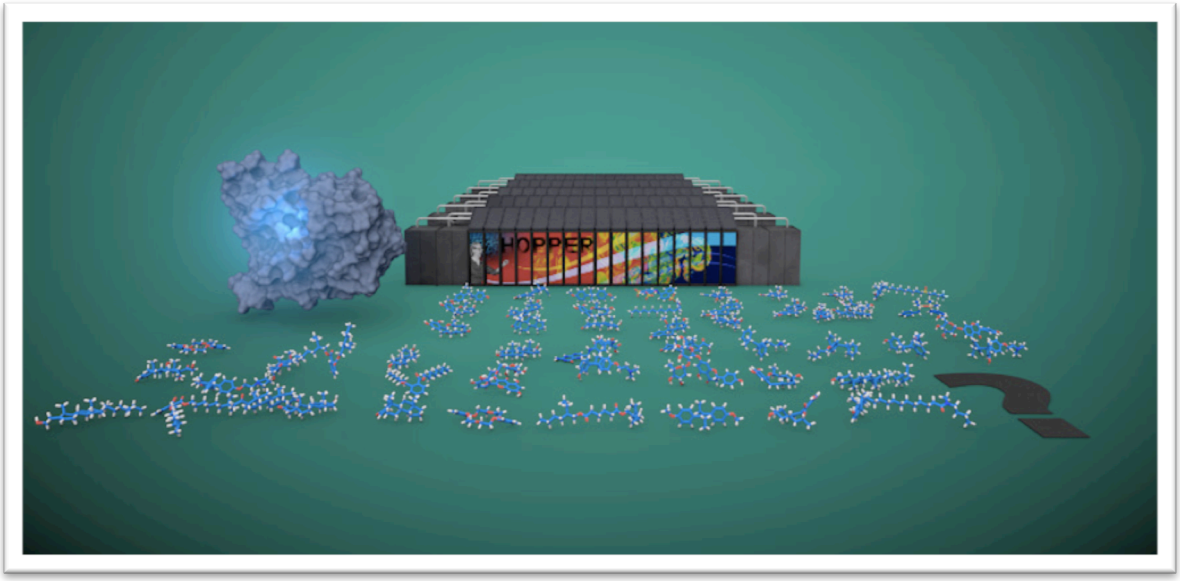
# CBSB12

# From Computational Biophysics to Systems Biology

## Knoxville, TN

## June 3-5, 2012



# Program Schedule

**Saturday, June 2, 2012**

| | |
|---|---|
| Afternoon | Arrival in Knoxville |
| 5:00-7:00 pm | Check-in |

**Sunday, June 3, 2012**

| | |
|---|---|
| 8:30-8:55 am | Registration |
| 8:55-9:00 am | Welcome |
| 9:00-10:00 am | **Ron Levy** (Keynote)<br>Exploring Landscapes for Protein Folding and Binding Using Replica Exchange Dynamics, Kinetic Networks and Markov State Models |
| 10:00-10:30 am | Break |
| 10:30-11:00 am | **Phanourios Tamamis** (CBSB12 Outstanding Young Researcher Talk)<br>Development of Regulatory Compounds for the Complement System by MD Simulations and Experiments |
| 11:00-11:20 am | **Nicholas Bodmer**<br>Molecular Investigations into the Mechanics of a Muscle Anchoring Complex |
| 11:20-11:40 pm | **Gia Maisuradze**<br>Protein Folding by Coarse-Grained Molecular Dynamics |
| 11:40-12:10 pm | Panel Discussion |
| 12:10-2:00 pm | Lunch |

| | |
|---|---|
| 2:00-2:30 pm | **Jens Meiler** (Invited Talk)<br>A Computational Platform for Protein Structure Determination Integrating Limited Experimental Data |
| 2:30-3:00 pm | **Julie Mitchell** (Invited Talk)<br>Knowledge-Based Structural Approaches for Predicting Hot Spots of Protein Binding and Allostery |
| 3:00-3:30 pm | **Ewa Golas** (CBSB12 Outstanding Young Researcher Talk)<br>Molecular Dynamics of the Hsp70 Chaperone in Response to Nucleotide and Substrate: A Coarse-Grained Perspective |
| 3:30-4:00 pm | Break |
| 4:00-4:30 pm | **Xiaolin Cheng** (Invited Talk)<br>Simulating the Transport of a Cellulose Chain Through the Cellulose Catalytic Tunnel |
| 4:30-4:50 pm | **Jun Fan**<br>Molecular Dynamics Simulation and Coarse-Graining Study on Cofilin Remodeling Actin filaments |
| 4:50-5:10 pm | **Amitava Roy**<br>Long-Distance Correlations of Rhinovirus Capsid Dynamics Contribute to Uncoating and Antiviral Activity |
| 5:10-5:40 pm | Panel Discussion |
| 5:40-6:00 pm | Break |
| 6:00-7:30 pm | Poster Session |

**Monday, June 4, 2012**

| | |
|---|---|
| 9:00-9:30 am | **Celeste Sagui** (Invited Talk)<br>Free Energy Landscapes of Polyproline and Polyglutamine Peptides |
| 9:30-10:00 am | **Garrett Goh** (CBSB12 Outstanding Young Researcher Talk)<br>pH-Coupled Simulations of RNA in Explicit Solvent |
| 10:00-10:30 am | Break |
| 10:30-11:00 am | **Arjan van der Vaart** (Invited Talk)<br>Enhanced Sampling Simulations of DNA and Protein-DNA Complexes |
| 11:00-11:20 am | **Jeff Wereszczynski**<br>The Calculation of Free Energies Over Vast Length Scales Through Conventional, Enhanced Sampling, and Free Energy Molecular Dynamics Simulations |
| 11:20-11:40 am | **Sigurdur Jonsson**<br>Distinct Phases of Free Alpha-Synuclein - A Flat-Histogram Monte Carlo Study |
| 11:40-12:10 pm | Panel Discussion |
| 12:10-2:00 pm | Lunch |
| 2:00-3:00 pm | **Gregory Voth** (Keynote)<br>Theory and Simulation of Biomolecular Systems: Surmounting the Challenge of Bridging the Scales |
| 3:00-3:30 pm | **Chunli Yan** (CBSB12 Outstanding Young Researcher Talk)<br>Coupling of DNA Binding and Architectural Remodeling Drives the Function of Human RPA |

| | |
|---|---|
| 3:30-4:00 pm | Break |
| 4:00-4:30 pm | **Sandor Vajda** (Invited Talk)<br>High-Throughput Identification and Druggability Analysis of Protein Binding Sites |
| 4:30-4:50 pm | **Jodian Brown**<br>Using Molecular Dynamics to Understand the Molecular Mechanisms Underlying the Application of Combination Therapies to Inhibit Hepatitis C Virus Polymerase |
| 4:50-5:10 pm | **Igor Drobnak**<br>Modeling Autotransporter Secretion |
| 5:10-5:40 pm | Panel Discussion |
| 5:40-6:00 pm | Break |
| 6:00-8:00 pm | Dinner |

**Tuesday, June 5, 2012**

| | |
|---|---|
| 9:00-9:30 am | **Jana Shen** (Invited Talk)<br>Recent Development and Application of Continuous Constant pH Molecular Dynamics |
| 9:30-10:00 am | **Virginia Burger** (CBSB12 Outstanding Young Researcher Talk)<br>Characterizing Pathways between Metastable States of Intrinsically Disordered Proteins |
| 10:00-10:30 am | Break |
| 10:30-11:00 am | **Collin Stultz** (Invited Talk)<br>Modeling Disordered States of Proteins: Are Structural Models of the Unfolded State Correct? |

| | |
|---|---|
| 11:00-11:20 am | **Stephanie DeLuca**<br>RosettaEPR: An Integrated Tool for Protein Structure Determination from Sparse EPR Data |
| 11:20-11:40 am | **Reza Salari**<br>Direct Observations of Shifts in the Î²-Sheet Register of a Protein-Peptide Complex Using Explicit Solvent Simulations |
| 11:40-12:10 pm | Panel Discussion |
| 12:10-2:00 pm | Lunch |
| 2:00-2:30 pm | **Jerome Baudry** (Invited Talk)<br>From Computational Docking to Exploration of Bichemical Pathways |
| 2:30-3:15 pm | **John Straub** (Invited Talk)<br>Role of Hydration and Confinement in Protein Folding and Aggregation |
| 3:15-3:30 pm | Concluding Remarks |
| | *Conference ends* |

# Keynote Speakers

1. Ronald Levy
   Rutgers University

2. Gregory Voth
   University of Chicago

# Invited Speakers

1. Jerome Baudry
   University of Tennessee, Knoxville

2. Xiaolin Cheng
   University of Tennessee, Knoxville
   Oak Ridge National Lab

3. Jens Meiler
   Vanderbilt University

4. Julie Mitchell
   University of Wisconsin

5. Celeste Sagui
   North Carolina State University

6. Jana Shen
   University of Oklahoma

7. John Straub
   Boston University

8. Collin Stultz
   Massachusetts Institute of Technology

9. Arjan van der Vaart
   University of South Florida

10. Sandor Vajda
    Boston University

## Ronald Levy

*Department of Chemistry and Chemical Biology*
*BioMaPS Institute for Quantitative Biology*
*Rutgers University, New Jersey, United States*
*ronlevy@lutece.rutgers.edu*


### Exploring Landscapes for Protein Folding and Binding Using Replica Exchange Dynamics, Kinetic Networks and Markov State Models

Advances in computational biophysics depend critically on the development of accurate effective potentials and powerful sampling methods to traverse the rugged energy landscapes that govern protein folding, binding and fitness. I will review work in my lab over the last few years concerning the construction of all-atom effective potentials for proteins and multi-scale methods for simulating their folding and binding on long time scales. Replica exchange (RE) is a generalized ensemble molecular simulation method for accelerating the exploration of free-energy landscapes which define many challenging problems in computational biophysics, including protein folding and binding. We have clarified some of the obstacles to obtaining converged thermodynamic information from RE simulations. I will discuss these issues and new multi-scale approaches to recover protein folding rates and pathways for folding and binding using the combined power of replica exchange, kinetic network models with flux analysis, and effective stochastic dynamics.


References:

**1**.W. Zheng, M. Andrec, E. Gallicchio, R.M. Levy, Proc. Natl. Acad. Sci. USA, 104, 15340-15345 (2007) **2**. E. Gallicchio, K. Paris, R.M. Levy, J. Chem. Theory Comp., 5, 2544-2564 (2009) **3**. W. Zheng, M. Andrec, E. Gallicchio, R.M. Levy, J. Phys. Chem. B, 113, 11702-11709 (2009) **4**. W. Zheng,, E. Gallicchio, N. Deng, M. Andrec, R.M. Levy, J. Phys. Chem. B, 115, 1512-1523 (2011) **5**. N. Deng, W. Zheng, E. Gallicchio, R.M. Levy, J. Am. Chem. Soc., 133, 9387-9394 (2011) **6.** E. Gallicchio, M. Lapelosa, R.M.Levy, J. Chem. Theory Comp., 6, 2961-2977 (2010) **7.** E. Gallicchio, R.M. Levy, Curr. Opin. Struct. Biol., 21, 161-166 (2011)



## Gregory Voth

*Department of Chemistry*
*James Franck Institute, Institute for Biophysical Dynamics, and Computation Institute*
*University of Chicago, Chicago, IL*
*gavoth@uchicago.edu*


### Theory and Simulation of Biomolecular Systems: Surmounting the Challenge of Bridging the Scales

A multiscale theoretical and computational methodology will be presented for studying biomolecular systems across multiple length and time scales. The approach provides a systematic connection between all-atom molecular dynamics, coarse-grained modeling, and mesoscopic phenomena. At the heart of the approach is a method for deriving coarse-grained models from protein structures and their underlying molecular-scale interactions. This particular aspect of the work has strong connections to the theory of renormalization, but it is more broadly developed and implemented for heterogeneous systems. A critical component of the methodology is also its connection to experimental structural data such as cryo-EM or x-ray, thus making it "hybrid" in its character. Applications this overall multiscale approach to study key features of large multi-protein complexes such the HIV-1 virus capsid, the entire HIV-1 immature virion, actin filaments, and protein-mediated membrane remodeling will be presented as time allows.

## Xiaolin Cheng

*University of Tennessee, Knoxville, TN*
*Oak Ridge National Laboratory, Oak Ridge*
*chengx@ornl.gov*


### Simulating the transport of a cellulose chain through the cellulose catalytic tunnel

The degradation of cellulosic biomass to sugars both in nature and in biorefineries is primarily accomplished by enzymes such as the Family 7 cellobiohydrolase (Cel7A) from Trichoderma reesei. Therefore, a molecular-level understanding of the mechanisms of cellulose-degrading enzymes is critical to developing improved technologies for biofuel production. We have run extensive molecular dynamics simulations of a single cellulose chain inside the Cel7A catalytic tunnel to understand the structural basis for processivity. In particular, we have investigated the initial binding of a cellulose chain into the catalytic tunnel of Cel7A. In multiple unbiased simulations, the cellulose chain spontaneously diffuses into the tunnel by the length of a cellobiose unit. Further free energy calculations reveal an overall downhill free energy profile for the initial threading, which becomes progressively flat and rugged toward the active site, highlighting a strong interplay between processivity and catalysis. The free energy profiles for different cellulose chain orientations show a clear thermodynamic preference for the reducing end binding at the -5 subsite, suggesting that the selective hydrolysis of cellulose from the reducing end might be partially achieved through the preferential initial binding of cellulose to Cel7A.


## Jens Meiler

*Computational Chemical and Structural Biology*
*Vanderbilt University, Nashville, TN*
*jens.meiler@vanderbilt.edu*


### A computational platform for protein structure determination integrating limited experimental data

I will present BCL::Fold, a new algorithm for de novo prediction of complex and large protein topologies by assembly of secondary structure elements. The method was designed to integrate experimental data from NMR, EPR, EM, SAXS experiments, or combinations thereof. These data sets often provide more readily restraints for regions of defined secondary structure. I will present examples for atomic-detail structure elucidation from medium resolution cryo-EM density maps (Figure 1) and paramagnetic restraints from NMR spectroscopy. Briefly: Computational de novo protein structure prediction is limited to small proteins of simple topology. The present work explores an approach to extend beyond the current limitations through assembling protein topologies from idealized α-helices and β-strands. The algorithm performs a Monte Carlo Metropolis simulated annealing folding simulation. It optimizes a knowledge-based potential that analyzes radius of gyration, β-strand pairing, secondary structure element packing, amino acid pair potential, amino acid environment, and loop closure. Discontinuation of the protein chain favors sampling of non-local contacts and thereby creation of complex protein topologies. The folding simulation is accelerated through exclusion of flexible loop regions further reducing the size of the conformational search space. The algorithm is benchmarked on 66 proteins with lengths between 83 and 293 amino acids. For 61 out of these proteins the best SSE-only models obtained have an RMSD100 below 8.0 Å and recover more than 20% of the native contacts. The algorithm assembles protein topologies with up to 215 residues and a relative contact order of 0.46. BCL::Fold includes a modified scoring

function for the assembly of membrane proteins. BCL::Fold is typically combined with Rosetta refinement algorithms to arrive at proteins models accurate at atomic detail.


