Modeling of Biomolecular Systems Interactions, Dynamics, and Allostery: Bridging Experiments and Computations

SEPTEMBER 10–14, 2014 | ISTANBUL, TURKEY

Biophysical Society

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September 2014

Dear Colleagues,

It is our great pleasure to welcome you to the Biophysical Society Thematic Meeting on *Modeling of Biomolecular Systems Interactions, Dynamics, and Allostery: Bridging Experiments and Computations*. We have an exciting program, covering a broad range of topics, with the participation of leading scientists, both experimental and computational, in the field. We strongly hope that the meeting will not only provide a venue for sharing our recent progress, findings and pending questions, but also foster new collaborations and many stimulating discussions toward gaining deeper insights into challenging problems of molecular biophysics and systems biology. We also encourage you to take part in social and cultural activities, because Istanbul has a lot to offer.

Thank you all for joining our meeting, and we look forward to having a very enjoyable four day event together.

Best regards,

Ivet & Ozlem

Ivet Bahar, Ph.D.
Distinguished Professor and J. K. Vries Chair
Dept. of Computational & Systems Biology
School of Medicine
University of Pittsburgh
United States

Ozlem Keskin, Ph.D.
Professor
Chemical and Biological Engineering Dept.
Koc University
Turkey

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GENERAL INFORMATION

Registration Hours

The registration desk is located in the lobby of Hall A at the American Hospital. Registration hours are as follows:

Wednesday, September 10	2:00 PM – 7:00 PM
Thursday, September 11	8:00 AM – 3:00 PM
Friday, September 12	8:00 AM – 1:15 PM
Saturday, September 13	8:00 AM – 6:20 PM
Sunday, September 14	8:00 AM – 11:30 AM

Instructions for Presentations

(1) Presentation Facilities:

A data projector will be made available in Hall A. Speakers are required to bring their laptops. Speakers are advised to preview their final presentations before the start of each session.

(2) Poster Session:

1) All poster sessions will held in Hall B.

2) A display board measuring 85cm (2.7 feet) wide by 170 cm (5.5 feet) high will be provided for each poster. Poster boards are numbered according to the same numbering scheme as in the abstract book.

3) Posters being presented on Thursday, September 11, should be set up on the morning of September 11 and removed by 6:00 PM on September 11. Posters being presented on Saturday, September 13, should be set up on the morning of September 13 and removed by 6:00 PM on September 13.

4) During the poster session, presenters are requested to remain in front of their poster boards to meet with attendees.

5) All posters left uncollected at the end of the meeting will be disposed of.

Coffee Breaks

Coffee breaks will be held in the lobby of Hall A where tea, coffee, and snacks will be provided.

Internet

Wifi is available in the lobby and meeting rooms of the American Hospital.

Smoking

Please be advised that smoking is not permitted inside the American Hospital.

Meals

Lunches (September 11-13) are included in the registration fee and the Gala Reception/Dinner for those that confirmed attendance in advance. The Welcome Reception/Gala Dinner will be held at the Divan Hotel. Buffet lunch will be provided in the cafeteria on the 7th floor of the American Hospital on September 11-13.

Sightseeing Tours

There will be two optional tours organized for attendees and/or accompanying guests.

1. Visit to the Old City on Friday, September 12, at 2:00 PM. *(Tentative fee is $50 per person, including transportation, entry tickets to Haghia Sophia, Topkapi, Blue Mosque, dinner and an English-speaking guide)*

2. Bosphorus Boat Tour on Saturday, September 13, at 6:30 PM. (*Fee is $55 per person, including dinner)*

Pre-registration is required for the tours. If you have signed up for the tours, please pay the fees at the on-site registration desk.

Name Badges

Name badges are required to enter all scientific sessions and poster sessions. Please wear your badge throughout the conference.

Contact

If you have any further requirements during the meeting, please contact the meeting staff at the registration desk from September 10-14 during registration hours.

In case of emergency, you may contact the following organizers/staff:

Dorothy Chaconas: dchaconas@biophysics.org
Ozlem Keskin: okeskin@ku.edu.tr
Ivet Bahar: bahar@pitt.edu

Or call the Divan Hotel at 90 (212) 315 55 00 and ask to leave a message in their room.

Modeling of Biomolecular Systems Interactions, Dynamics, and Allostery: Bridging Experiments and Computations

Istanbul, Turkey
September 10-14, 2014

PROGRAM

All functions will be held in Hall A of the American Hospital unless otherwise noted.

Wednesday, September 10, 2014

Welcome & Session I:	**Allosteric Transition in Proteins and How They Relate to Function** Co-Chairs: Amnon Horovitz, Weizmann Institute of Science, Israel Rebecca Wade, Heidelberg University, Germany
4:00 - 4:20 PM	Welcome/Opening Remarks: Ivet Bahar, University of Pittsburgh School of Medicine, USA Ozlem Keskin, Koc University, Turkey
4:20 - 4:50 PM	Amnon Horovitz, Weizmann Institute of Science, Israel *Distinguishing between Allosteric Mechanisms Using Structural Mass-Spectrometry Is Demonstrated for the Chaperonin GroEL*
4:50 - 5:20 PM	Ruth Nussinov, Tel-Aviv University, Israel, and NIH, USA *Ras: A Structural Biologist View and Questions*
5:20 - 5:50 PM	Rebecca Wade, Heidelberg University, Germany *Organism-adapted Specificity of Allosteric Regulation of Central Metabolic Enzymes in Lactic Acid Bacteria*
5:50 - 6:10 PM	Tom McLeish, Durham University, United Kingdom* *Predicting Global Low Frequency Protein Motions in Allostery without Conformational Change: Application to CRP/FNR Family Transcription Factors*
6:10 - 6:30 PM	Vanessa Ortiz, Columbia University, USA* *Quantifying Signal Propagation and Conformational Changes in Allosteric Proteins*

**Short talks selected from among submitted abstracts*

6:30 - 7:00 PM	Banu Ozkan, University of Arizona, USA *Mechanism of Protein Evolution: Conformationaly Dynamics and Allostery*	
7:15 - 9:30 PM	Welcome Reception/Gala Dinner	**Divan Hotel**

Thursday, September 11, 2014

Session II:	**Protein Interactions and Complex Systems Modeling I: Evolution and Function** Co-Chairs: Janet Thornton, European Bioinformatics Institute (EMBL-EBI), United Kingdom Burkhard Rost, Technical University of Munich, Germany	
8:45 - 9:15 AM	Janet Thornton, European Bioinformatics Institute (EMBL-EBI), United Kingdom *The Evolution of Enzyme Mechanisms and Functional Diversity*	
9:15 - 9:45 AM	Anne-Claude Gavin, EMBL Heidelberg, Germany *Lipid-Protein Networks*	
9:45 - 10:00 AM	Sebnem Essiz Gokhan, Kadir Has University, Turkey* *Soman Induced Conformational Changes of Human Acetylcholine Esterase*	
10:00 - 10:30 AM	Burkhard Rost, Technical University of Munich, Germany *Evolution Teaches Predicting Protein Interactions from Sequence*	
10:30 - 10:45 AM	Andrew Pohorille, NASA Ames Research Center, USA* *Proteins with Novel Function, Structure and Dynamics*	
10:45 - 11:00 AM	Srinath Krishnamurthy, National University of Singapore, Singapore* *How Enzymes Access Caged Substrates? Phosphodiesterase-Protein Kinase A Interactions Mediate Hydrolysis of PKA Receptor Bound Cyclic AMP*	
11:00 - 11:30 AM	**Coffee Break**	**Hall A Lobby**
Session III:	**Protein Interactions and Complex Systems Modeling II: Cell Regulatory and Signaling Mechanisms** Co-Chairs: Leslie Loew, University of Connecticut Health Center, USA Anna Panchenko, National Center for Biotechnology Information, NIH, USA	

**Short talks selected from among submitted abstracts*

11:30 AM - 12:00 PM	Leslie Loew, University of Connecticut Health Center, USA *Clusters and Comets: Regulation of Actin Assembly*
12:00 - 12:30 PM	Anna Panchenko, National Center for Biotechnology Information, NIH, USA *Regulation of Protein-Protein Binding and Pathway Crosstalk*
12:30 - 1:00 PM	Zaida Luthey-Schulten, University of Illinois at Urbana-Champaign, USA *Stochastic Simulations of Cellular Processes: from Single Cells to Colonies*
1:00 - 1:15 PM	Yaman Arkun, Koc University, Turkey* *Modeling and Dynamic Analysis of Feedback Loops of the Insulin and Angiotensin II Signalling Systems*
1:15 - 3:00 PM	**Lunch & Poster Session I** **Hall B**
3:00 PM	Free Afternoon

Friday, September 12, 2014

Session IV:	**Structure and Dynamics I: From Molecular Fluctuations to Supramolecular Machinery** Co-Chairs: Ada Yonath, Weizmann Institute of Science, Israel Klaus Schulten, Beckman Institute, University of Illinois at Urbana-Champaign, USA
9:00 - 9:30 AM	Ada Yonath, Weizmann Institute of Science, Israel *What Was First, the Genetic Code or Its Products?*
9:30 - 10:00 AM	Klaus Schulten, Beckman Institute, University of Illinois at Urbana-Champaign, USA *The Photosynthetic Membrane of Purple Bacteria as a Clockwork of Atomic and Electronic Level Processes*
10:00 - 10:30 AM	Canan Atilgan, Sabanci University, Turkey *How Much Can Local Dynamical Features of Proteins Can Inform on Conformational Possibilities?*
10:30 - 10:45 AM	Savas Tay, ETH Zurich, Switzerland* *Molecular Noise Facilitates NF-κB Entrainment under Complex Dynamic Inputs*

**Short talks selected from among submitted abstracts*

10:45 - 11:00 AM	Günther Peters, Technical University of Denmark, Denmark* *Self-assembly of a Glucagon-like Peptide 1 Analogue: Bridging Experiment and Simulations*
11:00 - 11:30 AM	**Coffee Break** **Hall A Lobby**
Session V:	**Membrane Proteins II: Interactions and (Neuro) Signal Transduction** Co-Chairs: Lukas Tamm, University of Virginia, USA Christine Ziegler, The Max Planck Institute for Biophysics, Germany
11:30 AM - 12:00 PM	Lukas Tamm, University of Virginia, USA *Fold to Fuse: The F2F Code of SNAREs on Membranes*
12:00 - 12:30 PM	Ingo Greger, MRC Laboratory of Molecular Biology, United Kingdom *Dynamics and Function of the AMPA Receptor N-Terminal Domain*
12:30 - 1:00 PM	Christine Ziegler, The Max Planck Institute for Biophysics, Germany *Two Is Better than One: Molecular Mechanism of Sodium Coupling in the Betaine Transporter BetP*
1:00 - 1:15 PM	Liviu Movileanu, Syracuse University, USA* *Engineered Protein Nanopores for Challenging Tasks in Molecular Diagnosis*
1:15 - 2:00 PM	**Lunch** **Cafeteria**
2:00 PM	Free Afternoon

Saturday, September 13, 2014

Session VI:	**Drug Discovery I: From Molecular Modeling to Systems Pharmacology** Co-Chairs: John Overington, European Bioinformatics Institute (EMBL-EBI), United Kingdom Celia Schiffer, University of Massachusetts Medical School, USA

**Short talks selected from among submitted abstracts*

8:45 - 9:15 AM	John Overington, European Bioinformatics Institute (EMBL-EBI), United Kingdom *Data Mining Large-Scale Bioactivity Datasets to Find Patterns in Ligand Recognition*
9:15 - 9:45 AM	Celia Schiffer, University of Massachusetts Medical School, USA *Combating Drug Resistance: Lessons from the Viral Proteases of HIV and HCV*
9:45 - 10:00 AM	Serdar Durdagi, Bahcesehir University, Turkey* *In Silico Studies on K-RAS-PDEδ Interaction Inhibition to Design Novel Anti-Cancer Drugs*
10:00 - 10:30 AM	William Eaton, NIDDK, NIH, USA *Sickle Cell Hemoglobin: Allostery, Aggregation Kinetics, and Search for a Drug*
10:30 - 10:45 AM	Serdar Kuyucak, University of Sydney, Australia* *Computational Design of Drugs for Autoimmune Diseases from Peptide Toxins*
10:45 - 11:00 AM	Judith Klein-Seetharaman, University of Warwick, United Kingdom* *Molecular Speciation, Dynamics and Interactions of Lipid Droplets with Proteins*
11:00 - 11:30 AM	**Coffee Break** **Hall A Lobby**
Session VII:	**Protein-Protein Interactions** Co-Chairs: Zhiping Weng, University of Massachusetts Medical School, USA Nikolay Dokholyan, University of North Carolina at Chapel Hill, USA
11:30 AM - 12:00 PM	Zhiping Weng, University of Massachusetts Medical School, USA *Protein-Protein Docking and Design*
12:00 - 12:30 PM	Attila Gursoy, Koc University, Turkey *Structural Networks of Signaling Pathways on Proteome Scale: Challenges and Opportunities*
12:30 - 1:00 PM	Nikolay Dokholyan, University of North Carolina at Chapel Hill, USA *Controlling Allosteric Networks in Proteins*

**Short talks selected from among submitted abstracts*

1:00 - 1:15 PM	Lee-Wei Yang, National Tsing Hua University, Taiwan* *Intramolecular Communication Based on Time-dependent Linear Response Theories*
1:15 - 3:00 PM	**Lunch & Poster Session II** **Hall B**
Session VIII:	**Protein Structure & Dynamics II: From Molecular Fluctuations to Supramolecular Machinery** ***In Honor of Professor Erman – Celebrating 40 Years of Science*** Co-Chairs: Ken A. Dill, SUNY at Stony Brook, USA Turkan Haliloglu, Bogazici University, Turkey
3:15 - 3:30 PM	Introduction Ivet Bahar, University of Pittsburgh School of Medicine, USA
3:30 - 4:00 PM	Ken A. Dill, SUNY at Stony Brook, USA *Integrative Modeling of Proteins*
4:00 - 4:20 PM	Batu Erman, Sabanci University, Turkey *Protein-Protein Interactions that Inhibit the Activity of the p53 Tumor Suppressor*
4:20 - 4:40 PM	Andrzej Kloczkowski, The Ohio State University, USA *From Polymer Rubberlike Elasticity to Protein Dynamics – How Simple Physical Models of Rubber Influenced Modern Biophysics*
4:40 - 5:05 PM	Malcolm Walkinshaw, University of Edinburgh, United Kingdom *Allosteric Regulation of the Glycolytic Pathway in Mammals and Trypanosomes*
5:05 - 5:30 PM	Turkan Haliloglu, Bogazici University, Turkey *Sequence Variations and Allosteric Dynamics in Binding*
5:30 - 5:55 PM	Robert Jernigan, Iowa State University, USA *Extracting Dynamics Information from Multiple Molecular Structures and Computationally Generating Their Transition Pathways*
5:55 - 6:20 PM	Burak Erman, Koc University, Turkey *Fractal Structure of Interaction Pathways in Proteins and Prediction of Allosteric Paths*

**Short talks selected from among submitted abstracts*

Sunday, September 14, 2014

Session IX:	**Protein Interactions, Dynamics, and Dysfunction** Co-Chairs: Shoshana Wodak, The Hospital for Sick Children, and University of Toronto, Canada David Perahia, CNRS, France
9:00 - 9:30 AM	Shoshana Wodak, The Hospital for Sick Children, and University of Toronto, Canada *Role of Histidine Protonation in the pH Induced Changes in Prion Protein Stability*
9:30 - 9:45 AM	Pemra Doruker, Bogazici University, Turkey *Effect of Ligand Binding on Enzyme Global Dynamics and Functions*
9:45 - 10:15 AM	David Perahia, CNRS, France *A New Approach for Exploring Free Energy Landscapes of Large Structural Changes: Molecular Dynamics with Excited Collective Motions (MDeNM)*
10:15 - 10:30 AM	Erik Marklund, University of Oxford, United Kingdom* *Restraining Molecular Dynamics and Modeling With Ion-Mobility Mass Spectrometry*
10:30 - 10:45 AM	Jocelyne Vreede, University of Amsterdam, The Netherlands* *Modeling the Binding of H-NS to AT-rich DNA*
10:45 - 11:00 AM	Yaakov (Koby) Levy, Weizmann Institute of Science, Israel* *Protein-DNA Interactions: Fine Balance between High Affinity and Fast Kinetics*
11:00 - 11:30 AM	Closing Remarks and *Biophysical Journal* Poster Awards

**Short talks selected from among submitted abstracts*

SPEAKER ABSTRACTS

Distinguishing between Allosteric Mechanisms Using Structural Mass-Spectrometry Is Demonstrated for the Chaperonin GroEL

Amnon Horovitz.
Weizmann Institute of Science, Rehovot, Israel.

Allosteric regulation is often described by the concerted Monod–Wyman–Changeux or sequential Koshland–Némethy–Filmer models of cooperativity. In general, however, it has been impossible to distinguish between these allosteric models using ensemble measurements of ligand binding in bulk protein solutions. In this talk, a new structural mass-spectrometry approach will be described that breaks this impasse by providing the full distribution of ligand-bound states of a protein complex. Given this distribution, it is possible to determine all the binding constants of a ligand to a highly multimeric cooperative system and, thus, infer its allosteric mechanism. The approach will be demonstrated for the chaperonin GroEL that consists of two back-to-back stacked heptameric rings with a cavity at each end where protein folding can take place. GroEL displays intra-ring positive cooperativity and inter-ring negative cooperativity in ATP binding, with respect to ATP, that are crucial for its function. It will be shown that this new approach provides evidence for a concerted mechanism of allosteric switching and information on the ATP-loading pathway. The impact of the concerted nature of the intra-ring allosteric transitions of GroEL on its folding function will be discussed.

Ras: a Structural Biologist View and Questions

Ruth Nussinov.
NCI, Frederick, MD, USA.

Ras proteins are small GTPases that act as signal transducers between cell surface receptors and several intracellular signaling cascades. KRas4B is among the frequently mutated oncogenes in human tumors. Ras proteins consist of highly homologous catalytic domains, and flexible C-terminal hypervariable regions (HVRs) that differ significantly across Ras isoforms. We have been focusing on key mechanistic questions in Ras biology from the structural standpoint. These include whether Ras forms dimers, and if so what is their structural landscape; how do Ras dimers activate Raf, a key Ras effector in a major signaling pathway; how calmodulin inhibit Raf signaling, and the potential role of the hypervariable region and its membrane anchoring regulation. We believe that structural biology, computations and experiment, are uniquely able to tackle these fascinating questions.

Organism-adapted Specificity of Allosteric Regulation of Central Metabolic Enzymes in Lactic Acid Bacteria

Rebecca C. Wade[1,2], Stefan Henrich[1], Anna Feldman-Salit[1,2], Nadine Veith[1,2], Ursula Kummer[2], Vlad Cojocaru[1,3].
[2]Heidelberg University, Heidelberg, Germany, [1]Heidelberg Institute for Theoretical Studies, Heidelberg, Germany, [3]Max Planck Institute for Molecular Biomedicine, Münster, Germany.

Allosteric regulation provides one important strategy for adaptation of an organism to its environment and for cross-talk between metabolic pathways. Lactic acid bacteria have adapted to a range of different environments where they have diverse effects ranging from being antibiotic-resistant pathogens causing severe disease to healthy probiotics used in the food industry. We focused on the enzymes, lactate dehydrogenase [1] and pyruvate kinase [2], which both play central roles in the metabolism of lactic acid bacteria. These enzymes need to react quickly to changes in the environment and, therefore, their activity is strictly regulated. We developed and applied computational techniques to predict the allosteric modulators that are responsible for the activation or inhibition of these enzymes in four different bacteria, and to explore the effects of environmental conditions on the allosteric regulation. The studies of both proteins show how enzymes with high sequence similarity can have subtle but significant differences in allosteric regulation in related organisms that must function in different environments.

[1] Anna Feldman-Salit, Silvio Hering, Hanan L Messhia, Nadine Veith, Vlad Cojocaru, Antje Sieg, Hans V. Westerhoff, Bernd Kriekemeyer, Rebecca C Wade and Tomas Fiedler. Regulation of the activity of lactate dehydrogenases from four lactic acid bacteria. J. Biol. Chem., (2013) 288:21295-306.
[2] Nadine Veith, Anna Feldman-Salit, Vlad Cojocaru, Stefan Henrich, Ursula Kummer and Rebecca C Wade. Organism-adapted specificity of the allosteric regulation of pyruvate kinase in lactic acid bacteria. PLoS Comput Biol., (2013) 9:e1003159.

Funding from the Klaus Tschira Foundation and the German Federal Ministry of Education and Research (BMBF, SysMO-LAB2 Projects 0313979A, 0315788B) is gratefully acknowledged.

Predicting Global Low Frequency Protein Motions in Allostery without Conformational Change: Application to CRP/FNR Family Transcription Factors

Tom C. McLeish[1], David Burnell[1], Martin J. Cann[1], Emkhe Pohl[1], Shane A. Richards[1], Thomas L. Rogers[2], Philip D. Townsend[1], Mark R. Wilson[1].
[1]Durham University, Durham, United Kingdom, [2]University of Manchester, Manchester, United Kingdom.

Our objective is a foundational theory for how allostery can occur as a function of thermally-excited low frequency dynamics without a change in protein structure, together with predictive tools for protein design and modification [1-4].

We have generated coarse-grained models that describe the protein backbone motions of the homodimeric CRP/FNR family transcription factors, Catabolite Activated Protein (CAP) of Escherichia coli and GlxR of Corynebacterium glutamicum [3]. We demonstrate that binding the first molecule of cAMP ligand modulates the global normal modes resulting in negative co-operativity for binding the second cAMP ligand without a change in mean structure. Crucially, the value of the co-operativity is itself controlled by the interactions around a set of third allosteric “control sites”.

The theory makes key experimental predictions, validated by analysis of variant proteins by a combination of structural biology and isothermal calorimetry. A quantitative description of allostery as a free energy landscape revealed a protein ‘design space’ that identified the key inter- and intramolecular regulatory parameters that frame CRP/FNR family allostery. Furthermore, by analyzing naturally occurring CAP variants from diverse species, we demonstrate an evolutionary selection pressure to conserve residues crucial for allosteric control. The methodology establishes the means to engineer allosteric mechanisms that are driven by low frequency dynamics [5].

[1] R.J. Hawkins and T.C.B. McLeish, Phys. Rev. Letts, 2004, 93, 098104
[2] R. J. Hawkins and T. C. B. McLeish, Biophys. J., 2006, 91, 2055-2062
[3] H. Toncrova and T.C.B. McLeish, Biophys. J., 2010 98, 2317-2326
[4] T. C. B. McLeish, T. L. Rodgers and M. R. Wilson, Phys. Biol. 2013 10 056004
[5] T.L. Rogers et al., PLOS-Biology, 2013 11(9): e1001651

Quantifying Signal Propagation and Conformational Changes in Allosteric Proteins

Andre A. S. T. Ribeiro, **Vanessa Ortiz**.
Columbia University, New York, NY, USA.

Allostery connects subtle changes in a protein's potential energy surface to significant changes in its function. Understanding this phenomenon and predicting its occurrence are major goals of current research in biophysics and molecular biology. At the microscopic level, protein energetics is characterized by a balance between different inter-atomic interactions, with small perturbations at specific sites potentially leading to major changes in conformational distributions. Therefore, a thorough characterization of allostery requires understanding of two aspects: (1) how energy propagates through the protein structure, and (2) which regions of the protein are likely to suffer structural deformations as a response to the applied perturbation.

On the first aspect, we have developed a new energy-based network analysis method, which allows characterization of signaling pathways in proteins. The method assumes that signals travel more efficiently through residues that have strong inter-atomic interactions, and is able to correctly identify important residues for allosteric signal propagation in the allosteric enzyme imidazole glycerol phosphate synthase. In addition, we introduce a quantity named energetic coupling, which is able to discriminate allosterically active mutants of a known allosterically regulated protein, the lactose repressor (LacI). Commonly used protein structure networks based on correlation coefficients or number of inter-residue contacts, are not able to reproduce our results.

On the second aspect, we show that the calculation and analysis of atomic elastic constants of LacI, highlights regions that are particularly prone to suffer structural deformation, and are experimentally linked to allosteric function. The calculations are based on a high resolution, all-atom description of the protein, but are computationally inexpensive when compared to methods employing the same resolution. Lower resolution models are shown to yield qualitatively different results, indicating the importance of adequately describing the local environment surrounding the different parts of the protein.

Mechanism of Protein Evolution: Conformationaly Dynamics and Allostery

Banu Ozkan.
Arizona State University, Tempe, AZ, USA.

The first crystal structure was solved in late 1950, which revolutionized our ability to understand protein function. However, much more revolutionary information came after, when we learned that proteins dynamically interconvert between conformations in the native state. Indeed, the critical role of protein dynamics has become well recognized in various biological functions, including allosteric signaling and protein ligand recognition electron transfer etc. Likewise in protein evolution, the classical view of the one sequence-one structure-one function paradigm (the Pauling and Landsteiner proposal) is now being extended to a new view: an ensemble of conformations in equilibrium that can evolve new functions. Therefore, understanding inherent structural dynamics are crucial to obtain a more complete picture of protein evolution. A small local structural change due to a single mutation can lead to a large difference in conformational dynamics, even at quite distant residues due to allostery. We have recently analyzed conformational dynamics evolution of different protein families including GFP proteins, beta-lactamase inhibitors and nuclear receptors, and observed that alteration of conformational dynamics through allosteric regulations leads to functional changes. Moreover, proteome-wide conformational dynamics analysis of over 100 human proteins shows a strong correlations between dynamic profile, and corresponding evolutionary rate of each position. Indeed, the preservation of dynamic properties of residues in a protein structure is critical for maintaining the biological function at a proteome scale.

The Evolution of Enzyme Mechanisms and Functional Diversity

Janet M. Thornton[1], Gemma L. Holliday[2], Syed Asad Rahman[1], Nicholas Furnham[3], Sergio Martinez Cuesta[1].
[1]European Bioinformatics Institute (EMBL-EBI), United Kingdom, [2]University of California, San Francisco, USA, [3]London School of Hygiene & Tropical Medicine, United Kingdom.

Enzyme activity is essential for almost all aspects of life. With completely sequenced genomes, the full complement of enzymes in an organism can be defined, and 3D structures have been determined for many enzyme families. Traditionally each enzyme has been studied individually, but as more enzymes are characterised it is now timely to revisit the molecular basis of catalysis, by comparing different enzymes and their mechanisms, and to consider how complex pathways and networks may have evolved. New approaches to understanding enzymes mechanisms and how enzyme families evolve functional diversity will be described.

References

1. Furnham, N, Sillitoe, I, Holliday, GL, Cuff, AL, Laskowski, RA, Orengo, CA, and Thornton, JM. Exploring the Evolution of Novel Enzyme Functions within Structurally Defined Protein Superfamilies. 2012, PLoS Comput. Biol. 8, e1002403.
2. Rahman, Syed A., Cuesta Sergio M., Furnham Nicholas, Holliday Gemma L., and Thornton Janet M. EC-BLAST: a tool to automatically search and compare enzyme reactions. Nature methods. Volume 11, (2014), p.171-4
3. Gemma L. Holliday, Asad Syed Rahman, Nicholas Furnham, and Janet M. Thornton. Exploring the biological and chemical complexity of the ligases (2014), J. Mol. Biol. In Press

Lipid-Protein Networks

Anne-Claude Gavin.
European Molecular Biology Laboratory, Heidelberg, Germany.

Eukaryotic cells use membrane-bounded organelles with unique lipid and protein compositions to regulate and spatially organize cellular functions and signalling. As part of this tight control, many proteins are regulated by lipids. In humans, the importance of these regulatory circuits is evident from the variety of disorders arising from altered protein–lipid interactions, which constitute attractive targets for pharmaceutical drug development. However, the full repertoire of interactions remains poorly explored and exploited because their detection is still difficult to achieve on a large, systematic scale. I will describe a series of chemical biology approaches to characterize in vivo assembled, stable protein-lipid complexes(1) and to study lipid interactions with peripheral membrane proteins(2). Data from yeast and human cell lines reveal surprising insights, such as the discovery of a new family of oxysterol-binding protein, conserved in humans (where it has been linked to several diseases) with unexpected specificities for an important signaling lipid, phosphatidylserine. The assays are scalable to the proteome and/or lipidome levels and are easily adapted to the study of small-molecules that disrupt protein–lipid interactions.

Soman Induced Conformational Changes of Human Acetylcholine Esterase

Sebnem Essiz Gokhan[1], Brian Bennion[2], Edmond Y. Lau[2], Felice C. Lightstone[2].
[1]Kadir Has University, Istanbul, Turkey, [2]Lawrence Livermore National Lab, Livermore, CA, USA.

