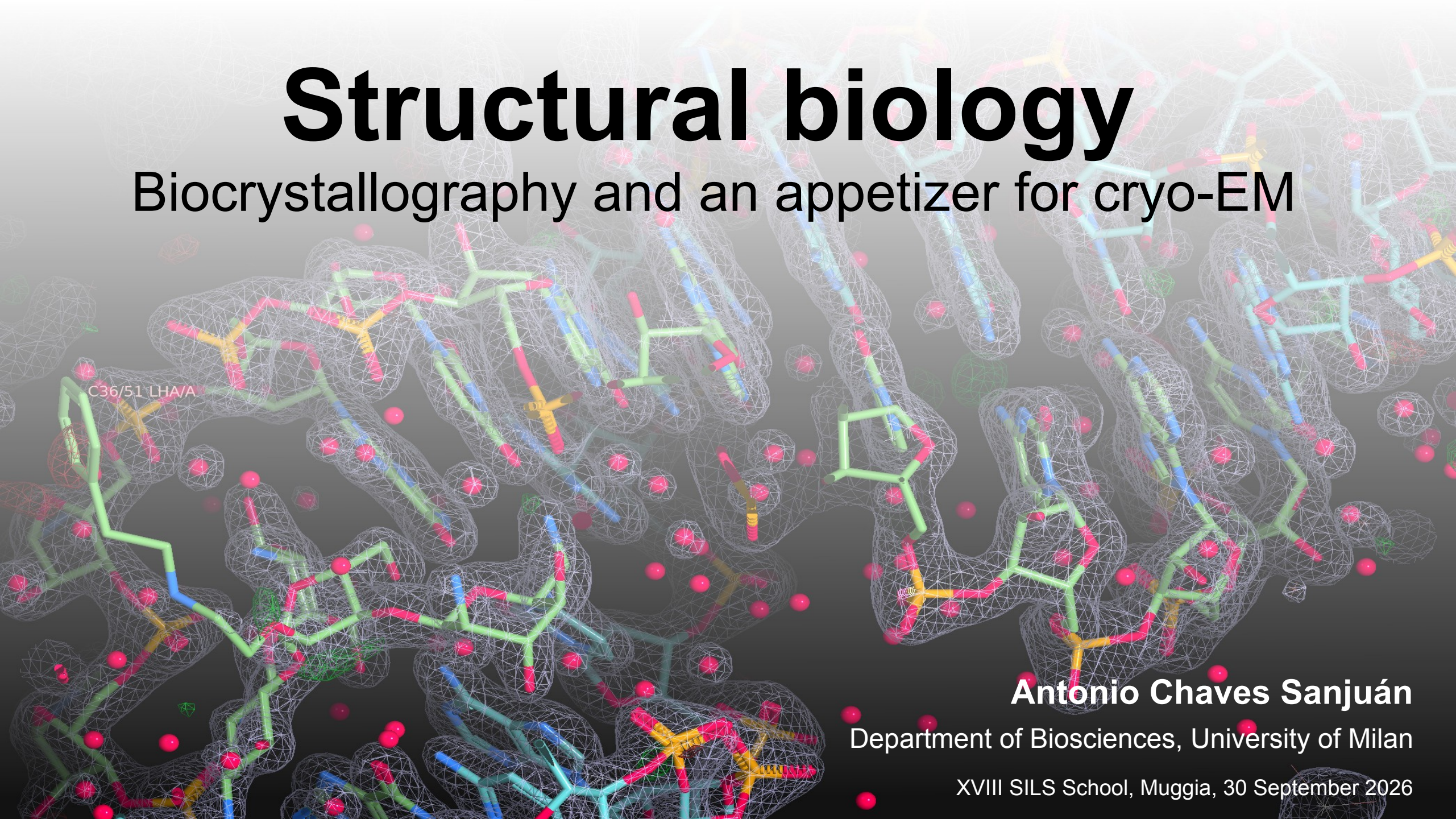


Structural biology

Biocrystallography and an appetizer for cryo-EM



Antonio Chaves Sanjuán

Department of Biosciences, University of Milan

XVIII SILS School, Muggia, 30 September 2026

The synchrotron as a scientific zoo



XRD1 / XRD2: Structural biology (MX)

XAFS / XRF: chemistry and elements

APE / BACH: electrons and magnetism

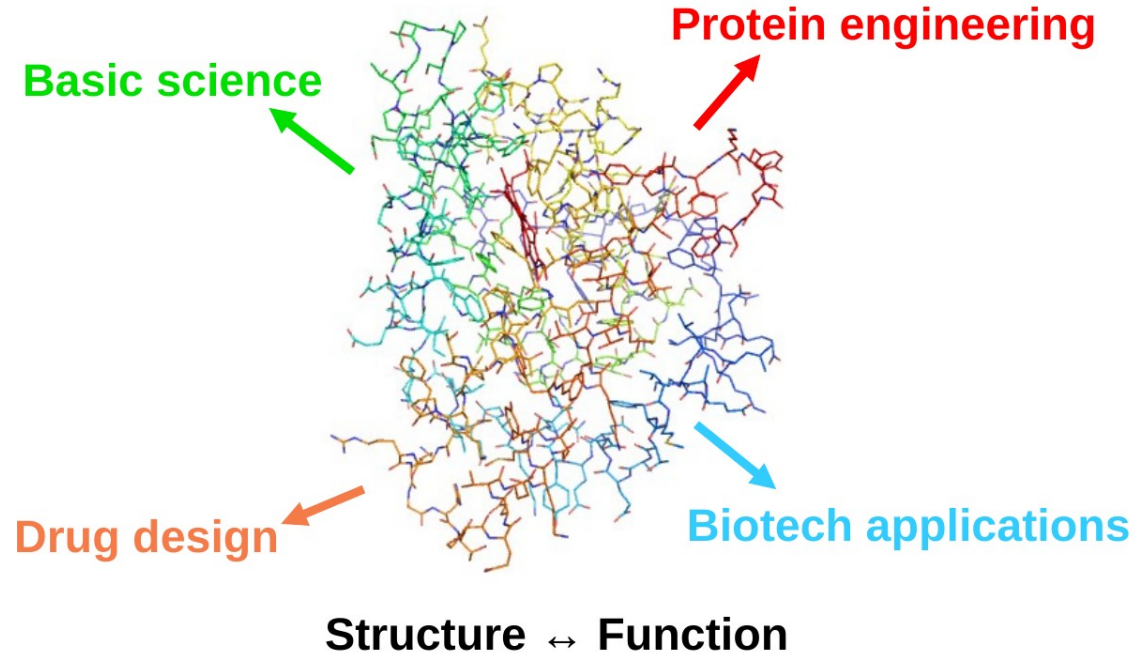
SYRMEP / TwinMic: imaging

MCX / Xpress: materials and pressure

SAXS / SISSI: soft matter and biology

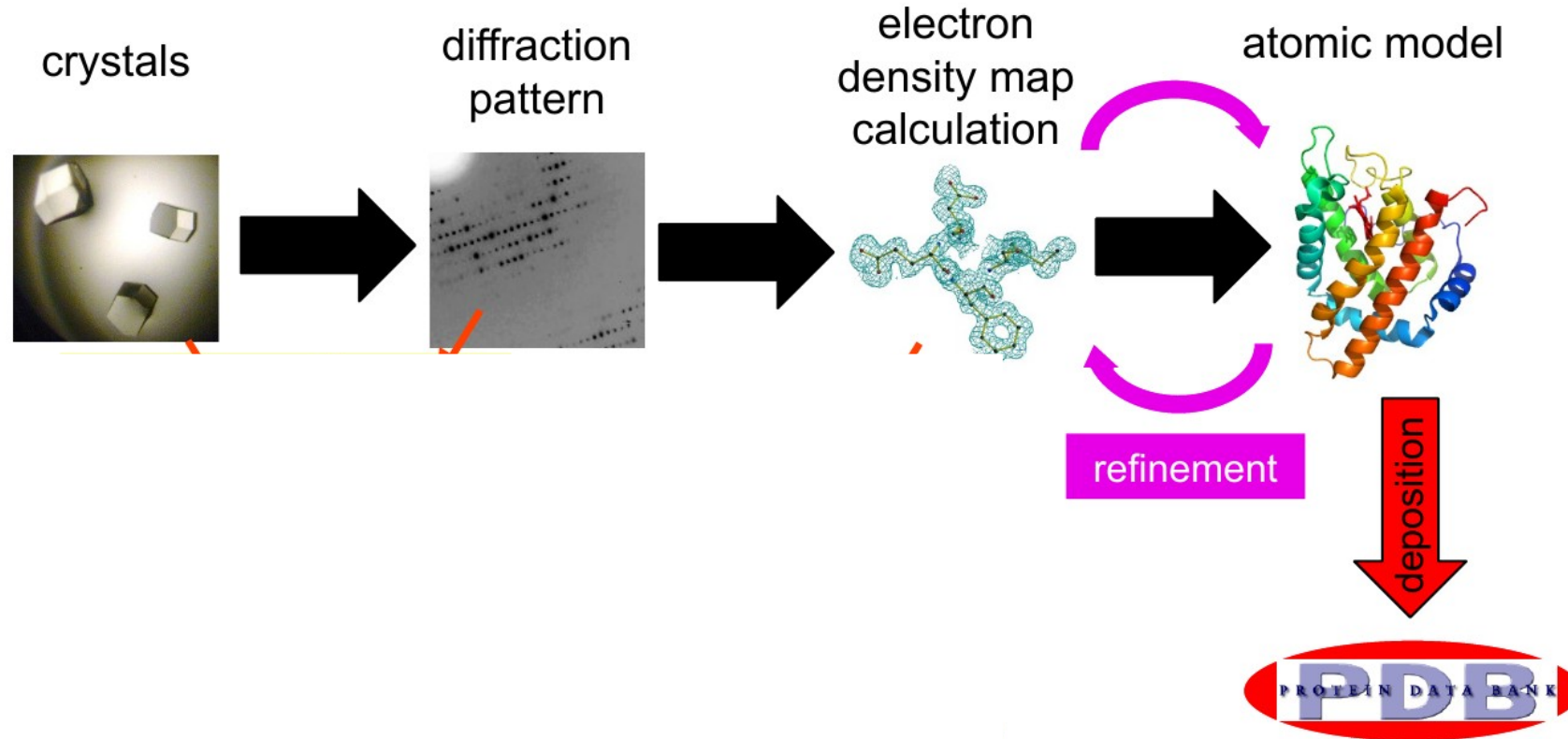
What does this molecule or macromolecular complex do?

