1992-1994  Structural Biology Center founded by Sam Wilson, E. Brad Thompson and James C. Lee

1995-2002  David Gorenstein, Inaugural Director

2002-2005  James C. Lee, Interim Director

2005-2010  Vincent Hilser, Director

2010-2012  Wayne D. Bolen, Interim Director

2012-present B. Montgomery Pettitt, Director
20th Anniversary Structural Biology Symposium  2015

May 2, 2015
Sealy Center for Structural Biology and Molecular Biophysics
Symposium
The University of Texas Medical Branch

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Gabrielle Rudenko

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Junji Iwahara
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Javier Navarro
B. Montgomery Pettitt
Gabrielle Rudenko
Svetla StoiLOVA-McPhie
Scott Weaver
20th Annual Sealy Center for Structural Biology Symposium

Scientific Program
University of Texas Medical Branch
Levin Hall Main Auditorium
Galveston, TX

7:30 - 8:45 am  Registration, Breakfast - Foyer
                Poster Set-up – Foyer

8:45 - 9:00 am  Opening Remarks and Welcome address – B. Montgomery Pettitt
                Welcome Address—Sealy Foundation Family

Morning Session

Session Chair     Jim Lee

9:00 - 9:55 am   **Susan S. Taylor**
University of California, San Diego, CA
Howard Hughes Medical Institute, San Diego, CA
“Dynamic Assembly of Macromolecular Signaling Complexes”

Session Chair     Vince Hilser

9:55 - 10:50 am  **Lewis Kay**
University of Toronto, Toronto, ON
“Seeing the Invisible by NMR Spectroscopy”

10:50 - 11:05 am Break (Foyer)

Session Chair     Brad Thompson

11:05 - 12:00 pm **A. Keith Dunker**
Indiana University, School of Medicine, Indianapolis, IN
“Intrinsically Disordered Protein and the Origins of Complex Multicellular Organisms”
12:00 - 12:45 pm  Lunch – *Levin Hall Dining Room*

12:45 - 1:50 pm  Posters/Judging – *Foyer*

Afternoon Session

Session Chair  David Gorenstein

1:50 - 2:45 pm  **Junji Iwahara**
University of Texas Medical Branch, Galveston, TX
“*Protein Dynamics in DNA Recognition and Scanning*”

Session Chair  Samuel Wilson

2:45 - 3:35 pm  **Robert D. Wells**
Texas A&M Health Science Center, Houston, TX
“*DNA Structure Matters*”

3:35 - 4:15 pm  Break & Posters

Session Chair Wayne Bolen

4:15 - 5:10 pm  **George Rose**
Johns Hopkins University, Baltimore, MD
“*Conformational Entropy: The Neglected Free Energy Term in Protein Folding*”

5:10 - 5:20 pm  Poster Awards and Closing Remarks
Mark White and Marc Morais

5:20 - 6:00 pm  Pre- and Post-Doc Speaker Mixer – *Dining Room*

6:00 pm  Cocktails & Banquet
**Mario’s Italian Restaurant**
628 Seawall Blvd.
Galveston, TX  77550
The Gulf Coast Consortia (GCC) brings together the strengths of its member institutions to build interdisciplinary collaborative research teams and training programs in biological sciences at their intersection with the computational, chemical, mathematical, and physical sciences. GCC is comprised of seven prominent and geographically proximate Houston-Galveston area institutions, Baylor College of Medicine, Rice University, University of Houston, University of Texas Health Science Center at Houston, University of Texas Medical Branch at Galveston, University of Texas MD Anderson Cancer Center and the Institute of Biosciences & Technology at Texas A&M Health Science Center. GCC provides a unique, cutting edge collaborative training environment and research infrastructure beyond the capability of any single institution. It's mission is to train the next generation of biomedical scientists and to enable scientists to ask and answer questions that cross scientific disciplines to address the challenging biological issues of our time and, ultimately, to apply the resulting expertise and knowledge to the treatment and prevention of disease.

The training arm of the GCC, the Keck Center for Quantitative Bioscience Training, currently supports over 50 trainees and has over 400 affiliated training faculty through competitive grants from federal and state agencies. Within the Keck Center, the emphasis is on continuing its 25-year successful tradition of fostering interdisciplinary and multi-institutional training. The Keck Center provides a unique intellectual and physical setting in which to train the next generation of scientists with expertise in multiple disciplines, able to reach across boundaries to advance insight and understanding. The Keck Executive Committee formulates training policy in terms of didactic courses, seminars, workshops, retreats, selecting trainees, and advising Keck Fellows and mentors, while leaving individual program directors latitude to tailor the implementation of these to the unique needs of their program.

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 22 core members, 14 associate members and 6 managers (from 4 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.
SPONSORS
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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Keck Center for Interdisciplinary Bioscience Training

Molecular Dimension
Protein kinases have evolved to be dynamic, highly regulated molecular switches, and PKA serves as a prototype for this large gene family that regulates so much of biology. In addition to revealing the bi-lobal fold, the initial PKA structure showed how conserved sequence motifs cluster around the active site and how a single phosphate nucleates an active conformation. Searching later for spatially conserved motifs revealed two highly conserved hydrophobic “spines”. The assembled regulatory (R) spine is the hallmark signature of an active kinase, and the essential elements of the kinase switch are embedded within the assembly of the R-spine. To validate the importance of the spines and the switch mechanism we turned to cancer genes where in BRaf single hydrophobic mutations can drive assembly of the spines to create functional pseudo-kinases or constitutively active kinases that are unregulated.

While most kinases are regulated by assembly/disassembly of the R-spine, PKA is packaged as a holoenzyme whose activity is regulated by cAMP. There are four functionally non-redundant regulatory subunits, and the R$_2$C$_2$ holoenzymes are assembled as part of targeted macromolecular “signalosomes”. Surprisingly the four holoenzymes are structurally very different, which allows us to appreciate how the R$_2$C$_2$ holoenzymes are assembled in an isoform-specific way that leaves them dynamically poised for allostERIC activation by cAMP. Mosaic brain imaging of RIIb and RIIb further highlights the importance of isoform specificity.
An understanding of the role played by a protein in cellular function requires a detailed picture of its three-dimensional structure as well as an appreciation of how the structure varies as a function of time due to molecular dynamics. Proteins are not static and often interconvert to states higher in energy than the ground conformation that play important roles in biological function. These so called excited states are often ‘invisible’ in biophysical studies because of their low population and transient formation. Here I will describe NMR approaches for studying invisible states at atomic resolution. A number of examples will be presented including include protein folding, where the pathway from unfolded to folded protein proceeds through formation of excited intermediate states, and protein misfolding, where partially folded high energy intermediates have been implicated as the starting points for aggregation and formation of cytotoxic oligomers that are involved in a host of human diseases.
“Intrinsically Disordered Protein and the Origins of Complex Multicellular Organisms”

A. Keith Dunker1*, Karl J. Niklas2, Sarah E. Bondos3, Fei Huang1, Christopher J. Oldfield1
1Indiana University School of Medicine, Center for Computational Biology and Bioinformatics, Indianapolis, IN 46202, USA; 2Cornell University School of Integrative Plant Science, Plant Biology Section, Ithaca, NY 14853 USA, 3Texas A&M Health Science Center, Department of Molecular and Cellular Medicine, College Station, TX 77843 USA
* Corresponding Author kedunker@iupui.edu

Abstract

DNA sequencing has enabled the widespread construction of phylogenetic trees, revealing that multicellular organisms evolved independently from unicellular ancestors about 25 times among prokaryotes and eukaryotes. Multicellular organisms can be classified as simple, in which all of the cells are in direct contact with the surrounding milieu, or complex, in which some cells are completely surrounded by other cells. Current phylogenetic trees indicate that complex multicellular organisms evolved independently from unicellular ancestors about 10 times, and only among the eukaryotes, including once for animals, twice each for green, red, and brown algae, and thrice for fungi.

Given these multiple independent evolutionary lineages, we asked two questions: 1. Which molecular functions underpinned the evolution of multicellular organisms?; and, 2. Which of these molecular functions depend on intrinsically disordered proteins (IDPs, reviewed in [1])? The former requires the advent of molecules for cellular adhesion, for cell-cell communication and for developmental programs. In addition, the developmental programs need to be regulated over space and time. Finally, each multicellular organism has cell-specific biochemistry. To answer the second question we used Key-words in Swiss Protein ranked for associations with predictions of protein structure or disorder. With a Z-score of 18.8 compared to random-function proteins, “differentiation” was the biological process most strongly associated with IDPs. As expected from this result, large numbers of individual proteins associated with differentiation exhibit substantial regions of predicted disorder [2]. All five of the underpinning molecular functions for multicellularity were found to depend critically on IDP-based mechanisms [3].

These new findings necessitate a rethinking of the gene regulatory network models currently used to explain cellular differentiation and the evolution of complex multicellular organisms [4].

Junji Iwahara

Associate Professor, Department of Biochemistry & Molecular Biology, Sealy Center for Structural Biology & Molecular Biophysics, University of Texas Medical Branch

When transcription factors and DNA repair/modifying enzymes perform their function, these molecules must first locate their specific target sites in the vast presence of nonspecific but structurally similar sites on DNA. We seek to better understand DNA recognition and scanning by proteins. Using NMR spectroscopy, fluorescence spectroscopy, biochemical assays, and computational simulations, we study how proteins move on DNA at both molecular and atomic levels. Our research on the Egr-1 zinc-finger and Antp homeodomain proteins reveals the kinetic and thermodynamic importance of dynamic conformational ensembles of the proteins in their DNA recognition and scanning processes. In my talk, I will introduce our recent results on the dynamics of the intermolecular ion pairs at protein-DNA interfaces as well as on the inter-domain dynamics of the zinc-finger proteins in the DNA-scanning process. Insights from these studies provide strategies for engineering of artificial transcription factors, DNA modifying enzymes, and DNA itself.
I convey my congratulations and appreciation to Dr. Sam H. Wilson for his administrative and research accomplishments on this 20th anniversary of his founding of the UTMB Structural Biology Symposium.

I work on non-B DNA conformations, mutagenesis, and human diseases. A group of >20 hereditary neurological diseases are associated with simple DNA sequence amplifications. The dynamic mutations are the molecular basis for myotonic dystrophy, Huntington disease, fragile X syndrome, ALS, and Friedreich ataxia. For type 2 diseases, the expansions are massive (to thousands of repeats) whereas in type 1 diseases, the triplet repeats are in coding regions and elicit a modest expansion of a polyamino acid tract (usually polyglutamine). More severe neurological syndromes are found in patients with longer repeat tracts. We have shown that expansions and deletions are mediated by DNA replication, repair, and recombination in a variety of in vitro and in vivo systems, probably acting in concert. The slippage of the DNA complementary strands to form non-B DNA structures, such as hairpin loops or slipped conformations, with differing relative stabilities are important components in the mechanisms.

Also, simple repeating DNA tracts in chromosomes adopt non-B DNA structures such as triplexes, cruciforms, slipped structures, left-handed Z-DNA, and tetraplexes which are mutagenic. The mutagenesis is due to the non-B DNA conformations rather than to the DNA sequence per se in the orthodox right-handed Watson-Crick B-form. The human genetic consequences of these non-B structures are ~20 neurological diseases and ~50 genomic disorders, such as polycystic kidney disease, adrenoleukodystrophy, and spermatogenic failure (caused by gross deletions, inversions, duplications and translocations).

The recent convergence of bioinformatics, molecular biology, genetics, and genomic studies has enabled a new paradigm implicating the non-B DNA conformations, not the sequences as such, as the mutagenesis specificity determinants.

“Conformational Entropy: The Neglected Free Energy Term in Protein Folding”

George Rose
Johns Hopkins University, Jenkins Department of Biophysics, 3400 N. Charles Street, Baltimore, MD 21218 grose@jhu.edu

Typical protein folding studies focus on side chain interactions that stabilize the folded state, the hydrophobic effect especially. Such studies are anchored in the plausible viewpoint that (i) the unfolded state lacks any significant degree of organization and (ii) conformational discrimination must arise via interactions between and among side chains, that component of the structure which differs from one residue to the next. In contrast to this viewpoint, protecting osmolytes, that force folding, operate predominately on the polar backbone in the unfolded state. Osmolytes are small, ubiquitous, organic molecules that have no net charge. A diverse repertoire of such compounds is utilized throughout all three kingdoms of life. We speculate that under folding conditions, most conceivable protein conformations are highly disfavored because they encounter steric clashes or result in backbone polar groups with dangling hydrogen bonds. These two factors winnow the population to a comparatively small subset of thermodynamically viable conformations, at which point sequence differences discriminate among the remaining conformational alternatives.
Map to the Banquet at Mario’s Italian Restaurant

1001-1099 Market St
Galveston, TX 77550

1. Head east on Avenue D toward 10th St
2. Turn right onto 6th St/University Blvd
   - Continue to follow 6th St
   - Destination will be on the right

Mario’s Seawall Italian Restaurant
628 Seawall Blvd, Galveston, TX 77550
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P-3 Meenakumari Muthuramalingam Initiation of Structural Studies of ParDE Toxin-Antitoxin Systems

P-4 Tim Dosey TRPV Channel Structure Determined By Cryo-EM

P-5 Prem Raj Joseph Solution NMR Characterization of Chemokine CXCL8 Monomer and Dimer Binding to Glycosaminoglycans: Structural Plasticity Mediates Differential Binding Interactions

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P-7 Thomas Lucas Overcoming the Protein Misfolding and Aggregation of Mitochondrial Polymerase I

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P-12 David Marciano Cooperativity Enhances Mutational Robustness of a Negative Auto-regulation Transcription Factor

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P-14 Tahani Alshammari Genetic Deletion of Fgf14 Recapitulates Molecular, Cellular and Circuit Alterations Underlying Cognitive Impairment Associated With

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Structure Poster Number P-1

Discovery of a Small Molecule that Specifically Binds the Histone mRNA Stem-Loop of the Malarial Parasite *Plasmodium Falciparum*

Roopa Thapar\(^1,2,3\), Sai P. Velagapudi\(^4\), Edward P. Nikonowicz\(^1\), Mark A. Titus\(^5\), & Matthew D. Disney\(^4\)

(1) BioSciences at Rice, Rice University (2) Hauptman-Woodward Medical Research Institute (3) Department of Structural Biology, SUNY at Buffalo, NY 14203, USA (4) The Scripps Research Institute, Jupiter, FL 33458, USA (5) Department of Genitourinary Medical Oncology, The University of Texas M.D. Anderson Cancer Center, Houston TX 77030, USA

