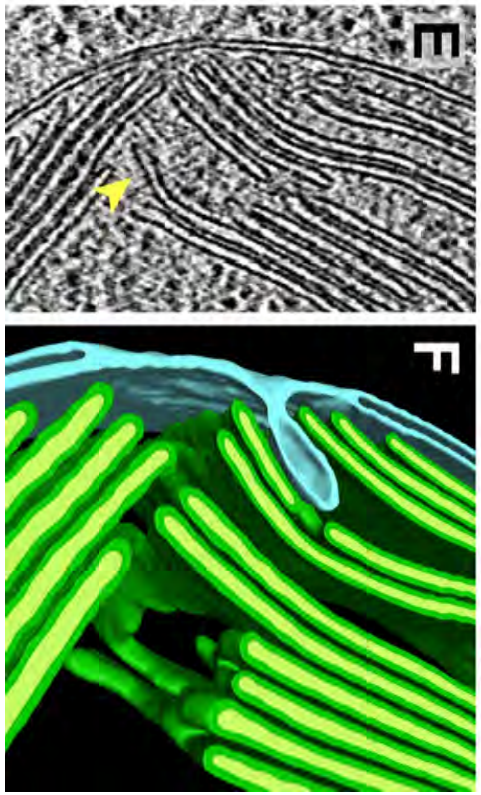
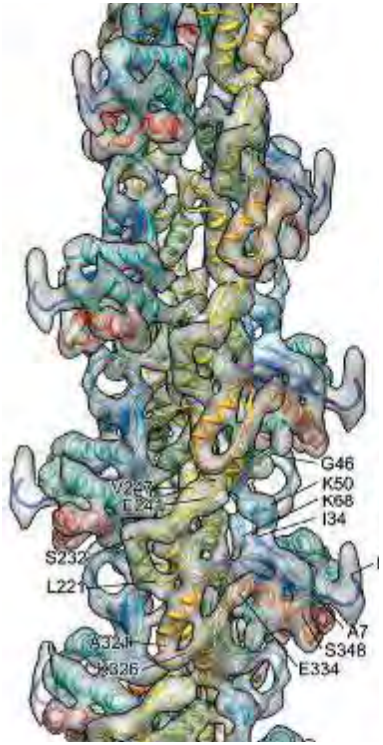
Cryo-electron microscopy

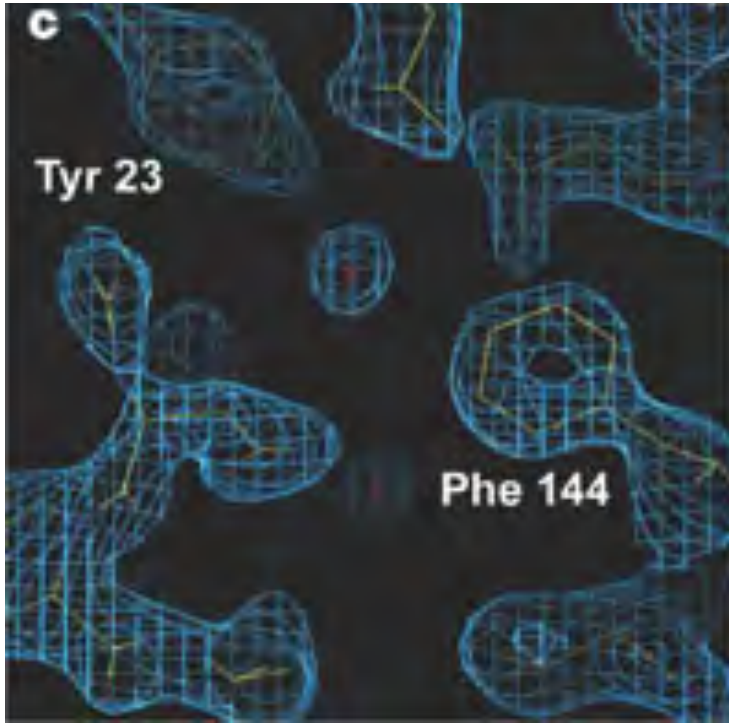
John Briggs

3DEM. Why and when?

Structure across range of resolutions and samples

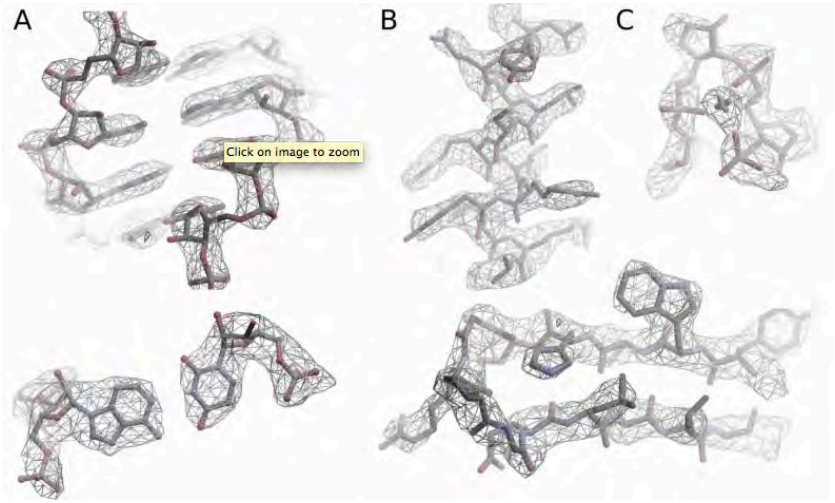






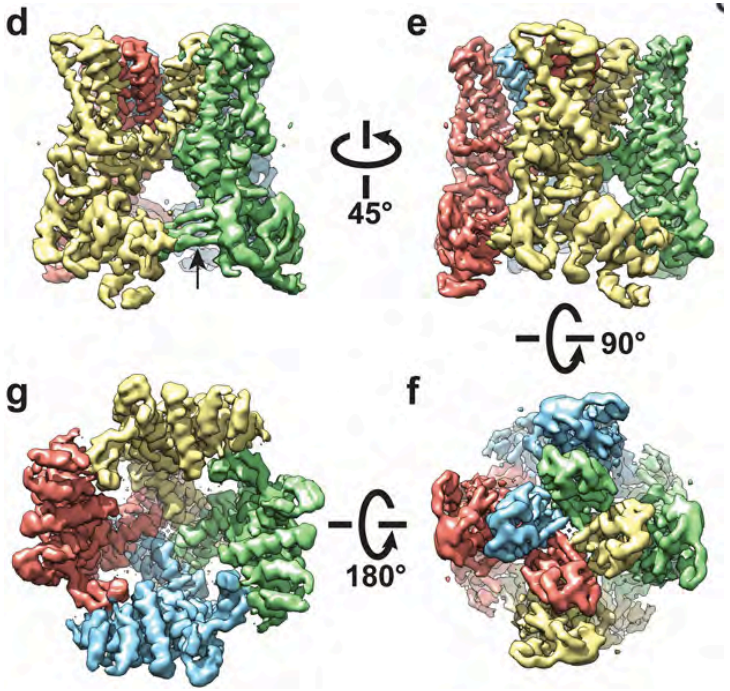
3DEM. Why and when?

Easier than crystallography?



Yeast mitochondrial ribosome
Amunts et al. Science 2014

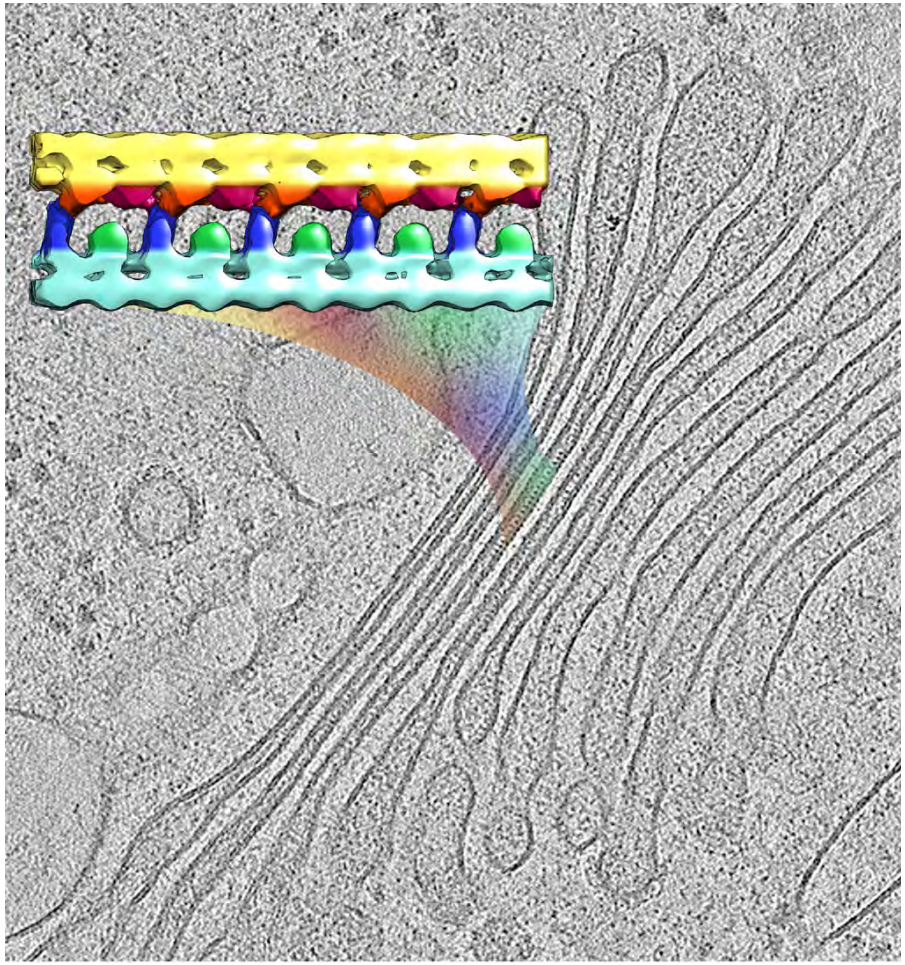
Membrane Proteins



TRPV1 ion channel
Liao et al Nature 2013

3DEM. Why and when?

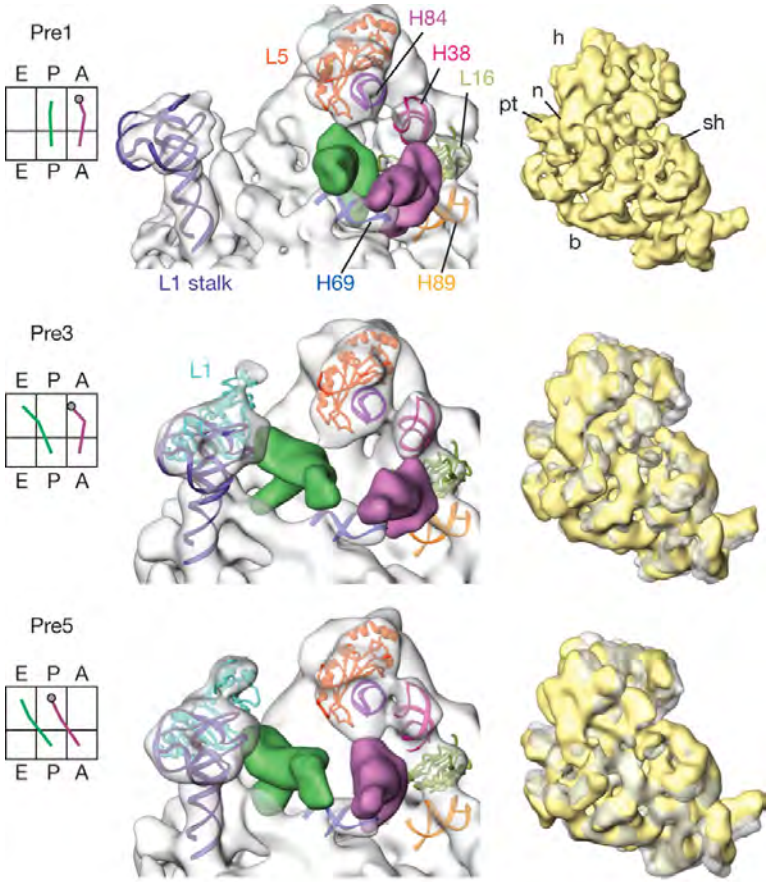
Solve structure “in situ”



B. D. Engel, M. Schaffer, S. Albert, S. Asano, J. M. Plitzko and W. Baumeister: In situ structural analysis of Golgi intracisternal protein arrays. *Proceedings of the National Academy of Sciences USA*, September 8, 2015

3DEM. Why and when?

Mixed samples, populations



N Fischer *et al. Nature* 2010

Five things we need for 3D EM

1. Preparation methods which preserve 3D structure

2. A microscope which allows us to image the sample

3. Different views of the object

4. Computational approaches for producing 3D reconstructions from projections

5. Approaches for assessing our 3D reconstruction and interpreting it in terms of biology

Sample preparation methods

Requirements for sample preparation:

3D structure preserved at appropriate resolution

Sample thin enough for electron beam preparation

Sample stable in a vacuum

3DEM. Sample prep (brief)

1. Negative staining
2. Vitrification (for cryo-EM)

VOL. 34 (1959) BIOPHYSICA ACTA

A NEGATIVE STAINING METHOD FOR HIGH RESOLUTION ELECTRON MICROSCOPY OF VIRUSES

S. BRENNER AND R. W. HORNE

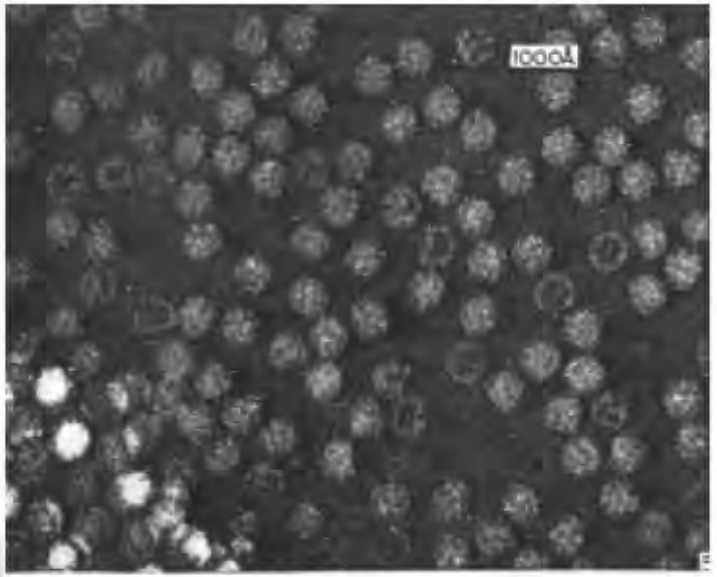
Medical Research Council Unit for Molecular Biology and Electron Microscopy Group, Cavendish Laboratory, Cambridge (Great Britain)

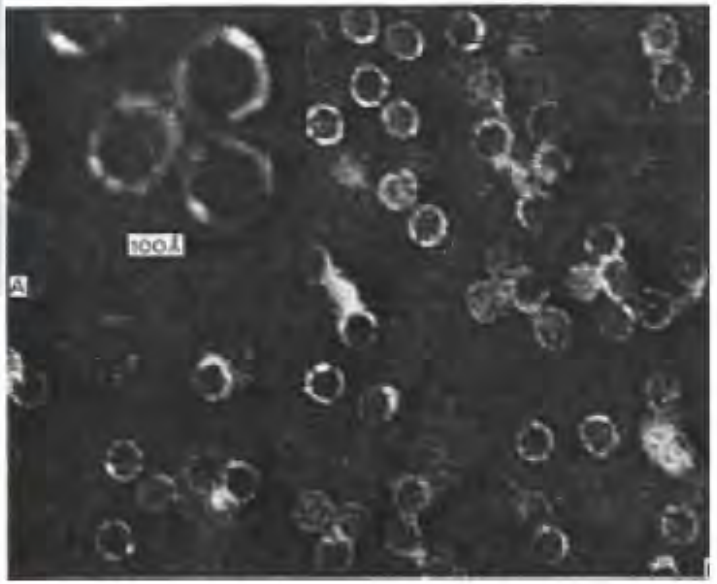
(Received November 14th, 1958)

SUMMARY

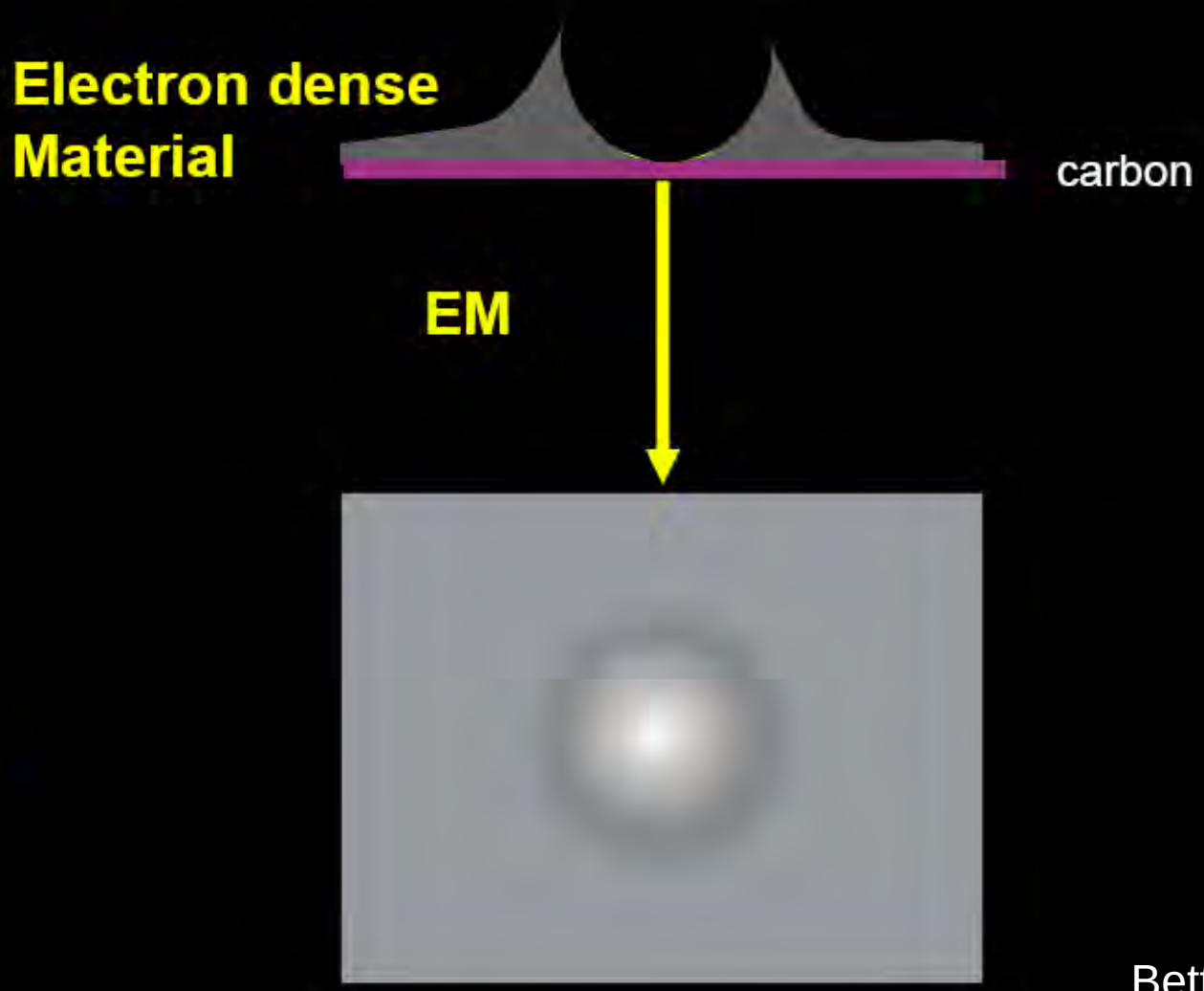
A simple technique has been developed for the study of the external form and structure of virus particles. High contrast with good preservation is obtained by mixing virus preparations with 1% phosphotungstic acid adjusted to pH 7.5 and spraying directly onto electron microscope supporting films made from evaporated carbon. The application of the technique to tobacco mosaic virus and turnip yellow mosaic virus is described. Structural details suggested by X-ray diffraction methods have been resolved.

