



**The 27th Annual Gibbs Conference on
Biothermodynamics**

October 5-8, 2013

**Organized by
James L. Cole and Aron W. Fenton**

**Touch of Nature Environmental Center
Southern Illinois University, Carbondale, IL**

**Sponsored by
Amgen • Avanti • Aviv • Horiba
ISS • Jasco • NanoTemper • UMMC**

Table of Contents

The 27th Gibbs Conference on Biothermodynamics



Introduction

<i>Map of Touch of Nature</i>	<i>2</i>
<i>History and List of Meetings.....</i>	<i>3-4</i>
<i>Gibbs Society Governance – Incorporation, Officers, Committees.....</i>	<i>5</i>
<i>The Gary K. Ackers Lecture in Biothermodynamics</i>	<i>6</i>

Meeting schedule

<i>Saturday, October 5.....</i>	<i>7</i>
<i>Sunday, October 6</i>	<i>8 - 9</i>
<i>Monday, October 7</i>	<i>10 - 11</i>
<i>Tuesday, October 8</i>	<i>12</i>

List of posters

<i>Poster information.....</i>	<i>13</i>
<i>Session I - Sunday.....</i>	<i>13</i>
<i>Session II - Monday</i>	<i>17</i>

Abstracts

<i>Speakers.....</i>	<i>21</i>
<i>Posters</i>	<i>52</i>

List of participants

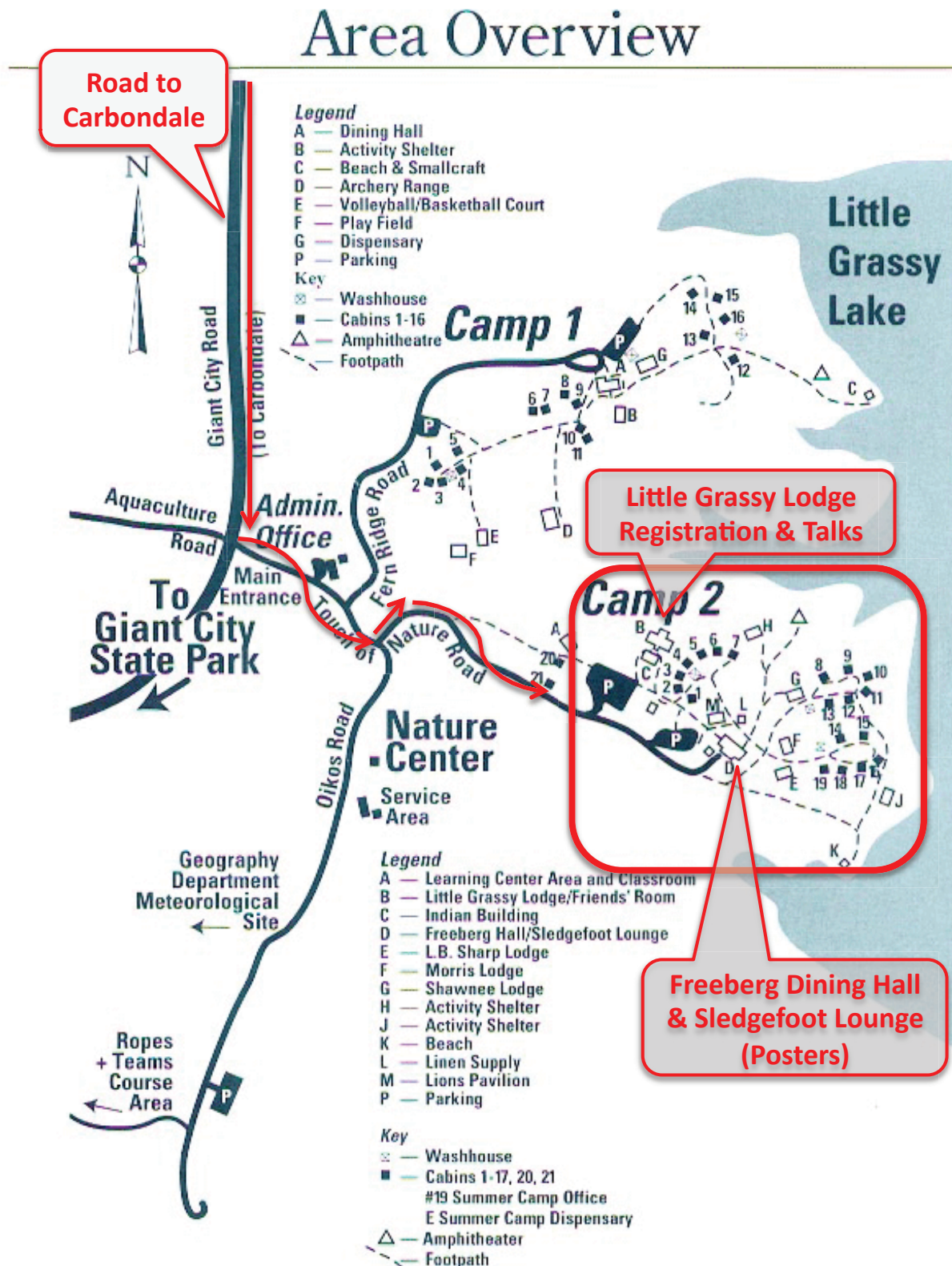
<i>Alphabetical list of participants.....</i>	<i>157</i>
<i>Participants by lab.....</i>	<i>171</i>

Sponsors

<i>Listing and Product Information</i>	<i>175</i>
--	------------

Map of Touch of Nature Environmental Center

Most of the Gibbs Conference activities will be held in "Camp 2" as shown in the map below. Cell phone reception is extremely limited; parking lots are popular places for making calls.



The Gibbs Conference on Biothermodynamics

History

Fall, 1986

Discussion of the discipline: Thermodynamics in Biological Systems

At the Gill residence in Vail, Colorado

Gary Ackers, Wayne Bolen, Ernesto Freire, Stan Gill, Jim Lee

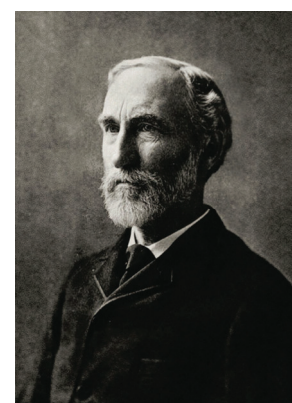
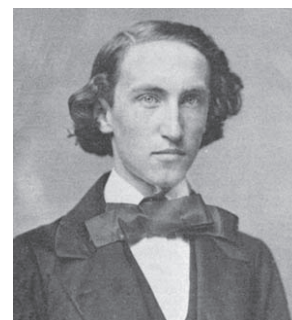
February, 1987

Discussion of the discipline: Thermodynamics in Biological Systems

The Gumbo Shop, New Orleans, LA during the 31st annual Biophysical Society Meeting - Gary Ackers, Norma Allewell, Wayne Bolen, Ken Breslauer, Ken Dill, Ernesto Freire, Stan Gill, Jim Lee

A history of the first ten years of the meeting was provided by Ackers GK and Bolen DW, The Gibbs Conference on Biothermodynamics: Origins and Evolution. *Biophysical Chemistry* **64** (1997) 3-5 (doi:10.1016/S0301-4622(96)02246-6)

An update is provided by Shea, MA, Correia, JJ, and Brenowitz, MD
Introduction: Twenty five years of the Gibbs Conference on Biothermodynamics
Biophysical Chemistry **159** (2011) 1-5 (doi:10.1016/j.bpc.2011.07.002)



Meetings

All meetings have been held at the Touch of Nature Environmental Center associated with Southern Illinois University – Carbondale. From 1987 through 1993, all of the speakers in the scientific sessions were students or postdoctoral fellows.

- | | |
|------|---|
| 1987 | Organizers: Jim Lee and Wayne Bolen
Philosophical Talks: Gary K. Ackers and Ken Dill |
| 1988 | Organizers: Gary Ackers and Michael Johnson |
| 1989 | Organizers: Susan G. Frasier and Michael Johnson |
| 1990 | Organizers: Michael Johnson and Marty Straume |
| 1991 | Organizers: Gary Ackers and Tim Lohman
Keynote Speaker: Ernesto Freire |
| 1992 | Organizers: Jim Lee and Tomasz Heyduk
Keynote Speakers: Serge Timasheff and John Schellman |
| 1993 | Organizers: Maurice Eftink and Glen Ramsay
Keynote Speakers: Peter von Hippel and Julian Sturtevant |
| 1994 | Organizers: Enrico Di Cera and Madeline Shea
Keynote Speakers: Gary Ackers and Kathleen S. Matthews |
| 1995 | Organizers: Kenneth P. Murphy and Michael D. Brenowitz
Keynote Speakers: Victor Bloomfield and Mario Amzel |

- 1996 Organizers: Jonathan B. Chaires and Michael L. Doyle
Keynote Speakers: J. Michael Schurr and Allen Minton
- 1997 Organizers: Dorothy Beckett and Jack Correia
Keynote Speaker: Adrian Parsegian
- 1998 Organizer: Andy Robertson
Keynote Speaker: David Draper
- 1999 Organizers: Bertrand Garcia-Moreno E. and John Shriver
Keynote Speakers: Wayne Bolen and Gary Ackers
- 2000 Organizers: George Turner and Kim Sharp
Keynote Speaker: Steve White
- 2001 Organizers: Margaret A. Daugherty and Luis A. Marky
Keynote Speaker: George Rose
- 2002 Organizers: Michael Mossing and George Makhatadze
Keynote Speaker: Rodney Biltonen
- 2003 Organizers: Vince Hilser and Dick Sheardy
Keynote Speaker: Jim Lee
- 2004 Organizers: Doug Barrick and Kathleen Hall
Keynote Speaker: Nacho Tinoco
- 2005 Organizers: Trevor Creamer and Clay Clark
Keynote Speaker: Carl Frieden
- 2006 Organizers: Karen Fleming and Rohit Pappu
Keynote Speakers: Madeline A. Shea and Timothy Lohman
- 2007 Organizers: Brian M. Baker and Michael T. Henzl
Keynote Speaker: Jamie Williamson
- 2008 Organizers: Jannette Carey and David Bain
Keynote Speakers: Dorothy Beckett and Ken Dill
- 2009 Organizers: Nathan Baker and Liskin Swint-Kruse
Keynote Speaker: Linda Jen-Jacobson
The Gary K. Ackers Lecture in Biothermodynamics: Michael Brenowitz
- 2010 Organizers: Elisar Barbar and Vince LiCata
Keynote Speaker: C. Nick Pace
The Gary K. Ackers Lecture in Biothermodynamics: Timothy Lohman
- 2011 Organizers: Gibbs Society of Board of Directors
Keynote Speaker: Bertrand Garcia-Moreno E.
The Gary K. Ackers Lecture in Biothermodynamics: Madeline Shea
Editors of Special Issue of *Biophysical Chemistry* – Enrico Di Cera, Tim Lohman, Jack Correia
- 2012 Organizers: Aaron L. Lucius and Patricia L. Clark
Keynote Speaker: Terry G. Oas
The Gary K. Ackers Lecture in Biothermodynamics: Enrico Di Cera
- 2013 Organizers: James L. Cole and Aron W. Fenton
Keynote Speaker: Doug Barrick
The Gary K. Ackers Lecture in Biothermodynamics: Bertrand Garcia-Moreno E.

Gibbs Society Governance

Incorporation

In 2002, the *Gibbs Society of Biological Thermodynamics* incorporated in the Commonwealth of Virginia, under the guidance of Michael L. Johnson, then Treasurer of the Society and originator of the Society website. Articles of Incorporation and By Laws are available here: <http://www.jhu.edu/~gibbs>.

Current Officers

- ❖ President: David Bain, 2012-2013
- ❖ Vice President: Michael L. Johnson, 2011 - 2013
- ❖ Secretary: Margaret A. Daugherty, 2004 – 2013
- ❖ Treasurer: John J. Correia, March 2011 – October, 2016

Board of Directors, listed alphabetically

- ❖ David Bain, President
- ❖ Douglas Barrick, Past President
- ❖ John J. Correia, Treasurer
- ❖ Margaret Daugherty, Secretary
- ❖ Michael L. Johnson, Vice President
- ❖ George Makhatadze, President Elect
- ❖ Madeline Shea

Past Presidents

2001-2002	Gary Ackers
2002-2003	Jack Correia
2003-2004	D. Wayne Bolen
2004-2005	Madeline Shea
2005-2006	Dorothy Beckett
2006-2007	Jonathan (Brad) Chaires
2007-2008	Tim Lohman
2008-2009	Luis Marky
2009-2010	Bertrand Garcia-Moreno E.
2010-2011	Karen Fleming
2011-2012	Doug Barrick

Past Treasurer

2001-2011	Michael L. Johnson
-----------	--------------------

Committees & Other Contributions

Ackers Lecturer Selection Committee – Micheal Brenowitz, Chair
Gibbs Society Website Hosting – Karen Fleming (2010 -)
GoogleDocs Application/Registration & PayPal – Nathan Baker and Jack Correia
Mailing List – Margaret Daugherty
Saturday Night Thermo Organizers – Liskin Swint-Kruse and Vincent J. LiCata
With thanks to Alan Teska at the Touch of Nature Conference Center

5th Annual Gary K. Ackers Lecture

2013 Lecturer – *Bertrand Garcia-Moreno E., Department of Biophysics, Johns Hopkins University*

This lecture honors the scientific contributions of Gary K. Ackers (1939-2011) to the field of Biological Thermodynamics. He served on the faculty at the University of Virginia, Johns Hopkins University and the Washington University School of Medicine. He was a Fellow of the Biophysical Society and was one of the founding organizers of the Gibbs Conference.

Gary demonstrated a lifelong commitment to the growth and development of an intellectual community of scholars devoted to furthering the field of biothermodynamics. Gary was an active member of the Biophysical Society throughout his career and served as President of the Society, as well as Organizer of the annual meeting. While on the faculty of the University of Virginia, he was a leader in the graduate biophysics training program. When on the faculty in the Department of Biology at the Johns Hopkins University, he conceived and organized the *Institute for Biophysical Studies of Macromolecular Assemblies*, a university-wide training program in molecular biophysics that has continued for decades. While at Johns Hopkins, he also played a leading role in the establishment of the Gibbs Conference on Biothermodynamics, an annual meeting organized to promote innovative development of biophysical principles applied to current problems in biology and to train the next generation of molecular biophysicists to tackle hard problems rigorously. After moving to St. Louis to chair the Department of Biochemistry and Molecular Biophysics at Washington University, he spearheaded a new graduate program in biophysics and hired many faculty who have joined the community of regular contributors to the Gibbs Conference.

Gary was a pioneer in the development of methods and application of principles of equilibrium thermodynamics to the study of linkage in complex macromolecular assemblies. Studies from his laboratory on the energetics of self-association and ligand binding in human hemoglobin proved unequivocally that the classic and elegant MWC model of intersubunit allostery was insufficient to explain cooperative oxygen binding: the position, as well as the number, of ligands matters. His contributions in this area greatly enhanced our understanding of the relationship between structure, energy and function in hemoglobin, and in multimeric allosteric systems in general. By probing ever more deeply into the molecular mechanism of cooperativity, he demonstrated a beautiful, useful, and general strategy for dissecting functional energetics in macromolecular assemblies.

His quantitative study of the interactions between proteins and nucleic acids in the bacteriophage lambda system included the development of quantitative DNase footprinting methods for measuring free energies of repressor-operator interactions. The footprinting assay remains an effective tool for measuring the extremely tight binding constants that are often encountered in site-specific interactions between proteins and nucleic acids. Those studies paved the way for similar methods to study protein-nucleic acid interactions in more complex systems, including time-resolved studies of the kinetics of RNA folding. Based on his experimental studies of phage lambda, his group developed statistical thermodynamic models to simulate the lysogenic-to-lytic growth switch: the series of macromolecular events that determine the fate of bacteriophage lambda during infection of *E. coli*. This work demonstrated how a complex biological function could be predicted quantitatively, strictly from the kinetics of transcription and translation, and the Gibbs free energy of interactions between the key macromolecular components in the genetic switch.

During Gary's early career, he developed methods to measure association constants in self-associating systems based on analytical gel permeation chromatography. Those methods have since become standard tools in the field. His group was also responsible for modifications of the cryo-gel electrophoresis methods, moving from applying them to hemoglobin to protein-DNA interactions. These contributions focused on developing the capacity to quantify intermediate states that are only transiently populated during the course of a biochemical process. His more than 200 articles and chapters changed our view of the molecular mechanisms that govern complex biochemical reactions.

△Gibbs₂₇ • Saturday Evening • October 5, 2013

4:00 – 10:00 pm **Check-in at Little Grassy Lodge**

7:30 – 10:00 pm **Open Reception in Indian Lodge – Light refreshments, beer, wine and soft drinks**
Participants are expected to make dinner arrangements independently

Gibbs Mugs Bazaar – pick up mugs purchased at registration

Saturday night thermo – Event for trainees only

Faculty Organizers

Vince LiCata, Louisiana State University & Liskin Swint-Kruse, University of Kansas Medical Center

Trainee Moderators: Jaycob Warfel, LiCata Lab, Louisiana State University

Kate Henderson, Emerson Lab, Mississippi State University

5:30 pm **Freeberg Hall – Dinner for trainees who registered in advance**

6:00 – 7:00 pm **Flash Talks (Poster Introductions) – Session open to all trainees**

1. **The annexin-lipid party line: Communication between protein and membrane**
Anika Rannikko, Hinderliter Lab, University of Minnesota - Duluth
2. **Structural and dynamic features underlie the switch of ligand binding specificity in a Tiam1 PDZ domain mutant**
Xu Liu, Fuentes Lab, University of Iowa
3. **Cyclipostin derivatives as inhibitors of rat hormone sensitive lipase**
Elena Vasilieva, Dupureur Lab, University of Missouri – St. Louis
4. **Domain interactions in VA I RNA mediate high-affinity PKR binding**
Katherine Launer-Felty, Cole Lab, University of Connecticut
5. **Distinctive solvation patterns make renal osmolytes diverse**
Prem Sinha, Rösigen Lab, Penn State College of Medicine
6. **Are proton binding reactions in proteins coupled to local conformational fluctuations?**
Daniel Richman, Garcia-Moreno Lab, Johns Hopkins University
7. **Calmodulin recognition of anti-parallel nested binding sites in the neuronal voltage-dependent sodium channel Na_v1.2**
Mark Miller, Shea Lab, University of Iowa
8. **Contributions of alanine residues to the hydrodynamic radius of p53(1-93)**
Romel Perez, Whitten Lab, Texas State University

7:00 – 7:15 pm **Refreshment break**

7:15 – 8:15 pm **Career panel – Session open to all trainees**
Andrew Robertson, National Psoriasis Foundation
Ana-Maria Soto, Towson University
Catherine Royer, Rensselaer Polytechnic Institute

8:15 pm **Adjourn to reception in Indian Lodge**

ΔGibbs₂₇ • Sunday Morning • October 6, 2013

7:00 - 8:15 am **Breakfast served in Freeberg Hall**

Macromolecular Folding and Stability

8:30 - 8:35 am **Welcome by David Bain**, Gibbs Society President

Moderator: Giselle Jacobson, Clark Lab, University of Notre Dame

8:35 - 8:50 am **Introduction to the 27th Annual Gibbs Conference Keynote Speaker**
George Makhatadze, Rensselaer Polytechnic Institute

8:50 – 9:30 am **Keynote Lecture**
Measuring coupling energies in simple proteins and extrapolating to complicated proteins
Doug Barrick, Johns Hopkins University

9:30 – 9:45 am **Capped G-quadruplexes: A model for a looped out motif flanked by duplex DNA**
Vu Le, Lewis Lab, Mississippi State University

9:45 – 10:00 am **Probing N-H - - N hydrogen bond “strain” in a conserved N-capping motif**
Matthew Preimesberger, Lecomte Lab, Johns Hopkins University

10:00 – 10:30 am **Break – Refreshments in Indian Lodge**

10:30 – 11:00 am **Studying the folding of individual residues in a small protein**
Nicholas Fitzkee, Mississippi State University

11:00 – 11:15 am **Exchange of putrescine²⁺ and Mg²⁺ in the ion atmosphere of RNA: Contributions to folding and gene regulation**
Robert Trachman, Draper Lab, Johns Hopkins University

11:15 – 11:45 am **Tug-of-war between thermodynamic stability and actin-binding function of tandem calponin-homology domains**
Krishna Mallela, University of Colorado Anschutz Medical Campus

11:45 am **Conference photo near Freeberg Hall**

11:50 am **Lunch in Freeberg Hall**

Free Time until Late Afternoon Session

Information about local parks & attractions is available near the entrance to Little Grassy Lodge.

ΔGibbs₂₇ • Sunday Afternoon • October 6, 2013

Nucleic Acids and Their Binding Partners

Moderator: Irine Khutsishvili, Marky Lab, University of Nebraska Medical Center

3:00 – 3:30 pm **Structured mRNA induces the ribosome into a hyper-rotated state**
Peter Cornish, University of Missouri

3:30 – 3:45 pm **How do the different DNA-binding modes of *E. coli* SSB affect function?**
Vincent Waldman, Lohman Lab, Washington University

3:45 – 4:15 pm **Looking at multicomponent systems with small angle x-ray scattering: Ions around DNA; DNA around proteins**
Lois Pollack, Cornell University

4:15 – 4:35 pm **Break – Refreshments in Indian Lodge**

4:35 – 4:50 pm **Binding and salt linkages of HIV-1 reverse transcriptase with different DNA and RNA substrates reflect polymerization versus RNase H compatible modes of interaction**
Hiromi Brown, LiCata Lab, Louisiana State University

4:50 – 5:20 pm **DNA binding behavior of sequence specific polyamides**
Cynthia Dupureur, University of Missouri - St Louis

5:20 – 5:35 pm **Yeast Rap1 interacts with telomeric sequences with multiple binding modes**
Erik Feldmann, Galletto Lab, Washington University

5:35 – 6:05 pm **Substrate recognition by human O6-alkylguanine DNA alkyltransferase**
Michael Fried, University of Kentucky

6:05 – 6:15 pm **General Discussion**

6:15 pm **Dinner in Freeberg Hall**

ΔGibbs₂₇ • Sunday Evening • October 6, 2013

8:00 – 10:00 pm **Poster Session I in Sledgefoot (lower level) & Freeberg (upper level)**
Presenters with last name A to L
Please remove posters before midnight to make room for Monday presenters.

Sponsors displays in Freeberg (upper level) – near beer, wine and soft drinks

△Gibbs₂₇ • Monday Morning • October 7, 2013

Posters to be presented on Monday night may be mounted as soon as space is available on Sunday night.

Airport Ride Board will be available in Little Grassy Lodge, near check-in window

7:00 - 8:15 am **Breakfast served in Freeberg Hall**

Structure / Thermodynamic Correlations

8:30 – 8:35 am **Announcements by organizers**

Moderator: Keith Connaghan, Bain Lab, University of Colorado Anschutz Medical Campus

8:35 – 8:50 am **Introduction to the Gary K. Ackers Lecture in Biothermodynamics**
David Bain, University of Colorado Anschutz Medical Campus

8:50 – 9:30 am **5th Annual Gary K. Ackers Lecture**
pH-Dependent processes in proteins
Bertrand Garcia-Moreno E., Johns Hopkins University

9:30 – 9:45 am **Impact of pH on the structure and function of neural cadherin**
Matt Dukes, Pedigo Lab, University of Mississippi

9:45 – 10:00 am **Revisiting thermodynamic cooperativity: Studying TRBP interactions with double stranded RNA**
Roderico Acevedo, Showalter Lab, Pennsylvania State University

10:00 – 10:20 am **Break – Refreshments in Indian Lodge**

10:20 – 10:50 am **Molecular switches in signaling and disease**
Linda Nicholson, Cornell University

10:50 – 11:05 am **Thermodynamics of cationic and anionic surfactant interaction**
Vytautas Petrauskas, Matulis Lab, Vilnius University

11:05 – 11:35 am **Structure, dynamics and inhibition of Tiam1 PDZ domain complexes**
Ernesto Fuentes, University of Iowa

11:35 – 11:40 am **Vendor introduction**

11:40 am **Lunch in Freeberg Hall**

1:00 – 2:00 pm **Meeting of past organizers - Indian Building**
Refreshment area will be unavailable to other meeting attendees during this time

Trainee funding panel

2:00 – 3:00 pm **Discussion of trainee fellowship applications - open to all attendees**

Trevor Creamer, University of Kentucky
Sarah Bondos, Texas A&M Health Science Center
Dorothy Beckett, University of Maryland

ΔGibbs₂₇ • Monday Afternoon • October 7, 2013

Macromolecular Interactions

Moderator: Clarissa Weaver, Lucius Lab, University of Alabama at Birmingham

3:15 – 3:45 pm **Transition metal homeostasis: Allosteric switching by metal sensor proteins**
David Giedroc, Indiana University

3:45 – 4:00 pm **Heterotropic linkage between protein-protein and protein RNA interactions determines protein partitioning in the snRNPs**
Sandra Williams, Hall Lab, Washington University

4:00 – 4:30 pm **Engineering linked-equilibria into protein interfaces to allow regulation of protein interactions**
James Horn, Northern Illinois University

4:30 – 4:45 pm **Break – Refreshments in Indian Lodge**

4:45 – 5:15 pm **Biophysical studies on the A1 and A3 domain homologues of von Willebrand factor**
Matthew Auton, Mayo Clinic

5:15 – 5:45 pm **Structure and function of a novel phosphopeptide binding domain from the transcriptional corepressor MINT**
Rhett Kovall, University of Cincinnati College of Medicine

5:45 – 6:00 pm **Discussion**

6:00 pm **Dinner in Freeberg Hall**

ΔGibbs₂₇ • Monday Night • October 7, 2013

8:00 – 10:00 pm **Poster Session II in Sledgefoot (lower level) & Freeberg (upper level)**
Presenters with last name M to Z

Sponsors displays in Freeberg (upper level) – near beer, wine and soft drinks

ΔGibbs₂₇ • Tuesday Morning • October 8, 2013

Check-out : Please leave your room keys at the counter in the lobby of Little Grassy Lodge.
Airport Ride Board will be available in Little Grassy Lodge, near the check-in window

7:30 – 8:30 am **Breakfast in Freeberg Hall**

Modeling and Computation

8:40 – 8:45 am **Closing announcements by organizers**

Moderator: Catie Shelton, Herr Lab, University of Cincinnati College of Medicine

8:45 – 9:15 am **Computation and calorimetry: What can we learn from each other?**
Michael Gilson, University of California-San Diego

9:15 – 9:30 am **The tertiary architecture of LacI/GalR proteins accommodates multiple networks of functionally important positions**
Daniel Parente, Swint-Kruse Lab, University of Kansas Medical Center

9:30 – 10:00 am **Temperature effects on the hydrodynamic radius of intrinsically disordered proteins**
Steven Whitten, Texas State University

10:00 – 10:20 am **Break – Refreshments in Indian Lodge**

10:20 – 10:40 am **Rational design of two proteins with 80% sequence identity but different folds using sequence transplantation**
Lauren Porter, Potomac Affinity Proteins

10:40 – 10:55 am **Deletion of the connecting linker between kringle-1 and kringle-2 in prothrombin stabilizes the zymogen in a collapsed conformation**
Nicola Pozzi, Di Cera Lab, Saint Louis University

10:55 – 11:25 am **Molecular modeling of the ligand binding free energy and conformational rearrangements in AMPA type glutamate receptors**
Maria Kurnikova, Carnegie Mellon University

11:25 am **Box lunch available in Freeberg Hall**

Check-out Please leave your keys at the counter in Little Grassy Lodge

Posters Information

Posters will be presented in two evening sessions in Sledgefoot Hall (next to Freeberg Dining Hall). Session I will be held Sunday evening and will feature posters from presenters with last names A to L. Session II will be held Monday evening and will feature posters from presenters with last names M to Z. Both sessions will start at 8:00 pm; posters should be taken down before the talks start the next morning.

Session I (A-L)

1. **Intermolecular forces in DNA condensation with cationic dendrimers** Min An, Jason DeRouchey, Department of Chemistry, University of Kentucky
2. **Molecular Dynamics Studies of the Role of Protein Flexibility in Immunological Molecular Recognition** Cory M. Ayres, Daniel R. Scott, Brian M. Baker and Steven A. Corcelli, Department of Chemistry and Biochemistry, University of Notre Dame
3. **Thermodynamic and Structural Determinants of Differential Pdx1 Binding to Elements from the *Insulin* and *IAPP* Promoters** Monique Bastidas and Scott Showalter, Department of Chemistry, The Pennsylvania State University
4. **Functional coupling via communication of surface loop disorder-to-order transitions through a folded protein core** Dorothy Beckett, Christopher Eginton, Katie Marshall, Sharrol Bachas & Hershel Wade, Department of Chemistry & Biochemistry, University of Maryland College Park; Department of Biophysics and Biophysical Chemistry, Johns Hopkins School of Medicine
5. **Use of internal Asp and Glu residues to design a conformational switch sensitive to changes in physiological pH** Peregrine Bell-Upp, Ilaria Caturegli, Meredith Peck, Aaron C. Robinson, Bertrand García-Moreno E. T.C. Jenkins, Department of Biophysics, Johns Hopkins University
6. **Investigating Metal Ion Binding in HPCD** Or I. Benet, Kate L. Henderson, Joseph P. Emerson, Mississippi State University
7. **Analysis of co-localization of 5-hydroxymethylcytosine and G-quadruplex-forming regions in gene transcriptional start sites: might hydroxymethylation affect the C-rich i-motif structure?** Yogini P. Bhavsar-Jog, Eric Van Dornshuld, Gregory S. Tschumper, Randy M. Wadkins, Department of Chemistry and Biochemistry, The University of Mississippi
8. **Kinetic control in protein folding for light chain amyloidosis and the differential effects of somatic mutations** Luis. M. Blancas-Mejía, Alexander Tischer, James R. Thompson, Jonathan Tai, Lin Wang, Matthew Auton, Marina Ramirez-Alvarado, Department of Biochemistry and Molecular Biology, Division of Hematology and Department of Physiology and Biomedical Engineering, Mayo Clinic; Department of Chemistry, University of Illinois
9. **Physical Basis of Recognition of Antigens by T Cell Receptors** Sydney Jo Blevins, Kurt Piepenbrink, and Brian M. Baker, Department of Chemistry and Biochemistry, University of Notre Dame
10. **Influence of cavities on the stability and pressure sensitivity of proteins** José A. Caro, Mariano Dellarole, Jamie L. Schlessman, Catherine A. Royer, Bertrand García-Moreno E., Department of Biophysics, Johns Hopkins University, Centre de Biochimie Structurale, CNRS

11. **Assembly, Structural and Functional Studies of Polyubiquitin Chains** Carlos A. Castañeda^a, Susan Krueger^b, Ashton Cropp^c and David Fushman^a, University of Maryland; NIST Center for Neutron Research, Virginia Commonwealth University
12. **Metal Binding Specificity of the Staphylococcal Biofilm Protein Aap** Catherine T. Chaton and Andrew B. Herr, Department of Molecular Genetics, Biochemistry and Microbiology, University of Cincinnati College of Medicine
13. **Analysis of PKR dimerization by resonance energy transfer** Bushra Husain, Michael Bruno and James L. Cole, Department of Molecular and Cell Biology, University of Connecticut
14. **SPOC—A novel phospho-peptide binding domain important for repressing Notch target genes** Kelly J. Collins, Nassif H. Tabaja, and Rhett A. Kovall, Department of Molecular Genetics, Biochemistry, and Microbiology, University of Cincinnati
15. **Steroid Receptors Cooperatively Assemble at Promoters with Unique Dependence on Inter-site Distance** Keith D. Connaghan, Nicole T. Fazio, Qin Yang, Amie D. Moody, Michael T. Miura and David L. Bain, Department of Pharmaceutical Sciences, University of Colorado Anschutz Medical Campus
16. **Role of Primary Structure Variation in Structure and Function of Calcineurin** Erik C. Cook, Tori B. Dunlap, Praveen R. Juvvadi, William J. Steinbach, and Trevor P. Creamer, Center for Structural Biology and Department of Molecular and Cellular Biochemistry, University of Kentucky
17. **Studies of RNA recognition and catalytic domains in the conserved virulence factor A from *Streptococcus pyogenes*** Valeria A. Copello, Taylor D. Griffith, Catherine A. Carney, Fanny Chu, Kyu Hong, Cho, Brian M. Lee and Gabriela C. Pérez-Alvarado, Department of Chemistry and Biochemistry and Department of Microbiology, Southern Illinois University
18. **Steroid receptors partition their interaction energetics in parallel with their phylogenetic divergence** Rolando W De Angelis, Qin Yang, Michael T Miura and David L Bain, Department of Pharmaceutical Sciences, University of Colorado Anschutz Medical Campus
19. **RNA Target Discrimination by U1A and SNF Proteins** Gregory T. DeKoster, Kimberly J. Delaney, and Kathleen B. Hall, Department of Biochemistry & Molecular Biophysics, Washington University School of Medicine
20. **Understanding DNA Condensation: From Simple Ions to Protamine-DNA Packaging in Sperm** Jason DeRouchey, Department of Chemistry, University of Kentucky
21. **Backbone conformational reorganization as a determinant of pKa values of surface residues in proteins** Brian M. Doctrow, Kelli L. Baran, Jamie L. Schlessman, Ananya Majumdar, Bertrand García-Moreno, Department of Chemistry, United States Naval Academy; Department of Biophysics and Biomolecular NMR Facility, Johns Hopkins University
22. **Driving Forces for Protein Secretion Across the Bacterial Outer Membrane** Igor Drobnak and Patricia L. Clark, Department of Chemistry and Biochemistry, University of Notre Dame
23. **Calmodulin induced calcineurin conformational changes and activation** Tori B. Dunlap, Julie Rumi-Masante, Terrence E. Lester, Hou-fu Guo, Emily Holbrook, Hou-fu Guo, Farai I. Rusinga, Craig W. Vander Kooi, David D. Weis, and Trevor P. Creamer, Department of Molecular and Cellular Biochemistry, University of Kentucky; Department of Chemistry, University of Central Arkansas; Department of Chemistry, University of Kansas
24. **Examination of the thermodynamic mechanism for *E. coli* ClpAP assembly** Elizabeth C. Duran and Aaron L. Lucius, Department of Chemistry, University of Alabama at Birmingham
25. **Unfolding Thermodynamics of the DNA Sarcin-Ricin Loop Sequence** Nadera R. Elkarmi, Sarah E. Johnson and Luis A. Marky, University of Nebraska at Omaha, Department of Pharmaceutical Sciences, University of Nebraska Medical Center

26. **In search of techniques that will aid the development of “silent coupling” leads into allosteric drugs: H/DX-MS studies of pyruvate kinase** Charulata B. Prasannan, Maria T. Villar, Aleksandra Gmyrek, Antonio Artigues and Aron W. Fenton, The University of Texas Medical Branch at Galveston and The University of Kansas Medical Center
27. **Nucleosome Remodeling by the chromatin remodeler ISWI** Christopher J. Fischer, Gada Al-Ani, Koan Briggs, Department of Physics and Astronomy; University of Kansas Department of Molecular Biosciences, University of Kansas
28. **From dimer folding to nucleosome assembly** Lisa M. Gloss, Traci B. Topping, Paul J. Guyett and Anna M. Lindsay, School of Molecular Biosciences, Washington State University
29. **Investigating the Structural and Dynamic Features that Support Tiam2 PDZ Domain Binding Specificity** Lisa C. Golden, Xu Liu, and Ernesto J. Fuentes, Department of Biochemistry, University of Iowa
30. **Single-primer quadruplex priming amplification (QPA) for DNA diagnostics** David Gvarjaladze, Besik Kankia, Department of Chemistry and Biochemistry, The Ohio State University
31. **Enhanced peptide flexibility leads to diminished antigenicity and the failure of a cancer vaccine candidate** Lance M. Hellman, Andrew D. McKernan, and Brian M. Baker, Department of Chemistry and Biochemistry and Harper Cancer Research Center, University of Notre Dame
32. **Thermodynamics of Fe(II) Binding in Dioxygenase Enzymes** Kate L Henderson, Joseph P Emerson, Department of Chemistry, Mississippi State University
33. **Synaptotagmin linker: tuning of cooperativity in Ca^{2+} binding** Troy A. Hendrickson, Jacob W. Gauer, Komemba J. Lohese, Michael E. Fealey, R. Bryan Sutton, and Anne Hinderliter, Department of Chemistry and Biochemistry, University of Minnesota Duluth; Department of Cell Physiology, Texas Tech University Health Sciences Center
34. **Characterizing the Binding Affinity of the Tiam1-Talin Complex** Frances C. Hindt, Zhen Xu, and Ernesto J. Fuentes, Department of Biochemistry, University of Iowa
35. **Thermodynamic studies of full length amyloidogenic light chains** Timothy J. Horn, Luis Blancas-Mejia, Winter A. Okoth, Matthew Auton, Alexander Tischer, Marina Ramirez-Alvarado, Department of Biochemistry and Molecular Biology, Division of Hematology, Mayo
36. **mRNA splicing alters the function of Ubx, a Hox transcription factor** Hao-Ching Hsiao, Kelly Churion, Kathleen S. Matthews, Sarah E. Bondos, Department of Molecular and Cellular Medicine, Texas A&M University System Health Science Center; Department of Biochemistry and Cell Biology, Rice University
37. **Cisplatin Adduct Effects on DNA-DNA Interactions** James Hutchison, Jason DeRouchey, Department of Chemistry, University of Kentucky
38. **Probing the dynamics of nascent GFP chains on the ribosome** Giselle Jacobson, Srabasti Acharya, Basir Ahmad, Yujie Chen, Krastyu Ugrinov, Lisa Lapidus and Patricia L. Clark, Department of Physics and Astronomy, Michigan State University; Department of Chemistry and Biochemistry, University of Notre Dame
39. **Dissection of molecular driving forces of affinity maturation** Sriram Jakkaraju, Robert James Hoey IV and James R. Horn, Department of Chemistry and Biochemistry, Northern Illinois University
40. **Targeting of a DNA Complex with Joined Triplex-Duplex Motifs** Sarah E. Johnson, Hui-Ting Lee and Luis A. Marky, Department of Pharmaceutical Sciences, University of Nebraska Medical Center
41. **Transient partial unfolding in *E. coli* DHFR** Joseph R. Kasper and Chiwook Park, Department of Medicinal Chemistry and Molecular Pharmacology, Purdue University

42. **Hydration Effects in the Folding Thermodynamics of a Bent Oligonucleotide Duplex** Irine Khutsishvili and Luis A. Marky, Department of Pharmaceutical Sciences, University of Nebraska Medical Center
43. **Calcium-Mediated Regulation of Calcineurin by a Dynamic Duo of EF-Hand Proteins** Sean A. Klein, Susan O'Donnell, Brett C. Waite, Jesse B. Yoder, and Madeline A. Shea, Department of Biochemistry, University of Iowa
44. **Lipid and the Thermodynamics of Amyloid beta Fibril Formation** Alexandra Klinger and Paul Axelsen, Pharmacology, University of Pennsylvania
45. **The Effects of *E. coli* SSB Tail Mutations and Deletions on ssDNA Binding Properties and Binding Modes Transitions** Alexander G. Kozlov, Elizabeth A. Weiland and Timothy M. Lohman, Department of Biochemistry and Molecular Biophysics, Washington University School of Medicine
46. **Single molecule force spectroscopy analysis of alpha-synuclein misfolding** Alexey V. Krasnoslobodtsev, Ivan L. Volkov, Jean-Christophe Rochet and Yuri L. Lyubchenko, Department of Pharmaceutical Sciences, University of Nebraska Medical Center; Department of Medicinal Chemistry and Molecular Pharmacology, Purdue University
47. **Domain interactions in VA I RNA mediate high-affinity PKR binding**, Katherine D. Launer-Felty and James L. Cole, Department of Molecular and Cell Biology, University of Connecticut
48. **Allostery mediated by the intrinsic disordered domain governs the function of glucocorticoid receptor translational isoforms** Jing Li, James Sowers, E. Brad Thompson and Vincent J Hilser, Department of Biology and T.C. Jenkins Department of Biophysics and Department of Chemistry, Johns Hopkins University; Department of Biochemistry and Molecular Biology, University of Texas Medical Branch
49. **Molecular dynamics simulations of 441 two-residue peptides in aqueous solution: effects of sidechain interactions on backbone conformations** Shuxiang Li, Casey T. Andrews, Mark Miller, Stephen Siemonsma, Timothy Collingsworth, Isaac Rockafellow, Anh Nguyet, Brady Campbell, Reid Brown, Chengxuan Guo, Michael Schrodtt, Yu-Tsan Liu and Adrian H. Elcock, Department of Biochemistry, University of Iowa
50. **Thermodynamic and hydrodynamic examination of ClpB assembly** Jia Bei Lin and Aaron L. Lucius, Department of Chemistry, University of Alabama at Birmingham
51. **Structural and Dynamic Features Underlie the Switch of Ligand Binding Specificity in a Tiam1 PDZ Domain Mutant** Xu Liu, David C. Speckhard, Tyson R. Shepherd and Ernesto J. Fuentes, Department of Biochemistry, Department of Chemistry, University of Iowa
52. **Isothermal generation of DNA clones for next-generation sequencing** Levan Lomidze, Besik Kankia, Department of Chemistry and Biochemistry, The Ohio State University
53. **YKB: A Protein Biosensor Used to Detect Changes in Translation Rate** Jamie Luther and Patricia L. Clark, Department of Chemistry and Biochemistry, University of Notre Dame

Session II (M-Z)