Malaria is a life-threatening disease that kills millions of people worldwide. Malaria is caused by the parasite *Plasmodium falciparum* (*P. falciparum*) that is transmitted by insect vectors. Drug treatments for malaria are antiquated and development of drug resistance in the parasites is an enormous problem. Thus, there is an urgent need to identify novel drug targets to treat this deadly disease. Cellular pathways that differ between parasite and host represent possible sites of attack. We propose to exploit the differences in the *P. falciparum* and *H. sapiens* genomes, particularly in the RNA elements and RNA binding proteins that regulate histone biogenesis, as an avenue for design of novel anti-malarial drugs. In metazoans, the mRNAs that encode histone proteins are regulated posttranscriptionally, and while much is known about how these replication-dependent histone mRNAs are processed and degraded\(^4\), almost nothing is known about histone mRNA metabolism in *P. falciparum*. Intriguingly, genome sequencing projects and bioinformatics studies indicate that protozoan histone mRNAs are likely regulated in a manner reminiscent of that in humans, but with critical differences that may be exploitable for drug development. To identify small molecules that bind the histone H3 *P. falciparum* stem-loop, we used a lead identification strategy called Informa. The Informa computational approach identified a novel molecule we call Histone Loop Binder 1 (HLB-1) that preferentially bound the *P. falciparum* histone tetraloop compared to the *H. sapiens* tetraloop or a GNRA loop sequence. To gain structural insight into the mode of recognition of the *P. falciparum* H3 stem-loop (pfSL28) by HLB-1, we determined the solution structure of the free and HLB-1 bound *P. falciparum* histone H3 RNA stem-loop by NMR Spectroscopy. HLB-1 inhibits complex formation between *P. falciparum* SLBP and the *P. falciparum* histone H3 mRNA stem-loop in vitro, suggesting that the *P. falciparum* histone mRNA stem-loop/SLBP complex may be an attractive target for development of new drug leads in the treatment of malaria.
Structure and Functional Insights Revealed By the Docking Interface of the *Saccharomyces Cerevisiae* Histidine Phosphotransfer Protein Ypd1 and Its Downstream Response Regulator Ssk1

Katie Branscum¹, Smita Menon¹, Clay Foster¹, & Ann West¹
(1) Chemistry & Biochemistry, University of Oklahoma, 101 Stephenson Parkway, Norman, OK, 73019

Lower eukaryotes such as plants and fungi respond to environmental stresses using signal transduction pathways known as His-Asp multi-step phosphorelays, similar to bacterial two-component systems. The model yeast organism, *Saccharomyces cerevisiae*, uses the Sln1 pathway to respond to osmotic and oxidative stress. This pathway contains a membrane-bound hybrid histidine kinase (Sln1) that autophosphorylates and transfers a phosphoryl group to its C-terminal receiver domain. The phosphoryl group is then transferred to cytoplasmic histidine phosphotransfer (HPt) protein Ypd1, which can then shuttle phosphoryl groups to one of two downstream response regulators: Ssk1 or Skn7. This choice is environmentally dependent. There is evidence that Ypd1 uses the same hydrophobic docking site to bind the receiver domains of Sln1, Ssk1 and Skn7. However, residues that influence interaction specificity are unknown. In the current literature, structural data for response regulators in complex with their upstream phosphodonors is sparse. In this work, we present the co-crystal structure of Ypd1 in complex with the receiver domain of Ssk1 at a resolution of 2.8 Å and highlight unique characteristics that may explain differences in protein-protein interactions involving Ypd1.
Structure Poster Number P-3

Initiation of Structural Studies of ParDE Toxin-Antitoxin Systems

Meenakumari Muthuramalingam¹, & Christina R. Bourne¹
(1) Dept. of Chemistry & Biochemistry, University of Oklahoma, Norman, OK, 73019-6111

Toxin-antitoxin (TA) systems are present throughout the genome of bacteria and archaea. In general TA system consists of dicistronic operons encoding two genes; one for a toxic protein and another for antitoxin which neutralizes its target toxin (Hayes F, 2003 & Leplae et al., 2011). TA systems have been reported to play numerous physiological roles, including formation of persister cells (Maisonneuve et al., 2011), stress resistance (Christensen et al., 2001), protection from bacteriophages (Koga et al., 2011) and regulation of biofilm formation (Wang et al, 2011). Type II TA systems are highly abundant and increasingly recognized from genomic sequencing efforts.

The main goal of this project is to analyze type II TA system ParDE from different organisms at functional and structural level. Even though advanced computational methods are available, it is difficult to annotate or classify TA operons based on just the sequence due to low conservation and the lack of clearly recognizable sequence motifs. Hence it is important to characterize and validate the individual system experimentally. ParE toxin genes have been shown to inhibit cell growth and cause cell filamentation. Therefore the toxin-antitoxin genes from Pseudomonas aeruginosa, Vibrio cholera, Caulobacter crescentus and Agrobacterium tumefaciens were cloned in expression systems and large scale recombinant protein purification was undertaken. The toxin genes were also screened for filament formation, one indicator of an active ParE gene. Out of the toxin genes tested, C. crescentus parE and A. tumefaciens toxin genes (T-10, 12 and 13) showed filamentous phenotype. Here we present the methodology to characterize the individual proteins and aims to prepare them for crystallization to get detailed understanding of structural interaction between the toxins and antitoxin. The systematic correlation of the highly variable ParE sequences with functional and structural data will facilitate understanding how prevalent they are and begin to answer what role they may play in bacterial physiology.
TRPV Channel Structure Determined By Cryo-EM

Tim Dosey¹, Wang Zhao¹, Guizhen Fan¹, Irina Serysheva¹, Wah Chiu¹, & Theodore Wensel¹
(1) Dept. Department of Biochemistry and Molecular Biology, Baylor College of Medicine, Houston, TX, Baylor College of Medicine (2) Integrative Molecular and Biomedical Sciences Program, Baylor College of Medicine, Houston, TX (3) Dept. of Biochemistry and Molecular Biology, University of Texas Medical School, Houston, TX

In the Protein Data Bank there are over 100,000 available structures for soluble proteins, but only about 2,000 structures for transmembrane proteins (TMP). This large disparity is due to the difficulty in expressing and purifying milligram amounts of TMPs for x-ray crystallography as well as the huge physical barrier of forming crystals. The difficulty in solving TMP structures has created a significant unmet need in biomedical science, as an estimated 30% of the human genome encodes TMPs and over 50% of FDA approved pharmaceuticals target TMPs. Therefore, there is much opportunity for advancement in the field of TMP structure determination, and new methodologies should be more aggressively pursued in order to solve these structures which can assist in the design of new therapeutics.

An alternative method for solving molecular structures is single particle cryo-EM. With this method, the two major problems for solving TMPs structures are not an issue because crystallization of the protein is unnecessary and microgram quantities of the protein is sufficient. Although, until recently, cryoEM structures for TMPs seldom surpassed sub-nanometer resolution whereas x-ray crystallography could achieve at least near-atomic resolution for TMPs. However, recent advancements in electron-detecting camera technology and data processing software have enabled near-atomic resolution for TMPs to be achieved.

The TMPs we have focused on are Transient Receptor Potential (TRP) channels which form a superfamily of non-selective cation channels conserved throughout the eukaryotic domain. In mammals, TRP channels are best understood for their roles in sensory perception; however, not a single member of this ion channel superfamily has a solved crystal structure. Therefore, our understanding of how these channels function in response to a diverse range of stimuli has been greatly impeded. In our research, we have tested the ability to heterologously express and purify multiple TRP channels in a Saccharomyces Cerevisiae expression-system and we have found TRPV2 to be the most promising candidate for structural determination via cryoEM and we are currently working toward refining our structure to near-atomic resolution. Furthermore, in our pursuit of this structure, we have discovered a modification that can be made to multiple TRP channels which greatly increases their expression in this yeast expression system, perhaps opening the door for many future TRP channel structures and the possibility of using these purified proteins to screen for interacting small molecules with therapeutic potential.
Solution NMR Characterization of Chemokine CXCL8 Monomer and Dimer Binding to Glycosaminoglycans: Structural Plasticity Mediates Differential Binding Interactions

Prem Raj Joseph¹, Junji Iwahara¹, Umesh R. Desai², & Krishna Rajarathnam¹
(1) Biochemistry and Molecular Biology, University of Texas Medical Branch, Galveston, TX 77555 (2) Department of Medicinal Chemistry, Virginia Commonwealth University, Richmond, VA

Chemokine CXCL8 plays a crucial role in directing neutrophils and oligodendrocytes to combat infection/injury and tumor cells in metastasis development. CXCL8 exists as monomers and dimers, whose interactions with glycosaminoglycans (GAGs) mediate these diverse cellular processes. Very little is known regarding the structural basis underlying CXCL8-GAG interactions. Moreover, there are conflicting reports on the affinities, geometry, and whether the monomer or dimer is the high-affinity GAG ligand. To resolve these issues, we characterized the binding of a series of heparin-derived oligosaccharides (dp2, dp4, dp8, and dp14) to the WT dimer and a designed monomer using solution NMR spectroscopy. The pattern and extent of binding-induced chemical shift perturbation varied between dimer and monomer and between longer and shorter oligosaccharides. NMR-based structural models showed that GAGs adopted multiple geometries, and that the nature of interactions varied between monomer and dimer and between shorter and longer oligosaccharides. GAG binding studies carried out under conditions where WT CXCL8 exists as monomers and dimers provide unambiguous evidence that dimer is the high-affinity GAG ligand. Together, our data indicate that a set of core residues function as the major recognition/binding site and a set of peripheral residues define the various binding geometries, and that the structural plasticity of the individual GAG-binding residues allows multiplicity of binding interactions. We conclude that structural plasticity is an intrinsic property of GAG-binding residues, and that this plasticity regulates in vivo CXCL8 monomer/dimer-GAG interactions and function.
Structure Poster Number P-6

Biochemical and Structural Characterization of Aryl-Alcohol Oxidase (GloA) From the Thermophilic Fungus Myceliophthora Thermophile

Marco Antonio Seiki Kadowaki¹,², & Mariana Ortiz de Godoy¹, Igor Polikarpov², & Rolf Alexander Prade¹
(¹) Microbiology and Molecular Genetics, Oklahoma State University (²) Physics Institute of São Carlos. São Paulo University. São Carlos. São Paulo-BR

Lignocellulosic polymers are a massive, renewable, and available source for production of biofuels and biochemicals. Fungi such as *Myceliophthora thermophila* are able to secrete an array of hydrolytic and oxidative enzymes responsible for biodegradation. Oxidative enzymes such as aryl-alcohol (GloA) oxidases are specially involved in biomass degradation by hydrogen peroxide production. In this work the flavoenzyme GloA was cloned and expressed using the close expression system *Aspergillus nidulans*. The enzyme was purified using two simple steps (ionic exchange and size exclusion chromatographic) and identified by mass spectrometry (MALD-TOF) prior to enzymatic and structural studies. The UV-visible absorption spectra shows two well define peaks (368 and 454 nm) known as FAD containing enzyme signature. Enzymatic assays showed that GloA prefers aromatic alcohols with an optimum pH and temperature of 50 °C and 7.0. The low-resolution molecular envelope in solution accessed by Small Angle X-ray Scattering indicates a monomeric globular shaped enzyme with presumed polar glycosylation loop extensions. GloA was crystallized in two different forms and the SAXS data are been used for initial phase calculations.
Structure Poster Number P-7

Overcoming the Protein Misfolding and Aggregation of Mitochondrial Polymerase I

Thomas Lucas¹, & Whitney Yin¹
(¹) Department of Biochemistry and Molecular Biology, The University of Texas Medical Branch at Galveston, Galveston, TX, 77555

Mitochondrial Polymerase I (MIP1) is the only polymerase found inside the mitochondria of S. cerevisiae. It is responsible for all replication and repair of the mitochondrial genome. MIP1 is unlike most other known mitochondrial polymerases in that it functions as a monomer whereas the mitochondrial polymerases of higher order eukaryotes require additional accessory proteins in order to function. Our lab is specifically interested in the unique C-terminal extension domain of MIP1, which appears to possess its own distinct structure and functions as an intramolecular accessory domain. However, the investigation of this protein has been hindered due to issues of protein misfolding and subsequent problems with the purification process which have been overcome. Our lab was able to use low temperature induction with arctic cells to correct the misfolding of the protein and the addition of a DNA affinity step in the presence of a stabilizing agent betaine to successfully isolate pure and active MIP1.
Probing the Structural and Molecular Basis of Nucleotide Selectivity By Human Mitochondrial Polymerase γ

Michal R. Szymanski¹², Christal D. Sohl³, Sheida Amiralaei⁴, Ray Schinazi sup>4, Karen S. Anderson³, & Whitney Yin¹²

¹ Department of Pharmacology, The University of Texas Medical Branch at Galveston  (2) Sealy Center for Structural Biology, University of Texas Medical Branch Galveston, Texas 77555, USA.  
³ Department of Pharmacology, Yale University School of Medicine, New Haven, Connecticut 06520, USA.  
⁴ Center for AIDS Research, Department of Pediatrics, Emory University School of Medicine, Atlanta, Georgia 30322, USA.

Nucleoside analog reverse transcriptase inhibitors (NRTIs) are the essential components of highly active antiretroviral (HAART) therapy targeting HIV reverse transcriptase (RT). NRTI triphosphates (NRTI-TP), the biologically active forms, act as chain terminators of viral DNA synthesis. Unfortunately, NRTIs also inhibit human mitochondrial DNA polymerase (Pol γ), causing unwanted mitochondrial toxicity. Understanding the structural and mechanistic differences between Pol γ and RT in response to NRTIs will provide invaluable insight to aid in designing more effective drugs with lower toxicity. The NRTIs emtricitabine [(-)-2,3'-dideoxy-5-fluoro-3'-thiacytidine, (-)-FTC] and lamivudine, [(-)-2,3'-dideoxy-3'-thiacytidine, (-)-3TC] are both potent RT inhibitors, but Pol γ discriminates against (-)-FTC-TP by two orders of magnitude better than (-)-3TC-TP. Furthermore, while (-)-FTC-TP is only slightly more potent against HIV RT than its enantiomer (+)-FTC-TP, it is discriminated by human Pol γ four orders of magnitude more efficiently than (+)-FTC-TP. As a result, (-)-FTC is a much less toxic NRTI. Here, we present the structural and kinetic basis for this striking difference by identifying the discriminator residues of drug selectivity in both viral and human enzymes responsible for substrate selection and inhibitor specificity. This work, for the first time, illuminates the mechanism of (-)-FTC-TP differential selectivity and provides a structural scaffold for development of novel NRTIs with lower toxicity.
Structure Poster Number P-9

**Full-Length Dengue Virus Nonstructural Protein 5 (NS5) Assembles into a Dimer With a Unique Methyltransferase and Polymerase Interface**

Valerie Klema¹, Mengyi Ye¹, Aditya Hindpur¹, Tadahisa Teramoto², Keerthi Gottipati¹, Radhakrishnan Padmanabhan², & Kyung H. Choi¹

(1) Dept. of Biochemistry & Molecular Biology, University of Texas Medical Branch, Galveston, TX (2) Dept. of Microbiology & Immunology, Georgetown University School of Medicine, Washington D.C., 20057

Flavivirus nonstructural protein 5 (NS5) catalyzes three essential yet distinct chemical reactions required for viral genome replication. The methyltransferase (MTase) domain of NS5 is required for both RNA capping and methylation to form a cap I structure at the 5’-end of the RNA genome, whereas the RNA-dependent RNA polymerase (RdRp) domain carries out RNA synthesis. The physical linkage of the MTase and RdRp domains within a single polypeptide chain suggests that RNA synthesis and 5’-RNA capping/methylation may be coupled during genome replication.

