# Molecular Biophysics in the Northeast 2019

November 9<sup>th</sup>, 2019 Northeastern University

Program

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### **Event Description**

The first *Molecular Biophysics in the Northeast* (MBN2019) meeting will provide an opportunity for members of the experimental and theoretical molecular biophysics community to discuss current research topics. This event will emphasize the engagement of trainees (from undergraduates to post-docs) through oral presentations (selected from abstracts) and posters. The majority of the oral presentations will be given by students, and there will be more than 50 posters presented throughout the day. This year, we have 127 registered participants, representing over 20 universities and research institutes. It is our hope that, by bringing together a diverse group of researchers, this event will help the field bridge our understanding of the relationships between physical principles, molecular structure and biological function.

We would like to acknowledge support from the Departments of Physics and Bioengineering at Northeastern University, which made this event possible.

Organizing Committee: Jianhan Chen – University of Massachusetts Michael Hagan – Brandeis University Andrei Korostelev – UMass Medical School Eric May – University of Connecticut Paul Whitford – Northeastern University

### Arrival Information: Event Location and Parking Details

All MBN2019 activities will be in the new Interdisciplinary Science and Engineering Complex (ISEC), on the Northeastern University campus. The ISEC building (right) is located at 805 Columbus Ave, in Boston. Talks will be in the main auditorium, which is located on the ground floor. Posters will be on display throughout the day in the atrium, which is adjacent to the auditorium.



*Arriving by public transit:* If you are arriving via the T, the closest stops are Ruggles (Orange Line) and Northeastern (Green Line). See map below.

Arriving by car: Street parking is sometimes available. The closest paid parking is available, on a first-come first-serve basis, at the Renaissance Parking Garage (835 Columbus Ave, Boston, MA 02120). If you pre-purchased a parking pass, please park in the Renaissance Garage and take a ticket upon entry. When you check in for MBN2019, you will receive your parking pass. When you depart, present your parking ticket and pass, in order to exit the garage.



## Schedule

9-9:30 Check-in, coffee and poster setup

**Session 1: Characterizing Biomolecular Interactions**. Chair: Mary Jo Ondrechen - Northeastern University

9:30-9:50 **Min Chen** – University of Massachusetts, Amherst A ClyA nanopore tweezer to differentiate anomeric sugar bound states of MBP

9:55-10:07 **Morgan R. Packer** – Northeastern University *Raf promotes dimerization of the Ras G-domain* 

10:10- 10:22 **Christopher Nordyke** – University of New Hampshire Structural and functional studies of the polar organizing protein Z from Caulobacter crescentus using solution NMR spectroscopy

10:30 - 11:00 Coffee break - posters on display

Session 2: Bridging Computational and Experimental Biophysical Techniques. Chair: John Straub - Boston University

11:00-11:20 **Yu-Shan Lin** – Tufts University *Understanding and designing cyclic peptides* 

11:25-11:37 **Christopher Myers** – SUNY Albany Accounting for electrostatic polarization in gas-phase simulations of ion mobility spectroscopy

11:40- 11:52 **Koushik Kasavajhala** – SUNY Stony Brook Using structure reservoirs to accelerate biomolecular simulations

11:55- 12:07 **Jacob Remington** – University of Vermont Molecular dynamics simulations reveal molecular basis of PAC1 receptor activation and internalization

12:10-2:00 Lunch and Posters: 12:30-1:15, even number posters. 1:15-2:00, odd posters

**Session 3: Dynamics of Ribonucleoprotein Assemblies**. Chair: Craig Martin – University of Massachusetts, Amherst

2:00-2:20 Dmitri Ermolenko – University of Rochester Medical School

Making ends meet: A new role of mRNA secondary structure in translation

2:25-2:37 **Christine Carbone** – UMass Medical School Complex dynamics of ArfB-mediated ribosome rescue revealed by cryo-EM

#### 2:40- 2:52 Ailun Wang – Boston College

How diffuse ions regulate conformational dynamics of ribonucleoprotein assemblies

**Session 4: Collective Dynamics at Large Length Scales**. Chair: Michael Hagan - Brandeis University

3:00-3:20 Nicolas Fawzi – Brown University Structural biology of RNA-binding protein phase separation in health and disease

3:25-3:37 **Searle Duay** – University of Connecticut Exploring the pH-dependent interactions of the antimicrobial peptide clavanin A with E. coli outer membrane using molecular dynamics simulations

3:40-3:52 **Wenjun Xie** – MIT Characterizing chromatin folding coordinate and landscape with deep learning

4:00-4:30 Coffee break. Remove posters.

4:30 Introduction of Keynote Speaker: Carla Mattos - Northeastern University

4:35-5:35 Keynote Speaker: Martin Karplus – Harvard University

Closing remarks

### **Keynote Speaker**



© Martin Karplus

Martin Karplus is the Theodore William Richards Professor of Chemistry Emeritus at Harvard University. He was born in Vienna and escaped to Switzerland shortly after Hitler's entrance into Austria. He was able to move with his parents and brother to the United States shortly after that. A graduate of Harvard College '51, he received his Ph.D. in chemistry from the California Institute of Technology under two-time Nobel laureate Linus Pauling in 1953. Karplus started teaching at Harvard in 1966, where he continues to do research. He received the Nobel Prize in Chemistry in 2013.

## **Poster Titles**

Below is the list of presenters and poster titles. Full author lists and abstracts may be found at the end of this document.

#	Presenter	Institution	Title
1	Aidan B Fike	Tufts University	Rapid cyclic peptide structural prediction using machine learning methods
2	Matthew Yarnall	Amherst College	Allosteric-site residues govern the stabilities of classical protein tyrosine phosphatase domains
3	Jonathan Sreter	University of New Hampshire	Structural and Functional Characterization of Antifreeze Protein ApAFP752
4	Jasmin Portelinha	University of Connecticut	Biophysical methods reveal two high affinity Cu2+ binding sites that potentiate the broad-spectrum activity of the antimicrobial peptide Gad-1
5	Michael Morse	Northeastern University	Anti-viral protein APOBEC3G binds single stranded DNA in multiple steps and conformations to enable efficient search and deamination
6	Nejc Nagelj	Amherst College	Native-HXMS: a method to directly measure protein folding and stability under native conditions
7	Xiping Gong	University of Massachusetts, Amherst	Accelerating the GBMV2/SA Implicit Solvent Model Using Graphic Processing Units
8	Nikita Malik	UMASS Amherst	Changes in bacterial chemoreceptor structure and dynamics in response to adaptational modification
9	Farri Mohajerani	Brandeis University	The role of scaffolding peptides in microcompartment assembly
10	Sanjib Paul	University of New hampshire	Self-assembly of lobed particles into porous structures.
11	Jiayuan Miao	Tufts University	Beta-Branched Amino Acids Stabilize Specific Conformations of Cyclic Hexapeptides
12	He Huang	Tufts University	Effects of Cyclization on Properties of Peptides Backbones and the Length Dependence
13	Erin Reinhart	Dartmouth College	Structural and biochemical characterization of the FYVE domain of ALFY
14	Denis Susorov	UMass Medical School	Nanoluciferase maturation upon release from the ribosome
15	Zhiguang Jia	UMass	Chloride channels or phospholipid scramblase: a gatekeeper story
16	Lori Goldner	UMass Amherst	The Nanomechanics of Cellulose Synthesis
17	Ryan Puterbaugh	University of New Hampshire	Structural Studies of the Regulators of a G-protein Signaling Protein
18	Michael Schwabe	Northeastern University	Dynamics of the Rap1A Active Site Uncovered by Molecular Dynamic Simulations

19	Mahmoud Sharawy	University of Connecticut	Computational studies on the effect of the chemical environment of the DNA guanine quadruplex on the free energy of binding Na and K cations
20	Monica Ojeda	Northeastern University	Determining conformational dynamics of the allosteric site in HRas R97G
21	Omeir Khan	Tufts University	Simulation Guided Design of a Cyclic Peptide Growth Hormone Receptor Inhibitor
22	Dmitrij Golovenko	UMass Medical School/Weizmann Institute of Science	DNA shape recognition by 'guardian of the genome' p53
23	Mary Jo Ondrechen	Northeastern University	Can we mimic evolution for the rapid design of novel enzymes?
24	Ali Mehdizadeh	Northeastern University	Continuum Electrostatics of Bio-Membranes: Multiscale Modeling of Boundary Condition Using a Solvation-Layer Interface Condition (SLIC)
25	Xiaorong Liu	UMass Amherst	Modulation of p53 transactivation domain conformations by ligand binding and cancer- associated mutations
26	Andrew Latham	МІТ	Maximum Entropy Optimized Force Field for Intrinsically Disordered Proteins
27	Jason G Pattis	Temple University	Large Structural Changes During the Lassa Virus Nucleoprotein Trimer to Monomer Transition Revealed by Markov State Modeling
28	Ben Cashen	Northeastern University	Single molecule binding dynamics of LINE-1 ORF1p to ssDNA
29	Craig Martin	University of Massachusetts Amherst	Novel Approaches to RNA Synthesis by T7 RNA Polymerase
30	Koushik kasavajhala	Stony Brook University	Using Structure Reservoirs to Accelerate Biomolecular Simulations
31	Anna Bock	Brown University	N-terminal acetylation affects phase separation and aggregation of the low complexity domain of RNA-binding protein FUS
33	Joshua Dudley	Wesleyan University	Analysis of Enhanced NMR Peak Fitting Method
34	Zhongling Jiang	Massachusetts Institute of Technology	Theory of active chromatin remodeling
35	Yong Liu	University of New Hampshire	Dynamics and water transport in artificial water channels
36	Xingcheng Lin	Massachusetts Institute of Technology	Deciphering PRC2-mediated Nucleosomal Interactions for Controlling Gene Regulation
37	Jasper Flint and Isabella Han	Amherst College	Validation of Hydrogen Exchange Mass Spectroscopy (HXMS) Native-State Protein Stability Method
38	Amritha Anup	Amherst College	Unfolding Nature's Origami: Acetonitrile's Role as a Protein Denaturant

39	Spencer Shorkey	UMass Amherst	Nanopore Tweezer Analysis of Ubiquitin Chains
40	Lev Levintov	University of New Hampshire	Conformational heterogeneity and its role in ligand recognition by RNA molecules
41	Yang Wang	Boston College	How diffuse ions regulate conformational dynamics of ribonucleoprotein assemblies
42	Kaylee Mathews	Brown University	Structural and biophysical characterization of splicing-associated assemblies of the SMN protein
43	Alfredo Angeles- Boza	University of Connecticut	What have we learned from metal-binding AMPs? Inorganic lessons from nature
44	Xinqiang Ding	MIT	Deciphering protein evolution, fitness landscapes and stability with latent space models
45	Colin Smith	Wesleyan University	Simulation of Isomerization/Quenching in de novo Designed Mini Fluorescent Proteins
46	Trinity Cookis	Northeastern University	Distinct Behavior of the Two KRas Splice Variants
47	Erik Nordauist	UMass Amherst	Understanding the Origins of DnaK's Selective Promiscuity with Physics-based Statistical Modeling
48	Lauren Phung	Harvard Medical School and Northeastern University	Inhibition of histone demethylase Lsd1 presents a potential epigenetic approach for Atoh1 upregulation in cochlear hair cell regeneration
49	Asis Jana	University of Connecticut	Investigating Cell Entry Mechanisms of Non- enveloped Viruses using Computer Simulations
50	Mosavverul Hassan	Stony Brook University	Ab-Initio SSE Construction
51	Asem Hassan	Northeastern University	Using simulations to identify precise single- molecule probes for ribosome dynamics
52	Mariana Levi	Northeastern University	Identifying steric components in the ribosome that control tRNA hybrid-state formation
53	Mahdieh Yazdani	UMass-Amherst	Nonspecific Membrane Interaction Modulation of BK Channel Gating
54	Sarah Duggan	Amherst College	Characterizing the Interplay between Dynamics and Regulation in the Trypsinogen/Trypsin Protease System
55	Dongyue Liang	Boston University	Dynamic Changes in the Electric Double Layer Complicate the Use of Zeta Potential in Predicting Binding Properties of Nanoparticles
56	Gudfridur Bjorg Moller	Northeastern University	Intercalation of Small Rhodium Complexes into Matched and Mismatched DNA
57	Yifeng Qi	МІТ	Data-driven polymer model for mechanistic exploration of diploid genome organization

### **Presenter Instructions**

#### **Oral Presenters**

All speakers should check in with the session chair 5-10 minutes before the start of their session.

*Invited Talks:* Invited talks will be 20 minutes. We have scheduled 5 minutes for transitions afterwards, which will be used for questions. It is requested that all presentations be pre-loaded, in Powerpoint format, to the common PC that is connected to the projection system. All pre-loaded presentations will be deleted from the display computer immediately after MBN2019 ends. If you choose to use your own laptop, the projection system is equipped with VGA and HDMI connections, in addition to a variety of adapters. If you are presenting in the first two sessions, please arrive by 9am to test your presentation with the projector system. If you are presenting in the afternoon, please upload/test your talk during the lunch/poster session.

*Selected Talks:* In an effort to maximize the number of selected student speakers, we have to limit the presentations to 12 minutes. There is a 3-minute transition period, which will be used for questions, while the next speaker prepares. Given the tight schedule, selected speakers must send their presentation to Paul Whitford (paul.whitford@gmail.com), no later than Friday at 5pm. You may share the files via email, Google drive, or Dropbox. Any copies of the presentations will be deleted immediately after MBN2019 completes.

#### **Poster Presenters**

Posters will be on display throughout the day. Please set up your poster between 9:00 and 9:30 am, before the event begins. This will allow the posters to be viewed during both coffee breaks, lunch and during the poster session. Posters will be mounted on 3' x 4' boards. Pushpins and tape will be available for mounting your posters. We will ask that you stand by your poster for (at least) half of the poster session.

