

# Frederick National Laboratory for Cancer Research



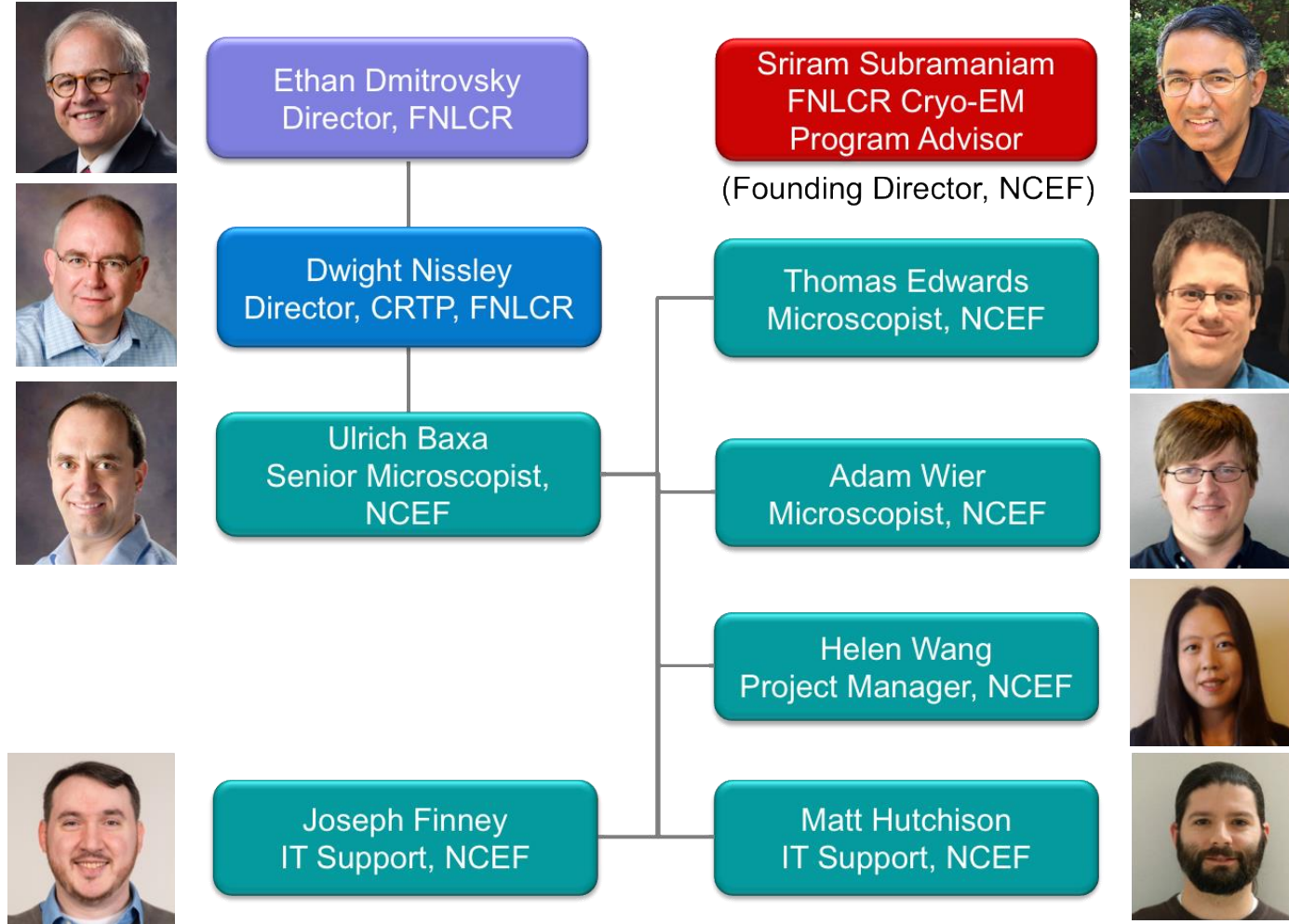
## NCEF Update

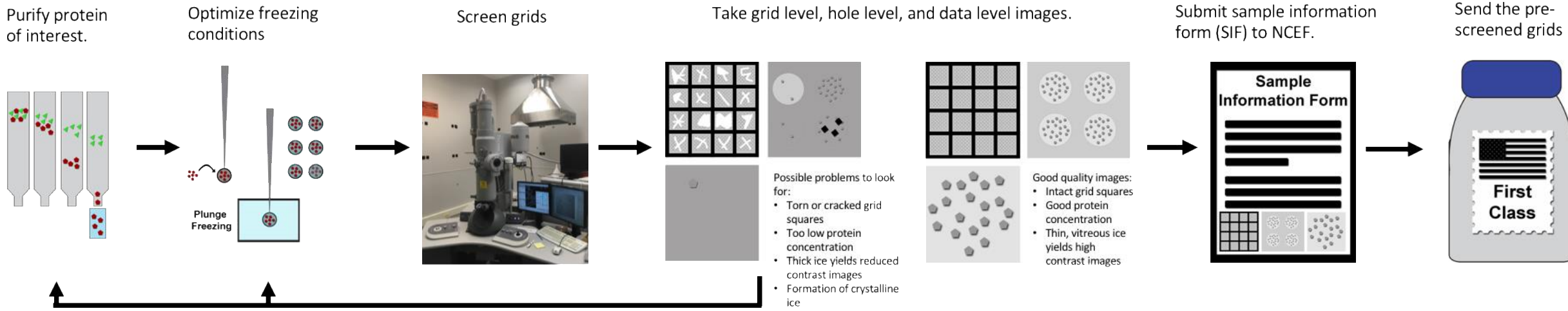
June 27, 2019

# NCEF Overview and Team Members

- Mission: address gap between need for cryo-EM and access to expensive instrumentation
- Opened in May 2017 with one Titan Krios microscope; Glacios operational in Dec 2018; second Titan Krios operational in May 2019
- 250 runs from 32 institutions across US have been completed; feedback has been very positive
- User publications appeared in *Nature*, *Cell*, *Nature Communications*, *PNAS*, *Nature Structural and Molecular Biology*, and others

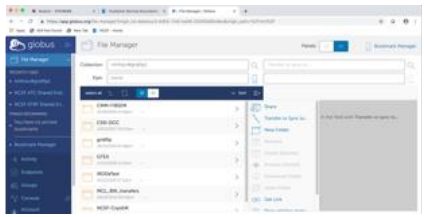
## NCEF Personnel





- Possible problems to look for:
- Torn or cracked grid squares
  - Too low protein concentration
  - Thick ice yields reduced contrast images
  - Formation of crystalline ice

- Good quality images:
- Intact grid squares
  - Good protein concentration
  - Thin, vitreous ice yields high contrast images

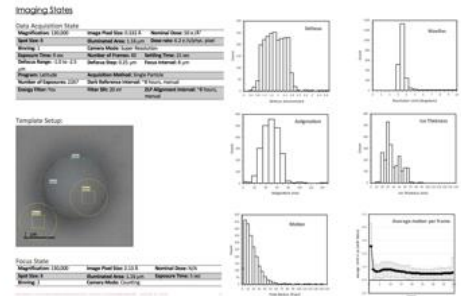


Download of data through globus

Micrographs

ID	Micrograph	Shift/Plot	PadFile	Defocus(µm)	Alignment(µm)	Max. Res(Å)
740				1.30	0.012	3.27
739				1.58	0.025	3.36
738				1.87	0.022	3.57

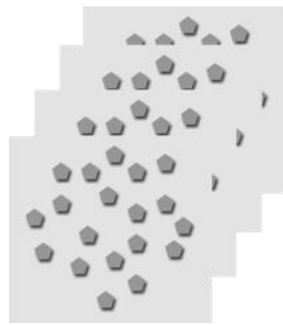
Monitoring website for Users



Notify user with data report, feedback, and instructions



Compress movies and move to storage server



Acquire movies on detector.



