Molecular Biophysics Symposium in Honor of the Grand Opening of the MSU Cryo-EM Core Facility

Friday, October 4

8:00-8:30 Registration/breakfast – Radiology Atrium

8:30-8:35 Acknowledgements – Lisa Lapidus

8:35-8:45 Introduction - Phil Duxbuy – Radiology Auditorium

8:45-9:00 Introduction - Doug Gage

9:00-9:45 James Conway - *Resolving protein structure at high resolution by cryo-electron microscopy - from viruses to crystals*

9:45-10:30 Aaron Frank - *Data-Driven Solutions to Challenges in Biophysics*

10:30 Coffee Break

10:45-11:30 Vinny Manohoran- *Watching individual RNA viruses self-assemble*

11:30-11:50 Jens Schmidt - *Mechanism of processive telomerase catalysis revealed by optical tweezers*

11:50-1:00 Lunch – Radiology Atrium

1:15-4:00 Poster session and Cryo-EM Facility Tour – Engineering Research Facility, Rm D115

Saturday, October 5

8:00-9:00 Breakfast – Radiology Atrium

9:00-9:45 Tom Walz - *Electron Microscopy Approaches to Studying Lipid-Protein Interactions*

9:45-10:30 Yann Chemla - *Probing nature's nano-machines with single-molecule techniques*

10:30-10:50 coffee break

10:50-11:10 Dipali Sashital - *Memory Formation by the Cas4-Cas1-Cas2 Complex During CRISPR Adaptation*

11:10-11:55 Nils Walter - *The RNA Nanomachines of Gene Expression Dissected at the Single Molecule Level*

11:55- 12:15 Susan Hafenstein- *Transferrin receptor binds virus capsids with dynamic motion - receptor and virus rock and roll*

12:15-1:45 Lunch

1:45-2:30 Mark Foster - *Population Shifts from Allosteric Coupling of RNA and Tryptophan in the Gene-Regulating Ring-Shaped Protein TRAP*

2:30-2:50 Kelly Kim - *Characterization of membrane proteins using Cryo-EM: a story of insect olfactory receptor Orco*

2:50-3:35 Liz Kellogg - *Cryo-EM structure of the P element transposase strand-transfer complex*

3:35 – Closing remarks – Lisa Lapidus

Probing nature's nano-machines with single-molecule techniques

Yann R. Chemla
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The living cell is a highly organized 'factory' of molecular machines that carry out specialized tasks, as diverse as moving cargo around the cell or maintaining the cell's genome. The emergence of single-molecule techniques has revolutionized our understanding of such systems, shedding light on their dynamics and functions. In this talk, I will discuss our work developing new single-molecule methods and integrating them with atomic-scale simulations to provide us unprecedented access into structure-function relationships in molecular machines. I will discuss various applications of these approaches, in particular our measurements of DNA helicases, which use the energy of ATP hydrolysis to separate the strands of nucleic acid duplexes and participate in such essential cellular processes as DNA replication, recombination, and repair. Our work shows how prototype members of the largest structural family of helicases unwind DNA and possess molecular switches that regulate their activity. Our findings provide new insights on the mechanisms by which these molecular machines function in the cell.

Resolving protein structure at high resolution by cryo-electron microscopy: from viruses to crystals

James Conway
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Cryo-electron microscopy (cryoEM) has developed into a powerful tool for studying the structure of protein complexes. A number of advances in microscope and camera technology coupled with increasing powerful image analysis techniques have extended the capability of cryoEM-based structure determination to resolutions where atomic-modeling may be performed with high confidence. Particular advantages include study of complete and functional complexes, conformationally heterogeneous populations, and membrane proteins. I will briefly review some of the background of cryoEM, discuss an example of a bacteriophage family that has a close connection to a human virus, and introduce the recent blossoming of electron diffraction.

Molecular mechanisms of *Shigella* phage Sf6 host range expansion

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Bacteriophages infecting Gram-negative bacteria must overcome two membrane layers to gain access to their host. The lipopolysaccharide (LPS) on the outer membrane often mediates the first, reversible interaction between phage and host. Subsequent binding to a secondary receptor, such as outer membrane protein C (OmpC), then triggers phage genome ejection to initiate infection. Compared to long-tailed myo- and siphoviruses, these stages are poorly characterized in short-tailed podoviruses. Previously, it was known that podovirus tailspikes bind to and degrade LPS, bringing the particle closer to the secondary receptor. Another unidentified tail protein was then hypothesized to interact with this second receptor to initiate infection.

To examine the host recognition and initial infection processes in a podovirus, we conducted a series of experimental evolution and targeted genetic studies to determine how a specialist phage, *Shigella* phage Sf6, can gain the ability to infect a new host. Phage Sf6 infects only *Shigella flexneri* serotype Y (strain PE577), with a very low efficiency of plaque formation on serotype 2a (strain CFS100). By serial passaging in a mixture of host types, nine mutant phages that gained the ability to infect serotype 2a were isolated. Genetic and biochemical results indicate that: 1) tailspikes can bind both LPS *and* OmpC, and 2) LPS alone can inactivate phage particles prior to genome translocation. The first result suggests that tailspike is the singular critical protein responsible for infection initiation. This indicates a novel function for the protein, which was previously only known to interact with LPS. Using a series of *S. flexneri* deletion mutants, we also determined that Sf6 specifically requires multiple components of LPS for infection, as the disruption of genes in two independent LPS biosynthesis pathways completely block infection. Finally, based on our results showing that LPS alone can inactivate phage particles, we hypothesize that LPS may be used as a passive weapon against phages. Even low levels of "incompatible" LPS will bind to and block phage tailspikes, rendering the particles uninfectious. This may serve as community-level immunity for bacteria with significant ecological implications, as mixtures of susceptible and non-susceptible bacteria in an environment can reduce the overall presence of infectious phages.

