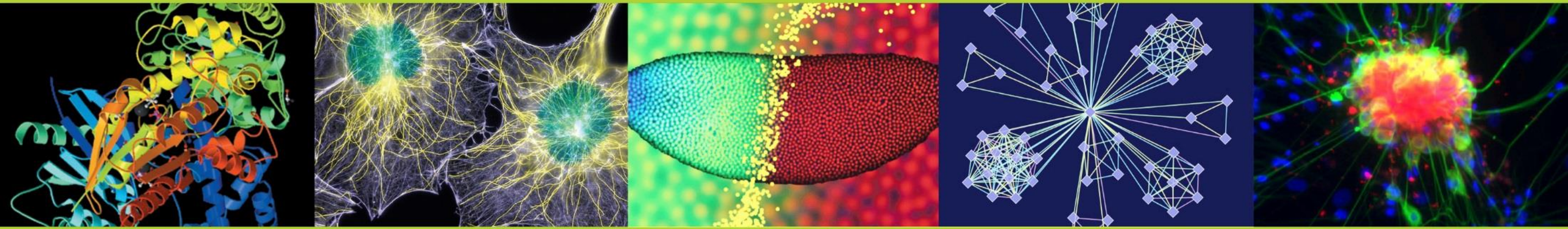
Transformative Potential of High Resolution Cryo-Electron Microscopy

Sponsoring ICOs: NIGMS, NEI, NHLBI, NIDDK, NINDS, ORIP

Interested ICOs: NCI, NIAID, NIDA

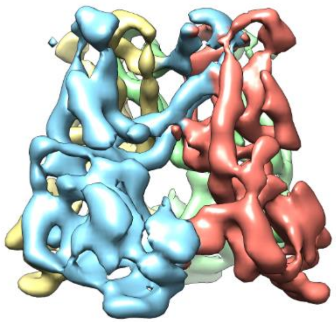


National Institute of General Medical Sciences

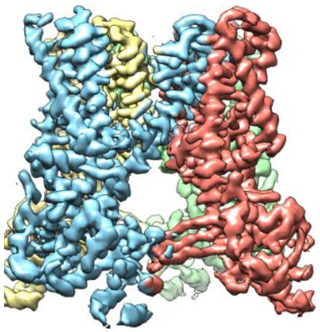
Why Now? New Technological Breakthroughs in Cryo-EM

1) **New electron microscopy technology dramatically improves our ability to see biological molecules**

Old Methods

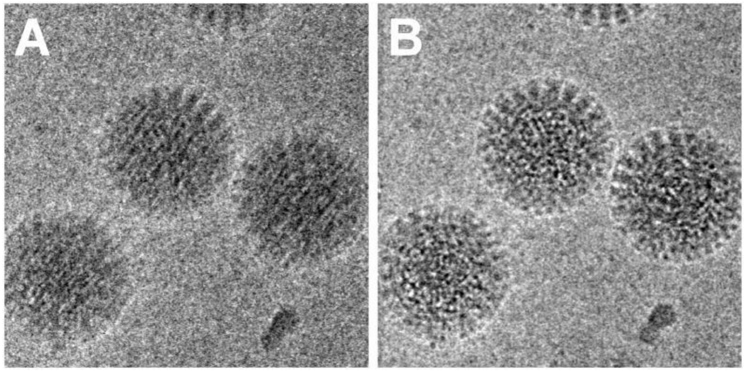


New Methods



TRPV1 Ion Channel:
Mediates burn sensation,
Yifan Cheng UCSF

2) **New motion correction methods resolve blurring of images due to movement of particles in electron beam**



Rotavirus Particles
Niko Grigorieff, Janelia Farms

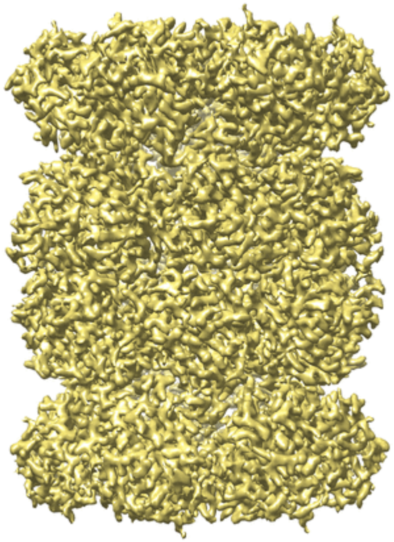
Scientific Opportunities through Cryo-EM

