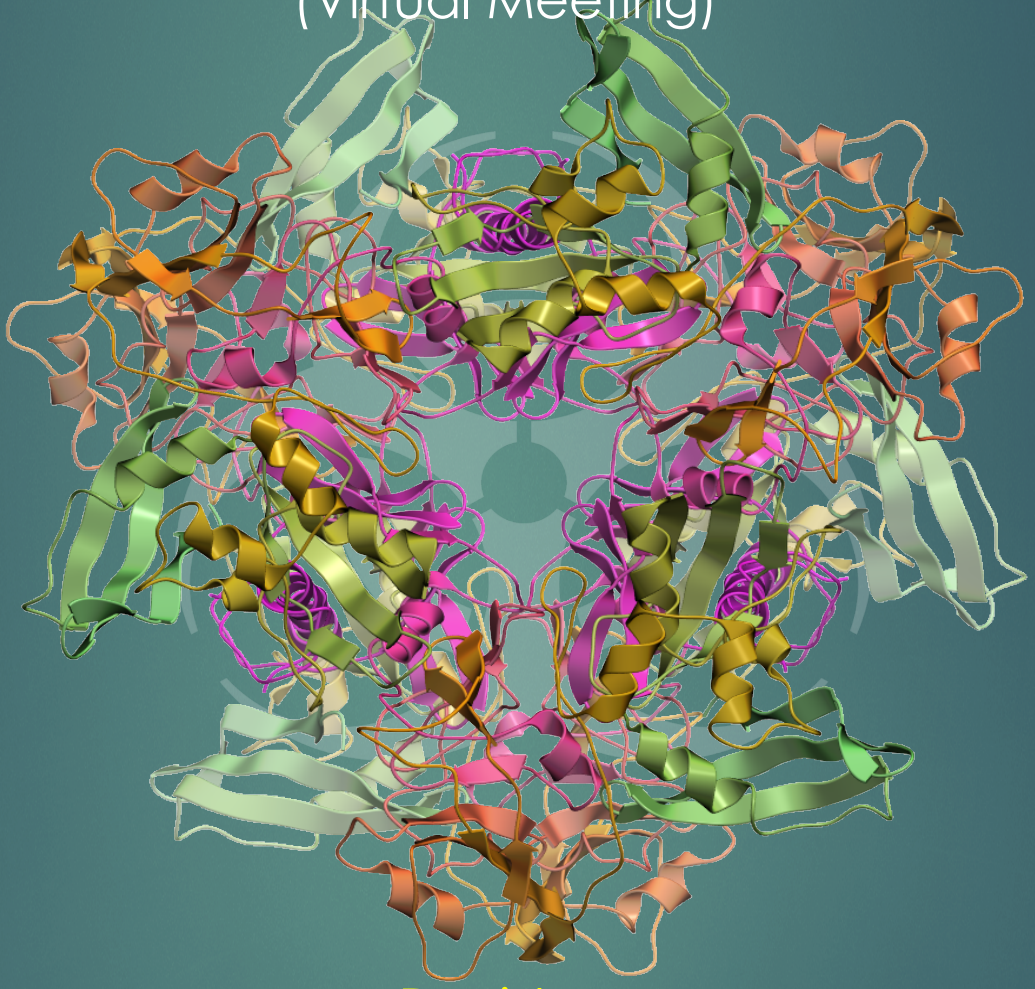
NESS 2020

STRUCTURAL BIOLOGY OF HOST-PATHOGEN INTERACTIONS

October 24, 2020 | UCONN | STORRS, CT

(Virtual Meeting)



Register:

http://pesb.uconn.edu/ness-2020

Organizers:

James Cole | UCONN | james.cole@uconn.edu

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UCONN | Partnership for Excellence in Structural Biology

General Information

The meeting will open on-line on October 24, 2020 at 9:00 am EST. All posters will be viewable to attendees continuously from October 23 - 30, 2020.

To encourage sharing of unpublished data at NESS 2020, recording screenshots and/or videos or downloading material is strictly prohibited.

1. Be polite and respectful of others when using the microphone or chat functions.
2. Do not transmit photographs of slides or posters under any circumstances.
3. Attendees should refrain from posting content from talks for posters to social media (Tweeting, Facebook, etc.).

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Irina Bezsonova, PhD
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North Eastern Structure Symposium

The annual North Eastern Structure Symposium (NESS) series brings together a spectrum of Structural Biology researchers from the North East region, and distinguished speakers from further afield. This series is intended to provide a venue for discussing new developments in Structural Biology and for sharing expertise on new methodologies, as well as to offer a special opportunity for students and senior investigators to interact and foster new collaborations. Each year's symposium covers a defined topic in Structural Biology.

We look forward to your participation in this exciting and thought-provoking symposium and ask that you please share the information about NESS with your colleagues.

https://pesb.uconn.edu/ness-north-eastern-structure-symposium/

NESS 2020 Organizers

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Professor of Molecular & Cell Biology and Structural Biology, Biochemistry and Biophysics
University of Connecticut, Storrs, CT

Melissa J. Caimano, PhD
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UConn Partnership for Excellence in Structural Biology

The UConn Partnership for Excellence in Structural Biology brings together faculty, staff, and students to form an interactive research and training environment. We have active research programs in the structural analysis of macromolecular assemblies and membrane proteins, computational and experimental methods development, computational modeling, and analysis. Our major facilities include NMR, X-ray crystallography, Analytical Ultracentrifugation, and Proteomics & Metabolomics core facilities. The partnership supports collaborative research projects, interdepartmental graduate training and an annual symposium (NESS).

https://pesb.uconn.edu/

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PROGRAM

INTRODUCTION

9:15 - 9:25 **Juli Wade**. Dean, College of Liberal Arts and Sciences

PLENARY SESSION

Chair**: Jim Cole**

9:25 **Ian Wilson**, *Structural insights into antibody responses to SARS-CoV-2*

Break (10 min)

Session 1: Host-Viral Interactions

Chair: **Olga Vinogradova**

10:15 **Sandra Weller**, *Viral Nucleases as Novel Antiviral Targets: from Herpes to Coronavirus*

10:45 **Gaya Amarasinghe**, *Mechanisms of Filoviral Immune Evasion*

11:15 **Jason McLellan**, *Structure-Function Studies of the SARS-CoV-2 Spike and Development of COVID-19 Interventions*

Lunch / Poster viewing

11:45 - 1:00 https://pesb.uconn.edu/ness-2020-posters/

Session 2: Host-Bacterial Interactions

Chair: **Amit Luthra**

1:00 **Jorge Galán**, *The type III secretion protein injection machine*

1:30 **Kelly Hawley**, The Rationale and Building Blocks of "NucleVar"

1:45 **Alexei Savchenko**, Bacteria-encoded ubiquitin protein (E3) ligases: beating eukaryotes at their own game

2:15 **Neha Verma,** Structural Modeling of t6A Biosynthesis System and Flexibility Analysis of TsaC2 in *Borrelia*

Break (10 min)

Session 3: Host Responses

Chair: **Jianbin Ruan**

2:40 **Alexei Korennykh**, *Railroad Crossing Gate of Translation: dsRNA Surveillance by the RNase L System*

3:10 **Louis Hollingsworth**, DPP9 directly sequesters the NLRP1 C-terminus to repress inflammasome activation

3:25 **Erik Nordquist**, Physical Origins of DnaK-Substrate Binding Revealed through Physics-based Modeling

3:40 **Melissa J. Caimano** Poster prizes / Closing remarks

SPEAKERS

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NESS 2020 Poster Session

Posters will be displayed October 23 – 30, 2020
https://pesb.uconn.edu/ness-2020-posters/

Please contact presenters directly with questions.

