

Frederick National Laboratory for Cancer Research

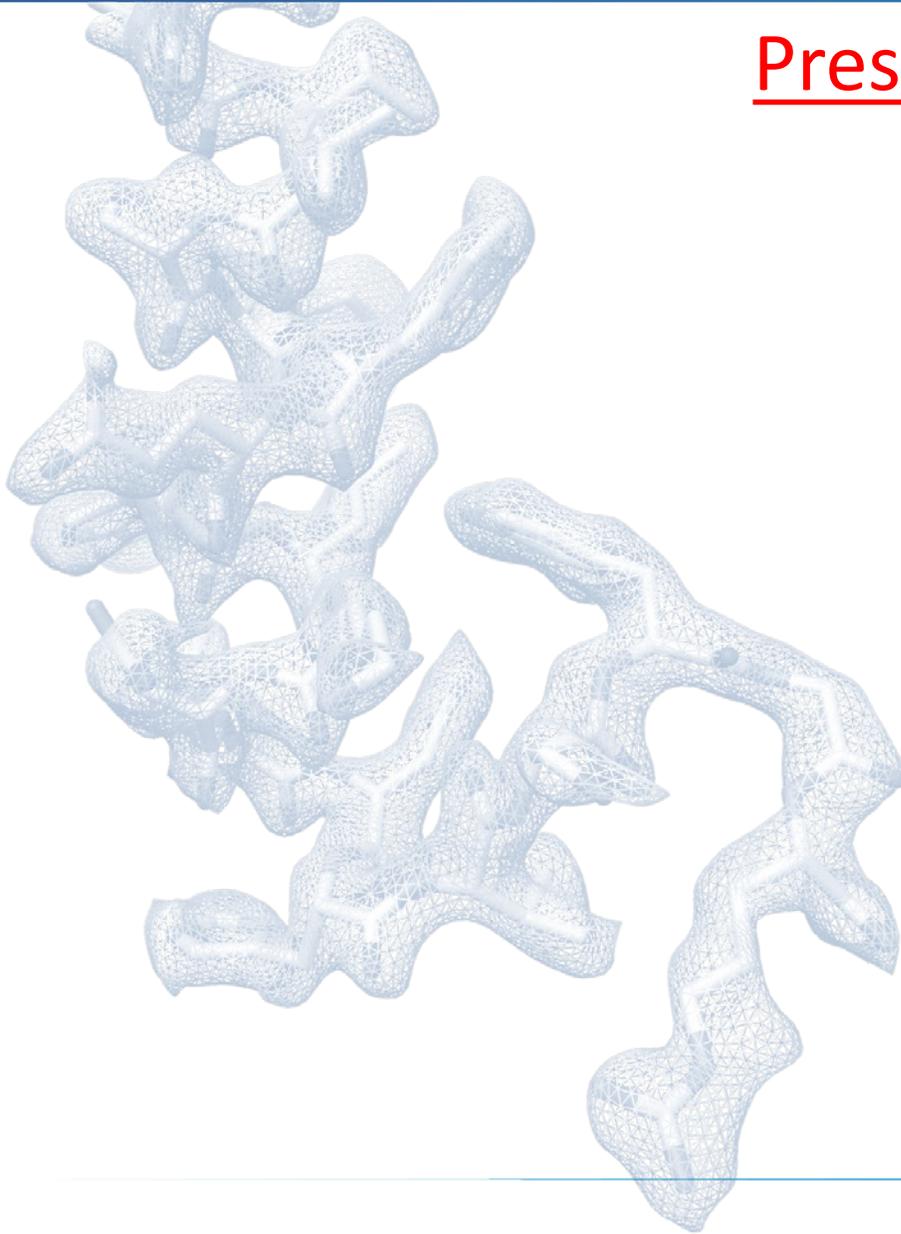


Update National Cryo-EM Facility FNLAC Meeting

October 30, 2017

Presentation Outline: National Cryo-EM Facility

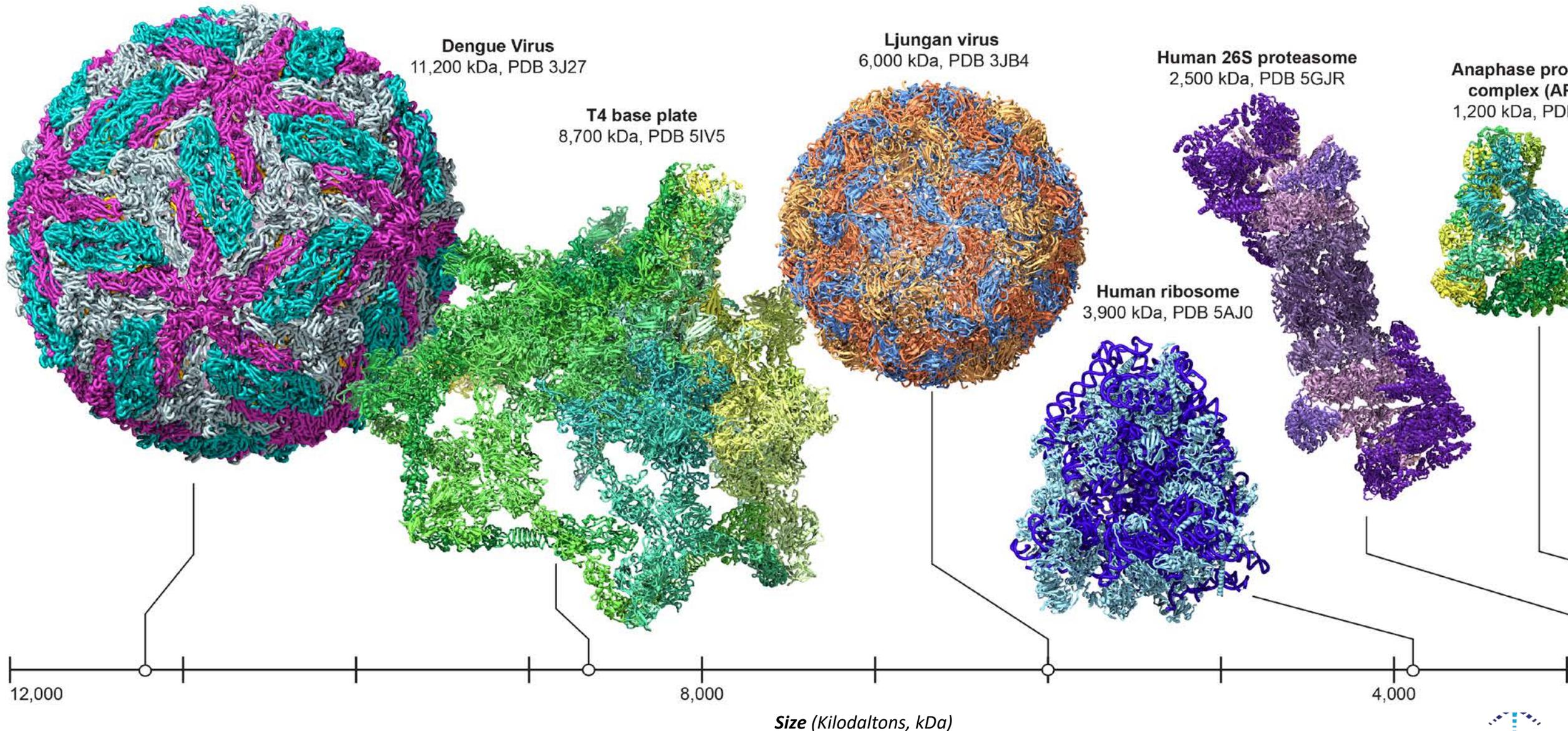
1. The cryo-EM workflow
2. What can we do now that we couldn't do before?
3. Applications to cancer research
4. NCEF infrastructure and operational details
5. Performance metrics
6. Plans for FY 2018