Protein 3D structure



Let's look at the structure

Single crystal diffraction - crystallography workflow



Prof. Macchi's lecture "X-ray diffraction" → intensities, reciprocal space, structure factors, phases
Today → what changes when the crystal contains macromolecules?
Why does MX need synchrotron radiation?

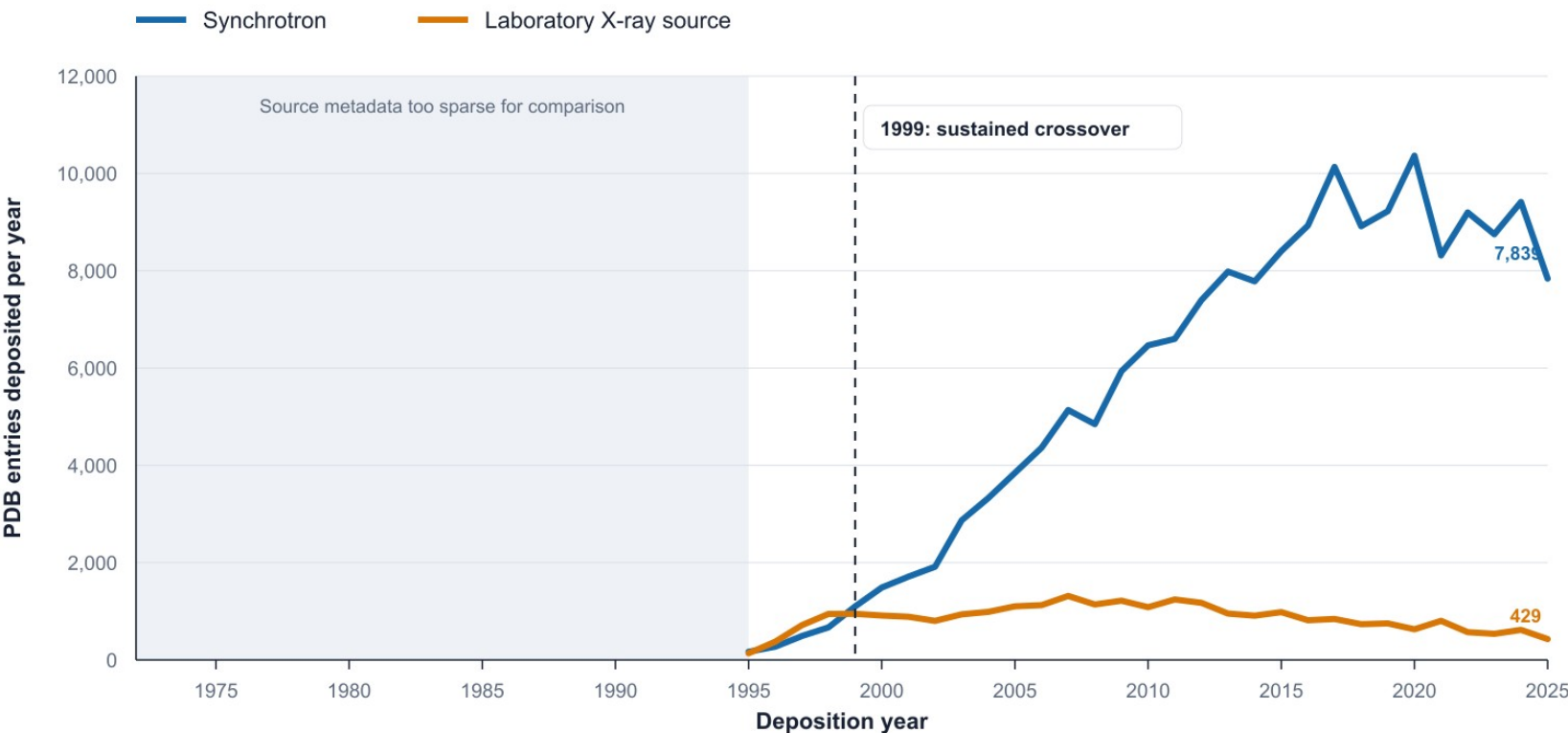
How synchrotrons became part of the workflow

1958	Kendrew: myoglobin, the first protein structure solved
1960s	Early protein structures used laboratory sources
1970s	Pioneering synchrotron diffraction
1981–2008	SRS Daresbury: MX user operation in dedicated beamlines
Later decades	Specialist MX beamlines and shared user facilities

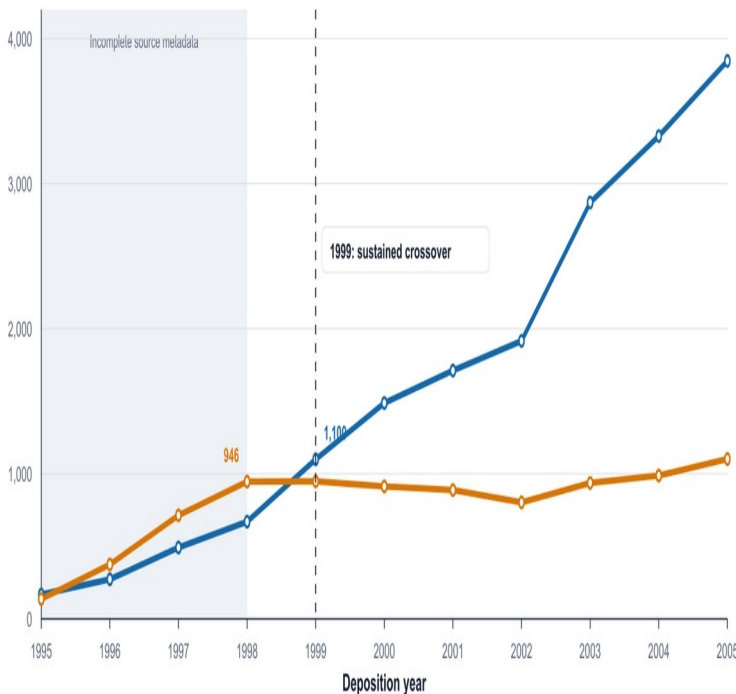
Synchrotron and laboratory sources in the PDB

Synchrotron radiation became the dominant X-ray source for the PDB

Annual X-ray structures deposited in the PDB, by reported radiation source · 1972–2025