54. **Temperature Dependence of the Thermodynamics for Binding Histone H10, its Carboxyl Domain (H10-C), or its Globular Domain (H10-G) to Calf-Thymus DNA** Venkata Machha, Anna L. Turner, and Edwin A. Lewis, Department of Chemistry, Mississippi State University
55. **Dysferlin; Small Change Big Flex** Ryan W. Mahling, Anne M. Rice, R. Bryan Sutton, and Anne Hinderliter, Texas Tech University Health Sciences Center; University of Minnesota Duluth
56. **Dose response assays can measure accurate equilibrium DNA binding parameters *in situ*** Karl Maluf and David L. Bain, Department of Pharmaceutical Sciences, University of Colorado Anschutz Medical Campus
57. **Substrate binding to Mn-, Fe-, and Co-HPCD** Amy E. Martin, Kate L. Henderson, Joseph P. Emerson, Mississippi State University
58. **Bifurcation of Human Sodium Channels Based on Calmodulin Discrimination of IQ Motifs** Dagan C. Marx, Kristin M. Tefft, Mark S. Miller, Jesse B. Yoder, Elaine Kim, Liam Hovey, Sterling C. Martin, Andrew Tarleton, Brett C. Waite, Michael D. Feldkamp, and Madeline A. Shea, Department of Biochemistry, Carver College of Medicine, University of Iowa
59. **The Pitfalls of Intrinsic Thermodynamics – Structure Correlations of Lead Binding to Target Proteins** Asta Zubrienė, Lina Baranauskienė, Egidijus Kazlauskas, Joana Gylytė, David D. Timm, Vaida Morkūnaitė, Miglė Kišonaitė, Edita Čapkauskaitė, Virginija Dudutienė, Vaida Jogaitė, Justina Kazokaitė, Aistė Kasiliauskaitė, Vilma Michailovienė, Aurelija Mickevičiūtė, Sandra Bakšytė, Vilma Petrikaitė, Vytautas Petrauskas, Alexey Smirnov, Lena Manakova, Saulius Gražulis, Visvaldas Kairys, Jurgita Matulienė, and Daumantas Matulis, Department of Biothermodynamics and Drug Design, Institute of Biotechnology, Vilnius University, Lithuania
60. **The Thermodynamics of Translocation of the Ribosome** Drew E. Menke, Peter V. Cornish, Department of Biochemistry, University of Missouri Columbia
61. **Molecular Interactions that Control Protein Assembly into Nanofiber Structures** Reagan J. Meredith and Steven T. Whitten, Department of Chemistry and Biochemistry, Texas State University
62. **Calmodulin Recognition of Anti-Parallel Nested Binding Sites in the Neuronal Voltage-Dependent Sodium Channel NaV1.2** Mark S. Miller, Liam Hovey, C. Andrew Fowler, Dagan C. Marx, Jesse B. Yoder, Elaine Kim, Shuxiang Li, Kristin M. Tefft, Brett C. Waite, Michael D. Feldkamp, Liping Yu, and Madeline A. Shea, Department of Biochemistry, Carver College of Medicine, University of Iowa
63. **Broad ligand specificity in the small multidrug resistance transporter EmrE** Emma A. Morrison and Katherine A. Henzler-Wildman, Department of Biochemistry and Molecular Biophysics, Washington University in St. Louis
64. **Osmolyte Effects on the Self-Association of Concanavalin A: Testing the Tanford Transfer Model** Thomas W. Silvers and Jeffrey K. Myers, Department of Chemistry, Davidson College
65. **Probing the second high affinity binding site in human carbonic anhydrase II** Whitnee Nettles, Amy Martin, William Kennon, Joseph Emerson, Department of Chemistry, Mississippi State University
66. **Dynamics of Human Replication Protein A (RPA) Bound to Single Stranded DNA** Binh Nguyen, Joshua Sokoloski, Marc S. Wold, Roberto Galletto, Timothy M. Lohman, Department of Biochemistry & Molecular Biophysics, School of Medicine, Washington University, Department of Biochemistry, University of Iowa

67. **Apparent stability of PrPSc fibrils from natural host species as a tool in the understanding and differentiation of prion disease strains** Catherine E. Vrentas, Justin J. Greenlee, and Eric M. Nicholson, Virus and Prion Diseases Research Unit, National Animal Disease Center, U.S. Dept. of Agriculture, Agricultural Research Service
68. **Multiple Recognition Motifs Provide Rigidity to Stabilize LC8 Complexes** Afua Nyarko, Yujuan Song, & Elisar Barbar, Department of Biochemistry and Biophysics, Oregon State University
69. **Two-primer exponential quadruples priming amplification for diagnostics** Tamar Partskhaladze and Besik Kankia, Department of Chemistry and Biochemistry, The Ohio State University
70. **Contributions of alanine residues to the hydrodynamic radius of p53(1-93)** Romel B. Perez and Steven T. Whitten, Department of Chemistry and Biochemistry, Texas State University
71. **The Annexin-Lipid Party Line: Communication Between Protein and Membrane** Anika Rannikko, Samantha Jaworski, Anne Rice, Michael Fealey, and Anne Hinderliter, University of Minnesota-Duluth
72. **Loop Effects on the Stability and Targeting of DNA Pseudoknots** Calliste Reiling and Luis A. Marky, Department of Pharmaceutical Sciences, University of Nebraska Medical Center
73. **Are proton binding reactions in proteins coupled to local conformational fluctuations?** Daniel E. Richman, Ananya Majumdar, Bertrand Garcia-Moreno E., Dept. of Physics and Astronomy and Biomolecular NMR Center and Dept. of Biophysics, Johns Hopkins University
74. **Ion pairs buried in the hydrophobic interior of proteins: Are they charged and are they stabilizing?** Aaron Robinson, Ananya Majumdar, Jamie Schlessman and Bertrand García-Moreno E., Department of Biophysics, Johns Hopkins University
75. **The Role of Stability in the Function of the *Staphylococcus epidermidis* Biofilm Protein Aap** Catie Shelton and Andrew B. Herr, Department of Molecular Genetics, Biochemistry and Microbiology, University of Cincinnati College of Medicine Cincinnati
76. **Quantitative Biophysical Characterization of Intrinsically Disordered Proteins: NMR Structure Ensembles and the Thermodynamics of Folding-Up-on-Binding** Scott A. Showalter, Chad Lawrence, Anthony Zidell, and Debashish Sahu, Department of Chemistry, The Pennsylvania State University
77. **Molecular Dynamics Simulations of the Conformational Behavior of Tripeptides in Aqueous Solution** Scott A. Shubert, Oluwatoni J. Olayiwola, Aakash Jain, Shuxiang Li, Casey T. Andrews, Adrian H. Elcock, Department of Biochemistry, University of Iowa
78. **The Kinetic Mechanism of RecBCD Binding to and Melting Blunt End DNA** Michael J. Simon and Timothy M. Lohman, Biochemistry and Molecular Biophysics, Washington University School of Medicine
79. **Distinctive solvation patterns make renal osmolytes diverse** Prem Kumar Sinha, Ruby Jackson-Atogi, and Joerg Rösger, Department of Biochemistry and Molecular Biology, Penn State College of Medicine
80. **Development of a Selectively Fluorescent VHH Dimer Variant for Small Molecule Detection** Christopher Smith and James R. Horn, Northern Illinois University
81. **Engineering of protein conformational switches sensitive to changes in physiological pH: Design with internal Lys residues** J. L. Sorenson, E. Wheeler, I. Caturegli, M. Peck, J. L. Schlessman, A. Robinson, and B. García-Moreno E., Department of Biophysics, Johns Hopkins University

82. **The specific interaction of mRNA with CvfA protein through the KH domain in *Streptococcus pyogenes*** Gavin Sowa, Bingxin Yuan, Jinsai Shang, Bohui Jiang, Fanny Chu, Stephanie Geiser, Rebecca C. Weber, Gabriela C. Pérez-Alvarado, Kyu Hong Cho and Brian M. Lee, Department of Chemistry and Biochemistry, Southern Illinois University
83. **Using Sedimentation Velocity to Assay the Self-assembly of ClpAΔE286/565A in the Presence and Absence of Nucleotide** Ryan Stafford and Aaron Lucius, Department of Chemistry, University of Alabama at Birmingham
84. **Structural and biophysical characterization of the CSL-RITA complex, a novel mechanism to regulate transcription in the Notch signaling pathway** Nassif Tabaja and Rhett Kovall, Department of Molecular Genetics, Biochemistry, and Microbiology, University of Cincinnati College of Medicine
85. **Prediction of Crystallographic B-factors for the backbone atoms of the protein Calbindin D9k using Molecular Dynamics Simulations** Mahendra Thapa, Mark Rance, Department of Molecular Genetics, Biochemistry and Microbiology and Department of Physics, University of Cincinnati
86. **Breaking the disulfide bond induces a molten globule intermediate state in the von Willebrand Factor A1 domain that strengthens platelet adhesion** Alexander Tischer, Pranathi Madde and Matthew Auton, Department of Hematology, Mayo Clinic
87. **Biophysical Studies of the *S. aureus* SrrB-SrrA Two-component Signal Transduction System Involved in Toxic Shock Syndrome** Nitija Tiwari, Jonathan W. Willett, Caroline M. Linke, John R. Kirby and Ernesto J. Fuentes, Department of Biochemistry and Department of Microbiology, University of Iowa
88. **Effect of Mutations on the Conformation of A-site RNA Hairpins** Amber R. Truitt and Ana-Maria Soto, Department of Chemistry, Towson University
89. **Defining the Chemical Rules for Successful Biomaterials Functionalization Via Gene Fusion** Shang-Pu Tsai, Hao-Ching Hsiao, Sarah. E. Bondos, Department of Molecular and Cellular Medicine, Texas A&M Health Science Center
90. **Cyclipostin derivatives as Inhibitors of Rat Hormone Sensitive Lipase** Elena Vasilieva, Raj Malla, Supratik Dutta, Ben Martin, Cynthia M. Dupureur, Christopher D. Spilling, Department of Chemistry & Biochemistry and Center for Nanoscience, University of Missouri-St.Louis
91. **Two step bending of DNA in binding site recognition by Integration Host Factor** Yogambigai Velmurugu, Paula Vivas, Serguei V. Kuznetsov, Phoebe A. Rice and Anjum Ansari, Department of Physics and Department of Bioengineering, University of Illinois at Chicago; Department of Biochemistry and Molecular Biology, University of Chicago
92. **Conformational Selection is a Dominant Mechanism of Ligand Binding** Austin D. Vogt and Enrico Di Cera, Edward A. Doisy, Department of Biochemistry and Molecular Biology, Saint Louis University School of Medicine
93. **Determining Association Rates of Protein-protein Interactions Using a Quantitative Bead Aggregation Assay** Nagamani Vunnam, N. René Phongam, C. Ashton Walters, Nathan I. Hammer and Susan Pedigo, Department of Chemistry and Biochemistry, University of Mississippi
94. **Ligand-Control in MDR protein functions** Herschel Wade, Sharrol Bachas, Brian Kohr and John Froehlig, The John Hopkins University School of Medicine
95. **Folding and Hydrodynamics of the DNA i-motif from the c-MYC Promoter** Randy M. Wadkins, Samantha M. Reilly, Daniel Lyons, John J. Correia, and David M. Jameson, Department of Chemistry and Biochemistry; Department of Biochemistry, University of Mississippi; Department of Cell and Molecular Biology, University of Hawaii at Manoa,

96. **A Two-Step Model for Protein-Gold Nanoparticle Interactions** Ailin Wang, Karthik Vangala, Dongmao Zhang, and Nicholas C. Fitzkee, Department of Chemistry, Mississippi State University
97. **Thermodynamics of Single-stranded and Double-stranded DNA binding by *Deinococcus radiodurans* and *Escherichia coli* RecA homologues** Jaycob D. Warfel and Vince J. LiCata, Department of Biological Sciences, Louisiana State University
98. **Kinetic mechanism of *Escherichia coli* ClpA catalyzed ATP hydrolysis and thermodynamic linkage to macromolecular assembly** Clarissa L. Weaver and Aaron L. Lucius, Department of Chemistry, University of Alabama at Birmingham
99. **Mechanical Unfolding Studies of RNA Hairpins** Huizhong Xu, Muwen Kong, Ryan Slack, Krishna Murari Agarwal, Maumita Mandal, Department of Physics, Chemistry and Biomedical Engineering and Molecular Biophysics and Structural Biology Program, Carnegie Mellon University
100. **The self-assembly of a linear molecule** Yujia Xu, Rebecca Strawn and Parminderjeet Kaur, Department of Chemistry, Hunter College of the City, University of New York
101. **Characterizing the Folding and Unfolding Pathways for a Small Model Protein** Dinesh Kumar Yadav and Nicholas C. Fitzkee, Department of Chemistry, Mississippi State University
102. **Probe and Nanoparticles diffusion in polymer gels** Xiaolu Zhang, Nathanael Stocke, J. Zach Hilt and Jason DeRouchey, Department of Chemistry and Department of Chemical and Materials Engineering, University of Kentucky
103. **How Disordered? Assessing NMR-based Studies of Denatured Proteins** Yue Zhang and Nicholas C. Fitzkee, Department of Chemistry, Mississippi State University
104. **Structural Homology $\neq$ Thermodynamic Equivalence** Michael T. Zimmermann, Alexander Tischer, Steve Whitten, and Matthew Auton, Division of Biomedical Statistics and Informatics, Departments of Internal Medicine and Biochemistry and Molecular Biology, Mayo Clinic; Departments of Chemistry and Biochemistry, Texas State University

Speaker Abstracts

in order of presentation

Macromolecular Folding and Stability

Introduction to Keynote Lecture: George Makhatadze, Rensselaer Polytechnic Institute

Measuring coupling energies in simple proteins and extrapolating to complicated proteins

Keynote Lecture

Doug Barrick, Thuy Dao, Jake Marold, Katie Geiger, Tural Aksel, Katie Tripp

Department of Biophysics, Johns Hopkins University, 3400 N. Charles St, Baltimore, MD 21298.

One of the most striking features of the protein folding process is cooperativity. Although the structures of the native states of proteins comprise many short- and long-range elements that might appear to be independent (in a thermodynamic sense), these elements are in fact highly coupled, populating not as independent subsets but approximating a whole. Cooperativity in folding is likely to use the same underlying interactions, and thus, have the same thermodynamic features as functional properties such as allostery and assembly. Despite the importance of cooperativity in folding, our analysis of it remains largely at the "yes" or "no" (i.e., two-state versus multistate) level.

We have been using linear repeat proteins to extend our description of cooperativity in folding to a quantitative (thermodynamic) level. By constructing an array of 33-residue ankyrin repeats that have varying length but identical (consensus) sequence, we have resolved the high cooperativity of unfolding into local versus nearest-neighbor energy, entropy, and heat capacity. This approach has revealed an extremely strong coupling free energy between repeats. We find this coupling to be entropically driven at low temperatures, consistent with hydrophobic desolvation, and to be highly sensitive to histidine protonation state and to salt concentration, suggesting an electrostatic contribution to interface formation. This high level of coupling is considerably stronger than that seen in a set of consensus TPR helical repeats. Recent results with a novel, naturally occurring 42 residue TPR-like polypeptide (that shows nearly identical sequence from unit to unit) is of lower stability than the 34 residue TPR motifs, perhaps resulting from weaker coupling between repeats. For β -sheet leucine-rich repeat proteins, we find that a high level of cooperativity and stability can also be achieved with consensus repeats, although stability contributions are also made from variable capping motifs.

Linear repeat proteins are also well-suited for kinetic studies of folding. Due to the high level of structural symmetry of naturally-occurring (sequence-variable) repeat proteins, and the additional sequence symmetry of consensus repeat counterparts, we can examine the effects of chain-length on folding rates, the presence of parallel pathways, and the relationship between stability variation and cooperativity in determining rates and pathways. In kinetic studies with consensus ankyrin repeat folding, we find multiple parallel pathways with a transition state that appears to have around 1.5-2 repeats folded but not yet paired (i.e., coupled). These kinetic data can be fitted to a one-dimensional kinetic Ising model, which captures both the parallel and stepwise nature of folding and unfolding kinetics. This model provides a direct measurement of nucleation and propagation rates folding.

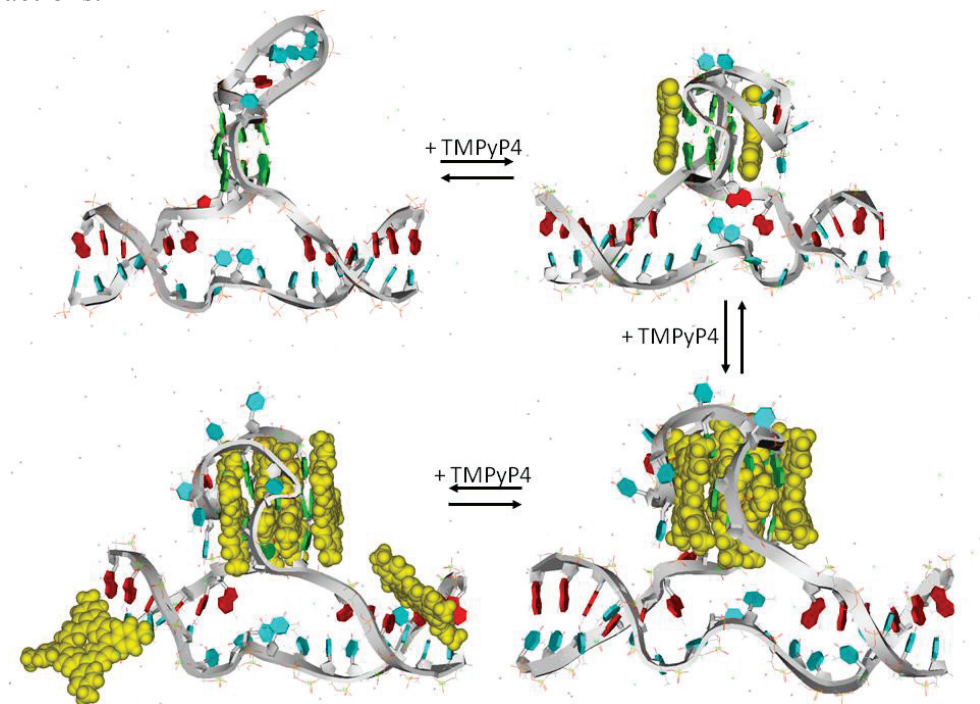
In contrast to arrays of identical repeats, we find that most naturally occurring repeat proteins fold through a single dominant pathway that corresponds to a region of lowest stability, and will illustrate this with folding and HX studies on a LRR protein. From the recurrent observation that the local free energy variation (1-2 kcal/mol) of naturally occurring repeat proteins is enough to collapse parallel folding into a single dominant pathway, it is tempting to generalize that folding by parallel pathways may be the exception rather than the rule, perhaps even more for globular proteins than for repeat proteins.

Capped G-quadruplexes: a Model for Looped out Motif Flanked by Duplex DNA

Vu H. Le, Matt McGuire, Meredith Blynn, Edwin A. Lewis

Department of Chemistry, Mississippi State University

G-quadruplex forming sequences are present in or near the regulatory regions of over 40% of all genes, including known oncogenes. To date studies performed on the G-quadruplex have been limited to single stranded oligo constructs that are potentially limited in their physiological relevance. We report here the results of a biophysical characterization of an ensemble of single stranded and “capped” ds intramolecular G-quadruplexes that model the polypurine sequence in the human c-Myc P1 promoter. CD results demonstrate that the spectra for the “capped” constructs are consistent with that of parallel intramolecular G-quadruplexes and that thermal melting of the “capped” retained G-quadruplex CD signature up to 95°C. DSC studies of “capped” constructs indicate a change in relative percentage of each conformation when compared to the uncapped constructs. The binding interactions between a probe ligand TMPyP4 and the “capped” constructs are very complex. The number of binding modes for TMPyP4 was increased with the addition of capping sequence from two to three as described by end-stacking, intercalation, and non-specific binding to the flanking duplex regions. The initial non-binding reaction observed in the ITC thermogram is attributed to be the restructuring transition of partially folded to completely folded G-quadruplex induced by TMPyP4 prior to binding reactions.



Probing N-H - - N hydrogen bond “strain” in a conserved N-capping motif

Matthew R. Preimesberger, Ananya Majumdar, and Juliette T.J. Lecomte
Department of Biophysics, Johns Hopkins University, Baltimore, MD 21218 USA

Hydrogen bonds have long been recognized for their contribution to the stability and self-assembly of macromolecules. However, the presence of a hydrogen bond is most often inferred indirectly. Methodological advances in nuclear magnetic resonance (NMR) have enabled site-resolved direct identification of each atom involved in a hydrogen bond through use of hydrogen bond scalar couplings (HBCs). Since the seminal observation of N-H - - N type HBCs in Watson-Crick base pairs, these measurements have become the method of choice for unambiguous determination of hydrogen bond arrangement in nucleic acids. Additionally, since the magnitude of the HBC is related to the covalent character of the hydrogen bond, valuable insight can be obtained by correlation with data derived from crystallographic or thermodynamic measurements. In biological systems, the study of N-H - - N type hydrogen bonds has been limited primarily to nucleic acids, in contrast, very few examples in proteins have been reported.

Here, we report the identification and characterization of a conserved hydrogen bond within the Group I truncated hemoglobin (Hb) lineage. HNN 2D NMR correlation experiments were specifically tailored to identify the donor amide Ser76 N-H and acceptor His79 N δ , which initiate the G helix within *Chlamydomonas eugametos* (*Chlamy*) chloroplastic Hb. We also observed a similar interaction between Asn80 N-H and His83 N δ within *Synechocystis* hemoglobin (GlbN), a cyanobacterial protein sharing 42% identity with the algal *Chlamy* globin. *Synechocystis* GlbN has the distinction among essentially all other hemoglobins in that it is capable of a spontaneous posttranslational modification (PTM) which covalently links the heme 2-substituent with His117 (forming GlbN-A). Additionally, a nonnative linkage to the 4-substituent can be engineered in the Leu79His variant to produce an unprecedented double histidine crosslinked heme (forming GlbN-AB). NMR data indicated that the N-capping hydrogen bond was maintained in all forms of each protein.

We then measured the N-H - - N HBCs and examined the effects of temperature, cyanide binding, and heme crosslinking on the value. Compared to most nucleic acid base pairs ($^2J_{\text{NN}} = 6\text{-}10$ Hz), the histidine-amide HBCs in *Chlamy* Hb and GlbN were at the low end of the scale with $^2J_{\text{NN}} \sim 5 \pm 0.1$ Hz, suggesting relatively longer H-bonds. Interestingly, all nonnatively crosslinked samples (L79H/H117A GlbN-B, L79H GlbN-AB and L75H *Chlamy* Hb-B) exhibited significantly lowered $^2J_{\text{NN}}$ values (4.5, 4.3, 4.0 $\pm$ 0.1 Hz respectively), consistent with H-bond straining upon nonnative PTM. Correlations between the measured HBCs and other observables such as NMR chemical shift, ^{15}N R_{ex} (CPMG), histidine pK_{a} , and crystallographic heavy atom distances will be discussed. Overall, our NMR observations expand the experimental knowledge of N-H - - N type hydrogen bonds and enable new comparisons between H-bonds found in different types of macromolecules.

Studying the Folding of Individual Residues in a Small Protein

Nicholas C. Fitzkee

Department of Chemistry, Mississippi State University

Despite decades of intense study, several key questions remain about the thermodynamics and mechanism of protein folding. Many small proteins fold using a two-state mechanism, but it is commonly thought that multiple disordered ensembles exist depending on the experimental conditions. For example, the urea-unfolded state likely differs from the thermally-unfolded state or the unfolded state under native conditions. In this view, intrinsically disordered proteins are simply another feature on the vast landscape of many possible disordered states. Experimentally, it has been difficult to prove this idea, because ensemble-based measurements do not typically offer details of folding at the resolution of individual residues. Furthermore, structure-based NMR measurements, which have traditionally excelled at resolving individual residues, may be insensitive to high levels of organization in the disordered state. Here, we propose an alternative method for characterizing the folding transition using ZZ-exchange NMR spectroscopy. This approach allows the measurement of thermodynamic and kinetic parameters under equilibrium conditions where both folded and unfolded states are significantly populated. These parameters can be measured for individual residues, and additional information, such as residue-specific m -values, can be derived as well. We demonstrate this approach for GB3, the third IgG-binding domain from streptococcal protein G. Differences in the parameters allow us to make hypotheses about the folding pathway of this small protein, and it is possible to speculate where organization (if any) might exist in the unfolded state. Work is ongoing to apply this approach to other disordered systems, with the goal of developing a unifying model for protein disordered states.

Exchange of Putrescine²⁺ and Mg²⁺ in the Ion Atmosphere of RNA: Contributions to Folding and Gene Regulation.

Robert J. Trachman and David E. Draper

The Johns Hopkins University
Departments of Biophysics and Chemistry

RNA folding in vivo is driven by a diverse set of interactions with cytoplasmic components. Of these interactions, the screening of negatively charged phosphates on the RNA backbone by cations is critical for folding to the native structure. Given their high intracellular concentrations, the positive charges of Mg²⁺ and putrescine²⁺ (totaling 100mM and 60mM respectively) neutralize a large fraction of negative charges possessed by structured RNA. Using a technique analogous to equilibrium dialysis we observe the competitive interactions of putrescine²⁺ and Mg²⁺ in an RNA ion atmosphere for both native and intermediate states of RNAs. These interactions are related to a folding free energy landscape for three different RNAs. In the presence of Mg²⁺, putrescine²⁺ is found to be either stabilizing or destabilizing depending on the fold of the RNA. RNAs that chelate Mg²⁺ are destabilized in the presence of putrescine²⁺ while non-chelators are stabilized by putrescine²⁺. For some RNA molecules putrescine²⁺ has an apparent synergistic effect on excess Mg²⁺ while other RNAs show an antagonistic effect. These results can be attributed to a difference in the manner in which putrescine²⁺ and Mg²⁺ populate the ion atmosphere and affect RNA conformation.

Tug-of-war between thermodynamic stability and actin-binding function of tandem calponin-homology domains

Swati Bandi, Surinder M. Singh, Geoffrey Armstrong, and Krishna M.G. Mallela*

Department of Pharmaceutical Sciences, Skaggs School of Pharmacy and
Pharmaceutical Sciences, University of Colorado Anschutz Medical Campus, 12850 E
Montview Blvd, C238, Aurora, CO 80045. *E-mail: krishna.mallela@ucdenver.edu

Tandem calponin-homology (CH) domains constitute a major class of actin-binding domains that include dystrophin and utrophin, the two key proteins involved in muscular dystrophy. Despite their importance, how their structure controls their function is not understood. Here, we study the contribution of individual CH domains to the actin-binding function of utrophin's tandem CH domain. Actin co-sedimentation assays indicate that the C-terminal CH2 domain binds weakly to F-actin when compared with the full-length tandem CH domain, consistent with the published results on tandem CH domains. However, the surprise came from the CH1 domain. Isolated CH1 binds strongly to F-actin when compared with the full-length tandem CH domain. These results indicate that CH2 has a negative influence on actin-binding when it is linked with CH1. Thus, the obvious question that arises is why tandem CH domains require CH2, when CH2 is reducing their actin-binding efficiency. To answer, we probed the thermodynamic stabilities of individual CH domains. Isolated CH1 domain is unstable and is prone to serious aggregation. Isolated CH2 is very stable, even more stable than that of the full-length tandem CH domain. This makes utrophin's tandem CH domain as the first example where an isolated domain is more stable than the full-length protein. In addition, the CH2 domain, which is more stable, is less functional. These results indicate that the main function of CH2 is to stabilize CH1 at the expense of decreasing the actin-binding efficiency. Consistently, the proposed structure of utrophin's tandem CH domain based on earlier X-ray studies indicates a close proximity between the C-terminal helix of CH2 and the N-terminal helix of CH1, and this helix in CH2 becomes more dynamic in the full-length protein when compared with that in the absence of CH1, suggesting the mechanism by which CH2 stabilizes CH1 despite the decrease in actin-binding function.

Nucleic Acids and Their Binding Partners

Structured mRNA induces the ribosome into a hyper-rotated state

Peter V. Cornish^{1,2,3}, Peiwu Qin¹, Dongmei Yu^{2,3}, Xiaobing Zuo⁴

¹Department of Biochemistry, University of Missouri, Columbia, Missouri, 65211, USA.

²Department of Biological Engineering, University of Missouri, Columbia, Missouri, 65211, USA. ³Informatics Institute, University of Missouri, Columbia, Missouri, ⁴X-ray Science Division, Argonne National Laboratory, Argonne, IL, 60439, USA.

During protein synthesis, mRNA and tRNA are moved through the ribosome by the process of translocation. The small diameter of the mRNA entrance tunnel only permits unstructured mRNA to pass through. However, there are structured elements within mRNA that present a barrier for translocation that must be unwound. The ribosome has been shown to unwind RNA in the absence of additional factors, but the mechanism remains unclear. Here, we show using single molecule Förster resonance energy transfer and small angle X-ray scattering experiments a new global conformational state of the ribosome. In the presence of the frameshift inducing *dnaX* hairpin, we observed that the ribosomal subunits were driven into a hyper-rotated state and the L1 stalk was predominantly in an open conformation. This previously unobserved conformational state provides structural insight into the helicase activity of the ribosome and may have important implications for understanding the mechanism of ribosomal frameshifting.

Binding and Salt Linkages of HIV-1 Reverse Transcriptase with Different DNA and RNA Substrates Reflect Polymerization versus RNase H Compatible Modes of Interaction

Hiromi S. Brown and Vince J. LiCata

Department of Biological Sciences, Louisiana State University, Baton Rouge, LA 70803

HIV reverse transcriptase (RT) is a multifunctional protein that carries out a complex, multi-step reaction to convert the retroviral single-stranded RNA genome into double-stranded DNA. RT performs RNA- and DNA-dependent DNA polymerization and removes the RNA template from DNA/RNA heteroduplexes using RNase H activity. During the HIV life cycle, RT encounters all possible combinations of primer/template substrates comprised of RNA and DNA. When RNA-dependent DNA polymerization is stalled, the heteroduplex product can be a substrate for the RNase H activity. The two catalytic sites reside in the P66 subunit of the P66/P51 heterodimer, and a nucleic acid binding cleft runs between the sites.

Herein we have characterized the basic binding equilibria for HIV-RT binding to DNA/DNA, RNA/RNA, DNA/RNA, and RNA/DNA primer/template substrates at various KCl concentrations using a fluorescence anisotropy assay, and have quantitated the linked ion releases upon formation of the different binary complexes. Our results show that HIV-RT can distinguish between the homoduplex and heteroduplex substrates, as reflected by affinity, the differences in linked ion release, and salt sensitivity. The binding of heteroduplex substrates (DNA/RNA and RNA/DNA) by HIV-RT is tighter at any KCl concentration, and is linked to the release of at least two additional ions upon complex formation than upon binding of either the DNA/DNA or RNA/RNA homoduplexes. These differences can be correlated with observed structural differences in the substrate interactions of HIV-RT complexed with DNA/DNA (PDB ID: 1RTD) and with DNA/RNA (PDB ID: 4B3O) showing the polymerization and RNase H compatible modes of substrate binding, respectively. While there are currently no structures of RNA/RNA bound HIV-RT, the thermodynamics of RNA/RNA binding was nearly equivalent to the DNA/DNA binding profile, which suggests that RNA/RNA binding will also be in the polymerase compatible mode despite the fact that there is a difference in nucleic acid geometry between DNA/DNA and RNA/RNA (B-form versus A-form).

How do the Different DNA-Binding Modes of *E. coli* SSB Affect Function?

Vincent M. Waldman, Elizabeth A. Weiland, Timothy M. Lohman

Department of Biochemistry and Molecular Biophysics, Washington University

The essential single-stranded DNA binding (SSB) proteins bind with high affinity to single-stranded DNAs (ssDNA) that are generated during DNA replication, recombination, and repair. This interaction protects ssDNA and the intrinsically disordered C-terminal tail of SSB is used to recruit over a dozen enzymes to the DNA. Therefore, understanding SSB and ssDNA interactions is vital to understanding genomic biology.

The functional unit of the well-studied SSB protein of *E. coli* (*EcSSB*) is a homotetramer. Each subunit has an OB-fold that binds to ssDNA and *EcSSB* binds to ssDNA in several binding modes that differ in the number of OB-folds that contact DNA. In the (SSB)₆₅ mode, ssDNA interacts with all four subunits covering ~65 nucleotides of ssDNA. In another binding mode, (SSB)₃₅, ssDNA contacts an average of two subunits of the tetramer and can form cooperative protein-DNA filaments. Formation of (SSB)₃₅ is regulated, in part, by negative cooperativity in ssDNA binding to the first two OB-folds relative to the second two. The stabilities of these modes, *in vitro*, are sensitive to solution conditions and it has been proposed that the different modes are used for different processes *in vivo*. However, it is unknown whether only one or both of these dramatically different binding modes are functionally important *in vivo*.

To address this issue we have created *EcSSB* mutants that are designed to stabilize the (SSB)₃₅ mode. We have recently created *EcSSB* mutants where two of the four subunits are covalently linked. This construct forms a homodimer in solution meaning the four OB-folds are maintained. This 'linked dimer' (LD) construct behaves similarly to *EcSSB* in solution and functionally complements loss of *EcSSB* in *E. coli*. To bias the intrinsic propensity of the LD construct to form the (SSB)₃₅ mode we have introduced mutations designed to destabilize the (SSB)₆₅ mode. Our strategy has been to disrupt DNA binding in two, but not all four, of the OB-folds. Interestingly, these mutants, with only two wild-type like OB-folds, are able to functionally complement loss of *EcSSB* *in vivo* and strains expressing these mutants do not have hypersensitivity to DNA damaging agents. Using fluorescence titrations we have demonstrated that these proteins have decreased affinities for ssDNA in general and have a marked increase in negative cooperativity for ssDNA binding to the second two OB-folds relative to the first two. Ongoing research will further characterize the cooperative ssDNA binding properties of these mutants and further probe their functional consequences.

(Supported by NIH Grant: GM030498)

Looking at multicomponent systems with small angle x-ray scattering: Ions around DNA; DNA around proteins

Lois Pollack, Joshua Blose, Yujie Chen, Steve Meisburger, Suzette Pabit, Joshua Tokuda, Traci Topping and Lisa Gloss

Department of Applied and Engineering Physics, Cornell University

In this talk I will discuss recent biological applications of contrast variation methods in small angle x-ray scattering. In a multi-component system (ions around nucleic acids, or protein-nucleic acid complexes), it is of great interest to measure the structure of each component independently by separating the scattering signals from the different components. This separation can be accomplished in two different ways. Anomalous small angle scattering works by changing the scattering strength of one component, for example the ions in an ion-DNA system. In this case the energy of the incident x-ray beam is changed until it approaches the absorption edge of a particular element (the ions). At the edge the x-ray energy equals the binding energy of an inner electron. By measuring the scattering at two different energies near the edge, it is possible to highlight the contribution to the scattering signal that arises from the ions alone. New analysis methods allow us to count the number of excess ions present, and to determine their spatial distribution around the nucleic acid. The ready availability of powerful x-ray sources and sophisticated data analysis packages also enhances the application of traditional, solution based contrast variation methods that focus on separating the scattering from the protein and nucleic acid parts of a protein-nucleic acid complex. This separation is accomplished by raising the electron density of the solvent until it equals that of the protein phase (the nucleic acid is slightly more electron dense). We recently applied this method to study the nucleosome core particle. Results that highlight the strengths of both methods will be discussed.

DNA Binding Behavior of Sequence Specific Polyamides

Cynthia M. Dupureur, Gaofei He, Kevin J. Koeller, G. Davis Harris, James K. Bashkin
Department of Chemistry & Biochemistry, Center for Nanoscience, University of Missouri-St. Louis, One University Blvd., St. Louis, MO

Polyamides (1) are minor groove DNA binding ligands derived from the smaller and naturally occurring distamycin A. Studies of sequence-specific DNA interactions of 8-ring hairpin polyamides that vary in sequence and composition will be presented. Techniques applied include quantitative DNaseI footprinting with capillary electrophoresis and fluorescence spectroscopy. Using the former technique with a 500 bp A/T rich duplex, perfect and mismatch binding sequences were detected with large differences in K_d. A simple fluorescence assay has been applied to confirm K_ds using a TAMRA-labeled hairpin and also to study binding kinetics for various PAs. The results inform the subtleties of established recognition rules and underlying kinetics that influence affinity.

This research has been supported by NIH (AI083803).

References

(1) P.B. Dervan, B.S. Edelson, (2003). Recognition of the DNA minor groove by pyrrole-imidazole polyamides, *Curr. Opin. Struct. Biol.* 13, 284-299.

Yeast Rap1 interacts with telomeric sequences in multiple binding modes

Erik A. Feldmann and Roberto Galletto

Department of Biochemistry and Molecular Biophysics, Washington University in St. Louis – School of Medicine

The ends of eukaryotic chromosomes contain a region of large nucleoprotein complexes called telomeres. Improper maintenance of telomeres is associated with chromosome instability and oncogenic processes. In the budding yeast *Saccharomyces cerevisiae*, telomeric DNA contains multiple repeats of Rap1 recognition sequences that serve to specifically target Rap1 at the end of chromosomes. When bound at the telomere, Rap1 interacts with the Rap1 interacting factors (Rif1 and Rif2) to form the Shelterin-like complex. Our studies show that Rap1 binding to DNA is more complex than the simple 1:1 interaction accepted for this system. Analytical sedimentation velocity and equilibrium ultracentrifugation and equilibrium fluorescence titrations with model DNA substrates show that the DNA binding domain of Rap1 binds to its target sequence with stoichiometries higher than 1:1. We also provide evidence suggesting that access to these higher stoichiometries is regulated in the context of the full-length Rap1 protein. We propose that Rap1 binds dsDNA in multiple binding modes and when in high concentrations, is capable of oligomerizing on its target DNA substrate. We also suggest that the interactions of Rap1 interacting factors with Rap1 play a role in modulating the stoichiometry of telomere-bound Rap1.

Substrate Recognition by Human O⁶-Alkylguanine DNA Alkyltransferase

Michael G. Fried, Manana Melikishvili and Ingrid Tessmer

Department of Molecular and Cellular Biochemistry
University of Kentucky, Lexington, KY 40536

Ph: (859) 323-1205 michael.fried[at]uky.edu

Human cells contain DNA alkyltransferases that protect genomic integrity under normal conditions but also defend tumor cells against chemotherapeutic alkylating agents. Here we explore how structural features of the DNA substrate affect the binding and repair activities of the human O⁶-alkylguanine-DNA alkyltransferase (AGT).

In vitro, cooperative binding results in all-or-nothing association on short templates. A requirement for contact with 4 DNA base-pairs results in oscillation of average binding site size S_{app} and cooperativity factor ω with template length. Models in which protein molecules overlap along the DNA contour predict that protein-protein contacts will be optimal when the DNA is torsionally relaxed. Supporting this prediction, topoisomerase assays show that AGT binding is accompanied by a small-but-measurable net unwinding (~7 deg/protein). A torsional free energy model couples this unwinding to observed limits in cooperative cluster size. These results predict that AGT will partition in favor of torsionally-relaxed, relatively protein-free DNA structures like those near replication forks.

AGT binds O⁶-methylG-C and O⁶-methylG-T lesions with a specificity ratio (K_S/K_N) far too low for efficient lesion search. This suggests that other factors are needed to direct AGT to lesion sites. Recently we have found that AGT binds the human MutS β homologue and PCNA proteins. We propose that these proteins target AGT to sites of mismatch repair (including those containing O⁶-methylG-T pairs) and to sites near the replication fork, where O⁶-methylG lesions may be repaired before their potential for mutagenesis can take effect.

This work was supported by NIH grant GM070662 to MGF.

Structure / Thermodynamic Correlations

Introduction to the Gary K. Ackers Lecture in Biothermodynamics:

David Bain, University of Colorado Anschutz Medical Campus

pH-dependent processes in proteins

5th Annual Gary K. Ackers Lecture in Biothermodynamics

Bertrand García-Moreno E.

Department of Biophysics, Johns Hopkins University, Baltimore MD 21218

Tight regulation of intracellular pH is one of the hallmarks of all living systems. Not surprisingly, many proteins have evolved to respond to changes or fluctuations in physiological pH as signals that modulate their biological functions. Understanding these pH dependent processes is of great interest. They are essential for the normal functioning of living systems. The disruption of intracellular and extracellular pH homeostasis that occurs under many conditions (cancer, stress, etc) can lead to global physiological changes that are triggered by the effects of changes in pH on proteins. The thermodynamic basis of pH effects on macromolecules is well understood. It involves either an alteration of acid/base chemistry or pH-driven shifts in macromolecular equilibria (e.g. stability, binding, conformational reorganization) governed by the pK_a values of ionizable groups or by differences in pK_a values in different states of the macromolecule. What are not well understood are the myriad factors that determine the pK_a values of ionizable groups in macromolecules, and how electrostatic and non-electrostatic contributions to free energy are balanced. I will describe on-going experiments in our lab aimed at characterizing the molecular determinants of pK_a values and at understanding mechanism of pH-driven conformational switching in proteins. I will also discuss implications of our results for the development and stringent benchmarking of computational methods for structure-based calculations and for the evolution and engineering of pH-sensing motifs in proteins.

Impact of pH on The Structure and Function of Neural Cadherin

Matthew P. Dukes, Jared M. Jungles, Nagamani Vunnam, Susan Pedigo
The University of Mississippi, Department of Chemistry and Biochemistry

Neural cadherin is a primary cell adhesion protein within synaptic adherens junctions. It is responsible for cell-cell adhesion through calcium-dependent dimerization of proteins on adjoining synaptic membranes. During neurotransmission, activity-dependent fluctuations in extracellular pH occur that result from the release of vesicular protons into the synaptic cleft. Because of the localization of adherens junctions at the transmission zone, neural cadherin will be directly exposed to proton flux during periods of increased synaptic activity. Because synaptic acidosis has numerous implications to the fields of brain physiology and pathophysiology, we carried out experiments that explore the effect of pH on the linked equilibria of calcium binding and dimerization.

N-cadherin has 5 modular extracellular domains that bind 3 calcium ions between the modules. Calcium binding is required for dimerization. We will consider the impact of pH on the linked equilibria of the folding and stability of the apo state, binding of calcium and then formation of dimer for NCAD12, a two-domain construct. Thermal unfolding studies of the apo protein reveal that there is a small increase in stability as solution pH is reduced, consistent with a pH-dependent reduction of electrostatic repulsion through neutralization of anionic residues. Since NCAD12 contains two tryptophans (W2 and W113), one in each of the two domains, utilization of single tryptophan mutants W2A and W113F presents a unique opportunity to monitor site-specific responses of calcium-binding. Calcium titrations of these mutants monitored by W2 fluorescence are pH sensitive, whereas those monitored by W113 are not. Since W2 is a key component in dimerization, we hypothesized that the dimerization affinity of NCAD12 will be pH dependent. To test this hypothesis, two types of dimers can be studied, a calcium-saturated species, and a kinetically-trapped apo species. We used SEC to show that the kinetically-trapped apo dimer is disassembled by a decrease in pH from 7.4 to 7.0. This striking sensitivity of dimer disassembly to pH is supported by recently published reports of synapse duration in cultured cells. Thus, our simple chromatographic technique is a viable means to detect physiologically relevant equilibria. Analytical ultracentrifugation studies are currently underway to determine the impact of pH on the dimerization affinity of the calcium-saturated species. These in vitro studies indicate that N-cadherin experiences regional differences in sensitivity to pH that affect calcium binding events involving the dimer interface. In summary, our studies suggest that NCAD functions as a pH sensor during periods of transient synaptic acidosis.

Revisiting Thermodynamic Cooperativity in TRBP Interactions with Double-Stranded RNA

Roderico Acevedo, Debashish Sahu, Nichole Orench Rivera, Kaycee A. Quarles, and Scott A. Showalter

Department of Chemistry, The Pennsylvania State University, 104 Chemistry Building, University Park, PA 16802, USA

MicroRNAs are critical post-transcriptional regulators of gene expression and their precursors have an A-form helical geometry, which prevents proteins from identifying the sequence-determining hydrogen bonding groups on the nucleobases. This suggests that global structural features (e.g., bulges, mismatches) play a central role in specific double-stranded RNA (dsRNA) selection from cellular dsRNA pools by double-stranded RNA binding domain (dsRBD) containing proteins. Furthermore, the processing enzymes in the maturation pathway require tandem-dsRBD cofactor proteins for optimal function, suggesting a mechanistic role for cooperative intra-chain binding of dsRNA between domains. Here, we focus on one tandem-dsRBD, TRBP, which has been shown to tightly bind perfect dsRNA duplexes. It is our hypothesis that although dsRBDs bind dsRNA in a non-sequence specific manner, their presence is essential for the enzymes in the maturation pathway to efficiently locate the proper cleavage sites along the RNA. We present a combination of circular dichroism, electrophoretic mobility shift assays, and isothermal titration calorimetry based assays demonstrating that TRBP binds dsRNA cooperatively, due to intra-chain interactions. Application of multiple nucleic acid lattice models to our data suggests that the microstates accessed by an individual dsRBD binding to RNA are indeed different from those accessed by the tandem-dsRBD-construct; furthermore, allosteric effects may be present in the tandem construct.

