Frederick National Laboratory
for Cancer Research

sponsored by the National Cancer Institute

Update on the National Cryo-EM Facility (NCEF)

Dwight V. Nissley, PhD - Director, Cancer Research Technology Program, FNLCR

October 18, 2021

DEPARTMENT OF HEALTH AND HUMAN SERVICES • National Institutes of Health • National Cancer Institute

The Frederick National Laboratory is a Federally Funded Research and Development Center operated by Leidos Biomedical Research, Inc., for the National Cancer Institute

FNLCR Cancer Research Technology Program (CRTP)

Frederick National Laboratory for Cancer Research

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

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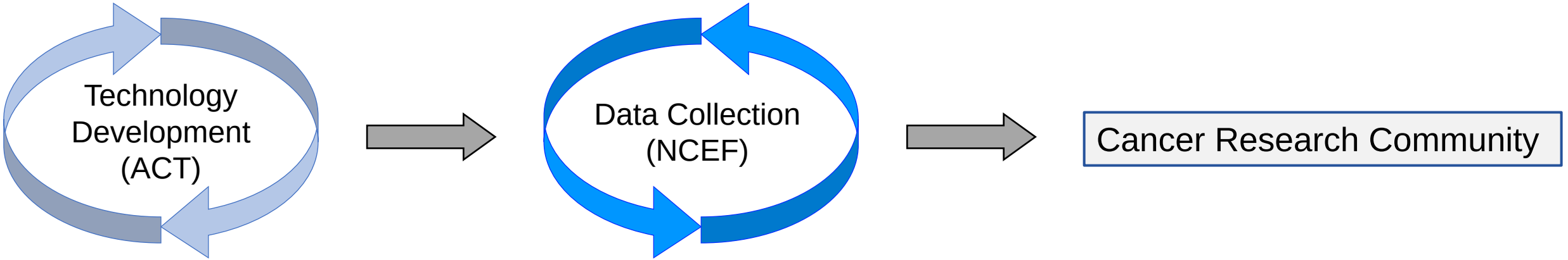
1. National Cryo-EM Facility 2017-present
   - Extramural user facility for cryo-EM data collection,
   - Ongoing expansion of scope, bandwidth and turn around

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
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- Group III. Biologists with interest in important biomedical problems
  - interested in adding cryo-EM methods to their toolkit
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* As defined by Sriram Subramaniam, 2017

NCEF and ACT Instrumentation

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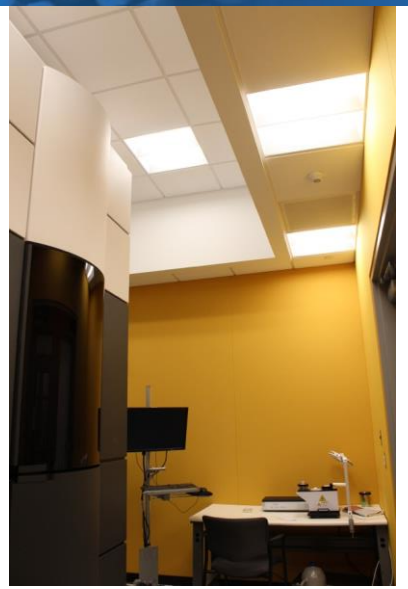
- NCEF provides no-cost access to two Titan Krios 300 kV X-FEG cryo-electron microscopes. These microscopes are equipped with:
  - Thermo Fisher Falcon 3C direct electron detectors
  - Gatan Bioquantum energy filters
  - Gatan K3 direct electron detector cameras
  - Volta Phase Plate (Yellow microscope only)
- Users with cancer related projects can gain access to imaging slots through our access portal: https://ncef.submittable.com/login
- 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.



National Cryo-EM Facility (NCEF) - Four Years of Operation

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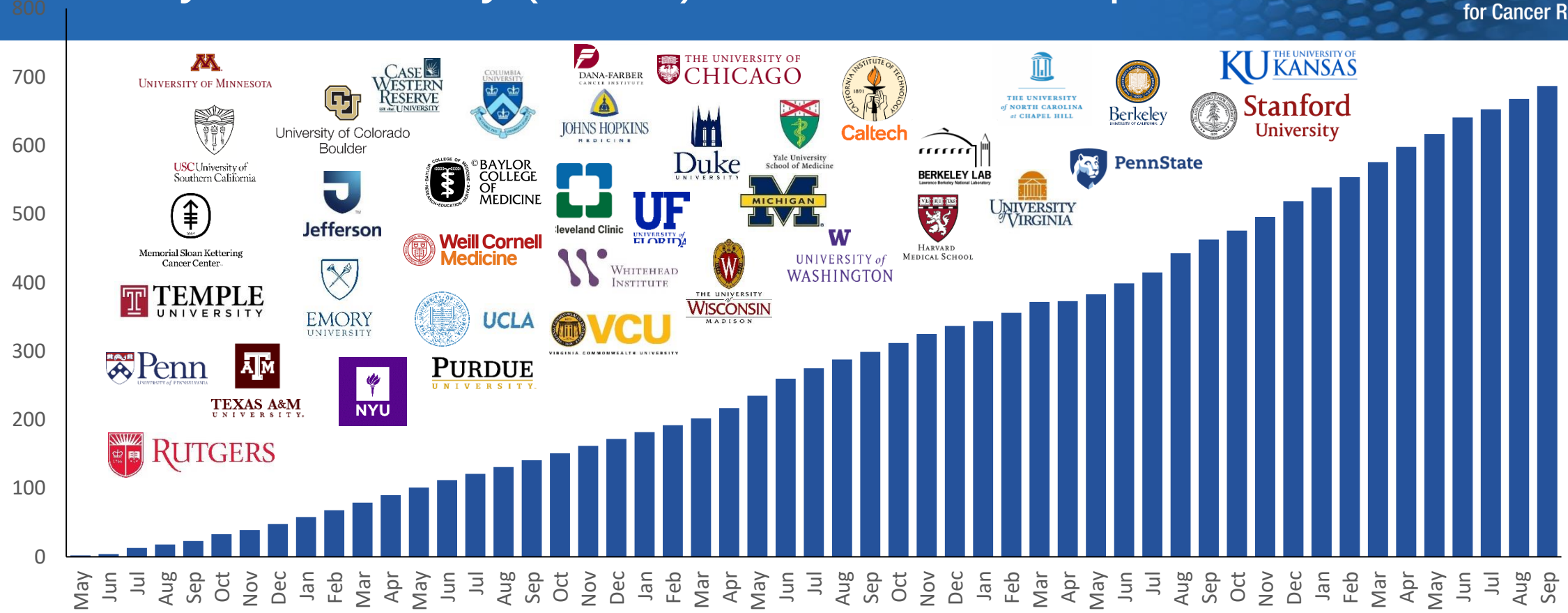