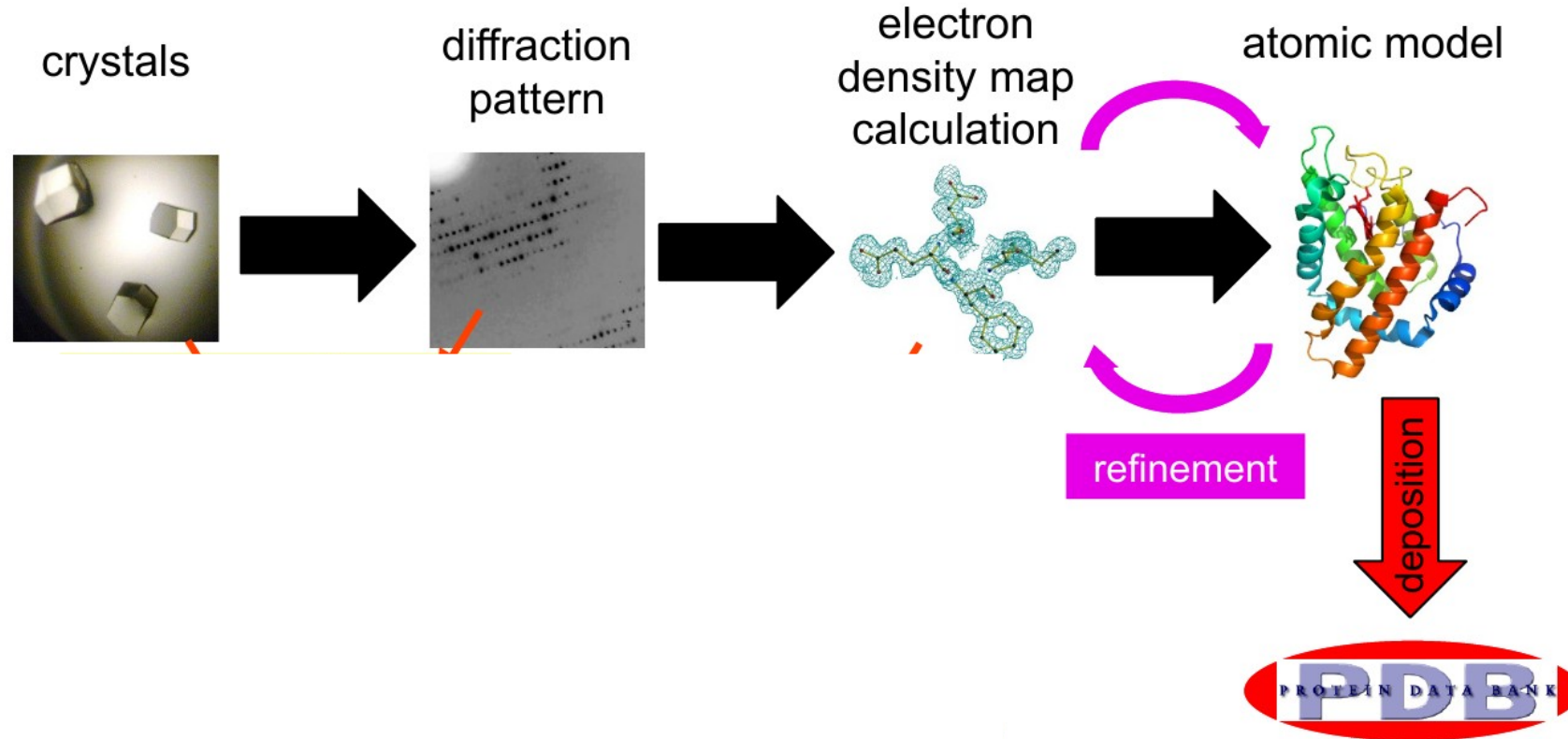
Source: RCSB PDB Search API, queried 31 Aug 2026. Known reported sources only; XFEL, other and unreported sources excluded. 2026 is incomplete and omitted.



Source: RCSB PDB Search API, queried 31 Aug 2026. Known reported sources only; XFEL, other and unreported sources excluded. 2026 is incomplete and omitted.

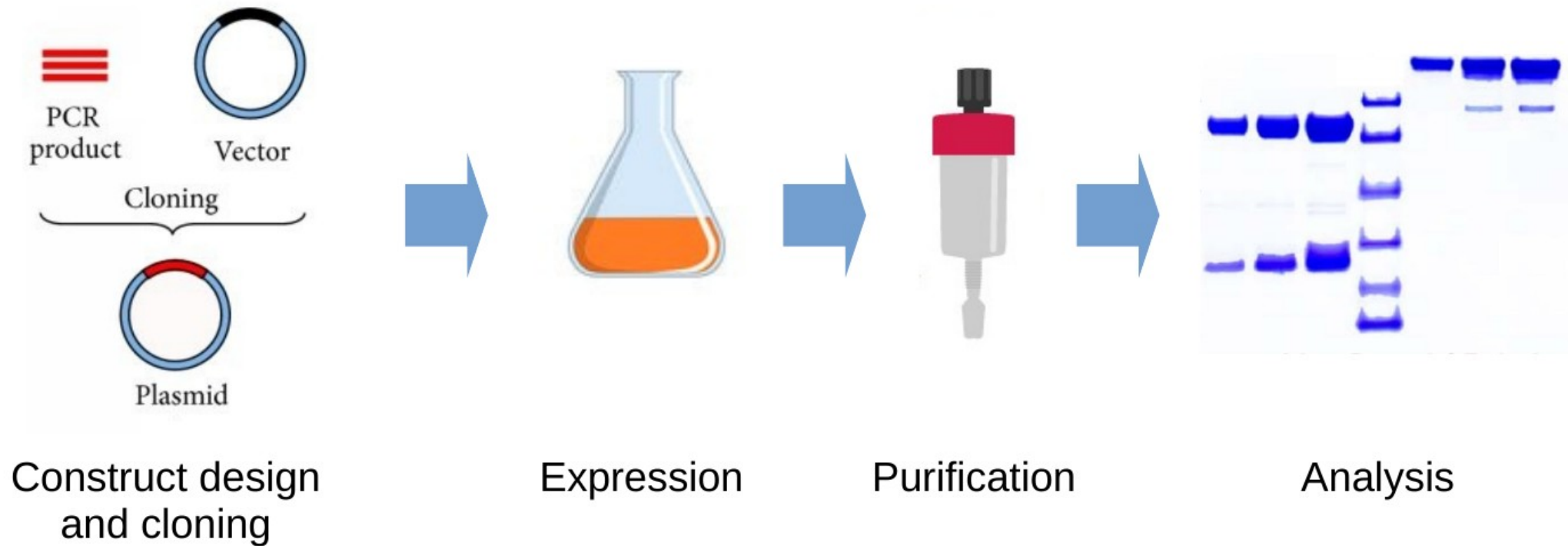
Deposition year. Archived RCSB query, 31 Aug 2026.

The macromolecular crystallography workflow



The workflow begins with a suitable biological sample.

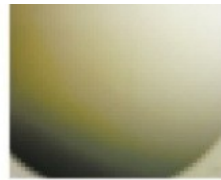
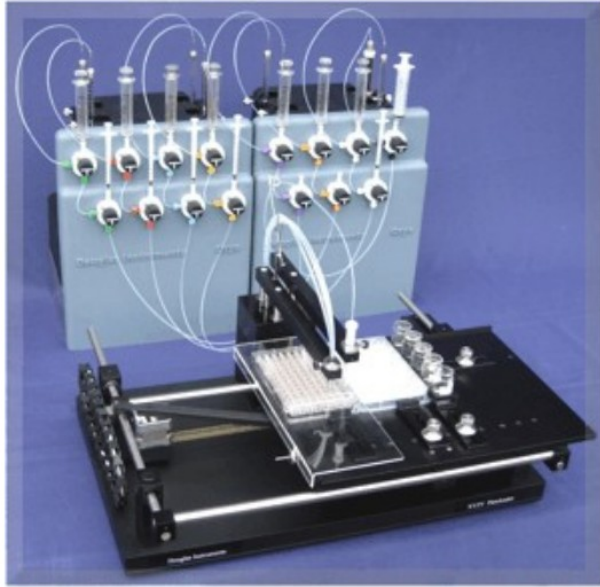
A good experiment begins with the protein



