

Execution and Dissemination Plan

Sriram Subramaniam
Ulrich Baxa

Outline of presentation

Introduction to NCEF (Sriram Subramaniam)

- Scientific origin and brief history of NCEF
- NCEF mission and strategy
- Budget, infrastructure and future expansion plans

Execution Plan (Ulrich Baxa)

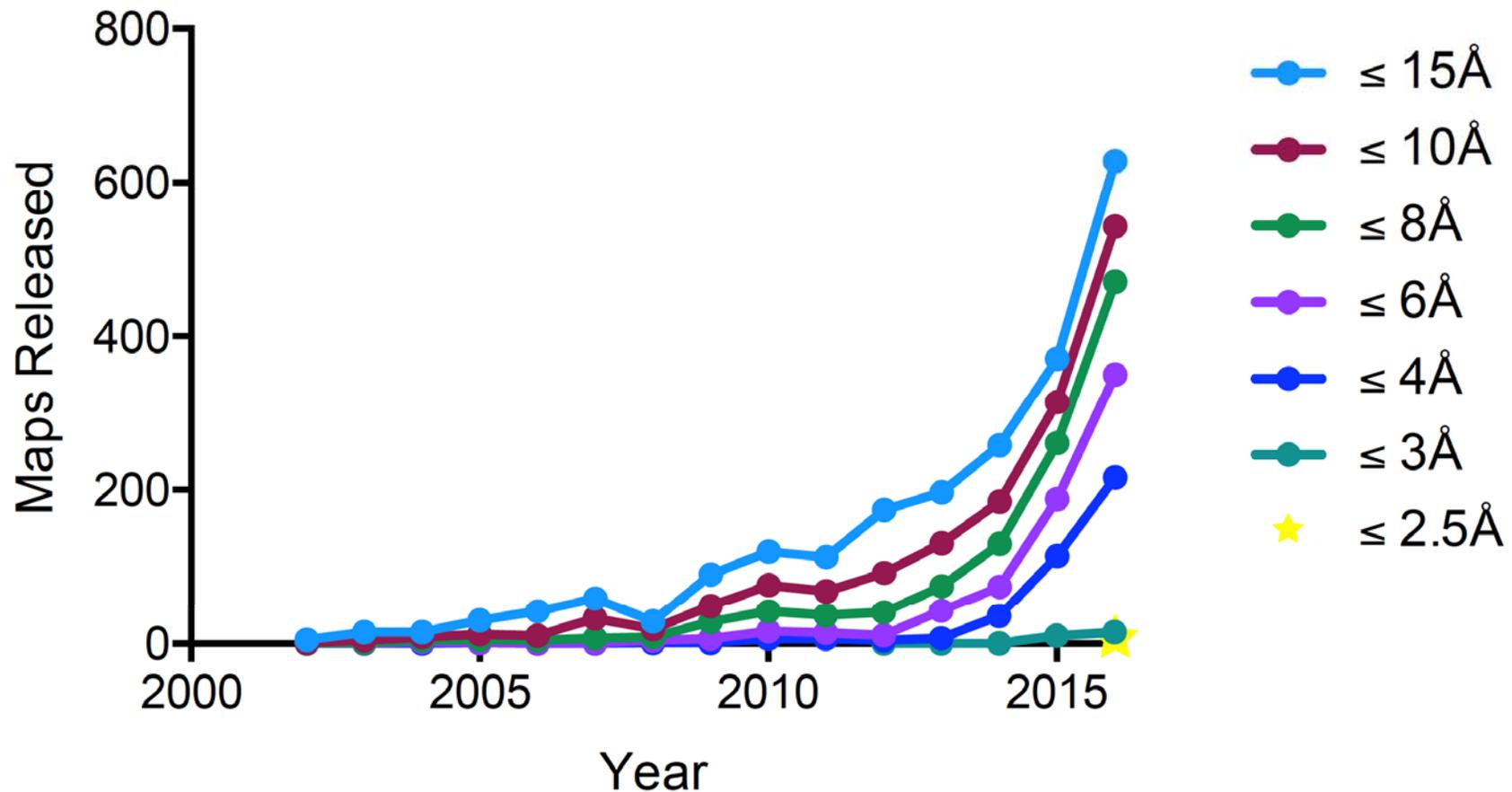
- Personnel
- Operational plan
- User access to NCEF
- Data transfer plan
- Suggestions/comments for NCEF operation
- Recommendations for performance metrics