- **Determine structures more rapidly and easily**

> Venki Ramakrishnan: *"It's safe to predict that cryo-EM will largely supersede crystallography."* ***Nature*** (2015)

- **Direct visualization of subcellular structures, *in situ***

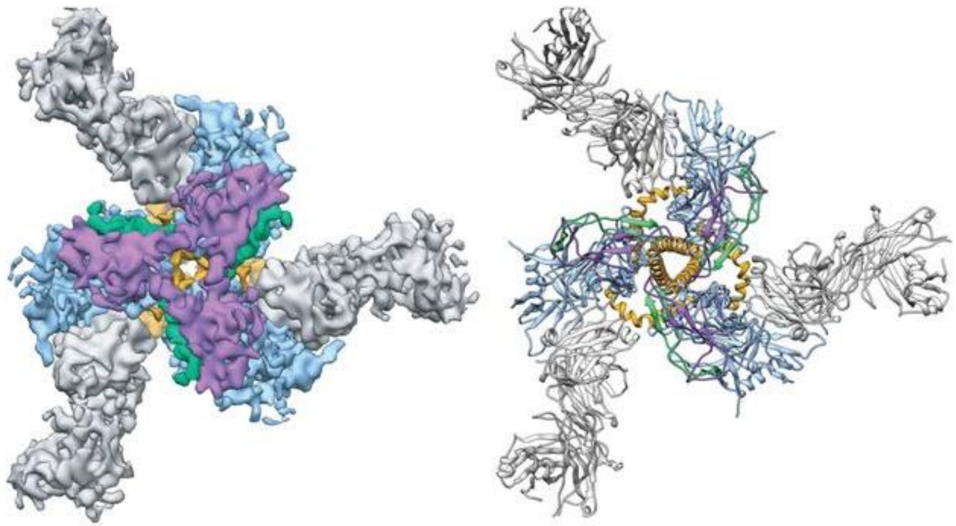
> Richard Henderson: *"If it carries on, and all the technical problems are solved, cryo-EM could indeed become, not just a first choice, but a dominant technology. We are probably halfway there."* ***Nature*** (2015)



2.8 Å structure of proteasome.
Campbell et. al, eLife (2015)

Impacts on Research: Structures of hard to crystallize and complex molecules, such as channels and receptors; elucidating conformational changes in complexes; rapid determination of effects of mutations on structure; structural basis of drug action; structures of molecules determined inside of (or on) cells.

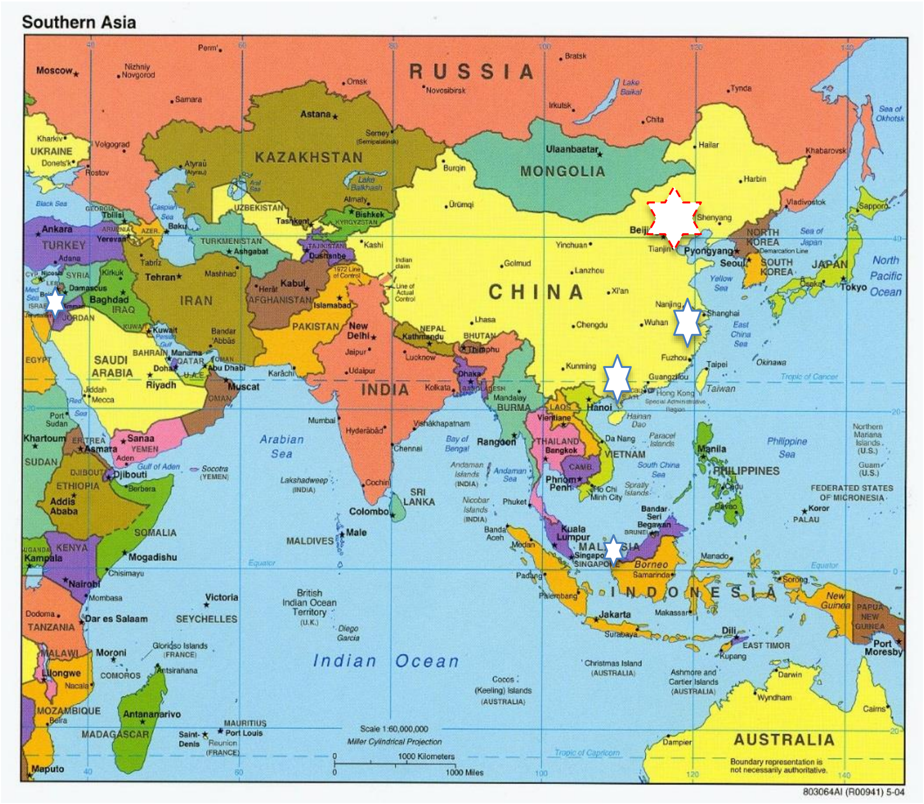
Cryo-EM Was Crucial for Recent Advances Towards an HIV Vaccine



