

Physics of Life

PHYS-468

Electron Microscopy in Life Sciences

Henning Stahlberg,
LBEM, IPHYS, SB, EPFL

**Biology:
Parkinson's
Disease**

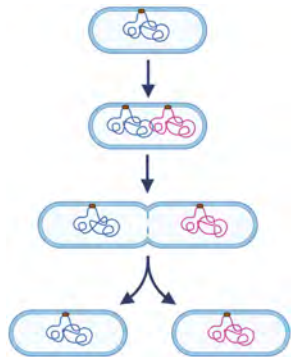
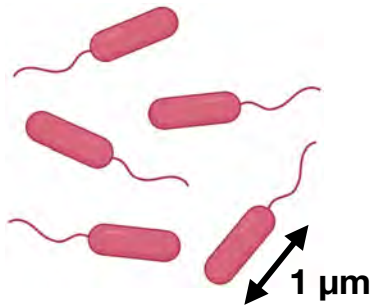


**Physics:
Improving
Cryo-EM**

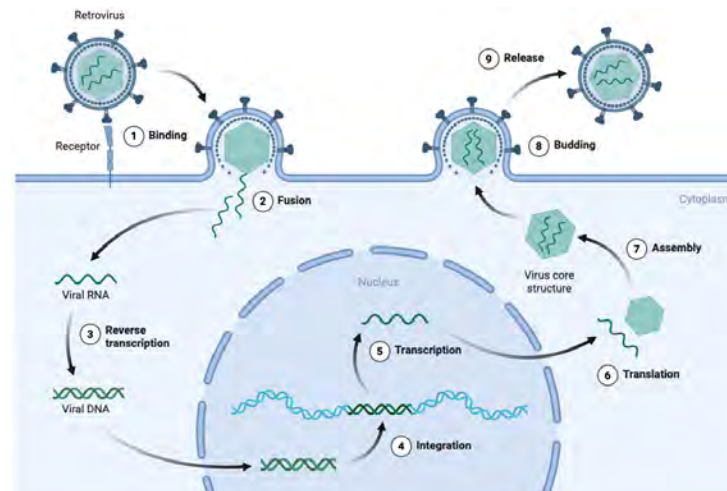
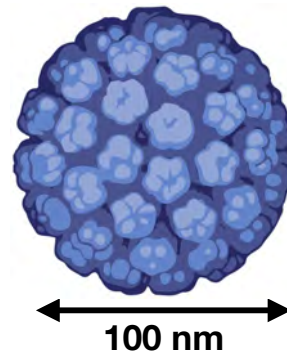


Bacteria, Viruses, and Prions

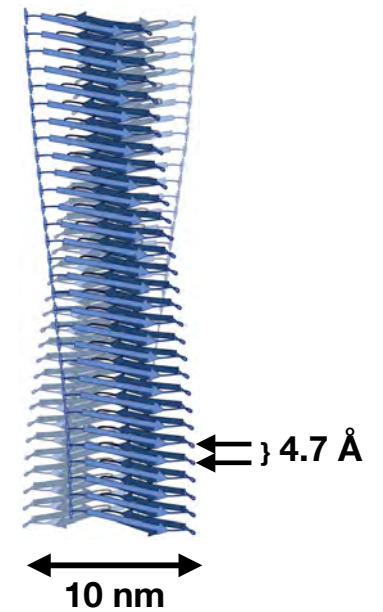
Bacteria



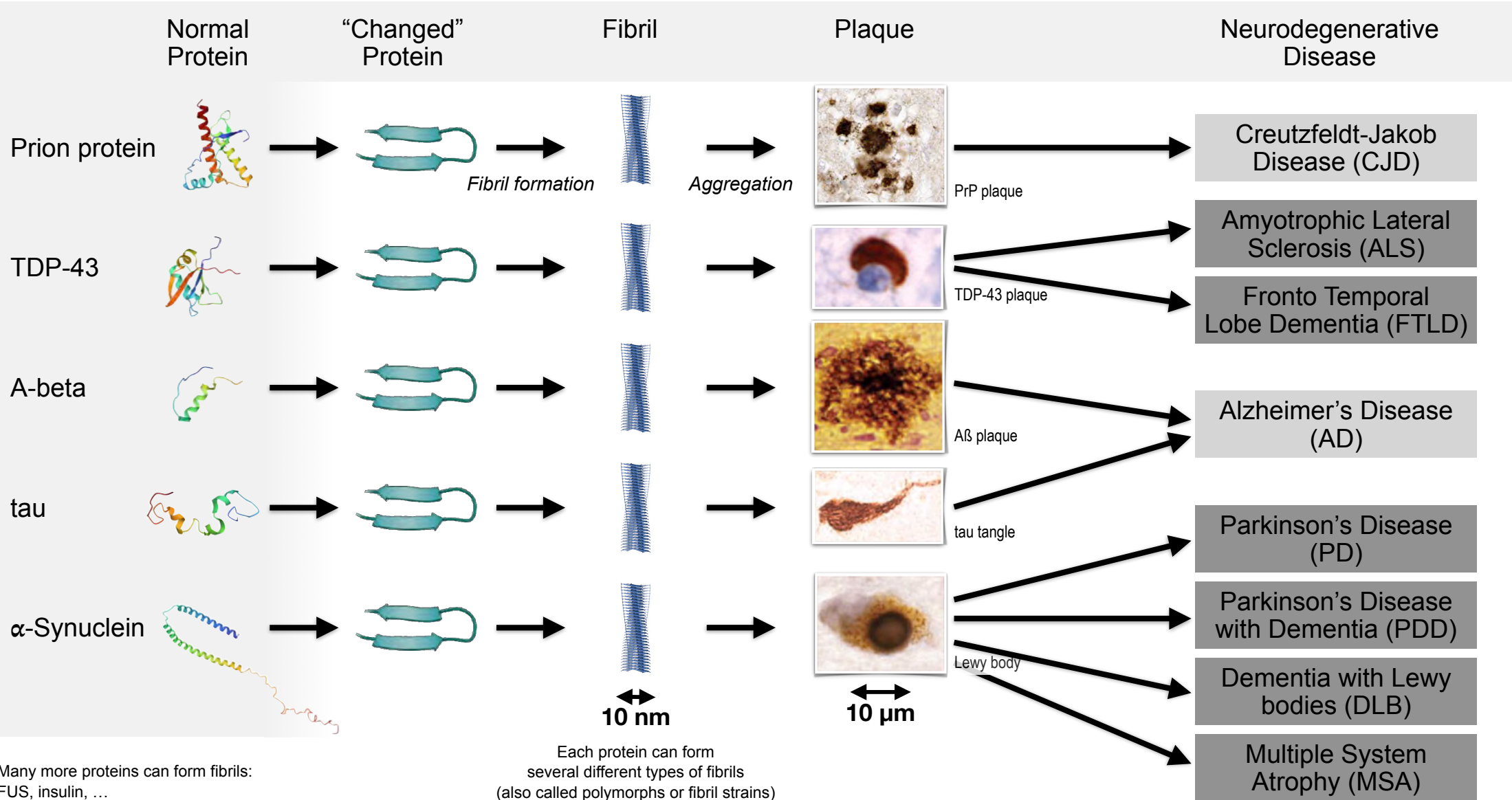
Viruses



Fibrils



Prionoid fibril growth: Fibrils form plaques, which correlate with (cause?) disease



Prionoid fibril growth: Fibrils form plaques, which correlate with (cause?) disease

Normal Protein

"Changed" Protein

Fibril

Plaque

Neurodegenerative Disease



Creutzfeldt-Jakob Disease (CJD)

Amyotrophic Lateral Sclerosis (ALS)

Fronto Temporal Lobe Dementia (FTLD)

Alzheimer's Disease (AD)

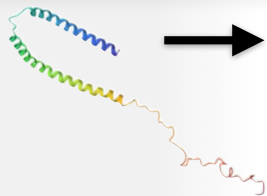
Parkinson's Disease (PD)

Parkinson's Disease with Dementia (PDD)

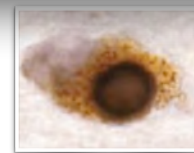
Dementia with Lewy bodies (DLB)

Multiple System Atrophy (MSA)

α -Synuclein



10 nm



10 μ m

Lewy body

Many more proteins can form fibrils:
FUS, insulin, ...

Each protein can form
several different types of fibrils
(also called polymorphs or fibril strains)

Parkinson's Disease: Lewy bodies contain the protein alpha-synuclein



<https://www.youtube.com/watch?v=vZZ4Vj3HinA>; 19.4.2017



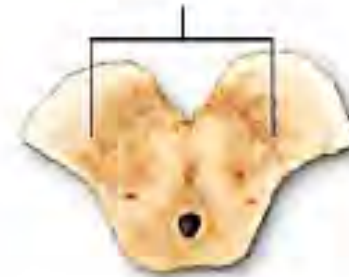
Cut section
of the midbrain
where a portion
of the substantia
nigra is visible



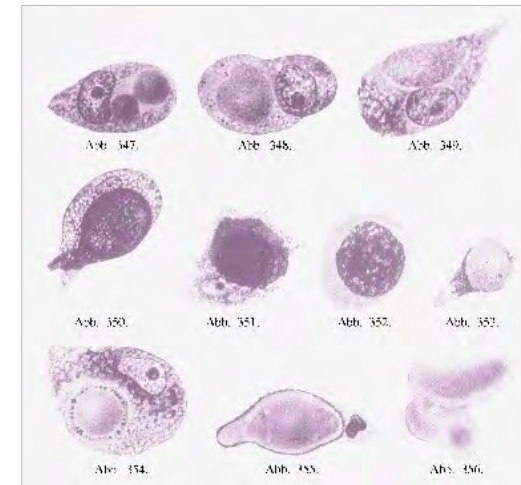
Substantia nigra



Diminished substantia nigra
as seen in Parkinson's disease



Friedrich Heinrich Lewy
(1885 – 1950)



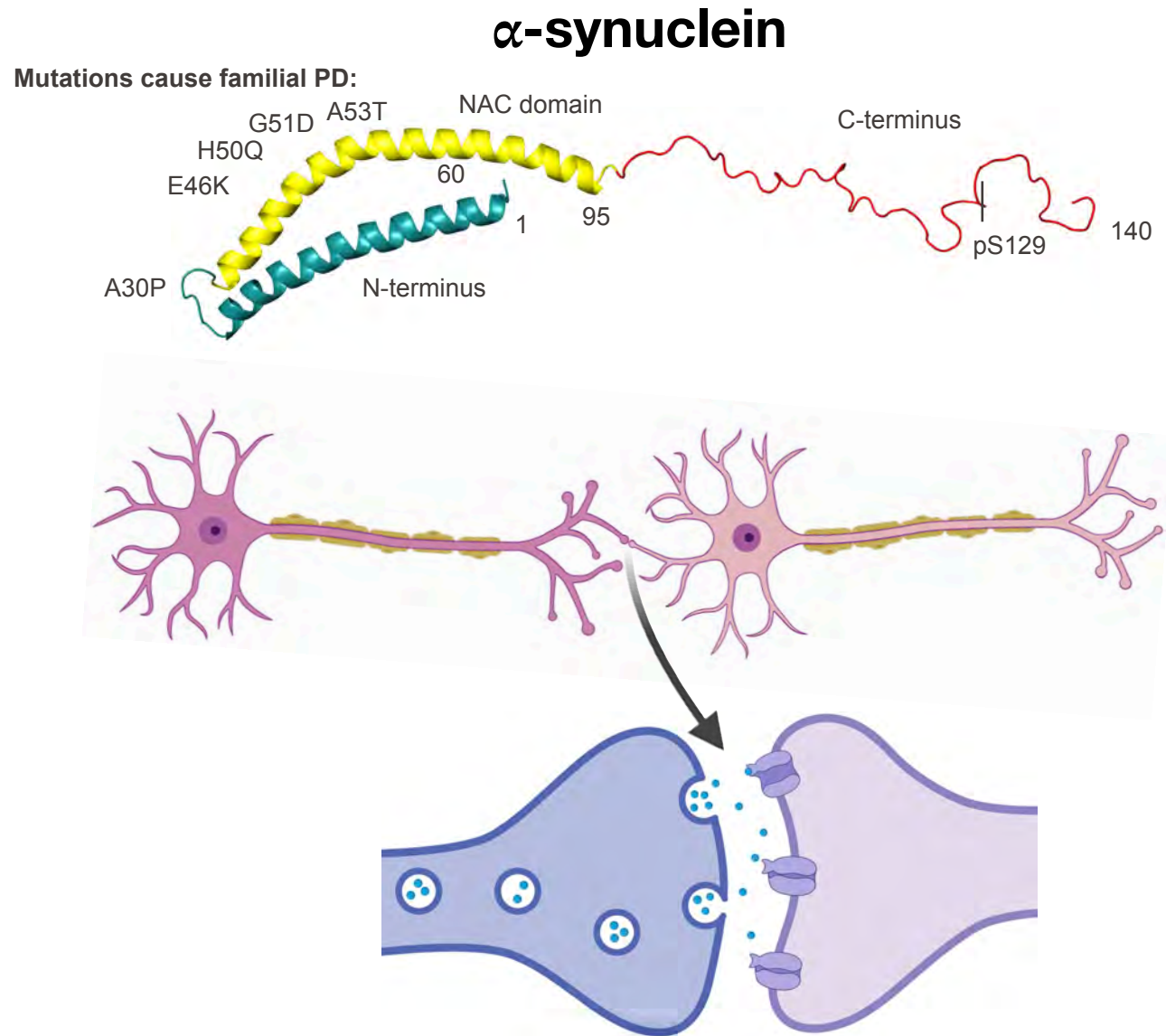
A figure from Lewy's *Tonus und Bewegung*
(1923), showing images of Lewy bodies

“Lewy” bodies in the brain.
These are full of alpha-synuclein.

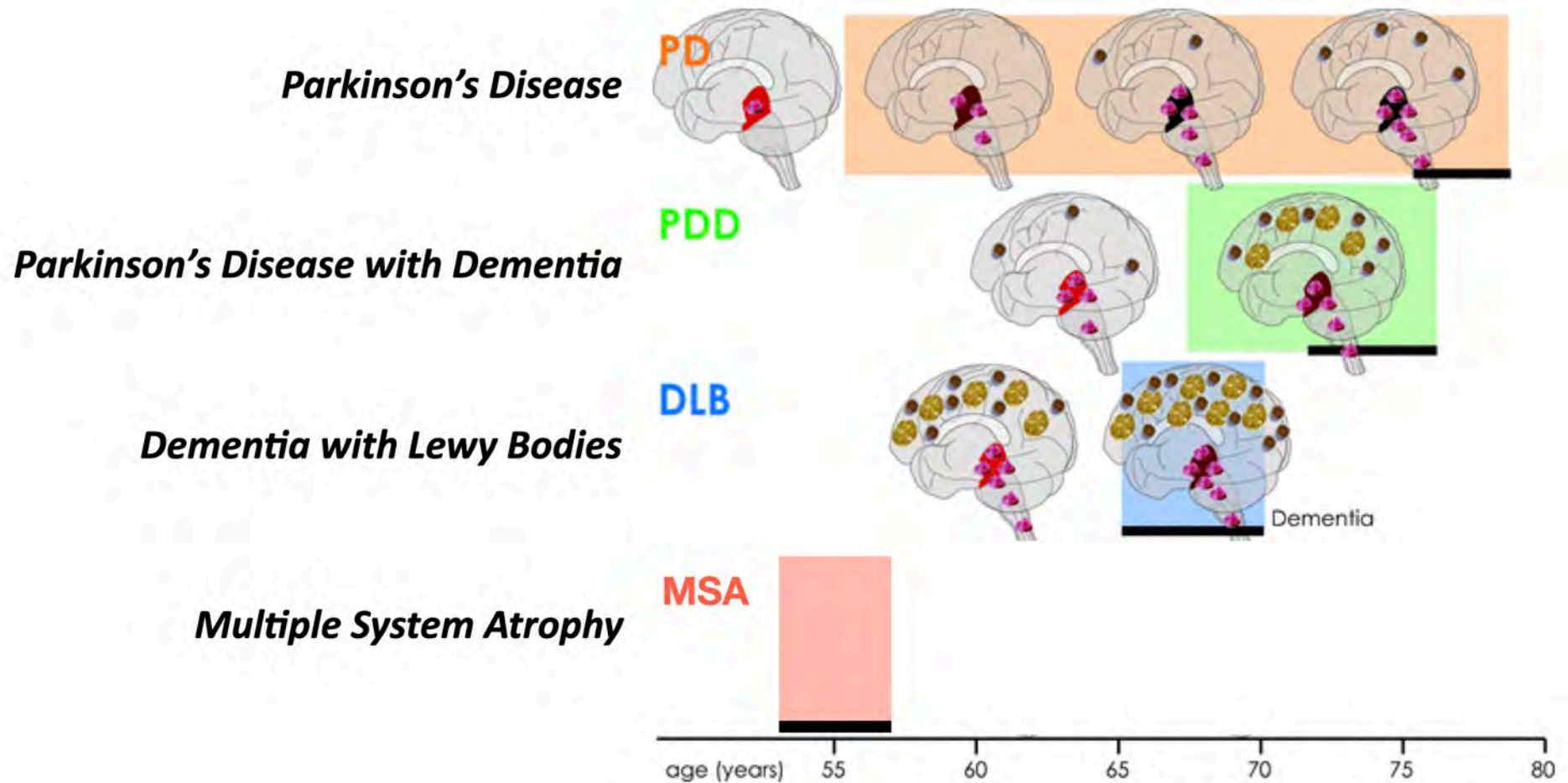


alpha-synuclein is a small protein, found at high concentration in Lewy bodies

- 14 kDa weight
- 140 amino acids
- Natively unfolded
- Membrane interacting (Nanodisks? Pores?)
- α -helical in contact with membranes
- β -sheet in fibrils
- Mutations can cause familial PD (A30P, E46K, H50Q, G51D, A53T)



Synucleinopathies: Different “strains” of α -synuclein fibrils may cause different diseases



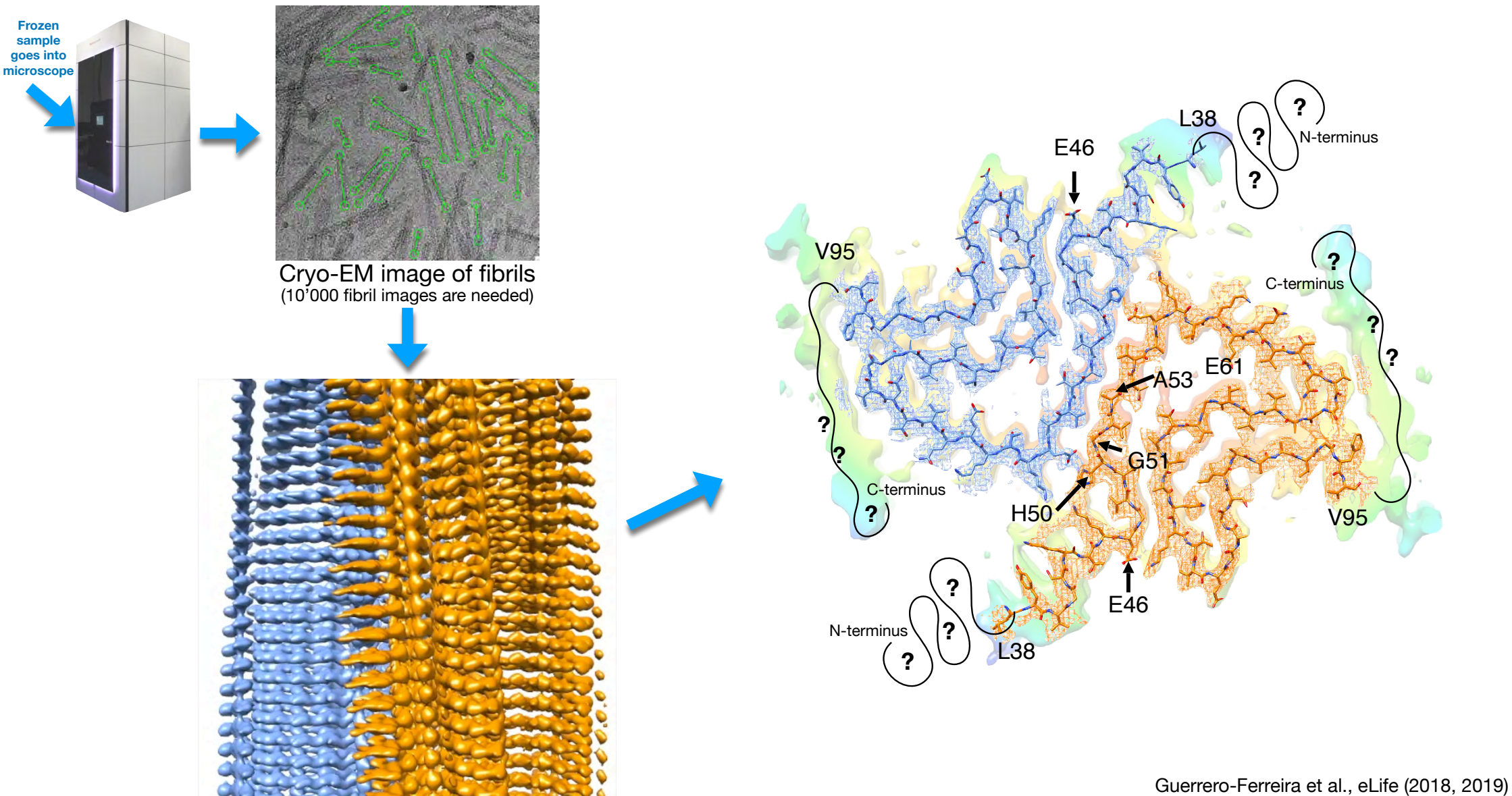
Other diseases related to α Syn fibrils

- Lewy Body variant of Alzheimer's Disease (AD)
- Neurodegeneration with Brain Iron Accumulation (NBIA) Type I
- Pure Autonomic Failure (PAF) Disease

Modified from: Halliday & McCann, Annals of the New York Academy of Sciences 1184(1), 188-195 (2010)

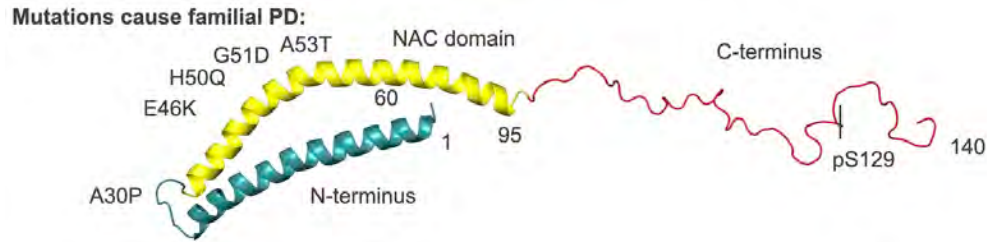
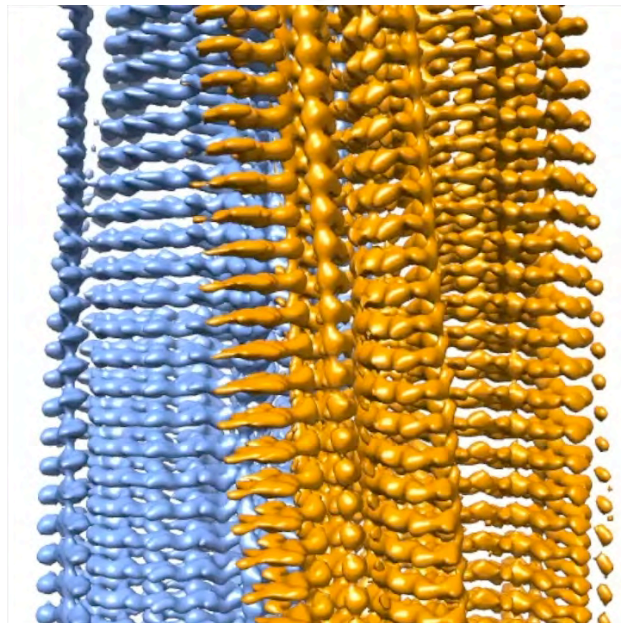
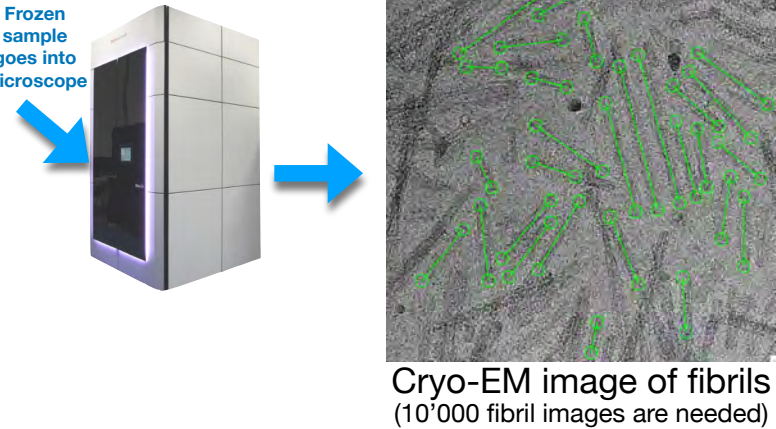
In vitro generated aSyn fibrils