Consultation about the parameters



Load and screen grids





# User Project Workflow – Prof. Cynthia Wolberger (Johns Hopkins)

September 6, 2018 – Sample Information Form was submitted

September 6, 2018 – Submission was approved

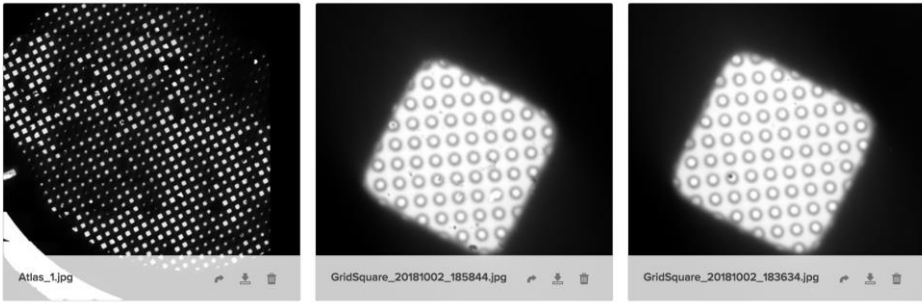
October 31, 2018 – Sample delivered in person for imaging

# User Project Workflow – Prof. Cynthia Wolberger (Johns Hopkins)

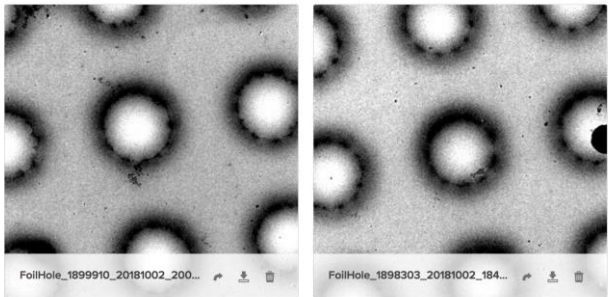
← Back to list | < 2 of 3 > | Regulation of Histone Methylation | Accepted ✓ | Info | Assign 4 | Labels | More ▾

NEEDS TO BE REVIEWED | ULRICH APPROVE

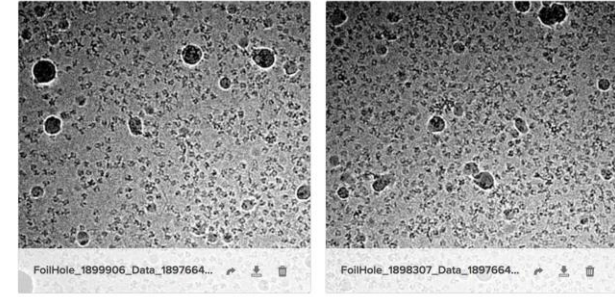
Low Magnification Pre-Screening Image



Medium Magnification Pre-Screening Image



High Magnification Pre-Screening Image



**Activity & Rating** | Messages

Type a note...

Admins Only | Add

all | notes | assignments | ratings | labels

10 months ago VIEWABLE BY SUBMITTER

Automated submission response sent to **Evan Worden**

**Response:**  
(VIEWABLE BY SUBMITTER)

Dear Evan ,

We have received your SIF with pre-screening images for NCEF review. We will follow up with you shortly. If you have any further questions, please contact NCI-NCEF@mail.nih.gov or visit our website at www.cancer.gov/research/resources/cryoEM.

All the Best,  
The National Cryo-EM Facility Team at Frederick National Laboratory for Cancer Research

