MICROCRYSTAL ELECTRON DIFFRACTION: THEORY AND APPLICATION

Cryo-EM Course at the Laboratory for BioMolecular Structure (LBMS)

Brookhaven National Laboratory

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There’s plenty of room at the bottom

Richard Feynman at the American Physical Society meeting 1959

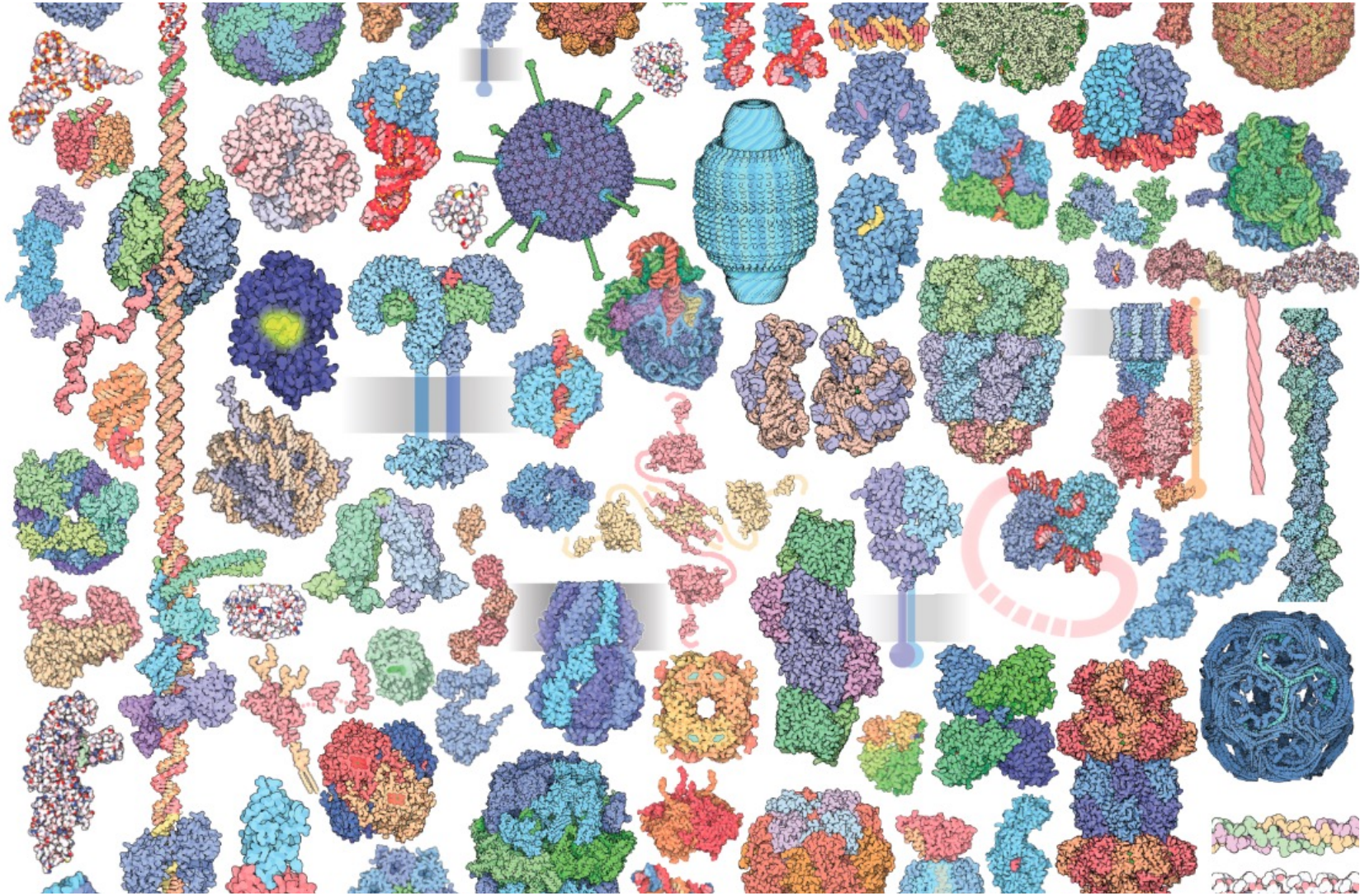
“What you should do in order for *us* to make more rapid progress is to make the electron microscope 100 times better.”

“It is very easy to answer many of these fundamental biological questions; you just look at the thing!”

“It would be very easy to make an analysis of any complicated chemical substance; all one would have to do is look at it and see where all the atoms are!”

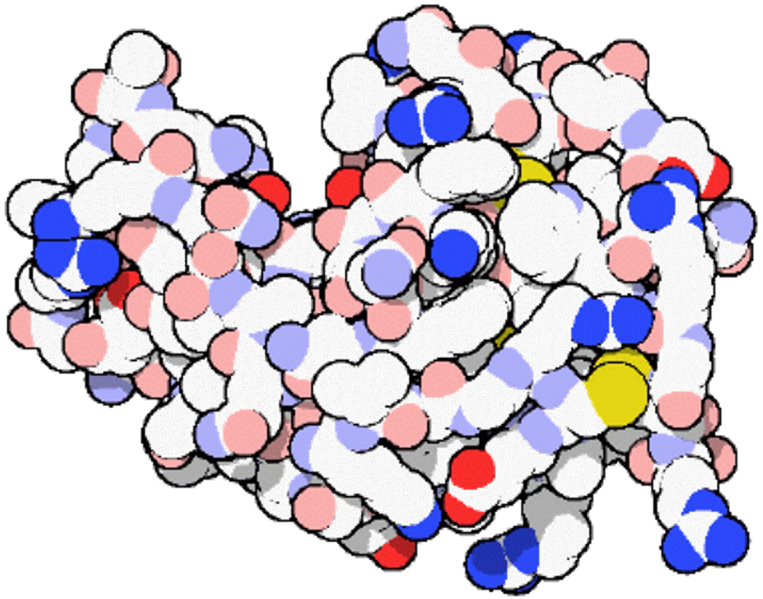
https://www.nobelprize.org/

Structure and Function

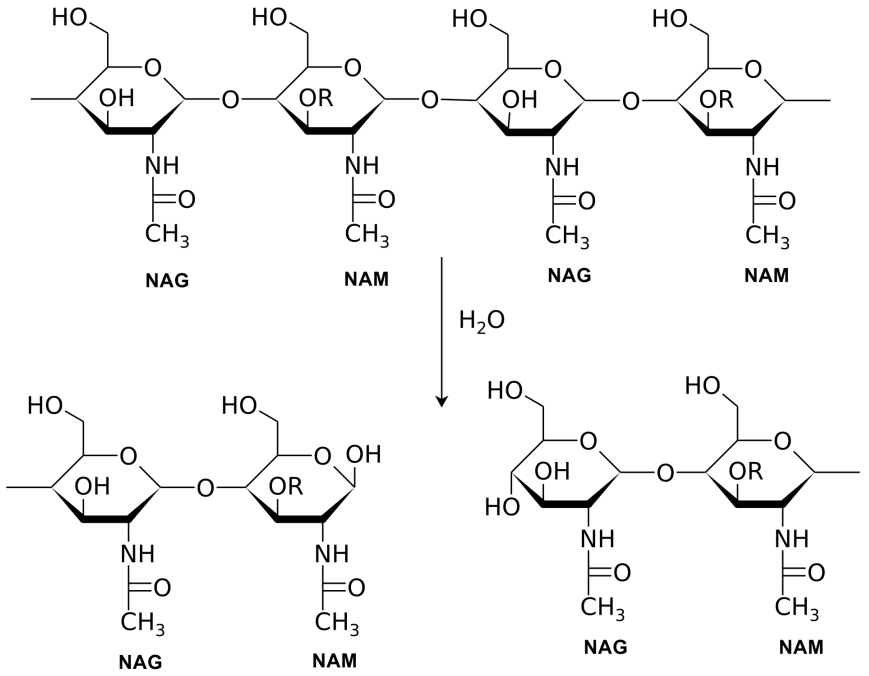


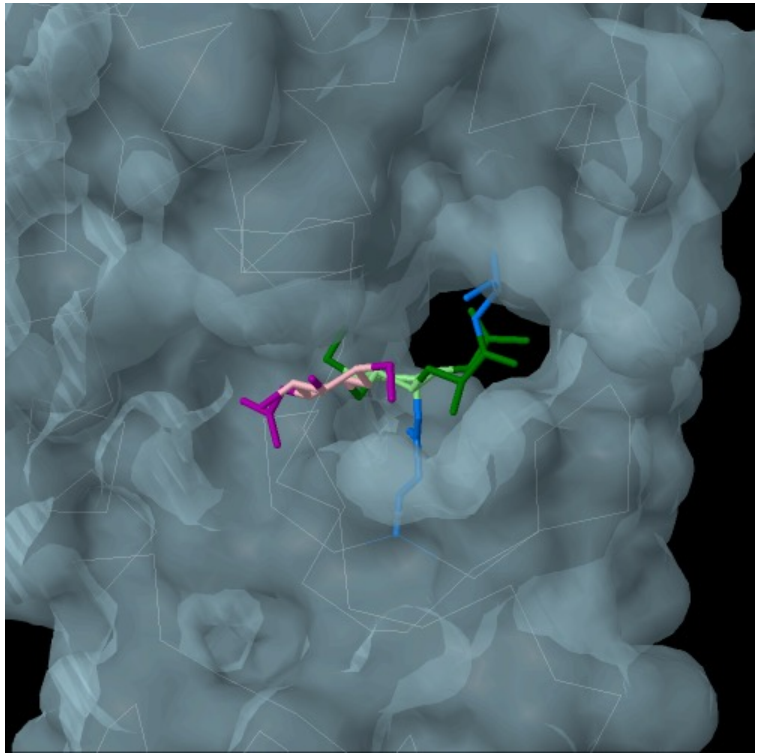
https://pdb101.rcsb.org/motm/motm-about

Structure and Function



PDB 2LYZ





https://pdb101.rcsb.org/
https://en.wikipedia.org/wiki/Lysozyme/

Much better microscopes

Transmission Electron Microscopy of Beryllium

We have prepared thin films of beryllium in which it is possible to observe directly the arrangements of dislocations and other structural defects by transmission electron microscopy. The films were made by two methods.

Beryllium was deposited as thin (0·03–0·05 mm.) polycrystalline flake on cool surfaces from metal melted by electron bombardment. This flake was etched in a dilute aqueous solution of hydrofluoric and nitric acids to give specimens suitable for examination. The grain-size was about 1μ. Single-crystal specimens were prepared by cleaving a beryllium crystal parallel to the basal plane to obtain a flake which was ground and polished down to a thickness of 0·02 mm. and then etched. Specimens were examined using 70-kV. electrons in the prototype Metropolitan-Vickers *E.M.*6 electron microscope.

Grain boundaries and single perfect dislocations were clearly visible in the evaporated flake. Many grains were structurally perfect while others contained several dislocations. From the electron-optical fringes on the grain boundaries the films were shown to be several thousand angstrom units thick.

A low-magnification electron micrograph of a single-

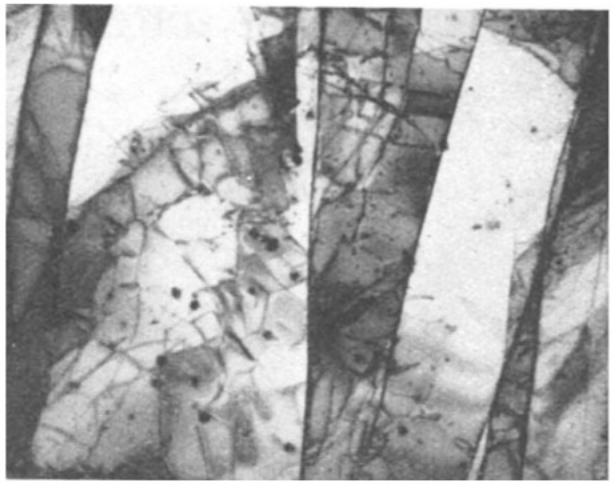


Fig. 2. Bend planes, dislocations and dislocation loops in beryllium. (×40,000)

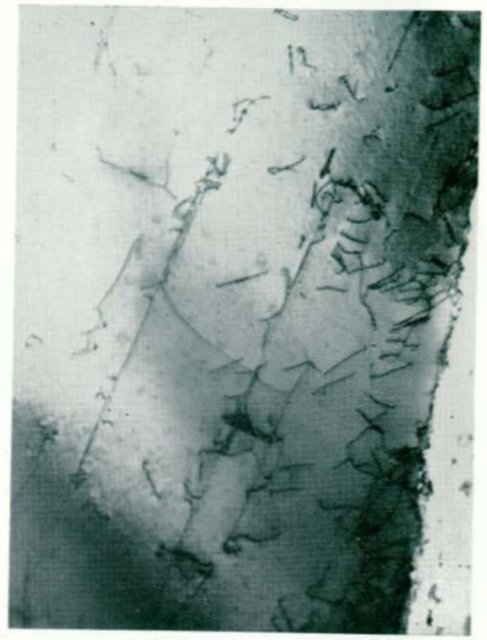
evidence with the present observations one may conclude that the boundaries consist of edge dislocations stacked normal to the basal plane. It is yet to be determined whether these boundaries ('bend planes') are formed during the growth of the crystal from the melt or during cleavage.

Baird et al. Nature (1958)

An Electro-Polishing Technique for the Preparation of Metal Specimens for Transmission Electron Microscopy†

By Heather M. Tomlinson (*née* Murphy)
Crystallographic Laboratory, Cavendish Laboratory, Cambridge

[Received April 24, 1958]



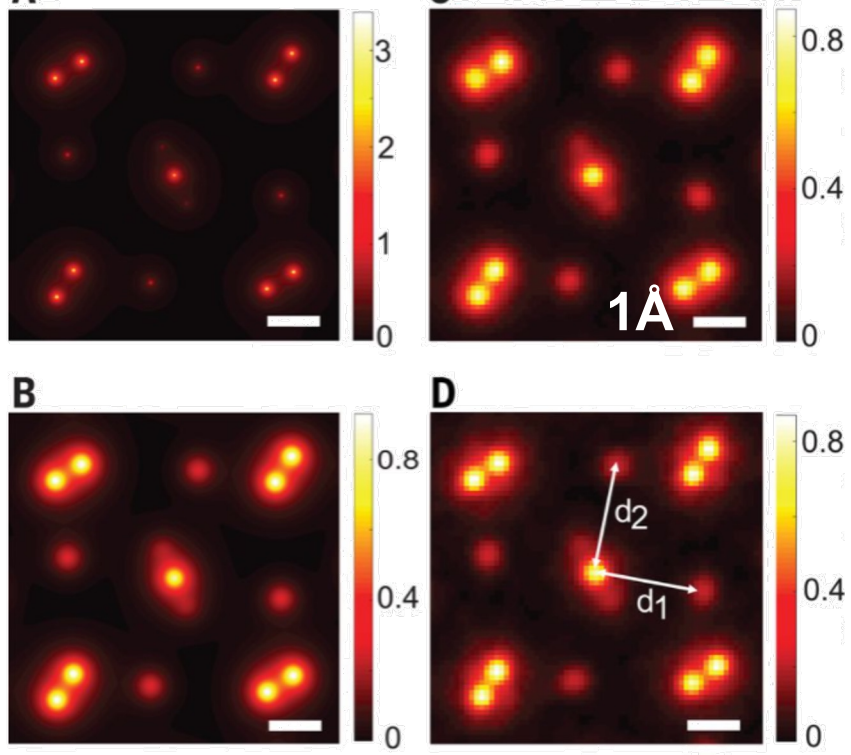
Dislocations arranged on slip planes in polycrystalline α-brass, deformed 1% by rolling at room temperature. Small regions of stacking faults can be seen in various areas. (Magn. ×40 000, 100 kv.)

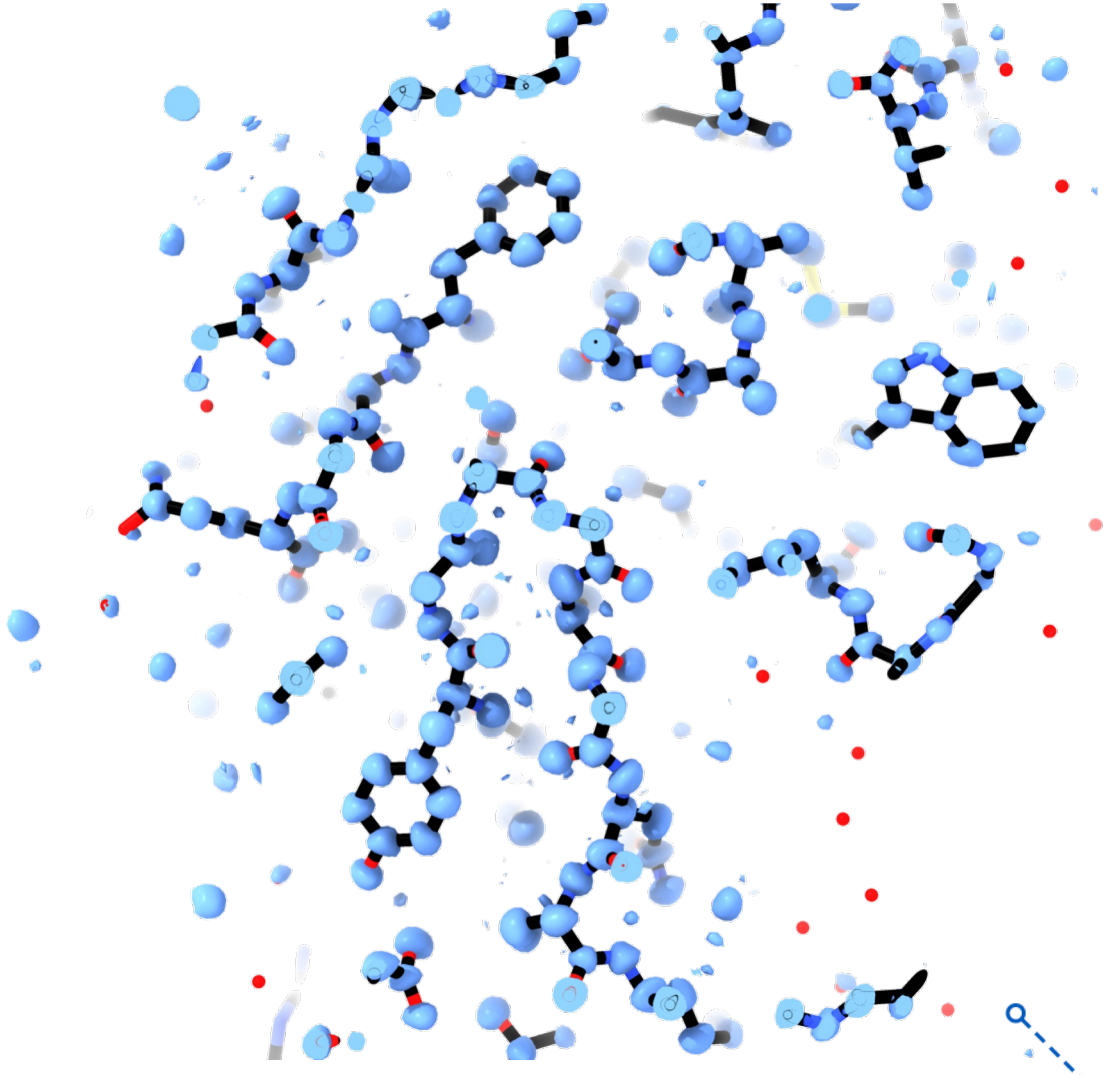
Much better microscopes

ELECTRON MICROSCOPY

Electron ptychography achieves atomic-resolution limits set by lattice vibrations

Zhen Chen[1]*, Yi Jiang[2], Yu-Tsun Shao[1], Megan E. Holtz[3]†, Michal Odstrčil[4]‡, Manuel Guizar-Sicairos[4], Isabelle Hanke[5], Steffen Ganschow[5], Darrell G. Schlom[3,5,6], David A. Muller[1,6]*



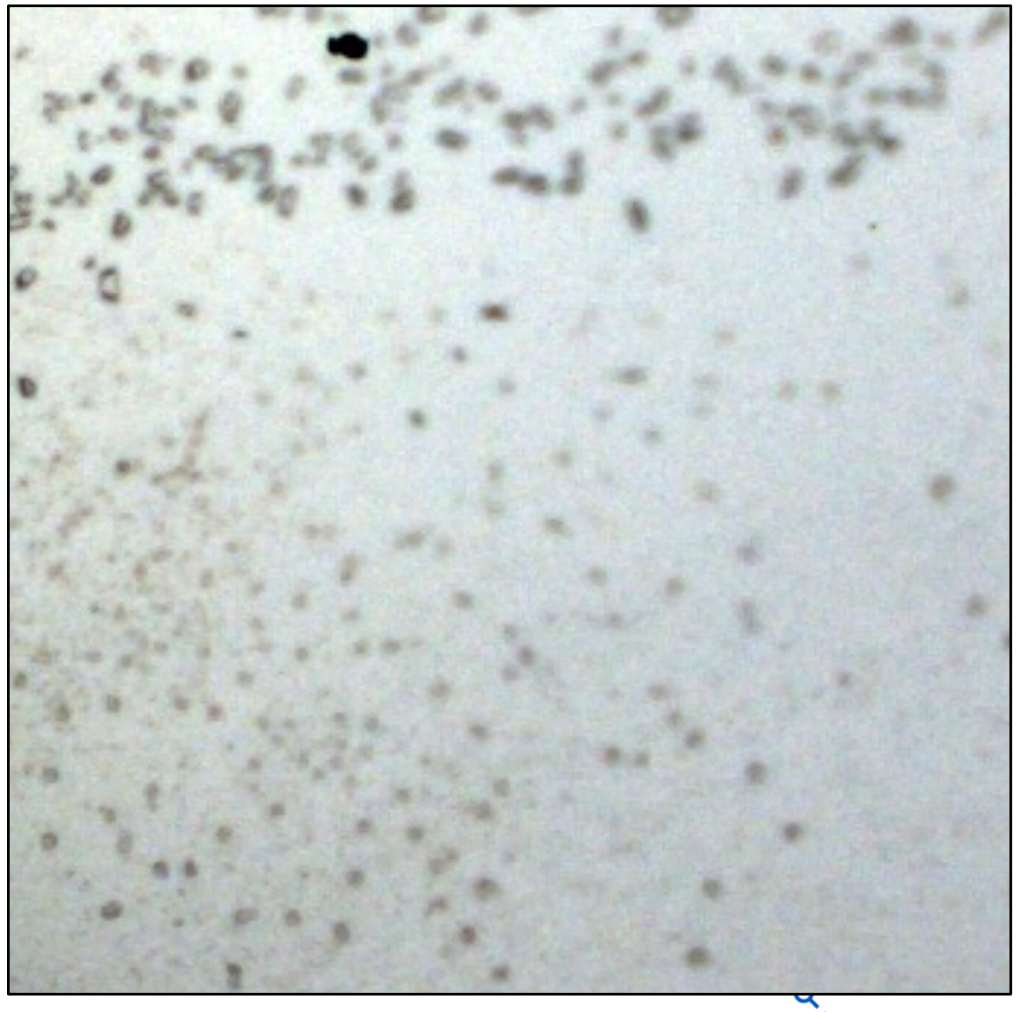


Chen et al. Science (2021)

Martynowycz et al. Nature Methods (2021)

Why are we talking about electrons?

- X-ray crystallography has been the main method for high-resolution structure determination
- In conventional X-ray crystallography, large crystals are needed
- Large crystals can extremely difficult and time consuming to grow
- There is a need in crystallography to develop methods for micro and nanocrystals

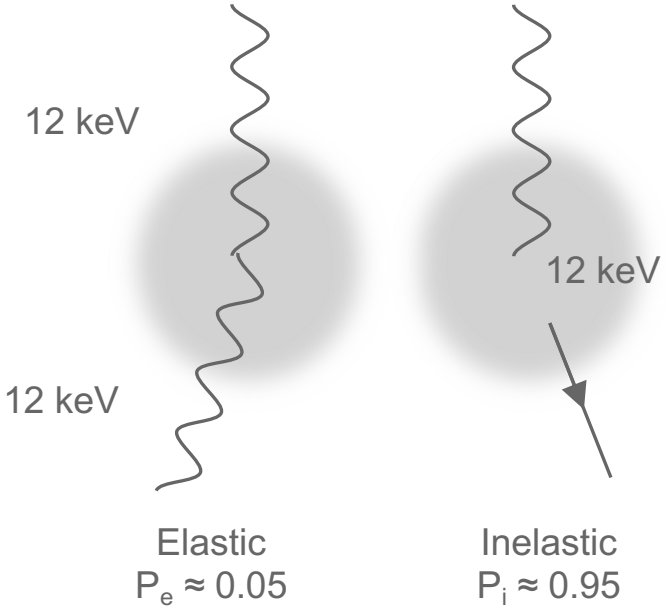


Henderson, Quarterly reviews of biophysics (1995)

Why are we talking about electrons?

