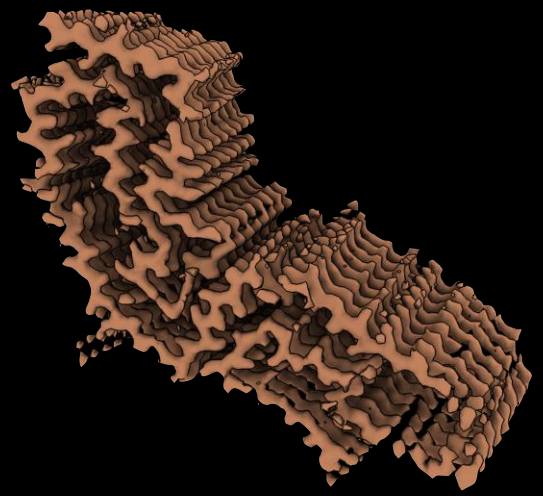
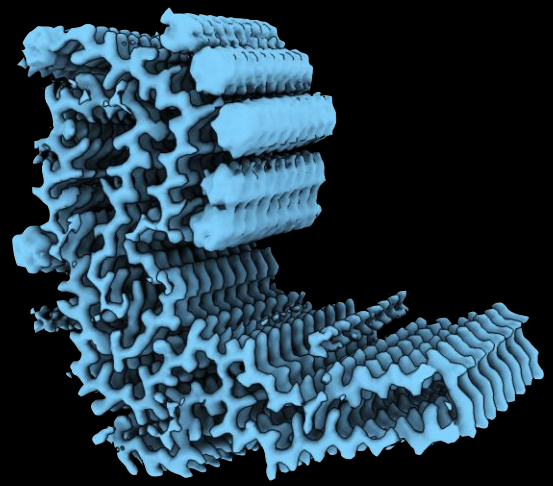

Cryo-correlative imaging of prion strains
in neurons
Thomas Trainer

263K



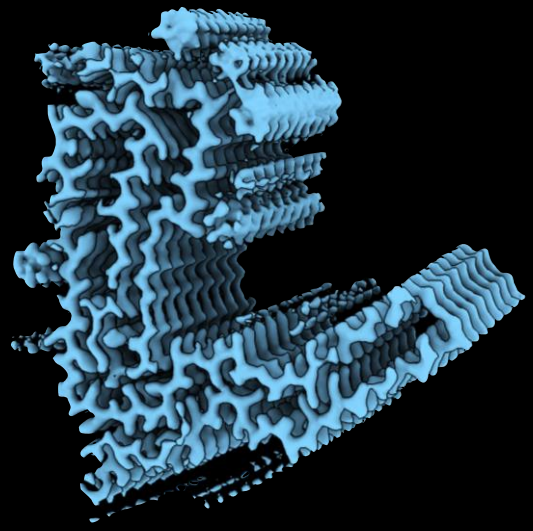
Kraus et al. 2021

RML



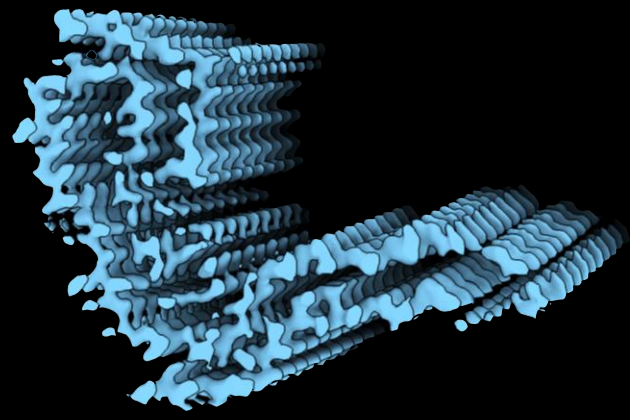
Manka et al. 2022
Hoyt et al. 2022

ME7



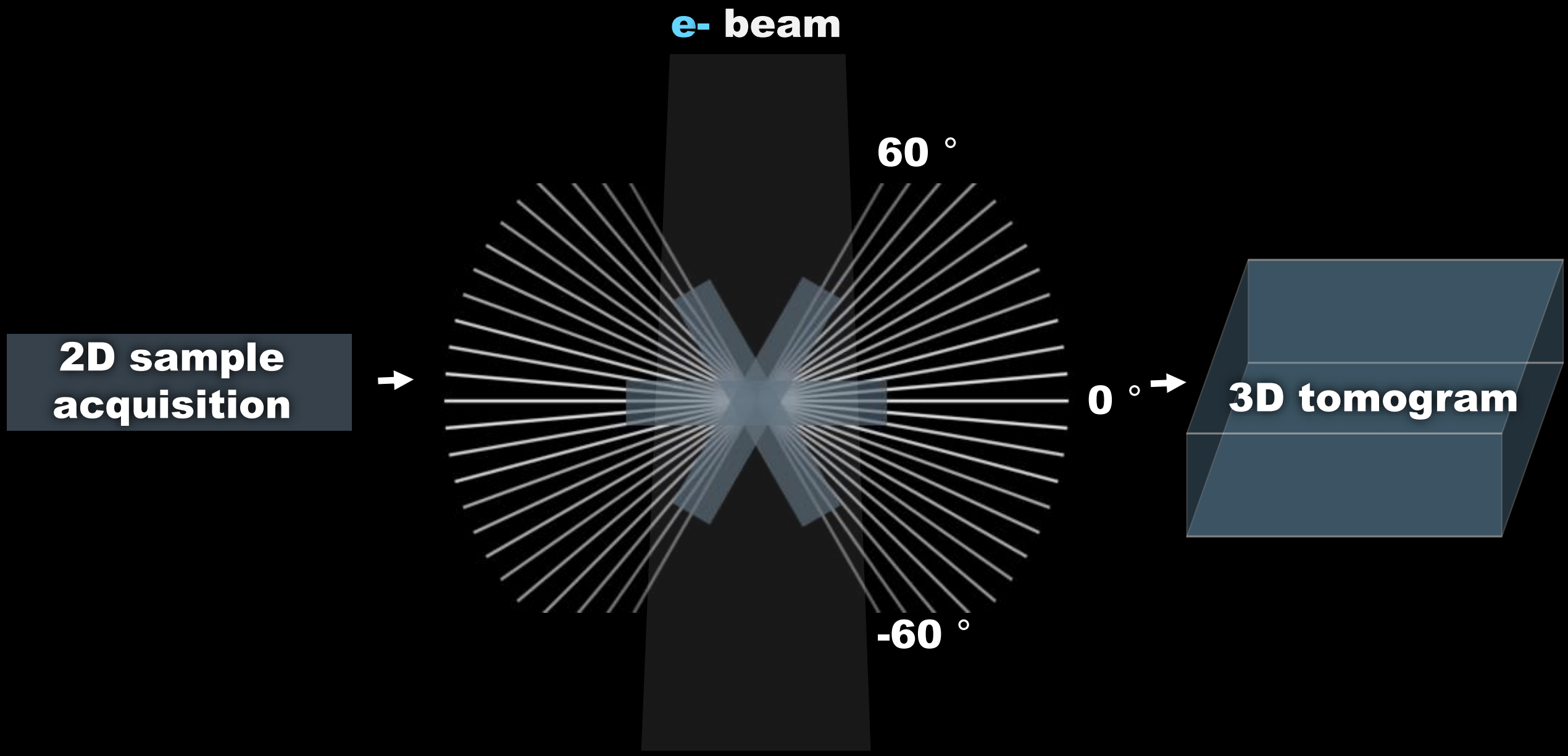
Manka et al. 2023

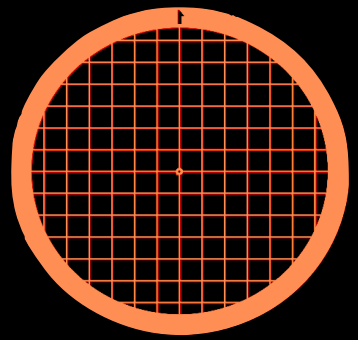
a22L



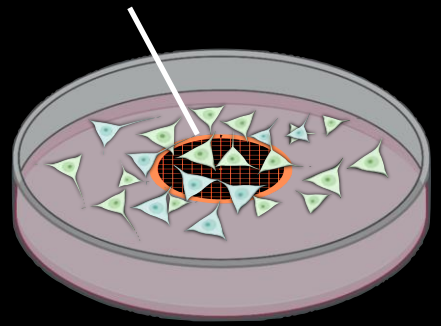
Hoyt et al. 2023

Cryo-electron tomography (Cryo-ET)



