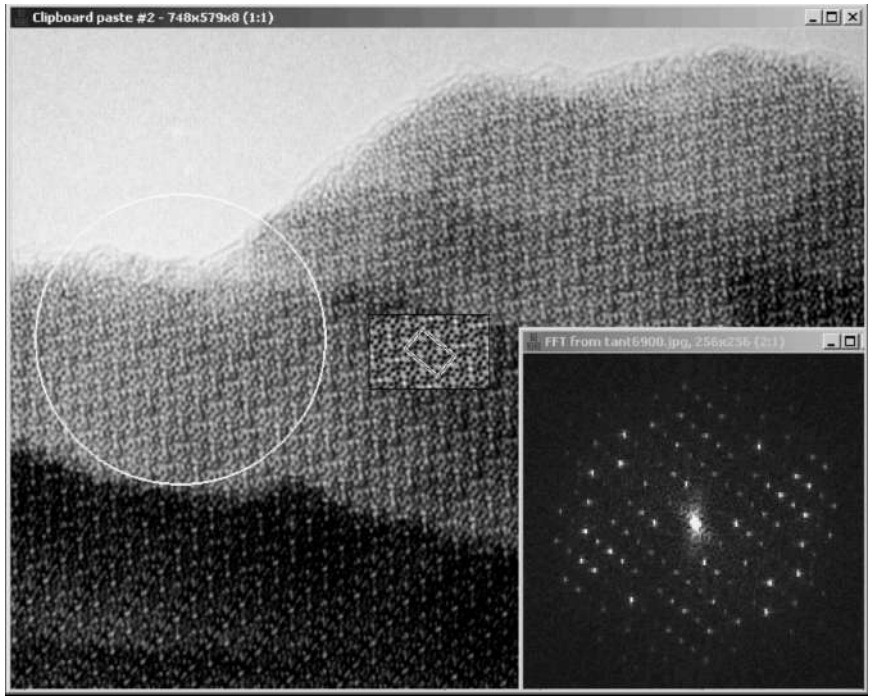
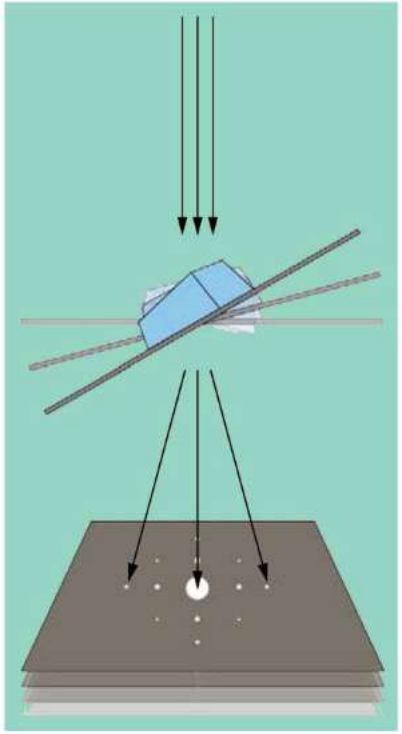
MicroED and 2D Crystallography

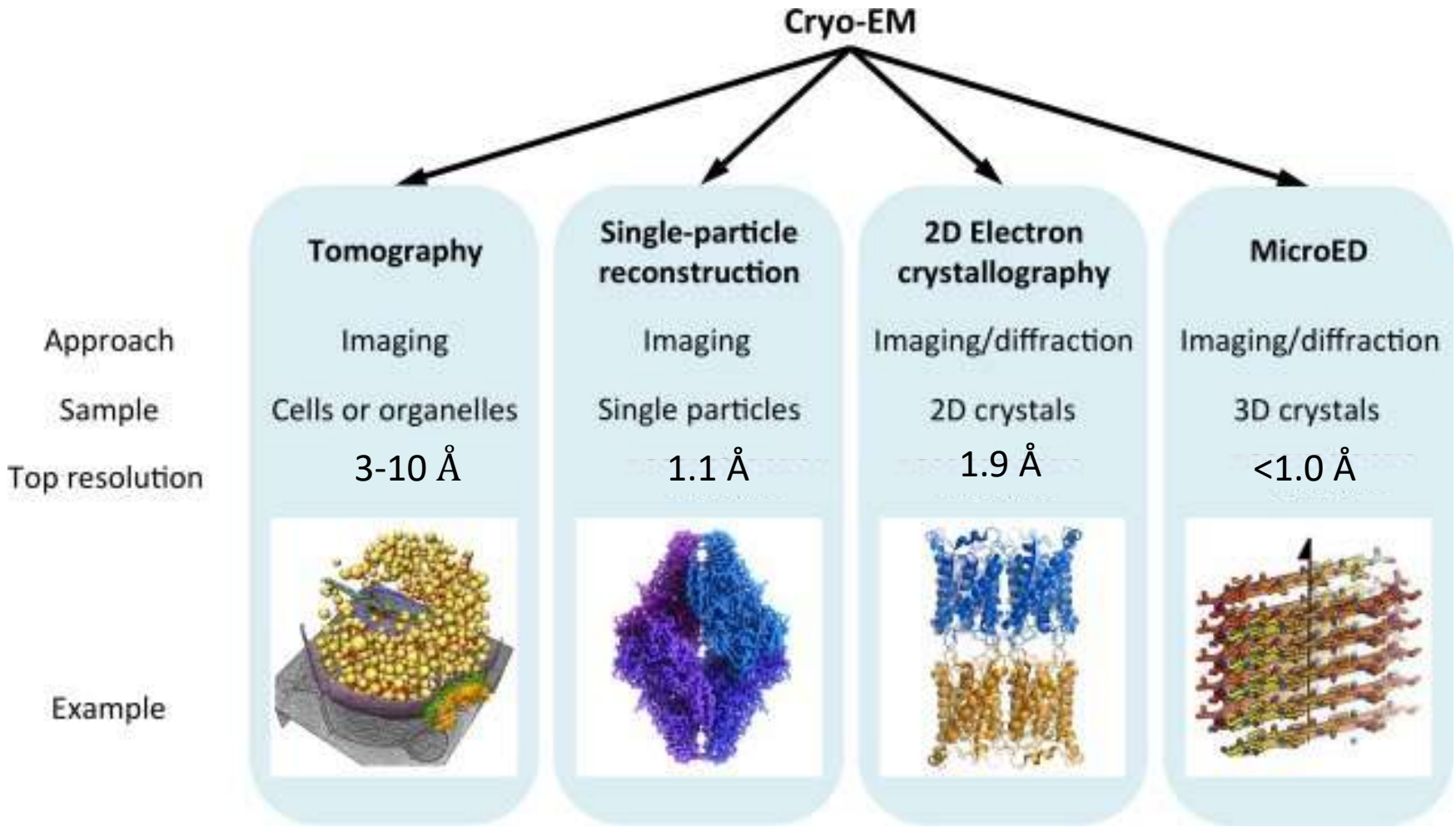
Feb. 10, 2025

William Rice, NYU Langone

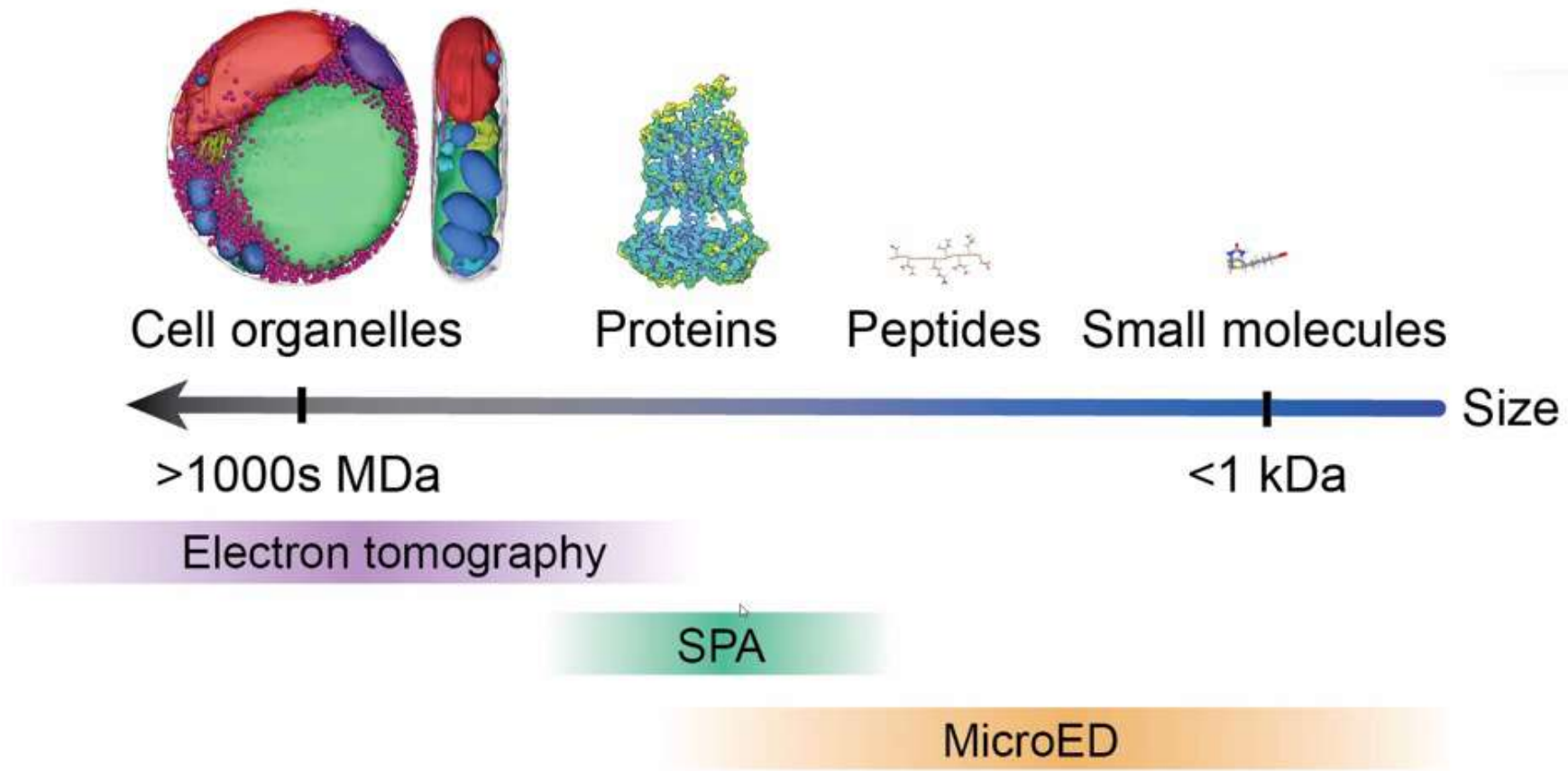




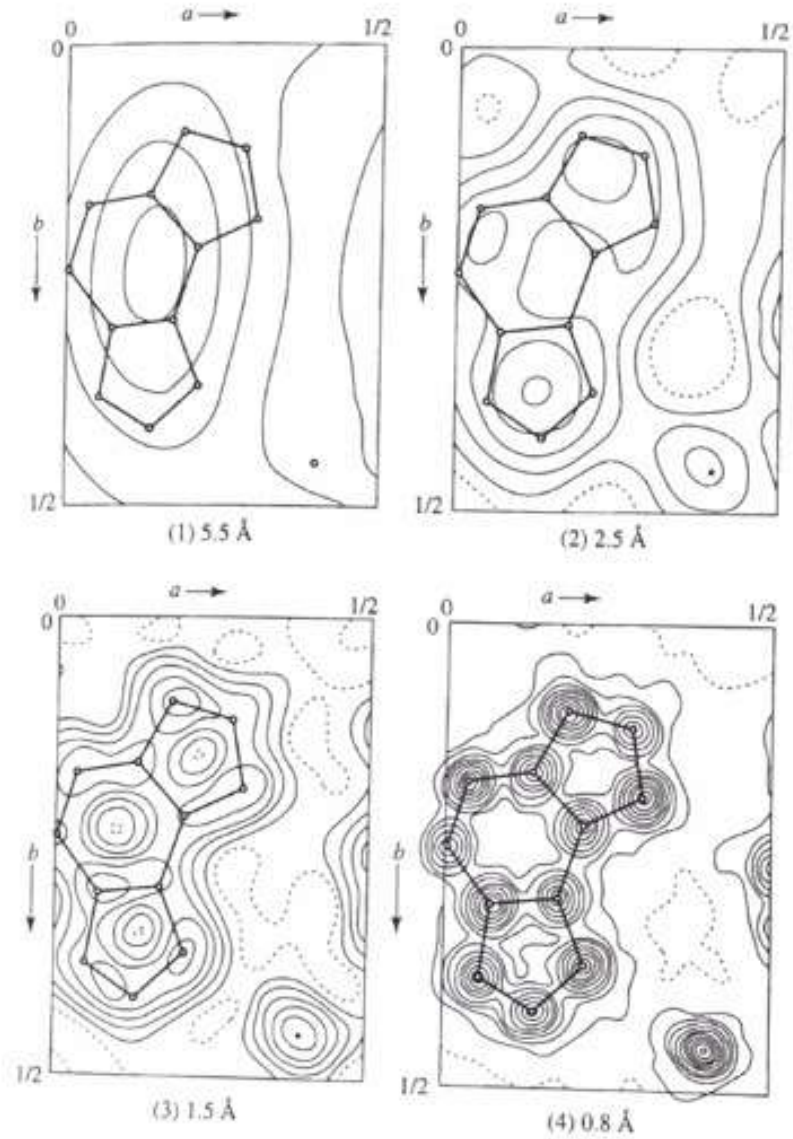
Best Resolution from EM Techniques



Sample Size


Cell organelles
Proteins
Peptides
Small molecules
Size
>1000s MDa
<1 kDa
Electron tomography
SPA
MicroED

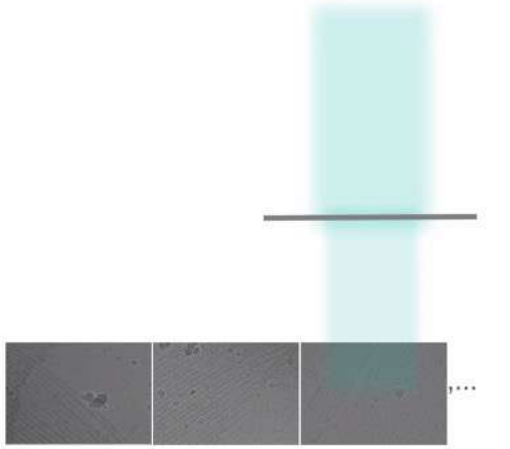
Practical example of resolution of a small molecule



The only “atomic” example

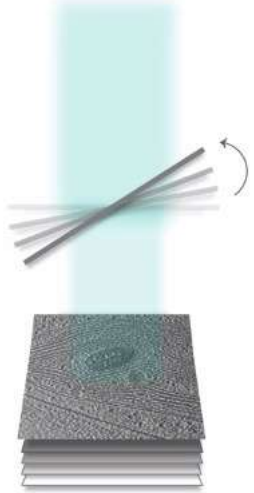
EM Techniques: Collection Strategy

Single Particle Acquisition



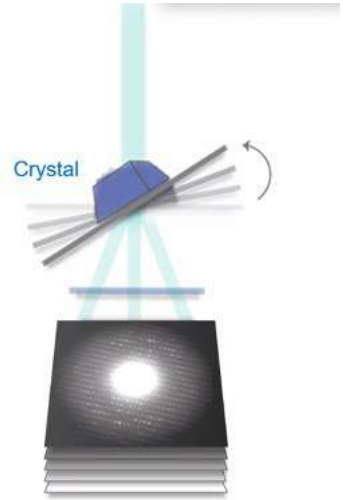
Capture → Move to new location → Capture,…
Imaging

Tomography



Capture → Tilt → Capture,…
Imaging

Micro ED

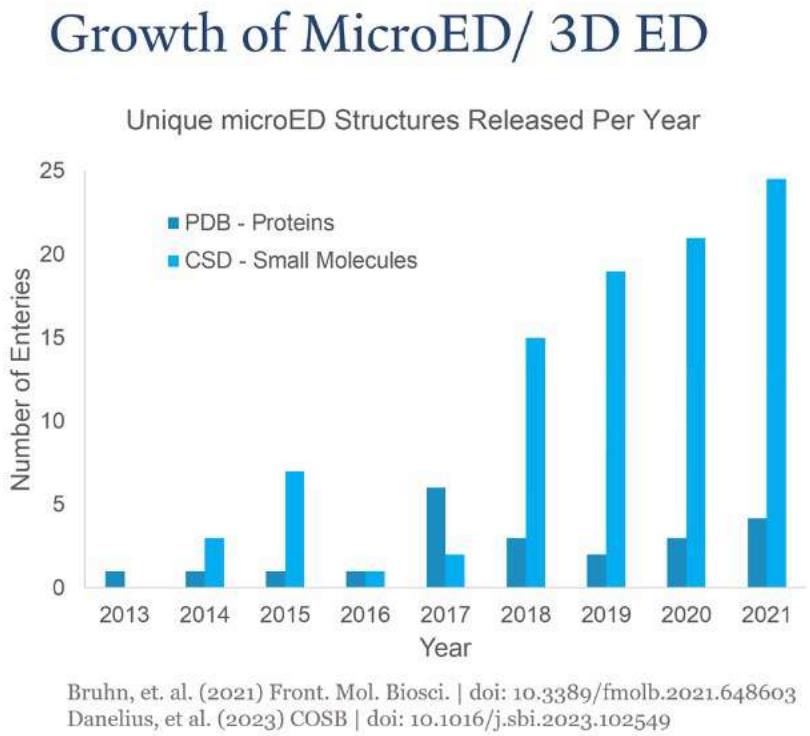


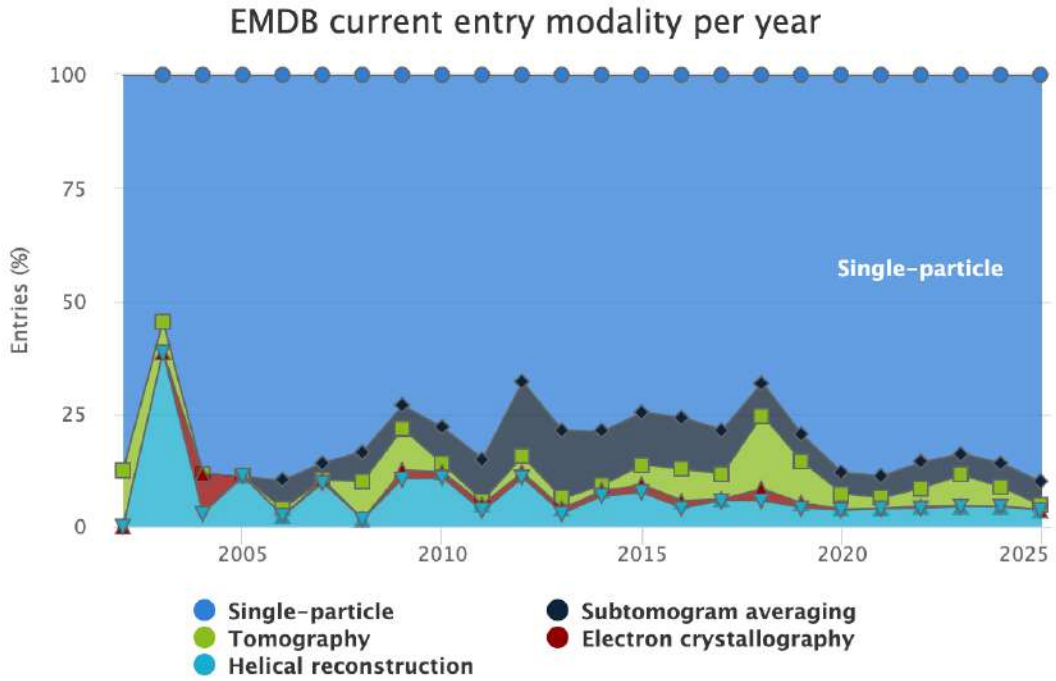
Capture & Tilt,…
Diffraction

Timeline of Electron Diffraction and Structure Determination

- In 1927, Davisson and Germer used a heated tungsten filament to fire a collimated beam of electrons at a polished chunk of crystalline nickel
- Wide range of experiments in the century between the birth of electron crystallography to today
- Structure determination by X-ray diffraction dominated from the mid-20th century to today
- In the mid 2000's, several groups began to publish structures determined by electron diffraction
- Resolution revolution in single particle CryoEM around 2013
- MicroED in 2013