Ricardo Guerrero, with Markus Britschgi (Roche) and Roland Riek (ETHZ)



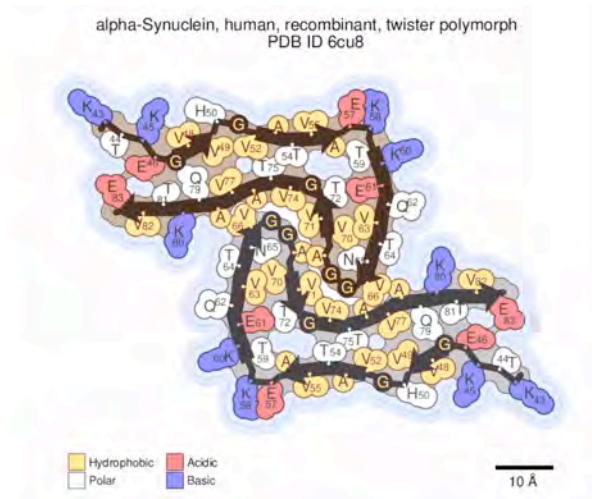
Guerrero-Ferreira et al., eLife (2018, 2019)

aSyn fibril polymorphism



129 known aSyn fibril structures (as of Dec. 2024)

<https://people.mbi.ucla.edu/sawaya/amyloidatlas/>

[illegible]

Alpha-Synuclein Fibril Polymorphism

In vitro, wt

In vitro, wt

In vitro, Ac-E46K

MSA brain purified

In vitro, pTYR39

PD & MSA brain seeded (PMCA)

Polymorph 1a

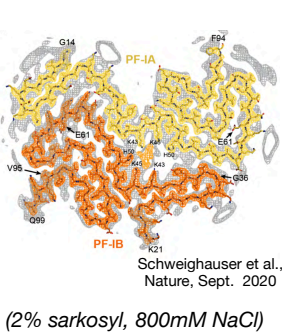
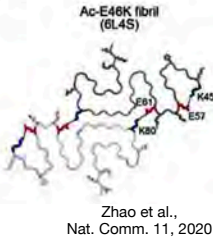
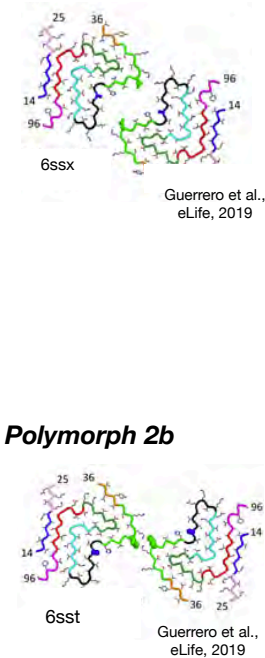
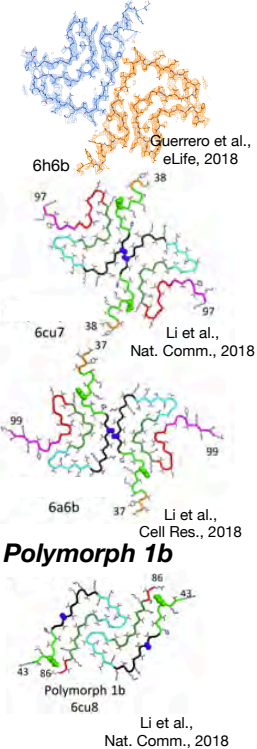
Polymorph 2a

Polymorph 3a

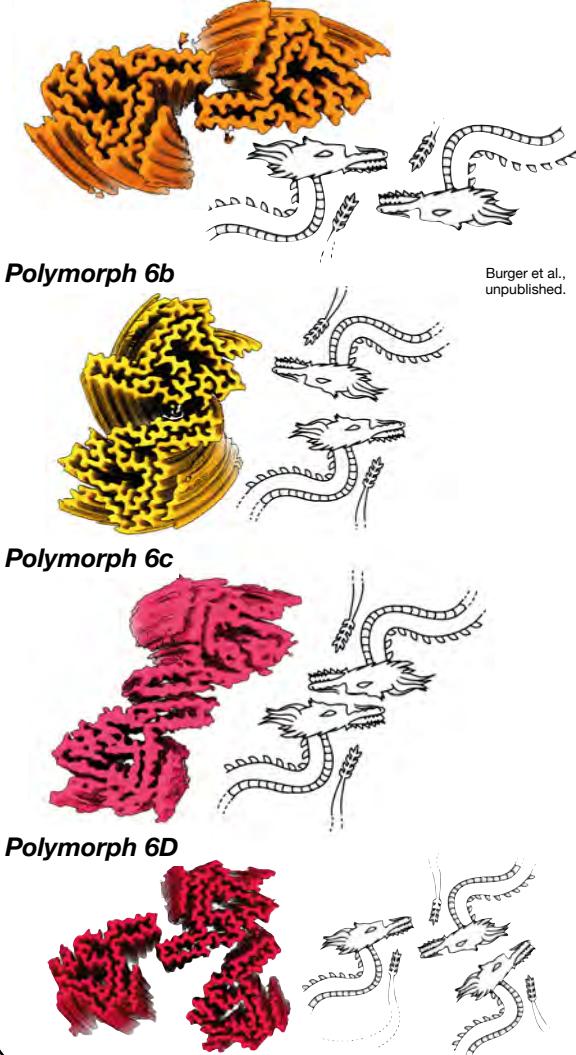
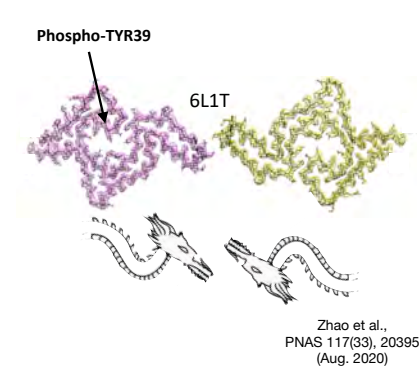
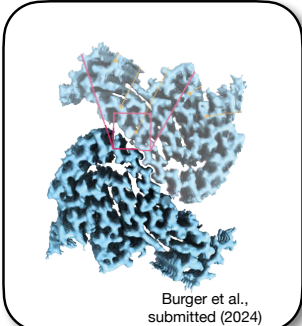
Polymorph 4a

Polymorph 5a

Polymorph 6a



“1B”, causing MSA in mice





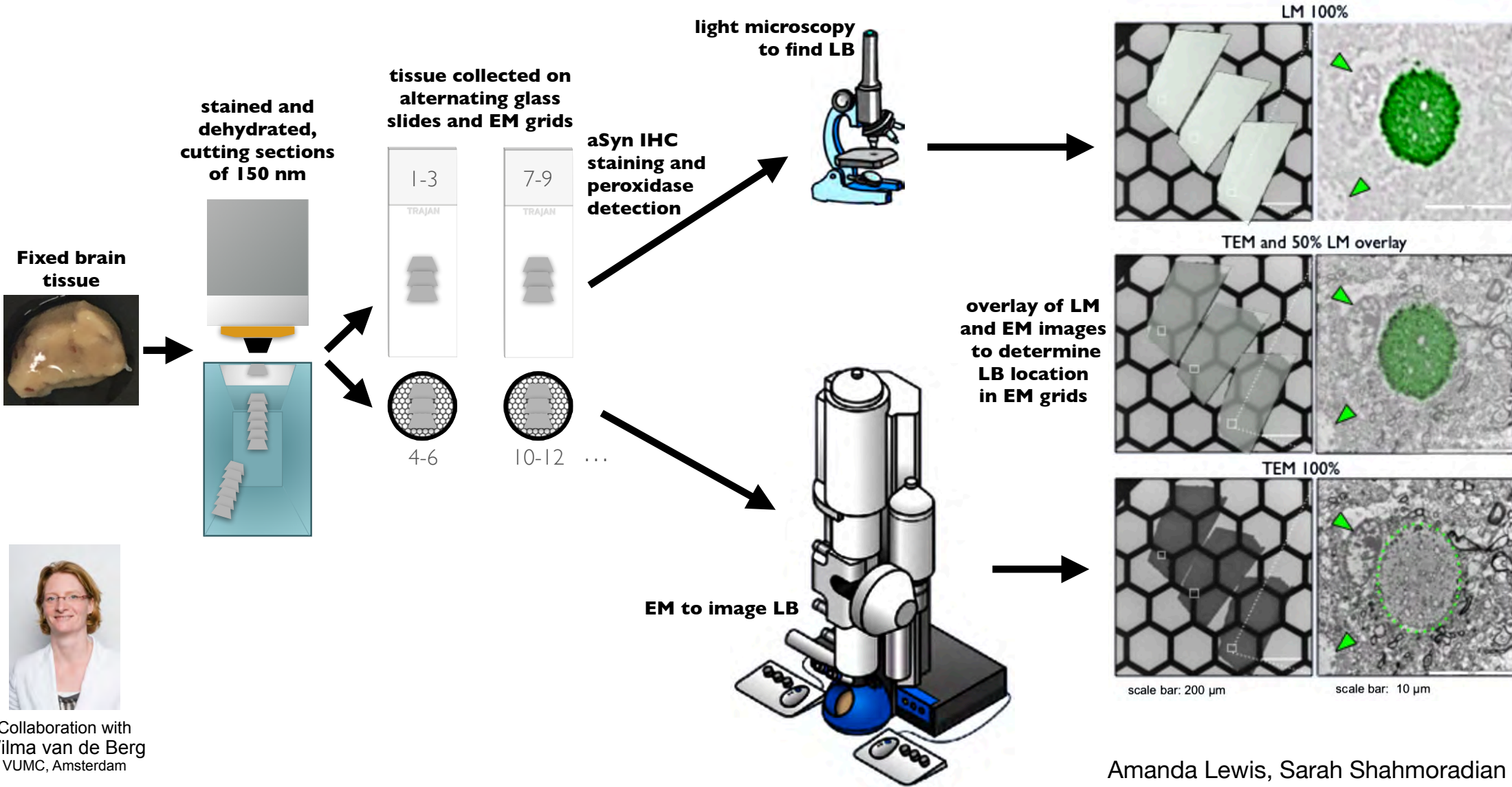
What is the mechanism of neurodegeneration in the human brain ?



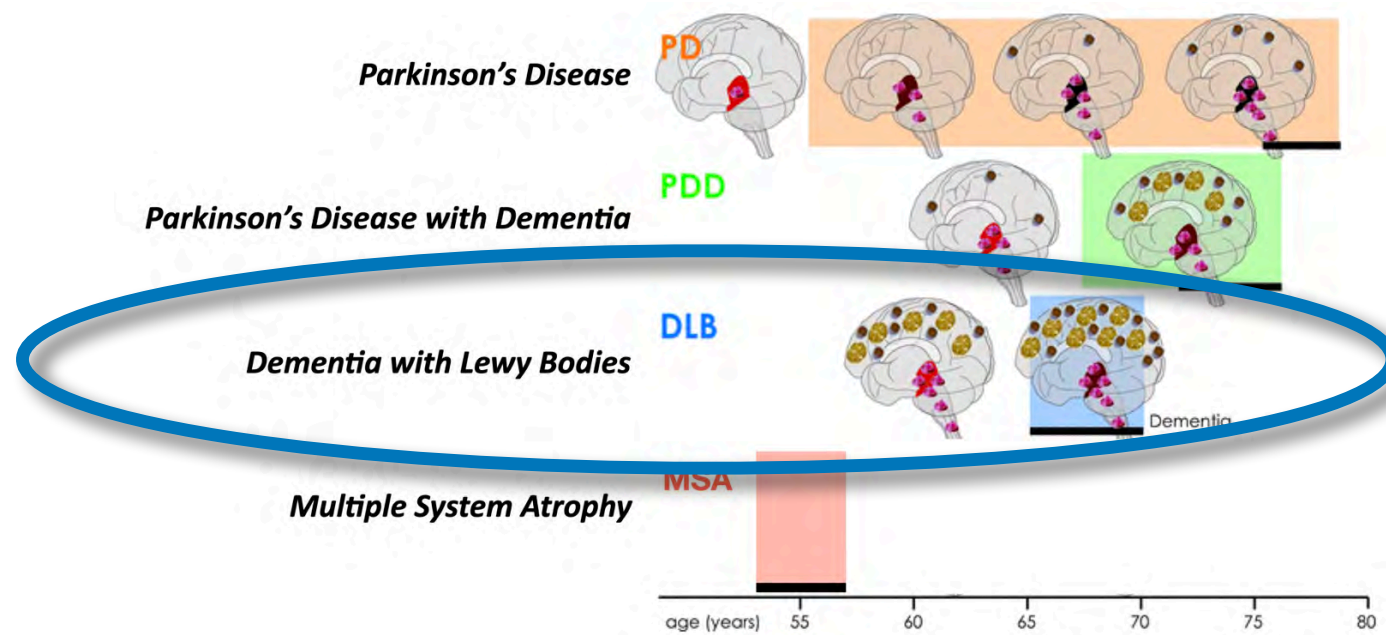
Collaboration with
Wilma van de Berg
VUMC, Amsterdam

Autopsy
of patient tissue donors
at the VUMC Amsterdam
to retrieve fresh human brain.

CLEM - Correlative Light and Electron Microscopy

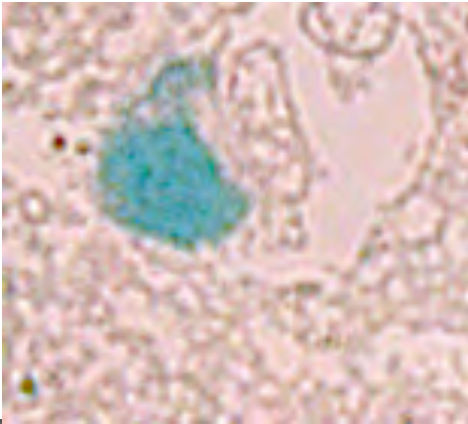


Dementia with Lewy bodies (DLB)



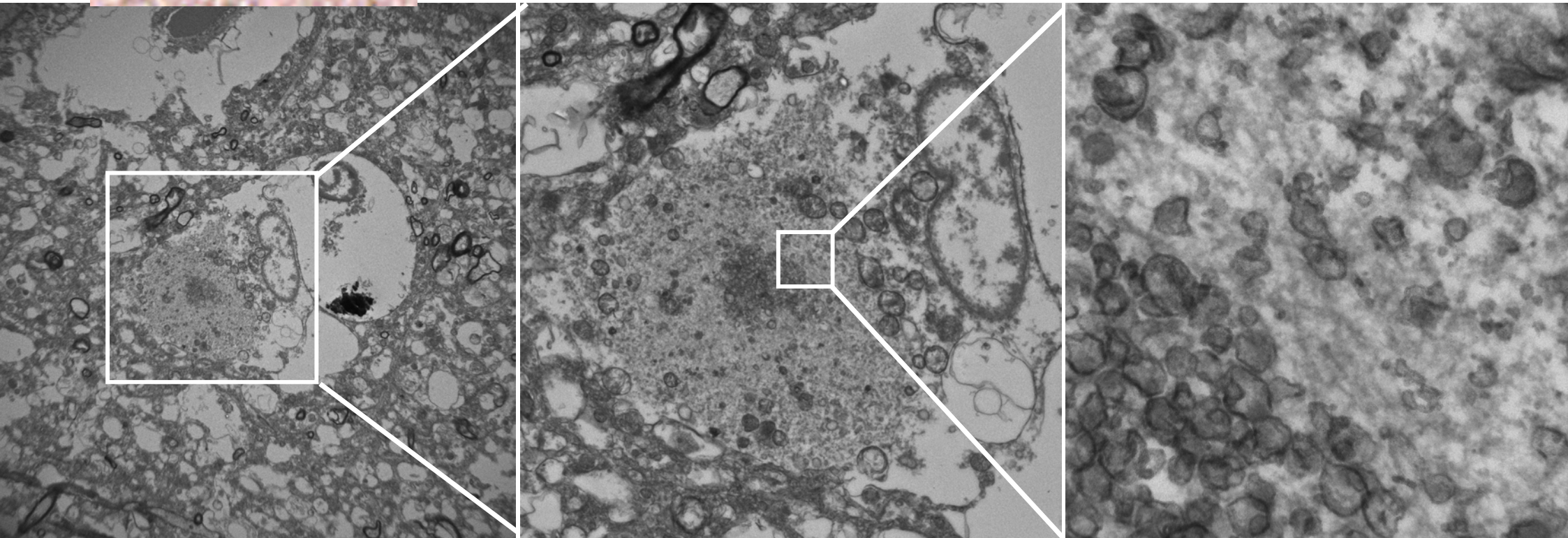
DLB: CLEM image of a Cingulate Gyrus of a DLB patient

Notash Shafiei, LBEM

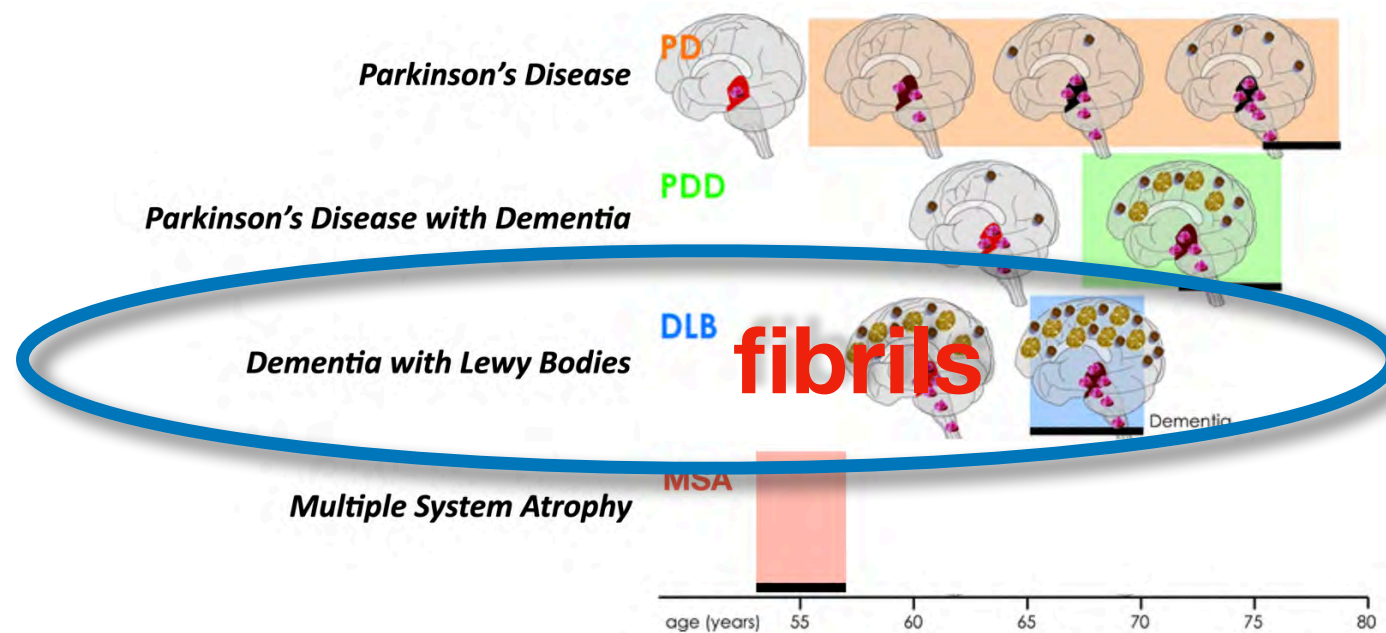


Light Microscopy

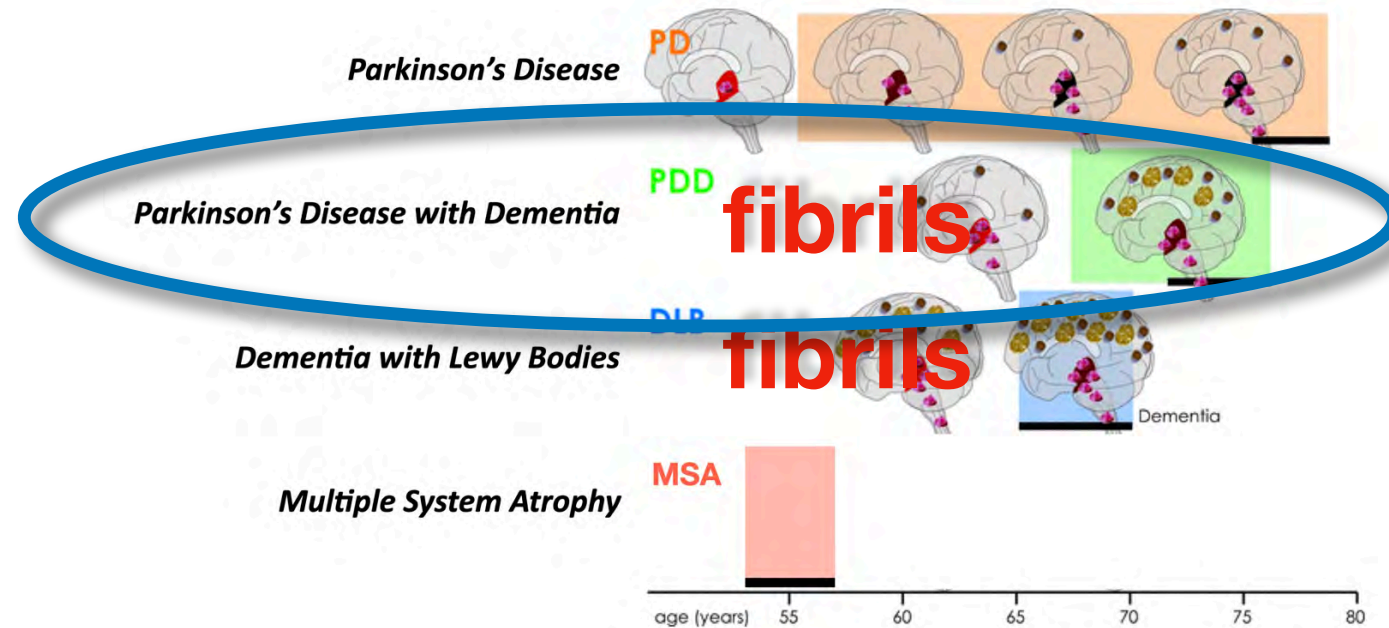
Electron Microscopy



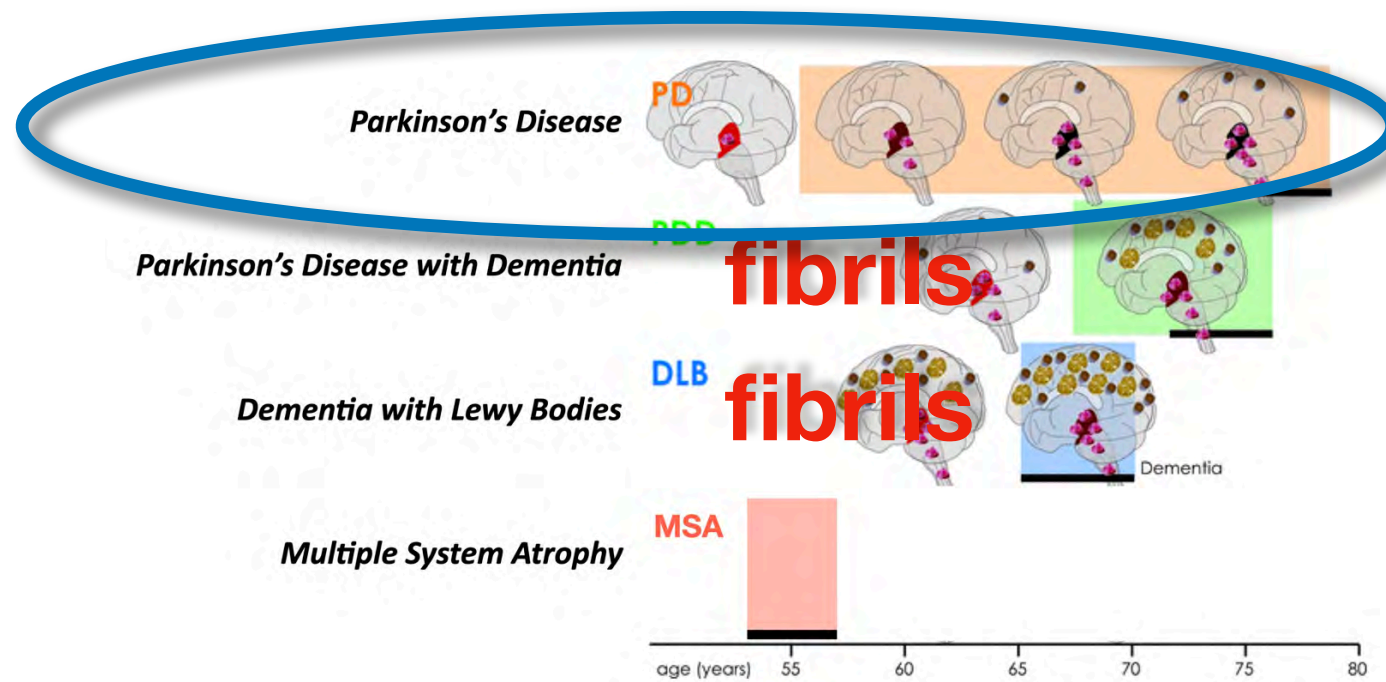
Dementia with Lewy bodies (DLB)



