2019 First Triangle Area Cryo-EM Symposium

December 16-17th, 2019

Research Triangle Park Headquarters
12 Davis Drive, RTP, NC 27709

sites.duke.edu/cryoemsymposium

Agenda

December 16th, 2019

9:30-10:20 AM – Opening Session

9:30-9:40 AM – Opening remarks – Dr. Darryl Zeldin, Scientific Director, NIEHS

9:40-9:50 AM – Welcome remarks – Dr. Colin Duckett, Vice Dean for Research, School of Medicine, Duke

9:50-10:00 AM – TBA – Dr. Richard Brennan – Chair, Department of Biochemistry, Duke

10:00-10:10 AM – "Cryo-EM in HIV vaccine design" – Dr. Bart Haynes, Director, Duke Human Vaccine Institute

10:10-10:20 AM – "How it all got started" – Dr. Jack Griffith, Professor of Microbiology and Immunology, UNC-CH

10:20-11:00 AM – *Coffee break and poster setup*

11:00-12:20 PM – Membrane Proteins

11:00-11:20 AM – Ying Yin, Duke, "Structural basis of cooling agent and lipid sensing by the cold-activated TRPM8 channel"

11:20-11:40 AM – Jonathan Fay, UNC-CH, "Cryo-EM visualization of a high open probability CFTR ion channel"

11:40-12:00 PM – Yang Suo, Duke, "Structural insights into electrophile irritant sensing by the human TRPA1 channel"

12:00-12:20 PM – Allen Hsu, NIEHS, “Translocation mechanism of folded protein Is suggested by structures of AAA protein translocase Bcs1”

12:20-2:00 PM – *Lunch and posters*

2:00-3:00 PM – **Macromolecular Complexes I**

2:00-2:20 PM – Jack Griffith, UNC-CH, “Molecular microscopy of DNA at a single molecule level”

2:20-2:40 PM – Maria Schumacher, Duke, “*Francisella tularensis* pathogenesis is controlled by a virulence specific RNA polymerase.”

2:40-3:00 PM – Rory Henderson, Duke, “Structural characterization of the HIV-1 glycan-V3 targeting DH270 broadly neutralizing antibody lineage”

3:00-4:00 PM – *Coffee break and posters*

4:00-5:00 PM Plenary Lecture

“G-Protein coupled receptors and their interactions with beta-arrestins”
Robert J. Lefkowitz, MD
James B. Duke Professor of Medicine
Duke University

5:00-7:00 PM – Happy hour

December 17th, 2019

9:30-10:30 AM – **Macromolecular Complexes II**

9:30-9:50 AM – Shivesh Kumar, Duke, “Cryo-EM analysis of a master regulator of the plant defense signaling network”

9:50-10:10 AM – Rob McGinty, UNC-CH, “Structural basis for activation of Dot1L methyltransferase by H2B ubiquitylation”

10:10-10:30 AM – Robert Edwards, Duke, “Fab-dimerized glycan-binding antibodies”

10:30-11:00 AM – *Coffee break and posters*

11:00-11:40 AM – **Validation and Interpretation**

11:00-11:20 AM – Jane Richardson, Duke, “Assessing at EMDB model challenges: strengths, weaknesses, and rapid improvement.”

11:20-11:40 AM – Christopher Williams, Duke, “What is CaBLAM and how does it help improve cryoEM models?”

11:40-1:30 PM – *Lunch and posters*

1:30-2:50 PM – **Enzymes**

1:30-1:50 PM – Kathryn Gunn, UNC-CH, “A new twist in lipase storage: the cryo-EM structure of helical LPL”

1:50-2:10 PM – Yu-Hua Lo, NIEHS, “Structural and functional analyses of AAA ATPase Rix7 in the process of unfolding substrate”

2:10-2:30 PM – Monica Pillon, NIEHS, “Cryo-EM reveals active site coordination within a multienzyme pre-rRNA processing complex”

2:30-2:50 PM – Brad Klemm, NIEHS, “Structure-function studies of a SAMHD1 homolog from bacteria reveal a novel mode of regulation”

2:50-3:30 PM – *Coffee break and posters*

3:30-5:00 PM – Method Development and Closing Remarks

3:30-3:50 PM – Jonathan Bouvette, NIEHS, “Accelerating data collection in Cryo-ET: working towards high-resolution in situ structures”

3:50-4:10 PM – Ye Zhou, Duke, “Improved resolution achieved by unsupervised data-driven protocols for particle sorting”

4:10-4:30 PM – Nicholas Brown, UNC-CH, “Quantifying the heterogeneity of macromolecular machines by mass photometry”

4:30-4:50 PM – Joshua Strauss, UNC-CH, “Organization of platelet microtubule marginal band microtubules”

4:50-5:00 PM – Closing Remarks

Organizers

Priyamvada Acharya (Duke)	Mario Borgnia (NIEHS)	Saskia Neher (UNC-CH)
Alberto Bartesaghi (Duke)	Robin Stanley (NIEHS)	Matthew Redinbo (UNC-CH)