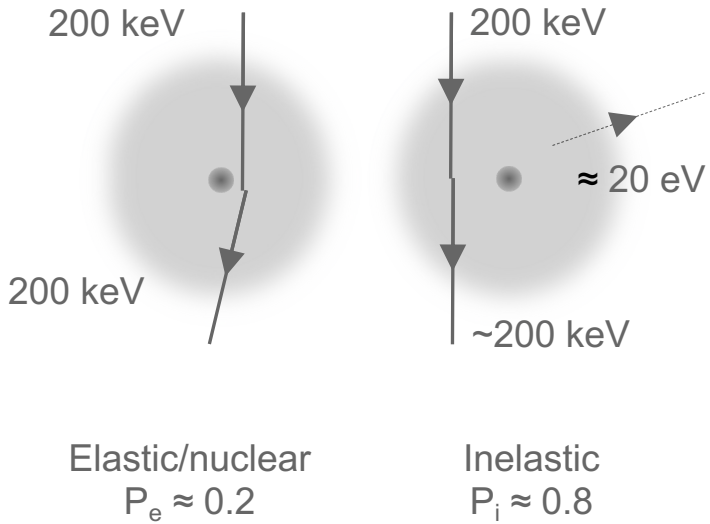
Electrons: less damage per interaction than X-rays

X-rays



≈ 200 keV collateral damage
per useful diffracted quantum

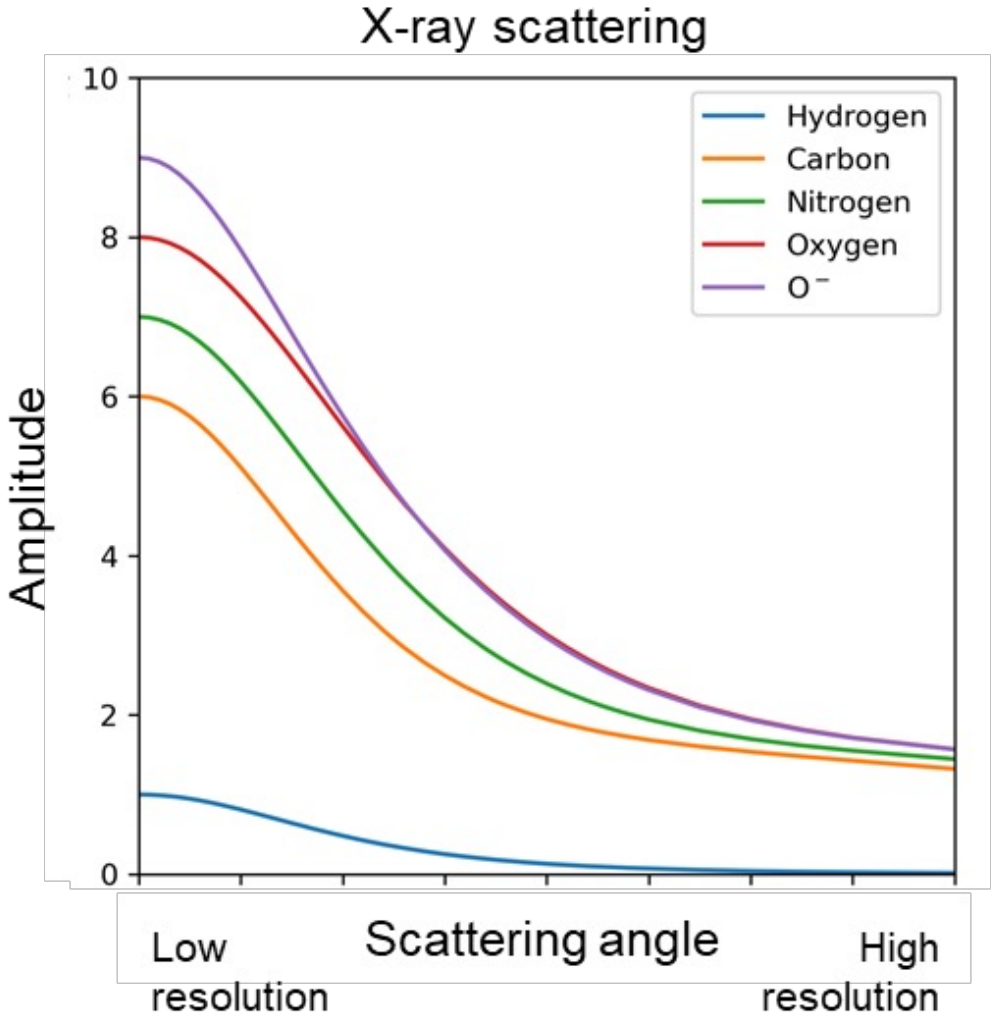
Electrons

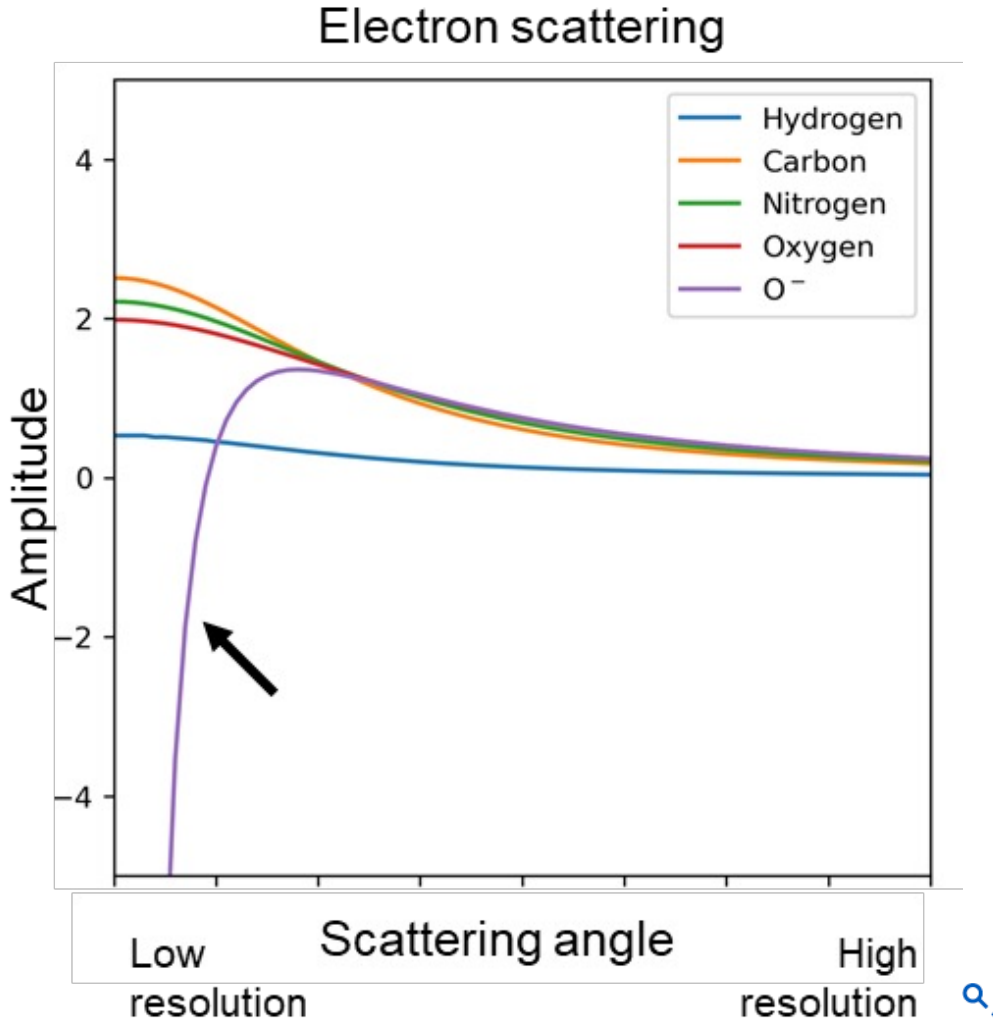


≈ 20 eV collateral damage
per useful diffracted quantum

Henderson, Quarterly reviews of biophysics (1995)

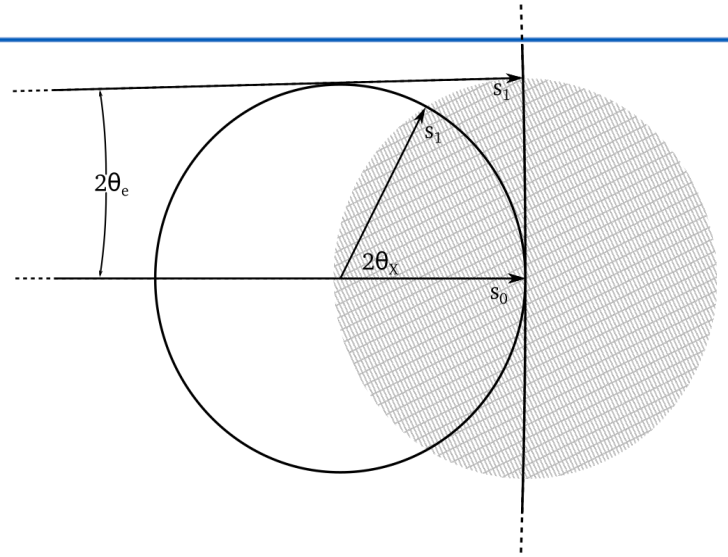
X-rays and electrons scatter differently

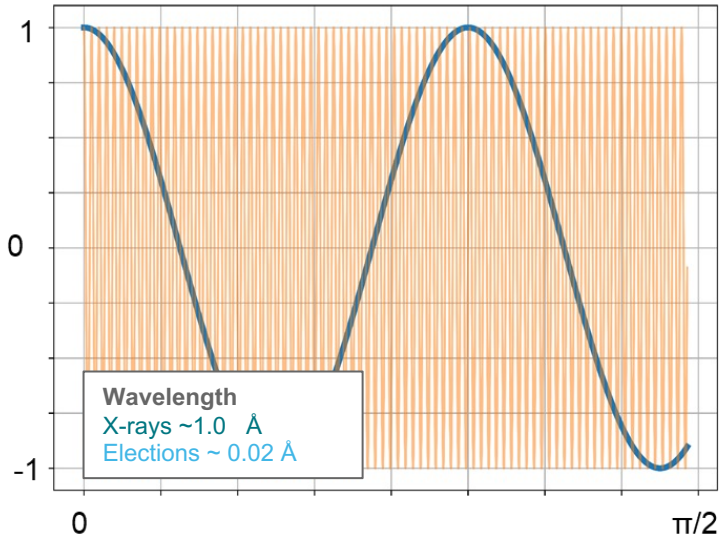




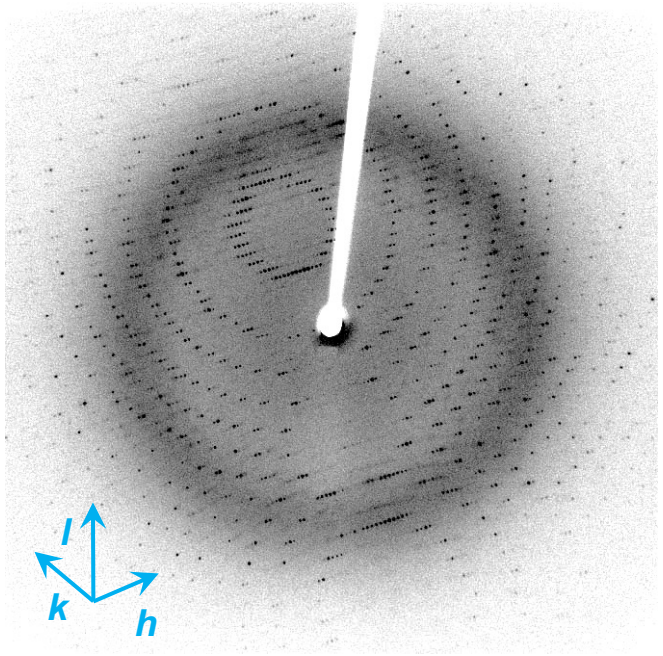
Martynowycz and Gonen, Physics Today (2022)

Electron scattering geometry



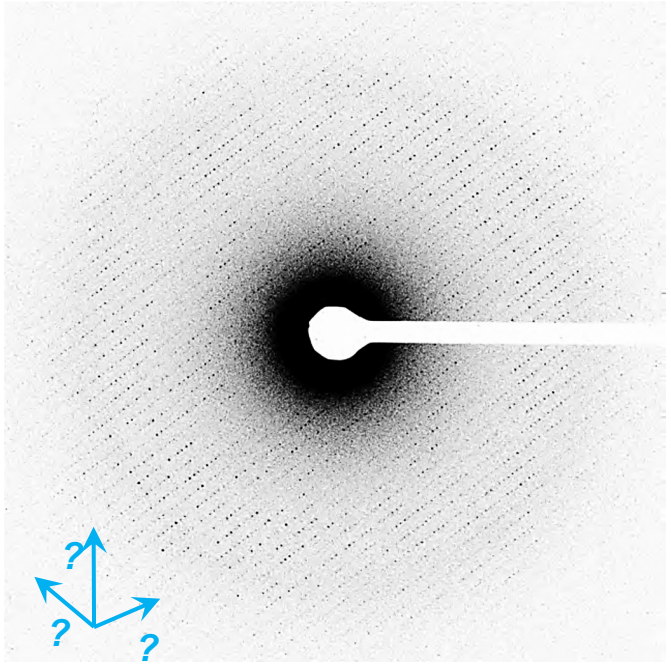


X-ray diffraction

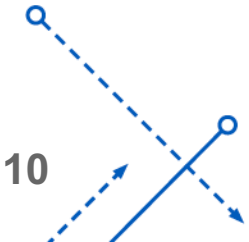


12 keV X-ray photons, λ = 1.0332 Å
Scattering angle $2\theta_X \approx 62.2°$ at 1 Å

Electron diffraction

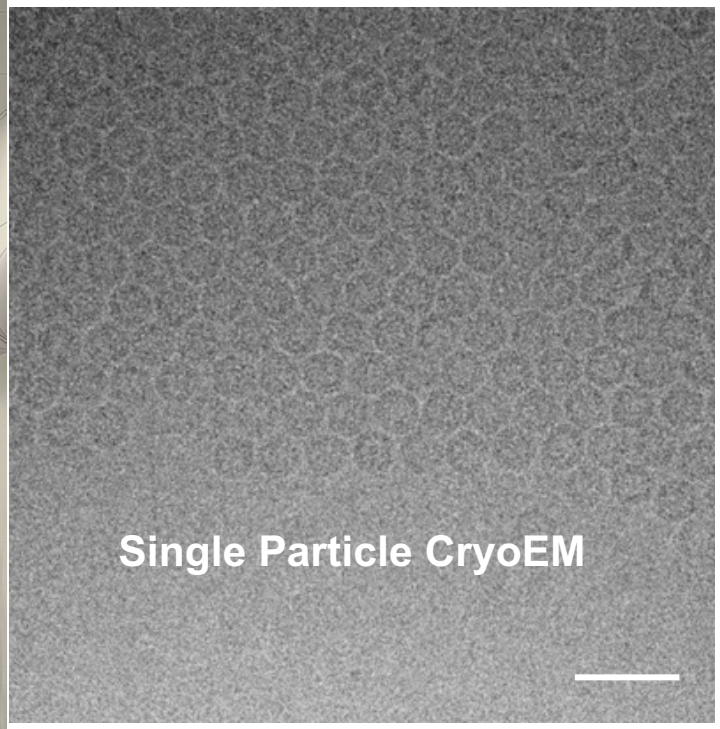


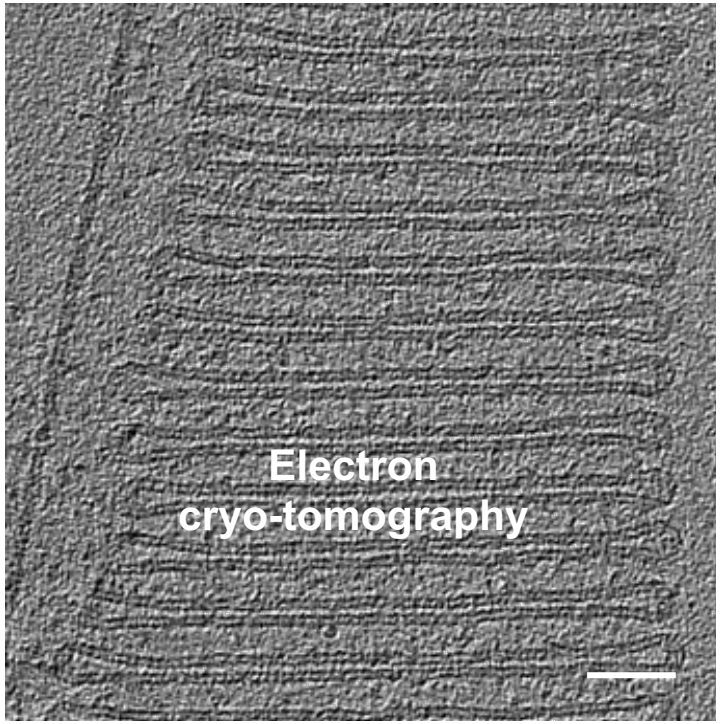
200 kV electrons, λ = 0.0251 Å
Scattering angle $2\theta_e \approx 1.4°$ at 1 Å



Martynowycz and Gonen, Physics Today (2022)

Modern Cryo-EM methods





Martynowycz and Gonen, Physics Today (2022)

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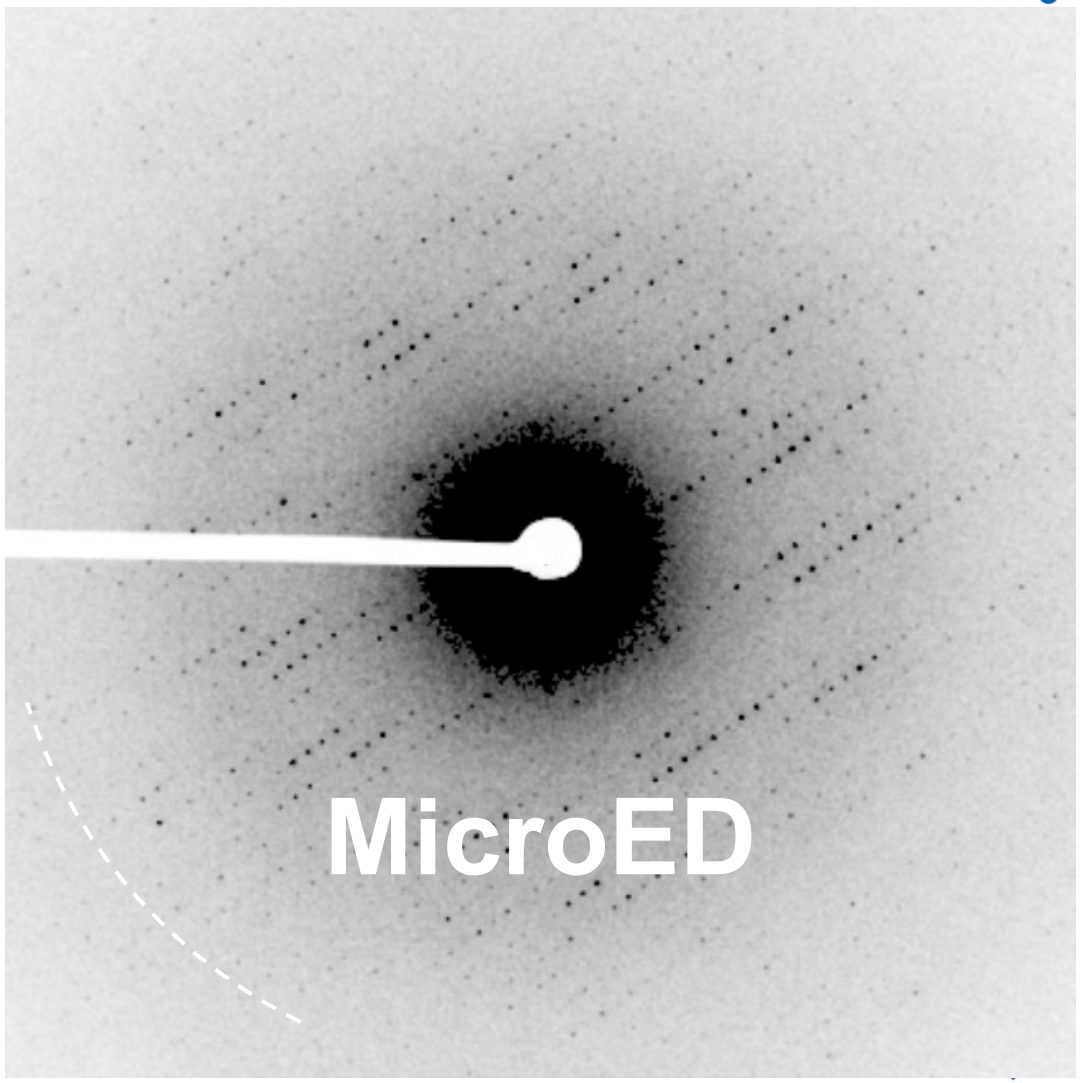
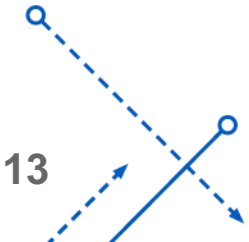


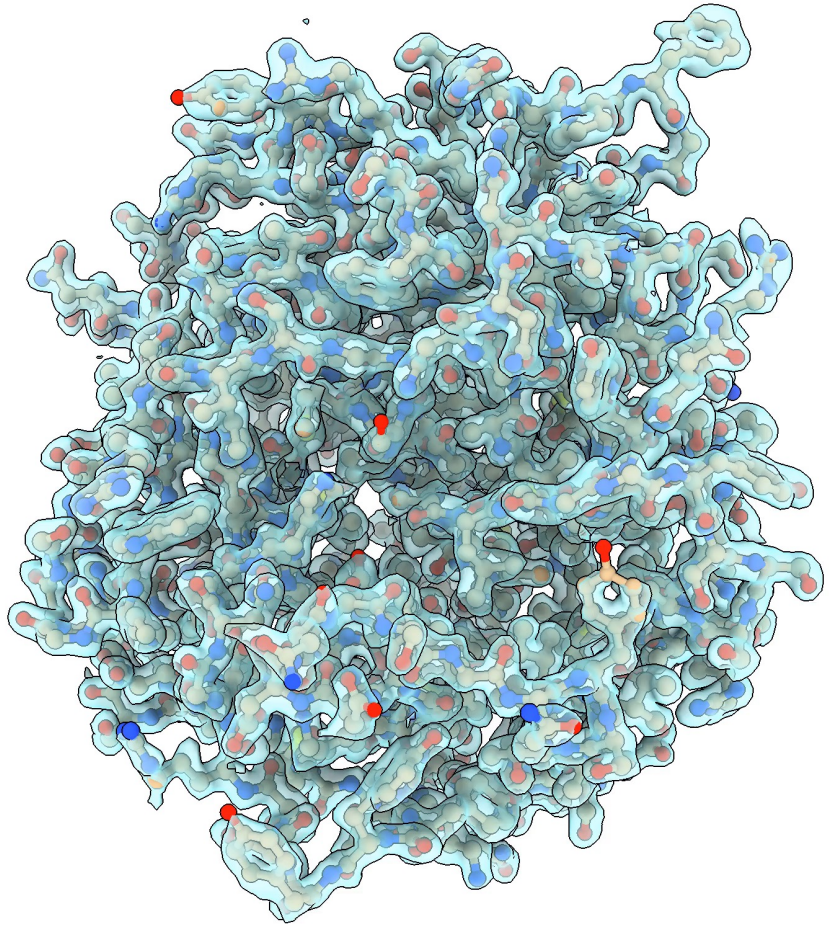
Martynowycz and Gonen, Physics Today (2022)