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Interactions of PIP and RIR motifs in TLS Polymerases with PCNA and Rev1-CT

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Translesion synthesis (TLS) is a mechanism utilized by cells to circumvent accumulated structural damage to DNA which would otherwise impede DNA replication. In TLS, proliferating cell nuclear antigen (PCNA) is monoubiquitinated, which recruits the specialized TLS polymerases (pols): pol η, pol ι, pol κ, pol ζ and Rev1 to DNA. These bind to PCNA via its ubiquitin binding domains and to the interdomain connector loop (IDCL) via their "PCNA interacting Peptide" or PIP motif. The TLS pols also bind to the c-terminal (CT) domain of Rev1, which also serves as a scaffold for the TLS complex, via their "Rev1 Interacting Regions" or RIR motifs. Both PIP and RIR motifs feature two adjacent hydrophobic residues (typically 'FF') which insert into hydrophobic pockets in Rev1-CT or the PCNA IDCL. Certain motifs in yeast have also shown capacity to bind both scPCNA and scRev1-CT, contributing to the hypothesis that these are not distinct domains, but in fact, a single family of "PIP" like motifs. To better validate this claim and further elucidate of the mechanism of TLS pol binding in the human system, we sought to compare relative binding affinities of each of the motifs to PCNA or Rev1-CT by performing isothermal titration calorimetry (ITC) and nuclear magnetic resonance (NMR) titrations. We demonstrate that PIP and RIR motifs retained specificity for their canonical partners. However, we have also identified several motifs with overlapping specificities for both PCNA and Rev1-CT. These findings suggest that the protein-protein interactions governing the assembly of the TLS complex may be more complicated than previously assumed and supports the notion of dual acting "PIP-RIR" motifs.

The Development of Parameters for Modified Amino Acids to Study Protein Dynamics

Kellon A. A Belfon, Chuan Tian, Lakshan Manathunga, Lauren Raguette, James A. Maier, and Carlos Simmerling

Departments of Chemistry, Laufer Center for Physical and Quantitative Biology, Stony Brook University, NY

Modified amino acids are used as probes in spectroscopic techniques to study protein dynamics and function. The modified amino acids are placed at specific locations in proteins to extract conformational changes over time. However, the spectroscopic data often do not reveal atomistic details of the underlying structure, or how the system behaves in the absence of the added probes. Molecular Dynamics (MD) simulation can be a complementary tool to spectroscopy by providing deeper insight on the experimentally observed conformational changes. Accurate force field parameters for the engineered, modified amino acid are pivotal. For example, incorrect dihedral parameters can cause incorrect distance distributions and lead to the inability to match Electron Paramagnetic Resonance (EPR) data. In this project, our goal is to develop accurate dihedral parameters for five popular modified amino acids: acetylated lysine, cyano-phenylalanine, azido-phenylalanine, methanethiosulfonate spin-label, and selenomethionine to study protein dynamics. We utilized our genetic algorithm, RAGTAG: Rapid Amber Gpu Torsion pArameter Generator, to fit the dihedral parameters to Quantum Mechanics (QM) energies. We calculated QM reference energies for over 4,000 conformations of the modified amino acids at the MP2/6-311+G** level. The dihedral parameters were generated by fitting the MM energies to the QM reference energies with an average absolute error that ranged from 0.89 to 1.16 kcal/mol. The partial charges were generated by doing a restrained fit to electrostatic potential calculated at the HF/6-31G* level. Additionally, we added support for the use of selenium in Amber by developing VDW, bond, and angle parameters fitted to enthalpy heat of vaporization and QM bond dissociation energies, respectively. We show that the newly parameterized modified amino acids have good agreement with experimental data from EPF and FRET spectroscopy on T4-Lysozyme, HIV-1 protease, and IAPP.

Diverse Environment Related (Der) protein is a novel OMP85 subfamily present in free-living bacteria and pathogenic *Leptospira* spp.

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Leptospirosis is an emerging zoonotic spirochaetal disease with a worldwide distribution. The incidence of acute leptospirosis is estimated to be >1 million cases annually, with more than ~59,000 deaths. Pathogenic *Leptospira* spp., the causative agent of leptospirosis, are maintained in nature within zoonotic cycles. Infection begins when a naïve host, including humans, comes into direct contact with water or soil contaminated with urine from an infected reservoir host. Like their non-pathogenic saprophytic counterparts, pathogenic leptospires may survive for weeks outside of the host in water and soil. Little is known about the survival programs and gene products required for "environmental adaptation" and the extent to which these processes differ between saprophytic and pathogenic *Leptospira* spp. D15/Oma87/Omp85-like (Omp85) proteins are outer membrane β-barrels widely distributed in Gram-negative bacteria. The hallmark feature of Omp85 is the presence of an evolutionarily conserved C-terminal membrane-embedded β-barrel domain. The Omp85 superfamily can be further divided into at least 10 subfamilies based on the domain architecture of their N-terminal (periplasmic) regions. Omp85 proteins have functions in the assembly of outer membrane proteins (*e.g.*, BamA) and protein translocation (*e.g.*, FhaC). This study aimed to understand the distribution of Omp85 proteins in *Leptospira* spp. The domain composition of *L. interrogans* proteome available in Pfam database describes four proteins containing the conserved C-terminal Omp85 domain (PF01103). One (LIC11623) contains canonical N-terminal POTRA domains (PF07244). Based on bipartite domain organization and structural modeling, we propose that LIC11623 encodes the leptospiral BamA homolog. The other three proteins (LIC12252, LIC12254 and LIC12258) contain no previously defined N-terminal domain. Using ThreaDomEx server we predicted the boundaries for an extra domain of unknown function (DUF) in their periplasmic N-terminal region. *In silico* prediction of DUF secondary structure suggested an α1-α2-β1- β2-α3-β3-α4-β4 topology. As identified by RT-PCR, the novel OMP85 are upregulated in infected mouse urine compared to *in vitro* and DMCs. LIC12258 presented the highest transcriptomic changes, with a urine-upregulation of 4.67- and 6.58-fold compared to DMCs and *in vitro* cultures, respectively. Mice infected with a *L. interrogans* mutant, containing a transposon insertion in a novel OMP85 (LIC12254) site, excreted 1-$\log_{10}$ less bacteria in urine over time. Protein HMMER (pHMMER) ortholog searches using each DUF sequence as queries identified 19 common hits with high residues conservation. All found matches were associated with C-terminal OMP85 domains. Interestingly, identified orthologs are from free-living species, most of them from Bacteroidetes and Proteobacteria phyla and isolated from harsh environments. That fact led us to name the novel OMP85 as Diverse Environment Related (Der) proteins. We analyzed the Omp85 composition in 26 different *Leptospira* spp. While BamA sequence and locus is conserved in all analyzed species, Leptospiral Der Proteins (Ldp) evolved differently in pathogenic/saprophytic *Leptospira*. Phylogenetic analysis using full-length Ldp sequences demonstrate the existence of five Ldp orthologues, with only one present in pathogenic and saprophytic species, suggesting evolutionary adaptations for each bacteria-environment interaction. Despite the observed relation between Der proteins and environment fitness, their function(s) remains to be determined. Diversity in Omp85 composition could have allowed *Leptospira* spp. to evolve a more diverse outer membrane proteome and to adapt to its changing environments. Future studies, including mutagenesis sand biophysical analyses, are needed to establish the putative function(s) of these intriguing leptospiral Der proteins.