Brenner and Horne BBA 1959: „ A simple technique has been developed for the study of the external forms and structures of virus particles“



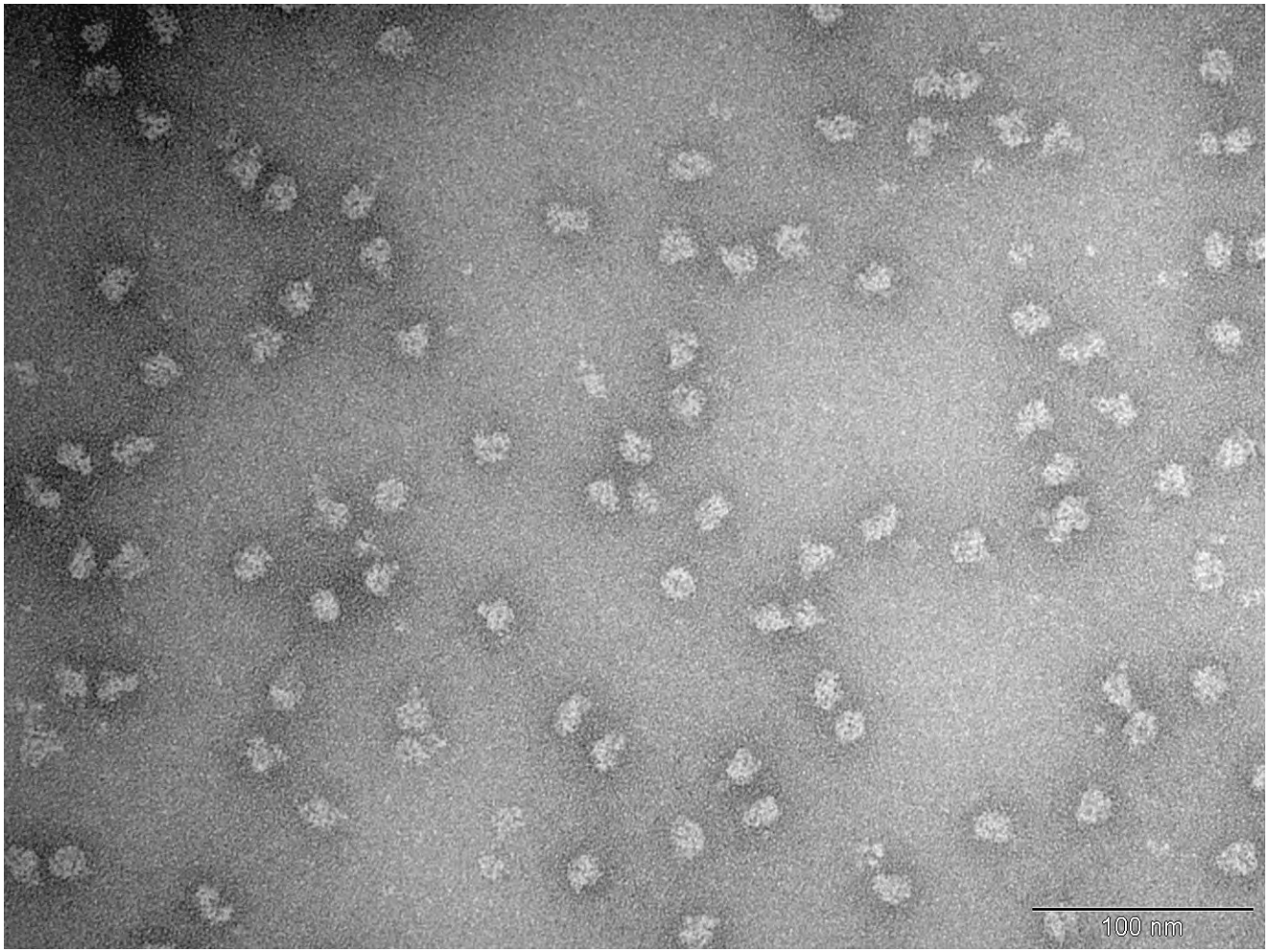


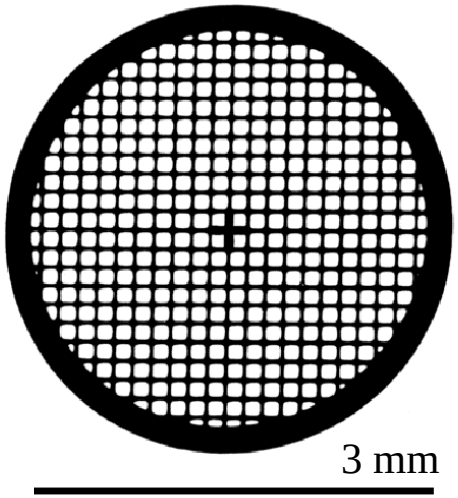
Negative-Staining

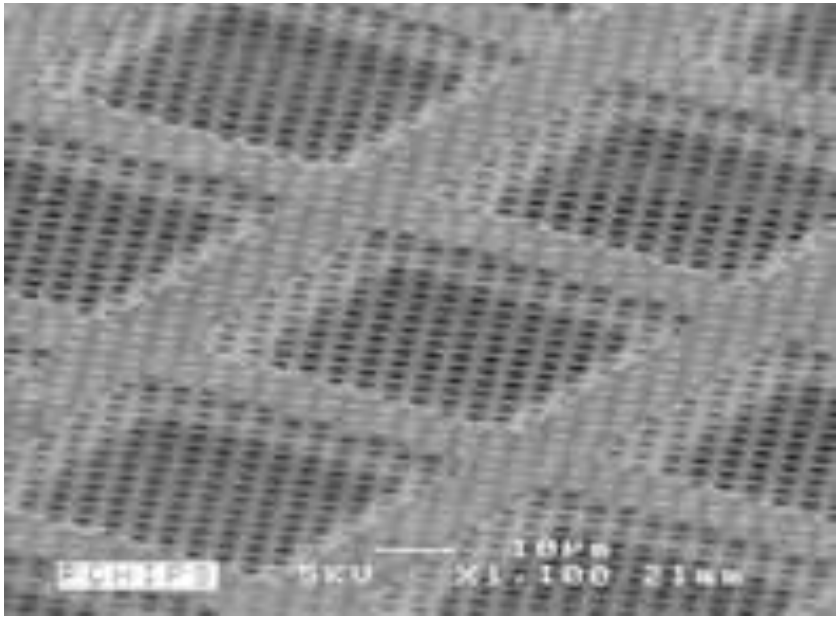


Bettina Boettcher

Micrograph of negatively stained sample

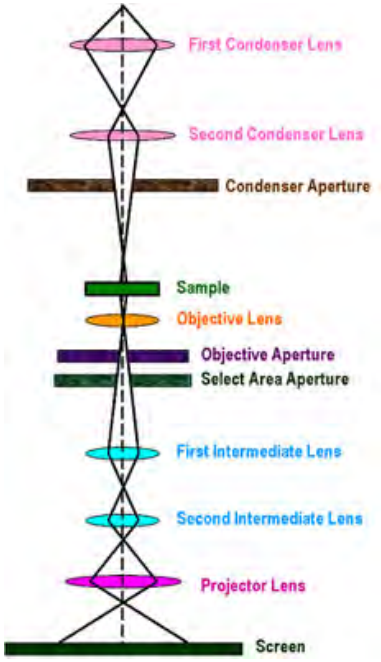




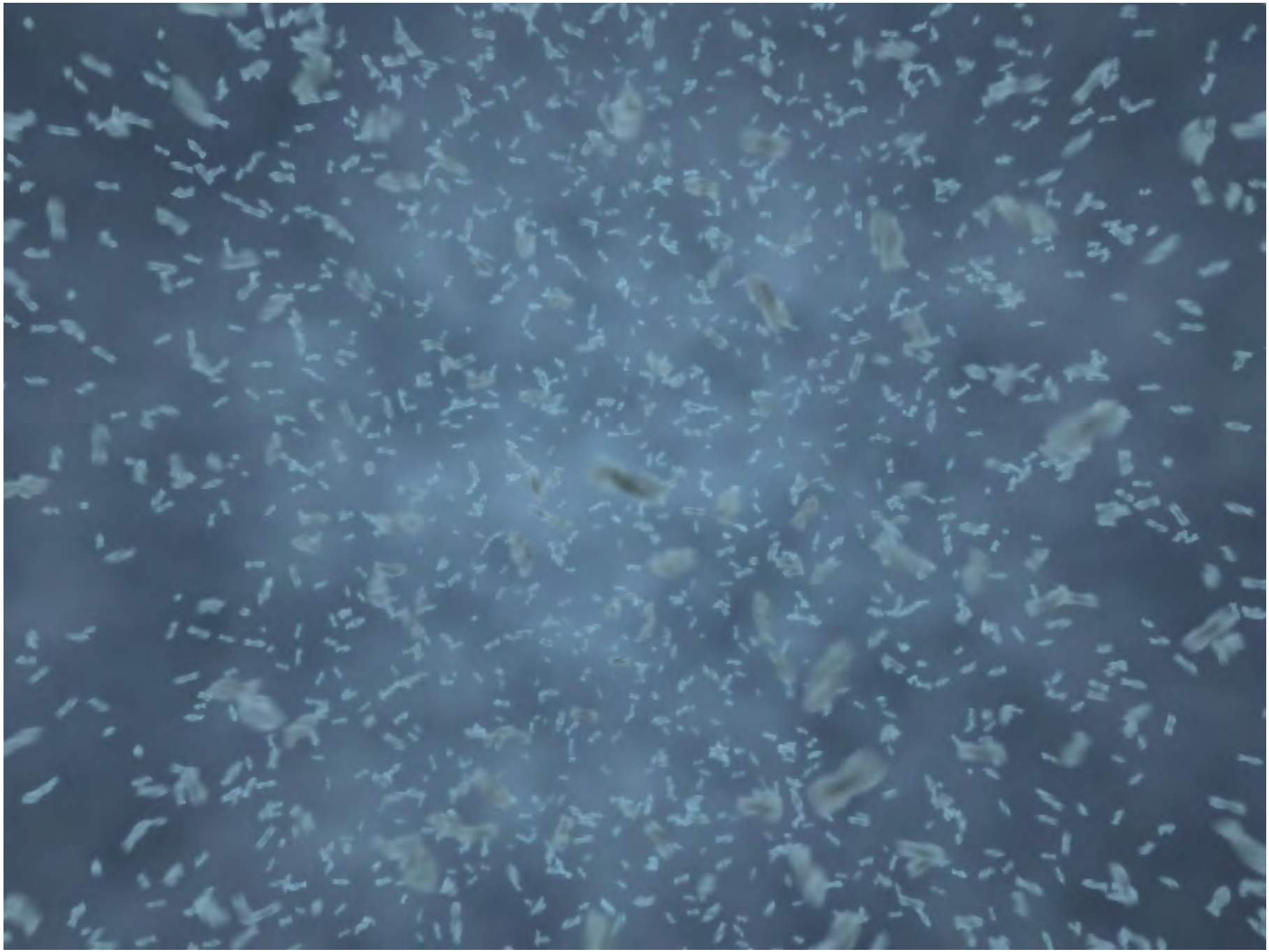


Bridget Carragher

The microscope



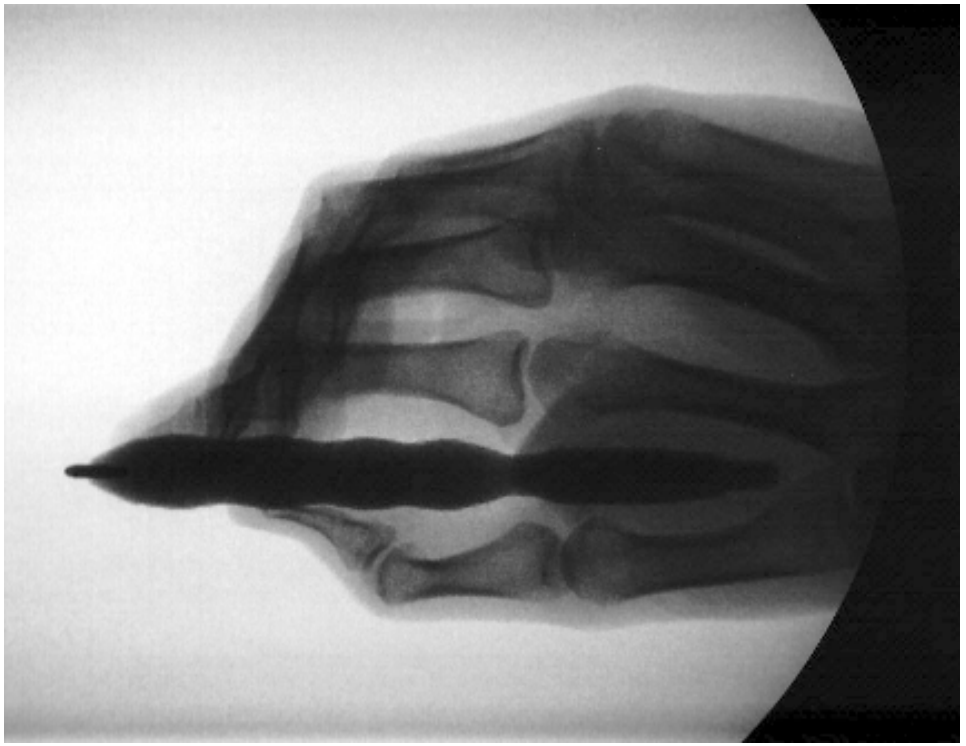
Titan Krios electron microscope



Movie from the Baumeister lab, Max-Planck Institute for Biochemistry

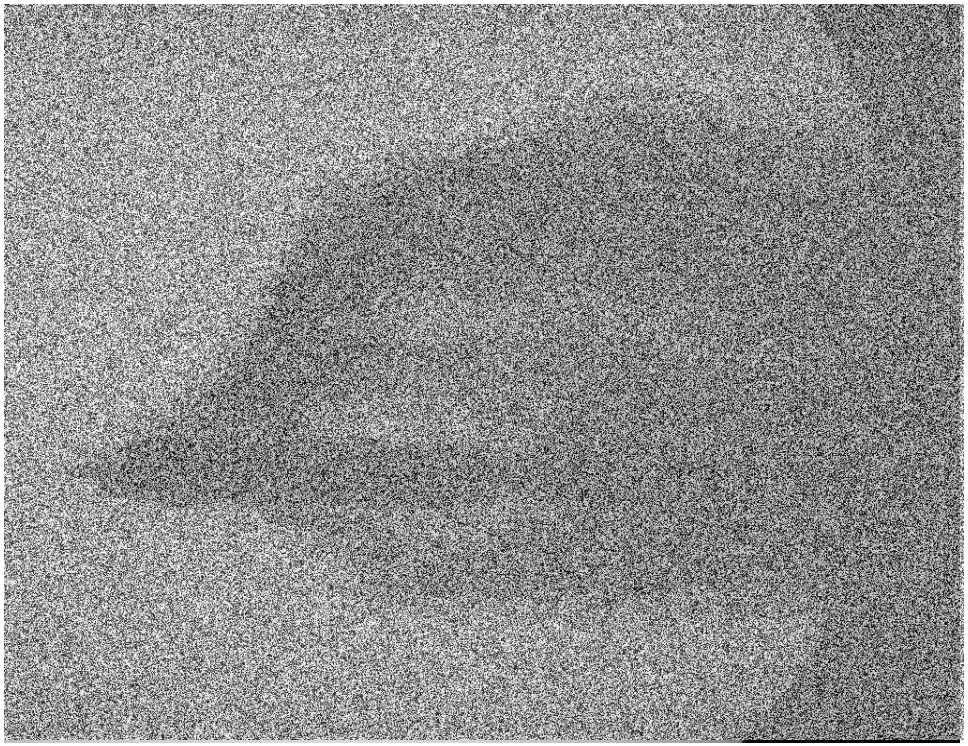
What is a cryo-EM image?