Homogeneous sample means >99.9% purity

Crystallisation is a BRUTE FORCE search

Crystallisation is often a bottleneck



Clear Drop



Precipitate/Phase



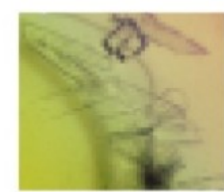
Needle Cluster



Skin/Precipitate



Quasi Crystals



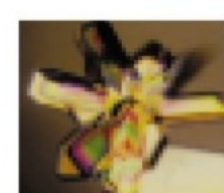
Plates



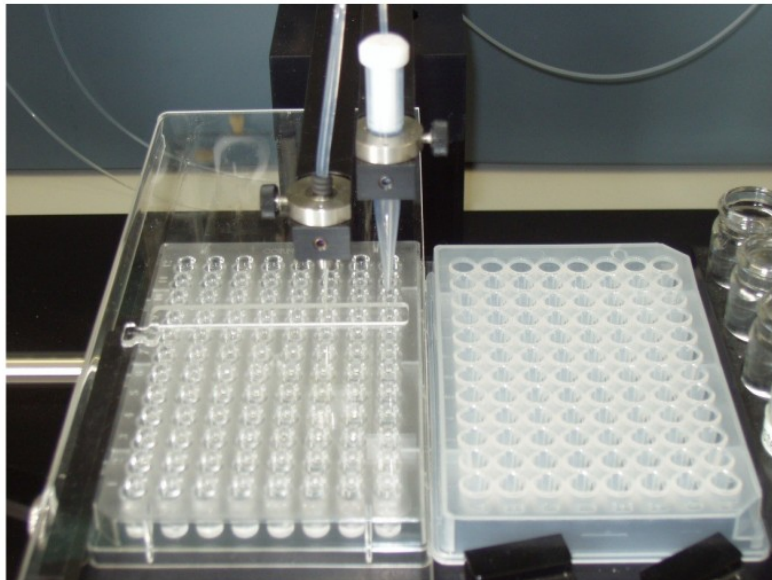
Precipitate



Microcrystals

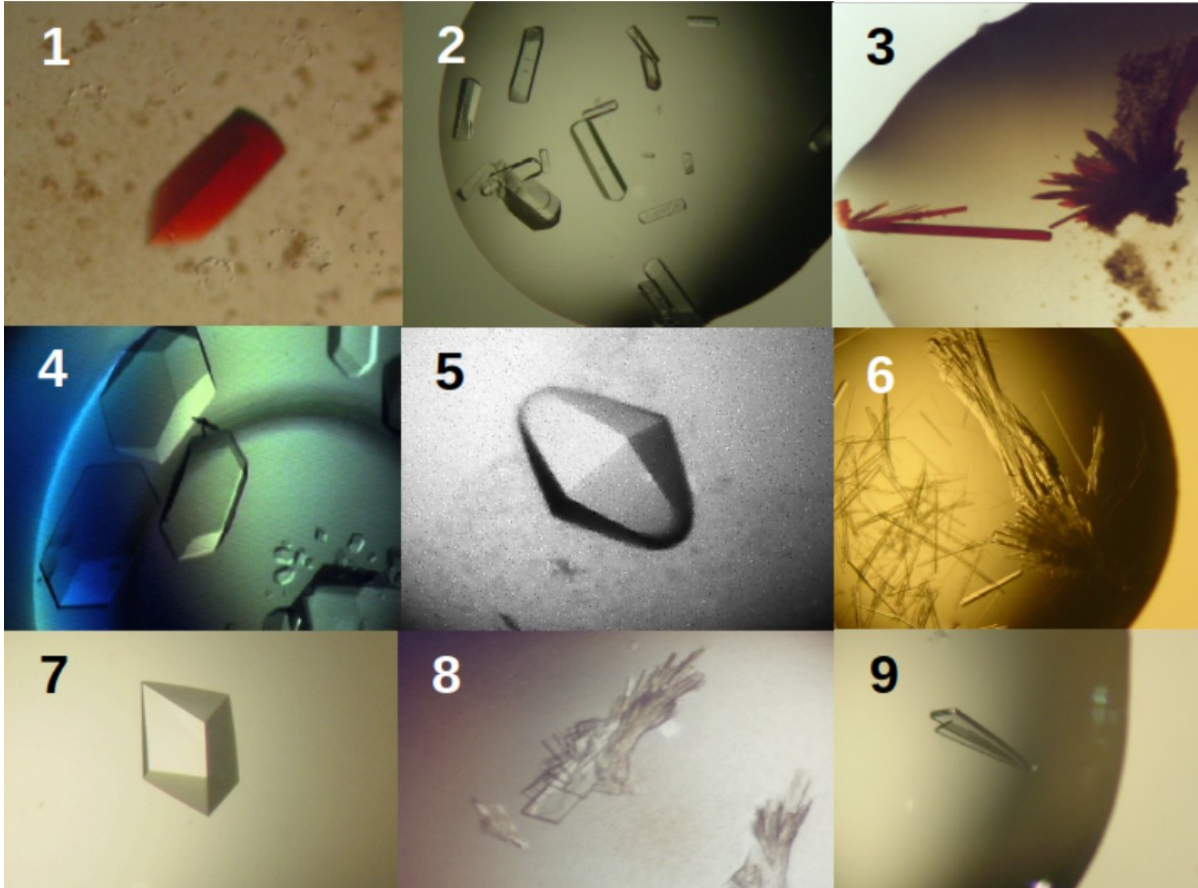


Rod Cluster



A search for conditions that support ordered growth

Protein crystals

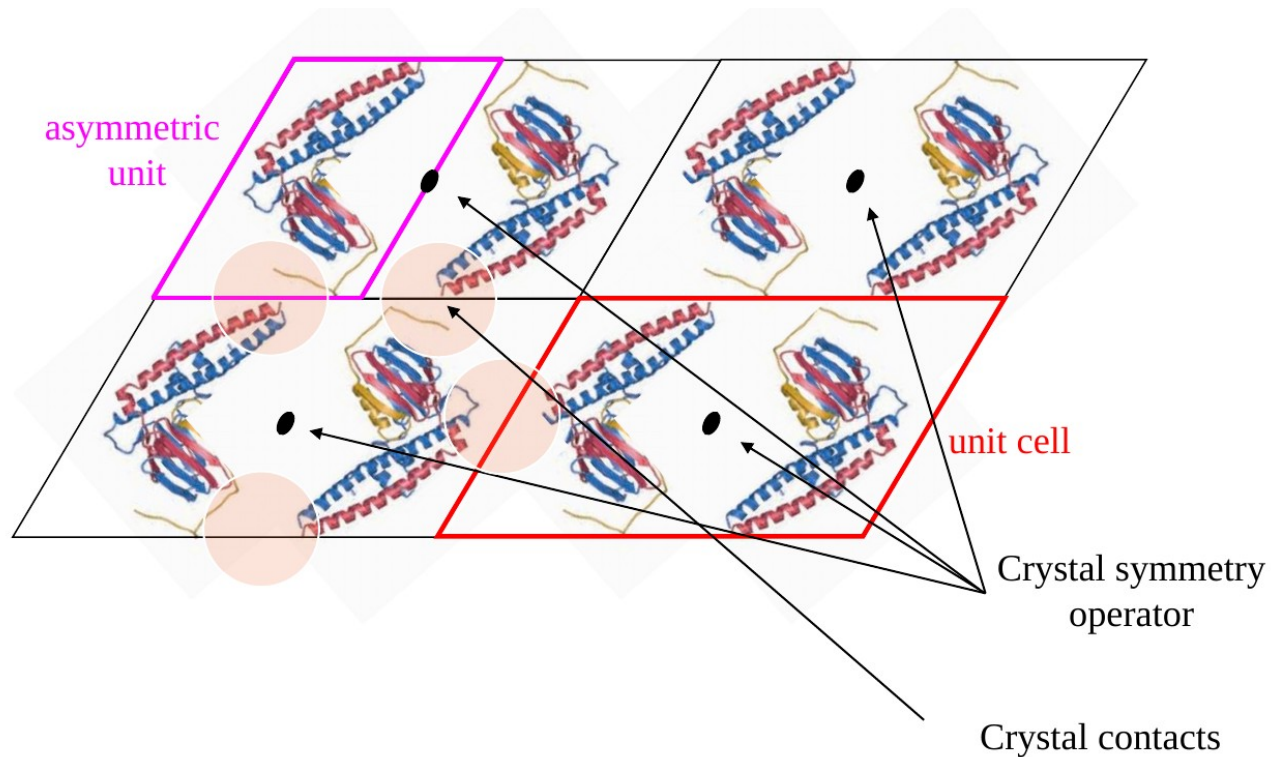


Macromolecular crystals are small

High intensity X-rays amplifies the crystal signal (at high resolution)

Beam size matched to useful crystal volume

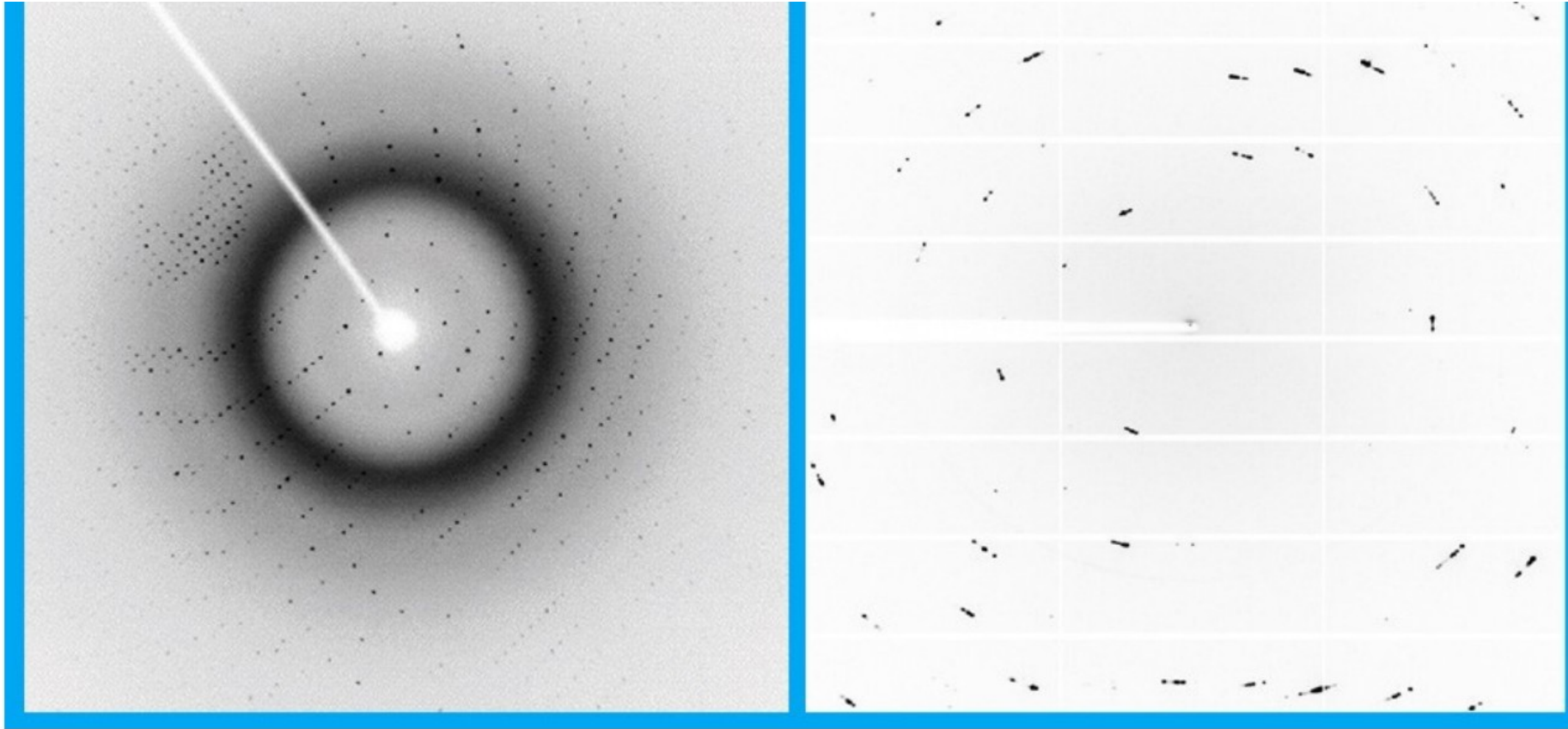
Crystal contacts and biological contacts



