



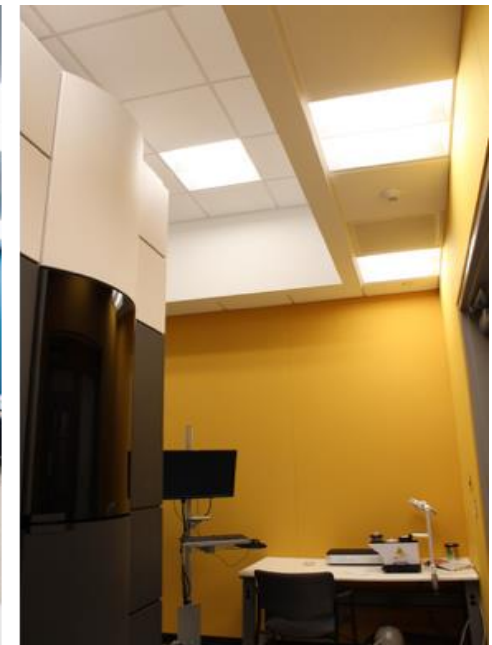
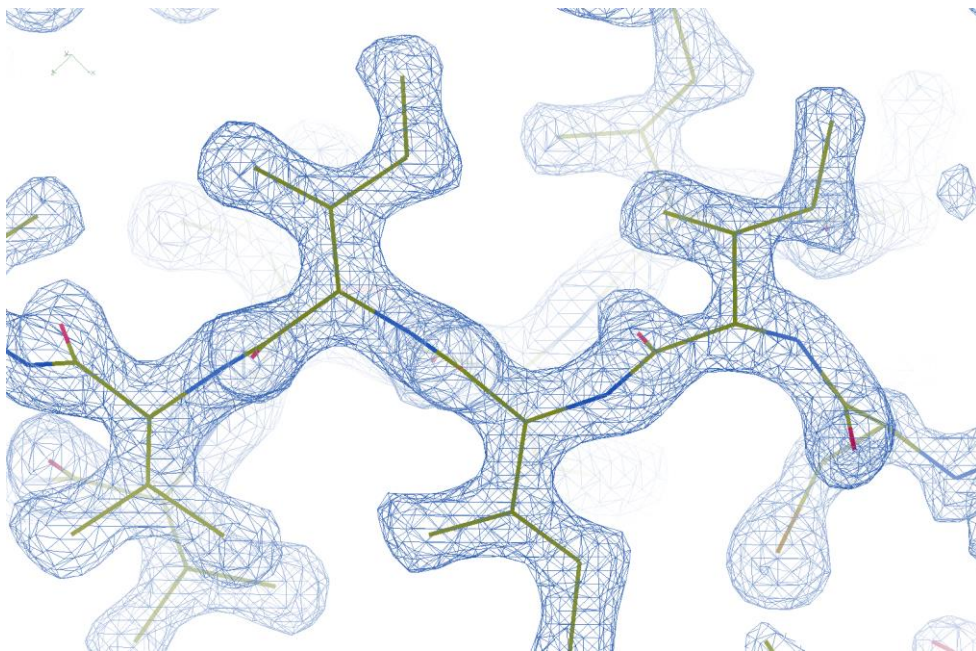
The National Cryo-EM Program (NCEP): Enabling Structural Biology in the Extramural Community

Dwight V. Nissley, PhD

Director, Cancer Research Technology Program

Frederick National Laboratory for Cancer Research

October 23, 2024





FNLCR / Cancer Research Technology Program

Operational Models

Technology Support for NIH/NCI

Cryo-EM, TEM, Volume EM & Optical Microscopy

Protein Expression and Characterization

Genomics & Proteomics, CLIA

Technology Evaluation (imaging CyTOF)

Basic Research

Molecular Pharmacology Program

National Missions (FNLAC)

NCI RAS Initiative

National Cryo-EM Program

NCI-DOE Collaboration

Extramural Enabling

Nanotechnology Characterization Lab

Antibody Characterization Lab



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 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



