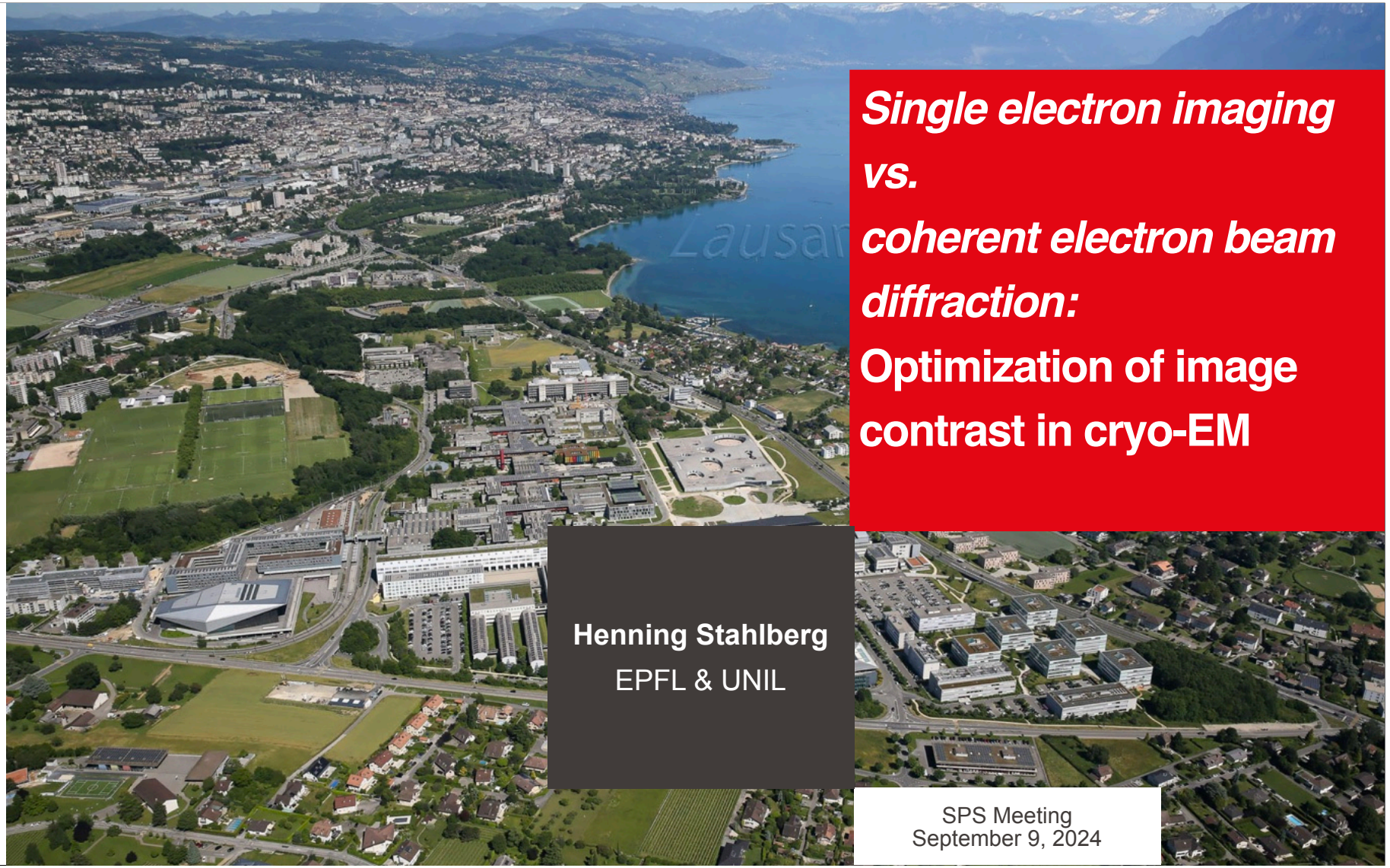
EPFL

UNIL | Université de Lausanne

Laboratory of Biological Electron Microscopy

LBEM



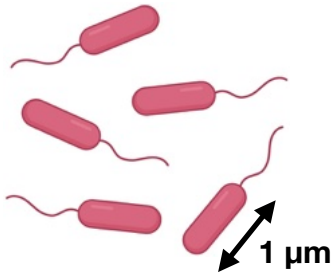
Single electron imaging vs. coherent electron beam diffraction: Optimization of image contrast in cryo-EM

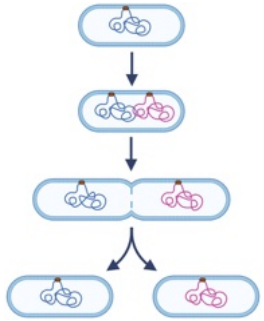
Henning Stahlberg
EPFL & UNIL

SPS Meeting
September 9, 2024

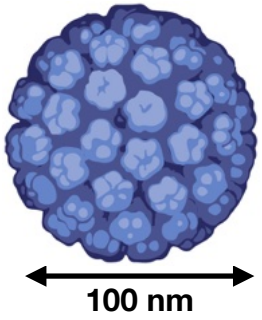
Bacteria, Viruses, and Prions

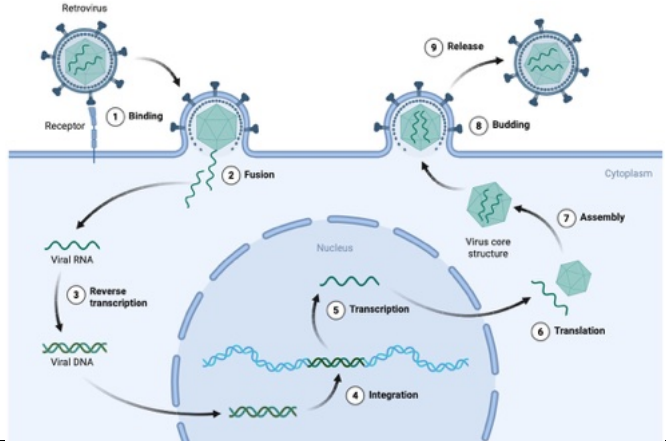
Bacteria



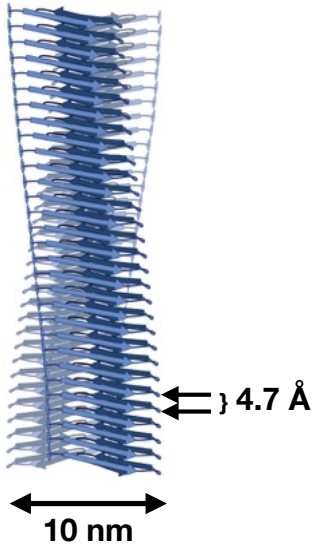


Viruses



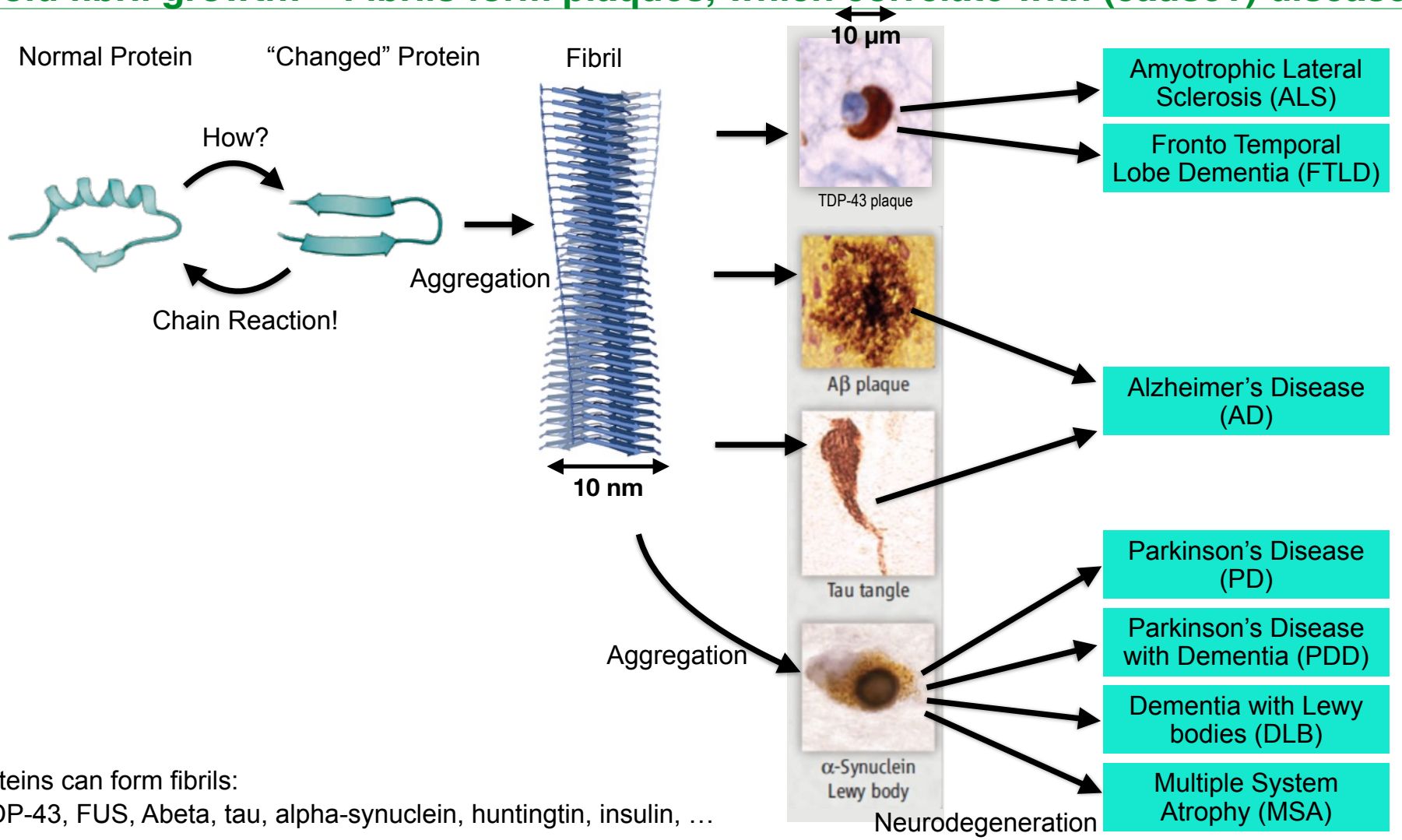


Fibrils



Created with BioRender.com

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

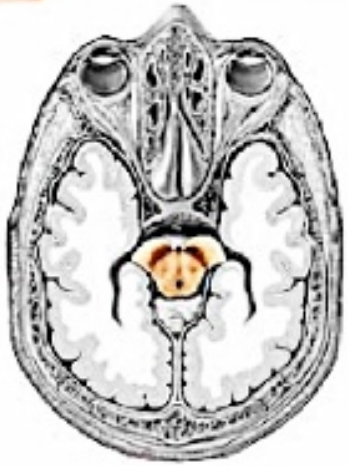


Many proteins can form fibrils:
PrP^{SC}, TDP-43, FUS, Abeta, tau, alpha-synuclein, huntingtin, insulin, …

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

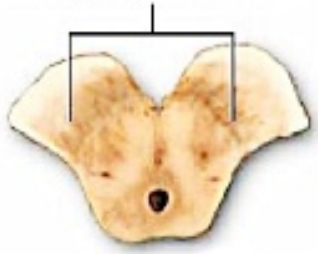
https://www.youtube.com/watch?v=wZZ4Vf3HinA: 19.4.2017

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

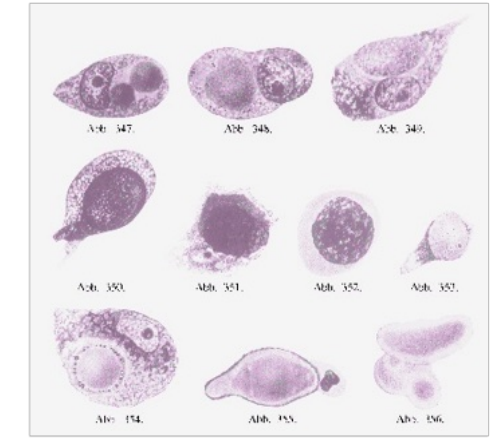


Substantia nigra

Diminished substantia nigra as seen in Parkinson’s disease



Friedrich Heinrich Lewy
(1885 – 1950)

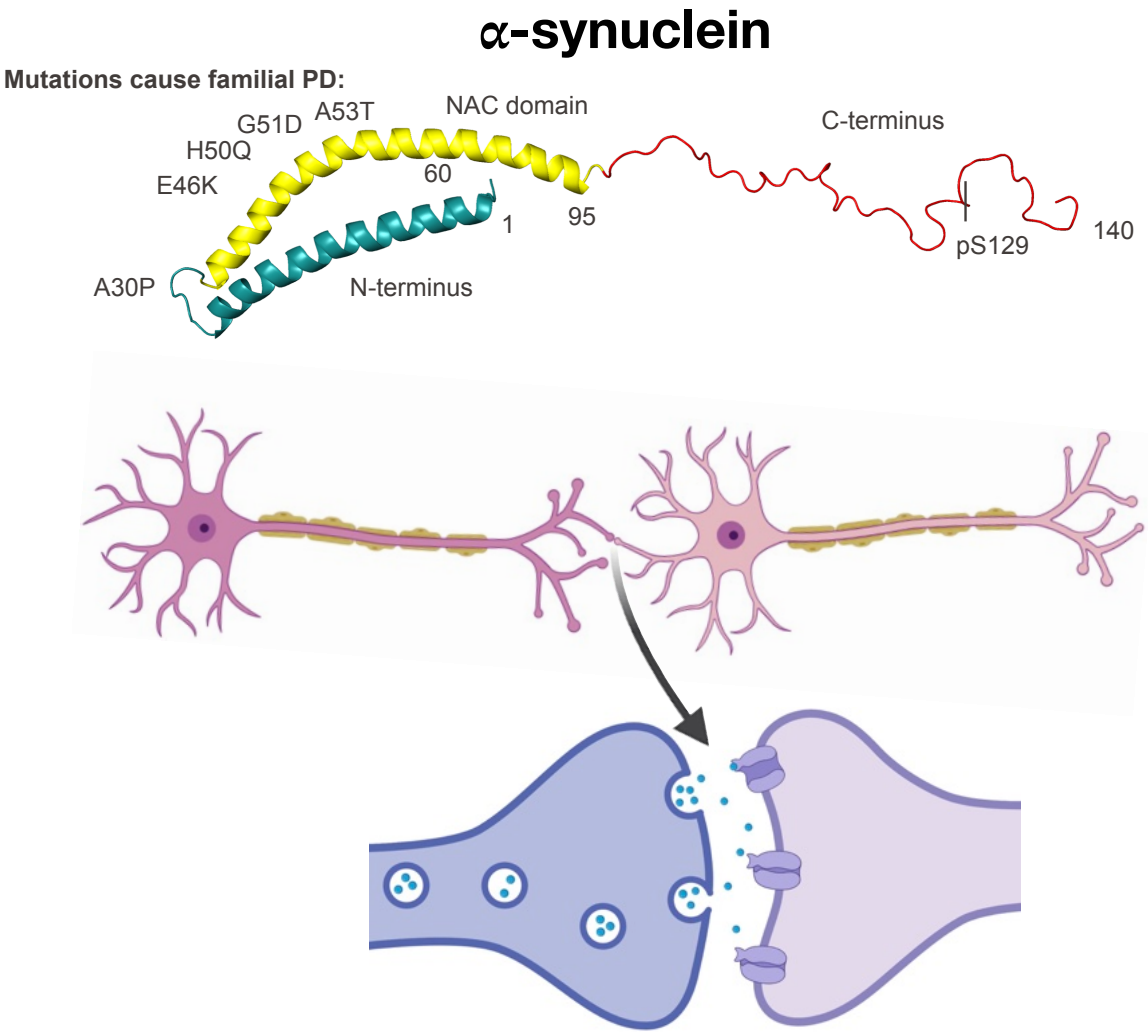


A figure from Lewy's *Tonus und Bewegung* (1923), showing images of Lewy bodies

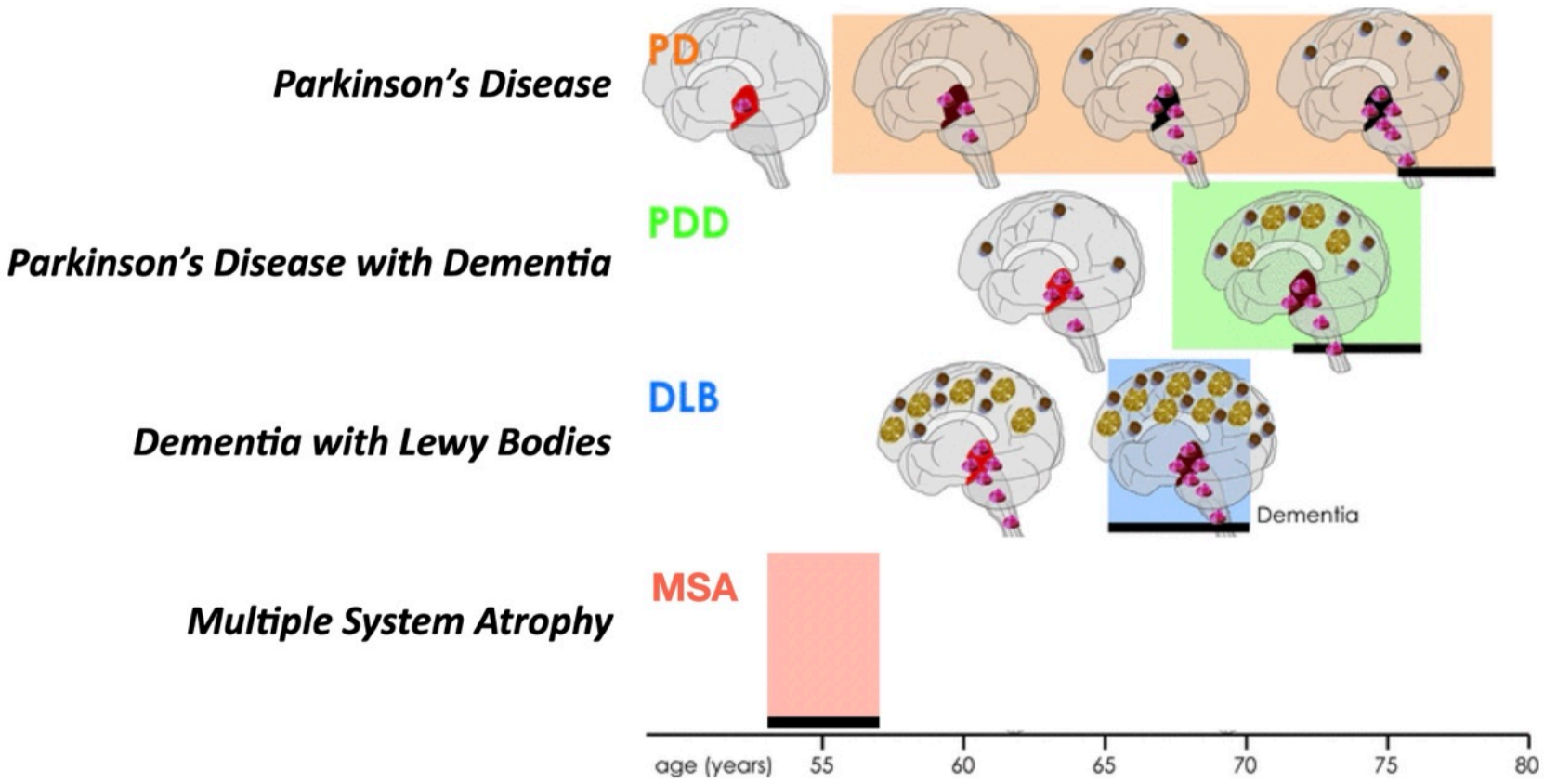
“Lewy” bodies in the brain.
These are full of alpha-synuclein.

