

Frederick National Laboratory for Cancer Research



Sriram Subramaniam

FNLAC meeting, May 8 2018

NCI National Cryo-EM Facility

<https://www.cancer.gov/research/resources/cryoem>

1-800-4-CANCER Live Chat Publications Dictionary

ABOUT CANCER CANCER TYPES **RESEARCH** GRANTS & TRAINING NEWS & EVENTS ABOUT NCI search 🔍

Home > Research > Research Tools, Specimens, and Data

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RESEARCH TOOLS, SPECIMENS, AND DATA

Conducting Clinical Trials

Statistical Tools and Data

Terminology Resources +

NCI Data Catalog

Cryo-EM

Access

High Resolution Cryo-EM



National Cryo-Electron Microscopy Facility

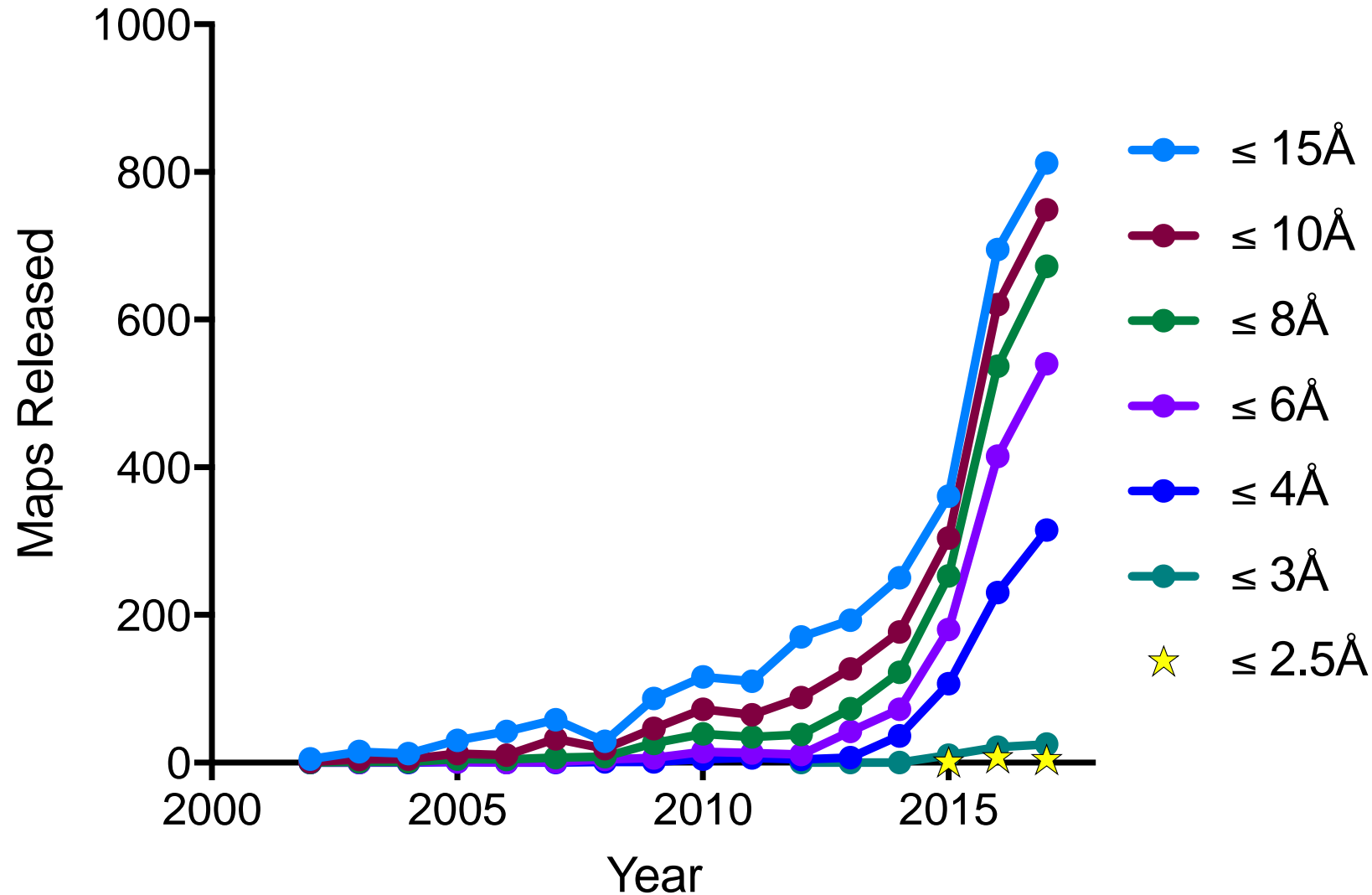
The mission of the National Cryo-Electron Microscopy Facility (NCEF) at NCI is to provide cancer researchers access to the latest cryo-EM technology for high resolution imaging. The National Cryo-EM is a service facility under the umbrella of the [Frederick National Laboratory for Cancer Research](#).

Launched
May 2017

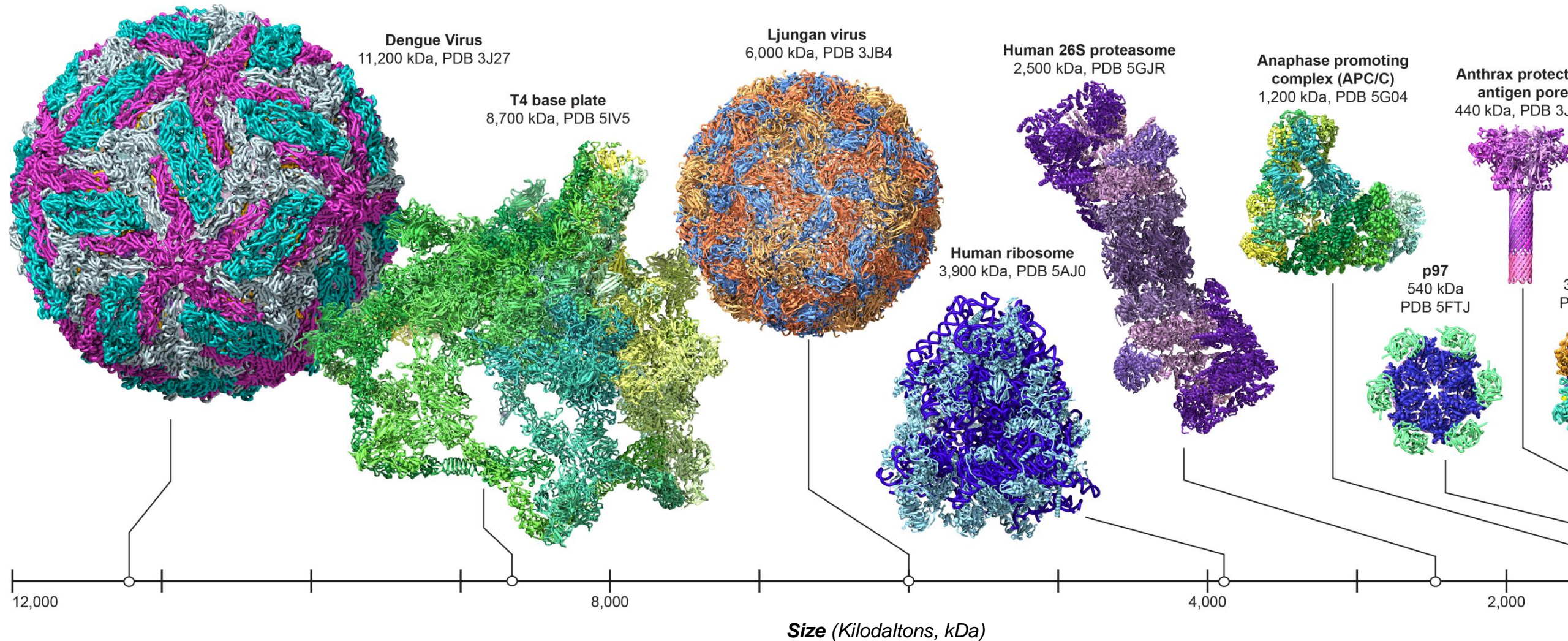
Talk Outline

1. Brief introduction to cryo-EM growth and some examples from recent work at NCI
2. National Cryo-EM Facility performance over last year
3. Discussion on plans for coming year