We report the crystal structure of full-length dengue virus NS5. Eight molecules of NS5 are arranged as four independent dimers in the crystallographic asymmetric unit. The relative orientation of each monomer within the dimer, as well as the orientation of the MTase and RdRp domains within each monomer, is conserved, suggesting that these structural arrangements represent the biologically relevant conformation and assembly of this multifunctional enzyme. Furthermore, many NS5 residues known to reduce viral replication are located at either the inter-domain interface within a monomer or at the inter-molecular interface within a dimer. The X-ray structure of NS5 presented here suggests not only that the enzyme functions as a dimer during viral genome replication, but also that the MTase and RdRp activities could be structurally coupled. These results provide new insights to aid the development of novel antiviral inhibitors targeting both dengue virus itself as well as other members of Flaviviridae.
Structure Poster Number P-10

Oxidative Damage Changes the Structure and Function of Human Mitochondrial Single Stranded DNA Binding Protein

Andrew Anderson¹, & Whitney Yin²
(1) Structural Computational Biology and Molecular Biophysics, Baylor College of Medicine (2) Dept. of Pharmacology & Toxicology, UTMB, Galveston, TX, 77555

The human mitochondrial genome contains the genes for 13 essential proteins, 2 rRNAs, and 22 vital tRNAs. Human mitochondrial Single Stranded DNA Binding Protein (SSB) is responsible for the stimulation of multiple elements of the mitochondrial replisome, including at the DNA Polymerase (Pol γ) and helicase. The mitochondrial matrix is host to significant quantities of reactive oxygen species, which are generated continuously by the electron transport chain even while operating normally. Oxidation of human mitochondrial single stranded binding protein has a marked effect on the ssDNA binding affinity and binding modes exhibited. This is due to disruption of the charge based, arginine-rich, protein-ligand interface rather than a catastrophic disruption of protein structure. A 3-fold loss of affinity, in the oxidized form, is coupled with a shift in binding mode, and considering that SSB binding is synergistic over large strands of ssDNA, this oxidation will cause a large shift in binding.
Structure Poster Number P-11

PS-GC Nanodiscs Assembly for Structural Studies of Coagulation Proteins and Their Complexes.

Kirill Grushin¹, & Svetla Stoilova-McPhie¹²,
(¹) Dept. of Neuroscience & Cell Biology, UTMB (²) Sealy Center for Structural Biology and Molecular Biophysics, UTMB, Galveston, TX, 77555

Nanodiscs (ND) are well known as a lipid membrane platform for structural and functional studies of transmembrane proteins but they can be also helpful for structural studies of membrane associated proteins. Factor VIII active form (FVIIIa) within the Factor VIIIa - Factor IXa (Tenase) complex is crucial for normal hemostasis and requires binding to the surface of the negatively charged activated platelet membrane. Despite the critical role of FVIII for coagulation process, the knowledge of its membrane-bound organization alone or within the Tenase complex is incomplete which hampers drug discovery for effective regulation of the complex activity and thus design of new pro- and anti-coagulant drugs. In this study we present our work on ND designed for structural studies of membrane-bound FVIII by transmission electron microscopy (TEM) and single-particle analysis (SPA). ND assembled from galactosylceramide (GC) and negatively charged phosphatidylserine (PS) lipid mixtures, and with two different membrane scaffolding proteins forms (MSP1D1 and MSP1E3D1) were characterized by negatively stained TEM showing that their size and homogeneity strongly depended on the lipid composition and lipid to membrane scaffolding protein ratio. Our results show that the ND with highest PS content (80%) and average diameter of 12 nm are the most suitable for structure determination of the membrane-bound FVIII molecules by single particle analysis. Our preliminary 3D reconstruction of the FVIII bound to the PS containing ND demonstrates the suitability of the optimized ND for structural studies by TEM and SPA. Further assembly of the FVIIIa and the whole FVIIIa-FIXa complex on ND will help to identify the protein-protein and protein-membrane interfaces critical for the Tenase complex assembly and function.
Cooperativity Enhances Mutational Robustness of a Negative Autoregulation Transcription Factor

David C. Marciano1*, Rhonald C. Lua1*, Christophe Herman1, & Olivier Lichtarge1,2,3
(1) Molecular & Human Genetics, Baylor College of Medicine (2) Verna & Marrs McLean Department of Biochemistry & Molecular Biology, Baylor College of Medicine (3) Computational & Integrative Biomedical Research Center, Baylor College of Medicine; * These authors contributed equally

Biological systems are known to be robust to external perturbations and intrinsic fluctuations. In the bacterium *Escherichia coli*, transcriptional factors often repress their own expression to form a negative-feedback network motif that enables robustness to changes in biochemical parameters. However, the response of negative feedback networks to deleterious mutations in the transcription factor have not been treated theoretically and tested experimentally. Here we present a simple phenomenological model of a negative feedback transcription factor repressing both itself and another target gene. Analysis of the model shows that the target gene levels are robust to mutations of the transcription factor, and that the robustness improves as the degree of cooperativity in self-repression increases (modeled as a Hill coefficient). The prediction is tested in the LexA transcriptional network of *E. coli* by altering cooperativity of the system. Indeed, the robustness of target gene expression to deleterious mutations in LexA is dependent upon the Hill coefficient of the negative feedback system. Considering the proposed importance of gene regulation in speciation, parameters governing a transcription factor's robustness to mutation may have significant influence on an organism's capacity to evolve.
Regulation of Adult Neurogenesis By FGF14 as a Potential Mechanistic Link to Human Brain Disorders

Musaad Alshammari\textsuperscript{1,2,4}, Tahani Alshammari\textsuperscript{1,2,4}, & Fernanda Laezza\textsuperscript{3,4}
(1) Department of Pharmacology & Toxicology, The University of Texas Medical Branch, Galveston, TX, 77555, (2) King Saud University Graduate Studies Abroad Program, KSU, Saudi Arabia, (3) Mitchell Center for Neurodegenerative diseases, UTMB, Galveston, TX, 77555, (4) Dept. of Pharmacology & Toxicology, UTMB, Galveston, TX, 77555

Adult neurogenesis, the production of mature neurons from progenitor cells in the adult mammalian brain, is linked to the etiology of neurodegenerative and psychiatric disorders. However, a thorough understanding of the molecular elements at the base of adult neurogenesis remains elusive. Identifying new factors required for neural stem cell proliferation, migration, maturation and integration into the synaptic hippocampal circuit could inform the pathogenesis of a variety of brain disorders. Using a combination of BrdU incorporation studies and confocal imaging, we discovered that genetic deletion of fibroblast growth factor 14 (fgf14), a brain disease-associated factor that controls neuronal excitability and synaptic plasticity, leads to previously undescribed alterations in adult neurogenesis in the dentate gyrus (DG) of the hippocampal region. We show that fgf14\textsuperscript{-/-} mice exhibit an increase in the immature population of doublecortin and calretinin positive neurons and a reduction in mature neurons expressing calbindin, while early progenitor stem cells in the DG remained intact. In humans mutations of the fgf14 gene are the genetic cause of spinocerebellar ataxia 27, a complex neurodegenerative disorder associated with cognitive and motor deficits. Thus, while providing evidence for a novel regulator of adult neurogenesis, this study provides potential new insights to the complex pathology associated with disrupted FGF14 function in human diseases.
Biochemistry Poster Number P-14

Genetic Deletion of Fgf14 Recapitulates Molecular, Cellular and Circuit Alterations Underlying Cognitive Impairment Associated With Human Psychiatric Disorders

Tahani Alshammari\textsuperscript{1,2,8}, Musaad Alshammari\textsuperscript{1,2,8}, Miroslav Nenov\textsuperscript{8} Eriola Hoxha\textsuperscript{3}, Andrea Marcinno\textsuperscript{3}, Marco Cambiaghi\textsuperscript{3}, Thomas James\textsuperscript{7}, Benedetto Sacchetti\textsuperscript{3}, Filippo Tempia\textsuperscript{3,8}, & Fernanda Laezza\textsuperscript{4,5,6,8}

(1) Department of Pharmacology and Toxicology, The University of Texas Medical Branch (2) King Saud University Graduate Studies Abroad Program, King Saud University, Saudi Arabia (3) Department of Neuroscience, University of Torino, Italy (4) Mitchell Center for Neurodegenerative Diseases, (5) Center for Addiction Research, (6) Center for Biomedical Engineering, (7) Department of Neuroscience, (8) Department of Pharmacology and Toxicology, University of Texas Medical Branch, Galveston, TX, 77555

Cognitive processing is highly dependent on the functional integrity of gamma-amino-butyric acid (GABA) interneurons in the brain. These cells regulate excitability of principal neurons balancing the excitatory/inhibitory tone of cortical networks. Reduced function of parvalbumin interneurons (PVIs) and disruption of GABAergic synapses result in desynchronized cortical circuitry associated with cognitive impairment across many psychiatric disorders including schizophrenia, bipolar disease, and depression. Yet, the mechanisms underlying these phenotypes are still poorly understood. Here, we show that in animal models genetic deletion of fibroblast growth factor 14 (Fgf14), a resident protein of the axonal initial segment (AIS), regulator of neuronal excitability and synaptic transmission, and an emerging brain disease-associated factor, leads to loss of PVIs hippocampal CA1 region, a critical area for cognitive function. This cellular phenotype associates with decreased expression of glutamic acid decarboxylase 67 (GAD67) and vesicular GABA transporter (VGAT) at GABAergic presynaptic puncta. Coincides with loss in frequency and amplitude of spontaneous and miniature inhibitory synaptic events in CA1 pyramidal neurons, reduced in vivo gamma frequency oscillations and impaired working memory. Together these phenotypes recapitulate salient molecular, cellular, functional and behavioral features associated with cognitive impairment in complex brain disorders, adding FGF14 to the repertoire of potential risk factors for psychiatric disorders.
Biochemistry Poster Number P-15

Targeting Protein: Protein Interaction Sites “Hotspots” Within the Macromolecular Complex of the Voltage-Gated Sodium Channels as a Novel Drug Development Approach

Syed R Ali¹, Zhiqing Liu¹, Miroslav N. Nenov¹, Neli I. Panova-Elektro¹, Jia Zhou¹, & Fernanda Laezza¹
(1) Department of Pharmacology and Toxicology, The University of Texas Medical Branch Galveston, TX, 77555

Fibroblast growth factor 14 (FGF14) is a functionally relevant accessory protein of the neuronal Nav channel. Through a monomeric interaction with the intracellular C-terminus of Nav channels, FGF14 modulates Na+ currents in a Nav isoform-specific manner serving as a fine-tuning regulator of excitability. In previous studies we have reconstituted the FGF14:Nav1.6 complex in live cells using the split-luciferase complementation assay (LCA) and through site-direct mutagenesis identified “hot-spots” at the FGF14 surface critical for binding to Nav1.6. Based on in silico studies, we have designed short peptide fragments that align with the FGF14 β12-strand and β8-β9 loop and validated their in-cell activity as inhibitors of the FGF14:Nav1.6 complex. One peptide, Fpep1, we have generated novel peptidomimetics that are currently being evaluated. Small molecule inhibitors (SMI) and/or peptidomimetics targeting druggable pockets at the FGF14 β8-β9 and β12 might give rise to a new class of unconventional protein: protein interaction-based allosteric modulators of Nav channels. We expect our studies to have a broad impact in the drug design against a wide range of still untreatable brain disorders associated with Nav channel dysfunction.
A Cell-Free Investigation of the Relationship Between Myoglobin Expression, Globin Stability and Heme Affinity

Premila P. Samuel¹, George N. Phillips¹, & John S. Olson¹

(1) Biosciences, Rice University, 6100 Main St, Houston, TX 77005

We have successfully developed a wheat germ extract cell-free assay to analyze the relationship between myoglobin (Mb) expression levels and both globin stability and heme affinity. A major advantage of this assay over measurements of Mb production in *E. coli* is the decoupling of cellular homeostasis with protein expression. Mb variants were selected to include highly stable Mbs of deep diving mammals, unstable Mbs of terrestrial mammals, and Mb mutants with apolar mutations in the heme pocket. Based on our in vitro assay, there is a strong linear correlation between the quantified in vitro expression levels of fully folded Mb variants and their corresponding apoMb unfolding parameters measured independently with purified proteins. ApoMb stability was more critical for holoMb expression than hemin affinity. Higher expression levels were observed for Mb mutants with heme pocket apolar mutations that significantly increase apoMb stability but decrease heme affinity. These results confirm previous qualitative and anecdotal studies of holoMb expression in *E. coli* by both Hargrove et al. ((1994) Biochemistry 33, 11767-11775) and Scott et al. ((2001) J. Biol. Chem., 275, 27129-27136) and the conclusions about higher muscle Mb concentration in deep diving mammals by Mirceta et al. ((2013) Science 14, 1324-1327). Our main goal is to implement this assay for high throughput library screening of globins for enhanced stability. The results of the screens will then be used to engineer more robust recombinant hemoglobins that could potentially be used as oxygen carriers in transfusion medicine and in tissue engineering applications. The assay could also be developed for general protein stability engineering.
Biochemistry Poster Number P-17

Molecular Architecture of the Synaptic Organizer Calsyntenin 3 and Its Interaction With Neurexin 1 Alpha