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



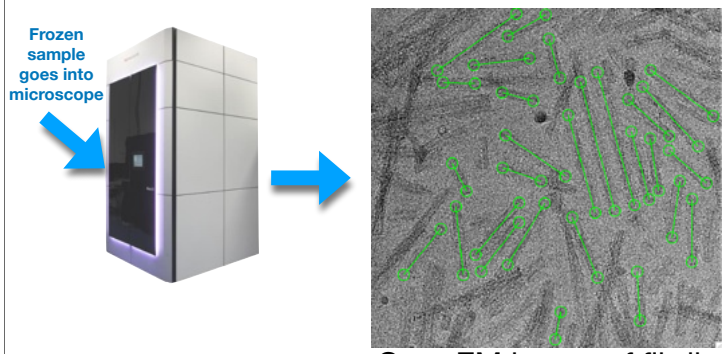
Other diseases related to aSyn fibrils

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

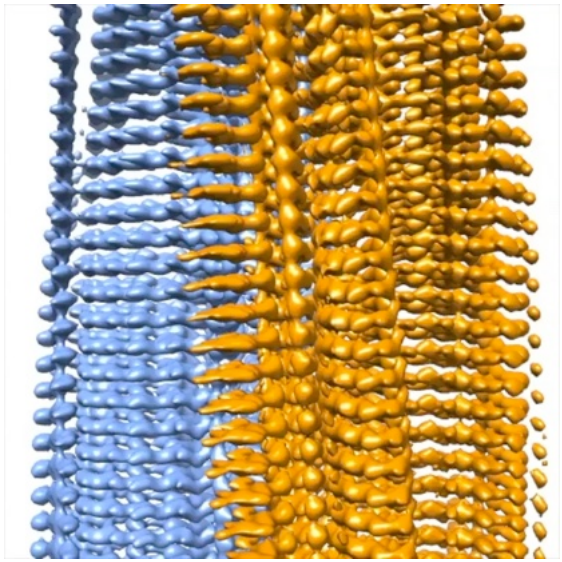
Modified from: Halliday & McCann, Annals of the New York Academy of Sciences 1184(1), 188-195 (2010)

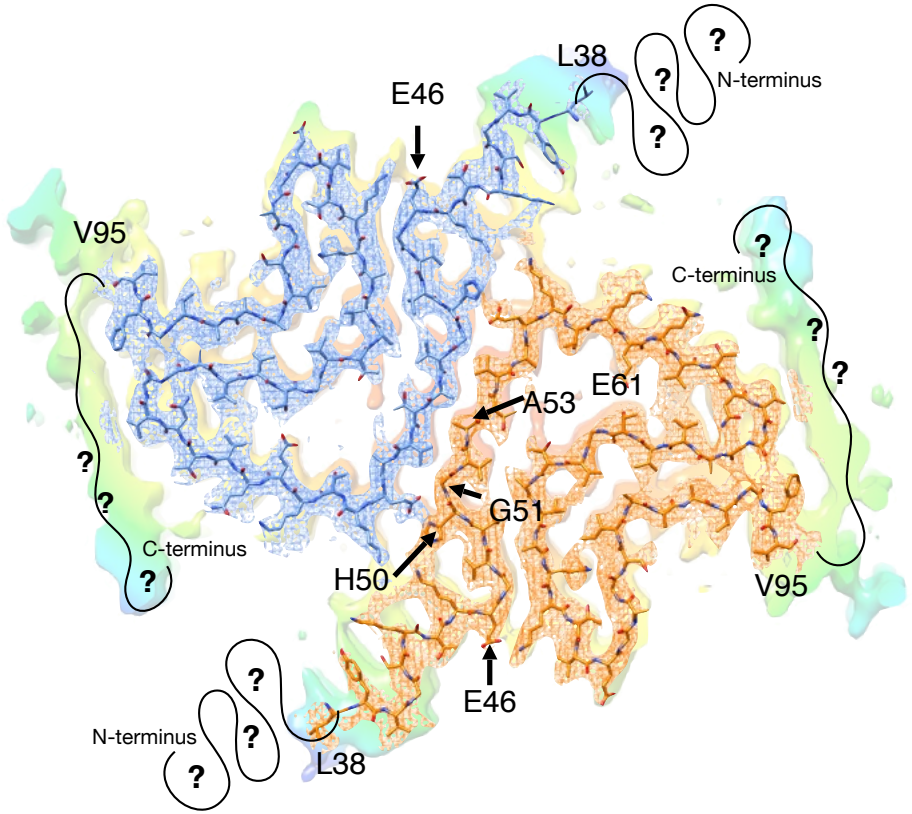
In vitro generated aSyn fibrils

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



Cryo-EM image of fibrils
(10'000 fibril images are needed)



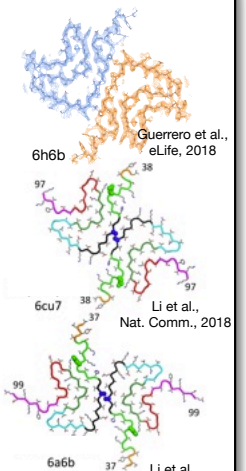


Guerrero-Ferreira et al., eLife (2018, 2019)

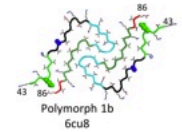
Alpha-Synuclein Fibril Polymorphism

In vitro, wt

Polymorph 1a



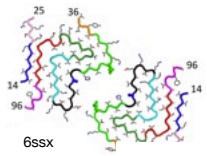
Polymorph 1b



Li et al., Nat. Comm., 2018

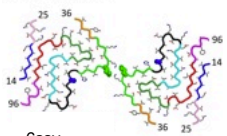
In vitro, wt

Polymorph 2a



Guerrero et al., eLife, 2019

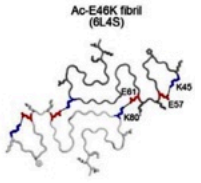
Polymorph 2b



Guerrero et al., eLife, 2019

In vitro, Ac-E46K

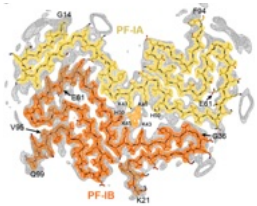
Polymorph 3a



Zhao et al., Nat. Comm. 11, 2020

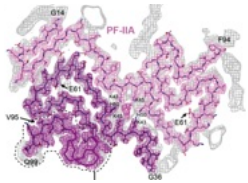
*MSA **brain** purified*

Polymorph 4a



Schweighauser et al., Nature, Sept. 2020

Polymorph 4b

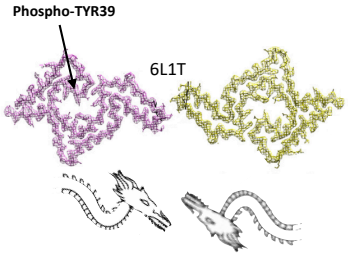


Schweighauser et al., Nature, Sept. 2020

(sarkosyl, ultrasound)

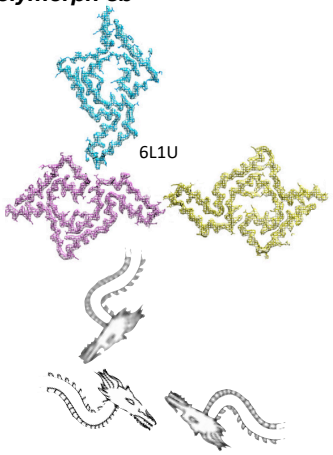
In vitro, pTYR39

Polymorph 5a



Zhao et al., PNAS 117(33), 20395 (Aug. 2020)

Polymorph 5b



Zhao et al., PNAS 117(33), 20395 (Aug. 2020)

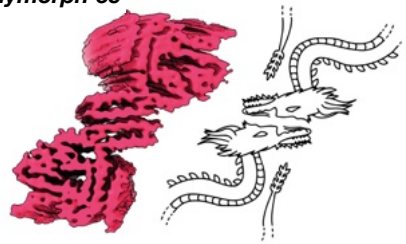
*PD & MSA **brain** seeded (PMCA)*

Polymorph 6a

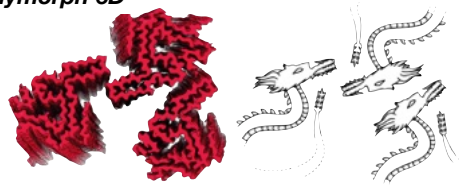
Polymorph 6b

Domenic Burger (EDNE)

Polymorph 6c



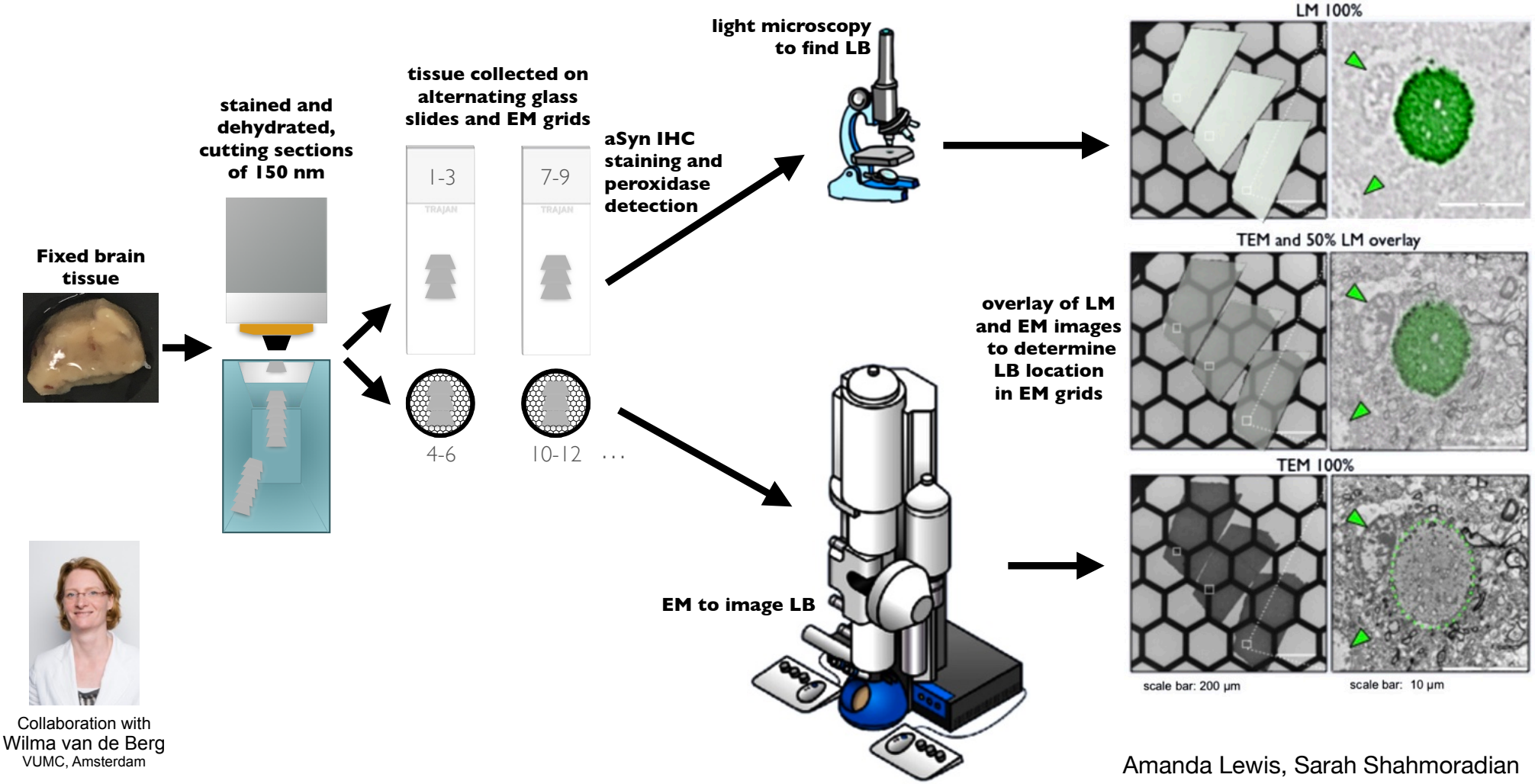
Polymorph 6D



Cryo-EM of the human brain suffering from neurodegeneration

What is the mechanism of neurodegeneration in the human brain ?

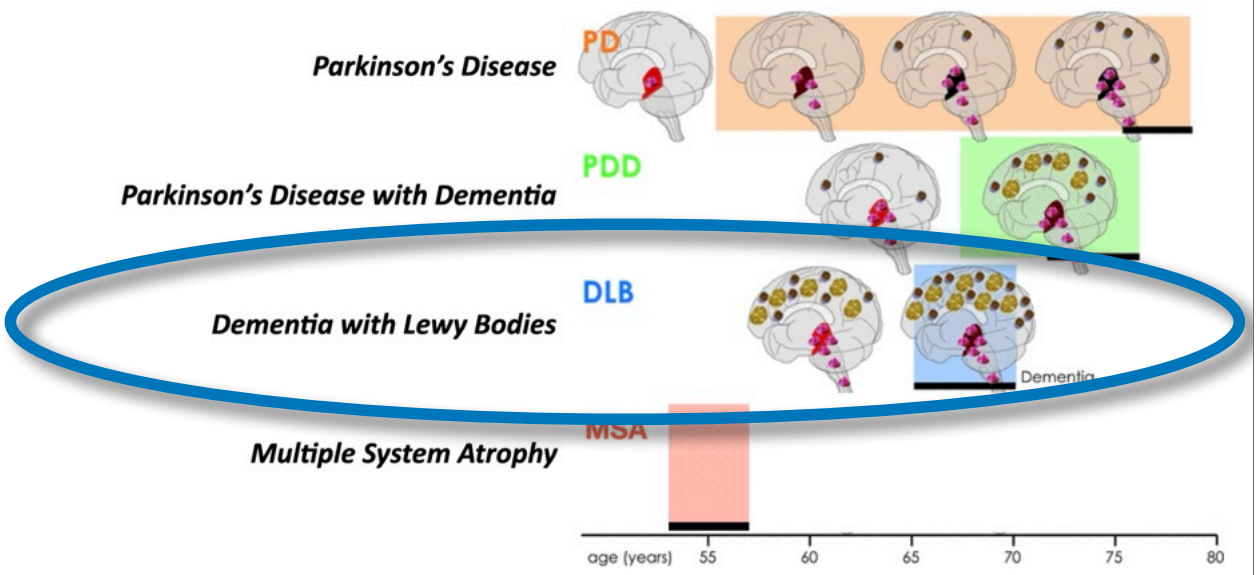
CLEM - Correlative Light and Electron Microscopy



Collaboration with
Wilma van de Berg
VUMC, Amsterdam

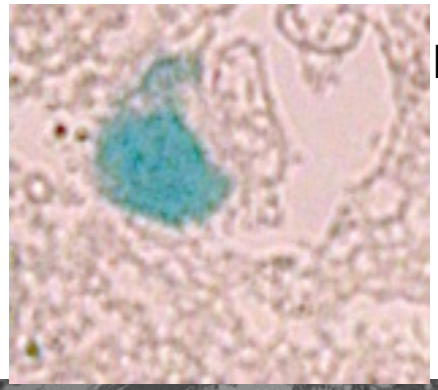
Amanda Lewis, Sarah Shahmoradian

Dementia with Lewy bodies (DLB)



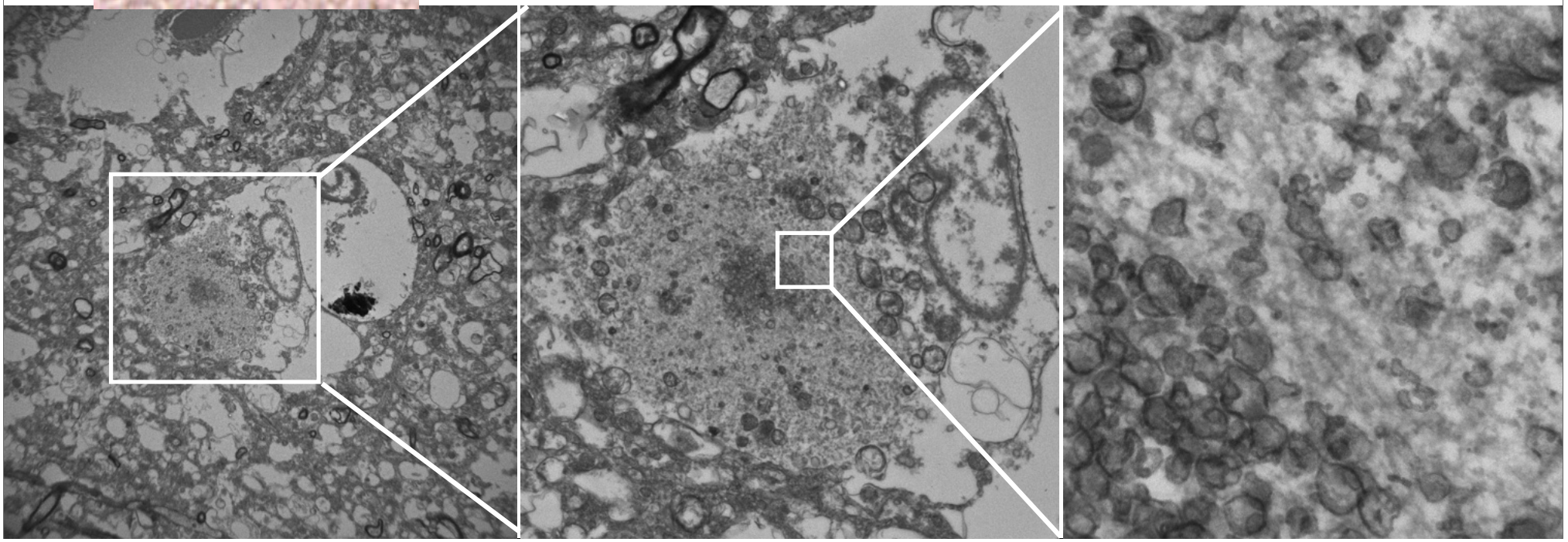
DLB: CLEM image of a Cingulate Gyrus of a DLB patient