1) Small surface contacts hold the lattice together → xtal fragility

2) Macromolecules are big molecules: 2-20nm → Huge unit cells

Protein and salt diffraction

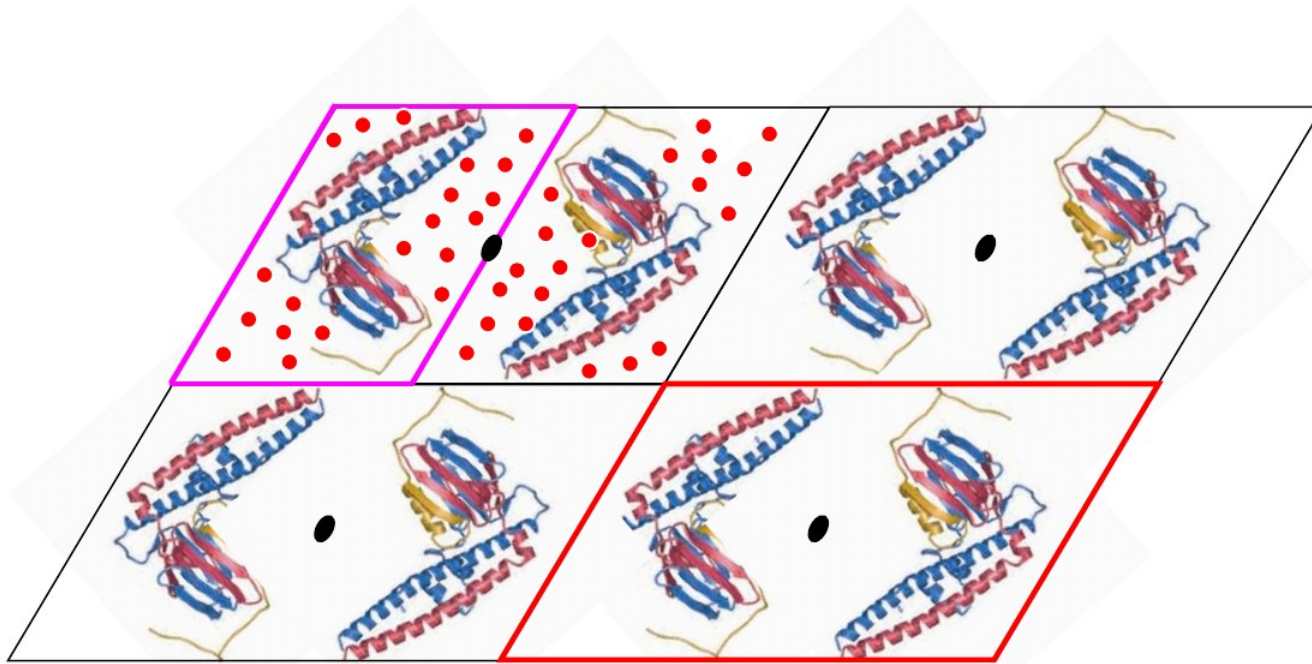


Protein: large unit cells, closely spaced reflections
Salt: smaller unit cells, more widely spaced reflections

Illustrative orthogonal cells: $a^* = 1/a$

Low beam divergence helps separate reflections → **Synchrotron radiation helps (collimation)**

A crystal with a lot of water



Hydrated molecules and solvent channels

- 3) High solvent content. Often roughly 30–80% solvent
 - High disorder
 - Radiation damage

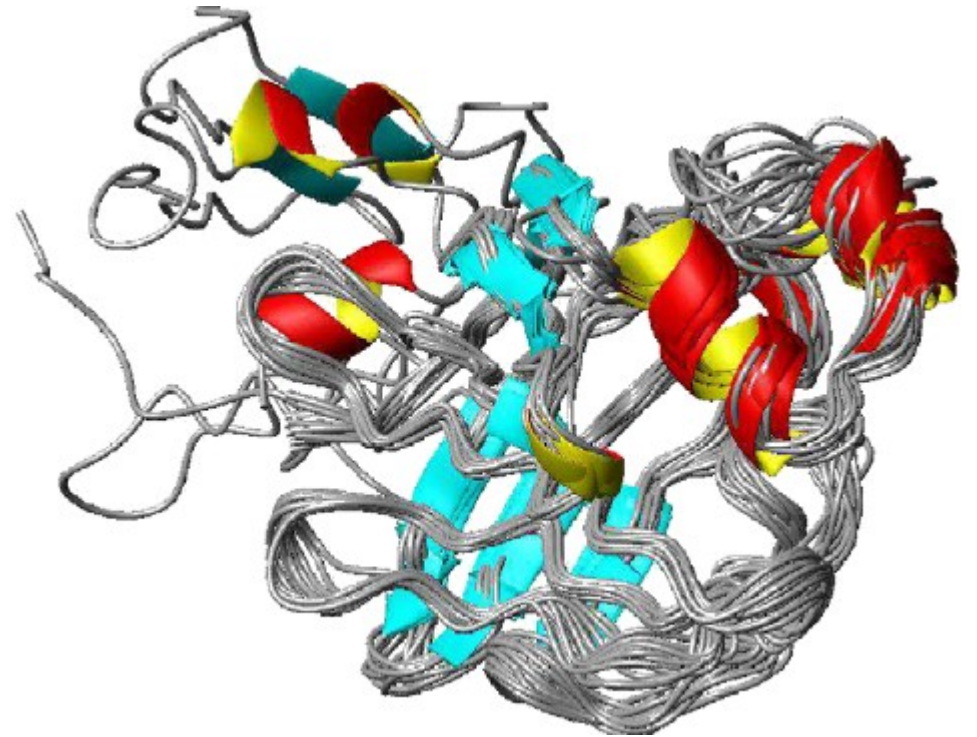
Disorder limits the information

Disorder weakens high-resolution information

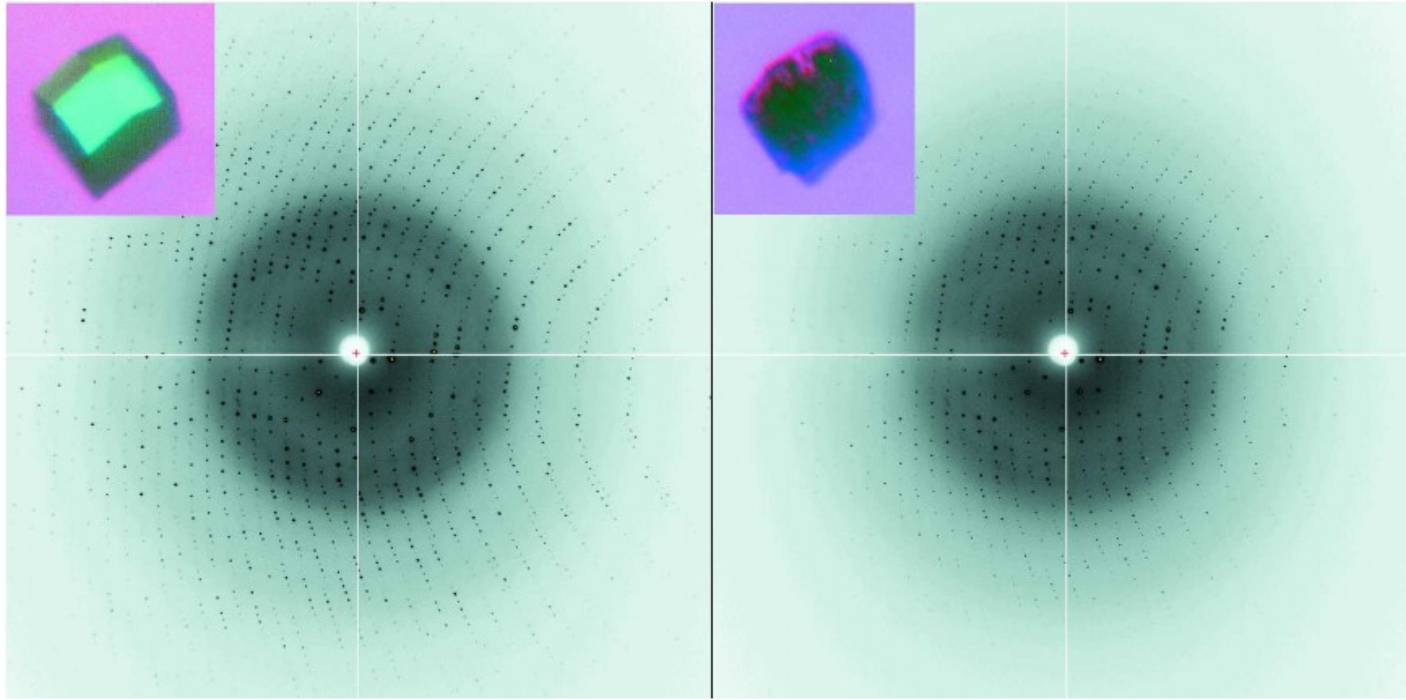
- Solvent is disordered
- Macromolecules contain flexible parts
- Mosaicity