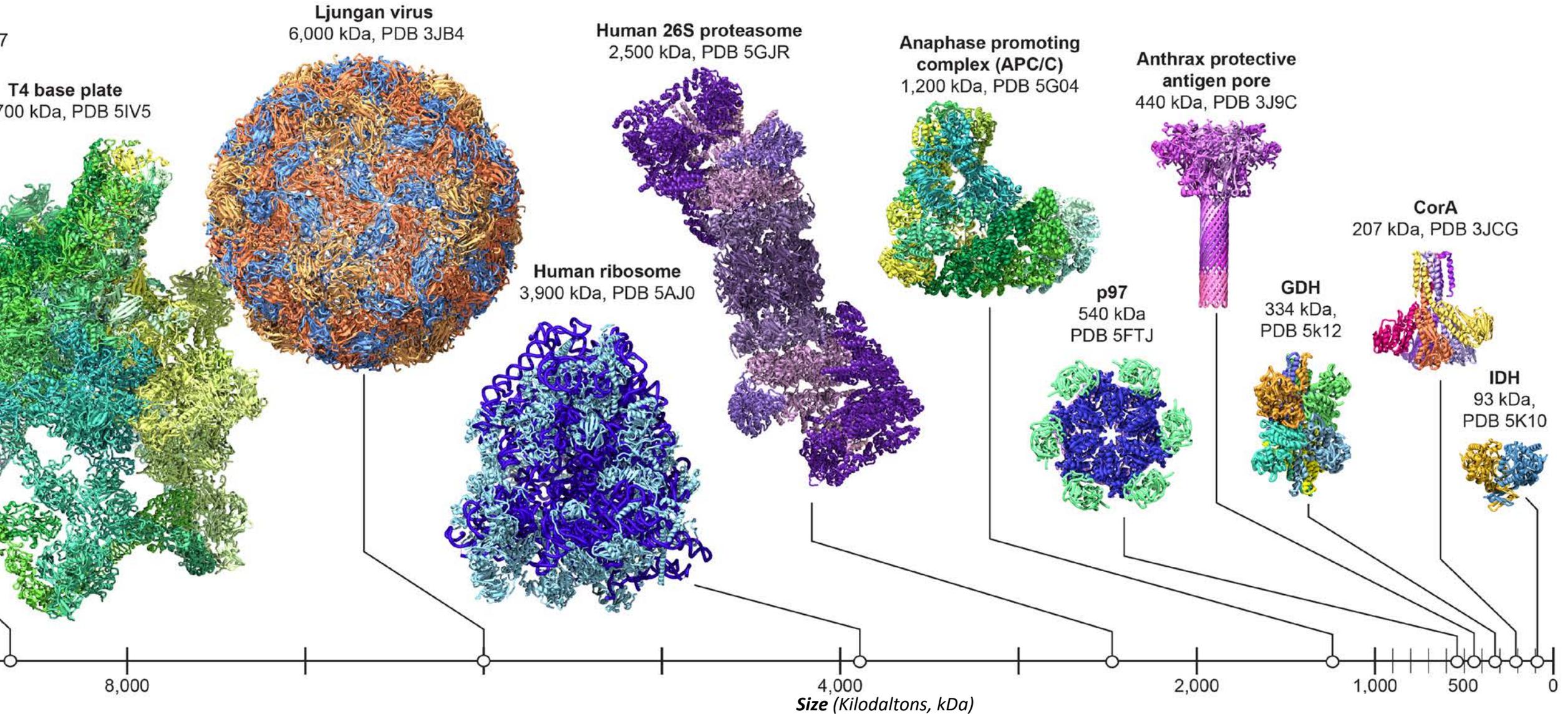
Single-Particle Cryo-Electron Microscopy