Conformational exchange at a C_2H_2 zinc-binding site facilitates redox sensing by the PML protein

Thomas Bregnard[1], Dmitry Korzhnev[1], Sandra Weller[1] and Irina Bezsonova[1]

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The promyelocytic leukemia protein, PML, is a small, ubiquitin-like modifier (SUMO) ligase that plays a vital role in various cellular stress responses, including the response to oxidative stress. Oxidation promotes formation of PML-rich structures in the nucleus called PML nuclear bodies, which coordinate cellular stress responses through SUMO signaling. Recruitment of various proteins to a PML nuclear body may trigger their post-translational modification, activation, sequestration, or degradation through the ubiquitin-proteasome pathway. In response to oxidation, PML multimerizes and forms mesh-like structures that serve as the outer shell of PML nuclear bodies. PML multimerization is the key event in formation of nuclear bodies; however, its molecular mechanism remains poorly understood. In this work we identify redox-sensitive domains and specific amino acid residues of PML that mediate its response to oxidation. A molecule of PML contains between 24 and 29 cysteine residues, making it a potential sensor of the cellular redox state; however, most of the cysteines are structurally important and engaged in zinc coordination within the N-terminal RING, B-box1 and B-box2 domains of PML. Using *in vitro* oxidation assays, we have compared oxidation sensitivity of the isolated RING and B-box1 domains and show that B-box1 is more sensitive to oxidation. NMR studies of the structure and conformational dynamics of B-box1 show that one of its two zinc-binding sites undergoes significant conformational exchange on a μs-to-ms timescale, revealing a hotspot for potential loss of coordinated zinc and exposure of reactive cysteines. Overall, our results suggest that the increased sensitivity of B-box1 to oxidative stress makes this domain an important redox-sensing component of PML.

The Rationale and Building Blocks of "NucleVar": an Automated, "Genomics-to-Structural Vaccinology" Approach to Expedite Vaccine Design against Syphilis

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Syphilis, caused by the pathogenic spirochete *Treponema pallidum* subsp. *pallidum* (*T. pallidum*) poses a growing threat to global health; the World Health Organization estimates an annual worldwide incidence of approximately six million cases. These alarming trends emphasize the pressing need for a vaccine against syphilis, which must target surface exposed (i.e., antibody-accessible) extracellular loops (ECLs) of outer membrane proteins (OMPs) expressed by geographically diverse strains of *T. pallidum*. The vaccine development efforts need to proceed cooperatively along three fronts. One is to perform topologically and structurally characterization of predicted OM-embedded β-barrels in *T. pallidum*, coupled with their protection studies in the experimental rabbit model. The second is to implement advanced whole-genome sequencing (WGS) methodologies to catalog sequence variability among β-barrels encoded by geographically diverse *T. pallidum* clinical isolates. The third is to localize sequence variability onto 3D structural models of β-barrels to investigate its effect on functionality, antigenicity and immune evasion. Recently, significant progress has been made in characterizing β-barrels (Radolf JD *et al*., Curr Top Microbiol Immunol. 2018) and developing enrichment methods for WGS of *T. pallidum* directly from syphilis chancres (Chen W *et al*., JID 2020). To expedite the design of a ECL-driven vaccine against syphilis, where sequence variability largely contributes to immune evasion, we developed a pipeline to manually curate OMP-encoding genes from 74 geographically diverse *T. pallidum* strains and then performed Multiple Sequence Alignments (MSAs) on the corresponding amino acid sequences using Clustal Omega. Absolute site variabilities (ASVs) were computed from each MSA using the Shannon Entropy method of the PVS (Protein Variability Server) program. ASVs then were superimposed and visualized on 3D models by using custom PyMOL scripts. From these analyses emerged a wide spectrum of variability within *T. pallidum* OMPs, ranging from fully conserved to highly variable. In many cases, variability localized to ECLs containing predicted B-cell epitopes, thus providing evidence for adaptive evolution in *T. pallidum*. However, all steps in our bioinformatics analysis, from the manual curation of genes to mapping amino acid substitution on 3D models, require multiple programs. The use of various programs is a time-intensive and error-prone method, which prompted us to compile all scripts into a single automated platform. Therefore, we have initiated the design of a complied program "**NucleVar**" which will automate all steps from **Nucle**otides (gene) curation to **Var**iability's (amino acid substitution) mapping on the 3D structures. The **NucleVar** platform will be an effective tool that follows a "Genomics-to-Structural Vaccinology" approach to select correct antigens for an effective vaccine against, not only syphilis, but also other infectious diseases caused by different pathogens.

Characterizing Structural Heterogeneity in Computationally Designed Miniproteins.

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Compared to small molecules, *de novo* Rosetta-designed miniproteins have the potential to dock to the surface of other proteins with higher target specificity. Previous work has shown success of such designs, delivered by nasal spray in mice, in treating botulism and influenza, though a more thorough understanding of their structure and dynamics is needed. Here we use Nuclear magnetic resonance (NMR) techniques to investigate one example, EHEE_rd2_0005. A new method, FitNMR, was used to fit a ^{15}N relaxation series obtained for this miniprotein and has shown the ability of the algorithm to resolve underlying scalar couplings which were overlooked by other methods. The scalar couplings agree with the designed secondary structure but peak doubling, also observed in these spectra, may be suggestive of structural heterogeneity, dynamic exchange, or a combination of each. Variable temperature HSQC studies have suggested the protein undergoes a global unfolding event upon heating, and a molecular dynamics simulation shows a tryptophan flip, either of which may cause the peak doubling. Current work is aimed at trapping specific states through heat shocking and separation via Size Exclusion Chromatography (SEC). The species present in the chromatogram are being studied by HSQC to map changes in peak intensities and positions to further explain the features present in the NMR spectra. We hope this work will insight the design of miniproteins to ensure the intended functions are carried out in the cell.

