

Why Cryo-EM?

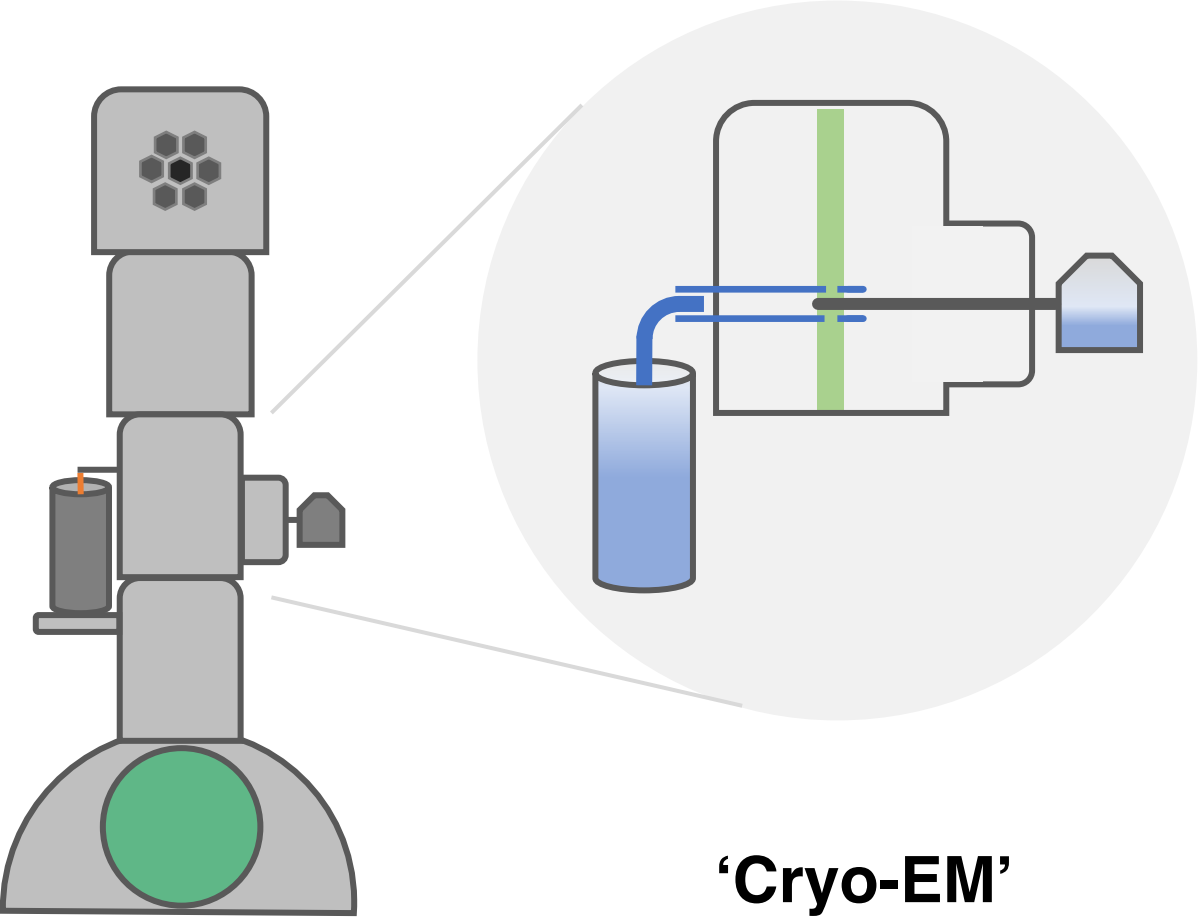
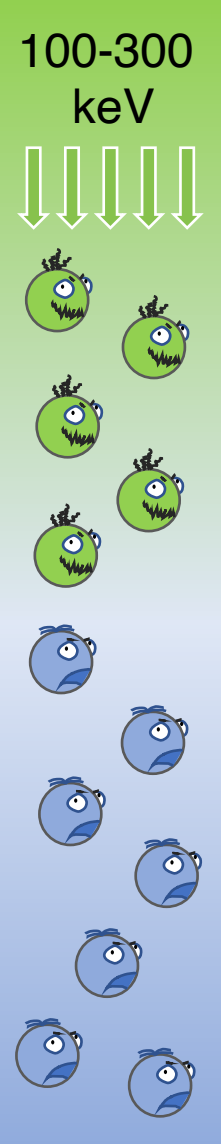
Kyle Morris, Ph.D.

eBIC, Diamond Light Source, UK

Senior Scientist & Training Coordinator

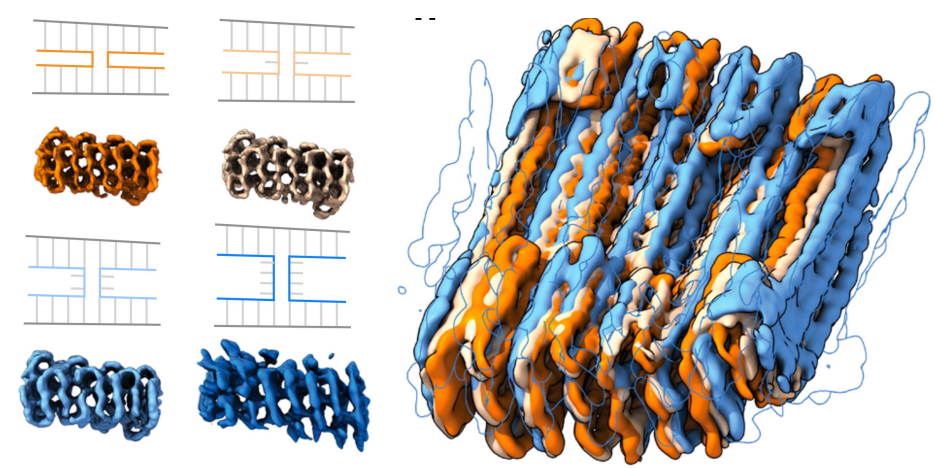
Image credit: Veronica Falconieri, National Cancer Institute

Cryo-Electron Microscopy : Electron Cryo-Microscopy



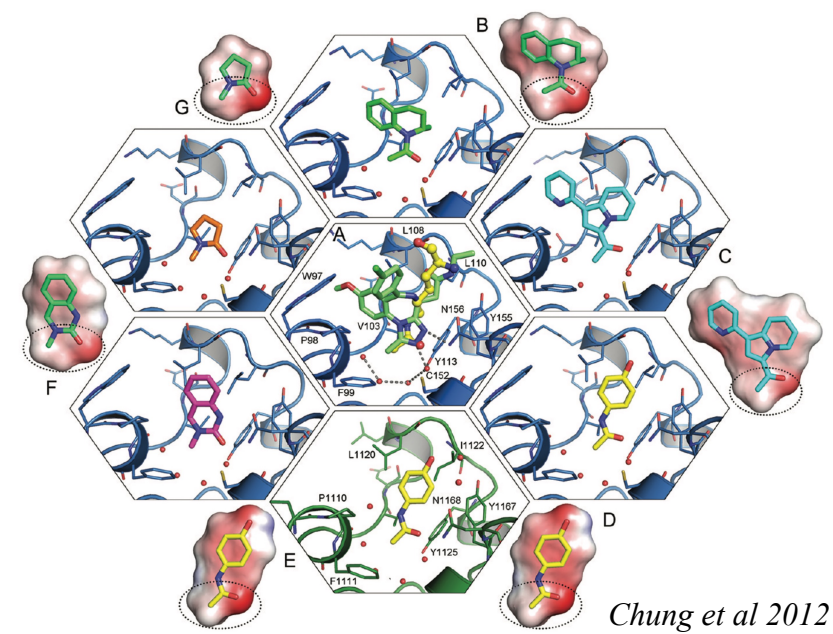
Rational design

"precision design needs precision validation"