It is a projection image

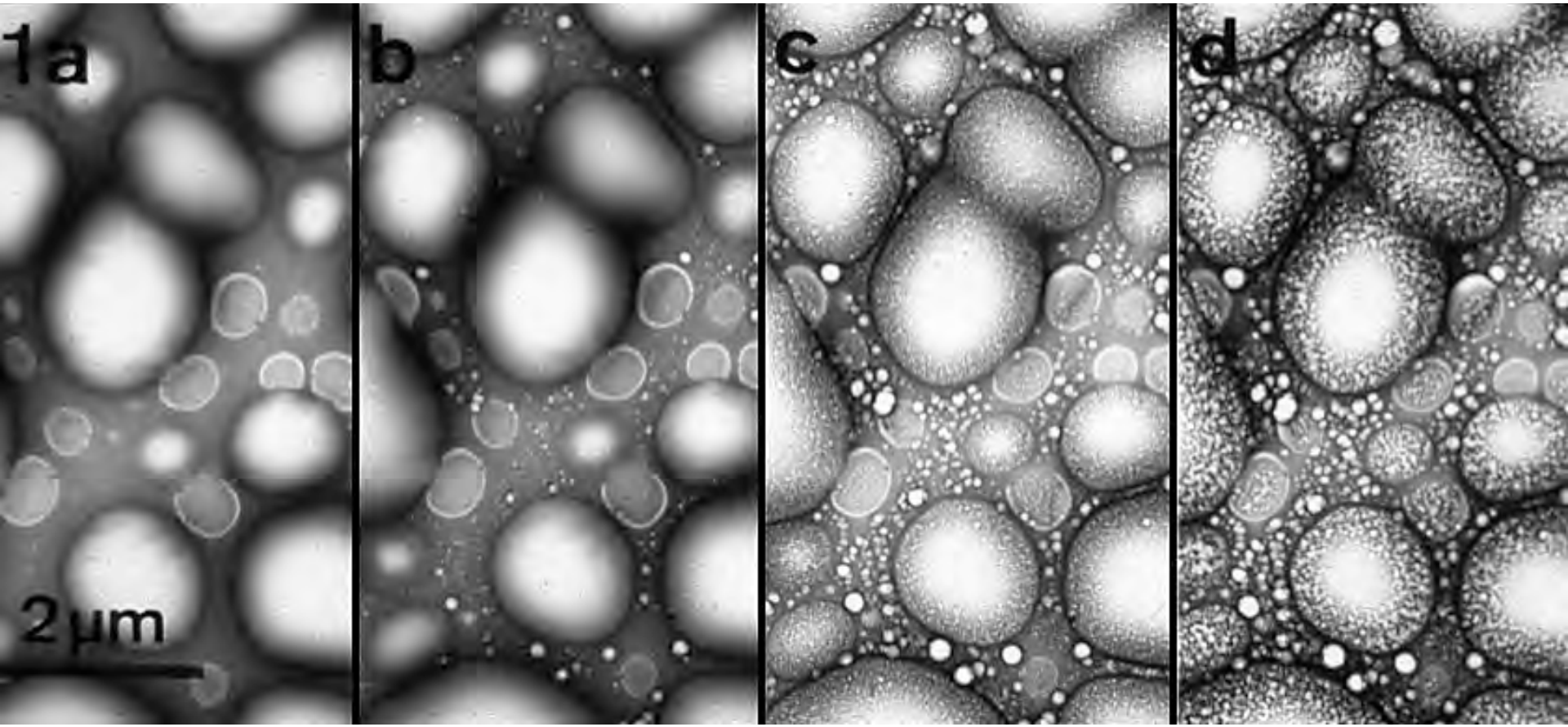


What is a cryo-EM image?

It is a noisy projection image

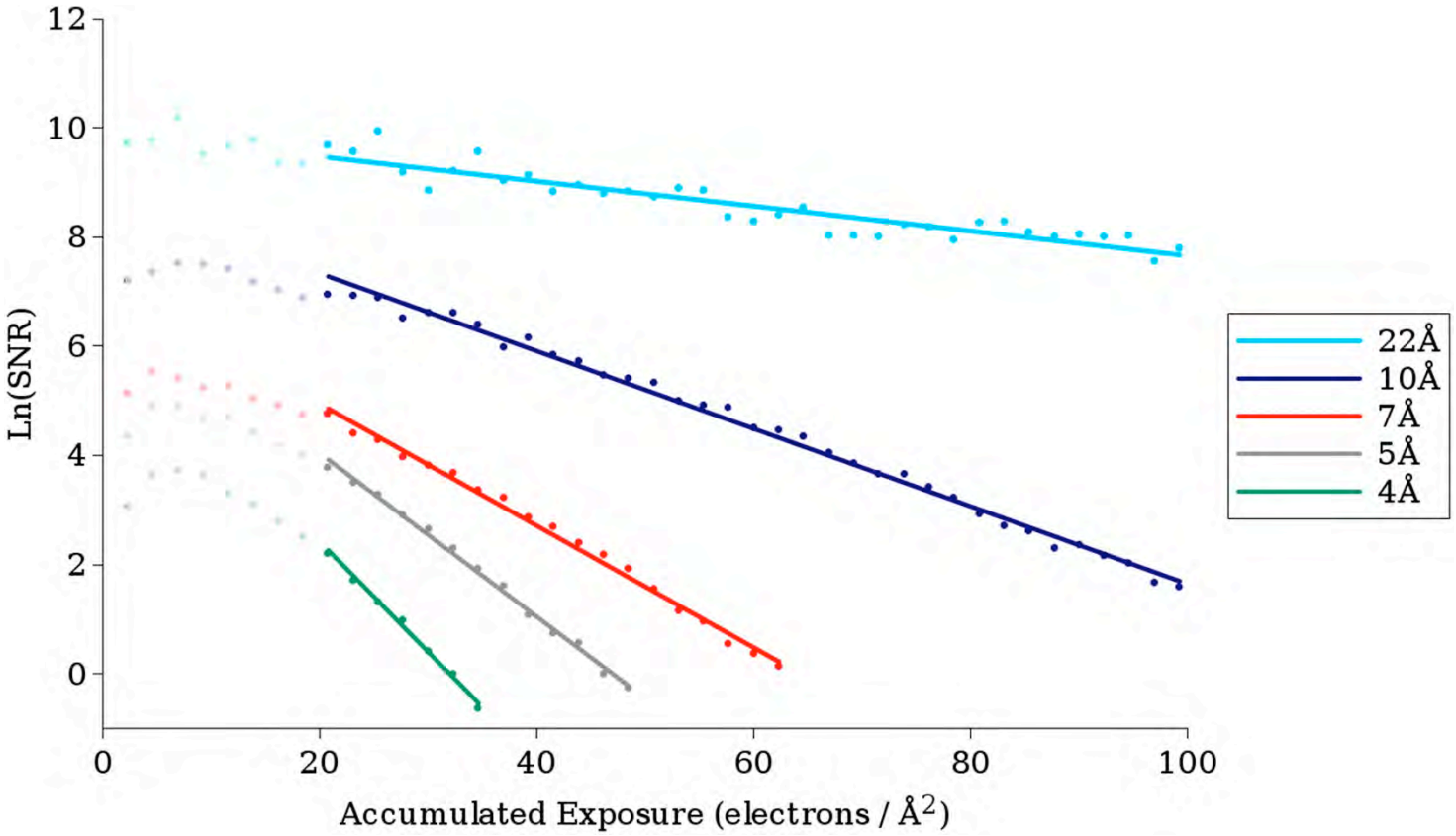


It is noisy because of limited electron dose



Slide: Andy Hoenger

Limited exposure

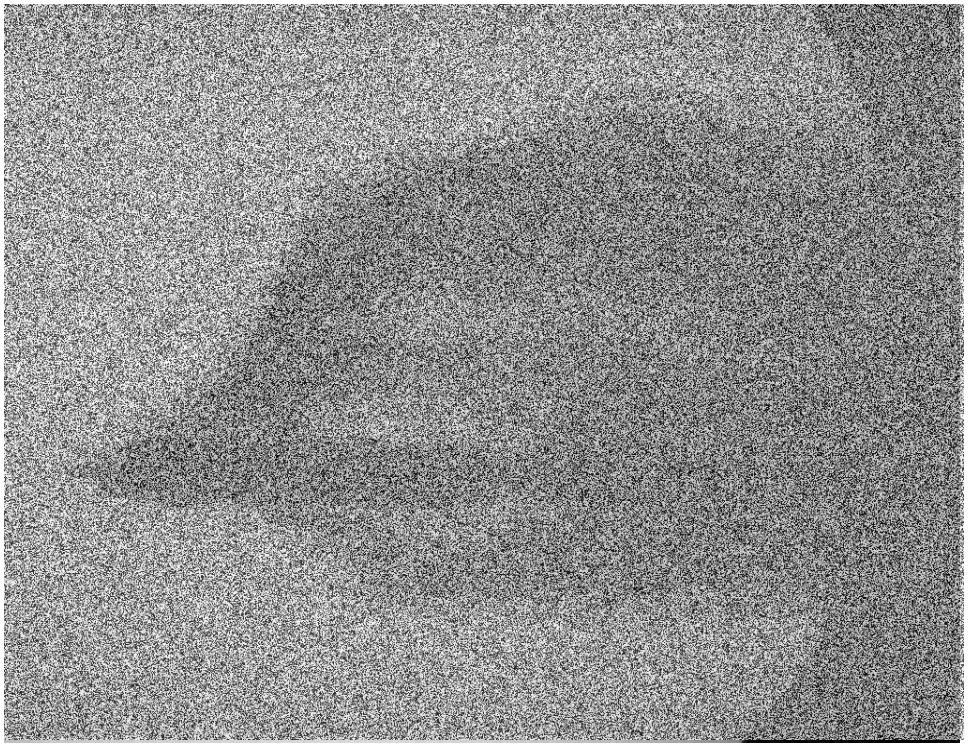


Grant and Grigorieff. eLife 2015;4:e06980. DOI: 10.7554/eLife.06980

High-resolution information is lost as exposure accumulates

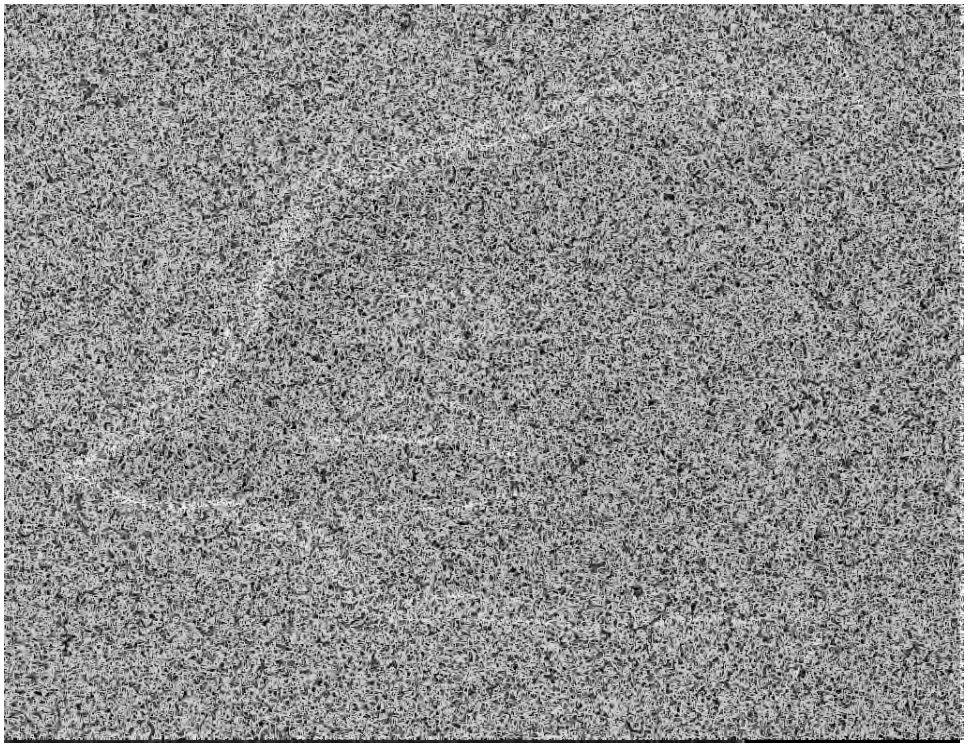
What is a cryo-EM image?

It is a noisy projection image



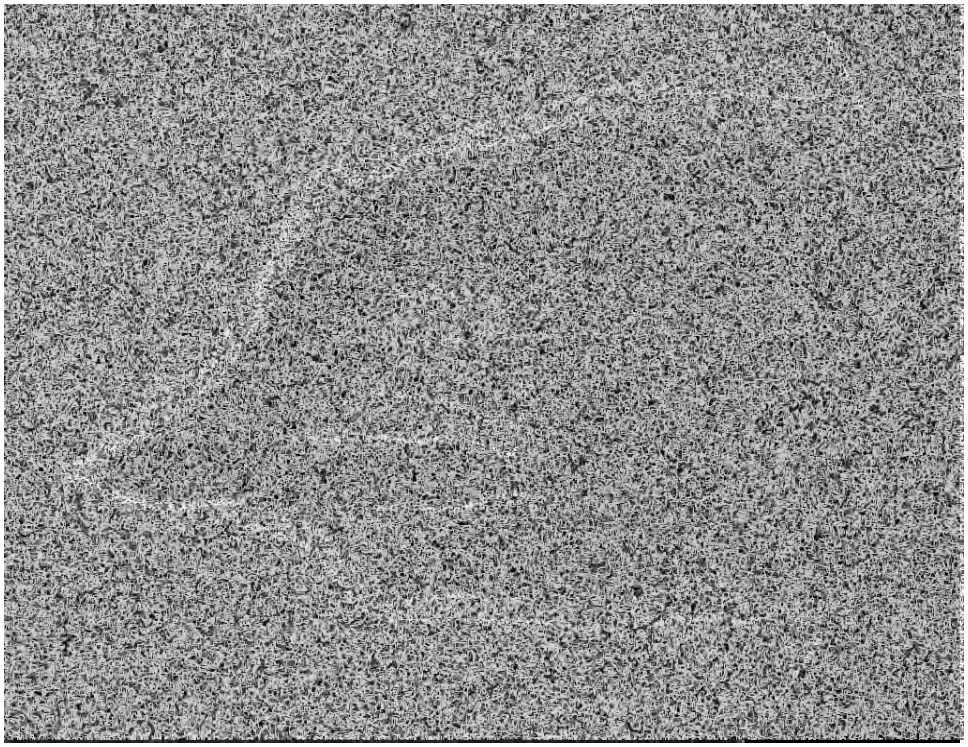
What is a cryo-EM image?

It is a noisy projection image modulated by a transfer function



What is a cryo-EM image?

How to go from this to a 3D reconstruction?

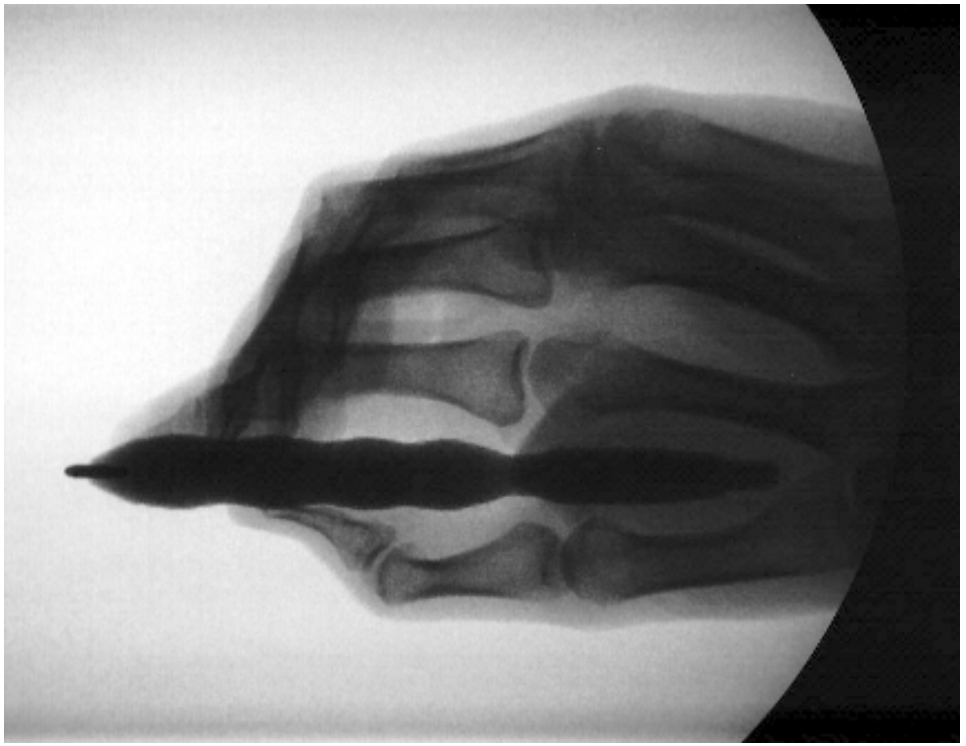


From 2D image to 3D structure

Key concept

We need different views of our object.