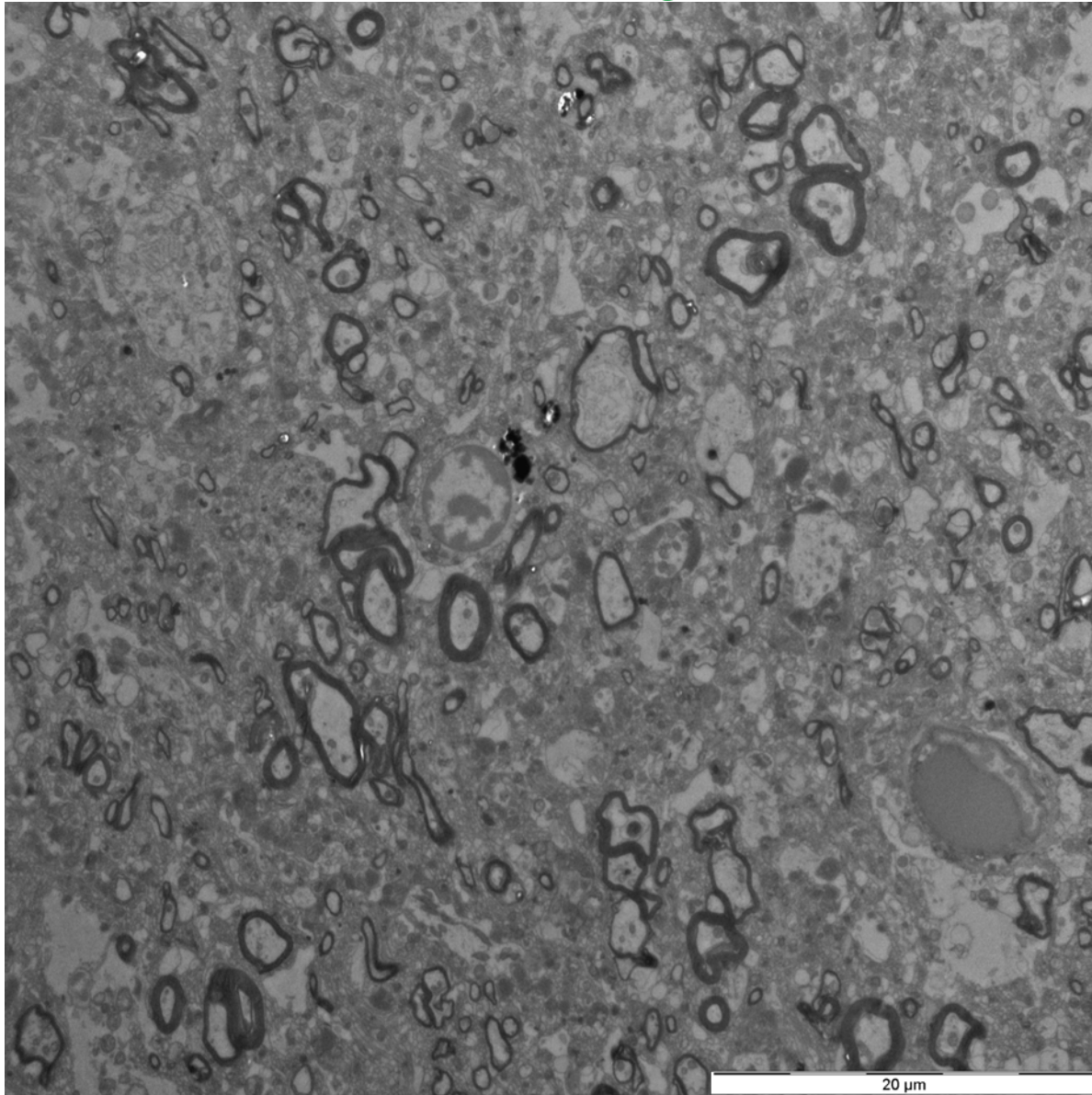
Parkinson's Disease with Dementia (PDD)



Parkinson's Disease (PD)



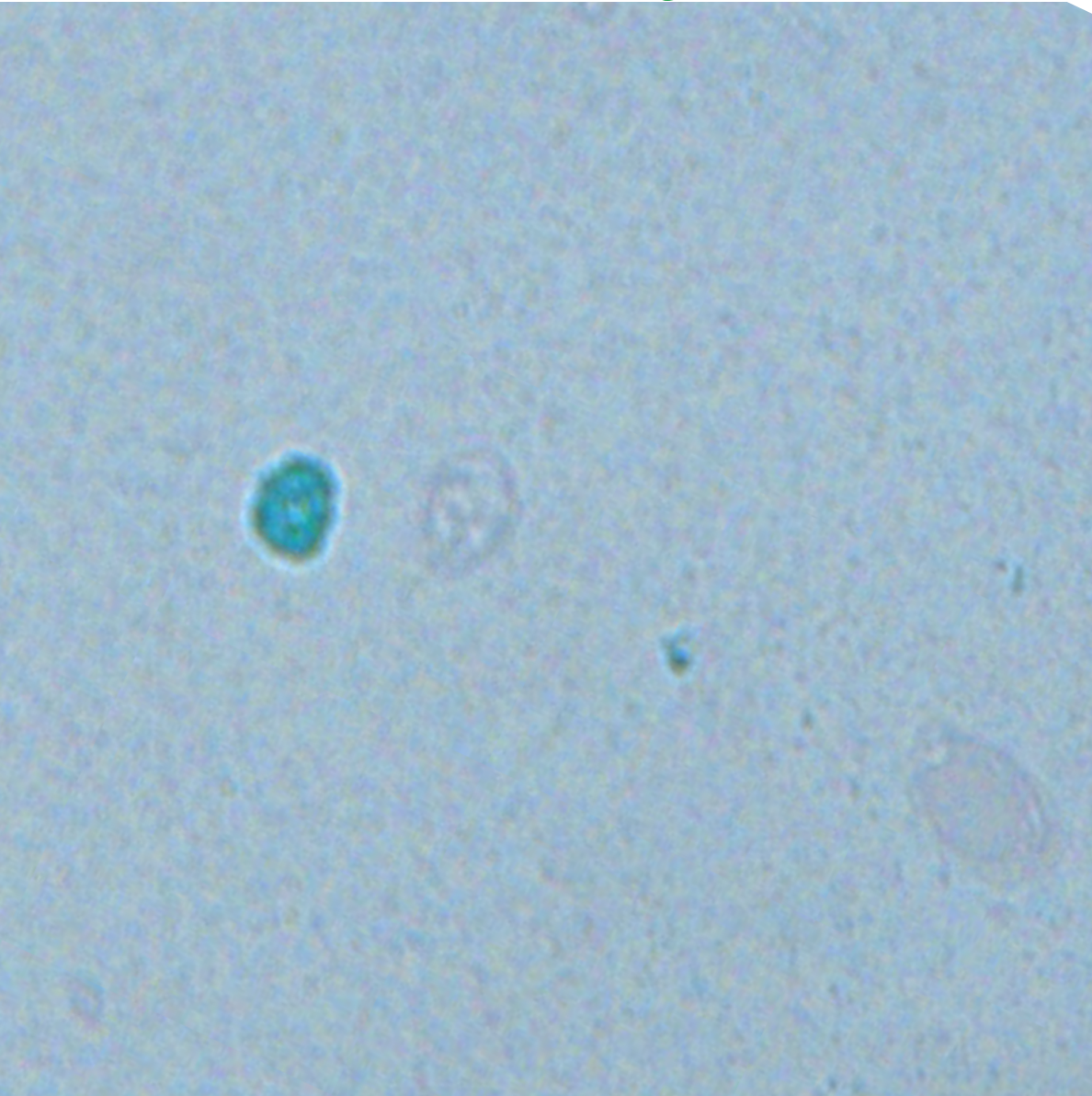
PD: CLEM - Correlative Light and Electron Microscopy



Parkinson's Disease (PD)

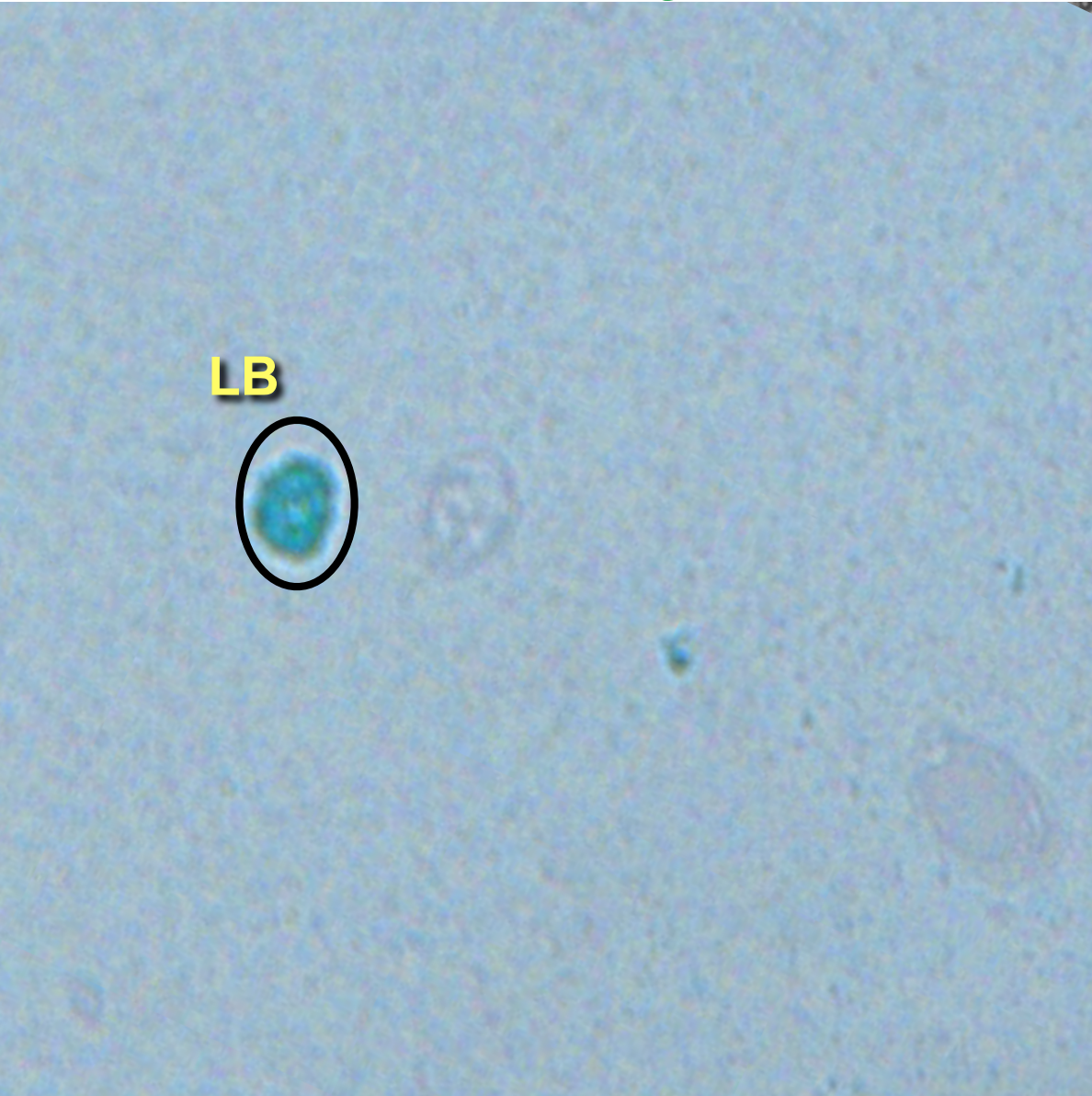
Shahmoradian *et al.*, Nature Neurosc. **22**, 1099-1109 (2019)

PD: CLEM - Correlative Light and Electron Microscopy



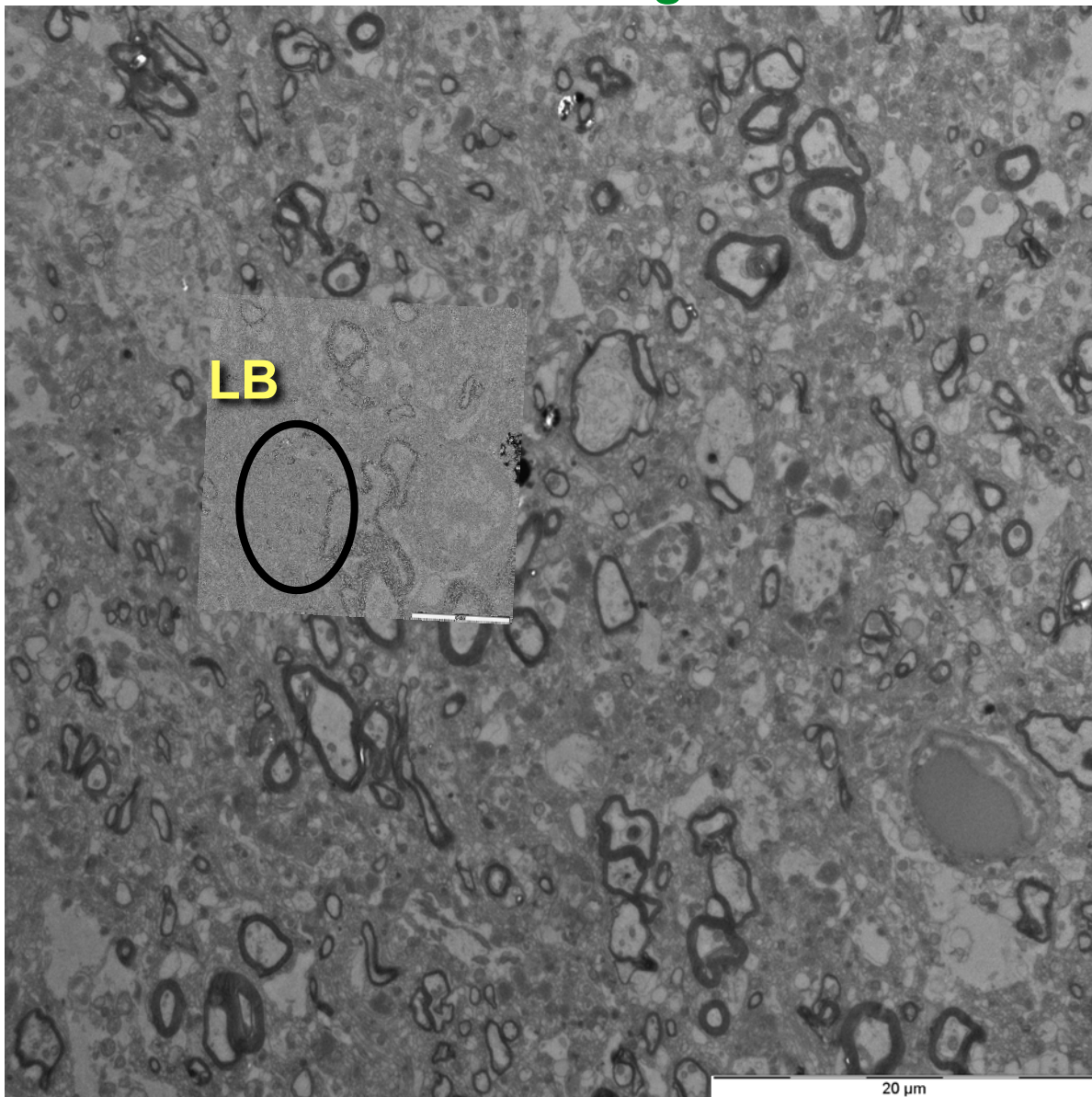
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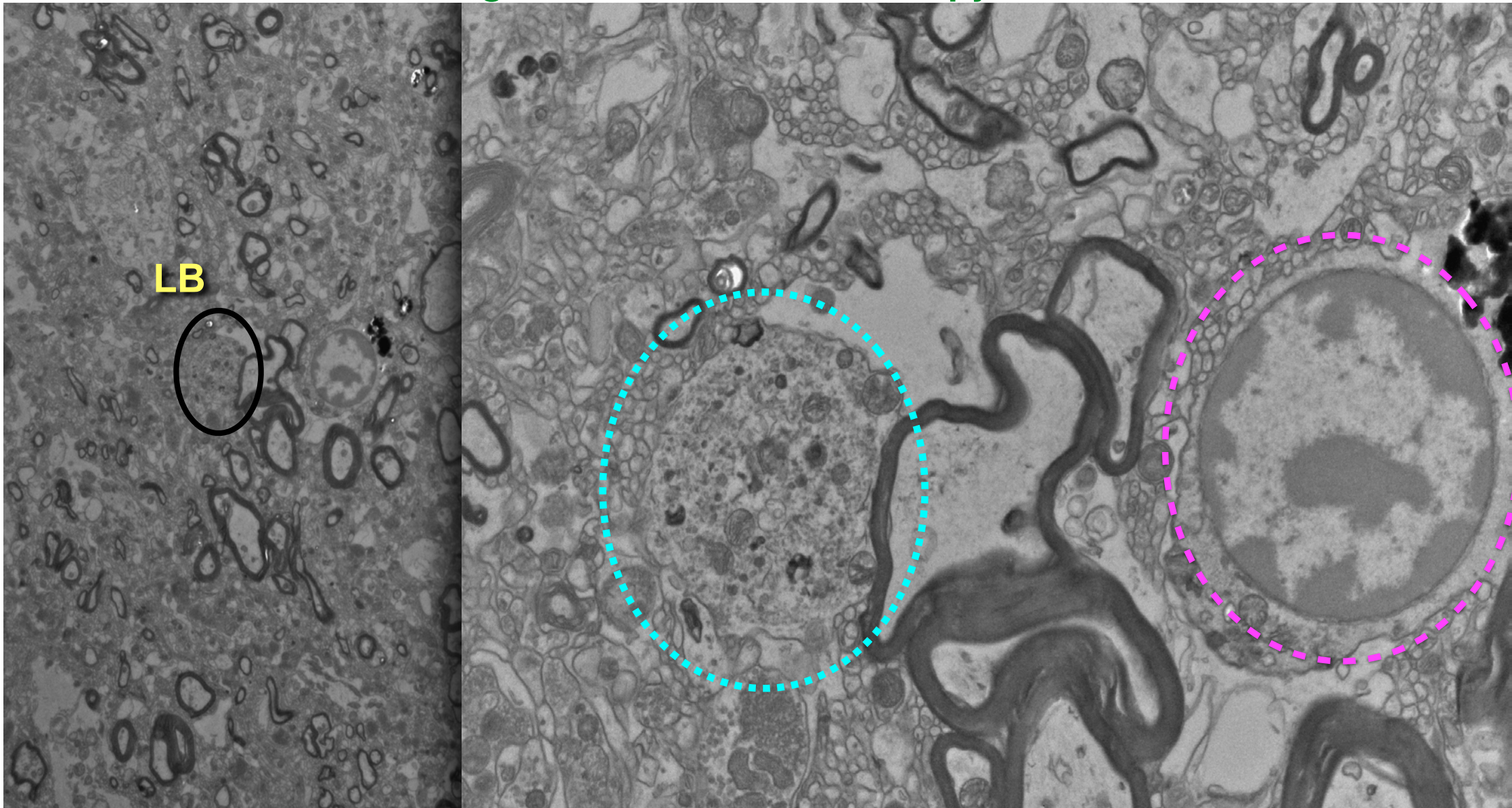
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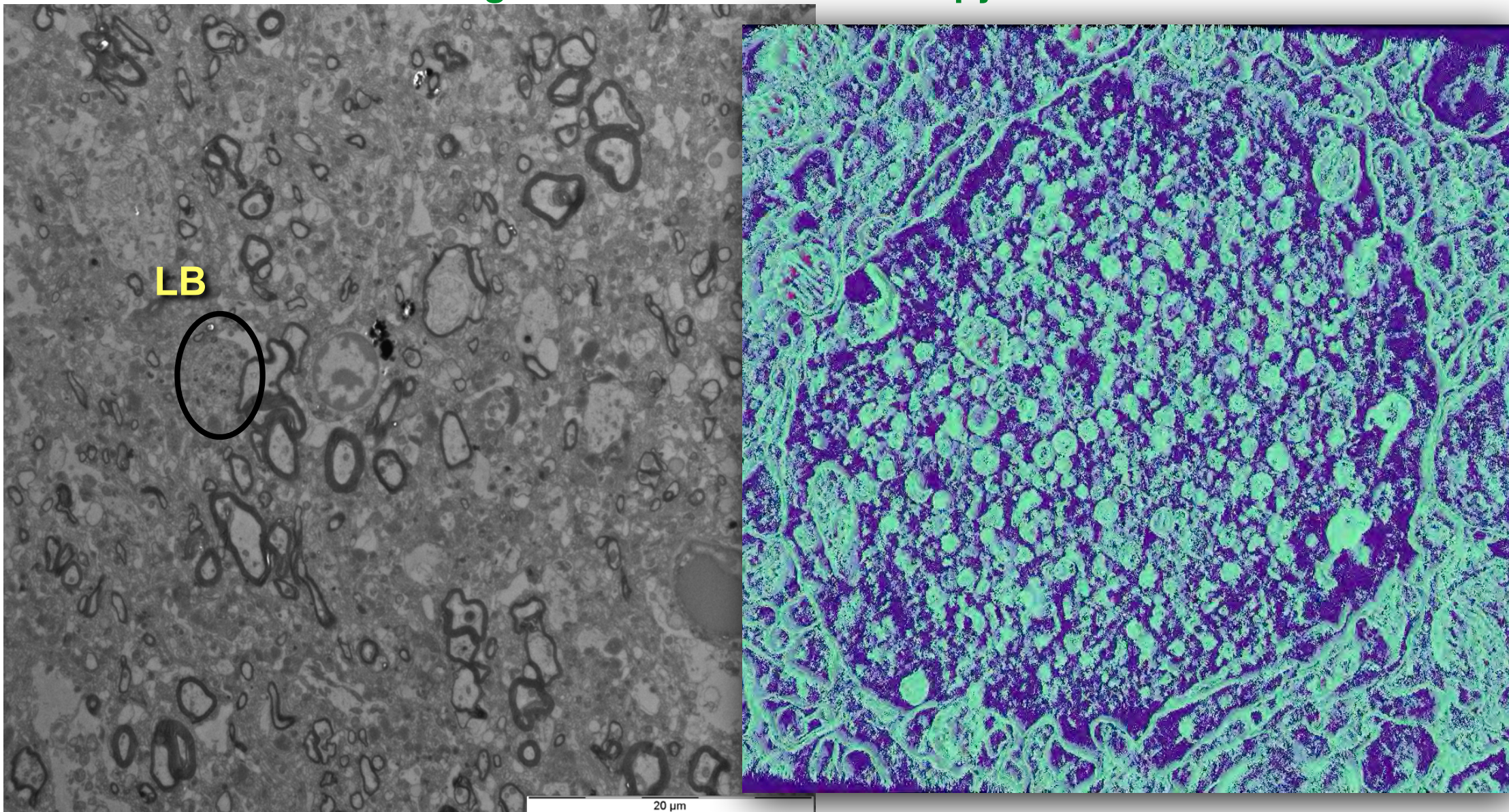


Shahmoradian *et al.*, Nature Neurosc. **22**, 1099-1109 (2019)

PD: CLEM - Correlative Light and Electron Microscopy



PD: CLEM - Correlative Light and Electron Microscopy



PD:

Lewy body, *Substantia nigra*

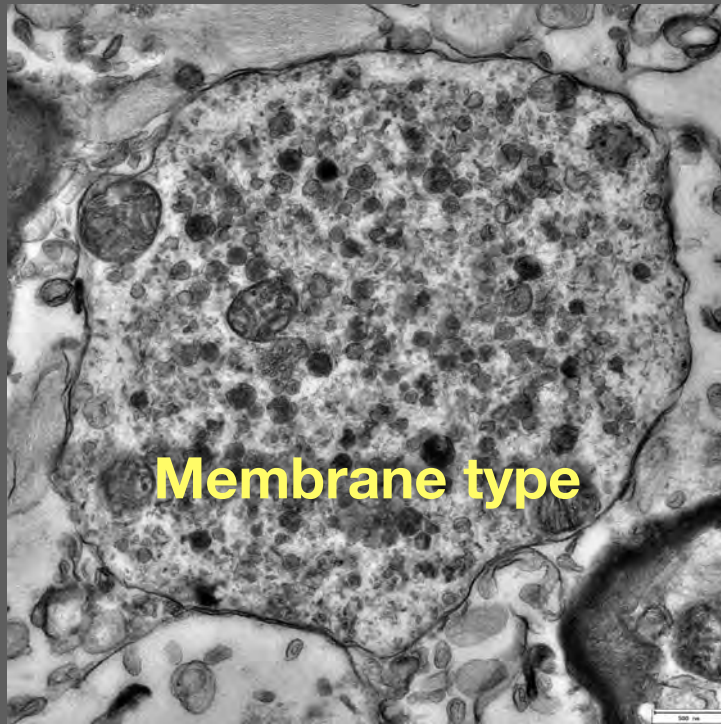
Amanda Lewis, LBEM



LB #1

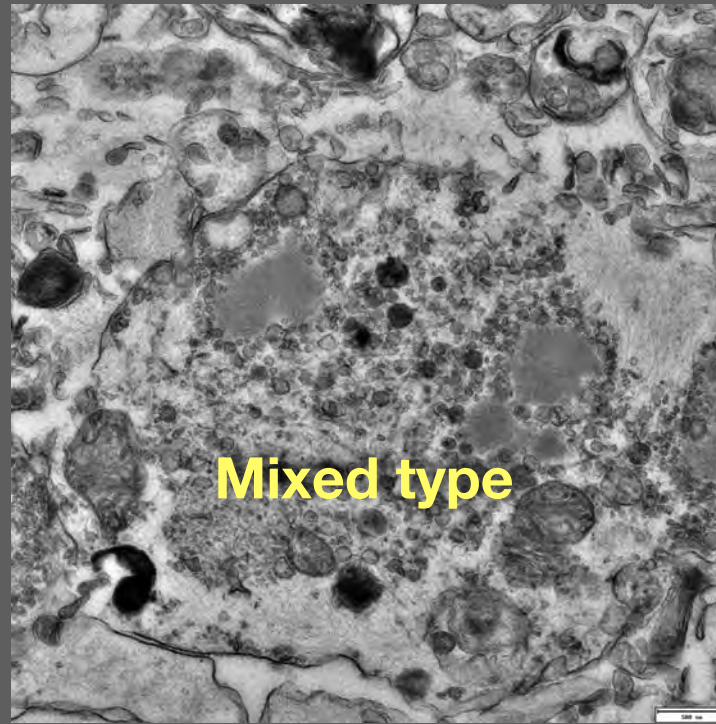
LB #2

LB #3



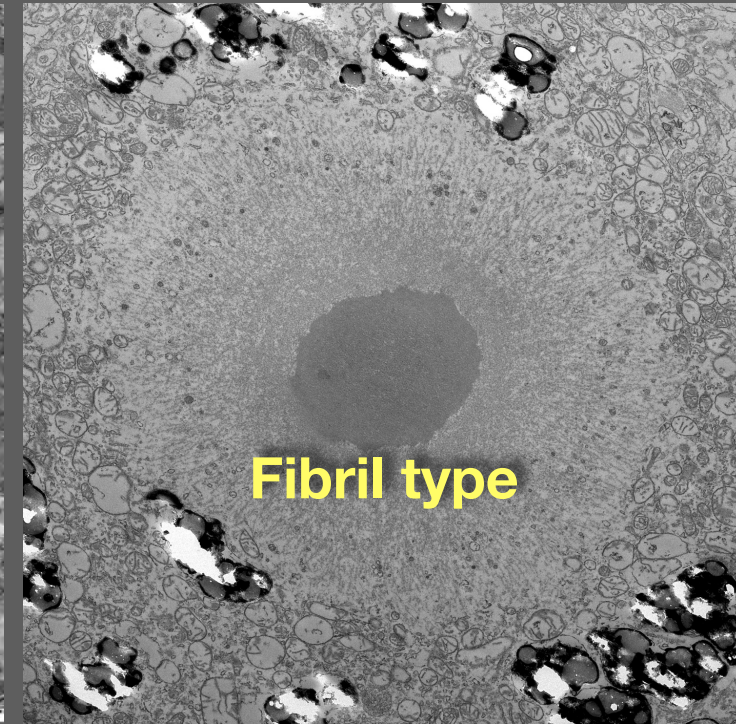
Membrane type

500 nm



Mixed type

500 nm



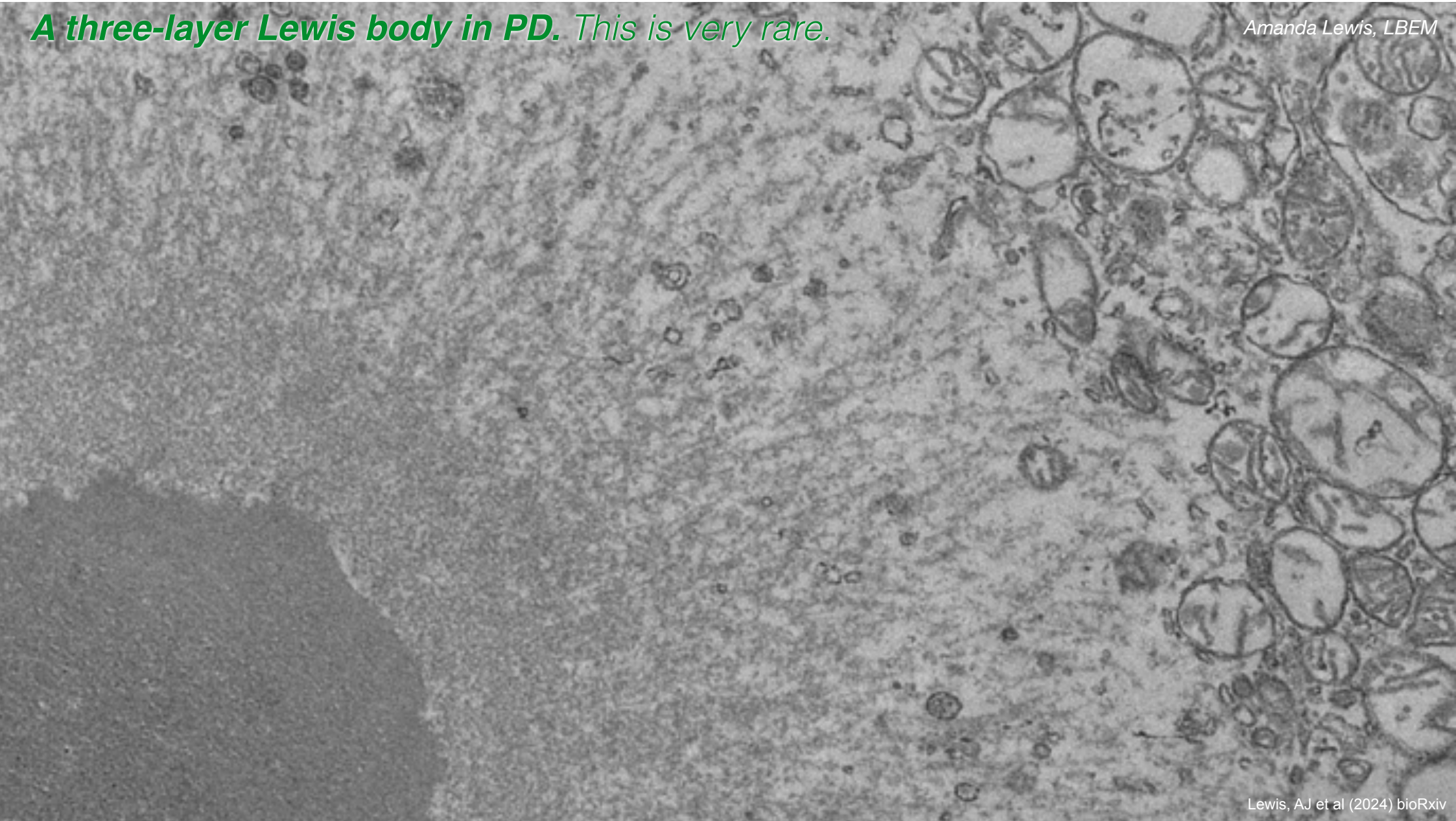
Fibril type

- organelles
- vesicles
- membranes
- filaments (peripheral+central, interspersed)
- dense granular regions

**The majority of Lewy bodies in PD
are primarily composed of
membrane fragments**

A three-layer Lewis body in PD. This is very rare.

Amanda Lewis, LBEM

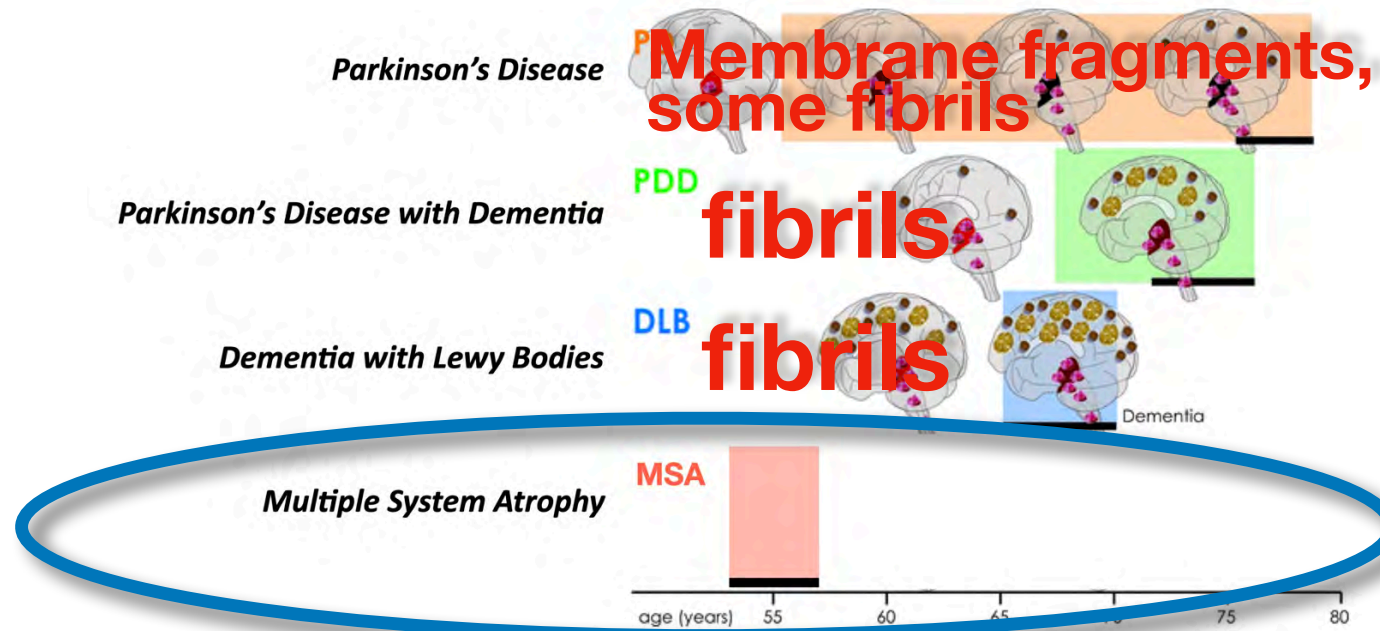


Lewis, AJ et al (2024) bioRxiv

Multiple System Atrophy (MSA)

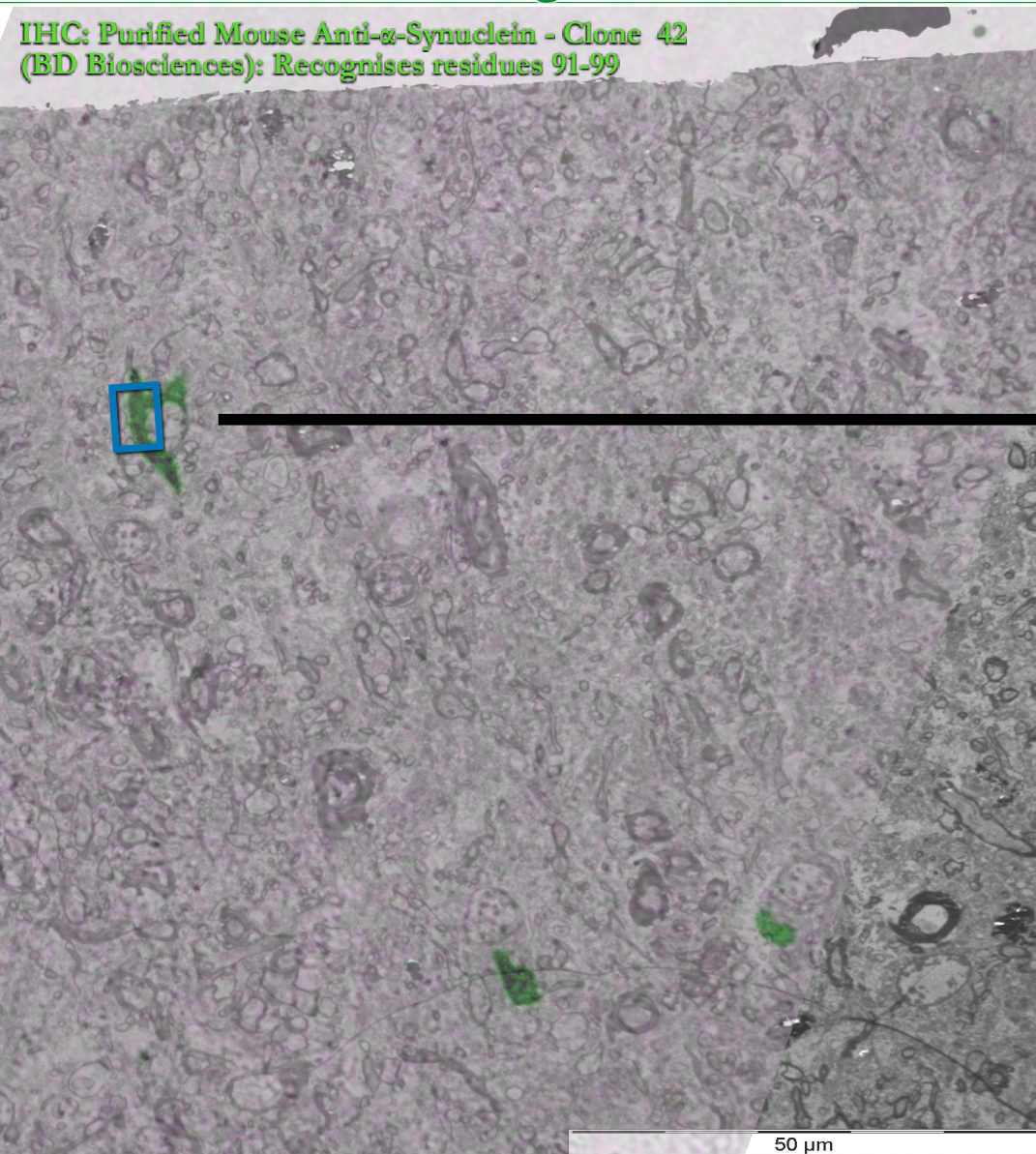


<https://www.multiplesystematrophy.org/>

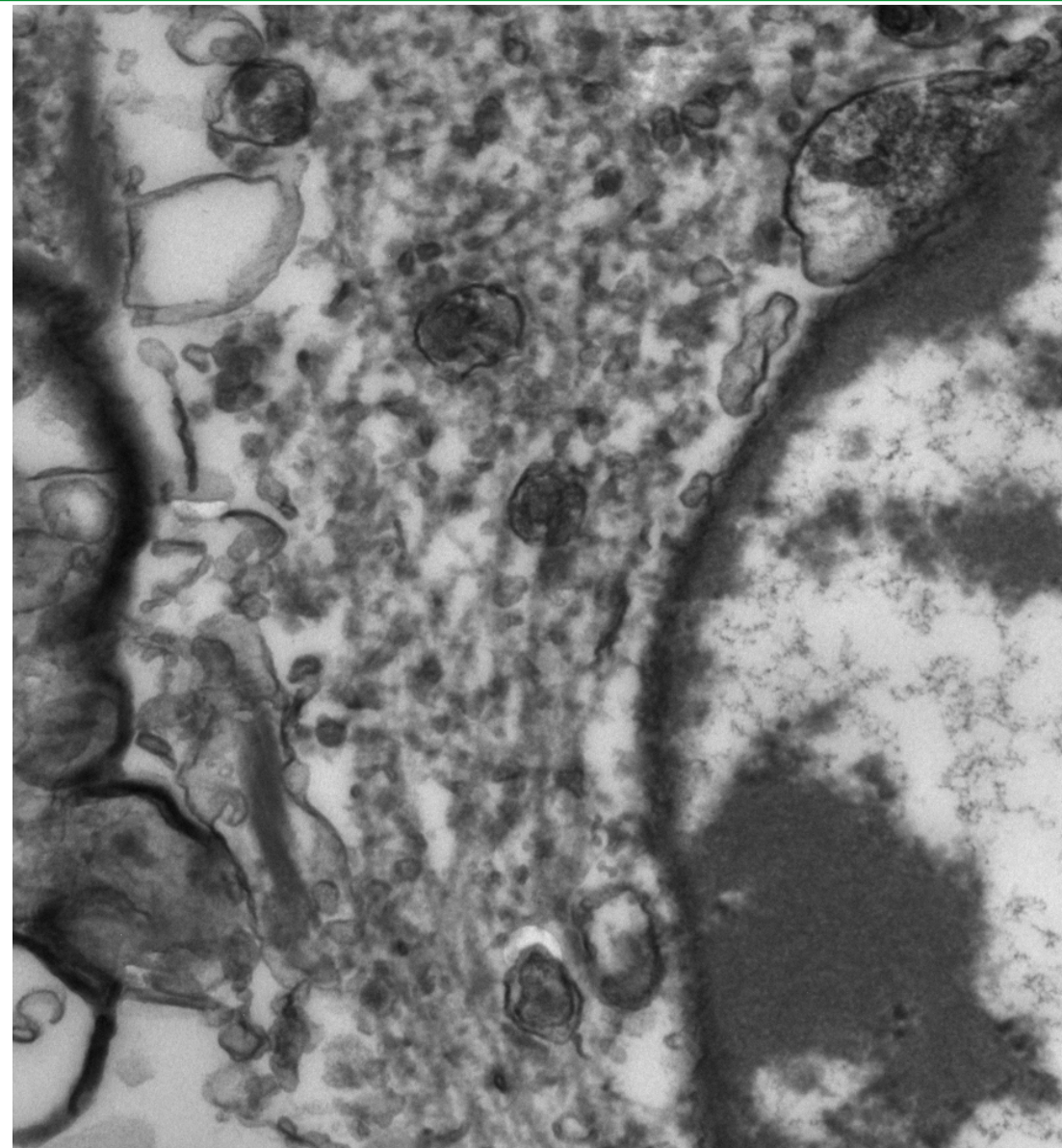


MSA: *Substantia Nigra*

IHC: Purified Mouse Anti- α -Synuclein - Clone 42
(BD Biosciences): Recognises residues 91-99

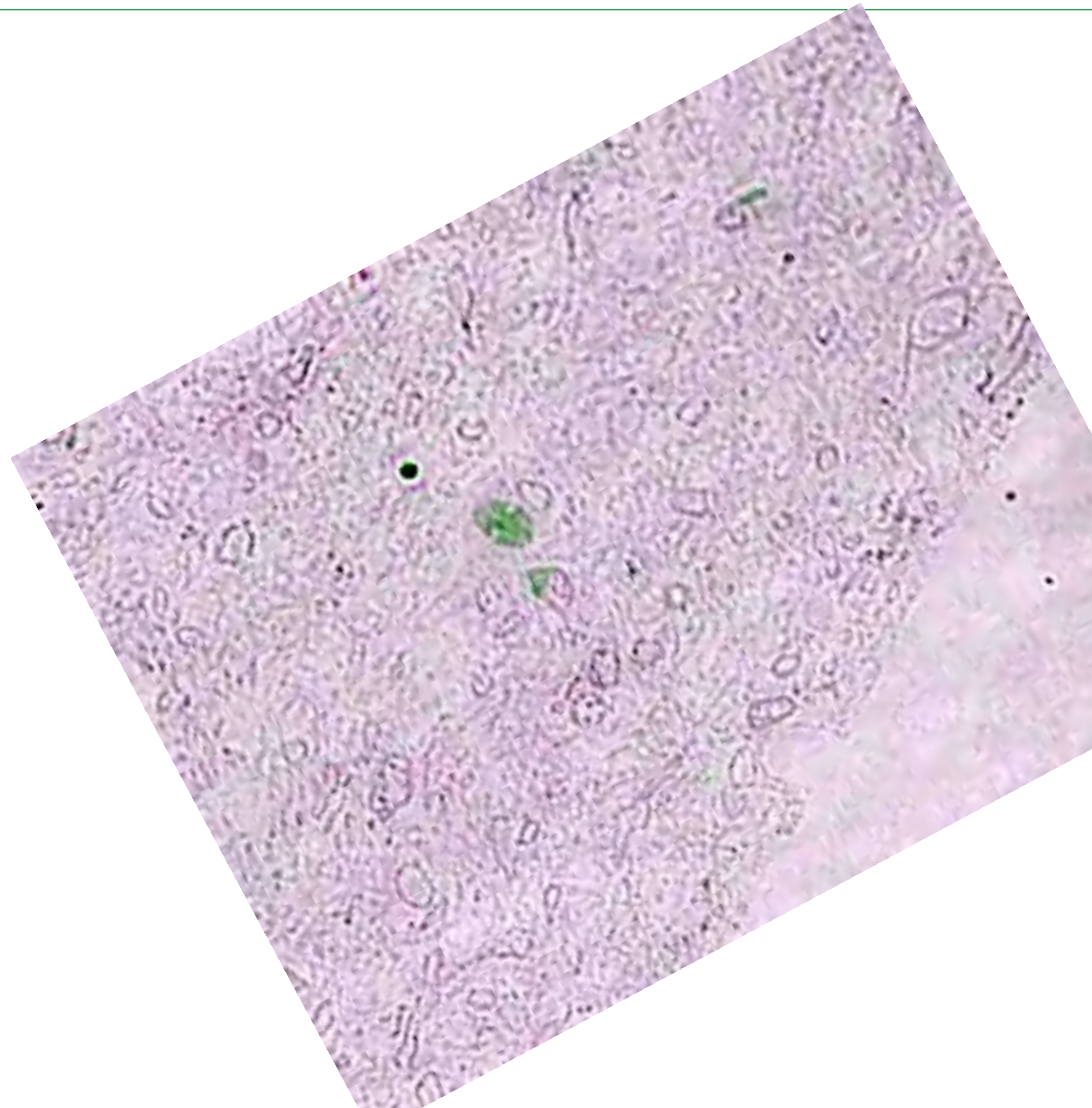
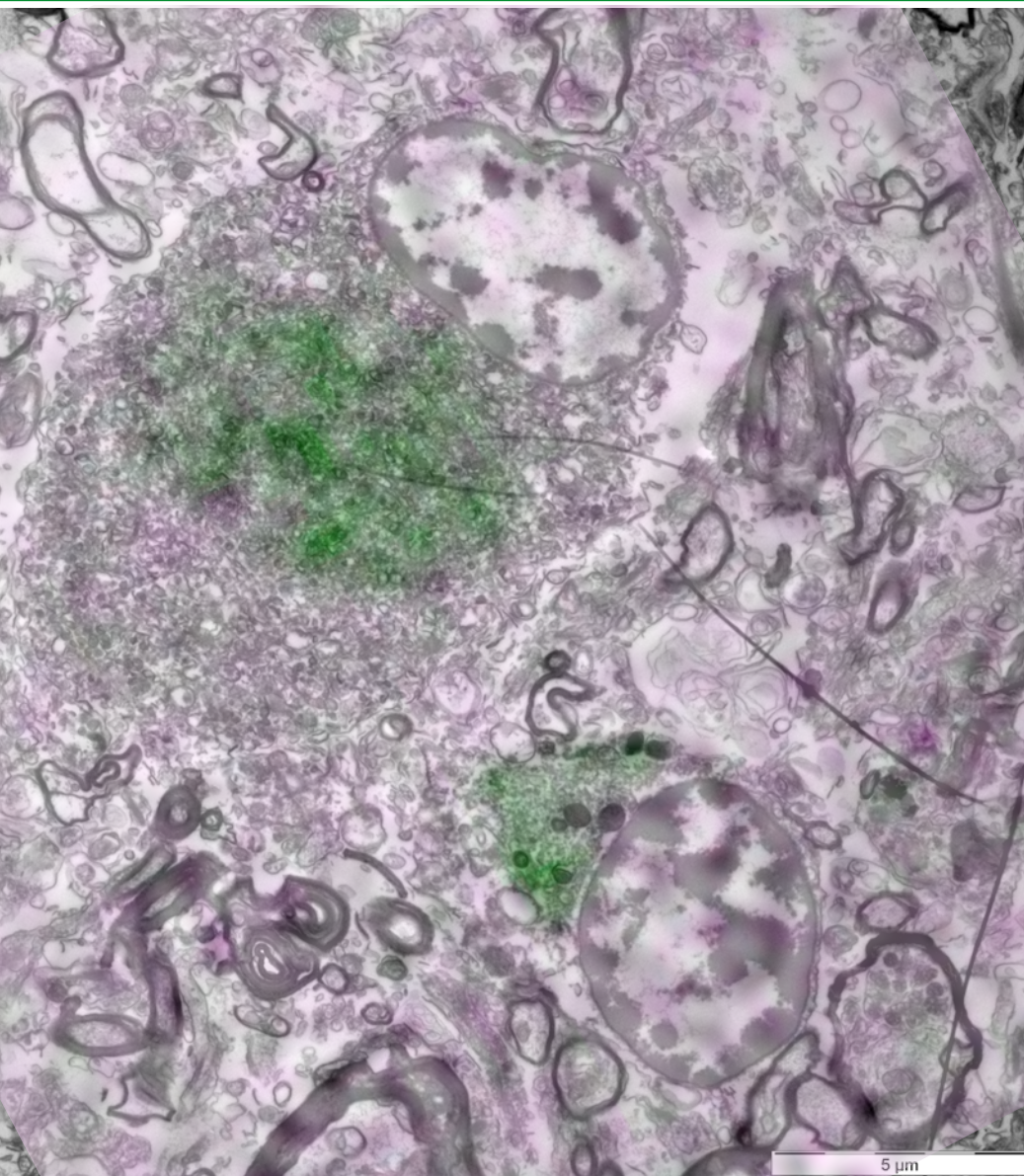