Kube et al 2020 Nat Comm

Drug development

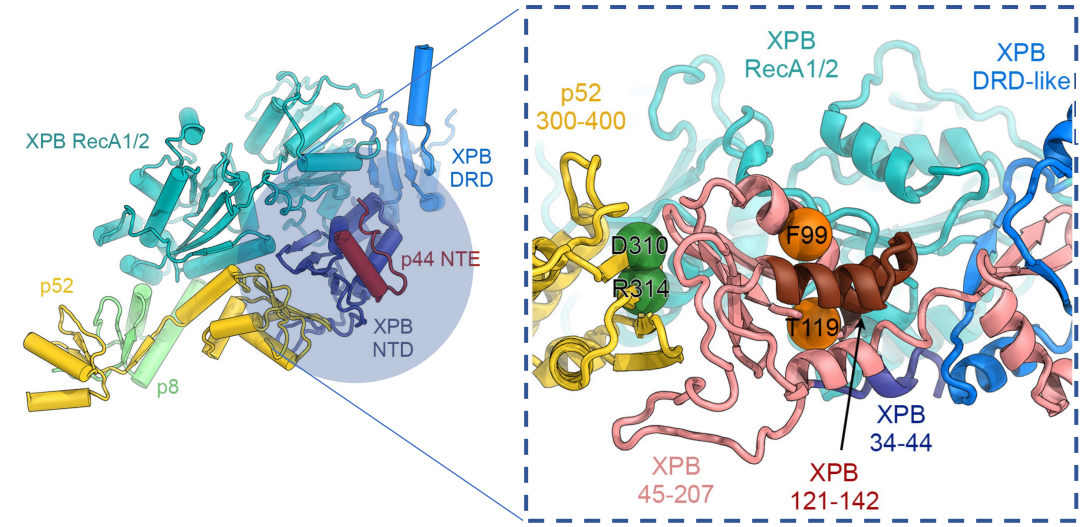


Chung et al 2012 JMC

Protein Structure

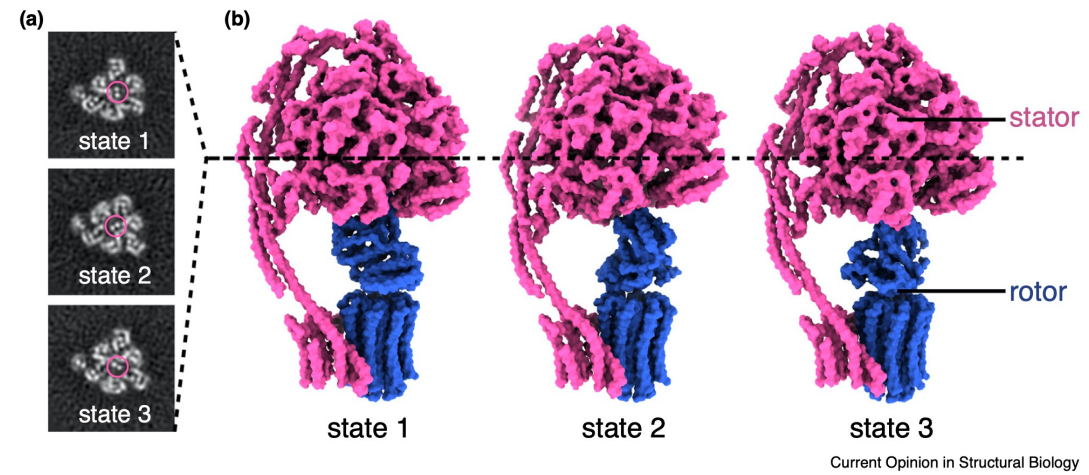


Greber et al 2020 Nat Comm



Mechanistic understanding of disease

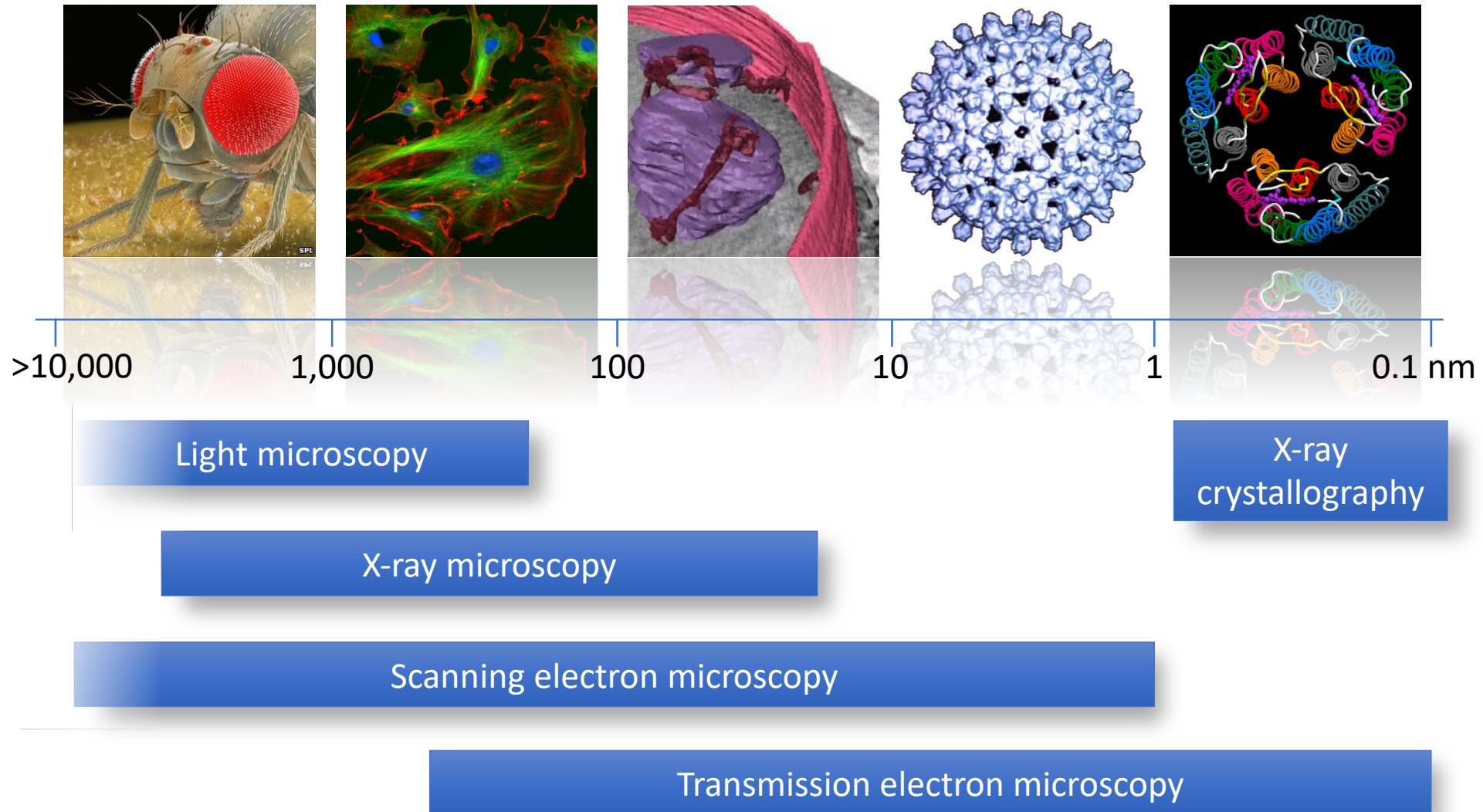
Determination



Current Opinion in Structural Biology

Fundamental understanding of biological machines

Biology exists across multiple length scales



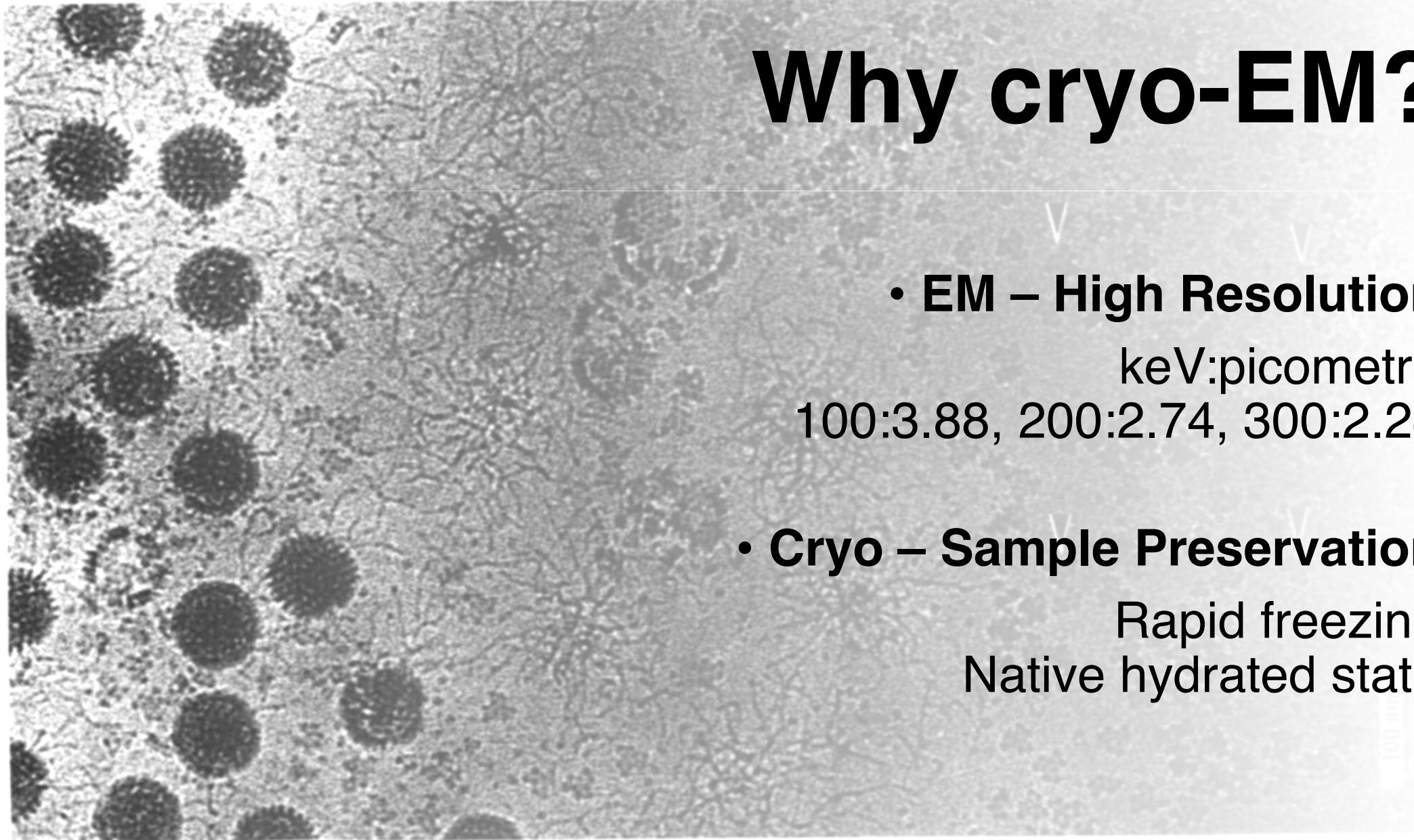
Why cryo-EM?

- **EM – High Resolution**

keV:picometre
100:3.88, 200:2.74, 300:2.24

- **Cryo – Sample Preservation**

Rapid freezing
Native hydrated state

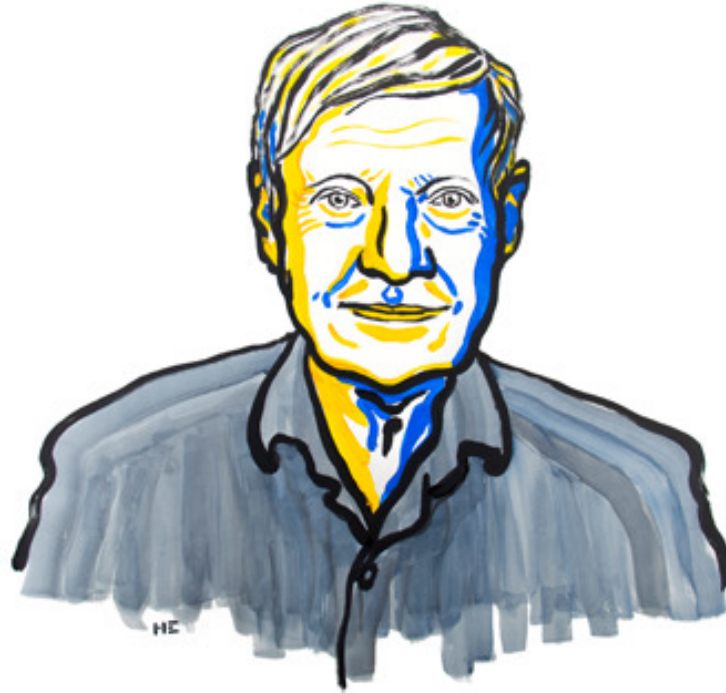


Thin vitrified layer of a solution of partially disrupted adenovirus. Many groups of nine (Go) are visible; some have been marked by an arrow.
(From Dubochet *et al.* 1985.)

The Nobel Prize in Chemistry 2017 was awarded to **Jacques Dubochet, Joachim Frank and Richard Henderson** "*for developing cryo-electron microscopy for the high-resolution structure determination of biomolecules in solution*".