Veera Venkata Vijaya Sekhar Reddy Manukonda¹, Zhuoyang Lu², Yun Wang³, Fang Chen³, Huimin Tong², Lin Luo⁴, Suchithra Seshadrinathan¹, Lei Zhang², Luis Marcelo F. Holthauzen¹, Ann Marie Craig⁴, Gang Ren², & Gabby Rudenko³

(1) Dept. of Pharmacology and Toxicology, University of Texas Medical Branch, Galveston, TX (2) Lawrence Berkeley National Laboratory, Berkeley, CA (3) University of Michigan, Ann Arbor, MI (4) University of British Columbia, Canada

Calsyntenin 3 (Cstn3 or Clstn3), a recently identified synaptic organizer, promotes the development of synapses. Cstn3 localizes to the postsynaptic membrane and triggers presynaptic differentiation. Calsyntenin members play an evolutionarily conserved role in memory and learning. Cstn3 was recently shown in cell-based assays to interact with neurexin 1alpha (n1α), a synaptic organizer that is implicated in neuropsychiatric disease. Interaction would permit Cstn3 and n1α to form a trans-synaptic complex and promote synaptic differentiation. However, it is contentious whether Cstn3 binds n1α directly. To understand the structure and function of Cstn3 we determined its architecture by electron microscopy and delineated the interaction between Cstn3 and n1α biochemically and biophysically. We show that Cstn3 ectodomains form monomers as well as tetramers that are stabilized by disulfide bonds and Ca2+, and both are likely flexible in solution. We show further that the extracellular domains of Cstn3 and n1α interact directly and that both Cstn3 monomers and tetramers bind n1α with nanomolar affinity. The interaction is promoted by Ca2+ and requires minimally the LNS domain of Cstn3. Furthermore, Cstn3 uses a fundamentally different mechanism to bind n1α compared to other neurexin partners such as the synaptic organizer neuroligin 2 (NL2), because Cstn3 does not strictly require the sixth LNS domain of n1α. Our structural data suggest how Cstn3 as a synaptic organizer on the postsynaptic membrane, particularly in tetrameric form, may assemble radially symmetric trans-synaptic bridges with the presynaptic synaptic organizer n1α to recruit and spatially organize proteins into networks essential for synaptic function.
Biochemistry Poster Number P-18

Preparation of 2-(Propylamino)Ethanethiol 2-Chlorotrityl (PAET) Resin for the Synthesis of Peptide Thioesters

Robert Fox¹, & Kris F. Tesh¹
(1) Biology & Biochemistry, The University of Houston

The preparation of peptide thioesters using Fmoc chemistry requires their synthesis as amides flowed by an N -> S-acyl shift reaction. Recently, bis(2-sulfanylethyl)amino-trityl-polystyrene (SEA) resin has been reported (1, 2), where the peptide is synthesized as a secondary amide, and the N -> S-acyl shift occurs by reaction of one of the two 2-sulfanylethyl groups. The PAET resin described herein is easily synthesized and results in di-substituted amide peptide that promotes the N -> S-acyl shift reaction when the single 2-sulfanylethyl isomerizes to the correct position. The rate of N -> S shift is superior to that of the cystamine adduct. A peptide-pea adduct supported a native chemical ligation (NCL) reaction in the presence of MesNa. The presence of a secondary amide with a single 2-sulfanylethyl is sufficient for the efficient production of peptide thioesters.

Biochemistry Poster Number P-19

An Alternate Route for Repairing Ionizing Radiation Induced Double Strand Breaks in Cancer Cells

Arijit Dutta¹, Sanjay Adhikari², Pavana M Hegde², Aye Su Hlaing³, Miaw-Sheue Tsai³, Michael Weinfeld⁴, Muralidhar Hegde², & Sankar Mitra¹²
(1) Department of Biochemistry and Molecular Biology, University of Texas Medical Branch, 301 University Blvd., Galveston, TX 77555 (2) Department of Radiation Oncology, Houston Methodist Research Institute, Houston, TX 77030 (3) Department of Cell and Molecular Biology, Lawrence Berkeley National Laboratory, Berkeley, CA 94720 (4) Department of Oncology, University of Alberta, Cross Cancer Institute, 11560 University Avenue, Edmonton, Alberta, T6G 1Z2
Ionizing radiation (IR) induces damage clusters in the genome that include DNA double-strand breaks (DSB), and more frequently oxidized bases and single-strand breaks. The DSBs mostly have blocked termini such as 3’-phosphate, 3’-phosphoglycolate, 5’-phosphodeoxyribose which require processing to generate 3’OH and 5’Phosphate for repair. Moreover, repair of closely spaced oxidized bases could generate additional DSB intermediates with blocked ends. Although DSBs are predominantly repaired via DNA-PK-dependent non-homologous end joining (NHEJ), recently error-prone microhomology dependent alternative end joining (Alt-EJ) for DSB has been reported. Alt-EJ requires SSB/base excision repair (SSBR/BER) proteins such as XRCC1, PARP1 and DNA ligase 3. Relative contribution of these pathways at IR-induced complex genome damage has not been elucidated. While current DSB repair (DSBR) studies mostly examine DSBs with undamaged termini, we have generated a novel linearized reporter plasmid with 3’-blocked ends, mimicking IR-induced termini, whose in-cell repair may recapitulate in vivo DSBR. Furthermore, the presence of microhomology sequences flanking the DSB in the substrate allows quantitation of NHEJ vs. Alt-EJ by sequence analysis of repair joints in the circularized plasmids recovered from cancer cells. While the contribution of Alt-EJ is significantly lower than that of NHEJ, it was significantly enhanced in pre-irradiated cancer cells. Alt-EJ was enhanced due to DNA-PK inhibition and was decreased by XRCC1 depletion. Combined deficiency of XRCC1 and DNA-PK caused significantly additive DSB accumulation and radiosensitivity. In-cell DSBR in PNKP down-regulated cells also showed enhancement of Alt-EJ, suggesting that Alt-EJ takes over when NHEJ is suppressed because of the deficiency in 3’ end cleaning in the substrate. We observed recruitment of XRCC1 at IR-induced clustered damage but not at etoposide-induced DSBs. These observation together indicate substantial role of XRCC1-mediated Alt-EJ at IR-induced genome damage. We also found enhanced interaction of XRCC1 with Mre11 and CtIP that promote Alt-EJ by end-resection in irradiated cancer cells. Finally XRCC1-immunocomplex isolated from U2OS cells with ectopic XRCC1-FLAG can carry out Alt-EJ in vitro. Thus our study documents for the first time that Alt-EJ is enhanced after irradiation and that the XRCC1 immunocomplex can carry out DSB repair in vitro and underscores a critical role of the back-up DSBR pathway in IR-induced damage repair whose targeting could enhance radiosensitivity of cancer cells. (Research supported by USPHS grant R01 CA158910)
Biochemistry Poster Number P-20

A High-Throughput Assay to Monitor Tyrosyl-tRNA Synthetase Activity

Eric First¹, & Charles Richardson¹
(1) Biochemistry and Molecular Biology, LSU Health Sciences Center in Shreveport, 1501 Kings Highway, Shreveport, LA, 71103

Tyrosyl-tRNA synthetase catalyzes the attachment of tyrosine to the 3’ end of tRNA\textsuperscript{Tyr}, releasing AMP, pyrophosphate, and L-tyrosyl-tRNA as products. As this enzyme plays a central role in protein synthesis, it has garnered attention as a potential target for the development of novel antimicrobial agents. Although high-throughput assays that monitor tyrosyl-tRNA synthetase activity have been described, tRNA is generally the limiting substrate in these assays. Here, we describe an alternate approach, in which the Tyr-tRNA product is cleaved, regenerating the free tRNA substrate. We show that cyclodityrosine synthase from Mycobacterium tuberculosis can be used to cleave the L-Tyr-tRNA product, regenerating the tRNA\textsuperscript{Tyr} substrate. As tyrosyl-tRNA synthetase can use both L- and D-tyrosine as substrates, we replaced the cyclodityrosine synthase in the assay with D-tyrosyl-tRNA deacetylase, which cleaves D-Tyr-tRNA. This substitution allowed us to use the tyrosyl-tRNA synthetase assay to monitor the aminoacylation of tRNA\textsuperscript{Tyr} by D-tyrosine. Furthermore, by making Tyr-tRNA cleavage the rate-limiting step, the assay has been used to monitor the activities of cyclodityrosine synthetase and D-tyrosyl-tRNA deacetylase. This assay can be adapted to monitor both aminoacylation and post-transfer editing activities in other aminoacyl-tRNA synthetases.
Biochemistry Poster Number P-21

Mnk1/2 Kinase Regulates Translation of Capped MRNAs With Structured 5'-UTR

Nadejda Korneeva\textsuperscript{1,2}, Anren Song\textsuperscript{3}, Robert E. Rhoads\textsuperscript{2}, Thomas Arnold\textsuperscript{1}, & Mary Ann Edens\textsuperscript{1}

(1) Department of Emergency Medicine, Louisiana State University Health Sciences Center-Shreveport (2) Department of Biochemistry and Molecular Biology, Louisiana State University Health Sciences Center-Shreveport, Shreveport, LA 71103 (3) University of Texas, Health Sciences Center at Houston, Dental Branch, Houston, Texas 77054

The mitogen-activated protein kinase-interacting kinases 1 and 2 (MNK1 and MNK2) are activated by extracellular-signal-regulated kinases 1 and 2 (ERK1/2) or p38 in response to cellular stress and extracellular stimuli that include growth factors, cytokines, and hormones. Modulation of MNK activity affects translation of mRNAs involved in the cell cycle, cancer progression, and cell survival. However, the mechanism by which MNK selectively affects translation of these mRNAs is not understood. MNK binds eIF4G and phosphorylates the cap-binding protein eIF4E. Phosphorylation of eIF4E can be prevented either with a kinase inhibitor, CGP57380, or with a fragment of eIF4G, eIF4G(1357-1600), that blocks MNK binding to intact eIF4G. We used a cell-free translation system from rabbit reticulocyte lysate (RRL) programmed with mRNAs containing different 5’-ends to show that CGP57380 and eIF4G(1357-1600) affect translation of only mRNAs that contain both a cap and a highly structured 5’-untranslated region (UTR). Polysomal analysis of carcinoma cells followed by real-time PCR demonstrated that CGP treatment shifted ODC and VEGF mRNAs into slower sedimenting complexes, suggesting inhibition of the initiation step of translation. Analysis of proteins bound to m7GTP-Sepharose revealed that MNK inhibition decreases binding of eIF4E to eIF4G in both RRL and breast carcinoma cells. Interestingly, this did not increase binding of eIF4E to 4E-BP1, which binds the same residues in eIF4E that make up the eIF4G interaction site. We also observed decreased accumulation of eIF4E in the nucleus after CGP treatment, suggesting sequestration of eIF4E in the cytosol. These data suggest that MNK stimulates translation of capped mRNAs with structured 5’-UTRs by enhancing eIF4E binding to eIF4G. RER was supported by grant R01GM20818 from the NIGMS.
Effect of Phosphorylation on Structure of C-Terminal Segment of AMPA Receptor

Caitlin Nurik1, David Cooper2, Swarna Ramaswamy1, Christy Landes2, & Vasanthi Jayaraman1

(1) Biochemistry and Molecular Biology, University of Texas Health Science Center Medical School (2) Dept. of Chemistry, Rice University, Houston, TX, 77030

The α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor is the primary contributor to fast excitatory transmission in neurons. The AMPA receptor can be divided into four domains. Extracellularly, there are the amino-terminal and ligand-binding domains. The transmembrane domain serves as the actual ion-channel pore and, of course, links the extracellular domains to the cytoplasmic domain. Of these four domains, the structure of the outermost three has been shown in detailed crystal structures of the tetramer. However, very little is known about the structure of the cytoplasmic domain. Although it is widely thought that this segment is highly disordered, it is unknown whether local order (higher levels of secondary and/or tertiary structure) exists in the cytoplasmic terminus, or whether structural changes may occur as conformational shifts in the terminal due to functional modifications. Previous studies have established phosphorylation sites at residues S818, S831, and T840 in the GluA1 subtype receptor. Our studies examined a representative membrane-proximal section of the GluA1 c-terminus comprising residues 809-841 in order to consider structural changes brought about by these phosphorylation events. The peptide was examined using circular dichroism (CD) investigation, which showed no global conversion to greater helix content in the phosphomimetic sample. Single molecule fluorescence resonance energy transfer (smFRET) was used to examine the peptide in both the unphosphorylated state and in the PKCα-phosphorylated state, in order to gauge the distance between two native cysteines in the peptide. Studies were also conducted using constructs with cysteines bookending all three PKC phosphorylation sites. These studies indicate that lipids have a significant effect on the local structure of the GluA1 c-terminus, and that the effect is most notable when the c-terminus is phosphorylated, and in the presence of charged lipid. FTIR studies agree with these notions, and ensemble FRET suggests that local structural changes that may occur in the molecule do not increase the overall distance between the molecule and the membrane.

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Biophysics Poster Number P-23

Effects of Peptide Concentration, Bilayer Composition and Nanoparticles on the Dynamics and Stability of H-Ras Peptide Nanoclusters

Xubo Lin¹, Zhenlong Li¹, Alemayehu A. Gorfe¹*
(1) The Department of Integrative Biology and Pharmacology (IBP), The University of Texas Medical School at Houston Department of Integrative Biology and Pharmacology, Medical School, The University of Texas Health Science Center at Houston, 6431 Fannin St., Houston, Texas 77030, United States

The dynamics and stability of nanoclusters of lipid-anchored Ras proteins play an important role in Ras signaling. However, how changes in protein concentration and membrane domain stability might affect the stability of Ras nanoclusters is far from clear. Here we used coarse-grained molecular dynamics simulations to examine the effects of peptide concentration, cholesterol concentration and nanoparticles (C60) on the dynamics and stability of peptides representing the H-Ras lipid anchor that form dynamic clusters in a two-domain lipid bilayer. Combined with our previous studies [Janosi L. et al. Proc. Natl. Acad. Sci. U.S.A. 2012, 109, 8097; Li Z. et al. J. Am. Chem. Soc. 2012, 134, 17278], the current simulations indicate a reversible effect of peptide/cholesterol concentrations on the dynamics and stability of H-Ras nanoclusters, and suggest a correlation between the stabilities of lipid domains and peptide clusters. We further show that C60 nanoparticles penetrate into the bilayer core and localize in the liquid-disordered domain; they destabilize the domain boundary and thereby the H-Ras clusters. Taken together, these results suggest that the stability of the boundary-prone H-Ras nanoclusters is largely determined by the stability of the domain boundary.
Biophysics Poster Number P-24

Computational Modeling and Design of Orthogonal GPCR-Mediated Signaling Complexes

Melvin Young¹, Kuang-Yui Chen¹, & Patrick Barth¹,²,³
(1) Biochemistry & Molecular Biology, Baylor College of Medicine (2) Pharmacology, Baylor College of Medicine, Houston, TX (3) Structural and Computational Biology & Molecular Biophysics Graduate Program, Baylor College of Medicine

G protein-coupled receptors (GPCRs) commonly exhibit an inherently high level of promiscuity in ligand and effector binding and activation that hinders accurate understanding of their regulation and specific role in diseases. Consequently, many cell and gene therapy strategies are limited by the inability to reprogram receptor signaling properties without affecting endogenous cellular signaling pathways. We hypothesize that orthogonal, highly specific, GPCR-mediated signaling complexes will allow for the properties of specific receptor and downstream effector systems to be finely tuned without perturbing the function of related receptors and alternative pathways. A combination of Rosetta's multi-state design (MSD) and docking protocols have been used to evolve and select in-silico novel and highly specific binding interfaces unrelated to native GPCR/G protein complexes. The MSD protocol does not allow for a flexible backbone, limiting the available sequence space. To compensate for this limitation, we use the docking protocol to produce a larger diversity of GPCR/G protein binding interfaces that allows access to a substantially larger sequence space than MSD alone.