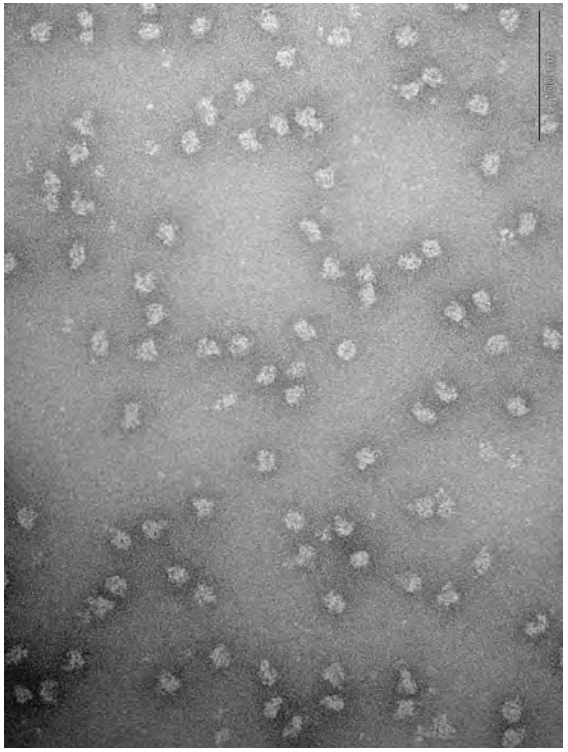
From 2D image to 3D structure



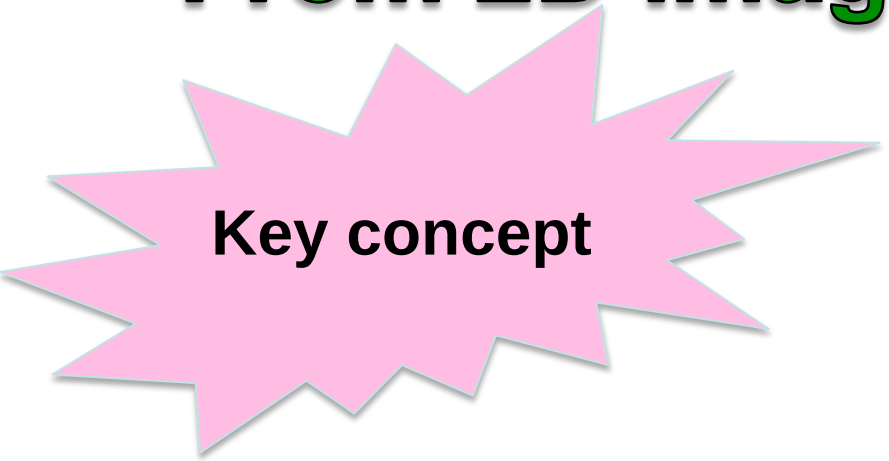
From 2D image to 3D structure

Single particle EM

The particles are randomly oriented. Each image of the particle gives us a different view



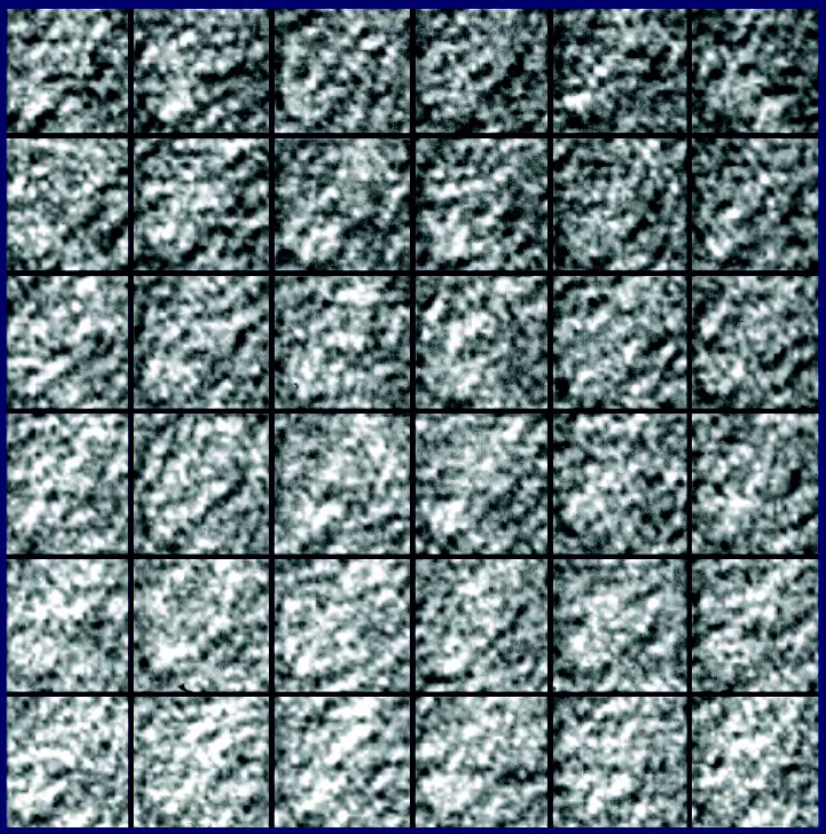
From 2D image to 3D structure



We need to average many images

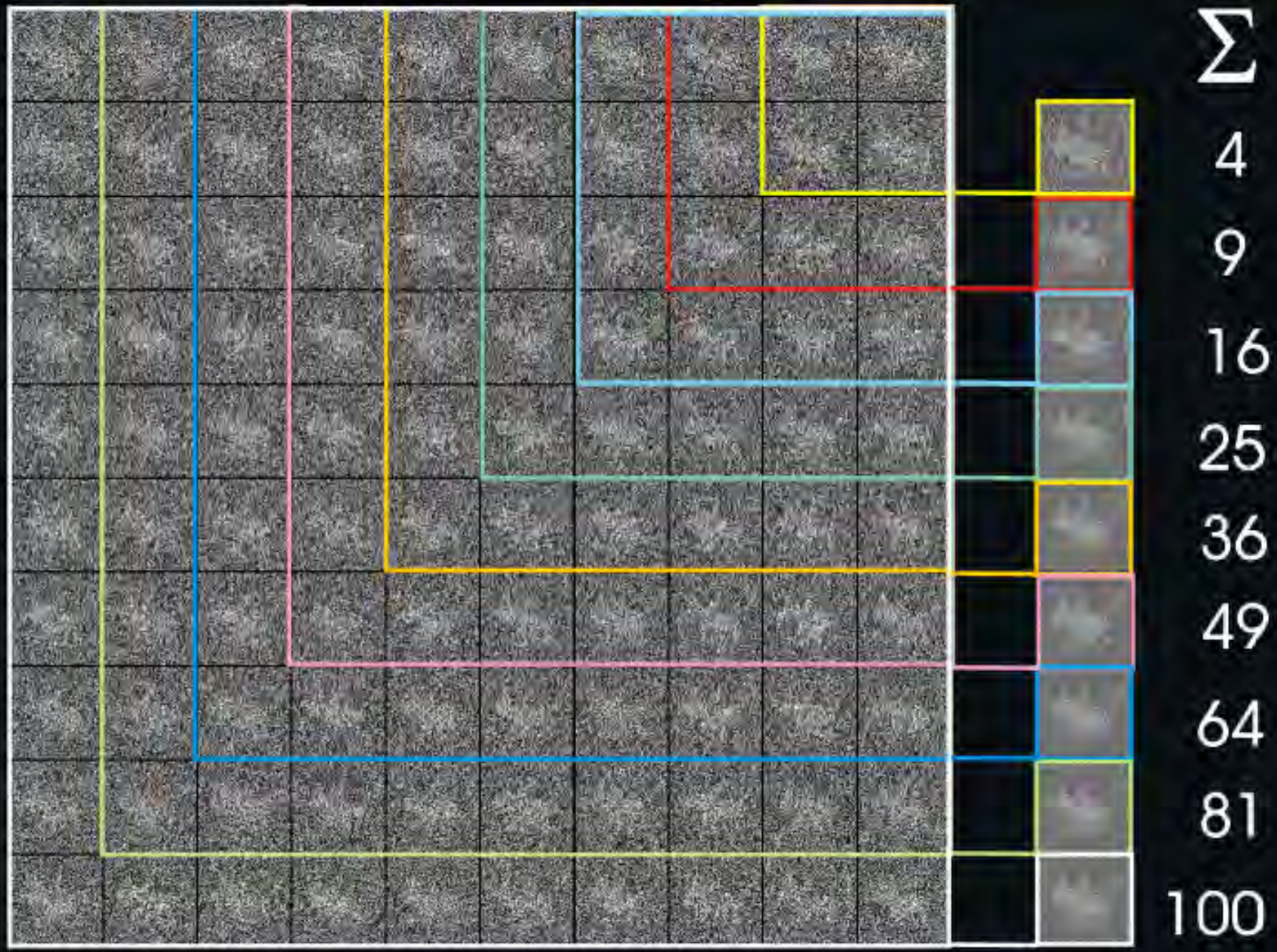
averaging of noisy identical image elements

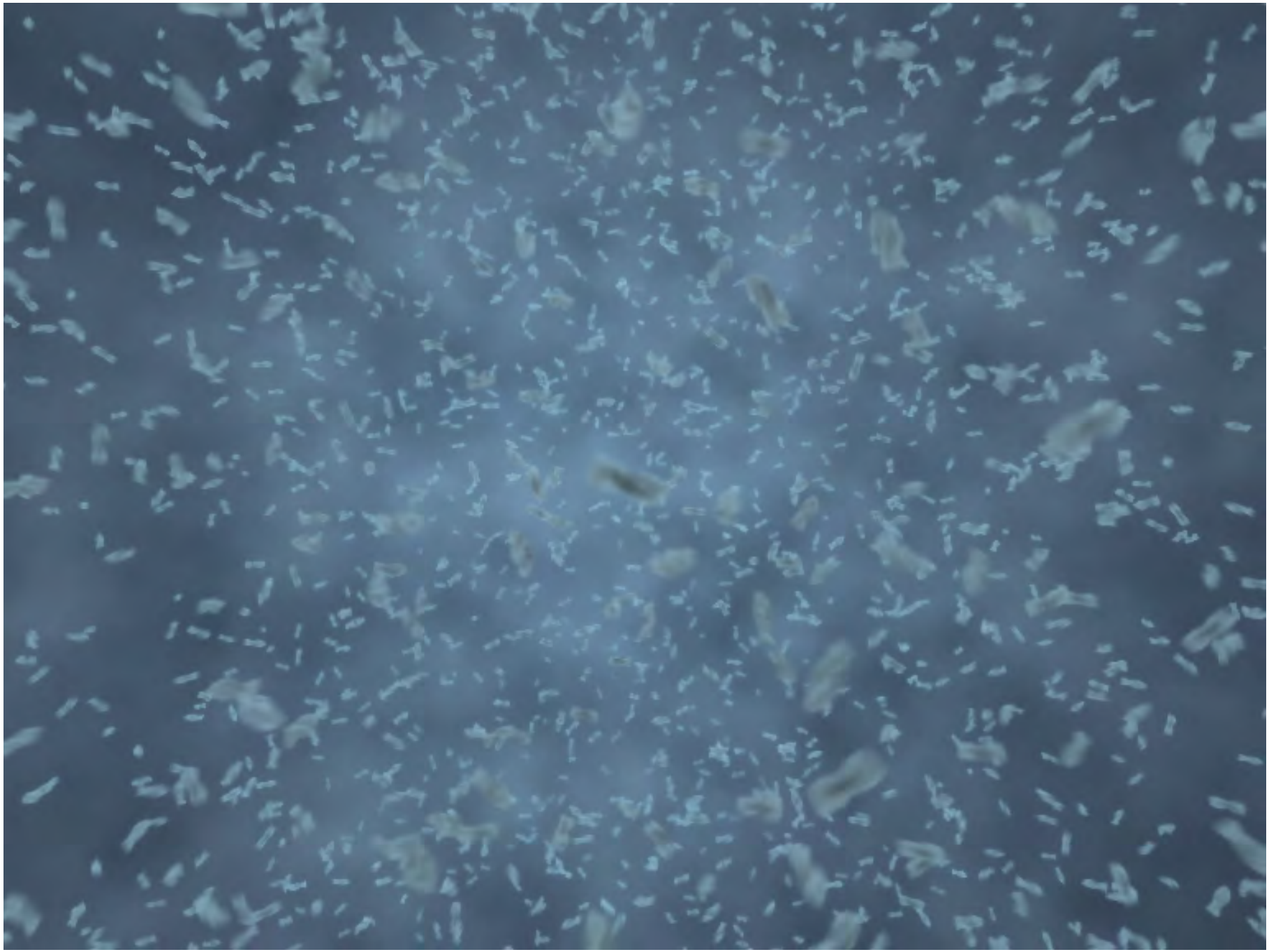
Slide: Andy Hoenger



Averaging

Slide: Bettina Boettcher





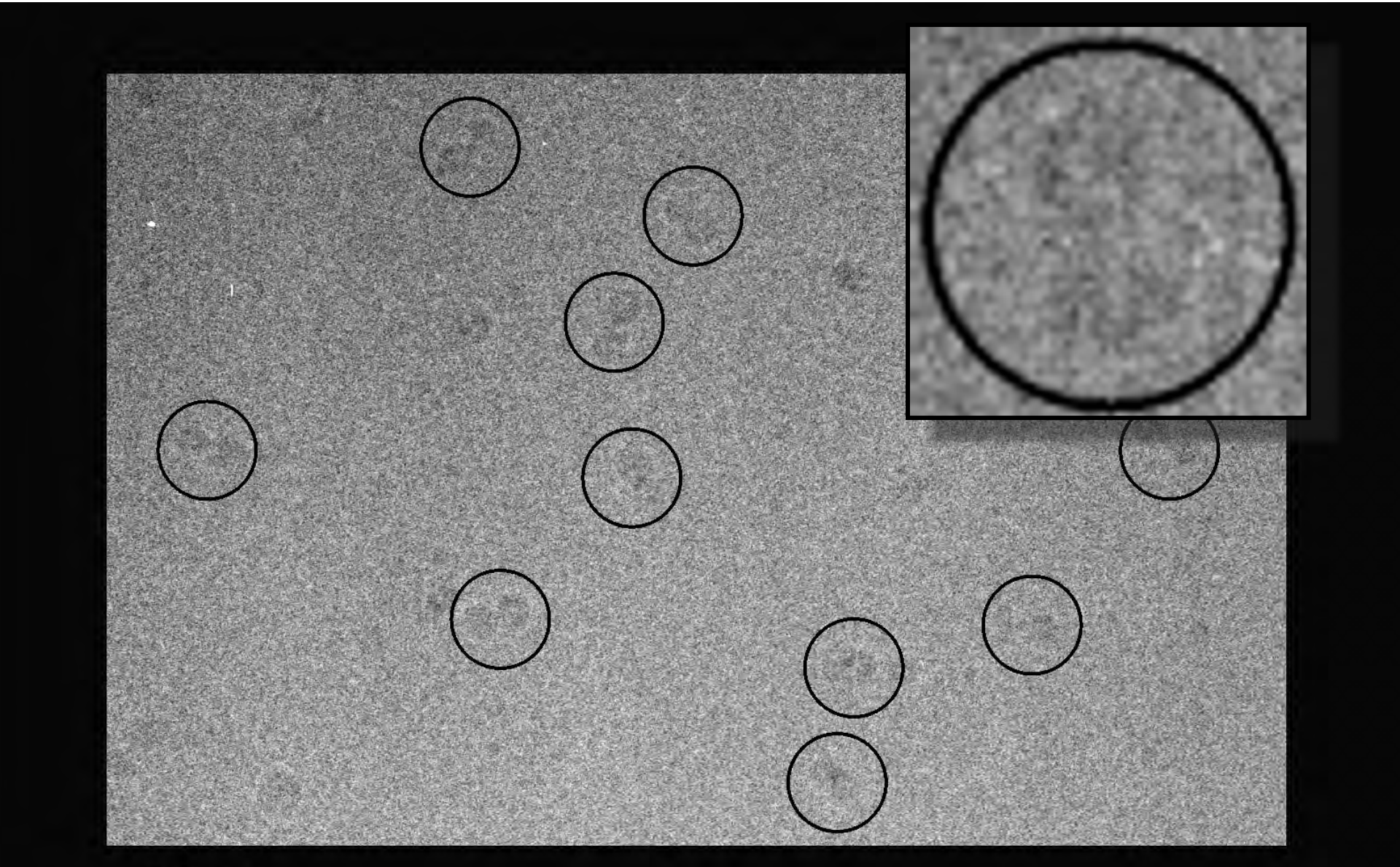
Movie from the Baumeister lab, Max-Planck Institute for Biochemistry

From 2D image to 3D structure

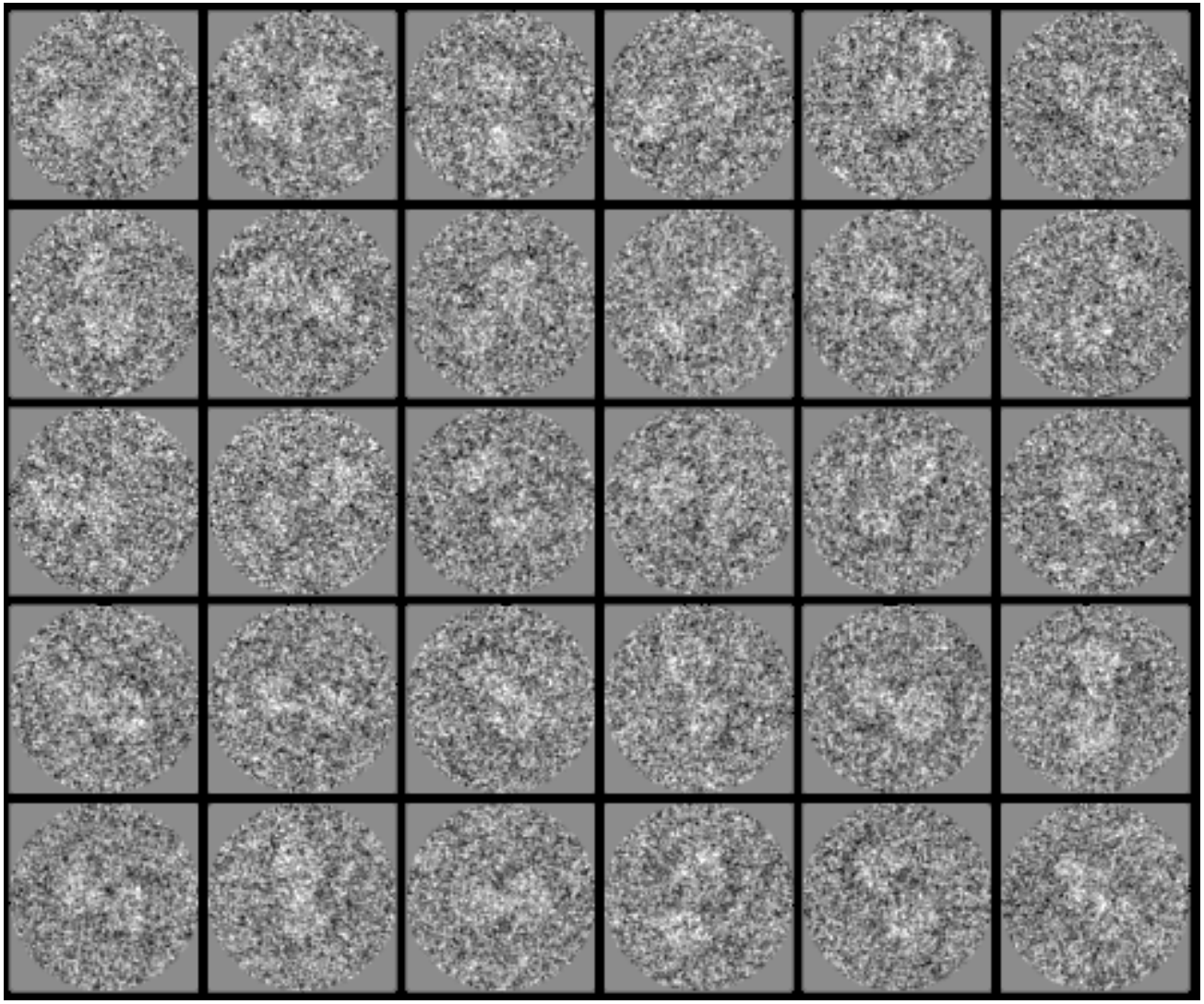
Computational tools for producing a 3D reconstruction from projections

CTF correction
Filtering
Alignment
Classification
Averaging
Angle assignment
3D reconstruction
Refinement
Sharpening/weighting