NCI National Cryo-EM Program

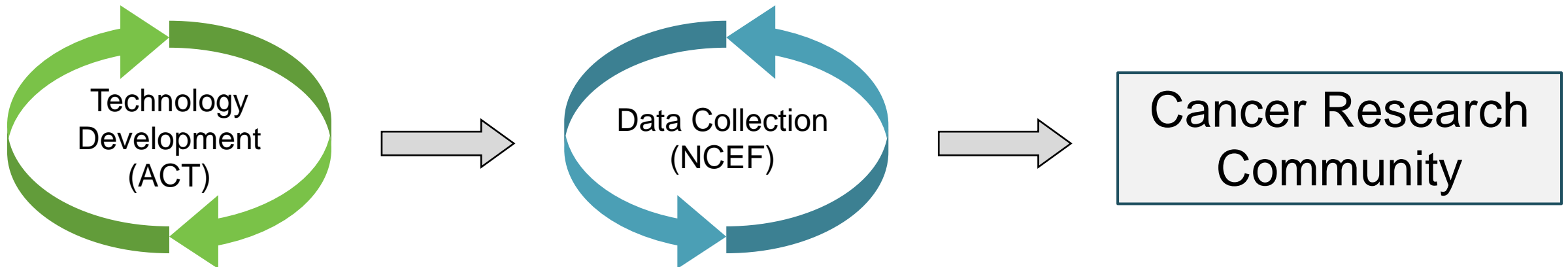
- **National Cryo-EM Facility 2017-present**