10 months ago VIEWABLE BY SUBMITTER  
Submitted by **Evan Worden**

9 am October 31, 2018 to – Setup for data collection

9 am November 2, 2018 – Data collection stopped; 2284 movies collected

November 6, 2018 – Report and download link provided

# User Project Workflow – Prof. Cynthia Wolberger (Johns Hopkins)

## Frederick National Laboratory for Cancer Research

sponsored by the National Cancer Institute

### National Cryo-Electron Microscopy Facility Customer Report

November 6, 2018



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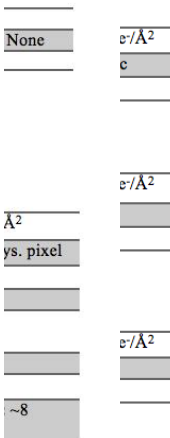
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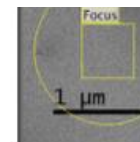
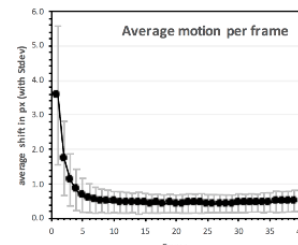
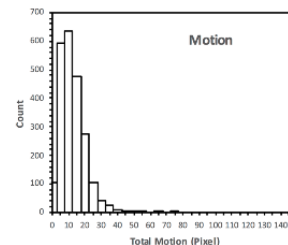
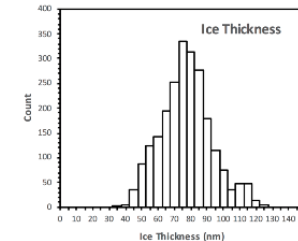
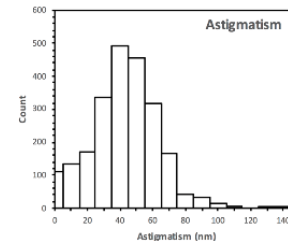
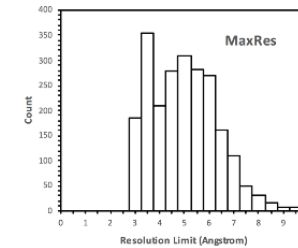
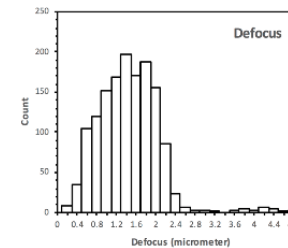
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### Data Acq Magnificat

### Grid Inve

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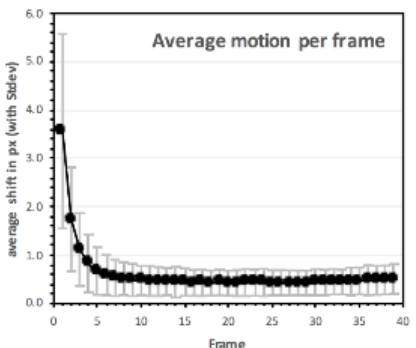
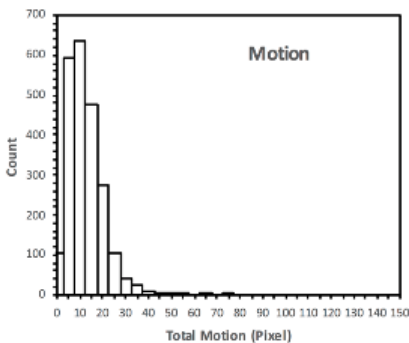
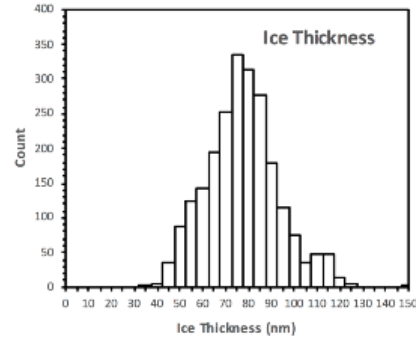
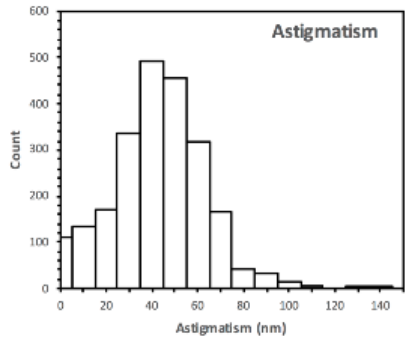
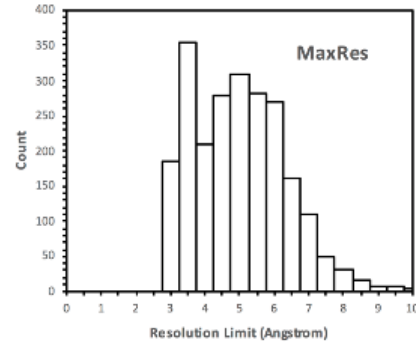
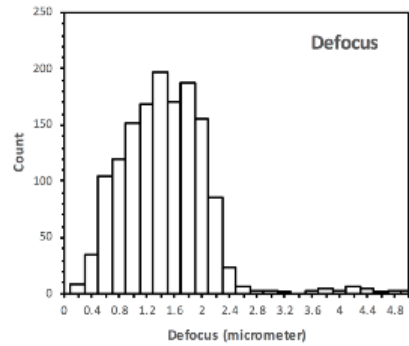
Project Number:  
NCEF-037-002-00152

Primary Investigator:  
Dr. Cynthia Wolberger

Point of Contact:  
Dr. Evan Worden



# User Project Workflow - Dot1L methyltransferase complex nucleosomes



## Report includes

- All imaging settings and parameters
- Feedback on sample quality
- recommendations for optimization for future data collection
- Summary of Quality Control

## Global motion correction and CTF fit

- Results can be used to QC data
- Data can be used to check microscope performance (astigmatism, mag anisotropy, etc.)