Molecular Switches in Signaling and Disease

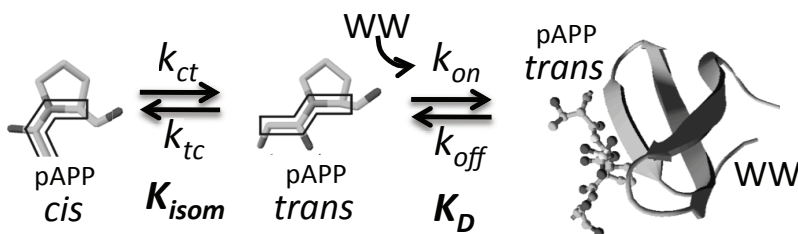
Linda K. Nicholson¹, Soumya De², Monique Rogals¹, Evgenii Kovrigin³, Kun Ping Lu⁴,
and Alex Greenwood¹

¹Department of Molecular Biology and Genetics, Cornell University, Ithaca, NY 14853, United States

²Department of Biochemistry and Molecular Biology, Department of Chemistry, and Michael Smith Laboratories, The 993 University of British Columbia, Vancouver BC, V6T 1Z3, 994 Canada.

³Department of Chemistry, Marquette University, Milwaukee, Wisconsin 53201, United States

⁴Cancer Biology Program and Biology of Aging Program, Department of Medicine, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Massachusetts 02215, United States



The *cis* and *trans* isomers of a prolyl peptide bond offer a simple two-state molecular switch, with chemically identical but structurally distinct states that exchange at an intrinsically slow rate, with an exchange time constant typically on the order of several minutes. Peptidyl prolyl isomerase enzymes accelerate this exchange rate, often shortening the exchange time constant down to the millisecond range. In the absence of isomerase activity, the *cis* and *trans* states of an X-Pro peptide bond (where X is any residue) are effectively isolated from each other in terms of timescales relevant for rapid cellular signaling. For an isomer-specific interaction, the amount of bound complex formed on the millisecond timescale (e.g. after sudden introduction of the binding partner) will depend on both the equilibrium constant for the bimolecular interaction, K_D , and the equilibrium constant for the isomerization reaction, K_{isom} . The implications of this for the role of isomerases in modulation of affinity and regulation of flux between otherwise isolated interaction networks will be discussed. The combination of phosphorylation and isomerization will be highlighted as a condition-dependent type of molecular switch in the context of the proteolytic processing of the amyloid precursor protein. Additionally, the combination of oligomerization and prolyl *cis-trans* isomerization will be highlighted as another type of conditional molecular switch in the context of linking an activated receptor signaling complex to cytoskeletal remodeling.

Thermodynamics of cationic and anionic surfactant interaction

Vytautas Petrauskas, Povilas Norvaišas, and Daumantas Matulis

vytautas.petrauskas@bti.vu.lt, matulis@ibt.lt

Department of Biothermodynamics and Drug Design, Institute of Biotechnology, Vilnius University, Graičiūno 8, Vilnius, Lithuania

The interaction between positively and negatively charged linear surfactants is an interesting system for the understanding of fundamental interplay of hydrophobic and ionic forces in lipid membranes and proteins. Despite the biological and industrial importance (Stellner et al. 1988) of surfactant systems, many questions concerning their thermodynamics have remained unanswered since the first demonstration that oppositely charged surfactants bind with large exothermic enthalpy (Papenmeier & Campagnoli 1969). In our investigation (Norvaišas et al. 2012), isothermal titration calorimetry was utilized to dissect the Gibbs free energies, enthalpies, entropies, and heat capacities of interaction into hydrophobic and ionic contributions for alkylamine interaction with alkyl sulfates and alkane sulfonates. Dependence on aliphatic chain length, surfactant concentration, temperature, and ionic strength provided a detailed thermodynamic description of this interaction. Results imply that the reactions of surfactants with tails longer than approximately 10 carbon atoms were primarily driven by enthalpy changes arising from solid-phase interactions between aliphatic tails, whereas entropic contribution was relatively small. All thermodynamic parameters depended linearly on aliphatic chain length. Gibbs free energy of aggregation exhibited clear positive correlation with increased concentration, thus confirming the previous findings (Matulis 2001). Depending on temperature, liquid or solid aggregates of surfactants were formed. Thermodynamic dissection yielded the parameters of the phase transition.

References:

- Matulis, D. 2001. Thermodynamics of the hydrophobic effect. III. Condensation and aggregation of alkanes, alcohols, and alkylamines. *Biophysical chemistry*, **93**: 67-82.
- Norvaišas P., Petrauskas V., and Matulis D. 2012. Thermodynamics of cationic and anionic surfactant interaction. *Journal of Physical Chemistry B*, **116**: 2138-2144.
- Papenmeier, G. J. and Campagnoli, J. M. 1969. Microcalorimetry. Thermodynamics of the Reaction of an Anionic Detergent with a Cationic Detergent. *Journal of the American Chemical Society*, **91**: 6579-6584.
- Stellner, K., Amante, J., Scamehorn, J. and Harwell, J. 1988. Precipitation phenomena in mixtures of anionic and cationic surfactants in aqueous solutions. *Journal of Colloid and Interface Science*, **123**: 186-200.

Structure, dynamics and inhibition of Tiam1 PDZ domain complexes

Ernesto J. Fuentes

Department of Biochemistry, University of Iowa, Iowa City, IA, 52242

The T-cell lymphoma invasion and metastasis 1 (Tiam1) is a guanine nucleotide exchange factor (GEF) for the Rho-family GTPase Rac1 that is critical for cell-cell adhesion and cell migration. Tiam1/Rac1 deregulation leads to various malignancies, including cancer. We have previously shown that the cell adhesion receptor syndecan1 binds to the Tiam1 PDZ domain and that this interaction is important for cell adhesion and migration. However, little is known about the specificity of the PDZ domain with other syndecan family members. Here, we used equilibrium binding experiments to show that the Tiam1 PDZ domain specifically interacts with syndecan1, phosphorylated syndecan1 and syndecan3, but not syndecan2 and syndecan4. We determined the crystal structures of the Tiam1 PDZ domain in complex with the phosphorylated and native syndecan1 peptide, which revealed the structural basis for specificity. Remarkably, comparison of the PDZ/syndecan1 and PDZ/phosphorylated syndecan1 structures showed that a new specificity pocket was used to accommodate the phosphate group. Moreover, side chain methyl ($^{13}\text{CH}_2\text{D}$) NMR spin-relaxation experiments of the syndecan1- and phosphorylated syndecan1-bound PDZ domain revealed distinct patterns of dynamics propagation within these complexes. Finally, we combined structure-based *in silico* docking screening and unbiased *AlphaScreen*-based high-throughput screening to discover inhibitors targeting Tiam1 PDZ/syndecan1 interaction. Collectively, our results provide insight into the specificity, structure and dynamics of the Tiam1 PDZ domain/syndecan1 complex and identify several lead compounds that target this complex.

Macromolecular Interactions

Transition metal homeostasis: Allosteric switching by metal sensor proteins

David P. Giedroc

Department of Chemistry, Indiana University, Bloomington, IN 47405-7102

All cells are capable of responding to a changing landscape of transition metal availability by controlling the expression of genes encoding membrane transporters (uptake and efflux systems) and metal trafficking/sequestration proteins that collectively manage the intracellular availability of essential metals. This homeostatic control is mediated by a panel of specialized metal-binding proteins termed metalloregulatory or “metal sensor” proteins that function as metal-specific allosteric switches. We have employed a simple coupled equilibrium model as an artificial yet powerful construct to understand molecular determinants of allosteric regulation of DNA operator binding (ΔG_c) in a number of metalloregulatory protein families, distinct from those determinants that dictate the affinity (K_{Me}) and specificity of cognate vs. non-cognate metal (Me) binding. A key feature of these regulatory networks that emerges from our studies is that the first metal coordination shell dictates metal selectivity and likely tunes K_{Me} in an appropriate range, while the second coordination shell, organized only upon binding a cognate metal, drives biological regulation in the cell. Proper tuning of K_{Me} may well allow weakly competitive transition metals, *e.g.*, iron and manganese, to perform their specialized functions in the presence of highly competitive metals, *e.g.*, copper(I) and zinc. Parallel structural, thermodynamic and cell biological approaches will be discussed with a focus on zinc homeostasis and copper resistance in the Gram-positive respiratory pathogen, *Streptococcus pneumoniae*. Supported by the US National Institutes of Health (GM042569).

Heterotropic linkage between Protein-Protein and Protein-RNA interactions determines protein partitioning in snRNPs

Sandra G. Williams and Kathleen B. Hall

Washington University, St Louis

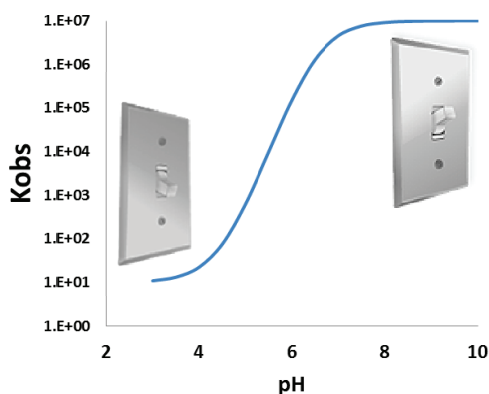
U1A, U2B", and *Drosophila* SNF are highly related proteins in the U1 and U2 snRNPs. These proteins use an RNA recognition motif (RRM) to bind their RNA targets with unusual specificity. This RRM can also bind to a leucine rich repeat protein, U2A', which is a U2 snRNP-specific protein. While U1A, U2B", and SNF have high sequence identity, as do their RNA ligands, they partition in different ways to the U1 and U2 snRNPs: U1A is present only in the U1 snRNP, U2B" is present only in the U2 snRNP, and SNF localizes to both the U1 and U2 snRNPs. U2A', which binds the RRM on the opposite face from the RNA binding surface, only localizes to the U2 snRNP. We show that snRNP localization of both the RRM proteins and U2A' is due to differences in heterotropic linkage effects between protein-protein and protein-RNA binding. Using both fluorescence-based binding assays and ITC, we find that protein-protein and protein-RNA binding can have large positive linkage (~ 200 -fold increase in binding affinity, or a $\Delta g_{\text{linkage}} = -3 \text{ kcal/mol}$) but that the extent of linkage is highly dependent on both the RRM and the RNA. Additional results suggest the importance of coupled conformational transitions in both the ligands and RRM in determining linkage effects.

Engineering Linked-Equilibria into Protein Interfaces to Allow Regulation of Protein Interactions

James R. Horn, Megan L. Murtaugh, Sean W. Fanning, Tressa M. Sharma, and Alexandra M. Terry

Department of Chemistry and Biochemistry, Northern Illinois University, DeKalb IL 60115, jrhorn@niu.edu

The goal of protein engineering frequently involves enhancing affinity or stability. Here, we explore new directions in the design of protein interactions by introducing linked equilibria with the goal of engineering protein regulation. Despite the importance of linked equilibria in regulating biological events and its potential use in applications using proteins, introducing such regulation into a protein interface has not been well explored, mainly due to complex set of interactions across protein-protein interfaces. In the context of single domain antibodies, we have explored two methods to regulate protein interactions. Using synthetic combinatorial histidine-scanning libraries, which allow the production of billions of protein variants that cover significant energetic and structural space, we have produced single domain VHH antibody variants that exhibit significant pH- or metal ion-dependent binding. Several of the resulting antibody variants have been subjected to detailed thermodynamic and structural studies, which have helped reveal how the single domain VHH antibodies achieved new function, while maintaining near wild-type affinity for the target antigen. The engineered pH-dependent VHH antibodies were found to tolerate the incorporation of multiple histidine residues across the small VHH interface, with up to three histidine groups participating in the observed pH-dependence. The engineered metal ion-dependent antibody, possessing bi-specific target recognition, exhibited significant conformational flexibility in CDR1 in order to bind antigen or metal ion. Overall, these results are expected to be transferable to a wide range of biomolecular interactions and applications, as reversible control of high affinity protein interactions (e.g., protein reagents) is expected to be a highly desirable quality. Further, the designed proteins serve as excellent models to better understand the fundamental nature of protein interactions and commonly observed linked equilibria.



Biophysical studies on the A1 and A3 domain homologues of von Willebrand Factor.

Matthew Auton

Mayo Clinic, Division of Hematology, Depts. of Internal Medicine and Biochemistry & Molecular Biology, Rochester MN 55905

The von Willebrand factor A1 and A3 domains are structurally homologous (average backbone RMSD = $1.8 \text{ \AA}$) with somewhat comparable sequences comprising 19% identity and 38% similarity. Yet, despite their homology, they display very different thermodynamics properties of unfolding. These thermodynamic differences are key to their biological role in hemostasis to bind subendothelial collagen, capture platelets, and contribute to the arrest of bleeding under the vascular shear stress of blood flow. While the A3 domain has a classical two-state unfolding character, the A1 domain exhibits three state character with a well-defined stable intermediate. At moderate temperatures, chemical denaturation of these domains is reversible; however, the native to intermediate thermal transition of A1 is irreversible. This irreversible transition in the A1 domain is affected by known clinical mutations that result in von Willebrand disease, the most common inherited bleeding disorder in man.

As the spirit of the Gibbs conference is educational, the goal of this talk will be to illustrate the principles we have learned from studying these two homologues and the methods used to identify these principles. With respect to the A1 domain, we focus on the relationship between conformational thermodynamics and the physics of platelet adhesion dynamics under shear flow and how clinical mutations affect these properties. I will also cover methods of distinguishing between two-state and three-state unfolding and between reversible and kinetically irreversible unfolding. I will also present a urea-temperature phase diagram method that deciphers thermodynamic contributions of denatured state expansion from protein unfolding of the reversible A3 domain. And if time permits, I will cover a new computational method based on transfer free energies that can potentially decipher structural regions that contribute to thermodynamic deviations from two-state character in homologues.

Structure and Function of a Novel Phosphopeptide Binding Domain from the Transcriptional Corepressor MINT

Rhett A. Kovall

University of Cincinnati College of Medicine, Department of Molecular Genetics,
Biochemistry and Microbiology, Cincinnati, Ohio

The recognition of phosphorylated residues, such as phosphoserine/threonine and phosphotyrosine, by specific protein binding domains is integral to the process of signal transduction in cells. Examples of phosphopeptide binding domains include SH2, PTB, POLO-box, 14-3-3, WD40, and BRCT. These domains play important roles in fundamental cellular processes, such as cell cycle control, DNA repair, transcriptional regulation, metabolism/cell growth, and apoptosis, to name a few. As part of our ongoing structure-function studies of transcription complexes that regulate signaling in the Notch pathway, we have recently identified a new phosphopeptide binding domain, termed SPOC, within the C-terminus of the transcriptional corepressor MINT. MINT is conserved from *Drosophila* to humans and functions as a transcriptional corepressor in the Notch pathway, as well as other signaling pathways, such as nuclear hormone receptor signaling. Through its SPOC domain, MINT is thought to bind other ubiquitous transcriptional repressor proteins, *e.g.* SMRT/NCoR and CtIP/CtBP, and recruit these multiprotein repression complexes to target genes, effectively silencing gene expression at these sites. However, the molecular details of how SPOC interacts with other repressor proteins, and whether these interactions are regulated, are currently unknown. Here, using ITC we convincingly show that SPOC only binds NCoR peptides that are phosphorylated on two conserved serine residues; and using X-ray crystallography, we determine the 1.5 Å structure of SPOC in complex with phosphoNCoR. Taken together, our results suggest a phospho-switch mechanism for SPOC to interact with multiple binding partners in a regulated manner and that members of the greater SPOC domain family are previously unidentified phosphopeptide binding domains.

Computation and Calorimetry: What Can We Learn from Each Other?

Michael K. Gilson, Hari Muddana and Andrew Fenley

Skaggs School of Pharmacy and Pharmaceutical Sciences

Molecular dynamics simulations of unprecedented duration are providing new insights into biomolecular mechanisms, and are also reaching a point where direct comparisons between computation and calorimetry can be used to test and ultimately improve simulations.

I will first discuss the analysis of a 1-ms molecular dynamics simulation of the small protein bovine pancreatic trypsin inhibitor, which reveals that its main conformations have different thermodynamic profiles and that perturbation of a torsion angle, for example, can easily select for occupancy of a particular conformational state. These observations establish the plausibility of a mechanism that we term entropy-enthalpy transduction (EET), in which the thermodynamic character of a local perturbation, such as enthalpic binding of a small molecule, is camouflaged by the thermodynamics of a global conformational adjustment. The EET mechanism can make measured entropies and enthalpies of folding and binding unreliable indicators of actual thermodynamic driving forces, and it may be a physical mechanism underlying many cases of entropy-enthalpy compensation.

Time permitting, I will also discuss our efforts towards what may be termed computational calorimetry. In particular, it appears that we can now compute rigorously converged estimates of host-guest binding enthalpies. Such calculations, combined with existing methods of computing binding free energies, establish a basis for direct comparison of simulations with calorimetry. This capability opens new opportunities for testing and improving physics-based modeling of molecular recognition, with implications for the improvement of computational tools for computer-aided drug design.

The tertiary architecture of LacI/GalR proteins accommodates multiple networks of functionally important positions

Daniel J. Parente and Liskin Swint-Kruse

Department of Biochemistry and Molecular Biology, University of Kansas Medical Center, Kansas City, KS 66160

Proteins could evolve new functions by at least two different means: First, the amino acids at key positions could be changed to alter function, such as when amino acids are changed in ligand binding sites to alter specificity. Second, the locations of key functional positions could move on the protein structure. We explored these possibilities by using co-evolution analysis to identify non-conserved positions that are under evolutionary constraint, a signature of functional importance.

As a model system, we used the family of LacI/GalR proteins. The LacI/GalR family is composed of several subfamilies which regulate diverse aspects of bacterial metabolism. Homologs drawn from different subfamilies have similar tertiary structures but low sequence identity. For example, the *Escherichia coli* homologs from the LacI and PurR subfamilies structurally align with RMSD of 1.75 Å, but have only 26% sequence identity. Within each subfamily, homologs are more closely related (>40%). We analyzed the six largest subfamilies of the LacI/GalR transcription regulator family with five different co-evolutionary algorithms. All results showed that the co-evolution networks of each subfamily were highly dissimilar from the others. That is, the strongest co-evolving positions were unique to each subfamily. Furthermore, each subfamily showed subfamily-specific conserved positions that were in unique locations. Therefore, the common tertiary architecture of LacI/GalR proteins can robustly accommodate multiple networks of functionally important positions. Correlations with available structural/functional data and implications for protein engineering will be discussed.

Temperature effects on the hydrodynamic radius of intrinsically disordered protein.

Steven T. Whitten

Department of Chemistry and Biochemistry, Texas State University

Intrinsically disordered proteins (IDP's) are often characterized in terms of the hydrodynamic radius, R_h . The R_h values of IDP's are known to depend on fractional proline content and net charge, where increased numbers of proline residues and increased net charge cause larger R_h . Though sequence and charge effects on the R_h of IDP's have been studied, the temperature sensitivity of R_h has been noted only briefly. Discussed here are R_h values that were measured by dynamic light scattering in the temperature range of 5°C to 75°C for the intrinsically disordered N-terminal region of the p53 protein, p53(1-93). Of note, the R_h of this protein fragment was highly sensitive to temperature, ranging from 35 Å at 5°C to 26 Å at 75°C. Computer generated simulations of conformationally dynamic and disordered polypeptide chains were performed to provide a hypothesis for the heat-induced compaction of R_h , which was opposite to heat effects on the R_h of a folded protein. The simulations demonstrated that heat caused R_h to trend toward the R_h expected for a statistical coil, indicating that the opposite heat effects that were observed could be interpreted as thermal denaturation for both proteins. The simulation data also suggested that net charge rather the proline content contributes dominantly to the native R_h of p53(1-93).

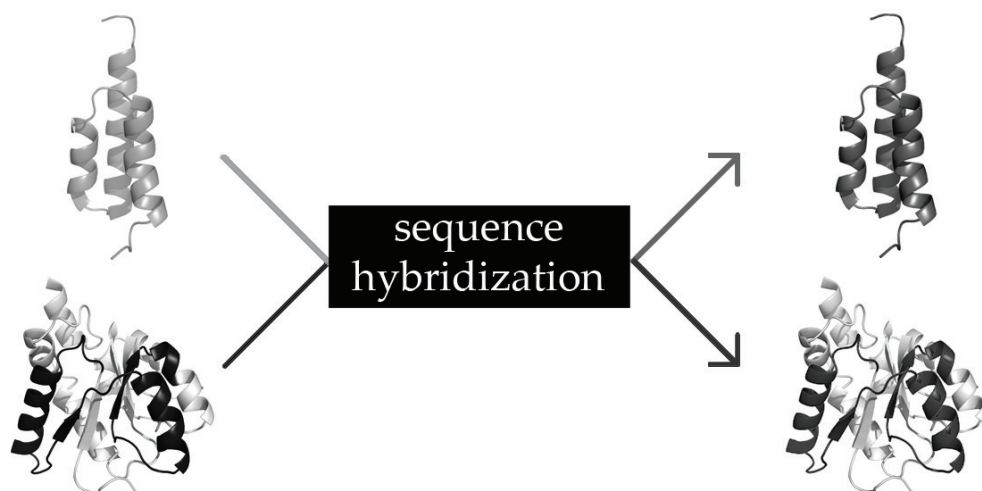
Rational design of two proteins with 80% sequence identity but different folds using sequence transplantation

Lauren L. Porter¹, Yihong He², John Orban², and Philip N. Bryan^{1,2}

1. Potomac Affinity Proteins 9601 Medical Center Dr. #330 Rockville, MD 20850

2. Institute for Bioscience and Biotechnology Research 9600 Gudelsky Dr.
Rockville, MD 20850

We present a method successfully used to engineer two proteins with 80% sequence identity but different folds. This method, called sequence transplantation, involves substituting the amino acid sequence encoding a substructure from a large protein with a sequence chimera. The chimera is a hybrid of the subsequence and another sequence that encodes a structurally dissimilar domain. In this case, the sequence of a segment of maltose binding protein (MBP) that adopts the Rossman fold was hybridized with the sequence of the human serum albumin binding domain of protein G (G_A), a three-helix bundle. We mutated both G_A and the subsequence of MBP until the two had 80% sequence identity. We call the resulting variants G_{A80} and MBP_{80} , respectively. Far-UV CD spectra, thermal denaturation curves, and NMR spectra suggest that both MBP_{80} and G_{A80} are folded yet adopt different topologies. These variants of MBP and G_A are currently the endpoints of a trajectory of mutations computationally designed using a set of simple principles. All variants along this trajectory were folded and raised the identity level between MBP and G_A . To test the generality of these principles, we designed variants of G_A with > 40% sequence identity to wildtype segments of Internalin B (α/β superhelix) and OspA (β -sheet). Both variants have helical CD spectra and unfold cooperatively in response to heat, suggesting that they are folded. The high level of identity between G_{A80} and MBP_{80} corroborates previous studies showing that only a handful of mutations in a given amino acid sequence are needed to switch one protein topology to another. Our combined results also suggest that designing proteins with high sequence identity might not be as difficult as previously anticipated.



Deletion of the connecting linker between kringle-1 and kringle-2 in prothrombin stabilizes the zymogen in a collapsed conformation

Nicola Pozzi, Zhiwei Chen, Leslie A Pelc, Daniel Shropshire and Enrico Di Cera

Edward A. Doisy Department of Biochemistry and Molecular Biology, Medical School, Saint Louis University

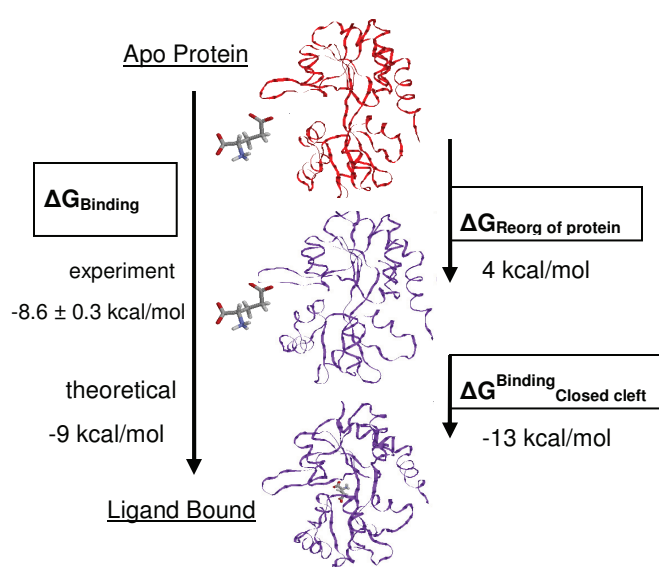
The zymogen prothrombin is a 72 kDa multi-domain protein and is converted to thrombin by the prothrombinase complex assembled on the surface of activated platelets or red blood cells. Prothrombin is composed of fragment 1, fragment 2 and a protease domain. Fragment 1 contains a Gla-domain (residues 1-46) and kringle-1 (residues 65-143), fragment 2 contains kringle-2 (residues 170-248), and the protease domain contains the A chain (residues 272-320) and the catalytic B chain (residues 321- 579). Three linker domains connect kringle-1 to the Gla-domain (residues 47-64), the two kringles (residues 144-169) and kringle-2 to the A chain (residues 249-284), respectively. Our recent x-ray structure of prothrombin, along with LRET measurements in solution, reveal a highly flexible conformation. Prothrombin exists in at least two conformations, in a 3:2 ratio, with the distance between the two kringles either fully extended or partially collapsed due to the intrinsic flexibility of the linker region connecting the two kringles. We therefore tested the role of this linker in the zymogen to protease conversion of prothrombin. The deletion mutant, ProT Δ 145-168, readily crystallized under several solution conditions and its structure was solved in both the Ca²⁺-free (2.8 Å) and Ca²⁺-bound (3.5 Å) forms. LRET studies and MD simulations suggest that the mutant is stabilized in the collapsed conformation. Remarkably, activation by the prothrombinase complex still proceeds through the same intermediates as wild-type prothrombin and with comparable kinetic rates. These findings prompt a re-evaluation of current models of prothrombin activation where the zymogen is thought to assume a fully extended conformation for optimal interaction with the prothrombinase complex.

Molecular Modeling of the Ligand Binding Free Energy and Conformational Rearrangements in AMPA type Glutamate Receptors

Maria Kurnikova, Kirill Speranskiy, Tatyana Mamonova, Michael Yonkunas

Chemistry Department, Carnegie Mellon University, Pittsburgh, PA 15217

AMPA type glutamate receptors are responsible for initiation of excitatory signal transmission in brain. Thermodynamics of AMPA receptor ligand binding domain (LBD) interaction with its agonist ligand L-glutamate, and its subsequent conformational reorganization was modeled using a pathway combining:



i) Molecular Dynamics/Poisson-Boltzmann/Surface Area (MD/PBSA) technique for absolute ligand binding free energy estimate;

ii) a Hamiltonian tempering, via Thermodynamic Integration (TI), and

iii) enhanced sampling via Umbrella Sampling technique (US) for subsequent conformational rearrangement of the LBD domain.

The role of charged residues in the binding site will be discussed in detail. Specifically formation of salt-bridges and hydrogen bonds play key role not only in ligand binding but also for

conformational rearrangement of the protein. Modeling of the protein is validated via quantitative comparison of computed binding energies and vibrational frequencies of the ligand with the calorimetric and FTIR experiments. Also, we will present results of simulations and computing of energetics LBD dimer interactions and the transmembrane domain (TM) pore opening as well as discuss their role in the AMPA receptor ion channel gating.

References:

1. K. Speranskiy, M. Kurnikova, *Biochemistry* Vol. 44, pp. 11508-11517 (2005)
2. T. Mamonova, M. Yonkunas, and M. Kurnikova, *Biochemistry*, Vol. 47(42), pp 11077-11085 (2008)
3. M. Yonkunas and M. Kurnikova, *Biophys. J.*, Vol 100(2), pp L5 -L7 (2011)

Poster Abstracts

alphabetical by presenting author's last name

Intermolecular forces in DNA condensation with cationic dendrimers

Min An, Jason DeRouchey

Department of Chemistry, University of Kentucky

In order to compact DNA, linear cations are believed to bind in DNA grooves and to interact with the phosphate backbone of apposing helices. The DeRouchey group has previously shown a length dependent attraction resulting in higher packaging densities with increasing charge for linear cations. Hyperbranched polycations, such as polycationic dendrimers, presumably would not be able to bind to DNA and correlate their charges with the phosphates of adjoining DNA in the same manner as linear cations. We use osmotic stress coupled with x-ray scattering to study fundamental molecular scale interactions and supramolecular self-assembly of low generation poly(amido amine) (PAMAM) dendrimer-DNA complexes. Dendrimer-DNA assembly displays fundamentally different physical behavior; higher dendrimer generation number give lower DNA packaging densities. The salt and pH dependence of packaging in dendrimer-DNA complexes was also measured. These studies begin to elucidate the role of cation topography in DNA condensation.

Molecular Dynamics Studies of the Role of Protein Flexibility in Immunological Molecular Recognition

Cory M. Ayres, Daniel R. Scott, Brian M. Baker and Steven A. Corcelli

Computational Molecular Science and Engineering Laboratory, Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, IN 46556 USA

Activation of the cellular immune response involves the recognition of an antigenic peptide presented by either the class I or class II major histocompatibility complex (MHC) by T-cell receptors (TCRs). Because of the large population of potential antigenic peptides in comparison to the number of available TCRs, TCRs must be cross-reactive, yet must also maintain a degree of specificity to avoid indiscriminate T cell activation. A commonly implicated factor for TCR specificity and cross reactivity, as well as the current focus for this study, involves the flexibility of TCR complementarity-determining region (CDR) loops. This study focuses on the DMF5 TCR, which is specific for epitopes of the MART-1 protein, upregulated in the majority of melanomas. Beyond its potential utility in cancer therapy, this particular TCR is of interest because of the apparent rigidity of its CDR loops, which contrasts with what traditionally has been expected of TCR binding loops. Molecular Dynamics simulations of both the unligated and ligated DMF5 TCR were performed in order to assess its flexibility and to gain insight into the roles of mobility in DMF5 binding.

Thermodynamic and Structural Determinants of Differential Pdx1 Binding to Elements from the *Insulin* and *IAPP* Promoters

Monique Bastidas and Scott Showalter

Department of Chemistry, The Pennsylvania State University, University Park, PA

In adult mammals, the production of insulin and other peptide hormones, such as the islet amyloid polypeptide (IAPP), is limited to β -cells due to restricted expression of a set of tissue-specific transcription factors, the most well known of which is Pdx1. Like homeodomain transcription factors from several gene clusters, Pdx1 binds to a core DNA recognition sequence containing the tetranucleotide TAAT, and fully reported as 5'-CTCTAAT(T/G)AG-3'. Describing the mechanism of target site selection by Pdx1 requires a complete thermodynamic profile of Pdx1 binding to near-consensus and natural promoter sequences. Strikingly, while Pdx1 regulatory elements in the human *insulin* promoter conform to the pentanucleotide 5'-CTAAT-3' sequence, the Pdx1 regulatory elements in the human *iapp* promoter all contain a substitution to 5'-TTAAT-3'. The available crystal structure of Pdx1 bound to the consensus nucleotide sequence does not explain how Pdx1 would be able to identify this natural variation, if it does at all. Here we report a combination of isothermal calorimetric titrations, NMR spectroscopy, and multi-microsecond molecular dynamics calculations of Pdx1 that define its interactions with a panel of natural promoter elements and DNA sequences derived from the consensus sequence. Our results show a small preference of Pdx1 for cytidine in the 5'-position with respect to the core TAAT promoter element. Molecular mechanics calculations, corroborated by experimental NMR data, lead to a rational explanation for sequence discrimination at this position. Taken together, our results suggest a molecular mechanism for differential Pdx1 affinity to elements from the *insulin* and *IAPP* promoter sequences.

Functional coupling *via* communication of surface loop disorder-to-order transitions through a folded protein core

¹Dorothy Beckett, ¹Christopher Eginton, ¹Katie Marshall, ²Sharrol Bachas & ²Hershel Wade

¹Department of Chemistry & Biochemistry, University of Maryland College Park, MD 20742

²Department of Biophysics and Biophysical Chemistry, Johns Hopkins School of Medicine, Baltimore, MD

Disorder-to-order transitions in surface loops play critical roles in regulating protein function. Corepressor bio-5'-AMP binding to the *E. coli* biotin repressor promotes homodimerization that is a prerequisite to sequence specific DNA binding. This assembly process results in repression of transcription initiation at the biotin biosynthetic operon. Folding of a loop segment comprised of residues 210-222 in the corepressor binding site is required to obtain the full -4.0 kcal/mole energetic coupling between bio-5'-AMP binding and dimerization. Several additional loops, some of which are disordered in the unliganded monomer, participate directly in the homodimer interface of the corepressor-bound protein. Alanine scanning mutagenesis has demonstrated the functional significance of these loops for dimerization. Replacement of a single glycine at position 142 in loop 140-146 with alanine results in complete abolition of coupling of corepressor binding to dimerization. Consistent with solution measurements and in contrast to wild type BirA and, the structure of this variant solved by x-ray crystallography reveals a monomeric liganded protein. Moreover, the alanine replacement results in adoption of an alternative conformation by the 140-146 loop. Furthermore, distinct from wild type corepressor-bound BirA, both a neighboring dimer interface loop comprised of residues 193-199 and the 210-222 loop that is located 33 Å away from the alanine replacement are disordered in the liganded variant. These results suggest that energetic coupling between ligand binding and dimerization in the biotin repressor is achieved through communication of distant disorder-to-order transitions through the folded protein core.

Use of internal Asp and Glu residues to design a conformational switch sensitive to changes in physiological pH

Peregrine Bell-Upp, Ilaria Caturegli, Meredith Peck, Aaron C. Robinson, Bertrand García-Moreno E.

T.C. Jenkins Department of Biophysics, Johns Hopkins University

Many proteins are known to undergo conformational and functional transitions in response to slight changes to pH in the physiological pH range. These pH-driven switches have the potential to be useful as platforms for therapeutic or biotechnological purposes. One of the problems with rational engineering of pH-sensitive switches that are active in the physiological pH range is that most ionizable residues do not titrate in this range. We have explored the utility of internal Glu and Asp residues for the engineering of pH-driven switches active near pH 7. The basis of this design is that the majority of pK_a values of Glu and Asp residues buried in hydrophobic environments are shifted to values of 7 or higher. The design exercise was based on our database of pK_a values of Asp and Glu residues at 25 internal positions in staphylococcal nuclease. With internal Asp and Glu residues the stability of the protein decreases with increasing pH. By matching the component of stability that is not related to the shifted pK_a values of the internal Glu and Asp with the component that is determined by the shifts in pK_a values, we hoped to be able to engineer a protein that unfolded cooperatively and reversibly near pH 7. This represents one of the first instances of rational engineering of pH-switching behavior tailored to respond to changes to pH in a desired physiological range. The underlying thermodynamic principles necessary for the engineering of such a switch will be discussed.

Investigating Metal Ion Binding in HPCD

Or I. Benet, Kate L. Henderson, Joseph P. Emerson

Mississippi State University

Homoprotocatechuate 2,3-dioxygenase (HPCD) is a nonheme metalloenzyme that uses dioxygen to catalyze an oxidative ring opening reaction. HPCD has been shown to be active with three distinctive metal ions: Mn^{2+} , Fe^{2+} , and Co^{2+} . We aim to uncover the underlying thermodynamics associated with metal ion selectivity and stability in HPCD by studying these variants under partially- and fully-denaturing conditions using a combination of spectroscopic methods (CD, fluorescence, and UV-vis) and thermochemical techniques (ITC and DSC). These results will help to improve our understanding of the unique metal binding behavior of HPCD and give us insight into how thermodynamics play a role in determining transition metal ion selectivity in vivo. In addition, this study will highlight a number of common biophysical methods that have been successfully adapted to probe protein-metal ion interactions.

Analysis of co-localization of 5-hydroxymethylcytosine and G-quadruplex-forming regions in gene transcriptional start sites: might hydroxymethylation affect the C-rich i-motif structure?

Yogini P. Bhavsar-Jog, Eric Van Dornshuld, Gregory S. Tschumper Randy M. Wadkins

Department of Chemistry and Biochemistry, The University of Mississippi, MS-38677

E-mail: bhavsar.yogini@gmail.com

5-hydroxymethylation of cytosine bases (5hmC) in genomic DNA of stem cells is an epigenetic modification occurring at the transcriptional start sites (TSS) of numerous genes. The 5hmC is known to be associated with gene enhancers, suggesting a potential function of the modified cytidine in gene regulation. Additionally, G-quadruplexes (in G-rich strands) and i-motifs (in C-rich strands) are topological variants (secondary structures) of DNA that are also known to be clustered in close proximity to TSS and regulate transcription. Genome analysis of G-rich sequences with a greater potential to form quadruplex structures suggests that these structures are present in higher abundance in proto-oncogenes as opposed to other classes of genes. Hence, both 5hmC and quadruplex-forming sequences are regulatory elements that occur at TSS of many genes. However, it is still unknown whether 5hmC enriched regions of a particular gene overlaps with, or is related to, the probability of occurrence of number of G-quadruplexes in its vicinity. Here, we present statistical analyses of the co-localization of these two elements in an effort to explore the hypothesis that enrichment of 5hmC occurs in the C-rich strands complimentary to G-rich strands with quadruplex potential in and around a TSS. Our results indicate that the G4/iM-forming gene with high 5hmC content is primarily associated with cell differentiation and proliferation.

Kinetic control in protein folding for light chain amyloidosis and the differential effects of somatic mutations.

Luis. M. Blancas-Mejía¹, Alexander Tischer^{1,2}, James R. Thompson³, Jonathan Tai⁴, Lin Wang¹, Matthew Auton^{1,2}, Marina Ramirez-Alvarado¹.

¹Department of Biochemistry and Molecular Biology, ²Division of Hematology, ³Department of Physiology and Biomedical Engineering, Mayo Clinic, 200 First St. SW, Rochester, MN 55905. ⁴Department of Chemistry, University of Illinois, Urbana, IL 61801. ramirezalvarado.marina@mayo.edu

Light chain amyloidosis is a devastating disease where immunoglobulin light chains form amyloid fibrils, resulting in organ dysfunction and death. Previous work by our group and others has shown a direct correlation between the protein thermodynamic stability and the propensity for amyloid formation for some proteins involved in light chain amyloidosis. Here we investigate the role of somatic mutations in the protein stability and *in vitro* fibril formation in the protein AL-103 by studying single and double restorative mutants compared to the wild type germline control protein.

A scan rate dependence and hysteresis in the thermal unfolding and refolding was observed for all proteins. This indicates that the folding reaction is kinetically determined with different kinetic constants for unfolding and refolding, even though this process remains experimentally reversible. We fit the thermal scan data using a simple kinetic model for scan rate dependence within a reversible two-state process. In this model, heating and cooling curves at each scan rate were analyzed independently with a two-state irreversible model to obtain the temperature dependence of the forward and reverse rate constants for unfolding and refolding. The equilibrium constant was calculated from the ratio of these kinetic rate constants to obtain the thermodynamic stability at equilibrium. Our structural analysis of AL-103 and AL-103 delP95aIns suggests a kinetic coupling of the unfolding/refolding process with *cis-trans* prolyl isomerization. Our data reveal that deletion of Proline 95a (AL-103 delP95aIns) that removes the *trans cis* di-proline motif present in the patient protein AL-103, results in a dramatic increment in the thermodynamic stability and a significant delay in fibril formation kinetics with respect to AL-103. Fibril formation is pH dependent; all proteins form fibrils at pH 2; reactions become slower and more stochastic as the pH increases up to pH 7.

To the best of our knowledge, this is the first time that a reversible folding process that is kinetically controlled has been described for amyloidogenic light chains. Our results lead the proposal that in addition to thermodynamic stability, kinetic stability (possibly influenced by the *cis* Proline 95a), plays a major role in the AL-103 amyloid fibril formation process.

Physical Basis of Recognition of Antigens by T Cell Receptors

Sydney Jo Blevins, Kurt Piepenbrink, and Brian M. Baker

Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, IN
46556

The Adaptive Immune Response is the body's way of identifying and destroying foreign pathogens. This process involves a recognition event utilizing two major proteins: a T cell receptor (TCR) and a Major Histocompatibility Complex (MHC) protein presenting a peptide. The complete mechanism by which a TCR recognizes an antigen (peptide-bound MHC) and proceeds to initiate the signaling cascade to destroy infected cells is still unknown. Even less clear is how a particular T cell receptor distinguishes between foreign- and self-peptides presented on an MHC. To better study this recognition event, several TCR-pMHC interactions have been studied using double mutant cycles to determine the free energy of interaction ($\Delta G^\circ_{\text{int}}$) existing between pairs of residues. Three TCRs that utilize the same V α chain (TRAV12-2*01) have been studied for their interactions with three unique pMHCs. Additionally, the well-studied human TCR A6 has been used to determine the role that conserved residues play in maintaining structural integrity and forming communication networks within the receptor.

Influence of cavities on the stability and pressure sensitivity of proteins

José A. Caro¹, Mariano Dellarole², Jamie L. Schlessman¹, Catherine A. Royer²,
Bertrand García-Moreno E^{1*}.

¹Department of Biophysics, Johns Hopkins University, 3400 N Charles St., Baltimore MD, USA. ²Centre de Biochimie Structurale, CNRS UMR 5048, Montpellier, France.

It has been proposed that cavities in the folded state of proteins are the main determinants of the volume change, ΔV , responsible for the pressure unfolding of proteins (Roche J, et al. (2012) Cavities determine the pressure unfolding of proteins. *Proc Natl Acad Sci USA* 109:6945–6950). In that study, the natural cavity observed in the crystal structure of staphylococcal nuclease (SNase) was increased in size by single alanine substitutions. Pressure unfolding experiments monitored by fluorescence and NMR spectroscopy of the single Ala variants always resulted in ΔV values larger in magnitude than that of the parent protein. To test the extent to which cavities determine volume changes, we performed three different types of experiments. **(1)** We engineered double and triple Ala variants of SNase with very large cavities as observed in crystal structures. The pressure unfolding of the multiple Ala variants showed that as the observed volume of the engineered cavities increased, so did the ΔV measured by fluorescence and NMR spectroscopy. **(2)** A protein without detectable cavities should still report a significant ΔV owing to packing defects. To test this possibility we engineered a set of variants with substitutions that introduce bulky residues in the protein core in attempts to block the natural cavity in SNase. Pressure unfolding measurements monitored by fluorescence of all but one of these variants resulted in no significant change in ΔV . The exception was a variant with a decrease in ΔV of about 25%. This suggests that a basal level of ΔV is determined by unavoidable packing defects. **(3)** The state of hydration of internal cavities of proteins is usually unknown. Internal hydration could lower the volume of the folded state and should be favored at higher pressures. In an attempt to hydrate internal cavities, a third set of variants was engineered to line the internal cavity with polar groups that can readily hydrogen bond to internal water molecules. Although the crystal structures show internal hydration, the ΔV values of these variants remained high. Our systematic study suggests that cavities do represent a dominant contribution to ΔV , the volume difference between the folded and unfolded states of proteins. Packing defects too small to fit a water molecule also seem to contribute to a baseline ΔV value. Polar and apolar cavities of identical size contribute similarly to ΔV , suggesting that the large ΔV measured already accounts for the hydration of internal cavities.