Investigation into the Effect of Dimerization on PKR Activation with Enhanced Sampling Molecular Dynamics Methods

Aaron G. Feinstein, James L. Cole and Eric R. May

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Human protein kinase R (PKR) is a serine/threonine kinase which is centrally involved in the cell's innate immune response to viral infection. Upon binding viral dsRNA transcripts, PKR dimerizes and self-activates through autophosphorylation, enabling the downstream phosphorylation of eukaryotic translation initiation factor 2 subunit alpha (eIF2α). In its phosphorylated state eIF2α inhibits the translation of viral RNA products, arresting viral proliferation. The prevailing model of the self-activating dimerization event has PKR's protomers forming a back-to- back (BTB) homodimer, with their activation loops on opposite sides of the complex. In the B2B model, the protomers are autophosphorylated in a cis fashion when an intra subunit contact is formed between the activation loop and the ATP binding site. We propose that activation in fact occurs in a three-step process: back-to-back dimerization, which induces an active-like conformation of the protomers, followed by a second front-to-front trans event in which a third "substrate" protomer is phosphorylated by the active dimer. Here we focus on the first stage of activation, how back-to-back dimerization can induce an active conformation of the protomer. We use non-equilibrium molecular dynamics simulations to generate the activation pathways of PKR molecules in both monomer and back-to-back dimer states. A hallmark of activation in ser/thr kinases is the repositioning of their α-c-helix toward the enzyme's catalytic center, which is the coordinate we use to define the activation transition. From our pathways we perform extensive bias-exchange umbrella sampling simulations to sample and compute robust estimates of the free energy landscape for activation. From these free energy profiles we are able to compare barriers and overall free energy changes to evaluate how dimerization affects the energetics of activation.

PlzA is a unique dual function c-di-GMP effector protein required for survival of the Lyme disease spirochete, *Borrelia burgdorferi*, within its arthropod vector and mammalian host

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**these authors contributed equally to this work*

Lyme disease, the most prevalent arthropod-borne disease in the United States, is caused by the spirochete *Borrelia burgdorferi* (*Bb*). *Bb* cycles between a hard tick vector, *Ixodes scapularis*, and a mammalian reservoir host, usually small rodents. In order to maintain this enzootic cycle, *Bb* must sense and respond to myriad signals encountered in these vastly different niches. Two-component systems (TCS) are important mechanisms by which bacteria adapt to their surroundings. *Bb* encodes only two TCSs, Hk1/Rrp1 and Hk2/Rrp2. While the contribution of Hk2 remains unclear, Rrp2 is part of a regulatory pathway involving the spirochete's alternative sigma factors, RpoN and RpoS. Genes within the Rrp2/RpoN/RpoS regulon function to promote tick transmission and early infection. Activation of the other TCS, Hk1/Rrp1, results in production of the second messenger cyclic dimeric guanosine monophosphate (c-di-GMP) by Rrp1, a diguanylate cyclase (DGC). Hk1/Rrp1 is thought to work exclusively in ticks. c-di-GMP exerts its effector function either allosterically or transcriptionally (riboswitches) and is involved in a wide range of adaptive programs in bacteria, including biofilm formation, motility and virulence. The only known c-di-GMP effector protein in *Bb* is PlzA, which contains a C-terminal c-di-GMP binding PilZ domain. Inactivation of both *plzA* and *rrp1* renders *Bb* unable to survive in ticks. In mice, Δ*rrp1* is fully virulent, leading us to propose that mammals represent a c-di-GMP-free environment for the spirochete. Surprisingly, Δ*plzA* strain is avirulent in mice, suggesting that PlzA has both c-di-GMP-dependent and -independent functions. Indeed, complementation of Δ*plzA* with a *plzA* mutant allele that is unable to bind c-di-GMP is virulent in mice. Cultivation of Δ*plzA* mutant in a rat peritoneal cavity prior to murine infection partially circumvents the virulence defect observed with the null mutant, suggesting a role for unbound PlzA early following infection. Using a *Bb* strain expressing a constitutively active DGC, we demonstrated that bound-PlzA interferes with RpoS mediated transcription, but not with RpoS mRNA or protein levels. An extended sequence and structure analysis revealed that *Bb* PlzA has a bipartite domain organization. The C-terminus contain a prototypical PilZ domain that includes a six-strand beta barrel with a C-terminal alpha helix and an extended N-terminal loop containing c-di-GMP binding motifs (RxxxR and [D/N]*h*SxxG). The N-terminal region of PlzA contains a divergent PilZ domain named PilZN3. PilZN3 retains the six-strand β-barrel but lacks the c-di-GMP binding motif. In addition, PilZN3 carries unique features compared to bipartite PilZ-like domains from other bacteria: C-terminal helix is conserved and includes 3 additional α helices, 2 at N-terminal portion and one between β3 and β4. The unique structure of PlzA combined to a predicted conformational change from “bound” to “unbound” states license PlzA to exert different functions, one in ticks and another in mammals.

The global *Treponema pallidum* OMPeome: a structural platform for deciphering stealth pathogenicity and developing a syphilis vaccine with worldwide efficacy

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The outer membrane (OM) of *Treponema pallidum* (*Tp*) serves as the permeability barrier and interface between the syphilis spirochete, an extracellular bacterium with extremely limited biosynthetic capacity, and its obligate human host. It is also the key to developing a syphilis vaccine with worldwide efficacy. The molecular architecture and composition of the *Tp* OM differ markedly from those of prototypical Gram-negative bacteria; recently we have used structural and bioinformatics computational tools to delineate the repertoire of β-barrel forming outer membrane proteins ('OMPeome') in the Nichols strain. The *Tp* OMPeome consists of two 'stand-alone' proteins (BamA and LptD) involved in OM biogenesis and four paralogous families (8-stranded barrel, long-chain fatty acid transport protein (FadL), OM factor of efflux pump (OMF), and *Tp* repeat protein (Tpr) OMPs) involved in influx and efflux of small molecules. The β-barrel assembly machinery (BAM) in *E. coli* consists of BamA and four accessory lipoproteins (BamB/C/D/E). *T. pallidum*'s BAM differs markedly. *Tp* has a BamA (TP0326) but lacks orthologs for BamB/C/D/E; this is consistent with (i) the presence of a gain-of-function mutation (*E. coli* E470 → TP0326 K484) in the BamA β-barrel domain, (ii) the absence of BamB/C/D/E interacting residues in BamA's polypeptide transport-associated motifs (POTRA)1-5 and (iii) a hybrid BAM system in which POTRA1-5 interacts with the DUF domain of TamB (TP0325). SAXS analysis of TP0326 POTRA1-5 revealed a three-state ensemble of compact, bent, and intermediate conformations, highlighting this region's flexibility and providing a model of BamA POTRA-TamB interactions. Although *Tp* does not produce lipopolysaccharide, surprisingly, its genome encodes a nearly complete Lpt-like apparatus for transport and insertion of an unidentified OM constituent, presumably glycolipids. Interestingly, the *Tp* LptD ortholog (TP0515) contains a large unstructured C-terminal domain, which models unbiasedly inside its β-barrel, like LptE of prototypes. *Tp* has four sequentially unique eight-stranded β-barrels containing positively charged extracellular loops believed to aid the spirochete in dissemination. Surprisingly, five orthologs of FadL (14-stranded β-barrel) were found in the *Tp* genome; all have hatch domains and NPA motifs inside the barrel, characteristic features of Gram-negative FadLs. The *Tp* genome encodes Mac and RND tripartite efflux pumps that achieve remarkable combinatorial diversity by co-expression of four paralogous OMFs. Lastly, we confirmed the 'bipartitism' of Tpr paralogs by solving the solution structure of N-terminal periplasmic domains of TprK and *T. denticola* MOSP, the parental ortholog of the Tpr family, and building 3D models of C-terminal β-barrel domains from ten Tprs. Overall, *Tp* appears to have evolved a unique OMP repertoire that balances the spirochete's physiological needs with its parasitic strategy as a stealth pathogen. The Nichols OMPeome provides a structural platform to (i) elucidate *Tp*'s enigmatic parasitic strategies for 'making a living' in the human host, (ii) decipher the targets of natural immunity, and (iii) select candidate vaccinogens for the development of a broadly protective syphilis vaccine for a spirochete that has afflicted humankind for centuries.

