National Cryo-EM Program Update

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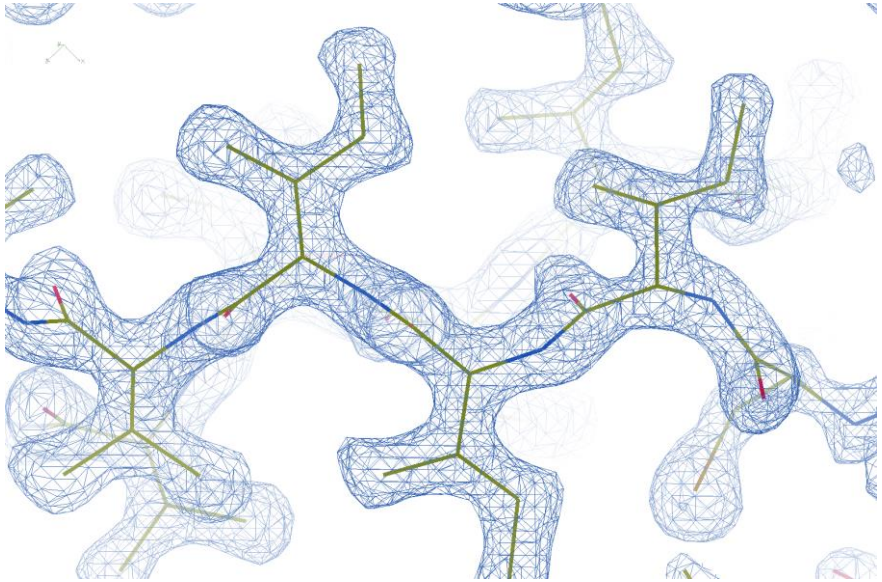
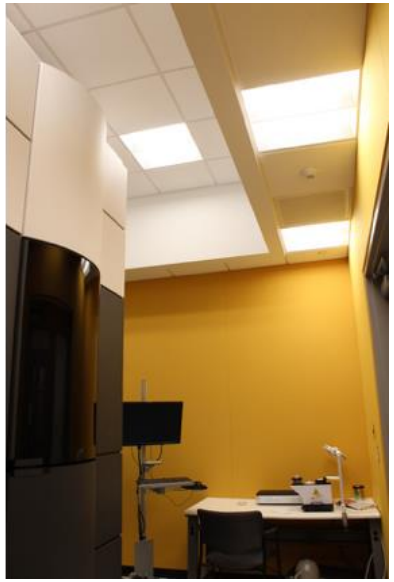
Director, Cancer Research Technology Program, FNLCR

October 19, 2021





FNLCR Operational Models

Dr. Ethan Dmitrovsky
Laboratory Director, FNLCR

Dr. Leonard P. Freedman
Chief Science Officer

Dr. Dwight V. Nissley
Directorate Head, CRTP

National Missions

NCI RAS Initiative

National Cryo-EM Program

NCI-DOE Collaboration

Extramural Enabling

Nanotechnology Characterization Lab (NCL)

Antibody Characterization Lab (ACL)

Technology for NIH/NCI

Cryo-EM, TEM and Optical Microscopy

Protein Expression and Characterization

Genomics and Proteomics

NCI National Cryo-EM Program

1. **National Cryo-EM Facility 2017-present**

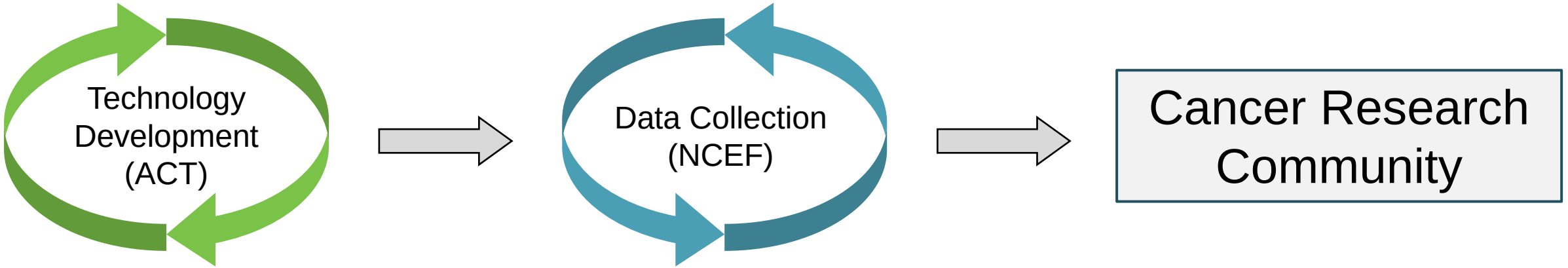
   Extramural user facility for cryo-EM data collection

   Ongoing expansion of scope, bandwidth and turn around

2. **Cryo-EM Research and Development 2019-present**

   Newly created component to explore new platforms

   Methods and technology development for cryo-EM field



NCEP - User Communities and Mission

- **Group I: Research groups with experience in cryo-EM technology**
  - have some access to local screening microscopes
  - inadequate access to high-end instrumentation
  - are key drivers of growth of cryo-EM in the US
- **Group II: Structural biologists in adjacent disciplines (X-ray, NMR)**
  - see value in using cryo-EM
  - have expertise in protein biochemistry
  - need training in cryo-EM specimen preparation, data collection, and processing
- **Group III. Biologists with interest in important biomedical problems**
  - interested in adding cryo-EM methods to their toolkit
  - need training and collaboration in all aspects of the workflow from protein purification to the final interpretation of the structures

National Cryo-EM Facility (NCEF)

National Cryo-EM Facility Organization

Thomas Edwards
Senior Scientist
NCEF

Adam Wier
Electron Microscopist
NCEF

Helen Wang
Program Manger
NCEF

Tara Fox
Electron Microscopist
NCEF

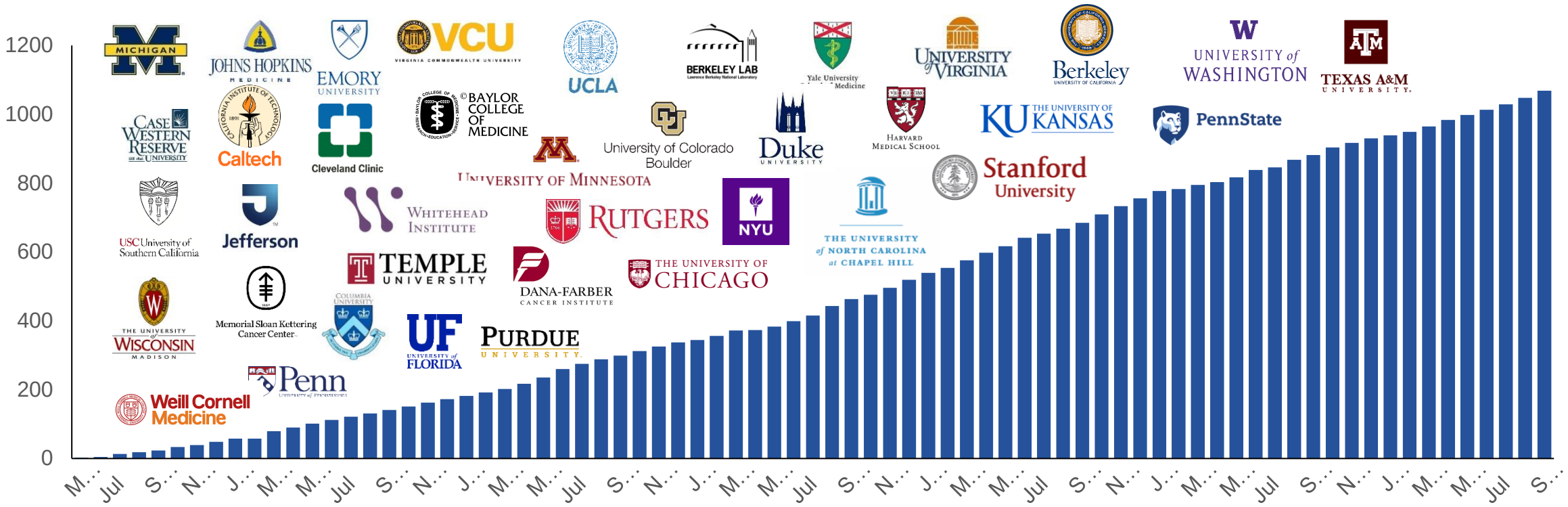
Joseph Finney
Software Developer
NCEF

Jenny Wang
Electron Microscopist
NCEF

Vladimir Veremeychik
Software Developer
NCEF

- NCEF opened in May 2017 to address access issues to high-end imaging platforms
- Performed over 1050 imaging sessions resulting in 106 publications to date
- 145 primary investigators from over 50 institutions have utilized NCEF
- Onboarded a new Software Developer this year

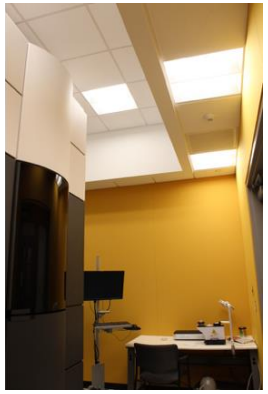
NCI National Cryo-EM Facility Operational Metrics

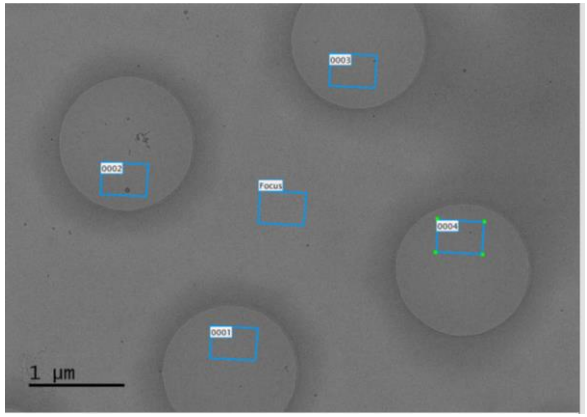


- Total of 145 investigators from over 50 institutions
- 1050 imaging sessions have been completed over the past 6.5 years, with an average of 15.6 imaging sessions per month over the previous year.
- 106 publications in 6.5 years, with 26 publications in the past year.
- Publications are in high-impact journals such as Science, Nature, Nature Communications, etc.
- Over 210 structures deposited in the EMDB

