Update on the National Cryo-EM Facility (NCEF)
Dwight V. Nissley, PhD - Director, Cancer Research Technology Program, FNLCR
October 18, 2021
FNLCR Cancer Research Technology Program (CRTP)

Dr. Ethan Dmitrovsky
Laboratory Director, FNLCR

Dr. Len P. Freedman
Chief Science officer

Dr. Dwight V. Nissley
Directorate Head, CRTP

National Missions
- NCI RAS Initiative
- National Cryo-EM Facility

Extramural Enabling
- Nanotechnology Characterization Lab (NCL)
- Antibody Characterization Lab (ACL)

Technology Support 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
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

* As defined by Sriram Subramaniam, 2017
NCEF and ACT Instrumentation

• NCEF provides **no-cost access** to two Titan Krios 300 kV X-FEG cryo-electron microscopes. These microscopes are equipped with:
  - Thermo Fisher Falcon 3C direct electron detectors
  - Gatan Bioquantum energy filters
  - Gatan K3 direct electron detector cameras
  - Volta Phase Plate (Yellow microscope only)

• Users with cancer related projects can gain access to imaging slots through our access portal: [https://ncef.submittable.com/login](https://ncef.submittable.com/login)

• Users are provided reports containing all images, imaging parameters and statistics to evaluate their samples.

• The Advanced Cryo-EM Technology group has two 200 kV cryo-electron microscopes to evaluate cutting edge and lower-cost technologies for high resolution structure determination.
  - JEOL CryoARM 200 is equipped with a cold-FEG, Omega energy filter, and Gatan K3 direct electron detector.
  - Thermo Fisher Glacios is equipped with Falcon 3C and a Direct Electron DE-64 cameras.
NCI National Cryo-EM Facility Associated Publications - 2021

More than 50 publications, primarily in high-impact journals

19 publications thus far in 2021

- Ultrapotent antibodies against diverse and highly transmissible SARS-CoV-2 variants
  Wang L, ..., Misasi J.  *Science*. 2021

- Cryo-EM structure of the periplasmic tunnel of T7 DNA-ejectosome at 2.7 Å resolution
  Swanson NA, ..., Cingolani G.  *Mol Cell*. 2021

- Structural insight on assembly-line catalysis in terpene biosynthesis
  Faylo JL, ..., Christianson DW.  *Nat Commun*. 2021

- Mechanistic insight into substrate processing and allosteric inhibition of human p97
  Pan M, ..., Zhao M.  *Nat Struct Mol Biol*. 2021

- Structures of the mycobacterial membrane protein MmpL3 reveal its mechanism of lipid transport
  Su CC, ..., Yu EW.  *Plos Biol*. 2021

- SARS-CoV-2 S2P spike ages through distinct states with altered immunogenicity
  Olia AS, ..., Kwong PD.  *J Biol Chem*. 2021

- Expression and characterization of SARS-CoV-2 spike proteins
  Schaub JM, ..., Finkelstein IJ.  *Nat Protoc*. 2021

- Nanobodies from camelid mice ad llamas neutralize SARS-CoV-2 variants
  Xu J, ..., Casellas R.  *Nature*. 2021

- DPP9 sequesters the C terminus of NLRP1 to repress inflammasome activation
  Hollingsworth LR, ..., Wu H.  *Nature*. 2021

- Structural basis of ribosomal RNA transcription regulation
  Shin Y, Qayyum MZ, ...Murakami KS.  *Nat Commun*. 2021

- Purification and cryoelectron microscopy structure determination of human V-ATPase
  Wang L, Chen Z, Wu H, Fu TM.  *STAR Protoc*. 2021

- A 'Build and Retrieve' methodology to simultaneously solve cryo-EM structures of membrane proteins
  Su CC, ... Robinson CV, Yu EW.  *Nat Methods*. 2021

- Seesaw conformations of Npl4 in the human p97 complex and the inhibitory mechanism of a disulfiram derivative
  Pan M, ..., Zhao M.  *Nat Commun*. 2021

