

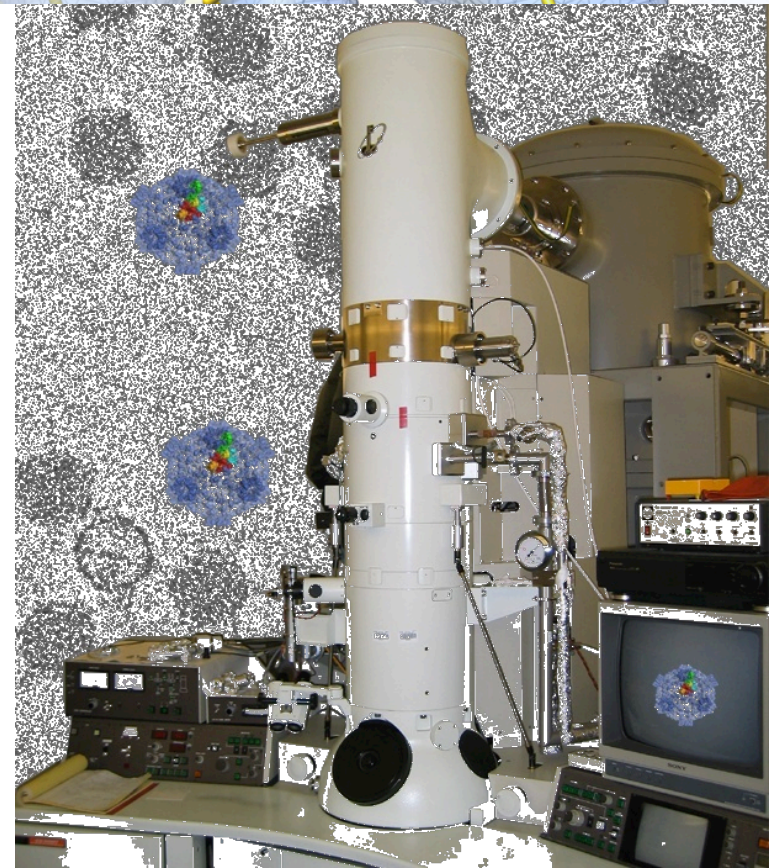
# **N C M I**

## **National Center for Macromolecular Imaging**

**Wah Chiu  
Baylor College of Medicine  
Houston, Texas**

wah@bcm.edu

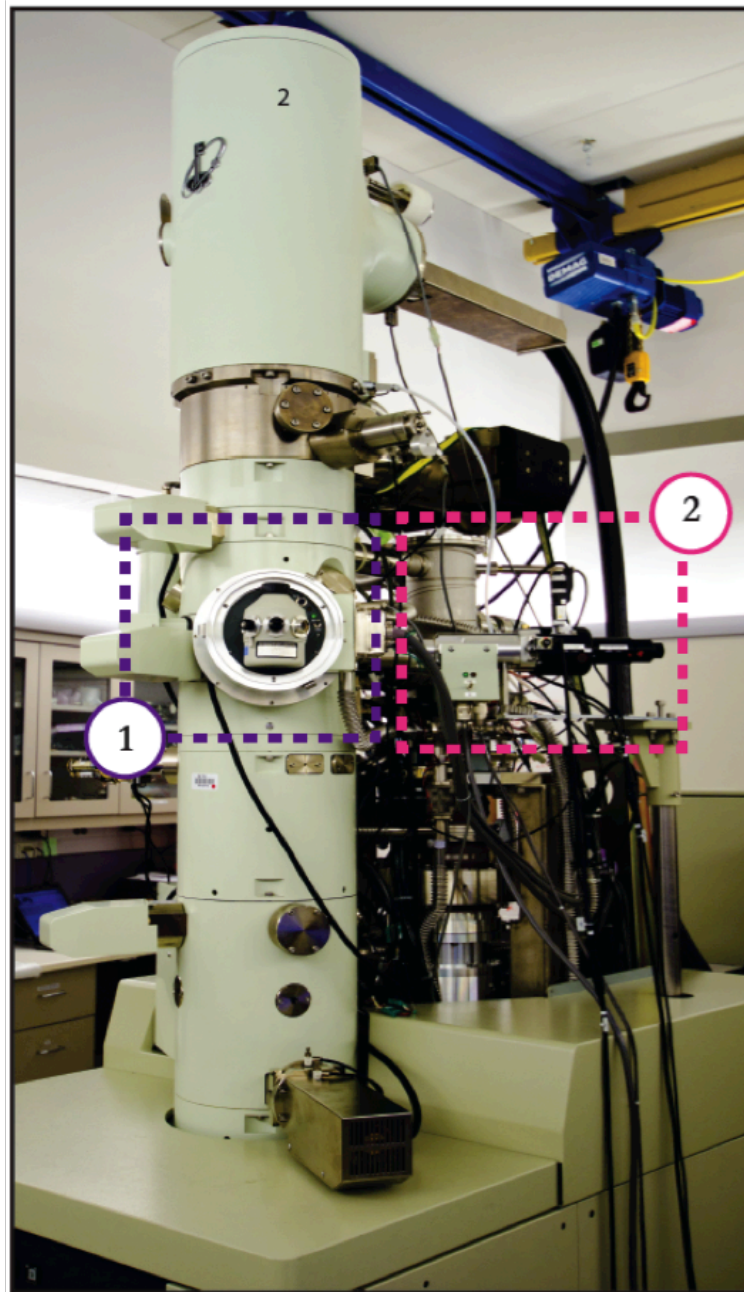
NIH P41GM103832



# Questions From Bridget, Clint and Ron

- What are the advantages of using phase plates for recording tomographic data?
- Do they improve resolution and / or SNR.
- Are there limits on the resolution that can be attained?
- Should we all get one?
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# Zernike Phase Plate Electron Cryo-Microscope



