2023

WEST COAST STRUCTURAL BIOLOGY WORKSHOP

MARCH 19-22, 2023
Asilomar Conference Center
Pacific Grove, CA

March 19 – 22, 2023
Asilomar Conference Center
Pacific Grove, California

The WCSBW organizers acknowledge our presence on the traditional, ancestral and unceded territory of the Ohlone, Rumsen and Esselen peoples

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WELCOME

After a pandemic hiatus, the West Coast Structural Biology Workshop (WCSBW) has return. March 2023 will present the 25th meeting of this historic and influential workshop focusing on methods developments in structural biology. Driven by rapid advances in the field, the workshop will encompass the latest methods for structural analysis. Posters and oral presentations will emphasize the technical challenges and achievements enabling successful structural analysis. In addition to research talks and posters, the meeting will feature two hands-on workshops on: (1) advances in cryo-EM structure determination protocols, and (2) an introduction to coding neural networks. As in past years, the meeting will be held at the beautiful Asilomar Conference Center in Pacific Grove, CA. This idyllic venue and the interactive spirit of the WCSBW meeting offer wonderful opportunities for interactions between current and future leaders in structural biology.

On behalf of the 2023 WCSBW organizers, welcome.

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SCHEDULE

2023 West Coast Structural Biology Workshop
March 19th – 22th Asilomar, California

SUNDAY

Check in & Registration	**4:00 – 6:00 PM**	Room check-in in the **Asilomar Social Hall** Pick up your name badge and program in the lobby of **Asilomar Main Lodge**
Dinner	**6:00 – 7:00 PM**	**Crocker Dining Hall**

[All talks to be held in Chapel Hall]

Welcome	**7:10 – 7:15 PM**	**Welcome and introductions by Prof. Todd O. Yeates**
SESSION 1	**7:15 – 8:30 PM**	**KEYNOTE presentation by Robert Stroud** "Caught in the act! Building the wall, ... - again!"
Sponsor Talks	**8:30 – 9:20 PM**	**Short talks by Sponsors** *Chair: Roger Castells Graells, UCLA*
1. Joyce Frank, *MiTeGen*		Developments in Sample Preparation and Handling Technology for CryoEM and Crystallography
2. Tim Booth, *SPT Labtech*		Enhanced Tools for the Routine Optimization of cryoEM samples with *Chameleon*
3. Joseph Pesavento, *ThermoFisher*		Advancing cryoEM technologies to enable scientific breakthroughs
4. Robert Monteverde, *Direct Electron*		A Novel Event-Based Direct Detector for cryoEM
Reception	**9:30 – 11:00 PM**	**Drinks and Snacks, Fred Farr Forum**

MONDAY

Breakfast **7:30 – 9:00 AM** **Crocker Dining Hall**

SESSION 2 **9:00 – 10:15 AM** **Membrane structure and function – Understanding transport, signaling, and membrane remodeling**
Chair: Yuntao Liu, UCLA

1. **Alan Blakely** Dynamic conformations of the insulin receptor as activated by a venom-derived insulin.
2. **Yi Xiao Jiang** Amyloid fibrils in frontotemporal lobar degeneration with TDP-43 inclusions (FTLD-TDP) are composed of TMEM106B, rather than TDP-43
3. **Laurie Wang** Visualizing small molecule inhibition of the Sec61 translocon by cryo-EM
4. **Steven C. Wilson** Organizing Structural Principles of the IL-17 Ligand–Receptor Axis

Break **10:15 – 10:45 AM** **Break and Sponsor Expos**

SESSION 3 **10:45 – NOON** **Serial X-ray diffraction & other approaches to investigate structural ensembles and kinetic processes**
Chair: Yuntao Liu, UCLA

1. **Asmit Bhowmick** Investigating structural changes in Photosystem II using time-resolved X-ray free-electron laser (XFEL) crystallography
2. **Jonathan Philpott** PERIOD phosphorylation leads to feedback inhibition of CK1 activity to control circadian period
3. **Oanh T.N. Tran** Metal-substituted P450cam investigates substrate interaction in the P450cam active site.
4. **David Moses** Structural biases in disordered proteins are prevalent in the cell

Lunch **12:00 – 1:00 PM** **Crocker Dining Hall**

Flash Talks 1 **1:30 – 2:00 PM** **Poster-related 2-minute talks in Chapel Hall**
Chair: Maria Flores, UCLA

Amber Vogel, Ambarneil Saha, Ashraya Ravikumar, Becky Jenkins, Billy Poon, Joshua Dolinsky, Aldo Munoz, Justin Miller, Jeff Qu

Workshop I **2:00 – 4:00 PM** **Pythia:** "A Deep Learning Primer For Applications in Structural Biology" by Mohamed El Hibouri

Poster Session 1 **4:00 – 6:00 PM** **Fred Farr Forum**

Dinner **6:00 – 7:00 PM** **Crocker Dining Hall**

SESSION 4 **7:15 – 8:30 PM** **KEYNOTE presentation by Juli Feigon**
"Structural Biology of Regulatory RNPs: telomerase and 7SK"

8:30 – 9:00 PM **Short talk session**
Chair: Maria Flores, UCLA

1. **Onellah Weerakoon** Structural Characterization of AAV-AAVR Interactions by cryo-EM
2. **Jiahui Lu** Cryo-EM structures of the D290V variant of the hnRNPA2 low complexity domain suggest the mechanism of its pathogenicity
3. **Matthias Malago** Designing Protein Binders Using Next Generation Artificial Intelligence
4. **Yuanyuan Chen** Structural basis of mitochondrial protein import by the TIM23 complex

Reception **9:00 – 11:00 PM** **Drinks and Snacks at Fred Farr Forum**

TUESDAY

Breakfast **7:30 – 9:00 AM** **Crocker Dining Hall**

SESSION 5 **9:00 – 10:15 AM** **Nucleic acids – Mechanisms of information processing and novel cellular roles for RNA**
Chair, Dorothée Liebschner, LBNL

1. **Baocheng Liu** Structure of active human telomerase with telomere shelterin protein TPP1
2. **Tilini Wijeratne** Structural modes of B-Myb DNA binding domain bound to nucleosomes
3. **Tamara Christiani** Characterization of bacteriophage P4 portal protein structure using cryo-EM and protein prediction software
4. **Yao He** Structure of telomerase-bound CST with Polymerase α-Primase

Break **10:15 – 10:45 AM** **Break and Sponsor Expos**

SESSION 6 **10:45 – NOON** **New EM methods, instruments and cool structures – breaking barriers in resolution, size, and speed**
Chair, Dorothée Liebschner, LBNL

1. **Rebeccah Warmack** Structural consequences of turnover-induced homocitrate loss in nitrogenase
2. **Roger Castells Graells** A Designed Imaging Scaffold Breaks the Barrier to High-Resolution Structure Determination of Small Proteins by Cryo-EM
3. **Yuntao Liu** Isotropic reconstruction for electron tomography with deep learning
4. **Jacob Summers** Time-resolved Cryo-Electron Tomography of Surface Layer Lattice and Membrane Dynamics of Gram-Negative Bacteria upon pH Jump

Lunch **12:00 – 1:00 PM** **Crocker Dining Hall**

Flash Talks 2 **1:30 – 2:00 PM** **Poster-related 2-minute talks in Chapel Hall**
Chair, Rebecca Warmack, Caltech

Karina Guadalupe, Lily Taylor, Mengfan Lyu, Michael Sawaya, Xiaoying Cai, Natalie Schibrowsky, Niko Vlahakis, Jon Philpott,

Workshop II **2:00 – 3:30 PM** **Thermo Fisher Scientific:** "Automated Cryo-EM data collection workshop: *SmartEPU* and Embedded *CryoSPARC* Live"
by Francis Reyes

Flash Talks 3 **3:30 – 4:00 PM** **Poster-related 2-minute talks in Chapel Hall**
Chair, Rebecca Warmack, Caltech

Peter Ngoi, Robyn Stanfield, Nina Harpell, Scott D. Pegan Silvia Russi, Sivasankar Putta, Stephanie Wankowicz, Vitor Hugo Balasco Serrão, Shivansh Mahajan

Poster Session 2 **4:00 – 6:00 PM** **Fred Farr Forum**

Dinner **6:00 – 7:00 PM** **Crocker Dining Hall**

SESSION 7 **7:15 – 9:00 PM** **Electron diffraction & other new cryo-EM methodologies and challenges for determining novel structures**
Chair, Rebecca Warmack, Caltech

1. **Logan Richards** Structural characterization of human LECT2 amyloids, drivers of kidney amyloidosis
2. **Dorothee Liebschner** Using predicted protein models in Phenix
3. **Muyuan Chen** Improving resolution and resolvability of single particle CryoEM using Gaussian representation
4. **Oleg Sobolev** A global Ramachandran score identifies protein structures with unlikely stereochemistry

Reception **9:00 – 11:00 PM** **Drinks and Snacks at Fred Farr Forum**

WEDNESDAY

Breakfast **7:30 – 9:00 AM** **Crocker Dining Hall**

SESSION 8 **9:00 – 10:15 AM** **Structures that illuminate cell function**
Chair, Roger Castells Graells, UCLA

1. **Nathanael Caveney** Cytokine Masquerading as a Hormone? Structure of the Leptin Receptor Complex Guides Ligand Engineering
2. **Maria Flores** Molecular Snapshots of the Mammalian Prion CPEB3
3. **Heather Liu** Structural Basis for Evasion of IgA Immunity by *Streptococcus pyogenes* Revealed in the Complex of Arp4 with Human IgA
4. **Mohammed Kaplan** Unique structural adaptations occur within the microbial predator *Bdellovibrio bacteriovorus* during prey invasion

Break **10:15 – 10:45 AM** **Break & Sponsor Expos**

SESSION 9 **10:45 – NOON** **Hybrid method structure determination and automation – sourcing structural data from machine learning and other methods; applications of protein prediction to structure determination**
Chair, Roger Castells Graells, UCLA

1. **Jenny Tang** Development of a Stabilizer for 14-3-3 Interaction with ERα
2. **Chengcheng Fan** Neutralizing monoclonal antibodies elicited by mosaic RBD nanoparticles bind conserved sarbecovirus epitopes
3. **Sonya Bharathkar** Structure-based engineering of secretory immunoglobulin A provides new approaches for targeting mucosal pathogens.
4. **David Gonzalez** Defining the structure and function of newly identified Tamdy nairovirus vOTUs to examine the evolutionary trends within the nairovirus genus for those nairoviruses of human consequence

Concluding Remarks & Lunch **12:00 – 1:00 PM** **Boxed lunch and departure**

KEYNOTE SPEAKER

Professor Robert Stroud

University of California San Francisco

Professor Robert Stroud was born in Stockport, England. He was influenced in his youth by early exposure to the sciences, experimenting in elementary astronomy and electronics. At university in Cambridge, he studied physics and mathematics, and first learned of crystallography. Professor Stroud did his doctoral research at Birkbeck College in London, working with the pioneering crystallographer J.D. Bernal. There, his research studies involved computational crystallography and structure determination of an anti-cancer compound by non-centrosymmetric direct methods. Stimulated in part by David Phillips' determination of the structure of the enzyme lysozyme, Stroud turned to the study of protein structure, accepting a postdoctoral position in the U.S. with Richard Dickerson at Cal Tech in 1968. After a stint on the faculty at Cal Tech, he was recruited to UCSF in 1976 to help develop a program there in structural biology.

At UCSF, Professor Stroud began groundbreaking research on the structure and function of membrane proteins, whose structures presented tremendous experimental challenges. His achievements in the area of membrane proteins include early structural elucidations of the acetylcholine receptor, the first structures of aquaporins and the ammonia channel, and structure determination of the signal recognition particle (SRP), a key protein complex involved in membrane protein targeting to the ER. Professor Stroud's research has also emphasized the role of structure in in the discovery and design of novel drug molecules.

Among his notable awards, Professor Stroud is a member of the U.S National Academy of Sciences and a Fellow of the Royal Society of Medicine.

KEYNOTE SPEAKER

Professor Juli Feigon

University of California Los Angeles

Professor Juli Feigon received her B.A. from Occidental College and her M.S. and Ph.D. from the University of California, San Diego where she studied with Dr. David Kearns. Her postdoctoral work was completed at the Massachusetts Institute of Technology, where she was a Damon Runyon-Walter Winchell Cancer Fund Postdoctoral Fellow with Dr. Alexander Rich. Dr. Feigon has been a UCLA faculty member since 1985, when she was appointed the first female Assistant Professor in the Department of Chemistry and Biochemistry. She currently holds the rank of Distinguished Professor of Biochemistry and the Christopher Foote Term Chair. Her awards and honors include the Dupont Young Faculty Award (1985-1986), Presidential Young Investigator Award of the National Science Foundation (1989-1994), Camille and Henry Dreyfus Teacher/Scholar Award (1990), Glenn T. Seaborg Research Award (1992), Herbert Newby McCoy Award, (1993, 2009, 2022), elected Fellow of the American Association for the Advancement of Science (AAAS) (2002), Elected Member, National Academy of Sciences (2009), Dorothy Crowfoot Hodgkin award of the Protein Society (2017), Biophysical Society Founders Award (2018), and UCLA Academic Senate Diversity, Equity, and Inclusion Research Award (2020), and Finalist, Excellence in Postdoctoral Mentoring Award (2022).

Professor Feigon's research interests are in structural biology of nucleic acids and their complexes. She pioneered the application of macromolecular NMR spectroscopy to the study of DNA and RNA structure, folding, and interactions with cations, drugs, and proteins. Her laboratory published the first high resolution structures of DNA triplexes, quadruplexes, and aptamers, and her work has provided fundamental insights into DNA A-tract and protein induced bending, cation interactions with DNA, Hoogsteen base pairs, and drug binding to DNA. She has made major contributions to understanding RNA folding and function, including studies of RNA aptamers, ribozymes, and riboswitches, and recognition of RNA by proteins. Her laboratory solved the first NMR structure of a riboswitch and set the standard for high-resolution RNA structures determined by NMR. For all of this work, she developed new NMR methods and applications for studying nucleic acids structure and dynamics, including assignments and detecting cation interactions. Her work has combined structural and functional studies of RNA–protein complexes to reveal essential determinants of protein recognition of single stranded and of double stranded RNA by RRMs and dsRBDs, respectively. Currently, the Feigon laboratory employs an integrative structural biology approach combining NMR spectroscopy, X-ray crystallography, and electron microscopy (EM) along with biochemistry to study structure, dynamics, assembly, and function of non-coding RNA and RNA-protein complexes, in particular 7SK RNP and telomerase. Over the past two decades, her laboratory pioneered structural studies of telomerase, from solution NMR and X-ray crystal structure and dynamics studies of telomerase RNA and RNA-protein domains of human and Tetrahymena telomerase to the first structure of a telomerase holoenzyme, by negative stain EM at 25 Å resolution, and cryo-EM studies telomerase and associated proteins at increasingly higher resolution.

SPONSOR TALKS

Developments in Sample Preparation and Handling Technology for Crystallography and Cryo-EM

Joyce Frank

(joyce@mitegen.com)
MiTeGen

Sample preparation for Crystallography and Cryo-EM is a multistage process that is time consuming and fraught with inefficiencies. During our talk, Developments in Sample Preparation and Handling Technology for Crystallography and Cryo-EM, we will discuss tools and technologies that can aid the sample preparation process. This will include: How to decrease the barriers of entry to get started; The development of technologies to improve chances of success; How to increase the ease of the sample preparation workflow; The use of a one-stop-shop for the majority of materials, consumables, and equipment needed.

Enhanced Tools for the Routine Optimization of CryoEM Samples with *Chameleon*

Tim Booth
(tim.booth@sptlabtech.com)
SPT Labtech

Conventional cryoEM sample preparation workflows are still of the “guess-and-check” type, using the electron microscope to determine the outcome. There are few optimization workflows specific to sample type beyond common sense decision making. Only a consensus that optimization is required for most samples and that approximately ten different methods will be attempted in combination before improvement is seen [1]. The chameleon instrument and self-wicking grids represent a paradigm shift towards a routine, automated, fast-plunge future for sample optimization [2]. Alternative film materials and novel self-wicking grid geometries allow for a wider array of freezing conditions to be evaluated at once, moving downstream feedback forward in the workflow, reducing the time-to-structure loop and dependence on screening instrumentation.

References: 1. B. Carragher, et al. *J. Microsc* 276 (2019); 2. T. Levitz, et al. *Front. Mol. Biosci.* Vol. 9 (2022)

Advancing cryoEM technologies to enable scientific breakthroughs

Joseph Pesavento
(joseph.pesavento@thermofisher.com)
ThermoFisher

To fully understand biological processes, and how they fail in disease, it is vital to obtain structural information for the relevant biological machinery. Notably, it is becoming increasingly apparent that proteins, the key biological players in fundamental biology or disease mechanisms, often adopt multiple conformations or act in complexes with other proteins. These large and/or dynamic systems present a challenge to traditional methods of 3D structural determination.

To address these challenges, Cryo-EM has been firmly established as a must-have technique for structural biology, in addition to X-Ray crystallography and NMR. Recent advancements in Cryo-EM technology, such as the introduction of direct electron detectors, energy filtering, and automated sample handling, have made adoption of Cryo-EM for single particle analysis and structural biology more accessible than ever. In addition, the recent introduction of the SmartEPU software platform has brought about improved data analysis and throughput benefits.

Additional relevant applications of Cryo-EM including Cryo-electron tomography (Cryo-ET) and microcrystal electron diffraction (MicroED) have also been demonstrated to be a great add-on to the Cryo-EM researcher's toolkit. In this presentation, we will discuss these latest advancements in Cryo-EM technology and methods including Cryo-ET and MicroED, as well share some specific examples of recent scientific achievements.

Apollo: A Novel Event-Based Direct Detector for Cryo-EM

Robert Monteverde
(bmonteverde@directelectron.com)
Direct Electron

Over the past 10 years, electron counting with direct detection cameras has become the de facto standard for cryo-EM data acquisition. However, since its initial demonstration in 2009, the technology for electron counting has remained fundamentally unchanged: Electron counting is performed computationally, by thresholding and centroiding blobs on each of many integrating mode frames acquired under a strictly limited TEM beam current.

One of the most significant bottlenecks for cryo-EM is the restrictive imaging conditions imposed by electron counting. Maintaining sparse illumination within each frame from the camera is necessary to avoid coincidence loss stemming from the inability to discriminate multiple coincident electrons as separate events. The limited exposure rate imposed by current cameras has two consequences: it places an upper limit on throughput, and it eliminates the microscopist's flexibility to optimize imaging conditions for new methods.

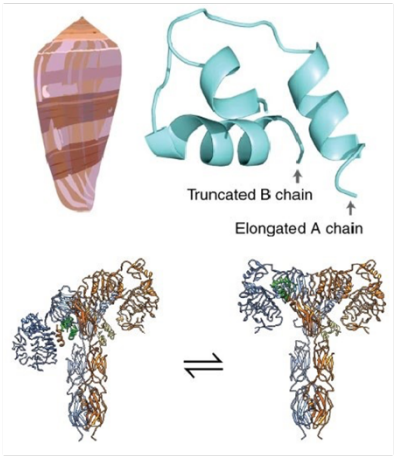
A new TEM camera, Apollo, is based on new direct detection technology that—for the first time—performs electron counting in hardware with a large-format sensor, enabling high-quality image acquisition across a wide range of exposure rates with minimal coincidence loss. Apollo's novel sparse-binary-readout direct detection sensor performs correlated-double sampling (CDS), thresholding, and identification of event "blobs" automatically on-chip, at a significantly higher readout rate than has been achieved with conventional direct detection sensors. Subsequently, super-resolution centroiding is performed in FPGA hardware in real-time, forming 8192×8192 dose-fractionated frames for motion correction and dose filtering. The performance of Apollo has been verified for both single particle cryoEM and for micro electron diffraction (microED).

SHORT TALKS

Dynamic conformations of the insulin receptor as activated by a venom-derived insulin.

Alan Blakely
Department of Biochemistry, University of Utah

Management of type-1 diabetes remains a difficult task due to the tight dosing margins required to achieve optimal blood glucose levels. One reason for this is the slow onset of action following subcutaneous injection. Insulin self-associates into dimers and hexamers, the latter of which is thought to diffuse slowly from the injection site. Efforts to engineer insulins with reduced self association have yielded some commonly used fast acting insulins. Still, there is room for improvement over the current 15-30 minute delay in action for the fastest insulin therapeutics.



The recent discoveries of insulin-like peptides in cone snail venoms that lack conserved dimerizing elements have provided the opportunity to re-examine the long-standing challenge of insulin-self association. Experiments show that one such insulin based on cone snail venom is much more monomeric than a previously designed, so-called “monomeric” insulin, suggesting the potential for even faster action.

The mechanism of binding of this venom-derived insulin was examined by cryo-EM, revealing how this class of venoms compensates for the loss of receptor binding residues conserved among vertebrate insulins, as well as conformational dynamics of the receptor revealed by 3D variability analysis which suggest an activation mechanism based on multistep insulin binding.

References: 1. Xiong and Blakely et al. Nat. Chem. Bio. 2022

Amyloid fibrils in frontotemporal lobar degeneration with TDP-43 inclusions (FTLD-TDP) are composed of TMEM106B, rather than TDP-43

POSTER

Yi Xiao Jiang[1,2†], Qin Cao[1,2,3†], Michael R. Sawaya[1,2], Romany Abskharon[1,2], Peng Ge[1,2], Michael DeTure[2], Dennis W. Dickson[2], Janine Y. Fu[1], Rachel R. Ogorzalek Loo[1], Joseph A. Loo[1], David S. Eisenberg[1,2].

1. Department of Biological Chemistry, UCLA-DOE Institute, Molecular Biology Institute, UCLA, Los Angeles, CA, USA.; 2.Howard Hughes Medical Institute, UCLA, Los Angeles, CA, USA.; 3. Bio-X Institutes, Key Laboratory for the Genetics of Developmental and Neuropsychiatric Disorders, Ministry of Education, Shanghai Jiao Tong University, Shanghai, 200030, China; 4. Mayo Clinic, Jacksonville, FL, USA; † These authors contributed equally to this work.

Frontotemporal lobar degeneration (FTLD) is the third most common neurodegenerative condition after Alzheimer’s and Parkinson’s diseases[1]. FTLD typically presents in 45 to 64 year olds with behavioral changes or progressive decline of language skills[2]. The subtype FTLD-TDP is characterized by pathological neuronal inclusions with TAR DNA-binding protein (TDP-43) immunoreactivity[3]. Here we extracted amyloid fibrils from brains of four patients, representing four out of the five FTLD-TDP subclasses, and determined their structures by cryo-electron microscopy. Unexpectedly, all amyloid fibrils examined were composed of a 135-residue carboxy-terminal fragment of transmembrane protein 106B (TMEM106B), a lysosomal membrane protein previously implicated as a genetic risk factor for FTLD-TDP[4]. Three fibril polymorphs were identified from each patient, comprised of protofilaments that share a stable, conserved, golf-course-like fold. In addition to TMEM106B fibrils, we detected abundant non-fibrillar aggregated TDP-43 by immunogold labeling. Our observations confirm that FTLD-TDP is associated with amyloid fibrils, and that the fibrils are formed by TMEM106B rather than TDP-43.

