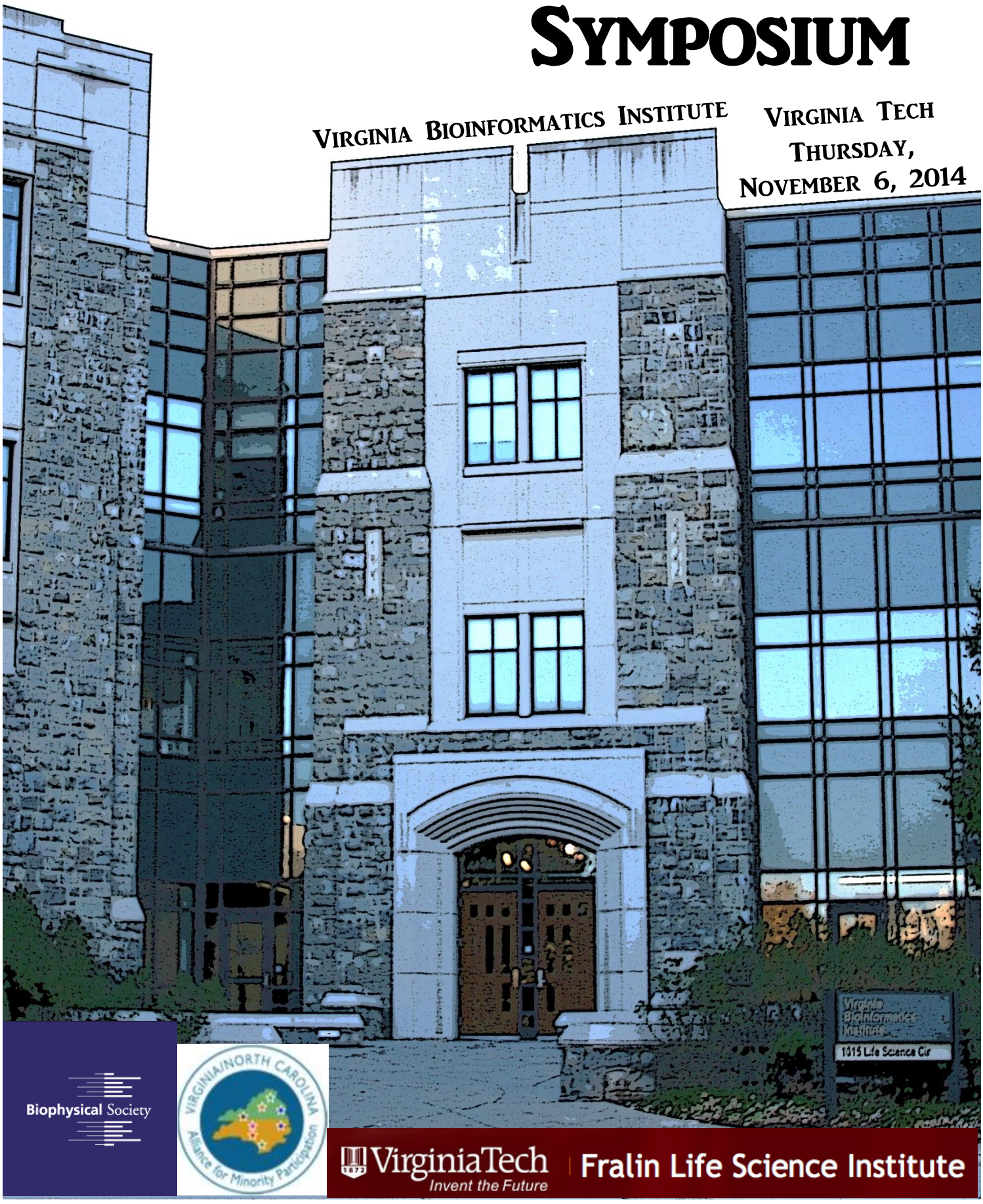
Molecular Biophysics Symposium

Virginia Bioinformatics Institute

Virginia Tech

Thursday,

November 6, 2014



Biophysical Society

Virginia/North Carolina Alliance for Minority Participation

VirginiaTech Invent the Future | Fralin Life Science Institute

Molecular Biophysics Symposium

A conference hosted by Virginia
Bioinformatics Institute at Virginia Tech

Keynote Speakers

Dr. Andrew L. Lee – University of North Carolina Chapel Hill
Dr. Kylie Walters – Center for Cancer Research, National Cancer Institute, NIH

Session Chairs

Session 1: Mary Kate Brannon (Capelluto lab) and Danielle Miller (White lab)
Session 2: Anne Brown (Bevan lab) and Paul Velander (Xu lab)

Organizing Committee

Dr. Daniel Capelluto
Mary Kate Brannon
Xiaolin Zhao
Elizabeth Brown
Kristen Fread
Sam Berk
Davia Blake
Mike Finlan
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Acknowledgements

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The Biophysical Society
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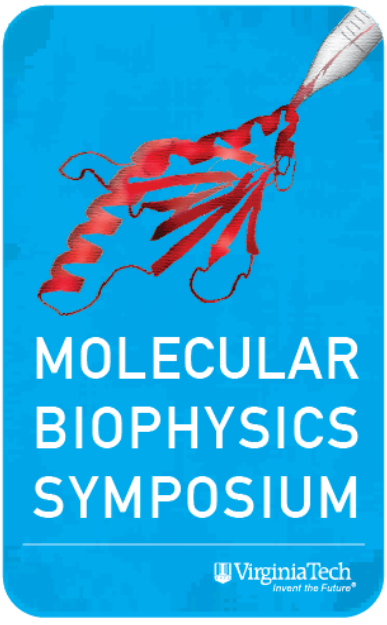

PROGRAM DESCRIPTION

7:30 AM *Registration and Breakfast*

Session 1
Chairs: Mary Kate Brannon and Danielle Miller

8:30 AM Welcome **Daniel Capelluto** (Virginia Tech)

8:40 AM **Dr. Kylie Walters,** National Cancer Institute, National Institutes of Health
Applying molecular biophysics to reveal how proteasome recognizes substrates

9:30 AM **Ryan Godwin,** Wake Forest University
The zinc-finger NEMO: A molecular dynamics study

9:50 AM **Arthur Sikora,** University of Virginia
Using CW-EPR to explore the mechanism of TonB-dependent transport in BtuB and FhuA

10:10 – 10:30 AM *Coffee Break*

Session 2
Chairs: Anne Brown and Paul Velander

10:30 AM **Dr. Andrew L. Lee,** University of North Carolina Chapel Hill
Protein side-chain dynamics in ligand binding and allostery

11:20 AM **Xiaolin Zhao,** Virginia Tech
Structural basis of phosphoinositide (PIP) recognition by the TIRAP PIP-biding motif

11:40 AM **Rachel Parker,** Virginia Tech
Combinational and statistical approaches to protein structure and function: Synthetic motifs that selectively recognize bacterial cell wall peptidoglycans

12:00 – 1:00 PM *Lunch*

Session 3

1:00 - 2:30 PM Poster Session

2:35 PM Poster Awards announced

3:00 PM Group photo with speakers

Session 4

3:15 Meeting of appointed undergraduates with biophysicists and structural biologists

ABSTRACTS

I. KEYNOTE SPEAKERS

Kylie Walters
National Cancer Institute, National Institutes of Health

APPLYING MOLECULAR BIOPHYSICS TO REVEAL HOW PROTEASOME RECOGNIZES SUBSTRATES

The compact 76 amino acid protein ubiquitin is used to signal for a broad spectrum of cellular events, including protein degradation by proteasome. Ubiquitination is diversified by expansion into a ubiquitin polymer, formed through eight possible linkages. Ubiquitin receptors contribute to determining the outcome of ubiquitination by their specificity for distinct ubiquitin polymers. This talk will present new interactions involving ubiquitin receptors and ubiquitin polymers, as well as functional implications.