Assembly, Structural and Functional Studies of Polyubiquitin Chains

Carlos A. Castañeda^a, Susan Krueger^b, Ashton Cropp^c and David Fushman^a

^a University of Maryland, College Park, MD

^b NIST Center for Neutron Research, Gaithersburg, MD

^c Virginia Commonwealth University, Richmond, VA

Ubiquitination is a vital post-translational modification that signals a wide variety of biological events, including proteasomal degradation, DNA repair, cell cycle regulation, T-cell activation in the immune response, among others. The diverse array of signaling outcomes stems from the ability of ubiquitin, a small 76-aa protein, to form polyubiquitin chains linked between the C-terminus of one Ub and the ϵ -NH₂ amino group on one of seven different lysine residues (K6, K11, K27, K29, K33, K48 and K63) on a second Ub. We hypothesize that the molecular basis of polyubiquitin's signaling diversity is rooted in the variability of the structural and dynamical properties of the different types of polyubiquitin chains. To study the solution properties of polyubiquitin chains comprised of every Lys linkage type, we have devised a chemical assembly method ⁽¹⁾ that has enabled us to construct ubiquitin chains of any linkage, any length and any isotopic labeling scheme.

Using NMR spectroscopy and small angle-scattering, we have begun biophysical characterization of each of these chains in solution. NMR studies have shown that each of these chains exhibits a unique set of chemical shift perturbations and residual dipolar couplings, which reveal distinct Ub-Ub interactions and Ub-Ub orientations for each linkage type. Of particular interest, we observed that K11-linked diubiquitin (Ub₂) exhibits increased Ub-Ub interactions in the presence of physiological salt concentration. Small angle-scattering studies also confirmed that the conformation of K11-linked Ub₂ is more compact in the presence of salt. Furthermore, the structures of K11-linked Ub₂ in solution are quite distinct from two recent crystal structures of K11-linked Ub₂ ⁽²⁾. These studies suggest that the conformations of polyubiquitin chains may be modulated extensively, and that determining the conformational ensembles of these chains will be important to elucidating how each chain is recognized by downstream receptor proteins. Our studies will provide the framework necessary in understanding the mechanism by which each of these chains elicits unique biochemical signals in the cell.

1. Castañeda et al., *JACS* 133, 17855-17868 (2011).

2. Bremm, A., Freund, S.M.V. & Komander, D. *Nat Struct Mol Biol* 17, 939-947 (2010);
Matsumoto, M.L. et al. *Mol Cell* 39, 477-484 (2010).

Metal Binding Specificity of the Staphylococcal Biofilm Protein Aap

Catherine T. Chaton and Andrew B. Herr

Department of Molecular Genetics, Biochemistry and Microbiology, University of Cincinnati College of Medicine, Cincinnati, OH 45267

Staphylococcal infections in hospitals kill almost a hundred thousand patients every year in the United States alone. It is estimated that as many as 80% of these infections involve the formation of a biofilm, a specialized surface-adherent colony that is highly resistant to antibiotic treatment and host immune responses. In *S. epidermidis*, the attachment-associated protein (Aap) has been found to be necessary for biofilm formation, as it mediates intercellular adhesion through zinc-induced self-assembly. Aap is an elongated protein covalently attached to the cell wall whose C-terminal half contains 5-17 tandem copies of the 'B-repeat,' a 128-amino acid sequences comprising two subdomains. The B-repeat region is necessary and sufficient for zinc-induced intercellular adhesion in staphylococcal biofilms. We previously solved the crystal structure of a minimal B-repeat construct (Brpt1.5) that forms a zinc-dependent dimer. The Zn^{2+} ion is coordinated in trans by two Brpt1.5 protomers, with residues H75, D21, and E203 acting as ligands in the primary binding mode. While the ability of zinc to induce B-repeat dimerization is known, we are studying the effects of other divalent cations and their ability to bind or dimerize the Brpt1.5 construct to better understand Aap's metal coordination. Sedimentation velocity interference analytical ultracentrifugation (AUC) experiments show the effect of various divalent cations on Brpt1.5 self-assembly as well as potential inhibition of zinc-induced assembly through competitive binding. Circular dichroism (CD) allows us to follow the unfolding of Brpt1.5 as a function of temperature to investigate the stability imparted upon Brpt1.5 by metal binding. The mutant Brpt1.5 E203A is capable of binding zinc but lacks the ability to dimerize. Therefore, it allows us to study the effects of metal binding separate from those of assembly in both AUC and CD. By better understanding the role of divalent cation coordination in biofilm formation, including metal specificity, we are hoping to build the foundational knowledge necessary for developing targeted therapeutics.

Analysis of PKR dimerization by resonance energy transfer

Bushra Husain, Michael Bruno and James L. Cole

Department of Molecular and Cell Biology, University of Connecticut, Storrs, CT

Protein kinase R (PKR) is a critical component of the interferon-induced innate immunity pathway. PKR is expressed in a latent inactive form and becomes activated upon binding dsRNA to undergo autophosphorylation. PKR contains a C-terminal kinase domain and two tandem dsRNA binding motifs. It is believed that dimerization plays a critical role in the mechanism of activation of PKR by dsRNA. Activation requires dsRNA long enough to accommodate at least two PKRs. In addition, the kinase domain has been crystalized as a dimer and the structure suggests a mechanism linking the dimer interface with the active site. However, there is no direct evidence for dimerization of PKR on dsRNA lattices and some RNAs that bind multiple PKRs fail to activate. Therefore, we have developed a fluorescence assay to quantitatively probe PKR dimerization upon binding to dsRNA. Using unnatural amino acid mutagenesis, *p*-azidophenylalanine was selectively incorporated into PKR at a site that lies near the kinase dimer interface. This residue was labeled with an Alexa Fluor 488 - alkyne conjugate using click chemistry. This fluorophore has a small Stokes shift and is capable of homo Förster Resonance Energy Transfer (homo-FRET). The fluorescence anisotropy of this probe decreases upon PKR binding to an activating 40 bp dsRNA due to depolarization induced by homo-FRET. The anisotropy increases upon further addition of the dsRNA due to dilution of the PKR monomers onto separate dsRNAs. Hetero-FRET between Alexa Fluor 488- and Cy3-labeled PKR confirms that depolarization is due to dimerization. PKR binding to nonactivating dsRNAs does not induce a decrease in anisotropy, indicating the absence of kinase dimer formation.

SPOC—A novel phospho-peptide binding domain important for repressing Notch target genes

Kelly J. Collins, Nassif H. Tabaja, and Rhett A. Kovall

University of Cincinnati, Department of Molecular Genetics, Biochemistry, and Microbiology

The Notch pathway is a highly conserved cell-to-cell signaling process with essential roles in embryonic development and tissue maintenance. Notch signaling ultimately leads to transcriptional activation of Notch target genes. The sole Notch nuclear effector, CSL, is a DNA binding protein that, upon Notch pathway activation, serves as a docking site for co-activator proteins, resulting in active transcription of Notch target genes. In the absence of a Notch signal, CSL functions to keep Notch target genes repressed through interactions with co-repressor proteins, such as MINT. MINT is a large, multi-domain protein that mediates interactions with different nuclear factors through distinct domains. MINT has been shown to have a high affinity interaction with CSL through its CSL-interaction domain; in addition, the highly conserved C-terminal SPOC domain of MINT has been shown to facilitate interactions with the ubiquitous corepressors, NCoR and SMRT, which are necessary components of chromatin remodeling machinery, such as histone-deacetylases (HDACs). Although a direct interaction between the SPOC domain of MINT and NCoR has been suggested in multiple studies, we were initially unable to measure a direct interaction through isothermal titration calorimetry (ITC). Interestingly, two independent, global phosphorylation studies demonstrated that NCoR is phosphorylated on two conserved serine residues in its C-terminus. We hypothesized that NCoR requires phosphorylation of these serine residues in order to interact with the SPOC domain of MINT. Using X-ray crystallography and ITC, we have structurally and functionally characterized how phosphorylated NCoR contributes to the SPOC-NCoR interaction. Our studies suggest that NCoR requires phosphorylation on both serine residues in order to achieve a high affinity interaction with the SPOC domain of MINT. This interesting finding identifies the SPOC domain of MINT as a new phospho-peptide binding domain. Further, these findings implicate phosphorylation of different corepressor proteins as a novel mechanism in Notch signaling for regulating how larger, HDAC containing complexes assemble at Notch target genes.

Steroid Receptors Cooperatively Assemble at Promoters with Unique Dependence on Inter-site Distance

Keith D. Connaghan, Nicole T. Fazio, Qin Yang, Amie D. Moody, Michael T. Miura and David L. Bain

Department of Pharmaceutical Sciences
University of Colorado Anschutz Medical Campus
Aurora, CO 80045

Transcriptional activation involves the interactions of at least 50 proteins plus DNA. Despite this complexity, activation occurs only at specific genes and only at precise times. The basis of specificity and precision are not well understood. For example, the steroid receptor family activates transcription by assembling at promoters, followed by recruitment of coactivators and the transcriptional machinery. However, each receptor activates a subset of all genes. To identify the factors responsible for specific activation we are quantitatively characterizing receptor assembly at promoters. Previously we have shown that each receptor partitions its promoter assembly energetics differently between dimerization and cooperativity; and that this partitioning enhances the stability of specific receptor-promoter combinations, which then generates promoter-specific occupancy. We hypothesize that receptors assemble at target-gene promoters with enhanced stability, such that receptors discriminate among promoters and only assemble at their targets – thus achieving specific activation. Evidence supports the idea that a receptor's ability to assemble cooperatively varies with promoter architecture, thereby selectively enhancing stability. To further test this idea, we measured the cooperativity of three receptors as a function of distance between two promoter-binding sites. We found: 1) Progesterone receptor-B assembles with enhanced cooperativity on promoters where binding sites are closer together and in-phase; 2) glucocorticoid receptor, assembles with two regions of enhanced cooperativity; and 3) estrogen receptor- α is non-cooperatively on all promoters. These results provide strong support for the hypothesis that receptors have unique energetics that allow for targeted promoter-assembly. Lastly, changes in dimerization globally affect assembly at all promoters; whereas cooperativity affects assembly locally for only the specific receptor-promoter combination. Therefore, cooperativity allows for more diversity in selectively enhancing stability. Interestingly, the advent of cooperativity – and weakening of dimerization – parallels the evolution of the steroid receptor family. Perhaps, cooperativity evolved at the expense of dimerization to provide receptors the means to discriminate among more promoters and allow for more diverse functions.

Role of Primary Structure Variation in Structure and Function of Calcineurin

Erik C. Cook, Tori B. Dunlap, Praveen R. Juvvadi, William J. Steinbach, and Trevor P. Creamer

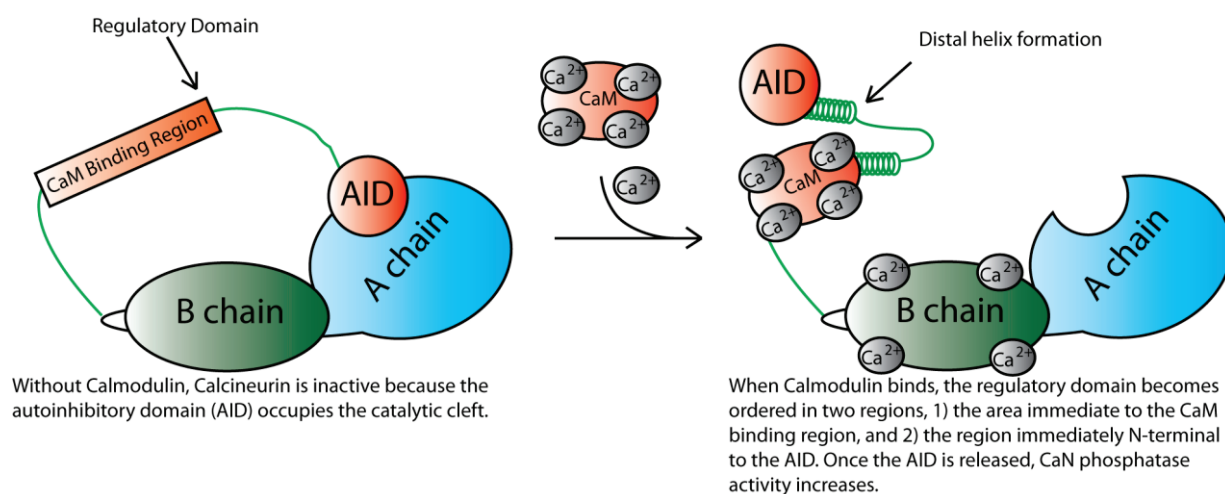
Center for Structural Biology and Department of Molecular and Cellular Biochemistry

University of Kentucky

741 South Limestone Street BBSRB 278A

Lexington, KY 40536-0509

A. fumigatus is one of the leading causes of invasive fungal infections in solid organ transplant and adult stem cell transplant recipients. Here, we study the heterodimeric phosphatase calcineurin that is critical for the virulence and pathogenicity of this organism. CaN is activated when calcium-loaded calmodulin (**CaM**) binds the regulatory domain (**RD**) of the A subunit. Upon binding to CaM, two separate regions of the RD of **human** calcineurin (the CaM-binding site and another region downstream which we call the distal helix site) transitions from a disordered state to an ordered state. These ordered states are predicted to contain a high alpha helical content from circular dichroism studies (Rumi-Mansante, 2012). The downstream distal helix that forms upon CaM binding has also been predicted to be amphipathic of which the hydrophobic face may fold back onto the CaM-RD complex (Dunlap, 2013). Dunlap et. al. has shown that this distal helix can be destabilized by mutating residues on the hydrophobic face to hydrophilic residues and that this full-length CaN mutant cannot achieve full activity. In *A. fumigatus* and other organisms (such as *D. melanogaster* and *S. cerevisiae*) the sequence for this distal helix is deleted. Hence, the goal of our research is to elucidate the mechanism behind the activation of CaN in these organisms to understand how evolution has acted through sequence variations to affect higher order structure.



Studies of RNA recognition and catalytic domains in the conserved virulence factor A from *Streptococcus pyogenes*

Valeria A. Copello^{*,1}, Taylor D. Griffith^{*,1}, Catherine A. Carney¹, Fanny Chu¹, Kyu Hong Cho², Brian M. Lee¹ and Gabriela C. Pérez-Alvarado¹

¹Department of Chemistry & Biochemistry, Southern Illinois University, Carbondale

²Department of Microbiology, Southern Illinois University, Carbondale

*Both authors contributed equally

The conserved virulence factor A (CvfA) protein from *Streptococcus pyogenes* is involved in the regulation of gene expression of virulence factors and glycolytic enzymes as a result of nutrient availability. CvfA has four predicted conserved structural domains: a transmembrane helix, a predicted coiled coil region, an hnRNP K (KH) D domain which is an RNA binding domain, and a metal-dependent His/Asp phosphohydrolase (HD) domain. HD domains present variable overall folds, metal-coordination, catalytic metal-dependence and substrate-specificity directly associated with their biological functions. Proteins including both the RNA recognition domain (KH domain) and the catalytic HD domain of CvfA have been overexpressed, purified and characterized. These proteins are being used to study the metal ion dependence controlling catalytic activity as well as the effects of RNA-recognition on enzymatic function. (Research supported by 1R15 GM101603-01 MPI).

Dissection of androgen receptor-promoter interactions: Steroid receptors partition their interaction energetics in parallel with their phylogenetic divergence

Rolando W De Angelis, Qin Yang, Michael T Miura and David L Bain

Department of Pharmaceutical Sciences
University of Colorado Anschutz Medical Campus
Aurora, CO 80045

Steroid receptors comprise a homologous family of ligand-activated transcription factors. The members include androgen receptor (AR), estrogen receptor (ER), glucocorticoid receptor (GR), mineralocorticoid receptor (MR) and progesterone receptor (PR). Phylogenetic studies demonstrate that AR, GR, MR and PR are most closely related, falling into subgroup 3C. ER is more distantly related, falling into subgroup 3A. To determine the quantitative principles by which receptors generate their unique functional responses, we are systematically dissecting the promoter-binding energetics of all receptors under “standard state” conditions. Here we examine the self-assembly and promoter-binding energetics of full-length AR and a mutant associated with prostate cancer, T877A. We first demonstrate that both constructs exist only as monomers, showing no evidence of dimerization. Although this result contradicts the traditional understanding that steroid receptors dimerize in the absence of DNA, it is fully consistent with our previous work demonstrating that GR and two PR isoforms either do not dimerize or dimerize only weakly. Moreover, both AR constructs also exhibit strong inter-site cooperativity, again as seen previously for GR and PR. In direct contrast, the more distantly related ER- α generates dimerization energetics so strong they can only be measured indirectly, yet generates negligible cooperativity. Thus homologous receptors partition their promoter-binding energetics quite differently. Moreover, since receptors most closely related in phylogeny partition their energetics most similarly, such partitioning appears to be evolutionarily conserved. We speculate that these differences in interaction energetics, coupled with different promoter architectures, serve as the basis for generating receptor-specific promoter occupancy and thus function.

RNA Target Discrimination by U1A and SNF Proteins

Gregory T. DeKoster, Kimberly J. Delaney, and Kathleen B. Hall

Washington University School of Medicine,
Department of Biochemistry & Molecular Biophysics, St. Louis, MO 63110

Human U1A and *Drosophila* SNF proteins are conserved RNA binding proteins. They consist of two RNA recognition motifs (RRMs) joined by a long flexible linker. The RRM1s have an α/β fold, and RNA binding occurs on the surface of the β -sheet. U1A and SNF are components of snRNPs, but while U1A is found in only U1 snRNP, SNF is present in both U1 and U2 snRNPs. Their different distributions are due in large part to their different RNA binding properties that arise from their distinct RNA binding mechanisms. Human U1A binds U1 snRNA stemloop II (SLII) with high affinity and specificity, but although the U2 snRNA SLIV and U1 SLII display many of the same nucleotides in the same structural context, U1A does not bind SLIV. SNF binds to both SLII and SLIV. Since the RNA Recognition Motif (RRM1) of U1A and SNF are 74% identical and their structures are virtually identical, the molecular basis of their discrimination is not immediately apparent. Previously, we have shown thermodynamic coupling between highly conserved U1A beta-sheet surface residues Tyr13, Gln54, and Phe56.¹ These residues form an interacting network to create the RNA binding site that also encompasses a proximal protein loop (loop3). Using a variety of NMR methods to compare the two RRM1s, we find that their backbone dynamics are distinctly different on both rapid (ps-ns) and intermediate (μ s-ms) timescales, and that the Tyr-Gln-Phe residues on their RNA binding surfaces do not have the same mobility/interactions. We posit that these fundamental RRM1 properties allow SNF to bind two RNAs, but restrict U1A to bind only one RNA stemloop. Supported by NIH R01 GM096444.

1. Kranz and Hall, *JMB* **1999** 285, 215.

Understanding DNA Condensation: From Simple Ions to Protamine-DNA Packaging in Sperm

Jason DeRouchey

Department of Chemistry, University of Kentucky

DNA in nature exists primarily in a highly compacted state critical for most biological functions. DNA condensation, however, remains poorly understood at the molecular level. We are interested in understanding the fundamental interactions, molecular scale forces and elucidating mechanisms by which polycations interact with DNA *in vitro* and *in vivo*. We use osmotic stress coupled with x-ray scattering, to study packaging densities and compaction energies between DNA helices in the presence of various cations. In this talk, we will discuss from simple ions to complex proteins and how these cations modulate both the attractive and repulsive forces between DNA helices. Lastly, the biological implications of these forces will be discussed with regards to spermatogenesis where chromatin histones are replaced by arginine-rich protamines to densely compact DNA in sperm heads. Tight packaging from spermatogenesis is considered essential for both successful transport as well as to protect DNA from damage.

Backbone conformational reorganization as a determinant of pK_a values of surface residues in proteins

Brian M. Doctrow, Kelli L. Baran, Jamie L. Schlessman¹, Ananya Majumdar²,
Bertrand García-Moreno

Department of Biophysics, Johns Hopkins University, ¹Department of Chemistry,
United States Naval Academy, ²Biomolecular NMR Facility, Johns Hopkins
University

The pK_a values of surface ionizable residues in proteins are usually similar to the pK_a values of ionizable residues in water. This is still difficult to reproduce using structure-based electrostatics calculations with static structures, which tend to exaggerate the shifts in pK_a values because the magnitude of Coulomb effects is exaggerated and the balance between Coulomb and dehydration effects is not reproduced correctly. These problems can be minimized by using artificially high protein dielectric constants, but sometimes the discrepancy persists even when the protein interior is treated with the dielectric constant of water. This suggests that pK_a values are affected by the conformational dynamics of the protein, which are not reflected in the crystal structure. Molecular dynamics or Monte Carlo simulations can be used to attempt to reproduce these dynamic effects implicitly, but there are no useful data for testing this approach directly. To examine the role of backbone reorganization in determining pK_a values, NMR spectroscopy was used to measure the pK_a values of all 20 Asp and Glu residues in variants of staphylococcal nuclease with Gly substituted at select locations. These substitutions were intended to facilitate backbone reorganization without affecting the overall protein structure. Some Gly substitutions detectably shifted the pK_a values of some carboxylic groups. Crystal structures of these variants showed no significant changes relative to the structure of the reference protein, but hydrogen exchange measurements indicated increased propensity for local unfolding near the residues with shifted pK_a values. Calculations with continuum methods using these crystal structures do not reproduce the measured shifts in pK_a values. Our results suggest that the high apparent polarizability of proteins might be due to subtle structural reorganization that is difficult to reproduce computationally.

Driving Forces for Protein Secretion Across the Bacterial Outer Membrane

Igor Drobnak & Patricia L. Clark

Department of Chemistry & Biochemistry
University of Notre Dame, Notre Dame, IN 46556 USA

Autotransporters are a large and diverse class of monomeric virulence proteins secreted from Gram-negative bacterial pathogens. Secretion across the outer membrane is facilitated by the C-terminal translocator domain that creates a pore in the outer membrane, through which the N-terminal passenger (the functional, extracellular part of the protein) exits the cell. However, there is no ATP in the periplasm nor an ion gradient across the outer membrane. It is therefore not clear where the free energy comes from to drive the transport of the passenger through the pore. Using a computational model of secretion kinetics, we show that the free energy of passenger folding could be used as a driving force for secretion, provided that the passenger does not fold prematurely in the periplasm. We have tested this model experimentally by reversibly stalling secretion and probing the periplasmic conformation of the stalled protein. Our results show that the passenger remains unfolded in the periplasm, confirming that the major requirement for coupling folding to secretion is met. This model is also able to describe the overall secretion kinetics reasonably well. Further work is in progress to experimentally characterize the interplay between autotransporter passenger folding and secretion.

Calmodulin induced calcineurin conformational changes and activation

Tori B. Dunlap^{1,2}, Julie Rumi-Masante¹, Terrence E. Lester¹, Hou-fu Guo¹, Emily Holbrook¹, Hou-fu Guo¹, Farai I. Rusinga³, Craig W. Vander Kooi¹, David D. Weis³, and Trevor P. Creamer¹

¹Center for Structural Biology, Department of Molecular and Cellular Biochemistry, University of Kentucky, 741 S. Limestone St., Lexington, KY 40536-0509

²Department of Chemistry, University of Central Arkansas, 201 Donaghey Ave., Conway, AR 72035

³ Department of Chemistry, University of Kansas, 1251 Wescoe Hall Drive, Lawrence, KA 66045

Calcineurin (CaN) is a highly regulated Ser/Thr protein phosphatase that plays critical roles in learning and memory, cardiac development and function, and immune system activation. Alterations in CaN regulation contribute to multiple disease states such as Down syndrome, cardiac hypertrophy, Alzheimer's disease, and autoimmune disease. In addition, CaN is the target of the immunosuppressant drugs FK506 and cyclosporin A. Despite its importance, CaN regulation is not well understood on a molecular level. Full CaN activation requires binding of calcium-loaded calmodulin (CaM). Previous work has demonstrated that the regulatory domain of CaN (RD) is disordered. The binding of CaM to CaN results in RD folding. Folding of the RD in turn causes the autoinhibitory domain (AID) located C-terminal to the RD to be ejected from CaN's active site. This binding-induced disorder-to-order transition is responsible for the activation of CaN by CaM. In this work, using size-exclusion chromatography and crystallography, we demonstrate that a single CaM molecule is responsible for binding to and activating CaN. We also determine that CaM binding to CaN induces an amphipathic helix (the distal helix) C-terminal to the CaM binding region. The distal helix undergoes a hairpin-like chain reversal in order to interact with the surface of CaM, resulting in the removal of the AID from CaN's active site. We employ site-directed mutagenesis, circular dichroism spectroscopy, and phosphatase assays to observe the distal helix and determine its impact on CaM's activation of CaN.

Examination of the thermodynamic mechanism for *E. coli* ClpAP assembly

Elizabeth C. Duran and Aaron L. Lucius

University of Alabama at Birmingham, Department of Chemistry, 1530 3rd Ave S,
Birmingham, AL 35294-1240

The ClpAP system in *E. coli* is responsible for the removal of damaged or misfolded proteins and serves as a prokaryotic model to study highly conserved, ATP-dependent proteolysis in eukaryotes. *E. coli* ClpA is a AAA+ (ATPase Associated with diverse cellular Activities) chaperone that catalyzes the ATP-dependent unfolding and translocation of substrate proteins targeted for degradation by the cell into a proteolytic protease, ClpP. ClpA hexamers associate with one or both ends of the ClpP tetradecamer to form either 1:1 or 2:1, ClpAP complexes, based on relative concentrations of the two components. Although the overall mechanism of protein degradation by ClpAP has been examined using steady-state kinetics, the affinity of the association between ClpP tetradecamers and ClpA hexamers remains unclear. Quantitatively determining these affinities is essential because the interpretation of kinetic data hinge on knowing the population of 1:1 and 2:1 complexes. Here we use a fluorescently modified ClpP construct to monitor the association of ClpA hexamers with tetradecameric ClpP. The affinity of ClpAP complex formation is examined with binding isotherms constructed from changes in fluorescence emission as a function of ClpA concentration. Determining the affinity of the association of ClpA to ClpP is a crucial step in the development of a complete thermodynamic mechanism for ClpAP assembly as well as interpretation of kinetic data.

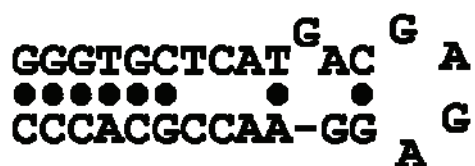
Unfolding Thermodynamics of the DNA Sarcin-Ricin Loop Sequence

Nadera R. Elkarmi^{*}, Sarah E. Johnson[†] and Luis A. Marky[†]

^{*}University of Nebraska at Omaha,

[†]Department of Pharmaceutical Sciences, University of Nebraska Medical Center,
986025 Nebraska Medical Center, Omaha, NE, 68198-6025

The sarcin-ricin loop (SRL) is the part of the ribosome that interacts with elongation factors, allowing for the synthesis of proteins. The NMR and X-ray crystal structure of the RNA SRL sequence showed the folded of a hairpin loop containing 8 W-C base pairs, four mismatches, and a bulged G nucleotide in the stem that is closed by a GNRA tetraloop (5'-GAGA-3'). In this work, we use the analog DNA sequence of SRL (*DNA-SRL*):



and investigated its unfolding thermodynamics. Specifically, we used a combination of differential scanning calorimetry (DSC) and temperature-dependent UV spectroscopy techniques to determine thermodynamic profiles of the *DNA-SRL*. UV melting curves show monophasic transitions with transitions temperatures ($\sim 40.9^\circ\text{C}$) independent of strand concentration, indicating the unfolding of an intramolecular complex. DSC curves also showed monophasic transitions with a van't Hoff enthalpy of 54.5 kcal/mol and calorimetric enthalpy of 67.9 kcal/mol. The ratio of these two enthalpies ($= 0.80$) indicates a non-two-state transition i.e., the transition involves intermediate states. We also measured indirectly a heat capacity effect ($\Delta C_p = 840 \text{ cal/mol-}^\circ\text{C}$). The favorable folding of this molecule ($\Delta G_{(20)}^\circ = -4.5 \text{ kcal/mol}$) takes place through the characteristic favorable enthalpy-unfavorable entropy compensation. The large magnitude of the folding enthalpy and the non-two state nature of the transition is consistent with the stacking of 8 base pairs and 4 mismatches in the stem. Supported by Grant MCB-1122029 from the National Science Foundation.

In search of techniques that will aid the development of “silent coupling” leads into allosteric drugs: H/DX-MS studies of pyruvate kinase

Charulata B. Prasannan, Maria T. Villar, Aleksandra Gmyrek, Antonio Artigues , and

Aron W. Fenton

The University of Kansas Medical Center, Kansas City, Kansas and The University of Texas Medical Branch at Galveston, Galveston, Texas

A recent suggestion is that effectors that cause “silent coupling” are the ideal outcome of small molecule library screens aimed at developing allosteric drugs. Such silent effectors modify how the enzyme binds substrate, but the outcome of this change is substrate binding with the same affinity observed when the effector is not present. Given that this concept has not often been considered in the literature, we include a theoretical consideration using energy diagrams. Starting with silent effector leads, subtle chemical modifications can generate either allosteric activators or allosteric inhibitors. Unfortunately, there is currently no example of a structure/function study that has provided insights that can, in turn, be used to direct rational design of silent effectors towards allosteric drugs.

In our previous study of rabbit muscle pyruvate kinase (M₁-PYK) with small angle X-ray scattering (SAXS), we included alanine as a non-allosteric analogue to contrast with the changes caused by binding of the allosteric inhibitor, phenylalanine. This use is based on the fact that alanine binds competitively with phenylalanine, but causes little to no change in the affinity of the protein for substrate, phosphoenolpyruvate (PEP). To our surprise, the presence of alanine modifies the SAXS detected structural changes caused by PEP binding.

The SAXS structural evidence for alanine-PEP coupling has now been confirmed by the use of ITC to show that ΔH for PEP binding is greatly altered when alanine is present. To determine the areas of the protein that facilitate the “silent coupling” between PEP and alanine binding, we have initiated a hydrogen/deuterium exchange study evaluated by mass spectrometry (H/DX-MS). Peak assignment/interpretation is ongoing. However, our initial evaluation indicates that the areas of the protein that play roles in alanine-PEP silent coupling are the same areas highlighted in a previous H/DX-MS study. In that previous study, we followed the phenylalanine and alanine comparison used in the SAXS study: We attempted to identify which changes were caused by phenylalanine that were in addition to those caused by alanine. Since the same areas of the protein appear to play roles in “silent coupling” by alanine and in allosteric inhibition by phenylalanine, we can suggest that very subtle differences must dictate the two outcomes. Nonetheless since these changes all occur on one side of the effector binding site, the outcome of the H/DX-MS study would direct which parts of the effector molecule should be a targeted to convert the silent effector into an allosteric drug. Funded by NIH DK78076.

Nucleosome Remodeling by the chromatin remodeler ISWI

Christopher J. Fischer¹, Gada Al-Ani², Koan Briggs¹

¹University of Kansas Department of Physics and Astronomy, ²University of Kansas
Department of Molecular Biosciences.
Lawrence, KS 66045

The regulation of chromatin structure is controlled by a family of molecular motors called chromatin remodelers. The ability of these enzymes to remodel chromatin structure, through the repositioning and sliding of nucleosomes, is dependent on their ability to hydrolyze ATP. While many studies have been conducted, the exact mechanism by which these motors couple the binding and the hydrolysis of ATP into the mechanical work that drives the nucleosome repositioning remains unclear. In this study we characterized the interaction of the chromatin remodeler ISWI to fluorophore labeled nucleosome substrates by monitoring the changes in the fluorescence anisotropy of the fluorophore upon ISWI binding. We then used this information to analyze the nucleosome repositioning activity of ISWI as a function of the fraction of each bound species to determine the activity associated with these species. We found that a monomer of ISWI was sufficient for nucleosome repositioning and was responsible for the nucleosome stimulated ATPase activity of ISWI.

From dimer folding to nucleosome assembly

Lisa M. Gloss, Traci B. Topping, Paul J. Guyett and Anna M. Lindsay

School of Molecular Biosciences
Washington State University
Pullman, WA 99164-7520

The Gloss lab has focused on the H2A-H2B histone heterodimer of the nucleosome core particle as a model system for dimeric protein folding to elucidate the impact of the transient population of monomeric and dimeric intermediates with non-native structure on the kinetic landscape. Partially folded monomers associate with a nearly diffusion-limited rate to form a dimeric ensemble (I_2) characterized by non-native structure. We have previously characterized the I_2 species, and this study examines the structures of the H2A and H2B monomers that facilitate the rapid association to form I_2 , despite the formation of non-native interactions in the monomeric precursors. For comparison, we also examined early folding events in the homodimeric archaeal histones, hMfB and hPyA1, which fold more slowly through fewer transient intermediates. Moving from protein folding studies to macromolecular assembly, we are poised to use our suite of H2A-H2B mutations to characterize the interactions between the histone dimer and histone chaperones such as NAP-1 and their effects on the stability and dynamics of the nucleosome.

Investigating the Structural and Dynamic Features that Support Tiam2 PDZ Domain Binding Specificity

Lisa C. Golden¹, Xu Liu, and Dr. Ernesto J. Fuentes

¹Undergraduate student

Department of Biochemistry, University of Iowa, Iowa City, IA, 52242, USA

T-Cell lymphoma invasion and metastasis proteins, Tiam1/2, are guanine nucleotide exchange factors that activate the Rho GTPase Rac1, and serve an important role in cell adhesion, migration and neuronal growth and development. Both Tiam1 and Tiam2 contain several protein-protein interaction domains, including a PDZ domain. The PDZ domain is responsible for binding the C-terminus of other proteins to build scaffolding and signaling complexes. In previous experiments, four different residues in the Tiam1 PDZ domain were mutated to those found in the Tiam2 PDZ domain, resulting in a switch of binding specificity. Here, we describe the reciprocal experiment, where the same four residues in the Tiam2 PDZ domain were mutated to those in the Tiam1 PDZ domain (M911L, E912K, F915L and V920L). The binding specificity for several ligands of the Tiam2 PDZ quadruple mutant (QM) was determined using fluorescence anisotropy experiments using the C-termini of several ligands (e.g. syndecan1-4, Caspr4, neuexin1 and CADM1). The determined dissociation constants (K_d) showed that Tiam2 QM PDZ specificity was switched to that of the Tiam1 PDZ domain. Surprisingly, the Tiam2 QM PDZ bound syndecan ligands with higher affinity than the wild type Tiam1 PDZ domain. In order to understand the structural and dynamic basis for the Tiam2 QM PDZ domain specificity switch, we have begun structural analysis by NMR. We will present the backbone assignments of Tiam2 QM alone and in complex with syndecan1. Preliminary results indicate that several regions are dynamic to the extent that resonances are broadened beyond detection. To summarize, the quadruple mutant PDZ domain is able to switch the binding specificity of wild type Tiam2. The molecular basis for the increase in syndecan family binding affinity and dynamic properties will be studied through single and double mutant analysis.

Single-primer quadruplex priming amplification (QPA) for DNA diagnostics

David Gvarjaladze, Besik Kankia

Department of Chemistry and Biochemistry, The Ohio State University, Columbus, OH
43210

The polymerase chain reaction (PCR) is one of the main tools of modern molecular biology, which is able to amplify a single or few copies of a DNA across several orders of magnitude. However, PCR is extremely difficult due to many variables including temperature cycling and a complicated quantification mechanism. The overall aim of our lab is to design amplification system free from the current shortcomings of PCR. Here we employ quadruplex priming amplification (QPA) using only one primer

The feasibility of single-primer QPA was demonstrated earlier using a 59-nt stem-loop construct containing a 21-nt recognition sequence in the loop (1). Here we propose a different approach, where the recognition sequence or pathogen complement is placed at the 3'-end. As a result, quadruplex and its complement (PBS) are directly connected to each other. This allows (i) amplification of only quadruplex+PBS (i.e., amplification of an additional loop sequences required in traditional RT-PCR as a probe binding site, is not necessary in QPA since fluorescence comes from the primers); (ii) use of an incomplete set of dNTPs (only dGTP and dCTP if quadruplex with C-loops is used); and (iii) destabilization of the monomolecular hairpin duplex (due to the unfolding of at least two G·C bp to make a loop) to facilitated priming. Thus, the single-primer QPA has the potential to be the simplest amplification system for gene expression (quadruplex+PBS can be incorporated into cDNA during reverse transcription by attaching to mRNA primers) or in diagnostics where the recognition process is separated from amplification. We performed thermodynamic and optical studies of the 22-nt sequence 3'-CCCGCCCGGGCGGGTGGGTGGG-5', which depending on K⁺ concentration forms two different structures: hairpin and/or quadruplex. A combination of UV, CD and fluorescence techniques was employed to investigate the structure of the construct. In addition, QPA using 14-nt primer (GGGTGGG(3MI)GGGCGG) was performed. Currently we are able to amplify 100fM concentration of templates isothermally in less than 50 min.

Supported by Bill & Melinda Gates Foundation.

References:

1. Kankia, B.I. (2011) Self-dissociative primers for nucleic acid amplification and detection based on DNA quadruplexes with intrinsic fluorescence. *Anal Biochem*, **409**, 59-65.
2. Bordelon, H., Adams, N.M., Klemm, A.S., Russ, P.K., Williams, J.V., Talbot, H.K., Wright, D.W. and Haselton, F.R. (2011) Development of a low-resource RNA extraction cassette based on surface tension valves. *ACS Appl Mater Interfaces*, **3**, 2161-2168.
3. Burbulis, I., Yamaguchi, K., Yu, R., Resnekov, O. and Brent, R. (2007) Quantifying small numbers of antibodies with a 'near-universal' protein-DNA chimera. *Nat Methods*, **4**, 1011-1013.

Enhanced peptide flexibility leads to diminished antigenicity and the failure of a cancer vaccine candidate

Lance M. Hellman¹, Andrew D. McKernan¹, & Brian M. Baker^{1,2}

¹Department of Chemistry & Biochemistry, University of Notre Dame

²Harper Cancer Research Center, University of Notre Dame

Abstract

Peptide-based cancer vaccines are commonly modified at the peptide primary anchor positions to enhance peptide binding to the major histocompatibility complex (MHC) proteins. However, enhanced peptide binding to the MHC does not always translate to improved recognition by T-cell receptors (TCR). Anchor modification of the MART1₂₇₋₃₅ melanoma antigen AAGIGILTV (AAG), to ALGIGILTV (ALG) significantly impairs binding affinity of the DMF5 TCR, with reported K_D 's of 70 μ M and ≥ 300 μ M, respectively (1). However, recognition of ALG is recovered by a high-affinity variant of DMF5, α D26W/ β L98W (DMF5_{WW}), with a K_D of 16 μ M. Previous studies have shown that both AAG and ALG both adopt a flipped (extended) conformation when bound in the MHC. However, ALG also samples a “non-flipped” conformation (1,2). It has been postulated that the structural heterogeneity in the ALG peptide reduces TCR binding affinity through entropic means. To investigate, we examined the thermodynamics of DMF5_{WW} binding to AAG/HLA-A2 and ALG/HLA-A2 by van't Hoff analysis. Consistent with our earlier hypothesis, our data indicates that the loss in binding affinity towards ALG is entropic in nature, with a $T\Delta\Delta S^\circ$ of nearly -9 kcal/mol. Surprisingly, this is compensated by a favorable $\Delta\Delta H^\circ$ of -6 kcal/mol. These results confirm that anchor modification of the AAG peptide introduces greater structural heterogeneity into the peptide, resulting in weaker receptor binding and diminished antigenicity. These findings have implications for the design and optimization of peptide-based cancer vaccines.

- 1) Insaiddoo FK, Borbuleyevch OY, Hossain M, Santhanagopalan SM, Baxter TK, Baker BM. 2011. *J. Biol. Chem.* 286: 40163-73
- 2) Silz P, Michielin O, Cerottini J-C, Luescher I, Romero P, Karpus M, Wiley DC. 2001. *J. Immunol.* 167: 3276-84
- 3) Sturtevant JM. 1977. *Proc. Natl. Acad. Sci. USA* 74: 2236-40
- 4) Pabhu NV, Sharp KA. 2005. *Annu. Rev. Phys. Chem.* 56: 521-48

Thermodynamics of Fe(II) Binding in Dioxygenase Enzymes

Kate L Henderson, Joseph P Emerson

Department of Chemistry, Mississippi State University

Many non-heme iron (II) dioxygenases contain a similar metal binding motif at the enzyme active site center. This facial triad motif can contain two histidine residues (His99 and His255) and a carboxylate residue (Asp101) as observed in Taurine Dioxygenase (TauD); or three histidine residues (His62, His64 and His104) as seen in β -Diketone-Cleaving Dioxygenase (Dke1). Both facial triad motifs afford an octahedral geometry of the iron (II) center, where binding of substrate and incorporation of dioxygen occurs. In this study, the thermodynamics of metal coordination to these dioxygenase enzymes is explored using isothermal titration calorimetry. Here, iron (II) is incorporated into the active site and changes in enthalpy and entropy for the system are observed. Comparisons are made between the two dioxygenases, and the thermodynamic parameters that drive metal binding are discussed.

Synaptotagmin linker: tuning of cooperativity in Ca^{2+} binding

Troy A. Hendrickson¹, Jacob W. Gauer¹, Komemba J. Lohese¹, Michael E. Fealey¹, R. Bryan Sutton², and Anne Hinderliter¹

¹Department of Chemistry and Biochemistry, University of Minnesota Duluth, Duluth, Minnesota, United States of America. ²Department of Cell Physiology, Texas Tech University Health Sciences Center, Lubbock, Texas, United States of America.

