April 8, 2011
Sealy Center for Structural Biology and Molecular Biophysics Symposium
The University of Texas Medical Branch

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16th Annual Sealy Center for Structural Biology Symposium
Hotel Galvez
2024 Seawall Blvd.
Galveston, Texas 77550

Friday, April 8, 2011

Scientific Program at Hotel Galvez, Galveston, TX
All Lectures will be in the Music Hall

7:00-8:30 am	Registration—Promenade Poster Set-up—East & West Parlors
8:30-8:45 am	Opening Remarks – Dr. Wayne Bolen Welcome address – Dr. Regino Perez-Polo Introduction to Keynote Address – Werner Braun
8:45 -9:40 am	**Keynote Speaker** **THEODORE JARDETZKY** SCHOOL OF MEDICINE, STANFORD UNIVERSITY, STANFORD, CA **"STRUCTURAL INSIGHTS INTO ENVELOPED VIRUS ENTRY AND INHIBITION"**

Morning Session

Session Chair:	**Scott Weaver**
9:40-10:25 am	**G. MARIUS CLORE** NIDDK, NATIONAL INSTITUTES OF HEALTH, BETHESDA, MD **"EXPLORING SPARSELY-POPULATED STATES OF MACROMOLECULES BY PARAMAGNETIC NMR "**
10:25-11:00 am	***Break (Foyer) & Posters (East & West Parlors)***
11:00-11:45 am	**MEI HONG** IOWA STATE UNIVERSITY, AMES, IA **"STRUCTURE AND MECHANISM OF THE INFLUENZA M2 PROTEIN FROM SOLID-STATE NMR"**
11:45 –1:45 pm	***Lunch (Veranda) & Posters (East & West Parlors)***

Afternoon Session

Session Chair: **Ted Wensel**

1:45 – 2:30 pm

CHARLES SANDERS
SCHOOL OF MEDICINE, VANDERBILT UNIVERSITY, NASHVILLE, TN
"STRUCTURAL BASIS FOR MODULATION OF VOLTAGE-GATED POTASSIUM CHANNELS BY KCNE FAMILY MEMBRANE PROTEINS"

2:30 – 3:15 pm

FRANCIS T.F. TSAI
BAYLOR COLLEGE OF MEDICINE, HOUSTON, TX
"STRUCTURE, FUNCTION AND MECHANISM OF A PROTEIN DISAGGREGATING MACHINE"

3:15 – 3:45 pm ***Break (Foyer) & Posters (East & West Parlors)***

Session Chair: **Junji Iwahara**

3:45 – 4:30 pm

ERIC C. GREENE
HOWARD HUGHES MEDICAL INSTITUTE, COLUMBIA UNIVERSITY, NEW YORK, NY
"HIGH-THROUGHPUT SINGLE MOLECULE IMAGING OF PROTEIN-DNA INTERACTIONS"

4:30 –5:15 pm

LAWRENCE C. SOWERS
UNIVERSITY OF TEXAS MEDICAL BRANCH, GALVESTON, TX
"STRUCTURE AND BIOLOGY OF INFLAMMATION-MEDIATED DNA DAMAGE PRODUCTS"

5:15 – 5:30 pm ***Poster Awards and Closing remarks- Mark White and Wayne Bolen***

6:00 pm ***Banquet & Cocktails (Hotel Galvez)***

HOPE TO SEE YOU NEXT YEAR AT THE

17th ANNUAL SCSB SYMPOSIUM

Sealy Center for Structural Biology & Molecular Biophysics

Structure-based studies of proteins and other biological molecules are a key aspect to understanding the molecular basis for disease, as well as for designing drugs to treat disease. The Sealy Center for Structural Biology and Molecular Biophysics (SCSB) was established in 1995 to provide UTMB with state-of-the-art resources for structural and functional studies of biological macromolecules. However, unlike traditional structural biology centers, whose research efforts are directed almost entirely toward structure determination, SCSB was founded on the principle that the success of structural biology in medical research is predicated on an understanding of how structure is linked to function. Consequently, in addition to traditional research programs that use X-ray crystallography and nuclear magnetic resonance (NMR), recruitment in SCSB also included faculty whose expertise cover experimental and theoretical biophysics, as well as computational biology. Today the SCSB consists of 20 core members, 14 associate members and 4 managers (from 8 departments), and the breadth of research spans all aspects of molecular biophysics and biochemistry, addressing such fundamental issues as molecular recognition, signal transduction, allosteric regulation, protein folding, systems biology, and drug design.

Gulf Coast Consortia: Interdisciplinary Bioscience Research and Training

The Gulf Coast Consortia (GCC), created in March 2001, arose from the interactions among a number of faculty in the major research institutions of the Houston / Galveston region. The aspiration of the GCC is to facilitate interactions and leverage strengths to foster innovative training and research collaborations at the frontiers formed by the interface of the biosciences with the computational, mathematical, physical, chemical, and engineering sciences. The vision and engagement of faculty from the GCC member institutions, Baylor College of Medicine, Rice University, University of Houston, University of Texas Health Science Center at Houston, the University of Texas Medical Branch at Galveston, and the University of Texas M.D. Anderson Cancer Center, have contributed to the GCC's growth to include six research consortia, one center and eight interdisciplinary training programs.

The individual consortia, which form the research arm of the GCC, serve to catalyze interactions and develop interdisciplinary collaborative communities of faculty interested in research in a range of interdisciplinary fields. The GCC provides a supportive environment for the encouragement of research programs and acquisition of shared equipment beyond the reach of a single institution, enhancing the research capacity and capabilities of the Gulf Coast region and the GCC's member institutions. Currently, the GCC supports research consortia in protein crystallography, magnetic resonance, bioinformatics, chemical genomics, membrane biology, theoretical and computational neuroscience, and a center for computational cancer. For more information visit: www.gulfcoastconsortia.org

Keck Center: Interdisciplinary Bioscience Training

The Keck Center for Interdisciplinary Bioscience Research and Training was established in 1990 with support from the W. M. Keck Foundation. From its roots as the W. M. Keck Center for Computational and Structural Biology with founding institutions, Rice University and Baylor College of Medicine, the Keck Center has now grown to include the six major public and private institutions that comprise the Gulf Coast Consortia. Driving the formation of the Keck Center was the realization that major advances in the biological sciences, such as the DNA sequence of the human genome, would be driven by the integration of biology and computer science. The partners realized, however, that most biological scientists were not prepared to capitalize on novel approaches to visualization, analysis and interpretation of experimental data made possible by rapid advances in computing technology. Moreover, most researchers in computer programming and analysis systems did not have adequate knowledge about biology and biological systems. The Keck Center was explicitly designed to bridge this gap between biological and computational sciences by fostering collaborations among biologists, biomedical researchers, mathematicians, bioengineers, physicists, and computer scientists through specially designed research and training programs.

Building on this foundation in computational biology, the Keck Center has established its expertise in multidisciplinary inter-institutional programs, and has become the training arm of the Gulf Coast Consortia, supporting training programs in computational biology and medicine, molecular biophysics, pharmacoinformatics, and nanobiology. The Keck Center hosts a number of educational events, including a weekly seminar during the spring and fall semesters, available via webcast, and the Keck Annual Research Conference in the fall.

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On behalf of everyone attending the Symposium, the Organizing Committee thanks those who have provided us with financial support. We are grateful to the following organizations for their generous assistance.

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Gulf Coast Consortia for Quantitative Biomedical Sciences

Keck Center for Interdisciplinary Bioscience Training

Lecture L-1

Structural Insights into Enveloped Virus Entry and Inhibition

Theodore S. Jardetzky, Ph.D.
Department of Structural Biology, Stanford University School of Medicine, Stanford, California 94305

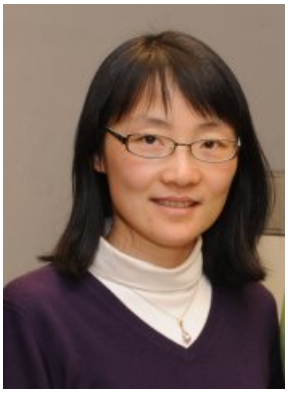
Enveloped viruses must fuse their own lipid bilayer with that of a target host cell to initiate infection and replication. This viral entry process requires multiple steps, including targeting, attachment, specific activation and membrane fusion. In some viruses, such as influenza virus, all of these functions are encoded in a single glycoprotein, while other viruses, such as the herpesviruses and paramyxoviruses, distribute these functions over multiple glycoproteins. For example, Epstein-Barr virus (EBV) is a gamma herpesvirus that infects B cells and epithelial cells and that has been linked to tumor growth *in vivo*. EBV utilizes different host receptors and 3-4 viral glycoproteins to regulate its infection of B cells and epithelial cells. For the paramyxoviruses, two viral glycoproteins mediate this entry process – an attachment protein referred to as HN, H, or G, depending on the virus, and the fusion (F) glycoprotein. Structural and functional studies of viral glycoproteins have yielded new insights into the mechanisms that determine cell tropism and activate virus entry, and also opened up new avenues for the development of virus entry inhibitors. Specific examples drawn from our own work on herpesvirus and paramyxovirus entry glycoproteins will be presented.

Lecture L-2

Exploring Sparsely-Populated States of Macromolecules by Paramagnetic NMR

G. Marius Clore, Ph.D.
Laboratory of Chemical Physics, NIDDK, National Institutes of Health, Bethesda, MD 20892-0520

Sparsely-populated states of macromolecules, characterized by short lifetimes and high free-energies relative to the predominant ground state, often play a key role in many biological, chemical and biophysical processes. In this talk we will briefly summarize various new developments in NMR spectroscopy that permit these heretofore invisible, sparsely-populated states to be detected, characterized and in some instances visualized. In the fast exchange regime (time scale less than ~250-500 ms) the footprint of sparsely -populated states can be observed on paramagnetic relaxation enhancement profiles measured on the resonances of the major species, thereby yielding structural information that is directly related to paramagnetic center-nuclei distances, from which it is possible, under suitable circumstances, to compute a structure or ensemble of structures for the minor species. In the slow exchange regime, differential transverse relaxation measurements coupled with dark-state saturation difference spectroscopy can be used to probe exchange processes between NMR-visible and high-molecular weight NMR-invisible ('dark') species and to probe dynamics within the 'dark' states. Examples of these various approaches will be presented.



Lecture L-3

Structure and Mechanism of the Influenza M2 Protein from Solid-state NMR

Mei Hong, Ph.D.
Department of Chemistry, Iowa State University, Ames, IA 50011

The structure and dynamics of the influenza M2 protein in phospholipid bilayers are investigated in detail using solid-state NMR spectroscopy. The protein forms a drug-sensitive tetrameric proton channel that is important for the virus lifecycle. To understand how the channel conducts protons, we examined the structure, dynamics and hydrogen bonding of the proton-selective residue, histidine-37 (His37) in virus-mimetic lipid membranes. ^{15}N chemical shifts, proton exchange, and dynamic NMR experiments show that at acidic pH, cationic His37 shuttles protons by imidazole protonation and deprotonation events on the functional timescale of ~3000 per second. The proton transfer between imidazole and water is facilitated by microsecond reorientations of the rings, whose energy barrier is consistent with the barrier for proton conduction. The antiviral drug, adamantane, inhibits the channel by binding to the N-terminal region of the pore, both obstructing the water pathway and shifting the His37 proton dissociation constant. Distance measurements between ^{13}C labeled protein and ^{2}H-labeled drug yielded a 0.3 Å-resolution structure of the drug-complexed region of the protein. It also revealed the existence of a second, non-specific binding site on the surface of the channel that is unrelated to the pharmacology of the amantadine class of anti-flu drugs. These results illustrate the complexity of membrane protein structure and dynamics and the power of solid-state NMR to elucidate crystallographically inaccessible atomic structure and dynamics information in a near-native biological environment.

Lecture L-4

Structural Basis for Modulation of Voltage-Gated Potassium Channels by KCNE Family Membrane Proteins

Charles R. Sanders[1], Wade Van Horn[1],
Carlos Vanoye[1] Jarrod Smith[1], Jens Meiler[1], and Frank Sönnichsen[2]

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[2] *Dept. of Chemistry, Christian Albrechts University of Kiel, Germany.*

The human KCNE family of single span membrane proteins modulate the function of the KCNQ1 voltage-gated potassium channel. KCNE1 (MinK) acts to both delay channel opening and enhance the conductance of the open state, as is necessary for hearing and for the cardiac action potential. KCEN3, on the other hand, does not impact the kinetics of channel opening, but does enhance open state conductance. The structural basis for how KCNE1 and KCNE3 modulate channel function was investigated using NMR to determine the structure and dynamics of these two membrane proteins. This was followed by experimentally-restrained docking of the structures to the open and closed KCNQ1 channel states. Based on these studies mechanisms are proposed for how the KCNE subunits modulate KCNQ1 potassium channel function. The results also explain why KCNE1 alters the kinetics of channel opening while KCNE3 does not. *This work was supported by NIH/NIDCD grant RO1 DC007416.*

References

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Lecture L-5

Structure, Function, and Mechanism of a Protein Disaggregating Machine

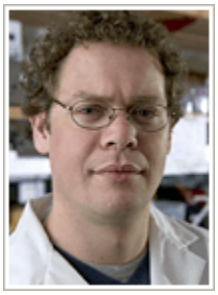
Francis T.F. Tsai, Ph.D.
Verna and Marrs McLean Department of Biochemistry and Molecular Biology, and Department of Molecular and Cellular Biology, Baylor College of Medicine, Houston, TX 77030.

AAA+ machines are a group of evolutionary conserved, ring-forming, ATP-dependent molecular machines that convert metabolic energy into mechanical work to mediate diverse biological activities. Notable members include molecular chaperones and energy-dependent proteases as well as DNA helicases and translocases. While common mechanisms are apparent, distinct structural features exist that confer specific functions.

Yeast Hsp104 and its bacterial ortholog ClpB are ATP-dependent protein disaggregases, which, together with the cognate Hsp70 system, have the remarkable ability to rescue stress-damaged proteins from a previously aggregated state. The ability to do so is strictly dependent on the M-domain that is a hallmark of the ClpB/Hsp104 family. Surprisingly, it has been proposed that the location of the M-domain is different in Hsp104 and ClpB. To address this issue, we determined the hexamer structures of two Hsp104 variants, in addition to the structure of an engineered Hsp104 chimera harboring T4 lysozyme (T4L) within the M-domain of Hsp104. The fitted cryoEM structure of the Hsp104 hexamer places the M-domains on the Hsp104 exterior, as they are in ClpB, where they can potentially interact with large, aggregated proteins. Perhaps most astonishingly, our engineered Hsp104-T4L chimera not only is fully functional biochemically but also has gained the ability to solubilize heat-aggregated protein substrates in the absence of the Hsp70 system. Our work suggests a novel regulatory role for Hsp70, in addition to its known chaperone function, which is required to unleash the protein remodeling activity of Hsp104.

At this symposium, I will present our latest research findings on ATP-dependent protein disaggregases and other AAA+ machines.

Work in the Tsai lab is supported by grants from the National Institutes of Health (AI076239, GM067672, DK071505, and RR002250), the Robert A. Welch Foundation (Q-1530), the Department of Defense, and by a Research Scholar Award from the American Cancer Society.



Lecture L-6

High-throughput Single Molecule Imaging of Protein-DNA Interactions

Eric C. Greene, Ph.D.
Howard Hughes Medical Institute, Columbia University

Our group uses single-molecule optical microscopy to study fundamental interactions between proteins and nucleic acids - we literally watch individual protein molecules or protein complexes as they interact with their DNA substrates. Our overall goal is to reveal the molecular mechanisms that cells use to repair, maintain, and decode their genetic information. This research combines aspects of biochemistry, physics, and nanoscale technology to answer questions about complex biological problems that simply can not be addressed through traditional biochemical approaches. The primary advantages of our approaches are that we can actually see what proteins are bound to DNA, where they are bound, how they move, and how they influence other components of the system - all in real-time, at the level of a single reaction. Our research program is focused on studying the regulation and activity of proteins that are involved in repairing damaged chromosomes. We are particularly interested in determining the physical basis for the mechanisms that proteins use to survey DNA molecules for damage and initiate repair processes, and how these initial steps are coordinated with downstream events that lead to completion of repair. As part of our work, we are also actively pursuing the development of novel experimental tools that can be used to facilitate the study of single biochemical reactions. In particular, we are applying techniques derived from nanotechnology to our biological research, and using nano- and micro-scale engineering to facilitate the development of new, robust experimental platforms that enable "high throughput" single molecule imaging.

Lecture L-7

Structure and Biology of Inflammation-mediated DNA Damage Products

Lawrence C. Sowers, Ph.D.
Department of Pharmacology and Toxicology, University of Texas Medical Branch, School of Medicine, Galveston, Texas.

DNA in living cells is persistently damaged by reactive molecules produced through normal metabolic pathways. In addition, cellular DNA can be damaged by reactive molecules generated through activation of innate immunity. Activated neutrophils can generate HOCl and activated eosinophils can generate HOBr which can react with many biological molecules including DNA. Many damaged bases are formed in DNA including 5-chlorouracil (ClU), 5-chlorocytosine (ClC) and 5-bromocytosine (BrC). NMR data will be presented on structures of bases pairs formed by ClU in oligonucleotides, and a model will be proposed to explain the mutagenicity of halouracil analogs. NMR data will also be presented on ClC bases pairs. A model will be proposed suggesting that both ClC and BrC can act as fraudulent epigenetic signals, inducing methylation of cytosine residues in the DNA. Aberrant methylation within promoter regions of key tumor suppressor genes has long been associated with gene silencing. Together, these findings suggest a possible mechanism that might explain the long-standing association between inflammation and the development of cancer.

treatment for a multitude of diseases.
Acknowledgement: This work was supported by a training fellowship from the Houston Area Molecular Biophysics Program (HAMBP) though NIH Molecular Biophysics Training Grant (T32 GM008280)

Structure Poster Number P-13

Biochemical and Biophysical Characterization of the Capsid Assembly and RNA Transcription Mechanism of a Small DsRNA Virus

Aaron Collier[1], Junhua Pan[2], Jane Tao[1]
(1) Biochemistry and Cell Biology, Rice University (2) Department of Biological Chemistry & Molecular Pharmacology, Harvard Medical School, Boston, MA

Double-stranded (ds) RNA viruses include members from at least seven virus families, with many of them being pathogens of health and agricultural importance. Most known dsRNA viruses share structural similarity by having a characteristic, 120-subunit capsid surrounding the viral genome. Such a capsid provides protection for the genome and the enzymes involved in RNA synthesis. One such enzyme is the viral RNA-dependent RNA polymerase (RdRp). The RdRps of dsRNA viruses possesses several unique structural features that allow them to recognize the positive sense (+sense) transcripts of the viral genome in the cytosol during packaging, attach themselves to the interior of the capsid during capsid assembly, and to synthesize negative sense (-sense) RNA using the +sense as a template to form dsRNA. In mature virions, the RdRps then synthesize additional +sense transcripts utilizing a either a conservative or semi-conservative mechanism using the -sense strand of the dsRNA as the template. The +sense transcripts must then exit the capsid to further propagate the viral genome.
The Penicillium stoloniferum virus F (PsV-F) contains a single capsid protein and there is only one dsRNA segment packaged in each virion, making it an excellent model for studying dsRNA virus structure and function. Our project is to use biophysical and biochemical methods to elucidate the mechanisms for viral RdRp packaging, viral RNA transcription, and viral capsid assembly for this virus.
This work was supported by a training fellowship from the Houston Area Molecular Biophysics Program under the National Institute of General Medical Sciences Grant No. 2T32 GM008280-21A1. Funding was also provided by the National Institute of Health (Grant AIO67638).