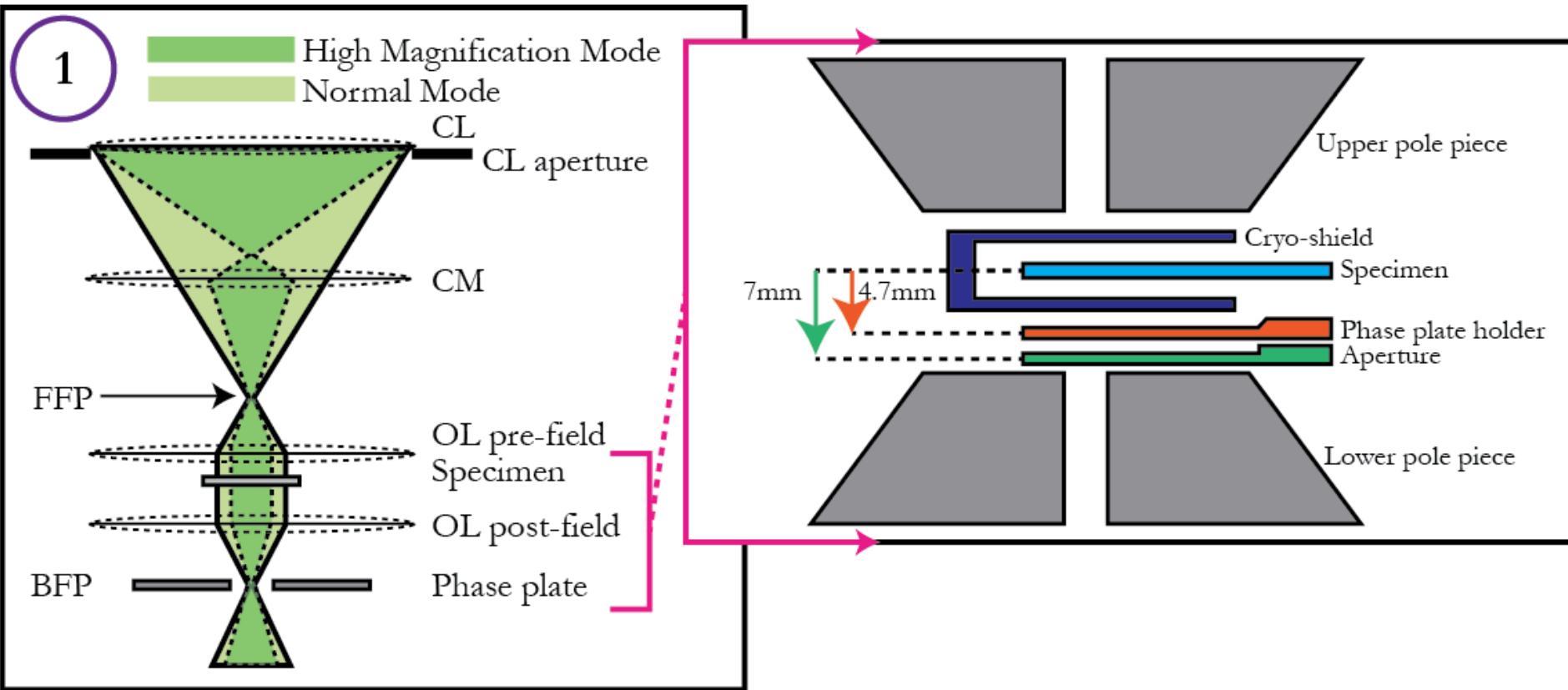
JEM2200FS

In-Column Energy Filter

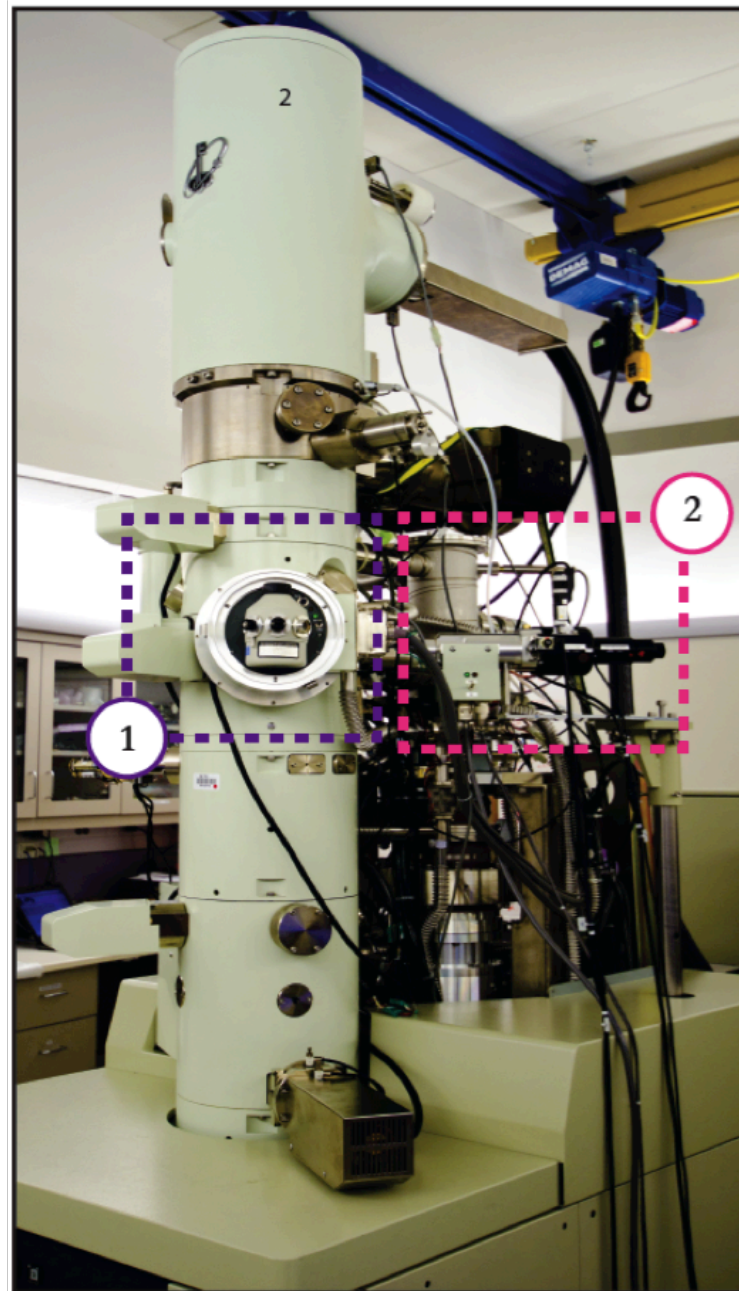
Gatan 4k CCD

DE12

# Zernike Phase Plate Electron Cryo-Microscope



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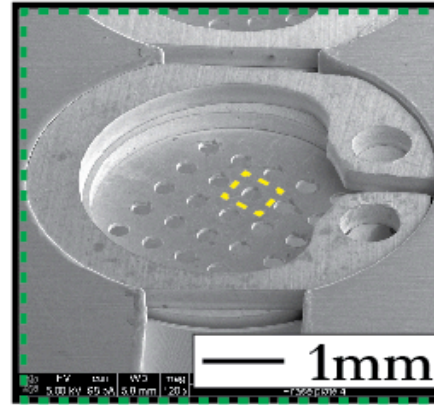


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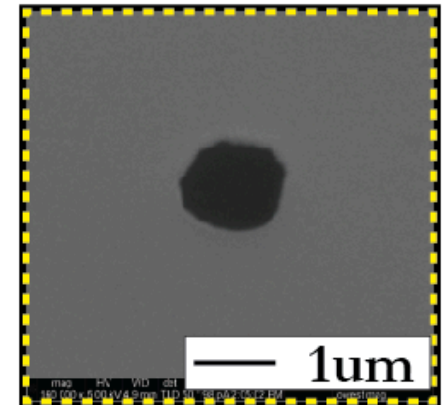
2