# **Oral Presentation Abstracts**

#### A ClyA nanopore tweezer to differentiate anomeric sugar bound states of MBP

Min Chen Department of Chemistry UMASS Amherst

Conformational changes of proteins are essential to their functions. Yet it remains challenging to measure the amplitudes and timescales of protein motions. Here we show that the ClyA nanopore was used as a molecular tweezer to trap a single maltose-binding protein (MBP) within its lumen, which allows conformation changes to be monitored as electrical current fluctuations in real time. In contrast to the current two state binding model, the current measurements revealed three distinct ligand-bound states for MBP in the presence of reducing saccharides. Our analysis reveal that these three states represented MBP bound to different isomers of reducing sugars. These findings shed light on the mechanism of substrate recognition by MBP and illustrate that the nanopore tweezer is a powerful, label-free, single-molecule approach for studying protein conformational dynamics under functional conditions.

#### Understanding and designing cyclic peptides

Yu-Shan Lin Department of Chemistry Tufts University

Protein-protein interactions play critical roles in many important and disease-relevant biological processes. The ability to modulate protein–protein interactions thus provides a means to control diverse cellular functions for both fundamental research and therapeutic intervention. Unfortunately, protein–protein interfaces are challenging targets for traditional small molecules because the interfaces are relatively large and flat. Cyclic peptides offer a promising solution for targeting protein-protein interactions, owing to their inherently large surface area and their ability to easily mimic functional groups and structures at protein interfaces. However, the high potential applicability of cyclic peptides is currently severely limited by our poor capacity to accurately predict cyclic-peptide structures. We recently developed an enhanced sampling method that uses metadynamics to target the coupled two-dihedral motions during conformational switches of cyclic peptides. This technique enables efficient simulations of cyclic peptides using even all-atom force fields and explicit solvent models and thus allows more accurate description of cyclic peptide structures and energetics to be used during their rational design. In this talk, we describe how we used molecular dynamics simulations and enhanced sampling methods to achieve the first accurate de novo structure prediction of 5- and 6membered cyclic peptides.

#### Making ends meet: a new role of mRNA secondary structure in translation

Dmitri Ermolenko Department of Biochemistry and Biophysics University of Rochester

The 5' and 3' termini of RNA play important roles in many cellular processes. Using Förster resonance energy transfer (FRET), we show that mRNAs and lncRNAs have an intrinsic propensity to fold in the absence of proteins into structures in which the 5' end and 3' end are ≤7 nm apart irrespective of RNA length. Computational estimates suggest that the inherent proximity of the ends is a universal property of most mRNA and lncRNA sequences. Only guanosine-depleted RNA sequences with low sequence complexity are unstructured and exhibit end-to-end distances expected for the random coil conformation of RNA. Our studies provide the basis for measuring, computing and manipulating end-to-end distances and secondary structure in RNA in research and biotechnology.

While the biological implications remain to be explored, short end-to-end distances could facilitate the binding of protein factors that regulate translation initiation by bridging mRNA 5' and 3' ends. The 5' cap and 3' poly(A) tail of mRNA are known to synergistically stimulate translation initiation via the formation of the cap•elF4E•elF4G•PABP•poly(A) complex. The inherent compactness of mRNAs may stabilize the cap•elF4E•elF4G•PABP•poly(A) complex and enhance cap-poly(A) translational synergy. We test this hypothesis by introducing intrinsically unstructured sequences into the 5' or 3' UTRs of model mRNAs. We found that the introduction of unstructured sequences into the 3' UTR (but not the 5' UTR) decreases mRNA translation in wheat germ extract without affecting mRNA stability. The observed reduction in protein synthesis results from the diminished ability of the poly(A) tail to stimulate translation through synergistic interaction with the 5' cap. These results suggest that base pair formation by the 3' UTR enhances the cap-poly(A) synergy in translation initiation.

#### Structural biology of RNA-binding protein phase separation in health and disease

Nicolas Lux Fawzi Department of Molecular Pharmacology, Physiology, and Biotechnology Brown University

The mechanistic basis for disordered domain functional liquid-liquid phase separation (LLPS) and protein inclusion formation in neurodegenerative disease remains poorly understood. This gap persists due to a lack of direct, atomically-detailed structural information. Here, I present results using NMR spectroscopy and simulation to visualize structure and interactions of several human RNA-binding proteins with aggregation-prone low complexity (LC) domains that undergo LLPS and aggregate in ALS and frontotemporal dementia. First, we focus on sequence factors regulating LLPS. In the Fused in Sarcoma (FUS) LC domain, we find that hydrogen bonding and hydrophobic/pi interactions mediate contacts while phosphorylation disrupts LLPS. In hnRNPA2, asymmetric dimethylation of arginine residues disrupts arginine-aromatic residue contacts and decreases LLPS. Because many phase separating RNA-binding proteins contain phosphorylation and methylation sites, understanding the biophysical chemistry of modifications will be important. Second, we examine factors distinguishing LLPS and aggregation. The hnRNPA2 P298L disease variant removes a conserved,  $\beta$ -sheet breaking proline to enhance aggregation, suggesting an important role for prolines in aggregation-prone LC domains. Third, we examine the role of structured domains in modulating LC domain LLPS. In contrast to disorder of FUS and hnRNPA2 LC domains after LLPS, LLPS of TDP-43 is mediated in part by partially-structured  $\alpha$ helical assembly that is disrupted by ALS-associated mutations. Furthermore, LLPS of full-length TDP-43 is enhanced by weak linear polymerization of the globular N-terminal domain. Substitution at a conserved phospho-site decreases LLPS in vitro and in cells and disrupts splicing activity. Our work points to the potential for post-translational modification to alter assembly, function, and pathological interactions of disease-associated disordered domains.

# Molecular dynamics simulations reveal molecular basis of PAC1 receptor activation and internalization

Jacob M Remington and Jianing Li Department of Chemistry University of Vermont

Pituitary adenylate cyclase-activating polypeptide's (PACAP) receptor (PAC1R) is G proteincoupled receptor involved in many facets of physiology including regulation of the circadian rhythm, insulin secretion, immunity and inflammation, stress, and brain cell development. PAC1R expressed in the brain takes on two dominate isoforms that lack or contain the HOP cassette insert in the third intracellular loop. To investigate the potentially bifurcating role of this insert, homology modeling and all-atom molecular dynamics simulations of PACAP bound PAC1R embedded in a cell membrane and complexed with beta-arrestin are described. Microsecond length simulations reveal that the HOP insert forms stable contacts with the N-domain of betaarrestin that are lacking in the PAC1R model with no HOP insert. This novel interaction was found to ratchet the C-edge of the beta-arrestin into the membrane for the remainder of the simulation. This orientation of beta-arrestin could potentially enhance caltherin binding and ultimately receptor internalization. These results are complemented by in vitro experiments that reveal differences in the temporal response of receptor internalization and receptor-mediated extracellular signal-regulated kinase activation upon PACAP exposure. Combining experiments with theory suggests the variable expression of the PAC1R isoforms in neuronal tissue may affect neuroplasticity and the maladaptive consequences of chronic pain and stress.

#### Complex Dynamics of ArfB-Mediated Ribosome Rescue Revealed by Cryo-EM

<u>Christine Carbone</u>, Egor Svidritskiy and Andrei Korostelev RNA Therapeutics Institute UMass Medical School

A translating ribosome stalls when it encounters the end of a non-stop mRNA, truncated during cellular stress or other conditions (Hayes and Keiler, 2010; Keiler, 2015). Alternative rescue factor B (ArfB) rescues stalled ribosomes by catalyzing peptide release from peptidyl-tRNA (Chadani et al., 2011). Crystallographic work (Gagnon et al., 2012) showed that the C-terminal  $\alpha$ -helix of ArfB binds in the mRNA entry channel, allowing the catalytic N-terminal domain to reach the A site of the peptidyl transferase center. Thus, binding of ArfB should be incompatible with the mRNAs extending beyond the A-site. However, biochemical data demonstrate that ArfB can act on ribosomes stalled on a rare codon cluster, and it is unclear how the ribosome can accommodate both ArfB and mRNA in this case (Handa et al., 2011). In this work, we sought to clarify the mechanism for ArfB depends on mRNA length rather than sequence identity, yet ArfB remains efficient on mRNAs extending beyond the A site. Using single particle cryo-EM, we identified multiple states that suggest different mRNA-length-dependent structural mechanisms for ArfB-mediated ribosome rescue.

## Exploring the pH-dependent interactions of the antimicrobial peptide Clavanin A with E. coli outer membrane using molecular dynamics simulations

<u>Searle S. Duay<sup>1</sup>, Alfredo M. Angeles-Boza<sup>1</sup></u>, and Eric R. May<sup>2</sup> <sup>1</sup>Department of Chemistry, <sup>2</sup>Department of Molecular and Cell Biology University of Connecticut

Antimicrobial peptides (AMPs) are an important class of molecules because of their ability to kill bacteria using a variety of mechanisms, such as membrane pore formation and interference with internal cell targets. Their ability to exert different mechanisms of action depending on their local environment offers a promising advantage to develop novel antibiotics that are less susceptible to antibiotic resistance. Clavanin A (ClavA) is a naturally occurring AMP that is found in the phagocytic hemocytes of Styela clava, where the pH ranges from ~7.4 to ~5. Interestingly, ClavA is more active against E. coli at pH 5.5, than at the physiological pH 7.4, in vitro. The current hypothesis is that the mechanism of action of ClavA is pH-dependent, pointing to membrane disruption via pore formation at pH 7.4 and internal cell target at pH 5.5. This is based upon experimental evidence that ClavA causes membrane depolarization at pH 7.4 and at pH 5, confocal microscopy experiments show internalization of ClavA in E. coli cells with DNA being the most likely target. In this study, we employed microsecond timescale all-atom molecular dynamics (MD) simulations to explore the pH-dependent interaction of the ClavA with a complex model of an E. coli outer membrane. In its transmembrane state at pH 7, we observed that it facilitates transport of water molecules across the membrane by favorable polar-polar interactions between water and its polar residues. In a pH 7 simulation initiated from a transmembrane, random coil conformation of ClavA, the spontaneous breaking of interaction between His21 and the lower leaflet phosphates prompted the transition to a surface-bound state. Once surface-bound, water transport across the membrane is drastically reduced. Currently simulations of pH 5 systems that parallel the pH 7 systems are being conducted. Also, we aim to present results from Hamiltonian replica exchange MD to evaluate pH-effects on the free energy difference between transmembrane and surface-bound states. This will provide insight into the difference in probability of pore formation between the two pHs. Overall, we aim to understand how differences in local environment factors, such as pH, affects the interaction of ClavA with membranes which influences its mechanism of action.

# Accounting for electrostatic polarization in gas-phase simulations of ion mobility spectrometry

Christopher A. Myers<sup>1</sup> and Alan A. Chen<sup>2,3</sup> <sup>1</sup>Physics Department, <sup>2</sup> Chemistry Department, <sup>3</sup> RNA Institute University at Albany (SUNY) Albany, NY 12222

Molecular dynamics (MD) simulations coupled with ion mobility spectrometry (IMS), a gas-phase extension to mass spectrometry that further filters the analytes by their topology, allows researchers to perform three dimensional structural elucidation of nucleic acids. Recently combined IMS-MD studies of short DNA duplexes (Porrini et al, 2017) and hairpins (Lippens et al, 2016), however, have struggled with obtaining accurately compact structures that agree with the experimentally determined collision cross sections for parts of their work. Because IMS experiments are performed on charged molecules, long range electrostatic interactions, as well as the changes in charge density due to protonated phosphate sites along the backbone, play a crucial role in understanding how nucleic acids behave in a gaseous environment and can benefit from a detailed quantum chemical analysis. In the work presented here, we explore what a properly tuned electrostatic force field for nucleic acids might look like. Based off of density functional theory calculations of small oligonucleotides, we examine how one could adjust the partial charges in an MD force field to more accurately replicate Coulomb interactions for charged and protonated nucleic acids.

#### References

Porrini, M., Rosu, F., Rabin, C., Darré, L., Gómez, H., Orozco, M., & Gabelica, V. (2017). Compaction of Duplex Nucleic Acids upon Native Electrospray Mass Spectrometry. ACS Central Science 2017 3 (5). 454-461.

Lippens, J. L., Ranganathan, S. V., D'Esposito, R. J., & Fabris, D. (2016). Modular calibrant sets for the structural analysis of nucleic acids by ion mobility spectrometry mass spectrometry. Analyst 141, 4084–4099.

#### Characterizing chromatin folding coordinate and landscape with deep learning

#### Wenjun Xie, Yifeng Qi and Bin Zhang Department of Chemistry MIT

Genome organization is instrumental in setting up the spatial environment of gene transcription, and significant progress has been made in its characterization. The underlying molecular mechanism for its establishment is much less understood. We applied a deep learning approach, variational autoencoder (VAE), to analyze the fluctuation and heterogeneity of chromatin structures revealed by single-cell super-resolution imaging and to identify a reaction coordinate for chromatin folding. This coordinate monitors the progression of topologically-associateddomain (TAD) formation, and connects the seemingly random structures observed in individual cohesin-depleted cells as intermediate states along the chromatin folding pathway. Analysis of the folding landscape derived from VAE suggests that well-folded structures similar to those found in wild-type cells remain energetically favorable in cohesin-depleted cells. The interaction energies, however, are not strong enough to overcome the entropic penalty, leading to the formation of only partially folded structures and the disappearance of TADs from contact maps upon averaging. Implications of these results for the molecular driving forces of chromatin folding are discussed.

#### Using structure reservoirs to accelerate biomolecular simulations

<u>Koushik Kasavajhala</u> and Carlos Simmerling Department of Chemistry Stony Brook University

Molecular Dynamics (MD) simulations have become an indispensable tool to understand biological processes such as protein folding, drug binding, and protein-protein interactions besides many others. However, standard MD is limited by the slow sampling of conformational space and obtaining converged Boltzmann-weighted conformational ensembles still takes a long time (weeks to months) even on the latest computational hardware. To alleviate this problem, our lab previously developed the Reservoir Replica Exchange MD (Reservoir REMD) to accelerate convergence of biomolecular simulations. In Reservoir REMD, an unweighted (non-Boltzmann) set of pre-generated structure snapshots representing different local minima are used as a reservoir. Monte Carlo moves are then attempted between the reservoir structures and the REMD replicas.

