

# CRYOEM 001 : INTRO TO CRYOEM

NCCAT Embedded Training — Master Class series

August 20, 2020

NATIONAL CENTER FOR  
CRYOEM ACCESS & TRAINING



New York Structural  
Biology Center

SIMONS ELECTRON  
MICROSCOPY CENTER



# NCCAT CROSS-TRAINING



knowledge



capability



capacity



productivity



performance

Training is teaching, or developing in oneself or others, any skills and knowledge that relate to specific useful competencies. Training has specific goals of improving one's capability, capacity, productivity and performance.

<https://en.wikipedia.org/wiki/Training>

# THE STARTING POINT — WHAT IS YOUR LEVEL?

## Core EM knowledge

Beginner  
Intermediate  
Advanced

## Sample preparation

### Negative stain

Beginner  
Intermediate  
Advanced

### CryoEM

Beginner  
Intermediate  
Advanced

## Microscope operations

### Screeners

Beginner  
Intermediate  
Advanced

### High-end

Beginner  
Intermediate  
Advanced

## Data Collection

Beginner  
Intermediate  
Advanced

## Data Processing/Analysis

### Quality assessment

Beginner  
Intermediate  
Advanced

### Structural biology

Beginner  
Intermediate  
Advanced

# NCCAT TRAINING DOCUMENTATION

<https://nccat.nysbc.org/activities/nccat-cross-training/>

<https://nccat.nysbc.org/activities/nccat-remote-learning/>

<https://nccat.nysbc.org/activities/nccat-cross-training/remote-embedded-cross-training/>



National Center for CryoEM Access and Training

HOME

ABOUT

NEWS

ACCESS

TRAINING

ACKNOWLEDGEMENTS

JOBS

## TRAINING

NCCAT Workshops  
and Short courses

> SPA Short course  
2020

NCCAT Cross-training  
Programs

NCCAT Remote  
Learning

> Remote Office  
Hours

> Online Classroom

> Previous EM  
Courses

> Curriculum  
Partners

## Workshops and Cross-training

NCCAT provides access to state-of-the-art equipment, including high-end microscopes and direct detectors, as well as specimen preparation robots, screening microscopes and all the other ancillary equipment required to solve structures to the highest possible resolution using cryo electron microscopy (cryoEM) methods.

An already established cross-training program provides training across a wide variety of skill levels and career goals. Our workshops and forums provide opportunities to target specific areas of education and professional development.



Workshops & Courses

[Learn with us. >](#)



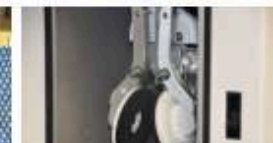
Embedded cross-training

[Join our community. >](#)



Remote Learning Central

[Distance education. >](#)



# NCCAT CROSS-TRAINING RESOURCES

## Remote Cross-training Program

The screenshot shows the NCCAT website with the 'Remote Cross-training Program' page. The header includes the NCCAT logo and the text 'National Center for CryoEM Access and Training'. The navigation bar has links for HOME, ABOUT, NEWS, ACCESS, TRAINING, ACKNOWLEDGEMENTS, and JOBS. The main content area features a sidebar with links to TRAINING, NCCAT Workshops and Short courses, SPA Short course 2020, NCCAT Cross-training Programs, NCCAT Remote Learning, Remote Office Hours, Online Classroom, Previous EM Courses, and Curriculum Partners. The main content area has a section for 'Embedded Cross-training Modules' with a grid of four modules: Sample preparation, Microscope operations, Data acquisition, and Data processing. Below this is a section for 'Remote Microscope Operation Module' with a description of the module and a list of resources. At the bottom, there is a section for 'Leginon Remote - Remote Krios operations' with a description and a list of resources.

## Remote Learning Central

The screenshot shows the NCCAT website with the 'Remote Learning Central' page. The header includes the NCCAT logo and the text 'National Center for CryoEM Access and Training'. The navigation bar has links for HOME, ABOUT, NEWS, ACCESS, TRAINING, ACKNOWLEDGEMENTS, and JOBS. The main content area features a sidebar with links to TRAINING, NCCAT Workshops and Short courses, SPA Short course 2020, NCCAT Cross-training Programs, NCCAT Remote Learning, Remote Office Hours, Online Classroom, Previous EM Courses, and Curriculum Partners. The main content area has a section for 'NCCAT Remote Learning Central' with a description of the program and a grid of resources. The resources are organized into three columns: Video conferencing, Online workshops, and Other resources. The Video conferencing column includes Office hours, Online classes, and Thoughts on Food. The Online workshops column includes SPA course, EM classes, and Science outreach. The Other resources column includes Reading list, YouTube, and Additional links.

## SEMC meetings

Lab meetings

Project meetings

Office hours

## NCCAT workshops

Roundtables

Appion

## Remote learning modules

Core knowledge

Sample preparation

Microscope operations

Data collection

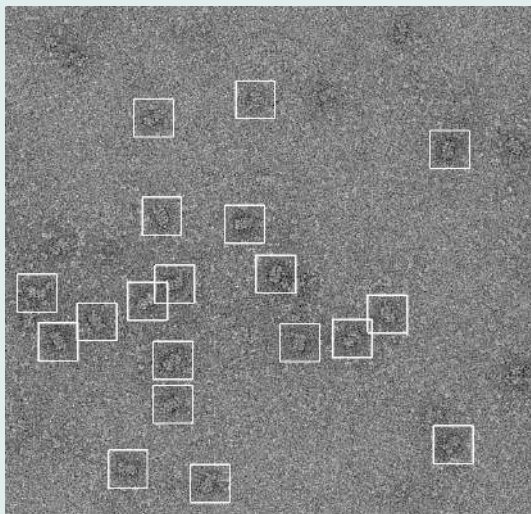
Data processing

# APPION WORKSHOPS — AN OVERVIEW

## *Single particle*

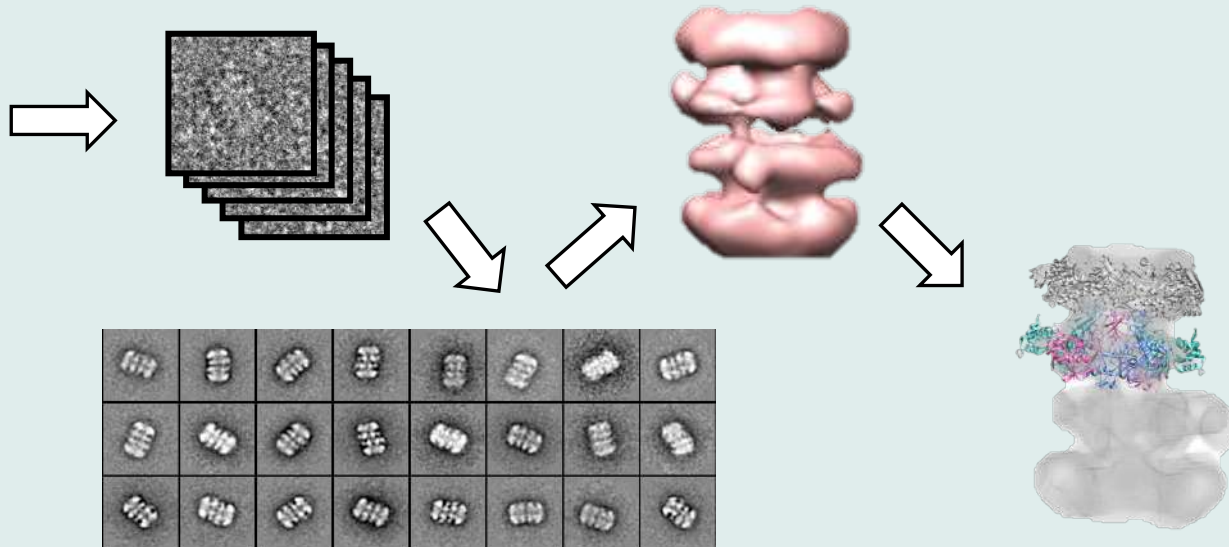
Part I

Tools to generate a particle stack from micrographs and obtain an initial assessment of the sample.