## Julie Mitchell

*Departments of Mathematics and Biochemistry*
*University of Wisconsin, Madison*
*mitchell@math.wisc.edu*


### Knowledge based structural approaches for predicting hot spots of protein binding and allostery

Using information derived from protein structures, it is possible to predict amino acid positions where mutations will have a deleterious effect on protein binding or allosteric communication. The KFC2 model captures 80% of alanine scanning mutagenesis hot spots, which result in a binding energy increase of at least 2 kcal/mol. A unique feature of the model is a local plasticity feature that suggests whether a change in sequence can be accommodated through local sidechain rearrangements. A different plasticity measure, known as local structural entropy, is a dominant feature in our AlloSIND model for allosteric hot spots that lie between the effector and active sites of allosteric proteins. One possible interpretation is that rigidity of internal protein secondary structure prevents an allosteric protein from absorbing the impact of effector binding locally, resulting in longer-range conformation effects.


## Celeste Sagui

*Department of Physics*
*North Carolina State University*
*Celeste_Sagui@ncsu.edu*


### Free energy landscape of polyproline and polyglutamine peptides

We use enhanced sampling techniques (the Adaptively Biased Molecular Dynamics (ABMD) method, multiple walkers, replica exchange, steered MD, and various combinations thereof) to study peptide systems whose conformational space cannot be sampled with regular MD simulations. These include transitions between the PPII and PPI forms of polyproline (polyP); proline-rich guest/host peptides; polyglutamine (polyQ), and polyQ-polyP systems. Several statistical techniques allow us to explore the atomic mechanisms that underlie various experimental observations: the apparent PPII propensity of guest amino acids in polyP-rich peptides; the properties that may favor aggregation in polyQ systems; and the suppression of aggregation of polyQ by the addition of a C-terminal polyP peptide.

## Jana Shen

*Department of Chemistry, University of Oklahoma*
*jana.k.shen@ou.edu*


### Recent development and application of continuous constant pH molecular dynamics

Development of the constant pH molecular dynamics techniques has opened a door to atomically detailed studies of dynamic processes coupled to protonation/deprotonation. Here we discuss the most recent development of the continuous constant pH molecular dynamics (CpHMD) technique and application studies for gaining novel insights into ionization-coupled conformational phenomena in biology and chemistry. We show that CpHMD simulations offer, for the first time, thermodynamic description of coupled protonation and conformational equilibria for proteins. We will also discuss other applications such as pH titration of micelles and pH-dependent phase transitions.


## John Straub

*Department of Chemistry*
*Boston University*
*straub@bu.edu*


### Role of hydration and confinement in protein folding and aggregation

Reverse micelles provide an environment in which the number of water molecules and overall cavity size may be "tuned," by adjusting the water-to-surfactant ratio, allowing, in principle, the role of hydration and confinement on protein folding and aggregation to be systematically studied. We have used molecular dynamics simulations to explore the structure and dynamics of the alanine-rich AKA2 peptide and aggregation-prone fragments of amyloid proteins in reverse micelle environments. The dependence of the peptide-micelle interaction on capping of the N- and C- termini and the nature of the force field is explored. The time scales for fluctuations in the reverse micelle shape and surface area are characterized and compared with the results of more idealized spherical micelle models. The results suggest that an understanding of the detailed nature of protein-surfactant interactions can be essential to the interpretation of studies of protein folding and aggregation in reverse micelle environments.


## Jerome Baudry

*Biochemistry, Cellular and Molecular Biology Department*
*University of Tennessee, Knoxville, TN*
*jbaudry@utk.edu*


### From computational docking to exploration of biochemical pathways

Virtual (in-silico) docking of small molecules in protein targets is a popular and successful approach to discover molecules capable of binding in proteins. We describe how docking is used to investigate biochemical pathways, focusing on how P450s can turn environmental molecules into estrogenic pollutants by increasing their binding to the estrogen receptor alpha target. We also describe ongoing developmental work to use supercomputing architectures efficiently to perform massive docking of very large chemical databases against a large number of protein targets.

## Collin Stultz

*EECS and HST*
*Massachusetts Institute of Technology, Cambridge, MA*
*cmstultz@mit.edu*


### Modeling disordered states of proteins: Are structural models of the unfolded state correct?

The characterization of intrinsically disordered proteins is challenging because accurate models of these systems require a description of both their thermally accessible conformers and the associated relative stabilities or population weights. These structures and weights are typically chosen such that calculated ensemble averages agree with some set of pre-specified experimental measurements; however, the large number of degrees of freedom in these systems typically leads to multiple conformational ensembles that are degenerate with respect to any given set of experimental observables. Moreover, our recent work demonstrates that estimates of the relative stabilities of conformers within an ensemble are often incorrect when one does not account for the underlying uncertainty in the estimates themselves. Therefore, we have developed a method for modeling the conformational properties of disordered proteins that estimates the uncertainty in the weights of each conformer. A unique and powerful feature of the approach is that it provides a built-in error measure that allows one to assess the accuracy of the ensemble. Using this approach we constructed an ensemble that characterizes the accessible states of the IDPs, tau protein, alpha-synuclein and abeta; i.e., proteins that play a role in several neurodegenerative disorders. These data led to new insights into intramolecular interactions that may play a role in promoting IDP self-association – a process which has been linked to neuronal death and dysfunction in patients with Alzheimer's disease. We further demonstrate that these data may be used in the initial stages of a strategy to design ligands that prevent IDP aggregation. More generally, we derive an order parameter that quantifies the extent of disorder within a protein. Although protein disorder is normally thought of as a binary phenomenon (i.e., a protein is either disordered or not), we suggest that the concept of protein disorder should be treated like a continuous variable, and that not all unfolded states are created equal.


## Arjan van der Vaart

*Department of Chemistry*
*University of South Florida*
*avandervaart@usf.edu*


### Enhanced sampling simulations of DNA and DNA-complexes

The flexibility of long DNA is well described by the worm-like chain model, but at the small-length scales this model breaks down. Moreover, several experiments suggest that short DNA has an increased flexibility. We performed enhanced sampling simulations of short DNA strands to assess its flexibility. Our results indicate that the stiffness of DNA decreases upon strong bending. We will also discuss enhanced sampling simulations to show how proteins and ligands modulate the DNA structure.

**Sandor Vajda**
*Department of Biomedical Engineering and Chemistry*
*Boston University*
*vajda@bu.edu*

## High-throughput identification and druggability analysis of protein binding sites

Our lab has developed the computational solvent mapping method of determining binding hot spots of proteins (1). The method globally samples the surface of a target protein using small organic molecules as probes, finds favorable positions, clusters the conformations, and ranks the clusters on the basis of the average energy. The regions that bind several probe clusters predict the binding hot spots, in good agreement with experimental results. Solvent mapping can be used to solve two important problems. First, it achieves higher accuracy than any other method in the identification of ligand binding sites on unbound protein structures (2). Second, the mapping results provide information for assessing druggability, i.e., the ability of a protein to bind drug sized ligands with high affinity (3). While both applications were very successful, they required lengthy calculations and hence were originally demonstrated on small benchmark sets. We describe our work toward modifying the methods such that applications to large sets of proteins would become computationally feasible, enabling some general conclusions on the binding properties of proteins.

**1.** Brenke, R., Kozakov, D., Chuang, G.Y., Beglov, D., Hall, D., Landon, M.R., Mattos, C. and Vajda, S. (2009) Bioinformatics, 25, 621-627**. 2.** Ngan, C.H., Hall, D.R., Zerbe, B., Grove, L.E., Kozakov, D. and Vajda, S. (2012) Bioinformatics, 28, 286-287. **3**. Kozakov, D., Hall, D.R., Chuang, G.Y., Cencic, R., Brenke, R., Grove, L.E., Beglov, D., Pelletier, J., Whitty, A. and Vajda, S. (2011) Proc. Natl. Acad. Sci. U S A, 108, 13528-13533.

# CBSB12 Outstanding Young Researchers

1. Virginia Burger
2. Garrett Goh
3. Ewa Golas
4. Phanourios Tamamis
5. Chunli Yan

**Virginia Burger**
*Computational Biology*
*University of Pittsburg*
*vmb12@pitt.edu*

## Characterizing Pathways between Metastable States of Intrinsically Disordered Proteins

Intrinsically disordered proteins (IDPs) lack well-defined secondary and tertiary structure. These proteins make up about 50% of signaling proteins and are implicated in diseases such as cancer and neurodegenerative disorders. IDPs often undergo synergistic folding upon binding, but range from being completely random coil to having a small degree of residual structure while unbound. Capturing the unbound state of IDPs is a particular challenge for traditional molecular dynamics simulations. Increasingly, atomistic simulations can adequately sample the conformational landscape of small peptides, but analysis techniques for characterization of metastable substates in IDPs are lacking. Here, we present a novel method for multi-scale spatio-temporal organization of long time-scale IDP simulation data.

We demonstrate our method on the nuclear co-activator binding domain (NCBD) of CBP (CREB binding protein). NCBD is a 59-residue molten globule peptide containing three helices which unfold and refold to form diverse bound configurations. Six experimental structures of this peptide, both bound to co-activators and unbound, have been determined with three distinct folds. To characterize the conformational space of NCBD, we generated 40-microseconds of all-atom explicit water MD simulation of unbound NCBD at equilibrium, which comes within 1 - 4 Angstroms of each experimental structure. We clustered the simulated conformers into conformationally similar states using a hierarchical random-walk based grouping scheme. Analogous to Markov State Models, we use spectral methods to group conformational states into kinetically similar metastable states. However, here anharmonic conformational analysis is applied to obtain energetically coherent conformational states, and the number of states is determined implicitly by the landscape. The hierarchical nature of our clustering scheme demonstrates multi-scale conformational similarities, in that finely separated clusters with fast interconversions are identified at early hierarchy levels, while coarsely grouped conformations with slow interconversions are provided by later hierarchy levels. To characterize the transition pathways between the clusters, we identify kinetic bottlenecks and short time-scale transitions between the distinct conformational states using spectral perturbation theory. We find pathways and rates describing the transition of unbound NCBD between its conformational states and identify rate-limiting steps separating the six experimental conformations. This multi-scale spatio-temporal analysis can be used to describe the conformational space of IDPs in general, e.g. for identifying druggable metastable states.

## Garrett Goh

*Chemistry*
*University of Michigan*
*gbgoh@umich.edu*


### pH-coupled simulations of RNA in explicit solvent

From serving as catalytic residues in ribozymes to triggering pH-dependent conformation changes, the role of protonated nucleotides in modulating RNA dynamics and function is fast emerging as one of the key unanswered questions in the study of RNA biology. Conventional molecular dynamics (MD) methods can only partially address these questions because the protonation states are modeled as fixed states, which are reliant on prior knowledge of the identity and pKa values of key residues. With the work presented here, we establish the framework and demonstrate the first constant pH MD simulations (CPHMD^MSλD) of nucleic acids in explicit solvent, where the protonation states are modeled as dynamic variables that are coupled to the dynamics of the RNA via λ-dynamics. We adopted a recently developed functional form, λNexp, for λ within the framework of multi-site λ-dynamics (MSλD) which improves sampling efficiency in the λ-space 10-fold over existing explicit solvent CPHMD methods. Calculated pKa values of simple nucleotides are in an excellent agreement with experiment, with an average unsigned error of 0.24 pKa units. Using lead-dependent ribozyme as a model RNA system, we show that CPHMD^MSλD simulations accurately reproduce the direction of pKa shifts and provide reasonably accurate recapitulation of experimental pKa values, demonstrating the potential for applying this approach to explore pH-dependent processes in complex RNA molecules. We anticipate that CPHMD^MSλD simulations will be used as a powerful tool applied in conjunction with existing experimental techniques to investigate various pH-dependent phenomena of nucleic
acids.


## Ewa Golas

*Molecular Modeling Group, Theoretical Chemistry*
*University of Gdansk, Poland*
*Ewa.golas.chem@gmail.com*


### Molecular Dynamics of the Hsp70 Chaperone in response to nucleotide and substrate: a coarse-grained perspective

The 70 KDa heat-shock (Hsp70) proteins form a class of chaperones recognized for their diverse and essential roles in the domain of protein repair, folding assistance, and agglomerate prevention. The present study examines the mechanism of Hsp70 function via molecular dynamics, employing the coarse-grained UNRES model and forcefield in a series of canonical Langevin molecular dynamics simulations. The effect of implicit nucleotide in the nucleotide-binding domain (NBD) and substrate in the substrate-binding domain (SBD) was investigated for the Hsp70 from *E. Coli*, DNAK (PDB 2KHO). Three binding pathways of the SBD to the NBD were observed, along with a dominating effect of substrate. Implications of both nucleotide and substrate towards the Hsp70 cycle are discussed.

**Phanourios Tamamis**
*Department of Physics*
*University of Cyprus, Cyprus*
*tamamis@ucy.ac.cy*

## Development of Regulatory Compounds for the Complement System by MD Simulations and Experiments

The complement system (CS) provides the first line of defense against the invasion of foreign pathogens. Nevertheless, its inappropriate or excessive activation may cause or aggravate several pathological conditions, such as age-related macular degeneration (AMD). Therefore, the development of drugs tackling chemical pathways for the control of inappropriate or excessive complement activation is of significant medical interest. Compstatin and PMX53 are two peptide-based promising therapeutic compounds as they bind, respectively, on key proteins C3 and C5aR of CS and inhibit complement activation. Simulations and experiments have been employed to optimize the binding efficacy and inhibitory activity of both compounds.

Using Molecular Dynamics (MD) simulations, we have recently suggested an interpretation for the species specificity of Compstatin [1] (its activity against human C3 and inactivity against lower-mammal C3), and succeeded in designing a modified "transgenic" mouse protein aiming at testing AMD disease models in non-primates [2]. Furthermore, using a combination of de novo drug design and MD simulations we recently proposed new compstatin analogs with optimized binding affinity and solubility, relative to known compstatin analogs [3]. The most promising compounds constitute the most potent inhibitors of AMD drusenoid bound-C5b9 complexes in running experiments with human retinal pigmented epithelial assays. In addition, we have recently implemented a combined protocol, consisting of ligand-docking, implicit-membrane MD simulations and binding free energy calculations, to generate and assess an exhaustive ensemble of structural models for the complex between the key GPCR protein of the
complement-system key C5aR and its most potent antagonist, hexapeptide PMX53. The most promising complex contains interactions in line with available experimental data from site-directed mutagenesis [4] and provides insights into PMX53 residues which confer antagonist activity. Novel compounds based on PMX53 are now computationally and experimentally investigated aiming at optimizing the peptide inhibitory activity.