In this work, we built on the method and show that structure reservoirs can be used to reproduce accurate Boltzmann-weighted ensembles obtained by much more expensive conventional REMD, with at least 25x faster convergence rates even for larger proteins (>50 amino acids) compared to conventional REMD. We have also tested the method on RNA systems and obtained similar convergence speeds. Furthermore, we also show that structure reservoirs can be used to predict accuracy of new force fields, thereby, accelerating force field development. Finally, structures obtained from non-MD methods such as homology-based models or Rosetta-based models and structures obtained from other enhanced sampling techniques can be integrated into this method, making it a powerful tool that combines physics-based approaches with non-physics-based approaches leading to better structure predictions of biomolecules. The GPU Reservoir REMD code is now available for use with the AMBER suite of programs.

#### How diffuse ions regulate conformational dynamics of ribonucleoprotein assemblies

#### <u>Ailun Wang</u><sup>1</sup>, Yang Wang<sup>1</sup>, Mariana Levi<sup>2</sup>, Udayan Mohanty<sup>1</sup> and Paul C. Whitford<sup>2</sup> <sup>1</sup> Department of Chemistry, Boston College <sup>2</sup> Department of Physics, Northeastern University

Structure-based model (SBM) allows millisecond effective timescale simulations of large biomolecular assemblies such as ribosome, which provides opportunities to study the dynamics of biomolecular assemblies at relative long time-scale. Diffuse ions, especially divalent cations, play a significant role in the dynamic behaviors of RNA, which regulates the conformational dynamics of ribosome. Therefore, explicit treatment of diffuse ions and electrostatic between ions and biomolecules is necessary in simulations. Here, we have developed an all-atom structure-based model in which non-hydrogen atoms, monovalent (K+, Cl-) and divalent ions (Mg2+) and electrostatics are described explicitly. The effective potentials for ion-ion, ion-RNA and ion-protein interactions are constructed to treat the solvation implicitly. The parameters in the effective potentials are refined with an iterative protocol for correct accumulation of ions near the RNA with reference from both explicit solvent simulation and experiment measurement. The same protocol has been applied on the refinement of parameters for ion-protein interactions. After the refinement, this transferable set of effective potentials for ion-ion and ion-RNA/protein interactions is then used in the SBM simulation of ribosome to study the ionic effect on dynamic behaviors of ribosome, such as the subunit rotation and dynamic motions of the stalk regions. This model provides insights into how diffuse ion atmosphere, outer-sphere ions, sitebound ions and flexibility drive conformation fluctuations in ribonucleoprotein assemblies.

# Structural and functional studies of the polar organizing Protein Z from Caulobacter crescentus using solution NMR spectroscopy

<u>Christopher Nordyke</u> and Krisztina Varga Department of Molecular, Cellular and Biomedical Sciences University of New Hampshire

The bacterial cytoplasm is a highly organized compartment despite containing over two million proteins. This organization is due, in part, to multiple proteins forming macromolecular assemblies imperative for cellular function. Cell poles are prominent examples of macromolecular assembly sites, and proteins that assemble at these locations control a variety of functions including chromosome replication and segregation, cell-cycle dependent gene expression, motility, and biofilm formation. One such protein is the Polar Organizing Protein Z (PopZ), which forms 3D polymeric superstructures at the cell poles of the Caulobacter crescentus bacterium, and it is required for recruiting and organizing regulatory proteins from the cytoplasm to the poles. Our previous studies have shown that the PopZ scaffold interacts directly with at least eight different target proteins. Current studies include utilizing solution nuclear magnetic resonance (NMR) spectroscopy to investigate interactions of PopZ with its binding partners and structurally characterizing a truncated, monomeric version of PopZ. PopZ adopts an intrinsically disordered conformation, with the exception of a short alpha-helical segment near the Nterminus which is a part of the binding motif. These results contribute to our understanding of bacterial hub proteins and to developing a more generalized mechanism for complex bacterial network organization.

#### Raf promotes dimerization of the Ras G-domain

<u>Morgan R. Packer</u><sup>1</sup>, Jillian A. Parker<sup>1</sup>, Jean K. Chung<sup>2</sup>, Zhenlu Li<sup>3</sup>, Young Kwang Lee<sup>2</sup>, Hugo Guterres<sup>1</sup>, Amin Hossain<sup>1</sup>, Daniel P. Donnelly<sup>1</sup>, Jeffrey N. Agar<sup>1</sup>, Lee Makowski<sup>1</sup>, Matthias Buck<sup>3</sup>, Jay T. Groves<sup>2</sup>, and Carla Mattos<sup>1</sup> <sup>1</sup>Northeastern University <sup>2</sup>University of California Berkeley <sup>3</sup>Case Western Reserve University

The Ras/Raf/MEK/ERK pathway is a major regulator of cell proliferation and is highly implicated in human cancers. Ras dimerization was first proposed thirty years ago, and it is known that Ras dimerization is essential for Raf activation, yet the mechanism through which this occurs has remained elusive. Attempts to detect Ras dimers on supported membranes have led to the conclusion that Ras does not dimerize in the absence of other factors. Here we show that the Ras binding domain of Raf (Raf-RBD) is the key element prompting Ras dimerization. A small amount of Ras/Raf-RBD dimer is seen in solution, while the addition of Raf-RBD to Ras on supported lipid membranes produces robust levels of dimers. On the membrane, Ras/Raf-RBD binding and Ras dimerization appear to be concerted events that lead to a high-affinity signaling complex. Molecular dynamics simulations of Ras and Raf-RBD alone, in a complex, and in the dimer reveal that the proteins become increasingly connected with higher levels of allosteric networks. This is apparent in the increasing connectivity between the helices involved in dimerization, helices 4 and 5, developing more consistent communication with the phosphate binding loop in the Ras active site. These data reveal a novel mode of regulation of the mitogenic Ras/Raf/MEK/ERK pathway and further supports the role of allosteric modulation of Ras GTPase activity in the context of Raf-RBD signaling.

# **Poster Abstracts**

#### Poster # 1 Rapid cyclic peptide structural prediction using machine learning methods

<u>Aidan B Fike</u> and Yu-Shan Lin Department of Physics Tufts University

Cyclic peptides are a promising class of drug molecules, but their sequence–structure relationships are still not well understood. Although it is currently possible to use a molecular dynamics simulation to calculate the conformations that a given cyclic peptide will take, this method requires a large amount of computational power. To decrease the complexity of this problem, I attempt to use a neural network to learn from a large set of simulation data and find key sequence–structure relationships. Once this neural network is trained, it is able to rapidly predict which conformations any given cyclic peptide will take without needing to run a time-consuming simulation.

#### Poster # 2 Allosteric-site residues govern the stabilities of classical protein tyrosine phosphatase domains

#### Matthew Yarnall and Anthony Bishop Department of Biochemistry/Biophysics Amherst College

Allosteric-site targeting small molecules possess incredible pharmaceutical applications for the inhibition of oncogenic proteins. Such compounds hold special promise for the inhibition of proteins with conserved active site structure—because they could theoretically allow for target specificity between catalytic domains of near identical peptide sequence. One class of proteins which constitutes a viable candidate for allosteric research are protein tyrosine phosphates (PTPs). PTPs are a class of signaling enzymes which—when mutated in humans— have been connected to a host of genetic diseases, including cancer. The inability to specifically target oncogenic PTPs through traditional active-site directed mechanisms has constituted the greatest impediment to pharmaceutical cancer treatment.

Due to their pharmaceutical promise, significant research has been done to identify PTP allosteric sites and potential small-molecule inhibitors. However, the structural effects of allosteric sites on overall PTP stability–compared to proteins of near-identical sequence but lacking in allosteric site residues–remains an unexplored field. Because allosteric inhibitors act by either destabilizing a PTP's active conformation or stabilizing an inactive conformation, the intrinsic contribution of allosteric sites to overall protein stability remains a critical phenomenon to examine.

My research begins by examining the structural and catalytic effects of an allosteric site in the fourth domain of the human PTP SHP2. I find that SHP2 is rendered less stable than traditional PTPs lacking this allosteric site; this research nuances previous findings in the Bishop lab, which identified that this cite can be targeted by allosteric-acting small molecules with significant inhibitory potency and minimal off-target effects. Building off this finding in SHP2, I broaden my research to other allosteric sites identified in the existing PTP literature. These findings will begin to provide a macroscopic understanding of the intrinsic stability effects of allosteric sites in human PTPs.

#### Poster # 3 Structural and functional characterization of antifreeze protein ApAFP752

Jonathan Sreter and Krisztina Varga Department of Molecular, Cellular, and Biomedical Sciences University of New Hampshire

Antifreeze proteins (AFPs) are specialized adaptations by certain organisms, including fish, plants, fungi, bacteria, and insects, that live in harsh, cold climates. AFPs contribute to freeze resistance by binding to the surface of ice crystals, preventing their growth, and inhibiting ice recrystallization (i.e. small crystals restructuring into large crystals) during temperature fluctuations. AFPs also have the unique property of thermal hysteresis, which is the lowering of the freezing point of water without significantly changing the melting point. The exact mechanism of ice-binding and ice-growth inhibition by AFPs at the molecular level is not fully understood in spite of significant efforts over the past four decades. Here we are presenting structural and functional characterization of an antifreeze protein from the desert beetle Anatolica polita utilizing nuclear magnetic resonance (NMR) spectroscopy and other biophysical methods.

#### Poster # 4 Biophysical methods reveal two high affinity Cu2+ binding sites that potentiate the broadspectrum activity of the antimicrobial peptide Gad-1

#### <u>Jasmin Portelinha</u> and Alfredo Angeles-Boza Department of Chemistry University of Connecticut

Antimicrobial peptides (AMPs) have broad spectrum antimicrobial activity and are predominately short (11-100 amino acid), amphipathic,  $\alpha$ -helical, and cationic peptides. Marine AMPs commonly contain multiple histidine residues, which can comprise metal binding motifs including the amino terminal copper and nickel binding unit (ATCUN) and the HXXXH motif that can potentiate the antimicrobial activity of the AMPs. The ATCUN motif is found in many natural AMPs and consists of an XXH motif at the N-terminus, while the HXXXH motif, coordinates divalent metal ions when in an  $\alpha$ -helix. Gaduscidin 1 (Gad-1, FIHHIIGWISHGVRAIHRAIH-NH2), an AMP found in Atlantic cod fish phagocytic granules, has both an ATCUN and an HXXXH motif that can both bind to Cu2+. Herein, the interplay of Cu2+ ions and Gad-1 was investigated by examining the binding and thermodynamic parameters, the coordination of binding, and the effect on the antimicrobial activity and mechanism in the presence of Cu2+. Isothermal titration calorimetry (ITC) experiments revealed that there is strong binding to Cu2+ at both sites under physiological pH and low pH. Holo-Gad-1 potentiates activity against Escherichia coli eight fold when compared to apo-Gad-1. The mechanism of this potentiation was shown to be lipid peroxidation via Cu-induced ROS formation in the bilayer that was coupled with pore formation, the suspected mechanism of apo-Gad-1. Moreover, electron paramagnetic resonance (EPR) and nuclear magnetic resonance (NMR) evidence was collected to confirm the bound residues and examine the coordination of Cu2+ at both binding sites. These techniques confirmed there are two binding sites and EPR data showed that there was typical square planar binding at the ATCUN site, while the HXXXH motif appeared to bind in a square planar manner via two nitrogen atoms and two oxygen atoms, likely contributed by the imidazole ring from His17 and His21 and water. NMR data showed that His4 may be weakly coordinated to the ATCUN motif to further stabilize binding. Taken together, the inclusion of Gad-1 in phagocytic granules appears to be fortuitous in that during an infection there is an oxidative burst and high levels of Cu2+ present inside of acidic granules. Gad-1 can contribute to the pathogen clearance by binding Cu2+ to potentiate antimicrobial activity and kill intracellularly or extracellularly when there is degranulation.

#### Poster # 5 Anti-viral protein APOBEC3G binds single stranded DNA in multiple steps and conformations to enable efficient search and deamination

<u>Michael Morse</u> and Mark Williams Department of Physics Northeastern University

APOBEC3G (A3G), an innate immune protein expressed in humans and other primates, has the potential to inhibit human immunodeficiency virus type 1 (HIV-1) infectivity in the absence of viral infectivity factor (Vif). A3G is a single-stranded DNA (ssDNA) deoxycytidine deaminase with two domains, a catalytically active, weakly ssDNA binding C-terminal domain (CTD) and a catalytically inactive, strongly ssDNA binding N-terminal domain (NTD). Using optical tweezers, we measure A3G binding a single, long ssDNA substrate under various applied forces to characterize the binding interaction. A3G binds ssDNA in two distinct conformations, distinguished by degree of ssDNA contraction. Quantitative analysis of the force dependence of A3G-ssDNA binding kinetics reveal that A3G binds the substrate in multiple steps. A3G stabilizes formation of ssDNA loops that naturally form in the absence of a strong extending force due to the high flexibility of ssDNA. This binding mode is displayed by individual A3G monomers and does not require A3G oligomerization, suggesting the two domains can bind in an intersegmental conformation. Our data suggests A3G securely binds ssDNA through the NTD, while the CTD samples and potentially deaminates the substrate. Oligomerization of A3G stabilizes ssDNA binding but inhibits the CTD's search function. These processes explain A3G's ability to efficiently deaminate numerous sites across a 10,000 base viral genome during the reverse transcription process.