Macromolecular crystals diffract at low resolution

More photons cannot restore missing crystal order



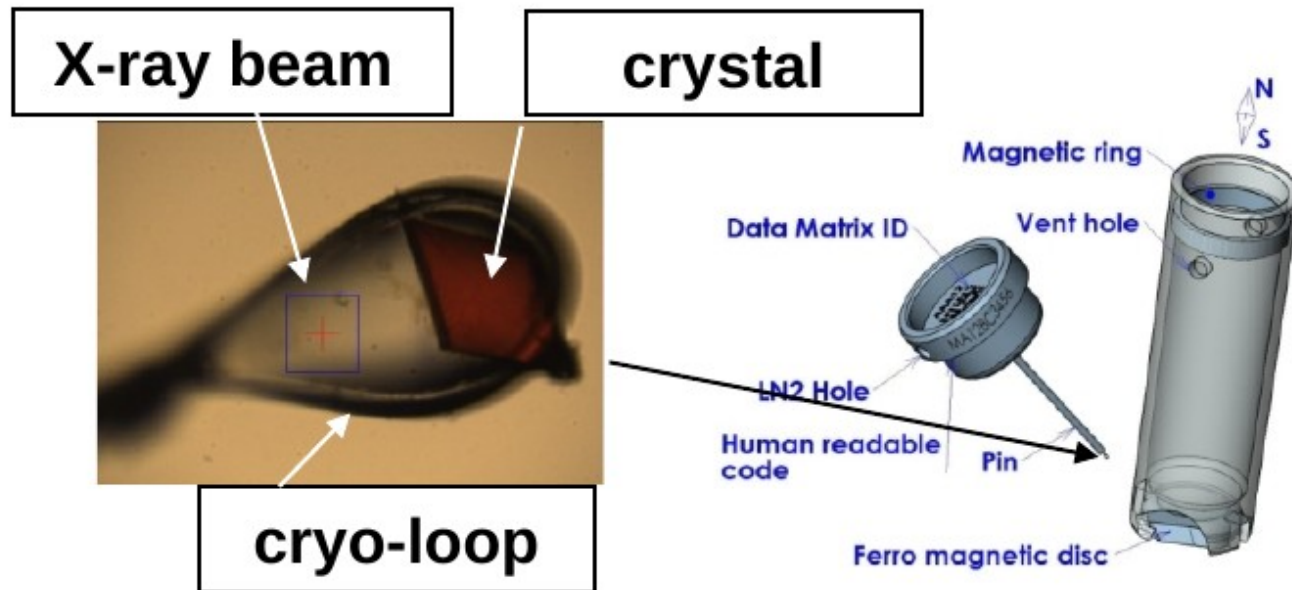
The measurement damages the sample



X-rays are ionizing radiation→ Radiolysis of water molecules produces ROS

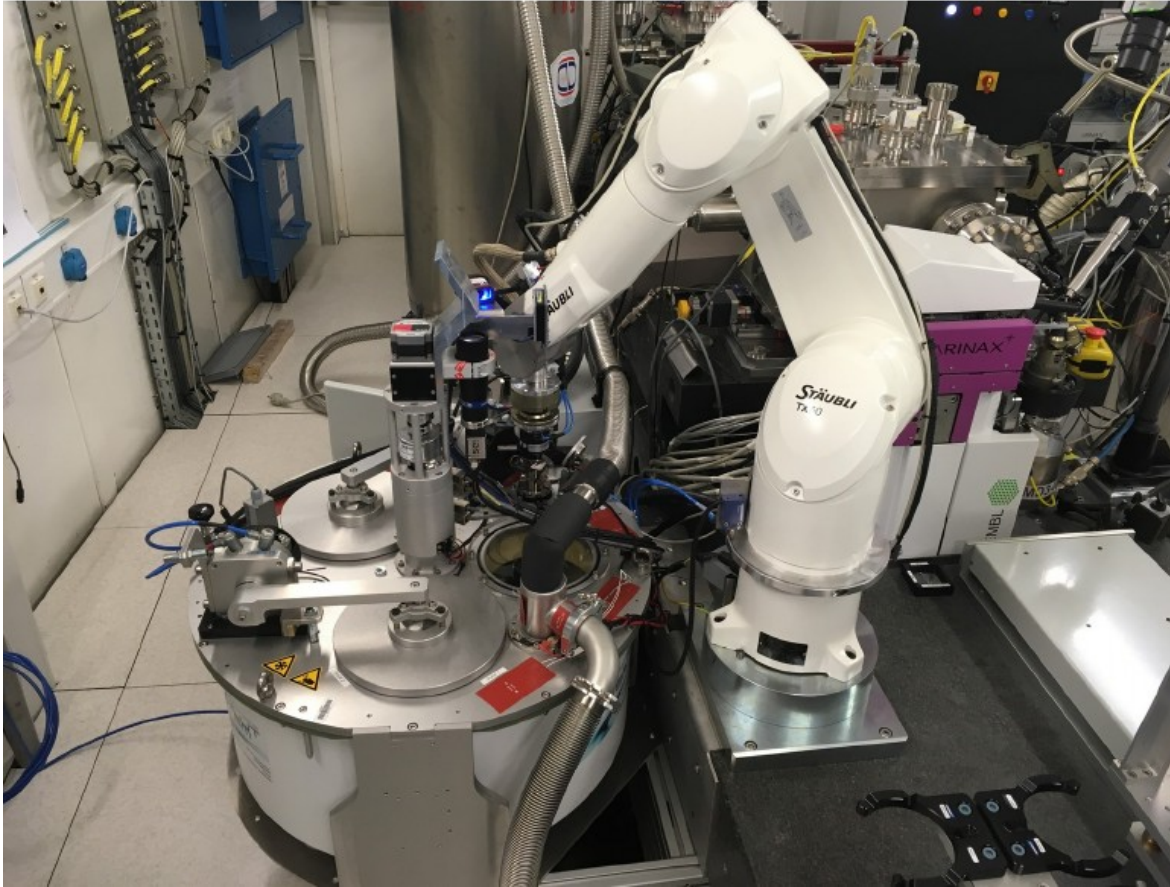
ROS diffuse within the xtal and chemically react (damage) with macromolecules

Cryocooling and dose management



Cooling at LN2 slows ROS diffusion → It delays, but it do not eliminate, radiation damage