Andrew L. Lee
University of North Carolina Chapel Hill

PROTEIN SIDE-CHAIN DYNAMICS IN LIGAND BINDING AND ALLOSTERY

We are carrying out NMR studies of PDZ domains to reveal relationships between protein dynamics and function. PDZ (post synaptic density-95, discs large, zo-1) domains are small, protein-protein binding modules that typically recognize C-terminal tail residues of target proteins. They are commonly found in multidomain signaling proteins and play a role in providing a scaffold for recruitment of multiple factors. PDZ domains have been extensively studied for their intradomain signaling properties and extradomain structural motifs. NMR spectroscopy is ideally suited for characterizing molecular dynamics over a wide range of motional timescales. Analysis of nuclear spin relaxation rates in the third PDZ domain of postsynaptic density-95 (PSD-95) has revealed a role for picosecond-nanosecond motions in ligand binding. Specifically, methyl containing side-chain motions can be quite sensitive to various perturbations to the domain, such as the presence of an extradomain helix. The dynamics are affected near and far from the perturbation and results in significant changes in conformational entropy, which modulates binding affinity. These findings underscore the potential importance of “dynamic allostery” proteins.

II. SHORT TALKS

Ryan Godwin[1], William Gmeiner[2], and Freddie R Salsbury Jr.[1]
[1]- Department of Physics, Wake Forest University, Winston-Salem, NC 27109
[2]- Department of Cancer Biology, Wake Forest University Health Sciences, Winston-Salem, NC 27107

THE ZINC-FINGER NEMO: A MOLECULAR DYNAMICS STUDY

Molecular dynamics simulations are becoming an increasingly valuable tool as computational power combined with parallel optimization is pushing simulation capability into unprecedented territory. Now, with accelerated GPU's, microsecond simulations are very attainable for small to medium sized systems (hundreds of thousands of atoms.) A detailed study of the zinc-binding protein, NEMO, will be presented. NEMO contains a ubiquitin-binding domain, and plays a role in the apoptotic signaling pathway, as well as oncogenesis and immune responses. NEMO is characterized by three cysteines and a histidine at the zinc-binding active site. Results reported are from all-atom simulations with explicit water, run using the CHARMM 22 force field. Simulations across different timescales show that microsecond-regime trajectories highlight rare-events that are often missed in nanosecond-regime simulations. Thus, longer simulations increase the conformational sampling of the protein, and highlight changes that occur on more biologically significant timescales. Simulations of structural variations on the active site cysteines suggest that zinc plays an important role in stabilizing the folded structure and suggests that, while zinc-binding is energetically inexpensive, the (de)-protonation of the cysteines provides the larger energy barrier when transitioning between a protonated protein without zinc and a zinc-bound, deprotonated protein.

Arthur Sikora and David S. Cafiso
Chemistry Department, University of Virginia, Charlottesville, VA 22901

USING CW-EPR TO EXPLORE THE MECHANISM OF TONB-DEPENDENT TRANSPORT IN BTUB AND FHUA

TonB dependent transporters are essential for the success of many pathogenic bacteria, making these interesting targets for new antibiotics. These proteins are a family of outer membrane β-barrels that Gram-negative bacteria use to transport essential nutrients such as vitamin B12 or iron. Although high-resolution crystal structures have been obtained for many of these proteins, the mechanism of substrate transport is still unclear. Utilizing a combination of site-directed spin labeling electron paramagnetic resonance (SDSL-EPR) and chemical denaturation we are able examine whether portions of the proteins' core unfold more readily than others. We also examine the effects of substrate on this differential unfolding. Understanding the thermal stability of the core will shed light onto possible rearrangement of this domain during substrate transport.

Xiaolin Zhao[1], Shuyan Xiao[1], Sam Berk[1], Anne M. Brown[2], David R. Bevan[2], Geoffrey Armstrong[3], and Daniel G. S. Capelluto[1]
[1]Protein Signaling Domains, Department of Biological Sciences and [2]Department of Biochemistry, Virginia Tech, Blacksburg VA, 24061, USA; [3]Department of Chemistry and Biochemistry, University of Colorado, Boulder CO 80309, USA.

STRUCTURAL BASIS OF PHOSPHOINOSITIDE (PIP) RECOGNITION BY THE TIRAP PIP-BINDING MOTIF

Toll-like receptors (TLRs) provide early immune system recognition and response to infection. TLRs activated by pathogens consequentially initiate a cytoplasmic signaling cascade though adaptor proteins, the first being the modular TIR domain-containing adaptor protein (TIRAP). TIRAP contains a C-terminal TIR domain, which is responsible for association with TLRs and other adaptors including the myeloid differentiation primary response gene88 (MyD88) protein. Membrane recruitment of TIRAP is mediated by its N-terminal PIP-binding motif (PBM). Upon ligand-mediated activation, TLRs are recruited to the PIP-enriched regions where TIRAP resides. At these sites, TIRAP recruits MyD88 to the membrane by bridging MyD88 to activate the TLR signaling pathway. To understand the mechanism of membrane targeting of TIRAP and the basis for its regulation, we functionally and structurally characterized its PBM using experimental and computational studies. TIRAP PBM adopts a folded conformation in membrane mimics, such as dodecylphosphocholine micelles, and binds PIPs. Structural rearrangements of TIRAP PBM were influenced by membrane interaction, with monodispersed PIPs inducing helical structure in the peptide. In contrast, monodispersed phosphatidylinositol and inositol trisphosphate did not promote structural changes in TIRAP PBM. NMR spectra reveal that TIRAP PBM binds PIPs in a fast exchange regime with a moderate affinity through two conserved basic regions. Solution NMR structure of TIRAP PBM shows a central short helix, and paramagnetic studies indicate that this region is close to the micelle core. Molecular dynamics simulations studies indicated that TIRAP PBM diffused to and interacted with a model membrane composed of palmitoyl oleoyl phosphatidylcholine and phosphatidylinositol 4,5-bisphosphate. Thus, we propose that two sets of basic residues contact both the head group and acyl chains of PIPs, whereas the central helix is responsible for membrane insertion.

Rachael Parker[1], Ana Mercedes and Tijana Z. Grove[1]
[1]Dept. of Chemistry, Virginia Tech, Blacksburg, VA 24061

COMBINATORIAL AND STATISTICAL APPROACHES TO PROTEIN STRUCTURE AND FUNCTION: SYNTHETIC MOTIFS THAT SELECTIVELY RECOGNIZE BACTERIAL CELL WALL PEPTIDOGLYCANS

Repeat proteins have recently emerged as especially well-suited alternative binding scaffolds due to their modular architecture and biophysical properties. Here we present the design of a scaffold based on the consensus sequence of the leucine rich repeat (LRR) domain of the NOD family of cytoplasmic innate immune system receptors. Consensus sequence design has emerged as a protein design tool to create de novo proteins that capture sequence-structure relationships and interactions present in nature. The multiple sequence alignment of 311 individual LRRs, which are the putative ligand-recognition domain in NOD proteins, resulted in a consensus sequence protein containing two internal and N- and C- capping repeats named CLRR2. CLRR2 protein is a stable, monomeric, and cysteine free scaffold that without any affinity maturation displays micromolar binding to muramyl dipeptide, a bacterial cell wall fragment. To our knowledge, this is the first report of direct interaction of a NOD LRR with a physiologically relevant ligand. CLRR2 will be used as a scaffold to incorporate binding affinity for other nonproteinaceous ligands.