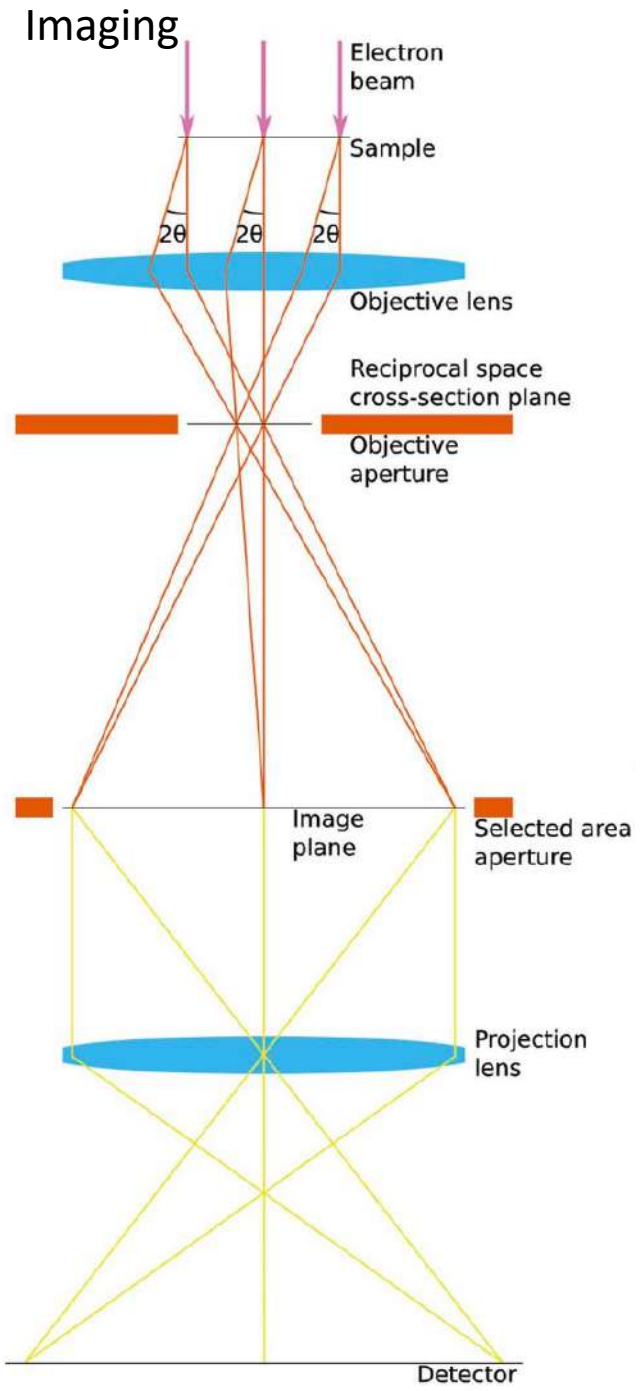
Growth of the MicroED Technique

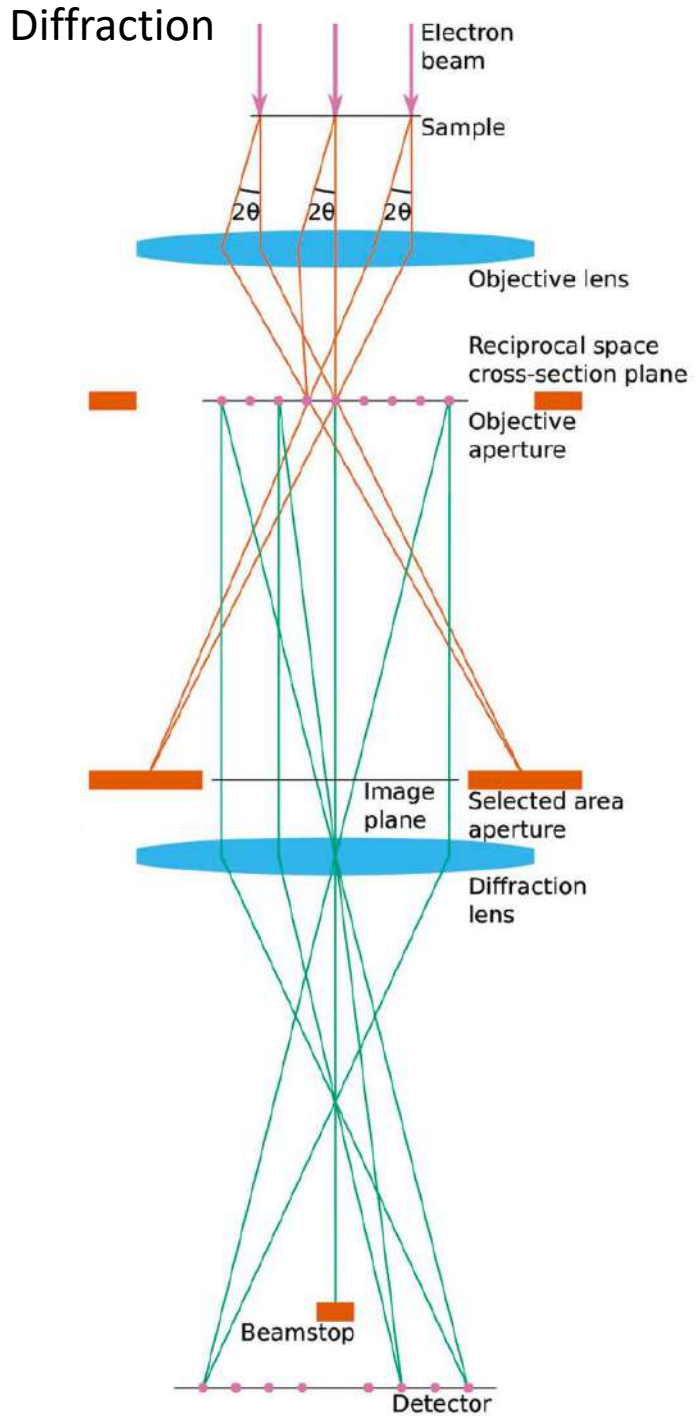




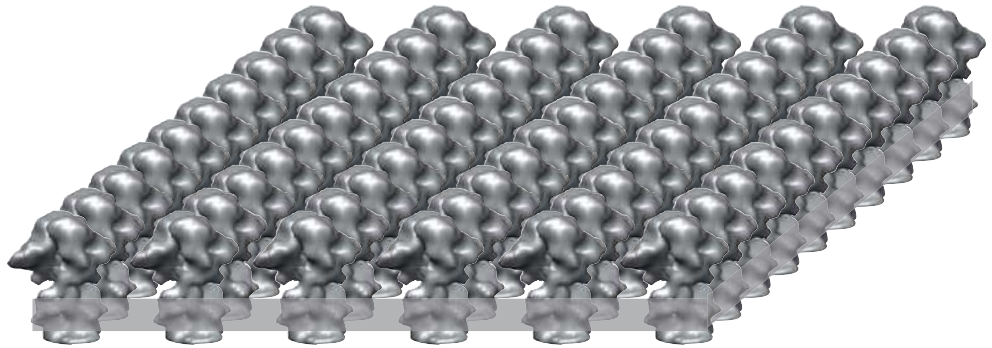
Electron microscope setup for diffraction

Electron Optics





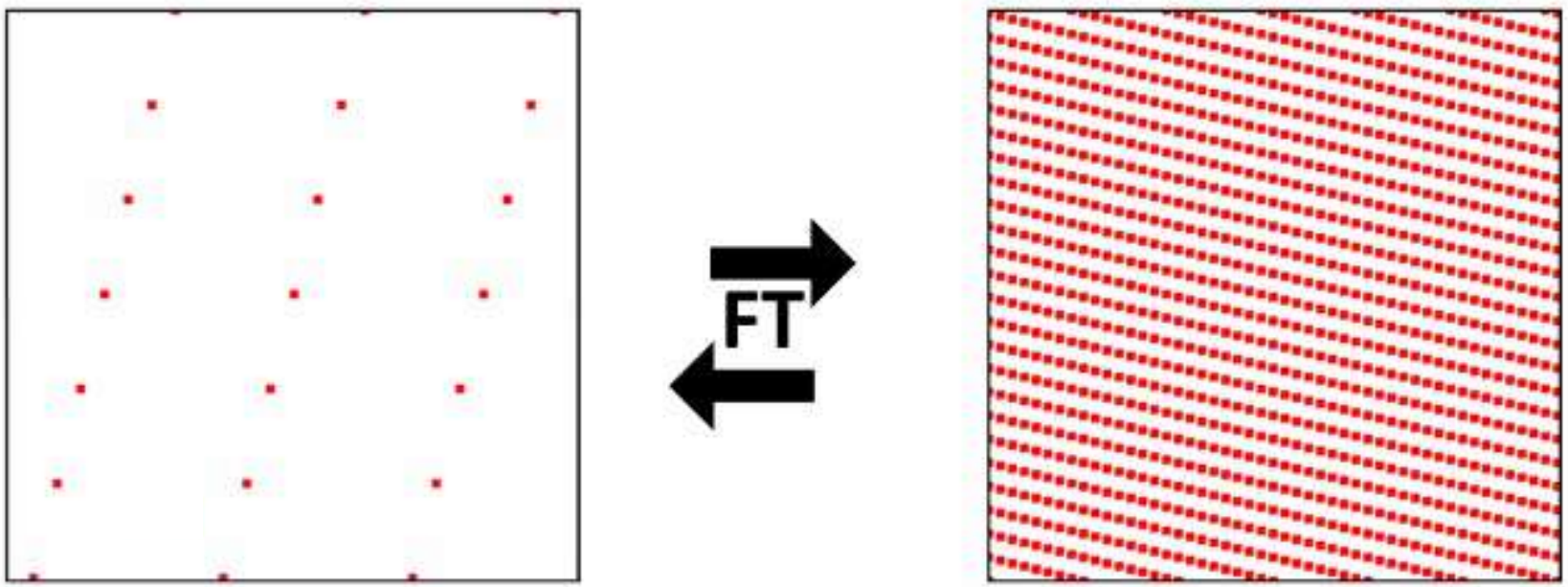
Hattne et al, 2015



2D Crystallography

The first high resolution Cryo-EM method, mostly for membrane proteins

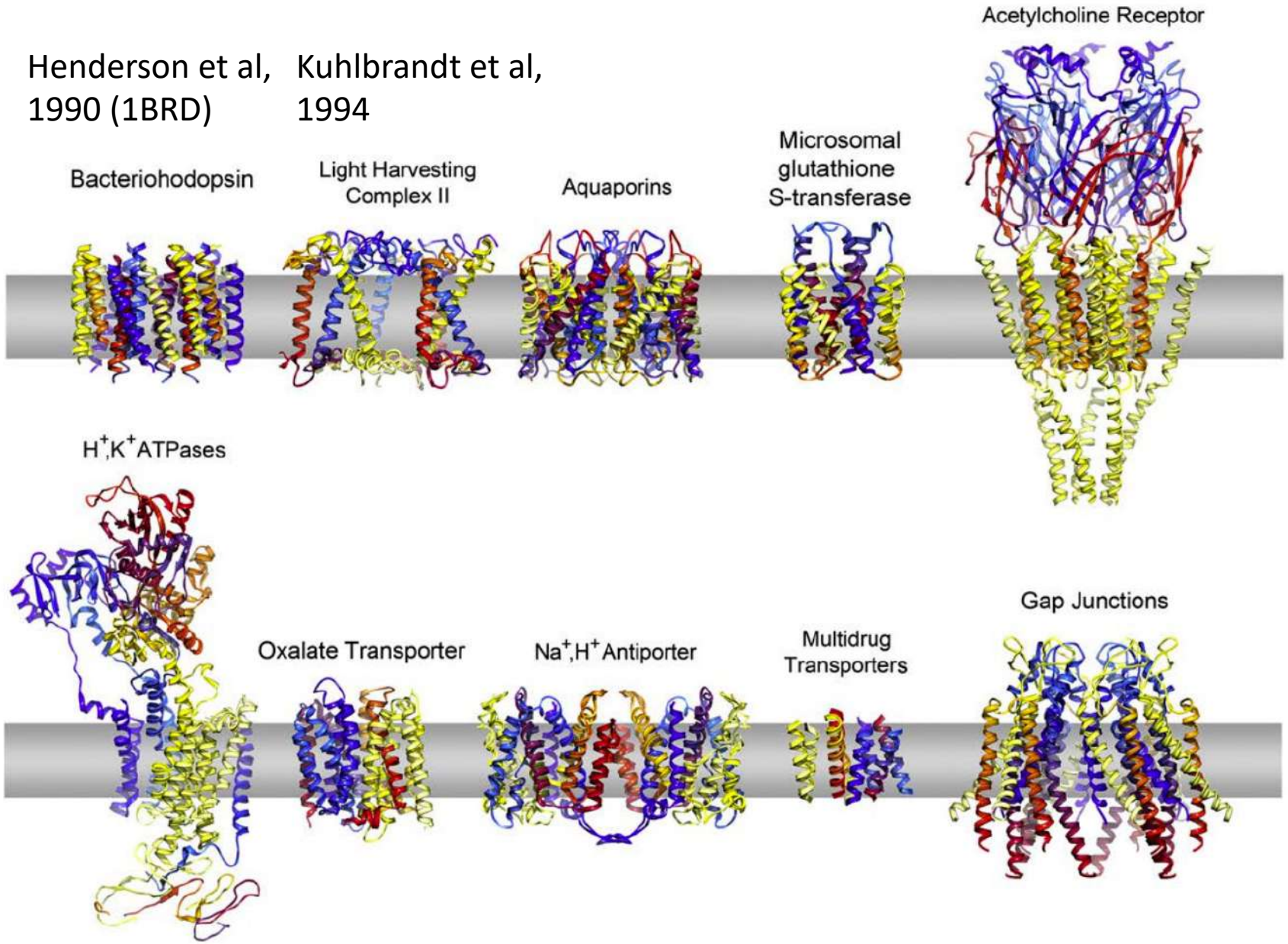
Diffraction (FT) of a 2D lattice



Spacing d

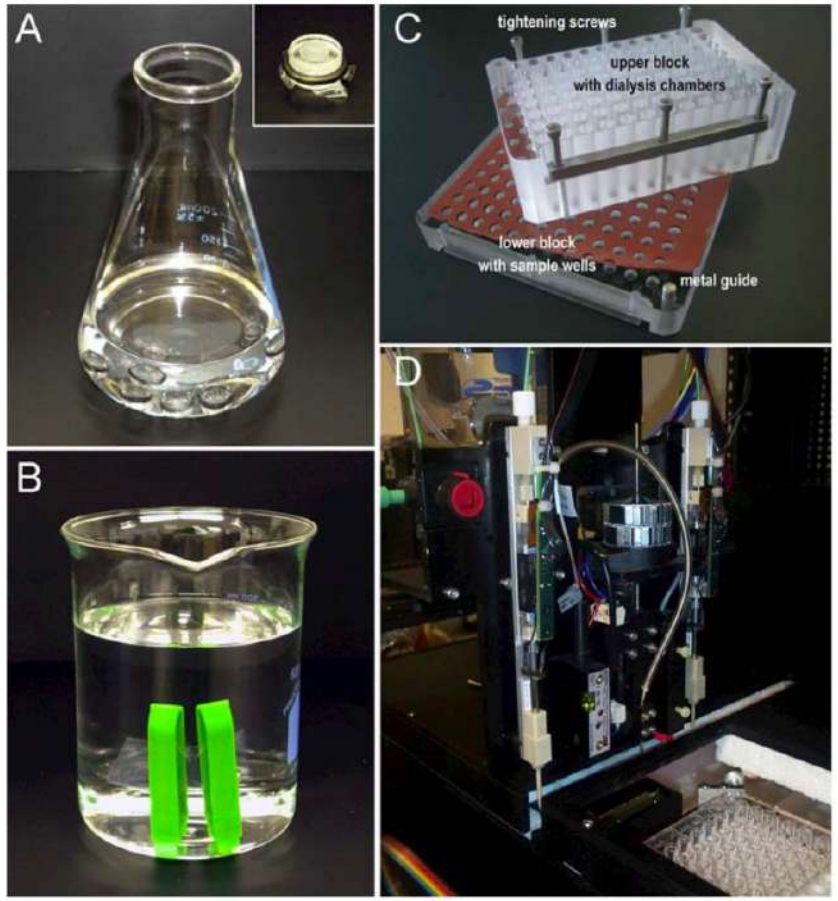
Spacing 1/d

High Resolution Structures from 2D Crystallography



Wisedchaisri et al, 2011

Preparation of 2D crystals: Remove detergent and put into lipid bilayer



- A: dialysis buttons
- B: Dialysis tubing
- C: 96-well dialysis block
- D: Robot for cyclodextrin mediated detergent removal