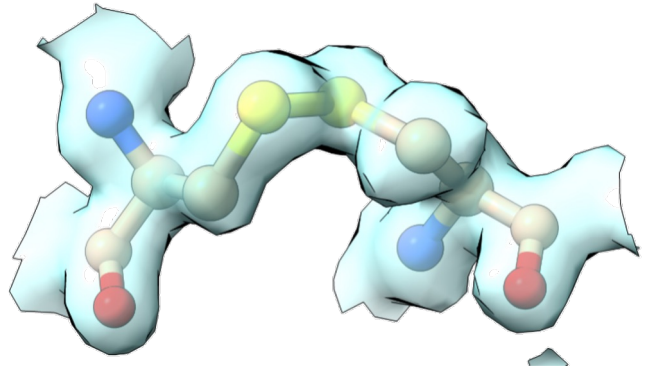
Microcrystal electron diffraction



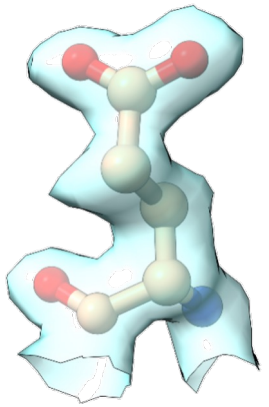
https://www.thermofisher.com/us/en/home/electron-microscopy/life-sciences/microed.html

Microcrystal electron diffraction

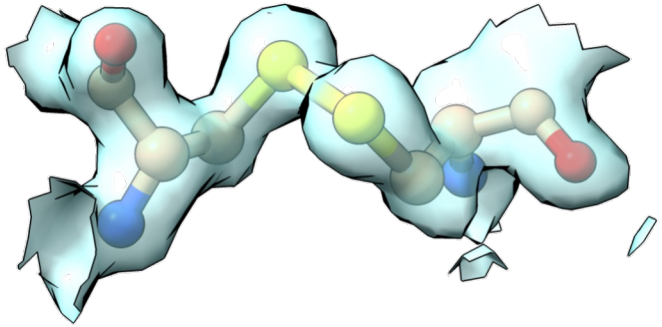




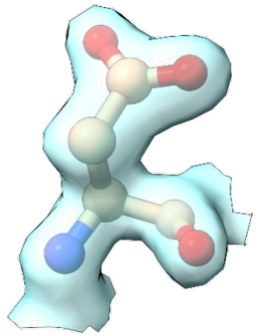
$CYS^{139} – CYS^{238}$



GLU^{148}



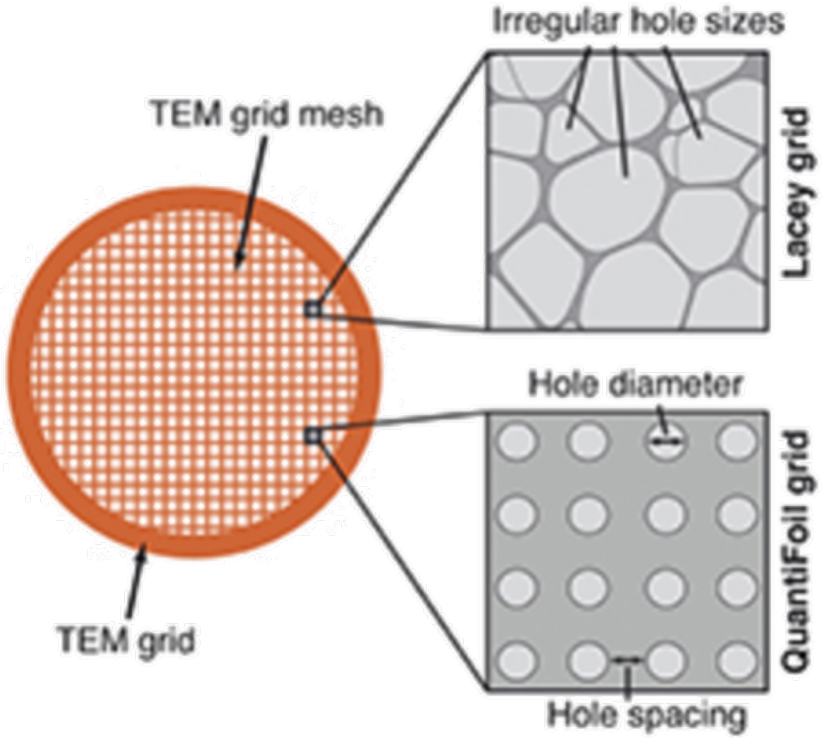
$CYS^{283} – CYS^{354}$

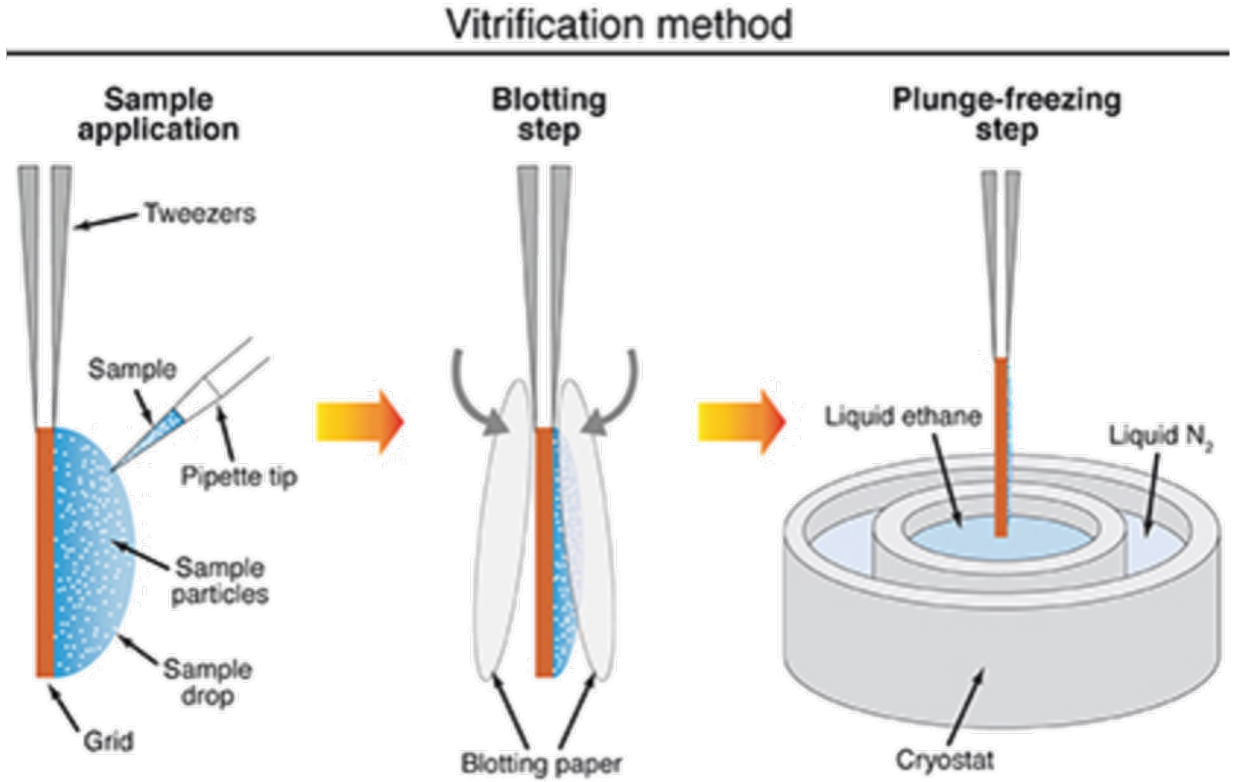


ASP^{131}

Hattne et al. Structure (2018)

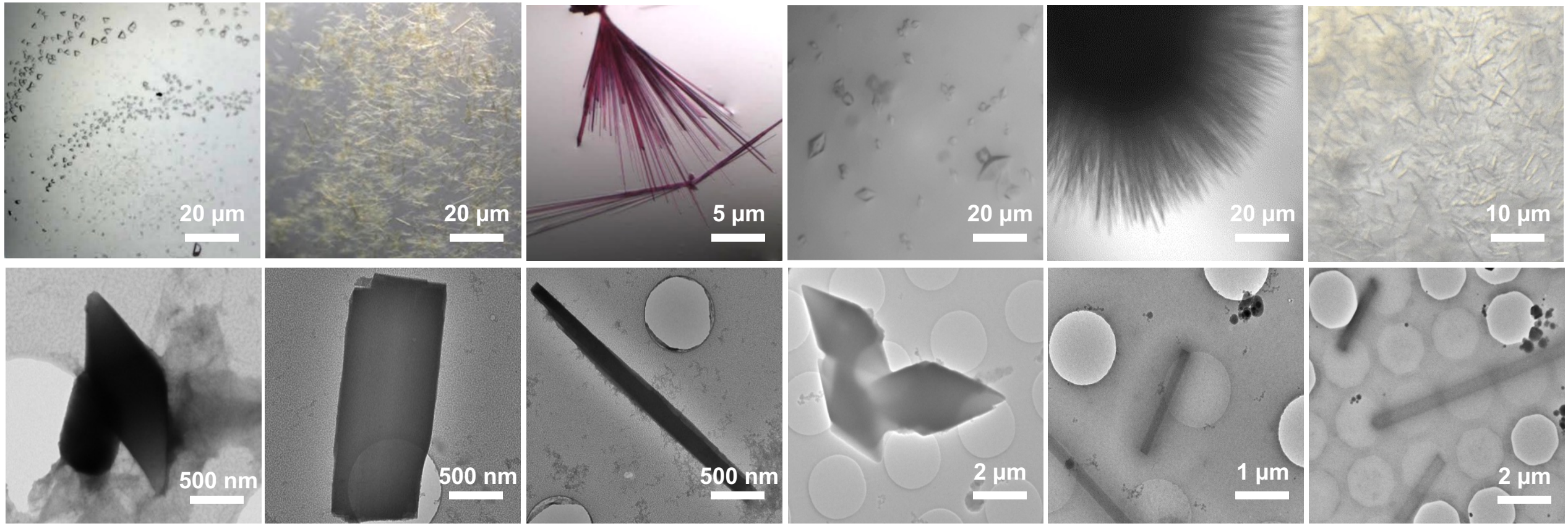
Microcrystal electron diffraction



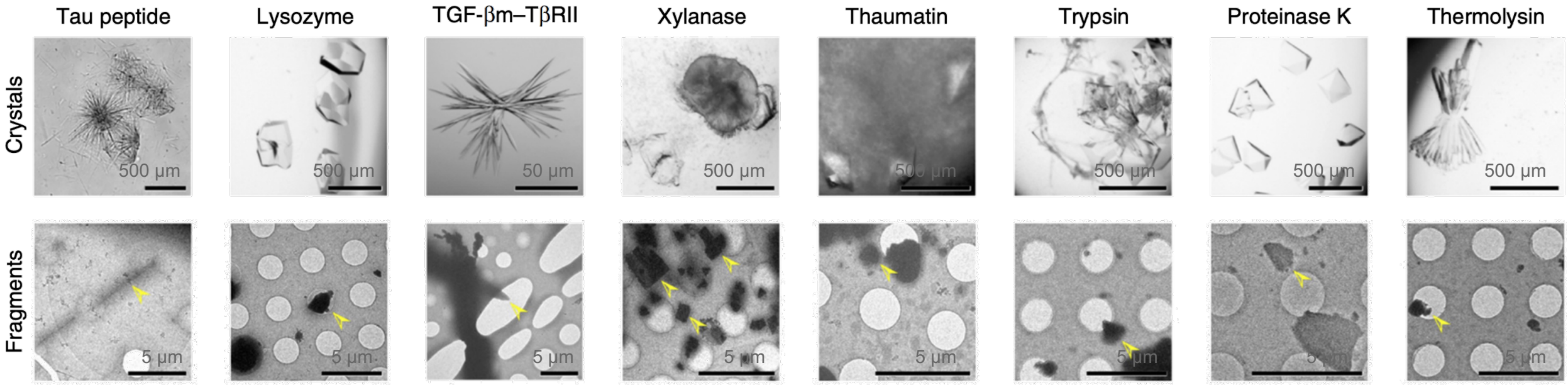


de la Cruz et al. Nature Methods (2017)
Sgro GG & Costa TR Frontiers in molecular biosciences (2018)

Microcrystal electron diffraction



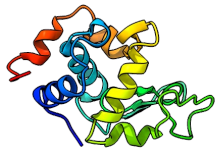
Microcrystal electron diffraction



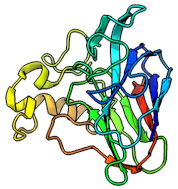
de la Cruz et al. Nature Methods (2017)
Sgro GG & Costa TR Frontiers in molecular biosciences (2018)

Microcrystal electron diffraction

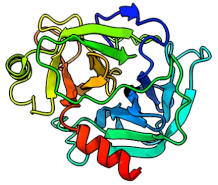
Lysozyme (3J6K)



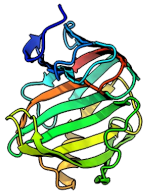
Thaumatin (5K7Q)



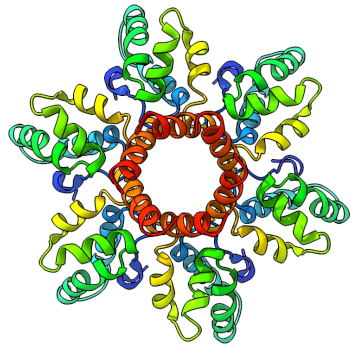
Trypsin (5K7R)



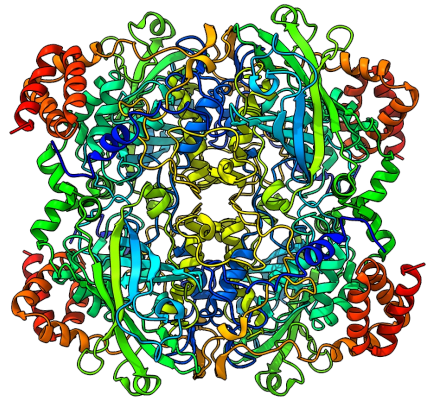
Xylanase (5K7P)



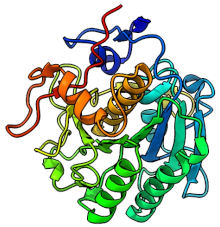
HIV1 Gag (6N3U)



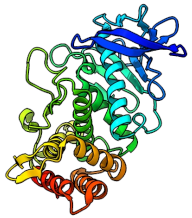
Catalase (3J7B)



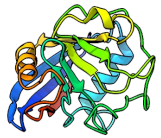
Proteinase K (6CL7)



CypA (6U5G)

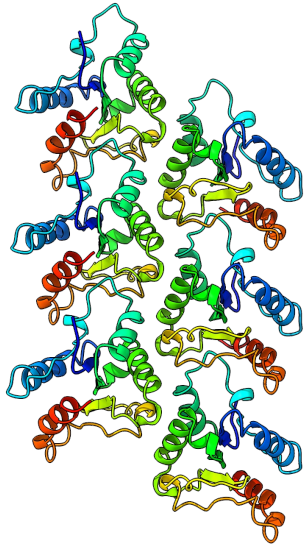


Thermolysin (5K7T)

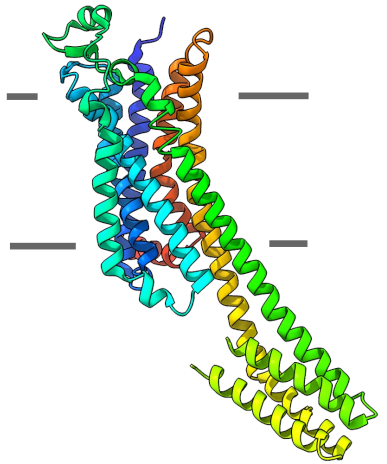


HCA II (6YMA)

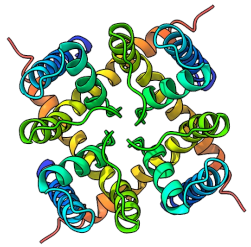
MyD88^{TIR} (7BEQ)



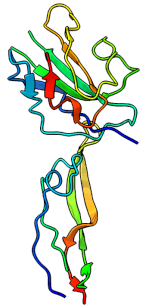
A_{2A} receptor (7RM5)



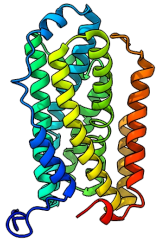
NaK channel (6CPV)



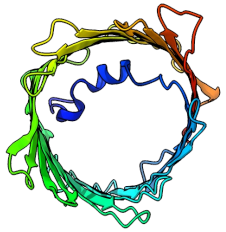
TGF-βm-TβRII (5TY4)



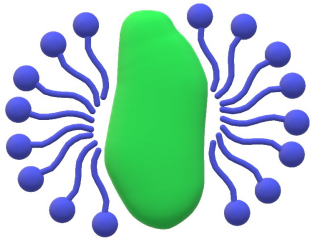
R2lox (6QRZ)



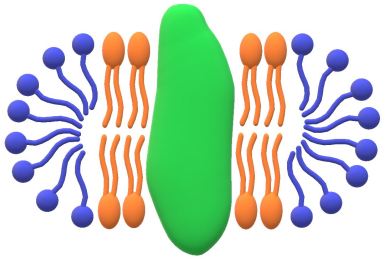
mVDAC (7KUH)



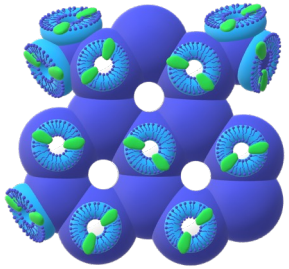
Electron crystallography of membrane proteins



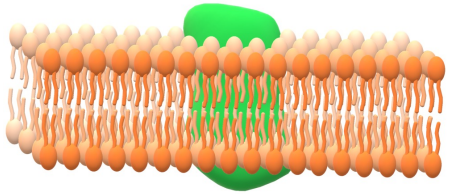
Detergent micelle



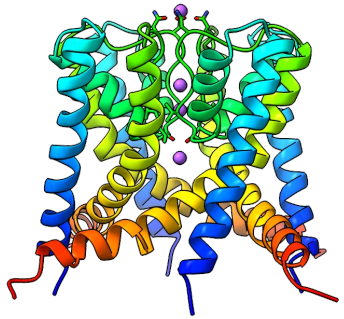
Detergent-lipid bicelle

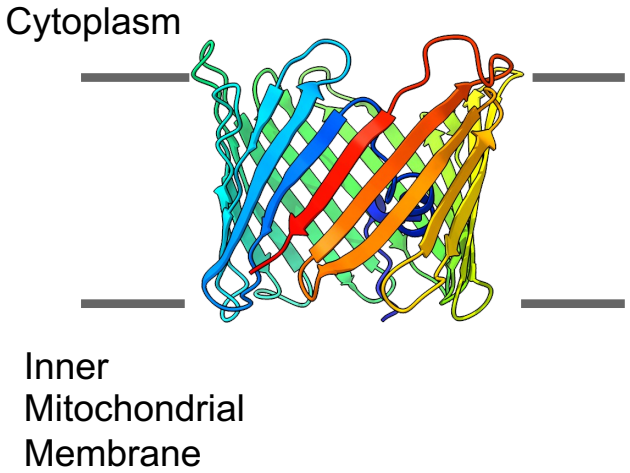


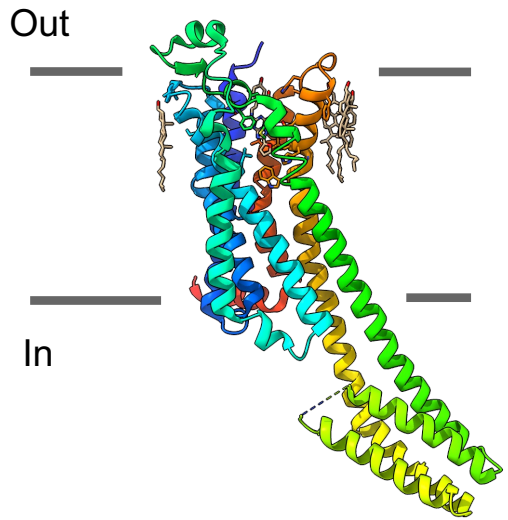
Lipidic cubic phase (LCP)