MEMBRANE PROTEINS

Structural basis of cooling agent and lipid sensing by the cold-activated TRPM8 channel

Ying Yin (Duke University)

Transient receptor potential melastatin member 8 (TRPM8) is a calcium permeable cation channel that serves as the primary cold and menthol sensor in humans. Activation of TRPM8 by cooling compounds relies on allosteric actions of agonist and membrane lipid phosphatidylinositol-4,5-bisphosphate (PIP2), but lack of structural information has thus far precluded a mechanistic understanding of ligand and lipid sensing by TRPM8. Using single particle cryo-electron microscopy, we determined two structures of TRPM8 in complex with the synthetic cooling compound icilin, PIP2, and Ca2+ and in complex with the menthol analog WS-12 and PIP2 to ~3.4 Å and ~4 Å resolution, respectively. Our study reveals the binding sites for cooling agonists and PIP2 in TRPM8 and illustrates the mechanism of allosteric coupling between PIP2 and agonists, thereby providing a platform for understanding the molecular mechanism of TRPM8 activation by cooling agents.

Cryo-EM visualization of a high open probability CFTR ion channel

Johnathan Fay (UNC-CH)

Jonathan F. Fay[1], Luba A. Aleksandrov[1], Timothy J. Jensen[1], Liying L. Cui[1], Joseph N. Kousouros[1], Lihua He[1], Andrei A. Aleksandrov[1], Drew S. Gingerich[2], John R. Riordan[1] and James Z. Chen[2]
[1]University of North Carolina, Chapel Hill
[2]Oregon Health & Science University

The cystic fibrosis transmembrane conductance regulator (CFTR) anion channel is a highly dynamic protein with marginal thermal stability (Tm ~44oC). It is a unique member of the large family of ATP-binding cassette transport proteins, as it is a channel. Its role as a channel is critical for proper maintenance of epithelial salt and water homeostasis. CFTR gene mutations cause cystic fibrosis. Phosphorylation and nucleotide binding are essential for channel regulation and activity. Recently, the first 3D structures of dephosphorylated, ATP-free and phosphorylated ATP-bound CFTR have been reported. However, the structural changes that allow for the transitions between active and inactive functional states are not yet clear. We had previously purified and reconstituted a thermally stabilized (Tm ~53oC) active, non-hydrolytic form of human CFTR that has high channel open probability when phosphorylated by protein kinase A (Aleksandrov, Jensen et al. 2015). Here we studied a further modified CFTR variant that had a still higher Tm (~58oC). This homogeneous purified protein, reconstituted in proteoliposomes, also shifts from an inactive to a high open probability state on phosphorylation by PKA. Using cryo-EM we have determined structures of dephosphorylated and phosphorylated states of this thermally stabilized CFTR with very high channel open probability when phosphorylated. The unique repositioning of the transmembrane helices and R domain density that we observe in our cryo-EM maps may provide new insights into the structural changes that occur between active and inactive functional states of CFTR.

Structural insights into electrophile irritant sensing by the human TRPA1 channel
Yang Suo (Duke University)

Transient receptor potential Ankyrin subfamily member 1 (TRPA1) is the primary sensor of environmental stimuli and noxious algogens such as allyl isothiocyanate, cinnamaldehyde and propofol. Notably, a variety of TRPA1 agonists are electrophiles that bind and activates TRPA1 by covalent modification. In 2014, the first cryo-EM structure was reported at 4.2 Å resolution. However, the detailed mechanism of electrophile sensing by TRPA1 has been limited due to a lack of high-resolution structure. Here we present high-resolution cryo-EM structures of nanodisc-reconstituted TRPA1 in ligand-free, and in complex with two covalent agonists. Our structural studies, together with functional studies not only provide mechanistic insights into electrophile recognition by the TRPA1 channel but also provide a platform for novel analgesic development targeting the TRPA1 channel.

Translocation mechanism of folded protein Is suggested by structures of AAA protein translocase Bcs1
Allen Hsu (NIEHS)

The mitochondrial membrane-bound AAA protein Bcs1 has been shown to translocate substrates across the membrane without prior unfolding. One substrate of Bcs1 is the iron-sulfur protein (ISP), a subunit of the respiratory Complex III. How Bcs1 translocates ISP across the membrane is unclear. Here, we report the structures of Bcs1 in two different conformations, representing three nucleotide states. The apo and ADP-bound structures reveal a homo-heptamer and show a putative large subunit binding cavity accessible to the matrix space. We observe that ATP binding drives a contraction of the cavity by concerted motion of the ATPase domains, which could be used to push substrate across the membrane. Our findings not only shed light on the mechanism of translocating folded proteins across a membrane, but also offer insights into the assembly process of Complex III and perplexing clinical manifestations of Bcs1 mutants.