Cryo Imaging

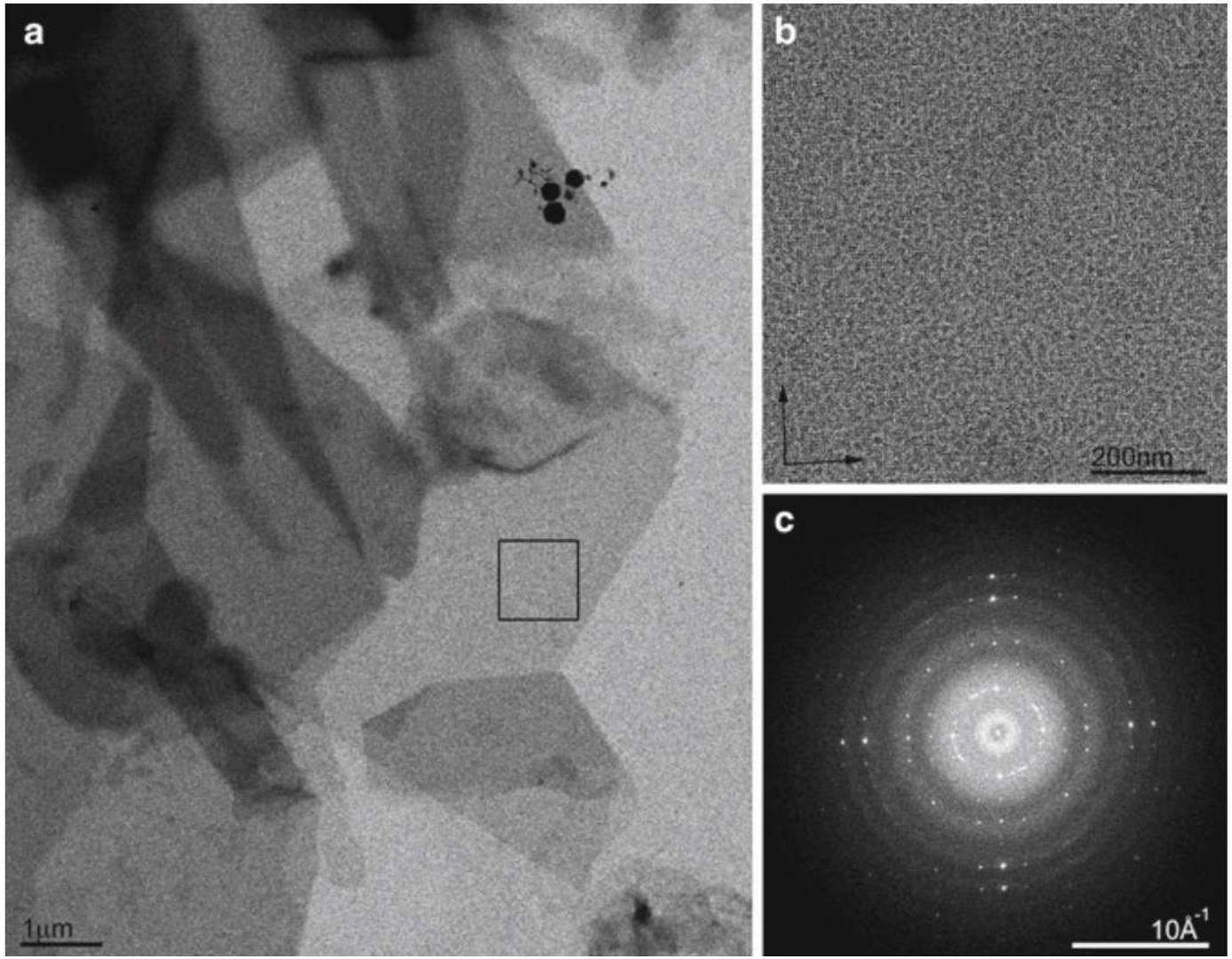
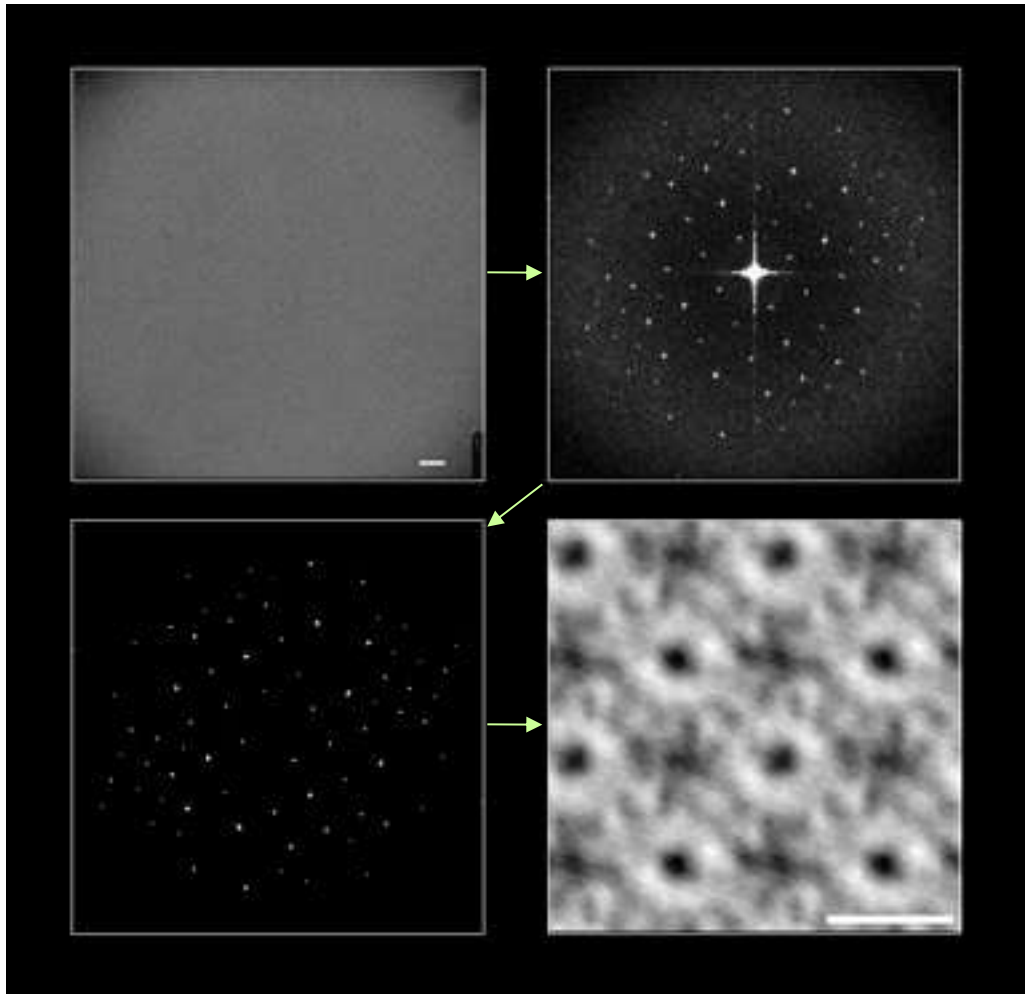


Fig. 3. Cryo EM of two-dimensional crystals. (**a**) Crystals of the water channel aquaporin-0 are large and have sharp edges attesting to the degree of order within. (**b**) High-resolution image of the crystal area highlighted by a *box* in (**a**). (**c**) Fourier transform of the image in (**b**) showing strong and sharp spots to ~6Å resolution. These crystals are ready for analysis by electron diffraction because the crystals appear uniformly *grey* on the grid. The spots in the Fourier transform are sharp and extend to ~6Å resolution without unbending. At this stage the sample should be frozen and the microscope setup should be changed to diffraction and data collected.

Gonen, 2011

Fourier analysis of images of 2D crystals

Original image

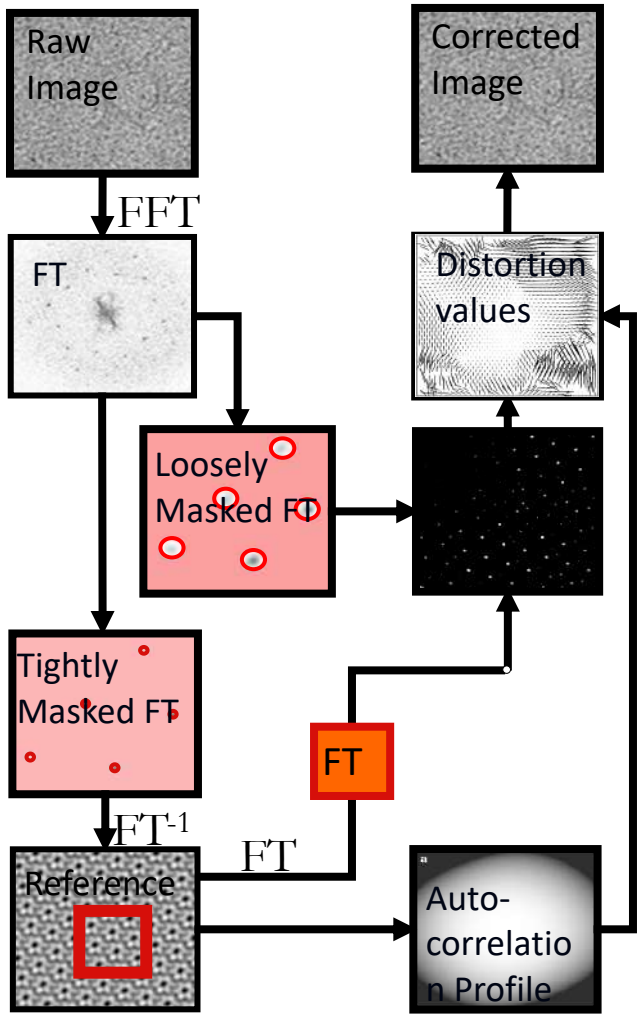


Fourier transform

Extraction and correction of Fourier components

Fourier synthesis

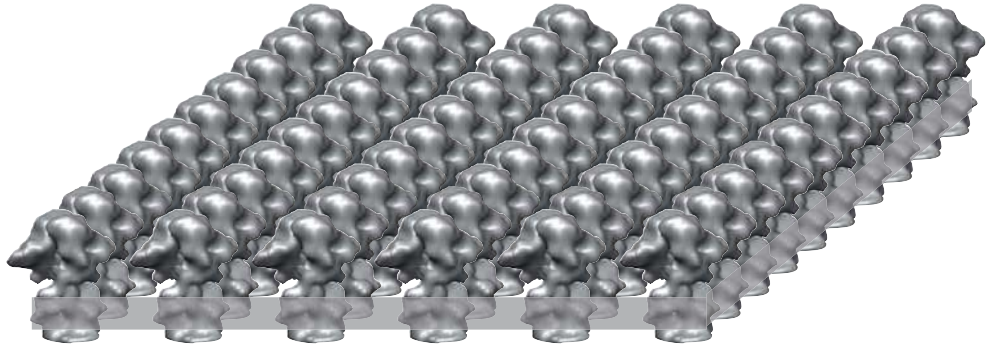
Unbending: Removal of lattice distortions

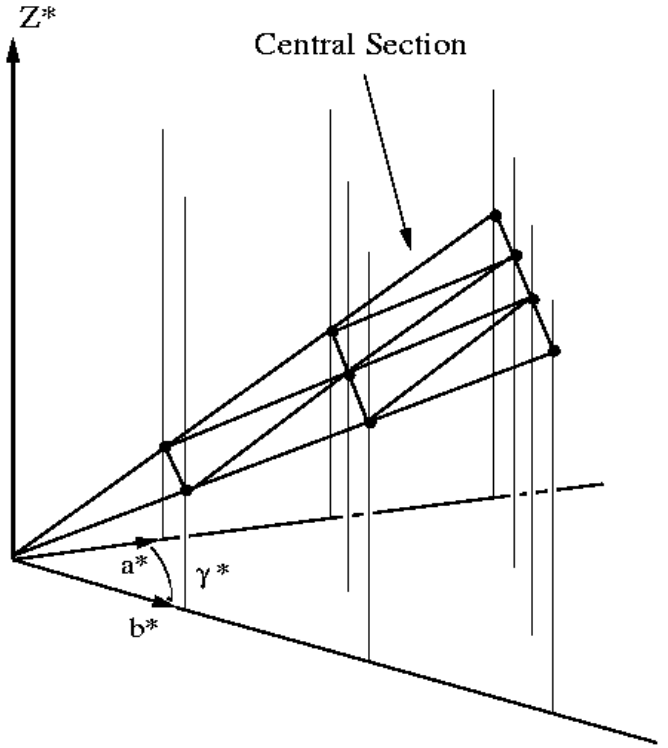


- Define a reference with good contrast
- Enhance the distortion at a similar contrast as the reference
- Generate Cross-Correlation map
- Create auto-correlation profile of the reference
- Quantify the distortion
- Make the correction

Anchi Cheng

For 3D information, we need to collect images of tilted crystals





Difficult:

Crystal may not be flat

Beam induced specimen motion is greater at higher tilt angles

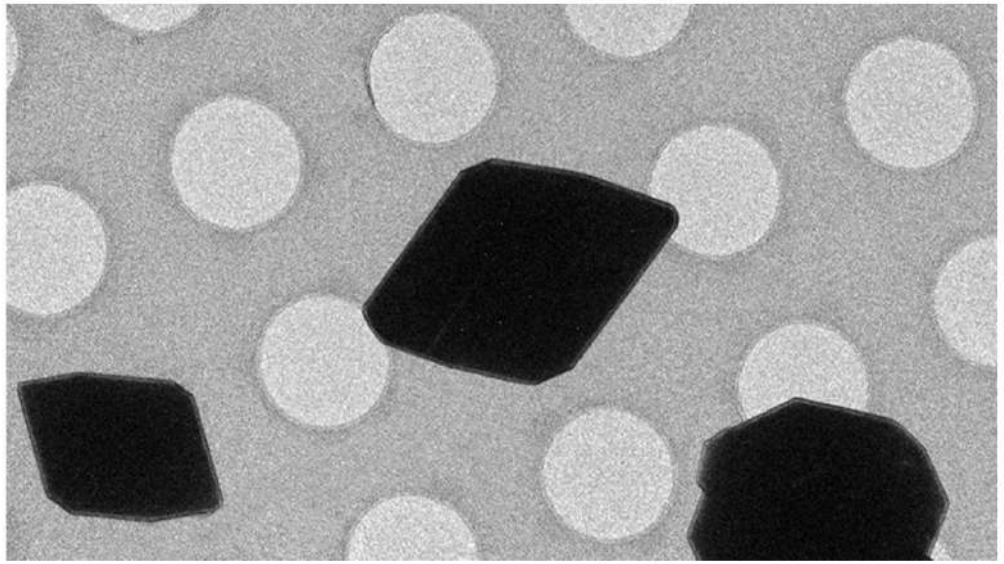
Difficulties in 2D crystallography

- Screening
  - Setting up conditions
  - Screening one by one
  - Large factorial surface (buffer, additives, lipid, detergent, speed of detergent removal)
- Samples
  - Need to be extremely flat over a large area
  - Need to be very well ordered
- Collection
  - Need to merge crystals at different tilts to get 3D reconstructions
  - Collect images as well as diffraction data
  - Hard to collect high quality tilted images
  - Manual collection
- Software
  - Difficult to use: until 2dx, command-line driven scripts

Resolution Revolution

- With the development of direct detectors, electron counting, and better software such as Relion and CryoSPARC, single particle analysis can routinely reach 3 Å or better resolution, down to ~1 Å
- Minimum sample size ~50 kDa for single particle analysis (so far)
- 2D crystallography has been abandoned for the most part, apart from helical analysis (next week!)
- However, 3D crystallographic techniques appeared coincidentally at about the same time as direct detectors…

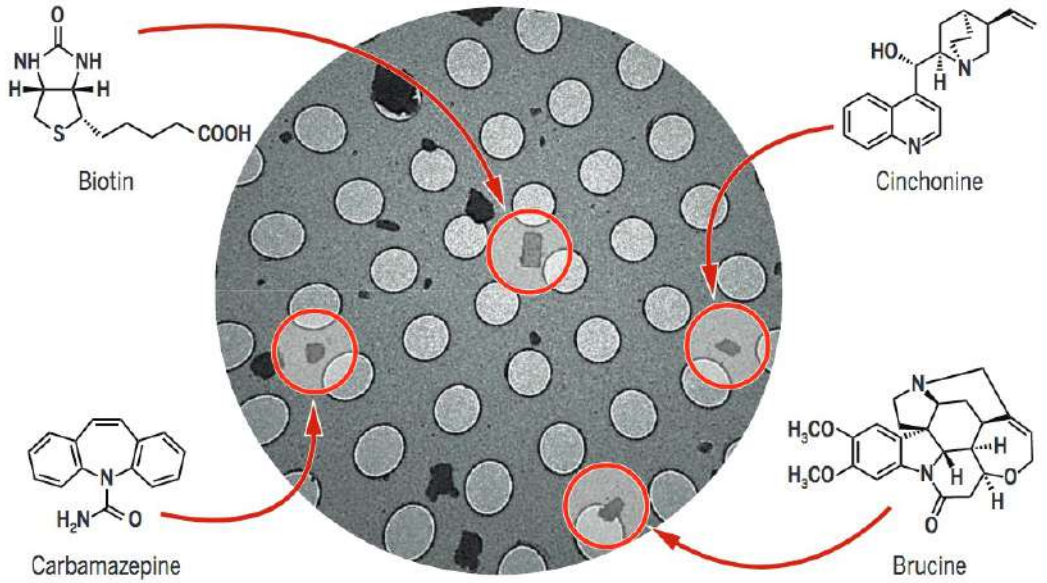
Molecular structures made simple



Structures can now be gleaned from micrometer-size crystals (black), seen here on an electron microscope slide. (GONEN LAB)

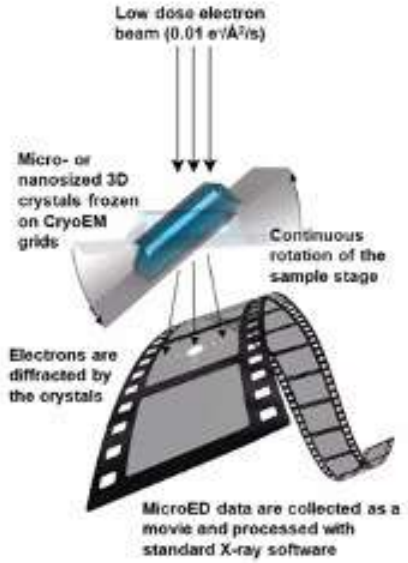
Structures from a mix of microcrystals

A new technique identified structures of four compounds from tiny crystals on an electron microscope slide.

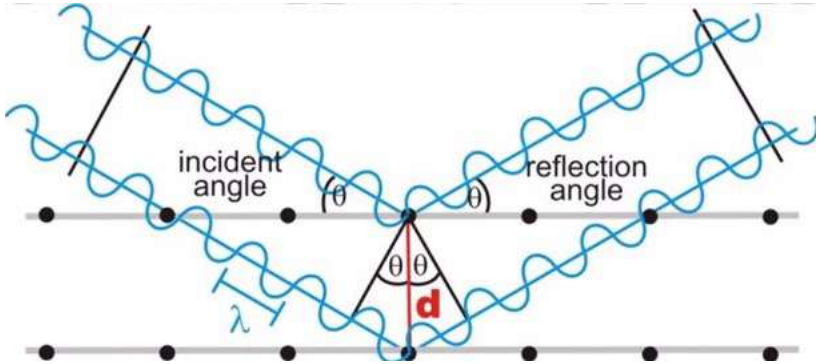


MicroED

Runner-up for Science's 2018 Breakthrough of the Year



Bragg's Law of Diffraction

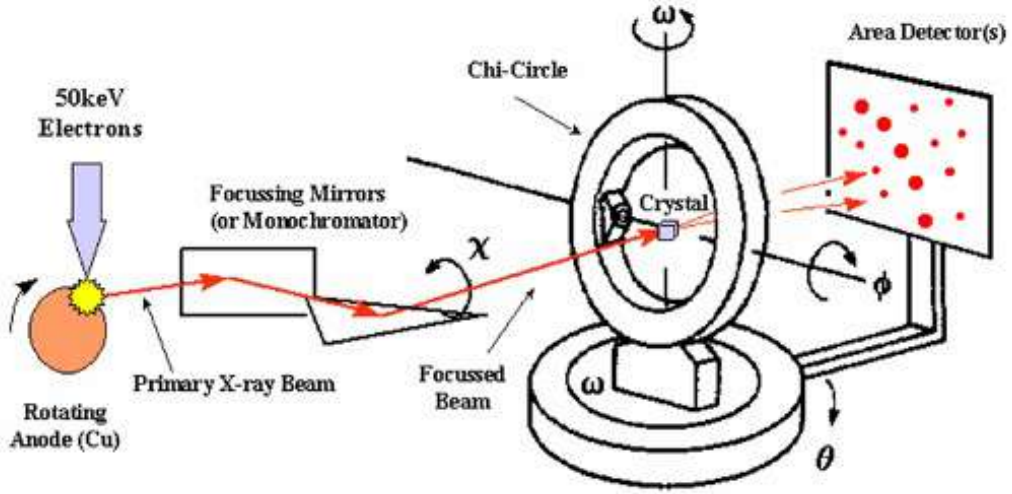


Bragg: $n\lambda = 2d \sin \theta$

Wavelengths

- X-ray
  - λ=70.9 pm (Ag Ka)
  - λ =154 pm (Cu Ka)
- EM
  - 80 keV: 4.18 pm
  - 120 keV: 3.35 pm
  - 200 keV: 2.51 pm
  - 300 keV: 1.97 pm

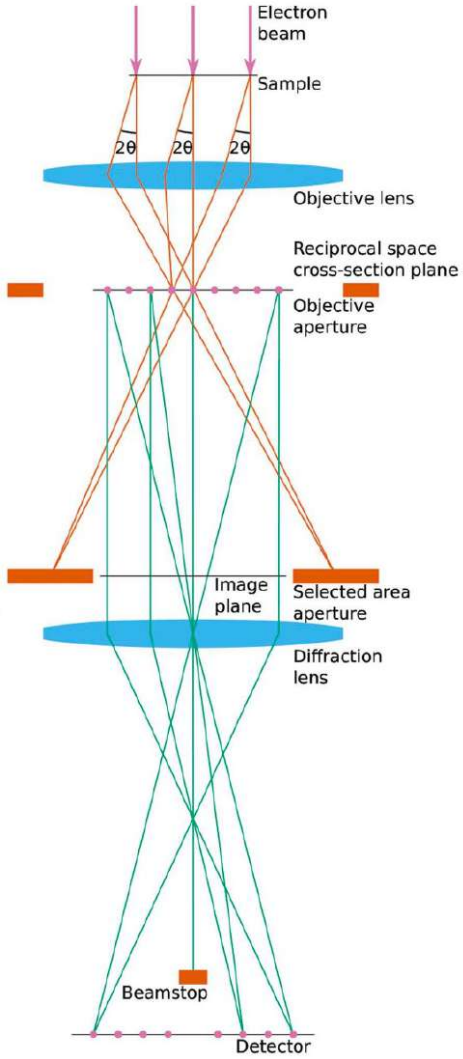
X-ray Diffraction



4-Circle Goniometer (Eulerian or Kappa Geometry)