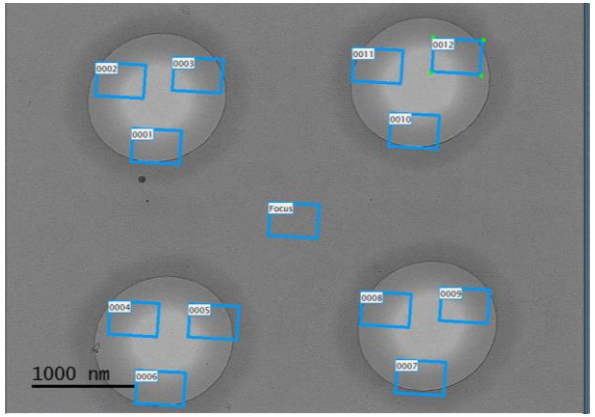
NCEF Capabilities

- **Two Titan Krios Microscopes**
- **Each is equipped with Gatan K3 Direct Detector and BioQuantum Energy Filter**
- **Current general imaging collects at 180 images/hour. 6000-7000 images for a two-day session**
- **Completed upgrades to both microscopes both for Windows 10 and Fringe-Free Imaging (FFI)**
  - FFI has already facilitated increased thruput in data collection
- **With new software and workflow, NCEF aims to double thruput in the coming year**
- **Testing VitroJet automated grid preparation machine for user access program**





Without FFI, a single target per hole

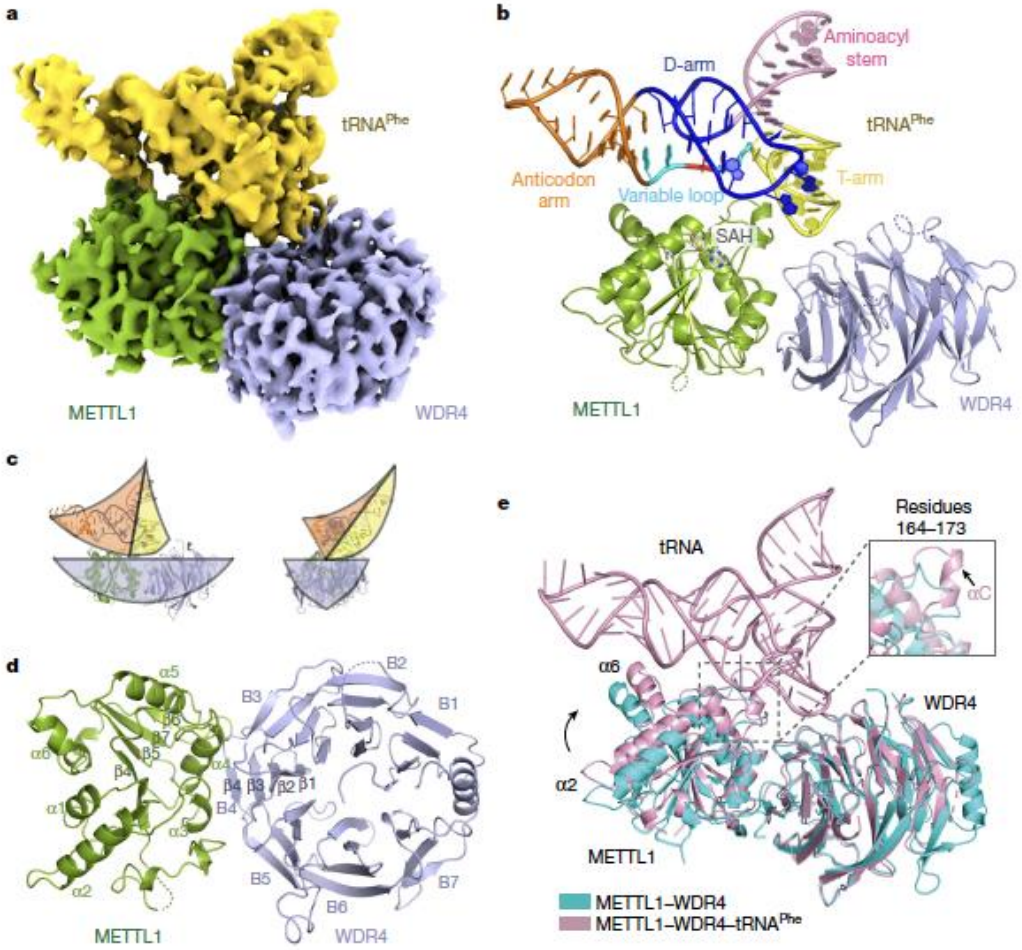


With FFI, multiple targets per hole

Grid Preparation Program Development

- **NCEF is developing a grid preparation and screening service**
- **Access to latest generation of grid freezing technology**
  - Using VitroJet platform for reproducibility of grid preparation
  - Sample sent and frozen by NCEF staff
  - Limited iterations (concentration and freezing time manipulation) to control how much resources are used
  - Grids to be screened on an NCEF microscope
- **NCEF is currently testing workflows for intaking and freezing samples**
  - Consistent production of grids at given settings
- **Screening will be performed on Glacios equipped with Falcon 4 camera**
  - Test automated screening software such as SmartScope, Multigrid.
- **Development of sample tracking software**
  - Using modified GP2S from GenenTech
- **Developing reporting framework to provide user with useful feedback to continue their experiments**
- **Targeting users that lack sufficient cryo-EM infrastructure**
  - Developing application process.

NCEF User Success: METTL1-WDR4-tRNA^{phe}

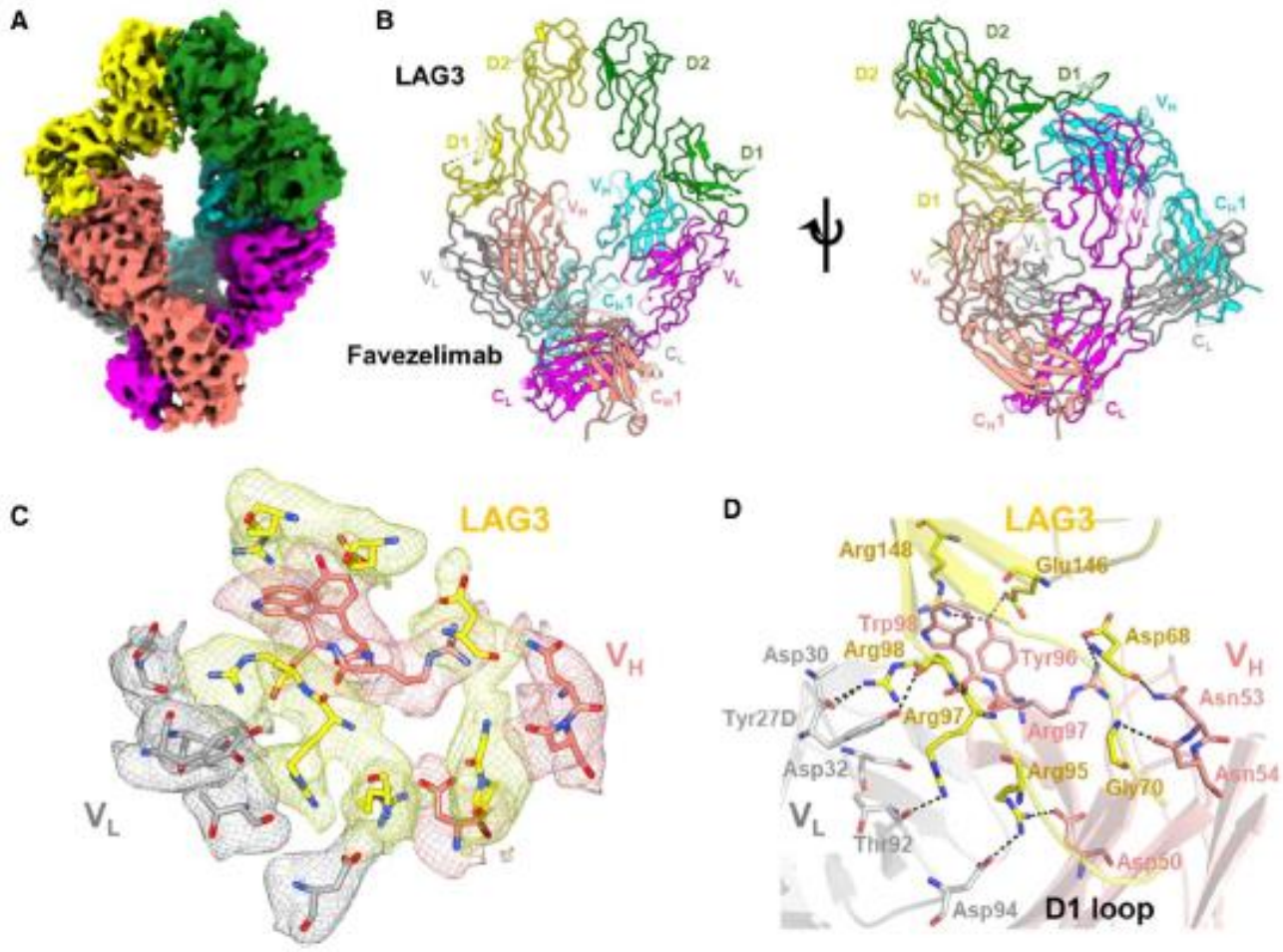


- METTL1-WDR4 is important for proper tRNA maturation
- Mutation of METTL1-WDR4 plays a role in developmental disorders
- Dysregulation of METTL1-WDR4 plays a role in tumorigenesis.
- METTL1-WDR4 complex with a truncated tRNA substrate structure (METTL1-WDR4-tRNA^{phe}) was solved to 3.3 Å
- Structure contributes to understanding of dysregulation of methyltransferase activity for tRNAs in oncogenesis
- Submitting PI: Richard Gregory, Harvard University