Permanent inhibition of acetylcholine esterase, AChE, results in “runaway” neurotransmission leading to cognitive deficiencies, seizures, paralysis, and eventually death depending on the exposure. We present data from quantum mechanics/molecular mechanics (QM/MM) and 100 ns (MD) simulations of the apo and soman-adducted forms of hAChE to investigate the effects on the dynamics and protein structure when the catalytic Serine 203 is phosphonylated. By using correlation and principal component analysis of MD trajectories, we identified the allosteric sites in addition to segments of the protein, which are loosing flexibility due to the presence of soman in the binding pocket. The altered motions and resulting structures provide for alternative pathways into and out of the enzyme active site through the side-door in the soman-adducted protein.

Evolution Teaches Predicting Protein Interactions from Sequence

Burkhard Rost.
Technical University of Munich, Garching, Germany.

The physical protein-protein interaction (PPI) between two proteins A and B can be predicted from sequence alone. However, methods perform poorly on this difficult task when both proteins A and B have not been in the training set. Tobias Hamp in our group has developed a new approach that improves significantly over state-of-the-art methods. We machine learned highly reliable human PPIs from the Hippie resource through a new profile-kernel based SVM. This use of evolutionary information in combination with predicted sub-cellular localization raises precision even for low recall levels (most reliable predicted few interactions). A new rigorous way to reduce PPI redundancy reveals that only a fraction of the available PPIs is needed to build more accurate classifiers.

Proteins with Novel Function, Structure and Dynamics

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Recently, our collaborators evolved in vitro a small enzyme that ligates two RNA fragments with the rate of 1,000,000 above background (Seelig and Szostak, Nature 448:828-831, 2007). This enzyme does not resemble any contemporary protein (Chao et al., Nature Chem. Biol. 9:81-83, 2013). It consists of a dynamic, catalytic loop, a small, rigid core containing two zinc ions coordinated by neighboring amino acids, and two highly flexible tails that might be unimportant for protein function. In contrast to other proteins, this enzyme does not contain ordered secondary structure elements, such as α-helix or β-sheet. The loop is kept together by just two interactions of a charged residue and a histidine with a zinc ion, which they coordinate on the opposite side of the loop. Such structure appears to be very fragile. Surprisingly, computer simulations indicate otherwise. As the coordinating, charged residue is mutated to alanine, another, nearby charged residue takes its place, thus keeping the structure nearly intact. If this residue is also substituted by alanine a salt bridge involving two other, charged residues on the opposite sides of the loop keeps the loop in place. These adjustments are facilitated by high flexibility of the protein. Computational predictions have been confirmed experimentally, as both mutants retain full activity and overall structure. These results challenge our notions about what is required for protein activity and about the relationship between protein dynamics, stability and robustness. We hypothesize that small, highly dynamic proteins could be both active and fault tolerant in ways that many other proteins are not, i.e. they can adjust to retain their structure and activity even if subjected to mutations in structurally critical regions. This opens the doors for designing proteins with novel functions, structures and dynamics that have not been yet considered.

How Enzymes Access Caged Substrates? Phosphodiesterase-Protein Kinase A Interactions Mediate Hydrolysis of PKA Receptor Bound Cyclic AMP

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CAMP dependent-Protein Kinase (PKA) signaling is a fundamental regulatory pathway for mediating cellular responses to hormonal stimuli. The pathway is activated by association of cAMP with regulatory subunit of PKA and signal termination is achieved upon cAMP dissociation from PKA. While steps in the activation phase are well understood, little is known on how signal termination/resetting occurs. Due to the high affinity of cAMP to PKA (KD ~ low nM), bound cAMP does not readily dissociate from PKA, thus begging the question of how bound cAMP dissociates from PKA to reset its signaling state to respond to subsequent stimuli. We specifically set out to determine how cAMP-bound to the regulatory subunit is hydrolyzed to return PKA to an inactive state and the role of phosphodiesterases in resetting of the system. We report discovery of a novel signaling complex between phosphodiesterase (PDE8) and PKA Regulatory subunit (RIα) in mammalian cAMP signaling by a combination of Structural Mass spectrometry, specifically Amide hydrogen/deuterium exchange mass spectrometry (HDXMS), peptide arrays and computational docking. Using experimental data as input, a computational model for the complex was derived. This model reveals the phosphodiesterase active site in close proximity to the cAMP binding site on PKA and highlights a role for substrate channeling in the PDE-dependent dissociation and hydrolysis of cAMP bound to PKA. Real time reaction monitoring by Structural Mass spectrometry and fluorescence polarization assays provides further evidence for substrate channeling. This is the first instance of PDEs directly interacting with a cAMP-receptor protein in mammalian systems and highlights an entirely new class of binding partners for RIα. Furthermore, this introduces molecular channeling as a new paradigm for macromolecular assemblies in microdomains and localized stimulus-response cycles in cell signaling.

Clusters and Comets: Regulation of Actin Assembly

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The dynamics of the actin cytoskeleton underlies cellular processes as migration, cytokinesis, endocytosis, and the invasion of pathogenic microbes; it also controls dynamic morphological features of cells such as dendritic spines in neurons and the foot processes of kidney podocytes. Actin polymerization is regulated in specific ways to shape these diverse functions. We have combined experiments and mathematical modeling to try to understand the upstream regulation of actin assembly. One approach has been to use the Virtual Cell modeling platform to develop comprehensive spatial models of actin dynamics. This approach has been used to understand actin dynamics at the leading edge of migrating cells triggered by nucleation promoting factors such as N-WASp. We have also used this approach to analyze how the adaptor protein, Nck recruits N-WASp and other key signaling molecules in the comet tails that propel invading microbial pathogens. But traditional modeling approaches, which track each species, cannot deal with the combinatorial complexity associated with polymerization and aggregation, both of which are key process in cytoskeletal signaling. Recently, we have begun to address the special role of molecular aggregates and clusters in cell biology. We have developed an efficient non-spatial algorithm, based on classical polymer theory developed by Flory and Stockmayer, that efficiently predicts the dynamic composition and sol-gel transition of molecular aggregates. We have also developed a novel spatial stochastic algorithm based on Langevin dynamics to accurately describe clustering of multivalent biological molecules. Both of these algorithms are being applied to signaling systems that trigger actin dynamics. (Supported by NIH grants P41GM103313 TR01DK087660 and RO1 CA82258).

Regulation of Protein-Protein Binding and Pathway Crosstalk

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Phosphorylation offers a dynamic way to regulate protein activity and subcellular localization, which is achieved through reversibility and fast kinetics of posttranslational modifications. Adding or removing a dianionic phosphate group on a protein often changes protein's structural properties, its stability and dynamics. We estimate the effect of phosphorylation on protein binding and function for different types of complexes from human proteome. We find that phosphorylation sites tend to be located on protein-protein binding interfaces and may orthosterically modulate the strength of interactions. We study the effect of phosphorylation on protein-protein binding in relation to intrinsic disorder and observe the coupling between phosphorylation events and protein-protein binding through disorder-order or order-disorder transitions. Finally we investigate how different phosphorylation patterns may mediate dynamic regulation of cellular processes and may provide the biological cross-talk between different biochemical pathways.

Stochastic Simulations of Cellular Processes: from Single Cells to Colonies

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High-performance computing now allows integration of data from structural, single-molecule, and biochemical studies into coherent computational models of cells and cellular processes under *in vivo* conditions. Here we analyze the stochastic reaction-diffusion dynamics of a genetic switch, ribosome assembly, and metabolic responses of *Escherichia coli* cells. Using our GPU based Lattice Microbe software, we simulate the dynamics for an entire cell cycle and compare the mRNA/protein distributions to those observed in single molecule experiments. We show how such distributions can be integrated into a flux balance analysis of genome scale models of metabolic networks. The distribution of growth rates calculated for a colony of bacteria are analyzed and correlated to changes in fluxes through the metabolic network for various subpopulations. Finally, reaction-diffusion kinetics of the surrounding medium are coupled with the cellular metabolic networks to demonstrate how small colonies of interacting bacterial cells differentially respond to the competition for resources according to their position in the colony.

Modeling and Dynamic Analysis of Feedback Loops of the Insulin and Angiotensin II Signalling Systems

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This work applies the tools of systems theory to analyze the structure and the dynamics of the complex cellular networks associated with diabetes and hypertension. The primary research focus is the interaction between Angiotensin II (Ang II) and Insulin AKT signalling pathways. We provide a better understanding of design and operating principles of processes such as glucose uptake, cell proliferation, and blood pressure control by developing mathematical models of interactions at the system level. The overall model is a dynamic nonlinear model that includes mass-action kinetics and conservation laws. System behavior is analyzed within the context of signalling pathways and feedback regulation. We show that complex signalling pathways that govern the cross-talk between hypertension and diabetes are regulated by a nested set of feedback loops that are organized in hierarchical fashion. Using the dynamic models we develop, we simulate different scenarios to elucidate the functions of these feedback structures. While doing so, dominant steady-state and dynamic characteristics that determine the normal and diseased states are revealed. In particular parametric sensitivity and bistability of these feedback loops are shown to affect the regulatory mechanisms such as glucose uptake and vasodilation-vasoconstriction balance.

What Was First, the Genetic Code or Its Products?

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Ribosomes, the universal cellular machines for translation of the genetic code into proteins, possess spectacular architecture accompanied by inherent mobility, allowing for their smooth performance as polymerases that translate the genetic code into proteins. The site for peptide bond formation is located within a universal internal semi-symmetrical region. The high conservation of this region implies its existence irrespective of environmental conditions and indicates that it may represent an ancient RNA machine. Hence, it could be the kernel around which life originated. The mechanistic and genetic applications of this finding will be discussed.

The Photosynthetic Membrane of Purple Bacteria as a Clockwork of Atomic and Electronic Level Processes

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The chromatophore of purple bacteria is a spherical bioenergetic membrane of 70 nm diameter with (by area) 90% protein content involving about 130 large protein complexes. With each chromatophore generated through invagination of the inner bacterial membrane, hundreds of chromatophores provide a bacterium with energy in the form of ATP, the synthesis of ATP being driven by sun light. The overall function in each chromatophore comes about through a clockwork of intertwined physical processes organized through a multi-million atom macromolecular structure. Recent progress has lead to a surprisingly rigorous description of key aspects of chromatophore biology: the huge overall structure got resolved down to its atomic, even electronic level, components; the physical mechanisms underlying the different participating processes have been largely identified and proven through computer simulations; the coupling of the different processes leading to a clockwork with robust and optimal photosynthetic function has been described in principle. This clockwork involves: (1) the quantum biological processes of light absorption, exciton formation, and coherent excitation transfer arising in so-called light harvesting proteins; (2) coupled electron-proton transfer and charging of the quinone-quinole pool achieved in a protein complex called the reaction center; (3) discharging of the quinone-quinole pool and charging of the membrane voltage achieved through electron-proton transfer realized in the bc1 protein complex; and (4) use of the membrane voltage by a protein complex called ATP synthase. The lecture exploits the most advanced molecular graphics achievable today (using the author's program VMD), and the most rigorous computational description of the subprocesses possible today (using the author's programs NAMD and PHI) offering views of the processes described as well as advanced and detailed computational (in particular also quantum chemical) descriptions.

How Much Can Local Dynamical Features of Proteins Can Inform on Conformational Possibilities?

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It is not merely the protein structure, but also accompanying dynamics that are key in deciphering potential local sites to manipulate function. Based-on the assumption that target sites other than the direct binding region exist on the protein surface and that these are allosteric modifiers of binding-region dynamics, we have developed a methodology (termed perturbation-response scanning, PRS) whereby perturbations in the form of forces are introduced at selected sites and propensity of the protein to ease into other conformations under the influence of this force is quantified(1,2). Residue-by-residue scanning proteins by such perturbations and recording the subset of residues whose perturbation potentially leads to another known conformation, we map potential target sites on the surface of the protein. We show, through molecular dynamics simulations on sample proteins, that acting on these candidate sites either directly by mutations(3) or indirectly by lowering the pH(5), conformational change may be achieved on time scales shorter than measured experimentally under uniform environmental conditions. Even in the absence of conformational change, selected point mutations manipulate functional dynamics by altering the electrostatic distribution which in turn induces subtle differences in residue fluctuations around their identical average positions. Thus, residue fluctuations in the protein are greatly altered due to effective propagation of perturbation and presence of remotely controlling residues. It is plausible that certain residues have evolved to occupy positions in electrostatically susceptible and mechanically effective positions. PRS is an efficient method that can pinpoint such positions.

1. Atilgan C, Atilgan AR. 2009. PLoS Comput. Biol. 5: e1000544
2. Atilgan C, Gerek ZN, Ozkan SB, Atilgan AR. 2010. Biophysical Journal 99: 933-43
3. Aykut AO, Atilgan AR, Atilgan C. 2013. PLoS Comput. Biol. 9: e1003366

Molecular Noise Facilitates NF-κB Entrainment under Complex Dynamic Inputs

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Biological systems use oscillations for time-keeping and transcriptional regulation, with prominent examples in circadian rhythms, brain waves and developmental patterning. NF-κB is a signalling pathway central to immunity and many diseases that shows oscillations even under constant inputs, with significant cell-to-cell variability. NF-κB oscillation dynamics help determine the specificity and timing of gene expression. Upstream oscillatory pathways or signalling waves in tissue can result in periodic inputs to cells that can lead to their entrainment, where normally out-of-phase oscillators phase-lock to the input and become synchronized. Whether NF-κB can be entrained and its implications for the population response have been unclear. Here we use high-throughput microfluidic live-cell imaging, quantitative gene expression analysis and mathematical modelling to characterize the frequency response of NF-κB at the single-cell level over 48 hours, and we find that periodic modulation of the TNF-α input readily leads to synchronization and entrainment, causing significantly reduced NF-κB and mRNA variability between cells. We measure a much broader entrainment frequency range (Arnold Tongues) than what is expected from deterministic calculations, and stochastic simulations show that intrinsic molecular fluctuations in the transcription of negative-feedback genes IKBα and IKBε cause this enhanced bandwidth. Individual cells show diverse locking responses, with cells responding to fractions of the input frequency as well as cells "hopping" between locking modes. Oscillations in the expression of early and late genes appear with NF-κB entrainment, indicating the synchronization of gene expression between individual cells. Furthermore, efficient entrainment leads to increased mRNA production at the population level. Our results suggest a surprising role for intrinsic noise in reducing population variability under dynamical input signals.

Self-assembly of a Glucagon-like Peptide 1 Analogue: Bridging Experiment and Simulations

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In the present study, we have studied the self-assembly of the glucagon-like peptide 1 (GLP-1) analogue, liraglutide, which is an agonist and is used in the treatment of type 2 Diabetes Mellitus. Compared to GLP-1, liraglutide has an added fatty acid moiety and a gamma-glutamic acid linker on lysine-26, and a lysine to arginine substitution at residue 34. The modification on lysine-26 causes concentration-independent heptamerisation of liraglutide as suggested by small-angle X-ray scattering (SAXS) data. Using the SAXS data as input for *ab initio* shape determination suggested global shape of the heptamer. The orientation of the fatty acid chains could, however, not be deduced. To bridge the global shape information to an atomistic description of the heptamer, full-atomic and coarse grained molecular dynamics simulations are applied. A reverse transformation from coarse grained to atomistic description allows to access a large time scale in the simulations and at the same time to study the intermolecular interactions on the molecular level as well as the dynamics and stability of the complexes. Different start conformations of heptamers are generated and simulated. To validate the simulated complexes, theoretical SAXS curved are calculated and compared with the experimental SAXS profiles.

Fold to Fuse: The F2F Code of SNAREs on Membranes

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Soluble N-ethylmaleimide-sensitive factor attachment protein receptors (SNAREs) mediate membrane fusion in intracellular membrane traffic. Cognate SNAREs on the surface of vesicles (v-SNAREs) and target membranes (t-SNAREs) assemble in a highly exothermic reaction to form an intertwined 4-helix bundle. The energy gained from this folding reaction is used to force membranes into close proximity, bend them, remove water between them, and ultimately merge them into a single membrane. But, how exactly does the SNARE folding machine perform work on membranes?
In this presentation I will show how the structures of v- and t-SNAREs change upon assembling with each other and when zippering towards their respective membrane-spanning transmembrane domains. A surprising, but important result is that the starting conformations of SNAREs are different on membranes than in solution and, moreover, that they depend on membrane curvature. These factors modulate the energy gain upon folding and thus also the minimal number of SNAREs that is required for generating a single productive fusion pore. These results were obtained by a combination of NMR, EPR, and single molecule fluorescence spectroscopy experiments, which helped us with dissecting the inner workings of this highly specialized and rather exquisite molecular machine.

Ellena, Liang, Wiktor, Cafiso, Jahn, Tamm (2009) Dynamic structure of lipid-bound synaptobrevin suggests a nucleation-propagation mechanism for trans-SNARE complex formation. Proc. Natl. Acad. Sci. USA 106:20306.

Kiessling, Ahmed, Domanska, Holt, Jahn, Tamm (2013) Rapid calcium-regulated fusion of synaptic vesicles with reconstituted target SNARE membranes. Biophys. J. 104:1950.

Liang, Kiessling, Tamm (2013) Prefusion solution NMR structure of neuronal SNARE syntaxin. Proc. Natl. Acad. Sci. USA 110:19384.

Liang, Davidowski, Ellena, Tamm*, Cafiso* (2014) The SNARE motif of synaptobrevin exhibits an aqueous-interfacial partitioning that is modulated by membrane curvature. Biochemistry 53:1485.

Dynamics and Function of the AMPA Receptor N-Terminal Domain

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AMPA receptors (AMPARs) are glutamate-gated cation channels that mediate the majority of excitatory neurotransmission in the brain. AMPAR malfunction underlies a variety of neurological disorders rendering them a central drug target. AMPARs consist of four core subunits (GluAs) and a variety of auxiliary subunits that modulate receptor gating. The GluA core comprises two layers at the extracellular face of the receptor – the membrane-proximal ligand-binding domain (LBD) and the distal N-terminal domain (NTD), clamshell-shaped structures belonging to the periplasmic-binding protein family. The NTD is the most sequence-diverse portion and encompasses 50% of primary polypeptide sequence; the precise role of this domain is nevertheless obscure.
Our work has highlighted NTD allosteric capacity, which appeared pronounced in the GluA3 subunit [1, 2]. We have solved numerous GluA3 NTD structures in an attempt to co-crystallize small-molecule NTD modulators. This assemble of high-resolution structures reveal a spectrum of NTD dimer assemblies giving rise to two dominant modes of motion: i) a shearing motion, described by a rotation axis running through the upper lobe of the clamshell dimer-interface, resulting in a 18 angstrom displacement of the clamshell lower lobes, and ii) an opening motion, resulting in the splaying apart of the lower lobe dimer interface. These motions can be recapitulated in coarse grain simulations using the anisotropic network model [1, 2] and in all-atom molecular dynamics simulations further implying allosteric capacity for the NTD. Moreover, we describe an unexpected interaction of the NTD with AMPAR auxiliary subunits. We show that these proteins bridge the loosely connected NTD layer to the rest of the receptor, creating a continuous allosteric path from the NTD to modulate channel activity.

References:
[1] Sukumaran et al., (2011). *EMBO J*. 30, p 972.
[2] Dutta et al., (2012). *Structure* 20, p. 1838.

Two Is Better than One: Molecular Mechanism of Sodium Coupling in the Betaine Transporter BetP

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BetP is a Na^+-coupled symporter that shares the highly conserved LeuT-like fold of two-inverted structural repeats with other sequence unrelated secondary transporters, e.g., the medical important neurotransmitter transporters. Recently, we have obtained atomic structures from BetP in distinct conformational states, which elucidated the alternating access mechanism of BetP, suggesting a common mechanistic principle in LeuT-like fold transporters. Our structural data in combination with molecular dynamics simulations reveal key features of the formation of the central betaine-binding site and the two sodium-binding sites. We suggest a sequential Na^+/substrate binding process in which the sodium ions are loosely associated with partially formed sites in the outward-open state, in the absence of substrate. Subsequent binding of substrate to the central binding site results in an improved coordination of the two ions. Whereas one of the sodium ions seems to be responsible for closing the periplasmic pathway after substrate binding, the other one controls the opening of the inward-facing pathway. Na^+ binding and release are the key factors in guiding the alternating-access cycle in a pseudo symmetrical fashion and therefore the different roles of the two sodium ions observed for BetP are of particular interest for sodium-coupled secondary transporters.

Engineered Protein Nanopores for Challenging Tasks in Molecular Diagnosis

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Protein nanopore-based sensing elements represent a pressing need in molecular biomedical diagnosis. However, the integration of protein nanopores with other solid-state nanofluidic devices is a challenging task. This is especially true if we consider that isolated single proteins are in general fragile and unstable under harsh conditions of detection. Here, I will present a strategy for improving the stability of a redesigned nanopore using ferric hydroxamate uptake component A (FhuA), a beta-barrel membrane protein channel of E. coli (Mohammad, Iyer, Howard, McPike, Borer & Movileanu, 2012). The primary function of FhuA is to facilitate the energy-driven, high-affinity Fe^{3+} uptake complexed by the siderophore ferrichrome. The key ingredient of this strategy was the coupling of direct genetic engineering of FhuA with a fast-dilution refolding approach to obtain an unusually stable protein nanopore under a broad range of experimentation. These advantageous characteristics were recently demonstrated by examining proteolytic activity of an enzyme at a highly acidic pH, a condition at which majority of beta-barrel protein nanopores are normally gated or unfolded. Future membrane protein design work will not only reveal a better understanding of the processes employed in membrane protein folding and stability, but will also serve as a platform for the integration of robust protein components into devices.

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References

M.M. Mohammad, R. Iyer, K. R. Howard, M. P. McPike, P. N. Borer & L. Movileanu (2012). Engineering a Rigid Protein Tunnel for Biomolecular Detection. J. Am. Chem. Soc. 134, 9521-9531.

Data Mining Large-Scale Bioactivity Datasets to Find Patterns in Ligand Recognition

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We have built a large-scale open data resource, ChEMBL; this contains in excess of 1.4 million compound structures, and over 10 million associated bioactivities. Where possible the data is linked to molecular targets, and further annotation performed to provide deeper indexing and organisation of the data. This has become an important resource for the community in developing data-driven approaches to a number of important problems in drug design and safety, including predicting targets and 'off-targets' for novel compounds, quantifying the drug-likeness of compounds, and in the design of novel bioactive molecules. This data has been recently complemented by a new resource, SureChEMBL, containing automatically text-mined data from patent sources, signficantly increasing coverage of chemical and target space and diversity. The presentation will present the principles in the organisation and features of ChEMBL, and then some approaches addressing the difference between allosteric and non-allosteric ligands, target predictions from phenotypic data, and finally analysis of polypharmacology - the binding of a ligand to many components of a cell.

Combating Drug Resistance: Lessons from the Viral Proteases of HIV and HCV

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Drug resistance negatively impacts the lives of millions of patients and costs our society billions of dollars by limiting the longevity of many of our most potent drugs. Drug resistance can be caused by a change in the balance of molecular recognition events that selectively weakens inhibitor binding but maintains the biological function of the target. To reduce the likelihood of drug resistance, a detailed understanding of the target’s function is necessary. Both structure at atomic resolution and evolutionarily constraints on its variation is required. “Resilient” targets are less susceptible to drug resistance due to their key location in a particular pathway. This rationale was derived from our lab’s experience with substrate recognition and drug resistance in HIV-1 protease and Hepatitis C (HCV) NS3/4A. Both HIV-1 protease and HCV NS3/4A protease are potentially “resilient” targets where resistant mutations occur outside of the substrate binding site. These principals are likely more generally applicable to other quickly evolving diseases where drug resistance is quickly evolving.

In Silico Studies on K-RAS-PDEδ Interaction Inhibition to Design Novel Anti-Cancer Drugs

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The family of RAS (rat sarcoma viral oncogene homolog) proteins works as a signal transduction pathway network that transfer information from the extra-cellular environment to the nucleus. RAS proteins are cytoplasmic proteins that translocate to the plasma membrane where they transmit growth factor signals and drive cell proliferation. However, it's well-known that direct inhibition of Kirsten RAS (K-RAS) is not enough for clinically useful drugs.[1,2] Recently, Zimmermann *et al.*[1] reported binding of mammalian phosphodiesterase D subunit (PDEδ) to K-RAS by means of small molecule inhibitors which are suppress oncogenic RAS signalling by altering its localization to endomembranes. These available inhibitor-bound high-resolution X-ray structures from Zimmermann are used to better understand and compare the molecular mechanisms of different inhibitors. We used 6 ligands from Zimmerman *et al.* [1] and performed long molecular dynamics (MD) simulations (~0.1 μs) for the complex systems at physiological conditions to understand their molecular mechanism at atomistic level and design novel inhibitors based on MD simulations results. These results are used to design novel small molecule inhibitors for imparing the K-RAS-PDEδ interaction. *In silico* cardiotoxicity of novel designed compounds are also tested using hERG potassium channel models.

References:
et al Nature 497, 638 (2013)
[2] J. Downward Nature Rev. Cancer 3, 11 (2003)

Sickle Cell Hemoglobin: Allostery, Aggregation Kinetics, and Search for a Drug

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Unlike other protein aggregation diseases, the molecular pathogenesis of sickle cell anemia is well understood. If sickle hemoglobin were at equilibrium in the tissues with respect to fiber formation, patients would not survive once fetal hemoglobin disappears after the first few weeks of life. The disease is survivable because of the highly unusual kinetics of fiber formation. Like amyloid formation, there is a delay period prior to fiber formation, which allows the vast majority of cells to escape the narrow vessels of the tissues before fibers make the red cells less deformable and cause vaso-occlusion. However, unlike amyloid formation, there is an enormous sensitivity of the kinetics to solution conditions, with the delay time inversely proportional to up to the 40^{th} power of the initial sickle hemoglobin concentration and a nucleation rate proportional to up to the 80^{th} power (no typos). The kinetics can be explained by a double nucleation mechanism, in which nucleation of new fibers on the surface of pre-existing fibers results in an autocatalytic time course that produces the delay period. In spite of our understanding of the thermodynamics and kinetics and their relation to pathogenesis, there is still only one partially successful drug, hydroxyurea, discovered more than 20 years ago, which replaces about 20% of sickle hemoglobin with fetal hemoglobin in up to 80% of cells. Hydroxyurea is therapeutic because it dilutes the sickle hemoglobin and increases the delay time. What is needed is a drug that acts on all cells. We have developed an automated laser-photolysis/image-analysis assay to screen for anti-sickling compounds. Our initial screen is being carried out on compounds already approved by the FDA for other human diseases. Compounds active in this screen would be rapidly approved for clinical trials.

Computational Design of Drugs for Autoimmune Diseases from Peptide Toxins

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Developing drugs from natural products such as toxins has a great potential but progress has been slow due to complexity of the problem. Combination of experimental methods with accurate simulations of protein-peptide complexes could help to improve this situation. Two main computational challenges are construction of accurate models of complexes and prediction of reliable binding free energies. The former can be achieved using docking programs, followed by refinement via molecular dynamics (MD) simulations. Binding free energies can be obtained from the potential of mean force (PMF) calculations. We showed the feasibility of this approach in a potassium channel-charybdotoxin complex, where the complex structure is known from NMR [1]. As a real-life application, we considered ShK toxin, which binds to Kv1.3 channels with very high affinity, and therefore, it is developed as an immunosuppressant drug. However, it also binds to Kv1.1 with similar affinity, and it essential to find analogues of ShK with increased selectivity for Kv1.3. We have developed accurate models for Kv1.1-1.3 channels in complex with ShK, which are validated by comparing with available mutagenesis data and binding free energies [2]. The complex structures of ShK indicated several mutations on ShK (e.g. K18A, R29A) that could enhance its Kv1.3/Kv1.1 selectivity. Free energy perturbation and PMF calculations of the K18A mutation on ShK yielded about 2 kcal/mol improvement on its Kv1.3/Kv1.1 selectivity, which has been confirmed in subsequent experiments [3]. The computational methods considered here would be very useful in rational drug design, especially in solving selectivity problems for unintended targets.

[1] P.C. Chen and S. Kuyucak. 2011. Biophys. J. 100:2466-2474.
[2] M.H. Rashid and S. Kuyucak. 2012. J. Phys. Chem. B 116:4812-4822.
[3] M.H. Rashid et al. 2013. PloS ONE 8:e78712.