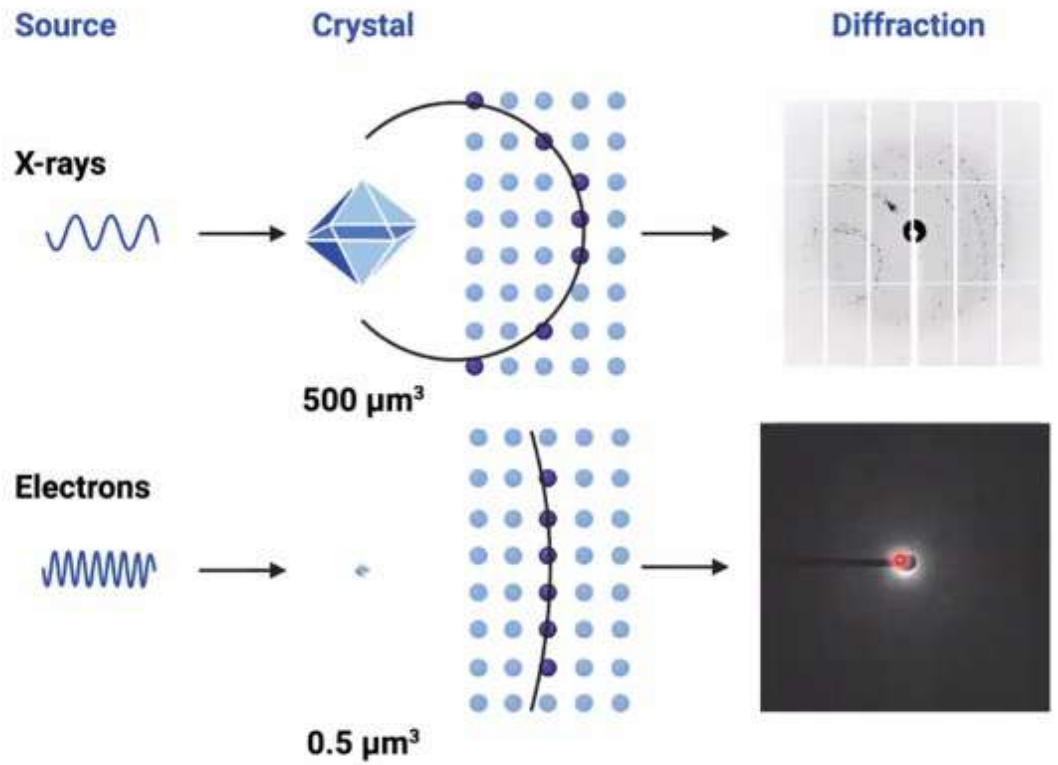
Electron Diffraction

120-300 keV electrons

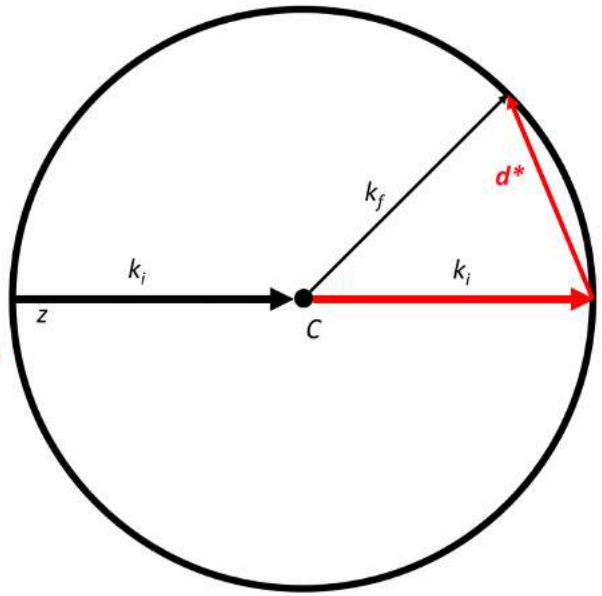


Differences between x-ray and electron diffraction

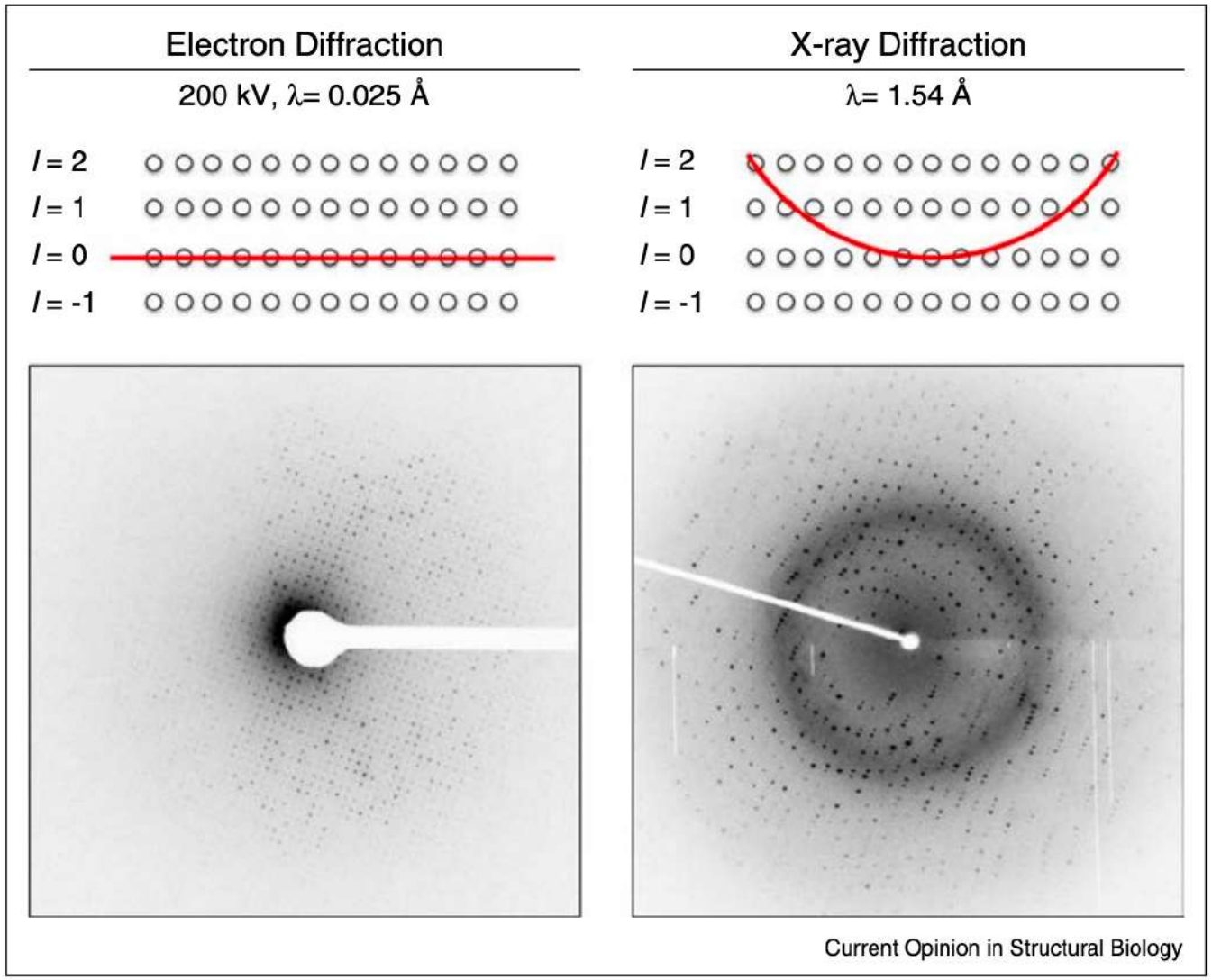
- Radiation source
  - Electrons interact more strongly with matter than X-rays
  - Ratio of elastic/inelastic scattering is 1:3 for e^-; 1:10 for X-rays
- Crystal Size
  - Electron diffraction requires much smaller crystals
- Diffraction pattern
  - Wavelength of electrons is very small
    - 0.025 Å at 200 kV, versus 1.5 Å
    - Flat Ewald sphere



Ewald Sphere

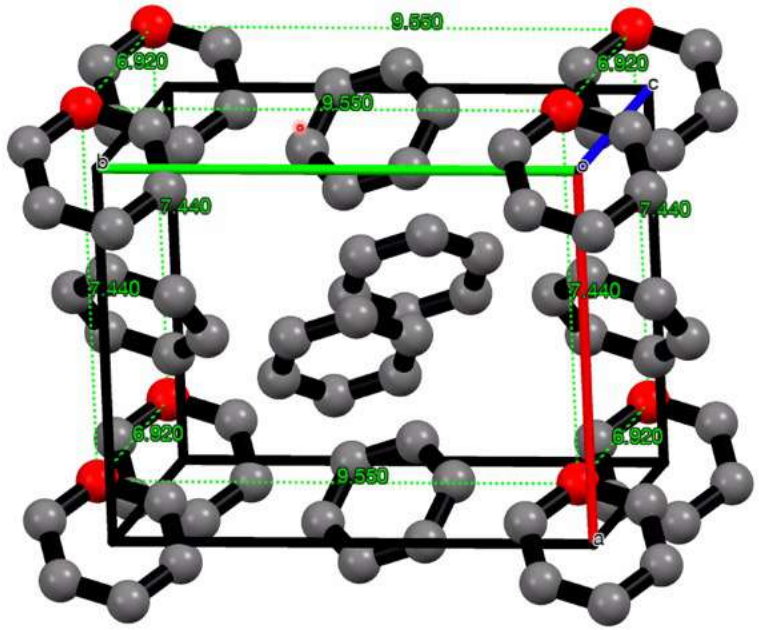


Ewald circle (or sphere in 3D) has a radius of **1/λ**



Comparison of diffraction data obtained from lysozyme crystals by electron diffraction and X-ray diffraction. Because the wavelength of the diffracting electrons is so short, the resulting Ewald sphere (left, red line) is essentially a plane when compared to the Ewald sphere for X-ray diffraction (right, red line). Diffraction only occurs when the Ewald sphere contacts a reflection in reciprocal space (top panels, white circles represent reflections in reciprocal space). Therefore, because the Ewald sphere is so flat, the patterns produced from electron diffraction (bottom left) appear as planar 2-dimensional slices through the 3-dimensional volume of reflections, whereas the patterns from X-ray diffraction (bottom right) appear as circular 2-dimensional projections of the sphere on the detector.

3D Crystal of a simple molecule

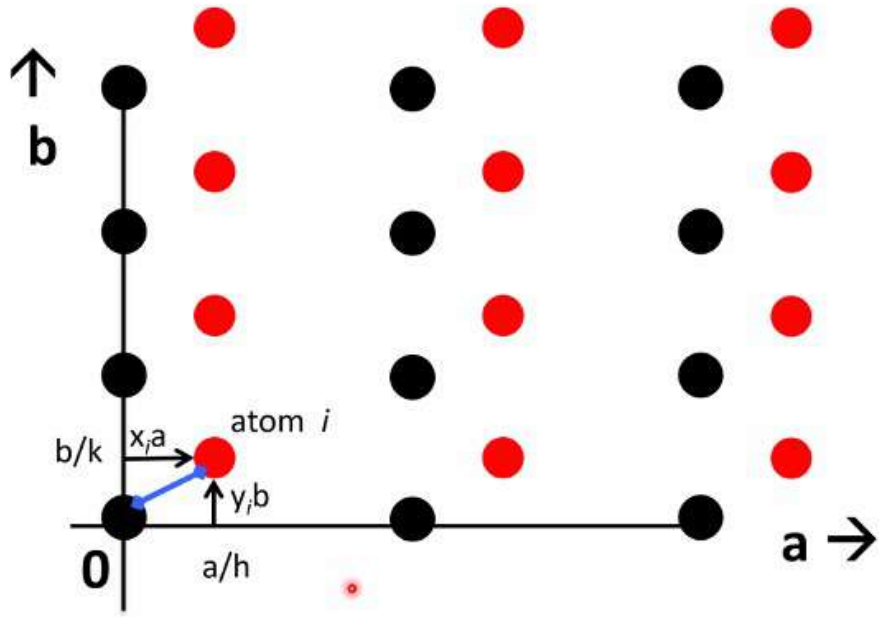


Pbca

a = 7.440 Å
b = 9.550 Å
c = 6.920 Å

α = 90°
β = 90°
γ = 90°

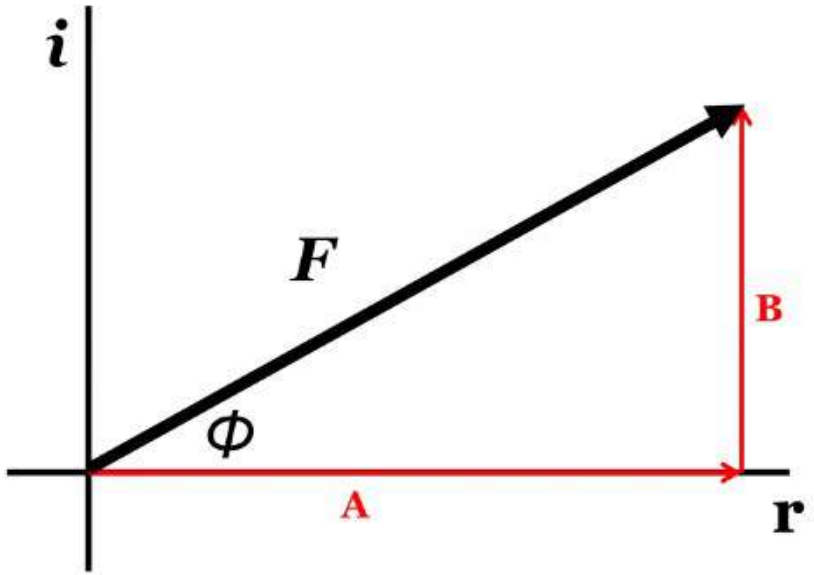
Shift a lattice to add a second atom



Move some fraction:
a/h along a
b/k along b
c/l along l (not shown)

$$\Delta\Phi_{i(a)}=2\pi\frac{x_i a}{a/h};\ \Delta\Phi_{i(a)}=2\pi\frac{y_i b}{b/k};\ \Delta\Phi_{i(a)}=2\pi\frac{z_i c}{c/l}$$

Use a complex plane to describe the phase shift from atom to atom



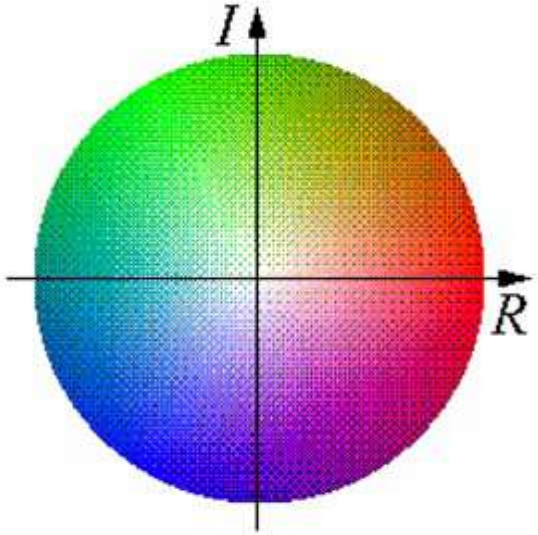
We cannot directly measure the magnitude of F

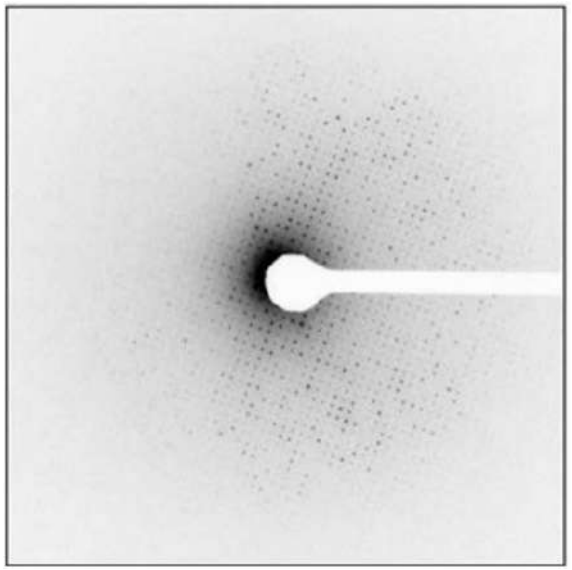
$I \propto F^2$

$$\phi = \arctan\frac{\mathrm{B}}{\mathrm{A}} \qquad |F| = \sqrt{\mathrm{A}^2 + \mathrm{B}^2}$$

$$F_c = \sum_j F_j(cos2\pi(hx + ky + lz) + i\, sin2\pi(hx + ky + lz))$$

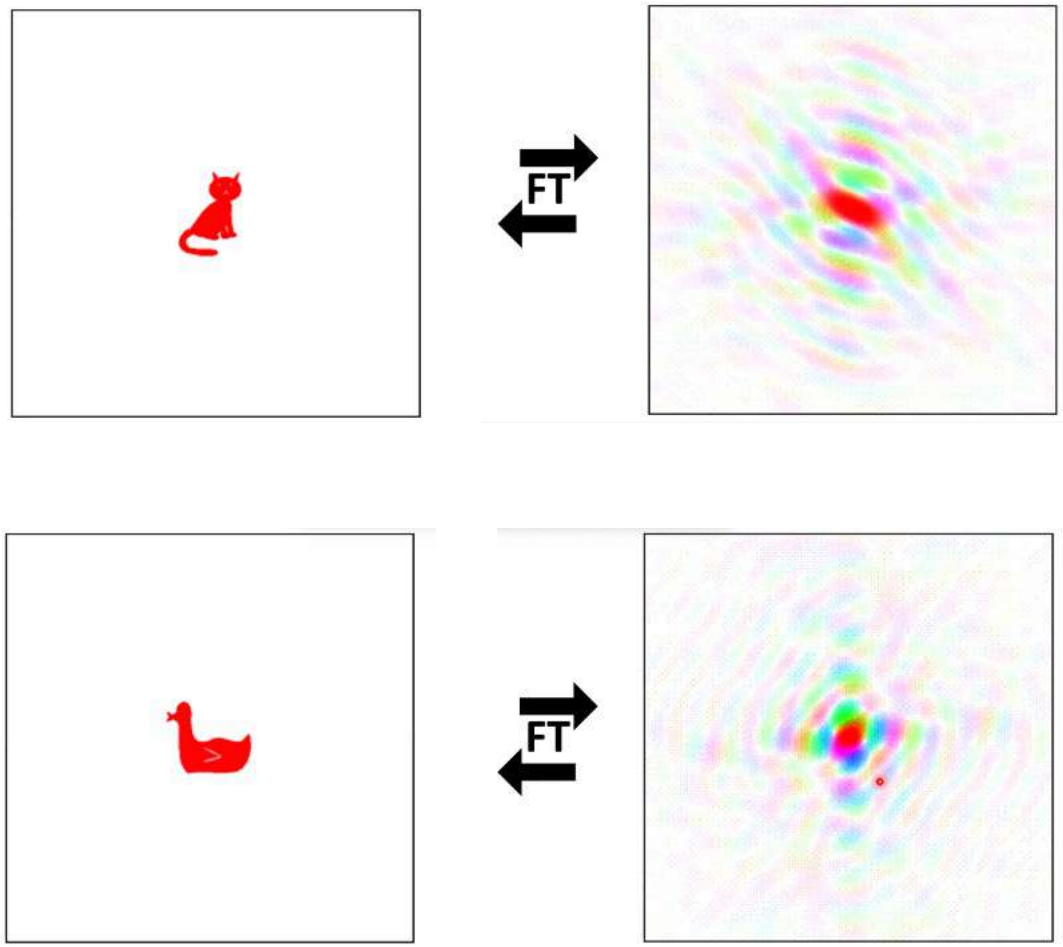
Phase and Magnitude



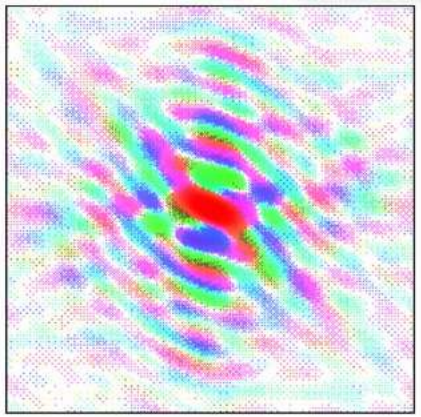


- The magnitude is represented by saturation and brightness
- The phase is represented by color
- When we collect diffraction data, we collect the intensity, which is magnitude^2
- Phase information is lost