Structure Poster Number P-14

Protein Structure-Based Analysis of NsSNPs in the Cancer Genome Atlas

Susan McGovern[1], John N. Weinstein[2], and Wah Chiu[1]
(1) Biochemistry, Baylor College of Medicine (2) Department of Bioinformatics and Computational Biology, UT MD Anderson Cancer Center, Houston, TX

Numerous mutations in cancer cells enable them to grow or to evade therapy. An especially important type of mutation is the non-synonymous single nucleotide polymorphism (nsSNP), which produces a single amino

acid change in the protein encoded by a gene. Deep sequencing results from cancer genomics projects such as The Cancer Genome Atlas (TCGA) are becoming increasingly available, and informatics tools for analysis of large volumes of nsSNP data are needed. By illuminating three-dimensional relationships not apparent from analysis of a one-dimensional sequence, structural biology may provide a useful framework for interpreting these data. Our central hypothesis is that visualizing nsSNPs from multiple cancer patients on the three-dimensional protein structure will allow us to identify functionally relevant mutations in cancer. To test this hypothesis, nsSNPs in 465 glioblastoma and 588 ovarian adenocarcioma samples in TCGA were analyzed. nsSNPs from p53, PTEN, and EGFR were mapped onto their respective three dimensional atomic resolution structure. For the tumor suppressors p53 and PTEN, mutations were significantly concentrated within a 10 Å radius of the ligand binding site ($p < 0.0001$ vs. random distribution). For the oncogene EGFR, mutations were not enriched around the ligand binding site ($p = 0.28$) but were primarily contained within a 10 Å depth along the dimerization interface ($p < 0.001$ vs. random distribution). These results suggest that most nsSNPs in p53, PTEN, and EGFR in glioblastoma and ovarian adenocarcinoma do not occur randomly but are instead enriched in structurally and functionally significant locations. Moreover, the interaction surface that is preferentially mutated differs depending on the function of the protein in carcinogenesis.

Structure Poster Number P-15

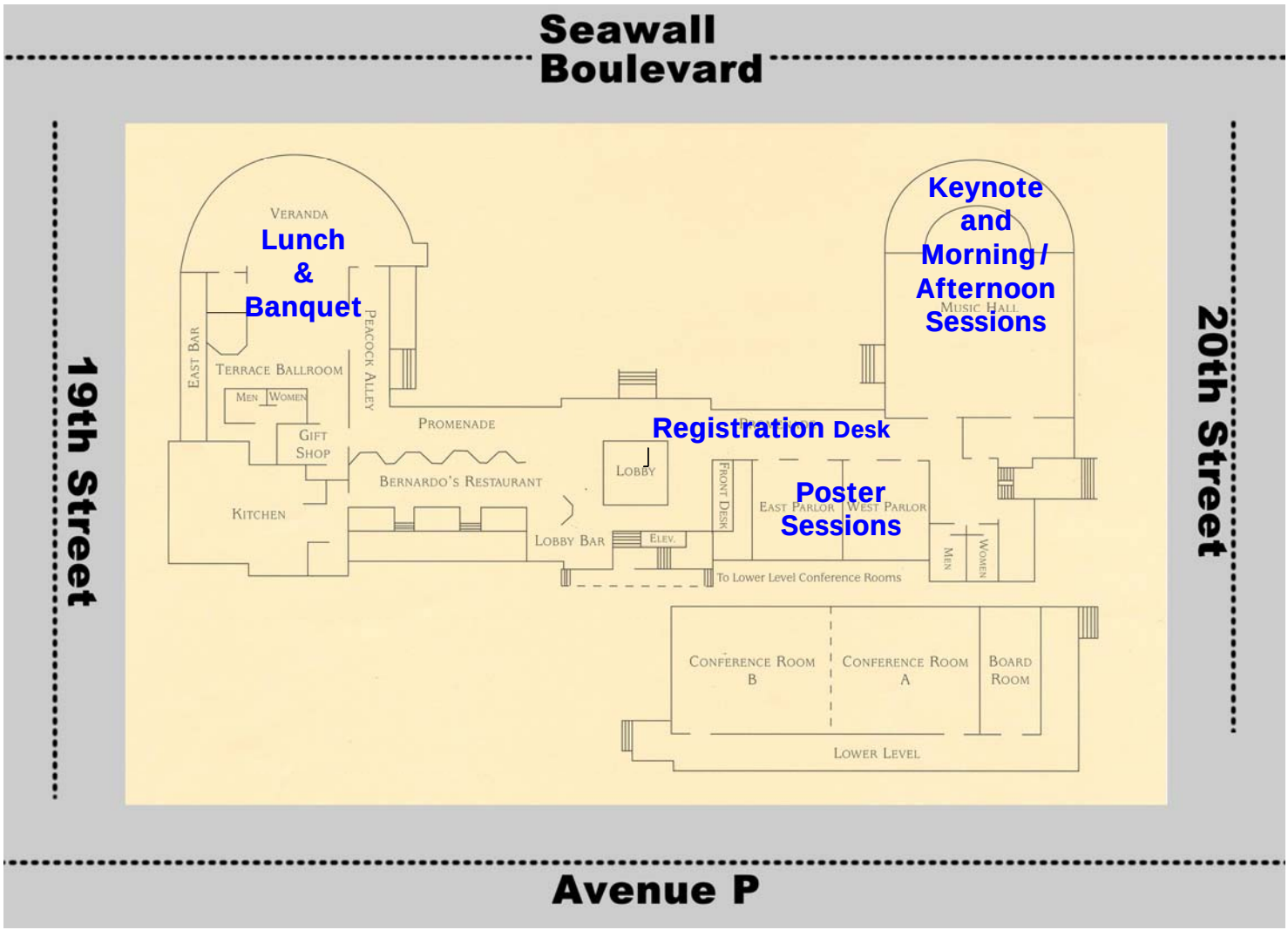
Structural Characterization of Interleukin-8 Monomer and Dimer Interactions With Glycosaminoglycans: Implications for in Vivo Neutrophil Recruitment

Prem Raj Joseph[1], and Krishna Rajarathnam[1]
(1) BMB, UTMB Department of Biochemistry and Molecular Biology & Sealy Center for Structural Biology and Molecular Biophysics, UTMB, Galveston, TX 77555

Interactions of chemokine interleukin-8 (IL-8) with cell surface glycosaminoglycans (GAGs) play a crucial role in mobilizing neutrophils to the site of infection or injury. IL-8 exists reversibly as both monomers and dimers, and both recruit neutrophils but in a highly differential manner. We hypothesize that the monomer-dimer equilibrium of the free and GAG-bound IL-8 are coupled and tightly regulated, and that this process is critical for IL-8 function.

GAGs, such as heparan sulfate and heparin, are highly negatively charged linear polysaccharides, and are the most complex and diverse biomolecules expressed in humans. So it is not surprising that there is very little consensus regarding the structural basis and the molecular mechanisms underlying IL-8–GAG interactions. For instance, there are conflicting reports regarding stoichiometry, geometry and orientation of GAG in the bound complex, and whether monomer or dimer is the high affinity GAG binding ligand. Further, structure determination faces many difficulties, as GAG-protein interactions are transient and weak, and native GAGs are chemically heterogeneous and conformationally flexible.

We have now characterized the interactions of IL-8 monomer and dimer to heparin derived oligosaccharides using NMR spectroscopy and HADDOCK docking. Our data show that (i) both monomers and dimers bind GAG with similar low μM affinities; (ii) monomer and dimer bind GAG with 1:1 and 1:2 stoichiometry, respectively; (iii) GAG binds to a groove (parallel to the C-helix) flanked by the basic residues of the C-helix and 310 helix of N-loop; (iv) GAG binds only to a monomer of the dimer, ruling out the perpendicular orientation model; (v) the structure of GAG-bound monomer and dimer are similar, but the underlying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Women
To Lower Level Conference Rooms
Music Hall
Keynote and Morning / Afternoon Sessions
Conference Room B
Conference Room A
Board Room
Lower Level

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P-3	Wei Dai	Visualizing the Production of Syn5 Cyanophage From Its Host Cyanobacteria By Electron Tomography
P-4	Ryan Rochat	Zernike Phase Contrast Cryo-EM Reveals the Structure of the Genome Packaging Apparatus in Herpes Simplex Virus
P-5	Rossi Irobalieva	Pushing the Limits of Both NMR and Cryo-Electron Tomography: the Structure of the 42.8 KDA Encapsidation Signal of MoMuLV
P-6	Mariah Baker	PATHWALKER: a Sequence-Free Method for De Novo Backbone Modeling
P-7	Andria Denmon	Structural and Stability Effects of Base Modifications on the Tyrosyl-Anticodon Stem-Loop From Bacillus Subtilis
P-8	Emilio ReyesAldrete	Towards a High Resolution Model of the Phage Phi29 DNA-Packaging Motor
P-9	Michael Sherman	CryoEM At UTMB
P-10	Christina R. Bourne	Optimizing the Anti-Folate Profile of Dihydrophthalazine Compounds Targeting Bacterial Dihydrofolate Reductase
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P-14	Susan McGovern	Protein Structure-Based Analysis of NsSNPs in the Cancer Genome Atlas
P-15	Prem Raj Joseph	Structural Characterization of Interleukin-8 Monomer and Dimer Interactions With Glycosaminoglycans: Implications for in Vivo Neutrophil Recruitment
P-16	David Power	A Structural Comparison of a 3D- Model of the Peanut Allergen Ara h 2 and the Recently Released X-Ray Crystal Structure
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P-18	Svetla Stoilova-McPhie	Membrane-Bound Organization of Coagualtion Factor VIII: Impilcation for the Tenase Complex Assembly and Function
P-19	Keerthi Gottipati	Crystal Structure of N^{Pro}: a Novel Cysteine Auto-Protease in Classical Swine Fever Virus
P-20	Xiaowei Zhao	Flagellar Stator Revealed in Situ
P-21	Bo Hu	Molecular Architecture of Intact Flagellar Motor and Chemotaxis Array Revealed By Cryo-Electron Tomography of Motile and Tiny Escherichia Coli
P-22	Seonghee Paek	Structural and Biochemical Studies of the Novel Lipoprotein Cj0090 From *Campylobacter Jejuni*
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P-24	Marc Morais	The Mechanism of DNA Ejection in the Bacillus Anthracis Spore-Binding Phage 8a Revealed By Cryo-Electron Tomography
P-25	Cecile Bussetta	Structural Study of the Interaction Between the Methyltransferase and the Polymerase Domains of Dengue Virus NS5
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P-36	Luis Holthauzen	The SCSB Solution Biophysics Laboratory
P-37	Kurtis Anderson	Counterion Condensation Around Thioated Oligonucleotides
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Structure Poster Number P-1

Modulating Chaperonin Interactions as a Potential Therapeutic Target

Benjamin Bammes[1], Moses Kasembeli[2], Susan L. McGovern[1], Judith Frydman[3], David J. Tweardy[2], Wah Chiu[1]

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Chaperonins are a class of heat shock proteins (Hsp) that promote the native folding of newly-translated or stress-denatured proteins. In eukaryotes, the primary cytosolic chaperonin is the TCP1 Ring Complex (TRiC), which is necessary for proper folding of many essential proteins, including cytoskeletal proteins such as actin and tubulin, and tumor suppressors such as the von Hippel Lindau protein (pVHL). Modulating interactions between TRiC and its substrates may provide a therapeutic strategy for diseases caused either by increased activity or misfolding and aggregation of cellular proteins.

Signal transducer and activator of transcription 3 (Stat3) is a transcription factor with anti-apoptotic activity. Stat3 activity is increased in ~50-70% of cancers, and inhibition of Stat3 in many cancers induces apoptosis. Alternatively, Stat3 up-regulation is of interest for ischemia/reperfusion injuries, where apoptosis is undesirable. Altering the interaction between Stat3 and TRiC may provide an opportunity for regulating Stat3 activity in vivo, by either increasing or decreasing the folding efficiency of Stat3.

We have found that Stat3 directly interacts with TRiC. Immunodepletion of TRiC inhibits synthesis of Stat3 in rabbit reticulocyte lysates, and Stat3 folding is restored by re-addition of TRiC. To test if this interaction can be artificially strengthened, we inserted the TRiC-binding domain (TBD) from pVHL at the N-terminal of Stat3. Addition of the TBD strengthened both the interaction between TRiC and Stat3, as well as Stat3 function, as measured by Stat3 binding to phosphopeptide ligand (EGFR pY1068).

Electron cryo-microscopy (cryo-EM) of the TRiC+Stat3-TBD complex revealed that Stat3-TBD binds to TRiC in three separate regions across the apical domains of the TRiC subunits. Based on comparison between cryo-EM structures of TRiC+Stat3-TBD and TRiC+pVHL, we conclude that wild-type Stat3 interacts with TRiC through at least two binding sites, and addition of the pVHL TBD strengthens this interaction by adding an additional TRiC binding site to Stat3. Cryo-EM of TRiC+Stat3 confirms this hypothesis, showing an interaction between only two regions of TRiC to Stat3. Therefore, we have demonstrated that TRiC-substate interactions can be artificially modulated by affecting the contact between TRiC and the substrate during initial binding.

This work is supported by the National Institutes of Health through a training fellowship from the National Library of Medicine through the Keck Center of the Gulf Coast Consortia (T15LM007093), the Center for Protein Folding Machinery (5PN2EY016525-05), and the National Center for Macromolecular Imaging (5P41RR002250-24).

Structure Poster Number P-2

The Effect of Macromolecular Crowding, Ionic Strength and Calcium Binding on Calmodulin Dynamics

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The flexibility in the structure of calmodulin (CaM) allows its binding to over 300 target proteins in the cell. To investigate the structure-function relationship of CaM in response to the changing intracellular environment, we use a combined method of computer simulation and experiments based on circular dichroism (CD) to investigate the structural characteristics of CaM that influence its target recognition in crowded cell-like conditions. The conformation, helicity and EF hand orientation of CaM are analyzed computationally to address the effect of macromolecular crowding, ionic strength and calcium binding in the experiments. We applied a unique solution of charges computed from quantum mechanics/molecular mechanics (QM/MM) to accurately represent the charge distribution in the transition from apoCaM to holoCaM upon calcium binding. Computationally, we found that a high level of macromolecular crowding, in addition to calcium binding and ionic strength typical of that found inside cells, can impact the conformation, helicity and the EF hand orientation of CaM. Because EF hand orientation impacts the specificity of CaM's target selection, our result may provide unique insight into understanding the promiscuous behavior of calmodulin in target selection inside.

Structure Poster Number P-3

Visualizing the Production of Syn5 Cyanophage From Its Host Cyanobacteria By Electron Tomography

Wei Dai[1], Desislava Raytcheva[2,3], Caroline Fu[1], Jacqueline Piret[3], Jonathan King[2], & Wah Chiu[1]

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Marine phages that infect cyanobacteria (are also called cyanophages) play an important role in mediating marine ecosystem by controlling the cyanobacteria community structure and lateral gene transfer between hosts. The later may pose unpredictable detrimental effects on human health. Cyanophage Syn5 infects marine Synechococcus strain WH8109. Syn5 virions consist of an 60nm icosahedral capsid, a short tail assembly, and a novel “horn” sitting on top of the vertex that is at the direct opposite side of the capsid where the tail is located. The capsid and the tail assembly display remarked morphology similarity and sequence

homology to T7-like phages. The "horn" is a filamentous structure unique to Syn5. It is about 50 nm long, 10nm wide at the base, and tapers to only 2-5 nm wide at the tip. How/when the horn is assembled onto the capsid, and what are the possible functions are largely unknown.
A well-accepted assembly pathway for icosahedral tailed-phages is that a dodecameric portal ring forms first, and the capsid subunits are recruited to the portal ring nucleus to form the procapsid. This model explains well that only a single portal complex is included in the capsid. However, since this model suggested that the remaining eleven vertexes of the capsid are all identical, it failed to account for the fact that the "horn" exists, and only exists in the direct opposite vertex of the tail assembly. We have infected the host with Syn5 phages, and used electron tomography to capture maturing virions inside the host. We were able to identify three different kinds of maturation intermediate particles from infected cells. The procapsid is a class of spherical particles with a diameter that is about 15% smaller than mature capsids. They have a scaffolding core located in the middle. The second class of particles is morphologically undistinguishable to the mature phage. They show clear icosahedral symmetry. DNA has been packed in, and "horn" has been assembled. The third class of maturation product has all the protein structural elements of the second class, but DNA is missing. By analyzing Syn5 procapsid and maturation intermediates we will elucidate at what stage the "horn" is assembled onto the vertex at direct opposite of the tail, and formulate a new model for how the horn is added to the procapsid during maturation.
This work was supported by a training fellowship from the National Library of Medicine to the Keck Center for Interdisciplinary Bioscience Training of the Gulf Coast Consortia (NLM Grant No. T15 LM007093).

Structure Poster Number P-4

Zernike Phase Contrast Cryo-EM Reveals the Structure of the Genome Packaging Apparatus in Herpes Simplex Virus

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High-resolution structural studies of large spherical viruses have become a routine procedure using single-particle cryo-electron Microscopy (cryo-EM). One limitation of this technology is in studying the small structural components of viruses that are not icosahedrally organized. While there are several algorithms capable of resolving these non-icosahedral features, many of these protein complexes remain unsolved for very large viruses such as the herpes simplex virus type I (HSV-1).
We have used a Zernike phase contrast electron cryomicroscope to collect images of ice embedded HSV-1 B-capsids. This instrument is equipped with a thin carbon film phase plate that is capable of phase-shifting electrons, scattered by the sample, $\pi/2$ out of phase with the un-scattered beam. The phase plate consists of a 500nm thin carbon film aperture suspended on a rigid support system held in the back focal plane of the objective lens. As the scattered electrons are a quarter-wavelength out of phase, the resulting CTF is transformed from a sine to a cosine curve, resulting in little to no loss of information at low spatial

frequencies and removing the need to correct the CTF of the data.
The HSV-1 virion is an enveloped virus approximately 200nm in diameter comprised of glycoproteins, tegument and a dsDNA filled capsid. The capsid is a T=16 icosahedron 125nm in diameter that is formed by four distinct viral proteins. Using image-processing software recently developed at the NCMI, we asymmetrically reconstructed the phase contrast images of the HSV-1 B capsid, revealing for the first time the genome packaging apparatus (the portal protein) situated beneath one of the capsid's 12 pentameric vertices. These studies demonstrate how recent advances in electron optics have been used to reconstruct the portal complex from within HSV-1, a feature that until now has remained elusive to the cryo-electron microscopist. This poster will outline in detail not only the biological significance of this work but also the practical application of such a device in cryo-EM.

