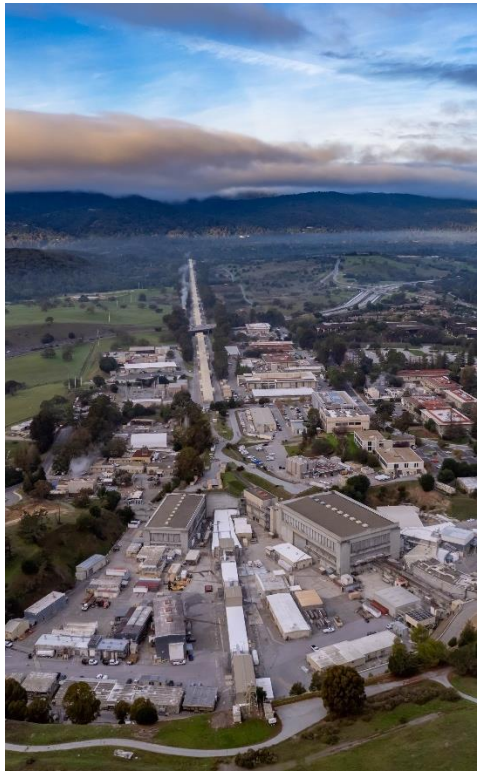
SLAC NATIONAL ACCELERATOR LABORATORY

Correlating cryogenic light and electron microscopy at the nanoscale for a deeper understanding of biological systems



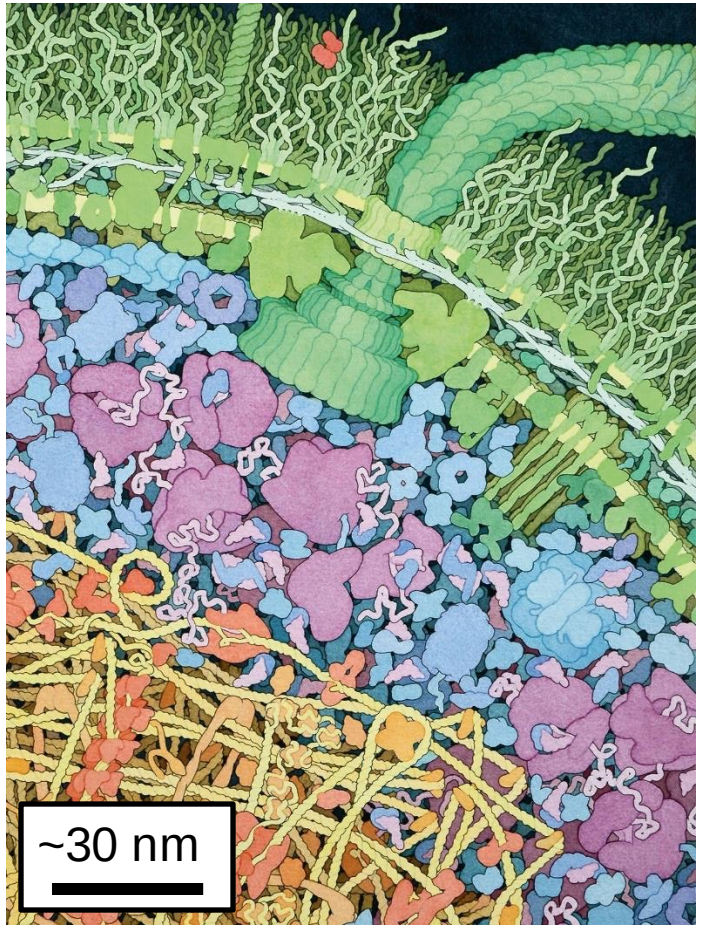
Peter D. Dahlberg

SLAC National Accelerator Laboratory
SSRL

Committee on Atomic, Molecular, and Optical Sciences
2024 Fall Meeting

October 10, 2024

Want to observe the many biomolecules working in concert *in situ*

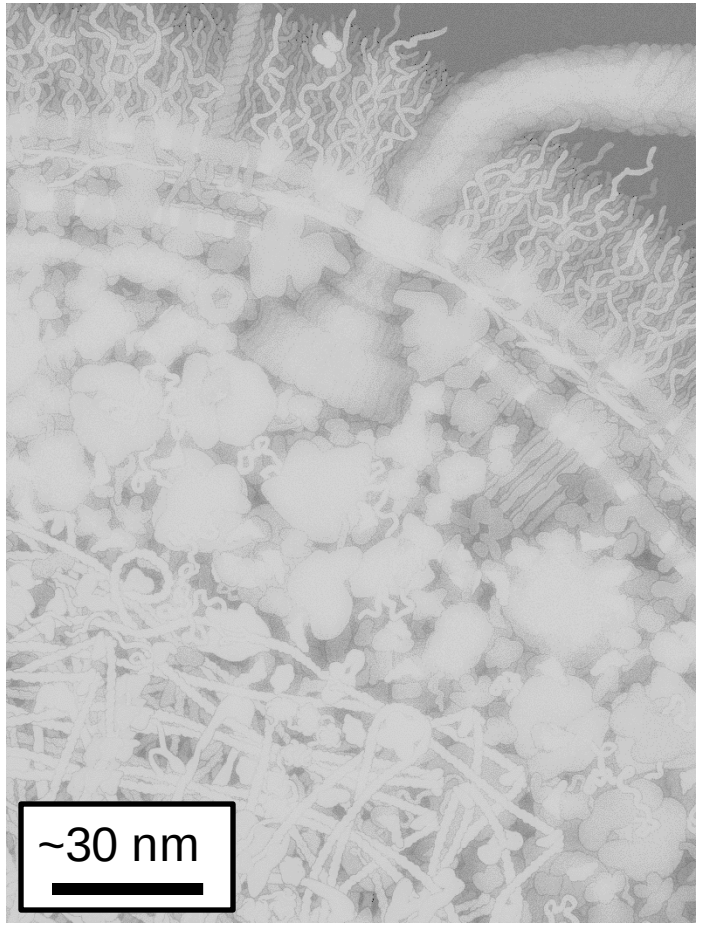


E. coli illustration: David Goodsell (2009) *The Machinery of Life*

Many different biomolecules acting together

- DNA
- RNA
- Protein
- Lipids
- …

Strengths of super-resolution are the weaknesses of electron microscopy and vice-versa



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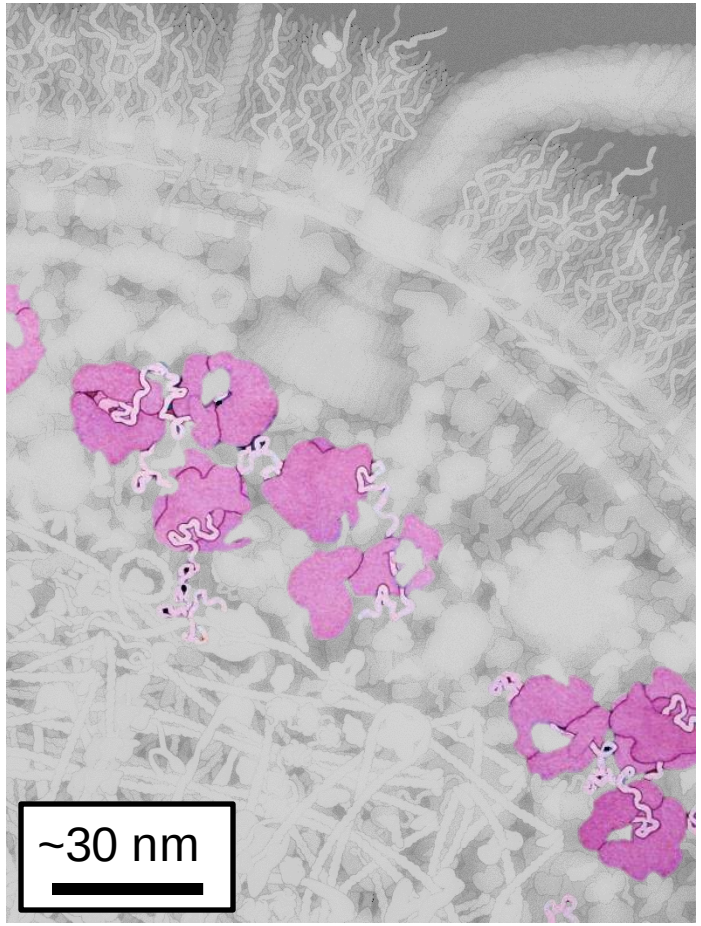
Cryogenic Electron Tomography:
molecular-scale resolution and cellular context

Single-Molecule Fluorescence:
localizations with single-molecule sensitivity and specificity

Goal: single-molecule fluorescence localizations for annotations of high-resolution electron tomography



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Cryogenic Electron Tomography: molecular-scale resolution and cellular context