Notash Shafiei, LBEM

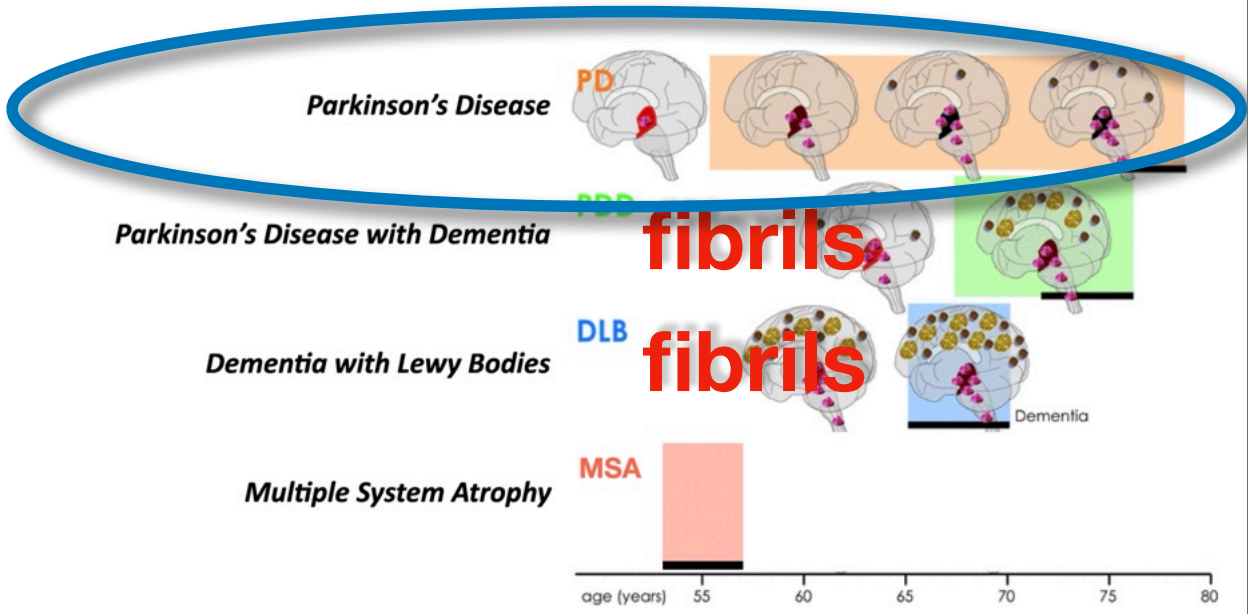


Light Microscopy

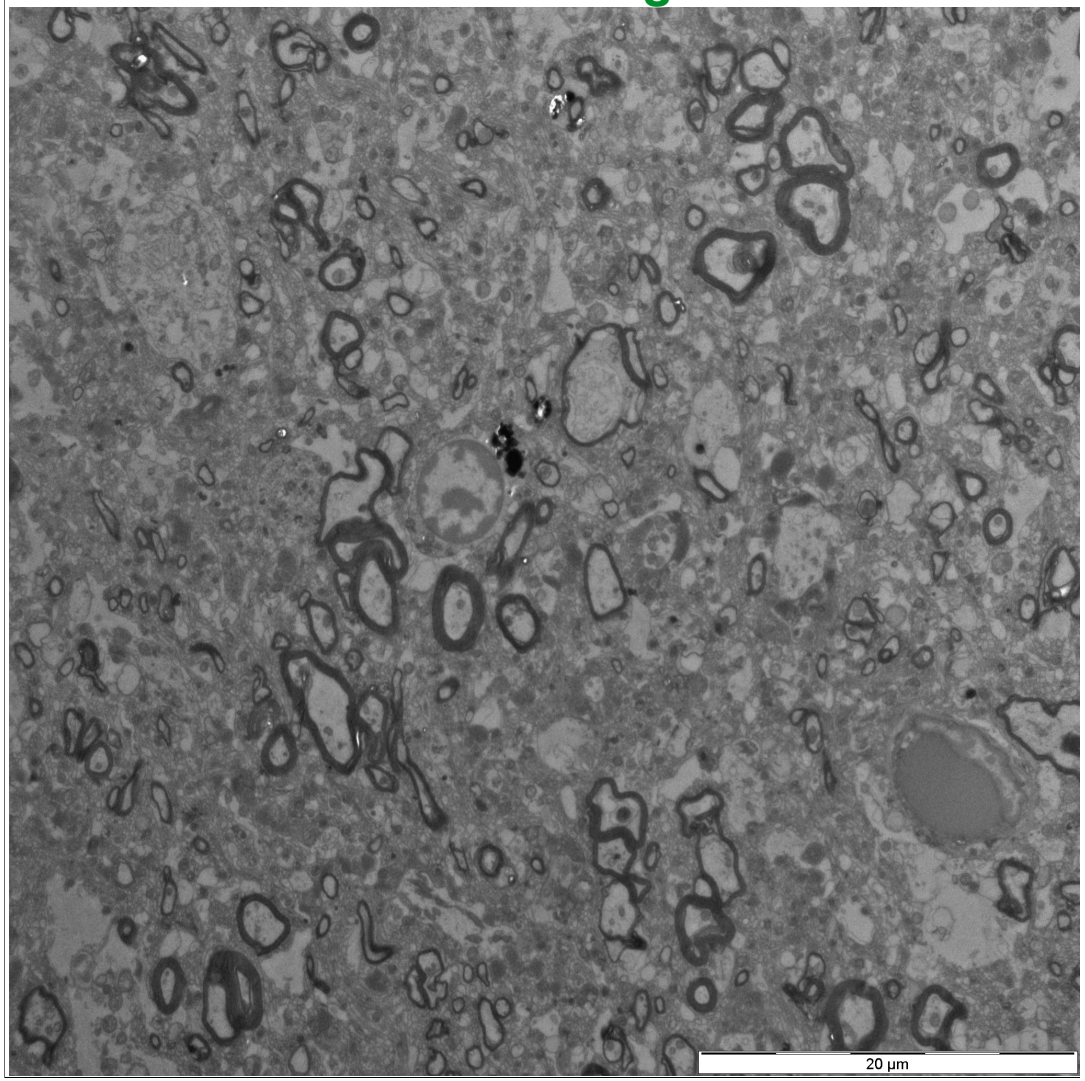
Electron Microscopy



Parkinson's Disease (PD)



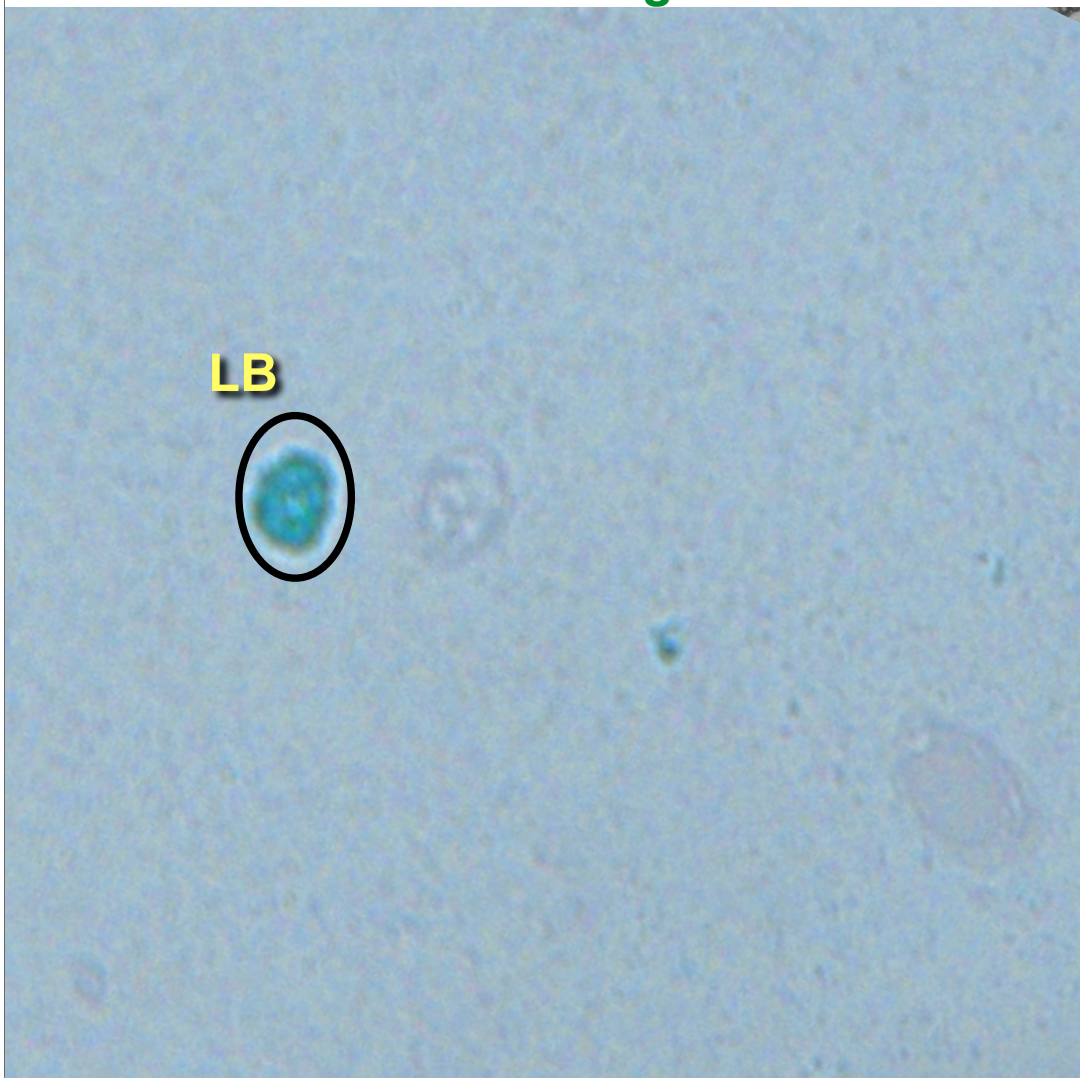
PD: CLEM - Correlative Light and Electron Microscopy



Parkinson's Disease (PD)

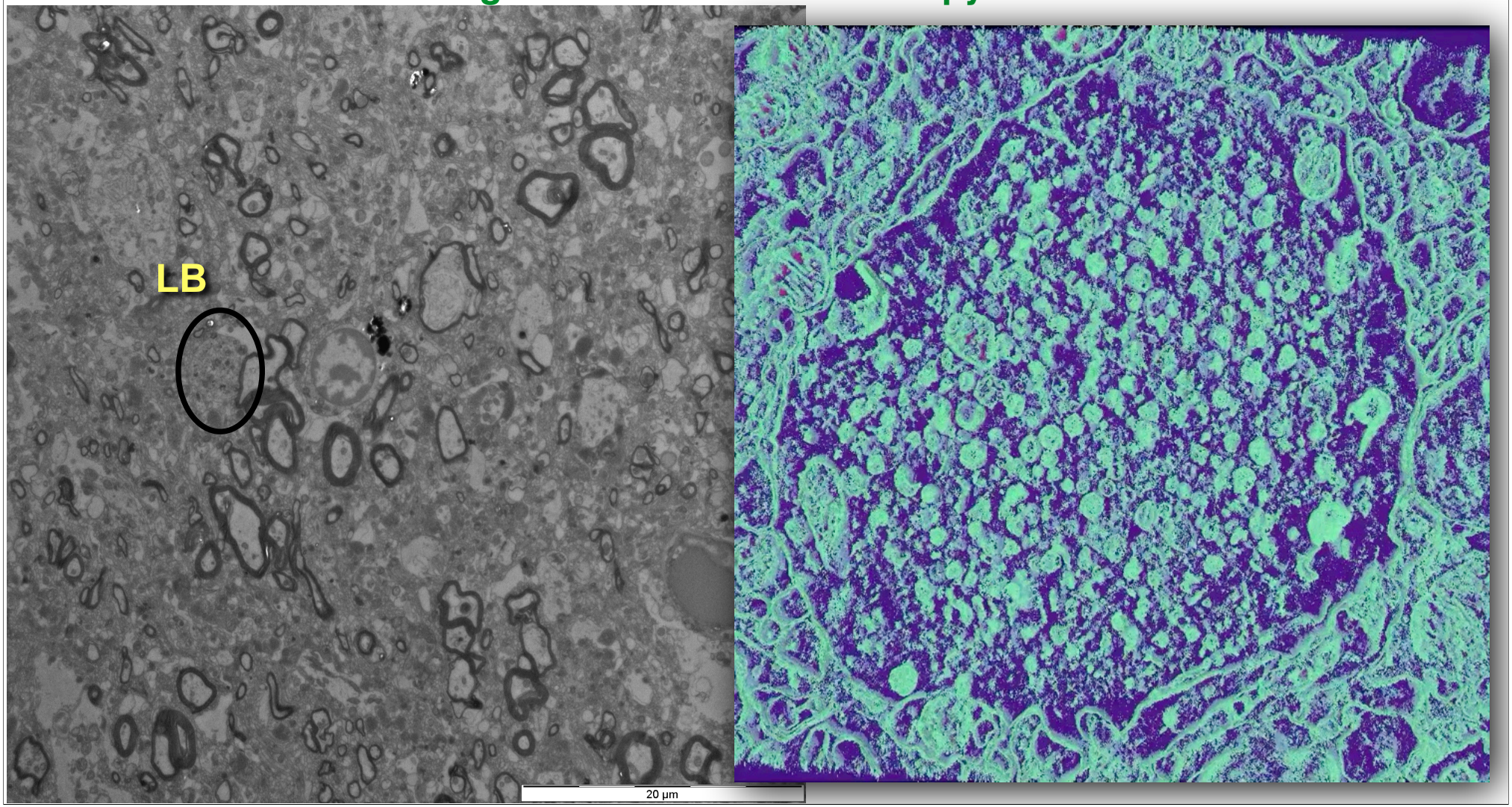
Shahmoradian *et al.,* Nature Neurosc. **22**, 1099-1109 (2019)

PD: CLEM - Correlative Light and Electron Microscopy



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

PD: CLEM - Correlative Light and Electron Microscopy

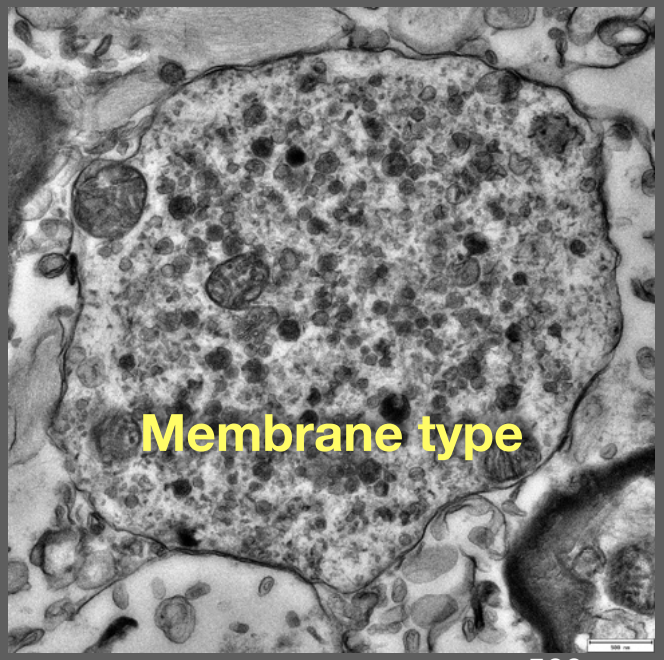


PD:

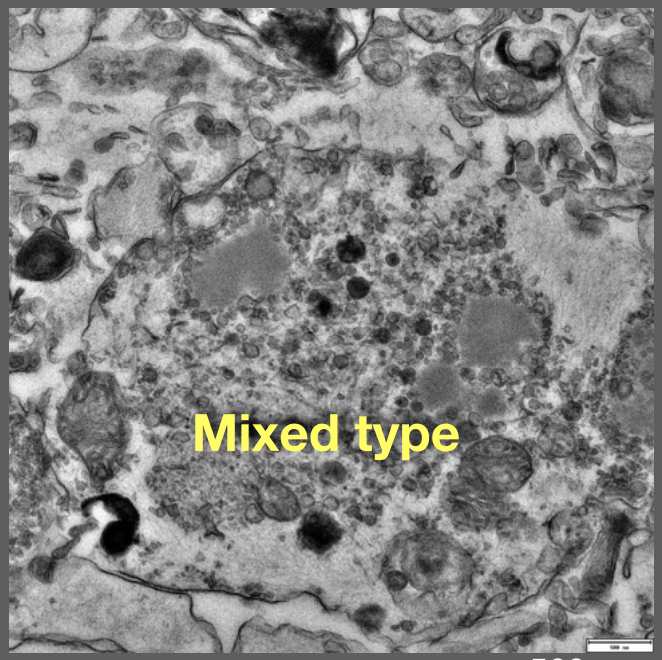
Lewy body, *Substantia nigra*

Amanda Lewis, LBEM

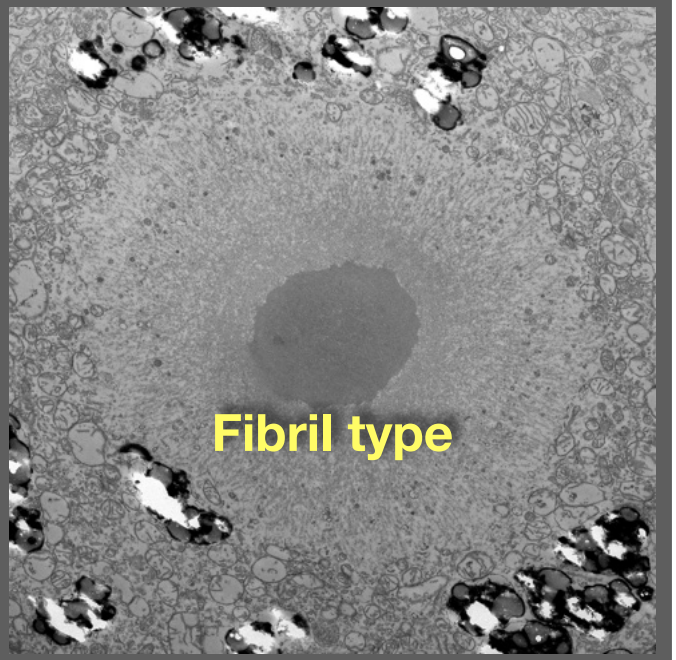
LB #1



LB #2



LB #3



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

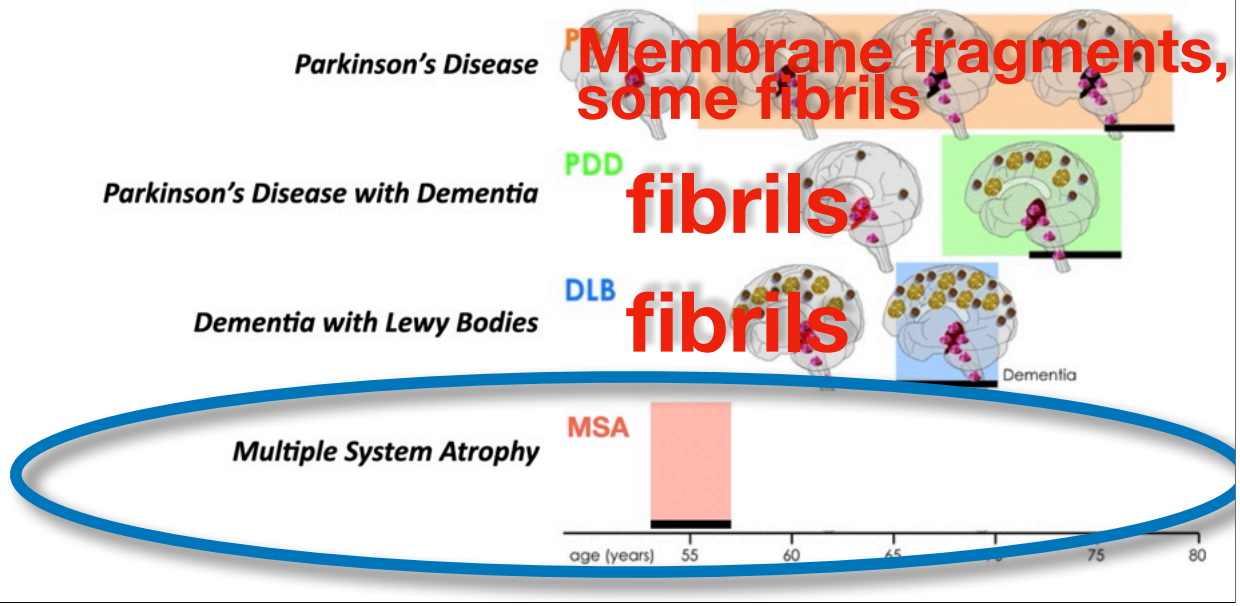
The majority of Lewy bodies in PD are primarily composed of membrane fragments