The most promising computationally designed orthogonal GPCR/G protein complex has been experimentally cross validated by monitoring agonist-induced specific activation of design and WT complexes using cell-based assays. Our activation assay shows that the change in membrane potential with the designed GPCR and designed G protein pair compares favorably with the WT/WT pair, while both design/WT pairs have drastically lower activation as intended by the design process. This suggests that our methodology should greatly aid in the creation of highly specific GPCR-mediated signaling complexes.

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Biophysics Poster Number P-25

Force Field Dependent Solution Properties of Glycine Oligomers

Justin Drake$^{1,2}$, & B. Montgomery Pettitt$^{1,2}$

(1) Biochemistry and Molecular Biology, University of Texas Medical Branch, (2) Sealy Center for Structural Biology and Molecular Biophysics, UTMB, Galveston, TX 77555

Molecular simulations can be used to model disordered polypeptide systems. It provides a temporal and spatial resolution for a model that often surpasses experimental biophysics techniques. As the number of disordered protein systems investigated with molecular simulations increase, it is important to understand how particular force fields effect the distribution of conformers and how these effects propagate to longer polypeptides. We use all-atom molecular dynamics (MD) to simulate two polyglycine peptides, a model disorder system, which differ in their number of amino acids. Gly3 and Gly10 were simulated in explicit aqueous solvent using the CHARMM27 (C27), CHARMM36 (C36), and Amber ff12SB force fields. By characterizing the distributions of conformers with a variety of structural metrics (e.g. end-to-end distance, radius of gyration, solvent accessible surface area, dihedral angles) we reveal force-field dependent solution properties of polyglycine. The helical bias noted with C27 has been considerably reduced in C36, which largely samples polyproline-II regions, while ff12SB more evenly samples the major regions of Ramachandran space. A greater population of extended structures is observed with C36 followed by ff12SB, and to a much lesser extent C27. Furthermore these structural differences are accentuated with the Gly10 simulations compared to Gly3. Interestingly, the J-coupling constants calculated from the trajectories of Gly3 match those measured experimentally by NMR despite the fact that each force field produces different distributions of Gly3 conformers. Finally, we note that care must be taken when making mechanistic inferences from simulations of disordered polypeptides using any one particular force field as the biases we discovered may lead to different conclusions.
NMR Investigation of Energy Barriers for Hydrogen-Bond Breakage of Protein Side-Chain NH$_3^+$ Groups

Levani Zandarashvili$^1$, & Junji Iwahara$^1$

(1) Dept. of Biochemistry and Molecular Biology, Sealy Center for Structural Biology and Mol. Biophysics, Univ. of Texas Medical Branch, Galveston, TX 77555

Although charged side chains play important roles in protein function, their dynamic properties are not well understood. Nuclear magnetic resonance methods for investigating the dynamics of lysine side-chain NH$_3^+$ groups were established recently. Using this methodology, we have studied the temperature dependence of the internal motions of the lysine side-chain NH$_3^+$ groups that form ion pairs with DNA phosphate groups in the HoxD9 homeodomain-DNA complex. For these NH$_3^+$ groups, we determined order parameters and correlation times for bond rotations and reorientations at 15, 22, 28, and 35 °C. The order parameters were found to be virtually constant in this temperature range. In contrast, the bond-rotation correlation times of the NH$_3^+$ groups were found to depend strongly on temperature. On the basis of transition state theory, the energy barriers for NH$_3^+$ rotations were analyzed and compared to those for CH$_3$ rotations. Enthalpies of activation for NH$_3^+$ rotations were found to be significantly higher than those for CH$_3$ rotations, which can be attributed to the requirement of hydrogen bond breakage. However, entropies of activation substantially reduce the overall free energies of activation for NH$_3^+$ rotations to a level comparable to those for CH$_3$ rotations. This entropic reduction in energy barriers may accelerate molecular processes requiring hydrogen bond breakage and play a kinetically important role in protein function.
Structural Basis of Chemokine Heterodimers and Their Glycosaminoglycan-Complexes: An NMR Approach to Characterize Systems of Up to Ten Species

Aaron Brown¹, Krishna M. Sepuru¹, & Krishna Rajarathnam¹,²
(1) Biochemistry and Molecular Biology, University of Texas Medical Branch (2) Sealy Center for Structural Biology and Molecular Biophysics, UTMB, Galveston, TX 77555

Protein-protein interactions play a fundamental role in life, and characterizing these interactions at a structural level is vital to understanding their underlying roles in biology. For instance, characterizing interactions between two proteins is straight forward; however, characterizing proteins that also exist as dimers becomes a daunting task. Such a system has five species: two monomers, two homodimers, and a heterodimer, and their relative amounts depend on the total protein concentration and the three equilibrium constants: of the two homodimers and a heterodimer. Characterizing such a system becomes all the more challenging if we take into consideration their interactions with other macromolecules, in which case, we have a system in which ten species coexist. Traditional methods such as pull-down assays and mass spectrometry can distinguish between homodimers and heterodimers, but cannot provide a structural basis or provide a rigorous molecular basis of the various interactions. Nuclear Magnetic Resonance (NMR), however, is the perfect tool to obtain this information. Here we illustrate how NMR can be used to structurally characterize a model system of two chemokines which can form homodimers and heterodimers and also bind glycosaminoglycans, increasing the number of species to ten. NMR allows for selective labeling of one species or another, separating half of the species immediately. In addition, chemical shifts are extremely sensitive to secondary, quaternary and tertiary structure, which allows for detection of the remaining six species. Under conditions of fast exchange, the NMR spectra is further simplified from six to three (weight average between the free and bound form). Using these data, we show the relative amounts of the various species that coexist in solution and relative affinities for GAG. Our studies provide proof-of-concept of the feasibility and also provide important insights into how these various species mediate in vivo function, and could also provide valuable leads as drug targets for inhibiting protein-protein interactions for various diseases.
Kinetic Impact of Semi-Specific DNA Sites During Target Location By a Sequence-Specific DNA Binding Protein

Catherine Kemme¹, Alexandre Esadze¹, Anatoly B. Kolomeisky², & Junji Iwahara¹
(1) Dept. of Biochemistry and Molecular Biology, Sealy Center for Structural Biology and Mol. Biophysics, University of Texas Medical Branch, Galveston, TX 77555 (2) Dept. of Chemistry and Center for Theoretical Biological Physics, Rice University, Houston, TX 77030

Many transcription factors function by creating sequence specific complexes within the regulatory regions of genes. Although chromosomal DNA contains numerous target sites for every transcription factor, the presence of similar base pair sequences to the target site can cause off-target association. It is not well understood to what degree these ‘semi-specific’ sites influence the function of the transcription factors. In this work, we have studied the kinetic impact of semi-specific DNA sites on the efficiency of target location by the inducible transcription factor Egr-1, which recognizes 9 base pairs (bp). Using stopped flow fluorescence kinetic assays, we measured the kinetics of Egr-1's association with a target site on 143 bp DNA in the presence of nonspecific and semi-specific DNA duplexes under various conditions. The presence of the semi-specific sites significantly slowed the target association, depending strongly on their concentration and affinity, as well as the degree of saturation of their binding to the protein. To quantitatively describe the kinetic impact of the semi-specific sites, we derived an analytical expression for the pseudo-first-order kinetic rate constant for the target association and used it for fitting to the experimental kinetic data. Our analysis demonstrates that the kinetic impact of semi-specific sites on the efficiency of target search by a sequence-specific DNA-binding protein can be quantitatively described in terms of trapping of the protein at semi-specific sites.
Biophysics Poster Number P-29

The Role of Hydrodynamic Interactions in the Rate of Protein Folding

Mohammadmehdi Ezzatabadipour¹,² & Margaret S. Cheung¹,²
(1) Department of Physics, University of Houston, 4800 Calhoun Rd., Houston, TX, 77004 (2) Center of theoretical biological physics, Rice University, Houston, TX, 77005

Hydrodynamic interactions (HI) affect the diffusivity of protein dynamics. However, a computationally quantitative assessment of its role in protein folding has been an underexplored research field. A lack of HI in molecular dynamics simulations fails to accurately contrast the experimentally measurement of translational and rotational diffusion coefficients when HI is no longer negligible, such as inside a crowded cell. In our study, we incorporated hydrodynamic interactions into the equations of motion from the Brownian dynamics by computing the diffusion correlation matrices between each residue. We used a Cα-only and a structure-based model, to investigate folding of chymo-trypsin inhibitor 2 (CI2), an experimentally and theoretically well-studied protein. We observed the rate of helical formation in a fraction of CI2 increases by 30 percent in the presence of hydrodynamics interactions. We aim to compare the folding rates of the whole CI2 in the absence and in the presence of hydrodynamic interactions. In addition, we will compare them with the folding rates predicted by the Energy Landscape Theory. The difference in the simulation and the theory will allow us better develop a computational framework for protein folding in crowded cells.
Biophysics Poster Number P-30

Speeding Up Target Location By Sequence Specific DNA Binding Proteins

Ali Alexandre Esadze¹, Levani Zandarashvili¹, Catherine A. Kemme¹, & Junji Iwahara¹
(1) Department of Biochemistry and Molecular Biology, Sealy Center for Structural Biology, University of Texas Medical Branch at Galveston, Galvston, TX 77555

Transcription factors and DNA repair/modifying enzymes must locate the target sites through stochastic scanning of DNA in the vast presence of nonspecific sites. In this work, we demonstrate that the kinetic efficiency in target search by these proteins can be improved via engineering based on structural dynamic knowledge of the DNA-scanning process. Our data for the Egr-1 zinc-finger protein and its derivatives reveal a kinetic role of the dynamic equilibrium between two modes in the DNA-scanning process: one suitable for search and the other for recognition. A shift of this equilibrium toward the recognition mode increases affinities for DNA, but reduces the search speed. We show that optimizing the balance between the search and recognition modes improves the kinetic properties of zinc-finger proteins. A pursuit of high affinity in conventional zinc-finger technology for artificial gene control may lead to kinetically deficient proteins that spend too much time on nonspecific DNA.
Biophysics Poster Number P-31

Specificity and Diversity of Glycosaminoglycan Interactions With Neutrophil Activating Chemokines: Insights From Solution NMR Studies

Krishna Mohan Sepuru¹, Junji Iwahara¹, & Krishna Rajarathnam¹
(1) Department of Biochemistry and Molecular Biology, The University of Texas Medical Branch at Galveston Department of Biochemistry and Molecular Biology, and Sealy Center for Structural Biology and Molecular Biophysics, University of Texas Medical Branch, Galveston, Texas, USA.

Glycosaminoglycan (GAG)-bound chemokine gradients in the vasculature and extracellular matrix play crucial roles in rapidly mobilizing neutrophils to the site of tissue infection and injury. GAGs, such as heparan sulfate and heparin, are highly sulfated polysaccharides, and structure-function studies have established that electrostatic interactions between basic lysine, arginine, and histidine residues and GAG sulfates mediate binding. However, very little is known regarding the structural mechanisms by which these interactions impart specificity, affinity, and function. We have now characterized the binding of chemokines CXCL5, and CXCL1 to a panel of heparin-derived oligosaccharides (dp4 to dp26) using solution NMR spectroscopy. Our data indicate that for a given chemokine, a single binding geometry cannot simultaneously engage all of the GAG-binding residues indicating multiple binding modes must exist. Our data also show that distribution of GAG-binding residues in the context of tertiary and quaternary structures vary between related chemokines, which allows diverse binding geometries. Not surprisingly, shared binding geometries and chemokine-specific binding geometries were observed. NMR studies under conditions where both monomers and dimers exist indicated dimer is the high-affinity GAG ligand. Interestingly, our data show that GAG-binding and receptor-binding sites overlap suggesting that the GAG-bound chemokine cannot activate the receptor. We conclude that GAG-binding interactions are highly context-dependent and chemokine-dependent, and that the GAG-bound dimer indirectly influences monomer-mediated neutrophil trafficking.
NMR Research At UTMB

Tianzhi Wang¹
(1) Sealy Center for Structural Biology & Molecular Biophysics, University of Texas Medical Branch at Galveston, Galveston, TX 77555

Nuclear magnetic resonance (NMR) spectroscopy is a powerful tool in the studies of Structural Biology, Proteomics, Metabolomics, Nutrition, Toxicology, 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 or 4D triple resonance NMR experiments. The SCSB NMR instrumentation includes the state-of-the-art high field Bruker Avance III 800MHz (with a TCI CryoProbe), 750MHz and 600 MHz (with a QCI CryoProbe, which is capable of various multi-dimensional 1H/2H/13C/15N/31P NMR experiments) NMR spectrometers with 31P NMR capability. The core Research laboratory offers instrumentation, training, software and assistance in designing experiments. The research though NMR laboratory is focusing on NMR methods, structure determination, protein dynamics & function, and metabolomics.
Dithioation of DNA phosphate is known to enhance binding affinities, at least for some proteins. We mechanistically characterized this phenomenon for the Antennapedia (Antp) homeodomain - DNA complex by integrated use of fluorescence, isothermal titration calorimetry (ITC), NMR spectroscopy, and X-ray crystallography. By fluorescence and ITC, we found that this affinity enhancement is entropy-driven. By NMR, we investigated the ionic hydrogen bonds and internal motions of lysine side-chain NH3+ groups involved in ion pairs with DNA. By X-ray crystallography, we compared the structures of the complexes with and without dithioation of the phosphate. Our NMR and X-ray data demonstrate that the lysine side chain in contact with the DNA phosphate becomes more dynamic upon dithioation. Our analysis shows that the affinity enhancement by the oxygen-to-sulfur substitution in DNA phosphate is due to an entropic gain relevant to the dynamics of the ionic interactions between protein and DNA.
Biophysics Poster Number P-34

