Update National Cryo-EM Facility
FN LAC Meeting
October 30, 2017
Presentation Outline: National Cryo-EM Facility

1. The cryo-EM workflow
2. What can we do now that we couldn’t do before?
3. Applications to cancer research
4. NCEF infrastructure and operational details
5. Performance metrics
6. Plans for FY 2018
Single-Particle Cryo-Electron Microscopy Technique Overview
“Traditional” cryo-EM targets

- Dengue Virus, 11,200 kDa, PDB 3J27
- T4 base plate, 8,700 kDa, PDB 5IV5
- Ljungan virus, 6,000 kDa, PDB 3JB4
- Human 26S proteasome, 2,500 kDa, PDB 5GJR
- Human ribosome, 3,900 kDa, PDB 5AJ0
- Anaphase proteasome complex (APC), 1,200 kDa, PDB 5Q6
Growing diversity of cryo-EM targets

- **Ljungan virus**: 6,000 kDa, PDB 3JB4
- **Human ribosome**: 3,900 kDa, PDB 5AJ0
- **Human 26S proteasome**: 2,500 kDa, PDB 5GJR
- **Anaphase promoting complex (APC/C)**: 1,200 kDa, PDB 5G04
- **Anthrax protective antigen pore**: 440 kDa, PDB 3J9C
- **CorA**: 207 kDa, PDB 3JCG
- **p97**: 540 kDa, PDB 5FTJ
- **GDH**: 334 kDa, PDB 5k12
- **IDH**: 93 kDa, PDB 5K10

**Size (Kilodaltons, kDa)**
2.2 Å resolution cryo-EM structure of β-galactosidase in complex with a cell-permeant inhibitor

Alberto Bartesaghi,¹⁺ Alan Merk,¹⁺ Soojay Banerjee,¹ Doreen Matthies,¹ Xiongwu Wu,² Jacqueline L. S. Milne,¹ Sriram Subramaniam¹†
2.3 Å resolution cryo-EM structure of human p97 and mechanism of allosteric inhibition

Soojay Banerjee,1,∗ Alberto Bartesaghi,1,∗ Alan Merk,1 Prashant Rao,1 Stacie L. Bulfer,2 Yongzhao Yan,3 Neal Green,4 Barbara Mroczkowski,5 R. Jeffrey Neitz,2 Peter Wipf,3 Veronica Falconieri,1 Raymond J. Deshaies,6 Jacqueline L. S. Milne,1 Donna Huryn,3 Michelle Arkin,2 Sriram Subramaniam1†
Detailed structure of inhibitor-binding site
An atomic structure of the human 26S proteasome

Xiuliang Huang¹⁻⁴, Bai Luan¹⁻⁴, Jianping Wu¹⁻⁴ & Yigong Shi¹⁻³
Human 26S Proteasome

- Detailed understanding asymmetric docking of the regulatory particle onto the core
Molecular mechanism of APC/C activation by mitotic phosphorylation

Suyang Zhang*, Leifu Chang*, Claudio Alfieri1, Ziqiao Zhang1, Jing Yang1, Sarah Maslen1, Mark Skehel1 & David Barford1
Structural basis of kainate subtype glutamate receptor desensitization

Joel Meyerson, Sagar Chittori, Alan Merk, Prashant Rao, Tae Hee Han, Mihaela Serpe, Mark Mayer and Sriram Subramaniam

2016

Desensitized kainate receptor at 3.8 Å resolution
Cryo-EM Structures Reveal Mechanism and Inhibition of DNA Targeting by a CRISPR-Cas Surveillance Complex

Tai Wei Guo\textsuperscript{1,6}, Alberto Bartsaghi\textsuperscript{1,7}, Hui Yang\textsuperscript{2,6}, Veronica Falconieri\textsuperscript{1}, Prashant Rao\textsuperscript{3}, Alan Merk\textsuperscript{3}, Edward T. Eng\textsuperscript{3}, Ashleigh M. Raczkowski\textsuperscript{3}, Tara Fox\textsuperscript{4,5}, Lesley A. Earl\textsuperscript{1}, Dinshaw Patel\textsuperscript{1} and Sriram Subramaniam\textsuperscript{1,4}.

Type I-F Csy complex bound to DNA/inhibitors in different functional states

The battle between protection against foreign DNA vs infection
Growth of cryo-EM structures

Year

Maps Released

≤ 15Å
≤ 10Å
≤ 8Å
≤ 6Å
≤ 4Å
≤ 3Å
≤ 2.5Å

Initial NCEF discussions
NCEF Personnel

David Heimbrook
Lab Director, FNLCR

Dwight Nissley
Director, CRTP, FNLCR

Ulrich Baxa
Senior Microscopist, NCEF

Sriram Subramaniam
FNLCR Cryo-EM Program Advisor
(Founding Director, NCEF)

Thomas Edwards
Junior Microscopist, NCEF

Helen Wang
Project Manager, NCEF

New Hire
IT and Microscopy Support, NCEF

Operational since May 15, 2017

To be appointed
FNLAC Ad Hoc NCEF Working Group

– 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; FNLAC member)
– Dr. Stephen Harrison (Harvard University School of Medicine)
– Dr. Sara Hook (National Cancer Institute)
– Dr. Grant Jensen (Caltech)
– Dr. Piermaria Oddone (Fermilab; FNLAC member)
– Dr. Hong Zhou (UCLA)
Operations – NCEF Perspective

1. Receive request
2. Receive grids
   - **ACCEPT**: Communicate decision within 48 hours
   - **REJECT**: Provide feedback and advice as needed
Operations – NCEF Perspective

1. Receive request
2. Receive grids
3. Schedule imaging slot
4. Collect data
5. Deliver data

- Enter into queue
- Engage with user and reach agreement on imaging conditions
- Communicate with user throughout session
- Transfer data immediately after session

Current wait time < 1 month, but will get progressively longer over the coming year
NCEF Cumulative Projects Since May 15 Launch

Extramural user projects

Software/hardware installation and methods testing

May 15 | June | July | August | September | October

CUMULATIVE PROJECTS

0 20 40
NCEF User feedback

- Application process: 100% satisfied
- Shipping: 100% satisfied
- Data collection: 100% satisfied
- Data transfer: 100% satisfied
- Data quality: 100% satisfied

Other comments

- “very satisfactory”
- “images were of very high quality”
- “images [were of] outstanding quality”
- “[data] is at resolution of 4 Å”
- “impressed with high quality of data”
- “staff is very knowledgeable and helpful”
- “very satisfied with your service”
- “allowed us to reconstruct [structure] down to ~3.4 Å”
- “[operational model] is bound to promote and accelerate the best possible research”
Plans for FY 2018

• Construction of new microscope facility at ATRF will be completed by June 2018
• Installation of Krios #2 will start in July 2018
• Krios #1 will be moved to ATRF once Krios #2 is operational
• 1-2 new personnel will be added by summer 2018
• Continued efforts to provide access to latest technologies
• Addition of third microscope in 2019 if demand continues to grow
• Consideration of new directions for increasing impact of NCEF

We welcome your suggestions and advice!