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

Lewis, AJ et al (2024) bioRxiv

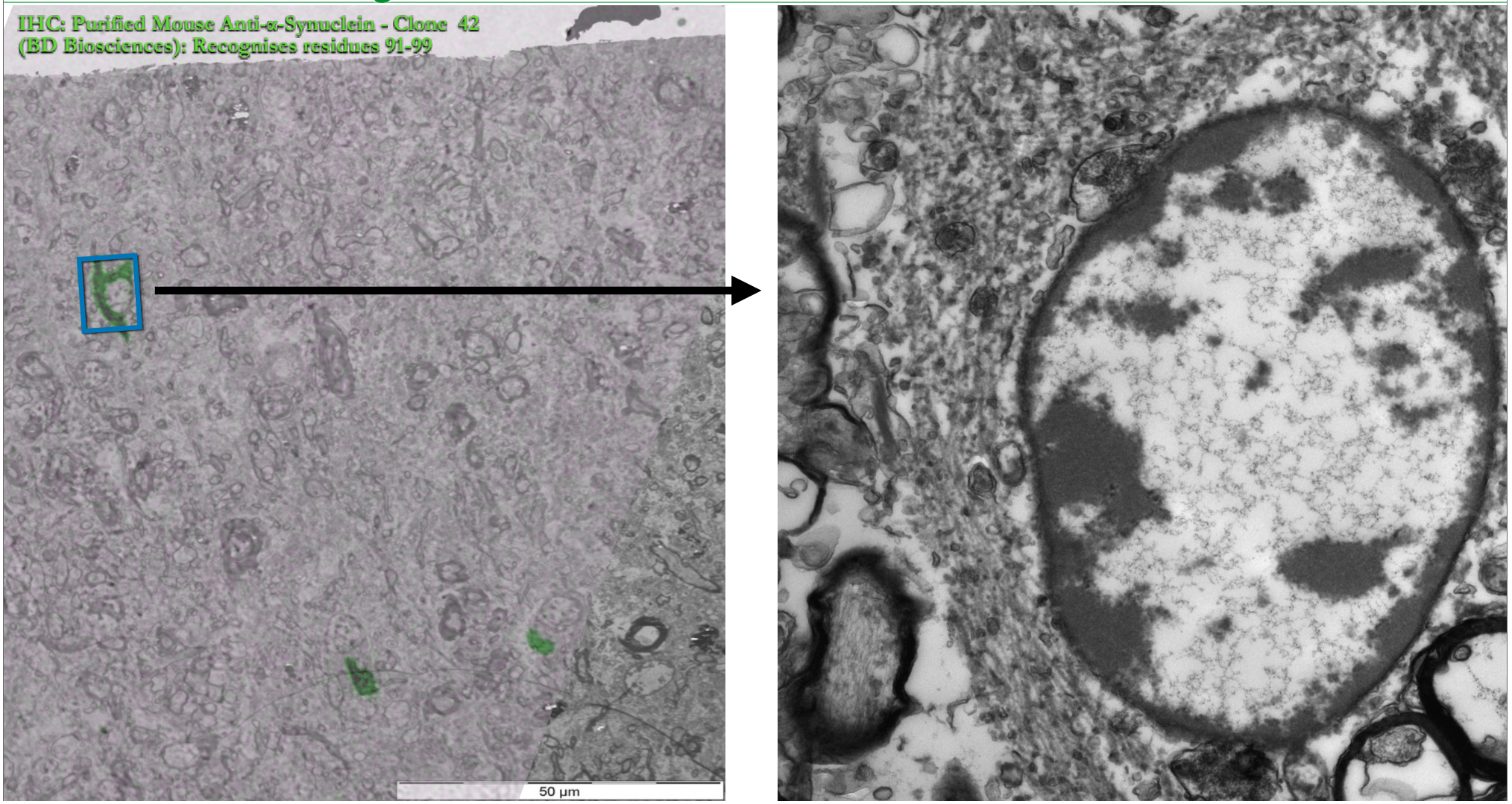
Multiple System Atrophy (MSA)

https://www.multiplesystematrophy.org/

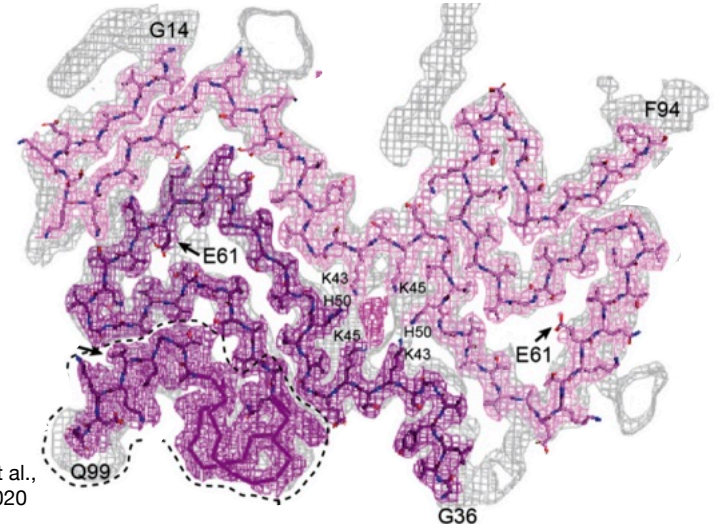


MSA: *Substantia Nigra*

Amanda Lewis, Domenic Burger, Carolin Böing



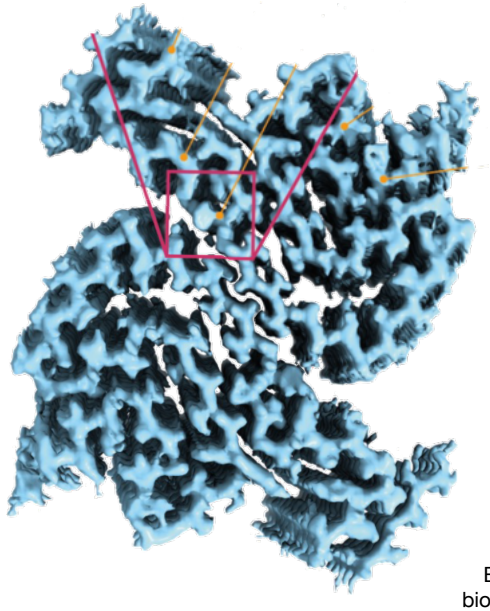
Multiple System Atrophy: A specific alpha-synuclein fibril strain



Schweighauser et al.,
Nature, Sept. 2020

Purified from human brain of MSA patients

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

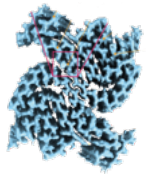


Burger et al.,
bioRxiv.org, 2024

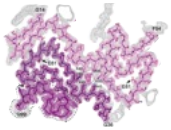
Synthetic “1B” fibril, causing MSA in mice

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

Multiple System Atrophy: A specific alpha-synuclein fibril strain

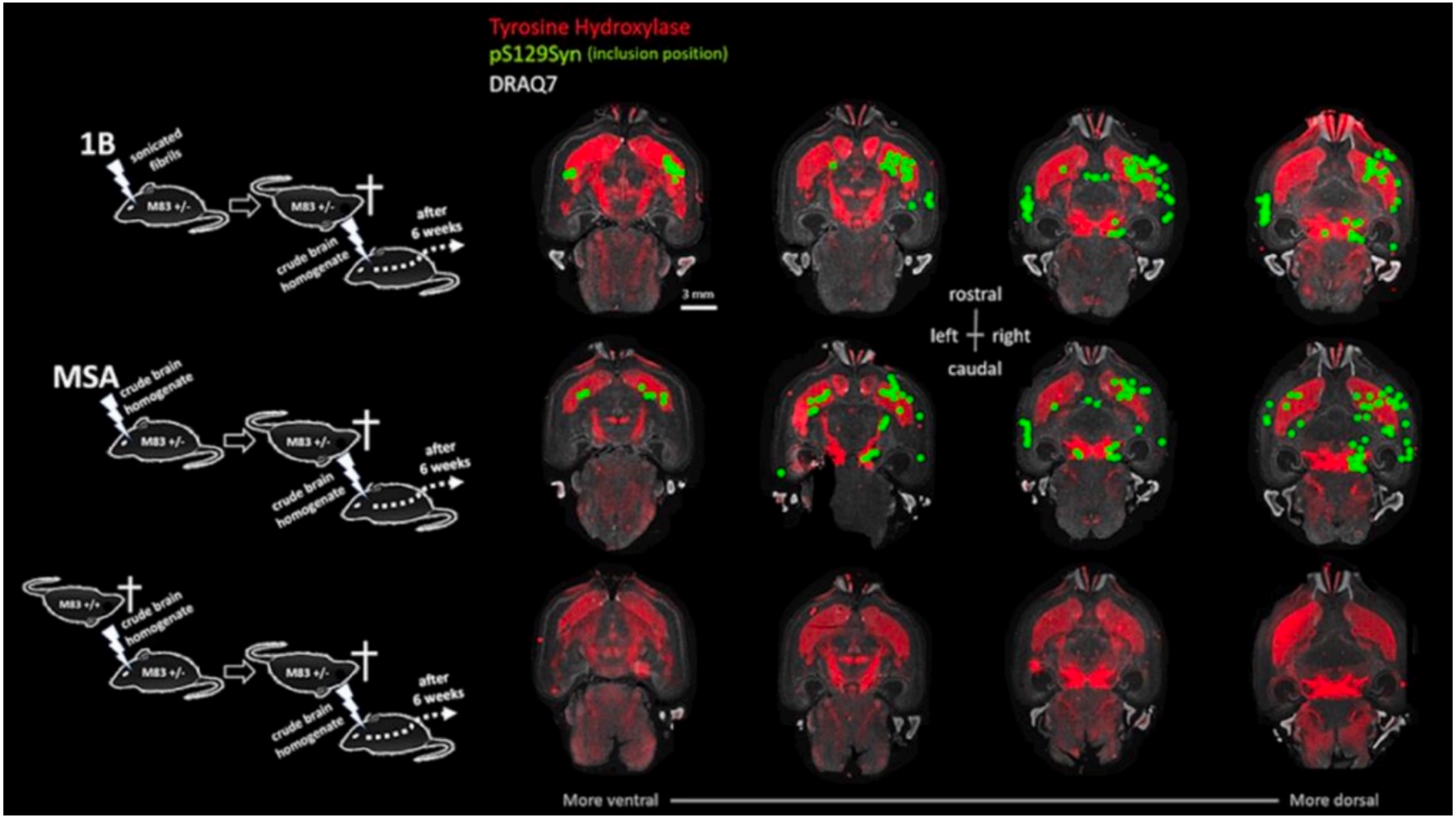


Synthetic “1B” fibril, causing MSA in mice



Purified from human brain of MSA patients

M83 mice express human A53T aSyn



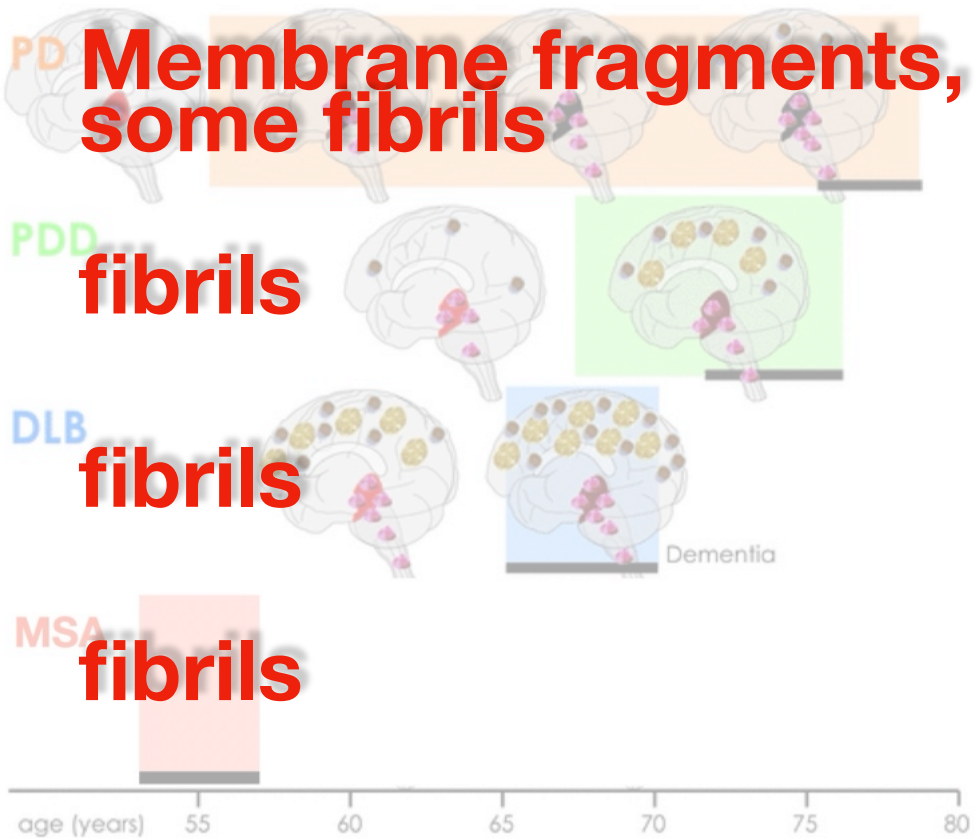
Synucleinopathies: Different “strains” of α-synuclein fibrils may cause different diseases

Parkinson’s Disease

Parkinson’s Disease with Dementia

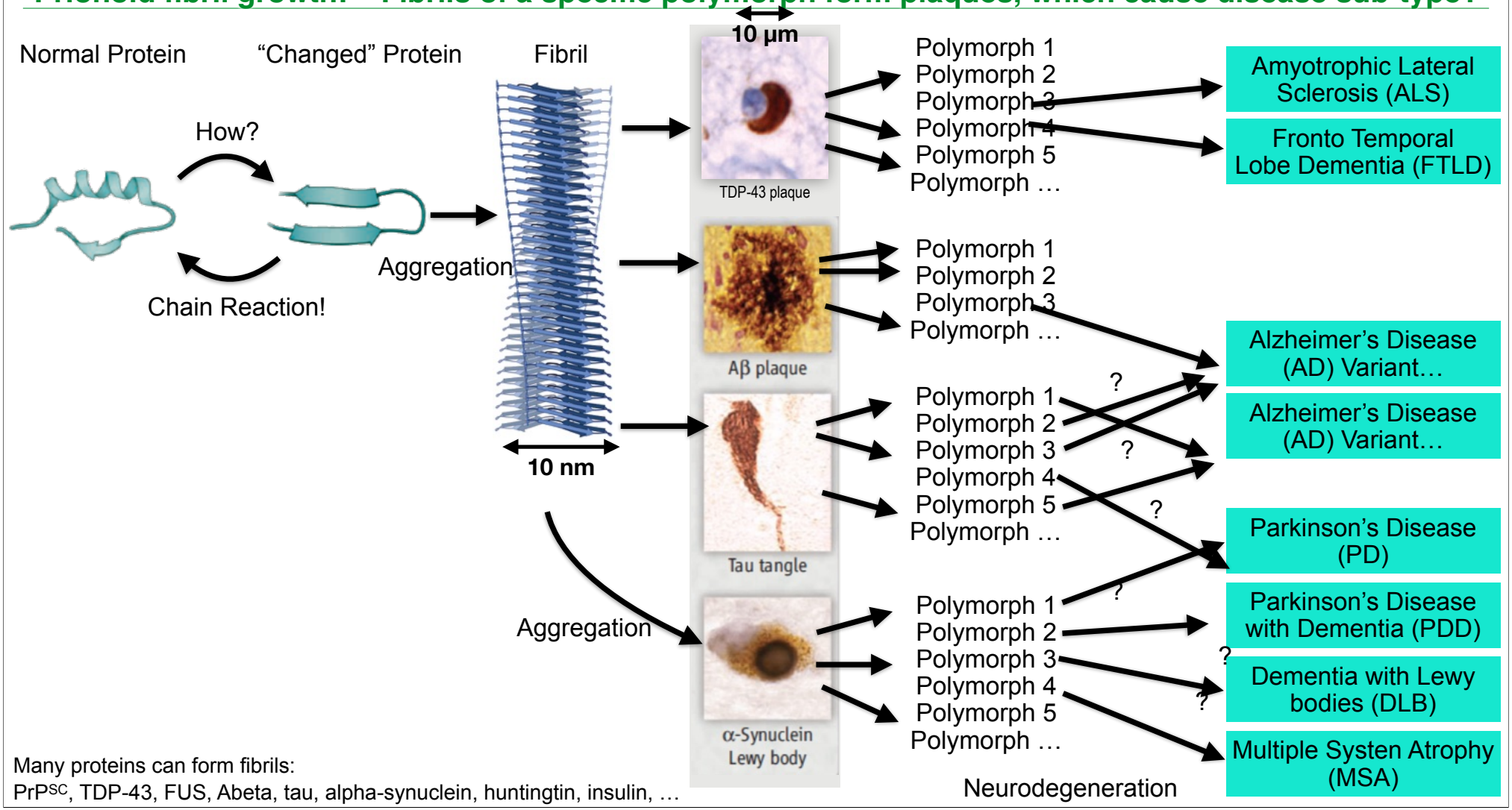
Dementia with Lewy Bodies

Multiple System Atrophy



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

Prionoid fibril growth: Fibrils of a specific polymorph form plaques, which cause disease sub-type?



Many proteins can form fibrils:
PrP^{SC}, TDP-43, FUS, Abeta, tau, alpha-synuclein, huntingtin, insulin, …

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

"Sh*t happens"

Protein "XYZ" → Fibril

Where does the first fibril come come from?

? ?

Mitochondrial damage

Pesticide

Which Polymorph or Polymorphs?

Which type of brain cells are damaged?
(How are they damaged?)

How does it spread?
(What spreads?)

"Disease Name"

Benjamin Stecher: https://tmrwedition.com/

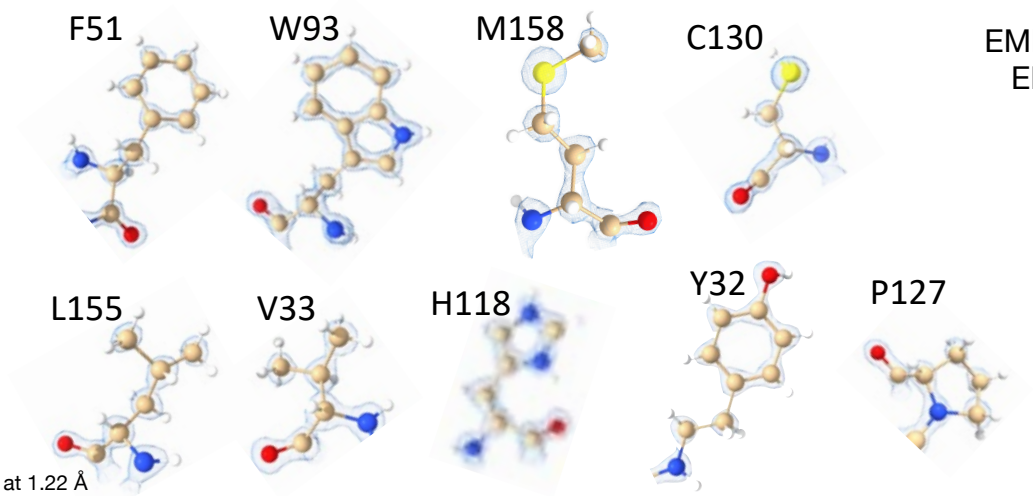
Structural Investigations of Neurodegeneration

Biology: Parkinson's Disease

We need more "contrast" in cryo-Electron Microscopy.