Jacques Dubochet,
Lausanne, Switzerland



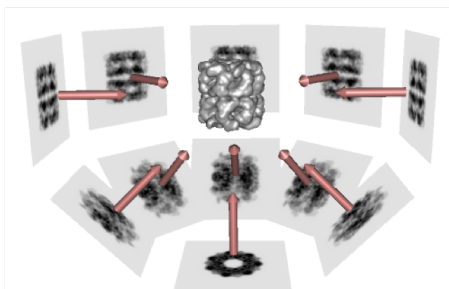
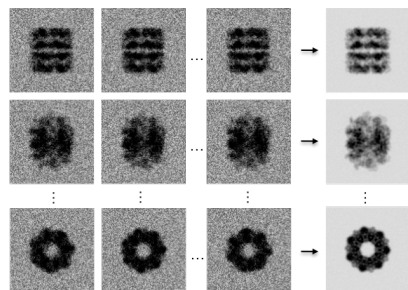
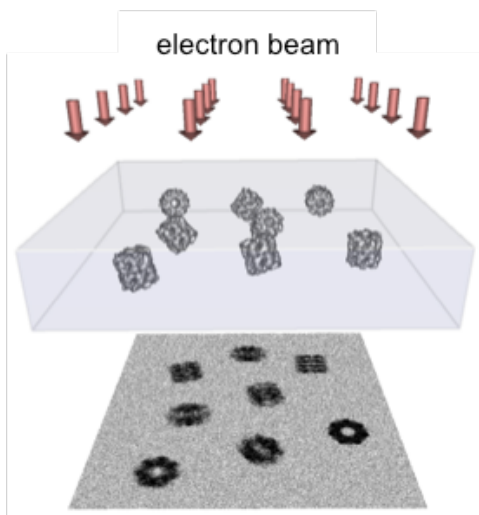
Joachim Frank,
New York, USA



Richard Henderson,
Cambridge, UK

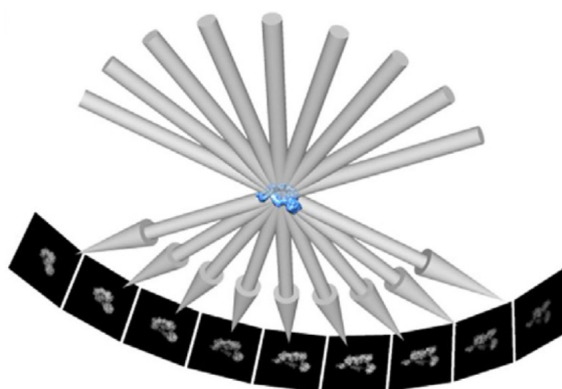
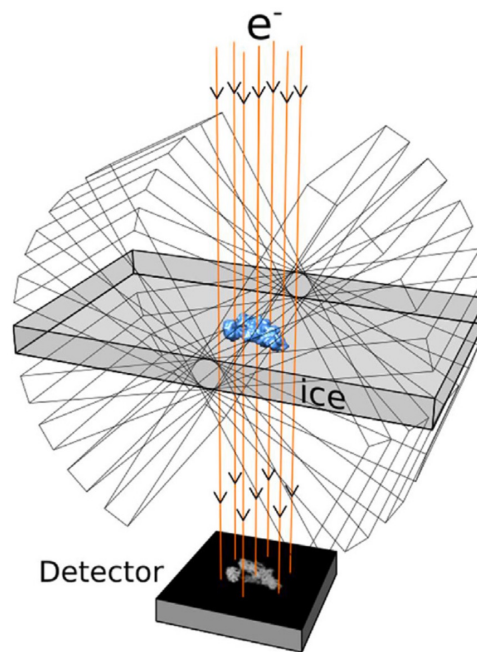
Illustrations: Niklas Elmehed. Copyright: Nobel Media AB 2017

Single Particle Analysis



Greg Pintille

Cryo-electron tomography

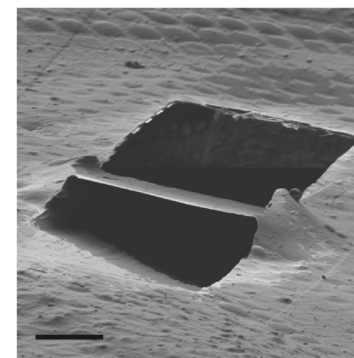
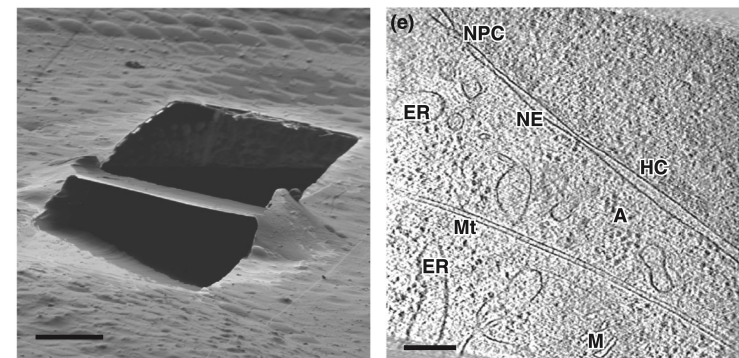


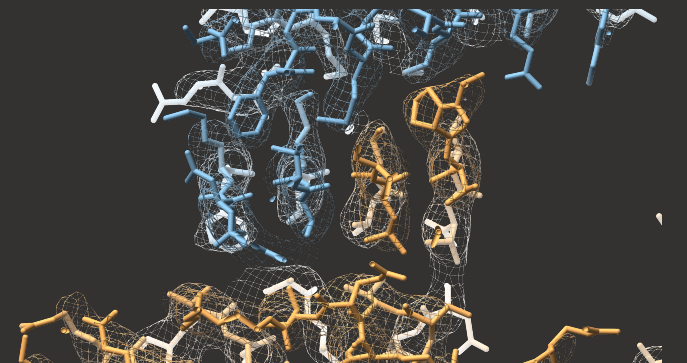
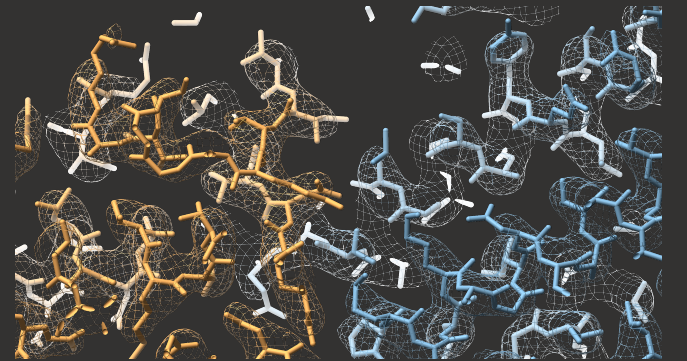
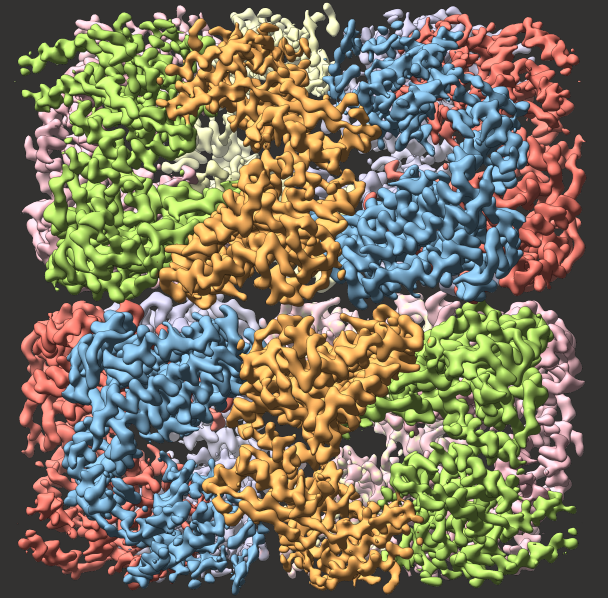
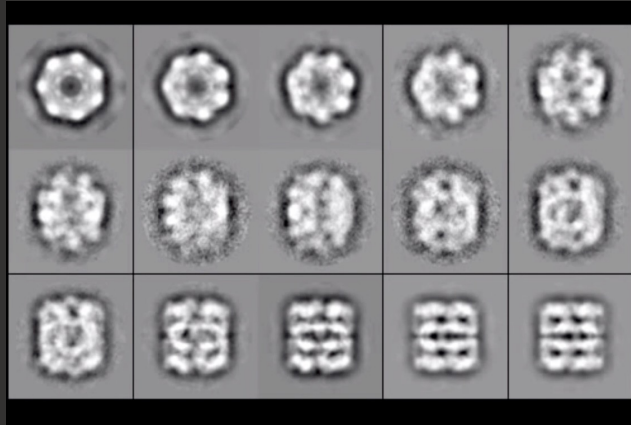
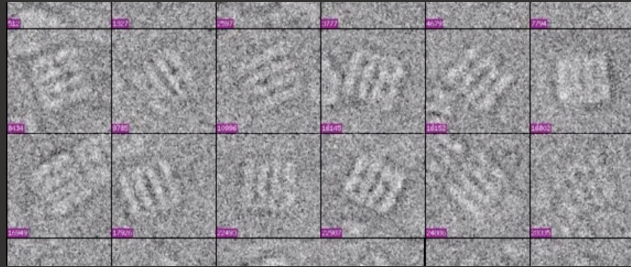
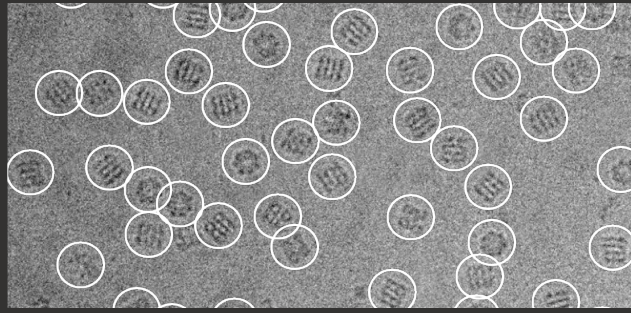
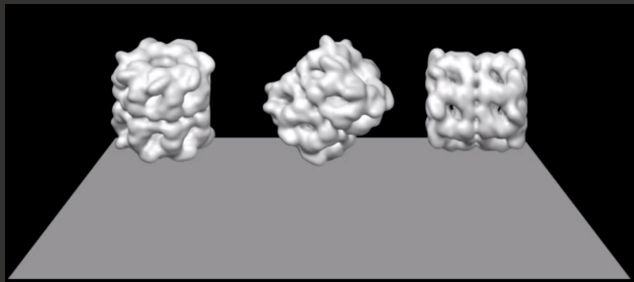
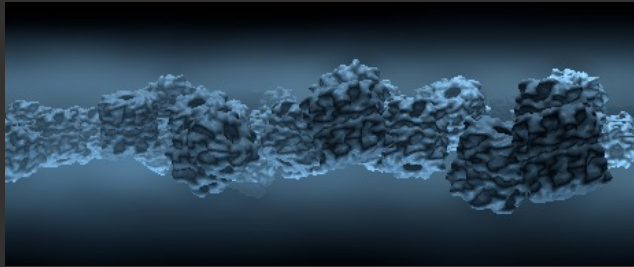
Koning et al 2018