Synaptotagmin I (Syt I) contain two Ca^{2+} binding domains that function in the regulated exocytosis of neurotransmitters. Syt I participates in the sensing of the calcium influx in nerve cells during signal propagation. The two C2 domains are linked to each other with C2A, the domain closest to the vesicle, having a linker to connect with a vesicle. The C2A domain was studied along with the portion of the linker between the vesicle and C2A. We propose that the lower free energy of stability found in the linker-associated domain, as compared to the domain without the linker, allows C2A more conformations for a finer tuning of its Ca^{2+} binding. Although the linker is not in close proximity to the Ca^{2+} binding loops, it is still able to affect the cooperativity of the Ca^{2+} binding sites. For experimental purposes the linker was broken into three parts: residues 141-268 (C2A Short), residues 96-265 (C2A Long), and the 83-265 (C2A Long Long). The inclusion of the extra piece of the linker going from C2A Short to C2A Long causes a lower free energy of stability. The free energy of the C2A Long construct was determined by globally fitting the construct to a two-state model of unfolding. A low free energy of stability suggests the protein structure is more flexible and more responsive to changes in its environment. The longer construct thus allows for a wider range of responsiveness by Syt I to changes in Ca^{2+} concentrations within its environment. The lower stability associated with the C2A Long affects the cooperativity of the two Ca^{2+} binding sites in C2A. The impact of Syt I construct on Ca^{2+} binding cooperativity was defined using isothermal titration calorimetry. To test the stability of Syt I, a combination of differential scanning calorimetry and fluorescence lifetime spectroscopy were used to study that free energy of stability. From here, the next experimental step will be to include the full tether and vesicle to determine their effect on the stability of Syt I. We predict that the inclusion of the vesicle will lead to a decrease in Syt I stability allowing accessibility to a wider variety of cooperativity and conformers in calcium response. This research will provide a deeper understanding of the neuronal response to stimuli by providing more information on how a nerve cell responds to a calcium influx.

Characterizing the Binding Affinity of the Tiam1-Talin Complex

Frances C. Hindt¹, Zhen Xu, and Ernesto J. Fuentes

¹Undergraduate student

Department of Biochemistry, University of Iowa, Iowa City, IA, 52242, USA

The T-cell lymphoma invasion and metastasis gene 1 (Tiam1) is a guanine nucleotide exchange factor (GEF) that regulates the Rho-family guanine triphosphatase (GTPase) Rac1. Tiam1 is important for several essential cellular processes, such as cell migration, adhesion, and polarity. Tiam1 has been identified to interact with the cytoskeletal protein Talin. The Tiam1-Talin interaction has been shown to be essential in Rac1 activation and migration of cells, as well as involved in regulating focal adhesion turnover.

The N-terminal FERM domain of Talin contains three subdomains – F1, F2, and F3 – and mediates the Tiam1-Talin interaction. In order to explore the dynamic and structural properties of the Talin-Tiam1 interaction, HIS- and GST-tagged vectors containing Talin F1F2F3, F2F3, and F3 subdomains were produced. The GST-tagged constructs of the Talin FERM subdomains were purified and used in pull-down assays with the Tiam1 PH-PH domain fragment. The results of multiple pull-down assays showed that the Tiam1 PH-PH fragment bound to Talin F2F3 and F3 of the FERM domain, confirming the Tiam1-Talin interaction *in vitro*. Ongoing ITC experiments are being used to quantify the binding affinity and determine the thermodynamic properties of the Tiam1-Talin interaction. These methods will ultimately be used to identify the minimal fragment of Tiam1 that binds Talin, which will be used to guide future structural and cell biological studies.

Thermodynamic studies of full length amyloidogenic light chains

Timothy J. Horn^{1,3}, Luis Blancas-Mejia¹, Winter A. Okoth¹, Matthew Auton^{1,2}, Alexander Tischer^{1,2}, Marina Ramirez-Alvarado¹.

¹Department of Biochemistry and Molecular Biology, ²Division of Hematology, Mayo Clinic, Rochester, MN 55905 ³University of Minnesota Rochester.
ramirezalvarado.marina@mayo.edu

Light chain (AL) amyloidosis is a fatal disease where monoclonal immunoglobulin light chains (LC) deposit as insoluble amyloid fibrils. The process of deposition causes tissue degeneration, organ failure, and death. An immunoglobulin LC consists of one variable and one constant domain. The variable region presents somatic mutations and as a result, each AL amyloidosis patient has a unique monoclonal protein sequence expressed and deposited in vital organs. Amyloidogenic light chain AL-09 is a patient derived protein that belongs to the kl O18/O8 (IGKV 1-33) germline sequence. In previous studies, the variable domain of AL-09 has been identified as an unstable protein, and fast fibril former, when compared to the germline variable domain [1] [2].

Our laboratory and others have demonstrated a direct correlation between the protein thermodynamic stability and the protein's propensity to form amyloid fibrils [3]. For many years, AL amyloidosis researchers have conducted their biochemical and biophysical studies with the variable domain based on a report that stated that amyloid deposits from AL amyloidosis patients were formed primarily by the variable domain and small regions of the constant domain [4]. However, in 2008, proteomic studies conducted with amyloid deposits from fat aspirates [5] found full length (FL) light chains as part of the amyloid deposits. This was later confirmed by a proteomics study using biopsy samples from affected tissues [6]. The function of the constant domain in the pathophysiology of AL amyloidosis is not well established. Only recently, the role of constant domain in thermal stability and aggregation has been characterized for a lambda-6a (IGLV 6-57) full length AL protein and its corresponding variable and constant domain proteins [7]. We are currently performing thermodynamic and structural studies of FL amyloidogenic light chains to compare them to the variable domain studies conducted previously. This will allow us to determine the role that the constant domain plays in the protein stability and ability to form amyloid fibrils.

The thermal unfolding reaction of AL-09 FL is irreversible, precluding us from calculating thermodynamic parameters as has been reported before. AL-09 has characteristic scan rate dependence during thermal denaturation. Circular dichroism measurements of scan rates can be used to calculate thermodynamic parameters [8].

1. Baden, E.M., et al., J. Biol. Chem., 2008. **283**(23): p. 15853-60.

2. Martin, D.J. and M. Ramirez-Alvarado, Amyloid, 2010. **17**(3-4): p. 129-36.

3. Blancas-Mejia, L.M. and M. Ramirez-Alvarado, Annu. Rev. Biochem., 2013. **82**(1): p. 745-774.

4. Olsen, K.E., K. Sletten, and P. Westermark, Biochem. Biophys. Res. Commun., 1998. **251**(2): p. 642-7.

5. Lavatelli, F., et al., Molecular & cellular proteomics : MCP, 2008. **7**(8): p. 1570-83.

6. Vrana, J.A., et al., Blood, 2009. **114**(24): p. 4957-9.

7. Klimentchuk, E.S., et al., Biochemistry, 2010. **49**(45): p. 9848-9857.

8. Tischer, A., M.A. Cruz, and M. Auton, Protein Sci., 2013. **22**(8): p. 1049-1059.

mRNA splicing alters the function of Ubx, a Hox transcription factor

Hao-Ching Hsiao¹, Kelly Churion¹, Kathleen S. Matthews², Sarah E. Bondos¹

1. Department of Molecular and Cellular Medicine, Texas A&M University System
Health Science Center, College Station, TX77840

2. Department of Biochemistry and Cell Biology, Rice University, Houston, TX77005

In bilaterally symmetric animals, Hox transcription factors differentiate body segments by driving the development of different organs and appendages, and regionalizing systems, such as skin, gut, musculature, and nervous system, that span the length of the animal. Hox proteins, which are hundreds of amino acids long, all contain a conserved 60 amino acid DNA binding domain termed the homeodomain. The homeodomain includes three α helices connected by loops and a structurally disordered N-terminal arm. Hox homeodomains have high DNA-binding affinity (~ 60 pM) but poor specificity. There are several mechanisms which may improve DNA specificity *in vivo*: DNA binding site sequence variation, cooperative protein-protein interactions, and influence from non-homeodomain regions. Our lab recently identified a region outside the homeodomain of the *Drosophila melanogaster* Hox protein Ultrabithorax (Ubx) that appears to directly bind the homeodomain to alter both binding affinity and specificity. This region includes 3 alternatively spliced microexons. During *Drosophila* development, alternative *ubx* mRNA splicing creates different Ubx isoforms, in a stage- and tissue-specific manner. Therefore, alternative splicing has the potential to regulate Ubx function in a tissue-specific manner. We investigated the role of alternative splicing in altering protein interactions, Ubx stability, and DNA binding. Our preliminary data suggests that alternative splicing alters DNA binding *in vivo* by i) by changing the distance between the autoinhibitory YPWM motif and homeodomain, ii) altering Ubx stability, iii) altering the dynamics of interactions between the N-terminal arm of the homeodomain and the minor groove of DNA, and iv) altering interactions of transcription factors that form higher order complexes with Ubx and DNA.

Cisplatin Adduct Effects on DNA-DNA Interactions

James Hutchison, Jason DeRouchey

Department of Chemistry, University of Kentucky, Lexington KY 40506

Small drug molecules that bind directly to DNA are known to influence vital cell functions such as transcription and DNA replication. Cisplatin is a well-studied anti-tumor drug which has been used extensively to fight various forms of cancer. The high rate of cell apoptosis incurred has been attributed to the 1,2 intrastrand adducts formed between DNA and cisplatin. Currently, little is known about how cisplatin interacts with highly packaged DNA that is typically found within cells. We use osmotic stress coupled with x-ray scattering to study fundamental molecular scale interactions in DNA arrays. Inductively coupled plasma atomic emission spectroscopy (ICP-AES) was used to monitor unreacted cisplatin concentrations. DNA-DNA forces were measured as a function of cisplatin concentration. Large disruptions of the DNA-DNA spacing are observed with increasing cisplatin. These studies begin to shed light on how cisplatin interacts with condensed DNA.

Probing the dynamics of nascent GFP chains on the ribosome

Giselle Jacobson¹, Srabasti Acharya², Basir Ahmad², Yujie Chen², Krastyu Ugrinov¹, Lisa Lapidus² & Patricia L. Clark¹

¹Department of Chemistry and Biochemistry,
University of Notre Dame, Notre Dame, IN 46556

²Department of Physics and Astronomy
Michigan State University, East Lansing, MI 48824

Proteins can begin to fold co-translationally as they are synthesized by the ribosome, but little is known about how proximity to the ribosome surface might alter nascent chain motions versus corresponding free polypeptide chains. Altered nascent chain mobility could significantly affect nascent chain folding kinetics and/or mechanisms. We seek to investigate how polypeptide chain dynamics change as nascent chains elongate and move away from the ribosome surface. The sequence of GFP was modified to contain only a single probe and quenching moiety. Stalled ribosomes bearing nascent chains of different lengths were prepared, representing a series of translation 'snapshots' with which to measure the intramolecular diffusion of the probes as the GFP nascent chain elongates. The decay rate of the tryptophan triplet state decreases as the nascent chain increases in length, indicating proximity to the ribosome surface affects the diffusion rate of the nascent chain.

Dissection of molecular driving forces of affinity maturation

Sriram Jakkaraju, Robert James Hoey IV and James R. Horn

Department of Chemistry and Biochemistry, Northern Illinois University, 60015, Dekalb,
IL

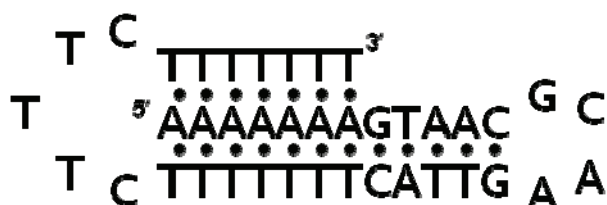
Antibody fragments are exciting alternatives to conventional antibodies for both therapeutic and diagnostic applications, Protein engineering methods such as library based techniques yielded affinity matured antibodies. Improved selection outcome require better understanding of biophysical properties. But, very less is known about thermodynamic origins of enhanced affinity. Currently we are trying to understand thermodynamic origins of ~100 fold enhanced affinity of an in vitro affinity matured anti-RNase A VHH Antibody (VHH-mat) compared to in vivo generated VHH-wt. $\Delta\Delta\Delta G$ value for nine interface residues were calculated from alanine scanning mutagenesis, four of nine residues were critical for VHH-mat's increased in affinity. Binding and stability studies were conducted on Individual, double, triple, CDR1 only, CDR 3 only variants generated from changing wild type residues with VHH-mat residues. Interesting trends were observed, positive & negative cooperativity was observed between CDR3 loop residues Y101D/T107R & E102A/R104V respectively. In addition additive affects were observed in between CDR1 & 3 loops. Thermal stability studies were also conducted using circular dichroism, addition of charged residues resulted in T_M destabilization & stabilization after addition. These insights might help in ultimate goal of developing affinity matured antibodies.

Targeting of a DNA Complex with Joined Triplex-Duplex Motifs

Sarah E. Johnson, Hui-Ting Lee and Luis A. Marky

Department of Pharmaceutical Sciences, University of Nebraska Medical Center,
986025 Nebraska Medical Center, Omaha, NE, 68198-6025

Our laboratory is interested in developing methods that can be used for the control of gene expression. To this end, we are investigating the reaction of intramolecular DNA complexes with partially complementary strands. We used a combination of isothermal titration (ITC), differential scanning calorimetry (DSC) and temperature-dependent UV spectroscopy to determine complete thermodynamic profiles for these targeting reactions. Specifically, we have targeted the complex shown below



with the following single strands: A₇GA₃GA₇ and A₇GA₃GA₇GTA₂CT₂GCG, which are partially complementary to the complex, starting from the 3' end. Both reactions investigated by ITC yielded exothermic heats of -66.3 kcal/mol and -82.8 kcal/mol. However, by investigating the unfolding of reactants and products of each reaction and establishing Hess cycles yielded reaction enthalpies of -69.3 kcal/mol to -81.9 kcal/mol. These favorable heat contributions result from the formation of base-pair stacks involving the unpaired bases of the loops, indicating that each complementary strand was able to invade and disrupt the secondary structure. This research is supported by National Science Foundation Grant MCB-1122029.

Transient partial unfolding in *E. coli* DHFR

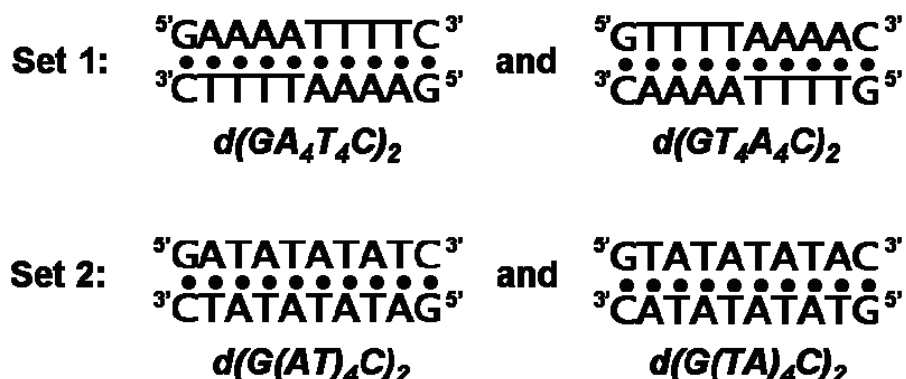
Joseph R. Kasper and Chiwook Park

Department of Medicinal Chemistry and Molecular Pharmacology
Purdue University, West Lafayette, IN 47907

A protein's conformational energy landscape underlies many functional processes including catalysis, binding, allostery, and folding. In agreement with this view, both experiment and computation provide evidence that a protein explores a range of conformations under native conditions, albeit in very small numbers. These less-populated conformations have proven to be functionally relevant. The hierarchy of structure formation during the folding process parallels the hierarchy of partially unfolded forms present under native conditions. We have used native-state proteolysis and protein engineering to resolve the relationship between the folding process and a partially unfolded form of *E. coli* dihydrofolate reductase (DHFR). The stability of the partially unfolded form has a clear dependence on urea, with an m -value of 0.5 kcal/(mol·M) compared to 2.4 kcal/(mol·M) for global unfolding. From this comparison, we estimate that 20% of the protein's buried surface is exposed upon partial unfolding. We introduced a series of destabilizing mutations to find which elements of structure are unfolded in this partially unfolded form. The region of unfolding appears to encompass the loops of the discontinuous loop domain as well as the residues of the central β -sheet contacting those loops. This pattern is in agreement with and further refines the structure of a late kinetic folding intermediate. We also investigated the effect of NADP⁺ binding on partial unfolding of DHFR to make connections between different regions of the conformational energy landscape, because binding of ligands to DHFR is accompanied by conformational change of the native protein and altered protein dynamics. When bound by NADP⁺, DHFR can partially unfold without releasing the ligand, revealing a partially unfolded form distinct from the partially unfolded form of apo protein. Using the same destabilizing mutations to probe the pattern of unfolding, we discovered that partial unfolding of the ligand-bound protein occurs within a region overlapping with the unfolded region of the partially unfolded form of apo protein.

Irine Khutsishvili and Luis A. Marky

Considerable attention has been given to the description of sequence-dependent curvature of nucleic acids and protein-nucleic acid complexes. We used a combination of spectroscopic, calorimetric, and density techniques to determine complete thermodynamic profiles, including hydration effects, for the unfolding (or folding) of two sets of two self-complementary DNA duplexes:



UV melting curves of each duplex show monophasic transitions with transition temperatures, T_M s, dependent on strand concentration. This confirms their bimolecular formation. Thermodynamic profiles obtained from differential scanning calorimetry experiments indicate that the favorable folding of each duplex results from the typical compensation of favorable enthalpy and unfavorable entropy contributions, uptake of counterions and negative heat capacity effects. Pressure Perturbation Calorimetric curves yielded positive folding volumes, which corresponded to releases of water molecules.

In the first set of duplexes, $d(GA_4T_4C)_2$ is more stable than $d(GT_4A_4C)_2$, by a ΔG° of -2.7 kcal/mol, due to a more favorable heat contribution of -15.3 kcal/mol. Its folding volume is lower, by 14 cm³/mol, while the differential heat capacity change and ion binding are similar. On the other hand, we obtained similar thermodynamic profiles for the duplexes of the second set. Therefore, the thermodynamic differences of the bent $d(GA_4T_4C)_2$ duplex are due to the proper phasing of its A₄-tracts rather than to differences in the central base-pair stack. Supported by Grant MCB-1122029 from the NSF.

Calcium-Mediated Regulation of Calcineurin by a Dynamic Duo of EF-Hand Proteins

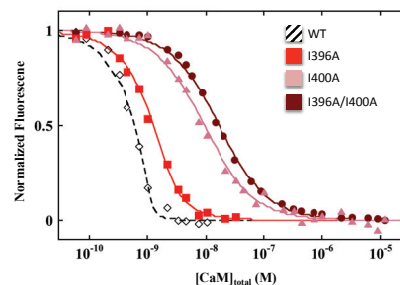
Sean A. Klein, Susan O'Donnell, Brett C. Waite, Jesse B. Yoder, and Madeline A. Shea
Department of Biochemistry, Carver College of Medicine, University of Iowa
Iowa City, IA 52242-1109

Calcium-mediated regulation of early stages of heart development critically depends on calcineurin (CaN), a heterodimeric, calcium-activated phosphatase. CaN dephosphorylates NF-AT transcription factors, allowing them to enter the nucleus and launch a transcription program that controls cardiac muscle growth. Although CaN activity is required for other pathways in development, embryonic heart development is the earliest and most sensitive responder to changes in CaN activity. This pathway also regulates commitment of cells to repair the adult heart. However, CaN is inhibited by immunosuppressant drugs (e.g., cyclosporine and FK506) that are commonly used to disrupt regulation of the immune system to mitigate host-graft rejection. Thus, these drugs cause unwanted side effects, especially during pregnancy. The primary vulnerability of the developing heart to fluctuations in CaN activity propels our research program to understand calcium-dependent regulation of CaN. Calcium partially activates CaN by binding to CaN_B, its intrinsic regulatory subunit. Greater activity is achieved by the action of calmodulin (CaM), a ubiquitous human calcium receptor that recognizes a canonical CaM-binding domain (CaMBD) in a regulatory sequence between the binding site for CaN_B and auto-inhibitory domain that occludes the active site.

To probe the sequential nature of CaN activation by CaN_B and CaM, we determined that CaM binding to CaN_A causes a thousand-fold increase in calcium-binding affinity of the N- and C-domains of CaM while preserving their 10-fold difference (calcium binds to the C-domain preferentially). To explore this linkage, we studied interactions between site-knockout mutants of CaM and CaN_B with CaN, and mutagenized the CaMBD, a 1-5-8-14 BAA-motif that has picomolar affinity for (Ca²⁺)₄-CaM. Affinities of CaM for the CaMBD were measured using a biosensor protein composed of the target sequence embedded between a pair of auto-fluorescent proteins (donor/CFP, acceptor/YFP) that undergo FRET which is disrupted by CaM binding in proportion to occupancy, allowing estimates of equilibrium binding constants. Knockout mutants of Ca²⁺-binding sites in CaM showed that perturbations of sites II and IV have larger effects on the CaN-CaM interaction than do changes in sites I and III.

Mutations in the CaMBD indicate that the energetic contributions of residues at the 1-5-8-14 positions are dramatically different; alanine substitution of the Ile at position 5 was more detrimental to binding (by ~ 3 kcal/mol) than the same substitution at positions 1 or 8. Further, alanine substitution of an aromatic residue (F410) hypothesized by some laboratories to be an “anchor residue” of the CaMBD has almost no effect on binding of WT CaM. Conventional motif classifications for CaM targets are not sufficient to indicate energetic roles at interfaces. In the current study, we are pursuing connections between these protein-protein binding affinities and the effects of immunosuppressant drugs on enzymatic activity of CaN to understand how effects on transplant patients may lead to improper development of the cardiac muscle.

Supported by AHA Grants to SEO and MAS.



Lipid and the Thermodynamics of Amyloid beta Fibril Formation

Alexandra Klinger and Paul Axelsen

Pharmacology, University of Pennsylvania, Philadelphia, PA

The amyloid hypothesis states that the pathology of Alzheimer's disease (AD) is driven by an accumulation of abnormally folded amyloid beta protein ($A\beta$). $A\beta$ is a 39 to 43 amino acid proteolytic byproduct of the transmembrane protein, amyloid precursor protein (APP). In AD, these normally soluble $A\beta$ peptides misfold to aggregate into fibrils with a dramatic conformational change. These fibril then deposit outside the neurons to form the amyloid plaque, which are characteristically found in the brains of AD patients. Thermodynamic and kinetic studies of fibril formation and stability in vitro cannot explain how or why fibrils form in vivo. $A\beta$ concentrations in cerebral spinal fluid are several orders of magnitude lower than the micromolar concentrations of monomeric $A\beta_{40}$ that have been reported by several groups to be in equilibrium with fibrillar $A\beta_{40}$. One possible explanation for in vivo aggregation is that chemical modification of the protein by lipid oxidation products and/or metal complex formation allows the formation of "seeds" that nucleate fibrillization. Herein, we present the effects of $A\beta$ modification by the peroxidative lipid products 4-hydroxy-2-nonenal (HNE) and 4-hydroxy-2-hexenal (HHE). Both HNE and HHE bind to the three His residues of $A\beta$ proteins and rapidly accelerate fibril formation. Also, we discuss the effects of such lipid modification on monomer Cu^{2+} binding and resulting fibril topology.

The Effects of *E. coli* SSB Tail Mutations and Deletions on ssDNA Binding Properties and Binding Modes Transitions

Alexander G. Kozlov*, Elizabeth A. Weiland and Timothy M. Lohman

Department of Biochemistry and Molecular Biophysics
Washington University School of Medicine, 660 S. Euclid Ave.
St. Louis, MO 63110

ABSTRACT

E. coli single strand DNA binding protein (SSB) is one of the key proteins in DNA replication, recombination and repair. SSB functions as a homotetramer with each subunit (177 amino acids) possessing two domains: a DNA binding domain containing an oligonucleotide/oligosaccharide binding (OB)-fold (residues 1-112) and an intrinsically disordered C-terminal tail (65 residues), of which the last nine amino acids provide the site for interaction with more than a dozen other metabolic proteins. Upon ssDNA binding an SSB tetramer can form different binding modes with varying number of subunits involved in DNA interaction and distinct cooperativities. It has been hypothesized that the different modes are involved in different metabolic activities providing versatility of SSB functioning. Here we examine SSB constructs possessing different C-terminal tail mutations and deletions (excluding the very end 9 amino acids, responsible for protein-protein binding) in order to understand its role in DNA binding. Using complementation assays *in vivo* we find that *E.coli* expressing SSB with various C-terminus deletions survive, although the replacement of amino acids following SSB core can be detrimental. We find that these observations correlate with the changes in DNA binding properties and binding mode transitions and discuss it in context of possible biological function.

Single molecule force spectroscopy analysis of alpha-synuclein misfolding.

Alexey V. Krasnoslobodtsev¹, Ivan L. Volkov¹, Jean-Christophe Rochet² and Yuri L. Lyubchenko¹.

² Department of Pharmaceutical Sciences, University of Nebraska Medical Center, 986025 Nebraska Medical Center, Omaha, NE 68198, United States

¹ Department of Medicinal Chemistry and Molecular Pharmacology, Purdue University, West Lafayette, IN 47907-2091, United States

Protein misfolding is a transient state during self-assembly into aggregates defining molecular mechanism of the development of neurodegenerative diseases. Misfolding and subsequent aggregation of alpha-synuclein (α -Syn) protein is critically involved in the development of Parkinson's disease (PD). Three familial single point mutations, A30P, E46K, and A53T, correlate with early-onset PD; however the molecular mechanism of the effects of these mutations on the structural properties of α -Syn and its propensity to misfold remains unclear. We address this issue utilizing a single molecule AFM force spectroscopy approach to probe transient misfolded states of α -Syn measuring pair-wise interactions between individual α -Syn molecules at conditions that induce conformational transitions associated with enhanced aggregation. Using SMFS we detected and characterized misfolded dimers of α -Syn and structural details of the dimers formed by all four variants of α -Syn. Our results demonstrate that more than one segment within the protein molecule is responsible for the initial association of α -Syn into dimers and potentially into higher-order oligomers and fibrils. The interactions are not limited to the central non-amyloid-beta component (NAC) of the protein, but rather expand beyond this segment. This finding suggests that even the first step of α -Syn self-assembly (dimerization) possesses a certain degree of heterogeneity. We hypothesize that these different misfolded conformations can lead to different types of oligomers and define the aggregation pathway. All three mutations of α -Syn alter the protein's folding and interaction patterns affecting interactions far beyond their immediate locations. The marked differences in the misfolding patterns between WT α -Syn and single point mutants might be responsible for the higher propensity of the mutants to aggregate and cause early-onset PD.

Acknowledgements: The work was supported by grants to YLL from National Institutes of Health (1P01GM091743-01A1 and 1 R01 GM096039-01A1), U.S. Department of Energy Grant DE-FG02-08ER64579, National Science Foundation (EPS – 1004094) and the Nebraska Research Initiative.

Domain interactions in VA I RNA mediate high-affinity PKR binding

Katherine D. Launer-Felty and James L. Cole

Department of Molecular and Cell Biology, University of Connecticut, Storrs, CT

Adenovirus virus-associated RNA I (VA I) is a short, non-coding transcript whose major function is to inhibit the activity of PKR, a component of the innate immunity antiviral pathway. PKR recognizes duplex RNA regions to undergo dimerization and activation by autophosphorylation. VA I contains three domains: an apical stem-loop, a highly-structured central domain, and a terminal stem. Previous analysis localized PKR binding to the apical stem and central domain. However, the molecular mechanism for inhibition of PKR is not known. We have characterized the stoichiometry and affinity of PKR binding to VA I and several domain constructs using analytical ultracentrifugation. In the presence of divalent ion, PKR binds to VA I with a 1:1 stoichiometry and a surprisingly high affinity ($K_d = 334$ nM). Although divalent ion does not affect the secondary and tertiary structure of VA I, it strongly modulates PKR binding. PKR is capable of binding the isolated terminal stem, but deletion of this domain from VA I does not affect PKR binding affinity or inhibition of autophosphorylation. Consistent with this observation, chemical probing data show that the structures of the apical stem and central domain are preserved upon removal of the terminal stem. PKR binds weakly to the isolated apical stem and does not bind to the isolated central domain. These results indicate that interactions between the apical stem and the central domain are necessary to form a high affinity PKR binding site. Our data support a model whereby VA I functions as a PKR inhibitor because it binds a monomer tightly but does not permit PKR dimerization.

Allostery mediated by the intrinsic disordered domain governs the function of glucocorticoid receptor translational isoforms

Jing Li¹, James Sowers², E. Brad Thompson³ and Vincent J Hilser¹

1.Department of Biology and T.C. Jenkins Department of Biophysics, Johns Hopkins University, Baltimore, MD 21218; 2.Department of Chemistry, Johns Hopkins University, Baltimore, MD 21218; 3.Department of Biochemistry and Molecular Biology, University of Texas Medical Branch, Galveston, Texas 77555

Intrinsically disordered (ID) proteins have challenged the dominant view of protein structure-function relationships. Allosteric coupling often governs the function of proteins containing ID regions, for example, in steroid hormone receptors (SHRs). However, little is known about the role of intrinsic disorder in mediating allosteric coupling and how this coupling regulates the function of the ID region. Recently, our group has developed a theoretical model demonstrating that site-to-site allosteric coupling is maximized when intrinsic disorder is present in the domains or segments containing one or both of the coupled binding sites. In this study, through thermodynamic characterization and luciferase reporter assay, we found that the ID GR NTD is composed of two functionally distinct regions coupled with each other, and both of these regions are coupled to the DNA binding domain. GR uses competing energetic couplings, which can be modulated through production of different isoforms, to provide a broad spectrum of translation efficiencies and responses to environmental cues. Moreover, figuring out the underlying molecular basis for the coupling raises the potential for development of allosteric drugs, treating diseases caused by the malfunction in SHRs.

Molecular dynamics simulations of 441 two-residue peptides in aqueous solution: effects of sidechain interactions on backbone conformations

Shuxiang Li, Casey T. Andrews, Mark Miller, Stephen Siemonsma, Timothy

Collingsworth, Isaac Rockafellow, Anh Nguyet, Brady Campbell, Reid Brown,

Chengxuan Guo, Michael Schrodtt, Yu-Tsan Liu and Adrian H. Elcock

Department of Biochemistry, University of Iowa, Iowa City, Iowa 52242, United States

The conformational preferences of amino acids are well known to have consequences for protein structure. One way to obtain a detailed structural view of local interactions is to perform all-atom molecular dynamics (MD) simulations of short peptides in explicit solvent. To this end, we report here the results of 441 long MD simulations of all possible two-residue peptides that contain the 20 standard amino acids with histidine modeled in both its neutral and protonated states. The 882 Ramachandran maps that result show clear influences from sidechain interactions and provide a comprehensive view of the effects of such interactions on the backbone preferences of two-residue peptides.

Thermodynamic and hydrodynamic examination of ClpB assembly

Jia Bei Lin and Aaron L. Lucius

University of Alabama at Birmingham, Department of Chemistry, 1530 3rd Ave S,
Birmingham, AL 35294-1240

E. coli ClpB is a heat shock protein that belongs to the AAA+ protein family. Studies have shown that ClpB and its eukaryotic homologue, Hsp104, can disaggregate denatured proteins by themselves or cooperate with the DnaK chaperone system *in vivo*. It is thought that ClpB requires binding of nucleoside triphosphate to assemble into hexameric rings with protein binding activity and hexamer is the only conformation of ClpB in the presence of nucleotides. In contrast to this conclusion, our sedimentation velocity data show that decreasing NaCl concentration in solution can lead to ClpB hexamer formation in the absence of nucleotide. Further, adding nucleoside triphosphate to ClpB does not always drive ClpB assembly. In addition, information on the kinetics of ClpB oligomer dissociation can be obtained from sedimentation velocity experiments. Our data indicate that ClpB hexamers reside in fast subunit exchange in the absence of nucleoside triphosphate, while the exchange rates decrease in the presence of a large excess of ATPγS (a slowly hydrolysable ATP analog). We anticipate our studies on ClpB assembly to be a starting point for understanding ClpB-assembly linked protein-aggregate binding and disaggregation.

Structural and Dynamic Features Underlie the Switch of Ligand Binding Specificity in a Tiam1 PDZ Domain Mutant

Xu Liu¹, David C. Speckhard², Tyson R. Shepherd¹ and Ernesto J. Fuentes¹

¹Department of Biochemistry, University of Iowa, Iowa City, IA, 52242, USA.

²Department of Chemistry, Loras College, Dubuque, IA, 52001, USA.

The T-cell lymphoma invasion and metastasis (Tiam) family of proteins are guanine exchange factors (GEFs) for the Rho-family GTPase Rac1 crucial for cell-cell adhesion and cell migration. Deregulation of Tiam1/Rac1 signaling leads to various malignancies, including cardiovascular disease and cancer. Tiam proteins contain several protein–protein interaction domains, in particular a PDZ domain. Previously we found that the Tiam1 and Tiam2 PDZ domains had distinct binding specificities. Intriguingly, four residues in the ligand binding pocket were not conserved between the Tiam1 and Tiam2 PDZ domains. To test their importance in specificity, we engineered a quadruple mutant of the Tiam1 PDZ domain (PDZ-QM), where four residues in the Tiam1 PDZ domain were substituted for those in Tiam2. Remarkably, the Tiam1 PDZ-QM binding preference was changed to that of Tiam2. Here, we used equilibrium binding experiments and structural analyses to investigate the origins for this altered specificity. Ligand-free and -bound PDZ-QM crystal structures showed that enlarged P₀ and P₋₂ ligand binding pockets and a favorable electrostatic interaction at the P₋₄ sub-pocket were critical for the changed specificity. Biochemical studies indicated that Tiam1 PDZ-QM was less thermally stable than the WT, while NMR studies showed that a set of residues explored multiple conformations. Backbone (¹⁵N) and side chain methyl (¹³C) NMR relaxation studies confirmed the dynamic features of the ligand free Tiam1 PDZ-QM domain. In the presence of ligand, however, PDZ-QM dynamics were dampened. These studies provide novel insights into the structural and dynamic basis for Tiam1 and Tiam2 PDZ domain specificity.

Isothermal generation of DNA clones for next-generation sequencing

Levan Lomidze, Besik Kankia

Department of Chemistry and Biochemistry, The Ohio State University, Columbus, OH 43210

Next-generation sequencing (NGS) is massively parallel and requires DNA clusters immobilized to a solid support. After breaking genomic DNA into smaller sizes, ligating universal adapters, and separating DNA into single strands, molecules are ready for immobilization and amplification. The two most common methods in use today are emulsion PCR (emPCR) and solid-phase bridge PCR (bPCR). Both methods use thermo-cycling PCR and solution-cycling PCR, respectively, which have very low efficiency due to early PCR plateaus caused by the monomolecular nature of interactions at a solid support. Recently a new “wildfire” method (5500xl W, Life Technologies) was developed, which uses immobilized and free primers. After initial template hybridization to immobilized AT-rich primers and replication at 37 °C, amplification is continued at 60 °C, where terminal AT-segments start “breathing” or unfolding. As a result the template is able to “walk” to new primers, however the efficiency of the assay is very low (less than 80,000-fold amplification in 1 h) due to absence of a driving force. In addition, unfolding of other (mobile) end has disadvantages such as strand diffusion, which can contaminate clones or initiate unwanted new clones. Our laboratory has previously designed quadruplex priming amplification (QPA), which (i) allows having isothermally active and passive primers; (ii) perfectly fits to “wildfire” design (template “walking” is fueled by quadruplexes, while mobile end of product is securely folded) and (iii) can generate DNA clones very efficiently in truly isothermal way. Here we present data of the role of loop sequence, attachments and immobilization to solid surface on thermodynamic of GGGTGGGTGGGTGGG quadruplex. The data reveals strong influence of loop sequence and attachments on thermal stability of the quadruplex. We also present data on isothermal generation of DNA clones using immobilized quadruplex primers.

Supported by Bill & Melinda Gates Foundation.

YKB: A Protein Biosensor Used to Detect Changes in Translation Rate

Jamie Luther and Patricia L. Clark

University of Notre Dame, Department of Chemistry and Biochemistry
Notre Dame, IN 46556

Anfinsen's principle asserts that all of the relevant information required for a protein to fold into its final native state is encoded by the primary amino acid sequence. More recently it has been shown that many proteins begin to fold while still tethered to the ribosome. This cotranslational folding can influence the pathway a protein takes to its final native state *in vivo*. Additionally, altering translation rate could further impact cotranslational folding mechanisms. Currently, however, we have limited understanding of the cellular factors that control translation rate and how these might modulate cotranslational folding. To observe the effects of translation rate on final protein structure, synonymous codon mutations can be introduced at the mRNA level. While most amino acids are encoded by multiple synonymous codons, they are not used with equal frequencies. Rare codons are generally translated more slowly than common codons and can cause pauses in translation. To measure changes in translation rate *in vivo* I

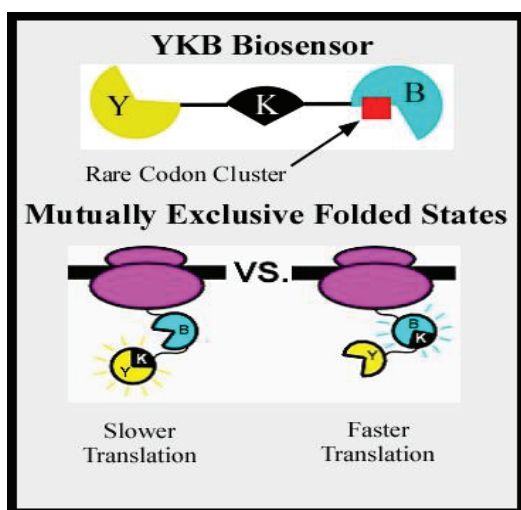


Figure 1 - YKB Biosensor

use the fluorescent protein biosensor, YKB, developed by Ian Sander in the Clark lab (Figure 1). YKB can assume one of two mutually exclusive folded states as it is translated. Synthesis of YKB from N to C terminus can produce either the YK folded state or the KB folded state. The ratio of YK (yellow) to KB (blue) fluorescence allows me to directly gauge any changes in translation rate *in vivo*. Specifically, increases in the YK to KB ratio suggest that translation rate is slowing down under some given conditions. My main goal is to use the YKB protein biosensor to gain a better understanding of how codon usage and growth conditions affect translation rate.

Temperature Dependence of the Thermodynamics for Binding Histone H1⁰, its Carboxyl Domain (H1⁰-C), or its Globular Domain (H1⁰-G) to Calf-Thymus DNA

Venkata Machha, Anna L. Turner, and Edwin A. Lewis

Department of Chemistry, Mississippi State University, Starkville, MS

Linker histones (H1) are the basic proteins in higher eukaryotes that are responsible for the final condensation of chromatin. In contrast to the nucleosome core histone proteins, the role of H1 in compacting DNA is not clearly understood. In this study we used isothermal titration Calorimetry (ITC), to directly measure the binding constant, enthalpy change, and stoichiometry or binding site size at different temperatures (i.e., 15°C, 20°C, 25°C, 30°C, and 35°C) for binding of H1⁰ or its C-terminal and globular domains to highly polymerized calf-thymus DNA.

The temperature studies allowed us to estimate the ΔC_p values for the binding of H1⁰ and its carboxyl (H1⁰-C) and globular domains (H1⁰-G) to CT-DNA. The enthalpy changes for binding H1⁰, H1⁰-C, or H1⁰-G to CT-DNA became more exothermic with increasing temperature over the range 15°C to 35°C. The interaction between H1⁰-G with CT-DNA was observed to be calorimetrically silent at 25°C. At higher temperatures, ΔH for the H1⁰-G/CT-DNA interaction becomes exothermic (e.g. $\Delta H \approx -5$ kcal/(mol H1⁰-G) at 35 °C).

The thermodynamic parameters observed for binding either the whole H1⁰ protein or the C-terminal domain, H1⁰-C were very similar. Although the thermodynamic parameters observed for binding H1⁰-G to CT-DNA are quite different than for either H1⁰ or H1⁰-C, the ΔH for binding H1⁰ is approximately equal to the sum of the ΔH values for binding H1⁰-C and H1⁰-G. The binding site size for interaction of the complete protein (36 bp) is also approximately equal to the sum of the bps covered in binding H1⁰-C (28 bp) and H1⁰-G (7bp).

Dysferlin; Small Change Big Flex

Ryan W. Mahling¹, Anne M. Rice¹, R. Bryan Sutton², and Anne Hinderliter¹

¹ University of Minnesota Duluth, ² Texas Tech University Health Sciences Center

Muscle cells act to generate force; this force places a high amount of stress on the plasma membranes of these cells and leads to formation of micro lesions within the membrane. In order for the cell to survive, these lesions must be rapidly and effectively repaired. The repair process has been proposed to occur through the patch mechanism, in which the influx of calcium ions into the cell after membrane damage signals the fusion of intracellular vesicles with the plasma membrane to form a patch, which reseals the lesion. One protein thought to be critical for this process is Dysferlin. Through the use bioinformatics seven C2 domains (C2A-C2G) have been identified within Dysferlin. Only the C2A domain of has been found to exist in two isoforms is the C2A, the canonical C2A and variant 1 or C2Av1. Given that C2 domains are a calcium ion and lipid binding motif found in eukaryotes, dysferlin may sense the influx of calcium ions upon membrane damage to enhance membrane binding and facilitate fusion of its attached intracellular vesicle to the lesion site. Both isoforms of the C2A domain are co-expressed in most tissues, however, the level of expression of each varies between tissue types. The differential levels of co-expression between the two isoforms have led us to hypothesize that these isoforms, although only differing by 30 amino acids, function in tandem in the membrane repair process. As such, we predict that each would show different responses to their endogenous ligands of calcium and phospholipid. We investigated the impact of the ligands on both the stability, and the ability of both isoforms to bind calcium ions and membrane. Both the canonical C2A and C2Av1 have free energies of stability half that of thermal energy, suggesting highly flexible structures that are extremely sensitive to changes in their local environment such as the influx of calcium ions. Surprisingly, canonical C2A shows a high affinity for calcium in solution, but extremely low affinity for acidic phospholipid in both the presence and absence of calcium. The C2Av1 domain however, has a robust affinity for acidic phospholipid, but no affinity for calcium in the absence of membrane. However, in the presence of acidic phospholipids the C2Av1 is able to bind calcium leading to a decrease in its ability to interact with the membrane. This data suggests that upon calcium ion influx the C2Av1 would dissociate from the membrane allowing it to potentially act as a scaffold to recruit other proteins involved in the repair process, while the canonical instead interacts with the membrane and may link the incoming vesicle with the plasma membrane.

Dose response assays can measure accurate equilibrium DNA binding parameters *in situ*

Karl Maluf and David L. Bain

Department of Pharmaceutical Sciences
University of Colorado Anschutz Medical Campus
Aurora, CO 80045

Gene expression is controlled primarily through the binding of transcription factors to specific DNA sequences in promoters, which in turn either activates or suppresses transcription. Thus, a major experimental goal is to develop an assay which can measure affinity of transcription factor binding to their DNA sites *in situ*. Recent work from our lab showed a strong correlation between DNA binding affinity for the glucocorticoid receptor (GR) measured *in vitro* using rigorous quantitative DNase footprinting and its apparent affinity measured *in situ* using a transient transfection “dose-response” assay. In fact, the correlation was so strong we were able to model it quantitatively using a simple equilibrium molecular model. This model assumed that the timescale for GR binding to its DNA site is rapid relative to the timescale for recruitment of the general transcriptional machinery, i.e. GR is in rapid equilibrium with its DNA site relative to the initiation of transcription. In addition, this model assumed that occupancy is directly proportional to gene expression. While the model does an excellent job describing the *in situ* data, it is perhaps surprising that it works given the complexity of the cellular environment. Therefore, we have adapted current mathematical models that describe the kinetics of gene expression to help understand how dose response assays can report on valid equilibrium parameters *in situ*. Our work shows that these models, which take into account the kinetics of mRNA production and translation, as well as mRNA and protein degradation, in fact do predict a direct proportionality between transcription factor occupancy and the *in situ* concentration of the gene product. We conclude that dose response assays can provide accurate quantitative information on *in situ* equilibrium DNA binding parameters for the GR receptor.