Intermolecular Interactions Between Calmodulin and Intrinsically Disordered Protein Neurogranin Alter Calcium Binding Sites

Pengzhi Zhang\textsuperscript{1,2}, Swarnendu Tripathi\textsuperscript{1,2}, & Margaret S. Cheung\textsuperscript{1,2}
\textsuperscript{(1) Dept. of Physics, University of Houston, Houston, TX, 77004 (2) Center for Theoretical Biological Physics, Rice University, Houston, TX, 77005}

Neurogranin (Ng) is abundant in neurons in the brain and has essential role in learning and memory formation. Ng binds to calmodulin (CaM) at low Ca\textsuperscript{2+} level and releases CaM when Ca\textsuperscript{2+} level rises, allowing CaM to bind with Ca\textsuperscript{2+} and activate other targets. Ng is an intrinsically disordered protein (IDP), which lacks stable tertiary structures under physiological conditions. Because the binding between CaM and Ng is rather weak, study of the binding mechanism and determination of the bound complex are beyond the capacity of current experimental techniques, as well as theoretical work. We investigated binding of CaM and the Ng using a sidechain-\textit{C}_\alpha\textit{ model that was calibrated to balance intrinsic helical propensities of Ng and intermolecular interactions based on the binding affinities measured by fluorescence experiments. We employed a statistical dihedral angle model to sufficiently sample the conformational ensemble of Ng. We performed molecular simulations with umbrella sampling method to enhance the sampling of the structures from the weakly-bound complexes. Using the change in chemical shifts of CaM based on the experimental results as a reference, we selected a cluster of bound CaM-Ng complex structures. From the bound complex ensemble, we found the interaction between Ng and Calcium binding loops on N-domain of CaM (nCaM) disrupts the interaction between the Calcium binding loops. We hypothecate that this competition between Ng and nCaM could be related to the mechanism of Ca\textsuperscript{2+} release from CaM, and increase of Ca\textsuperscript{2+} level would help dissociate Ng from CaM. These hypotheses based on the structures of the bound complex from our coarse-grained simulations can be further justified by physics-based atomistic simulations or experiments.
Identification and Characterization of the Interaction Between BLIP-II and *Staphylococcus Aureus* PBP2a

Carolyn Adamski\(^1\), Dar-Chone Chow\(^2\), Nicholas G. Brown\(^{1,2}\), & Timothy Palzkill\(^{1,2}\)

\(^{(1)}\) Verna and Marrs McLean Department of Biochemistry and Microbiology, Baylor College of Medicine \(^{(2)}\) Department of Pharmacology, Baylor College of Medicine, Houston, Texas 77030

The prediction and manipulation of protein-protein interactions remains a difficult task. Model systems such as the complex of β-lactamase inhibitory protein II (BLIP-II) with β-lactamases have been used to investigate the principles of protein-protein interactions. Previous studies have focused on the determinants of binding affinity and specificity between BLIP-II and class A β-lactamases. However, interactions between BLIP-II and other proteins have yet to be explored. Here, we show that BLIP-II binds penicillin binding protein 2a (PBP2a) from methicillin resistant *Staphylococcus aureus* (MRSA) with a binding affinity in the low micromolar range. Alanine scanning of the interface residues of BLIP-II was performed and variants were analyzed using surface plasmon resonance experiments to determine how binding is achieved between BLIP-II and PBP2a. The results suggest that charged residues on the periphery of the BLIP-II interface play a critical role in binding PBP2a while an inner ring of aromatic residues on the binding surface is primarily responsible for the high affinity interaction of BLIP-II with β-lactamases. Interestingly, changes in BLIP-II binding affinity for PBP2a were found to be largely due to changes in both \(k_{on}\) and \(k_{off}\) while changes in binding affinity for β-lactamases were previously shown to be primarily mediated by \(k_{off}\). In summary, the results of the study reveal BLIP-II binds PBP2a a million-fold weaker than it binds β-lactamases and provides insights into how BLIP-II employs different combinations of hot spot residues to bind a wide range of target proteins.
Investigations of the Structural Mechanism of Modulation of the NMDA Receptor

Rita Sirrieh¹, David M. MacLean¹, & Vasanthi Jayaraman¹
(1) Biochemistry and Molecular Biology, The University of Texas Health Science Center at Houston 6431 Fannin St., Houston, TX, 77030

The NMDA receptor, one of the three main types of glutamate receptors found on the postsynaptic side of a neuronal synapse, is involved in the processes of learning and memory formation. Upon activation by the agonists glutamate and glycine, the NMDA receptor forms a cation selective pore that passes sodium and calcium ions into the cell. The receptor is an obligate heterotetramer typically composed of GluN1 and GluN2 subunits. The GluN2 subunits can be one of four subtypes A-D. Each subunit is organized into distinct domains, the intracellular carboxy-terminal domain, the transmembrane pore forming region, and extracellularly the agonist binding domain and the amino-terminal domain (ATD). The ATDs of the NMDA receptor contain the binding site for a number of modulators. Inhibitors and potentiators bind the ATDs with specificity for a particular GluN2 subtype. Zinc inhibits the receptor and has highest affinity for the GluN2A subtype and intermediate affinity for the GluN2B subtype. The synthetic compound ifenprodil inhibits receptors that contain the GluN2B subtype, and spermine potentiates receptors that also contain the GluN2B subtype. Extensive studies have focused on the mechanism of zinc inhibition, and previous work from our lab and others has shown that zinc inhibition proceeds via a cleft-closure conformational change. To determine if the mechanism employed by zinc was a common mechanism of inhibition, we used luminescence resonance energy transfer to map the conformational changes that the receptor undergoes upon binding of ifenprodil in GluN2B inhibition. Additionally, we monitored the conformational changes when the potentiator spermine binds. Interestingly, spermine potentiation of agonist-evoked current in GluN1-GluN2B containing receptors seems to proceed through an opposite structural mechanism to inhibition; both the GluN1 and GluN2B ATDs seem to be stabilized in an open conformational state. Additionally, structural studies suggest that the lower lobe of the GluN2 ATD twists in addition to moving towards or away from the upper lobe of the ATD. Future studies will be aimed at understanding how conformational changes at the ATDs are propagated towards the agonist binding domain and ultimately the pore of the receptor to modulate function.
Photoreceptor Dynamics: Understanding the Reaction Intermediates of a Photocycle

Jonathan Clinger¹, Jason Tenboer², Mark Andrew White³, Sethe Burgie⁴, Richard Vierstra⁴, Marius Schmidt², George Phillips¹

¹Department of Biosciences, Rice University ²Department of Physics, UW-Milwaukee, Milwaukee, WI, 53211 ³Department of Biochemistry and Molecular Biology & The Sealy Center for Structural Biology and Molecular Biophysics, UTMB, Galveston, TX, 77555 ⁴Department of Genetics, UW-Madison, Madison, WI, 53706

TePixJ, a blue and green light sensing photoreceptor from photosynthetic bacteria, drives photo taxis via isomerization of a bilin chromophore. The light absorption state information is transmitted through multiple domains to a phosphorylation scaffold domain, which initiates a kinase pathway to drive the flagella. The dynamic intermediate state, which involves the excitation and relaxation of the chromophore, is not well understood, and how the protein utilizes the light absorption state to drive signaling via structural changes has not been previously studied. We are using multiple biophysical methods to understand the chromophore and overall protein movement. These include flash photolysis spectroscopy, small and wide angle scattering (SAXS and WAXS), and serial femto-second crystallography (SFX).

In collaboration with the Schmidt lab at UW-Milwaukee, I am probing the previously unstudied photocycle of the chromophore in both directions. The heme-derived chromophore is related to phytochromes found in plants and other bacteria, but features a unique attachment to the protein backbone, utilizing two covalent bonds to Cys sidechains instead of the typical one. Using a ns tunable laser, we follow the reaction path from 50ns to 5ms and monitor the chromophore evolution. In addition to being important in its own right, the chromophore state provides context for the protein’s structural state, which is studied using small and wide angle scattering and serial femto-second crystallography.

In collaboration with Mark White at UTMB, I am using SAXS to study protein structure in solution. SAXS and WAXS preliminary scattering curves have been collected to determine experimental conditions and feasibility of time resolved SAXS/WAXS at either a synchrotron or the LCLS. In these studies, TePixJ has been found to be stable under the x-ray beam and mono dispersed at 8mg/mL concentration. Time resolved SAXS and WAXS will allow me to study large conformational shifts in the protein that are unobservable in SFX studies.

In collaboration with Allen Orville, LBNL, and the Vierstra lab, UW-Madison, I am developing micro crystals of TePixJ for data collection at LCLS. Micro crystals are necessary for data collection because each x-ray pulse at the LCLS destroys the crystal that is in the beam. Thus, many smaller crystals are used instead of a single large one. Current micro crystals are grown using Li2SO4 and PEG 400 without the need for micro-seeding, which is advantageous for large scale production. I am continuing to search for alternative crystal forms, which may diffract or photo convert better than the current formula. In conclusion, I am using a number of biophysical methods to study the dynamics involved in the photo conversion of TePixJ.
Biophysics Poster Number P-38

Predicting the Biochemical Parameters of An Antibiotic Efflux Pump Using a Mathematical Model

Anisha Perez¹, Erin O’Brien¹, Marcella Gomez¹, Matthew R. Bennett¹, & Yousif Shamoo¹
(1) Dept. of BioSciences, Rice University, 1600 Main Street, Houston, TX, 77251-1892 (2) Dept. of Systems, Synthetic, and Physical Biology, Rice University, Houston, TX, 77030-1400

The Centers for Disease Control and Prevention report that at least 2 million people in the United States will become ill due to antibiotic resistant pathogens leading to 23,000 deaths each year. In order to circumvent these resistance mechanisms, it is essential to quantitatively understand how the function of the protein(s) involved relates directly to resistance. Integral membrane efflux pumps are known determinants of single-drug and multidrug-resistance in a wide variety of pathogenic organisms. These transporters are membrane proteins and characterization typically requires reconstitution in an artificial membrane. Subsequently, these important proteins are difficult to characterize by traditional in vitro studies.

My project aims to determine the physicochemical parameters of the efflux pump TetB utilizing molecular biology and mathematical modeling. TetB is composed of 12 transmembrane (TM) alpha helices and is found within the inner membrane of Gram-negative bacteria. This protein allows for the efflux of tetracycline (TET), doxycycline (DOX), and minocycline (MCN) from the cytoplasm into the periplasm. Tetracycline class of antibiotics are bacteriostatic and function by inhibiting protein translation. For cells grown in tetracyclines, the efflux mechanism of TetB allows for cytosolic antibiotic concentration to decrease and rate of protein translation to increase.

I have inserted a tet(B) expression system into the E. coli chromosome and have determined its growth profile under various concentrations of TET, MCN, and DOX using high-throughput 96-well plates. The growth rate profiles correlate with TetB pumping rates for each drug. TetB more readily pumps out TET compared with DOX and MCN and we observe that cells expressing TetB can grow at higher TET concentrations compared with DOX and MCN. We are currently working on understanding how efflux expression effects bacterial growth by testing ribosome binding site (RBS) sequences of varying strengths in our tet(B) expression system. The shapes of the growth rate profiles produced in the different drugs give insight into the physicochemical mechanism of TetB. We are currently working on building a mathematical model that can simulate these growth profiles and predict efflux pump physicochemical parameters. Future works are geared toward modeling more complex efflux pump such as the tripartite pumps which traverse both bacterial membranes and cause multi-drug resistance. Collectively, this project aims to build an in vivo system will allow for the characterization of a variety of efflux pumps without the arduous tasks of protein purification and efflux pump reconstitution.

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Biophysics Poster Number P-39

Structural Characterization of a Noncanonical Interaction Between Bax and Bcl-2 BH4 Domain

Khushboo Singh¹ & James M. Briggs¹
(1) Department of Biology and Biochemistry, University of Houston, Houston, TX, 77204

Diffuse large B-cell Lymphoma (DLBCL) is an aggressive Non-Hodgkin Lymphoma accounting for 70,800 newly diagnosed cases and 18,990 deaths in the United States of America in 2014. Frequent Bcl-2 mutations were identified in germinal center B-cell (GCB) lymphoma patients (a molecular subtype of DLBCL); preferentially clustered in the BH4 domain and in the folded loop domain (FLD). The BH4 domain of Bcl-2 is a relatively poorly conserved amphipathic alpha helix known to interact with more than 20 signaling proteins. However, the role of Bcl-2 BH4 domain mutations in the etiology of the disease and on drug response is poorly understood. Bcl-2 is a negative regulator of programmed cell death, involved in the canonical anti-apoptotic mechanism of sequestering activated pro-apoptotic protein Bax by its hydrophobic groove. Recently, an unexpected finding of synthetic Bcl-2 BH4 domain interaction with the full-length Bax was reported, suggesting a non-canonical interaction mechanism. This interaction blocks the N-terminal conformational changes required for Bax activation and its translocation to the mitochondrial outer membrane. Our study explores the structural characterization and dynamics of Bax-Bcl-2 BH4 domain interaction. The binding site of the BH4 domain was investigated by performing docking calculations using the CABS-dock web server, which is based on the coarse grained CABS protein model and utilizes Replica Exchange Monte Carlo dynamics. Binding dynamics of the complex was further studied using molecular dynamics simulations. The long-term goal of the project is to study the structure function implication of BH4 mutants on Bax binding.
Confined DNA Thermodynamics: Structure, Pressure, Elasticity

Christopher Myers\textsuperscript{1,2}, & B. Montgomery Pettitt\textsuperscript{1,2}
(1) Structural and Computational Biology and Molecular Biophysics (SCBMB) Program, Baylor College of Medicine (2) Sealy Center for Structural Biology and Molecular Biophysics, University of Texas Medical Branch (UTMB), Galveston, TX 77555-0144