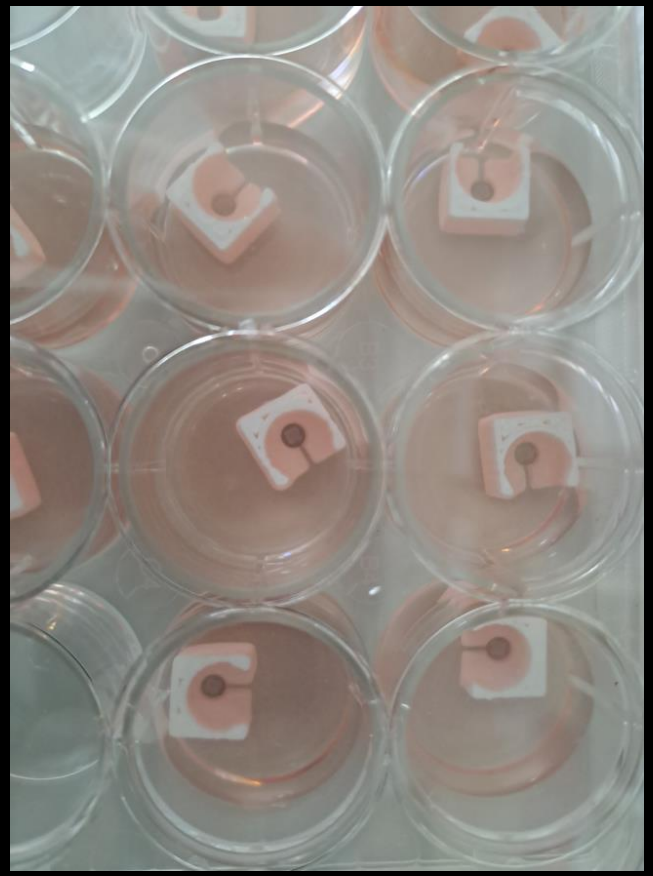


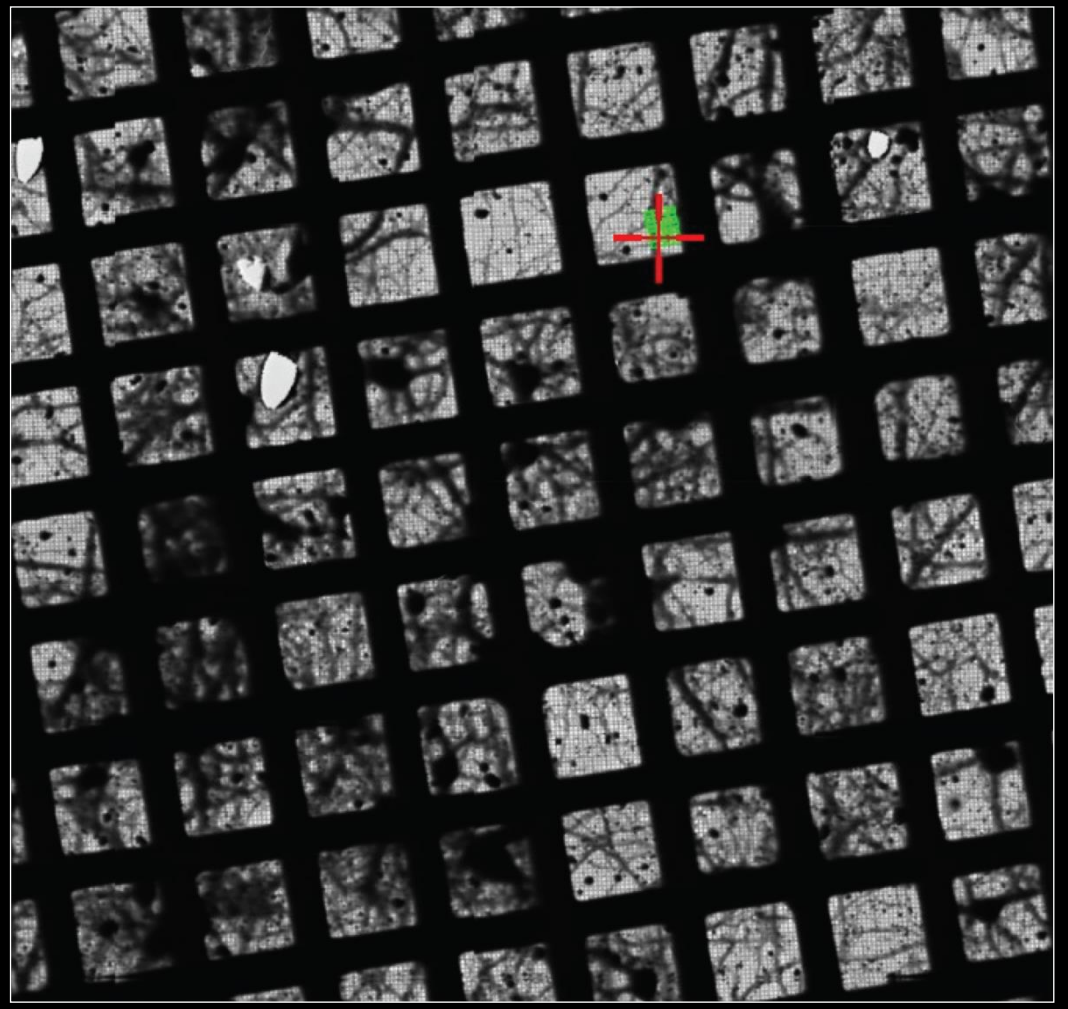
EM grid

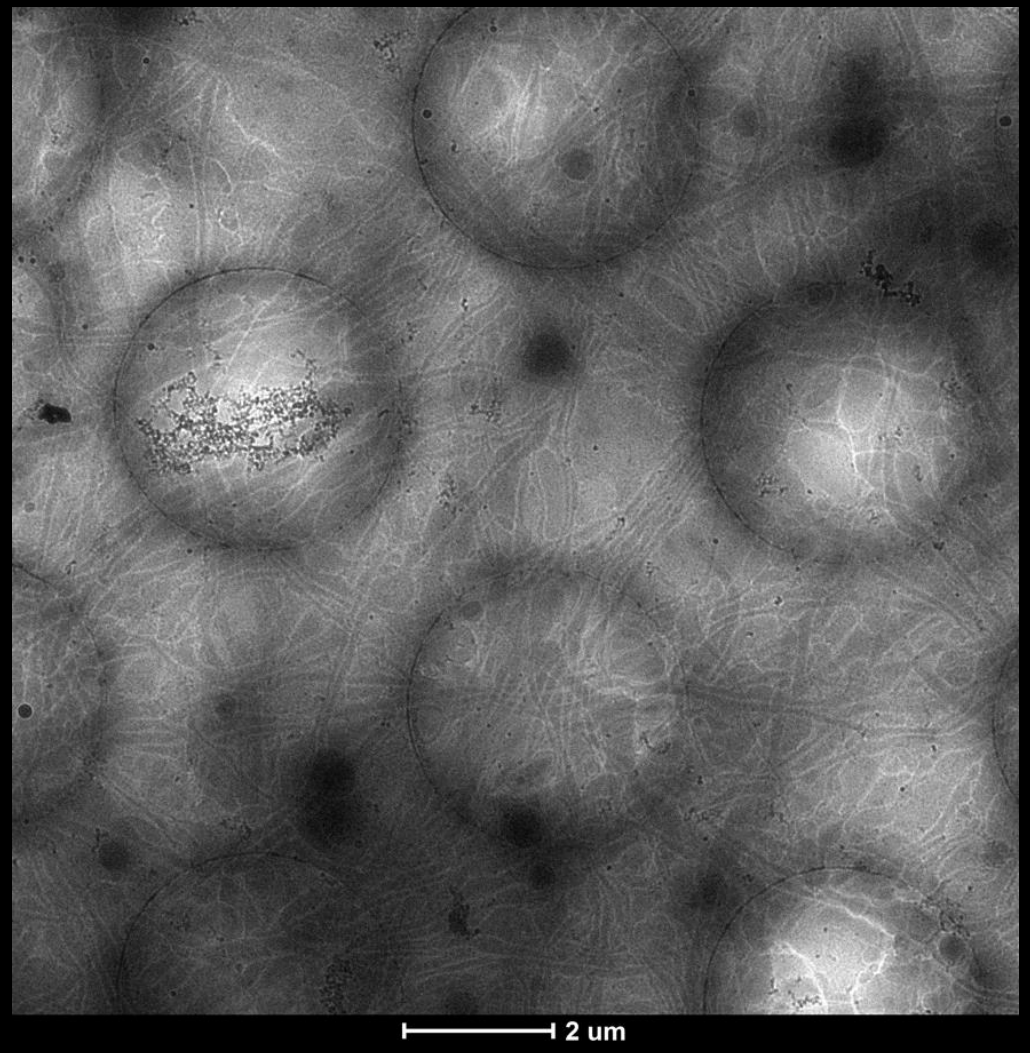


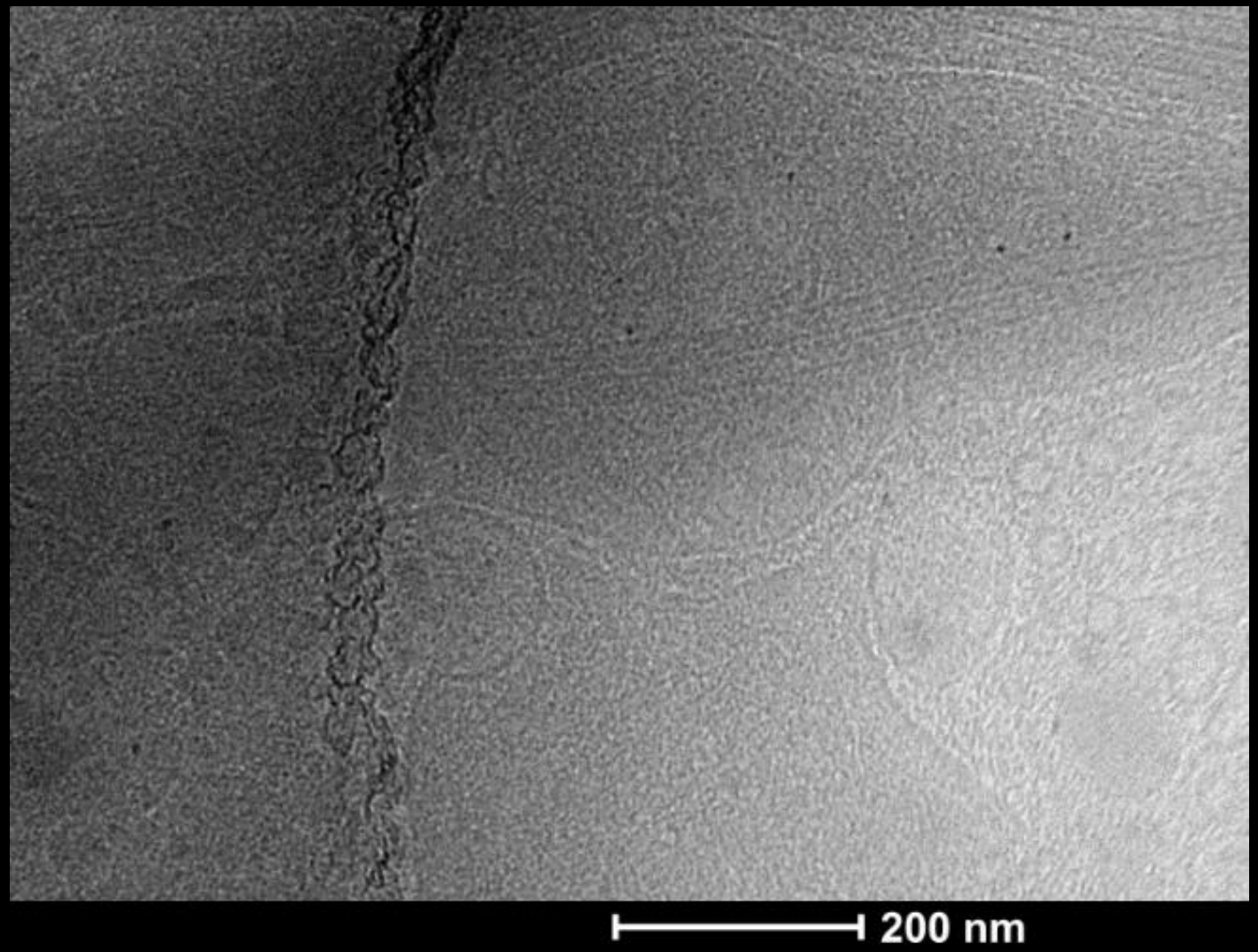
E17 mouse embryo

Primary neurons





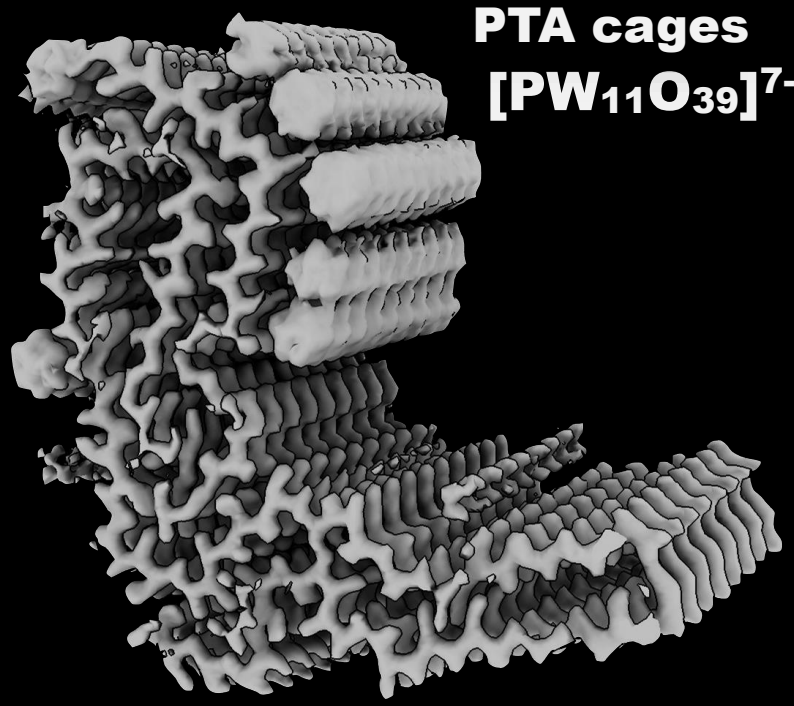




Allows direct visualisation of proteins within neurons

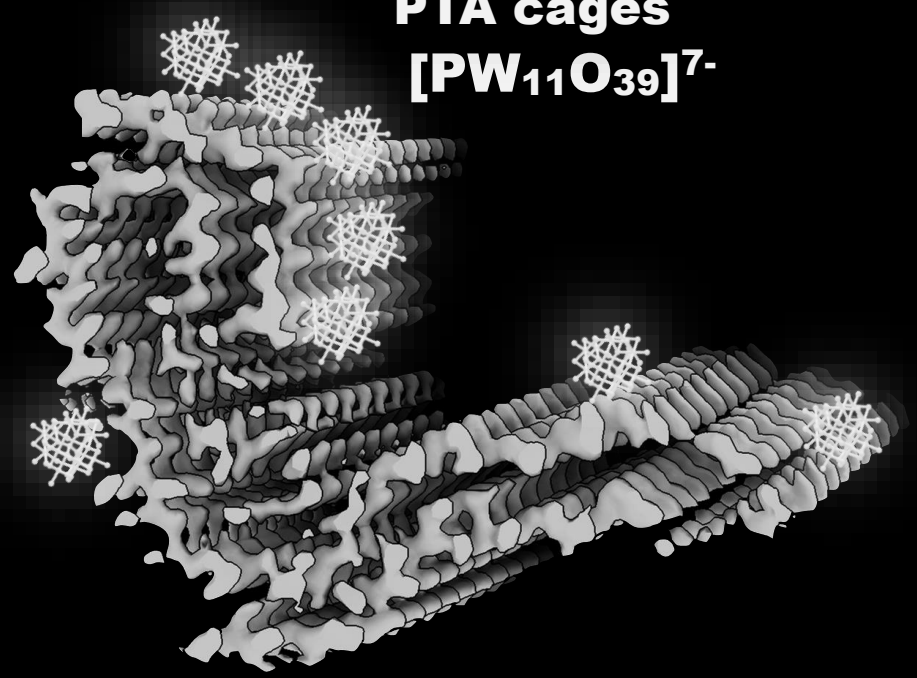
But how to target prions?