Part II

**3D reconstruction,  
refinement and  
validation.**



## *Tomography*

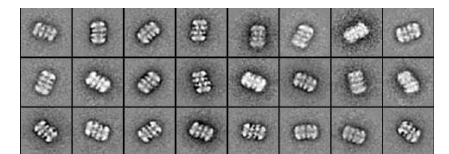
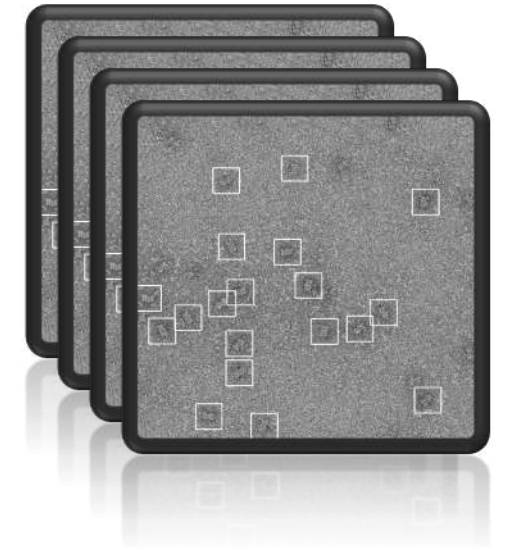
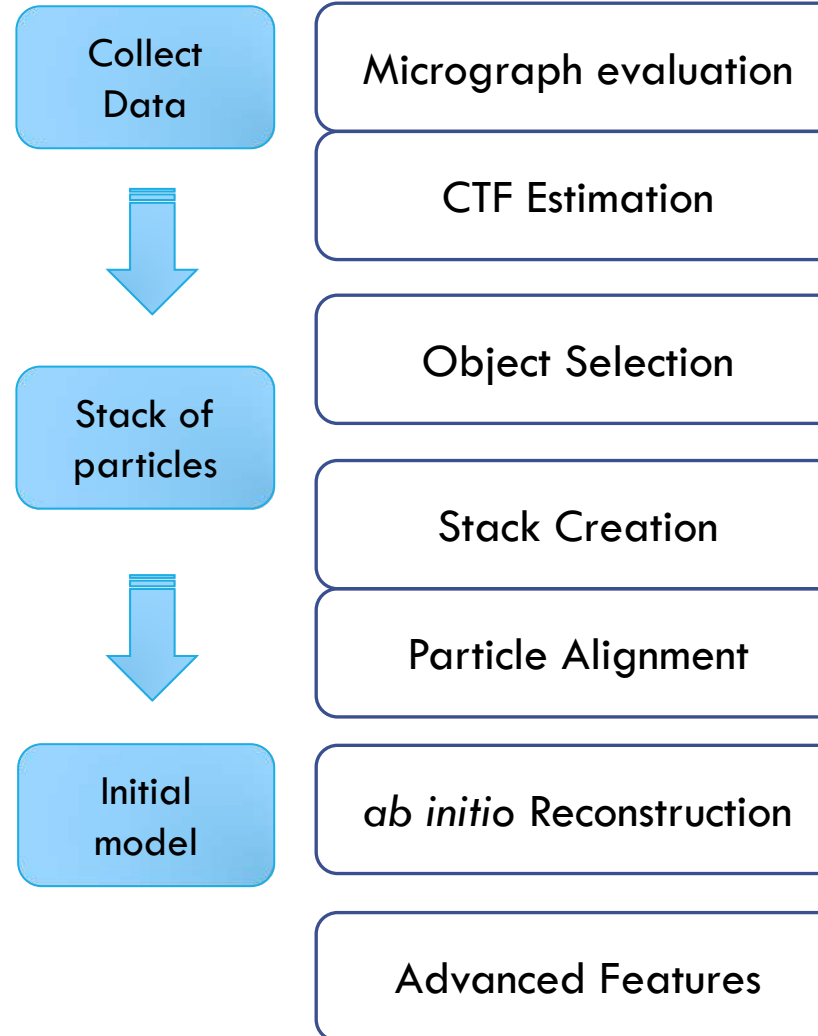
Part III

Tomography tools.

# DEBRIEF ON APPIONI

JULY 29-30, 2020 2PM-4PM

PRACTICAL WORKSHOP WHERE STUDENTS WILL WORK IN PAIRS ON A TEST DATASET. AFTER EACH LECTURER GIVES AN OVERVIEW OF THE TOPIC THE ATTENDEES WILL HAVE HANDS-ON TIME TO USE THE INTERFACE.





001

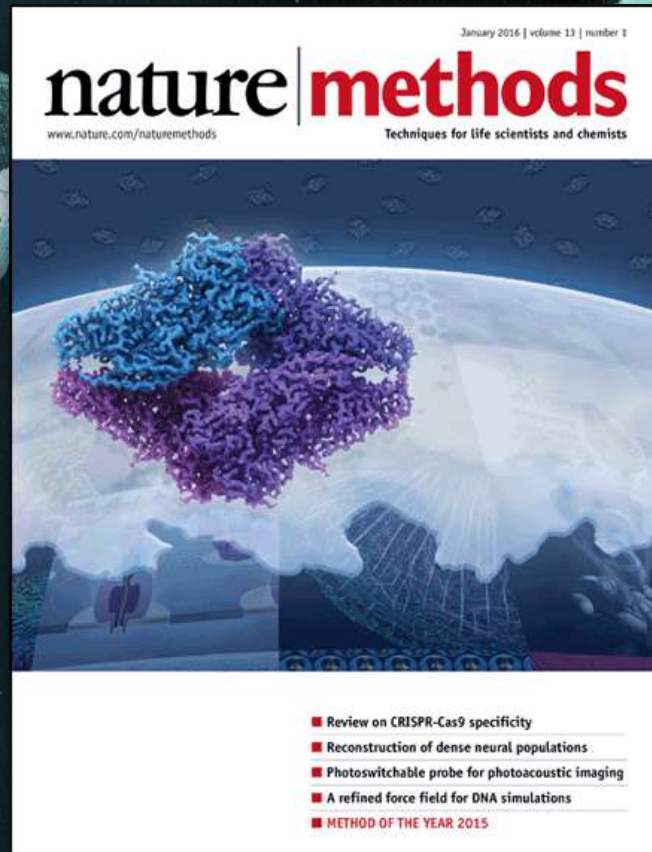
CRYOEM 101

START  
HERE...

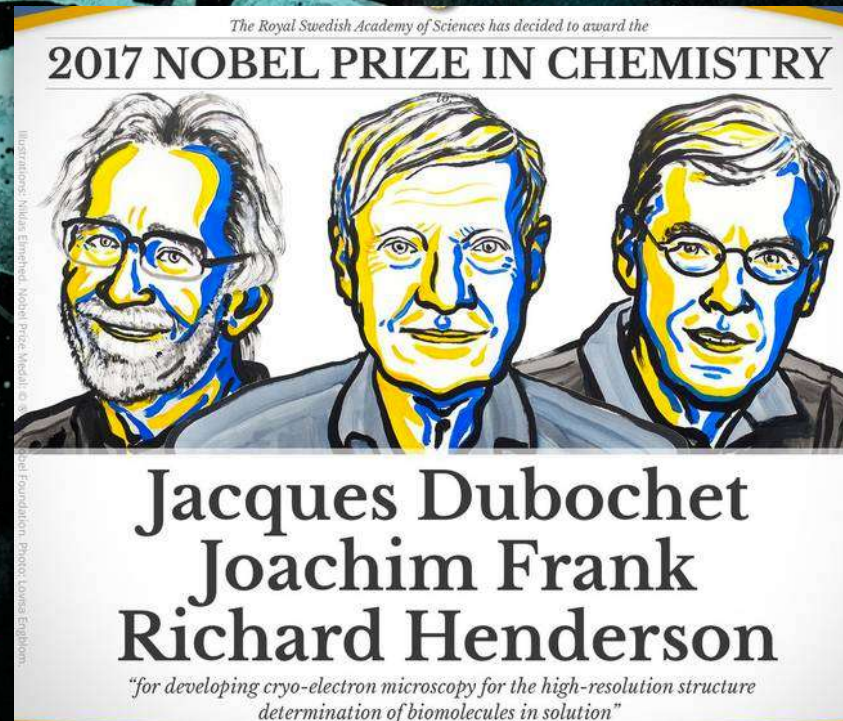


# CRYOEM: TECHNOLOGY ON THE RISE

Single-particle cryo-electron microscopy (cryo-EM)  
is the Method of the Year 2015