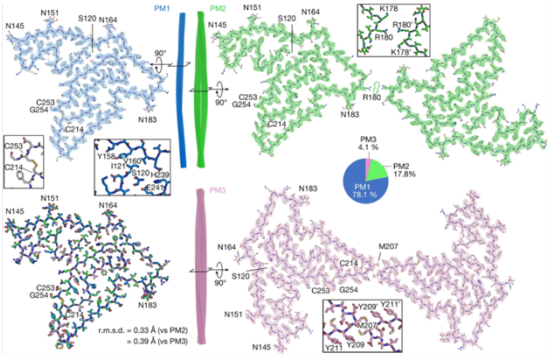


Figure 1. Cryo-EM structures of TMEM106B fibrils from FTLD-TDP donor 1

References: 1. Mohandas, E. & Rajmohan, V. Frontotemporal dementia: an updated overview. Indian J. Psychiatry 51, S65– S69 (2009); 2. Neary, D. et al. Frontotemporal lobar degeneration: a consensus on clinical diagnostic criteria. Neurology 51, 1546–1554 (1998); 3. Neumann, M. et al. Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. Science 314, 130–133 (2006); 4. Van Deerlin, V. M. et al. Common variants at 7p21 are associated with frontotemporal lobar degeneration with TDP-43 inclusions. Nat. Genet. 42, 234–239 (2010).

Visualizing small molecule inhibition of the Sec61 translocon by cryo-EM

Sam Itskanov[1], Laurie Wang[2], Tina Junne[3], Rumi Sherriff[2], Li Xiao[4], Nicolas Blanchard[5], Wei Q. Shi[6], Craig Forsyth[4], Dominic Hoepfner[7], Martin Spiess[3], and Eunyong Park[2,8]

[1]Biophysics Graduate Program, University of California, Berkeley, Berkeley, CA 94720, USA ; [2]Department of Molecular and Cell Biology, University of California, Berkeley, CA 94720, USA ; [3]Biozentrum, University of Basel, CH-4056, Basel, Switzerland [4]Department of Chemistry and Biochemistry, The Ohio State University, Columbus, Ohio 43210, USA; [5]CNRS, LIMA, UMR 7042, Université de Haute-Alsace, Université de Strasbourg, Mulhouse, France; [6]Department of Chemistry, Ball State University, Muncie, IN 47306, USA; [7]Novartis Institutes for BioMedical Research, Novartis Pharma AG, Forum 1 Novartis Campus, CH-4056, Basel, Switzerland [8]California Institute for Quantitative Biosciences, University of California, Berkeley, CA 94720

The Sec61 complex is a protein conducting channel at the endoplasmic reticulum and is required for the proper production of both soluble and membrane proteins. Previous studies have identified several structurally unrelated natural product and synthetic inhibitors of the channel which selectively block its protein conducting activity, though the molecular mechanism underlying inhibition has remained unclear. In this study, we present structures of the Sec61 channel inhibited by a panel of small molecule inhibitors: cotransin, decatransin, apratoxin F, ipomoeassin F,mycolactone, cyclotriazadisulfonamide (CADA), and eeyarestatin I (ESI). We found that all inhibitors block Sec61's partially open lateral gate and plug domain through a common lipid-exposed pocket, stabilizing the plug domain in a closed state. In cellulo mutations of inhibitor-interacting polar residues in the human channel conferred resistance, and a mutagenesis screen in yeast show that resistance-conferring mutations clustered around this binding pocket. Taken together, this study reveals molecular interactions between Sec61 and its various inhibitors and provides a structural framework towards designing therapeutics targeting the translocon.

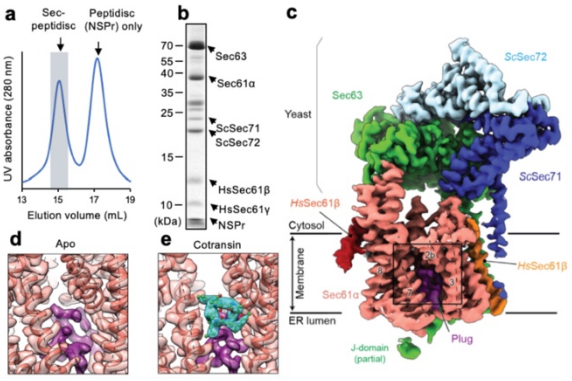


Figure 1. Purification and structural determination of the inhibited human Sec61 complex. a, Size exclusion chromatography elution profile of Sec61 complex **b,** Coomassie-stained SDS gel of the peak fraction **c,** 2.7Å resolution cryo-EM map of the apo form of the chimeric Sec complex with human channel **d-e,** views of the inhibitor-binding site of Sec61α for apo and cotransin structures

References: 1. Itskanov, S., Wang, L., Junne, T., Sherriff, R., Xiao, L., Blanchard, N., Shi, W. Q., Forsyth, C., Hoepfner, D., Spiess, M., Park, E. (2022) A common mechanism of Sec61 translocon inhibition by small molecules. bioRxiv. https://doi.org/10.1101/2022.08.11.503542; 2. Rapoport, T. A., Li, L., & Park, E. (2017). Structural and Mechanistic Insights into Protein Translocation. Annual review of cell and developmental biology, 33, 369–390. https://doi.org/10.1146/annurev-cellbio-100616-060439; 3. Luesch, H. & Paavilainen, V. O. (2020) Natural products as modulators of eukaryotic protein secretion. Nat Prod Rep 37, 717-736. https://doi.org/10.1039/C9NP00066F.

Organizing Structural Principles of the IL-17 Ligand–Receptor Axis

Steven C. Wilson[1,2,5], Nathanael A. Caveney[1,2,5], Michelle Yen[1,2], Christoph Pollmann[3], Xinyu Xiang[1,2], Kevin M. Jude[1,2,4], Maximillian Hafer[3], Naotaka Tsutsumi[1,2,4], Jacob Piehler[3] & K. Christopher Garcia[1,2,4,*]

[1]Department of Molecular and Cellular Physiology, Stanford University School of Medicine, Stanford, CA, USA. [2]Department of Structural Biology, Stanford University School of Medicine, Stanford, CA, USA. [3]Divison of Biophysics, Department of Biology, University of Osnabrück, Osnabrück, Germany. [4]Howard Hughes Medical Institute, Stanford University School of Medicine, Stanford, CA, USA. [5]Equal contribution. *Corresponding author.

The IL-17 family of cytokines and receptors have central roles in host defense against infection and development of inflammatory diseases[1]. The compositions and structures of functional IL-17 family ligand– receptor signaling assemblies remain unclear. IL-17E (also known as IL-25) is a key regulator of type 2 immune responses and driver of inflammatory diseases, such as allergic asthma, and requires both IL-17 receptor A (IL-17RA) and IL-17RB to elicit functional responses[2]. Here we studied IL-25–IL-17RB binary and IL-25–IL-17RB–IL-17RA ternary complexes using a combination of cryo-electron microscopy, singlemolecule imaging and cell-based signaling approaches. The IL-25–IL-17RB–IL-17RA ternary signaling assembly is a C2-symmetric complex in which the IL-25–IL-17RB homodimer is flanked by two 'winglike' IL-17RA co-receptors through a 'tip-to-tip' geometry that is the key receptor–receptor interaction required for initiation of signal transduction. IL-25 interacts solely with IL-17RB to allosterically promote the formation of the IL-17RB–IL-17RA tip-to-tip interface. The resulting large separation between the receptors at the membrane-proximal level may reflect proximity constraints imposed by the intracellular domains for signaling. Cryo-electron microscopy structures of IL-17A–IL-17RA and IL-17A–IL-17RA– IL-17RC complexes reveal that this tip-to-tip architecture is a key organizing principle of the IL-17 receptor family. Furthermore, these studies reveal dual actions for IL-17RA sharing among IL-17 cytokine complexes, by either directly engaging IL-17 cytokines or alternatively functioning as a co-receptor.

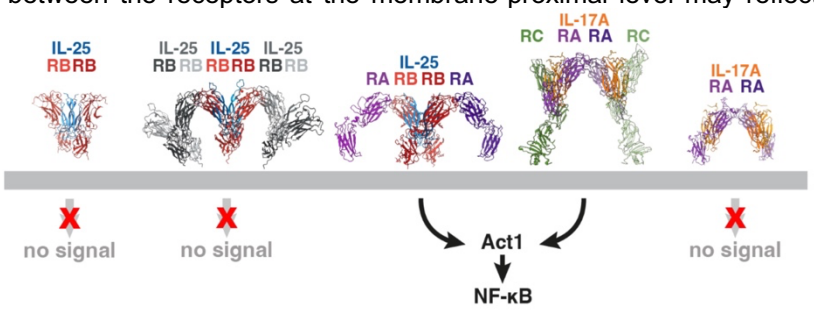


Figure 1: Cryo-EM structures reveal organizing principles of the IL-17–IL-17R cytokine–receptor signaling axis.

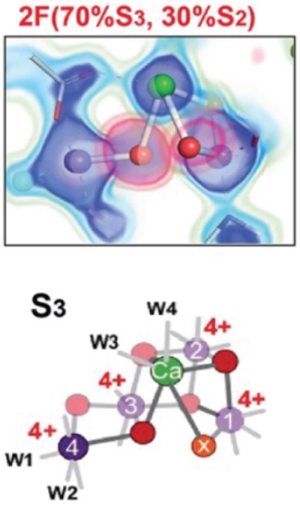
References: 1. McGeachy, M. J., Cua, D. J. & Gaffen, S. L. The IL-17 family of cytokines in health and disease. Immunity **50**, 892–906 (2019); 2. Rickel, E. A. et al. Identification of functional roles for both IL-17RB and IL-17RA in mediating IL-25-induced activities. J. Immunol. **181**, 4299–4310 (2008).

Investigating structural changes in Photosystem II using time-resolved X-ray free-electron laser (XFEL) crystallography

Asmit Bhowmick[1], Rana Hussein[2], Isabel Bogacz[1], Philipp S. Simon[1], Mohamed Ibrahim[2], Johannes Messinger[3,4], Athina Zouni[2], Jan Kern[1], Vittal K. Yachandra[1], Junko Yano[1]

1 Molecular Biophysics and Integrated Bioimaging Division, Lawrence Berkeley National Laboratory, Berkeley; 2 Humboldt-Universität zu Berlin, Department of Biology, 10099 Berlin, Germany; 3 Molecular Biomimetics, Department of Chemistry- Ångström, Molecular Biomimetics, Uppsala University, SE 75120 Uppsala, Sweden; 4 Department of Chemistry, Umeå University, SE 90187 Umeå, Sweden.

The water oxidation complex in Photosystem II is the catalyst for the transformation of two water molecules to molecular oxygen, releasing four electrons and four protons in the process. Starting from the ground state, the complex gets progressively oxidized and moves along the Kok cycle (S_0-S_4 intermediate states) upon absorption of a photon in each step. In the last state (S_4), the complex releases its stored oxidative power to catalyze the reaction and return to the ground state. Here we present our results on time-resolved structural studies done at room temperature on Photosystem II using X-ray free-electron laser (XFEL) crystallography of the various stable intermediates along the Kok cycle as well as timepoints in between these intermediates. The data shows the sequence of events leading to the insertion of a new substrate water bridging Ca and Mn1 atom in the $S_2 \rightarrow S_3$ transition. The water insertion is accompanied by distance changes within the cluster as well as side-chain coordination with the complex. In addition, we observed critical structural changes in the different water and proton channels that are coordinated with the substrate water insertion and tell us about their functional role in this transition. We also discuss preliminary results on the final transition that leads to formation of molecular oxygen (i.e. $S_3 \rightarrow [S_4] \rightarrow S_0$ transition).



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PERIOD phosphorylation leads to feedback inhibition of CK1 activity to control circadian period

Jonathan M. Philpott[1], Alfred M. Freeberg[1], Jiyoung Park[2], Kwangjun Lee[2], Clarisse G. Ricci[3], Sabrina R. Hunt[1], Rajesh Narasimamurthy[4], David H. Segal[1], Rafael Robles[1], Yao Cai[5], Sarvind Tripathi[1], J. Andrew McCammon[3,6], David M. Virshup[4,7], Joanna C. Chiu[5], Choogon Lee[2]*, Carrie L. Partch[1,8,9,]*

[1]Department of Chemistry and Biochemistry, University of California Santa Cruz, Santa Cruz, CA 95064; [2]Department of Biomedical Sciences, College of Medicine, Florida State University; [3]Department of Chemistry and Biochemistry, University of California San Diego; [4]Program in Cancer and Stem Cell Biology, Duke-NUS Medical School, Singapore ; [5]Department of Entomology and Nematology, University of California Davis; [6]Department of Pharmacology, University of California San Diego; [7]Department of Pediatrics, Duke University Medical Center ; [8]Center for Circadian Biology, University of California San Diego; ‡Current address: D.E. Shaw Research, New York, NY 10036 [9]Lead contact; *Correspondence: cpartch@ucsc.edu, choogon.lee@med.fsu.edu

PERIOD (PER) and Casein Kinase 1δ regulate circadian rhythms through a phosphoswitch that controls PER stability and repressive activity in the molecular clock. CK1δ phosphorylation of the Familial Advanced Sleep Phase (FASP) serine cluster embedded within the Casein Kinase 1 binding domain (CK1BD) of mammalian PER1/2 inhibits its activity on phosphodegrons to stabilize PER and extend circadian period. Here, we show that the phosphorylated FASP region (pFASP) of PER2 directly interacts with and inhibits CK1δ. Cocrystal structures in conjunction with accelerated molecular dynamics simulations reveal how pFASP phosphoserines dock into conserved anion binding sites near the active site of CK1δ. Limiting phosphorylation of the FASP serine cluster reduces product inhibition, decreasing PER2 stability and shortening circadian period in human cells. We found that *Drosophila* PER also regulates CK1δ via feedback inhibition through the phosphorylated PER-Short domain, revealing a conserved mechanism by which PER phosphorylation near the CK1BD regulates CK1 kinase activity.

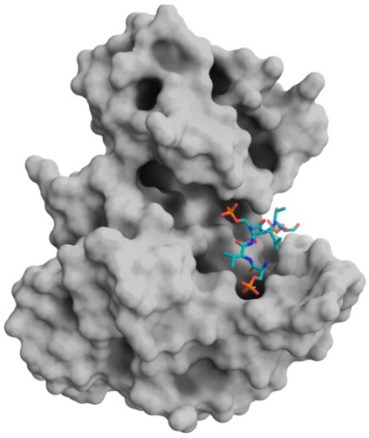

Figure 1. Phosphorylated FASP peptide bound to CK1δ catalytic domain

Metal-substituted P450cam investigates substrate interaction in the P450cam active site.

Oanh T. N. Tran[1], Jose Amaya[1], Sarvind Tripathi[1], Thomas L. Poulos[1]

[1] University of California, Irvine, Department of Chemistry

Cytochrome P450 (CYP) is a family of heme proteins that play a role in many biological processes. Such as facilitating the metabolism of xenobiotics in the body. Cytochrome P450cam, a model for CYP, helps understand how cytochrome P450 catalyzes the hydroxylation reaction(1). As part of the monooxygenase system, P450cam hydroxylates Camphor to 5-Exo-Hydroxycamphor, with the help of its redox partners, Putidaredoxin (Pdx) and Putidaredoxin Reductase (PdR) (2). This system uses the Catalytic cycle for oxidation activation and shuttles protons and electrons to make a highly active Compound I. Through mutagenesis,Tripathi *et al*. (2013) crosslinked and crystallized P450cam to Pdx to visualize the role of Camphor in initiating conformation changes that result in catalytic activity (3). Their crystallographic data showed only 5-Exo-Hydroxycamphor docked in the active site. Since the heme in P450cam facilitates the catalytic reaction, my project utilizes alternate metal protoporphyrins, such as Cobalt, to halt the catalytic cycle and visualize how Pdx and the substrate initiate structural changes that form a highly reactive Compound I. Thus by incorporating Cobalt, I plan to halt the catalytic activity of P450cam and visualize how Camphor interactions with P450cam can initiate conformational changes that lead to oxidation reaction.

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Structural biases in disordered proteins are prevalent in the cell

POSTER

David Moses, Karina Guadalupe, Shahar Sukenik

University of California Merced, Merced, CA, Chemistry and Biochemistry

Intrinsically disordered proteins and protein regions (IDPs) are essential to cellular function in all proteomes. Unlike folded proteins, IDPs exist in an ensemble of rapidly interchanging conformations. IDP sequences encode interactions that create structural biases within the ensemble. Such structural biases determine the three-dimensional shape of IDP ensembles and can affect their activity. However, the plasticity and sensitivity of IDP ensembles means that structural biases, often measured *in vitro*, may differ in the dynamic and heterogeneous intracellular environment. Here we reveal that structural biases found *in vitro* in well-studied IDPs persist inside human-derived cells. We further show that a subset of IDPs are able to sense changes in cellular physical-chemical composition and modulate their ensemble in response. We propose that IDP ensembles can evolve to sense and respond to intracellular physicochemical changes, or to resist them. This property can be leveraged for biological function, be the underlying cause of IDP-driven pathology, or be leveraged for the design of disorder-based biosensors and actuators.

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Structural Characterization of AAV-AAVR Interactions by cryo-EM

Onellah Weerakoon[1], Edward E. Large[1], Grant Zane[1], Mark Silveria[1], Guiqing Hu[2], Nancy L. Meyer[3], Tommi A. White[1], Scott S. Stagg[2] and Michael S. Chapman[1]

Department of Biochemistry, University of Missouri; Institute of Biomolecular Physics, Florida State University; Pacific Northwest Center for Cryo-EM

Adeno-associated virus (AAV) is a 4.7kb, ssDNA virus that has been used in recombinant form as a successful *in vivo* gene therapy vector. Two AAV gene therapy drugs have been approved recently by the FDA for treatment of Spinal Muscular Atrophy (SMA-1) and Hemophilia B. Many other trials are underway, some more challenging, for which we would be helped by a better understanding of AAV's cellular entry pathway and the molecular interactions that are still not well understood. Structural studies of the AAV capsid can further improve the success as a therapeutic gene-delivery vector, as AAV primarily interacts with receptors, attachment factors, and antibodies through its capsid. The AAV receptor (AAVR) was identified through genome-wide screening, initiated in the Carette and Chapman laboratories. It is now understood to play an essential role in AAV receptor-mediated endocytosis and trafficking of AAV to the peri-nuclear *trans* Golgi network (TGN) for all AAV serotypes except AAV4. AAVR is a transmembrane protein, containing five polycistronic kidney disease domains (PKD1-5). Most serotypes (eg. AAV2) bind strongest to PKD2, but PKD1 appears to be the exclusive target of AAV5 and AAVGo.1, a goat AAV. Thus, while AAV2 binds primarily PKD2, PKD1 affects its transduction. By contrast, AAV5 and AAVGo.1 interact exclusively with PKD1. Furthermore, high resolution Cryo-Electron Microscopy (cryoEM) shows that, in spite of homology between the AAVR domains, the binding sites of PKD1 and PKD2 on AAV are completely distinct, while cryo-EM at 2.4 Å resolution of the AAVR-independent serotype AAV4 shows why it binds neither. The AAVR constructs used to make complexes contain both PKD1 and PKD2, but high resolution cryo-EM reveals only single domains, PKD1 for AAV5 / AAVGo.1, and PKD2 for AAV2 where these domains are strongly bound to the AAV surface. The second domains are only revealed by cryo-Electron Tomography (cryo-ET) at lower resolution, because they are pointing predominantly away from the virus surface in heterogeneous conformations. Nevertheless, consistent with the transduction data, enzyme-linked immunosorbent assays (ELISA) indicate an association between PKD1 and AAV2. Cryo-EM is being used to study the nature of these weaker secondary interactions of PKD1 with AAV2-like viruses. A comprehensive understanding of virus-receptor interactions is not only a foundation for the rational design of gene therapy vectors with more specific and efficient cell entry. It is also needed to understand which regions of the virus might be off-limits when modifying vectors to avoid a neutralizing antibody response.

Cryo-EM structures of the D290V variant of the hnRNPA2 low complexity domain suggest the mechanism of its pathogenicity

Jiahui Lu[1], Peng Ge[1], Michael R. Sawaya[1], Michael P. Hughes[2], David R. Boyer[1], Qin Cao[3], Romany Abskharon[1], Einav Tayeb-Fligelman[1], Duilio Cascio[1], David S. Eisenberg[1*]

[1]Departments of Chemistry and Biochemistry and Biological Chemistry, University of California, LosAngeles, Los Angeles, CA, USA, UCLA-DOE Institute, Molecular Biology Institute, Howard Hughes Medical Institute; [2]Department of Cell and Molecular Biology, St. Jude Children's Research Hospital, Memphis, TN, USA; [3]School of Life Sciences and Biotechnology, Shanghai JiaoTong Univeristy, Shanghai, Shanghai, China *Correspondence to david@mbi.ucla.edu

Human ribonucleoprotein hnRNPA2 belongs to a class of proteins that bind and shuttle RNA. They contain low-complexity domains (LCDs) composed of a limited repertoire of amino acid types. These LCDs aggregate into amyloid-like fibrils facilitating protein function in a separate liquid phase within the cell nucleus. Tens of mutations that are associated with neurodegenerative diseases are located in the LCDs of ribonucleoproteins and hundreds of pathogenic amyloid structures are determined to near-atomic resolutions. But none can give us a clear structural mechanism of amyloid pathogenicity. Here we investigate the D290V variant associated with MultiSystem Proteinopathy (MSP) by determining cryo-EM structures of the recombinant D290V LCD and we find that it forms three amyloid fibril polymorphs. In contrast to the wildtype fibrils, the PY-nuclear localization signals are masked in the fibril cores of all three variant polymorphs, rendering it inaccessible to chaperones for RNA trafficking and thereby interfering with function. Also, the variant fibrils are more stable than wildtype, reversible fibrils as judged by phase separation, thermal stability, and energetic calculations. Thus, these structures offer compelling evidence to explain how a single missense mutation that changes a charged residue (Asp) to a hydrophobic residue (Val) diverts the assembly of reversible, functional amyloid-like fibril into the assembly of pathogenic amyloid, and may shed light on analogous conversions occurring in other neurological diseases such as amyotrophic lateral sclerosis and frontotemporal dementia.

Designing Protein Binders Using Next Generation Artificial Intelligence

Matthias Malago

(matthias@pythialabs.com), Pythia Labs

The ability to design proteins with specific functions has broad applications in biotechnology, sustainability, and materials design. Despite recent progress, protein design remains challenging, and traditional approaches have low success rates. Pythia Labs is developing a platform that leverages next- generation AI to improve success rates and make it easier and faster to design novel proteins. In this talk, we will provide a glimpse into our solution and discuss how we can collaborate to generate the molecules of tomorrow.

PYTHIA

Structural basis of mitochondrial protein import by the TIM23 complex

Sue Im Sim[1,2,†], Yuanyuan Chen[1,2,†], Diane L. Lynch[3], James C. Gumbart[3], Eunyong Park[1,2,*]

[1]Department of Molecular and Cell Biology, University of California, Berkeley, CA 94720, USA; [2]California Institute for Quantitative Biosciences, University of California, Berkeley, CA 94720, USA.; [3]School of Physics and School of Chemistry and Biochemistry, Georgia Institute of Technology, Atlanta, GA 30332, USA; [*]Corresponding author. E-mail: eunyong_park@berkeley.edu [†]These authors contributed equally to this work.