#### Poster # 6 Native-HXMS: a method to directly measure protein folding and stability under native conditions

<u>Nejc Nagelj</u><sup>1</sup>, Minjee Kim<sup>2,3</sup>, Kaeli Mathias<sup>1,4</sup>, Dr. Sheila Jaswal<sup>2,3</sup> <sup>1</sup>Department of Chemistry, <sup>2</sup>Program in Biophysics and Biochemistry, Amherst College, Amherst MA, 01004, <sup>3</sup>Harvard Medical School, Boston, MA 02115, <sup>4</sup>St. Jude Children's Research Hospital,Memphis, TN 38105

Protein function is intimately linked to the dynamics and energetics of the native state. Most proteins need to fold into a specific conformation and maintain that structure in order to function properly. The conformations sampled, their thermodynamic stability differences, and the transition rates between them are defining features of the protein's landscape. Traditionally, these landscape parameters are extracted from equilibrium denaturation experiments coupled with spectroscopic methods. The aforementioned approach requires numerous measurements as a function of denaturant or temperature, only to then empirically extrapolate results to native conditions. Furthermore, this approach is limited to proteins which can unfold and refold reversibly without misfolding or aggregating under these harsh conditions. Many biologically interesting and disease-related proteins are hence not accessible to these methods. Hydrogen exchange mass spectrometry (HXMS) detects sampling of unfolded states from the native state even under non-denaturing conditions. Using protein L, a well-characterized model protein, we demonstrate that we can extract the free energy of unfolding ( $\Delta GU$ ) from a single HXMS experiment over 30 minutes with less than 10µl of 10µM protein in no denaturant. The HXMSderived  $\Delta GU$  matches that determined from traditional equilibrium denaturation monitored by fluorescence. We will discuss how we are modifying this approach to extract other landscape parameters and access a broader range of experimental conditions. This will allow us to apply N-HXMS to map a more diverse collection of proteins, expanding our understanding of the interplay between protein landscape and function.

#### Poster # 7 Accelerating the GBMV2/SA implicit solvent model using Graphic Processing Units

<u>Xiping Gong</u><sup>1</sup>, Mara Chiricotto<sup>1</sup>, Xiaorong Liu<sup>1</sup>, Erik Nordquist<sup>1</sup>, Michael Feig<sup>2</sup>, Charles L. Brooks III<sup>3</sup> and Jianhan Chen<sup>1,4\*</sup>

<sup>1</sup> Department of Chemistry, University of Massachusetts, Amherst, Amherst, MA 01003, United States, <sup>2</sup> Department of Biochemistry and Molecular Biology, Michigan State University, East Lansing, Michigan 48824, United States, <sup>3</sup> Department of Chemistry, University of Michigan, Ann Arbor, MI, 48109. United States, <sup>4</sup> Department of Biochemistry & Molecular Biology, University of Massachusetts, Amherst, Amherst, MA 01003, United States

Background: The Generalized Born with Molecular Volume (GBMV2) model is one of the best implicit solvent models because of its accurate description of molecular volume and the potential capabilities to describe both structural and flexible proteins. However, its computational efficiency is limited.

My work: The GPU-accelerated GBMV2/SA (GPU-GBMV2/SA) has been implemented in the CHARMM/OpenMM module. It follows the original GBMV2/SA model that provides an accurate computation of GB electrostatic energy and employs an approximate SASA model to describe the nonpolar solvation energy accurately.

Results & Conclusions: Preliminary tests show that the GPU-GBMV2/SA is fully equivalent to the original CPU-GBMV2/SA numerically and thus reproduces the results including the conformational energies and forces. Additionally, it offers more than 80-fold speed up on one regular GPU, indicating that it can enhance the applications in the implicit solvent simulations for larger systems with longer time lengths.

#### Poster # 8 Changes in bacterial chemoreceptor structure and dynamics in response to adaptational modification

#### <u>Nikita Malik</u> and Lynmarie Thompson Department of Chemistry UMASS Amherst

Bacterial chemoreceptors are arranged in arrays composed of helical receptors arranged as trimers of dimers, coupled to a histidine kinase CheA, and a coupling protein CheW. Ligand binding at the external domain generates a transmembrane signal that regulates the kinase activity leading to a change in the swimming behavior. The reaction is controlled by reversible methylation, and demethylation of specific glutamate residues. However, the exact mechanism of signal propagation through the helical receptor to the histidine kinase remains elusive. Dynamics of the receptor cytoplasmic domain are thought to play an important role in the signal transduction and the current models propose inverse dynamic changes in different regions of the receptor. We hypothesize that the adaptational modification controls the dynamics, which in turn modulates the binding of the kinase, CheA.

We investigated the difference in dynamics between the methylated and unmethylated states of the chemoreceptor using solid-state NMR. The unmethylated receptor (CF4E) shows increased flexibility and decreased rigidity relative to the methylation mimic (CF4Q). This combined with the previous HDX studies in our lab suggest destabilization throughout CF rather than inverse changes in dynamics of regions of the receptor. Experiments are in progress to determine the precise locations of the increased flexibility. To assess the structural changes related to the signaling states, 2D experiments (NCA and NCO) that report the regions with low mobility were employed. We estimate that ~25% of our protein is rigid enough to be observed in such experiments, and believe that it includes the protein interaction region of the receptor as it is stabilized due to the interactions with CheA and CheW. Indeed, we were able to obtain wellresolved resonances in the C-alpha and the carbonyl specific spectra that match the number of expected residues in this region. Additional experiments to obtain structural constraints in the receptor are in progress. Our preliminary findings with NMR combined with HDX data suggest that the interaction between the receptor tip and the histidine kinase is altered between the signaling states. The study aims to correlate the dynamic changes in the receptor to modulation of binding and activity of the kinase, to develop a detailed model for the long range signaling.

#### Poster # 9

#### The role of scaffolding peptides in microcompartment assembly

#### <u>Farri Mohajerani</u>, Evan Sayer and Michael F. Hagan Department of Physics Brandeis University

Bacterial Microcompartments (BMC) are icosahedral shells that sequester enzymes inside bacteria. Experimental and computational studies have identified different assembly pathways for assembly of BMCs and have identified the role of cargo interactions and phase separation in determining the assembly pathway. However, many aspects of BMC assembly remain unclear, including what factors control the size of assembled shells and what controls cargo phaseseparation and encapsulation. One such factor is scaffolding proteins, long polypeptides that drive aggregation of cargo and recruit shell subunits. We use coarse-grained computational and theoretical modeling to study the role of scaffolding proteins on BMC assembly. We model scaffolds as polymers of variable length and perform simulations with multi-domain-scaffolds to investigate the experimental observation that BMC shell size is correlated to the scaffold length. We found that shell assembly occurred by different pathways depending on whether or not the polymer-cargo interaction strength was large enough to drive phase separation of polymer-cargo complexes. For both types of pathways, short polymers caused shells to become smaller than the empty shells, while long polymers caused them to become larger, meaning that polymer length is a key factor controlling shell size. However, the phase-separating polymer-cargo complexes allowed complete shells to form over a wider range of interaction strengths than the non-phaseseparating cases, and the largest simulated microcompartments assembled around phaseseparating polymer-cargo complexes.

#### Poster # 10

#### Self-assembly of lobed particles into porous structures.

Sanjib Paul and Harish Vashisth Department of Chemical Engineering University of New hampshire

Colloidal patchy particles in recent years have emerged as novel building blocks which selfassemble into higher order structures dictated by their shapes and directional interactions. Depending on the specific techniques used and the choice of starting ingredients it has been possible to synthesize soft and hard type patchy particles at different length scales. Although a significant effort has been made to prepare these kind of colloidal particles, extensive study on their self-assembly, mainly for the lobed particles (patchy particles where patches appear as lobes around a seed) is yet to be explored. In this work, we have studied the self-assembly of seven different hard lobed particles of different shapes using coarse-grained Langevin dynamics simulations and show that various types of unique self-assembled morphologies can be achieved by tuning the attractive interactions between the lobes. The linear building blocks having two lobes around the seed show self-assembly only at higher attractive interaction and were found to form rings of different sizes in their self-assembled structures. The trigonal planar building blocks were found to generate cylindrical hollow tubes at lower interaction and two-dimensional sheets at higher attractive interaction. The square planar building blocks were found to form spherical clathrates. The tetrahedral, trigonal bipyramidal and octahedral shaped particles were found to form compact porous crystalline structures which are constituted by either hexagonal close packed or face centered cubic lattices. The pore size distributions are also estimated for the self-assembled structures. Depending on the higher order structures produced, these lobed particles may have potential applications in various fields like tissue engineering, in the study of host guest chemistry, adsorption, and catalysis.
# Poster # 11 Beta-branched amino acids stabilize specific conformations of cyclic hexapeptides

<u>Jiayuan Miao</u> and Yu-Shan Lin Department of Chemistry Tufts University

Cyclic peptides (CPs) are a promising class of molecules for drug development, particularly as inhibitors of protein-protein interactions. Predicting low-energy structures and global structural ensembles of individual CPs is critical for the design of bioactive molecules, but these are challenging to predict and difficult to verify experimentally. In our previous work, we used explicit-solvent molecular dynamics simulations with enhanced sampling methods to predict the global structural ensembles of cyclic hexapeptides containing different permutations of glycine, alanine, and valine. One peptide, cyclo-(VVGGVG) or P7, was predicted to be unusually well structured. In this work, we synthesized P7, along with a less well-structured control peptide, cyclo-(VVGVGG) or P6, and characterized their global structural ensembles in water using NMR spectroscopy. The NMR data revealed a structural ensemble similar to the prediction for P7 and showed that P6 was indeed much less well-structured than P7. We then simulated and experimentally characterized the global structural ensembles of several P7 analogs and discovered that  $\beta$ -branching at one critical position within P7 is important for overall structural stability. The simulations allowed deconvolution of thermodynamic factors that underlie this structural stabilization. Overall, the excellent correlation between simulation and experimental data indicates that our simulation platform will be a promising approach for designing wellstructured CPs and also for understanding the complex interactions that control the conformations of constrained peptides and other macrocycles.

# Poster # 12 Effects of cyclization on properties of peptides backbones and the length dependence

<u>He Huang</u> and Yu-Shan Lin Department of Chemistry Tufts University

Cyclization of peptides brings new perspectives to peptide properties and applications in drug design and new therapeutic discoveries. Cyclic peptides (CPs) are thought to be more enduring and potent in targeting and interfering protein-protein interactions. However, the fundamental mechanism of CPs' essential conformational transitions has not be understood clearly, which prohibits effective CP study and design. For peptides of various lengths, the underlying thermodynamic and kinetic differences between CPs and their linear counterparts (LPs) have not been investigated in a systematic manner, which is the main focus of this study. The effect of peptide lengths in both cyclic and linear has also been compared and characterized to enrich the understanding of their distinctions. Shed light by atomic-detailed molecular dynamics simulations in explicit waters, the CPs of smaller than 8-mers respectively manifest unique structural ensembles in terms of configurational entropies of backbone dihedral angles, while larger CPs gradually lose the uniqueness and behave more similar to their linear counterparts. The dynamics of both CPs and LPs, however, seems to share similar elementary mechanisms in internal dihedral hopping and peptide bond flipping, which could not explain the large end-toend distance changes in LPs. Therefore, more efforts have been paid into investigating the distinct essential conformational transitional motions between CPs and LPs. These findings overall are helpful in explaining the different behaviors and properties of CPs from LPs. Furthermore, the uniqueness of small CPs provides new instructive principles for which protein design could benefit from.

#### Poster # 13 Structural and biochemical characterization of the FYVE domain of ALFY

# <u>Erin F. Reinhart</u>, Maria Pellegrini, and Michael J. Ragusa Department of Chemistry Dartmouth College

Damaged or aggregated proteins can accumulate with age and contribute to various age-related protein pathologies including Alzheimer's, Parkinson's, and Huntington's diseases. Autophagy can facilitate the degradation of these protein aggregates by encapsulating them in a double membrane vesicle called the autophagosome and transporting them to the lysosome for degradation. ALFY (Autophagy-linked FYVE protein) is a 400-kDa scaffolding protein that is important for the selective degradation of protein aggregates via autophagy. ALFY consists of a number of domains capable of facilitating protein-protein interactions and contains a FYVE domain at its C-terminus. FYVE domains recognize phosphitylinositol-3-phosphate (PI(3)P) and anchor the proteins containing these domains to membranes enriched with PI(3)P, such as the endosomal and the autophagic membranes. Although ALFY contains a FYVE domain, this protein has a unique localization in cells as compared to other FYVE domain containing proteins. This suggests that the FYVE domain of ALFY may be distinct from other FYVE domains. To investigate this, we determined the structure of the FYVE domain of ALFY using nuclear magnetic resonance and performed liposome sedimentation assays to assess the ability of this domain to bind to PI(3)P. We identified a highly conserved glutamic acid residue that affects both liposome binding as well as localization.

### Nanoluciferase maturation upon release from the ribosome

Denis Susorov and Andrei Korostelev RNA Therapeutics Institute UMass Medical School

Denis Susorov and Andrei A. KorostelevNanoluciferase is widely used as a reporter for studying gene expression in cells. This small (19 kDa) enzyme emits light upon oxidizing its specific substrate furimazine. However, little is known about the timing of folding and maturation of nanoluciferase during its synthesis on the ribosome. It is therefore unclear whether the reporter gets active while only partially translated, so it could glow when translation or peptide release is stalled under certain cellular conditions. Here we use cell-free translation system and show that nanoluciferase is inactive while on the ribosome, but acquires activity nearly instantaneously upon release from the ribosome.

## Chloride channels or phospholipid scramblase: a gatekeeper story

Zhiguang Jia and Jianhan Chen Department of Chemistry UMass

Despite highly sequence and structural similarity, two members in transmembrane protein 16 family (TMEM16) were found to transport significant different cargoes cross membrane. TMEM16A is a Calcium-activated chloride channels (CaCCs) which are critical in regulating neural excitability, nociception, smooth muscle contraction, secretion and gut motility. On the other hand, TMEM16F is an enigmatic Ca2+-activated phospholipid scramblase (CaPLSase) that passively transports phospholipids down their chemical gradients and mediates blood coagulation, bone development and viral infection. Despite recent advances in the structure and function understanding of TMEM16 proteins, how mammalian TMEM16 CaCCs/CaPLSases open and close, or gate, their ligand permeation pathways remains unclear. Combining experimental and simulation techniques, we identified an inner activation gate in similar position of TMEM16A/F , which is established by three hydrophobic residues in the middle of the chloride/phospholipid permeation pathway. Strikingly, an analogous mutation of gate residue to TMEM16F in TMEM16A is sufficient to confer CaPLSase activity to the Ca2+-activated Cl- channel (CaCC). The identification of an inner activation gate can help elucidate the gating and permeation mechanism of TMEM16 CaCC/CaPLSases channels.