Chemistry Nobel prize 2017



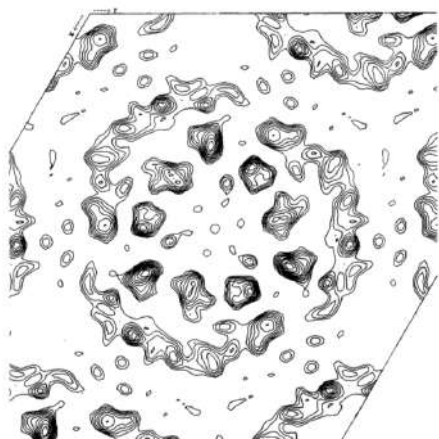
microED  
Science breakthrough of the year  
runner-up 2018





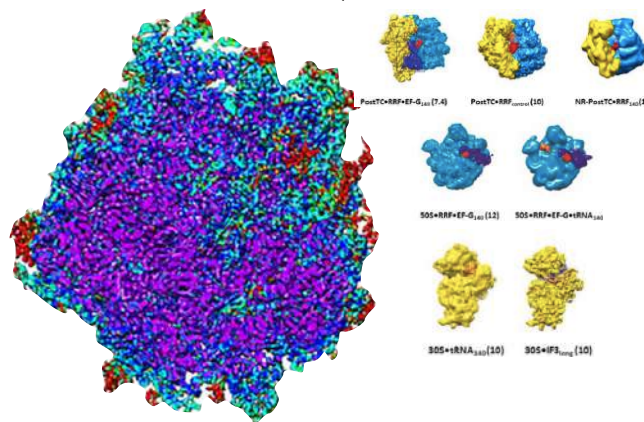
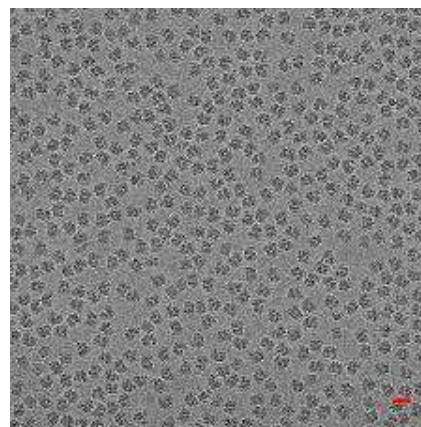
# CRYOEM: TECHNOLOGY ON THE RISE

1986



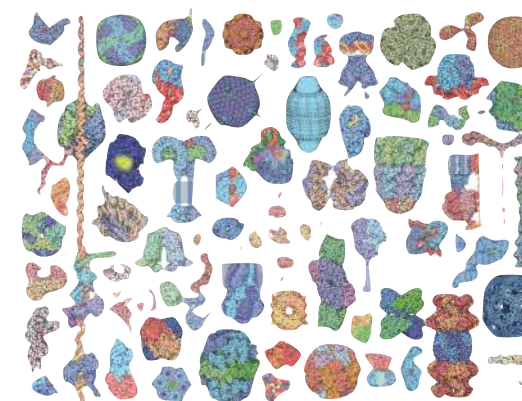
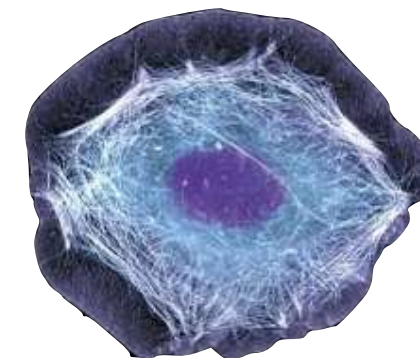
Henderson, et al. (1986)

2017



Frank (2017)

????

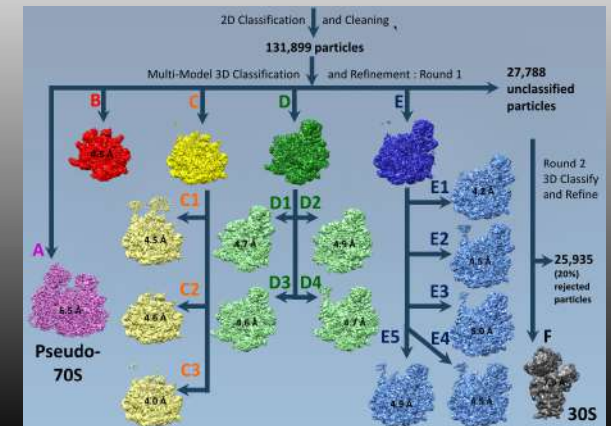


TBD (20??)

# Hardware



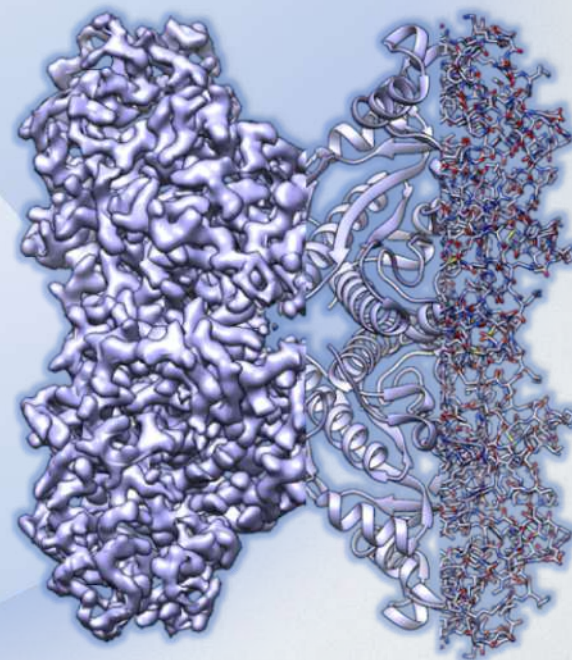
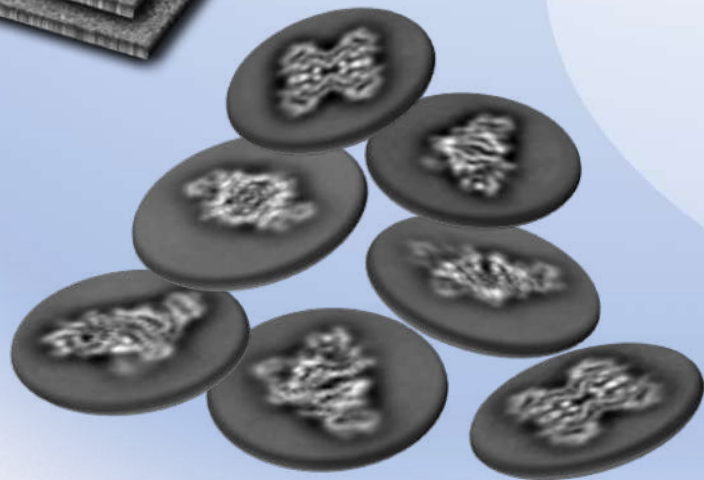
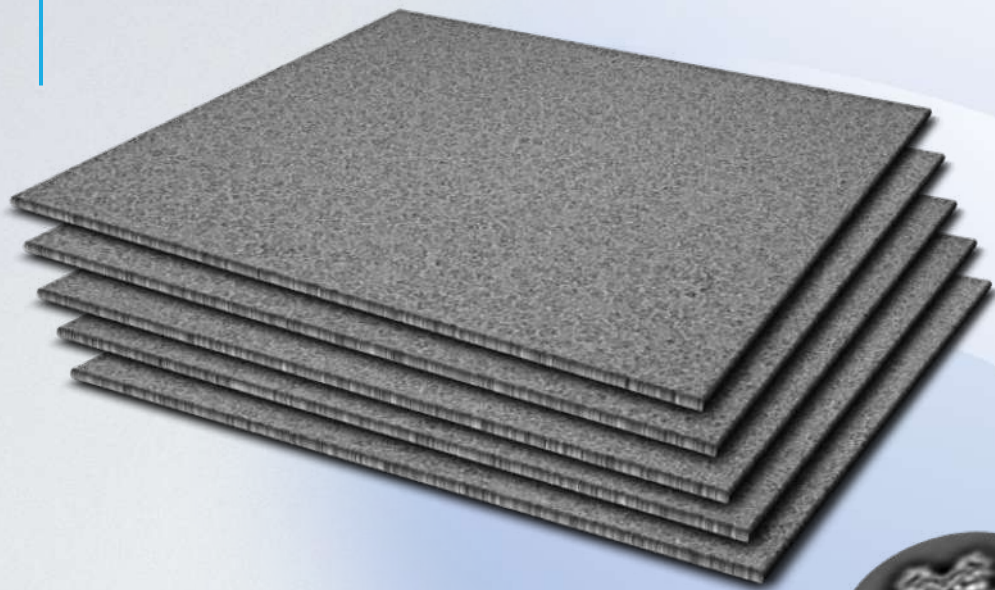
# Software