Phase Plate  
Air-lock

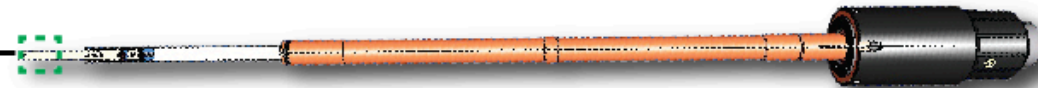
Phase Plate Grid



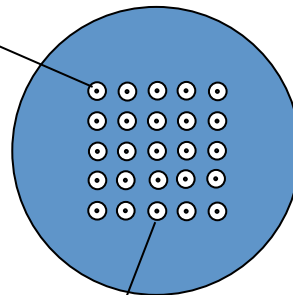
Phase Plate Hole



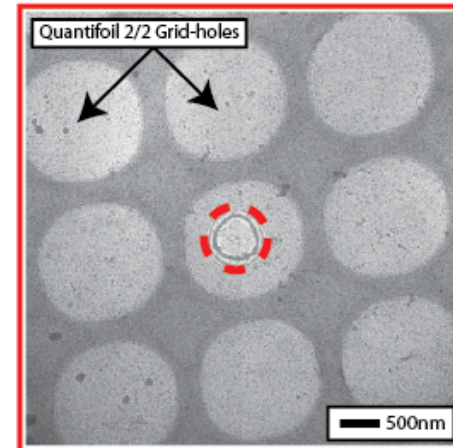
Phase Plate Holder



Phase plate  
 $\Phi 100 \mu\text{m}$

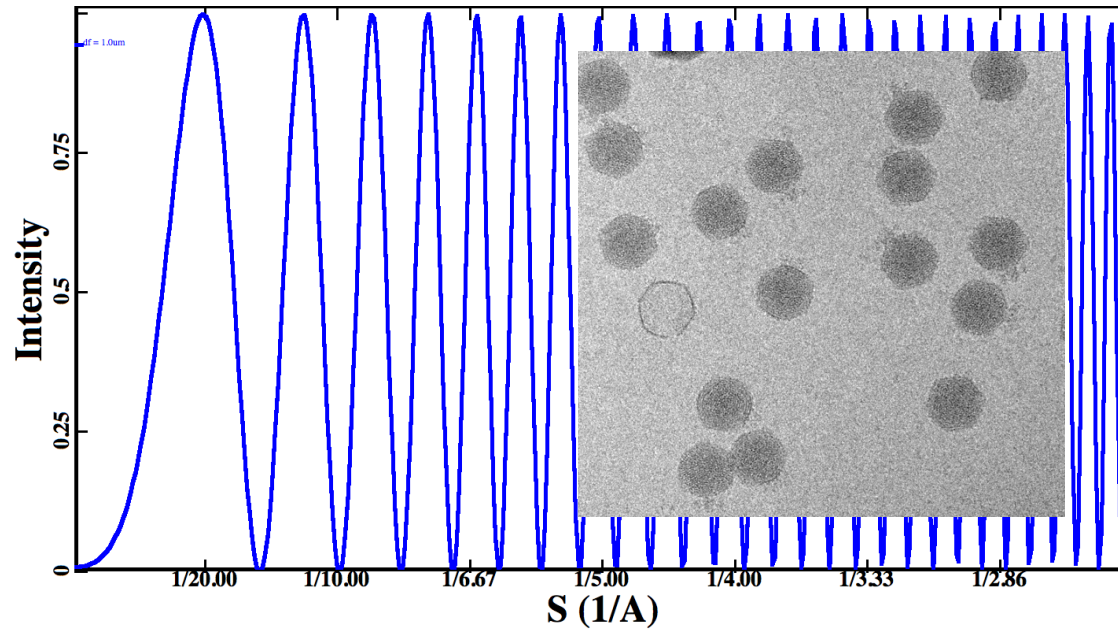


Phase Plate Hole  
 $\Phi 0.1 \sim 1 \mu\text{m}$



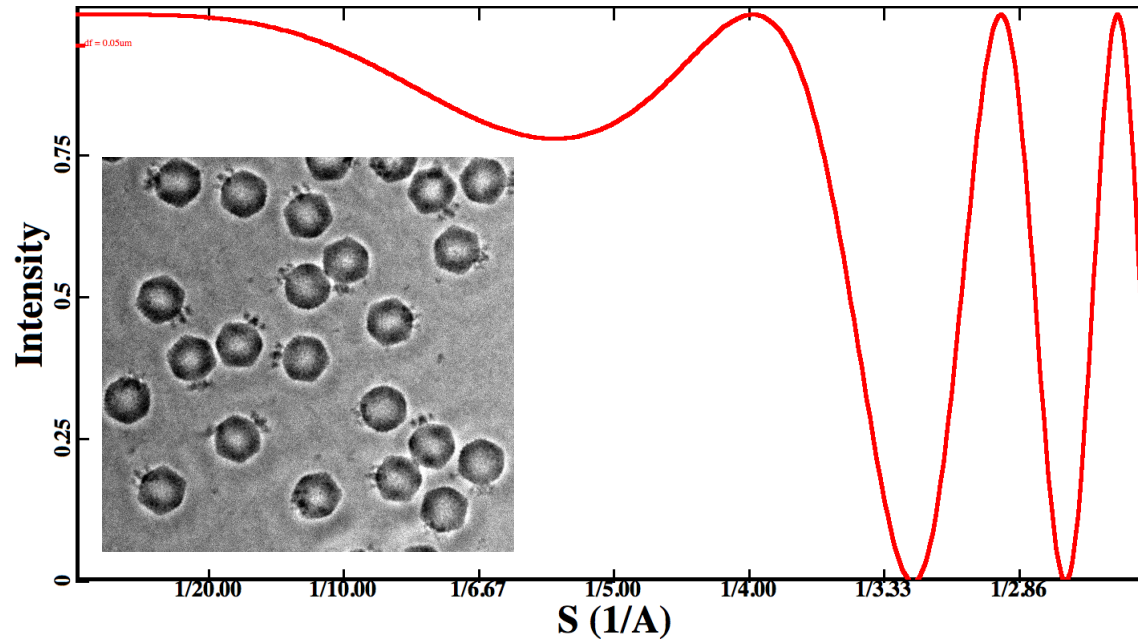
Phase Plate Hole seen in  
Defocused Diffraction Mode Image  
of a Quantifoil™ Grid