Focussed Ion Beam Scanning Electron Microscopy

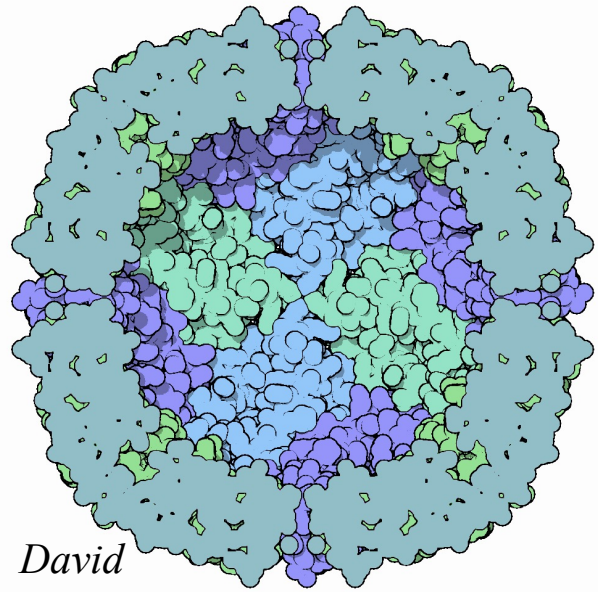
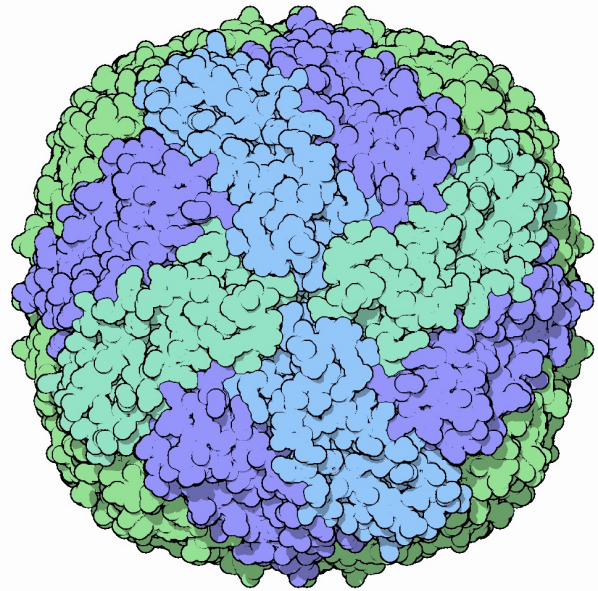


Villa et al 2015



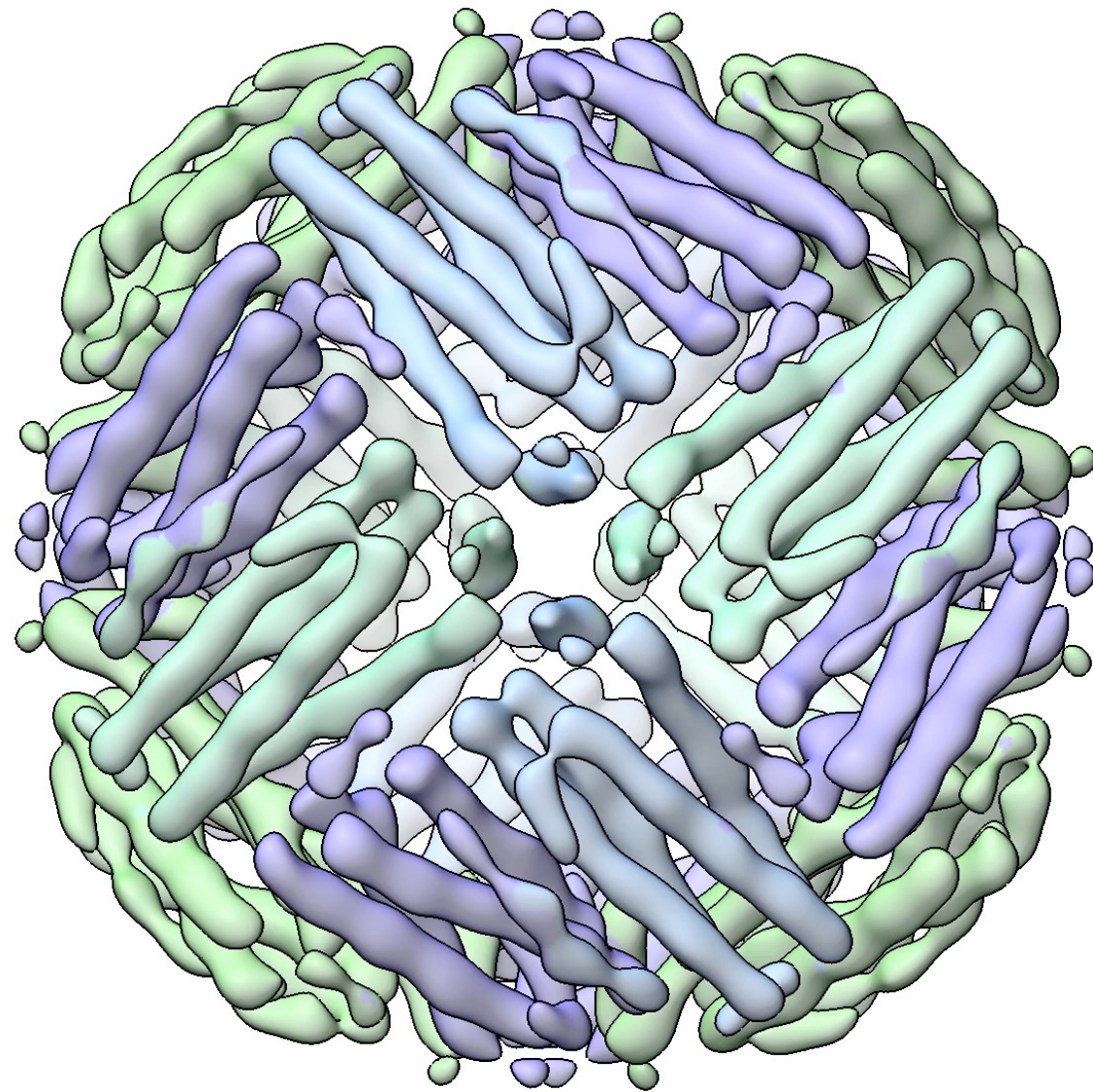


Ferritin (Iron storage protein cage)



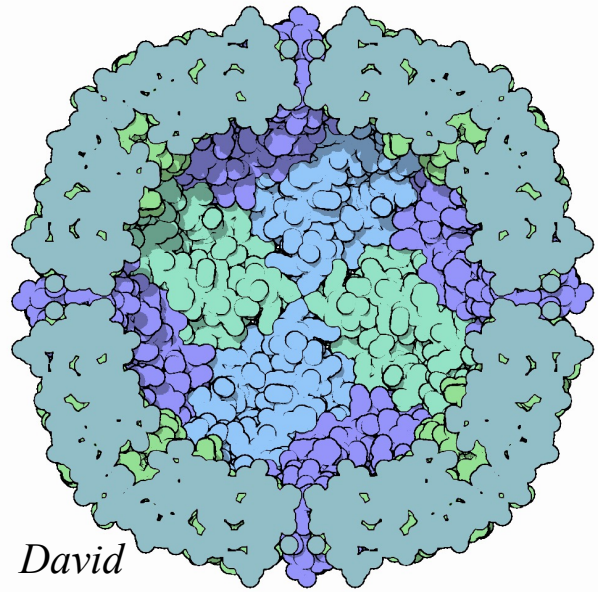
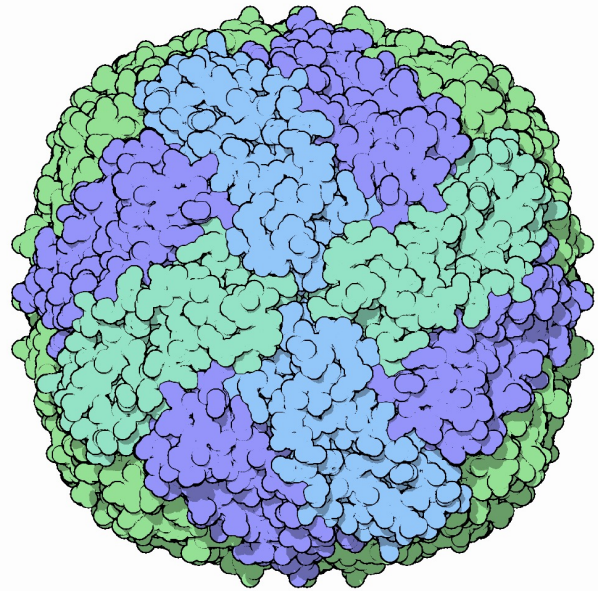
*David
Goodsell*

Apo ferritin structure, ion coordination at 4-fold channel



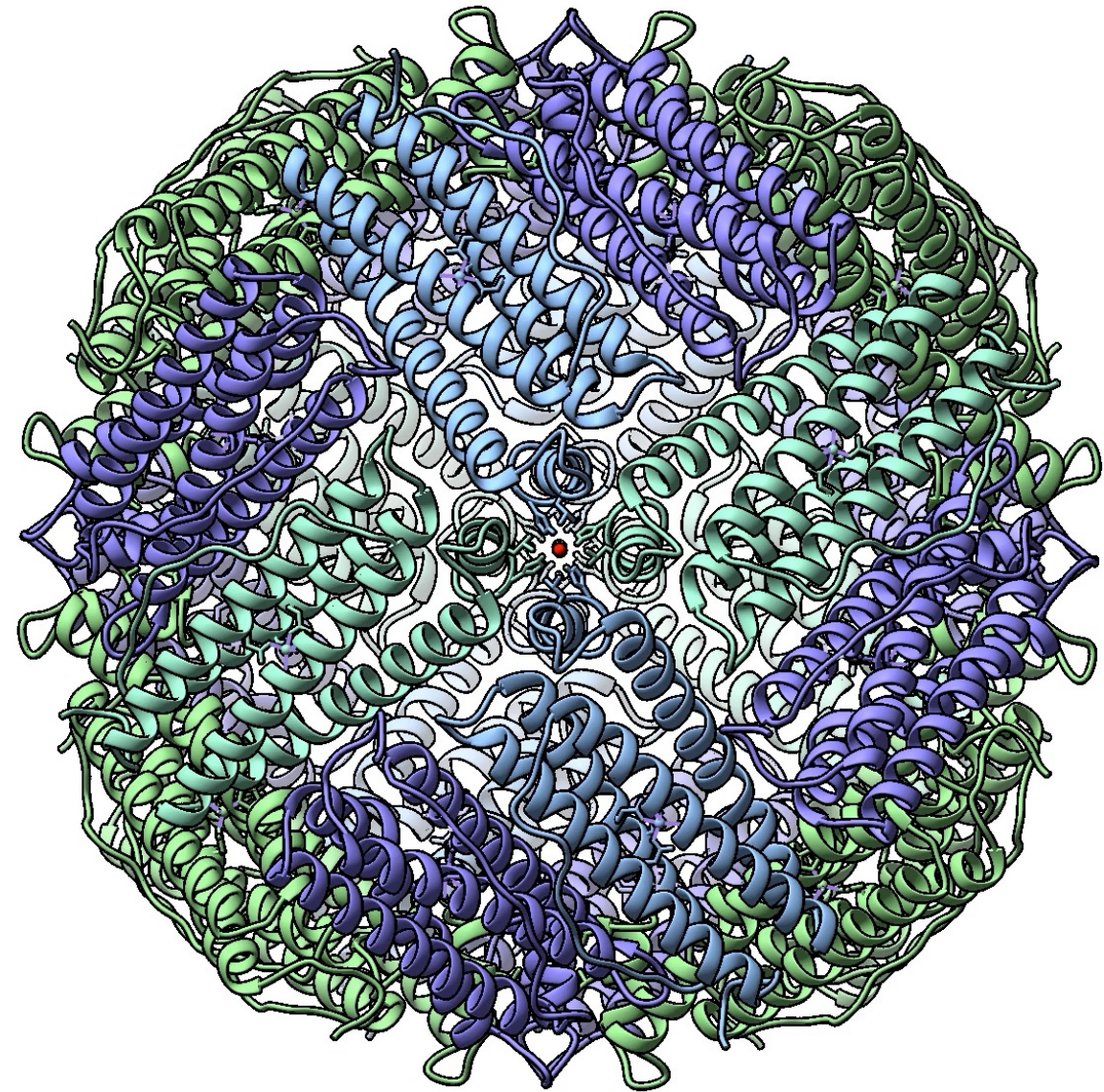
1.22Å resolution from cryo-EM – Nakane et al 2020 Nature