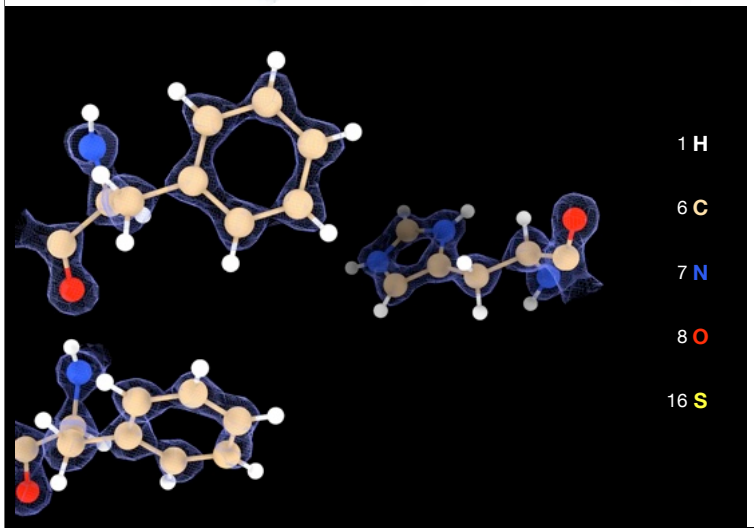
EPFL Unil UNIL | Université de Lausanne

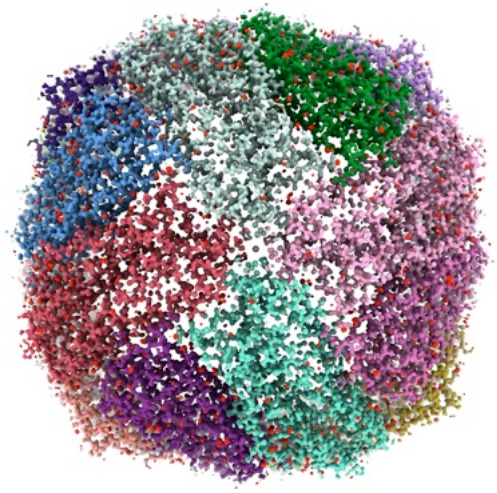
Dubochet Center for Imaging Lausanne: Apoferritin @ 1.09 (March 2024)



EMPIAR: 11866
EMDB: 19436
PDB: 8RQB

Here shown at 1.22 Å

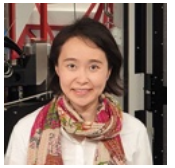




Alex Myasnikov
Technical Director

Christel Genoud
Executive Director

Bertrand Beckert



Emiko Uchikawa

Inay Mohamed

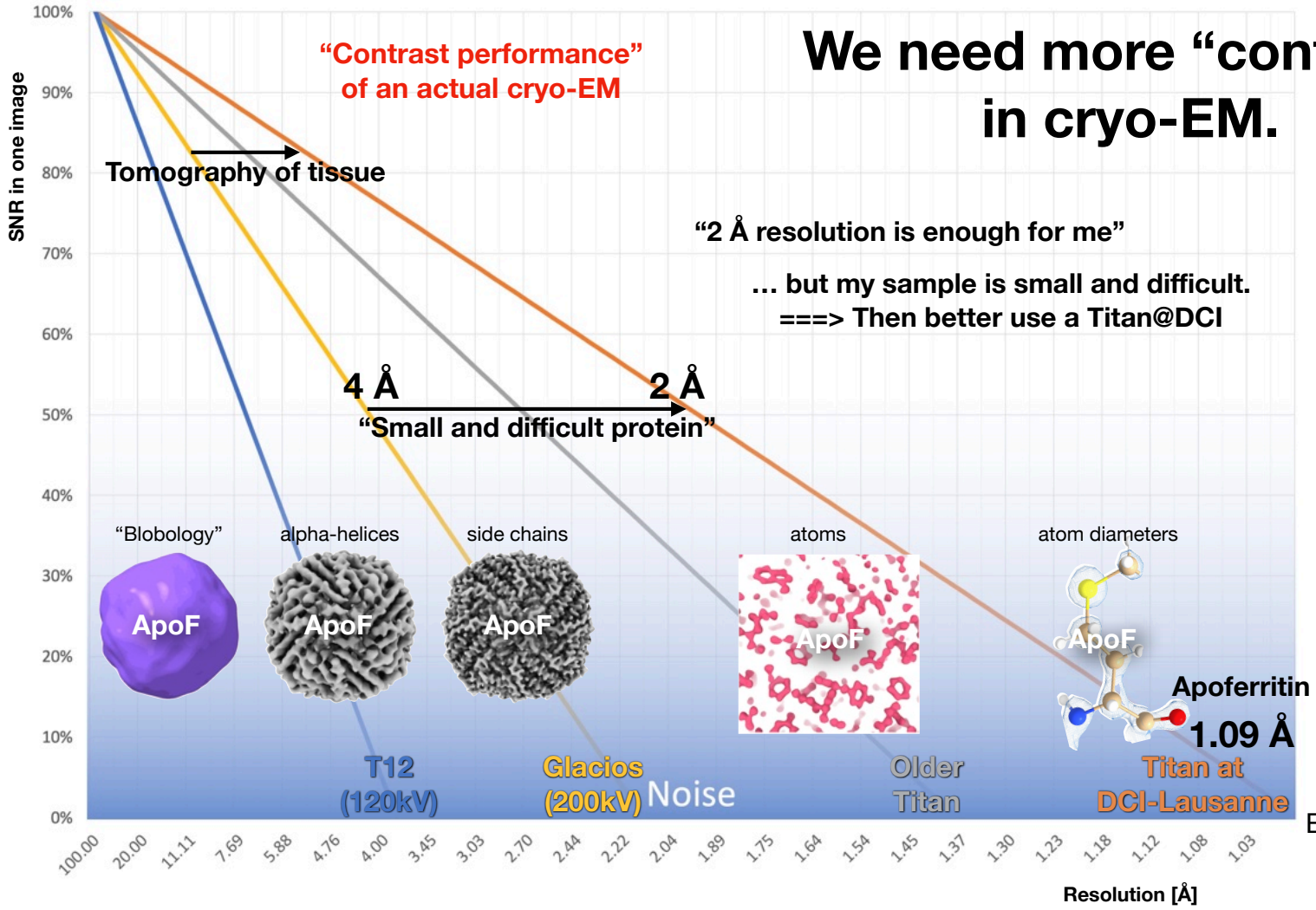
Sergey Nazarov

Mireille Fasmeyer

Florent Wenger

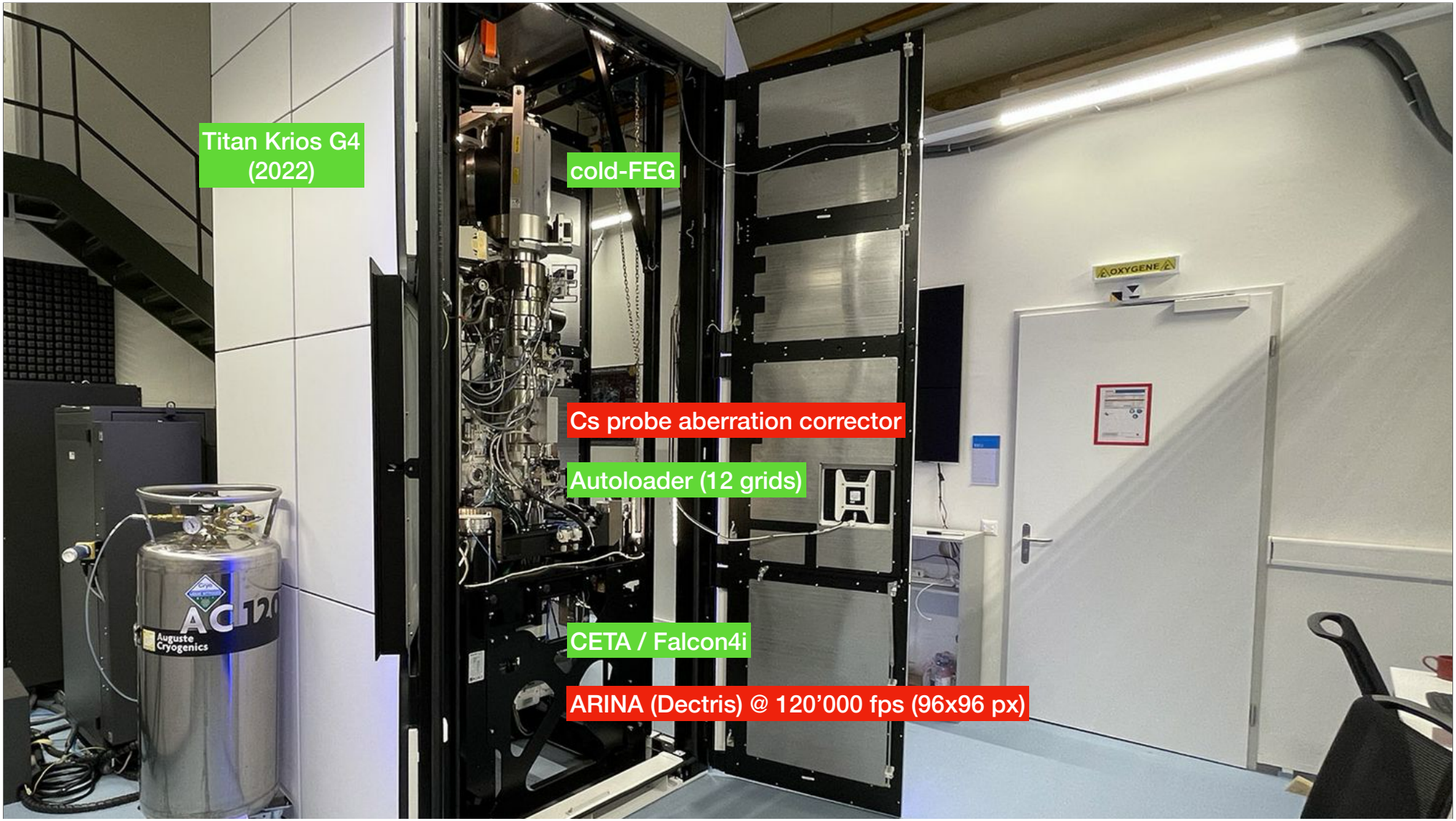
Sofya Laskina

High resolution is essential, even if you don't need it.



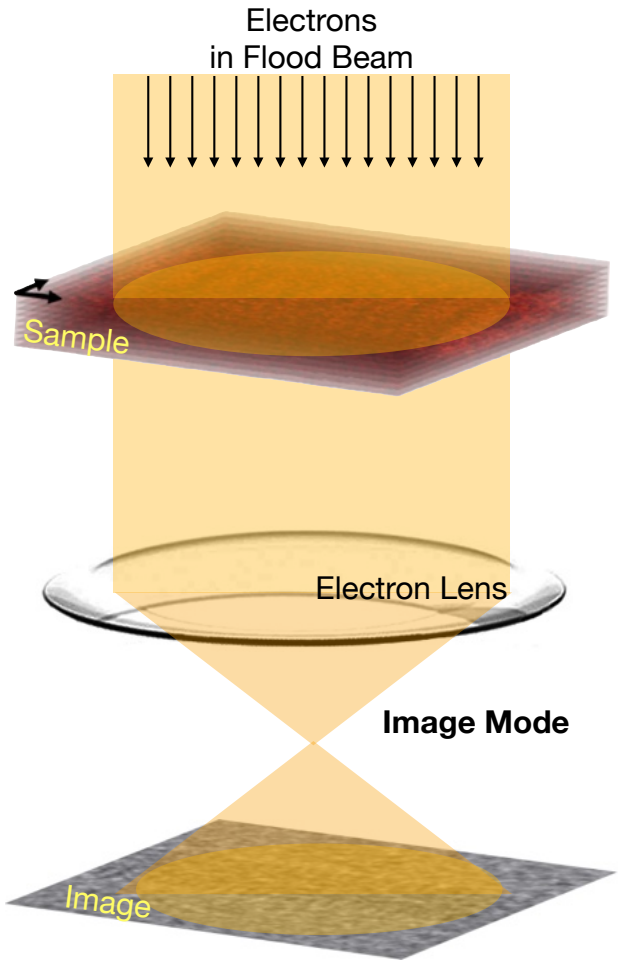
EMPIAR: 11866
EMDB: 19436
PDB: 8RQB

We need more “contrast”
in cryo-EM.

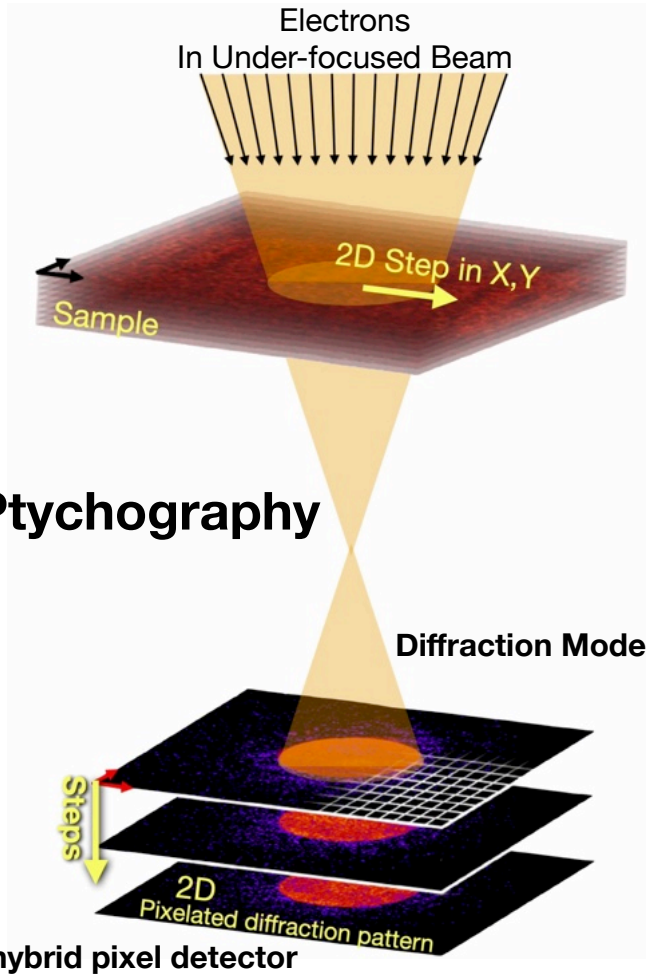

Titan Krios G4
(2022)
cold-FEG
Cs probe aberration corrector
Autoloader (12 grids)
CETA / Falcon4i
ARINA (Dectris) @ 120’000 fps (96x96 px)
OXYGENE
Auguste
Cryogenics

Cryo-Electron Microscopy of Proteins

Cryo-TEM

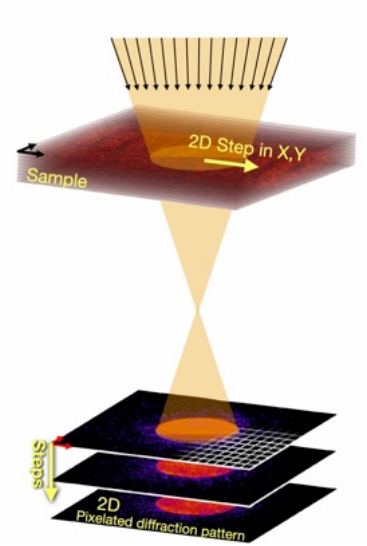


4D-STEM



Fast hybrid pixel detector

4D-STEM: Parallax (TcBF) vs. Ptychography reconstruction



512 x 512 beam positions
= 262'144 beam positions

= 2.4 GB
@ 120'000 fps => 2 seconds

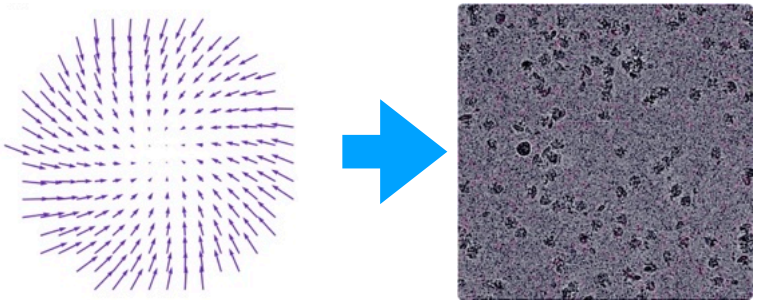
96 x 96 pixels on detector
(262'144 diffraction patterns)

py4Dstem axXiv:2309.05250v1 (2023)

Iterative Phase Retrieval Algorithms for Scanning Transmission Electron Microscopy

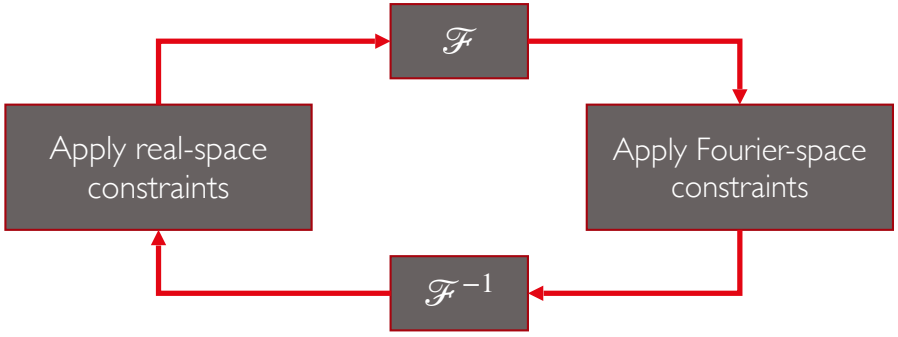
GEORGIOS VARNAVIDES,[1,2,†,*] STEPHANIE M. RIBET,[2,3,4,†] STEVEN E. ZELTMANN,[5,6] YUE YU,[7,8] BENJAMIN H. SAVITZKY,[2] VINAYAK P. DRAVID,[3,4,9] MARY C. SCOTT,[2,5] AND COLIN OPHUS[2,*]

Parallax



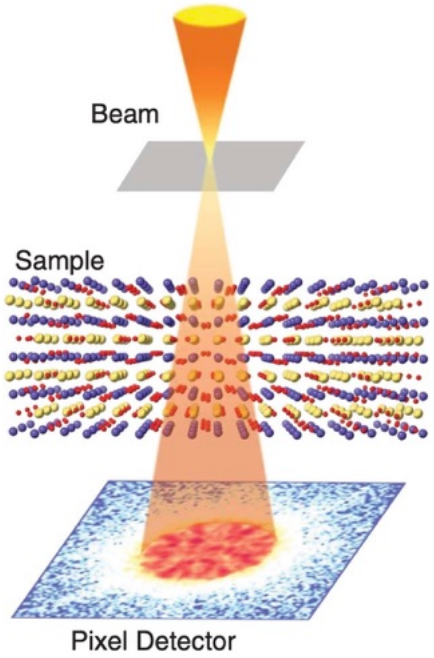
Images are aligned to each other **and averaged**