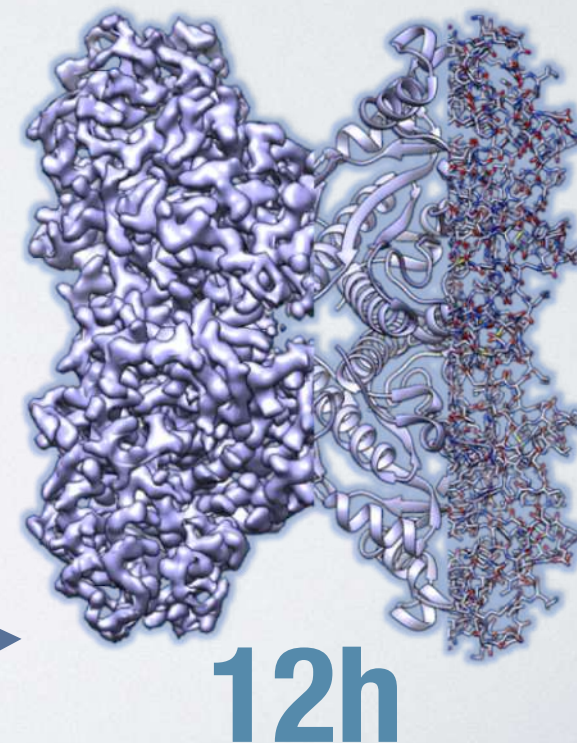
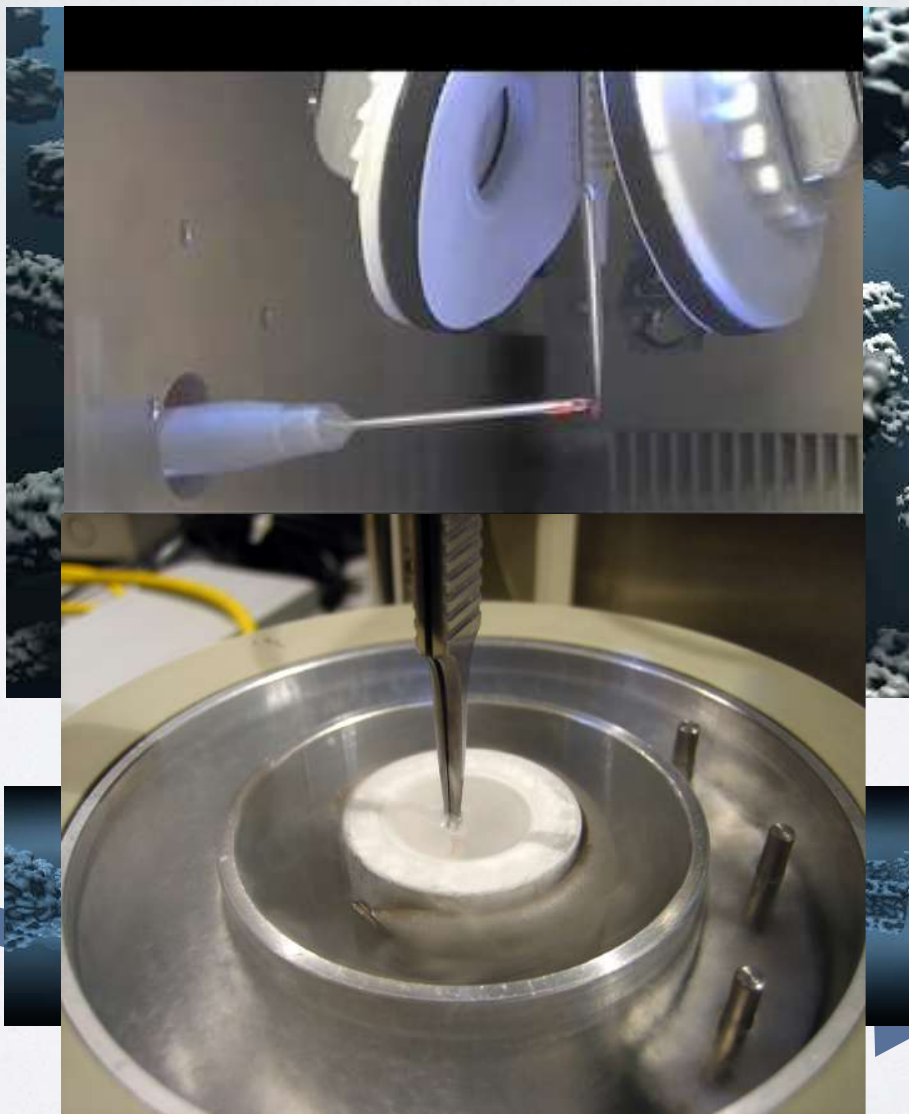
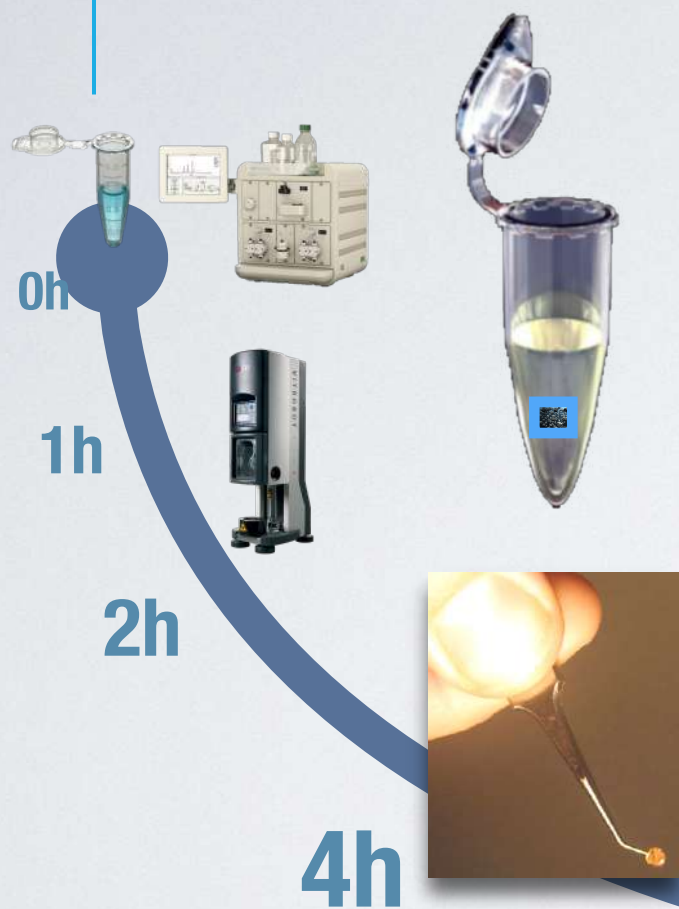
# WHAT IS POSSIBLE TODAY?