Molecular Speciation, Dynamics and Interactions of Lipid Droplets with Proteins

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Lipid droplets (LDs) control intracellular fat storage and allow mobilization of lipids in response to metabolic demands, playing critical roles in obesity and other metabolic disorders. They are spherical particles surrounded by a phospholipid monolayer filled with triacylglycerols, cholesterol esters and free fatty acids including oxidizable polyunsaturated molecular species. They interact with a number of proteins, which are involved in LD shape, size and lipid enzymatic reactions. To date, little is known about the biophysical properties of LD and the molecular events involved in these functions are unknown. Here, we used coarse-grained molecular dynamics simulations to shed light on structure and dynamics of LD depending on lipid composition. We developed the first computational model of a lipid droplet and optimized the parameters used based on experimental findings. We then studied its interaction with CIDEA, a protein found highly overexpressed in brown adipocytes responsible for causing enlargement of LDs. Our simulations predict CIDEA's C-terminal helix may interact with LDs, which was experimentally confirmed by biochemical means. Our model was also useful to study the effect of lipid composition on LD structure and function. It was shown experimentally using mass spectrometry that peroxidized species are present in LDs under hypoxic conditions such as observed during dyslipidemia and cancer. Both, oxidatively-truncated forms and hydroxy-derivatives of triacyl glycerides were the prevailing oxidized lipid species. Our simulations indicate that both types of oxidized species partition preferentially into the outer monolayer surface, where they can affect essential metabolic pathways and undergo conversions possibly leading to the formation of oxygenated lipid mediators.

Protein-Protein Docking and Design

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Protein-protein interaction is highly important for many cellular processes. We have built computational algorithms and benchmarks to predict the 3D structures of protein-protein complexes (protein-protein docking). I will present our recent work on both scoring function and search algorithm to improve protein-protein docking accuracy. In addition, we have built computational algorithms and an affinity benchmark for designing proteins to achieve stronger and more specific binding to another protein (protein design). We have thus far focused on designing T cell receptors and I will report on our recent progress.

Structural Networks of Signaling Pathways on Proteome Scale: Challenges and Opportunities

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Recent advances in high-throughput techniques have resulted in large amount of data on protein structure and protein-protein interactions. Networks of protein–protein interactions provide valuable information in understanding of cellular functions and biological processes. However, these networks lack structural (3D) details of most interactions, and these structural details are the key components usually for understanding the function of proteins. Augmenting protein interaction networks with structural data at proteome scale (3D interactome) is a challenging task, at the same time, extremely important because it allows prediction of protein function, helps drug discovery and takes steps toward genome-wide structural systems biology. In this talk, the challenges in building such networks will be discussed and a computationally feasible way towards building them using protein interfaces will be presented. Structural protein interaction networks can indicate which binding partners can interact simultaneously and which are competitive, how signals coming from different upstream pathways merge and propagate downstream, how multi-subunit signaling complexes form. They can help drug discovery along the line of emerging network medicine paradigm, and can help forecasting potentially harmful drug side effects.

Controlling Allosteric Networks in Proteins

Nikolay Dokholyan.
University of North Carolina at Chapel Hill, Chapel Hill, NC, USA.

We present a novel methodology for delineating allosteric pathways in proteins. We use this methodology to uncover the structural mechanisms responsible for coupling of distal sites on proteins and utilize it for allosteric modulation of proteins. We will present examples where inference of allosteric networks and its rewiring allows us to “rescue” cystic fibrosis transmembrane conductance regulator (CFTR), a protein associated with fatal genetic disease cystic fibrosis. We also use our methodology to control protein function allosterically. We design a novel protein domain that can be inserted into identified allosteric site of target protein. Using a drug that binds to our domain, we alter the function of the target protein. We successfully tested this methodology in vitro, in living cells and in zebrafish. We further demonstrate transferability of our allosteric modulation methodology to other systems and extend it to become ligh-activatable.

Intramolecular Communication Based on Time-dependent Linear Response Theories

Lee-Wei Yang, Ban-Chiech Huang.
National Tsing Hua University, Hsinchu, Taiwan.

It has been an established idea in recent years that protein is a physiochemically connected network. Allostery, understood in this new context, is a manifestation of residue communicating to remote parts in a molecule, and hence a rising interest to identify communication pathways within such a network. In this short review, we first bring the attention to marked evidence demonstrating that site-directed mutation 19Å away from the functional site, could substantially impact the enzymatic activity of dihydrofolate reductase (DHFR) and these chemically sensitive remote sites are identified along the signal propagation pathways initiated from the catalytic center. We then outline a few new and notable approaches that characterize the pathways chemically, physically, geometrically (per inter-residue connectivity) and/or evolutionarily. Our new development using time-dependent linear response theories (LRTs) combined with normal mode analysis (NMA) and Langevin damping is introduced with clear physics providing time-resolved physical changes within molecules. Illustrative results are shown to demonstrate the capabilities of our method that assumes promising agreement with physical observables and point-mutation-caused functional changes. We close the discussion by commenting the advantageous distinctions of our method from others and its applicable future.

Integrative Modeling of Proteins

Ken Dill, Justin MacCallum, Alberto Perez.
Stony Brook University, Stony Brook, NY, USA.

We are developing methods for combining physical forcefield-based MD simulations of proteins with external information, such as from experiments, heuristics, or database inferences. The method (MELD) is based on a statistical mechanical approach to applying spring-forces in a forgiving and adaptive way throughout an REMD simulation. We are finding it useful for refining protein structures based on NMR data, for finding peptide binding poses to receptor proteins, and for using heuristics to guide protein-structure prediction.

Protein-Protein Interactions that Inhibit the Activity of the p53 Tumor Suppressor

Batu Erman.
Sabanci University, Turkey.

Through homology modeling, in silico docking and functional studies we identified that a BTB-Zinc Finger transcription factor, PATZ1 interacts with the tumor suppressor protein p53. PATZ1 is a ubiquitously expressed protein with known transcriptional suppressor functions. PATZ1 interacts with p53 and suppresses its transcriptional activity by competing with DNA binding by p53. Genome wide analysis of genes that are controlled by PATZ1 by RNA Sequencing revealed that PATZ1 can not only inhibit p53 activity but in some cases can activate the expression of p53 target genes. Thus we identified PATZ1 as a context dependent regulator of p53.

From Polymer Rubberlike Elasticity to Protein Dynamics – How Simple Physical Models of Rubber Influenced Modern Biophysics

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Elastic Network Models of proteins have their origins in the theory of rubberlike elasticity of polymer networks. I will discuss the historical context of development of the theory of rubberlike materials and contributions of Professor Burak Erman to this field. I will show how simple models of Gaussian phantom polymer networks were applied to study protein dynamics, with the specific emphasis on significant involvement and major contributions of Burak Erman and his collaborators to this area of research. In the later part of my talk I'll show how fluctuational dynamics of proteins described by the normal mode analysis and elastic network models controls protein dynamics and can explain variety of physical processes, such as the order of breaking contacts during the mechanical unfolding of proteins, and the process of structural refinement of protein models in protein structure prediction.

Allosteric Regulation of the Glycolytic Pathway in Mammals and Trypanosomes

Malcolm Walkinshaw.
Edinburgh University, Edinburgh, United Kingdom.

The two most highly regulated enzymes in the glycolytic pathway are phosphofructokinase (PFK) and pyruvate kinase (PYK). Both enzymes are allosterically activated by a range of metabolites as well as by a number of poorly characterised covalent modifications including acetylation, phosphorylation and nitrosylation. In a dividing cell, glucose metabolites are required for protein and DNA synthesis. A delicate balance must be reached between allowing the pathway to burn glucose to generate ATP and providing building blocks for the growing cell. Both cancer cells and unicellular parasite share a craving for glucose to allow unrestrained growth.

The enzyme mechanisms of PFK and PYK are closely conserved between trypanosomes and mammals however the control mechanisms regulating their activities have diverged significantly and make use of different classes of molecules including hormones, amino acids and nucleotides to allosterically activate or inhibit their enzyme activities. We have been studying the structures and kinetics of human and parasite PYK and PFK in order to understand the regulatory mechanisms at a molecular level and use this insight to design potential new therapeutic approaches to tackle proliferative and infective diseases.

Sequence Variations and Allosteric Dynamics in Binding

Turkan Haliloglu.
Bogazici University, Istanbul, Turkey.

Proteins are of highly dynamic nature with a complex interrelation between structural dynamics and their binding behavior. With the capacity of assuming an ensemble of conformations, they perform local to global fluctuations to interact with others in a dynamic infrastructure adopted to functional motion. On the exemplary case studies, it will be presented how the sequence variations at hinge regions of most cooperative movements could be instrumental to allosterically affect the binding site dynamics or dispose alternative binding modes with a change in functionality. The long-range dissemination of the perturbations in local chemistry or physical interactions through an impact on global dynamics can reform the allosteric dynamics. The findings posits an aspect for the coupling of structural dynamics and evolvability in the modulation of protein interactions.

Extracting Dynamics Information from Multiple Molecular Structures and Computationally Generating Their Transition Pathways

Robert Jernigan[1], Kannan Sankar[1], Kejue Jia[1], Jie Liu[1], Yuan Wang[1], Ataur Katebi[1], Michael Zimmermann[2,1].
[1]Iowa State University, Ames, IA, USA, [2]Mayo Clinic College of Medicine, Rochester, MN, USA.

Meaningful dynamics information can be extracted from multiple experimental structures of the same, or closely related, proteins or RNAs. The covariance matrix of atom positions is decomposable into principal components. Usually only a few principal components describe the motions of the structures, and these usually relate to the functional dynamics. This dynamics information provides strong evidence for the plasticity of protein and RNA structures, and also suggests that these structures almost always have a highly limited repertoire of motions. In some cases, such as many enzymes the dominant motions are opening and closing over the active site. For myoglobin the changes are smalle, reflecting in part the small changes in sequence, but nonetheless they show characteristic details in their motions that are species dependent.

We are computing pathways for transitions between different conformations, by generating structures with a Metropolis Monte Carlo method, using free energies for structural intermediates computed using our 4-body potentials and entropies from elastic network models. These provide effective pathways that traverse the space of the experimental structures. In some other cases transitions can be initiated by exothermic reactions, and these transitions can be effected by application of forces at or near the reaction center. This work provides new tools that can be used to understand the sets of available structures.

Fractal Structure of Interaction Pathways in Proteins and Prediction of Allosteric Paths

Burak Erman.
Koc University, Sariyer Istanbul, Turkey.

Information from one point to another in a protein proceeds along fractal paths. The problem is that of a random walk on fractal structures. We propose a simple computational method to determine the minimum number of steps to move between two distant points in the protein, which leads to the Hausdorff dimension of interaction pathways. The magnitude of the dimension depends on the range of interactions. We define the range of interaction as the radius of a sphere in which a central residue interacts with other residues inside this sphere. At short interaction length scales the Hausdorff dimension approaches 2.0 which is below the fractal dimension 2.55 of a liquid just above the glass transition temperature. At length scales above 6.8 Angstroms, the fractal dimension of interaction pathways in proteins exhibits a constant universal value around 1.3. The fractal path problem in a protein is equivalent to the bond percolation problem. We propose a step by step method, based on the successive powers of the contact matrix of a protein, to determine percolation clusters and the residues on the most probable fractal path between two points. The problem is of special interest for studying allosteric paths in proteins. Sample calculations on several proteins show that residues on most probable paths determined by the present model are mostly conserved residues.

Role of Histidine Protonation in the pH Induced Changes in Prion Protein Stability

Shoshana J. Wodak, Anatoly Malevanets, Andrew Chong, Flemming Hansen, Julie Forman-Kay.
Hospital for Sick Children, Toronto, Canada.

Nuclear Magnetic Resonance (NMR) is employed to investigate origins of the greater pH susceptibility of the PrP protein from golden hamster (GHaPrP), relative to its homolog from rabbit (RaPrP). Titration experiments were performed to measure the pKa of the five His residues in the considered PrP domains (residues 90-231). In addition, proton-exchange rates of amide groups across the two PrP domains were measured at pH 5 and pH 7, respectively, in absence of denaturants. Results revealed a single buried His residue (H187 in GHaPrP, and H186 in RaPrP) to have a markedly down shifted pKa~5. On the other hand, significantly larger pH-induced shifts in exchange rates were detected for the hamster protein compared to the rabbit homolog. Using an extension of the classical proton exchange model where a mixture of protein sub-states contribute to the observed exchange rates, evidence is provided that protonation of the buried histidine is the primary event responsible for the pH-induced destabilization of both PrP variants. The marked difference in the pH susceptibility between hamster and rabbit PrP is then related to the difference between the global intrinsic stability of the two proteins. The folded state of the more stable rabbit PrP is only marginally perturbed by His protonation, whereas protonation significantly perturbs hamster PrP, resulting in a considerably less stable conformational state. Our findings link the protonation of a single buried histidine to PrP intrinsic stability, as the underlying mechanism for PrP pH-induced destabilization and its likely implication in disease susceptibility.

Effect of Ligand Binding on Enzyme Global Dynamics and Function

Pemra Doruker.
Bogazici University, Istanbul, Turkey.

The link between enzyme dynamics and catalytic activity can be facilitated through computational means. Such studies based on elastic network models (ENM) and molecular dynamics (MD) simulations will be presented in this talk. In the case of enzymes with functional loops that close over the active site during catalysis, the seemingly localized conformational changes of these loops are in fact coupled to the global ENM or essential MD modes of motion. For the specific case of triosephosphate isomerase (TIM), the functional, dimeric topology drives loop opening/closure as opposed to the monomeric TIM with reduced catalytic activity. Thus, collective modes dictated by the enzyme topology seem to guide its functional loop dynamics, which is critical for substrate entrance, product release and catalysis. Binding of ligands to specific sites on an enzyme act as constraints on its network of interactions, thereby affecting its vibrational dynamics to various extents. For example, binding of certain allosteric inhibitors at TIM's subunit interface alters global modes and enzyme activity significantly. In this respect, the extent to which different type of ligands, including allosteric and orthosteric ones, modify the low-frequency vibrational modes of enzymes can be determined by mixed resolution ENM. Such an approach can be further utilized for isolating among alternative ligand binding positions/poses those that have an effect on enzyme global dynamics.

A New Approach for Exploring Free Energy Landscapes of Large Structural Changes: Molecular Dynamics with Excited Collective Motions (MDeNM)

Mauricio G. S. Costa[1,2], Paulo Ricardo Batista[2], Paulo Mascarello Bisch[3], **David Perahia**[1].
[1]CNRS, Cachan, France, [2]Fundação Oswaldo Cruz, Rio de Janeiro, Brazil, [3]Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil.

The lowest frequency normal modes (NM) describe the most collective motions of a molecule. A large diversity of structural changes can be obtained by exploring the space defined by these modes. For macromolecules or their complexes the exploration of large conformational changes would be computationally very demanding by carrying out solely conventional molecular dynamics (MD) simulations. I will present a new method that we developed very recently using a combined use of NM and MD allowing an extended conformational exploration. Its principle is based on the kinetic activation of motions along linear combinations of NMs in MD simulations accounting for different time scales, going from pico to milliseconds. It allows the obtaining of unbiased large scale conformational distributions for macromolecules that are necessary for a fine description of their structural variability and preferences, as well as for getting free energy landscapes along relevant reaction coordinates. We demonstrated the computational efficiency of our approach compared to conventional molecular dynamics simulations, and its usefulness for the establishment of structure, dynamics and activity relationships in macromolecules, for performing docking simulations in which the receptor and ligand undergo large conformational changes upon association, for understanding the dissociation processes, and for studying channel opening in membrane proteins. I will give different examples of the application of our method.

Restraining Molecular Dynamics and Modeling with Ion-Mobility Mass Spectrometry

Erik Marklund, Matteo Degiacomi, Carol Robinson, Andrew Baldwin, Justin Benesch.
University of Oxford, Oxford, United Kingdom.

The dynamical variability of protein assemblies is a major stumbling block for structural biology and biophysical tools that provide only an ensemble average. Recent developments in ion mobility coupled to mass spectrometry (IM-MS) have rendered it an attractive new approach for studying the structure and dynamics of biomolecular complexes.

From drift times measured by IM-MS the collisional cross sections (CCS) of proteins can be inferred, a single measurement reporting on all mass-separated species in a sample, such as multiple oligomeric states of polydisperse proteins. Since IM-MS separation takes place within milliseconds, the observed CCS distribution accurately represents the solution conditions. Furthermore, with the ability to perform time-resolved experiments, IM-MS is well suited for studying protein interactions and dynamics. As shown in this study, CCS hold structural information that is distinct from other geometric parameters of proteins, such as gyration radii.

Making use of IM-MS data however requires computational modeling, for example comparison of CCS from candidate structures with those observed in experiments. This approach has been limited by the demanding calculation of macromolecular CCS. To overcome this limitation we have developed a new algorithm (IMPACT) for estimating CCS of structure models at unprecedented speed, with potential to transform the IM-MS-based modeling and for the first time allowing for directly restraining molecular dynamics simulations with IM-MS data. We have analyzed conformational ensembles and show how modern IM instrumentation is capable of assessing not only the structure of proteins, but also their conformational dynamics.

Our analysis of all biological assemblies in the Protein Data Bank and other structural databases furthermore reveals how protein complexes of similar mass frequently have sufficiently different cross sections to be distinguishable by IM, which translates to proteome-wide applicability of IM-MS for the study of biomolecular structure and dynamics.

Modeling the binding of H-NS to AT-rich DNA

Eva C. Van Mastbergen, **Jocelyne Vreede**.
University of Amsterdam, Amsterdam, The Netherlands.

Bacteria organize their chromosomal DNA within a structure called the nucleoid, by employing nucleoid-associated proteins. The histone-like nucleoid structuring protein (H-NS) can form bridges between two DNA duplexes, therefore locally reducing the effective volume. H-NS contains an oligomerization region and a DNA-binding domain. H-NS prefers to bind to the minor groove of AT-rich DNA with a loop containing a motif that consists of Glutamine112, Glycine113 and Arginine114. Molecular simulation can complement experiments by modeling the dynamical time evolution of biomolecular systems in atomistic detail. To study the binding mechanism of H-NS to DNA, we performed advanced molecular simulations on a system containing the H-NS DNA binding domain and an AT-rich dsDNA sequence. First, we performed straightforward Molecular Dynamics simulations, followed by biased sampling of association/dissociation using metadynamics. Finally, we obtained unbiased transition paths using transition path sampling. Our results indicate that H-NS binds to the minor groove with residues Q112, G113 and R114, in agreement with experiment. Furthermore, we identified two mechanisms; Q112 binds first, followed by G113 and R114; or R114 binds first, followed by G113 and Q112. The hydrogen bond between G113 and a thymine base is essential in the association and dissociation of H-NS and DNA.

Protein-DNA Interactions: Fine Balance between High Affinity and Fast Kinetics

Yaakov (Koby) Levy.
Weizmann Institute of Science, Rehovot, Israel.

Interactions between proteins and nucleic acids are ubiquitous and central to the life of cells. The remarkable efficiency and specificity of protein-DNA recognition presents a major theoretical puzzle given the size of the genome, the large number of molecular species in vivo at a given time, and the crowded environment they inhabit. Our research is motivated at quantitatively advancing our understanding of the kinetics and mechanisms of protein-DNA recognition, the molecular and physical principles of fast association, and protein recruitment by DNA. For the first time, we have visualized protein sliding along DNA where the protein binds DNA nonspecifically and performs a helical motion when it is placed in the major groove. Using coarse-grained models we found that the spiral motion along the sugar-phosphate rail is typical to various DNA-binding motifs. This stochastic dynamics that is governed by electrostatic forces has similar structural features to the specific binding mode of the protein with the DNA. In our study, we address the question of the linkage between the molecular architecture of DNA-binding proteins and the search mechanism. We have explored the interplay between the molecular characteristics of the proteins (e.g., DNA recognition motifs, degree of flexibility, and oligomeric states) and the nature of sliding, intersegment transfer events and the overall efficiency of the DNA search. Another important aspect of the search is how the in-vivo conditions (for example, crowding in the cell or coverage of DNA by nucleosomes) affect the efficiency of DNA search.

POSTER ABSTRACTS

THURSDAY POSTER SESSION

1:15 PM – 3:00 PM, Hall B

Posters being presented on Thursday, September 11 should be set up on the morning of September 11 and removed by 6:00 PM on September 11.

Abbas, Sherif	1-POS	Board 1
Abdizadeh, Haleh	2-POS	Board 2
Adiguzel, Yekbun	3-POS	Board 3
Akdas, Basak	4-POS	Board 4
Akten, Ebru	5-POS	Board 5
Alaybeyoglu, Begüm	6-POS	Board 6
Aldaais, Ebtisam	7-POS	Board 7
Anand, Ganesh	8-POS	Board 8
Arouri, Ahmad	9-POS	Board 9
Aykut, Ayse	10-POS	Board 10
Aykut, Ayse	11-POS	Board 11
Bahadur, Ranjit	12-POS	Board 12
Balog, Erika	13-POS	Board 13
Barrozo, Alexandre	14-POS	Board 14
Basak, Jolly	15-POS	Board 15
Bastug, Turgut	16-POS	Board 16
Bekcioglu, Gül	17-POS	Board 17
Bignucolo, Olivier	18-POS	Board 18
Biswas, Ansuman (Abstract Withdrawn)	19-POS	Board 19
Budak, Maral	20-POS	Board 20
Chaban, Vitaly	21-POS	Board 21
Chakroun, Nesrine	22-POS	Board 22
Chang, Chun-Chun	23-POS	Board 23
Chung, Yi	24-POS	Board 24
Corridon, Peter	25-POS	Board 25
Dalgicdir, Cahit	26-POS	Board 26
Demir, Ozlem	27-POS	Board 27
Dinler-Doganay, Gizem	28-POS	Board 28
Eaton, William	29-POS	Board 29
Ersahin, Tulin	30-POS	Board 30
Eskici, Gozde	31-POS	Board 31
Ettrich, Rudiger	32-POS	Board 32
Fuglebakk, Edvin	33-POS	Board 33
Gandhi, Neha	34-POS	Board 34
Garip, Sebnem	35-POS	Board 35
Gavrilov, Yulian	36-POS	Board 36

Girodat, Dylan	37-POS	Board 37
Globisch, Christoph	38-POS	Board 38
Güçlü, Tandaç Fürkan	39-POS	Board 39
Guven-Maiorov, Emine	40-POS	Board 40
Haas, Juergen	41-POS	Board 41
Hungyo, Kharerin	42-POS	Board 42
Iqbal, Samir	43-POS	Board 43
Jain, Alok	44-POS	Board 44
Johnson, Margaret	45-POS	Board 45
Kale, Seyit	46-POS	Board 46
Karahan, Nilay	47-POS	Board 47

1-POS Board 1

Estimation of Protein Secondary Structure from FTIR Spectra Using Wavelet Analysis and Neural Networks

Sherif Abbas[1,3], Parvez Haris[2], Feride Severcan[1], Mete Severcan[1].
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In order to improve the accuracy to predict protein secondary structure from Fourier transform infrared (FTIR) spectra, a novel method for extraction of Protein FTIR features using wavelet transform analysis was developed. Recording of proteins FTIR spectra was performed in aqueous solution using spectrum using BRUKER Vectra 200 FT-IR Spectrometer. The protein spectra preprocessd using Matlab and OPUS 5.5 software. Amide I region (1700 - 1600 cm-1) is utilized and its wavelet analysis has been done in order to do the secondary structure analysis of proteins from FTIR spectra . The secondary stucture of this proteins has been calculated also from thier x-ray crystal structure using PDBsum bioinformatics tool. This calculated structure has been used as atarget for our Artificial Neural Network (ANN). These results indicate that the methodology introduced is effective and estimation accuracies are in some cases better than those previously reported in the literature.

2-POS Board 2

A Computational Approach to Iron Release Dynamics in Human Serum Transferrin

Haleh Abdizadeh, Ali Rana Atilgan, Canan Atilgan.
Sabanci University, Istanbul, Turkey.

Human serum transferrin(hTF) transports ferric ions in blood serum and delivers them to cells via receptor mediated endocytosis[1]. Transferrin is folded into two homologous lobes; each is further divided into two similar sized subdomains. Four major populations, namely holo, two monoferric and apo hTf are suggested, based-on the iron occupancy of the deep cleft within the subdomains in each lobe[2]. Many factors affect distribution of conformational substates of hTf, including pH, and anion concentration. The mechanisms leading to conformational changes prior to iron release are not known. The specialized role of the two lobes and whether communication between them leads to a controllable release mechanism is also debated in literature[3]. Here, we study the dynamics of the full structure, separate lobes, and monoferric states of holo form of hTf under the nearly neutral pH conditions in blood serum and the more acidic one in the endosome. Results are in remarkable agreement with experimental observations and underscore the distinguishing effect of pH on the dynamics of hTf[4]. Furthermore, in a grand total of 1 μs molecular dynamics simulations of holo form of hTf at different pH values, residue fluctuations and cross-correlations elucidate the cross talk between the two lobes communicated by the bridging linker. Finally, we use a combination of solvent accessible surface area, Poisson-Boltzmann and radial distribution function calculations to decipher the so-called kinetically significant anion binding(KISAB) sites at the exposed surface of the protein. Some KISAB sites have been determined experimentally and were proposed to contribute to the iron release rate by interacting with non-synergistic anions[5].

[1]M.W. Hentze et al.,Cell,142,24-38(2010).
[2]A.B. Mason et al. Protein Expres. Purif.,23,142-150(2001).
[3]S.L. Byrne et al. J. Mol. Biol.,396,130-140(2010).
[4]A.N. Steere et al. Biochim. Biophysic. Acta-General Subj.,1820,326-333(2012).
[5]S.A. Kretchmar, K.N. Raymond, Inorg. Chem.,27,1436-1441(1988).

3-POS **Board 3**

Web-Based Structure Prediction as Supplementary Tools to Protein Secondary Structure Analysis with FT-IR Spectroscopy

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Protein studies with Fourier Transform Infrared (FT-IR) spectroscopy include protein secondary structure determination and investigation of protein dynamics through structural changes. The common approach involves curve fitting of the protein-sourced regions of the FT-IR spectrum. The success of this approach relies on several criteria, among which, proper selection of the initial parameters is of utmost importance. Information derived from high resolution structures are tended to be used for this purpose, in order to perform protein studies with FT-IR. However, web-based resources and techniques that serve for protein secondary structure prediction of the proteins with known amino acid sequences can well compensate the necessary information, when high resolution structures are not available or hard to obtain. Therefore, those resources are evaluated as potential supplementary tools for protein studies with FT-IR, through this work. Secondary structure of basic fibroblast growth factor was inspected in this study, as proof of the concept. This protein is of biological and physiological relevance. It binds to heparin, performs wide range of mitogenic and angiogenic activities, and involves in processes like development of the limbs and neural system. Protein secondary structure information of this protein from DSSP (the database for secondary structure assignment in Protein Data Bank) reveals 6.90% helix structure, 41.1% sheets, 19.4% turns, and 32.6% random coils. Seven distinct models that enable protein secondary structure prediction through web-based protein structure prediction servers were tested. Among those, secondary structure prediction by self-optimized prediction method (SOPM) yielded the best results. Accordingly, SOPM results of the basic fibroblast growth factor protein sequence resulted in 6.20% helices, 41.1% sheets, 17.8% turns, and 34.9% random coils. RMSD is 1.23, which is good enough for setting parameters during curve-fitting of FT-IR data for protein studies.

4-POS **Board 4**

Exploring Alternative Signaling Pathways between Decoding Center of Bacterial Ribosome and Active Site of EF-Tu – tRNA – GDP Complex

Basak Akdas, Ozge Kurkcuoglu Levitas.
Istanbul Technical University, Istanbul, Turkey.

Supramolecule ribosome, responsible for protein synthesis, consists of small and large subunits, respectively called 30S and 50S in bacteria. During protein synthesis, communication of distant functional sites on the complex is maintained via signal transmission throughout the structure, such as between the decoding center (DC) on 30S decoding the genetic code and elongation factor (EF) Tu assisting aminoacyl-transfer RNA (tRNA)[1]. Revealing major and minor communication pathways between functional sites is critical to understand the allosteric mechanisms employed by the complex for a successful translation. In this study, Yen's Algorithm[2] is employed with the elastic network approach[3] on Thermus Thermophilus ribosome structure to reveal twenty shortest paths between DC and EF-Tu. The ribosome structure is represented as a network of nodes and edges. Nodes are placed at alpha-carbon and phosphor atoms of residues, and lengths of edges are calculated based on atom-atom interactions. Major and minor signaling pathways exist between DC and GTPase center of EF-Tu and pass through functional regions such as Sarcin-Ricin Loop (SRL), aminoacyl-tRNA[1] and intersubunit bridge B2a, which was proposed to bear an important role in enzymatic steps of translation[4]. These findings suggest important hints for understanding functional activities of the ribosome and identifying new target sites for drug design.

[1] Schmeing, T.M.; Voorhees, R M.; Kelley, A.C.; Gao, Y.-G.; Murphy, F.V. IV; Weir, J.R.; Ramakrishnan, V. Science, 2009, 326(5953), 688.
[2] Yen, J.Y. Management Science, 1971, 17, 712.
[3] Kurkcuoglu, O.; Turgut, O.T.; Cansu, S.; Jernigan, R.L.; Doruker, P. Biophysical Journal, 2009, 97, 1178.
[4] Kipper, K.; Hetenyi, C.; Sild, S.; Remme, J.; Liiv, A. J Molecular Biology, 2009, 385, 405.