**1**. P. Tamamis, D. Morikis, C. A. Floudas and G. Archontis. Species Specificity of the Complement Inhibitor Compstatin investigated by All-atom Molecular Dynamics Simulations. Proteins: Structure, Function and Bioinformatics 78, 2655-2667 (2010). **2**. P. Tamamis, P. Pierou, S. Mytidou, C. A. Floudas, D. Morikis and G. Archontis. Design of a modified mouse protein with ligand binding properties of its human analog by molecular dynamics simulations: the case of C3 inhibition by compstatin. Proteins: Structure, Function and Bioinformatics, 79: 3166-3179 (2011). **3**. P. Tamamis, A. López de Victoria, R. D. Gorham Jr., M. L. Bellows, P. Pierou, C. A. Floudas, D. Morikis and G. Archontis. Molecular Dynamics Simulations in Drug Design: New Generations of Compstatin Analogs. Chemical Biology & Drug Design, 79: 703-718 (2012). **4**. A. Higginbottom, S. A. Cain, T. M. Woodruff, L. M. Proctor, P. K. Madala, J. D. A. Tyndall, S. M. Taylor, D. P. Fairlie, and P. N. Monk. Comparative Agonist/Antagonist Responses in Mutant Human C5a Receptors Define the Ligand Binding Site. J. Biol. Chem. 280, 17831-17840 (2005).

**Chunli Yan**
*Chemistry*
*Georgia State University*
*utchunliyan@gmail.com*

## Coupling of DNA binding and architectural remodeling drives the function of Human RPA

The eukaryotic single-stranded (ss) DNA-binding protein (SSB), replication protein A (RPA), plays a central role in replication, recombination and repair. Human RPA is a heterotrimer with three subunits of ~70, 32 and 14 kDa, which are referred to as RPA70 (ABC), RPA32 and RPA14, respectively. In DNA-processing events, RPA also interacts with many additional nuclear proteins, and this interaction both regulates, and is regulated by, an interaction with ssDNA. Binding of ssDNA is critical for shielding DNA strands from endonuclease activity and preventing the formation of disruptive secondary structures. Using additional binding surfaces, RPA recruits DNA processing factors, thereby providing a platform for their organization and managing their access to the ssDNA. Engaging ssDNA and subsequent transfer from RPA to other DNA processing proteins are crucial events for the progression of DNA processing machinery; however, the physical basis for these transactions are not well understood.

RPA binds to DNA with a specific polarity and has at least three major binding modes characterized by the length of ssDNA that it contacts 8¨C10, 12¨C23 and 28¨C30 nucleotides (nt). The first mode, which is considered to be a major one, is characterized by an occluded binding site of ~30 nt. This binding mode exhibits high affinity and low cooperativity. The second mode, which is less stable and may be a precursor for the 30 nt mode, has an 8¨C10 nt binding site and exhibits a lower affinity and a higher cooperativity. The transition from the 8 to the 30 nt mode is thought to be a functionally important event implicated in DNA unwinding. A more stable intermediate binding of 12¨C23 nt mode occurs with the additional involvement of RPA70C. The association constant of the binding ranges from 108 to 1011 M-1 depending on the sequence and length of the substrate. These three modes of binding ssDNA proposed for RPA, involving contacts to RPA70AB, RPA70ABC, and RPA70ABC/32D, respectively, should modify the organization of RPA domains. Small angle X-ray and neutron scattering (SAXS/SANS) experiments with all-atom molecular dynamics simulation were performed to determine the effects of ssDNA binding on the architecture of RPA. The SAXS data combined with molecular dynamics simulations revealed two not three transitions as RPA binds ssDNA. There is no evidence for an intermediate state corresponding to the ~20 nt binding mode. The revised view of the DNA-bound states combined with evidence of significant residual motion leads to a new model for RPA-ssDNA interactions and provides insight into binding and release by RPA in DNA processing pathways.

# CBSB12 Contributed Talks

1. Nicholas Bodmer
2. Jodian Brown
3. Stephanie DeLuca
4. Igor Drobnak
5. Jun Fan
6. Sigurdur Jonsson
7. Gia Maisuradze
8. Amitava Roy
9. Reza Salari
10. Jeff Wereszczynski

## Nicholas Bodmer

*Department of Chemistry, University of Cincinnati*
*bodmernk@mail.uc.edu*


### Molecular Investigations into the Mechanics of a Muscle Anchoring Complex

Titin governs elasticity within muscular contractile units. Telethonin plays a critical role in anchoring titin repeats in the Z-disk of the sarcomere. Given the all b-strand structure of the complex, hydrogen bonding is crucial for structural stability, however it is unclear what part of the hydrogen bonding network is responsible for the function of the complex(1). Moreover atomic force microscopy experiments indicate that the titin-telethonin complex can withstand the highest measured force in a globular protein when pulled in the physiological C-terminal direction (2). However, when pulled along other directions, the complex fails at forces corresponding to the mechanical stability of typical immunoglobulin domains(3). Previous attempts to account for this phenomenon in silico have met with limited success due to a set up that deviates substantially from its experimental counterpart (4). We employed a different approach focused on a coarse grained description of the protein to follow the dynamics of the titin-telthonin complex under forces applied to mimic the AFM experiments (5) Due to the large size of the system and the long timescales involved, to be able to follow the behavior of the complex on experimentally relevant hundreds of ms, we implemented the simulations on graphics processing units (6). Our most exciting finding is that this system is finely tuned to the force load regime such that an order of magnitude change in the loading rate results in significant changes in behavior. Our results shed light on the structural underpinnings of the previous experimental results(2) and clarify the role of the hydrogen bond network in the mechanical behavior of titin-telethonin. Moreover our investigations show how a system comprised entirely of immunoglobulin proteins can display behavior that is significantly different from its building blocks.


**1**.Zou et al, 2006, Nature, 439:229 **2**. Bertz et al, 2009, PNAS, 103:13307 **3.** Rief et al, 1997, Science, 276:1109 **4.** Lee et al, 2006, Structure, 14:497 **5.** Hyeon et al, 2006, Structure, 14:1633 **6**. Zhmurov et al, 2010, Proteins, 78:2984



## Jodian Brown

*Department of Chemistry and Biochemistry*
*University of Maryland, Baltimore*
*Jobrown1@umbc.edu*


### Using Molecular Dynamics to Understand the Molecular Mechanisms Underlying the Application of Combination Therapies to Inhibit Hepatitis C Virus Polymerase

The RNA-dependent RNA polymerase (NS5B) of the hepatitis C virus (HCV) is a vital component of viral replication. In addition, there is no known mammalian homolog of HCV NS5B enzyme, making it a promising target for clinical investigation. A major challenge of treating HCV is the emergence of resistance to current treatment regiments. An approach to reducing the rate of drug resistance is to increase the inhibitory effects of small molecule inhibitors by using them in combination. This proposal focuses on understanding how multiple allosteric ligands can be used to synergistically inhibit the enzyme. The primary goal is to use molecular dynamics simulation to understand the dynamic and thermodynamic changes that result from the binding of multiple ligands to NS5B. Understanding the molecular mechanisms that mediate the binding of multiple

inhibitors to NS5B may allow us to optimize the inhibitory activity of these compounds against the enzyme. Furthermore, the knowledge gained can provide fundamental insight into how multiple ligands bind to proteins. Such information has direct applications in the areas of drug discovery, regulation of metabolic pathways, and other signal transduction processes.


## Stephanie DeLuca

*Center for Structural Biology*
*Vanderbilt University*
*stephanie.h.deluca@vanderbilt.edu*


### ROSETTAEPR: An Integrated Tool for Protein Structure Determination from Sparse EPR Data

Membrane proteins remain a particular challenge in structural biology. Only about 1.5% of reported tertiary structures and 60 unique membrane protein topologies consisting of more than one transmembrane span are represented in the PDB. However, these proteins make up an estimated 30-40% of the entire proteome, and over half of all therapeutics target this group. Site-directed spin labeling electron paramagnetic resonance (SDSL-EPR) is often used for the structural characterization of proteins that elude other techniques, such as X-ray crystallography and NMR. However, high-resolution structures are difficult to obtain due to uncertainty in the spin label location and sparseness of experimental data. ROSETTAEPR has been designed to improve high-resolution protein structure prediction using sparse SDSL-EPR distance data. The "motion-on-a-cone" spin label model is converted into a knowledge-based potential, which was implemented as a scoring term in ROSETTA. We have demonstrated the feasibility of using ROSETTAEPR with soluble proteins by benchmarking the method on T4-lysozyme. ROSETTAEPR increased the fractions of correctly folded models (RMSDCα < 7.5Å) and models accurate at medium resolution (RMSDCα < 3.5Å) by 25%. After full-atom refinement, ROSETTAEPR yielded a 1.7Å model of T4-lysozyme, thus indicating that atomic detail models can be achieved by combining sparse EPR data with ROSETTA. ROSETTAEPR was also benchmarked on a set of membrane proteins of known structure. If EPR experimental data were not available, simulated data were derived from the existing structures. It was generally observed that de novo folding in the presence of EPR restraints enriched the recovery of the proteins' correct topology compared to when folding with no restraints.


## Igor Drobnak

*Department of Chemistry and Biochemistry*
*University of Notre Dame*
*idrobnak@nd.edu*


### Modeling Autotransporter Secretion

Autotransporters are a class of proteins in Gram-negative bacteria that are secreted across the outer membrane and are often involved in pathogenic functions. They are transported across the inner membrane cotranslationally by the ATP-driven Sec machinery. To cross the outer membrane, however, they need an alternative energy source, since ATP is not available outside of the cytosol. It has been proposed that the autotransporter remains unfolded in the periplasm, but folds on reaching the extracelluar space, which would prevent it from backsliding. We have constructed a computational model that uses a stochastic simulation algorithm to simulate the kinetic behavior of an autotransporter system given different equilibrium and rate constants for folding and outer membrane translocation. Computational modeling provides useful insight into the secretion process even in the absence of experimental data - the ability or inability of the folded

autotransporter to cross the outer membrane is shown to be a crucial determinant of the system, along with the rate of folding in the periplasm. Extracellular protein folding can be used as a driving force for secretion only if translocation of the folded protein across the outer membrane is very slow. In this case, any folding in the periplasm represents a "dead-end" side reaction, the rate of which needs to be minimized if secretion is to be efficient. On the other hand, a high rate of translocation for the folded protein results in equilibration of the system, with the consequence that an external difference in free energy is needed to favor secretion. No such energy source is known to date. Efforts are currently underway to experimentally determine the kinetic parameters that control autotransporter secretion.

## Jun Fan

*Department of Chemistry*
*University of Chicago*
*junfan@uchicago.edu*

### Molecular dynamics simulation and coarse-graining study on cofilin remodeling actin filaments

Actin filaments consist of actin proteins, which are abundant in eukaryotic cells. These filaments form mesh-like structures to provide mechanical support and determine the shape of cells. Actin filaments also play important roles in cell mobility, cell division, endocytosis and intracellular transportation. Cofilin proteins, actin depolymerization factor, play key roles in the dynamics of actin filaments. The binding of cofilin modifies the structure, conformational dynamics and mechanical properties of actin filaments. We investigate these modifications using molecular dynamics (MD) simulations and coarse-grained (CG) analysis. In MD simulations, we observe that twist, tilt, cross-over length of actin filaments differ due to cofilin binding, consistent with experimental data. Moreover, mechanical properties, including the persistence length and torsional stiffness, decrease significantly after cofilin binding, in agreement with experiments. This decrease is caused by the weakened longitudinal and lateral interactions within the filament. To quantify this, we apply the CG analysis method to the MD data. CG analysis results reveal that the longitudinal distance between DNase-I binding loop and subdomain 1 of the neighboring subunit increases twice as far thus the effective interactions decrease remarkably. Another longitudinal contact between subdomain 3 and subdomain 4 of the neighboring subunit also become weaker even though the distance between these two subdomains does not change dramatically. Meanwhile, the lateral contact distances between hydrophobic loop and neighboring subunit vary slightly while the effective interaction strengths are still compromised with cofilin binding. These results provide a molecular interpretation for the effect cofilin binding has on the structure and properties of an actin filament.

## Sigurdur Jónsson

*Department of Astronomy and Theoretical Physics*
*Lund University, Sweden*
*Sigurdur.aegir@thep.lu.se*

### Distinct phases of free alpha-synuclein - a flat-histogram Monte Carlo study

The alpha-synuclein protein, implicated in Parkinson's disease, shows conformational versatility. It aggregates into beta-sheet-rich fibrils, occurs in helical membrane-bound forms, is disordered as a free monomer, and has recently been suggested to have a folded helical tetramer as its main physiological form. Here I present an implicit solvent all-atom Monte Carlo study of the conformational ensemble sampled by the free alpha-synuclein monomer [1]. We analyze secondary-structure propensities, size and topological properties, and compare with

existing experimental data. Our study suggests that free alpha-synuclein has two distinct phases. One phase has the expected disordered character. The other phase also shows large conformational variability. However, in this phase, the beta-strand content is substantial, and the backbone fold shows statistical similarities with that in alpha fibrils. Presence of this phase is consistent with data from low-temperature experiments. Conversion of disordered alpha-synuclein to this fibril-like form requires the crossing of a rather large apparent free-energy barrier. The presence of the free-energy barrier makes simulating this 140-residue protein a challenge. To tackle this problem, we use a two-step simulation procedure based on the Wang-Landau and the multicanonical methods [2].

1. Distinct phases of free alpha-synuclein -- a Monte Carlo study, S.A. Jónsson, S. Mohanty and A. Irbäck (submitted to Proteins)
2. Accelerating atomic-level protein simulations by flat-histogram techniques, S.A. Jónsson, S. Mohanty and A. Irbäck, Journal of Chemical Physics 135, 125102 (2011).