III. POSTER PRESENTATIONS

1 Steven M. Shell[1,2] and Walter J. Chazin[2]
[1]Department of Natural Sciences, University of Virginia's College at Wise, Wise, VA 24293
[2]Department of Biochemistry, Center for Structural Biology, Vanderbilt University, Nashville,
TN 37232

ISOLATION AND CHARACTERIZATION OF THE DNA BINDING APPARATUS FROM THE HUMAN DNA DAMAGE SENSING PROTEIN XPC

XPC serves as the primary sensor of damaged DNA in the nucleotide excision repair pathway in humans. XPC binds to both ssDNA and dsDNA with nanomolar affinity, sensing the ssDNA character induced in dsDNA by the presence of a lesion to identify damaged DNA. Crystal structures of the homologous *Saccharomyces cerevisiae* protein Rad4 show the DNA binding apparatus consists of four domains linked via short hinges that adopt a compact architecture in the absence of DNA. Upon encountering damaged DNA the TGD and BHD1 form a dsDNA clamp, and the hairpin of BHD3 is inserted between the DNA strands at the damage site. However, critical questions remain regarding human XPC. XPC contains a 180-residue insertion relative to Rad4 bisecting the TGD. Moreover, the compact architecture of Rad4 is seemingly at odds with the tight affinity of XPC for unmodified dsDNA. In order to gain further insight we isolated the DNA binding apparatus from human XPC (XPCDNA). Biochemical analysis indicates that elimination of the insertion in the TGD had no effect on the DNA binding activity of XPCDNA compared to full-length XPC. Small-angle x-ray scattering (SAXS) analysis indicates that XPCDNA has a modular architecture and adopts an elongated conformation in the absence of DNA. Binding of XPCDNA to a damaged DNA substrate results in changes to the domain architecture similar to that observed for Rad4. Together, these results suggest that the DNA binding apparatus of XPC is remarkably plastic and is remodeled to accommodate binding to a variety of DNA substrates.

2 Shuo Qian
Biology and Soft Matter Division & Center for Structural Molecular Biology, Oak Ridge National Laboratory

APPLICATION OF SMALL ANGLE NEUTRON SCATTERING ON THE STRUCTURE AND FUNCTION OF BIOLOGICAL COMPLEX

The structure-function relationships remain a critical theme in understanding many important biological processes. Small Angle Neutron Scattering (SANS) has developed as one of the tools suitable for such studies, with significant progress in recent years. Because of the naturally occurred difference in contrast, termed as neutron scattering length density, between different biomolecules such as proteins, lipids, RNA/DNA etc., SANS affords researchers to study structures of individual components in a complex biological systems and in a more relevant condition. Further, the high penetration power of neutrons and the lack of radiation damage make SANS well-suited to the study of large, multi-component

biological complexes both in-situ and in-vivo, by using neutron contrast variation techniques with selective deuterium labeling. Here I will present a few examples of SANS studies on protein structures, protein-RNA complex, and peptide-lipid interaction. Also I will give a brief introduction to an open-access user facility Bio-SANS, a high neutron flux and low experimental background SANS instrument at Oak Ridge National Laboratory dedicated for biological research.

3 Catherine Klancher, Reeder Robinson and Pablo Sobrado
Department of Biochemistry, Virginia Tech, Blacksburg, VA 24061

CHARACTERIZATION OF MUTANT ISOFORMS OF SIDEROPHORE A FROM *ASPERGILLUS FUMIGATUS*

The ability of some pathogenic organisms to grow relies on iron sequestration from the host. One such organism is the fungus *Aspergillus fumigatus.* It chelates free iron from a mammalian host by producing siderophores. The production of siderophores relies on the gene product siderophore A (SidA), which is a flavin-containing monooxygenase. It catalyzes the NADPH-and-oxygen-dependent hydroxylation of L-ornithine to contribute to the biosynthesis of siderophores. The structure of SidA suggests that residues T405 and Y407 may contribute to NADPH binding. These residues are part of the VATGY motif that form a hydrophobic pocket found in several flavin-dependent monooxygenases. The role of this motif has yet to be characterized. To probe the function of these residues, site-directed mutagenesis was used to replace threonine at position 405 to alanine and tyrosine at position 407 to alanine and phenylalanine. The resulting mutant enzymes were expressed, purified, and characterized using steady state kinetic assays.

4 Lu Zhai, Robert Burnham, Feng Li, Ling Wu, and Bin Xu
Department of Biochemistry and Center for Drug Discovery, Virginia Tech, Blacksburg, VA 24061

PROTEOME-SCALE STRUCTURAL MIMIC SCREEN FOR PATHOGEN IMMUNE EVASION

Large DNA viruses (herpesviruses, adenoviruses, poxviruses, etc.) have the ability to evade host innate and adaptive immune responses by encoding immunomodulatory gene products (viral evasion proteins). A thorough understanding of viral evasion is of vital importance not only for describing or discovering viral pathogenesis mechanisms, but also for developing new therapeutic strategies against these pathogens. Here we identified novel and remote viral homologs of host immune proteins (MHC I molecules, NK receptors, chemokines, cytokines etc) through a novel, structure-based, and high throughput computational analysis of the entire human herpesvirus proteome. We further developed an ultrafast method for refolding inclusion-body-based, recombinant cell surface immunoreceptors, viral receptors, and other proteins of therapeutic values within one day from the point of harvesting culture, a speed equivalent to soluble protein purification. This method can be easily adapted into high throughput format for screening of viral-host protein interactions and other structure biology, chemical biology, and drug screening applications.

5 Paul Velander^, Ling Wu^, Rebekah Watkins, David Bevan, Bin Xu*
Department of Biochemistry and Center for Drug Discovery, Virginia Tech, Blacksburg, Virginia 24061 ^ equal contribution

DISCOVERY AND MECHANISMS OF NATURAL PRODUCT INHIBITORS AGAINST PROTEIN AMYLOIDOSIS IN TYPE II DIABETES AND ALZHEIMER'S DISEASE

Human islet amyloid polypeptide (IAPP or amylin) is an established hallmark of type 2 diabetes (T2D) and presents in up to 90% of patients with T2D at autopsy(1). IAPP plaques were recently found in Alzheimer's patients with T2D(2). It is therefore proposed as a novel, second amyloid in the brain (the other is beta amyloid protein) that links diabetes and Alzheimer's disease. In pursuit of finding natural compound leads that exhibit anti amyloid activity pertaining to Type II diabetes and Alzheimers disease, our lab has recently initiated the characterization of a collection of natural compounds including flavonoids, polyphenols, and terpenoids for their anti-aggregation effects against IAPP. Utilizing a combination of *in vitro* thioflavin T (ThT) fluorescence assay as well as pancreatic beta cell- and neuronal cell-based assays in high throughput fashion to monitor hIAPP-induced amyloid formation and cytotoxicity, we have identified several lead compounds for further structure activity relationship (SAR) investigations. Our data demonstrates a novel relationship between anti amyloid activity and the presence of two essential hydroxyl groups at conserved positions within oleuropein, its downstream metabolites and structurally related analogues. Validation of anti-amyloid activity observed *in vitro* is currently underway using transmission electron microscopy (TEM) and photo-induced cross-linking of unmodified proteins (PICUP). Mechanistic insights are presently being investigated via additional cell-based assays and computational molecular simulations related to cell apoptosis and amyloid-induced membrane permeability.

6 Manisha Shrestha and Florian D. Schubot
Department of Biological Sciences, Virginia Tech, Blacksburg ,VA 24061

MOLECULAR STUDIES TO UNDERSTAND THE INTERACTIONS BETWEEN TRANSCRIPTION ACTIVATOR EXSA AND ANTI-ACTIVATOR EXSD THAT REGULATE THE TYPE THREE SECRETION SYSTEM (T3SS) IN *PSEUDOMONAS AERUGINOSA.*

Pseudomonas aeruginosa is an important opportunistic pathogen associated with both chronic and acute infections, especially in immunocompromised individuals. One important mechanism of infection is the Type Three Secretion System (T3SS), which is present in almost all pathogenic Gram negative bacteria. The T3SS functions as a molecular syringe; puncturing cell membranes during infection and releasing cytotoxic proteins directly into the cytoplasm of the host cell. Expression of genes related to the T3SS is regulated by a signaling cascade involving the proteins ExsA, ExsC, ExsD, and ExsE. The transcription factor ExsA, which is a member of AraC/XylS family of proteins, is responsible for activating transcription of all T3SS genes, including its own gene. ExsA activity is inhibited by the binding of ExsD, which acts as an anti-activator of T3SS

expression. The ExsA-ExsD interaction is unique for among AraC-type regulators, which are typically regulated by small molecule ligands. The direct interaction of ExsD with ExsA is believed to either prevent the dimerization of ExsA or preventing the binding of ExsA to its promoter. It is also possible that ExsD functions via a combination of both mechanisms. *In vitro* studies have shown that ExsD alone is capable of inhibiting ExsA dependent transcription and that this inhibition is temperature dependent. My current research focuses on understanding this interaction at the molecular level. Using a combination of structure-guided site-directed mutagenesis and *in vitro* transcription experiments we are systematically mapping the ExsA-ExsD interface to facilitate future efforts to develop drugs that can mimic ExsD and thus control T3SS related infections.