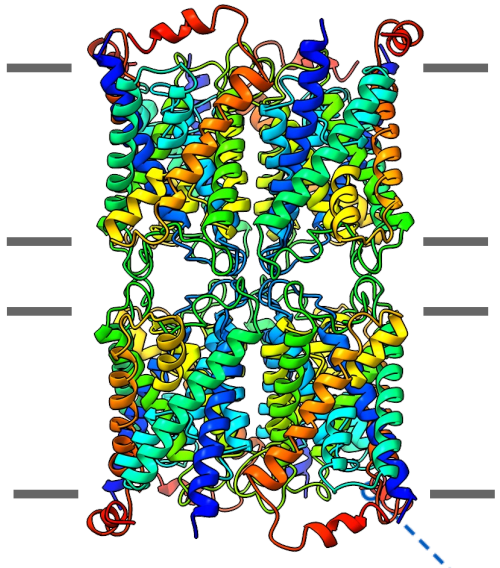


Lipid bilayer

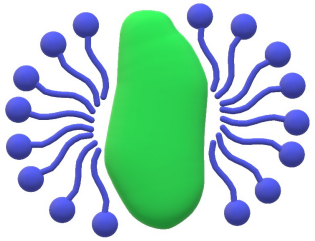




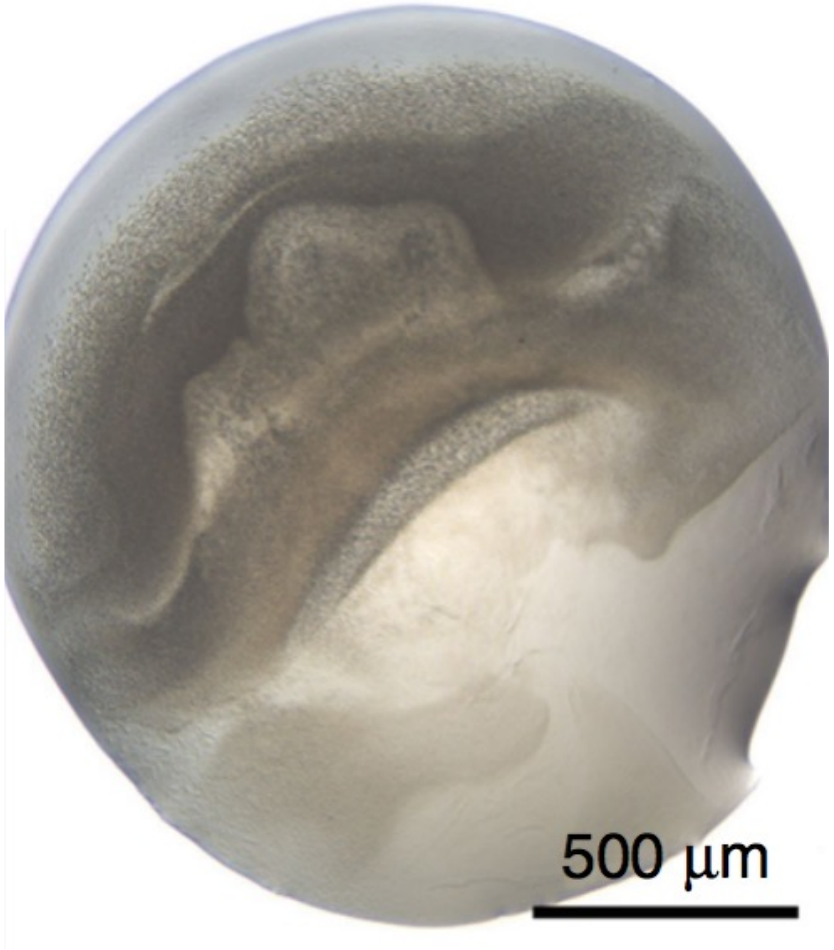


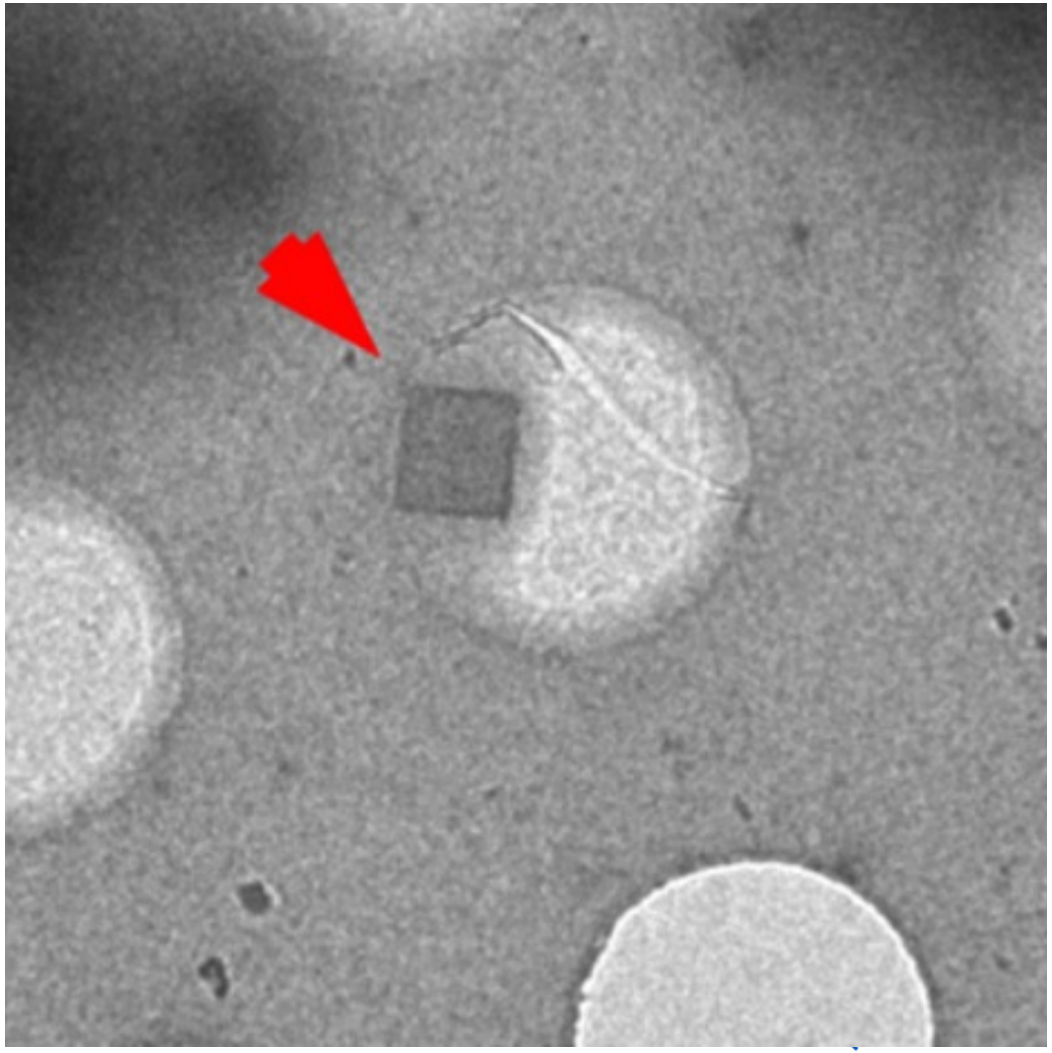


Non-selective cation channel - NaK



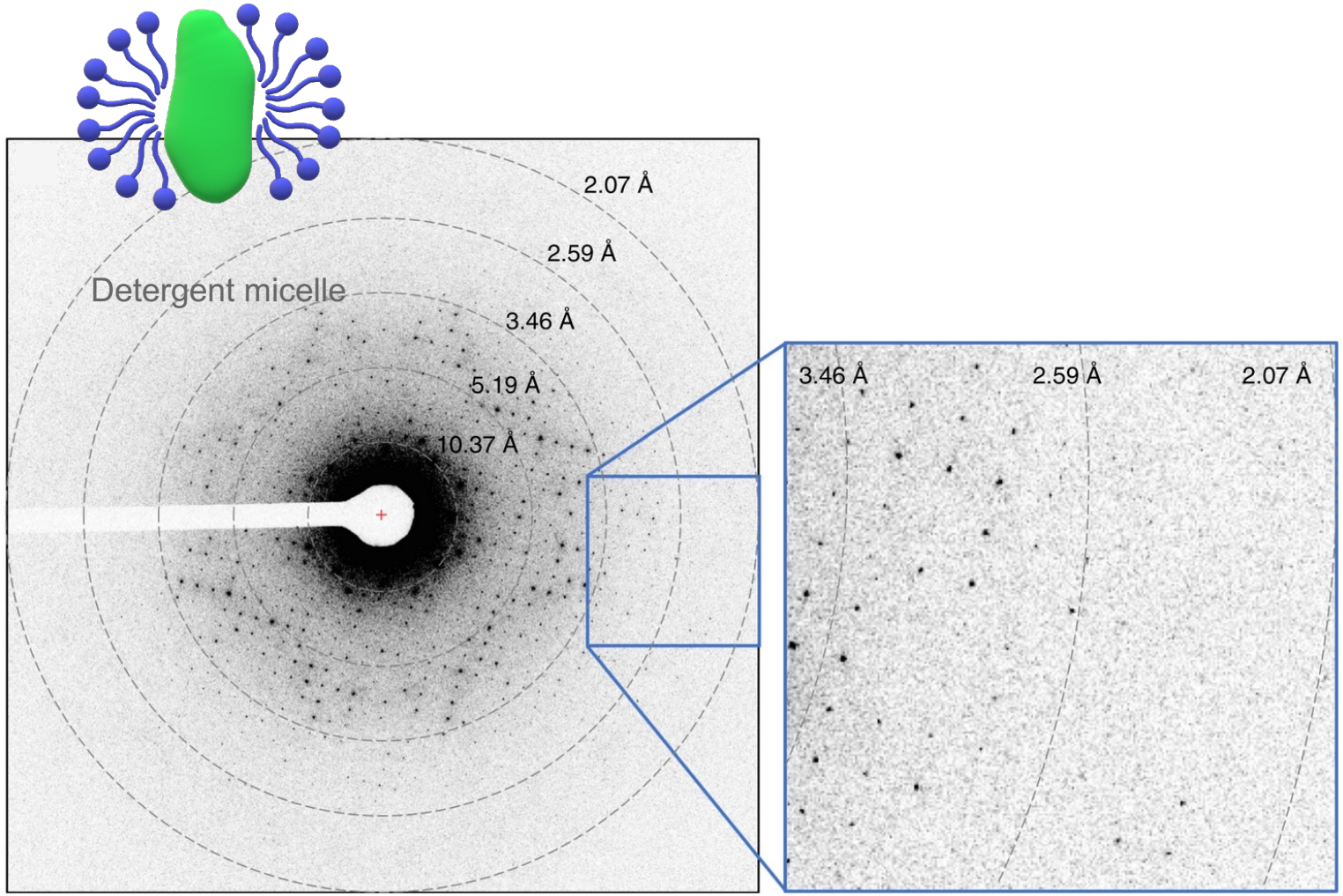
Detergent micelle

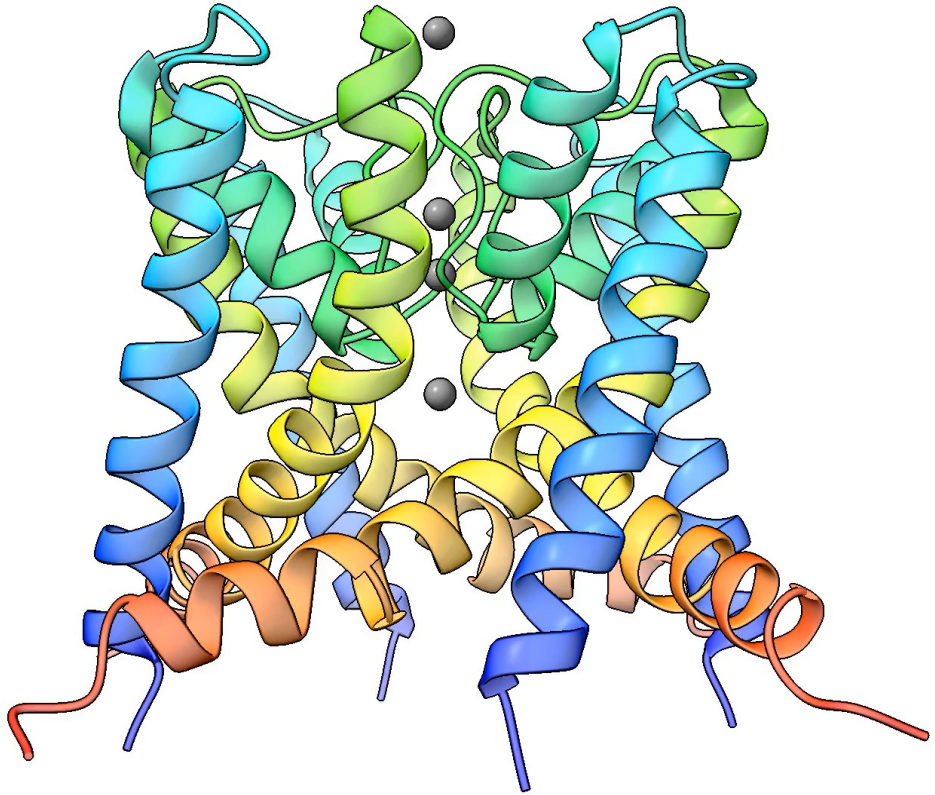




Liu and Gonen. Communications Biology (2018)

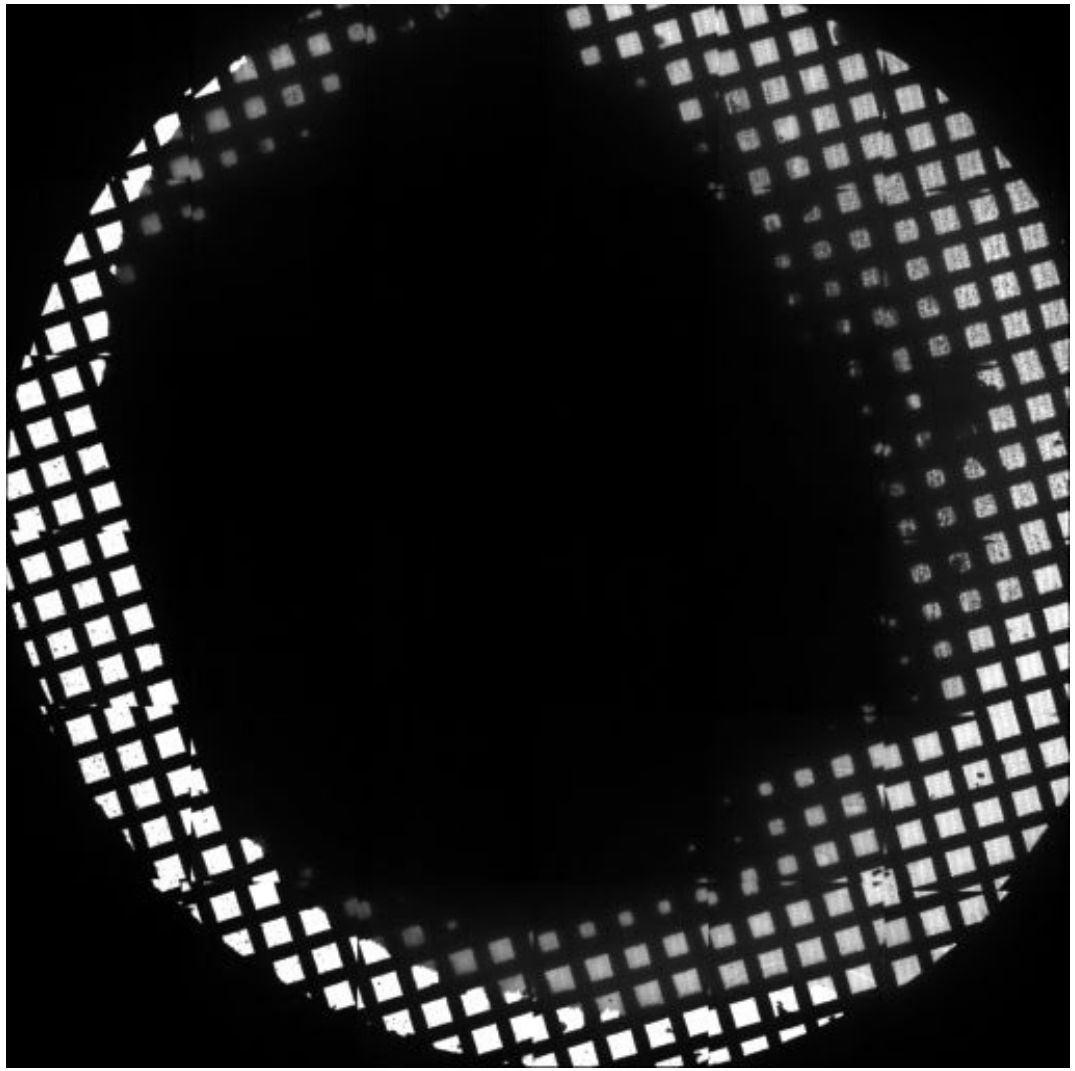
Non-selective cation channel - NaK

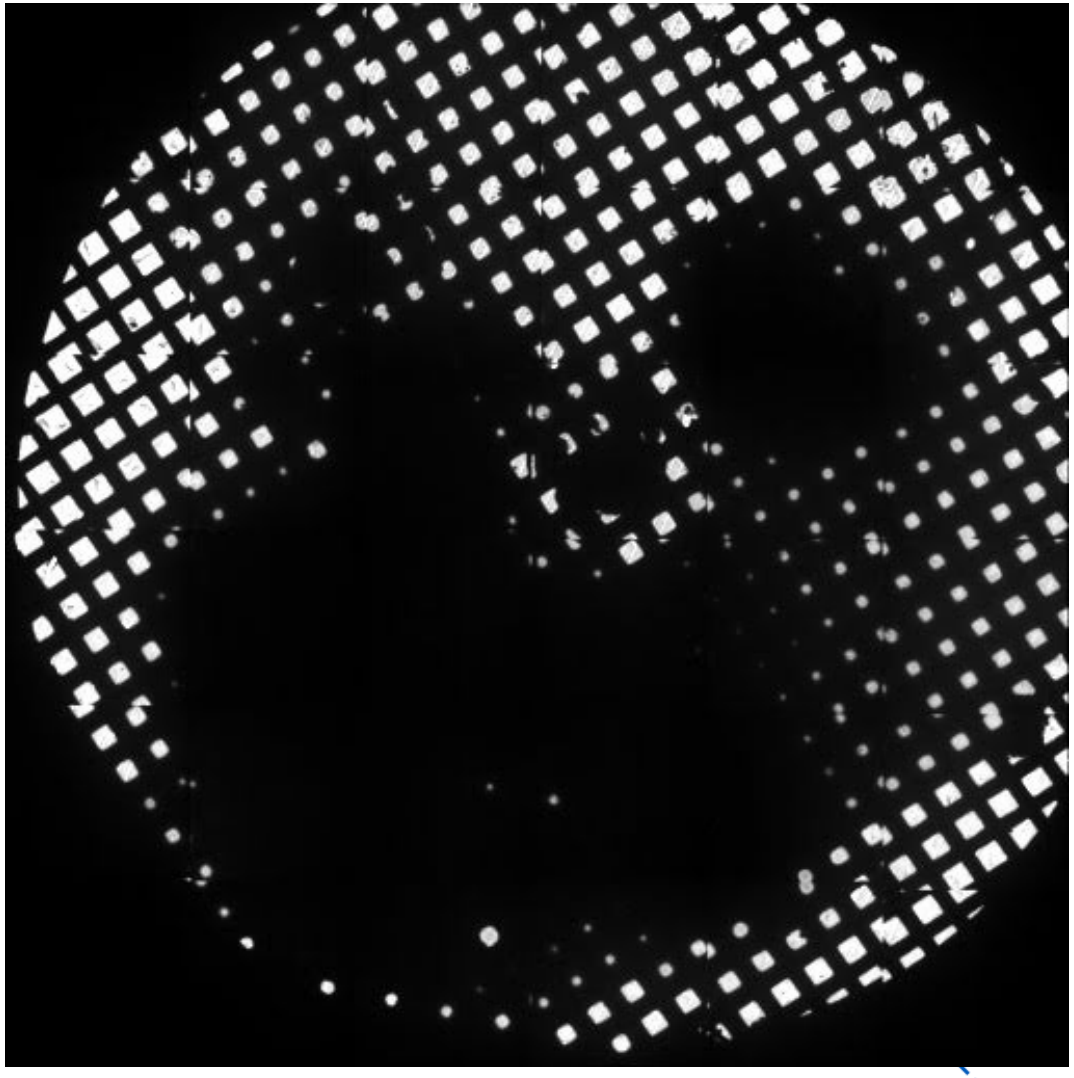




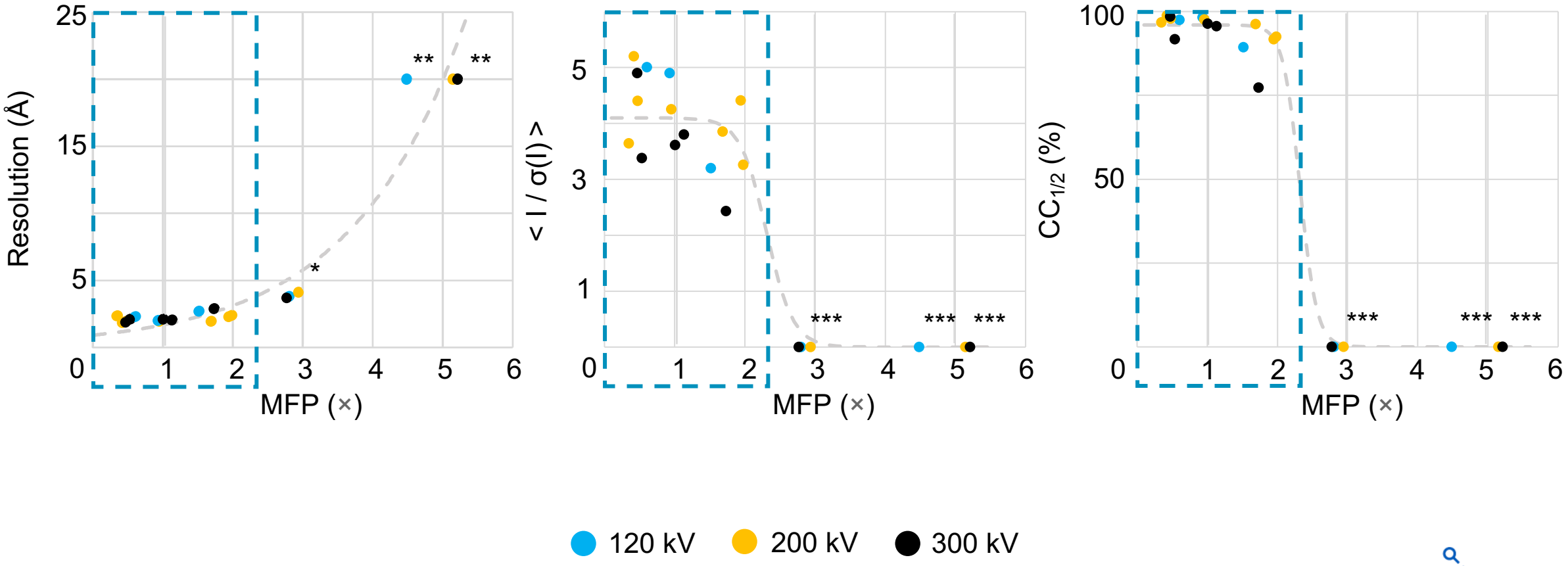
Liu and Gonen. Communications Biology (2018)

Where are my crystals?



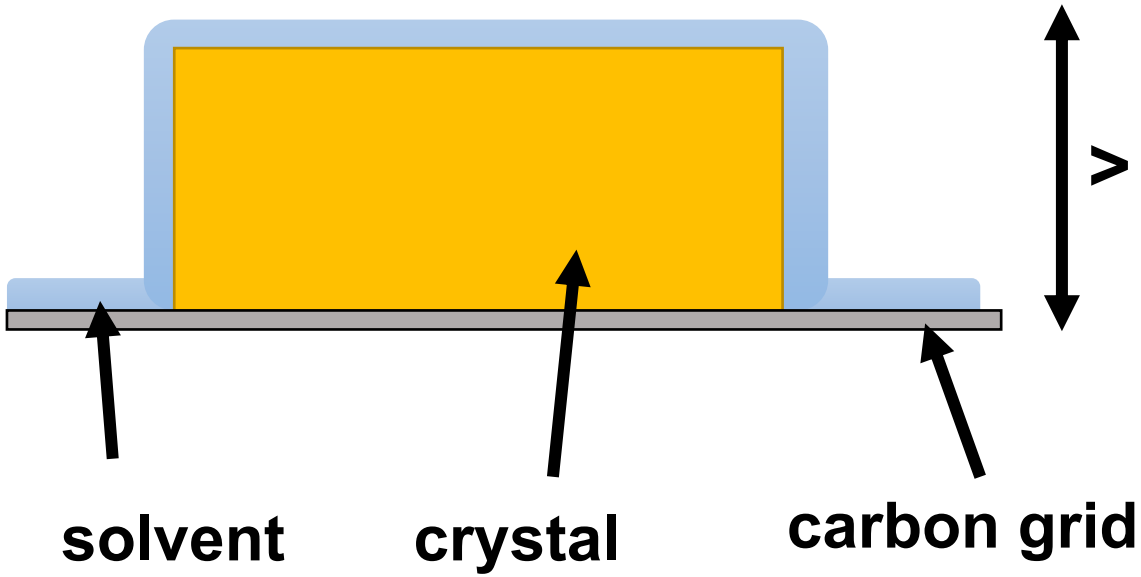


Where are my crystals?

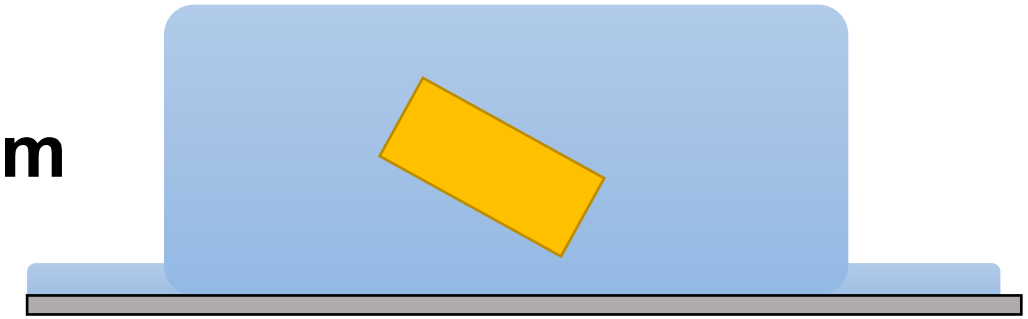


Where are my crystals?