Structure Poster Number P-5

Pushing the Limits of Both NMR and Cryo-Electron Tomography: the Structure of the 42.8 KDA Encapsidation Signal of MoMuLV

Rossi Irobalieva[1], Rossitza Irobalieva[1], Yasuyuki Miyazaki[1], Blanton Tolbert[1], Adjoa Smalls-Mantey[1], Kilali Iyalla[1], Kelsey Loeliger[1], Victoria D'Souza[1], Htet Khant[1], Michael F Schmid[1], Eric Garcia[1], Alice Telesnitsky[1], Wah Chiu[1], Michael F Summers[1]
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Cryo-electron tomography (cryo-ET) is a technique that can reveal the location and low-resolution structures of nanomachines in a variety of 3D cellular environments. So far, cryo-ET has been used primarily to characterize large structures, such as cells, organelles, and can also be used to average multiple instances of viruses or other large protein complexes. Here, for the first time, we apply cryo-ET to study a much smaller and heterogeneous specimen - nucleic acid extracted from Moloney Murine Leukemia Virus (MoMuLV). Proper genome packaging in retroviruses requires a conserved dimeric RNA structure that is organized in a "double hairpin motif" (ΨCD). The hairpins (SL-C and SL-D) are capable of forming "kissing" interactions stabilized by two intermolecular G-C base pairs. We have determined the three-dimensional structure of the double hairpin from the Moloney Murine Leukemia Virus (MoMuLV) ([ΨCD]2, 132-nucleotides, 42.8 kDaltons) using cryoET which confirmed the overall global structure. In addition, we applied a 2H-edited NMR spectroscopy-based approach, which enabled the detection of interactions that were not observed in previous studies of isolated SL-C and SL-D hairpin RNAs using traditional NMR methods. The hairpins participate in intermolecular cross-kissing interactions (SL-C to SL-D' and SLC' to SL-D), and stack in an end-to-end manner that gives rise to an elongated overall shape (ca. 95 Å by 45 Å by 25 Å). [ΨCD]2 is simultaneously the smallest RNA to be structurally characterized to date by cryo-ET and among the largest to be determined by NMR.

Structure Poster Number P-6

PATHWALKER: a Sequence-Free Method for De Novo Backbone Modeling

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Electron cryo-microscopy (cryoEM) is an emerging technology for imaging macromolecular assemblies under near native conditions. Routine structure determination can achieve subnanometer resolutions with many structures approaching near atomic resolutions. Currently, analysis of cryoEM density maps is supplemented by fitting previously determined atomic structures of individual protein or domains. However, even if the structure of a protein component is known, structures of fully assembled components are likely different from those in isolation. Structural information can also be gleaned directly from density maps using feature recognition tools without the need for previously determined structures or structural homologues. At subnanometer resolutions (<10Å) secondary structure elements (SSE), alpha helices and beta sheets, appear as distinct shapes and can be recognized computationally. One method in determining structural information utilizes SSE correspondence, whereby sequence predicted SSEs are matched with SSEs derived directly from the density map. However, this process requires accurate prediction of the secondary structure elements and works best in single domain proteins with multiple alpha helices. This project aims to address these shortcomings and develop new tools and methodologies for computational modeling 3D density maps at subnanometer to near atomic resolutions. We have recently developed a new technique that builds models without sequence correspondences, called Pathwalker. Pseudoatoms, corresponding to the number of atoms in a protein, are semi-automatically placed at regular distances within the density map. Using a modified traveling salesman problem approach, the optimal connectivity of these atoms is scored and an initial model produced. Visual evaluation and iterative refinement of the connectivity was used to construct models for Aquoporin, a subunit for the 30S ribosome, rotavirus VP6 and gp7 of epsilon15. Construction of these models typically took one day, though Pathwalker runs only require a few seconds of computational time. Future studies include adding additional constraints based on allowable angles and preserving previously defined SSE connectivity.

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Structure Poster Number P-7

Structural and Stability Effects of Base Modifications on the Tyrosyl-Anticodon Stem-Loop From Bacillus Subtilis

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Nucleoside base modifications play a critical role in RNA structure and stability. Base modifications are especially important in tRNA molecules since they improve translation fidelity and efficiency. Additionally, base modification to the anticodon stem-loop (ASL) of tRNA molecules may play a significant role in transcriptional regulation through the T-box regulatory system. The Bacillus subtilis tyrosyl-anticodon stem-loop (ASLTyr) has three modified residues. The first is the Queuine modification at position 34, which is added by tRNA-guanine transglycosylase. Residue 37 is hyper-modified by MiaA and MiaB to make (2-thiomethyl)-N6-dimethylallyl (ms2i6A37). Finally, the pseudouridine (Y) modification is added by TruA at position 39. NMR spectroscopy was employed to examine the effects of partial base modification on the Bacillus subtilis ASLTyr structure. NMR data indicates that the unmodified and [Y39]-ASLTyr form a protonated C-A+ Watson-Crick-like base pair instead of a bifurcated C-A+ interaction. Additionally, the loop regions of the unmodified and [Y39]- ASLTyr molecules are well ordered. Interestingly, the [i6A37]- and [i6A37;Y39]-ASLTyr molecules do not form a protonated C-A+ base pair and the bases of the loop region are not well ordered. The NMR data also suggests that the unmodified and partially modified molecules do not adopt the canonical U-turn structure. The structures of the unmodified, [Y39]-, and [i6A37;Y39]-ASLTyr molecules do not depend on the presence of Mg2+, but the structure of the [i6A37]-ASLTyr molecule does depend on the presence of divalent cations. In the absence of divalent cations, differential scanning calorimetry data indicates that the Y39 modification improves the molecular stability of the ASL.

Structure Poster Number P-8

Towards a High Resolution Model of the Phage Phi29 DNA-Packaging Motor

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Bacilllus subtilis bacteriophage phi29 has long been used as a model system to study the process of genome packaging in dsDNA viruses, which package their genomes into a preformed protein shell. This process of genome encapsidation by phi29 and other dsDNA viruses is remarkable considering the electrostatic, bending, and entropic energies that must be surmounted in order to package DNA to near-crystalline densities. To overcome these energetic barriers, phi29 has evolved one of the most powerful molecular motors known in nature, capable of producing forces in excess of 57pN.
The phi29 DNA-packaging motor is a large multimeric complex made up of two proteins and a virally-encoded RNA. The first component, the connector, is a dodecameric protein assembly that forms an open

channel in the preformed capsid. The second component, a virally encoded packaging RNA (pRNA), assembles as a pentameric ring that encases the connector channel and recruits assembly of the final component. This third and final component, a multimeric ATPAse, uses energy from hydrolysis of ATP to drive insertion of genomic DNA into the preformed capsid. High resolution structures exist for the connector (2002) and pRNA (Ke and Grimes, personal communication) components of the phi29 packaging motor. Hence, only the atomic resolution structure of the ATPase component remains unkown. We have thus undertaken the task of obtaining an atomic-resolution structure of a packaging ATPase from the phi29 family of phages. Here, we present preliminary functional and structural studies of a packaging ATPase from the phi29 relative phage asccphi28. Combined results from sedimentation equilibrium analytical ultracentrifugation, small angle X-ray scattering, electron microscopy, and initial X-ray diffraction experiments suggest that the recombinantly-expressed asccphi28 ATPase is active as a multimeric ring structure. Native gel electrophoresis and size-exclusion chromatrography suggest that four different assemblies may be present in solution.

Structure Poster Number P-9

CryoEM At UTMB

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The Laboratory is a unique research and training facility dedicated to studying EM high-resolution structure of proteins, nucleic acids and their complexes as well as host-pathogen interactions, replication, pathogenesis, and NIH Category A-C bioagents and emerging infectious pathogens. This new facility, the first U.S. laboratory of its kind, includes state-of-the-art 200 keV cryo-electron microscope in a biosafety level 3 containment environment to study viruses and other pathogenic microorganisms. Remote imaging capabilities allow researches worldwide to directly access this facility and its instrumentation. This Laboratory provides unique research opportunities to study highly pathogenic viral and bacterial diseases and to train young microbiologists for productive careers developing new vaccines and antimicrobial therapies to combat emerging and reemerging infectious diseases.
The Laboratory serves as an intellectual core drawing together and helping researchers at UTMB, and around the country. The Laboratory promotes extensive and productive collaborations between molecular- and cell-biologists, virologists, microbiologists and electron microscopists to obtain and analyze high resolution structures of infectious agents to understand the mechanism of their infectivity and virulence. The state-of-the -art equipment in the Laboratory allows to study not only infectious agents but also protein-protein-, protein-lipid- or protein-nucleic acid complexes. We also aim at studying cell organelles and even whole cells.
The Laboratory provides user training to perform regular- and cryo-EM.

Structure Poster Number P-10

Optimizing the Anti-Folate Profile of Dihydrophthalazine Compounds Targeting Bacterial Dihydrofolate Reductase

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The infectious burden on human health is exacerbated by the emergence of drug-resistance, necessitating new antimicrobial treatments. We have focused on the antibacterial target dihydrofolate reductase (DHFR) to combat a wide spectrum of human pathogens. Inhibitory compounds were built on diaminopyrimidine and methoxybenzyl ring structures, as in the antibiotic trimethoprim, which target a conserved binding surface on the protein. This framework was coupled to an acryloyl linker and large dihydrophthalazine moiety bearing varied constituents at a chiral center. Results are a collection of inhibitors with broad-spectrum activity against microorganisms including *B. anthracis* and *S. aureus*. We find potent inhibition exemplified by low minimum inhibitory concentrations (MIC's) $\leq$ 16 μg / mL and IC_{50} values in the nM range. Co-crystal structures revealed previously unappreciated details in the volume of bacterial DHFR binding sites and highlighted a unique surface cavity in *S. aureus* DHFR that is susceptible to inhibitor binding. These studies also provide a rationalization for the biochemical activity of separated enantiomers. Unlike the natural substrate or analogs thereof, the phthalazine ring provides a large hydrophobic anchor that embeds within the DHFR active site and increases inhibitory activity relative to trimethoprim. Current details are contributing to future modifications of dihydrophthalazine derivatives, which now have demonstrated anti-microbial performance.

Structure Poster Number P-11

The Structure of Androcam, a Specialized Myosin VI Light Chain

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Despite the structural plasticity, target binding versatility, and tunable Ca^{2+} affinity of calmodulin, metazoans also express many essential calmodulin-like proteins that perform tissue specific functions. We are studying androcam, an essential protein in D.melanogaster that has 67% sequence identity to calmodulin, in order to determine how its structure, Ca^{2+} binding, and target recognition properties differ from those of calmodulin and contribute to its unique function. Our NMR structures solved at high and low calcium show that unlike calmodulin, which switches its conformation with changes in [Ca^{2+}], each lobe of androcam is locked in a single fold over the entire physiological range of [Ca^{2+}]. In the C lobe, androcam is structurally similar to calmodulin and has two EF hands that each coordinate a Ca^{2+} ion. However, the C lobe of androcam binds Ca^{2+} more tightly than calmodulin and is therefore constitutively present in the Ca^{2+} bound "open" conformation. The N

lobe of androcam does not bind Ca^{2+} at physiological concentrations but is nevertheless well structured and constitutively adopts a "closed" conformation similar to Ca^{2+}-free calmodulin. 2D N-HSQC spectra have established differences in the binding modes of calmodulin and androcam to the unique 'Insert2' peptide of myosin VI. Further, the clustering on one face of the N lobe of androcam of residues that differ from calmodulin indicates the role of other binding partners in the interaction of androcam with myosin VI. Our results indicate that the androcam sequence has been optimized by evolution starting from the highly versatile calmodulin sequence to adopt only one of the many conformations that calmodulin can sample. We propose that many other calmodulin-like proteins might have also evolved to be specialists for a unique functional state using subsets of the plethora of conformations that calmodulin is known to populate

Structure Poster Number P-12

Reprogramming the Conformational Regulation of GPCR Signaling

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A fundamental condition that living organisms must address is communication across lipid membranes in which extracellular stimuli is sensed and transmitted to generate appropriate cellular responses. G-protein coupled receptors (GPCRs) are one of the largest families of membrane-embedded receptors that serve this function and do so through long-range allosteric conformational changes. Despite extensive genomic and functional studies on GPCRs, it was only in the past couple of years that new structural data have made accurately evaluating mechanistic and structural changes possible at the molecular level. My project is part of a general effort to uncover at the molecular level the sequence, structure, and energetic determinants governing GPCR signaling. I hypothesize that GPCR signaling is driven by conformational changes that allow them to toggle between functional states; computational designs that stabilize receptors in different states will allow us to reprogram their signaling properties and provide a molecular-level understanding of the underlying conformational regulation.

To address the relationship between protein stability and conformational plasticity in allosteric receptors, we have combined sequence bioinformatics techniques with the design mode of RosettaMembrane to deconstruct the sequence space governing GPCR conformational stability. Sites predicted to be suboptimal for the stability of the resting state GPCR are targeted for design, and mutations that stabilize the receptor's inactive conformation are selected. The mutants are characterized via radioligand binding assays to measure apparent thermal stability. For this study, I will focus on beta-adrenergic receptors, which are extensively studied representative members of the largest class of GPCRs. Thus far, several potentially stabilizing mutations have been computationally identified, and we have begun experimentally validating these designs. Our initial designs of the beta-1-adrenergic receptor (B1AR) have yielded mutants with increased apparent thermostability in comparison to a B1AR variant used in solving the B1AR structure (Warne et al. 2008).

Additionally we are working to modulate and switch the functional states of GPCRs. The goal is to apply our techniques to study GPCRs with unknown structures. With over eight hundred GPCRs in the human genome, many of which have been implicated in disease studies, it is apparent that there is a critical need for atomic-resolution structural and mechanistic understanding of their signaling. The bridging of computational molecular biophysics and molecular biology in the GPCR research field is rapidly developing and will contribute to the basic scientific knowledge of receptor signaling as well as provide a means to develop better

molecular mechanisms must be different, since some of the helical GAG residues are structured in the dimer and unstructured in the monomer; and (vi) GAG-binding and receptor-binding interfaces overlap, and so GAG-bound chemokine cannot bind and activate the receptor.
Our observations are novel, and has provided critical insights into the structural basis and molecular mechanisms by which IL-8 monomers and dimers interactions with GAGs could regulate in vivo neutrophil recruitment.

Structure Poster Number P-16

A Structural Comparison of a 3D- Model of the Peanut Allergen Ara h 2 and the Recently Released X-Ray Crystal Structure

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Understanding the molecular characteristics of allergenic proteins is important for guiding the treatment of patients, for reducing allergens in our food and air, and eventually for designing hypoallergenic proteins for use in foods and immunotherapy. Currently we have gene/protein sequence information for more than 1000 protein allergens, however experimentally determined 3D structures are known for about 70 allergens. We have shown previously that a large fraction of all allergens with no experimental 3D structure can be reliably modelled with current 3D prediction tools [1] and we made those 3D models publicly available in our SDAP web server[2].
With the recently determined X-ray crystal structure of the peanut allergen Ara h 2 [3](PDB code 3OB4) we can now assess the quality of our 3D model for that allergen. The template structure used in the 3D modeling, RicC3, a 2S albumin protein from the seeds of castor beans, has a sequence identity of only 22% to Ara h 2 indicating a challenging modeling project. Both sets of 3D co-ordinates have missing residues, the X-ray crystal structure is lacking15 continuous residues in a large loop region probably due to enhanced disorder in the crystal lattice, and a segment of 8 residues are not determined in our 3D model, as these residues could not be aligned to the template.
Ara h 2 protein is a four helix bundle, and the helix packing of the four helices is identical in the 3D model and the X-ray crystal structure. The alignment of our 3D model with the X-ray crystal structure using the pairwise structural alignment program DaliLite yields a backbone RMSD of 3.1 Angstroms and a Z-Score of 3.7. Major structural differences are seen in the conformation of the kinked helix 1, and the loop regions between helices. The presentation will describe details of the structural comparison between the template, model and crystal structure as well as comparisons to other structurally similar proteins.

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Structure Poster Number P-17

Developing "Structure Space" Parameters Using Point Mutation Data

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The "structure space" of an RNA or protein molecule is defined as all the functional (valid) primary sequence variations that exist, or could exist, for that molecule. Provided a difference in primary sequence does not interfere with the structural or functional properties of the molecule; these variations have the potential to add to the fitness of the organism and, if fully incorporated over time, provide a pathway through which the molecule and, hence, organism may evolve. Understanding the nature of real structure spaces could provide significant insight to how evolution occurs at the molecular level. As a result, a number of studies have been undertaken using small RNAs as model molecules. However, these studies only consider Watson-Crick secondary structure and ignore tertiary interactions, and interactions with other molecules, altogether. A more realistic model system is required in order to make further progress.
To this end, the Fox laboratory has sought to collect the data needed to obtain a realistic model of a local region of the RNA structure space centered on the *Vibrio proteolyticus* 5S ribosomal RNA sequence. It was hypothesized that knowledge of the effect of point mutations at every position in the molecule could be used, in combination with tertiary structure data and comparisons with related sequences, to generate a realistic model sequence space. To this end, over 200 point mutations and over 400 multiple mutations of the *V. proteolyticus* sequence have been made and their effect on function characterized.
It is expected that individual variants will function in the same manner in closely related sequences. That is to say, when *V. proteolyticus* is compared with a closely related sequence, one can expect that all the individual differences seen in the second sequence would be functional, if introduced in *V. proteolyticus* one at a time. The obvious exception would be paired positions that likely will need to be considered as a double mutation unit. The key question is the definition of closely related; e.g. how far away in the sequence space can the second sequence be with the prediction largely holding true.
To address this, the available *Vibrio* 5s rRNA sequences were compared to the *V. proteolyticus* primary sequence. The differences between *V. proteolyticus* and other *Vibrio* sequences can essentially be looked at as point mutations in the *V. proteolyticus* sequence. This information was then cross-referenced with data from earlier, in vivo, point mutational studies done with *V. proteolyticus*, in order to determine how useful point mutational data is in predicting functional variants within this species. In general, the sequences with fewer variations contained a higher percentage of functional variants. Sequences with a larger number of variations contained a higher percentage of non-functional or undetermined variants. While further validation using the in vivo studies is needed, with respect to the undetermined variants, these initial results suggest that point mutational data may be useful in predicting functional and non-functional variants of the *V. proteolyticus* sequence, containing as many as 9-10 changes, with very high reliability.
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Structure Poster Number P-18

Membrane-Bound Organization of Coagulation Factor VIII: Impilcation for the Tenase Complex Assembly and Function

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The focus of this study is to follow the structural changes of two human recombinant Factor VIII (FVIII) forms: full length (FVIII-FL) and B domain deleted (FVIII-BDD) upon binding to phosphatidylserine (PS) containing phospholipid (PL) vesicles and nanotubes (LNT) by combining Cryo-electron microscopy (Cryo-EM), circular dichroism (CD) and Molecular Modeling studies. FVIII or antihemophilic factor is a multidomain plasma glycoprotein of 170 - 280 kDa, essential for blood coagulation. Activated FVIII (FVIIIa) is a cofactor to the serine protease Factor IXa (FIXa) in the Tenase complex assembled onto the negatively charged platelet surface. Despite the fact that Human recombinant FVIII (both BDD and FL) has been successfully employed as a drug against Hemophilia type A for the last 10 years, little is known about its conformation and action mechanism at the membrane-surface. Cryo-EM is unique in its ability to visualize the structure of macromolecules within an ensemble at subnanometer resolution and closest to physiological conditions. Our Cryo-EM data show strong protein-membrane and protein-protein interactions, induced upon membrane-binding and leading to fast aggregation of the membrane-bound FVIII-BDD form. No significant changes in the Far-UV CD spectra of FVIII have been observed upon binding of the protein to PL vesicles. However, a difference in the CD spectra in the 220 – 190 nm region has been observed between the FL and BDD form due to the presence of parts of the B domain in the heavy chain of the molecule. We are currently calculating an intermediate resolution structure of membrane-bound FVIII to LNT at 1.5 – 1.7 nm resolution. Our preliminary Cryo-EM and Cryo-electron tomography (Cryo-ET) data further support our hypothesis that the existing FVIII-BDD X-ray structure in solution is altered upon binding to the platelet membrane to form the Tenase complex with FIXa. Defining the membrane-bound structure of FVIII and its interaction with FIXa at the atomic level will help us design novel recombinant FVIII molecules capable to enhance or reduce the proteolytic activity of FIXa; thus altering the rate of Thrombin generation and hemostasis.
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Structure Poster Number P-19

Crystal Structure of N^{Pro}: a Novel Cysteine Auto-Protease in Classical Swine Fever Virus

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Classical swine fever virus (CSFV) is a single-strand plus-sense RNA virus that belongs to the genus pestivirus within the *Flaviviridae* family. CSFV N-terminal protease N^{pro} is essential for viral polyprotein processing and plays a crucial role in subverting the host immune system. N^{pro} is a cysteine protease that cleaves itself off from the capsid protein C, thus enabling further polyprotein processing. After initial cleavage, N^{pro} is no longer active as a protease and no trans-activity has been observed. Cleaved N^{pro} also suppresses the host's type-1 interferon (IFN) α/β-mediated apoptosis, the main innate immune response against viral infection. N^{pro} binds interferon regulatory factor-3 (IRF3), an activator of the IFN α/β pathway, and targets it for proteasomal degradation. Although the protease activity itself is not essential for binding IRF3, mutations in either the N or C-terminal regions of the protein disrupt this interaction.
We report the first crystal structure of CSFV N^{pro} determined to 1.5Å resolution. N^{pro} consists of two domains, a cysteine protease domain and a zinc-binding domain. The cysteine protease domain has a new protein fold that is different from other known proteases. The C-terminus is not only bound in the active site, but also contributes a strand to the beta-sheet that makes up the active site. Thus, this structure explains the auto-catalytic, and subsequent auto-inhibition mechanism of N^{pro} (i.e., why there is no trans-activity), and provides insight into its role in subversion of host immune response, including the interaction between N^{pro} and IRF3.