5-POS **Board 5**

Investigating the Allosteric Mechanism in Human β2-adrenergic Receptor (β2AR) upon Activation/Inactivation via Constrained Molecular Dynamics (MD) Simulation

Canan Özgür[1], Pemra Doruker[2], **Ebru D. Akten**[3].
[3]Kadir Has University, Istanbul, Turkey.[2]Bogazici University, Istanbul, Turkey, [1]Bogazici University, Istanbul, Turkey

As the member of G-protein coupled receptor (GPCR) superfamily β2AR is involved in a wide range of physiological processes such as the regulation of heart rate and muscle relaxation in lung tissues. In this study, the allosteric coupling that involves the intra- and extracellular parts of the receptor will be investigated via MD simulations under various distance constraints.

Several crystallographic structures of both active and inactive states of β2AR were revealed, yet none included the intracellular loop 3 (ICL3), which was cleaved prior to experiments to facilitate crystallization. This critical region of 32-residues was generated through homology modeling and incorporated in the receptor model due to its potential effect on the intrinsic dynamics of β2AR as previously reported in our MD study. Several MD runs under various distance constraints applied to selected residues were performed using NAMD in order to reveal the allosteric coupling. The receptor was embedded in a POPC membrane bilayer with explicit water, to generate a system of 68,000 atoms.

Allosteric coupling between intra- and extracellular parts of the receptor was revealed during 500 ns long MD runs under various constraints. Previously, a 1 μs long MD simulation conducted without any constraints showed that the closure of the G protein binding site as a result of a tightly packed ICL3 under the receptor was paired with the expansion of the ligand binding site as indicated by the increase in Ser207-Asp113 distance. In constrained MD runs, same coupling was once more confirmed. As the binding site is kept open, ICL3 is guided to a closed position, and vice versa. Furthermore, the closure of ICL3 under the receptor provided a new inactive state, which becomes a potential target for drug design studies.

6-POS Board 6

Translocation across the Membrane Analyzed by Dimensionality Reduction

Begüm Alaybeyoglu, Elif Ozkirimli Olmez.
Bogazici University, Istanbul, Turkey.

Peptide drugs are promising drug leads due to their high specificity and affinity to targets, but their use as therapeutics against intracellular targets is limited because of difficulty in delivery into the cell. In the general context of drug delivery, cell-penetrating peptides (CPPs) were discovered based on translocating ability of some proteins. Computational studies such as molecular dynamics (MD) simulations of peptide – membrane systems have focused on the behavior and effects of the peptide inside the membrane or to examine whether a spontaneous adsorption and insertion into the membrane would occur. On the other hand, steered molecular dynamics (SMD) simulations, have been favored to obtain time dependent atomic level information on long time scale events such as drug binding, water transport across aquaporin and unfolding. The computational simulation of membrane translocation is a complex process and gives a huge number of conformations in the form of Cartesian coordinates for each of the atoms. In order to quantitatively characterize a computer simulation and extract the important information of the motion, a low-dimensional embedding such that the properties of the underlying manifold is preserved should be defined. Finding a set of coordinates in which very few of them show significant variation and the others may considered almost constant is mathematically called dimensionality reduction problem. In an effort to characterize the translocation of the 18-residue long cell-penetrating peptide pVEC (LLIILRRRIRKQAHAHSK) and its variants through a lipid bilayer, we performed steered molecular dynamic (SMD) simulations, in which force is applied on the peptide to move it from one side of the membrane to the other. Here, we present an approach for the theoretical characterization of the nonlinear process of membrane uptake based on the idea of dimensionality reduction.

7-POS Board 7

A Theoretical Study of the Coupling between Chemical Equilibrium and Physical Interactions that Determine Self-Organization in End-Grafted Polyelectrolytes for Tissue-Material Applications

Ebtisam Aldaais[1,2], Mark J. Uline[1].
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A variety of interactions between implanted materials and local tissues impact clinical outcomes in terms of both therapeutic action and biological response. Tissue-material adhesion is a specific mode of interaction that is the therapeutic basis for many clinical material applications. Understanding the competition of interactions in highly inhomogeneous environments such as those relevant in tissue engineering, nanotechnology, and those responsible for biological cell function is critical to the further development of design platforms for such systems. We use a three dimensional mean-field theory to study the competition between electrostatic, van der Waals and steric interactions in determining the molecular organization of end-grafted polyacids. The polyelectrolyte layers spontaneously form self-assembled aggregates whose morphologies are manipulated by the composition of the solution in contact with the film. These theoretical calculations show that chemical equilibrium and the relevant physical interactions present in responsive polymer layers couple in a highly non-additive manner.

We find that charge regulation stabilizes micellar domains over a wide range of pH by reducing the local charge in the aggregate at the cost of chemical free energy and gaining in the van der Waals attractive interactions. The balance of interactions in this highly inhomogeneous environment determines the boundaries between different aggregate morphologies. We predict the formation of domains based on the proper choice of solution pH and salt concentration, and one can use these predictions to provide design guidelines for the creation of responsive polymer layers presenting self-organized patterns with the desired functional properties.

8-POS Board 8

Distinguishing Binding and Allostery in Protein-Ligand Interactions Using Amide H/D Exchange Mass Spectrometry

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Amide H/D exchange mass spectrometry (HDXMS) is a method that is highly suited for monitoring protein-ligand interactions and has important advantages including small sample sizes, automation and user-friendly visual interfaces, ability to map protein-ligand interactions in solution with no size limits of target proteins. We tested suitability of HDXMS to map interactions of ligands of varying affinity for Hsp90 (Radicicol, allylamino-demethoxygeldanamycin (AAG) (KDs of 19, 33 nM respectively) to low affinity geldanamycin derivatives with affinities of 490 and 570 μM). Amide exchange was initiated by incubating Hsp90 alone and in the presence of the above ligands in deuterated (D2O) buffer (pHread ~7.0) for a time series (0.5-10 min) and the reaction quenched by lowering the pHread to 2.5. A combination of LC-ESI mass spectrometry and pepsin proteolytic fragmentation was then carried out.Our results reveal that HDXMS shows high sensitivity to map interactions mediated by low affinity ligands (binding affinity ~490 μmicroM) which show similar magnitude changes in deuterium exchange in a subset of overlapping regions of Hsp90 as the high affinity inhibitor Radicicol (binding affinity ~19 nM). More importantly analysis of deuterium exchange kinetics enabled distinguishing between direct binding sites and allosteric sites distal to the predicted binding site- based on the crystal structure of Hsp90 bound to the low affinity ligand. Ligand interactions at the active site are reflected in decreased exchange at early time points and allosteric changes at distal sites are reflected in deuterium exchange decreases at later time points. This highlights a powerful application of HDXMS in protein-ligand interactions and development of specific allosteric target molecules for drug discovery.

9-POS Board 9

Phospholipase A_2 Action on Lipid-based Drug Delivery Systems

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Lipid-based delivery systems have intensively been used to enhance the physico-chemical properties and to modify the pharmacokinetic profiles of drugs, with many successful attempts. In anticancer drug delivery, the small size of the carriers can promote their accumulation is leaky cancer tissues. However, the need to construct liposomes that are stable during storage and in the blood stream is very often associated with a modest and slow release of the payload at the target. This calls for "smarter" strategies to actively unload the drug precisely at the target.

Our platform is based on benefiting from secretory phospholipase A_2 ($sPLA_2$) overexpressed in several cancer types to actively release anticancer drugs. The generated hydrolysis products, i.e. fatty acids and lysolipids, can also serve as cytotoxic agents and permeability enhancers, both at the carrier and target cell membranes. This approach opened new venues for designing lipase-sensitive liposome-forming lipid prodrugs, where the fatty acid at position *sn*-2 has been replaced by anticancer drugs. The enzymatic action of $sPLA_2$ on lipid carriers is strongly influenced by the lipid (substrate) chemical structure, lipid membrane composition, payload, and lipid/enzyme ratio.

References: T. L. Andresen, S. S. Jensen, and K. Jørgensen, Prog. Lipid Res. 44, 197–224 (2005); A. Arouri and O. G. Mouritsen, J. Liposome Res. 21, 296-305 (2011); A. Arouri and O. G. Mouritsen, Eur. J. Pharm. Sci. 45, 408-420 (2012); A. Arouri and O. G. Mouritsen, Prog. Lipid Res. 52, 130-140 (2013); A. Arouri, A. H. Hansen, T. E. Rasmussen, and O. G. Mouritsen, Curr. Opin. Colloid Interface Sci. 18, 419-431 (2013).

10-POS Board 10

Local and Allosteric Effects Due to Displacement, Force and Mutation Induced Perturbations on Calmodulin and Hsp70

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We investigate how perturbations that may be experienced by proteins in their fluctuating environments may be invoked to facilitate their access to different microstates, using the example of calmodulin[1] and Hsp70[2]. We introduce perturbations that are explored within the neighborhood of the local minima of the protein by (i) exerting external forces applied (PRS)[3], (ii) selected displacements[4], (iii) mutating each residue to alanine[2]. In (i), we operate under the linear response assumption and the kernel of this approach is the variance-covariance matrix obtained from all-atom molecular dynamics simulations. In (ii), external displacements are introduced such that the observed changes remain in the linear response regime. By minimizing the protein-water system under this constraint, all other atoms are allowed to re-arrange around the perturbed geometry. In (iii), selected residues are mutated to alanine, followed by energy minimization. This procedure also allows the protein-water system to respond around the perturbed geometry, revising the local interactions occurring due to the mutated residue. In all approaches, residues that led to the best overlaps with the experimentally determined conformational change are further analyzed to explore their relation to protein structure and function. Each method has a different perspective of inherent assumptions and the interactions accentuated by each vary. The differences between these methods are compared in terms of interactions, dominating forces in the response of the system, and key residues on the protein structure that are implicated by each approach.

[1] A.O. Aykut, A.R. Atilgan, and C. Atilgan, PLoS Comput. Biol., 9, 12, (2013).
[2] G. Ozbaykal, C. Atilgan, and A. R. Atilgan, to appear.(2014).
[3] C. Baysal and A. R. Atilgan, Proteins, 45, 62 (2001).
[4] C. Atilgan and A. R. Atilgan, PLoS Comput. Biol., 5, e1000544 (2009).

11-POS Board 11

Homology Modelling of DapE from S.enterica: Insight into Inhibition and Cooperativity

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There is an urgent need to discover and develop novel antibiotics to fill the repertoire of drugs available to fight infection. The dapE-encoded N-succinyl-L,L-diaminopimelic acid desuccinylase (DapE) is an essential enzyme of the anabolic pathway toward lysine in many bacteria and thus a promising target for antibiotic drug-design. The understanding of the exact enzymatic mechanism of this enzyme may thus facilitate the development of novel effective antimicrobial drugs. L-captopril has been shown to exhibit promising inhibitory activity in vitro against DapE however does not target DapE effectively in vivo. We have built homology models for S.enterica DapE in their dimeric apo and holo forms based on different template states, and docked L-captopril into both apo- and holo-model. The apo-model with ligand provides a plausible qualitative explanation for the metal-dependent selectivity of L-captopril inhibition: L-captopril targets only the Zn2+-metallo-isoform of the enzyme, whereas the Mn2+-enzyme, which is also a physiologically-relevant isoform in bacteria, is not inhibited. Here we show that DapE of the pathogen Salmonella enterica exhibits positive kinetic cooperativity, which is associated to a domain-movement in the enzyme dimer to form an ionic interaction between a conserved arginine of the dimerisation domain and a carboxylate of the substrate or substrate analog-inhibitor. Such cooperativity leads to the counterproductive effect of effective activation of enzyme activity by suboptimal dosage of competitive inhibitors occurring within a range of substrate concentration that is expected to be physiologically relevant. The holo model of DapE shows large domain movements that appear during catalysis, which are considered to give rise to the positive cooperativity in DapE. Understanding the mechanisms of inhibition and cooperativity from a structural perspective is an important step towards the discovery of new inhibitory lead compounds with improved properties.

12-POS Board 12

Hydration of Protein-RNA Recognition Sites

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We investigate the role of water molecules in 89 protein-RNA complexes taken from the Protein Data Bank. Those with tRNA and single-stranded RNA are less hydrated than with duplex or ribosomal proteins. Protein-RNA interfaces are hydrated less than protein-DNA interfaces, but more than protein-protein interfaces. Majority of the waters at protein-RNA interfaces makes multiple H-bonds, however, a fraction do not make any. Those making H-bonds have preferences for the polar groups of RNA than its partner protein. The spatial distribution of waters makes interfaces with ribosomal proteins and single-stranded RNA relatively ‘dry’ than interfaces with tRNA and duplex RNA. In contrast to protein-DNA interfaces, mainly due to the presence of the 2′OH, the ribose in protein-RNA interfaces is hydrated more than the phosphate or the bases. The minor groove in protein-RNA interfaces is hydrated more than the major groove, while in protein-DNA interfaces it is reverse. The strands make the highest number of water-mediated H-bonds per unit interface area followed by the helices and the non-regular structures. The conserved waters at protein-RNA interfaces make higher number of H-bonds than the other waters. Preserved waters contribute towards the affinity in protein-RNA recognition and should be carefully treated while engineering protein-RNA interfaces.

13-POS **Board 13**

An Allosteric Signaling Pathway of Human 3-phosphoglycerate Kinase from MD Simulations and Froce Distribution Analysis

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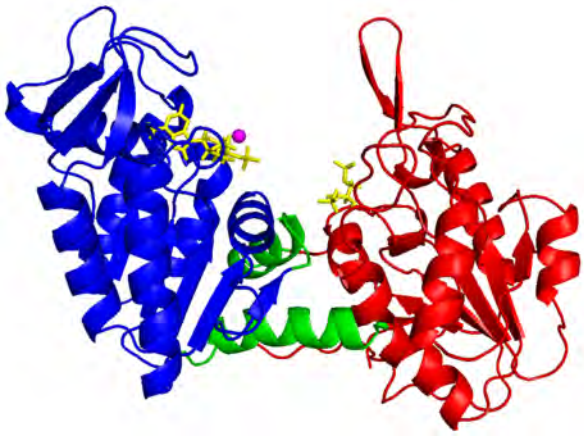
3-Phosphoglycerate kinase (PGK) catalyzes the phospho-transfer reaction between 1,3-bisphosphoglycerate and ADP. It is a two domain enzyme, with the two substrates bound to the two separate domains. In order to perform its function the enzyme has to undergo a large conformational change involving a hinge bending to bring the substrates into close proximity. The allosteric pathway from the open non-reactive state of PGK to the closed reactive state as triggered by substrate binding has only been partially uncovered by experimental studies. Using Molecular Dynamics simulations combined with Force Distribution Analysis we describe a complete allosteric pathway, which connects the substrate binding sites to the interdomain hinge region. While previously identified key residues involved in PGK domain closure are part of this pathway, we here fill the numerous gaps in the pathway by identifying newly uncovered residues and interesting candidates for future mutational studies.



14-POS Board 14

Catalytic Promiscuity and Evolution in the Alkaline Phosphatase Superfamily

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It has been observed that many enzymes are able to facilitate the turnover of more than one chemically distinct substrate (catalytic promiscuity). This feature has been related to enzyme evolution, with highly promiscuous ancestor enzymes evolving under evolutionary pressure to current specialists, while still retaining some level of their former promiscuous activities[1]. Such theory has been extensively tested by various experiments using in vitro evolution[2]. The alkaline phosphatase superfamily members provide a particularly attractive showcase for studying enzyme promiscuity, as they often show reciprocal promiscuity, in that the native reaction for one member is often a side-reaction for another[3]. While deceptively similar, their catalyzed reactions (cleavage of P-O and S-O bonds) proceed via distinct transition states and protonation requirements[4,5]. We present detailed computational studies of the promiscuous catalytic activity of two members: the phosphonate monoester hydrolases from *Burkholderia caryophili*[6] and *Rhizobium leguminosarum*[7]. We also make comparison with an evolutionary related member: the arylsulfatase from *Pseudomonas aeruginosa*[5]. By tracking their structural and electrostatic features, and comparing to other known members of the superfamily, we provide an atomic-level map for functional evolution within this superfamily.

1. R.A. Jensen, Annu. Rev. Microbiol. 30, 409 (1976)
2. O. Khersonsky, D.S. Tawfik. Annu. Rev. Biochem. 79, 471 (2010)
3. S. Jonas, F. Hollfelder. Pure Appl. Chem. 81, 713 (2009)
4. S.C.L. Kamerlin. J. Org. Chem. 72, 9228 (2011).
5. J. Luo, B. van Loo, S.C.L. Kamerlin, FEBS Lett. 586, 1211 (2012)
6. B. van Loo et al. Proc. Natl. Acad. Sci. USA. 107, 2740 (2010)
7. S. Jonas et al. J. Mol. Biol. 384, 120 (2008)

15-POS Board 15

Computational Prediction of miRNAs and Their Targets in *Phaseolus Vulgaris* Using Simple Sequence Repeat Signatures

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MicroRNAs (miRNAs) are endogenous, noncoding, short RNAs directly involved in regulating gene expression at the post-transcriptional level. In spite of immense importance, limited information of *P. vulgaris* miRNAs and their expression patterns prompted us to identify new miRNAs in *P. vulgaris* by computational methods. Besides conventional approaches, we have used the simple sequence repeat (SSR) signatures as one of the prediction parameter. The presence of SSRs in pre-miRNAs is already established, although their role in pre-miRs is unknown. Conserved SSR signatures are a potential parameter in predicting new miRNAs. In this study, we have used the conserved SSR signatures for the first time as a prediction parameter. There is no universal SSR that is conserved among all precursors of Viridiplantae, but conserved SSR exists within a miRNA family and is used as a signature in our prediction. Moreover, for all other parameters including normalized Shannon-entropy, normalized base-pairing index and normalized base-pair distance, instead of taking a fixed cut-off value, we have used 99% probability range derived from the available data. We have identified 208 mature miRNAs in *P. vulgaris* belonging to 118 families, of which 201 are novel. miRNA distribution varies between the families and the most populated ones are MIR1533, MIR1527, MIR5021 and MIR848 with 15, 10, 10 and 7 members, respectively. The length of mature miRNAs varies between 15-24 nucleotides. A total of 1305 target sequences were identified for 130 miRNAs. Using 80% sequence identity cut-off, proteins coded by 563 targets were identified. Our findings will contribute to the present knowledge of miRNAs and their targets in *P. vulgaris*. The new approaches and modifications of existing methods is not only restricted to *P. vulgaris* but can be applied to any species of Viridiplantae.

16-POS Board 16

Energetics of Single- and Multi-Ion Permeation in Sodium and Calcium Channels

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Recent determination of the molecular structures of voltage gated NavAB and CavAB channels from x-ray crystallography has led to a renewed interest in these ion channels. The pore architecture of them suggests a conduction pathway involving transitions between two main states with one or two hydrated Na^{+}/Ca^{2+} ions bound in the selectivity filter. Molecular dynamics methods are the only physically valid methods for studying the structure function relations in ion channels. Applications of these methods to sodium and calcium channels are presented, which illustrate the multi-ion nature of the permeation mechanism in selective biological channels.

17-POS Board 17

Ab-Initio Molecular Dynamics Simulation of Photoacids: Competition between Excited State Proton and OH^- Transport via a Water Wire

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Biophysical processes often take place based on proton relay along a hydrogen bonded chain [1]. Such proton transfer reactions along "water wires" are difficult to observe directly inside a protein. Photosensitive acid/base systems provide a method to control and study ultrafast proton transport via infrared spectroscopy [2]. Hydroxyquinolones (HQ), are simultaneously photoacids and photobases.
We elucidate excited state proton exchange mechanisms along short chains of water molecules (water wires) by means of ab-initio calculations. We exploit the specific geometry of 7-Hydroxyquinoline, a combined photoacid/-base, to establish well-defined proton donor/acceptor sites, linked by a water wire of three water molecules [3]. In addition, departing from our successful simulation of the excited state dynamics and fluorescence shift of the related N-methyl-6-quinolone [4], we also study the ground and excited state aqueous solvation of different HQs, focussing on the identification of water wires and excited state protonation dynamics.

[1] H. Luecke, H.-T. Richter, J. K. Lanyi, Science 280 (1998), 1934.

[2] O. F. Mohammed, D. Pines, J. Dreyer, E. Pines and E. T. J. Nibbering., Science 310 (2005), 5745.

[3] G. Bekcioglu, C. Allolio, and D. Sebastiani (submitted).

[4] C. Allolio, M. Sajadi, N. P. Ernsting and D. Sebastiani, Angew. Chem. Int. Ed. 52 (2013), 1813-1816.

18-POS **Board 18**

Aromatic Amino Acids Promote Peptide Folding by Locally Reducing Backbone Hydration

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The relation between the sequence of a protein and its tridimensional structure remains largely unknown. We investigated peptides of sequence EGAAXAASS (X = Gly, Ile, Tyr, Trp) through molecular dynamics (MD) simulations and NMR residual dipolar coupling (RDC) measurements. The RDC patterns of peptides with X= Gly or Ile are rather flat, suggesting extended, unfolded peptides, while the contrasted patterns for peptides with X = Tyr or Trp suggest compact folded structures. The MD simulations show that the formation of internal hydrogen bonds underlying helical-turns is key to reproduce the experimental RDC values for the peptides containing aromatic residues. The simulations further reveal that the driving force leading to such helical-turn conformation arises from the lack of hydration of the peptide chain on either side of the bulky aromatic side chain, which can potentially act as a nucleation point initiating the folding process. These results provide a starting point to understand the amino acid code underlying the mechanism of protein folding.

19-POS **Board 19**

Abstract Withdrawn

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20-POS Board 20

The Effects of The Mutations of Arylsulfatase A on Its Structure And Function

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Arylsulfatase A (ASA) is a lysosomal enzyme catalyzing the hydrolysis of sulfate ester bonds. Its major substrate is cerebroside-3 sulphate and the product of the hydrolysis reaction, cerebroside, is the major constituent of myelin sheats. In case of the deficiency of this enzyme, myelin sheath cannot be produced, as a result demyelination of neurons occurs leading to MLD (Metachromatic leukodystrophy disease). MLD has various lethal neurological symptoms, such as difficulties in walking and swallowing, spasticity etc. Özkara and coworkers (200x) identified some MLD patients with mutations E307K, T391S, W318C, N350S on ASA, but almost no ASA activity.

Our aim is to find the mechanism, which changes the ASA activity and functionality, as a cause of stated mutations. First, data mining about ASA's structure and function, activators and MLD were performed by Ayşe Eren and Maral Budak. Secondly, 3D structure of ASA was investigated in order to get a better understanding of the sites of the mutations and their closeness to the active site, and it is found out that they are rather far from there. Next, BLAST and COBALT were used to investigate whether the interest mutations are on the conserved sites or not, by comparing ASA with phylogenetical relatives. The free energies of the wild type and mutated types of ASA have been calculated using FoldX. It is predicted that these mutations detoriate the enzyme energy and maybe cause another minimum for the enzyme to fold in another conformation or some emergent intermolecular interactions, caused by the mutated residues effect enzyme's proper activity, thus it becomes inactive or cannot dimerize or octamerize.

21-POS Board 21

Prediction of Hydrophobe Extrusion Out of Bilayer

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The biological membranes can swallow a large variety of hydrophobic molecules floating nearby in vivo. Many of these molecules exhibit a limited solubility in the phospholipid bilayers. Therefore, separate phases can emerge between the leaflets. As new molecules join the encapsulated phase, its size finally exceeds certain limit (specific for each bilayer composition) and the separation takes place. Depending on the affinity between the phospholipids and the hydrophobe particles, the confined phase can be a droplet (in case of low attraction), a disc in case of stronger attraction) or can be distributed throughout the bilayer plane (in case of significant solubility).
We introduce a method to predict the critical linear sizes of encapsulated hydrophobic phase, which trigger curvature-driven extrusion. The phase extrudes when the projected force of its repulsion from the bilayer exceeds the force of phospholipids attraction within the bilayer. The larger is the curvature of the phase, the larger force (repulsive energy) it generates in the system. One can consider a range of relatively small (spatially) phases inside the bilayer and record the force corresponding to the bilayer resistance. The obtained function of penalty force versus phase volume must be extrapolated to zero force. Zero force, by definition, corresponds to a free motion of an object, i.e. spontaneous separation of two phases.
The proposed method can be applied to a variety of lipid bilayers and various encapsulated hydrophobes to get information about their separation and the durability of the bilayer curvature. The method allows the investigation of length scales, which are inaccessible for direct computer simulations with an explicit molecular resolution

22-POS Board 22

Computational Analysis of Biopharmaceuticals Stability for Early Prediction of Manufacturability

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Biopharmaceuticals or therapeutically relevant proteins have become one of the fastest growing parts of the pharmaceutical industry. These innovative molecules are more complex than conventional drugs and their processing is much more demanding.The analytical characterization of these new drugs is a fundamental step in the early prediction of their behavior in bioprocesses. This research project aims to develop a framework to improve candidate design and selection at early stages of development by establishing a set of critical analysis and identifying key properties (intrinsic and extrinsic) allowing the prediction of candidates behaviour in large-scale bioprocesses.
Our multidisciplinary approach combines the computational analysis (sequence analysis, Molecular Dynamics simulations and docking) and the biophysical characterization of a set of Fragment antibody (Fab) mutants. This allowed the identification of several regions of unstable structure which could be targeted to enhance candidate's stability. The effect of formulation was also investigated highlighting the role of electrostatics in Fab stability and folding. Additionally, aggregation kinetics studies were carried out at a wide range of temperature, pH and ionic strength allowing the determination of a model for Fab aggregation. These data are used as early indicators for protein stability and to create indices for product manufacturability

23-POS Board 23

Structural Analysis of Putative HCV Glycoprotein E2 Binding Sites on Human CD81 in Compared with Their Mouse Counterparts

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Hepatitis C virus (HCV), formally identified in 1989, is an enveloped virus with a single positive stranded genomic RNA. HCV infection in patients often results in chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma. However, the treatments for HVC infection are still limited. This might be due to the fact that the current cell and animal model systems are not sufficient for studying the HCV infection in details.
In this study, we are firstly aiming to investigate the interactions between HCV E2 protein and CD81 of host cells, which have been proved crucial for HCV entry into host cells, using molecular docking. From the bioinformatic results, we should be able to develop the potential peptide drugs for interrupting HCV E2/CD81 interactions to prevent the HCV infection into host cells. Previous studies showed that HCV can bind to human CD81 with high specificity. For comparison, homology modeling was also performed to obtain the structure of mouse CD81 using human CD81 as a template with high similarity up to 93%. So far, the docking results showed that the short helices near C-terminal domain of HCV E2 bind to the head regions of dimeric human CD81 with hydrophobic contacts, while the binding sites are altered when the HCV E2 are docked to mouse CD81. Two peptides were designed based on the docking prediction and the work were followed by the synthesis of fluorescent dye 5-carboxyfluorescein labelled peptides and flow cytometry in order to measure the binding efficiency of the synthetic peptides onto host cells.

24-POS Board 24

In Silico Analysis and Experiments of Potential Anti-Inflammatory Peptides Inhibiting the Binding of Chemokine CXCL8 with Its Receptors CXCR1 and 2

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Chemokine CXCL8 secreted by macrophages, endothelial cells, epithelial cells, mast cells and fibroblasts plays a central role in human immune and inflammatory response by binding to and activating its cognate G-protein coupled receptors CXCR1 and CXCR2. Upon binding of CXCL8, CXC receptors undergo conformational changes resulting in downstream signal transduction. The chemokine receptors have been identified as attractive targets for therapeutic intervention in various diseases due to the role of chemokine in immune and inflammatory responses.
The purpose of this study was to use molecular docking to determine the potential anti-inflammatory peptides to inhibit CXCL8 binding to its receptors CXCR1 and 2. The docking results showed that monomeric CXCL8 initially binds to CXCR1 and 2 at similar binding sites dominated by electrostatic interactions. The binding complex systems including monomeric and short peptide CXCL8 with CXCR1 and 2 were then embedded into the POPC lipid bilayers for 300 ns MD simulations respectively. The binding free energy (ΔG_{bind}) calculated by MM/PBSA technique indicated that the ΔG_{bind} of monomeric CXCL8 to CXCR1 is much lower than that of monomeric CXCL8 to CXCR2. On the other hand, the ΔG_{bind} for short peptide of CXCL8 to CXCR1 is also lower than that for monomeric CXCL8 to CXCR1, indicating that the determined potential peptide has a better competitive binding advantage to CXCR1 and 2 than that of monomeric CXCL8. The bio-assay experiments further verified that the synthesized potential peptide can significantly decrease LPS and CXCL8 induced monocyte adhesion to endothelial cells and inhibit monocytic migration induced by inflammation.