Substrate binding to Mn-, Fe-, and Co-HPCD

Amy E. Martin, Kate L. Henderson, Joseph P. Emerson

Mississippi State University

Homoprotocatechuate 2,3-dioxygenase (HPCD) is a nonheme iron enzyme that catalyzes a dioxygen dependent reaction that oxidizes catechol moieties to generate ring-opened products. Although this enzyme has been shown to be active with Mn^{2+} , Fe^{2+} , and Co^{2+} , the reaction mechanisms appear to pass through slightly different pathways. Here we are probing the thermochemistry associated with the initial event within these mechanisms: substrate binding. Isothermal titration calorimetry (ITC) was used to monitor the native substrate (HPCA) and a substrate analog (4-NC) as they bind to Mn-, Fe-, and Co-HPCD. Then using the ionization energies associated with different buffer systems, we also probed proton release during these binding events. The thermodynamic parameters as well as the change in protonation state associated with these binding events are compared.

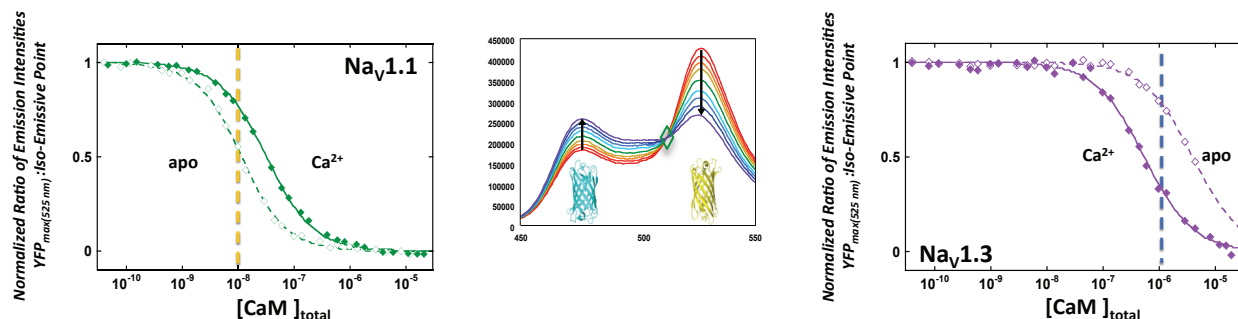
Bifurcation of Human Sodium Channels Based on Calmodulin Discrimination of IQ Motifs

Dagan C. Marx, Kristin M. Tefft, Mark S. Miller, Jesse B. Yoder, Elaine Kim, Liam Hovey, Sterling C. Martin, Andrew Tarleton, Brett C. Waite, Michael D. Feldkamp, and Madeline A. Shea,
Department of Biochemistry, Carver College of Medicine, University of Iowa
Iowa City, Iowa 52242-1109, USA

Voltage-dependent sodium channels (denoted VDSC or Na_v1.X) control the rising phase of the action potential in excitable cells. Channelopathies associated with these channels include Dravet syndrome, epilepsy, Long QT syndrome type 3, ventricular fibrillation, familial autism, and pain insensitivity. This family of ion channels is regulated by calmodulin (CaM), a small (148 a.a.) essential eukaryotic calcium sensor that contains two highly homologous domains (N and C) with 10-fold different affinities for calcium. CaM recognizes at least two regions of the channel including (a) the inactivation gate between transmembrane domains III and IV, and (b) an IQ motif (IQxxxBGxxxB, where B is Lys/Arg) located in the intracellular C-terminal tail of the pore-forming alpha subunit of Na_v. A genetic screen of *Paramecium* by Kung and coworkers showed the first biological role for differences in the two CaM domains by identifying mutations in the N-domain of CaM that specifically altered calcium-dependent sodium channels. However, the molecular mechanism is not yet understood.

Our laboratory has previously characterized in detail the interactions of human and *Paramecium* CaM with the IQ motif of neuronal Na_v1.2 (Feldkamp et al, *Structure* 2011, and Theoharis et al, *Biochemistry* 2008). In resting cells, apo (calcium-free) CaM binds to the IQ motif exclusively *via* its C-domain. Calcium binding to CaM changes its conformation, and lowers its affinity for the IQ motif. To probe the complexity of calcium-mediated feedback control, we have conducted thermodynamic analyses of CaM binding to the IQ motif sequences representing all 9 members of the human Na_v family, and the inactivation gate that is nearly identical among the channels. CaM association was detected by monitoring the loss of intensity of a biosensor containing the IQ motif sandwiched between two fluorescent reporter proteins (i.e., YFP-IQ-CFP).

We have discovered that despite a high degree of sequence homology, the 9 sodium channels split into two classes defined by their total free energy of binding and discrimination between apo and calcium-saturated CaM binding to the IQ motif. We are exploring the role of the residue that is commonly a glycine in canonical IQ motifs, and modeling competition between the IQ motif and inactivation gate sequence. These studies suggest that recognition of the IQ motif is not sufficient to explain the roles of both CaM domains in channel inactivation. Support: NIH R01 GM57001, Carver Charitable Trust Grant 01-224.



The Pitfalls of Intrinsic Thermodynamics – Structure Correlations of Lead Binding to Target Proteins

Asta Zubrienė, Lina Baranauskienė, Egidijus Kazlauskas, Joana Gylytė, David D. Timm, Vaida Morkūnaitė, Miglė Kišonaitė, Edita Čapkauskaitė, Virginija Dudutienė, Vaida Jogaitė, Justina Kazokaitė, Aistė Kasiliauskaitė, Vilma Michailovienė, Aurelija Mickevičiūtė, Sandra Bakšytė, Vilma Petrikaitė, Vytautas Petrauskas, Alexey Smirnov, Lena Manakova, Saulius Gražulis, Visvaldas Kairys, Jurgita Matulienė, and Daumantas Matulis (matulis@ibt.lt)

Department of Biothermodynamics and Drug Design, Institute of Biotechnology, Vilnius University, Graičiūno 8, Vilnius, Lithuania

The lead design in drug discovery is usually based on selecting the highest affinity compound that binds and inhibits the target protein function. However, the affinity alone provides limited information on the forces driving the binding event. Compounds with similar affinities often have very different enthalpic and entropic contributions due to the enthalpy-entropy compensation phenomenon. Such compounds have different reasons for binding. In the stages of optimizing the lead compound, it is important to take into consideration as many energetic contributions to the binding reaction as possible, including the volume of binding, a relatively rarely used thermodynamic parameter of binding. The enthalpic term is a direct measure of the net change in the number and strength of the non-covalent interactions upon binding.

Enzymatic inhibition methods are usually used to determine the potency of lead compound effect on the target protein. Similarly, anticancer compound efficiency is determined by obtaining the growth inhibition of the cancer cell lines. These tests are limited by the concentration of the target protein. If the target protein concentration, necessary for the assay, is higher than the K_d , then the true K_d cannot be determined. Therefore, isothermal titration calorimetry (ITC) is a method of choice in the pharmaceutical industry for determination of lead compound equilibrium binding enthalpy, entropy, and the Gibbs free energy to the therapeutic target in a single experiment. The method is very powerful for determination of intrinsic binding parameters that could be used in structure-energetics correlations. It is important to complement ITC results with thermal shift assay (TSA, ThermoFluor, TF) in order to avoid the narrow window of ITC K_d measurements. It is also important to determine the intrinsic enthalpy of binding and intrinsic K_d that are dissected from contributions occurring in solution due to linked reactions. Only the intrinsic K_d may be correlated with the structure of protein-ligand complex. The ITC and TSA K_d s should be compared with the enzymatic inhibition methods in order to confirm the binding properties in the presence of the substrate.

The methods are illustrated using carbonic anhydrase (CA) and chaperone Hsp90 inhibitor development. CA isozymes are important targets for the design of inhibitors as antiglaucoma, antitumor, antiobesity or anticonvulsant drugs. There are 12 active CA isoforms in human. However, most inhibitors are non-specific towards desired isoform and exhibit strong undesired side-effects. Here we have designed several series of aromatic sulfonamide inhibitors and tested their affinities and specificities towards selected CA isoforms. Binding affinities and intrinsic thermodynamics were determined by TF and ITC. Crystallographic structures of selected compounds bound to several isoforms were determined. Hsp90 chaperone is over-expressed in cancer cells and Hsp90 inhibitors have shown selectivity for cancer cells. Therefore, small molecule inhibitors are being developed as anticancer therapeutics.

Acknowledgements: This research was funded by a grant (No. LIG-09/2012) from the Research Council of Lithuania. The authors also acknowledge FP7- REGPOT-2009-1 grant “MoBiLi”, Agreement No.: 245721, and the COST projects TD0905 and CM0804.

The Thermodynamics of Translocation of the Ribosome

Drew E. Menke¹, Peter V. Cornish¹

¹ Department of Biochemistry, University of Missouri Columbia, MO 65211, USA

The ribosome is responsible for the synthesis of protein in all living organisms and has implications in the treatment of bacterial infections through the development of novel antibiotics and the treatment of cancer through the development of drugs used to inhibit cancer growth. A better understanding of the mechanisms by which proteins synthesis occurs will allows us to address many of these problems. Translocation, the process by which the ribosome advances along its mRNA and the associated energetics are not fully understood, this makes it an ideal candidate for investigation. By probing the energy landscape of the ribosome using single molecule Forster Resonance Energy Transfer (smFRET) during translocation and observing key dynamic motions at a range of different temperatures, we will be able to address key controversies associated with the energy needed to drive these processes. It will also allow us to determine the energy of activation, kinetic rates and the contributions of dynamic motions to translocation of the ribosome. This study will result in a complete elucidation of the energetics of translocation of the ribosome and a better understanding of its mechanism.

Molecular Interactions that Control Protein Assembly into Nanofiber Structures.

Reagan J. Meredith and Steven T. Whitten

Department of Chemistry and Biochemistry, Texas State University

The prion diseases are a class of neurodegenerative disorders associated with the conversion of natively folded prion protein to amyloid particles. To investigate this structural transition, small peptide ligands were developed that interact with recombinant prion protein to induce misfolding to amyloid particles under native conditions. Observations from these studies revealed that: 1) the ligand:protein interaction is sequence specific, 2) ligand-induced conversion of natively folded recombinant protein to amyloid involves multiple intermediates, 3) the intermediates form quickly (~ immediately) whereas amyloid fibrils form slowly (hours), 4) fibrils are able to self-propagate misfolding without the ligand present, and 5) sequence homology of the ligand to RF-amide neuropeptides suggests that physiological cofactors may play a role in prion diseases, similar to the hypothetical protein X suggested by Prusiner.

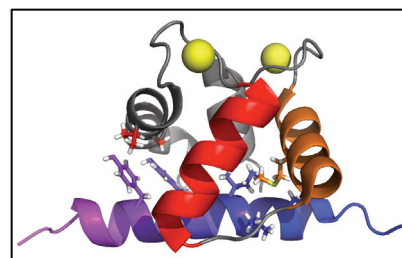
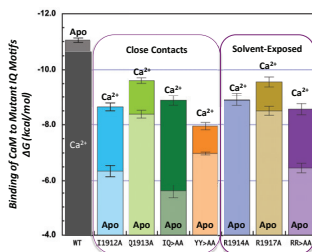
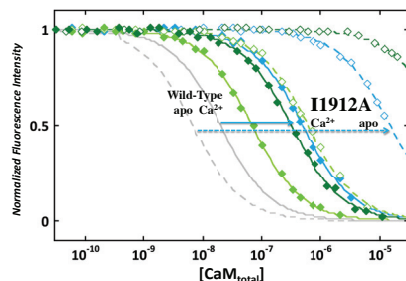
Calmodulin Recognition of Anti-Parallel Nested Binding Sites in the Neuronal Voltage-Dependent Sodium Channel Na_v1.2

Mark S. Miller, Liam Hovey, C. Andrew Fowler, Dagan C. Marx, Jesse B. Yoder, Elaine Kim, Shuxiang Li, Kristin M. Tefft, Brett C. Waite, Michael D. Feldkamp, Liping Yu, and Madeline A. Shea, Department of Biochemistry, Carver College of Medicine, University of Iowa, Iowa City, IA 52242-1109

Calmodulin (CaM) is an essential, eukaryotic calcium sensor that regulates the inactivation of the neuronal voltage-gated sodium channel (Na_v1.2) by binding to an IQ motif [IQxxFBGFxxB, where F is aromatic, and B is basic] that is embedded in the C-terminal tail of the channel and an inactivation gate near the plasma membrane. The IQ motif binds with high affinity ($K_d < 10$ nM) to the C-domain of CaM in both its apo (calcium-free) and calcium-saturated states. The major goal of this study was to understand how calcium triggers a change in the interface between CaM and the ion channel to regulate inactivation. We undertook a mutational perturbation study based on our solution structure (2KXW) of the C-domain of apo-CaM bound to the helical IQ motif of Na_v1.2 [IQRAYRRYLLK]. The cleft of semi-open CaM made many van der Waals contacts with the Ile and both Tyr residues, while an interhelical turn made a network of hydrogen bonds with the Gln residue. CaM made few contacts with highly conserved but solvent-exposed Arg residues in the IQ motif.

Energetic contributions of these residues were tested by monitoring changes in CaM binding to alanine-substituted biosensors containing the Na_v1.2 IQ motif bracketed by a pair of auto-fluorescent proteins (YFP, CFP); CaM binding disrupts FRET in proportion to fractional saturation. Mutations in the IQ motif lowered its affinity for calcium-saturated CaM by up to 2 orders of magnitude, but even larger changes were seen for apo CaM. Thermodynamic linkage analysis indicated a large coupling energy between I and Q but modest to negligible coupling between the solvent-exposed R residues. The directions and magnitudes of shifts indicated that there is a large calcium-triggered change difference in the CaM-IQ interface.

Our new solution (NMR) structure of the open C-domain of calcium-saturated CaM bound to the IQ motif of Na_v1.2 is the first example of apo and Ca²⁺-saturated CaM bound to the same IQ motif and explains the large energetic differences. The orientation of CaM is anti-parallel to that observed in the apo structure: CaM has pivoted on the Ile residue, binding to a surface nested within the canonical IQ motif but interacts primarily with only one Tyr residues and makes minimal contacts with the Gln residue. Binding in a reversed orientation rotates the location of the N-domain of CaM and has exciting implications for channel regulation. A single CaM molecule is expected to dissociate from the IQ motif before re-binding. This release would allow for transient interactions with the ion channel inactivation loop near the membrane. *NIH R01 GM57001*



Broad ligand specificity in the small multidrug resistance transporter EmrE

Emma A. Morrison and Katherine A. Henzler-Wildman

Department of Biochemistry and Molecular Biophysics, Washington University in St. Louis, Saint Louis, MO, USA

EmrE, an *E. coli* small multidrug resistance transporter, exports a broad range of toxic polyaromatic cations, thus imparting resistance to drug compounds of this type. EmrE interconverts between inward- and outward-facing structures during the transport cycle, using the import of two protons across the inner membrane to drive the export of one substrate molecule. As a multidrug resistance transporter, EmrE has particularly broad specificity and a large variation in affinities for these transported substrates, yet all substrates must trigger the same conformational change between inward- and outward-facing states in order for transport to occur. Our research focuses on this coupling between substrate binding and conformational exchange and the functional outcome of this coupling: transport.

EmrE transports polyaromatic cations that vary in geometry (ie. planar vs. tetrahedral), size, and charge (ie. $+1$ vs. $+2$). I have taken advantage of this broad specificity to compare the conformational changes induced in EmrE upon binding a variety of substrates. In particular, I focus on two ligand series: i) a series of related tetrahedral ligands with an identical charge of $+1$, and ii) a series of planar ligands that vary in size and charge. We have directly monitored conformational exchange in substrate-bound EmrE using ZZ-exchange NMR spectroscopy and found that the rate of conformational exchange varies significantly with the identity of the bound ligand. Since this conformational change is critical to the transport cycle, these studies test how broad ligand specificity is accommodated within the simple single-site alternating access model for transport. In conjunction with isothermal titration calorimetric measures of binding affinities, kinetic studies for microscopic rate constants, and liposomal assays of transport rate, this will enable more detailed mechanistic insight into the transport cycle.

Research supported by NSF GRFP, Searle Scholars Program, and NIH NIGMS.

Osmolyte Effects on the Self-Association of Concanavalin A: Testing the Tanford Transfer Model

Thomas W. Silvers and Jeffrey K. Myers*

Department of Chemistry, Davidson College, Box 7120, Davidson, NC 28035

*jemyers@davidson.edu

BACKGROUND: A large body of literature exists describing the effect of osmolytes (small organic molecules accumulated by cells to protect against osmotic stress) on protein folding. However, very few studies address their effect on protein binding. The formation and stability of protein-protein interfaces is of obvious biological importance. The plant lectin concanavalin A (con A) was used as a model system to investigate the influence of nine osmolytes on protein self-association. Con A undergoes a reversible tetramer to dimer equilibrium as a function of pH. The stabilizing or destabilizing impacts of the osmolytes were evaluated from pH titrations combined with circular dichroism (CD) spectroscopy.

RESULTS: Relative to the dimer, trimethylamine N-oxide, betaine, proline, sarcosine, sorbitol, sucrose and trehalose all stabilized the con A tetramer to varying extents. Glycerol had a negligible effect, and urea destabilized the tetramer. From multiple titrations in different osmolyte concentrations, an m-value (a thermodynamic parameter describing the change in the association free energy per molar of osmolyte) was determined for each osmolyte. Experimental m-values were compared with those calculated using the Tanford transfer model and transfer free energies determined by Bolen and co-workers. The model failed to accurately predict the m values in most cases.

CONCLUSIONS: Most of the osmolytes tested stabilized the con A dimer-dimer interface. Further experimental work is needed to determine if there is a general stabilization by osmolytes of protein-protein interfaces. Although the Tanford transfer model has been used successfully to predict m-values for protein folding, it demonstrated only limited quantitative applicability to con A association. Further theoretical work is needed to develop a thermodynamic model to predict the effect of osmolytes on such interfaces.

Probing the second high affinity binding site in human carbonic anhydrase II

Whitnee Nettles, Amy Martin, William Kennon, Joseph Emerson

Mississippi State University
Department of Chemistry, Room 1115
310 President's Circle
Mississippi State University
Mississippi State, MS 39762-9573

A second high affinity binding site for Cu(II) in human carbonic anhydrase II (CA) has been observed by isothermal titration calorimetry (ITC). However, the location and identity of the side-chain residues participating in the Cu binding have not yet been identified. Here we report data collected from a series of HSQC NMR experiments that have allowed us to localize this high affinity Cu(II) binding to two small peptides, we have identified several of the key players associated with this novel metal binding site.

Dynamics of Human Replication Protein A (RPA) Bound to Single Stranded DNA

Binh Nguyen,¹ Joshua Sokoloski,¹ Marc S. Wold,² Roberto Galletto,¹ Timothy M. Lohman¹

¹Department of Biochemistry & Molecular Biophysics,
School of Medicine, Washington University in St. Louis, St. Louis, MO 63110

²Department of Biochemistry, University of Iowa, Iowa City, IA 52242

Replication Protein A (RPA) is an essential hetero-trimeric single stranded (ss) DNA binding protein that functions in DNA replication, repair and recombination in eukaryotic organisms. RPA binds to the transient ssDNA that forms during nearly all aspects of DNA metabolism and also interacts with a large array of other proteins in the cell. Even though RPA binds with very high affinity to single stranded DNA, it must be removed from or redistributed along ssDNA during DNA replication. We have used single molecule fluorescence techniques to examine the dynamics of Human RPA (hRPA) while bound to fluorescently labeled ssDNA. These experiments show that hRPA is able to diffuse along ssDNA while remaining tightly bound. Furthermore, hRPA can transiently melt DNA hairpin structures by diffusing in from ssDNA that is adjacent to the DNA hairpin. This ability of hRPA to diffuse along ssDNA means that it should be able to be readily redistributed along ssDNA by being pushed by a directional motor such as a polymerase or other translocase motor. The bacterial analogue of RPA, the *E. coli* SSB protein, has also been shown to be able to diffuse along ssDNA and thus this important class of DNA replication proteins shares this property. (GM030498 (TML), GM098509 (RG), GM044721 (MSW))

Apparent stability of PrP^{Sc} fibrils from natural host species as a tool in the understanding and differentiation of prion disease strains

Catherine E. Vrentas, Justin J. Greenlee, and Eric M. Nicholson

Virus and Prion Diseases Research Unit, National Animal Disease Center, U.S. Dept. of Agriculture, Agricultural Research Service, 1920 Dayton Ave., Ames, IA 50010
Eric.Nicholson@ars.usda.gov

Transmissible spongiform encephalopathies (TSEs) or prion diseases are a class of transmissible neurodegenerative diseases, caused by an infectious protein, that afflict humans and other mammals. The infectious agent is a host encoded protein known as PrP that has adopted a misfolded conformation termed PrP^{Sc}. Despite the protein only nature of the infectious agent, the various prion diseases exist in many different strains, which in the context of a protein only disease must arise due to differences in conformation. Depending upon the strain in question, the strains can be differentiated by tissue distribution, brain lesion profiles, as well as glycoform analysis and molecular weight profile using western blot. In rodent adapted models, prion strains have also been shown to confer differences in the apparent stability of PrP^{Sc} as determined using GdnHCl denaturation. In addition, PrP^{Sc} stability has been shown to correlate well with incubation time such that the shortest incubation times result from the least stable prion strains. It is postulated that less stable prion fibrils will more readily fragment resulting in a higher concentration of template for the recruitment of additional PrP. In natural host species, *a priori* prediction of incubation time based upon an *in vitro* assay are relevant for studies of pathogenesis and holds practical implications for disease management. Toward this goal, we assess the apparent stability of PrP^{Sc} derived from sheep experimentally inoculated with 2 strains of scrapie, each with a substantially different incubation time, in order to provide evidence for the stability/incubation time correlation in a natural host system. We also demonstrate the utility of the stability methodology in characterizing these scrapie isolates throughout serial passage in sheep. We then apply the same methodology to PrP^{Sc} from age matched Holstein steers inoculated with 3 different strains of bovine spongiform encephalopathy (BSE) (H-type, L-type, and classical) as well as cattle passaged transmissible mink encephalopathy (TME). Each BSE strain is differentiable using glycoform analysis and molecular weight profile on a western blot, and the observed incubation time for TME, H-type BSE, and L-type BSE is considerably shorter than that for classical BSE. We compared the PrP^{Sc} stability determined using GdnHCl unfolding of PrP^{Sc} to the observed incubation time. In the case of BSE strains, the stability/incubation time correlation appears invalid. The apparent stability we report is not a true conformational stability as the unfolding PrP^{Sc} is not reversible and the PrP^{Sc} fibrils are not a single species. These and other factors that may influence the ability to correlate the apparent PrP^{Sc} stability and incubation time in a manner analogous to that observed for sheep and rodent models of TSEs need to be considered. Approaches to addressing these factors are discussed and include evaluation of the unfolding rate and fractionation of the PrP^{Sc} present in brain homogenate.

*All work involving research animals was performed with the approval of the institutional animal care and use committee.

Multiple Recognition Motifs Provide Rigidity to Stabilize LC8 Complexes

Afua Nyarko, Yujuan Song, & Elisar Barbar

Department of Biochemistry and Biophysics, Oregon State University, Corvallis, OR 97331

Hub proteins have a large number of interacting partners and are of special importance in organizing protein-protein networks. LC8, a 10 kDa dimeric component of the cytoplasmic dynein biological motor complex has a relatively large number of interacting partners and fits the description of a hub. Partner proteins of LC8 include the intermediate chain (IC) subunit of cytoplasmic dynein, Chica, a mitotic spindle-associated protein, Nup159, a component of the yeast nuclear pore complex, as well as a wide array of proteins with roles in apoptosis, enzyme regulation and viral pathogenesis. Linear motifs mediate binding between LC8 and its partner proteins, but while partner proteins such as dynein IC have only one recognition motif, others such as Chica and Nup159 are enriched with multiple interacting motifs.

To better understand the importance of multiple recognition motifs in the LC8 interaction network, we have initiated structural and thermodynamic studies of the LC8/Chica and LC8/Nup159 complexes. Chica and Nup159 have four and six putative recognition motifs respectively, nestled within segments that are intrinsically disordered. Our results indicate that LC8 forms a dynamic complex with both proteins, binding only three (Chica) or five (Nup159) of the putative recognition motifs. Furthermore, we show that only three LC8 dimers are needed for optimal stability of the Nup159/LC8 complex, and suggest that the evolutionary adaptation of multiple LC8 recognition motifs imparts to the complex other properties such as rigidity. These findings extend the repertoire of functions of intrinsically disordered proteins to fine-tuning and versatile assembly of higher order macromolecular complexes.

Two-primer exponential quadruples priming amplification for diagnostics

Tamar Partskhaladze, Besik Kankia

Department of Chemistry and Biochemistry, The Ohio State University, Columbus, OH

Nucleic acid amplification technologies are used as leading methods in detecting and analyzing a small quantity of nucleic acids. The polymerase chain reaction (PCR) is the most widely used method for DNA amplification for detection and identification of infectious diseases, genetic disorders and other research purposes. However, disadvantage of this method is a thermo cycling procedures required for DNA strand separation. To address these limitations, we have devised two-primer exponential quadruplex priming amplification (QPA) reaction, which relies on specifically designed guanine-rich primers. In this approach, sample (i.e., patient DNA) is immobilized to magnetic beads, processed and moved to QPA recognition and amplification buffers. The probe molecules contain pathogen complements and a universal QPA construct. If the sample contains pathogen, probe will hybridize to it and will be transported to the adjacent solution containing the universal amplification buffer.

A mechanism of two-primer exponential QPA performed in the amplification buffer is following: initially the QPA primer binds to the probe and replicates it. After spontaneous quadruplex formation, the next QPA priming/elongation occurs, which is accompanied by displacement of the first amplicon. The displaced strand contains a freely available primer-binding site for the 2nd primer. The primer binds and replicates the amplicon including the quadruplex at the 5'-end. After another step of amplification, a short duplex containing primer-binding sites for both primers is created and the reaction becomes exponential. In order to select favorable condition for rapid amplification, different experimental conditions and polymerases were tested. It was shown that using Vent (exo-) polymerase, DNA concentration of 100aM can be reproducibly differentiated from background signal. It is a highly sensitive chain reaction that can be specifically triggered by given DNA sequences and can achieve amplifications of 10⁹-fold in less than 40 min. The efficiency of QPA strongly depends on thermodynamic properties of primer/PBS and product DNA, which will be discussed.

Contributions of alanine residues to the hydrodynamic radius of p53(1-93).

Romel B. Perez and Steven T. Whitten

Department of Chemistry and Biochemistry, Texas State University

The hydrodynamic radius, R_h , is an important constraint for assessing the structure of intrinsically disordered proteins (IDP's). NMR can provide local structural details in IDP's, but the wide conformational freedom observed by most residue positions limits structural resolution. Thus, global parameters like R_h are needed for molecular models of IDP structure. The R_h values of IDP's are known to depend on fractional proline content and net charge, where increased numbers of prolines and increased net charge cause larger R_h . Here we assess alanine contributions to R_h in IDP's since alanine, like proline, favors extended polyproline II helical structures in unfolded protein.

The Annexin-Lipid Party Line: Communication Between Protein and Membrane

Anika Rannikko, Samantha Jaworski, Anne Rice, Michael Fealey, and Anne Hinderliter

University of Minnesota Duluth

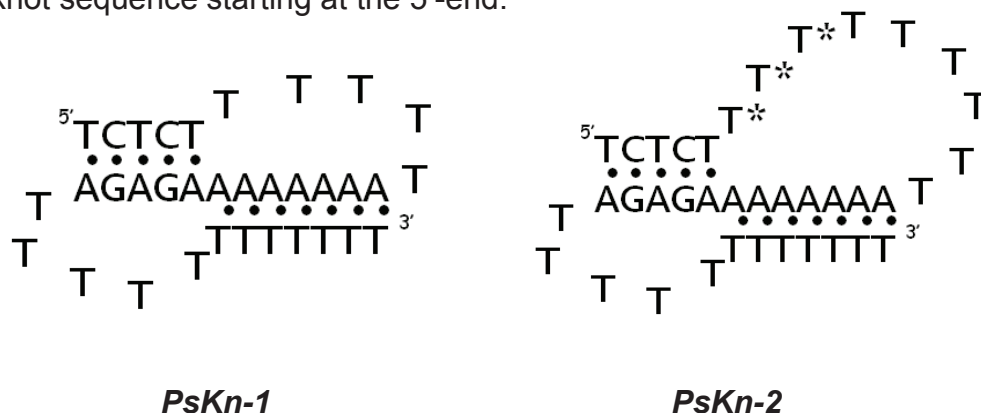
The annexin protein family constitutes two percent of all eukaryotic proteins within the cell, yet despite the fact that they are so prevalent, their exact functions are still under investigation. Multiple binding partners have been characterized for the annexins ranging from small molecules like Ca^{2+} and phospholipids, to a variety of proteins. Through calcium interactions and association with acidic phospholipids, we hypothesize these proteins are capable of sensing and modulating lipid distributions. Stressors on the cell redistribute the lipids within the leaflet of the membrane; this can affect cellular signaling, protein function, and the effectiveness of the membrane as a barrier. We hypothesize that the annexins can sense and respond to these changes and regulate the lipid distribution in the bilayer such that the permeability of the membrane decreases. This effect on permeability could impact the cell's ability to transduce signaling information such as calcium levels. A carboxyfluorescein (CF) release assay was performed to determine annexin a5's effect on membrane permeability using a model membrane of acidic and neutral lipids containing CF buffer. In order to simulate the release of molecules from the membrane under stress, vesicles were perturbed through their phase transition by decreasing their temperature, causing CF to leak from the vesicles. The fluorescent signal associated with this leak was measured across this phase transition over time. The change in efflux of CF from the vesicles in the presence and absence of annexin implies annexin's ability to impact membrane permeability. The efflux of CF from the vesicles in the presence of annexin a5 without Ca^{2+} showed only a slight decrease compared to the control of vesicles alone. However, there was a larger signal decrease in the presence of annexin a5 and Ca^{2+} . This suggests that in the presence of Ca^{2+} , annexin a5 works to reduce the permeability of the membrane. These studies were repeated with samples composed of increasing mole fractions of cholesterol while keeping the ratio of other lipids consistent. This allows for the determination of the effects of both cholesterol and annexin a5 on membrane permeability. These cholesterol-containing lipid vesicles showed a decrease in permeability as less CF was released from the vesicles, suggesting that annexin a5 prevents movement of molecules across the membrane in the presence of Ca^{2+} and cholesterol. Additionally through the use of isothermal titration calorimetry (ITC) we determined that not only does annexin a5 impact the function of the membrane, but the membrane in turn impacts annexin. This is evidenced a change in the Ca^{2+} binding parameters and stoichiometry of annexin a5 in the presence of cholesterol-containing membranes. The CF efflux data, combined with previous ITC data, leads us to hypothesize that annexins are sensors of the distribution of lipids in the membrane and as such help communicate changes in the membrane environment as a function of their calcium binding ability.

Loop Effects on the Stability and Targeting of DNA Pseudoknots

Calliste Reiling and Luis A. Marky

Department of Pharmaceutical Sciences, University of Nebraska Medical Center,
986025 Nebraska Medical Center, Omaha, NE, 68198-6025

Pseudoknots belong to a common motif of RNA molecules and are involved in a variety of cellular processes, including ribosomal frameshifting. To this end, our laboratory is investigating the unfolding of RNA structural motifs and mimicking the targeting of RNA by reacting DNA stem-loop motifs with partially complementary strands. We used a combination of differential scanning calorimetry (DSC), temperature-dependent UV spectroscopy, and isothermal titration calorimetry (ITC) techniques to investigate the unfolding of the DNA pseudoknots shown below, with the longer loop predicted to form a triplex stack adding to the overall stability of the molecule and their reaction with single strands ($5'\text{-CT}_8\text{A}_n\text{AGAGA}$, where $n = 5$ or 9) that are partially complementary to the pseudoknot sequence starting at the 5'-end.



Both pseudoknots unfold through biphasic intramolecular transitions; **PsKn-2** unfolds with a higher enthalpy of 33 kcal/mol. This heat corresponds to the formation of three T*AT base triplets (two T*AT/T*AT base triplet stacks) at the center of **PsKn-2**, increasing its overall stability by a ΔG° of -6.1 kcal. We also used DSC to determine the unfolding thermodynamics for the duplex products of each reaction. The unfolding data is then used to create thermodynamic (Hess) cycles that correspond to each targeting reaction. The resulting enthalpies are compared with the reaction enthalpies obtained directly from ITC experiments. All reactions yielded favorable free energy contributions, in excess of -4 kcal/mol, that were enthalpy driven. These favorable heat contributions result from the formation of base-pair stacks involving the unpaired bases of the loops, indicating that each complementary strand was able to invade and disrupt the secondary structure of the pseudoknots. Supported by Grants MCB-1122029 from NSF and GAANN- P200A120231 (C.R.) from the U.S. Department of Education.

Are proton binding reactions in proteins coupled to local conformational fluctuations?

Daniel E. Richman¹, Ananya Majumdar², Bertrand Garcia-Moreno E.³

¹Dept. of Physics and Astronomy, ²Biomolecular NMR Center, ³Dept. of Biophysics
Johns Hopkins University, Baltimore MD 21218

Acid can unfold a protein when more protons (H^+) are bound by the acid-unfolded state (U_A) than by the folded native state (N). Acid unfolding implies that ionizable residues that titrate in the acidic range of pH (Asp and Glu) have pK_a values close to normal values in water in the U_A state and depressed pK_a values in the N state. This is consistent with the observation that surface ionizable groups in the N state are under the influence of net stabilizing Coulomb interactions. To examine the origins of the cooperative acid unfolding transition we examined the hypothesis that when local conformational fluctuations allow a carboxylic group to sample two microenvironments, one in which its pK_a value is normal and one in which it is depressed, then at pH values below the apparent pK_a the locally unfolded state in which the pK_a value is normal becomes dominant. NMR experiments with staphylococcal nuclease revealed increased backbone fluctuations near carboxylic residues as pH decreases from the neutral into the acidic range. Hydrogen exchange measurements suggest that conformational fluctuations promoted by decreasing pH reflect local unfolding near some carboxylic groups, which in turn leads to normalization of their pK_a values. With decreasing pH the conformational ensemble becomes biased toward increasingly unfolded states, even under pre-denaturational conditions. These results also suggest that local conformational fluctuations and local stabilities are important determinants of the apparent pK_a values of Asp and Glu residues.

Ion pairs buried in the hydrophobic interior of proteins: Are they charged and are they stabilizing?

Aaron Robinson, Ananya Majumdar, Jamie Schlessman and Bertrand García-Moreno E.

Department of Biophysics, Johns Hopkins University, Biomolecular NMR Center, Johns Hopkins University and Chemistry Department, US Naval Academy.

Internal ion pairs buried in hydrophobic environments in proteins are essential for many important biochemical processes including H^+ transport, e^- transfer, ion homeostasis, and catalysis. Despite the importance of these internal ion pairs, their properties remain poorly understood. These motifs are of special interest not only for their functional roles, but also because they can be used as probes to examine the balance between Coulomb and hydration energies in dehydrated environments. In these environments the pK_a values of isolated internal ionizable residues may be highly anomalous and very different from the normal pK_a values in water. The precise tuning of these pK_a values is necessary for proton transfer or general acid/base chemistry, and it is not clear how the pairing of buried ion pairs affects the pK_a values. To examine molecular determinants of pK_a values of internal ionizable groups, and the properties of internal ion pairs in particular, we engineered multiple variants of staphylococcal nuclease with pairs of internal ionizable residues of opposite polarity (Glu-Lys; Asp-His) that could, in principle, form favorable interactions. X-ray crystallography, NMR spectroscopy and equilibrium thermodynamic experiments were used to characterize the microenvironments of each pair and to determine the pK_a values and charge states of the ionizable moieties, and their effects on thermodynamic stability of the protein. We have shown that stable charge pairs can be engineered in the protein interior without the need for specialized structural adaptations. The inherent polarity and plasticity of the protein can be sufficient to stabilize the interaction. However, the charge state of a particular ion pair depends on nuances of the microenvironment that are difficult to predict *a priori*. These data constitute a set of benchmarks that will allow stringent evaluation of structure-based electrostatics calculations.

The Role of Stability in the Function of the *Staphylococcus epidermidis* Biofilm Protein Aap

Catie Shelton & Andrew B. Herr

Department of Molecular Genetics, Biochemistry and Microbiology
University of Cincinnati College of Medicine Cincinnati, OH

Staphylococcus epidermidis, though a component of the normal skin flora, causes persistent medical-device associated infections. This organism has been directly associated with infections of ventricular assist devices¹, heart valves, intravascular catheters and joint replacements.² Persistent infections are due to the formation of biofilms, bacterial communities that are resistant to antibiotic treatment, on the medical device. The accumulation-associated protein (Aap) is required for the formation of a biofilm by *S. epidermidis*.³ Aap is a surface protein that contains 5-12 nearly identical tandem B-repeats, with each repeat 128-amino acids in length. The B-repeat region is specifically implicated in intercellular adhesion in nascent *S. epidermidis* biofilms. Our data suggests that Aap on adjacent cells hold the cells together in a zinc-mediated fashion⁴, and forms amyloid-like fibers that, along with other factors, resiliently hold the biofilm together.

We are inherently interested in the stability of this protein since the Eisenberg lab (UCLA) has demonstrated the role of local unfolding in the formation of amyloid fibers.⁵ Previous work by our lab demonstrated that, in a truncated construct containing 1.5 repeats, the interface between repeat modules was responsible for providing stability to the overall protein fold.⁶ As a result, we hypothesized that additional repeat modules beyond 1.5 repeats would produce a concomitant increase in protein stability, as measured by circular dichroism (CD) temperature melts. We found that this was true until the number of repeats exceeded 3.5, above which point the melting temperature (T_m) plateaued.

Intriguingly, the temperature plateau occurred in longer constructs containing a conserved variant set of amino acids that differed from those in repeats 1-3. These conserved variant residues are nested against the interface between repeat modules, which we know is responsible for protein stability. We are currently investigating whether the conserved variant amino acids in longer repeats are responsible for “tuning” the stability of the protein fold, effectively retaining the possibility for local unfolding events required for amyloid formation. To test this hypothesis we are generating 100% identical repeats and determining the effect on T_m of various repeat lengths.

1. Gordon *et al* (2012) *Journal of Infectious Disease* 205 (9): 1391-1398
2. Rogers *et al* (2009) *Infectious Disease Clinics of North America* 23 (1): 73-98
3. Hussain *et al* (1997) *Infection and Immunity* 65 (2): 519-524
4. Conrady *et al* (2008) *PNAS* 105 (49): 19456-19461
5. Goldschmidt *et al* (2010) *PNAS* 107 (8):3487-3492
6. Conrady *et al* (2013), *PNAS* 110 (3): E202-11

Quantitative Biophysical Characterization of Intrinsically Disordered Proteins: NMR Structure Ensembles and the Thermodynamics of Folding-Upon-Binding

Scott A. Showalter, Chad Lawrence, Anthony Zidell, and Debashish Sahu

Department of Chemistry, The Pennsylvania State University, University Park, PA
16802, USA

Current methods for protein structure determination and models for interaction mechanisms are primarily formulated under the assumption that a protein's folded state is temporally stable. In contrast, intrinsically disordered proteins (IDPs) adopt spatially and temporally dynamic ensembles that are poorly characterized by contemporary structure-determination methods. Furthermore, while these proteins are disordered when alone in solution, many undergo a disorder-to-order transition when interacting with a binding partner. This has lead many investigators to propose a significant loss of chain entropy must oppose the binding process, thus explaining the generally weak (micromolar) binding affinities experimentally measured for IDP-mediated interactions. When applied without validation, such assumptions can be damaging. We aim to develop semi-quantitative models for the solution ensembles of IDPs through novel NMR spectroscopic methods and to apply thermodynamically rigorous tests of existing folding-upon-binding models using titration calorimetry. Here we report a thorough case study of the C-terminal regulatory domain of the phosphatase FCP1. Our results show that very few residues in FCP1 experience an increase in backbone order upon forming a functionally essential complex with the winged-helix domain from RAP74. Our data suggest that the moderation of binding affinity associated with chain entropy in folding-upon-binding mechanisms may be generally less severe than previously indicated. In order to better understand the thermodynamics of the FCP1-RAP74 interactions we have performed extensive temperature dependent isothermal titration calorimetry experiments in both aqueous solution and in the presence of multiple osmolytes, in addition to differential scanning calorimetry experiments investigating stabilization of the FCP1-RAP74 complex. Our calorimetric data indicate a large and negative heat capacity change is associated with the binding event, with a transition from entropically driven (endothermic) binding to enthalpically favored binding that occurs around 293K. Overall, the calorimetric data strongly favors a binding model in which an increase in solvent entropy associated with the burial of hydrophobic surface dominates the binding event and dictates whether entropy or enthalpy is the dominant driver of binding at a given temperature. General implications for folding-upon-binding and its role in mediating cellular signaling will be discussed.

Molecular Dynamics Simulations of the Conformational Behavior of Tripeptides in Aqueous Solution.

Scott A. Shubert, Oluwatoni J. Olayiwola, Aakash Jain, Shuxiang Li, Casey T. Andrews, Adrian H. Elcock

Department of Biochemistry, University of Iowa, Iowa City, Iowa 52242, United States

Recent interest in intrinsically disordered proteins has led to investigation of the conformational behavior of short host-guest GXG peptides. Experimental studies have indicated that end groups and pH can significantly affect the conformational properties of these peptides as reflected, for example, in their $^3J(H_n, H_a)$ NMR coupling constants. Here we describe our efforts to understand and rationalize these data using long explicit solvent molecular dynamics simulations of a variety of GXG peptides. The results highlight the potential benefits and challenges associated with using molecular simulations to interpret experimental data on the conformational behavior of biological molecules.

The Kinetic Mechanism of RecBCD Binding to and Melting Blunt End DNA

Michael J. Simon and Timothy M. Lohman

Washington University School of Medicine, Biochemistry and Molecular Biophysics

RecBCD is a bacterial helicase, translocase, and nuclease. It is involved in the double-stranded break repair of damaged DNA in *E. coli*. RecBCD has three polypeptides: RecB, a 3'→5' translocase with a nuclease domain, RecD a 5'→3' translocase, and RecC, a processivity and structural factor that binds both RecB and RecD. The complex binds to DNA blunt ends, and has been shown to melt the terminal 5-6 base pairs in a Mg^{2+} dependent and ATP independent manner before unwinding DNA in an ATP dependent manner and processing the ends for homologous recombination.