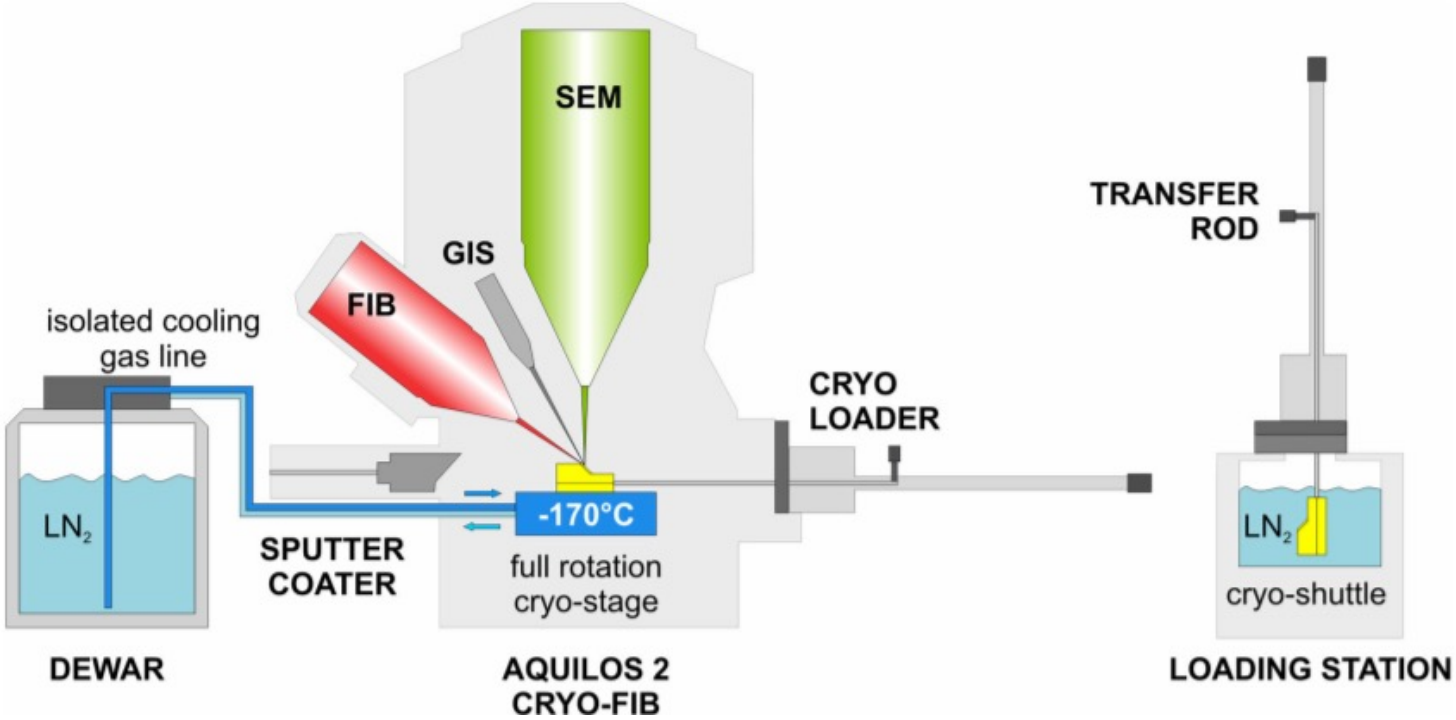
Crystal too big



Crystal embedded in media

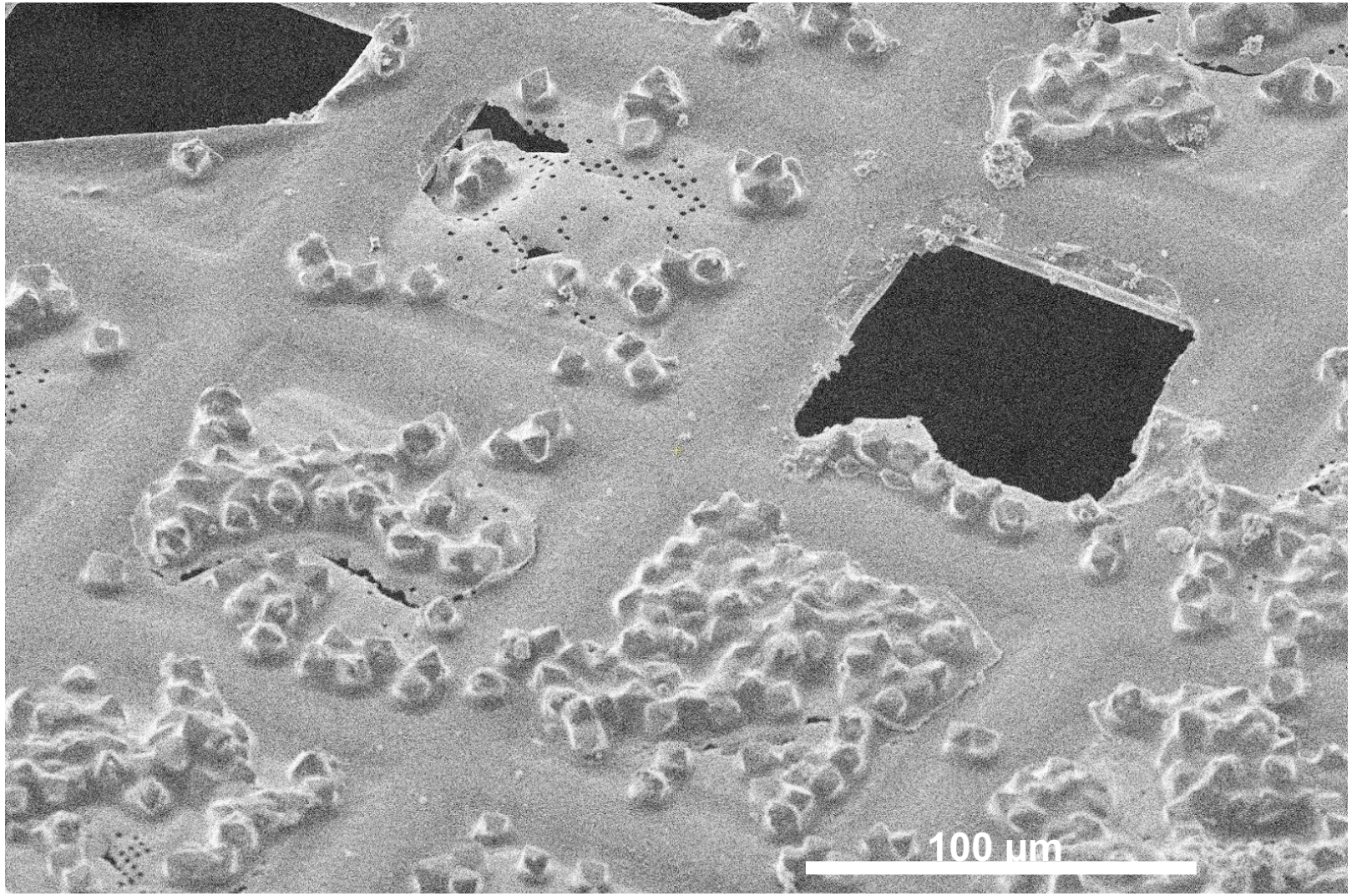


Where are my crystals?

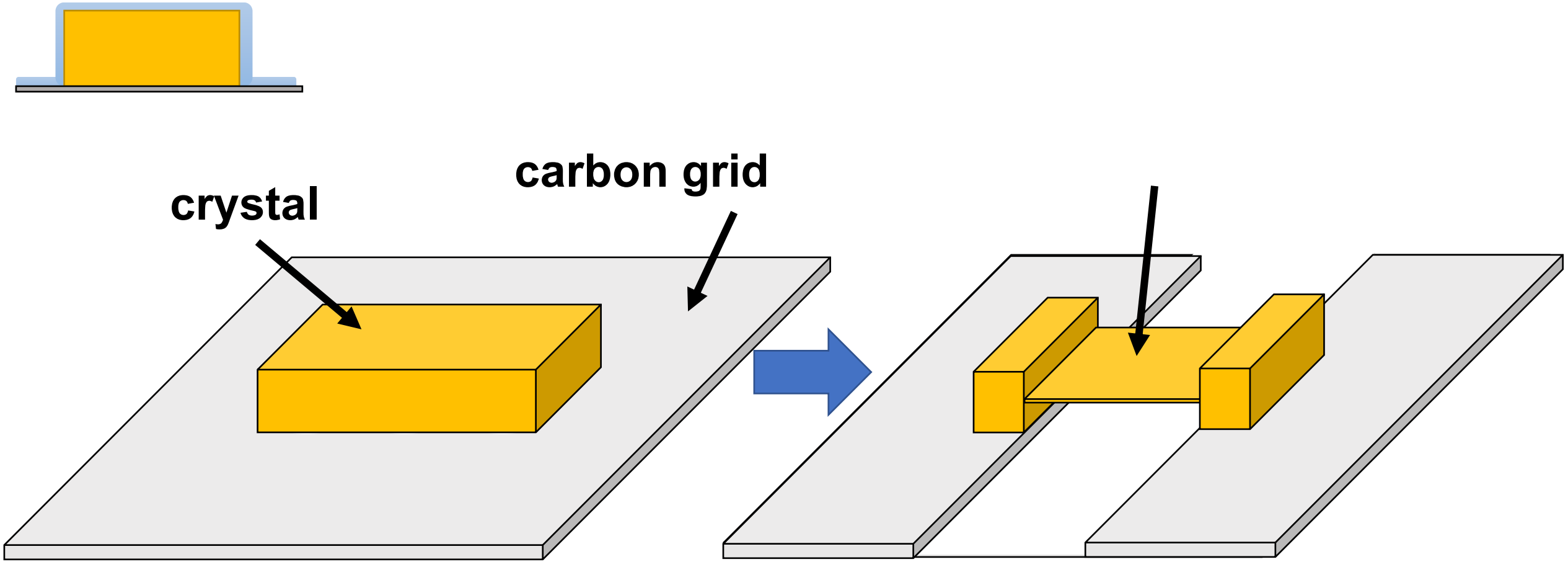


Kuba et al. Journal of Microscopy (2020)

Where are my crystals?

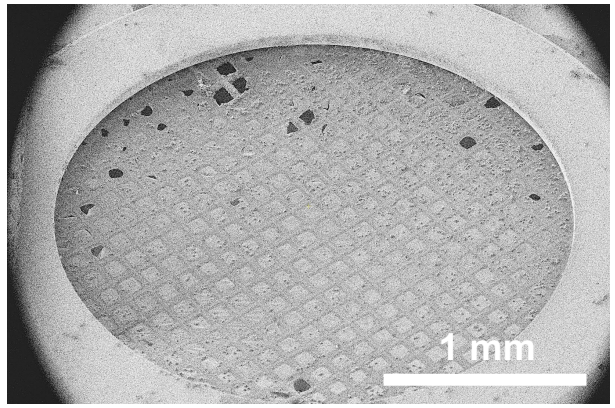


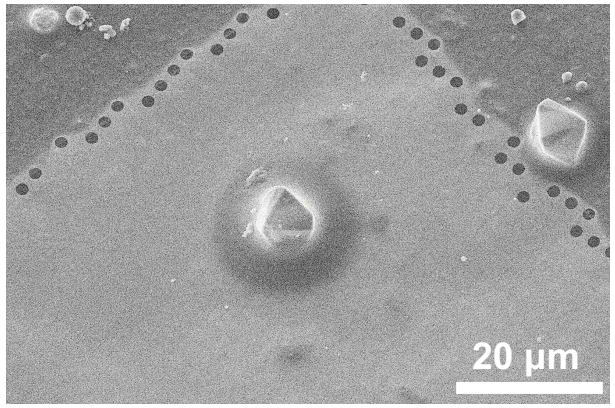
Making lamellanade

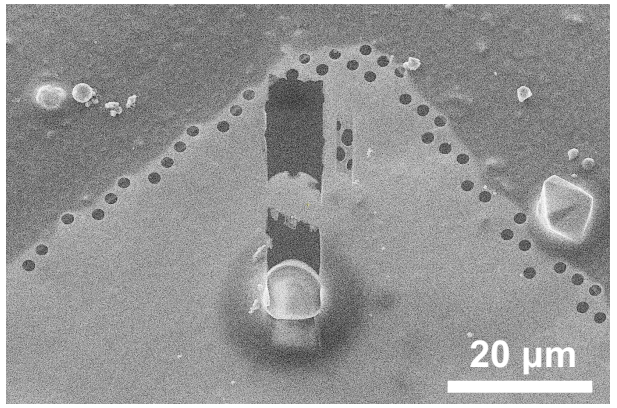


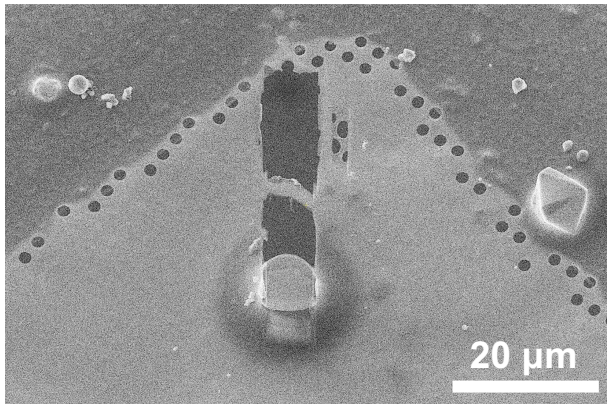
Making lamellanade

SEM

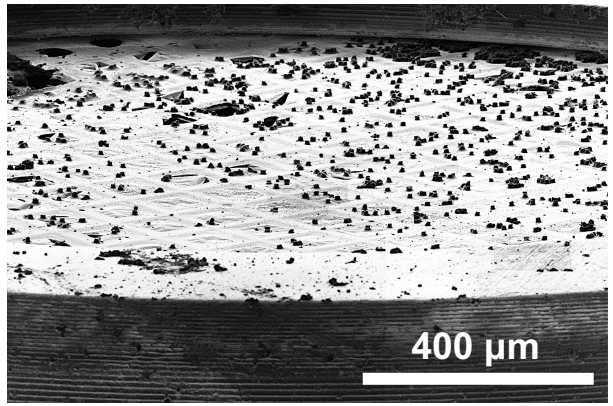


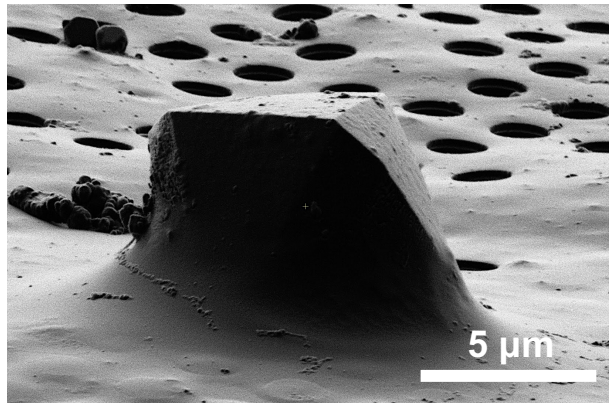


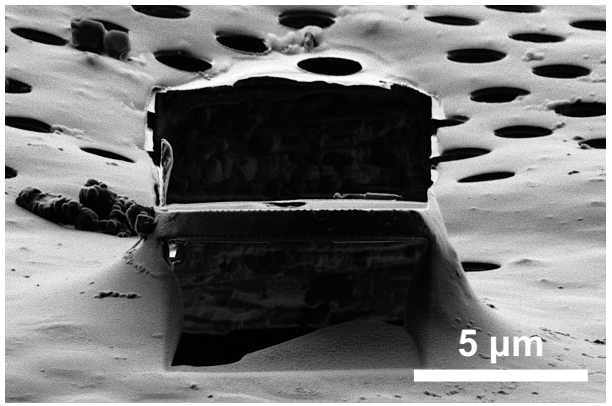


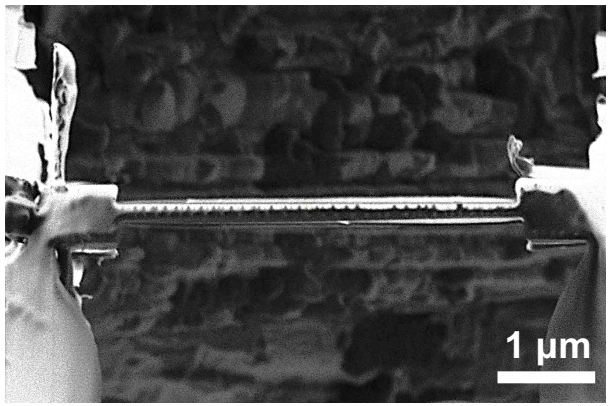


FIB



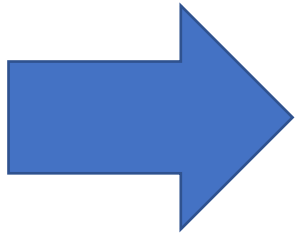




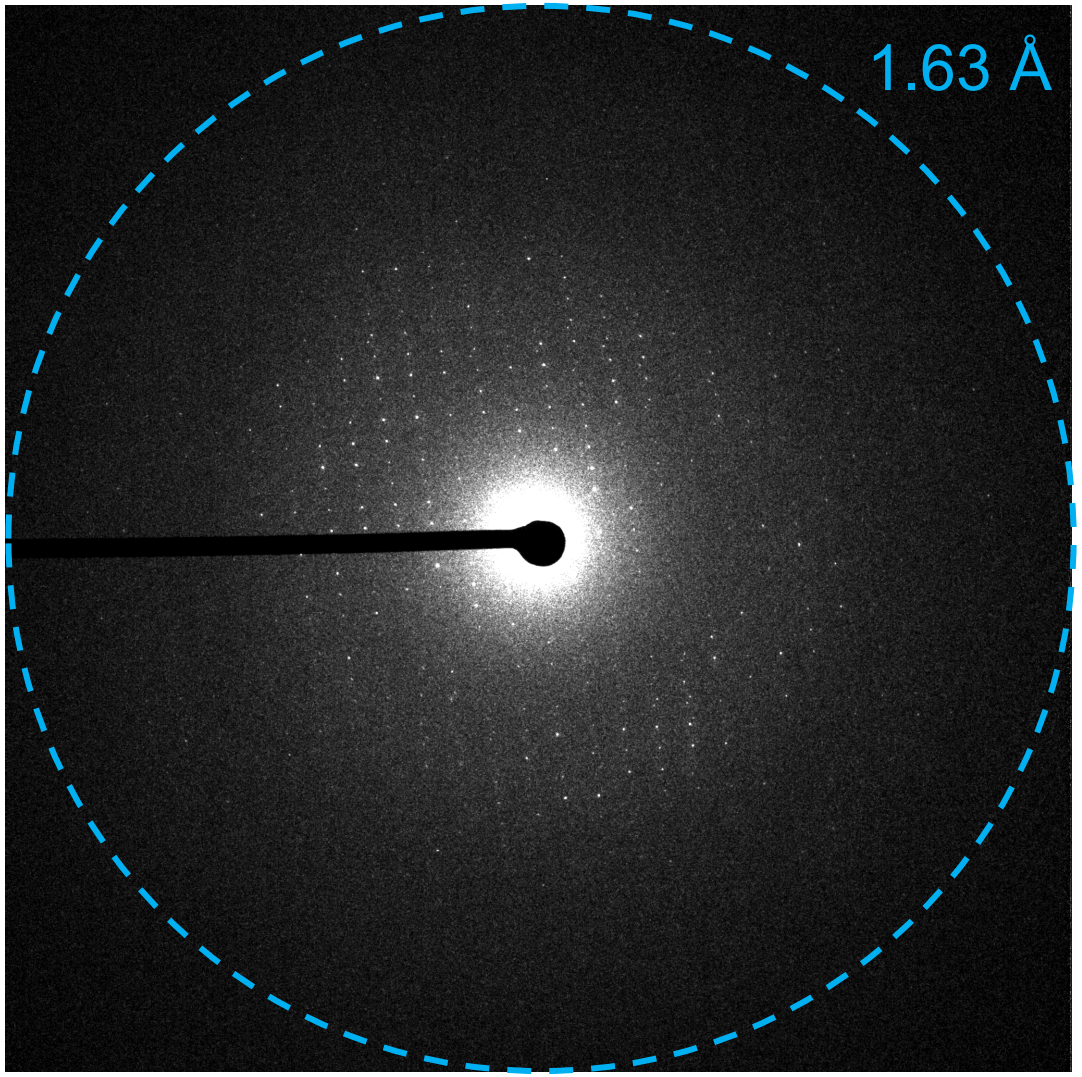


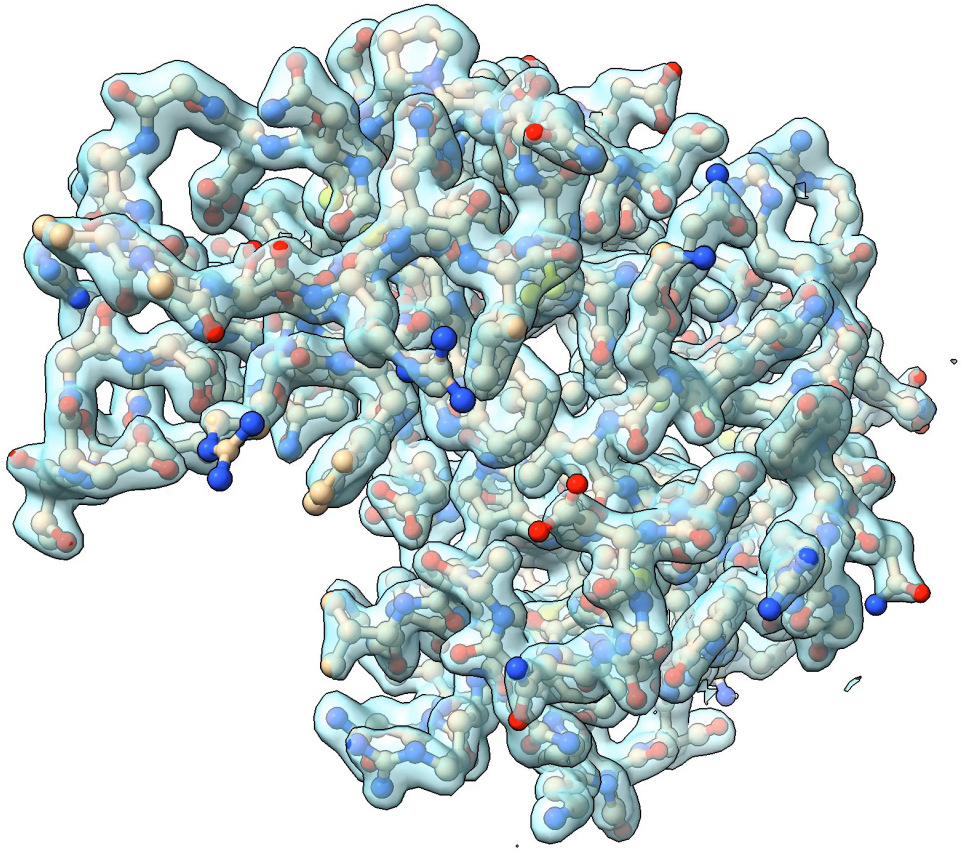
Martynowycz & Gonen. Methods in Molecular Biology (2021)