Structure Poster Number P-20

Flagellar Stator Revealed in Situ

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The bacterial motility and its driving force, the flagellar motor, are important virulence factors of Lyme disease spirochete Borrelia burgdorferi and many other pathogens. Powered by the proton gradient across the cytoplasmic membrane, the flagellar motor converts electrochemical energy into torque through an interaction between a central rotor and a surrounding stator complex that is composed of MotA and MotB. The coupling of the proton gradient to mechanical rotation is one of the most fascinating features of this molecular machine, yet the mechanism remains elusive at the molecular level, mainly because the lack of structural information about the stator and its interaction with the rotor during flagellar rotation.
Recently, a high-throughput cryo-electron tomography system was utilized to determine the 3-D structure of the intact flagellar motor in living cells at a level of detail that has not been observed previously. To further

characterize the stator and its interaction with the rotor, a motB mutant was constructed by a newly developed non-polar gene inactivation system. The motB mutant synthesizes periplasmic flagella but is paralyzed and lacks the density corresponding to the MotA/B complex. The motility is restored after successful complementation in trans. The three-dimensional structure of the stator was revealed for the first time based on the comparative analysis of flagellar motor structures from WT, motB mutant and motB complemented cells. Sixteen MotA/MotB complexes form a stator ring assembled around the rotor. The absence of the stator complexes in motB mutant cells results in an apparent rearrangement of the densities corresponding to the location of FliG. This unexpected conformational change indicates that stator-rotor interactions have profound structure effects on C ring. The study here provides novel structural insights into the fundamental mechanism of flagellar rotation and bacterial motility.

Structure Poster Number P-21

Molecular Architecture of Intact Flagellar Motor and Chemotaxis Array Revealed By Cryo-Electron Tomography of Motile and Tiny *Escherichia coli*

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Motility and chemotaxis are vital functions for many bacteria. Although different swimming styles exist, most are based on the rotation of bacterial flagella. The direction (CCW/CW) of which is controlled by chemotaxis arrays that sense environmental signals. This fundamental system enables bacteria to swim towards the optimum environment for growth and survival. E. coli has been a pre-eminent model system to study both the flagellar assembly and the chemotactic signaling pathway. The proteins responsible for flagellar assembly and chemotactic signaling have been identified and extensively characterized. However, the 3-D structures of the basic signaling unit (chemotaxis receptor) and the torque generator (stator), which are essential for understanding the molecular mechanism of the signal transduction and the flagellar rotation, remain elusive.

In this study, we constructed a mreB mutant of E. coli with cell diameter as small as 0.2μm. Additional inactivation of clpX and overexpression of flhD/flhC genes produced highly motile cells with multiple flagellar motors and chemotaxis receptor arrays embedded in the cytoplasmic membrane. This tiny E. coli organism is an ideal model system for in situ structural studies of the ternary receptor signaling complex, as well as the flagellar motor by using high throughput cryo-electron tomography (cryo-ET). Cryo-ET and image analysis of those tiny cells resulted in high resolution three-dimensional (3D) structures of the intact E. coli flagellar motor and the chemotaxis receptor array for the first time, providing the clearest avenue yet available to understanding of the molecular mechanism of the fundamental biological processes: flagellar rotation and chemotaxis signaling.

Structure Poster Number P-22

Structural and Biochemical Studies of the Novel Lipoprotein Cj0090 From *Campylobacter Jejuni*

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Campylobacter jejuni, a Gram-negative motile bacterium, is an important food-borne pathogen causing human gastrointestinal infections worldwide. The mechanism of *C. jejuni* mediated enteritis is poorly understood and appears to be multifactorial. Lipoproteins are universal components of bacterial membranes and of great interest in understanding molecular pathogenesis of *C. jejuni*. Previously, bioinformatics analyses predicted about 30 putative lipoproteins in the *C. jejuni* genome sequence. Interestingly, most of these lipoproteins have no sequence similarity to other known proteins in the database. To discover novel structural and biochemical properties of *C. jejuni* lipoproteins, we have initiated a structural lipoproteomics project. One of our targets, Cj0090, is a small protein (~14 kDa) and contains a Cys residue and the lipobox motif [-Leu(Ala/Val)$_{-4}$-Leu$_{-3}$-Ala(Ser)$_{-2}$-Gly(Ala)$_{-1}$-Cys$_{+1}$-], indicating a lipoprotein. We first showed that Cj0090 is indeed translocated to the outer membrane and secreted mostly to the extracellular space. To decipher the structure-function relationship of Cj0090, we set out to solve the structure of Cj0090. The Cj0090 crystals belonged to space group $P4_12_12_1$ with cell dimension a=b=51.1Å and c=186.5 Å. Crystals contained 2 molecules per asymmetric unit and diffracted to a resolution of 1.9 Å at the 19ID beamline of the Structural Biology Center at APS. The structure was solved by SAD phasing using a Br soaked Cj0090 crystal. Model building and refinement are in progress. In addition, using phage display, we are investigating novel peptide ligands that specifically interact with Cj0090. Our findings will provide framework for new strategies for the rational design of small molecule inhibitors efficiently targeting Cj0090.

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Structure Poster Number P-23

The Many Conformations of Epac2: a Cyclic-AMP Sensing Cellular Regulator Studied via Solution X-Ray Scattering (SAXS) & Hydrogen Deuterium Exchange Mass Spectrometry

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Epac2 is a guanine nucleotide exchange factor which directly activated by cAMP. According to the model of Epac activation, a localized "hinge" motion is a major change in the Epac structure upon cAMP binding. In this study, we test the functional importance of hinge bending for Epac activation by targeted mutagenesis. We show that substitution of the conserved residue phenylalanine 435 by glycine facilitates the hinge bending. As a result, Epac2-F435G mutant is constitutively active and stimulates nucleotide exchange in the absence of cAMP. In contrast, substitution of the same residue with a bulkier side chain, tryptophan, impedes the hinge motion and results in a dramatic decrease in Epac2 catalytic activity. Structural parameters for wild type Epac and two of its mutants determined by small-angle X-ray scattering (SAXS) further confirms the importance of hinge motion in Epac activation. In addition, peptide amide hydrogen/deuterium exchange mass spectrometry (DXMS) was used to probe the solution structural and conformational dynamics of full length Epac2 in the presence and absence of cAMP. Our study also suggests that the side-chain size of the amino acid at the position 435 is a key to Epac functioning. It seems that phenylalanine at this position has the optimal size to prevent "hinge" bending and keep Epac closed and inactive in the absence of cAMP while still allowing the proper hinge motion for full Epac activation in the presence of cAMP. The DXMS results also support this mechanism in which cAMP-induced Epac2 activation is mediated by a major hinge motion centered on the C-terminus of the second cAMP binding domain. This conformational change realigns the regulatory components of Epac2 away from the catalytic core making the later available for effector binding. Furthermore, the interface between the first and second cAMP binding domains is found to be highly dynamic, providing an explanation of how cAMP gains access to the ligand binding sites, that in the crystal structure, are seen to be mutually occluded by the opposing cAMP binding domain. Moreover, cAMP induced conformational changes are observed at the ionic latch/hairpin structure, which is directly involved in RAP1 binding. These results suggest that in addition to relieving the steric hindrance imposed upon the catalytic lobe by the regulatory lobe, cAMP may also be an allosteric modulator directly affecting the interaction between Epac2 and RAP1. Finally, cAMP binding also induces significant conformational changes in the Dishevelled/Egl/Pleckstrin (DEP) domain, a conserved structural motif that, while missing from the active Epac2 crystal structure, is important for Epac subcellular targeting and *in vivo* functions.

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Structure Poster Number P-24

The Mechanism of DNA Ejection in the Bacillus Anthracis Spore-Binding Phage 8a Revealed By Cryo-Electron Tomography

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The three-dimensional structure of the recently discovered Bacillus anthracis spore-binding phage 8a (SBP8a) has been determined by cryo-electron tomography (cryo-ET). The resulting reconstruction indicates that the capsomer-capsomer distances on the phage head surface are consistent with an icosahedral lattice constructed from protein subunits utilizing the HK97 capsid protein fold. A contractile tail is attached to the head via a head-tail connector, or portal protein, whose cone-like shape suggests structural homology with other phage portal proteins. The tail consists of a six-start helical sheath that surrounds the central non-contractile tail tube, and a baseplate at the distal end of the tail that recognizes and attaches to surface receptors on the outer membrane of Bacillus anthracis host cells. Analysis of 2518 intact phage particles indicate four distinct conformations of the phage are present that may correspond to four sequential states of the DNA ejection process during host cell infection. Comparison of the four observed conformations suggest the following mechanism for DNA ejection: 1) recognition of the host cell receptor causes a reorganization of the protein subunits in the baseplate from a closed, "fist"-like structure to a more open structure; 2) this rearrangement triggers tail sheath contraction, which drives the tail tube through the cell envelope and initiates injection of the phage genome into the host cell; 3) contraction of tail sheath also triggers a "gate" in the neck protein between the head and the tail to "open", allowing DNA to exit the head and travel through the tail tube en route to the host cell cytoplasm. This is the first detailed analysis of the bacteriophage DNA ejection process using high-throughput cryo-ET.

Structure Poster Number P-25

Structural Study of the Interaction Between the Methyltransferase and the Polymerase Domains of Dengue Virus NS5

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Many members of the Flavivirus are important human pathogens, including four serotypes of Dengue virus (DV-1 to DV-4). DV NS5 (Non Structural protein 5) is a two domain protein that plays a key role in viral replication; NS5 contains activities required for capping and synthesis of the viral RNA genome. The N-

terminal domain of NS5 is an S-adenosyl-L-methionine-dependent methyltransferase (MTase), responsible for the two methyltransferase activities need for the synthesis of a type-1 cap-structure ($^{7Me}GpppA_{2'OMe}$). The C-terminal domain is an RNA-dependent RNA polymerase (RdRp), and is the central domain of the replication complex. Because of these two essential activities in the replication process, NS5 is an attractive target for antiviral drugs. Although the crystal structures of the two separate MTase and RdRp domains have been solved, the full-length NS5 polymerase structure has not been determined, and thus the relative orientation of the two domains in NS5 is still unknown The aim of this study is to obtain structural information regarding the full-length NS5 including the potential contact surface between the MTase and RdRp domains. We carried out the first structural analysis of NS5 in solution, using Small Angle X-ray Scattering (SAXS) in combination with X-ray crystallography.

Biochemistry Poster Number P-26

Structural Plasticity Within the Postsynaptic Density

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The postsynaptic density (PSD) is a large membrane-associated protein complex which works to cluster neurotransmitter receptors at the synapse and organize the intracellular signaling molecules needed for synaptic transmission. Little is known about the structure and composition of PSDs isolated from different regions of the brain. It is likely that different brain regions may require different synaptic transmission and the structure and protein composition of the PSD may change in order to achieve the required synaptic transmission for each region. In order to understand how the PSD may allow for differential synaptic transmission, PSDs were isolated from rat cerebellum, hippocampus and cortex. Cryo-tomography and immunogold labeling are currently being utilized to examine the composition and structure of these cytoskeletal specializations. The labeling densities for PSD-95, αCaMKII, and βCaMKII differed dramatically among the PSD types, indicating that PSD protein levels vary between the different regions of the brain. The range of surface areas also differed between the different PSDs types. Cerebellar PSDs were found to have the widest range of surface areas, with cortical PSDs having the smallest range. The protein composition of cerebellar PSDs was also found to be highly variable unlike the protein composition of hippocampal and cortical PSDs. Hippocampal and cerebellar PSDs were found to have similar labeling densities for PSD-95, an important scaffold protein, yet cerebellar PSDs were found to have clusters of PSD-95, unlike the other PSD types. Cortical and hippocampal PSDs had similar labeling densities for αCaMKII, while cerebellar PSDs had notably low labeling for the protein. All three regions were found to have significantly different labeling densities for βCaMKII, and all regions had moderate to high labeling for βCaMKII, illustrating the importance of βCaMKII to the PSD structure. These results support the idea that the composition and structure of the PSD change with brain region, possibly to achieve the specific synaptic transmission required to complete each of the numerous tasks required of the brain.

Biochemistry Poster Number P-27

High Throughput Analysis of Estrogen Receptor-Alpha Identifies Novel Factors Influencing Gene Regulation

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Estrogen receptor-alpha (ER) is a type I nuclear receptor important in normal reproductive development and diseases such as breast cancer and osteoporosis. Ligand-activated ER can regulate gene expression through binding of estrogen response elements primarily located upstream of target genes. To visualize the mechanisms involved in ER mediated gene regulation, we have created and validated a microscopy-based approach to quantify coregulator recruitment to an ER-occupied promoter locus (Sharp et al, 2006, J Cell Sci; Berno et al, 2008, PloSOne; Ashcroft et al, 2011, Gene, in press) as part of a platform that is amenable to high content analysis and screening (HCA/HCS). This system exploits an engineered dual stable cell line harboring a microscopically-visible, multicopy integration of the ER-regulated prolactin promoter array and GFP-ER in HeLa (PRL-HeLa). The goal of the current project is to identify the full spectrum of ER coregulators in order to create a more complete picture of ER-regulated transcription in a cellular context. Further, we have performed an antibody screen of >1000 transcriptionally relevant antibodies by HCA, and have identified members of the mediator complex and other basal transcription machinery that target the PRL array following treatment to estradiol (10-8M), tamoxifen (10-8M) or bisphenol A (10-6M). In parallel, we have also performed GFP-ER immunoprecipitation in estradiol-treated (10-8M) PRL-HeLa extracts followed by mass spectrometry (IP-MS) to identify ER interactors. IP-MS identified known and novel interactors including SRC-3 and sirtuin, respectively. These screening methods coupled with a siRNA screen to look for functional consequences of loss of coregulators or nuclear receptors have identified multiple novel factors impacting the PRL array or ER stability. We have identified helicases, ubiquitination enzymes, and nuclear receptors as possible regulators of ER-regulated transcription. Future studies centering on ER ligands and endocrine disruptors will utilize HCA to differentially classify effectors of ER function at a mechanistic level whereby ER or CoR functions are regulated through direct or indirect interactions and/or post-translational modifications.

Biochemistry Poster Number P-28

Beta 2-Adrenergic Receptor Elicits a Calcium Signal in HEK-293 Cells

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G-protein coupled receptors (GPCRs) are encoded by more than 800 genes of the human genome. These receptors account for approximately 40% of current drug targets and are the most abundant family of transmembrane signaling proteins. GPCRs are involved in many cell signaling processes, among which is the regulation of intracellular calcium levels. Changes in the concentration and distribution of calcium ions

play an important role in regulating all aspects of cell function. Monitoring of intracellular calcium levels in real time using a fluorescent indicator dye revealed that an endogenous receptor in HEK-293 cells responds to the adrenergic agonist norepinephrine, by a delayed rise in intracellular [Ca2+]. Pharmacological analysis revealed that the response is blocked by propanolol, an antagonist selective for beta-2 adrenergic receptor (β2 -AR), and that epinephrine is more potent than norepinephrine, consistent with β2-AR being the receptor responsible. Treatment with thapsigargin, an inhibitor of the SERCA Ca2+ pump of the endoplasmic reticulum, or with extracellular EGTA, revealed that the source of the Ca2+ is release from intracellular stores, not entry from outside the cell. These results reveal a novel pathway by which β2-AR, classically thought to signal primarily through cAMP production, can trigger release of Ca2+ from intracellular stores. Experiments in progress are directed at determining the molecular mechanisms by which this response occurs, and the biophysical properties of the underlying protein-protein, and small-molecule-protein interactions that mediate it.

Biochemistry Poster Number P-29

MIRFOCUS for Proteomic and miRNAome Integral Profiling Analysis

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miRNAs have now been accepted as signatures of human diseases, especially cancers, which remain as a major thread for human vitality. In protein and miRNA profiling studies of cancer samples, our interests reside in pathway-guided analyses in these, establishing the models for decoding the complexity of cancer cellular biology. Abundant information exists linking specific miRNA with a particular cancer or a subtype of a cancer. We will describe our method of utilizing pathway analysis for identification of specific miRNAs as reporters of tumorgenesis pathways; we will experimentally demonstrate the mirfocus findings. The method has great potential in defining new molecular diagnostic (MDx) panels for population-based preventive examination of cancers.

Biochemistry Poster Number P-30

Immunogenicity of a PCP-Consensus Dengue Envelope Domain III Protein Representing All Four Types

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Dengue viruses 1-4 are a major public health threat, with an estimated 50-100 million infections and tens of thousands of deaths annually. Despite this large global disease burden, there is no approved vaccine for any of these viruses. One major concern when designing a dengue vaccine is the need to elicit high levels of

neutralizing antibodies against all four viruses to avoid the possibility of antibody dependent enhancement. Subunit vaccines based on domain III of the envelope protein (EIII) are appealing because this protein is the target of many highly specific, strongly neutralizing antibodies. A physicochemical property (PCP-) consensus EIII sequence (7P8), representing all four dengue viruses, was designed using over 600 unique sequences selected from the Flavitrack database, expressed in E. coli, and purified. This 7P8 protein was reactive with mouse immune ascites fluid raised against representative strains of all four dengue viruses. Mouse sera raised against 7P8 was able to bind to EIII proteins representing all four dengue viruses using an ELISA test and Western Blotting. The anti-7P8 sera was also able to inhibit the growth of all four Dengue virus types, in varying degrees, in a PRNT assay. Multiple dosing strategies are under way involving mice and rabbits. This strategy shows promise for designing a single protein capable of protecting against all four dengue viruses.