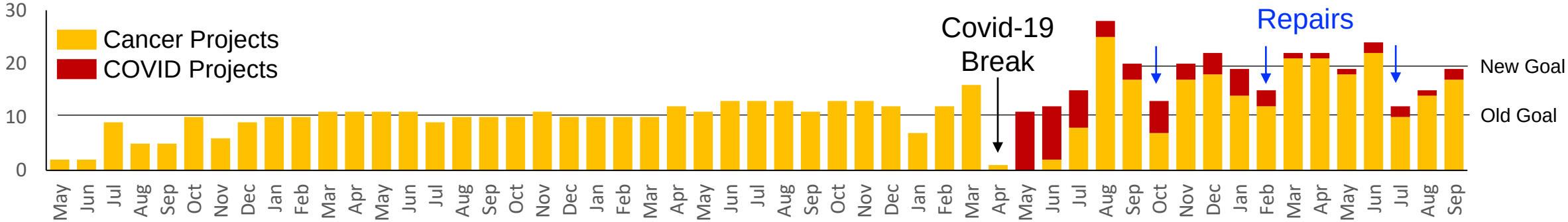

NCI National Cryo-EM Facility Associated Publications - 2021

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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
Steimle S,…, Daldal F. 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
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Plan to Provide Sample Grid Freezing

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- 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)

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Planned Training Course at FNL (Summer 2022)

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

Advance Cryo-EM Technology (ACT)

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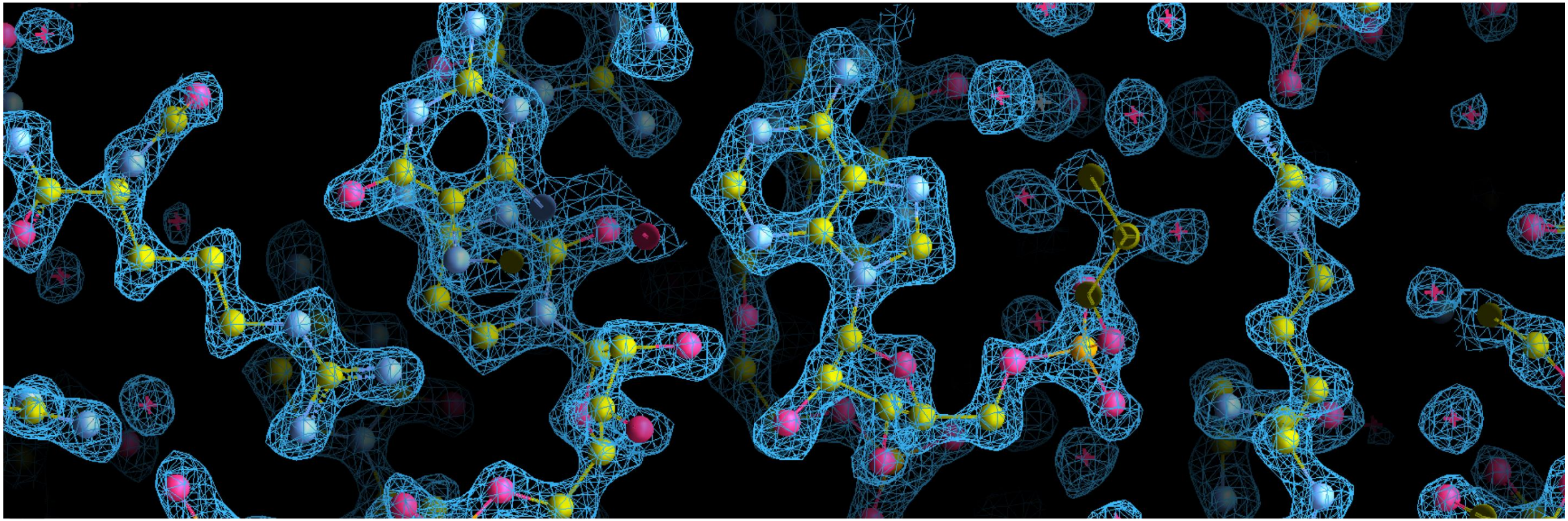

Three Areas of Focus for Technology Development

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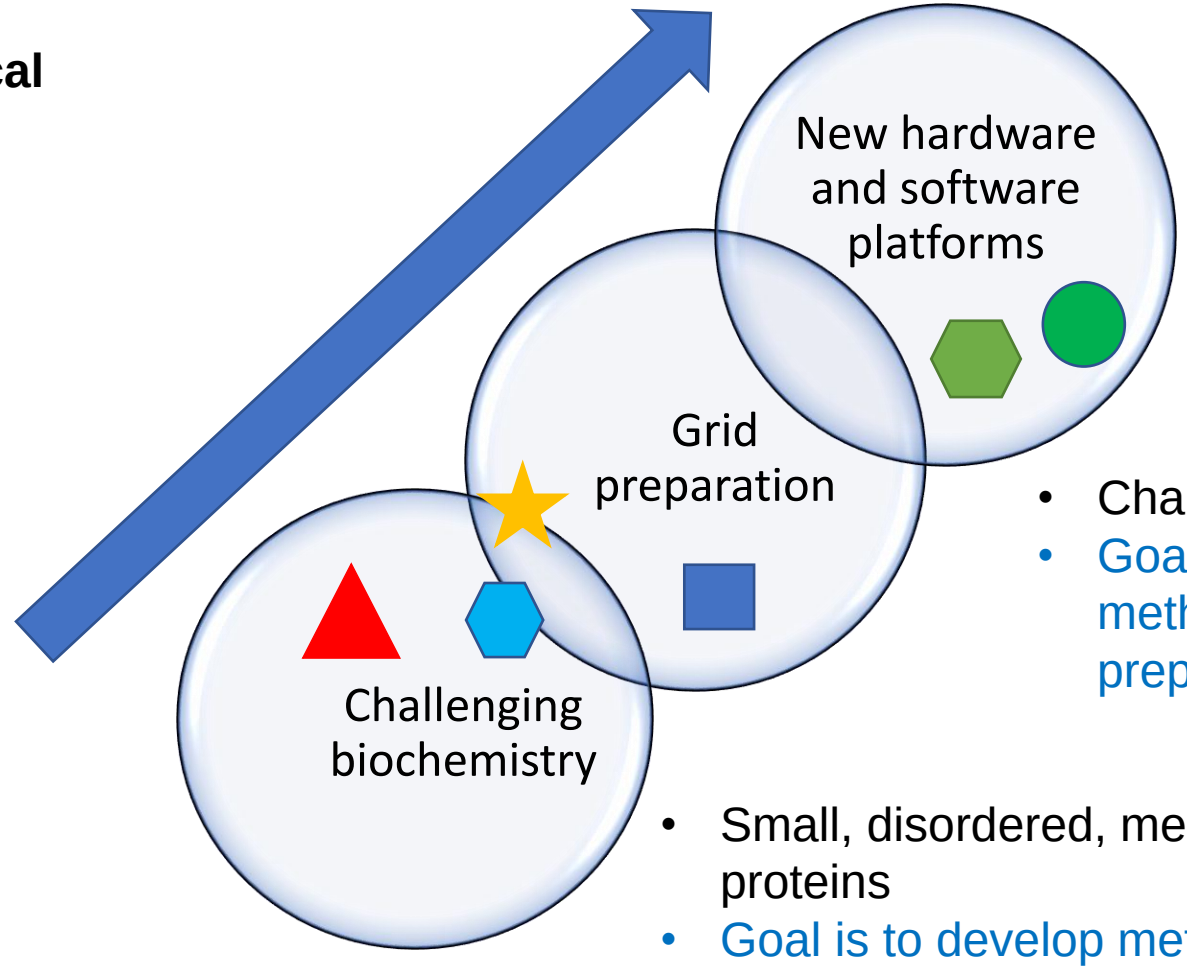
Mapping specific biological projects to current and future NCEP portfolio