# CTF with Zernike Phase Plate CryoEM



Conventional  
CTF

The C film shifts the  
electron phase  $\pi/2$



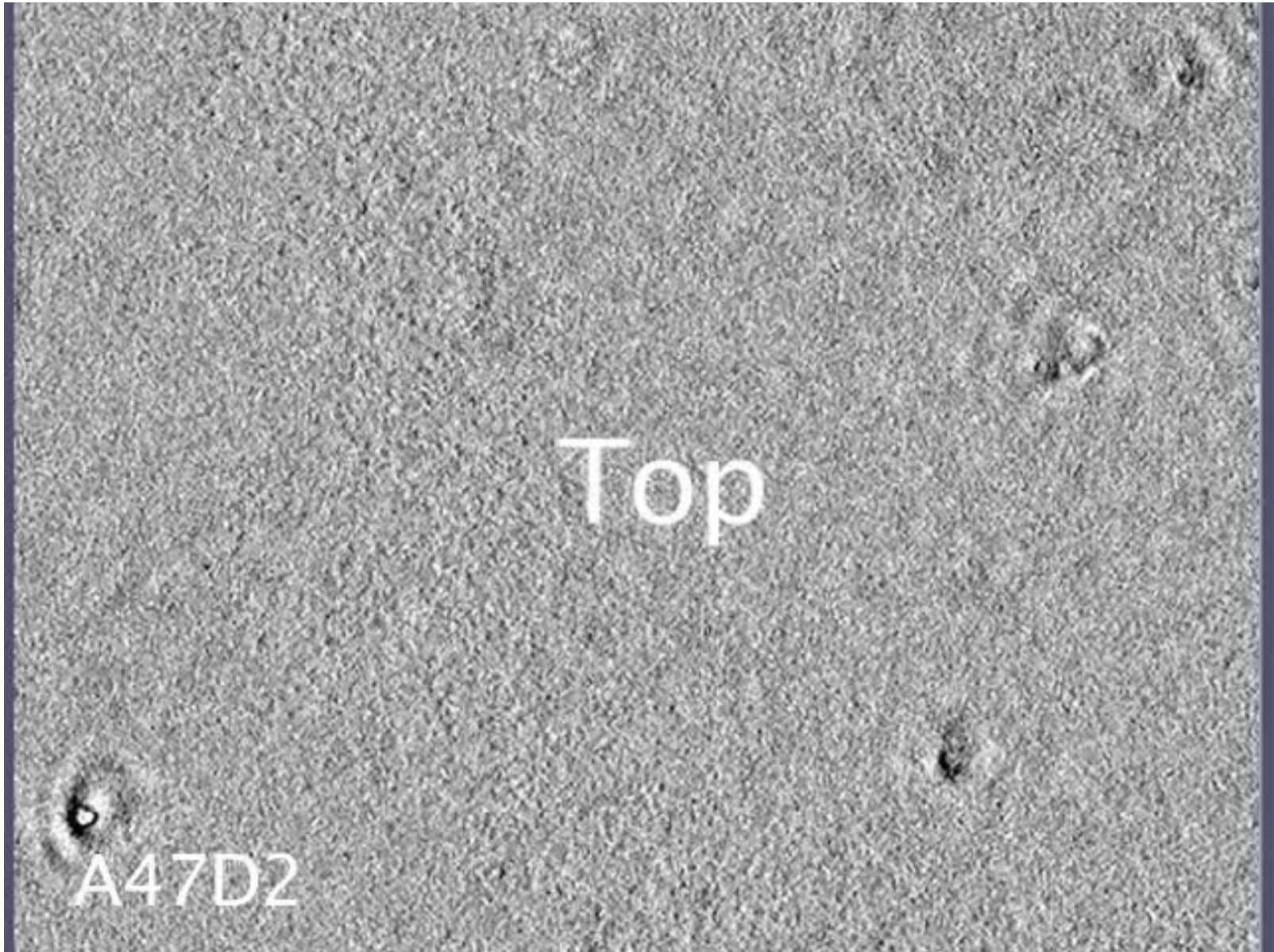
Phase Plate  
CTF

# Questions From Bridget, Clint and Ron

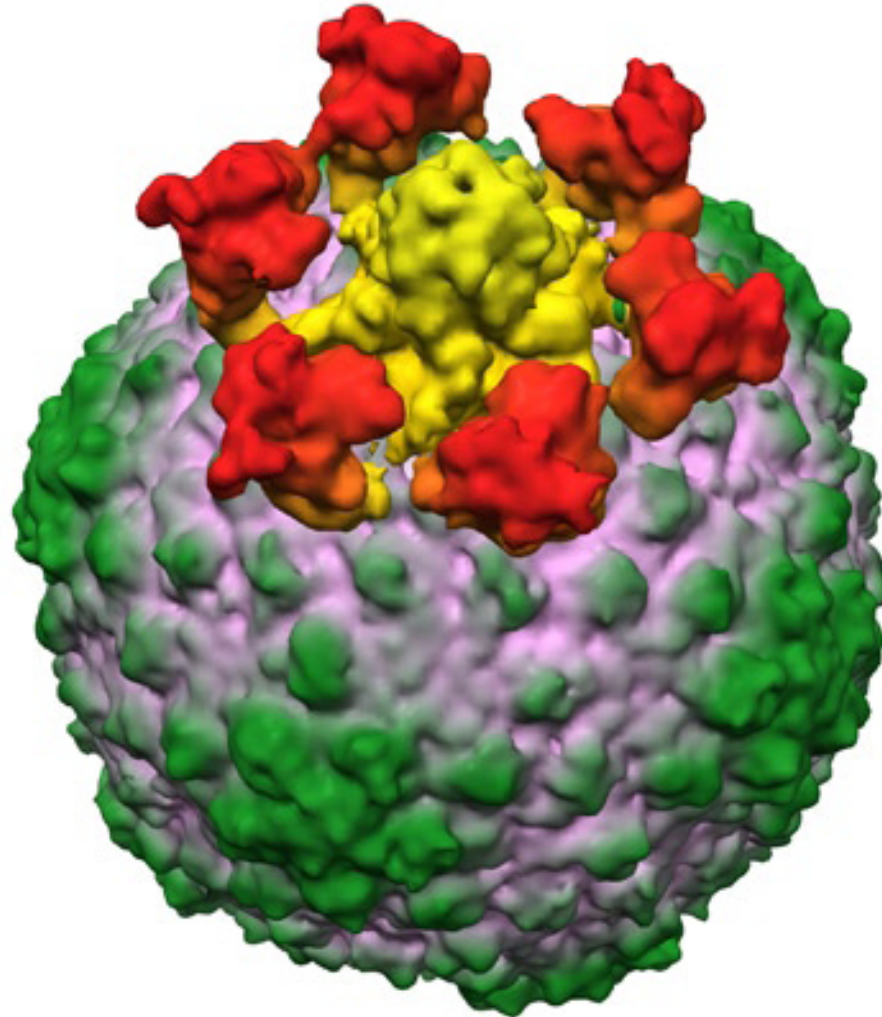
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# ZPP CryoET of $\epsilon 15$ Phage

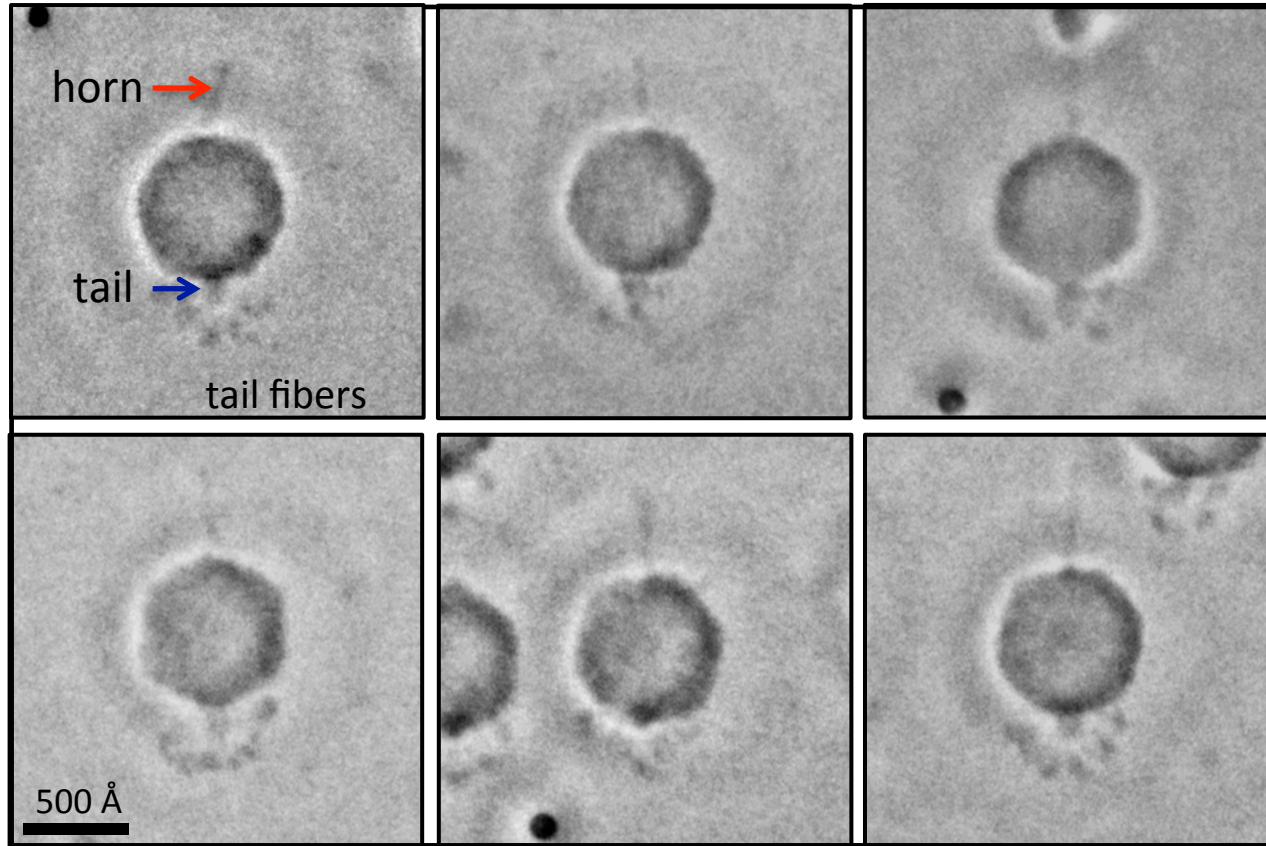


# Sub-Tomogram Average of $\epsilon 15$ Phage from Zernike Phase Plate Cryo-ET



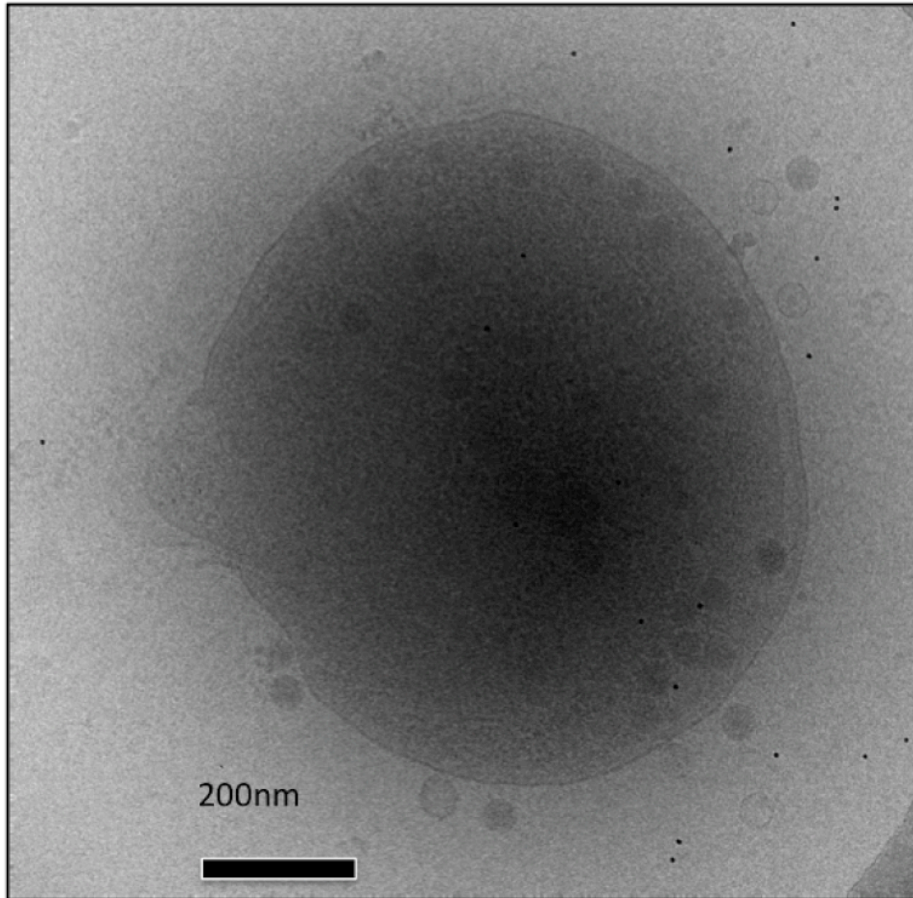
- 30 Å resolution
- 80 particles in one tomogram