- Structural mechanism of heat-induced opening of a temperature-sensitive TRP channel
  Nadezhdin KD, ..., Sobolevsky AI.  *Nat Struct Mol Biol*. 2021

- Structural analysis of cross α-helical nanotubes provides insight into the designability of filamentous peptide nanomaterials
  Wang F, ...Egelman EH, Conticello VP.  *Nat Commun*. 2021

- Cryo-EM structures of engineered active bc1-cbb3 type CIII2CIV super-complexes and electronic communication between the complexes
  Matyszewski M, ..., Sohn J.  *Nat Commun*. 2021

- Distinct axial and lateral interactions within homologous filaments dictate the signaling specificity and order of the AIM2-ASC inflammasome
  Matyszewski M, ..., Sohn J.  *Nat Commun*. 2021

- Potent neutralizing nanobodies resist convergent circulating variants of SARS-CoV-2 by targeting diverse and conserved epitopes
  Sun D, ..., Shi Y.  *Nat Commun*. 2021

- Distinct axial and lateral interactions within homologous filaments dictate the signaling specificity and order of the AIM2-ASC inflammasome
  Matyszewski M, ..., Sohn J.  *Nat Commun*. 2021
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
Plan to Provide Sample Grid Freezing

- Grid preparation and screening are major bottle necks for investigators
- Biologists/biochemists with no cryo-EM infrastructure at home institutions are particularly limited
- New grid freezing technology is highly automated, controls for more variables and also addresses preferred orientation and other air-water interface problems with millisecond scale freezing times
- Provide the latest grid freezing technology (Chameleon, Vitrojet)
- Samples frozen and screened at NCEF
- Final imaging on a higher-end cryo-electron microscope (Titan Krios)
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
# Planned Training Course at FNL (Summer 2022)

<table>
<thead>
<tr>
<th>Topic</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Day 5</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Morning Lectures</strong></td>
<td>Grid Preparation</td>
<td>Alternative Grid Preparation Platforms</td>
<td>Screening &amp; Data Collection</td>
<td>Data Processing</td>
<td>Model Building &amp; Validation</td>
</tr>
<tr>
<td></td>
<td>Cryo-EM sample preparation</td>
<td>Problems at the air/water interface and how to minimize its effects</td>
<td>Aspects of TEM projects</td>
<td>RELION apoferritin data set (4h)</td>
<td>Refmac lecture + demo (2h)</td>
</tr>
<tr>
<td></td>
<td>• Introduction (30 min)</td>
<td>• Grid selection (1 h)</td>
<td>• Operation and overview of TEM (1h)</td>
<td></td>
<td>COOT (1h)</td>
</tr>
<tr>
<td></td>
<td>• Brief cryo-EM overview (30 min)</td>
<td>• Air/water interface (30 min)</td>
<td>• Grid quality assessment (30 min)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Sample preparation (30 min)</td>
<td>• Problems at the air/water interface and how to minimize its effects</td>
<td>• Automated acquisition software (1h)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Negative staining (30 min)</td>
<td>• Grid selection (1 h)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Sample vitrification using conventional plunge-freezing devices (1h)</td>
<td>• Air/water interface (30 min)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Leica plunge freezer (30 min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Afternoon Hands-on</strong></td>
<td>Work on plunge-freezing using Vitrobot instruments</td>
<td>Grid clipping and loading</td>
<td>Demonstration on Titan Krios microscope</td>
<td>CryoSPARC proteasome data set (4h)</td>
<td>Chimera / ISOLDE</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Phenix lecture + demo (2h)</td>
</tr>
</tbody>
</table>
Three Areas of Focus for Technology Development

Mapping specific biological projects to current and future NCEP portfolio

- β-gal
- Receptors
- Cancer targets
- NF1
- Raf/Ras
- Viruses (HIV/HPV)

New hardware and software platforms

- JEOL and DE-64
  - Goal is to establish cryoEM workflows with alternative platforms that could lower costs

Grid preparation

- Chameleon, Vitrojet
  - Goal is to test better methods for specimen preparation