“Another major advance toward developing an effective HIV vaccine came in 2013 when a team of researchers led by John Moore at Weill Cornell Medical College in New York City and Ian Wilson at the Scripps Research Institute in La Jolla, California, obtained an atomic-level image of the HIV envelope trimer, the principal target for broadly neutralizing antibodies.”

-Wayne Koff, *The Scientist*, May 1, 2015

The U.S. Is Falling Behind Asia and Europe in Cryo-EM



- Initial Investment, 1-2 Cryo-EM microscopes, shared facility
- Moderate Investment, 3-4 Cryo-EM microscopes, regional facility
- Significant Investment, 5+ Cryo-EM microscopes, HTP user facility

Challenges for Researchers Today

Infrastructure

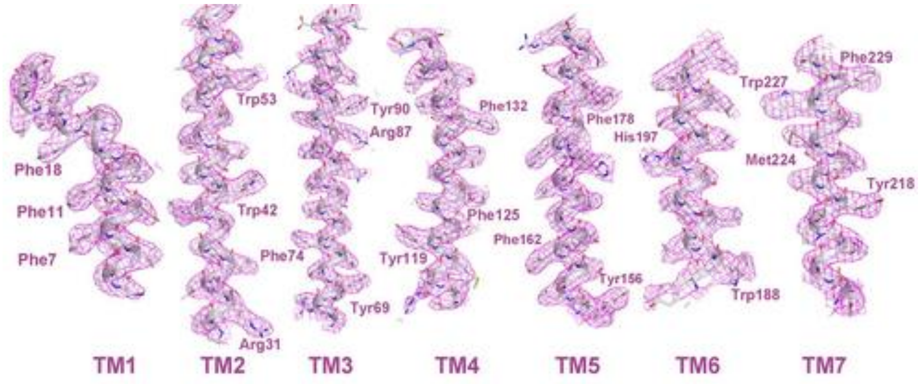
- Current technology only available to a few experts
- Inadequate to take advantage of scientific opportunity

Investigator base

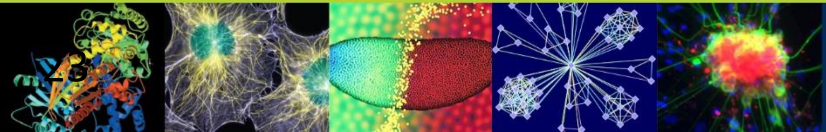
- Workforce bottleneck: major training need
- Crystallographers want to move to EM