7 Mary K. Brannon[1], Shuyan Xiao[1], Geoffrey Armstrong[3], Kristen Fread[1], Carla V. Finkielstein[2], and Daniel G. S. Capelluto[1]
[1]Protein Signaling Domains and [2]Integrated Cellular Responses laboratories, Department of Biological Sciences, Virginia Tech, Blacksburg VA, 24061, USA;
[3]Department of Chemistry and Biochemistry, Boulder CO, 80309, USA.

STRUCTURAL AND FUNCTIONAL BASIS OF TOLLIP ASSOCIATION TO THE ENDOSOMAL ADAPTOR PROTEIN TOM1

Adaptor proteins are often committed to cellular processes that involve cargo internalization from the plasma membrane. Ubiquitinated cargo is internalized by endocytosis and delivered to early endosomes *via* intracellular vesicles. Cargo is then sorted to late endosomes/multivesicular bodies followed by, in most cases, degradation in the lysosomal compartments. Adaptor proteins, such as Tollip and Tom1, facilitate cargo sorting through their ubiquitin-binding domains. Tollip is localized to early endosomes, through binding to phosphatidylinositol 3-phosphate (PtdIns(3)P). Tom1 can also bind ubiquitin-conjugated cargo and is recruited to the endosomal membranes through its association with Tollip. The interaction of these two proteins is proposed to be involved in the lysosomal degradation of polyubiquitinated cargo. In this work, we demonstrate that binding of Tollip to PtdIns(3)P is negatively modulated by interaction with Tom1. Structural studies determine that the Tom1-binding domain (TBD) of Tollip is intrinsically disorded and folds upon binding to the Tom1 GAT domain, which also undergoes a conformational change upon binding. Intermolecular NOEs of the Tollip TBD-Tom1 GAT complex indicate that association is mainly driven by hydrophobic contacts with very high affinity. Ubiquitin binds to the Tom1 GAT domain at a site that does not overlap with that for the Tollip TBD, but the binding events are mutually exclusive and are likely driven by conformational changes in the GAT domain. Endosomal localization of Tom1 depends on the presence of Tollip in this compartment. Using fluorescence microscopy, we show that mutations in the binding interphase of the Tom1 GAT and Tollip TBD complex leads to a dissociation of the proteins and triggers cytosolic localization of Tom1. Consequently, we propose that association of Tom1 to Tollip helps to release Tollip from endosomal membranes, allowing Tollip to commit to endosomal ubiquitinated cargo trafficking.

8 Ashton Brock, Shelby Lipes, and Linda Columbus
Department of Chemistry, University of Virginia, Charlottesville, VA

SYSTEMATIC PERTURBATIONS OF MICELLE PROPERTIES TO INVESTIGATE THE STABILIZATION OF MEMBRANE PROTEIN STRUCTURE AND FUNCTION

Membrane protein research is hampered by the difficulty in selecting a membrane mimic that solubilizes and stabilizes protein fold and function. While detergents are often utilized, identifying the appropriate detergent composition to maintain protein solubilization and stability is an expensive, time-consuming, empirical process that is often unsuccessful. The goal of this research is to understand the interactions between detergent and protein by relating physical surfactant properties with membrane protein fold, function, and stability, enabling rational detergent selection. To determine important micelle and protein characteristics, outer membrane phospholipase A1 (OMPLA), the protease OmpT, and the lipid A palmitoyltransferase PagP were purified in different detergent micelles with varying properties such as alkyl chain length, charge, and head group. The overall protein structure and function were evaluated in many pure micelles to identify trends with detergent characteristics and protein function. Upon determination of kinetic parameters for several β-barrel proteins in pure micelles, protein function and structure will be investigated with mixed micelles, to test hypotheses generated by the trends observed in pure micelles. This research will provide a logical rationale for the selection of detergents based on the physical properties of membrane proteins and detergents.

9 Jennifer N. Martin[1], Ryan H. Lo[1], Alison K. Criss[2], and Linda Columbus[1]
[1]Department of Chemistry, University of Virginia, Charlottesville VA
[2]Department of Microbiology, Immunology and Cancer Biology, University of Virginia School of Medicine, Charlottesville, VA

INSIGHTS INTO THE SPECIFICITY OF *NEISSERIAL* OPA PROTEIN INTERACTIONS WITH HUMAN RECEPTORS

Pathogenic *Neisseria gonorrhoeae* (Gc), the causative agent of gonorrhea, possess a family of outer membrane proteins referred to as opacity-associated (Opa) proteins. These Opa proteins are β-barrel outer membrane proteins that bind to human host cell receptors, inducing engulfment of the bacterium. To date, there have been over 300 distinct *opa* alleles sequenced. The differences in sequence have stemmed primarily from recombination events, and are most pronounced in two regions of the protein: the second and third extracellular loops (termed hypervariable loops, HV1 and HV2). These HV regions are responsible for determining host cell receptor specificity. The most abundant Opa family engages human CEACAM receptors (carcino-embryonic antigen-like cellular adhesion molecules). While the Opa protein family has conserved structural elements, the molecular determinants of the receptor interactions are unknown. We aim to compare the function of a variety of Opa proteins reconstituted into liposomes in order to define how differences in Opa sequence and structure determine receptor binding. To investigate the structural motif for Opa-receptor recognition and engagement, we are employing techniques that will yield both low and high resolution models of the Opa-receptor complex. Affinities for a variety of CEACAM-binding Opa family members were determined using fluorescence

polarization, with dissociation constant values ranging from ~1-10 nM. To determine which specific amino acids are involved in receptor recognition, competition assays were performed using different peptides that mimic segments of the HV regions of Opa proteins. For a higher resolution understanding of the Opa-CEACAM interaction, x-ray crystallography was used. Together, these results will provide insights into the specific molecular determinants of Opa protein interactions with host receptors.

10 Marissa Kieber[1], Tsega Solomon[1], and Linda Columbus[1]
Department of Chemistry, [1]University of Virginia, McCormick Rd., Charlottesville, VA

NEISSERIAL OPA PROTEIN CONFORMATIONAL DYNAMICS AND MECHANISM OF INTERACTION WITH HOST CEACAM RECEPTORS

Human pathogens *Neisseria gonorrhoeae* and *N. meningitides* are unique in their utilization of opacity-associated (Opa) proteins to mediate bacterial uptake into non-phagocytic cells. Opa proteins engage either heparan sulfate proteoglycan (HSPG) receptors or carcinoembryonic antigen-related cellular adhesion molecules (CEACAMs) to hijack host cellular mechanisms, which induces bacterial engulfment. The Opa family of proteins are eight stranded β-barrels with four extracellular loops. Regions in loops two and three contain hypervariable sequences among Opa variants and dictate receptor specificity. We aim to reveal the structural determinants of Opa-receptor interactions. Overall loop dynamics of Opa_{60}, a CEACAM-binding Opa variant, were determined using CW-EPR and combined with the limited NMR relaxation data. Results indicate that the loops and hypervariable regions are highly mobile on the nanosecond timescale. Preliminary DEER experiments provide distance measurements between Opa_{60} and CEACAM in the complex and preliminary models will be presented. Elucidating the mechanism of interaction between Opa and CEACAM will provide an understanding of the molecular determinants behind the entry of a foreign body into non-phagocytic cells.