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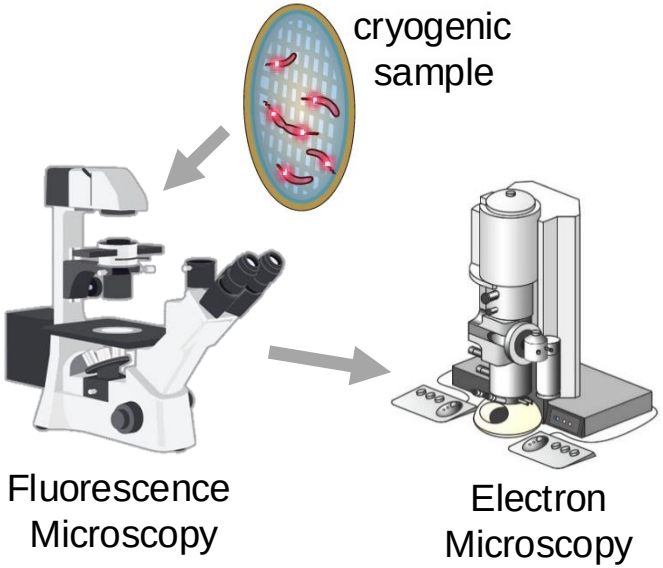
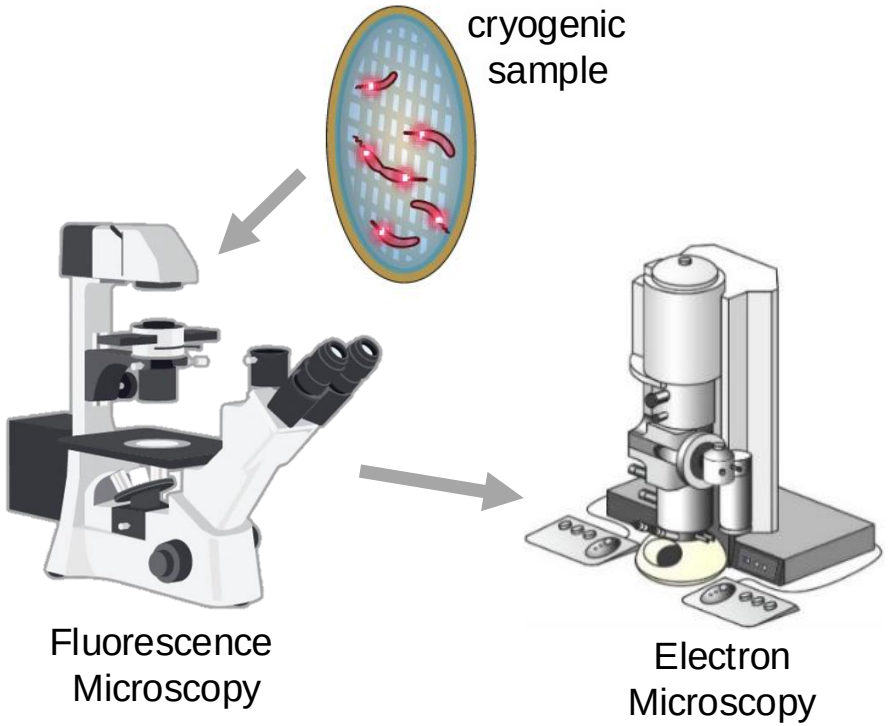
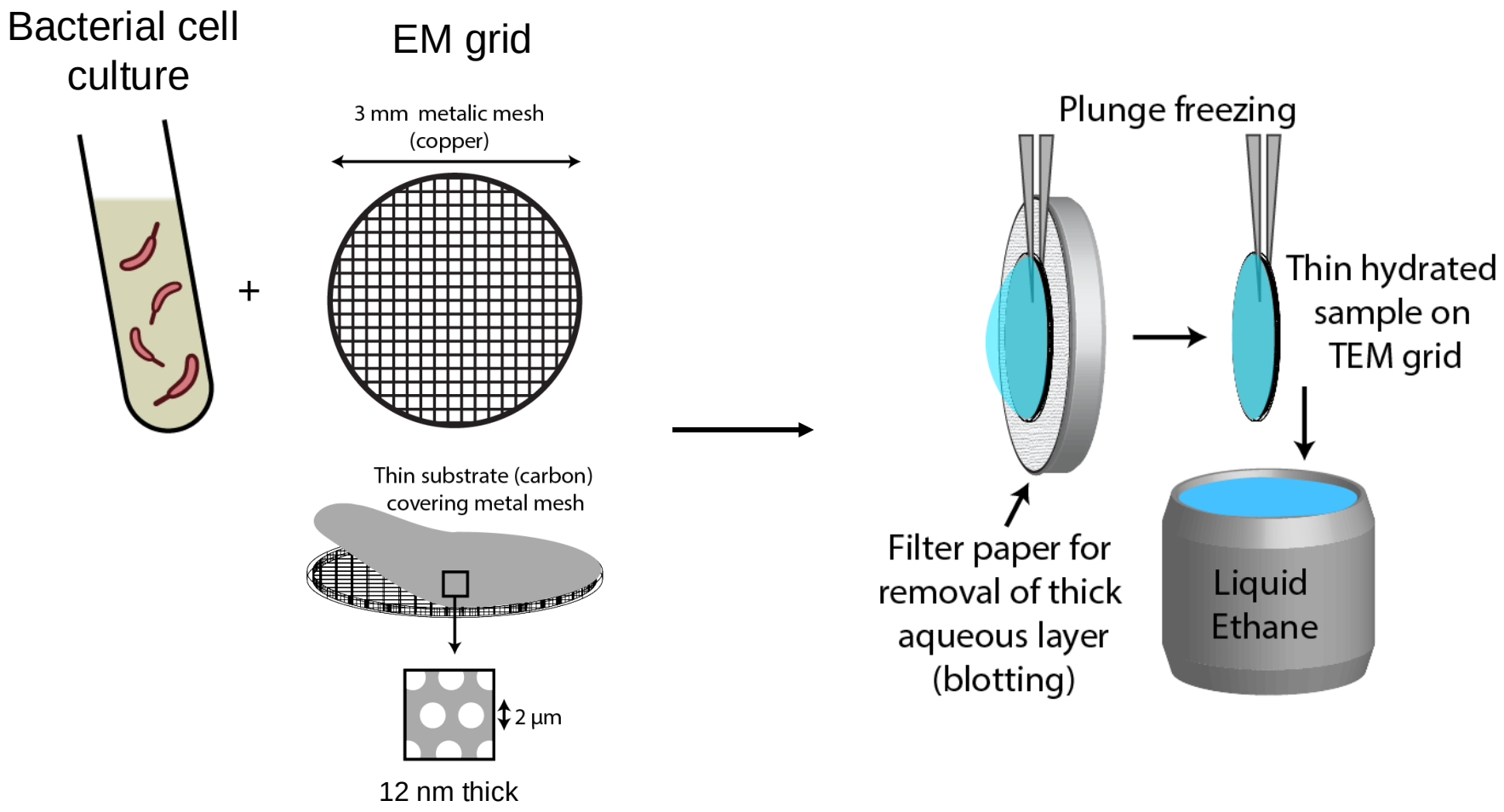


Overview

- Overview of cryogenic electron tomography and single-molecule super-resolution
  - challenges we face adapting super-resolution to cryogenic temperatures
- Results in the bacterium *Caulobacter crescentus*
- Improvements that have led to better cryogenic super-resolution
  - Hardware
  - Photophysical understanding
- Brief perspective on the future of these methods

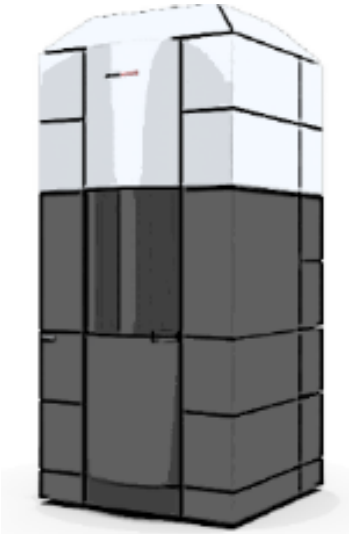


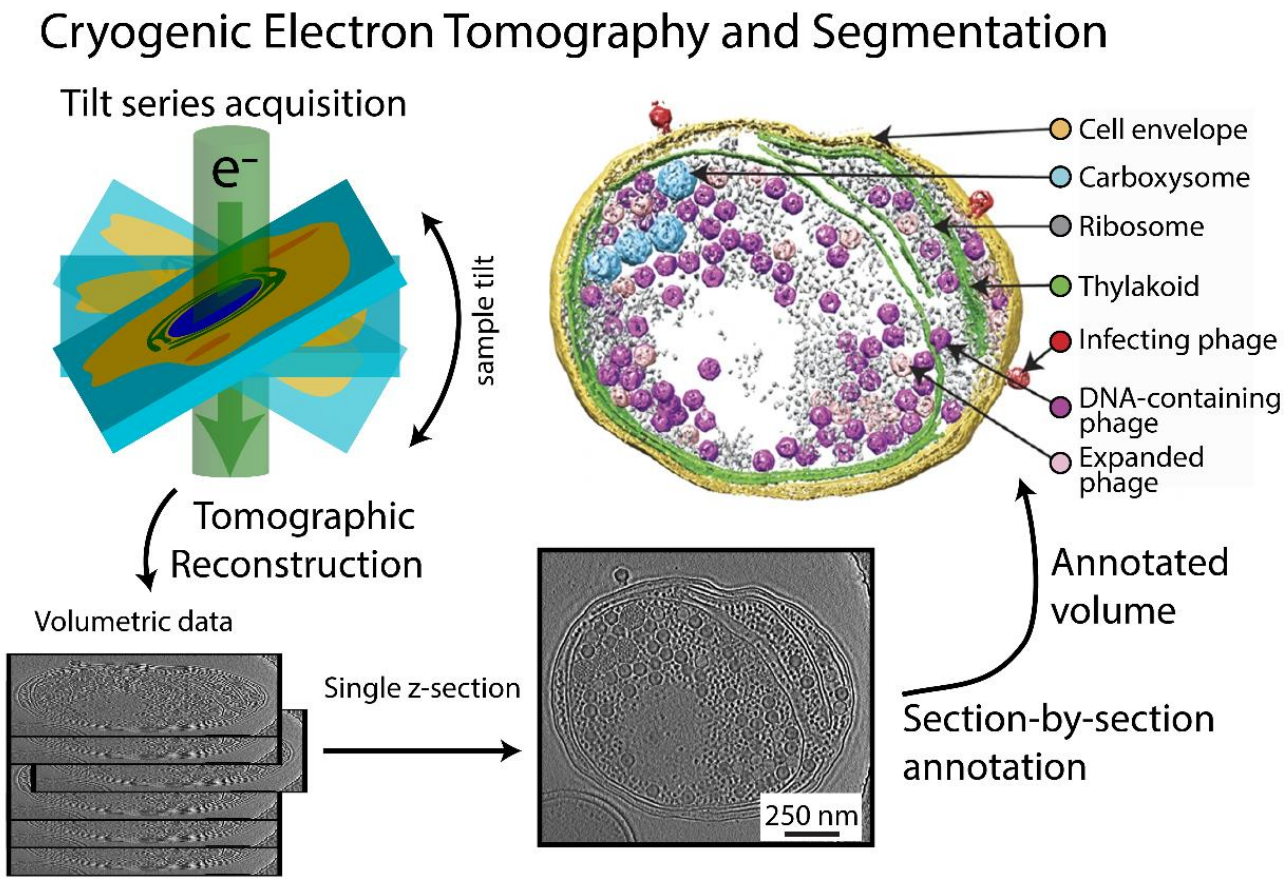
Workflow for Cryogenic Electron Tomography (CryoET)



Workflow for Cryogenic Electron Tomography (CryoET)

Load cryogenic sample into cryogenic electron microscope

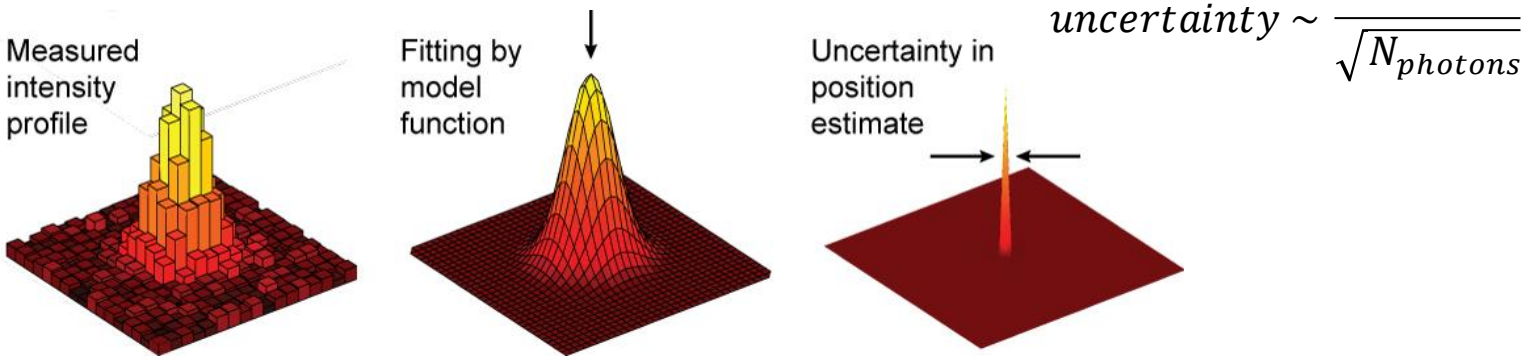




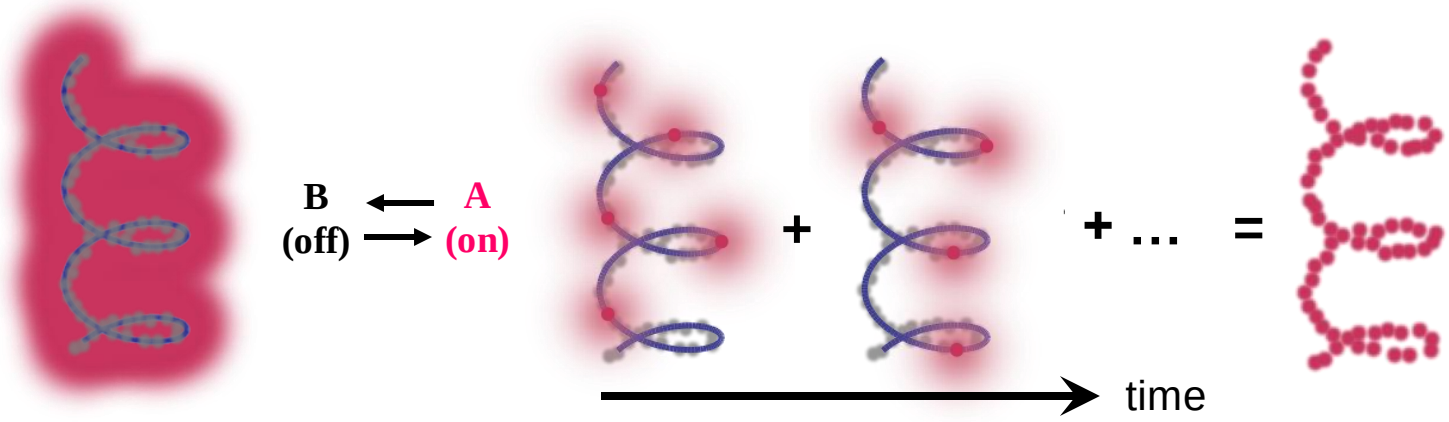
Single-molecule-based super-resolution surpasses the diffraction limit by using two key concepts