Ferritin (Iron storage protein cage)



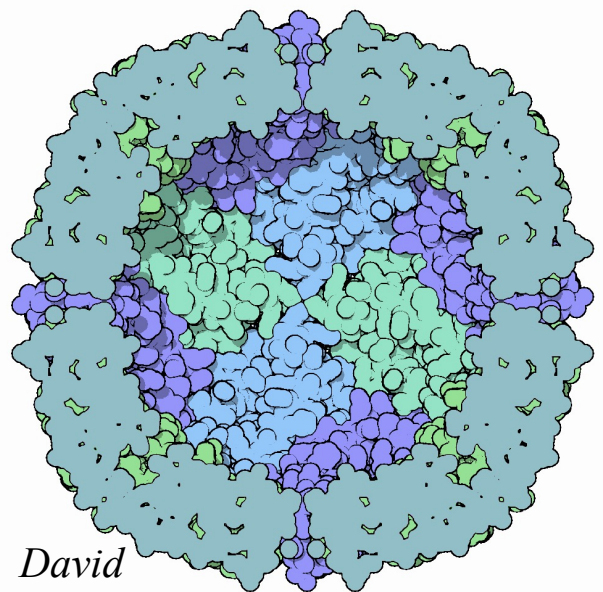
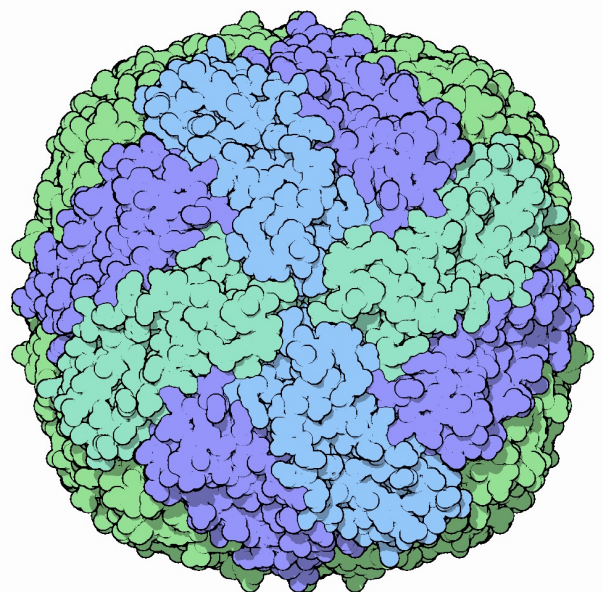
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Goodsell*

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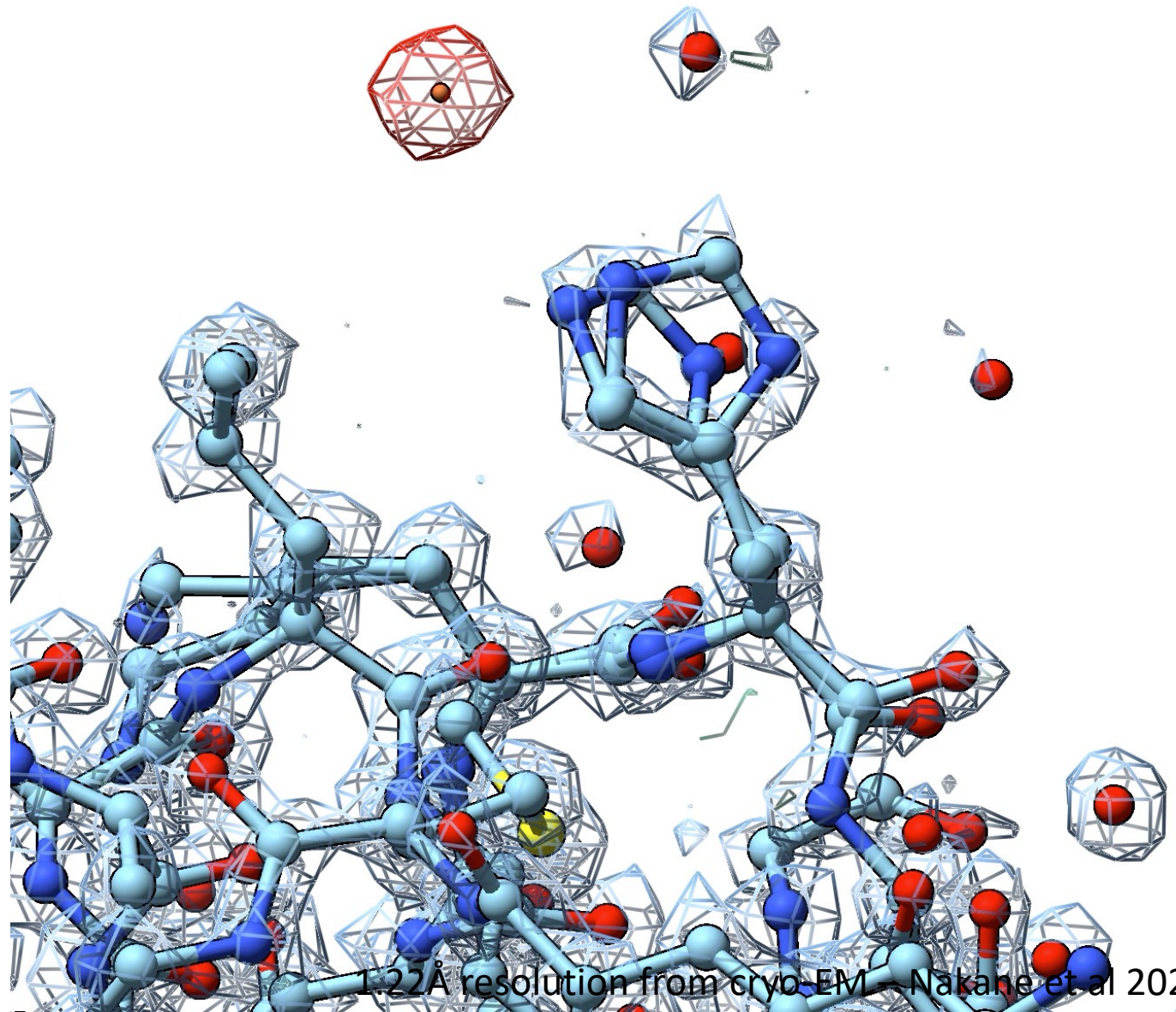
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Ferritin (Iron storage protein cage)



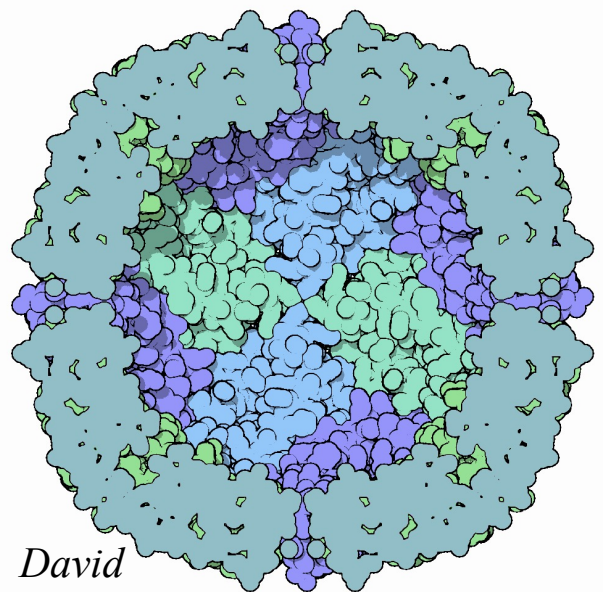
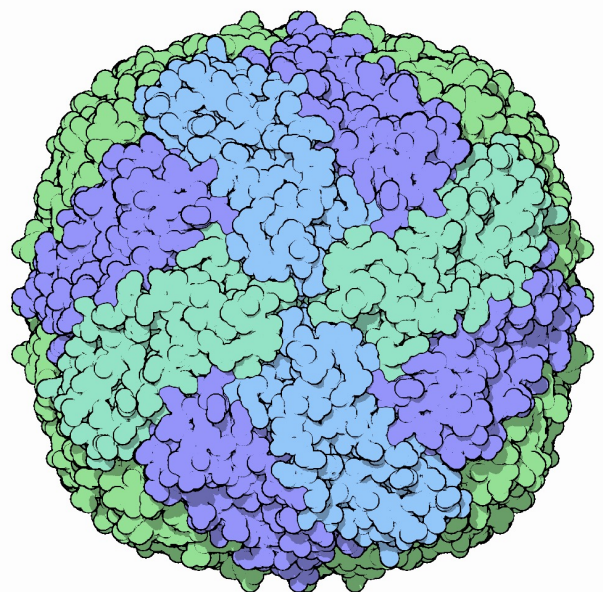
David
Goodsell

Apo ferritin structure, ion coordination at 4-fold channel



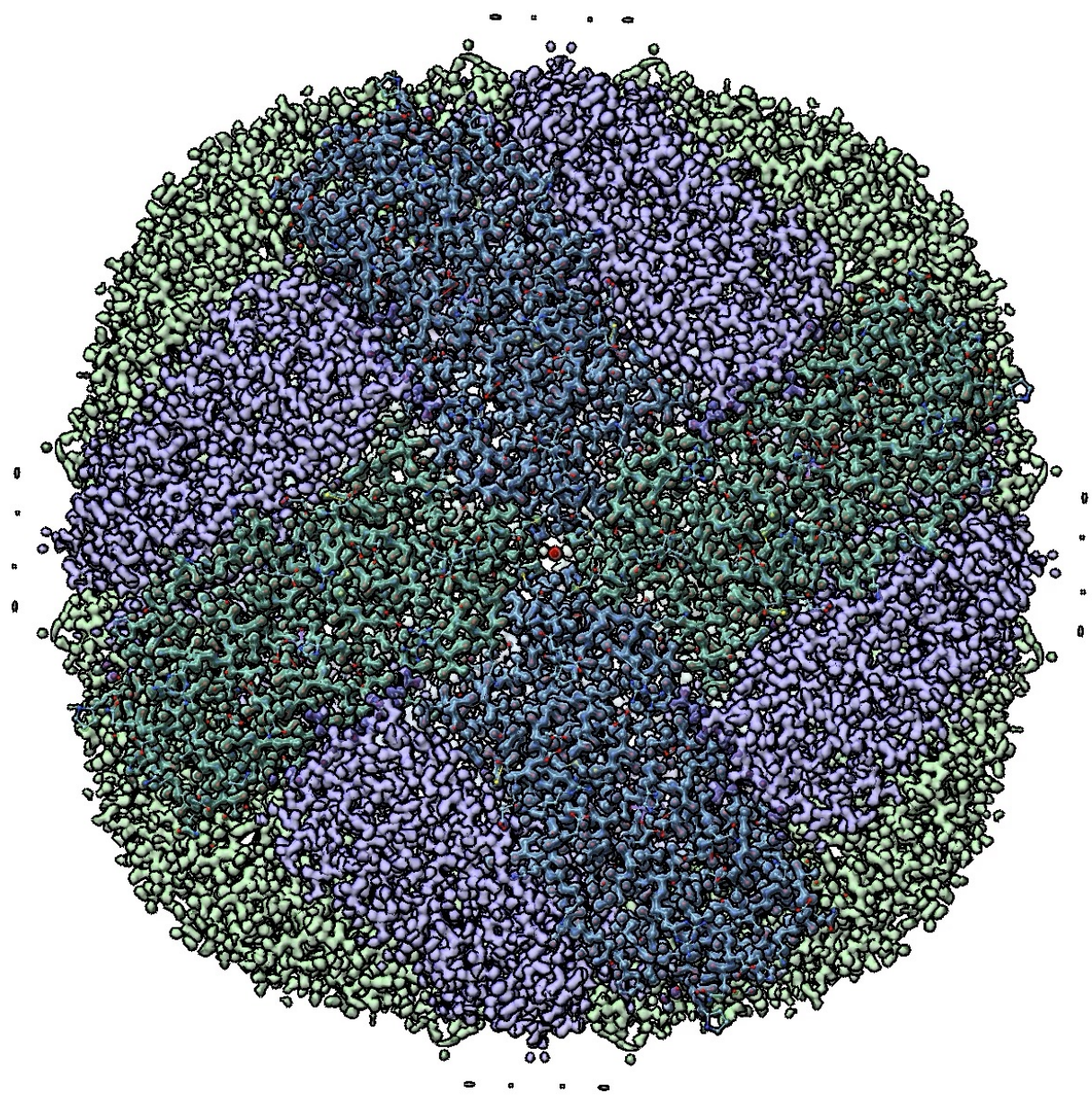
1.22Å resolution from cryo-EM - Nakane et al 2020 Nature