MACROMOLECULAR COMPLEXES I

Title TBA
Jack Griffith (UNC-CH)

Abstract TBA

Francisella tularensis pathogenesis is controlled by a virulence specific RNA polymerase
Maria Schumacher (Duke University)

The bacterium, Francisella tularensis (Ft) is one of the most infectious agents known and classified as a category A bioweapon. Ft virulence is controlled by a unique set of transcription regulators, the MglA-SspA heterodimer and PigR, and the stress signal, ppGpp. These factors drive expression of the Francisella pathogenicity island (FPI), which is required for virulence. Interestingly, MglA-SspA is expressed during infection and was shown to form a constitutive subunit of RNA Polymerase (RNAP). How these components collaborate to activate transcription of the FPI, however, is unknown. Here we report cryo-EM structures of FtRNAP(MglA-SspA) and FtRNAP-(MglA-SspA)-ppGpp-PigR-complexes bound to FPI promoter DNA. The FtRNAP(MglA-SspA)-DNA structures suggests an unexpected PigR-independent contribution to virulence supported by in vivo studies. The FtRNAP-(MglA-SspA)-ppGpp-PigR-DNA structure reveals that ppGpp binds to MglA-SspA to tether the DNA-binding activator, PigR, to the FPI promoter. Strikingly, PigR then recruits the two distinct FtRNAP alpha-CTD subunits to DNA UP elements that sandwich the PigR DNA-binding-site to generate a stable initiation complex. Thus, these studies unveil a novel paradigm for pathogenesis in Ft involving a virulence-specific bacterial RNAP that employs two MglA-SspA-based strategies to activate virulence genes.

Structural characterization of the HIV-1 glycan-V3 targeting DH270 broadly neutralizing antibody lineage
Rory Henderson (Duke University)

Background: The HIV-1 broadly neutralizing, glycan-V3 targeting DH270 lineage represents an attractive target for rational, lineage-based vaccine design because of its relatively limited rate of somatic mutation and our understanding of the details regarding its maturation. In order to enable computational and structure-based design of HIV-1 Env immunogens, we determined high-resolution CH848 SOSIP-associated Fab structures of each lineage member. Additionally, in order to examine the unliganded glycan-V3 epitope configuration, we determined structures for several autologous CH848 virus SOSIPs with varying V1 sequences and lengths bound to the CD4 binding site VRC01 bnAb Fab. **Methods**: Antibody bound Env SOSIP cryo-electron microscopy (cryo-EM) images were collected on a FEI Titan Krios microscope fitted with a GATAN K3 or K2 camera. Map reconstruction was carried out in cryoSPARC following movie frame alignment and CTF correction. Flexibility analysis and focused refinement were performed in Relion. Fitting of coordinates was carried out using a combination of manual fitting in Coot and automated refinement in Phenix and Rosetta. Visual analysis was carried out in PyMol with geometric analysis of antibody, SOSIP, and antibody-SOSIP performed using VMD and custom scripts.

Results: A total of nineteen structures, including all DH270 lineage members bound to CH848 10.17 DS SOSIP, excluding the DH270 UCA3 and DH270 UCA G57R structure which were complexed with CH848 10.17 DS DT, a structure of DH270.6 complexed with CH848 0526.25 DS, a 32 amino acid length V1 containing Env, and five autologous CH848 SOSIPs complex with VRC01 were determined in this study. Examination of the fitted coordinates of the DH270 lineage antibodies revealed a remarkable array of shifts in the relative orientations of the antibody to the Env gp120, the antibody VH-VL orientations, and antibody elbow angles. Concomitant changes in the V1 loop and interactive glycans with the acquired somatic mutations revealed an intricate procession toward the development of neutralization breadth. **Conclusions**: Together, with observations in the free glycan-V3 epitope, these results reveal for the first time, at atomic resolution, the details of the development of a broadly neutralizing antibody and the defense and reflexive response of HIV-1 to its maturation.

MACROMOLECULAR COMPLEXES II

Cryo-EM analysis of a master regulator of the plant defense signaling network

Shivesh Kumar (Duke University)

The systemic acquired resistance allows plants to initiate the innate immune response in cells away from infection sites. NPR1 is a key transcriptional coregulator in plant defense responses and has a fundamental role in response to pathogenic challenges in plants. NPR1 overexpressing crops has provided new insights regarding the role of NPR1 in both biotic and abiotic stresses in several plant species. NPR1 plays a significant role in the establishment of systemic acquired resistance (SAR) as well as induced systemic resistance (ISR), it acts as the master regulator of the plant defense signaling network, mediating crosstalk between the salicylic acid (SA) and jasmonic acid/ethylene responses. The importance of dissecting the mode of action of SA in plant defense responses underlies in the physiological processes that require SA-directed signaling networks and its crosstalk to other pathways. NPR1 has been shown to be a SA receptor. NPR1 harbors a BTB/POZ domain followed by ankyrin repeats at its N-terminal. Also, the C-terminal region is predicted to be unstructured and contains two closely located cysteine residues (C521/C529), which has been shown to be involved in copper mediated SA binding. Despite extensive progress has been made in understanding the signaling networks, there is no structural information available to date on the regulation of NPR1 protein in the presence of SA. Given the critical roles of SA in plant defense and the lack of a complete understanding of SA signaling in plant defense responses, we sought to investigate the structure and function of NPR1 using Cryo-EM analysis, which is expected to lead to a better understanding of its overall architecture and functional regulation.