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



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

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

- Small, disordered, membrane-associated proteins
- Goal is to develop methods to streamline purification protocols optimized for cryoEM

Cryo-EM statistics and trends

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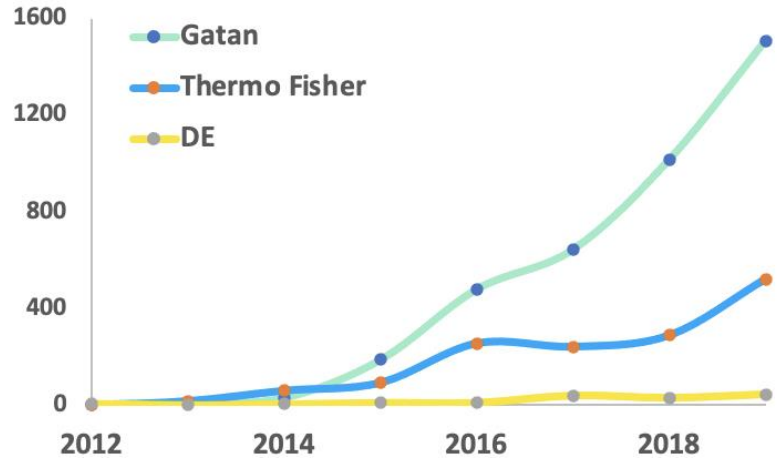
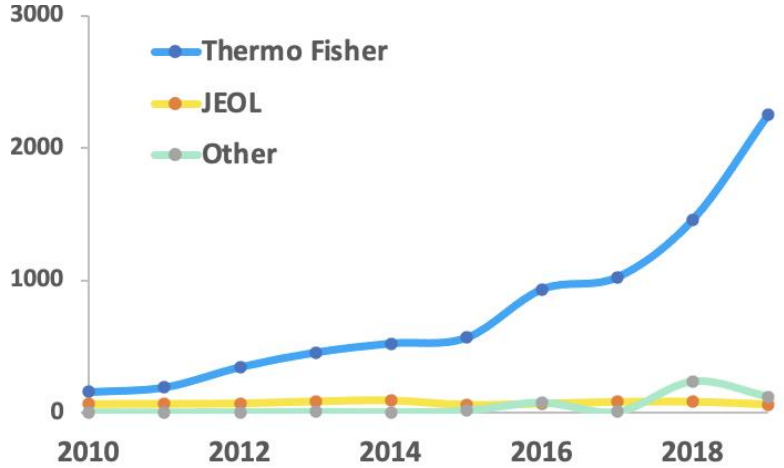
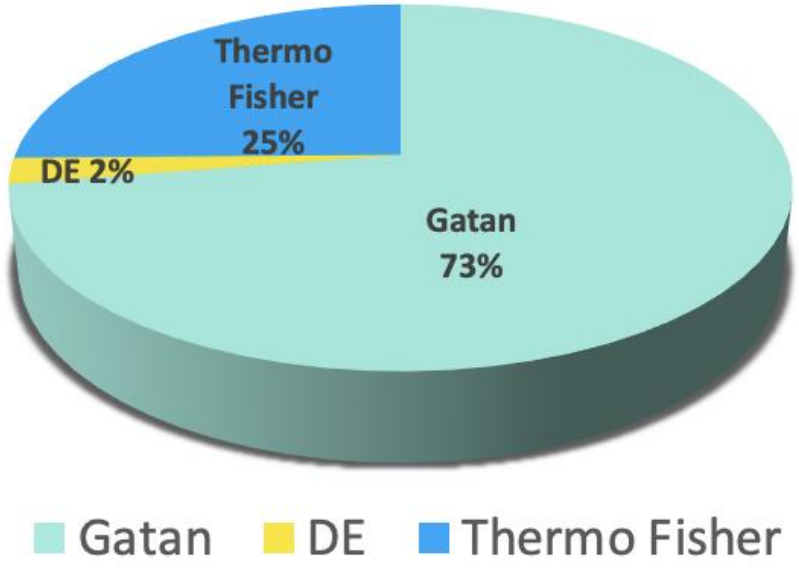
Trend in direct electron detector usage distribution