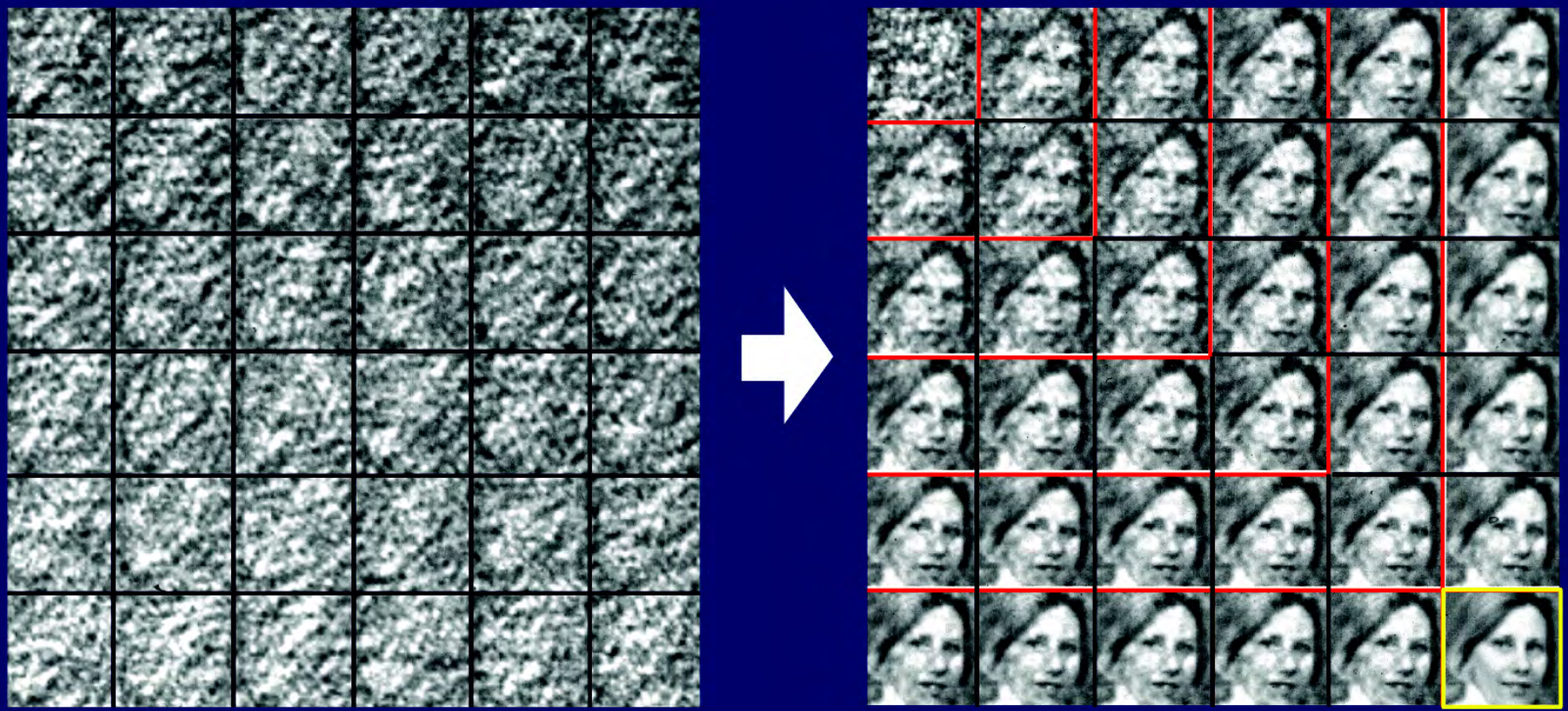
Find the particles within the image



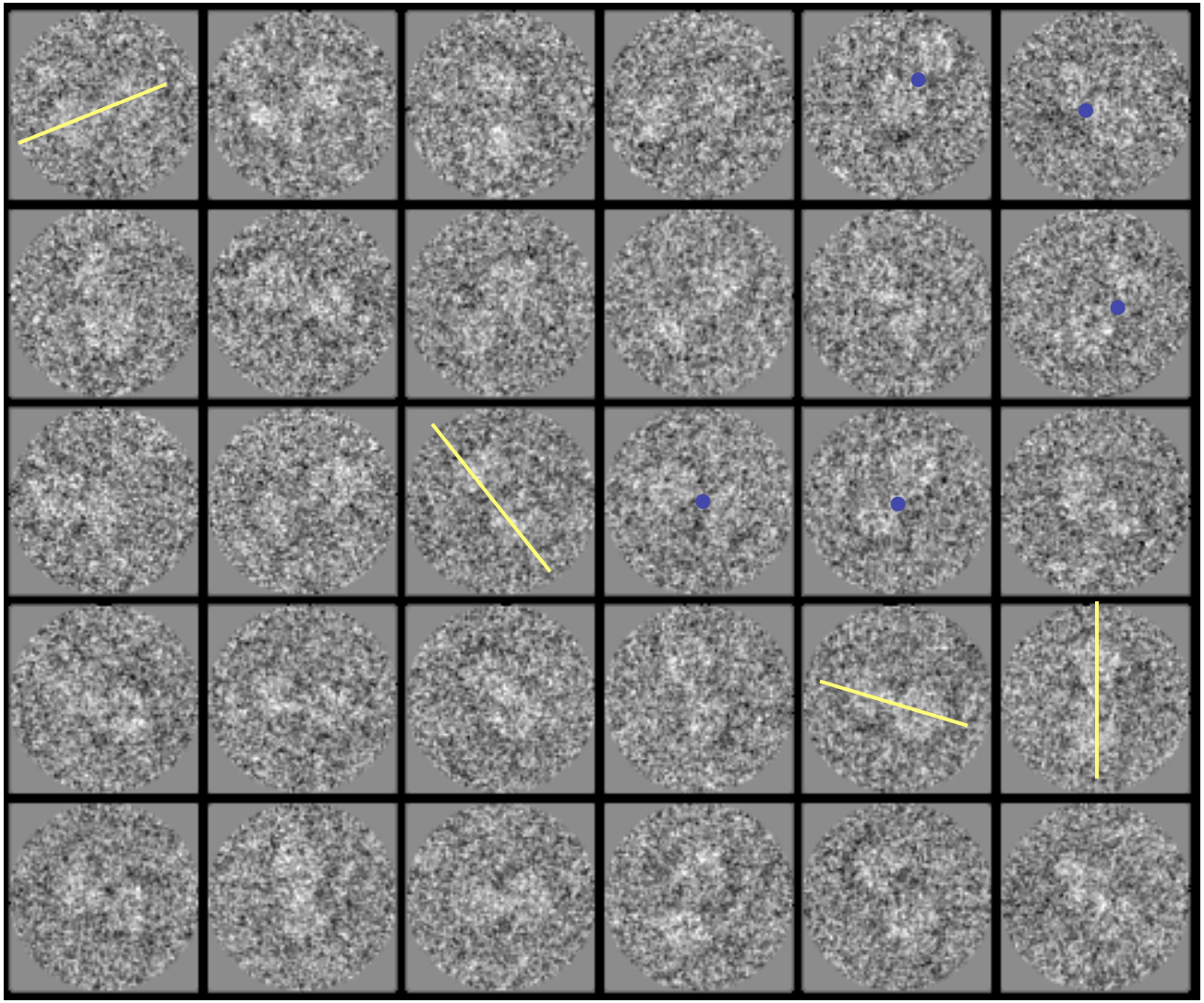
Extract the particles from the image



Averaging is going to require alignment…

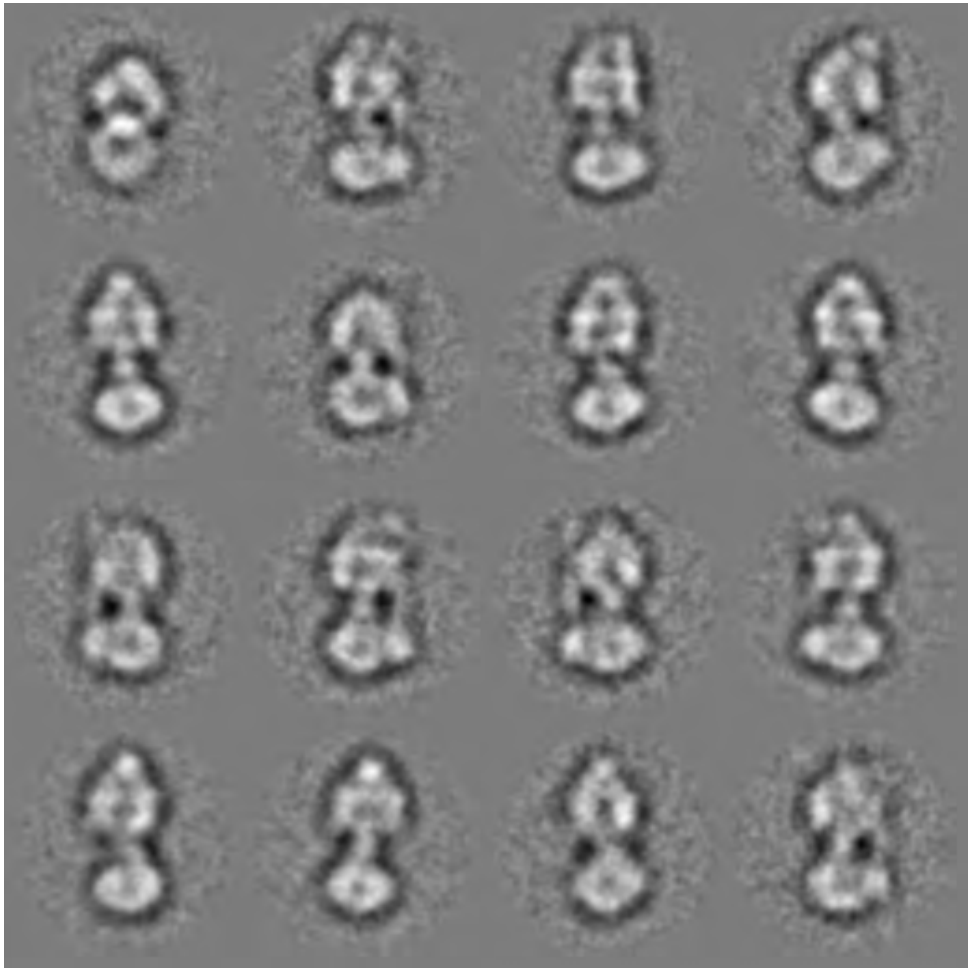


Align the particles – rotationally and translationally



Bettina Boettcher

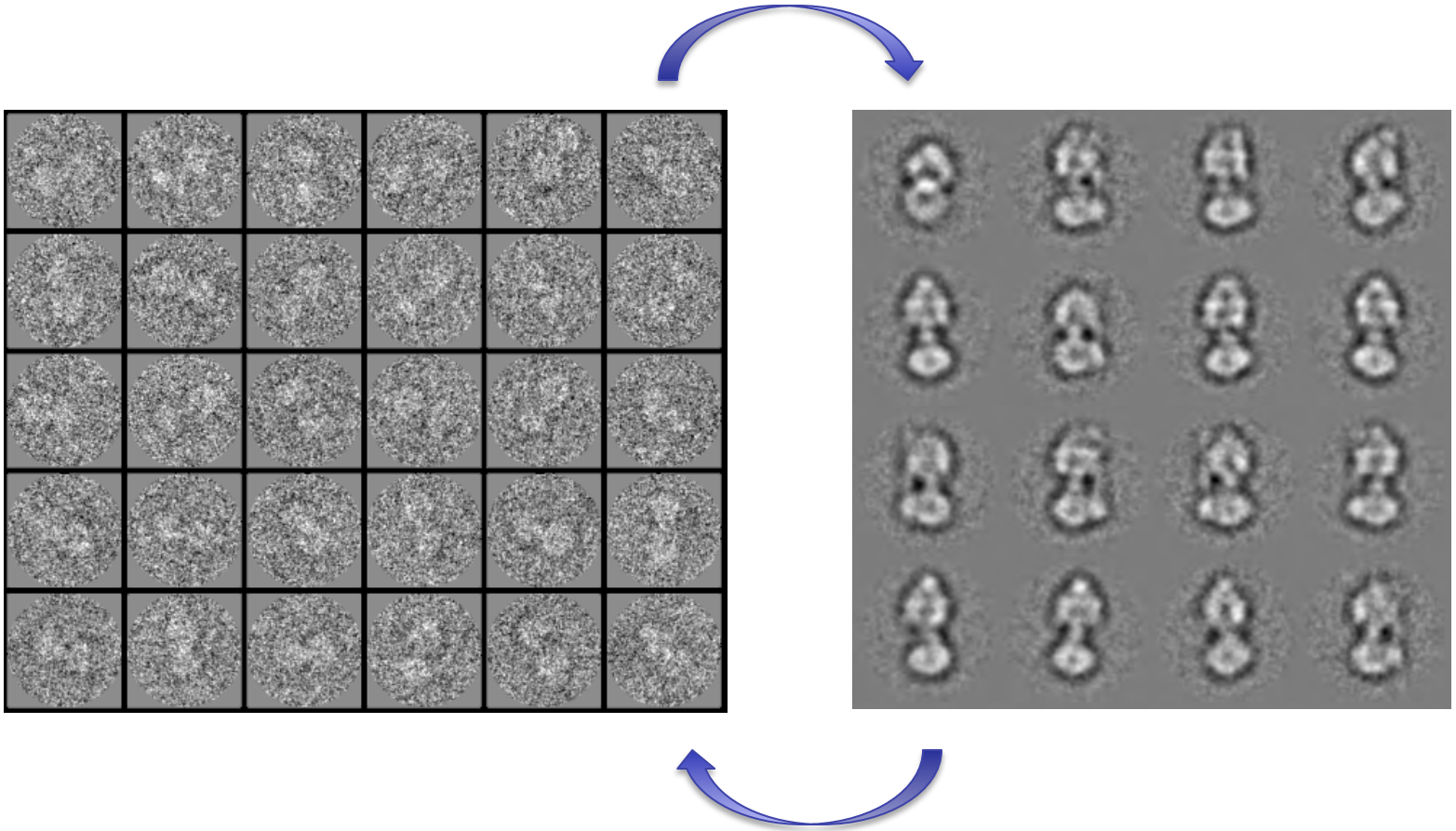
Classify and average similar images:
Example: F-type ATP-Synthase, Bettina Boettcher



Bettina
Boettcher

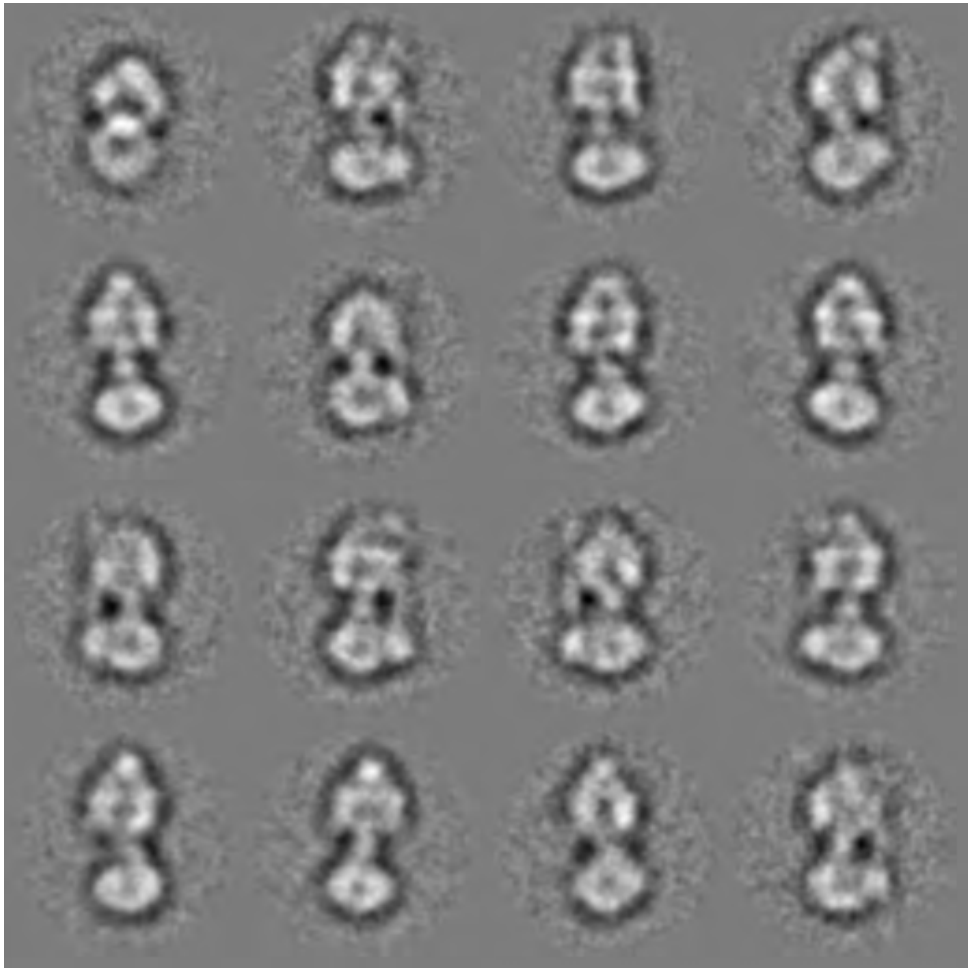
Classify and average similar images:
Example: F-type ATP-Synthase, Bettina Boettcher

And then use to improve the alignment… iteration

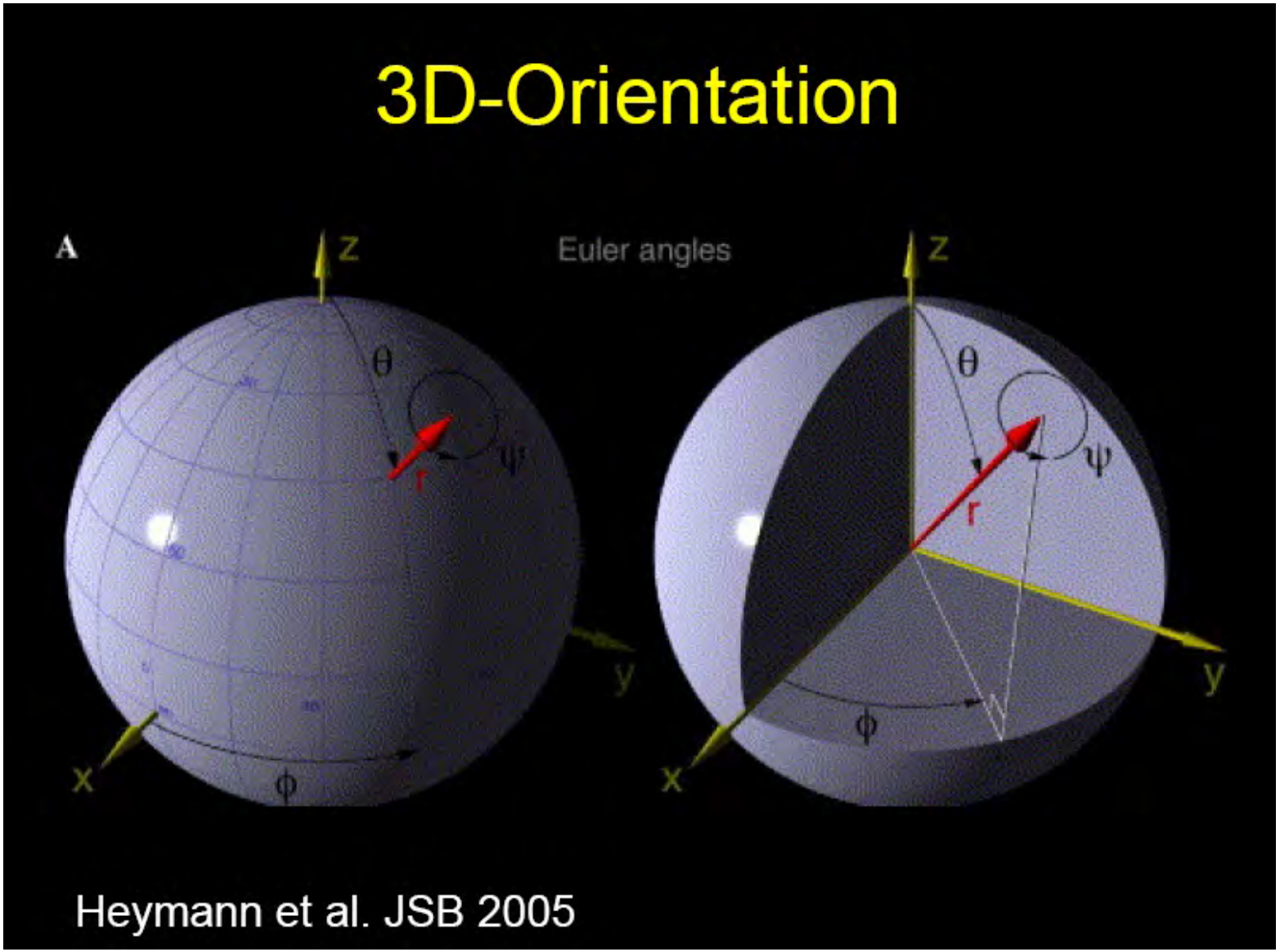


Angle assignment

How to these views all relate to each other?