RML



PTA cages
$[PW_{11}O_{39}]^{7-}$

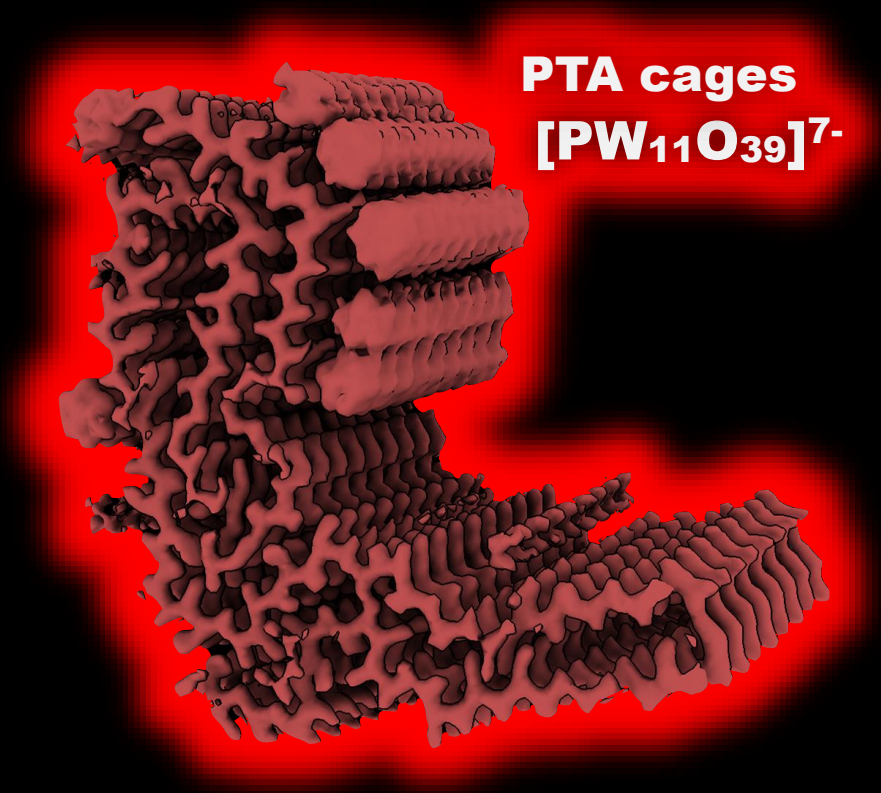
22L



PTA cages
$[PW_{11}O_{39}]^{7-}$

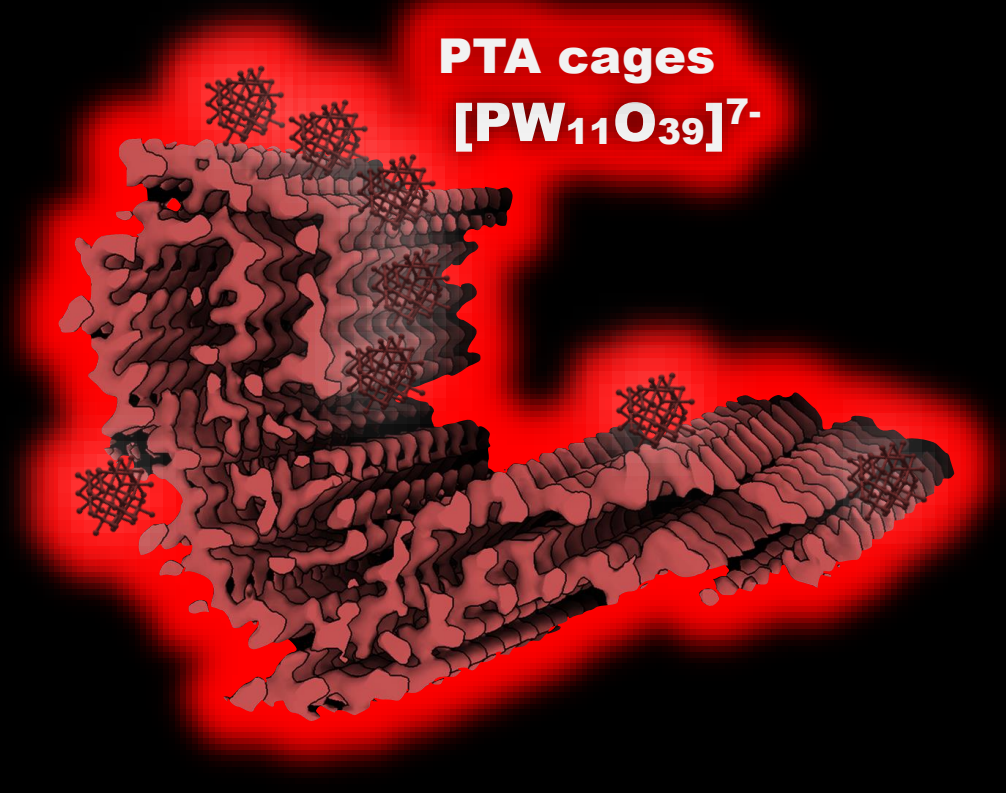
AlexaFluor546-NHS-ester

RML

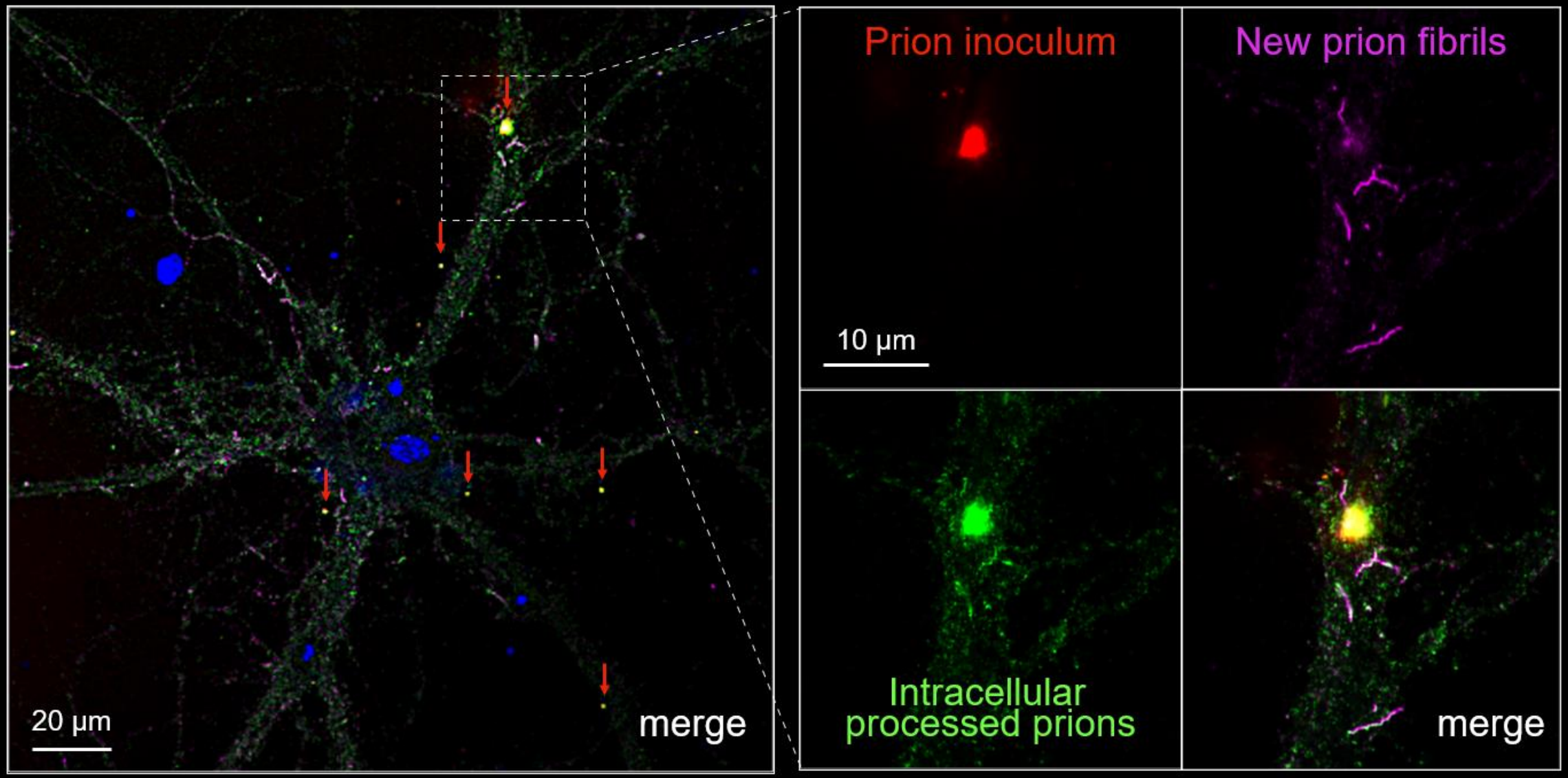


+

22L



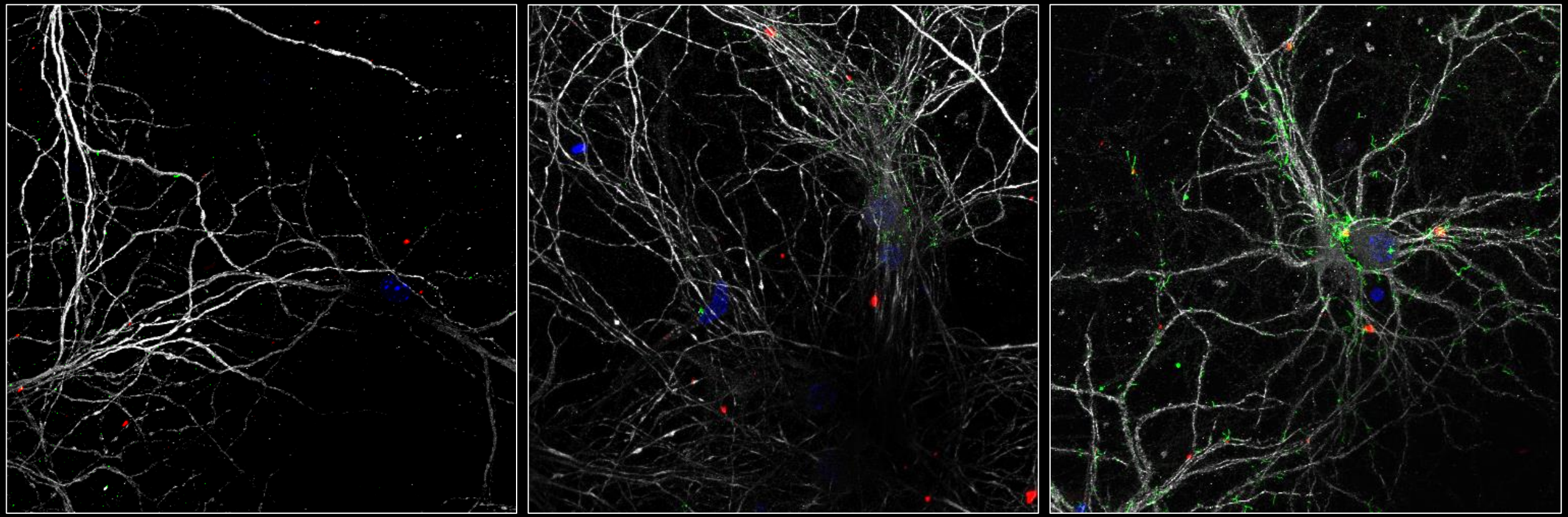
Selective Abs:
6D11
5B2
Ribes et al. 2023



