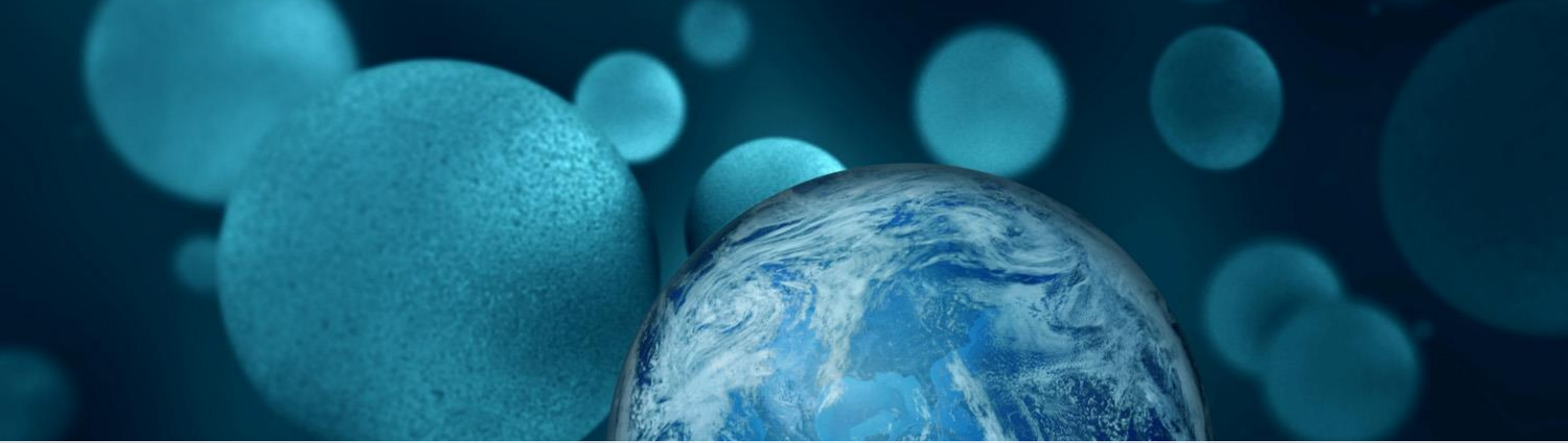
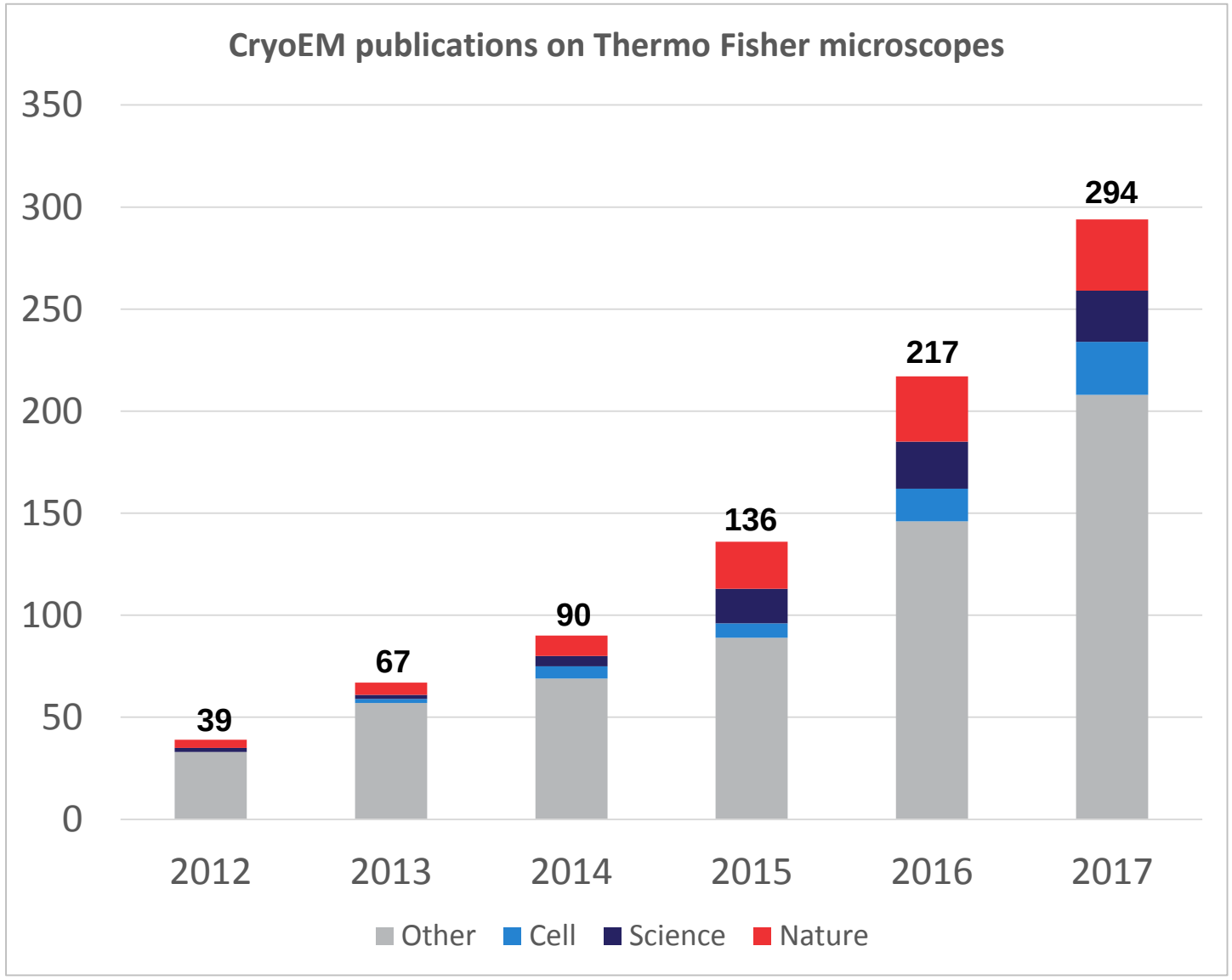


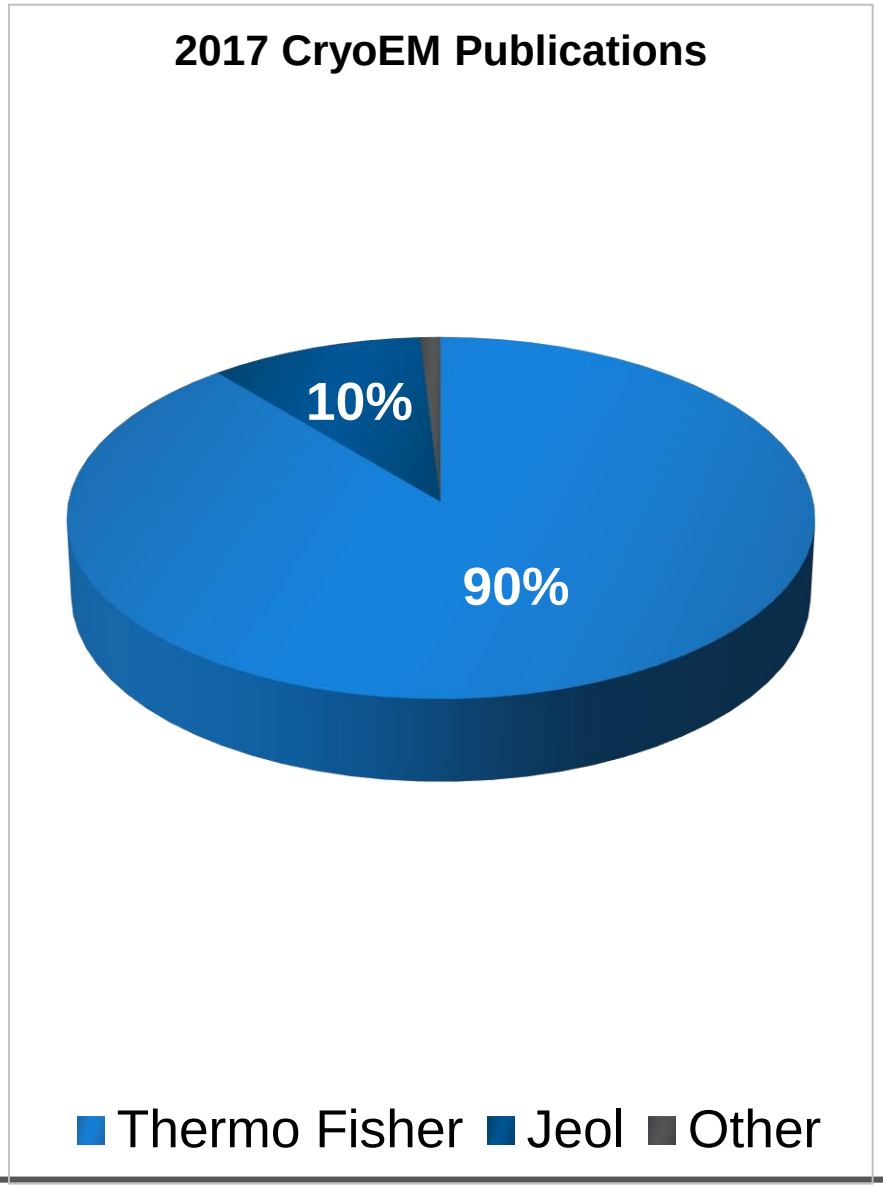
ThermoFisher SCIENTIFIC

Single Particle Analysis (SPA) Workflow
Understanding molecular structures – faster and more reliably with CryoEM

Medinprot, Budapest
21-04-2018
Max Maletta

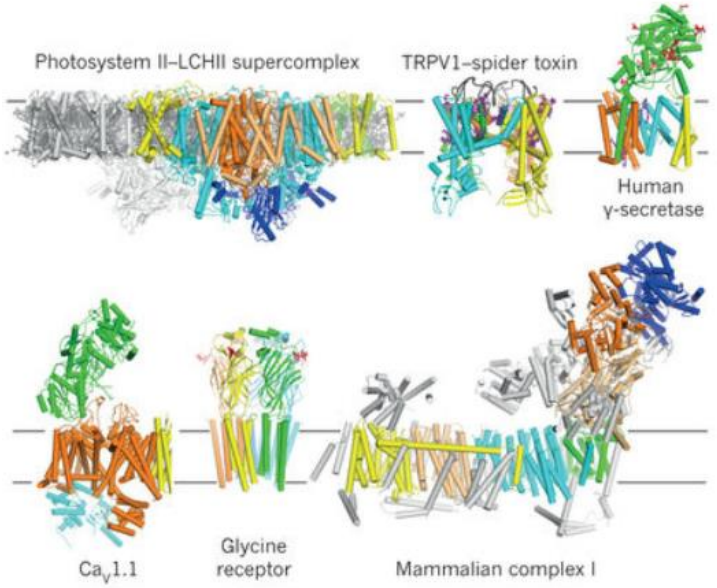
Growth in structural biology CryoEM over the past 5 years