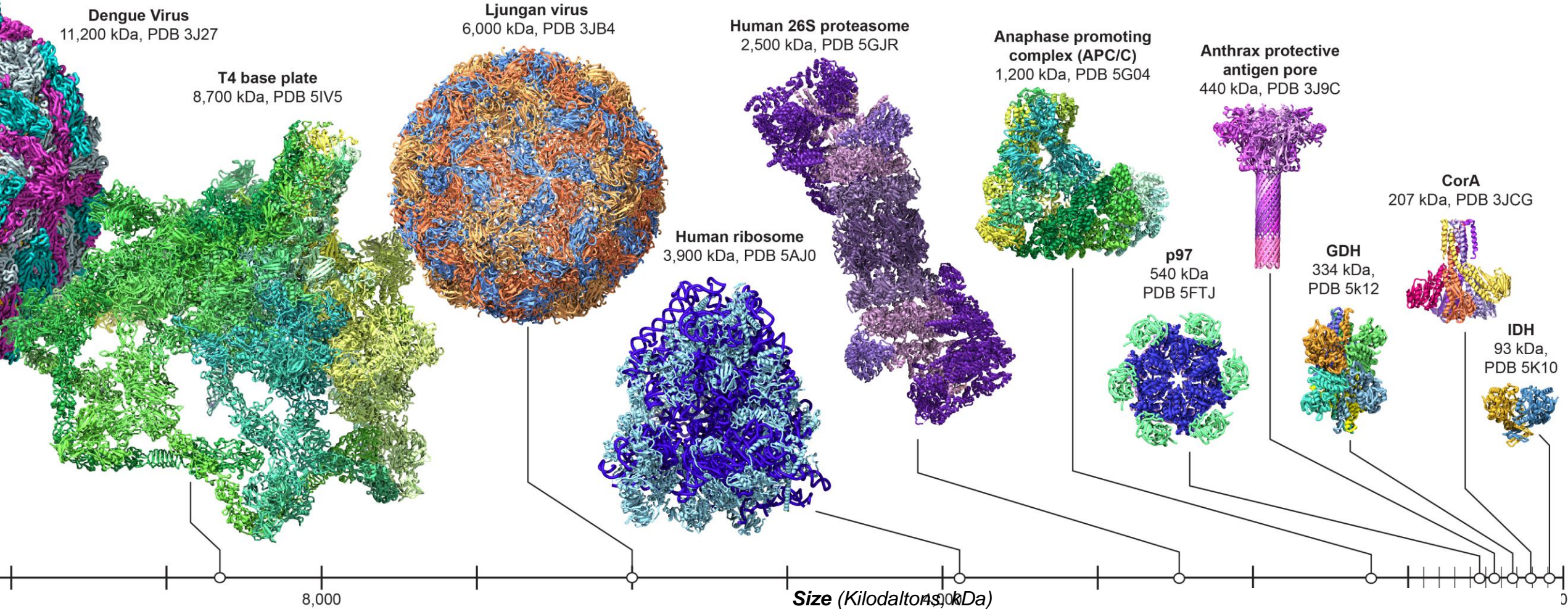
Progress in Single Particle Cryo-EM



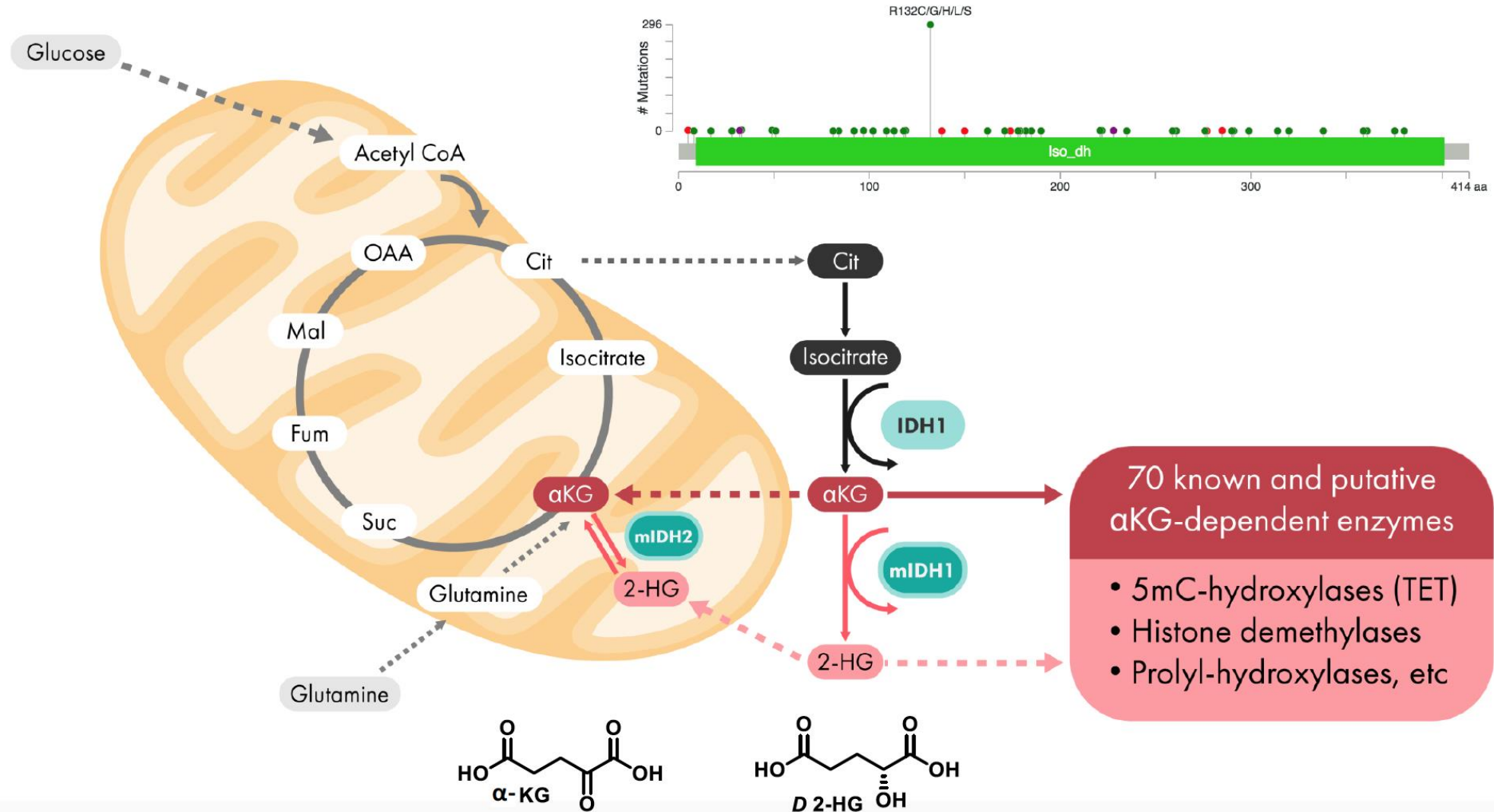
“Traditional” cryo-EM targets



Growing diversity of cryo-EM targets



2-HG oncometabolite production by IDH1



(from NCATS, NIH)

The logo for the journal Cell, featuring the word "Cell" in white on a dark blue rectangular background.

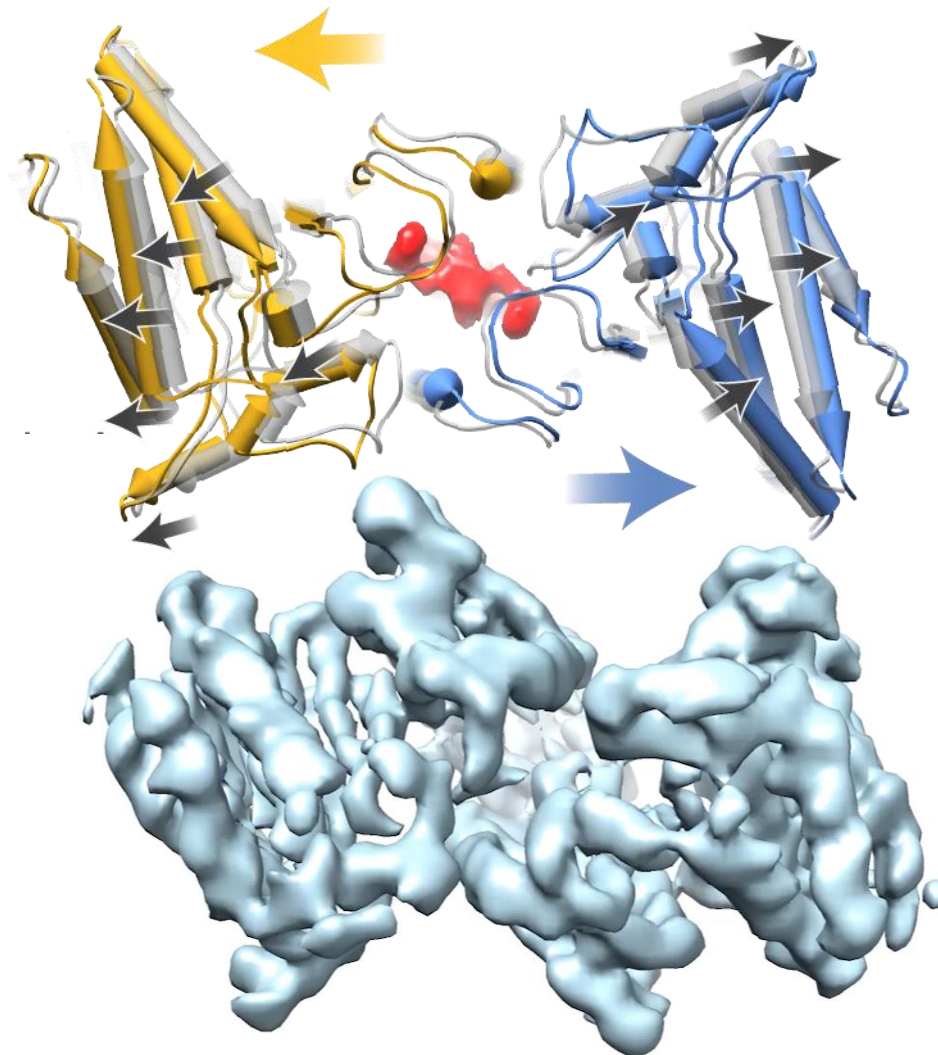
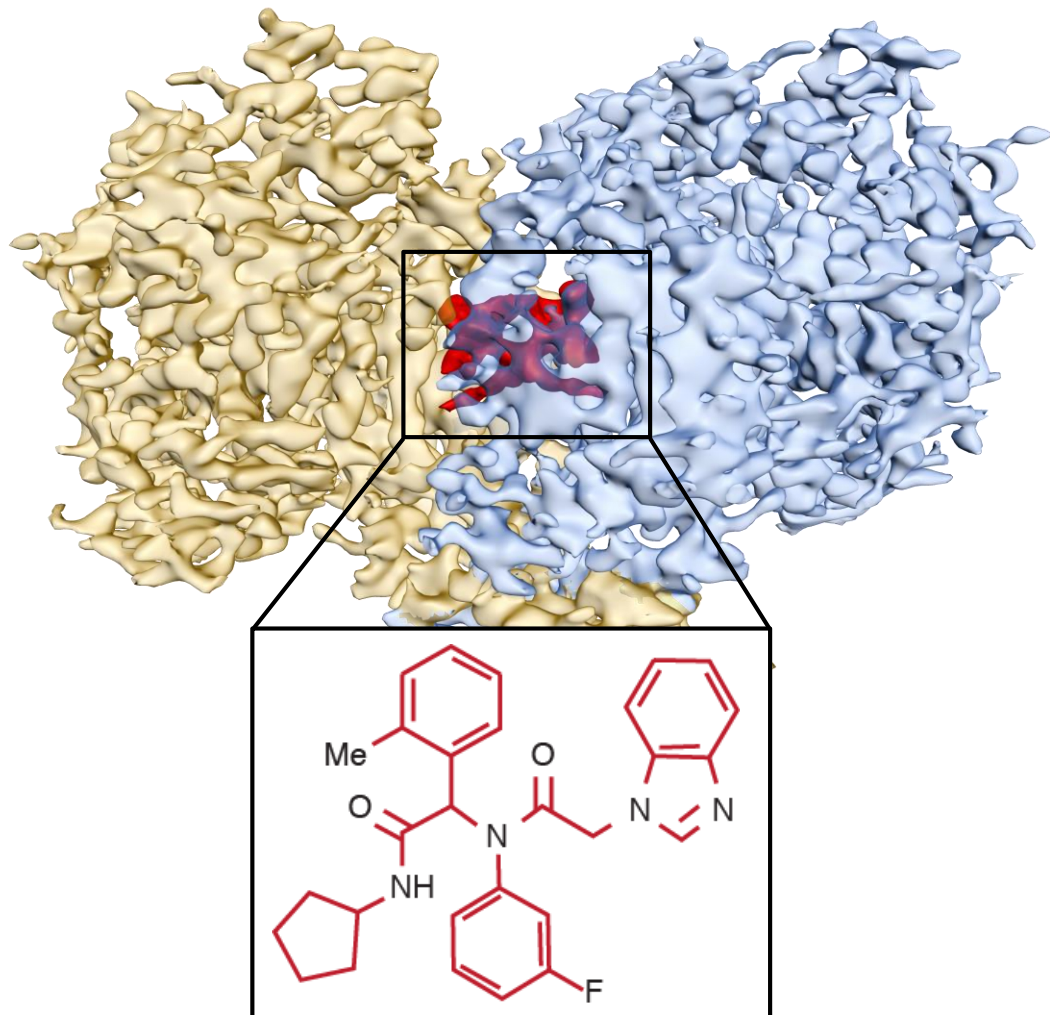
2016