We are studying the thermodynamics of DNA packaging within viral capsids. Specifically, we are using physics-based simulations to predict the internal hydrostatic / osmotic pressures and structural data of double-stranded DNA packaged within bacteriophages. Biophysical prediction of the pressure and structures of DNA confined within phage capsids will aid prediction of DNA expression in vivo, and is also essential for better design of phage sequence-delivery methods. We have already shown that excluded volume, electrostatic forces and surface-induced correlations are sufficient to predict most of the major features of the current cryo-electron microscopy (cryo-EM) structural data of DNA packaged within viral capsids without assuming any elastic conformational ordering. Current models assume highly-ordered, even spooled conformations, based on interpretations of cryo-EM density maps. We have shown that surface-induced ordering of unconnected DNA polymer segments is the only necessary ingredient in creating ringed densities consistent with experimental density maps. This implies a larger ensemble of possible conformations of the DNA polymer within the capsid could be consistent with such cryo-EM data in contrast to the traditional assumptions of a highly-ordered spool conformation. This suggests a more disordered, entropically-driven view of phage packaging may be possible. Based on current insight into DNA flexibility, we hypothesize that non-linear elastic anomalies might be responsible for the reduction of overall elastic bending energies, allowing more disordered conformations to be free-energetically favorable, especially those induced by DNA twisting. Experimental techniques of other labs have shown phage DNA is negatively-twisted upon packaging. We are employing Monte Carlo path sampling techniques, in conjunction with twist and sequence-dependent DNA thermodynamic predictions, to accurately model the elastic bending contributions. Using such techniques, we are currently incorporating models of DNA flexibility that take into account the twist-bend coupling of DNA flexibility to better predict the pressure and thermodynamics of viral DNA packaging.
Computational Poster Number P-41

Explicit Solvent Simulation of Influenza Hemagglutinin At Low PH Environment

Xingcheng Lin\textsuperscript{1,2}, Jeffrey Noel\textsuperscript{1,2}, Nathan Eddy\textsuperscript{1,2}, Jianpeng Ma\textsuperscript{3,6}, & Jose' Onuchic\textsuperscript{1,2,4,5}

(1) Center for Theoretical Biological Physics, Rice University (2) Dept. of Physics & Astronomy, Rice University, (3) Dept. of Bioengineering, Rice University, (4) Dept. of Chemistry, Rice University, (5) Dept. of Biochemistry & Cell Biology, Rice University, Houston, TX, 77005 (6) Dept. of Biochemistry & Molecular Biology, Baylor College of Medicine, Houston, TX, 77030

Influenza hemagglutinin (HA) is a representative viral membrane fusion protein necessary for the entry of viruses into human host cell. Previous study with a dual-basin structure-based model has identified several generic kinetic events and has mapped out the landscape of HA conformational change. However, the initial extrusion of HA fusion peptides remains as a question and the pH change has been suggested as the trigger. To address this problem, we performed an explicit solvent simulation of HA stem domain in the low pH environment. It is found the decrease of pH and the associated change of electrostatics played a vital role in HA dynamics. By introducing some frustration into the buried cavity of fusion peptides, it led to its final falling off. Some other phenomena, like the "cracking" observed in previous structure-based model simulation, have also been observed in explicit-solvent simulation.
Computational Poster Number P-42

Predicting Binding Modes of Large Peptides to MHC-I Receptors

Dinler A. Antunes1, Ankur Dhanik1, John S. McMurray2, & Lydia E. Kavraki1
(1) Dept. of Computer Science, Rice University, Houston, TX, 77054. (2) Dept. of Experimental Therapeutics, MD Anderson Cancer Center, Houston, TX, 77030.

The endogenous antigen presenting pathway plays a central role in mounting cellular immunological responses. It allows peptides derived from cytosolic proteins to be presented at the surface, bound to class I Major Histocompatibility Complex (MHC-I). These “peptide:MHC” (pMHC-I) complexes can be then recognized by lymphocytes through the T cell receptor (TCR), a specific interaction which can trigger the elimination of diseased cells (e.g. infected by a virus). Structural analysis of pMHC-I complexes has several applications, from vaccine development to autoimmunity research. However, genetic variability of MHC family (receptor) prevents large-scale use of experimental methods, and the size of the ligands (large and highly flexible) makes this a challenging task for docking methods. Here we propose the use of DINC, a novel AutoDock-based incremental docking protocol for large ligands (http://dinc.kavrakilab.org/). Instead of considering all degrees of freedom at once, DINC starts with a fragment of the ligand, for which Autodock can provide fast and reliable results. This fragment is incrementally extended, up to cover the entire ligand, but exploring only 6 degrees of freedom in each step. A pilot study redocking 73 large ligands with different receptors was able to show a great performance increase in relation to Autodock 4, with equivalent accuracy. Our next goal is to apply DINC to a set of MHC allotypes of major biomedical interest, including ligands with more than 35 degrees of freedom. Additionally, DINC protocol can be refined to consider specific features of different MHC supertypes.
Computational Poster Number P-43

Ion Transport and Single Strained DNA Conformational Dynamics in the Alpha-Hemolysin and Mutated Protein Nanopores.

Olga Samoylova¹, Suren Markosyan², Pablo De Biase², Eric Ervin³, Pritwish Pal³, Geoffrey Barral³, & Sergei Noskov²
(1) Dept. of Chemistry and Biochemistry, Baylor University, Waco, TX 76798 (2) Dept. of Biological Sciences, University of Calgary, Calgary, AB, T2N 1N4 (3) Electronic BioSciences, San Diego, CA, 92121

A simple and inexpensive technique for DNA sequencing is the electrophoretically driven DNA transfer through the biological nanopore. To be able to characterize current modulations produced by various nucleotides while single strained DNA is transferring through the pore, and recognize particular nucleotide, it is necessary to clearly understand the interactions of the nucleotides with various protein pore residues.

We applied computational approach through all-atom Molecular Dynamics and Grand-Canonical Monte-Carlo/Brownian Dynamics simulations to investigate ss-DNA translocation and interactions with the biological alpha-hemolysin and mutated nanopores. We studied translocations of poly(dA)₄₀ and poly(dC)₄₀ ss-DNA within wild type hemolysin and its mutated modification for two different T regimes to screen protein - nucleotides interaction along the pores and ionic currents through the pores.

We report significant influence of DNA conformational dynamics on the residual currents, ion distributions around the DNA and nanopore solvent properties.
Computational Poster Number P-44

Exploring Protein Conformational Space Using Robotic Path Planning Techniques

**Didier Devaurs¹, Anastasia Novinskaya¹, Jayvee Abella¹, Dinler Antunes¹, Bryant Gipson¹, Corey Hryc², Mark Moll¹, Wah Chiu², & Lydia Kavraki¹**

(1) Computer Science, Rice University, Houston, TX 77005 (2) NCMI, Baylor College of Medicine, Houston, TX 77030

Proteins play a critical role in numerous biological processes, often performing complex tasks as the result of large-scale changes in their structure. Describing the exact details of these conformational changes remains a central challenge for computational structural biology, due to the enormous computational requirements of this problem. This has lead to the development of a rich variety of methods designed to answer specific questions at different levels of spatial, temporal, and energetic resolution. These methods fall largely into two classes: 1) physically accurate, but computationally demanding methods, and 2) fast, approximate methods. Here, we present a hybrid modeling framework, the Structured Intuitive Move Selector (SIMS), designed to bridge the divide between these two classes, while allowing the benefits of both to be seamlessly integrated into a single method. This is achieved by applying a modern path planning algorithm, borrowed from the field of robotics, in tandem with a well-established protein modeling library. SIMS can combine precise energy calculations with approximate or specialized conformational sampling routines to produce rapid, yet accurate, analysis of the large-scale conformational variability of protein systems. It features several key advancements, including the abstract use of generically defined moves (i.e., conformational sampling methods), as well as an expansive and probabilistic conformational exploration. SIMS represents a fast and accurate tool for the analysis of a wide range of challenging protein-flexibility problems, such as the determination of conformational changes involving long-range coordinated motions, or the discovery of transient conformational states.
Intrinsic Disorder Contributions to Binding and Allostery

Danielle Stuhlsatz\textsuperscript{1,2}, & B. Montgomery Pettitt\textsuperscript{2,1}

(1) Structural Computational Biology and Molecular Biophysics, Baylor College of Medicine, Houston, Texas 77030 (2) Sealy Center for Structural Biology and Molecular Biophysics, University of Texas Medical Branch at Galveston, Galveston, Texas

Misfolded and unstructured domains of proteins represent important examples of disease states where the understanding of the recognition, or folding process has important potential therapeutic implications. The mechanisms governing molecular recognition and the transition of proteins from their unfolded state to their native state remain related but unanswered fundamental biophysical questions. Refolding of protein domains often occurs during DNA binding. Specifically, LacI contains a hinge region that is disordered without DNA or when nonspecifically bound, but becomes ordered when bound to the correct DNA sequence. Experimentally, the hinge region has a measurable disorder to order transition when binding its specific sequence. This system has a nontrivial sequence and allows us to test the importance of the protein folding transition versus that of the contributions of DNA bending and DNA protein interactions separately. The hinge-helix sequence of the LacI system will be computationally analyzed through simulations in various multicomponent aqueous solutions.
Intermolecular Ion Pairs in Protein-DNA Complexes

Chuanying Chen¹, Alexandre Esadze², Levani Zandarashvili², Dan Nguyen², Junji Iwahara¹,² & B. Montgomery Pettitt¹,²,
(1) Sealy Center for Structural Biology and Molecular Biophysics, The University of Texas Medical Branch at Galveston (2) Dept. of Biochemistry and Molecular Biology, UTMB, Galveston, TX, 77555

For many molecular processes, ion pairs are of fundamental importance, yet their dynamic properties are not well understood in context of protein-DNA recognition and function. Crystal structures show either solvent-separated ion pair (SIP) or contact ion pair (CIP) states for each intermolecular ion pair; only the SIP state has been observed for eukaryotic transcription factors Antennapedia (Antp) homeodomain protein Lys46 and Egr-1 zinc-finger Lys79 with DNA phosphates. However, the NMR data of hydrogen-bond scalar coupling between ¹⁵N-³¹P nuclei clearly indicate the presence of the CIP state for these Lys NH³⁺ groups. In this study, we performed 600 ns molecular dynamics (MD) simulations to characterize the dynamics of the ion pairs of protein side-chain NH³⁺ and DNA phosphate groups in the specific DNA complexes of Antp and Egr-1 proteins. Our current study confirms dynamic transitions between the CIP and SIP states, typically on a picosecond - nanosecond timescale, for Lys NH³⁺ groups at the protein-DNA interfaces. Computations of the dynamic intermolecular ion pairs are consistent with NMR data of the three-bond ¹⁵Nζ-¹³Cγ scalar coupling constants relevant to the χ₄ torsion angle and ¹⁵N-relaxation-derived order parameters for the Cε-Nζ bonds of the interfacial Lys side chains. The results illustrate the highly dynamic equilibrium of the intermolecular ion pairs in protein-DNA complexes important for recognition.
A light harvesting molecular triad is a synthetic molecule with covalent bonding between pyrrole-fullerene (C60), diaryl-porphyrin (P), and carotenoid polyene (C) components. This triad is a donor-acceptor molecule that is capable of absorbing incident light in the visible region.\textsuperscript{1} Charge separation upon electron excitation results in a dipole moment of ~150 D with a lifetime of ~300 ns which is highly desirable for real life photonic applications.\textsuperscript{2} Previous molecular dynamics simulations employing non-polarizable force field have shown the structural stability of the triad for the ground and excited states. While non-polarizable force fields describe thermodynamics and structural properties well they do not account for the induced polarization effects. We perform molecular dynamics simulations for the triad in explicit solvent to study the polarization effects on structural stability of the triad in its ground state. Quantum chemistry studies were performed first for the individual components of the triad and solvent followed by parameterization of partial atomic charges utilizing RESP methodology.\textsuperscript{3} Atomic charges were parameterized based on the Merz Kolman\textsuperscript{4} approach at B3LYP/cc-pVTZ//HF/6-31G* level. Thole-linear polarizable model\textsuperscript{5,6} was employed in the fitting. Good agreement with experimental data has been reached on a number of properties including the gas phase geometries and dipole moments for each component. A series of molecular dynamics simulations was then performed for the neat solvent or triad/solvent mixture to characterize the influence of induced polarization effects on the structural stability of the molecular triad.
Molecular Dynamic Simulations of Tissue Factor Cytoplasmic Region / Pin-1 WW Domain Complex

Amir Ali\textsuperscript{1}, James M. Briggs\textsuperscript{1}, & John Craft\textsuperscript{1}

(1) Department of Biology and Biochemistry, University of Houston, 4500 Calhoun Road, Houston, TX, 77004

Tissue Factor's (TF) role in coagulation, angiogenesis, metastasis, and cardiovascular disease has made it an attractive drug target. Phosphorylation of TF alters cell signaling, migration, and angiogenesis as well as TF expression. Since both the phosphorylation sites of TF reside in the cytoplasmic region, experiments were performed to determine the structure of TF and its intracellular binding partners. A known binding partner TF’s cytoplasmic region (TFCR) is Pin-1, a Proline isomerase. Filtered NMR experiments, conducted by Dr. John Craft and collaborators, were performed to elucidate the structure of TFCR in complex with Pin-1 WW domain to better understand the regulatory role of TF. These experiments were able to identify the residues in the interface of Pin-1 WW domain and TFCR; however, they were not able to unambiguously determine the orientation of the peptide in the binding cleft. To build on this NMR information, protein refinement with NOE constraints and modeling of the peptide-protein interactions in AMBER produced viable configurations between the two. An ensemble of twenty-five configurations was generated using a Monte Carlo methodology. Molecular dynamics simulations of the solvated complex were performed in NAMD and examined to confirm the hypothesized binding orientation of the complex. Using these multiple sources of information, we can propose a structure or a set of probable structures for the protein complex, which can be used as a starting point for drug design or further investigation.
Understanding Structure, Dynamics and Mechanism of PTEN and Its Possible Genotype-Phenotype Correlations in Endometriosis and Cancer By Molecular Modelling and Simulations

Iris Smith¹, & James M. Briggs¹
(1) Biology and Biochemistry, University of Houston, 4500 Calhoun Road, Houston, TX, 77004