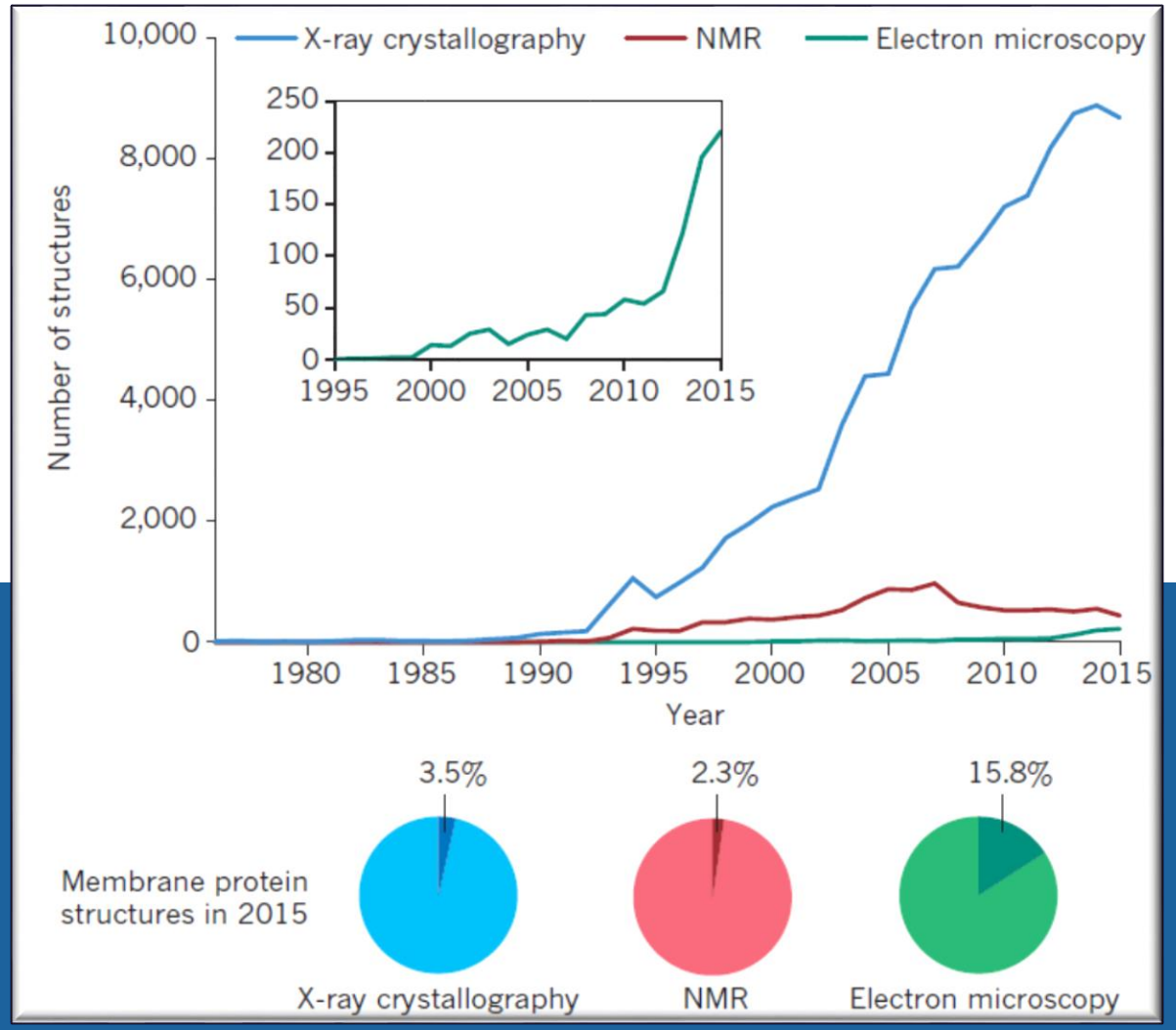
ThermoFisher SCIENTIFIC

Growth in structural biology over the past 40 years



Examples of membrane protein structures that were determined through cryo-EM

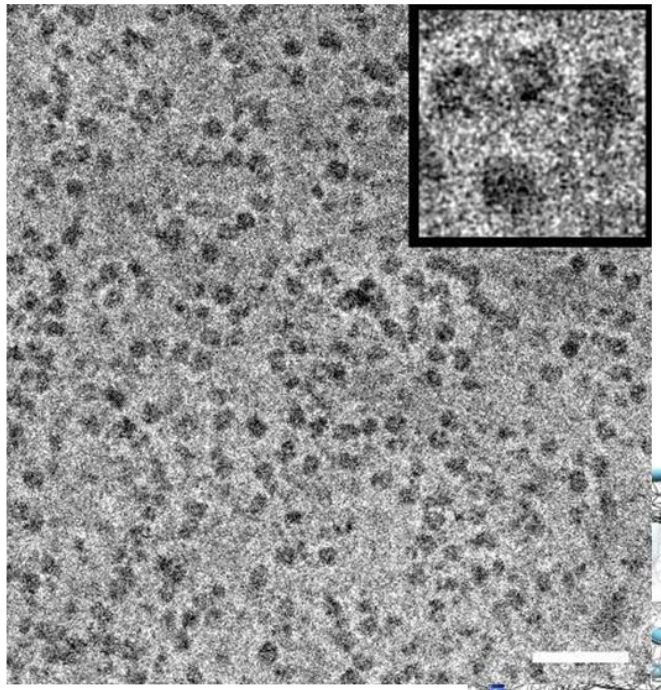
"Developments in the electron microscopy are providing unprecedented opportunities for the structural characterization of biological macromolecules. This is resulting in a wave of information about processes in the cell that were impossible to characterize with existing techniques in structural biology. "



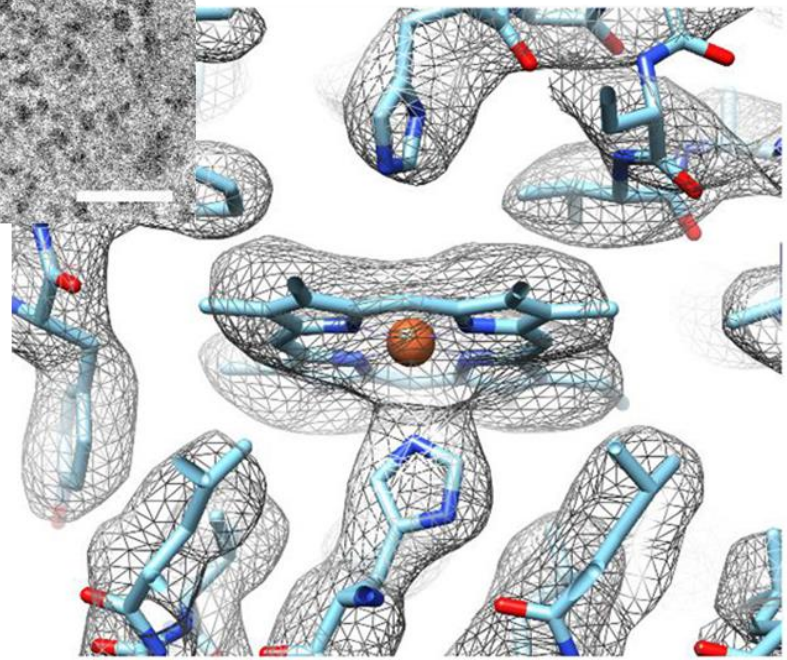
R. Fernandez-Leiro & S. H. W. Scheres Nature 2016

ThermoFisher SCIENTIFIC

Resolution revolution from “blob-ology” to atomic resolution



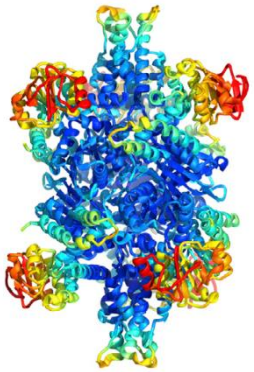
Titan Krios with Volta Phase Plate was tested for the most challenging small particles – hemoglobin (**MW 64 kDa** and C2 symmetry)



Cryo-EM structure of haemoglobin at 3.2 Å determined with the Volta phase plate
bioRxiv preprint 2016
Submitted to Nature 2016
M. Khoshouei, M. Radjainia, W.Baumeister & R.Danev

Titan Krios TEM 300kV

- Proven performance < 2Å
  < 100kDa

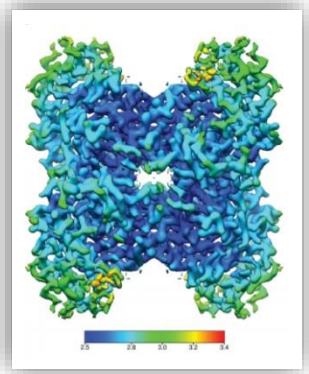


Glutamate dehydrogenase
- 334 kDa
- **1.8Å resolution**

Breaking Cryo-EM Resolution Barriers to Facilitate Drug Discovery
Cell, 2016
A.Merk, A. Bartesaghi, S. Banerjee, L. A. Earl, J. Milne, S. Subramaniam

Talos Arctica TEM 200kV

Proven performance <3Å

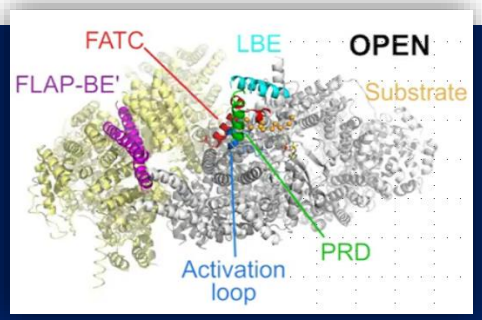


Rabbit muscle aldolase
- 150 kDa
- **2.6Å resolution**

Merk, A. et al. (2016), *Cell*, 165: 1698
Herzik, M.A. Jr et al (2017), *preprint on biorxiv*, http://dx.doi.org/10.1101/141994

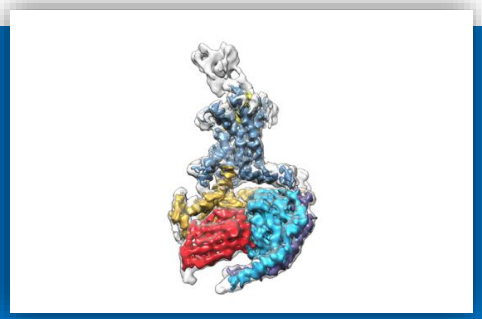
CryoEM SPA is complementary to traditional structural biology techniques

Study of **dynamic** biological processes



Human ATM
Multiple conformations

Structures that are difficult to solve with other techniques: **membrane** proteins and ion channels



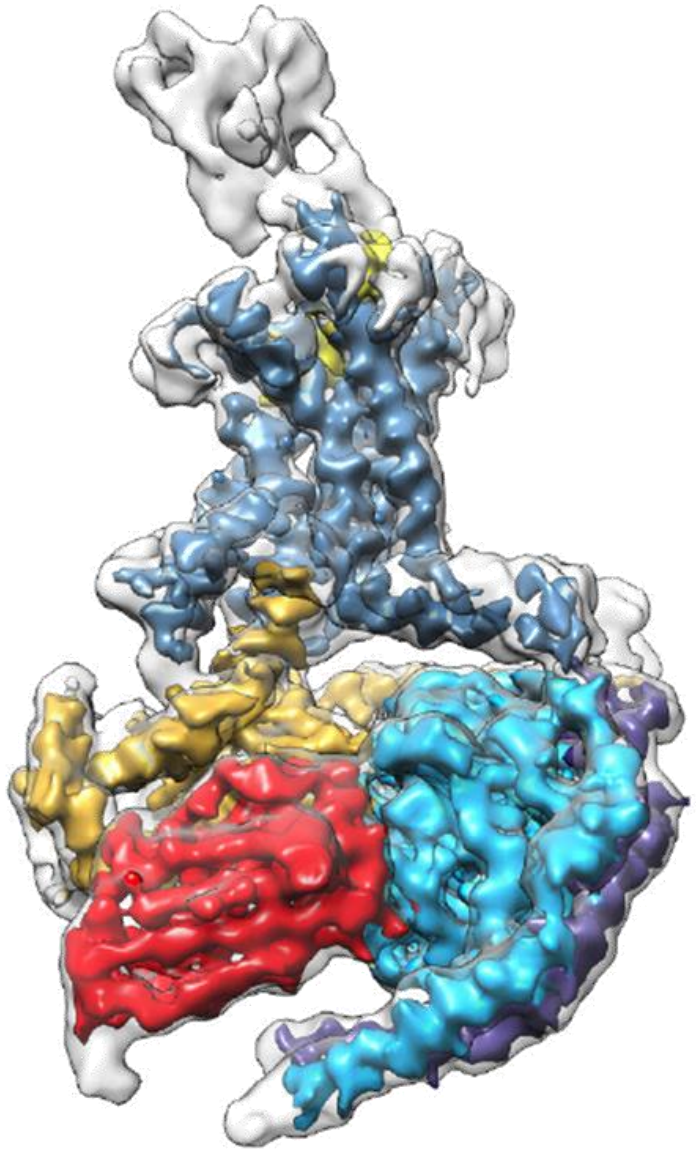
GPCRs
Large membrane protein

Atomic resolution structures of protein **complexes**, aggregates and large virus assemblies

TAU protein
Aggregation of a protein complex

Structure of active GPCR at atomic resolution

membrane



Liang et al. (2017), *Nature* **546**, 118–123, doi:10.1038/nature22327

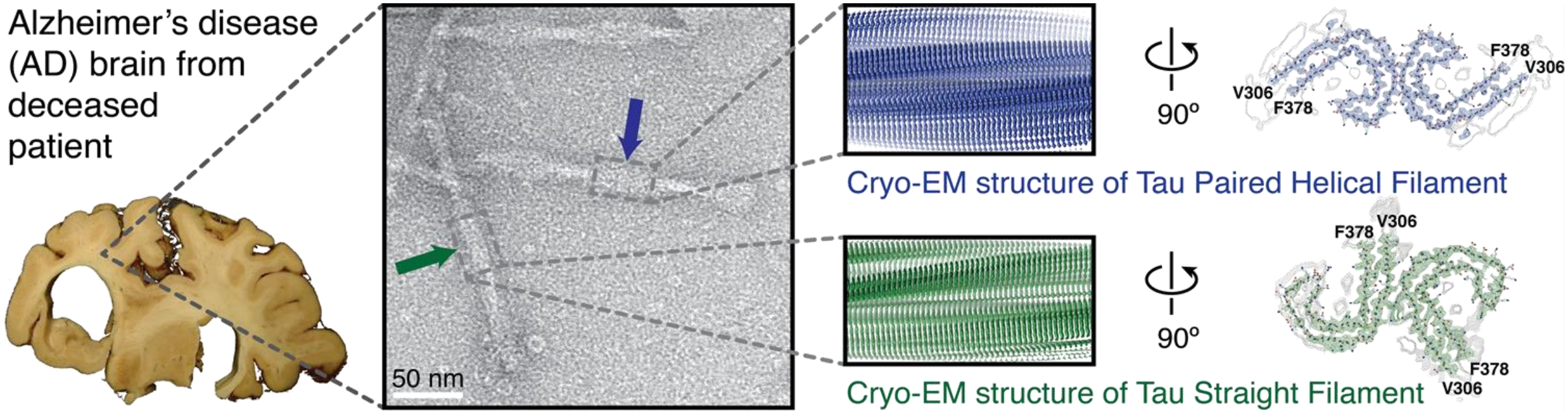
- G protein-coupled receptors (GPCRs) are major targets for treatment of chronic diseases
- Research objective
  Understand mechanism of membrane trafficking – “functional states”
- Unique
  CryoEM allows to visualize the structure of an *activated* GPCR complex (bound to proteins)
- **This information supports the design of better drugs to treat diabetes, obesity, osteoporosis and migraine**

CryoEM of Tau protein filaments provides insight in aggregation mechanism

complex

- Alzheimer's disease caused by Tau protein filaments aggregating in the brain (protein stacks → plaque)
- <u>Research objective</u>
  Understand mechanism of protein aggregation

- <u>Unique</u>
  CryoEM revealed structure of 2 distinct types of filament – small tweaks in how monomers associate with one another has drastic effects on the fibril shape
- **This understanding of the aggregation mechanism contributes to future drug design**