Biological and synthetic platforms for cobalt biomineralization using microbial nanowires

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Since its characterization as a dissimilatory metal reducer, *Geobacter sulfurreducens* has been studied for its genetic system and production of a pilin nanowire [1,2]. These appendages facilitate a wealth of biomineralization processes, electrosyntrophic interactions, bioremediation of contaminated waters, and allow for the formation of electroactive biofilms along electrodes [3]. Across the genus many metals have been characterized to interact with pilin nanowires, including uranium and iron oxides. Pilin assemblages have also been shown to conduct electrons between a gold electrode and soluble cobalt (II), leading to the formation of cobalt nanoparticles along a putative metal trap [4]. Building off these studies, our research demonstrates that *G. sulfurreducens* has the capacity to resist high concentrations of Co^{2+} and, furthermore, that this resistance seems to rely on the proper cycling of pili and suggests their role in heavy metal tolerance. These findings outline a mechanism for microbial biomineralization of Co for biotechnical and industrial applications in the cycling and detection of this valuable metal.

[1] M. V. Coppi, C. Leang, S. J. Sandler, and D. R. Lovely. Microbiology, 67(7), 3180–3187. (2001)
[2] G. Reguera, K. D. McCarthy, T. Mehta, J. S. Nicoll, M. T. Tuominen, and D. R. Lovely. Nature, 435(7045), 1098–1101. (2005)
[3] G. Reguera, and K. Kashefi. Adv. Microb. Physiol., 74, 1–96. (2019)
[4] K. M. Cosert and G. Reguera. J Ind Microbiol Biotechnol. (2019)

RNA Condensation in Sub-Cellular Scale Coarse-Grained Simulations

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Condensation of RNA has been observed by many studies before. However, the mechanism and determinants of this process are not well known. In this study, we performed molecular dynamics (MD) simulations to characterize phase separation events of RNA at the sub-cellular scale using a coarse-grained model. Firstly, we simulated a bacterial cytoplasm model and observed that tRNA molecules are phase separated by the effects of ribosome and positively charged proteins. After that, we defined model systems with three components, which are snRNA, positively charged proteins and ribosome to investigate the determinants of the phase separation. Similar to tRNA in the bacterial cytoplasm simulations, we also observed phase separation of snRNA in the three component system. Then, for this model system, the effects of the charges of RP and proteins, the size of the system and the presence of crowders were studied in more detail.

Structural analysis of *Helicobacter pylori* VacA Toxin Reveals Insights into Oligomerization

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Helicobacter pylori is a Gram-negative bacterium that persistently colonizes the stomachs of >50% of the human population, with a prevalence as high as 90% in developing nations [1,2]. *H. pylori* infection causes gastritis and can lead to the development of peptic ulcer disease and gastric cancer [3,4].

An important *H. pylori* virulence factor implicated in these diseases is the pore-forming toxin vacuolating cytotoxin A (VacA) [5,6]. VacA has been reported to cause multiple cellular effects including cell vacuolation, membrane permeabilization, mitochondrial dysfunction, cell death, autophagy, T cell inhibition, and other immunomodulatory effects [7].

H. pylori secretes VacA as an 88 kDa monomer (p88). p88 binds to the surface of gastric epithelial cells, oligomerizes, and forms anion-selective membrane channels [7]. p88 contains two domains (p33 and p55) [8]. The N-terminal p33 domain contains a hydrophobic region required for channel formation and regions within both the p33 and p55 domains mediate VacA oligomerization and binding to host cells [7].

We have determined low-resolution structures of a VacA dodecamer and heptamer and a 3.8-Å structure of the VacA hexamer using single-particle cryo-electron microscopy [9]. From these analyses, we observe that VacA p88 consists predominantly of a right-handed beta-helix extending from the p55 domain into the majority of the p33 domain. We map the regions of p33 and p55 involved in hexamer assembly, model how interactions between protomers allow heptamer formation, and identify loops that likely contact membrane. This work provides new insights into the basis of VacA oligomerization and defines regions that are likely important for VacA interactions with cell membranes, two functions required for VacA activity.

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[2] Parsonnet, J. Infect. Dis. Clin. North Am. 12, 185 (1998).
[3] Marshall, B. J., and Warren, J. R. Lancet 1, 1311 (1984).
[4] Suerbaum, S., and Michetti, P. N. Engl. J. Med. 347, 1175 (2002).
[5] Atherton, J. C., et al., J. Biol. Chem. 270, 17771 (1995).
[6] Cover, T. L. M. Bio. 7, e01869 (2016).
[7] Foegeding, N. J., et al., Toxins (Basel) 8 (2016).
[8] Telford, J. L., et al.,, J. Exp. Med. 179, 1653 (1994).
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Data-Driven Solutions to Challenges in Biophysics

Aaron Frank
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Machine learning, as an embodiment of artificial intelligence, has revolutionized all aspects of our lives. In the sciences, modern, data-driven machine learning techniques are now used to tackle a variety of challenges, and biophysics is no exception. Using examples from my research, I will demonstrate how machine learning can be used to address problems ranging from biomolecular structure prediction to biomolecular design.

Fibril structure of Aβ-amyloid from Alzheimer's disease by CryoEM and Solid-state NMR

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The formation of amyloid fibrils from normally soluble proteins and peptides is a common form of self-assembly phenomenon and are associated with a range of debilitating human disorders like Alzheimer's, Parkinson's diseases. Although amyloid fibrils are formed by a wide variety of proteins, they possess highly similar generic structures with some minor variations in local packing leading to morphological differences. From the structural point of view, amyloids are unbranched highly ordered protein aggregates containing an array of β-sheets oriented perpendicular to the fibril axis. Determining the structures for these filamentous aggregates is important to understanding their formation, spreading and clearance in the human body.