Ferritin (Iron storage protein cage)



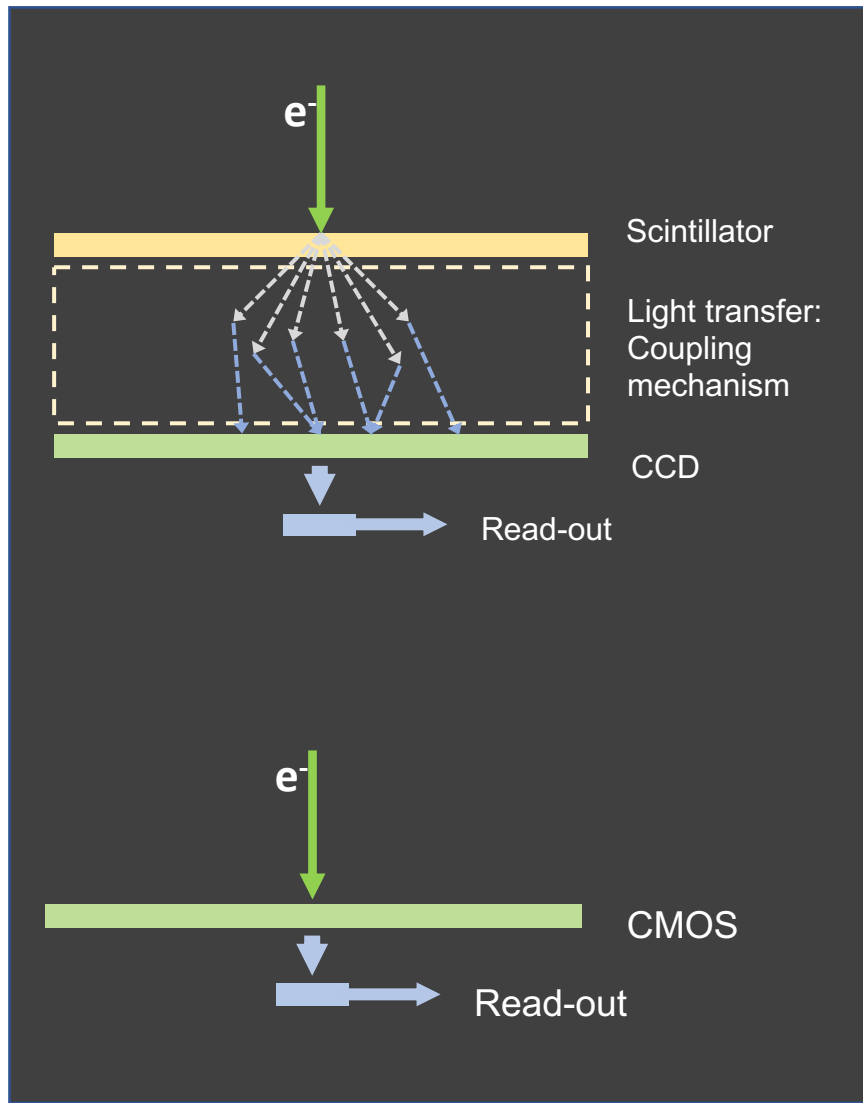
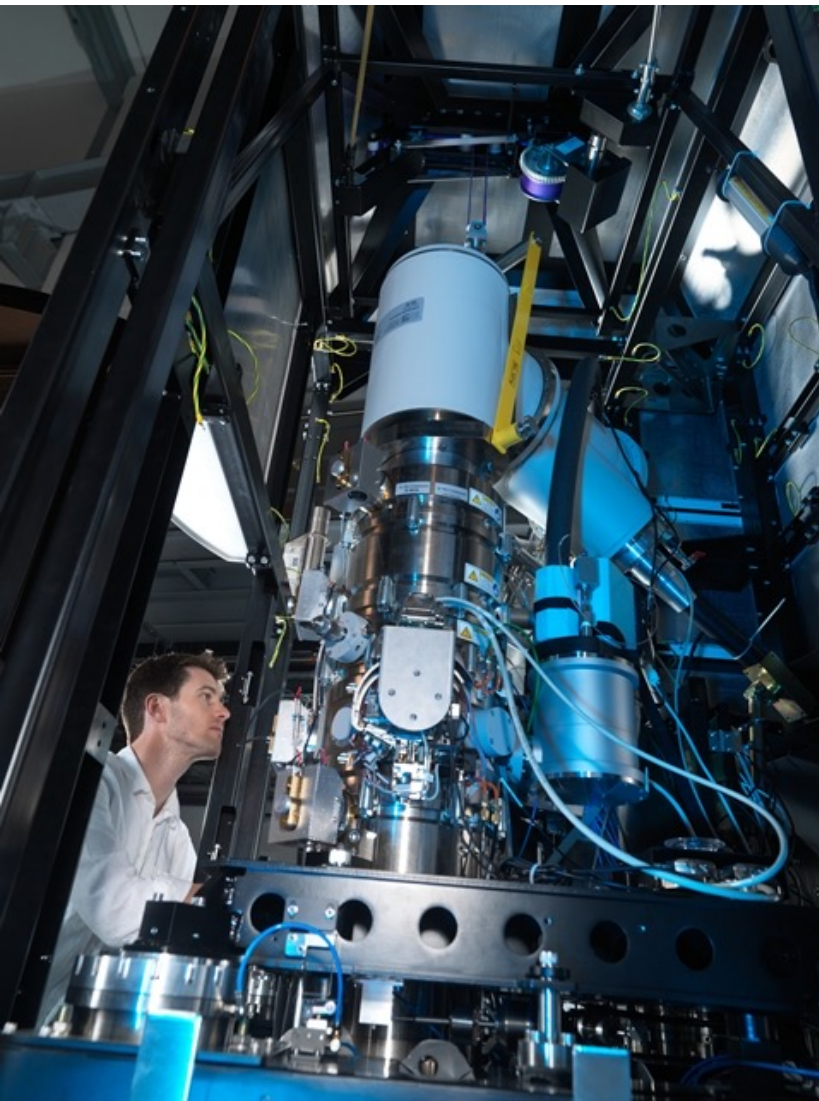
David
Goodsell

Apoferritin structure, ion coordination at 4-fold channel



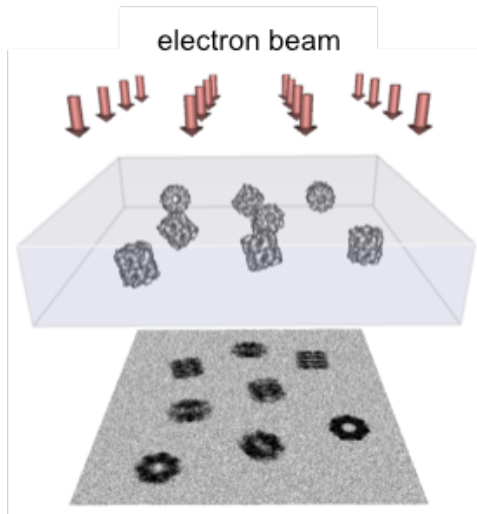
1.22Å resolution from cryo-EM – Nakane et al 2020 Nature