- **Single-Molecule localization**: localize an individual emitter more precisely than the width of the point-spread-function it generates

$$uncertainty \sim \frac{1}{\sqrt{N_{photons}}}$$

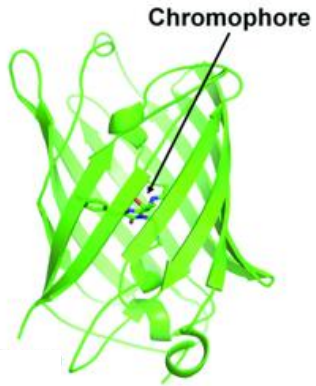


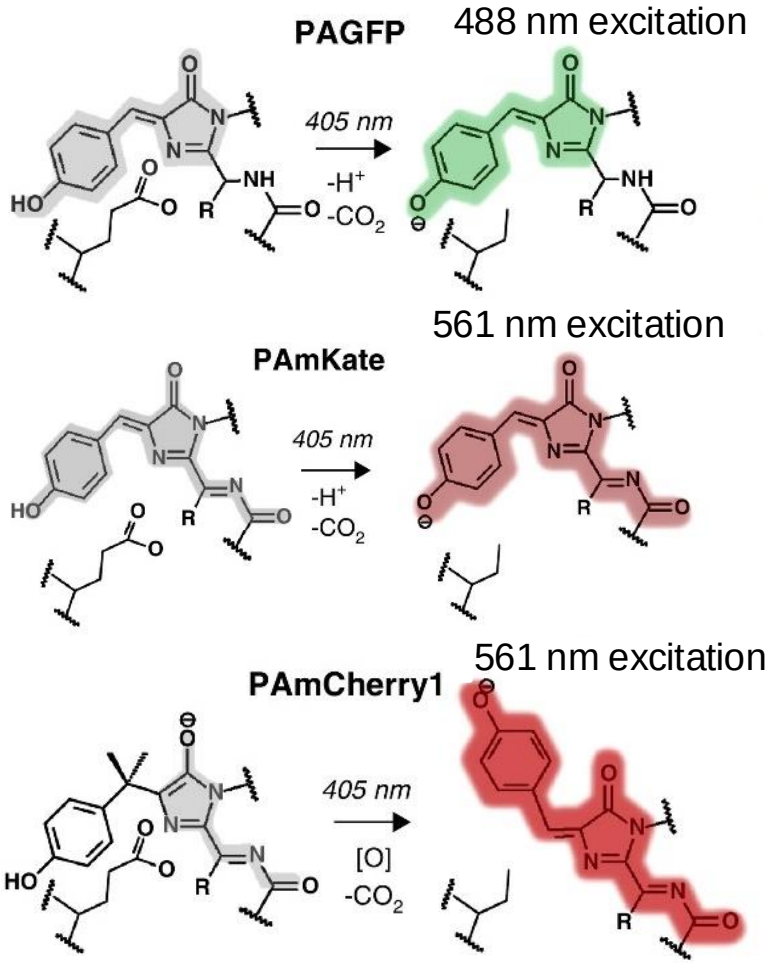
- **Active control:** only subset of emitters is ‘on’ and subsets are imaged sequentially to prevent PSF overlap

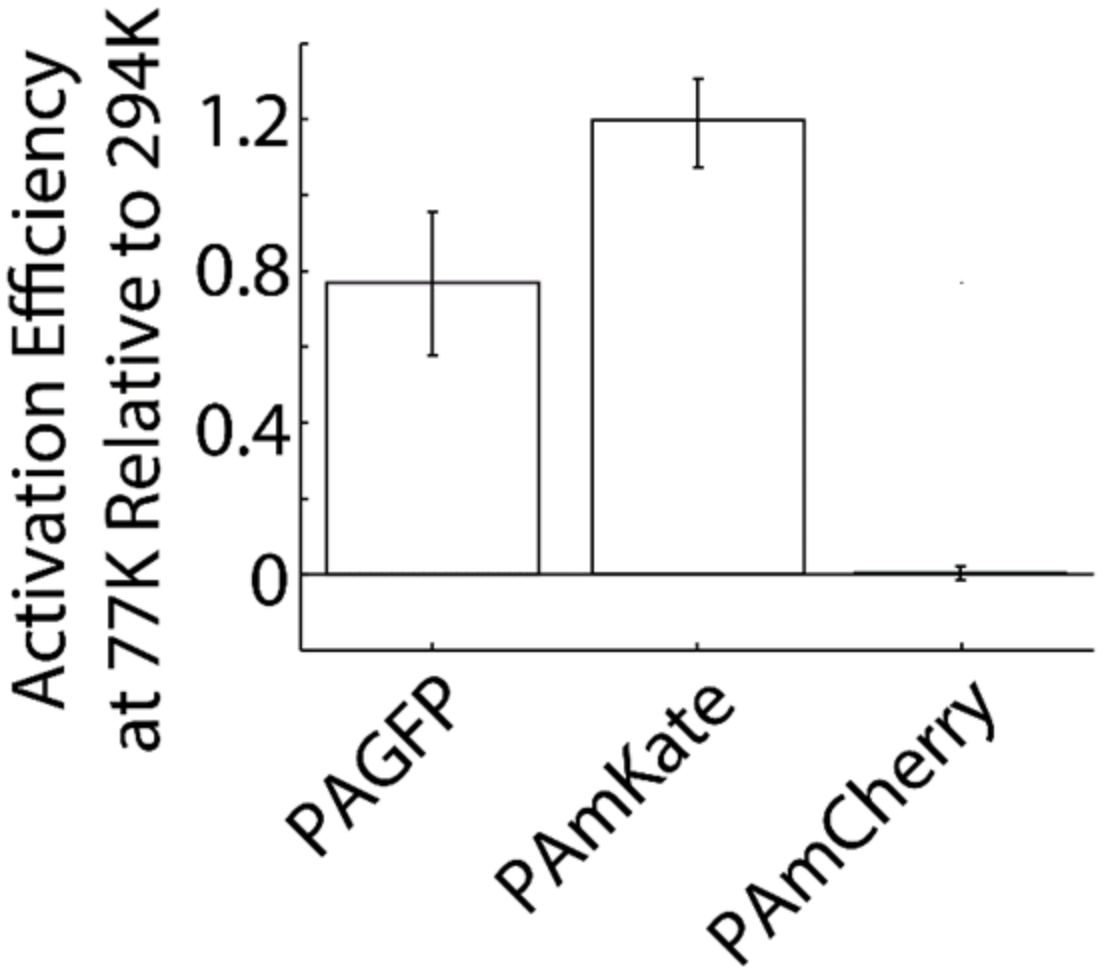


Figures: A. Gahlmann and W. E. Moerner, *Nat. Rev. Microbiol.*, 2013 and M. A. Thompson *et al.*, 2010.