Technique Overview

“Traditional” cryo-EM targets



Growing diversity of cryo-EM targets

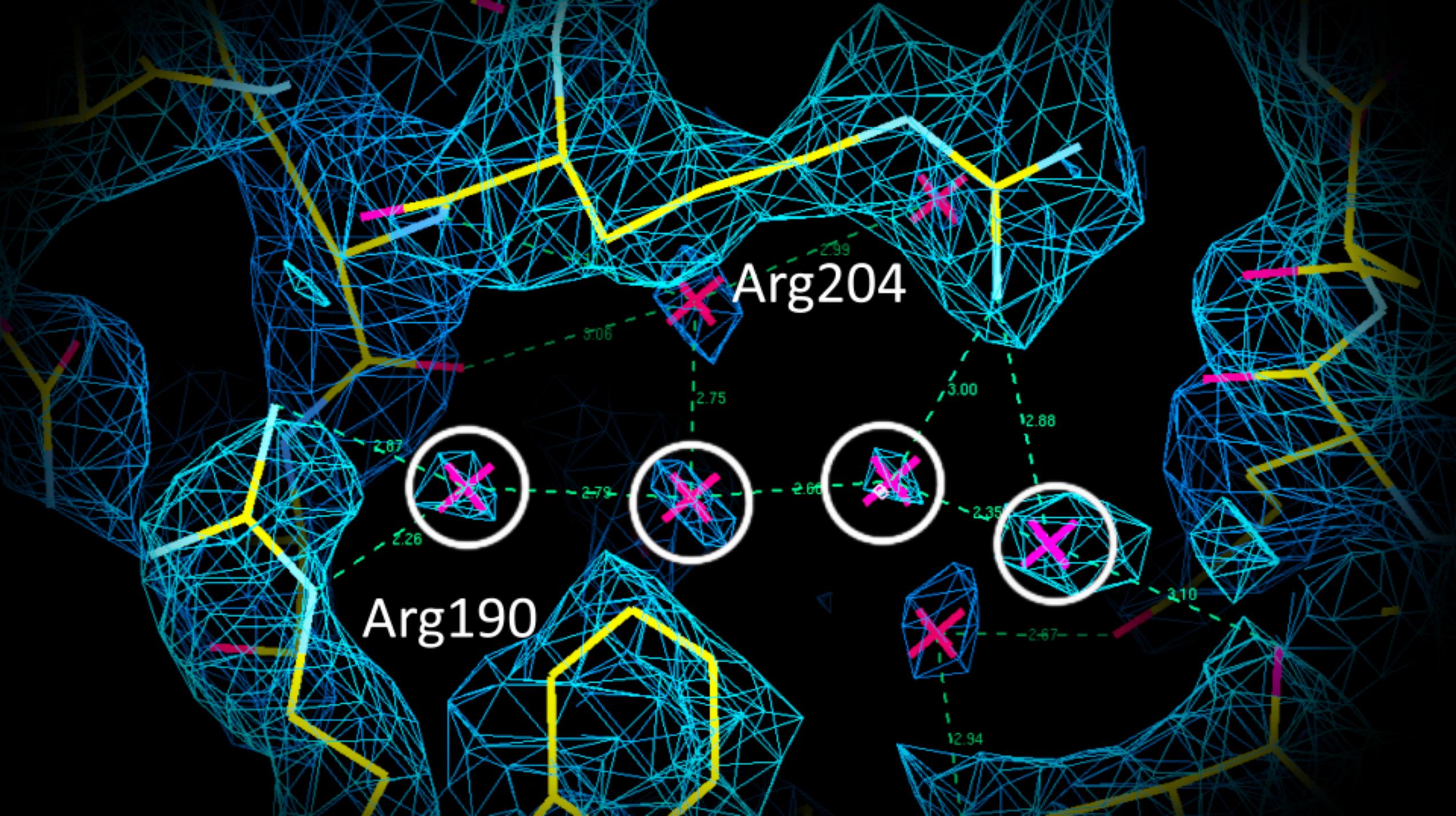



Science AAAS**2015**

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 Subramaniam^{1†}





Arg204

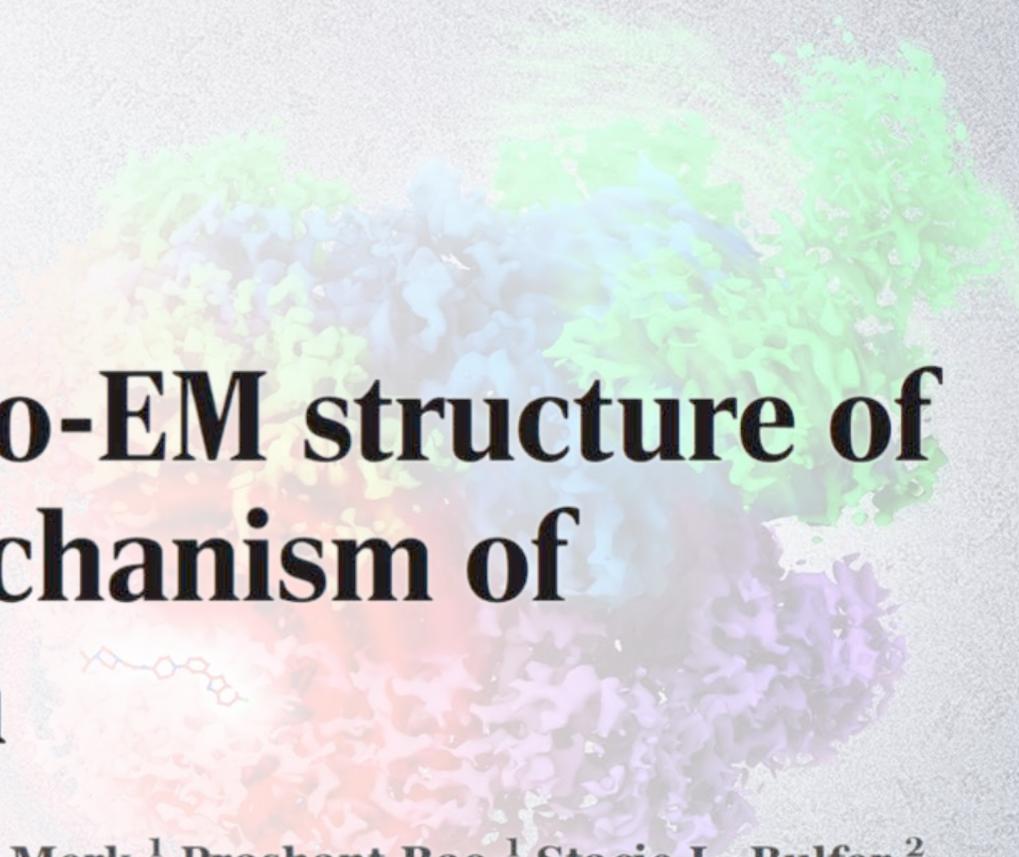
Arg190


Science AAAS

2016

STRUCTURAL BIOLOGY

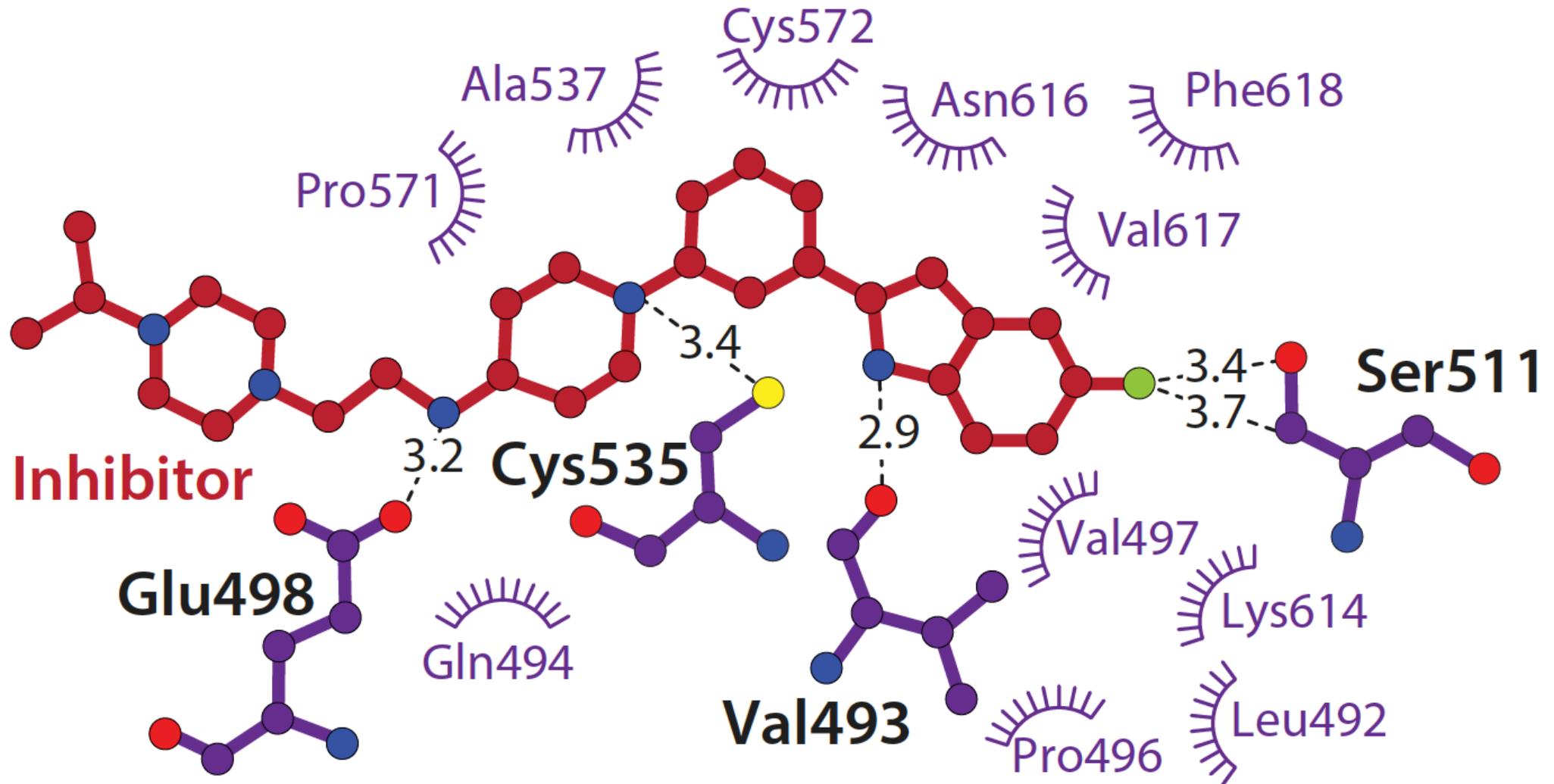
2.3 Å resolution cryo-EM structure of human p97 and mechanism of allosteric inhibition



