problem that is relevant to an understanding of the molecular basis of infectious diseases. The cytotoxin colicin E1 uses the outer membrane/trans-periplasmic drug export protein, TolC, for its import. TolC consists of a 12 strand OM β-barrel connected to a 12 strand α-helical tunnel that defines a pathway through the peptidoglycan barrier in the periplasm to the cytoplasmic membrane in which the C-terminal domain of colicin E1 inserts and forms a depolarizing ion channel. Previous studies with planar bilayers showed that colicin E1 occludes TolC channels, as do the translocation [T]-domain peptides of certain colicins. The nature of the interaction of the colicin with TolC the mechanism of its translocation through the TolC, across at least half of the periplasmic space, is not known.

In vivo protection of sensitive E. coli from colicin E1 by a series of N-terminal colicin peptides was used to probe the interaction of the colicin with TolC, with the goal of defining the sites of TolC-colicin interaction and the mechanism of colicin passage across at least half of the periplasm. N-terminal segments '1-40','1-81', and '1-100' of the colicin did not provide cytotoxic protection, nor occlude TolC channels. Segments '1-120', '1-140', 1-190', as well as '41-190' and '57-190' protected efficiently in vivo and occluded TolC channels formed in planar bilayer membranes. Occlusion required a negative electrical potential and was irreversible. Coelution of the colicin peptides with TolC on a Superdex 200 column was shown for '41-190', but not for '1-81'. In addition to the correlation with protection in vivo from killingby colicin E1, occlusion efficiency also correlated with a basic pI between colicin residues 82 and 140.

Fusion protein TM-TM interactions: Modulators of pre-fusion protein stability

Stacy Webb and Rebecca Dutch (University of Kentucky)

Membrane associated domains of proteins are often under-studied because of their lipid environment, however recent studies suggest that protein transmembrane domains (TMDs) are important for cell signaling events, protein oligomerization, and other functions. Enveloped viruses utilize fusion proteins (F) studding the envelope of the virus to promote viral entry via fusion. To drive fusion, the F proteins transition from a meta-stable pre-fusion conformation to a more thermodynamically stable post-fusion conformation; understanding the elements which control stability of the pre-fusion state and the initiation of conformational changes is important for understanding the function of F proteins. Mutations in F protein TMDs have implicated the TMD in the fusion process, but the molecular details of the role of TMDs in fusion remains unclear. Using the paramyxovirus F protein TMDs as models, we previously utilized ultracentrifugation to demonstrate that isolated TMDs of Hendra virus F associate in a monomer-trimer equilibrium. To determine elements critical for this association, we examined the Hendra virus F sequence. Directly upstream of the TMD is a heptad repeat domain that contains a leu/ile zipper (LIZ) which continues in frame into the TMD. We found that a TMD replacing four L/I residues with alanine dramatically reduced TM-TM association, implicating the LIZ in TM-TM interactions.

Studies with the LIZ mutations in the whole protein indicate decreased protein stability, suggesting that TM-TM interactions promoted by the LIZ may be critical for pre-fusion stability. Previous studies have shown that the pre-fusion conformation of F can be thermally triggered to its post-fusion conformation. We have used this characteristic of F to demonstrate that reduced TM-TM association in the LIZ F mutant results in reduced stability of F in its pre-fusion conformation.

Overexpression and Purification of Human Connexins 43 and 46 from Bacterial Expression Host

Ty E. Whisenant and Luis G. Cuello (Texas Tech University)

Human Connexin 43 (Cx43) and Connexin 46 (Cx46) protein expression levels in bacteria were evaluated following changes to numerous growth variables in a step-wise fashion to determine optimal conditions. XL E. coli strains proved to consistently express higher quantities of protein while grown in LB media. Also, short durations of induction at 37*C increased protein amounts. Metal affinity and size exclusion chromatography were then used for purification and expression.

Optineurin(OPTN) is genetically linked to glaucoma and ALS

Johnny Wise (Purdue University)

Optineurin(OPTN) is genetically linked to glaucoma and ALS. Multiple functional roles have been identified, including in vesicle trafficking, Golgi apparatus organization, induction of macroautophagy and cell cycle. Recent data showed OPTN in aggregates of several neurodegenerative diseases, including frontotemporal lobar degeneration, Alzheimer's, Huntington's, and Parkinson's disease(PD). Major pathological hallmarks in PD include aggregation of α-synuclein into Lewy bodies and progressive loss of dopaminergic (DA) neurons in the substantia nigra (SN). Macroautophagy is a primary route for degradation of protein aggregates. This pathway is dysregulated in PD. Currently, data on roles for OPTN in the pathogenesis of PD is very limited. Given OPTN's involvement in many cellular pathways that are also implicated in PD pathogenesis, we characterized basal brain expression and response to DA neurotoxicants. Here we show evidence that OPTN is enriched in DA neurons in the SN. Using primary rat mesencephalic cultures that contain DA and nonDA neurons and glia, OPTN expression increased after acute exposure to methamphetamine, paraquat, or overexpression of α-synuclein (both mutant and wild type), compared to control, assessed by quantitative immunofluorescence. These data demonstrate that OPTN has high expression in DA neurons, and its expression is increased when PD is modeled by toxicant insult or genetically. Ongoing experiments are examining OPTN expression in SN of rats exposed to rotenone used to model early- and late-stage PD. Here, brains have been sampled prior to overt neurodegeneration, representing a 'preclinical' sampling point. Our next steps are to investigate the interaction between OPTN and macroautophagy after PD-relevant insults.