Mitochondria import nearly all their ~1,000–2,000 constituent proteins from the cytosol across their double-membrane envelope. Genetic and biochemical studies have shown that the conserved protein translocase, termed the TIM23 complex, mediates import of presequence-containing proteins (preproteins) into the mitochondrial matrix and inner membrane. Among about ten different subunits of the TIM23 complex, the essential multipass membrane protein Tim23, together with the evolutionarily related protein Tim17, has long been postulated to form a protein-conducting channel. However, the mechanism by which these subunits form a translocation path in the membrane and enable the import process remained unclear due to a lack of structural information. Here, we have determined the cryo-electron microscopy (cryo-EM) structure of the core TIM23 complex (heterotrimeric Tim17–Tim23–Tim44) from Saccharomyces cerevisiae. Contrary to the prevailing model, Tim23 and Tim17 themselves do not form a water-filled channel, but instead have separate, lipid-exposed concave cavities that face in opposite directions. Our structural and biochemical analyses show that surprisingly, the cavity of Tim17, not Tim23, forms the protein translocation path whereas Tim23 is likely to play a structural role. The results further suggest that, during translocation of substrate polypeptides, the nonessential subunit Mgr2 seals the lateral opening of the Tim17 cavity to facilitate the translocation process. We propose a new model for the TIM23-mediated protein import and sorting mechanism, a central pathway in mitochondrial biogenesis.

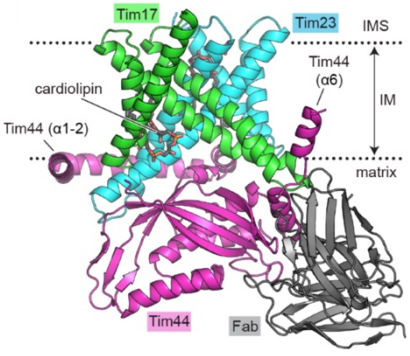

Figure 1. Atomic model of core TIM23 complex from *S. cerevisiae.*

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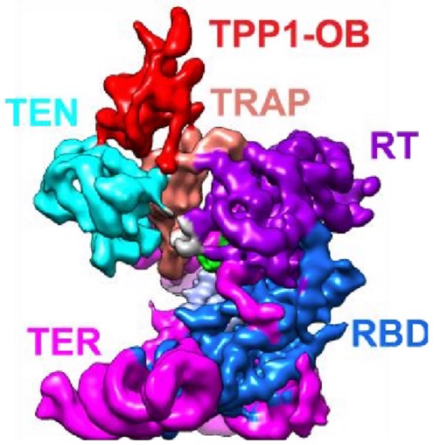
Structure of active human telomerase with telomere shelterin protein TPP1

Baocheng Liu1, Yao He[1,2], Yaqiang Wang[1], He Song[1], Z. Hong Zhou[2,3], Juli Feigon[1]

[1] Department of Chemistry and Biochemistry, University of California, Los Angeles, Los Angeles, CA, USA; [2] Department of Microbiology, Immunology, and Molecular Genetics, University of California, Los Angeles, Los Angeles, USA; [3] California NanoSystems Institute, University of California, Los Angeles, Los Angeles, CA, USA.

Human telomerase is an RNA-protein complex that extends the 3′end of linear chromosomes by synthesis of multiple copies of the telomeric repeat TTAGGG. Its activity is a determinant of cancer progression, stem cell renewal, and cellular aging. Telomerase is recruited to telomeres and activated for telomere repeat synthesis by the telomere shelterin protein TPP1. Human telomerase has a bilobal structure with a catalytic core RNP and an H/ACA RNP. Here we report cryo-EM structures of human telomerase catalytic core of telomerase reverse transcriptase (TERT) and telomerase RNA (TER or hTR) at 3.3Å and the first structure with the shelterin protein TPP1, at 3.5Å resolution. TPP1 forms a structured interface with the TERTunique TEN and TRAP domains and conformational dynamics of TEN–TRAP are damped upon TPP1 binding, defining the requirements for recruitment and activation. The structures further reveal that elements of TERT and TER involved in template and telomeric DNA handling, including TEN domain and TRAP–thumb helix channel, are largely structurally homologous to those in Tetrahymena telomerase, and provide unique insights into mechanism. The binding site of telomerase inhibitor BIBR1532 overlaps a critical interaction between TER pseudoknot and TERT thumb domain. Numerous mutations leading to telomeropathies are located at the TERT–TER and TEN–TRAP–TPP1 interfaces, highlighting the importance of TER–TERT and TPP1 interactions for telomerase activity, recruitment, and as drug targets.



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Structural modes of B-Myb DNA binding domain bound to nucleosomes

Tilini Wijeratne, Vitor Serrao, Seth Rubin

University of California, Santa Cruz

B-Myb is a key transcription factor critical for cell-cycle progression through mitotic gene activation. B-Myb DBD is highly conserved across the Myb family of transcription factors. It is known to bind weakly to linear DNA in a sequence specific manner with a 1-10 μM affinity[1]. However, B-Myb DNA binding domain (DBD) has three Myb repeats that are often found in chromatin or nucleosome binding proteins like TRF2[2]. Through fluorescence polarization (FP) assay we determined that B-Myb binds Widom 601 nucleosomes with high affinity. These nucleosomes have the canonical Myb binding site the DNA. Through the FP assay we found that the B-Myb DNA binding domain (DBD) binds Widom 601 nucleosomes with ~100 nM nanomolar affinity; however, it shows weak (~10 μM) affinity to linear Widom 601 DNA. Previous studies have identified two mutations on the DBD (K128A, K183E) that completely disrupt DNA binding[1]. I used the FP assay to verify that these two mutants do not bind nucleosomes. I concluded that the B-Myb DBD has specific affinity for the nucleosome structure. Therefore, we hypothesize that that B-Myb binds the bent DNA and possibly the histone octamer with high affinity through its DBD and that this interaction stabilizes the MuvB complex at promoters to activate mitotic genes. The cryo-EM model of the B-Myb DBD bound to nucleosome shows that it interacts primarily with the nucleosomal DNA carrying the canonical Myb binding site (Fig. 1). Mutating the Myb binding site at the DNA show weaker to binding nucleosomes suggesting that B-Myb DBD might be interacting with the histone octamer too. In-vitro binding data with the histone H2A-H2B dimer shows that B-Myb DBD can interact with the histones. To test whether B-Myb interaction with DNA is supported by the interaction with histones, we will tile the Myb binding site around the Widom 601 DNA thus changing the position of the sequence relative to the histone dimer would show a reduction in affinity compared to the WT nucleosomes. B-Myb binds MuvB to activate mitotic gene transcription which are repressed by MuvB at the chromatinized gene promoters[3]. Through electrophoretic mobility shift assays, we show that B-Myb-nucleosome interaction is tighter than MuvB-nucleosome interaction and B-Myb can compete MuvB interaction with nucleosomes. Future studies with chromatin immunoprecipitation will show how B-Myb DBD mutants that do not interact with nucleosome will affect the mitotic gene expression. These results will altogether inform how an oncogenic transcription factor recognizes nucleosomes and establish a mechanism of how B-Myb cooperates with MuvB to drive cell-cycle progression.

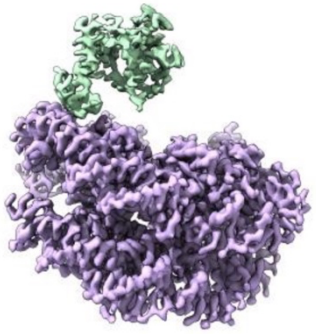

Figure 1. 2.7 Å cryo-EM map of B-Myb DNA binding domain (green) bound to Widom 601 nucleosome (purple).

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Characterization of bacteriophage P4 portal protein structure using cryo-EM and protein prediction software

Tamara Christiani[1], Fei Guo[2], Mariel Vazquez[1,3], Javier Arsuaga[3,5]

[1]Microbiology & Molecular Genetics; [2]Biological Electron Microscopy Facility; [3]Mathematics; [4]Molecular & Cellular Biology; University of California Davis

Portal proteins of double-stranded DNA bacteriophages sit at the vertex of an icosahedral capsid and allow bidirectional translocation of the viral genome. The maturation of this protein, which switches its function from DNA packing to tail assembly, is a critical step in the production pathway. Using cryo-electron microscopy and protein structure prediction programs, such as AlphaFold and HSYMDOCK, we present structures of the bacteriophage P4 portal protein during ejection of its viral genome. Our cryo-EM density shows the range of varying diameters in the channel of the portal protein which situated in the bacteriophage capsid. The predicted protein structure displays the charge distribution in the portal channel which suggest how the viral DNA may be interacting with the protein during ejection.

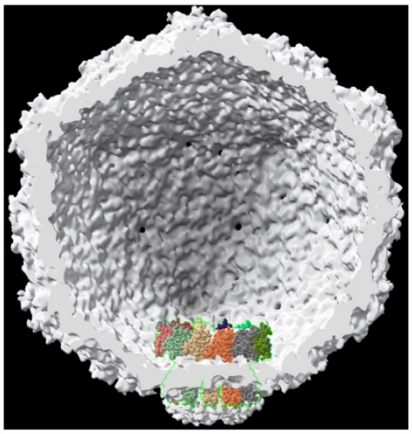


Figure 1. Portal protein. The predicted structure of the portal protein is overlayed onto a cut-out cryo-EM density of bacteriophage P4's capsid during DNA ejection.

POSTER

Structure of telomerase-bound CST with Polymerase α-Primase

Yao He[1,2], He Song[1], Henry Chan[1], Baocheng Liu[1], Yaqiang Wang[1], Lukas Susac[1], Z. Hong Zhou[2,3], Juli Feigon[1]

[1]Department of Chemistry and Biochemistry, University of California, Los Angeles, Los Angeles, CA, USA; [2]Department of Microbiology, Immunology, and Molecular Genetics, University of California, Los Angeles, Los Angeles, CA, USA; [3]California NanoSystems Institute, University of California, Los Angeles, Los Angeles, CA, USA.

Telomeres are the physical ends of linear chromosomes, composed of short repeating sequences (e.g. TTGGGG in Tetrahymena for the G-strand) of double-stranded DNA with a singlestrand 3'-overhang of the G-strand and a group of proteins called shelterin. Among these, TPP1 and POT1 associate with the 3'-overhang, with POT1 binding the G-strand and TPP1 recruiting telomerase via interaction with telomerase reverse transcriptase (TERT). The ends of the telomeric DNA are replicated and maintained by telomerase, for the G-strand, and subsequently DNA Polymerase α-Primase (PolαPrim), for the Cstrand. PolαPrim is stimulated by CTC1–STN1–TEN1 (CST), but the structural basis of both PolαPrim and CST recruitment to telomere ends remains unknown. Here we report cryo-EM structures of *Tetrahymena* CST in the context of telomerase holoenzyme, both in the absence and presence of PolαPrim, as well as of PolαPrim alone. Ctc1 binds telomerase subunit p50, a TPP1 ortholog, on a flexible Ctc1 binding motif unveiled jointly by cryo-EM and NMR spectroscopy. PolαPrim subunits are arranged in a catalytically competent conformation, in contrast to previously reported auto-inhibited conformation. Polymerase POLA1 binds Ctc1 and Stn1, and its interface with Ctc1 forms an entry port for G-strand DNA to the POLA1 active site. Together, we obtained a snapshot of four key players required for telomeric DNA synthesis in a single complex—telomerase core RNP, p50/TPP1, CST and PolαPrim—that provides unprecedented insights into CST and PolαPrim recruitment and handoff between G-strand and C-strand synthesis.

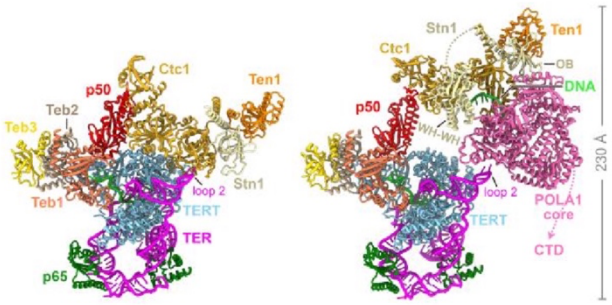


Figure 1. cryo-EM structure of *Tetrahymena* telomerase (left) and telomerase-PolαPrim (right).

References: **He, Y.***, Song, H.*, Chen, H., Liu, B., Wang, Y., Sušac, L., Zhou, Z. H., & Feigon, J. (2022) Structure of Tetrahymena telomerase-bound CST with Polymerase α-Primase. ***Nature***, 608(7924), 813-818.

Structural consequences of turnover-induced homocitrate loss in nitrogenase

POSTER

Rebeccah A. Warmack[1], Ailiena O. Maggiolo[1], Andres Orta[2], Belinda B. Wenke[1], James B. Howard[3], and Douglas C. Rees[1,4]

[1]Division of Chemistry and Chemical Engineering 147-75 California Institute of Technology Pasadena, California 91125, United States; [2]Biochemistry and Molecular Biophysics Graduate Program California Institute of Technology Pasadena, California 91125, United States; [3]Department of Biochemistry, University of Minnesota, Minneapolis, Minnesota 55455, United States [4]Howard Hughes Medical Institute, California Institute of Technology, United States

Nitrogenase catalyzes the ATP-dependent reduction of dinitrogen to ammonia during the process of biological nitrogen fixation that is essential for sustaining life. The active site FeMo-cofactor contains a [7Fe:1Mo:9S:1C] metallocluster coordinated with an *R*-homocitrate (HCA) molecule. We have established through single particle cryoEM and chemical analysis of two forms of the *Azotobacter vinelandii* MoFe-protein – a high pH turnover inactivated species and a ΔNifV variant that cannot synthesize HCA – that loss of HCA is coupled to α-subunit domain and FeMo-cofactor disordering, and formation of a histidine coordination site. We have further found a population of the ΔNifV variant complexed to an endogenous protein identified through structural and proteomic approaches as the uncharacterized protein NafT. Recognition by endogenous NafT demonstrates the physiological relevance of the HCA-compromised form, perhaps for cofactor insertion or repair. Our results point towards a dynamic active site in which HCA plays a role in enabling nitrogenase catalysis by facilitating activation of the FeMo-cofactor from a relatively stable form to a state capable of reducing dinitrogen under ambient conditions.

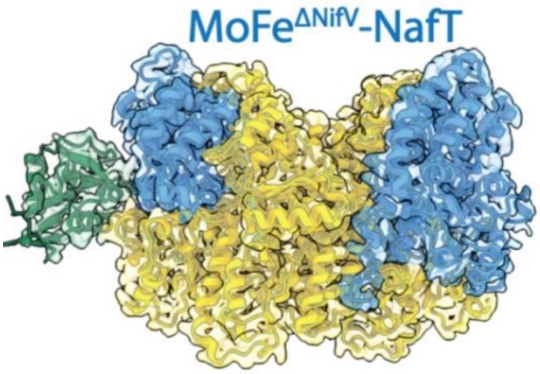


Figure 1. Structure of the nitrogenase MoFe- protein in complex with the previously uncharacterized NafT protein.

A Designed Imaging Scaffold Breaks the Barrier to High-Resolution Structure Determination of Small Proteins by Cryo-EM

POSTER

Roger Castells-Graells[1], Kyle Meador[2], Mark A. Arbing[1], Michael R. Sawaya[1], Morgan Gee[2], Duilio Cascio[1], Emma Gleave[3], Judit É. Debreczeni[3], Jason Breed[3], Karoline Leopold[4], Ankoor Patel[4], Dushyant Jahagirdar[4], Bronwyn Lyons[4], Sriram Subramaniam[4], Chris Phillips[3], Todd O. Yeates[1,2]

[1] UCLA-DOE Institute for Genomics and Proteomics; Los Angeles, US; [2] UCLA Department of Chemistry and Biochemistry; Los Angeles, US; [3] Discovery Sciences, R&D, AstraZeneca; Cambridge, UK [4] Gandeeva Therapeutics, Inc., Burnaby; Canada

Recent technical advances have made cryo-electron microscopy (cryo-EM) an attractive method for atomic structure determination, but problems of low signal-to-noise prevent routine structure determination of proteins smaller than about 50 kDa. We have developed symmetric protein imaging scaffolds to display and solve the structure of small proteins. In earlier work (Liu Y, et al., 2019), we demonstrated the design of a novel protein cage scaffold with sufficient rigidity and modularity to reach an imaging resolution of 3.8 Å for a 26 kDa protein. In the present work, we use molecular engineering techniques to further rigidify a new cryo-EM imaging scaffold, enabling 3 Å or better resolution imaging to be achieved, even for very small proteins. We apply this system to the key cancer signaling protein KRAS (19 kDa in size), obtaining four structures of oncogenic mutational variants by cryo-EM. Importantly, a structure for the key G12C mutant bound to an inhibitor drug (AMG510) reveals significant conformational differences compared to prior data in the crystalline state. The findings highlight the promise of cryo-EM scaffolds for advancing the design of drug molecules against small therapeutic protein targets in cancer and other human diseases.

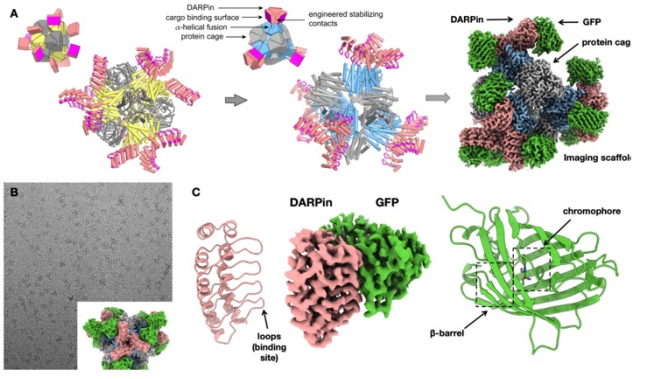


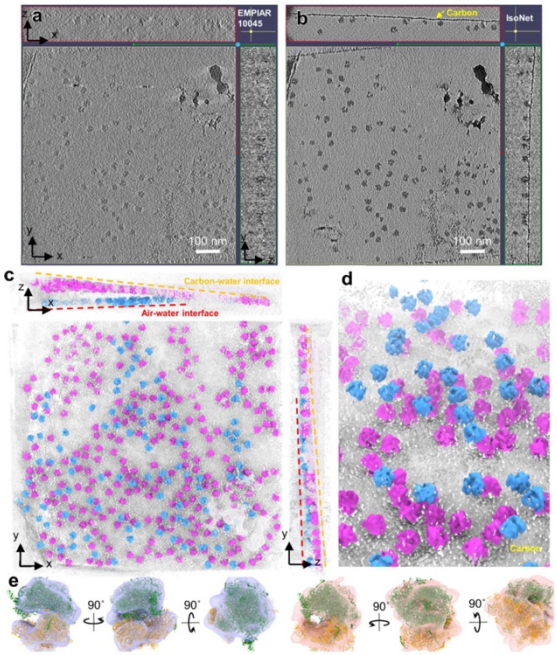
Figure 1. Rigidified modular cryo-EM imaging scaffolds. A. (left) A scheme for a previously described scaffold (Liu, et al. 2018; Liu et al. 2019), based on a self-assembling protein cage, displayed protruding DARPin domains as modular binders via continuous alpha helical fusions. B. Cryo-EM micrograph of the rigidified imaging scaffold bound to GFP (model shown in inset) C. (middle) A view of the final density map covering the DARPin and its bound GFP protein. Ribbon models of the two components are shown on the sides.

References: 1. Y. Liu, D. T. Huynh, T. O. Yeates, A 3.8 Å resolution cryo-EM structure of a small protein bound to an imaging scaffold. Nature Communications. 10, 1864 (2019); 2.T. O. Yeates, M. P. Agdanowski, Y. Liu, Development of imaging scaffolds for cryoelectron microscopy. Current Opinion in Structural Biology. 60, 142–149 (2020).; 3. Castells-Graells, R., Meador, K., Arbing, M.A., Sawaya, M.R., Gee, M., Cascio, D., Gleave, E., Debreczeni, J.É., Breed, J., Phillips, C., Yeates, T.O. Rigidified Scaffolds for 3 Angstrom Resolution Cryo-EM of Small Therapeutic Protein Targets. BioRxiv. (2022).

Isotropic reconstruction for electron tomography with deep learning

Yun-Tao Liu, Heng Zhang, Hui Wang, Chang-Lu Tao, Guo-Qiang Bi & Z. Hong Zhou
California NanoSystems Institute, University of California, Los Angeles (UCLA), Division of Life Sciences and Medicine, University of Science and Technology of China

Cryogenic electron tomography (cryoET) allows visualization of cellular structures in situ. However, anisotropic resolution arising from the intrinsic "missing-wedge" problem has presented major challenges in visualization and interpretation of tomograms. Here, we have developed IsoNet, a deep learning-based software package that iteratively reconstructs the missing-wedge information and increases signal-to-noise ratio, using the knowledge learned from raw tomograms. Without the need for sub-tomogram averaging, IsoNet generates tomograms with significantly reduced resolution anisotropy. Applications of IsoNet to three representative types of cryoET data demonstrate greatly improved structural interpretability: resolving lattice defects in immature HIV particles, establishing architecture of the paraflagellar rod in Eukaryotic flagella, and identifying heptagoncontaining clathrin cages inside a neuronal synapse of cultured cells. Therefore, by overcoming two fundamental limitations of cryoET, IsoNet enables functional interpretation of cellular tomograms without sub-tomogram averaging. Its application to high-resolution cellular tomograms should also help identify differently oriented complexes of the same kind for sub-tomogram averaging.



References: Liu, YT., Zhang, H., Wang, H. et al. Isotropic reconstruction for electron tomography with deep learning. Nat Commun 13, 6482 (2022). https://doi.org/10.1038/s41467-022-33957-8

Time-resolved Cryo-Electron Tomography of Surface Layer Lattice and Membrane Dynamics of Gram-Negative Bacteria upon pH Jump

Jacob Summers [1,2], Joey Yoniles [1,2], Kara Zielinski [3], Max Di Perna [2] Lois Pollack [3], Chris Kupitz [4], Mark Hunter [4], Wah Chiu [1,2,4], Soichi Wakatsuki [1,2,4], Peter Dahlberg [4]
[1] Stanford University School of Medicine, [2] Stanford University, [3] Cornell University, [4] SLAC National Accelerator Laboratory

Cryogenic electron tomography (cryo-ET) is a powerful method that provides pristine snapshots of cells or cellular fragments preserved by rapid freezing in their native hydrated state[1]. The most common method of rapid fixation is plunge freezing that can preserve specimen integrity; however, it lacks temporal context. It is difficult with this approach to capture rapid transient phenomena and dynamics. Here, we report our recent development of a time-resolved cryo-freezing apparatus for microbiology that uses microfluidic mixing devices to both stimulate and aerosolize the sample[2] with sub-second precision. As a proof-of-concept experiment, we performed time-resolved cryo-ET experiments to observe the response of *Caulobacter crescentus* (a Gram-negative bacterium) to a rapid pH jump. Previous research has shown that low pH degrades the surface layer protein (SLP) lattice[3]. Through this approach, we observe and characterize structural dynamics of the SLP lattice, outer membrane deformation, and changes in the ribosomal distribution as a function of time (Figure 1). In the future, this work will be extended for a wider range of microbiology and cell biology applications with a robust sample delivery, smooth transition of reaction time ranges, and coupling with cryogenic correlative light and electron microscopy[4].

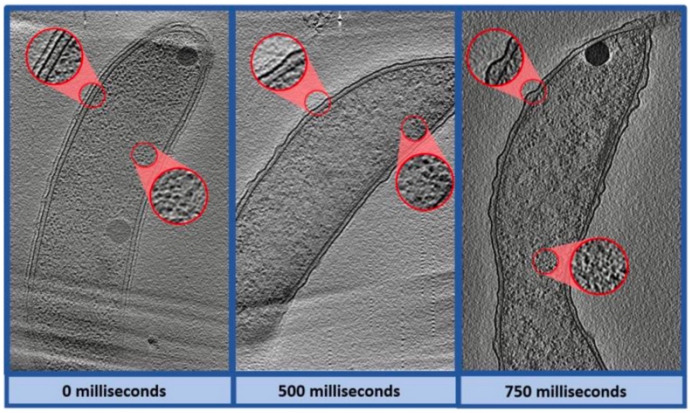


Figure 1: Time-resolved cryo-ET experiments involving reacting the Gram-negative bacteria *Caulobacter crescentus* with a low pH solution prior to vitrification allow us to observe and quantify morphological changes in SLP crystalline lattice shedding, outer membrane curvature, and ribosome variations over sub-second time scales.