Fibrillar aggregates of the amyloid-β protein (Aβ) are the major component of the senile plaques develop in the brain of the Alzheimer's disease patients. Recently, it has been shown that the patients diagnosed with typical Alzheimer's Disease (t-AD) develop a predominant polymorph in their brain tissues [1]. In this study, we determined the structure of this predominant Aβ fibril polymorph extracted from the brain-tissues of t-AD patients by cryo-electron microscopy (cryoEM) and solid-state NMR experiments. Unlike solid-state NMR, where we get the global structure from local structural constraints, in cryoEM we get the local structure from global structure. Here we have combined the merits of these two techniques, that is, incorporating the local structural constraints from NMR into the 3D electron density obtained from cryoEM. This hybrid approach is invaluable for complex systems like amyloids.

The structure of Aβ fibrils is composed of two complete extended protofilaments and two half-molecules that span the next cross-β layer as seen by cryoEM to 2.9 Å resolution and solid-state NMR. In the end we have combined the cryoEM density and solid-state NMR restraints to calculate the structure of the t-AD brain-derived Aβ fibrils. To our knowledge, this kind of hybrid approach has never been applied before, certainly not for amyloids.

[1] W. Qiang, W.M. Yau, J.X. Lu, J. Collinge and R. Tycko. Nature 541, 217 – 221 (2017

Antibody binding dynamics revealed by asymmetric Fab-virus complexes

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There is significant overlap on the surface of parvovirus capsids between the sites recognized by host immune molecules and by the host receptor, transferrin receptor type-1 (TfR). Thus antibody escape mutants may introduce changes within the TfR binding site that also alter recognition of specific host receptors, contributing to the ability of the virus to jump species. In order to understand this phenomenon, we have defined the structure of the canine parvovirus capsid and a monoclonal antibody fragment (Fab) that specifically binds and neutralizes canine parvovirus by recognizing the TfR-attachment site. This antibody, MAb 14, binds poorly to the closely related feline parvovirus. We solved atomic resolution structures of Fab-virus complex as well as a second structure that had been incubated with low molar amounts of Fab. This undersaturated Fab-virus complex was used to solve local structures of the Fab-bound and -unbound antigenic sites extracted from the same complex map. The comparison of these sub-capsid volumes revealed clear conformational changes within the binding site that were too small to resolve with traditional structural approaches. We implemented innovative approaches for assigning the order and position of attached Fabs that allowed assessment of complementarity between the Fabs bound to different positions. Our study provides a new understanding of icosahedral virus structures and functions associated with asymmetry of binding that has gone undetected in previous studies due to the use of symmetry averaging.

Transferrin receptor binds virus capsids with dynamic motion - receptor and virus rock and roll

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Canine parvovirus (CPV) is an important pathogen causing severe diseases in dogs, including acute hemorrhagic enteritis, myocarditis and cerebellar disease. Cross-species transmission of CPV occurs as a result of mutations on the viral capsid surface that alter the species-specific binding to the host receptor, transferrin receptor type-1 (TfR). The interaction between CPV and TfR has been extensively studied and previous analyses have suggested the CPV-TfR complex is asymmetric. To enhance the understanding of the underlying molecular mechanisms, we determined the CPV-TfR interaction using cryo electron microscopy to solve the icosahedral (3.0 Å resolution) and asymmetric (5.0 Å resolution) complex structures. Structural analyses revealed conformational variations of the TfR molecules relative to the binding site, which translated into dynamic molecular interactions between CPV and TfR. For the first time, the precise footprint of the receptor on the virus capsid was identified along with the identity of the amino acid residues in the virus-receptor interface. Our ‘rock and roll’ model provides a novel explanation for previous findings and gives new insights into species jumping and the variation in host ranges associated with new pandemics in dogs.

High-Accuracy Protein Structure Prediction via Physics-based Model Refinement

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Protein structure prediction has served as an alternative to experimental structure determination. Template-based modeling with experimental structures of homologous proteins provides fair models. Recently, structure predictions based on co-evolutionary analysis via machine learning have shown an ability to predict models without templates. Protein model refinement has been applied to protein models at the last step of the prediction to improve approximate models to higher accuracy. Molecular dynamics (MD) simulation-based method is one of the best approaches. It improved models consistently, but the improvements were moderate. One of the main challenges of the MD-based model refinement is conformational sampling from protein models to their experimental structures. In this presentation, we present recent advances in protein model refinement. First of all, a few critical obstacles in refinement were identified via extensive MD simulations. [1] Native states were at the lowest free energies in all the tested cases, and the corresponding structures had sub-Angstrom accuracies in many of the cases. However, refinement was prohibited by a rough energy landscape and significant kinetic barriers with at least μs time scales. Along with the identified refinement pathways, it required partial unfolding and re-folding in the intermediate states. A new MD-based refinement method was devised based on the lessons, and it was blindly tested during CASP13. [2] The new protocol achieved significant improvements with expanded conformational sampling. In addition, some models were considerably refined to near-experimental accuracy. Finally, we applied our physics-based refinement to models from AlphaFold, a state-of-the-art machine learning-based structure prediction method. [3] The resulting models had higher accuracy both in global and local similarity and outperformed any other prediction method tested in CASP13.

[1] L. Heo and M. Feig, Proc. Natl. Acad. Sci. USA 115(52), 13276–13281 (2018).
[2] L. Heo, C. F. Arbour, and M. Feig, Proteins (2019).
[3] L. Heo and M. Feig, bioRxiv, 731521 (2019).

Is the lipid bilayer a good solvent for folding of membrane proteins?