### The nanomechanics of cellulose synthesis

Lori Goldner Department of Physics UMass Amherst

Despite its ubiquity and technological importance, little is known about how plants produce cellulose. In plants, cellulose is crystalline fiber synthesized by complexes (CSCs) resident in the cell membranes. Each CSC produces multiple glucan chains, which then crystallize into the cellulose microfibrils that partially comprise the cell wall. CSCs are known to move slowly and in a linear fashion as they make cellulose, averaging between 100 and 400 nm/s. This movement is believed to be driven by the growth of the rigid cellulose microfibril, which has been modeled as a Brownian polymerization ratchet, rather than by a molecular motor. Careful study of CSC motion may therefore also reveal the dynamics or mechanical properties of the native microfibrils. We track the motion of individual CSCs in the cell membranes of living plants for up to 7 minutes with resolution of a few nanometers and time resolution down to 300 ms. We find evidence for a sub-diffusive mechanism and long timescale relaxation that are not explained by the current model.

## Poster # 17 Structural studies of the regulators of a G-protein signaling protein

<u>Ryan Puterbaugh</u> and Krisztina Varga Department of Molecular, Cellular, and Biomedical Science University of New Hampshire

G-protein coupled receptors (GPCRs) are involved in a host of cellular interaction and have been linked to cardiovascular, neurological, and visual disorders as well as many health risks such as diabetes and cancer. The regulators of G-protein signaling (RGSs) have become important targets for new therapeutic interventions, as the inhibition of these proteins could decelerate GTPase activity affecting the termination of GPCR signals within the cell. Thiadiazolidione (TDZD) analogues have been identified as allosteric inhibitors of RGSs, however the exact mechanism for how these small molecules bind to the RGS protein is not fully understood. Single turnover GTPase assays determined that potency of TDZD analogues in the presence of RGS4 and RGS8 decreased with the elimination of cysteines within the proteins suggesting that cysteines are critical for binding even though they are buried within the protein interior. This poses a problem for the TDZD cysteine binding model however, the formation of a dynamic pocket within the protein that exposes the cysteines to the exterior of the protein was proposed as an explanation for how the TDZD gains access to the cysteines. This hypothesis was supported by hydrogen deuterium exchange (HDX) experiments which have revealed that the  $\alpha$ 4 and  $\alpha$ 6 helices of RGS isoforms containing the cysteine residues are highly flexible. Additionally, molecular dynamics (MD) models suggest that a dynamic pocket with exposed cysteines could form, allowing TDZD to bind to the protein. The goal of the Varga research group is to characterize the ligand binding interface of RGS8 and experimentally characterize protein flexibility and dynamics utilizing nuclear magnetic resonance (NMR) spectroscopy. Successful characterization of the interactions between RGS8 and TDZD inhibitors will bring new understanding of the RGS proteins that can be applied to rational drug design for targeting specific proteins.

# Poster # 18 Dynamics of the Rap1A active site uncovered by molecular dynamic simulations

<u>Michael Schwabe<sup>1</sup></u>, Genevieve A. Holzapfel<sup>2</sup> and Carla Mattos<sup>1</sup> <sup>1</sup>Department of Chemistry & Chemical Biology, Northeastern University <sup>2</sup> North Carolina State University

GTPases are hydrolytic proteins that convert GTP into GDP and serve as bivalent switches, where the GTP-bound state is active and the GDP-bound state is inactive. Rap1A is a GTPase with 50% sequence homology and similar tertiary structure to the archetypal GTPase Ras, with a key difference occurring at position 61. In Ras Q61 participates in the hydrolysis mechanism and mutation leads to oncogenesis, while T61 in Rap1A is not thought to be involved in catalysis. The goal of this project is to explore the Rap1A active site and its mechanism of intrinsic hydrolysis. Two crystal structures were obtained with differing conformations of T61 in the Rap1A active site. Accelerated molecular dynamic simulations were run for 200 ns, starting with each of the structures, to sample the variety of motion present in Rap1A. Results indicate coordinated movements between the switch I, switch II, and helix 3 regions. Further, switch II appears less mobile than simulations with Ras isoforms.

## Poster # 19 Computational studies on the effect of the chemical environment of the DNA guanine quadruplex on the free energy of binding Na and K cations

<u>Mahmoud Sharawy</u> and Styliani Consta Department of Molecular and Cell Biology University of Connecticut

Guanine quadruplex (G-quadruplex) structures play a vital role in stabilizing the DNA genome and in protecting healthy cells from transforming into cancer cells. The structural stability of Gquadruplexes is greatly enhanced by the binding of monovalent cations, such as Na+ or K+, into the interior axial channel. We compute the free energy of binding of Na+ and K+ ions to two intramolecular G-quadruplexes that differ considerably in their degree of rigidity and the presence or absence of terminal nucleotides. The goal of our study is two-fold. One, we study the free energy of binding every ion, which complements the experimental findings that report the average free energy for replacing Na+ with K+ ions. Two, we examine the role of the Gquadruplex structure in the binding free energy. In the study, we employ all-atom molecular dynamics simulations and the alchemical transformation method for the computation of the free energies. Numerical results presented in our studies may offer reference values for the future design of cationic drug-like ligands that replace the metal ions in G-quadruplexes to stabilize its structure which may prevent the formation of cancer.

## Poster # 20 Determining conformational dynamics of the allosteric site in HRas R97G

Monica Ojeda, Christian Johnson and Carla Mattos Department of Chemistry and Chemical Biology Northeastern University

HRas is a small GTPase that acts as a molecular switch for the Raf/MEK/ERK pathway that leads to cell proliferation, survival, and differentiation. Oncogenic mutations in HRas renders it unable to hydrolyze GTP to GDP, resulting in constitutive activation of HRas and cell proliferation. Additionally, HRas contains an allosteric site that binds Ca2+ and a negatively charged ligand, promoting helix 3 to shift towards helix 4 and the ordering of switch II. This conformation is associated with intrinsic hydrolysis of HRas in the presence of Raf. Experiments have been performed in our lab to study the effects of point mutations in the allosteric site of HRas on intrinsic hydrolysis constants. When compared to wild-type, HRas R97G demonstrated increased intrinsic hydrolysis when alone and a significant decrease when in the presence of Raf. Molecular dynamics simulations were utilized to further gain insight on how the dynamics of the allosteric site differed in HRas R97G compared to the wild-type. These simulations demonstrated increased flexibility in the upper region of helix 3, with greater accessibility to the catalytic conformation for GTP hydrolysis.

# Poster # 21 Simulation guided design of a cyclic peptide growth hormone receptor inhibitor

<u>Omeir Khan</u> and Yu-Shan Lin Department of Chemistry Tufts University

Human growth hormone (hGH) is a peptide hormone that is produced in the pituitary gland. Human growth hormone receptor (hGHR) exists as a constitutive dimer and upon binding of hGH, the extracellular domains of the two hGHR chains interlock, initiating cell signaling pathways that induce cell proliferation and inhibit apoptosis. Excess production of hGH leads to an increase in signaling activity, resulting in acromegaly, a disorder that has been linked to a number of morbidities, including osteoarthritis, polyp formation, and cardiomyopathy. Currently an hGHR antagonist exists in the form of Pegvisomant, a hGH analog. However, due to its large size and proteinaceous nature, Pegvisomant lacks oral bioavailability and must be administered through daily subcutaneous injections. This study aims to provide an improved hGHR antagonist in the form of a novel cyclic peptide. Cyclic peptides are promising inhibitors of protein-protein interactions due to their potential to bind to a protein surface with a high specificity while also having improved bioavailability compared to their linear counterparts. A loop-mediated proteinprotein interaction, known as a "hot loop", that contributes to the interlocking of the dimerized hGHR complex has been identified. The observation of this hot loop has led us to hypothesize that a cyclic peptide mimicking the hot loop sequence and structure can be developed to inhibit interactions between hGHR dimer subunits, thus preventing hGHR activity. The approach to designing such a cyclic peptide involves linking the hot loop sequence in order to create a cyclized peptide, running molecular dynamics simulations in order to analyze the structural ensembles of newly created cyclic peptides, and then modifying linker sequences in order to create a new cyclic peptide sequence that is more likely to mimic the target hot loop structure. The project serves to provide new insights into the viability of cyclic peptide inhibitors for hGHR dimer subunit interactions while also providing support that validates computational approaches to rationally designing cyclic peptide inhibitors for specific protein–protein interactions.

### DNA shape recognition by 'guardian of the genome' p53

Dmitrij Golovenko<sup>1,2</sup> and Andrei Korostelev<sup>1</sup> <sup>1</sup>RNA Therapeutics Instutute, UMass Medical School <sup>2</sup>Zippora Shakked, Weizmann Institute of Science

Human tumor suppressor p53, also known as 'guardian of the genome', is a transcription factor that regulates hundreds of genes in response to the various cellular stress signals. Its main functional module is DNA-binding domain (DBD) which functions as homotetramer binding specifically to double stranded DNA response elements (REs). Each RE is composed of two decameric half-sites of general form RRRCWWGYYY (R = A, G; W = A, T; Y = C, T). It came as surprise that in the crystal structures of the DBD complexed with consensus RE that central A/T doublets within conservative CATG motifs (bold) in both half-sites, surprisingly have Hoogsteen base-pairing (HG) (Kitayner2010). Hence, all four A bases are in syn conformation, pushing adjacent C1' atoms in A/T base pairs closer comparing to one of Watson-Crick pairing (WC): 8.5 Å vs 10.4 Å, correspondingly. Interestingly, such base-pairing, was found to be transient (0.4%) in free DNA duplex in solution at physiological pH (Nikolova 2013).

Here we describe method to stop flip base-pairing of WC/HG geometry to characterize effect of response element shape on interactions with p53 both crystalographically and biochemically. Approach is in designing two distinct REs were both central A/T doublets are replaced by modified nucleotides in a way that both avoids introduction of bulky groups and preserves minor groove composition of corresponding natural WC/HG A:T base-pair. Thus, to shift natural equilibrium toward HG geometry Adenine bases were replaced by 2-oxo-Adenine. For 'locking' WC geometry instead of Adenine and Thymine bases were introduced Inosine and 5-methyl-Cytosine, correspondingly. Such modified REs were co-crystallized with DBD producing high-quality p53-DNA crystal structures, each in two different crystal forms. Both types of 'locked' REs have only designed HG (LHG) or 'locked' WC (LWC) type of base-pairing. Comparison of the structures revealed that DNA in both complexes mostly differ in ~2 Å narrower helix diameter of LHG DNA at the center of each half-site. Also, complexing LHG increased total buried surface of DBD tetramer by 300 Å2 vs one bound to LWC. This difference might explain relatively higher binding affinity of p53 to LHG DNA as well as increased half-life of such complex vs one with LWC DNA. These biochemical effects were consistent for p53 constructs of different length. Furthermore, we show that palindromic RE with two CATG motifs that is displaying all WC geometry in complex with non-symmetrical DBD tetramer, has to all four A:T base pairs in HG conformation when crystalized bound to symmetrical DBD tetramer forming continuous DNA duplex. Presented here crystal structures of p53-DNA complexes and biochemical analysis support the hypothesis that p53 prefer REs that are adopting rare Hoogsteen conformation highlighting role of DNA shape among mechanisms of specific DNA recognition.

## Can we mimic evolution for the rapid design of novel enzymes?

Mary Jo Ondrechen Department of Chemistry & Chemical Biology Northeastern University

Imagine a world where industrial chemical processes are catalyzed enzymatically at ambient temperature under neutral conditions, with lower energy consumption and producing fewer unwanted by-products than conventionally catalyzed or thermal processes. With few exceptions, an enzyme that has the specific desired capability for industrial application does not exist in nature. Currently the computational design of novel enzymes is able to produce stable folds but with very low catalytic activity; years of effort and many rounds of directed evolution are then required to produce a novel enzyme with catalytic activity on par with natural enzymes. The question then remains: What properties are imparted by directed evolution and can we build in these properties earlier in the design process? We analyze natural enzymes and evolving families of designed enzymes and report on the electrostatic and chemical properties that characterize those constructs with good catalytic activity. Interactions between catalytic residues and their neighboring amino acids are important features in natural enzymes. We show that these couplings increase with increasing activity in series of evolving artificial enzymes.

## Poster # 24 Continuum electrostatics of bio-membranes: Multiscale modeling of boundary condition using a Solvation-Layer Interface Condition (SLIC)

Ali Mehdizadeh<sup>1</sup>, Ming Gao<sup>2</sup> and Steven Lustig<sup>2</sup> <sup>1</sup>Mechanical Engineering and <sup>2</sup> Chemical Engineering, Northeastern University

Implicit solvent models have employed continuum theory to describe the electrostatic interactions between biomolecules and the surrounding solvents for years. These models are simpler to implement and orders of magnitude faster than explicit-solvent molecular-dynamics (MD). Macroscopic continuum theory, however, exhibits substantial deviations from experimental results when applied to systems of very short (atomistic or near-atomistic) length scales. The most significant errors, in particular, are due to the continuum theory's poor treatment of the first solvation shell of solvent molecules (the first layer of the solvent molecules surrounding the solute). SLIC (Solvation-Layer Interface Condition) has been shown to make predictions of solvation energies and thermodynamics in bio-molecules remarkably more accurate with a simple modification to the classical boundary condition. This talk will focus on the implication of SLIC for a large-scale problem and assesses the accuracy of the model in modeling the properties of water and ionic aqueous solutions in the vicinity of phospholipid bilayers. The influence of the shape of a bilayer membrane on the electrostatics of solvation and electrostatic potential of mean force between bilayer membranes and charged particles will also be discussed.