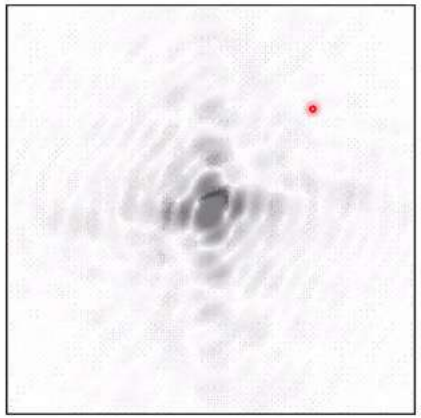
Computational Experiment: Importance of Phases and Amplitudes



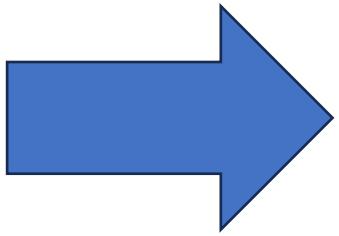
Combine phases and magnitudes

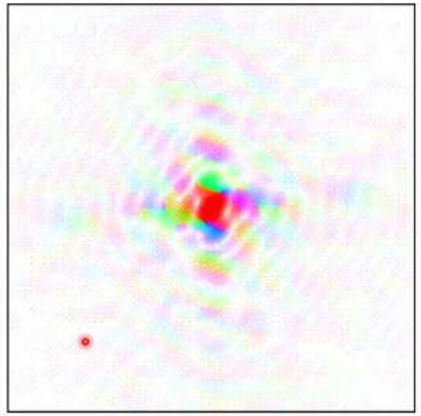


Phase of cat (equal amplitudes)

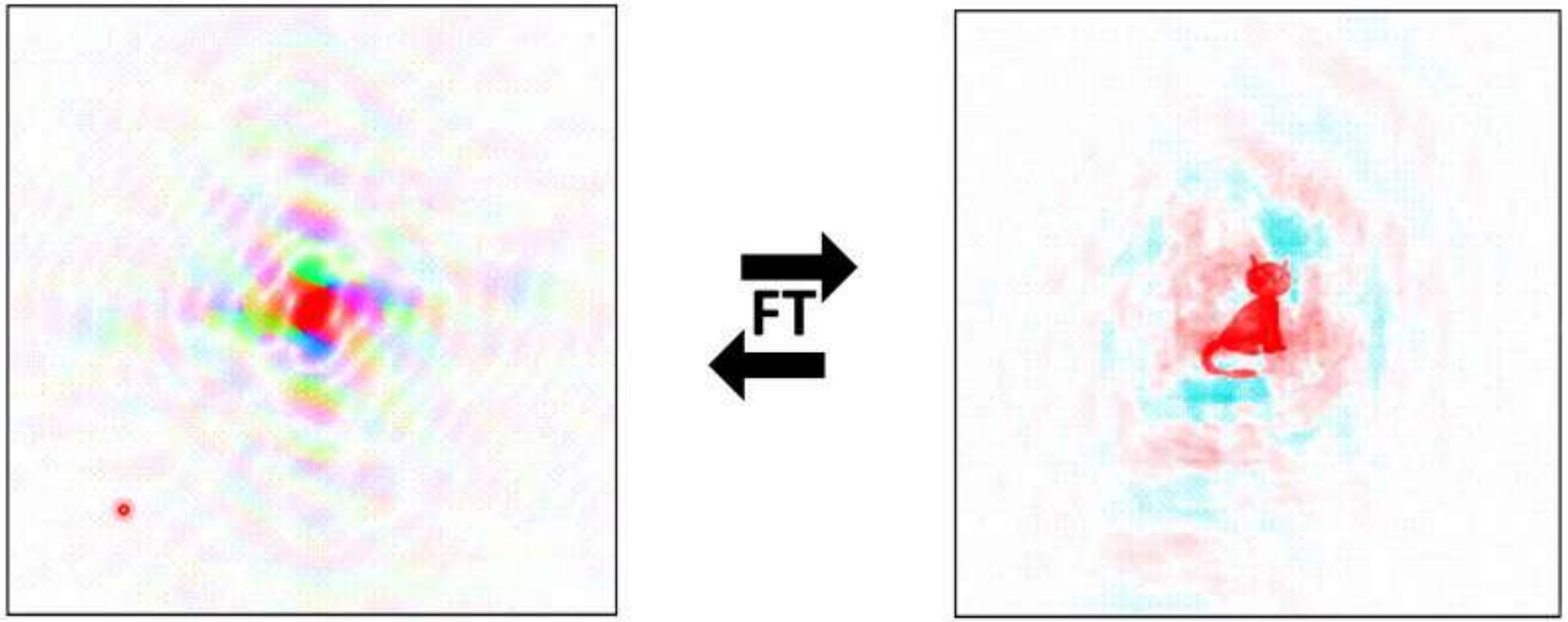


Amplitude of duck





The heart of crystallography



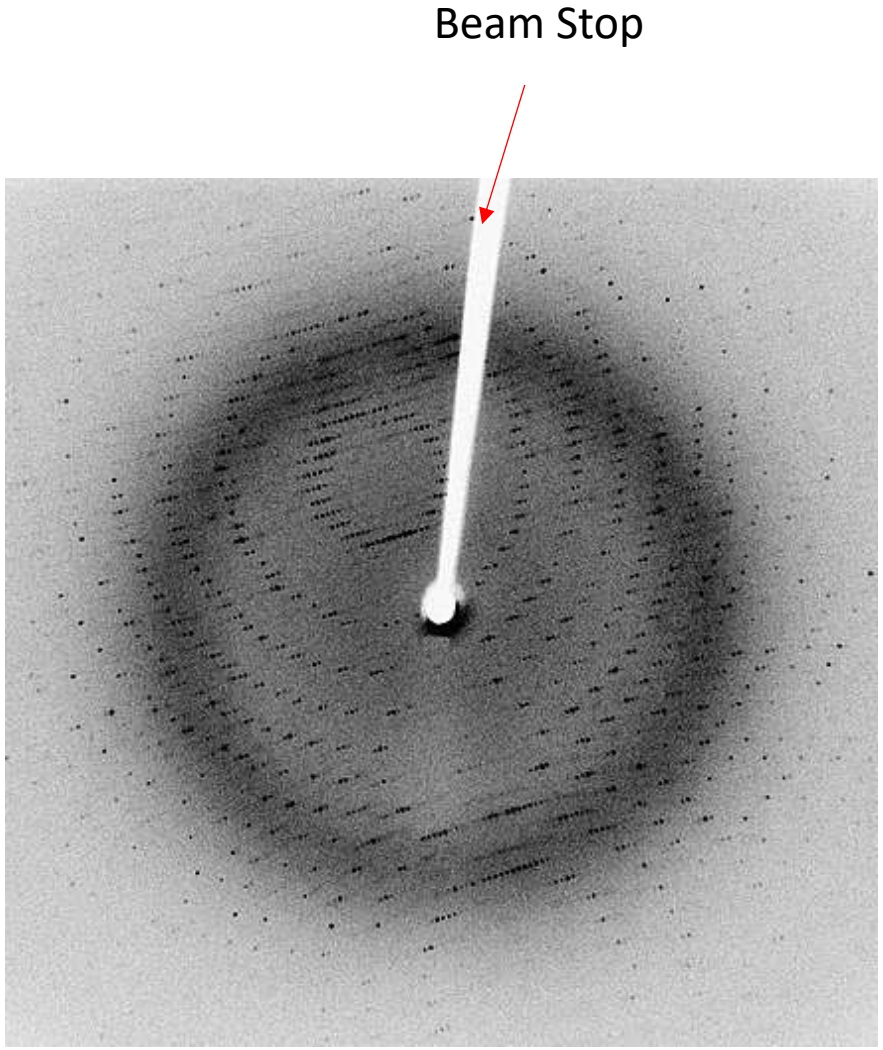
In diffraction, we only collect the amplitudes, and the phases are lost.
Unfortunately, the phases contain the bulk of the structural information.
This is the “phase problem” in crystallography: the phases must be solved correctly to get a structure.
This is the calculation in crystallography when “solving” a structure.

Strategies to solve the phases

- Direct methods
  - Calculate from the data itself
  - Need good data to 1Å or better
  - Generally for small molecules
- Molecular replacement
  - Start with the phases of a known similar molecule
  - Try an alphaFold model if an existing structure is unavailable
- MAD/SAD phasing
  - X-ray diffraction only, use SeMet
- Soak in heavy atom derivatives (eg. Pb)
  - X-ray diffraction only

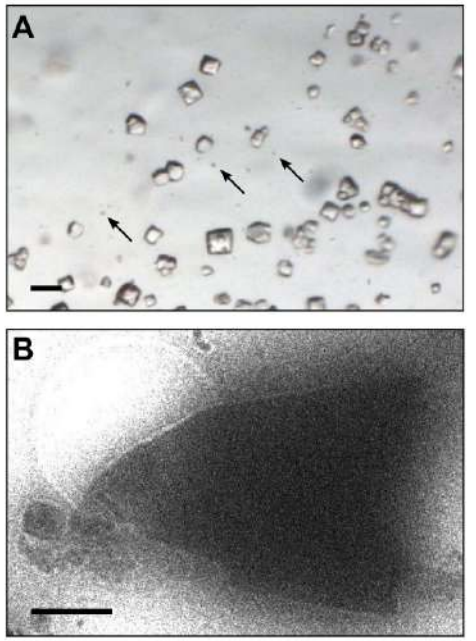
X-Ray Diffraction of crystals

- Mount crystal, expose to x-ray beam at defined wavelength
- Collect images of reflections on detector
- Only collect intensities and positions, not phases
- Rotate crystal (180 deg) to get all reflections
- From positions, get 3D lattice parameters
- Phasing
  - Ab initio (small, high resolution)
  - Heavy atom derivatives
  - MAD/SAD
  - Molecular replacement



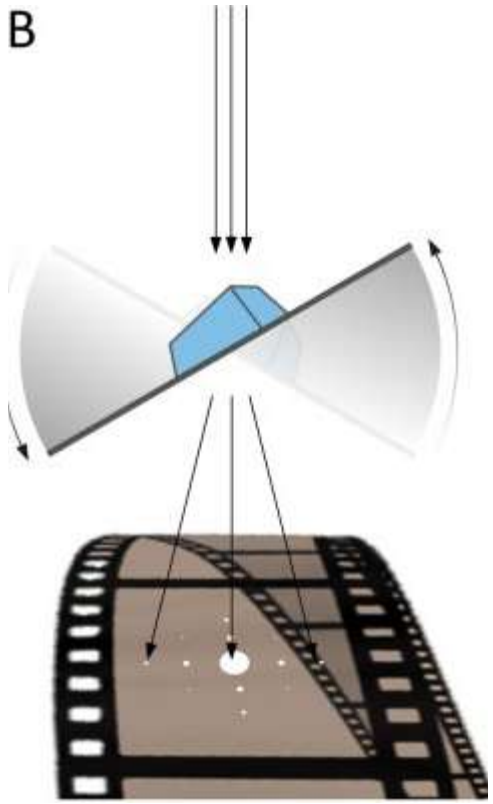
Electron Diffraction of Crystals
Original implementation (Shi et al, 2013)

- Image single images at various tilts (1 deg increment)
- Reflections recorded in this manner are generally partial reflections
  - Needed in-house scripts to index the data and group symmetry-related reflections
- Lysozyme at 2.9 Å resolution
  - 200 keV on TVIPS F416 CMOS detector
- Apply solution with crystals to grid
- Blot with Vitrobot

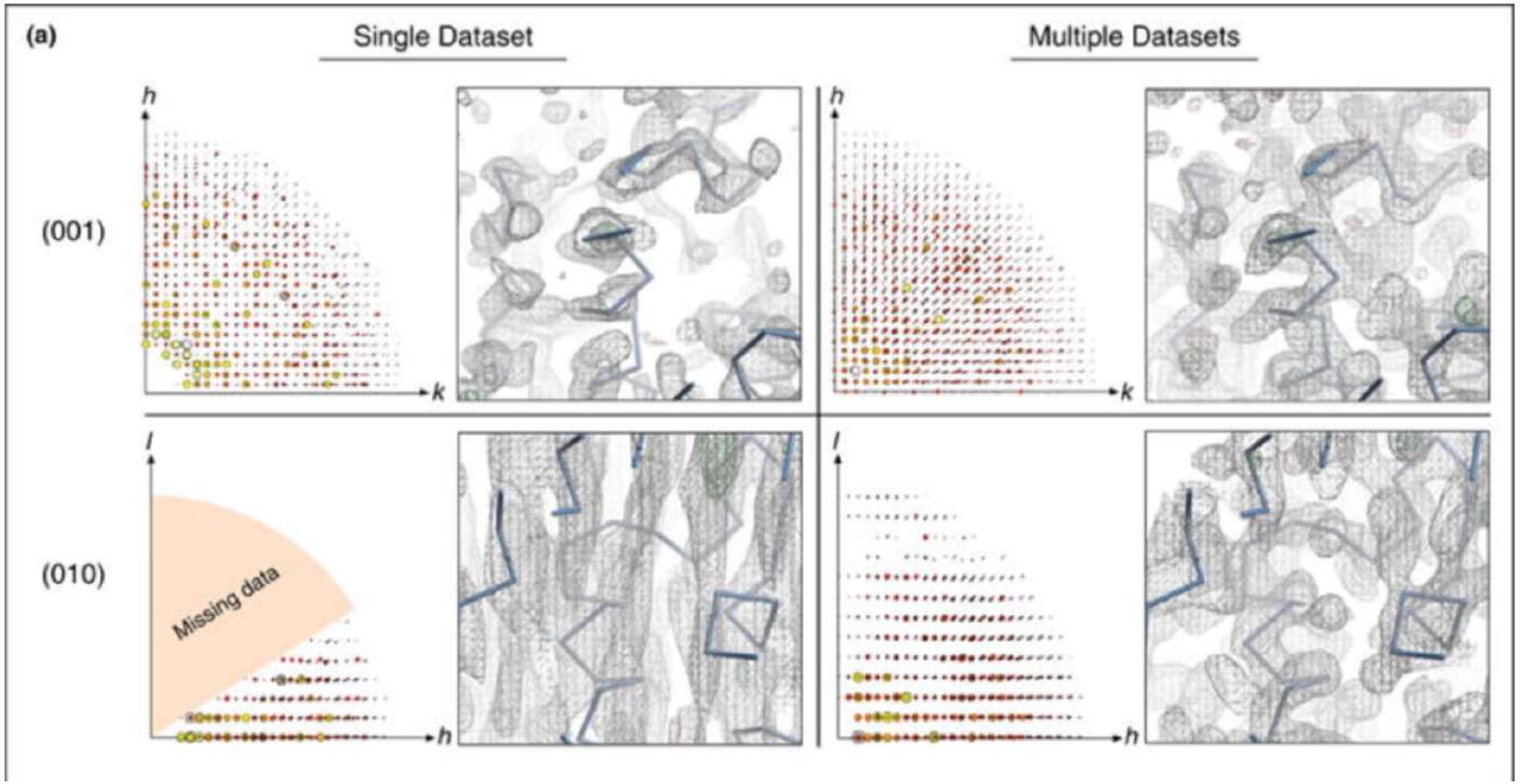


Electron Diffraction Collection: Continuous rotation

- Rotate stage at continuous rate
- Rotate to coordinate with exposure time
- Camera needs to be in continuous “rolling shutter” mode
- High rotation rate: increases the recorded reflection fraction on each frame
  - Too high: spot overlap
- Low rotation rate: makes weaker, high resolution reflections more visible
  - Too low: too few spots per image



Missing Wedge (-60 to +60 degrees)



Nannenga and Gonen, 2014

Sample preparation

Types of Microcrystals

Small Molecule

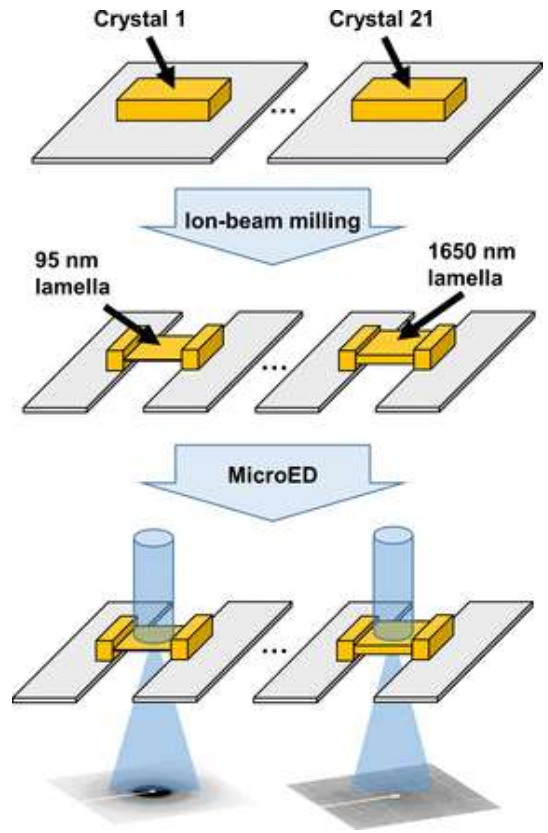
- Organic molecules often are dried after synthesis
- Dried powders often contain tiny crystals of the material, too small for X-ray diffraction
- Can be applied directly to EM grids at room temperature
- Can be cooled to cryo temperatures after insertion in the microscope
- Direct methods can be used for phasing

Macromolecules

- Protein should be crystallized
- Needs to be frozen onto a grid usually with standard plunge freezing
- Resolution is generally not good enough for direct methods to work
- Molecular replacement