Extramural user facility for cryo-EM data collection

Ongoing expansion of scope, bandwidth and turn around

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

Methods and technology development for cryo-EM field





Technology Development Team

Jana
Ognjenović



Kendra Leigh



Alan Merk



Dennis Winston



Bernard
Heymann

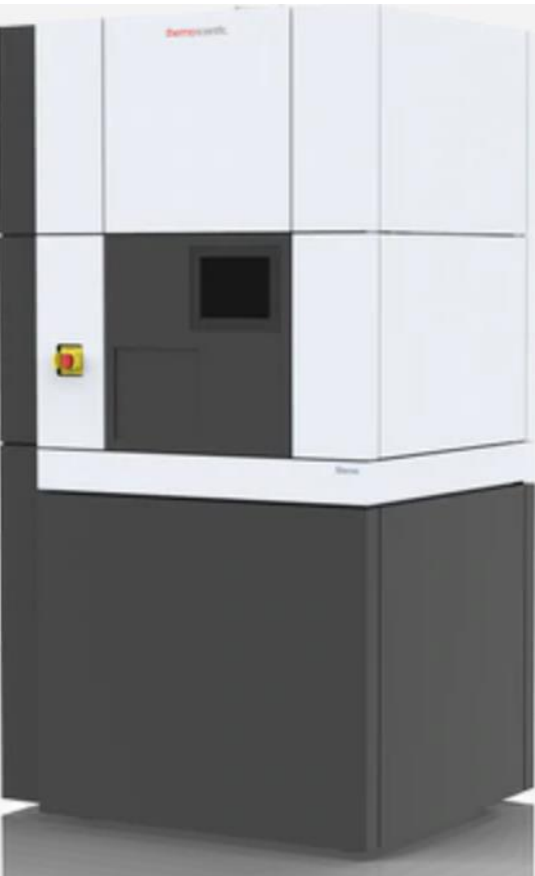


Daniel Cleary



Technology Development (ACT) Infrastructure

Glacios
Thermo Fisher



electron source
X-FEG
200 keV



DE-64
Direct Electron



Falcon4i
Thermo Fisher

CryoARM200
JEOL



electron source
cold-FEG
200 keV

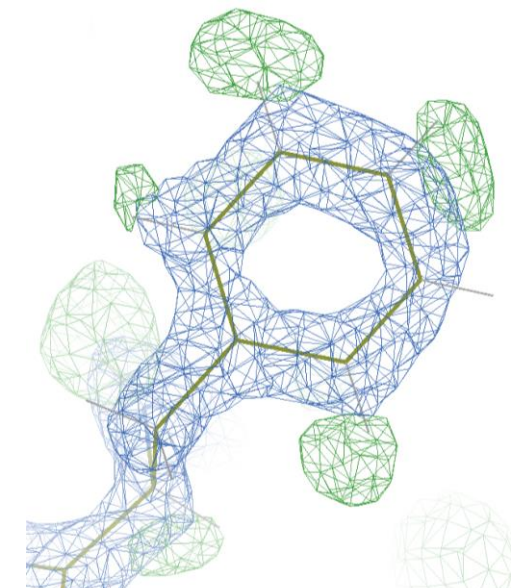
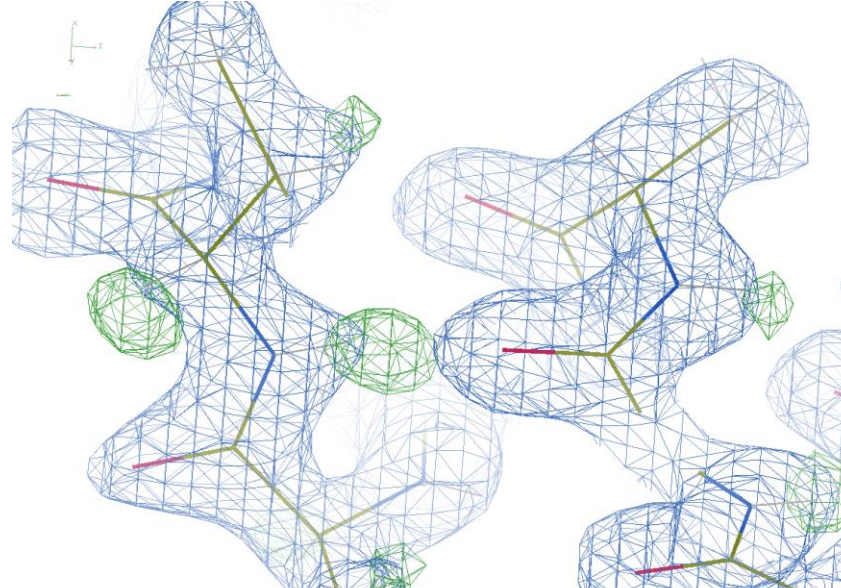
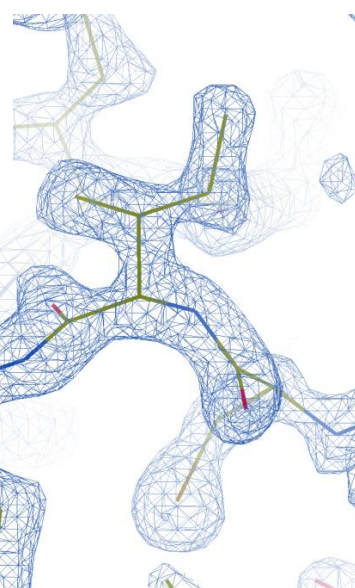
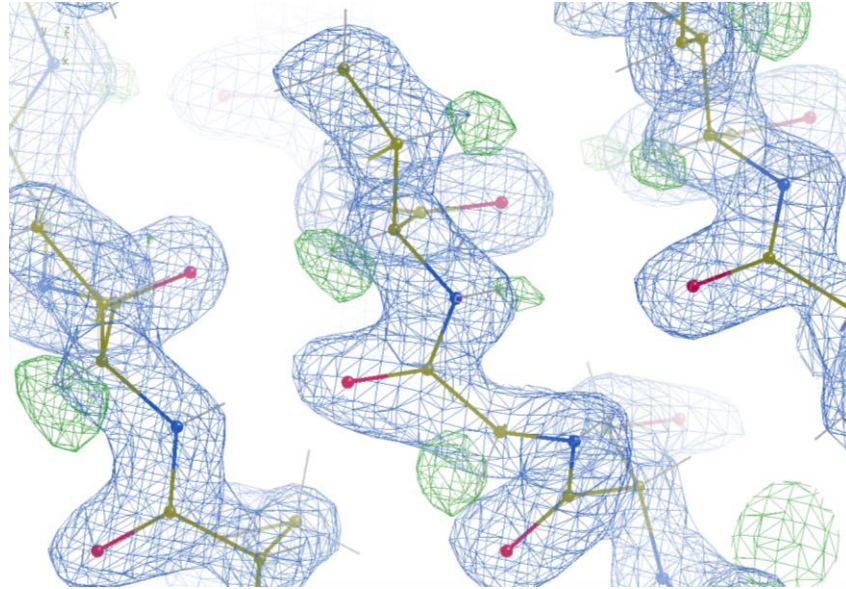
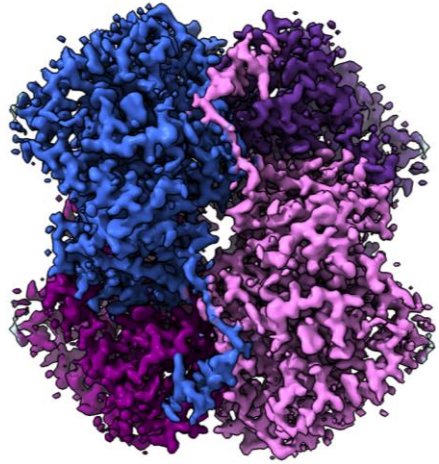
in-column Ω
energy filter