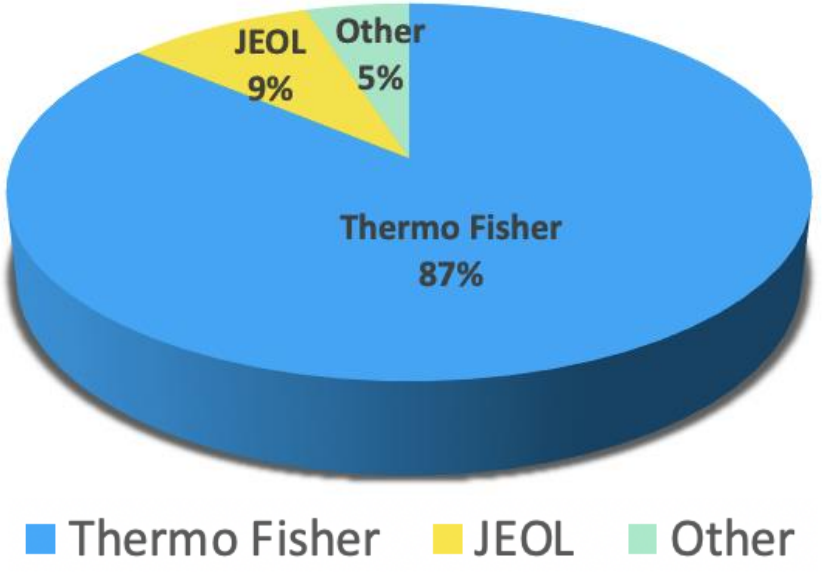
Trend in microscope usage distribution



Direct electron detector usage distribution



Microscope usage distribution



Advancing Cryo-EM – High Resolution at Lower Cost

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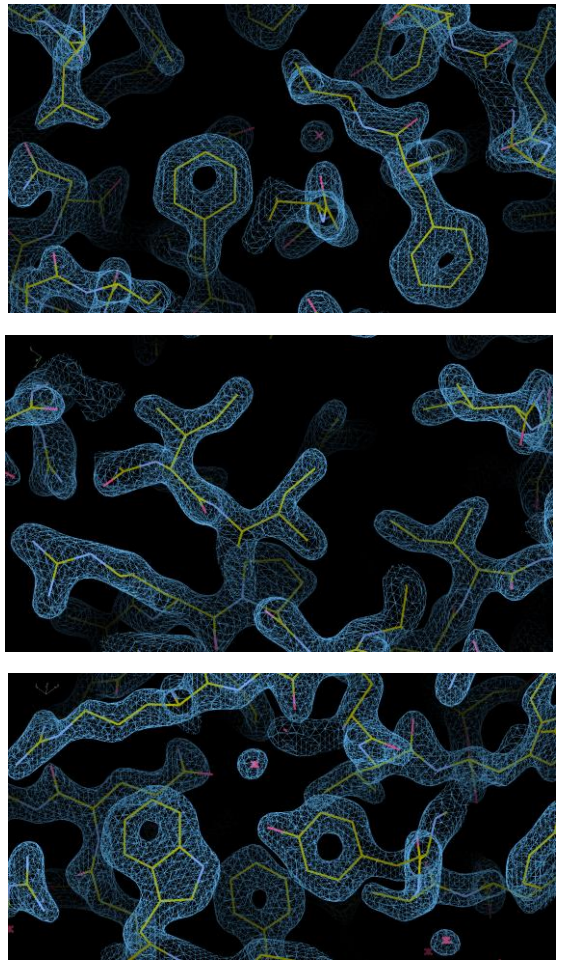
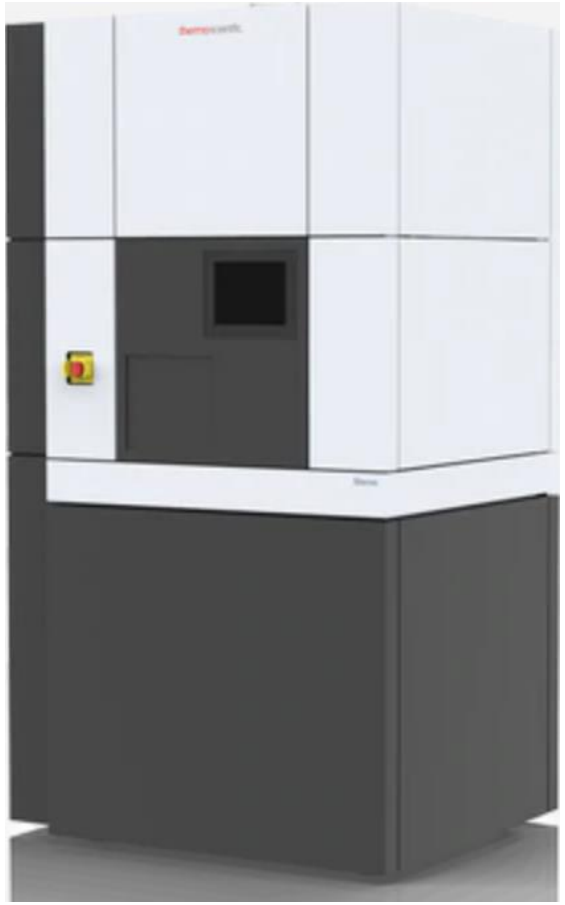
2.1 Å resolution



EMPIAR-10817

Thermo Fisher Glacios

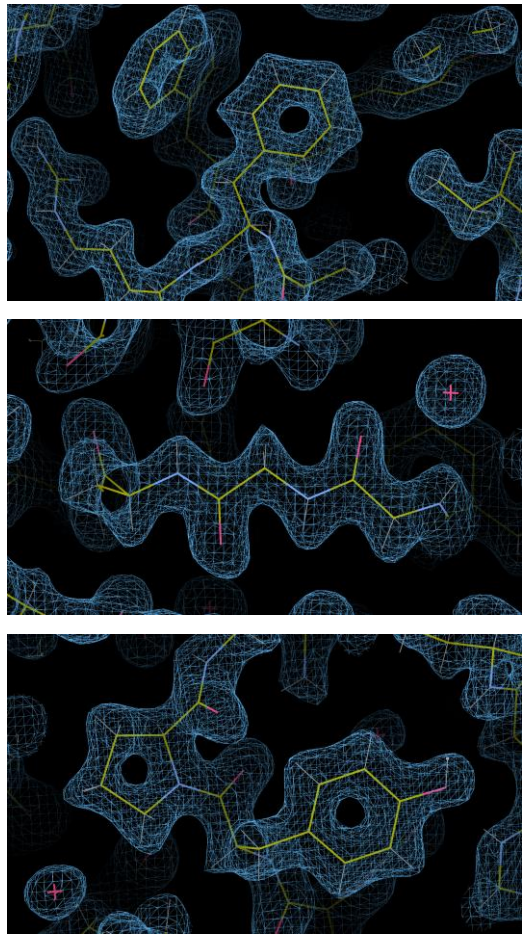


Falcon 3C and Direct Electron DE-64 cameras

JEOL CryoARM 200

Gatan K3 Camera

1.8 Å resolution



EMPIAR-10466

β-galactosidase at 1.61Å Resolution

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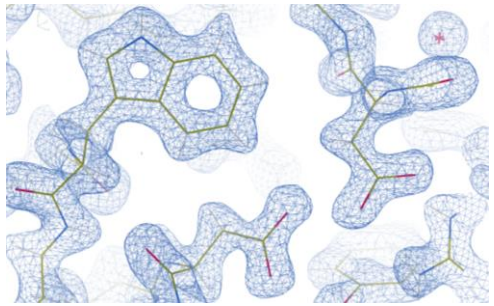