Equipment

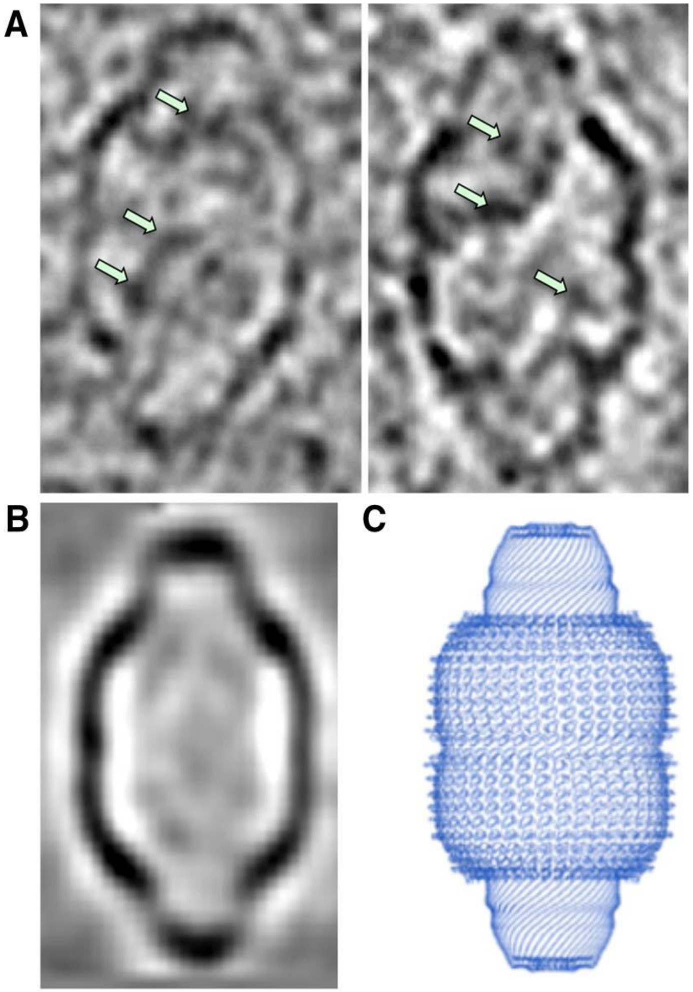
- Expensive, limited numbers
- Inaccessible to most potential users
- Highly inefficient for each institution to buy and maintain its own cryo-EM



3.4 Å EM density map for all seven transmembrane segments of the APH-1 component of γ-secretase. Bai et al., Nature (2015)



Technology Development Needed for Tomography



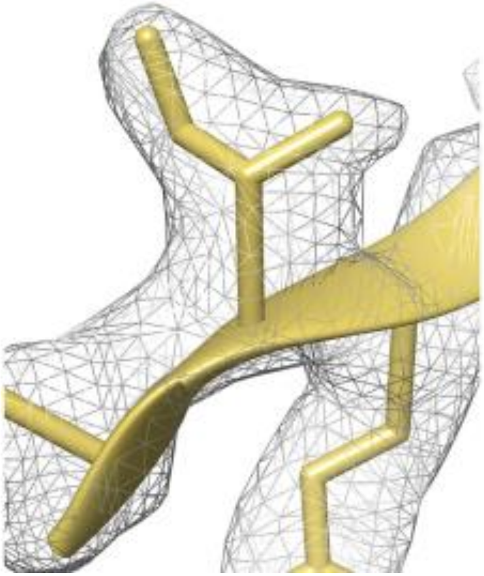
- Reconstruction of the structures of molecules inside of cells
- Recognition of molecules in tomograms is still done largely by eye
- More sensitive, automated, better resolution methods for tomography are needed

Crystal structure of purified rat liver vaults (~13 MDa).
Woodward et al. *Cell. Mol. Life Sci.* (2015)

Short-term Strategy: NIGMS Regional Consortia

NIGMS Regional Consortia Program (RFA- GM-16-001)

- Supports only equipment upgrades for expert laboratories
- No research assistance for screening or computational analysis
- No training



Unambiguous establishment of the rotameric conformation of an isoleucine residue in a 2.8 Å structure of *Thermoplasma acidophilum* 20S proteasome, , Campbel et al., *eLife* (2015)