References: 1. Wu, Gong-Her et al., Multi-scale 3D Cryo-Correlative Microscopy for Vitrified Cells. *Structure* **28(11)**, 1231-1237 (2020); 2. Calvey et al., Mixing injector enables time-resolved crystallography with high hit rate at X-ray free electron lasers. *Struct. Dyn.* **3(5)**:054301 (2016); 3. Hermann et al., A bacterial surface layer protein exploits multistep crystallization for rapid self-assembly. *PNAS* **177(1)**, 388-394 (2019); 4. P. Dahlberg and W. E. Moerner, Cryogenic Super-Resolution Fluorescence and Electron Microscopy Correlated at the Nanoscale. *Annu. Rev. Phys. Chem* **72:1**, 253-278 (2021).

Structural characterization of human LECT2 amyloids, drivers of kidney amyloidosis

Logan S. Richards[1], Maria D. Flores[1], Samantha Zink[1], Natalie A. Schibrowsky[1], Michael R. Sawaya[1] and Jose A. Rodriguez[1*]

[1] Department of Chemistry and Biochemistry; UCLA-DOE Institute for Genomics and Proteomics; STROBE, NSF Science and Technology Center; University of California, Los Angeles (UCLA); Los Angeles, CA 90095, USA.

ALECT2 is a type of systemic amyloidosis caused by deposition of the Leukocyte cell-derived chemotaxin-2 (LECT2) protein in the form of fibrils[1]. Accumulation of LECT2 fibrils in ALECT2 results in renal failure, and affected patients have no effective treatment options outside of renal transplant or dialysis[2]. While the structure of LECT2 in its globular form has been determined by X-ray crystallography [3], the structures of LECT2 amyloids remain unknown. Structures of LECT2 peptide segments, including one determined by fragment-based phasing methods from MicroED data (GSTVYAPFT), demonstrate the propensity of portions of the protein sequence to adopt amyloid-like conformations. A single particle cryo-EM reconstruction of recombinant human LECT2 amyloids reveals twisting fibrils with canonical amyloid features. At their core, LECT2 fibrils contain two mating protofilaments, each formed by a polypeptide spanning residues 55-75 of the LECT2 sequence. Each fibril is a multi-layer structure whose completely dry core is stabilized by a network of hydrophobic contact and a network of hydrogen-bonded uncharged polar residues, while its outer surface displays several charged residues. The LECT2 fibril is calculated to be an overall stable structure, in line with other pathogenic amyloids. This is supported by the limited dissolution of fibrils in 3M urea and the persistence of LECT2 fibril cores after mild treatment with proteinase K. Together, our structural data from LECT2 amyloids, including its recombinant fibril structure unveil characteristics of a persistent amyloid state and presents a potential target for treatments against ALECT2.

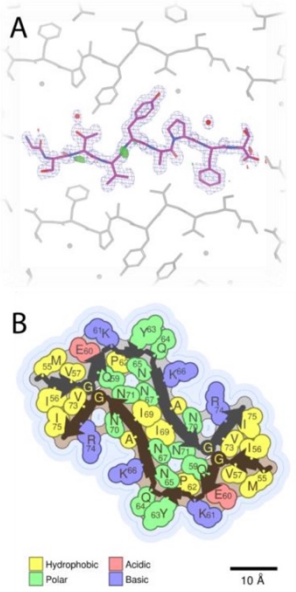


Figure 1. LECT2 amyloid structures overview. A) LECT2 segment GSTVYAPFT determined by MicroED. B) Cartoon map of the LECT2 fibril core determined by cryo-EM showing the distribution of polar and nonpolar residues.

References: 1. Benson, M.D., James, S., Scott, K., Liepnieks, J.J., and Kluve-Beckerman, B. (2008). Leukocyte chemotactic factor 2: A novel renal amyloid protein. Kidney International *74*, 218–222. 10.1038/ki.2008.152; 2. Mann, B.K., Bhandohal, J.S., Cobos, E., Chitturi, C., and Eppanapally, S. (2022). LECT-2 amyloidosis: what do we know? Journal of Investigative Medicine *70*, 348–353. 10.1136/jim-2021-002149; 3. Zheng, H., Miyakawa, T., Sawano, Y., Asano, A., Okumura, A., Yamagoe, S., and Tanokura, M. (2016). Crystal Structure of Human Leukocyte Cell-derived Chemotaxin 2 (LECT2) Reveals a Mechanistic Basis of Functional Evolution in a Mammalian Protein with an M23 Metalloendopeptidase Fold*. Journal of Biological Chemistry *291*, 17133–17142. 10.1074/jbc.M116.720375.

Using predicted protein models in Phenix

Dorothee Liebschner[a], Thomas C. Terwilliger[b,c], Paul D. Adams[a,d]

[a]Molecular Biophysics and Integrated Bioimaging Division, Lawrence Berkeley National Laboratory, Berkeley, 68 CA 94720, USA, [b]New Mexico Consortium, Los Alamos, NM 87544, USA, [c]Los Alamos National Laboratory, Los Alamos, NM 87545, 67 USA, [d]Department of Bioengineering, University of California, Berkeley, Berkeley, CA 94720, USA.

Recently developed artificial intelligence/machine learning methods – including AlphaFold (AF2)[1], OpenFold[2], and RosettaFold[3] – can predict protein 3D structures with impressive accuracy, sufficient to generate biological hypotheses. Although these advanced prediction methods are still unable to predict accurate structures of nucleic acids, conformational changes, or ligand-bound forms, they can kickstart experimental methods that provide the definitive atomic details.

Phenix[4] is a software package for macromolecular structure determination using crystallographic and electron cryo-microscopy data. We have developed new tools that can use predicted models from AF2 and other prediction software in the Phenix structure determination workflow. Using these models can be very helpful in structure determination because the models can be very accurate over much of their length and the models come with accuracy estimates that allow removal of poorly-predicted regions.

The *process_predicted_model* tool can prune unreliable parts of predicted models and split them into domains by clustering regions with low mutual predicted aligned error. This pruned model can then be used as a search model for molecular replacement or for docking in a cryo-EM reconstruction.

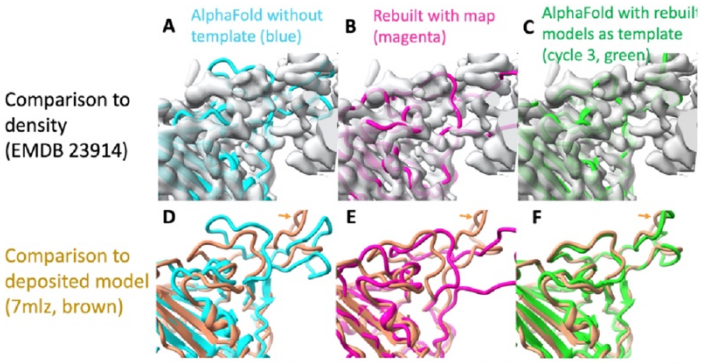


Figure 1: Iterative improvement of AlphaFold modeling for SARS-CoV-2 spike protein complex (cryo-EM, 3.7Å).

We also developed a new iterative model-improvement procedure that docks an AF2 model and adjusts it to better match a map before feeding the result back to AF2 as a template (Fig. 1). We have shown that this approach can greatly improve structure prediction[5,6]. The Phenix tool *predict_and_build* generates predicted models and uses them to solve an X-ray structure by MR or to interpret a cryo-EM map. The tool then carries out iterative model rebuilding and prediction to improve the models. The iterative procedure allows creating more accurate predicted models than can be obtained with a simple prediction. A fairly accurate model can be automatically generated starting from just a sequence file and either cryo-EM half-maps or X-ray data. Additionally, the tool provides morphed versions of unrefined predicted models that can be useful as reference models for refinement.

References: 1. Jumper, J. et al. Nature 596, 583–589 (2021); 2. Ahdritz, G. et al. BioRxiv (2022) doi:10.1101/2022.11.20.517210; 3. Baek, M. et al. Science 373, 871–876 (2021); 4. Liebschner, D. et al. Acta Crystallogr. D Struct. Biol. 75, 861–877 (2019); 5. Terwilliger, T. C. et al. BioRxiv (2022) doi:10.1101/2022.11.18.517112; 6. Terwilliger, T. C. et al. Nat. Methods 19, 1376–1382 (2022).

Improving resolution and resolvability of single particle CryoEM using Gaussian representation

Muyuan Chen, Wah Chiu

SLAC National Accelerator Laboratory

Single particle cryogenic electron microscopy (CryoEM) routinely determines protein structures at near atomic resolution, and has become one of the most popular tools in structural biology. To produce high resolution structures, the core computational process of the technique is to determine the projection orientation of the particles. While the process has been developed over decades, its performance can still be suboptimal particularly when the macromolecule contains flexible parts. Here, we present a novel method that uses Gaussian mixture models (GMMs), instead of voxel-based density maps, as references for the alignment. The usage of the GMM based representation eliminates artifacts caused by interpolation and masking, and also provides a robust way to focus the alignment on any local region of the protein. Furthermore, information about the continuous conformational change of the target protein can also be used to improve orientation assignment of particles, leading to better resolved features in the flexible domains. The method is tested on multiple public datasets,and shows improved “gold-standard” resolution and local feature resolvability in every scenario.

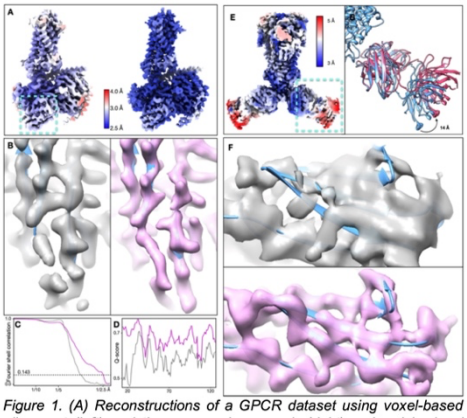


Figure 1. (A) Reconstructions of a GPCR dataset using voxel-based alignment (left) and the proposed approach (right), colored by local resolution. (B-D) Comparison of real space features (B), “gold-standard” Fourier shell correlation (C) and Q-score (D) from reconstructions in A. Gray – original; pink – GMM based refinement. (E) Left: reconstruction of an ABC transporter, with the cyan box highlighting the highly flexible Fab attached to the complex. Right: movement trajectory of the Fab, obtained using deep learning based heterogeneity analysis. (F) Comparison of protein features on the Fab in the original map (gray) and the refinement results after converting particle conformation to orientation differences.

A global Ramachandran score identifies protein structures with unlikely stereochemistry

POSTER

O. V. Sobolev[1], P. V. Afonine[1], N. W. Moriarty[1], M. L. Hekkelman[2,3], R. P. Joosten[2,3], A. Perrakis[2,3], P. D. Adams[1,4]

[1]Molecular Biosciences and Integrated Bioimaging Division, Lawrence Berkeley National Laboratory, USA; [2]Division of Biochemistry, The Netherlands Cancer Institute, the Netherlands; [3]Oncode Institute, Amsterdam, the Netherlands; [4]Department of Bioengineering, University of California, Berkeley, USA.

Ramachandran plots report the distribution of the (φ, ψ) torsion angles of the protein backbone and are one of the best quality metrics of experimental structure models. Typically, validation software reports the number of residues belonging to “outlier”, “allowed” and “favored” regions. While “zero outliers” is considered the “gold standard”, it can be misleading if deviations from expected distributions, even within the favored region, are not considered.

We therefore turned our attention to the Ramachandran Z-score (Rama-Z), a quality metric introduced three decades ago, but underutilized [1].

We re-implemented Rama-Z in CCTBX using modern high quality models from top8000 database used to derive Ramachandran plot contours in MolProbity. We advocate that Rama-Z is well suited to assess backbone geometry and highlight unusual distributions of (φ, ψ) angles on Ramachandran plot that otherwise can be unnoticed (Fig 1). The metric does not depend on experimental data (X-ray or Cryo-EM) used to derive atomic model.

The method is implemented and available in open-source CCTBX (mmtbx.rama_z) library as well as in Phenix as command line tool phenix.rama_z and also in various validation reports generated by Phenix. The tortoize implementation is available in PDB-REDO and will become available in the CCP4 and CCPEM suites in the near future.

We argue for a greater acceptance of this metric by the community. PDB-REDO has been reporting Rama-Z score since its inception. More discussion of this metric is published recently [2].

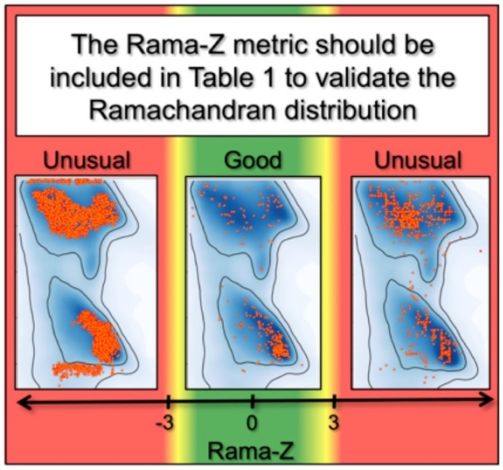


Figure 1. Rama-Z score examples and interpretation

This work was supported by the NIH (Project P01 GM063210), the Phenix Industrial Consortium and by the Netherlands Organization for Scientific Research (NWO; Vidi grant 723.013.003); References: 1. Hooft, R. W. W., Sander, C. & Vriend, G. (1997). *Bioinformatics*. **13**, 425–430; 2. Sobolev, O. V., Afonine, P. V., Moriarty, N. W., Hekkelman, M. L., Joosten, R. P., Perrakis, A., Adams, P. D. (2020). *Structure*. **28**. 1249-1258.

Cytokine Masquerading as a Hormone?
Structure of the Leptin Receptor Complex Guides Ligand Engineering

Nathanael Caveney[1,*], Robert Saxton[1,*], Maria Moya-Garzon[2], Karsten Householder[1], Grayson Rodriguez[1], Kylie Burdsall[1], Jonathon Long[2], and K Chris Garcia[1,3]

[1]Stanford Medicine, Department of Molecular and Cellular Physiology; [2]Stanford Medicine, Department of Pathology [3]Howard Hughes Medical Institute

Leptin is an adipocyte-derived protein hormone largely known for promoting satiety and energy homeostasis by activating the leptin receptor (LepR)–STAT3 signaling axis in the hypothalamus[1]. Leptin shows remarkable similarity to IL-6 family cytokines, and in turn has cytokine-like roles in wound healing. Both hormone- and cytokine-like leptin signaling are dysregulated in obesity, where the STAT3 effects of leptin are not potentiated, a state referred to as leptin resistance[2]. As a result, therapeutic use of leptin as an anti-obesity medication has been limited to rare cases of leptin deficiency[3]. To gain insight into the mechanisms of leptin signaling and resistance, we resolve the structure of the leptin–LepR signaling complex using cryoEM[4]. The structure reveals an asymmetric architecture in which a single leptin induces LepR dimerization via two distinct receptor-binding sites. Structure-based design of leptin mutants yield both partial and biased agonists that decouple stimulation of STAT3 from activation of negative regulators. Together, these results reveal the structural basis for LepR activation, provide insights into the differential plasticity of leptin signaling pathways, and form the basis for modulating leptin signaling in cases of leptin resistance.

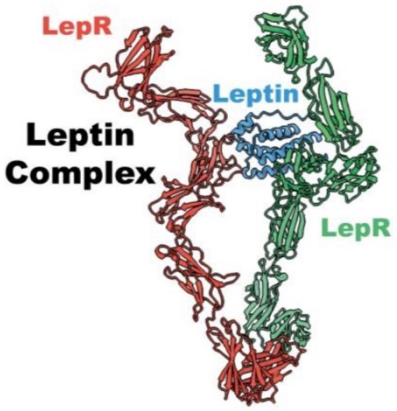


Figure 1. Structure of the signaling leptin receptor complex.

References: 1. Allison, M. B. & Myers, M. G., Jr. 20 years of leptin: connecting leptin signaling to biological function. *J Endocrinol* **223**, T25-35 (2014); 2. Frederich, R. C. *et al*. Leptin levels reflect body lipid content in mice: evidence for diet-induced resistance to leptin action. *Nat Med* **1**, 1311-1314 (1995); 3. Farooqi, I. S. *et al*. Effects of recombinant leptin therapy in a child with congenital leptin deficiency. *N Engl J Med* **341**, 879-884 (1999).; 4. Saxton, R.*, Caveney, N.*, *et al*. Structural insights into the mechanism of leptin receptor activation. *Nat Commun* in press.

Molecular Snapshots of the Mammalian Prion CPEB3

Maria D. Flores, Jose A. Rodriguez

Department of Chemistry and Biochemistry; UCLA-DOE Institute for Genomics and Proteomics; STROBE, NSF Science and Technology Center; University of California, Los Angeles (UCLA); Los Angeles, CA 90095, USA

The cytoplasmic polyadenylation element- binding protein 3 (CPEB3) is a functional prion, thought to modulate protein synthesis at synapses and enable consolidation of long-term memory in neurons. Here, we report that the prion-like domain 1 of CPEB3 self-assembles into labile amyloid fibrils *in vitro*. A cryoEM structure of these fibrils reveals an ordered 48-residue core, spanning L103 to F151. CPEB3 constructs lacking this amyloidogenic segment create abnormal puncta in cells when compared to wild type CPEB3, and displays reduced localization in dormant p-bodies and increased localization in stress granules. Removal of the amyloid core segment in CPEB3 also abolishes its ability to regulate protein synthesis in neurons. Finally, we interrogate the native structure of CPEB3 and uncover molecular snapshots of cytoplasmic inclusions in dormant neurons using cryo-FIB milling and cryo-ET. These data reveal CPEB3-GFP signal colocalized to lamella that contain multivesicular bodies (MVBs) that envelop dense, rope-like structures. Collectively, our data show that the newly identified amyloidogenic segment within the CPEB3 prion domain is important for its regulated aggregation in cells and suggest its involvement in regulating translational activity. Tomographic reconstructions begin to offer clues to how cells have evolved to safely store and transport CPEB3 prion-like assemblies and proteinaceous compartments during non-stimulated states.

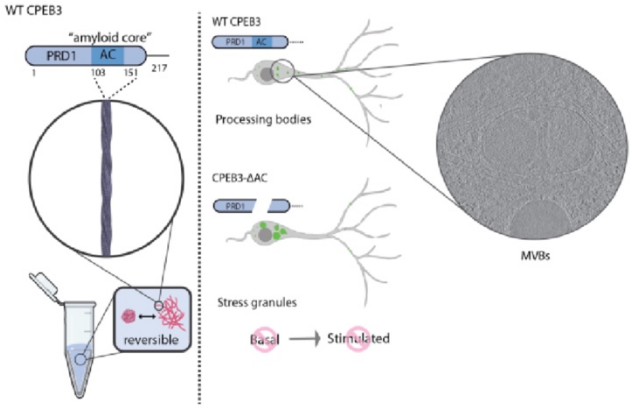


Figure 1. Model of CPEB3 aggregation, regulation and ultrastructural conformation.

Structural Basis for Evasion of IgA Immunity by *Streptococcus pyogenes* Revealed in the Complex of Arp4 with Human IgA

Qianqiao Liu[1] and Beth Stadtmueller[1,2]

[1]Department of Biochemistry, University of Illinois Urbana-Champaign, Champaign, IL, 61820, US; [2]Department of Biomedical and Translational Sciences, Carle Illinois College of Medicine, University of Illinois Urbana-Champaign, Champaign, IL, 61820, US.

Secretory (S) Immunoglobulin A (IgA) is the predominant mucosal antibody in mammals, which binds both pathogens and commensal microbes. Some bacteria have evolved “decoy proteins” to evade the pathogen clearance mechanism initiated by serum and mucosal forms of IgA. One of these proteins is Arp4, a surface protein found in *Streptococcus pyogenes* that has a 29-residue IgA binding region [1]. Published studies have shown that Arp4 can bind to the same region on IgA as the human IgA Fc receptor (CD89) thereby inhibiting binding of IgA to CD89 and downstream effector functions, such as respiratory burst [2]. We have determined the Cryo-EM structure of the complex of Arp4 dimer with human secretory IgA at 3.1 Å resolution. Movies were collected on a Titan Krios equipped with a Falcon4 camera at the Stanford-SLAC Cryo-EM Center (S^2C^2). To address preferred particle orientation in the data set, additional data were collected with the stage tilted at 30 degrees. Both data sets were processed in CryoSPARC and combined for 3D reconstruction and refinements. The SIgA-Arp4 structure reveals an extensive binding interface on SIgA, not only confirming the overlapping CD89 and Arp4 binding sites but also unveiling unique binding sites that have not been reported for other IgA receptors. Despite the reported accessibility of two receptor binding sites on SIgA, we observed 1:1 Arp4 to SIgA stoichiometry. These findings shed new light on host-pathogen evolution and guide strategies for therapeutic designs that could inhibit streptococcal proteins binding to SIgA without inhibiting host cell receptors binding.

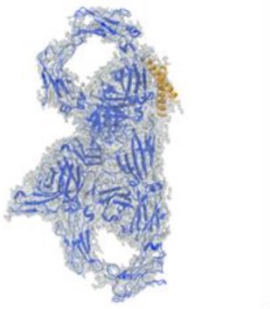


Figure 1. Cryo-EM structure of Arp4 (orange) in complex with human SIgA (blue); antigen binding fragments (Fabs) are disordered. Transparent map overlaid with the models are shown.

This project was funded by the University of Illinois and NIH grant R01-AI165570. The S^2C^2 is supported by the National Institutes of Health Common Fund Transformative High-Resolution Cryo-Electron Microscopy program (U24 GM129541). References: 1. Hedén, L.-O., Frithz, E. and Lindahl, G. (1991), Eur. J. Immunol., 21: 1481-1490. https://doi.org/10.1002/eji.1830210623; 2. Pleass, R. J., Areschoug, T., Lindahl, G., & Woof, J. M. (2001). Journal of Biological Chemistry, 276 (11), 8197–8204. https://doi.org/10.1074/jbc.M009396200

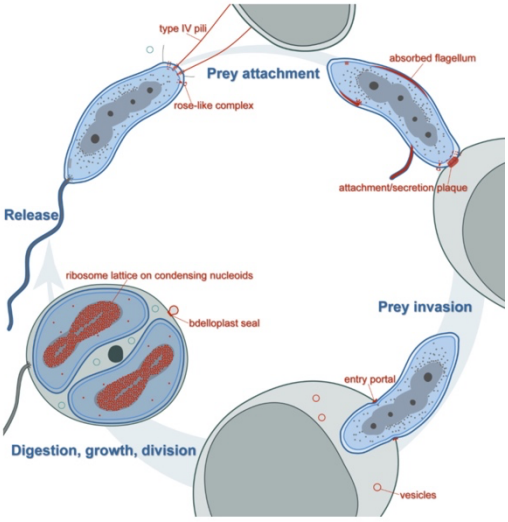
POSTER

Unique structural adaptations occur within the microbial predator *Bdellovibrio bacteriovorus* during prey invasion

Mohammed Kaplan[1,#], Yi-Wei Chang[1,2,#], Catherine M. Oikonomou[1], William J. Nicolas[1], Andrew I. Jewett[3], Stefan Kreida[1,4], Przemysław Dutka[1,5], Lee A. Rettberg[1], Stefano Maggi[1]and Grant J. Jensen[1,6,*]

[1]Division of Biology and Biological Engineering, California Institute of Technology, Pasadena, CA 91125, USA; [2]Current address: Department of Biochemistry and Biophysics, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA; [3]Department of Integrative Structural and Computational Biology, The Scripps Research Institute, La Jolla, CA, USA ; [4]Department of Microbiology, Tumor and Cell Biology, Karolinska Institute, 17177 Stockholm, Sweden; [5]Division od Chemistry and Chemical Engineering, California Institute of Technology, 1200 California Boulevard, Pasadena, CA 91125, USA; [6]Department of Chemistry and Biochemistry, Brigham Young University, Provo, UT 84604, USA

Bdellovibrio bacteriovorus is a microbial predator that offers promise as a living antibiotic for its ability to kill Gram-negative bacteria, including human pathogens. Even after six decades of study, fundamental details of its predation cycle remain mysterious. Here, we used cryo-electron tomography to comprehensively image the lifecycle of *B. bacteriovorus* at nanometer-scale resolution. In addition to providing the first high-resolution images of predation in a native (hydrated, unstained) state, we also discover several surprising features of the process, including novel macromolecular complexes involved in prey attachment/invasion and a flexible portal structure lining a hole in the prey peptidoglycan that tightly seals the prey outer membrane around the predator during entry. Unexpectedly, we find that *B. bacteriovorus* does not shed its flagellum during invasion, but rather resorbs it into its periplasm for degradation. Finally, following replication and division in the bdelloplast, we observe a transient and extensive ribosomal lattice on the condensed *B. bacteriovorus* nucleoid.