11 Andrew T. Lucas[1,2,*], Xiangping Fu[1,*], JingJing Liu[1], Mary K. Brannon[2], Jianhua Yang[1,3], Daniel G. S. Capelluto[2], and Carla V. Finkielstein[1]
[1]Integrated Cellular Responses Laboratory and [2]Protein Signaling Domains Laboratory, Virginia Bioinformatics Institute, Department of Biological Sciences, Virginia Tech, Blacksburg, VA 24061, USA

LIGAND BINDING REVEALS A ROLE FOR HEME IN TRANSLATIONALLY-CONTROLLED TUMOR PROTEIN DIMERIZATION

The *t*ranslationally-*c*ontrolled *t*umor *p*rotein (TCTP) is a highly conserved, ubiquitously expressed, abundant protein that is broadly distributed among eukaryotes. Its biological function spans numerous cellular processes ranging from regulation of the cell cycle and microtubule stabilization to cell growth, transformation, and death processes. In this work, we propose a new function for TCTP as a "buffer protein" controlling cellular homeostasis. We demonstrate that binding of hemin to TCTP is mediated by a conserved His-containing motif ($His^{76}His^{77}$) followed by dimerization, an event that involves ligand-mediated conformational changes and that is necessary to trigger TCTP's cytokine-like activity. Mutation in both His residues to Ala prevents hemin from binding and abrogates

oligomerization, suggesting that the ligand site localizes at the interface of the oligomer. Unlike heme, binding of Ca^{2+} ligand to TCTP does not alter its monomeric state; although, Ca^{2+} is able to destabilize an existing TCTP dimer created by hemin addition. In agreement with TCTP's proposed buffer function, ligand binding occurs at high concentration, allowing the "buffer" condition to be dissociated from TCTP's role as a component of signal transduction mechanisms.

12 Samuel J. Berk[1,2], Anne M. Brown[2], Xiaolin Zhao[1], Geoffrey Armstrong[3], Daniel G. S. Capelluto[1], and David R. Bevan[2]
[1]Protein Signaling Domains, Department of Biological Sciences and [2]Department of Biochemistry, Virginia Tech, Blacksburg VA, 24061, USA; [3]Department of Chemistry and Biochemistry, University of Colorado, Boulder CO 80309, USA.

MEMBRANE BINDING PROPERTIES OF THE INNATE IMMUNITY ADAPTOR TIRAP

The MyD88 pathway is one of the main mechanisms of innate immunity responses. Toll-interleukin-1 receptor domain-containing adaptor protein (TIRAP) has been shown to be the first event in the Toll-like receptor-2 (TLR-2) and TLR-4 signaling myeloid differentiation primary response gene 88 (MyD88)-dependent pathways, and is critical for MyD88 recruitment at the plasma membrane. TLR-2 and TIRAP-dependent signaling is viewed as a part of the "early warning system" in the heart activated in response to tissue injury. More importantly, TIRAP deficiency abolishes the cardio protective effects of ischemic preconditioning. TIRAP associates to the PIP2-enriched regions of the plasma membrane through a PIP-binding motif(PBM). However, the mechanism for membrane recruitment of TIRAP has yet to be fully identified. Molecular dynamics simulations were performed in order to investigate the binding of TIRAP to a phosphatidylinositol 4,5-bisphosphate ($PI(4,5)P_2$) enriched 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) bilayer through its Phosphoinositide Binding Motif (PBM). Specific positively charged amino acid residues were found to be critical for long-term peptide stability, with possible variances based on the order of contact. Strong binding of PBM to lipid bilayer membranes in all cases has led us to study the full-length TIRAP protein. Due to a lack of crystal structure, a homology model of TIRAP was built and evaluated by equilibration in solvent. Simulations of TIRAP dimer formation used to examine homology model validity are currently underway. These findings may provide important information as to the mechanism of membrane binding of TIRAP, and an understanding of the first intracellular event for innate immune responses.

13 Anne M. Brown, Justin A. Lemkul, Nicholas Schaum, and David R. Bevan
Department of Biochemistry, Virginia Tech, Blacksburg, VA 24061

SOLUTION CONDITIONS AND OXIDATION STATE OF METHIONINE-35 AFFECT THE AGGREGATION PROPERTIES OF AMYLOIDB-PEPTIDE (1-40)

The amyloid β-peptide (Aβ) is a 40-42 residue peptide that is the principal toxic species in Alzheimer's disease (AD). The oxidation of methionine-35 (Met35) to the sulfoxide form ($Met35^{ox}$) has been identified as potential modulator of Aβ aggregation. The role $Met35^{ox}$ plays in Aβ neurotoxicity differs among experimental studies, which may be due to inconsistent solution conditions (pH, buffer, temperature). We applied atomistic molecular dynamics (MD) simulations as a means to probe the dynamics of the monomeric 40-residue alloform of Aβ ($Aβ_{40}$) containing Met35 or $Met35^{ox}$ in an effort to resolve the conflicting experimental results. We found that Met35 oxidation decreases the β-strand content of the C-terminal hydrophobic region (residues 29-40), with a specific effect on the secondary structure of residues 33-35, thus potentially impeding aggregation. Further, there is an important interplay between oxidation state and solution conditions, with pH and salt concentration augmenting the effects of oxidation. The results presented here serve to rationalize the conflicting results seen in experimental studies and provide a fundamental biophysical characterization of monomeric $Aβ_{40}$ dynamics in both reduced and oxidized forms, providing insight into the biochemical mechanism of $Aβ_{40}$ and oxidative stress related to AD.

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ELUCIDATING THE ROLE OF A CONSERVED CYSTEINE IN ADENINE DEAMINASES

This study sought to characterize the functional role of a conserved cysteine (C127) found in adenine deaminase (Ade) from *Methanocaldococcus jannaschii*, as well as other archaeal and bacterial homologues. C127 was determined to be essential after site-directed mutagenesis to serine demonstrated complete loss of activity. Molecular docking and dynamics studies showed adenine was unable to properly orient in the active site due to distinct differences in active site conformation and rotation of D261 in the C127S Ade model structure. Thus, C127 was determined to be essential for maintaining active site conformation likely in all Ade.

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CHROMOSOME-NUCLEAR ENVELOPE INTERACTIONS HAVE MULTIPLE EFFECTS ON CHROMOSOME FOLDING DYNAMICS IN SIMULATION

It is well recognized that the chromosomes of eukaryotes fold into non-random configurations within the nucleus. In humans and fruit flies, chromosomes likely adopt a particular 3D configuration called the fractal globule (FG) which has multiple biologically significant properties. However, the fractal globule is a metastable state which, over time, transitions to a less biologically favorable state called the equilibrium globule. One of the key questions is how the FG state is stabilized in-vivo? We use simulations to study the effects of chromosome-nuclear envelope (Chr-NE) interactions on the dynamics of the fractal globule within a model of *Drosophila melanogaster* (fruit fly) interphase chromosomes. The computational model represents chromosomes as self-avoiding walks (SAW) bounded by the nuclear envelope (NE). Model parameters such as nucleus size, chromosome persistence length, and chromosome-nuclear envelope interactions are taken directly from experiment. Several key characteristics of the non-equilibrium FG state are monitored during each simulation's progress: chromosome territories, intra-chromosomal interaction probabilities, loci specific diffusion constants, and presence of the Rabl (polarized) chromosome arrangement. Next, we compare the outcomes of simulations which include or exclude Chr-NE interactions. We find that Chr-NE interactions reinforce the non-equilibrium properties such as chromosome territories, high intra-chromosome interaction probabilities, and the Rabl chromosome arrangement. In addition, Chr-NE interactions affect loci specific and averaged chromosomal diffusion. Based on these results we conclude that the presence of Chr-NE interactions may delay the decay of the biologically relevant fractal globule state in vivo.