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 Subramaniam^{1†}



Detailed structure of inhibitor-binding site



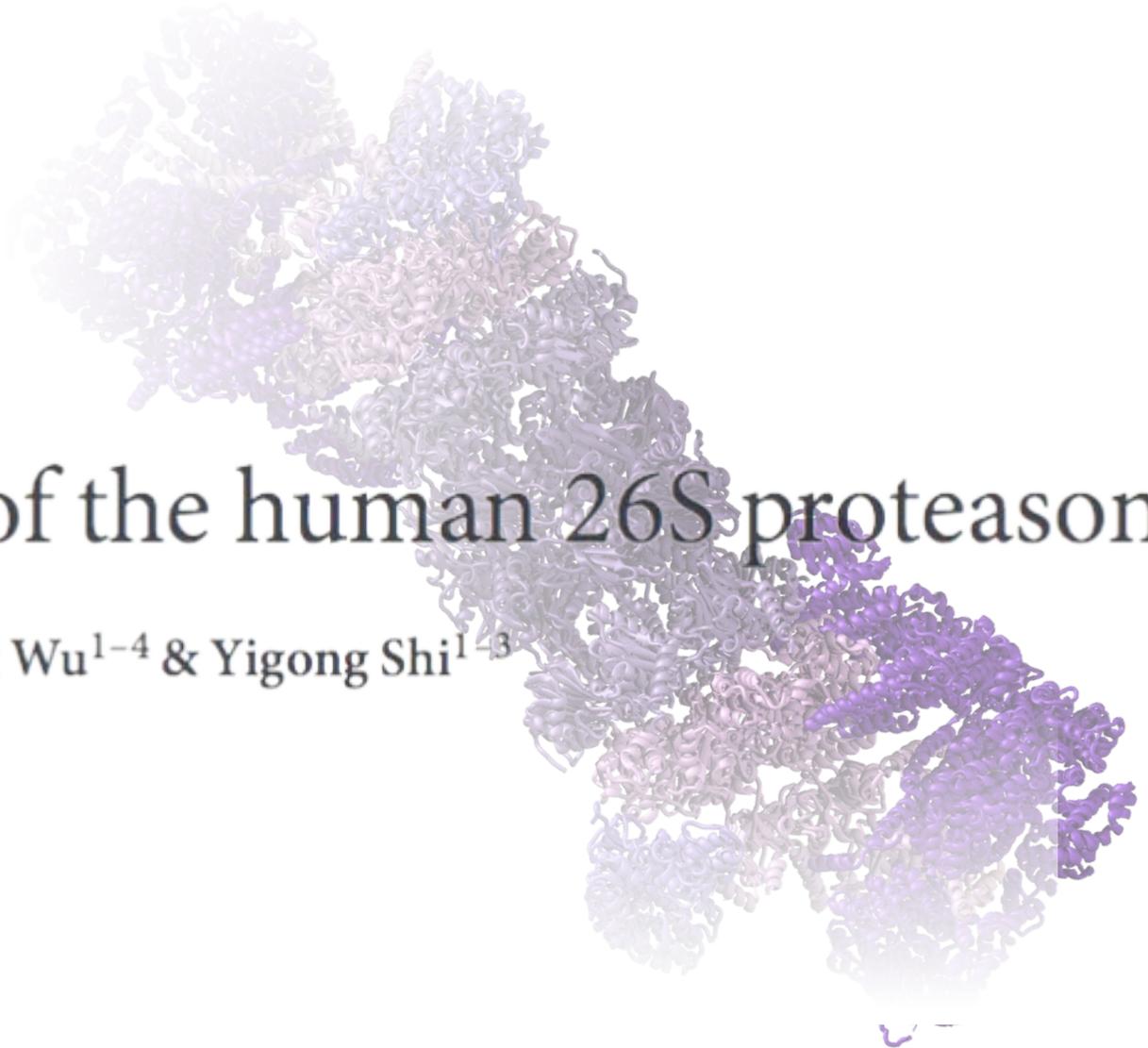


nature
structural &
molecular biology

2016

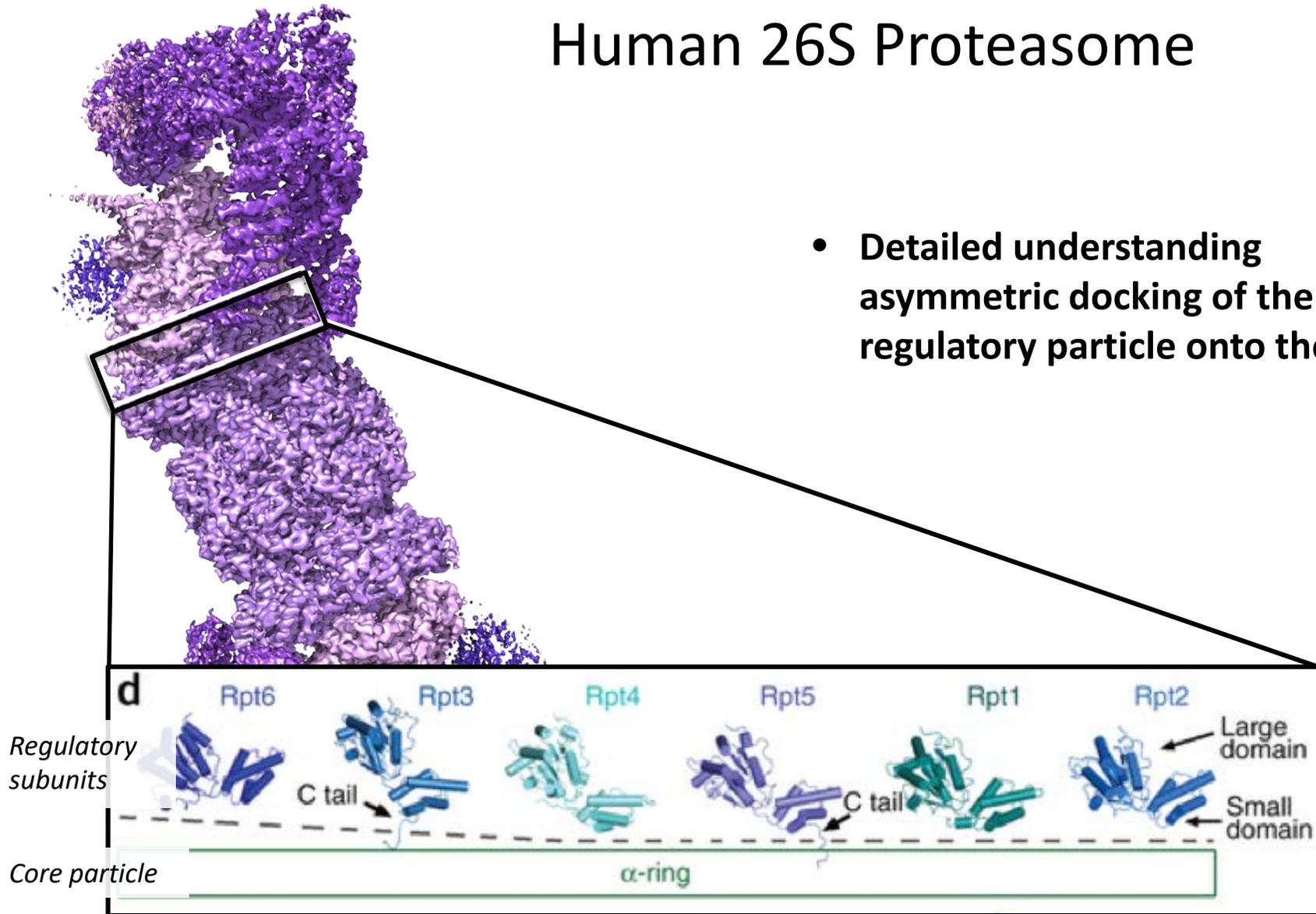
An atomic structure of the human 26S proteasome