7dpi
RML

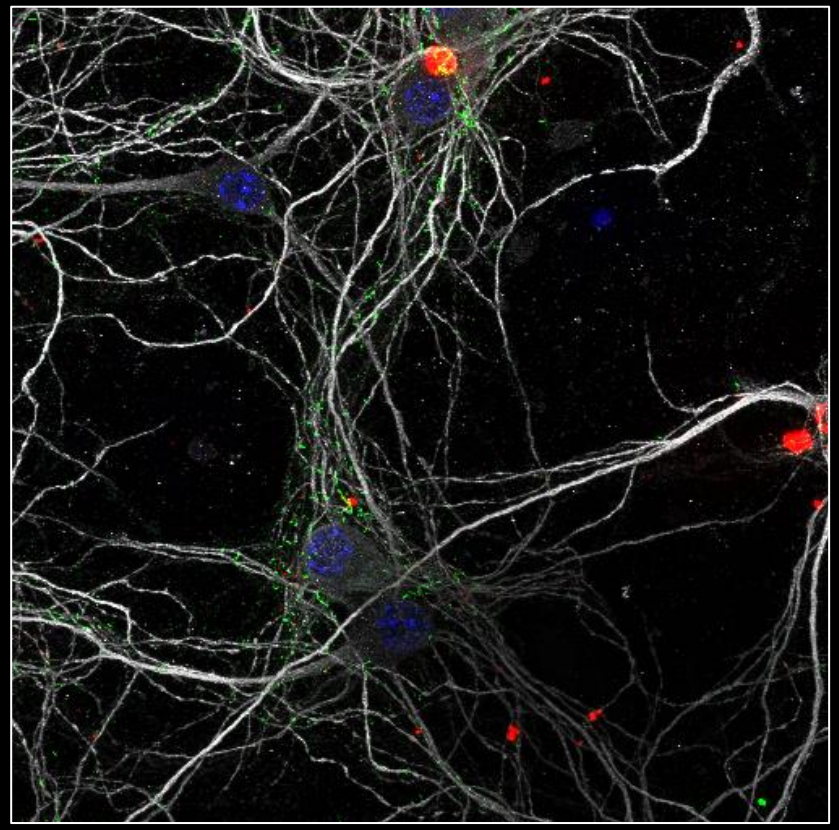
14dpi
RML

21dpi
RML

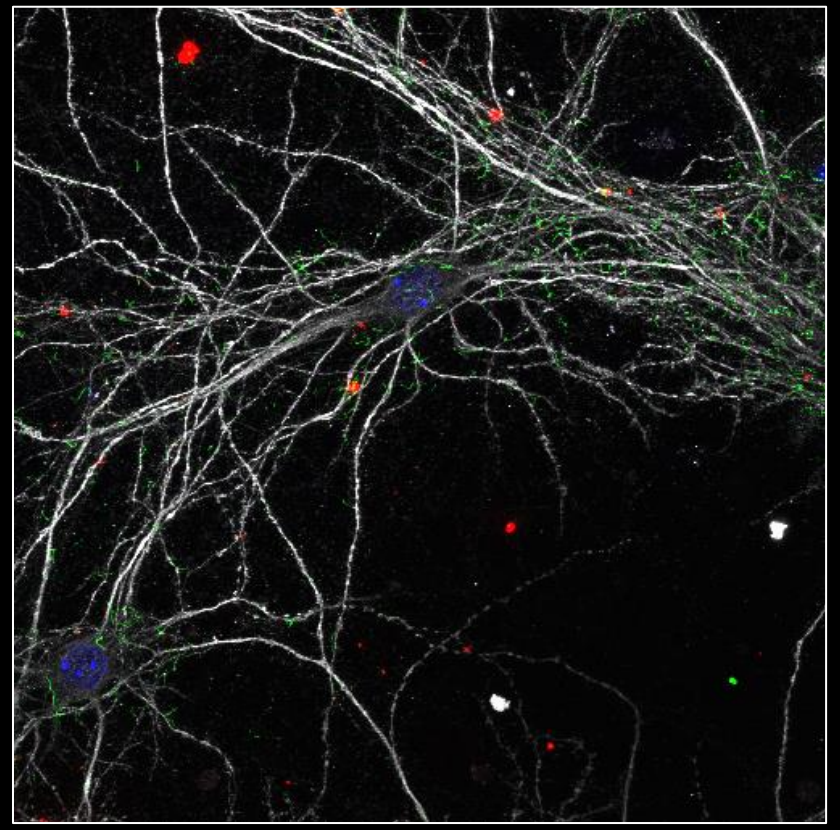


Fluorescence:
MAP2
5B2
546-pRML

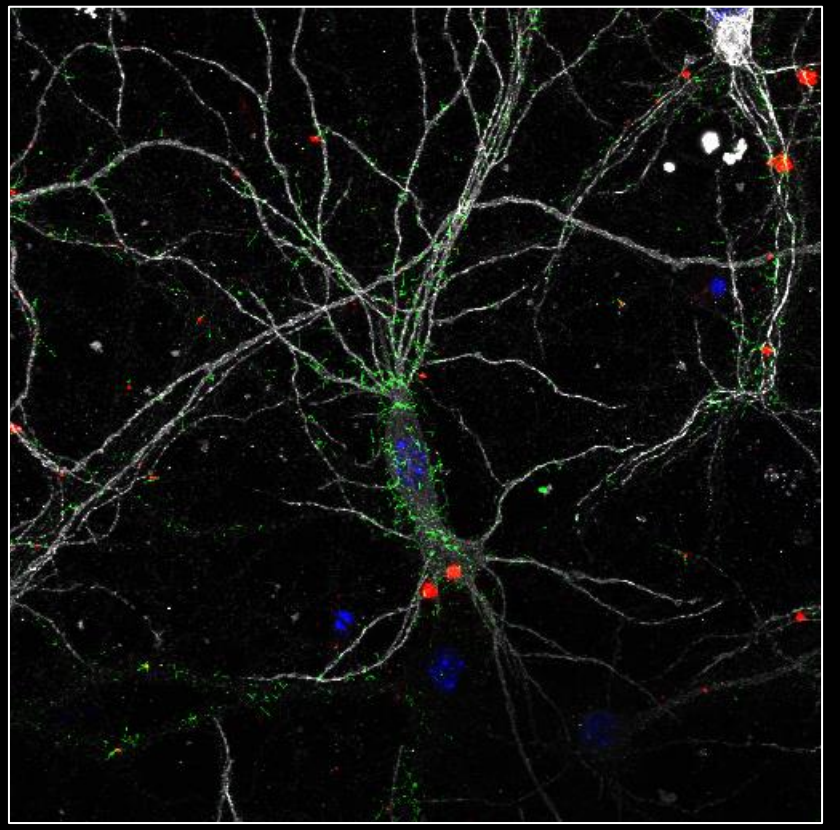
7dpi
22L



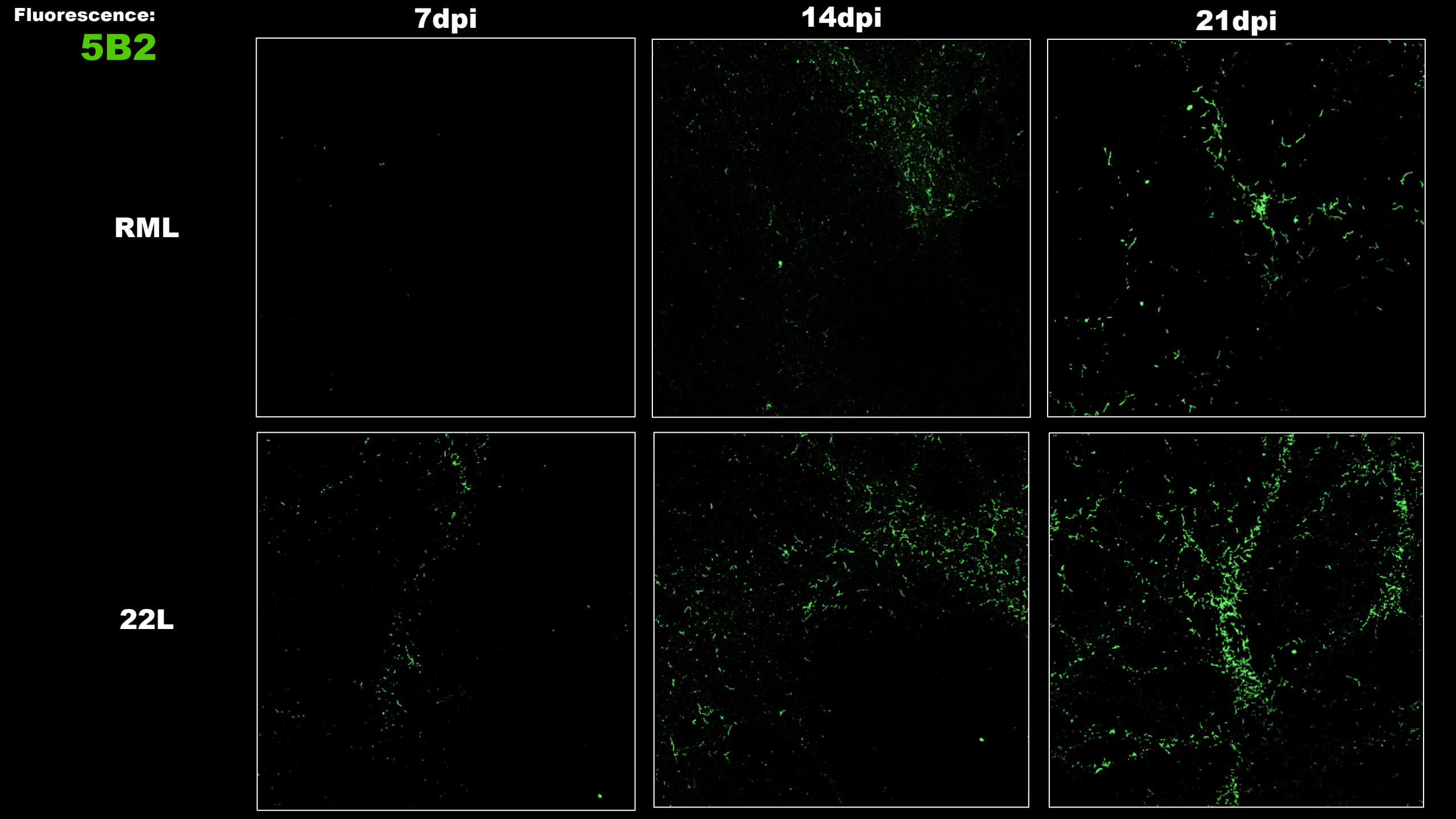
14dpi
22L



21dpi
22L



Fluorescence:
MAP2
5B2
546-pRML


Fluorescence:
5B2
7dpi
14dpi
21dpi
RML
22L

5B2

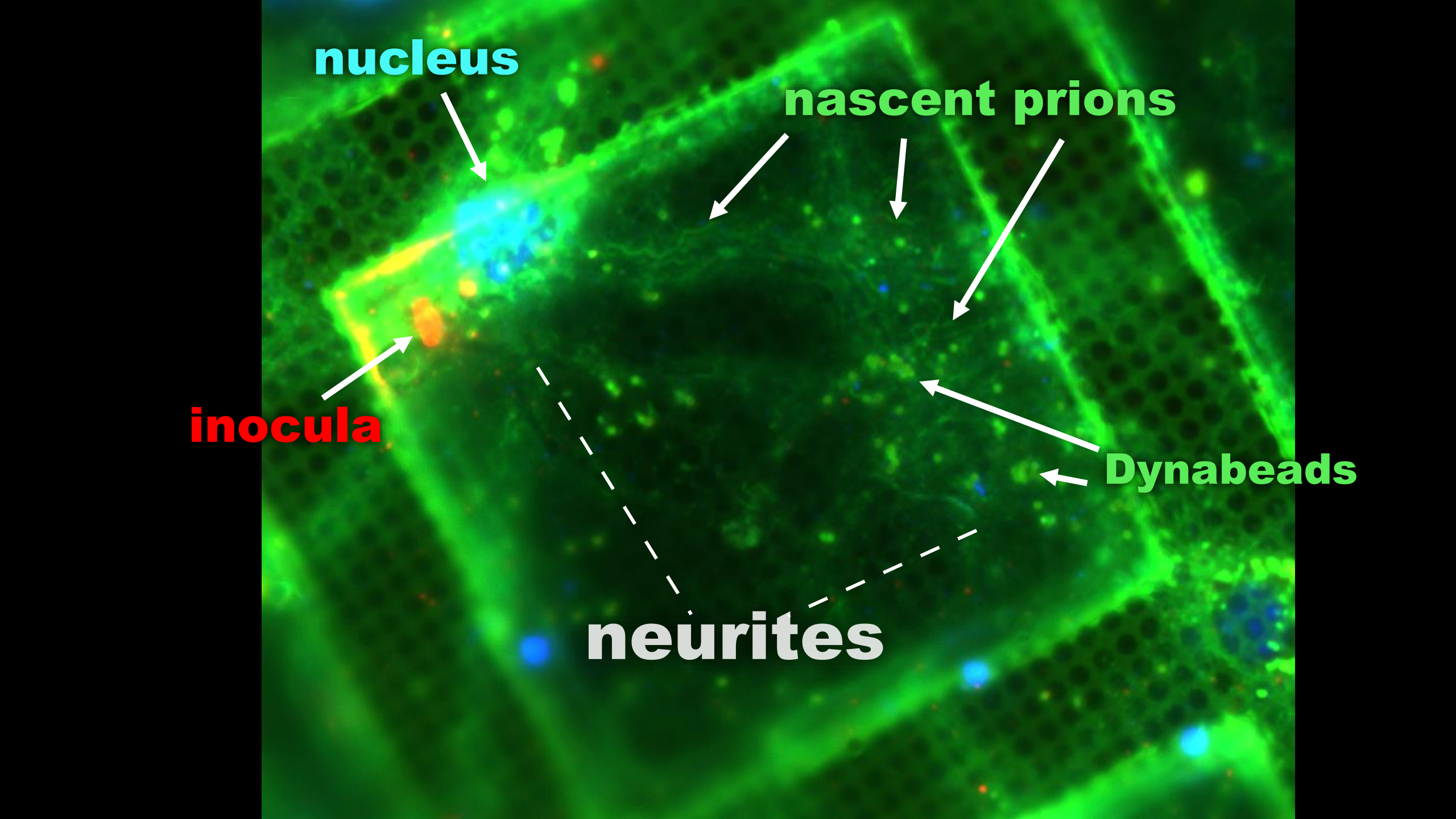
AlexaFluor488
primary
conjugation

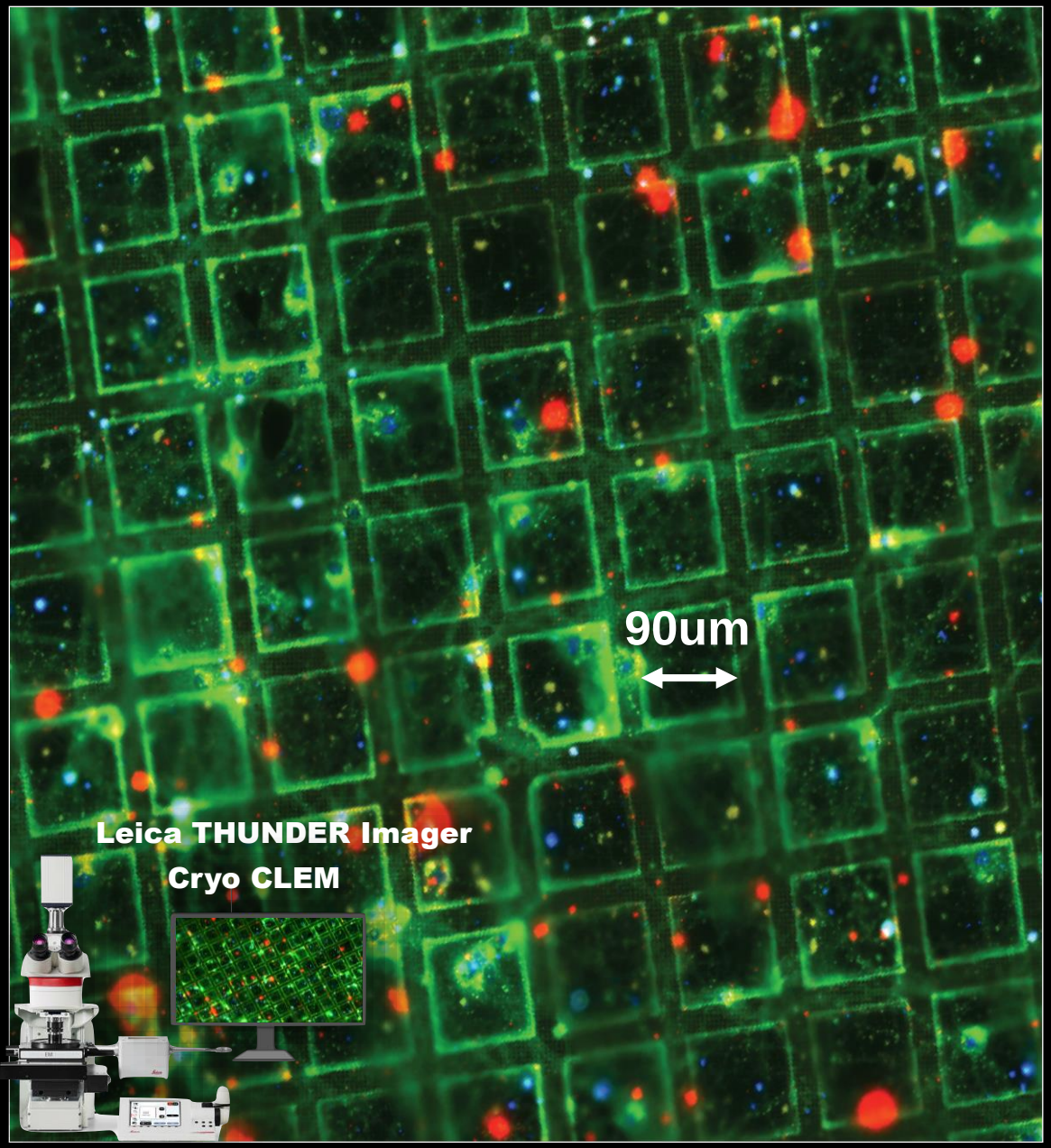
22L

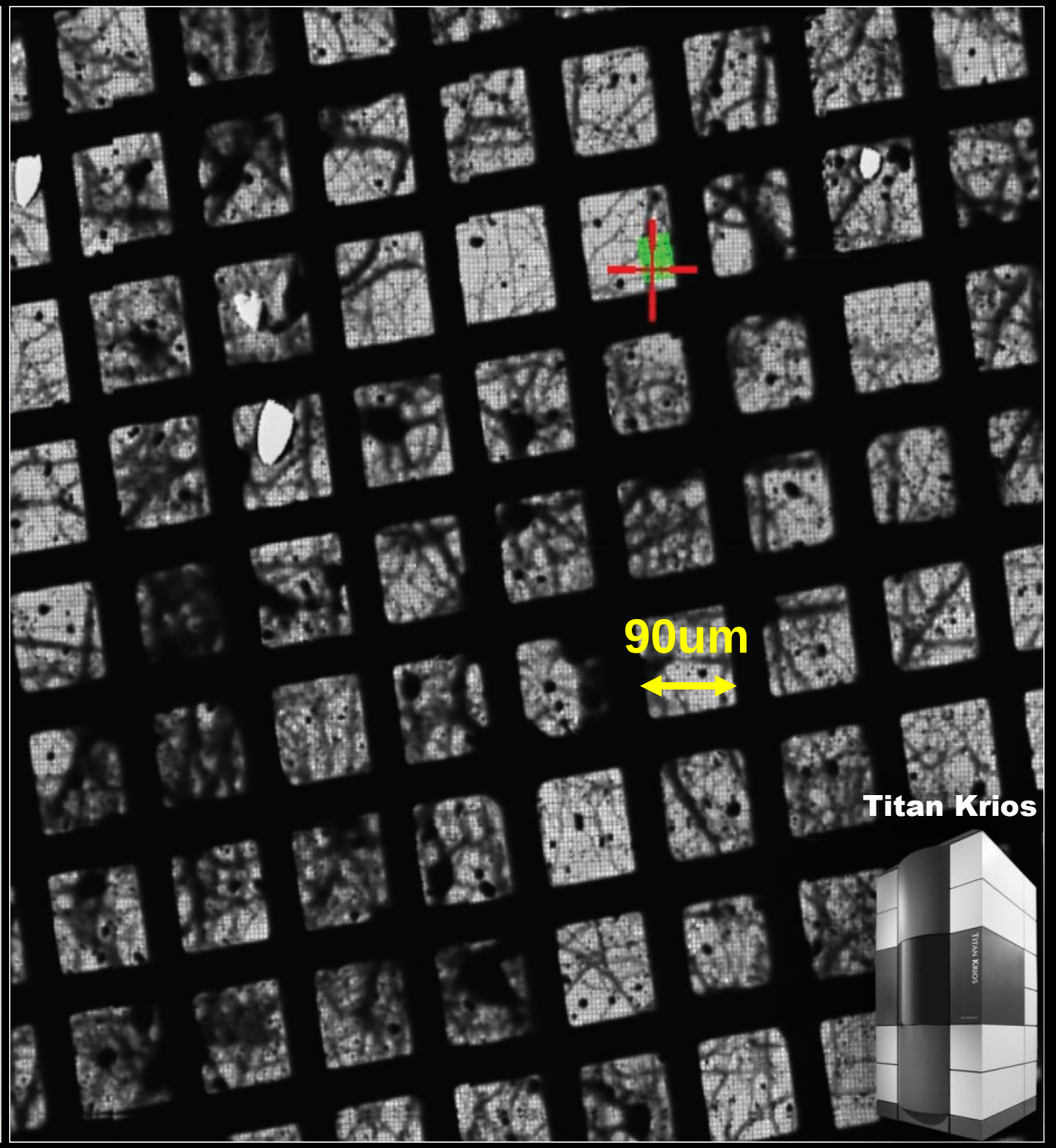
PTA cages
$[PW_{11}O_{39}]^{7-}$
+
AlexaFluor546-
NHS-ester