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Knowledge of the conformational properties of the denatured state ensemble (DSE) of proteins is essential to understand folding, chaperoning, degradation and translocation. Especially, it has been a central question whether the DSE is expanded or collapsed in aqueous solution. While a majority of studies have been focused on water-soluble proteins, the DSE of membrane proteins is poorly understood mainly because of the challenges in obtaining a large amount of the DSE in a lipid bilayer. Here we made a breakthrough in reconstituting the DSE of a foldable six helical-bundle membrane protein GlpG under native bilayer and solvent conditions using a steric trapping. The method couples spontaneous unfolding of a doubly biotin-tagged protein to competitive binding of bulky monovalent streptavidin. Using our novel paramagnetic biotin derivative conjugated to GlpG and double electron-electron resonance spectroscopy (DEER), we determined the interspin distances between specific biotinylated sites in the sterically trapped DSE's. To validate if DEER captures most of the interspin distances in the DSE, we employed *Upside*, a model for course-grained molecular dynamics simulation. *Upside* also provided the reference distance distributions at various strengths of inter-helical interactions to define the solvent quality of the lipid bilayers. In the large DMPC:DMPG:CHAPS bicelles, the trapped DSE's expanded by 30–60% relative to the native state in the length scale, but contracted to 65–84% relative to the fully expanded condition. Interestingly, the DSE's slightly more expanded in the native *E. coli* lipid bilayers, in which the degree of expansion reached 78–95% of the full expansion. Our result suggests that although the cell membranes may not be a good solvent for the DSE's of membrane proteins, they still allow expansion of the polypeptide chains preventing a nonspecific collapse during the folding within the membranes.

Dynamics-Function Relationships in Catalytic RNA

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Conformational dynamics are a required underpinning to many recognition and catalytic events in functional RNA molecules. A full exploration of these effects requires both an identification and analysis of the dynamic modes themselves and consequent functional probing in dynamics-attenuated variants, which we refer to as a “dynamics-function” study. We use an integrated approach of NMR spin relaxation studies with targeted metabolic ^{13}C labeling to detect minor conformers (so-called “invisible states”), substitution of covalently conformation-restricted nucleotide analogs for functional probing, and contemporary molecular dynamics computations to elicit atomic details of the functional dynamics. Results in model systems demonstrate a picture in which tertiary RNA potential energy landscapes are characterized by multiple deep, narrow potential wells separated by high barriers, and function is often limited by the ability of the molecule to sample the right part of the landscape. These effects are illustrated by striking gain-of-function effects upon conformational restriction away from the ground state in the lead-dependent ribozyme. We are now applying this approach to the docking transition in the hairpin ribozyme, a tertiary-only RNA-RNA interface formation. We have hypothesized this interaction to be an example of double conformational capture, in which only collisions between binding partners each of which is independently sampling a minor conformation are productive for binding. Unrestrained molecular dynamics simulations suggest the sampling of many features of a docked-like state by an isolated construct of one loop, and we are now integrating NMR and functional probing data to examine the experimental realization and functional relevance of those states.

Characterization of membrane proteins using Cryo-EM: a story of insect olfactory receptor Orco

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Determining membrane protein structures has been a challenging task as they are often difficult to overexpress and/or crystallize. In recent years, however, the quantum leap in single-particle cryo-EM has revolutionized the field of structural biology, and has led to structure determination of numerous long-sought membrane proteins such as the insect olfactory receptor Orco. Orco is a highly conserved small (50 kDa) membrane protein that interacts with a sequentially diverse odorant receptor (OR) to form a family of odorant-gated ion channels responsible for odor detection in insects. As these proteins have no homology to other known protein families and their structure determination had been elusive, many of their elementary characteristics had remained unknown. Recently, using single-particle cryo-EM, we determined the structure of an Orco homomer from the parasitic fig wasp *Apocrypta bakeri* at 3.5Å resolution. Our study shows Orco forms a homotetramer and possesses novel channel architecture. The structural analysis of Orco, complemented by functional studies, sheds light on how Orco can accommodate remarkable sequence diversity of OR, thereby facilitating the evolution of odor tuning.

Automated preprocessing pipeline for single-particle cryo-EM

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The growth of cryo-EM into a mainstream structural biology tool has led to its widespread adoption for users across a range of expertise, where experts represent a continually shrinking fraction of cryo-EM users. Considering the manual and subjective decisions involved in solving a structure, such as the programs, parameters and determination of good micrographs and good 2D class averages, cryo-EM frustrates many users. To make cryo-EM data processing more user-friendly and more reproducible, we have developed an automated pipeline for cryo-EM data analysis and assessment using a combination of deep learning and image analysis tools to help streamline the process of cryo-EM structure determination. Our workflow can automatically detect bad micrographs, determine the best parameters for particle picking and 2D classification, and identify the good class averages that can be used in 3D reconstruction. In the workflow, the subjective user decisions are replaced with statistical models based on the features extracted with image processing methods and convolutional neural networks, along with the expert knowledge. Specifically, we have built a deep-learning based generic micrograph classifier that can assess the quality of a micrograph with an accuracy of 97%, allowing bad micrographs to be removed without any user decision. We have also built a 2D class average classifier that can evaluate the quality of 2D class averages, and automatically search for the optimal mask diameter. We have verified the performance of this workflow on a number of EMPIAR datasets as well as a new dataset from a challenging sample (C1 symmetry, 180 kDa) [1]. We propose that our automatic workflow will make cryo-EM data preprocessing more convenient and more reproducible for the users from a range of backgrounds.

[1] J. Cash et al. Science Advances (2019).

Watching individual RNA viruses self-assemble

Vinothan Manoharan
Harvard University

Nearly 60 years ago, Caspar and Klug coined the term "self-assembly" to describe the formation of a virus from its constituent parts (Cold Spring Harbor Symposia on Quantitative Biology 1962). But we still don't understand how this process occurs even in the simplest viruses, positive-sense RNA viruses. Such viruses consists of proteins that form a highly-ordered protective shell (called a capsid) around the viral RNA. Viral particles can self-assemble spontaneously in a mixture of RNA and coat protein in a buffer, in the absence of any host factors. The yield and fidelity of the assembly is particularly remarkable in viruses with a triangulation number of 3 or higher, in which case some of the proteins must find their way to 5-fold coordinated sites and others to 6-fold coordinated sites on the same shell. To understand how such systems assemble, we have developed an interferometric technique that allows us to measure the scattering of individual assembling viral particles (MS2 bacteriophage) on time scales ranging from 1 ms to 1000 s. By comparing the scattered intensity to that of the wild-type virus, we infer the mass of proteins that have attached to the central RNA as a function of time. We find that individual particles grow to nearly full size in a short time following a much longer delay period. The distribution of delay times suggests that the assembly follows a nucleation-and-growth pathway. I will discuss how such a pathway might allow the virus to assemble so robustly.