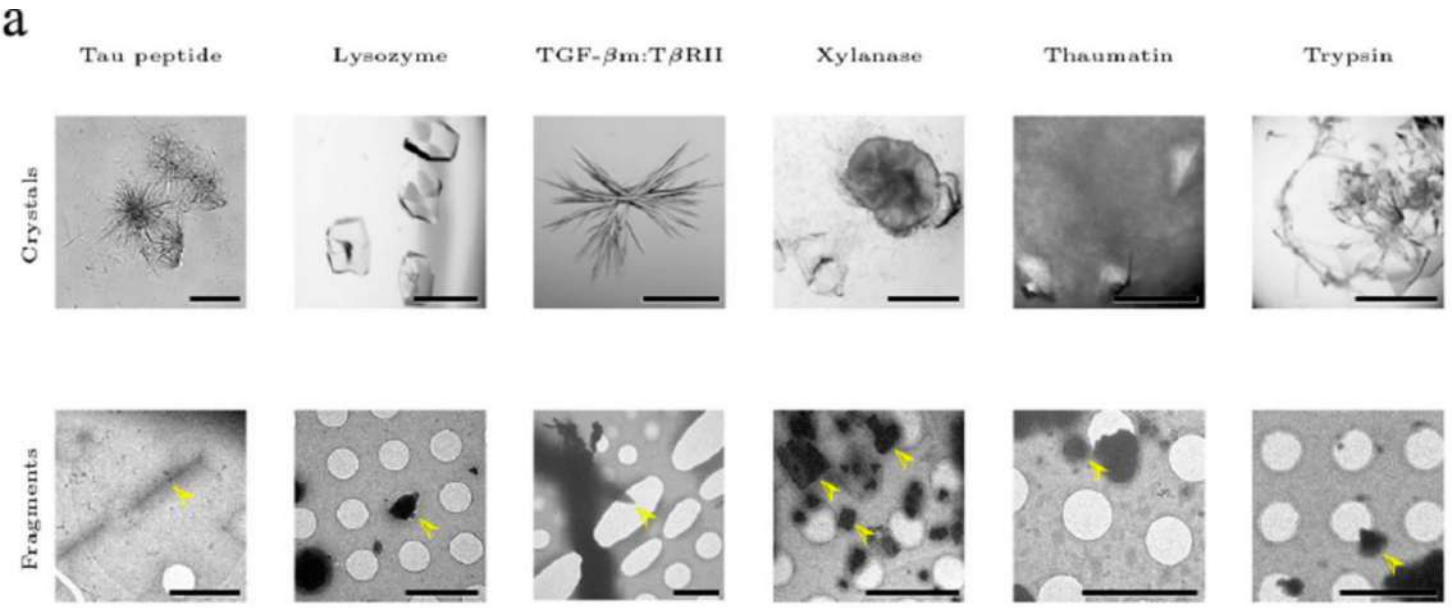
Crystal Thickness

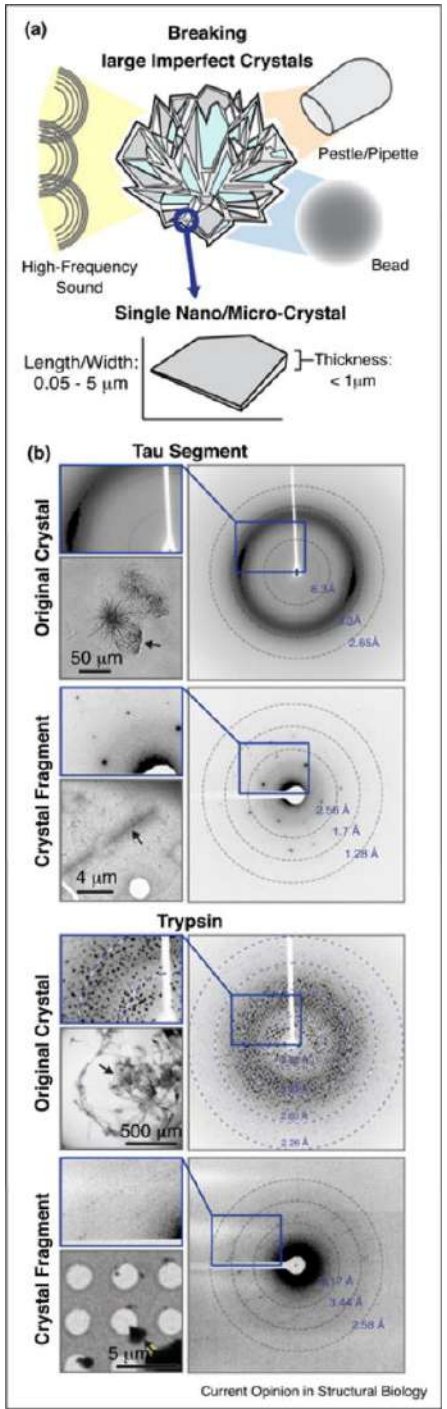
- Lysozyme: crystals thicker then 500 nm unusable
- Martynowycz MW, Clabbers MTB, Unge J, Hattne J, Gonen T. Benchmarking the ideal sample thickness in cryo-EM. Proc Natl Acad Sci U S A. 2021 Dec 7;118(49):e2108884118. doi: 10.1073/pnas.2108884118. PMID: 34873060; PMCID: PMC8670461.
- Maximum usable thickness ~ 2X mean free path of electrons
  - 120 kev: 430 nm
  - 200 kev: 540 nm
  - 300 keV: 640 nm



Larger (imperfect) crystals

→Break them up

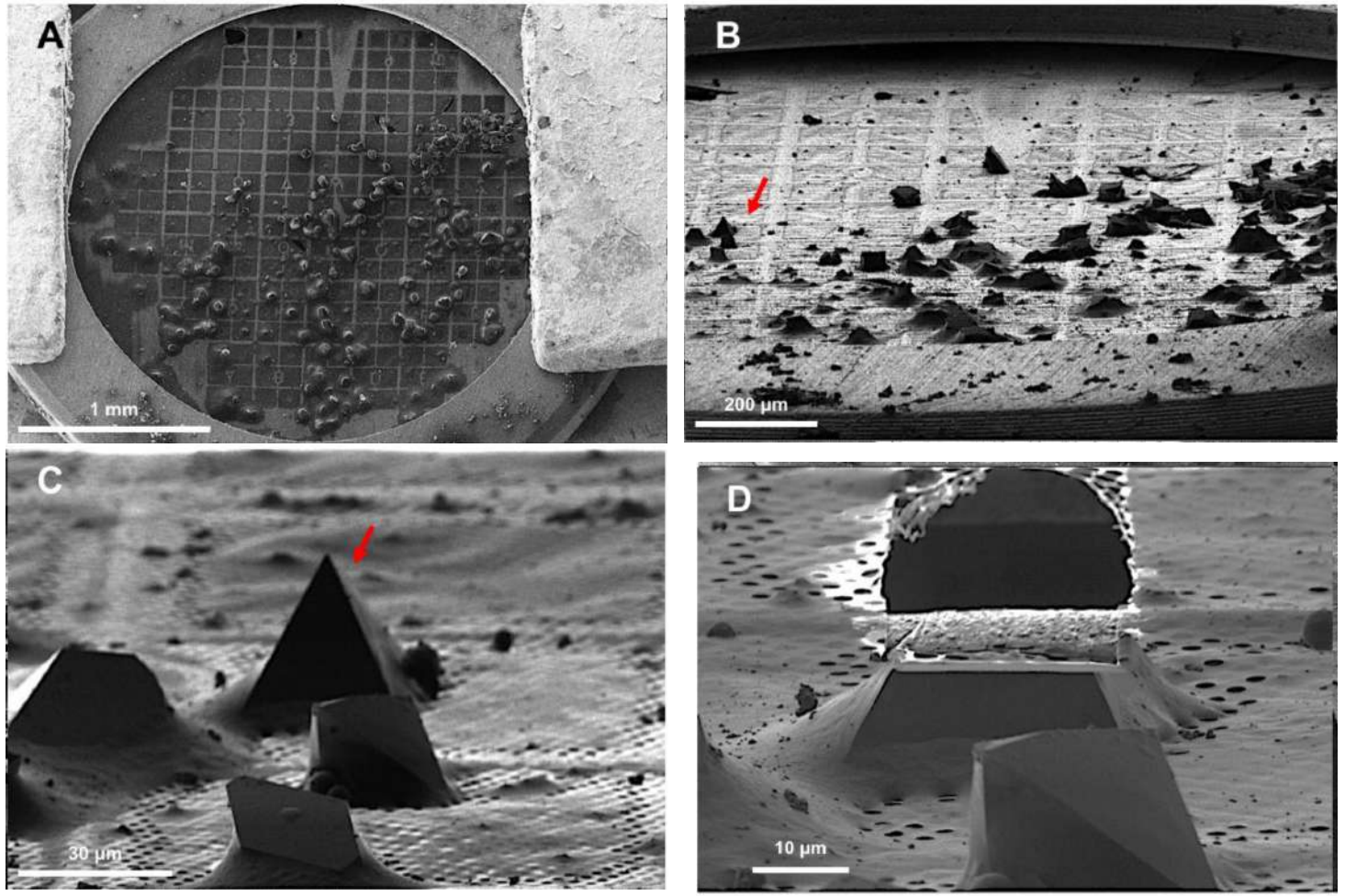




Nannenga and Gonen, 2014
de la Cruz et al, 2017

Larger (imperfect) crystals

→Use a FIB to thin them



Martynowcyz et al, 2018

Small Molecules

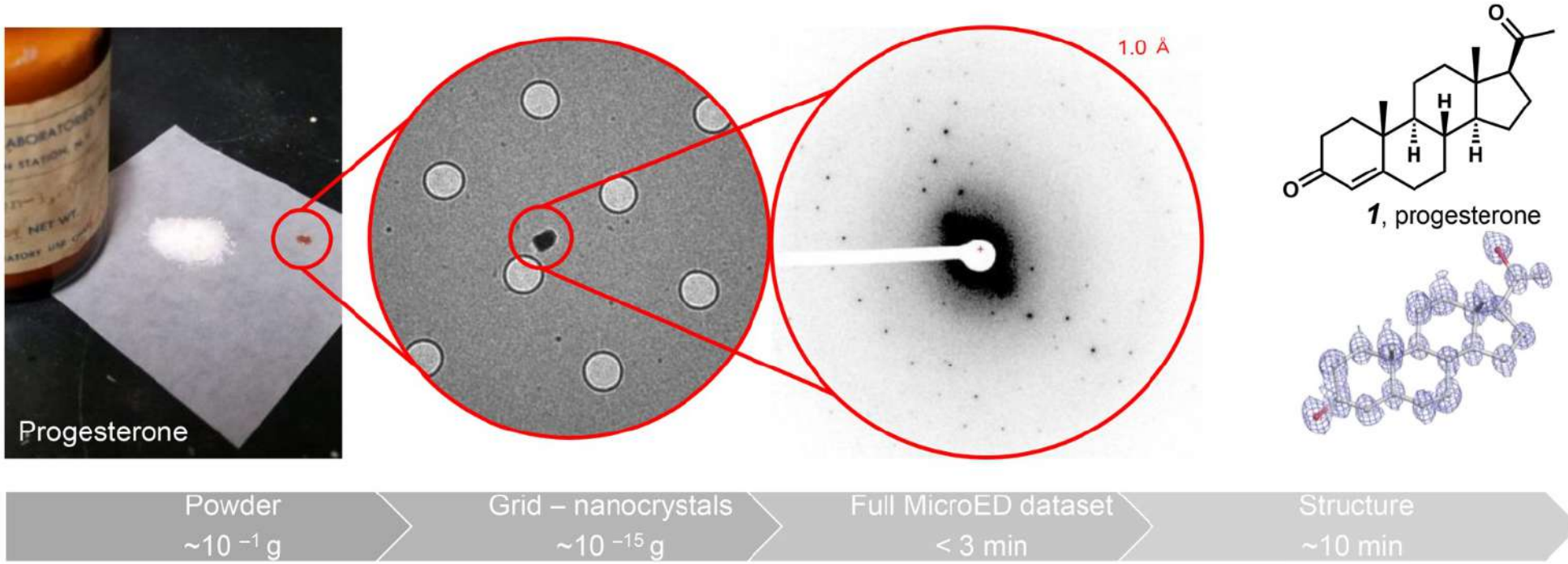


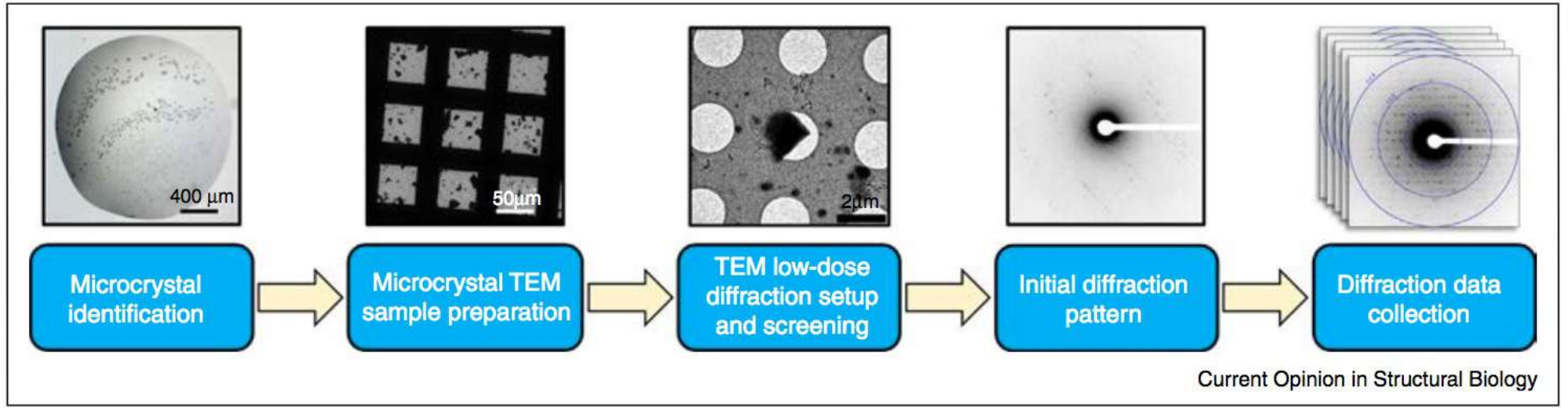
Figure 1. Process of applying MicroED to small molecule structural analysis. Here commercial progesterone (**1**) was analyzed, and an atomic resolution structure was determined at 1 Å resolution. Grid holes are 1 μm in diameter.

Cool in the vacuum of the microscope to avoid contaminating ice crystals

Jones et al, 2018

Data Collection Overview

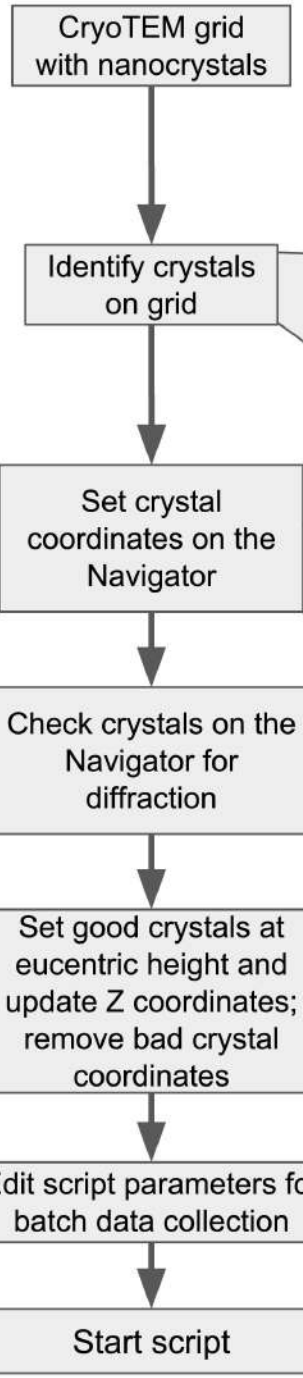
Workflow Overview

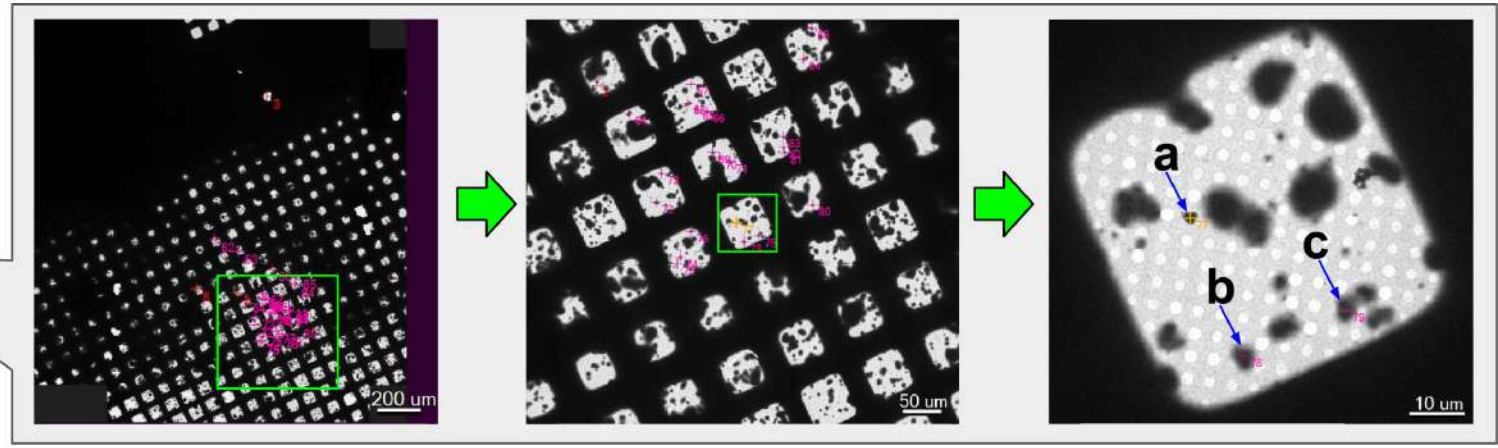


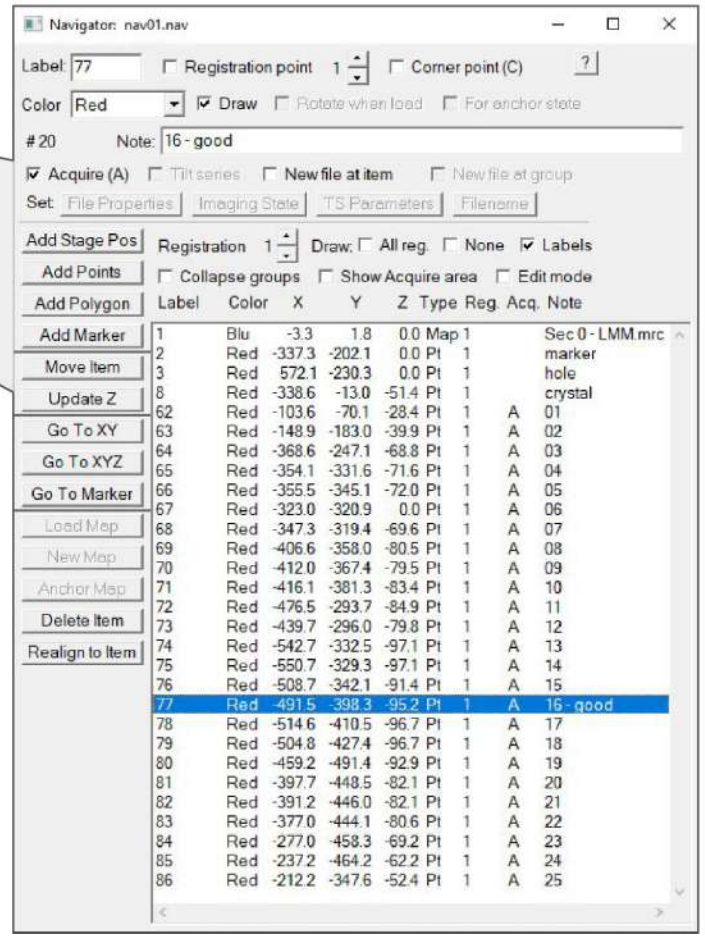
Nannenga and Gonen, 2014

Automated Collection

- SerialEM Script
- Leginon App
- Commercial: EPU-D, Latitude D
- In-house app (?)







de la Cruz et al., 2019

Equipment

Electron Source

LaB_6

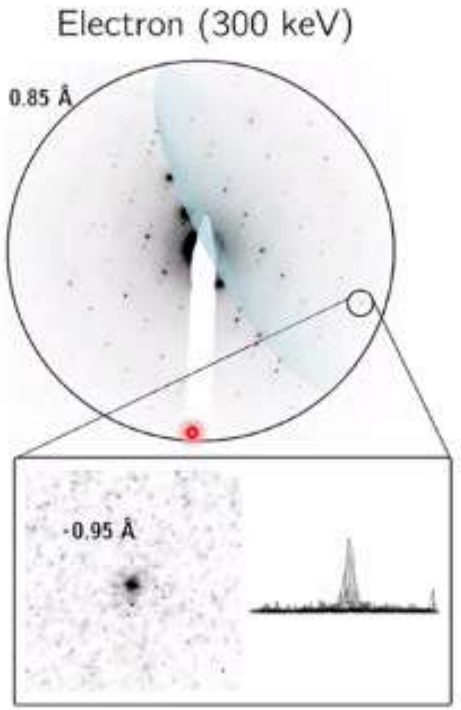
- Modern medium-end TEM with a pointed lanthanium hexabromide (LaB_6) source
- Voltage range 80-200 kV
- Less sensitive to damage from impacting positive ions
- Requires vacuum level $<10^{-6}$ mbar
- Microscope cost $0.5-1.5M
- Filament replacement cost $1500