Emergence of cryo-EM



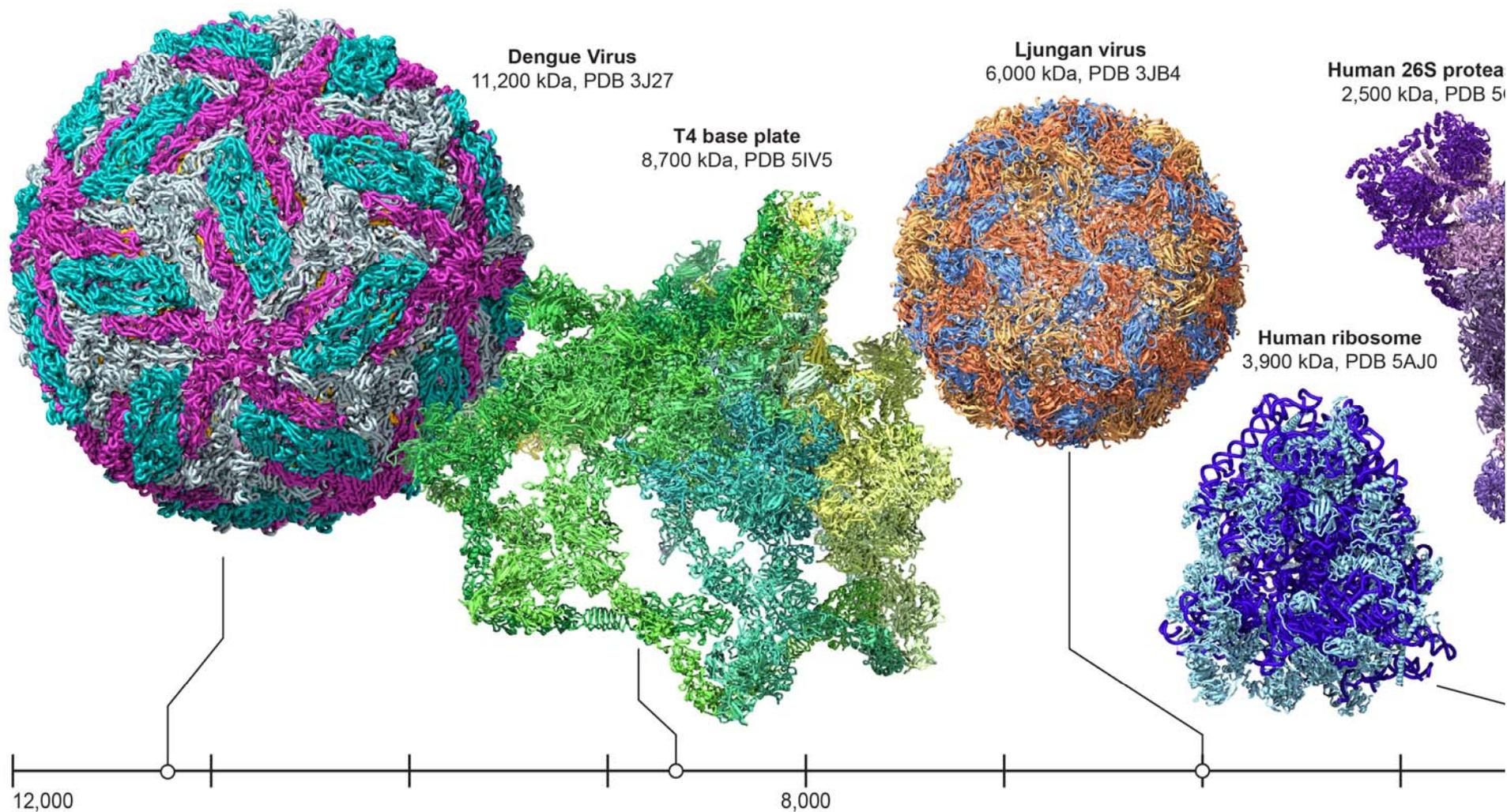
Cryo-EM: Method of the Year

Growth of cryo-EM structures

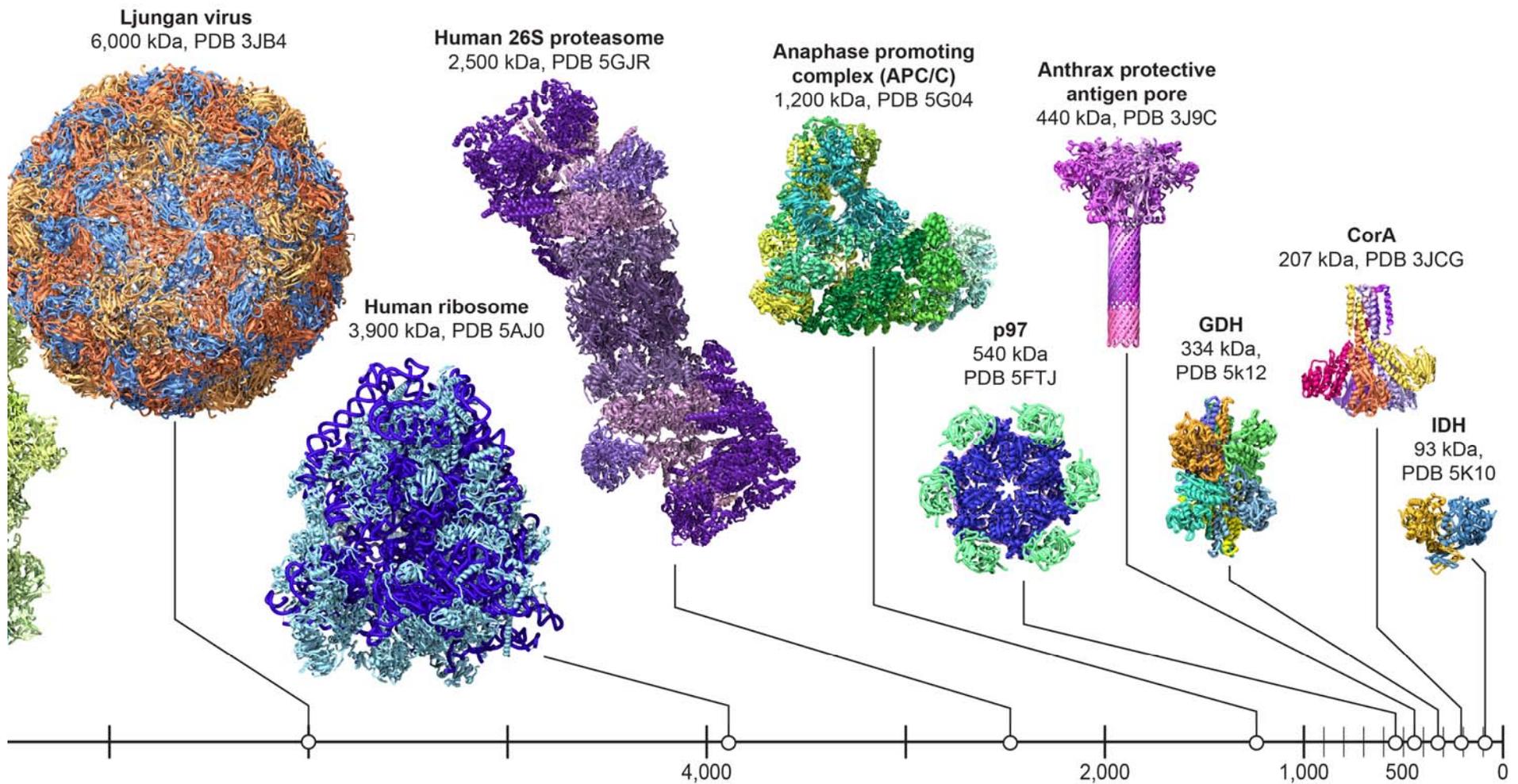


From the Protein Data Bank website: pdbe.org/emstats

Traditional cryo-EM targets



Growing diversity of cryo-EM targets



Chronology of efforts to launch cryo-EM at FNL

Spring-Summer 2014: Initial discussions between Subramaniam and FNLAC leadership (Joe Gray, Dave Heimbrook, and Harold Varmus)