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QUANTIFYING THE STABILITY OF ACRIDINES TO RIBOSOMAL G-QUADRUPLEXES

G-quadruplexes are involved in fundamental regulatory processes, including those associated with cancer. Previous studies primarily focused on telomeric and oncogenic DNA G-quadruplexes, but recent studies have demonstrated that G-quadruplexes in ribosomal DNA or ribosomal RNA (rDNA or rRNA), may be an alternative target with a significant clinical potential through the inhibition of RNA polymerase I (Pol I) in ribosome biogenesis. The structures of ribosomal G-quadruplexes at atomic resolution are unknown and very little biophysical characterization has been performed on them. In our present study, we modeled putative ribosomal G-quadruplexes that were previously predicted using

a bioinformatics algorithm and verified using CD spectroscopy to exist in a predominantly parallel topology. To quantify the relative stabilities of the acridine:G-quadruplex structures, we introduce two novel metrics for quantifying the relative stability of the G-quadruplex tetrads: 1) the center of mass base-to-base distance between diagonal guanines, and 2) the torsional angle between four guanines. Our relative free energy profiles show that the rDNA G-quadruplex structures with shorter loops are more stable for parallel topologies. The antiparallel topology was determined adopt a disordered configuration due to the lack of planarity after the simulation. To evaluate acridine molecules as a viable small molecule chemotherapeutic agent, we then rationally designed several acridine molecules. Each has a polyaromatic face that binds to the top-most G-tetrad and has amino or carboxyl side-chains to selectively optimize the shape and electrostatic complementarity. We then docked acridines to the rDNA G-quadruplexes and performed MD simulations. We identified several acridines with amino groups, and the nature of their specificity and relative stabilities depend on the rDNA G-quadruplex loop structures.

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STRONGLY BENT DNA: RECONCILING THEORY AND EXPERIMENT

The majority of double stranded DNA (dsDNA) in living cells is strongly bent. There is little doubt that standard models that assume harmonic bending energy (Hooke's law, worm-like chain) with effective persistence length of around 50 nm accurately describe the weak bending of DNA. However, it is not clear if these commonly used approximations are still accurate for the poorly understood strong bending regime, which is most relevant to biology. The available experimental evidence is controversial, with the most recent cyclization experiments of ~100 base-pair DNA fragments pointing to strongly bent dsDNA being considerably more flexible than expected. We present a general concept of polymer bending that naturally allows for a transition from the weak, harmonic deformation regime into the "softer" mode at large deformations. In this model, convexity properties of the bending energy potential of individual monomer sub-units of the polymer give rise to the transition between two bending modes. Namely, if the effective monomer deformation energy as a function of bending angle has a non-convex (concave up) region, then the deformation energy of the polymer is a linear function of the bending angle. The dsDNA effective bending potential determined from the angular distribution of base-pairs from PDB structures of DNA-protein complexes is in agreement with our model: it is harmonic in the weak bending regime and non-convex for strong bends. We propose a simple expression that replaces the harmonic approximation in the strong bending regime. The theory shows quantitative agreement with recent cyclization experiments of short dsDNA fragments. Atomistic molecular dynamics simulations point to short-range Lennard-Jones interactions as responsible for the onset of the linear bending regime of dsDNA.

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INSIGHTS FROM ALL-ATOM MOLECULAR DYNAMICS (MD) SIMULATIONS OF 40 NUCLEOSOME CHROMATIN FIBER

Gene expression is regulated, in part, by the organization of chromatin fiber, which is the next hierarchical level of chromatin compaction beyond the nucleosome. Due to the large size of the fiber – millions of atoms – computational studies investigating its organization have typically used coarsegrain simulations. Such simulations use customized, relatively unproven, force fields, and fail to elicit the finer details of the atom level structure. We use multiscale all-atom simulations of 40 nucleosome (over 1 million atom) chromatin fiber to study its structure and response to modifications such as post-translational modifications implicated in chromatin remodeling. The multiscale method used, Hierarchical Charge Partitioning (HCP), exploits the natural organization of biomolecules (atoms, groups, chains, and complexes), to speedup implicit solvent simulations of the fiber by over 2 orders of magnitude. The method uses the proven Amber force field to compute interactions between nearby atoms, while approximating interactions with distant components by a smaller number of charges that optimally reproduce the low order multipole moments of these components. We use the novel technique to gain insight into the organization of the more flexible regions of the fiber, such as linker DNA and histone tails.

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BUILDING WATER MODELS: A DIFFERENT APPROACH

Simplified classical water models are an indispensable component in practical atomistic simulations, especially in biology and chemistry where hydration effects are critical. Yet, despite several decades of intense research, these models are still far from perfect. Presented here is an alternative approach to constructing widely used point charge water models. In contrast to the conventional approach, we do not impose any geometry constraints on the model other than symmetry. Instead, we optimize the distribution of point charges to best describe the "electrostatics" of the water molecule, which is key to many unusual properties of liquid water. The search for the optimal charge distribution is performed in 2D parameter space of key lowest multipole moments of the model, to find best fit to a small set of bulk water properties at room temperature. A virtually exhaustive search is enabled via analytical equations that relate the charge distribution to the multipole moments. The resulting "optimal" 3-charge, 4-point rigid water model (OPC) reproduces a comprehensive set of bulk water properties significantly more accurately than commonly used rigid models: average error relative to experiment is 0.76%. Close agreement with experiment holds over a wide range of temperatures, well outside the ambient conditions at which the fit to experiment was performed. The improvements in the proposed water model extend beyond bulk properties: compared to the common rigid models, predicted hydration free energies of small molecules in OPC water are uniformly closer to experiment, with root-mean-square error < 1 kcal/mol.

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WHY DOUBLE-STRANDED RNA RESISTS CONDENSATION: A MECHANISM FOR TRIVALENT ION INDUCED CONDENSATION OF NUCLEIC ACID DUPLEXES.

The addition of small amounts of multivalent cations to solutions containing double-stranded DNA leads to attraction between the negatively charged helices and eventually to condensation. Surprisingly, this effect is suppressed in double-stranded RNA, which carries the same charge as the DNA. We combine atomistic molecular dynamics simulations and theory to propose a mechanism that explains the variations in condensation propensities of short (25 base-pairs) nucleic acid (NA) duplexes, from B-like form of homopolymeric DNA to A-like mixed sequence RNA. The mechanism connects the NA condensation propensity with the spatial variation of trivalent cobalt hexammine (CoHex) ion binding at the NA surface. The emerged atomistic picture of CoHex distributions around NA reveals two major NA-CoHex binding modes - internal and external - distinguished by the proximity of bound CoHex to the duplex helical axis. We suggest that the differences in the condensation propensities are connected with a variation of a single quantity: the fraction of CoHex ions in the external binding mode. This fraction determines both the number of ions participating in inter-duplex attraction and the external mode ion binding affinity - a major driving force of the NA condensation. Thus, while the CoHex induced NA-NA attraction depends on a complex interplay between various structural and sequence features, our new model proposes a single parameter which connects the NA condensation propensity with duplex geometry and sequence dependence of CoHex binding.

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COMPUTATIONAL AND EXPERIMENTAL CHARACTERIZATIONS OF SILVER NANOPARTICLE-APOLIPOPROTEIN BIOCORONA

With the advancement of nanotoxicology and nanomedicine, it has been realized that nanoparticles (NPs) interact readily with biomolecular species and other chemical and organic matter to result in biocorona formation. The field of the environmental health and safety of nanotechnology, or NanoEHS, is currently lacking significant molecular-resolution data, and we set out to characterize biocorona formation through electron microscopy imaging and circular dichroism spectroscopy that inspired a novel approach for molecular dynamics (MD) simulations of protein-NP interactions. In our present study, we developed a novel GPU-optimized coarse-grained MD simulation methodology for the study of biocorona formation, a first in the field. Specifically, we performed MD simulations of a spherical, negatively charged citrate-covered silver nanoparticle (AgNP) interacting with 15 apolipoproteins. At low ion concentrations, we observed the formation of an AgNP-apolipoprotein biocorona. Consistent with the CD spectra, we observed a

decrease in α-helices coupled with an increase in β-sheets in apolipoprotein upon biocorona formation.