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





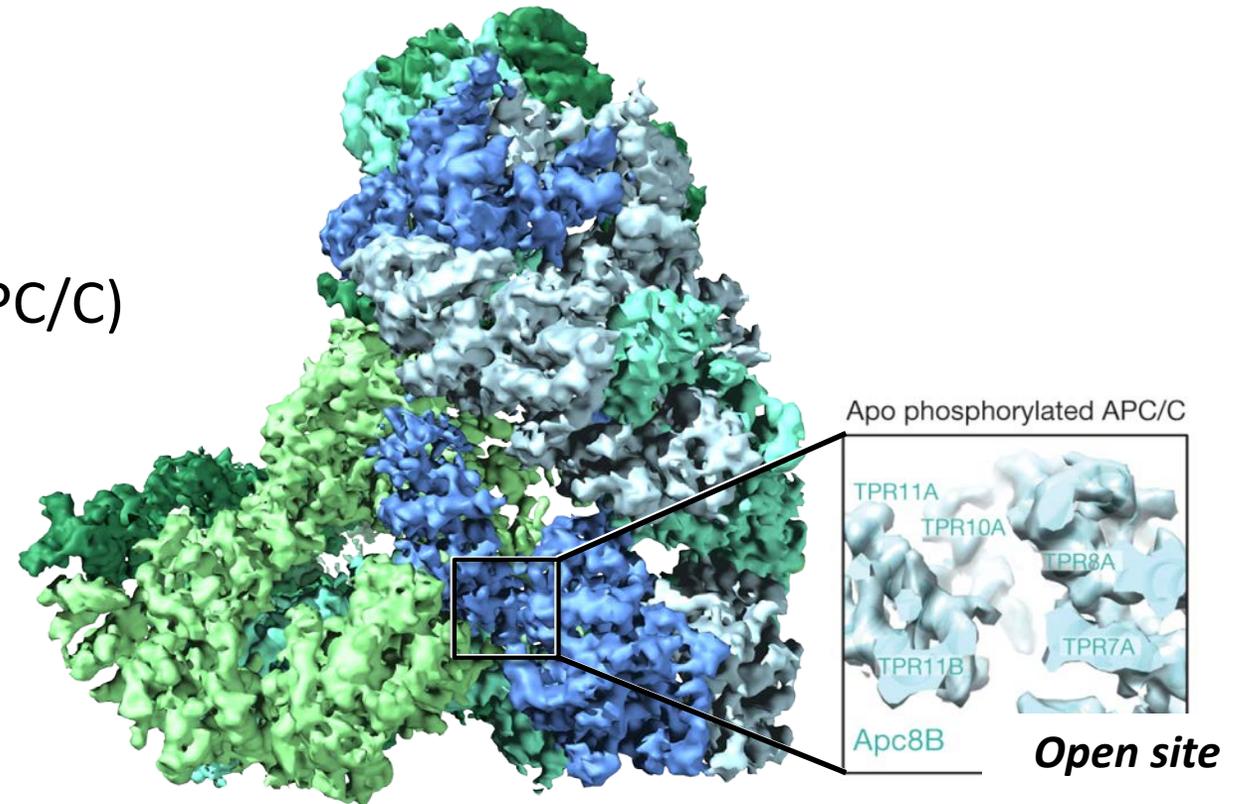
nature International weekly journal of science

Molecular mechanism of APC/C activation by mitotic phosphorylation

2016

Suyang Zhang^{1*}, Leifu Chang^{1*}, Claudio Alfieri¹, Ziguo Zhang¹, Jing Yang¹, Sarah Maslen¹, Mark Skehel¹ & David Barford¹

Anaphase Promoting Complex (APC/C)