Amanda Lewis, Carolin Böing, LBEM



MSA: Putamen

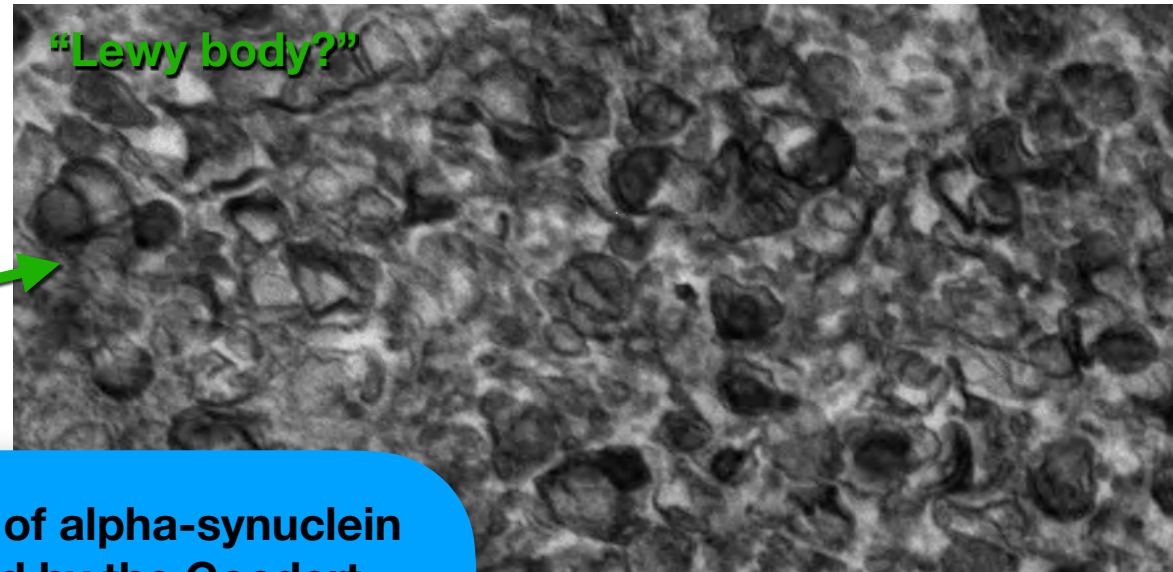
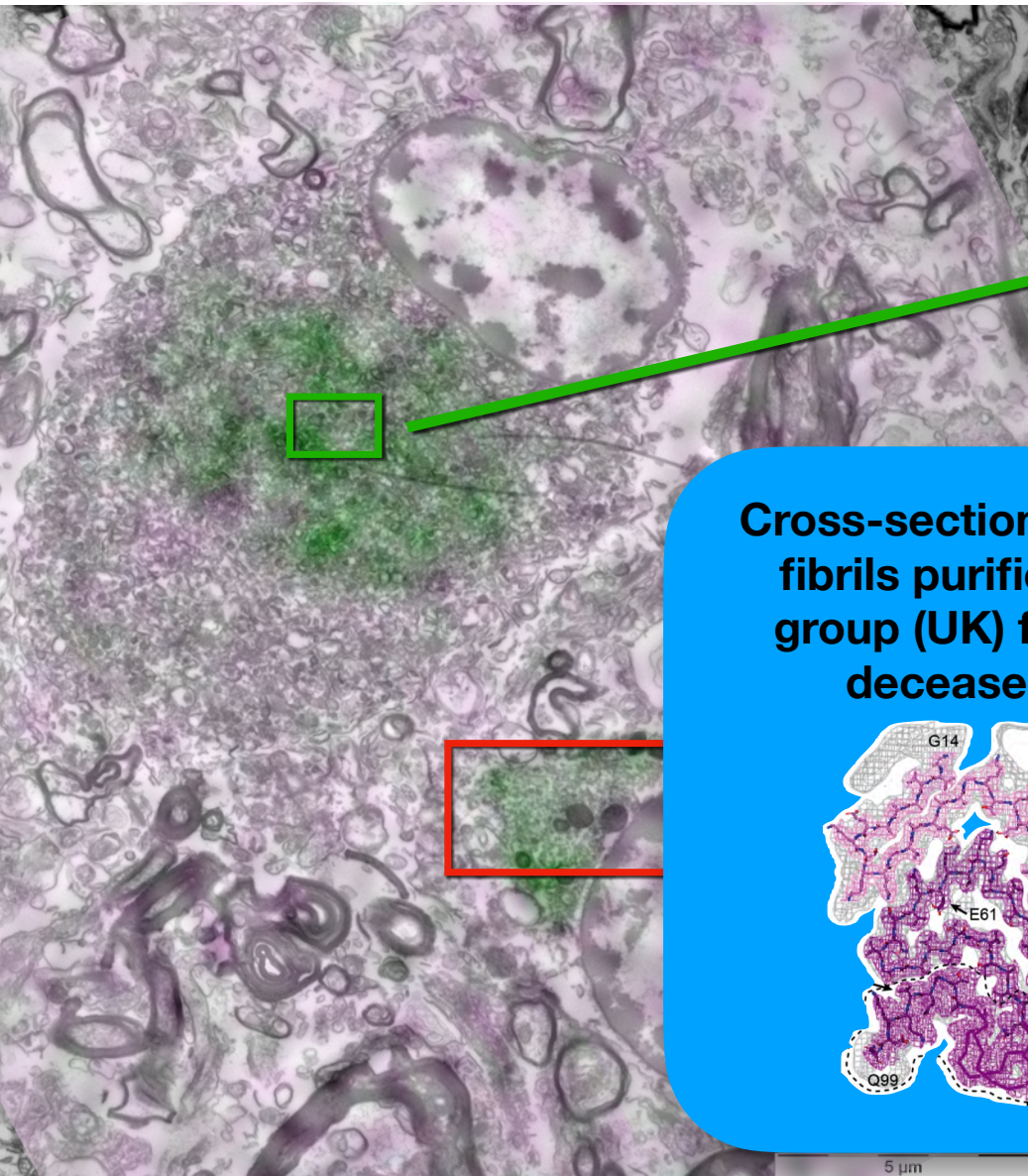
Amanda Lewis, Carolin Böing, LBEM



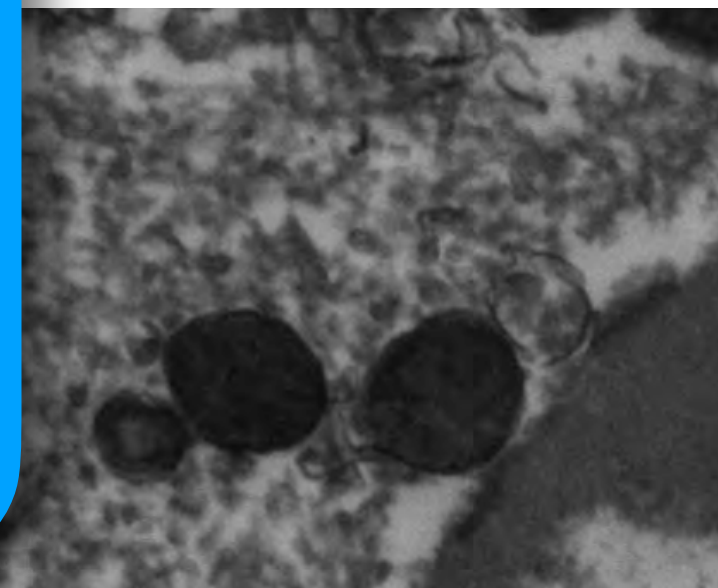
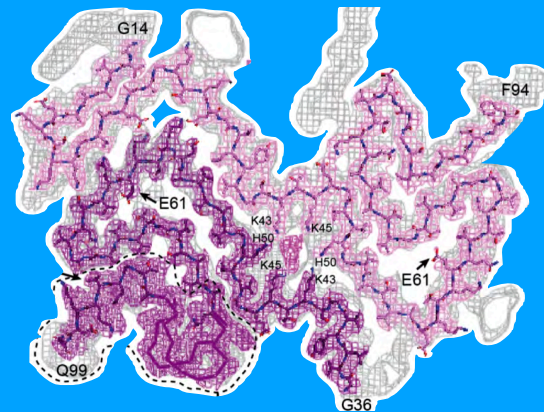
MSA: Putamen

“PD Lewy body” next to MSA GCI ?

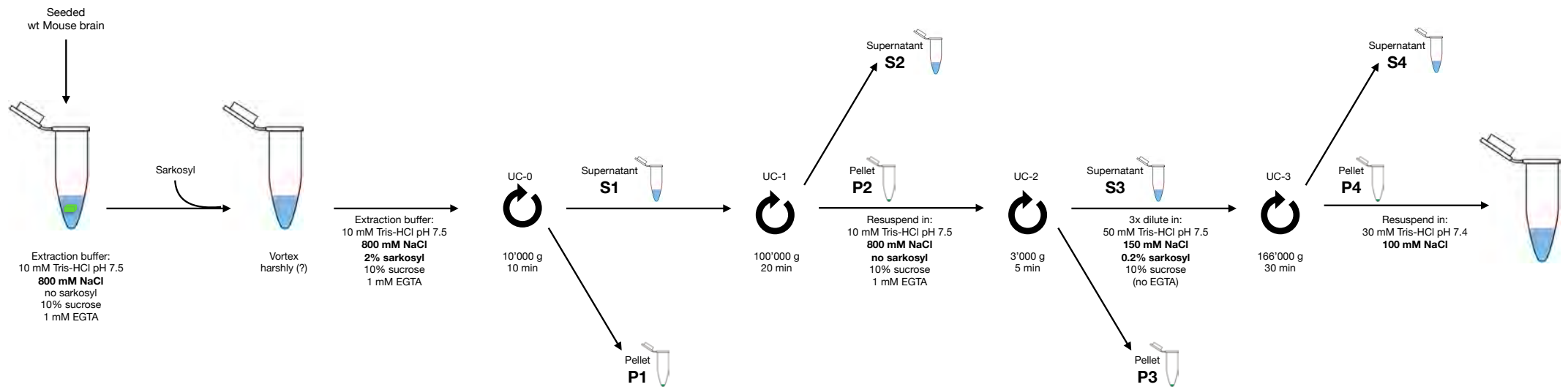
Amanda Lewis, Carolin Böing, LBEM



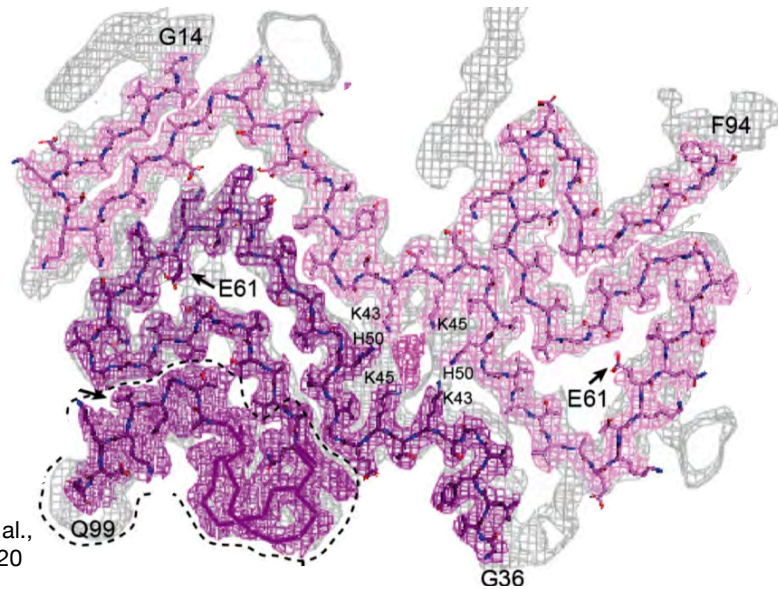
Cross-section of alpha-synuclein fibrils purified by the Goedert group (UK) from the brain of a deceased MSA patient.



Fibril Purification



Multiple System Atrophy: A specific alpha-synuclein fibril strain

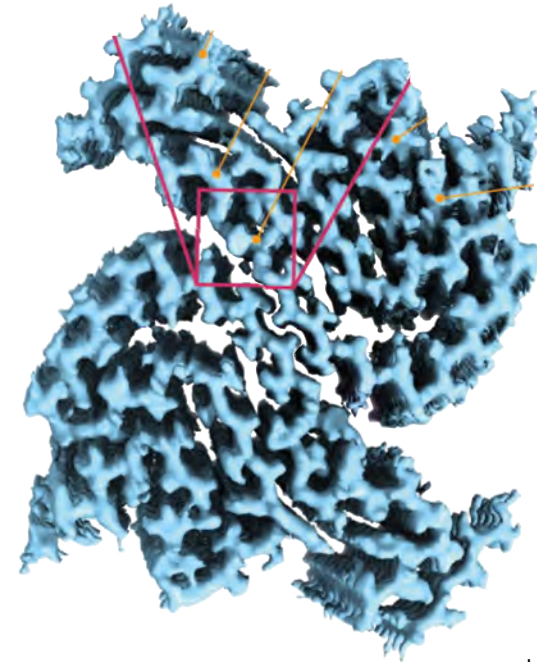


Schweighauser et al.,
Nature, Sept. 2020

- 2 % Sarkosyl detergent
- 800 mM NaCl
- 37°C
- 30 min shaking

**Purified from human brain
of MSA patients**

(Goedert and Scheres labs, MRC, Cambridge, UK)

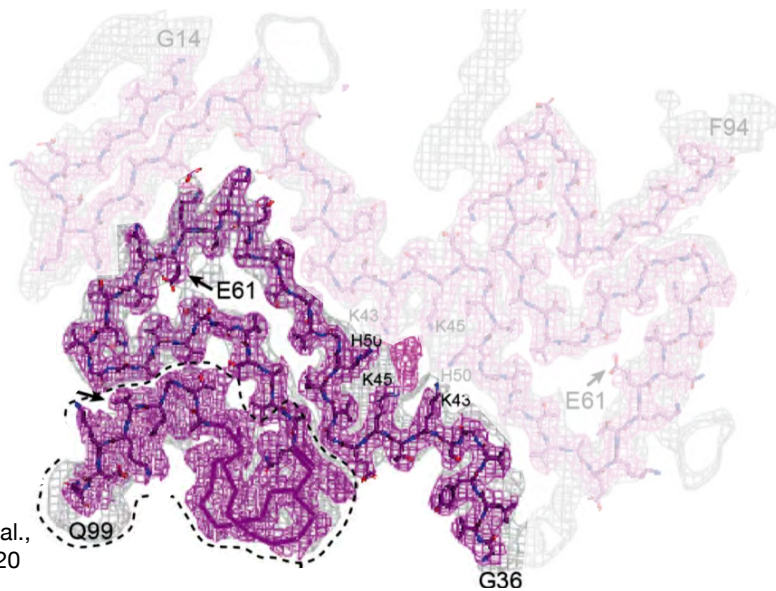


Burger et al.,
bioRxiv.org, 2024

**Synthetic “1B” fibril,
causing MSA in mice**

(LBEM, collaboration with François Ichas, Bordeaux, France)

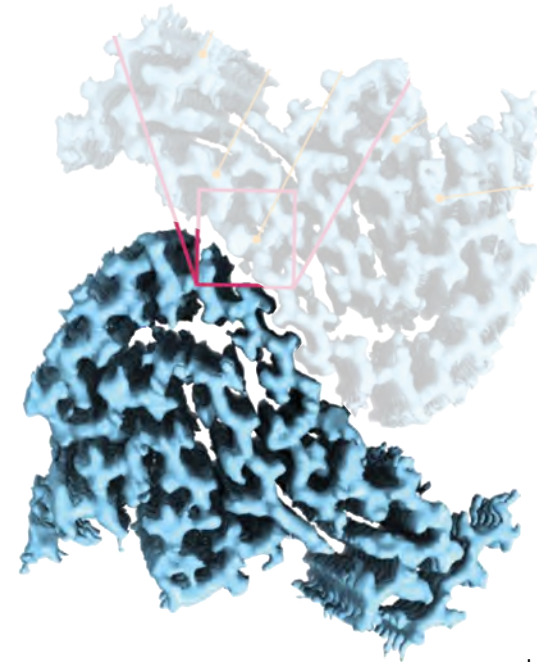
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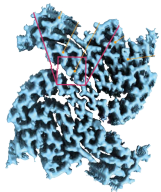
**Purified from human brain
of MSA patients**



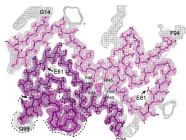
Burger et al.,
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**Synthetic “1B” fibril,
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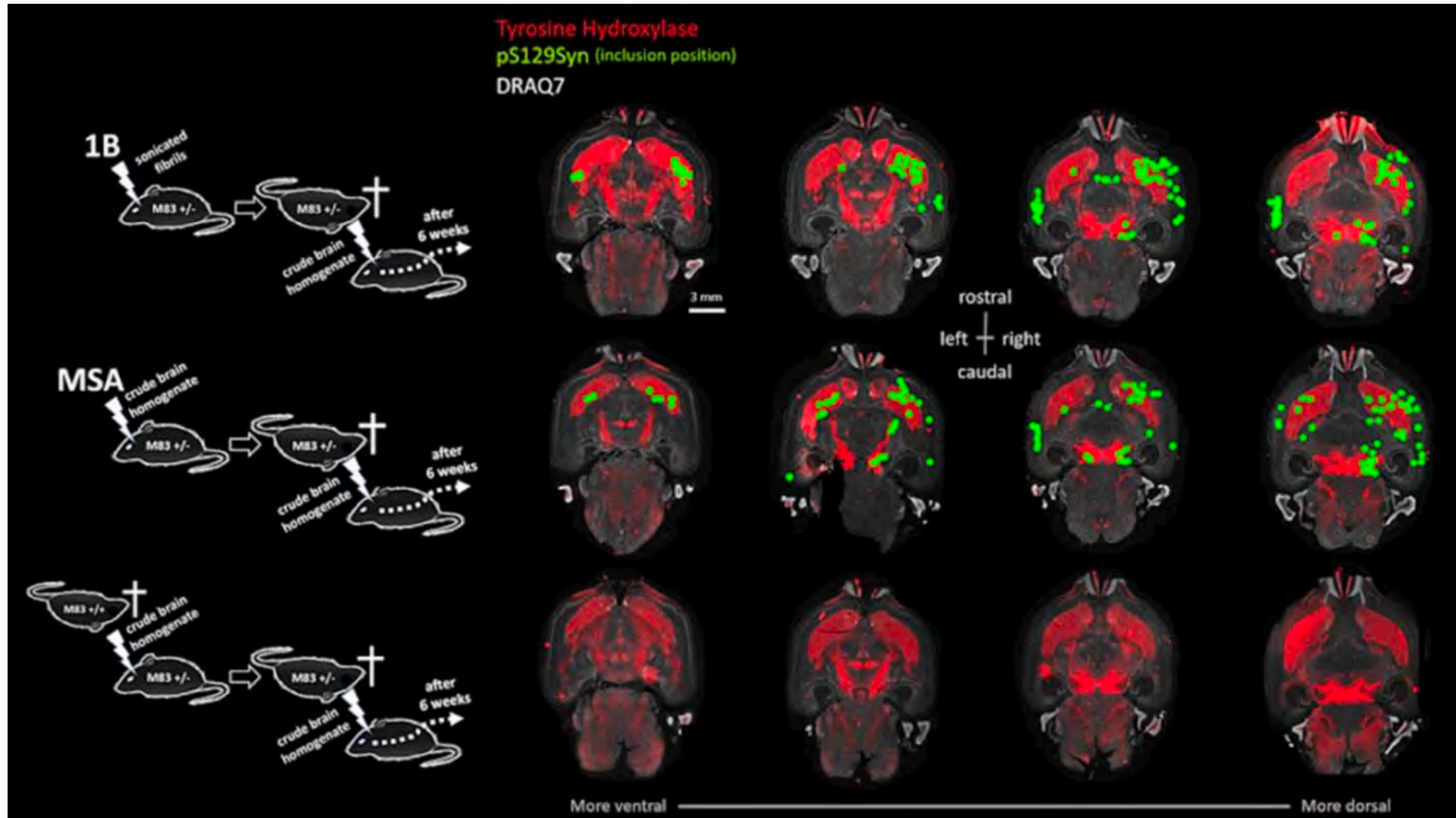


Synthetic "1B" fibril,
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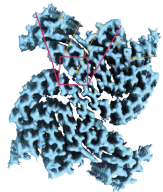


Purified from human brain
of MSA patients

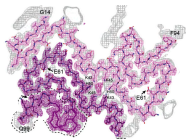
M83 mice
express
human A53T
aSyn



Multiple System Atrophy: A specific alpha-synuclein fibril strain

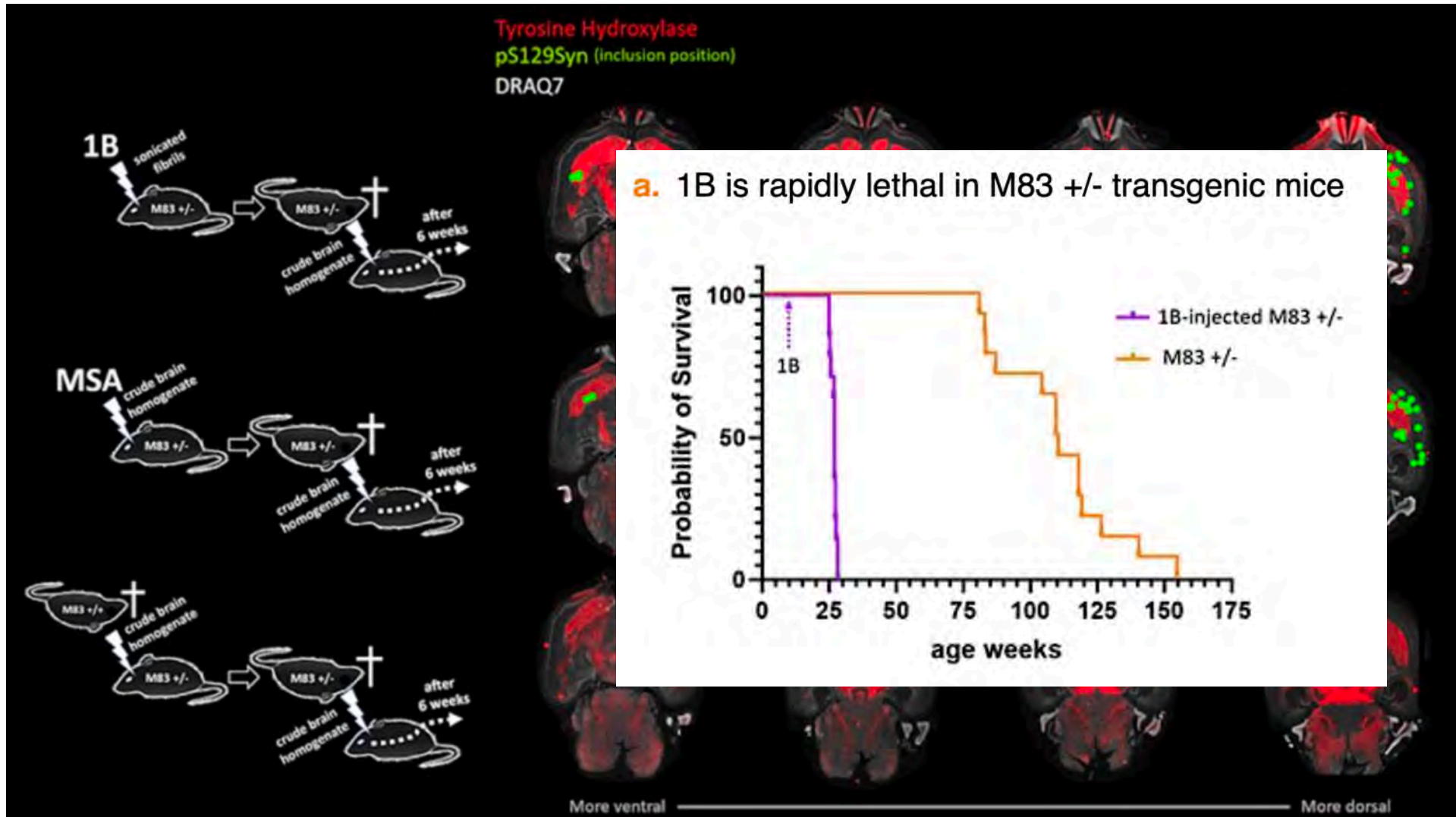


Synthetic "1B" fibril, causing MSA in mice



Purified from human brain of MSA patients

M83 mice express human A53T α Syn



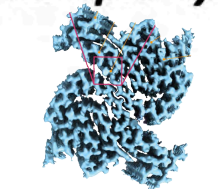
Synucleinopathies: Different “strains” of α -synuclein fibrils may cause different diseases