## Poster # 25 Modulation of p53 transactivation domain conformations by ligand binding and cancerassociated mutations

# <u>Xiaorong Liu</u> and Jianhan Chen Department of Chemistry UMass Amherst

Intrinsically disordered proteins (IDPs) are functional proteins that lack well-defined 3D structures under physiological conditions. Mutations and/or changes in their cellular concentrations are often associated with numerous human diseases. A particularly interesting example is tumor suppressor p53, one of the most frequently mutated proteins in human cancers. The stability and activity of p53 are tightly controlled by its interactions with key regulators, which are mainly mediated by its intrinsically disordered transactivation domain (TAD). The disordered ensemble of p53-TAD is poised to respond sensitively to all sorts of cellular signals, including posttranslational modifications, mutations, and ligand binding, thus acting as a central conduit of p53 regulation. However, it remains challenging to establish the sequencedisordered ensemble-function-disease relationship of p53-TAD, since it requires detailed characterization of the structural ensemble of p53-TAD which doesn't lend itself to traditional experimental measurements. In this work, we utilized physics-based atomistic force field a99SBdisp in combination with replica exchange with solute tempering (REST2) enhanced sampling technique to characterize the conformational ensemble of p53-TAD and study how it was modulated by four cancer-associated mutations (K24N, D49Y, W53G and N29K/N30D) and a wellknown anti-cancer drug epigallocatechin gallate (EGCG). Our results suggest that mutation N29K/N30D and binding of EGCG could induce compaction of p53-TAD, which shields its functional site and inhibits its recognition of targets. The current study enhances our understanding of how ligand binding and cancer-associated mutations modulate the structure and function of p53-TAD. It also demonstrates that atomistic simulations provide a viable approach for studying the sequence-disordered ensemble-function-disease relationships of IDPs.

# Poster # 26 Maximum entropy optimized force field for intrinsically disordered proteins

Andrew Latham and Bin Zhang Department of Chemistry MIT

Intrinsically disordered proteins (IDPs) perform critical roles in a variety of biological processes, including the formation of membraneless organelles through liquid-liquid phase separation. As they cannot be described by a single structure, IDPs are often studied as ensembles of conformations. Therefore, efforts have tried to detail IDP ensembles through computer simulation; however, systematic improvement of such models remains challenging. As a result, we present an algorithm to parameterize coarse-grained force fields by combining maximum entropy optimization and least squares regression. We have successfully created and applied our new maximum entropy optimized force field (MOFF) to study IDP structures. We anticipate the accuracy and efficiency of MOFF will enable future study of IDP phase separation.

## Poster # 27 Large structural changes during the Lassa Virus nucleoprotein trimer to monomer transition revealed by Markov State Modeling

Jason G Pattis and Vincent Voelz Department of Chemistry Temple University

Lassa virus is an enveloped negative strand RNA virus that causes a severe hemorrhagic fever, Lassa fever, which leads to about 5000 deaths per year. Lassa contains a nucleoprotein (NP) which encapsulates the viral genomic RNA forming the ribonucleoprotein (RNP). The first crystal structure solved of the Lassa virus NP, was a trimer structure with no exposed RNA binding site. A subsequent crystal structure of only the N-terminal domain was co-crystalized with RNA bound showing several large conformational changes, including the opening of helix 6, the loss of helicity of helix 5 and shifting down of a loop. The suggested model is that NP forms a trimer to keep the RNA gate closed, preventing off target binding. Previous work has investigated helix 6 opening and has suggested that RNA may make contact with a partially open NP, which may facilitate full binding. The current work investigates the scenario in which the trimer is disrupted to observe if a monomeric NP undergoes a conformational change that would allow for increased access to the RNA binding pocket. We have conducted molecular dynamics simulations using long time scale simulations on specialized hardware and a two stage adaptive sampling scheme to sample this transition. From the trajectories, a Markov State Model was constructed to describe the energetics of the transition, which reveals an energetically favorable conformational change. The most significant changes occur at the domain interface including the shifting out of helix 8 and 9 which may allow room for RNA to contact the RNA binding pocket, supporting a model in which significant structural reorganization of the NP is required for RNP formation.

### Single molecule binding dynamics of LINE-1 ORF1p to ssDNA

M. Nabuan Naufer<sup>1</sup>, <u>Ben A. Cashen<sup>1</sup></u>, Charlie E. Jones<sup>2</sup>, Anthony V. Furano<sup>2</sup>, Mark C. Williams<sup>1</sup>

<sup>1</sup> Northeastern University, Department of Physics, Boston, MA 02115, USA <sup>2</sup> The Laboratory of Molecular and Cellular Biology, NIDDK, NIH, Bethesda, MD, 20892, USA

Long interspersed nuclear element 1 (LINE-1 or L1) is an intragenomic parasitic element that makes up ~20% of the human genome. It amplifies in the host genome by copying its RNA transcript into genomic DNA through a process called retrotransposition. L1 encodes two proteins, ORF1p and ORF2p, that associate with their parent transcript to form a ribonucleoprotein complex (L1 RNP), an essential intermediate in L1 retrotransposition. Detailed mechanistic understanding of L1 retrotransposition is sparse, particularly with respect to the function of ORF1p, a coiled coil-mediated homotrimeric nucleic acid chaperone that can form tightly packed oligomers on nucleic acids. Here we studied three ORF1 proteins: a modern human one (111p), an inactive mutant (m15) wherein a single residue substitution within its coiled coil domain halts retrotransposition activity, and an active mutant (m14) with a largely modern coiled coil. We characterize ORF1p-nucleic acid interactions using an ~8 kbp ssDNA molecule and measure its binding kinetics while the ssDNA is held at constant force. We show that ORF1p binds to ssDNA in a biphasic manner, in which a rapid decrease in ssDNA extension is followed by a relatively slow elongation of the ORF1p-ssDNA complex. The biphasic behavior observed here qualitatively mimics E. coli SSB binding dynamics to ssDNA. Therefore, for the first time, this study provides compelling evidence that ORF1p wraps ssDNA in multiple conformations. In addition to wrapping ssDNA, we show that the ORF1p-ssDNA complex undergoes secondary compaction due to well-established protein-protein interactions. We present preliminary data which suggest that the ability to form stable oligomers on ssDNA is relatively deficient in the inactive m15 mutant when compared to the modern human 111p and the active m14 mutant, providing novel insights into ORF1p function.

### Novel approaches to RNA synthesis by T7 RNA polymerase

Craig Martin Department of Chemistry University of Massachusetts Amherst

Mechanistic studies of RNA function require highly pure RNA, yet T7 RNA polymerase, the preferred vehicle for in vitro RNA synthesis, is well-known to generate products other than the length directly encoded by the DNA template. This is particularly problematic for "high yield" syntheses (most end users). In this work, we confirm the origins of a major class of impurities and demonstrate approaches that generate dramatically improved RNA, both high purity and higher yield than conventional approaches. In this work, we also introduce RNA-Seq as an analytical tool to characterize transcription reaction products. This approach also opens up new studies of mechanism and structure.

#### 30 Using structure reservoirs to accelerate biomolecular simulations

# <u>Koushik Kasavajhala</u> and Carlos Simmerling Department of Chemistry Stony Brook University

Molecular Dynamics (MD) simulations have become an indispensable tool to understand biological processes such as protein folding, drug binding, and protein-protein interactions besides many others. However, standard MD is limited by the slow sampling of conformational space and obtaining converged Boltzmann-weighted conformational ensembles still takes a long time (weeks to months) even on the latest computational hardware. To alleviate this problem, our lab previously developed the Reservoir Replica Exchange MD (Reservoir REMD) to accelerate convergence of biomolecular simulations. In Reservoir REMD, an unweighted (non-Boltzmann) set of pre-generated structure snapshots representing different local minima are used as a reservoir. Monte Carlo moves are then attempted between the reservoir structures and the REMD replicas.

In this work, we built on the method and show that structure reservoirs can be used to reproduce accurate Boltzmann-weighted ensembles obtained by much more expensive conventional REMD, with at least 25x faster convergence rates even for larger proteins (>50 amino acids) compared to conventional REMD. We have also tested the method on RNA systems and obtained similar convergence speeds. Furthermore, we also show that structure reservoirs can be used to predict accuracy of new force fields, thereby, accelerating force field development. Finally, structures obtained from non-MD methods such as homology-based models or Rosetta-based models and structures obtained from other enhanced sampling techniques can be integrated into this method, making it a powerful tool that combines physics-based approaches with non-physics-based approaches leading to better structure predictions of biomolecules. The GPU Reservoir REMD code is now available for use with the AMBER suite of programs.

Poster # 30

## Poster # 31 N-terminal acetylation affects phase separation and aggregation of the low complexity domain of RNA-binding protein FUS

<u>Anna Bock</u> and Nicolas Fawzi Biotechnology Graduate Program Brown University

N-terminal acetylation, the most common post-translational modification (PTM) in mammals, alters the charge and interactions of the N-terminus of proteins. This PTM has been shown to promote helicity(Maltsev, Ying, & Bax, 2012) and protect from degradation(Arnesen et al., 2010), yet its effect is unknown for most proteins. FUS (Fused in Sarcoma) is a ubiquitous protein with roles in RNA metabolism and processing(Yamaguchi & Takanashi, 2016). FUS is physiologically acetylated at the N-terminus and is known to assemble via liquid-liquid phase separation (LLPS) into functional RNA granules and to aggregate into ALS-associated neuronal inclusions. Importantly, N-terminal tags are known to alter the behavior of FUS, yet N-terminal acetylation has been absent from previous in vitro studies using recombinant protein. Here we tested the effect of N-terminal acetylation on the structure, LLPS, and aggregation of the disordered, prionlike domain comprising the first 163 residues (FUS LC). Using NMR spectroscopy, we find that Nterminal acetylation has no significant enhancement of helical structure and slight slowing of local motion of the first 3 residues of FUS LC. Conversely, we found that acetylated FUS LC phase separates more avidly than unacetylated FUS LC. This difference in LLPS may arise due to Nterminal acetylation removing the positively charged NH3 of the nearly uncharged (only 2 negatively charged residues, no positively charged residues) FUS LC. We also report our preliminary findings regarding the effect of N-terminal acetylation on FUS LC aggregation and the phase separation and aggregation.

### Analysis of enhanced NMR peak fitting method

<u>Joshua Dudley</u> and Colin Smith Department of Chemistry Wesleyan University

Nuclear magnetic resonance (NMR) offers incredible potential for insight into the structure and dynamics of proteins. Obtaining this molecular information requires reliable extraction of peaks parameters from an NMR spectra, including their intensity, position, and volume. However, substantial difficulty can arise in crowded regions due to overlapping and coalesced signals. Using traditional methods, the degree of signal overlap, in particular, limits the information that can be derived. A new method, FITNMR, has been developed, compiled in an R package, and applied to computationally separate signals. The traditional approaches serve as a benchmark to compared the determined parameters. Values were calculated from both simulations at varying noise levels and raw experimental data to compare each of the methodologies. When minimal preprocessing, such as apodization, is applied to NMR data, the novel method is more accurate in quantifying increasingly small scalar couplings. Each method was evaluated using the root mean squared error (RMSE) in the parameters across the noise levels introduced to the simulated data. Experimental data sets were also used to compare scalar couplings calculated from the new method against structure based scalar couplings determined from the Karplus relationship.

### Theory of active chromatin remodeling

Zhongling Jiang and Bin Zhang Department of Chemistry Massachusetts Institute of Technology

Nucleosome positioning controls the accessible regions of chromatin and plays essential roles in DNA-templated processes. ATP driven remodeling enzymes are known to be crucial for its establishment, but their non-equilibrium nature has hindered the development of a unified theoretical framework for nucleosome positioning. Using a perturbation theory, we show that the effect of these enzymes can be well approximated by effective equilibrium models with rescaled temperatures and interactions. Numerical simulations support the accuracy of the theory in predicting both kinetic and steady-state quantities, including the effective temperature and the radial distribution function, in biologically relevant regimes. The energy landscape view emerging from our study provides an intuitive understanding for the impact of remodeling enzymes in either reinforcing or overwriting intrinsic signals for nucleosome positioning, and may help improve the accuracy of computational models for its prediction in silico.

### Dynamics and water transport in artificial water channels

Yong Liu and Harish Vashisth\* Department of Chemical Engineering University of New Hampshire, 33 Academic Way, Durham, NH03824 \*Harish.Vashisth@unh.edu

Peptide appended pillar[5]arene (PAP) is an artificial water channel resembling biological water channel proteins, which has shown promise for designing bioinspired water purification systems. Insertion of PAP channels into a membrane matrix is required for applications in separation devices, which unavoidably results in membrane perturbations due to channel-membrane interactions and may affect the water dynamics and transport in artificial water channels.

To better understand the nature of interactions between PAP channels and membranes, we have carried out atomistic MD simulation studies of a single PAP channel embedded in a lipid as well as a polymeric membrane matrix. Specifically, we have characterized PAP behaviors and identified several metastable states highlighting differences in channel dynamics in biological (lipid) and synthetic (polymeric) membranes. We will also present results on the functional transport characteristics of PAP channels where we observed wetting/dewetting transitions indicating a gating mechanism.

# Poster # 36 Deciphering PRC2-mediated nucleosomal interactions for controlling gene regulation

<u>Xingcheng Lin</u> and Bin Zhang Department of Chemistry Massachusetts Institute of Technology

Polycomb Repressive Complex 2 (PRC2) is a chromatin modification protein responsible for epigenetic silencing of genes. The mechanism underpinning this PRC2-induced gene regulation, however, is unclear. Recently, our single-molecule force spectroscopy revealed unexpected physical contacts between PRC2 and non-adjacent nucleosomes. To further probe into the underlying mechanism behind these long-range interactions, we harness tools of molecular modeling based on a newly developed coarse-grained protein-DNA model to investigate the molecular interactions between PRC2 and a tetranucleosome system. Our results not only confirm the PRC2-mediated non-adjacent nucleosomal interactions driven by electrostatic forces but also establish a detailed thermodynamic landscape uncovering the potential of PRC2 in regulating the thermodynamic stability of chromatin systems.

### Poster # 37 Validation of Hydrogen Exchange Mass Spectroscopy (HXMS) Native-State Protein Stability Method

# Jasper Flint, Isabella Han and Sheila Jaswal Department of Chemistry Amherst College

Protein function depends on the structure, stability and dynamics of the folded, or native state. All of these are captured in a free energy landscape model of a protein. Traditionally, the thermodynamic stability and rates of conversion between protein conformational states have been investigated through denaturing the native protein. Here we describe advances we have made in extracting quantitative landscape measures from hydrogen exchange mass spectrometry (HXMS) applied to native state proteins, without extrapolation from denaturing conditions. We use a numerical simulations approach integrating a Monte Carlo Markov Chain algorithm to fit native HXMS time-courses. This method captures a wide range of native-state dynamics ranging from residue-level fluctuations to cooperative global unfolding. We first validated our simulations approach by benchmarking against analytically calculated results for a simple two-state system. We have used the simulations tool to detect on-pathway intermediates populated under native conditions for the amyloidogenic protein b2M and the bacterial toxin CcdB. With the ability to accurately capture native state behavior using our combined experimental and computational HXMS method, we are poised to explore the landscapes of a more diverse set of proteins.