Desensitized kainate receptor at 3.8 Å resolution

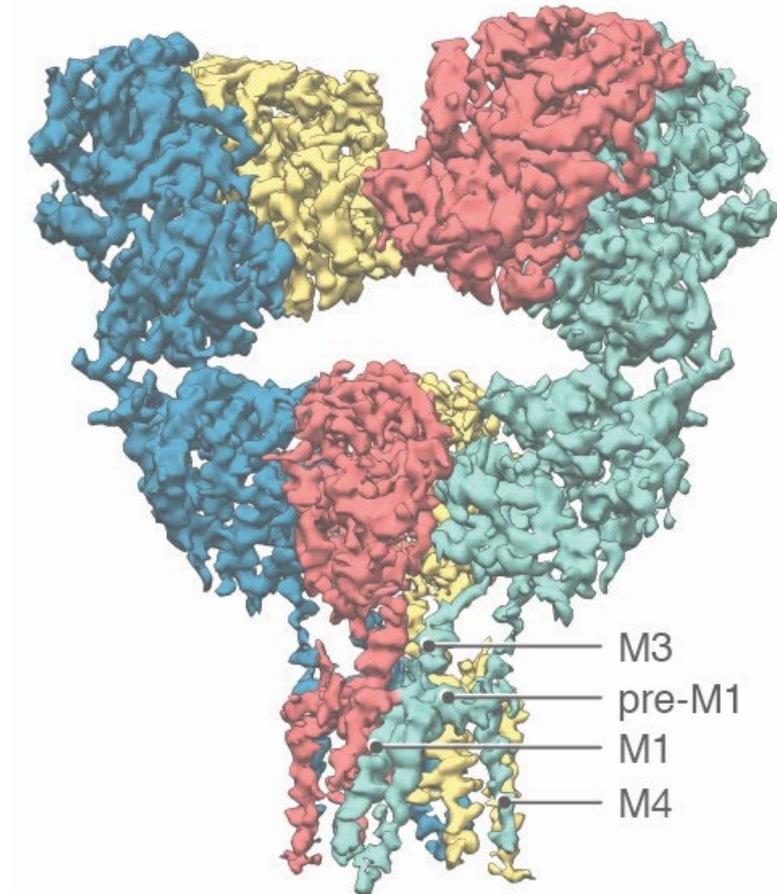
nature

International weekly journal of science

2016

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

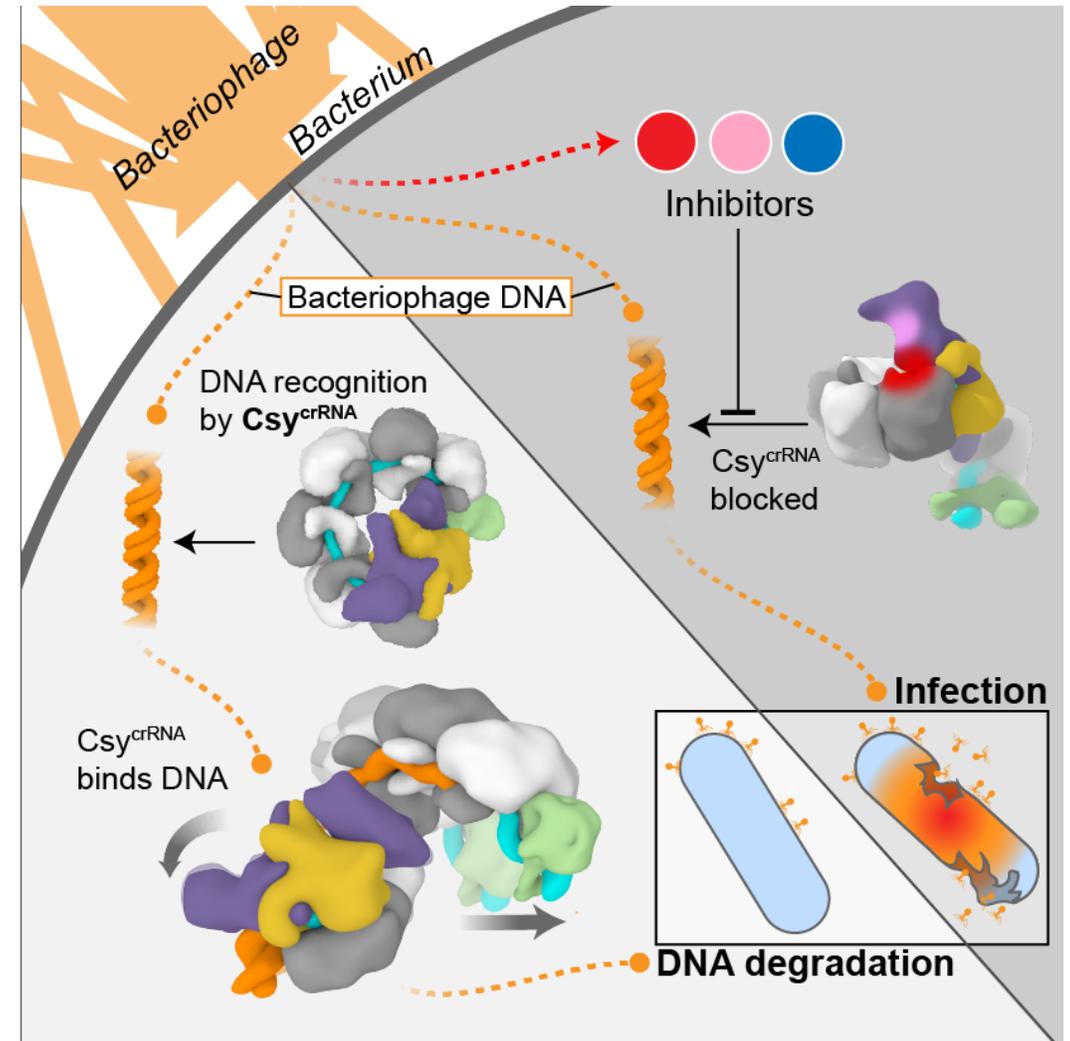


Cell 2017

Cryo-EM Structures Reveal Mechanism and Inhibition of DNA Targeting by a CRISPR-Cas Surveillance Complex

Tai Wei Guo^{1*}, Alberto Bartesaghi^{1*}, Hui Yang^{2*}, Veronica Falconieri¹, Prashant Rao¹, Alan Merk¹, Edward T. Eng³, Ashleigh M. Raczowski³, Tara Fox^{4,5}, Lesley A. Earl¹, Dinshaw Patel² and Sriram Subramaniam^{1,4}

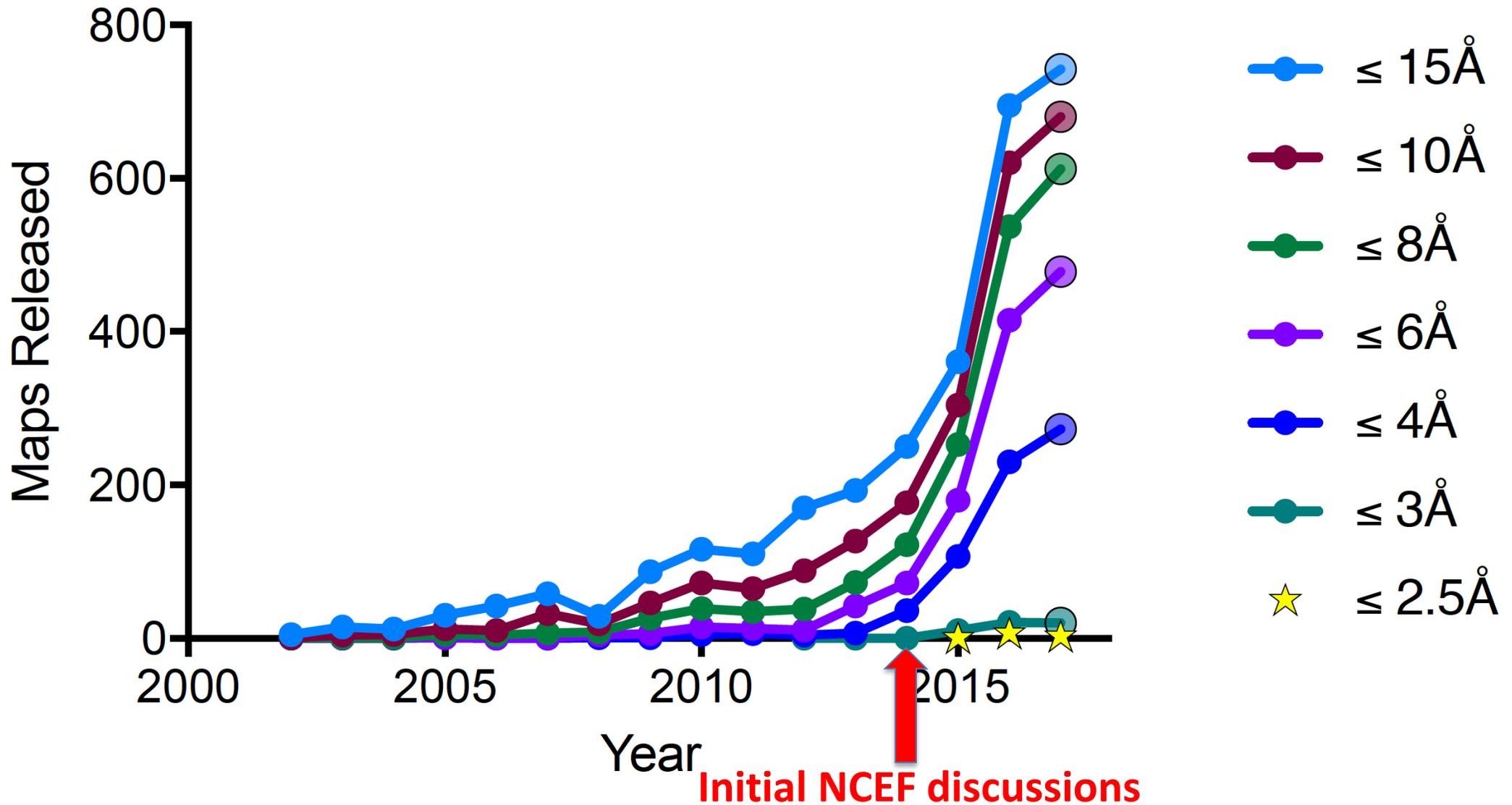
Type I-F Csy complex bound to DNA/inhibitors in different functional states