K3 direct detector
GATAN



Visualizing hydrogen atoms in human LDH



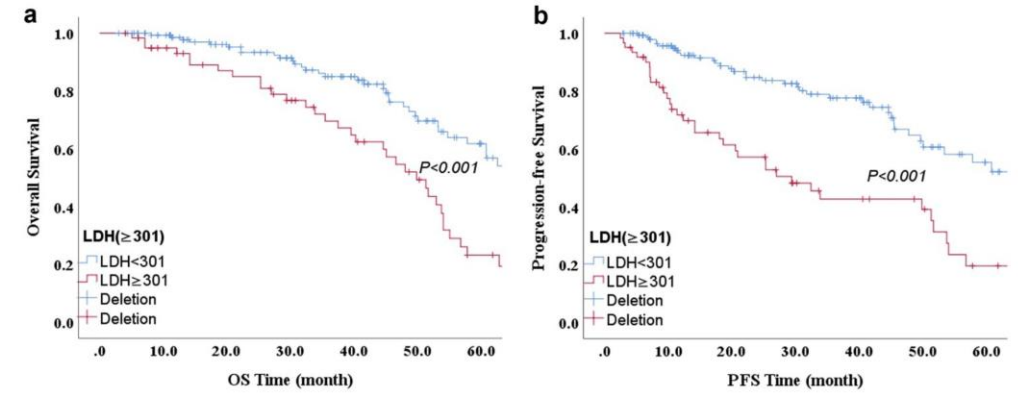
145 kDa, D2 symmetry

Currently at 1.8Å resolution

Plays role in the Warburg effect

no FDA-approved drug

Figure 2. Kaplan-Meier survival analysis of LDH. Overall survival (a) and progression-free survival (b) according to LDH in DLBCL patients.



<https://doi.org/10.1080/16078454.2023.2293514>



Data Collection Team (NCEF)