A fast cross-validation method for assessment of alignment quality of electron tomography images based on Beer-Lambert Law

Rui Yan, Thomas Edwards, Logan Pankratz, Jason Lanman, Richard Kuhn, Wen Jiang (Purdue University)

Alignment accuracy of 2D tilt series is a critical prerequisite to achieve 3D reconstruction with high resolution in electron tomography. However, it is still challenging to quantitatively evaluate the quality of alignment before reconstruction. We develop a fast and reliable method to determine the alignment quality on the basis of Beer-Lambert law. The method can globally measure the alignment quality by means of the linear pattern of intensity in log mode generated from tilt series. The experimental results, including negative staining and cryo datasets, illustrate that the method is able to successfully assess the alignment quality, furthermore, can be cross-validated with the resolution of reconstructed tomograms. In the future, it is promising that the method can serve as a scoring function to improve the alignment accuracy.

A less biased analysis of metalloproteins reveals novel zinc coordination geometries

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Zinc metalloproteins are involved in many biological processes and play crucial biochemical roles across all domains of life. Local structure around the zinc ion, especially the coordination geometry (CG), is dictated by the protein sequence and is often directly related to the function(s) of the protein. Current methodologies in characterizing zinc metalloproteins' CG consider only previously reported CG (canonical CG) models based mainly on non-biological chemical context. Exceptions to these canonical CG models are either misclassified or discarded as outliers.
Methods
We developed a less-biased method that directly handles potential exceptions without pre-assuming any canonical CG models. Zinc metalloproteins were acquired from the worldwide Protein Data Bank (wwPDB). We determined binding ligands for each zinc site using ligand-zinc bond lengths criteria. Zinc sites with a compressed ligand-zinc-ligand angle (about 58 or 38 degrees) were separated from normal zinc sites. K-means was then applied on normal and compressed classes separately to differentiate the CG based on angle statistics. Assignments of clusters to canonical and novel CGs were based on cluster centers, 3D structures, and average $\chi 2$ probabilities together. We also cross-validated our k-means cluster results against functional annotations derived from InterProScan.
Results and Conclusion
Our study shows that thousands of exceptions to canonical CGs could actually be classified, and that new CG models are needed to characterize them. Also, these new CG models are cross-validated by strong correlation between independent structural and functional annotation distance metrics, which is lost if these new CGs models are ignored. Furthermore, these new CG models exhibit functional propensities distinct from the canonical CG models.

Single-step Antibody-based Affinity Cryo-Electron Microscopy for Imaging and Structural Analysis of Macromolecular Assemblies

Guimei Yu, Wen Jiang (Purdue University)

Single particle cryo-electron microscopy (cryo-EM) is an emerging powerful tool for structural studies of macromolecular assemblies. Although single particle cryo-EM requires less concentrated and smaller amounts of samples than X-ray crystallography, it remains challenging to study specimens that are low-abundance, low-yield, or short-lived. The recent development of affinity grid techniques can potentially further extend single particle cryo-EM to these challenging samples by combining sample purification and cryo-EM grid preparation into a single step. Here we report a new design of affinity cryo-EM approach, cryo-SPIEM, that applies a traditional pathogen diagnosis tool Solid Phase Immune Electron Microscopy (SPIEM) to the single particle cryo-EM method. This approach provides an alternative, largely simplified and easier to use affinity grid that directly works with most native macromolecular complexes with established antibodies, and enables cryo-EM studies of native samples directly from cell cultures.

Modeling a system with intrinsic disorder: An MD/NMR study of peptide-protein encounter complex

Tairan Yuwen, Yi Xue, Nikolai Skrynnikov (Purdue University)

Intrinsically disordered proteins (IDPs) often rely on electrostatic interactions to bind with their structured targets, encounter complex is one commonly formed intermediate state in many protein interactions involved with IDPs. The properties about two peptide-protein complexes formed between a ten-residue peptide Sos (PPPVPPRRRR) and c-Crk N-terminal SH3 domain have been studied, which represent tight-binding and encounter complexes respectively. The dynamic properties of Sos peptide within these two complexes have been studied with MD simulation, NMR 15N-relaxation and several other biophysical approaches. In the encounter complex state, Sos peptide displays significant amount of motional freedom which causes the large conformational heterogeneity of this complex. This property has also been observed on some other well-studied electrostatic encounter complexes based on folded proteins.