EMD-26990 PDB 8CTH

Li et al. *Nature* 613, 391-397 2023

NCEF User Success: Favezelimab:LAG3

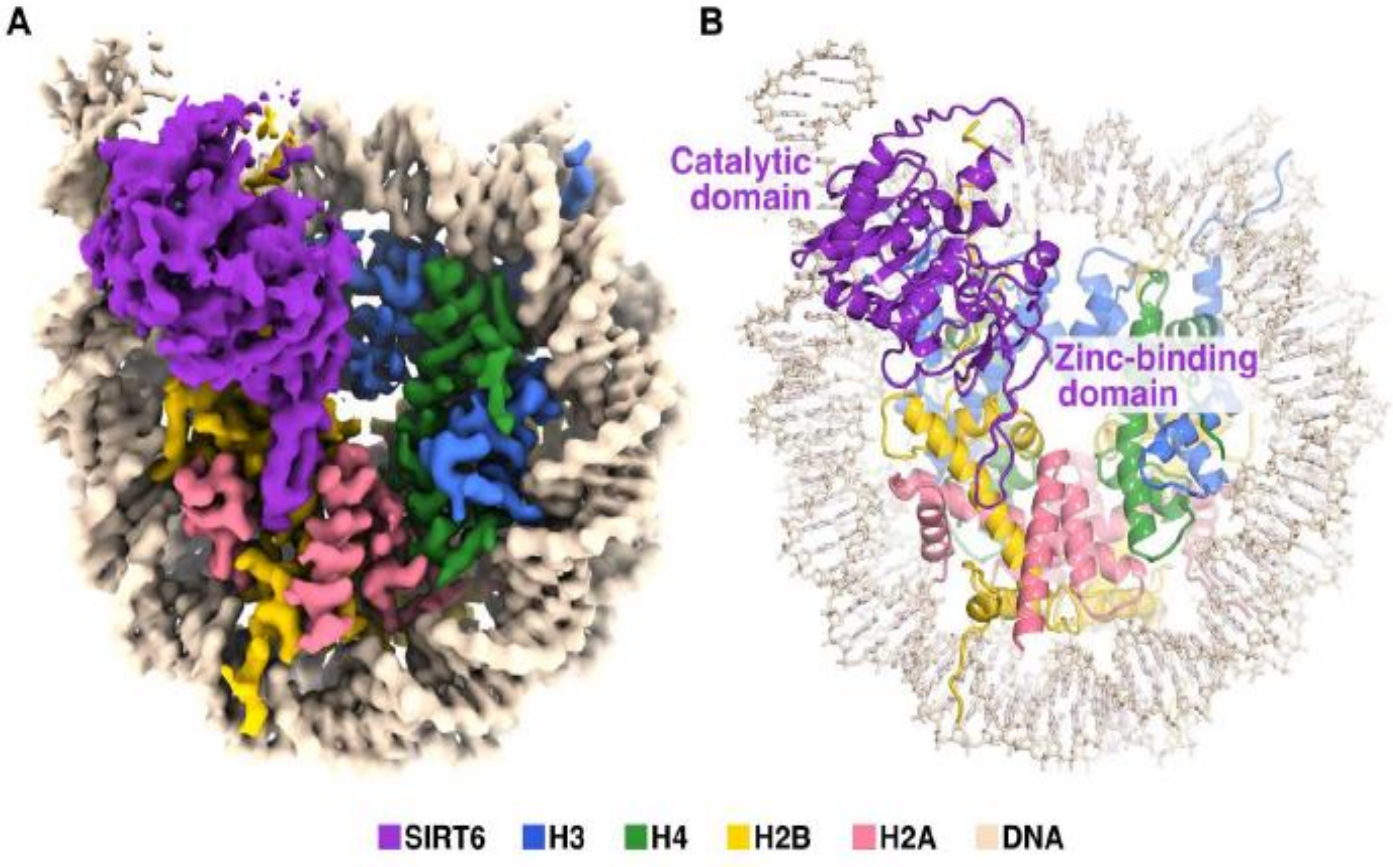


- Lymphocyte activation gene 3 (LAG3 is an immune inhibitory receptor whose presence can lead to reduced immune response in tumor environments
- Favezelimab is an antibody that suppresses LAG3
- Due to small size of antibody FAb to target, dimeric FAb was generated that resulted in a 3.5 Å structure
- Generating dimeric FAbs may aide in other antibody-epitope mapping for future targets.
- Submitting PI: Roy Mariuzza, University of Maryland

EMD-40646 PDB: 8SO3

Mishra et al. *Structure*, 31, 1149-1157 2023

NCEF User Success: Sirtuin-6-Histone

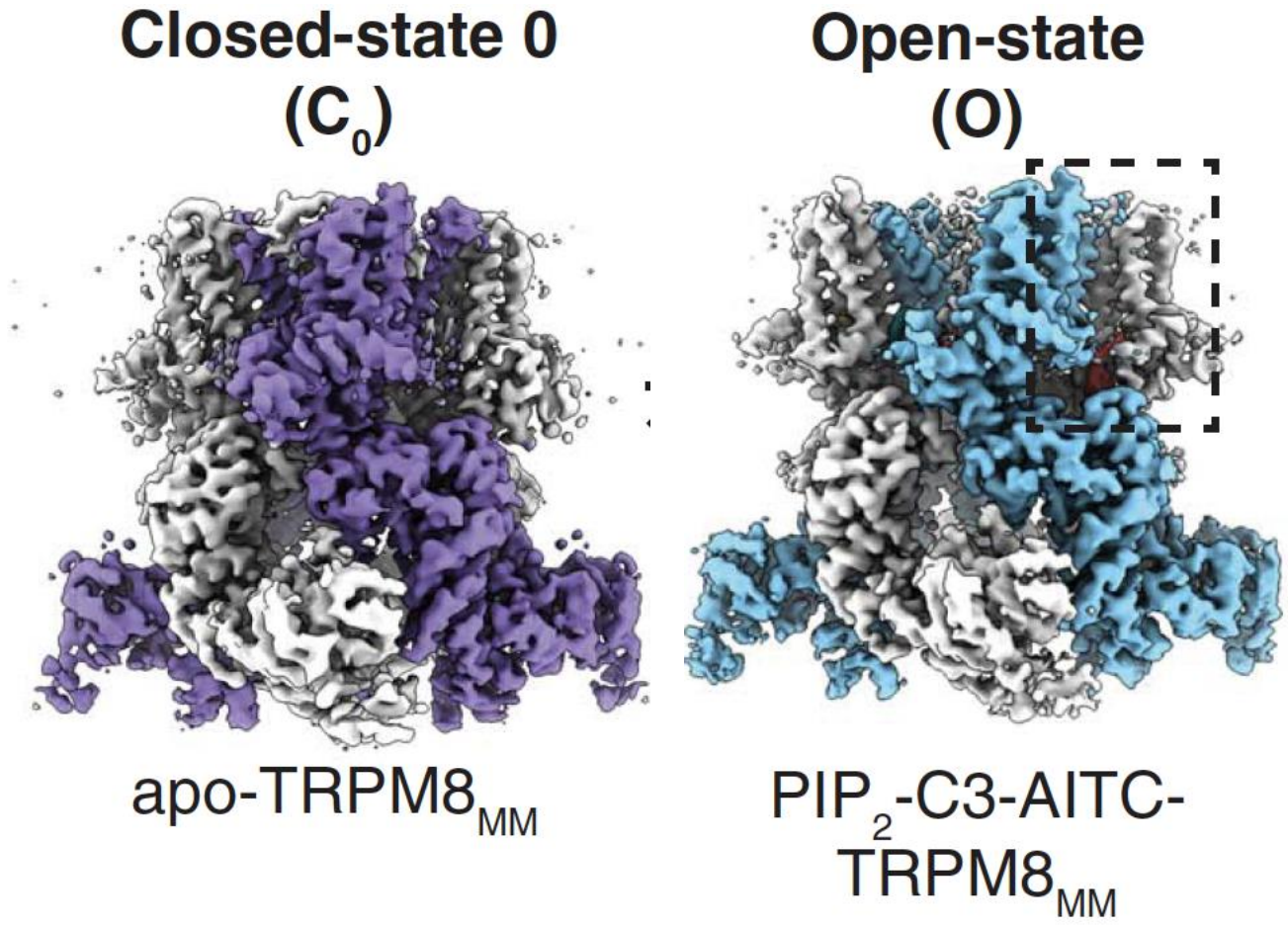


- Sirtuin-6 (SIRT6) has roles in tumorigenesis and ageing
- Structure of SIRT6 in complex with a 172 bp nucleosome was solved to 3.1 Å.
- Structure shows preference to deacetylate histone H3 K9.
- Submitting PI: Jean-Paul Armache, Pennsylvania State University

EMD-29735 PDB: 8G57

Chio et al. *Sci. Adv.,* 9, eadf7856, 2023

NCEF User Success: TRPM8



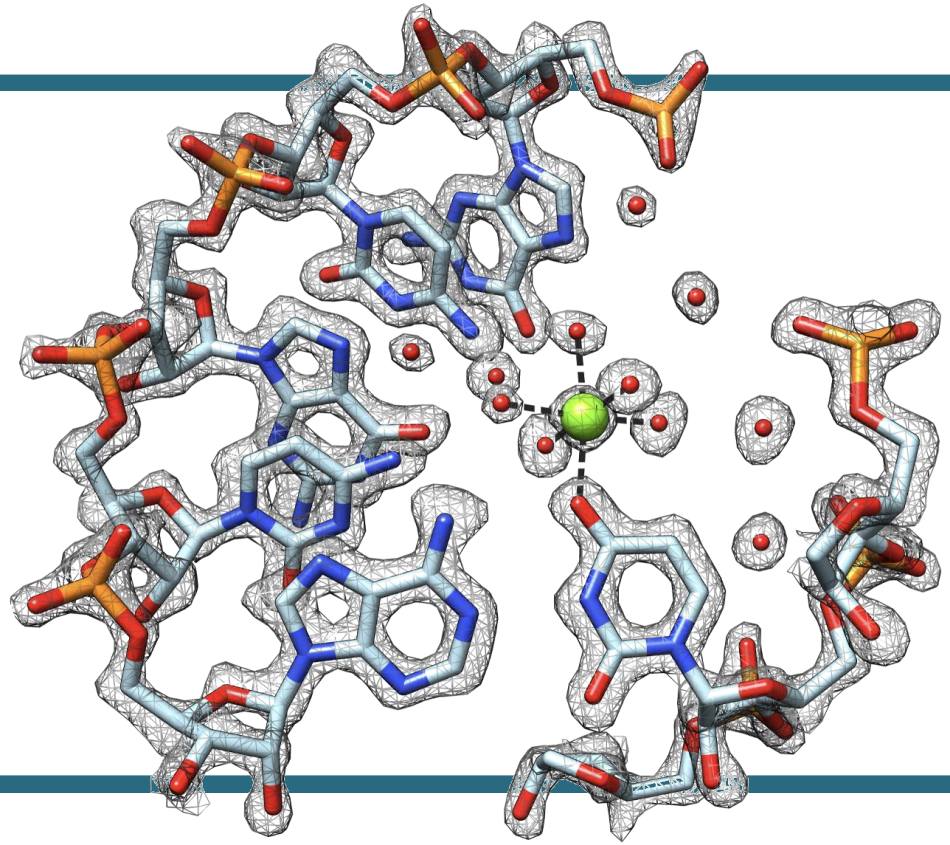
EMD-27895 PDB: 8E4P

EMD-27891 PDB: 8E4L

- TRPM8 is a receptor responsible for cold sensation
- Manipulation of the receptor may lead to alternative inflammation or pain therapeutics.
- Multiple state structures were determined in this paper including three states from NCEF data. Apo-TRPM8 and TRPM8 in the presence of lipid and agonist shown here at 3.6 Å and 3.3 Å respectively.
- Submitting PI: Seok-Yong Lee, Duke University