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SMALL FDU STRAND EXHIBITS SALT-DEPENDENT STABILITY

As shown in our previous work, F10 (a 10mer of Fdu – 5-fluoro-2'-deoxyuridine-5'-O-monophosphate) has a 40% likelihood of folding into a stable hairpin structure when Magnesium ions are used to neutralize the simulated system. Investigations of other counter ions revealed that F10 folds less than 10% of the time when neutralized with ions with a +1 charge and 40% of the time when neutralized with ions with a +2 charge. When simulated with biologically relevant combinations of K^{1+} , Na^{1+} and Mg^{2+} ions, F10 folds into this hairpin structure approximately 10% of the time; however, once the strand of Fdu enters this folded state, it is 96% likely to stay in this kinetic trap. Here we present the data from microsecond timescale simulations and hypotheses on F10's behavior in the presence of various counter ions.

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SASCALC—A FAST AND COMPREHENSIVE SCATTERING CALCULATOR FOR CHEMICAL AND BIOLOGICAL SYSTEMS

Increasingly atomistic models are being used to interpret small-angle X-ray and neutron scattering studies. Often, large structure ensembles ($>10^5$ configurations) are required for thorough analysis. Existing calculators, while robust and widely used, are mainly limited to study biological systems over a limited range in momentum transfer, Q. Recently, a new method to account for excess scattering density at molecular surfaces through calibration using atomistic simulations has been developed that extends the range and accuracy of scattering to a wider range of Q. Thus, there is a need to develop an open-source high-throughput accurate small-angle scattering calculator to enable the use of atomistic models to model scattering data. We have developed a new program, SasCalc, that can be used to calculate small-angle scattering profiles for chemical and biochemical systems. The program utilizes a simple rapid real-space algorithm to calculate scattering profiles using the golden vector method. This method has been shown to scale linearly with the number of atoms and is as accurate as established multipole expansion methods. Solvent contribution has been implemented using Hypred and is being expanded to handle generic co-solvents and conditions. Currently, SasCalc is implemented using CUDA and therefore accesses modern graphical processing units to dramatically reduce the time to calculate accurate scattering profiles for ensembles of structures. SasCal is available in the atomistic scattering analysis package SASSIE.

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IMPROVING SOLVATION MODELING BY ACCURATE EVALUATION OF CHARGE HYDRATION ASYMMETRY

Charge hydration asymmetry (CHA) - the characteristic dependence of hydration free energy upon the sign of solute charges – quantifies the asymmetric response of water to electric field at microscopic level. Accurate estimates of CHA are critical for understanding and prediction of hydration effects ubiquitous in chemistry and biology. CHA manifests itself in the significant difference in the observed hydration free energies of ions of similar size, such as the K+/F- pair. However, experimental estimations of CHA from ion hydration energies lack consensus, with roughly 3-fold uncertainty - tens of kcal/mol - due to a wide spread of the available experimental ion hydration data. Yet, fundamental difficulties associated with measuring and interpreting hydration energies of charged species lead to unacceptably large (up to 300 %) variation in the available estimations of the CHA effect from ion hydration data. We circumvent these difficulties by extracting an accurate estimate of the relative intrinsic CHA propensity of water from experimentally measured hydration free energies of neutral polar molecules, which are themselves highly accurate. Specifically, from a set of 504 small molecules, we identify two pairs that are analogous, with respect to CHA, to the K+ /F− pair of ions – a classical probe for the effect. We use these "CHA-conjugate" pairs of molecules to quantify the intrinsic charge-asymmetric response of water to microscopic charge perturbations: the asymmetry of the response is strong, 48 to 49% of the response (charge hydration free energy) itself. The ability of widely used water models to predict hydration free energies of small molecules strongly correlates with their ability to predict CHA.

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OPTICAL FIBER BIOSENSOR WITH SELF-ASSEMBLED NANOSCALE COATINGS FOR RAPID DETECTION OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS*

Methicillin-sensitive staphylococcus aureus (MSSA) is a frequent source of skin and soft tissue infections and can be easily treated with antibiotics. An antibiotic-resistant strain, methicillin-resistant staphylococcus aureus (MRSA), developed shortly after the introduction of penicillin and has been on the rise in the intervening decades. While MRSA used to be present almost exclusively in healthcare settings, recently more virulent community-acquired strains have been gaining ground. A rapid diagnostic test to distinguish MRSA infections from MSSA is needed to ensure that patients receive proper therapies, but current standard techniques take several days. We present a novel, rapid optical biosensor for identification of MRSA using long period gratings (LPGs) in optical fibers combined with nanoscale coatings. The transmission spectra for LPGs undergo environment-sensitive attenuation at resonant wavelengths. Our gratings are tuned to a

"turn-around point," where the system exhibits heightened sensitivity and single-wavelength simplicity, by adsorbing ionic self-assembled multilayers (ISAMs) to the fiber surface. The ISAM surface is then functionalized with receptors to form a rapid, sensitive, and specific bioaffinity sensor for MRSA with the potential for rapid diagnostic clinical applications. We have demonstrated the ability of the sensor system to distinguish 36 strains of methicillin-resistant staphylococci from 25 strains of methicillin-sensitive staphylococci and other bacterial species, all at concentrations of 10,000 CFU/ml.

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DYNAMICS OF CFTR AGGREGATION ARE ALTERED UPON PROTEASOME INHIBITION AS MEASURED BY N&B ANALYSIS

The Cystic Fibrosis Transmembrane conductance Regulator (CFTR) is a polytopic protein that traffics to the apical surface of epithelial cells where it transports chloride. The folding and synthesis of CFTR in the endoplasmic reticulum (ER) is inefficient, and up to 80% of wild type protein may be degraded. A common mutation in CFTR is the deletion of a single amino acid that enhances misfolding, causes complete degradation of the protein, and is a major cause of Cystic Fibrosis (CF). Prolonged inhibition of the proteasome results in the accumulation of CFTR into perinuclear insoluble aggregates termed aggresomes. The composition of CFTR aggresomes has been well studied but there is scant information on the dynamics of aggresome formation. We have employed number and brightness (N&B) analysis to investigate the dynamics of early CFTR misfolding intermediates. We expressed low levels of EGFP-CFTR in a renal epithelial cell line, and monitored the CFTR oligomeric status in the absence and presence of the proteasome inhibitor, MG132. We found EGFP-CFTR formed clusters that contained on average 6 fluorescent molecules and cluster sizes increased to ~15 fluorescent molecules after several hours of proteasome inhibition. Cluster formation in proteasome inhibited cells occurred at 5 fold lower expression levels compared to control cells. CFTR clusters colocalized with the ER marker Sec61-RFP but not with a marker for ER exit sites and were not perinuclear. CFTR clusters were reduced after ~3 hours of MG132 washout, suggesting CFTR puncta were not insoluble aggresomes but could represent “pre-aggresome” intermediates.

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EXPLORING SIGNALING PATHWAYS IN SKBR3 BREAST CANCER CELLS FOR IDENTIFYING NOVEL BIOMARKERS AND DRUG TARGETS

Mass spectrometry (MS) is widely accepted for its high sensitivity and resolution capabilities that enable the analysis of complex biological samples. In this study, we used MS-based proteomic approaches for identifying putative biomarkers and drug targets for cancer detection and therapy. Breast cancer cells (Her2+) were cultured in adequate media with 10 % FBS, arrested in the cell cycle by serum deprivation, stimulated with growth factors or hormones, harvested, and separated into nuclear and cytoplasmic cell fractions. The protein extracts were digested with trypsin, and prepared for nano-liquid chromatography (LC) analysis and MS detection with a linear ion trap mass spectrometer. After statistical data processing for selecting only high-confidence proteins, a range of bioinformatics tools were used for interpreting the data in biological context. Typically, over 1000 proteins could be identified in each cell fraction, and signaling pathways of highest relevance to cancer cell proliferation such as ErbB, Jak-STAT, PI3K, mTor, TGF-beta, could be mapped by as many as 5-20 proteins. These proteins were searched for posttranslational modifications and structural motifs that would enable them to be used alone, or in combination, as biomarker sets for cancer diagnosis, or as target molecules for developing novel anti-cancer therapies.