Biochemistry Poster Number P-31

Modeling of Small Peptide Fragments as Inhibitors of the FGF14:Nav Channel Complex Formation

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The pore-forming alpha subunit of the voltage-gated Na+ (Nav) channels provides the basis for electrical excitability in the brain. Dysfunction of specific Nav channels is linked to a plethora of excitability-driven human disorders that mostly lack effective therapeutic options. Current medications targeting Nav channels are designed toward highly conserved channel domains and, as such, lack specificity. Of the intracellular fibroblast growth factors (FGF11-14), FGF14 is the most potent and specific regulator of the Nav channel function. Through a monomeric interaction with the intracellular C-terminal tail of Nav channel alpha subunits, FGF14 acts as multivalent chaperone molecule promoting gating, stability and trafficking of native neuronal Nav channels. The rich FGF14:Nav channel interface provides a novel opportunity for drug development against Nav channelopathies.

We have aligned the FGF14 model with the crystal structure of the FGF13 to define the FGF14:Nav channel interface. Three model-based peptide fragments, Fpep1, Epep1 and Ppep1, that best matched the interface, were selected as potential candidate disrupters of the FGF14:Nav channel complex. The efficacy of the synthesized peptides in reducing the FGF14:Nav channel complex formation was evaluated using the split-luciferase complementation assay and co-immunoprecipitation. Our results indicate that Fpep1 inhibits significantly the association of FGF14 with the Nav channel. Molecular modeling revealed that Fpep1 overlays with amino acid residues critical for FGF14 binding to Nav channel. Further docking analysis will be employed to optimize potency and selectivity of this fragment as a platform for probe development and drug discovery against neuronal Nav channels.

Biochemistry Poster Number P-32

Investigating the Role of Naturally Occurring Variations in Ribosomal Elongation Rates

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Most proteins must adopt a folded structure in order to be functional, and the information necessary for this to occur is included in the amino acid sequence. However, the directional nature of the translation process temporally and spatially separates the N terminus from the more C terminal residues so the entire sequence is not available for folding until the polypeptide had completely emerged from the ribosome, rendering segments of incomplete polypeptide chains susceptible to misfolding. Ribosomal movement along mRNA is not uniform and is influenced by the codon content of the message, the physical-chemical nature of the codon -anticodon interaction, and the tRNA availability of the organism expressing that message. Recent experiments in our lab indicate that local folding events and polypeptide elongation rates are coupled, and Wobble-based substitutions can affect both of these processes. We are currently investigating whether a variety of correlations found among regions of naturally occurring variations in ribosomal elongation rates affect folding of nascent chain segments.

Biophysics Poster Number P-33

The Effect of Electromagnetic Properties on the Rotation of Fo-ATP Synthase

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The electromagnetic properties of Fo, a subunit of rotary motor protein complex ATP synthase, were investigated by a combined approach of molecular dynamics simulation and semi-empirical quantum chemistry calculations. A quantitative analysis of the electric field, which is hypothesized to scale with the number of c-ring proton binding sites and the proton motive force, was performed to characterize the necessary torque that rotates the FoF1-ATP synthase. This force, acting on the activation site on the Fo, is driven by the potential difference between the two conducting half channels through a dipolar field on the a-subunit. Semi-empirical quantum calculations were applied to characterize the charge distribution of the protonated and deprotonated states of the Fo subunit, followed by the calculation of electric potentials by solving the Poisson-Boltzmann equation. We determined the electrostatic potential and charge distribution at a residue level dependent on the dielectric constants across the membrane. The results, revealing changes in electrostatic potential along cross sections of Fo due to asymmetrically protonated half-channels in the a-subunit, underpin the driving forces acting on Fo.

Biophysics Poster Number P-34

Determination of Tether Threshold Forces, Interlayer Viscosity, and Thermal Area Expansivity Using a Magnetic Force Transducer

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Membrane tether experiments, which involve applying point forces to membranes to form thin tubes of the membrane, provide a powerful method to drastically alter membrane curvature and study membrane mechanics. To study the formation of membrane tethers, we have designed and built a magnetic force transducer (MFT) using microfabrication techniques. Our initial results indicate our ability to control the amplitude of the applied force up to 20 pN over a distance of 50 microns. We have used this device to precisely measure the tether formation force under dynamic loading conditions. Tether formation forces from microaspirated POPC giant unilamellar vesicles (GUVs) containing 0.5-5 mol% biotinylated lipid were found to range from 2 pN up to 19 pN, depending on the concentration of biotin. During the tether retraction phase the applied force is zero and a mechanical model can be applied to calculate the coefficient of interlayer viscosity that opposes relative motion between the inner and outer leaflets of the GUVs. Our results confirm earlier measurements that a significant portion of energy involved in dynamic tether formation and retraction goes into viscous loss. Finally, we noticed that activation of the MFT induced areal strain in the vesicles and studied this at different levels of current. By comparing these measurements to a thermal conduction model of the system, we determined the thermal area expansivity of SOPC GUVs to be 1.7x10-3/˚C in the temperature range of 22 to 50˚C.

Biophysics Poster Number P-35

The Achievements of the UTMB SCSB NMR Facility

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Nuclear magnetic resonance (NMR) spectroscopy is a powerful tool in the studies of Structural Biology, Protemics, Metabolomics, Nutrition, Toxcology, Clinical Research Solutions, Functional Genomics, Structure/Activity Relationship, Drug Discovery and Development, Macromolecule Analysis and Small Molecule Analysis through a number of methods, including relaxation measurements, chemical shift mapping, hydrogen exchange, conformational exchange, transfer NOE, Saturation Transfer Difference(STD), dynamics and 3D solution structure determination. The SCSB NMR instrumentation includes the state-of-the -art high field Varian VNMR system 800MHz (with a HCN Cold Probe), VNMR system 750MHz and Inova 600 MHz NMR spectrometer with 31P NMR capability. Both of the 800 and 750 MHz instruments are capable of HCN triple-resonance experiments with 2H decoupling. The core facility offers instrumentation,

training, software and assistance in designing experiments. The research though NMR faciliy is focusing on NMR methods, structure determination, protein dynamics and function, and metabolomics.

Biophysics Poster Number P-36

The SCSB Solution Biophysics Laboratory

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The Solution Biophysics Laboratory (SBL), located in rooms 5.148 and 5.150 on the fifth floor of the Medical Research Building, was established in 1995. The purpose of the lab is to enhance solution biophysics research of SCSB core faculty members and UTMB collaborators. Instruction is given in the use of a variety of SBL instrumentation, including: analytical ultracentrifugation, fluorescence, MALDI-TOF mass spectrometry, surface plasmon resonance, titration micro-calorimetry, and high throughput screening using thermal denaturation curves.

Biophysics Poster Number P-37

Counterion Condensation Around Thioated Oligonucleotides

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In the past decade oligonucleotide-based ligands (aptamers) have become increasingly important tools in targeted therapeutic delivery and nanovectorization. With their large size and diverse chemistry aptamers are ammenable to conjugation and provide researchers an alternative approach in scaffold-based drug discovery platforms. Aptamers can be developed towards any target through in-vitro selection processes and new sequences targeting biological molecules are being produced at a rapid pace. Although their oligonucleotide-character makes aptamers susceptible to human serum and cellular nucleases, several methods for subverting these defense mechanisms and enhancing bioavailability are described. Our lab focuses on the backbone chemistry of aptamers to reduce nuclease cleavage through modifications incorporating sulfur atoms at non-bridging phosphoryl oxygen positions in the phosphate backbone. Although enhancing bioavailability was the motive, we and others have demonstrated that oligonucleotides containing sulfur substitutions (phosphoro -mono/di-thioates) show higher binding affinities to protein than unmodified oligos containing normal phosphate backbones. Importantly, the position and quantity of backbone thioation is critical for specificity and we focus on the substitution's effect on the steps involved in molecular association of protein-DNA complexes. Since DNA is a highly-charged anion, counterion release and redistribution of the counterion atmosphere around oligonucleotides in solution is a critical energetic barrier during molecular recognition.

Here we present information on the counterion atmosphere around thioated and normal DNA duplexes probed by inductively-coupled plasma atomic emission spectroscopy (ICP-AES) in monovalent and divalent cation mixtures. We compare these findings to classical electrostatic counterion condensation theory and discuss how the counterion atmosphere affects aptamer design.

Biophysics Poster Number P-38

Prestin Self-Association Detected By FLIM-FRET Techniques

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The mammalian sense of hearing possesses a high degree of sensitivity and selectivity that is provided by active mechanical amplification of vibrations within the cochlea. The transmembrane protein prestin forms an integral piece of this process by providing the driving force for the electromotility of the outer hair cell, a specialized cell that drives the cochlear amplifier. It has been observed that the prestin molecule forms oligomers of several sizes, but the molecular scale activity of these complexes within the context of cochlear electromotility is largely unknown. Previous work using Forster resonance energy transfer (FRET) in our lab assayed the degree of prestin self-association via two similar methodologies that reported positive, but inconsistent, data. Forster resonance energy transfer (FRET) is a nanometer scale phenomenon that allows one fluorophore to transfer energy to another, with an efficiency that depends upon distance. Here we expand upon this work by utilizing fluorescence lifetime imaging (FLIM) detected by time correlated single photon counting (TCSPC), the most accurate FRET measurement technique available. FLIM techniques measure the characteristic fluorescence decay profiles of reporters; changes in this characteristic profile can be caused by FRET activity. Human embryonic kidney cells are used to host prestin molecules that are genetically encoded with either cyan fluorescent protein (CFP) or yellow fluorescent protein (YFP), a standard FRET pair.

Biophysics Poster Number P-39

Investigation of T7 ATP Dependant Ligase By NMR and Fluorescence Spectroscopy

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Many cancer and neurodegenerative diseases are related to DNA damage. Under normal circumstances damaged DNA is repaired by highly specialized DNA repair enzymes, one of which is DNA Ligase. Ligases finds broken phosphodiester bonds in DNA and seal the nick through phosphoril transfer reaction. DNA Ligases are able to locate and repair these sites efficiently, even though the concentration of nonspecific sites is much higher than that of specific (nicked). A Logical question is how the enzyme manages to perform the specific target search so effectively. One of the most important parts in nick sealing mechanism is the process of stochastic protein-DNA interaction through nonspecific binding. In this work we investigated binding

affinity of typical ATP dependant DNA Ligase (T7 Ligase) under wide range of conditions by means of NMR and fluorescence spectroscopy. Specifically we measured the binding affinities of adenilated or apo T7 DNA Ligase to 25pb nicked or sealed DNA, and found that binding of adenilated protein to nicked DNA is only 5-10 fold higher than in other cases. We also investigated stability and effect of divalent ion on the adenilated state of the enzyme; in conclusion we propose a possible passway and mechanism of efficient nick sealing by T7 DNA Ligase.

Biophysics Poster Number P-40

NMR Studies of Kinetics of Protein / Cellulose Interactions: Insights into Direct Transfer

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In order to increase production and efficiency of biofuels from biomass, understanding the kinetics of interactions between cellulase (enzyme hydrolyzing cellulose) and cellulose is important. Typically, a cellulase protein contains a catalytic domain and a cellulose binding domain (CBD). The CBD plays an important role to keep the enzyme in close proximity from the substrate. For efficient hydrolysis by cellulase, CBDs should be able to rapidly change its location on cellulose molecules. Here we studied direct transfer in the protein / cellulose interactions involving the CBDs from Cellulomonas fimi cellulase 9B (Cel9B) and Clostridium cellulovorrans cellulase (Cel5A). In the direct transfer process, which has been observed for protein translocation on DNA, proteins can move from one site on the ligand to another via collisions between a complex and a free ligand. Using NMR line-shape analysis for TROSY spectra recorded for $^{2}H/^{15}N$-labeled Cel5A and Cel9B CBDs in the presence of cello-oligosaccharides at some different concentrations, we have determined the kinetic rate constants for direct transfer, association, and dissociation processes. Our NMR data on Cel9B CBD suggest that direct transfer is significantly more efficient than the two-step translocation via dissociation followed by re-association under the current experimental conditions. On the other hand, our preliminary data implies that direct transfer is slower than two-step translocation for Cel5A CBD under the same conditions.

Biophysics Poster Number P-41

A Combined Quantum Mechanics/Molecular Mechanics and Molecular Dynamics Study of a Light-Harvesting Molecular Triad

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We investigated the effect of solvation on an artificial photosynthetic molecular triad in nanoconfinement of various sizes using a combined approach of Quantum Mechanics/Molecular Mechanics (QM/MM) method and all-atomistic molecular dynamics simulations with explicit water models. We used the Replica Exchange

Method Dynamics (REMD) to enhance the sampling efficiency and investigated the effect of confinement on the distribution of the ensemble structures and the energy landscape of triad. Using a Progress Variable Clustering (PVC) algorithm, we determined the dominant structures in an ensemble. The relationship of the charge distribution computed from QM/MM and the radial distribution function of water molecules in close proximity of triad will be discussed. The work presented here has profound implications on the photosynthetic function of triad. The knowledge gained from this project will provide an opportunity for better design of the molecular device for green energy.

Biophysics Poster Number P-42

Predicting Pressure of Capsid-Confined DNA

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Predicting the pressure necessary to confine DNA—a highly-negatively charged, elastic polymer—into viral capsids (over a 250-fold compaction) is a problem with implications in therapeutic nucleic acid delivery which we address by a theoretical, physics-based approach. A detailed physical understanding of this phenomenon is also relevant to in vivo mechanisms of tightly-stressed or confined DNA transcription, replication and nucleosomal packing. Experimental measurements of phage DNA confinement includes osmotic pressure ejection-inhibition experiments and single-molecule loading force measurements that provide pressure data for validation of theoretical models. Structural insight into DNA packaging is also aided by cryo-electron microscopy data, such as the asymmetric reconstructions done at the NCMI with Wah Chiu.

Most current models of phage packing assume DNA behaves as a linearly elastic polymer that bends uniformly and isotropically under stress, often assuming a highly-ordered ‘inverse spool’ model. The assumption of such spooled conformations is based primarily on interpretations of cryo-EM density maps, obtained by averaging thousands of structures, which show 'rings' of density, especially near the capsid surface. Because phage genomes are around ten kilobasepairs long, we have begun by employing a coarse-grained model of double-stranded DNA (already successfully applied to microarray experiments) as a chain of surface-charged, ion-penetrable spheres. Simulations of unconnected DNA coarse grained polymer beads of around 6 basepairs in capsid-like confinement clearly show ringed density distributions consistent with cryo-EM data. This demonstrates that virtually any connected polymer path may be consistent with such data, and indicates an ensemble of more entropically-driven, disordered conformations of DNA may be possible. We test this by incorporating non-linear elastic 'kinking' of DNA. Simulations have yielded precise determinations of pressure. By applying Monte-Carlo path-sampling “polymerization” techniques to these monomeric systems, we are able to test model hypotheses related to the amount of disorder, ion screening and the extent of DNA-protein interactions.

Biophysics Poster Number P-43

Observing the Effects of Osmolytes At the Single-Protein Level

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Polycystin-1 is a large transmembrane protein, which, when mutated, cause autosomal dominant polycystic kidney disease, one of the most common life-threatening genetic diseases which is a leading cause of kidney failure. PC1 is a large membrane protein that is expressed along the renal tubule and exposed to a wide range of concentrations of urea. Urea is known as a common denaturing osmolyte that affects protein function by destabilizing their structure. On the other hand, it is known that the native conformation of proteins can be stabilized by protecting osmolytes which are found in the mammalian kidney. PC1 has an unusually long ectodomain with a multimodular structure including 16 Ig-like polycystic kidney disease (PKD) domains. Here we used single-molecule force spectroscopy to directly study the effects of several naturally occurring osmolytes on the mechanical properties of PKD domains. This experimental approach more closely mimics the conditions found in vivo. We show that upon increasing the concentration of urea there is a remarkable decrease in the mechanical stability of human PKD domains. We found that protecting osmolytes such as sorbitol and TMAO can counteract the denaturing effect of urea. Moreover, we found that the refolding rate of a structurally homologous archaeal PKD domain is significantly slowed down in urea, and this effect was counteracted by sorbitol. Our results demonstrate that naturally occurring osmolytes can have profound effects on the mechanical unfolding and refolding pathways of PKD domains. Based on these findings, we hypothesize that osmolytes such as urea or sorbitol may modulate PC1 mechanical properties and may lead to changes in the activation of the associated Polycystin-2 channel or other intracellular events mediated by PC1.
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Biophysics Poster Number P-44

Structure-Based Design of a Z-Protein Variant to Inhibit Lassa Fever Virus Replication

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Lassa fever virus belongs to the large family of Arenaviradae, which also includes human pathogens such as Guanarito, Junin, and Machupo virus. The virus causes 100,000-500,000 cases of clinically apparent Lassa fever in West Africa annually, with up to 20% of these patients developing hemorrhagic fever with a mortality rate of 10-15% (CDC, 2010). Of particular concern is the potential of the virus to be employed as a weapon of bioterrorism. Lassa Fever virus is therefore an emerging infectious disease as well as a deleterious

bioterrorist threat agent.
The arena virus Z-protein (11-kDa) is essential for the replication and propagation of the Lassa fever virus because of its role in budding. The protein is 99 amino acids in length and is membrane associated via Gly2 myristoylation. Sequence alignment suggests the protein contains a 45-residue RING finger domain that coordinates Zn^{2+}. Related RING finger proteins have disordered N- and C- terminal regions flanking the central Zn^{2+} binding domain. Based upon previously published data, we hypothesize that these N- and C-terminal regions are responsible for Z-protein body assembly-oligomerization (*Borden, et. al*). Oligomerization is believed to aid in recruitment of nucleoprotein (NP) encapsidated ambisense, single-stranded genome to the budding site via direct interaction with the viral NP as well as coordinating the assembly of the mature virion at the plasma membrane. However, the role of oligomerization in this process is not well understood. The C-terminus (when N-terminus is fused with plasma membrane) may be disordered prior to its interaction with capsid proteins implicating the protein as a structural component of the virus. Structural and biophysical characterization of the protein will be necessary for successful design of antiviral strategies. Our long-term goal of the project is to develop an inhibitor of Lassa fever virus production in cells that inhibits the budding process. We will develop a Z-protein variant that will poison Z-protein oligomerization during budding by making some oligomer interactions but not others. Our aims are to: (1) produce Z-protein variants that are deficient in full oligomerization (Z-bodies), (2) investigate Z-protein oligomerization in silico to identify interaction surfaces, and (3) test these variants in cells challenged with the virus. Initially, Z-WT was purified under denaturing conditions (6M guanidine) and subsequently refolded by dialysis in the presence of sufficient Zn^{2+}. Size-exclusion data indicated higher order species (24-mers and tetramers) as well as monomers. The protein was then expressed in soluble form. Gel filtration experiments demonstrated this protein to be a monomer. To fully understand the behavior of Z-WT in vivo, we will isolate Z-WT from virally challenged cells and analyze the oligomeric state via native PAGE and gel filtration. This will offer conclusive results on the assembly behavior of the LFV Z-protein and whether the design of a dominant-negative mutant to disrupt higher-order assembly is warranted.
This work was supported by a training fellowship from the Keck Center Computational and
Structural Biology in Biodefense Training Program of the Gulf Coast Consortia (NIH Grant T32 AI065396-04)
Reference: Kentsis A., Gordon R.E., and Borden K.L. (2002). *Self-assembly Properties of a Model RING Domain. PNAS, 99(2):667-72.*

Biophysics Poster Number P-45

Hydrogen Bonding Dynamics of Lysine Side-Chains Studied By NMR Spectroscopy

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Protein side-chains play vital roles in molecular functions such as enzymatic catalysis, protein-protein and protein-nucleic acid interactions. Although there are many NMR techniques for investigations of protein dynamics, the vast majority is applicable only to the backbone or methyl groups. Recently we have developed NMR methods for investigating dynamics of lysine side-chain amino groups. Our new methodology based on the ^{15}N relaxation and long-range ^{15}N-^{13}C J-coupling provides unique information about hydrogen bonding dynamics of lysine side-chains. In this poster presentation, I will explain the principle of the methodology and our recent data on functionally important lysine side-chains in human ubiquitin.