Breaking Cryo-EM Resolution Barriers to Facilitate Drug Discovery

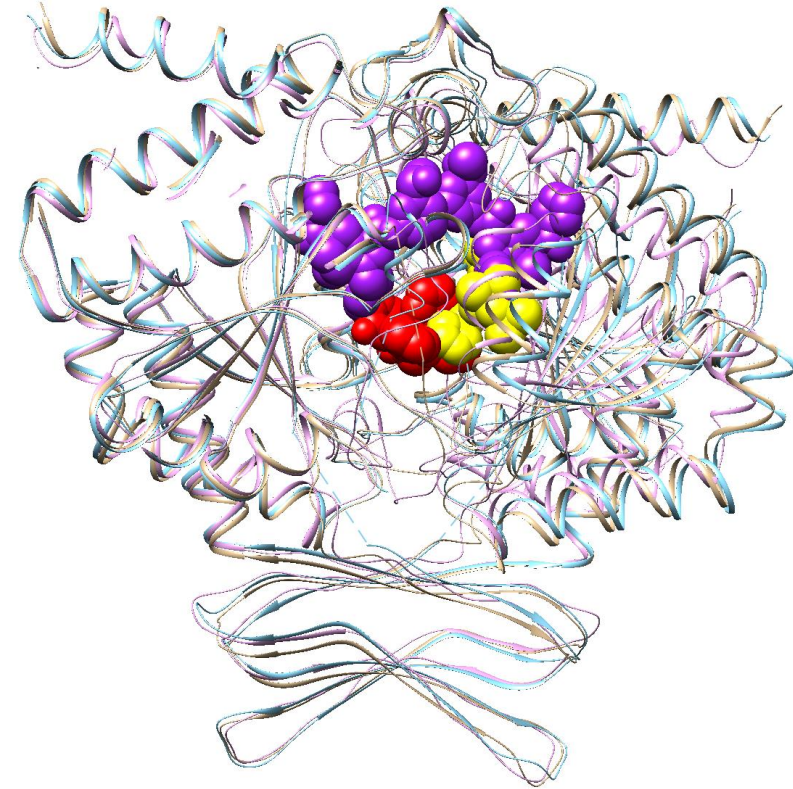
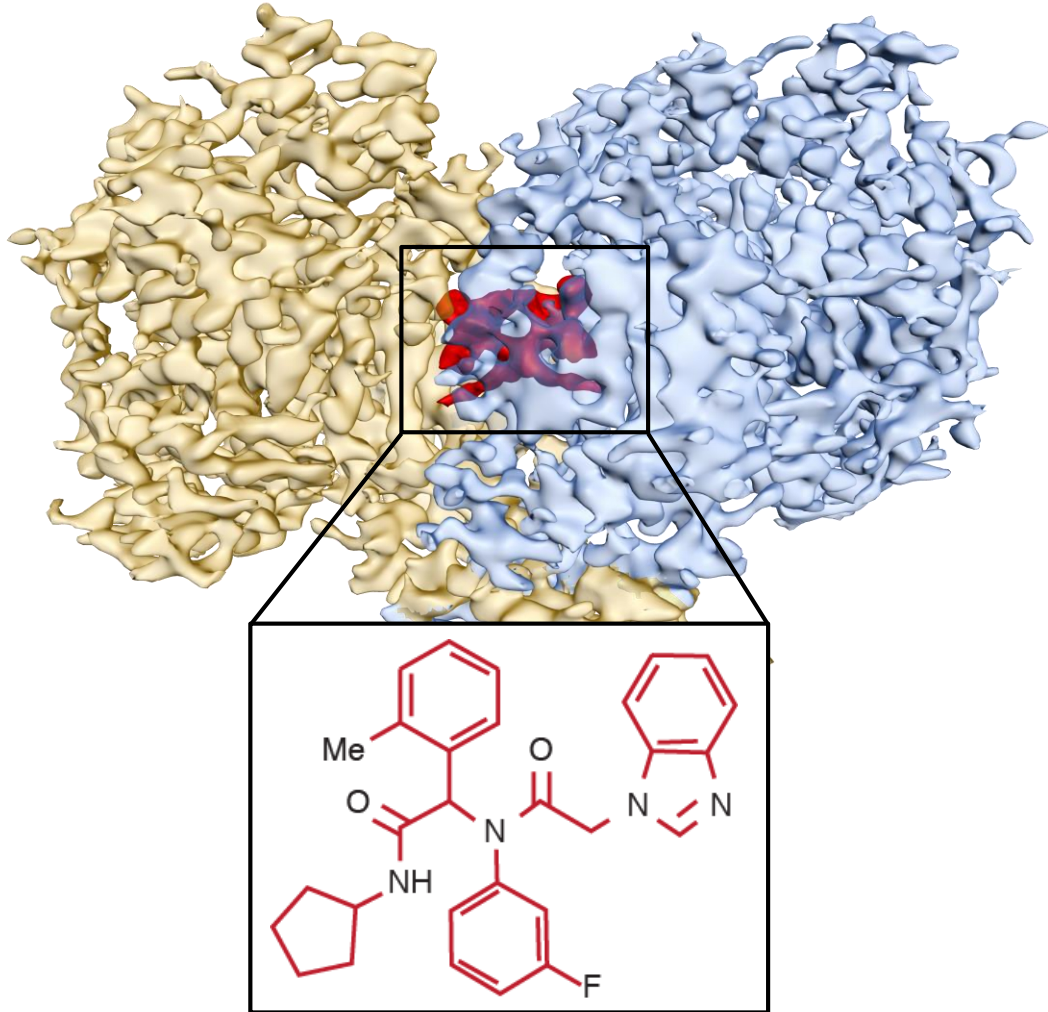
Alan Merk,^{1,3} Alberto Bartesaghi,^{1,3} Soojay Banerjee,^{1,3} Veronica Falconieri,¹ Prashant Rao,¹ Mindy I. Davis,² Rajan Pragani,² Matthew B. Boxer,² Lesley A. Earl,¹ Jacqueline L.S. Milne,¹ and Sriram Subramaniam^{1,*}

First report of cryo-EM structures and conformational states of glutamate, lactate and isocitrate dehydrogenase

Drug Localization by Cryo-EM in mIDH1



Drug Localization by Cryo-EM in mIDH1



Binding sites of different inhibitors:
GSK321A (GSK); **Compound 1 (Sanofi)** and **ML309 (NCATS/NIH)**