PAmKate remains efficiently photoactivatable at cryogenic temperatures



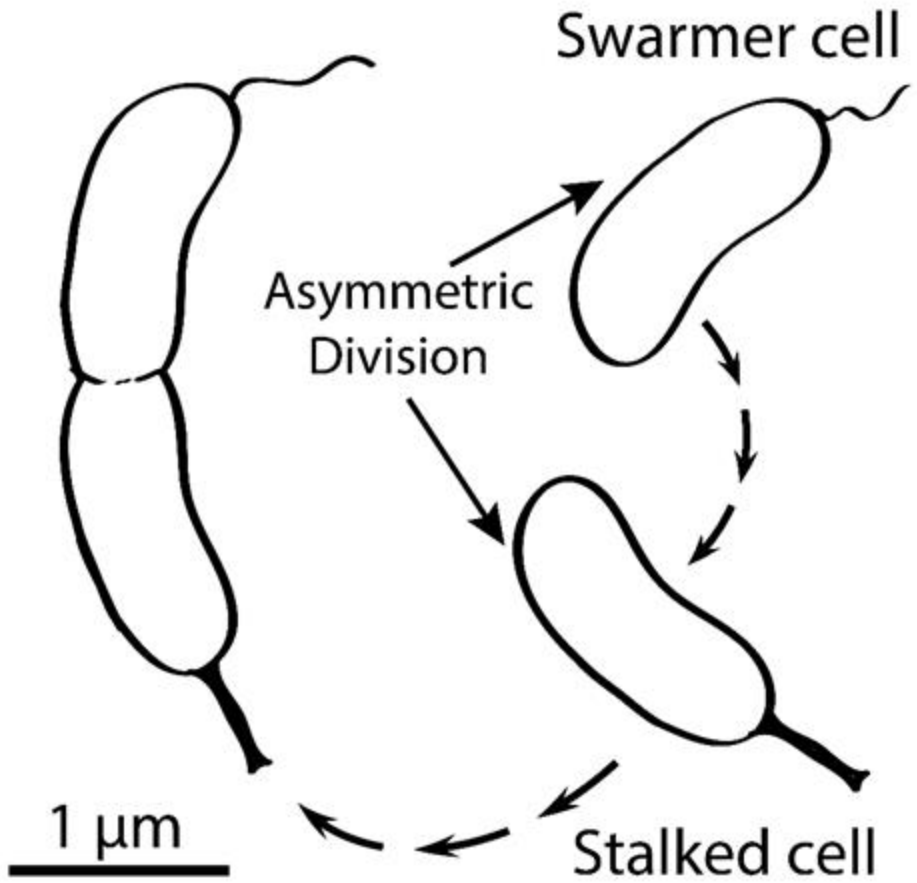




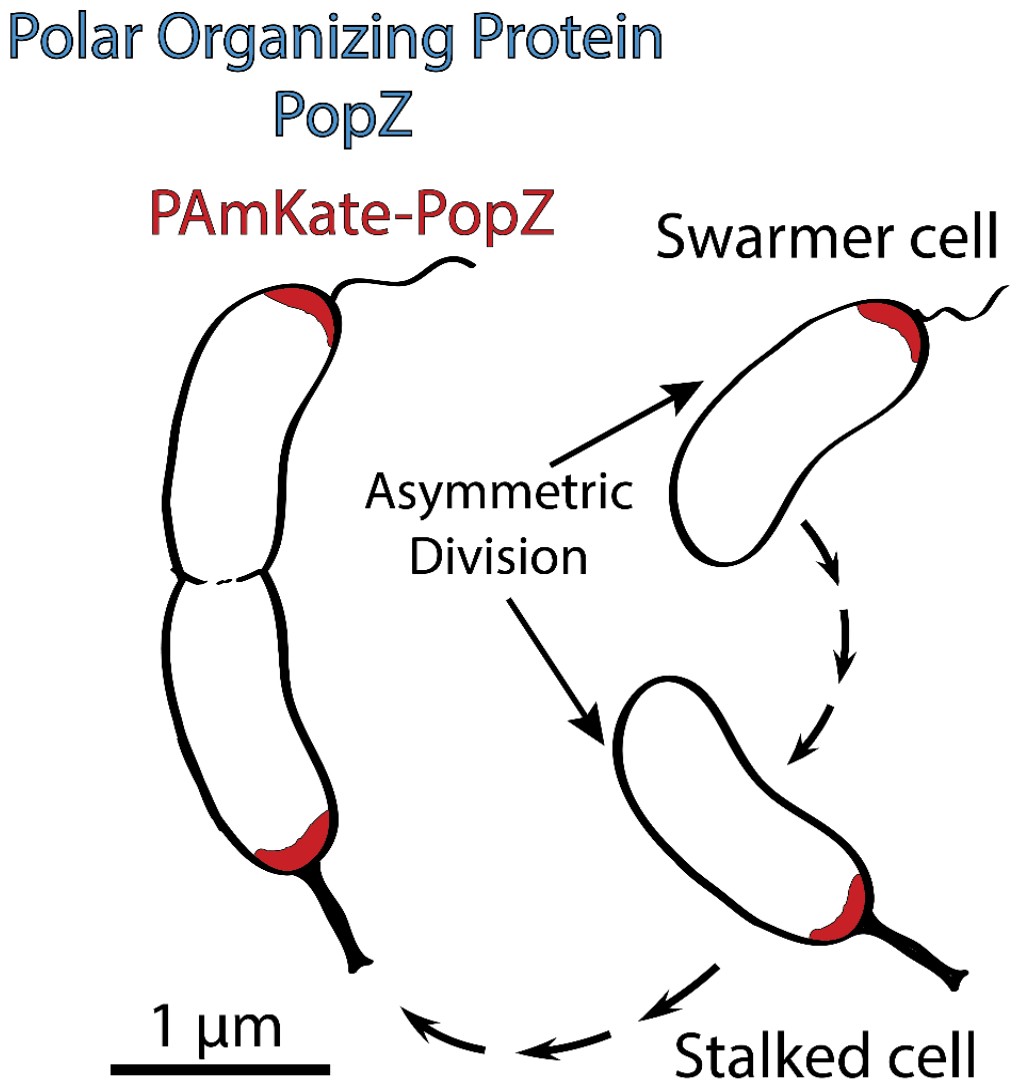
- Y.-W. Chang, et al., *Nat. Methods*, 11, (2014): 737–739
- Daria M Shcherbakova, Vladislav V Verkhusha, *Current Opinion in Chemical Biology*, (2014), : 60-68
- M. S. Gunewardene, et al. *Biophys. J.*, 101.6, (2011):1522-1528

Dahlberg et al. *JACS* **130**, 12310 (2018)

Caulobacter crescentus is a model system for asymmetric division

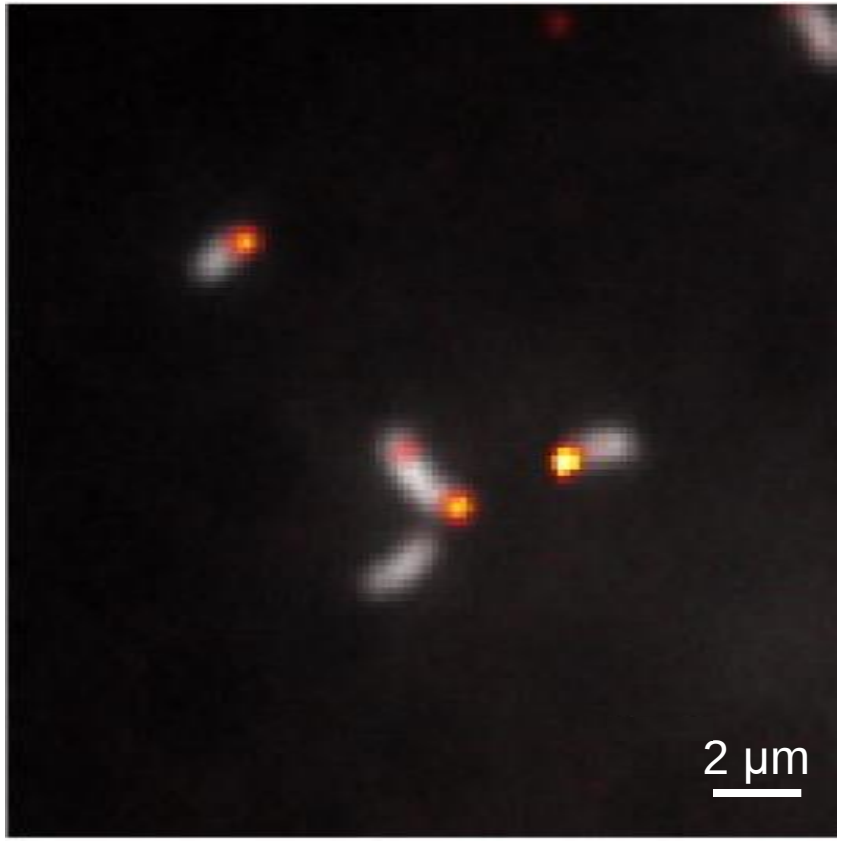


Polar Organizing Protein Z (PopZ): 17 kDa protein that forms a phase separated droplet



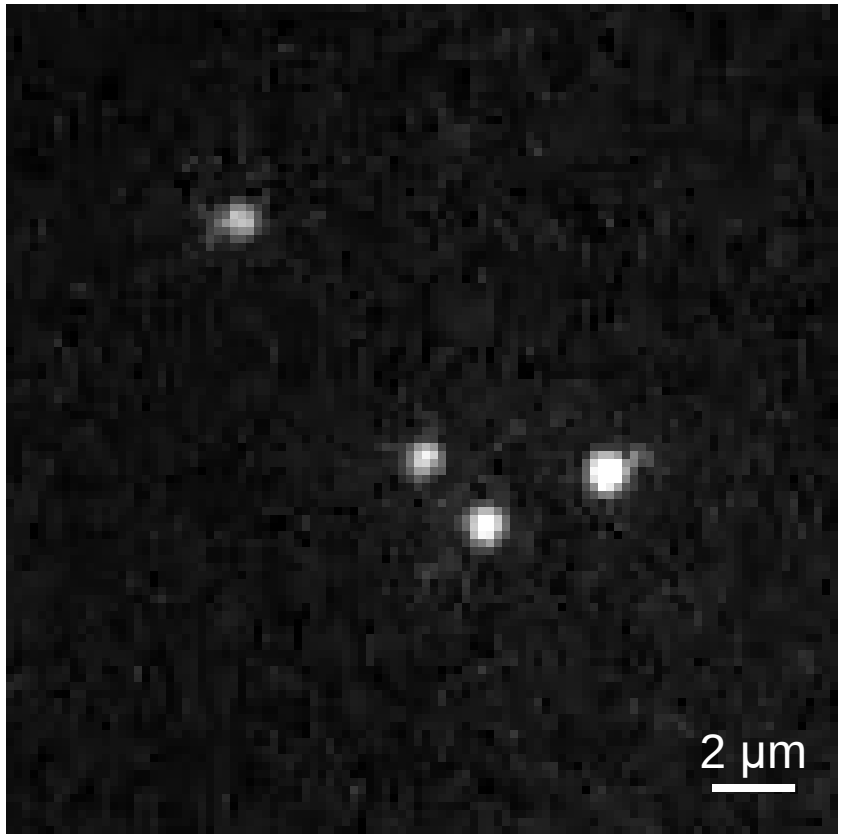
Single molecules of PAmKate protein fusions imaged at 77 Kelvin

Autofluorescence from 405 nm excitation (gray)
PAmKate fluorescence from 561 nm excitation (red)



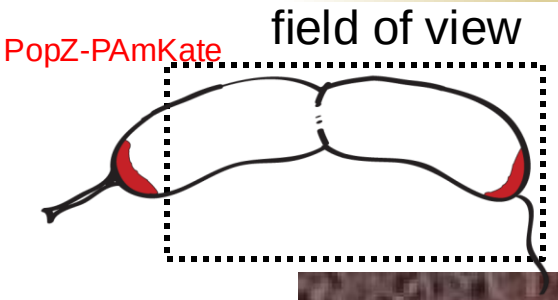
Long on-times at cryogenic temperatures due in part to reduced photobleaching