Structural basis for activation of Dot1L methyltransferase by H2B ubiquitylation

Robert McGinty (UNC-CH)

Cathy J. Anderson[1], Matthew R. Baird[2], Allen L. Hsu[3], Emily H. Barbour[2], Yuka Koyama[2], Mario J. Borgnia[3], and Robert K. McGinty[1,2]
[1]Department of Biochemistry and Biophysics, UNC School of Medicine, Chapel Hill, NC
[2]Division of Chemical Biology and Medicinal Chemistry, UNC Eshelman School of Pharmacy, Chapel Hill, NC
[3]Genome Integrity and Structural Biology Laboratory, National Institute of Environmental Health Sciences, Research Triangle Park, NC

Histone H3 lysine 79 (H3K79) methylation is a marker of actively transcribed genes, and its activity is required for the pathogenesis of MLL-rearranged leukemia. Methylation of H3K79 on the structured disk face of the nucleosome is mediated by the Dot1L methyltransferase. Dot1L activity is part of a trans-histone crosstalk pathway, requiring prior histone H2B ubiquitylation of lysine 120 (H2BK120ub) for optimal activity. Yet, the molecular details describing both how Dot1L binds to the nucleosome and why Dot1L is activated by H2BK120 ubiquitylation were previously unknown. We have solved a 3.9 Å cryo-EM structure of Dot1L bound to a nucleosome reconstituted with site-specifically ubiquitylated H2BK120. The structure reveals that Dot1L

engages the nucleosome acidic patch using a variant arginine anchor and occupies a conformation poised for methylation. In this conformation, Dot1L and ubiquitin interact directly through complementary hydrophobic surfaces. Using our structure, we identify point mutations that disrupt the nucleosome-specific and ubiquitin-dependent activities of Dot1L. This study establishes a path to better understand Dot1L function in normal and leukemia cells.

Fab-dimerized glycan-binding antibodies

Robert Edwards (Duke University)

Robert Edwards[1,2], Wilton B. Williams[1,2], R. Ryan Meyerhoff[1], Rory Henderson[1], Ye Zhou[4], Allen Hsu[5], Katayoun Mansouri[1], Katarzyna Janowska[1], Hui Li[6], Mario Borgnia[5], Alberto Bartesaghi[4], Kevin Saunders[1,3], George Shaw[6], Barton Haynes[1,2], Priyamvada Acharya[1,3]
[1]Duke Human Vaccine Institute
Departments of Medicine[2], Surgery[3], and Computer Science[4], Duke University
[5]National Institute of Environmental Health Sciences, NIH
[6]Perelmen School of Medicine, University of Pennsylvania

2G12 is an anti-HIV antibody that binds to surface glycans of the HIV Env protein. In 2G12, the heavy chains are domain-swapped, resulting in dimerization of the two Fab arms to give an I-shaped antibody. Because of its unusual structure, 2G12 has been considered one of a kind; and it is unclear whether, or how, similar antibodies might be elicited in the search for an effective HIV vaccine. Here we present cryo-EM structures for two novel glycan-binding antibodies bound to solubilized Env. Similar to 2G12, these antibodies form I-shaped structures with intramolecular Fab-dimers. Although negative stain of the antibodies alone show a mixture of I-shaped antibodies and traditional Y-shaped antibodies, cryo-EM of the Fab-Env complex shows that only the Fab-dimer form binds to Env. Similar to 2G12, the Fab-dimer binds to terminal residues of the Env glycans. Unlike 2G12 however, neither antibody is domain-swapped. For both antibodies, the Fab-Env complex showed considerable heterogeneity, including binding to multiple glycan epitopes on the Env protein. Hetero-refinements or multi-body refinements also indicated considerable movement of the Fab-dimer relative to the Env. The relative movement of the two parts limited the resolution for the entire complex, but resolution was improved by local refinement of the separate parts. For one of the antibodies, the Fab-dimer interface was very similar to 2G12 and involved some of the same residues. For the other antibody, the two Fabs were skewed at a different angle relative to one another, yielding a different interface that nevertheless also involved some of the same key residues. The structures presented here, together with negative stain surveys of other I-shaped Fab-dimerized antibodies, indicate that the glycan-binding and broad neutralization first observed in 2G12 may not be as unique as previously thought, and may represent an unexplored path towards development of an HIV vaccine.