Structural Studies of Hetero-Amyloid Signaling Complexes Using SSNMR

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Amyloids are protein aggerates that have a fibrillar morphology with beta-sheet secondary structures. Many amyloids are pathogenic and associated with multiple human diseases, while some of them play indispensable roles in the biological system, termed as functional amyloids. Our lab is specifically interested in the functional amyloids that are responsible for signaling.

Elucidating the Transport Mechanism of Endotoxin and Xenobiotics

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MsbA and P-glycoprotein (P-gp) are structurally, functionally, and mechanistically similar ABC transporter proteins. MsbA is found in gram-negative bacteria, while P-gp is found in intestinal and blood-brain membranes. The endogenous substrate for MsbA is the essential membrane component lipopolysaccharide (LPS), whereas P-gp exports foreign xenobiotics, being responsible for drug efflux activity in acquired multidrug resistance to chemotherapeutics. Co-crystal structures have been determined for P-gp with bound ligand, but MsbA has yet to be solved in the presence of antimicrobials or LPS. Elucidating the chemical characteristics of substrates and their protein binding pockets provides insight for designing inhibitors to combat cellular drug resistance. We constructed three-dimensional models at key points along the LPS translocation pathway. A mechanism for transport is revealed involving clusters of basic residues at the entry and exit portions of the transmembrane chamber. Lastly, sequence and structural analysis of P-gp revealed the location of putative cholesterol binding sites that are likely to influence drug transport.

DPP9 directly sequesters the NLRP1 C-terminus to repress inflammasome activation

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Abstract

Canonical inflammasomes are highly regulated, germline-encoded cytosolic signaling complexes that form in response to diverse pathogenic and endogenous danger signals to induce pyroptotic cell death and cytokine release. Gain-of-function mutations in NLRP1, one such sensor, cause skin inflammatory diseases including carcinoma, keratosis, and papillomatosis. NLRP1 contains a unique function-to-find domain (FIIND) that autoproteolyzes into noncovalently associated subdomains. Proteasomal degradation of the autoinhibitory N-terminal fragment (NT) activates NLRP1 by releasing the inflammatory C-terminal fragment (CT). Cytosolic dipeptidyl peptidases 8 and 9 (DPP8/9) interact with NLRP1, and small-molecule DPP8/9 inhibitors activate NLRP1 by poorly characterized mechanisms. We report cryo-EM structures of the human NLRP1-DPP9 complex, alone and in complex with the DPP8/9 inhibitor Val-boroPro (VbP). Surprisingly, NLRP1-DPP9 forms a ternary complex comprised of DPP9, one intact FIIND of a non-degraded full-length NLRP1 (NLRP1-FL), and one NLRP1-CT freed by NT degradation. Structure-based mutagenesis reveals that the binding of NLRP1-CT to DPP9 requires NLRP1-FL and vice versa, and inflammasome activation by ectopic NLRP1-CT expression is rescued by co-expressing autoproteolysis-deficient NLRP1-FL. Collectively, these data indicate that DPP9 functions as a "bomb-diffuser" to prevent NLRP1-CTs from inducing aberrant inflammation during homeostatic protein turnover.

Structural analysis of influenza A virus morphology using convolutional neural networks

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Influenza A virus (IAV) is a human respiratory pathogen that causes millions of cases and tens of thousands of deaths yearly in the US. IAV is pleomorphic, in both shape and size and also in the distribution and number of its surface glycoproteins, hemagglutinin (HA) and neuraminidase (NA). HA and NA serve complementary roles in the viral life cycle, with HA mediating cellular entry and NA allowing viral egress. Investigating HA/NA balance is thus an important question for better understanding of viral fitness and vaccine development. While cryo-electron tomography (cryo-ET) can depict the 3D organization of pleomorphic IAV, characterizing the viral surface landscape is difficult due to cryo-ET's low signal-to-noise ratio, and the substantial amount of manual effort it requires. Leveraging recent technological and analytical advancements in cryo-ET, we characterized the morphological architecture of more than 300 virions of the A/PR/8/34 (H1N1) strain. We applied the EMAN2 convolutional neural network package to identify viral structural components including glycoproteins. Neural networks successfully identified glycoproteins in tomograms, which were used to extract particles for subtomogram averaging and analysis of glycoprotein density. Overall, we developed a pipeline to efficiently segment IAV and characterize its surface morphology, which strengthens investigations of structure-fitness relationships.

Deciphering structure-activity relationship of USP7 enzyme

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Viral infection characteristically involves hijacking and use of host cellular machinery for propagation of viral progeny. Viruses, in particular encode for specialized enzymes that either mimic or bring about dysregulation of the hosts' ubiquitin machinery. Ubiquitin machinery (Ubiquitination proteasome pathway (UPP)) is notably a two-component system; involving an astounding number of enzymes that facilitate (de)-ubiquitination of proteins. Host cell utilizes UPP to mark the proteins for disposal by appending structural elements recognized by the T cells. However, viruses can manipulate the proteasome degradation machinery thereby affecting antigen presentation mechanism by class-I major histocompatibility complex (MHC), finally escaping T cell recognition. Thus, UPP forms an integral part of (a) adaptive and innate immune response, (b) Host-virus infection and (c) several other processes like cellular trafficking, gene expression & DNA repair. Thus, there is substantial cross talk among viral proteins & UPP proteins at different hierarchical levels, while the UPP dysregulation or lapse forms major etiological factor for viral infection.