Development of a Stabilizer for 14-3-3 Interaction with ERα

Jenny Tang, Mark Knapp, Kelly Yan, Tiffany Tsang, John Fuller, Liliana Pedro, Andreas Frank, LynnMcGregor, Irina Alimov, Chelsea Edwin, Rama Jain, Aregahegn Yifru, Qingming Zhu, Wolfgang Jahnke, Colin Skepper
Novartis Institutes for BioMedical Research

14-3-3 proteins are a family of regulatory proteins that are involved in key cellular functions. They have hundreds of known binding partners and regulate a wide variety of cellular processes, such as metabolism, apoptosis, and transcription. The human 14-3-3 family has seven isoforms, each with nine conserved α-helices forming an amphipathic groove that functions as phospho-serine/threonine binder[1]. One interaction partner is Estrogen receptor alpha (ERα). ERα dimerizes upon activation, which is highly associated with cancer formation [2,3]. Binding of 14-3-3 to ERα, and stabilization of this complex with the natural product fusicoccin A, has been shown to suppress ERα dimerization and transcriptional activity. The present study aims to identify LMW stabilizers of the 14-3-3/ERα interaction as potential starting points for a novel therapeutic approach to breast cancer, and more broadly as a proof-of-concept for targeted stabilization of this PPI. High-throughput screening of in-house libraries was performed using a fluorescence polarization (FP) assay, and primary hits were further validated by surface plasmon resonance (SPR), NMR and native MS. Binding modes of validated hit compounds were then elucidated by X-ray crystallography. Two unique chemical entities were identified and characterized. One forms a covalent interaction with 14-3-3σ at residue Cys38, exploiting favorable interactions with a hydrophobic pocket and surrounding waters. The second compound exploits a unique non-covalent binding mode, and does not occupy the conventional fusicoccin binding pocket. These results provide evidence that stabilization of the 14-3-3/ERα interaction can be achieved with two structurally distinct, non-fusicoccin small molecules.

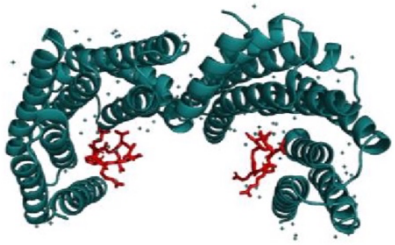


Figure 1. 14-3-3 sigma (green) bound to C-terminal of ERα (red), shown as homodimer.

References: 1. Pennington, K. L., Chan, T. Y., Torres, M. P., & Andersen, J. (2018). The dynamic and stress-adaptive signaling hub of 14-3-3: emerging mechanisms of regulation and context-dependent protein–protein interactions. Oncogene, 37(42), 5587-5604; 2. Liu, Y., Ma, H., & Yao, J. (2020). ERα, a key target for cancer therapy: A review. *OncoTargets and therapy,* 13, 2183; 3. De Vries-van Leeuwen, I. J., da Costa Pereira, D., Flach, K. D., Piersma, S. R., Haase, C., Bier, D., ... & de Boer, A. H. (2013). Interaction of 14-3-3 proteins with the estrogen receptor alpha F domain provides a drug target interface. *Proceedings of the National Academy of Sciences,* 110(22), 8894-8899.

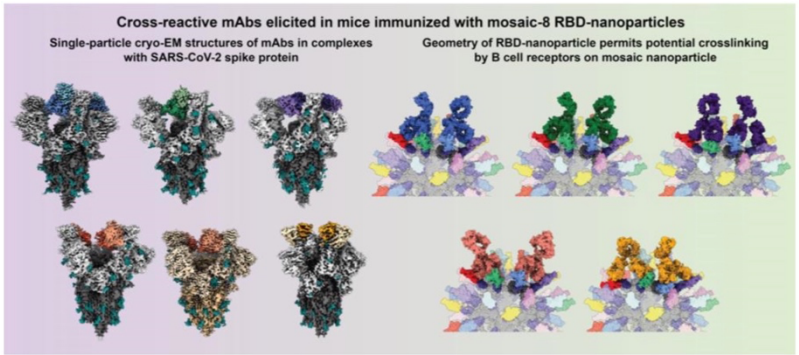
Neutralizing monoclonal antibodies elicited by mosaic RBD nanoparticles bind conserved sarbecovirus epitopes

POSTER

Chengcheng Fan[1], Alexander A. Cohen[1], Miso Park[2], Alfur Fu-Hsin Hung[2,3], Jennifer R. Keeffe[1], Priyanthi N.P. Gnanapragasam[1], Yu E. Lee[1,4], Han Gao[1], Leesa M. Kakutani[1], Ziyan Wu[1], Harry Kleanthous[5], Kathryn E. Malecek[1], John C. Williams[2], and Pamela J. Bjorkman[1,6*]

[1]Division of Biology and Biological Engineering, California Institute of Technology, Pasadena, CA 91125, USA; [2]Department of Molecular Medicine, City of Hope, Duarte, CA 91010, USA; [3]Present address: Rakuten Medical Inc., San Diego, CA 92121, USA; [4]Present address: Department of Biology, Stanford University, Stanford, CA 94305, USA; [5]Bill and Melinda Gates Foundation, Seattle, WA 98109, USA

Increased immune evasion by SARS-CoV-2 variants of concern highlights the need for new therapeutic neutralizing antibodies. Immunization with nanoparticles co-displaying spike receptor-binding domains (RBDs) from eight sarbecoviruses (mosaic-8 RBD-nanoparticles) efficiently elicits cross-reactive polyclonal antibodies against conserved sarbecovirus RBD epitopes. Here, we identified monoclonal antibodies (mAbs) capable of cross-reactive binding and neutralization of animal sarbecoviruses and SARS-CoV-2 variants by screening single mouse B-cells secreting IgGs that bind two or more sarbecovirus RBDs. Single-particle cryo-EM structures of antibody–spike complexes, including a Fab-Omicron complex, mapped neutralizing mAbs to conserved class 1/4 RBD epitopes. Structural analyses revealed neutralization mechanisms, potentials for intra-spike trimer crosslinking by IgGs, and induced changes in trimer upon Fab binding. In addition, we identified a mAb resembling Bebtelovimab, an EUA-approved human class 3 anti-RBD mAb. These results support using mosaic RBD-nanoparticle vaccination to generate and identify therapeutic pan-sarbecovirus and pan-variant mAbs.



Structure-based engineering of secretory immunoglobulin A provides new approaches for targeting mucosal pathogens.

Sonya Kumar Bharathkar[1]* and Beth M. Stadtmueller[1,2]

[1]Department of Biochemistry, University of Illinois, Urbana, Illinois 61801 USA; [2]Department of Biomedical and Translational Sciences, Carle Illinois College of Medicine, University of Illinois Urbana-Champaign

Secretory (S) Immunoglobin (Ig) A is the predominant antibody secreted into host mucosa where it binds antigens, as well as host and microbial proteins, in order to mediate interactions with commensal and pathogenic microbes. Despite significance, SIgA structure-function relationships and therapeutic potential remain poorly explored. In 2020 we reported a cryo electron microscopy (Cryo-EM) structure of SIgA, which revealed two IgA monomers connected through one joining-chain (JC) and bound by one secretory component (SC). [1]. In SIgA, the two IgAs adopted a bent and asymmetric conformation with JC co-folded at its core, and the SC bound to one face of dimeric (d) IgA leaving the five Ig-like domains of SC (D1-D5) solvent accessible, and of the five domains its second domain (D2) protruding farthest from the center of the complex (Fig.1). These results led us to hypothesize that D2 could be modified or replaced with alternative domains in order to explore SIgA effector mechanisms or build bi-specific SIgAs. Accordingly, we replaced D2 with single domain antibodies (sdAb) that bind antigens of the mucosal pathogen *Clostridioides difficile*, thereby creating chimeric (c) SC that we incorporated into recombinantly produced cSIgA. The cSC and cSIgA can bind and neutralize *C. difficile* toxins *in vitro* and in cell culture-based assays, and in the case of cSIgA, can be combined with antigen binding fragments targeting diverse antigens. In order to visualize SIgA-microbe interactions we also created a cSC in which D2 was replaced with a fluorescent protein (FP), allowing us to visualize the outcomes SIgA interactions with *C. difficile*. These results demonstrate that SC can tolerate D2-replacement and suggest that virtually any sdAb or FP could be incorporated into cSC and its function integrated into cSIgA. Broadly, our findings open the door for engineering cSIgA to study and/or treat pathogenic infections, modulate host microbiota and/or investigate SIgA effector mechanisms in animal models.

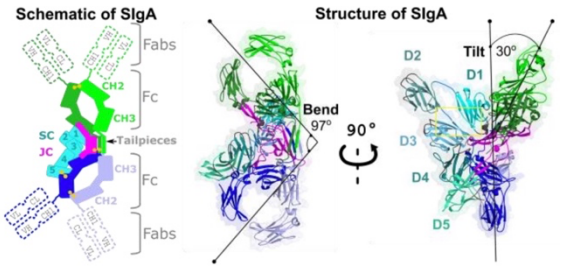


Fig1: The Schematic and structure of SIgA

References: 1. Kumar Bharathkar, S., B.W. Parker, A.G. Malyutin, N. Haloi, K.E. Huey-Tubman, E. Tajkhorshid, and B.M. Stadtmueller, *The structures of secretory and dimeric immunoglobulin A.* Elife, 2020. **9.** Funding**:** University of Illinois and NIH grant R01-AI165570. Disclosures: This work is included in a U.S. and International Patent Application entitled "Chimeric Secretory component polypeptides and uses thereof"

Defining the structure and function of newly identified Tamdy nairovirus vOTUs to examine the evolutionary trends within the nairovirus genus for those nairoviruses of human consequence.

David S Gonzalez[1], Vanessa Moresco[1], Jonathan Nguyen[1], Bryan Torres[1], Éric Bergeron[1], Scott D Pegan[1,3]

[1]Division of Biomedical Sciences, University of California Riverside, USA; [2]Viral Special Pathogens Branch, Division of High-Consequence Pathogens and Pathology, Centers for Disease Control and Prevention, USA; [3]Department of Chemistry & Life Science, United States Military Academy, United States

Nairoviruses are a tick-borne virus genus of the arbovirus Family Bunyaviridae that are emerging as dangerous human pathogens. Of the 39 negative (-) sense RNA nairoviruses known prior to 2021, several nairovirus were observed to be of human consequence. With nosocomial transmission known as well as mortality rates 5-40% typical and as high as 80%, Crimean-Congo hemorrhagic fever virus (CCHFV) is currently the most dangerous emerging nairovirus. However, others Issyk-kul virus, Dugbe virus, and Erve virus cause human disease of varying severity. This includes the Erve virus being linked to thunderclap headaches. With no FDA-approved vaccines or therapeutics for CCHFV and other nairoviruses, the WHO and others considered these to be dangerous emerging human pathogens. Within the last year, two newly discovered viruses belonging to the Nairoviridae species Tamdy have been found in Japan and China, Yezo and Songling nairoviruses respectively, to cause febrile illness in humans,. Concerning for the U.S. a third nairoivirus, Pacific Coast Tick nairovirus was found that year in Mendocino County, California within a tick species that is known to transmit viruses to humans. Previous reports suggest a conserved evasion mechanism colocalized within the L protein known as the viral homologue of the ovarian tumor protease (vOTU) superfamily. These proteases possess deubiquitinating and species-specific deISGylating activity that is key to the nairoviruses immune evasion as well as potentially zoonotic drift. Here we obtained the first 1.2 Å X-ray crystallographic structure of a human Tamdy nairovirus revealing evolutionary structural changes within the nairovirus genus. This coupled with enzymatic analysis of substrate preferences in emerging Tamdy species compared to various vOTUs from the existing nairovirus genus provides new insights on how the vOTUs have been evolving and to what consequence for humans.

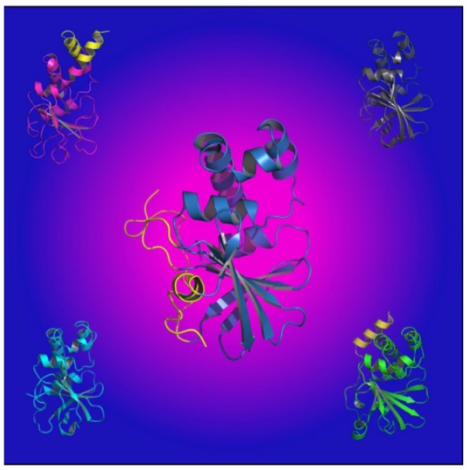


Figure 1. Center. A 1.2 Å crystal structure of a viral ovarian tumor domain protease originating from the emerging and human infecting Tamdy species of nairovirus. Surrounded by vOTUs originating from Farallon, Qalybus, CCHF, and Taggert nairoviruses.

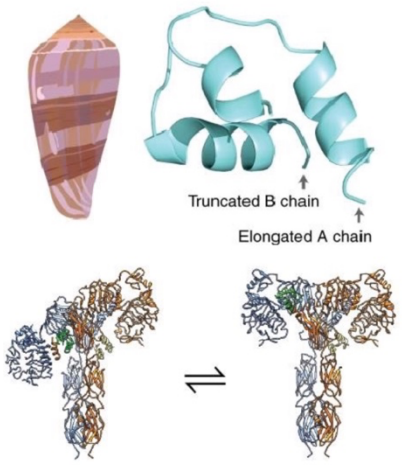
FLASH TALKS and POSTERS

A therapeutically promising mechanism of insulin receptor activation by a venom-derived insulin

Alan Blakely, Amber Vogel, Chris Hill

POSTER

Although the discovery of insulin therapeutics transformed a diagnosis of type 1 diabetes from a death sentence to a manageable disease, glycemic control for type 1 diabetics remains challenging and dangerous. Insulins on the market do not recapitulate the seamless response of native human insulin to changes in glucose concentrations due to slow absorption and dissociation of formulated insulin hexamers and dimers post injection. In principle, deletion of insulin B-chain C-terminal residues which form the dimer interface would reduce oligomerization, allowing for faster diffusion from the injection site and insulin receptor (IR) activation. Unfortunately, these same residues are critical for IR binding and activation.



Recently, discovery of a venomous insulin from cone snails has provided a framework for development of faster acting insulins. This venomous insulin lacks the B-chain C-terminus, leaving the mechanism of IR activation unclear. In this study, the activation mechanism of a hybrid venom-human insulin (Vh-Ins) is explained using CryoEM, AUC, and *in vivo* phosphorylation assays. We reveal that activation of IR by Vh-Ins lacking the Bchain C-terminus occurs through compensating interactions of an elongated A-chain in a tunable manner. The absence of B-chain residues allows Vh-Ins to evade oligomerization, enhancing its activation speed. Intriguingly, structural characterization reveals a previously uncharacterized ensemble of asymmetric conformations of IR upon activation, highlighting the complexity and obscurity of the IR activation mechanism. Our work paves the way for development of faster acting insulins with desirable therapeutic properties.

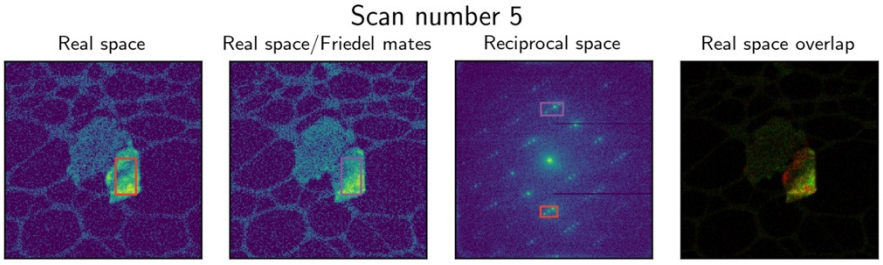
References: 1. Xiong and Blakely et al. Nat. Chem. Bio. 2022

Beyond MicroED: *Ab Initio* Crystal Structures with 4D-STEM

POSTER

Ambarneil Saha, Alexander J. Pattison, Peter Ercius, Jose A. Rodriguez
Department of Chemistry and Biochemistry, University of California, Los Angeles, California 90095, United States
National Center for Electron Microscopy, Lawrence Berkeley National Laboratory, Berkeley, California 94720, United States

Microcrystal electron diffraction (microED) has recently morphed into an increasingly mainstream technique in structural chemistry. MicroED's ability to interrogate nanocrystals orders of magnitude too small for conventional X-ray diffraction has enabled solid-state structure elucidation of several species previously considered impossible to solve using X-ray crystallography. Nevertheless, selected area aperture-enabled microED remains thwarted by the presence of disordered, overlapping, or otherwise poorly diffracting domains, all of which routinely conspire to mar data quality. Just as insufficient crystal size historically stymied conventional X-ray methods, these nanoscale defects frequently prohibit structure solution using well-rehearsed microED approaches. To overcome this, we apply the 4D scanning electron microscopy (4D-STEM) technique nanobeam electron diffraction tomography (nanoEDT). Our results represent the first 4D-STEM structures phased *ab initio* by direct methods. Critically, 4D-STEM's unique ability to pinpoint a specific nanoscale volume for data analysis enables pixel-by-pixel spatial exclusion of unwanted signal from Bragg-silent regions, empowering us to simply pick and choose whichever coherently diffracting zones (CDZs) of crystal generate the highest-quality diffraction patterns. Three strategies to parse these data—*k*-means clustering, center-of-mass analysis, and directly indexing sparse diffraction patterns using algorithms originally designed for XFEL experiments—will be discussed. Our analysis unveils that CDZs form an intricate topography of unpredictably distributed striations. CDZs appear as randomly shaped slivers often embedded within relatively Bragg-silent domains, even in nanocrystals anticipated to contain well-ordered, monolithic lattices. The ubiquity of these imperfections indicates sharp deviation from the defect-free, monolithic crystals assumed in computational simulations of electron diffraction patterns, providing an explanation for the current chasm between theory and experiment.



Effects of mutations on protein conformational heterogeneity

Ashraya Ravikumar, Stephanie Wankowicz, James Fraser
Department of Bioengineering and Therapeutics, University of California, San Francisco

How mutations affect protein activity, including binding and catalysis, is difficult to predict. While predictions can be improved by incorporating structural and evolutionary information, these methods do not take into account protein conformational dynamics. By using multiconformer models of stringently matched wild type and mutant structure pairs from X-ray datasets deposited in the PDB, we compare how patterns of conformational heterogeneity are affected by mutation. We sample the multiple conformations that are consistent with the electron density distribution and quantify the conformational heterogeneity by metrics such as B-factors, alternative conformations of sidechains, and crystallographic order parameters. We highlight the similarity and differences in heterogeneity in regions near and far from the site of mutation. We hypothesize that many mutations exert a deleterious effect by allosterically altering conformational heterogeneity throughout the protein, with major effects at functional sites. Revealing these patterns will suggest mechanisms to selectively stabilize and destabilize aspects of the conformational ensembles, providing new routes to exert allosteric control on proteins.

Analysis of Alpha-synuclein Amyloids Linked to Familial Neurodegenerative Disorders

Becky A. Jenkins[1], Jose A. Rodriguez[1]
[1] Department of Chemistry and Biochemistry; UCLA-DOE Institute for Genomics and Proteomics; STROBE, NSF Science and Technology Center; University of California, Los Angeles (UCLA)

Alpha-synuclein is an intrinsically disordered neuronal protein, derived from the SNCA gene, that has the capability to misfold and polymerize to form beta sheet rich aggregates termed fibrils. These fibrils have been found in brain inclusions of patients with Lewy body diseases (LBD) such as Parkinson's (PD) and are thought to directly and/or indirectly contribute to disease pathology. Clinical signs of LBD typically appear in the later stages of life at the age of 50 or older although autosomal dominant mutations in the SNCA gene have been associated with earlier onset of disease. On the other side of the age spectrum, recent studies have discovered the presence of alpha-synuclein enriched inclusions in mouse models and human brains afflicted with Krabbe disease, an autosomal recessive disorder that results in death before the age of 2 years old[1]. Pathogenesis of this particular disease results from the deficiency of the enzyme GALC that promotes the accumulation of the cytotoxic glycolipid, psychosine[2]. The presence of alpha-synuclein in this disease suggests dysregulation of metabolic processes as a potential molecular mechanism driving pathogenic fibril formation. However, the relationship between fibril structures and cellular pathology is not yet well understood in the case of both synucleinopathies. To aid our understanding, we have selected two systems of interest for structural and functional characterization: **(1)** A sequence variant of alphasynuclein that contains a threonine to methionine substitution (T72M) in the aggregation prone domain of alpha-synuclein and may be associated with late-onset PD[3] and **(2)** wild-type alpha-synuclein fibrils formed in the presence of psychosine. Using Cryo-EM, we investigate T72M variant fibrils and wild-type alphasynuclein fibrils grown in the presence of psychosine to study differences in their fold compared to previously determined synuclein fibril structures. To link the structural properties of these fibrils to pathological phenotypes, fibril formation is analyzed through *in vitro* kinetic assays and seeding is tracked within mammalian cell lines that model an aggregation prone alpha-synuclein mutation to evaluate prionlike spread[4] (Figure 1). Probing structural synuclein variation and its effects on pathogenic activity will further our understanding of alpha-synuclein in familial forms of alpha-synucleinopathies, and allows for the design of structure-based therapeutics. References

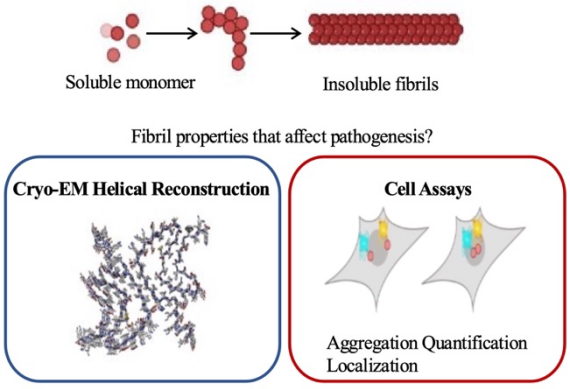


Figure 1. Characterization of aggregated alpha -synuclein assemblies (fibrils) with high -resolution structural methods and the tracking of assembly formation and propagation in living cells.