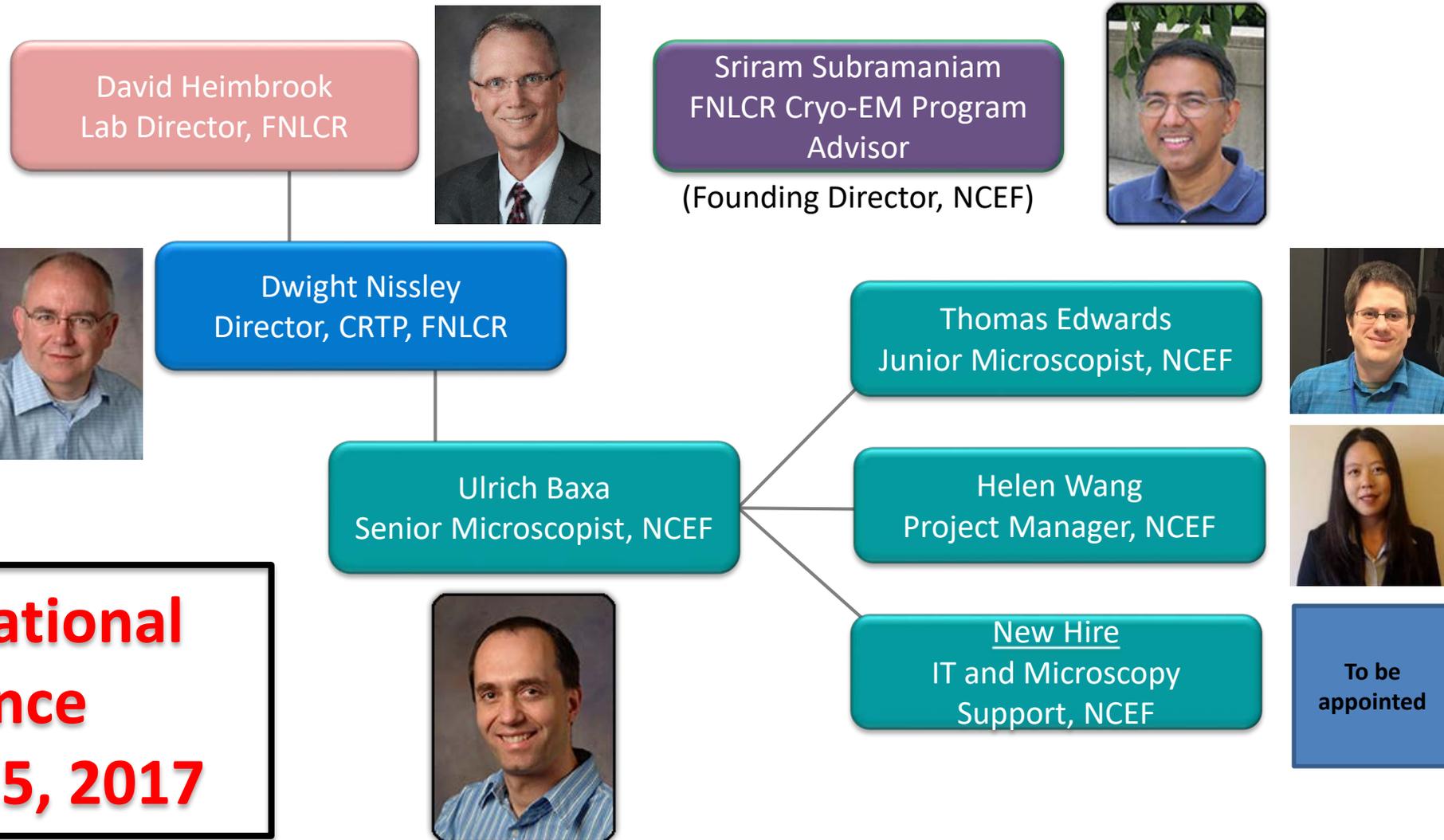
The battle between protection against foreign DNA vs infection