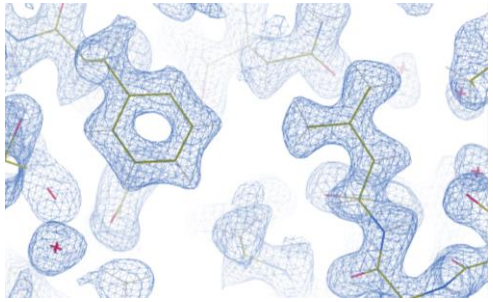

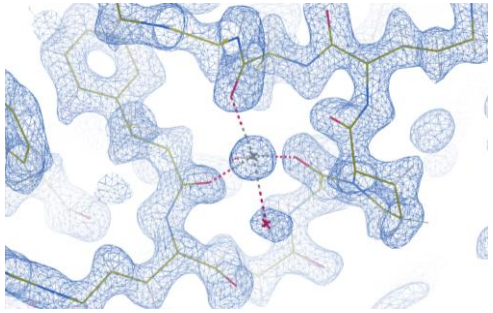

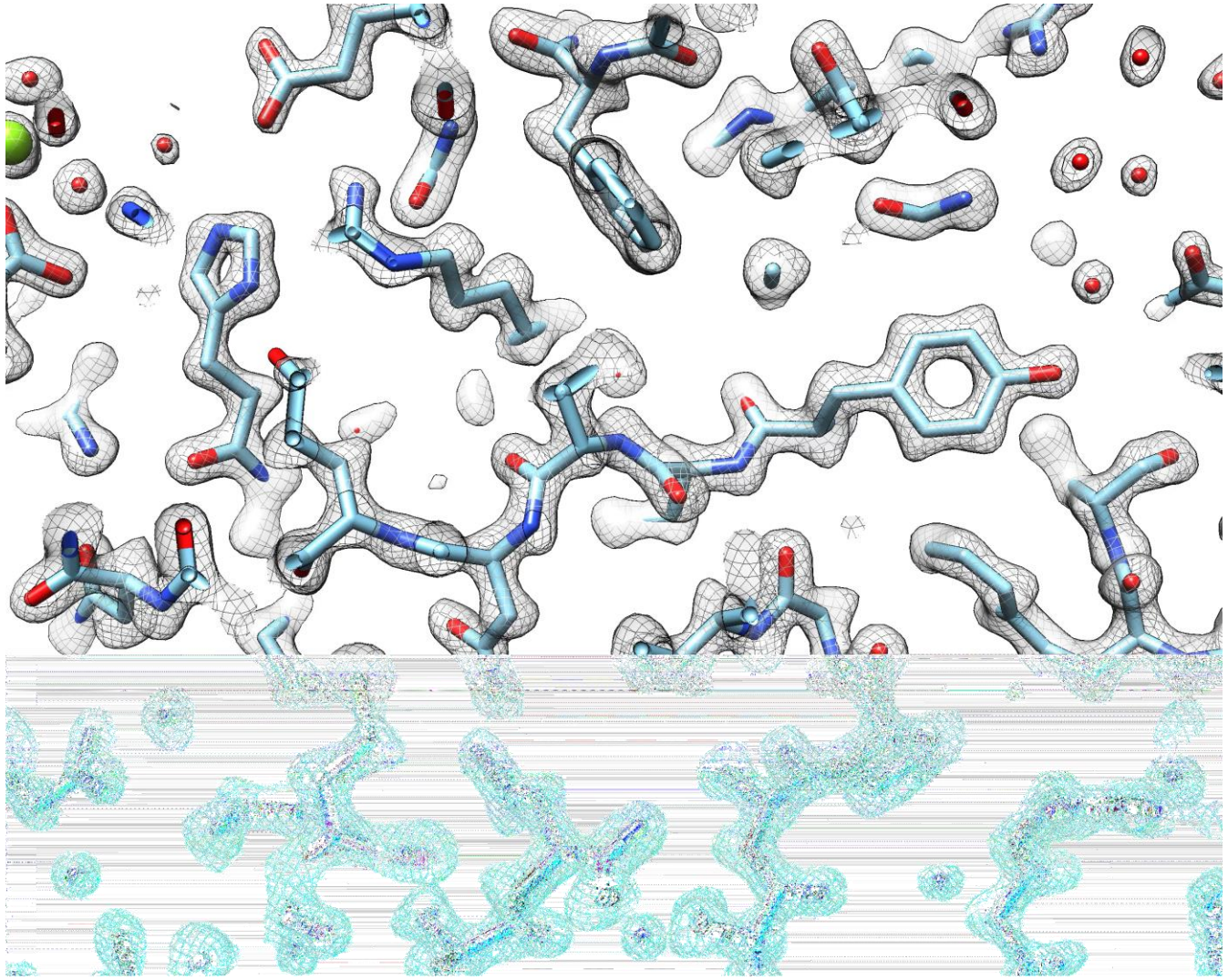

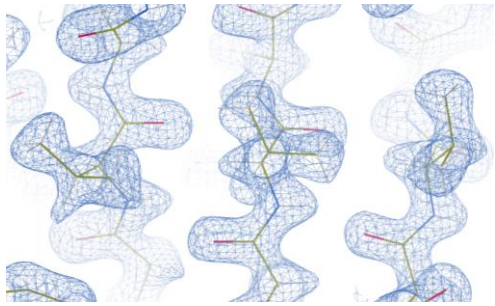

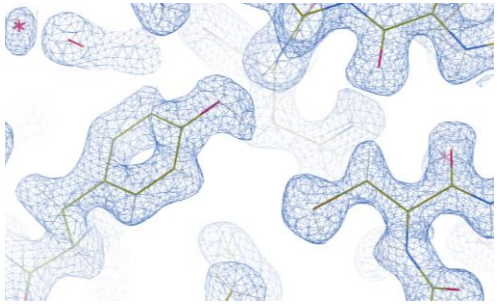

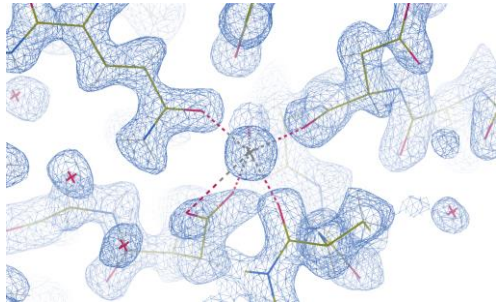

Neurofibromin (NF1)