Charge and Polar Interactions Determine Protein Diffusion in A Cytosol-like Solution – A Computer Simulation Study

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Recent experiments indicate that proteins in a living cell experience different degrees of reduction of their translational and rotational diffusion depending on the subcellular location. Nevertheless, processes underlying this effect are still far-off from being well understood because of limitations of experimental methods. By employing molecular dynamics simulations we provided detailed insight into diffusion and interactions of a small protein, villin, in a self-crowded dens solution, as a simple model of the cellular environment.[1] Although villins do not form strong or specific interactions we have found that contacts between them persist on sub-microsecond time scales during which the proteins undergo collective motions. We showed that weighting diffusion coefficients of different protein clusters with their distribution from the simulations largely reproduces the overall diffusion. It indicates the transient cluster formation as a primary determinant of protein diffusion in a concentrated solution. Further, we have found that formation of intermolecular contacts between charged and polar groups strongly correlates with protein rotational diffusion retardation.[2] This observation suggests that composition of amino acids at the protein surface mainly determines its propensity for interactions with surrounding proteins, and thus, its diffusive properties in a solution.

[1] G. Nawrocki, A. Karaboga, Y. Sugita, M. Feig, Phys. Chem. Chem. Phys., 21, 876-883 (2019).
[2] G. Nawrocki, P. Wang, I. Yu, Y. Sugita, M. Feig J. Phys. Chem. B, 121,11072-11084 (2017).

CryoEM Studies of the Phage Sf14

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The goal of this study is to achieve a high-resolution structure of the capsid of phage Sf14. Phage Sf14 is a *Shigella* phage that was found in the Red Cedar River at Michigan State University. Sf14 is interesting because it has a T number of 9, which is only found in two other viruses to date, but it also has decorator proteins that have two Ig-like domains ([1]). Another unique thing about Sf14 is that its phage genome falls in the range of 85.0-95.0 kbp, which is only true of about 2% of phage genomes in the NCBI RefSeq ([1]). Since there is a unique geometry to Sf14, we thought that the decorator proteins would bind to the surface differently than the T=7 capsids that we had seen many times before. The only way to understand these interactions would be through finding a structure. To solve a high-resolution structure of the Sf14 capsid and the decorator protein we used cryo electron microscopy and three-dimensional image analysis. We used different image processing programs to compare which one would achieve the highest resolution structure. Some of the programs include auto3DEM, RELION, and cisTEM. All were put through the same process of importing the micrographs into the software, and using the available tools to attain the highest possible resolution for our Sf14 structure. Our input included 57 micrographs with a total of 410 boxed particles. A comparison was done by using the original box coordinates of the particles on Robem, and then moving those over to cisTEM and RELION. We then compared the resolution of the resulting structures.

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^{13}C isotopic labeling and NMR in membrane of a large soluble ectodomain construct of the HIV gp41 membrane fusion protein

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Abstract:
An initial step in human immunodeficiency virus (HIV) infection of target white blood cells is joining (fusion) of the HIV and host cell membranes, with consequent deposition of the viral nucleocapsid in the cytoplasm. Fusion is catalyzed by the HIV gp41 protein which is a single-pass integral viral membrane protein, and which contains a ~150-residue soluble domain outside of the virus [1,2]. This soluble ectodomain (SE) adopts a helix-turn-helix structure under some solution conditions, with antiparallel alignment and van der Waals contact between the two helices. For a subset of these conditions, three hairpins associate together as a six-helix bundle.

There are competing hypotheses about the catalytic role in HIV/cell fusion of the SE hairpin structure. One hypothesis is that the hairpin is a “post-fusion” structure with no role in catalysis, whereas another hypothesis postulates an important role for the hairpin in catalysis which is associated with binding to the membrane. The latter hypothesis is supported by rapid vesicle fusion induced by the hairpin under some conditions [1]. The experiments of the present study are designed to determine the location of the SE hairpin in the membrane.

A large SE construct was expressed in minimal media using either ^{13}C-glucose and ^{13}C-glycerol, with dilution of ^{13}C labeling done by mixing with unlabeled glucose or by selectively-labeled glycerol [3]. A centrifugation protocol resulted in multi-mg purified yield of protein. Rotational-echo double-resonance (REDOR) solid-state NMR was applied to probe proximity between ^{13}C-labeled protein and ^{2}H labels at the center of the membrane [4]. Negligible REDOR dephasing for this sample indicates that the soluble ectodomain has interfacial rather than deep location in the membrane.

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Memory Formation by the Cas4-Cas1-Cas2 Complex During CRISPR Adaptation