The phosphatase and tensin homolog deleted on chromosome ten PTEN gene encodes a tumor suppressor phosphatase frequently mutated in a variety of human cancers. It exerts its function by dephosphorylating phosphatidylinositol 3,4,5-triphosphate (PIP3), converting it to phosphatidylinositol 4,5-bisphosphate (PIP2). PIP3 activates a variety of downstream effectors that turn on the PI3K/Akt oncogenic pathway leading to unregulated cell proliferation and tumorigenesis. Loss of function and somatic missense mutations of PTEN have recently been found in patients with endometriosis, endometrial cancer and ovarian cancer although no structural information on these mutations is currently available. This proposed study explores the interplay of the structural and dynamic effects that the identified PTEN mutations have on the relationship between genotype and phenotype. Understanding the functional impact missense mutations have on the structure of PTEN is essential to elucidating the molecular mechanism of endometriosis and malignant transformation. We propose that the phosphatase domain of PTEN may define a region within the active site wherein a small mutation subset possibly correlate with phenotypes. To study the influence of the mutations in greater detail, a combination of structural analysis, molecular dynamics simulations and normal mode analysis (NMA) utilizing both elastic network models (ENMs) and principal component analysis (PCA) to characterize atomic interactions. Each of the identified PTEN mutations were constructed and analyzed. The effects of the mutations reveal that the local structure and interactions affect polarity, conformational flexibility and electrostatic surface potential. Our data suggest that mutations within the active site disrupt the electrostatic interaction thus potentially affecting P loop conformation. One distinct residue, R130, has mutations implicated in each phenotype, a residue that potentially participates in mutation-driven allosteric regulation. Molecular dynamics simulations was utilized to examine the clustered mutations to characterize their effects on P loop conformations and the functional distortions they may impose within the active site as well as mutations within the C2 domain that may affect PTEN's function. Coarse-grained models of both WT PTEN and mutants were constructed using ENMs, such as Gaussian Network Model (GNM) and Anisotropic Network Model (ANM) to explain the influence that each of the mutants have on the global dynamics defined by the tertiary structure. Principal component analysis also served as a useful tool to further explain the global motions of both the WT and mutant systems. The combined approaches outlined in this research have the potential of identifying the mechanistic role of PTEN associated with endometriosis, endometrial cancer and ovarian cancer. The results from our study will aid in a better clinical-molecular classification of the resulting phenotypes and allow translation into new diagnostic and therapeutic approaches.
Gene Profiling of Metagenomic Samples

K. Khanipov*, G. Golovko*, L. Albayrak*, M. Rojas, Y*. Fofanov#
*Department of Pharmacology and Toxicology, #Sealy Center for Structural Biology, University of Texas Medical Branch, Galveston, TX 77555

Metagenomes hold valuable insight to the functionality of microbiota in all natural, engineered and medical systems. Analysis of microbiota can devolve key information that is vital to public and environmental health. Currently analysis of metagenomics samples relies on metabarcode markers to identify the make-up. However, this is limited by the availability of the specific marker sequences in the reference database, it is not able to distinguish between certain strains of organism or detect viruses and plasmids. Recent advances in High Throughput Sequencing (HTS) has opened a possibility to analyze and study microbiomes on the level of functionality of the gene composition in the sample. In order to perform the analysis all available genes were downloaded from the NCBI RefSeq database and clustered together to minimize redundancy and decrease the run time of the analysis. Mapping the sequenced data from the samples to the clustered gene database shows the gene level makeup of the sample from which presence of organisms and their function can be derived. The developed approach was evaluated on a study of the mouse model of colitis with 20 samples. By using gene profiling we observed changes in expression of certain genes in different organisms present and plasmids present, which would not have been possible by using metabarcode markers. Such approach proved to be effective in performing high resolution analysis of metagenomic samples.
Next Generation Sequencing-based Pathogen Detection in Clinical Samples

Levent Albayrak*, Mark Rojas*, Kamil Khanipov*, Georgiy Golovko*, Yuriy Fofanov*#

*Department of Pharmacology and Toxicology, #Sealy Center for Structural Biology, University of Texas Medical Branch, Galveston, TX 77555

Clinical microbiology labs currently rely on culture and biochemical tests to detect, identify and characterize human pathogens. Such methods are often too slow to be of help to guiding patient care, and many pathogens are unable to be cultured at all. Over the last several years Next Generation Sequencing (NGS) technologies have improved dramatically, both in terms of cost and the quality of data output. The decrease in the price of the sequencing instruments has already moved sequencing process from being exclusively conducted in large sequencing centers to being routinely used by Universities and even individual labs. Several challenges however, must be resolved in order to transform NGS technology from a powerful research tool to instruments that are routinely used for clinical diagnostics, including: 1) The absence of standard data analysis algorithms and reference databases; 2) The large and complicated output of available HTS data analysis pipelines, which usually require PhD level scientist to analyze and interpret the results; 3) The computational complexity of the available HTS analysis tools often require costly, high-maintenance computational infrastructure. These challenges are further magnified in the identification of bacterial pathogens, owing to their larger genome size and lack of available reference genomes, as compared to identification of viral pathogens. Here, we present our proposed approach to enable a fast and accurate diagnostics tool for pathogen detection used to evaluate a collection of both real and synthetic samples and that can be used by technicians and non-experts in clinical and field diagnostic settings.
Harnessing the Heat Shock Response to Protect Against TDP-43 Pathology in Neurodegenerative Diseases

Oluwarotimi Folorunso¹,²,³, Pei-Yi Lin¹,²,³, Anson Pierce¹,²,³
¹George and Cynthia Woods Mitchell Center for Neurodegenerative Diseases at The University of Texas Medical Branch, Galveston, TX 77555, USA ²Sealy Center for Vaccine Development at The University of Texas Medical Branch, Galveston, TX 77555, USA ³Department of Biochemistry and Molecular Biology

Despite the diversity in clinical manifestation, a common feature of many neurodegenerative diseases is the accumulation of misfolded protein aggregates, which can disrupt protein homeostasis and ultimately lead to neuronal cell death. In 2006, transactivation response element DNA/RNA binding protein-43 (TDP-43) was identified in aggregates found in postmortem tissues of ALS and frontotemporal dementia (FTD) patients. TDP-43 is a nuclear protein that has a disordered glycine-rich C-terminal region containing most missense mutations linking it genetically in multiple proteinopathies. The presence of soluble TDP-43 oligomers, C-terminal fragments (CTF), and aggregates have all been shown to contribute to its pathogenicity and could sequester functional TDP-43. It is known that the CTF can form aggregates, but studies showing the contribution of TDP-43 CTF oligomeric species to disease pathogenicity are limited. We have purified a TDP-43 fragment (277-414a.a) oligomer fraction (with an N-terminal His tag) that forms a predominantly pentameric species using dynamic light scattering, and we have shown that it causes toxicity in neuronal-like (SH-SY5Y) cells in a dose-dependent manner at nanomolar concentrations.

Therapeutic possibilities that eliminate/reduce these neurotoxic TDP-43 species could alleviate pathogenesis. Heat shock proteins (HSPs) such as HSP70, recognize misfolded or aggregated proteins and refold, disaggregate, or turn them over and are up-regulated by the master transcription factor heat shock factor 1 (HSF1). Here, we explored the effect of HSF1 overexpression on proteotoxic stress-related alterations in TDP-43 solubility, proteolytic processing, and cytotoxicity and the mechanism of HSF1-mediated protection. We show that enhancing the heat shock response by increased expression HSF1 has a protective effect via up-regulating multiple hsps against TDP-43 stress from transfecting human HSF1 overexpressing mouse embryonic fibroblast (MEF) cells with human TDP-43, which was coupled with a reduction in TDP-43 inclusions. Using the proteasome inhibitor MG132 to induce TDP-43 aggregation and cleavage, we observed that HSF1 overexpression mitigates the cleavage and aggregation of TDP-43 in SH-SY5Y cells, and reduces toxicity. We saw that inhibiting HSF1 activity or HSF1 knockout reversed these observations. Furthermore, pharmacological activation of HSF1 via celastrol protected against toxicity associated with overexpression of TDP-43 C-terminal fragment. Finally, we show that HSF1 activation does not increase the activity of protein clearance pathways such as ubiquitin proteasome system (UPS) and macro-autophagy, suggesting that protective effects of HSF1 is due to up-regulation of neuroprotective hsps. Our results support enhancing heat shock response via HSF1 up-regulation or activation as a possible therapeutic against TDP-43 proteinopathies.
Targeting The Laminin Receptor / PEDF Interface to Treat Prostate Cancer Cells

C.S. Umbaugh¹, A.O. Diaz-Quiñones¹, J. Zhou¹, X. Cheng², and M.L. Figueiredo¹
¹Pharmacology and Toxicology, University Of Texas Medical Branch, Galveston TX
²Integrative Biology and Pharmacology, University Of Texas Health Science Center At Houston, Houston TX

The non-integrin laminin receptor (LAMR1) is a multifunctional protein localized in the cytosol and at the membrane. Recently, the potent antiangiogenic protein required for retinal development and maintenance, pigment epithelium derived factor (PEDF), was confirmed as a binding partner for LAMR1. LAMR1 is frequently overexpressed in cancer cells. Our lab and others have demonstrated that PEDF abolishes neovascularure in prostate tumors, resulting in decreased tumor growth and burden and abatement of metastatic potential.

Using crystal structures for PEDF and LAMR1, we mapped the interaction interface and screened the Maybridge HitFinder Database \textit{in silico} (>14,000 compounds) using Docking@UTMB and AutoDock Vina. We selected seven hits and conducted a medium throughput screen in multiple cell lines. IC₅₀s were generated using a six point dose curve and a four parameter non-linear regression. Orthogonal validation was performed using luciferase assays against cancer specific promoters.

Finally, we identified the 67 kDa species of LAMR1 and successfully knocked endogenous protein down using siRNA duplexes. Our phenotypic screen revealed three putative laminin antagonists, two agonists, and two ineffective compounds. We established that PEDF and the peptide P18 can mediate changes in gene expression. One compound significantly reduced Fas Ligand promoter activity and phenocopied PEDF, suggesting that these compounds may modify the tumor microenvironment. Next, we will confirm LamR specificity using cell-surface binding assays and siRNA. Funding: UTMB CAR P50DA033935 Pilot Grant and Startup Funds.
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The Effects of Hydro-Priming on Seed Germination and Early Seedling Growth of Some Iranian Melon Varieties Under Salinity Stress

Behrooz Sarabi1, Sahebali Bolandnazar1, Nasser Ghaderi2, & Seyed Jalal Tabatabaei1
(1) Department of Horticulture, Faculty of Agriculture, University of Tabriz, Tabriz, Iran (2) Department of Horticultural Sciences, Faculty of Agriculture, University of Kurdistan, Sanandaj, Iran

Melon (Cucumis melo L.) is an important horticultural crop often cultivated in arid and semiarid regions of the world where salinity threatens to become, or already is, a problem. Although salt stress affects all growth stages of a plant, seed germination and seedling growth stages are known to be more sensitive for most plant species. The inhibitory effect of salinity stress on seed germination is due to an osmotic effect and/or ion toxicity. Hydro-priming is a method to improve germination and emergence in the seeds of many crops, under saline conditions. Hydro-priming involved soaking of seed in water before sowing. Although the effects of hydro-priming treatment on germination of some seed crops have been studied, but relatively little information is available on the invigorating of melon seed under salt stress. The aim of this study was to evaluate the effects of hydro-priming on seed germination and early seedling growth of five Iranian melon varieties and one melon cultivar ‘Galia F1’ in response to salinity. A factorial experiment was performed as a completely randomized design with four replications (25 seeds per petri dish). The first factor was six varieties of melon (Khatooni, Suski-Sabz, Kashan, Samsuri, Ghobadlu and Galia F1) and five levels of salinity. For this purpose, the seeds of melons were soaked in distilled water for 24 h at 25 °C, as hydro-priming treatment. After imbibition, the seeds were transferred into 9 cm sterile petri dishes on filter paper and then were wetted with 10 ml distilled water (control) or saline water solution at 20, 40, 60 and 80 mM NaCl. The petri dishes were incubated in a growth chamber under 16/8 h light/dark cycle at 25±2°C and relative humidity of 65±5%. Computation of germinated seeds was done daily until the end of the fourteenth day. All data were analysed by SPSS software and Duncan’s Multiple Range Test was used to determine significance of differences between variables (p<0.05). The results indicated that salinity treatments significantly decreased the shoot length, root length, shoot fresh weight, root fresh weight, shoot dry weight, root dry weight, seedling fresh weight, seedling dry weight, germination rate, seed vigour indicator (SVI) and stress tolerance index whereas mean germination time (MGT) was increased significantly. It is worthwhile that the decrease in seedling dry weight percent and the germination percentage was not significant. Simple correlation coefficient analyses showed the existence of significant positive and negative correlations among characteristics. For example, seedling fresh weight and shoot fresh weight had the highest positive correlation (r = 0.985**) and MGT with germination rate had the highest negative correlation (r = -0.717**). In addition, the results of this study demonstrated a significant difference among investigated varieties. Key words: Melon, Salinity Stress, Hydro-priming, Seed Germination
Challenges of Living With An Ostomy in Iranian Patients

Nasrin Sarabi 1, Hasan Navipour2, & Eesa Mohammadi3
(1) PhD Student of nursing, School of Nursing and Midwifery, Tarbiat Modares University, Tehran, Iran (2) Assistant Professor of Nursing, Nursing Department, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran (3) Associate Professor of Nursing, Nursing Department, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran

Background & Aims: The purpose of an ostomy is to provide a diversion for the elimination of waste material, either urine, faeces or intestinal effluent. Complications following ostomy surgery can diminish quality of life for individuals living with an ostomy. This study was performed to explain perception of the negative effects of ostomy complications on the Iranian ostomy patients’ life. Method: The conventional content analysis was used in the study in 2014. The participants were 27 ostomy patients in “Iran Ostomy Association” in Tehran. Purposive sampling began and continued up to data saturation. Unstructured interview was the main method for data collection. Result: During the process of analysis, three themes were revealed that indicate the nature and dimension of Ostomy patients' perception related to the effect of ostomy in the process of their adaptation. These themes were included: 1. Difficult to control physical effects in dimensions: “sexual dysfunction”, “inability to odor control”, “annoying leak”, “unwelcome Excretion of gas”, “insomnia Suffering”, “Skin irritation” 2. loss of life opportunities in dimensions: “depart Compulsory work” and “Losing opportunity of marry” and 3. humiliated Feel include: “Negative body image”, “Discomfort of treatment negligence of problems result to ostomy”, “discomfort of Contagious perspective to ostomy”, “Friability”, “unhappiness of Pity sense of others”. Conclusion: The results have shown that Ostomy complications cause physical, social and psychological limitations and decrease the patients’ QQL. This new information may potentially lead to the development of interventions that will improve care and quality of life for individuals living with an ostomy. keywords: Ostomy complication, Quality of life, Content analysis