We are studying the kinetic mechanism of the binding and melting activity of RecBCD, using fast-mixing kinetic methods to observe pre-equilibrium RecBCD-DNA complexes. We have used small (60 bp) dsDNA substrates with blunt ends fluorescently labeled to report changes in the local environment due to interaction of the DNA substrate with RecBCD using the fluorescence stopped-flow method. The interaction of RecBCD and substrates labeled with Cy3 shows a two-step kinetic process, suggesting a bimolecular binding step and an isomerization step that may be due to melting. Further experiments observing the fluorescent signal from substrates labeled with a FRET donor-acceptor pair on separate strands suggest that the second rate is due to strand separation, therefore a product of melting. Additionally, these signals attributed to strand separation are also observed in conditions that lack Mg^{2+} , indicating some partial melting even in the absence of Mg^{2+} . Quenched-flow experiments exploring this melting process are also underway, taking advantage of the ability of permanganate to oxidize unpaired thymine residues to report on blunt end strand separation.

Distinctive solvation patterns make renal osmolytes diverse

Authors: Prem Kumar Sinha, Ruby Jackson-Atogi, and Joerg Roesgen

Affiliation: Department of Biochemistry and Molecular Biology, Penn State College of Medicine, 500 University Drive, Hershey, PA 17033

Abstract: The aqueous solution within living cells is highly nonideal. The intracellular concentrations of some small molecules (osmolytes) are particularly high in organisms and organs that have adapted to different biochemical stress conditions, such as urea stress in the mammalian kidney. There exists a need to understand the physics of these osmolytes as they play protective role in several important normal and abnormal cellular processes.

With the objective of understanding the action of osmolytes we experimentally measured the partial molar volumes and osmotic activity coefficients for all renal osmolytes that have not yet been quantified, including myo-inositol, taurine and glycerophosphocholine. The osmotic coefficient is a measure of the deviation of the water chemical activity from ideal behavior. We derive the solvation properties of the osmolytes from these measured data. The result points towards three distinct categories of osmolytes: two extreme groups of osmolytes that either tend to self-associate (urea, taurine, myo-inositol) or show pure volume exclusion effects (trimethylamines), and a group that has intermediate properties. The self-association tendency of taurine and myo-inositol, which are protein stabilizers, is in contrast to the notion that self-associating osmolytes are denaturants. It is likely, though, that these osmolytes may interact in a different way with proteins than with other biomolecules. To test this idea, we are in the process of doing high-throughput experiments on the interactions between osmolytes and different macromolecules.

Development of a Selectively Fluorescent VHH Dimer Variant for Small Molecule Detection

Christopher Smith and James R. Horn, Northern Illinois University

Antibodies, which recognize target antigens with high affinity and specificity, have a variety of uses for in vitro applications. While intrinsic fluorescence-based ligand detection is not uncommon, this method requires the ligand to interact with aromatic residues of the antibody, which is not always possible without additional antibody engineering. In this study, we explore a homodimeric anti-caffeine VHH as a model system for developing a novel GFP-based fluorescent binding event. The construct consists of a single polypeptide containing both VHH domains, as well as two halves of a green fluorescent protein. The protein displays soluble expressed E. coli expression and exhibits a change in fluorescent signal upon binding to caffeine through bimolecular fluorescence complementarity. Isothermal titration calorimetry demonstrates that the caffeine binding of this variant is comparable to that of the wild-type anti-caffeine VHH. The structure of the wild-type VHH complex was analyzed to identify suitable residues in the VHH dimer interface of the GFP variant that may be mutated to weaken dimerization in the absence of caffeine to improve the fluorescent signal. Overall, this GFP-based construct may be generally useful for a quick visual screening of high affinity variants in a library design, and also for detection of specific ligands of interest in dipstick assays.

Engineering of protein conformational switches
sensitive to changes in physiological pH:
Design with internal Lys residues

J. L. Sorenson, E. Wheeler, I. Caturegli, M. Peck, J. L. Schlessman*, A. Robinson, and
B. García-Moreno E.

Department of Biophysics, Johns Hopkins University, Baltimore MD

*U.S. Naval Academy, Annapolis MD

The function of many proteins is modulated physiologically by conformational changes triggered by modest changes in pH. The pH-sensing element in naturally occurring pH-sensitive switches usually involves numerous ionizable groups, each with different pK_a values in the exposed and buried conformations. Rational engineering of pH-sensitive switches that respond to pH changes in the physiological range is challenging for two main reasons. First, it is difficult to tamper with multiple ionizable groups simultaneously without a corresponding loss of thermodynamic stability. Second, it is difficult to engineer proteins that respond to changes near neutral pH because very few ionizable groups in proteins titrate in this pH range. Unfortunately histidine, whose pK_a in water is 6.3, presents engineering challenges. Here we show how internal Lys residues buried in the hydrophobic interior of proteins can be used to engineer conformational switches that are activated by small pH changes in the physiological range. This approach works because these internal Lys residues can titrate with highly anomalous pK_a values depressed from their value in water, 10.4, all the way to the physiological pH range of 6 to 7. Our engineering efforts took advantage of knowledge of the pK_a values of Lys in 25 internal positions of staphylococcal nuclease. Using internal Lys residues we successfully engineered a variant of staphylococcal nuclease that unfolded completely and cooperatively over a small pH range centered around physiological pH.

The specific interaction of mRNA with CvfA protein through the KH domain in *Streptococcus pyogenes*

Gavin Sowa, Bingxin Yuan, Jinsai Shang, Bohui Jiang, Fanny Chu, Stephanie Geiser, Rebecca C. Weber, Gabriela C. Pérez-Alvarado, Kyu Hong Cho and Brian M. Lee

Department of Chemistry and Biochemistry, Southern Illinois University, Carbondale, Illinois

Abstract: *Streptococcus pyogenes* is responsible for causing a wide variety of human diseases such as scarlet fever, impetigo, pharyngitis, necrotizing fasciitis, streptococcal toxic shock syndrome and the post- streptococcal sequelae of rheumatic fever. It is a strictly human pathogen that can cause mild to life-threatening infections in a wide variety of tissues that present unique environmental and nutritional challenges. In order to thrive in these various conditions, *S. pyogenes* must somehow regulate gene expression to optimize the utilization of what is available in its surroundings. The conserved virulence factor A (CvfA) protein has four domains: a transmembrane domain at the N terminus, a coiled-coil domain, an hnRNP K homology (KH) domain towards the middle, and a histidine-aspartate-containing phosphohydrolase (HD) domain near the C terminus. We hypothesize that the CvfA protein of *S. pyogenes* uses its KH domain to target specific mRNA for degradation via cleavage by the HD domain. This mRNA decay is designed to control gene expression related to nutrient utilization and virulence based on nutrient availability. We have developed an effective strategy to express and purify the KH domain. This purified protein was used as a target for Systematic Evolution of Ligands by Exponential Enrichment (SELEX) studies to identify sequences with a high affinity for the KH domain. RNA binding affinity is measured by electrophoretic mobility shift assay (EMSA). Our future work will focus on using NMR chemical shift mapping to determine the structural details of how the KH domain recognizes mRNA.

Using Sedimentation Velocity to Assay the Self-assembly of ClpA Δ E286/565A in the Presence and Absence of Nucleotide

Ryan Stafford and Aaron Lucius

University of Alabama at Birmingham, Department of Chemistry, 1530 3rd Ave S,
Birmingham, AL 35294-1240

The AAA+ (ATPases Associated with various cellular Activities) group of proteins is a large superfamily whose members are present in single-celled and multi-celled organisms. Members of this superfamily are implicated with a myriad of cellular processes including membrane fusion and transport, DNA recombination and repair, DNA replication, protein disaggregation, and proteolysis of damaged proteins. This family is subdivided into two classes; Class I ATPases contain two ATPase domains per monomer while Class II ATPases contain one ATPase per monomer. Both classes utilize ATP binding and hydrolysis to undergo conformational changes that allow them to perform mechanical work. Further, most members of the AAA+ family exist as oligomers, typically as hexamers. ClpA is a Class I AAA+ protein. In order to study many of the structural and mechanistic aspects of wild-type ClpA, others have used ClpA variants that are deficient in hydrolysis activity at ATPase Domain I, II, or both Domain I and Domain II. Many approaches to investigating the enzymatic activities of ClpA require knowledge of the concentration of ClpA hexamers. Comparisons of the enzymatic activities for wild-type ClpA to any of the variants can be impacted if the population of hexamers for wild-type and variant are different. Here we show that the self-assembly of the hydrolysis deficient mutant ClpA Δ E286/565A is different from that of wild-type ClpA. Further, ClpA Δ E286/565A appears to assemble into hexamers in the absence of nucleotide and polypeptide substrate. In contrast, wild-type ClpA exists in a monomer-tetramer equilibrium in the absence of nucleotide and polypeptide substrate. These results suggest that the population of ClpA variant hexamers present at any given concentration of nucleotide will be different from the population of wild-type hexamers for the same nucleotide concentration. We anticipate the results of this study will set the stage for a better understanding of ClpA Δ E286/565A and how information obtained from examining this variant can be used to understand wild-type ClpA.

Structural and biophysical characterization of the CSL-RITA complex, a novel mechanism to regulate transcription in the Notch signaling pathway

Nassif Tabaja and Rhett Kovall

University of Cincinnati College of Medicine, Department of Molecular Genetics, Biochemistry, and Microbiology

The Notch pathway is an essential component of metazoan development and tissue homeostasis. Dysfunctional Notch signaling has been linked with cardiovascular disease, birth defects, and various cancers. Notch signaling ultimately results in changes in gene expression, which is regulated by the DNA binding protein CSL. CSL functions as both a repressor and activator of transcription from Notch target genes by interacting with transcriptional corepressors and coactivators, respectively. Recently, a new transcriptional coregulator has been identified, termed RITA, which binds CSL and facilitates its export out of the nucleus. RITA is thought to function as a corepressor by preventing the assembly of active transcription complexes at Notch target genes. However, the molecular details of the CSL-RITA complex are unknown. Here, using a combination of biophysical, biochemical/cellular, and structural techniques, we characterize the interaction between CSL and RITA. Using purified recombinant proteins, we show (1) a high affinity interaction between CSL and RITA using ITC; (2) the regions of RITA and CSL that are necessary and sufficient for complex formation by ITC; and (3) promising preliminary crystals of the CSL-RITA complex that will be important for future structural studies. Taken together, these data begin to elucidate at the molecular level the novel mechanism of Notch regulation mediated by RITA.

Prediction of Crystallographic B-factors for the backbone atoms of the protein Calbindin D_{9k} using Molecular Dynamics Simulations

Mahendra Thapa¹, Mark Rance²

¹University of Cincinnati, Department of Physics, Cincinnati, OH

²University of Cincinnati, Department of Molecular Genetics, Biochemistry & Microbiology, Cincinnati, OH

Molecular Dynamics (MD) simulation of a protein helps to study motion and its development with time which may not be studied experimentally in details. We used AMBER 12.0 software suit on GPU to simulate a protein Calbindin D_{9k}, which is involved in the uptake and transport of the calcium, on its four calcium binding states: a doubly-loaded state, two singly-loaded states and an apo state. Force field ff12SB and water model TIP3P at NVE condition were chosen in the simulation. The crystallographic B-factor is an indicator of relative vibrational motion of different atoms of a protein. The B-factors calculated from the analysis of the data obtained from MD for the doubly loaded state of the protein for its backbone atoms were compared with the corresponding experimental data and previously published results based on MD. Such B-factors were also calculated from MD generated data for these two singly loaded states and the apo state of the protein and then analyzed to study fluctuation and dynamical properties.

Breaking the disulfide bond induces a molten globule intermediate state in the von Willebrand Factor A1 domain that strengthens platelet adhesion.

Alexander Tischer, Pranathi Madde and Matthew Auton

Mayo Clinic Rochester/MN; Department of Hematology

The interaction of the platelet-receptor GPIb α with the A1-domain of the von Willebrand factor (vWF) is essential for an adhesion of platelets under shear stress to sites of vascular injury. Over the last two decades clinical mutations in the A1-domain have been described that affect the disulfide-bond which connects N- and C-termini of the domain. We have shown that the loss of the disulfide-bond unfolds the A1-domain to its intermediate state. Counter-intuitively, this unfolding results in increased affinity for GPIb α (Auton, Zhu, & Cruz, Biophys J. 2010).

This study rigorously compares native A1 with its intermediate state by using carboxyamidated A1 (RCAM A1) that lacks the single disulfide bond. Our results confirm earlier results (Miyata & Ruggeri, J Biol Chem. 1999) by showing the increased binding affinity using a new and improved platelet flow chamber approach. While normal platelets translocating on surface captured native A1 domain retain the catch bond character of pause times that increase as a function of shear rate at low shear and decrease as a function of shear rate at high shear, platelets that interact with RCAM A1 remain stably attached and do not translocate. In addition, solution biophysical studies (intrinsic protein fluorescence, ANS fluorescence, and fluorescence quenching by acrylamide) show that RCAM A1 exists as a molten globule that lacks tertiary structure or hydrophobic core. However, circular dichroism indicates that stable secondary structure elements remain and are likely responsible for the interaction with GPIb α . Based on our findings we propose that the extremely sensitive regulation of the A1-GPIb α interaction is due to folding of the tertiary structure of this domain. Removal of the tertiary structure by disrupting the disulfide bond destroys the regulatory mechanism of the A1-GPIb α interaction resulting in high strength interactions between platelets and vWF A1.

Biophysical Studies of the *S. aureus* SrrB-SrrA Two-component Signal Transduction System Involved in Toxic Shock Syndrome

Nitija Tiwari¹, Jonathan W. Willett², Caroline M. Linke², John R. Kirby² and Ernesto J. Fuentes¹

University of Iowa ¹Department of Biochemistry and ²Department of Microbiology

Bacterial two-component systems (TCS), composed of histidine kinases (HK) and a response regulator (RR), connect environmental stimuli to the appropriate cellular response. HKs are composed of an N-terminal extracellular sensing domain followed by a dimerization and phosphotransfer (DHp) domain and an ATP-binding catalytic (CA) domain. Upon ligand binding to the sensing domain, the CA domain transfers a phosphoryl group to the conserved histidine residue in the DHp domain. The phosphorylated HK then transfers the phosphoryl group onto a conserved aspartate residue in the receiver (REC) domain of the RR. The phosphorylated response regulator generates the output, which typically involves DNA binding to affect gene expression. Two-component signal transduction systems contribute to critical bacterial functions including virulence, resistance mechanisms and survival.

The SrrB-SrrA two-component system in *Staphylococcus aureus* regulates the production of the superantigen toxic shock syndrome toxin-1 (TSST-1), which is an important causative agent of Toxic Shock Syndrome. SrrB-SrrA has been found to negatively regulate exotoxin production in the absence of oxygen. The goal of our study is to determine the biochemical and structural mechanisms by which the SrrB-SrrA TCS regulates the production of TSST-1. Here we describe preliminary data towards this goal. Both SrrB and SrrA are multi-domain proteins. We have expressed and purified full-length and truncated SrrB and SrrA proteins and showed that they are biochemically active. Moreover, SrrB contains the typical HK (DHp-CA) domain and an N-terminal PAS (Per-Arnt-Sim) domain. We found that the PAS domain binds heme, suggesting that this domain may act as an O₂ sensor that plays an important role in regulation of the SrrB-SrrA system. These studies are beginning to reveal the regulatory mechanisms of the SrrB-SrrA TCS that might ultimately be exploited to develop novel strategies for inhibiting the production of the TSST-1 toxin.

Effect of Mutations on the Conformation of A-site RNA Hairpins

Amber R. Truitt and Ana-Maria Soto

Department of Chemistry, Towson University, 8000 York Rd., Towson, MD 21252

Aminoglycoside antibiotics bind to a ribosomal RNA (rRNA) region called the A-site and affect protein synthesis. Mutations on the bacterial rRNA A-site correlate with aminoglycoside antibiotic resistance. To better understand the effect of mutations on the binding properties of the A-site, we have investigated the stability, conformation and binding properties of model RNA hairpins.

Using the A-site of *M. tuberculosis* as a model system, we have compared the wild type sequence with a mutation associated with kanamycin resistance. Our results show that although the mutation is well tolerated in our A-site model, it significantly decreases the model's stability. The mutant decreases the T_m by 3.3 °C when inserted in a typical A-site model and more than 12 °C when the A-site model has short helical stems.

Binding experiments using circular dichroism show that the mutant binds kanamycin with similar or higher affinity, compared to the wild-type. However, UV-melts in the presence and absence of kanamycin suggest that the mutant and wild type may have different conformations. Hence, to avoid artifacts arising from different global conformations, we are investigating the binding of aminoglycoside antibiotics using fluorescence spectroscopy. Our results so far indicate that an aminopurine (AP) tag can be used to monitor the binding of kanamycin and paromomycin to the wild type A-site. However, the tag affects the conformation of the molecule such that the molecule containing an AP base binds less effectively than the untagged molecule. Results using different tag positions for the mutant and wild type will be discussed.

Our overall results indicate that the presence of base substitutions (mutations or fluorescent tags) on the A-site of *M. tuberculosis* affects the conformation around the conserved adenine bulge, which in turn affects the way aminoglycosides bind to this bulge.

This work was partially funded by Research Fellowships from the Fisher College of Science and Mathematics (ART) and the Towson University Undergraduate Research Committee (ART).

Defining the Chemical Rules for Successful Biomaterials Functionalization Via Gene Fusion

Shang-Pu Tsai, Hao-Ching Hsiao, Sarah. E. Bondos

Department of Molecular and Cellular Medicine, Texas A&M Health Science Center

Constructing replacements for damaged or diseased organs or tissues requires scaffolds that support cell growth in 3-dimensions and factors that regulate cell behavior. The existing methods (physical trapping, chemical crosslinking, etc.) to generate biomaterials are often performed in harsh conditions and usually harm sensitive molecules, such as proteins. Therefore, an effective and mild approach to generate the functional scaffold is needed.

We have discovered that the *Drosophila melanogaster* protein Ultrabithorax (Ubx) rapidly self-assembles into materials in mild buffers. *In vivo*, Ubx regulates transcription of crucial genes in animal development. *In vitro*, Ubx spontaneously assembles to form materials under gentle aqueous conditions at the air-liquid interface at low concentrations and fast timescales relative to other protein-based biomaterials. Consequently, we are able to functionalize these materials by fusing the Ubx gene with the gene of a functional protein. These constructs generate fused proteins that can both self-assemble and provide the function of interest.

One can imagine that the properties of the fused protein may determine whether its Ubx fusion can successfully form materials. To determine the impact of protein fusions on Ubx expression, solubility, and assembly, we have fused to Ubx more than 20 proteins that have different sizes, charges, and structural conformations. By testing the expression level, solubility and the ability to form material of all these fusions, we aim to define the limits of the gene fusion approach for Ubx materials. Hopefully, these efforts will enable us to predict whether an untested functional protein is likely to be successfully incorporated as a fusion protein into Ubx materials.

Cyclipostin derivatives as Inhibitors of Rat Hormone Sensitive Lipase

Elena Vasilieva, Raj Malla, Supratik Dutta, Ben Martin, Cynthia M. Dupureur,

Christopher D. Spilling.

Department of Chemistry & Biochemistry, Center for Nanoscience,

University of Missouri-St.Louis

Hormone sensitive lipase (HSL) is a protein involved in the hydrolysis of triglycerides to glycerol and free fatty acids (FFA) in cells. HSL is down regulated by insulin; in the case of type II diabetics, HSL is always activated. This leads to FFA accumulation in the bloodstream, which promotes cardiovascular disease. This problem makes HSL a drug target for type II diabetes. Rat HSL was cloned into a baculovirus expression system and purified using affinity chromatography. Cyclipostins, natural products, were found to be active low nM inhibitors of rat HSL using triolein as substrate (Laszlo Vertesy, et al., J. Antibiotics 2002, 55, 480-494). Cyclipostins consist of the bicyclic cyclophostin ring and an acyl tail off the phosphate. Cyclipostin R and P and its phosphonate analogs were synthesized and tested using radioactive lipase assay with triolein as a substrate (Hans Tornqvist et al., J. Lip. Res. 1972, 13, 424-426). Both isomers of cyclophostin were found to have high uM IC_{50} s. Cyclipostin P shows a low nM range inhibition against rat HSL with stereo selective preference for the trans diastereoisomer. Cyclipostin R shows low nM inhibition against rat HSL with stereo selectivity towards the cis diastereoisomer. Phosphonate analogs of cyclipostin P showed nM range of IC_{50} s, with HSL stereo selectivity towards cis diastereomer. IC_{50} s for phosphonate analogs of cyclipostin R are in nM range; HSL has no stereo selectivity towards either of diastereomers. Kinetic analysis was performed of cyclipostin P trans. The binding affinity of cyclipostin P trans is near the IC_{50} for this compound, about 10 nM. The inactivation rate for cyclipostin P trans is about 0.2 min^{-1} , meaning that it inhibits HSL quickly upon binding to its active site. The rate constant for reversibility of activation is close to zero, meaning the inhibitor is irreversible. Another set of monocyclic phosphonate compounds were tested on HSL. All compounds showed uM IC_{50} s.

Two step bending of DNA in binding site recognition by Integration

Host Factor

Yogambigai Velmurugu¹, Paula Vivas^{1,a}, Serguei V. Kuznetsov¹, Phoebe A. Rice²

and Anjum Ansari^{1,3}

¹Department of Physics, University of Illinois at Chicago, Chicago, IL 60607, USA;

²Department of Biochemistry & Molecular Biology, University of Chicago, Chicago IL 60637, USA;

³Department of Bioengineering, University of Illinois at Chicago, Chicago, IL 60607, USA;

^a Present address: Department of Molecular Virology, Immunology, and Medical Genetics, Ohio State University Wexner Medical Center, Columbus, OH 43210.

Many proteins that bind to specific sites on DNA locate these sites by first binding to DNA in a nonspecific way, and then scanning nearby sites for their specific binding site. Characterizing the rates and mechanism by which proteins switch from a nonspecific to their specific binding mode has been a challenge in the field. Here we demonstrate how the enhanced sensitivity and time-resolution of laser temperature-jump (T-jump) approach allows us to follow the detailed kinetics of DNA bending during binding site recognition by Integration Host Factor (IHF), an architectural protein ubiquitous in eubacterial systems. For IHF binding to one of its cognate sites on DNA, we can resolve at least two distinct steps in DNA bending in response to a T-jump perturbation. A rapid phase occurring on timescales of $\sim 100 \mu\text{s}$ is found to be independent of the DNA sequence, while a slower phase occurring on timescales of 1-10 ms is sequence-dependent. The amplitude of the fast component diminishes with increasing [salt] and is essentially absent at $[\text{KCl}] \approx 200 \text{ mM}$. We assign the fast component to nonspecific DNA bending by IHF prior to the formation of the specific complex; the slow component is assigned to the ultimate recognition step. These results represent the first observation of sub-millisecond DNA bending kinetics during binding site recognition that may bridge the gap between relatively slow ($> \text{ms}$) formation of specific complexes and relatively rapid ($< \text{ms}$) one-dimensional diffusion times measured for proteins scanning on DNA.

Conformational Selection is a Dominant Mechanism of Ligand Binding

Austin D. Vogt and Enrico Di Cera

Edward A. Doisy Department of Biochemistry and Molecular Biology
Saint Louis University School of Medicine, St. Louis, MO 63104

Molecular recognition in biological macromolecules is achieved by binding interactions coupled to conformational transitions that precede or follow the binding step, two limiting mechanisms known as conformational selection and induced fit, respectively. Sorting out the contribution of these mechanisms to any binding interaction remains a challenging task of general interest in biochemistry. Here we show that kinetics can never disprove conformational selection as a mechanism of ligand binding. Conformational selection is associated with a vast repertoire of kinetic behaviors and is sufficient to explain the relaxation kinetics documented experimentally for a large number of systems. On the other hand, induced fit features a narrow spectrum of kinetic behaviors and can be disproved in many cases. A paradigm shift emerges where conformational selection acquires pre-eminence as a mechanism of ligand binding, either alone or in combination with induced fit. This offers direct functional support to the emerging structural view of the macromolecule as a conformational ensemble from which ligands select the initial optimal fit to produce a biological response.

Determining Association Rates of Protein-protein Interactions Using a Quantitative Bead Aggregation Assay

Nagamani Vunnam, N. René Phongam, C. Ashton Walters, Nathan I. Hammer and Susan Pedigo

University of Mississippi, Department of Chemistry and Biochemistry, University, MS 38677

Developing new quantitative methods for studying the interactions of cell surface proteins is imperative to advance understanding of the molecular determinants of cell adhesion. Our interest in the dimerization kinetics of cadherin, a calcium-dependent cell-cell adhesion molecule, has motivated us to develop a quantitative bead aggregation assay to study the effects of calcium concentration on the kinetics of association. Although bead aggregation has been used for some time to study protein-protein interactions, the data are usually only interpreted qualitatively. Here we establish a quantitative method for studying the aggregation of beads that are coated with neural-cadherin. Data indicated exponential growth in aggregate size as a function of time. Time constants of association ranged from 18 to 3 minutes over a 100 fold increase in calcium concentration. We used this method to study the slow association kinetics of a mutant cadherin (NCAD1/R14E). These studies yielded a time constant of 28 minutes, which indicates that the association rate of mutant is slower than wild type (9 minutes). Experimental factors were controlled to ensure precision in the data including protein binding to beads, incubation time in the presence of calcium, mixing of the beads prior to placing on the slide and the residence time on the slide before data were acquired. This method is the first report of experimental determination of the kinetics of the association of proteins tethered to a surface, and is broadly applicable to study the kinetics of protein-protein interactions.

Ligand-Control in MDR protein functions

Herschel Wade, Sharrol Bachas, Brian Kohr and John Froehlig

The John Hopkins University School of Medicine, Baltimore, MD

BmrR is allosteric, multidrug-sensing switch that mediates the major efflux-related MDR response in *B. subtilis*. It does so by regulating the expression of the Bmr efflux pump in response to a diverse array of lipophilic cationic and neutral compounds. BmrR has been widely regarded as a prototype for studying MD recognition and MDR functions. However, until recently, a poor understanding of its drug recognition properties prevented insights into multispecific allostery and signal control. Using an elaborated in vitro transcription assay, we can now interrogate ligand-directed allostery in BmrR. Analyses of allostery in BmrR rely heavily on the determination of ligand-activation profiles. Crucial information is provided via solution and other electrophoretic-based techniques. The transcription data are well described by a Hill model that informs signal recognition, signal control and cooperative interactions. For a diverse array of ligands, the transcription profiles reveal an array of signal recognition and signal control properties. The ligands exhibit unique Hill coefficients (typically greater than one) implicating ligand-control at the level of BmrR-RNAP interactions. Analyses of ligand influences on BmrR action have enabled insights into both the ligand requirements and mechanism of transcription activation. Finally, we observe an atypical interplay between signal recognition and signal control. In contrast to highly-specific ligand responses, BmrR doesn't show the tighter binding—higher response relationship observed for many biological switch systems. Instead, to ensure an efficient, robust responses to various ligand-based signals, BmrR displays higher response transcriptional levels to weakly binding effectors, whereas high affinity binding elicits lower response levels. It is likely the other allosteric MDR systems will exhibits similar characteristics.

Folding and Hydrodynamics of the DNA i-motif from the c-MYC Promoter

Randy M. Wadkins^{†,*}, Samantha M. Reilly[†], Daniel Lyons[‡], John J. Correia[‡],
and David M. Jameson[§]

[†]Department of Chemistry and Biochemistry, University of Mississippi,
University, MS 38677

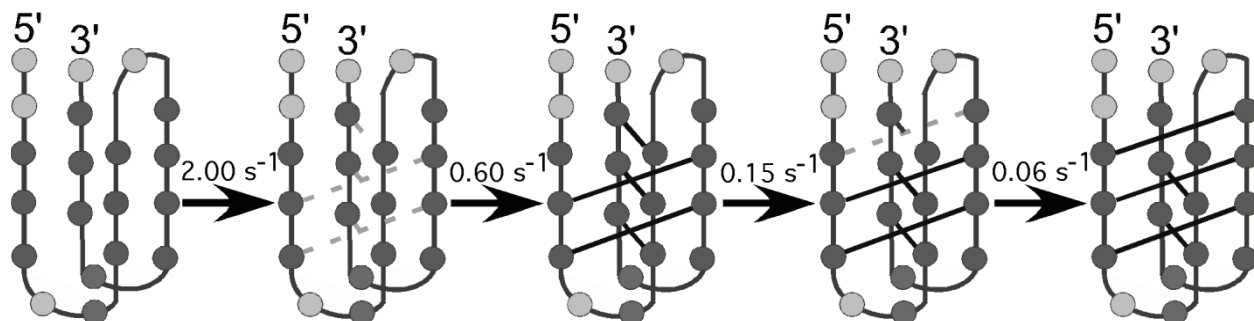
[‡]Department of Biochemistry, University of Mississippi Medical Center,
Jackson, MS 39216

[§]Department of Cell and Molecular Biology, University of Hawaii at Manoa,
Honolulu, HI 96822

*Corresponding Author: rwadkins@olemiss.edu

Abstract

In addition to its Watson-Crick conformation, DNA can adopt a variety of other structures such as G-quadruplexes (G4s) and i-motifs (iMs). G4s form in guanine-rich strands, whereas iMs form in cytosine-rich strands. Due to their topological variance from duplex DNA, their connection to transcription regulation, and their association with oncogene promoters, G4s and iMs have potential as unique drug-binding targets. Since iMs require protonation to form, they have not been studied to the extent that G4s have. Recently, however, it has been reported that iMs can form at neutral pH under molecular crowding conditions (Rajendran, et al., Chem. Commun. 46, 1299-1301 (2010)). Insight into the conformation dynamics, such as iM movement and shape in solution, can provide more details on how to target these structures. We have used circular dichroism, fluorescence anisotropy decay, fluorescence quenching, and ultracentrifugation data to characterize the folding pathway and final equilibrium conformation of the C-rich strand of the NHE III₁ promoter from the human c-MYC gene (Sequence: 5'-CTTCCCCACCCTCCCCACCCTCCCCA-3'). We labeled the strand with five individual 1,3-diaza-2-oxophenoxazine (tC⁰) substitutions (four of which participate in hydrogen-bonding and one in the loop). Using these oligos, we mapped the mechanism of iM folding and detail the hydrodynamics of the folded structure, as shown in the figure below.



A Two-Step Model for Protein-Gold Nanoparticle Interactions

Ailin Wang, Karthik Vangala, Dongmao Zhang, and Nicholas C. Fitzkee

Department of Chemistry, Mississippi State University

Protein-functionalized gold nanoparticles (AuNPs) can be widely used in biomedicine, and applications include drug delivery and cancer detection. While protein-AuNP interactions have been studied for years, the physical determinants of adsorption remain elusive. Determining the stoichiometry of binding has proven a challenge, and therefore interaction energies are difficult to interpret. We developed a straightforward 1D NMR method to quantitatively characterize the protein-AuNP interaction and to understand the mechanism of adsorption. Using a dataset of five proteins, ranging in size from three to 583 residues, we measured the stoichiometry of binding to AuNPs with a diameter of 13nm. We find that the stoichiometry of binding can be predicted based on simple geometric considerations assuming that proteins remain globular on the AuNP surface. We next tested the strength of the interaction by performing a competition assay with 2-mercaptobenzimidazole (MBI). GB3 is not able to displace MBI, but MBI can displace WT GB3 even after 24 hours of incubation at room temperature. Our data indicates that protein-AuNP interaction involves two processes: In the first phase of adsorption, binding is reversible and perturbations to the protein structure are relatively minor. In the second phase, which occurs much more slowly, the protein forms irreversible covalent attachments to the AuNP, effectively “hardening” on the surface.

Thermodynamics of Single-stranded and Double-stranded DNA binding by *Deinococcus radiodurans* and *Escherichia coli* RecA homologues

Jaycob D. Warfel and Vince J. LiCata

Department of Biological Sciences, Louisiana State University, Baton Rouge, LA 70803

Thermodynamic DNA binding studies were performed with RecA homologues from the organisms *Deinococcus radiodurans* and *Escherichia coli*. *D. radiodurans* has been shown to have a significantly more robust DNA repair capacity than *E. coli*, which helps it survive extremely high doses of ionizing radiation, as well as prolonged periods of desiccation. *D. radiodurans* RecA plays an integral part in the DNA repair process, and is proposed to perform homologous recombination via an inverse pathway from that found within *E. coli*. Fluorescence anisotropy assays were used to study RecA binding of DNA substrates at various concentrations of KCl and Kacetate. Both affinity and cooperativity were examined for twenty nucleotide long segments of either single stranded (ss) or double stranded (ds) DNA substrates using both Hill and McGhee-von Hippel analyses. Initial results indicate that *D. radiodurans* RecA binds to both ssDNA and dsDNA with roughly 10 fold higher affinity than *E. coli* RecA under equivalent solution conditions, with dissociation constants in the range of ~30nM versus ~300nM, respectively. *D. radiodurans* RecA also shows significantly higher cooperativity of binding to both ssDNA and dsDNA substrates, as assessed by both Hill and McGhee-von Hippel analyses. As regards substrate selectivity by the two different RecA species, initial analyses at low salt concentrations suggest that *E. coli* RecA shows greater affinity differences between ssDNA versus dsDNA binding than does *D. radiodurans* RecA. These results begin to provide thermodynamic evidence for some of the mechanistic differences in homologous recombination proposed for the *E. coli* versus *D. radiodurans* RecA homologues.

Kinetic mechanism of *Escherichia coli* ClpA catalyzed ATP hydrolysis and thermodynamic linkage to macromolecular assembly

Clarissa L. Weaver and Aaron L. Lucius

University of Alabama at Birmingham, Department of Chemistry, 1530 3rd Ave S,
Birmingham, AL 35294-1240

Escherichia coli ClpA is a motor protein that plays an important role in cell homeostasis by unfolding proteins either for degradation by proteolytic ClpP or for remodeling of misfolded proteins. Only hexameric ClpA, which forms upon nucleotide binding, can perform processive translocation required to unfold stable proteins. In the absence of nucleotide, ClpA exists in a dynamic equilibrium of lower order oligomeric states that may also hydrolyze ATP. A complete examination of the kinetic mechanism of ClpA catalyzed ATP hydrolysis requires analysis that incorporates the thermodynamic linkage to macromolecular assembly. The population of oligomeric states and therefore the activity of ClpA must be linked to ClpA concentration. Here we report, in contrast to published results, that the steady state hydrolysis of ATP by ClpA displays positive cooperativity. Additional experiments are underway to characterize the concentration dependence of additional kinetic parameters. We hypothesize that applying the methods used here to ClpA variants will reveal information on the respective roles of the two ATPase domains of the ClpA monomer. Furthermore, a deeper understanding of the ClpA mechanism will be generally applicable to the examination of other protein translocases, which are involved in a variety of important biological functions, as well as to examination of the 26 S proteasome, the eukaryotic homologue to prokaryotic ClpAP complex.

Mechanical Unfolding Studies of RNA Hairpins

Huizhong Xu¹, Muwen Kong⁴, Ryan Slack⁴, Krishna Murari Agarwal^{2,5}, Maumita Mandal^{1,2,3,4}

Department of ¹Physics, ²Chemistry and ³Biomedical Engineering, Carnegie Mellon University and ⁴Molecular Biophysics and Structural Biology Program, Pittsburgh, PA 15213, USA.

⁵Present Address: International Max Planck Research School, University of Hamburg, Germany.

RNA hairpin, the stem-loop structural motif, plays diverse roles in many biological processes such as transcription and translation. Here, we studied the folding of a model 22 bp RNA hairpin, P5ab, from *Tetrahymena thermophila* ribozyme [1, 2], using high resolution single-molecule optical tweezers. Typically the distances were accurately measured at sub nanometer range and forces within 0.1 pN. By mechanically unfolding and refolding the molecule under physiological conditions, we identified intermediate states towards the folding pathway of the hairpin. These intermediate states were then mapped onto the hairpin sequence by constant force measurements along with mutational strategies. Based on the measured probability distribution along the reaction coordinate, the free energy landscapes for P5ab and the mutants were constructed, which provide detailed information about the sequence-dependent folding kinetics and the transition barriers of the RNA hairpin structures.

REFERENCES

1. J. H. Cate, A. R. Gooding, E. Podell, K. Zhou, B. L. Golden, C. E. Kundrot, T. R. Cech, J. A. Doudna, Crystal structure of a group I ribozyme domain: principles of RNA packing. *Science* **273**, 1678 (1996).
2. J. Liphardt, B. Onoa, S. B. Smith, I. Tinoco Jr., C. Bustamante, Reversible unfolding of single RNA molecules by mechanical force. *Science* **292**, 733 (2001).

The self-assembly of a linear molecule

Yujia Xu, Rebecca Strawn and Parminderjeet Kaur

Department of Chemistry, Hunter College of the City University of New York, 695 Park Ave., New York, NY 10065

Our lab recently characterized a designed triple helical, collagen-mimetic peptide which forms native-like fibrils under physiological pH and temperature conditions. This work has implicated sequence repeats as being the minimal requirement for fibril formation; native collagen has inherent sequence repeats every 234 residues corresponding to its 67nm D-period. Proper self-assembly is crucial for collagen function, yet the mechanism of association is poorly understood. The characterized peptide is comprised of three repeating, identical sequences which are separated and flanked by stabilizing (GPP)₄ units. The fibrils form with a 35nm D-period, having a 10nm overlap and 25nm gap region, as visualized under TEM and AFM imaging. AUC and DLS data support that the fibrils are formed in solution, while computational and modeling work demonstrates that the fibrils are formed exclusively via repeat-unit staggering. New peptides comprised of only one and two sequence repeat units are currently being characterized to further test the role that sequence periodicity plays in fibril formation.

Characterizing the Folding and Unfolding Pathways for a Small Model Protein

Dinesh Kumar Yadav and Nicholas C. Fitzkee

Department of Chemistry, Mississippi State University

Kinetic and thermodynamic parameters can give a clear picture of protein folding-unfolding pathways. ZZ-Exchange NMR spectroscopy measures kinetic rates at each residue without altering the primary structure of the protein. This method measures rates of slow exchange by monitoring the ^1H - ^{15}N magnetization transfer during chemical exchange and can elucidate the energetic landscape of the folding-unfolding transition. We have collected the thermodynamic and kinetic parameters for a small IgG-binding domain of streptococcal protein G (GB3). Under partially denaturing conditions (when both the folded and unfolded states are significantly populated), we observe a 3-fold variation in folding rates, with the fastest rates occurring in the core of the protein. It is possible to develop this data into a model for folding in this small protein. In the future, these experiments will be used to determine whether the method of destabilization alters the folding pathway.

Probe and Nanoparticles diffusion in polymer gels

Xiaolu Zhang¹, Nathanael Stocke², J. Zach Hilt² Jason DeRouchey¹,

¹Department of Chemistry, University of Kentucky

²Department of Chemical and Materials Engineering, University of Kentucky

Controlled transport of nanoparticles (NPs) through biological gels, such as mucus or extracellular matrix (ECM), is critical to NP and drug delivery in cells. Using fluorescence correlation spectroscopy (FCS), we have studied transport and dynamics of probe molecules and charged dendrimers in polymeric gels. In the first study, transport of probe molecules was studied as a function of crosslinking density, crosslinker molecular weight, and water content during synthesis. In the second study, diffusion of cationic polyamidoamine (PAMAM) dendrimers in polymeric solutions was measured. Diffusion of PAMAM in the presence of charged polymer solutions greatly influenced the diffusion coefficients of PAMAM compared to the same uncharged polymer solution. The ability to control specific interactions to develop better mucus penetration nanoparticles and novel synthetic *in vitro* mucus model would be highly beneficial due to the significant work required to deliver drugs to tissues covered in this biological barrier to transport.

How Disordered? Assessing NMR-based Studies of Denatured Proteins

Yue Zhang and Nicholas C. Fitzkee

Department of Chemistry, Mississippi State University

The random-coil model has been the dominant model for unfolded proteins since the 1950's; however, some experiments have shown that unfolded proteins are biased toward specific conformations, which is apparently in conflict with the random-coil model. Recently, residual dipolar couplings (RDCs) and paramagnetic relaxation enhancement (PRE) rates have been applied to obtain a large amount of structural information on unfolded protein states. Typically, this data is interpreted in a framework of random-coil ensembles, and good agreement is observed between experiment and theoretical predictions from random coils. Here, we test whether nonrandom ensembles containing significant local organization can describe this data equally well. Using a complete set of RDC and PRE data for ubiquitin, we find no distinguishable difference between random-coil ensembles and ensembles containing 50% native structure. This number increases to 70% when inter-residue $^1\text{H}_\text{N}$ - $^1\text{H}_\text{N}$ RDCs are omitted, as is typically done. Thus, while it is important to measure as many RDCs are possible, even the best datasets may be insensitive to local organization in unfolded proteins.

Structural Homology ≠ Thermodynamic Equivalence

Michael T. Zimmermann¹, Alexander Tischer², Steve Whitten³, and Matthew Auton^{2*}

¹Division of Biomedical Statistics and Informatics, Department of Health Sciences Research, Mayo Clinic, Rochester, MN

²Division of Hematology, Departments of Internal Medicine and Biochemistry and Molecular Biology, Mayo Clinic, Rochester, MN

³Departments of Chemistry and Biochemistry, Texas State University, San Marcos, TX

*To whom correspondence is addressed, auton.matthew@mayo.edu.

The von Willebrand factor A1 and A3 domains are structurally homologous (average backbone RMSD = 1.6Å) yet, despite their homology, they display very different thermodynamics of protein unfolding. Urea and GndHCl denaturation show that while the A3 domain has a classical two-state unfolding character, the A1 domain exhibits three state character with a well-defined stable intermediate as determined from circular dichroism spectroscopy. To decipher the biophysical underpinnings of these experimental thermodynamic differences from their homologous structures, we have implemented an algorithm based on the structure-energy equivalence of domains (SEED; Porter and Rose, PNAS 2012), that parses the structures into residue specific contributions to the urea-induced unfolding *m*-values using transfer free energies. The algorithm calculates the ratio of the *m*-value for all fragments in isolation (excised) and within the context of structures (in-situ). The median *m*-values across all fragment lengths relative to the central amino acid of the fragment defines each residue's contribution to the cooperativity of unfolding, *excised* and *in-situ*. The median qualifying ratio ($QR = m_{excised}/m_{in-situ}$) identifies regions of high and low folding cooperativity and provides a discriminating thermodynamic scale for discerning the sequence and structural dependence of stability. Using the A3 domain as the reference structure, the difference in median QR's (A1-A3) identifies potential structural regions that contribute to thermodynamic deviations from two-state character in the A1 domain. These deviations correlate poorly with regions of sequence or spatial divergence, and cluster primarily around the A1-GPIb α binding interface. The largest differences occur within helices 2 and 3 which flank the β -strand that contacts the GPIb α β -hairpin. Interestingly this region of the A1 structure is known to harbor four clinically identified loss of function mutations that inhibit the A1-GPIb α interaction causing the bleeding diathesis, von Willebrand disease. The computed differences between structural homologues presented here establish the feasibility of predicting general features of a proteins thermodynamic folding landscape from a representative structure.