Adam Wier

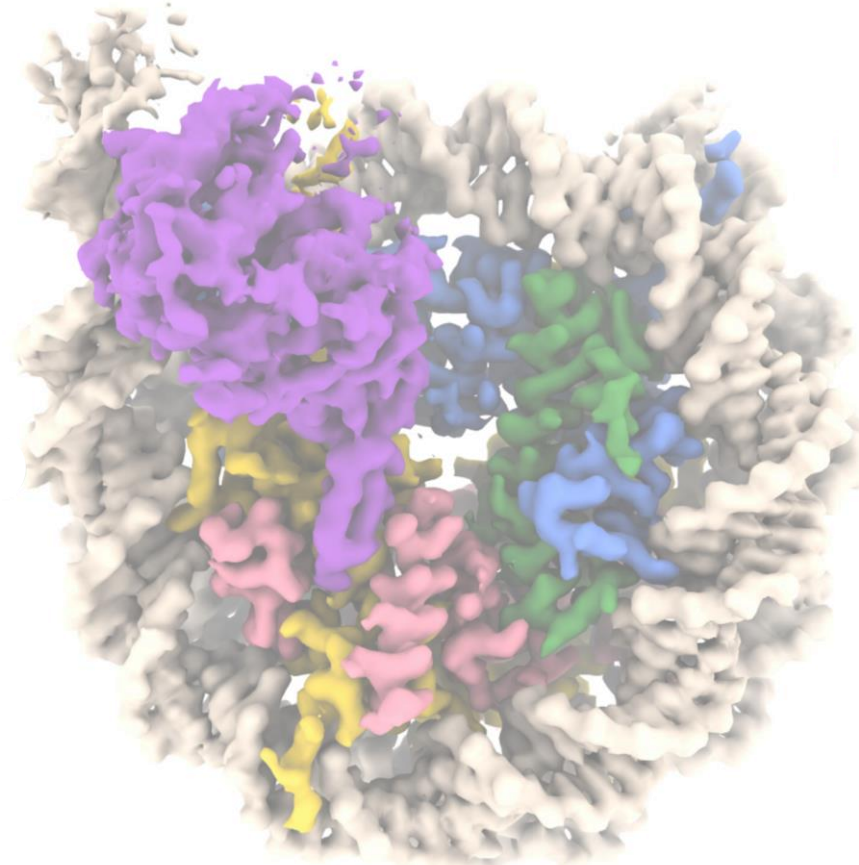


Thomas Edwards



Helen Wang

Jenny Wang



Tara Fox

Vladimir
Veremeychik



Joseph Finney



NCEF Capabilities

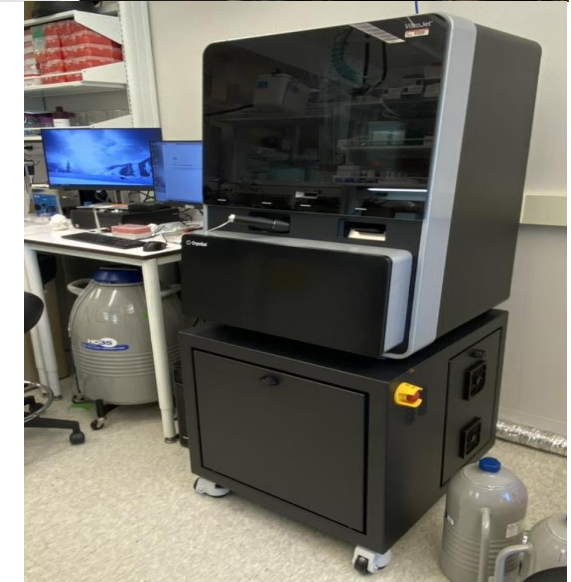
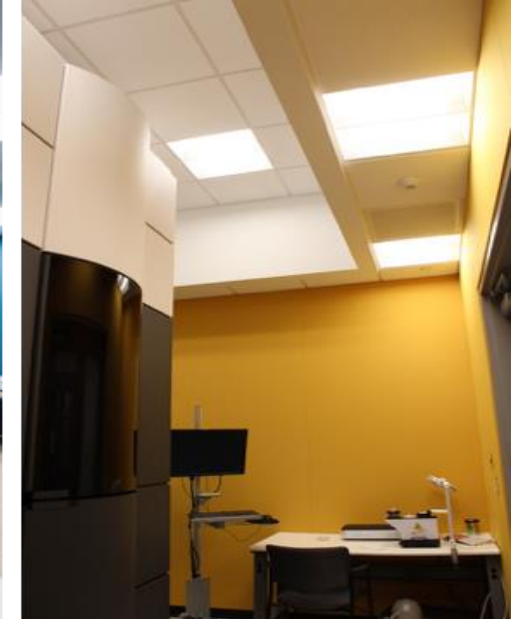
Two Titan Krios Microscopes for regular imaging

Each is equipped with Gatan K3 Direct Detector and BioQuantum Energy Filter and fringe free alignments

Current general imaging collects at 250-350 images/hour. 7000-10000 images for a two-day session

Glacios microscope equipped with multigrid and Athena platform for automated screening