21dpi

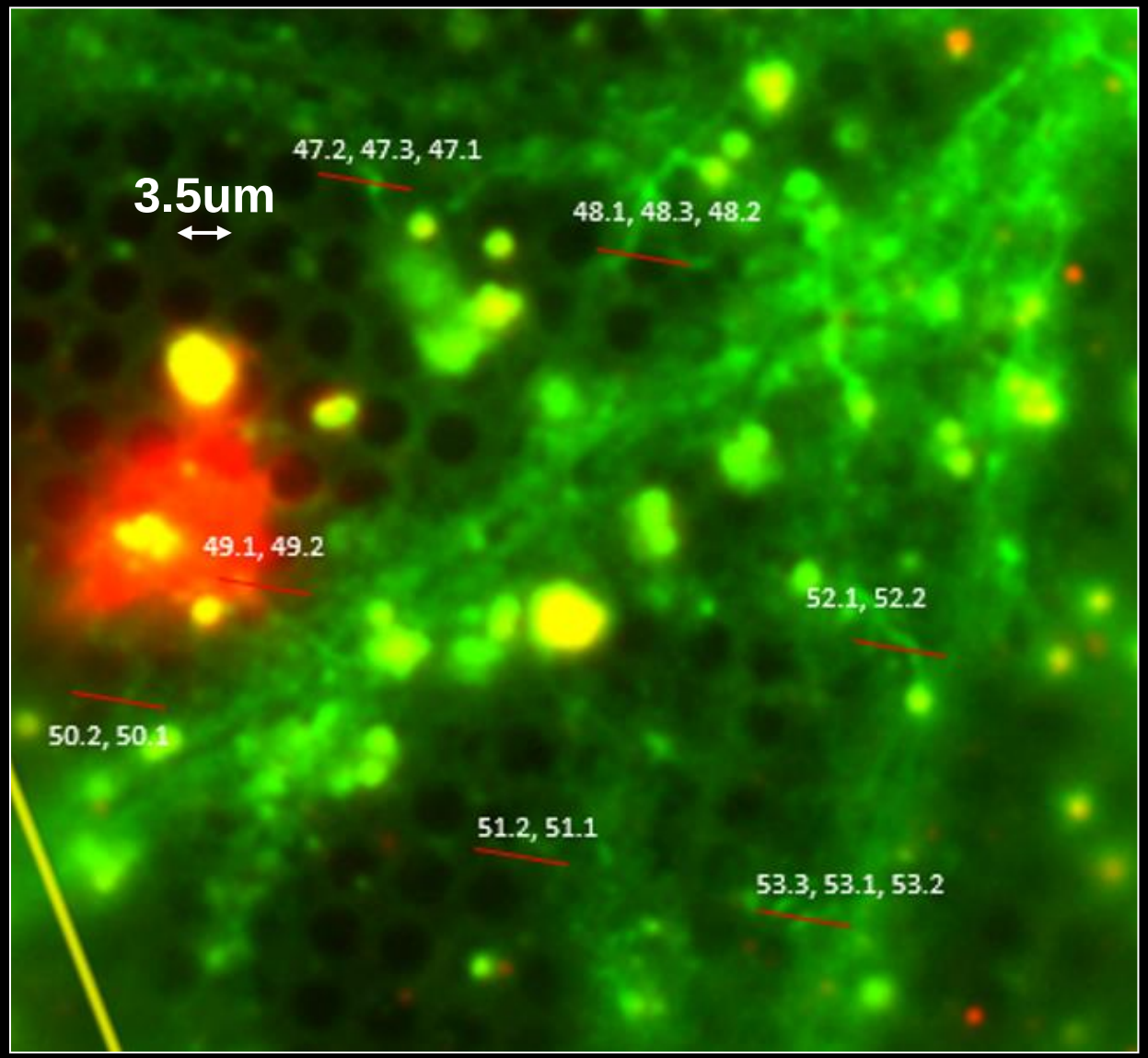
Plunge-freezing


nucleus
nascent prions
inocula
Dynabeads
neurites

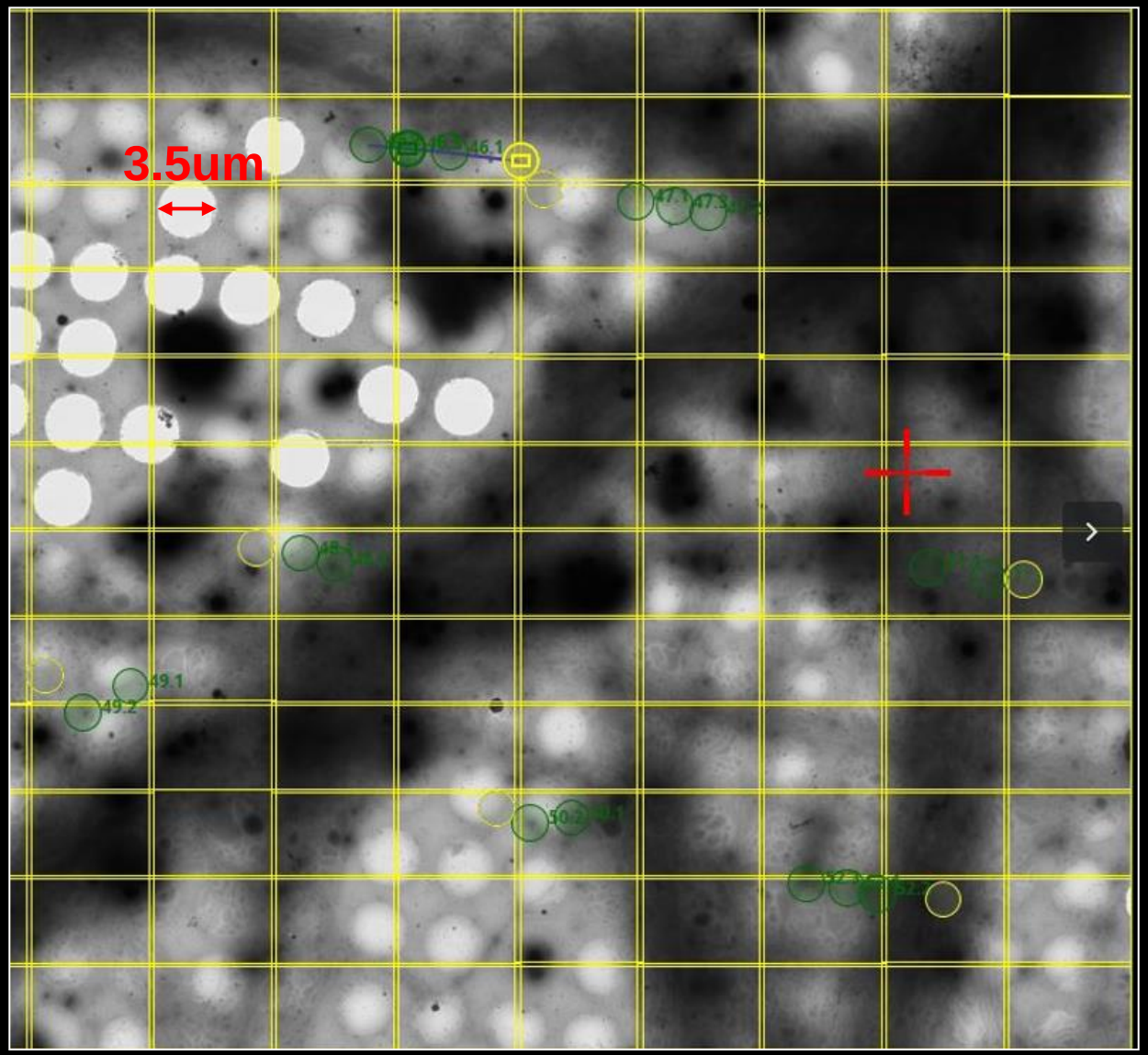

90um
Leica THUNDER Imager
Cryo CLEM


90um
Titan Krios

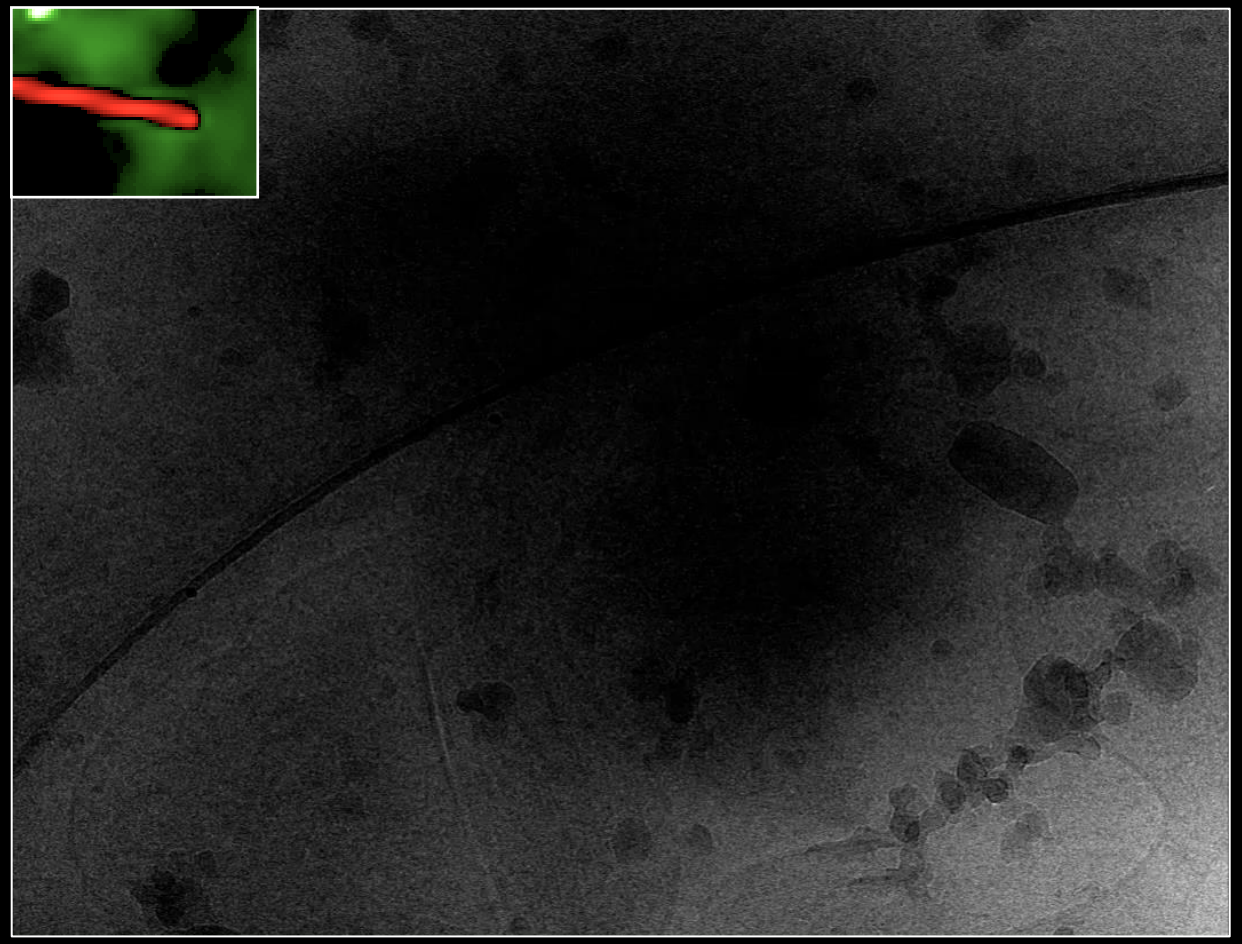
Cryo-widefield



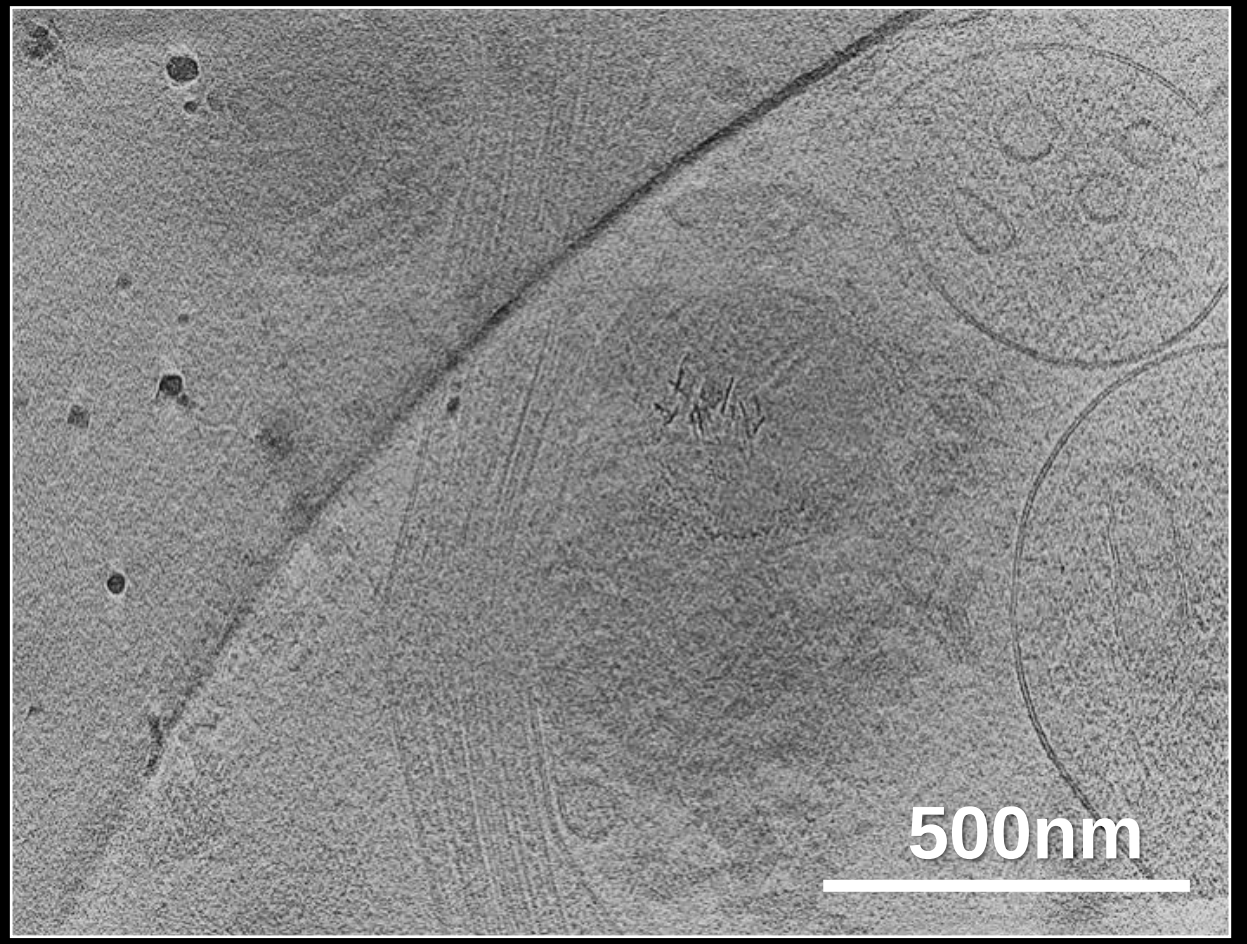
Cryo-EM

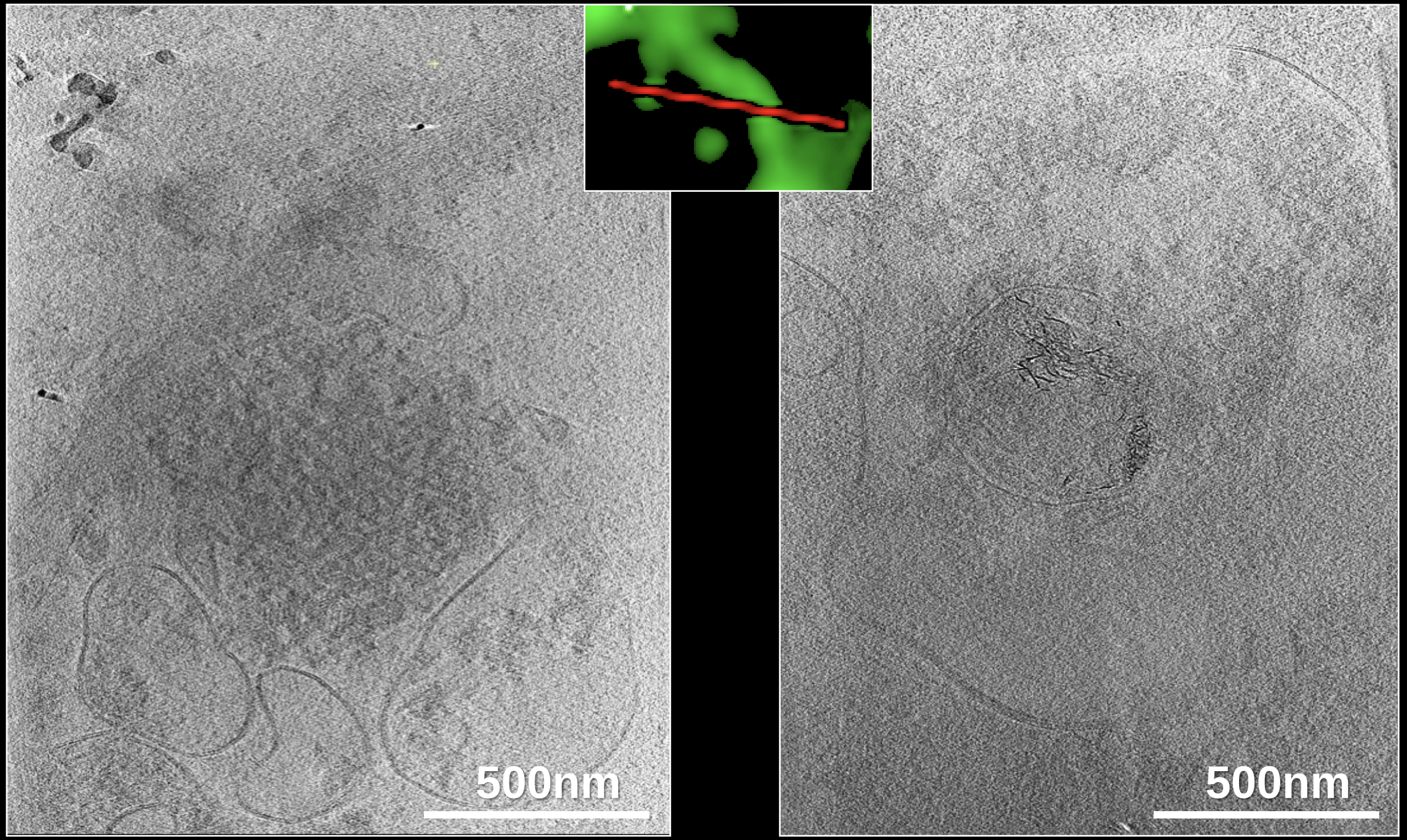


Tilt-series alignment