Biophysics Poster Number P-46

Engineering Novel Proteins With Enhanced Mechanical Strength

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Engineering novel proteins with specific properties remains a difficult goal to achieve, mainly because the understanding of the relationship between protein sequences and certain structures or properties is still incomprehensive. For the case of mechanical proteins, even though few successes are accomplished to enhance the mechanical strengths of protein domains, there are no rational and systematic methods to engineer proteins with specific mechanical properties. It has been shown that the Ig-like domains in the I-band of Titin need hierarchical external forces to unfold. We have analyzed the sequences of the weak and strong Ig-like domains from different species, and identified unique motifs which may be important for the hierarchical strengths of Ig-like domains. In this study, we engineered four novel proteins by swapping motifs or segments from strong domain I27 to weak domain I1. The hypothesis is that the unfolding forces of the mutants will be increased. We tested their unfolding forces by single-molecule Atomic Force Microscopy (AFM) experiments to verify the enhancements of mechanical strengths. Preliminary data indicate that the unfolding forces of some engineered mutants are increased to the level of I27 domains. Steered Molecular Dynamics (SMD) simulations for these mutants are in process to analyze the conformational changes during unfolding. The change of backbone hydrogen bonds of A-B, A'-G strands and their side-chain interactions with neighboring residues are monitored, since they were reported to be the key determinants of mechanical strengths for Ig-like domains in titin.

Biophysics Poster Number P-47

Development of An α7 NAChR Targeted MRI Contrast Agent

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Alzheimer's Disease (AD) is the 7th leading cause of death in the United States. Because there is no method of early diagnosis characterization of early indicators for targeted imaging is essential. The α7 nicotinic acetylcholine receptor is an indicator of AD progression, but knowledge of its timeline of change has yet to be well determined. We hypothesize that α7 nAChR concentration changes are indicative of AD onset and progression, and that this can be assayed non-invasively using MRI. We expect to show with that α7 receptor concentration first falls at the same time or before amyloid β plaque deposition.
To image these changes we are developing an α7 receptor targeted MRI contrast agent. To show receptor concentration in vivo we must have specific and high affinity binding as well as very good contrast. We have made a proof of concept targeted contrast agent using α-bungarotoxin to target the α7 receptor and gadolinium sequestered in diethylene triamine pentaacetic acid (DTPA) to provide contrast in the MRI. We

have demonstrated binding of the labeled α-bungarotoxin using Torpedo californica membranes. We plan on assessing the change of α7 concentration throughout the progression of AD using the Tg2576 mouse model. This model will be essential for measuring α7 concentration because it has all the hallmarks of AD except for neurodegeneration. Characterizing α7 as an early indicator for AD could eventually lead to diagnosis and treatment strategies to improve the quality of life of millions.

Biophysics Poster Number P-48

The Escherichia Coli PriA Helicase Specifically Recognizes Gapped DNA Substrates

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The primosome is a multi-protein-DNA complex that catalyzes the priming of the DNA during the replication process. In *Escherichia coli*, PriA helicase plays a fundamental role in the initiation of the ordered assembly of the primosome. PriA is involved in recombination and repair processes being a major factor that initiates the restart of the stalled replication fork at the damaged DNA sites. This happens, presumably, through the recognition of the damaged DNA site structure though the nature of this recognition process is unknown. Here we present quantitative studies of the ssDNA gap recognition by the PriA helicase and the effect of the nucleotide cofactors on the recognition process. The data indicate a surprisingly low minimum total site size of the enzyme in the gap complex, which is ~7 nucleotides or bp as compared with the site size of ~20 nucleotides of the enzyme-ssDNA complex. The low stoichiometry indicates that the helicase exclusively engages the strong DNA-binding subsite in the gap complex and assumes a very different orientation, compared with the complex with the ssDNA. The PriA helicase binds the ssDNA gaps with 4–5 nucleotides with the highest affinity without engaging in cooperative interactions with the enzyme molecules associated with the surrounding dsDNA. Binding of ADP to strong and weak nucleotide-binding sites of the enzyme profoundly affects the affinity and stoichiometry of the helicase-gapped DNA complex. These observations are of fundamental importance for understanding of the enzyme mechanisms in both replication and recombination processes.

Biophysics Poster Number P-49

The New UTMB SCSB Biological SAXS Facility

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The Sealy Center for Structural Biology has purchased new x-ray diffraction and solution scattering instrumentation for its crystallography lab. The new equipment includes the Rigaku Ultimate Home Lab® which comprises the unsurpassed high-brilliance FR-E^{++}DW Superbright x-ray generator with both Cu and Cr optics, and the industry standard RAXIS-IV^{++} crystallography system. In addition, we are acquiring the innovative Rigaku BioSAXS-1000, the first SAXS instrument dedicated to biological research in the southern mid-western states, once again marking UTMB as a leader in structural biology resources.

You are invited to join the Gulf Coast Protein Crystallography Consortium's Small Angle X-ray & Neutron Scattering interest group: The SAXNS. This is a group of researchers currently using or intending to apply SAXS or SANS to conquer difficult structural biology problems. The group is organized by Xiaodong Cheng, and the SCSB at UTMB, but includes members from most GCC institutions. For more information or to be informed of upcoming events and workshops please see the SAXNS website http://xray.utmb.edu/SAXNS.

Biophysics Poster Number P-50

DNA Repair Protein Kinetics Quantification Before and After Gamma-Irradiation Using Fluorescence Techniques

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Deficiencies in DNA-damage repair pathways are fundamental cause to most of human cancers. The kinetics of most proteins involved in DNA damage sensing, signaling and repair following ionizing radiation exposure cannot be quantified by current live cell fluorescence microscopy methods. This is because most of these proteins, with only few notable exceptions, do not attach in large numbers at DNA damage sites to form easily detectable foci in microscopy images. As a result a high fluorescence background from freely moving and immobile fluorescent proteins in the nucleus masks the aggregation of proteins at DNA damage sites. Currently, the kinetics of these repair proteins are studied by laser-induced damage and Fluorescence Recovery After Photobleaching that rely on the detectability of high fluorescence intensity spots of clustered DNA damage. We report on the use of imaging techniques, Raster-scan Image Correlation Spectroscopy (RICS) and Number and Brightness (N&B) analysis methods as a means to monitor kinetics of DNA repair proteins during sparse DNA damage created by gamma-irradiation, which is more relevant to cancer treatment than laser-induced clustered damage. We use two key double strand break repair proteins, namely Ku and DNA-PKcs as specific examples to showcase the feasibility of the proposed methods to quantify dose -dependent kinetics for DNA repair proteins after exposure to gamma-radiation.

Computational Poster Number P-51

Spatial Reasoning With Multiple Intrinsic Frames of Reference

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The FORMS spatial module for ACT-R 6 is an implementation of the FORMS (Frame Of Reference-based Maps of Salience) theory of human spatial cognition (Wang, Johnson & Zhang, 2001) within the framework of a unified theory of human cognition, ACT-R (Anderson et al., 2004). FORMS stresses two aspects of

spatial cognition: space as experienced from multiple frames of reference and selection among those frames of reference (FORs) and locations within those FORs by salience. The module takes as its input visual representations from ACT-R and parses them into symbolic, primitive bearing and distance representations. It also remaps spatial representations (SRs) from the egocentric FOR (EFOR) to be in terms intrinsic to an exogenous anchor object (the intrinsic FOR, or "IFOR"). The spatial module then makes these symbolic representations of spatial information available to the rest of cognition as theorized and instantiated by ACT-R.

Crucial to FORMS' success as a theory of human spatial cognition is the hand-in-hand interaction of the specifically-spatial part, FORMS itself, with the rest of cognition, which is what ACT-R provides. No account of spatial cognition can be complete without both components working together. Using a computational cognitive architecture is very important for reasons that have been enumerated elsewhere (Anderson et al., 2004; Byrne, 2001; Byrne & Kirlik, 2005; Gunzelmann, Anderson & Douglass, 2004; Newell, 1973), but the multi-FOR aspect of spatial reasoning has not heretofore been addressed. Using this approach we can elucidate the ways in which multiple FOR representations interact with each other and with other cognitive mechanisms to produce the emergent spatial behavior. Previous studies of spatial reasoning have addressed the role that FORs play, but not much has been done in accounts that integrate across FORs, a prerequisite for complex spatial reasoning such as might be the case in many tasks ranging from hide-and-go-seek to local and remote vehicle operation and even combat. Building a useful theory of multi-FOR spatial reasoning behavior within the constraints of a cognitive architecture will likely benefit applications in domains that draw upon that kind of reasoning, such as in the tasks just named. Eventually the computational nature of such an account built within an architecture like ACT-R can be useful as high-fidelity cognitive modeling (Gray, Schoelles & Myers, 2004).

Computational Poster Number P-52

The Potential Role of Positive-Feedback Loop in the Sensitivity of Memory Consolidation and Reconsolidation to Inhibitors of Protein Synthesis and Kinases

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Memory consolidation and reconsolidation require kinase activation and protein synthesis. Blocking either process during or shortly after training or recall disrupts memory stabilization, which suggests the existence of a critical time window during which these processes are necessary. Using a computational model of kinase synthesis and activation, we investigated the ways in which the dynamics of molecular positive-feedback loops may contribute to the time window for memory stabilization and memory maintenance.

In the present study, we used a modified version of the Smolen et al. (2009) model. The model has interlinked, dual-time positive feedback loops. One loop is a fast auto-activation loop that represents activation of a kinase. It is coupled to a second loop that represents kinase synthesis. This two-loop model is bistable (i.e., the level of kinase activity and total kinase can exist in either a lower or upper steady state). We heuristically equated an increase to a stable upper state of kinase activity, A, and total amount of kinase, B, with consolidation of a new memory. This two-loop model was used to examine whether positive feedback loops were responsible for the empirically obtained time windows for sensitivity of memory consolidation

and reconsolidation to protein synthesis inhibition (PSI) with physiological "dosages" (e.g., 95% ~ 98% PSI, Milekic and Alberini 2002; Squire and Davis 1975). Simulation results show that PSI starting from before to 40 min after training blocked or delayed consolidation. Beyond 40 min, substantial (>95%) PSI had little effect despite the fact that the elevated amount of kinase was maintained by increased protein synthesis. However, PSI made established memories labile to perturbations. We also found that this model reproduced a time window for sensitivity of memory consolidation to kinase inhibition. To examine whether these time windows require both fast and slow feedback loops, we eliminated kinase auto-activation to generate a one-loop model that had only slow feedback. The simulation results in the one-loop model indicate that a single feedback loop involving protein synthesis suffices to generate resistance of memory consolidation to PSI (≥95%). Similar properties were found in several other models that also included positive-feedback loops.
Even though our models are based on simplifications of the actual mechanisms of molecular consolidation, they illustrate the practical difficulty of empirically measuring "time windows" for consolidation. This is particularly true when consolidation and reconsolidation of memory depends, in part, on the dynamics of molecular positive-feedback loops.

Computational Poster Number P-53

A Site Under Selective Pressure in the LexA Repressor is Required for Control of the DNA Damage Response

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The LexA repressor of the bacterial DNA damage response or its interaction with the RecA recombinase may be good drug targets for combating the emergence of antibiotic resistance. LexA structure-function relationships can help guide the development of LexA inhibitors. LexA has several crystal structures and functional studies available and yet, the mechanism of RecA-mediated LexA hydrolysis is uncertain. Here we use a computational tool for sequence analysis to identify a new functional site on LexA and provide genetic evidence indicating its importance in repressor function. LexA residues were ranked according to each position's variation pattern as it relates to the protein family tree using the Evolutionary Trace program (http://mammoth.bcm.tmc.edu/). Core residues of the known auto-proteolytic active site, cleavage site and DNA binding site are within the top 10^{th} percentile; indicating accuracy in the identification of functional sites. Eighteen highly ranked residues were targeted for site-directed mutagenesis and their effect upon resistance to DNA damage and in vivo repressor function was measured. Five of six mutations in a site adjacent to the dimerization interface improved resistance to DNA damage; suggesting a constitutive or sensitized response to DNA damage. This site contributes towards repressor function and steady-state protein expression levels as indicated by a LexA-repressed GFP reporter assay and Western blot analysis. This modulation site on LexA may influence RecA-mediated hydrolysis and prove amenable to pharmacological inhibition that can delay the appearance and spread of antibiotic resistance determinates.
Supported by training fellowships from the National Institutes of Health and the National Library of Medicine to the Keck Center Pharmacoinformatics and Interdisciplinary Bioscience Training Programs of the Gulf Coast Consortia to PK and DCM (NIH Grant T90DK070109-05 & NLM Grant T15LM007093).

Computational Poster Number P-54

Copy Number Aberrations Predict Progression Free Survival of Pediatric Patients With Ependymoma

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Background. Ependymomas account for ~10% of intracranial tumors in children, typically presenting in the first five years of life. Despite recent advances in neurosurgical and radiotherapy techniques in the management of this disease, 5 year overall survival remains around 50%. Progression free survival is worse, and late recurrences are not uncommon. These abysmal statistics stem from our profound lack of understanding about the biology of ependymoma. Currently the role of chemotherapy is not established and no compelling drug targets are known.

Materials & Methods. In an attempt to identify molecular markers that could predict clinical outcome, we analyzed 124 cases of pediatric intracranial ependymomas collected from Texas Children's Hospital (n = 38) and Children's Oncology Group (n = 86 from a single clinical protocol) using Affymetrix 250k StyI SNP arrays for copy number aberrations (CNAs) and loss of heterozygosity (LOH). Subjects ranged between 19 months and 19 years of age, with 44% under the age of four. Clinical features of this patient cohort are consistent with the disease epidemiology, with the ratio of infratentorial to supratentorial tumors being 1.95, and 47% of the cases were classified as anaplastic (WHO grade III). All but 6 patients received postoperative radiotherapy, and >75% received complete resection during their initial surgery. CNA calls were made based on a reference panel of matched blood samples from over half of these subjects, using the recursive segmentation algorithm distributed by Partek, Inc.

Results. Visualization of these CNAs by unsupervised hierarchical clustering demonstrated three distinct groups with different clinical outcomes. The first group, High Copy Number (HCN), includes patients with multiple aberrations involving whole chromosomes or arms, with deletion of chromosome 6 being the most common abnormality. A second group dominated by gain of chromosome 1q separated from the remaining cases with balanced karyotype or small focal CNAs. Patients with 1q gain had significantly worse progression free survival (PFS), even after correction for all available clinical factors (Cox model HR = 3.223, p = 0.0054). Membership in the 1q gain group was as predictive of outcome as tumor location and degree of surgical resection. Further investigation into gene expression within these 1q gains revealed gene transcripts (CHD1L – 1q21.11, PARP1 – 1q42.12) that could better identify high risk patients. This expression-based classifier was validated using 30 independent EPN tumors.

Conclusions. Using DNA copy number profiling, we have developed a molecular classification that has prognostic significance. Additionally, transcript over-expression of two genes within the CNA-based classifier was more predictive of PFS, and was validated using an independent sample. Both of these genes, PARP1 and CHD1L, are novel EPN related genes, and are currently targetable for chemotherapy.

Computational Poster Number P-55

Computational Prediction of Active Motifs in PCG-Regulating Long Noncoding RNA

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Polycomb group (PcG) and Trithorax Group (TrxG) proteins are regulatory factors involved in the maintenance of epigenetic cellular memory, pluripotency, and stem cell self-renewal. It is currently believed that PcG protein complexes PRC1 and PRC2 are recruited to PRE regions by DNA-binding proteins such as YY1 and noncoding RNA such as HOTAIR. When many RNAs of similar function are known, the active site -finding problem is often reduced to a motif-finding one. Unfortunately, the information content in primary RNA sequence alone seems to be relatively low when compared to DNA and protein. We have developed a motif-finding procedure for RNA based on searching for sequence similarities in predicted stem-loops and simple pseudoknot structures.

Computational Poster Number P-56

Pharmacophore Based Virtual Screening as a Method to Identify Function of Small Metabolite Binding Proteins

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We have developed a pharmacophore-based virtual screening method, which can be used to predict the function of proteins whose structure is available but whose function is unknown. The method was evaluated using five test cases spanning two different folds, four superfamilies and three enzyme classes. The first step involves predicting the ligand binding site using a combination of two methods – CASTp and THEMATICS. In the next step, the protein binding site is mapped using chemical probes (e.g., hydrogen-bond donor, acceptor and/or hydrophobic) to search for favorable interacting positions (pharmacophore features). A combination of two or three such features is termed as a pharmacophore model. A database of 13,058 metabolites was searched to identify compounds that fit the pharmacophore models and thus are candidate cognate ligands. Subsequently, knowledge-based filters were used to enrich the hits with substrate-like compounds. Finally, a physics-based scoring function was used to rank the compounds. This method retains substrate or substrate like compounds within top 5% of the hits from search of the metabolite database. Pharmacophore-based virtual screening is a time efficient method that can be used to predict the probable function of a protein of unknown function.

Computational Poster Number P-57

Finding Critical Proteins in Metabolic Pathways

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Determining which proteins are critical to a pathway's function would allow for identification of efficient drug targets and improved interpretation of the effect of genetic variation. In this experiment, such proteins will be found in a sets of *S. cerevisiae* and *E. coli* pathways by using a computational algorithm with experimental validation. Because protein residues are known to show evolutionary pressure if they are critical for protein function, the Evolutionary Trace algorithm, which finds conserved residues in individual proteins, has here been used to instead find conserved residues across a set of functionally related proteins in various metabolic pathways. Proteins are then assigned a relative importance based on their density of highly invariant residues compared to other proteins. These results have been compared to alternative methods, such as mutation rate, to show an improvement in ability to predict which proteins are the least dispensable, as measured by experimental validation. Future work will include further experimental validation in other organisms and pathway types, as well as combing with and comparing to additional alternative computational methods.
Supported by a training fellowship from the Keck Center NLM Training Program in Biomedical Informatics of the Gulf Coast Consortia (NLM Grant No. T15LM007093).