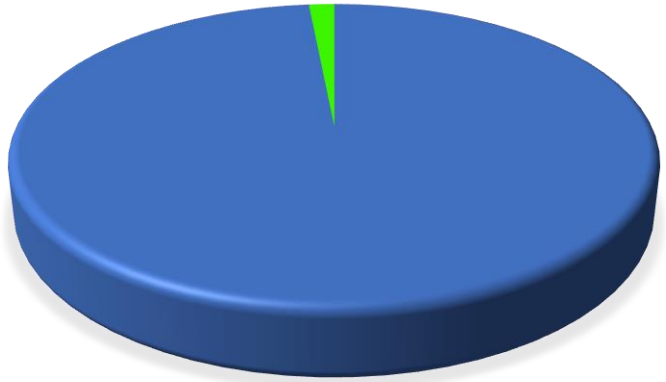
Yin et al. *Science*, 378, eadd1268, 2022

Advance Cryo-EM Technology Team



Cryo-EM Statistics & Technology Gap

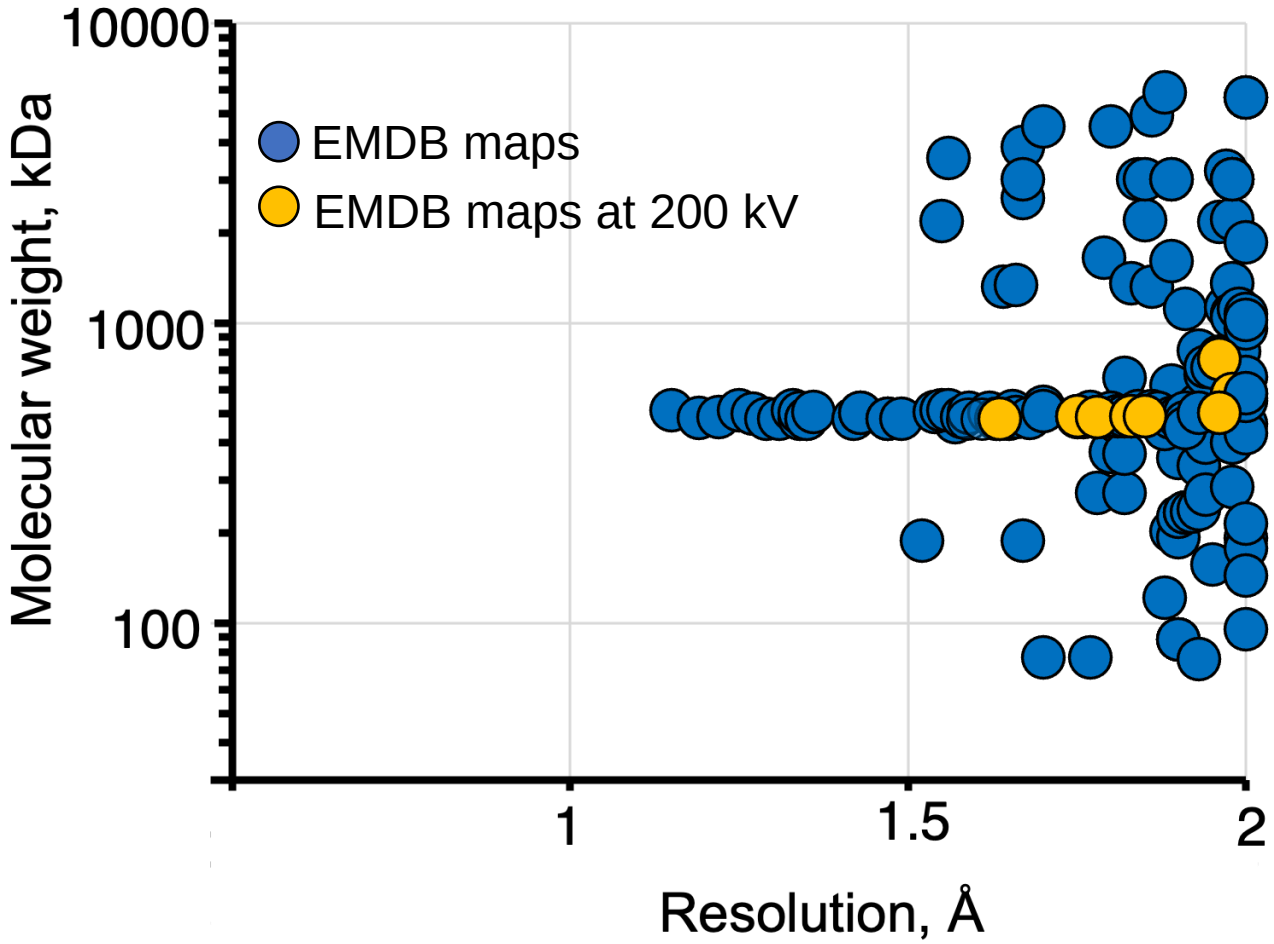
Comparing microscope usage in EMDB data



JEOL

Thermo Fisher

Cryo-EM resolution advancements



Cryo-EM Statistics & Technology Gap

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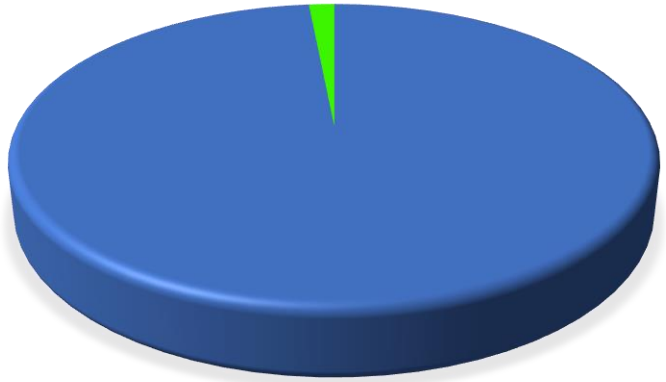
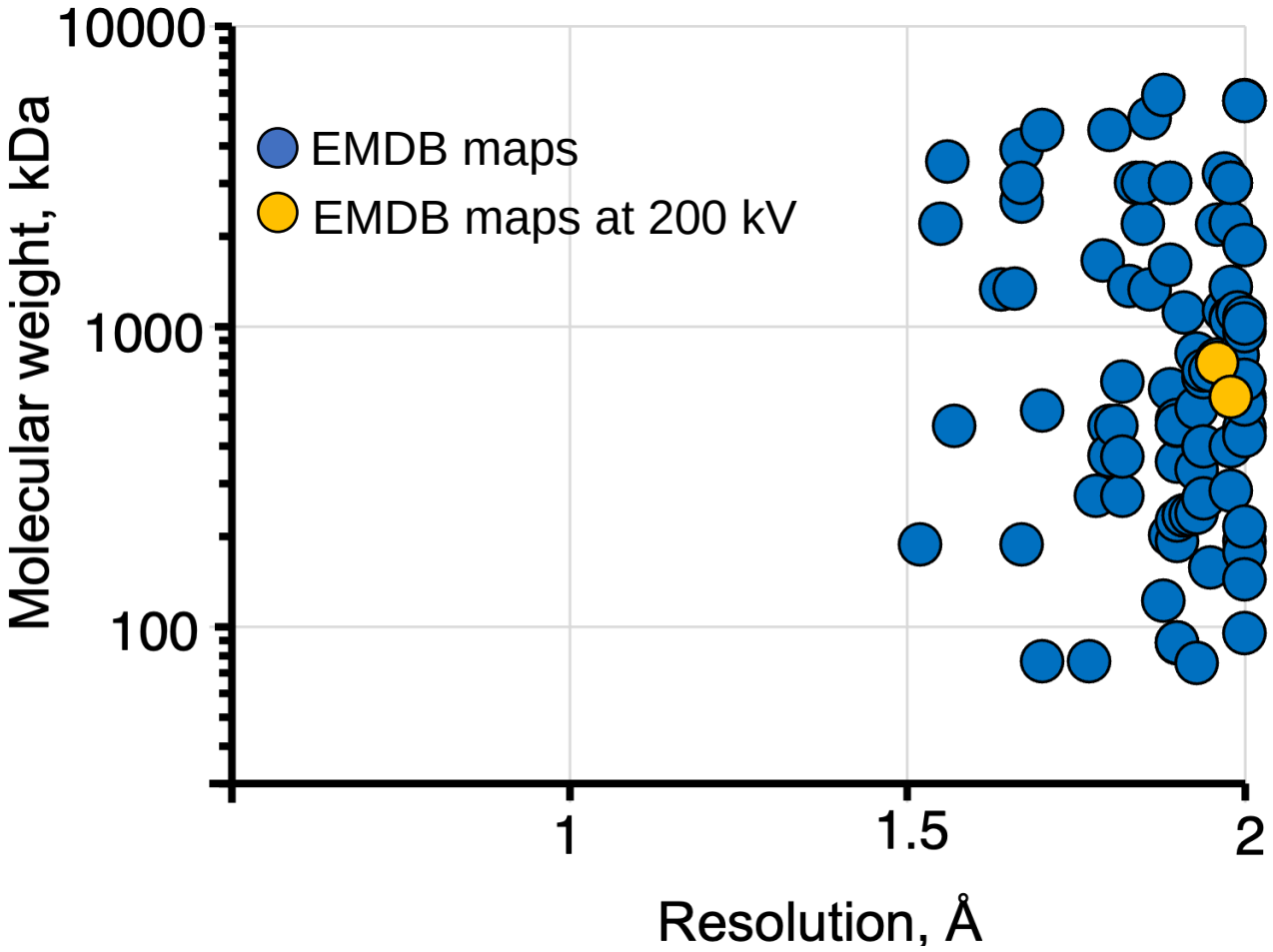