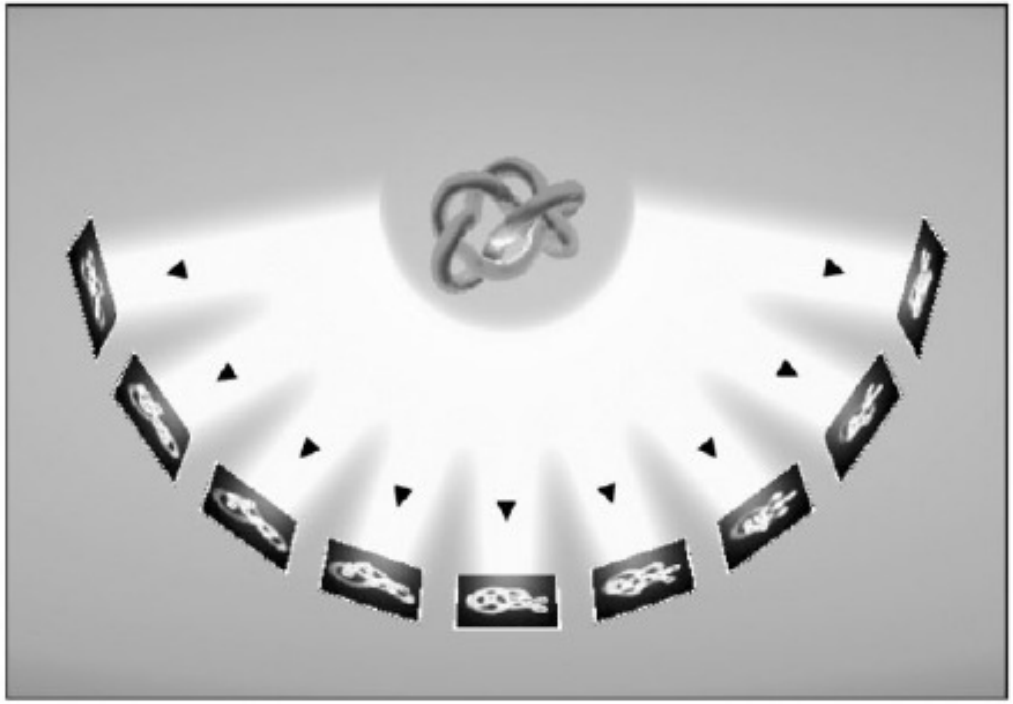
Angle assignment



Slide: Bettina Boettcher

If you have a starting model:

Projecting a model and compare images to the projections

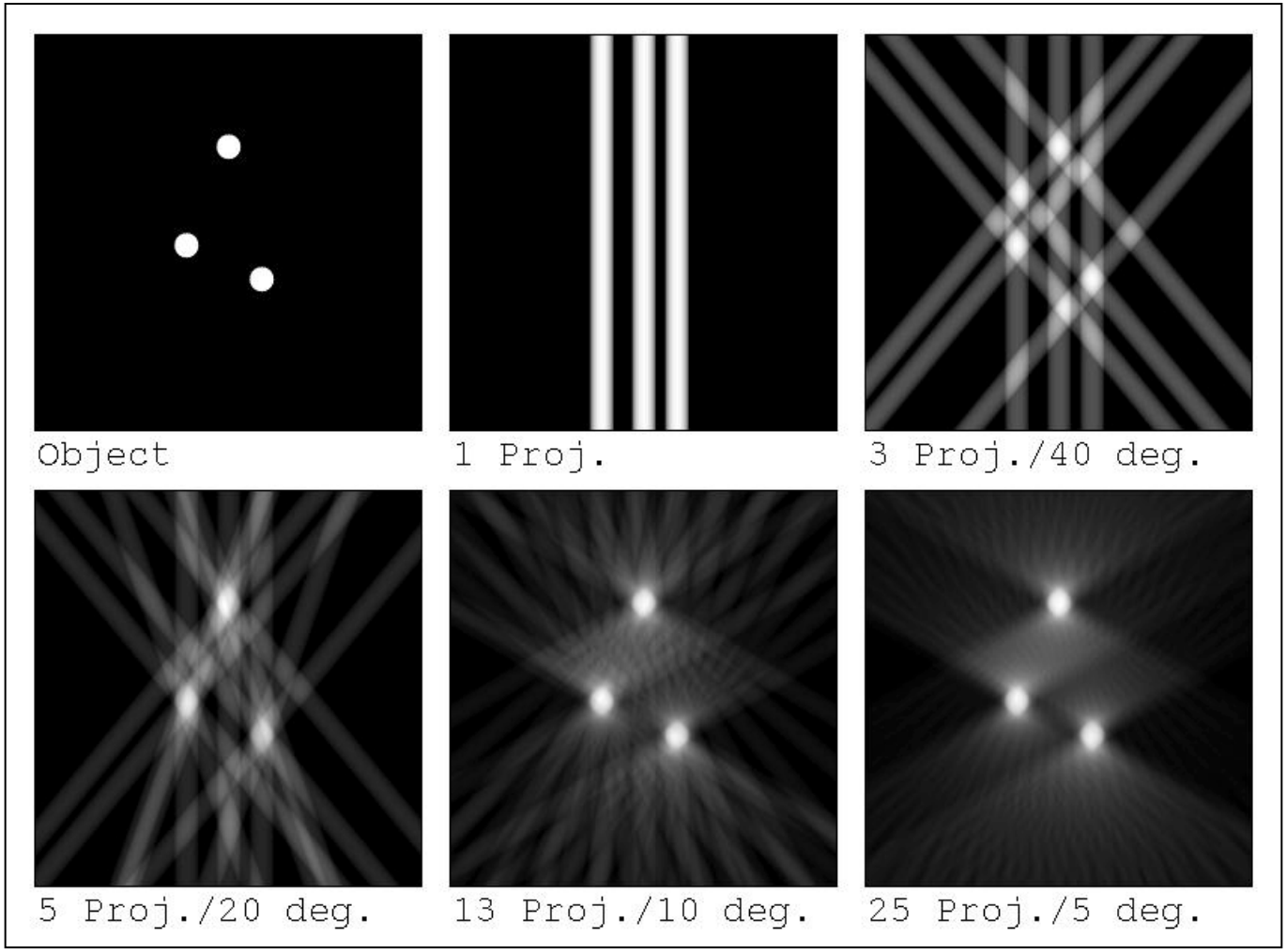


(projection matching)

Next – generate the 3D reconstruction

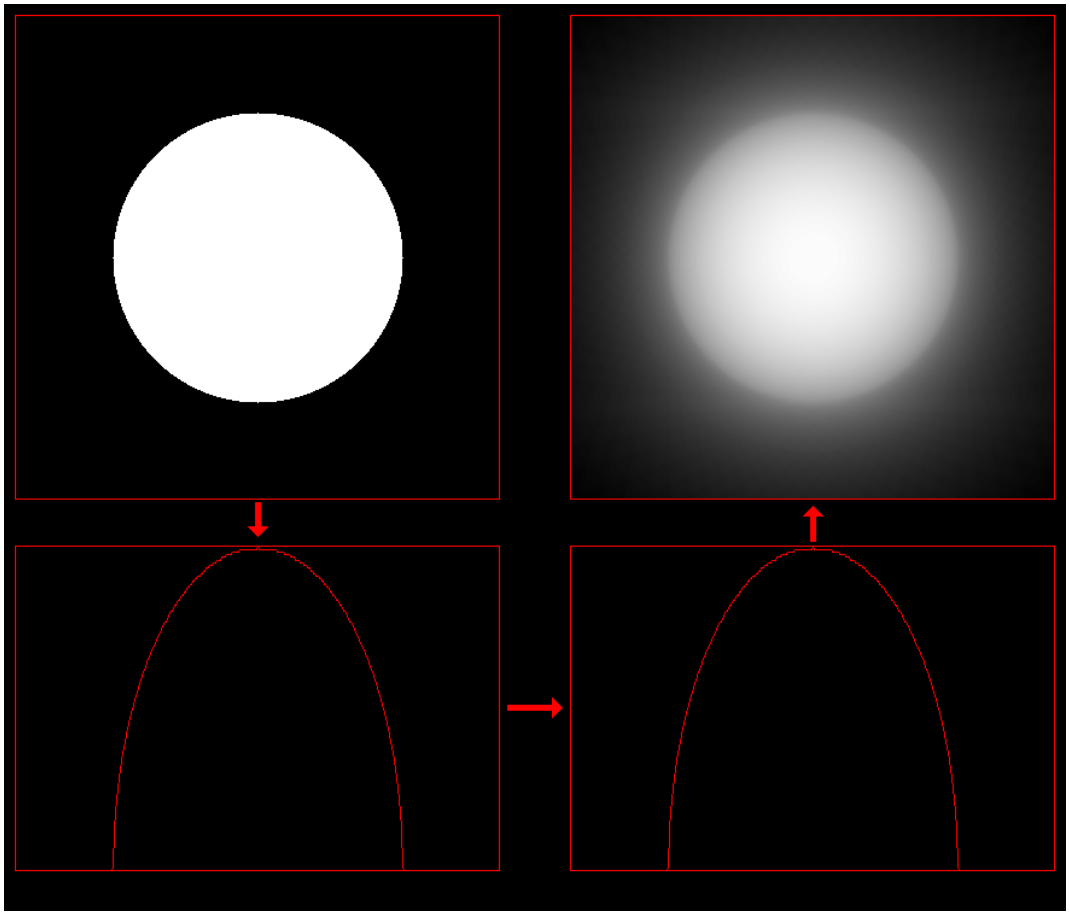
In real space or in Fourier space.

3D reconstruction by back projection



A. Frangakis

3D reconstruction by back projection

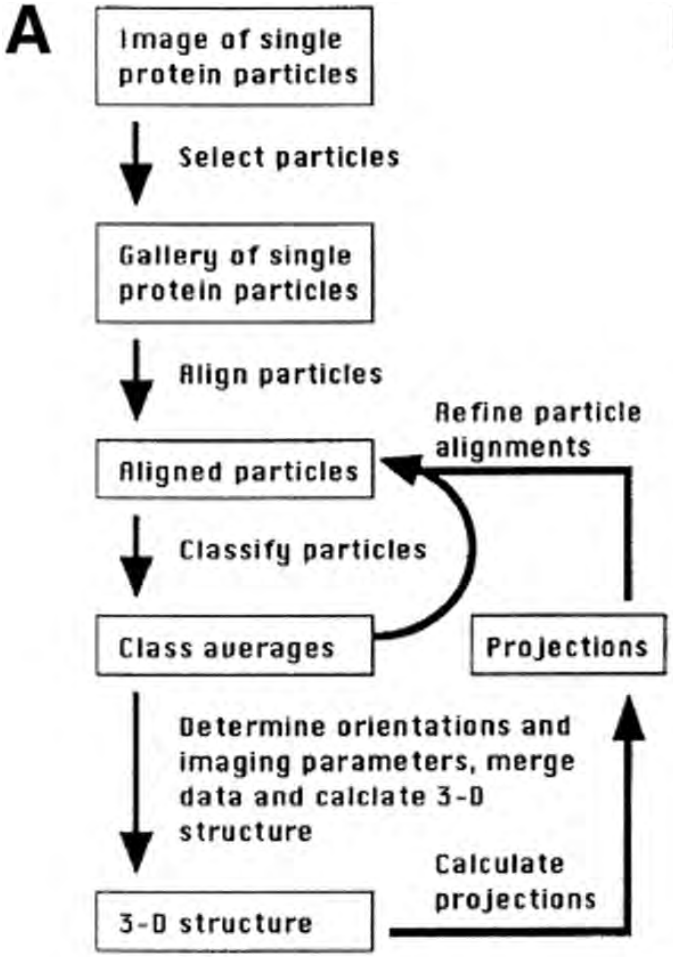


A. Frangakis

Once we have a model, we can compare new images to the model to work out their orientation… and refine our structure to high resolution.

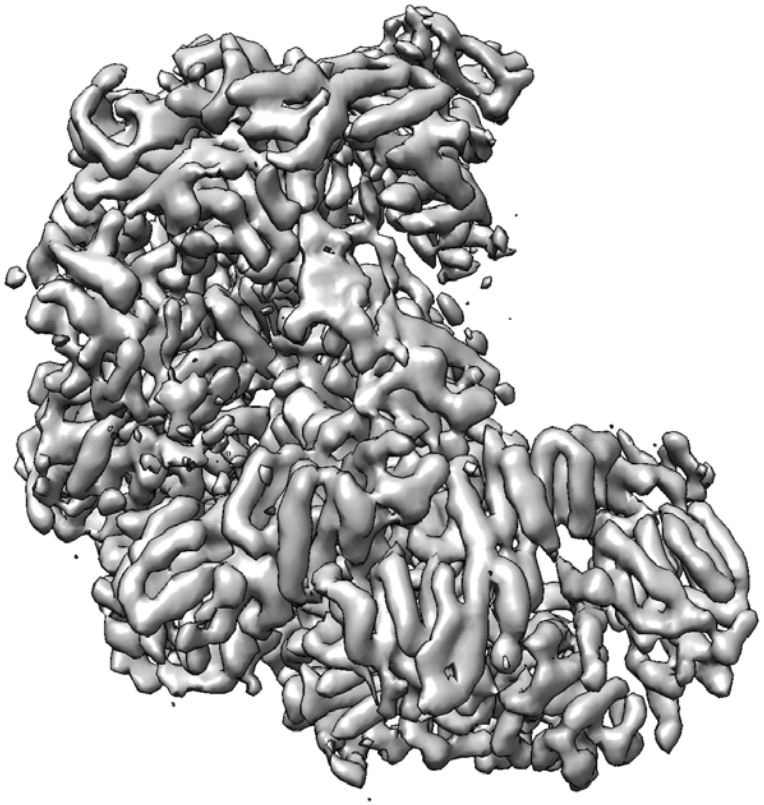
(projection matching)