25-POS **Board 25**

Combining Intravital Imaging and Gene Delivery to Better Understanding Protein Dysregulation, Degradation and Upregulation for the Generation of Improved Renal Therapeutics

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Renal ischemic injury manifests in substantial protein dysregulation and degradation. Conventionally, there have been many limitations to improving the fundamental understanding of this diseased state. In vivo techniques, like two-photon intravital imaging, provide a unique platform to conduct such investigations with meaningful spatial and temporal resolutions. Using this approach, in conjunction with a novel hydrodynamic gene delivery technique, we were able to simultaneously investigate structural and functional changes that occur in the live rat kidney. Specifically, an effective use of hydrodynamic fluid forces has enabled efficient and reliable renal gene delivery that can overcome traditional hindrances to these investigations. By facilitating the expression of fluorescent actin, we were able to track this biomarker in normal in vivo conformations and modulations it undergoes during ischemic injury and repair. We also utilized this gene delivery technique to genetically alter the mitochondrial proteome. These alterations conferred with native enzyme upregulation that resulted in both the treatment of and protection against ischemic injury in the rat kidney. Overall, this novel and powerful combination of intravital imaging and gene delivery appears to be an exciting basis that can foster further biological advances in renal physiology.

26-POS Board 26

A Transferable Coarse-Grained Model for Diphenylalanine: How to Represent an Environment Driven Conformational Transition

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Coarse-grained (CG) simulation models allow longer simulation times and larger systems due to their reduced degrees of freedom compared to the all-atomistic (AA) models. However standard CG methods yield state-dependent potentials whereas biological processes such as folding/unfolding upon interaction with an interface or upon aggregation, involve multiple states. The present study investigates the challenge of transferability for CG models and aims to generate a CG model that is able to represent such processes.

Diphenylalanine is a zwitterionic dipeptide that displays a transition from a trans-like to a cis-like conformation upon aggregation as well as upon transfer from bulk water to the cyclohexane/water interface. The nonbonded interactions are obtained by mimicking solvation free energies of structurally relevant counterparts where each solvent molecule is represented with a single bead and the peptide with a total of four beads. The success of the model strongly depends on nontrivial decisions one has to make to capture the delicate balance between the bonded and nonbonded interactions.

We show that one can construct a coarse-grained model that is able to reproduce the bulk and interface conformational behavior and the segregation between the bulk and hydrophobic/hydrophilic medium. We found that the cyclohexane/water interaction potential, an interaction that does not involve the peptide can influence the conformation of the peptide as well as the properties of the hydrophobic/hydrophilic interface. Furthermore, we show that a small modification to improve the structural/conformational properties of the CG model could dramatically alter the thermodynamic properties.[1]

[1]Dalgicdir, C., Sensoy, O., Peter, C., & Sayar, M. (2013). A transferable coarse-grained model for diphenylalanine: How to represent an environment driven conformational transition. *The Journal of chemical physics*, 139(23), 234115.

27-POS Board 27

Discovery of a Cryptic Druggable Pocket in Human p53

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The tumor suppressor p53 has a major role in the defense of a cell against cancer. Cancer can only proceed after p53 or its pathways are inactivated by mutations. Thus, reactivation of mutant p53 with small-molecules have been a long-standing idea for potential cancer treatment. We explored the dynamic ensemble of many p53 mutants as well as the wild-type protein using molecular dynamics (MD) simulations using NAMD suite. In the MD-generated ensembles of p53 mutants, we have identified a transiently open binding pocket occluded in the available crystal structures. Virtual screening against different conformations of this cryptic pocket identified 45 promising compounds among which stictic acid emerged as a potential p53 reactivation compound. Stictic acid demonstrated dose-dependent p21 activation in human osteosarcoma cells with R175H mutant of p53. Encouraged by this result, we have performed virtual screening of 1.7 million compounds against a single pocket-open conformation of p53. Among the top 1% the compounds with the highest binding scores, we used machine learning to select a set of 138 diverse compounds out of which 15 compounds (~11% hit rate) were found to be potential p53 reactivation compounds by biological assays. Our findings highlight this cryptic druggable pocket as a promising pharmaceutical target for p53 reactivation.

28-POS Board 28

His226 is Important to Control the Linker Induced Open and Closed States of the ATPase Domain of DnaK

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Hsp70 proteins have essential roles in cells such as de novo folding of newly synthesized proteins, refolding or ubiquitination of denatured proteins, protein tarfficking and translocation through membranes. DnaK, Escherichia coli homolog of Hsp70 molecular chaperone, is comprised an N-terminal ATPase domain (NBD), a C-terminal substrate-binding domain (SBD) and a partially conserved hydrophobic linker that connects the domains. Substrate-binding affinities on SBD are driven by ATP-ADP conversion cycles in NBD. Allosteric communication between the two domains is provided by the conserved 389VLLL392 sequence on the linker region. Previous studies done using truncated DnaK(1-392) construct, containing the 389VLLL392 sequence, showed a pH-dependent enhanced ATPase activity, similar to the substrate-stimulated activity of the full-length protein; whereas construct lacking this sequence, DnaK(1-388) showed an activity resembling to the unstimulated-form of full-length. In the same study, it was proposed that linker binding to the ATPase domain causes a change in the slow step of the ATP hydrolysis reaction by rearranging the ATPase domain to a conformation where ADP release becomes the rate-limiting step. Here, we are investigating the molecular details of the reason of pH dependence and enhanced ATPase activity upon linker interactions with the domain using ATPase domain constructs both in in vitro and in silico. We observed with molecular dynamic simulations significant upshift in the pKa values of Asp194 and Asp201 compared to their expected pKa values as negatively charged residues, and it seems like that protonation states of these residues at different nucleotide-bound forms are important in the linker derived conformational changes leading, speculatively, variations in the Pi and ADP affinities. Our results will be discussed with overall ATPase rate as well as ADP off-rate measurements on DnaK(1-388) and DnaK(1-392) constructs.

29-POS Board 29

Protein Folding Transition Paths from Simulations, Theory, and Experiment

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The transition-path is the tiny fraction of an equilibrium, single-molecule trajectory when the transition over a free-energy barrier occurs between two states. In the case of protein folding, the distribution of transition paths contains all of the mechanistic information on how a protein folds and unfolds. Transition path distributions can now be predicted for fast folding proteins by all-atom molecular dynamics simulations and by an Ising-like theoretical model (1,2). Experimental information on transition paths should provide the most demanding test of both simulations and theoretical models. However, transition-paths for barrier crossings have never been observed experimentally for any molecular system in solution. Because it is a single molecule property, even determining the average transition-path time is challenging. In this presentation, I will discuss how we use measurements of Foerster resonance energy transfer in single molecule fluorescence experiments and a photon-by-photon analysis to measure average transition path times for proteins of different topology and folding rate coefficients using the Gopich/Szabo maximum likelihood method (3.4). These results, which are surprisingly interesting, are just the first, but important, steps toward measuring intra-molecular distances during individual transition paths.

1. R. B. Best, G. Hummer, and W.A. Eaton. “Native contacts determine protein folding mechanisms
in atomistic simulations.” *Proc. Natl. Acad. Sci. USA* 110, (2013)
2. E.R. Henry, R.B. Best and W.A. Eaton. “Comparing a simple theoretical model for protein folding with all-atom molecular dynamics simulations.” *Proc. Natl. Acad. Sci. USA* 110, 17880-17885 (2013).
3. H.S Chung, K. McHale, J.M. Louis, and W.A. Eaton. “Single-molecule fluorescence experiments determine protein folding transition path times.” *Science* 335, 981-984 (2012).
4. H.S. Chung, and W.A. Eaton. "Single molecule fluorescence probes dynamics of barrier crossing." *Nature* 502, 685–688 (2013)

30-POS Board 30

Transcriptomic Profiling to Reveal Therapeutic Targets and Cellular Mechanisms of Drug Resistance in Liver Cancer

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Liver cancer is one of the leading causes of cancer-related deaths worldwide with limited therapeutic options for advanced Hepatocellular carcinoma (HCC). Yet, the only FDA-approved therapeutic agent, Sorafenib, which targets RAF/MEK/ERK pathway, fails to prevent tumor recurrence due to persistent signaling through alternative PI3K/AKT/mTOR pathway. In this study, we show that targeting of PI3K/AKT/mTOR signaling by small molecule inhibitors is cytotoxic in HCC cells, the underlying transcriptomic response is dependent on Akt activation status, and combination of inhibitors of PI3K or AKT and Sorafenib leads to synergistic growth inhibition and enhanced cell death. Cytotoxic activities of nine small molecule inhibitors of PI3K/AKT/mTOR pathway were shown by SRB and RT-CES assays in HCC cell lines having normal or hyper-active AKT protein. Apoptotic cell death, and suppression of cell cycle progression and migration were shown by flow cytometry, wound healing, immunofluorescence and western blots experiments. When used in combination with Sorafenib, two most potent inhibitors, PI3Ki-alpha and Akti-2, showed synergistic actions on HCC cells in vitro and in vivo mice xenografts. Computational analysis of microarray and RNA-seq data acquired in the presence of these agents alone or in combination with Sorafenib identified specific gene sets. KEGG pathways were used to visualize drug-mediated regulation of cellular signaling in molecular level in Cytoscape. Our transcriptomic analysis identified direct enzymatic targets and key downstream effector proteins. Comparison of the transcriptomic profiles of Sorafenib, PI3Ki-alpha and Akti-2 as single and combined agents revealed proteins that are responsible for enhanced cytotoxic activity and suggested new targets.

31-POS Board 31

Simulated Amyloid Fibril Nucleation in Reverse Micelles

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A recently published FTIR study has shown that the 40-residue amyloid beta (Abeta) protein forms extended beta-strands in reverse micelles, while an analogue with a scrambled sequence does not. This result suggests that the Abeta sequence is inherently amyloidogenic, and that its amyloidogenicity is enhanced in a crowded confined membrane-like environment of a reverse micelle. This result is significant because it suggests that these factors may nucleate or otherwise promote the formation of amyloid fibrils in the human brain in Alzheimer's disease. We have conducted molecular dynamics simulations of wild-type and scrambled-sequence Abeta protein in reverse micelles of the same composition studied experimentally to gain insight into the physicochemical factors that promote beta structure in wild type, but not scrambled sequence protein. Preliminary results show that the wild-type sequence does indeed form extended beta structure, while the scrambled sequence does not. The interactions stabilizing beta structure in the wild type sequence appears to be hydrogen bond formation involving amino acid side chains.

32-POS Board 32

Molecular Dynamics Comparison of E. coli WrbA Apoprotein and Holoprotein

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WrbA is a novel multimeric flavodoxin-like protein of unknown function. A recent high-resolution X-ray crystal structure of E. coli WrbA holoprotein revealed a methionine sulfoxide residue with full occupancy in the FMN-binding site, a finding that was confirmed by mass spectrometry [1]. In an effort to evaluate whether methionine sulfoxide may have a role in WrbA function, the present analyses were undertaken using molecular dynamics simulations in combination with further mass spectrometry of the protein. Methionine sulfoxide formation upon reconstitution of purified apoWrbA with oxidized FMN is fast as judged by kinetic mass spectrometry, being complete in ~5 hours and resulting in complete conversion at the active-site methionine with partial conversion at second, heterogeneous sites. Analysis of methionine oxidation states during purification of holoWrbA from bacterial cells reveals that methionine is not oxidized prior to reconstitution, indicating that methionine sulfoxide is unlikely to be relevant to the function of WrbA in vivo. Although the simulation results, the first reported for WrbA, led to no hypotheses about the role of methionine sulfoxide that could be tested experimentally, they elucidate the origins of the two major differences between apo- and holoWrbA crystal structures, an alteration of inter-subunit distance and a rotational shift within the tetrameric assembly.

[1] I Kishko, J Carey, David Reha, J Brynda, R Winkler, B Harish, R Guerra, O Ettrichova, Z Kukacka, O Sheryemyetyeva, P Novak, M Kuty, I Kuta Smatanova, R Ettrich, M Lapkouski (2013) 1.2 Å-resolution crystal structure of Escherichia coli WrbA holoprotein. Acta Crystallographica Section D: Biological Crystallography 69: 9. 1748-1757.

33-POS Board 33

Evaluating B-factors for Benchmarking Models of Collective Motion

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The interpretation of crystallographic B-factors in terms of thermal motion is prevalent in many branches of protein science, and commonly used to validate or benchmark computational models of protein motion. This practice implicitly assumes that known limitations to the thermal interpretation of B-factors can be safely ignored. One common criticism against using B-factors as a standard for validating modes is that they are influenced by many non-thermal factors. Another concern is that the thermal component of B-factors arises from motion in a highly restrictive crystalline environment. This environment is expected to dampen collective motion, the kind of motion often involved in functional motion like conformational change. I will present recently published results that reveal potential problems with using B-factors as a model of thermal motion of solvated proteins (Fuglebakk et al., JCTC, 2013). We have compared the collective motions of several elastic network models, a kind of protein model commonly validated and parameterized against B-factors. We obtained collective motion predictions from elastic network models and compared them with molecular dynamics simulations for seven solvated protein structures. We find that models that give good reproduction of B-factors are severely compromised in their ability to recapitulate collective motions. Moreover, we compare the elastic network models with a null model with restricted collective motion, and find that models parameterized to reproduce B-factors are in close agreement with this null model. We therefore find it important to consider the effect of the crystalline environment when interpreting B-factors, and avoid them altogether when doing quantitative comparisons.

34-POS Board 34

Influence of Phosphorylation State on the Conformation of Antibody Epitopes from the Polyproline Region of the Tau Protein: A Molecular Dynamics Simulation Study

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Tau is a highly soluble microtubule-associated protein (MAP) and its hyperphosphorylation is linked to several tauopathies. The role of Tau phosphorylation and dephosphorylation during microtubule (MT) stabilization and the formation of neurofibrillary tangles in Alzheimer's disease (AD) is poorly understood. The presence of many hyperphosphorylation sites in the proline-rich region (PRR) of the Tau protein suggests that the PRR domain may be important in phosphorylation-induced conformational changes.[1] Phosphorylations at both Thr231/Ser235 and Ser202/Thr205 in the PRR abolishes the ability of the Tau protein to polymerize tubulin into MT. We have characterised the structural impact of phosphorylation of the Tau protein by molecular dynamics (MD) simulations of functional fragments of the AT180 epitope. Salt bridges, hydrogen bonding and secondary structures were monitored and it was found that phosphorylation of the Thr231 and Ser235 residues stabilizes a short α-helix that runs from Ser237 until the first MT binding repeat, in agreement with NMR data.[2] Anti-PHF-tau mAb AT8 recognizes an epitope doubly phosphorylated at Ser202 and Thr205 in the PRR.[3] We have also characterised the conformation of the AT8 epitope using conventional and scaled MD simulations, with preliminary results suggesting the presence of turn propensity in the region pThr205-Arg209. The conformational properties of both epitopes are in stark contrast to circular dichroism data.[4,5] This structural characterisation will help in understanding of how phosphorylation regulates Tau's physiological and pathological aspects by disturbing local conformations.

References:
1. Buee et al., Brain Res Brain Res Rev, 2000; 33:95-130.
2. Sibille et al., Proteins, 2012; 80: 454–462.
3. Porzig et al., Biochem Biophys Res Commun, 2007; 358: 644–649.
4. Bielska AA and Zondlo NJ. Biochemistry, 2006;45(17):5527-37.
5. Brister et al., J Am Chem Soc, 2014; 136 : 3803-3816.

35-POS Board 35

Epileptic Seizure-induced Structural Changes in Genetically Epileptic Rat Bone Tissues: A Synchrotron-Fourier Transform Infrared Imaging Study

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It is previously reported that anti-epileptic drugs (AEDs) may cause osteopenia, osteoporosis, and fractures in epilepsy patients. However, it cannot be determined whether the bone disorders in epileptic patients are due to AED therapy and/or to epilepsy and epileptic seizures. The current study provides the first report on determination of the possible effects of epilepsy and epileptic seizures on bone tissues including intra-bone variations. The experiments performed on genetically epileptic and healthy rats, give the advantage of studying the effects of epileptic seizures alone without interfering with anti-epileptic drugs. Spine tissues were investigated by synchrotron-Fourier Transform Infrared microspectroscopy (SR-FTIRM) to get information about the site-specific effects of seizures on cortical part of spines. According to SR-FTIRM studies, mineral content was found to be decreased in epileptic group compared to the healthy control. B-type carbonate content which substitutes for phosphate groups in the mineral part of bone, was shown to be increased in epileptic group compared to the control in all parts of cortical bones. In addition, relative amount of nonreducible (mature) to reducible (immature) types of collagen cross-links, was found to be changed critically in epileptic group, indicating an increase in immature collagen crosslinks in the bones of that group. Furthermore, crystallinity value indicating crystal size was found to be increased in epileptic group compared to the healthy control which was due to the effect of epilepsy and epileptic seizures on bones. In conclusion; epilepsy and epileptic seizures caused a decrease in the strength of bone without any anti-epileptic treatment. The most affected cortical parts in spines, were mid-cortical and endosteum (inner cortical) according to SR-FTIR studies. This result may point to an alteration in the osteoclastic endosteal bone resorption due to epilepsy.

36-POS Board 36

Modulating of Protein Stability by Modifications

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A quite conventional way to change the thermodynamic stability of a protein is to control the unfolded state entropy and the folded state enthalpy. In principle, it should also be possible to regulate the enthalpy of the unfolded state and the entropy of the folded state. Here, we present several studies to illustrate that various protein modifications (PM) can change the thermodynamic stability of a protein in different ways. Using computational and experimental techniques we showed that shortening the L4 loop of the hmACP protein by six residues results in a strong stabilization. Surprisingly this effect was due to increasing the entropy of the folded state. Furthermore, we demonstrated that this mechanism is not exclusive for this system. In fact also the ubiquitination on the Ubc7 protein results in entropic destabilization. This is mostly due to reduction of the conformational flexibility of the loop region. In both studies the effect on the protein loop regions was critical. Glycosylation usually results in the stabilization of the protein but experimentally it was shown that the MM1 protein was destabilized by attaching of N-acetylgalactosamine. Using computational tools we found that attached sugars strongly interact with the protein, which leads to the disruption of the intramolecular native contacts. Most likely, the observed destabilization is related to these conformational changes.
Experiments have revealed that the effect of modifications of the PinWW protein depends on the type (Glycosylation/PEGylation) and site of the modification. Our computational study shed light on the molecular origin of experimentally reported effect of each modification of the stability of the PinWW. These examples illustrate the complex effect of modifications on the thermodynamic stability of proteins and may provide some principle for manipulating protein properties.

37-POS Board 37

Charting the Thermodynamic Landscape of Nucleotide Binding of the Universally Conserved Molecular Switch, Elongation Factor Tu.

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Protein design currently can produce scaffold structures that have the ability to bind to a specific ligand with a particular affinity. However, rational design of a protein with the ability to bind to two very similar ligands with a specific affinity is still a challenge. In order for this to become a routine process we have to improve our understanding of how the structure and dynamics of proteins have evolved to achieve fine-tuning of affinities as well as specificity for ligand selection. As a model system we have studied the universally conserved GTPase Elongation Factor Tu (EF-Tu). EF-Tu has the capability to bind to both GTP and GDP. Surprisingly, EF-Tu binds to GDP with a 40 fold higher affinity. This is unexpected, as the extra interactions between EF-Tu and the additional phosphate group on GTP should favor the latter interaction.
Here we report using rapid kinetics approaches the thermodynamic parameters that govern nucleotide binding for both GDP and GTP of EF-Tu. Interestingly EF-Tu has evolved in such a way that GTP and GDP binding differ based on the energy barriers of dissociation and not association. We also find that the EF-Tu•GDP complex is enthalpically favored while the EF-Tu•GTP complex is entropically favored. To investigate the thermodynamic parameters further we performed Molecular Dynamic simulations of EF-Tu bound to the respective nucleotide. These simulations allowed us to identify the dynamic features of EF-Tu that are likely to give rise to the reported thermodynamic parameters. We identify a hydrogen bonding network within EF-Tu that stabilizes the GDP conformation where as differences in water coordination favor the GTP conformation. Our findings provide for the first time the dynamic and thermodynamic properties that govern EF-Tu's nucleotide binding properties.

38-POS Board 38

CCMV Capsid Deformation Studied by Multi-Scale Simulation Techniques - Link towards Understanding of the Aggregation Process

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Here we report on our coarse graining efforts of CCMV (Cowpea Chlorotic Mottle Virus), an icosahedrally symmetric plant virus consisting of 180 identical protein monomers.

We utilize atomistic simulations of dimers for construction and optimization of a supportive elastic network used with a MARTINI-level CG model. This approach allows us to predict inter-protein conformational flexibility and properties of larger capsid fragments and reproduces experimental (Atomic Force Microscopy) indentation measurements of the entire viral capsid.

Later on we extend the AFM mimicking simulations to look into the breaking process of the virus until its ultimate structural failure and develop an automated method to analyze these huge trajectories. The method approaches the virus at different resolution levels and allows for classification of the capsomer interfaces in terms of symmetry classes and structure deformation at the protein level but can also track down to the residue level.

The symmetry-classes differ substantially in their stability and appear to backtrack the putative assembly pathway: the reverse stability order resembles the believed sequence. Dimers and pentamers of dimers (first and second assembly step) never fail while hexamers of dimers (last assembly step) do. While the wild type capsid fortifies this location with a cooperatively formed 6-stranded beta-barrel motif, the mutant we employed in our studies misses this part. Therefore we hypothesize that the assembly order is regulated by the strengths of the interfacial binding, but the late and weak spots may be reinforced by cooperative motifs that form post-assembly.

39-POS Board 39

Comparative Study of Functional Dynamics in Mutant von Hippel-Lindau Tumor Suppressor Protein Structures

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The von Hippel-Lindau cancer syndrome, which is an autosomal dominant disease characterized by development of tumors in different organs including renal cell carcinoma, is associated with the mutations on the von Hippel-Lindau tumor suppressor protein (pVHL). The mutation on pVHL bound to ElonginC-ElonginB complex activates the production of the hypoxia inducible factor (HIF) and the vascular endothelial growth factor (VEGF) which in turn leads to tumor growth. In this work, mutant structures of the pVHL bound to Elongin C and HIF which were experimentally demonstrated to have variable stabilities and binding affinities are built for further computational structural dynamics analysis. The dynamics of the wild-type, the designed Y98N mutant, and the designed Y98N-G123F double mutant structures are assessed comparatively using the Anisotropic Network Model (ANM), which predicts the magnitudes and directionalities of the collective motions by a harmonic vibrational analysis based on normal modes. In a previous Molecular Dynamics (MD) study, Y98N mutant was shown to be associated with the VHL disease whereas Y98N-G123F double mutant was shown to exhibit similar stability with the wild-type structure. Here, the analyses of the orientation of fluctuations and the correlations between fluctuations in the most cooperative functional modes of ANM also show that the wild-type dynamic behavior is similar to the Y98N-G123F double mutant compared to the Y98N mutant structure. The directions of fluctuations differ mainly around the dynamically key regions, i.e. the hinge regions in the minima of the mode shapes, justifying that the dynamic behavior of hinge residues should correlate with the functioning of proteins. Overall, this study would help in the drug design studies for the VHL disease by explaining the structural dynamics basis of molecular recognition and evolutionary optimization in the pVHL system.

40-POS Board 40

Structural Toll-like Receptor Pathway May Illuminate Its Roles in Inflammation and Cancer Crosstalk

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Inflammation is crucial for defense against pathogens, maintain homeostasis and heal wounds. Inflammation should be strictly regulated; if not finely tuned, it can lead to oncogenesis. Toll-like receptor (TLR) pathway orchestrates both innate and adaptive immune systems with an essential role in inflammation. Although extremely useful, the classical representation of pathways in terms of nodes-and-edges is incomplete: they exhibit which proteins interact but not how. Also, atomic details of interactions elucidate which parallel pathways can co-exist, how mutations affect the protein interactions and change the cellular outcome and support malignancies. TLR pathway plays a central role in inflammation and cancer crosstalk and construction of their structural pathway provides insights on their mechanism of action in tumor microenvironment. Here, we constructed the structural TLR pathway by employing a powerful algorithm, PRISM (PRotein Interactions by Structural Matching), mapped clinically observed oncogenic mutations of the structures of key adaptor molecules in the pathway. Structural analysis revealed that parallel pathways of TLR network are mutually exclusive due to shared binding sites: TRAF6, TRAF3, and FADD – which induce pro-inflammatory cytokines, interferons and anti-inflammatory cytokine IL-10, and apoptosis, respectively – compete to bind to the overlapping interfaces on MyD88. We also found that C27* nonsense mutation on FADD protein abolishes its interaction with MyD88 and thus prevents apoptosis. If FADD can no longer occupy MyD88 binding site, TRAF6 is free to bind, allowing constitutive activation of MAPKs and production of pro-inflammatory cytokines. And this may explain how C27* mutation on FADD contributes to initiation or progression of tumor.

41-POS Board 41

The ProteinModelPortal - How Good is My Prediction? –First Results from The Continuous Automated Model EvaluatiOn (CAMEO)

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Protein structure modeling is widely used in the life science community to build models for proteins, where no experimental structures are available. The ProteinModelPortal (PMP, http://www.proteinmodelportal.org) contains more than 21 million models from various modeling servers for more than 5.1 million distinct UniProt sequences and is regularly updated. Depending on the difficulty of the target protein the various modeling approaches differ in performance and we established the Continuous Automated Model EvaluatiOn (CAMEO, http://www.cameo3d.org) platform assessing the performance of servers predicting protein structures in its 3D category. Based on the weekly PDB pre-release, protein sequences (targets) are submitted to participating servers. After the release of the 3D coordinates four days later, the returned predictions are then compared to the corresponding PDB structures (references). Within 121 weeks 49837 protein structure predictions by 34 servers for 2129 targets were assessed in the 3D category. Besides 3D predictions, CAMEO assesses ligand binding residues predictions in proteins (16 servers registered). Since the quality of models ultimately determine their utility, a new category "Quality Estimation of protein structures models" is being introduced to CAMEO. So far two servers and 4 stand-alone versions of the most popular quality estimation methods along with a custom naïve predictor based on CAMEO 3D data are being evaluated. Continuous assessment of prediction servers allows to retrospectively analyze the performance of a given server - with implications for PMP, as the quality of the predictions may vary significantly among different servers depending on the specific target protein and the chosen approach. The blind predictions obtained by CAMEO are directly suited for publication and the straightforward modular extension of CAMEO allows new categories and scores to be added on demand of the respective communities.

42-POS Board 42

Multiple Pre-Existence of Conformations Underpinning Allosteric Activation of Gal3p in Galactose Signal Transduction

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Conformational dynamics of protein has been considered as one of the key driving forces regulating cellular signaling. Signaling proteins have been shown to pre-exist predominantly in inactive conformation (I) untill a ligand or mutation induces a population shift in favor of the formerly less populated active conformation (A). However, the transition(s) between inactive and active conformational states has not been clearly elucidated. Here we investigate the dynamic stability and the role of amino acids involved in stabilization and intra-domain signal communications in Gal3p, a signal transducer protein in *GAL* genetic switch of *Saccharomyces cerevisiae*. Gal3p is known to exist in multiple conformational states (I and A). In response to galactose, Gal3p switches from open conformation (I) to closed conformation (A), to activate transcription of *GAL* genes. Closed and open conformer dynamics using canonical molecular dynamics (CMD) simulations were carried out for the wild type protein and its mutant variants. Distribution of the domain-lip distance from closed and open conformer dynamics shows a bimodal behavior for the former, while demonstrating a gaussian unimodal for the latter. Using hydrogen bond (H-bond) network analysis from CMD, we identified set(s) of conserved H-bonded amino acid pairs that are spaced far apart in the primary structure of the protein(s). Our results suggest the presence of a typical signature of H-bonded network specific to the state of the protein (open or closed) as well as varying H-bonded network for different mutant variants which could play a crucial role in determining their dynamic stability. We also used targeted molecular dynamics (TMD) simulations to study the free energy landscape along the transitional pathways from state 'I' to state 'A' in order to identify the transitional structures (local minima) encountered during the structural switching.

43-POS Board 43

Electrostatic Denaturation of Proteins during Solid-State Nanopore Translocation

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Protein translocation through solid-sate nanopores is promising emerging technique for identification of specific molecules at low concentrations. In the experiments, a voltage bias is applied across the nanopore and the ionic current is measured through the nanopore. As soon as a molecule travels through the nanopore, a dip in current is registered, called a pulse. The interactions in the confinements of a nanopore and biological molecules are still less understood. Experimental work at such scales is extremely difficult and the results are statistical in nature. Molecular dynamics simulations can predict important parameters to achieve required sensitivity and selectivity in detecting proteins. We report Nanoscale Molecular Dynamics simulations performing all-atom physical interactions between the nanopore walls and the proteins along with externally applied forces. The potential across the 6 nm thick nanopore was varied gradually from 50 to 500 mV and the conformational changes were investigated temporally for 10 ns of simulation time. The deviation of the protein structure from its initial form was quantified with root mean square deviations and also from changes in the energy states of the system. The otherwise stable protein structures were seen to be enormously disrupted, probably loosing functionalities also. The gradual unfolding of the protein molecules was observed both at the nanopore opening and inside the pore. The protein size, molecular weight and amino acid chain length also affected the conformational variation. Such changes can affect the outputs in proteomic studies at hand, by large margin, as any elongation or unfolding in the structure can change the supposedly “signature pulses” of the molecules. These results add towards better understanding of the protein behavior when passing through the nanopore and thus assisting its detection. Theoretical assessment of these phenomena is crucial before drawing experimental conclusions.