Parkinson's Disease

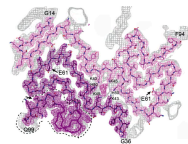
Parkinson's Disease with Dementia

Dementia with Lewy Bodies

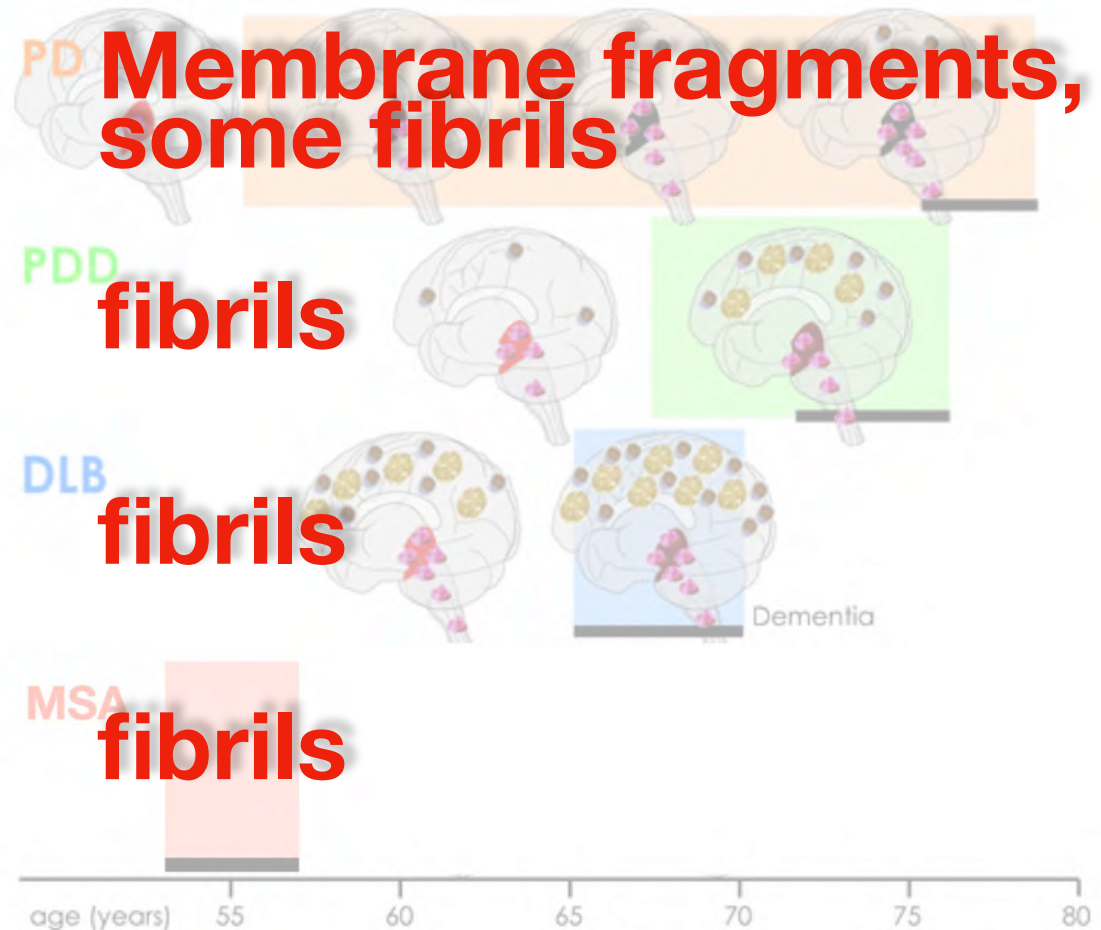
Multiple System Atrophy



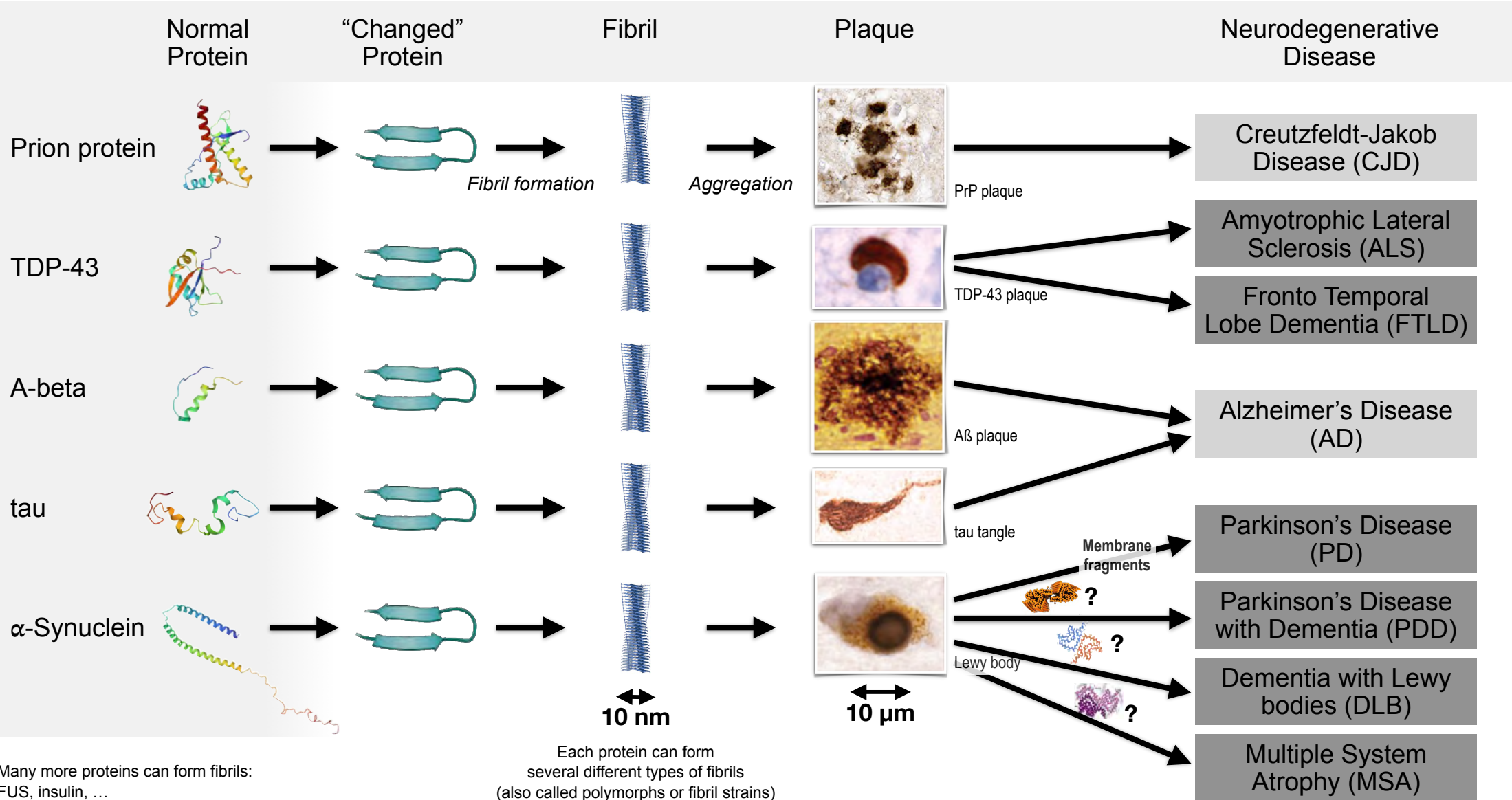
Synthetic “1B” fibril,
causing MSA in mice



Purified from human brain
of MSA patients



Prionoid fibril growth: Fibrils form plaques, which correlate with (cause?) disease

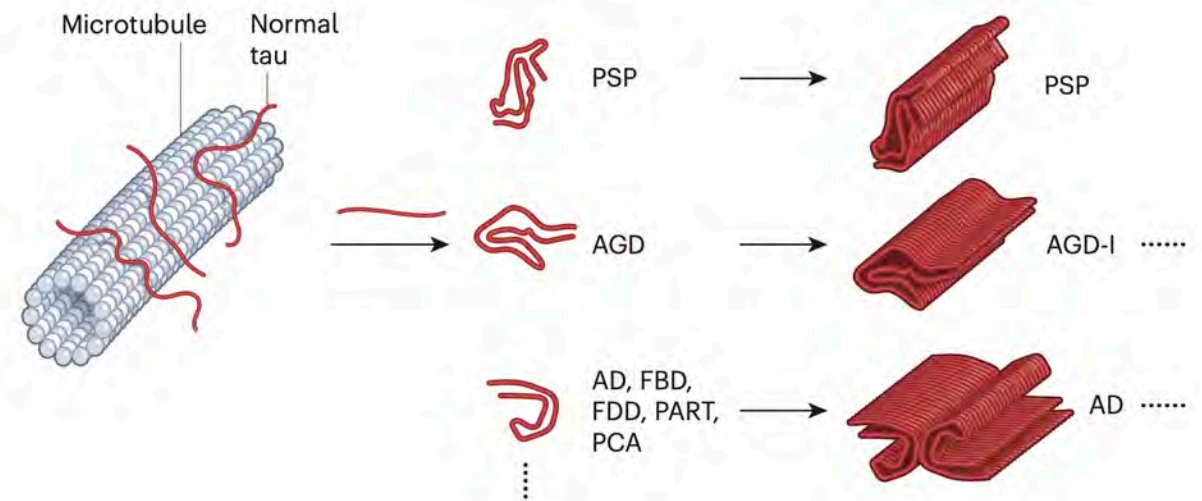


tau tangles in Alzheimer's Disease

Tau Fibril Polymorphism

Work by the groups of Michel Goedert and Sjors Scheres, LMB, Cambridge, UK

Different tau fibril “strains”
cause
different diseases?



The international journal of science / 14 October 2021

nature

PROTEIN MISFOLDS

Neurodegenerative diseases classified by shapes of tau filaments

Under fire
How scientists are harassed for speaking up about COVID-19

Mass market
Electric vehicles must be lighter to give greatest benefits

Carbon costs
Estimating the global energy spend for a warmer world

Tau Fibril Polymorphism

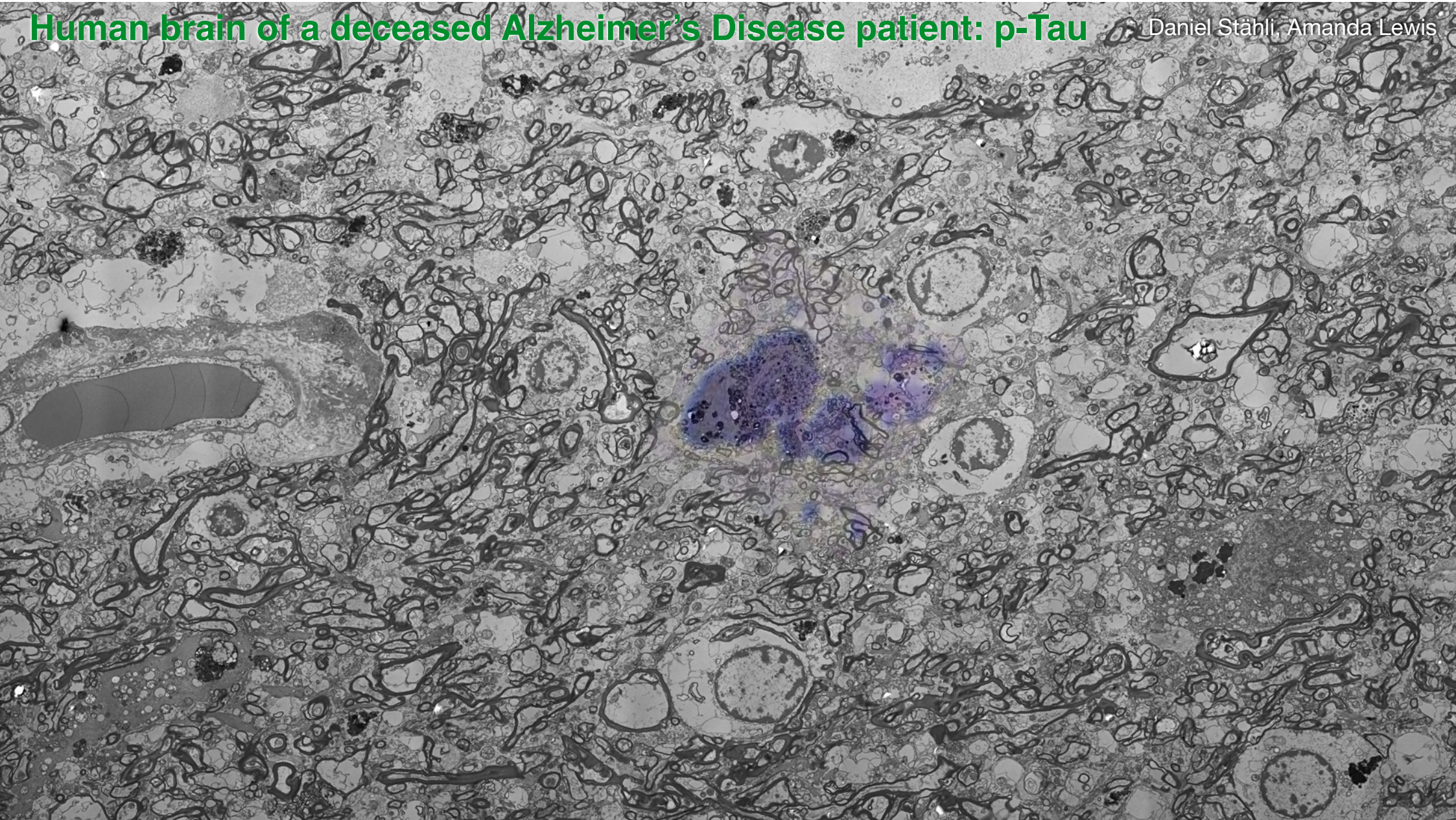
Work by the groups of Michel Goedert and Sjors Scheres, LMB, Cambridge, UK

[illegible]

**Different tau fibril “strains”
correlate with (cause?)
different diseases.**

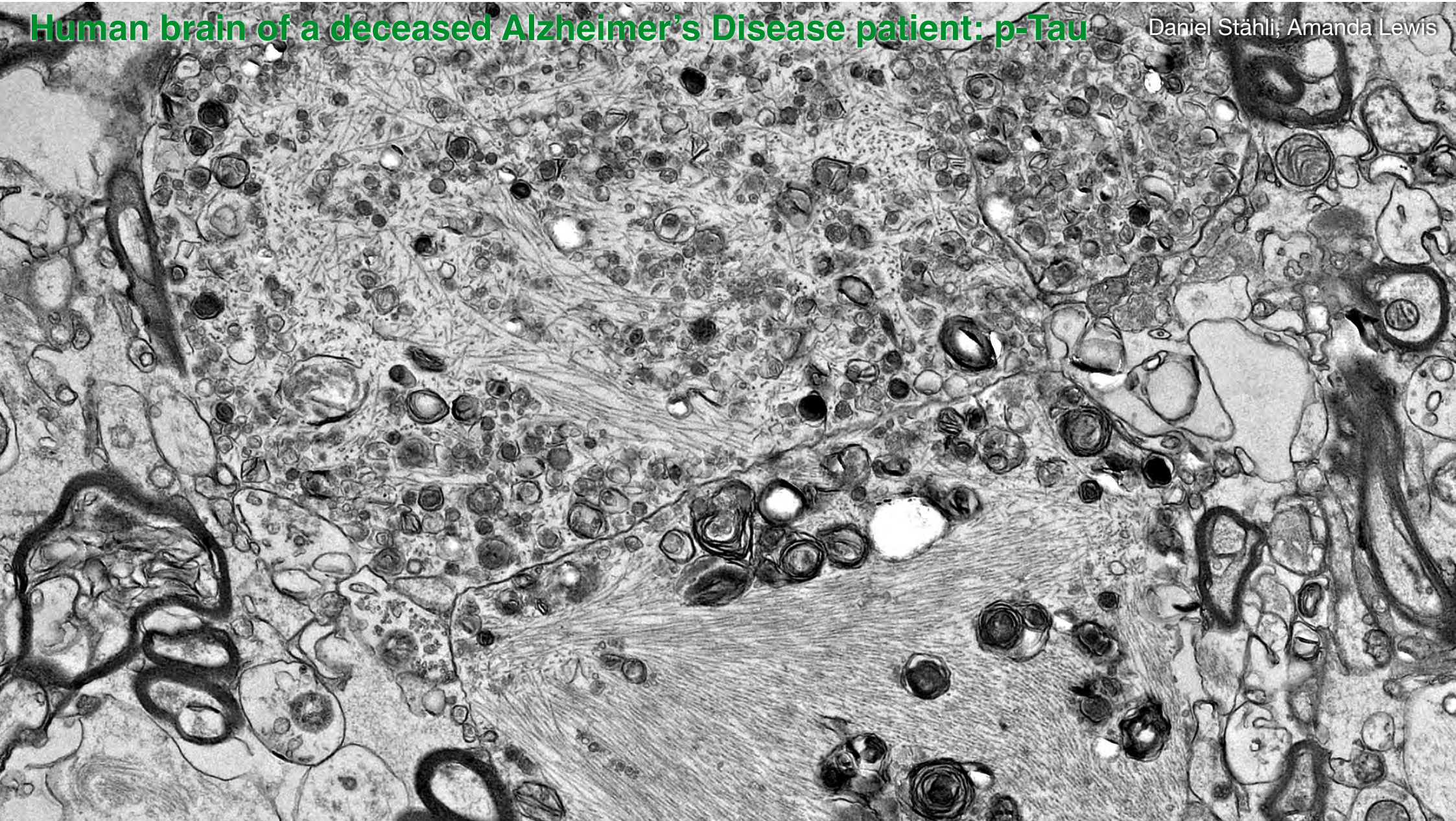
Human brain of a deceased Alzheimer's Disease patient: p-Tau

Daniel Stähli, Amanda Lewis

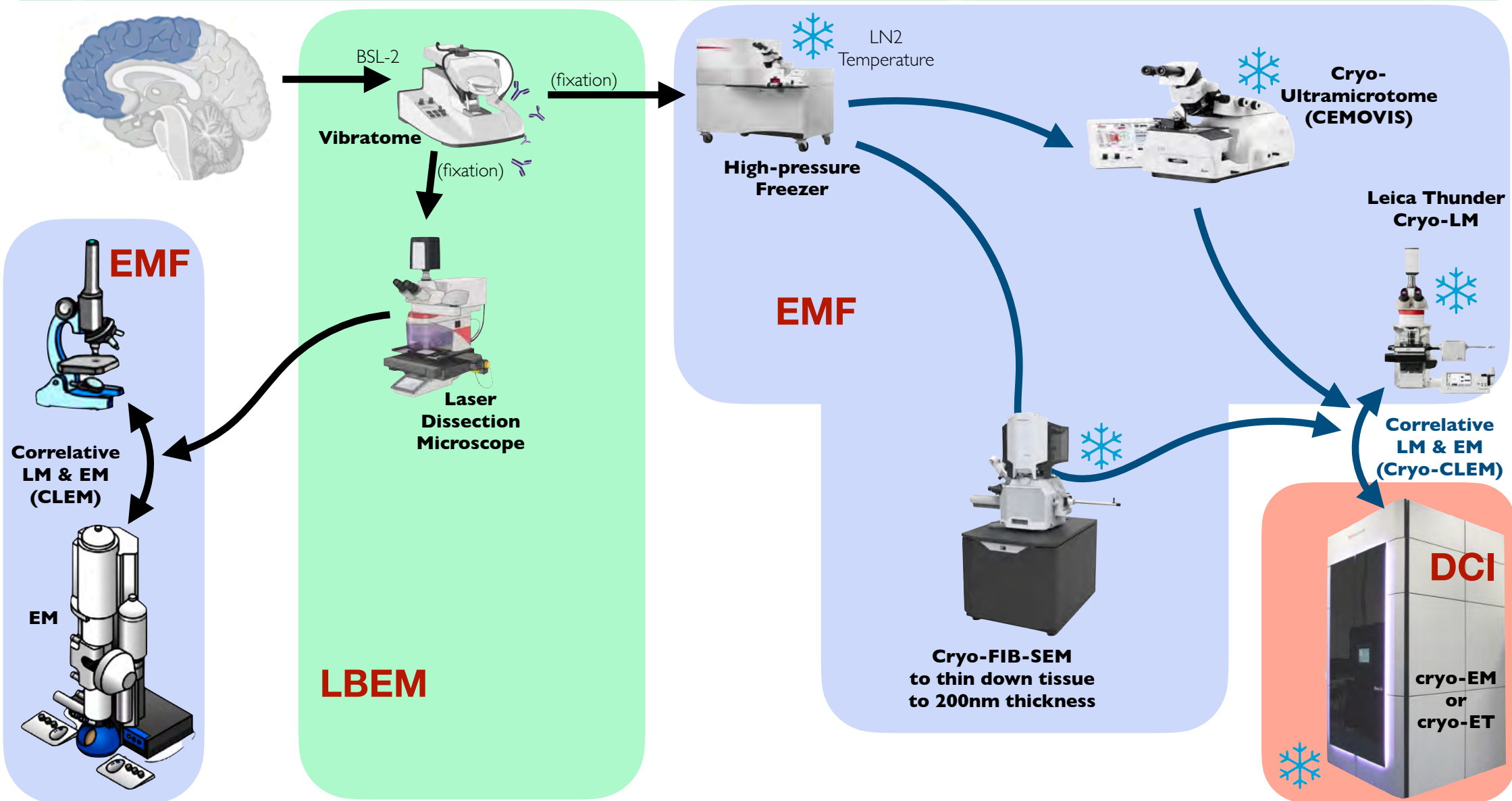


Human brain of a deceased Alzheimer's Disease patient: p-Tau

Daniel Stähli, Amanda Lewis



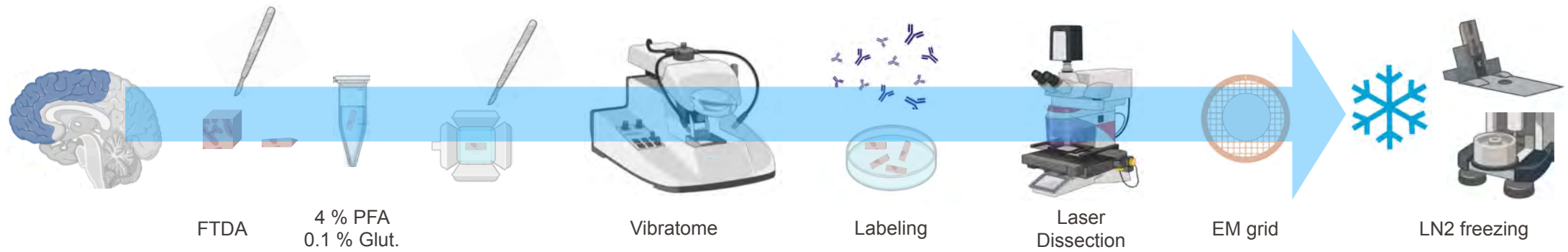
Cryo-EM of the human brain suffering from neurodegeneration



**Cryo-FIB-SEM
of high-pressure frozen
human brain**

Cryo-FIB-SEM of human brain

Meltem Tatli et al.