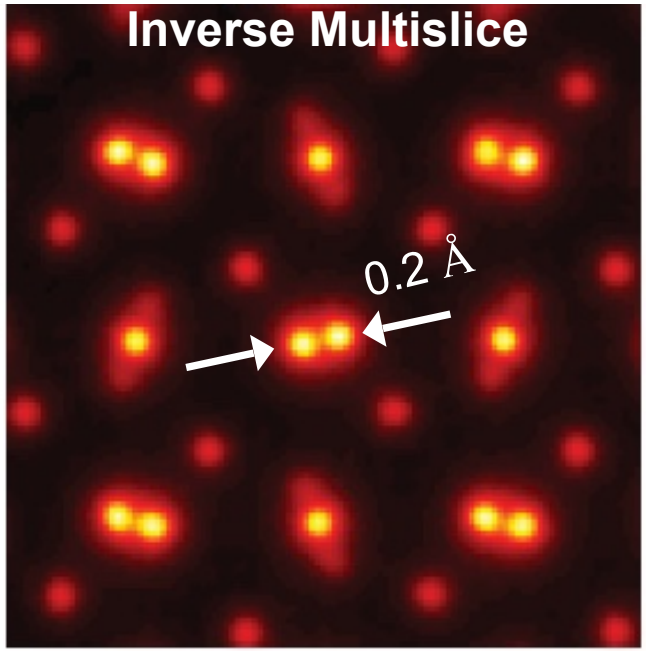
Ptychography



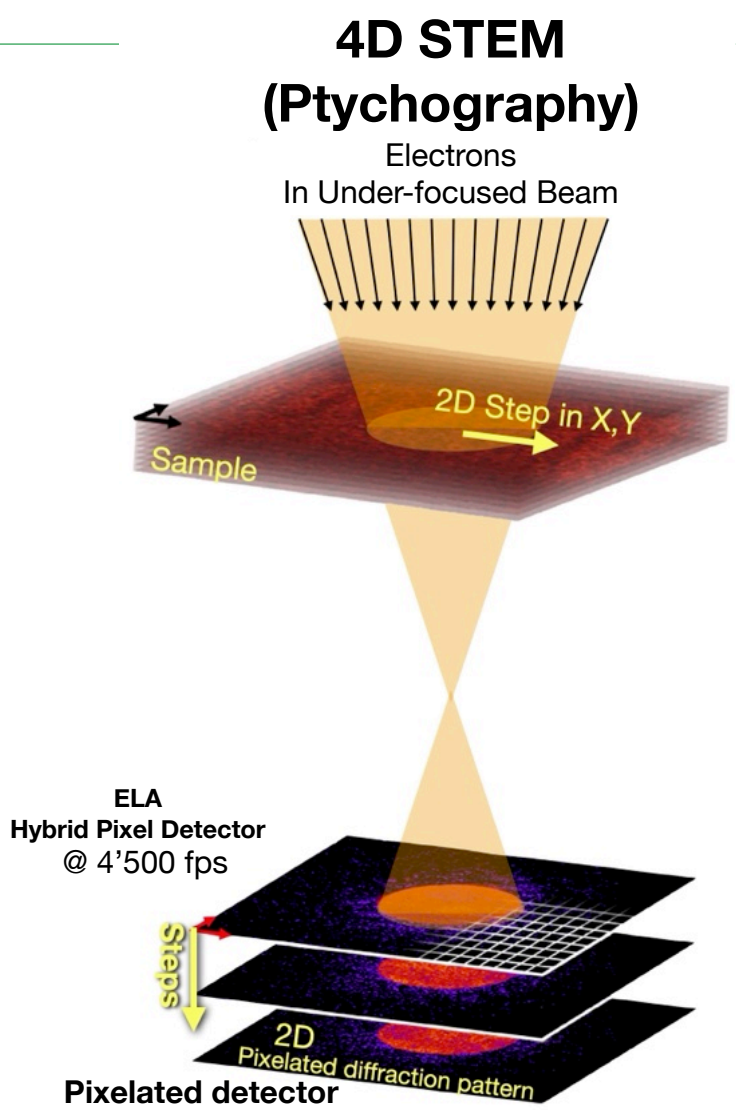
4D STEM in Materials Sciences: Ptychography

Resolution of 0.2 Å



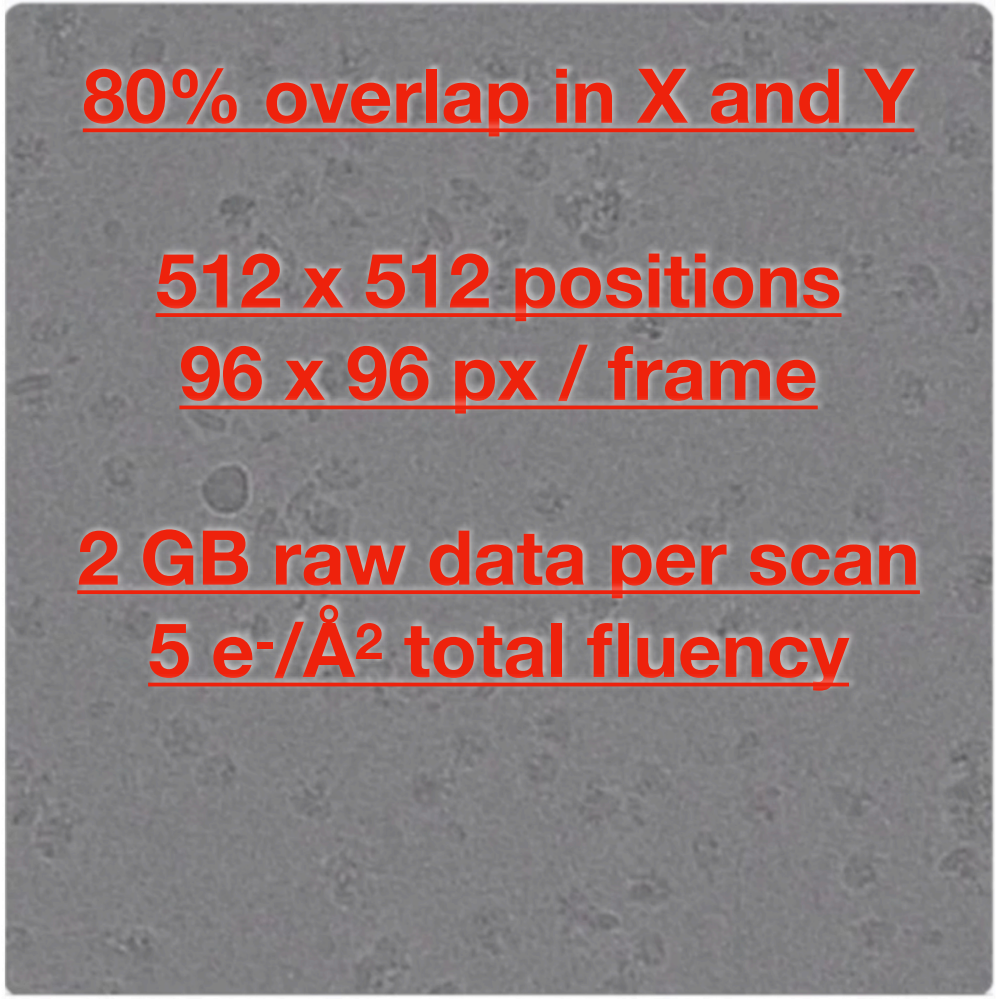


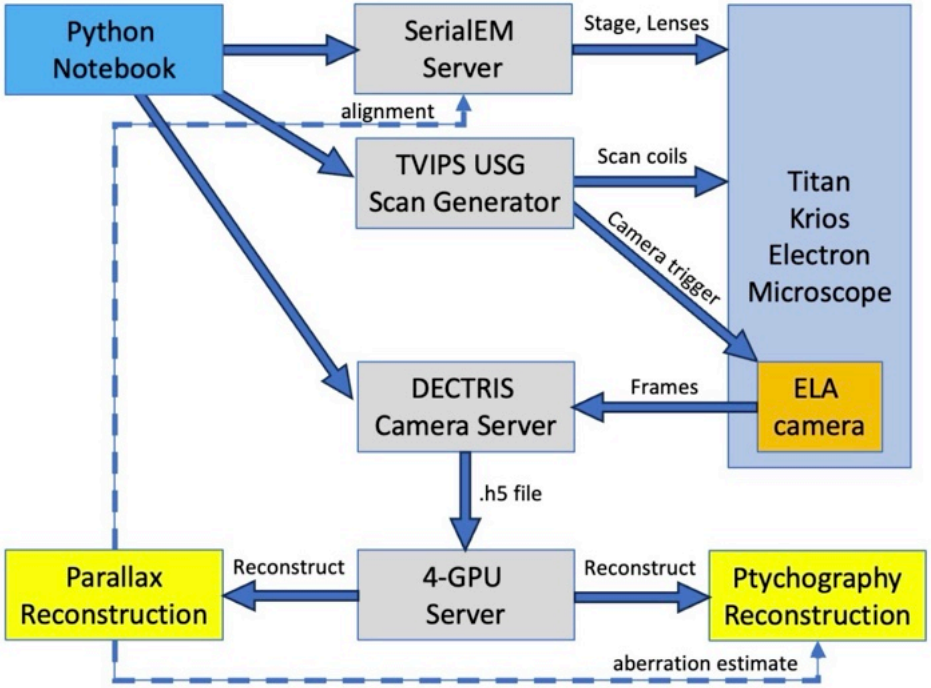
Chen, ..., Muller:
Science 2021



4D STEM

B. Küçükoğlu et al., (2024) bioRxiv.org 2024.02.12.579607

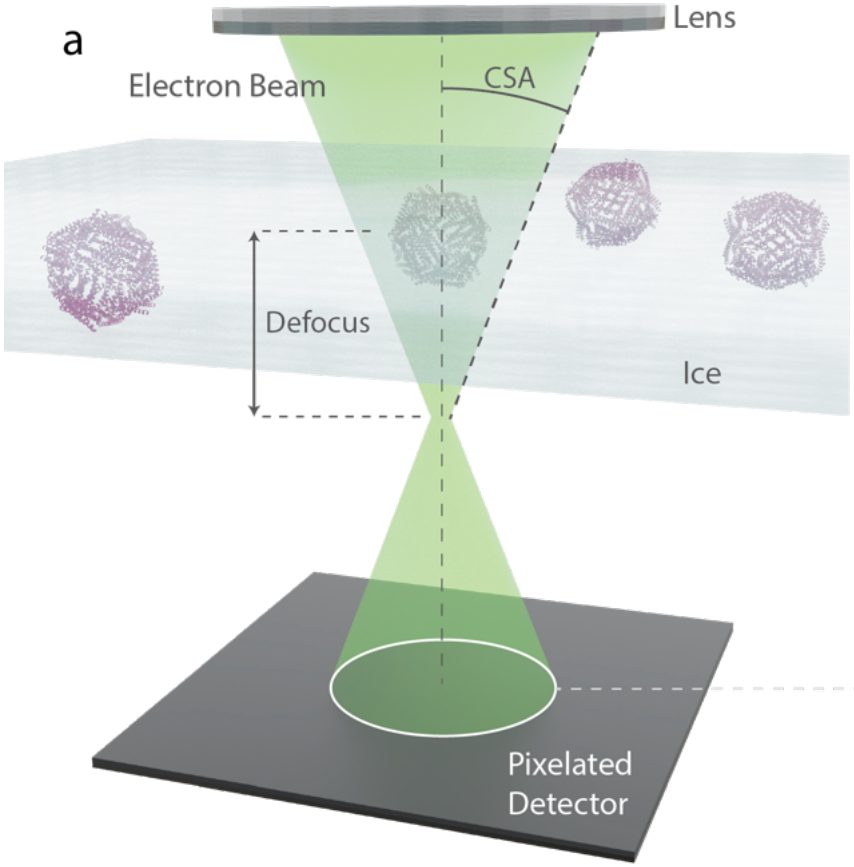


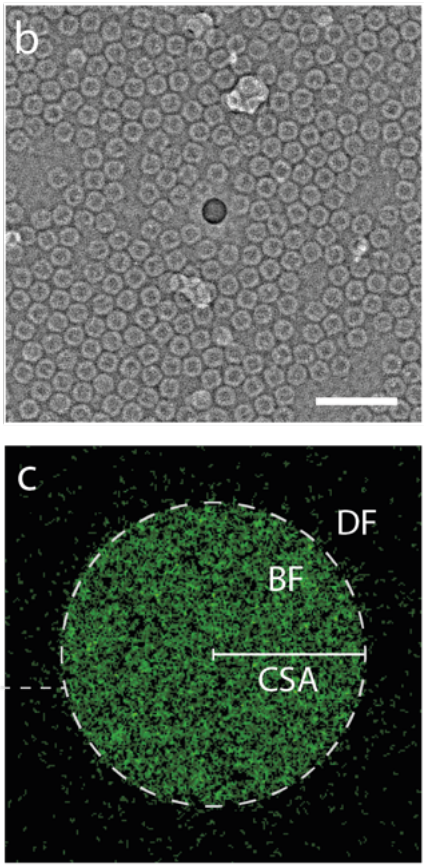


Thanks for help with SerialEM integration from
Michael Elbaum and Shahar Seifer, Weizmann, Israel

4D STEM

B. Küçükoğlu et al., (2024) bioRxiv.org 2024.02.12.579607





(Sum of 3 recorded frames)
CSA = 4 mrad

Titan Krios G4:
- 300kV cold-FEG
- Probe correction Cs=0mm
- TVIPS USG
- ELA detector

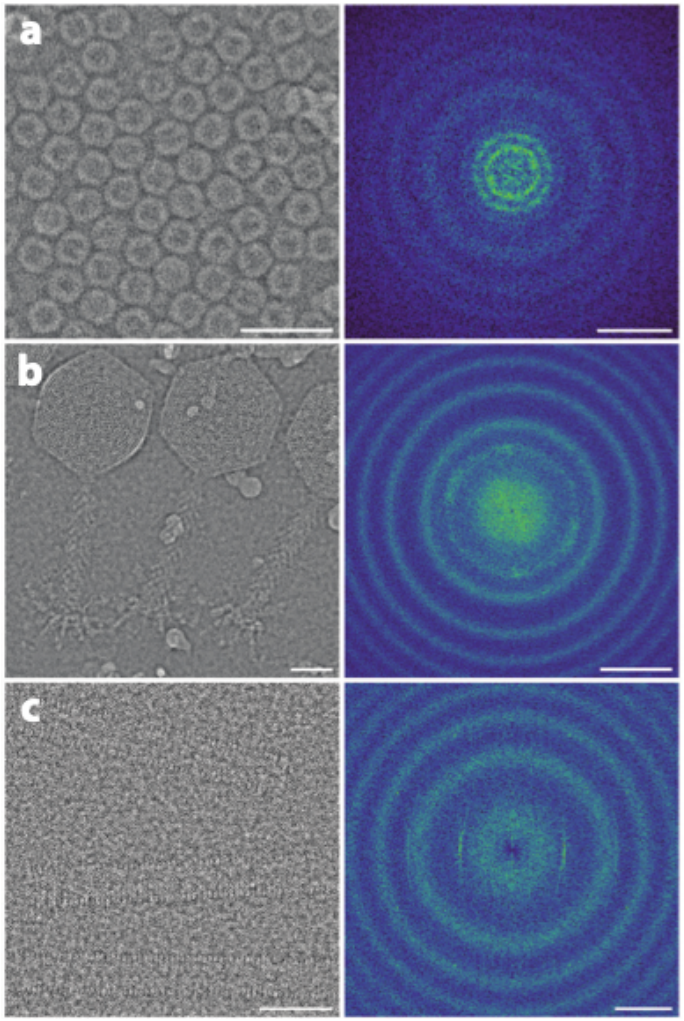
Illumination:
- CSA: 4.0 to 6.0 mrad
- Defocus: 1.0 to 2.0 µm
- Beam diameter: 8 to 24 nm

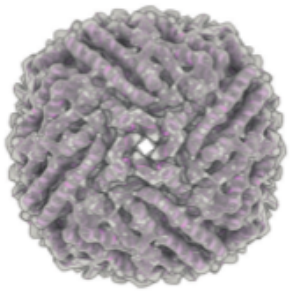
Low-dose conditions:
- 35…50 $e^-/Å^2$

Specimens:
- Apoferritin
- Phi92 Bacteriophage
- Tobacco Mosaic Virus
- Bacteriorhodopsin 2D crystals

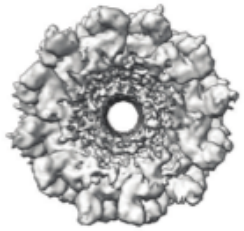
Cryo-electron ptychography of proteins

B. Küçükoğlu et al., (2024) bioRxiv.org 2024.02.12.579607

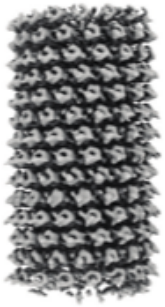




Apoferritin
CSA: 4.0 mrad
Dose: 35e-/Å2
Number particles: 11'552
Final Resolution: 5.8 Å



Phi92 stalk
CSA: 5.1 mrad
Dose: 49e-/Å2
Number particles: 1'600
Final Resolution: 8.4 Å

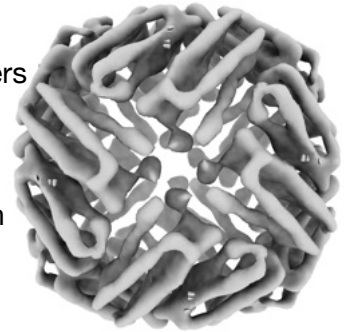


TMV
CSA: 6.1 mrad
Dose: 32e-/Å2
Number particles: 2'120
Final Resolution: 6.4 Å

Cryo-electron ptychography vs. Cryo-EM

ToDo:
- Dose fractionation
- Motion correction
- Dose-dep. resolution filters

- Larger CSA
- Less defocus
- Multi-slice reconstruction

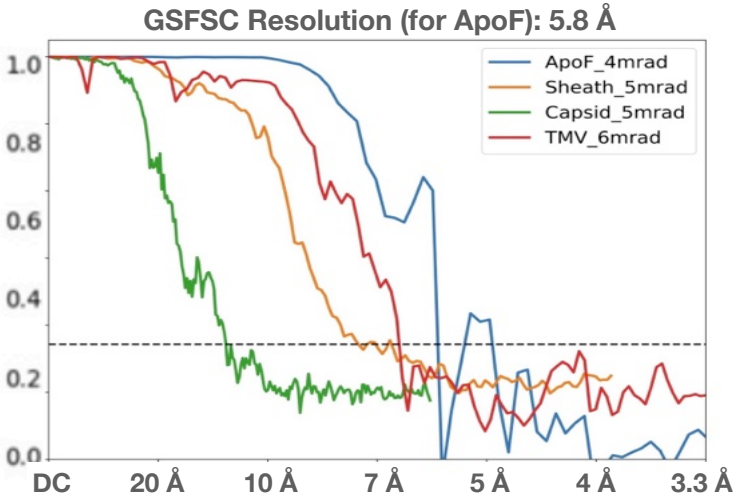


Implemented:
- Dose fractionation
- Motion correction
- Dose-dep. resolution filters

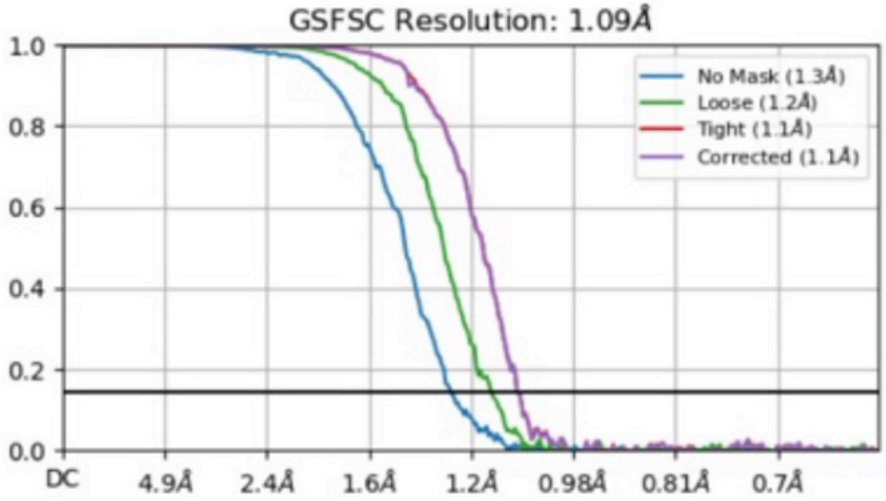
Alex Myasnikov
Inay Mohammed
Sergey Nazarov