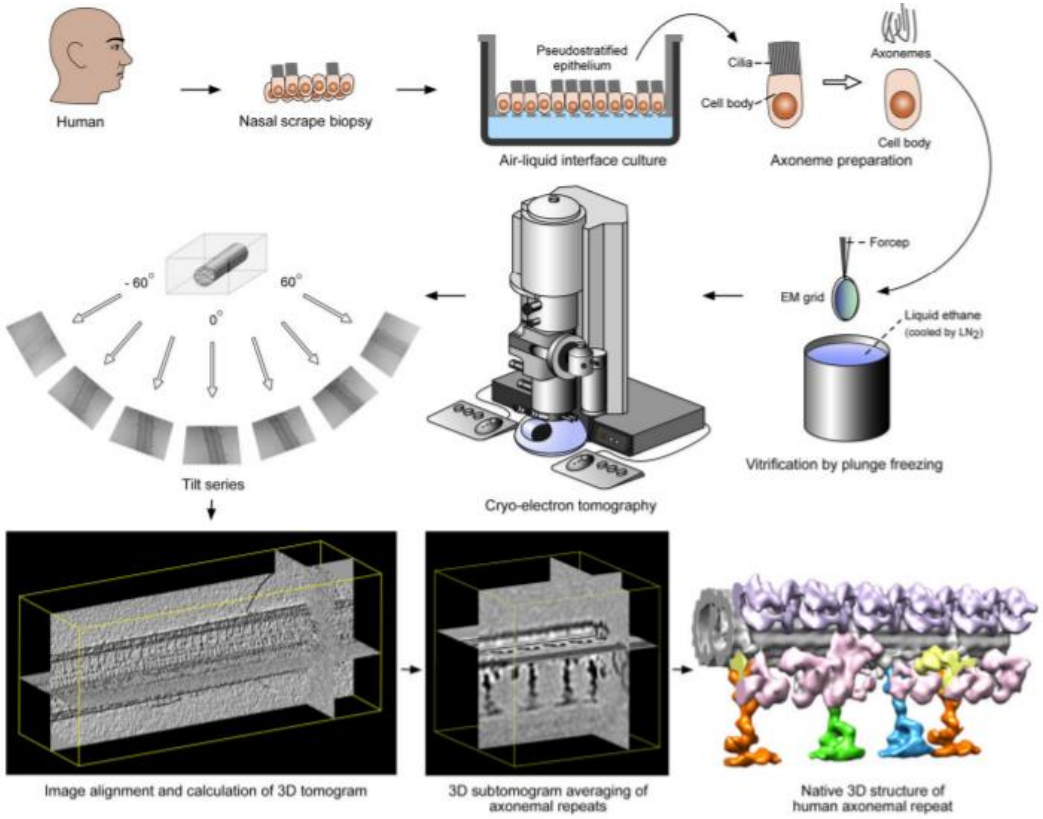
Fitzpatrick et.al. (2017) *Nature* **547**, 185-190 | doi:10.1038/nature23002

ThermoFisher SCIENTIFIC

Structural biology from human derived sample

Cryo-electron tomography reveals ciliary defects underlying human *RSPH1* primary ciliary dyskinesia

Jianfeng Lin[1], Weining Yin[2], Maria C. Smith[1], Kangkang Song[1], Margaret W. Leigh[5], Maimoona A. Zariwala[3], Michael R. Knowles[4], Lawrence E. Ostrowski[2,*], and Daniela Nicastro[1,*]



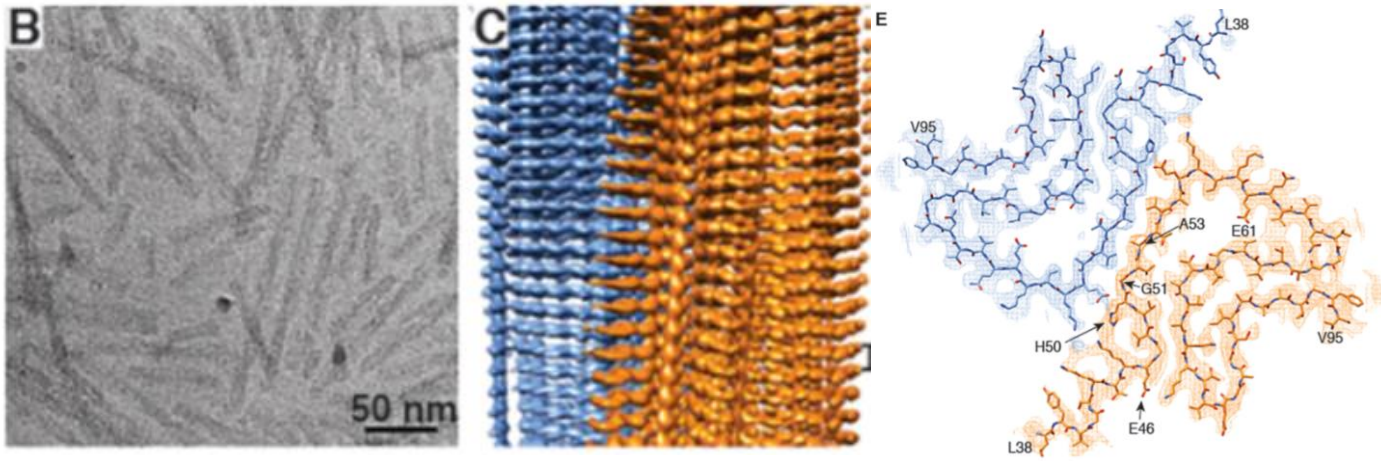
STRUCTURAL BIOLOGY

Fibril structure of amyloid-β(1–42) by cryo–electron microscopy

Lothar Gremer,[1,2] Daniel Schölzel,[1,2] Carla Schenk,[1] Elke Reinartz,[2] Jörg Labahn,[1,2,3] Raimond B. G. Ravelli,[4] Markus Tusche,[1] Carmen Lopez-Iglesias,[4] Wolfgang Hoyer,[1,2] Henrike Heise,[1,2] Dieter Willbold,[1,2]* Gunnar F. Schröder[1,5]*

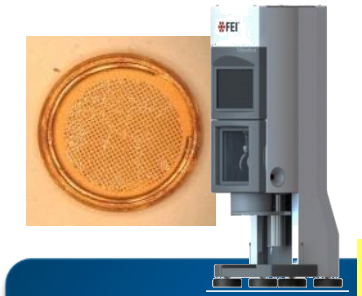
Cryo-EM structure of alpha-synuclein fibrils

Ricardo Guerrero-Ferreira[1], Nicholas M.I. Taylor[1]†, Daniel Mona[2], Philippe Ringler[1], Matthias E. Lauer[3], Roland Riek[4], Markus Britschgi[2], and Henning Stahlberg[1,*].

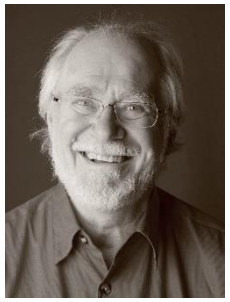


ThermoFisher SCIENTIFIC

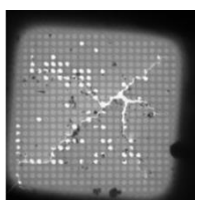
2017 Breaking News: Nobel Prize for CryoEM SPA Workflow



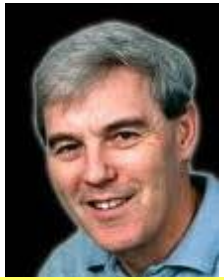
Vitrification



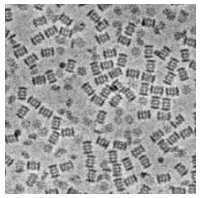
Sample prep



Screening

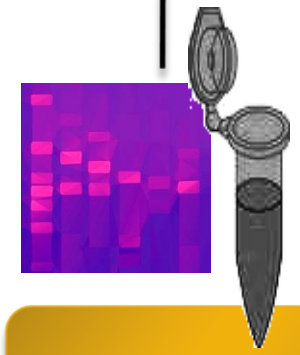


High Resolution Acquisition

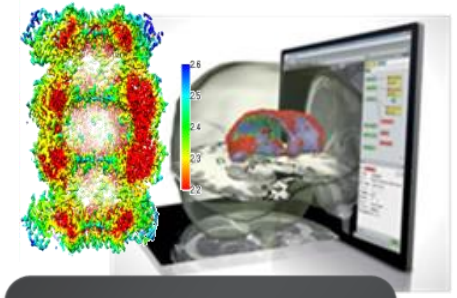


High resolution data acquisition

Data Processing



Biochemistry



Processing: 3D reconstruction

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Single Particle Analysis (SPA) Workflow Challenges

Biochemistry and vitrification complexity

- Efficient and affordable screening & sample optimization

Reproducibility of best performance

- Ease-of-use through extensive automation

Access to expertise and productivity

- Customized systematic approach to integrated service and applications support

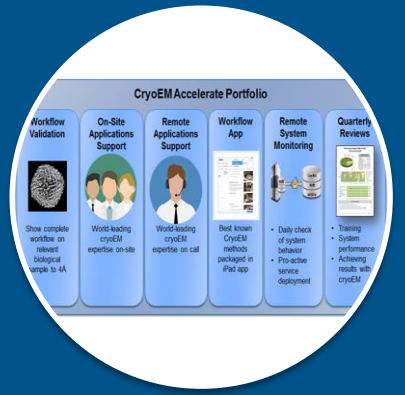
New in the Single Particle Analysis (SPA) Portfolio

Glacios Cryo-TEM
200 kV FEG + AutoLoader

- Efficient and affordable screening solution

Krios G3i Cryo-TEM
300 kV FEG + AutoLoader

- Ease-of-use through extensive automation



Accelerate portfolio for customer success

- Individualized systematic approach to integrated service and applications support

ThermoFisher SCIENTIFIC

Thermo Scientific Krios G3i Cryo-TEM – State-of-the-Art Cryo-EM

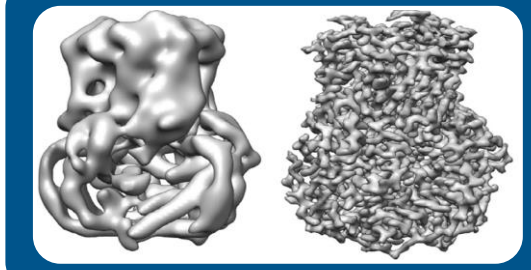
NEW

Access the next level state-of-the-art cryo electron microscopy, with improved ease-of-use and performance

- Optimal tool performance always guaranteed
- Automation and guidance simplifies experiment set up
- Highest resolution data at maximum throughput

ThermoFisher SCIENTIFIC

Krios G3i Cryo-TEM – Workflow Performance Improvements

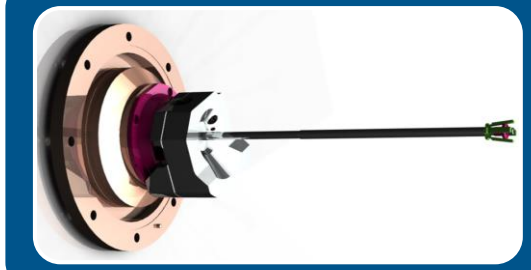


Data quality → 3D model resolution

- Guaranteed low anisotropic magnification distortion: < 1%
- Magnification range: 20kX → 150 kX

Throughput

- Improved drift after transfer
- Atlas speed improvement: immediately after transfer and data acquisition within minutes
- Eucentricity improvement for tomography: 1x1x3 μm^3



Sample life time

- Improved cryo box with anti contaminator
- 3 days guaranteed (column)
- 5 days in Autoloader

Grant, T. et al. (2015), *J. Structural Biology*, 192(2): 204

Thermo Scientific Glacios Cryo-TEM – Efficient Screening and Data Acquisition

NEW

- ***Throughput acceleration by efficient cryo-screening of particle distribution and ice quality***
- ***An affordable and complete solution for routine SPA data acquisition***

- Optimal tool performance always guaranteed
- Automation and guidance simplifies experiment set up
- Cryo, Autoloader, 200 kV electron optics on a brand new hardware platform
- Seamless connectivity to Krios TEM and small footprint for reduced installation requirements
- Smaller footprint

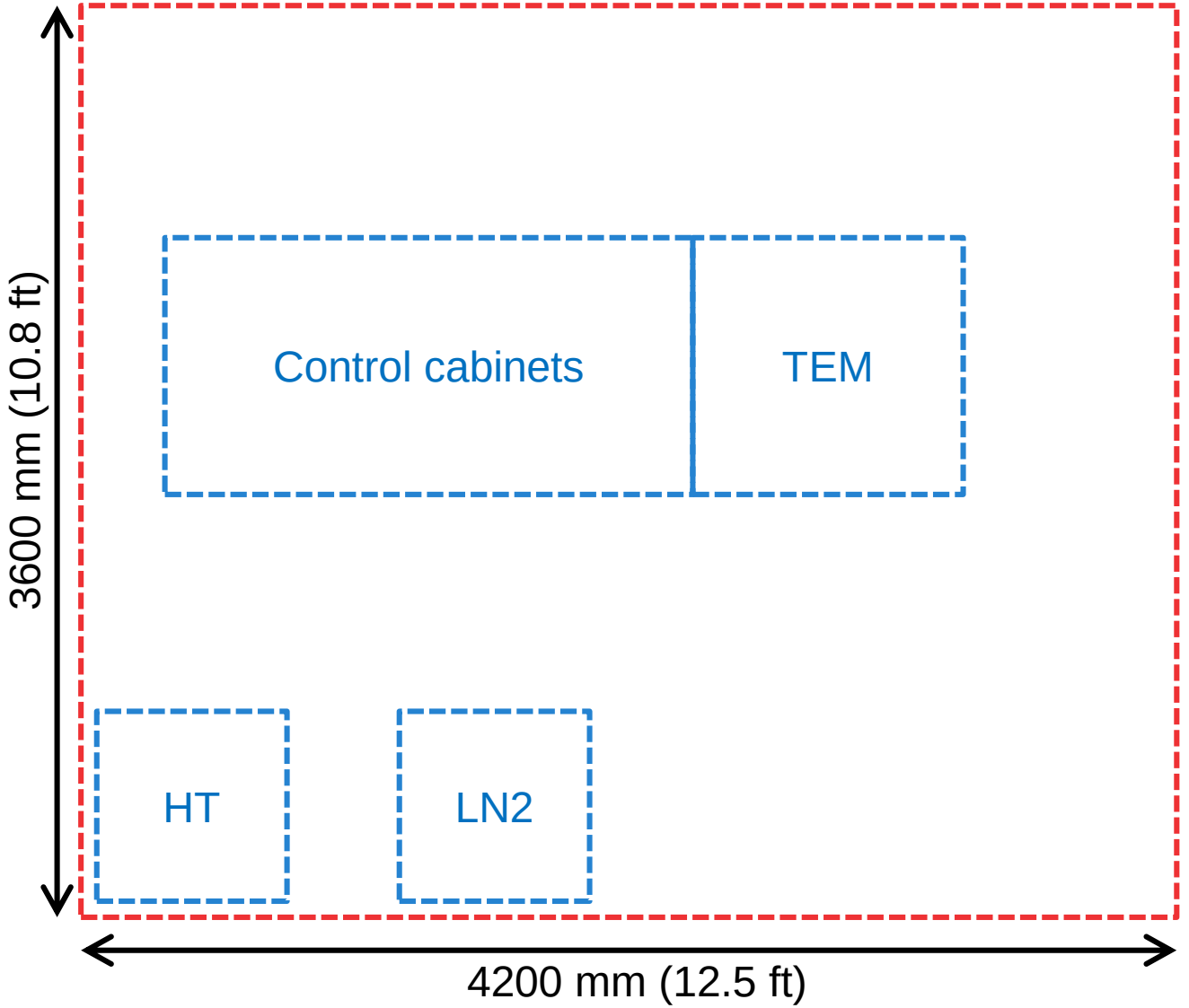
ThermoFisher SCIENTIFIC

Small Footprint and Easy Access for Reduced Glacios Installation Requirements

- New, more modular hardware platform

- Room floor space: 3800 mm x 3300 mm
- Room height requirement: 2800 mm (9.2 ft)