Under the purview of Human- viral infections, Human ubiquitin-specific protease 7 (USP7) is often manipulated by viruses such as Herpes Simplex Virus, Human Immunodeficiency Virus, Epstein-Barr Virus, Cytomegalovirus, Kaposi's Sarcoma Herpesvirus & many more, because it stabilizes proteins that play critical roles in the anti-viral responses. USP7 is the most crucial deubiquitinating enzyme (DUB) that is also vital for regulation, stabilization, activity or localization of substrate proteins involved in epigenetics, tumor suppression, the DNA damage response & other pathways. Molecular attenuation of USP7 by viral proteins would allow viral infection & favor its persistence by dampening/ deregulating cellular processes mediated by USP7. Though several studies in the recent past have focused on manipulation of USP7 by viral proteins, still a considerable gap in knowledge exists regarding structure-activity relationship of USP7. Complete understanding of underlying molecular mechanisms is absolutely necessary to advance our knowledge of host-virus survival interactions and aim for future development of therapeutics to combat infection and downstream pathogenesis caused by viruses.

A molecule of USP7 (~130 kDa) is composed of seven domains including N- terminal TRAF-like domain followed by the catalytic domain (CD) and five ubiquitin-like domains. Remarkably, even small C-terminal truncations diminish the activity of the enzyme, suggesting that the C-terminus regulates USP7 activity via a yet unknown mechanism. According to the current model, USP7 adopts an L-shaped structure with N- and C-termini separated by 80-100 Å. This model, however, fails to explain regulation of CD by C-terminus of USP7. Speculatively, C-terminus and CD are brought together in close proximity to create an active conformation, although conclusive structural evidences are still missing, while the available biochemical and structural data are contradictory. Despite successful crystallography studies of various fragments of USP7, structural studies of the full length (FL) protein have been unsuccessful. Here we report our progress in structural and functional characterization FL-USP7 enzyme using integrational approach combining enzymatic activity assays with NMR spectroscopy, Cryo-Electron Microscopy, Small Angle X-ray Scattering and computational molecular docking. A newly developed docking algorithm in HADDOCK allowed us to incorporate SAXS-derived spatial restrains into the modeling of the FL enzyme. Mapping of the inter-domain interaction sites using NMR chemical shift perturbation experiments validated the HADDOCK models. In our integrational FL-USP7 model the catalytic domain and the regulatory C-terminal region of USP7 are located in close proximity, supporting previously hypothesized molecular mechanism of USP7 auto-activation.

GCN Sensitive Protein Translation in Yeast

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GCN codons are overrepresented in the initial codons of ORFs (the ramp region), particularly in highly expressed genes. Molecular Dynamics (MD) simulations have revealed an interaction surface in the mRNA entrance tunnel of ribosomes near the A site decoding center. This CAR interaction surface consists of 16S/18S rRNA C1054, A1196 (*E. coli* numbering), and yeast ribosomal protein Rps3 R146. We observe H-bonding between the CAR interface and GCN in the mRNA +1 codon, the codon about to enter the A site. We hypothesize that this mRNA-ribosome interaction can lead to modulation in protein translation, and under different conditions (e.g. stress conditions) or sequence contexts, the mRNA-ribosome interactions can serve as a mode of regulation for protein translation. Our wet lab experiments have shown that mutations that deviate from the GCN periodicity in the ramp region lead to changes in protein expression levels. We similarly made mutations in the mRNA in MD simulations of the ribosome to observe how the mRNA-ribosome interactions may change. We observed that deviations from GCN led to decreased interactions between the CAR interface and the mRNA +1 codon. Indeed, A-rich and CGN codons show particularly weak CAR interactions. We hypothesize that the codon identity and the degree of conformance to the GCN periodicity of the codons in the ramp region determine the level of mRNA-CAR interaction and hence the level of protein expression.

Kinetics and NMR Studies on Xylanase A mutant to elucidate Thumb Flexibility in Enzymatic Mechanism.

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Protein activity is traditionally studied with major focus on the active site and protein dynamics which contribute to activity. However, the activity of several enzymes, such as Xylanase, has also been linked to the flexibility of adjacent regions, warranting more exploration into the contributions of these distal regions to their mechanisms. Xylanase A (XylA), from Bacillus circulans, cleaves xylan polymers or hemicellulose found in tree bark to xylose or heteroxylans by hydrolysis at internal β-1,4-xylosidic linkages as shown below. XylA contains a “thumb” region which has been correlated in prior studies to substrate binding and product release through its flexibility. However, a double mutation D11F/R122D, which has already been documented to affect the activity and to change the flexibility of the thumb, maintains significant activity even when held in a more open thumb conformation. Michaelis-Menten enzyme kinetics is being studied to evaluate activity perturbations of this double mutation. Following, NMR studies are being used to reveal protein dynamics at an atomic level in the double mutant relative to the wild-type with specific focus being placed on changes in flexibility in the thumb. More specifically, order parameters and chemical shift deviations will be calculated to evaluate the enzyme’s dynamics in the presence of the two mutations. These, as well as molecular dynamics simulations, will aid in further characterizing the necessity of thumb flexibility in the enzymatic mechanism.

Assembly of *S. cerevisiae* Polymerase ζ

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Translesion synthesis (TLS) is a DNA damage tolerance pathway that allows replication over damaged DNA without repair, using specialized error-prone polymerases. TLS enables viability during genotoxic stress at the cost of increased genomic mutagenesis. During TLS, an inserter TLS polymerase, Rev1, Polι, Polη, or Polκ will insert nucleotides across the DNA lesion, and a second TLS polymerase will extend past the site of DNA damage. Polymerase ζ (Polζ) is the major TLS polymerase responsible for the extension step of TLS. Polζ is made of four subunits, Rev3, Rev7, and two accessory subunits, named Pol31 and Pol32 in yeast (PolD2 and PolD3 in humans). Rev3 is the catalytic subunit responsible for DNA synthesis, and Rev7 is the regulatory subunit. Although Rev7 has no catalytic function of its own, Rev7 is a critical subunit to Polζ function.

The catalytic activity of Rev3 is increased substantially when bound to Rev7, and Rev7 is integral for the localization of Polζ to DNA damage sites through its protein-protein interaction with the TLS scaffold, Rev1 C-terminal domain. Recently, a cryo-EM structure of *S. cerevisiae* Polζ was solved in its apo form and in complex with DNA by Lancey *et al*. This structure is groundbreaking to the TLS field because it is the first structure of the Polζ holoenzyme of any species, and *S. cerevisiae* is a model organism for studying TLS. Although a structure has been published, the assembly mechanism of the Rev3 and Rev7 complex is not understood. Rev7 is a member of the HORMA domain family, proteins capable of hetero- and homo- dimerization that bind its binding partners in a unique safety belt mechanism. Structural and biophysical studies of *H. sapiens* Rev7 report that Rev7 is a homodimer that binds two Rev7 binding motifs (RBMs) on Rev3 with a consensus sequence of PXXX(A/P)P, where X is any residue. In this study, we report that *S. cerevisiae* Rev7 exists as a monomer and exhibits an induced dimerization event upon binding to *S. cerevisiae* Rev3. We also report that *S. cerevisiae* Rev3 has two RBMs with a consensus sequence of two consecutive proline residues. However, Y2H and mass-spec assays suggest that RBM2 is the primary partner of Rev7 and that RBM1 exhibits weak interactions with Rev7, which is not observed in the human protein. In conclusion, *S. cerevisiae* Polζ displays a unique assembly mechanism where Rev7 dimerization and the Rev3-Rev7 complex formation is dependent on the RBM2-Rev7 binding interaction. This data furthers our understanding of translesion synthesis and could lead to further investigations into the mechanisms necessary for DNA damage tolerance to occur.