References 1. Smith, B. R. et al. Neuronal inclusions of α-synuclein contribute to the pathogenesis of Krabbe disease: Thioflavin-S-reactive inclusions in Krabbe disease. J. Pathol. 232, 509–521 (2014); 2)Hawkins-Salsbury, J. A. et al. Psychosine, the cytotoxic sphingolipid that accumulates in globoid cell leukodystrophy, alters membrane architecture. Journal of Lipid Research 54, 3303–3311 (2013); 3. Fevga, C. *et al.* A new alpha-synuclein missense variant (Thr72Met) in two Turkish families with Parkinson's disease. *Parkinsonism Related Disorders* **89**, 63–72 (2021); 4. Holmes, B. B. et al. Proteopathic tau seeding predicts tauopathy in vivo. Proceedings of the National Academy of Sciences 111, E4376–E4385 (2014).

New and Updated Phenix features

Billy K. Poon, Paul D. Adams, Pavel V. Afonine, Dorothee C. Liebschner,
Nigel W. Moriarty, Christopher J. Schlicksup, Oleg V. Sobolev
Molecular Biophysics and Integrated Bioimaging Division, Lawrence Berkeley National Laboratory, Berkeley, CA 94720, USA.

Phenix is a comprehensive software package for macromolecular structure determination that handles data from diffraction experiments and electron cryo-microscopy. Tasks performed with Phenix include data-quality assessment, map improvement, model building, the validation/rebuilding/refinement cycle and deposition. Each tool caters to the type of experimental data. The design of Phenix emphasizes the automation of procedures, where possible, to minimize repetitive and time-consuming manual tasks, while default parameters are chosen to encourage best practice. A graphical user interface provides access to many command-line features of Phenix and streamlines the transition between programs, project tracking and re-running of previous tasks. Some of the new features in Phenix, like utilizing predicted models from AlphaFold and a viewer for reciprocal space data, are highlighted in this poster.

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D-peptide Inhibitors of Tau Aggregation in Alzheimer's Disease

Ke Hou[1,2,3,4,5], Joshua Dolinsky[1,2,3,4,5], Carolyn Hu[1,2,3,4,5], Romany Abskharon[1,2,3,4,5],
Jeffrey Zhang[1,2,3,5], Xinyi Cheng[1,2,3,4,5], David Eisenberg[1,2,3,4,5]
[1]Department of Chemistry and Biochemistry, UCLA, Los Angeles, CA, USA. [2]Department of Biological Chemistry, UCLA, Los Angeles, CA, USA. [3] UCLA-DOE Institute, Los Angeles, CA, USA. [4]Molecular Biology Institute, UCLA, Los Angeles, CA, USA. [5]Howard Hughes Medical Institute, Los Angeles, CA, USA.

Tau is a microtubule-binding protein known to aggregate into amyloid fibrils in Alzheimer's Disease (AD). Aggregated tau is correlated with disease progression and neuronal death in AD.[1-3] Previously, our laboratory used *in silico* structure-based design methods to develop a six-residue D-peptide (D-TLKIVW) and a seven-residue D-peptide (D-TLKIVWC) that acted as tau aggregation inhibitors *in vitro.*[4,5] DTLKIVWC could also disaggregate AD-tau, prevent tau seeding in biosensor cells, reduce toxicity of tau fibrils in neuronal cells, and reverse neurological deficits in the PS19 mouse model of AD.[5] Building on this work, we further tested seven residue D-peptides with a variable seventh residue (D-TLKIVWX, where X= C, P, A, V, I, S, T, R, K, D, or E). These DP-X peptides were assayed on their abilities to prevent tau aggregation *in vitro*, disaggregate recombinant and brain-derived tau fibrils *in vitro,* prevent seeding of ADtau in tau biosensor cells, and protect neuronal cells from tau aggregation-induced toxicity. We find that hydrophobic D-peptides (DP-V, DP-I, and to a lesser extent DP-A) and polar D-peptides (DP-S and DP-T) reduce tau aggregation and its effects in all tested *in vitro* and cellular model systems. Interestingly, while cationic D-peptides (DP-R and DP-K) were effective tau aggregation inhibitors and disaggregators *in vitro,* we find that their disaggregation products are seed-competent and toxic to neuronal cells. This work uses *in vitro* and cellular assays to characterize *in silico* designed peptide-based drug candidate against AD-tau.

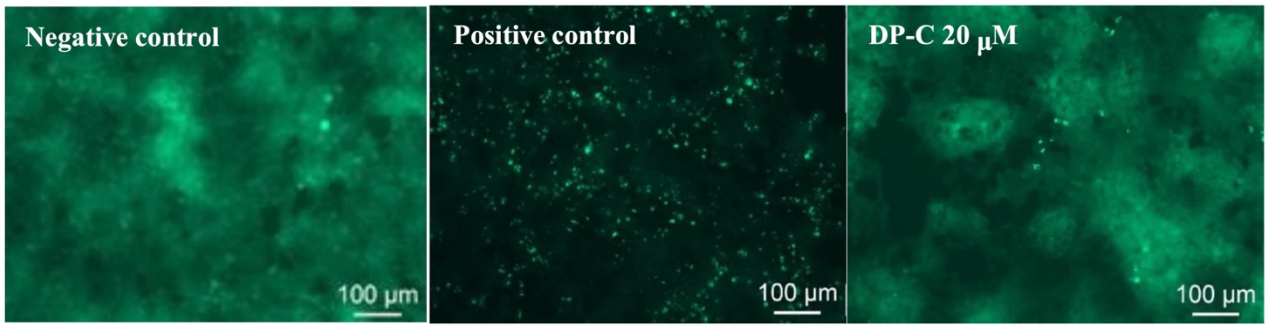

Figure 1. Example of D-peptide-based reduction of tau aggregation in HEK293T tau biosensor cells. When HEK293T biosensor cells overexpressing a tau K18-YFP construct are seeded with brain-derived AD-tau fibrils, the diffuse K18-YFP aggregates, visualized as puncta (left, middle). Overnight incubation of AD-tau fibrils with D-TLKIVWC (DP-C) almost completely abrogates tau K18 aggregation. Images taken at 100x zoom in the GFP channel three days after seeding.

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The Use of Conserved Cellular Receptors as Therapeutic Antibody Targets

Aldo Munoz, Jose Rodriguez
University of California, Los Angeles

The current, most widely used, strategies of treating/preventing viral infections depend on targeting surface viral glycoproteins. However, these strategies quickly become ineffective as antigenic drift and appearances of diverse viral lineages causes the antigens to be distinct from the original targeted virus. Therefore, targeting instead the more conserved cellular receptors that the viruses use to mediate cellular entry might be a viable alternative in the development of a broadly neutralizing inhibitor. Pathogenic New World Mammarenaviruses (NWM) contain six different etiological agents and are characterized as having less than 40% glycoprotein conservation between them. Despite this, they all target the human transferrin receptor 1 (hTfR1) for cellular entry. This makes NWM's a perfect model to study the effect of targeting the cellular receptor as a therapeutic target. A murine antibody, OKT9, which binds the apical domain of hTfR1, has already been shown to prevent the internalization of NWM pseudovirus[1] and increase survivability of mice infected with Junín (an etiological agent of NWM). However, a detailed molecular model of how OKT9 prevents pathogenesis is not known. The limitations being the receptor being a membrane protein, its homodimer state and the flexibility of the antibodies once bound. These limitations were overcome by the creation of a soluble transferrin receptor 1, a solubilized apical domain of hTfR1 (AP01)[2], and a single-chain or Fab construct of OKT9. Therefore, we aim to determine the structure of the scOKT9-AP01/OKT9 Fab-AP01 complex via crystallization efforts, mutate OKT9 residues along the binding interface to increase affinity, and test the efficacy/potency of newly designed OKT9 in mice when infected with Junín. The successful completion of our aims will not only establish the foundation for the development of therapeutics against NWM's but also a framework to target cellular receptors as therapeutic targets.

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AlphaFold-Assisted Molecular Replacement of a Novel Protein Domain

Justin E. Miller[1], Matthew P. Agdanowski[2], Joshua Dolinsky[1], Duilio Cascio[3], Todd O. Yeates[1,2,3]
UCLA Molecular Biology Institute[1], UCLA Department of Chemistry and Biochemistry[2], UCLA-DOE Institute for Genomics and Proteomics[3]

Macromolecular crystallography requires the recovery of missing phase information from diffraction data to reconstruct an electron density map of the crystallized molecule. Most recent structures have been solved using molecular replacement as a phasing method; this technique relies on the existence of an *a priori* structure that is closely related to the target protein to serve as a search model. When no such search model exists, molecular replacement is typically not feasible. New advances in computational machine learning methods, however, have resulted in major advances in protein structure predictions from sequence information. Notably, AlphaFold predicts the structure of many proteins to high degrees of accuracy, even those without homologues of known structure, providing a potentially powerful approach to molecular replacement. Taking advantage of these advances, we applied AlphaFold predictions to enable structure determination of a bacterial protein of unknown function (UniprotKB Q63NT7), based on diffraction data which had evaded phasing attempts by MIR and anomalous scattering methods. We were able to solve the structure of the main fragment of the protein – the domain for which AlphaFold predicted a three-dimensional fold with high reliability – using X-ray and micro-electron (microED) diffraction data. This provides an early example of a potentially general path for protein structure determination by diffraction methods.

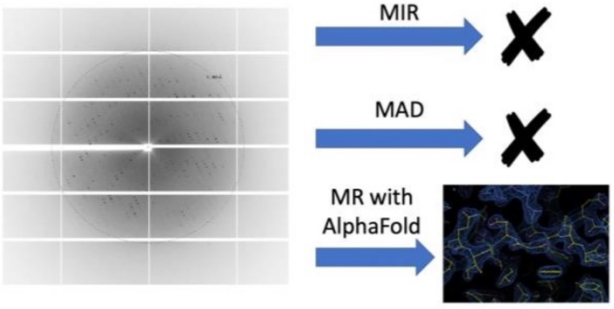

Figure 1. Graphical Abstract

Development and Evaluation of Machine Learning Tools that Predict Amyloid Propensity

POSTER

Jeff Qu, Samantha Zink, Niko Vlahakis, Eesha Shankar, Paulina Stanley, Thomas Cross, Jose Rodriguez
Department of Chemistry and Biochemistry; UCLA-DOE Institute for Genomics and Proteomics; STROBE, NSF Science and Technology Center; University of California, Los Angeles (UCLA)

The amyloid fold is linked to grave neurodegenerative disorders, including Alzheimer's disease (AD), Parkinson's disease (PD), and prion diseases. At their core, amyloid fibrils contain peptide segments assembled into tightly mated and interdigitated structures referred to as 'steric zippers'. Steric zippers help exclude water from fibril cores, rendering them resistant to denaturation or degradation, and promoting their aggregation in tissues[1]. It is therefore desirable to structurally characterize and predict the propensity for segments to form amyloids and ultimately predict their amyloid fold. Algorithms that rely on protein design approaches, such as secondary structure predictors and Rosetta modelers have accurately predicted amyloid propensity for short segments,[2] but are computationally expensive to apply on the scale of entire genomes. Machine learning offers a faster and potentially accurate alternative to the prediction of amyloid propensity. Here we analyze the amyloid-forming propensity of short peptide segments by training a neural network on Rosetta energy scores of computed structures. the calculated Rosetta energy scores of computed six-residue steric zipper structures. Training a network on this data leverages many hours of compute time already invested in zipper structure predictions and promises to replace the expensive process with fast evaluation by the trained network. The network can yield propensity scores for 6-residue segments in seconds rather than hours or days.

After training the network on one million structures, we evaluated its performance by scoring its accuracy against a computed set during training and assessed its speed of prediction when scoring entire genomes containing millions of hexapeptide sequences. Experimentally, we evaluate predicted scores from the network by probing the propensity of synthetic peptides to form steric zippers in solution. For those segments that form what appear to be amyloid assemblies, we characterize their structures by X-ray crystallography or electron diffraction and compare their structures and scores to those predicted by the network. These studies are a first step toward an improved understanding the amyloid fold and its prediction, with broad applicability.

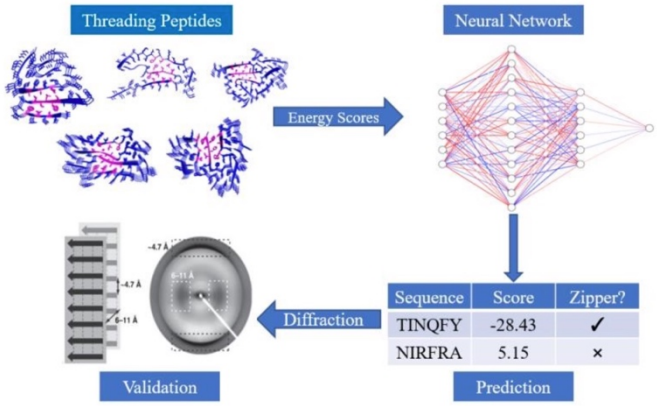

Figure 1. Prediction of amyloid propensity for hexapeptide segments using a machine learning pipeline trained on

Reference: Sawaya, Michael R., et al. "The expanding amyloid family: Structure, stability, function, and pathogenesis." Cell 184.19 (2021): 4857-4873; 2. Goldschmidt, Lukasz, et al. "Identifying the amylome, proteins capable of forming amyloid-like fibrils." Proceedings of the National Academy of Sciences 107.8 (2010): 3487-3492; 3. Greenwald, Jason and Roland Riek. "Biology of amyloid: structure, function, and regulation." Structure 18 10 (2010): 1244-60 .

Structural dynamics of intrinsically disordered proteins imaged in live cells

POSTER

Karina Guadalupe[1], Ryan J. Emenecker[2], Nora M. Shamoon[1], Alex S. Holehouse[2], Shahar Sukenik[1]
[1]Department of Chemistry and Biochemistry, University of California Merced, Merced, CA;
[2]Department of Biochemistry and Molecular Biophysics, Washington University School of Medicine, St. Louis, MO

The cellular environment is heterogenous and dynamic. Osmotic pressure, molecular crowding, and solution composition can change within seconds due to internal cellular events such as mitosis, motility, or apoptosis. Intrinsically disordered protein regions (IDPs) play critical roles in cellular regulation and homeostasis, and make up over 30% of the proteome. Unlike well-folded proteins, IDPs have a low number of intramolecular bonds and a high surface area. This makes them sensitive to the physical-chemical changes that occur in the cell, but our understanding of how their sequence determines their structure and sensitivity is poorly understood. Here we use live cell FRET microscopy to measure the ensembles of a synthetic library of dozens of IDPs, as well as their response to cellular composition changes. The IDP library is rationally designed to cover a wide range of chemically distinct sequences, while maintaining protein disorder. We find that despite having the same length, IDPs have remarkably diverse ensembles that can differ dramatically based on their sequence, with a significantly prominent effect for charge patterns in the sequence. In addition, we show that these divergent ensembles display a unique response to changes in the cellular environment, with more expanded ensembles having an overall higher sensitivity. Our findings show that IDP ensembles are encoded in their sequence and can be reshaped by the physicochemical environment of live cells, and this may play a key role in regulating their function in the dynamic intracellular environment.

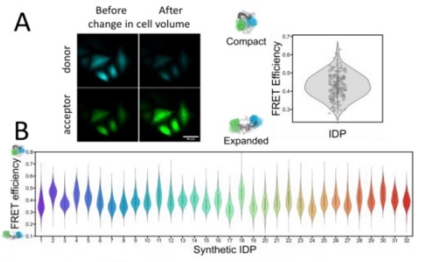

Figure 1. (A) Live-cell FRET microscopy quantifies IDR ensemble dimensions before and after cell volume modulation. (B) Synthetic IDP library displays sequence-encoded ensemble dimensions.

Toward Structural Insights into New World Hemorrhagic Fever Mammarenavirus Glycoprotein-Mediated Infection

Lily Taylor[1], Jose A. Rodriguez[1]

[1]Department of Chemistry and Biochemistry, UCLA-DOE Institute for Genomics and Proteomics, University of California Los Angeles, Los Angeles, California, USA

New World Hemorrhagic Fever Mammarenaviruses (NWMs) are responsible for causing life-threatening disease in humans; they include human pathogens: Junin, Machupo, Guanarito, Chapare, and Sabia.[1] These viruses can cause lethal hemorrhagic fevers as a result of zoonotic transmission from rodent to human hosts.[2] Infection is mediated by a tripartite glycoprotein complex (GPC) that decorates the surface of all Clade B NWM particles. Despite low sequence similarity across glycoproteins in this Clade, most bind a single conserved site on a cell surface receptor (human transferrin receptor, hTfR1) to mediate cellular entry and initiate infection, and therefore are assumed to adopt a conserved structural fold.[3]

Pathogenic Machupo and Junin NWM GPCs (70% sequence identity) display high sequence identity (6467%) to the nonpathogenic Tacaribe GPC, yet only Machupo and Junin can bind hTfR1 to elicit human infection. We aim to identify the key structural regions which modulate NWM pathogenicity through cryoEM single-particle analysis of NWM GPCs with varying capacities for human infection. We set out to map structural perturbations across pathogenic and nonpathogenic NWM GPCs onto residues that modulate receptor binding, with the ultimate goal of assessing how sequence variation manifests in differential hTfR1 compatibility. To this end, we have developed and expressed several constructs encoding the Machupo GPC ectodomain, residues 59-428, which can be efficiently isolated from cellular supernatants alone and in complex with antigen-binding fragments that share an epitope with hTfR1. In isolating an intact tripartite GPC, we are taking steps toward elucidation of its structure and a better understanding of its targeting in the quest to curb New World Hemorrhagic Fevers.

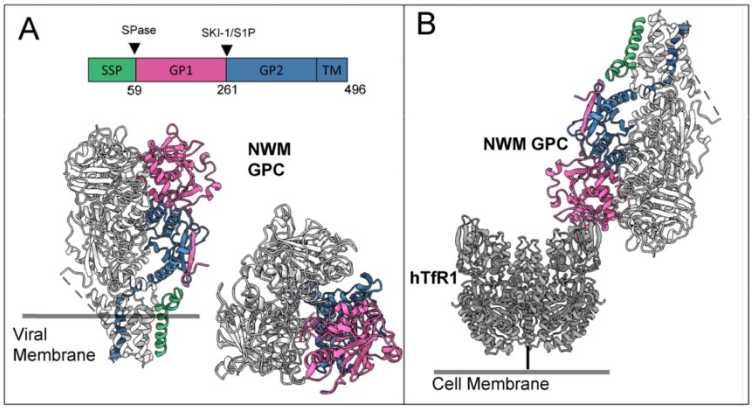

Figure 1: (A) NWM tripartite GPC is comprised of the GP1 (pink), GP2 (blue), and SSP (green). (B) NWM GP1 contacts hTfR1 to gain access to the host cell.

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The Cryo-electron Microscopy Structure of Teleost IgM Provides Insights on Polymeric Antibody Evolution, Assembly, and Function

Mengfan Lyu[1, *], Andrey G. Malyutin[3,4,5], Beth M. Stadtmueller[1,2]

[1]Department of Biochemistry, University of Illinois Urbana-Champaign, Urbana, Illinois 61801 USA; [2]Department of Biomedical and Translational Sciences, Carle Illinois College of Medicine, University of Illinois Urbana-Champaign, Urbana, Illinois 61801 USA; [3]Division of Biology and Biological Engineering, California Institute of Technology, Pasadena, CA91125 USA; [4]Beckman Institute, California Institute of Technology, Pasadena, CA 91125 USA

[5]Present address, Takeda Development Center Americas, San Diego, California 92121. *Presenting author contact: mlyu3@illinois.edu

Polymeric (p) immunoglobulins (Igs), including heavy-chain classes IgA and IgM, serve broad functions during vertebrate immune responses. Typically, pIgs contain between two and five Ig monomers; each Ig monomer consists of two antigen binding fragments (Fabs) and one fragment crystallization (Fc). Many pIgs also include a protein called the joining-chain (JC). However, JC incorporation and/or assembly into different-sized pIgs is poorly understood and varies with species, heavy-chain class, and isoform. In 2020, our group and others published mammalian polymeric IgA and IgM structures, revealing JC-dependent pIg assembly mechanisms and structure-function relationships [1-4]. Here, we report the cryo-electron microscopy structure of the Fc region of IgM from teleost (t) fish, which do not encode a JC. The structure revealed four tIgM Fcs linked through eight C-terminal tailpieces (Tps), which adopt a β-sandwich-like domain (Tp assembly) located between two Fcs. Remarkably, two of eight heavy chains fold uniquely, resulting in a structure distinct from mammalian IgM, which typically contains five IgM monomers linked through a centrally-located Tp assembly and one JC. Together, structural data and mutational analysis indicate that across species, pIgs share features such as a Tp- β-sandwich scaffold, yet their mechanisms of assembly and structures are variable and likely to impact downstream antibody effector functions.

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https://doi.org/10.1126/science.aaz5425

Navigating the World of Amyloid Fibrils with Amyloid Atlas and Amyloid Illustrator

POSTER

Michael Sawaya, Thomas Holton, David Eisenberg
Molecular Biology Institute, UCLA, Los Angeles, CA, USA, Howard Hughes Medical Institute, Los Angeles, CA, USA.

Amyloids fibrils are a type of protein assembly in which thousands of identical molecules align via β-sheet backbone hydrogen bonds. Many are disease-related, others are biologically functional, and almost any protein can form amyloid if coerced. Most of our knowledge of amyloid fibril structure has come to light only within the last 6 years. We are just beginning to uncover structural explanations for the many unusual characteristics of amyloid, including origins of pathogenicity, biological function, energetic stability, and propensity for polymorphism (1). To comprehend the growing number of amyloid structures (>230) and gain understanding of amyloid behavior we constructed the Amyloid Atlas database, which catalogs all amyloid structures and depicts them in physically insightful, and easy-to-grasp schematics. It facilitates comparisons between amyloid structures by using a consistent color scheme, size scale, and generous residue labeling. Moreover, it offers a numerical estimate of the fibril's energetic stability using an algorithm based on the standard free energy of solvation (2). We enable structural biologists to create insightful schematics from their own amyloid fibril coordinates using the Amyloid Illustrator web tool. Amyloid illustrator draws and labels amyloid residues expertly and automatically within minutes.