Computational Poster Number P-58

Scientific Workflow Archival and Analysis With EMEN2

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Structural and computational biologists frequently work with complex data sets assembled from diverse experimental sources, public resources, and analysis methods. Archiving and mining these data sets with their complicated interrelationships remains a persistent challenge, particularly with "open science" initiatives to make entire workflows, including all raw and intermediate data, available with publications.
To address these needs, we have developed EMEN2, an object-oriented scientific database and electronic notebook. EMEN2 uses a flexible schema based on plain text descriptions of experimental protocols. These protocols may be local and describe techniques and data within a single lab group, reference published ontologies (e.g. GO, NCBO BioPortal), or contain links to external resources (PDB, GenBank, etc.). Similarly, an EMEN2 installation can itself act as a resource, providing public access to selected protocols and data. While originally developed to serve the needs of the cryo-EM community, we believe EMEN2's architecture provides an excellent foundation for many other scientific endeavors.
EMEN2 is developed using all open-source technologies. The core database is written in the Python programming language, with BerkeleyDB providing a robust embedded database back-end. The infrastructure is highly modular, permitting new ontologies to be fully implemented using only it's "Web 2.0" interface. In addition, there is a remote API available for client applications. The included EMDash

program is a standalone GUI tool for equipment integration, currently used to upload data transparently from our electron microscopes as it is being collected, as well as integrate with other lab equipment. The EMEN2 server itself can be extended in a similar way by writing custom Python modules, which can expose additional views to the Web interface, or new methods to the API.
A full ontology for cryo-EM has been established for internal use and has been in active use at the NCMI for ~3 years. It is used to archive all data at the center, and currently provides services for over 750 users, with over 16 terabytes of data in 460,000 records. As an example of its extensibility and ontology mapping capabilities, we have developed a module for harvesting the database and producing PDB compliant XML files which can be used to seed a structure deposition to EMDatabank.org.
EMEN2 is available, with complete source, at our wiki: http://blake.grid.bcm.edu/emanwiki/EMEN2
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Computational Poster Number P-59

Binding Dynamics of the Bacterial Quorum Signal Destroying Bacillus Thuringiensis Lactonase

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The lactonase enzyme (AiiA) produced by Bacillus thuringiensis serves to degrade autoinducer-1 (AI-1) signaling molecules in what is proposed to be an evolved mechanism by which to compete with other bacteria. Bioassays have been performed to determine whether the AI-1 carbon tail lengths have any effect on AiiA's bioactivity, however, data to date are conflicting. Additionally, specific residue contributions to the catalytic activity of AiiA are in question. For example, it has been proposed that Y194 serves as an oxyanion hole to the substrate which is curious given the fact it spans across two Zn(2+) ions. To investigate these problems, multiple molecular dynamics simulations are performed across a family of seven acylated homoserine lactones (AHL). Distance analyses and binding energetics using the linear interaction energy method were performed and compared to current bioassay data. Our simulations show that AiiA degrades AHL in manner that is tail length independent. Only the presence of the tail is required for activity. Also, oxyanion contribution of Y194 towards the substrate is not seen in any of the trajectories.

Computational Poster Number P-60

A Smooth Structural Distribution of Evolutionary Pressure Characterizes the Determinants of Protein Function and Improves Function Annotation

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Motivation: The evolutionary constraints among sequence, structure and function are not fully understood. Defining this relationship can help to better identify key functional residues and localize functional sites, and thus to guide protein engineering and function prediction.
Methods and Results: Building on prior results, we introduce here, first, a formal analytic measure of the continuity of evolutionary importance of residues within protein structure, the quadratic form of the discrete Laplacian operator, and show that greater continuity correlates with better predictions of functional residues and sites. This justifies a novel Evolutionary Trace algorithm, designed specifically to improve the continuity of evolutionary importance among residue neighbors. As hoped, it yields greater continuity, better overlap of protein functional sites compared to ET, and improved protein function prediction.
Conclusions: This work shows that selective pressure operates smoothly within protein structures, in the precise sense that second spatial derivatives of evolutionary importance can be meaningfully computed across residue neighbors and are normally at a minimum. It explicitly ties the importance of residue to its structural interactions with neighbors. This improves our annotation of key functional residues, of the sites they define, and the recovery of function from the search for similar residues in other structures.

Computational Poster Number P-61

Biosensor Approach to Cultural Differences

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Data-driven Bayesian clustering was applied to functional magnetic resonance imaging (fMRI) data obtained during a multi-round exchange game played across and within cultures. The resulting three clusters are statistically significantly correlated with within-culture dyads, cross-cultural dyads aware of their cross-cultural status, and cross-cultural dyads unaware of their status. This shows that in simple interactions, a person's brain activity can act as a "biosensor" for cultural differences.

This result makes sense intuitively: since people mostly interact within their culture, they have developed more sophisticated patterns and techniques allowing them to handle such interactions. The opponent's behavior in within-culture interactions is more familiar, so it invokes these patterns and techniques -- and

thus, leads, on average, to a more intense brain activity. In contrast, cross-cultural interactions are less familiar and thus, invoke less of these techniques -- and therefore, lead to a somewhat less intense brain activity.
The differences between within-culture and cross-cultural interactions are subtle and while this subtlety is not fully captured by the Bayesian clustering of the behavioral data alone, the Bayesian analysis of the neural data does capture these differences. Our analysis shows cultural differences affect the social interactions so much that in an agnostic Bayesian clustering, the only clusters we have are the ones correlated with cultural differences. These differences appear even when the participants are not aware that they are interacting across cultures.

Our results demonstrate that simple economic games combined with fMRI data can parse social interaction in ways that behavioral analysis alone cannot; at least for simple economic exchange, emitted behavor alone does not capture the full story of the interaction.

Computational Poster Number P-62

Networks of Evolutionary Template Matches for Prediction of Enzymatic Function

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The Structural Genomics projects frequently target structures that lack sequence similarity to known structures. This makes annotation through sequence-based methods difficult, and as a result, nearly half of the protein structures solved by Structural Genomics projects have no known function. To fill this gap, Evolutionary Trace Annotation (ETA) selects evolutionarily important amino acids in protein structures and transfers annotations to proteins with similar amino acid configurations. To improve this method, we have pooled many ETA matches into a single network of enzymatic similarity and then allow annotations diffuse throughout the network, in a process inspired by physical diffusion. This network diffusion model naturally allows us to attach a confidence score to each prediction, allowing us to group our predictions by confidence. When we do so, we find that the 50% most confident predictions are 98.8% or 97.6% accurate, depending on the descriptiveness of the predicted functional label. These results are a significant improvement over conventional nearest neighbor network models. Our high accuracies have allowed us to predict enzymatic substrates in many cases. We have applied this method to unannotated proteins in the Protein Data Bank, and highlight a case study of an interesting and difficult novel prediction in the medically relevant organism Staphylococcus aureus.
This work was supported in part by a training fellowship from the Biomedical Discovery Training Program of the W. M Keck center for Interdisciplinary Bioscience Training of the Gulf Coast Consortia (NIH Grant No. T90 DA022885).

Computational Poster Number P-63

Measure Underlying Mutations and Diagnose Mitochondria Dysfunction Using Next-Generation Sequencing Techniques

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Cardiovascular disease is the number one killer of Americans leading to an estimated 80,000,000 people aged >20 with 831,272 in 2006 and the numbers are rising. Metabolic syndrome, obesity and type II diabetes contribute to cardiovascular disease. Mitochondrial ATP production in normal hearts is generated from 40% glucose and 60% fatty acid oxidation, however in diabetic heart tissue, fatty acid oxidation increases to 95% or more of the total fuel consumption. The shift in fuel signals a redistribution of metabolic pathways that leads to mitochondrial reactive oxygen species or ROS production. An increase in ROS production may lead to increased mitochondrial dysfunction. By observing mutations in the mtDNA, percentage of dysfunction can be calculated and used as an early measure of mitochondrial damage.

Collaboration efforts have been made to obtain and isolate mtDNA from multiple samples of two types of mitochondria from the cardiac muscle: subsarcolemmal mitochondria (SSM) and intermyofibrillar mitochondria (IFM). Deep sequencing using Next Generation Sequencing (NGS) techniques of three stressed mitochondria samples from Human diseased tissue and a comprehensive computational approach have allowed us to align approximately 1,800 sequenced reads to Human mitochondria per base pair. This analysis identified proportions of each type of genomic variation; however, a much higher level of insertions and deletions were exhibited than originally estimated throughout the length of the mitochondrial genome.

These findings raised a concern about the reliability and trustworthiness of SNP data generated from short reads without regard to sequence misalignment. Because mapping with mismatches requires sequence alignment, the possibility for misaligning a read to its target position on the reference genome may increase just because of the mismatch. When a read is misaligned it can incorrectly imply the presence of a SNP when in fact the SNP is a result of misalignment only. These SNP Identification Errors (SIEs) can arise from sequencing errors or errors in the alignment algorithm. If not accounted for SIEs can undermine the re-sequencing methods for SNP detection and cast doubt on SNP identification especially in low frequency SNP identification. Discerning SIEs from real SNPs is the primary obstacle in the development of a robust SNP calling algorithm for post-mapping analysis and a major focus of this research.

Computational Poster Number P-64

Designing a Human-Centered Visualization Platform for Anesthesia Preoperative Assessment

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Performing an anesthesia preoperative assessment is a data intensive task that is not well supported by traditional Electronic Medical Record (EMR) interfaces. Using a systematic process grounded in cognitive theory, we analyze the cognitive processes used by anesthesia residents to perform a preoperative assessment. The resulting data are then mapped to appropriate visual representations. A prototype for an alternative visual representation is then proposed to better support clinical decision making. Although focused on a specific task relevant to anesthesiologists, the resulting visual representations could potentially be applied to much wider use in clinical medicine.

Computational Poster Number P-65

Constrained Conformational Analysis: Protein Motion Planning With Cryo-Electron Microscopy

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Understanding the time-dependent behavior of macro-molecular protein systems remains a significant computational and experimental challenge. Over the last decade, Single Particle Cryo-Electron Mircoscopy (cryo-EM) has emerged as a reliable tool for the rapid determination of mid-resolution (4-6 Å) structures of proteins. In certain cases, it is even possible to produce atomic models of individual conformations (e.g., open and closed) --as in the case of the group II chaperonin Mm-cpn. While these results describe many important features of such a system, they provide only individual, typically energetically minimized, snapshots of conformations of the protein. Capturing the full nature of protein function requires an understanding of the dynamic behavior which drives it.

The central aim of this project is the development of a computational method which produces ensemble solutions that characterize the time-dependent behavior of large, optionally symmetric proteins or protein complexes, resolved at mid-resolution by single particle cryo-EM, in order to describe the functional mechanism of these proteins. This will be achieved by reducing the dimension of the problem by modeling inflexible stretches of the protein as rigid bodies, leaving others as free articulated joints. This dimension reduced system thus becomes an articulated robot navigating an energy landscape –allowing for time-dependent conformational exploration when recast as a generic robotic motion planning problem. Structures previously determined by single particle analysis thus become known "islands" of stability in the global conformational space which can then be used to guide the planning process and verify success. Once a desired description of the local conformational space is achieved, path smoothing methods can be used to insure energetic or steric boundaries for all returned results. Moreover, since this process is both random and graph based, it can be iteratively extended and searched, producing probability based ensemble path solutions about (or among) a given set of input states. The resulting system may then be used to answer specific time-

dependent questions about a protein under study or used generally to provide a more complete statistical overview of the system as a whole.

Computational Poster Number P-66

Structural Studies of Western Equine Encephalitis Virus for Vaccine Design

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Western equine encephalitis virus (WEEV) is a mosquito-borne enveloped, positive single stranded RNA virus of the Togaviridae family, genus Alpha-virus, which causes lethal infection in humans and equines. The genome of WEEV shows that it has a moderate to high sequence identity with other alphaviruses. The 5' end of the genome encodes four nonstructural proteins (nsP1, nsP2, nsP3 and nsP4) which are required for virus replication while the 3' end of the genome encodes structural proteins (capsid, E3, E2, 6k and E1). In alphaviruses, both envelope proteins E1 and E2 are required for virus entry. The E1 protein mediates the fusion between the viral and cellular membranes while the E2 protein binds to a cellular receptor and form spikes on the viral surface. WEEV has been classified as a biological safety level 3 (BSL-3) agent, which has prevented structural studies to examine the organization of the envelope proteins. In the current work we used homology modeling, cryo-electron microscopy and bioinformatics techniques to investigate the role of surface-exposed residues in the E1 and E2 proteins of WEEV. The MPACK program was used to generate homology models of the WEEV E1 and E2 envelope proteins, which were fitted into a 13-Å resolution cryo-electron density map of WEEV using rigid body fitting and molecular dynamics simulations. Results of the ongoing study will enable the development of new vaccine candidates and antivirals by designing peptides to target the E1-E2 interaction sites and receptor binding site.

Computational Poster Number P-67

Statistics Speaks of Function of Type II Cluster of Differentiation Proteins

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The application of statistics to biological systems and protein studies is becoming a more routine practice as the advancement of proteomics, genomics, and immunomics occurs. The high dimensionality of data obtained in this research drives the need for methodology that can reduce dimensionality into a number easily grasped by the human mind. Principal component analysis (PCA) is a multivariate statistical technique that can reduce dimensionality of input variables to an easily identifiable number as well as provide a quantitative description of the system that is otherwise not apparent. In this research we examine human Cluster of

Differentiation (CD) proteins. CD proteins are human leukocyte cell markers often involved in immune reactions through cell-cell communication and signal transduction. Probably the best known examples are CD4 and CD8. There are currently more than three hundred known CD proteins. Our study focuses specifically on the thirty six type II single transmembrane CD proteins. These thirty six proteins were examined for one hundred and twenty four biochemical and biophysical properties derived from primary amino acid sequences. Such characteristics as length, amino acid content, instability index, secondary structure content, hydropathicity etc. were collected for each region of the protein (extracellular, transmembrane, cytoplasmic) and analyzed using PCA. By application of PCA the dimensionality of 124 characteristics was reduced to two main principal components that account for 54% of the input data. To partition the CD proteins into homogeneous subgroups unsupervised K-means clustering was applied. What was found was the thirty six CD proteins can be clustered into two consistent subgroups. Surprisingly all proteins in group one contains binding functioning proteins such as ligands and receptors. The second group contains proteins having enzymatic function with the exception of CD71. CD71 is known to be a transferrin receptor responsible for the cellular uptake of iron. The inclusion of CD71 within the enzymatic protein group prompts our hypothesis that CD71 may have an enzymatic function not previously discovered. It will be interesting to confirm our hypothesis experimentally. This research is an excellent example of the relevance of statistics to protein studies. This quantitative approach could unlock the ability to predict protein function based on sequence which is easily obtainable thus significantly reducing time intensive protein studies.

Computational Poster Number P-68

Enzymatic Annotation of Proteins Through Structural Templates and Experimental Validation of Biological Relevance of Templates

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Many of the protein structures solved by Structural Genomics (SG) project are associated with partial or incomplete functional information. Therefore, the use of such proteins in structure-function based research is limited. To address this problem we developed Evolutionary Trace Annotation (ETA) pipeline, which infers the function of a protein from the structure based on local evolutionary and geometric similarities to proteins with known function using 3D templates composed of evolutionary important residues. Previously, we used computational benchmarking to show that ETA templates composed of six residues was extremely accurate in determining protein function. Recently, we improved ETA by generating smaller, and if possible, multiple templates for a single protein and tested this improved version in the case of over 1000 SG enzymes, where the function was described by Enzyme Commission (EC) number. These ETA improvements were shown to increase prediction coverage from 67% to 78% and 65% to 73% at the 3-digit EC level and 4-digit EC level respectively, whereas prediction accuracy is not substantially compromised (96% vs 91%) at the 3-digit EC level and (90% vs 84%) at the 4-digit EC level. To further test the efficacy of the new ETA protocol we generated a novel prediction for the un-annotated SG protein from Silicibacter sp. (PDB 2pbl). ETA predicts 2pbl as having carboxylesterase activity (EC 3.1.1.1) and our in vitro biochemical testing was able to validate this prediction. Additionally, we also tested whether the templates generated by ETA are composed of residues that are integral to protein function and/or stability. Our results show that mutation of any of the

template residues disrupts enzymatic activity, thereby, confirming that the template residues are in fact integral to protein function. These studies show that ETA is capable of making reliable novel predictions relying on functionally relevant structural motifs.

Computational Poster Number P-69

Segregation of Negatively Charged Phospholipids By the Polycationic and Farnesylated Membrane Anchor of Kras

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The Kras protein, a member of the Ras family of bio-switches that are frequently mutated in cancer and developmental disorders, becomes functional when anchored to the inner surface of the plasma membrane. It is well known that membrane attachment involves the farnesylated and poylcationic C-terminus of the protein. However, little is known about the structure of the complex and the specific protein-lipid interactions that are responsible for the binding. On the basis of data from extensive (>0.55 μs) molecular dynamics simulations of multiple Kras anchors in bilayers of POPC/POPG lipids (4:1 ratio), we show that, as expected, Kras is tethered to the bilayer surface by specific lysine-POPG salt bridges and by nonspecific farnesyl-phospholipid van der Waals interactions. Unexpectedly, however, only the C-terminal five of the eight Kras Lys side chains were found to directly interact with the bilayer, with the N-terminal ones staying in water. Furthermore, the positively charged Kras anchors pull the negatively charged POPG lipids together, leading to the clustering of the POPG lipids around the proteins. This selective Kras-POPG interaction is directly related to the specific geometry of the backbone, which exists in two major conformational states: 1), a stable native-like ensemble of structures characterized by an extended geometry with a pseudohelical turn; and 2), less stable nonnative ensembles of conformers characterized by severely bent geometries. Finally, although the interface-bound anchor has little effect on the overall structure of the bilayer, it induces local thinning within a persistence length of ~12 Å. Our results thus go beyond documenting how Kras attaches to a mixed bilayer of charged and neutral lipids; they highlight a fascinating process of protein-induced lipid sorting coupled with the (re)shaping of a surface-bound protein by the host lipids.