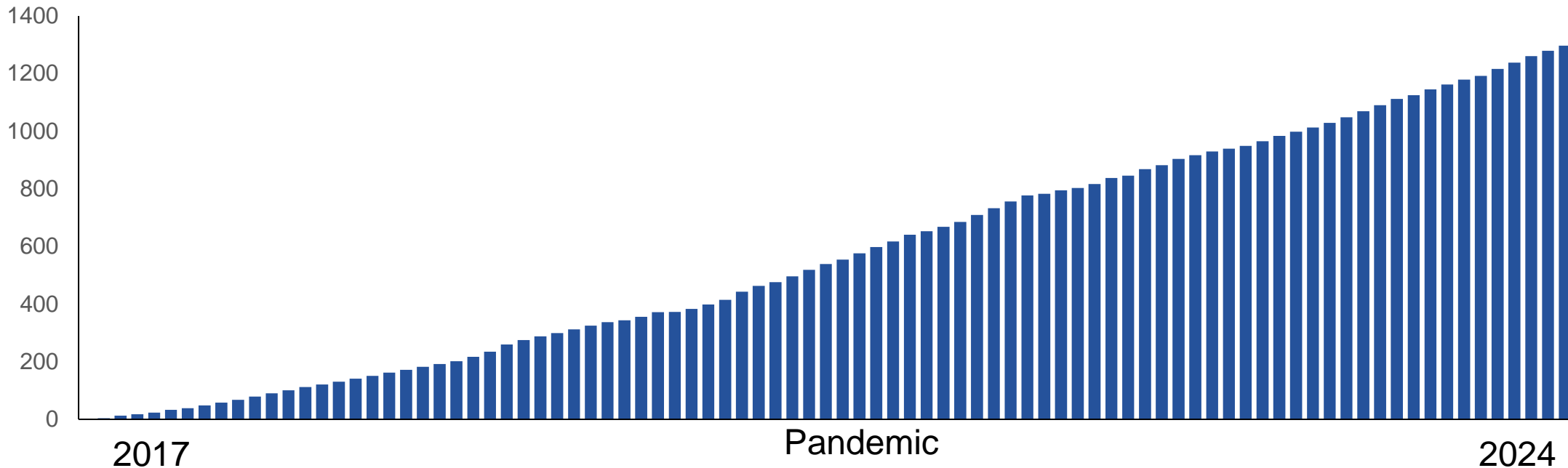
VitroJet automated grid freezing robot



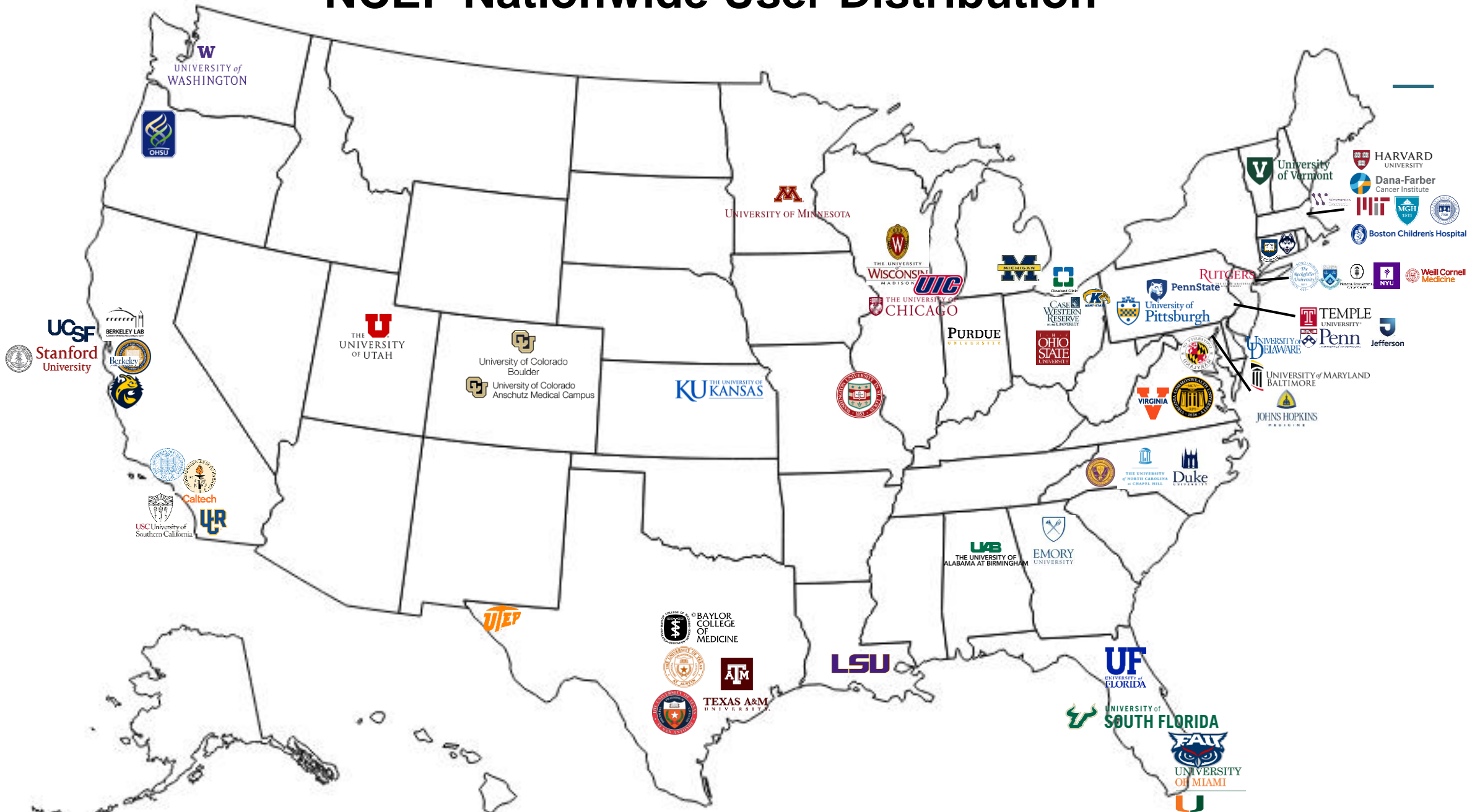


NCI National Cryo-EM Facility Operational Metrics

- Collected data for 160 investigators from 60+ institutions
- 1300 imaging sessions with an average of 19 imaging sessions per month this year.
- 128 publications in 7 years, with 21 publications in the past year.
- Publications are in high-impact journals such as Science, Nature, Cell, etc.
- Over 240 structures deposited in the EMDB



NCEF Nationwide User Distribution





Grid Preparation Program Development

Developing a grid preparation and screening service

Access to latest generation of grid freezing technology

- VitroJet automated freezing robot is centerpiece of program
- Sample will be frozen onsite with the help of NCEF staff
- Grids screened onsite

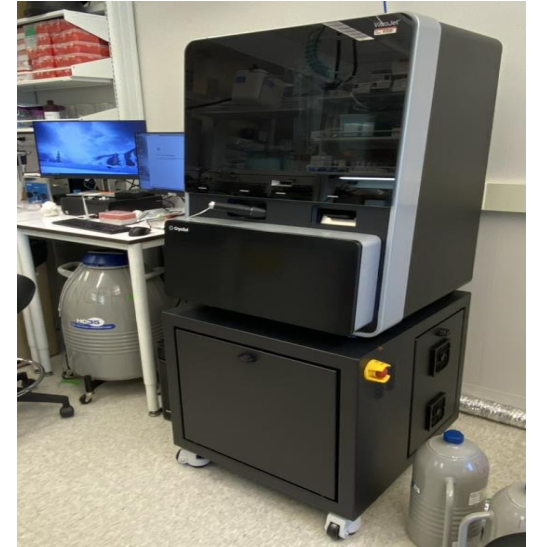
VitroJet platform validated and now generating consistent results

**Automated screening using Falcon 4i direct detector on Glacios microscope
(multigrid and Athena platform protocols)**

Beta testing using samples from internal programs and local extramural users

Developing reporting framework to provide user with feedback for grid optimization

Expected rollout to extramural community summer 2026





Software Development: NEMO

- **NanoFab Equipment and Management Operations (NEMO) originally developed at NIST. Free and open source software**
- **Adding features to specialize for Cryo-EM Facility management**
- **Current Features added by NCEF**
 - Management of SCIPION data pipelines
 - Extraction of metadata from imaging sessions
 - Automated report generation
 - Sample Inventory management
- **Plan to release modifications to public so that other centers may also use.**