Long Term Strategy – The Synchrotron Model

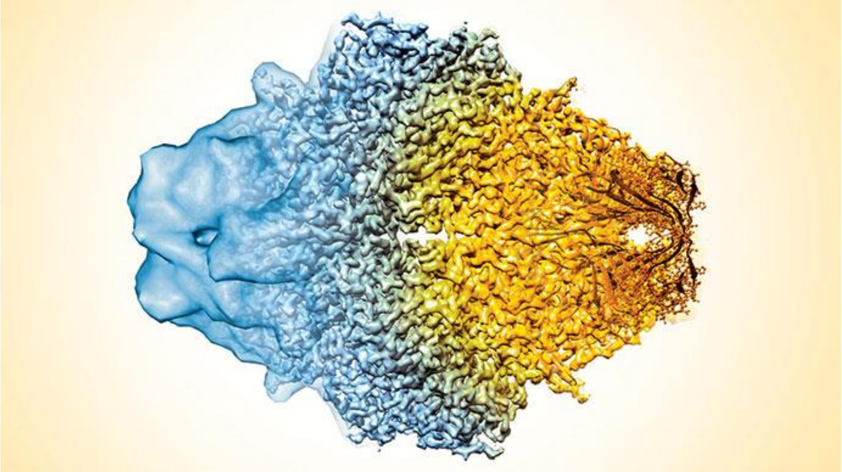
The Synchrotron Model for Cryo-EM

- State of the art regional user facilities
- Access open to all through peer review process
- Training for users
- Professional and technical staff to assist with data collection and analysis; maintain and upgrade equipment; provide training
- Wet lab facilities & lodging
- High-throughput and mail-in services

Advanced Photon Source, Argonne National Laboratory

Goals, Deliverables, Impact

- Move U.S. to the forefront of cryo-EM research
- Provide efficient and economical access to cryo-EM technologies and training: create economies of scale
- Develop new technologies and computational methods to lower cost, improve resolution, and increase throughput and ease of use
- Push the frontiers of *in situ* Cryo-EM (tomography)



BETTER RESOLUTION
This composite image of the protein β-galactosidase shows how cryo-EM has progressed over the years, from the indistinct blobs once obtained with the technique (left) to the nearly 2-Å-resolution structures possible today (right).
Credit: Sriram Subramaniam/NCI

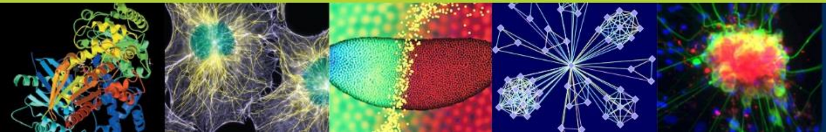
Draft Proposed Budget

3 Comprehensive Centers

		Year 1	Year 2	Year 3	Years 4-5	5 Year Total
Equipment	4 microscopes @ 3 centers	$22M	$22M	$ 22M	0	**$66M**
Operating Cost	Staff, facilities, maintenance	$4M	$6.4M	$8.7M	$7.1M	**$33.3M**
Training Cost	3 FTEs @ 3 centers	$0.6M	$1.2M	$1.8M	$1.8M	**$7.2M**
		$26.6M	**$29.6M**	**$32.5M**	**$8.9M**	**$106.5M**

Investigator-Initiated Research

	Activity	TC yearly	5 Year Total
Cryoelectron Tomography TR&D	R21, R01	$5M	**$25M**
Single Particle Analysis CryoEM TR&D	R21, R01	$2.5M	**$12.5M**
		$7.5M	**$37.5M**



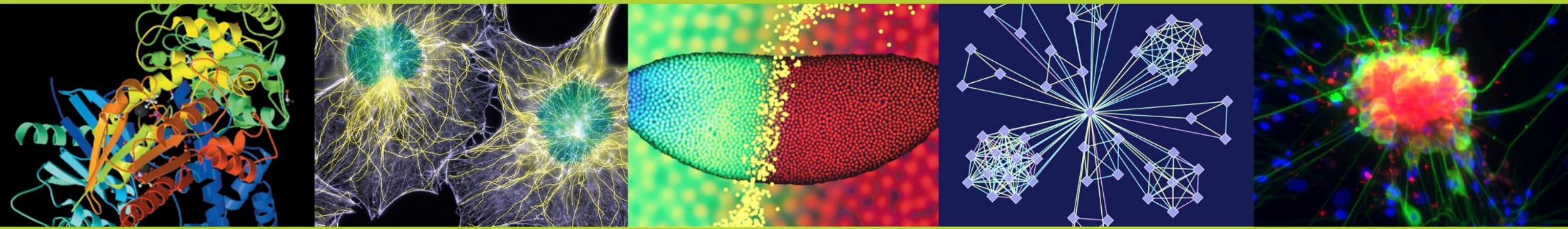
Sustainability Plan

- Depending on future needs and technological developments, we could enhance or expand the number of regional facilities in a second phase of Common Fund support.