Cryo-Light
Microscopy
(Leica Thunder)

Cryo-FIBSEM
thinning

Inside of cryo-
FIBSEM

Milling/
Thinning

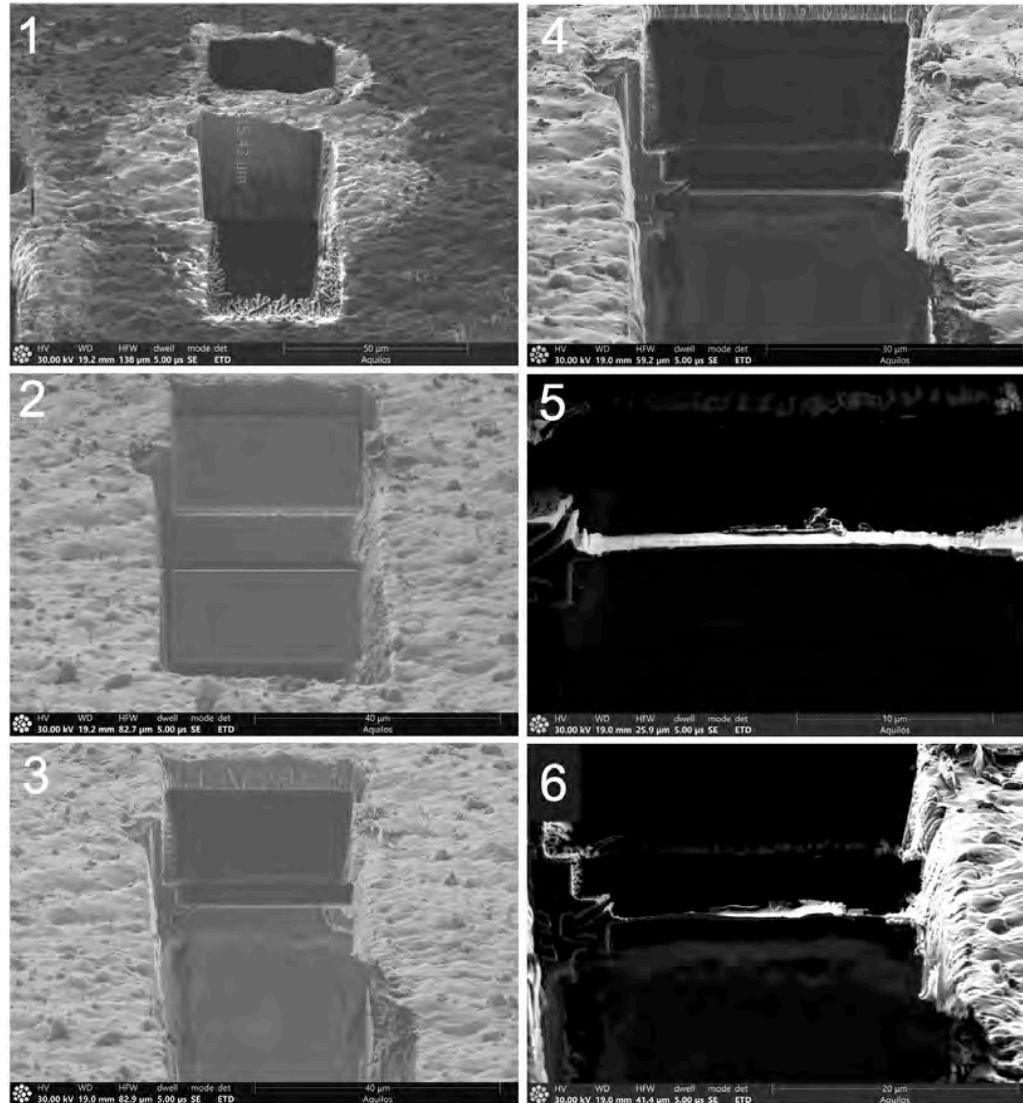


Cryo-FIB-SEM of human brain

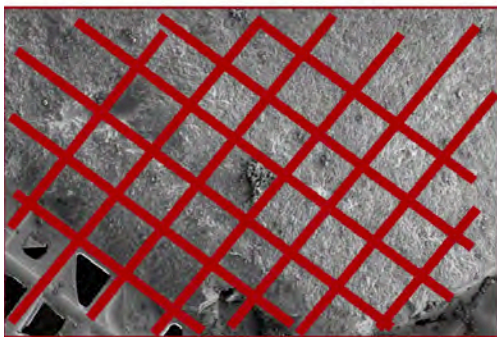
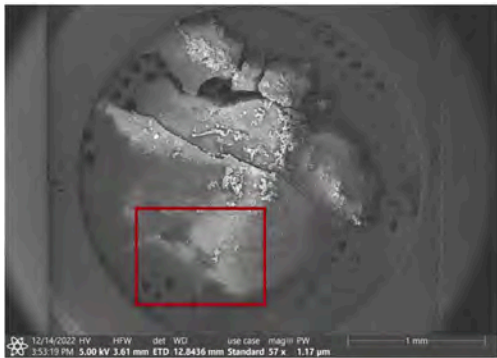
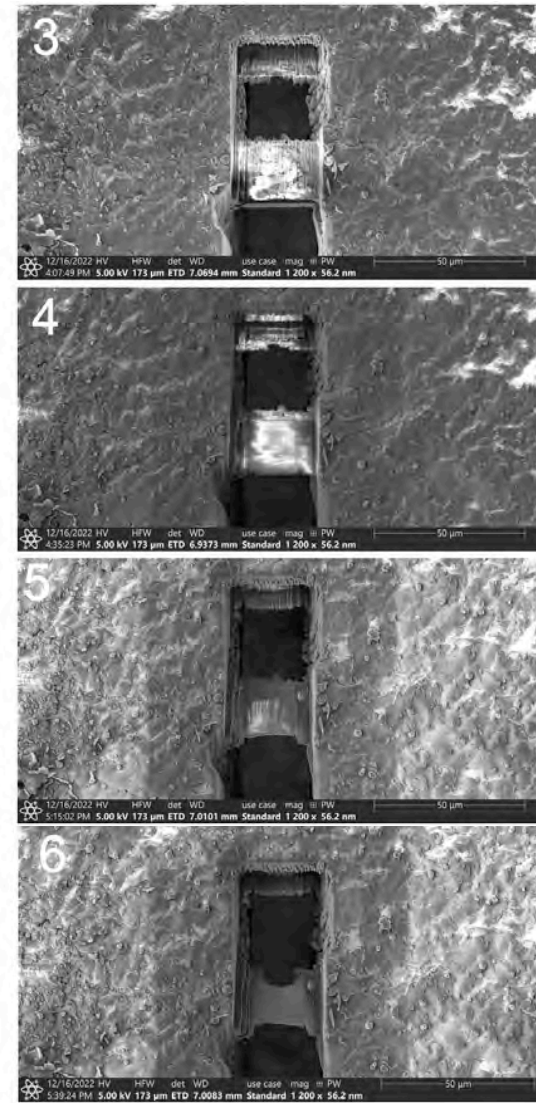
Meltem Tatli et al.



FIB (side-view)



SEM (top-view)



Cryo-FIB-SEM of human brain

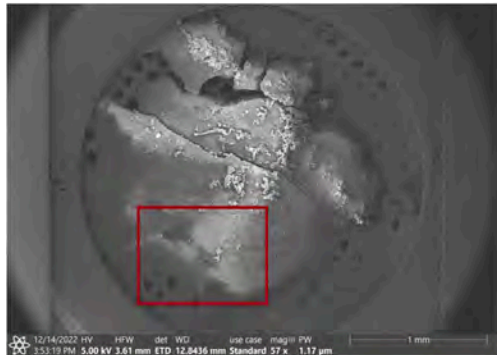
Frontotemporal lobar degeneration (FTLD) A

Meltem Tatli et al.

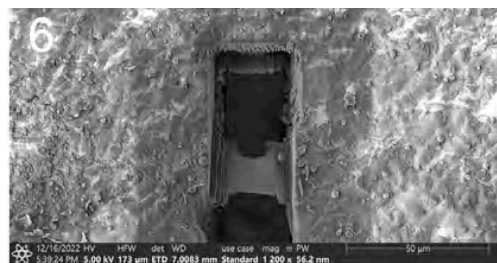
Cryo-Electron Tomography (@ DCI) with 3D reconstruction



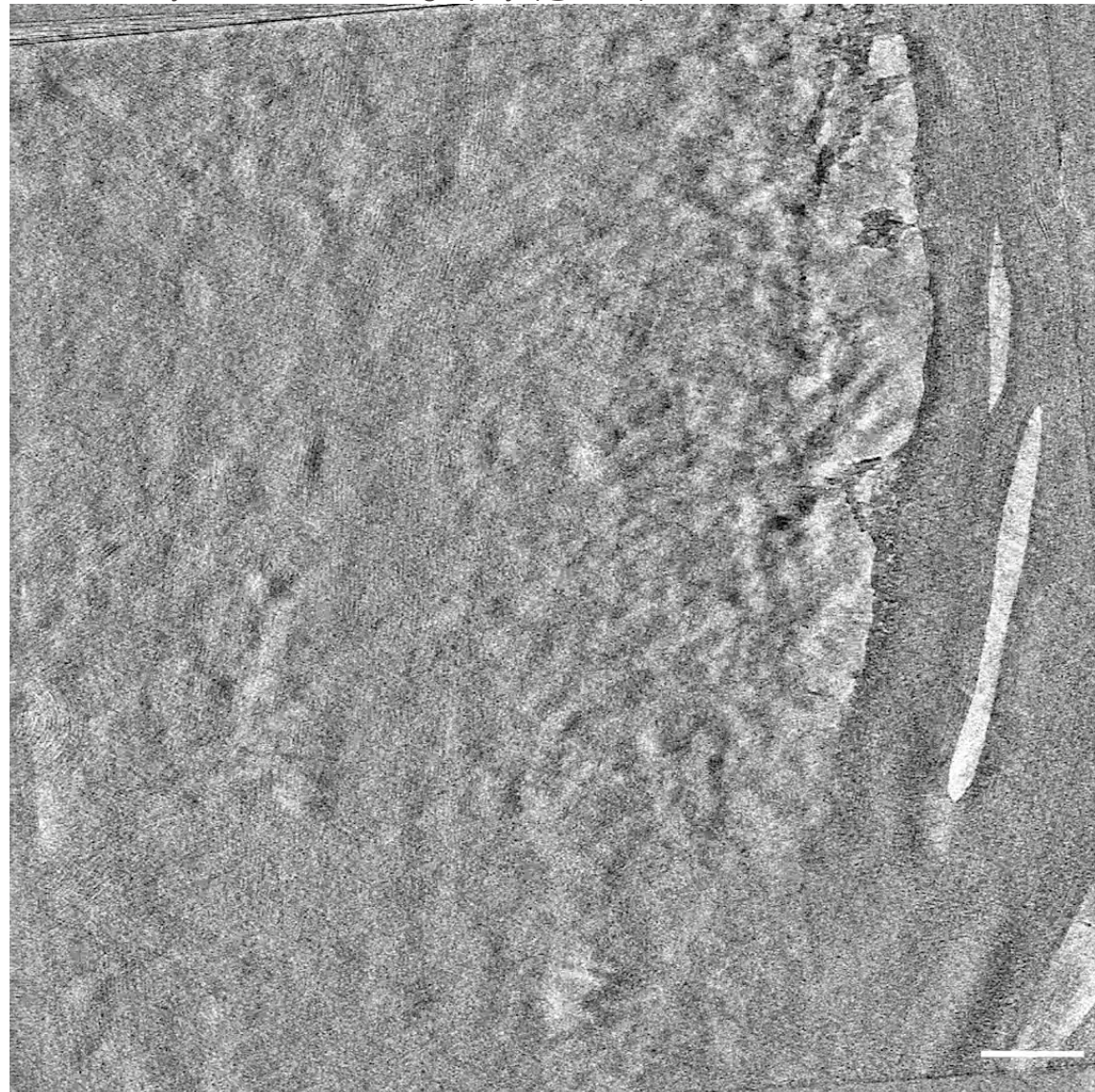
Aquilos2 FIB-SEM @ EMF



High-pressure frozen human brain on EM grid

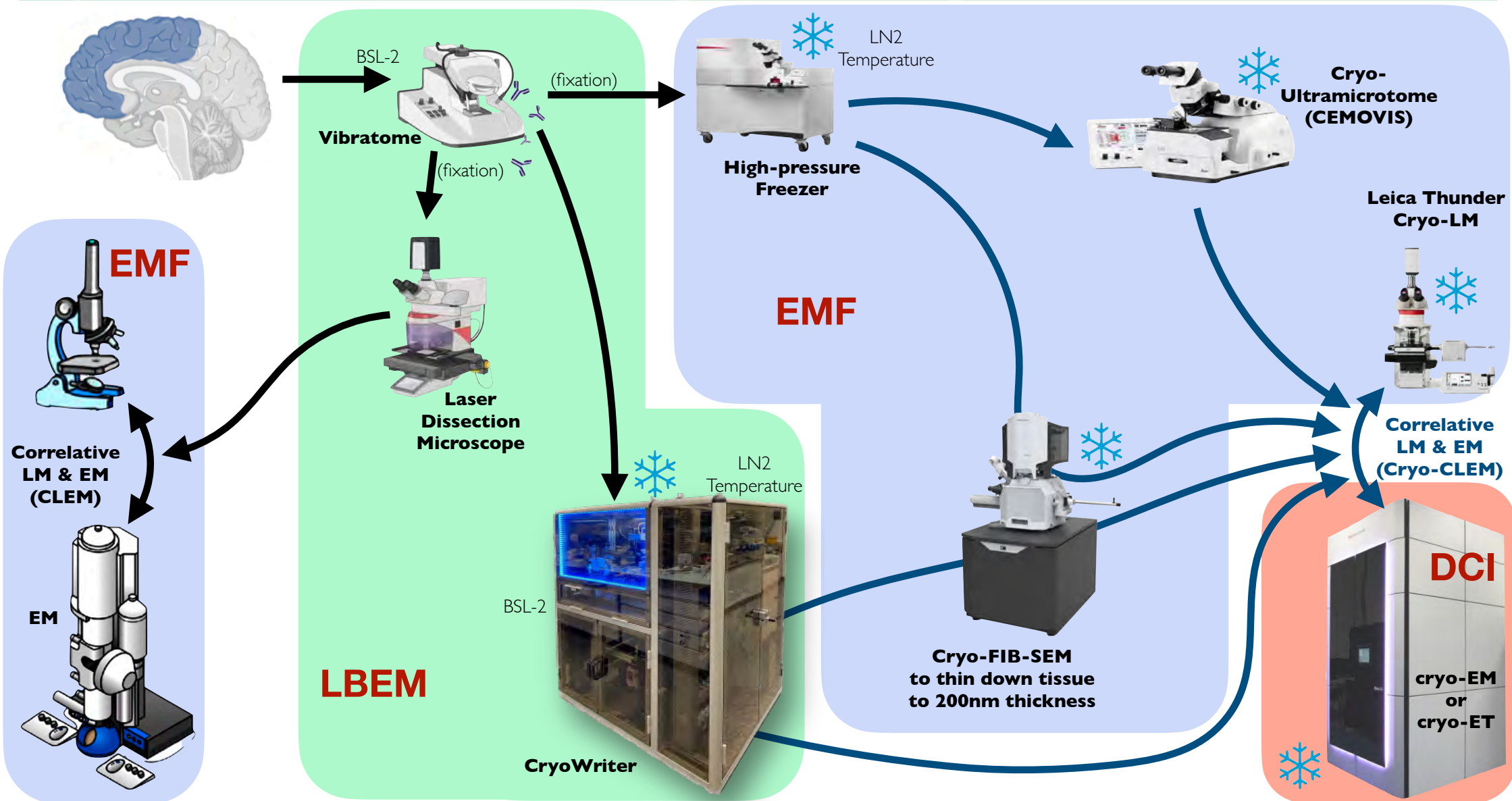


200nm lamella of frozen human brain



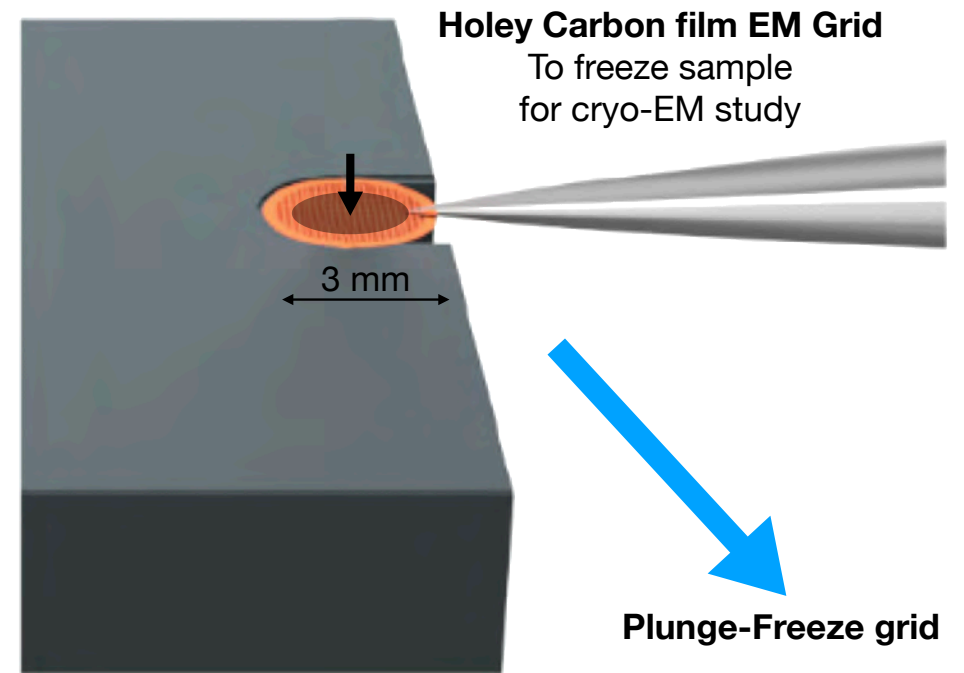
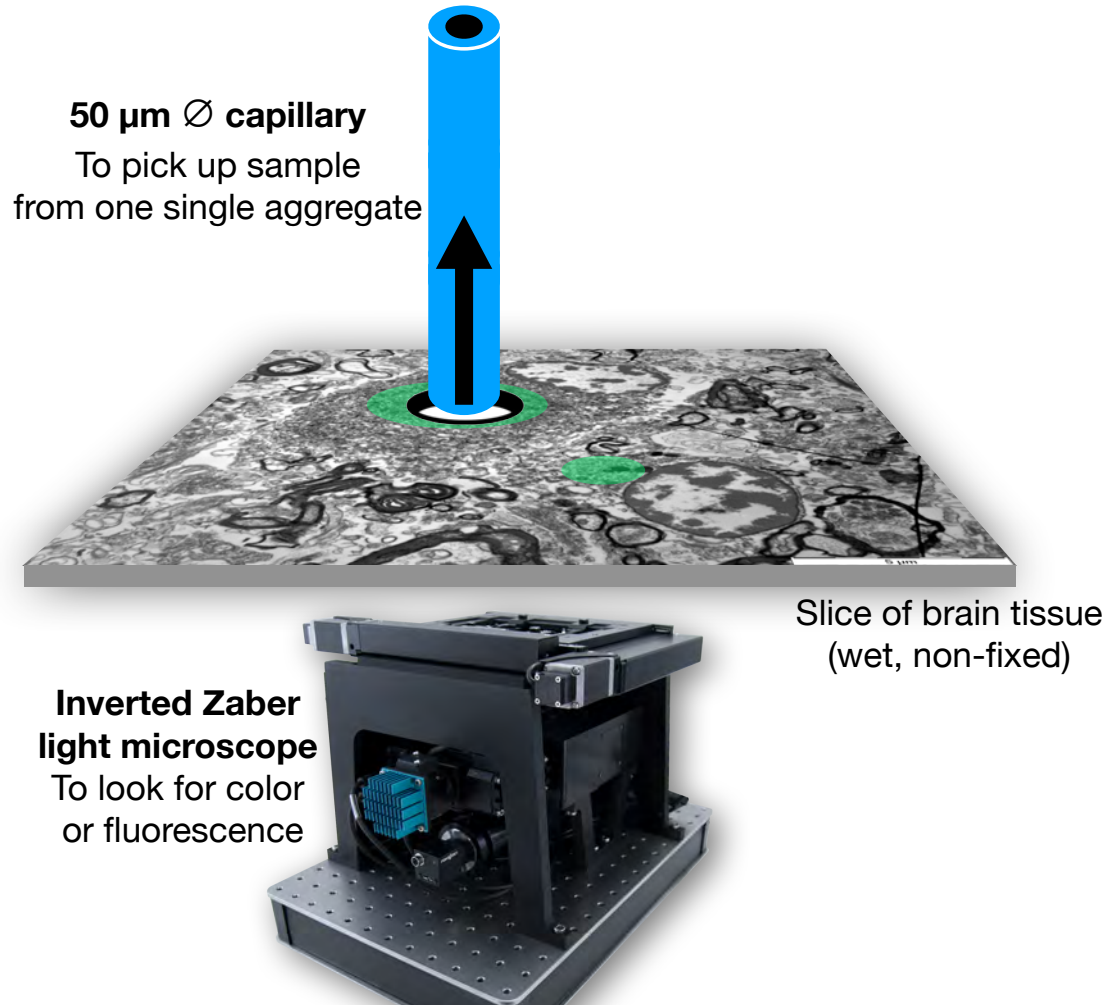
TDP-43
fibrils

Cryo-EM of the human brain suffering from neurodegeneration



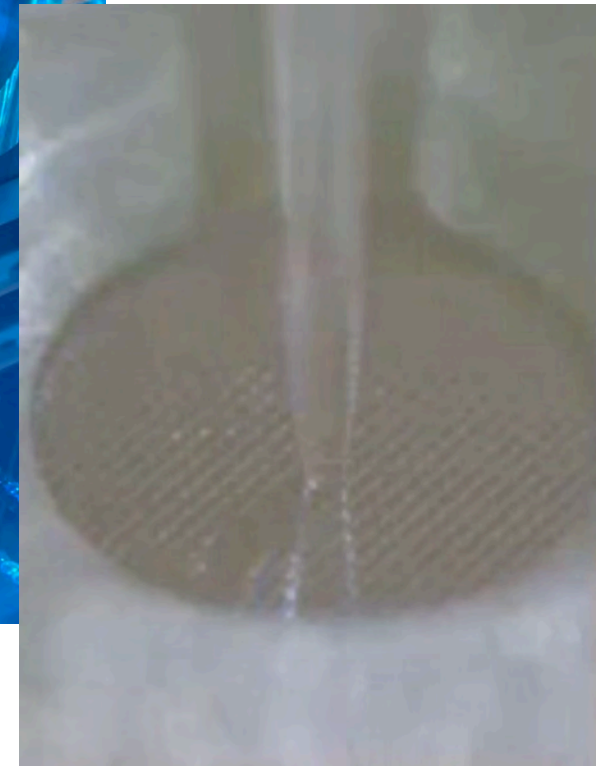
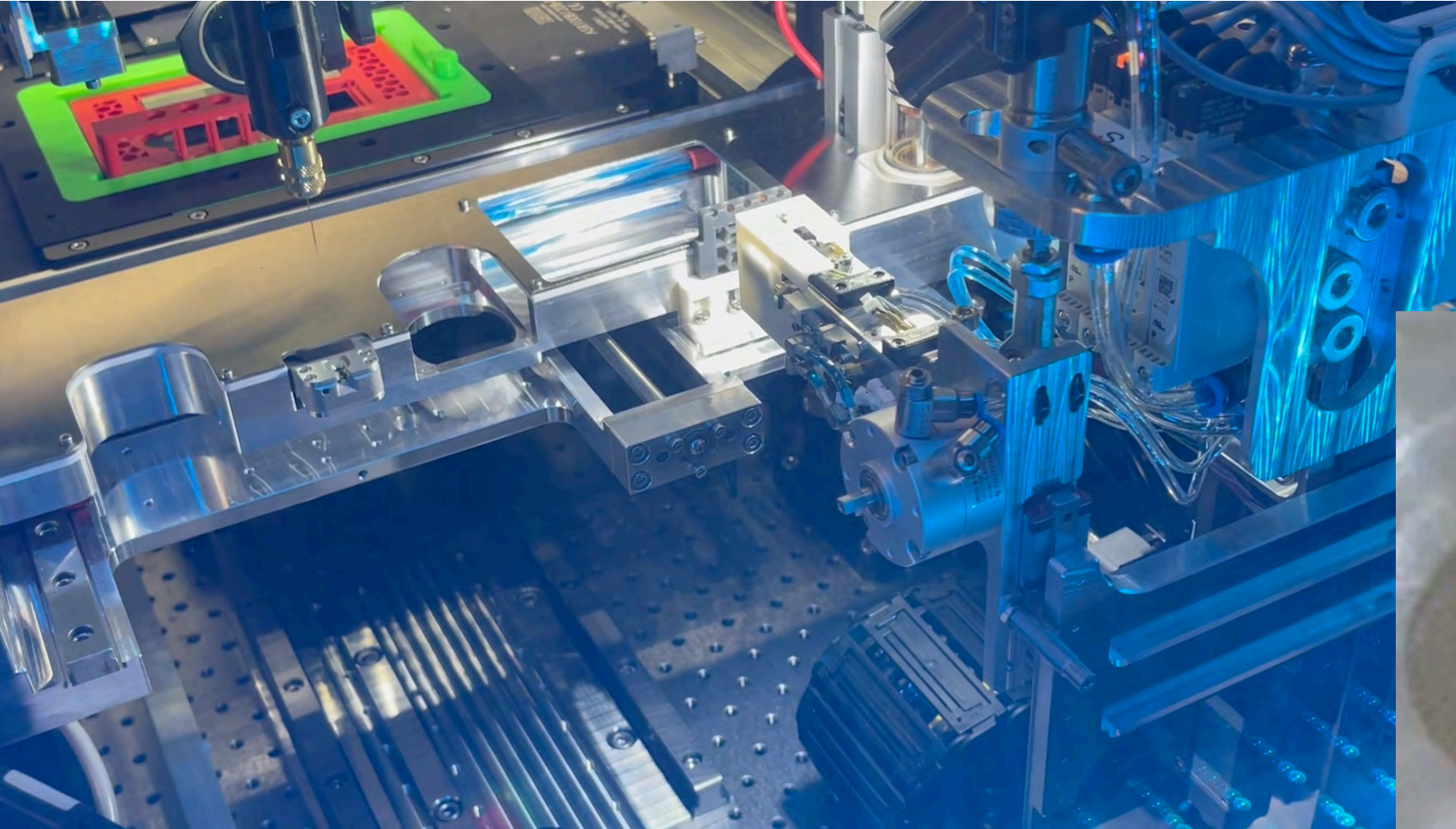
Studying fibrils from a single aggregate