JEOL

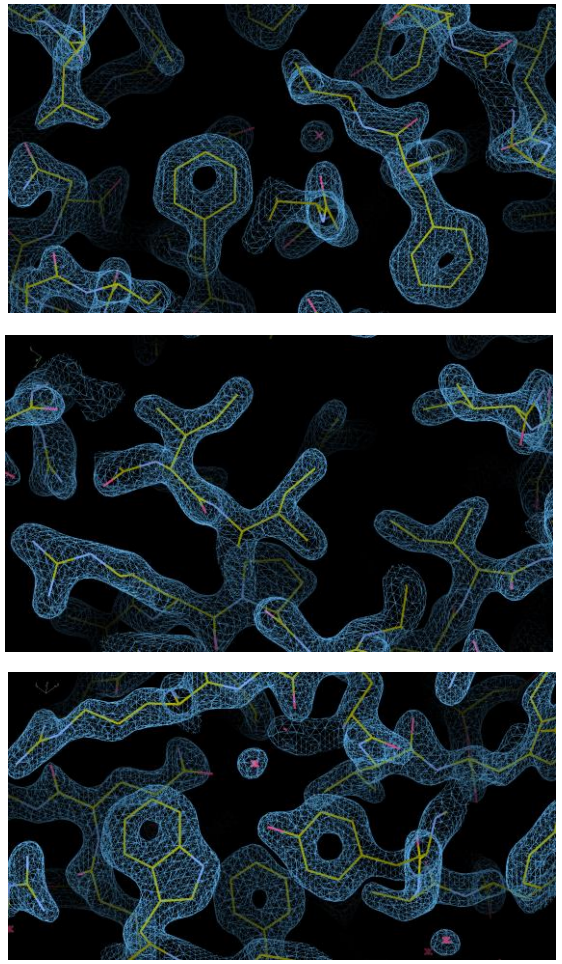
Thermo Fisher

Cryo-EM resolution advancements



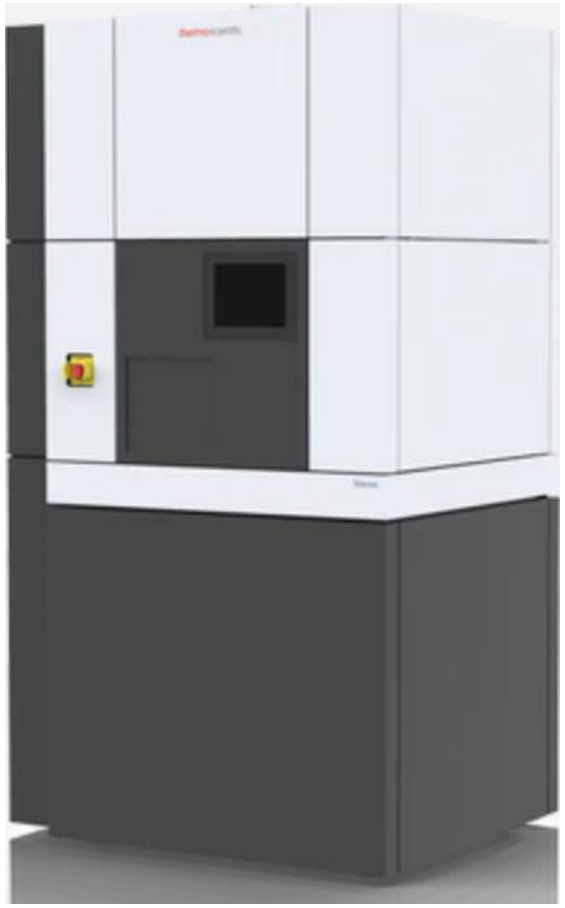
Advancing cryo-EM – High resolution at low cost

2.1 Å resolution



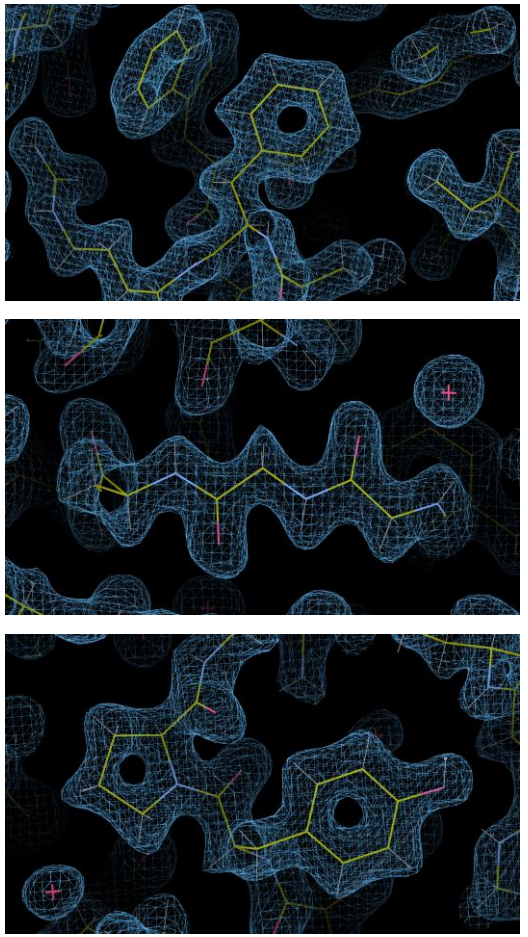
EMPIAR-10817

Thermo Fisher
Glacios



JEOL
CryoARM 200

1.8 Å resolution



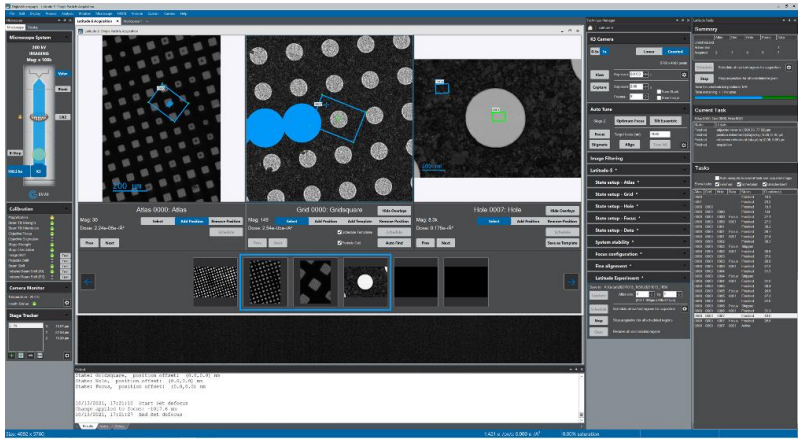
EMPIAR-10466

cCRADA with Gatan Inc

Cooperative Research and Development Agreements with Gatan Inc.

Focus on feature and performance testing of Latitude-S, a software platform for automated cryo-EM data collection

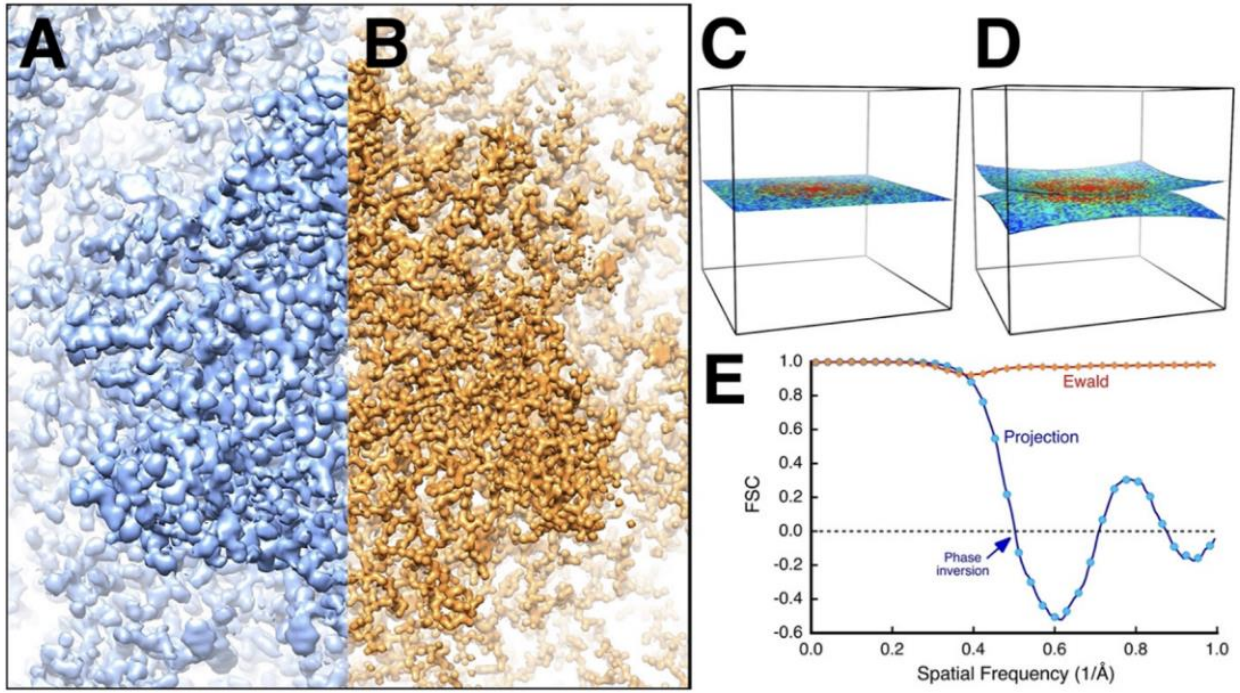
- to increase efficiency of Latitude so that the setup time required for an experiment takes less than 10 minutes not counting aperture centering
- to optimize the data acquisition process to increase imaging throughput to 300 images/hour
- to explore data collection efficiency using different experimental parameters. Parameters to be varied include the carbon grid hole pattern, magnification setting, and dose rate range
- evaluate a new version of Gatan cameras on the CryoARM200 microscope



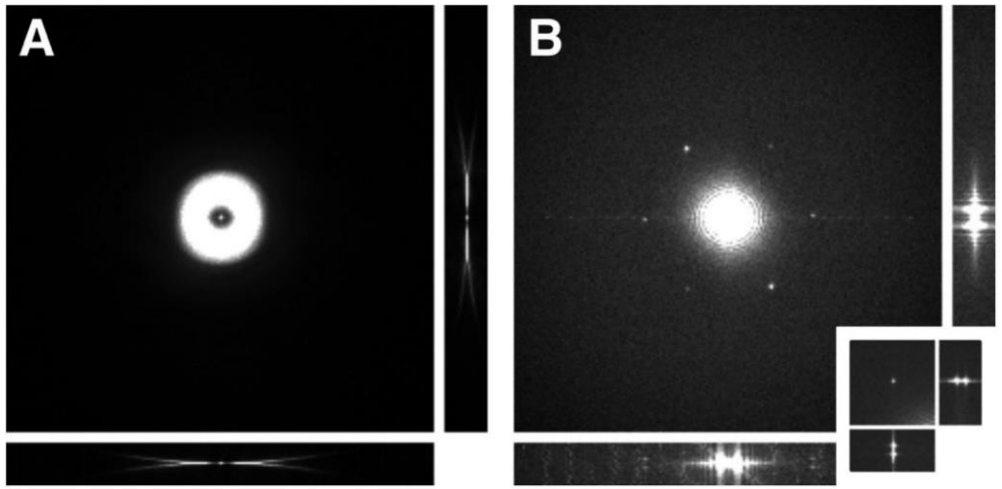
Alpine detector

Unsolved mysteries in cryo-EM

- Accounting for the **Ewald spheres** in cryo-EM reconstitutions and their relationship to 3D Fourier transforms of focal series