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A NOVEL MICROFLUIDIC APPROACH FOR THE STUDY OF PHOSPHO-SIGNALING EVENTS IN CELLS

In our laboratory, we use SKBR3, a widely studied HER2+ breast cancer cell line, as a model system. Cells are cultured in medium complemented with FBS, arrested in the cycle by FBS withdrawal, and then stimulated with EGF/FBS for re-entering the cell cycle. The cells are further processed by a protocol that includes protein extraction, proteolytic digestion, sample cleanup and nano-liquid chromatography (LC)-mass spectrometry (MS) analysis. Subsequently, more than 1000 proteins can be detected and confidently identified in each cell state. By comparing proteomic profiles, key proteins that are part of cancer-relevant Kegg pathways such as MAPK, P53, apoptosis, ErbB, and TGF-beta can be selected to infer protein interaction networks that contribute to excessive cell proliferation. A variety of transcriptional/translational activators, repressors of CDK inhibitors, mediators of cell survival and evasion of apoptosis, as well as cell adhesion and metastasis proteins were identified by this approach. To capitalize on our preliminary results generated with bench-top instrumentation, our next goal was to devise a microfluidic device that enables cell stimulation, digestion, enrichment in phospho-peptides and nano-LC separations. Fast cell lysis and sample transfer between various microfluidic functional elements, followed by efficient LC separations, are key features enabled by this device, and essential for

profiling phosphorylation events in a cell. The microfluidic strategy also facilitates the quantitation of proteins with detection at the ~1 fmol level with a dynamic range of ~10^3, with reagent consumption and analysis costs reduced ~100 and ~10-100-fold, respectively. The benefits, challenges and limitations of this microfluidic approach will be discussed

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AN INTRODUCTION TO THE ANALYSIS OF PHOSPHOPROTEINS BY MASS SPECTROMETRY TECHNOLOGIES

Protein phosphorylation represents one of the most important post-translational modifications (PTM) that occur in a biological system. This PTM "activates" a protein to perform a specific function. For example, proteins can be phosphorylated to initiate an intracellular signal cascade for a subsequent reaction to occur. In eukaryotic cells, protein phosphorylation affects mainly polar amino acids such as Serine, Threonine and Tyrosine. Mass spectrometry (MS) is a powerful technology that enables a detailed analysis of phosphorylation. The sequence of proteins and their peptide fragments, the presence of a PTM, as well as the site of the modification can be determined with accuracy. Though the process is straightforward, there are many challenges associated with the MS analysis of phosphoproteins, mainly related to the lability of the phosphate group, and the low abundance and transient nature of the modification itself. A common MS analysis strategy involves the following steps: performance of a biological experiment, extraction of proteins from cells, proteolytic digestion with an enzyme (typically trypsin), enrichment in phosphorylated peptides, sample cleanup, and nano-liquid chromatography (LC)-MS followed by bioinformatics analysis of the data. In this presentation we will highlight some of the breast cancer projects that are pursued in our laboratory. We are mainly interested in profiling cellular signaling networks that lead to uncontrolled proliferation of cancer cells. This type of research can impact the fight against cancer by identifying novel drug-targets and biomarkers that can contribute to finding resourceful ways to prevent the onset and development of this life-threatening disease.

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DETERMINING THE ROLE AND REGULATORY CONTROL OF POL IN PLANT STEM CELLS

All plant biomass originates from meristems, which contain discrete populations of stem cells. Maintenance of these stem cell populations is controlled by cell-cell signaling networks composed of a transmembrane receptor kinase (from the CLAVATA1 clade), a peptide ligand (from the CLE family), and a homeobox transcription factor (from the WOX family). The signaling cascade from this RK-WOX network is responsible for limiting stem cell proliferation and allowing differentiation. The two redundant class 2C protein phosphatases, Poltergeist (Pol) and Poltergeist-Like 1 (Pll1), negatively regulate the RK-

WOX pathways. Mutation in one of these genes causes no noticeable phenotype, but the *pol pll* double mutant is seedling lethal, indicating its importance in development. It is currently unknown how these proteins negatively regulate the signaling network, or how they are regulated themselves. Pol has two novel conserved domains, DI and DII. DI is enriched in serine residues, and twelve of the residues conform to a S-(A/G)-P-(L/I) phosphorylation consensus sequence, where the serine is phosphorylated. We hypothesize that the novel DI domain is involved in regulation, post-transcriptionally, via phosphorylation. To test this hypothesis, *Arabidopsis thaliana pol pll1/+* double mutants were transformed with four modified variants of the Pol protein. The modified variants consist of catalytically active (1) or dead (2) forms of the phosphatase, and aspartic acid (3) or alanine (4) substituted serine residues in the DI domain coupled to wild-type phosphatase activity. Using these modified lines, we hope to elucidate the function and regulation of the Poltergeist family of protein phosphatases.

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EXAMINING DIURNAL VARIATION IN AMPLITUDE OF ELECTRIC ORGAN DISCHARGE IN *PARAMORMYROPS KINGSLEYAE*

Mormyrid fish produce an electrical communication signal called an electric organ discharge (EOD). Temporal variation in EODs, such as differences in duration and complexity, convey important information between senders and receivers. EODs are generated through a mechanism similar to that of the action potential of a neuron, specifically using voltage-gated sodium channels. Recent work identified a sodium channel gene, *Scn4aa*, which is specifically expressed in electric organ of mormyrids. We hypothesize that the reduced expression of *Scn4aa* in the electric organ will lead to a decrease in the amplitude of the EOD. To test this hypothesis, we developed an EOD recording application to work with National Instruments data acquisition hardware using LabVIEW. We will use this application to determine if the mormyrid *Paramormyrops kingsleyae* exhibits natural diurnal variation in its EOD. Next, we will interfere with *Scn4aa* expression by injecting a morpholino into the electric organ. Morpholinos are small fragments of antisense RNA designed to perform targeted interference with protein translation. This interference could provide a link between gene expression and communication behavior in *Paramormyrops kingsleyae*.

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NOROVIRUS ANTIBODY PROFILING BY A LUCIFERASE IMMUNOPRECIPITATION SYSTEMS ASSAY

Norovirus is a major etiologic agent of acute gastroenteritis, causing about 200,000 deaths per year in children in developing countries. Because a cell culture system does not currently exist for norovirus, vaccine development and immunological studies of norovirus pathogenesis have been greatly hindered. In this study, we investigate whether the recently developed Luciferase Immunoprecipitation Systems (LIPS) assay, a novel high-throughput method of antigen generation and sera analysis also serves as a sensitive and specific immunological assay for profiling norovirus antibody responses in sera. The LIPS assay involves subcloning a gene encoding the protein antigen of interest into a plasmid vector encoding a reporter enzyme Renilla luciferase (Ruc), and quantifying antigen-specific antibody levels in tested sera through measurement of light production. We established a novel LIPS assay through the generation of three constructs using different norovirus antigenic domains of the GII.4/2006b variant: Ruc-VP1, Ruc-shell domain, and Ruc-protruding domain. Using these three fusion proteins, we analyzed antibody levels of hyperimmune guinea pig sera raised against the GII.4/2002 and GII.4/2006b norovirus variants to generate data for cross-variant and homotypic antibody responses, respectively. Overall, this study has important implications for new diagnostic methods in hospitals and during outbreaks, and for future studies elucidating the immunogenic role of the shell domain and protruding domain of norovirus to inform vaccine development.