Computational Poster Number P-70

Mechanism of Inositolphosphate-Mediated Allosteric Activation of the Clostridium Difficile Toxins

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Background: *Clostridium difficile* infection (CDI) causes antibiotic-associated diarrhea and potentially fatal inflammation of the colon. Much of the morbidity associated antibiotic resistant CDI is due to two toxins, TcdA and TcdB, which are allosterically regulated cysteine proteases. A small regulatory molecule, myo-inositol*hexakis*phosphate (IP6; also known as phytic acid), binds to a positively charged cleft abutting α flexible β flap in the cysteine protease domain, activating the toxin. Understanding this allosteric mechanism may lead to novel treatments for CDI.
Objective: To combine computational/structural analysis methods with experimental assays of IP6 related compounds, to elucidate the mechanism of allostery in these toxins.
Methods: Docking, with Autodock and GOLD, of inositol phosphate based compounds with various phosphates or pyrophosphates placed at different ring positions were used to analyze the binding mode of alternative activators of the TcdB cysteine protease. Inositol phosphate derivatives were purchased and tested for their ability to activate the cleavage of TcdB using SDS-PAGE analysis.
Results: The binding sites for IP6 on TcdA and TcdB models and crystal structures were analyzed. A library of inositol phosphate family members was constructed and docked to the IP6 binding site. The binding energies decreased linearly with the number of phosphates, and the greatest allosteric activity was predicted for the pyrophosphate inositol*heptakis*phosphate (IP7). Experimental assays of the activation of self cleavage by selected derivatives agreed reasonably with the docking energies for a series of IP6 related compound. Certain positions on the puckered ring are more solvent exposed than others and would seem to contribute less to the overall binding capabilities than others. Additional constraints on the binding, such as the role of metal ion binding in modulating the activity, are now being investigated.
Conclusions: The binding of IP6 derivatives indicate that certain ring positions are more important for binding and activation of the *C. difficile* toxins than others. This information can aid in designing compounds to control the activity of this toxin.
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Computational Poster Number P-71

Models of the Initial Insulin-Insulin Receptor Binding Step

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Insulin signaling starts with the binding of Insulin to its receptor (IR). This causes conformational changes of the extracellular ectodomain and the intracellular part of the IR and subsequently the activation of the intracellular tyrosine kinase domain. Chemical cross-linking, site specific mutagenesis and many other experiments revealed which residues on the Insulin and IR side are important for the binding process. However, the molecular details are still unknown. The crystal structure of Insulin is known since 1969 and in 2006 the crystal structure of the ectodomain α-chain of IR was solved. In 2010 the α-chain IR structure was further refined and included also the C-terminal of the α-chain, which is known to be involved in the Ins-IR binding. A complex structure of Ins-IR is however still elusive. Also, earlier models that were based on the initial structure of the IR are not very useful since in those models the insulin was placed at the position of the C-terminal.
Here, we report theoretical Insulin-IR α-chain complex models that represent the initial step of Insulin-IR binding. We generated these complexes based on the Insulin and the latest refined α-chain IR crystal structure. First, we generated a complete model of the α-chain IR using the refined crystal structure as template. We then docked Insulin into this model and generated 2000 solid body docking poses with ZDOCK. These poses we clustered using pair wise RMSD of the ligand Insulin. Finally, we energy minimized representatives of these clusters with AMBER.

Computational Poster Number P-72

Towards Allosteric Ras Inhibitors: Hitting a Moving Target

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Ras proteins are GTP-hydrolyzing molecular switches that mediate a variety of key signaling pathways. A somatic mutation at codon 12, 13 or 61 leads to constitutive activation and is associated with about 15% of all human tumors. Despite the obvious importance of Ras proteins as potential targets for anticancer therapy, no Ras-selective drugs are currently available. A major reason for this is the poor selectivity of ligands that bind to the catalytic or guanine nucleotide binding site—the traditional target for structure-based drug design. We hypothesized that there exist novel allosteric sites distal from the catalytic site that can be targeted by small molecule inhibitors. To identify such sites, we searched for potential ligand binding sites on the K-ras structure (PDB code 3GFT) using a "blind docking" procedure and a dataset of small molecules that were shown to bind to Ras via high throughput flow cytometry assays[1]. Analysis of the docked ligand poses and their preferred site of interaction on the protein surface led to the identification of two novel allosteric sites: at the back side of the effector binding switch and near the distal C-terminus. These results are consistent with

subsequent findings by others based on NMR and PRE experiments[2]. To further validate our results, we used an ensemble-based approach in which many K-ras structures derived from multiple molecular dynamics (MD) simulations were used to dock a novel compound (SRJ23) that has anticancer chemotherapeutic properties[3]. We found that SRJ23 primarily targets the back side of the effector loop. Binding at this site was possible only when the protein is in an inactive-like open conformation, which was sampled during the MD simulations. Binding at the back of the effector loop suggests that SRJ23 potentially interferes with the interactions of Ras with its effectors and activators. We are currently investigating the affinity of SRJ23 to its preferred binding sites using MD and thermodynamic integration, which will allow us to obtain accurate values for the free energy of binding.

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Computational Poster Number P-73

Molecular Simulations of Sequence-Specific Association of Transmembrane Proteins in Lipid Bilayers

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Association of membrane proteins is central in material and information flow across the cellular membranes with current drug designing efforts targeting regulation of this process. The amino-acid sequence and the membrane environment are critical factors controlling association, however, quantitative knowledge of such contributions is limited. Probing membrane-mediated and protein-protein interactions is a challenging task both for experimental and simulation methods. Herein we present a study on the dimerization of helices in lipid bilayers using extensive parallel Monte Carlo simulations with recently developed algorithms [1].
The dimerization of Glycophorin A was examined employing a coarse-grain model that retains amino-acid specificity, in three different phospholipid bilayers. Association is driven by a balance of protein-protein and lipid-induced interactions with the latter playing a major role at short separations. However, during protein recognition an ensemble of dimers is found, controlled by the free energy of tilting in the specific membrane environment. In all bilayers, sequence-specificity is evident by the formation of a clear interface as suggested in literature studies. Furthermore, the extracted estimates on the dimerization affinity of Glycophorin A are in excellent agreement with experimental data [2].
Following a different approach, the effect of amino-acid sequence was studied using the four transmembrane domains of the epidermal growth factor receptor family in identical lipid environments [3]. Detailed characterization of dimer formation and estimates of the free energy of association reveal that these transmembrane domains present significant affinity to self-associate in qualitative agreement with

experimental findings. However, certain dimers form non-specific interfaces with helices aligning parallel to the membrane normal. Lipid-mediated entropic contributions present a more-complex character than anticipated, favoring a decrease of protein separation or a parallel orientation depending on the interfacial residues. This result has major implications on the activity of the formed dimers; several studies support activity is a function of the interface formed.

Our studies provide significant insight into the dimerization of proteins in a single component lipid bilayer. Mammalian cell membranes are rich and diverse in proteins, lipids and carbohydrates. Future studies will aim to address the effect of lipid composition of multi-component membranes on the association of transmembrane proteins.

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Computational Poster Number P-74

Prediction of the Peptide Cross-Reactivity With the Mountain Cedar Allergen Jun a 1

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Allergic diseases include atopic dermatitis, urticaria, angioedema, asthma, sinusitis, and anaphylaxis due to food, insect, animal and pollen allergens. Allergen-specific immunotherapy (SIT) is an important strategy for allergy treatment. Modified forms of the allergenic protein, such as denatured proteins, hypoallergenic proteins obtained through mutations of the active allergens, recombinant allergens and recombinant allergen derivatives, T-cell epitope peptides, modifications of the immunoglobulin E (IgE) epitopes, and synthetic peptides derived from IgE epitopes can be used for SIT. We developed the Structural Database of Allergenic Proteins (SDAP, http://fermi.utmb.edu/SDAP/) [1-3] to help us predict the allergen cross-reactivity and the IgE-binding potential of genetically modified food proteins. To identify protein regions similar with known epitopes, SDAP uses a sequence similarity search that takes an epitope sequence and uses a sliding window algorithm to compare it with the sequences of all allergens in SDAP. This computational approach is based on the *PD* sequence similarity score calculated with our amino acids descriptors E_1-E_5 that represent the first five principal components from a collection of 237 amino acid scales. To evaluate the ability of the *PD* score in detecting peptides that could cross-react with known IgE epitopes, we designed a peptide library starting from three IgE epitopes (i.e., AFNQFGPNAGQR, MPRARYGL, and WRSTRDAFING) of Jun a 1, the dominant allergen from the mountain cedar pollen [4]. For each epitope, we generated peptides with *PD* values between 0 and 10. The peptides were synthesized on a membrane that was probed with a pool of sera from patients allergic to Jun a 1, and the experimental results were used to identify the peptide positions and

physicochemical properties that determine peptide cross-reactivity with Jun a 1. The peptide cross-reactivity with Jun a 1 was modeled with two machine learning algorithms, namely partial least square (PLS) and k-nearest neighbors (kNN). These two models represent two different approached in modeling immunoinformatics data, because PLS generates global models that consider all peptides, whereas kNN makes predictions based only on a small group of similar peptides. The peptide sequences are translated into an array of E_1-E_5 descriptors which are then used to establish sequence-activity models that highlight the main physicochemical properties of amino acids responsible for the cross-reactivity with Jun a 1.

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Additional Poster Number P-75

Identification of a Polyoxometalate Inhibitor of the DNA Binding Activity of Sox2

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Aberrant expression of transcription factors is a frequent cause of disease yet drugs that modulate transcription factor protein-DNA interactions are presently unavailable. To this end, the chemical tractability of the DNA binding domain of the stem cell inducer and oncogene Sox2 was explored in a high-throughput fluorescence anisotropy screen. The screening revealed a polyoxometalate as a direct and nanomolar inhibitor of the DNA binding activity of Sox2. The polyoxometalate was found to be selective for Sox2 and related Sox-HMG family members when compared to unrelated paired and zinc finger DNA binding domains. TROSY-NMR experiments coupled with computational docking studies using Autodock 4.0, revealed an interaction site of the polyoxometalate on the Sox2 surface that enabled the rationalization of its inhibitory activity. The unconventional molecular scaffold of the polyoxometalate and its inhibitory mode provides strategies for the development of drugs that modulate transcription factors.

Additional Poster Number P-76

Anther Culture Potential of Pepper (Capsicum Annum L.): Effects of Genotypes, Nutrient Media and Incubation Treatments on Callus Formation and Differentiation

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Anther Culture was used in recent years as a tool to fasten various breeding programs and crop improvement via broadening genetic variability. Calli/calluses produced successfully using the fresh buds of the two F1 pepper plants. Freshly collected buds were examine to identify the developmental stage of microspores and then placed in cold room at 4°C in dark for 24 h. After cold treatment, flower buds were excised and anthers were placed on C and N6 induction media. , 2 incubation temperatures 24 and 30°C, media without hormones as a control and 4 hormonal treatments for callus induction and R and N6 media, 6 hormonal treatments for regeneration stage Experiment was performed in factorial, based on CRD design with five replications. Results showed in callus induction 30 4°C and C media supplemented with (BA) (0.5 mg/L), (NAA) (0.5 mg/ L) and (2,4-D) (0.1 mg/L) produced the highest rate of callus. Producing inbred lines in short time help to increase genetic diversity and these lines have appropriate agronomic traits which can highly use in breeding.
Additional key words: Pepper, Anther Culture, Callusogenesis, Nutrient media, Plant Growth Regulators

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Additional Poster Number P-77

Synthesis of Acrylic Type Polymers and Poly Ether Contain Ibuprofen

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The nonsteroidal antiinflamatory of drug 2-(4-isobutylphenyl)-propionic acid (ibuprofen) were covalently linked to acrylic type polymers to obtain macromolecular prodrugs. The polymers of glycidyl methacrylate (GMA) and poly ether containing ibuprofen were synthesized by radical and cationic polymerization. The obtained polymers and prodrugs were characterized by FT.IR, HNMR and CNMR spectroscopies. The main objectives of designing such controlled and sustained release drug delivering systems are the elimination of undesired side effects and simultaneously purposeful drug therapy.
Key words: Ibuprofen, Glycidyl methacrylate, Nonsteroidal anti-inflammatory, Prodrugs

Additional Poster Number P-78

Biochemical Disorders in Children With Febrile Seizure

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Introduction: Convulsion in infants with the prevalence of 4 to 6 cases in 1000 is the most common neurological disorder in pediatrics. Febrile seizure is the most common of seizures in children of ten associated with tonic-clonic and a decrease in consciousness level as well as an upward gaze. This disorder is considered the most common cause of epilepsy along with severe neurological damage. Because of the rather high prevalence of convulsion, its psychosocial and cognitive importance this study aimed to assess the electrolyte disorder to control seizure.
Materials & methods: This was a descriptive- analytical study carried out on 172 children admitted for febrile seizure. In this study, the levels of potassium, glucose, sodium and calcium serum were measured.
Results: Out of 172 children studied, 57.2% were males and 42.8% females. 86.7% had been born naturally and 13.3% through cesarean section. Underlying factors of Febrile Seizure were upper respiratory infection (40%), gastroenteritis (3.4%), urinary infection (4%), pneumonia (12%), otitis (5%), septicemia (3.6%), and unidentified fever (5%). The results showed that, out of all the investigated cases, 30 children proved to suffer from hyponatremia, 7 from hypernatremia, and 135 cases showed a normal level of sodium. Furthermore, 6 children were suffering from hyperkalemia, 8 from hypokalemia, while 158 cases were enjoying a normal level of potassium. Besides, 26 children proved as hypocalcemic, 14 as hypercalcemic and 132 cases showed a normal level of calcium. Only one child was suffering from hypoglycemia.
Conclusion: According to the statistical achievements of this study, there can't be any significant relation between the seizure occurrences and electrolyte disorders in forms of hyper-or-hypomatremia, hyper-or-hypokalemia, and hyper-or-hypoglycemia ($P>0.05$). Therefore, such patients are suggested to undergo these chemical tests Just in case of presenting underlying specific symptoms of such disorders. Furthermore, careful examining planning should be performed for each infant; because diagnostic assessment influences treatment, family counseling, necessity of hospitalization, and particular follow-up of these patients.
Key words: febrile seizures, electrolyte disorders, consciousness level

Additional Poster Number P-79

Determination of Some Mineral Elements in Iranian Edible Wild Asparagus

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Asparagus officinalis L. is a well known vegetable widely cultivated as an important economic crop all over the temperate world. The young shoots of the plant, known as spears, are low in calories, and contain various essential nutrients. In order to determination of mineral elements, spears of edible wild asparagus were collected from their main natural growing regions across the Alborz Mountains of Iran. For comparison, the cultivar Mary Washington was included. Mineral elements (K, Ca, Na, Ba and Li) were analyzed by flame photometer and Mg, Fe, Cu, Zn and Mn were measured using atomic absorption spectrophotometer. Results showed that the means of percent of dry matter (16.74) and ash (1.35) of spears in Iranian asparagus genotypes were higher than that of Mary Washington cultivar (11.6 and 1.05 respectively). Also the amount of mineral elements include K, Ca, Mg, Fe, Cu, Zn, Mn, Na, Li and Ba in wild asparagus in mg per 100 g fresh weight were 139.4, 1.865, 53.311, 0.811, 0.214, 0.706, 0.239, 2.363, 0.767 and 0.004 respectively, which were higher than that of Mary Washington cultivar. The results of the present study show that Persian asparagus can be considered as an excellent source of these valuable food components for human diet.
Keywords: Spear, Mineral Elements, Flame Photometer, Atomic Absorption Spectrophotometer

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Additional Poster Number P-80

Moisture-Dependent Physical Properties of Seed of Iranian Edible Wild Asparagus

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A range of physical properties of seed of Iranian edible wild asparagus were determined as a function of moisture content. As the moisture content increased from 8.7 to 31.2% dry basis (d.b.), the average length, width, thickness and geometric mean diameter varied from 3.8 to 4.45, 3.36 to 3.93, 2.36 to 2.72 and 3.11 to 3.62 mm respectively. In the same moisture range, studies on rewetted asparagus seed showed that the sphericity, surface area and thousand seed mass increased from 0.807 to 0.822, 30.37 to 41.15 mm2 and 21.1 to 31.24 g respectively. As the moisture content increased from 8.7 to 31.2% d.b., the bulk density, true density and porosity were found to decrease from 630 to 530 kgm-3, 1360 to 1040 kgm-3 and 53.67 to 49.03%, whereas the terminal velocity, dynamic and static angle of repose were found to increase from 9.79 to 11.44 ms-1, 0.41 to 0.49° and 0.29 to 0.38°, respectively. The static coefficients of friction on rubber,

aluminum, plywood, glass, iron and galvanized iron sheet also increased linearly with increase in moisture content. The rubber and galvanized iron sheet offered the maximum and minimum friction respectively.
Keywords: Asparagus officinalis, Moisture content, Physical properties, Seed

Additional Poster Number P-81

Evaluation of Some Morphological Characteristics of Iranian Edible Wild Asparagus (Asparagus Officinalis L.)

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Asparagus (Asparagus officinalis L.) is an ancient vegetable originated from Europe, Asia and Africa. The germplasm of A. officinalis in Iran is limited and only some individual plants were found in Taleghan Mountains. In order to evaluate the morphological characteristics of this valuable vegetable, 58 individual plants within Taleghan population were studied. Results showed that the means of some evaluated traits such as plant height, number of first rate branches, diameter of main stem, length of first rate branches and number of scales under the first panicle branches were 133.81 cm, 38.38, 5.3 mm, 39.6 cm and 14.78 respectively. The evaluated genotypes were 68.97% male and 31.03% female. In studied plants, flowers were appeared from early May to early June and green fruits were formed about early July. The number of seeds per red mature berry fruit, which were collected around early September, was variable from 4 to 6 between evaluated plants. The simple correlation coefficient between measured characteristics showed that the spear diameter with length of spear scales had the highest positive correlation at 99% level. In factor analysis, the characteristics divided in four main factors that two first factors verify 44.74% of total variance and include 9 characteristics. Also the evaluated genotypes divided into five groups in cluster analysis and the means of all evaluated characteristics of third group, which completely consists of male genotypes, were higher than that of total mean. Results of this research showed that the wild asparagus genotypes had relatively high diversity and this locale could be considered as a natural origin of A. officinalis and the only genetic resource of this valuable plant should be preserved.
Keywords: Genotype, Spear, Correlation, Factor analysis, Cluster analysis

Additional Poster Number P-82

The Effects of Carbohydrate Source and Concentration on Somatic Embryogenesis of Strawberry (Fragaria x Ananassa Duch.)

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This experiment was performed to evaluate the effects of different types and concentrations of carbohydrate source on callus fresh weight, number of somatic embryos per embryonic explant and percentage of globular embryos developing into cotyledonary embryos from the leaf explants of three strawberry cultivars (Kurdistan, Paros and Camarosa). MS medium containing 1.0 mg/l 2,4-dichlorophenoxyacetic acid (2,4-D) and 0.5 mg/l 6-benzyladenine (BA) supplemented with three types of carbohydrates (sucrose, glucose and fructose) at concentration of 1.5%, 3%, 6%, 9% and 12% separately were tested. Among the different sugars tested, 6% sucrose was found superior not only for giving optimum embryo induction of embryonic culture but also uniform embryo developmental stages. Maximum number of 11.68, 12.68 and 13.35 globular embryos per explant, 81.53, 87.65 and 86.35% of globular embryos developing into cotyledonary embryos obtained for the cultivars of Kurdistan, Paros and Camarosa, respectively. Sucrose also was the best for proliferation of embryonic tissue with an optimal concentration of 3%.

Key words: Strawberry, Carbohydrates, Somatic embryogenesis

Additional Poster Number P-83

Effects of Mycorrhiza Fungi on Nutrient Uptake, Change of Chlorophyll and Visual Quality of Turfgrass in Different Concentrations of Humic Acid

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In these years, the using of mycorrhiza fungi (MF) as bio-fertilizer had been important. The aims of these factors are increasing of plant resistance to hard conditions. The aim of research was determination of inoculation with MF in different levels of humic acid (HA) on nutrient uptake changes of chlorophyll and visual quality of turfgrass plant in natural conditions in during 6o days. After autoclave of soils, the inoculums of MF including of Glomus intraradices was adding at pots and then seeds were cultivated. After of establishing of plants, HA was sprayed on leaves of plants at different levels (0, 100, 400, and 1000 mg/L) and measured up described on 9 weeks after starting of treatments. The results show that the concentration of

Fe, Zn, P and K and chlorophyll contents in plants inoculated by MF were better than non-MF plants and had a better visual quality. 100 and 400 mg/L treatments significantly increased the uptake of elements, and chlorophyll contents but with no effects on visual quality. Also 100,400 and 1000 mg/L treatments increased the amount of leaf K content rather than 0 mg/L. Moreover, time had significant effects on all the measurements.

Keywords: Humic acid, Glomus intraradices, Natural condition

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