✱ 2Å within a day



# WHAT IS POSSIBLE TODAY?

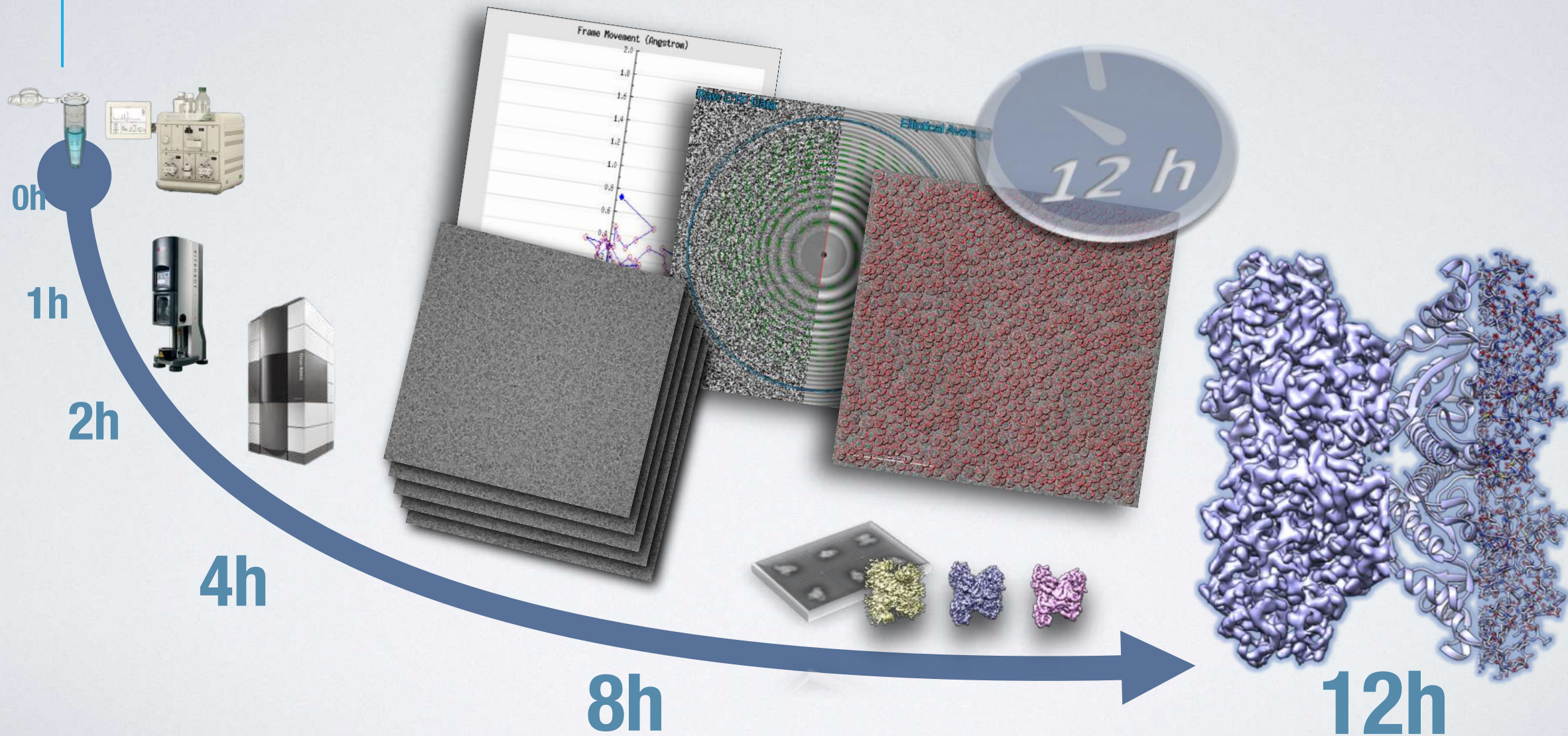
\*2Å within a day





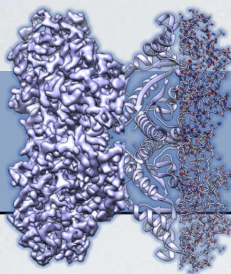
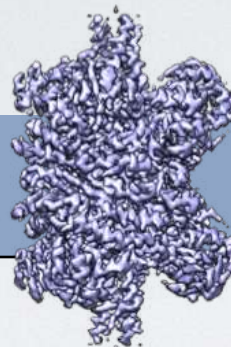
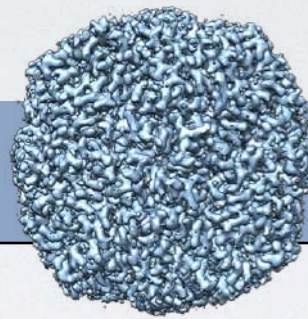
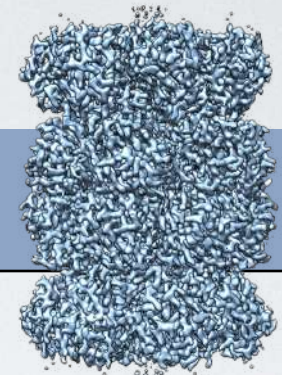
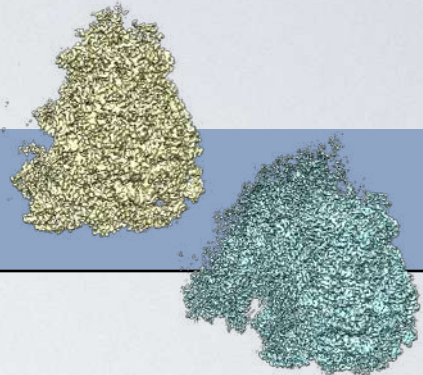
# WHAT IS POSSIBLE TODAY?

✱ 2Å within a day

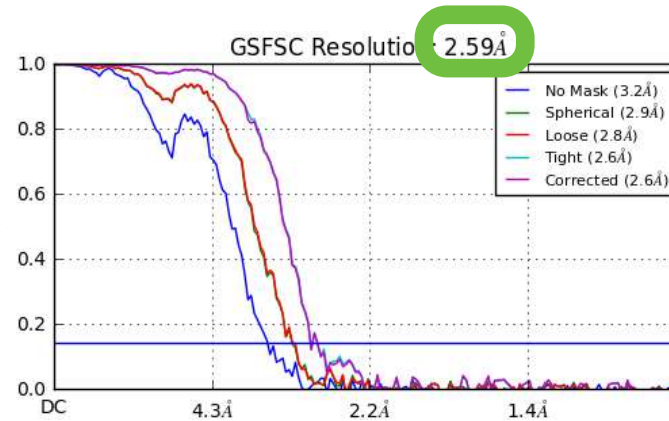
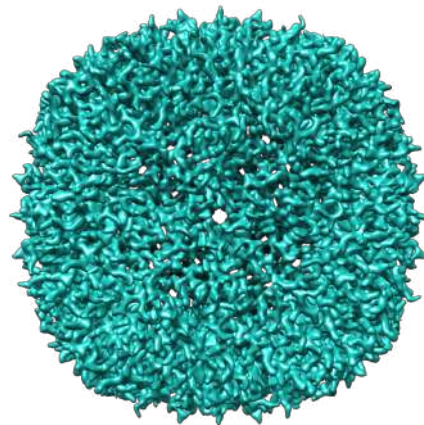
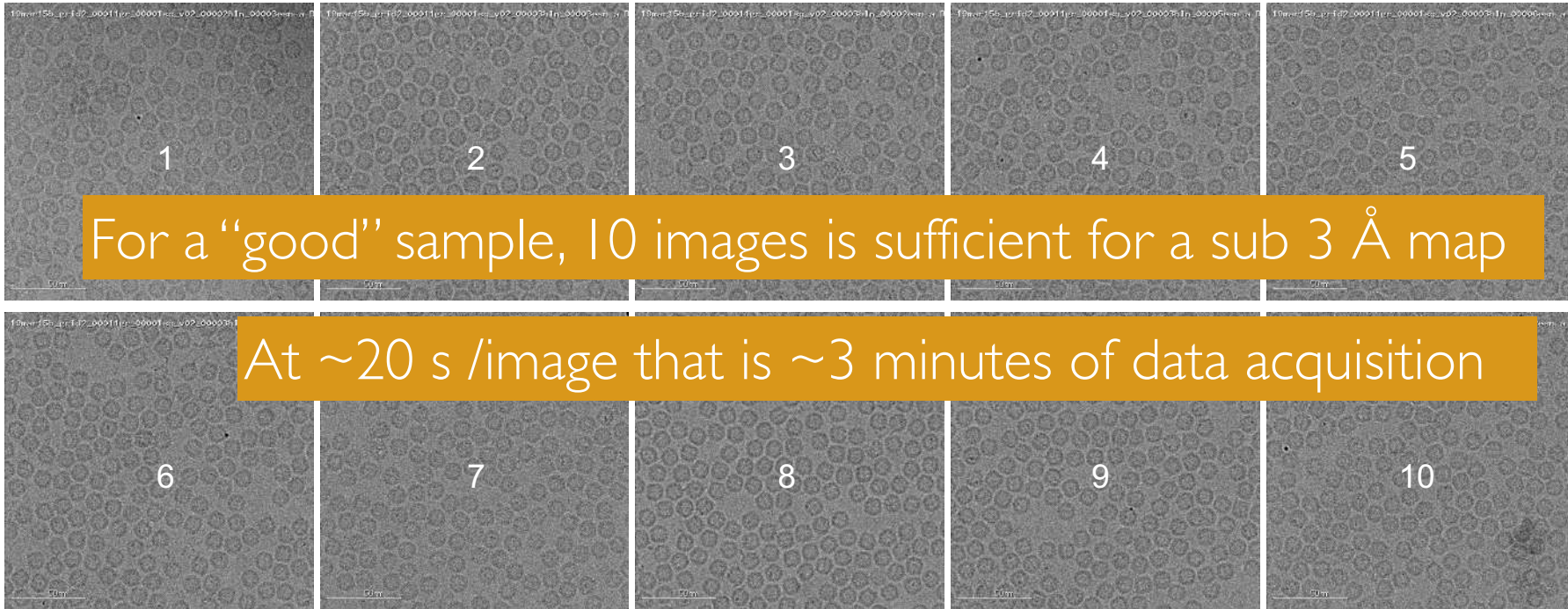