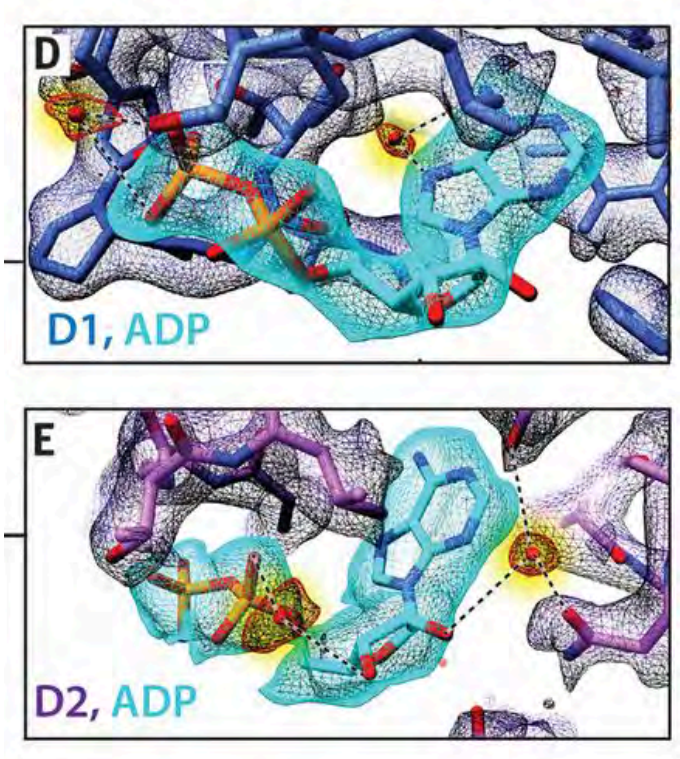
Iterate…



Nogales E , Grigorieff N J Cell Biol 2001;152:F1-F10

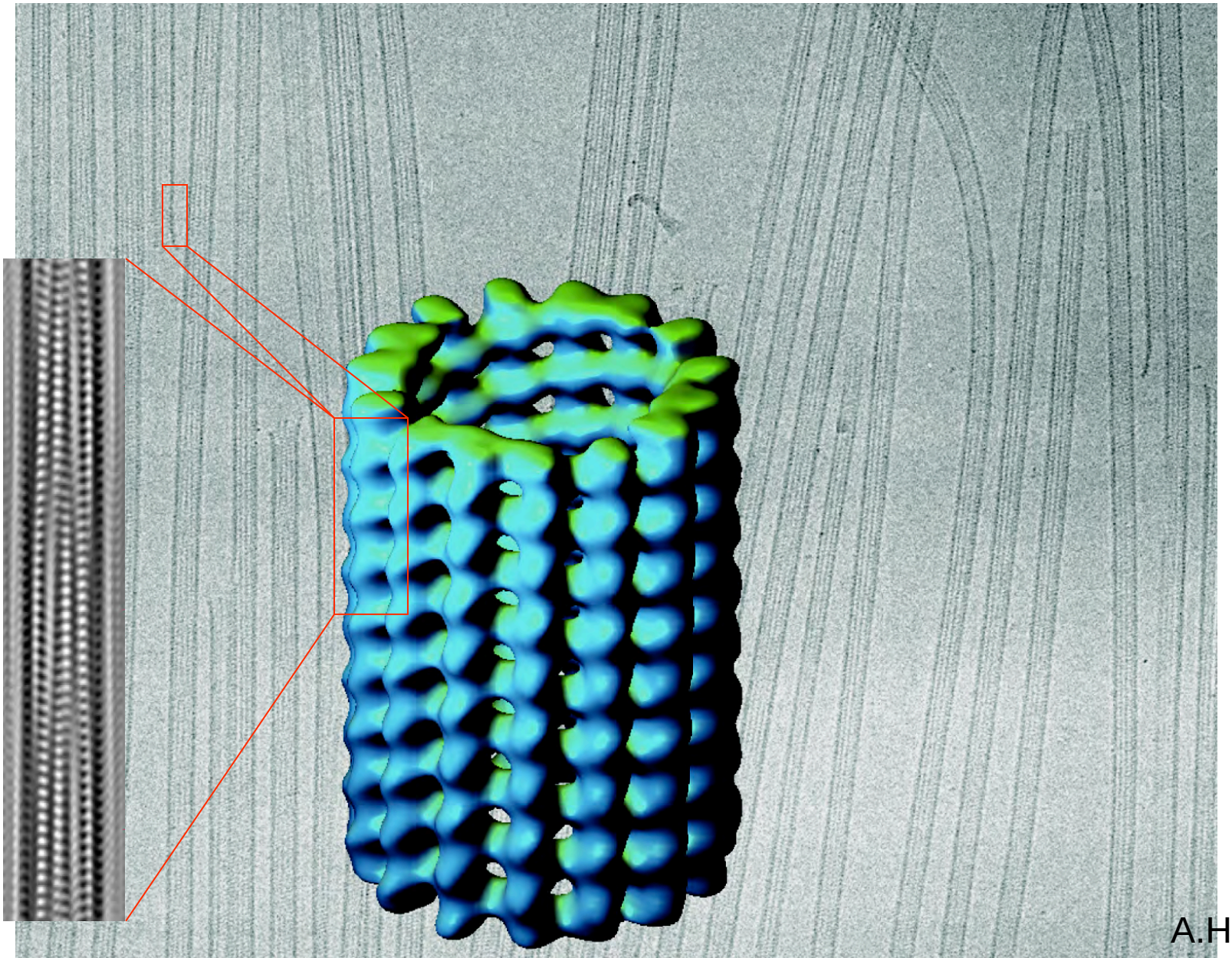
Interpreting structures





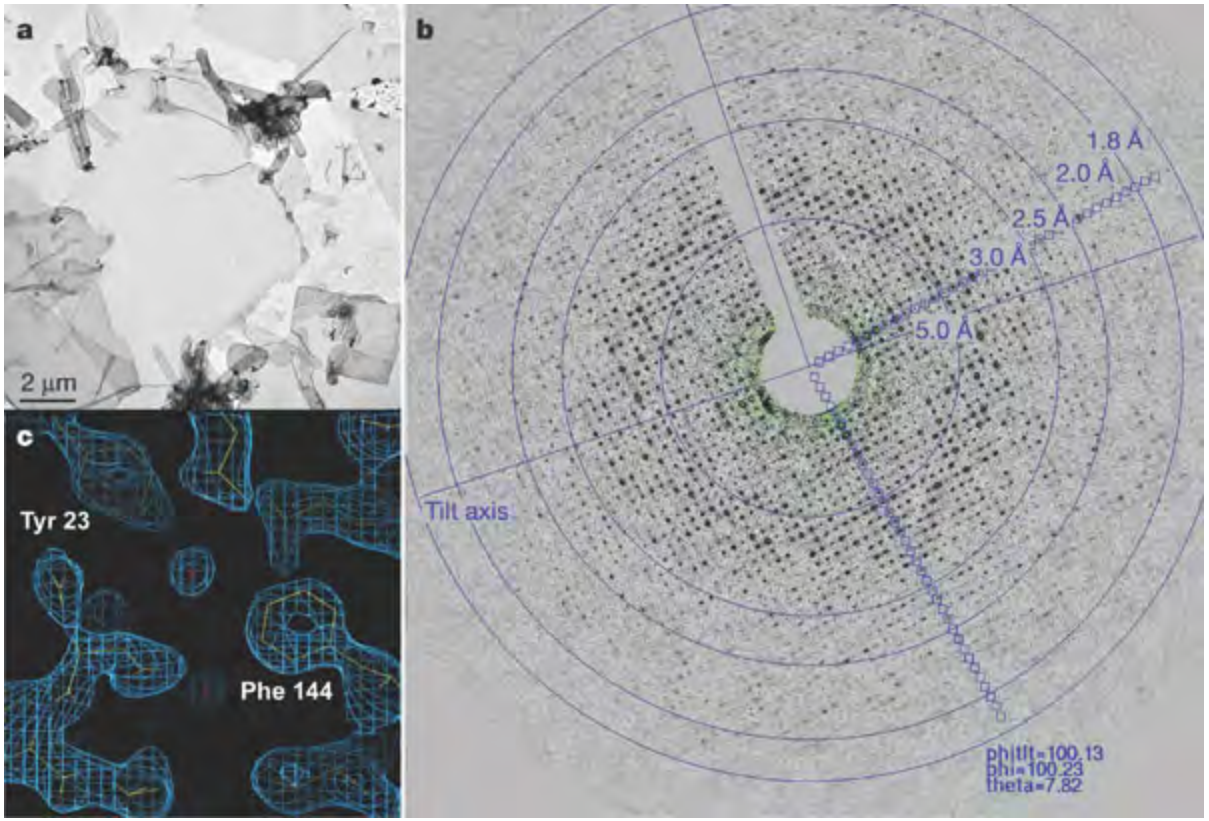
Barford lab

Subramaniam lab

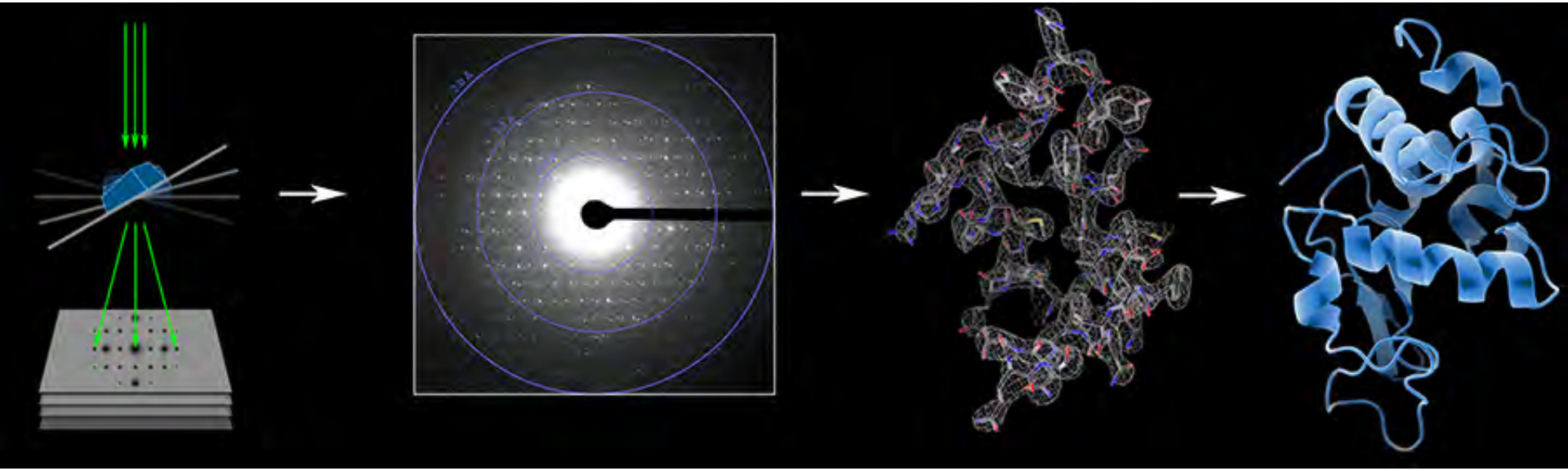


A.Hoenger

2D crystallography



MicroED



Tamir Gonen, Janelia/HHMI

In situ or in vitro?

Complex systems
Components in situ

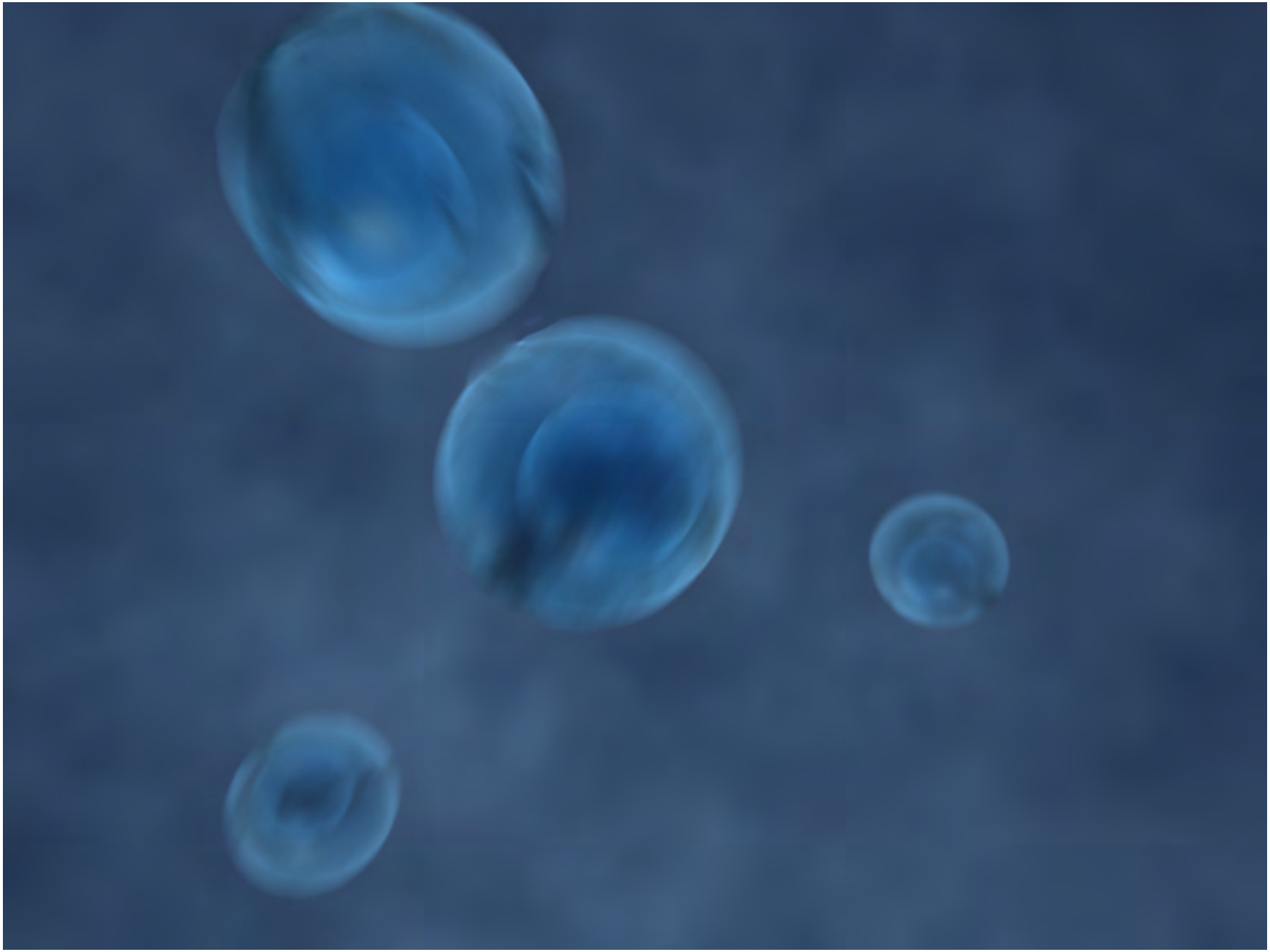
Simple systems
Purified components in vitro

Low-resolution “blobs”