September 2014: Subramaniam presentation at FNLAC meeting proposing creation of National lab for cryo-EM at Frederick

December 2014: Subramaniam and Varmus organize workshop with leading structural biologists, institutional heads, and NIH IC representatives

Workshop results in recommendation of an urgent need for national user facilities similar to the synchrotron facilities available for X-ray crystallography

February 2015: Subramaniam presentation at the FNLAC meeting with a revised plan for the National Microscopy Laboratory

March 2015: Subramaniam presentation to Francis Collins and all NIH IC Directors on rapid growth of cryo-EM field and national needs

September 2015: Unanimous approval from FNLAC for proposal to launch NCI funded National Cryo-EM Facility at Frederick National Laboratory

Defining user communities and NCEF 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

Budget, infrastructure and future expansion

- **Budget of ~ \$5M/year for 4 years**
- **FEI Titan Krios microscope has been installed and is now operational**
- **Microscopists and project manager recruited**
- **Projected date of opening for user access: May 15, 2017**
- **Construction of new microscope facility at ATRF by summer 2018**
- **Relocation of Krios #1 to ATRF and addition of Krios #2 (or equivalent) in 2018**
- **Possible addition of lower voltage (200 kV) microscope in 2017/2018**

Outline of presentation

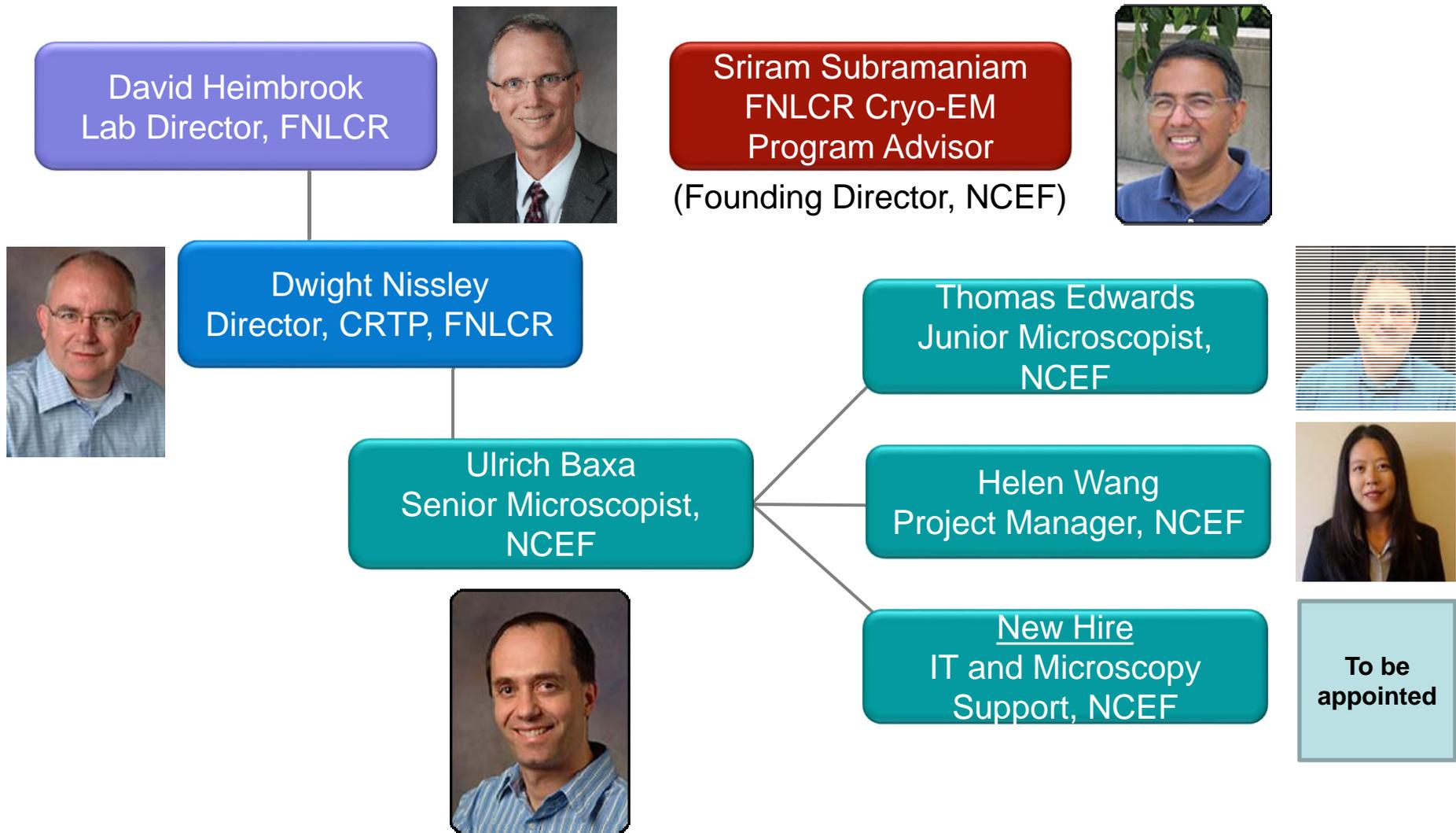
Introduction to NCEF (Sriram Subramaniam)