44-POS Board 44

Molecular Simulation Studies on Large Scale Aggregation of Self-Assembling Amphiphilic Peptides Reveal Factors Governing Biomineralization

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Biomineralization is the intricate process employed by living organisms to form minerals on preformed biological aggregates to build skeletal structures and shells. Rapaport and coworkers [1-3] have designed an important class of acidic residue-rich, self-assembling amphiphilic peptides that form hydrogels in bulk and, upon addition of ions to solution, enhance bone tissue regeneration.

Human orthopedic conditions, such as osteoporosis, are a direct consequence of poorly orchestrated biomineralization. Deciphering the molecular mechanism of this vital yet poorly understood process is thus essential for the development of therapeutic approaches. Our study fills this void by revealing the factors that might promote formation of stable aggregates, akin to the extracellular matrix (ECM), and subsequent biomineralization events.

We have used molecular dynamics simulations to obtain insight into the factors that govern the peptide aggregation and into the early stages of the biomineralization process. The effect of the various aspects of the peptide sequence on aggregate stability and ion-peptide interactions were studied. Our results reveal that peptides with proline as terminal residues formed more strongly ordered aggregates compared to those with phenylalanine. Aggregate stability was also found to be influenced by the nature of the side-chain groups of the peptides. Simulations in the presence of various ions showed how the ions influence aggregate stability in a side-chain-dependent manner. Our simulations also captured the crystallization events which might occur during the early stages of biomineralization.

[1] Rapaport, H.; Kjaer, K.; Jensen, T. R.; Leiserowitz, L.; Tirrell, D. A. *J. Am. Chem. Soc.* 2000, 122, 12523.
[2] Rapaport, H.; Grisaru, H.; Silberstein, T. *Adv. Funct. Mater.* 2008, 18, 2889.
[3] Amosi, N.; Zarzhitsky, S.; Gilsohn, E.; Salnikov, O.; Monsonego-Ornan, E.; Shahar, R.; Rapaport, H. *Acta Biomater.* 2012, 8, 2466.

45-POS Board 45

Modeling Multi-Protein Assembly Processes Using Single-Particle Reaction Diffusion

Margaret Johnson.
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Biological processes ranging from receptor mediated signaling to clathrin-mediated endocytosis depend on populations of distinct proteins competing and cooperating to stochastically form protein complexes at the membrane and in solution. The recruitment of proteins to both the membrane surface and to growing protein complexes can significantly alter a protein's dynamics and subsequent binding reactions. Building accurate models of these complex processes therefore requires tracking both the spatial and temporal evolution of proteins and their higher order assemblies. The challenge for these models lies in accurately reproducing experimentally known reaction rates between protein domains while correctly accounting for multi-protein complex formation at time scales of seconds or longer. We show how a recently developed algorithm for efficient simulation of single-particle protein-protein interactions can be extended to model protein recruitment and binding on the membrane, as well as specific protein-domain interactions. This free-propagator reweighting (FPR) method combines simple position updates to the proteins using free diffusion along with a trajectory reweighting method that allows us to recover the correct association rates for all binding interactions. The method extends readily from 3D solution to the 2D membrane, and the approach can correctly reproduce effects of rotational motion and orientational contraints on protein-protein interactions. With this level of spatial and structural resolution we are uniquely able to quantify the changes in protein binding dynamics that occur upon membrane binding and complex formation. With an assembly process such as occurs in the early stages of clathrin-mediated endocytosis, changes in the interaction dynamics could provide important controls for the successful formation of the clathrin protein coat, where protein motion is constrained by other proteins and by the membrane.

46-POS Board 46

Multi-layered, Iterative Protocols for Quantum Chemical Calculations

Seyit Kale[1], Benoit Roux[2], Jonathan Weare[3], Aaron Dinner[1,4].
[1]University of Chicago, Chicago, IL, USA, [2]University of Chicago, Chicago, IL, USA, [3]University of Chicago, Chicago, IL, USA, [4]University of Chicago, Chicago, IL, USA.

A common strategy in quantum chemical calculations is to start by modeling a system with a low level of theory and to progress to the desired (high) level of theory. While this seems intuitively reasonable, there is no formal reason that such a sequence is guaranteed to converge to the optimum for the desired level of theory. In fact, in cases in which the low and high levels of theory favor very different solutions, this approach could lead to local traps and slow down convergence. Here, we propose a theoretical framework for how one force field can be used to precondition another, so as to seamlessly accelerate convergence of the latter. We demonstrate this idea by applying it to geometry optimization and reaction path discovery for reactions of chemical and biological significance (hydrogen bond formation, proton transfer, Claisen rearrangement, and phosphate hydrolysis). Speedups of up to 3-5 fold are obtained.

47-POS Board 47

Investigating the Relationship between Characteristics of Protein-Protein Interfaces and Binding Affinity

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[1]Koc University, Istanbul, Turkey, [2]Arizona State University, Tempe, AZ, USA

Relating structure to function has been a fundamental issue in structural biology. Knowledge of structural details of protein-protein interactions is crucial in understanding protein function. However, to determine whether a protein complex actually exists under a given pH, temperature and concentration, and whether it is permanent or transient, knowledge of binding affinity is essential. Here, using a structure-based benchmark, we investigate whether the binding affinity correlates with the structural features of protein-protein interfaces. Proteins forming larger interfaces are observed to show a stronger binding, i.e. higher binding affinity. Additionally, higher number of critical residues, hot spots, implies a protein-protein interface with higher affinity. We also extend the contact order concept to analyze protein complexes and find that contact order of protein complexes correlate with binding affinity independent of the contact order of their unbound components. Finally, we investigate the organization of hot spot residues at protein-protein interfaces of benchmark complexes which show a large conformational change upon binding. Although protein interfaces undergo a large conformational change, there are some rigid residues which correspond to the computational hot spots at protein interfaces. Our findings would be crucial for predicting binding affinity based on features of protein interfaces as well as for docking studies.

SATURDAY POSTER SESSION

1:15 PM – 3:00 PM, Hall B

Posters being presented on Saturday, September 13, should be set up on the morning of September 13 and removed by 6:00 PM on September 13.

Kawabata, Takeshi	48-POS	Board 1
Kaya, Cihan	49-POS	Board 2
Kellett,Whitney	50-POS	Board 3
Korkmaz, Elif	51-POS	Board 4
Krieger, James	52-POS	Board 5
Kumar, Prasun	53-POS	Board 6
Kumar, Prasun	54-POS	Board 7
Kurkcuoglu, Zeynep	55-POS	Board 8
Lin, Milo	56-POS	Board 9
Malliavin, Therese	57-POS	Board 10
Marino, Kristen	58-POS	Board 11
Martin,Juliette	59-POS	Board 12
Merzel, Franci	60-POS	Board 13
Monkenbusch,Michael	61-POS	Board 14
Moreira, Irina	62-POS	Board 15
Muratcioglu, Serena	63-POS	Board 16
Nagel-Steger, Luitgard	64-POS	Board 17
Naithani, Ankita	65-POS	Board 18
Naumann, Renate	66-POS	Board 19
Ohue, Masahito	67-POS	Board 20
Ozbabacan, Ece	68-POS	Board 21
Ozbaykal, Gizem	69-POS	Board 22
Özbek, Pemra	70-POS	Board 23
Özcan, Gülin	71-POS	Board 24
Ozdemir Isik, Gonca	72-POS	Board 25
Ozisik, Rahmi	73-POS	Board 26
Pandey, Saurabh	74-POS	Board 27
Pérez-Villa, Andrea	75-POS	Board 28
Piepoli, Sofia	76-POS	Board 29
Pinamonti, Giovanni	77-POS	Board 30
Ramos, Javier	78-POS	Board 31
Sayers, Zehra	79-POS	Board 32
Sen, Emel	80-POS	Board 33
Serçinoglu, Onur	81-POS	Board 34
Shin, Seokmin	82-POS	Board 35
Sümbül, Fidan	83-POS	Board 36

Tatar, Gizem	84-POS	Board 37
Tay, Savas	85-POS	Board 38
Tuncbag, Nurcan	86-POS	Board 39
Turupcu, Aysegul	87-POS	Board 40
Uyar, Arzu	88-POS	Board 41
Uzun, Pelin	89-POS	Board 42
Vega, Juan	90-POS	Board 43
Weng, Wei-Hsiang	91-POS	Board 44
Wieden, Hans-Joachim	92-POS	Board 45
Xypnitou, Andromachi	93-POS	Board 46
Yenenler, Asli	94-POS	Board 47
Thukral, Lipi	95-POS	Board 48
Feher, Victoria	96-POS	Board 49

48-POS Board 1

Similarity-based 3D Modeling of Compound -Protein Complexes

Takeshi Kawabata, Akira Kinjo, Haruki Nakamura.
Osaka University, Suita, Osaka, Japan.

3D complex structures of proteins and other molecules provide a clue for mechanism of interaction. It is important to model them in computational methods because only small amount of 3D complex structures for known interacting molecular pairs are available. Similarity-based modeling (template-based modeling) of compound-protein complexes can be performed by similar way to model protein monomers. If the experimental complex 3D data of a similar compound to the target compound is available, the protein-bound structure of the target compound can be predicted by aligning the conformation of the target compound on the known 3D structure of the reference. We developed the flexible alignment program *fkcombu*, which aligns the target compound based on atomic correspondences with the reference compound. The correspondences are obtained by the MCS (maximum common substructure) of 2D chemical structures, using our build-up algorithm (Kawabata, *J.Chem.Info.Model.*, 2011,*51*,1775). The prediction performance was evaluated using many target-reference pairs of superimposed ligand 3D structures on the same protein in the PDB, with different ranges of chemical similarity. We found that the RMSD between the predicted and correct target conformations significantly correlates with the chemical similarities between target-reference molecules. Generally speaking, if the reference and target compounds have more than 70 % chemical similarity, then the average RMSD of 3D conformations is less than 2.0Å. The source codes of *KCOMBU* package are freely available. We also developed the server *HOMCOS* (http://homcos.pdbj.org) to search and model complex structures. The server separates component molecules of PDB files of complexes (such as proteins, nucleic acids, small chemical compounds), stores their binding relationships. It searches these molecules by *BLAST* and our chemical structure comparison program *KCOMBU*. Based on found similar complexes, simple template-based models of the complex can be generated.

49-POS Board 2

Spatio-Temporal Modelling of Neurotransmitter Transport

Cihan Kaya, Bing Liu, James R. Faeder, Ivet Bahar.
University of Pittsburgh, Pittsburgh, PA, USA

Learning and memory are two of the most fundamental and widely studied processes which underlie neuroscience. The mechanisms facilitating these processes are collectively known as synaptic plasticity. Through the modulation of synapse strength depending on the activity of neurons, synaptic plasticity reshapes the neural network over time. Synaptic plasticity is caused by the release of glutamate from pre-synaptic neurons and the activity of the synapse is mediated by glutamate receptors (AMPA and NMDA), excitatory amino acid transporters (EAAT), Calmodulin (CAM) and related protein kinases (CAMKII) and phosphatases (PP1). The crucial mechanism is that the ion transport into the post-synaptic compartment through ion transporters affects ion conductance and the number of ion transporters. In this study, parameters are estimated for a spatio-temporal model of the synapse and post-synaptic neuron. The effect of different initial concentrations of ions and neurotransmitters in different compartments are compared to understand the mechanism of severe neurodegenerative diseases such as Alzheimer's disease and Huntington disease. Overall, the modelling of the synapse and post-synaptic neuron complex will provide significant information about vulnerable targets for drug design.

50-POS Board 3

Computational and Experimental Insights into the Protein-Protein Cooperativity and Catalysis of the GTPase enzyme RhoA and Activating Protein Rho.GAP

Whitney F. Kellett, Nigel G. Richards.
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GTPase enzymes, which hydrolyze guanosine triphosphate (GTP) to guanosine diphosphate (GDP) and inorganic phosphate (Pi), are involved in a large number of critical cellular processes including proliferation. GTPase Activating Protein (GAP) is responsible for the regulation of GTPase. GTPase proteins, when poorly regulated, can signal for uncontrolled cellular growth and are indicated in oncogenesis [1]. The objective of this study is to marry X-ray crystallography, solution NMR and computational methodologies to reveal catalytic and dynamic properties of the GTPase protein RhoA bound to the regulating protein RhoA.GAP.

Using this computational model as well as experimental insight, we study both the transition state specifically and in context of the full catalytic cycle, using Quantum Mechanics (QM) and Mixed Quantum and Molecular Mechanics (QM/MM) methodologies. Additionally, we use long-time scale MM simulations to model the protein-protein interface, and use docking methodologies to score libraries of compounds suited for interfering with this interface.

These simulations provide evidence for a dissociative transition state, pairing well with NMR data. We suggest that the MgF_3- crystallographic additive is the best adduct to date to model the transition states of these types of enzymatic phosphoryl transfer reactions. These simulations also provide the first complete catalytic model of a GTPase based phosphoryl transfer mechanism. We also have identified structural effects of binding the RhoA.GAP protein to RhoA, and have preliminary results that this interface may be selectively "drug-able" based on initial compound screening. This model could be further exploited to identify ways to dissociate the errant GTPase:GAP complex by targeting the GTP binding site or even the protein-protein interface itself.

51-POS Board 4

Molecular Dynamics and X-Ray Crystallography Reveal the Role of the Skip Regions in Human Cardiac Muscle Protein Myosin

Elif N. Korkmaz[1,2], Keenan C. Taylor[3], Ivan Raymond[3], Qiang Cui[1,2].
[1]University of Wisconsin, Madison, Madison, WI, USA, [3]University of Wisconsin, Madison, Madison, WI, USA.[2]University of Wisconsin, Madison, Madison, WI, USA,

Cardiac and skeletal muscles contain interdigitated thick and thin filaments, which allow muscle contraction through sliding thick filaments past the actin-containing thin filaments. The globular N-terminal domains generate force through ATP hydrolysis and interactions with actin. The C-terminal region forms a long α-helix and dimerizes to form a coiled-coil (CC), which is known as the myosin rod. Mutations in this rod lead to a wide variety of skeletal and cardiac myopathies. Yet, the molecular organization of the myosin rods is still unresolved.

Our ultimate goal is to construct a high-resolution model for the thick filament consisting of 1935 amino acids. To tackle this challenge, a 'divide and conquer' type of approach has been employed. The rod includes four Skip residues that disrupt the α-helical heptad-repeat pattern typical for the CCs. We have concentrated on understanding the dynamics of these regions, the role of the Skip residues, and the importance of neighboring residues as a first step toward constructing a model for the thick filament from individual myosin rods.

Molecular dynamics simulations are carried out for all four skip regions using the X-Ray structures we recently solved and model structures formed via homology modeling. Several relevant mutants are also studied to dissect the role of the specific neighboring residues. Microsecond simulations are carried out via the AMBER-MD package using implicit solvent. Our results suggest that the Skip1, 2 and 3 determine how well the helices are intertwined. They provide flexibility through increasing the CC pitch with conserved nearby sequences ensuring stability, whereas Skip4 adopts a different behavior consistent with its different functionality. We have found that mutations that cause myopathies are generally located near the Skip residues and disrupt flexibility, and the self-assembly.

52-POS **Board 5**

Co-evolutionary Analysis of Ionotropic Glutamate Receptor N-Terminal Domain Function

James Krieger[1], Madhav Sukumaran[1], Anindita Dutta[2], Ivet Bahar[2], Ingo Greger[1].
[1]MRC Laboratory of Molecular Biology, Cambridge, United Kingdom, [2]University of Pittsburgh, Pittsburgh, PA, USA.

Ionotropic glutamate receptors (iGluRs) are key mediators of synaptic transmission and plasticity, especially those selective for alpha-amino-3-hydroxy-4-isoxazole propionic acid (AMPA) and N-methyl-D-aspartate (NMDA). They are composed of a common domain architecture with two extracellular periplasmic-binding protein (PBP)-like clamshell domains. The membrane-proximal ligand-binding domain closes around agonists such as glutamate to pull open the ion channel. The function of the distal N-terminal domain (NTD) is less clear though it has been implicated in receptor assembly, synapse formation and allosteric modulation of channel gating (at least for NMDA receptors). Here we have used various co-evolutionary methods (statistical coupling analysis [SCA], direct coupling analysis [DCA] and mutual information [MI]) to identify key residues and help elucidate the potential allosteric function of this domain. Other receptors such as metabotropic glutamate receptors (mGluRs) and atrial natriuretic peptide receptors (ANPRs) are known to use dimeric PBP-like domains as primary ligand binding domains that allosterically transmit signals to the rest of the structure. As these domains have similar sequences and structures to the iGluR NTD, these other receptors have been included in the co-evolution analysis to gain further insights through commonalities and differences. We believe that this PBP domain has evolved as useful scaffold for allostery and there may be common input and output regions that are used to different extents in different receptors. For example, mGluRs use the clamshell cleft of each protomer to bind glutamate and induce inter-protomer conformational changes while the ANPRs primarily use the dimer interface, which is also used by mGluRs to bind cations.

53-POS **Board 6**

Do Not Call Me α, I Am π-helix

Prasun Kumar, Manju Bansal.
Indian Institute of Science, Bangalore, India.

Existing secondary structure identification methods identify very few π-helices in the structures available in Protein Data Bank (PDB). The path traversed by Cα atoms is used to devise a new method for the identification of secondary structure elements (SSEs) in proteins and is designated as ASSP (Assignment of Secondary Structure in Proteins). Using ASSP to search a non-redundant subset of high-resolution and well-refined protein structures comprising of 3582 protein chains, we found total of 574 π-helices with average unit twist and rise being 85.2˚and 1.2 Å respectively. A total of 391 (68%) π-helices were found at the termini of α-helices with a majority (312) of them being present at the C-termini. The size of the dataset allowed us to analyze the position wise preference for the commonly occurring 20 amino acids within and around π-helices. They show certain positional amino acid preferences and these are different from those of α-helices. Amino acid propensities in π-helices were found to be context dependent, viz. occurring independently, or at N-terminus, C-terminus or middle of α helices. They also influence the preference of amino acids in the flanking α-helices. Majority of interspersed π-helices were found to be conserved across a large number of structures within a family and introduce a kink or distortion in the neighboring α-helices. In addition to hydrogen bonds, several other non-bonded interactions contributing to the stability of the π-helices have also been identified and studied in detail. Finally, functional and structural role of π-helices have been analyzed. Our analysis indicates that the conformation of the π-helix has evolved to provide unique structural and functional features within a variety of proteins.

54-POS Board 7

Intrinsic Variations in the Structure of Spacer Regions Can Critically Influence Transcription

Arvind Marathe, **Prasun Kumar**, Manju Bansal.
Indian Institute of Science, Bangalore, India.

TraR of Agrobacterium tumefaciens is a member of the LuxR family of transcriptional regulators, which regulates genes that control vegetative replication and conjugal transfer of the tumour-inducing (Ti) plasmid. It exists as a dimer in the solution with each monomer comprising of 234 residues. TraR is activated only when the N-terminal domain of each monomer binds to an autoinducer molecule and C-terminal domain binds to specific DNA sequences (traboxes) of the target promoters. In this study, we have carried out extensive (60 ns) molecular dynamics simulations of a free wild-type trabox d(GATGTGCAGATCTGCACATC), the same sequence with double mutation (G9→C and C12→G) in the spacer region, which eliminates transcription, a TraR-trabox complex (PDB ID: 1L3L) and unbound TraR. Structural variations in the dinucleotide step parameters, primarily slide and roll, dictate the binding of traR protein to the trabox. Mutations affect the groove width and the overall conformation of the trabox by introducing kinks in the spacer region, possibly making it unfavourable for binding to traR monomer/dimer. Principal component analysis of motion of each monomer of bound and unbound TraR shows that each monomer tend to have different conformations. Our analysis shows that TraR selects a suitably positioned trabox, and that the wild-type trabox is far more likely to assume such a suitable conformation as compared to the mutated trabox. This study highlights the influence on protein binding, of the intrinsic structural variations in regions of DNA that do not directly hydrogen bond to the protein.

55-POS **Board 8**

Generation of Atomistic Conformers Using Elastic Network Model for Proteins Undergoing Large Conformational Changes and Ribosome

Zeynep Kurkcuoglu, Pemra Doruker.
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Efficient computational algorithms are necessary to sample protein conformations for drug design studies, especially in the absence of ligand-bound structures. For this purpose, we developed an unbiased iterative conformational search algorithm based only the apo structure, which combines global modes from elastic network model, clustering and energy minimization with implicit solvation model. At the end of procedure, conformers having a radius of gyration larger than the apo state can be discarded in order to obtain a manageable set for docking applications. The algorithm is applied to five hinge-bending proteins (with conformational changes up to 15 Å RMSD), two proteins showing functional loop motions and the supramolecule ribosome. To assess the performance of generated conformers, known ligands were docked to both relatively closed and intermediate states for adenylate kinase (AK), LAO-binding protein and dipeptide-binding protein. Close-to-holo poses were obtained in all cases. Clustering with the available experimental x-ray and NMR structures (33 for AK, 160 for calmodulin, 24 for biotin carboxylase and 2 structures for the rest of the proteins) indicated that intermediate and/or closed states were sampled during conformation generation. Functional loop motions were also captured by applying the technique to two proteins; triosephosphate isomerase and murA. In order to illustrate the applicability of the algorithm to supramolecules, 70S ribosome conformers were generated embodying its experimentally reported functional motions. Using this computationally efficient method, it is possible to generate conformers for proteins of a wide spectrum, in terms of system size and magnitude of conformational change, which can further be utilized in docking studies.

56-POS Board 9

Dynamical Correlations and Long-Range Allostery Revealed by Intra-Protein Conditional Activity

Milo Lin.
University of California, Berkeley, Berkeley, CA, USA.

A new type of statistical measure, called the conditional activity, is introduced to quantify correlations in the waiting times between transitions amongst the degrees of freedom. The conditional activity is the dynamical analogue of the mutual information, and, for systems in thermal equilibrium, correspond to the instantaneous change of the free energy barrier of one degree of freedom following a transition in another degree of freedom. For three different proteins, we calculated the conditional activity of side-chain fluctuations from 3 microsecond-long all-atom molecular dynamics simulations. We show that the conditional activity reveals a rich dynamical architecture, and that the principle eigenvector of this measure can retrodict known pathways of dynamical allostery. The applicability of this method to predicting novel allosteric pathways in proteins, as well as to other types of networks, will be discussed.

57-POS Board 10

Activation of Bordetella Pertussis Adenyl Cyclase by Calmodulin

Therese Malliavin[1], Edithe Selwa[2,1], Marilyne Davi[1], Alexandre Chenal[1], Ana-Cristina Sotomayor-Perez[1], Elodie Laine[2], Daniel Ladant[1].
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The adenyl cyclase (AC) is an essential toxin from Bordetella pertussis able to invade eukaryotic cells where it is activated upon binding to calmodulin. Based on the crystal structure of the AC catalytic domain in complex with the C-terminal half of calmodulin (C-CaM), we previously carried out molecular dynamics simulations (Selwa et al, 2012) which suggested that three residues, Arg^{338}, Asn^{347} and Asp^{360}, might be important for the stabilization of the AC/CaM interaction. These residues belong to a loop-helix-loop motif, LHL, at the C-terminal end of AC, located at the interface between CaM and the AC catalytic loop. In the present work, we have characterized in silico and in vitro, three AC variants in which, one (Asn^{347}; ACm1A), two (Arg^{338} and Asp^{360}; ACm2A), or three residues (Arg^{338}, Asn^{347} and Asp^{360}; ACm3A) were modified to Ala. Biochemical studies revealed that the affinity for CaM of ACm1A and ACm2A was not significantly affected, while that of ACm3A was drastically reduced. To understand the effect of the modifications, molecular dynamics simulations were performed on the modified proteins. The MD trajectories recorded on the ACm3A/C-CaM complex revealed that the calcium-binding loops of C-CaM display large fluctuations that could be related to the weakened interaction of ACm3A with its activator. All together, our results suggest that the LHL motif at the C-terminal end of AC is crucial to link CaM-binding to the stabilization of the AC catalytic loop in an active configuration.

References:
Selwa et al. Model of the activation of Bordetella pertussis adenyl cyclase by calmodulin: molecular dynamics and mutagenesis studies. In revision at J Biol Chem.
Selwa et al. Differential role of Calmodulin and Calcium ions in the stabilization of the catalytic domain of adenyl cyclase CyaA from Bordetella pertussis. Proteins 2012;80:1028-40.

58-POS **Board 11**

Allosteric Communication within the B-Raf Dimer: The Effect of the V600E Mutation and Inhibitor Binding

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Kinase proteins play a fundamental role in cellular signaling: by transferring a phosphate group from ATP to specific target molecules they are able to modify their activity and consequently regulate cell function. Given the importance of kinases in regulating most of the cellular responses, it is not surprising that aberrant activation of kinases is one of the major causes of human diseases, including cancer, making them fundamental targets for drug design. Conformational transitions play a central role in kinase regulation. X-ray structures have shown that kinases adopt an active state that is maximally active and one or more inactive states that show minimal activity.
The V600E mutation of B-Raf is one of the most common in metastatic melanomas. This mutation is expected to shift the balance between the active and inactive forms of the kinase towards the former. Thus B-Raf appears to be a promising target in the treatment of melanoma. Complicating drug design efforts, some ATP-competitive B-Raf inhibitors induce a “paradoxical activation” of the Raf pathway. From experimental evidence it has been deduced that drug binding promotes dimerization, and that when a drug-bound monomer dimerizes with an apo-monomer, the apo-monomer adopts an active conformation. Unfortunately, a clear picture of B-Raf dimerization and allosteric communication within the dimer are lacking. Using all-atom molecular dynamics simulations and the metadynamics enhanced sampling method, we can identify the effect of the V600E mutation and drug binding on the allosteric networks within the dimer, which is difficult to obtain experimentally. This knowledge could greatly contribute to the development of more effective and selective drugs and to counter the emergence of resistance to drugs currently in clinical use.

59-POS **Board 12**

Are There any Structural Taboos in Protein-Protein Interactions? Insights from a Genome-Wide Study of Interacting and Non Interacting Protein Pairs.

Juliette Martin, Nicoletta Ceres, Guillaume Launay, Richard Lavery.
CNRS , Lyon, France.

Functional interactions take place between specific proteins in a healthy cell. Yet, the density of a cell in macromolecules is such that proteins are constantly in contact or in close proximity with other proteins that are not functional partners. How is the specificity of functional interactions maintained? In particular, is the ability to interact encrypted in the protein's 3D structure? To address this question we have performed an in silico large-scale experiment to explore the structural repertoire of interacting and non-interacting proteins pairs, and to see whether they differ.

We considered *yeast cerevisiae*, for which 3D structural models and protein-protein interaction data are available, as well as four different data sets of nominally “negative interactions”. Positive interactions, i.e. experimentally known interactions, were analyzed in parallel to serve as positive control. For each interaction (positive or negative), we considered the 3D structures of the proteins involved, and tried to determine whether the PDB contains a protein-protein complex where similar structures interact. When a complex was found in the PDB, we qualified the interaction as “supported” by the PDB.

We find that negative interactions selected on the basis of different sub-cellular localizations are supported by PDB protein-protein complexes to the same extent as positive interactions. This finding stresses the importance of physical sequestration to maintain interaction specificity. Finally, we have analyzed the structural features of the resulting 3D models of binary protein complexes and also their stability using our coarse-grain model PaLaCe. We found that the more sophisticated PaLaCe energy evaluation was necessary to distinguish interacting and non-interacting protein pairs. Interestingly, some nominally non-interacting pairs are nevertheless predicted to form stable complexes.

60-POS Board 13

Internal Dynamics of DHFR Revealed by Simulated Shock Waves Propagation

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It has been demonstrated that the adiabatic compressibility (AC) is a useful measure of the conformational flexibility of proteins in different functional forms. Binding of various ligands to dihydrofolate reductase (DHFR) gives rise to large differences in AC. Moreover, neutron scattering experiments have shown that binding of the cancer drug methotrexate softens the low-frequency vibrations of its target protein DHFR. Here, using non-equilibrium molecular dynamics simulations of the response of DHFR to the shock waves at various incident angles we explore the relationship between the population and directionality of the protein low frequency vibrational modes and AC. We identify protein dynamics characteristics that might be critical for enzyme function of DHFR.