# ZPP CryoEM of Biochemically Purified Cyanophage Syn5

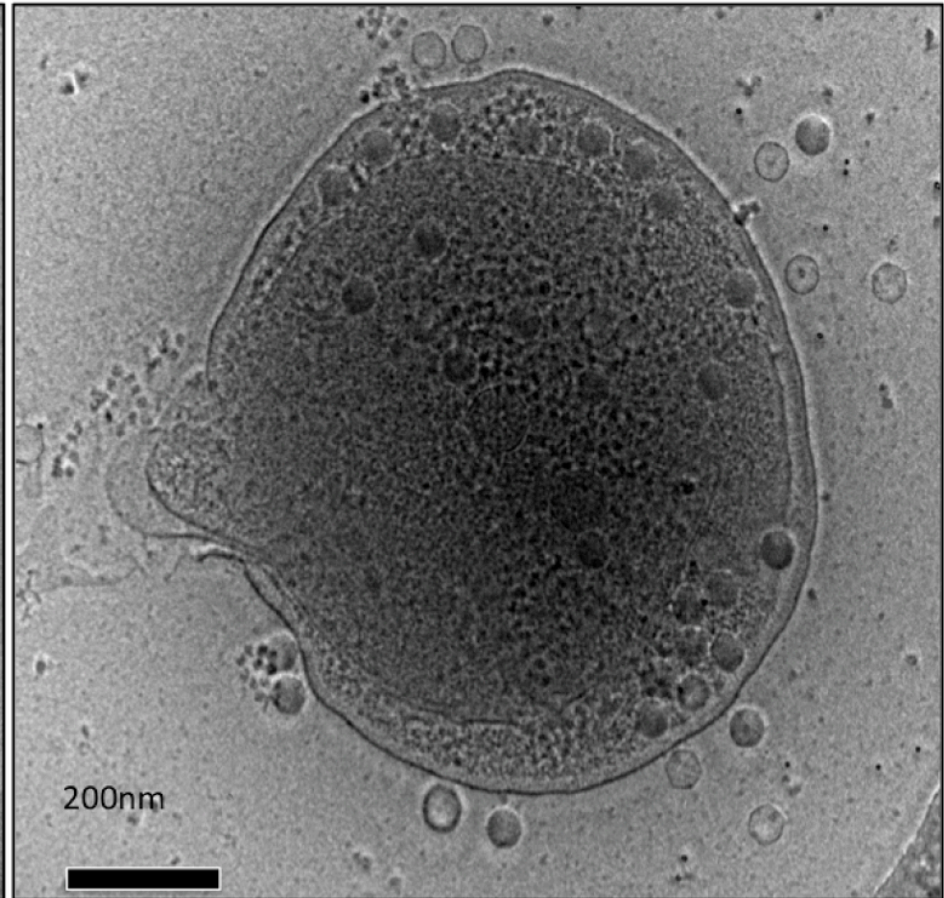


# Cyanobacterium Infected with Cyanophages

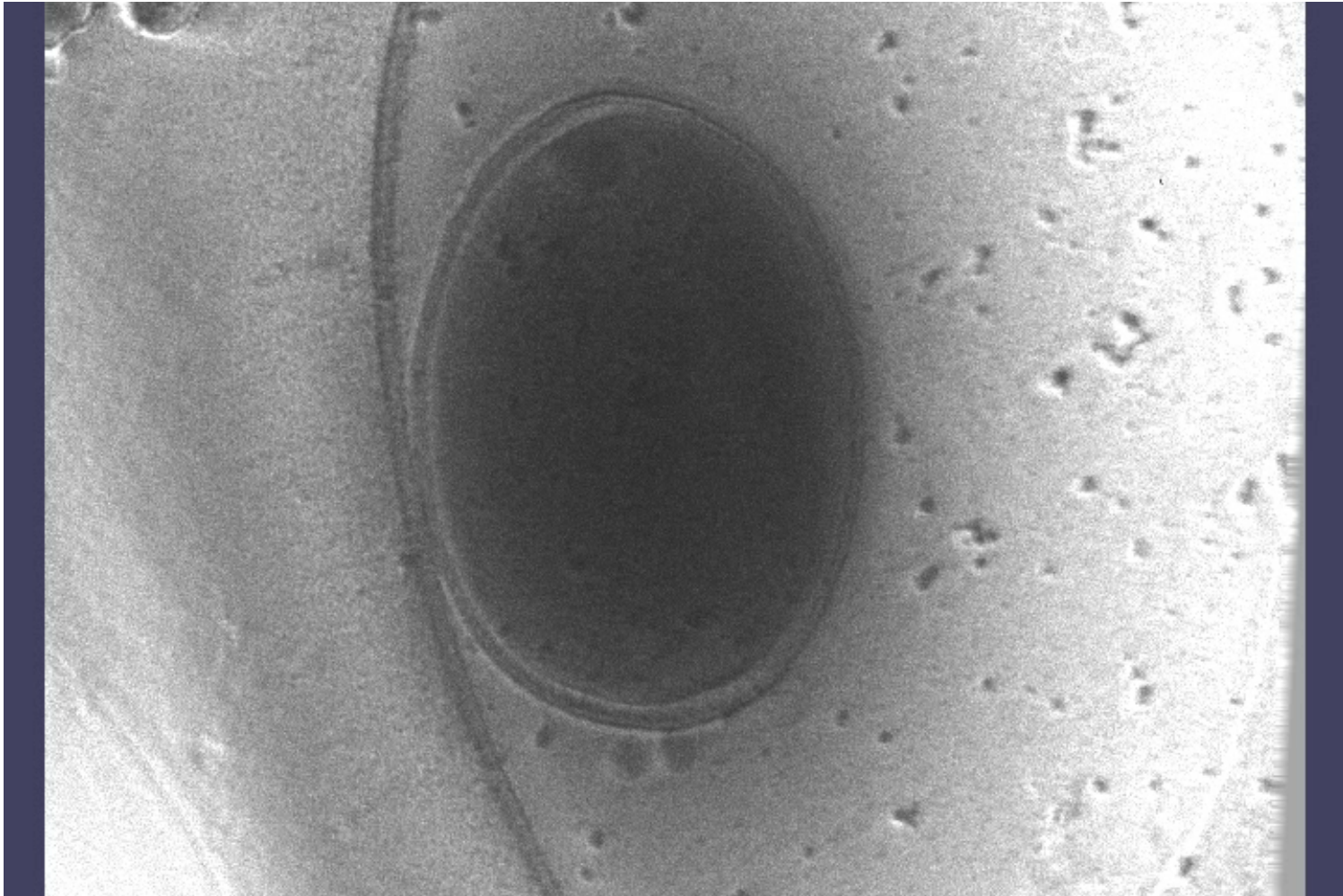
Conventional CryoEM



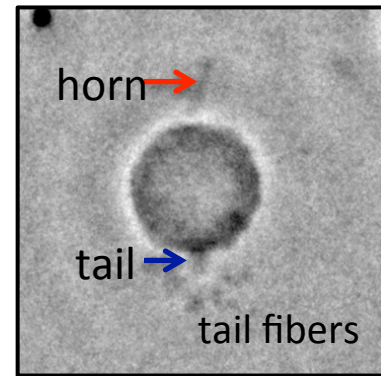
Zernike Phase Plate CryoEM



# ZPP CryoET of Syn5 Infected Cyanobacterium Early Stage of Infection

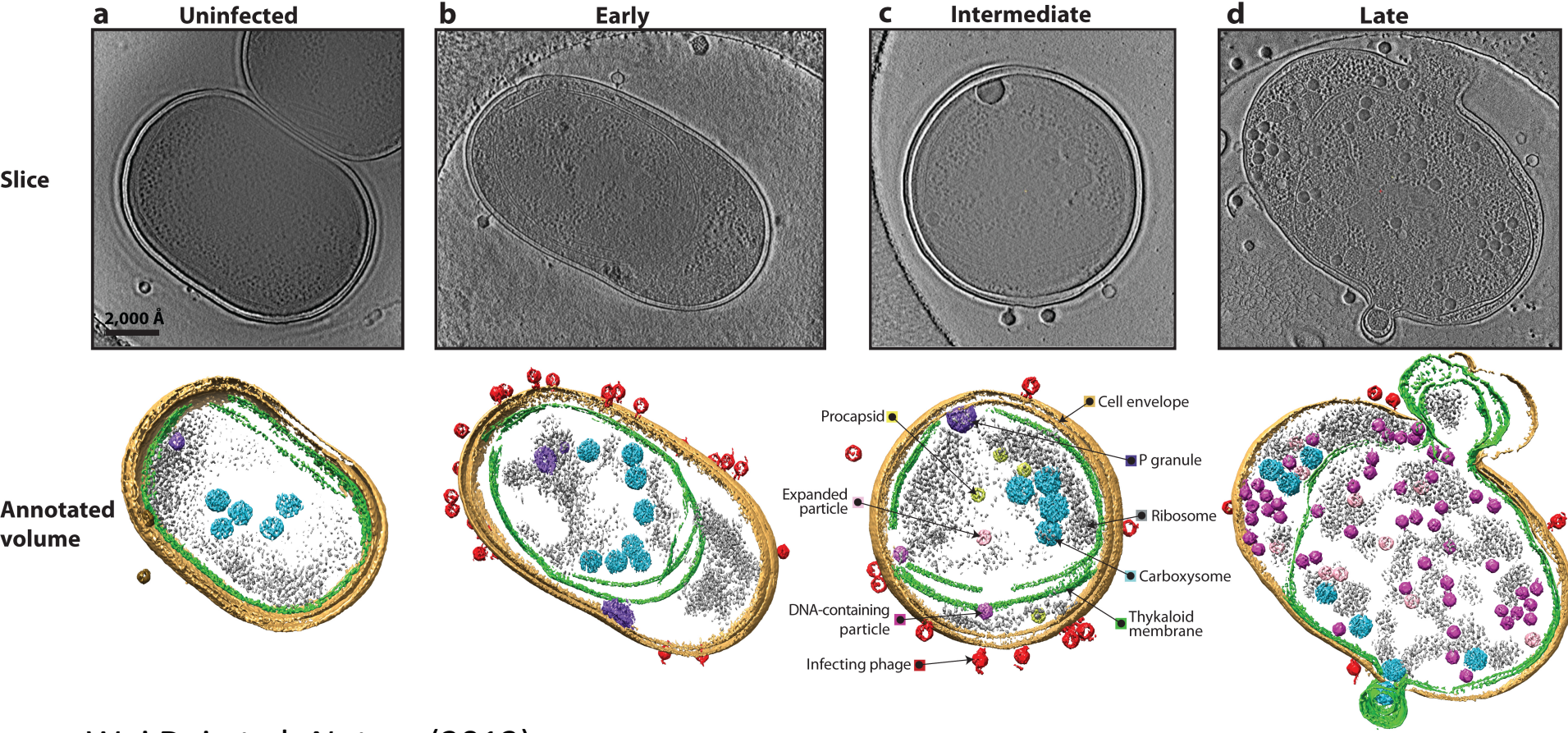


# Zernike Phase Plate CryoET of Cyanobacterium Infected with Syn5



Wei Dai, Caroline  
Fu, et al (2013)  
*Nature*

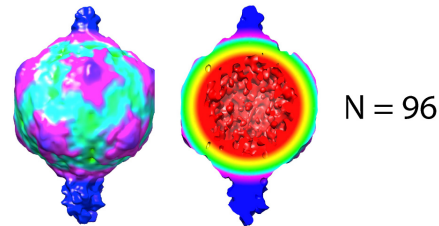
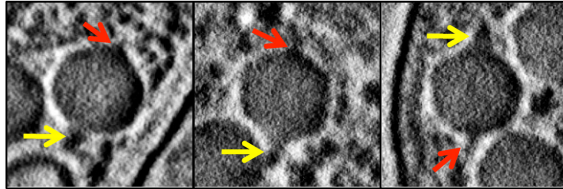