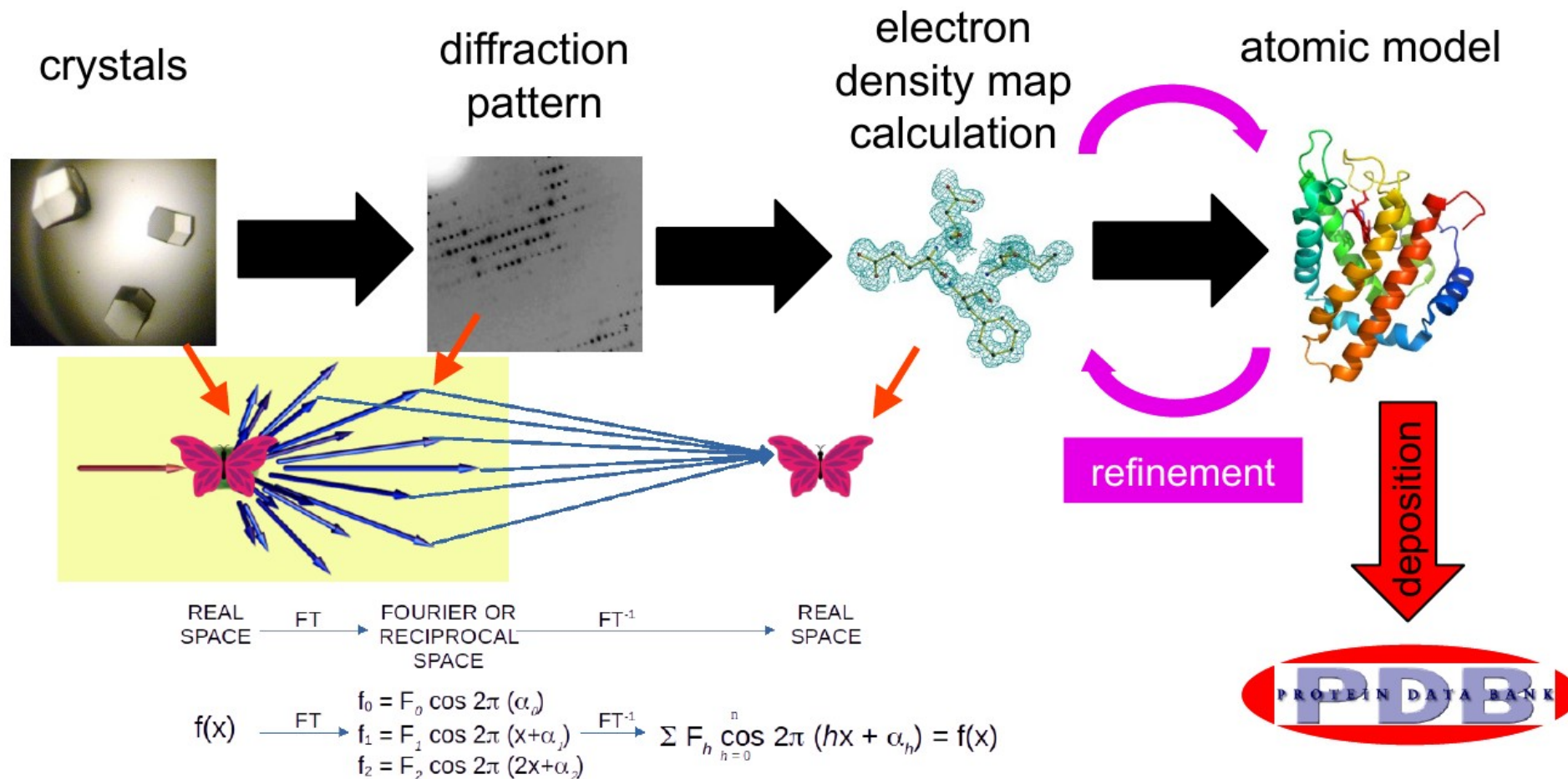
Automation at the beamline



Fast sample exchange and alignment

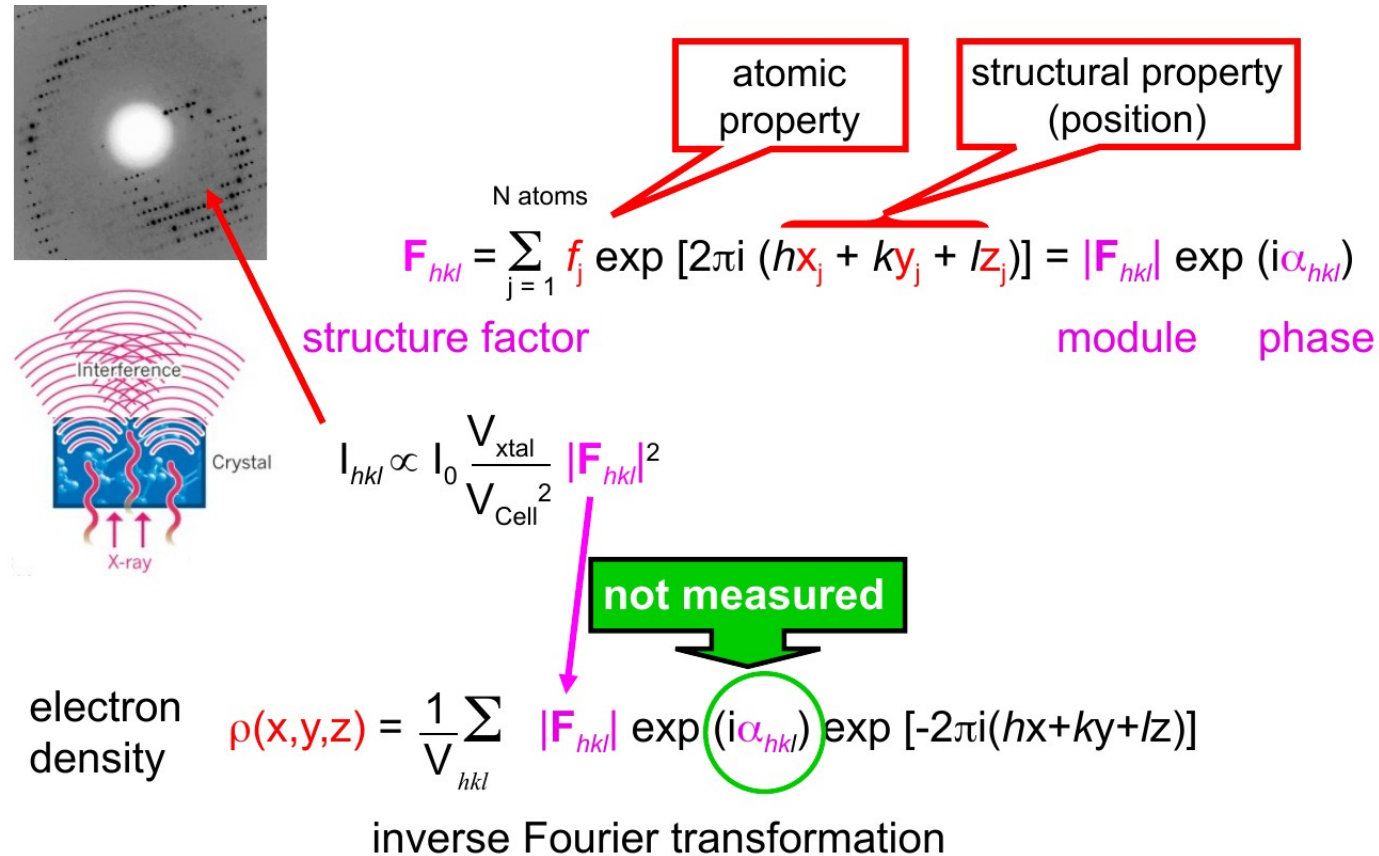
High photon flux speeds up data collection and enables high-throughput screening of many crystals.

Workflow checkpoint: diffraction data



We have intensities. We still need phases.

The phase problem



Measured intensities give structure-factor amplitudes
 An electron-density map also needs phases

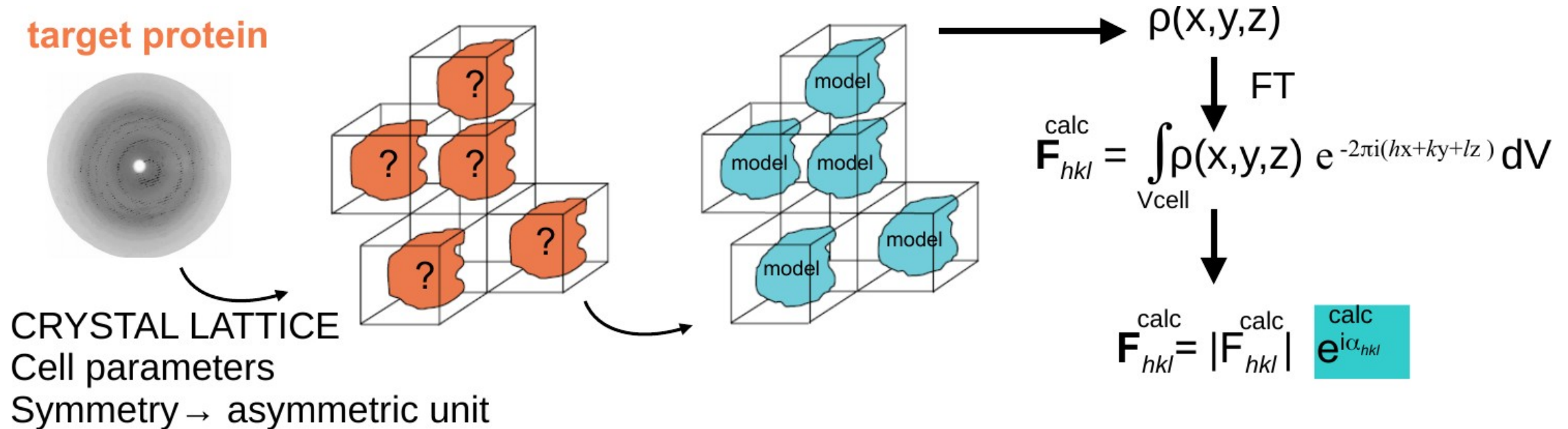
Where can the phase information come from?

Direct methods exploit statistical relationships among diffraction intensities to infer the missing phases. They work when the ratio of independent observations to unknown parameters is sufficiently high; in macromolecular crystallography, however, the large number of atoms, disorder and limited resolution produce too few independent data for too many unknowns, making the phase problem severely underdetermined.