61-POS Board 14

Neutron Spectroscopic Bbservation of Fast Motions in ADH with and without NAD in Aqueous Solution.

Michael Monkenbusch[1], Andreas Stadler[2], Biehl Ralf[2], Jacques Ollivier[3], Michaela Zamponi[4], Dieter Richter[2].
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Covering the range from nanoseconds to picoseconds and several nanometer to Angstroems high resolution inelastic neutron yields information on large scale domain motions in a protein as well as more local protein dynamics. Results from high resolution time-of-flight spectroscopy and neutron backscattering spectroscopy from a 5% solution of alcohol dehydrogenase ADH with and without NAD in a deuterated buffer solution are presented. Whereas the large scale domain motions are significant to facilitate incorporation of the NAD cofactor [Biel et al.,PRL 101, 138102] the question remains how the fast more local dynamical features correlate with function, resp. incorporation of NAD. Local protein dynamics on top of the large scale diffusional and domain motions have been observed. A fraction of about 1/3 of the non-exchangable protons in the protein show high mobility with large amplitudes of several Angstroems. Indications are seen that the association of NAD reduces this mobilty.

62-POS Board 15

Solvent Accessibility Affects Binding Hot-Spots at Protein Interfaces.

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REQUIMTE, Porto, Portugal.

Protein-protein interactions are the basis of many physiological processes and their specificity and affinity are critically important in heath and disease. Various studies demonstrated the existence of binding Hot-Spots (HS) at these interfaces.[1] HS are residues that potentially have a higher contribute for the overall binding free energy and are usually surrounded by a layer of residues (forming an O-ring structure) to better protect them from the bulk solvent. To test the application of this theory at protein-protein and protein-DNA interfaces we have performed Molecular Dynamic (MD) simulations of various complexes and analyzed the position and dynamics of water molecules as well as different features of Solvent Accessible Surface Area (SASA).[2-4] Our results clearly show a much lower number of water molecules surrounding a HS in comparison with a non-HS and a higher loss of SASA upon complex formation in both types of interfaces. The use of the various SASA features as HS differentiators was also statistically evaluated and the combination of them analyzed by a support vector machine method, which allowed us to establish a new accurate model for predicting hot-spots: SBHD (SASA-Based Hot-Spots Detection).[5] This method presents a Precision, Recall, and F1 score of 0.91, 0.73 and 0.81 for an independent test set.

[1] I.S. Moreira, P.A. Fernandes, M.J. Ramos, Proteins, 68 (2007) 803-812.
[2] I.S. Moreira, J.M. Martins, M.J. Ramos, P.A. Fernandes, M.J. Ramos, Biochem. Biophys. Acta, 1834 (2013) 401-414.
[3] I.S. Moreira, R.M. Ramos, J.M. Martins, P.A. Fernandes, M.J. Ramos, Journal of Biomolecular Structure and Dynamics, 32 (2013) 186-197.
[4] R.M. Ramos, L.F. Fernandes, I.S. Moreira, Computational Biology and Chemistry, 44 (2013) 31-39.
[5] J.M. Martins, R.M. Ramos, A.C. Pimenta, I.S. Moreira, Proteins: Structure, Function, and Bioinformatics, 82 (2014) 479-490.

63-POS Board 16

Identification of Effector Binding Sites on H-Ras Explains Signal Propagation

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Ras proteins (HRAS, NRAS and KRAS) are small GTPases that regulate diverse cellular processes. These proteins activate multiple signaling pathways with complex and divergent effects including cell cycle progression, cell differentiation and survival. Ras proteins cycle between two conformations: GDP-bound inactive and GTP-bound active forms. Active Ras proteins transmit the information through a physical interaction with its downstream effector proteins. Therefore, it is of capital importance to determine the complex structures of ras with these proteins to understand the pathways at the structural level. Here we show that Ras-GTP interacts with its downstream effector proteins through different interfaces. These interface regions include Switch I effector binding site and allosteric site. The predominant interface region consists of α1, β2, β3, β4 and β5.The effector proteins that bind to these regions are Raf-1, B-raf, PI3Kγ, PLCε, and Byr2 (crystal structures of the complexes are available), Cdc42, Ftase (the complexes are predicted) and RASSF1 (structure modeled and interface predicted). The second interface region populated by RAIN, RGS12, RGL1 (structure modeled and interface predicted) and TIAM proteins (the complexes are predicted) includes α2, α 3, β7, α4, β10 and α5. Few effector proteins such as AFAD, RIN1 and FAK bind to H-Ras through an interface region that partially overlaps both binding sites. Here we also identify mutually inclusive/exclusive interactions by predicting and comparing the interface regions of H-Ras with its partners. This may help us identify the pathways that can be activated simultaneously by active Ras proteins.

64-POS Board 17

Assembly of the Alzheimer's Disease Associated Amyloid beta Peptide during the Lag Phase of Fibril Formation

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One of the most important targets in Alzheimer’s therapy is still the proteolytic fragment of the amyloid precursor protein, called amyloid beta peptide. This fragment is between 39 and 43 amino acids long and is marked by its high tendency to self-associate. The self-assembly, which finally leads to the formation of amyloid fibrils, has to proceed via an unknown number of intermediate structures, among which a more potent therapeutic target than the fibril is suspected. Additionally other pathways might exist, leading also to the formation of small oligomeric species, which might as well as the on-pathway species be responsible for the toxic effects the Abeta peptide exerts on neuronal cells.
OBJECTIVE: Identification and characterization of early, distinct assemblies of the amyloid beta peptide in solution regarding size, shape and fraction.
METHODS: Sedimentation velocity centrifugation, complemented by density gradient centrifugation, atomic force microscopy, fluorescence assays and CD-spectroscopy were utilized.
RESULTS: Aside from the monomeric peptide a set of globular assemblies have been identified in solutions which were still in the lag phase of amyloid formation. These species had increased beta sheet content, were negative for thioflavin T staining and exhibited a stronger cytotoxicity than amyloid fibrils at the same mg/ml concentrations.
CONCLUSIONS: By sedimentation velocity centrifugation size-, shape information and fraction of distinct oligomeric species of the amyloid beta peptide could be retrieved simultaneously from the same sample solution.

65-POS **Board 18**

A Molecular Dynamics Study of the Allosteric Mechanism of Pyruvate Kinase

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There is a growing body of interest to understand the regulation of proteins by "allosteric communication" between different ligand binding sites. Pyruvate kinase from *Leishmania mexicana* catalyzes the final reaction of glycolysis and is allosterically activated by fructose-2, 6-bisphosphate (FBP). The presence of this allosteric site 40 Å away from the active site makes it an ideal target to study allosteric mechanisms and identify potential communication pathways. We have carried out Molecular Dynamics Simulations to enhance our knowledge of allostery and also gain insight into the structural and dynamical properties at the atomic level. Our preliminary results provide new and promising insights into the classical Monod-Wyman-Changeux model of allostery.

66-POS **Board 19**

A Kinetic Model of Proton Transport in a Multi-Redox Center Protein: Cytochrome *c* Oxidase

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Chemical reaction kinetics is employed to explore the stepwise electron and proton transfer reactions of cytochrome *c* oxidase (C*c*O) from *R. sphaeroides*. Proton transport coupled to electron transport is investigated in terms of a series of coupledprotonation-dependent redox reactions. Thereby, we assume fixed rather than shifting dissociation constants of the redox sites. Proton transport can thus be simulated particularly when separate proton uptake and release sites are assumed rather than the same proton pump site for every ET step. In order to test these assumptions, we make use of a model system introduced earlier, which allows to study direct ET of redox enzymes by electrochemistry. A four-electron transfer model of C*c*O has been used, according to which electrons are transferred from the electrode to Cu_A. Thereafter, electrons are transferred along the sequence heme *a*, heme a_3 and Cu_B. We consider protonation equilibria of the oxidized and reduced species for each of the four centers. Moreover, we add oxygen/H_2O as the terminal (fifth) redox couple including protonation of reduced oxygen to water. Finally we arrive at a kinetic model comprising five protonation-dependent redox couples. Simulations are compared with fast-scan voltammetry data obtained in the absence and presence of oxygen. These results are corroborated by fitting time-resolved FTIR spectra modulated by electrochemical excitation to the model. Summarizing, we can show that proton transport can be modeled in terms of protonation-dependent redox kinetics.

67-POS Board 20

The MEGADOCK Project: High-Performance Protein-Protein Interaction Prediction Tools on Supercomputing Environments

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Background
Protein-protein interaction (PPI) plays a core role in cellular functions. In recent years, PPI prediction methods based on protein docking have been developed and have been applied for large-scale PPI network prediction based on tertiary structures. However, such network prediction requires much computing resources, and a faster PPI prediction method is eagerly demanded.

Results
We have developed a high throughput PPI prediction system based on rigid-body protein docking, “MEGADOCK”. MEGADOCK can perform faster docking based on its original scoring function. Recently, MEGADOCK has been accelerated by using the general purpose graphics processing unit (GPGPU) technique and it is now released as MEGADOCK-GPU. MEGADOCK was also parallelized for massively parallel supercomputing environment using the hybrid parallelization (MPI/OpenMP) technique. The system, named MEGADOCK-K, achieved an excellent scalability on supercomputing environments, such as K computer, which has 705,024 Fujitsu SPARC64 VIIIfx CPU cores. We have already applied MEGADOCK system to a number of interactome analyses such as bacterial chemotaxis pathway, human apoptosis pathway and human epidermal growth factor receptor related pathway.

Conclusion
We present a new protein-protein docking engine aimed at exhaustive docking of millions of protein pairs. The system was shown to be scalable when running on thousands of nodes and multiple GPUs.

68-POS **Board 21**

The Structural Pathway of Interleukin 1 (IL-1) Initiated Signaling Reveals Mechanisms of Oncogenic Mutations and SNPs in Inflammation and Cancer

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Interleukin-1 (IL-1) is a large cytokine family closely related to innate immunity and inflammation. IL-1 proteins are key players in signaling pathways such as apoptosis, TLR, MAPK, NLR and NF-κB. The IL-1 pathway is also associated with cancer, and chronic inflammation increases the risk of tumor development via oncogenic mutations. Here we illustrate that the structures of interfaces between proteins in this pathway bearing the mutations may reveal how. Proteins are frequently regulated via their interactions, which can turn them ON or OFF. We show that oncogenic mutations are significantly at or adjoining interface regions, and can abolish (or enhance) the protein-protein interaction, making the protein constitutively active (or inactive, if it is a repressor). We combine known structures of protein-protein complexes and those that we have predicted for the IL-1 pathway, and integrate them with literature information. In the reconstructed pathway there are 104 interactions between proteins whose three dimensional structures are experimentally identified; only 15 have experimentally-determined structures of the interacting complexes. By predicting the protein-protein complexes throughout the pathway via the PRISM algorithm, the structural coverage increases from 15% to 71%. In silico mutagenesis and comparison of the predicted binding energies reveal the mechanisms of how oncogenic and single nucleotide polymorphism (SNP) mutations can abrogate the interactions or increase the binding affinity of the mutant to the native partner. Computational mapping of mutations on the interface of the predicted complexes may constitute a powerful strategy to explain the mechanisms of activation/inhibition. It can also help explain how an oncogenic mutation or SNP works.

69-POS **Board 22**

Computational Studies on Hsp70 Unify Experimental Findings and Reveal Additional Significant Residues for Further Study

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An Hsp70 protein orchestrates allosteric communication between its nucleotide and substrate binding domains. We employ the structure of *E. coli DnaK*, Hsp70, [1] and we propose the following methodology: (i) We arbitrarily pick an amino acid in the protein structure; (ii) we substitute the residue by an alanine and we minimize the altered structure; (iii) we record the positional changes that take place following minimization. This methodology quantifies to what extent a single-point mutation would affect each residue in the protein. We obtain the average displacement of a residue by averaging the displacements when all others are substituted by alanine. Moreover, we obtain the change in average reachability of residues by calculating the difference in average path length of a residue between the mutated and original networked structure. We propose that the sites of functional importance, such as those associated with binding or folding display large deviations from their original state. A cluster of residues (T428, Q471, and D490-E496) at the substrate binding cavity show pronounced differences which are confirmed by experimental studies.[2] Furthermore, we calculate the betweenness centrality to distinguish residues most traversed when a walk is performed between any two arbitrary residues in the networked structure. The walk is biased, based-on residue-residue contact energies. Results emphasize residues N522, D526, C529 and E530 located at the lid which plays role in an opening-closing movement so as to bind and release the substrate. They are found to affect the chaperone activities of Hsp70 either by changing its substrate binding affinity or by modifying folding mechanism.[3]

1 Kityk et al., Mol Cell,48,863(2012).

2 Swain et al., Mol Cell,26,27(2007).

3 Burkholder, et al., PNAS, 93,10632(1996); Suh et al., PNAS,95,15223(1998); Moro etal., J Biol Chem,279,19600(2004); Slepenkov et al., Biochemistry,42,5867(2004).

70-POS Board 23

Computational Investigation of the Energy Exchange Pathways in Peptide-loaded Major Histocompatibility Complex Proteins

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The dynamic behavior of peptide-loaded Major Histocompatibility Complex (pMHC) plays a role in defining the immunogenicity of the pMHC-T-cell Receptor interaction. However, due to the highly polymorphic nature of the antigen chain of the complex (Human Leukocyte Antigens, HLA) and the limitation of experimental and computational methods, studying the association between disease states and pMHC dynamics is difficult. In this work, we aim to perform an overall functional characterization of HLA alleles by employing computational methods with an emphasis on identifying energy exchange pathways within the residue interaction network. As an initial step, a dataset was constructed consisting of a total of 389 HLA proteins in complex with peptide epitopes using the Protein Data Bank and manually-curated Immune Epitope Database as sources. In addition, disease associations of alleles were identified using relevant information available from the literature. As a fast and efficient screening method to find energy exchange pathways, we employed a coarse-grained method based on the perturbation of Kirchhoff matrix of the Gaussian Network Model (GNM). In order to test the effectiveness and validity of this method, we performed initial 20 ns long atomistic molecular dynamics (MD) simulations of B-27:05 and B-27:09 subtypes which are differentially associated with Ankylosing Spondilitis using NAMD molecular dynamics software and CHARMM27 force-field. A Principal Component Analysis of the cross-correlations of residue fluctuations showed a large overlap between the predictions of the method and dynamics simulations. Our approach of GNM-based screening followed by MD simulations establishes a framework for our upcoming studies, in which we plan to extend our analysis to other allele and subtypes in our dataset and complement it with more exhaustive methods such as atomic packing analysis.

71-POS Board 24

Comparison of the Binding Behavior of HLA-B Alleles Related to Ankylosing Spondylitis Disease by Computational Methods

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Ankylosing Spondylitis (AS), which is an autoimmune disease affecting the axial skeleton, is associated with B*27 allele of Human Leukocyte Antigen (HLA). The single amino acid replacement (ASP116HIS), having a key role in pathogenesis pathway, distinguishes HLA-B*27:05 and HLA-B*27:09 alleles as non-associated and associated with AS, respectively. Although sharing the same peptide repertoire, both alleles interact with T cells differently. Hence, understanding the differences in binding behavior is of considerable interest both from structural and immunological points of view. In this study, molecular docking and molecular dynamics simulations are carried on aiming to comprehend the differences in the binding behavior of both alleles. Initially, a ‘re-docking’ experiment is applied on both HLA-B*2705 and HLA-B*27:09 alleles by Autodock Vina for the validation of the molecular docking protocol. This validated protocol is then performed on a library of modeled peptides formed upon single point mutations aiming to address the effect of 20 naturally occurring amino acids at the binding core peptide positions. The free binding energies (FBEs) obtained from computational docking experiments are compared within the peptide library and between the alleles. The amino acid preferences of each position are studied enlightening the role of each on binding. Based on the amino acid preferences of each position, 9 individual peptides are constructed by changing a single position while keeping the rest of the peptide fixed. Molecular dynamics simulations are performed on the docked structures for 2 ns aiming to see the effect of the mutations on the root mean square deviation (RMSD) and cross-correlations. As a result of this study, an informative guideline would be obtained for the binding behavior of the HLA-B*27 molecules in a comparative manner.

72-POS **Board 25**

Prediction of Substrate Specificity in NS3/4A Serine Protease by Threading Methodology

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Proteases are enzymes which recognize specific substrate sequences and catalyze the hydrolysis of designated peptide bonds to activate or degrade them. Due to the biological importance of proteases, it is particularly important to identify the recognition and binding mechanisms of protease-substrate complex structures in studies of drug design. Cleavage specificity in protease-substrate systems is generally determined by the amino acid profile, structural features and distinct molecular interactions. Besides experimental methods, computational tools for prediction of natural substrate cleavage sites has emerged as useful approaches that are helpful in understanding substrate specificity. In this work, the substrate variability and substrate specificity of the NS3/4A serine protease encoded by the Hepatitis C virus (HCV) is investigated by the biased sequence search threading (BSST) methodology. Other than the crystal structures of the bound NS3/4A protease used as templates, additional template structures are also created in silico by performing various peptide building and docking procedures followed by molecular dynamics (MD) simulations. For this, the crystal structures of the NS3/4A protease-substrate complexes which have missing substrate residues in their crystal structures with only the N-terminal cleavage products are used. BSST is performed starting with known binding and nonbinding peptides and low energy sequences are generated using low-resolution knowledge-based potentials. Then, target sequences of yet unidentified potential substrates are predicted by statistical probability approaches applied on the pool of low energy sequences. The results show that the majority of these predicted peptide positions correspond to the natural substrate sequences with conserved amino acid preferences, while some positions exhibit variability. This indicates that the BSST seems to provide a powerful methodology for predicting the substrate specificity for the NS3/4A protease, which is a target in drug discovery studies for HCV.

73-POS **Board 26**

Bond Orientation Order Parameters Explain the Influence of Contact Density and Protein Geometry on Fluctuations

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The Anisotropic Network Model (ANM) was used to investigate the fluctuations of 210 globular proteins. The results of ANM were compared with molecular dynamics (MD) simulations and experimental findings. The ANM results were analyzed using Bond Orientation Order (BOO) parameters, which were reformulated as a sum of contact density and geometrical distribution of contacts in space. This reformulation of BOO expression makes it possible to investigate the role of each individual component and identify cut–off ranges where each component dominates protein fluctuations. The results indicate that the two components of the BOO dominate protein fluctuations at different length scales: contact density at small length scales and geometric distribution of residues at length scales comparable to the protein size. In addition, their probability distributions change with respect to cut–off distance. The combination of these two effects (length scale and cut–off dependence) leads to a unique outcome such that at small length scales, where most simulations or analysis is performed, only the contact density's effect is seen even though the geometric component is most important for the overall stability of proteins.

74-POS **Board 27**

Binding-Competent States for L-arginine in E. coli Arginine Repressor Apoprotein

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Arginine repressor of E. coli is a multifunctional hexameric protein that provides feedback regulation of arginine metabolism upon activation by the negatively cooperative binding of L-arginine. A molecular mechanism of allostery has been described earlier in which conserved arginine and aspartate residues in each ligand-binding pocket promote rotational oscillation of the trimers within the hexameric domain that binds L-arginine by engagement and release of hydrogen-bonded salt bridges. Binding of exogenous L-arginine displaces resident arginine residues and arrests oscillation, shifting the equilibrium quaternary ensemble and promoting motions that maintain the configurational entropy of the system [1]. Interpretation of this complex system requires an understanding of the protein's conformational landscape. In this work the ~50 kDa hexameric C-terminal domain was studied by 100 ns molecular dynamics simulations in presence and absence of the six L-arg ligands that bind at the trimer-trimer interface. A rotational shift between trimers followed by rotational oscillation occurs in the production phase of the simulations only when L-arg is absent. Analysis of the system reveals that the degree of rotation is correlated with the number of hydrogen bonds across the trimer interface. The trajectory presents frames with one or more apparently open binding sites into which one L-arg could be docked successfully in three different instances, indicating that a binding-competent state of the system is occasionally sampled. Simulations of the resulting singly-liganded systems reveal for the first time that the binding of one L-arg results in a holoprotein-like conformational distribution.

[1] R Strawn, M Melichercik, M Green, T Stockner, J Carey, R Ettrich (2010) Symmetric allosteric mechanism of hexameric Escherichia coli arginine repressor exploits competition between L-arginine ligands and resident arginine residues. PLOS Computational Biology 6: 6. e1000801

75-POS **Board 28**

Hamiltonian Replica-Exchange Simulations of NS3 HCV Helicase Translocation along ssRNA

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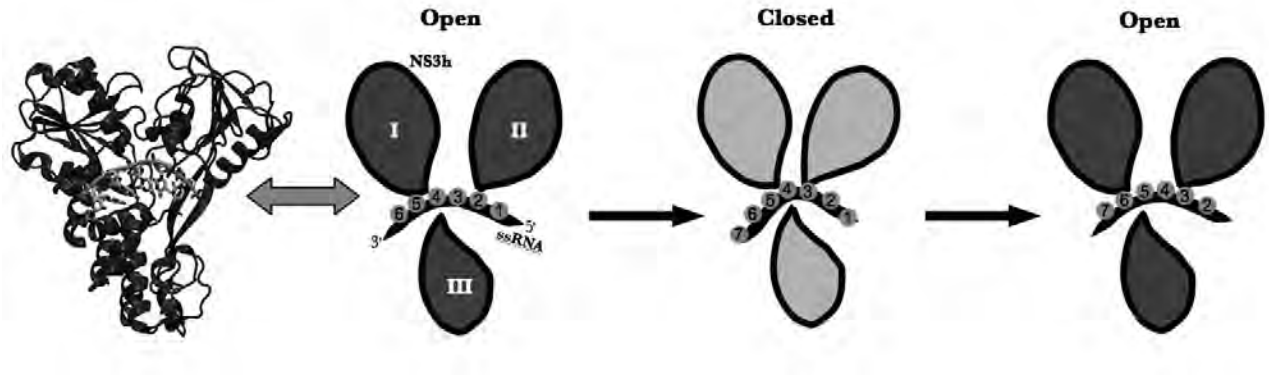
RNA helicases are enzymes crucial for RNA metabolism. One member of this group of proteins is the NS3 helicase from the Hepatitis C virus (NS3h HCV) which translocates along RNA hydrolyzing ATP. Single molecule experiments and crystallographic structures suggest an "inchworm" mechanism, with a processivity of one base pair unwinding per cycle. In spite of the available data in literature, a direct and clear evidence of the mechanism is still missing.

The aim of this study is to model the NS3h-ssRNA complex at atomistic detail and to compute the free-energy landscape associated to translocation. We employ well-tempered metadynamics in combination with Hamiltonian replica-exchange molecular dynamics in explicit solvent. Using these techniques we are able to observe the transition between the available experimental snapshots.



76-POS **Board 29**

Design of Target Specific Transcription Activator-like Effectors by Free Energy Perturbation Calculations

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Bacterial plant pathogens belonging to Xanthomonas genus infect cells by injecting Transcription Activator-Like effector (TALE) proteins to modify plant gene expression. DNA binding TALE proteins have been modified to generate site specific engineered TALENs (transcription activator-like nucleases). Crystal structure of the PthXo1 TALE protein bound to its DNA target reveals specificity of interactions between the two molecules at the sequence level. TALE DNA binding domains include ~17 repeats, each consisting of 33-34 conserved amino acids. Alignment of the sequence of the repeats shows perfect homology except for the amino acids at positions 12-13 (repeat variable diresidues-RVDs) which specifically bind to DNA bases according to the code: HD>C, NG>T, NI>A and NN>G in most cases, while there are some exceptions. Not all repeats have to perfectly contact DNA to bind and it is unclear how TALE pairs up with a target DNA sequence while avoiding off-target sites. To address this problem, we apply molecular dynamics-based tools to quantify specific binding stability, mimicking cellular conditions. We analyze selected point mutations introduced into the RVD region of the protein sequence and predict resulting free energy differences using detailed free-energy perturbation calculation. Identification of key residues for stability of binding at specific positions will allow computational design of TALEs that can be experimentally tested for binding affinity and off-target specificity. By systematically applying this method, we interpret the role of local interactions in contributing to the overall stability of the system. For example, we find that RVD mutations HD→HA and NI→NN results in ΔG=14.4 and -19.8 kcal/mol, respectively, as expected from concurrent statistical studies. We test the effect of the same mutation in different repeats and discuss the implication of our findings in the context of local structural arrangements and allosteric effects.

77-POS **Board 30**

Modeling RNA Dynamics Using Elastic Network Models

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The number of known fundamental functions of RNA in the cell has been steadily increasing in the last decades. The function of RNA molecules is often, though not always, intimately connected to their dynamics. For this reason, several experimental and computational efforts are being spent to characterize the internal dynamics of RNA. The scope of atomistic molecular dynamics (MD) simulations, which is presently limited due to its computational cost, can be aptly complemented by suitable coarse-grained models. These include elastic network models (ENM) where a biomolecule is represented as a set of harmonic oscillators. For proteins, ENM's predictions have been shown to successfully reproduce the principal components of motion derived from all-atoms MD simulations. As of today, a few works have studied the possibility to extend this modelling strategy to nucleic acid dynamics. We here considered four widely different RNA systems, testing the predictions of various ENMs, differing for the level of coarse-graining, against results obtained from the analysis of extensive MD trajectories. We report on the different performance of the considered ENMs and discuss their effectiveness in capturing RNA dynamics against MD references.

78-POS **Board 31**

Study of the Interaction of the Extracellular Domain in the ErbB2-ErbB2homodimer and ErbB2-Trastuzumab by Hydrodynamic Properties and Computational Models

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The ErbB2 receptor is one of the four members of the epidermal growth factor (EGFR) family of receptor tyrosine kinases (RTK), which also includes ErbB1/EGFR, ErbB3 and ErbB4. ErbB2 overexpression is associated with poor prognosis of breast cancer patients. It is known that ErbB2 receptors can exist on the cell surface as monomers, homodimers and heterodimers with ErbB1 or ErbB3. It has been shown that trastuzumab antibody, an approved therapeutics for treatment of ErbB2-overexpresing breast cancer, blocks ErbB2 homodimer activity [1].
In our study, we use a recombinant extracellular domain of the ErbB2, which has a His-tag motif of 10 His residues at the C-terminal. We study the formation of the ErbB2 homodimer and ErbB2/trastuzumab complexes by means of a combination of experimental techniques, such as dynamic and static light scattering as well as chromatography with tetradetection, along with simulations based on molecular dynamics and hydrodynamic models [2,3]. In this way, we are able to correlate the solution hydrodynamic data with macromolecular structural parameters. The experimental results indicate the presence of a variety of complexes between the ErbB2 extracellular domain and trastuzumab in aqueous phase. These complexes show a high degree of structural flexibility (measured by independent experiments in size- exclusion chromatography and dynamic light scattering), which is in agreement with our computational results. Under the experimental condition, our finding reveals the true dynamic features of the complexes in solution, an insight beyond what is known from the crystal structures. This flexibility does not allow us to perform 3D reconstruction imaging using TEM experiments [4].
References:
[1] Ghost R, et al. Cancer Res 2011, 71, 1871-1872
[2] Brandt J.P. et al. Biophysical J. 2010, 99, 905-913
[3] Brookes E. et al. Eur. Biophys J., 2010, 39(3), 423-435.
[4] Vicente-Alique E. et al. Eur. Biophys. J. 2011, 40, 835-842.

79-POS **Board 32**

Structural Features of the Gamma Subunit (AGG2) and Specificity of A. Thaliana Heterotrimeric G-protein Interactions

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Heterotrimeric G-proteins are involved in several essential signaling pathways in plants, including those controlling seed germination, growth and defense against pathogens. Despite detailed functional and structural information on mammalian G-proteins, structure-function characterization of the plant proteins is lacking. Moreover, although there are basic similarities in the signaling mechanisms, regulation of activity in the mammalian and plant G-proteins show important differences. We cloned and expressed A. thaliana subunits alpha, beta and gamma in E. coli for structural studies and here results on the gamma subunit AGG2 will be presented. Purified recombinant AGG2 shows a propensity to oligomerize in solution and as determined by SDS- and native-PAGE, size exclusion chromatography and dynamic light scattering a stable dimer is obtained only under fully reducing conditions. Small angle X-ray solution scattering (SAXS) measurements reveal that AGG2 dimer has an elongated flexible structure which can be modeled with the ensemble optimization method (EOM) used for intrinsically disordered proteins. Results indicate that AGG2 structure can be described as a population of inter-converting flexible conformers which all contain a rigid α-helix domain involved in the inter-molecular coiled coil interactions. Estimation of the secondary structure content from circular dichroism (CD) measurements agrees well with that predicted by homology modeling and SAXS models. Results on thermal stability of the protein monitored by CD measurements are also in agreement with the flexible structure predicted by EOM models. Structural models together with sequence analyses show the centrality of the AGG2 N-terminal region in establishing the coiled-coil interaction, crucial for binding to its natural partner AGB1. Our results also suggest that the disordered flexible regions may be part of the mechanism for conferring functional selectivity to the A. thaliana beta-gamma dimer at different location in the plant.