## Gia Maisuradze

*Chemistry and Chemical Biology*
*Cornell University*
*gm56@cornell.edu*

### Protein folding by coarse-grained molecular dynamics

In order to understand the kinetics and thermodynamics of protein folding it is important to know why proteins fold or do not fold, and what governs the way that proteins fold. To answer this general question, there are many aspects in folding which must be understood. For example, it is of interest to know (i) whether local fluctuations in a polypeptide chain play any role in the mechanism by which the chain folds to the native structure of a protein; the correlations between local and global motions; (iii) the key residues playing important role in folding; (iv) correlation between the side chain and main chain motions in native state and folding. All these aspects are addressed in this presentation by analyzing the folding and non-folding trajectories of the 37-residue triple beta-strand WW domain from the Formin binding protein 28 (FBP) [PDB: 1E0L], B-domain of staphylococcal protein A [PDB: 1BDD (alpha; 46 residues)], 46-residue alpha/beta model protein VA3 [PDB: 1ED0]. Molecular dynamics trajectories were generated with the coarse-grained united-residue (UNRES) and all-atom (OPLS) force fields.

## Amitava Roy

*Medicinal Chemistry and Molecular Pharmacology*
*Purdue University*
*amitroy@purdue.edu*

### Long-distance correlations of rhinovirus capsid dynamics contribute to uncoating and antiviral activity

Human rhinovirus (HRV), caustive of common cold, and other members of the enterovirus genus bind small-molecule antiviral compounds in a cavity buried within the viral capsid protein VP1. These compounds block the release of the viral protein VP4 and RNA from inside the capsid during the uncoating process. In addition, the antiviral compounds prevent “breathing” motions, the transient externalization of the N-terminal regions of VP1 and VP4 from the inside of intact viral capsid. The site for externalization of VP1/VP4 or release of RNA is likely between protomers, distant to the binding cavity for antiviral compounds. Molecular dynamics simulations were

conducted to explore how the antiviral compound, WIN 52084, alters properties of the HRV 14 capsid through long-distance effect. We developed an approach to analyze capsid dynamics in terms of correlated radial motion and the shortest-paths of correlated motions. In the absence of WIN, correlated radial motion is observed between residues separated by as much as 85 °A, a remarkably long distance. The most frequently populated path segments of the network were localized near the 5-fold symmetry axis and included those connecting the N-termini of VP1 and VP4 with other regions, in particular near 2-fold symmetry axes, of the capsid. The results provide evidence that the virus capsid exhibits concerted long-range dynamics, which have not been previously recognized. Moreover, the presence of WIN destroys this radial correlation network, suggesting that the underlying motions contribute to a mechanistic basis for the initial steps of VP1 and VP4 externalization and uncoating.

Concerted motion is essential for proteins to carry out their functions. Concerted motion implies correlated fluctuation of different parts of protein, which can be distance apart. Atomistic molecular dynamics (MD) simulation of proteins can be a powerful tool to reveal correlated motion in great details. However long-range correlation motion has so far been elusive in in-silico studies. The most widely used method to quantify correlated fluctuation is finding Pearson's correlation coefficient of displacement vector (DCC). DCC depends on the cosine of the angle between the vectors and is only sensitive to linear correlation. Radial correlation (RCC) and generalized correlation (GCC) coefficients have been used to quantify correlation to overcome shortcomings of DCC. However RCC is insensitive to azimuthal fluctuation and might not be useful where radial symmetry is not apparent. GCC requires components of a displacement vector to be independent of each other, a constraint which is usually violated. In this article we discuss merits and weaknesses of using DCC, RCC and GCC in analysis of MD trajectories of macromolecules and show a new correlation coefficient, distance correlation coefficient (DiCC) can capture both linear and non-linear correlation without imposing any assumption on the time series of vectors. We also discuss how dimension reduction based on multivariate analysis on linear covariances can be misleading and show that distance covariances can reduce dimension more effectively.

**Reza Salari**
*Department of Chemistry*
*University of Pittsburg*
*Res86@pitt.edu*

## Direct Observations of Shifts in the Î²-Sheet Register of a Protein-Peptide Complex Using Explicit Solvent Simulations

We report direct observations of rearrangements in an intermolecular protein-peptide Î²-sheet using explicit solvent simulations. The Î²-sheet is formed between the FHA domain of cancer marker protein Ki67 (Ki67FHA) and a peptide fragment of the hNIFK signaling protein. Simulations of rearrangements from a misregistered state to the native state were generated using a combination of large-scale distributed computing and supercomputing resources. We discuss a common mechanism that is shared by our resulting rearrangements. To our knowledge, these simulations provide the first atomically detailed visualizations of a mechanism by which nature might correct for errors in the alignment of intermolecular Î²-sheets.

**Jeff Wereszczynski**
*Chemistry and Biochemistry*
*University of California, San Diego*
*jmweresz@mccammon.ucsd.edu*

## The calculation of free energies over vast length scales through conventional, enhanced sampling, and free energy molecular dynamics simulations

The guided entry of tail-anchored proteins (GET) mechanism post-translationally targets tail-anchored (TA) protein to the endoplasmic reticulum membrane. At the center of this pathway is the ATPase Get3, a homodimer of approximately 700 residues that shuttles TA proteins from the cytosolic Get4/Get5 complex to the transmembrane Get1/Get2 complex for insertion. Crystallographic studies have shown that binding of ATP and ADP molecules induces large scale conformational rearrangements from the "open" state, which is observed in the nucleotide free structures, into "closed" conformations. Here, we present a series of all-atom molecular dynamics simulations that address the stability of these conformational states and the mechanism of transition between them. By combing results from conventional and accelerated MD simulations with rigorous free energy calculations, the thermodynamic landscape along the dimensions primarily responsible for the opening/closing transition are reconstructed for five possible nucleotide states. Results show good agreement with experiments on the propensity of the open and closed forms in the no-nucleotide, two ATP, and two ADP bound states, and reveal their relative populations in the asymmetric one ATP and one ADP states. In addition, the nucleotide-free case is shown to exist in an equilibrium of configurations, the recently revealed "semi-open" state is observed as an energy minimum in multiple nucleotide bound cases, and we present evidence for the novel "wide-open" conformation. Taken together, these results and have lead to a model of the nucleotide dependent mechanism of Get3 opening and closing and suggest new insights into Get3's function.

# Poster Presentations

1. Emal M. Alekozai
2. Bachir Aoun
3. Workalemahu Berhanu
4. Nicholas Callahan
5. Derek Cashman
6. Mihir Date
7. Samuel DeLuca
8. Sally R. Ellingson
9. Hao-Bo Guo
10. Russell Hanson
11. Jason Harris
12. Andrew Hirsh
13. Liang Hong
14. Ping Jiang
15. Alexander Johs
16. Andrea Kravats
17. Pawel Krupa*
18. Jianing Li
19. Peng Lian
20. Benjamin Linder
21. Yanxin Liu*
22. Buddhadev Maiti
23. Yinglong Miao
24. Magdalena Mozolewska*
25. Pincu Medeliene
26. Sayak Mukherjee
27. Hai Nguyen
28. Gungor Ozer
29. Xianghong Qi
30. Andrej Savol
31. Michael Schnieders
32. Roland Schulz
33. Ester Sesmero
34. Piotr Setny
35. Kelly Theisen
36. Sam Tonddast-Navaei
37. Sahin Uyaver*
38. Huan Wang
39. Tomasz Wirecki
40. Xiaojun Xu
41. Fatih Yasir
42. Zheng Yi
43. Hang Yu
44. He Zhang
45. Zhe Zhang*

*Fellowship

## Emal M. Alekozai 1

*Center for Molecular Biophysics*
*University of Tennessee*
*Emal.Alekozai@iwr.uni-heidelberg.de*


### Multiscale Sampling of the CBM and Lk Properties in the Cel7A-Cellulose Interaction

Cellulose, the most abundant biopolymer on earth (approx. 100 billion dry tons/ year) holds an enormous potential as a renewable energy source. It consists of sugar subunits which can be unlocked and fermented to produce bioethanol. Cellulase enzymes, in particular Cel7A, play an essential role in the cellulose degradation and carbon turnover in the biosphere. Cel7A consists of a carbohydrate binding module (CBM) and a catalytic domain which are hold together by a linker peptide (Lk). There is evidence that the CBM and the Lk are important for the Cel7A-cellulose interaction. In a two step simulation protocol Brownian (>40 ms) as well as molecular dynamic simulations (>5.3 microseconds) were conducted. Our results suggest that hydrophobic cellulose fiber faces are thermodynamically and kinetically favored. Our simulation results complement a large body of previous studies, providing detailed insights into the mechanism of the Cel7A-cellulose interaction.


## Bachir Aoun 2

*Laue Langevin Isere, Grenoble France*
*Bachir.aoun@e-aoun.com*


### nMOLDYN4 software

nMOLDYN is a modular program package for the analysis of Molecular Dynamics trajectories, especially designed for the computation and decomposition of neutron scattering spectra. The current release 3.11, it allows one to calculate the mean-square displacement, the velocity autocorrelation function as well as its Fourier Transform (the density of states) and its memory functions, the angular velocity autocorrelation function and its Fourier transform, the reorientational correlation function. Moreover it can compute several quantities related to neutron scattering: the coherent and incoherent intermediate scattering functions with their Fourier transforms and their memory functions, and the elastic incoherent structure factor. Additionally, the nMOLDYN package allows one to construct modified trajectories from an input trajectory; rigid-body trajectories, in which the internal motions of the molecules (or parts thereof) are eliminated, angular trajectories, which describe rigid-body motions by center-of-mass and orientational (quaternion) coordinates, frequency-filtered trajectories, from which motions outside a specified frequency interval are eliminated.


## Workalemahu Berhanu 3

*University of Oklahoma*
*wberhanu@chem.ufl.edu*


### Structure and dynamics of models of amyloid-β; segmental polymorphisms

Amyloid-beta (Ab) aggregates is considered to play a role in the pathogenesis of Alzheimer's disease. Ab molecules form b-sheet structures with multiple interaction sites within each Ab molecules forming various polymorphism. Ab polymorphism gives rise to differences in morphology, physico-chemical property and level of cellular toxicity. Eisenberg laboratory has determined the microcrystal structures of short, self-complementing

pairs of β-sheets (steric zippers) from segments of Aβ. Combining these atomic structures with previous NMR several fiber models have been proposed. We investigated the conformational stability of various segmental polymorphisms of Ab structures in solution using molecular dynamics simulations.
The structural comparison among the segmental polymorphism in aqueous environment shows the inter-sheet side chain-side chain interaction, hydrophobic interaction among the strands (β1 and β2) and salt bridges are important in stabilizing the aggregates. The segmental polymorph with smaller size of steric zipper shows a larger structural fluctuation while the one with larger size of steric zipper at the interface is very stable. Despite some difference in their structural stability the segmental polymorphic models of Ab the retained U-shaped architecture with smaller fluctuation in b-sheet region during the simulation showed. Residues at the edge and loop region showed higher mobility. The inter-peptide salt bridges between Asp23 and Lys28 were strong compared to intra-chain salt bridge and there is as exchange of the inter-chain salt-bridge with intra-chain salt bridge. The simulation showed the segmental polymorphs of Ab retain U-shaped architecture with smaller fluctuation in b-sheet region. Residues at the edge and loop region showed higher mobility. This knowledge of structural stability and aggregation behavior of Ab polymorphic forms may help to develop therapeutics for Alzheimer's disease.


## Nicholas Callahan 4

*Department of Chemistry, The Ohio State University*
*callahan.122@osu.edu*


### Mutual Information Study of Adenylate Kinase

In the course of protein evolution, the interactions between side chains are optimized for overall organism fitness. An enzyme must both be stable and fulfill its metabolic role within certain kinetic parameters. Because the preferred environment of organisms can vary widely across phylogenies, homologous enzymes can have similar backbones but different side chain packing, thereby fine-tuning interaction strength and dynamic mechanisms for environmental conditions. This evolution-driven varying of side chain packing can create positional correlations in multiple sequence alignments. It has been shown that these correlations may reflect stabilizing interactions, but do not necessarily do so. We present here correlation-guided mutations made in the adenylate kinase enzyme of Bacillus subtilis bacteria (bsADK) which, in the context of a destabilized variant, affect kinetic activity without altering stability. The folded Lid domain of bacterial ADK closes over the active site following substrate binding. This domain is stabilized by either a zinc-chelating motif in gram-positive species or by a network of hydrogen bonds in gram-negative species. Mutating the four chelating residues of bsADK to their counterparts in Escherichia coli ADK (ecADK), abolishing metalbinding, results in a severe drop in global stability and the loss of enzymatic activity. Using mutual information analysis, we located two positions in the lid domain strongly correlated to chelating motif. Further mutations were made in bsADK at these positions. These additional mutations were found to have little effect on global stability, but served to partially rescue enzymatic activity. Although it is understood that loss and gain of stability can directly alter the dynamics of adenylate kinase, the fact that these variants do not vary in stability suggests that they could serve as an experimental system for further studying the kinetic pathway of this enzyme. We are currently pursuing biophysical strategies to further characterize these variants and elaborate on their kinetic differences.

## Derek Cashman 5

*UT/ORNL Center for Molecular Biophysics*
*Oak Ridge National Laboratory*
*cashmandj@ornl.gov*


### Homology Modeling in the Twilight Zone: Can Molecular Dynamics or Monte Carlo Simulations Improve the Quality of Homology Models?

In order to assess the quality of homology modeling, models were constructed of the E. coli and T. maritime chemotaxis protein, CheW, two proteins with known NMR structures. A homology model of E. coli CheW was constructed based on its sequence aligned to the T. maritima NMR structure, and a model of T. maritime CheW was constructed based on its sequence aligned to the E. coli NMR structure. In other words, we are asking the question of whether it is possible to build a model of a protein of known structure using the template of a homologous protein, and then to use simulation methods to find the correct conformation of the original known protein that we are modeling. Three homology models of each protein were constructed and each structure was simulated in explicit solvent for 50 ns using the NAMD2 molecular dynamics software. Each of the three models was also simulated using the Library-Based Monte Carlo (LBMC) software package (Zuckerman et al., 2009). While the molecular dynamics simulations provide a good picture of the conformational fluctuations of the CheW protein in a time-dependent manner, the primary advantage of the Monte Carlo simulations lies in their random and time-independent nature, as well as in their ability to more rapidly sample the broad conformational space of proteins. Simulations were also performed using the starting NMR structures as a control. The root-mean-square deviation was calculated for each MD and LBMC trajectory individually, as well as between each individual structure in the homology-modeled trajectory versus each individual structure in the native trajectory. This measures the ability of each homology-modeled structure to "find" the native protein structure as well as the other homology-modeled structures in the conformational space and therefore provide an assessment of how closely each homology-modeled ensembles agree with the overall native protein ensemble. Our results indicate that there are significant fluctuations in the amine and carboxy termini of the proteins, both in the native and homology-modeled ensembles. However, when the protein core is aligne without these flexible regions, there is reasonably good agreement between the homology models and the native protein.