Science AAAS

2016

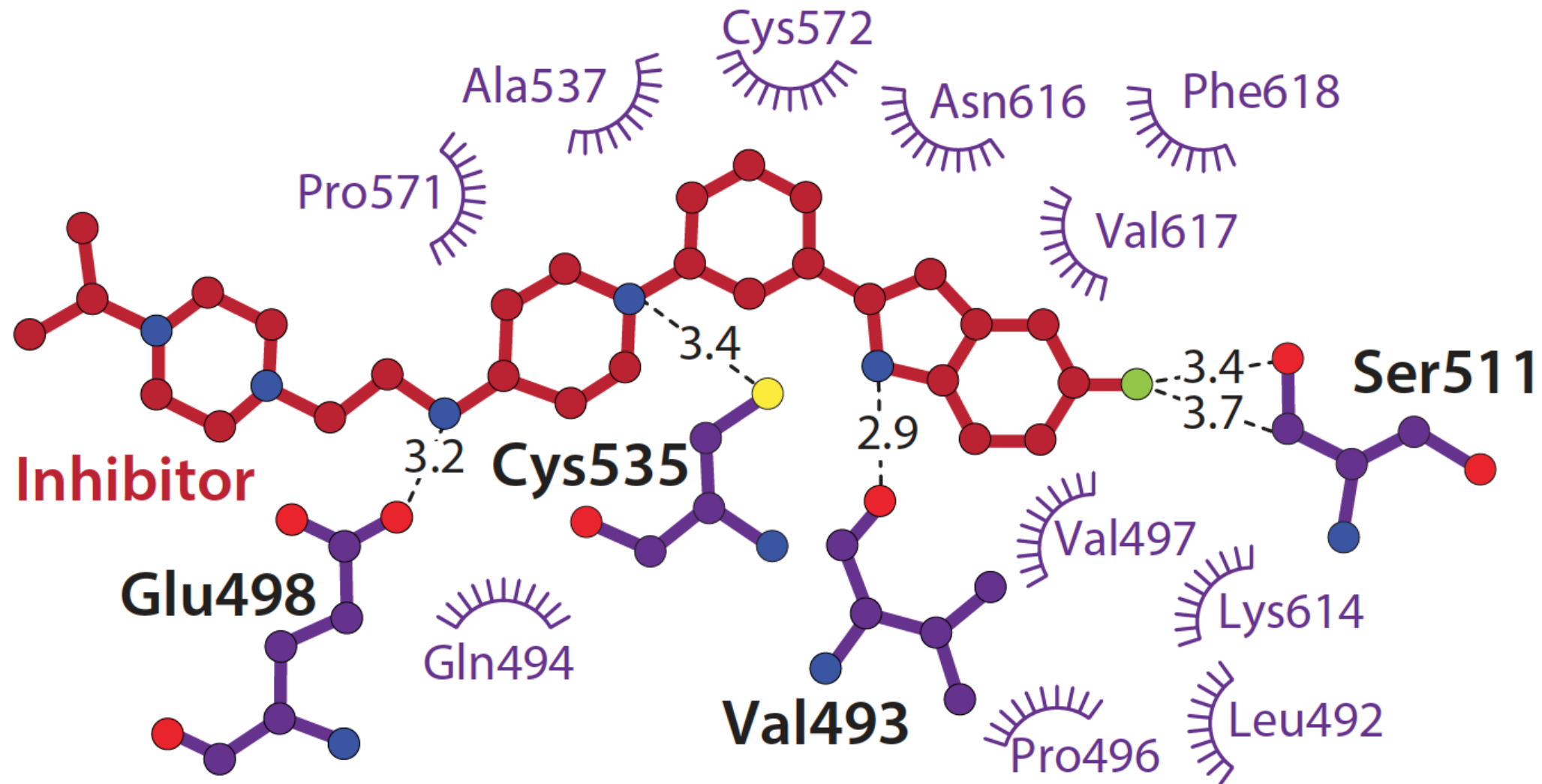
STRUCTURAL BIOLOGY

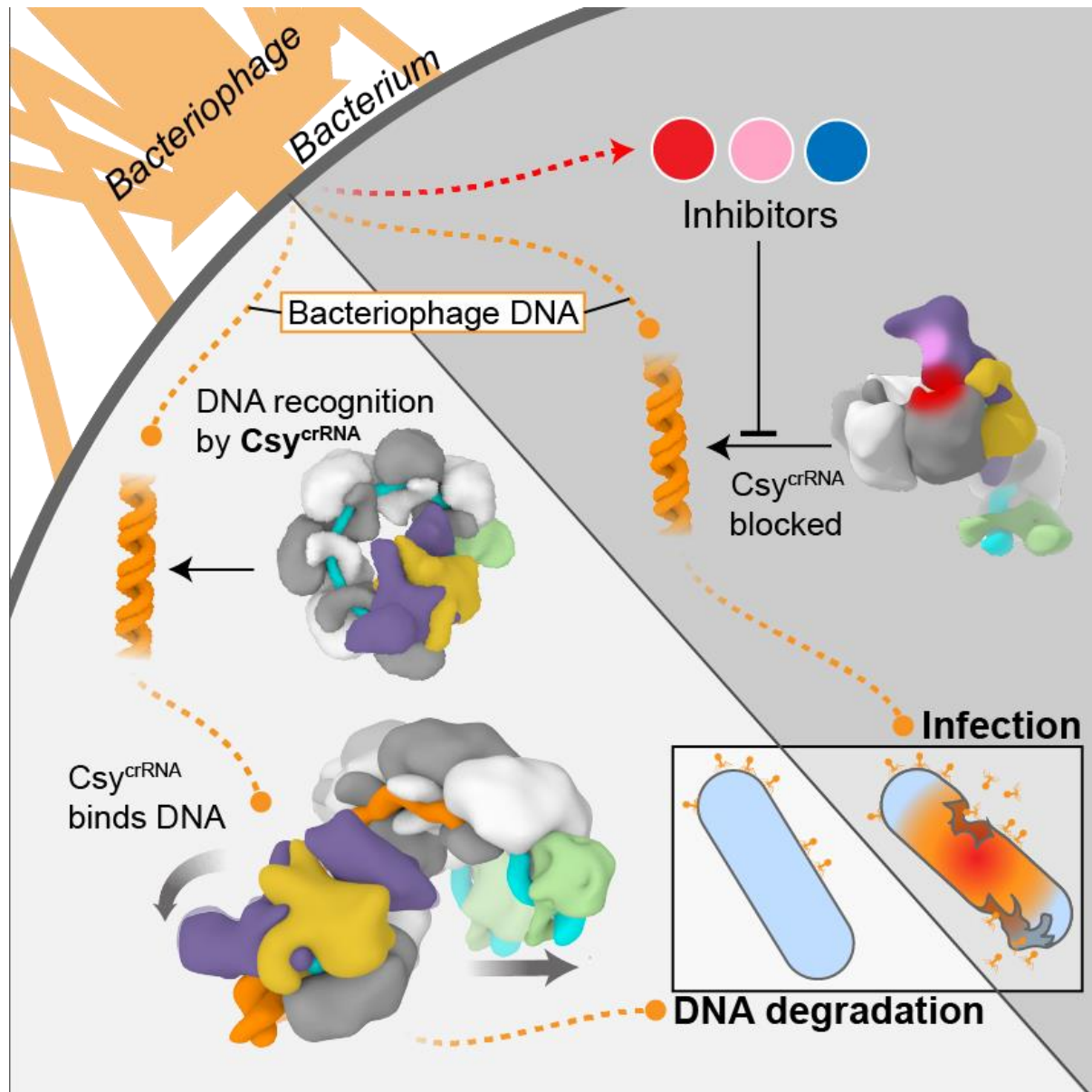
2.3 Å resolution cryo-EM structure of human p97 and mechanism of allosteric inhibition