- Access route width: 900 mm (3 ft)
- Access route height: 2300 mm (7.6 ft)



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Krios G3i and Glacios Cryo-TEMs – Reproducible and Optimal Performance

Data acquisition quality and efficiency

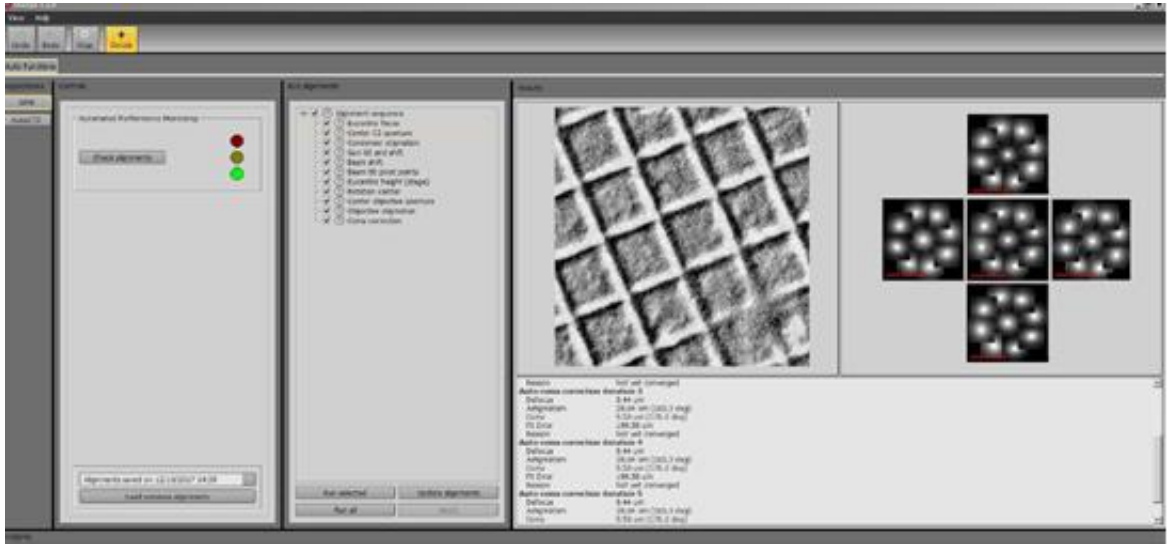
Depend on status of alignments – must always be optimal when starting data acquisition

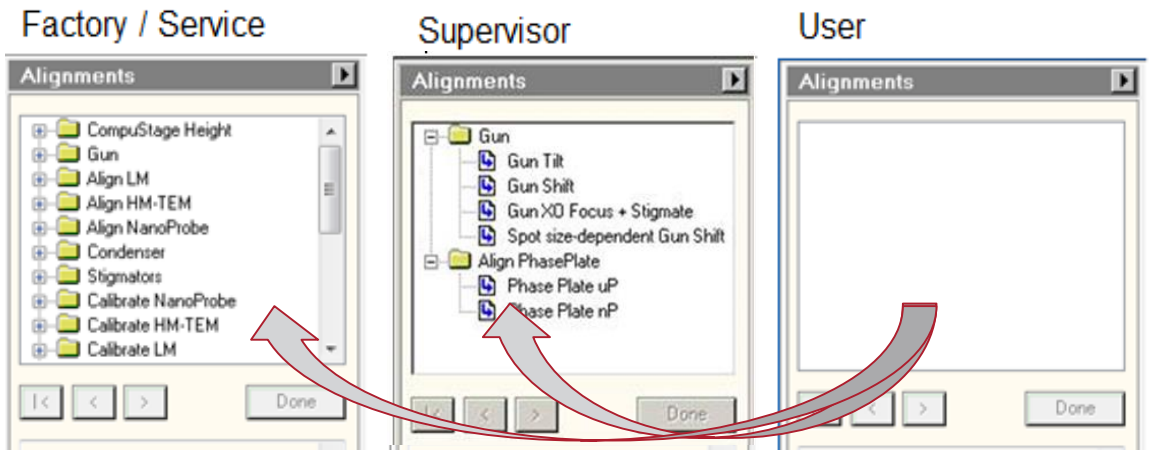
Self-assessment of the actual microscope status
Software for optimization of all alignments

Performance measurement and alignment automation

Reduce alignment complexity for users

Alignment automation and control access to unnecessary alignments





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Krios G3i and Glacios Cryo-TEMs – Simplified Experiment Setup

Full SPA workflow control

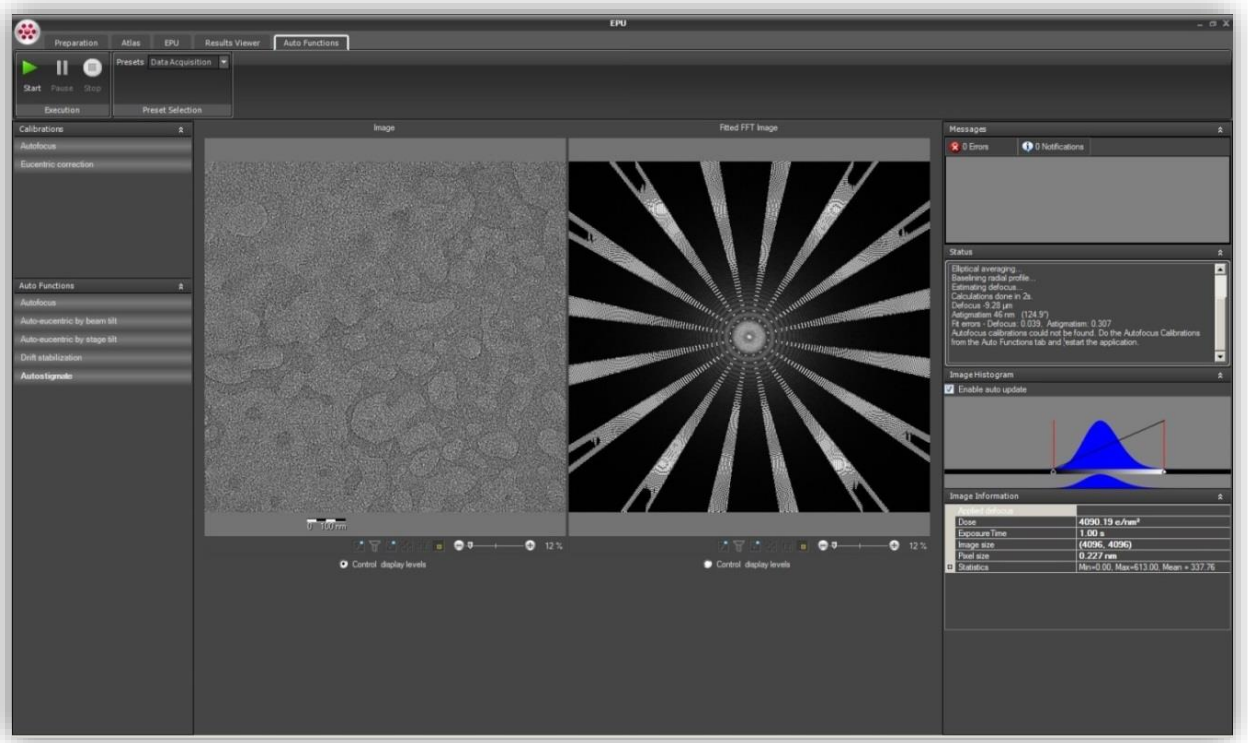
- Microscope setup
- Sample screening
- Data acquisition
- All controlled from EPU UI

One UI for "everything"

- Logical workflow guidance
- Automation in each step

Automated "daily" alignments

- Auto-eucentricity
- Auto-focus
- Auto-coma
- Auto-stigmate



Automated CTF fitting

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Krios G3i and Glacios Cryo-TEMs – Efficient Screening and Data Acquisition

Batch acquisition on all 12 grids

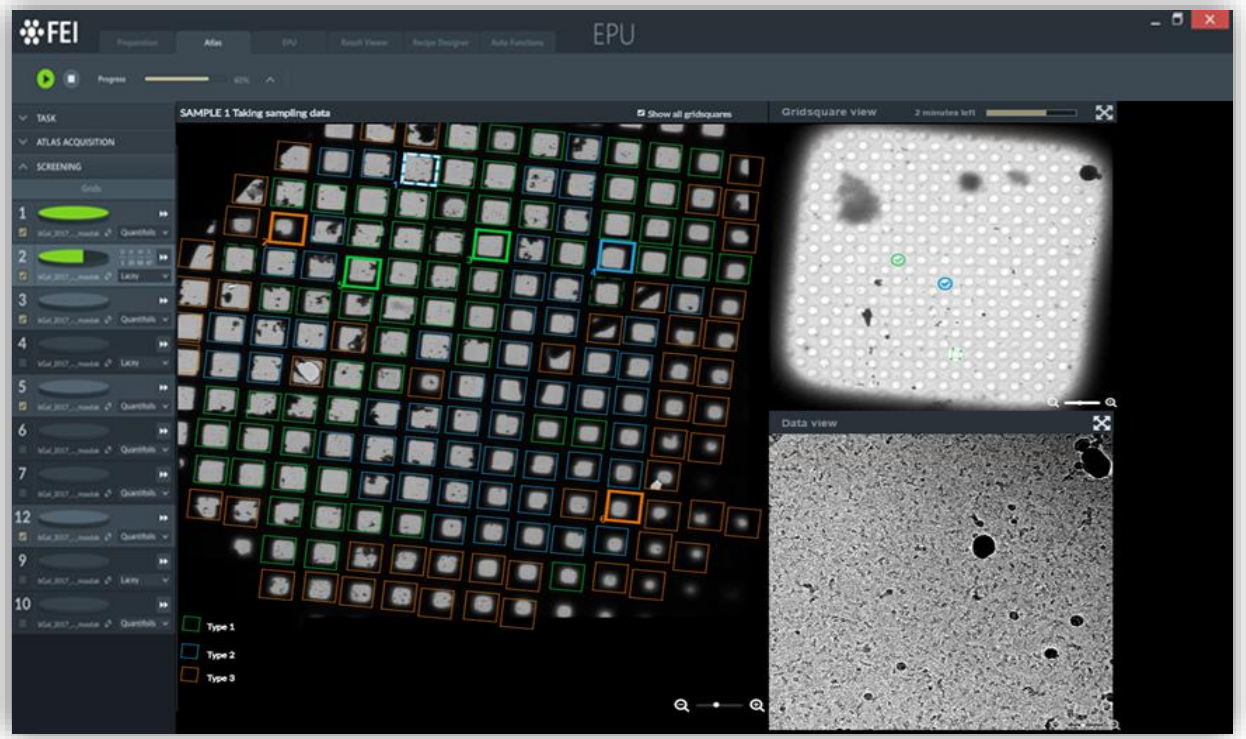
Autoloader control via EPU → automated grid atlas of all grids in a Cassette

Automated classification of ice

Grid screening on selected grids → automatically analyze Ice presence and thickness and classify in groups

Simplified acquisition of high magnification images for evaluation of ice quality and particle distribution/orientation

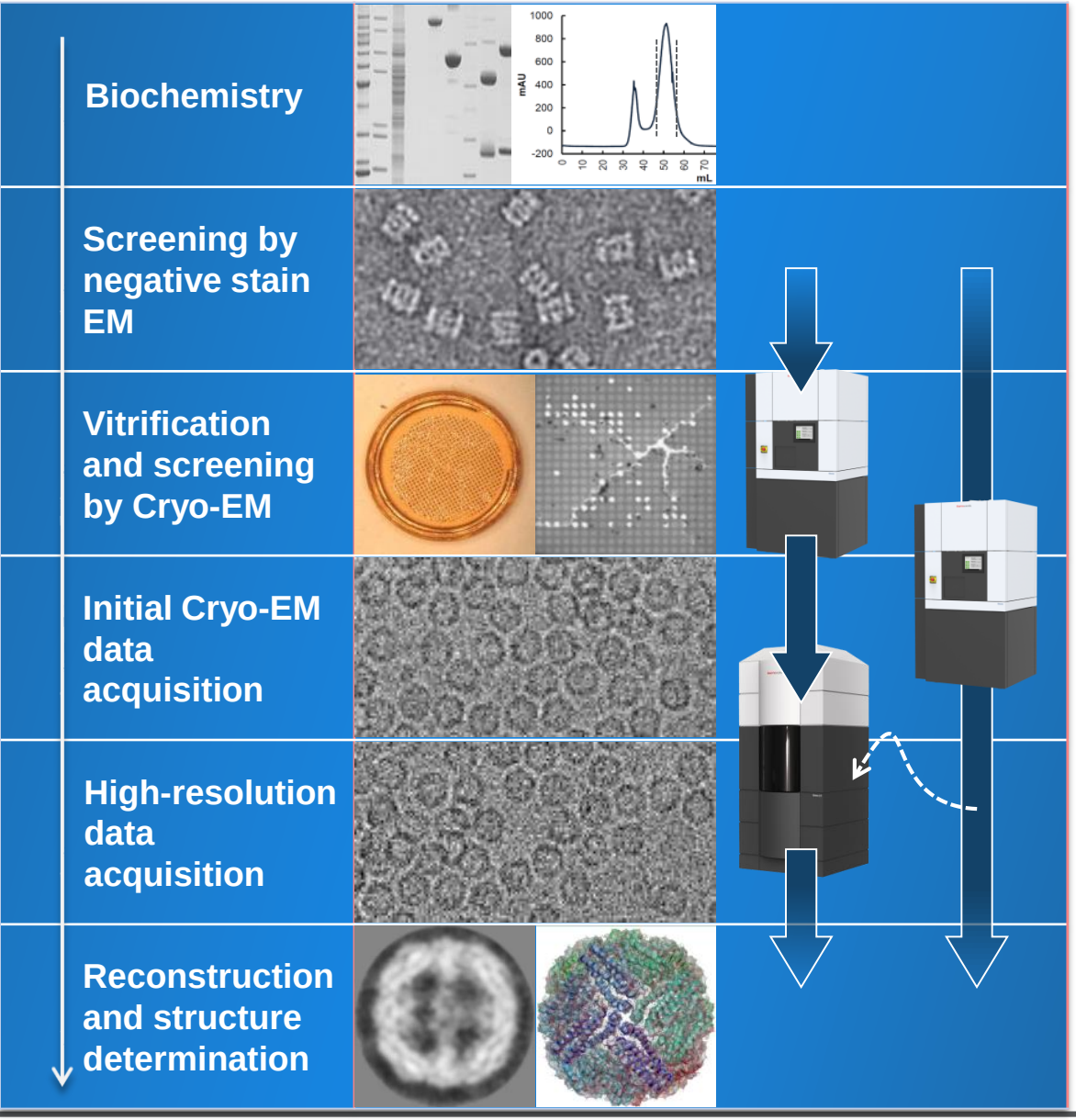
Single-click acquisition including autofocus outside the target field of view