Technology drivers



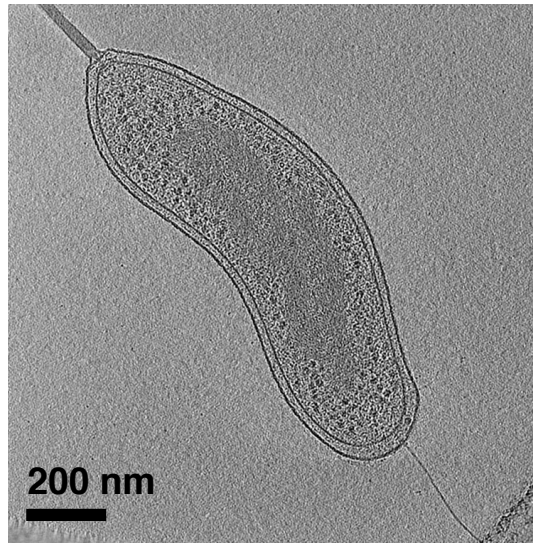
A note on possible samples

Molecules



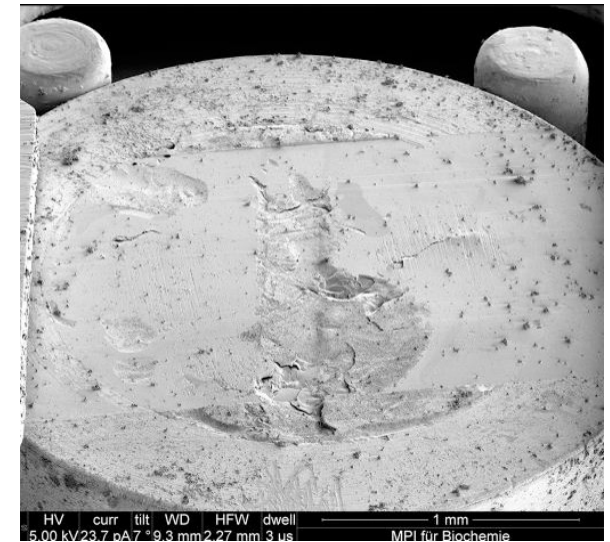
Greg Pintille

Cells



©Eikosi cc-by-sa-4.0

Tissues

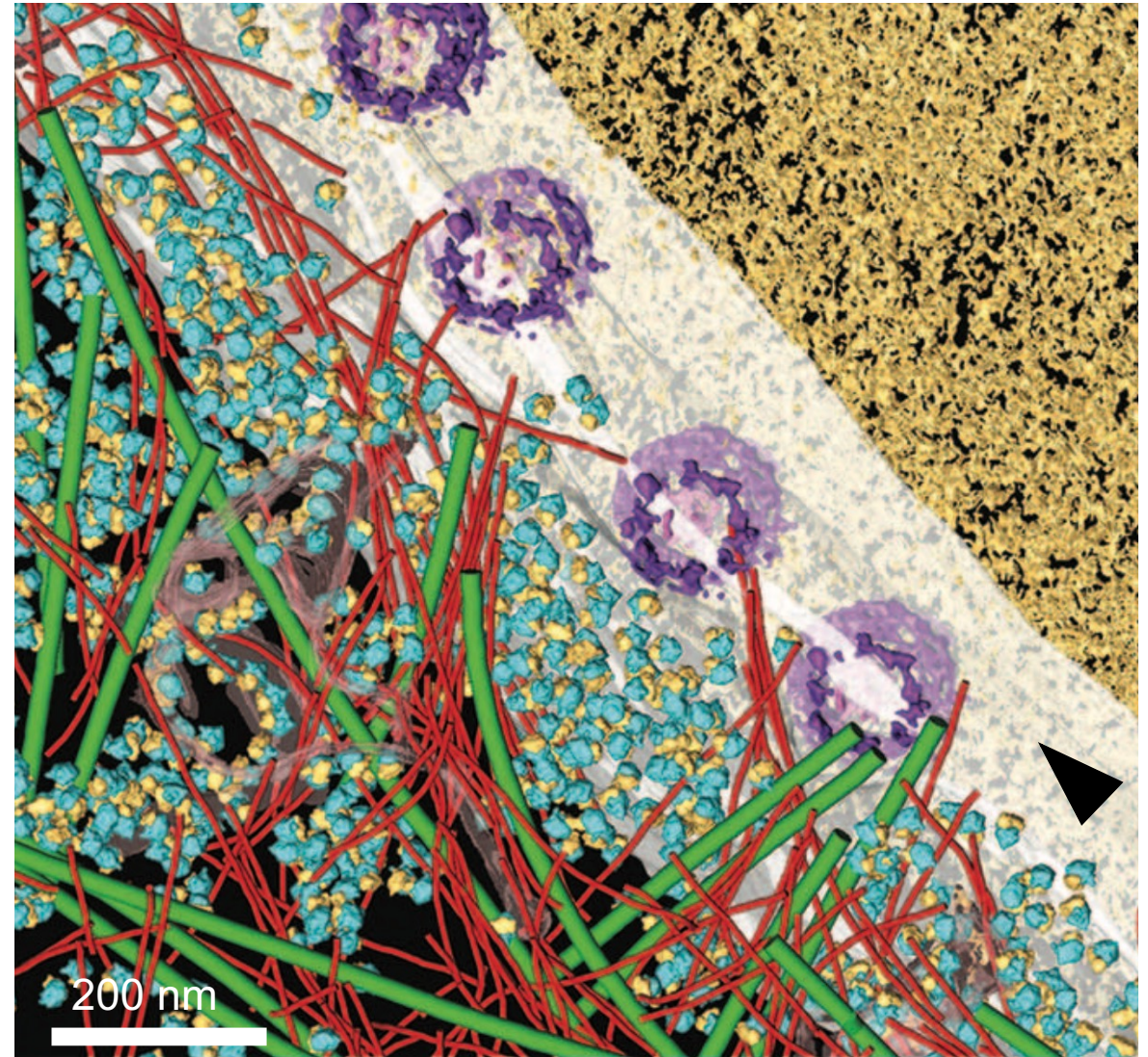
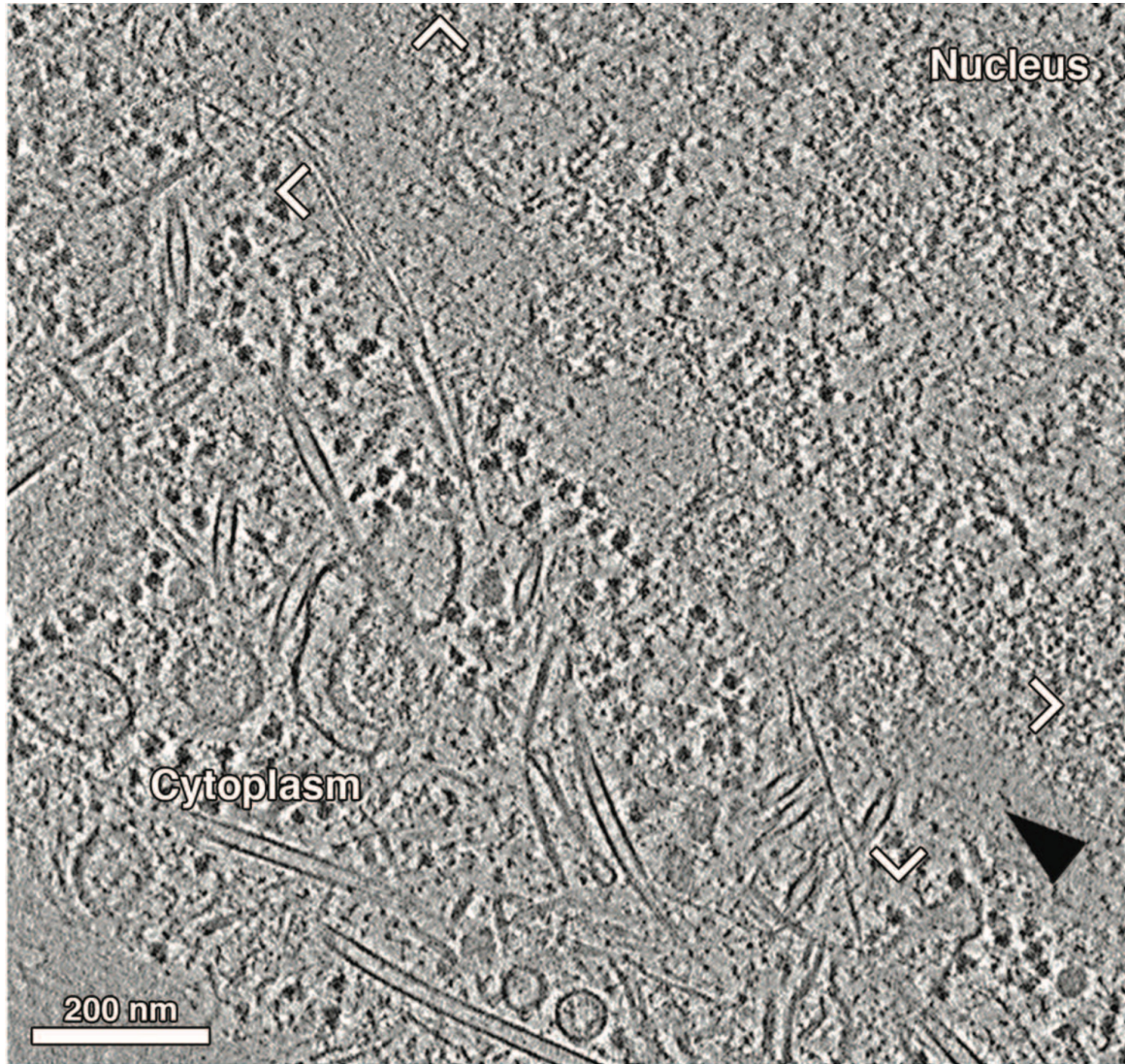


Schaffer et al 2019



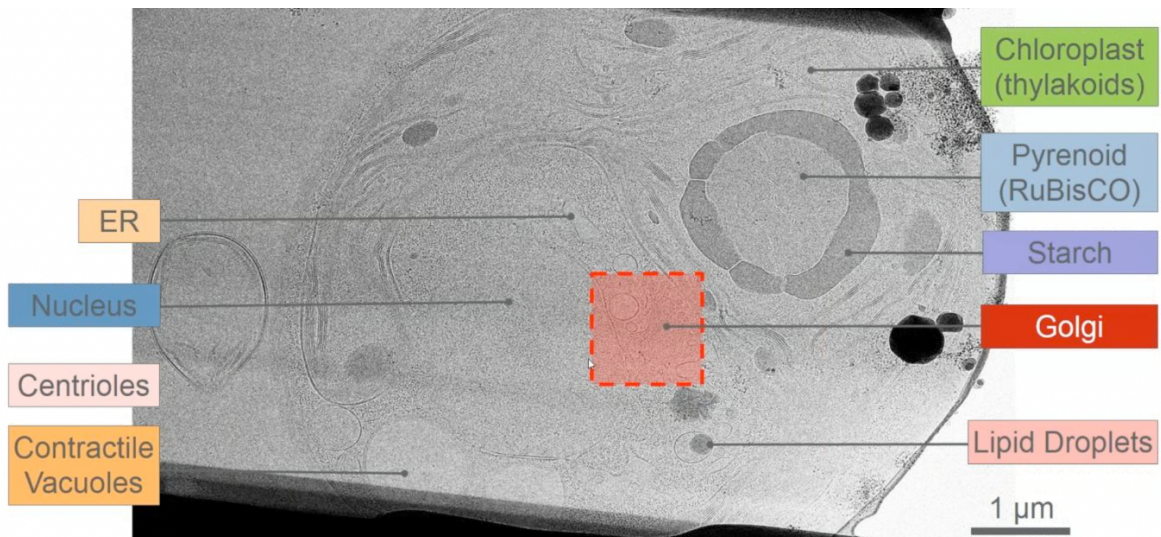
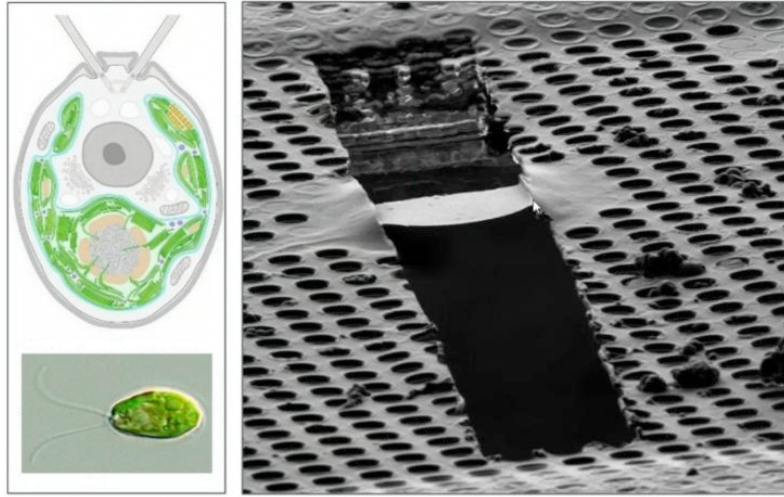
Villa et al 2015