#### # 38 Unfolding Nature's origami: Acetonitrile's role as a protein denaturant

Amritha Anup and Sheila Jaswal Department of Chemistry Amherst College

Protein function is determined not only by the structure of the folded, or native, conformation, but also by the stability and dynamics of the native state. To study folding, stability, and dynamics, denaturants such as Urea and GdmCl are used to perturb the native state, enabling detection of non-native conformations through spectroscopic methods. Linear extrapolation of multiple measurements as a function of denaturant concentration allows for extrapolation of  $\Delta$ GNU to conditions in the absence of denaturant. The volatile solvent acetonitrile (AcN) is known to denature proteins but has not been systematically investigated. Here we validate the ability of acetonitrile to cooperatively denature two model proteins, myoglobin and trypsinogen, yielding  $\Delta$ GNU values that closely match those obtained from traditional denaturants. In addition, we establish a method for accurately determining AcN concentration in denaturation samples, which is of critical importance given AcN's volatility. Together these results lay the foundation for use of AcN as a complementary denaturant that is compatible with hydrogen exchange mass spectrometry.

Poster # 38

### Nanopore tweezer analysis of Ubiquitin chains

Spencer Shorkey and Min Chen Department of Chemistry UMass Amherst

Nanopore-based sensing offers advantages as a portable, label-free, real-time approach with single-molecule sensitivity. Funnel-shaped nanopore geometries can be applied as single-molecule nanopore tweezers; where a voltage bias drives protein analyte entry into the nanopore funnel and traps it there. Measurements of the pore ion conductance gives information about trapped protein analyte size, shape, and charge over time, opening up several analytical possibilities. Here we use E. coli ClyA nanopore tweezers for direct analysis of ubiquitin (Ub) polymers, both as a model system for understanding and controlling protein trapping forces, and to develop a useful assay for different Ub polymer linkage types. The trapping of several Ub dimer linkage types exhibited conserved and unique current blockade levels, which may represent different Ub dimer conformations held in the nanopore tweezers.

# Poster # 40 Conformational heterogeneity and its role in ligand recognition by RNA molecules

Lev Levintov and Harish Vashisth Department of Chemical Engineering University of New Hampshire

RNAs are highly flexible molecules that can undergo conformational changes in response to environmental fluctuations or due to ligand binding. These conformational changes play an important role in many cellular functions and often lead to complex three-dimensional folds and an ensemble of structures. Viral RNA molecules are a good example of conformationally adapting molecules that have evolved to switch between many functions such as translation and replication. Specifically, the replication of human immunodeficiency virus Type-1 (HIV-1) results from a conformational change in the RNA transactivation response element (TAR RNA) elicited by the binding of the Tat protein. Since the discovery of TAR RNA, it has been explored as a potential target for inhibitors designed to block HIV-1 replication and many experimental structures have been resolved. However, the conformational dynamics of TAR-RNA with and without ligands/inhibitors has not been exhaustively sampled to date. In this work I will present results from long classical MD simulations of 14 uniquely resolved experimental TAR structures with and without ligands performed to provide a detailed mapping of the conformational space of HIV-1 TAR RNA that has relevance to ligand recognition.

#### Poster # 41 How diffuse ions regulate conformational dynamics of ribonucleoprotein assemblies

Ailun Wang<sup>1</sup>, Yang Wang<sup>1</sup>, Mariana Levi<sup>2</sup>, Udayan Mohanty<sup>1</sup> and Paul C. Whitford<sup>2</sup> <sup>1</sup> Department of Chemistry, Boston College <sup>2</sup> Department of Physics, Northeastern University

Structure-based model (SBM) allows millisecond effective timescale simulations of large biomolecular assemblies such as ribosome, which provides opportunities to study the dynamics of biomolecular assemblies at relative long time-scale. Diffuse ions, especially divalent cations, play a significant role in the dynamic behaviors of RNA, which regulates the conformational dynamics of ribosome. Therefore, explicit treatment of diffuse ions and electrostatic between ions and biomolecules is necessary in simulations. Here, we have developed an all-atom structure-based model in which non-hydrogen atoms, monovalent (K+, Cl-) and divalent ions (Mg2+) and electrostatics are described explicitly. The effective potentials for ion-ion, ion-RNA and ion-protein interactions are constructed to treat the solvation implicitly. The parameters in the effective potentials are refined with an iterative protocol for correct accumulation of ions near the RNA with reference from both explicit solvent simulation and experiment measurement. The same protocol has been applied on the refinement of parameters for ion-protein interactions. After the refinement, this transferable set of effective potentials for ion-ion and ion-RNA/protein interactions is then used in the SBM simulation of ribosome to study the ionic effect on dynamic behaviors of ribosome, such as the subunit rotation and dynamic motions of the stalk regions. This model provides insights into how diffuse ion atmosphere, outer-sphere ions, sitebound ions and flexibility drive conformation fluctuations in ribonucleoprotein assemblies.

## Poster # 42 Structural and biophysical characterization of splicing-associated assemblies of the SMN protein

<u>Kaylee L. Mathews</u><sup>1</sup>, Jacob Marglous<sup>2</sup> and Nicolas L. Fawzi<sup>2</sup> <sup>1</sup>Molecular Biology, Cell Biology, and Biochemistry Graduate Program <sup>2</sup>Molecular Pharmacology, Physiology, and Biotechnology Department Brown University, Providence, RI

Survival of motor neuron (SMN/Gemin1) plays an essential role in small nuclear ribonucleoprotein (snRNP) assembly and localizes to phase-separated bodies in both the nucleus and cytoplasm. Prior to snRNP assembly, SMN must self-assemble via its YG box, and missense mutations in SMN near the YG box cause a severe, neuromuscular degenerative disease called spinal muscular atrophy (SMA). However, the structure of the functional SMN oligomer and the mechanistic effect of the mutations on YG box assembly remains unknown. Here, we present a biophysical and biochemical characterization of the wild-type SMN oligomer. Using a combination of circular dichroism spectroscopy, multi-angle light scattering, and nuclear magnetic resonance (NMR) spectroscopy, we have also discovered the presence of a second assembly event mediated by the proline-rich region of SMN in addition to thermostable  $\alpha$ -helical character in the C-terminal domain of SMN. Furthermore, we have found that the SMN oligomer is larger in solution than has been reported previously. Using methyl-TROSY NMR spectroscopy, we have begun to structurally characterize the SMN oligomer. This work provides new data allowing us to further probe the normal structure of SMN and its dysfunction in disease.

# Poster # 43 What have we learned from metal-binding AMPs? Inorganic lessons from nature

Alfredo Angeles-Boza Department of Chemistry University of Connecticut

In recent years, my laboratory has been studying naturally occurring metal-binding antimicrobial peptides. Although we still do not have a complete picture on the advantages offered to the host immune system when an antimicrobial peptide binds metal ions such as Cu(II) and Zn(II), we have been able to learn some important lessons from nature. This presentation will enumerate these lessons and provide an avenue to exploit our knowledge for the design of new antimicrobial agents.

## Poster # 44 Deciphering protein evolution, fitness landscapes and stability with latent space models

Xingiang Ding and Bin Zhang Department of Chemistry MIT

The amount of protein sequence data has been increasing rapidly due to advances in sequencing technology. These protein sequences are rich in information about protein evolution, fitness landscapes, and stability. In this paper, we investigate how latent space models trained with variational auto-encoders can be used to infer information on these properties using protein sequences. We show that the low dimensional latent space representation of sequences from the model not only provides a way to visualize the sequence space of a protein family, but also captures both evolutionary and ancestral relationships between sequences. Together with experimental fitness data for the protein fitness landscape in a continuous low dimensional space. Moreover, the model is useful in predicting protein mutational stability landscapes and quantifying the importance of stability in shaping protein evolution. Finally, we illustrate that the application of advanced machine learning methodologies, such as the latent space models learned using variational auto-encoders, provide a mechanism for exploration of the rich data contained in protein multiple sequence alignments regarding evolution, fitness and stability and hence are well-suited to help guide protein engineering efforts.

# Poster # 45 Simulation of isomerization/quenching in de novo designed mini fluorescent proteins

Colin Smith Department of Chemistry Wesleyan University

The de novo computational design of proteins with a predefined three-dimensional structure is becoming much more routine due to advancements both in force fields and sampling algorithms. However, giving those molecules useful functions is often much more difficult. In that regard, the recent design of small beta barrel proteins that activate the fluorescence of an exogenous small molecule chromophore (DFHBI) is quite noteworthy. These proteins, termed mini Fluorescent Activating Proteins (mFAPs), have been shown increase the brightness of the chromophore by up to 100-fold upon binding to the designed ligand pocket. As a result of the design process, there is a large library of variants and associated brightness values, but no rational explanation of why one variant is brighter than another. Here we use quantum mechanics and molecular dynamics simulations to investigate how molecular flexibility in the ground and excited states influences brightness. We show that the ability of the protein to either resist or favor conformational isomerization/quenching of the chromophore is critical for predicting brightness. Our ultimate goal is to use this approach for rational design of brighter mFAP variants. In additional to improving protein function, this represents an ideal model system for studying how to design proteins that stabilize a particular ligand conformation, a critical aspect of enzyme design.

### Distinct behavior of the two KRas splice variants

# <u>Trinity Cookis</u>, Hugo Guterres, Kathleen Merritt, Spiro Pavlopoulos, and Carla Mattos Department of Chemistry and Chemical Biology Northeastern University

Ras GTPases are small dynamic signaling proteins that drive cellular pathways to control cell proliferation, differentiation, and survival. The HRas, NRas, and KRas genes encode four Ras isoforms that are frequently mutated at positions G12, G13, and Q61 in 20% of all human cancers. KRas is the most oncogenic isoform and can be alternatively spliced to generate both KRas4A and KRas4B splice variants. Because the four residues in the Ras G-domain that differentiate KRas4A from KRas4B are located in helix 5, oncogenic mutations remain unaffected by alternative splicing and will be expressed by both KRas4A and KRas4B transcripts. Recent findings have shown that both KRas4A and KRas4B are expressed in human cancers requiring studies of the previously neglected KRas4A isoform. Here we present the inactive and active state crystal structures of the KRas4A splice variant and have characterized the KRas4A G-domain behavior by accelerated molecular dynamics and 1D-H1NMR. We find that KRas4A and KRas4B isoforms exhibit distinct conformational sampling in Ras-effector binding regions and altered Ras G-domain dynamics. These findings suggest that KRas4A and KRas4B isoforms are structurally unique, requiring further investigation of the isoform-specific contributions that drive mutant KRas cancers.

# Poster # 47 Probing the origins of DnaK's selective promiscuity with physics-based modeling

# <u>Erik Nordquist</u><sup>1</sup>, Eugenia Clerico<sup>2</sup>, Lila Gierasch<sup>1,2</sup>, Jianhan Chen<sup>1,2</sup> <sup>1</sup> UMass Chemistry <sup>2</sup> UMass Biochemistry & Molecular Biology

The molecular chaperone DnaK, the *e. coli*. Hsp 70 variant, binds to a wide variety of substrates with relatively low binding affinity. Even so, it displays a few well-known preferences, especially for sequences of branched hydrophobics, which would tend to be exposed on unfolded proteins. Numerous crystal structures of DnaK bound to a host of substrate peptides reveal a highly conserved binding pose, which suggests that substrate residues are responsible for adopting specific interactions with the relatively static binding domain. We identified 5 sites of interaction between substrate residues and the binding domain, and probed them using molecular dynamics simulations constrained near the minima described by crystal structures. We hypothesize that these simulations contain sufficient information to build a physics-based model of DnaK binding which can recover information contained in peptide binding arrays.
### Poster # 48 Inhibition of histone demethylase Lsd1 presents a potential epigenetic approach for Atoh1 upregulation in cochlear hair cell regeneration

Lauren Phung, Nicolai Hathiramani, Dunia Abdul-Aziz, Niliksha Gunewardene, Albert Edge Department of Otolaryngology, Cell and Molecular Biology Harvard Medical School and Northeastern University

The Organ of Corti is the specialized sensory epithelium in the mammalian cochlea responsible for the mechanotransduction of sound into neural signals. It is composed of two types of mechanosensory hair cells (HCs): a row of inner hair cells (IHCs) and three rows of outer hair cells (OHCs) along with their associated supporting cells. In mammals, cochlear hair cells do not spontaneously regenerate to a large extent following loss, such that cumulative damage (as caused by noise, aging) leads to progressive, permanent sensorineural hearing loss. In contrast, in non-mammalian vertebrates, such as birds, and to a limited extent in the embryonic stage in mammals, hair cells are able to regenerate by differentiation of nearby supporting cells.

The expression of transcriptional factor Atoh1 has been shown to be necessary and sufficient for the conversion of supporting cells to hair cells. To achieve regeneration, supporting cells must reactivate Atoh1 expression and its downstream targets to transdifferentiate to hair cells. However, in the mature mammalian cochlea, Atoh1 is no longer able to induce differentiation and hair cells do not regenerate. We seek to understand the possible blocks to hair cell regeneration in mammals so that we can target them for therapeutic purposes. We hypothesize that the accessibility of Atoh1 and other hair cell genes, which is dictated by DNA and histone modifications, is fundamental to the block to regeneration in mammals. Lysine-specific demethylase 1 (Lsd1) is the first discovered histone demethylase, responsible for demethylating lysine 4 histone 3 (H3K4) resulting in gene repression. Lsd1 binds to the Atoh1 gene locus. It is expressed across inner ear progenitor cells and interacts with other chromatin modifying complexes. To date, the inhibition of Lsd1 has been shown to lead to neurogenesis in the inner ear, but its effect on hair cell differentiation has not been tested. We hypothesized that Lsd1 knockdown and inhibition would relieve Atoh1 gene repression, resulting in increased hair cells in the post-natal murine cochlea. Our experiments revealed that inhibition of Lsd1 led to an extra row of outer hair cells from the cochlear apex to the middle region. Collectively, these findings indicate that Lsd1 may be a potential epigenetic modifier regulating Atoh1 gene expression and hair cell regeneration.