VALIDATION AND INTERPRETATION

Assessing at EMDB Model Challenges: Strengths, weaknesses, and rapid improvement
Jane Richardson (Duke University)

Our lab at Duke runs the MolProbity model validation service, developed and successful for crystal structures of macromolecules at better than around 2.5Å resolution -- outlier markup guides corrections, and if all scores are good your model is sure to have pretty much the right answer. At 3 to 4Å, however, modeling is very much harder either for crystallography or for cryoEM, since the broader density is compatible with many distinct models, both right and wrong. For the last several years we have concentrated on learning the properties of single-particle cryoEM maps and models, how the models are built and refined, and what new validation criteria could more reliably locate local errors and help guide their correction. It turns out that refinement at these resolutions is restraining most of the traditional validation criteria, and can remove outliers without fixing the underlying problem. Worse yet, restraining multi-valued criteria such as Ramachandran can make the model worse by pulling local conformation into the wrong local minimum. We and others have developed new multi-residue validation criteria such as CaBLAM, EMRinger, and better secondary-structure analysis that are still sensitive to the problems. We are exploring modeling and refinement strategies that can minimize local errors and thereby make more serious errors less probable, such as incorrect connectivity or sequence misalignment. The good news is that every aspect of cryoEM structure solution is improving very rapidly, including these validation methods!

What is CaBLAM and how does it help improve cryoEM models?
Christopher Williams (Duke University)

CaBLAM is a validation tool for protein structure in MolProbity and Phenix. It analyzes the CA positions of a protein and extrapolates reasonable whole-backbone conformations from the CA trace. It then compares the modeled structure against these predictions. CaBLAM is a particularly useful validation for cryoEM because it provides suggested corrections in addition to identifying problems, because it is sensitive to modeling errors that are common in the 3-4A resolution regime, and because it is thus far resistant to being overfit in refinement. Here we show some practical examples of interpreting CaBLAM results and using them to guide model correction. We also debut a new visual markup for CaBLAM outliers to aid in this interpretation.

ENZYMES

A new twist in lipase storage: the cryo-EM structure of helical LPL
Kathryn Gunn (UNC-CH)

Lipases are enzymes necessary for the proper distribution and utilization of lipids in the human body. Lipoprotein lipase (LPL) is active in capillaries where it plays a crucial role in preventing dyslipidemia by hydrolyzing triglycerides from packaged lipoproteins. Thirty years ago, the existence of a condensed and inactive LPL oligomer was proposed. Although recent work has shed light on the structure of the LPL monomer, the inactive oligomer remained opaque. Here we present a cryo-EM reconstruction of a helical LPL oligomer at 3.8 Å resolution. Helix formation is concentration dependent and helices are composed of inactive dihedral LPL dimers. Heparin binding stabilizes LPL helices, and the presence of substrate triggers helix disassembly. Super-resolution fluorescent microscopy of endogenous LPL revealed that LPL adopts a filament-like distribution in vesicles. Taken together, this suggests that LPL is condensed into its inactive helical form for storage in intracellular vesicles.

Structural and functional analyses of AAA ATPase Rix7 in the process of unfolding substrate
Yu-Hua Lo (NIEHS)

Rix7 is a type II AAA-ATPase essential for the creation of the large ribosomal subunit. It has been suggested that Rix7 prompts the removal of assembly factors from pre-60S particles using ATP hydrolysis, but the exact release process is unknown. Recent publications have revealed the cryo-EM reconstruction of the tandem AAA domains (D1 and D2 AAA domains) of Rix7 that form an asymmetric stacked homohexameric ring. Five of the six Rix7 protomers grip the substrate through conserved pore loops (pore loop I and II) that line the central channel, suggesting that Rix7 functions as a molecular unfoldase. This project aims to elucidate the importance of each AAA domains, the central pore loop II and linker regions to the function of Rix7. We first performed yeast growth and proliferation assays to identify the effect of mutations on pore loop II in D1 and D2 AAA domains, which revealed that, in vivo, pore loop II in D1 AAA domain is strictly required for cell cycle progression as opposed to the less crucial D2 pore loop II. This result was striking because our previous studies showed that mutations in the D2 loop I were more lethal than mutations in the D1 domain loop I. To study inter-domain communication mechanism of Rix7, both the linker between the N-terminal domain and D1 AAA domain (NTD-D1) and the linker between the D1 and D2 AAA domains (D1-D2) were examined. Yeast growth and proliferation assays showed that the linker D1-D2 was essential for adequate growth and that the linker NTD-D1 showed inhibited growth for all but one mutation in a key residue. Mutagenesis studies revealed that although the pore loop I and II are equally important for substrate binding, these pore loop motifs take different roles in the two ATPase domains, suggesting that the loops act concertedly rather than sequentially. The deletion or mutation on linkers caused a severe growth defect in S. cerevisiae along with a defect in mature 60S formation, suggesting the conformational changes of core domain (D1 and D2) as well as alterations of NTD position that regulate the function of Rix7 and substrate engagement. We also solved the cryo-EM structure of Rix7 E602Q (Walker B mutant in D2 domain) at 4.3 Å resolution which revealed the power

stroke for substrate translocation is provided by ATP hydrolysis, which occurs sequentially in each ATPase domain around the Rix7 hexamer.