The CryoWriter (CryoWrite AG, Basel, Switzerland)

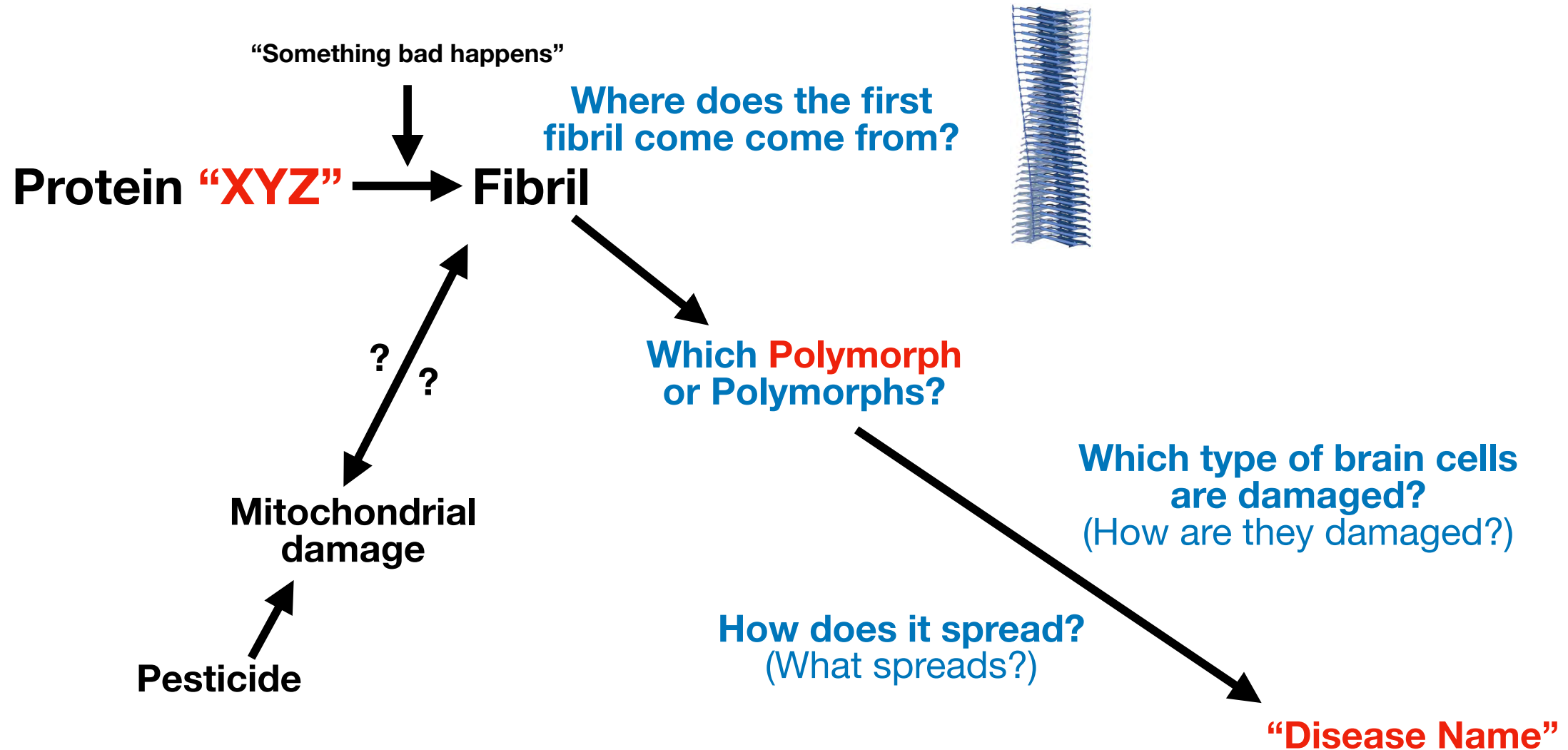


Studying fibrils from a single aggregate

The CryoWriter (CryoWrite AG, Basel, Switzerland)



Neurodegeneration: Alzheimer's, Parkinson's, DLB, PDD, MSA, ALS, FTLD, Creutzfeldt-Jakob, ...

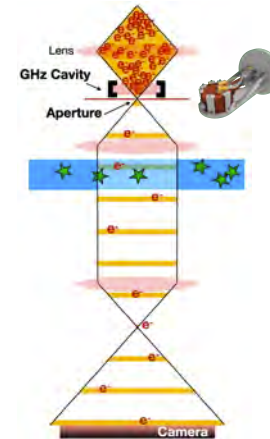


Biology: Parkinson's Disease



Physics: 4D-STEM

Stroboscopic
Single Electron Cryo-EM

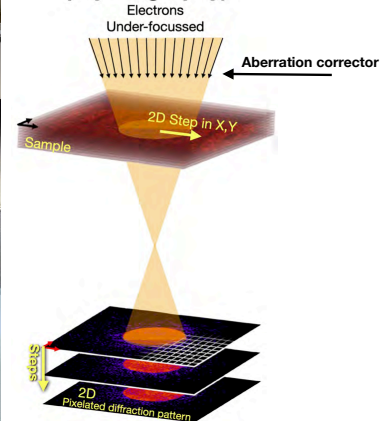


DrX.works RF Cavity
“Titan Chopper”

300kV Cold FEG



4D STEM
(Ptychography)



Cs Probe Corrector
ARINA (Dectris)

Titan Krios G4
(2022)

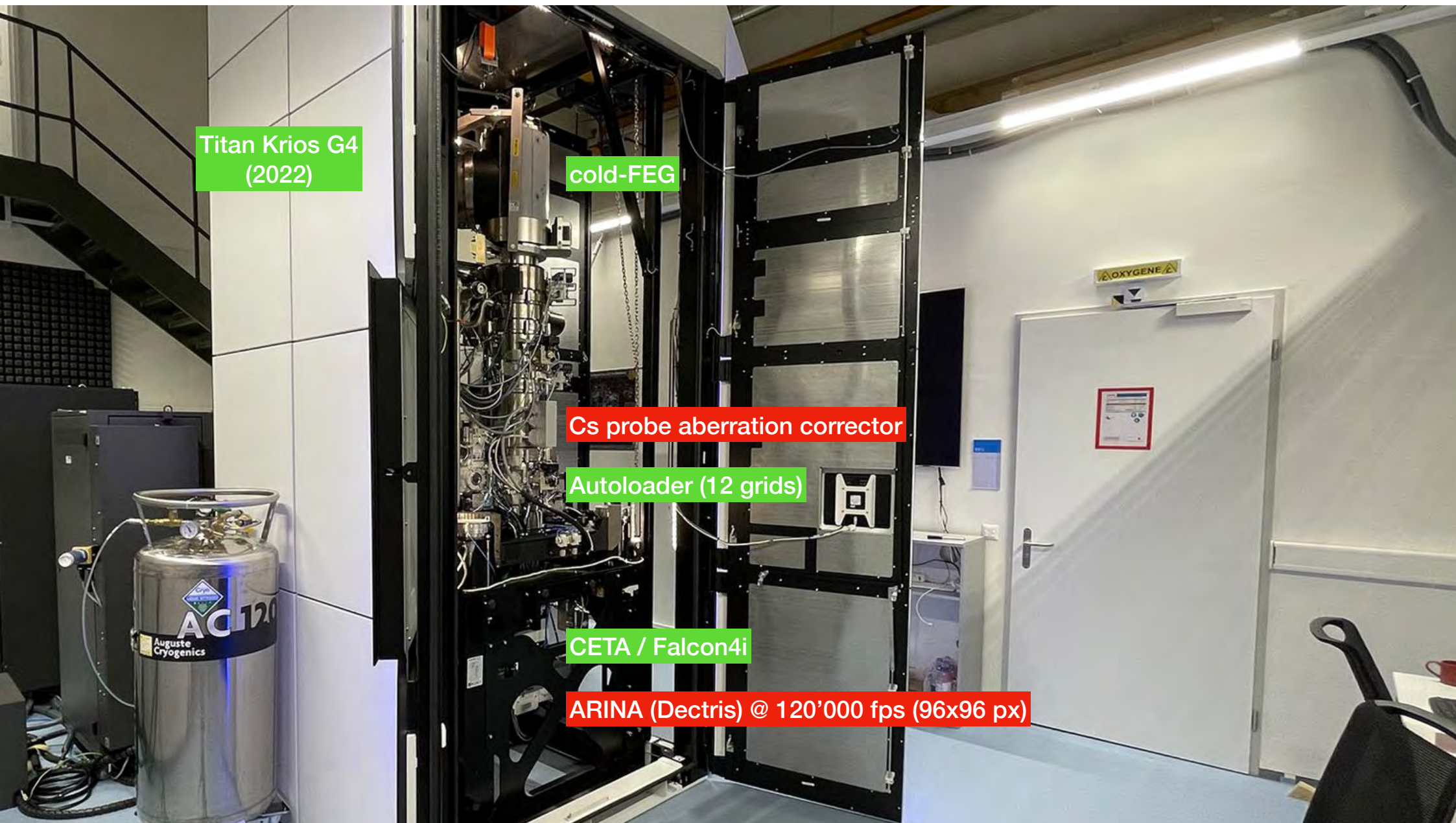
cold-FEG

Cs probe aberration corrector

Autoloader (12 grids)

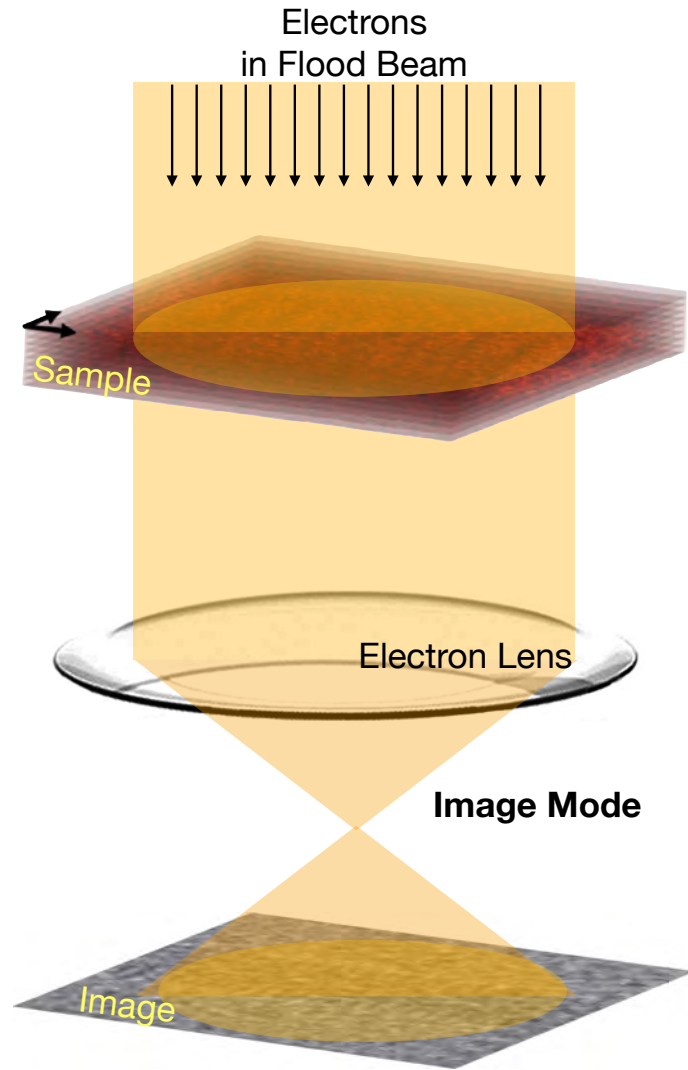
CETA / Falcon4i

ARINA (Dectris) @ 120'000 fps (96x96 px)

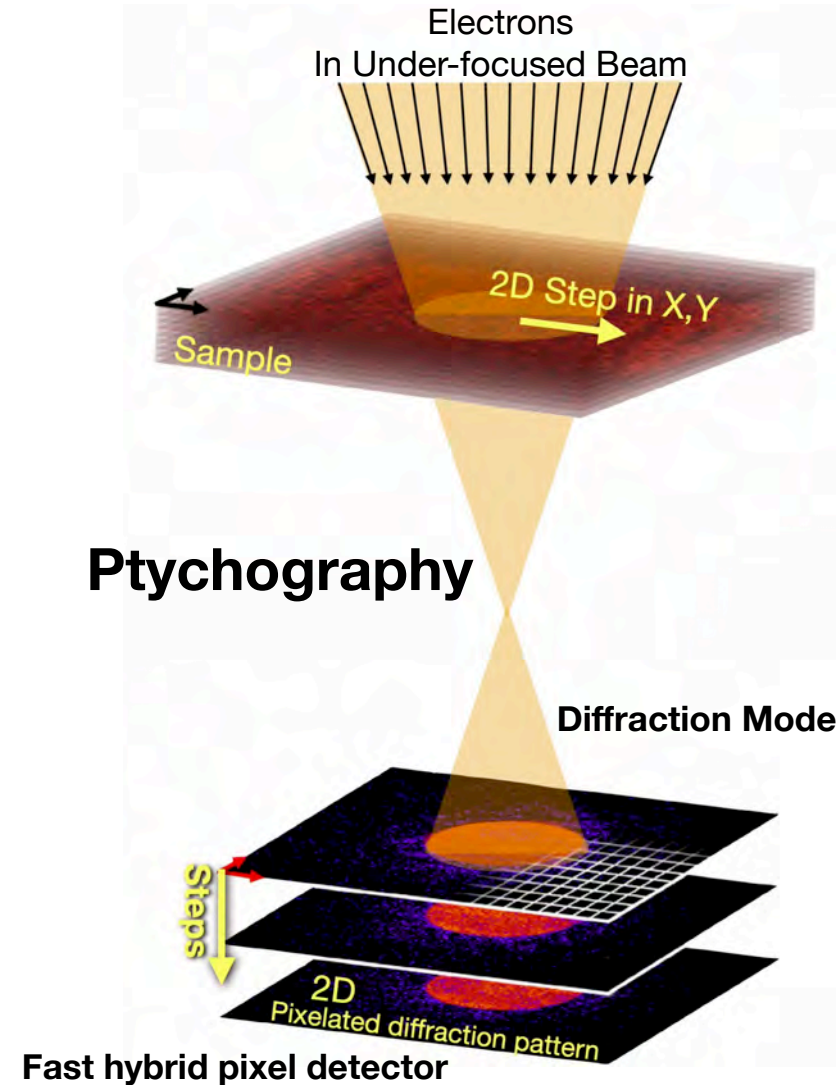


Cryo-Electron Microscopy of Proteins

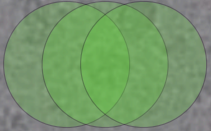
Cryo-TEM



4D-STEM



4D STEM in Life Sciences

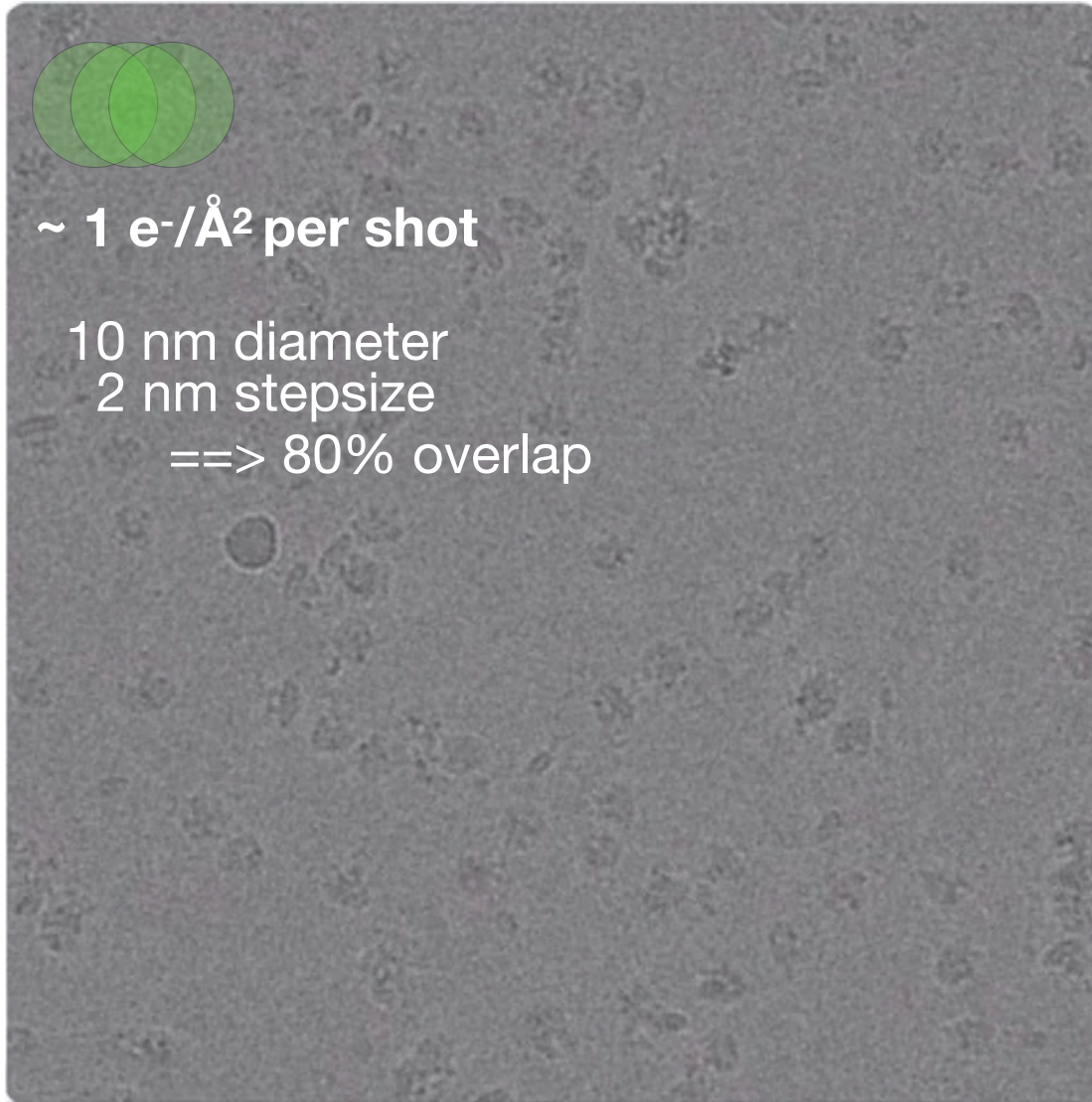


$\sim 1 \text{ e}/\text{\AA}^2$ per shot

10 nm diameter

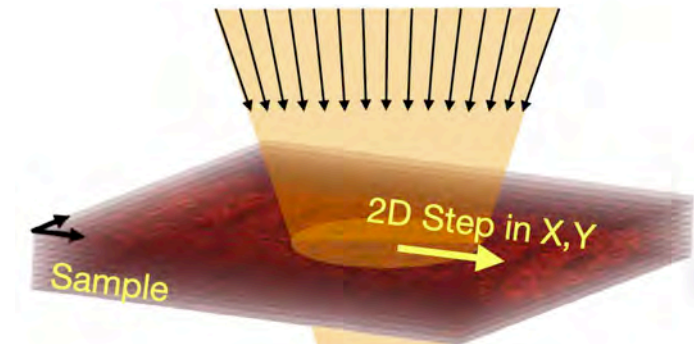
2 nm stepsize

$\Rightarrow$ 80% overlap



4D STEM (Ptychography)

Electrons
In Under-focused Beam

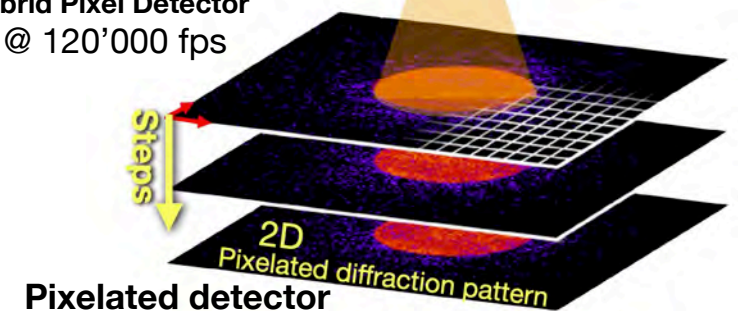


Sample

2D Step in X,Y

Arina
Hybrid Pixel Detector
@ 120'000 fps

Diffraction Mode



Steps

2D
Pixelated diffraction pattern

Pixelated detector

4D STEM in Life Sciences

See also: Zhou et al., Nat. Comm. (2020)11:2773



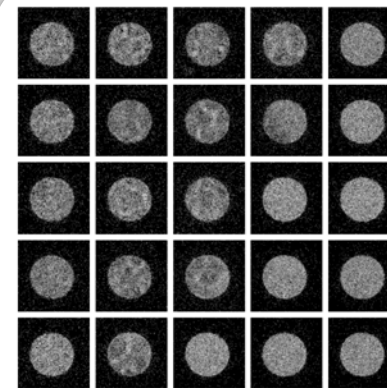
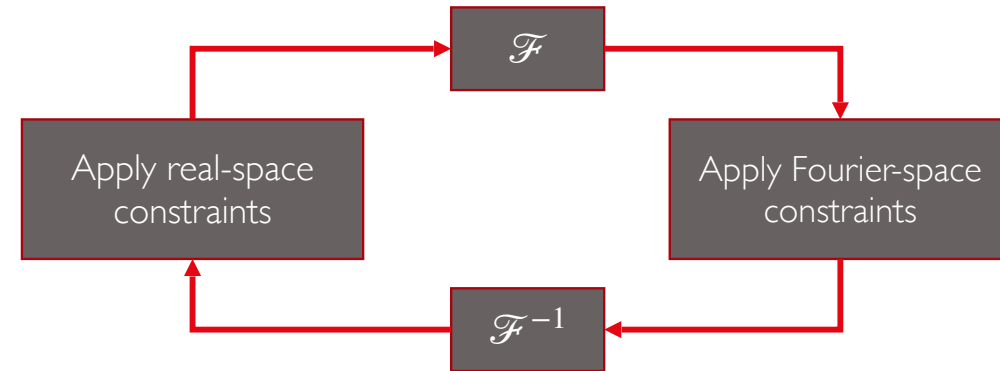
$\sim 1 \text{ e}/\text{\AA}^2$ per shot

10 nm diameter

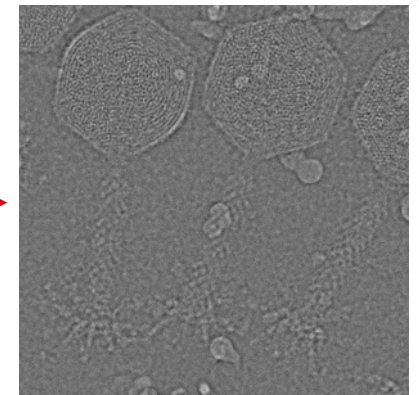
2 nm stepsize

$\Rightarrow$ 80% overlap

Software: Ptychography



Set of diffraction patterns



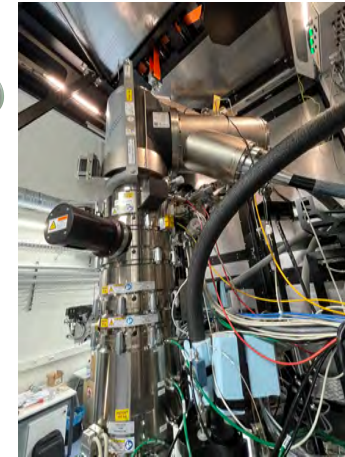
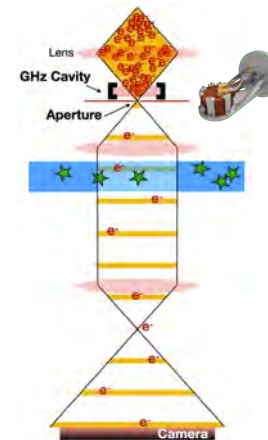
Reconstructed micrograph

Biology: Parkinson's Disease

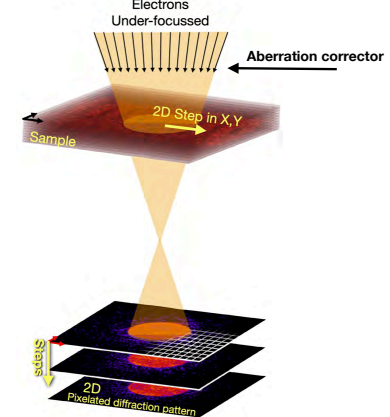


Physics: Improving Cryo-EM

Stroboscopic
Single Electron Cryo-EM



4D STEM
(Ptychography)



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