# Poster # 49 Investigating cell entry mechanisms of non-enveloped viruses using computer simulations

<u>Asis Jana</u> and Eric May Department of Molecular and Cell Biology University of Connecticut

The process by which non-enveloped viruses enter and infect host cells remains poorly understood. Many non-enveloped virus contain a membrane-active peptide, which is sequestered inside the capsid during much of the virus life cycle but becomes active under certain cellular conditions such as change in the environmental pH. Flock House Virus (FHV) is an excellent model system for studying non-enveloped viruses. Experimental studies have provided strong evidence that FHV replicates within the living cells through the acid-dependent endocytic pathway, where low pH inside endosome acts as a trigger for y liberation from the capsid interior, which can then disrupt host cell membrane and the externalization and activity of these y peptides is optimal at pH 6. However, the molecular mechanism that underlies this process still remains unclear and is particularly challenging to address by using current experimental techniques. All-atom molecular dynamics (MD) simulations, often termed a "computational microscope," have emerged as a powerful tool for studying viral systems. In the current study, we performed a series of atomically detailed simulations sampling over 20 µs to study the mechanism and energetics of the membrane lytic ( $\gamma$ ) peptides' liberation from the FHV capsid interior to the exterior and the role of pH in this process. Our simulation results qualitatively agree with the experimental findings and will help us to understand the mechanism of host cell membrane breaching by non-enveloped viruses in general.

Poster # 50

#### **Ab-Initio SSE construction**

Mosavverul Hassan<sup>1</sup>, Evangelos A. Coutsias<sup>1,2</sup> <sup>1</sup>Applied Mathematics and Statistics, Stony Brook University, Stony Brook NY 11794-3600. <sup>2</sup>Laufer Center for Physical and Quantitative Biology, Stony Brook University, Stony Brook NY 11794.

The relation of backbone dihedrals to their geometric characterization parameters is well studied and documented for the secondary structure elements (SSE's) of a protein, helices and  $\beta$ -strands. The backbone hydrogen-bond constrained geometrical flexibility of these elements is a primary concern in such characterizations. Assuming a canonical geometry, a simple assignment of a periodic pattern of backbone dihedrals produces a helical structure. However, a mathematical relationship providing backbone dihedral combinations leading to hydrogen-bonds is lacking. We present an analytical method based on Inverse Kinematics (IK) that derives the feasible  $\varphi$ ,  $\psi$ dihedral pairs consistent with the hydrogen-bond geometries observed in protein SSE's.

Our method utilizes a single design parameter  $\alpha$ , the angle defined by three successive backbone  $C_{\alpha}$ . A given value of the angle  $\alpha$  orients successive  $C_{\alpha}CNC_{\alpha}$  units rotatable about the  $C_{\alpha}$ - $C_{\alpha}$  virtual axis through the constrained  $\theta$ -angle ( $\angle NC_{\alpha}C$ ) at the middle  $C_{\alpha}$  atom. An algebraic formulation of this constraint in terms of torsions about the virtual axis joining successive  $C_{\alpha}$  atoms results in a polynomial, the Tetrahedral Equation (TE). For successive units, periodicity conditions couple the TE. By imposing hydrogen-bond formation as a geometrical design constraint on successive peptide units, we arrive at a version of a TE whose roots for each value of  $\alpha$  give hydrogen-bond forming configurations. In general, the roots of the TE as a function of  $\alpha$  encompass the range of all known helical structures, including the lesser-known five residues per turn helix of zero pitch.

Although the current implementation assumes a strict trans-amide bond geometry throughout, any deviation from such geometry is amenable within the kinematic formalism. This formalism may be modified to admit the design of super-helical structures or other deviations from a regular helical structure as desired while preserving stipulated hydrogen bond geometry. A natural consequence of our kinematic implementation also informed us of a  $\beta$ -strand geometry. Curved or pleated, regular  $\beta$ -strands may also be effected within this formalism since the  $\beta$ -strand geometry relies on the same kinematic principle.

# Poster # 51 Using simulations to identify precise single-molecule probes for ribosome dynamics

<u>Asem Hassan</u> and Paul C. Whitford Department of Physics Northeastern University

The ribosome is an ideal model system for investigating the dynamics and energetics of large biomolecular assemblies. It consists of a "large" subunit and a "small" subunit, which is further divided into a head domain and a body domain. Structural studies have shown that during mRNA-tRNA translocation, the small subunit exhibits rotational motion with respect to the large subunit. Further, the head domain rotates with respect to the body of the small subunit. In addition, simulations predict a complementary tilting motion that is also associated with the head domain [1]. The complexity of these processes make it unclear how to precisely distinguish between each motion in single-molecule experiments [2]. To address this, we performed statistical analysis of simulated molecular dynamics trajectories in which spontaneous translocation events occur. Using this data set, we have calculated the correlations between interprotein distances and head rearrangements. From this, we have identified residue-residue distances that can unambiguously distinguish between rotation and tilting of the head domain. This provides a physical-chemical foundation upon which single-molecule experiments may precisely measure the energetic properties of large-scale dynamics in the ribosome.

[1] K. Nguyen, P. C. Whitford. Steric interactions lead to collective tilting motion in the ribosome during mRNA-tRNA translocation. Nature Communications. 7, 10586, 2016.

[2] Wasserman M.R., Alejo J.L., Altman R.B., Blanchard S.C. Multiperspective smFRET reveals ratedetermining late intermediates of ribosomal translocation. Nat. Struct. Mol. Biol. 2016; 23:333– 341.

### Poster # 52 Identifying steric components in the ribosome that control tRNA hybrid-state formation

<u>Mariana Levi</u>, Kelsey Walak and Paul C. Whitford <sup>1</sup>NU Research Computing, Northeastern University <sup>2</sup>Department of Physics, Northeastern University

The task of protein production in the cell is performed by a biomolecular machine known as the ribosome. During this complex multi-step process, the ribosome undergoes large-scale molecular rearrangements to facilitate tRNA movement through the ribosomal binding sites. Here, we focus on the first step of translocation in the 70S bacterial ribosome, known as hybrid-state formation, which involves a ~20Å-40Å movement of the P-site tRNA between the ribosomal binding sites. This motion is facilitated by the rotation of the 30S body relative to the 50S by  $\sim$ 6°-10°. Abundance in structural studies of tRNA endpoints and intermediates motivated the exploration of the molecular mechanism governing P-site tRNA dynamics, which is poorly understood. In particular, it is unclear which structural and energetic factors in the ribosome may contribute to the free-energy landscape of hybrid-state formation. To study tRNA transitions between the classical P/P to the hybrid P/E configurations, we developed and simulated an all-atom structurebased model of the ribosome where the P-site tRNA can spontaneously interconvert between the P and E binding sites on the 50S. Using this model, we obtained over 120 P/P-P/E transitions upon subunit rotation, where multiple intermediates were identified, consistent with known cryo-EM data. With the simplified energetics description of our model, we were able to pinpoint key structural factors that control tRNA kinetics. Specifically, our analysis suggests that the structure of the ribosome alone is a rate-limiting factor of tRNA hybrid-state formation. Additionally, we investigate the extent to which specific interactions contribute to the underlying free-energy barrier by introducing free-energy perturbations to our models. Our calculations show that the tRNA must pass through a narrow three-residue "gate" on the 50S subunit to adopt a hybrid P/E configuration, where interactions with this gate significantly control tRNA kinetics. These findings offer a theoretical groundwork for the future design of experimental techniques that may exploit this gate in order to control translocation dynamics.

#### Poster # 53 Nonspecific membrane interaction modulation of BK channel gating

Mahdieh Yazdani and Jianhan Chen Department of Chemistry UMass-Amherst

The transmembrane (TM) and intracellular regulatory domains of TM channels and receptors are often connected through a single covalent linker. It has been under much debate whether the linker is largely an inert linkage or should be considered a key sensing element that participate in the control of TM protein function. To unambiguously address this question, we designed a linker permutation approach to suppress potential complex interplay of specific linker interactions with the rest of the protein, and demonstrated that a single source of force imposed on the C-linker of big potassium (BK) channels, namely, membrane anchoring effects, can directly modulate BK activation. Our finding strongly supports the notion that covalent linkers frequently play active roles in modulating TM protein function.

### Poster # 54 Characterizing the interplay between dynamics and regulation in the Trypsinogen/Trypsin Protease system

Sarah Duggan and Sheila Jaswal Department of Biochemistry Amherst College

Trypsin (Tn) is a digestive serine protease found in eukaryotes and prokaryotes. Tn is manufactured by the vertebrate pancreas as its proenzyme trypsinogen (Tgn) which includes an N-terminal six amino acid zymogen region. The zymogen form, Tgn, is disabled against cleavage of substrate until Tgn reaches the small intestine. Within the small intestine, enterokinase cleaves the inhibiting peptide from folded Tgn to release active Tn protease. Fluorescence experiments with bovine Tn demonstrate remarkably slow global unfolding, with a kinetic barrier to unfolding as large as kinetically stabilized bacterial homologues. However, Tn loses its catalytic activity under physiological conditions much faster than its homologues. Whereas bacterial homologues have evolved to resist proteolysis, thereby prolonging extracellular activity, mammalian Tgn/Tn is tightly regulated through a series of auto-cleavage events as it passes through the digestive system. We propose that Tgn/Tn experiences significant subglobal fluctuations, and that sampling of these semi-unfolded states under physiological conditions causes this rapid autolysis. To explore this, we will perform native-state hydrogen exchange mass spectrometry of a purified inactive mutant. Exploring the Tgn/Tn subglobal dynamics which expose the native-state to autolysis will grant understanding of the link between Tn dynamics and the regulation of its function through its specific sequence of self-cleavage events. By comprehensively characterizing the sampling of subglobally and globally unfolded states of Tgn/Tn, more can be inferred about the occurrence of non-native state protein aggregation and susceptibility to proteolytic attack. The Tgn/Tn system, when studied from the inactive Tgn/Tn mutant, allows direct assessment of the role of subglobal protein dynamics and the regulation of these dynamics in Tn folding disorders without the complication of autoproteolysis. The Tgn/Tn system will allow investigation of diseases characterized by abnormal protein aggregation and vulnerability to proteolytic attack, in addition to diseases directly related to Tn function such as pancreatitis.

### Poster # 55 Dynamic changes in the electric double layer complicate the use of zeta potential in predicting binding properties of nanoparticles

Dongyue Liang and Qiang Cui Department of Chemistry Boston University

Understanding nanoparticle's surface charge distribution, its experimental characterization and the impact on binding is critical to the design of nanoparticles (NPs). Zeta potential, as commonly measured in experiments, is not straightforward to interpret at the microscopic level. The electrical double layer consisting of counter ions could compensate the bare charges carried by the NP. Microscopic level analysis is required to understand whether zeta potential strongly reflects the capacity of NPs to bind charged biomolecules.

# Poster # 56 Intercalation of small Rhodium complexes into matched and mismatched DNA

<u>Guðfríður Björg Möller</u><sup>1</sup>, Liam Price<sup>1</sup>, Grace Ferris<sup>2</sup>, Micah J. McCauley<sup>1</sup>, Ioulia Rouzina<sup>3</sup>, Megan Núñez<sup>4</sup> and Mark C. Williams <sup>1</sup>

 <sup>1</sup> Northeastern University, Dept. of Physics, Boston, MA
<sup>2</sup> Lesley University, Dept. of Natural Sciences and Mathematics, Cambridge, MA
<sup>3</sup> The Ohio State University, Dept. of Chemistry and Biochemistry, Center for Retroviral Research, and Center for RNA Biology, Columbus, OH
<sup>4</sup> Wellesley College, Dept. of Chemistry, Wellesley, MA

We characterize the equilibrium binding and kinetics of the small rhodium based molecules, [Rh(bpy)2(chrysi)]3+ and [Rh(bpy)2(phi)]3+. Both motifs bind to DNA. Phi contains an aromatic ring system known to intercalate between adjacent bases. We expect intercalation to be weaker for chrysi due to the presence of an additional aromatic ring. Chrysi was developed as a potential anti-cancer drug that can selectively photo-cleave mismatched DNA in the mismatch-repair deficient cancer cells, while not affecting the undamaged DNA. We used optical tweezers on single DNA hairpin and long DNA molecules. Hairpin unfolding experiments on mismatchcontaining sequences show that chrysi binds preferentially to sequences containing mismatches, stabilizing the hairpin. In long double stranded DNA stretched in the presence of each ligand, a change in DNA extension is observed consistent with ligand intercalation. Under the influence of increasing force, intercalation of the phi ligand into matched DNA is observed as expected. Unexpectedly, we find that chrysi is also able to intercalate into matched DNA and does so with twice the binding affinity of phi. Furthermore, chrysi intercalates into matched DNA and stabilizes mismatched DNA with comparable affinity. The enhanced intercalation into mismatched DNA sites is driven by the mismatch-induced duplex destabilization that can also be achieved by dsDNA stretching. We hypothesize that similar intercalation enhancement can be achieved by DNA duplex destabilization in many alternative ways, including low salt or high temperature in vitro, DNA unwinding or local deformations by active helicases or other proteins, or defects in mismatch repair in vivo.

# Poster # 57 Predicting three-dimensional genome organization with chromatin states

Yifeng Qi and Bin Zhang Department of Chemistry MIT

We propose a data-driven mechanistic model for studying the human genome organization. In particular, a least-biased effective energy function whose parameters can be derived from chromosome conformation capture experiments along with a polymer model is used to simulate the ensemble of genome conformations. The model is built upon a few findings established in recent experiments. First, we take the one-dimensional A/B compartment profile determined from Hi-C representing active/repressed chromatin segments as input. Second, we explicitly take into consideration of the higher-order centromere clustering inferred from Hi-C contacts between pericentromeric genomic regions. Lastly, the model goes beyond a haploid genome to infer a diploid structure from Hi-C, which is challenging since in principle ensemble average bulk Hi-C data does not distinguish difference between homologous chromosomes. The model is able to reveal several notable features of the diploid genome, including patterns of cross-talk in homologous chromosomes as well as the known X chromosome inactivation.