Alphabetical List of Participants

Roderico Acevedo
Pennsylvania State University
Chemistry
110 Chemistry Building
University Park, PA 16801
rodito01@gmail.com

Ugur Akgun
Coe College
Physics
Cedar Rapids, IA 52402
uakgun@coe.edu

Min An
University of Kentucky
Department of Chemistry
Lexington, KY 40506
man223@uky.edu

Casey T. Andrews
University of Iowa
Biochemistry
Iowa City, IA 52242
casey-andrews@uiowa.edu

Matthew Auton
Mayo Clinic
Internal Medicine and Biochemistry and
Molecular Biology / Division of Hematology
Rochester, MN 55905
auton.matthew@mayo.edu

Cory Ayres
University of Notre Dame
Department of Chemistry and Biochemistry
Notre Dame, IN 46556
cayres@nd.edu

David Bain
University of Colorado
Anschutz Medical Campus
12850 E Montview Blvd
Aurora, CO 80045
david.bain@ucdenver.edu

Brian Baker
University of Notre Dame
Department of Chemistry and Biochemistry
Notre Dame, IN 46556
Brian-baker@nd.edu

John T. Baker
Louisiana State University
Biological Sciences
Life Sciences Building
Baton Rouge, LA 70803
jbake46@lsu.edu

Doug Barrick
Johns Hopkins University
Biophysics
Jenkins Hall, 3400 N. Charles St.
Baltimore, MD 21218
barrick@jhu.edu

Monique Bastidas
Pennsylvania State University
Chemistry
110 Chemistry Building
University Park, PA 16802
bastidasmq@gmail.com

Dorothy Beckett
University of Maryland
Chemistry & Biochemistry
College Park, MD 20742
dbeckett@umd.edu

Peregrine C. Bell-Upp
Johns Hopkins University
Rm. 004 Jenkins Hall
3400 N Charles Street
Baltimore, MD 21218
pbellup1@jhu.edu

Or I. Benet
Mississippi State University
Emerson Lab
Starkville, MS 39759
oib2@msstate.edu

Yogini Bhavsar-Jog
University of Mississippi
Chemistry and Biochemistry. Box 803
University, MS 38677
bhavsar.yogini@gmail.com

Luis M. Blancas-Mejia
Mayo Clinic
Biochemistry and Molecular Biology
200 First St. SW Guggenheim 1518
Rochester, MN 55905
blancasmejia.luis@mayo.edu

Sydney J. Blevins
Notre Dame
Chemistry and Biochemistry
Notre Dame, IN 46556
sblevins@nd.edu

Sarah E. Bondos
Texas A&M Health Science Center
Molecular and Cellular Medicine
College Station, TX 77845
sebondos@tamhsc.edu

Micayla A. Bowman
University of Notre Dame
Department of Chemistry and Biochemistry
Notre Dame, IN 46556
mbowman3@nd.edu

Hiromi S. Brown
Louisiana State University
Biological Sciences
Baton Rouge, LA 70803
hbrown5@tigers.lsu.edu

Reid Brown
University of Iowa
Biochemistry
Iowa City, IA 52242
reid-brown@uiowa.edu

Jose A. Caro
Johns Hopkins University
Biophysics
3621 Roland Ave #1
Baltimore, MD 21211
tuticarogmail.com

Carlos A. Castaneda
University of Maryland
Chemistry and Biochemistry, Bldg 296
Biomolecular Sciences
College Park, MD 20742
carlos.a.castaneda@gmail.com

Catherine T. Chaton
University of Cincinnati
Molecular Genetics ML0524
231 Albert Sabin Way
Cincinnati, OH 45267
chatonc@mail.uc.edu

Chen Chen
Purdue University
Medicinal Chemistry and Molecular
Pharmacology
221 W Wood ST Apt 9
West Lafayette, IN 47906
chen991@purdue.edu

Kelly A. Churion
Texas A&M University
Molecular and Cellular Medicine, 1114
440 Reynolds Medical Bldg
College Station, TX 77843
franceschi@neo.tamu.edu

Patricia L. Clark
University of Notre Dame
Chemistry & Biochemistry
251 Nieuwland Science Hall
Notre Dame, IN 46556
Pclark1@nd.edu

James L. Cole
University of Connecticut
MCB U-3125
91 N. Eagleville Road
Storrs, CT 06269
james.cole@uconn.edu

Kelly J. Collins
University of Cincinnati
Molecular Genetics, Biochemistry,
and Microbiology
Cincinnati, OH 45267
kelly.reneau.collins@gmail.com

Keith Connaghan
University of Colorado
Anschutz Medical Campus
Dept. of Pharmaceutical Sciences
Aurora, CO 80045
keith.connaghan@ucdenver.edu

Erik C. Cook
University of Kentucky
Molecular & Cellular Biochemistry, Box 228
741 S. Limestone
Lexington, KY 40536
ecco223@g.uky.edu

Valeria A. Copello
Southern Illinois University
Chemistry & Biochemistry, 4409
Neckers 224
1245 Lincoln Dr.
Carbondale, IL 62901
perez@siu.edu

Peter Cornish
University of Missouri
Biochemistry
117 Schweitzer Hall
Columbia, MO 65211
cornishp@missouri.edu

Jack Correia
University of Mississippi Medical Center
Biochemistry
2500 N State St
Jackson, MS 39216
jcorreia@umc.edu

Trevor P. Creamer
University of Kentucky
Molecular & Cellular Biochemistry
741 S. Limestone Street
Lexington, KY 40536
Trevor.Creamer@uky.edu

Margaret A. Daugherty
Colorado College
Chemistry and Biochemistry
14 E. Cache La Poudre ST
Colorado Springs, CO 80918
margaret.daugherty@coloradocollege.edu

Rolando W. De Angelis
University of Colorado
Anschutz Medical Campus
Dept. of Pharmaceutical Sciences, C238
12850 Montview Blvd
Aurora, CO 80045
rolando.deangelis@ucdenver.edu

Gregory DeKoster
Washington University School of Medicine
Dept. of Biochemistry & Molecular
Biophysics 8231
660 S. Euclid Ave.
St. Louis, MO 63110
dekoster@biochem.wustl.edu

Jason E. DeRouchey
University of Kentucky
Chemistry
125 Chem-Phys Bldg
Lexington, KY 40506
derouchey@uky.edu

Enrico Di Cera
Saint Louis University
Biochemistry and Molecular Biology
St Louis, MO 63104
enrico@slu.edu

John D. Dignam
University of Toledo College of Medicine
Biochemistry and Cancer Biology
3000 Arlington Ave.
Toledo, OH 43614
david.dignam@utoledo.edu

Brian M. Doctrow
Johns Hopkins University
Biophysics, Jenkins Hall
3400 N. Charles St.
Baltimore, MD 21218
bdoctrow@jhu.edu

David E. Draper
Johns Hopkins University
Chemistry
3400 N Charles St
Baltimore, MD 21210
draper@jhu.edu

Igor Drobnak
University of Notre Dame
Department of Chemistry and Biochemistry
251 Niewland Science Hall
Notre Dame, IN 46556
idrobnak@nd.edu

Matt Dukes
University of Mississippi
Chemistry
University, MS 38677
mpdukes@go.olemiss.edu

Tori Dunlap
University of Central Arkansas
Dept. of Chemistry, Laney Hall
201 Donaghey Ave.
Conway, AR 72035
toribo@gmail.com

Cynthia Dupureur
University Missouri St. Louis
Chemistry & Biochemistry, MC 27
One Univ. Blvd.
St. Louis, MO 63121
cdup@umsl.edu

Elizabeth C. Duran
University of Alabama at Birmingham
Chemistry
Birmingham, AL 35294
eduran@uab.edu

Nadera R. Elkarmi
University of Nebraska Omaha
College of Pharmacy
Omaha, NE 68198
naderaemail@gmail.com

Joe Emerson
Mississippi State University
Chemistry, Box 9573
1115 Hand Lab
Mississippi State, MS 39762
jemerson@chemistry.msstate.edu

Nicole Fazio
University of Colorado
Anschutz Medical Campus
12850 E Montview Blvd
Aurora, CO 80045
Nicole.Fazio@ColoradoCollege.edu

Erik A. Feldmann
Washington University School of Medicine
Biochemistry & Molecular Biophysics
660 S. Euclid Ave.
St. Louis, MO 63110
feldmanne@biochem.wustl.edu

Aron W. Fenton
University of Kansas Medical Center
Biochemistry and Molecular Biology, 3030
3901 Rainbow Boulevard
Kansas City, KS 66213
afenton@kumc.edu

Christopher J. Fischer
University of Kansas
Physics and Astronomy, 1082 Malott
1251 Wescoe Hall Dr.
Lawrence, KS 66045
shark@ku.edu

Nickolas Fitzkee
Mississippi State University
Department of Chemistry, Box 9573
Mississippi State, MS 39762
nfitzkee@chemistry.msstate.edu

Karen Fleming
Johns Hopkins University
Biophysics
3400 North Charles Street
Baltimore, MD 21218
Karen.Fleming@jhu.edu

Michael G. Fried
University of Kentucky
Molecular and Cellular Biochemistry
BBSRB Building
741 S. Limestone
Lexington, KY 40536
michael.fried@uky.edu

Ernesto J. Fuentes
University of Iowa
Biochemistry
51 Newton Rd
Iowa City, IA 52242
ernesto-fuentes@uiowa.edu

Roberto Galletto
Washington University School of Medicine
St. Louis, MO 63110
galletto@biochem.wustl.edu

Bertrand Garcia-Moreno E
Johns Hopkins University
Biophysics
3400 N. Charles St.
Baltimore, MD 21218
bertrand@jhu.edu

Kathryn R. Geiger
Johns Hopkins University
Molecular Biophysics
701 Cathedral St, Apt 1002
Baltimore, MD 21201
krgeiger@jhu.edu

David P. Giedroc
Indiana University
Chemistry
800 E. Kirkwood Avenue
Bloomington, IN 47405
giedroc@indiana.edu

Michael K. Gilson
University of California - San Diego
School of Pharmacy, 736
9500 Gilman Drive
La Jolla, CA 92093
mgilson@ucsd.edu

Lisa M. Gloss
Washington State University
School of Molecular Biosciences
Pullman, WA 99164
lmgloss@wsu.edu

Lisa C. Golden
University of Iowa
Department of Biochemistry
Iowa City, IA 52242
lisa-golden@uiowa.edu

Chengxuan Guo
University of Iowa
Biochemistry
Iowa City, IA 52242
chengxuan-guo@uiowa.edu
319-335-7894

David Gvarjaladze
The Ohio State University
Chemistry and Biochemistry
Columbus, OH 43210
gvarjaladze.1@osu.edu

Kathleen B. Hall
Washington University Medical School
Biochemistry/Molecular Biophysics
660 S Euclid Ave, CB 8231
St Louis, MO 63110
kathleenhal@gmail.com

Christine Harmston
NanoTemper Technologies, Inc
1001 Bayhill Drive, Suite 200
San Bruno, CA 94066
christine.harmston@nanotemper-
technologies.com

Lance M. Hellman
Notre Dame
Chemistry and Biochemistry
Notre Dame, IN 46556
lhellman@nd.edu

Kate L. Henderson
Mississippi State University
Department of Chemistry
Mississippi State, MS 39762
klh567@msstate.edu

Troy Hendrickson
University of Minnesota Duluth
Department of Chemistry and Biochemistry
Duluth, MN 55812
hendr681@d.umn.edu.

Andrew B. Herr
University of Cincinnati College of Medicine
Molecular Genetics, Biochemistry &
Microbiology, ML0524
231 Albert Sabin Way
Cincinnati, OH 45267
andrew.herr@uc.edu

Anne Hinderliter
University of Minnesota Duluth
Department of Chemistry and Biochemistry
Duluth, MN 55812
ahinderl@d.umn.edu

Frances C. Hindt
University of Iowa
Department of Biochemistry
Iowa City, IA 52242
frances-hindt@uiowa.edu

James R. Horn
Northern Illinois University
Dept. Chemistry and Biochemistry
DeKalb, IL 60115
jrhorn@niu.edu

Timothy J. Horn
Mayo Clinic
Biochemistry and Molecular Biology
Rochester, MN 55902
Horn.Timothy@mayo.edu

Liam Hovey
University of Iowa
Carver College of Medicine
Department of Biochemistry
4-403 Bowen Science Bldg.
51 Newton Road
Iowa City, IA 52242
liam-hovey@uiowa.edu

Hao Ching Hsiao
Texas A&M Health Sciences Center
Molecular and Cellular Medicine, 1114
440 Rynolds Medical Buidling
College Station, TX 77840
haoching@neo.tamu.edu

James M. Hutchison
University of Kentucky
Chemistry
Lexington, KY 40506
polohesse108@gmail.com

Giselle Jacobson
University of Notre Dame
Department of Chemistry and Biochemistry
251 Nieuwland Science Hall
Notre Dame, IN 46556
gjacobso@nd.edu

Sriram Jakkaraju
Northern Illinois University
Department of Chemistry and Biochemistry
60115
DeKalb, IL 60115
sriram.niu@gmail.com

Sarah E. Johnson
University of Nebraska
Pharmaceutical Sciences
Omaha, NE 68198
sarahe.johnson@unmc.edu

Besik Kankia
The Ohio State University
Chemistry and Biochemistry
Columbus, OH 43210
kankia.1@osu.edu

Joseph R. Kasper
Purdue
Medicinal Chemistry and Molecular
Pharmacology
575 Stadium Mall Stadium Mall Drive
West Lafayette, IN 47907
jkasper@purdue.edu

Irine Khutsishvili
University of Nebraska Medical Center
Pharmaceutical Sciences
986025 Nebraska Medical Center
Omaha, NE 68198
i.khutsishvili@unmc.edu

Sean A. Klein
University of Iowa
Carver College of Medicine
Department of Biochemistry
4-403 Bowen Science Bldg.
51 Newton Road
Iowa City, IA 52242
sean-klein@uiowa.edu

Alexandra Klinger
University of Pennsylvania
Pharmacology
3610 Hamilton Walk
Philadelphia, PA 19104
aklinger@upenn.edu

Katrina N. Koc
Washington University in Saint Louis
Biochemistry
660 S Euclid Ave
St. Louis, MO 63110
katrina.koc@go.wustl.edu

Rhett A. Kovall
University of Cincinnati College of Medicine
Molecular Genetics, Biochemistry and
Microbiology
231 Albert Sabin Way CARE4836
Cincinnati, OH 45267
kovallra@ucmail.uc.edu

Alex Kozlov
Washington University in St. Louis
Biochemistry and Molecular Biophysics
660 S. Euclid Ave., 8231
St. Louis, MO 63110
kozlov@wustl.edu

Alexey V. Krasnoslobodtsev
University of Nebraska Medical Center
Department of Pharmaceutical Sciences
Omaha, NE 68105
akrasnos@unmc.edu

Maria Kurnikova
Chemistry Department
Carnegie Mellon University
Pittsburgh, PA 15217
kurnikova@cmu.edu

Katherine D. Launer-Felty
University of Connecticut
Molecular and Cell Biology, U-3125
91 N. Eagleville Rd.
Storrs, CT 6269
katherine.launer-felty@uconn.edu

Vu H. Le
Mississippi State University
Chemistry
310 President Circle
Mississippi State, MS 39762
vhl11@msstate.edu

Juliette Lecomte
Johns Hopkins University
Biophysics
3400N Charles St.
Baltimore, MD 21218
lecomte_jtj@jhu.edu

Brian M. Lee
Southern Illinois University Carbondale
Chemistry and Biochemistry, MC 4409
1245 Lincoln Drive
Carbondale, IL 62901
brianlee@siu.edu

Edwin A. Lewis
Mississippi State University
Chemistry, 9573
Mississippi State, MS 39762
elewis@chemistry.msstate.edu

Jing Li
Johns Hopkins University
Biophysics
Mudd Hall 104, 3400 N. Charles St
Baltimore, MD 21218
jli75@jhu.edu

Shuxiang Li
University of Iowa
Department of Biochemistry
Iowa City, IA 52242
shuxiang-li@uiowa.edu

Vince LiCata
Louisiana State University
Biological Sciences
Life Sciences Building
Baton Rouge, LA 70803
licata@lsu.edu

Jiabei Lin
University of Alabama at Birmingham
Chemistry
Birmingham, AL 35294
jiabei@uab.edu

Xu Liu
University of Iowa
Department of Biochemistry
51 Newton Road
Iowa City, IA 52242
xu-liu@uiowa.edu

Timothy M. Lohman
Washington University
Biochemistry and Molecular Biophysics
660 S. Euclid Ave., 8231
St. Louis, MO 63110
lohman@biochem.wustl.edu

Levan Lomidze
The Ohio State University
Chemistry and Biochemistry
Columbus, OH 43210
lomidze.2@osu.edu
614-688-1232

Aaron L. Lucius
University of Alabama at Birmingham
Department of Chemistry
4404 Longwood Drive
Gardendale, AL 35071
allucius@uab.edu

Jamie Luther
University of Notre Dame
Chemistry & Biochemistry
251 Nieuwland Science Hall
Notre Dame, IN 46556
jluther2@nd.edu

Venkata Machha
Mississippi State University
Chemistry, 9573
Mississippi State, MS 39762
vm236@msstate.edu

Ryan W. Mahling
University of Minnesota Duluth
Chemistry and Biochemistry
Duluth, MN 55812
mahli010@d.umn.edu

George Makhatadze
Rensselaer Polytechnic Institute
Biology
110 8th Street
Troy, NY 12065
makhag@rpi.edu

Krishna Mallela
University of Colorado
Anschutz Medical Campus
Department of Pharmaceutical Sciences
12850 E Montview Blvd, C238
Aurora, CO 80045
krishna.mallela@ucdenver.edu

Nasib K. Maluf
University of Colorado Denver
Department of Pharmaceutical Sciences
12850 E. Montview Blvd, C238
Aurora, CO 80045
karl.maluf@ucdenver.edu

Luis A. Marky
University of Nebraska Medical Center
Pharmaceutical Sciences
986025 Nebraska Medical Center
Omaha, NE 68198
lmarky@unmc.edu

Amy E. Martin
Mississippi State University
Chemistry
1110 Old Mayhew Rd
Starkville, MS 39759
aem224@msstate.edu

Dagan C. Marx
University of Iowa
Carver College of Medicine
Department of Biochemistry
4-403 Bowen Science Bldg.
51 Newton Road
Iowa City, IA 52242
dagan-marx@uiowa.edu

Daumantas Matulis
Vilnius University
Biothermodynamics and Drug Design
Graiciuno 8
Vilnius, Lithuania -2241
matulis@ibt.lt

Drew E. Menke
University of Missouri Columbia
Biochemistry
117 Schweitzer Hall
Columbia, MO 65211
dem4p2@mail.missouri.edu

Reagan Meredith
Texas State University
Biochemistry
2024 Meadowview Dr.
San Marcos, TX 78666
Rm1353@txstate.edu

Mark S. Miller
University of Iowa
Carver College of Medicine
Department of Biochemistry
4-403 Bowen Science Bldg.
51 Newton Road
Iowa City, IA 52241
mark-s-miller@uiowa.edu

Emma A. Morrison
Washington University in St. Louis
Biochemistry and Molecular Biophysics
660 S. Euclid Ave, Box 8231
St. Louis, MO 63110
eamorris@wustl.edu

Jeffrey K. Myers
Davidson College
Chemistry, 7120
Davidson, NC 28035
jemyers@davidson.edu

Whitnee L. Nettles
Mississippi State University
Chemistry
Starkville, MS 39759
wlw99@msstate.edu

Binh Nguyen
Washington University in St. Louis
Biochemistry & Molecular Biophysics 8231
660 S. Euclid Ave
St. Louis, MO 63110
bnguyen@wustl.edu

Eric M. Nicholson
National Animal Disease Center, USDA
Virus and Prion Diseases Research Unit
1920 Dayton Ave., PO Box 70
Ames, IA 50010
eric.nicholson@ars.usda.gov

Linda Nicholson
Cornell University
Department of Molecular Biology and
Genetics
Cornell University, Ithaca, NY 14853
lkn2@cornell.edu

Afua Nyarko
Oregon State University
Biochemistry and Biophysics
2011 ALS bldg
Corvallis, OR 97331
nyarkoa@onid.orst.edu

Oluwatoni J. Olayiwola
University of Iowa
Biochemistry
Iowa City, IA 52242
oluwatoni-olayiwola@uiowa.edu

Daniel J. Parente
University of Kansas Medical Center
Biochemistry and Molecular Biology, 3030
3901 Rainbow Blvd
Kansas City, KS 66160
dparente@kumc.edu

Tamar Partskhaladze
The Ohio State University
Department of Chemistry and Biochemistry
Columbus, OH 43210
partskhaladze.1@osu.edu

Susan Pedigo
University of Mississippi
Chemistry and Biochemistry
Coulter Hall
University, MS 38677
spedigo@olemiss.edu

Romel B. Perez
Texas State University
Biochemistry
1400 Clarewood Dr. Apt. 432
San Marcos, TX 78666
rbp22@txstate.edu

Gabriela C. Perez-Alvarado
Southern Illinois University
Chemistry & Biochemistry
4409, Neckers 146I
1245 Lincoln Dr.
Carbondale, IL 62901
perez@siu.edu

Vytautas Petrauskas
Vilnius University Institute of Biotechnology
Biothermodynamics and Drug Design
Vilnius, Lithuania 2241
vytautas.petrauskas@bti.vu.lt

Lois Pollack
Cornell University
Applied & Engineering Physics
271 Clark Hall
Ithaca, NY 14853
lp26@cornell.edu
607-255-8695

Lauren L. Porter
Potomac Affinity Proteins
9601 Medical Center Dr., #330
Rockville, MD 20855
lperski2@jhu.edu

Nicola Pozzi
Saint Louis University
Edward A. Doisy Research Center
Dept. of Biochemistry & Molecular Biology
1100 South Grand Ave
St. Louis, MO 63104
nicolapozzi@gmail.com

Matthew R. Preimesberger
Johns Hopkins University
T.C. Jenkins Dept. of Biophysics
3400 N. Charles St.
Baltimore, MD 21218
matt.preimesberger@gmail.com

Anika M. Rannikko
University of Minnesota Duluth
Department of Chemistry and Biochemistry
Duluth, MN 55812
ranni005@d.umn.edu

Calliste Reiling
University of Nebraska Medical Center
Pharmaceutical Sciences
Omaha, NE 68198
calliste.reiling@unmc.edu

Daniel E. Richman
Johns Hopkins University
Biophysics
Baltimore, MD 21210
d.e.richman@jhu.edu

Aaron C. Robinson
Johns Hopkins University
Department of Biophysics
Jenkins Hall Room 110
3400 N Charles St
Baltimore, MD 21218
aaronr561@yahoo.com

Andrew Robertson
National Psoriasis Foundation
Research
405 S. Summit St.
Iowa City, IA 52240
arobertson@psoriasis.org

Catherine A. Royer
Rensselaer Polytechnic Institute
Biological Sciences
110 8th Street
Troy, NY 12180
catherine.royer@cbs.cnrs.fr

David Scott
School of Bioscience
University of Nottingham
Sutton Bonington Campus
Leicestershire, LE12 5RD, UK
david.scott@nottingham.ac.uk

Kevin A. Sforza
Johns Hopkins University
Biophysics
2961 Keswick Rd
Baltimore, MD 21211
sforza.kevin@gmail.com

Madeline A. Shea
University of Iowa
Carver College of Medicine
Department of Biochemistry
4-403 Bowen Science Bldg.
51 Newton Road
Iowa City, IA 52242
madeline-shea@uiowa.edu

Catie Shelton
University of Cincinnati College of Medicine
Molecular Genetics, Biochemistry &
Microbiology
Cincinnati, OH 45267
leimbacf@mail.uc.edu

Scott A. Showalter
The Pennsylvania State University
Department of Chemistry
104 Chemistry Building
University Park, PA 16802
sas76@psu.edu

Scott A. Shubert
University of Iowa
Biochemistry
Iowa City, IA 52242
scott-shubert@uiowa.edu

Michael J. Simon
Washington University in St. Louis
Biochemistry & Molecular Biophysics
660 S. Euclid Ave
St. Louis, MO 63109
simonm@biochem.wustl.edu

Prem K. Sinha
Penn State College of Medicine
Biochemistry and Molecular Biology, H171
500 University Drive
Hershey, PA 17033
pks14@psu.edu

Christopher Smith
Northern Illinois University
Department of Chemistry & Biochemistry
Dekalb, IL 60115
csmith57@niu.edu

Jaime Sorenson
Johns Hopkins University
Department of Biophysics
3400 N. Charles Street
Baltimore, MD 21218
jsorens6@jhu.edu

Ana-Maria Soto
Towson University
Dept. Chemistry
8000 York Road
Towson, MD 21252
asoto@towson.edu

Gavin M. Sowa
Southern Illinois University-Carbondale
Chemistry and Biochemistry MC 4409
1245 Lincoln Dr.
Carbondale, IL 62901
Gavin.Sowa@siu.edu

Ryan P Stafford
University of Alabama at Birmingham
Department of Chemistry
Birmingham, AL 35294
rstaff@uab.edu

Walter Stafford
Boston Biomedical Research Institute
AUCRL
64 Grove Street
Watertown, MA 02472
wstafford3@walterstafford.com

Wyatt Strutz
NanoTemper Technologies, Inc
1001 Bayhill Drive, Suite 200
San Bruno, CA 94066
christine.harmston@nanotemper-
technologies.com

Liskin Swint-Kruse
University of Kansas Medical Center
Biochem & Mol Biol MSN 3030
3901 Rainbow Blvd
Kansas City, KS 66160
lswint-kruse@kumc.edu

Nassif H. Tabaja
University of Cincinnati
Molecular Genetics, Biochemistry, and
Microbiology, CARE 4836
231 Albert Sabin Way
Cincinnati, OH 45267
tabajanf@mail.uc.edu

Kristin M. Tefft
University of Iowa
Carver College of Medicine
Department of Biochemistry
4-403 Bowen Science Building
51 Newton Road
Iowa City, IA 52242
kristin-tefft@uiowa.edu

Mahendra B. Thapa
University of Cincinnati
Physics
688 Riddle Rd., apt # 1400 F
Cincinnati, OH 45220
thapamb@mail.uc.edu

Alexander Tischer
Mayo Clinic
Hematology
Stabile Bldg 6th floor
Rochester, MN 55901
tischer.alexander@mayo.edu

Nitija Tiwari
University of Iowa
Biochemistry
51 Newton Road
Iowa City, IA 52242
nitija-tiwari@uiowa.edu

Robert J. Trachman
Johns Hopkins University
Biophysics
208 W. 29th St.
Baltimore, MD 21218
rtrachman@jhu.edu

Amber R. Truitt
Towson University
Chemistry
119 Olen Dr.
Glen Burnie, MD 21061
atruitt1@students.towson.edu

Shang-Pu Tsai
Texas A&M Health Science Center
Molecular and Cellular Medicine
440 Reynolds Medical Building
College Station, TX 77843
tsai@medicine.tamhsc.edu

Elena Vasilieva
University of Missouri St. Louis
Chemistry
10366 Sannois Drive Apt.4
St. Louis, MO 63146
evz7c@mail.umsl.edu

Yogambigai Velmurugu
University of Illinois, Chicago
Physics MC273
845 West Taylor street
Chicago, IL 60607
vyogam2@uic.edu

Austin Vogt
Saint Louis University
Edward A. Doisy Department of
Biochemistry and Molecular Biology
6114 Washington Blvd
St. Louis, MO 63112
advogt@gmail.com

Nagamani Vunnam
University of Mississippi
Chemistry and Biochemistry
122 Coulter Hall
University, MS 38677
nvunnam@olemiss.edu

Herschel V. Wade
Johns Hopkins University School of
Medicine
Biophys. Biophys. Chem
725 N Wolfe Street
Baltimore, MD 21205
herschel.wade@jhmi.edu

Vincent M. Waldman
Washington University
Biochemistry and Molecular Biophysics
St. Louis, MO 63118
waldmanv@biochem.wustl.edu

Jaycob D. Warfel
Louisiana State University
Biological Sciences
1788 Mullen Drive
Baton Rouge, LA 70810
jwarfe1@lsu.edu

Clarissa Weaver
University of Alabama at Birmingham
Chemistry
901 14th St S
Birmingham, AL 35294
cldurie@uab.edu

Steven T. Whitten
Texas State University
Chemistry and Biochemistry
601 University Drive
San Marcos, TX 78666
steve.whitten@txstate.edu

Sandra G. Williams
Washington University
Biochemistry & Molecular Biophysics
660 S Euclid Ave
St Louis, MO 63110
williamss@msnotes.wustl.edu

Robert Wright
University of Mississippi Medical Center
Biochemistry
Jackson, MS 39202
rtwright@umc.edu

Randy Wadkins
Department of Chemistry and Biochemistry
University of Mississippi
University, MS 38677
rwadkins@olemiss.edu

Huizhong Xu
Carnegie Mellon University
Chemistry
4400 Fifth Ave
Pittsburgh, PA 15213
hXu1@andrew.cmu.edu

Yujia Xu
Hunter College
Chemistry
695 Park Ave.
New York, NY 10065
yujia.xu@hunter.cuny.edu

Dinesh K. Yadav
Mississippi State University
Chemistry PO Box 9573
Mississippi State, MS 39762
dky12@msstate.edu

Xiaolu Zhang
University of Kentucky
Chemistry
505 Rose Street
Lexington, KY 40506
xiaolu.zhang@uky.edu

Yue Zhang
Mississippi State University
Department of Chemistry
Mississippi State, MS 39762
yz302@msstate.edu

Xinji Zhu
Louisiana State University
Biological Sciences
Life Sciences Building
Baton Rouge, LA 70803
xinjizhu1989@gmail.com

Valeria Zai-Rose
University of Mississippi Medical Center
Biochemistry
Jackson, MS 39216
vzairose@umc.edu

Michael T. Zimmermann
Mayo Clinic
Division of Biomedical Statistics and
Informatics
Department of Health Sciences Research
Rochester, MN 55905
Zimmermann.Michael@mayo.edu

Participants by Lab

Ugur Akgun Lab

Ugur Akgun

Anjum Ansari Lab

Yogambigal Velmurugu

Matthew Auton Lab

Matthew Auton

Alexander Tischer

Michael Zimmermann

Paul Axelsen Lab

Alexandra Klinger

David Bain Lab

David Bain

Keith Connaghan

Rolando De Angelis

Nasib Maluf

Nicole Fazio

Brian Baker Lab

Brian Baker

Cory Ayres

Sydney Blevins

Lance Hellman

Elisar Barbar Lab

Afua Nyarko

Doug Barrick Lab

Doug Barrick

Kathryn Geiger

Kevin Sforza

Dorothy Beckett Lab

Dorothy Beckett

Sarah Bondos Lab

Sarah Bondos

Kelly Churion

Hao Ching Hsiao

Shang-pu Tsai

Philip Bryan Lab

Lauren Porter

Patricia Clark Lab

Patricia Clark

Micayla Bowman

Igor Drobnak

Giselle Jacobson

Jamie Luther

Jim Cole Lab

Jim Cole

Katherine Launer-Felty

Peter Cornish Lab

Peter Cornish

Drew Menke

Jack Correia Lab

Jack Correia

Valeria Zai-Rose

Robert Wright

Trevor Creamer Lab

Trevor Creamer

Erik Cook

Peggy Daugherty Lab

Peggy Daugherty

Jason DeRouchey Lab

Jason DeRouchey

Min An

James Hutchison

Xiaolu Zhang

Enrico Di Cera Lab

Enrico Di Cera

Nicola Pozzi

Austing Vogt

John Dignam Lab

John Dignam

David Draper Lab

David Draper

Robert Trachman

Tori Dunlap Lab

Tori Dunlap

Cynthia Dupureur Lab

Cynthia Dupureur
Elena Vasilieva

Adrian Elcock Lab

Casey Andrews
Reid Brown
Chengxuan Guo
Shuxiang Li
Oluwatoni Olayiwola
Scott Shubert

Joe Emerson Lab

Joe Emerson
Or Benet
Kate Henderson
Amy Martin
Whitnee Nettles

Aron Fenton Lab

Aron Fenton

Christopher Fischer Lab

Christopher Fischer

Nicholas Fitzkee Lab

Nicholas Fitzkee
Dinesh Yadav
Yue Zhang

Karen Fleming Lab

Karen Fleming

Michael Fried Lab

Michael Fried

Ernesto Fuentes Lab

Ernesto Fuentes
Lisa Golden
Frances Hindt
Xu Liu
Nitija Tiwari

David Fushman Lab

Carlos Castaneda

Roberto Galleto Lab

Roberto Galleto
Erik Feldmann
Katrina Koc

Bertrand Garcia-Moreno E. Lab

Bertrand Garcia-Moreno E.
Peregrine Bell-Upp
Brian Doctrow
Daniel Richman
Aaron Robinson
Jaime Sorenson
Jose Caro

David Giedroc Lab

David Giedroc

Michael Gilson Lab

Michael Gilson

Lisa Gloss Lab

Lisa Gloss

Kathleen Hall Lab

Kathleen Hall
Gregory DeKoster
Sandra Williams

Katherine Hezler-Wildman Lab

Emma Morrison

Andrew Herr Lab

Andrew Herr
Catherine Chaton
Catie Shelton

Vincent Hilser Lab

Jing Li

Anne Hinderliter Lab

Anne Hinderliter
Ryan Mahling
Anika Rannikko
Troy Hendrickson

James Horn Lab

James Horn
Sriram Jakkaraju
Christopher Smith

Besik Kankia Lab

Besik Kankia
David Gvarjaladze
Levan Lomidze
Tamar Partskhaladze

Rhett Kovall Lab

Rhett Kovall
Kelly Collins
Nassif Tabaja

Maria Kurnikova Lab

Maria Kurnikova

Juliette Lecomte Lab

Juliette Lecomte
Matthew Preimesberger

Brian Lee Lab

Brian Lee
Gavin Sowa

Edwin Lewis Lab

Edwin Lewis
Vu Le
Venkata Machha

Vince LiCata Lab

Vince LiCata
Tod Baker
Hiromi Brown
Jaycob Warfel
Xinji Zhu

Timothy Lohman Lab

Timothy Lohman
Alex Kozlov
Binh Nguyen
Michael Simon
Vincent Waldman

Aaron Lucius Lab

Aaron Lucius
Elizabeth Duran
Jiabei Lin
Ryan Stafford
Clarissa Weaver

Yuri Lyubchenko Lab

Alexey Krasnoslobodtsev

George Makhatadze Lab

George Makhatadze

Krishna Mallela Lab

Krishna Mallela

Maumita Mandal Lab

Huizhong Xu

Luis Marky Lab

Luis Marky
Nadera Elkarmi
Sarah Johnson
Irine Khutsishvili
Calliste Reiling

Daumantas Matulis Lab

Daumantas Matulis
Vytautas Petrauskas

Jeffery Myers Lab

Jeffery Myers

NanoTemper Technologies, Inc.

Chrisine Harmston
Wyatt Strutz

Eric Nicholson Lab

Eric Nicholson

Linda Nicholson Lab

Linda Nicholson

Chiwook Park Lab

Chen Chen
Joseph Kasper

Susan Pedigo Lab

Susan Pedigo
Matt Dukes
Nagamani Vunnam

Gabriela Perez-Alvarado Lab

Gabriela Perez-Alvarado
Valeria Copello

Lois Pollack Lab

Lois Pollack

Marina Ramirez-Alvarado Lab

Luis Blancas-Mejia
Timothy Horn

Mark Rance Lab

Mahendra Thapa

Andrew Robertson Lab
Andrew Robertson

Jörg Rösken Lab
Prem Sinha

Catherine Royer Lab
Catherine Royer

David Scott Lab
David Scott

Madeline Shea Lab
Madeline Shea
Liam Hovey
Sean Klein
Dagan Marx
Mark Miller
Kristin Tefft

Scott Showalter Lab
Scott Showalter
Roderico Acevedo
Monique Bastidas

Ana-Maria Soto Lab
Ana-Maria Soto
Amber Truitt

Walter Stafford Lab
Walter Stafford

Liskin Swint-Kruse Lab
Liskin Swint-Kruse
Daniel Parente

Herschel Wade Lab
Herschel Wade

Randy Wadkins Lab
Randy Wadkins
Yogini Bhavsar-Jog

Steve Whitten Lab
Steve Whitten
Reagan Meredith
Romel Perez

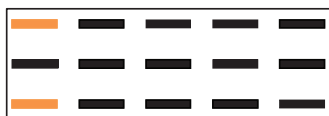
Yujia Xu Lab
Yujia Xu

Avanti Snoopers™

CHARACTERIZE PROTEIN-LIPID INTERACTION

Snoopers™ consist of various lipid-subtypes spotted individually on a solid support. Snoopers™ provide a protein-lipid interaction profile to identify cell signaling events, characterize lipid-binding antibodies, and identify novel lipid-interacting proteins of potential therapeutic value. With Snoopers™, lipid-protein interactions may be probed using standard chemiluminescent detection methods in order to better understand the molecular mechanism of interactions between cellular components.

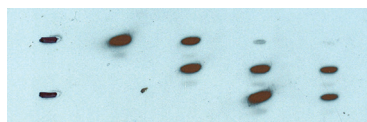
INOSITOL SNOOPERS™



Ref.	18:1 PI(3,5)P2	18:1 PI(4,5)P2	18:1 PI(3,4,5)P3	DMPC
Ref.	16:0 PI	18:1 PI	18:1 PI(3)P	18:1 PI(3,4)P2
Ref.	Liver PI	Soy PI	Brain PI(4)P	Brain PI(4,5)P2

Avanti Inositol Snoopers™ containing 1 µg of lipid per spot were probed with the WR304 antibody, which recognizes PIP and PIP₂.

Chemiluminescent Detection

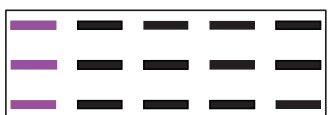


1 µg Lipid per spot

Avanti Number
330500

Primary Antibody:
WR304 - 1:1,000 (1,200 ng/ml)
Secondary Antibody:
Anti-Mouse HRP -
1:10,000 (35 ng/ml)

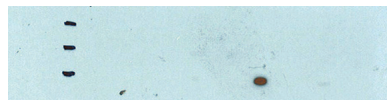
OXIDIZED PHOSPHOLIPID SNOOPERS™



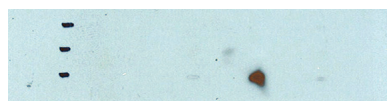
Ref.	Liver PI	16:0 PI	oxPAPS	DMPS
Ref.	oxPAPE	DMPE	oxPAPG	DMPG
Ref.	oxPAPA	DMPA	oxPAPC	DMPC

Avanti Ox-phospholipid Snoopers™ containing 0.5 µg or 1 µg of lipid per spot were probed with Avanti's E06 antibody, which recognizes the phosphocholine headgroup of oxidized phospholipid that is present in oxidized LDL. E06 does not bind to unoxidized PC or normal LDL.

Chemiluminescent Detection



0.5 µg Lipid per spot



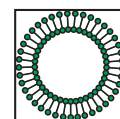
1 µg Lipid per spot

Avanti Number
330501

Primary Antibody:
E06 - 1:5,000 (200 ng/ml)
Secondary Antibody:
Anti-Mouse HRP -
1:10,000 (35 ng/ml)

CUSTOM SNOOPERS™

Please contact a Product Specialist to discuss your needs for Custom Lipid Snoopers™



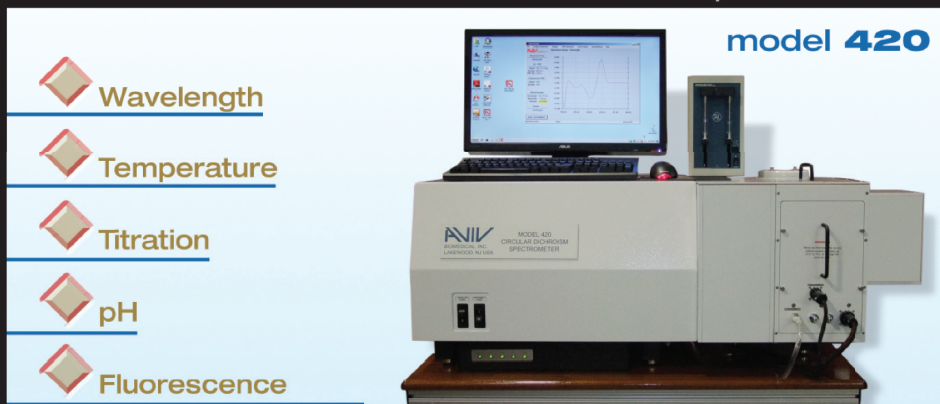
Avanti®
POLAR LIPIDS, INC.

MORE DETAILS AT AVANTILIPIDS.COM



biomedical, inc.

750 Vassar Avenue, Suite 2
Lakewood, New Jersey 08701-6929 USA



- Wavelength
- Temperature
- Titration
- pH
- Fluorescence

Phone (732) 370-1300 • Fax (732) 370-1303
info@avivbiomedical.com • www.avivbiomedical.com

HORIBA

Scientific



FluoroLog fluorometers combine superior performance, versatility and speed to produce accurate data from the widest range of samples. From our instruments to support to surprising affordability, HORIBA Scientific is dedicated to...

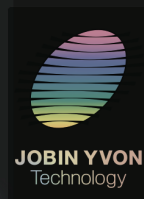
The art of perfection.



HORIBA Scientific Fluorologs:
Simply the best fluorometers built today.

www.fluorsolutions.com
email: info.sci@horiba.com

Solutions for:
Elemental Analysis
Fluorescence
Ellipsometry
Raman
OEM Spectrometry
Optical Components
Forensics
Particle Characterization



chronos

FLUORESCENCE

- Time-Resolved
- Steady-State
- TCSPC
- Phase & Modulation



CHRONOS FEATURES:

- TCSPC and/or Phase & Modulation
- Dual emission channels for T-format polarization and kinetics
- Fully computer-controlled
- Programmable sequence of measurements
- Upgradable to steady-state



Frequency-domain or time-domain fluorometer with picosecond resolution.

When you Think CD...Think *JASCO*

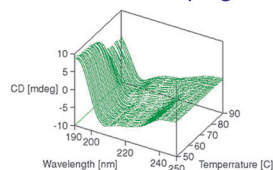
The World's Leading Circular Dichroism Spectrometer -- New! J-1500

JASCO's unparalleled optical performance and simultaneous measurement modes make the J-1500 a true "Chiro-optical Spectroscopy Workbench." With the ability to measure CD, Absorbance, and Fluorescence versus wavelength and temperature on up to six samples at a time, the J-1500 can provide considerable information to characterize samples faster than ever before. Also available are options for stopped-flow kinetics, auto titration, high-throughput CD and many others all controlled under the Spectra Manager II™ software platform.



J-1500 Circular Dichroism Spectrometer

Thermal Ramping



Multiple Position Peltier Cell Holder



MPTC-513



▶ **MicroScale Thermophoresis**

a technology by NanoTemper®

Instruments for the measurement of all biomolecular interactions

- ▶ Measure affinities without surface immobilization
- ▶ Measure label-free or with a sensitive fluorophore
- ▶ Measure with a few μl at pM concentration
- ▶ Measure in all buffers or in biofluids



Understand Biomolecular Interactions Better!

MST Technology is fast, sensitive, easy to use and affordable.

www.nanotemper-technologies.com



*The 27th Annual Gibbs Conference on Biothermodynamics
would like to offer special thanks for strong support from*



*University of Mississippi Medical Center
Department of Biochemistry <http://biochemistry.umc.edu/>*