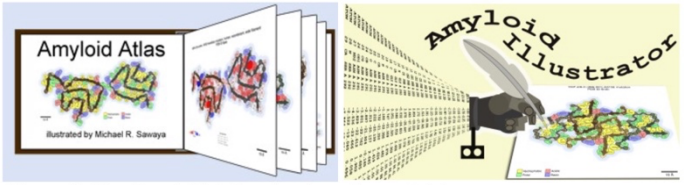

Figure 1. The Amyloid Atlas database is available at https://srv.mbi.ucla.edu/AmyloidAtlas/ and Amyloid illustrator is available at https://srv.mbi.ucla.edu/AmyloidAtlas/Illustrator/

References: 1.The expanding amyloid family: Structure, stability, function, and pathogenesis, M.R. Sawaya, M.P. Hughes, J.A. Rodriguez, R. Riek, D.S. Eisenberg, Cell, Vol 184, Issue 19, Sept. 16, 2021, pp. 4857-4873; 2. Solvation energy in protein folding and binding, D. Eisenberg, A.D. McLachlan, Nature, 319 (1986), pp. 199-203

Atomic structures of the diffocin, a contractile bacteriocin against Gram-positive bacteria

POSTER

Xiaoying Cai [1], Iris Yu [1,2], Yao He [1], Anthony Imani [1], Justin Bui [1,2], Wesley Shen [1,2], Dean Scholl [4], Jeff F. Miller [1,2], Z. Hong Zhou [1,2,3]
1. Department of Microbiology, Immunology & Molecular Genetics, University of California, Los Angeles, CA, USA; 2. California NanoSystems Institute, University of California, Los Angeles, Los Angeles, CA, USA; 3. Department of Bio-engineering, University of California, Los Angeles, Los Angeles, CA, USA [4] Pylum Biosciences, 100 Kimball Way S. San Francisco, CA, USA

Clostridoides difficile (CD) is a Gram-positive bacterium and one of the most prominent sources of nosocomial infection, responsible for half a million patients and several thousand deaths per year. Treatment of CD infection by regular antibiotics also results in the disruption of healthy gut microbiota and leaves a vacancy for more CD to proliferate, inducing serious forms of inflammation in the digestive system such as colitis. Contractile bactericidal nano-machines, also known as contractile bacteriocins, are cell-puncturing devices that share ancestry with contractile tail bacteriophages. Engineered contractile nano-machines based on R-type bacteriocins, such as the diffocins of CD and pyocins of *Psuedomonas aeruginosa*, hold promise for precision medicine applications to fight antibiotic resistance. These bacteriocins kill their target bacteria by dissipating ion (such as proton) gradients across cell envelopes that are needed to sustain metabolic activities of the bacterial cells. Several atomic-level studies have investigated the mechanism of action of contractile nanotubes including R-bacteriocins, phage tail structures, and T6SS[1-4]. However, all of these studies have focused on Gram negatives in which the structures bind to the outer membrane. Nothing is known about contractile nanotubes of Gram-positive bacteria, which have no outer membrane and a much different cell wall structure. Such differences call for atomic-level understanding of envelope-targeting bactericidal contractile nano-machines to be optimally engineered as precision antibiotics. Here, we report the atomic structures of diffocin, a contractile syringe-like molecular machine from CD, in three conformations, one in its pre-contraction state and two in its post-contraction state. We also report the resolved structure of the elusive tape measure protein that forms a coiled coil helix bundle. This first structural description for an Rtype bacteriocin of a Gram-positive bacterium offers major insights into the mechanism of contraction and shall inform design of potent protein-based precision antibiotics.

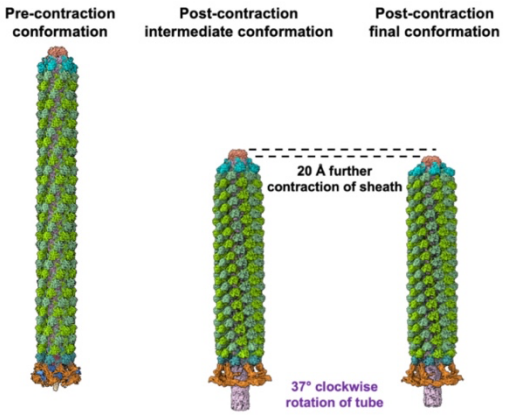

Figure 1. The cryoEM reconstructions of diffocin at different contraction states.

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We thank Ke Ding and Peng Ge for earlier efforts and discussion of this project.

Structure and pH-driven activation of cell-grown insecticidal protoxin Cry11Ba crystals

POSTER

Natalie A. Schibrowsky[1,2], Shervin Nia[1,2], Trevor Moser[3], Michael R. Sawaya[1], Dennis Bideshi[4], Hyun-Woo Park[4], Sarah R. Rudd[4], Brian Federici[4], Duilio Cascio[1], James Evans[3], Jose A. Rodriguez[1,2] 1

UCLA-DOE Institute for Genomics and Proteomics, University of California, Los Angeles, California 90095, United States; [2]Department of Chemistry and Biochemistry, University of California, Los Angeles, California 90095, United States; [3]Environmental Molecular Sciences Laboratory, Pacific Northwest National Laboratory, Richland, WA 99352; [4]Department of Entomology and Institute for Integrative Genome Biology, University of California, Riverside, CA 92521

Nature has evolved large, self-assembling protein complexes to perform various functions, including: storage, protection, and fortification. These self-assemblies range from filaments to full three-dimensional crystals and are pervasive across the tree of life. *Bacillus thuringiensis* (*Bt*) is a rod-shaped soil-dwelling bacteria that undergoes sporulation when stressed, and in doing so forms an endospore to protect dormant DNA and a paracrystalline inclusions.[1] These inclusions are of exceptionally interesting, since they naturally package insecticidal protoxins into a crystal in vivo and can be either cytosolic (Cyt), displaying hemolytic activity or crystalline (Cry), gaining activity after ingestion. The latter then form pores within the vector to cause mortality.[1] Cry11Ba is packaged into a crystalline inclusions and found to be one of the most effective[2] and toxic[3] pesticidal proteins. When these crystals are ingested by insect larval hosts, their Cry11Ba molecules switch from their packaged crystalline form to an active toxin at alkaline pH.[1] In recent work, we determined a *de novo* structure using serial femtosecond crystallography (SFX) approaches with X-ray free electron lasers (XFELs) and charted the crystals' pH sensitivity. [4] We now set out to study the Cry11Ba protoxins in alkaline environments as they are activated upon entering the gut of their target vector. We approached this by utilizing cryo-electron tomography (cryo-ET) and negative stain EM as ways to visualize the differences in the crystal's lattice that would be occurring from inducing with an alkaline pH. In tomograms, we observed dissolution of Cry11Ba crystals and the release of particles that we believed were Cry11Ba oligomers. We thus began negative stain screening to discover recurring particle species of the monomeric and multimeric states of Cry11Ba (Fig.1). Through negative stain EM, the particle species observed were multimeric and consistent with some theorized pore-forming active toxin models[5]. Through single particle analysis, we have begun constructing the multimeric structure of Cry11Ba within this alkaline environment in hopes to improve our understanding of the release and mode of action of these vital protoxins.

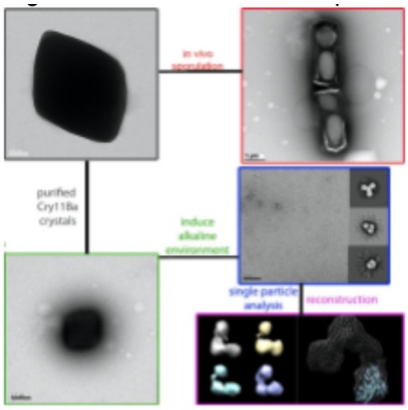


Fig. 1 Cry11Ba crystalline inclusions grown in vivo to purified Cry11Ba crystals activated upon introduction to alkaline environment for single particle analysis and reconstruction.

References:1. Palma, L., *et al*. *Bacillus thuringiensis* toxins: an overview of their biocidal activity. *Toxins*, *6*(12), 3296–3325 (2014); 2. Servant, P., *et al*. Production of Cry11A and Cry11Ba toxins in *Bacillus sphaericus* confers toxicity towards *Aedes aegypti* and resistant *Culex* populations. *AEM*, *65*(7), 3021–3026 (1999); 3. Park, H. W., *et al*. Recombinant strain of *Bacillus thuringiensis* producing Cyt1A, Cry11B, and the *Bacillus sphaericus* binary toxin. *AEM*, *69*(2), 1331–1334 (2003); 4. Tetreau, G., Sawaya, M.R., De Zitter, E. *et al*. De novo determination of mosquitocidal Cry11Aa and Cry11Ba structures from naturally-occurring nanocrystals. *Nat Commun* 13, 4376 (2022); 5. Xu, C., *et al*. Structural Insights into *Bacillus thuringiensis* Cry, Cyt and Parasporin Toxins. *Toxins* **2014**, *6*, 2732-2770.

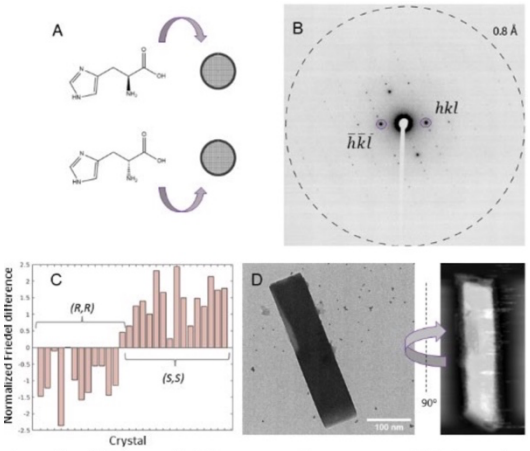
Characterization of chirality-associated scattering relationships in electron diffraction data

Niko Vlahakis[1,2], Ambarneil Saha[1,2], Jose Rodriguez[1,2]

[1] Department of Chemistry and Biochemistry, University of California, Los Angeles. [2] UCLA-DOE Institute for Genomics and Proteomics, University of California, Los Angeles.

POSTER

Determining the absolute configuration of molecules is fundamental to chemical synthesis and molecular analysis. Traditionally, this requires growing large pristine crystals for X-ray diffraction (XRD), but recent reports suggest that electron diffraction can be used to determine absolute structures from nanocrystalline powders.[1] While theory suggests electron diffraction is bereft of resonant scattering effects that allow chiral structure determination by XRD, reports have pointed to systematic intensity differences between Friedel symmetry-related reflections recorded by electron diffraction. Dynamical (multiple elastic) scattering is predicted to violate Friedel symmetry in electron diffraction,[2] and may therefore provide the key to distinguishing between enantiomers, but the impact of dynamical scattering effects on microED data collected from organic and biomolecular crystals has been demonstrably less than theory predicts.[3,4] To investigate if, in which cases, and with how much confidence dynamical scattering effects are reliable for informing on crystal chirality, we collected selected area diffraction data on enantiomeric pairs of chiral small molecule and peptide crystals representing a gradient of scattering cross section sizes by way of their atomic compositions. We assessed the impact of dynamical scattering on the data by quantifying differences in intensities between Friedel mates and intensities of symmetry-forbidden reflections. We consider the impact of crystal thickness and mosaicity on the degree of dynamical scattering measured in an electron diffraction experiment. We also consider metrics for assessing differential scattering between enantiomers, comparing X-ray and microED data. Studying how Friedel symmetry is violated in the X-ray diffraction case as a model, we attempt to identify quantitative relationships between Friedel intensity differences in microED data, the degree of multiple scattering artifacts present, and the likelihood of a successful absolute structure determination. These pre-refinement metrics are expected to clarify limitations of dynamical refinement protocols reported to clarify limitations of dynamical refinement protocols reported to determine chirality, and in certain cases could enable distinction between opposite enantiomers by ED even if a 3D structure cannot be solved.



intensity differences in ED patterns. From separate TEM samples of enantiomer pairs (A), ED data is collected and Friedel differences are measured (B) and assessed for significant differences due to chirality (C). Tomography is used to measure crystal thickness (D).

References: 1. Brázda P, Palatinus L, Babor M. *Science*. **2019** 364(6441), 667-669; 2. Kohra, K. *Journal of the Physical Society of Japan*. **1954** 9, 690-701; 3. Sawaya, M. R., Rodriguez, J., et al. *PNAS*. **2016** 113 (40), 11232-11236; 4. Martynowycz, M. W. et al. *bioRxiv*. **2017** 152504. https://doi.org/10.1101/152504.

PERIOD phosphorylation leads to feedback inhibition of CK1 activity to control circadian period

Jonathan M. Philpott[1], Alfred M. Freeberg[1], Jiyoung Park[2], Kwangjun Lee[2], Clarisse G. Ricci[3], Sabrina R. Hunt[1], Rajesh Narasimamurthy[4], David H. Segal[1], Rafael Robles[1], Yao Cai[5], Sarvind Tripathi[1], J. Andrew McCammon[3,6], David M. Virshup[4,7], Joanna C. Chiu[5], Choogon Lee[2]*, Carrie L. Partch[1,8,9,]*

[1]Department of Chemistry and Biochemistry, University of California Santa Cruz, Santa Cruz, CA 95064; [2]Department of Biomedical Sciences, College of Medicine, Florida State University; [3]Department of Chemistry and Biochemistry, University of California San Diego; [4]Program in Cancer and Stem Cell Biology, Duke-NUS Medical School, Singapore ; [5]Department of Entomology and Nematology, University of California Davis; [6]Department of Pharmacology, University of California San Diego; [7]Department of Pediatrics, Duke University Medical Center ; [8]Center for Circadian Biology, University of California San Diego; ‡Current address: D.E. Shaw Research, New York, NY 10036 [9]Lead contact; *Correspondence: cpartch@ucsc.edu, choogon.lee@med.fsu.edu

PERIOD (PER) and Casein Kinase 1δ regulate circadian rhythms through a phosphoswitch that controls PER stability and repressive activity in the molecular clock. CK1δ phosphorylation of the Familial Advanced Sleep Phase (FASP) serine cluster embedded within the Casein Kinase 1 binding domain (CK1BD) of mammalian PER1/2 inhibits its activity on phosphodegrons to stabilize PER and extend circadian period. Here, we show that the phosphorylated FASP region (pFASP) of PER2 directly interacts with and inhibits CK1δ. Cocrystal structures in conjunction with accelerated molecular dynamics simulations reveal how pFASP phosphoserines dock into conserved anion binding sites near the active site of CK1δ. Limiting phosphorylation of the FASP serine cluster reduces product inhibition, decreasing PER2 stability and shortening circadian period in human cells. We found that *Drosophila* PER also regulates CK1δ via feedback inhibition through the phosphorylated PER-Short domain, revealing a conserved mechanism by which PER phosphorylation near the CK1BD regulates CK1 kinase activity.

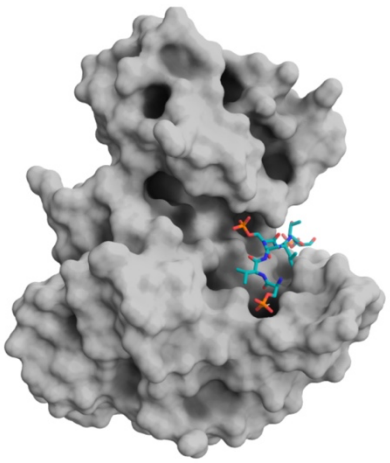


Figure 1. Phosphorylated FASP peptide bound to CK1δ catalytic domain

Structural Basis for Recognition of E2F Transcription Factor by the E3 Ligase Substrate Adaptor Cyclin F

POSTER

Peter Ngoi, Vitor Hugo Balasco Sarrão, and Seth Rubin
University of California, Santa Cruz

Virtually all cancerous cells have mutations in the Cdk-Rb-E2F pathway that contribute to uncontrolled cell division. E2F1-3 (E2F) are transcription factors that activate genes necessary for DNA synthesis during S phase of the cell cycle. Once DNA replication is complete, E2F is necessarily deactivated via phosphorylation by Cyclin A-Cdk2 and subsequent degradation by the ubiquitin proteosome system. Cyclin F has recently been identified as the substrate receptor protein that facilitates the ubiquitinoylation of E2F by the Skp1-Cullin-F box (SCF) E3 ubiquitin ligase. However, how Cyclin F recruits E2F and other substrates and how substrate recognition differs from Cyclin A is largely unknown. Using electron microscopy, we determined the structure of a Cyclin F-Skp1 complex bound to a peptide from E2F1, and we identified key differences between the Cyclin F and Cyclin A substrate-binding site. We hypothesize that these differences contribute to substrate specificity between Cyclin A and Cyclin F by recognizing substrate sequence elements that are outside of the canonical CY cyclin binding motif. Our rational for this work is to inform the development of novel cancer therapeutics targeting Cyclin F and activator E2Fs and to enhance our understanding of E2F regulation.

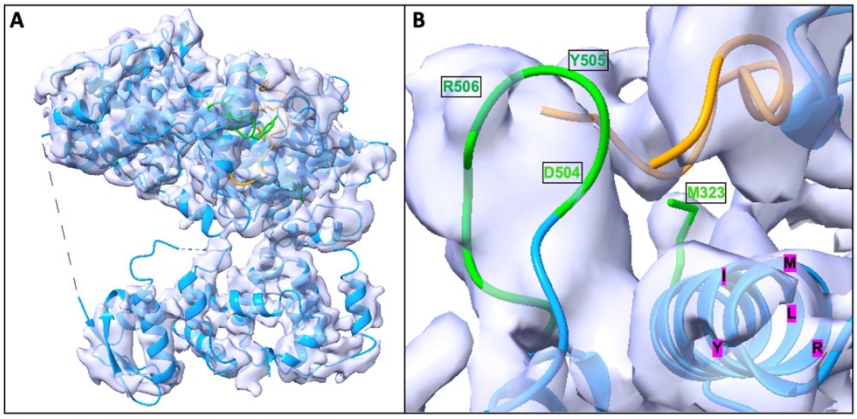


Figure 1. A) Model of E2F1-Cyclin F-Skp1 fit into the cryo-EM density map. **B)** E2F1-Cyclin F interface. E2F1 (orange) is in proximity to interact with Cyclin F (blue and green). Residues colored in green are outside of the canonical cyclin binding motif.

References: 1. Pagano, M. Cyclin F Controls Cell-Cycle Transcriptional Outputs by Directing the Degradation of the Three Activator E2Fs. *Molecular Cell* **2019**, *74* (6), 1264-1277.e7. https://doi.org/10.1016/j.molcel.2019.04.010; 2. Rubin, S. M.; Sage, J.; Skotheim, J. M. Integrating Old and New Paradigms of G1/S Control. *Molecular Cell* **2020**, *80* (2), 183–192. https://doi.org/10.1016/j.molcel.2020.08.020.

Bovine antibody recognition of SARS Cov-2 receptor binding domain

POSTER

Robyn Stanfield[1], Ruiqi Huang[2], Gabrielle Warner-Jenkins[2], Yunjeong Kim[3], Waithaka Mwangi[3], Kyeong-Ok Chang[3], Duncan McGregor[2], Vaughn, V. Smider[2,4], Ian A. Wilson[1]

[1]Department of Integrative Structural and Computational Biology, The Scripps Research Institute, La Jolla, CA 92037, USA; [2]Applied Biomedical Science Institute, San Diego, CA 92127, USA; [3]College of Veterinary Medicine, Department of Diagnostic Medicine and Pathobiology, Kansas State University, Manhattan, KS 66506, USA; [4]The Scripps Research Institute, Department of Molecular Medicine, The Scripps Research Institute, La Jolla, CA 92037, USA

It has been well known for many years that mice and humans produce antibodies that use 6 hypervariable loops (3 on each light or heavy chain) termed 'complementarity determining regions' (CDRs) to bind an unlimited number of different antigens. Of these 6 CDRs, the third heavy chain CDR (CDR H3) is the most variable in sequence and length, with sizes typically ranging from 12-15 amino acids in humans. Cows make similar antibodies, but also produce a subset of antibodies with extremely long CDR H3s of up to 70 amino acids. These ultralong CDR H3's fold into a small knob shaped domain supported by an extended β-ribbon stalk. The knob domains are highly variable in sequence and contain anywhere from 1-4 disulfide bonds, folding with 3 short, anti-parallel β-strands connected by loops of different lengths and structure, and with variable disulfide bond positions and connectivity. The previously determined structure of a cow Fab in complex with the HIV-1 envelope protein (1), revealed that only knob residues contacted antigen, with the rest of the Fab fragment acting as a scaffold to support the stalk/knob structure. We have now determined a structure for a neutralizing antibody isolated from a cow immunized with SARS Cov-2 spike protein in complex with the spike receptor binding domain (RBD). The crystal structure shows that the Fab contacts RBD with the distal surface of its CDR H3 knob, binding into a region near the binding site for its human receptor, ACE2. As seen for the complex of cow Fab with the HIV-1 envelope protein, only knob domain residues interact with the antigen, Knob domains can be produced as isolated fragments, suggesting their future utility as small protein therapeutics and reagents.

References: Stanfield, R.L., et al., *Structural basis of broad HIV neutralization by a vaccine-induced cow antibody.* Sci Adv, 2020. **6**(22): p. eaba0468.

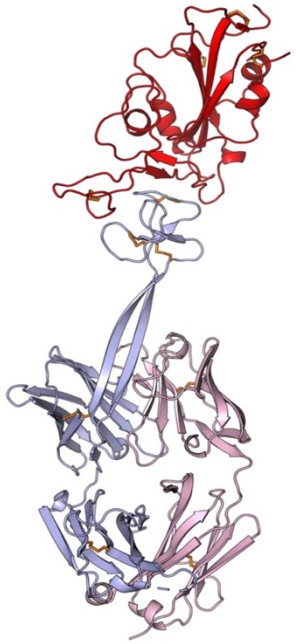


Figure 1. Cow Fab fragment bound to SARS Cov-2 receptor binding domain.

Structural determination of the 3' and 5' ends of Tobacco Mosaic Virus

POSTER

Nina Harpell, Anilu Duran-Meza, Maya Cornejo, Abigail Chapman, William Gelbart, Jose Rodriguez

Tobacco Mosaic Virus (TMV) has a remarkable history as the first virus ever isolated and has since been extensively studied through X-ray crystallography and cryoEM[1]. At present, there are several high-resolution structures of TMV deposited in the PDB that have identified key structural motifs and RNA-binding sites; however, due to current helical averaging techniques, variations across the capsid are averaged out in favor of a higher resolution overall structure[2]. This method eliminates the ability to identify any structural differences at the ends of the capsid in comparison to the center. And yet the ends of the capsid play an important role in cellular signaling and viral disassembly[3,4]. Since TMV is a cylindrical, helical, virus – with both the genomic RNA and the capsid proteins organized into perfectly-ordered helices – the 5' and 3' ends of the viral RNA sit at opposite ends of the capsid. Furthermore, the RNA content at either end differs significantly from that at the other end and from that at the center of the capsid. The 5' end completely lacks any guanine bases, which have the strongest interaction with capsid protein (CP) while the 3' end consists of 40% guanine bases. This indicates that the 5' end of the RNA may have a weaker interaction with the CP, leading to an alternative morphology of the capsid at each end. Additionally, due to the helical stacking of the CP in the capsid, the terminal "disks" of protein may experience reconstruction of the usual kind associated with the surfaces of crystals. To interrogate the relationship between capsid structure and RNA content, we have established a pipeline for differentiating each end of the viral capsid through gold-labeling of the 5' end of RNA, followed by packaging of it with TMV CP to form TMV virus-like particles (VLPs), cryoEM imaging, and structure determination. We show in particular that TMV RNA can be labeled with 1.4nm gold particles, followed by in vitro packaging with CP to form nuclease resistant TMV virus particles. We foresee this approach allowing analysis of the structure and stability of TMV at its 3' and 5' ends, and thereby providing a microscopic basis for its co- translational disassembly and other, related, steps in its infectivity/life cycle.

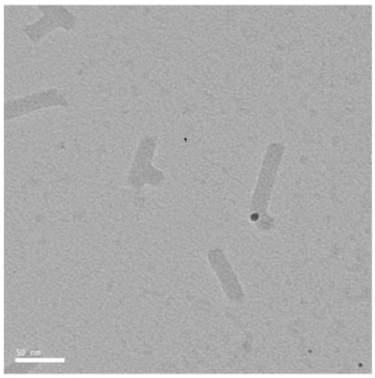


Figure 1: TMV labeled with gold

References: 1. C Sachse, et al., High-resolution electron microscopy of helical specimens: A fresh look at tobacco mosaic virus. J Mol Biol 371, 812–835 (2007); 2. Sachse, C.; Chen, J. Z.; Coureux, P.-D.; Stroupe, M. E.; Fändrich, M.; Grigorieff, N. High-Resolution Electron Microscopy of Helical Specimens: A Fresh Look at Tobacco Mosaic Virus. Journal of Molecular Biology 2007, 371(3), 812–835. https://doi.org/10.1016/j.jmb.2007.05.088; 3. Christensen, N.; Tilsner, J.; Bell, K.; Hammann, P.; Parton, R.; Lacomme, C.; Oparka, K. The 5′ Cap of Tobacco Mosaic Virus (TMV) Is Required for Virion Attachment to the Actin/Endoplasmic Reticulum Network During Early Infection. Traffic 2009, 10 (5), 536–551. https://doi.org/10.1111/j.1600-0854.2009.00889.x; 4. Wilson, T. M. Cotranslational Disassembly of Tobacco Mosaic Virus in Vitro. Virology 1984, 137 (2), 255–265. https://doi.org/10.1016/0042-6822(84)90217-4.