## Mihir Date 6

*Department of Chemistry, Clemson University*
*mdate@clemson.edu*


### Effect of salt on protein fold stability: Study through implicit as well as explicit solvation technique.

Solvent plays an important role in determining the structure and function of biological molecules. Hence an accurate representation of solvent is immensely essential for the accurate assessment of stability and dynamics of proteins and enzymes and their interaction with other solvated ions. Salt influences proteins and enzymes through the screening of intra and inter molecular electrostatic interactions as well as through Hofmeister effects. In this work, a continuum solvation model was developed to explore the impact of ionic strength on protein stability. This model combined a Poisson-Boltzmann equation for continuum electrostatic solvation forces with a surface area dependent term, containing a new salt concentration dependent microscopic surface tension function, to capture hydrophobic effects. The model was validated against experimentally determined salt effect data for cold-shock protein B and 27 of its mutants. It showed good qualitative as well as quantitative

agreement in matching experimental data. The approach was then applied to HIV-1 protease in order to explain the origins of its experimentally observed increased stability as a function of NaCl concentration. The effect of different ions in close vicinity to protein surfaces remains a valuable area of study, and may provide important information regarding protein folding and evolution. One way of capturing the Hofmeister effects on proteins is by quantifying how salts alter the association thermodynamics of waters and amino acids side chains on protein surfaces. Molecular dynamics simulations were performed on a model protein in a periodic box of TIP3P water molecules and Hofmeister salt ions at concentrations ranging from 0.5 to 3.0 mol/L. Radial distribution functions calculated for water molecules and salt ions and calculated coordination numbers between the protein and salt ions show an agreement between the rank for an ion in the Hofmeister series and its influence on solvation shell waters around the protein. On the other hand, the atom's positional correlation time calculated in different shells from protein surface and for different ions show that the rank of an ion has no correlation and influence on long range water structure making and breaking properties. With this result, one of the most important and new aspects of the influence of ions on proteins emerges as the salt impacts the structure of biological water and bulk water differently and the effect of salt on the later is not central to the Hofmeister effect.


## Samuel DeLuca 7

*Chemistry, Vanderbilt University*
*samuel.l.deluca@vanderbilt.edu*


### A Novel Method for Guiding High Throughput Protein-ligand docking with QSAR-Derived Pharmacophore Maps

Currently, QSAR and computational ligand docking studies are valuable but independently used tools for drug design. Data from Pharmacophore maps produced by tools such as COMFA are typically compared to the results of docking simulations by hand in a qualitative manner. RosettaLigand has been previously successful at predicting binding poses with high resolution (Kaufmann, et. al, Proteins, 2009). We are developing RosettaHTS, an extension to RosettaLigand which will integrate these two methods by using information from QSAR derived pharmacophore maps to guide the low resolution phase of ligand docking. Discrete cartesian grids describing the hydrogen bonding ability, electrostatics and shape of the ligand binding site are overlaid on the protein structure, and these grids are used to score the initial placement of the ligand prior to fine grained docking. Sampling of the ligand within this grid is guided by the chemical i As the scoring grids are precomputed, ligand scoring is extremely fast, and thorough Monte Carlo sampling of the ligand binding site can be rapidly performed before fine grained ligand docking using the high resolution Rosetta scoring function. This efficient and fast initial sampling makes it possible to distinguish between active and inactive compounds with less fine grained sampling, decreasing the amount of CPU time necessary to predict a single binding interaction, and increasing the practicality of structure based virtual High Throughput Screening (vHTS). The integration of structure based and ligand based vHTS techniques allows the full range of pharmacological information surrounding a target and drug scaffold to be considered in a single approach. This technique can be used to rapidly develop small focused libraries for High Throughput Screening, increasing the hit rate and decreasing the number of compounds that need to be purchased for testing.

## Sally R. Ellingson 8

*Genome Science and Technology and Center for Molecular Biophysics, UT/ORNL*
*sallyrose045@gmail.com*

### High-throughput Virtual Molecular Docking on High-Performance Computers

Many pharmaceuticals act by selectively binding to a specific protein and thus inhibiting a specific process relevant to a disease or illness. Because of this, the early stage of drug discovery consists of identifying potential compounds that bind to a protein of interest with a high affinity and specificity. Experimentally testing a very large number of these compounds is both costly and time consuming. Virtual high-throughput screening is an equivalent computational process that can reduce the time and cost of discovering new drugs. After a potential lead compound is identified in the drug discovery process further tests must be done in order to determine toxicity and side effects. Computational tools with the ability to virtually screen a lead compound against a very large number of different proteins to help predict these effects earlier in the drug discovery pipeline would be valuable. Here we discuss the screening of a million compound library on a petascale supercomputer and future directions to incorporate large libraries of proteins in high-throughput screens.

## Hao-Bo Guo 9

*UT/ORNL Center for Molecular Biophysics*
*Oak Ridge National Laboratory*
*guoh1@ornl.gov*

### Structural Modeling of the Outer Membrane Protein Igni1226 Nonamer of Ignicoccus hospitalis

Ignicoccus hospitalis, the smallest free-living Archaea, and Nanoarchaeum equitans, the smallest Archaea, form the smallest and the only purely archaeal symbiotic system known to date. N. equitans acquires metabolites and energy together with lipids and amino acids from its host, I. hospitalis. Previous studies suggested that an 85-residue protein Igni1226 may serve as the cell-to-cell transporter between the two Archaea. A recent electron tomography experiment showed that nine chains of Igni1226 form a nonamer spanning a 2 nm pore, through which materials such as the respiratory complexes and ions may transport among two Archaea and their environments. To understand how Igni1226 nonamer perform the transportation works, however, detailed structural knowledge would be demanded from either experimental or theoretical investigations, or both. In the present work, we constructed the undetermined, with yet unknown function Igni1226 pore structural model starting from its amino acid sequence. This model represents the electron tomography and reserves the predicted secondary structure within the presence of a membrane model. Molecular dynamics (MD) simulations of the Igni1226 nonamer model in the membrane environments, together with the normal mode analysis using the anisotropic network model (ANM), were performed to tackle the structural characteristics of the nonamer that may be correlated to the transportation between the two Archaea.

## Russell Hanson 10

*Harvard Medical School, Children's Hospital Boston*
*russellhanson@gmail.com*


### The use of immune epitope prediction and HLA subtype imputation to model cancer cell recognition by the immune system

It is well known that cancer is in part an immunological disease. In this study we present novel algorithmic and computational results for some of the largest cancer immunology studies done to date to predict and infer the immunological reactivity of different cancer point mutations as well as the susceptibility and protection status for different patient HLA subtypes. These studies are important for personalized medicine in that patients with different protected vs. susceptible types may need or require different treatment regimes. We present results across the largest cancer genome databases available for point mutations, HLA subtype imputations for from 3,000-4,000 patients, and indications for future work in the area of cancer immunology and immunome studies.


## Jason Harris 11

*Genome Science and Technology*
*University of Tennessee*
*jason.b.harris@gmail.com*


### Using high-performance supercomputing to find endocrine disrupters: A fast track to discovering new medicines and protecting the environment

Although endocrine disrupting chemicals (EDCs) are only a subset of the tens of thousands of chemicals known to mankind, their human health impacts can be enormous â"€ ranging from reproductive disorders, obesity, diabetes, immune dysfunction, neurodegenerative diseases, and increased incidences of some cancers (NIEHS 2010). Identifying EDCs occurs through expensive testing programs costing between $7,000 and $30,000 per chemical, yet there are still many chemicals to be tested that either already exist or are being synthesized on a regular basis (EDSTAC 1998). For this reason, it is critical to develop simple, fast and affordable experimental assays that can identify chemicals with endocrine disrupting activity before they. pose a health risk by entering the manufacturing stream. Moreover, new predictive models based on chemical binding to protein structures (e.g., protein docking), rather than models based only on the chemical structure (e.g., QSAR), are needed to identify undiscovered chemical-protein interactions with novel chemical features. The goal of this project is to build and validate a predictive protein-docking model that quickly, efficiently, and affordably identifies chemicals that bind directly to the human estrogen receptor alpha (hER-Î±) protein or indirectly through P450-mediated oxidation. Our multi-protein (hER-Î± and P450) approach integrates computational prioritization and experimental testing of compounds via computational docking models and activity measurements in bioluminescent-yeast-estrogen-receptor (BLYES) bioreporter assays. An additional advantage of this approach over previous methods such as QSAR (chemical similarity matching) is the ability to search for chemicals with novel binding modes and/or P450-mediated activity towards the hER-Î± protein.

## Andrew Hirsh 12

*Mechanical Engineering, University of Michigan*
*adhirsh@umich.edu*


### A Multiscale Model for T7 RNA Polymerase Initiation on Supercoiled DNA Minicircles

Because T7 RNA polymerase (RNAP) is a single subunit RNAP and shares biochemical characteristics with much larger and more complex multisubunit RNAPs, it remains a model system to study, mechanistically, fundamental aspects of polymerase function including transcription initiation, elongation, and termination. It is well known that many gene repression proteins can simultaneously bind two operator sites and tightly loop the intervening DNA, about 100 base pairs (bp). Not only do these DNA-repressor contacts interfere with transcription by directly blocking DNA, but the mechanics of the topologically constrained loop may also play an important role in repression. These factors, although not currently well understood, may affect T7-RNAP during initiation and prevent effective transition to the elongation phase. To understand how T7-RNAP initiation responds to highly stressed DNA, we construct a model for the RNAP complexed with a supercoiled (~100 bp) DNA minicircle. The current model represents the minicircle DNA with a continuum elastic rod model which relies heavily on boundary conditions derived from an atomistic model of DNA entering/exiting the RNAP. Preliminary results investigate two sets of boundary conditions derived from the rigid crystal structure and reveal significant differences depending on the boundary conditions. Therefore, in this study we describe a multiscale modeling approach in which the elastic rod model provides the conformation of the highly-stressed minicircle DNA as the initial condition to an all-atom MD simulation which accounts for the flexibility of the RNAP with the explicit goal of determining superior boundary conditions for further modeling efforts.


## Liang Hong 13

*UT/ORNL Center for Molecular Biophysics*
*Oak Ridge National Laboratory*
*hongl@ornl.gov*


### Combining molecular dynamics simulation with incoherent neutron scattering to illustrate the hydration effect on internal protein motions

Internal motions are crucial for protein function and folding, and depend on various external parameters, among which, hydration is of particular importance. Dehydrated enzymes lose bio-activity and the so-called dynamical transition, manifesting as an anharmonic onset in the mean-squared atomic displacement around 180-230 K, occurs only in hydrated proteins. Despite decades of study, the detailed microscopic mechanism of how water boosting the protein dynamics is still lacking. By combining incoherent neutron scattering and molecular dynamics simulation, the present work decomposed the internal motions of lysozyme on the ps-ns time scales into three components: localized single-well diffusion, methyl group rotation and non-methyl jumps, and showed that whereas methyl-group rotation is hydration independent and non-methyl jumps have weak hydration dependence, the localized diffusion is significantly boosted by hydration, manifesting as increase of diffusional amplitude. Further analysis revealed that the hydration effect on localized diffusion of protein atoms occurs on both the protein surface and in its dry core. This phenomenon can be attributed to the fact that these diffusive motions are strongly correlated, thus enabling the hydration effect to propagate into the protein interior.

## Ping Jiang 14

*Department of Chemistry and Biochemistry*
*University of Oklahoma, Norman, OK*


### Modeling Structural Flexibility of Proteins with Go-Models

Structure-based model is an efficient tool of folding of proteins, as by construction their energy landscape is minimal frustrated. However, their intrinsic drawback is a lack of structural flexibility as usually only one target structure is employed to construct the potentials. Hence, a Go-model may not capture differences in mutation-induced protein dynamics, if - as in the case of the disease-related A629P mutant of the Menkes protein ATP7A - the structural differences between mutant and wild type are small. We will introduce three implementations of Go-models that take into account the flexibility of proteins in NMR ensemble. Comparing the wild type and the mutant A629P of the 75-residue-large 6th domain of Menkes protein, we find that these new Go-potentials lead to broader distributions than Go-models relying on an arbitrarily chosen single member of the NMR ensemble. This allowed us to observe in our simulations the transient unfolding of a loosely formed β1β4-sheet in the mutant protein. The result is consistent with that of our previous simulations using physical force field in explicit solvents, and suggests a mechanism by which this mutation causes Menkes disease. In addition, the improved Go-models also suggest differences in folding pathway between wild type and mutant, an observation that was not accessible in simulations of this 75-residue protein with a physical all-atom force field and explicit solvent.


## Alexander Johs 15

*Environmental Sciences Division*
*Oak Ridge National Laboratory*
*johsa@ornl.gov*


### Structural characterization of intramolecular interactions of a metallochaperone domain in the mercuric reductase MerA

Many microbes possess the ability to deal with heavy metal toxicity through elaborate metal resistance mechanisms. One well known example, bacterial mercury resistance, is mediated by the mer operon, which encodes specific genes involved in the transfer and transformation of toxic Hg(II) species. The mercuric reductase MerA is a key component of the mer operon. MerA is an NADPH-dependent flavin-disulfide oxidoreductase and catalyzes the reduction of Hg(II) to Hg(0), which is relatively inert and passively diffuses from the bacterial cell. Here, we combine experimental biophysics and computer simulation techniques to investigate structural features important for Hg(II) transfer in MerA. All MerA proteins consist of a homodimeric catalytic core domain, and many also possess an N-terminal metallochaperone-like domain NmerA, which is tethered to the core by a ~30 amino acid linker of unknown fold. Prior studies using separately expressed NmerA and core domains showed that NmerA acquires Hg(II) from other mer proteins such as the organomercurial lyase, MerB, and the membrane transport protein, MerT, and delivers it to the MerA catalytic core for reduction. Here, we have applied small-angle X-ray scattering (SAXS), small-angle neutron scattering (SANS) and molecular dynamics simulations to characterize the interactions of NmerA and the core in full-length MerA in solution. Our data reveals the extent of spatial sampling of the two NmerA domains relative to the homodimeric catalytic core and identifies the inter-domain docking orientation that occurs during transient handoff of Hg(II) from a pair of cysteine residues on NmerA to a pair of cysteines on the C-terminus of the catalytic core.