EMPIAR: 11866
EMDB: 19436
PDB: 8RQB

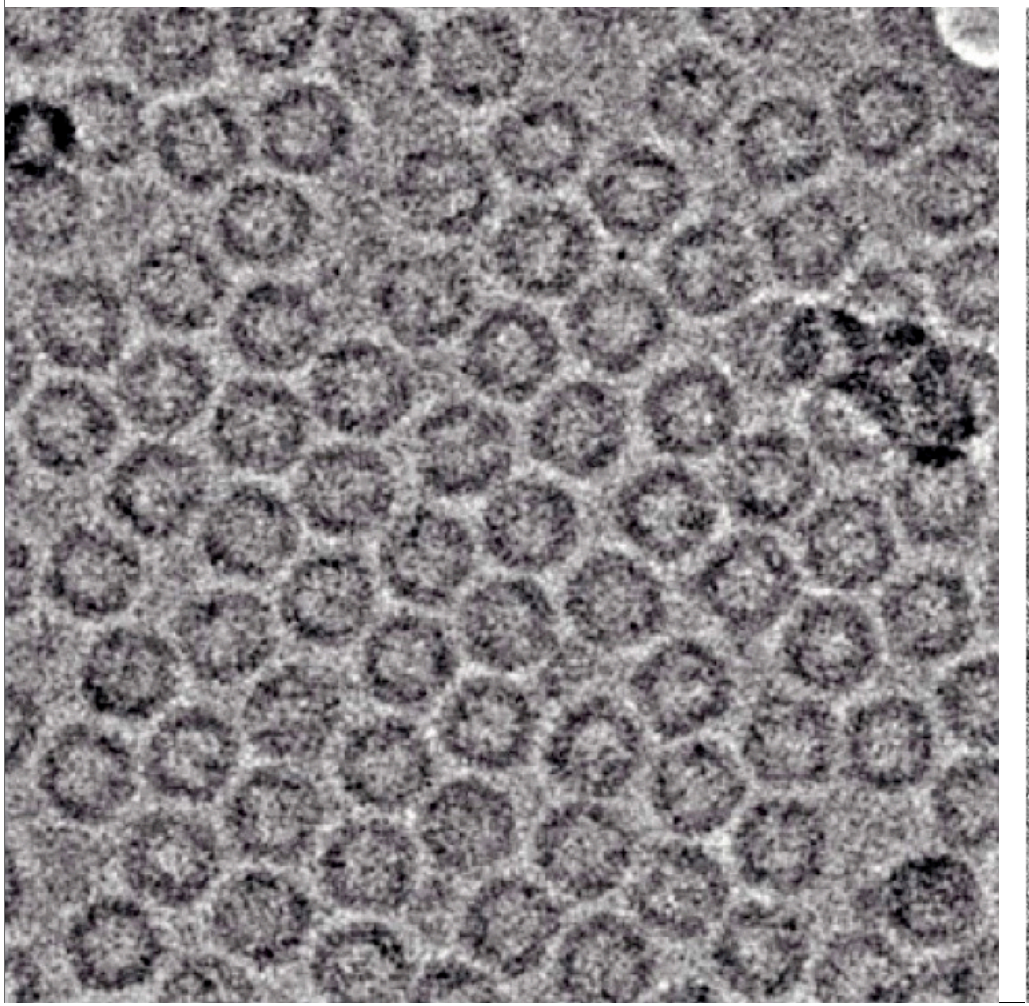
Titan Krios G4, Probe Corrector, ELA: 11’552 particles

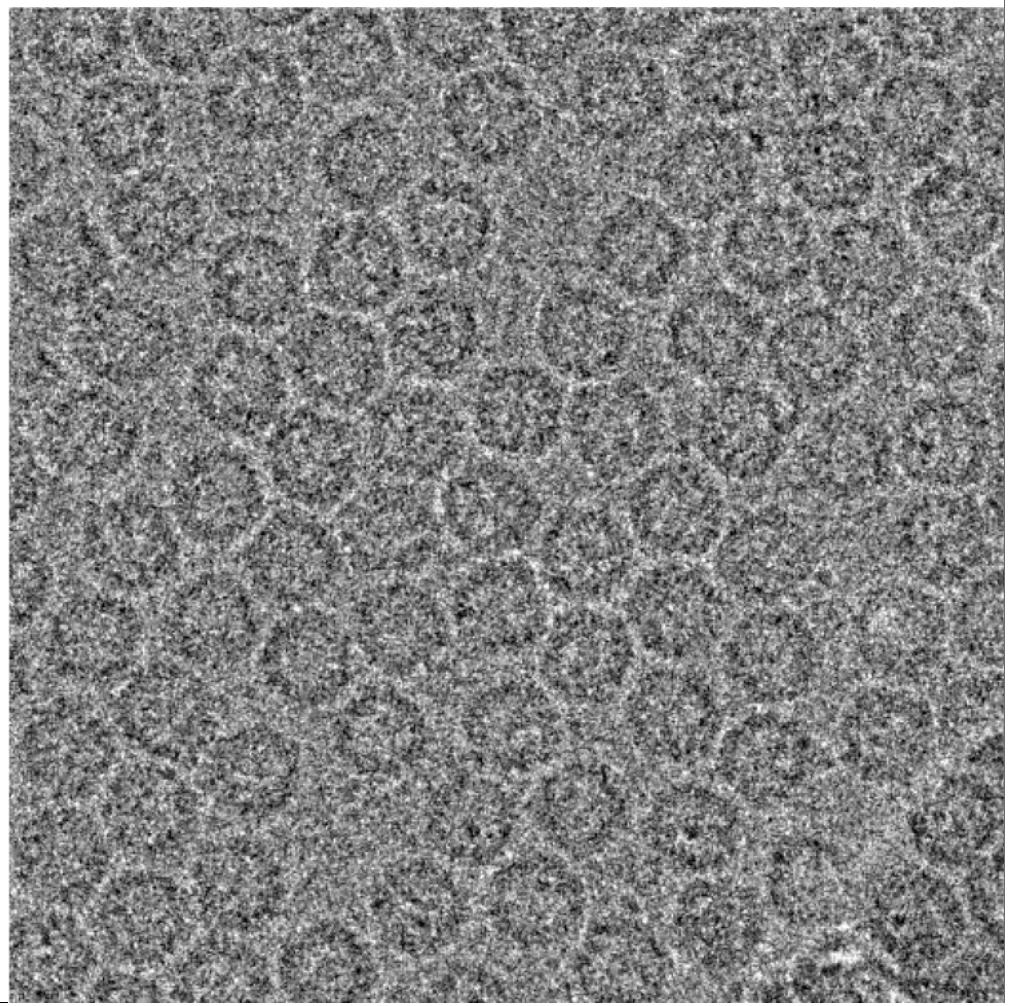


Titan Krios G4, Falcon4i: 411’705 particles



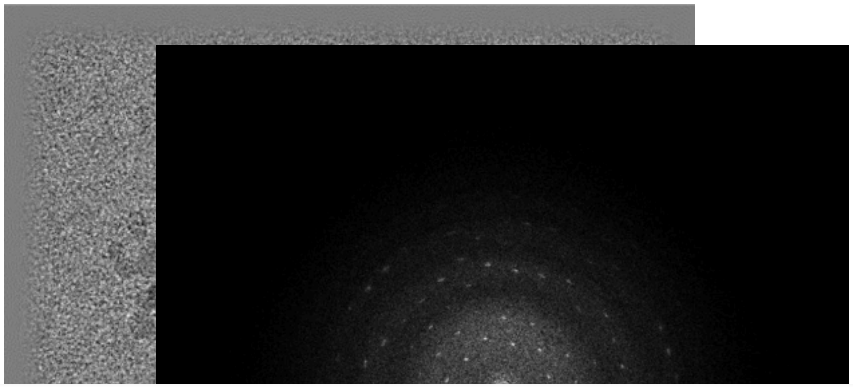
Cryo-electron ptychography vs. Cryo-EM

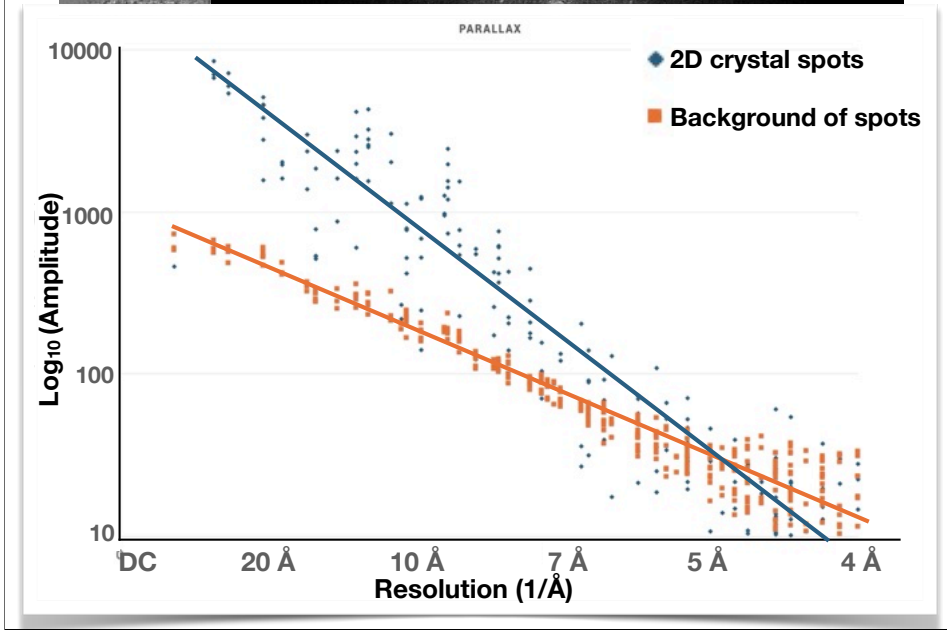




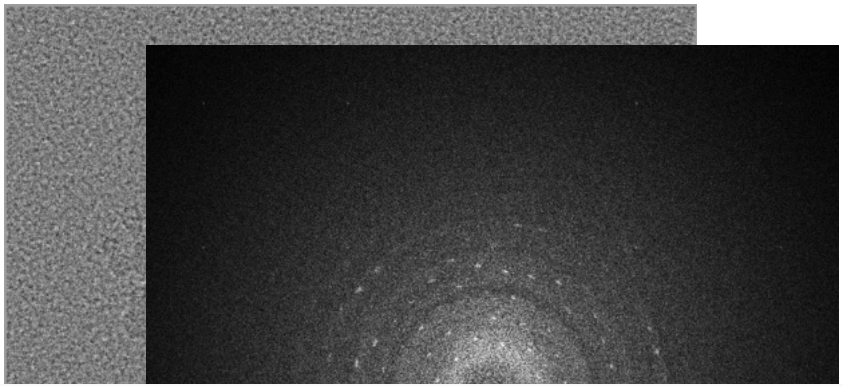
Cryo-electron ptychography of Bacteriorhodopsin 2D crystals

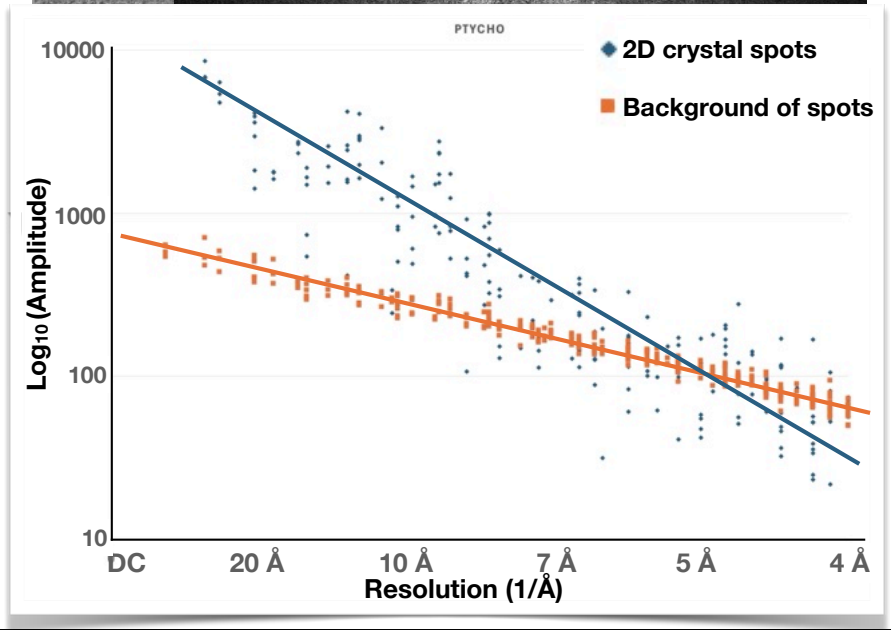
Parallax Reconstruction (py4Dstem)





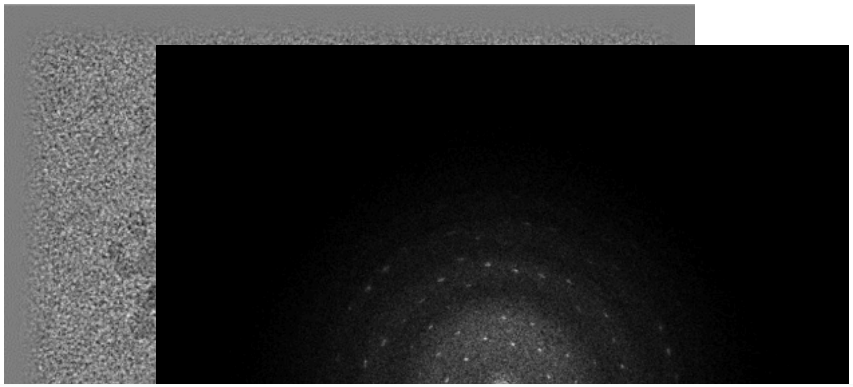
Ptychography Reconstruction (py4Dstem)

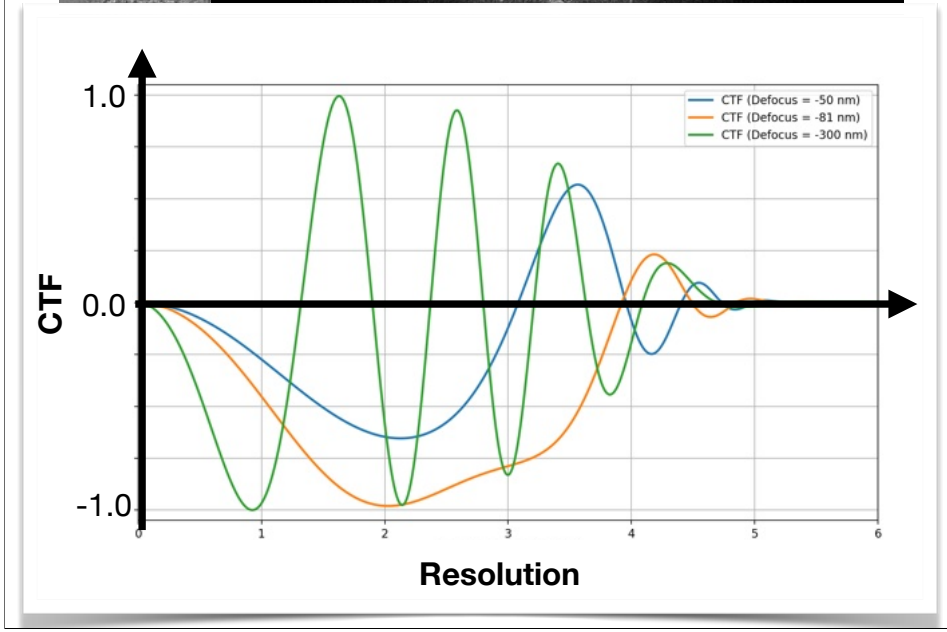




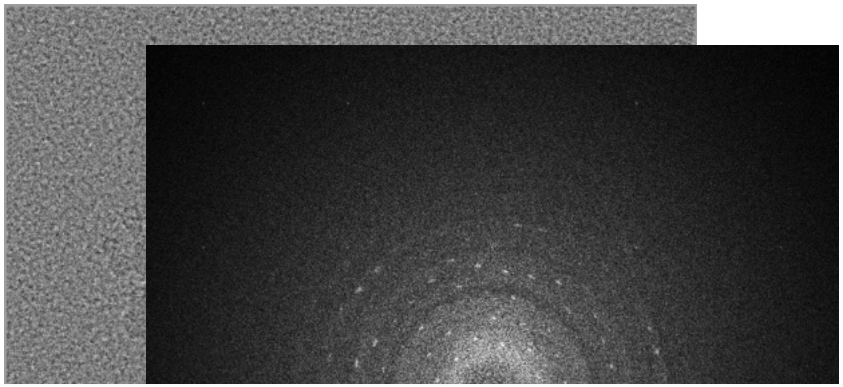
Cryo-electron ptychography of Bacteriorhodopsin 2D crystals

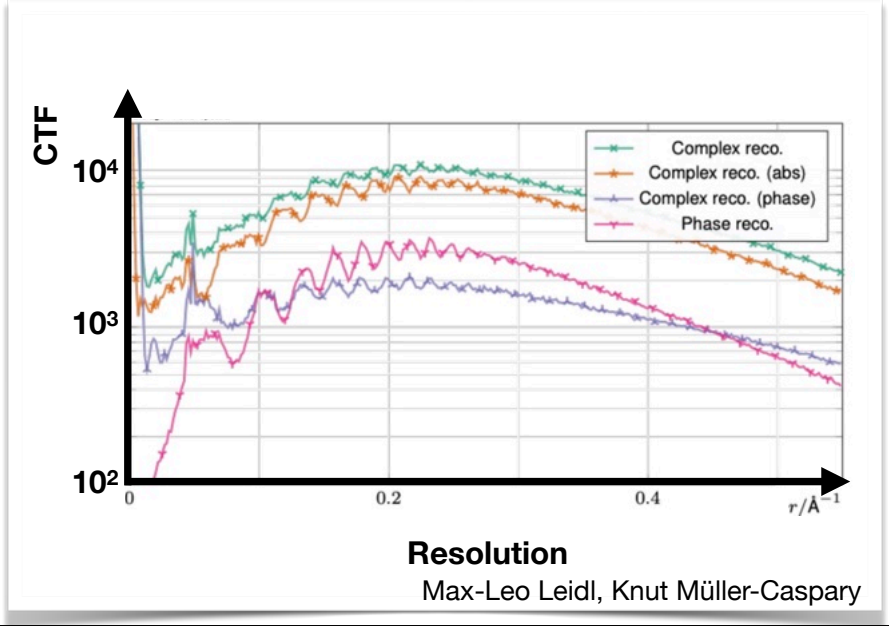
Parallax Reconstruction (py4Dstem)





Ptychography Reconstruction (py4Dstem)