Cryo-EM reveals active site coordination within a multienzyme pre-rRNA processing complex
Monica Pillon (NIEHS)

Ribosome assembly is a complex process reliant on the coordination of trans-acting enzymes to produce functional ribosomal subunits and secure the translational capacity of the cell. Las1 is a recently discovered endoribonuclease that assembles into a multienzyme complex with the Grc3 polynucleotide kinase to orchestrate RNA decay of a transcribed spacer (ITS2) from precursor ribosomal RNA (pre-rRNA). The essential Las1 endoribonuclease cleaves the ITS2 spacer at a defined site to initiate pre-rRNA processing. The Grc3 polynucleotide kinase subsequently phosphorylates the resulting 5'-hydroxyl RNA to signal for 5'- and 3'-exoribonucleases to degrade the ITS2. Disruption of mammalian Las1-Grc3 has been linked to congenital lethal motor neuron disease and X-linked intellectual disability disorders, thus highlighting its importance in human health; yet, its mechanism of action remains unclear. Here we report that the Las1 endoribonuclease assembles into a higher-order tetrameric complex with its binding partner the Grc3 polynucleotide kinase, which is essential for the activation of its nuclease and kinase functions. To understand how Las1-Grc3 achieves its strict nuclease specificity and coordinates its dual enzymes, we determined a series of high-resolution Las1-Grc3 structures in multiple conformational states. Structural characterization of Las1-Grc3 reveals its molecular architecture harboring a composite nuclease active site flanked by two discrete RNA kinase sites. Coupled with functional studies, we identify molecular features crucial for RNA specificity and two molecular switches that coordinate nuclease and kinase function. Together, our structures and corresponding functional studies establish how Las1-Grc3 couples its enzymatic functions to drive RNA decay during ribosome assembly.

Structure-function studies of a SAMHD1 homolog from bacteria reveal a novel mode of regulation
Brad Klemm (NIEHS)

Maintenance of a balanced dNTP pool is important for DNA polymerase fidelity, whereby variations from the normal dNTP levels lead to increased frequencies of DNA polymerase misincorporation. Thus, dNTP pool misregulation reduces DNA replication fidelity. The human SAMHD1 (Sterile Alpha Motif [SAM] domain and Histidine-Aspartate [HD] domain-containing protein 1) is a dNTPase, hydrolyzing dNTPs to deoxynucleosides and triphosphate. SAMHD1 activity is regulated via allosteric sites found between monomers in a dimer and between dimers in a tetrameric superstructure. SAMHD1 has recently been implicated in HIV restriction in some human cell types. In contrast to SAMHD1, some bacterial homologs are hexameric and highly-specific for dGTP. Here, we focus on the hexameric dGTPase from Leeuwenhoekiella blandensis, a member of the class of Flavobacteria, which are implicated in restoring ecosystem balance during and after harmful algal blooms by degrading the excess carbon sources. We investigated the L. blandensis dGTPase using a combination of X-ray crystallography, cryogenic electron microscopy, and in vitro biochemical assays to probe the structure and function of these

enzymes. We discovered a novel mode of allosteric regulation for HD-domain containing proteins. We identified a new binding pocket for nucleotide pool sensing, which is highly specific for dATP. Binding the dATP activator at this remote site transmits a conformation change to the active site, predisposing it for substrate binding. We have determined the highest-resolution crystal structure of a bacterial dNTPase (~1.8 Å), in the apo and metal-bound states. We also report the first cryo-EM structures of any SAMHD1 homolog (< 3.0 Å), including structures 1) in the apo state, 2) bound to the dGTP substrate, 3) bound to the dATP activator, and 4) bound to both ligands at once. These structures reveal the most complete active site organization bound to substrate for any SAMHD1 homolog to date. Whereas previous substrate-bound structures utilized metal-site mutations to prevent catalysis, we mutated the catalytic acid (histidine), allowing us to visualize the full metal coordination sphere and the correct positions of the substrate and the remaining protein side chains involved in substrate recognition. The structures also guided mutations in the active and allosteric sites to test the function of the enzyme and validate the structural observations.

METHOD DEVELOPMENT

Accelerating data collection in Cryo-ET: working towards high-resolution in situ structures
Jonathan Bouvette (NIEHS)