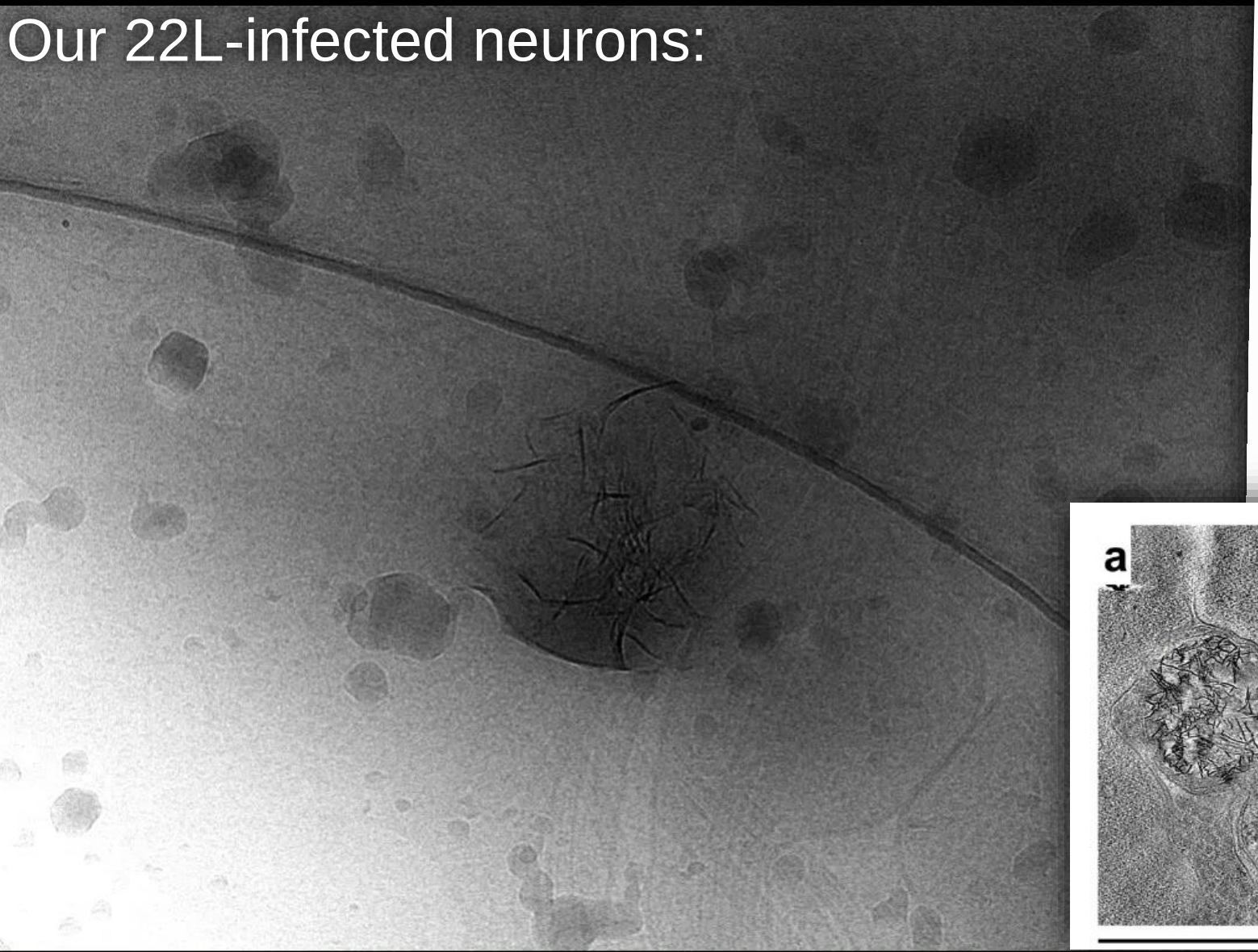
Reconstructed tomogram




500nm
500nm

Double membrane-bound compartments with sheet aggregates, shared in common with Huntington mutant and prion-infected neurons

Our 22L-infected neurons:



nature communications

Article

https://doi.org/10.1038/s41467-023-36096-w

CryoET reveals organelle phenotypes in huntington disease patient iPSC-derived and mouse primary neurons