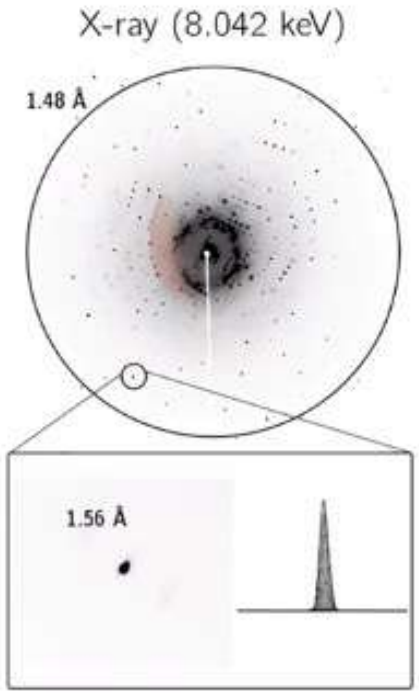
Field Emission Gun

- Highly parallel, coherent, bright beam
- Energies 200-300 keV, though 100 keV models are being introduced
- Highest vacuum requirements: 10^{-8} mbar or lower
- Filament is easily destroyed by vacuum loss: gun shutter
- Microscope cost $2.5M+
- Service contract

Energy Choice

Energy	Wavelength	2θ range (25 Å - 0.8 Å)
8 keV	1.54 Å (X-ray)	1.765° - 74.26°
120 keV	0.03349 Å (electron)	0.08° - 2.4°
200 keV	0.02508 Å (electron)	0.06° - 1.8°
300 keV	0.01969 Å (electron)	0.05° - 1.4°





Detector Choice

CMOS

- Fast readout
- Large area (2kX2K or 4K x 4K)
- Small pixel size (5-15 µm)
- Low background noise
- Many vendors: Thermo Fisher, TVIPS, Gatan
- CMOS detectors specialized for diffraction are available
- Suitable for imaging as well

Hybrid Pixel Detectors

- High dynamic range (20+ bits)
- Zero read-out time
- Zero read-out noise
- Small area: 256x 256, sometimes 512x512 by stitching
- Large pixel size (65 µm)
- Not suitable for imaging
- Many vendors: DECTRIS, Quantum Detectors, Gatan, Rigaku, X-Spectrum

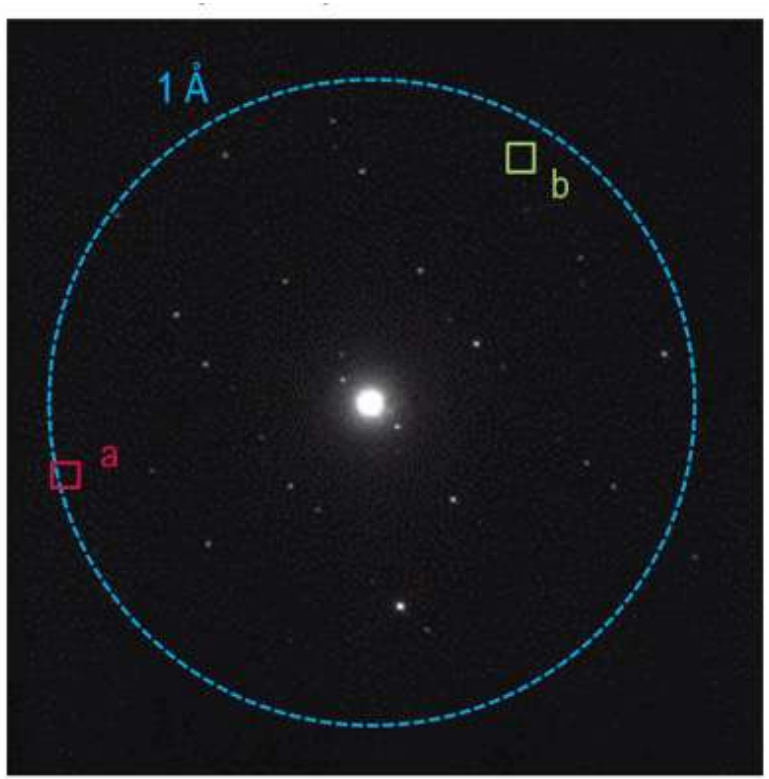
Detector Choice

Direct Electron Detectors

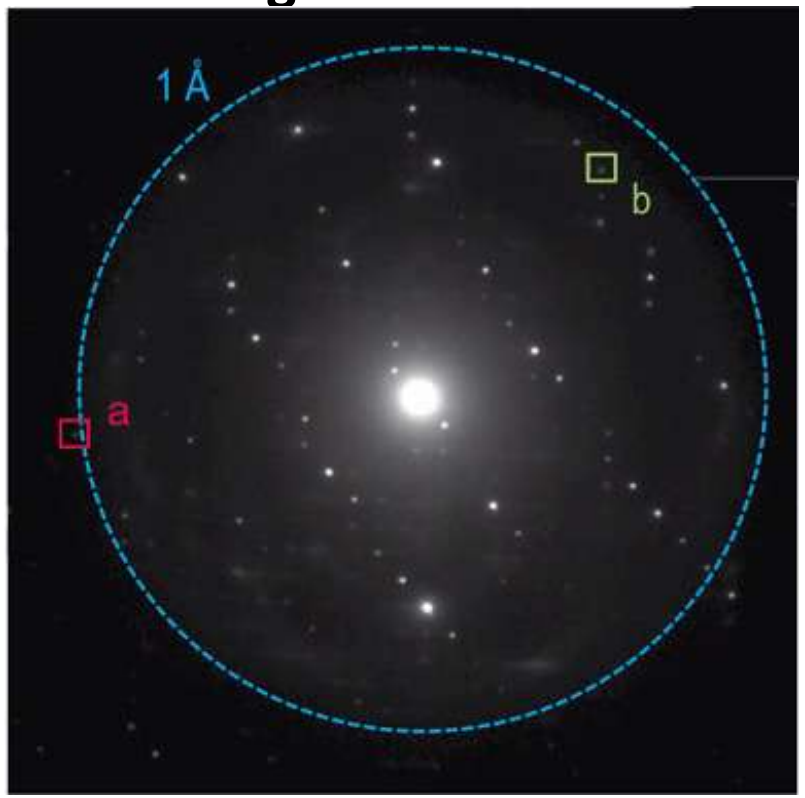
- Fast readout
- Large area (4K x 4K +)
- Low noise in counting mode
- TFS, Gatan, Direct Electron
- Commonly used in high resolution imaging
- Recent papers show much higher quality
- Ab initio phasing may be possible

Zeolite Crystal Example

CMOS Detector



DED in Counting Mode



GATAN
AMETEK

Direct Detectors

Energy	Detector type	Frame rate	DQE(0)	DQE(Ny)	Size
300 keV	Film (McMullan 2009)	n/a	33%	8%	~6k x 8k
300 keV	Falcon 3EC (STFC/RAL/LMB/TFS)	40 fps	95	34/53	4k x 4k
300 keV	Falcon 4/i (TFS)	300	90/95	28	4k x 4k
300 keV	DE 20	25	40	18	5k x 4k
300 keV	Apollo	60	95	45?	4k x 4k
300 keV	K2 (LBL/UCSF/Gatan)	400	80	24	4k x 4k
300 keV	K3 (LBL/UCSF/Gatan)	750	95	45	4k x 5k
300 keV	?????	10000?	98	60	10k x 10k ?

Instrumentation

Standard Cryo-EM instruments

Dedicated Electron Diffractometers

Standard Cryo-EM

Several programs are available

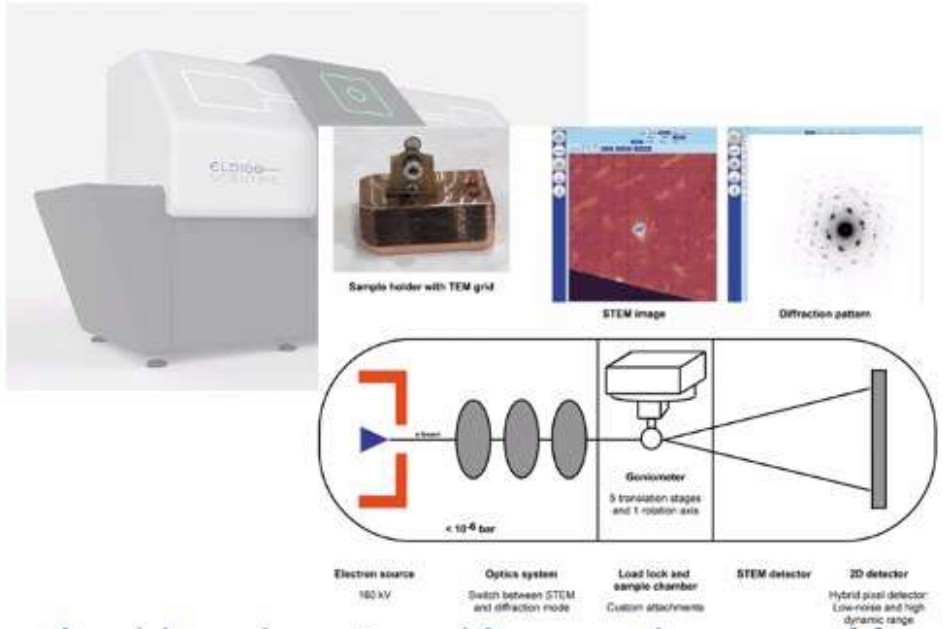
- Dedicated Commercial
  - EPU-D (TFS)
  - Latitude D (Gatan)
- Open source
  - SerialEM
  - Leginon

Autoloader (helpful)
CMOS or DED with shutter-free imaging
Tilt to +/- 60°
FEG

Data collection is fast: ~ 2 minutes for a tilt series
Software is to help with screening and automated collection
View diffraction patterns to identify high quality crystals

Dedicated Electron Diffractometers

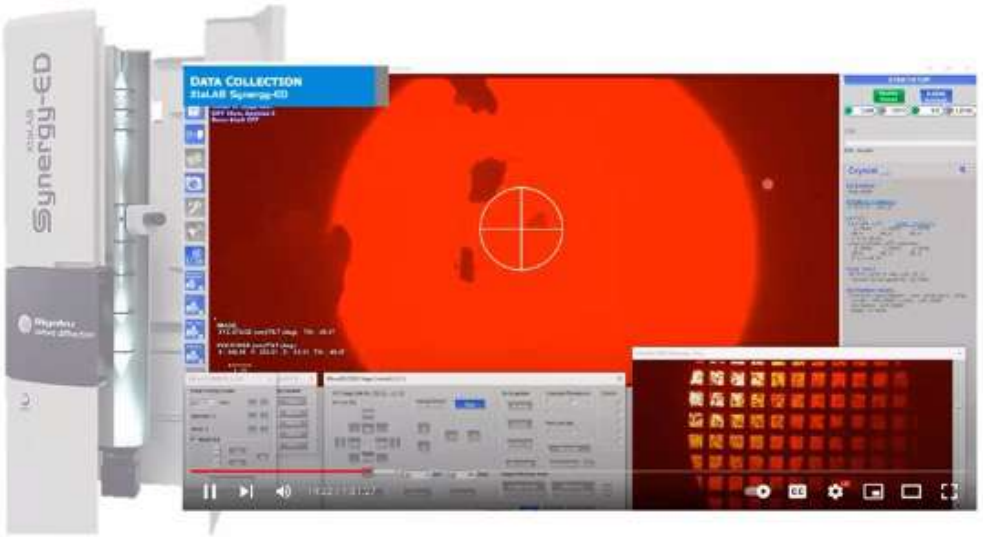
ELDICO ED-1



- A grid goniometer with more degrees of freedom
- Horizontal layout

DOI: 10.1107/S2056989023003109

XtaLAB Synergy-ED



- Mini condenser lens removed
- CrysAlisPro is the control AND data reduction software

DOI: 10.1039/D1CE01172C

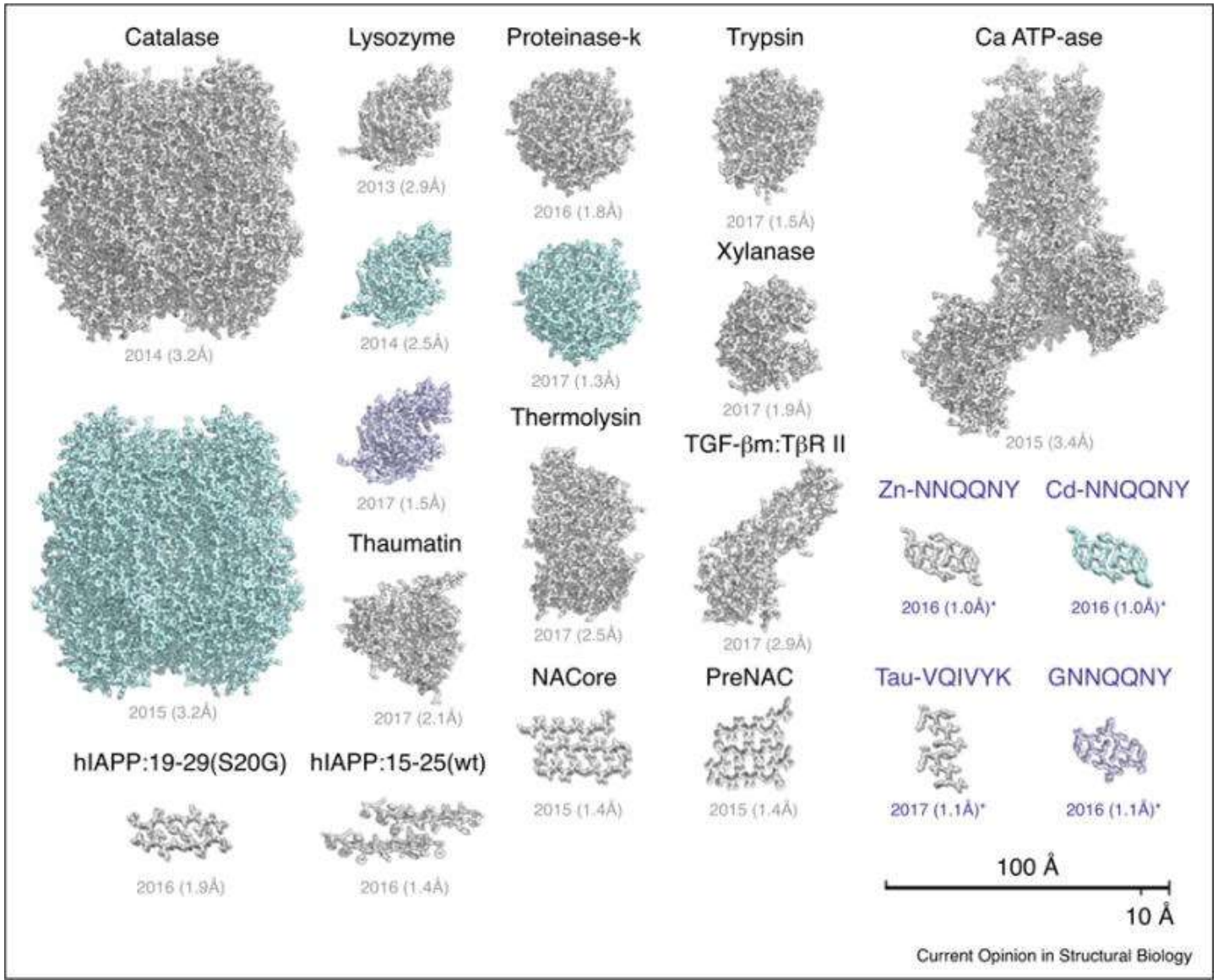
Processing

- Movies need to be converted to a format readable by X-ray crystallography packages such as DIALS (Waterman et al 2013), MOSFLM (Leslie and Powell, 2007), and XDS (Kabsch, 20100
- Super Marty View (SMV) can be read, conversion tools to SMV format exist
  - Interpretation of gain (ratio of variance to mean intensity in background regions)
  - Dead or hot pixels need to be flagged
  - Diffraction spots need to be in linear response region
- Most standard software needs a configuration file for the camera and microscope: camera length, wavelength, tilt axis

Phasing

- For protein structures, phasing was done through molecular replacement
- Standard X-ray crystallography tools
- CNS, Phaser, phenix.refine, REFMAC all have electron scattering factors built in
- Ab initio phasing: works well for small molecules
  - Need diffraction to 1 Å or better
  - Has now been used for proteinase K and lysozyme

Structures Solved by MicroED



Proteins (almost) all already solved by other means

Curr Opin Struct Biol. 2017 Oct; 46: 79–86.

Novel Structures

Solving a new R2lox protein structure by microcrystal electron diffraction

Hongyi Xu[1,*,†], Hugo Lebrette[2,†], Max T. B. Clabbers[1,†], Jingjing Zhao[1], Julia J. Griese[2,3], Xiaodong Zou[1,*] and Martin Högbom[2,*]

[1]*Department of Materials and Environmental Chemistry, Stockholm University, 10691 Stockholm, Sweden.*

[2]*Department of Biochemistry and Biophysics, Stockholm University, 10691 Stockholm, Sweden.*

[3]*Department of Cell and Molecular Biology, Uppsala University, 75124 Uppsala, Sweden.*

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↵† These authors contributed equally to this work.