Growth of cryo-EM structures



NCEF Personnel



**Operational
since
May 15, 2017**

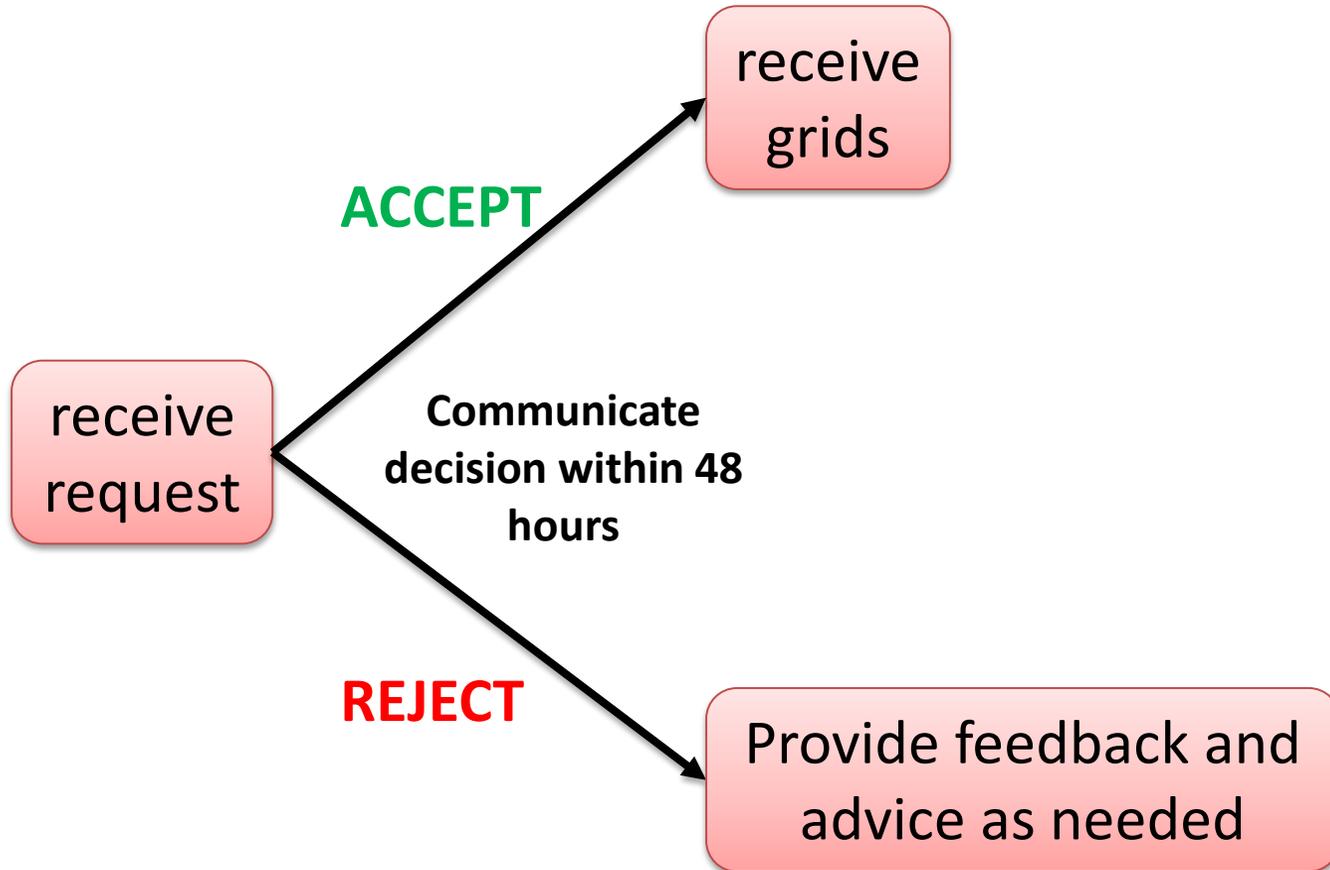


FNLAC Ad Hoc NCEF Working Group

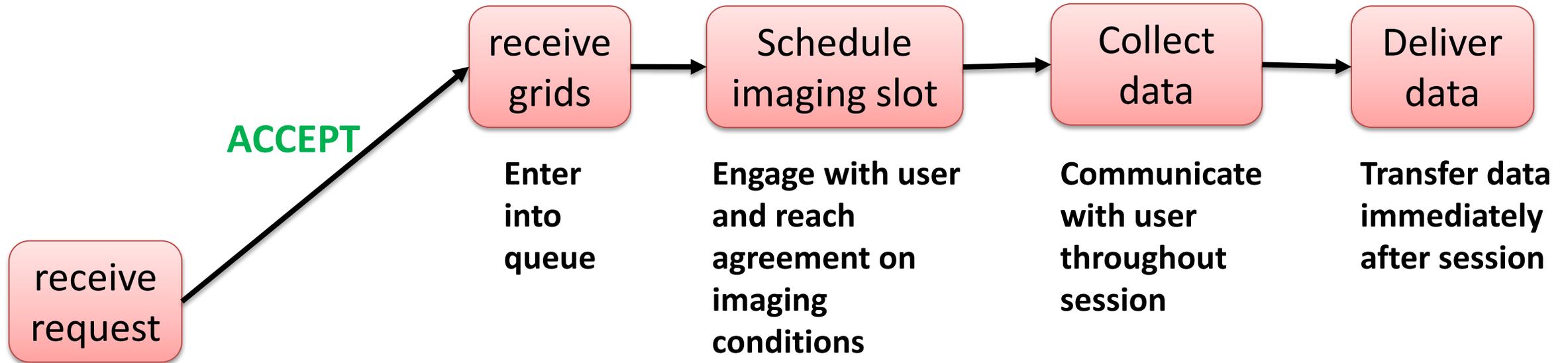
- Dr. Steven Ludtke (Baylor College of Medicine, Chair)
- Dr. Mario Amzel (Johns Hopkins University School of Medicine)
- Dr. Edward Egelman (University of Virginia)
- Dr. Angela Gronenborn (University of Pittsburgh; FNLAC member)
- Dr. Stephen Harrison (Harvard University School of Medicine)
- Dr. Sara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



Operations – NCEF Perspective



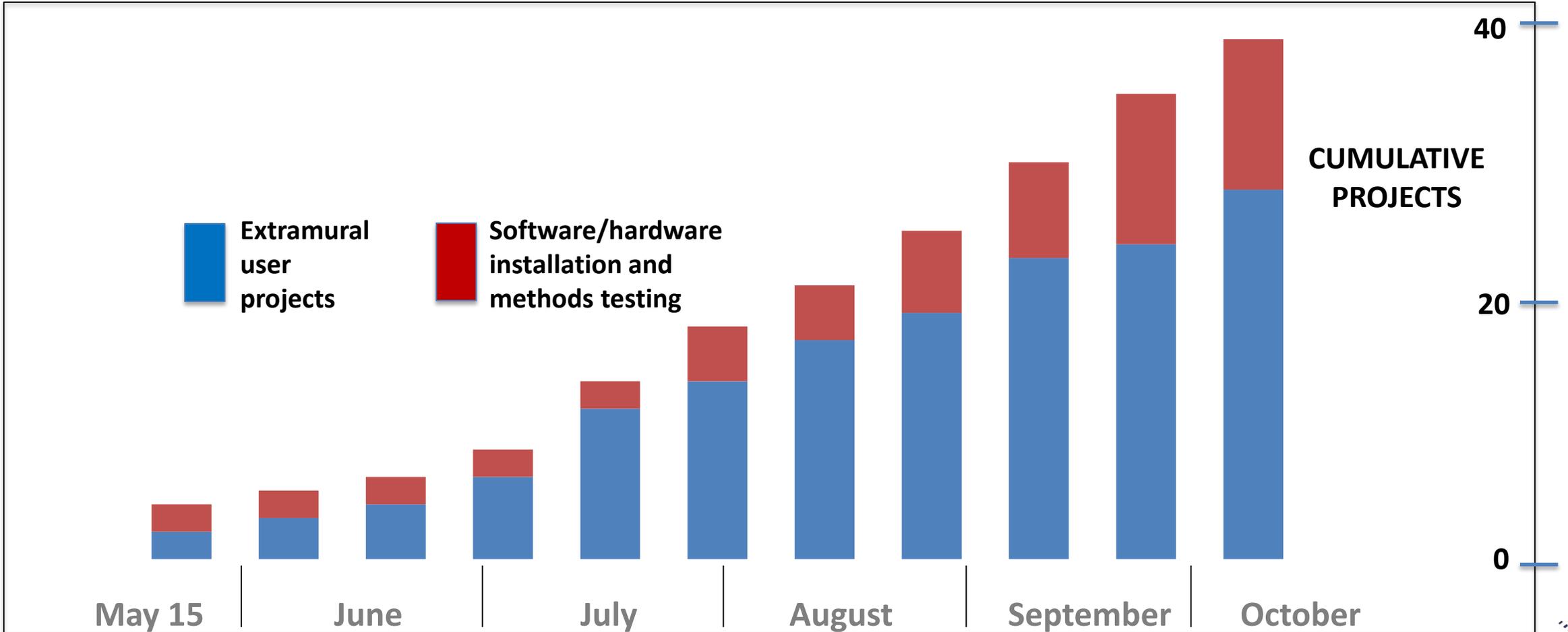
Operations – NCEF Perspective



Current wait time < 1 month, but will get progressively longer over the coming year



NCEF Cumulative Projects Since May 15 Launch



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!