Cryo-EM is now routinely used in structural biology to solve the structure of purified proteins and complexes. Single-particle Cryo-EM has been optimized for data quality speed of acquisition to reach atomic resolution efficiently. The technique requires averaging several thousands of projection images from isolated molecules recorded in an invariant context. Many complex biological systems are not amenable to study in isolation and their natural environment is difficult to reconstitute in vitro. In addition to facilitating the study of these labile systems, the ability to characterize macromolecules in their biological context is likely to reveal new information about their function. Cryo-electron tomography is the technique of choice when it comes to studying complexes associated to pleomorphic entities such as virions, liposomes, cells or tissues. This approach has been successfully used to acquire 3D maps and find macromolecules within such environments. Subvolumes containing the object of interest were then averaged leading to structures up to 30 A resolution. The limitation in resolution derives in part from the speed at which we can acquire tilt series, which allows for the collection hundreds to a few thousand images of the molecules of interest. Collection speed is reduced by the precision of the tracking of each tilt at high magnification. Attaining resolutions comparable with Single-particle Cryo-EM will require a reduction in pixel size, which translates into a reduction in the imaged volume, leading to an even smaller number of molecules. A method combining an increase in the speed of data acquisition in Cryo-electron tomography with improved tracking would yield more high-resolution volumes to average, allowing for in situ reconstruction of macromolecular complexes at sub-nanometer resolutions. Here, we describe a new strategy that makes use of beam shift / image shift to accelerate data acquisition in tomography. By linking the open source data collection package SerialEM with external python routines, we can provide real-time analysis of the tilt series to correct targeting and defocus. Preliminary results show that it is possible to track the center of the target area within a 5 nm margin of error and to image locations that are away from the tilt axis. This approach allows for very precise parallel acquisition of multiple tilt series at high magnification significantly enhancing speed and resolution.

Improved resolution achieved by unsupervised data-driven protocols for particle sorting
Ye Zhou (Duke University)

Technical advances in single-particle cryo-Electron Microscopy (EM) have transformed the technique into a popular tool for determining the structures of challenging biomolecules that are inaccessible to other technologies. While many samples can now be routinely analyzed using standard image processing protocols, significant intervention from expert users is still required. Here, we explore the use of unsupervised strategies for particle sorting that are compatible with autonomous high-resolution 3D structure refinement. By only relying on the statistics of particle alignment distributions while foregoing of human judgment, this method generated consistent and resolution improved results. For challenging samples that are outside the conventional range of satisfying conditions for single particles analysis (SPA), e.g. small size or flexible complexes,

user input remains a critical component for the success of the structure determination process. Here, we will also discuss some instances of these situations.

Quantifying the heterogeneity of macromolecular machines by mass photometry
Nicholas Brown (UNC-CH)

Sample purity is central to in vitro studies of protein function and regulation, as well as to the efficiency and success of structural studies requiring crystallization or computational alignment of individual molecules. Here, we show that mass photometry (MP) accurately reports on sample heterogeneity using minimal volumes with molecular resolution within minutes. We benchmark our approach by negative stain electron microscopy (nsEM), including workflows involving chemical crosslinking and multi-step purification of a multi-subunit ubiquitin ligase. When applied to proteasome stability, we detect and quantify assemblies invisible to nsEM. Our results illustrate the unique advantages of MP for rapid sample characterization, prioritization and optimization.

Organization of platelet microtubule marginal band microtubules
Joshua Strauss (UNC-CH)

Joshua D Strauss[1], Juleen Dickson[3], Wenchen Chen[2], Elizabeth R Wright[2], Renhao Li[2]
[1]Department of Biophysics and Biological Chemistry, University of North Carolina at Chapel Hill, Chapel Hill NC, 27516
[2]Aflac Cancer and Blood Disorders Center, Department of Pediatrics, Emory University School of Medicine, Atlanta GA, USA
[3]Department of Biochemistry, University of Wisconsin-Madison, Madison WI, USA

Platelet morphological and functional states are coupled to the organization of the cytoskeleton. Discoid resting platelets contain a circular bundle of dynamic microtubules (MTs) referred to as the marginal band (MB). Upon activation MTs depolymerize and the marginal band disassembles. Details describing the structural organization of the MTs is lacking at the molecular level. To gain deeper understanding into platelet ultrastructure we utilized whole-cellular cryo-electron tomography (cryoET) to directly visualize the native-like arrangement of MTs within the MB. We report that the MB consists of multiple MTs, the majority of which are shorter than the circumference of the entire MB, and MTs ends are not randomly distributed but rather form clusters.