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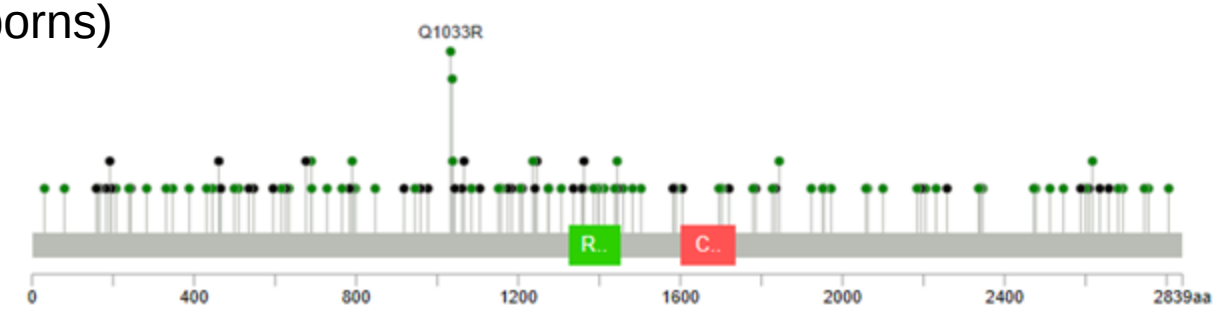
- mutated in at least 12% of human cancers (multiple missense and nonsense mutations)
- primary cause of neurofibromatosis type 1 (1:3500 newborns)
- 320 kDa protein (2818 amino acids)
- highly conserved from yeast to humans
- functions other than RAS-GAP mostly unknown



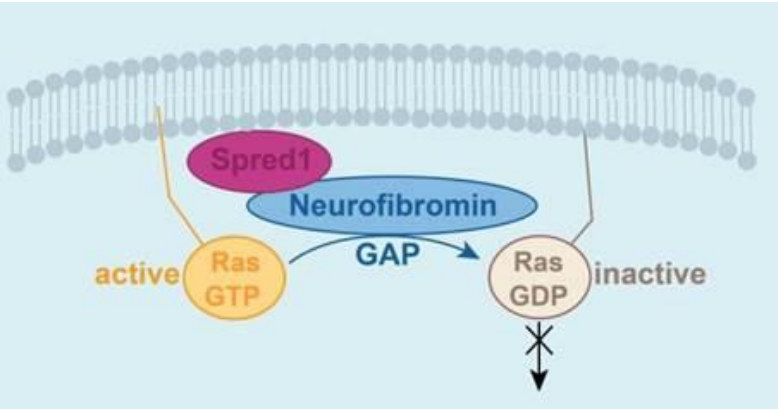
PNAS

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

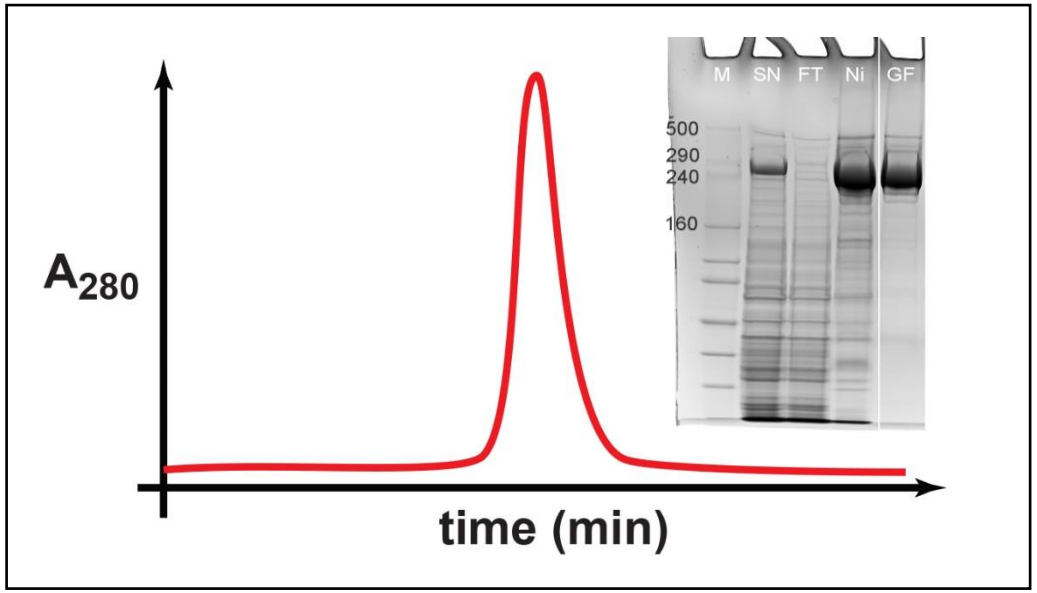
Theresia Dunzendorfer-Matt[a,1], Ellen L. Mercado[b,1], Karl Maly[c], Frank McCormick[b,2], and Klaus Scheffzek[a,2]

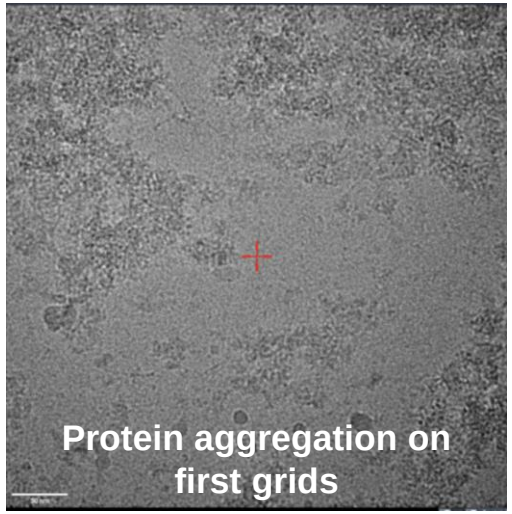
[a]Division of Biological Chemistry, Biocenter, Medical University of Innsbruck, 6020 Innsbruck, Austria; [b]Helen Diller Family Comprehensive Cancer Center, University of California, San Francisco, CA 94158; and [c]Division of Medical Biochemistry, Biocenter, Medical University of Innsbruck, 6020 Innsbruck, Austria