Challenging biochemistry

- Small, disordered, membrane-associated proteins
  - Goal is to develop methods to streamline purification protocols optimized for cryoEM
Cryo-EM statistics and trends

Trend in direct electron detector usage distribution

- Gatan
- Thermo Fisher
- DE

Direct electron detector usage distribution

Trend in microscope usage distribution

- Thermo Fisher
- JEOL
- Other

Microscope usage distribution
Advancing Cryo-EM – High Resolution at Lower Cost

2.1 Å resolution

EMPIAR-10817

Thermo Fisher Glacios

Falcon 3C and Direct Electron DE-64 cameras

JEOL CryoARM 200

Gatan K3 Camera

1.8 Å resolution

EMPIAR-10466
\( \beta \)-galactosidase at 1.61\( \text{Å} \) Resolution
Neurofibromin (NF1)

• mutated in at least 12% of human cancers (multiple missense and nonsense mutations)
• primary cause of neurofibromatosis type 1 (1:3500 newborns)
• 320 kDa protein (2818 amino acids)
• highly conserved from yeast to humans
• functions other than RAS-GAP mostly unknown

The neurofibromin recruitment factor Spred1 binds to the GAP related domain without affecting Ras inactivation

Theresia Dunzendorfer-Matt1, Ellen L. Mercado1,2, Karl Maly1, Frank McCormick1,2, and Klaus Scheffzek1,2
1Division of Biological Chemistry, Biocenter, Medical University of Innsbruck, 6020 Innsbruck, Austria; 2Helen Diller Family Comprehensive Cancer Center, University of California, San Francisco, CA 94158, and "Division of Medical Biochemistry, Biocenter, Medical University of Innsbruck, 6020 Innsbruck, Austria
Contributed by Frank McCormick, May 10, 2016 (sent for review November 3, 2015; reviewed by Jonathan Lisht and Nancy Katner)
Cryo-EM studies of NF1

Protein aggregation on first grids
Optimized cryo-EM grid

Initial 2D Classes
Cryo-EM studies of NF1

Proposed RAS-binding sites are occluded in the NF1 dimer
Advancing Cryo-EM through Technology Development

COVID-19

- Nsp1 of SARS-CoV-2 bound to 40S subunit of human ribosome at 2.0 Å resolution
- Technically-challenging sample
- Identified solvent-mediated contacts, including at interaction hot spot, which could be crucial for SBDD
- Highest resolution for SARS-CoV-2 protein by cryo-EM

Cancer

- Ribosome bound to omacetaxine (used to treat patients with CML) at 1.7 Å resolution
- Highest resolution for FDA-approved drug by cryo-EM
- Highest resolution structure of ribosome to date
- Allows visualization of PTMs, water molecules, metal ions, and even hydrogen atoms
Plan for Collaboration with Cancer Researchers

Extend NCEP resources to less experienced researchers including basic cell and cancer biologists that have limited structural biology expertise

• Hold workshop at FNL for extramural cancer researchers to describe cryo capabilities and to learn cancer biology community priorities for single particle analysis or cryo-ET.

• Establish a mechanism for visiting researchers to advance cancer-related projects while learning so they can do future cryo-EM work at their own institution.

  - This could include opportunities for postdocs with little or no structural biology expertise to work at FNL and their home institution to advance cancer projects.
National Cryo-EM Program Organization

Dwight Nissley  
Director, CRTP, FNLCR

Thomas Edwards  
Senior Microscopist  
NCEF

Adam Weir  
Electron Microscopist  
NCEF

Tara Fox  
Electron Microscopist  
NCEF

Helen Wang  
Program Manger  
NCEF

Matt Hutchison  
IT Support  
NCEF

Joseph Finney  
IT Support  
NCEF

National Cryo-EM Consultant  
(Open)

- Recognized thought leader in cryo-EM  
- Advise on emerging technologies  
- Extramural cancer research community

Jana Ognjenovic  
Senior Scientist  
ACT

Alan Merk  
Electron Microscopist  
ACT

Reinhard Grisshammer  
Biochemist  
ACT