Guided selection of grid squares

ThermoFisher SCIENTIFIC

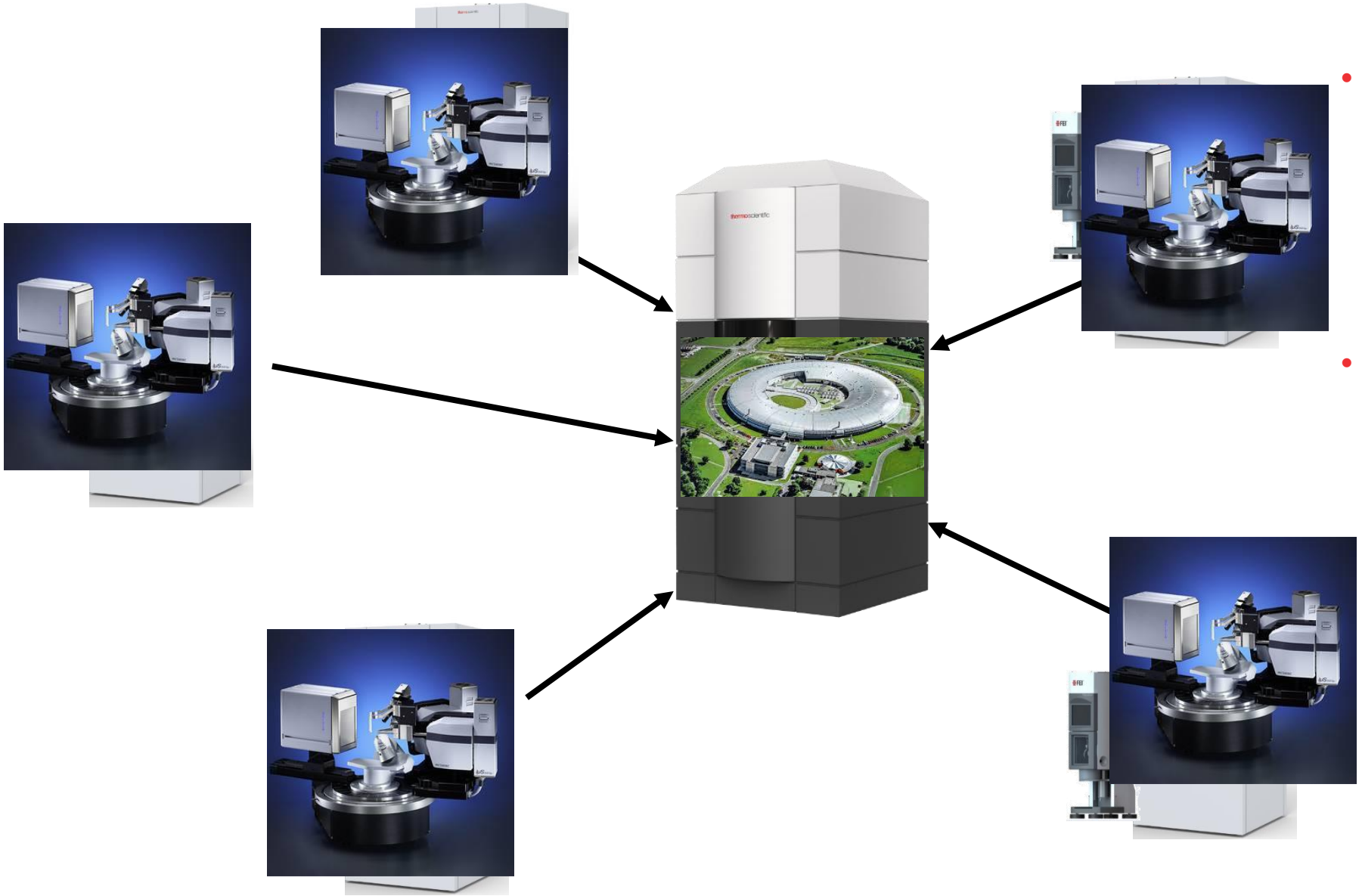
Connectivity Between Glacios and Krios/Arctica Systems for Improved Workflow Setup



- Contamination- and risk-free transfer
  - Using Autoloader Capsule
  - No manipulation of small parts (grids)
  - No exposure to environment
- Full compatibility:
  - Capsules – Autoloaders – microscopes

- Prescreening samples in Glacios system → high-resolution data acquisition in Krios system
- Glacios-based laboratories → pass exceptional samples to a Krios system for additional analysis

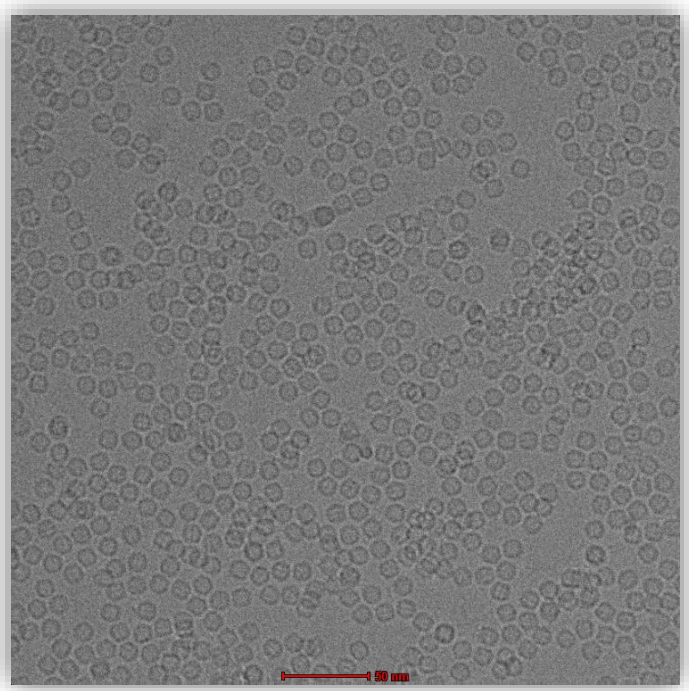
Cryo-EM adopt the “Synchrotron” model

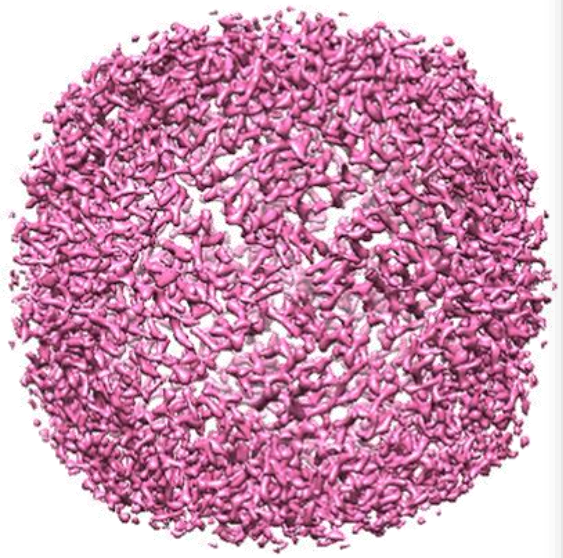


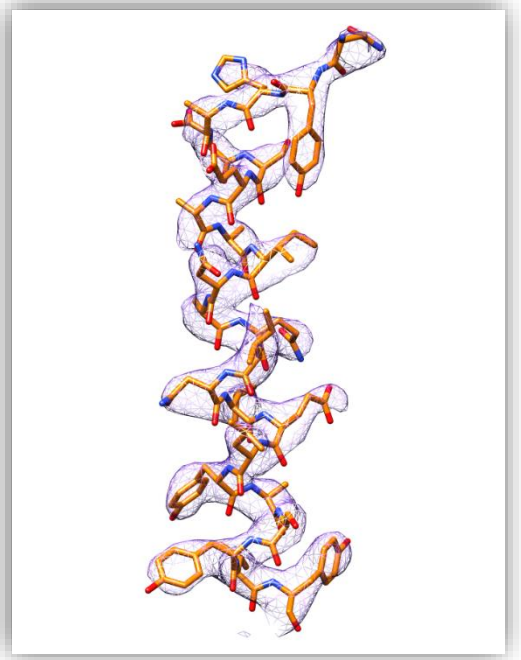
- Glacios for (cryo)sample quality evaluation and optimization
  (also room temp negative screening possible)
- Glacios for 2D-classes and initial model 3D model
  (required for users to get access to centralized facilities’ Krios)

Glacios for high-resolution 3D models (if no ultra-high resolution is required)

Performance: Apoferritin reconstruction at 3.3 Å resolution (Glacios/Falcon 3EC)







Timeline (days)

0

Automated data collection

~1

Data processing

~2

Apoferritin Reconstruction

@ 3.3 Å

Glacios / Falcon 3EC

Personalized Support via Dedicated and Expanding Teams

Customer Success Management

Site Survey Portfolio

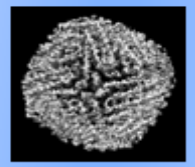
CryoEM Schools

4 wk cryoEM training EU, NA, APR
Supported by on-line training

IT Support

CryoEM Accelerate Portfolio

Workflow Validation



Show complete workflow on relevant biological sample to 4Å

On-Site Applications Support

World-leading cryoEM expertise on-site

Remote Applications Support

World-leading cryoEM expertise on call

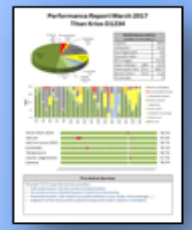
Workflow App

Best known CryoEM methods packaged in iPad app

Remote System Monitoring

- Daily check of system behavior
- Pro-active service deployment

Quarterly Reviews



- Training
- System performance
- Achieving results with cryoEM

On-site

- Assistance and training for all workflow components
- Support with the Workflow Assistant App

Remote

- General workflow assistance
- Support with the Workflow Assistant App
- Guided user assistance via RAPID system
- Offline support via email

CryoEM Single Particle Analysis: Summary

- Proven technology, best performance
  - → >90% of high resolution structures is based on Thermo Fisher microscope data
  - → Nearly 1020 structures deposited based on FEI microscope data (EMDB 2017)
  - → Nearly 294 high impact papers based on Titan Krios data (2017)
- Workflow connectivity
  - → 200+ installed base (Titan Krios and Talos Arctica)
  - → Entry level to high end, unified user experience (EPU) – switch from tool to tool easily
  - → Robust connectivity through AutoLoader sample handling (AutoGrids)
- Outcome based support structure
  - → 165+ cryoEM field service and application specialists
  - → Time-to-result after install – apps support, EM schools
- Multiple ways to start with CryoEM
  - → Full suite- full control on your process and timing
  - → Through CryoEM service facilities- waiting list

Micro Electron Diffraction (MED)

… Crystallography is back with revenge

Three-dimensional electron crystallography of protein microcrystals

Dan Shi†, Brent L Nannenga†, Matthew G Iadanza†, Tamir Gonen*

Janelia Farm Research Campus, Howard Hughes Medical Institute, Ashburn, United States

Abstract We demonstrate that it is feasible to determine high-resolution protein structures by electron crystallography of three-dimensional crystals in an electron cryo-microscope (CryoEM). Lysozyme microcrystals were frozen on an electron microscopy grid, and electron diffraction data collected to 1.7 Å resolution. We developed a data collection protocol to collect a full-tilt series in electron diffraction to atomic resolution. A single tilt series contains up to 90 individual diffraction patterns collected from a single crystal with tilt angle increment of 0.1–1° and a total accumulated electron dose less than 10 electrons per angstrom squared. We indexed the data from three crystals and used them for structure determination of lysozyme by molecular replacement followed by crystallographic refinement to 2.9 Å resolution. This proof of principle paves the way for the implementation of a new technique, which we name 'MicroED', that may have wide applicability in structural biology.