Modify pipeline

Pipeline: NZheng-NCEF-021-004-10574

Check logs

Location: Blue

Base path: /mnt/Blue-K3/20240402_0942

Microscope State

C1 Aperture Mode (μm): 2000

Camera: K3

Camera Mode: Counting

Imaging Mode: Nanoprobe EFTEM

Objective Aperture (μm): None

Select Area Aperture (μm): None

C2 Aperture Mode (μm): 100

Camera Configuration: 180°, flip about vertical axis

Cs (mm): 2.7

Microscope: Blue

Physical pixel size (μm): 5

Data Acquisition State

Acquisition Method: Single Particle

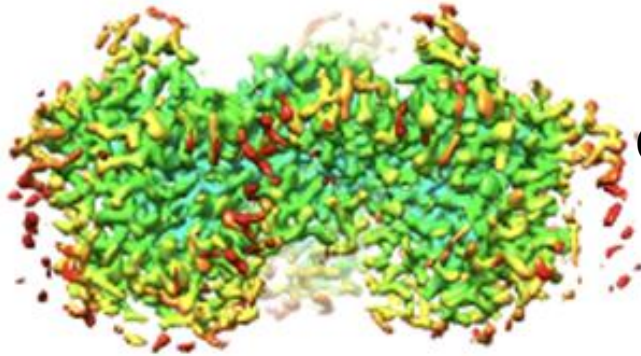
Camera Mode: Counting

Binning: 2

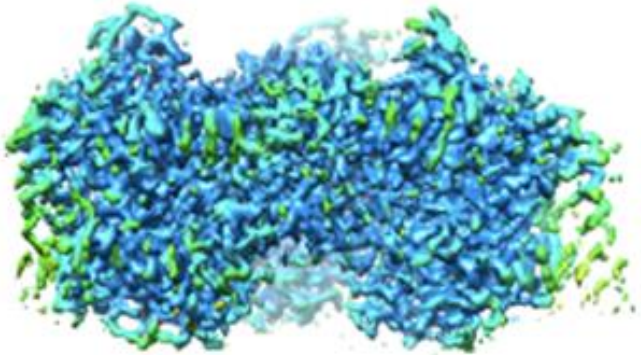
Dark Reference: 2



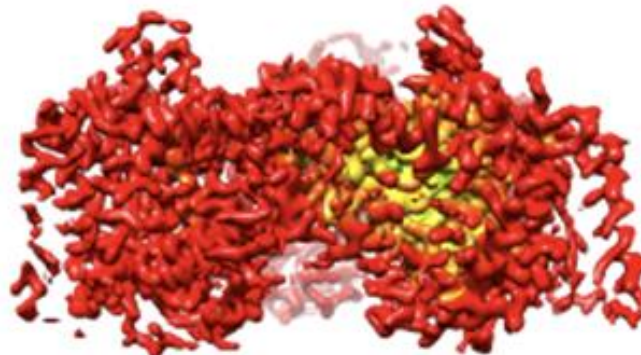
NCEF User Success: Concentrative Nucleoside Transporter 3



CNT3 – N⁴-hydroxycytidine
2.7 Å
EMD-41734 PDB: 8TZ5



CNT3 – GS-441524
2.3 Å
EMD-41732 PDB: 8TZ3



CNT3 – GS-441524 subset
3.2 Å
EMD-41733 PDB: 8TZ4

- CNT3 responsible for transport of nucleosides into the cell
- CNT3 Involved in nucleoside analog(NA) transport (antivirals and chemotherapeutics)
- This study uses multiple NAs and finds differences in their binding and transport properties that will inform future drug design
- Submitting PI: Seok-Yong Lee, Duke University



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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

38% (64)

Group II: Structural biologists in adjacent disciplines (X-ray, NMR)

- see value in using cryo-EM
- have expertise in protein biochemistry
- need training in specimen preparation, data collection, and processing

36% (60)

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

26% (43)

Discussion