- Scientific origin and brief history of NCEF
- NCEF mission and strategy
- Budget, infrastructure and future expansion plans

Execution Plan (Ulrich Baxa)

- Personnel
- Operational plan
- User access to NCEF and data transfer plan
- Benchmarks

NCEF Personnel



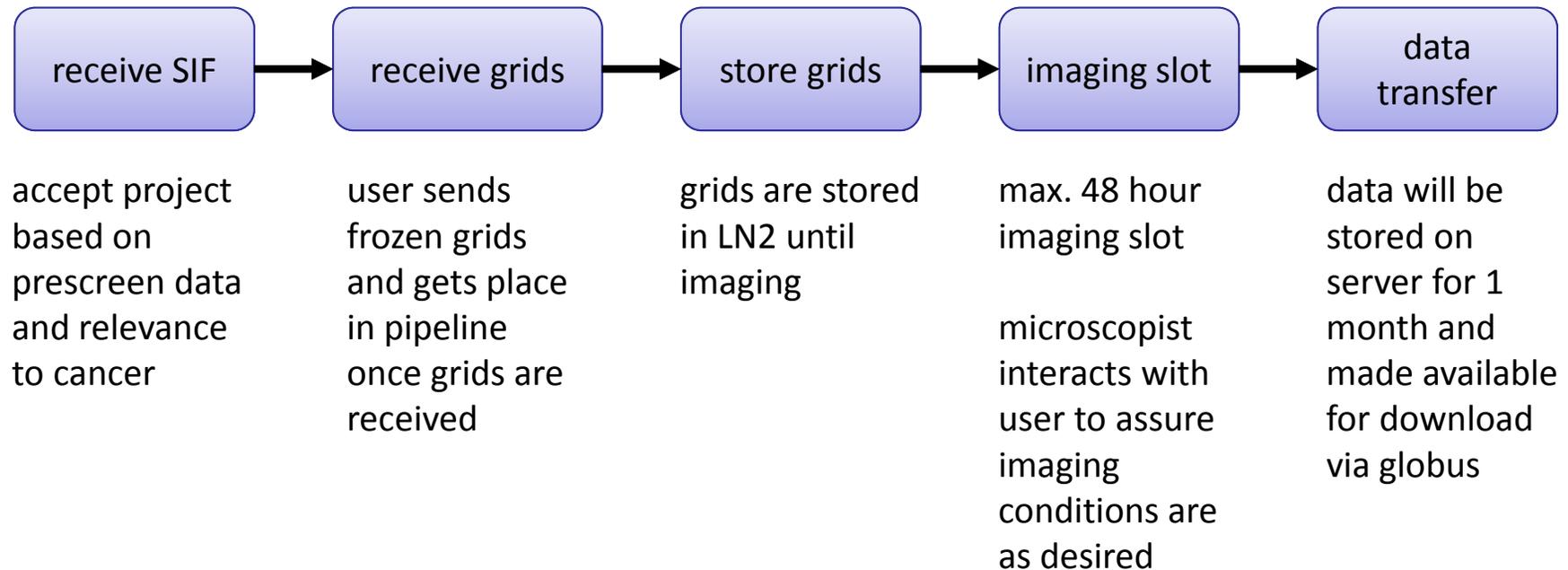
NCEF working group

- **Committee Members:**

- Dr. Steven Ludtke (Baylor College of Medicine, Chair),
- Dr. Mario Amzel (Johns Hopkins University School of Medicine),
- Dr. Edward Egelman (University of Virginia),
- Dr. Angela Gronenborn (University of Pittsburgh),
- Dr. Stephen Harrison (Harvard University School of Medicine),
- Dr. Grant Jensen (Caltech),
- Dr. Piermaria Oddone (Fermilab),
- Dr. Hong Zhou (UCLA)

Operational Plan

Our intention is to open NCEF on May 15, 2017



Sample Information Form

National Cryo-Electron Microscopy Facility (NCEF) Sample Information Form

The purpose of this application is to give us a general idea of what your sample looks like and what the imaging needs are. Your answers need not be comprehensive, but the more information you provide the better we can understand the project that you have in mind.

All details submitted in this form, all other communication, and data collected (if approved) will be kept confidential and are not shared with anyone outside the NCEF.

Please note that we can only accept samples at BSL1 level. No infectious material, toxins, or anything potentially dangerous can be accepted.

Please attach any relevant images or files to your email along with this document. Please send via email to nci-ncef@nih.gov

A. Contact Information

The information collected is for business purposes only and should not include any personal identifiable information.

Principal Investigator:
Institution/ Laboratory:
Point of Contact (POC):
POC email:
POC phone:

On the day of imaging we will need to contact you, so make sure to give us a contact we will be able to reach.

B. Short Project Description (Optional)

Note that we do not require any direct information on the project, except a one-sentence declaration of why it is relevant to cancer research. However, any additional information you provide on the scope of the research project and previous publications might help us provide better quality data.