# ZPP CryoET of Syn5 Infected Cyanobacteria at Different Stages of Infection



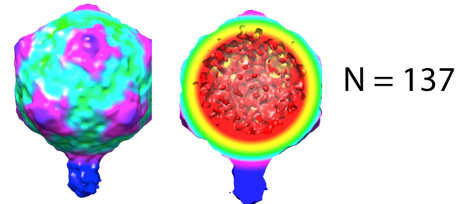
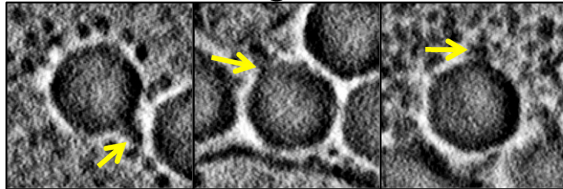
Wei Dai et al, *Nature* (2013)

# Five Types of Syn5 Particles Classified from Subtomograms

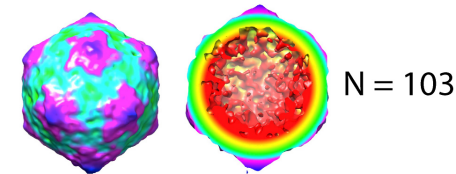
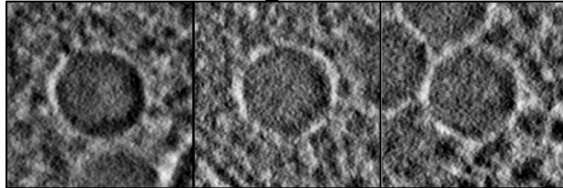
**a DNA-containing: with tail and horn**



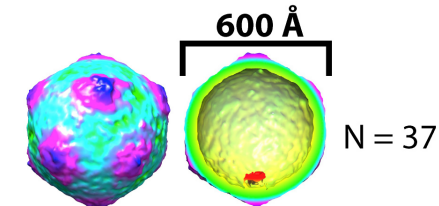
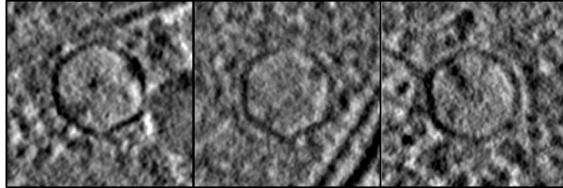
**b DNA-containing: with tail**



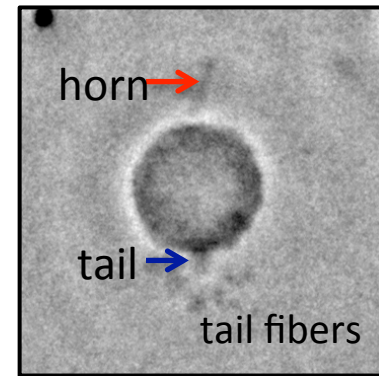
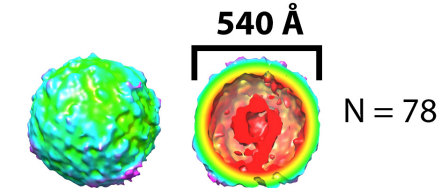
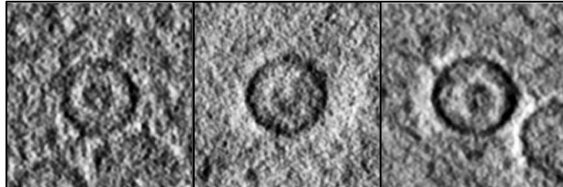
**c DNA-containing: no tail/horn**