Received: 26 March 2022

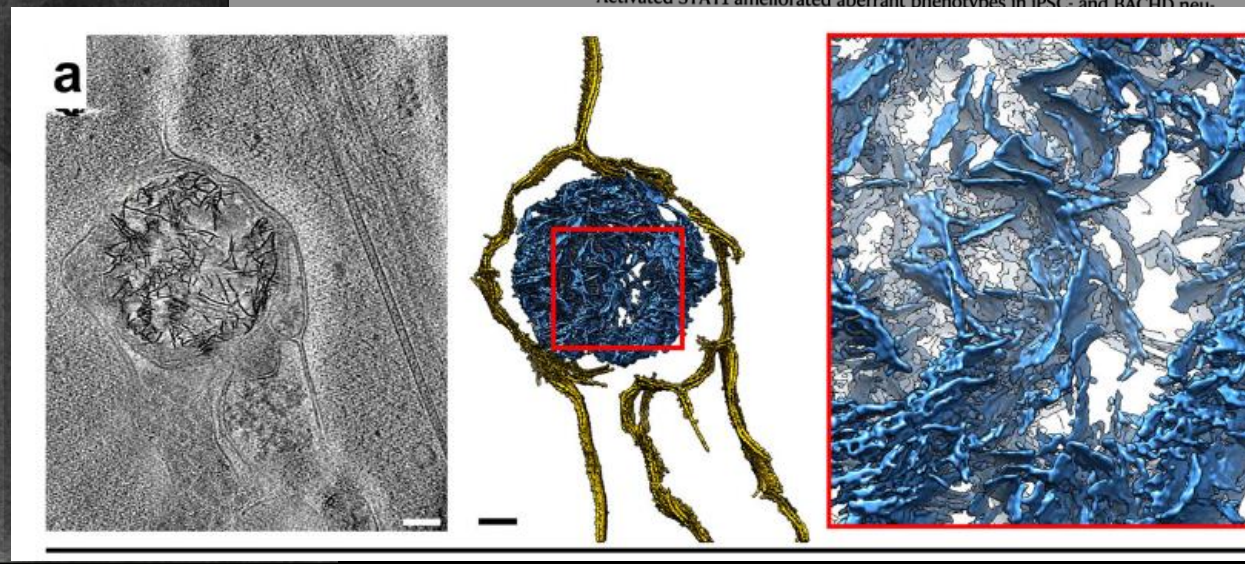
Accepted: 13 January 2023

Published online: 08 February 2023

Check for updates

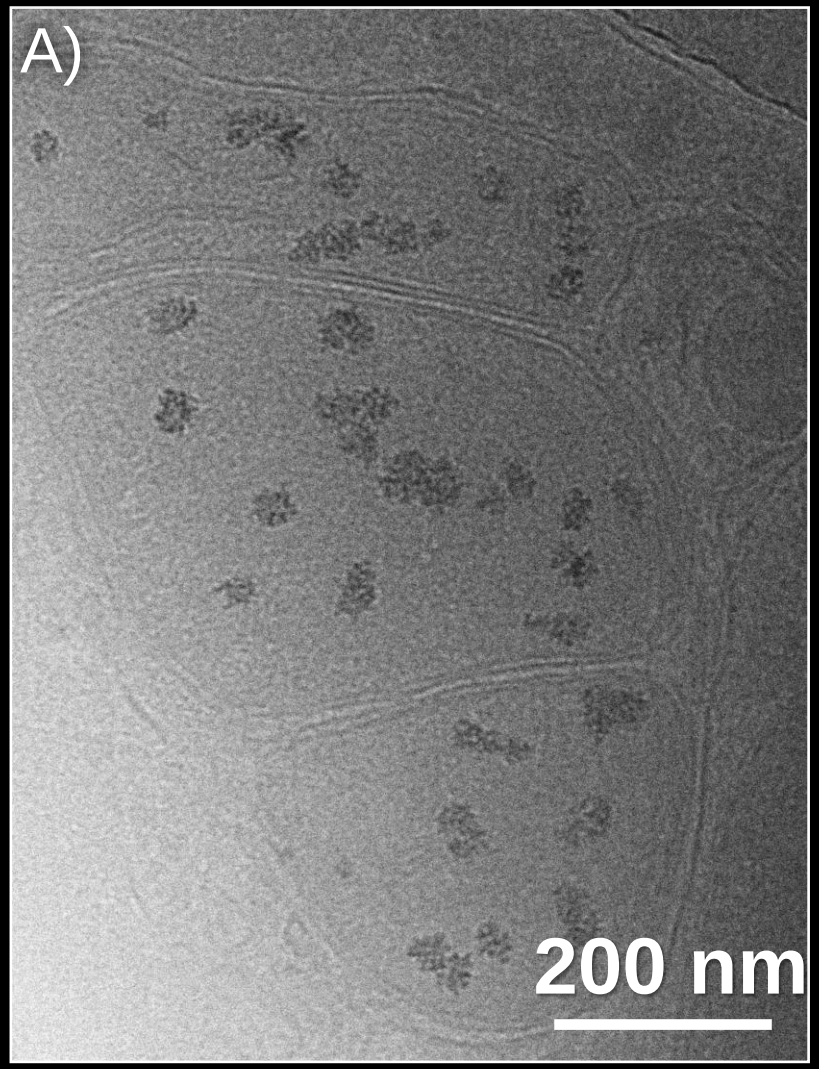
Gong-Her Wu[1,14], Charlene Smith-Geater[2,14], Jesús G. Galaz-Montoya[1], Yingli Gu[3], Sanket R. Gupte[4], Ranen Aviner[5], Patrick G. Mitchell[6], Joy Hsu[4], Ricardo Miramontes[7], Keona Q. Wang[8], Nicolette R. Geller[8], Cathy Hou[1], Cristina Danita[1], Lydia-Marie Joubert[6], Michael F. Schmid[6], Serena Yeung[4,9], Judith Frydman[5,10], William Mobley[3], Chengbiao Wu[3], Leslie M. Thompson[2,7,8,11,12,15] ✉ & Wah Chiu[1,6,13,15] ✉

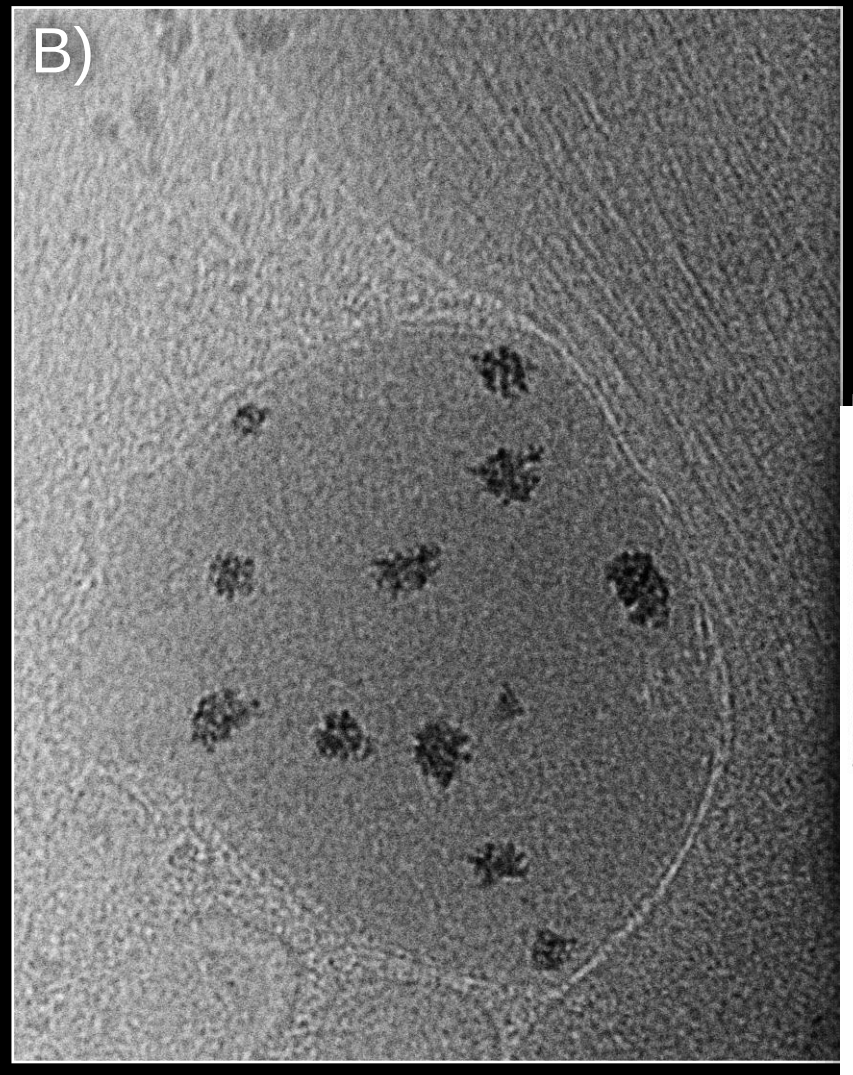
Huntington's disease (HD) is caused by an expanded CAG repeat in the huntingtin gene, yielding a Huntingtin protein with an expanded polyglutamine tract. While experiments with patient-derived induced pluripotent stem cells (iPSCs) can help understand disease, defining pathological biomarkers remains challenging. Here, we used cryogenic electron tomography to visualize neurites in HD patient iPSC-derived neurons with varying CAG repeats, and primary cortical neurons from BACHD, deltaN17-BACHD, and wild-type mice. In HD models, we discovered sheet aggregates in double membrane-bound organelles, and mitochondria with distorted cristae and enlarged granules, likely mitochondrial RNA granules. We used artificial intelligence to quantify mitochondrial granules, and proteomics experiments reveal differential protein content in isolated HD mitochondria. Knockdown of Protein Inhibitor of Activated STAT1 ameliorated aberrant phenotypes in iPSC- and BACHD neu-



Calcium phosphate granules accumulate in mitochondria, and cristae become poorly defined

22L-infected neurons:





nature communications

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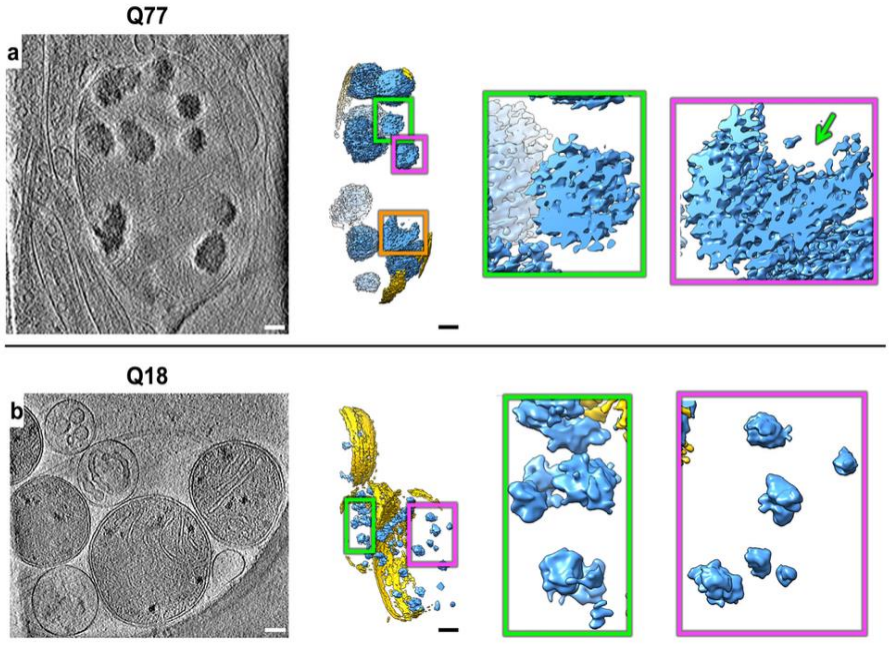

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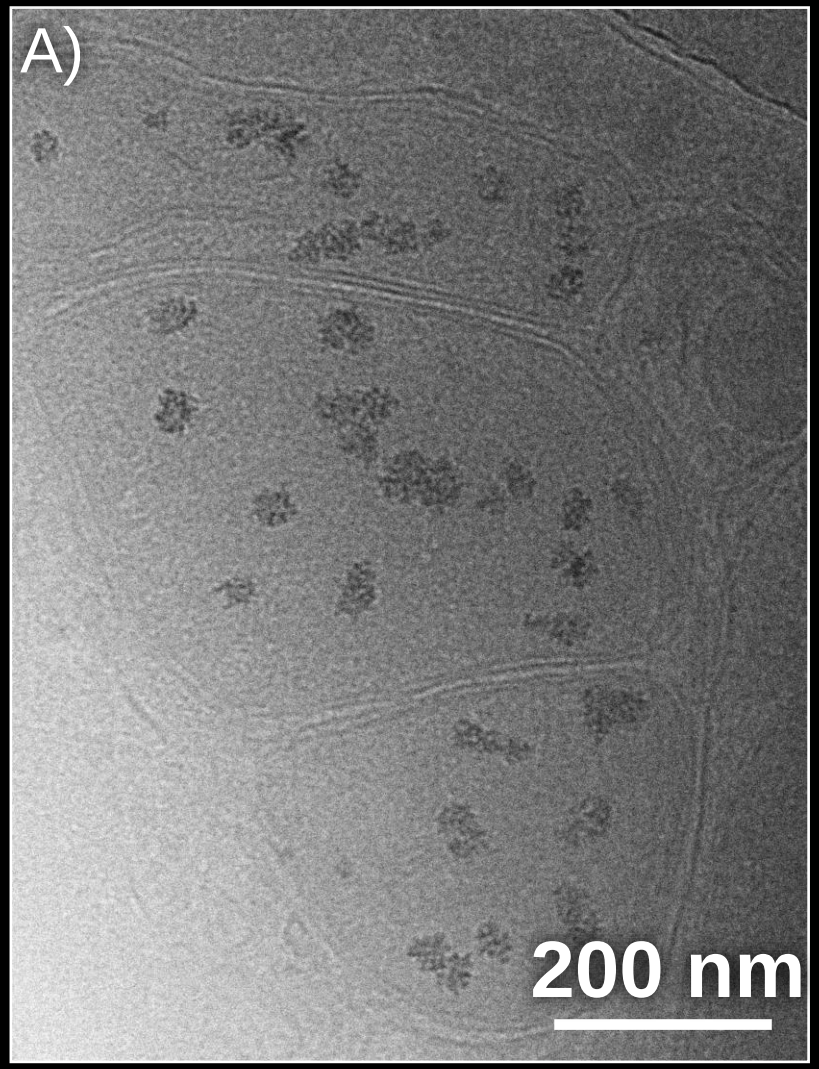
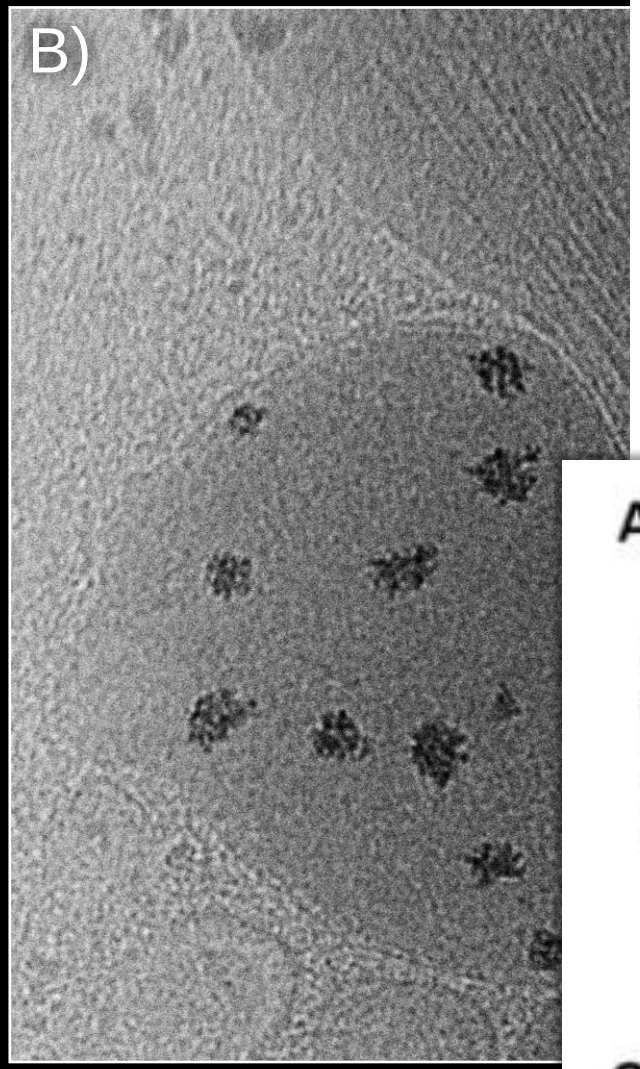