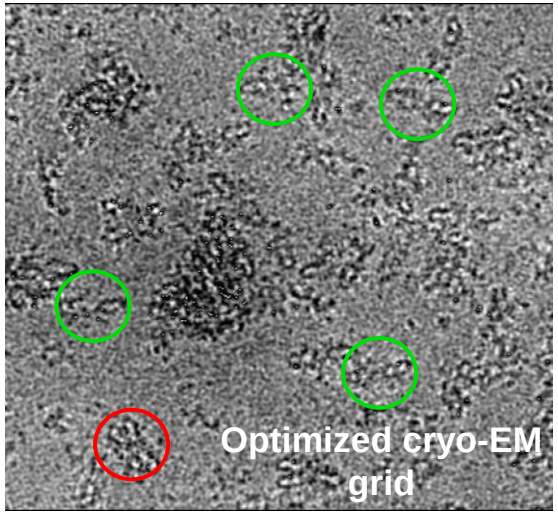
Contributed by Frank McCormick, May 10, 2016 (sent for review November 3, 2015; reviewed by Jonathan Licht and Nancy Ratner)

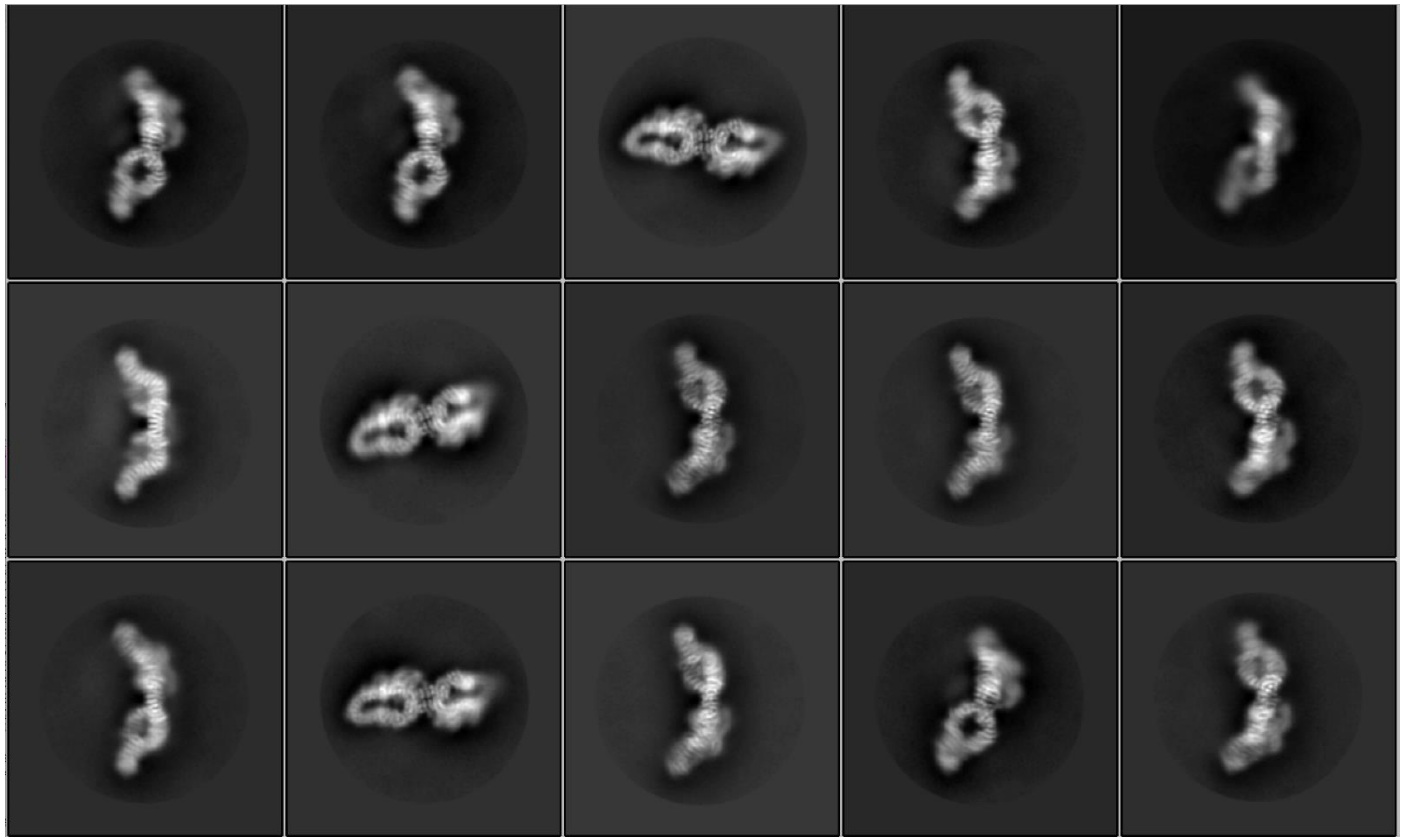


Cryo-EM studies of NF1









Initial 2D Classes

Cryo-EM studies of NF1

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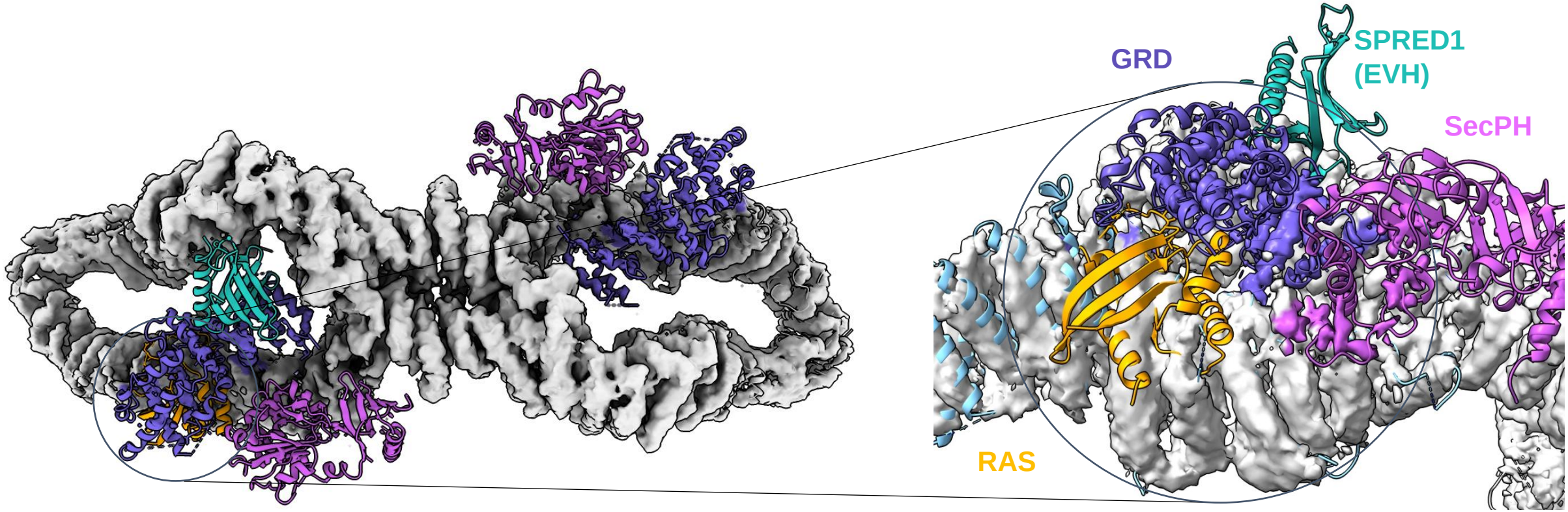