DOI: 10.7554/eLife.01345.001

Single Particle Analysis (SPA)

- >100kDa particles
- Protein Complex
- Membrane Protein
- Best resolution 2-4Å

NEW

3D-Crystallography (MED)

- 1-200kDa particles
- Single proteins
- Pharma Molecules
- Best resolution 1-2Å

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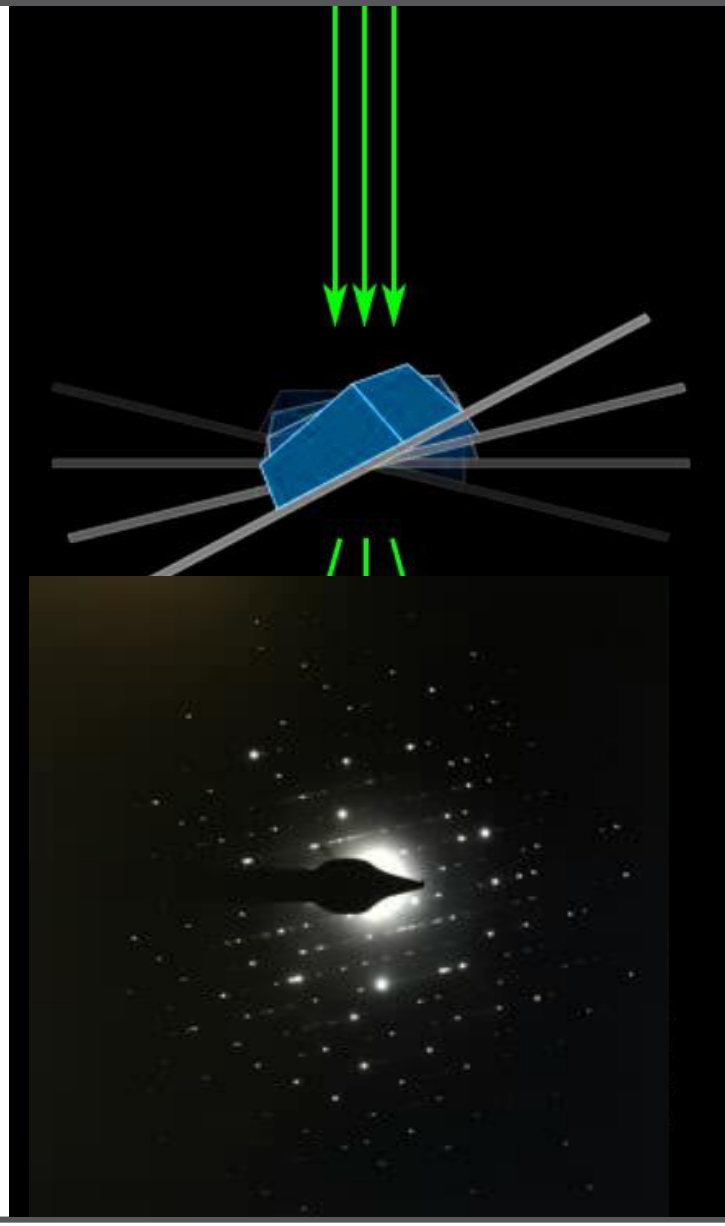
Introduction Micro Electron Diffraction

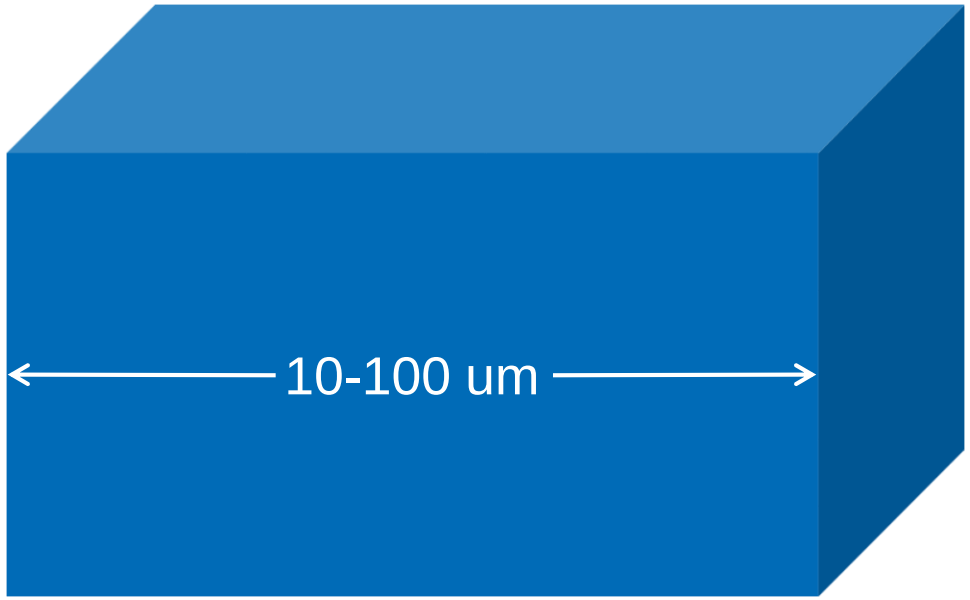
3D electron diffraction in a TEM on nano-crystals of bio-molecules using a continuous stage tilt

The 3D diffraction pattern can be used for high-resolution structure determination



Micro ED and X-ray crystallography

Micro Electron Diffraction can be done on crystal volumes which are a million times smaller than crystals used for X-ray crystallography.



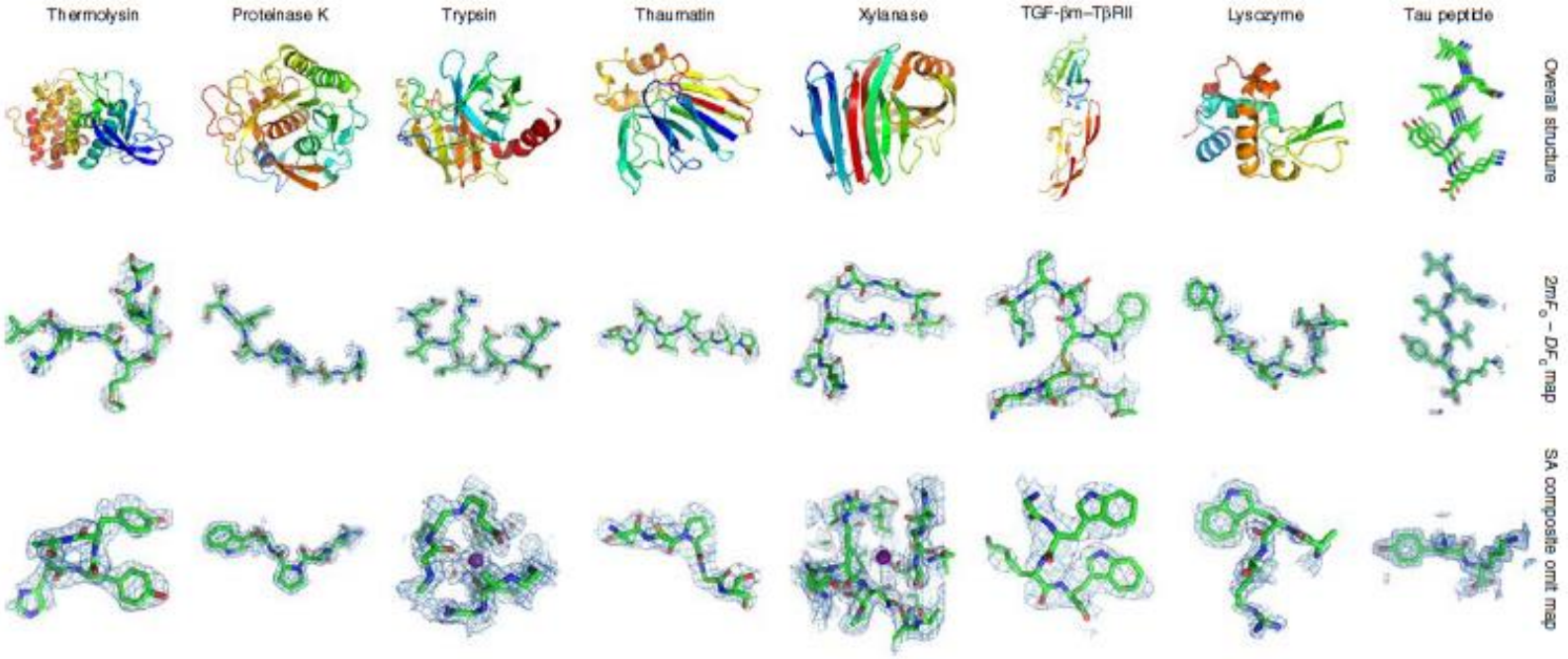
XFEL needs a bucket of nanocrystal to be injected in the system

MED needs between 2-10 nanocrystal

With a single image from a single crystal you can check if your crystal diffract nicely

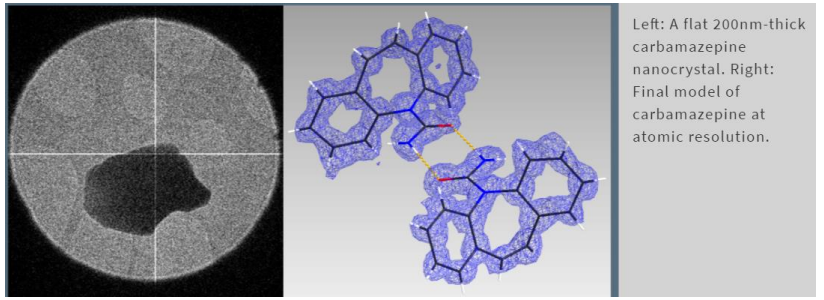
Main applications

Protein structure
1-3 Å resolution range

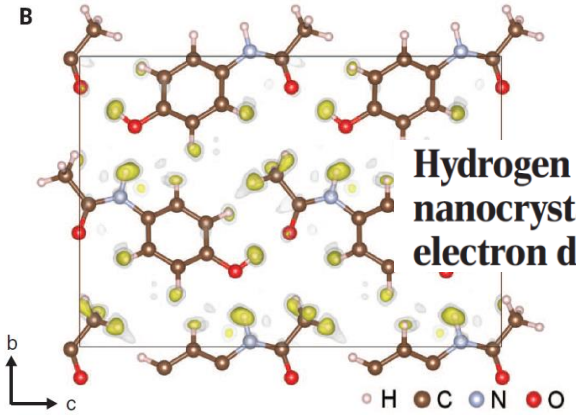


M. Jason de la Cruz *et al*, Atomic-resolution structures from fragmented protein crystals with the cryoEM method MicroED, Nature Methods 14, p.399 (2017)

Pharma molecules
<1 Å resolution range



J.Abrahams et al, Carbamazepine
https://c-cina.unibas.ch/research/ediff/roted/



Hydrogen positions in single nanocrystals revealed by electron diffraction

Palatinus *et al.*, *Science* **355**, 166-169 (2017) 13 January 2017

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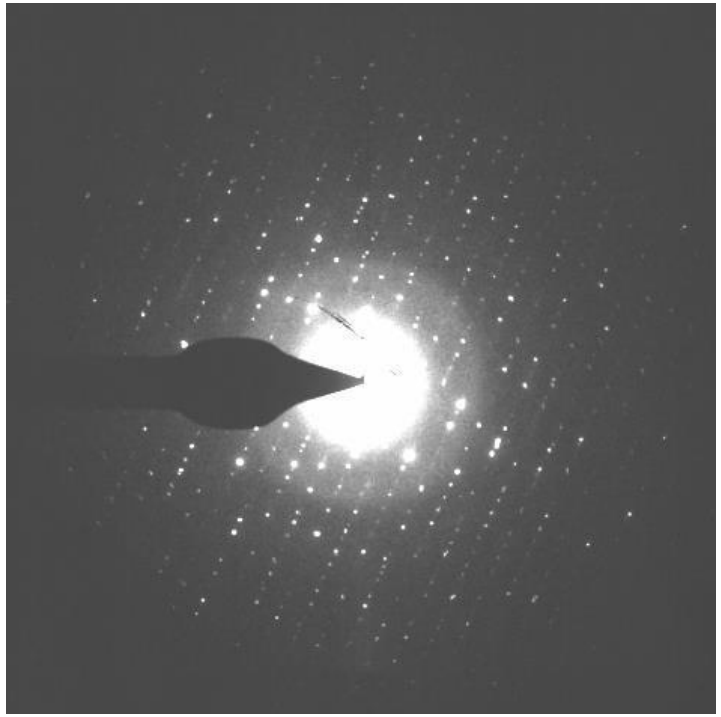
MED and Thermo Fisher

- Thermo Fisher scientific is collaborating with Tamir Gonen (UCLA) on Micro Electron Diffraction to further pioneer this technique
- Testing work will be conducted on Talos Arctica
- System has an accurate single-tilt stage and is equipped with a modified Ceta camera (CMOS, scintillator based detector)



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Join the resolution revolution!

Max Maletta
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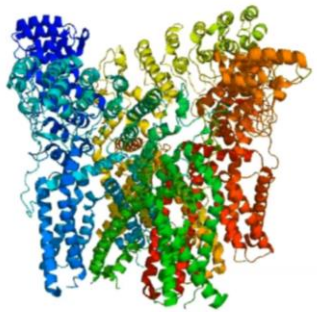
Cryo Tomography: Bridge protein structure with cellular function

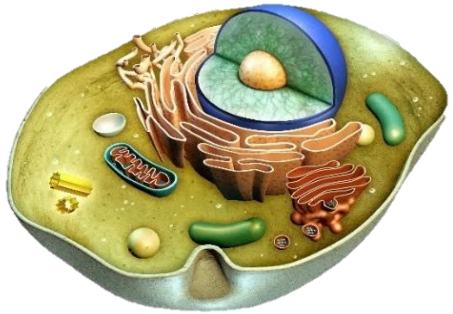
Protein coding Gene

Structure of folded Protein

Function of Protein in cells

Cellular organisation in tissue





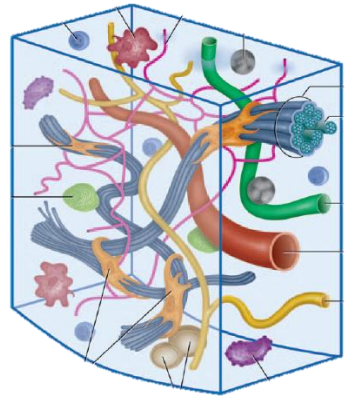


Illustration: Charis Tsevis (flickr)