**Andrea Kravats 16**
*Department of Chemistry, University of Cincinnati*
*akravats@gmail.com*

## Computer simulations of ATP-driven protein remodeling by the ClpY biological nanomachine

Selective destruction of misfolded proteins by the protein quality control system is critical to maintain cell viability. In bacteria, these activities are carried out by members of the AAA+ superfamily (ATP-ases associated with diverse cellular activities), known as Clp ATP-ases. We focus on ClpY, an unfoldase within this family which is well characterized crystallographically. ClpY forms a homohexameric ring structure with a narrow central pore which fluctuates between "open" and "closed" conformations of 18Å and 8Å, respectively. ATP hydrolysis induces large scale conformational changes of flexible central pore loops with a highly conserved GYVG motif. Substrate unfolding and translocation are effected using multiple cycles of ATP-driven allostery within the ClpY ring. Due to uneven binding affinity of subunits, ATP binding within ClpY is asymmetric. Experiments suggest non-concerted allostery; however, it remains unclear whether an ordered or random mechanism is favored. To elucidate the coupling between ClpY-assisted unfolding and translocation of a substrate protein (SP), we developed a coarse grained model of ClpY and a four helix bundle SP (1). We determined that unfolding and translocation occur on distinct timescales. Unfolding is achieved by unraveling from the C-terminus of the tagged SP, forming an obligatory non-native intermediate structure, a three-helix bundle. Although this intermediate structure is competent for initiation of translocation, we observe multiple translocation pathways following the initial unfolding event. Large portions of helical secondary structural elements are translocated simultaneously, indicating a powerstroke mechanism. These results are consistent with recent single molecule experiments suggesting discrete steps in translocation of a SP.

To investigate the effect of sequential intra-ring allosteric motions of ClpY, we performed Langevin dynamics simulations of ordered (clockwise and counterclockwise as determined on the proximal side of the pore) and random allostery. Our results suggest that clockwise intra-ring transitions are most efficacious in handling the substrate, successfully passing it from active loop to the next active loop during each allosteric transition (2). This results in the most effective SP translocation. In addition, we find the minimum requirement for unfolding and translocation is four active subunits, as predicted by experiments.

1. A. Kravats, M. Jayasinghe, G. Stan, Proc. Natl. Acad. Sci. USA 108, 2234-2239 (2011).
2. A. Kravats, S. Tonddast-Navaei, R. Bucher, G. Stan. Manuscript in preparation.

## Pawel Krupa 17

*Molecular Modeling*
*University of Gdansk, Poland*
*pawel.krupa@tlen.pl*


### Optimizing the time step in coarse-grained force field (UNRES) molecular dynamics simulations.

Force fields based on physical interactions are commonly used to perform all-atom simulations. This intuitive approach has many advantages; however, it enables us to investigate only small systems. The time step in these classical MD simulations should be smaller than the fastest vibration in the system; a consensus value is 1 fs. However, this value can be increased when the SHAKE/LINCS algorithms are used to constrain bond lengths and/or when the multiple-time step algorithms are applied. Typically, several million steps of molecular dynamics (MD) simulation per day can be run for a medium-size protein with explicit solvent, which translates into several nanosecond time of simulation, which is too short to observe the most interesting properties of proteins, as e.g. folding. However, use of dedicated machines, such as, e.g. ANTON increases this time even to milliseconds. One way to increase the time- and length-scale of simulations is to use coarse-grained force field. Such an approach not only reduces the CPU time per step (by orders of magnitude) because of reducing the number of interactions, but also enables us to use a greater time step. Because of reducing the representation of the system under study, the optimal time step is no longer related to the period of atom vibrations.
The purpose of our work was to determine the optimal length of the time step and to investigate the influence of time step on ensemble averaged calculated with the use of Replica Exchange Molecular Dynamics (REMD) and Multiplexing REMD simulations of the three small model proteins: 1BDD, 1L2Y, 2HEP. We used two different parameterizations of the UNRES force field and two algorithms to control the stability of the MD algorithm, namely the Variable Time Step (VTS) and Adaptive Multiple Time Step (A-MTS). The Weighted Histogram Analysis Method (WHAM) was used to calculate ensemble averages (average RMSD from the experimental structure and heat capacity) from the results of the simulations. We found that the time step can easily be increased to 10 fs and to 15-20 fs with the use of the A-MTS algorithm.


## Jianing Li 18

*Chemistry, University of Chicago*
*jianingli@uchicago.edu*


### Molecular dynamics insights into human adenosine A2A receptor activation and deactivation

The human adenosine A2A receptor (AA2AR) is a typical class A GPCR modulated by organic compounds acting as agonists or antagonists. Recently AA2AR ligands have been extensively tested as potent agents to treat a variety of diseases, but dynamic details of the ligand-dependent activation and deactivation are still incomplete. Using crystal structures of AA2AR in complex with three agonists and three antagonists, we constructed the membrane protein models and performed unbiased molecular dynamics and metadynamics simulations totaling over 5 μs. We have captured the distinct structural characteristics of the active and inactive states at atomic detail, including several key domains changing in a highly concerted manner. In particular, six conformational states of Trp246 induced by ligands with various efficacies have been revealed. Our findings suggest that during activation, Trp246 undergoes a rotameric transition, causing a series of coherent conformational changes to open the G-protein-binding site. Further metadynamics simulations have showed quantitative evidence for this mechanism, indicating that agonists and antagonists change the relative energy

and shift the equilibrium between the active and inactive states. Our analysis also identified a number of crucial residues in the ligand binding modes, enabling structure-based design targeting three distinct regions of the ligand-binding pocket. Generally our study provides a comprehensive picture of AA2AR-ligand interactions as well as dynamic insights of AA2AR activation and deactivation beyond crystal structures, shining a light on the path to design more effective and selective AA2AR ligands.


## Peng Lian 19

*UT/ORNL Center for Molecular Biophysics*
*Oak Ridge National Laboratory*
*penglian518@gmail.com*


### QM/MM Study on the Catalytic Mechanism of Cellulase TmCel12A

The efficiency of cellulase is the bottleneck of cellulosic ethanol production. Finding of cellulase 12A from Thermotoga maritime (TmCel12A) makes it possible to accelerate hydrolysis process via increasing the reaction temperature up to as high as 95 °C. However, atomic details of catalytic mechanism and hyperthermophilic nature of TmCel12A were previously unknown. In this study, free energy profile of the catalytic reaction was explored using umbrella-sampling method coupled with QM/MM (SCC-DFTB/MM) simulations. The retaining mechanism was confirmed, and free energy barrier for glycosylation and deglycosylation were 22.3 and 23.8 kcal/mol, respectively. In both steps, the glucose ring at position -1 inversed via E3 or 4H3 conformers. The charge population analysis suggested the existence of oxocarbonium in both transition states. A special character of TmCel12A is the low-barrier hydrogen bond (LBHB) between E116 and general base E134. This hydrogen bond decreases the energy barrier in deglycosylation step at the cost of increasing that of glycosylation. However, like other cellulases, the deglycosylation is still rate-limit step. Based on these findings we propose that the fact E116 interacts with E134 through LBHB may be an adaptation to the hyperthermophilic nature of TmCel12A.


## Benjamin Linder 20

*UT/ORNL Center for Molecular Biophysics*
*Oak Ridge National Laboratory*
*ben@benlabs.net*


### Calculation of Scattering Intensities from MD Simulation of Cellulose using a Massively Parallel Computer

Cellulose is a major component in the plant cell wall. It's abundance in nature makes it a prime target for sustainable production of biofuels. The crystalline quality of cellulose in nature can vary greatly and it shows strong correlation with it's recalcitrance to hydrolysis. Scattering experiments allow the structural and dynamical characterization of bulk cellulose samples. The calculation of scattering from molecular dynamics simulations allows to reconcile experiments with theoretical models of cellulose. Scattering calculations for biologically relevant samples, like cellulosic biomass, require enormous computational power, because the scattering intensities have to be orientationally averaged and/or a significant number of time points have to be analyzed. This requirement motivated the development of the software Sassena, which implements efficient parallel algorithms to compute the scattering intensities on a massively parallel computer in a timely manner. The software is shown to scale efficiently up to thousands of cores for different types of scattering calculations.

## Yanxin Liu 21

*Physics, University of Illinois, Urbana-Champaign*
*yliu@ks.uiuc.edu*


### Folding of a five-helix bundle protein from all-atom molecular dynamics simulations

The five-helix bundle lambda-repressor fragment is a fast-folding protein. A length of 80 amino acid residues puts it on the large end among all known microsecond folders and its size poses a computational challenge for molecular dynamics (MD) studies. We simulated the folding of lambda-repressor fast-folding mutants in explicit solvent using an all-atom description. By means of a recently developed tempering method, we observed reversible folding and unfolding of lambda-repressor in a 10-microsecond trajectory. Starting from an extended conformation, the folding kinetics was also investigated through constant temperature MD simulation with more than 100 microseconds duration. The protein was seen to fold into a native-like topology and a slow-folding pathway was identified. The simulation starting from high-temperature and high-pressure denatured state folded the protein in 30 microseconds, which confirmed the existence of the T-jump and P-jump induced fast-folding pathway. Our results also suggest new experimental observables for better monitoring the folding process, and a novel mutation expected to accelerate lambda-repressor folding


## Buddhadev Maiti 22

*Department of Chemistry*
*Georgia State University*
*iivanov@gsu.edu*


### Effect of Mutations and Calcium Ions Concentration on Connexin26 Hemichannel and Gap Junction Channel

Connexins or gap junction proteins, are a family of structurally related hundreds of intercellular communication channels that allow the passage of molecules such as ions, metabolites, nucleotides and small peptides. Each gap junction channel is composed by end-to-end docking of two hemichannels which are referred to as connexons. Each hemichannel constructed by six subunits with four transmembrane helices and two extracellular loops. Gap junctions have crucial roles in many biological processes including development, differentiation, cell synchronization, neuronal activity and immune responses. Mutations in connexins thus cause several human diseases, including neurodegenerative diseases, skin diseases, deafness and developmental abnormalities. In this context, we used all atom molecular dynamics (MD) simulations in an explicit solvent POPC membrane system for connexin26 hemichannel and gap junction channel to show the effect of conformational change by the mutation and calcium ion concentration. Our MD simulations provided the effect of calcium binding sites during mutations

## Yinglong Miao 23

*UT/ORNL Center for Molecular Biophysics*
*Oak Ridge National Laboratory*
*miaoy@ornl.gov*


### Coupled Dynamics Change in Cytochrome P450cam Substrate Binding Determined by Neutron Scattering, NMR and Molecular Dynamics Simulation

Neutron scattering and nuclear magnetic resonance (NMR) relaxation experiments are combined with molecular dynamics (MD) simulations in a novel approach to investigate the change in internal dynamics on substrate (camphor) binding to a protein (cytochrome P450cam). The MD simulations agree well with both the neutron scattering, which furnishes information on global flexibility, and the NMR data, which provide residue-specific order parameters. Decreased fluctuations are seen in the camphor-bound form using all three techniques, dominated by changes in specific regions of the protein. The combined experimental and simulation results permit a detailed description of the dynamical change, which involves modifications in the coupling between the dominant regions and concomitant substrate access channel closing, via specific salt-bridge, hydrogen-bonding and hydrophobic interactions. The work demonstrates how the combination of complementary experimental spectroscopies with MD simulation can provide an in-depth description of functional dynamical protein changes.


## Magdalena Mozolewska 24

*Molecular Modeling, University of Gdansk, Poland*
*magdalena.mozolewska@gmail.com*


### Molecular modeling of the interactions in the yeast Ssq1 - Jac1 - Isu1 system in the context of iron-sulfur cluster biogenesis.

Molecular modeling of the interactions in the yeast Ssq1 - Jac1 - Isu1 system in the context of iron-sulfur cluster biogenesis. Yeast proteins Isu1 (Iron Sulfur protein) and Jac1 (J-protein) are part of huge ATP system and both interact with the Ssq1 molecular chaperone. In bacteria, the equivalent proteins are IscU (for Isu1) and HscB (for Jac1), respectively. Isu1 has influence on iron homeostasis in the mitochondrion where it is involved in assembling of iron-sulfur proteins. It can also be involved in the repair of iron sulfur clusters. Jac1 is involved with Hsp70 and Isu1 in Fe-S cluster biogenesis in mitochondria. The iron-sulfur clusters are the most ancient co-factors of proteins involved in many essential processes such as catalysis and electron transfer. The release of the Fe/S cluster from Isu1, and its transfer and incorporation into recipient apoproteins (Apo) is facilitated by late components of the ISC assembly machinery including the ATP-dependent Hsp70 chaperone Ssq1, the DnaJ-like cochaperone Jac1, the nucleotide exchange factor Mge1, and the monothiol glutaredoxin Grx5. The disturbances of the balance of these processes can have very serious and dangerous consequences. In Homo sapiens, such disturbances can cause serious diseases such as cerebellar ataxia, myopathy, Friedreich's ataxia, microcytic anemia, tumor suppressor.

The aim of this work was to model the tertiary structure of Isu1 and to investigate the interactions between Isu1 and Jac1, which may be crucial to understand the machinery of yeast mitochondrial chaperone system. We also wanted to obtain support of the results of earlier experimental studies, which suggest that Jac1 interacts with Isu1 mainly by Leu105, Leu109 and Tyr163. WTo accomplish this, we modeled and assessed the stability of complexes of mutated Jac1 with Isu1, where we mutated the putative binding Isu1-binding residues of Jac1. To carry out simulations at a longer time scale, we used our coarse-grained UNRES force field. We used I-TASSER server and YASARA as tools to model, by means of homology modeling, the tertiary structure of Isu1. Docking of Isu1 to Jac1 was carried out using the ZDOK server. The structures of the proteins and complexes were

optimized by molecular dynamics (MD) simulations with explicit water. Analysis of interactions between Isu1 and Jac1 provides information about the residues playing crucial role in protein binding and determines the most probable conformations of the proteins.

## Sayak Mukherjee 25

*Nationwide Children's Hospital*
*Battelle Center for Mathematical Medicine, Columbus, Ohio*
*sayak.mukherjee@nationwidechilderns.org*

### Robustness of the Cell Signaling Network as a Means to Discriminate Among the Different Models of Itk Kinase Regulation in T cells.

The process that warrants the generation of self-tolerant peripheral T cells is called the thymocyte selection. During this maturation process, the overtly self reactive as well as un- responsive thymocytes are deleted from the cell population. The thymocytes equipped with T cell receptors (TCRs), capable of responding moderately to the self peptides are allowed to survive. Recently water soluble second messenger, inositol(1,3,4,5) tetrakisphosphate (IP4), has been implicated to play a crucial role in thymocyte positive selection (Huang et al.). It has been suggested that these IP4 molecules regulate the transient activation of the Tec- family protein tyrosine kinase Itk through a competing positive and negative feedbacks. The exact molecular mechanism involved in this feedback is however unclear. It is possible to con- struct more than one model with contrasting molecular mechanisms to explain the present body of experimental observations. This calls for criteria to choose among these models. Robustness in face of the variation of the parameters in a model has been ubiquitously used as a criterion for model discrimination. Here we have used the maximum entropy, calculated with the constraints imposed by the experiments as a measure of robustness. Our data indicates that the models which are maximally robust share a cooperative allosteric mode of Itk regulation involving dimeric PH domains.