Pyrococcus furiosus thioredoxin as a platform to express extracellular loops of *Treponema pallidum* outer membrane proteins to assess antigenicity and isolate antigen-specific B-cells: a new structural approach for syphilis vaccine development.

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Despite the availability of effective therapy and public health measures, the incidence of syphilis continues to increase in the US and globally. Development of an effective syphilis vaccine is a critical component of strategies to curtail this surging public health threat. Immune rabbit serum (IRS) enhances opsonophagocytosis of intact spirochetes by macrophages *in vitro,* which indicates the presence of potentially protective circulating antibodies targeting surface epitopes of *Treponema pallidum*. Bioinformatic and biophysical methods have enabled our group to identify *T. pallidum*'s repertoire of outer membrane proteins (OMPs): the *T. pallidum* repeat protein (Tpr), 8-stranded β-barrel, outer membrane factor of efflux pumps (OMF) and FadL paralogous families and two 'stand-alones', BamA and LptD, involved in outer membrane biogenesis. Our group has proposed that antibodies directed against extracellular loops (ECLs) of OMPs are responsible for the opsonic activity observed in IRS. Herein, we used *Pyrococcus furiosus* thioredoxin (PfTrx) as a scaffold to express ECLs for assessment of reactivity with syphilitic sera and as 'hooks' for isolation of antigen-specific B-cells for generation of ECL-specific monoclonal antibodies (mAbs). PfTrx was engineered to express BamA-ECL4 ($PfTrx^{BamA/ECL4}$) –a highly immunogenic and opsonic loop previously characterized by our group. N-terminal His6- and C-terminal Avi-tags were added to $PfTrx^{BamA/ECL4}$ for Ni-NTA purification and *in vivo* biotinylation, respectively. Reactivity of IRS and human syphilitic sera (HSS) with $PfTrx^{BamA/ECL4}$ but not with PfTrx alone was detected by immunoblotting. To confirm the antibody accessibility of the ECL4 epitope, a pull-down assay was performed by incubating $PfTrx^{BamA/ECL4}$ or PfTrx with IRS and protein G agarose beads, using normal rabbit serum (NRS) as a control. $PfTrx^{BamA/ECL4}$ was pulled down from IRS but not NRS, while PfTrx alone was not eluted from either IRS or NRS. $PfTrx^{BamA/ECL4}$ then was used to identify BamA/ECL4-specific rabbit B-cells by flow cytometry. Biotinylated $PfTrx^{BamA/ECL4}$ was conjugated with fluorochrome-labeled streptavidin (SP)-Alexa Flour-647 and Brilliant Violet-421, and biotinylated PfTrx was conjugated to APC-Cy7 as a control. After excluding cells that bind PfTrx alone, the frequency of rabbit B-cells that bind $PfTrx^{BamA/ECL4}$ was 1.66% of IgG^+ cells. *T. pallidum* contains five FadL paralogs, OMPs believed to be involved in uptake of hydrophobic nutrients. One of these, TP0856, reacts strongly with IRS and, therefore, could be an attractive vaccine candidate. ECL2 of TP0856, which contains a predicted B-cell epitope, also was expressed in PfTrx ($PfTrx^{TP0856/ECL2}$). Sera from three different immune rabbits strongly reacted against $PfTrx^{TP0856/ECL2}$ by immunoblotting, suggesting the presence of circulating B-cells that secrete antibodies against this epitope. BamA/ECL4 and TP0856/ECL2-specific mAbs generated from loop-specific single B-cells will be used to confirm surface localization of their respective epitopes in *T. pallidum* and assess opsonic activity using the rabbit macrophage opsonophagocytosis assay.

Physical Origins of Selective Promiscuity to Hsp70s Revealed Through Physics-Based Modeling

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Hsp70 molecular chaperones bind a wide variety of substrate sequences, yet retain preferences for particular amino acid motifs ("selective promiscuity"). Statistics-based analyses from peptide array data reveal that DnaK, the *E. coli* form of Hsp70, binds to sequences of 3-5 branched hydrophobic residues, often flanked by Arg, but they are agnostic to details like orientation or registry. Close inspection of available structures of DnaK-peptide complexes revealed a highly conserved substrate binding configuration and further suggests that DnaK's binding cleft consists of five largely independent sites for binding five central substrate residues. Surprisingly, the difference in backbone orientation between forward and reverse doesn't significantly affect sidechain interactions. We used molecular dynamics (MD) simulations to sample the interactions of all 20 amino acids in each of the 5 sites. These interaction terms form the basis of the Physics-based model of DnaK-Substrate Binding (Paladin). Trained using peptide array data, the new Paladin is as accurate as existing algorithms at distinguishing binders and nonbinders of DnaK, and further provides the capability of predicting the substrate binding configuration. Paladin is also the first model to consider peptide binding orientation, and can discriminate between peptides bound in both forward and reverse orientation, a novel result. The physical nature of the Paladin model allows one to examine how various physical interactions at the five binding sites together give rise to substrate selectivity.

Amber Force Field Parameters for Simulations of Phosphorylated Amino Acids

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Phosphorylated amino acids are highly involved in many cell regulatory networks and many research groups are studying proteins containing these post-translational modifications both experimentally and computationally. However, at this time there are no up to date Amber force field parameters that can model these amino acids in the same way as unmodified proteins. Current protein force fields are used to investigate a wide variety of structures and dynamics such as ligand binding, enzyme-reaction mechanisms, and protein folding events. ff14SB and ff19SB are the recommended protein force fields for Amber, but do not contain parameters for non-standard amino acids. The dihedral parameters for the side chains of the most common phosphorylated amino acids were parameterized following the ff14SB approach for multiple backbone conformations using QM and MM, and tested on various systems. Library files and corresponding frcmod parameter files were made available for use with both ff14SB and ff19SB.

A Conserved Arginine in Switch I is Critical for ppGpp Binding to the Prokaryotic Translational GTPase BipA

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BipA is a highly conserved translational GTPase that functions in a variety of cellular processes including fitness, pathogenicity and acclimation to adverse growth conditions. Structural and biochemical studies indicate that GTP and ppGpp compete for binding to BipA to promote differential association of the protein to either the 70S or 30S ribosomal species. Exactly how guanine nucleotide binding to BipA prompts a change in the association of this protein with the ribosome is not understood.