C. Sample information

We require that every sample we accept for imaging is prescreened by cryo-EM to show ice quality, particle distribution, and stability are acceptable. Prescreening should be done with grids made under identical conditions as the grids you are planning to ship to us. Please attach image of your screen (preferably low-mag, medium mag (holes), and high mag (particles))

D. Imaging Conditions

If you have any thoughts or wishes for imaging conditions (pixel size, dose rate and total dose, number of frames, frame exposure time and dose, etc.) please let us know about it here. You will get a detailed checklist from us once your project is approved, and on the day of imaging we will contact you to go over the imaging conditions in detail with recommendations based on our screening of grids. However, it might be useful to know about specific requirements beforehand.

E. Data transfer

Data will be made available for download through Globus (www.globus.org).

User access and data transfer

- **User access:**
 - public website with general information and link to “Sample Information Form” to submit a new project
 - website will publish regular updates on performance, upgrades, etc
 - Information on NCEF will be also be posted on social media (Twitter, Facebook, and LinkedIn) and on email listservers (3D-EM Listserv, Listserv of Microscopy Society of America (MSA), NIH Grantees List)
- **Data transfer:**
 - 100 TB storage on a blade system. Data will be made available for download through globus (www.globus.org)
 - data will be stored for one month to ensure adequate time for transfer to end user

Benchmarks

- **Data collection with automated workflows:**
 - Single Particle with Latitude on K2 up to 35 images/hour
 with EPU on K2 up to 24 image/hour
 with EPU on Falcon up to 34 images/hour
 - Tomography with Tomo on K2 up to 1 tiltseries in 55 min
 with Tomo on Falcon up to 1 tiltseries in 45 min
- **Data transfer/download**
 - several stable transfers of datasets were successfully performed with fast and slow internet connections (30 MB/s vs. 3.5 MB/s)