With GIF and Zero-loss imaging we can estimate the ice thickness on each image

- Provide ice thickness feedback to users
- Observe correlation of ice thickness and information limit
- make predictions about data quality in the future

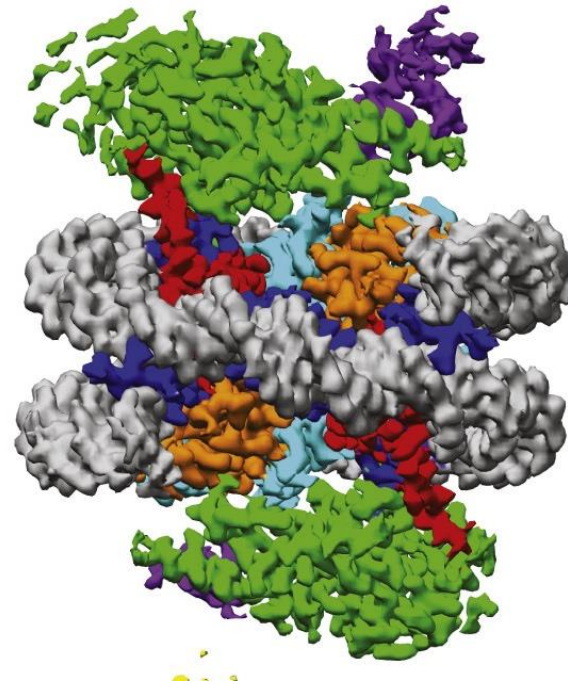


December 14, 2018 – Publication was submitted to *Cell*

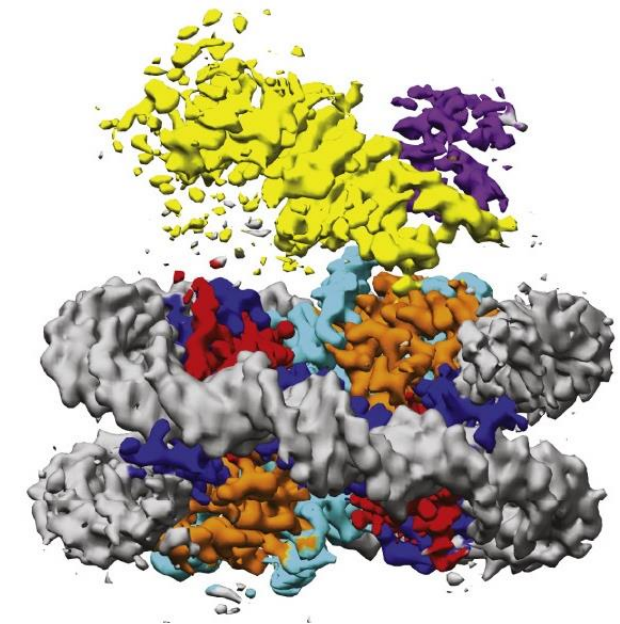
February 11, 2019 – Publication date

# User Project Workflow – Prof. Cynthia Wolberger (Johns Hopkins)

- Worden EJ, Hoffmann NA, Hicks CW, Wolberger: Mechanism of Cross-talk between H2B Ubiquitination and H3 Methylation by Dot1L. Cell. 2019 Mar 7;176(6):1490-1501.e12. Epub 2019 Feb 11.