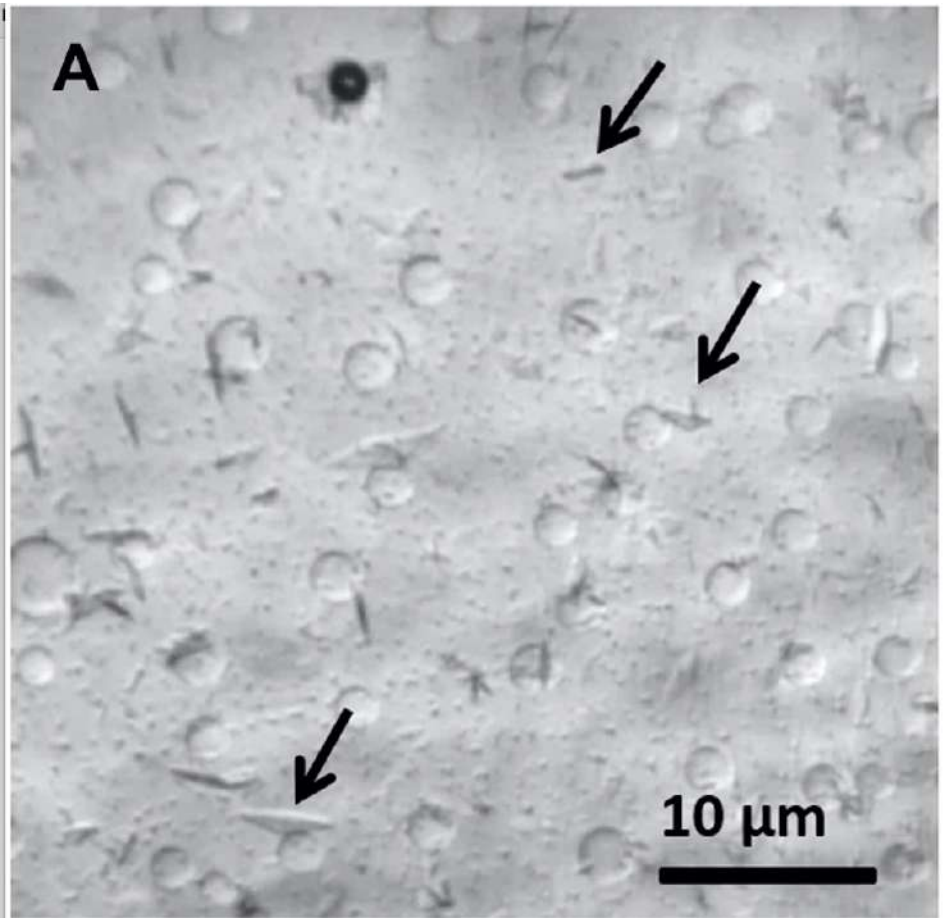
– Hide authors and affiliations

Science Advances 07 Aug 2019:
Vol. 5, no. 8, eaax4621
DOI: 10.1126/sciadv.aax4621

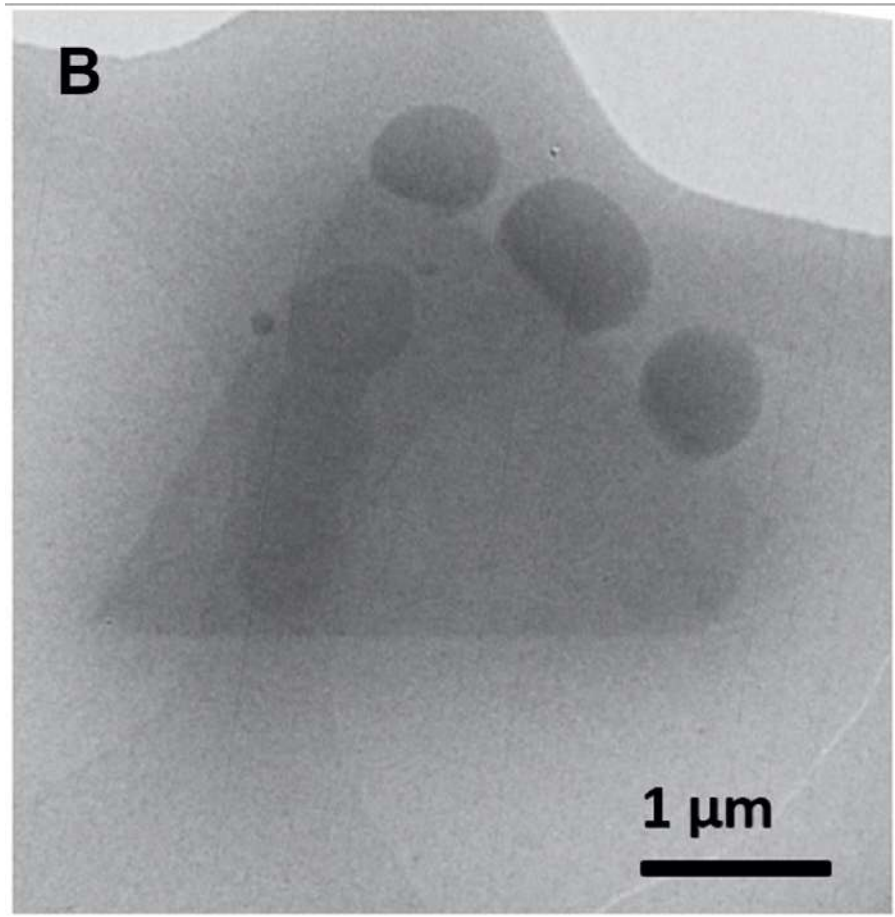
Continuous rotation method
JEM J2100 (200 keV, LaB6 filament)
Timepix hybrid pixel detector
Conventional software: XDS , phasing with phaser (used protein with 35% sequence identity), refinement (phenix.refine)

Solving a new R2lox protein structure by microcrystal electron diffraction

Hongyi Xu[1,*,†], Hugo Lebrette[2,†], Max T. B. Clabbers[1,†], Jingjing Zhao[1], Julia J. Griese[2,3], Xiaodong Zou[1,*] and Martin Högbom[2,*]



44% PEG 400; manual backside blot



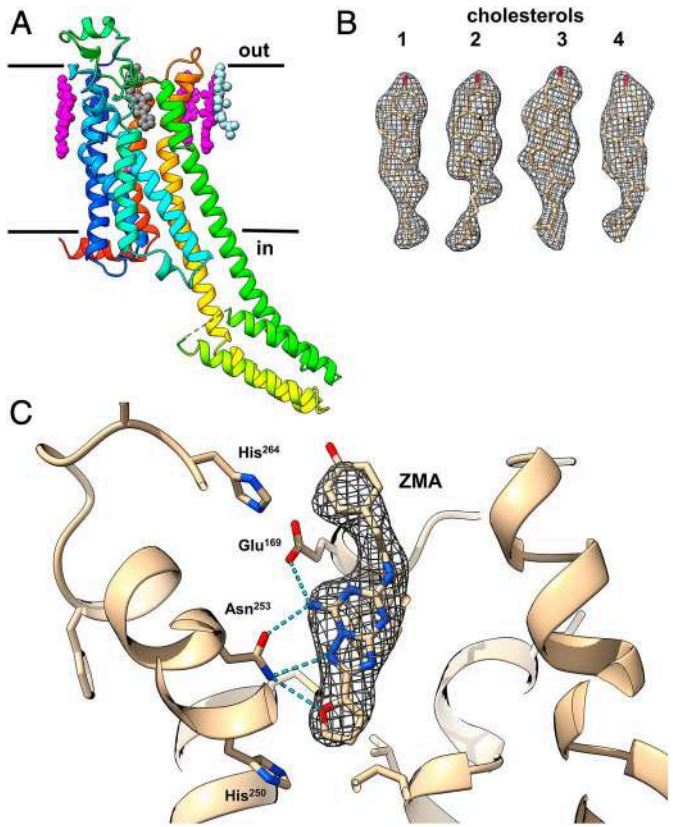
Thickness < 0.5 µm
Plate-like crystals had preferred orientation

MicroED structure of the human adenosine receptor determined from a single nanocrystal in LCP

Michael W. Martynowycz, Anna Shiriaeva, Xuanrui Ge, Johan Hattne, Brent L. Nannen…
+ See all authors and affiliations

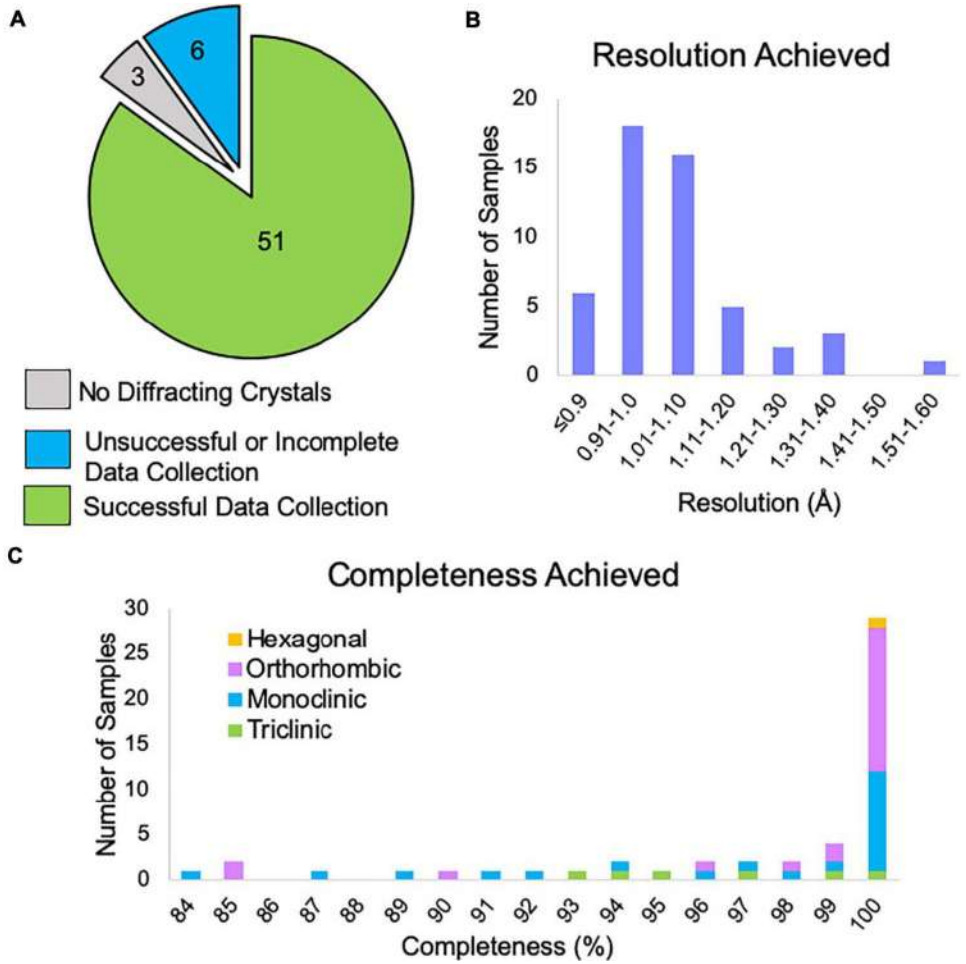
PNAS September 7, 2021 118 (36) e2106041118; https://doi.org/10.1073/pnas.2106041118

Edited by Yifan Cheng, University of California, San Francisco, CA, and approved July 20, 2021 (received for review March 29, 2021)



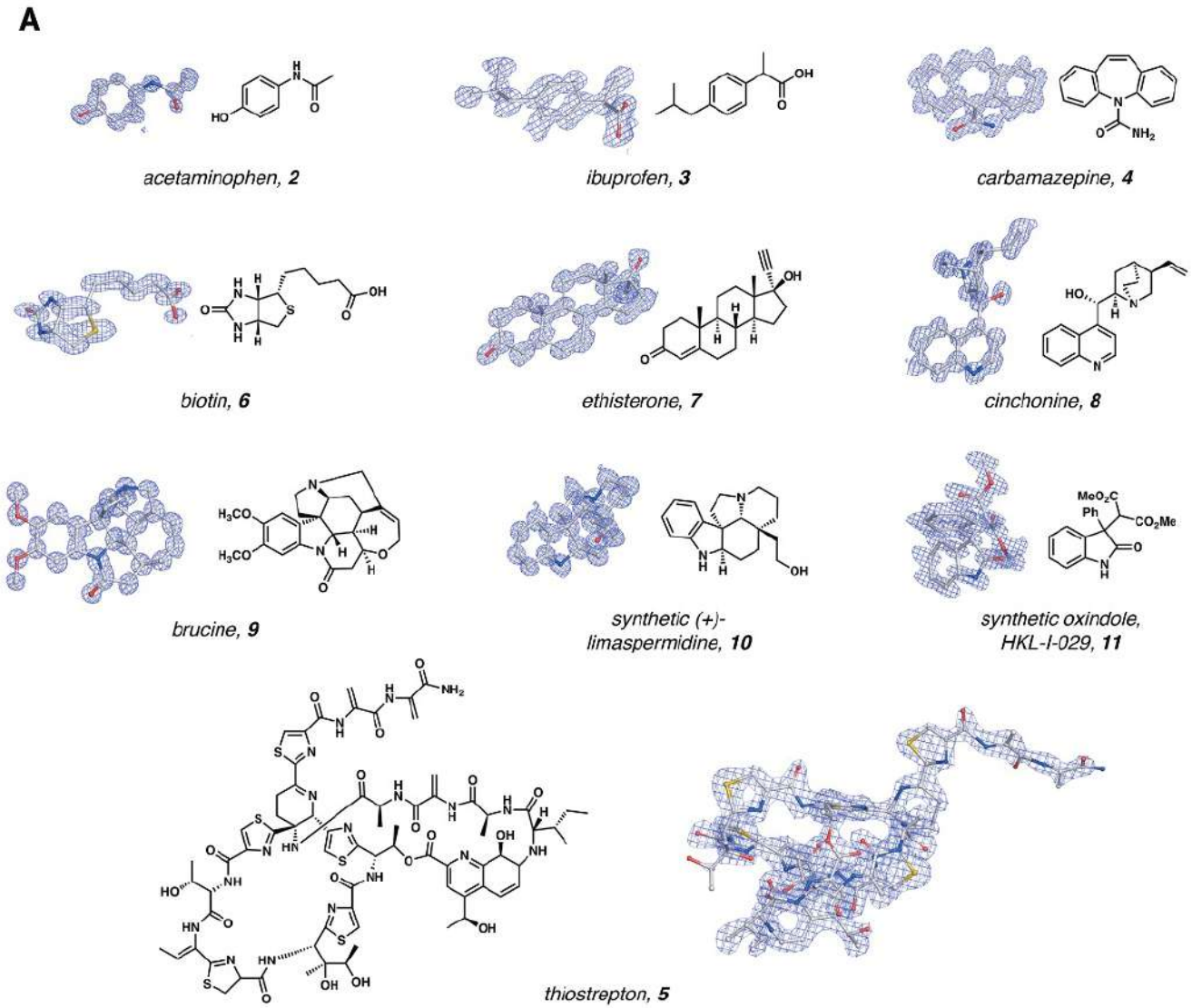
- The gel-phased lipidic cubic phase (LCP) was converted to the liquid-like sponge phase by mixing the LCP with a sponge phase-inducing agent
- Thick crystals were thinned using FIB milling

Collection of over 50 small molecule samples



Bruhn JF, Scapin G, Cheng A, Mercado BQ, Waterman DG, Ganesh T, Dallakyan S, Read BN, Nieusma T, Lucier KW, Mayer ML, Chiang NJ, Poweleit N, McGilvray PT, Wilson TS, Mashore M, Hennessy C, Thomson S, Wang B, Potter CS, Carragher B. Small Molecule Microcrystal Electron Diffraction for the Pharmaceutical Industry-Lessons Learned From Examining Over Fifty Samples. Front Mol Biosci. 2021 Jul 12;8:648603. doi: 10.3389/fmolb.2021.648603. PMID: 34327213; PMCID: PMC8313502.

Small Molecules



Jones et al, 2018

Why wasn’t it done sooner?

- Requires fast detectors (CMOS)
- Thin (few layer) 3D crystals had been studied using film detectors, but was computationally difficult and limited to 50-100 images per day
- The first generation of electronic detectors were based on CCD, which was too slow for continuous rotation (30s-1 min for 4Kx4K image) and had poor DQE (near zero at half-Nyquist resolution)
- Skepticism that dynamic scattering (more than one elastic scattering event) would result in uninterpretable patterns

Micro ED Summary

- Advantages
  - Mid-level microscope (200 keV, FEG better but not essential)
  - Highest resolution yet achieved by cryo-EM technique
  - Sample prep for small molecules is relatively simple
- Disadvantages
  - Crystals must be thin and randomly oriented
  - Sample prep and screening for proteins is difficult
    - FIB milling complicates even more
  - High quality camera and stage are essential
  - Phasing problem: molecular replacement needed for most protein structures
    - However, ab initio phasing has been used on counting DED data (lysozyme, proteinase K)
  - Processing may require more expertise than is generally needed in x-ray crystallography

Recommended Recent Protocols

nature protocols

https://doi.org/10.1038/s41596-024-01088-7

Protocol

Check for updates

Comprehensive microcrystal electron diffraction sample preparation for cryo-EM

William J. Nicolas[1,5], Cody Gillman[2,3,5], Sara J. Weaver[2], Max T. B. Clabbers[1,2], Anna Shiriaeva[2], Ampon Sae Her[2], Michael W. Martynowycz[2] & Tamir Gonen[1,2,3,4]

Abstract

Microcrystal electron diffraction (MicroED) has advanced structural methods across a range of sample types, from small molecules to proteins. This cryogenic electron microscopy (cryo-EM) technique involves the continuous rotation of small 3D crystals in the electron beam, while a high-speed camera captures diffraction data in the form of a movie. The crystal structure is subsequently determined by using established X-ray crystallographic software. MicroED is a technique still under development, and hands-on expertise in sample preparation, data acquisition and processing is not always readily accessible. This comprehensive guide on MicroED sample preparation addresses commonly used methods for various sample categories, including room temperature solid-state small molecules and soluble and membrane protein crystals. Beyond detailing the steps of sample preparation for new users, and because every crystal requires unique growth and sample-preparation conditions, this resource provides instructions and optimization strategies for MicroED sample preparation. The protocol is suitable for users with expertise in biochemistry, crystallography, general cryo-EM and crystallography data processing. MicroED experiments, from sample vitrification to final structure, can take anywhere from one workday to multiple weeks, especially when cryogenic focused ion beam milling is involved.

[1]Howard Hughes Medical Institute, University of California Los Angeles, Los Angeles, CA, USA. [2]Department of Biological Chemistry, University of California Los Angeles, Los Angeles, CA, USA. [3]Molecular Biology Institute, University of California, Los Angeles, CA, USA. [4]Department of Physiology, University of California Los Angeles,

Key points

- The preparation of microcrystal electron diffraction samples and the optimization of growth conditions enable the collection of diffraction data from a crystal on a transmission electron microscopy grid and rotated under a parallel electron beam.
- The crystals used for microcrystal electron diffraction have dimensions in the range of 50 nm × 200 nm × 200 nm, resulting in volumes 9 orders of magnitude smaller than a 100 µm × 100 µm × 100 µm crystal typically used in X-ray crystallography.

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Nicolas, W. J. et al. Preprint at *bioRxiv* (2024): https://doi.org/10.1101/2024.05.13.594022

Recommended Recent Protocols

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- Bruhn JF, Scapin G, Cheng A, Mercado BQ, Waterman DG, Ganesh T, Dallakyan S, Read BN, Nieusma T, Lucier KW, Mayer ML, Chiang NJ, Poweleit N, McGilvray PT, Wilson TS, Mashore M, Hennessy C, Thomson S, Wang B, Potter CS, Carragher B. Small Molecule Microcrystal Electron Diffraction for the Pharmaceutical Industry-Lessons Learned From Examining Over Fifty Samples. Front Mol Biosci. 2021 Jul 12;8:648603. doi: 10.3389/fmolb.2021.648603. PMID: 34327213; PMCID: PMC8313502.
- Martynowycz MW, Gonen T. Protocol for the use of focused ion-beam milling to prepare crystalline lamellae for microcrystal electron diffraction (MicroED). STAR Protoc. 2021 Jul 28;2(3):100686. doi: 10.1016/j.xpro.2021.100686. PMID: 34382014; PMCID: PMC8339237.
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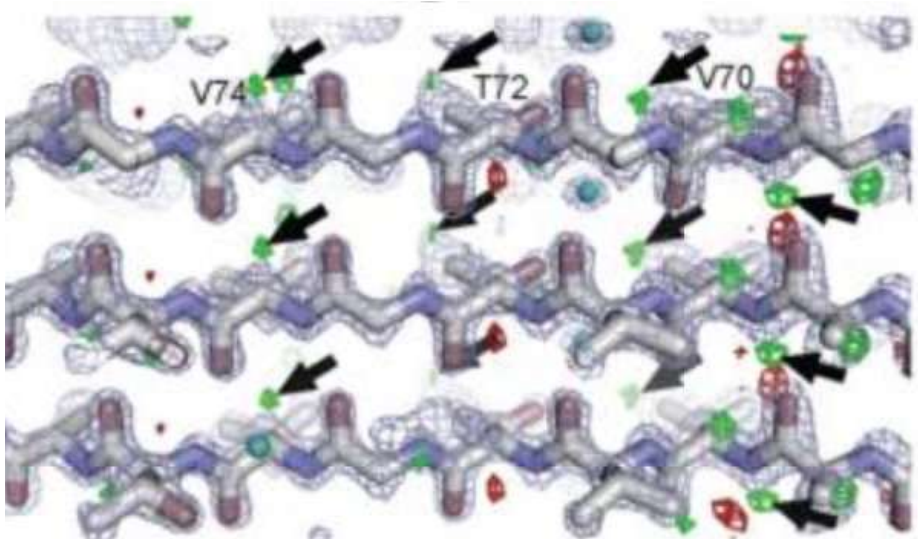
Questions



Acknowledgements

Some figures came from

- Brandon Q. Mercado, Yale Chemical and Biophysical Information Center
- Sarah EJ Bowman, Hauptman-Woodward Medical Research Institute
- Sahil Gulati, Gatan Inc. Ametek

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