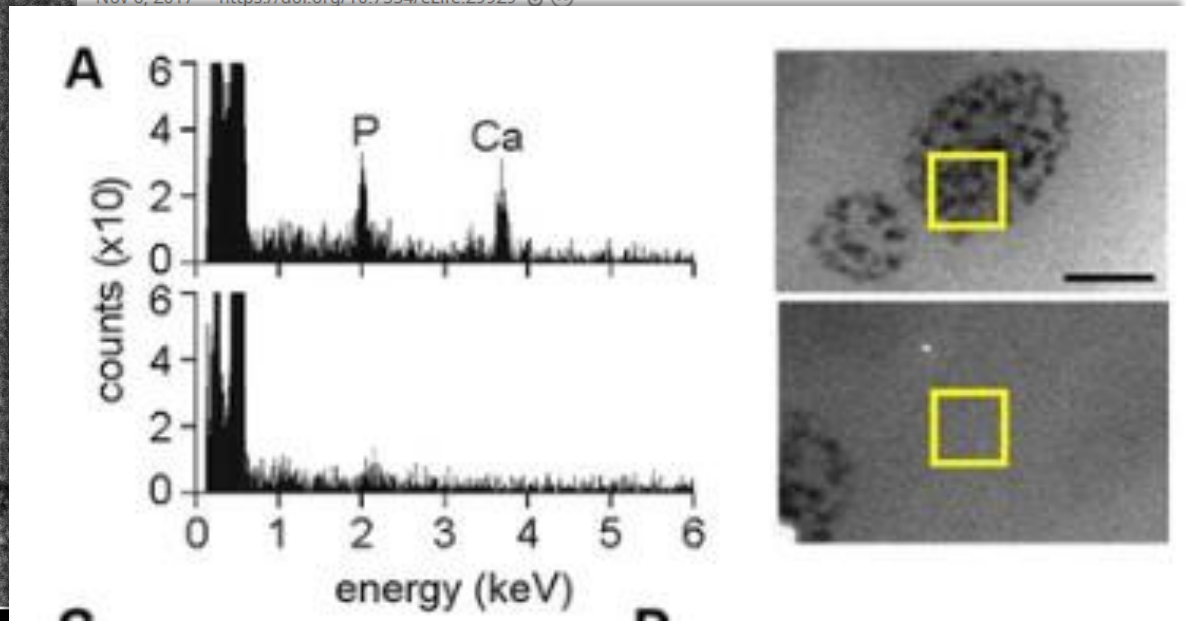
Research Article
Cell Biology

3D visualization of mitochondrial solid-phase calcium stores in whole cells

Sharon Grayer Wolf, Yael Mutsafi, Tali Dadosh, Tal Ilani, Zipora Lansky, Ben Horowitz, Sarah Rubin, Michael Elbaum, Deborah Fass

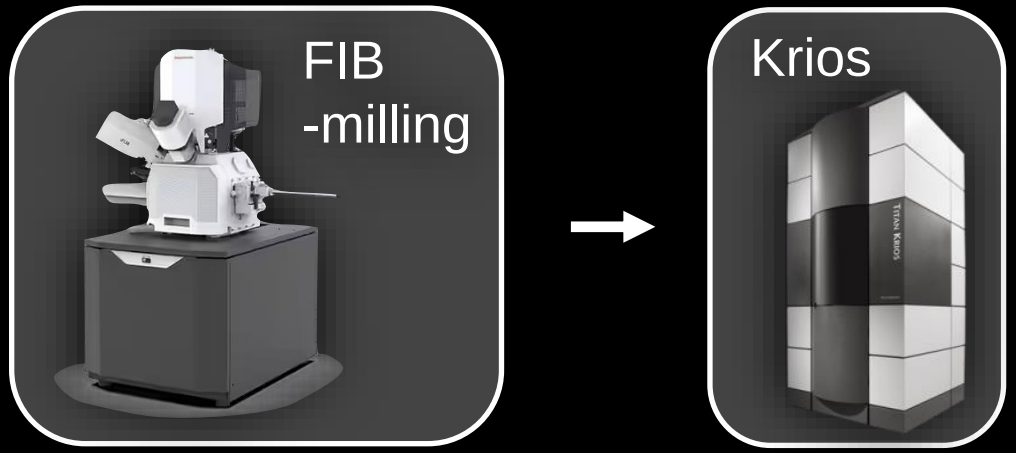
Weizmann Institute of Science, Israel

Nov 6, 2017 • https://doi.org/10.7554/eLife.29929



FURTHER STUDY:

Cryo-FIB-ET



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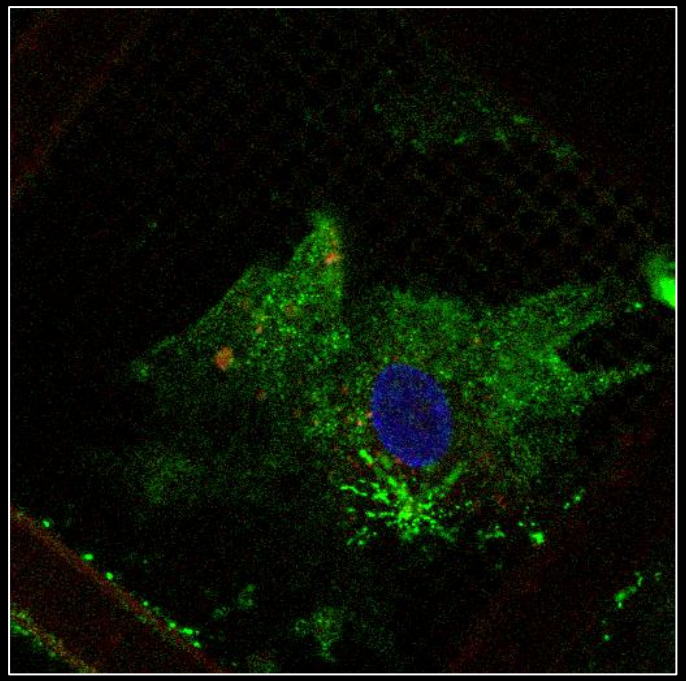
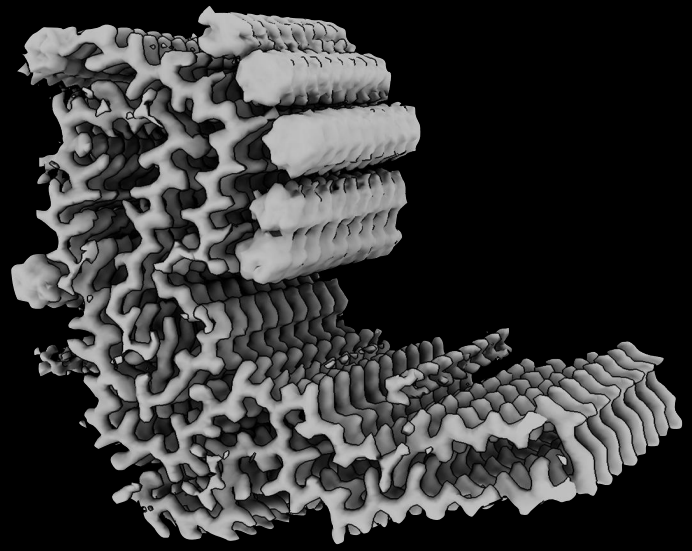
Prion infected astrocytes



RML



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cryo-SXT/CLEXM

SIRIUS XT

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Prion strains
Adam Wenborn
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Jonathan Wadsworth

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Dave Houldershaw
Shu Chen

Prion cell biology
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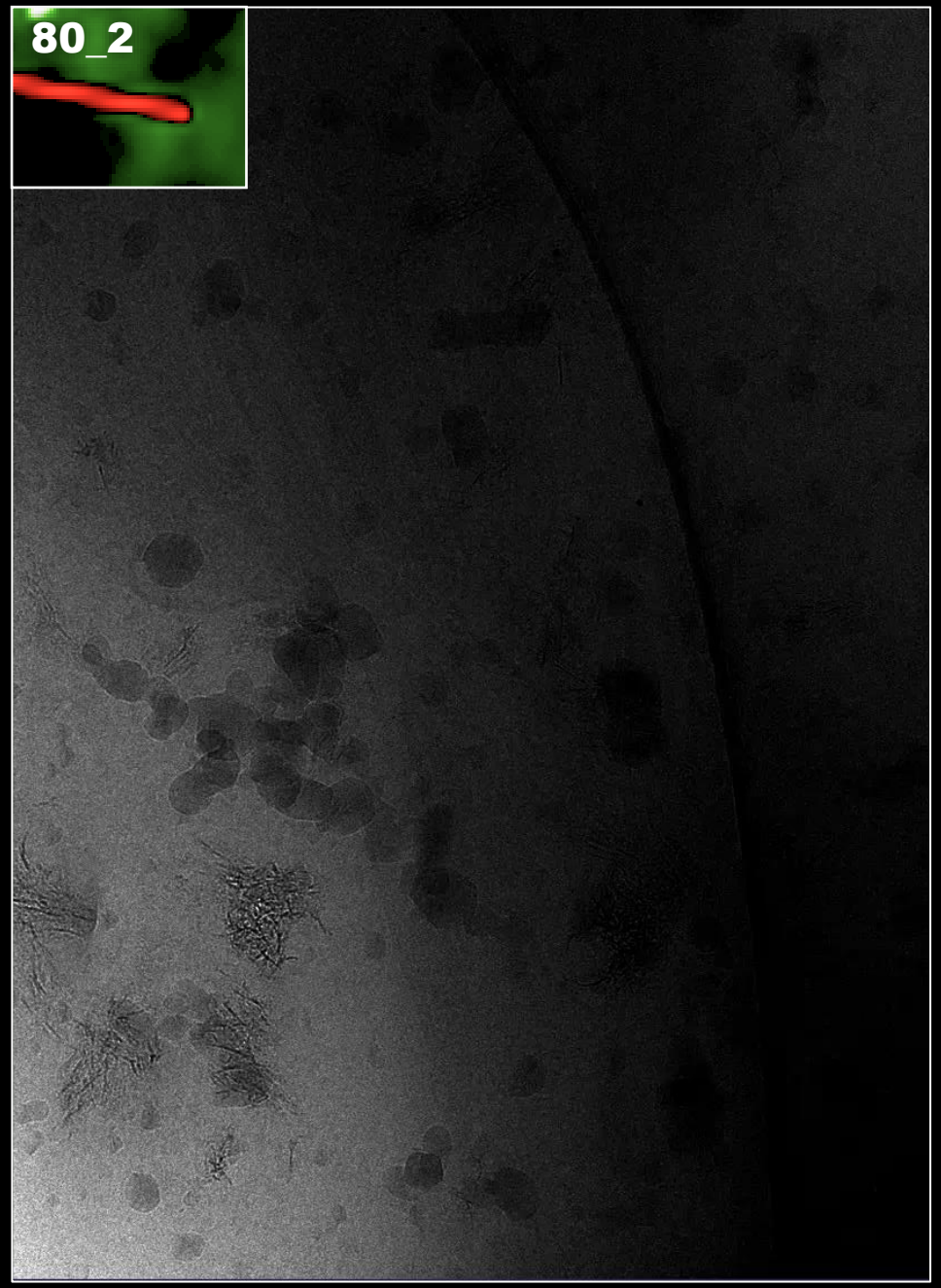
Aline Marinho
Mitali Patel
Juan RIbes
Peter Kloehn
Parmjit Jat

MRC Prion Unit, Director
John Collinge

Supervision
Szymon Manka

Tilt-series alignment



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