Seeing molecules inside cells

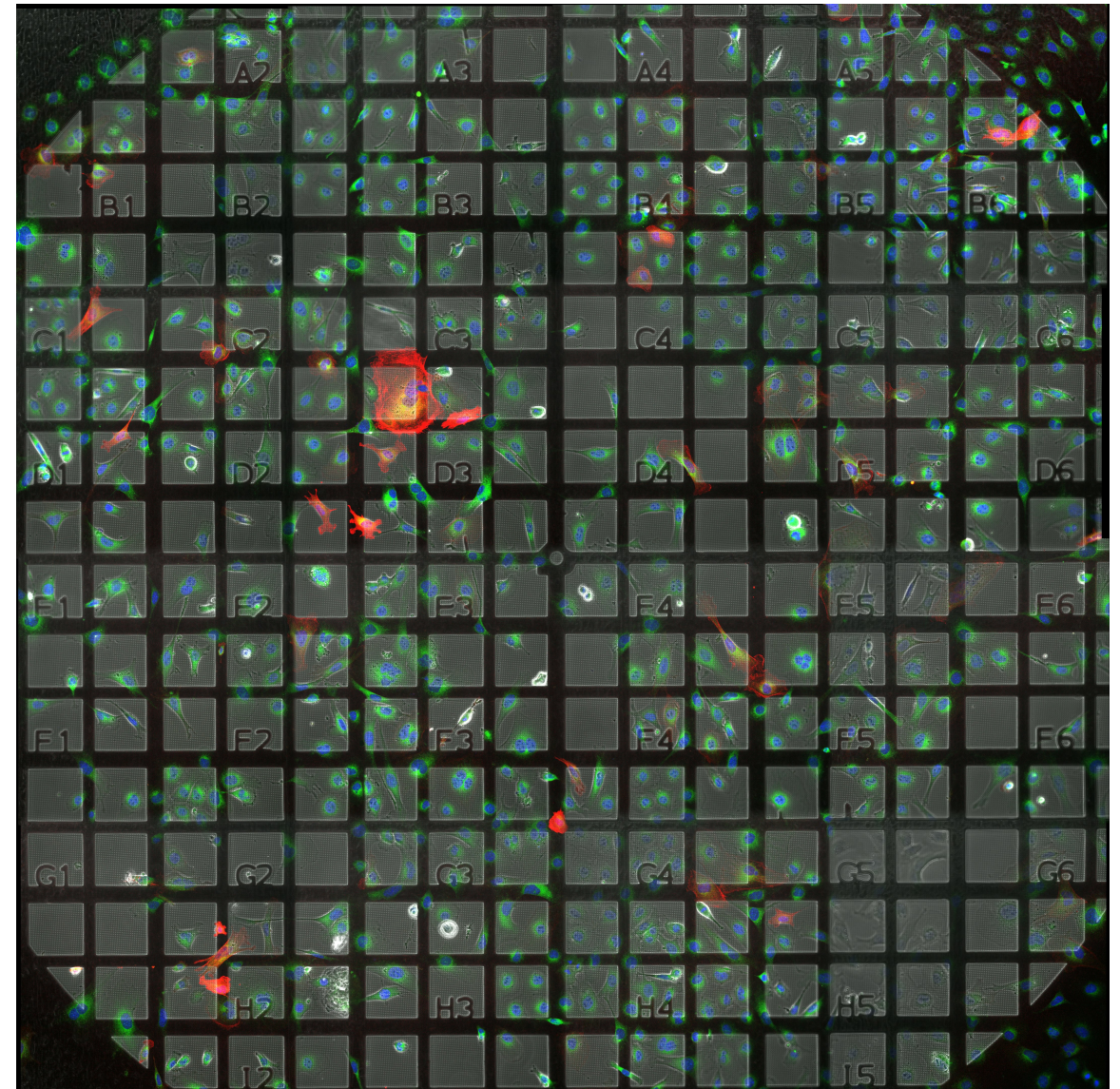


▶ NE Nuclear density NPC ER Actin & Intermediate filaments Microtubules Large & Small ribosomal subunits

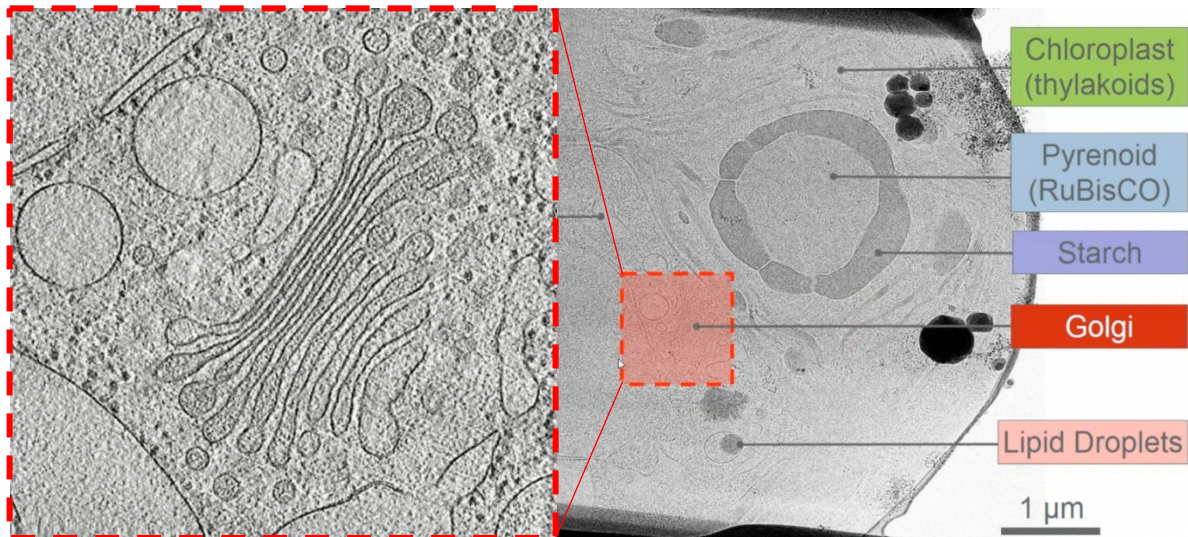
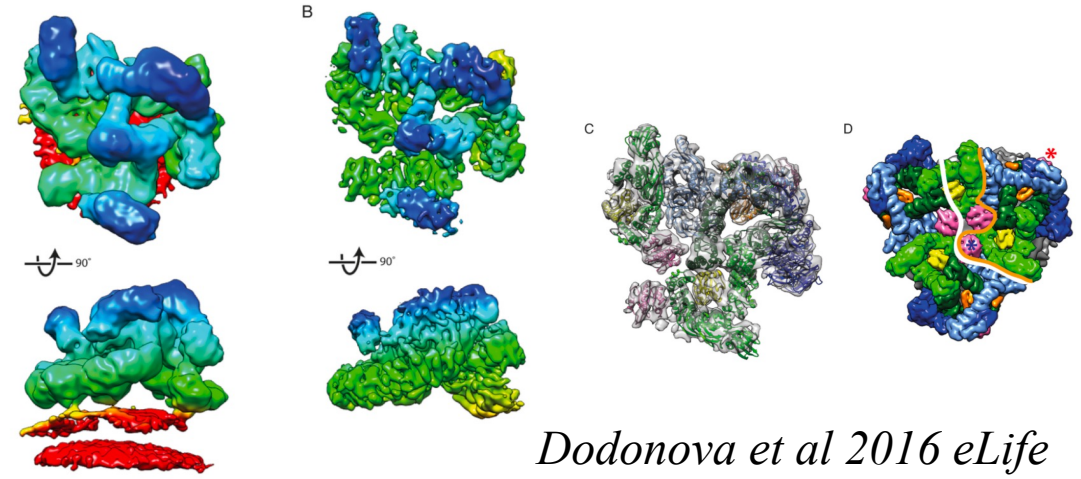
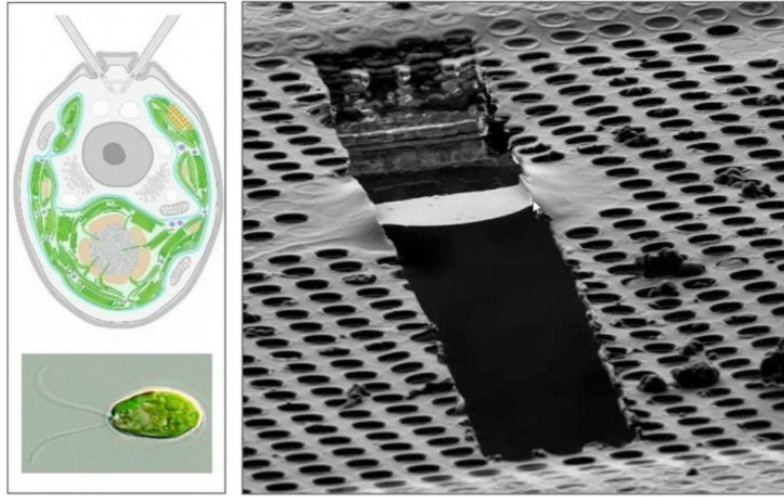
Cryo-ET of cells prepared by FIB-SEM



Schaffer and Engel



Cryo-ET of cells prepared by FIB-SEM



Summarise

- Cryo preservation excellent for preserving native states
- Preservation of molecules or cells or tissues all possible
- Cryo-EM enables multiple imaging modalities suited to specimen
- Explosion of tech dev in sample prep/microscopes/detectors/analysis
- Targets of many different natures and across scales may be imaged at ever increasing detail & resolution

Programme

Day 1: Monday, April 26th

09:45–10:00	Arrival: Introductions and welcome
10:00–10:30	Lecture 1: Why cryogenic electron microscopy (cryo-EM)? – Kyle Morris (eBIC)
10:30–11:15	Lecture 2: The electron microscope – Karen Davies (eBIC PBS)
11:15–12:00	Lecture 3: Sample preparation – Dimple Karia (Thermo Fisher Scientific)
12:00–13:00	Lunch break
13:00–16:00	Practical 1: Cryogenic sample preparation for cryo-EM – Kyle Morris (eBIC)

Day 2: Tuesday, April 27th

09:00–10:00	Lecture 4: Image formation – Dan Clare (eBIC PBS)
10:00–11:00	Lecture 5: Image processing – Colin Palmer (CCP-EM)
10:30–10:35	Tea break
11:00–12:00	Lecture 6: From 2D images to 3D density maps I: SPA – Chris Aylett (Imperial)
12:00–13:00	Lunch break
13:00–16:00	Practical 2: Screening samples in cryo-EM – Peter Harrison (eBIC PDRA)

Programme

Day 3: Wednesday, April 28th

09:00–10:00	Lecture 7: From 2D images to 3D density maps II: Cryo-ET – Tanmay Bharat (Oxford)
10:00–11:00	Lecture 8: Averaging and reconstructing 3D maps in cryo-ET – Ben Himes (HHMI/UMassMed)
10:30–10:35	Tea break
11:00–12:00	Lecture 9: Fitting and building of atomic models – Agnel-Praveen Joseph (CCP-EM)
12:00–13:00	Lunch break
13:00–16:00	Practical 3: Single-Particle Averaging data collection demonstration – Vinod Vogirala (eBIC)

Day 4: Thursday, April 29th

09:00–12:00	Practical 4: Cryo-ET data collection demonstration – Julika Radecke (eBIC)
12:00–13:00	Lunch break
13:00–14:00	Course WT DTP student talks and feedback
14:00–15:00	PDRA & PhD student talks
15:00–15:40	Lecture 10: Anatomy and Variations on the Theme Spike – Helen Duyvesteyn (STRUBI)
15:40–16:00	Wrap-up Q&A