- Support for regional facility operations and maintenance would shift from the Common Fund to ICs, other federal agencies (e.g., NSF, DoE, DoD), other funders (e.g., HHMI) and industry.
    - Analogous to current model for supporting synchrotrons

Thank You!

Questions?



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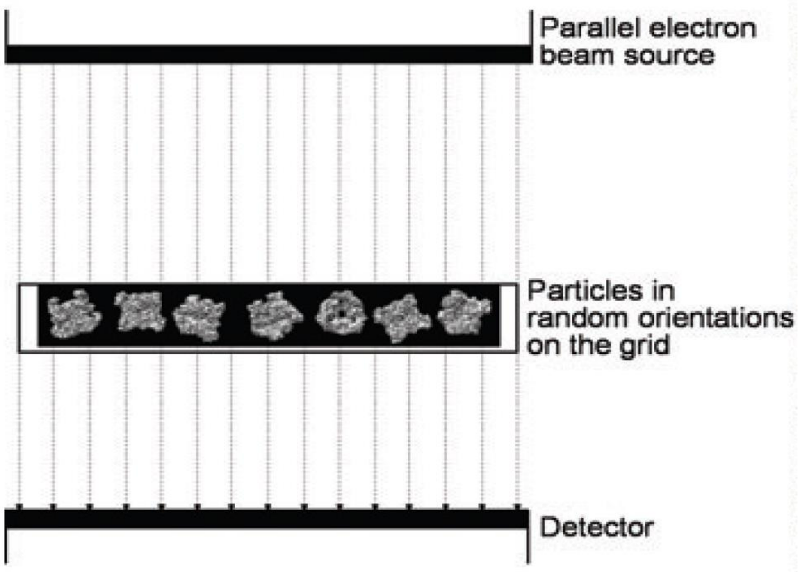
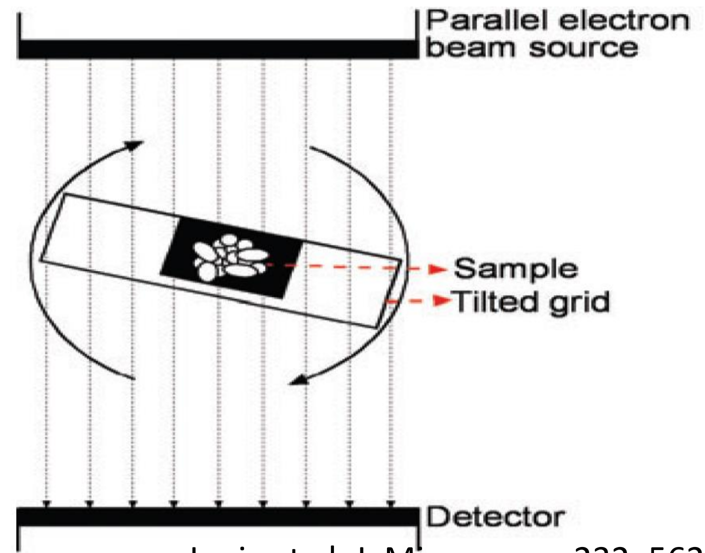
EXTRA SLIDE

Technology Development for Tomography





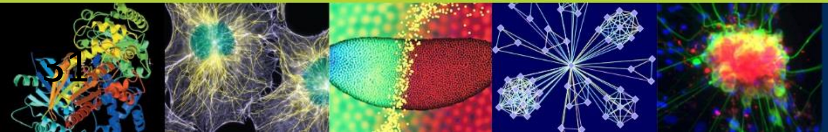
Jonic et al. J. Microscopy, 232, 562, 2008