- **High-precision**
- **Limited density of localizations**

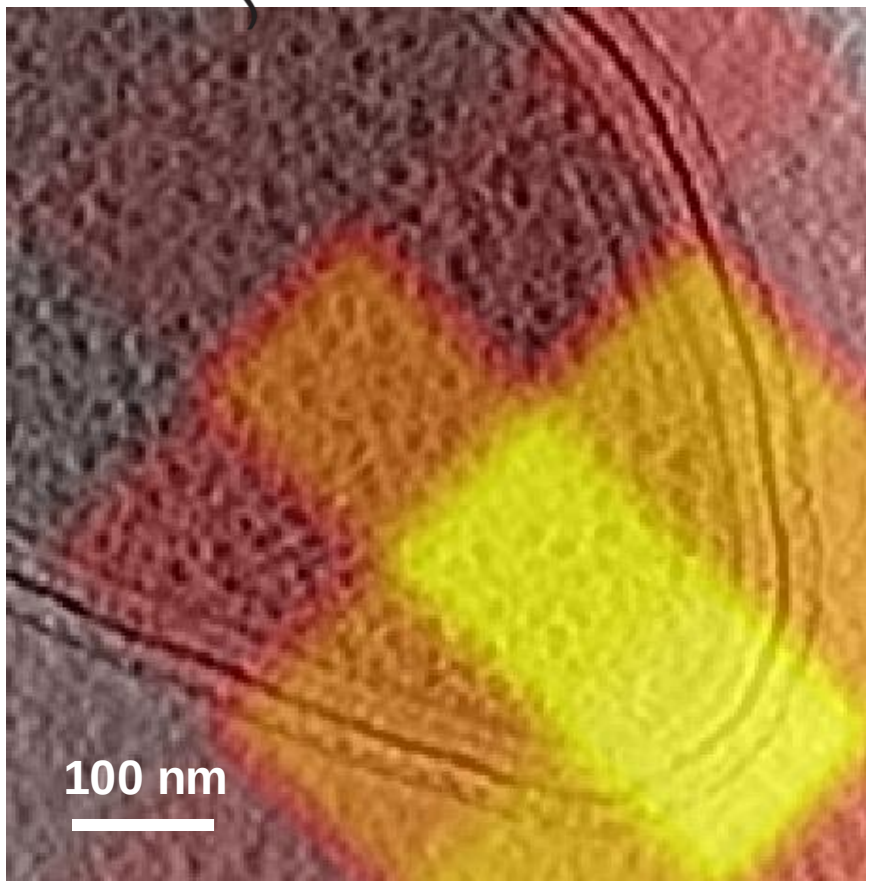


100x acquisition speed

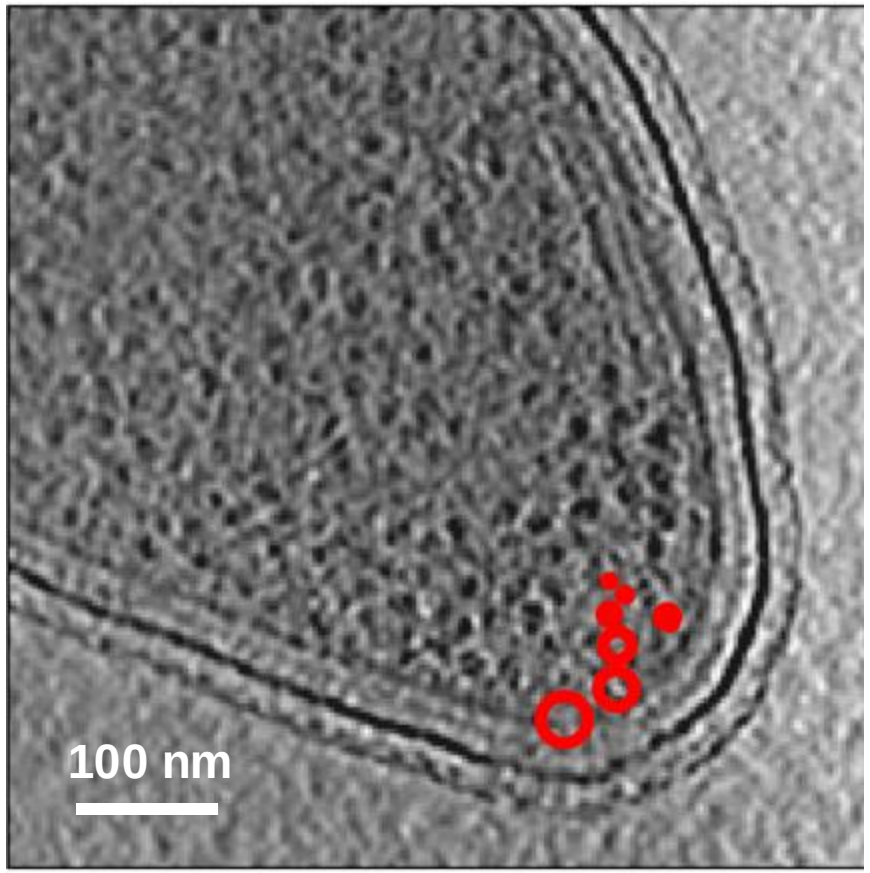
Correlative imaging can put locations of key proteins in high-resolution cellular context



Diffraction-limited
Cryogenic PopZ-PAmKate

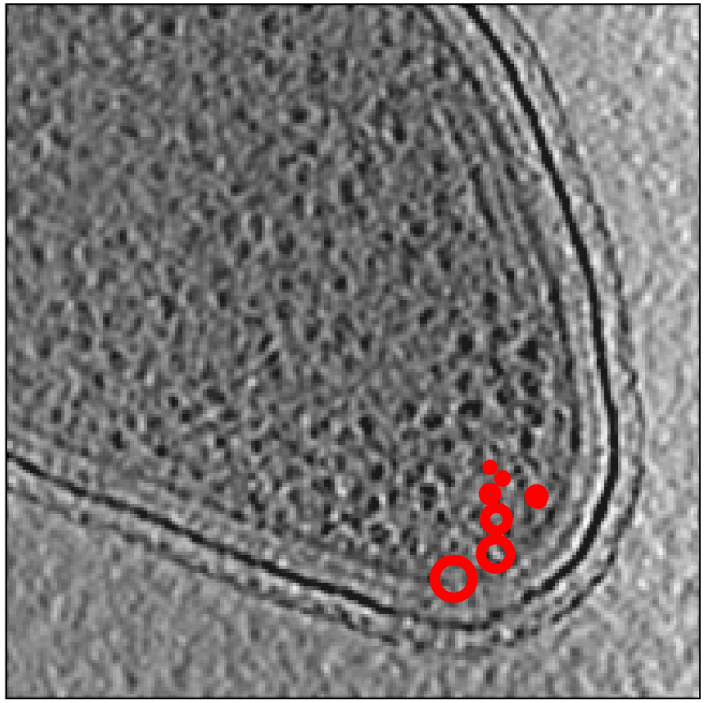


Mean radius = σ_{XY} ~ 9 nm
Mean photons ~ 12,000
Single-molecule localizations of
Cryogenic PopZ-PAmKate



Limited density of localizations due to long on-times of emitters and sample heating

Cryogenic PopZ Localizations



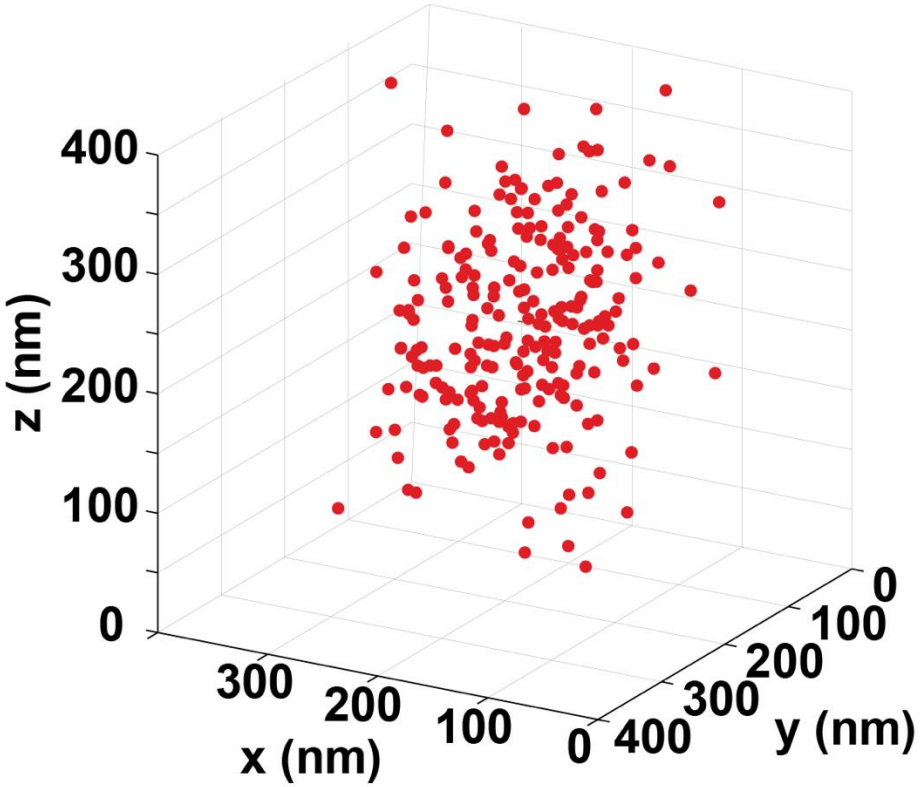
Limited density of localizations due to long on-times, 20+ minutes

Two effects:

1.) Reduced quantum yield of photobleaching

2.) Limited excitation intensity

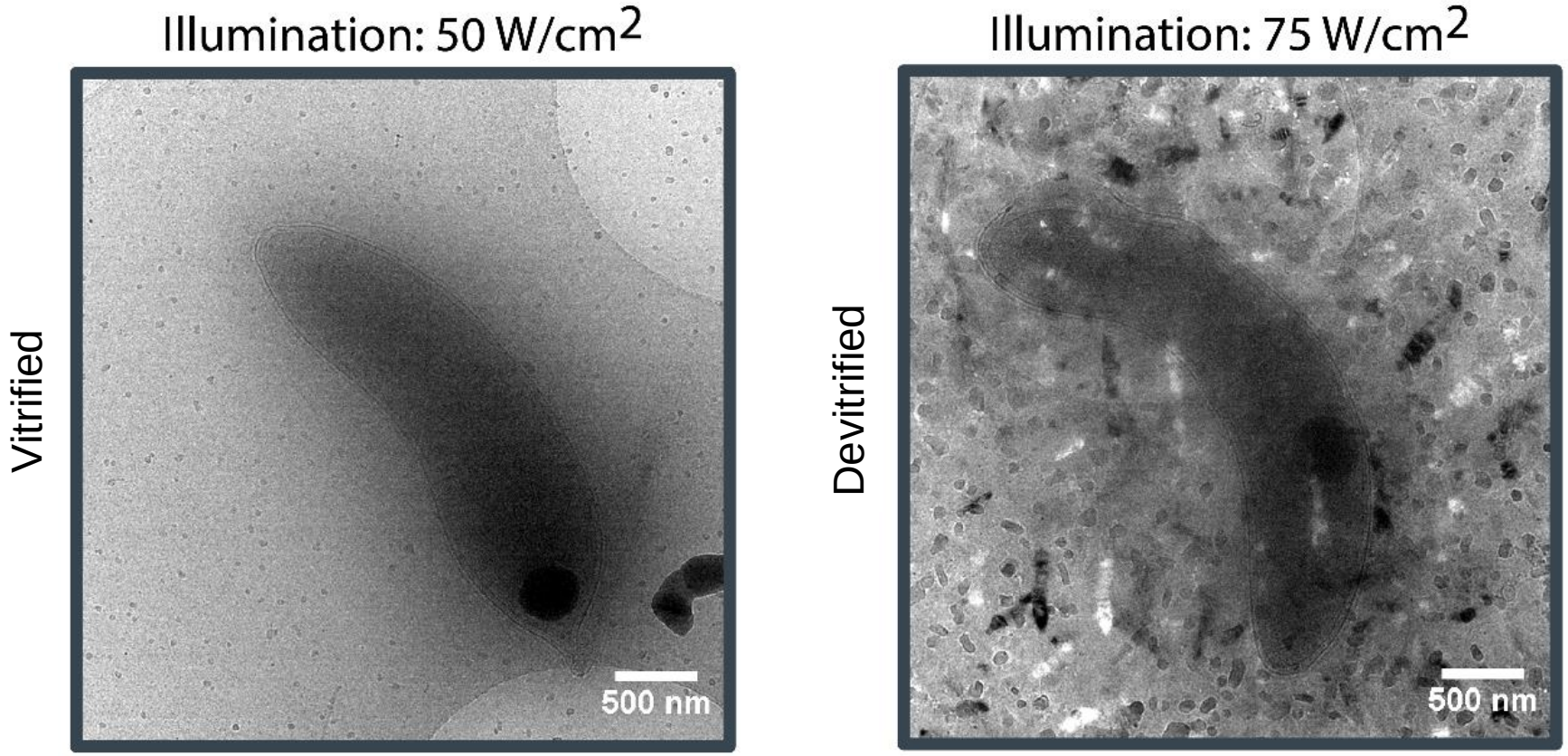
Room Temperature PopZ Localizations



Modified from Ptacin, Jerod L., et al. *PNAS*, *(2014)*

Heating of the sample limits usable excitation intensities

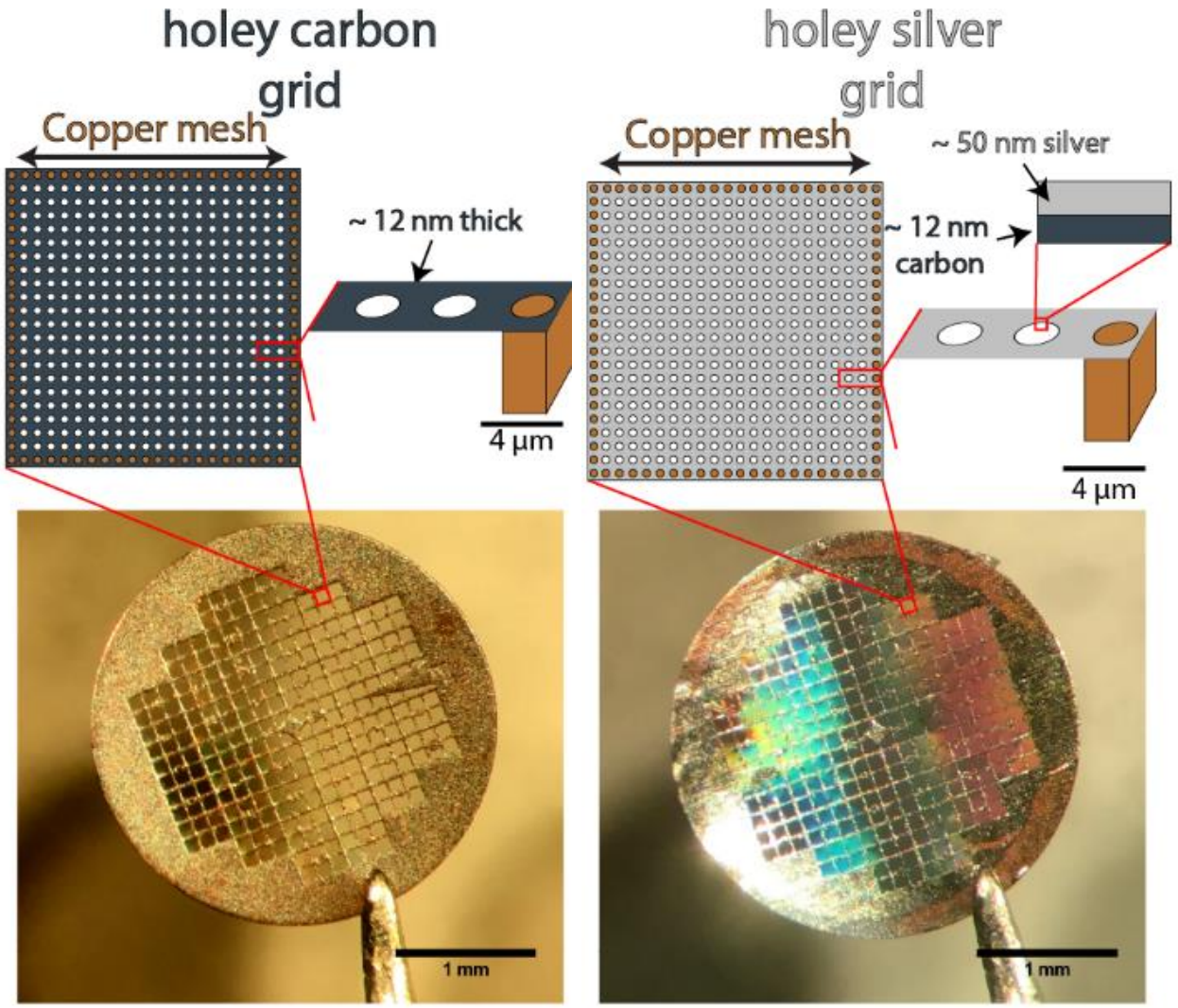
Onset of devitrification at ~50-75 W/cm^2 of optical pumping

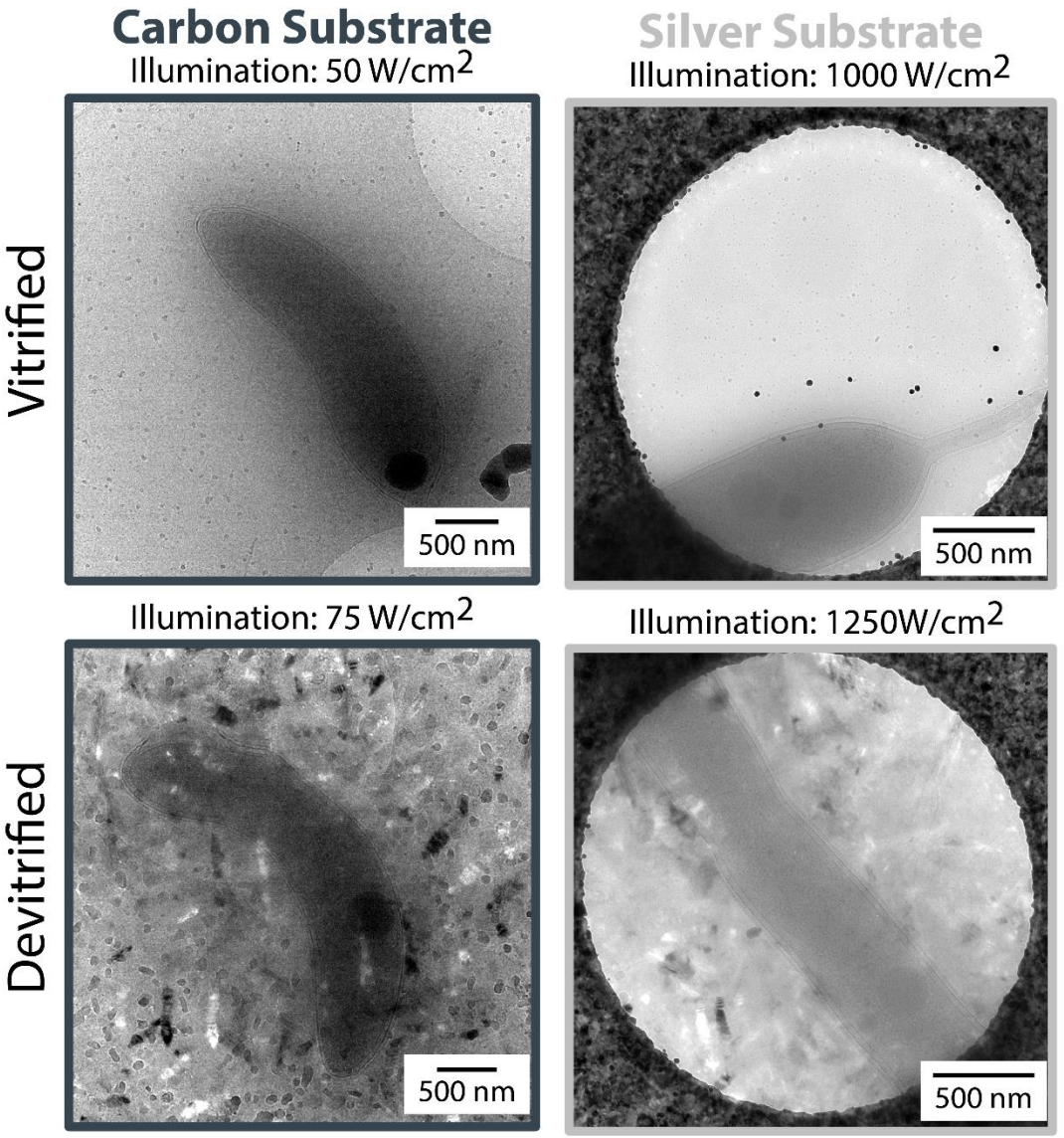


Room-temperature super-resolution typically uses kW/cm^2 for instance Gustavsson, A-K., et al. *Nat. Comm.* (2018) use 25 kW/cm^2

Want substrate with low absorptivity, high thermal conductivity, and high electrical conductivity

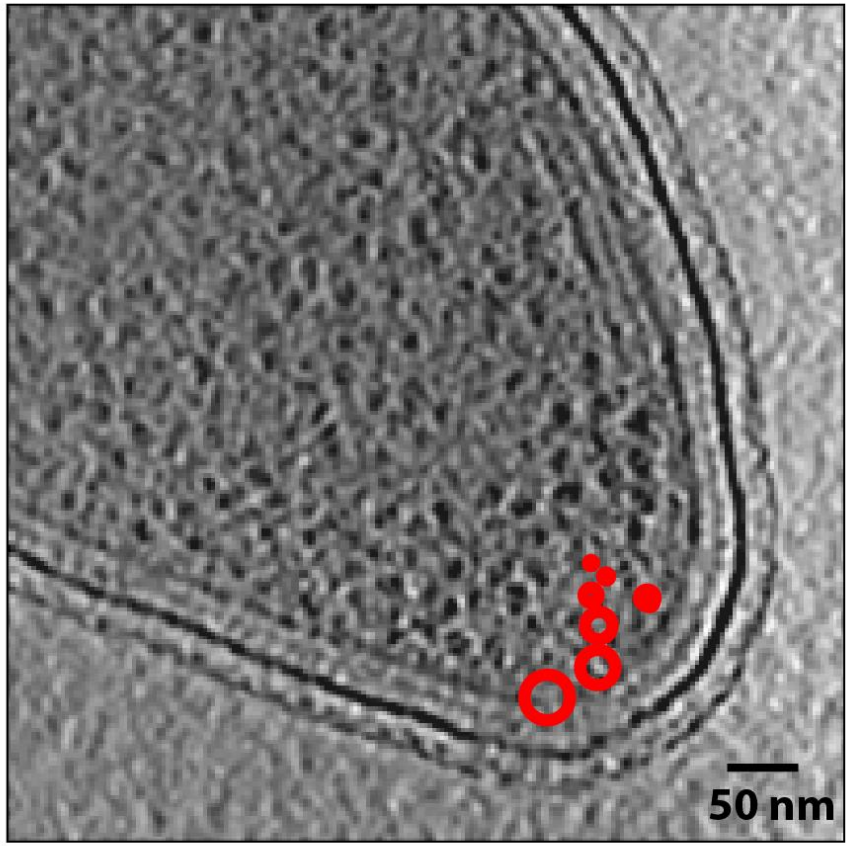
Changing the support film material properties improves cryogenic imaging





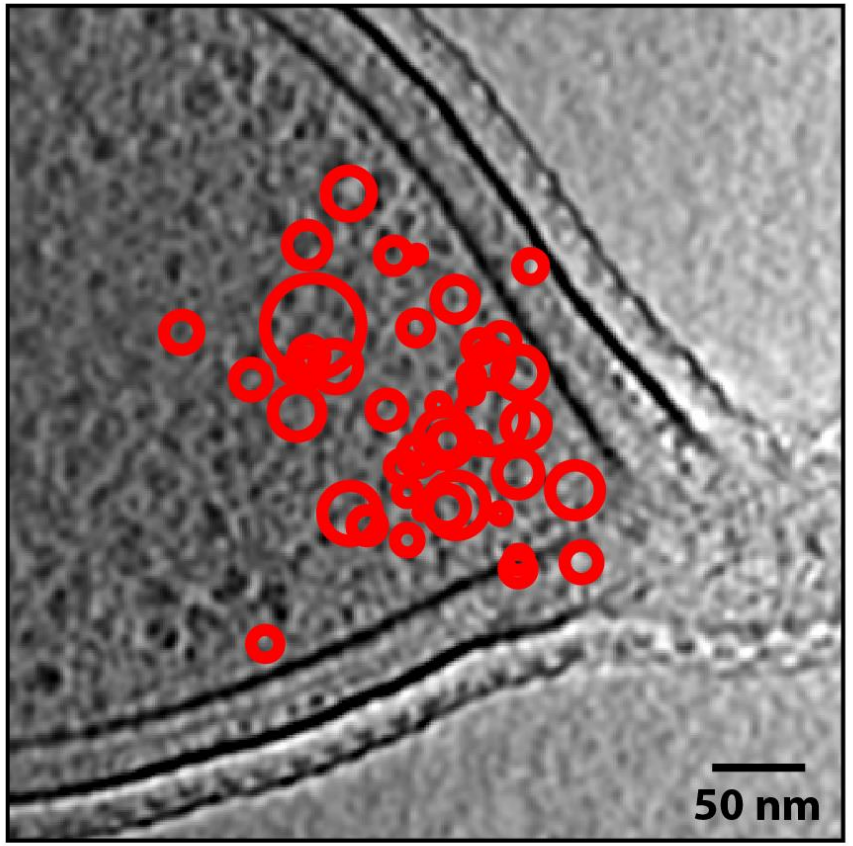
Density of localizations is substantially improved using silver grids