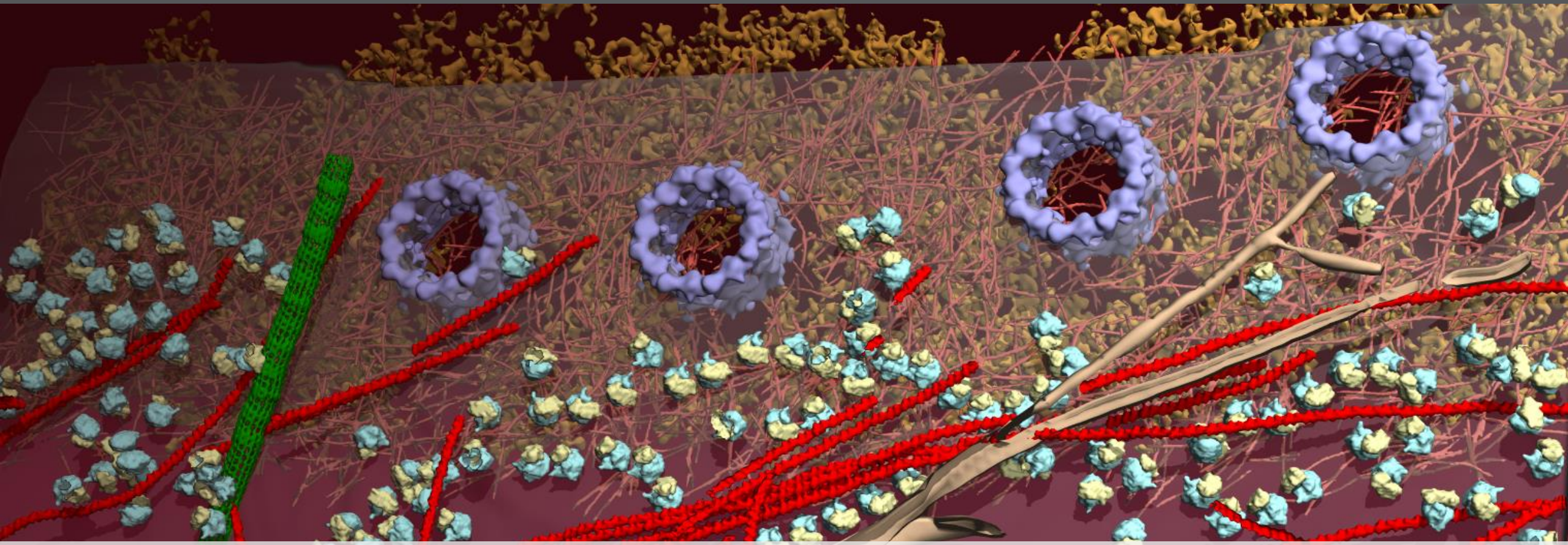
NextGen Sequencing

SPA

Cryo Tomography

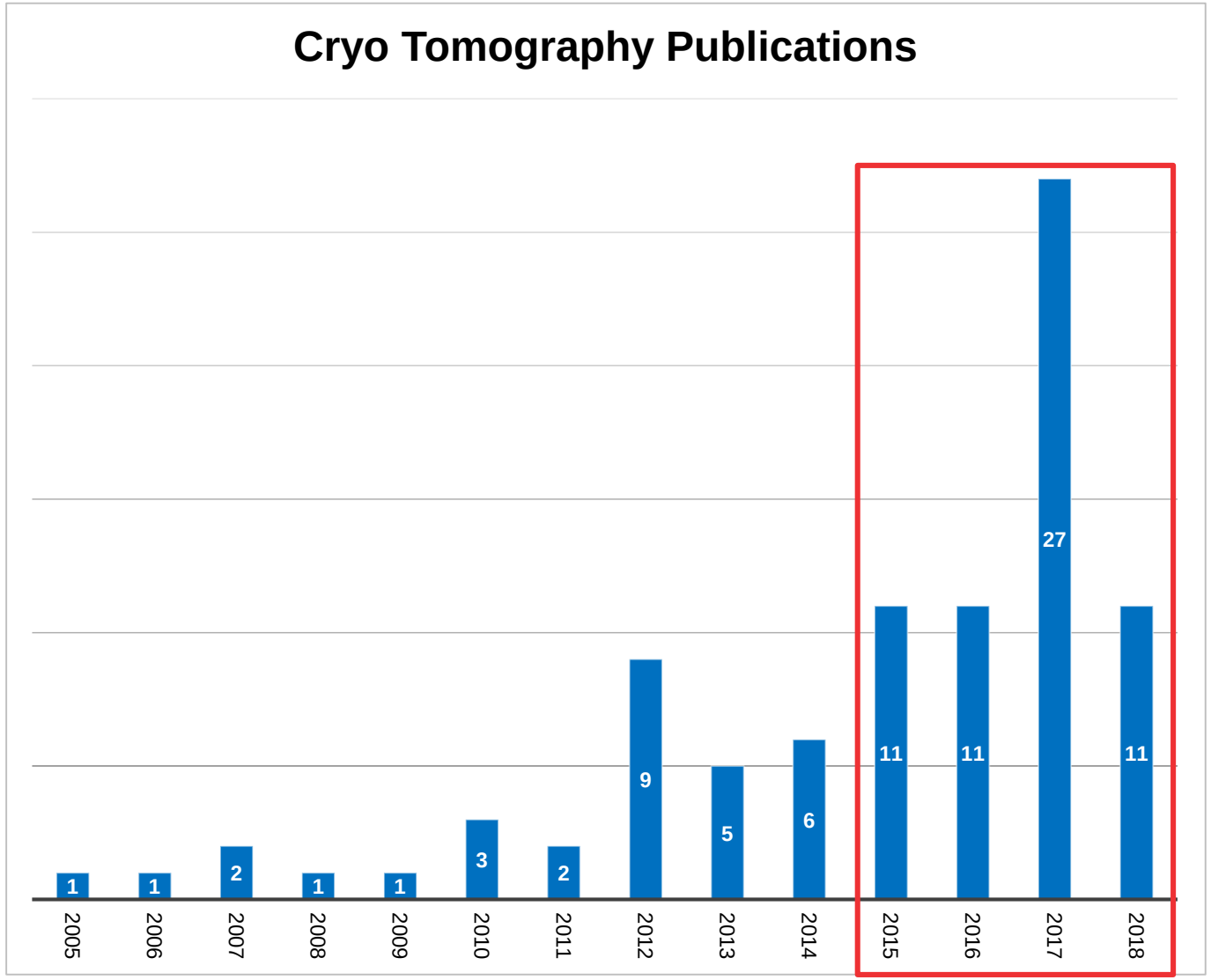
Large Volume Analysis

Cryo Tomography …



… is the only technique that is non-disruptive to cellular components
… visualizes cellular structures at unprecedented resolution in 3D
… resolves structures of proteins inside the cell via sub-tomogram averaging
… allows us to understand entire processes inside cells

of publications are rising exponentially



- 60 Publications 2015-2018:
- 23% (14) in widely read journals like Cell, Science, Nature, PNAS.
- 86% Thermo Fisher Scientific Instruments

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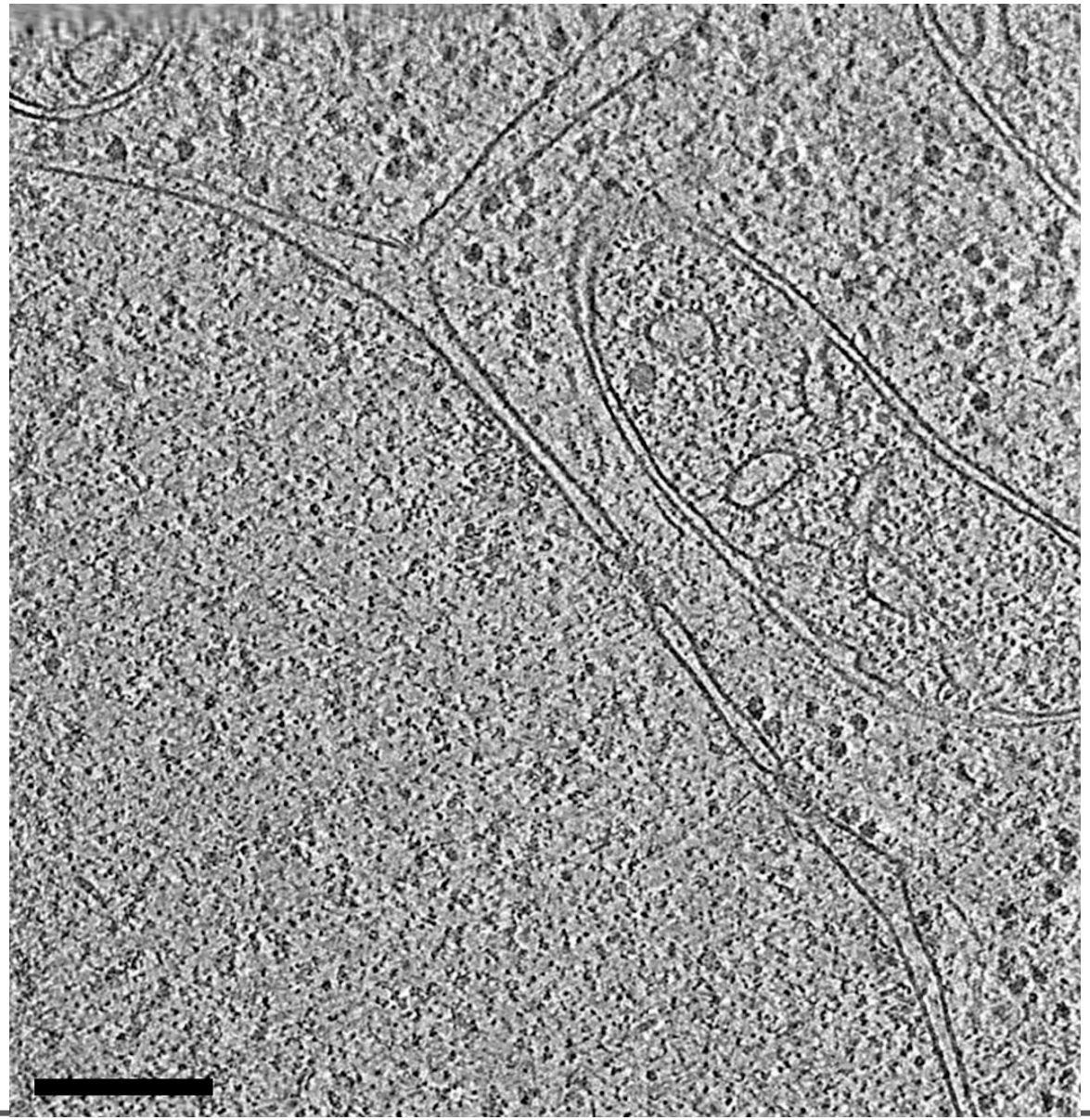
Proteasomes cluster around the NPC



The nuclear pore complex (NPC) selectively gates the transport of macromolecules between the nucleus and cytoplasm compartments

Research objective

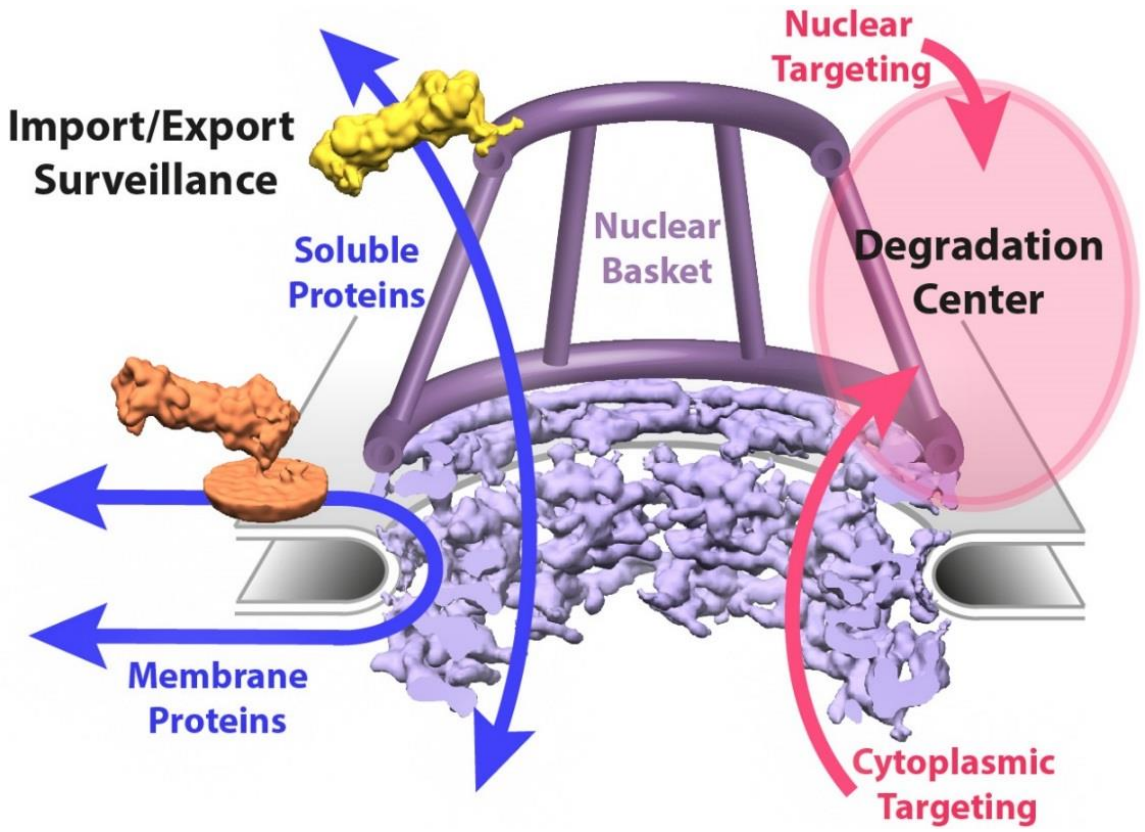
Understand if surveillance mechanisms exist to reinforce the selective gate function

Unique

- *in situ* cryo-electron tomography enables to image the native cellular environment
- to perform an extensive molecular-resolution structural survey of cytoplasmic and nuclear proteasomes