(**A**) Simulated reconstructions of an adeno-associated virus capsid (~290 Å diameter) and (**B**) integrating along the Ewald spheres.
(**C**) Illustration of a central section and (**D**) Ewald spheres within frequency space reconstruction boxes. (**E**) Fourier shell correlation of simulated reconstructions compared to the original map



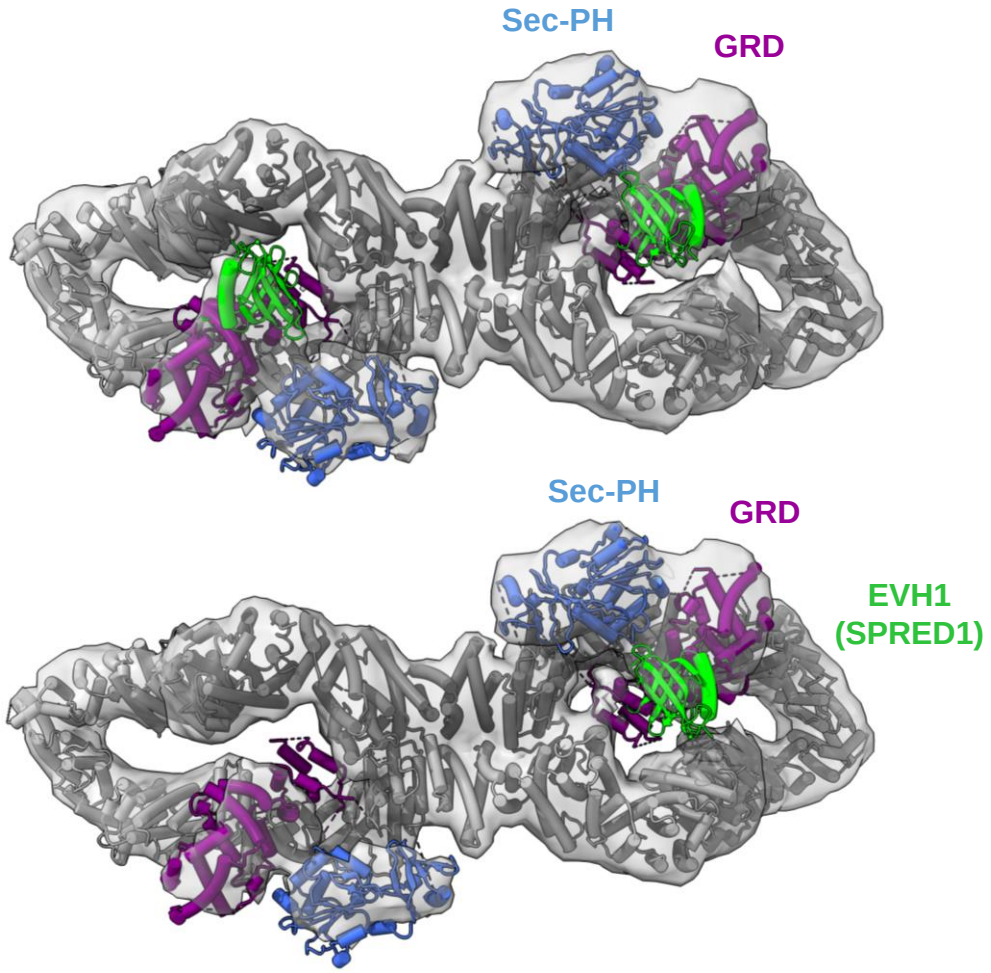
(**A**) Orthogonal views of the 3D power spectrum of a stacked focal series of micrographs of carbon, showing spheres that mimic Ewald spheres.

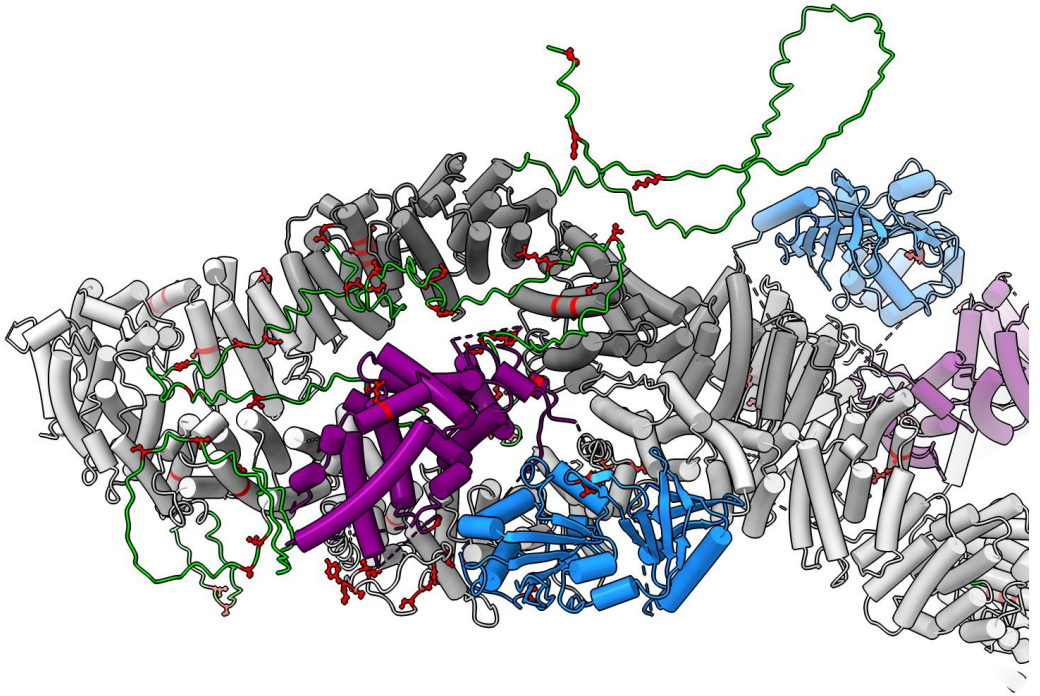
(**B**) Orthogonal views of the 3D power spectrum of stacked focal series of micrographs of graphene oxide. The inset shows orthogonal views of a reflection at 2.13Å with peaks on the apparent Ewald spheres.

Heymann, Merk, Ognjenovic (2023) Microscopy and Microanalysis

Harnessing power of Cryo-EM to study cancer-related targets

Visualizing NF1 complexes in the Ras-MAPK signaling pathway

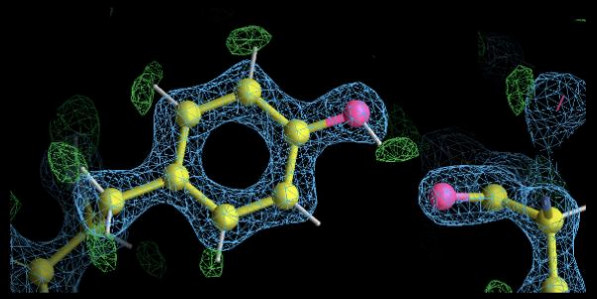


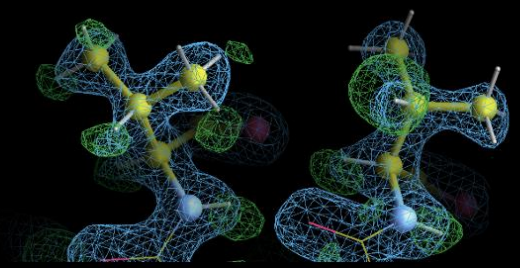


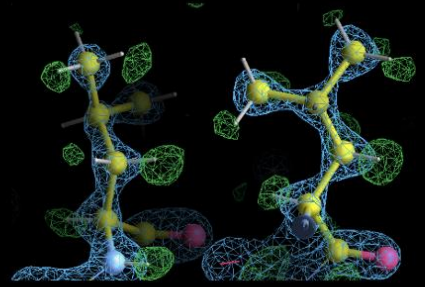
Assessing the importance of phosphorylation sites and flexible loops