High-resolution detail

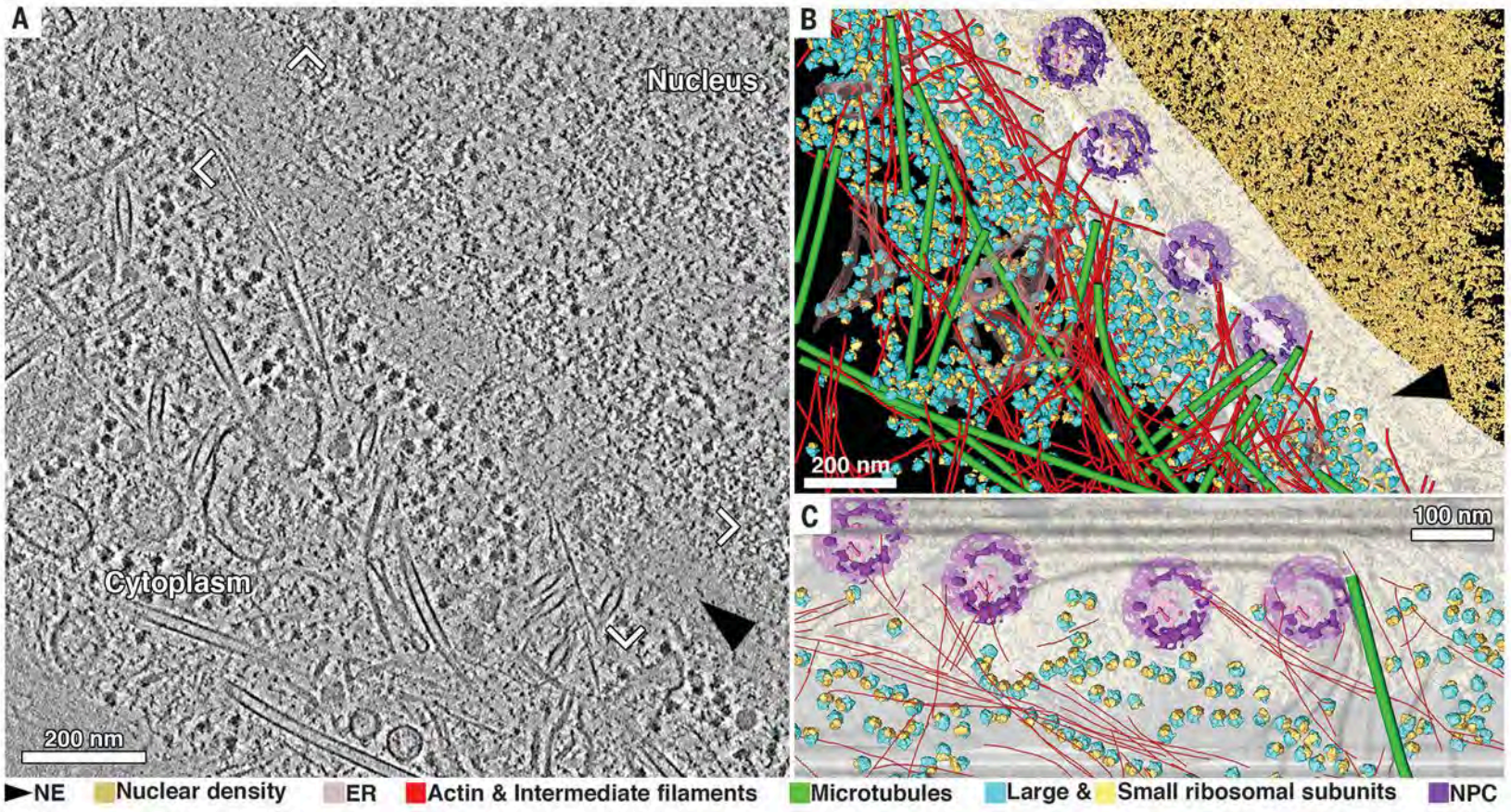
Cryo-electron tomography

Single-particle cryo-EM



Movie from the Baumeister lab, Max-Planck Institute for Biochemistry

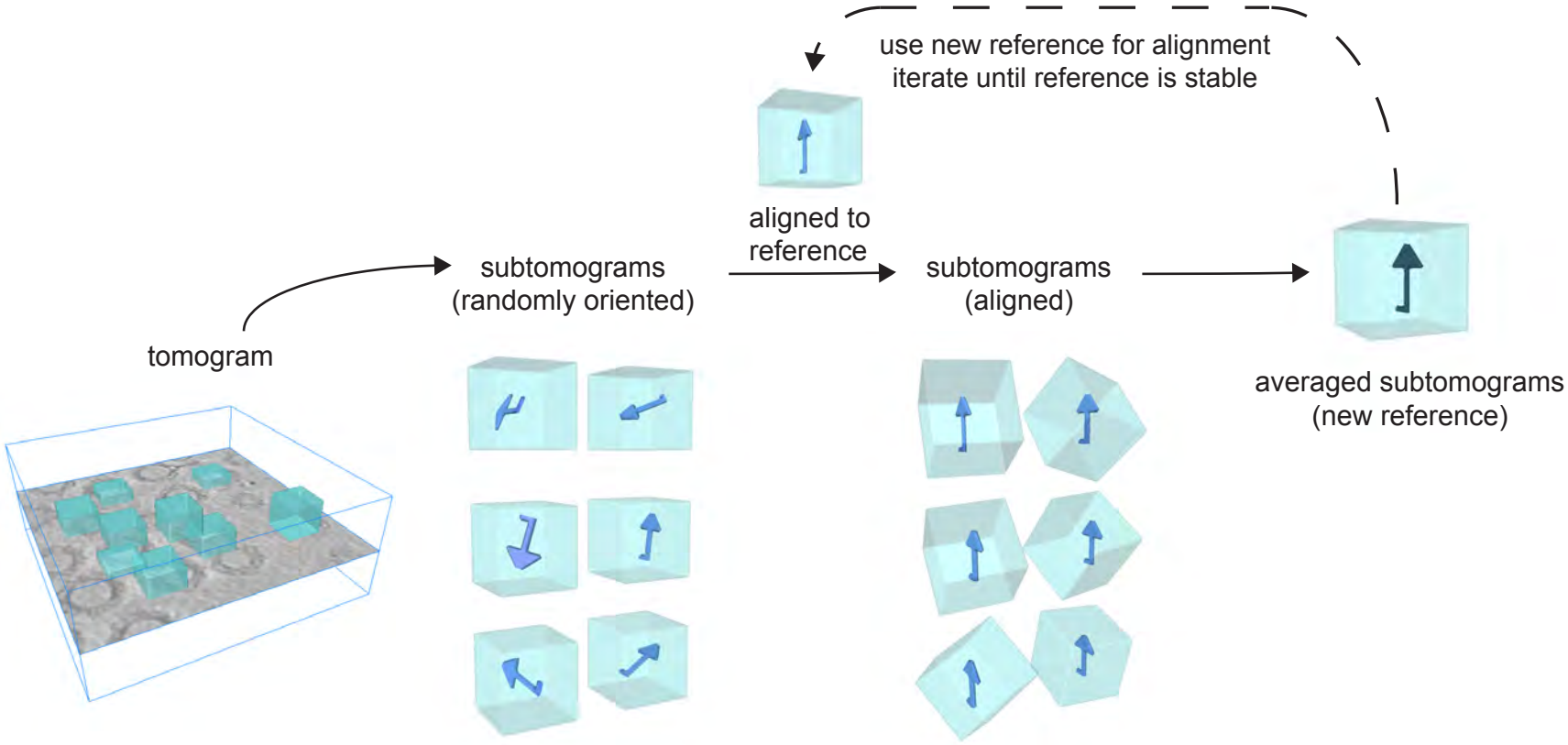
Cryo-electron tomography



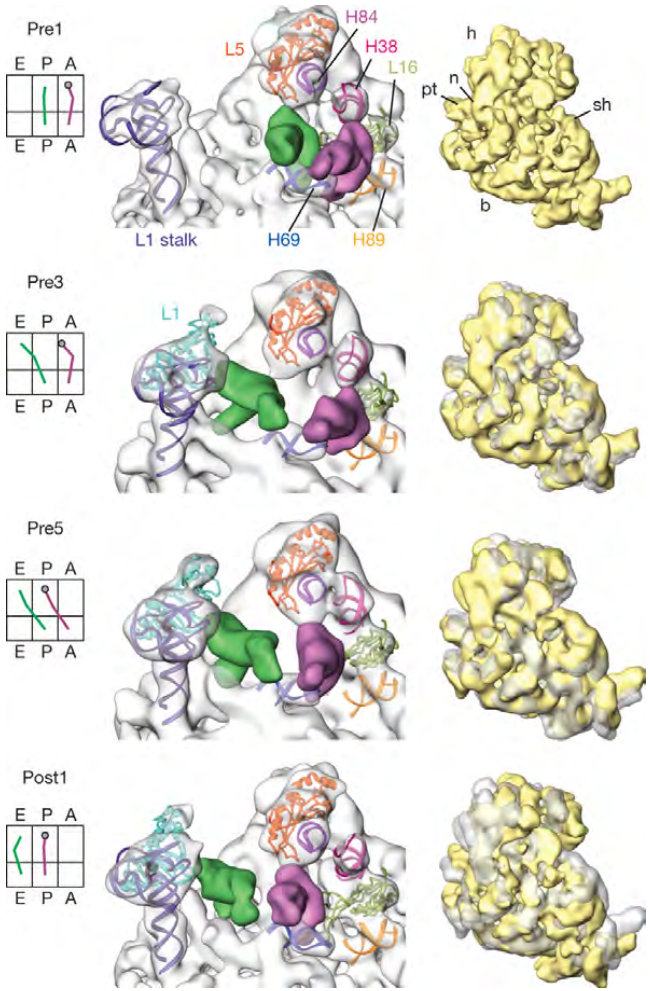
Julia Mahamid et al. Science 2016;351:969-972

Ribosomes at 28 Å resolution

Subtomogram averaging

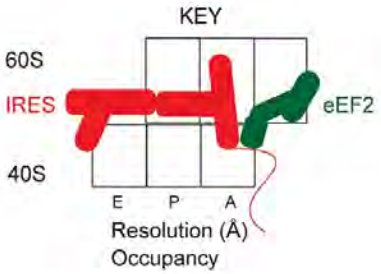


Multiple conformations/states



N Fischer *et al. Nature* 2010

Abeyrathna et al. eLife 2016;5:e14874

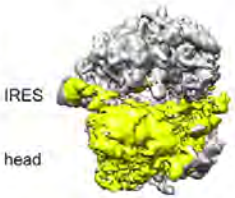


1,105,737 particles 2x binned stack

Alignment and Refinement (5 cycles)

3.5 Å
100%

3D Mask 4x binned stack - 15 classes (100 cycles)



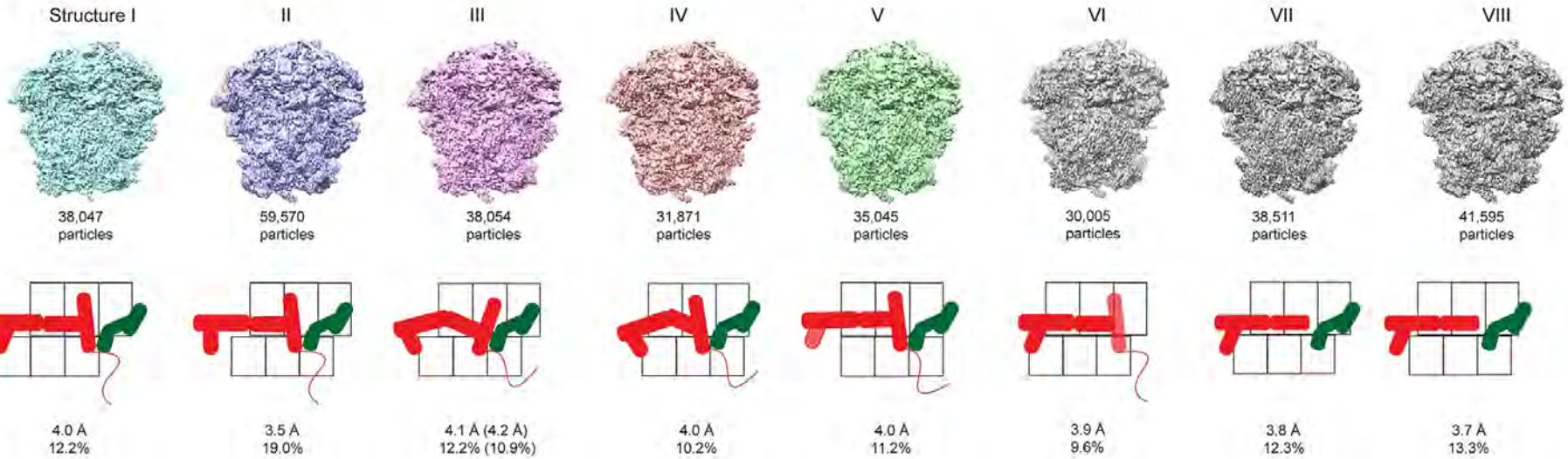
63,573 particles 7.2 Å 5.6%	58,258 particles 7.2 Å 5.3%	44,707 particles 7.4 Å 4.1%	43,839 particles 7.2 Å 4.0%	59,426 particles 7.2 Å 5.4%	42,895 particles 7.4 Å 4.0%	111,447 particles 7.9 Å 9.7%	73,830 particles 9.3 Å 6.7%	32,126 particles 8.2 Å 3.0%	50,759 particles 8.0 Å 4.6%	55,657 particles 7.6 Å 5.0%	55,660 particles 8.2 Å 5.0%	165,965 particles 7.1 Å 15.0%	158,711 particles 7.4 Å 14.3%	88,884 particles 7.6 Å 8.0%

312,698 particles

based on IRES and eEF2 occupancy

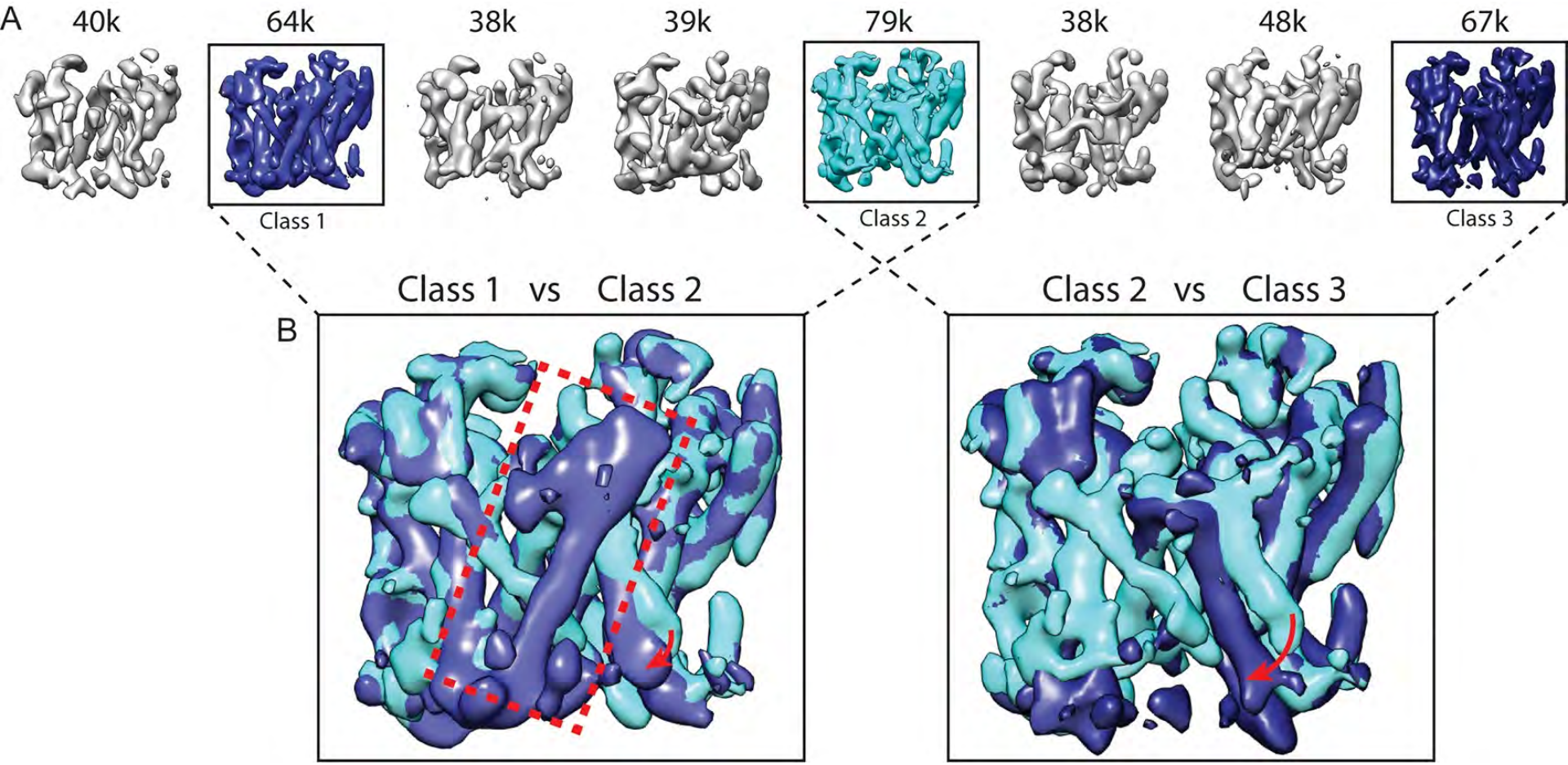
Domain IV eEF2 + PK I (45 Å radius)

Focused mask 4x binned stack - 8 classes (36 cycles)



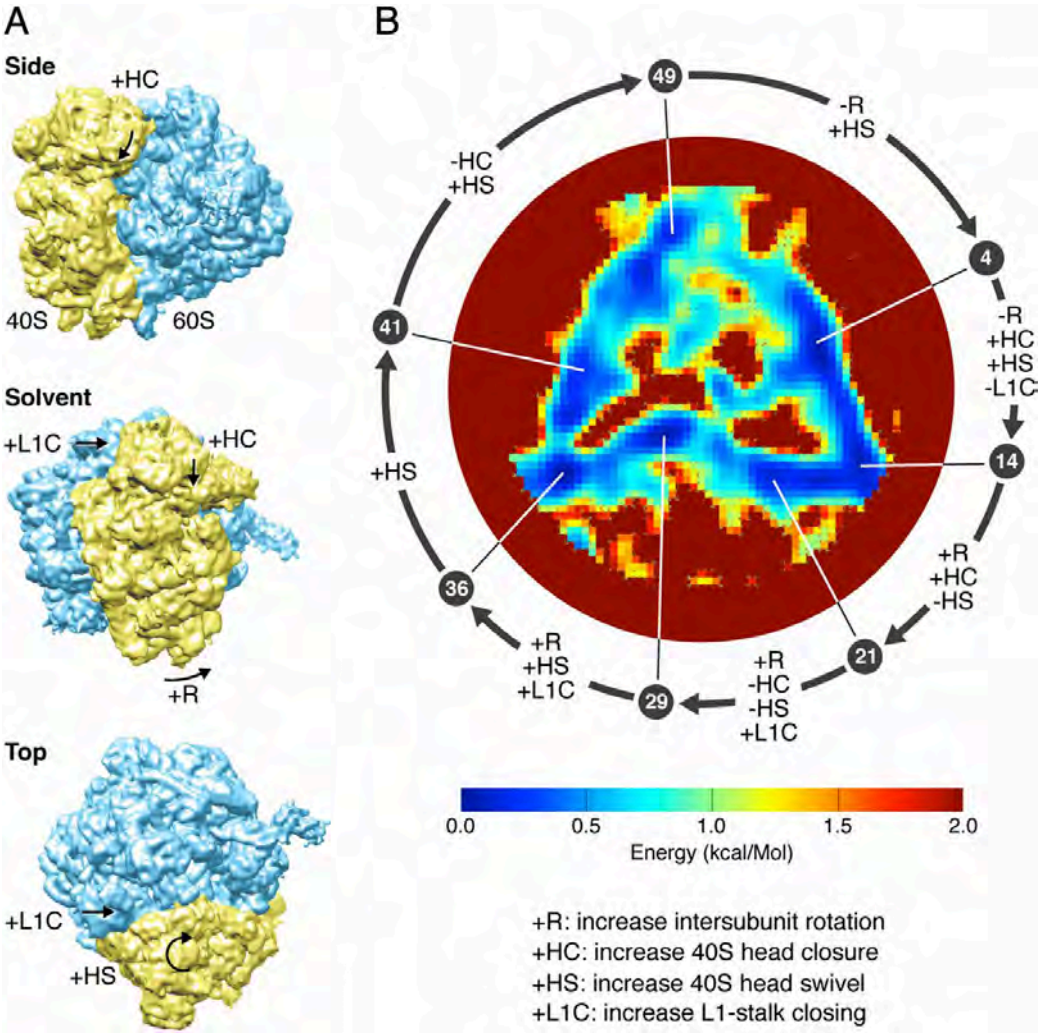
Structure I	II	III	IV	V	VI	VII	VIII
38,047 particles	59,570 particles	38,054 particles	31,871 particles	35,045 particles	30,005 particles	38,511 particles	41,595 particles
4.0 Å 12.2%	3.5 Å 19.0%	4.1 Å (4.2 Å) 12.2% (10.9%)	4.0 Å 10.2%	4.0 Å 11.2%	3.9 Å 9.6%	3.8 Å 12.3%	3.7 Å 13.3%

Multiple conformations/states



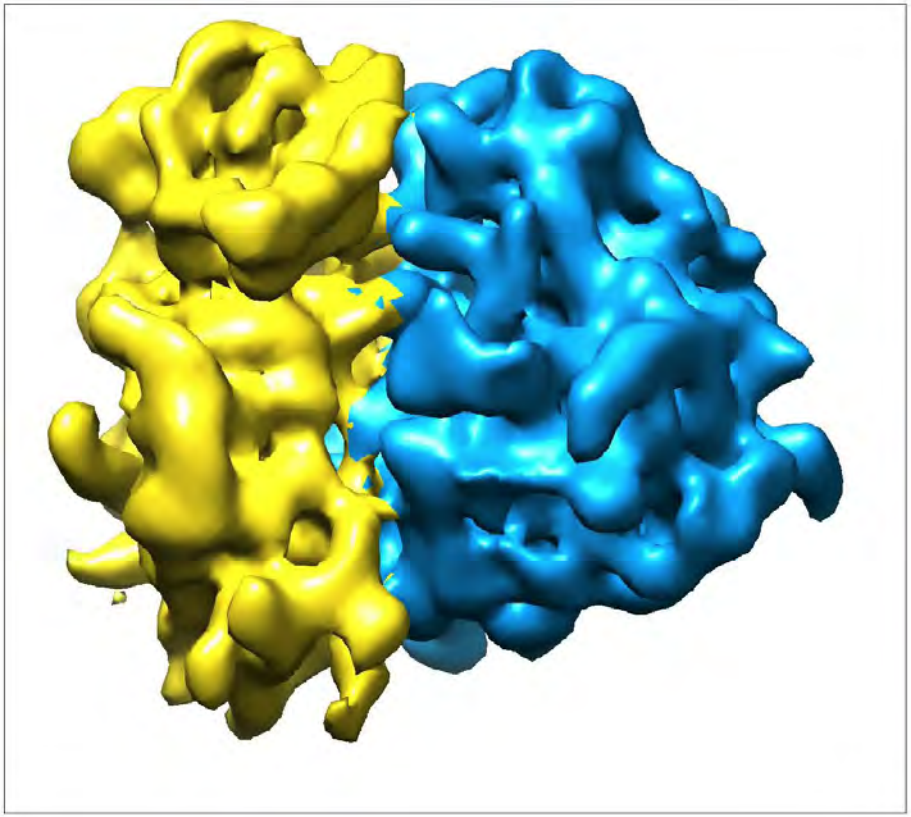
Bai et al. eLife 2015;4:e11182

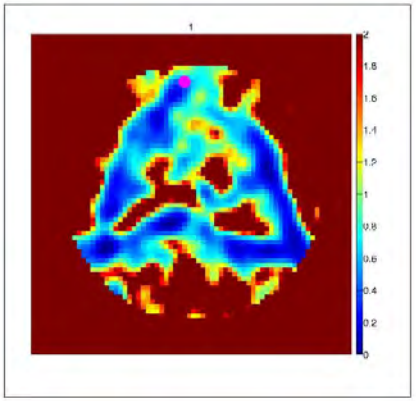
(A) Three views of a cryo-EM map of the 80S ribosome from yeast (32), with arrows indicating four key conformational changes associated with the elongation work cycle of the ribosome.



Ali Dashti et al. PNAS 2014;111:17492-17497

Multiple conformations/states





Ali Dashti et al. PNAS 2014;111:17492-17497

Summary

Cryo-EM can be used to:

Determine structures at a wide range of resolution

Determine structures within complex environments

Determine multiple structures from mixed samples

Determine conformational landscape of proteins, or energy landscape of proteins.