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





Cell

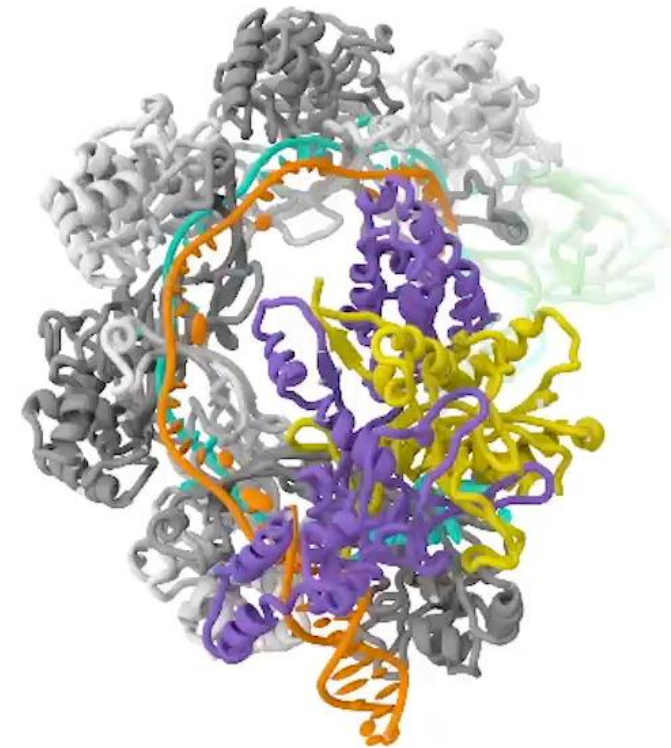
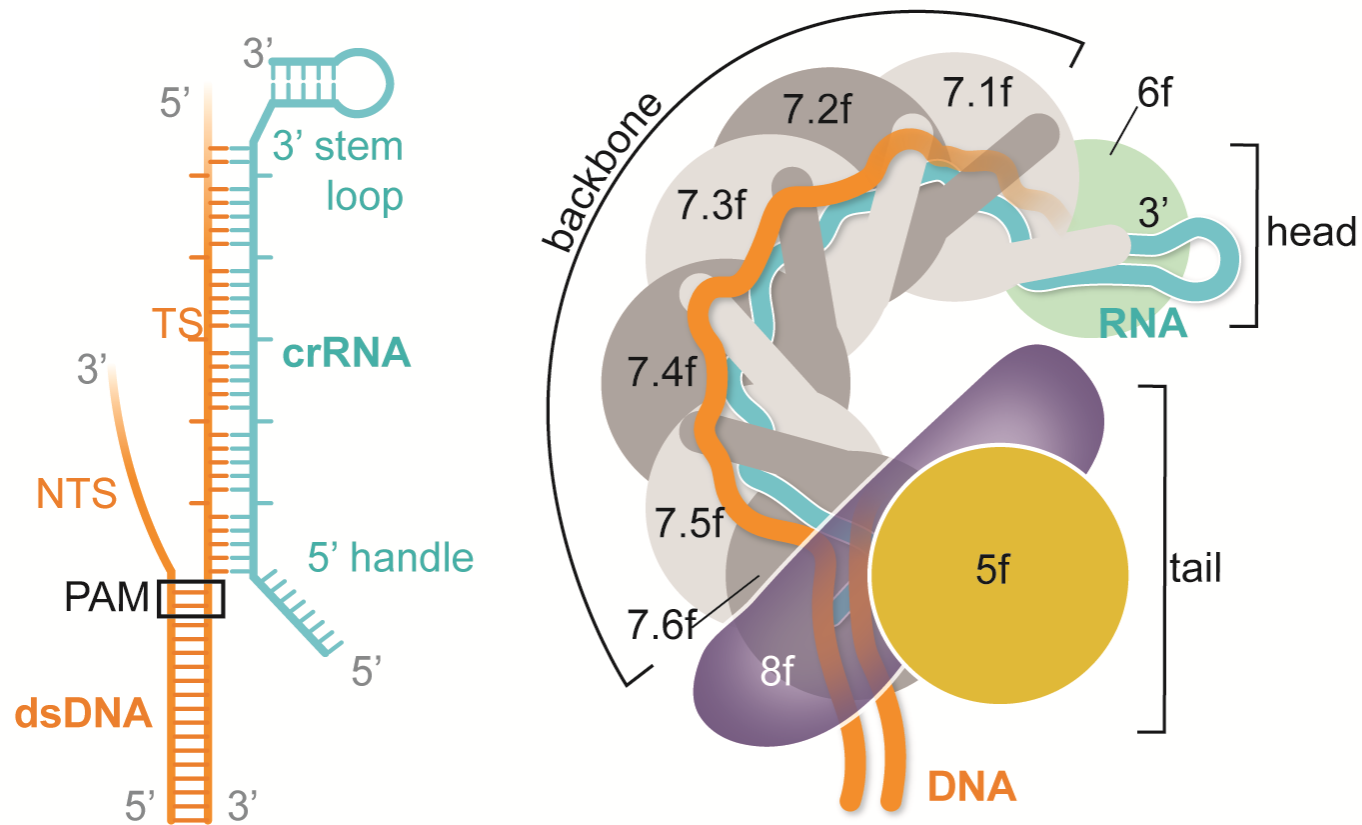
2017

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

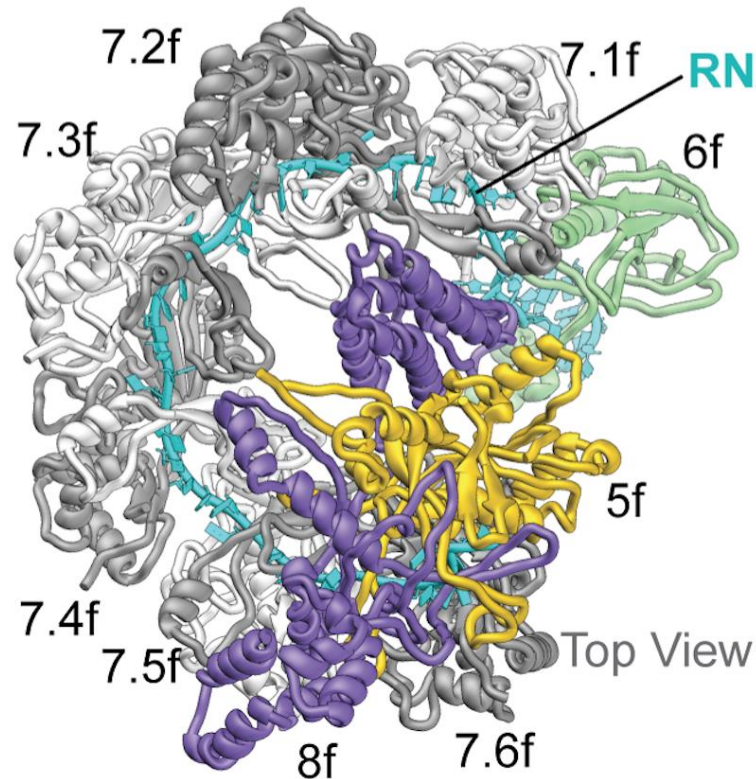
Cryo-EM structure of Csy CRISPR-Cas complex with bound dsDNA



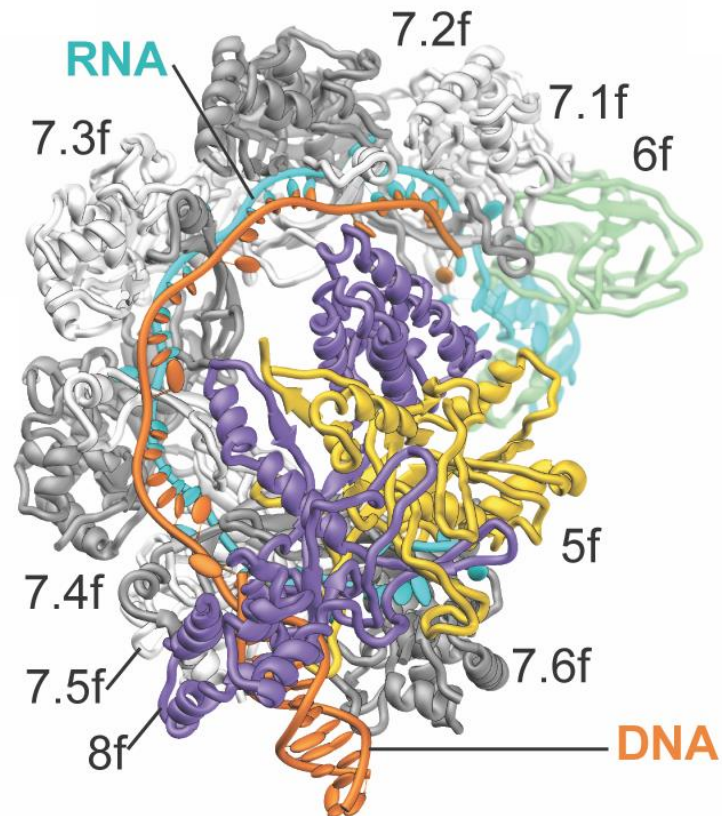
Asymmetric Type I-F surveillance
complex: 9 polypeptides + guide RNA +
dsDNA

2.9 Å resolution

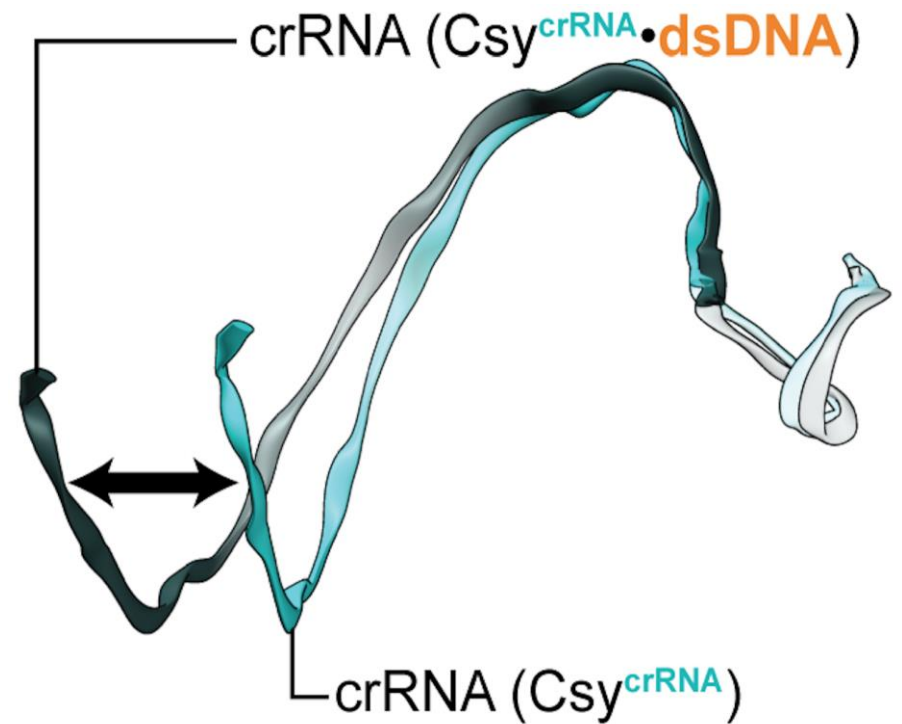
DNA binding induces dramatic change in pitch