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CRISPR (clustered regular interspaced short palindromic repeats) loci and *cas* (CRISPR associated) genes confer adaptive immunity in bacteria and archaea. Host cells adapt to infections by integrating short segments of invasive DNA into a CRISPR locus. This adaptation process results in the formation of a new “spacer” within the CRISPR, which immunizes the host against the invader and provides a heritable memory of the infection. Two proteins, Cas1 and Cas2, form a complex that is universally required for spacer integration in all CRISPR-Cas systems [1,2]. Cas4 proteins are also widespread and usually co-occur with *cas1* and *cas2* within *cas* operons, suggesting a role in adaptation [3]. However, the function of Cas4 has long remained unresolved. We recently discovered that Cas4 interacts with Cas1, enabling the formation of either Cas4-Cas1 or Cas4-Cas1-Cas2 complexes [4,5]. Structural analysis by negative-stain EM revealed that Cas4-Cas1 is a heterohexameric complex containing two Cas1 dimers and two Cas4 subunits, while Cas4-Cas1-Cas2 contains an additional Cas2 dimer. However, the architectures of the two complexes are mutually exclusive, and Cas4-Cas1 must fully disassemble to form the active Cas4-Cas1-Cas2 adaptation complex, suggesting a regulatory role for Cas4-Cas1. Within Cas4-Cas1-Cas2, Cas4 binds in close proximity to the Cas1 integrase active site, providing clues to the function of Cas4 during spacer capture and integration. Using complementary biochemical studies, we found that Cas4 is required for efficient prespacer processing prior to Cas1-Cas2-mediated integration. Upon activation by Cas1-Cas2, Cas4 processes single-stranded DNA through endonucleolytic cleavage. Cas4 cleaves precisely upstream of PAM sequences, short DNA motifs that are required for downstream function of the spacer sequence. Prior to cleavage, Cas4 blocks the Cas1 active site, preventing integration of unprocessed prespacers. Thus, Cas4 acts as a gatekeeper to ensure that only functional spacers will be integrated into the CRISPR array. Overall, our results reveal the critical role of Cas4 in maintaining fidelity during CRISPR adaptation, providing a structural and mechanistic model for prespacer processing and integration.

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Mechanism of processive telomerase catalysis revealed by optical tweezers

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Telomere maintenance by telomerase is essential for continuous proliferation of human cells and is vital for the survival of stem cells and ~90% of cancer cells . Telomerase is a reverse transcriptase composed of telomerase reverse transcriptase (TERT), telomerase RNA (TR), and several accessory proteins. To compensate for telomeric DNA lost during DNA replication, telomerase processively adds TTAGGG repeats to the single-stranded overhangs at chromosome ends by copying the template region within its RNA subunit. Between repeat additions, the RNA template must be recycled, which requires disrupting the base-pairing between TR and the substrate DNA. How telomerase remains associated with the substrate DNA during this critical translocation step remains unknown. Here, we demonstrate that stable substrate DNA binding at an anchor site within telomerase facilitates the processive synthesis of telomeric repeats. Using a newly developed single molecule telomerase activity assay utilizing high-resolution optical tweezers, we directly measured stepwise, processive telomerase activity. We found that telomerase tightly associates with its DNA substrate, synthesizing multiple telomeric repeats before releasing them in a single large step. The rate at which product is released from the anchor site closely corresponds to the overall rate of product dissociation from elongating telomerase, suggesting that it is a key parameter controlling telomerase processivity. We observed folding of the released product DNA into G-quadruplex structures. Our results provide detailed mechanistic insights into processive telomerase catalysis, a process critical for telomere length maintenance and therefore cancer cell survival.

Dropping Acid Makes You See Stars: Identifying Proteins Released During Giant Virus Genome Release

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Recently discovered, giant viruses (GV) are an understudied segment of virology and are potentially pathogenic. These viruses have been discovered in diverse ecosystems worldwide and can survive in harsh environments such as alkaline lakes [1] and frozen tundras [2]. To survive such extreme conditions, GV have developed extraordinary capsid stability. This stability allows GV to survive until they encounter a suitable host organism, but it also presents a thermodynamic barrier that must be overcome to induce the conformational changes in the capsid required for to genome release. To mediate this transition, GV use one of two types of seal complexes; the starfish-like seal of mimivirus [3] or the cork-like seal of Pithovirus [2]. Although the basic steps of GV genome release (*i.e.* seal disruption, capsid opening, etc.) have been hypothesized from thin section TEM data, the biomechanical mechanisms governing this process remain a mystery. This lack of knowledge largely stems from the complexity of the viruses themselves and the lack of an *in vitro* model system to study GV genome release.

Here we present the first *in vitro* system for studying GV genome release, using Samba virus, a GV isolated from Brazil [4]. Samba shares many of the traits common amongst mimivirus-like GV, including the use of a starfish-like seal complex to seal the particles prior to genome release [5]. We have been able to mimic the following distinct stages of the Samba genome release process *in vitro*: 1) Pre-Release, 2) Disruption of the Starfish Seal, 3) Nucleocapsid Release, 4) Completion. Particle morphologies at each stage were determined via cryo-electron microscopy, cryo-electron tomography, and scanning electron microscopy. Using differential mass spectrometry, we have also determined the proteins that have been released from the Samba viral particles at the early stages of infection. These experiments provide a glimpse of the biomechanical processes responsible for GV seal complex disruption/genome release.

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High-resolution optical tweezers and fluorescence measurements of human G-quadruplex DNA structures folding in equilibrium.

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Telomeres, the ends of human chromosomes, are composed GGGTTA DNA repeats produced by the telomerase reverse transcriptase ribonucleoprotein machine. Four adjacent repeats can fold into a G-quadruplex (GQ) structure, where four G's from adjacent strands bond non-canonically into a planar G-tetrad structure, and the full GQ structure contains three stacked G-tetrads. Folded GQ are very stable structures and have been shown to interfere with telomerase function. However, GQ exhibits a variety of alternate folding topologies with varying stability that may also interact differently with the telomerase machinery. We have performed high-resolution force spectroscopy of the folding and unfolding of GQ structures under equilibrium, low force conditions. Using a combination of simultaneous fluorescence and tweezers measurement we can unambiguously identify a set of single GQ folded structures and their properties. When we extend the DNA sequence to allow for up to four possible folded GQ, the individual GQ properties remain largely the same, with only adjacent strands folding to form GQ structures. These investigations allow for the detailed interpretation of single telomerase measurements under force.