The switch regions of most GTPases are highly flexible and cannot be observed by crystallography. Therefore, molecular dynamics simulations were used to visualize these regions of BipA in the various guanine nucleotide bound states. The ppGpp simulation suggests that a conserved arginine, located in the switch I region of the protein, makes direct contact with the alarmone. To examine whether this residue could be a specificity determinant for ppGpp binding, steady state kinetics and isothermal titration calorimetry measurements were done to assess how an alanine substitution at this site would impact the biochemical properties of BipA. These experiments revealed that although the GTP hydrolysis and GTP binding properties of the protein were unaltered, ppGpp binding was greatly diminished. In contrast to the wild-type protein, filter binding assays show that the alanine-substituted BipA does not associate with 30S ribosomes in the presence of ppGpp. Hydrogen deuterium exchange mass spectrometry confirm that the introduction of this mutation changes the overall dynamics of the protein away from the BipA:ppGpp state and thus selectively abolishing association with the ribosome. In bacteria, this mutant is unable to recover from the onset of stress, confirming the requirement of ppGpp binding by BipA for bacteria to mount an adaptive response via the formation of the 30S:BipA complex.

Reveal the Allosteric Pathway Regulating Alternate Ribosome Binding by the Translation Factor BipA

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BipA is a highly conserved translational GTPase that functions in a variety of cellular processes including fitness, pathogenicity and acclimation to adverse growth conditions. Structural and biochemical studies indicate that GTP and ppGpp compete for binding to BipA to promote differential association of BipA to either the 70S or 30S ribosomal species. Exactly how guanine nucleotide binding to BipA prompts a change in the association of this protein with the ribosome is not understood.

Guided by crystallographic models and amino acid conservation across the BipA family, site-directed mutagenesis was utilized to disrupt crucial interactions within the protein that stabilize inter-domain contacts. These include the interface between domains I and V as well as the surface between domains III, V, and the C-terminal domain. Steady state kinetics, isothermal titration calorimetry, ribosome binding assays and structural analysis were carried out to assess the impact of these substitutions on the biochemical properties of the protein. The intrinsic GTPase activity of all of the proteins was increased in comparison to wild-type BipA and several proteins did not exhibit ribosome-stimulated GTPase hydrolysis. This suggests either a disruption in the signaling pathway or an inability to form a complex with the ribosome. In vivo ribosome binding assays confirmed all but one BipA protein had lost their ability to bind to the ribosome. From these data, as well as accompanying computational modeling, we can propose a pathway of communication between the guanine nucleotide binding site and the C-terminal domain of the protein, previously shown to be required for ribosome binding. Our model also accounts for reverse flow of information required for the cellular role of the protein. Mapping the allosteric network within BipA that influences its' differential ribosome binding may expose a unique bacterial target that can be exploited for the development of antimicrobial therapies.

Structural Modeling of t^6A Biosynthesis System and Flexibility Analysis of TsaC2 in *Borrelia*

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The universal N(6)-threonylcarbamoyladenosine (t^6A) modification at position 37 of ANN-decoding tRNAs plays a vital role in translational fidelity through precise recognition of cognate codons and stabilization of codon-anticodon interactions. In typical bacteria, the t^6A biosynthesis starts with synthesizing an unstable intermediate threonylcarbamoyl-adenylate (TC-AMP), a reaction catalyzed by the universally conserved enzyme TsaC/TsaC2. TsaC is a one-domain protein, whereas TsaC2 has a bipartite architecture composed of an N-terminal domain, a linker region, and a C-terminal Sua5 domain. The threonylcarbamoyl (TC) moiety is then transferred to adenine-37 of tRNAs by a transfer complex comprised of three proteins (TsaB, TsaD, and TsaE). A recently conducted structural and biochemical study (Pichard-Kostuch *et al*. RNA 2018) established the essentiality of linker region and Sua5 domain in TsaC2 for the t6A biosynthesis. Of note, the N-terminal domain of TsaC2 is functionally-equivalent to TsaC. An intriguing question arising from the previous studies on TsaC2 is whether the two essential components (linker region and Sua5 domain) were gained or lost during the evolution. Therefore, we implemented a battery of bioinformatics tools to perform phylogenetic analysis to establish an evolutionary relationship between TsaC and TsaC2. This analysis resulted in a noteworthy observation that some strains of the *Borrelia burgdorferi* sensu lato complex (*Bb*), the causative agent of Lyme disease, encode two forms of TsaC2 (TsaC2-A and TsaC2-B). The Lyme disease spirochetes harbor up to 21 linear and circular plasmids along with its linear chromosome. TsaC2-A is encoded on the chromosome (*bb0734*), while two copies of TsaC2-B are plasmid-borne (*bbt06* and *bbu11*). Both forms of TsaC2 in *Bb* are highly identical, except TsaC2-B lacks the conserved functional motif (Pro-Gly-Met) present in the linker region. The linker region of TsaC2 was hypothesized to bring both domains in proximity for proper functioning. Therefore, to decipher the linker region's role, we cloned, expressed, purified the TsaC2-B and performed the SAXS (small-angle X-ray scattering) experiments in solution, an environment devoid of crystalline constraints. Scattering data revealed multiple conformations of TsaC2-B, in which the N-terminal and Sua5 domains fluctuate through the intrinsically disordered linker region. Next, we built homology models of all components (TsaB, TsaC2-A, TsaC2-B, TsaD, and TsaE) of t^6A modification machinery for Lyme disease *Borrelia* and Relapsing fever *Borrelia.* Structural comparison of 3D models of *Borrelia's* t^6A machinery with *Thermotoga maritima (*$TsaB_2D_2E_2$*) and Escherichia coli (*$TsaB_1D_1E_1$*)* suggests that *Bb* appears to have a divergent repertoire of t^6A components.

ALS-causing SOD1 mutations cause common perturbations to maturational free energy.

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WT-like SOD1 mutations are implicated in promoting or altering ALS disease progression, though the mechanism behind their effects is not yet understood. Using comprehensive backbone sampling from MD simulations and alchemical free energy calculations, we simulated how ten such mutations perturbed free energies at various stages of SOD1 maturation. Together, they provide insights into how these disparate, seemingly insubstantial mutations may cause disease.

NMR Mapping of Disordered Segments from a Viral Scaffolding Protein Enclosed in a 23 MDa Procapsid

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Scaffolding proteins are required for the capsid shell assembly of many tailed dsDNA bacteriophages, some archaeal viruses, herpesviruses, and adenoviruses. Despite their importance, only one high-resolution structure is available for scaffolding proteins within procapsids. Here we use the inherent size limit of NMR to identify mobile segments of the 303-residue phage P22 scaffolding protein free in solution and when incorporated into a ~23 MDa procapsid complex. Free scaffolding protein gives NMR signals from its acidic N-terminus (residues 1-40) and basic C-terminus (residues 264-303), while NMR signals from the middle segment (residues 41-263) are missing because of intermediate conformational exchange on the NMR chemical shift timescale. When scaffolding protein is incorporated into P22 procapsids, NMR signals from the C-terminal helix-turn-helix domain disappear due to binding to the procapsid interior. Signals from the N-terminal domain persist, indicating that this segment retains flexibility when bound to procapsids. The unstructured character of the N-terminus, coupled with its high content of negative charges, is likely important for dissociation and release of scaffolding protein during the dsDNA genome packaging step accompanying phage maturation.