PNAS Dec. 26 2017, Vol. 114, No.52 DOI: 10.1073/pnas.1716305114

Proteasomes cluster around the NPC

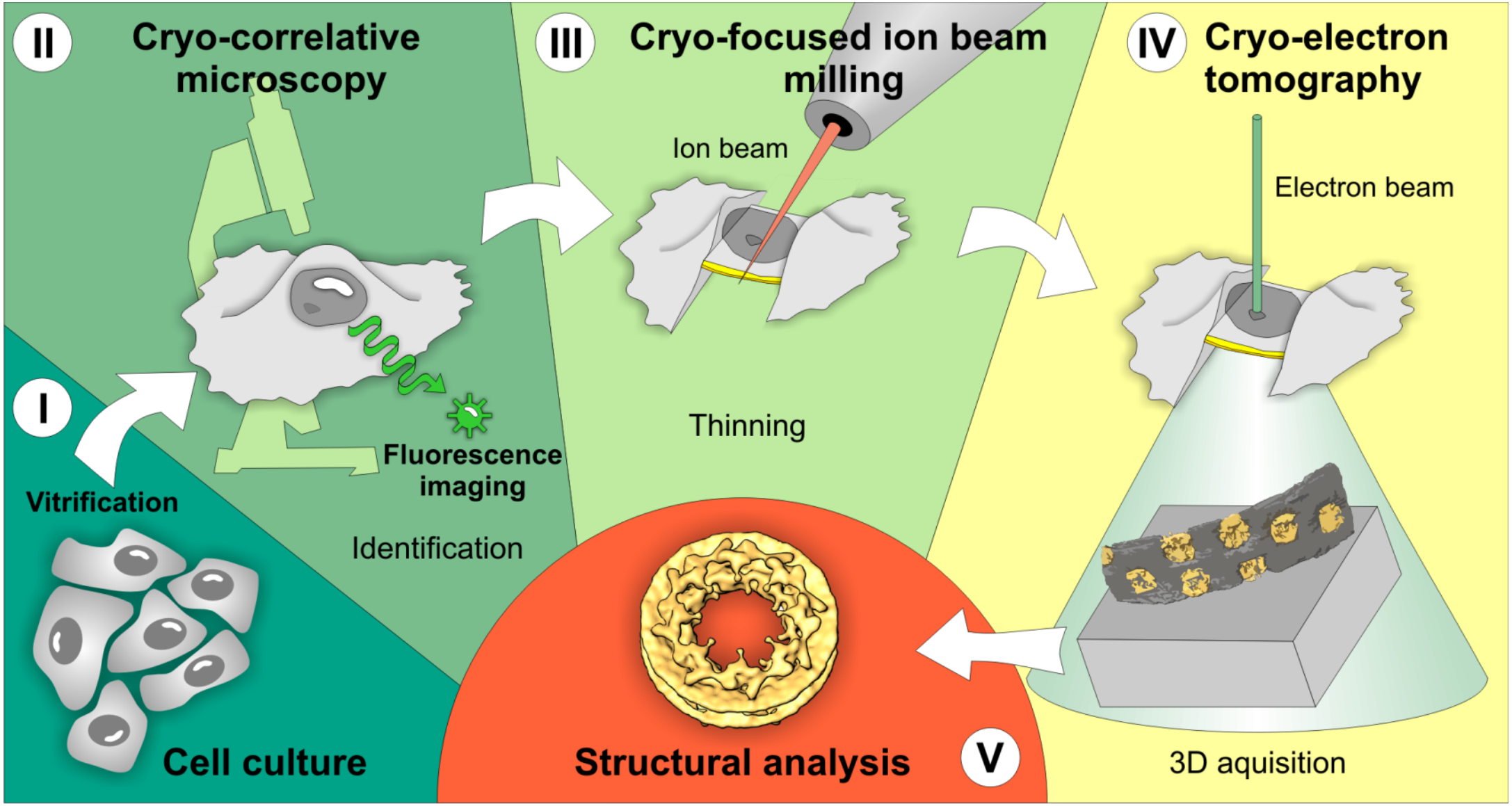


Results:

- the assembly states and functional states of proteasomes in each compartment were similar
- structural analysis revealed mechanistic details of the two NPC tethering interactions (nuclear basket ad nuclear membrane)
- proteasomes binds to NPC establishing a cellular hub for protein degradation at the gateway between the nucleus and cytoplasm

PNAS Dec. 26 2017, Vol. 114, No.52 DOI: 10.1073/pnas.1716305114

Cryo Tomography Workflow: Enabling In Situ Structural Biology



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Cryo-Tomography: Workflow challenges

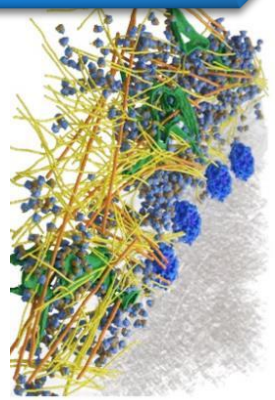
Sample preparation → Cryo-Fluorescence → Cryo-FIB Milling → Cryo-TEM Tomography → Visualization & Analysis

Vitrobot

Cryo-LM

Aquilos

Krios

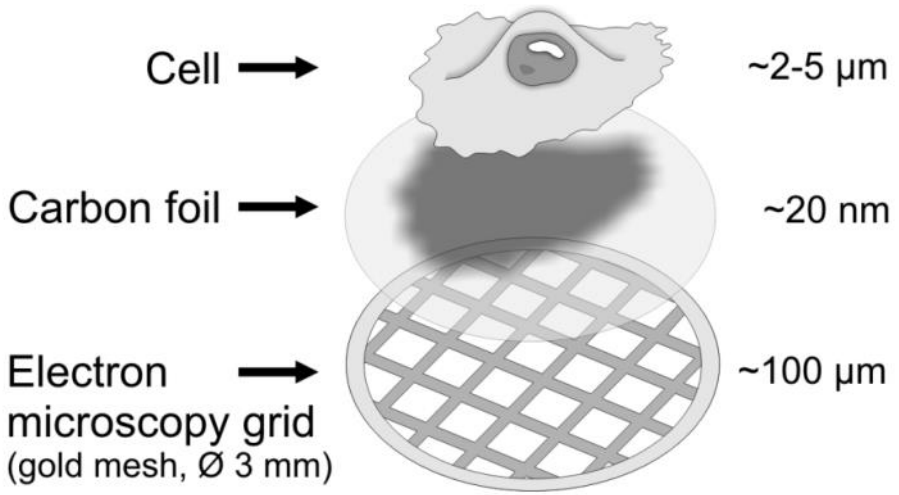


Amira

- Find area of interest
- Easy connectivity between instruments
- Lamella preparation fast and with minimum contamination

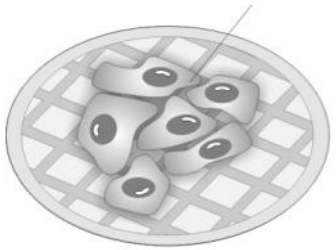
How to overcome the challenges?

Sample Preparation and connectivity



Easy connectivity between instruments

Vitrified cells on grid



Dedicated Cryo-FIB autogrid

EM grid carrier/support

- Provides stability to fragile grids
- Allows robotic handling (FEI Autoloader) and automated TEM loading

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Aquilos Cryo-FIB: A Complete Cryo-Sample Preparation Platform

Lamella preparation

GUIDED USER SOFTWARE

LN2 DEWAR

AQUILOS CRYO-FIB

SAMPLE LOADING STATION

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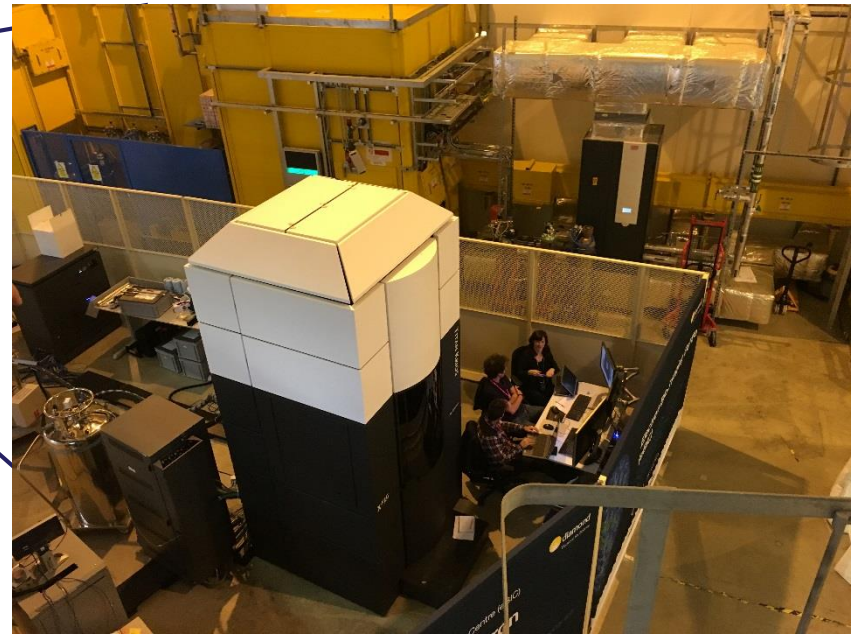
Lamella preparation

Unique Value Propositions-Summary

- Fast adoption of Cryo-Tomo
  - → 30 publication in 2017 (total 60 from 2015)
  - → 23% (14) in high impact papers and 83 % based on Thermo Fisher instruments
- Enabling *in Situ* Structural Biology with fully dedicated platform
  - → Dedicated sample prep tool for cryo-tomography workflow
  - → Best compatibility and connectivity within Thermo Scientific's cryo tomography workflow
  - → Minimized artifacts
  - → Full control over thickness
- Workflow connectivity
  - → Robust connectivity to TEM through AutoLoader sample handling (AutoGrids)
  - → MAPS easy correlation and targeting software

UK National Synchtron - EBIC



Diamond Synchrotron Statistics

User statistics

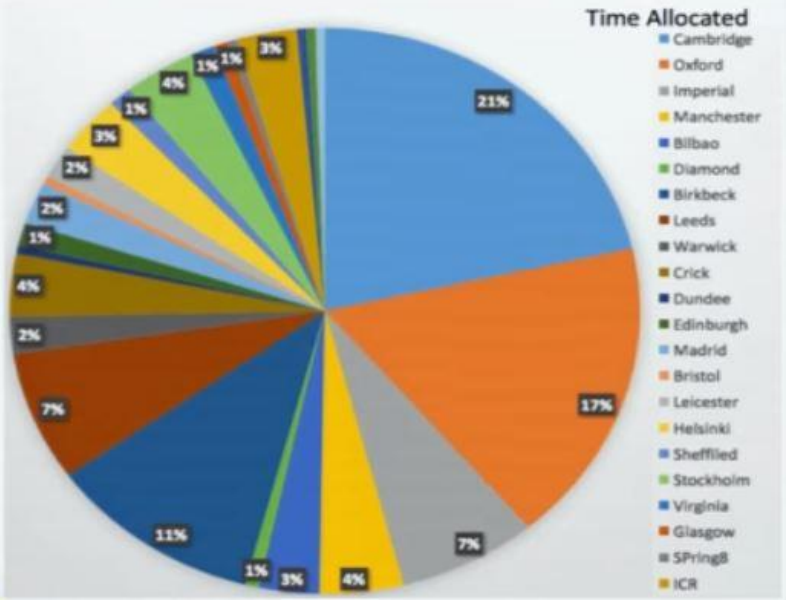
Krios I&II use: 597 days (7/15 – 4/17, 1792 shifts)

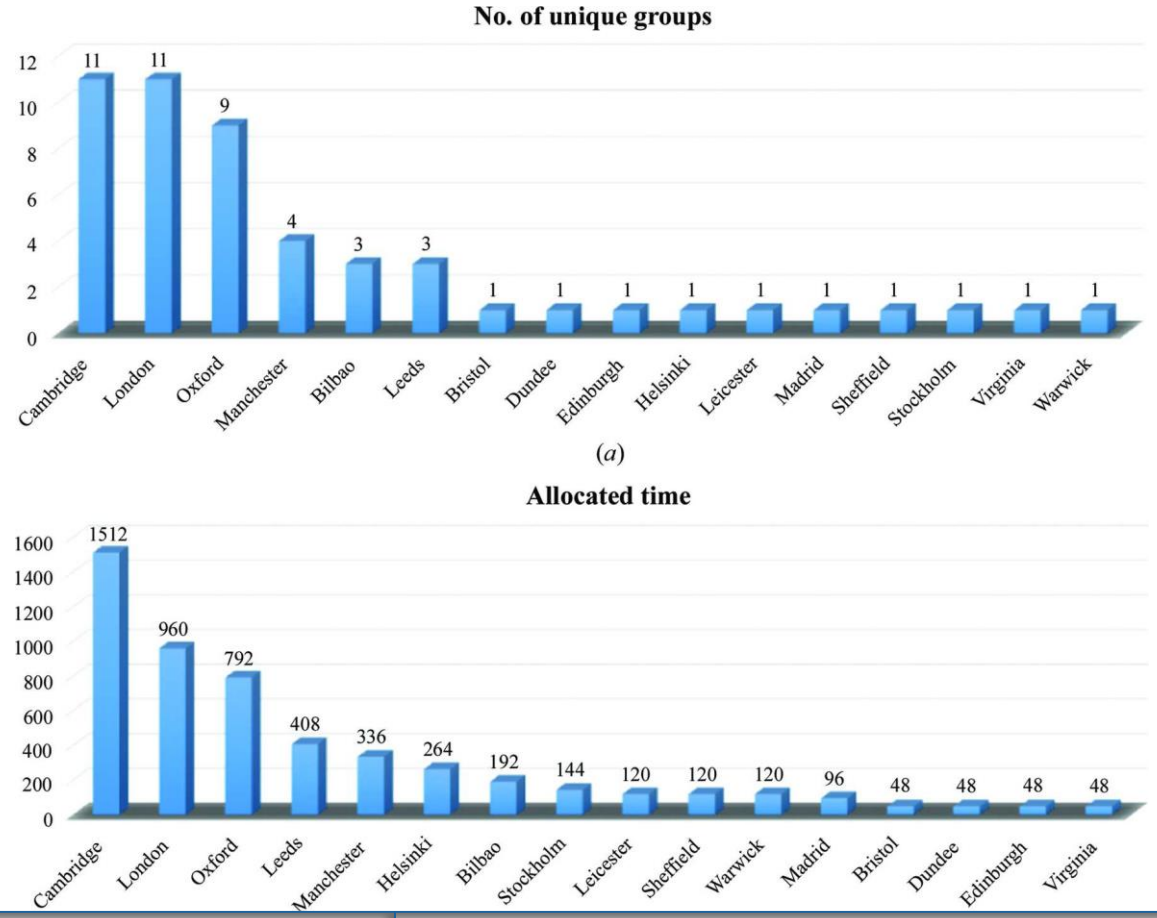
	Peer Reviewed	In House	Commissioning	Industrial	Training
Totals (shifts)	1307.375	281.875	140.75	45	17
Percentages	73.0	15.7	7.9	2.5	0.9

As of 3/2017:

- 149 proposals awarded
- 216 sessions allocated (incl. BAGs)

- UK Users: 86%
- EU Users 10.6%
- WW Users: 3.4%





- University of Oxford, and funded by the Wellcome Trust, the UK Medical
- Research Council (MRC) and the Biotechnology and Biological Sciences
- Research Council (BBSRC)

- Typical imaging time 48hrs
- 95 Separate Visits / 53 Individual Investigators
- 18 Total publications as of 2/2018

- First year of operation generation of 270TB of data via Krios 1
- Estimation will be up to 1 PB of data as Krios 4 comes online

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