Structural and Mechanistic Characterization of Protective Non-Neutralizing Antibodies Targeting Crimean-Congo Hemorrhagic Fever

POSTER

Ian A. Durie[1], Zahra R. Tehrani[2], Elif Karaaslan[3,4], Teresa E. Sorvillo[3], Jack McGuire[4], Joseph W. Golden[5], Stephen R. Welch[3], Markus H. Kainulainen[3], Jessica R. Harmon[3], Jarrod J. Mousa [6,7], David Gonzalez[4], Suzanne Enos[1], Iftihar Koksal[8], Gurdal Yilmaz[9], Hanife Nur Karakoc[10], Sanaz Hamidi[9], Cansu Albay[9], Jessica R. Spengler[3], Christina F. Spiropoulou[3], Aura R. Garrison[5], Mohammad M. Sajadi[2], Éric Bergeron[1,3*], Scott D. Pegan[4,11]

[1]Department of Pharmaceutical and Biomedical Sciences, University of Georgia, USA; [2]Division of Clinical Care and Research, Institute of Human Virology, University of Maryland School of Medicine, USA; [3]Viral Special Pathogens Branch, Division of High-Consequence Pathogens and Pathology, Centers for Disease Control and Prevention, USA; [4]Division of Biomedical Sciences, University of California Riverside, USA; [5]Virology Division, United States Army Medical Research Institute of Infectious Diseases, USA; [6] Department of Infectious Diseases, University of Georgia, USA [7]Center for Vaccines and Immunology, University of Georgia College, USA; [8]Department of Infectious Disease and Clinical Microbiology, Acibadem University Atakent Hospital, Turkey; [9]Department of Infectious Diseases, Karadeniz Technical University School of Medicine, Turkey; [10]Department of Infectious Disease and Clinical Microbiology, Bitlis State Hospital, Turkey [11]Department of Chemistry & Life Science, United States Military Academy, United States

Crimean-Congo hemorrhagic fever virus (CCHFV) causes a debilitating hemorrhagic fever with a mortality rate as high as 40%. With a 2017 outbreak in Spain illustrating CCHFV's continued ability to expand its endemic area and no approved vaccine or therapeutics available, CCHFV is viewed as a priority public health threat by the WHO. Recently, the non-neutralizing mouse monoclonal antibody (mAb) 13G8 has been shown to target the CCHFV non-structural glycoprotein GP38 and protect against lethality in a mouse model when challenged with several CCHFV strains. Here, we biochemically reveal how strain-strain differences among GP38s affect interactions with 13G8 as well as a new human mAb CC5-17. The latter was identified among 11 mAbs derived from the blood of a recovered CCHFV patient targeting CCHFV GP38. CC5-17 exhibits orders of magnitude superior binding affinity for GP38s, while being competitive with 13G8. To better understand the molecular origins of this phenomena, X-ray crystallography structures of GP38 from a human clinical isolate strain of CCHFV, alone and in complex with 13G8 and CC5-17, were obtained. This structural information not only identified GP38 regions that potentially serve as therapeutically relevant epitopes for protection against CCHFV, but also demonstrates a conserved region in diverse CCHFV strains that are also likely to be successful targeted using 13G8 or CC5-17. Mutational studies based on this data highlighted the broad spectrum potential and resiliency to viral evasion properties of these mAbs. This information coupled with *in vivo* efficacy data informs the development of monoclonal antibodies for effective prophylaxis and therapeutic use against CCHFV.

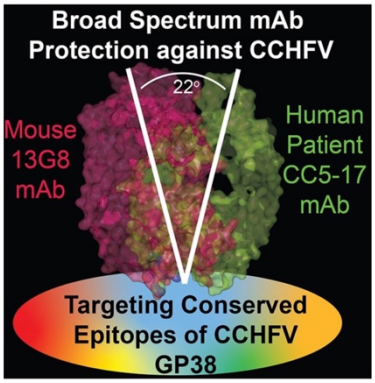


Figure 1. Two Fabs, one mouse the other human, binding to the same epitope on CCHFV GP38 but having different non- neutralizing and broad-spectrum protection properties. Structural insights into these phenomena

The New SSRL Remote Access Program for Elevated-Temperature and Humidity-Controlled Experiments

Silvia Russi [1]representing the SSRL Structural Molecular Biology Group
1. Stanford Synchrotron Radiation Lightsource, SLAC National Accelerator Laboratory, 2575 Sand Hill Rd., Menlo Park 94025, California, USA. e-mail: srussi@slac.stanford.edu

Expanding on its mature remote-access cryo-crystallography program, the Structural Molecular Biology (SMB) group at the Stanford Synchrotron Radiation Lightsource (SSRL) now supports remote user-access for diffraction experiments at controlled humidity conditions. During data collection at SSRL BL12-1, crystals can be maintained in a controlled temperature environment or at controlled humidity (and ambient temperature conditions) including stepwise crystal dehydration experiments. To enable robotic sample exchange at the beam line, ten samples on standard magnetic bases fit into a new SSRL in-situ plate. The plate has features of both a crystallization plate and a uni-puck, serving as a one-spot crystallization, sample storage and shipping container. Alternatively, to growing crystals in the plate, crystals in capillaries, MiTeGen sleeves, or certain microfluidic chips can shipped inside the plate, which serves as a secondary humidity chamber. For shock-free transport to the beam line, six SSRL in-situ plates fit inside an insulated thermal shipper which contains a phase-changing liquid to maintain the temperature for up to seven days (at around 20 or 5 ^{o}C) during transport using a standard courier service like FedEx. At the beam line, insitu plates are loaded into a humidity-controlled bay accessible to the sample exchange robot. To initiate an experiment, the Blu-Ice control software incorporates a new user interface to mount samples from inside the in-situ plates.

These novel tools and automation offer SSRL users a seamless home-lab to remote-lab experience, opening the door to more advanced remote access diffraction studies. Exciting opportunities enabled by this technology include exploring phase transitions, controlled dehydration to achieve higher resolution data, and studying protein structure and dynamics at near physiological temperatures. For example, the possibility to trigger chemical reactions within enzyme crystals during serial diffraction experiments can provide detailed mechanistic information. To support and accelerate these goals, a number of advanced options are available to the SSRL user community that build upon this new remote-access program.

Directions about this program and the sample compatibility is available online:
https://www-ssrl.slac.stanford.edu/smb-mc/content/users/manuals/remote-access-at-elevatedtemperatures-and-controlled-humidity

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Structural basis for how INK4 inhibits CDK6-Cyclin D complexes and confers drug resistance

Sivasankar Putta[1], Vitor Hugo Balasco Serrão[1,2], and Seth M. Rubin[1]
[1]University of California Santa Cruz, Department of Chemistry and Biochemistry;[2] Biomolecular cryo-Electron Microscopy Facility, University of California Santa Cruz

Cyclin-dependent kinases 4 and 6 (CDK4/6) phosphorylate target proteins to control the cell cycle. CDK4/6 activity is tightly regulated by activating cyclin D (CycD) proteins and inhibitory INK family proteins. An imbalance in the regulation of this activity results in uncontrolled growth that is a hallmark of cancer. A number of ATP competitive inhibitors (CDK4/6i) have been developed to target CDK4/6-CycD in breast cancer. However, these therapeutics suffer from acquired resistance, and other cancers are intrinsically resistant. Recently, it has been shown that binding of the INK4 protein p18 to the CDK6-CycD dimer does not completely inhibit kinase activity, and the trimer complex is resistant to CDK4/6i. We hypothesize that p18 remodels the complex such that the active site binds ATP somewhat weakly and does not bind compound. In order to test this hypothesis and gain insights into the mechanism of complex insensitivity to CDK4/6i, we performed single-particle cryo-EM of a p18INK4C–CDK6CycD1 complex. Initial data processing resulted in a reconstruction that reflects the expected model complex (Figure 1), and further refinement is ongoing.

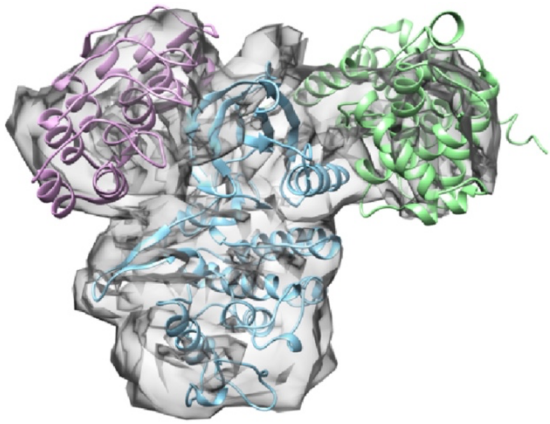

Figure 1. p18-CDK6-CycD1 model aligned to *ab initio* reconstruction from the cryo-EM data

References: 1. Guiley KZ, Stevenson JW, Lou K, Barkovich KJ, Kumarasamy V, Wijeratne TU, Bunch KL, Tripathi S, Knudsen ES, Witkiewicz AK, Shokat KM, Rubin SM. p27 allosterically activates cyclin-dependent kinase 4 and antagonizes palbociclib inhibition. Science. 2019, doi: 10.1126/science. aaw2106.; 2. Li Q, Jiang B, Guo J, Shao H, Del Priore IS, Chang Q, Kudo R, Li Z, Razavi P, Liu B, Boghossian AS, Rees MG, Ronan MM, Roth JA, Donovan KA, Palafox M, Reis-Filho JS, de Stanchina E, Fischer ES; 3. Rosen N, Serra V, Koff A, Chodera JD, Gray NS, Chandarlapaty S. INK4 Tumor Suppressor Proteins Mediate Resistance to CDK4/6 Kinase Inhibitors. Cancer Discovery. 2022, doi: 10.1158/2159-8290.CD20-1726.

Ligand binding remodels protein side-chain conformational heterogeneity

Stephanie A Wankowicz[1,2], Saulo H de Oliveira[3], Daniel W Hogan[1], Henry van den Bedem[1,3], James S Fraser[1]
1. Department of Bioengineering and Therapeutic Sciences, University of California, San Francisco, United States; 2. Biophysics Graduate Program, University of California San Francisco, United States; 3. Atomwise Inc., United States

While protein conformational heterogeneity plays an important role in many aspects of biological function, including ligand binding, its impact has been difficult to quantify. Macromolecular X-ray diffraction is commonly interpreted with a static structure, but it can provide information on both the anharmonic and harmonic contributions to conformational heterogeneity. Here, through multiconformer modeling of time- and space-averaged electron density, we measure conformational heterogeneity of 743 stringently matched pairs of crystallographic datasets that reflect unbound/apo and ligand-bound/holo states. When comparing the conformational heterogeneity of side chains, we observe that when binding site residues become more rigid upon ligand binding, distant residues tend to become more flexible, especially in non-solvent-exposed regions. Among ligand properties, we observe increased protein flexibility as the number of hydrogen bonds decreases and relative hydrophobicity increases. Across a series of 13 inhibitor-bound structures of CDK2, we find that conformational heterogeneity is correlated with inhibitor features and identify how conformational changes propagate differences in conformational heterogeneity away from the binding site. Collectively, our findings agree with models emerging from nuclear magnetic resonance studies suggesting that residual side-chain entropy can modulate affinity and point to the need to integrate both static conformational changes and conformational heterogeneity in models of ligand binding.

The Biomolecular cryo-EM Facility at UC Santa Cruz: What Should You Expect?

Vitor Hugo Balasco Serrão[1,2], Melissa Jurica[3,4], Sarah Loerch[1], Rebecca DuBois[5], Carrie Partch[1], Harry Noller[3,4], Jevgenij Raskatov[1], Alexander Ayzner[1], Joshua Arribere[3,4] and Seth Rubin[1]
[1]Department of Chemistry and Biochemistry - UCSC; [2]Biomolecular cryo-Electron Microscopy Facility - UCSC; [3]Department of Molecular Cell and Developmental Biology – UCSC; [4]Center for Molecular Biology of RNA – UCSC; [5]Department of Biomolecular Engineering - UCSC

Cryo-EM has become a highly effective tool in the field of structural biology. In 2013, the 'resolution revolution', initiated by a faster, more sensitive detector, drastically changed the field and pushed the visible details of molecules to atomic resolution. This cutting-edge approach offers the unique possibility of investigating biological molecules in their native state and revealing how these cellular machines function.

The Biomolecular cryo-EM facility at the University of California, Santa Cruz, is a leading provider of cutting-edge structural biology solutions using transmission electron microscopy. We provide access and guidance to a complete workflow from sample analysis and optimization to grid preparation, automated high-resolution imaging, data analysis, and tridimensional reconstruction, which allows structure determination via model construction and validation.

Our Glacios 200 kV is coupled with a Gatan K2 Summit direct detector, which allows sample preparation quality to be checked using our screening time blocks (4 hours, up to 8 grids). Low-magnification maps are initially collected using SerialEM, allowing fast selection of the top grids based on the overall ice quality. Pre-screened grids can be imaged and auto-processed to obtain high-resolution 3D reconstructions. Overnight data collection typically provides ~2,000-5,000 movies and thousands of particles that are automatically processed to optimize the efficiency and quality of the datasets. Using SerialEM coupled to cryoSPARC-Live, our team is capable of obtaining near-atomic resolution within ~16 hours of unsupervised data collection and processing. Our workflow allows preferential orientation and image and particle quality analysis, increasing the data quality and resulting in an initial 3D reconstruction in-live.

For data processing, our facility manages and grants access to one of our six GPU-based (NVIDIA RTX3090) workstations and storage. We have all of the most commonly used software packages for singleparticle and subtomogram averaging analysis (Relion 4.0, cryoSPARC 4.1, EMAN 2.91, cisTEM, Phenix, Coot, CCPEM, Dynamo, PEET, etc). Multiple levels of service and access are available and correspond with the type of analysis desired. On-site individual training is provided for users from different levels, with no geographical restrictions. We currently have 30+ ongoing projects, including collaborators from four different countries and companies.
www.ucsccryoem.org – cryoem@ucsc.edu

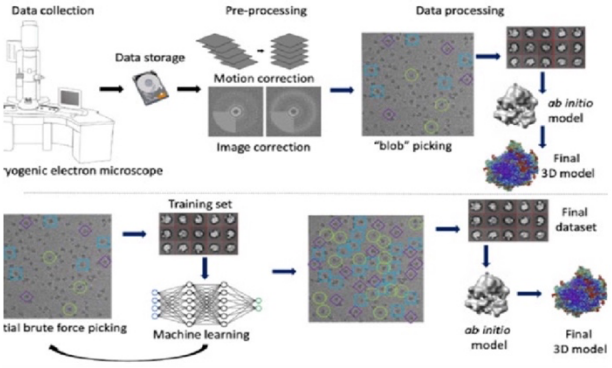

Figure 1. The biomolecular cryo-EM facility at UCSC workflow.

Towards mechanistic elucidation of the arsenite efflux pump ATPase ArsA from *Leptospirillum ferriphilum*

POSTER

Shivansh Mahajan, Douglas C. Rees & William M. Clemons

Division of Chemistry and Chemical Engineering, California Institute of Technology, 1200 E California Blvd, Pasadena, CA 91125

The arsenite-stimulated ATPase, ArsA is part of the arsenite efflux pump ArsAB that confers arsenite resistance in several bacterial species by coupling ATP hydrolysis to transport of arsenite via the integral membrane transporter ArsB. Elucidating the molecular basis of ArsAB pump would inspire synthetic biological arsenic bioremediation strategies. ArsA belongs to the 'Intradimeric Walker A' (IWA) family of P-loop NTPases, along with ATPases of diverse biological functions ranging from bacterial cell division regulation to nitrogen fixation. Unlike other IWA ATPases that function as dimers, ArsA is a pseudodimer with two homologous nucleotide binding domains connected by a flexible linker, and an arsenite binding site at the pseudodimer interface. Dramatic conformational rearrangements, particularly at the dimer interface, is a key mechanistic feature of IWA ATPases. The mechanism of ArsA is poorly understood as its conformational landscape is not well-characterized at the structural level. Moreover, the structural basis of how ATP binding and hydrolysis are coupled to arsenite binding awaits characterization. We cloned, expressed and purified ArsA from a thermotolerant and acidophilic bacteria *Leptospirillum ferriphilum*. Here, we discuss our ongoing efforts to structurally elucidate conformational changes in ArsA and their implications for its catalytic mechanism.

Towards the structural characterization of DPAGT1 and other essential NLG enzymes

POSTER

Jessica M. Ochoa & William M. Clemons

Division of Chemistry and Chemical Engineering, California Institute of Technology, 1200 E California Blvd, Pasadena, CA 91125

N-linked glycosylation (NLG) is a common posttranslational modification found in all domains of life. The process begins with the biosynthesis of a complex lipid-linked glycan. This process results in complex N-linked glycans that are synthesized on membrane-embedded dolicholpyrophosphate and relies on the accurate expression and function of a series of membrane-bound phosphotranferases and glycosyltransferases, some of which are thought to form functional complexes. Defects in glycosylation pathways have been linked to numerous congenital disorders, cancers and fetal lethalities. N-linked glycosylation relies on the accurate expression and function of a series of membrane-bound phosphotranferases and glycosyltransferases whose mechanisms remain poorly understood. In humans, the first membrane-committed step is performed by *N*-acetylglucosamine-phosphotransferase 1 which has homologs, including MraY and AglH, that are found in bacteria and archaea, respectively. Thus, defining mechanisms of catalysis will be broadly applicable for understanding N-linked glycosylation in all domains of life. We describe efforts towards the expression and purification of essential NLG enzymes including, human *N*-acetylglucosamine-phosphotransferase 1 (DPAGT1) and preliminary results towards the expression and purification of Alg14 and Alg13. We also present ongoing efforts to structurally characterize these NLG enzymes by single particle cryo-EM. Success of this work will provide a framework for the future development of novel therapeutics and antibiotics that target the essential enzymes of glycosylation pathways.

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Medium throughput	Dataset in 24 hours
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Applications	SPA

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Applications	SPA, MicroED, tomography

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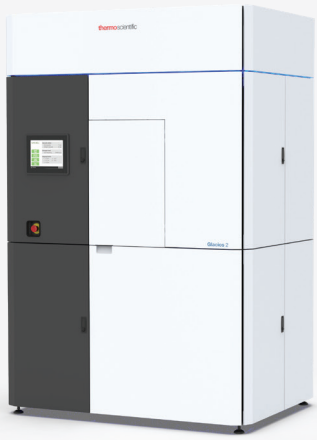
Highest throughput	Dataset in minutes
Sample type	Proteins, crystals, cells
Applications	SPA, MicroED, tomography

* Based on best published performance, actual results will depend on non-microscope factors such as sample and user experience. Not a promise of biological resolution performance.



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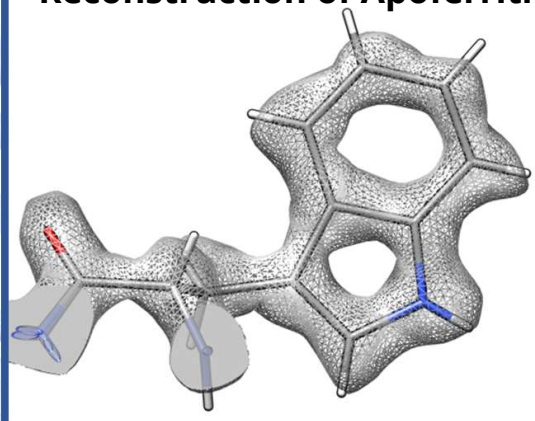
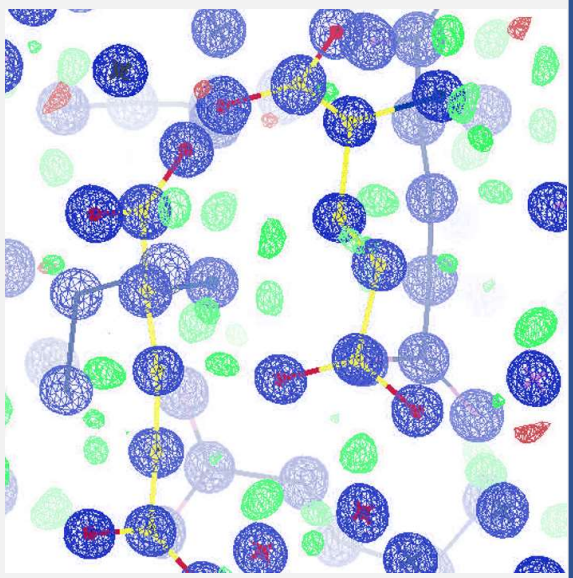
1.46 Å Single Particle CryoEM Reconstruction of Apoferritin



Courtesy of Miori Sakamoto, Takuma Fukumura, and Yuji Konyuba (JEOL Japan).

0.5 Å MicroED reconstruction of Monosodium Glutamate



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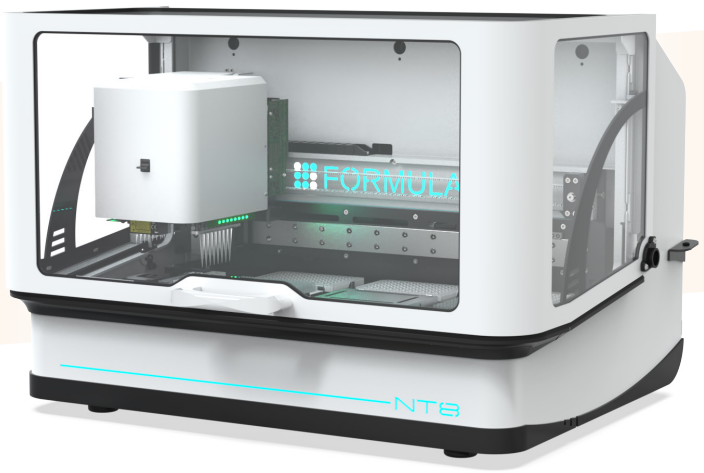

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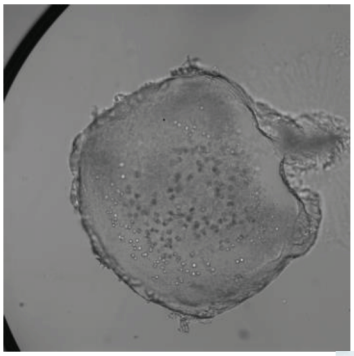

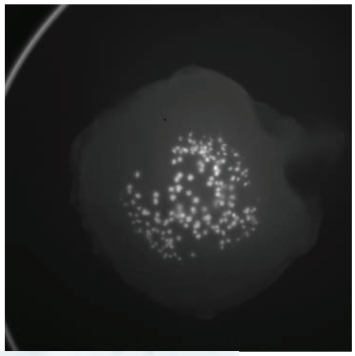

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THANK YOU

From the 2023 WCSBW Organizing Team,

Professor Todd O. Yeates
yeates@mbi.ucla.edu
UCLA

Professor Jose Rodriguez
jrodriguez@mbi.ucla.edu
UCLA

Dr. Jessica M. Ochoa
jmochoa@caltech.edu
Caltech

Dr. Pascal Egea
pegea@mednet.ucla.edu
UCLA

Dr. Duilio Cascio
cascio@mbi.ucla.edu
UCLA

Cindy Chau
cchau@mbi.ucla.edu
UCLA

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