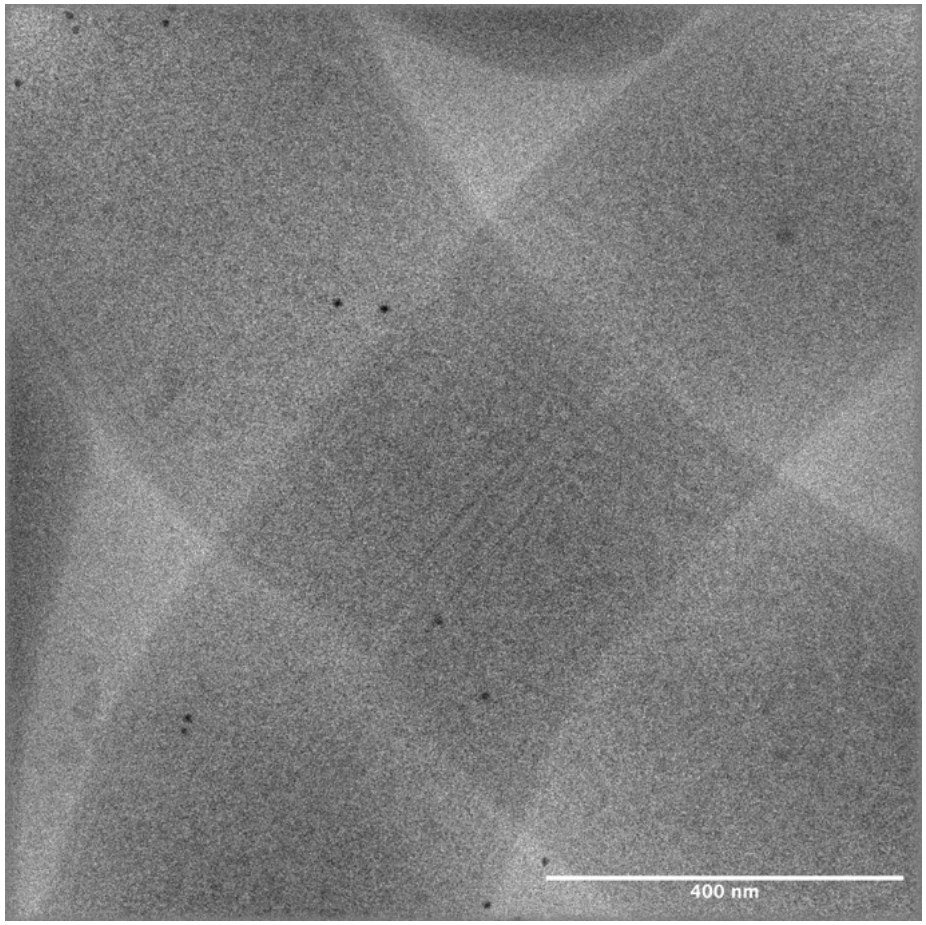




Max-Leo Leidl, Knut Müller-Caspary

Cryo-electron ptychography tomography

Berk Küçükoğlu, Massimo Kube, Julika Radecke



Thick Sample Tomography

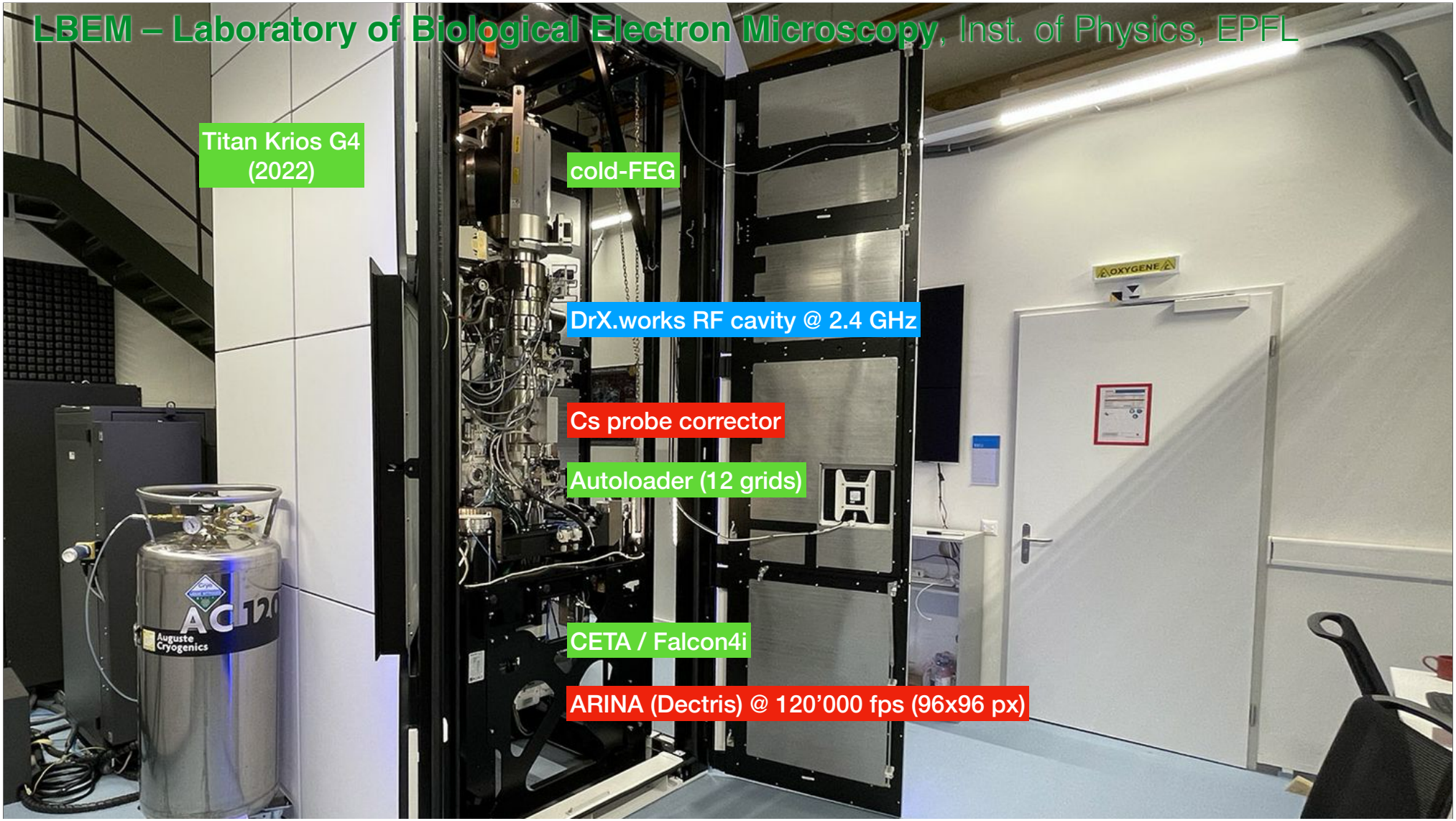
- Effects of dynamic scattering can partly be mitigated by **multi-slice ptychography**

  ===> Manuel Guizar-Sicairos, PSI & EPFL

- Larger tilt angle increments are possible due to thicker slabs in Fourier space (Crowther criterion)
- A 3D reconstruction with some modest data in the missing wedge can be computed.

Cryo-electron ptychography image of overlapping microbacteria (Sample thickness >600nm)

See also the work of Sharon Wolf, Michael Elbaum, or Dave Muller (Cornell) et al.


LBEM – Laboratory of Biological Electron Microscopy, Inst. of Physics, EPFL
Titan Krios G4 (2022)
cold-FEG
DrX.works RF cavity @ 2.4 GHz
Cs probe corrector
Autoloader (12 grids)
CETA / Falcon4i
ARINA (Dectris) @ 120’000 fps (96x96 px)
OXYGENE
Auguste Cryogenics

Louis de Broglie

Prince Louis-Victor Pierre Raymond de Broglie

Dualism:
Is the electron a wave?
Is the electron a particle?
==> It is both !

PhD Thesis 1924

RADIATIONS. — *Ondes et quanta* (¹).
Note de M. **Louis de Broglie**.

Considérons un mobile matériel de masse propre m_0 se mouvant par rapport à un observateur fixe avec une vitesse $v = \beta c$ ($\beta < 1$). D'après le principe de l'inertie de l'énergie, il doit posséder une énergie interne égale à $m_0 c^2$. D'autre part, le principe des quanta conduit à attribuer cette énergie interne à un phénomène périodique simple de fréquence ν_0 telle que

$$h\nu_0 = m_0 c^2,$$

c étant toujours la vitesse limite de la théorie de relativité et h la constante de Planck.

Pour l'observateur fixe, à l'énergie totale du mobile correspondra une fréquence $\nu = \frac{m_0 c^2}{h\sqrt{1-\beta^2}}$. Mais, si cet observateur fixe observe le phénomène périodique interne du mobile, il le verra ralenti et lui attribuera une fréquence $\nu_1 = \nu_0\sqrt{1-\beta^2}$; pour lui, ce phénomène varie donc comme

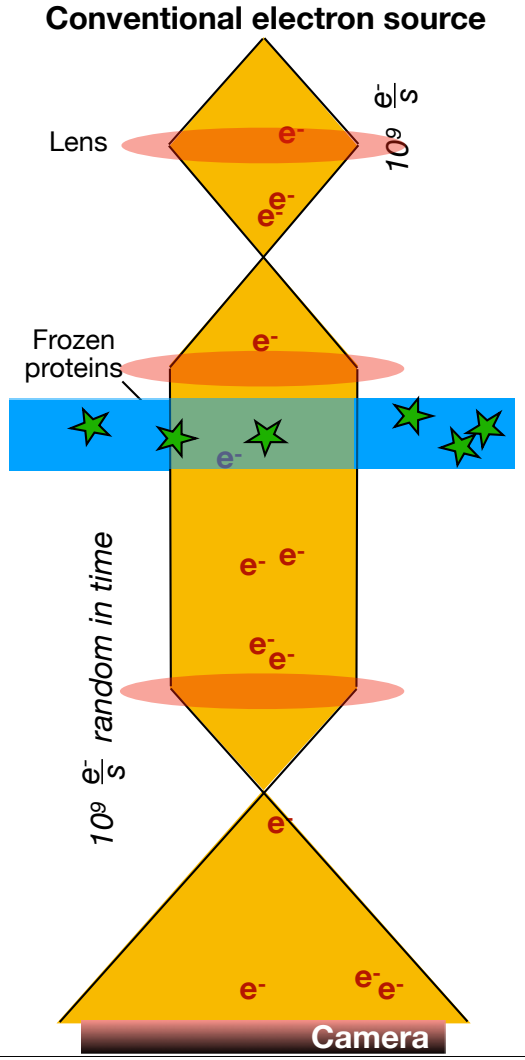
$$\sin 2\pi\nu_1 t.$$

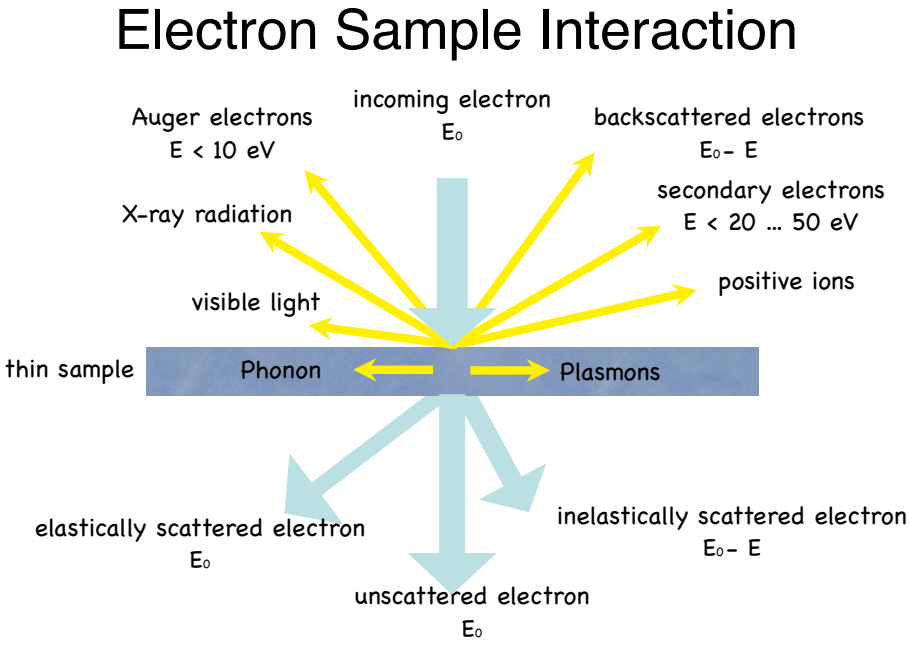
Supposons maintenant qu'au temps $t = 0$, le mobile coïncide dans l'espace avec une onde de fréquence ν ci-dessus définie se propageant dans la même direction que lui avec la vitesse $\frac{c}{\beta}$. Cette onde de vitesse plus grande que c ne peut correspondre à un transport d'énergie; nous la considérerons seulement comme une onde fictive associée au mouvement du mobile.

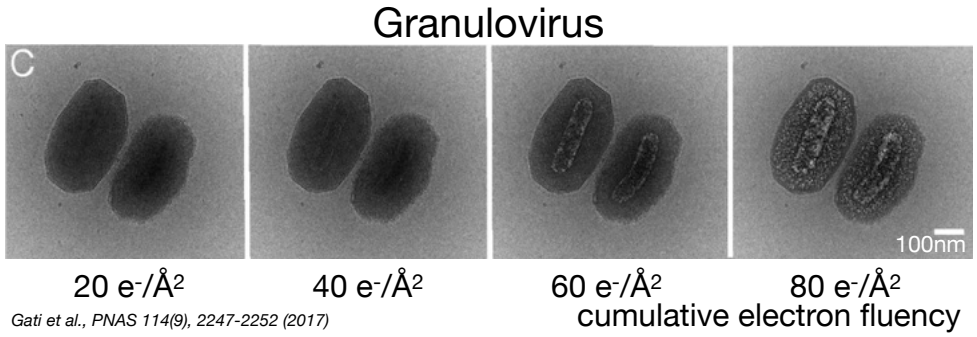
Je dis que, si au temps $t = 0$, il y a accord de phase entre les vecteurs de l'onde et le phénomène interne du mobile, cet accord de phase subsistera. En effet, au temps t le mobile est à une distance de l'origine égale à $vt = x$; son mouvement interne est alors représenté par $\sin 2\pi\nu_1 \frac{x}{v}$.

(¹) Au sujet de la présente Note, voir M. Brillouin, *Comptes rendus*, t. 168, 1919, p. 1318.

EM: Beam / Sample interaction

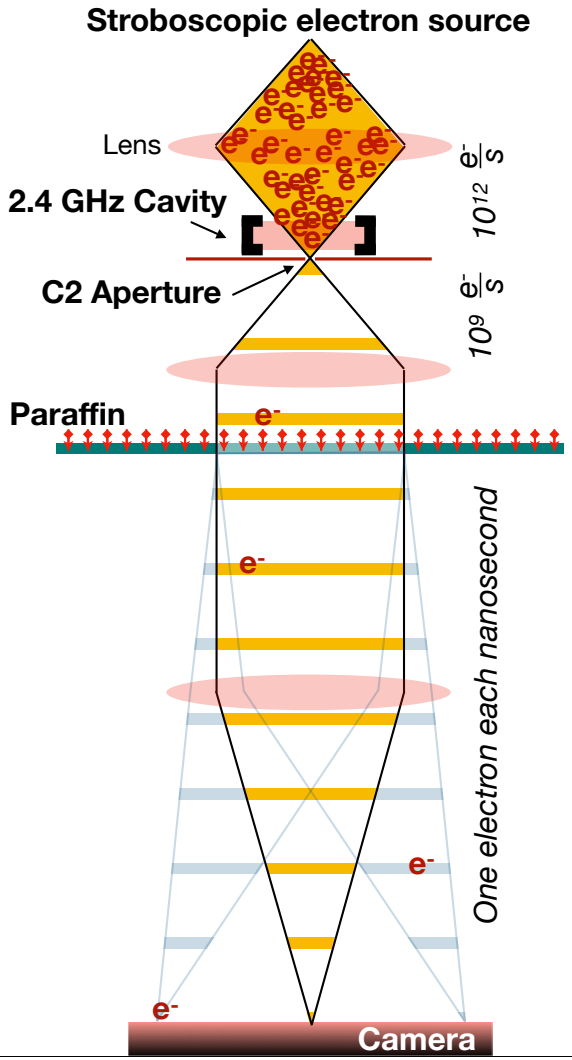


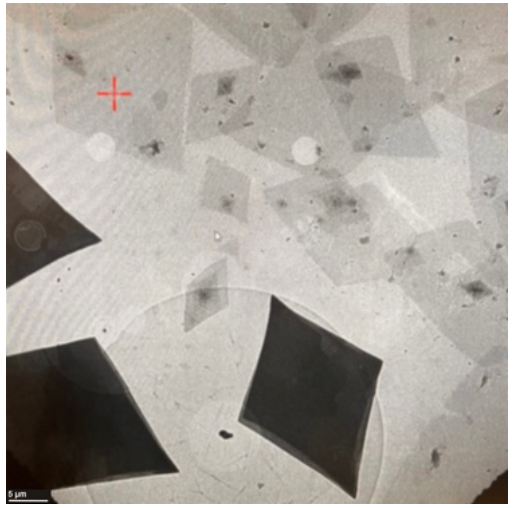




Gati et al., PNAS 114(9), 2247-2252 (2017)

Paraffin 2D Crystal under stroboscopic single electron diffraction

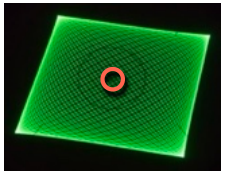




Electron diffraction

Tetratetracontane ($C_{44}H_{90}$)

Orthorhombic crystal structure



	X/Y Lissajous
Beam Frequency	75 MHz
Beam at gun	60.0 nA (!!!)
Beam on sample	4 pA
Beam diameter	548 nm
Fluency rate at sample	1.1 e⁻/A²/s
Pulse length	0.9 ps / 13.33 ns
On/Off ratio	1 : 15'000
Probability of 1 e- per gate	24.5%
Probability of 2 e- per gate	4.2%
Probability of 3 e- per gate	0.5%

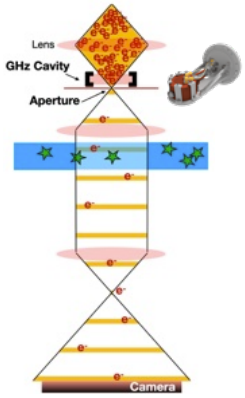
LBEM – Laboratory of Biological Electron Microscopy

EPFL Unil UNIL | Université de Lausanne

Biology: Parkinson's Disease

Physics: 4D-STEM

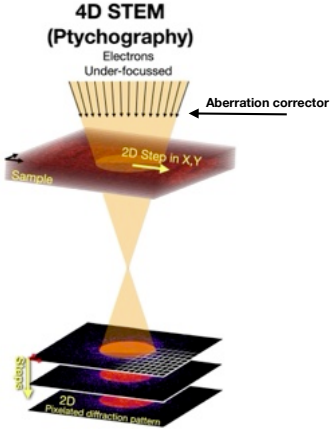
Stroboscopic
Single Electron Cryo-EM



DrX.works RF Cavity
"Titan Chopper"

300kV Cold FEG

4D STEM
(Ptychography)



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Acknowledgement

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Roland Riek
Paola Picotti
Tatiana Serdiuk

Uni Basel
Thomas Braun

CNRS Paris
Ronald Melki
Alexis Fenyi

VUMC, Amsterdam
Wilma van de Berg
Annemieke J.M. Rozemuller,

CNRS Bordeaux
François Ichas
Luc Bousset

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Hilal Lashuel

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Richard Henderson

TU München
Knut Müller-Caspary
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ER-C Jülich
Carsten Sachse

Weizmann, Israel
Michael Elbaum
Shahar Seifer

SLS Detector Group, PSI
Bernd Schmitt
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Hoffmann-La Roche, Basel
Matthias Lauer
Markus Britschgi

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