**d Expanded**



**e Procapsid**

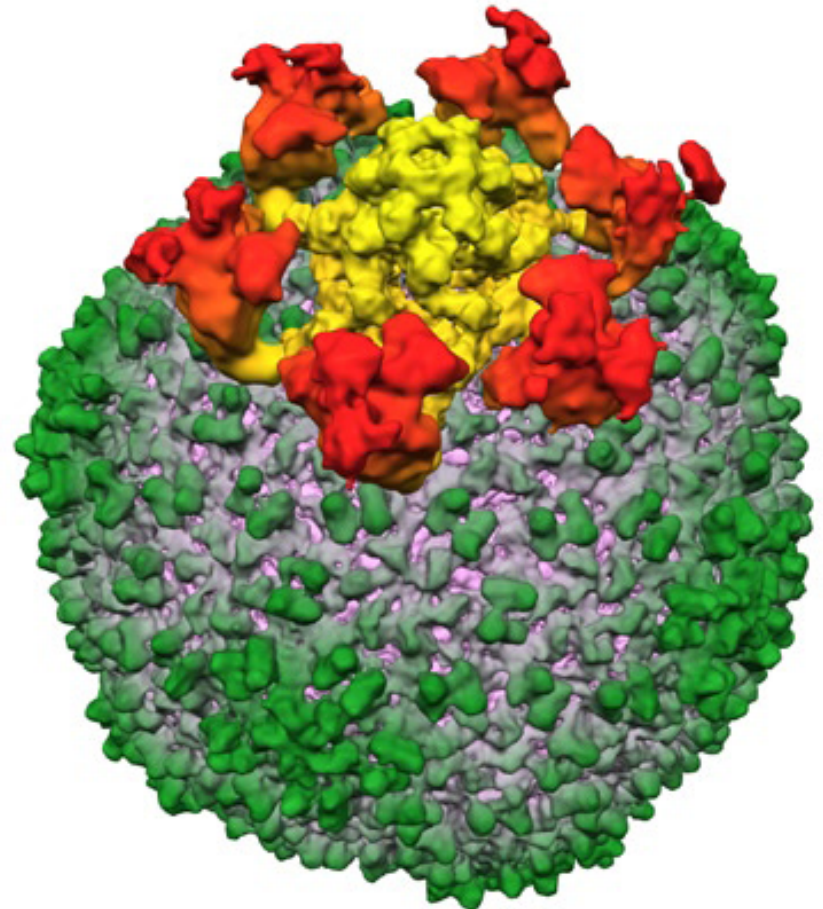
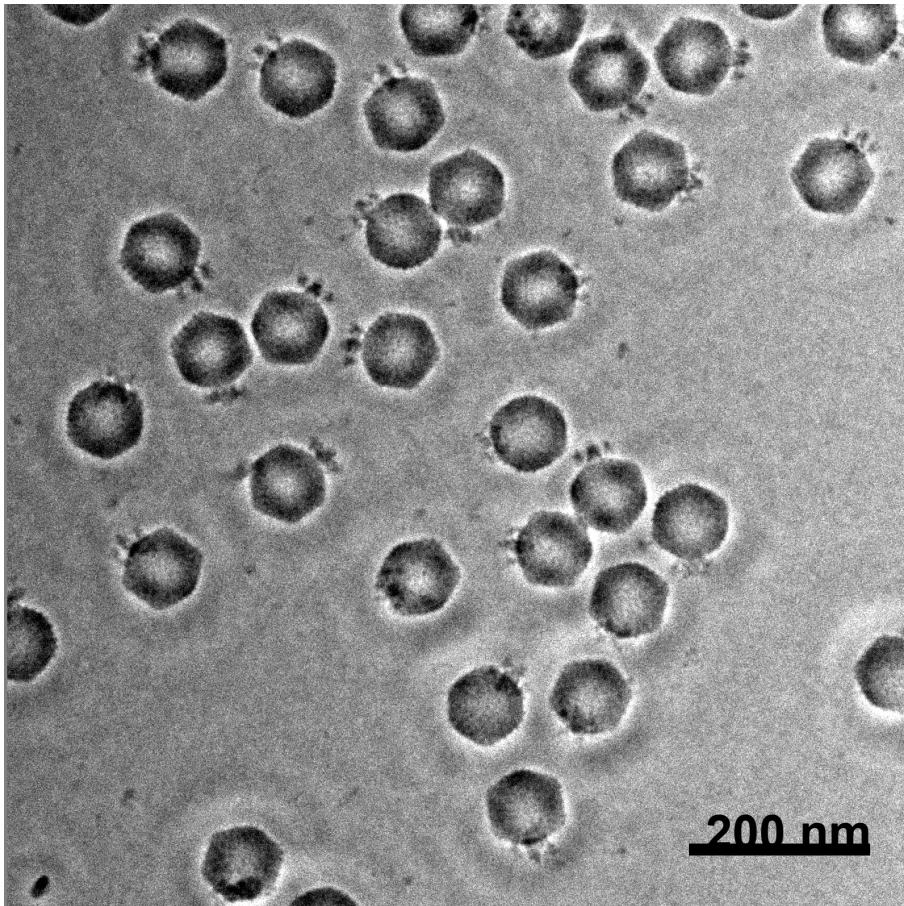




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# Symmetry-Free Reconstruction of ZPP CryoEM of $\epsilon 15$ Phage



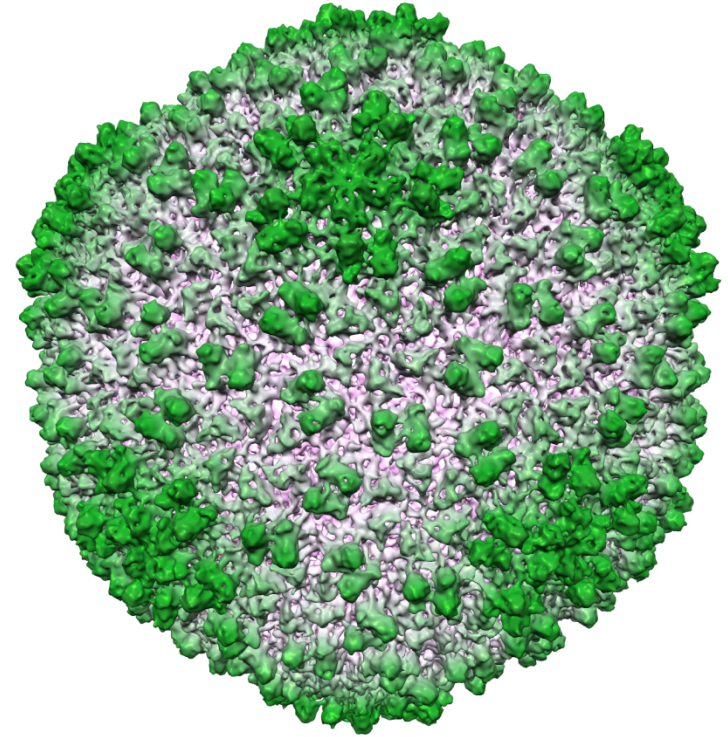
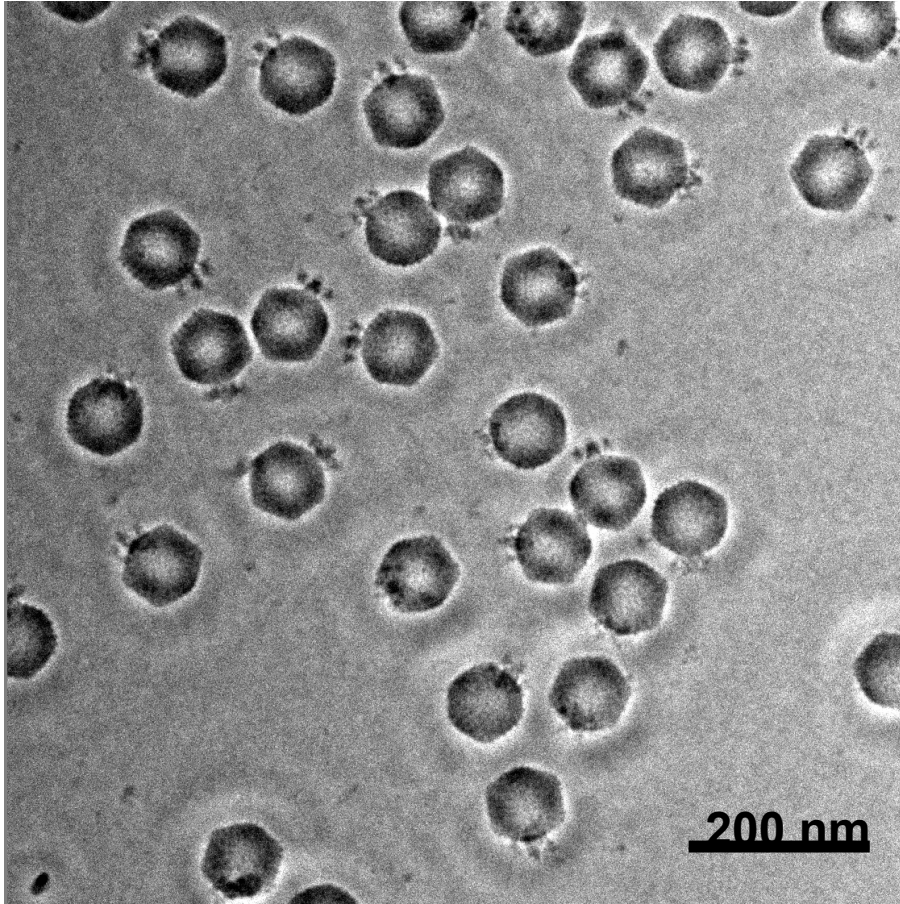
**5,015 particles**