Before DNA
binding



After DNA
binding

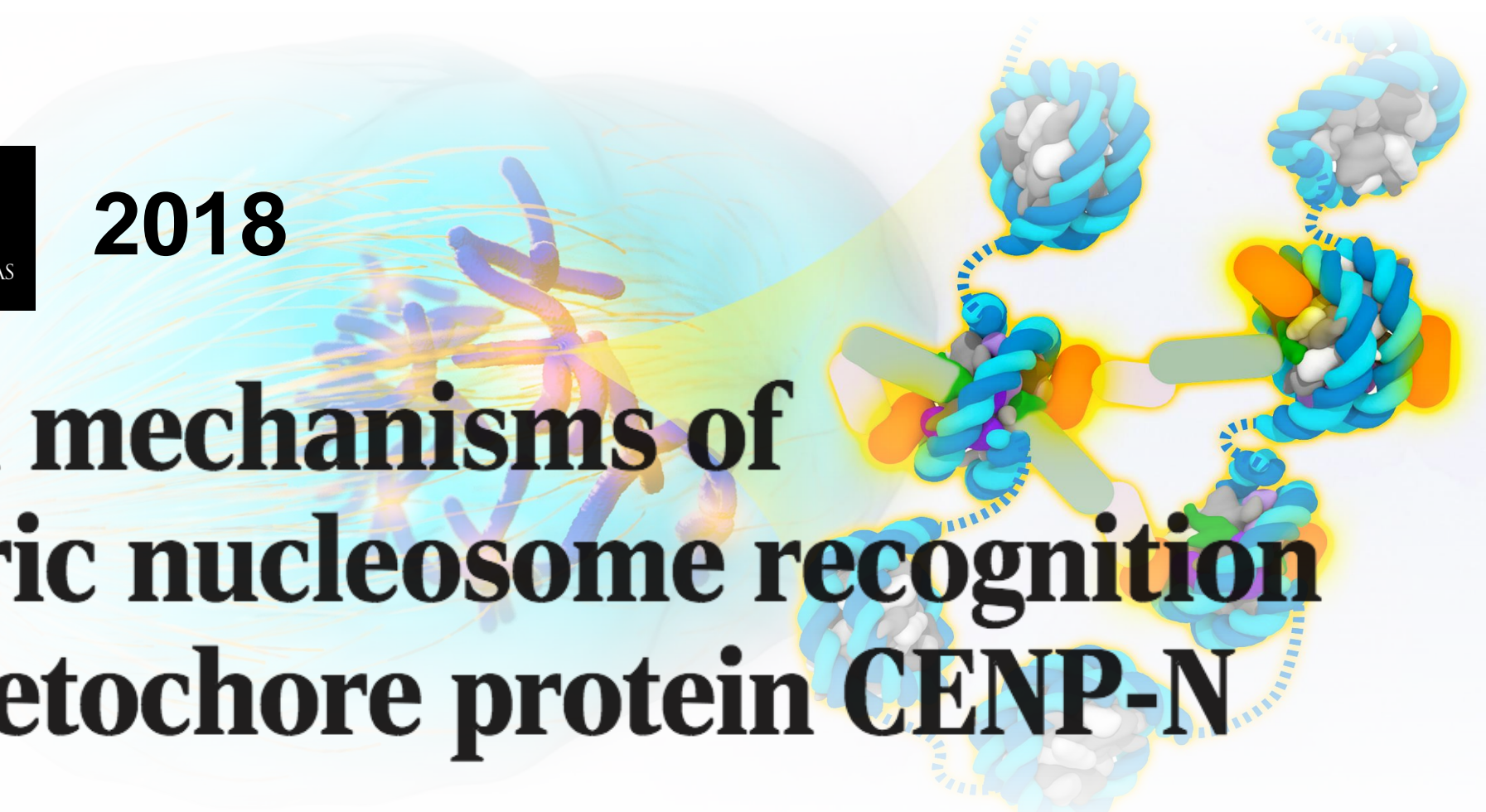


Science AAAS

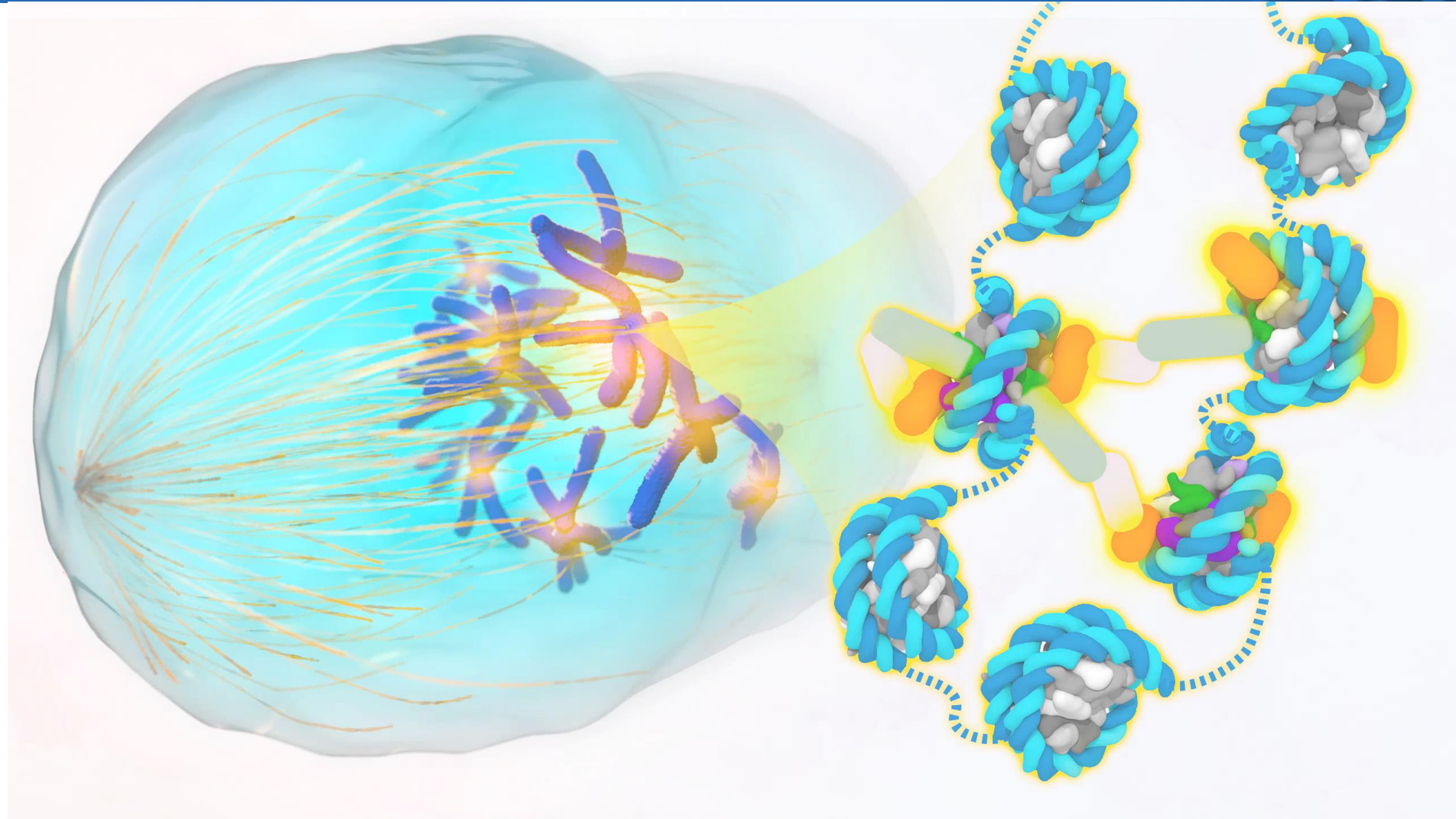
2018

Structural mechanisms of centromeric nucleosome recognition by the kinetochore protein CENP-N

**Sagar Chittori,^{1*} Jingjun Hong,^{2*} Hayden Saunders,² Hanqiao Feng,²
Rodolfo Ghirlando,³ Alexander E. Kelly,^{2†} Yawen Bai,^{2†} Sriram Subramaniam^{1†}**