Seeing holes and hydrogens for a range of samples

Standard samples

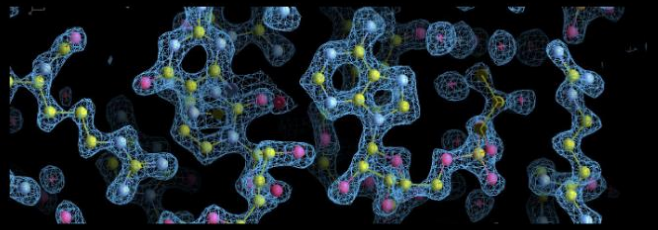




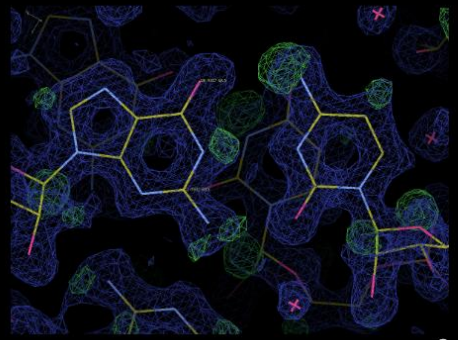


Beta gal at 1.6Å

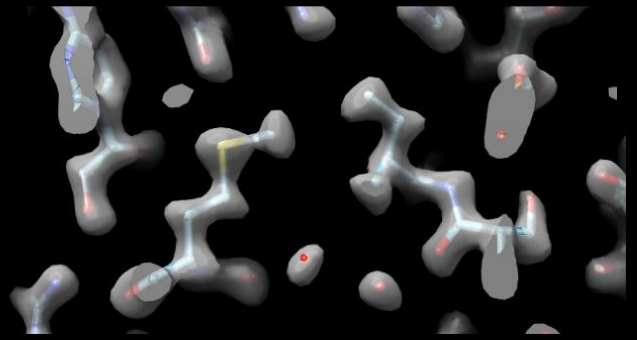
Common samples



Human Ribosome at 1.7Å

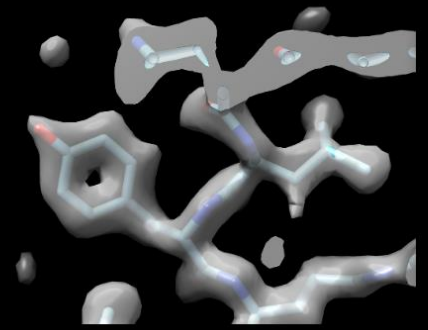


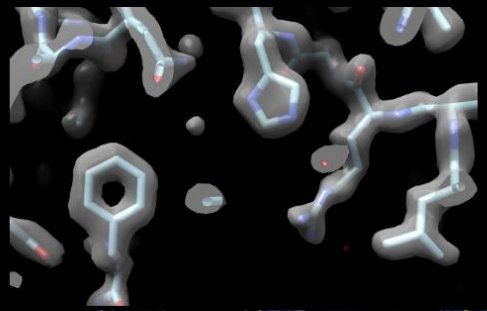
Rabbit Ribosome at 1.7Å

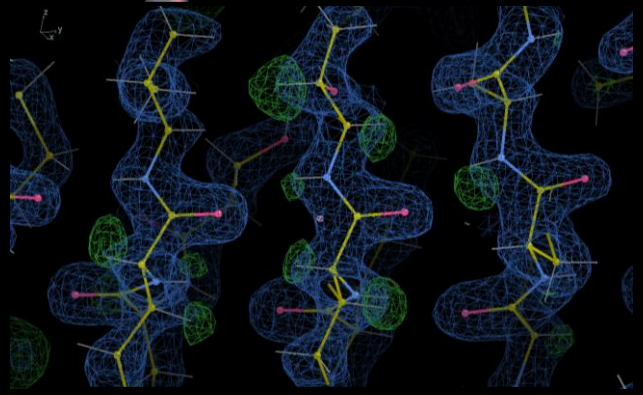


Bacterial Ribosome at 1.8Å

Challenging samples

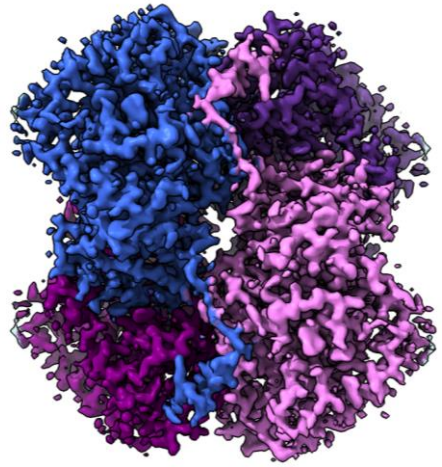


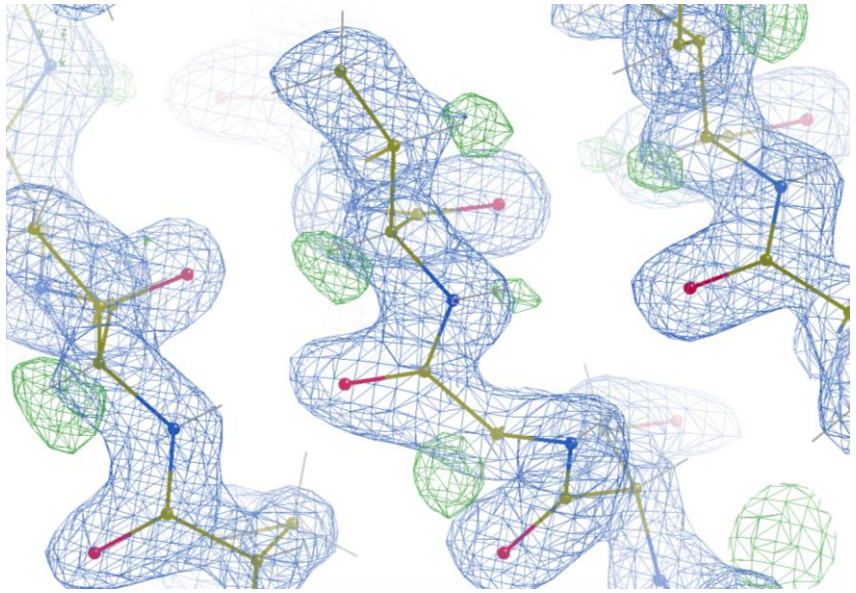




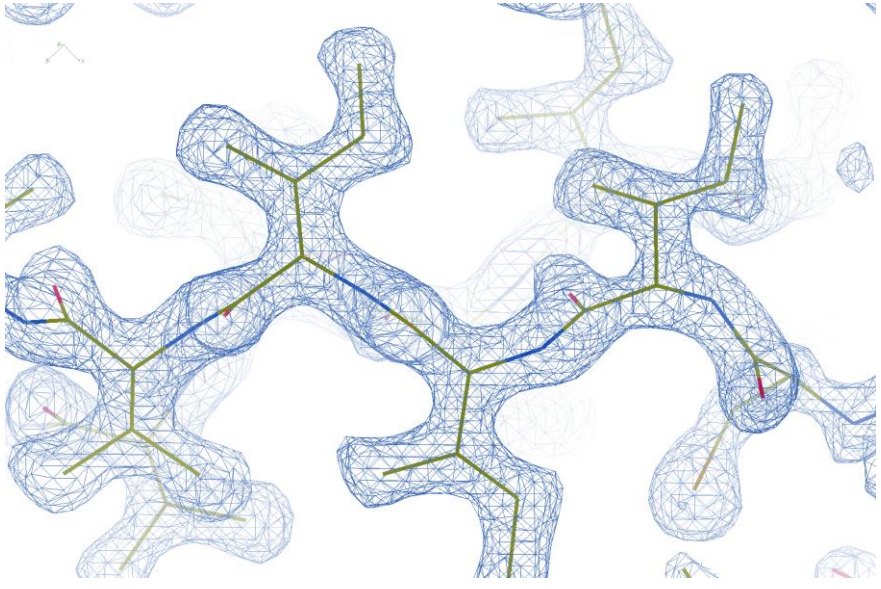
145 kDa protein at 1.8Å

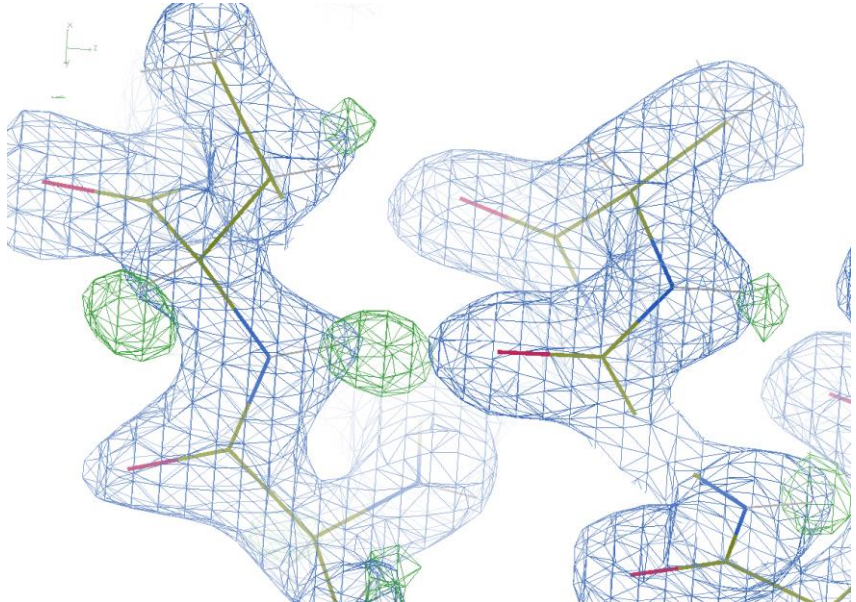
Visualizing hydrogen atoms in human LDH

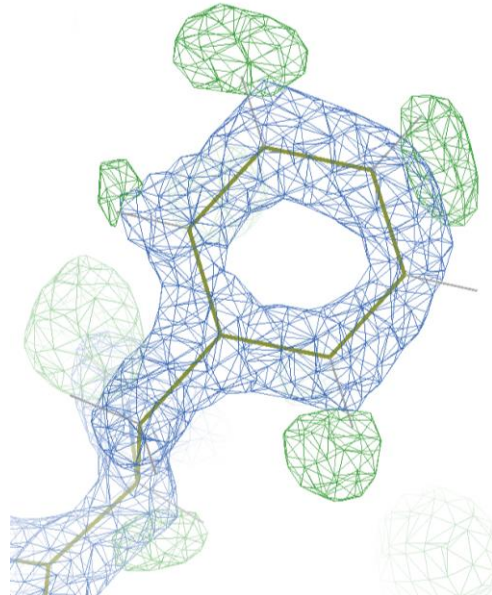




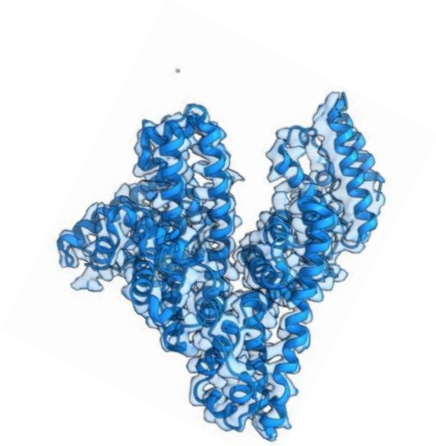
- 145 kDa, D2 symmetry
- Currently at 1.8Å resolution
- Plays a crucial role in the Warburg effect in cancer cells
- Despite its importance, there are currently no FDA-approved drugs that target LDH