Registered participants

Last Name	First Name	Affiliation	Email
Acharya	Priyamvada	Duke University	priyamvada.acharya@duke.edu
Anderson	Cathy	UNC-CH	catson4@email.unc.edu
Bartesaghi	Alberto	Duke University	alberto.bartesaghi@duke.edu
Barycki	Joe	NCSU	jjbaryck@ncsu.edu
Beese	Lorena	Duke University	lb12@duke.edu
Bell	Aaron	NCSU	abell01@hotmail.com
Borgnia	Mario	NIEHS	mborgnia@nih.gov
Bouvette	Jonathan	NIEHS	jonathan.bouvette@nih.gov
Brown	Nicholas	UNC-CH	nbrown1@med.unc.edu
Cao	Can	UNC-CH	caocan23@email.unc.edu
Chen	Vincent	Duke University	vbc3@duke.edu
Choe	Jun-yong	East Carolina University	choej18@ecu.edu
Copeland	Bill	NIEHS	copelan1@niehs.nih.gov
Edwards	Robert	Duke University	rj.edwards@duke.edu
Ehrhart	Catherine	Duke University	cie2@duke.edu
Ervin	Samantha	UNC-CH	smervin@live.unc.edu
Fay	Jonathan	UNC-CH	fayj@unc.edu
Frazier	Meredith	NIEHS	meredith.frazier@nih.gov
Gibbs	Morgan	UNC-CH	gibbsm@live.unc.edu
Gobeil	Sophie	NIEHS	sophie.gobeil@duke.edu
Gordon	Jacob	NIEHS	jacob.gordon@nih.gov
Gunn	Kathryn	UNC-CH	kgunn@med.unc.edu
Hao	Aili	Duke University	AILI.HAO@DUKE.ED
Hayne	Cassandra	NIEHS	cassandra.hayne@nih.gov
Haynes	Barton	Duke University	barton.haynes@duke.edu
Henderson	Rory	Duke University	rory.henderson@duke.edu
Hooks	Grace	Duke University	grace.hooks@duke.edu
Horng	James	NIEHS	jhorng@live.unc.edu
Hsu	Allen	NIEHS	allen.hsu@nih.gov
Iancu	Cristina	East Carolina University	cviancu@gmail.com
Ishtikhar	Mohd	East Carolina University	ishtikharm19@ecu.edu
Janowska	Katarzyna	Duke University	kj143@duke.edu
Kaminski	Andrea	Duke University	moon@niehs.nih.gov
Kasinadhuni	Ravitej	UNC-CH	jet3000@ad.unc.edu
Klemm	Brad	NIEHS	bradley.klemm@nih.gov
Kopp	Megan	Duke University	megan.kopp@duke.edu
Kumar	Shivesh	Duke University	shivesh.kumar@duke.edu
Lee	Seok-Yong	Duke University	seok-yong.lee@duke.edu
Liu	Zhiyong	Duke University	zhiyong.liu@duke.edu
Lo	Yu-Hua	NIEHS	yu-hua.lo@nih.gov
Manne	Kartik	Duke University	kmanne09@uab.edu
Mansouri	Katayoun	Duke University	katayoun.mansouri@duke.edu
McDowell	Shana	Duke University	shana.mcdowell@duke.edu

McGinty	Robert	UNC-CH	rmcginty@email.unc.edu
Min	Jungki	NIEHS	minj2@nih.gov
Mueller	Geoffrey	NIEHS	mueller3@niehs.nih.gov
Neher	Saskia	UNC-CH	neher@email.unc.edu
Perryman	Alexia	UNC-CH	Anplexia@email.unc.edu
Pillon	Monica	NIEHS	monica.pillon@nih.gov
Pittman	Nikea	UNC-CH	nikea@unc.edu
Qiu	Chen	NIEHS	qiuc2@niehs.nih.gov
Redinbo	Matt	UNC-CH	redinbo@unc.edu
Riccio	Amanda	NIEHS	riccioaa@nih.gov
Richardson	Jane	Duke University	jsr@kinemage.biochem.duke.edu
Richardson	Dave	Duke University	dcr@kinemage.biochem.duke.edu
Rodriguez	Yesenia	NIEHS	yesenia.rodriguez@nih.gov
Rose	Bob	NCSU	bob_rose@ncsu.edu
Roy	Sourav	UNC-CH	sourav@email.unc.edu
Schumacher	Maria	Duke University	maria.schumacher@duke.edu
Shi	Yuqian	Duke University	ys89@duke.edu
Sikkema	Andy	NIEHS	andrew.sikkema@nih.gov
Silva	Gustavo	Duke University	gustavo.silva@duke.edu
Sokratian	Arpine	Duke University	arpine.sokratian@duke.edu
Stalls	Victoria	Duke University	vds7@duke.edu
Stanley	Robin	NIEHS	robin.stanley@nih.gov
Strauss	Joshua	UNC-CH	Joshua_Strauss@med.unc.edu
Suo	Yang	Duke University	ys138@duke.edu
Tang	Qingyu	NCSU	qtang6@ncsu.edu
Walters	Mark	Duke University	mark.walters@duke.edu
Washington	Erica	Duke University	ew142@duke.edu
Wesley	Nate	UNC-CH	nwesley@live.unc.edu
Willcox	Smaranda	UNC-CH	smaranda_willcox@med.unc.edu
Williams	Christopher	Duke University	christopher.sci.williams@gmail.com
Winkler	Christopher	NCSU	crwinkler@ncsu.edu
Wright	Nicholas	Duke University	njw22@duke.edu
Xu	Guozhou	NCSU	gxu3@ncsu.edu
Yin	Ying	Duke University	ying.yin@duke.edu
Yoo	Jiho	Duke University	jiho.yoo@duke.edu
Zang	yunxiang	Duke University	yz432@duke.edu
Zeug	Matthias	East Carolina University	zeugm19@ecu.edu
Zhao	Yani	UNC-CH	yani61@live.unc.edu
Zhou	Pei	Duke University	peizhou@biochem.duke.edu
Zhou	Ye	Duke University	ye.zhou867@duke.edu
Zong	Guangning	NIEHS	guangning.zong@nih.gov