Cardiac Mitochondria Ultrastructural and Functional Changes Caused by Massive Calcium Loading Observed Using Cryo-EM and High-Resolution Respirometry

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Excessive calcium accumulation is the main cause of cardiac tissue and cell death during myocardial ischemia-reperfusion injury (IRI) and myocardial infarction (MI). Calcium dysregulation and excessive amounts of reactive oxygen species (ROS) lead to mitochondrial dysfunction, catastrophic energy failure, and opening of the mitochondrial permeability transition pore (mPTP). In the present study, we quantified the mitochondrial calcium sequestration and the formation of amorphous calcium phosphate (CaPi) granules in isolated cardiac mitochondria exposed to calcium boluses in the permeability transition range in the presence or absence of cyclosporine A (CsA). We acquired a time course series of images by cryo-electron microscopy (cryo-EM) to visualize the consequences of calcium overload in mitochondrial ultrastructure. In parallel studies, spectrofluorimetry, high-resolution respirometry, and other quantitative mitochondrial and biochemical assays were implemented to link changes in mitochondrial function with changes in ultrastructure. Exposing energized mitochondria to high calcium boluses induced the formation of CaPi granules of sizes in the range of 20–100 nm and inner/outer membrane (IMM/OMM) rupture. However, treating mitochondria with CsA induced the formation of CaPi granules of sizes in the range of 40–200 nm, aberrant morphologies, and increased cristae density. Despite loss of the OMM, ADP-stimulated mitochondrial respiration was preserved. Our preliminary data reveal a new and exciting link between calcium overload and mitochondrial ultrastructure that involves the formation of calcium phosphate granules, cristae remodeling, and inner mitochondrial membrane fragmentation, which decreases mitochondrial ATP synthesis rates.

goCTF: Geometrically optimized CTF Determination Supports cryo-ET using Volta Phase Plate

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Preferred particle orientation represents a recurring problem in single-particle cryogenic electron microcopy (cryo-EM). A specimen-independent approach through tilting has been attempted to increase particle orientation coverage, thus minimizing anisotropic three-dimensional (3D) reconstruction. The newly developed software tool, goCTF adopted a geometrically optimized approach to determine the global focus gradient. A novel strategy of determining contrast transfer function (CTF) parameters from a sector of the signal preserved power spectrum is applied to increase reliability. Subsequently, per-particle based local focus refinement is conducted in an iterative manner to further improve the defocus accuracy. Currently, the goCTF software development has been extended to support imaging with Volta phase plate (VPP). Encouraging preliminary data has shown great potential for goCTF to de-correlate VPP induced additional phase shift and the focus gradient at specimen tilt. The success of this technology development will break the technical barrel of utilizing new generation VPP, providing a much-needed tool to advance cryo-ET from nanometer to near-atomic resolution.

Structure of the *Shigella flexneri* podophage HRP29

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Short tailed phages (*Podoviridae*) are understudied compared to their long tailed phage counterparts, the *Myoviridae* and *Siphoviridae*. Podophages in *Salmonella* (P22), *E. coli* (T7), and *Shigella* (Sf6) have been structurally characterized and used as model systems to investigate capsid structure, morphogenesis, and stages of infection. However, many aspects of these processes are still unknown for a majority of environmental phage isolates. Recent interest in phage hunting activities has expanded the diversity and reportiore of phage isolates, and also provide new systems to investigate. As part of our phage hunting exercises to isolate *Shigella flexneri* phages from the environment, we have identified phages mostly belonging to the *Myoviridae* and *Siphoviridae* family [1], but to date only one short tailed, lytic podophage (HRP29) has been isolated. The genome sequence of HRP29 has only ~10 % average nucleotide identity to any known genome, and the nearest family members of HRP29 are not well studied. Although the structural genes of HRP29 follow a similar order to T7, it also encodes a full-length Sf6-like tail spike protein in addition to a partial T7-like tail fiber and utilizes completely different host receptors in comparison to Sf6. To understand the diverse mechanisms employed by the two different *S. flexneri*-infecting podophages Sf6 and HRP29, a high resolution structure of HRP29 is one of the key components. In my work, I will discuss the progress made in the process of determining HRP29's structure by cryo- electron microscopy and single particle analysis to generate three dimensional structures of the virion. Finally, the unique features of HRP29's structure and comparison to other known podophage will be discussed.

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Isolating OmpC-dependent *Shigella flexneri* phage from the Red Cedar River

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Many phages use specific receptor proteins embedded in their hosts' membranes to initiate infection. *Shigella* phages have not been well studied to date, especially regarding beginning stages of infection. However, these phages appear to have an unusual ability to utilize multiple receptor types. For example, some use outer membrane proteins C and A, as well as others, to identify and infect their host. It is difficult to study specific phage:host interactions when the phage can use multiple receptor types[1]. Therefore, isolating *Shigella* phages that rely solely on one receptor for host cell entry can help us examine the specific protein:protein interactions between the phage and the outer membrane receptor. Using techniques and methods from Doore et al. 2018 [2], an OmpC-dependent *Shigella* phage was isolated from river water on Michigan State University's campus. A series of assays to initially characterize the phage included host range, efficiency of plating (EOP), sequencing of the capsid and host adhesion proteins, and negative stain transmission electron microscopy. The phage was found to be morphologically T4-like and has some similarities to other known *Shigella* phages that were isolated in a previous year, yet also some differences.