# IS THIS ROUTINELY DONE?

				
Aldolase	Glutamate dehydrogenase	Apoferritin	20S proteasome	60S/80S ribosome
D2	D3	O	D7	C1
~150kDa	334kDa	443kDa	750kDa	~2-4MDa
rabbit muscle	cow liver	horse spleen	Thermoplasma or Mycoplasma	human

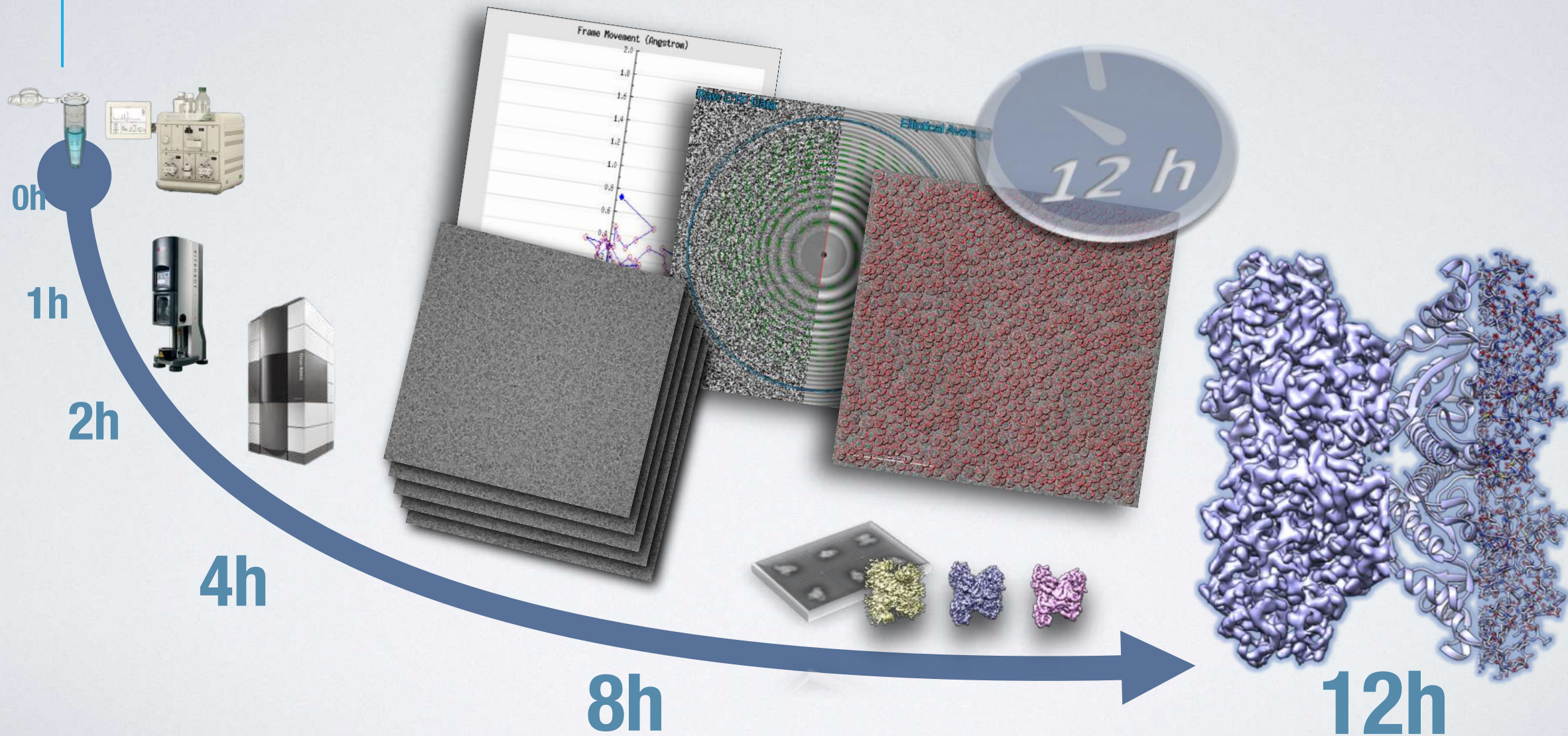
# HOW MANY IMAGES DO WE NEED?