## Hai Nguyen 26

*Department of Chemistry, Stony Brook University*
*hainm.comp@gmail.com*

### Improved Generalized Born Solvent Model Parameters for Protein Simulations

The generalized Born (GB) model is one of the fastest implicit solvent models and it has become widely adopted for Molecular Dynamics (MD) simulations. This speed comes with tradeoffs, and many reports in the literature have pointed out weaknesses with GB models. Because the quality of a GB model is heavily affected by empirical parameters used in calculating solvation energy, here, we have refit these parameters for GBNeck, a recently developed GB model, in order to improve the accuracy of solvation energy and effective radii calculation. The data sets used for fitting were significantly larger than those used in the past. Comparing to other pairwise GB models like GB-OBC and GB-Neck, the new GB model (GBNeck2) has better agreement to Poisson-Boltzmann (PB) in terms of reproducing solvation energies for a variety of systems ranging from peptides to proteins. Secondary structure preferences are also in much better agreement with explicit solvent simulations, as are protein MD simulations. We also obtain near-quantitative reproduction of experimental structure and thermal stability profiles for several model peptides.

## Gungor Ozer 27

*Department of Chemistry*
*Boston University*
*gungor@bu.edu*


### Enhanced sampling through the use of adaptive steered molecular dynamics and statistical temperature molecular dynamics

Conformational sampling of important events of biomolecules---such as folding and binding---using all-atom molecular dynamics has always been a challenge as in most cases atomistic-scale calculations is cost-prohibitive to observe such events in feasible simulation windows. In this context, many enhanced molecular dynamics algorithms have been developed to compensate for the substantial amount of computation including but not limited to steered MD (SMD) [J. Chem. Phys. 120, (2004)] and statistical temperature MD (STMD) [J. Chem. Phys. 126, (2007)]. The study presented herein aims to explore the foundations of these powerful algorithms as to develop and benchmark novel methodologies: i) SMD---in conjunction with Jarzynski's equality (JE)---is widely used to study energetics of the unfolding and binding/unbinding of proteins assuming prior knowledge of the reaction pathway. A staged integration of the SMD alogrithm, adaptive steered molecular dynamics (ASMD) [J. Chem. Theory Comput. 6, (2010)], has been recently formulated in which the reaction pathway is divided into stages so that the work distribution always exhibit good statistics (Gaussian nature) and the resulting PMF better represent the simulated nonequilibrium ensemble. ASMD is further explored, enhanced and benchmarked on several systems such as helix-coil transformation of decaalanine in vacuum and in explicit solvent. ii) STMD is a powerful flat energy distribution algorithm that updates statistical temperature estimate at each iteration. Generalized replica-exchange has also been recently integrated with the STMD method. STMD has been incorporated onto popular and fast MD integrators, CHARMM and NAMD, as to study more complex systems. STMD is currently being tested on CHARMM and NAMD on various previously benchmarked unfolding events of ww-domain, albumin-binding domain, and neuropeptide Y.


## Pincu Medeliene 28

*Department of Chemistry, University of California, Irvine*
*mpincu@uci.edu*


### Reactions of Cellobiose and Ionic Clusters

Ab initio molecular dynamics (DFT / BLYP with dispersion) is used to analyze the interaction between conformers of cellobiose (CB), cis and trans, with single proton and with micro-hydrated clusters of acids of different strengths, at ~300K. These are analyzed in terms of probabilities for proton transfer, degree of ionization as compared with that of the acids alone in the water cluster. Detailed information on the preferred protonation site of cellobiose and the competition between the sugar and water protonation are presented. The importance of conformer selectivity of reaction processes is discussed.

## Xianghong Qi 29

*UT/ORNL Center for Molecular Biophysics*
*Oak Ridge National Laboratory*
*xqi@utk.edu*


### Swimming motility is a key role in describing the cell clumping phenomenon

Flagellum swimming motility has been shown to modulate the initial interaction of bacteria with surfaces or with other cells and contribute to the emergence of macroscopic patterns. While the role of swimming motility in surface colonization has been analyzed in some detail, a quantitative physics analysis of transient interactions between motile cells is lacking. We present a physical model based on the motile bacterium Azospirillum brasilense to examine the dynamics of cells in a crowded environment. Especially, the effect of the swimming motility of the cells on the cell clumping is studied using simulations of motorized adhesive Brownian particles subjected to hydrodynamic interaction. We incorporate both equilibrium and active features, such as mechanical interaction (¡°stickiness'¡±) between cells, thermal noise, and swimming velocity into our model and investigate the clumping dynamics under several environmental and intrinsic factors. Our results show that the modulation of active motions of the cells is required for the initial aggregation of cells to occur at a realistic time scale. Slowing down flagellar motor rotation (and thus swimming speed) is correlated to the degree of clumping, which is consistent with the experimental results obtained for A. brasilense.


## Andrej Savol 30

*Computational and Systems Biology*
*University of Pittsburgh*
*ajs171@pitt.edu*


### Conformational Substate Discovery for Fast Folders

The kinetic relationship between metastable conformational intermediates and protein folding rates has challenged both computational and experimental methods. The present paradigm recognizes a rough free energy landscape (FEL) with minima corresponding to stable states and conformational transitions as energetic barriers. However, exceptions to this simple abstraction are necessary for both intrinsically disordered proteins (IDPs), whose FELs are necessarily 'flatter', and also 'ultra-fast' folders, which lack significant energy barriers during folding. Long-run all-atom simulations are increasingly capable of accessing the timescales required to observe folding events. Our approach provides the needed subsequent analysis: what conformational states were accessed, how long were they visited, and which ones facilitated or inhibited transition to the native state? We studied long molecular dynamics simulations of villin headpiece (VHP), one such 'ultra-fast folder', and identified non-native metastable states that function as waypoints within diverse folding trajectories. Rather than adopt structural similarity measures (RMSD, Rg) to probe structural transitions, we considered an embedded dihedral angle subspace where structural alignment bias is eliminated and overall dimensionality is drastically reduced. As documented, more dihedral-based modes are necessary to capture an equal quantity of variance as its Cartesian-based counterpart, but these internal (dihedral) modes provided excellent sensitivity to near-folded intermediates and rarely visited states. We additionally observed that commonly used folding order-parameters overlooked non-native contacts, even when such 'misfolding' is reproducibly necessary for achieving the eventual native state. Taken together, our results address several organizing principles for the conformational landscape of fast-folders.

## Michael Schneiders 31

*Biomedical Engineering*
*University of Texas, Austin*
*michael.schnieders@gmail.com*


### Refinement of DNA and Protein Structures from Neutron Crystallography Experiments: The Importance of Computational Biophysics

Oak Ridge National Laboratory is recognized as the world's leading center for Neutron Science due to the combination of the Spallation Neutron Source (SNS), High Flux Isotope Reactor (HFIR) and the Oak Ridge Electron Linear Accelerator Pulsed Neutron Source (ORELA). In particular, the SNS provides the most intense pulsed neutron beams in the world for performing neutron diffraction experiments on biological crystals. Unlike X-ray diffraction, the diffracted intensity of neutrons from hydrogen and deuterium atoms is as strong as that from heavier organic elements. Therefore, neutron diffraction is complementary to X-ray diffraction and provides critical information on protonation state, tautomerization and hydrogen-bonding networks. In this presentation, I'll discuss the joint X-ray/neutron refinement of DNA and protein crystals assisted by the prior chemical information in the polarizable atomic multipole AMOEBA force field evaluated using particle-mesh Ewald (PME) summation. As CBSB12 brings together leaders in neutron diffraction, force field development and high-performance electrostatics algorithms, this meeting represents an ideal opportunity to discuss the role of computational biophysics in the application and interpretation of neutron diffraction experiments.


## Roland Schulz 32

*UT/ORNL Center for Molecular Biophysics*
*Oak Ridge National Laboratory*
*roland@utk.edu*


### Molecular Dynamics Simulations of Lignocellulose on a Petascale Computer

The understanding of biomass recalcitrance is central to the efficient production of 2nd generation bio-ethanol. The performance and scaling of molecular dynamics (MD) simulation of multimillion-atom biological systems are important for the simulation of realistic models of biomass. In the era of petaflop supercomputers, such simulations are limited by the parallel efficiency of the MD algorithms. The bottleneck for highly parallel all-atom simulations is the computation of the electrostatic interactions. Our performance results show improved scaling for both reaction field and particle mesh Ewald and the importance of threading, IO, and load-balancing. The simulations show the mechanism of lignin collapse at low temperatures, and the origin of the preferred aggregation of lignin with crystalline cellulose.

## Ester Sesmero 33

*Chemistry and Biochemistry*
*University of Maryland, Baltimore*
*sesmero1@umbc.edu*


### Using Dynamic Importance Sampling to Explore Conformational Space in HCV Polymerase

Hepatitis C virus (HCV) is a wide spread health concern and causes approximately 35,000 new infections in the U.S. each year. Though there are treatments available, they cause many unpleasant effects and are not completely effective. HCV contains a positive sense single-stranded RNA genome and replicates with the aid of RNA dependent RNA polymerase (RdRp). This polymerase is known to have two different conformations: an open inactive conformation and a closed active conformation. Only the open conformation have been seen with an inhibitor bound. Our goal is to understand how this transition occurs in order to determine how allosteric inhibitors stop the replication of HCV. These inhibitors are termed "allosteric" because they bind to the enzyme at locations other than the active site. To accomplish this goal we employ the Dynamic Importance Sampling Algorithm (DIMS). DIMS is a pathway finding algorithm that gives information about the intermediate states between defined starting and ending points. In our case of study this starting and ending points are the coordinates of the open and close conformations obtained from the MD simulations we performed of RdRp previously. The DIMS algorithm will allow us to sample the conformations of intermediates between the open and close conformations so we can get a better understanding of how this transition takes place, what motions facilitate the transition and what role it plays in enzyme inhibition.


## Piotr Setny 34

*Department of Physics, Technical University of Munich, Germany*
*piotr.setny@tum.de*


### Protein-RNA docking with coarse grained force field

It is becoming increasingly evident that functional, non-coding (nc) RNAs play important roles in multiple cellular activities, such as RNA processing and modification, protein trafficking, chromosome maintenance or enzymatic catalysis. To perform their function, ncRNA molecules typically unite with protein partners, making ribonucleoprotein complexes. Structural insight into such assemblies is essential for our understanding of their mechanism of action and the ability to design new therapeutic strategies, yet the available structural data is still relatively sparse. Computational docking methods can complement particularly demanding ribonucleoprotein X ray crystallography and provide means for the refinement and integration of low resolution data coming from rapidly advancing methods such as cryoelectron microscopy.

We will present a new coarse-grained forcefield for protein-RNA docking. It is implemented within the framework of ATTRACT program, widely used for protein-protein docking. Complex structure prediction is based on energy minimization in rotational and translational degrees of freedom of binding partners, with possible extension to include structural flexibility. The coarse-grained representation allows for fast and efficient systematic docking search without any prior knowledge about complex geometry. The method gives very good results when both binding partners are submitted in their bound conformations, and reasonable predictions for unbound geometries. We will present different approaches to account for structural flexibility of the binding partners and show how they can improve predictions in unbound docking scenario.

**Kelly Theisen 35**
*Chemistry, University of Cincinnati*
*hainesky@mail.uc.edu*

## Multiscale modeling of the nanomechanics of microtubule protofilaments

Several microscopy studies have shown that microtubule (MT) depolymerization, which takes place on a milliseconds to seconds time scale, starts with the outward curling of protofilaments, forming "ram's horn" structures (1). The depolymerization products include circular structures formed by collections of many tubulin dimers (1,2), which may be caused by non-motile kinesins, such as kinesin-13(3). Conversely, MT severing by AAA-ATPases appears to result in the removal of a single tubulin dimer (4). We used molecular simulations of a self-organized polymer (SOP) model (5) of an MT protofilament to quantify structural changes associated with the stretching and bending that accompany protofilament depolymerization. Our simulations reveal that substantial bending of the protofilament can occur in both the outward direction, and towards the interior of the MT cylinder. Also, atomistic simulations revealed that the thermal bending of protofilaments has no directional preference (6). Most importantly, we found that only the bending in the outward direction leads to the detachment of long protofilament fragments, which suggests a mechanism for kinesin-13 (3). In contrast, interior bending leads to fragmentation of the protofilaments into individual tubulin dimers, which agrees well with the proposed mechanism of MT severing proteins (4). If multiple attachment point are used (7), the magnitude of the force required to sever the protofilaments compares well with the experimentally observed force applied by severing proteins (3,8).

**1.** Mandelkow, E., et al., 1991. J. Cell Biol., 114:977 **2**. Nogales, E., et al., 2003. Curr. Opin. Struct. Biol., 13:256 **3**. Moores, C., et al., 2002. Molec. Cell, 9:903 **4**. Diaz-Valencia, J., et al., 2011. Biophys. J., 100:2440 **5**. Hyeon, Changbong et al., 2006. Structure, 14:1633 **6**. Grafmuller, A. and Voth, G.A., 2011. Structure, 19:409 **7**. White, S., et al., 2007. J. Cell Biol., 176:995 **8**. Maier, B., et al., 2002. Proc. Natl. Acad. Sci. USA, 99:16012

## Sam Tonddast-Navaei 36

*Department of Chemistry, University of Cincinnati*
*tonddasm@mail.uc.edu*


### Substrate protein remodeling by the p97 nano-machine: Computational studies

The p97/VCP nanomachine, a double ring member of the AAA+ superfamily, is involved in substrate protein (SP) unfolding within the proteasomal degradation pathway. P97 has a homo-hexameric structure with two nucleotide binding domains, D1 and D2, per subunit that encloses a central pore. ATP hydrolysis leads to large scale conformational changes in D2 domain, which affects the topology of its pore. Conserved loops at the entrance of D2 pore are suggested to enable SP propagation through the pore via ATP-driven "paddling motion" of Trp551 and Phe552 residues. Two other essential residues inside the D2 pore, Arg586 and Arg599, contribute to the p97 function. However, it is unclear how p97 interacts with its substrate. We hypothesize that the SP, which enters through the D1 pore, binds to the Arg599 sites on the D2 cavity lining. Then, repetitive ATP-driven cycles of p97 mediate the complete translocation of the SP into the D2 pore.
To test this hypothesis, we perform implicit solvent simulations of the ssrA-ssrA peptide threading through p97. Our results confirm the role of Arg599 residues as the binding sites in the ATP bound state. These simulations reveal that these Arginines interact with the substrate primarily via electrostatic interaction with the peptide's backbone, indicating an interaction independent of the SP's sequence. By contrast, in the ADP bound state Glu554 has the primary role in releasing the SP by narrowing the pore radius which weakens the SP-Arg599 interaction (1). Using the results from implicit solvent simulations, we develop a coarse-grained model that extends our simulations to biologically relevant timescales. We investigate the coupling between ATP-DRIVEN conformational changes in the D2 domain of the p97 and remodeling of the SP ( four helix bundle protein fused with ssrA). The mechanism of binding to Arg599 and releasing followed by the force exerted by the D2 loops allows the unfolding and translocation of the SP through the pore (2).