Cryo-EM structure of CENP-N complex with CENP-A nucleosome



NCI National Cryo-EM Facility

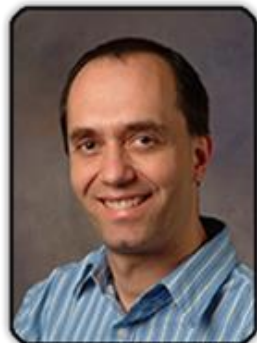


Ethan Dmitrovsky
Lab Director, FNLCR

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



Dwight Nissley
Director, CRTP, FNLCR



Ulrich Baxa
Senior Microscopist,
NCEF

Thomas Edwards
Microscopist, NCEF



Helen Wang
Project Manager, NCEF



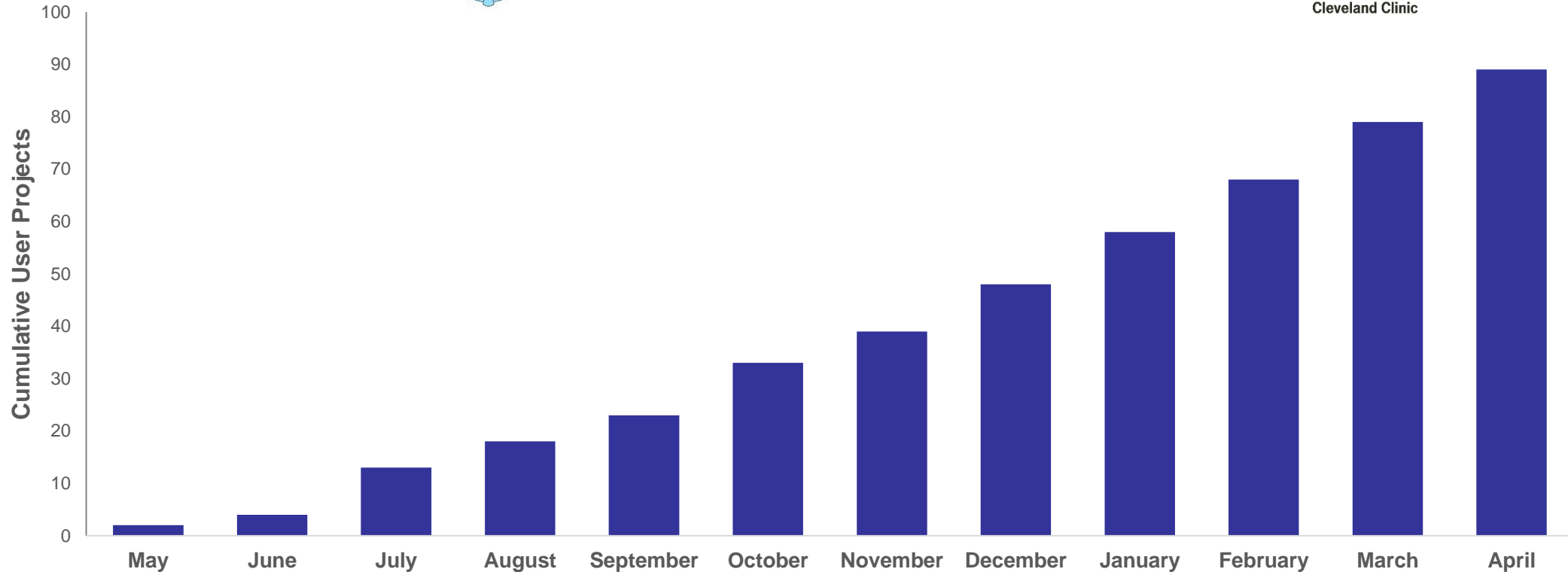
Matt Hutchison
IT Support, NCEF



NCI National Cryo-EM Facility

>90 projects so far from 20 institutions

Frederick
National
Laboratory
for Cancer Research



User publications have started to come out

Frederick
National
Laboratory
for Cancer Research



 Altmetric: 80


[More detail >>](#)

 Altmetric: 37

[More detail >>](#)

Article | [OPEN](#)

Cryo-EM structure of 5-HT_{3A} receptor in its resting conformation

Sandip Basak, Yvonne Gicheru, Amrita Samanta, Sudheer Kumar Molugu, Wei Huang, Maria la de Fuente, Taylor Hughes, Derek J. Taylor, Marvin T. Nieman, Vera Moiseenkova-Bell & Sudha Chakrapani 

Nature Communications **9**, Article number: 514
(2018)
doi:10.1038/s41467-018-02997-4

Received: 10 November 2017
Accepted: 10 January 2018
Published: 06 February 2018

Article

Cryo-EM structure of the gasdermin A3 membrane pore

Jianbin Ruan, Shiyu Xia, Xing Liu, Judy Lieberman & Hao Wu 

Nature **557**, 62–67 (2018)
doi:10.1038/s41586-018-0058-6
[Download Citation](#)

Received: 16 November 2017
Accepted: 08 March 2018
Published: 25 April 2018

New ATRF facility scheduled for completion in June 2018

Frederick
National
Laboratory
for Cancer Research



Community feedback from Year 1 and discussion of plans for future years

User comments

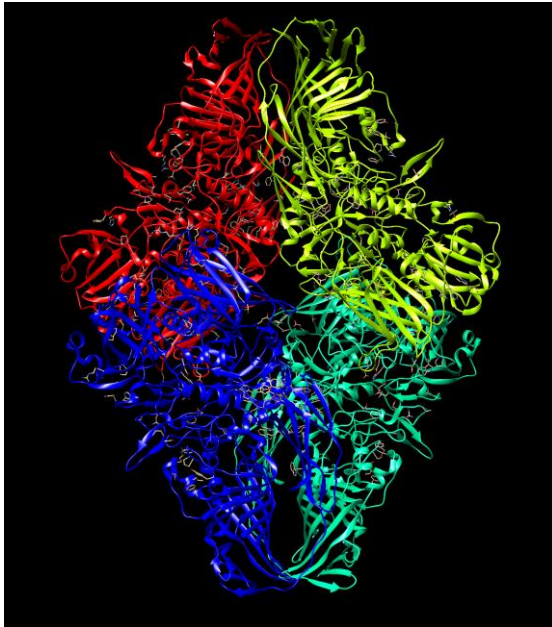
- **“Quite happy on the data collection efficiency”**
- **“We have been 110% satisfied with the promptness, professionalism, and quality of service you have provided for us these past few months”**
- **“The images are perfect! Most of them have defocus under 2 and the Thon ring are fit beyond 3 Å”**
- **“As always, we thank you immensely for your valuable service”**
- **“We have generated a reconstruction of ~ 3 Å...To date, the highest resolution reconstruction of our protein”**
- **“Experience was great for our data collection without any hurdles”**
- **“We always get prompt response on our SIF submissions”**
- **“The NCEF team is very professional in data collection. We always get high quality images”**

Panel Discussion

- **The next phase: Potential for increasing impact of NCEF with expansion of scope of activities**