PopZ localizations on Carbon grid



20x intensity
for 1/6 the time

PopZ localizations on silver grid

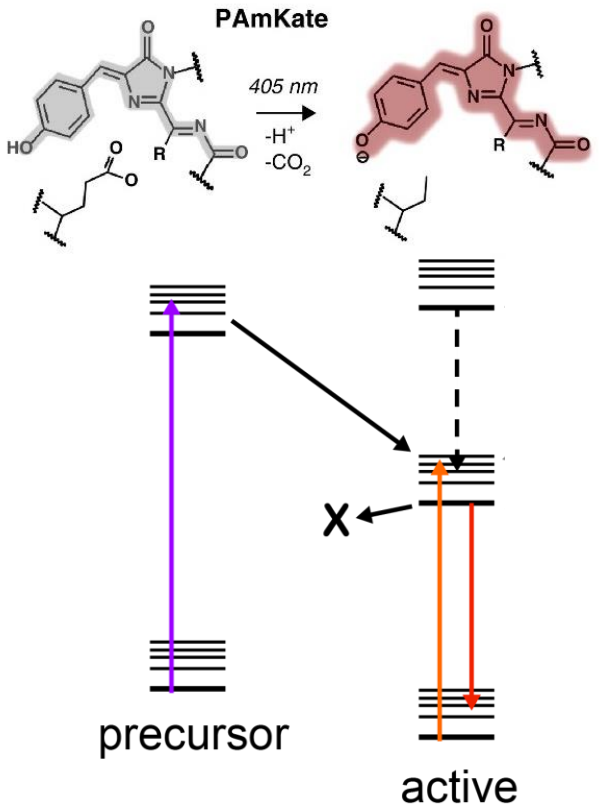


~20x improvement in localizations/second

Davis Perez

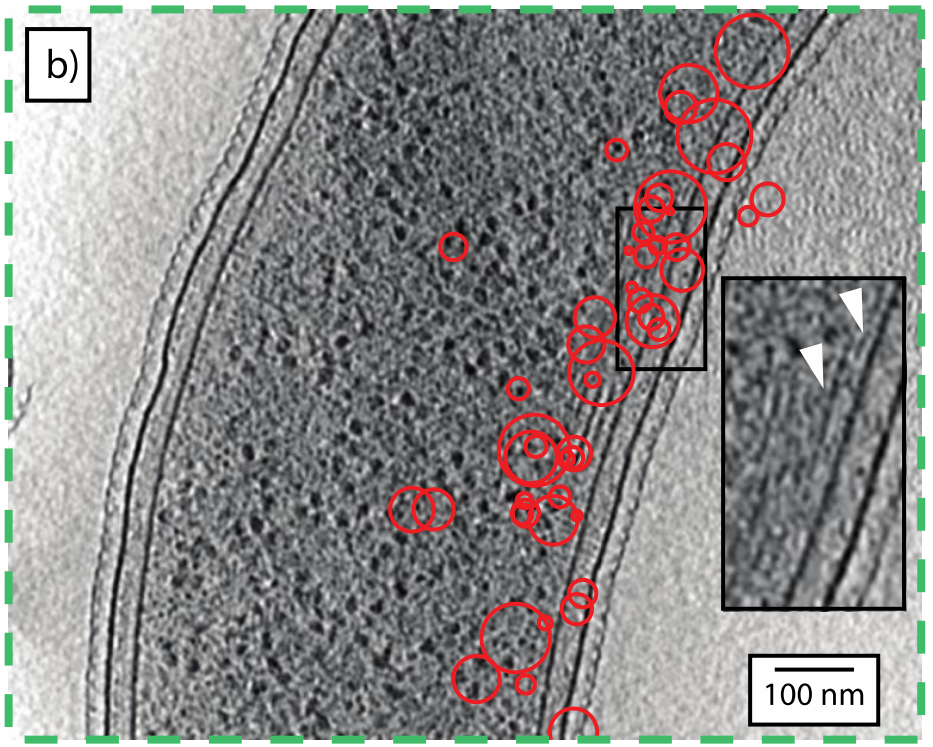
Photophysical understanding leads to better control and better super-resolution as well

Understanding of cryogenic energy landscape can be exploited using different colors and timed pulses to more efficiently turn emitters on and off.



- Dahlberg, Peter D., et al. *JACS* (2018)
- Sartor, Annina M., et al. *JPCB* (2023)
- Perez, Davis, et al. JACS (2024)

Just 10 minutes of data collection

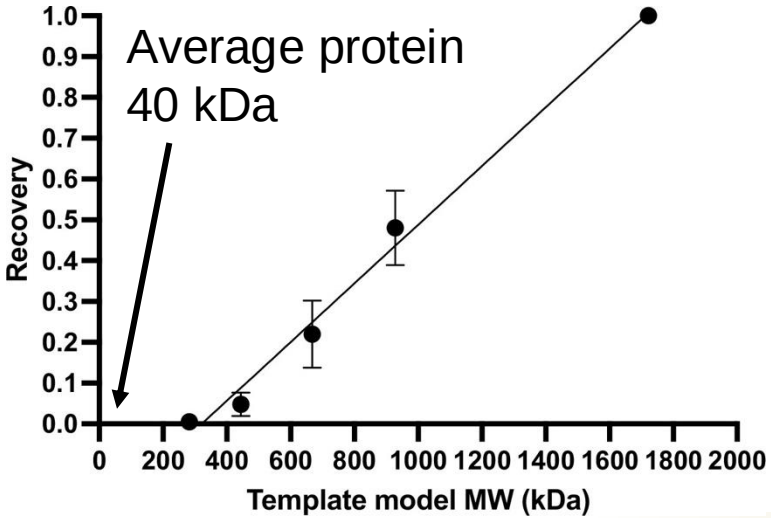


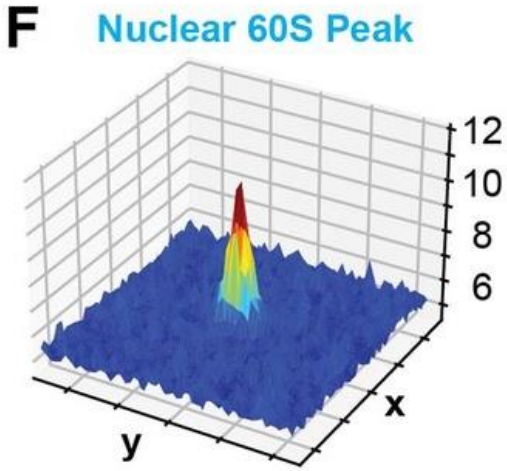
PAmKate Jablonski diagrams

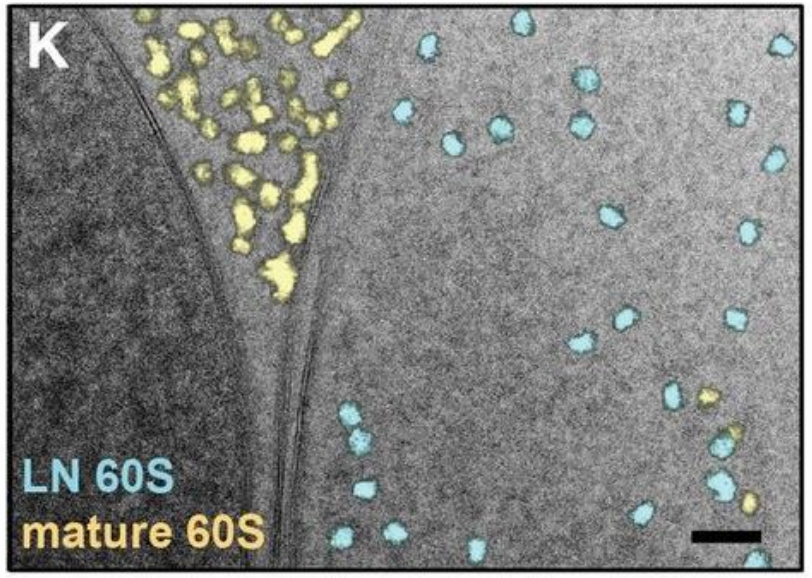
What is next? *Integration of super-resolution data into subtomogram averaging workflow*

- First step of subtomogram averaging is identification of your structures
  - Template matching scans the volume looking for regions of high correlation with known structure

- Problem: Most structures can not be identified without high numbers of false positives







Lucas, Bronwyn A., et al. *Elife* (2023) & Lucas, Bronwyn A., et al. (2022).

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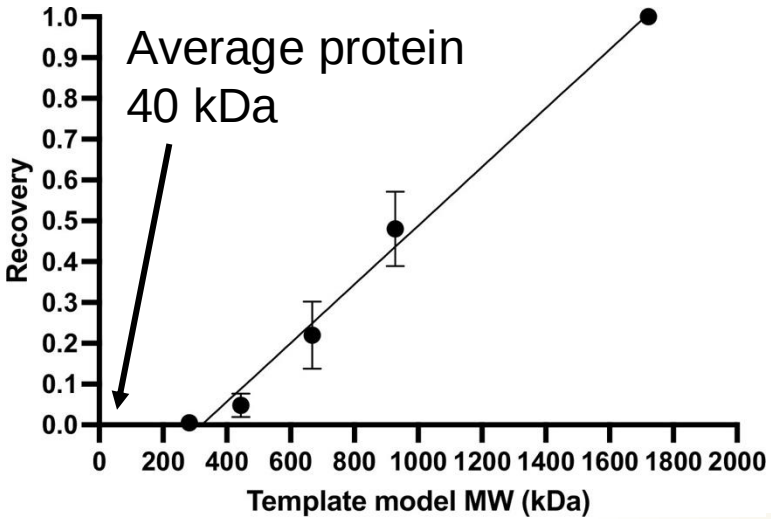
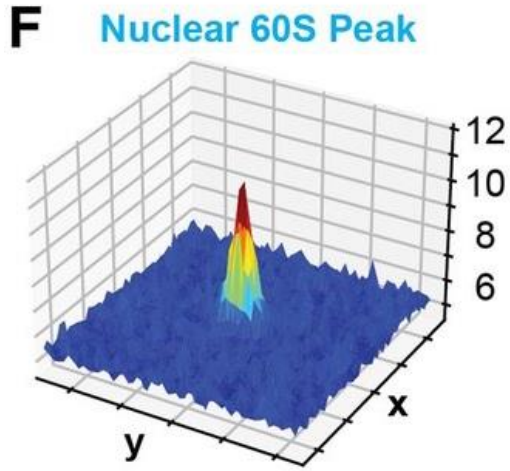
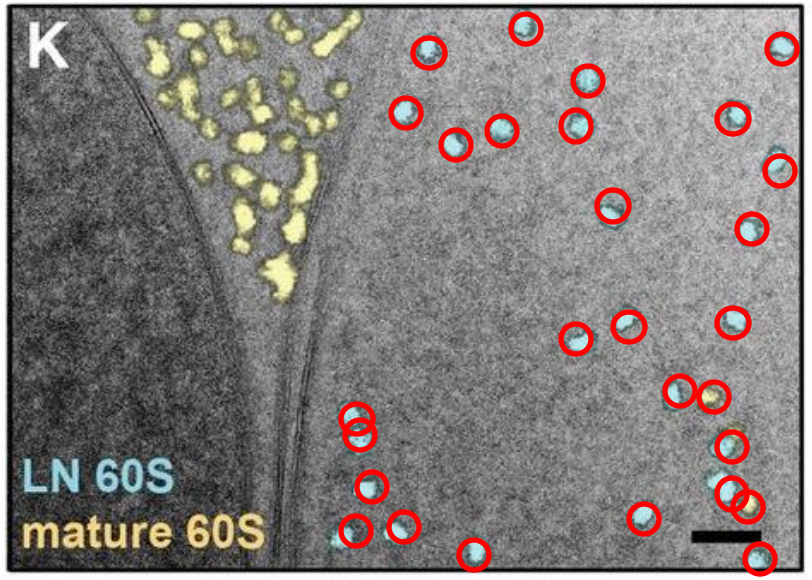