# WHAT IS POSSIBLE TODAY?

✱ 2Å within a day



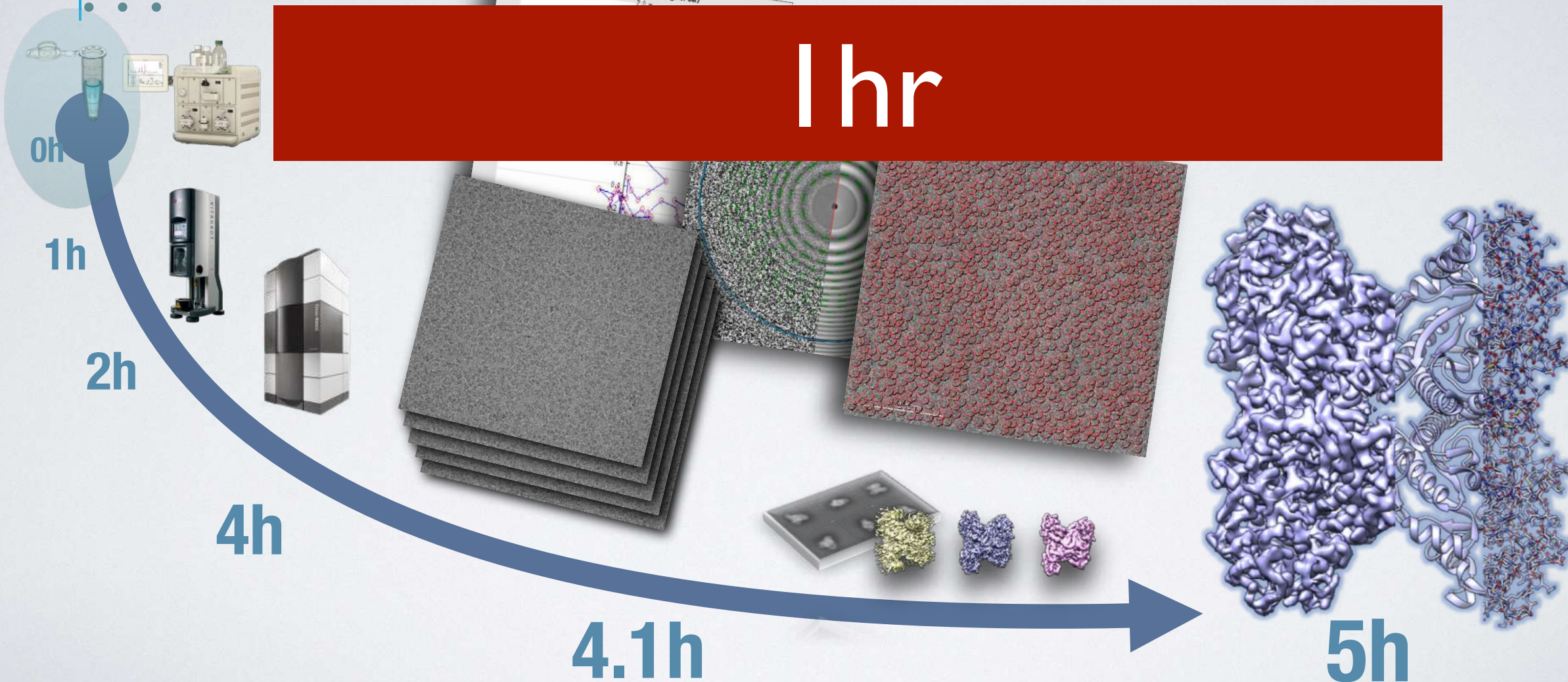


# WHAT IS POSSIBLE TODAY?

\*2Å within hours

???

1hr



# WHERE ARE THE BOTTLENECKS?

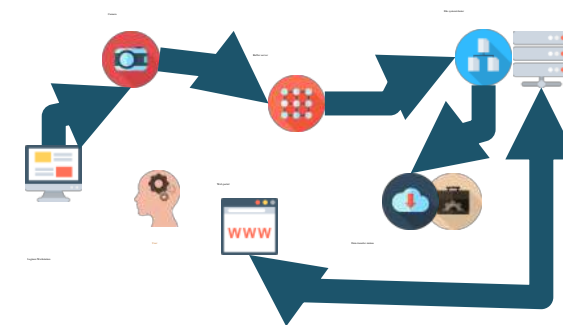
technology aimed towards completely automating the processes involved in solving macromolecular structure using cryo-electron microscopy (cryoEM)