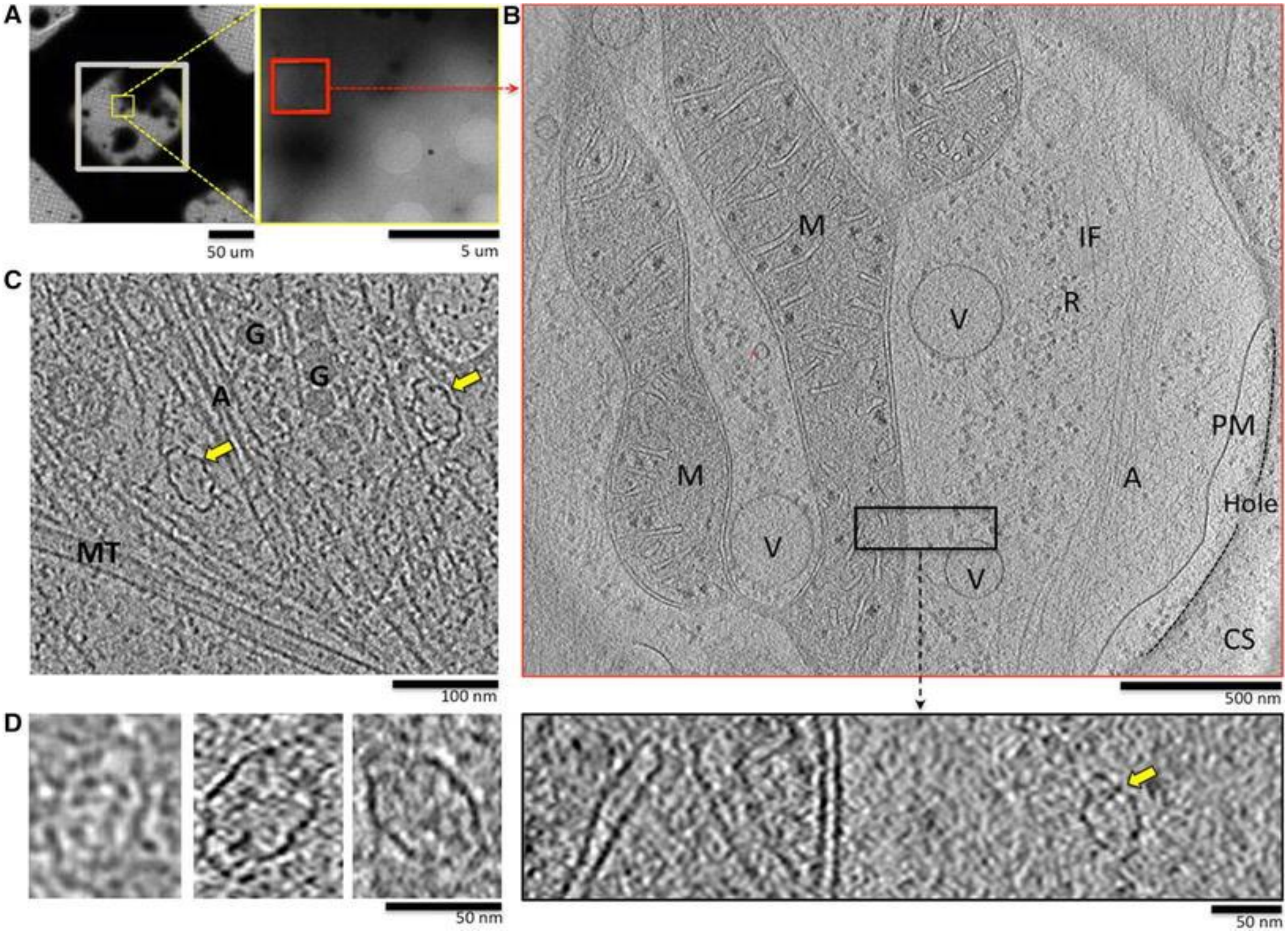
Single Particle Reconstruction

For molecules in ice. Many particles, one orientation and image per particle, low electron dose, high resolution. Particles must all be the same. Ultimate achievable resolution 2Å or better.

Tomographic Reconstruction

For frozen hydrated cells. All images (~100) are recorded from the same specimen. One-of-a-kind objects. High electron dose. Ultimate achievable resolution will be limited by radiation damage (15-20Å?).





Woodward et al. Cell. Mol. Life Sci. 72, 3401, 2015

Tomographic reconstruction of frozen hydrated human cells. *A* actin, *G* granule, *IF* intermediate filament, *M* mitochondria, *MT* microtubule, *PM* plasma membrane, *R* ribosomes, *V* vesicle, *CS* edge of carbon support hole, yellow arrows, vault particles.