Active state  
Result: 3.0 Å structure



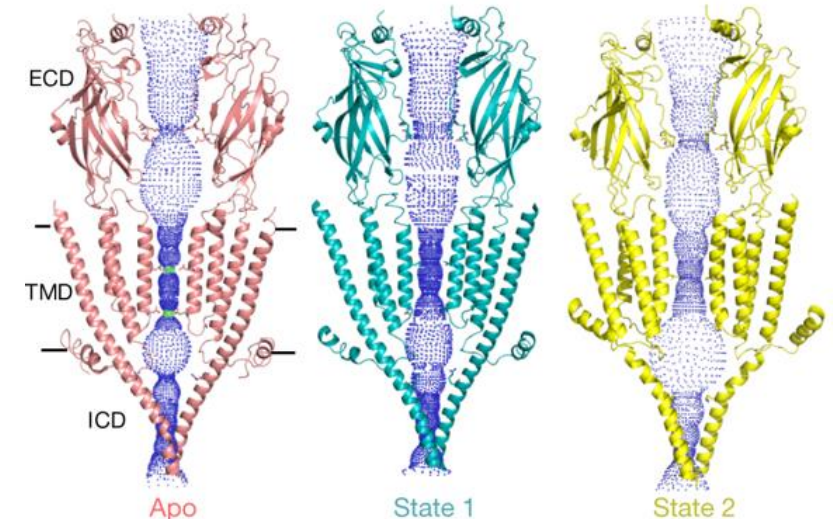
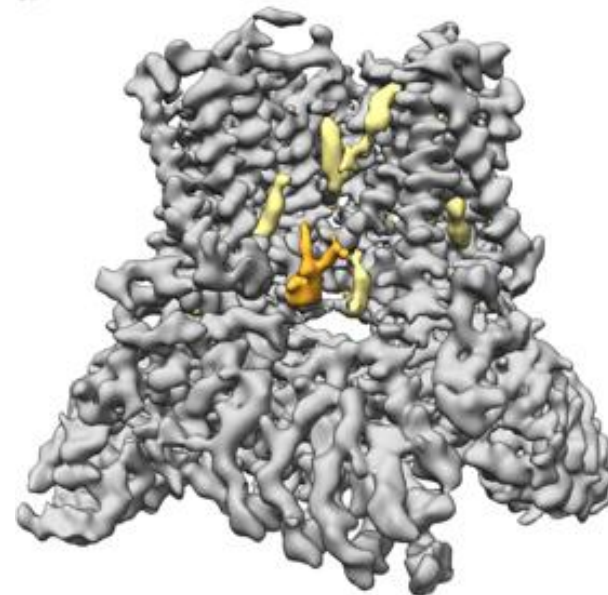
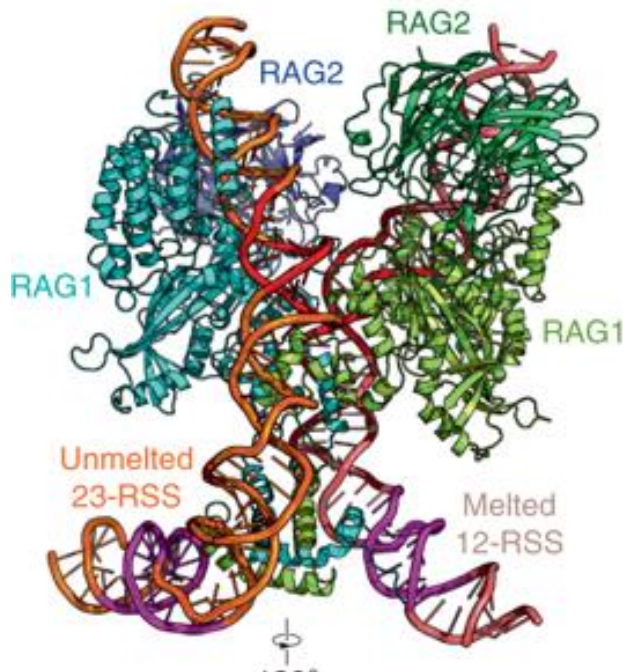
Poised state  
Result: 3.5 Å structure

# Other recent NCEF successes

- Dr. Hao Wu (Harvard University)  
RAG complex with DNA substrates
- Result: 3.9 Å structure
- Published: Ru et al. (2018) *Nat Struct Mol Biol* 25, 732-742

- Dr. Vera Moiseenkova-Bell (University of Pennsylvania)  
Complex of TRPV5 and CaM and PIP2
- Result: 3.9 Å structure
- Published: Hughes et al. (2018) *Nat Comm* 9, 4198

- Dr. Sudha Chakrapani (Case Western)  
Serotonin receptor (5-HT3AR)
- Result: 3.3 Å structure
- Published: Basak et al. (2018) *Nature* 563, 270-274