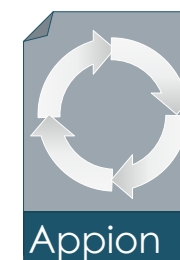
Sample preparation



Data collection



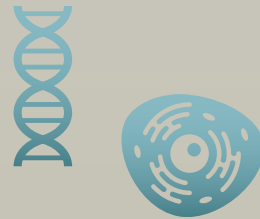
(pre-)Processing



# NCCAT CROSS-TRAINING FOCUS ON 4 AREAS



Core  
knowledge



Sample  
preparation



Microscope  
operations &  
Data  
collection



Processing  
&  
Data analysis

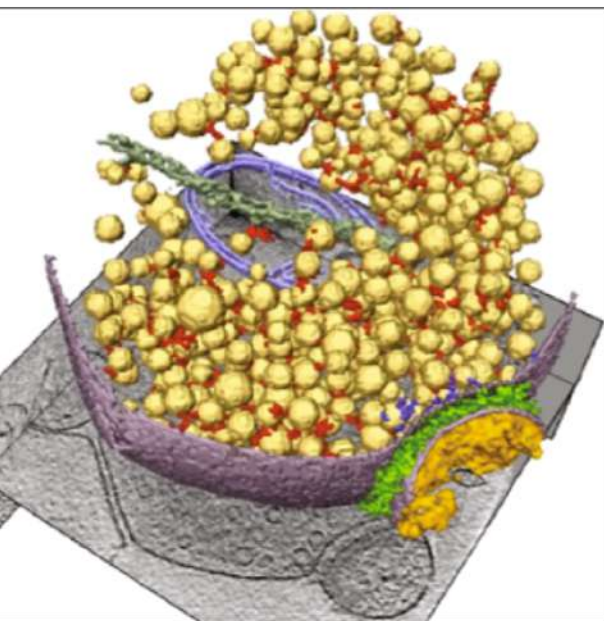


# NCCAT CROSS-TRAINING FOCUS ON 4 AREAS

## Cryo-EM methods

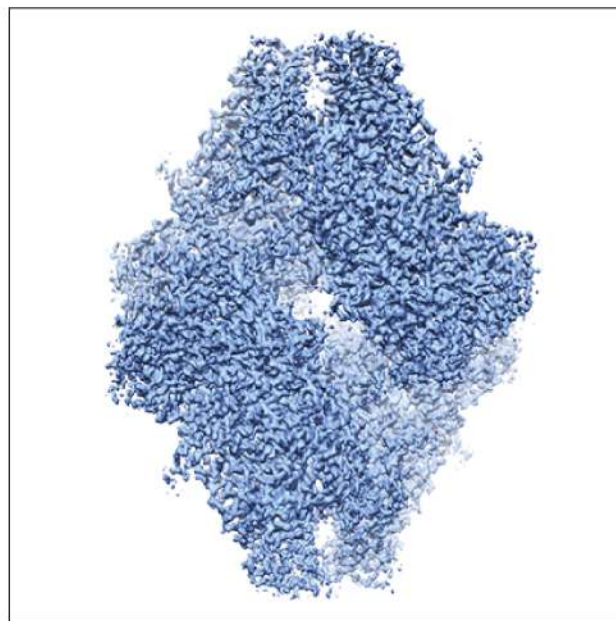
### Imaging

Electron tomography



Whole cells and organelles

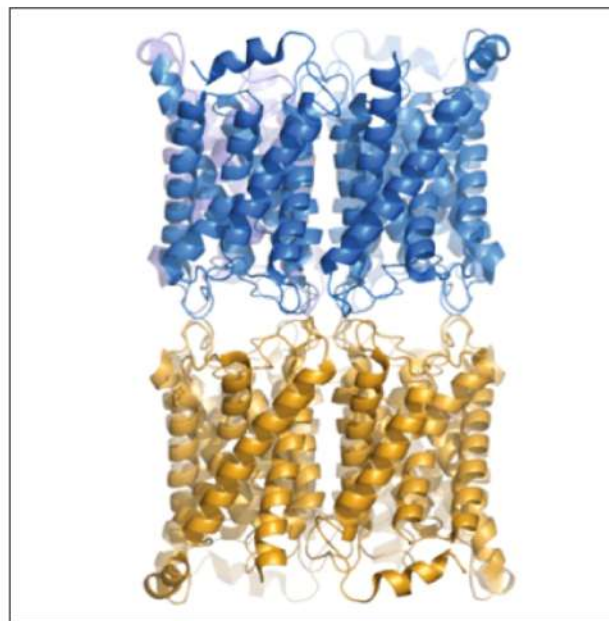
Single-particle reconstruction



Isolated single particles

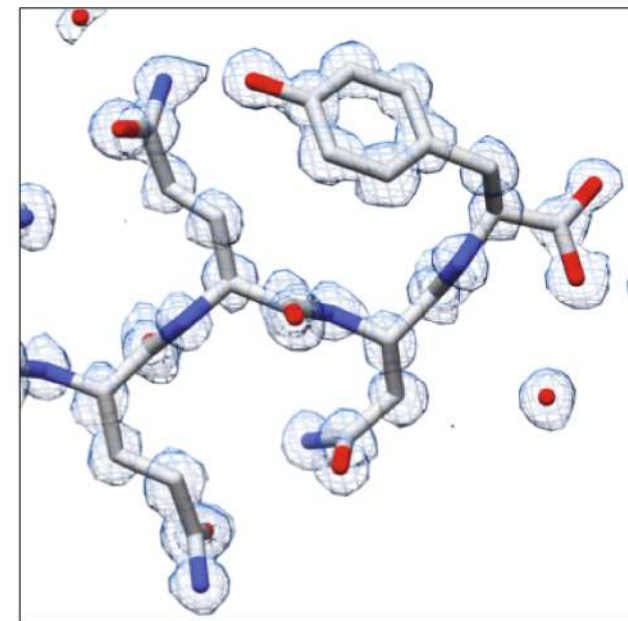
### Crystal-based

2D electron crystallography



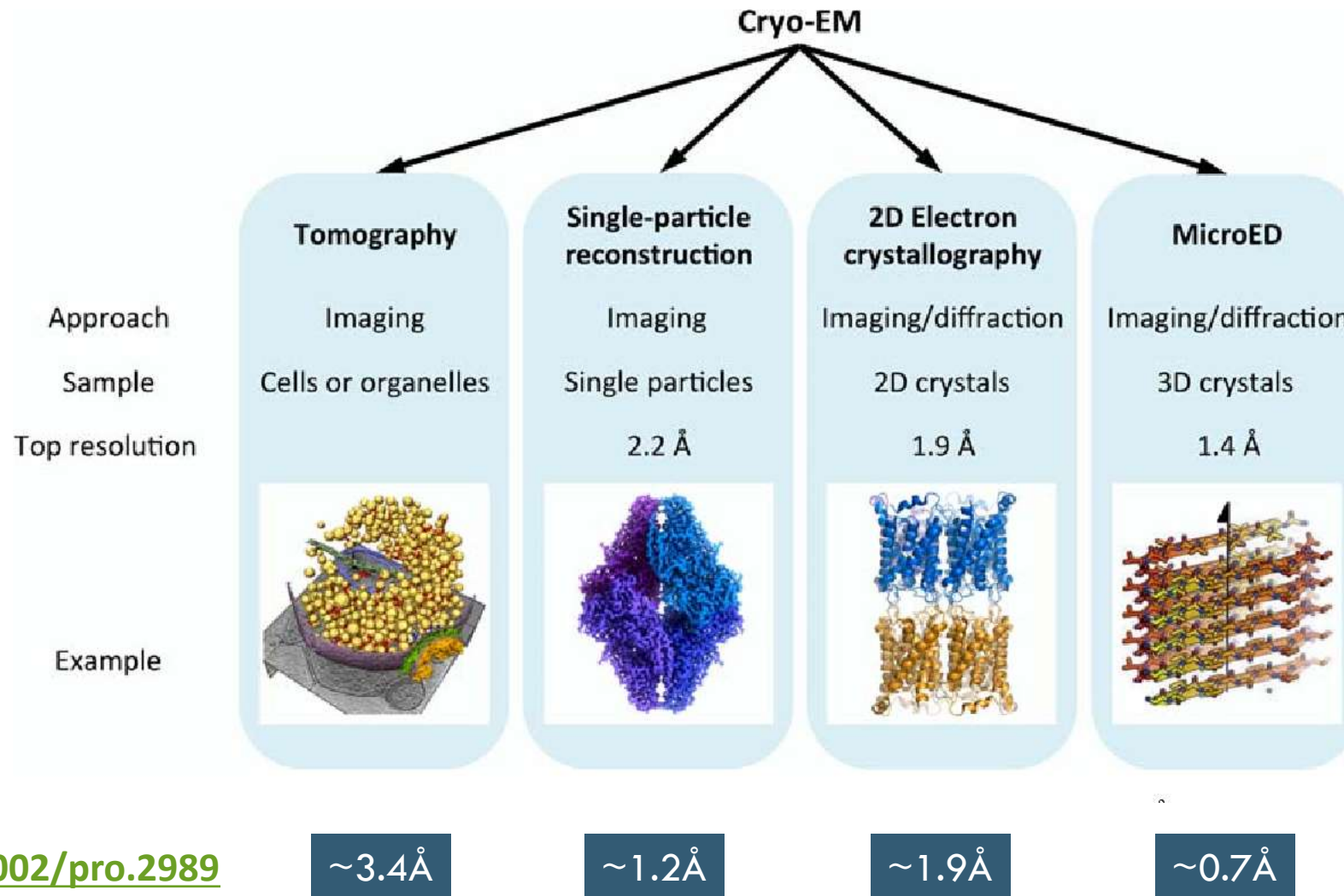
2D crystals

Microcrystal electron diffraction (MicroED)



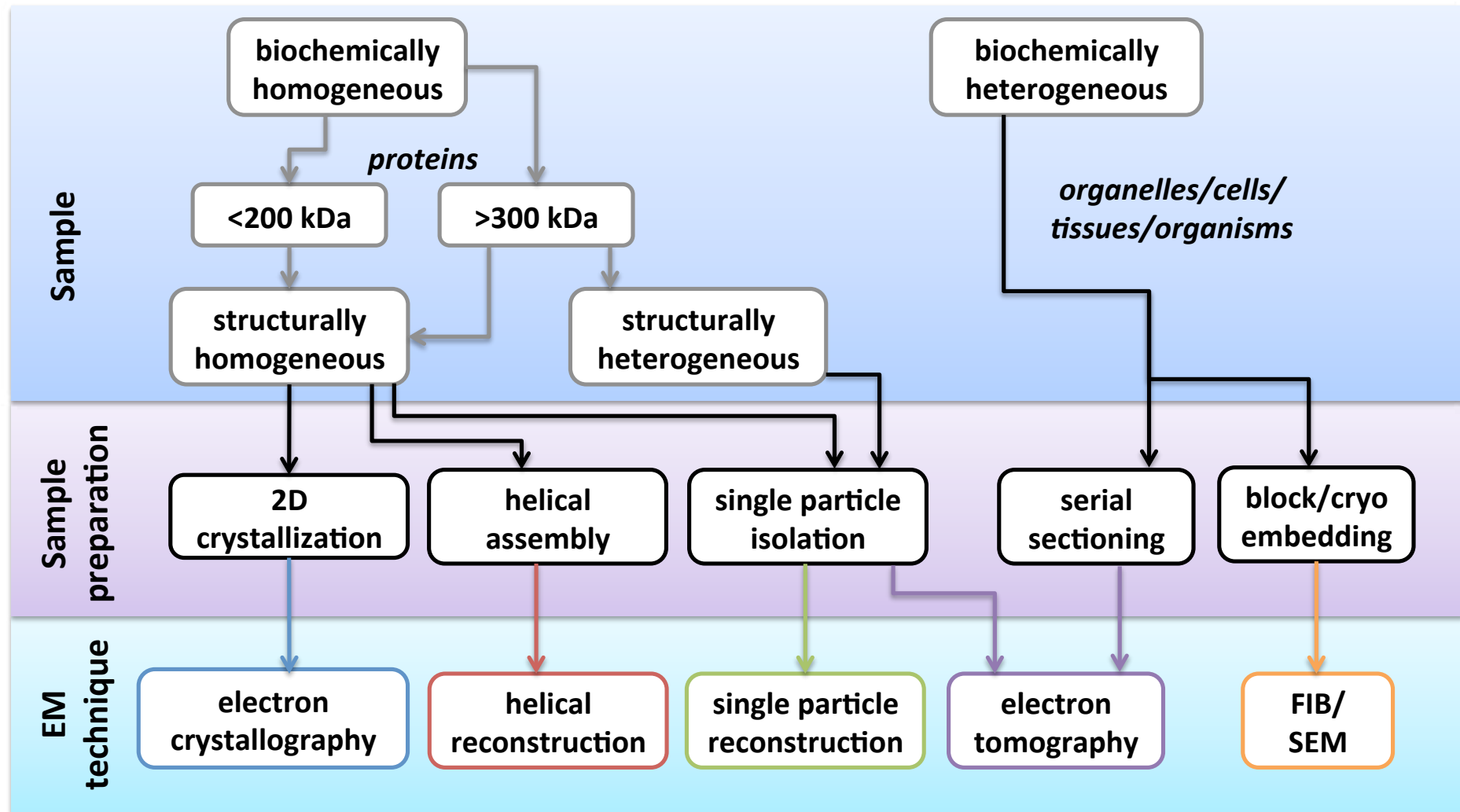
3D microcrystals

# THE FIELD IS CHANGING





# BUT STILL HAS BOTTLENECKS





WHAT NEXT?