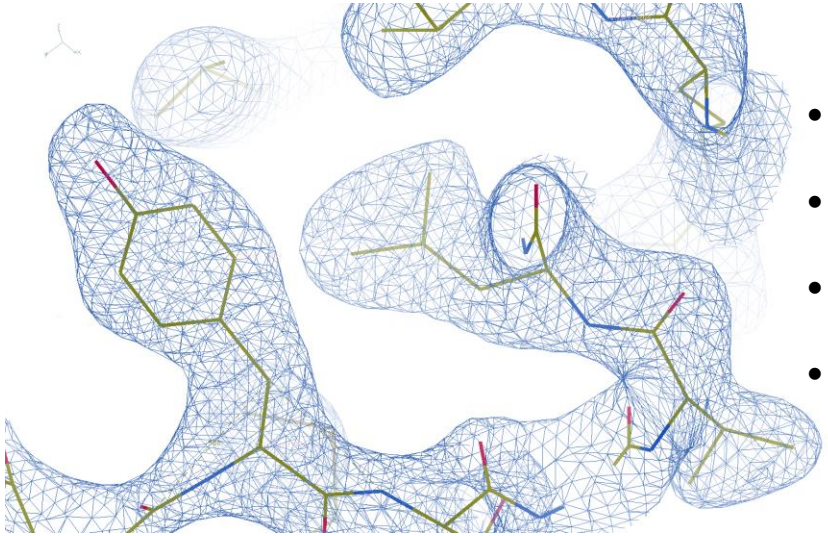


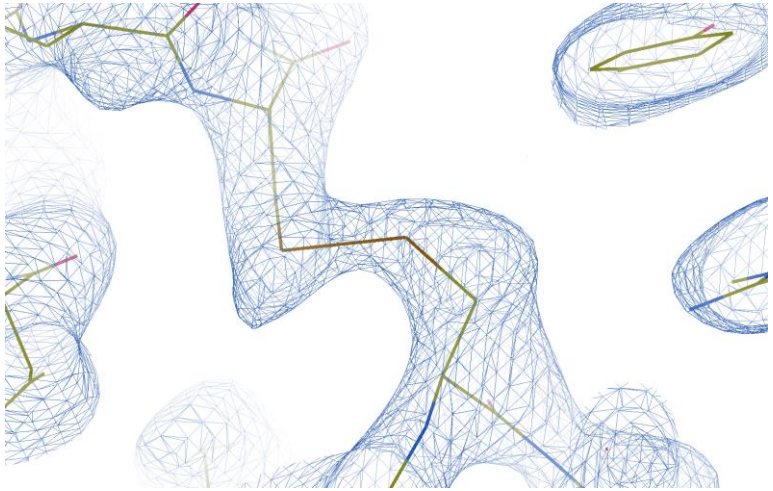


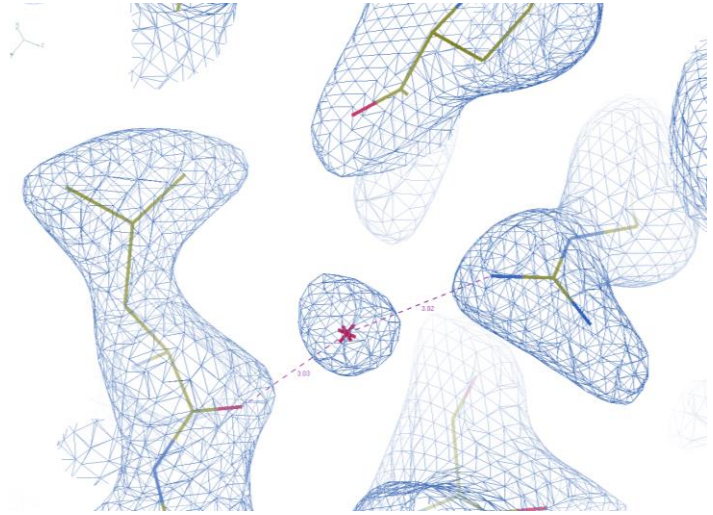


Human Serum Albumin (HSA)





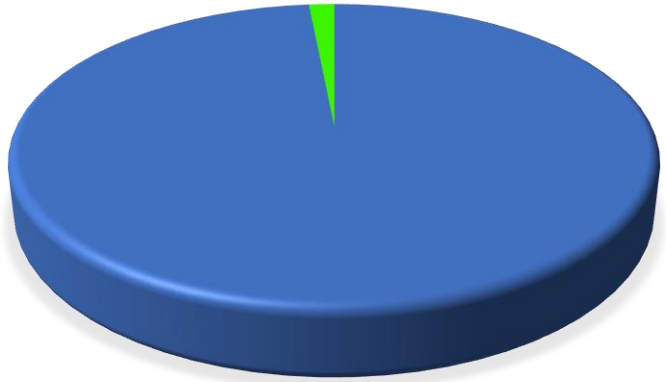




- 67 kDa, asymmetric
- Currently at 2.5Å resolution
- Most abundant protein in human plasma
- Has the ability to bind a wide variety of ligands with high affinity
- Binding of various ligands induces conformational changes and affects drug binding
- Post-translational modifications also modulate its binding properties

Cryo-EM Statistics & Technology Gap

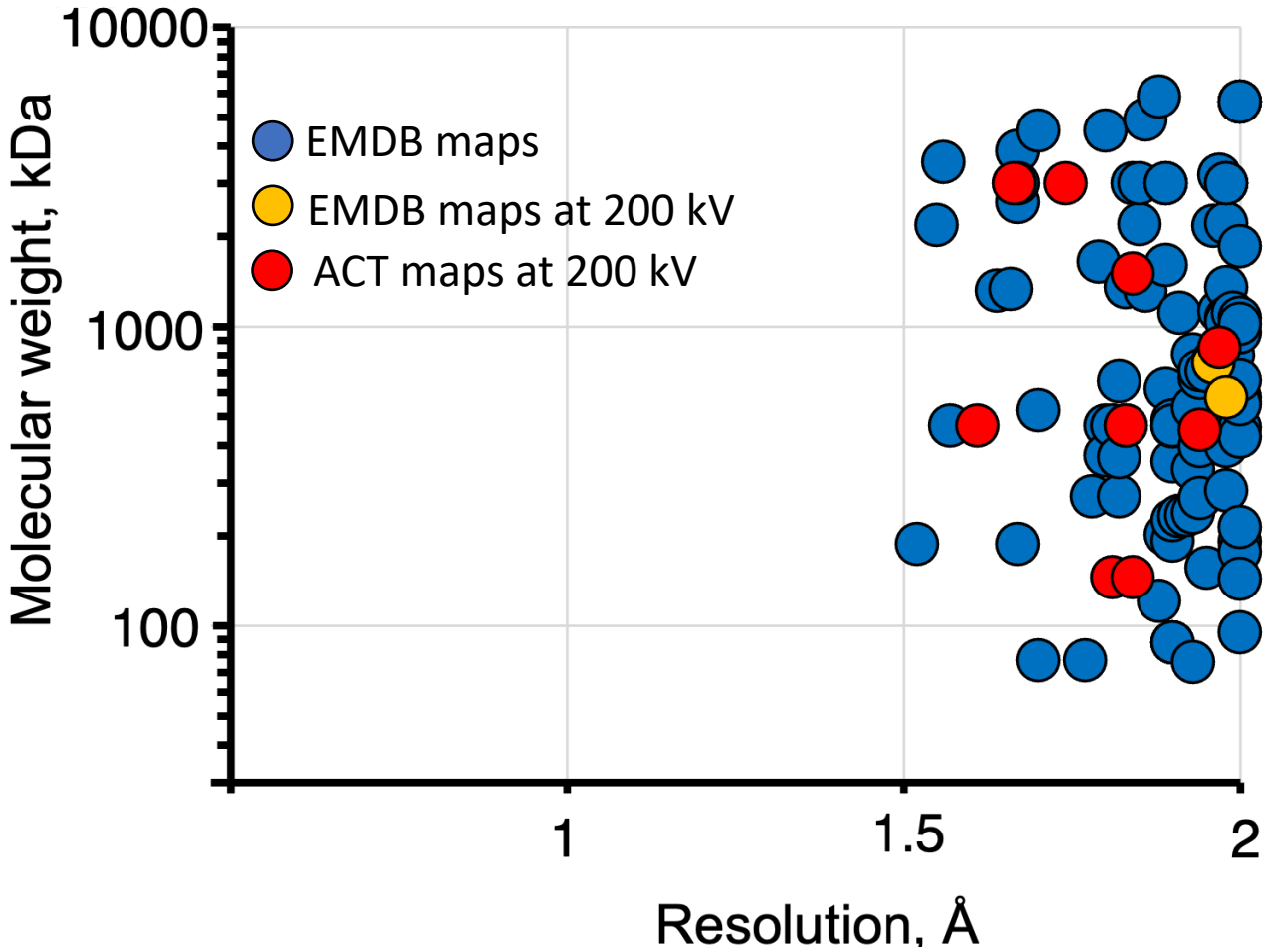
Comparing microscope usage in EMDB data



JEOL

Thermo Fisher

Cryo-EM resolution advancements



Future

Improved single-particle cryo-EM platform

Cost-effective microscope with minimized chromatic aberration for ultra high-resolution single-particle cryo-EM imaging

In situ tomography

Enabling the visualization of molecules within the cellular context providing deeper biological insights

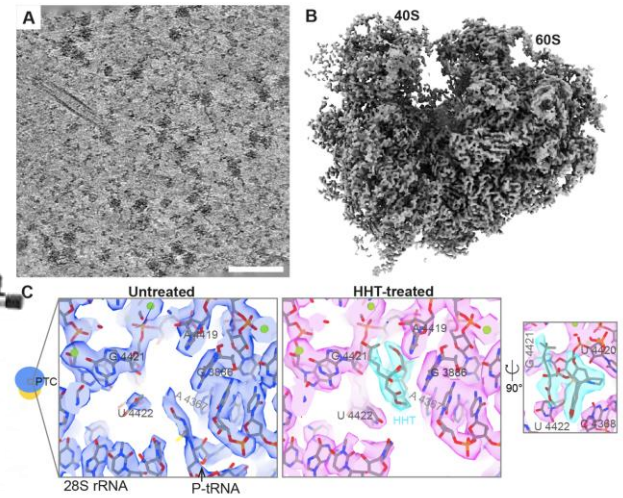


Fig. 1. 80S ribosome structures in human cells.

Xing et al. (2023), bioRxiv

ACT team

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Cryo-EM Training Program – Coming in Summer 2024

Event Overview

The inaugural NCEF Cryo-EM Training Program will be held in-person at the FNLCR's Advanced Technology Research Facility (ATRF) in Frederick, MD.

- Mornings will feature guest lecturers (FNL experts and invited faculties) who will provide extensive classroom learning on topics including sample preparation, grid screening, data collection and processing, structure determination and model building and validation.
- Afternoons will move into the National Cryo-EM Facility for comprehensive hands-on training in a laboratory setting.

FNLCR Technical Leads:

- **Jana Ognjenovic, PhD & Thomas Edwards, PhD**

2022 Training Program