80-POS **Board 33**

The Last 10 Residues of Human Sphingosine-1-Phosphate Receptor 1 Interacts with G-Protein Coupled Receptor Kinases in A Conserved Manner

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Sphingosine-1-phosphate receptors are a family of G-protein coupled receptors (GPCRs) highly expressed in human cells. They have roles in cellular proliferation, survival and migration. Similar to other GPCRs, Sphingosine-1-phosphate receptor 1 (S1PR1) C-terminal internalization via phosphorylation is an important intracellular mechanism for the downstream signaling. To better understand S1PR1 pathways, we synthesized a peptide consisting of the last 10 residues of the C-terminal of S1PR1, which we termed MNP301. We experimentally showed that MNP301 prevents S1PR1 internalization and uncouples the agonistic effect of phosphorylated FTY720, a modulator of S1PR1. However, how these last 10 residues prevent S1PR1 redistribution is still unclear. Several studies show that G-protein coupled receptor kinases (GRKs) are involved in the S1PR1 internalization and C-terminal phosphorylation. In this study, we investigated the specific interactions of these last 10 residues and GRKs by using three different docking tools. Our analysis shows that all GRKs available in Protein Data Bank (GRK1, GRK2, GRK3 and GRK6) bind to MNP301 from the same binding pocket with similar energy values. Our in silico results suggest that the peptide binds to GRKs in between their N-terminal regulator of G-protein signaling (RGS) domain and protein kinase domain. We will further use Molecular Dynamics (MD) and Perturbation Response Scanning (PRS) tools to give insight into the binding mechanism and allosteric residues of GRKs. A deeper comprehension of these interactions will contribute a better understanding of S1PR1 signaling pathways.

81-POS **Board 34**

An Investigation of the Effects of Model Simplification and Water Box Shell Size on the Molecular Dynamics Simulations of Peptide-loaded Major Histocompatibility Complex Proteins

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Peptide-loaded Major Histocompatibility Complex (pMHC) proteins play a key role in the transmission of molecular signals through the immune system via their interaction with T-cell receptors. Many alleles and sub-types of these proteins are found to be associated with autoimmune diseases; hence the importance of understanding the mechanism of signal transduction within the complex is crucial. As a computational tool, molecular dynamics (MD) simulations is commonly used for this purpose, however the high number of alleles/subtypes as well as the considerably large size of the pMHC complex limits the usage of this method in a large-scale comparative study. In this regard, reducing the size of the system by neglecting certain domains of the complex or decreasing the solvation shell size are two options previously investigated by researchers to obtain a computationally more manageable system without significant loss in accuracy. In this work, we performed 5 ns duplicate simulations of Human Leukocyte Antigen HLA*B-27:05 and HLA*B-27:09 sub-types binding 3 different peptide epitopes with simplified/full complex and different shell sizes of 10 Å and 20 Å. Simplification was done by excluding the α-3 domain of the antigen and β2-microglobulin. The overall Root Mean Square Deviation (RMSD) and Root Mean Square Fluctuation (RMSF) profiles were compared for each simulation setup. Between the sub-types, no significant difference was observed in terms of RMSD and RMSF profiles. However, we observed a peptide detachment in one simulation of the HLA*B-27:05 allele binding a peptide derived from Cathepsin A (pCatA) in a simplified setup with 20 Å shell size. Our results indicate the importance of checking peptide binding in parallel simulations before proceeding with longer MD simulations.

82-POS **Board 35**

Computer Simulations on Amyloid Formation

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Computational studies can provide basic understanding concerning the detailed mechanism of amyloid formation, the formation of amyloid fibrils and prefibrilar aggregates of misfolded proteins and peptides. Understanding amyloid formation is very important to elucidate possible causes for various neurodegenerative disorders. Systematic replica exchange molecular dynamics simulations have been performed on the formation of the oligomers and protofibrils of Aβ peptide and peptides from α-synuclein. We also performed MD simulations on different structural models of such fibrils including the effects of mutations. The mechanism of aggregation and growth of these peptides is examined from detailed analysis of such simulations. It was shown that the steric zipper contacts provided favorable interactions in the aggregation process and the structural flexibility might play important role for enhancing aggregation. Our results suggested that amyloid formation is basically a hierarchical process and different topologies are favored in the different stages of aggregation. Self-assembly of peptide-based building blocks has been shown to be useful in constructing artificial bionanostructures. We performed MD simulations on the proposed nanoring structures formed by the self-assembly of β-sheet forming peptides. The stability of the nanoring structure with respect to the size is investigated and several factors contributing to the stability are examined. We have also demonstrated that the sizes of nanorings can be controlled by introducing selective mutations.

83-POS Board 36

Structural and Dynamics Aspects of ASC Speck Assembly

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The inflammasome complexes are activated by rapid formation of ASC speck which acts as an adaptor that bridges procaspase-1 to the receptors. The resemblance between the ASC speck and aggresome raises the question whether the ASC speck formation is a result of specific interactions between PYD and CARD which both belong to the death fold superfamily or simply aggregation of hydrophobic patches of ASC proteins. To address this question, we performed structure and dynamics based analyses on the ASC protein using Gaussian Network Model (GNM) of PYD and CARD, and Molecular Dynamics (MD) simulations of the wild type and in-silico mutated PYD, with the mutational analysis on the ASC structure and its separated domains in human cells, we show that the ASC speck formation is an organized structure based on specific homophilic but not heterophilic interactions by PYD and CARD separately. PYD is able to use alternative interaction modes other than type I that might be important in compaction of the ASC speck. We propose a model in which filament formation is the first level of organization in the ASC speck and filaments further compact in a higher organization level.

84-POS **Board 37**

Elucidation of Interaction Mechanism of Coronavirus Proteins with Molecular Docking

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Coronavirus (CoV) is a very diverse virus family. They interact with host cells at several stages and also perform their infection and replication by using the various mechanisms of host cells. For example, in 2003, coronavirus phylogenetic typing work performed during an outbreak of Severe Acute Respiratory distress Syndrome (SARS), this virus family is characterized by frequently changing hosts were revealed. Therefore, It is necessary to know the important role factors in CoV replication-transcription. This important information will be developed in future vaccines and so it is important to develop treatments. The main aim of the study is illuminated the mechanism that a detailed between nucleocapsid protein (N) which has an important role the replication-transcription and collecting (assembly) of CoV and non structural protein (nsp3) which is thought to form the skeletal system of RTC, help of molecular modeling techniques.

In this study, primarily, Homology Modeling protocol is performed to obtain possible model structure of MHV proteins by using Discovery Studio (DS) 3.5 software. The obtained models are minimized by using Nanoscale Molecular Dynamics (NAMD) to be provide the stable form of the mentioned proteins. Then, protein-protein interactions mechanism have been analyzed through HADDOCK program. As a result of molecular docking, it will be providing contribution to the development of drugs and vaccines against CoV based on results of the experimental data.

85-POS **Board 38**

Microfluidic Single-Cell Analysis and Modeling of Cellular Information Processing

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Immune cells constantly receive signaling inputs such as pathogen-emitted molecules, use gene regulatory pathways to process these signals, and generate outputs by secreting signaling molecules. Characterizing the input-output relationship of a biological system allows building models to predict how the system will operate in complex physiological scenarios. A major obstacle here is that each cell contains its own, time-dependent composition of pathway components, generating distinct, time-varying outputs for the exact same inputs. Such variability makes time-dependent single-cell analysis crucial. Single-cell dynamical analysis, however, has been a low-throughput method due to technical challenges in isolating, manipulating and measuring individual cells. I will talk about how we address these limitations by developing automated, high-throughput, microfluidic/optofluidic single-cell analysis systems with unprecedented capabilities and measurement accuracy, and how we use them in understanding immune coordination during response to infection. Our recent efforts have resulted in a new set of technologies, including microfluidic systems to measure cytokine secretion dynamics from single-cells under complex time-varying signaling inputs, a cell culture system that creates programmable diffusion-based chemical gradients, a chip to measure cell-cell communication via secreted factors, and a new method for digital quantification of proteins and nucleic acids (mRNA and DNA) in the same cell. I will also talk about new biological insight from our experimental and modeling efforts about how single-cells detect and encode dose and frequency information using the immune pathway NF-κB, and how they create dynamic cytokine outputs under inflammatory stimuli. A primary goal in this combined technology/cell biology effort is to develop a computer model of tissue-level immune response through the NF-κB pathway, with particular focus on cytokine signal propagation mechanism (e.g. diffusion vs. waves), speed, range and duration.

86-POS Board 39

Revealing Clinically Relevant Targets across Various Glioblastoma Tumor Lines by Integrating Multiple Omic Data

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With the help of high-throughput technologies, we are able to comprehensively monitor quantitative molecular changes within signaling networks at many levels in response to perturbations or disease. Although a single omic dataset provides a wealth of information about a given biological problem, it is limited in the ability to fully capture a cellular pathway that is altered in a given experimental setting. Therefore, integrating multiple data sources together in a network context is crucial to find the omissions in each set, explore hidden entities and identify therapeutic targets. In this work, we reconstruct signaling networks across eight implemented xenograft models of Glioblastoma multiforme (GBM, the most aggressive type of malignant brain tumors) patients by integrating phosphoproteomic and interactome data. The prize-collecting Steiner forest (PCSF) algorithm has been used to reconstruct networks by taking into account confidence that the phosphoproteomic hits are significant and the probability that each reported protein-protein interaction is real. Simultaneous comparison of reconstructed networks led us to find out several common proteins and pathways across multiple tumor lines that were not identified in the phosphoproteomic dataset. For example, Nfkb signaling and mTOR pathways were common in most of the patients, as were proteins such as Mek1, Stat6, Erbb3. We have experimentally shown that Mek1 is stably over-phosphorylated in all tumor lines compared to normal human astrocytes. Another target, Numb, was found to be an invasiveness marker in this setup. Tumor lines having Numb present in their networks were in minimally invasive class while remaining were in highly invasive class. Overall, despite the heterogeneity across various tumor lines of GBM, common pathways and proteins revealed here have important outcomes in GBM and our integrative network modeling approach help in finding testable therapeutic targets.

87-POS **Board 40**

Structure and Dynamics of IL-1β and IL-1Ra Complexes with IL-1RI Examined by Molecular Dynamics Simulation

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Interleukin-1 (IL-1) family cytokines lead to inflammation and mediate immune responses. IL-1 family proteins have naturally occurring antagonist which distinguish them from other cytokine families. IL-1β triggers signaling via binding to IL-1 receptor which result in recruitment of IL-1 accessory protein (AcP) to the extracellular domain of the receptor. This heterotrimeric complex formation is necessary for intracellular signal transduction. IL-1 receptor antagonist (IL-1Ra) competes for the same site of the receptor and blocks signaling. Although IL-1β and IL-1Ra have homologous structures, their complex structures revealed several distinct regions. We performed 200 ns molecular dynamics simulation of IL1β - IL1RI, IL1Ra - IL1RI and IL1β-IL1RI-IL1RAcP complexes to elucidate differences upon binding of agonist and antagonist. We show that when the antagonist is bound to the receptor, immunoglobin-like domain 3 of the IL-1RI has larger B-factors than when the receptor binds to the IL1β which can have an effect on recruitment of IL1RAcP. In addition, we reported correlation between key residues which are validated by mutagenesis studies to understand the dynamics of these complexes. This can provide information for selecting target in designing an inhibitor for inflammatory diseases caused by IL1β.

88-POS **Board 41**

Generation of Conformational Transition Pathways and the Prediction of Closed Structure for Proteins

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Conformational transition pathways between open and closed crystal structures were produced for a set of 20 proteins using a hybrid simulation technique, named as ANM-MC. This iterative technique generates a targeted pathway between two conformations, where the collective modes from the anisotropic network model (ANM) are used for deformation at each iteration and the energy of the deformed structure is minimized via local moves using a knowledge-based Monte Carlo (MC) algorithm. Our data set consists of 9 hinge-bending-type proteins (initial RMSDs = 4-12 Å), 6 DNA-binding proteins (RMSD = 3-9 Å), and 5 enzymes with functional loop closure (loop RMSD = 3-14.5 Å). We observe successful approaches to target, with at least 50% decrease in RMSDs, for 17 of proteins studied. For proteins showing a significant change in radius of gyration (Rg) during the conformational transition, deforming along a single predominant mode (first or second) in the decreasing Rg direction leads to successful predictions of target structure for 7 out of 9 hinge-bending-type proteins (2.0-2.9 Å final RMSDs to target) and for two DNA-binding proteins (1.5 and 1.7 Å). Based on previously reported free energy surface of adenylate kinase, deformations along the first mode produced an energetically favorable path, which is interestingly facilitated by mode swapping at key points. Similar changes in slow mode shapes were also observed in other proteins during the transitions.

89-POS **Board 42**

Discovering Allosteric Communication Pathways on the Bacterial Ribosome: from Decoding Center to Peptidyl Transferase Center

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Ribosome, a molecular machine synthesizing proteins in the cell, is mainly composed of small and large subunits (called 30S and 50S in bacteria) connected by multiple intersubunit bridges. Elucidating the communication pathways responsible for information transport among distant functional sites on the subunits is critical both to understand the functional mechanisms of the complex and to develop new antibacterial therapeutics targeting the ribosome. Various studies indicate sophisticated allosteric communication mechanisms between numerous functional sites of the complex carefully employed during the translation process, such as between the decoding center (DC), which is responsible for decoding genetic code on 30S, and the peptidyl transferase center (PTC), which mediates the peptide bond synthesis on 50S[1]. In this study, three different crystal ribosome structures of Thermus Thermophilus are investigated to obtain twenty shortest signaling pathways between DC (residue A1492) and PTC (residues A2451 and G2251). For this purpose, k-shortest path algorithm[2] is used with the elastic network approach[3], describing the ribosome structure as a network of nodes linked by edges. Nodes are placed at alpha-carbon and phosphor atoms of residues, and length of edges between neighboring node-pairs are calculated based on atom-atom interactions. Results indicate that major and minor signaling paths exist between DC and PTC. These paths are composed of highly conserved and/or antibiotic binding residues, and pass through the B3 intersubunit bridge. Results suggest that B3 bear a critical position in signal transmission between DC and PTC, and give hints on new druggable sites in bacterial ribosome.

1) Rakauskaite, R; Dinman, J.D. Nucleic Acids Research, 2008, 36(5), 1497.
2) Yen, J. Y. Management Science, 1971, 17, 712.
3) Kurkcuoglu, O.; Turgut, O. T.; Cansu, S.; Jernigan, R. L.; Doruker, P. Biophysical Journal, 2009, 97, 1178.

90-POS **Board 43**

The Maturation Process of HPV16 Virus Like Particles as Revealed by Light Scattering, Z-Potential and Transmission Electron Microscopy

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Virus-like particles (VLPs) are envisaged as modularly engineered protein nanoparticles for their potential uses both as vaccine and in drug and nucleic acids delivery. Electrostatic interactions are known to be of fundamental importance for the function of viruses, as they are involved in features as assembly, receptor interaction, membrane diffusion, disassembly and delivery. Notwithstanding, these properties have not been yet explored in detail for VLPs. In order to obtain structurally stable VLPs for vaccine or drug carrier the electrostatic properties not only need to be under control, but also should provide an appropriate disassembly in the specific application conditions. A proper handling of thsee conditions might also allow one to design VLPs as delivery systems with specific cargos. We have studied the size, shape and surface electrostatic properties of Human Papilomavirus Type 16 (HPV16) by means of dynamic and static light scattering, Z-potential and transmission electron microscopy (using both stained and cryonized samples). These techniques have allowed us to obtain not only the hydrodynamic size, the molecular weight and the electrostatic features of the VLPs, but also the evolution of these physical properties during a post-production maturation process in different conditions. The initial HPV16 L1 proteins obtained directly through bioprocess yielded broadly distributed VLPs with a smaller size than the expected one for the virus. Additionally, the VLPs have shown poor electrostatic surface properties. During the maturation process an increase in the size of the VLPs has been observed. Additionally, we have observed that this increase is in parallel with significant changes in both the second virial coefficient and Z-potential, suggesting that the reorganization process of the L1 protein units within VLPs is related to structural changes that modify electrostatic interactions.

91-POS **Board 44**

In Silico Study on the Putative Methylation Mechanism Catalyzed by the Palmitic Acid Binding to the Catechol O-Methyltransferase

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Catechol O-methyltransferase (COMT) is an enzyme that catalyzes the donation of a methyl group from S-adenosyl methionine (SAM) to catecholamines. This transfer of methyl group initiates one of the major degradation pathways of catecholamine transmitters, such as dopamine, norepinephrine and epinephrine. In addition, COMT also plays a crucial role in the metabolism of catechol used in the treatment of hypertension, asthma, and Parkinson's disease. Previous report by Tony J.-F. Lee in 2011 has verified that palmitic acid methyl ester (PAME) can cause vasorelaxation through opening the voltage dependent K^+ channels on vascular smooth muscle cells.
Hence, we attempted to find out the methylation mechanism of palmitic acid (PA) catalyzed by COMT, using molecular docking combining MD simulations. Due to lack of the structure of PA binding to COMT, the complex structure of 3,5-dinitrocatechol (DNC) bound with SAM and COMT (PDB: 3A7E) was used as a template for molecular docking. Three various compounds including DNC (as an inhibitor), 3,4-Dihydroxyacetophenone (DHAP, as a positive control) and PA were docked with apo-COMT protein respectively for comparison. The most preferable complex structures were also determined to run 200 ns MD simulations under physiological conditions to study the interactions between them. The binding free energy (ΔG_{bind}) calculated by MM/PBSA technique showed that ΔG_{bind} of DNC is the lowest than that of DHAP and PA. The MD simulations showed that the distance between the hydroxyl group of PA and the methyl group near sulfur atom is increasingly approaching to form the possible methylation pathway. The proposed methylation mechanism of PA catalyzed by COMT helps to develop the therapeutic target for hypertension disease.

92-POS Board 45

Going through the Motions: Network Analyses of Molecular Dynamics Simulations Reveal Conserved Intramolecular Communication Pathways within EF-Tu Responsible for Ribosome Dependent GTPase Activation and Nucleotide Exchange.

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During the elongation cycle of bacterial protein synthesis elongation factor (EF) Tu delivers aminoacyl(aa)-tRNAs to the ribosome in a GTP-dependent manner. EF-Tu functions as a checkpoint, correct codon recognition is required to send an activating signal from the decoding centre on the 30S ribosomal subunit to the G domain of EF-Tu bound to the 50S ribosomal subunit. Following dissociation, EF-Tu•GDP is recycled into its GTP-bound form through the interaction with its nucleotide exchange factor EF-Ts. Both functions require the transmission of signals to facilitate either GTP hydrolysis or nucleotide release. Little is known about the dynamic features governing signal transmission within a highly conserved protein such as EF-Tu and its particular evolutionary constraints.
Here, molecular dynamics simulations of EF-Tu and variants with single amino acid substitutions were performed and subsequently interrogated using network analysis methods. Using these networks we identify communication pathways essential for efficient GTPase activation on the ribosome as well as EF-Ts-catalyzed nucleotide exchange. We find that single amino acid substitutions in EF-Tu can change the network organization dramatically and result in significantly reduced communication between domains across a set of universally conserved interdomain bridges. To validate our findings we have performed Michaelis-Menten analyses of ribosome-stimulated GTP hydrolysis in EF-Tu. Consistent with our network analysis, these results reveal that amino acid substitutions in domain II reduce the stimulatory effect of the 70S ribosome five-fold, while not affecting 50S-dependent stimulation. Variants of EF-Tu that disrupt the interdomain bridges reduce the stimulatory effect of the 70S ribosome to a similar extent. Analyses of the EF-Tu•EF-Ts complex revealed a different set of communication pathways promoting nucleotide exchange in EF-Tu which were validated using rapid-kinetics techniques.

93-POS **Board 46**

Hexokinase II as a Structure-based Cancer Target

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Hexokinase isoform II (HKII) catalyzes the first step of the glycolytic pathway, converting glucose to glucose-6-phosphate (Glc-6-P). This enzyme has been found to be implicated in many cancer types with an increased expression that maintains the highly glycolytic phenotype of malignant cells (Warburg effect). The present study aims to identify novel inhibitors of HKII using structure-based drug design. HKII is also inhibited by its product Glc-6-P. We are studying this potential allosteric mechanism as a route to the discovery of novel inhibitors.

94-POS **Board 47**

The Investigation of Activation Mechanism of BTL2 in Non-Aqueous Solvents through MD Simulations

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Microbial lipases are one of the largest classes of biocatalyst and are involved in hydrolysis, transesterification and esterification reactions . Microbial lipases offer new possibilities in industry with shift of thermodynamic equilibria by solvent engineering . In our project, bacterial thermoalkoliphilic lipase from Bacillus thermocatenulatus (pdb:2w22) is chosen to be used as biocatalyst in non-aqueous solvents. Overcoming change in destabilization of enzyme are essential to handle lipases in non-aqueous solvents. Before applying protein engineering approaches such as enzyme modification through site-directed mutagenesis, MD simulations have been performed to investigate the activation mechanism of BTL2 in non-aqueous solvents. Then, in-silico mutagenesis will be performed to strengthen the structurally vulnerable parts of the lipase .Up to now, 10ns of MD runs were performed for BTL2-toluene and BTL2-toluene/water complexes. Before 10ns simulations, 2000 steps of conjugate gradient minimization followed by 1ns equilibration run are performed at 310K. According to analysis of 10ns, it has been revealed that calcium coordination and lid domain of BTL2 moved more in toluene (2Å, 2.5Å backbone-rmsd) compared to toluene-water (50/50 v/v) (0.8Å, 1.5Å backbone-rmsd). Also, some buried parts of BTL2, playing crucial role in activation mechanism, are moving around 0.8Å (backbone-rmsd) towards the surface of the protein and trying to interact with toluene molecules which may lead to the destabilization. Moreover, surface exposed ligand binding part of the protein is less flexible in toluene (0.6Å backbone-rmsd) compare to toluene/water (1.6Å backbone-rmsd) and low flexibility creates problems for the function of the protein. To observe more conformational change, these simulations have been extended to 50ns and performed at 450K with 3 repeats. We believe the analysis of these runs will provide valuable insights about the stability and the functionality of this enzyme in toluene-water environment.

95-POS Board 48

Molecular Mechanism Underlying Recruitment of a Lipid-Anchored Protein

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Eukaryotic cells possess potential regulatory mechanisms to mediate many of its protein-membrane interactions in form of lipid-modified proteins. These highly specialized proteins have a unique architecture of a lipid anchor that enables them to peripherally dock onto negatively charged membrane surfaces in lipid-bound conformation. However, molecular events underlying this process of attachment to membrane is poorly understood. In this study, we investigate membrane recruitment of microtubule-associated protein light chain 3 (LC3) modified with phosphatidylethanolamine (PE) using multiple independent microsecond time-scale coarse-grain simulations. Spontaneous insertion events of lipid anchor were observed in multiple simulations, which allowed us to dissect the molecular mechanism of insertion with high statistical reliability. Positively charged residues in this helix especially ASN59, LYS65, and ARG68 ensures a stable and efficient delivery of PE chain into membrane. In addition, generation LC3 mutants showed distinct difference in puncta formation and localization when expressed *in vivo,* further substantiating the hypothesis. Thus, our study of active insertion of PE chain provides future avenues to investigate detailed regulatory aspects of this using biological process.

96-POS **Board 49**

Ligand Gating, Cavity Fluidity and Conformational Selection Observed for T4 Lysozyme Cavity Mutants Sampled by aMD Simulation.

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Native protein cavities, packing defects and hydrophobic core plasticity are thought to play an important role in protein stability, generation of conformational substates, function and allostery. Presently, we explore the conformational landscape of wild type and engineered N-terminal lobe cavities of T4 lysozyme as a model system with recently advanced long time-scale accelerated molecular dynamic simulation on GPU processors. Four systems were considered, each sampled for 1.2 – 1.6 microseconds; wild type, apo and benzene bound L99A mutant, and a triple mutant L99A/G113A/R119P representing a high energy L99A conformation that does not bind benzene. To facilitate conformational sub-state analysis, simulations were clustered on the basis of N-terminal lobe cavity volumes rather than RMSD.
Analysis and comparison of the conformational sub-states sampled by these different systems demonstrates cavity size impact on the number of conformational sub-states available to the protein, while maintaining native-like secondary structure. While it is not surprising that the apo form L99A mutant has the largest distribution of conformational sub-states, the distribution of cavity shapes is dramatic and will be compared with extensive published NMR dynamics studies. Rare event concerted side-chain rearrangement combined with secondary structure opening reveal several regions with gated-motions available for solvent exchange to the hydrophobic interior of the cavity mutant, however only one presents a large enough path for known hydrophobic ligands to exchange. Finally, cavity size appears to impact the frequency the inter-lobe hinge bending motion but not the magnitude.

Modeling of Biomolecular Systems Interactions, Dynamics, and Allostery: Bridging Experiments and Computations

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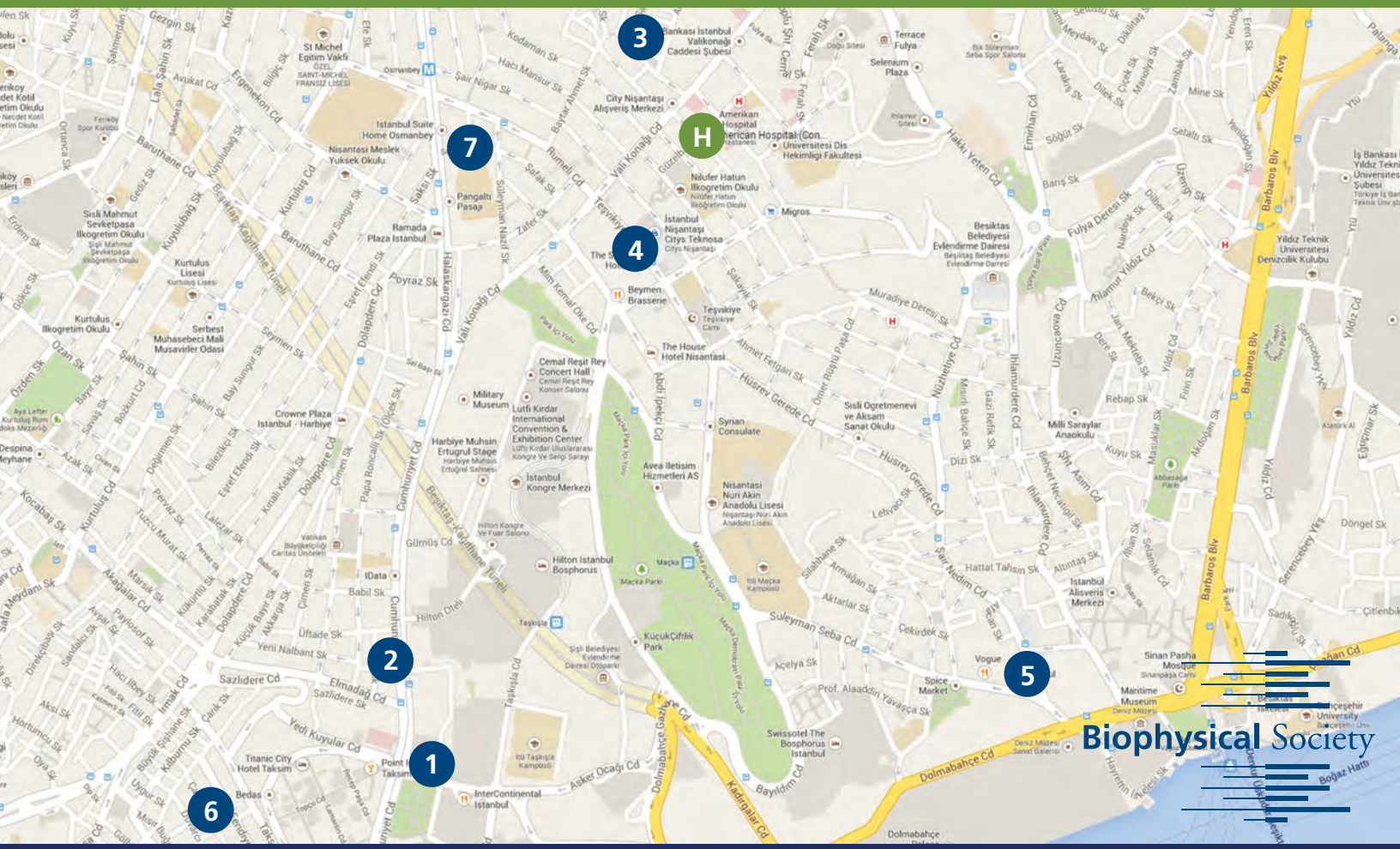

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