**13 Å**

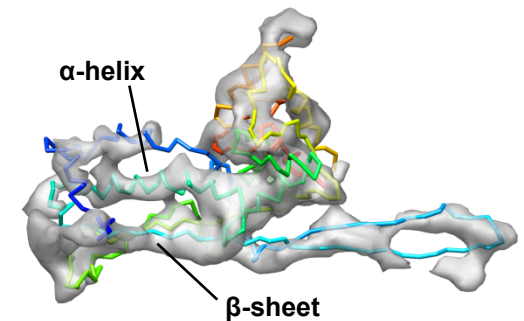
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# Subnanometer Resolution ZPP CryoEM of $\epsilon$ 15 Phage

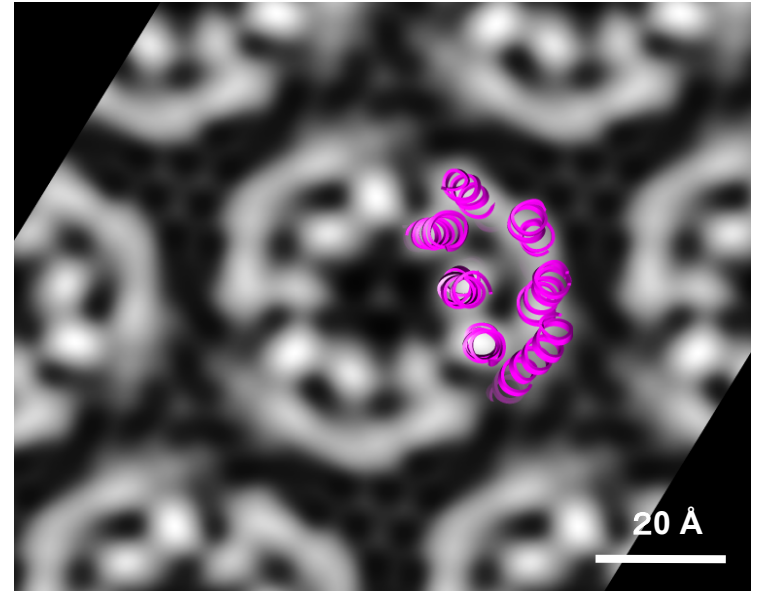
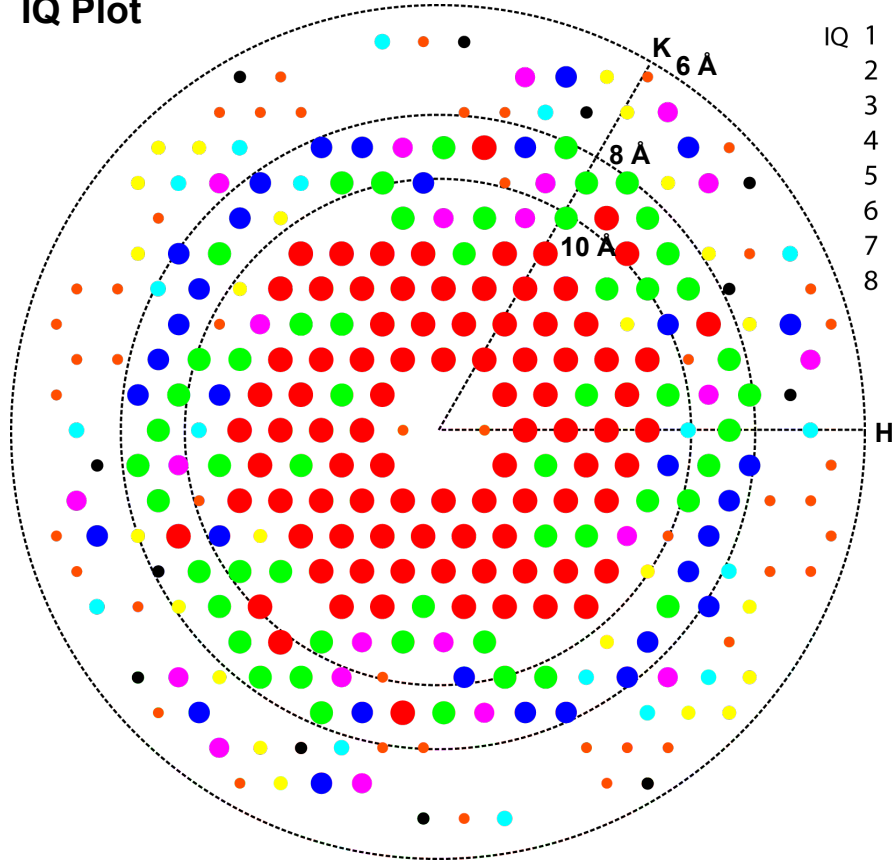


Asymmetric unit



# 8-Å Map of Bacteriorhodopsin from ZPP

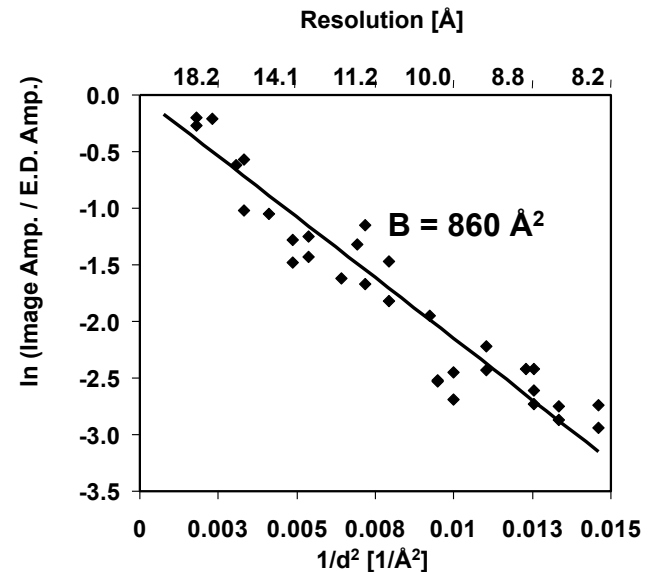
**IQ Plot**



**Amplitude's comparison with Electron diffraction**

**Phase error from the Henderson's projection map**

Resolution range (Å)	100-15	15-10	10-8	100-8
Phase error	7.2°	10.2°	11.2°	9.7°
S.D.	6.7°	9.3°	7.8°	8.1°



# Questions

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- How much money and effort is required ?
- Should we all get one?

# Phase Plate Statistics in Cyanobacteria and Syn5 Phage Progeny Project

- Total phase plate holes: 305
- Phase plate blades: 15
- 83 tilt series of cells completed: 71 collected manually, 12 by SerialEM.
- For each tilt series: needed to check 4 phase plate holes on average.

	Good	Charging	Radiation damaged	Incomplete	Total
Cell	57	5	5	20	87
Phage	9	7	0	0	16
Total	66	12	5	20	103

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# Further Developments Needed for Zernike Phase Plate

- More robust Zernike phase plate
- Optimize electron optics configuration
- Implement automatic data collection capability
- Incorporate DDD camera data collection