Making lamellanade

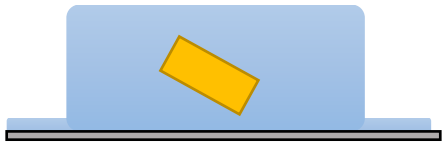


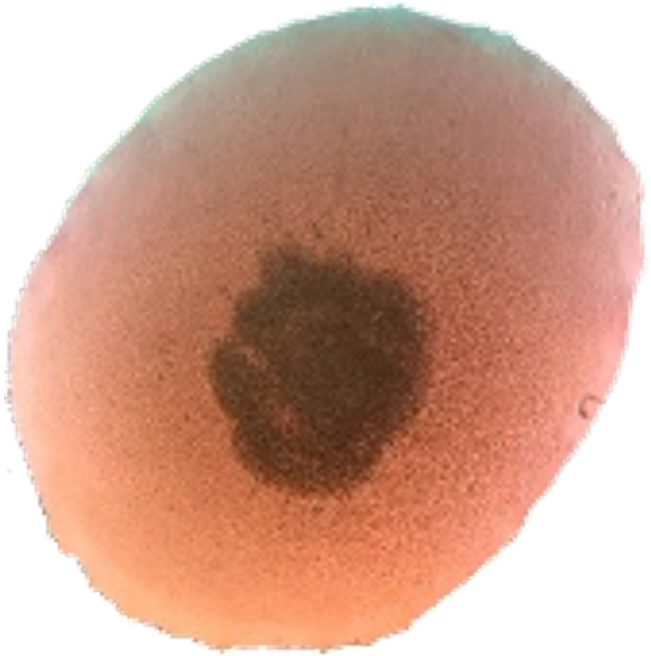
Making lamellanade

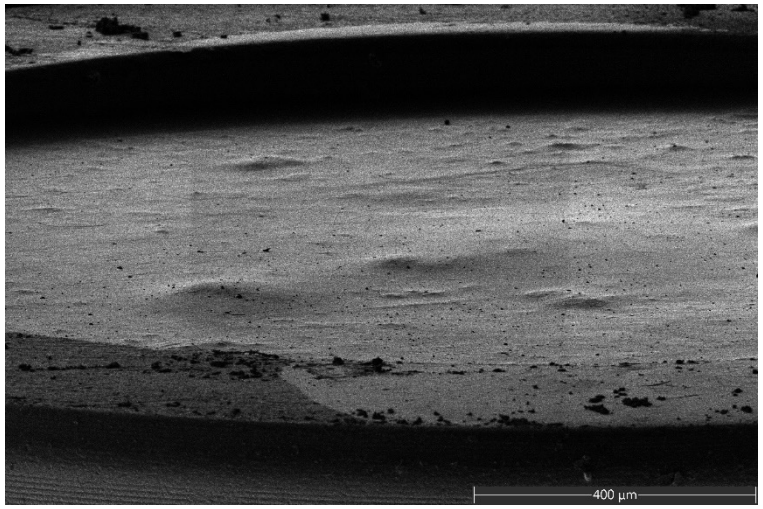


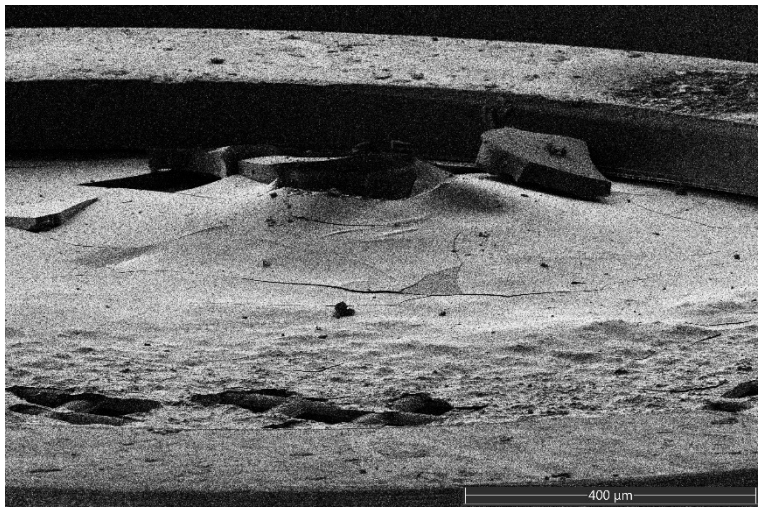


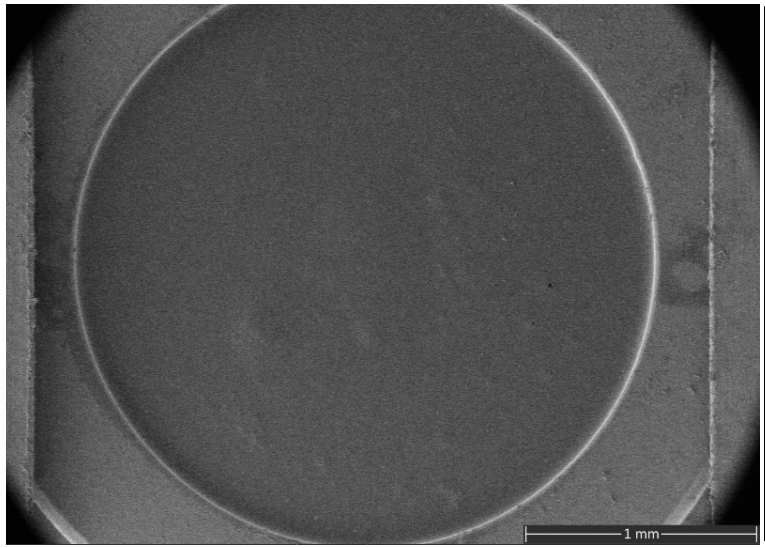
mVDAC

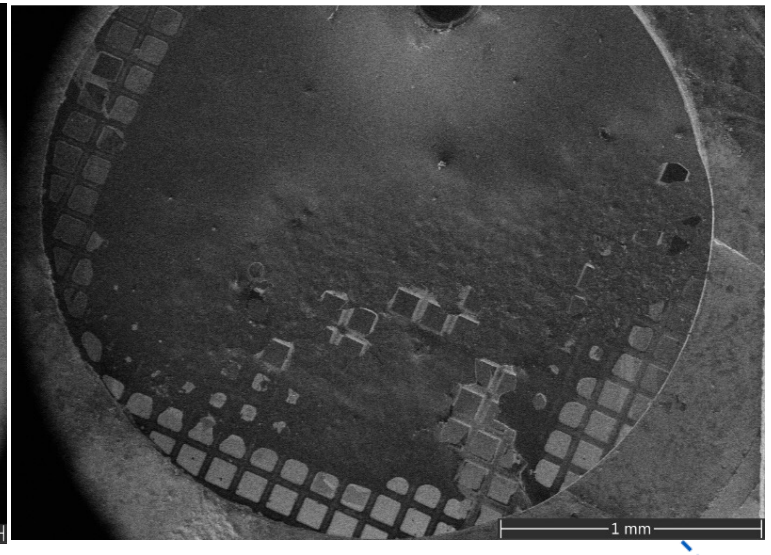






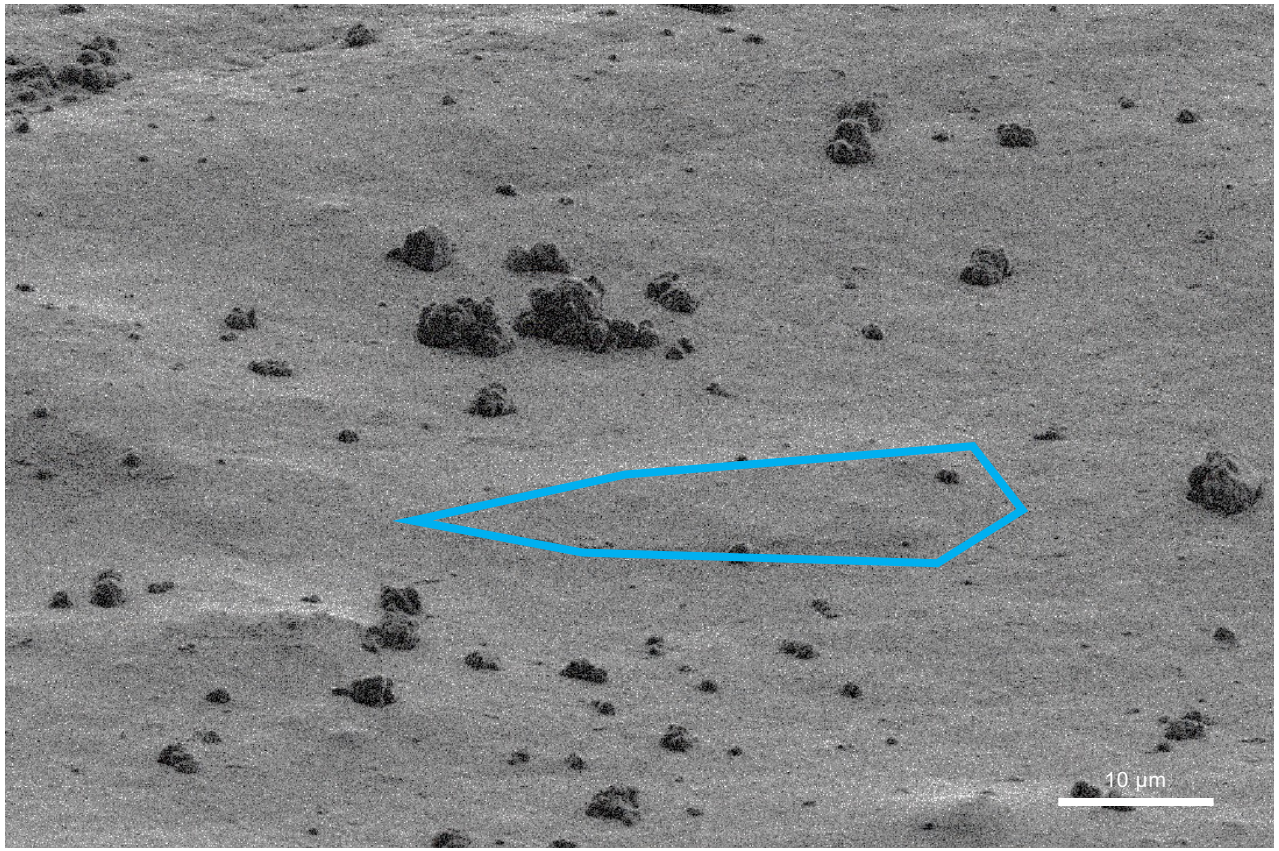


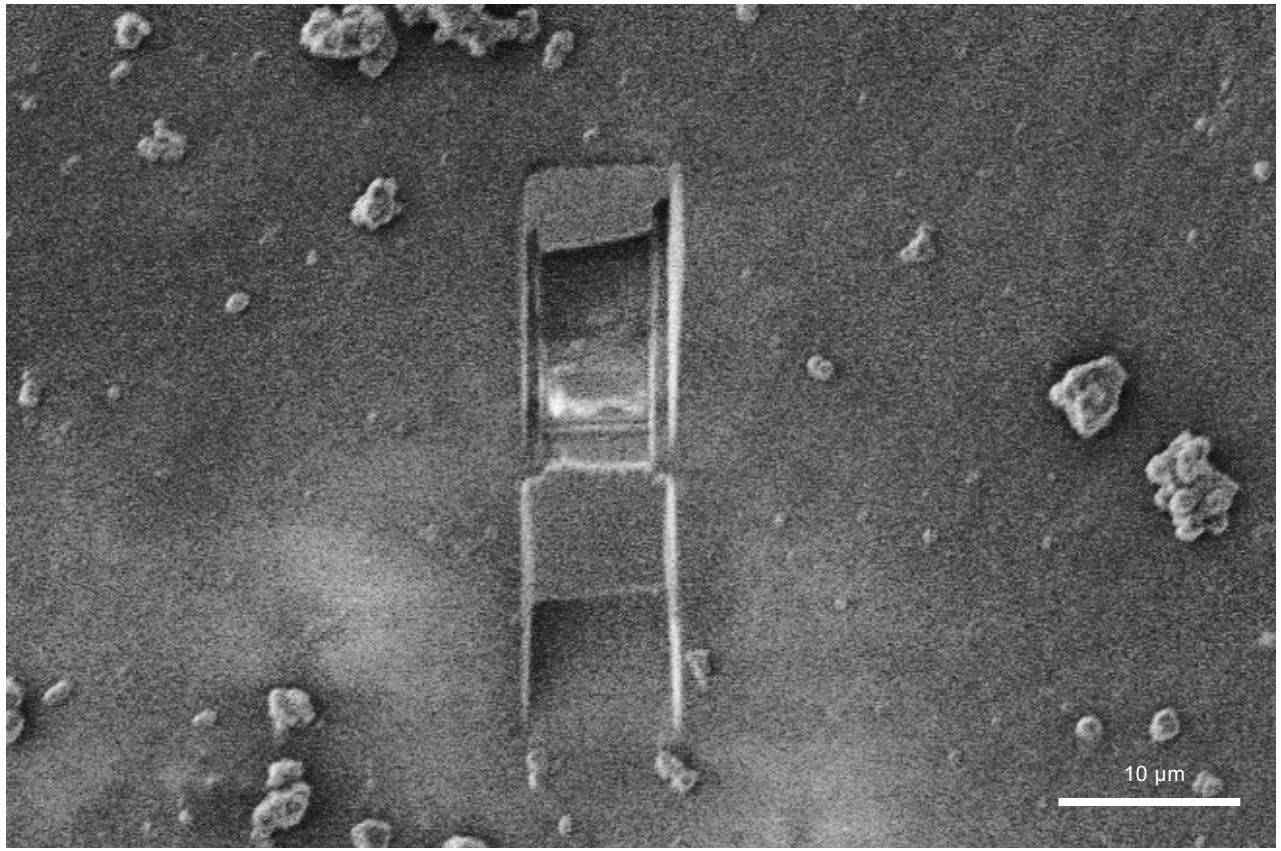




Martynowycz et al. PNAS (2020)

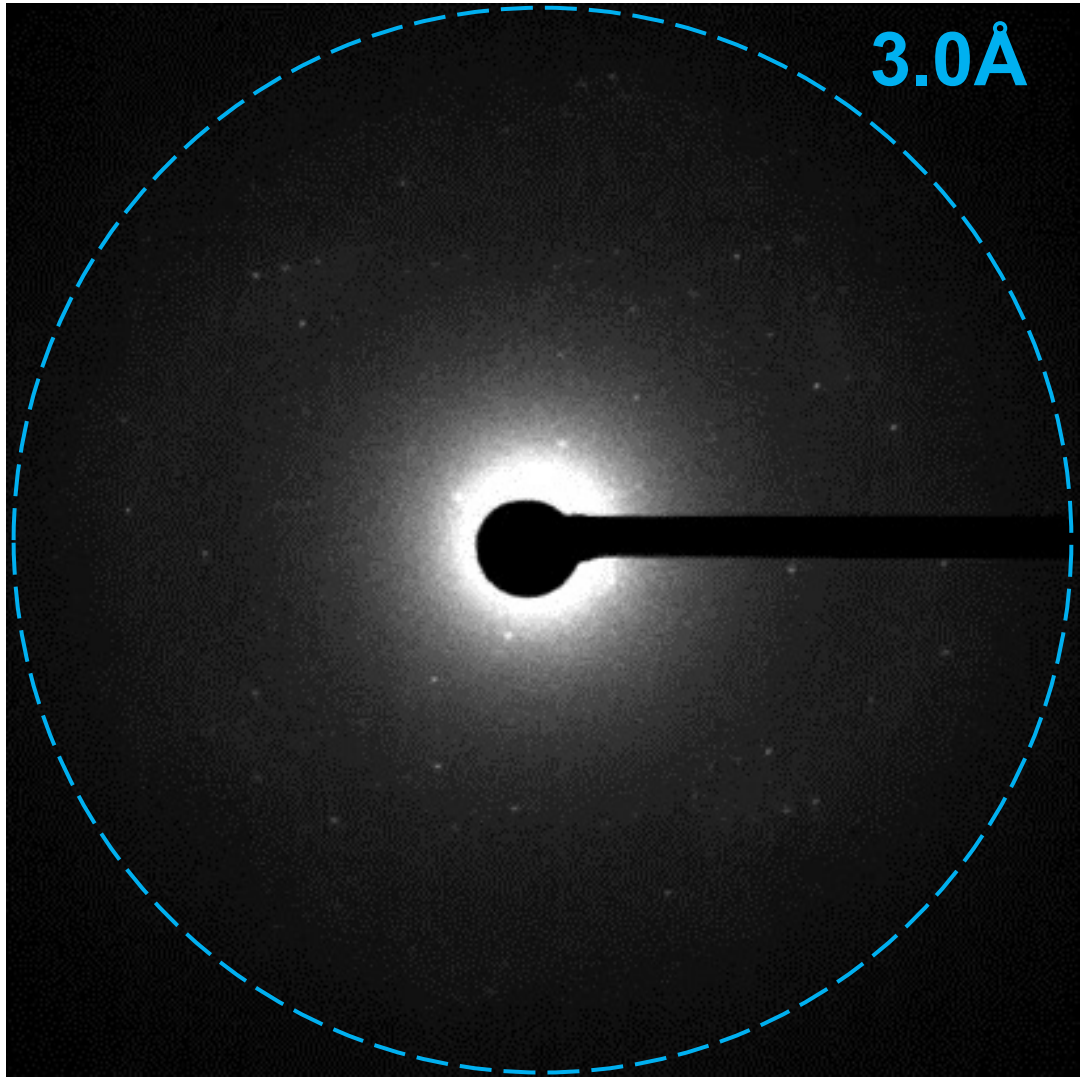
mVDAC

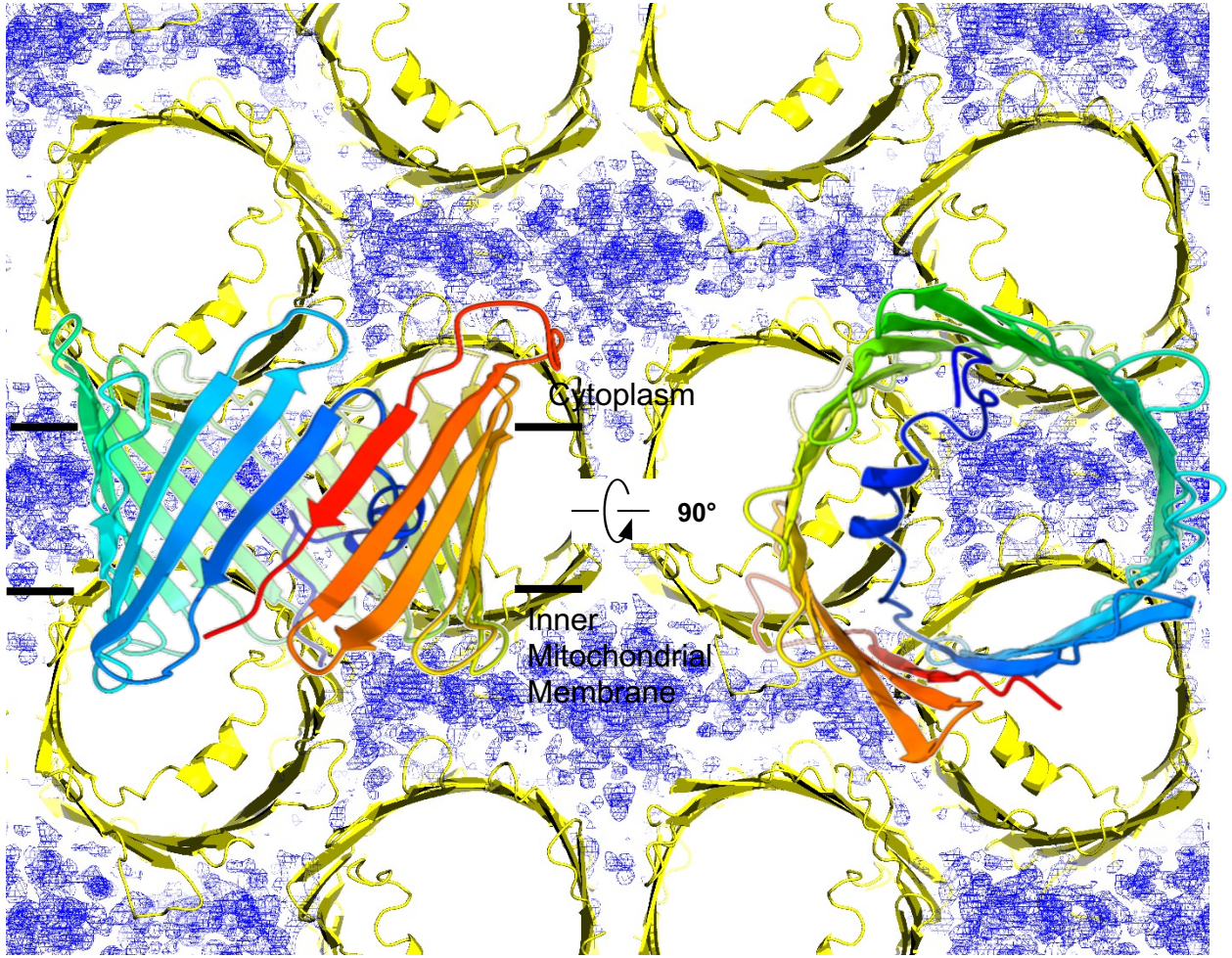




Martynowycz et al. PNAS (2020)

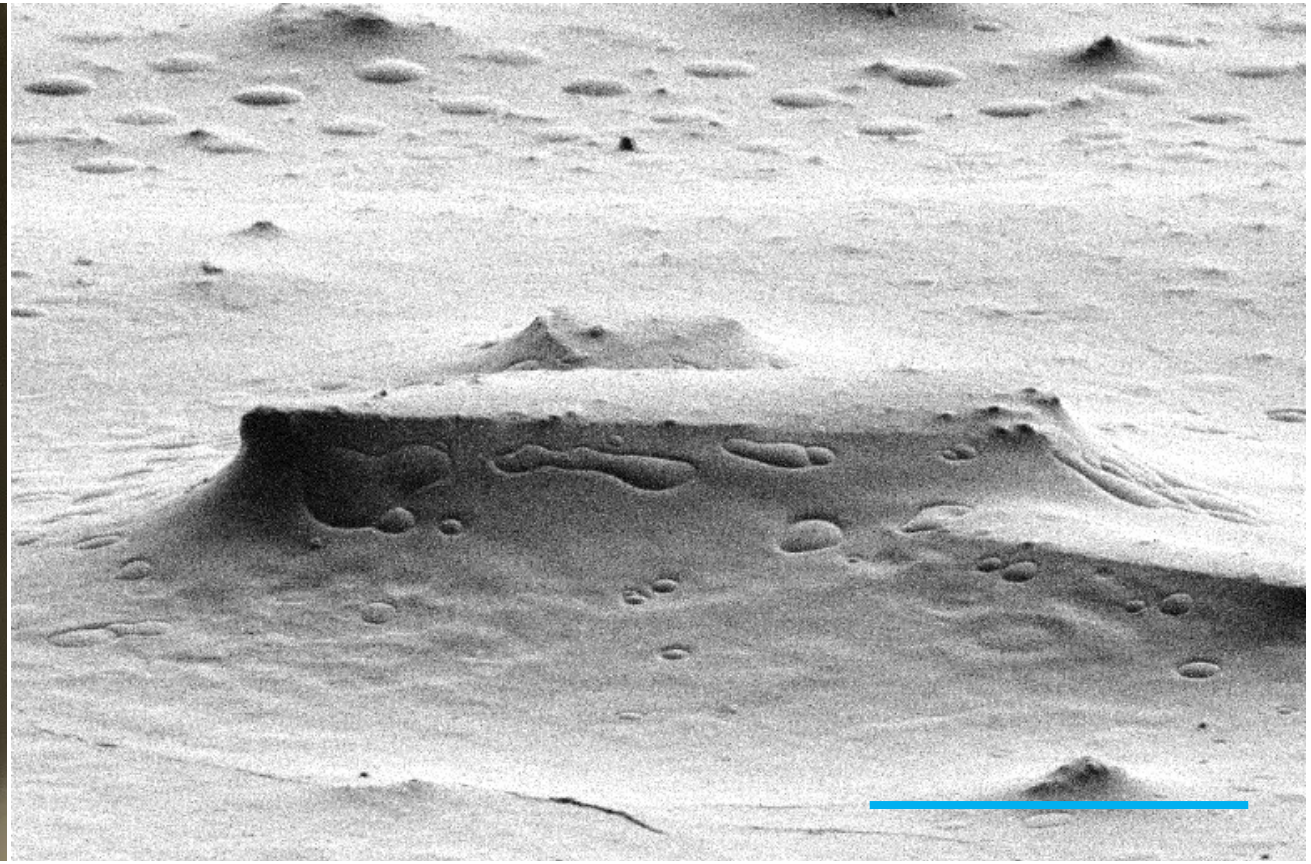
mVDAC





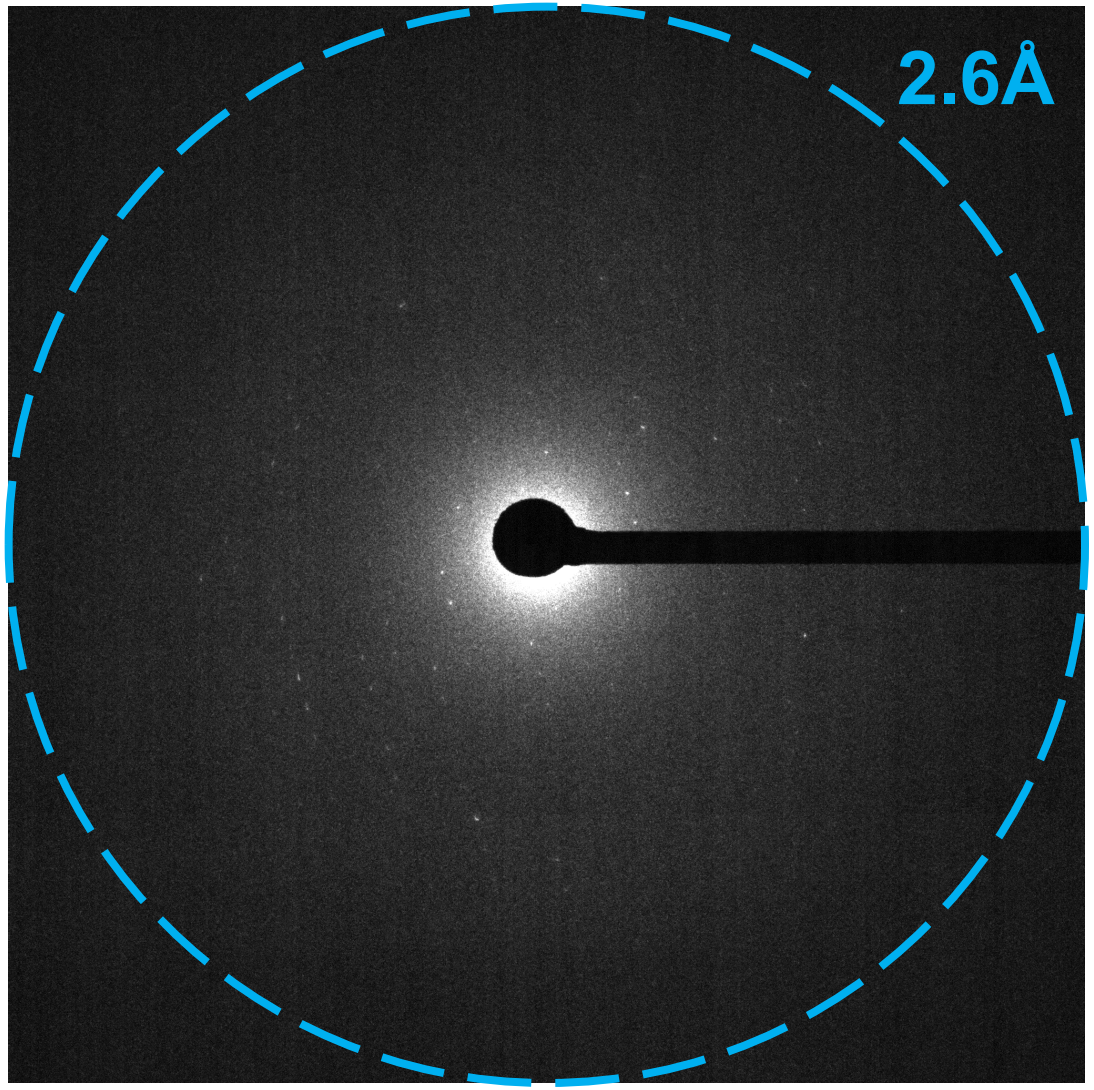
Martynowycz et al. PNAS (2020)