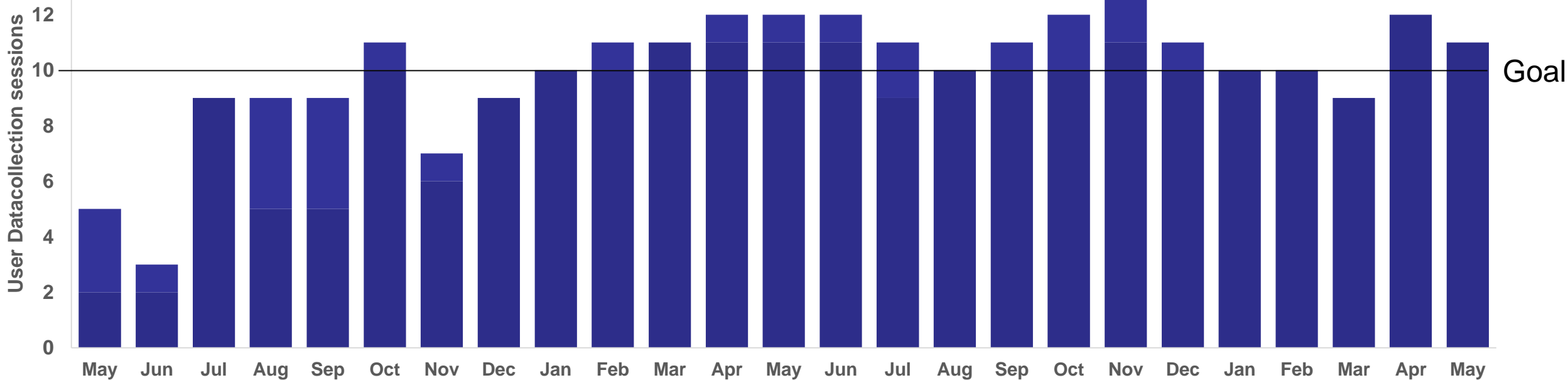




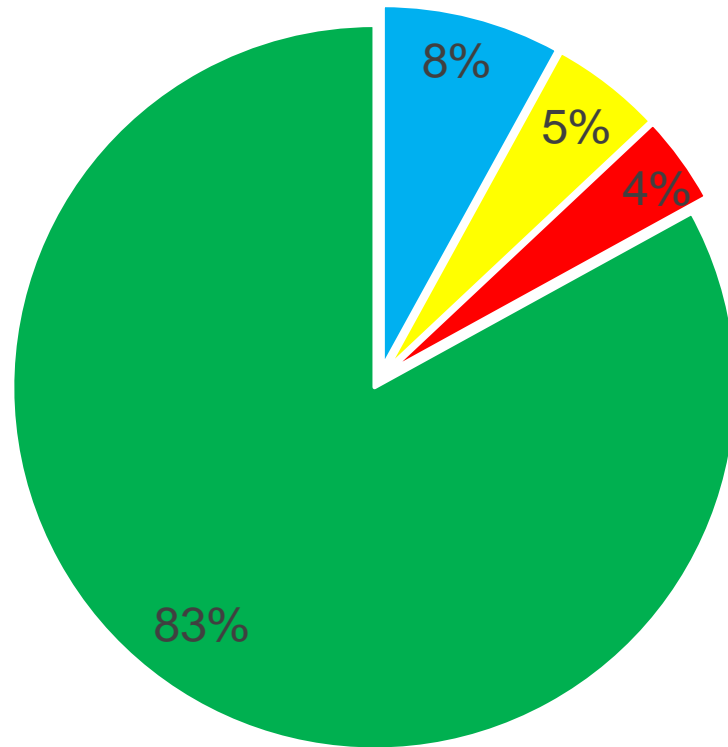
# NCI National Cryo-EM Facility

## 250 projects from 32 institutions

**Frederick  
National  
Laboratory**  
for Cancer Research







- Microscope operational 96% of the time
- During operational time, 90% is for imaging and 10% is for data setup/screening etc.

# Summary

- NCEF performed 250 data collection runs over the last 2 years
- Krios 2 is operational as of May 2019
- Krios 1 will be moved in July/August 2019 to its new home at the ATRF, and expected to be operational by October 2019 with a BioQuantum K3
- We plan to increase the number of user projects and implement several outreach efforts