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The RNA nanomachines of gene expression dissected at the single molecule level

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Over two decades, the Walter lab has contributed to the RNA field by building a broad research portfolio focused on dissecting the mechanisms of the nanoscale RNA machines of gene expression – ranging from small viroidal ribozymes and bacterial riboswitches to the eukaryotic spliceosome – by single molecule fluorescence microscopy. Leveraging this expertise, the two long-term goals of our current work are to: 1.) Apply our established mechanistic enzymology approaches to an ever broader set of RNAs involved in regulating transcription, translation and splicing, seizing the opportunities arising from the continuing discoveries of new functional RNAs. 2.) Push the limits of our approaches to be able to probe increasingly complex biological contexts and mechanisms since unexpected discoveries – as we found – often await where individual RNA nanomachines interact. In pursuit of these goals, we will address the overarching hypothesis that dynamic RNA structures are a major determinant of the outcomes of gene expression, often in ways that have been overlooked by a field that historically was rooted in genetics, where genes regularly were drawn as rectangular boxes, and function commonly was thought of as dictated by sequence rather than structure. Encapsulating the power of our pursuit, we recently combined single-molecule, biochemical and computational simulation approaches to show that transcriptional pausing at a site immediately downstream of a riboswitch requires a ligand-free pseudoknot in the nascent RNA, a precisely spaced consensus pause sequence, and electrostatic and steric interactions with the exit channel of bacterial RNA polymerase [1]. We posit that many more examples of such intimate structural and kinetic coupling between RNA folding and gene expression remain to be discovered, leading to the exquisite regulatory control and kinetic proofreading enabling all life processes. Thinking ahead, we are also developing tools to observe single RNA nanomachines in action within their natural habitat, inside living cells [2-8].

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Electron microscopy approaches to studying lipid–protein interactions

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In recent years it has been increasingly appreciated that the membrane environment can significantly affect the structure, function and organization of membrane proteins, indicating that the structure of membrane proteins is ideally studied in the context of a native lipid bilayer. Electron microscopy provides two approaches to determine high-resolution structures of membrane proteins in a membrane environment. Using electron crystallography, we determined structures of the water channel aquaporin-0 (AQP0) reconstituted into two-dimensional (2D) crystals with the quintessential raft lipids, sphingomyelin and cholesterol. The structures suggest principles that may underlie AQP0 array formation and potentially raft formation in general. We also used single-particle cryo-electron microscopy to study the structure of the mechanosensitive channel MscS reconstituted into nanodiscs with different lipids. These studies provide experimental support and a mechanistic basis for the 'lipids move first' model for mechanosensation by MscS.

Native Chemical Ligation Synthesis of Gp41 Ectodomain with Specific Isotopic Labeling and Solid-State NMR Structural Studies

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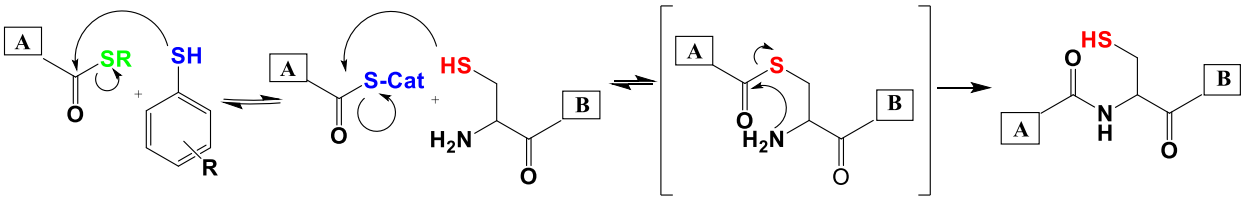
The native-chemical ligation[1] (NCL) approach is a potentially powerful tool for protein synthesis since it allows for the preparation of a specifically-labeled large protein. When combined with solid phase peptide synthesis, the NCL reaction can offer very precise control over the isotopic labeling of the individual amino acids that make up the full protein. Introducing specific isotopic labels into a protein is advantageous for unambiguous assignment of solid-state nuclear magnetic resonance spectra with broad linewidths. Gp41 is a viral membrane protein that contains an approximately 170-residue ectodomain which is important for initiating fusion between the virus and host cell. The gp41 ectodomain includes the fusion peptide (FP), N-helix, loop, C-helix, and viral membrane-proximal external region (MPER). There are currently little structural data regarding the location of the FP relative to the rest of the gp41 ectodomain in the membrane environment[2]. Combining the specific isotopic labeling with REDOR NMR allows for direct determination of close contact between the FP and other parts of the ectodomain.

This poster will discuss the details of the synthesis of the gp41 ectodomain including the FP by native chemical ligation and successful incorporation of carbon-13 and deuterium labels into the synthesized protein. The carbon-deuterium REDOR data acquired from the synthesized protein allows us to gain further insight into the structure of the protein in the membrane environment.



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IFP effects on membrane studied by ^{2}H paramagnetic relaxation enhancement (PRE) solid-state NMR

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Influenza is a very common infectious disease that can cause severe problems. There is no long-term effective vaccine to protect people from infection as the result of high mutation rate of influenza virus.

The hemagglutinin (HA) is glycoprotein of influenza virus with ~ 550 amino acid residues. It is responsible for binding the virus with the sialic acid receptor on the host membrane as well as fusion between host cell and virus. It turns out that the most conserved region of HA is the first 23 N-terminal amino acids of HA2 subunit referred as fusion peptide which is the most pivotal part for influenza virus fusion with host cell membrane.[1]

Researchers are taking a great effort to understand the mechanism of fusion. Based on what has been achieved in the past, it is clear that specific sites of influenza fusion peptide have closed contact with lipid acyl chain tail, which suggests that host membrane with influenza fusion peptide can adopt either membrane-spanning or lipid tail protrusion model.

In order to study whether the lipid tail protrusion is adopted during fusion, paramagnetic relaxation enhancement (PRE) developed from solid-state NMR was used as our main characterization to help us determine the topology of host membrane with influenza fusion peptide.

PRE is a useful tool to determine long-range distance of biomolecules up to 35 Å for the reason that the dipolar coupling between unpaired electron and target nuclear isotope increase the relaxation rate of the nucleus of interests and this increase is distance dependent. In our case, Mn^{2+} binding on the surface of lipid membrane served as the paramagnetic center. It produced the dipolar coupling between the nuclear of interest-deuteron and the unpaired electron of Mn^{2+} which has an impact on the relaxation rate. This enhancement is a distance- and orientation-dependent effect which can be used to explore structural information of biochemicals.[2] In general, the paramagnetic species enhance the relaxation of the nuclear spins at well-defined locations, resulting in line broadening.[3] The information can be exploited to determine the location of small peptides in lipid.

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