Solution: Restrict search space using single-molecule localizations

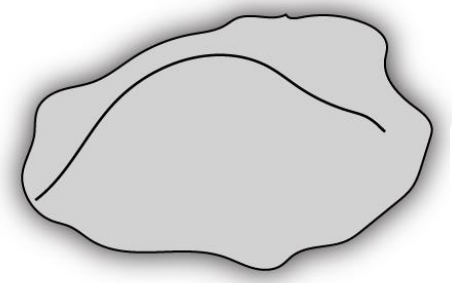




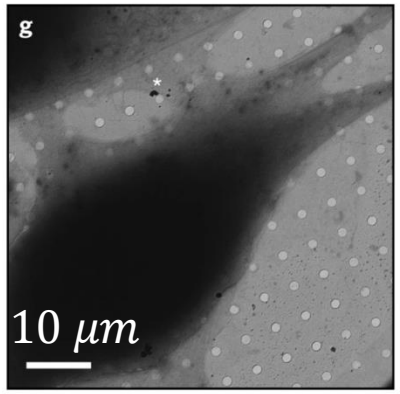
Lucas, Bronwyn A., et al. *Elife* (2023) & Lucas, Bronwyn A., et al. (2022).

What is next? *Integration into the FIB-SEM*

- Most cellular samples must be thinned to ~200 nm or less prior to tomography
  - Which section should be saved?
  - How can we take the most informative images of the final section?



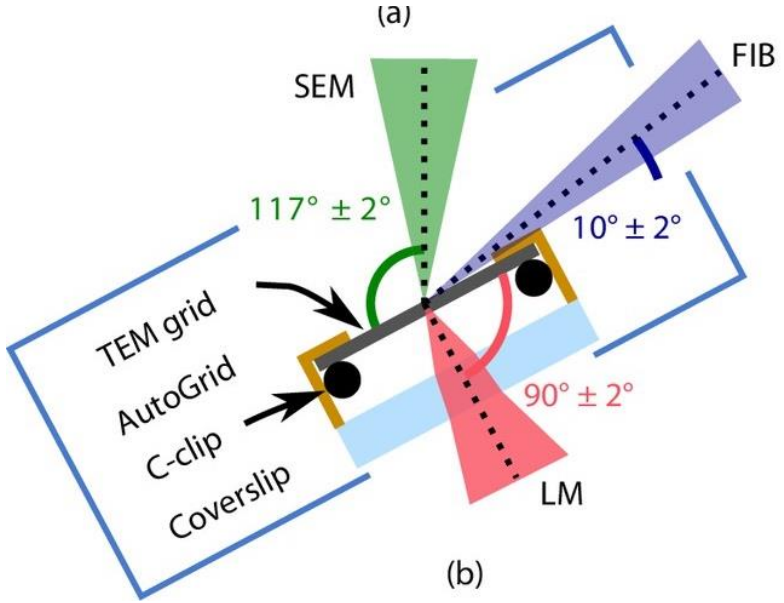
Cells on grid

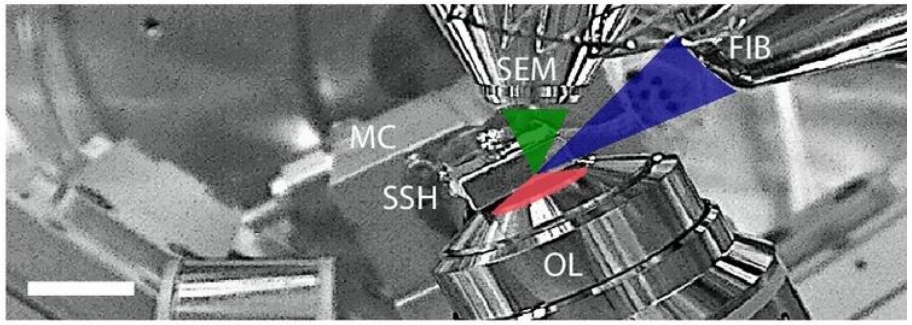


Wagner, Felix R., et al, Nature protocols (2020)

Phyllis Wang

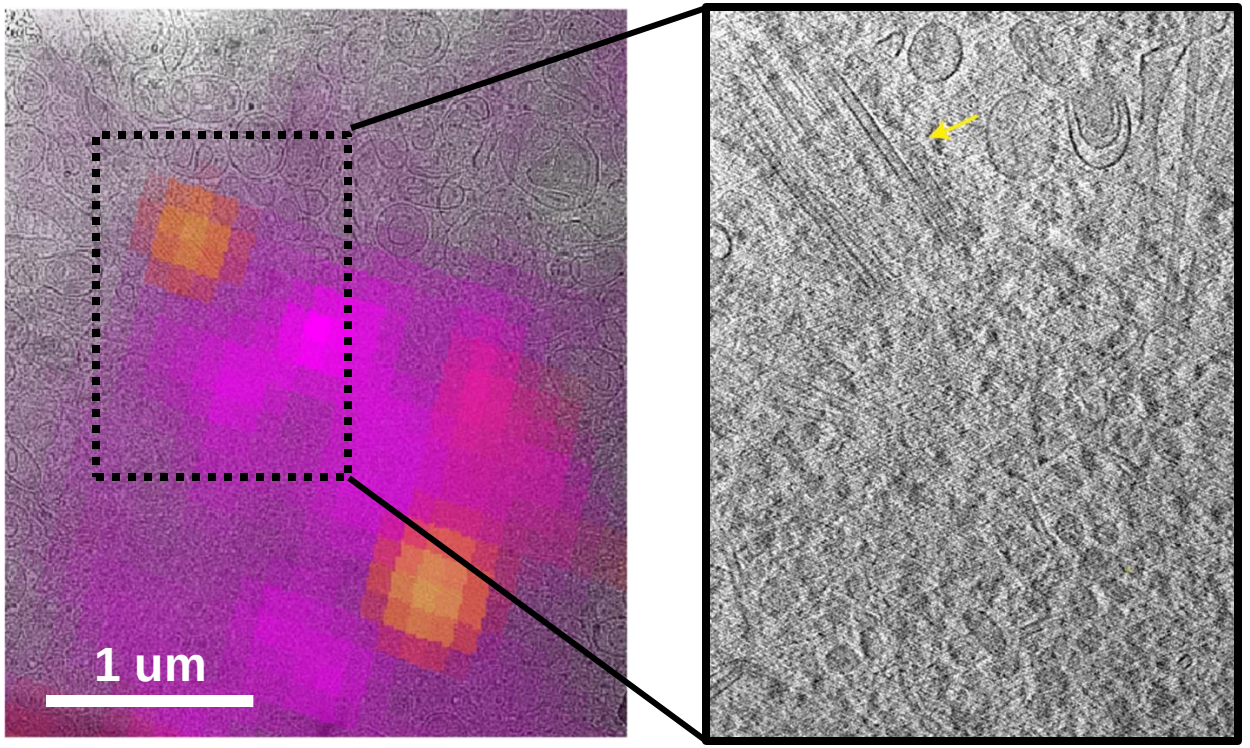
What is next? *Integration into the FIB-SEM*





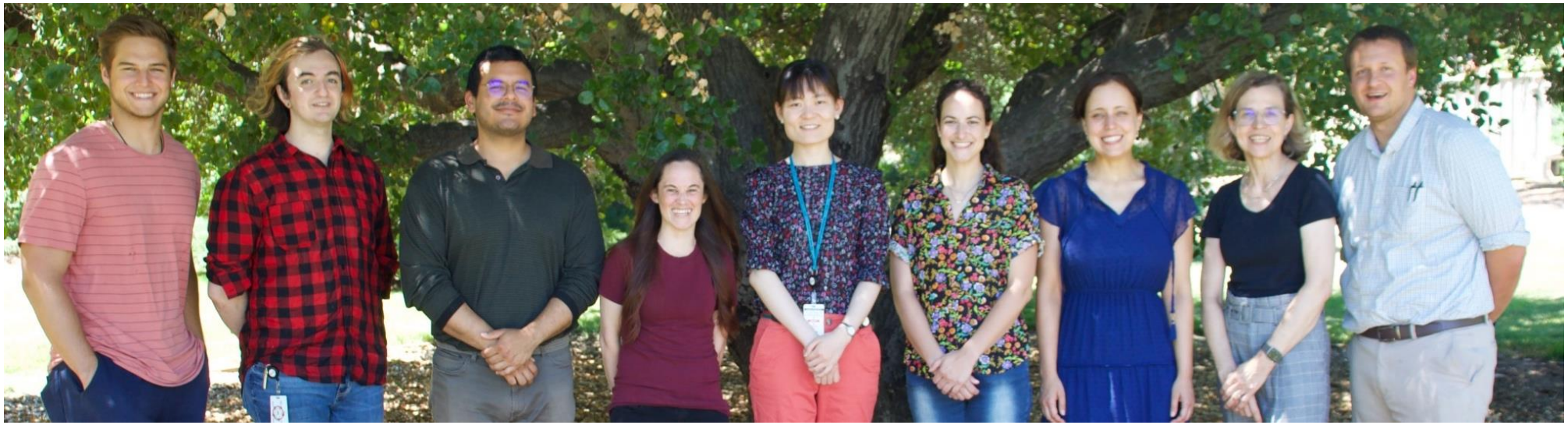
Boltje, Daan B., et al. *Elife* (2022)

- Fluorescence microscopy is an obvious choice for guiding milling
- Integration of light microscope into the FIB-SEM



Jue Wang et al. Under Review

Acknowledgements



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Questions?

Use SR-CryoCLEM to determine the locations of specific proteins of interest in the context of CryoET

Custom silver grids improve density of localizations by reducing sample heating

Future directions for correlative light and electron microscopy

- Improved subtomogram averaging
- Integration into FIB-SEM