Proposed RAS-binding sites are occluded in the NF1 dimer

Advancing Cryo-EM through Technology Development

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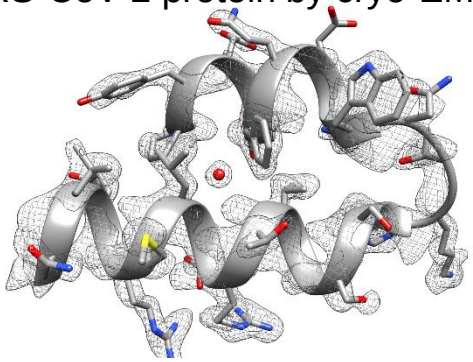
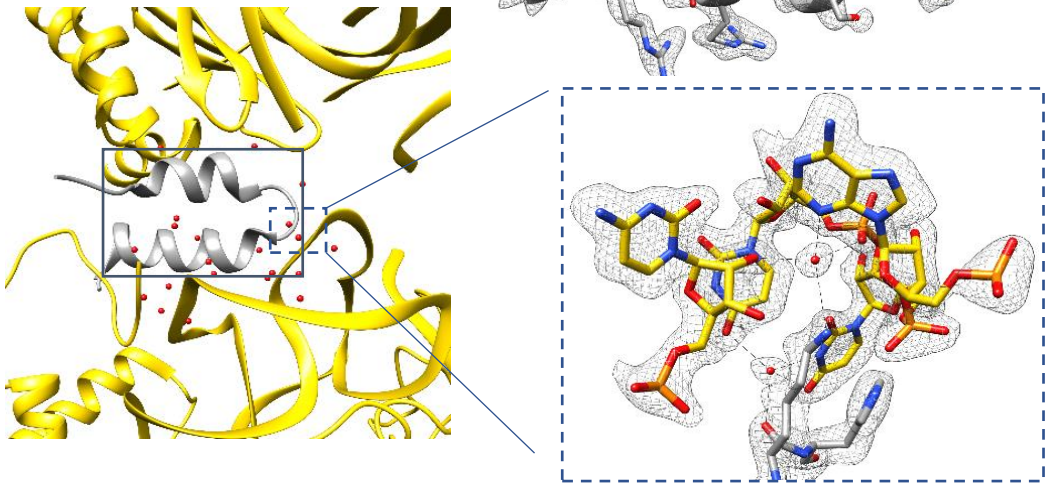
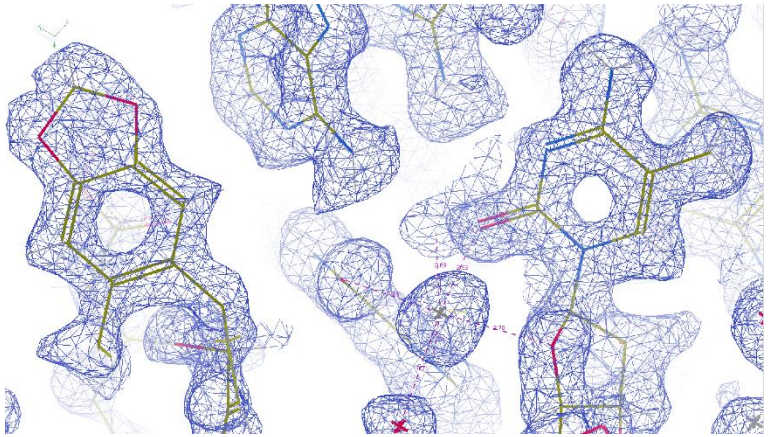
COVID-19

- 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

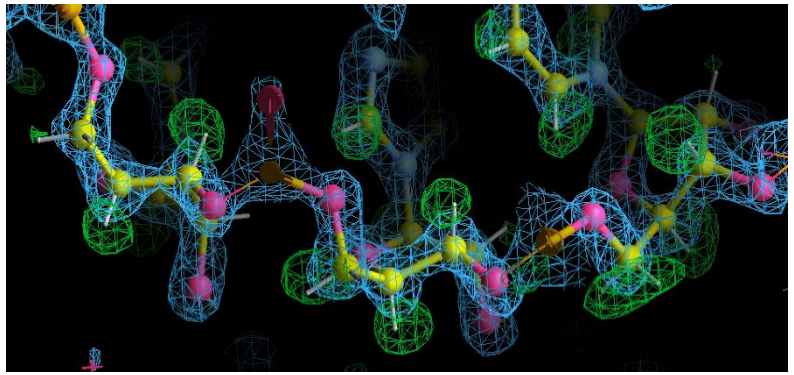


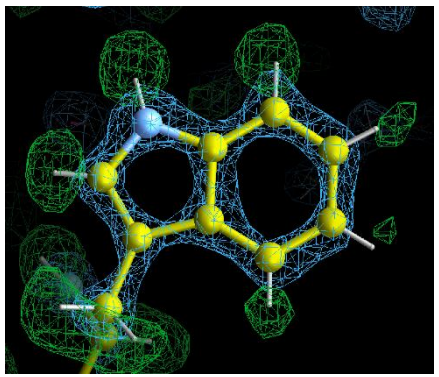


Cancer



- 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

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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

Frederick National Laboratory for Cancer Research

Dwight Nissley
Director, CRTP, FNLCR

National Cryo-EM Consultant
(Open)

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

Thomas Edwards
Senior Microscopist
NCEF

Helen Wang
Program Manger
NCEF

Jana Ognjenovic
Senior Scientist
ACT

Adam Weir
Electron Microscopist
NCEF

Matt Hutchison
IT Support
NCEF

Alan Merk
Electron Microscopist
ACT

Tara Fox
Electron Microscopist
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Joseph Finney
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Reinhard Grisshammer
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