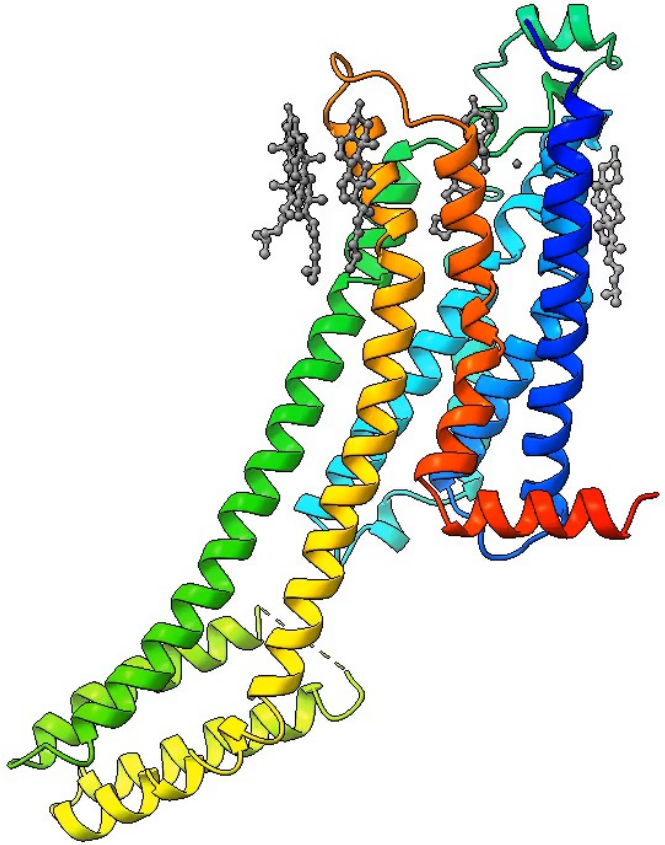
A2A Adenosine Receptor



Martynowycz et al. PNAS (2021b)

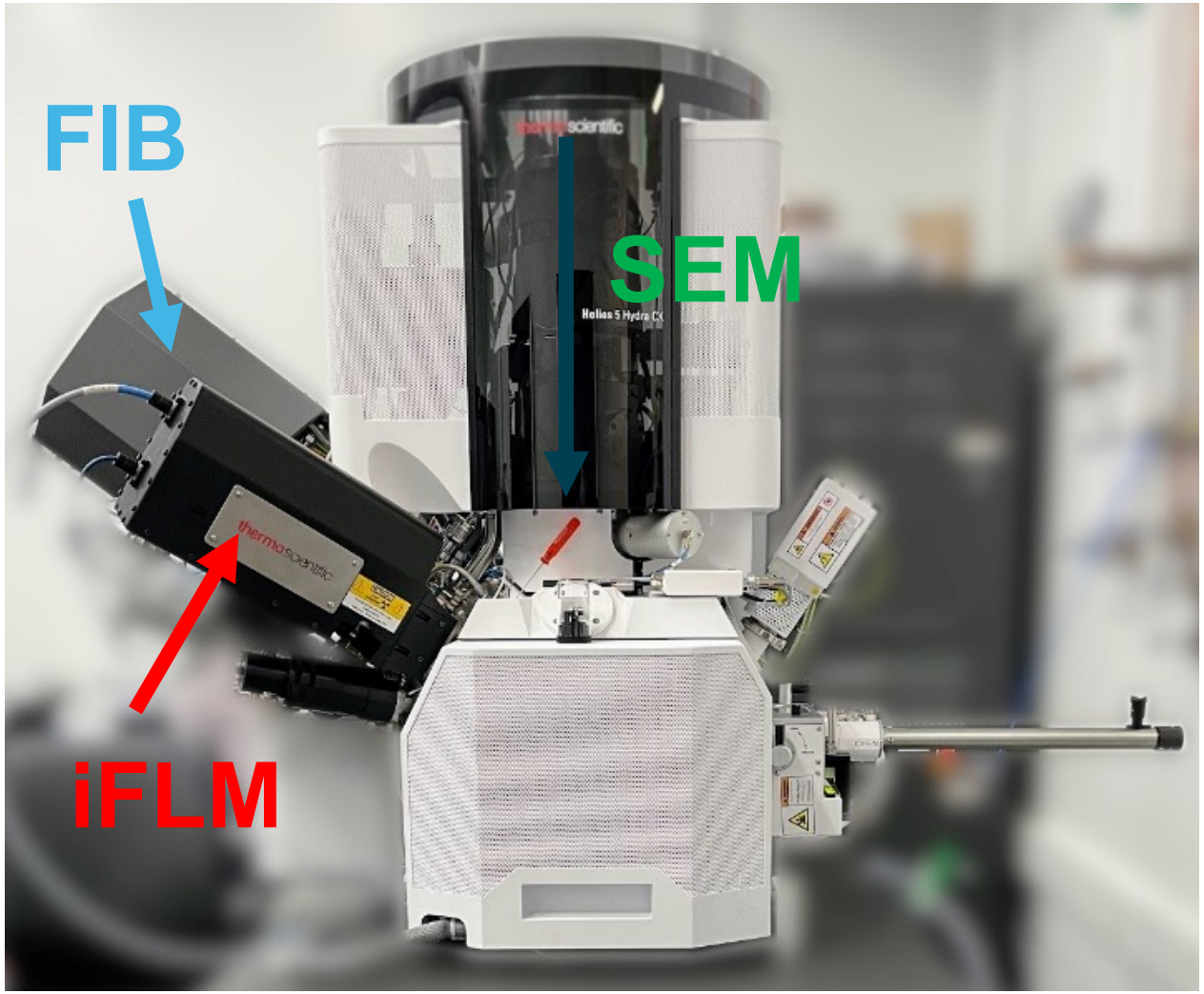
A2A Adenosine Receptor

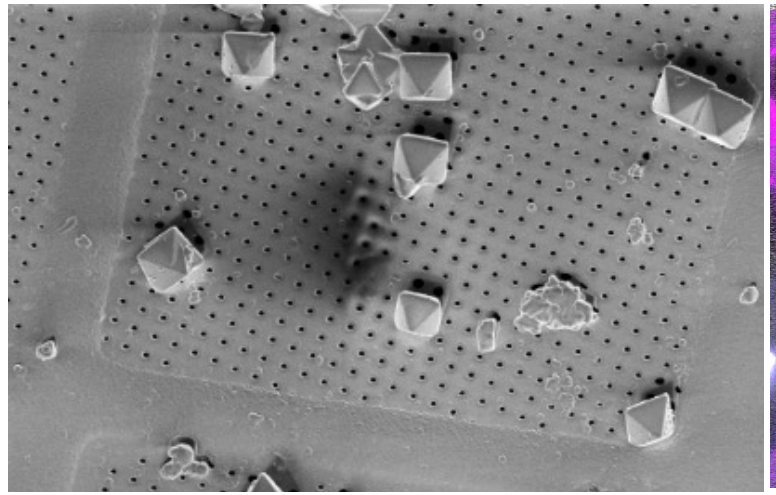


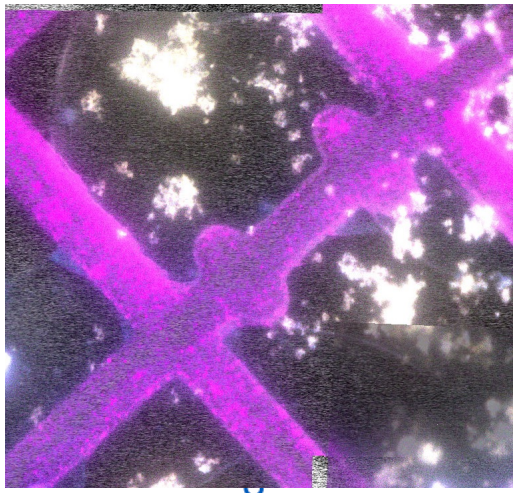


Martynowycz et al. PNAS (2021b)

Cutting deep: can we find a needle in a haystack?

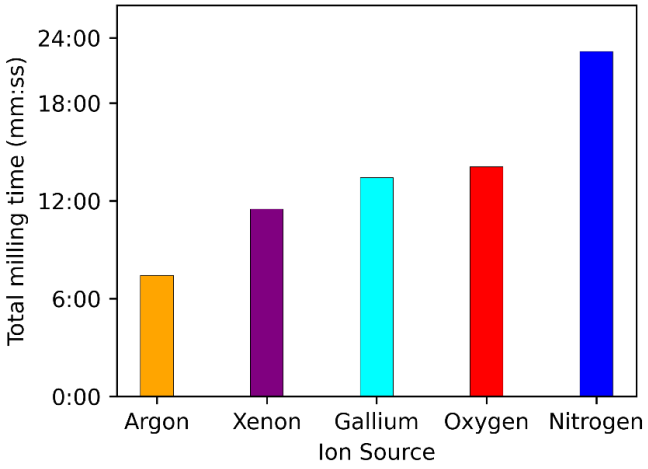


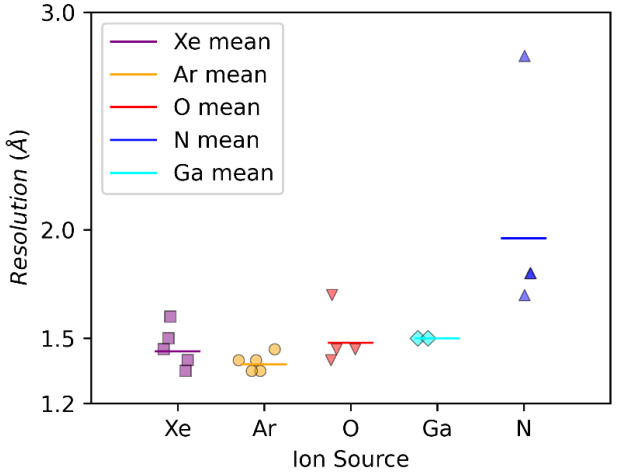


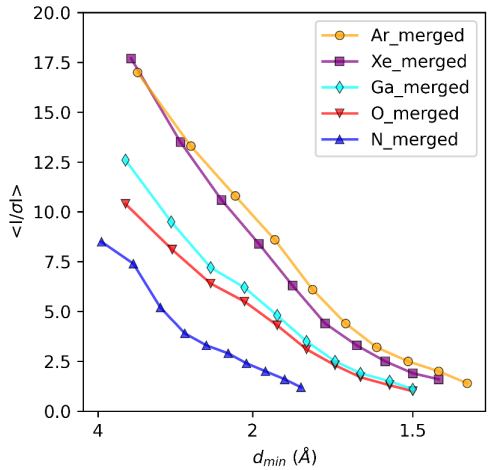


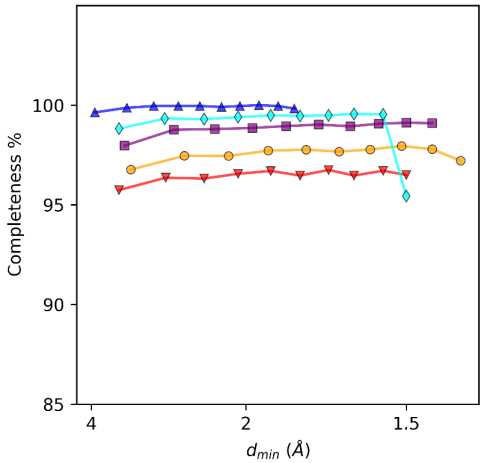
Martynowycz et al. Nature Communications (2023)

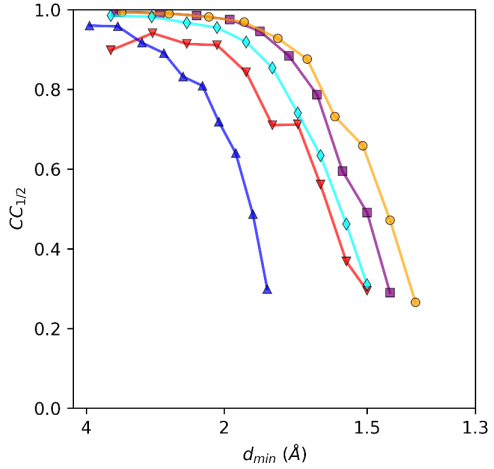
Does plasma even work?

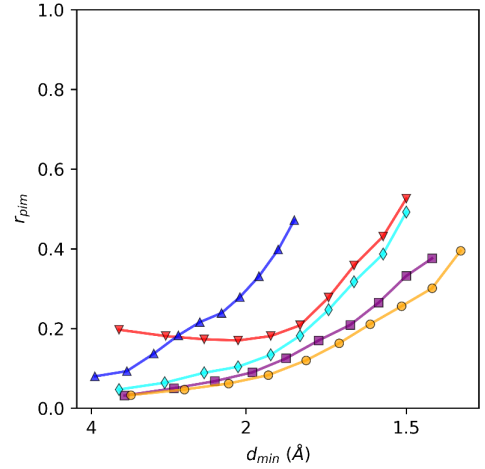






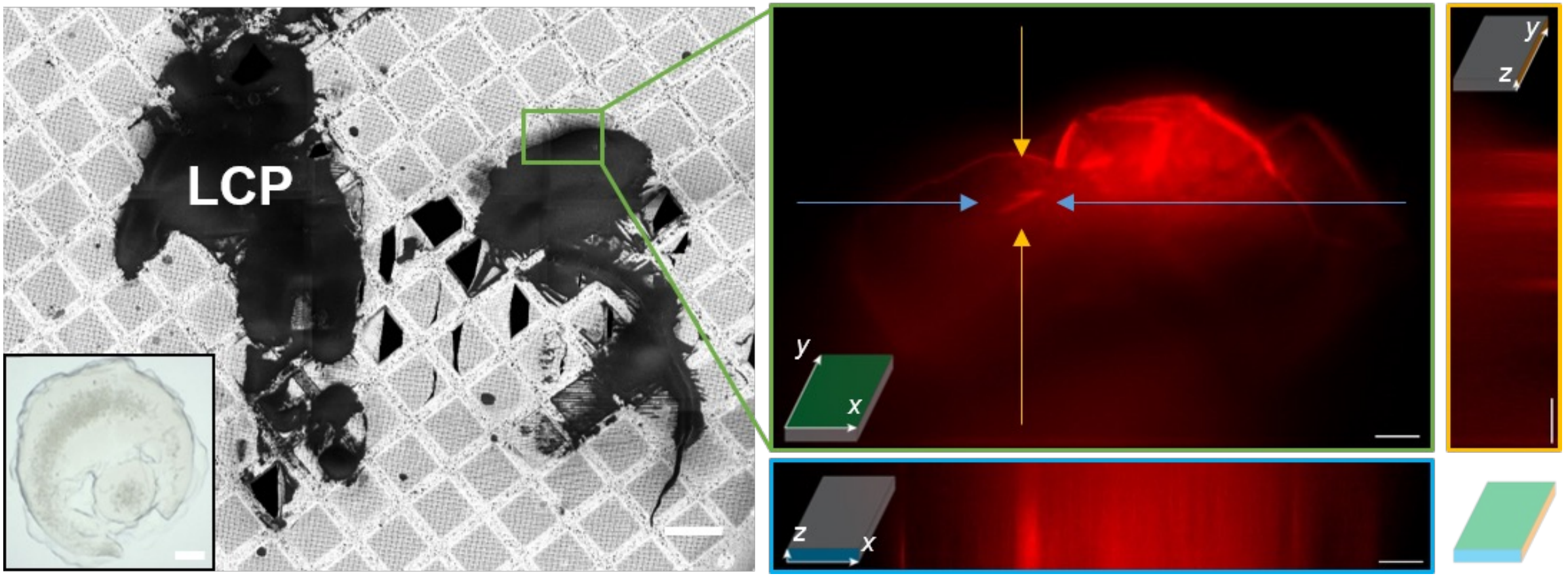




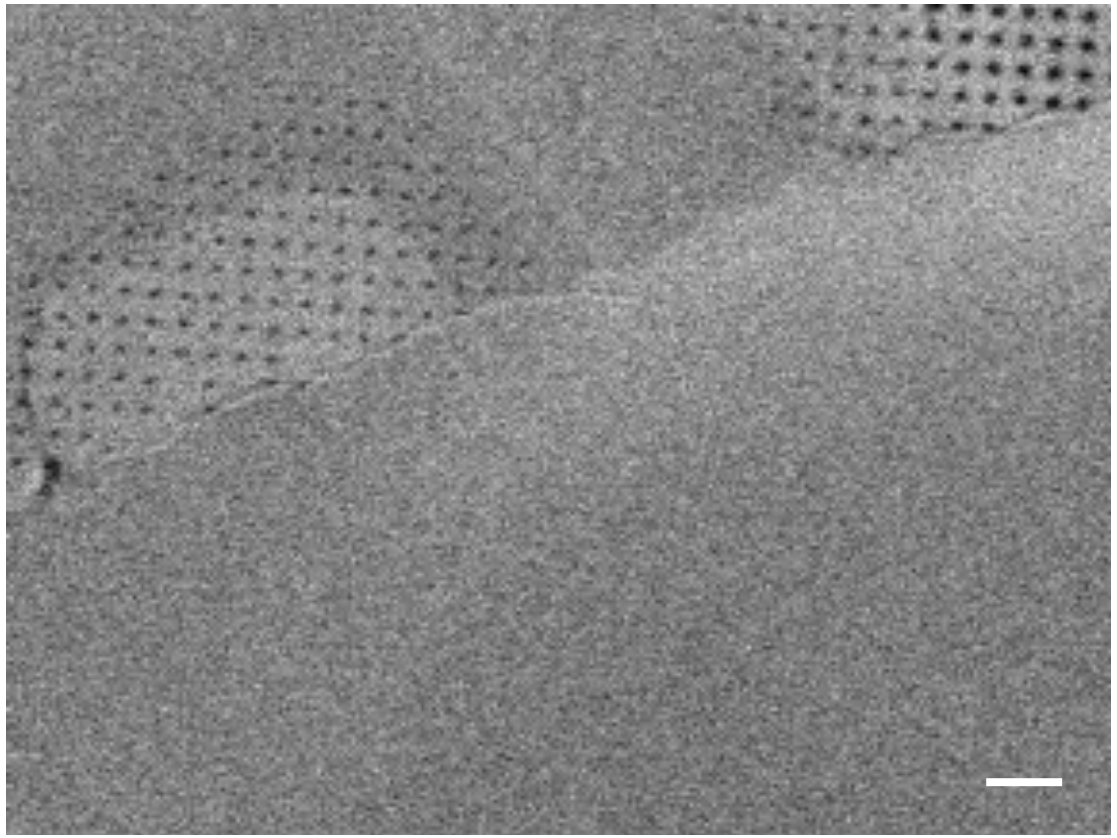


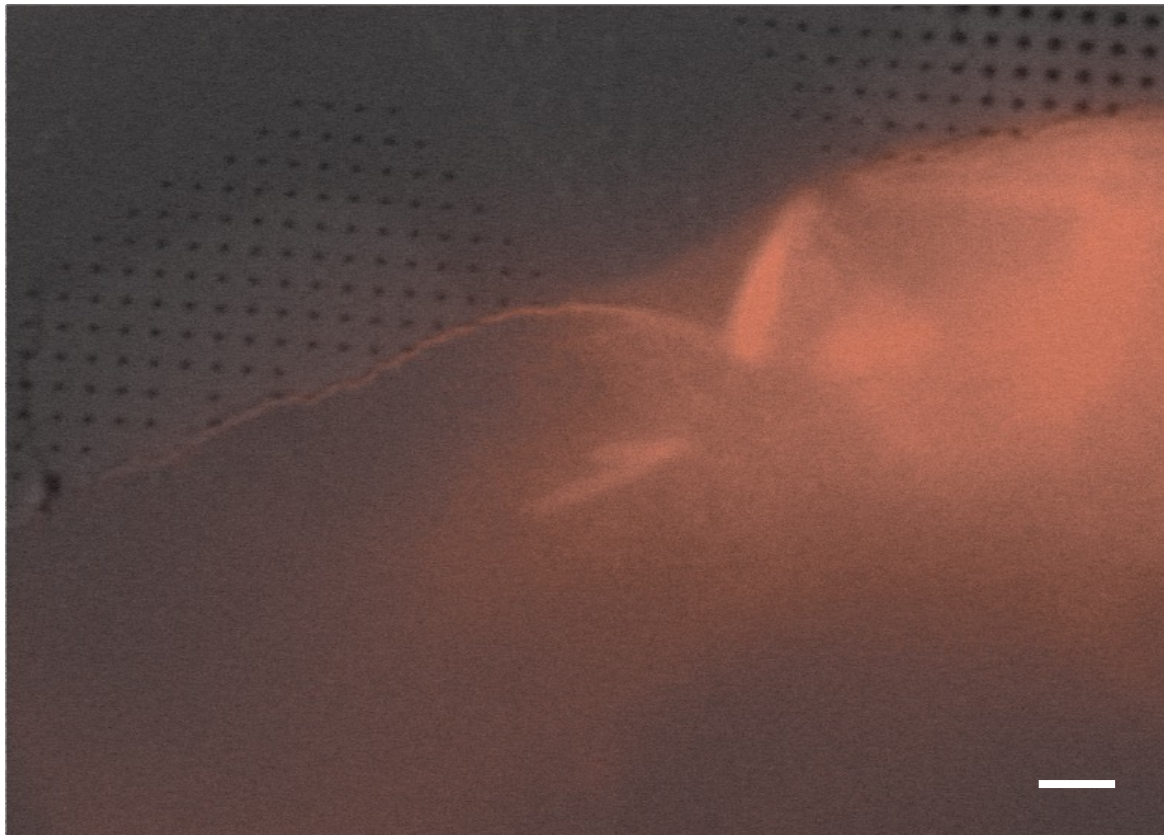
Martynowycz et al. Nature Communications (2023)

Cryo-fluorescence microscopy to screen whole grids



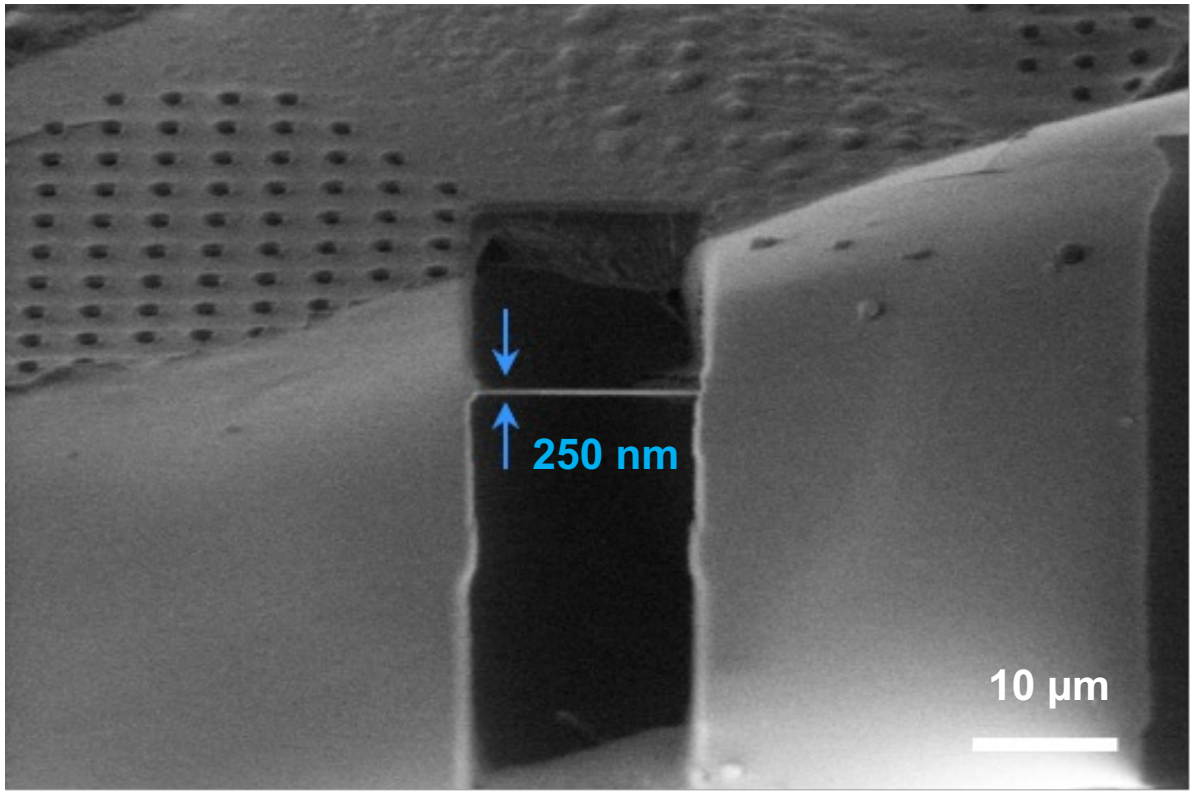
In chamber fluorescence microscopy of microcrystals