**1**.S. Tonddast-Navaei, G. Stan (manuscript in preparation) **2**.S. Tonddast-Navaei, A. Kravats, G. Stan (Manuscript in preparation)


## Sahin Uyaver 37

*Istanbul Commerce University Vocational School Istanbul Turkey*
*suyaver@iticu.edu.tr*


### Monte Carlo Simulation of a Polyelectrolyte Chain with Permuted Charge Distribution

The effect of a permuted charge distribution of a polyelectrolyte (PEL) chain is investigated by Monte Carlo simulations. The cascade transition of the PEL chain is studied at various charging degrees and in different solvent regimes. The scaling laws are applied. The effect of permuted charge distribution is investigated in comparison to the other charge distribution in the literature. It is observed that very compact structures occur in poor solvent regimes if the system is poorly charged. For some moderate charged case, globular structure is deformed, the charges are moved to a tail occurring. For higher charged case, PEL chain is quite stretched. The effect of permuting charge distribution is observed that pearl-necklace structures, which can occur in a poor solvent if the degree of charging is a light value or a moderate value, are most likely replaced with a deformed globule, a globule having with a tangling tail.

## Huan Wang 38

*Chemistry, University of Cincinnati*
*maryhasalittlelamb@gmail.com*


### Molecular dynamics simulations of the intra-ring allosteric mechanism of ClpY

Clp ATPases associate with its peptidase partner to degrade proteins in repetitive cycle of ATP hydrolysis. Clp ATPases use conformational changes coupled with ATP hydrolysis to effect the substrate protein to unfold and translocate through the central pore to the chambered peptidase for degradation. ClpY, one of the best structurally characterized member of ClpATPase, is a hexameric ring-shaped AAA+ motor with central pore formed by a highly conserved loop (GYVG motif) from each subunit. Structural and biochemical studies of AAA+ ATPases proteins have suggested several models (rotary, concerted and stochastic) of the progression of allosteric transition around the ring. We performed implicit solvent molecular dynamics simulations to investigate the effect of the ATP-driven conformational change of intra-ring coupling allosteric mechanism [1]. The largest effect of this allosteric motion is found on the neighboring subunit nearest to the active ATP binding pocket. High correlation at subunit-subunit interface near the active ATP binding site is due to networks of salt bridges formed by charged amino acids R393, E286, E80, E68, R321 residing in the interfaces. Based on these results, we propose that the intra-ring allostery proceeds in the clockwise direction (as determined by the axial view point from the proximal entrance of the ClpY pore). To elucidate the substrate remodeling mechanisms, we performed implicit solvent simulations that describe interaction between peptides with diverse secondary structure (alpha helix/beta turn/random coil) covalently attached to a degradation tag (ssrA) and the central channel of ClpY. We find that substrate binding is mediated by strong interaction at the Tyr91 site of ClpY and substrate release is effected by competing interactions at the neighboring Val92 site. In addition, we find that Tyr91 mutations (Y91A, Y91F, Y91W) reduce affinity of ClpY for the substrate due to the loss of capability to form transient hydrogen bonds. We find that the secondary structure of peptides is unraveled during translocation starting from both termini. Translocation of structured substrate proteins requires significantly longer timescale than unstructured substrate protein with unfolding as the rate-limiting step. In addition, the clockwise allosteric mechanism is found to be most effective due to strong coupling between steps.

**1**. Wang, H.; Jayasinghe, M.; Stan, G. Probing the directionality of ClpY's intra-ring allosteric mechanism, paper in preparation 2012


## Tomasz Wirecki 39

*Department of Molecular Modeling*
*University of Gdansk, Poland*
*fryzjer@chem.univ.gda.pl*


### Testing of the performance of Hybrid Monte Carlo methods

One of the most serious limitations of the canonical molecular dynamics (MD) method is the small size of the time step, which is limited by the period of the fastest vibrations of the system under study and usually is of the order of 1-2 fs. The canonical Metropolis Monte Carlo method, which is also used to study the behavior of dense systems (in particular biological macromolecules) does not suffer, in principle, from this shortcoming; however, the acceptance rate severely decreases with increasing the perturbation step size. Recently, methods resulting from combination of the MD and MC approaches, termed hybrid Monte Carlo methods, received considerable attention because they combine the best features of the MD (following the minimum-action direction) and MC (a possibility to take a step back if the chosen move leads to energy increase). In these methods, the Metropolis

criterion is applied to the new configuration of a system obtained from the old configuration after taking several large MD steps. In this work, a comparative study was carried out of three variants of the hybrid Monte Carlo methods: the original Hybrid Monte Carlo (HMC), the Shadow Hybrid Monte Carlo (SHMC) and the Separable Shadow Hybrid Monte Carlo (S2HMC) methods. A simple system composed of 108 Lennard-Jones particles in a periodic box was chosen for this purpose. The ergodicity of simulations and the dependence of the acceptance rate on the number of MD steps between Metropolis tests was studied. The acceptance rate decreases with an increase of the dt value. HMC and SHMC behave quite similarly, while S2HMC exhibits a significantly higher acceptance rates for higher dt values; consequently, it seems to be the best of the three approaches. S2HMC, however, has a higher acceptance rate than the other two methods. All the tested HMC methods present promising results. Implementation of the S2HMC algorithm in the united residue (UNRES) force field for large-scale simulations of protein structure and dynamics is pending.


## Xiaojun Xu 40

*Chemistry, Georgia State University*
*xxu1@student.gsu.edu*


### Structure and Dynamics of Repair Complexes of Flap Endonuclease 1 with PCNA and the Checkpoing Clamp 9-1-1

Processivity clamps such as PCNA and the checkpoint sliding clamp Rad9/Rad1/Hus1 (9-1-1) act as versatile scaffolds in the recruitment of proteins involved in replication, cell-cycle control and DNA repair. Structurally, both PCNA and 9-1-1 are composed of three subunits forming closed ring-shaped structures around DNA. While PCNA is a homotrimer, the 9-1-1 complex is heterotimeric, reflecting the differential involvement of the two clamps with protein partners and their distinct roles in coordinating DNA processing. A trimeric ring can provide multiple binding sites for replication and repair factors. Furthermore, competition among these bound proteins can lead to conformational switching and handoffs of these proteins. These are key processes in PCNA biology, which are incompletely understood from a structural perspective. Herein, we have chosen an integrative computational and experimental approach to model the assemblies of FEN1 with its double-flap DNA substrate and each of the two clamps. Fully atomistic models of the ternary DNA/hFEN1/h9-1-1 and DNA/hFEN1/hPCNA complexes were developed. The models were simulated with molecular dynamics (MD) in explicit solvent for 100ns to expose the conformational dynamics of the systems. Clustering analysis of the trajectories revealed the most dominant conformations accessible to the complexes. The cluster centroids were subsequently used in conjunction with single particle electron microscopy (EM) to generate an EM map of the h9-1-1/Fen1/DNA assembly to 18Å resolution. Finally, the atomistic models were refined by flexible fitting into the EM density resulting in a 3D structure of the 9-1-1/Fen1/DNA assembly.


## Fatih Yasar 41

*Department of Physics and Engineering*
*Haceteppe University, Ankara, Turkey*
*fatih@haceteppe.edu.tr*


### The Investigation of the Secondary Structure Propensities and Free-Energy Landscapes of Peptide Ligands by Replica Exchange Molecular Dynamics Simulations

The conformational states of three peptide sequences that bind to Staphylococcal enterotoxin B (SEB) are sampled by Replica-exchange molecular dynamic simulations (REMD) in explicit water. REMD simulations were treated with 52 replicas in the range of 280-501 K for each peptide. The conformational ensembles of each

peptide are dominated by random coil, bend and turn structures with a small amount of helical structures for each temperature. In addition, while an insignificant presence of β-bridge structures were observed for all peptides, the β-sheet structure was observed only for peptide II and III. The results obtained from simulations at 300 K are consistent with the experimental results obtained from circular dichroism (CD) spectroscopy. From the analysis of REMD results, we also calculated hydrophobic and hydrophilic solvent accessible surface areas (SASA) for all peptides and we observed that the hydrophobic segments of the peptides tend to form bend or turn structures. We have also obtained the free-energy landscapes of each peptide by principal component analysis, to understand how the secondary structural properties change according to their complex space. According to free-energy analysis, we have found several minima for each peptide when the temperature decreased. For these obvious minima of each peptide, it was observed that the random coil, bend and turn structures are still dominant and the helix, β-bridge or β-sheet structures can appear or disappear with respect to minima. On the other hand, when we compare the results of REMD with conventional MD simulations for these peptides, the configurations of peptide II might be trapped in energy minima during the conventional MD simulations.


## Zheng Yi 42

*UT/ORNL Center for Molecular Biophysics*
*Oak Ridge National Laboratory*
*zyi@utk.edu*


### Probing Conformational Dynamics with the Integration of Markov State Model and Dynamic Neutron and X-Ray Scattering

The conformational changes in biomolecules and the associated atomic motions up to the microsecond time scale can be directly probed by neutron and X-ray scattering experiments by measuring the time-dependence of the scattering signal and inferring relaxation times for the molecular processes. However, many underlying dynamic processes often exist on the same timescale, which makes it difficult to assign timescales seen in the experiment to particular structural rearrangements. Here, molecular dynamics simulation and Markov state modeling was used to connect the conformational changes directly to the exponential decay functions in the scattering spectra. This feat has been accomplished in two steps: First, Markov State Modeling was used to decompose the conformational state space into a number of long-lived metastable states, each having a distinct relaxation time. Then a mathematical framework was established for the direct computation of the intermediate scattering function from the eigenvectors and eigenvalues of the Markov model. This procedure allows the establishment of a complete set of the exponential decay functions and the full decomposition into their individual contributions, e.g. the contribution of every atom to each relaxation processes, which could be used to guide experimental designs of selective deuterium substitution to amplify certain processes.

## Hang Yu 43

*Biophysics and Computational Biology*
*University of Illinois, Urbana-Champaign*
*hangyu@ks.uiuc.edu*


### Computer Simulation of Membrane Tubulation by EFC F-BAR Domain Lattices

Cells are dynamically sculpted into many types of compartments by cellular membranes, in some cases with the help of BAR domain proteins. BAR domain proteins act under in vitro conditions are found to induce formation. of tubules. We have seen in coarse-grained molecular dynamics simulation stretching over 100 microseconds how a flat membrane is curved into a tube when F-BAR domain proteins are arranged on the membrane surface as a regular lattice of parallel rows. The simulations could also characterize the membrane bending properties of F-BAR domains in different lattice arrangements, showing membrane curvatures with radii ranging from 25 to 100 nm. Lastly, the simulations reveal two key structural features of F-BAR domain that facilitate efficient binding to membranes and membrane curving: (1) Curving is promoted by close contact between phosphoserine lipid head groups and clusters of cationic residues along the membrane facing surface of F-BAR domains, namely lysine and arginine residues 30, 33, 110, 113, 114, and 139, 140, 146, 150, respectively. (2) Within the 100 ns of contact, the F-BAR domain hinge region, through a 20 degree rotation of the helix moment of inertia, establishes a close contact between protein and membrane. (1) and (2) result in membrane bending on a microsecond-to-millisecond time scale.


## He Zhang 44

*Department of Chemistry*
*University of Wisconsin-Madison*
*Hzhang32@wisc.edu*


### A Lattice Monte Carlo Model of Biomass Degradation Systems

It has been decades for researchers to search for highly efficient cellulose deconstruction systems, from single enzymes to organisms. The complexity of these systems arises from the recalcitrance of biomass. Lignocellulosic biomass, as a sustainable source of renewable energy, is composed of crystalline cellulose fiber, crosslinked by hemicelluloses, coated by lignin and further surrounded by proteins and other biomolecules. Because there is no single enzyme can perform such a degradation job alone, nature has developed many biomass deconstruction systems involving hundreds of enzymes with different modularity and even higher level molecular architectures. In cellulose deconstruction problem, synergism involves combination of enzymatic activities, cooperation between catalytic modules and non-catalytic modules, and the way how they form a complex molecular architecture to increase the rate and yield of glucose released from cellulose. To study the synergy effect from molecular perspective, we built a Lattice Monte Carlo Model by calculating Reaction-diffusion processes with Metropolis algorithm. We investigated how cellulose morphology affects the synergy between different cellulases. The molecular basis for initial burst reaction is also studied.

**Zhe Zhang 45**
*Department of Physics, Clemson University*
*Universite Paris Diderot, Paris*
*zz@clemson.edu*

## In Silico Modeling the Effects of Disease-Causing Missense Mutations

Synder-Robinson Syndrome (SRS, OMIM, 309583) is a mental retardation disorder caused by three missense mutations (G56S, V132G and I150T) in spermine synthase protein (SMS). The molecular mechanism of these three missense mutations causing SRS will be presented. In addition, the mutability of the missense mutation sites was also explored. The results suggest that the mutability depends on the details of the structural and functional factors, and thus can't be predicted only based on the evolutionary information alone. Even the disease-causing sites can still harbor harmless missense mutations. Another typical X-linked mental retardation is creatine deficiency syndrome (CDS, OMIM, 300352), which is associated with the deficiency on creatine transporter (CRT) encoded by gene SLC6A8. CRT is a transmembrane protein taking up the creatine, which is playing a key role for energy supply in cardiac and skeletal muscle cells, from the outside of the cell. So far 17 missense mutations have been found clinically, 15 of which have been considered as disease-causing. Different from SMS, there is no experimental 3-D structure available, so we built the 3-D model of CRT in silico to investigate the molecular effects of the above mentioned missense mutations. During the above research, we developed a new methodology named sMMGB to predict protein stability changes due to a single point mutation. By testing sMMGB on a large database of 1109 mutants with experimental folding free energy change, our protocol achieved RMSD = 1.78 kcal/mol and slope = 1.04 of the linear regression between the predicted and experimental values.