A Potential Problem with the Accuracy of Membrane Protein Structures

S. D. Zakharov, S. Bhaduri, and W. A. Cramer (Purdue University)

Integral membrane proteins that span a biological membrane in a helical conformation are situated in the presence of trans-membrane electrical potentials of 100-150 mV across a low dielectric (lipid) medium of approximately 30 Å, yielding an electric field intensity of approximately 3 x105 V/cm. Atomic structures of > 500 independent integral membrane structures have been obtained in the absence of this field (1). The question arises as to whether the absence of this intra-membrane field results in a significant change in structures of membrane proteins, which are isolated in detergent, and crystallized in

detergent, lipid cubic phase, or bicelles, where the electric field is absent. The effect of this E-field on membrane protein structure was tested with the cytochrome b6f complex, from which a high resolution crystal structure (2.5 Å) was determined (2). The complex was incorporated into liposomes across which a valinomycin-dependent Nernst diffusion potential = -118 mV, was imposed. Characteristic times for formation and decay of the valinomycin-induced trans-membrane potential were determined using a potential-sensitive fluorescence probe. The dependence of the fluorescence yield and emission peak of tryptophan fluorescence from the liposome-embedded cytochrome complex was measured in the presence and absence of the Nernst potential. The emission peak generated by the tryptophan ensemble displayed a blue shift and a yield increase upon generation of the potential, implying that the structure of the cytochrome complex is perturbed in the presence of the trans-membrane electrical. [NIH GM-038323]. 1. http://blanco.biomol.uci.edu/mpstruc/ 2014; 2. Hasan, S. S. & W. A. Cramer, Structure (2104), 22, 1008-15.

EphA2 Cytoplasmic Phosphotyrosine Mapping, Dephosphorylation Profiling and Vav GEF interaction

Kaibo Zhang (Purdue University

The overexpression of the EphA2 receptor tyrosine kinase (RTK) and the dephosphorylation of its cytoplasmic phosphotyrosines by human cytoplasmic protein tyrosine phosphatase (HCPTP) are closely related to the metastasis and angiogenesis of human epithelial cancers, mediated by Vav guanine nucleotide exchange factors (GEFs). To illuminate the signaling significance of the EphA2 post-translational modification, the codon-optimized human EphA2 cytoplasmic domain (C0) has been expressed in E. coli. The dephosphorylation profile of C0 and its interaction with the Vav-SH2 domain have been probed with selected reaction monitoring mass spectrometry (SRM-MS) and isothermal titration calorimetry (ITC) respectively. The results indicate bacterially expressed EphA2 C0 is autophosphorylated with multiple phosphotyrosines that can be selectively dephosphorylated by HCPTP variants A and B, leading to distinct signaling consequences in cells. The ITC data using full-length C0 and synthetic peptides containing key phosphotyrosines suggests Vav binds to EphA2-C0 in vitro through the juxtamembrane (JM) phosphotyrosines, preferentially binding Y594. The structural basis of this interaction is under investigation.

A fluorescent, HTS-adaptable coupled-enzyme assay for measurement of human cytosolic sulfotransferase (SULT) 2B1b activity

Qing Zhou, and Andrew D. Mesecar (Purdue University)

Human cytosolic sulfotransferase (SULT) 2B1b, as well as other members in the SULT family, catalyzes the transfer of a sulfuryl moiety (-SO3) from the universal donor 3-phopho-adenosine 5'-phophosulfate (PAPS) to the hydroxyl groups of various substrates, which leads to a change in water solubility and function. SULT2B1b highly prefers 3β-hydroxysteroids, such as cholesterol, as its substrates. By switching the sulfonation state of its substrates, SULT2B1b is involved in the regulation of steroid synthesis, transportation

and signaling. Furthermore, SULT2B1b has been suggested as a potential drug target for prostate cancer. Therefore, we aim to identify small-molecule inhibitors from high-throughput screening (HTS), and utilize them to study the physiological roles of SULT2B1b in carcinogenesis and provide possible leads for drug discovery in the treatment of prostate cancer. Considering the lack of convenient and HTS-adaptable activity assays for SULT2B1b, we have developed a fluorescent coupled-enzyme assay. The real-time reading capacity of this assay allows it to be used in steady-state kinetics studies and the assay is amenable to HTS. Compared to commonly used radioactivity assays, this newly developed activity assay is suitable for regular activity measurements, kinetic study and HTS for SULT2B1b. Also, it can be easily adapted to work for other SULTs.

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