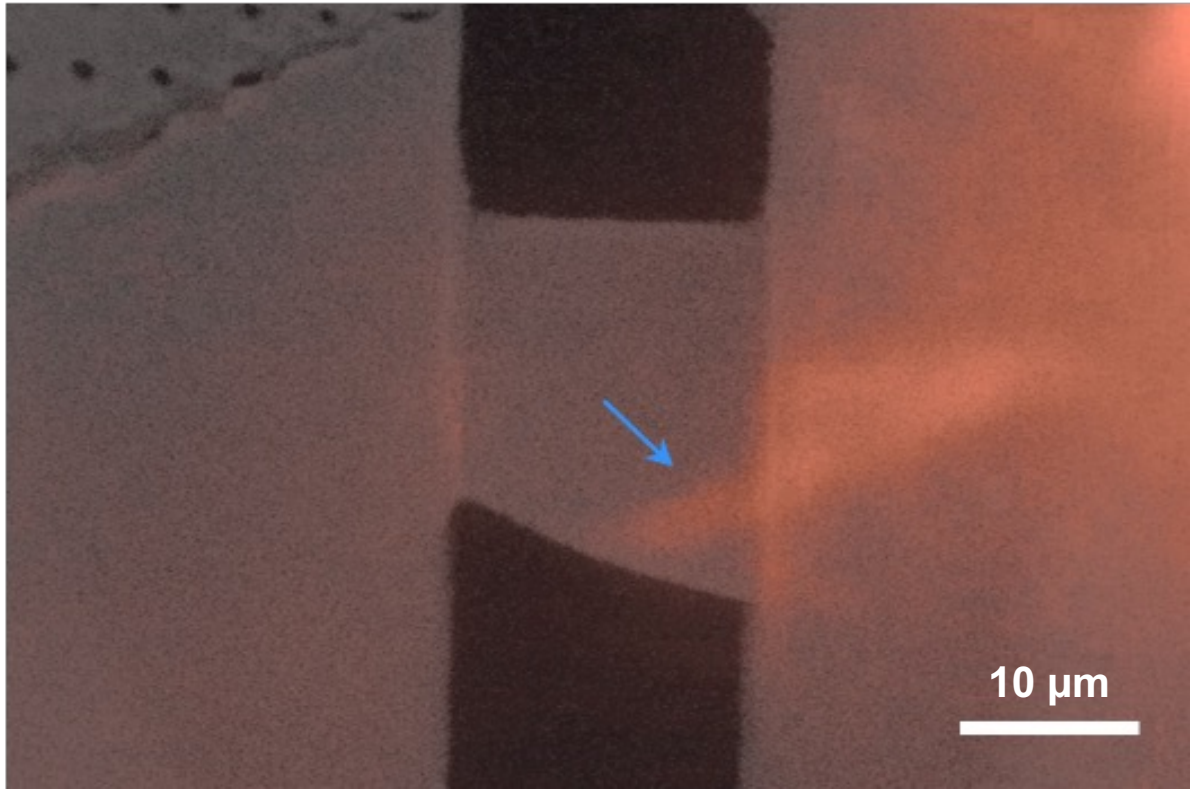


Martynowycz et al. Nature Communications (2023)

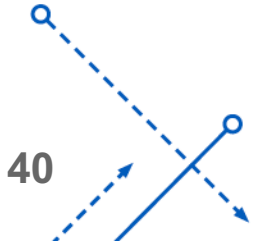
pFIB slices through 10s to 100s of microns of LCP



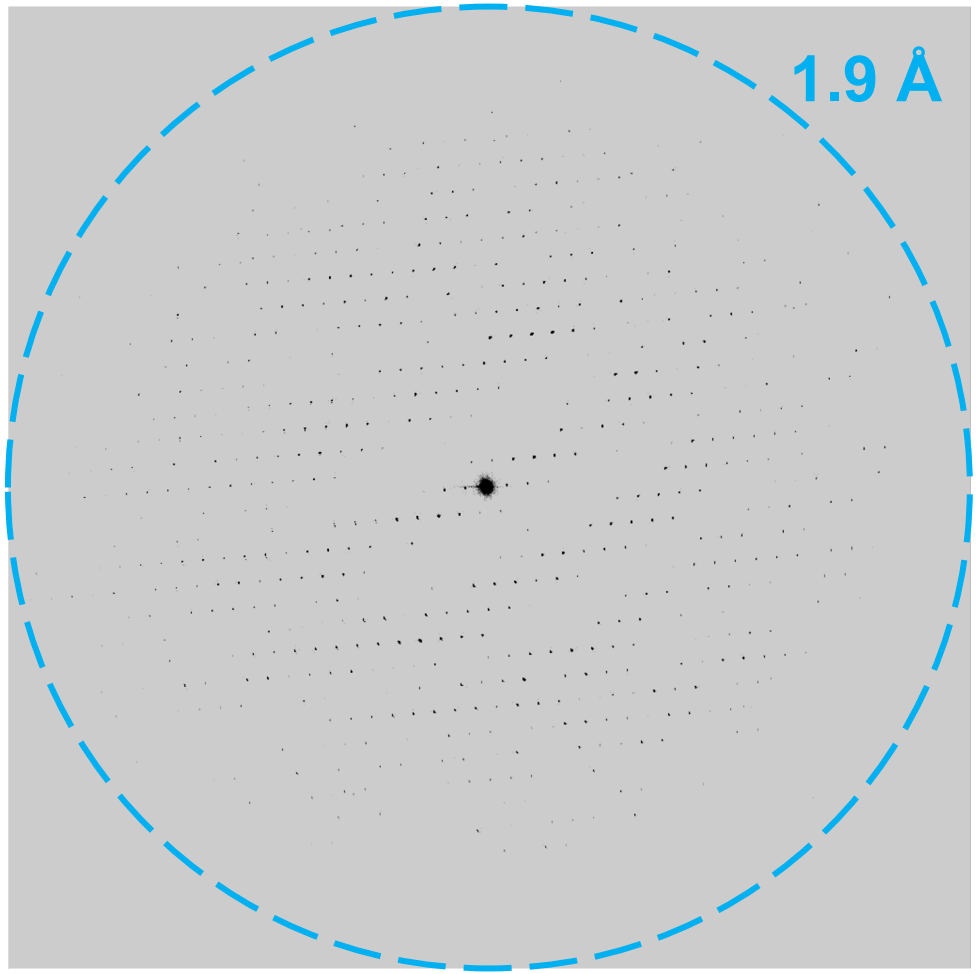
SEM

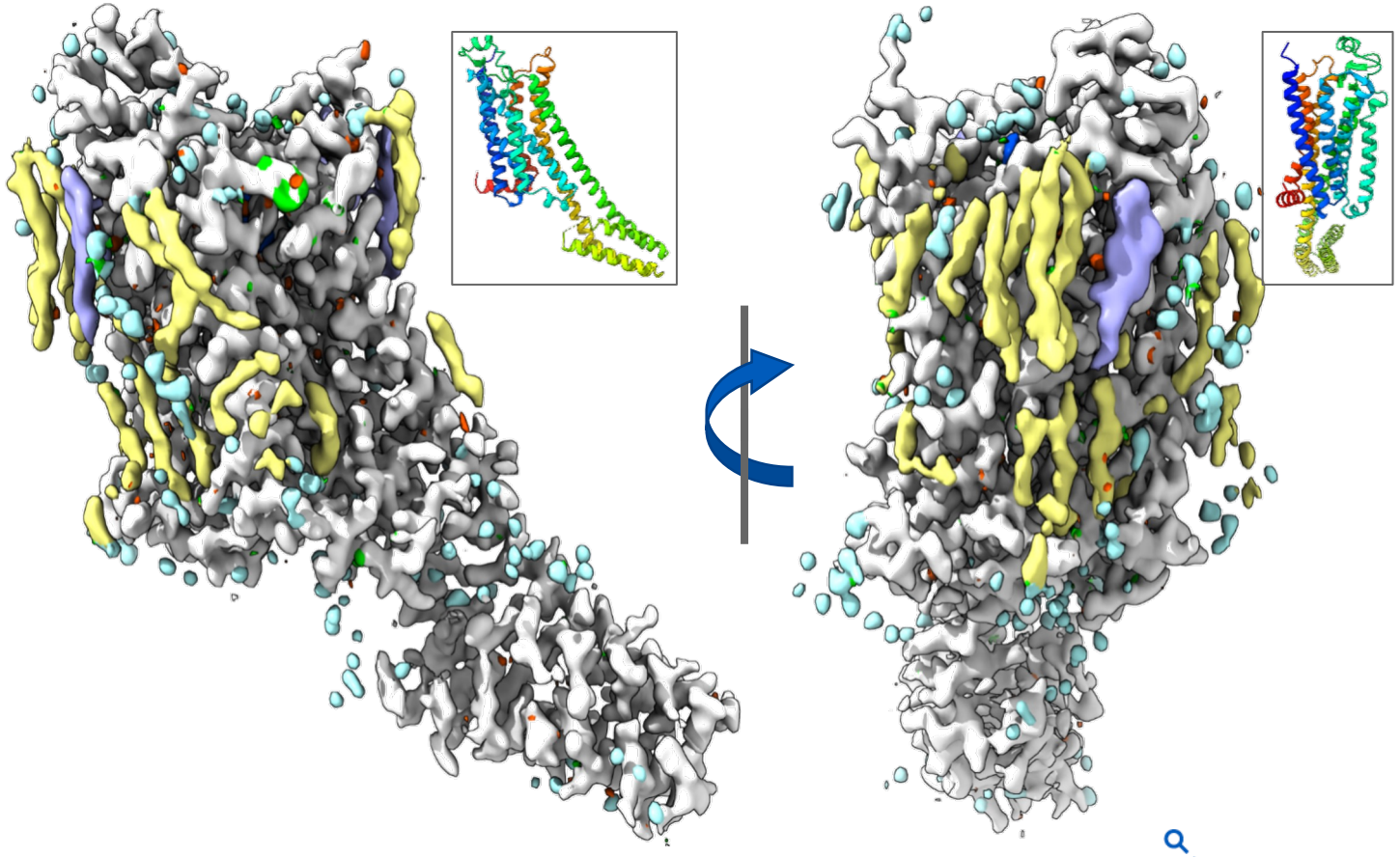


SEM+FLM



A high-resolution needle in a haystack

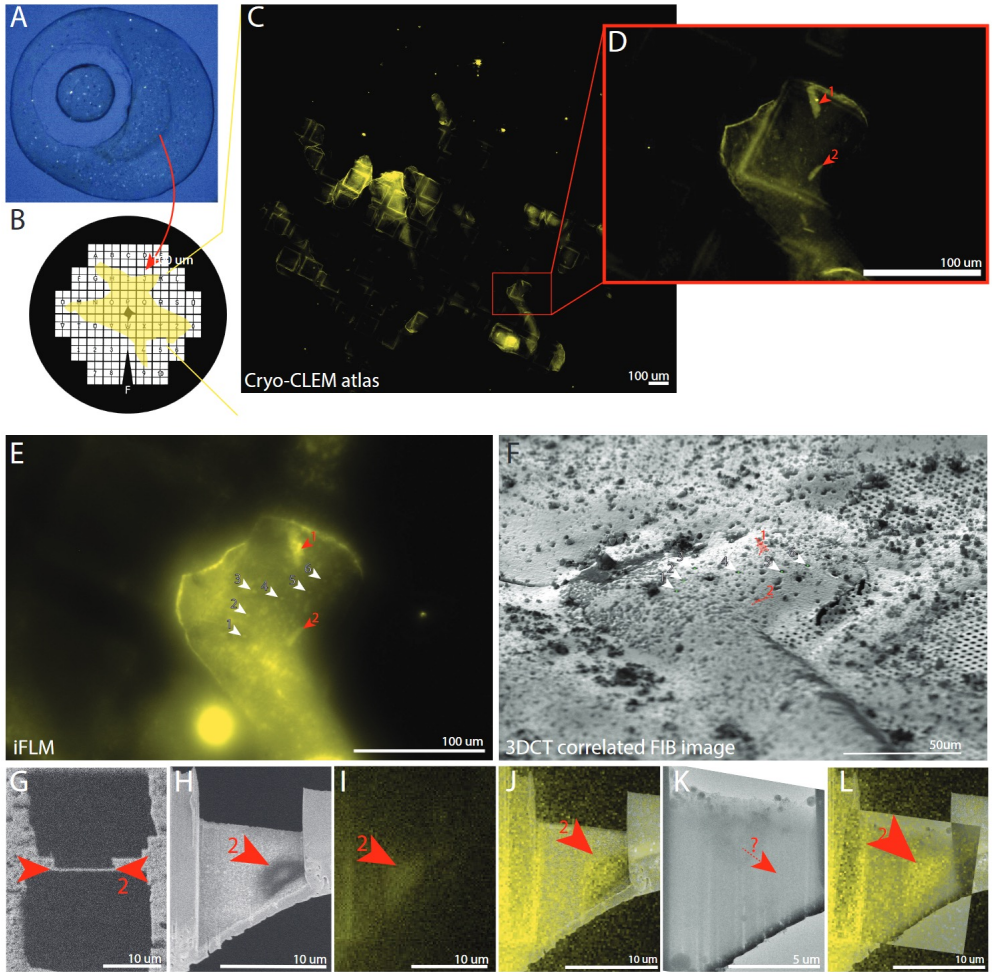


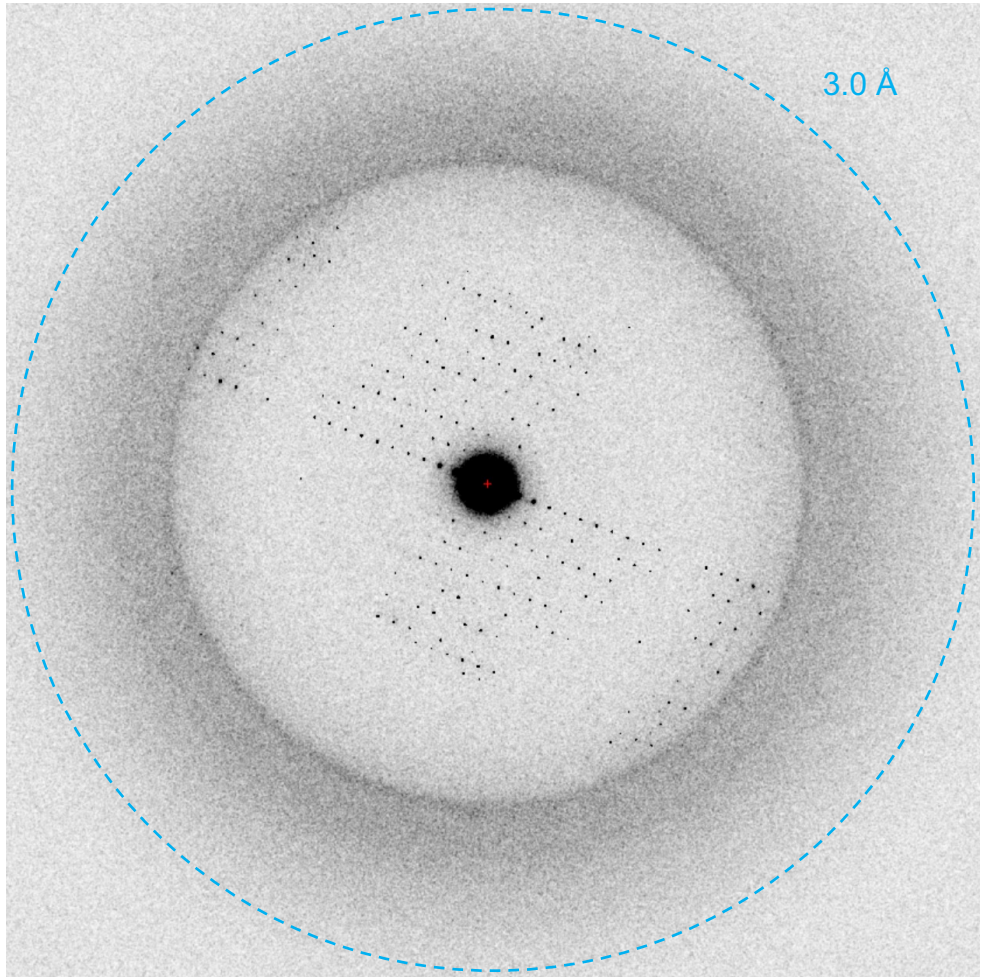


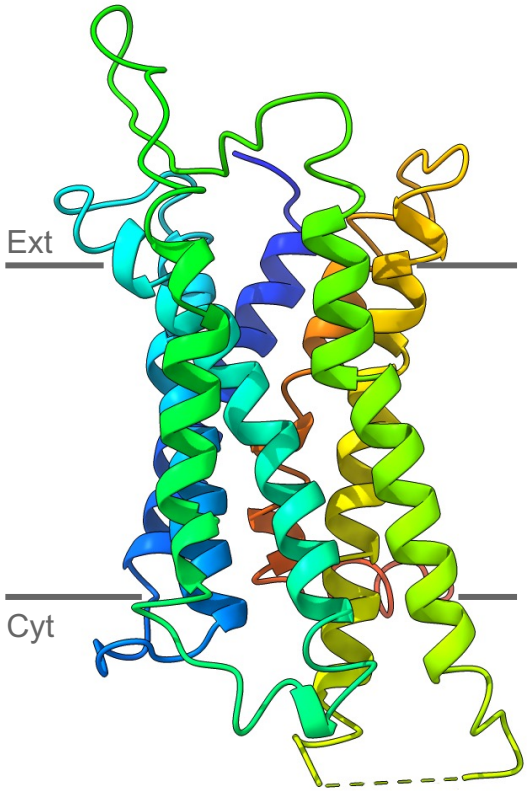
Martynowycz et al. Nature Communications (2023)

Not a fluke – novel structures!

A novel GPCR by MicroED: V1B



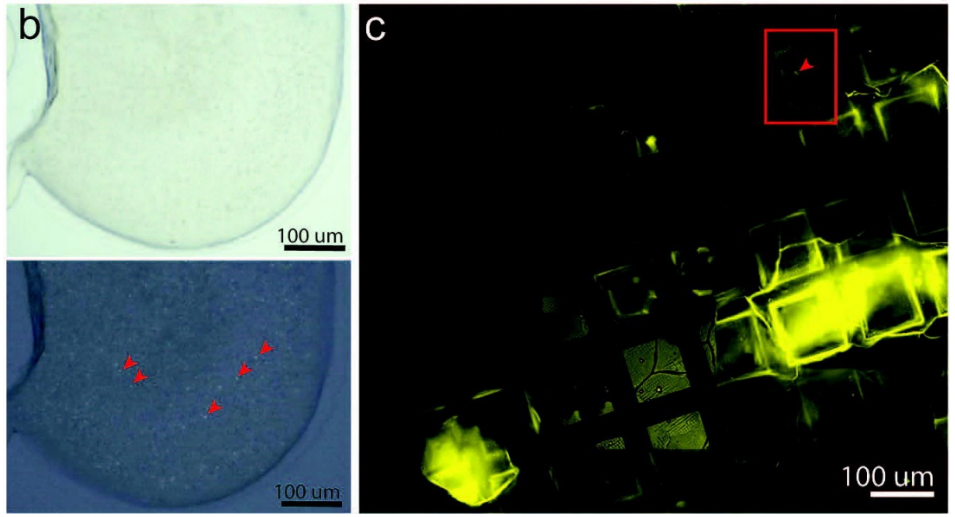


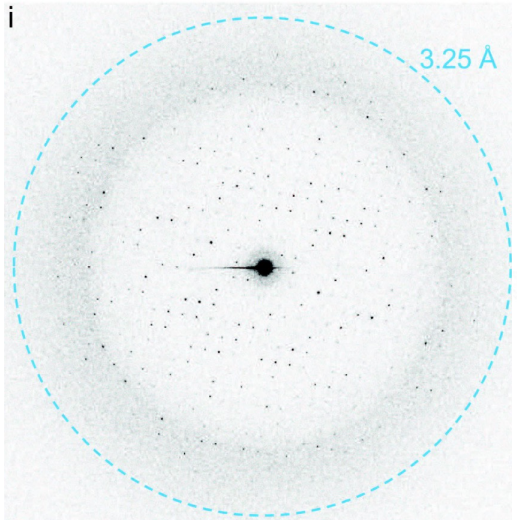


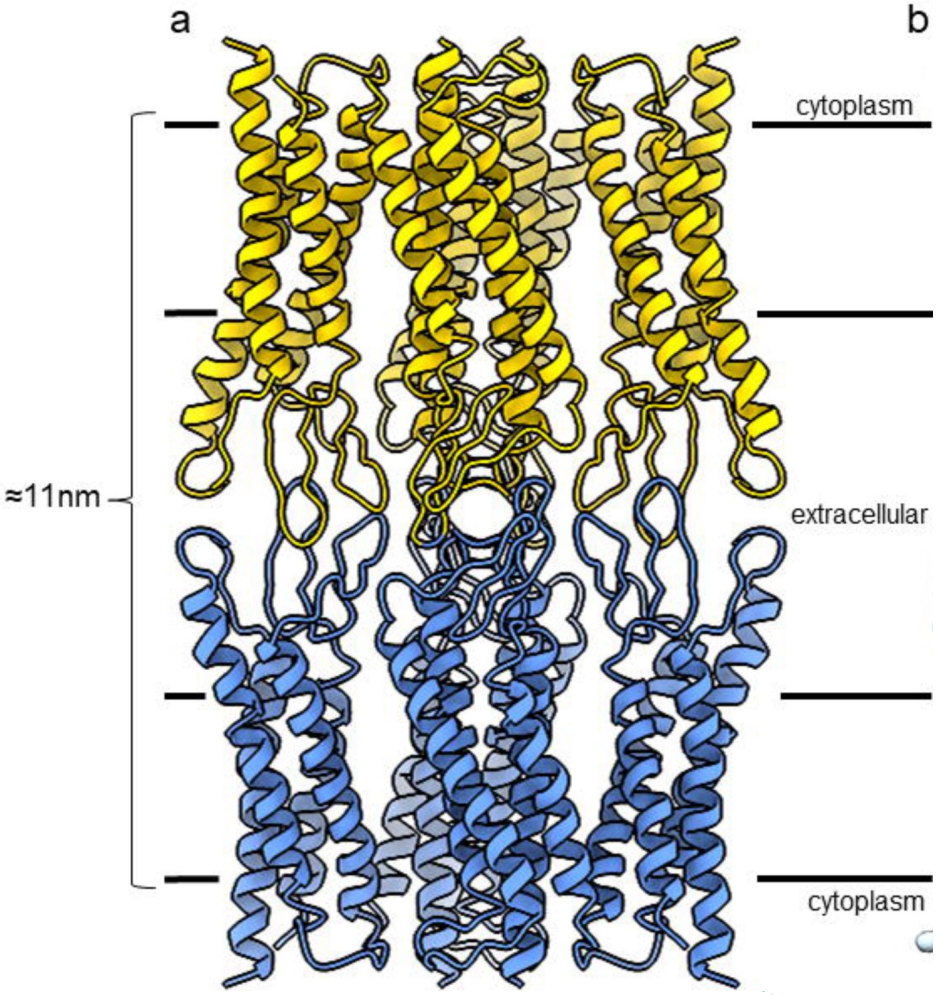
Shiriaeva and Martynowycz et al. BioRxiv (2024)

Not a fluke – novel structures!

A novel tetraspanin by MicroED: MP20







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