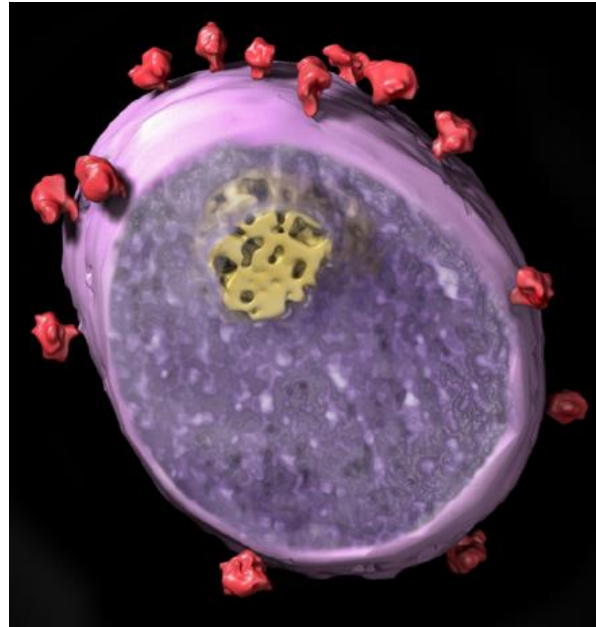
A 12-year program to bridge the gap

proteins



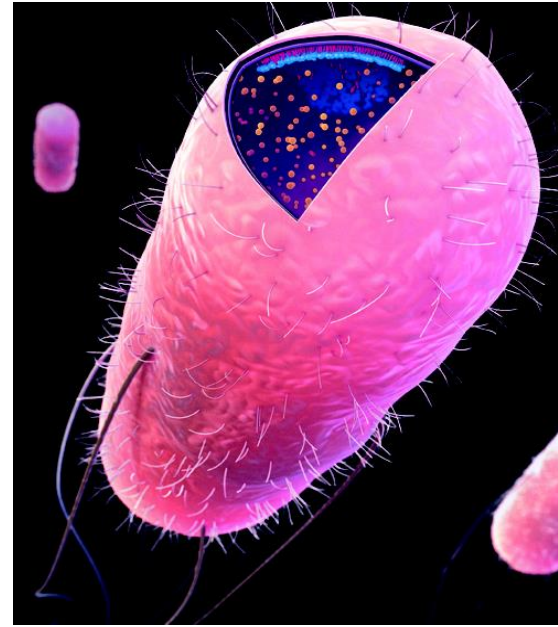
~ 15 nm

viruses



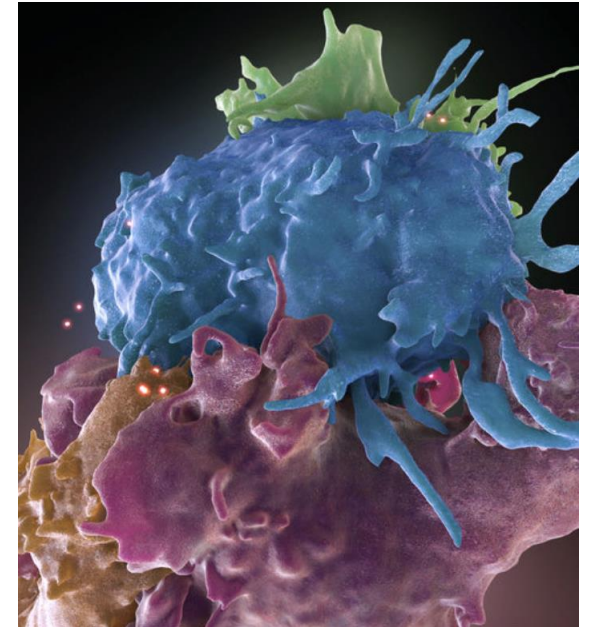
~ 150 nm

cells



~ 1500 nm

tissue



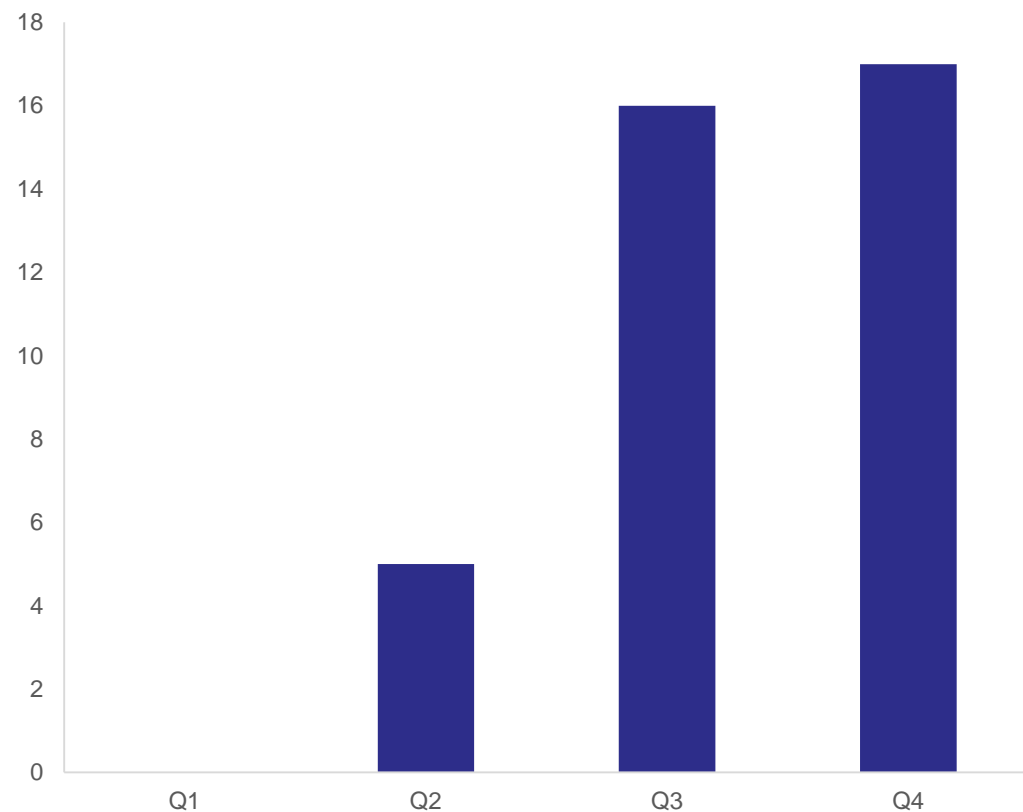
~ 15000 nm

Defining user communities and NCEF mission (as articulated when NCEF was launched)

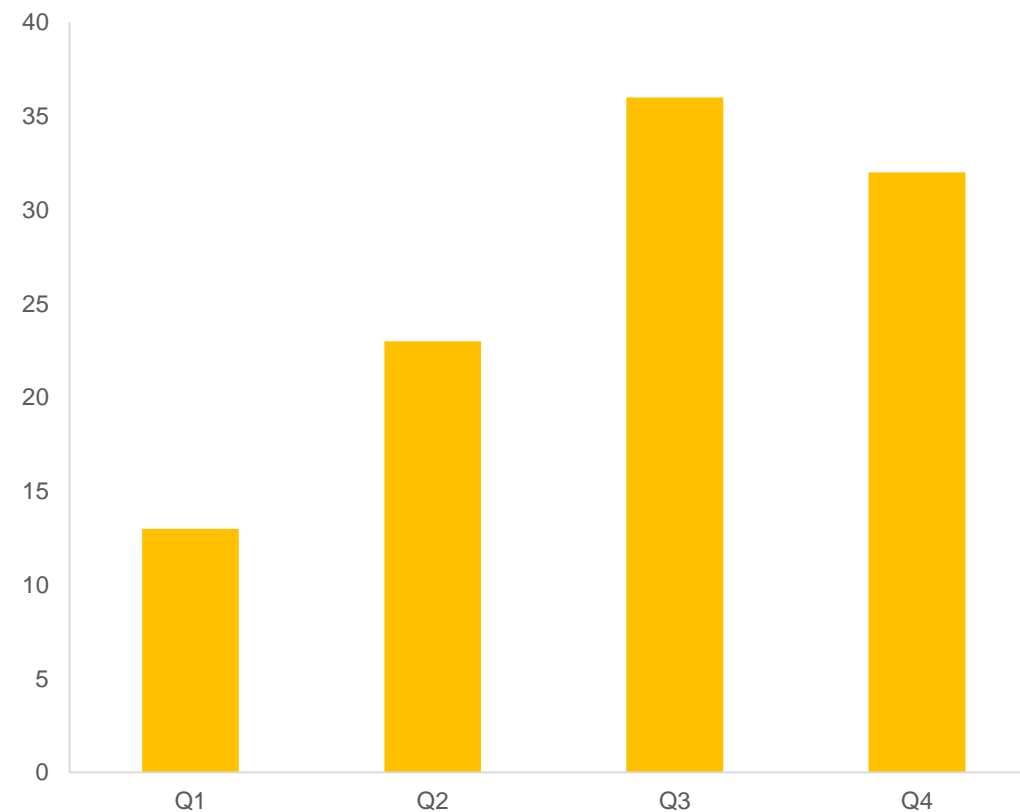
- **Group I: Research groups with experience in cryo-EM technology**
 - have some access to local screening microscopes
 - inadequate access to high-end instrumentation
 - are key drivers of growth of cryo-EM in the US
- **Group II: Structural biologists in adjacent disciplines (X-ray, NMR)**
 - see value in using cryo-EM
 - have expertise in protein biochemistry
 - have limited expertise 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
 - lack expertise in most aspects of cryo-EM workflow

Quarterly wait queue

Wait Queue at the End of Each Quarter

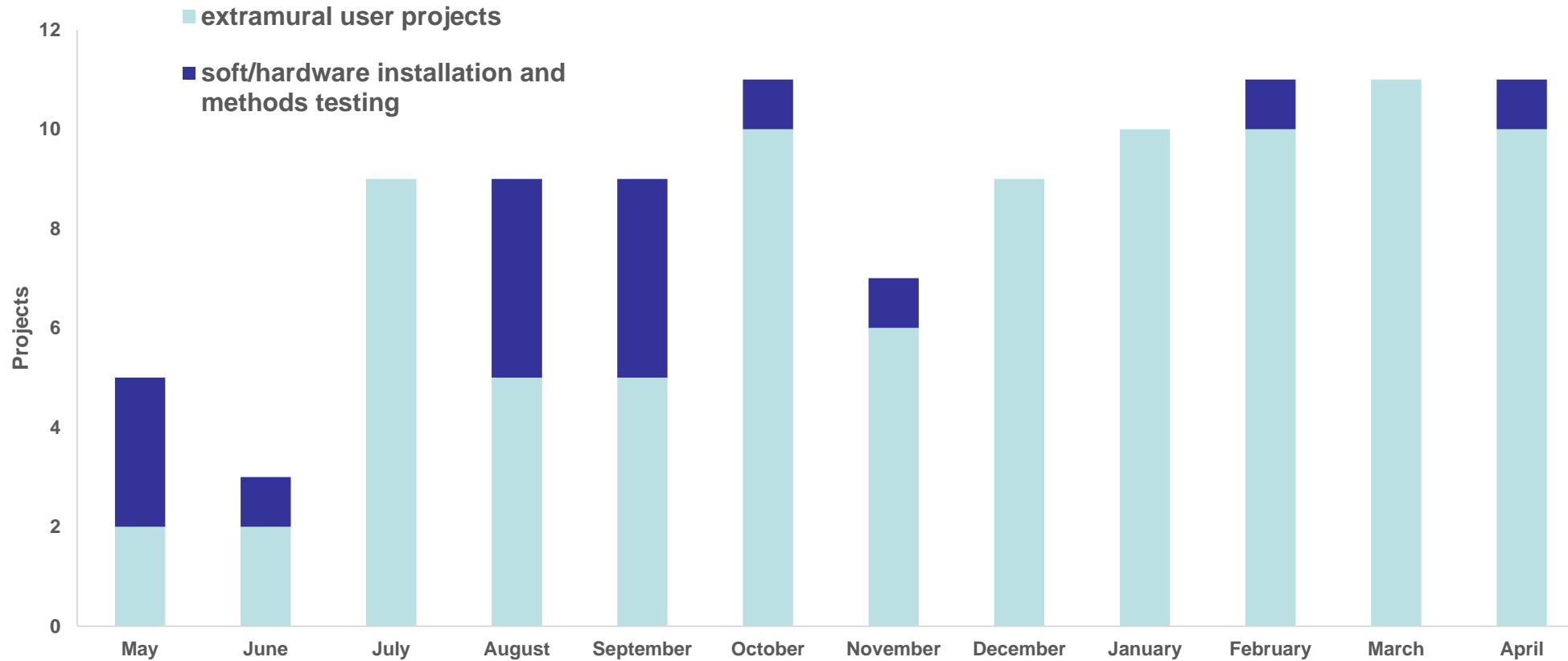


Project Submissions per Quarter



Monthly project list

projects per month



10 projects per month was the target we set when we launched NCEF