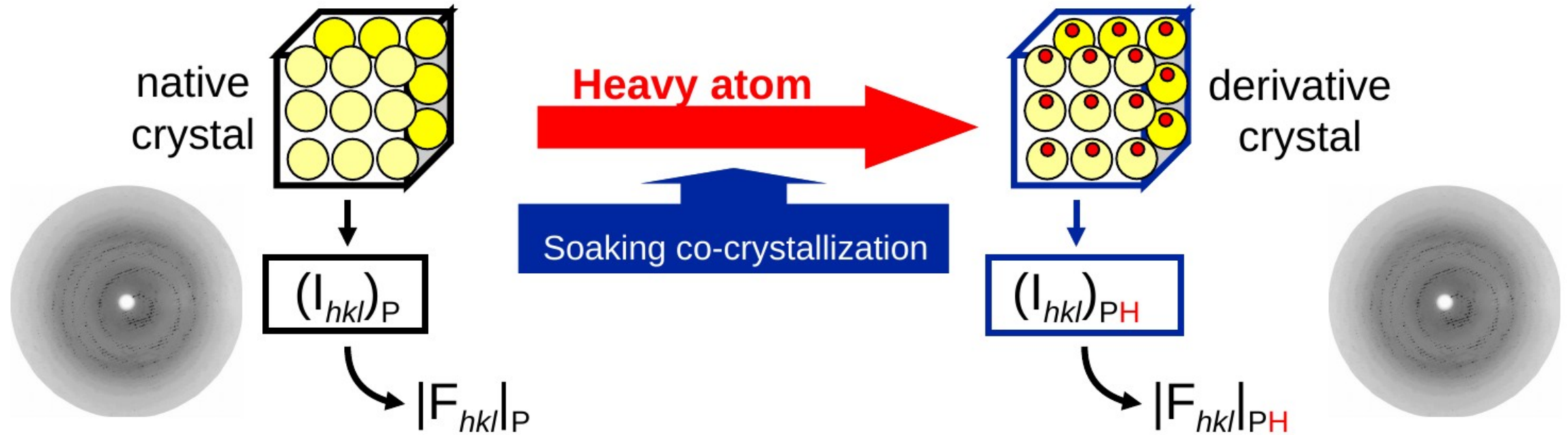
Too many unknowns, too few independent data

Molecular replacement



Place a related model in the new crystal
Use it to estimate starting phases

Isomorphous replacement



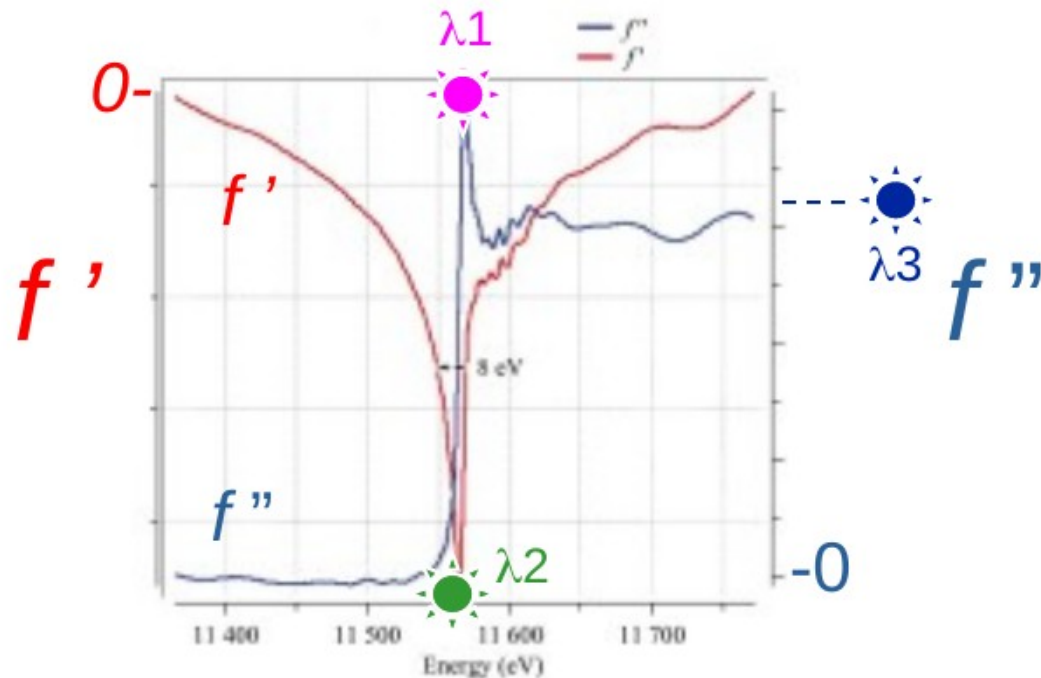
Compare native and heavy-atom derivative diffraction
MIR uses several derivatives

SAD and MAD

Diffraction is centro-symmetric → Friedel's law $|\mathbf{F}_{hkl}| = |\mathbf{F}_{-h-k-l}|$ and $\alpha_{hkl} = -\alpha_{-h-k-l}$

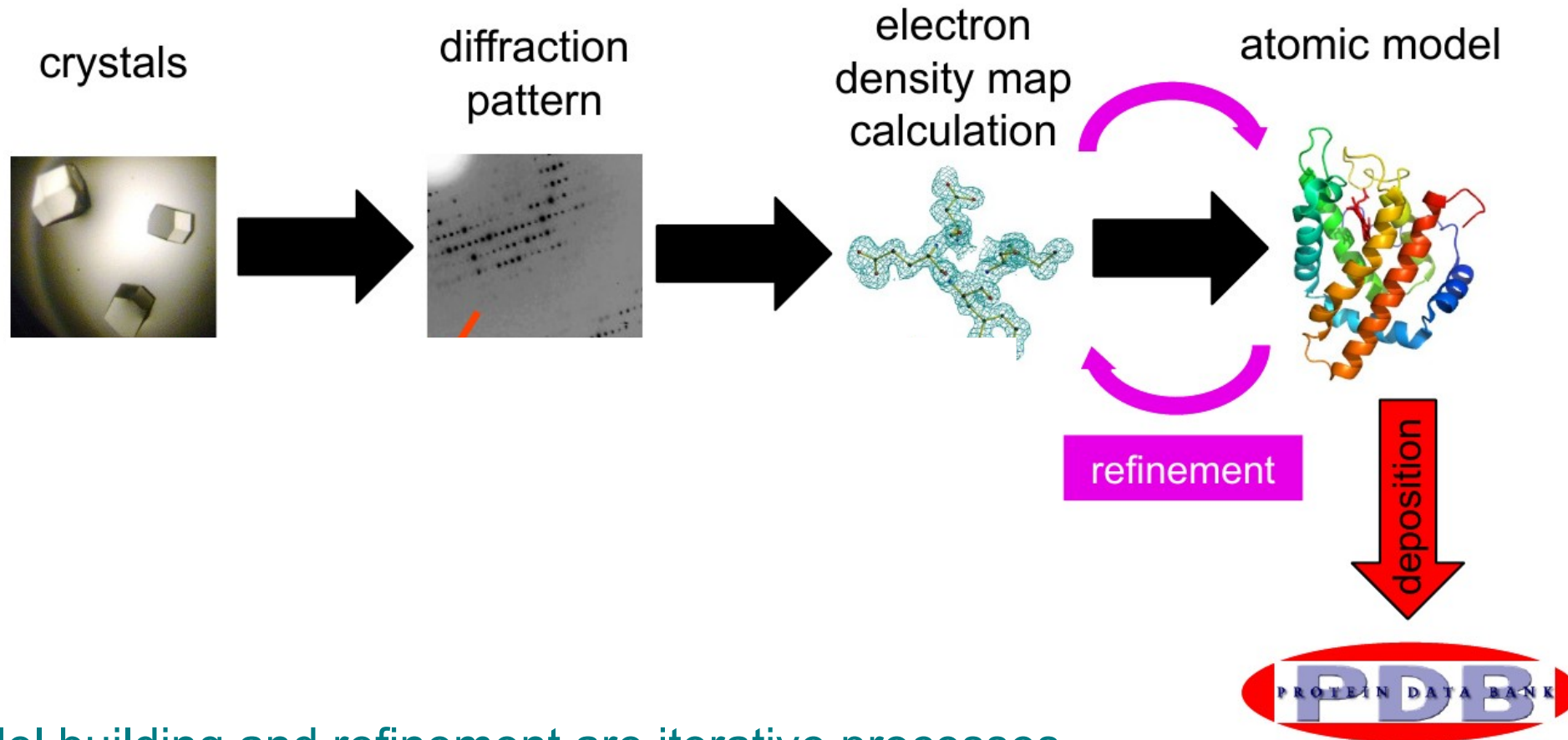
Near the absorption edge of a heavy atom, resonant scattering breaks Friedel's law.

Anomalous scattering → Friedel pairs (h,k,l) and $(-h, -k, -l)$ have different intensities



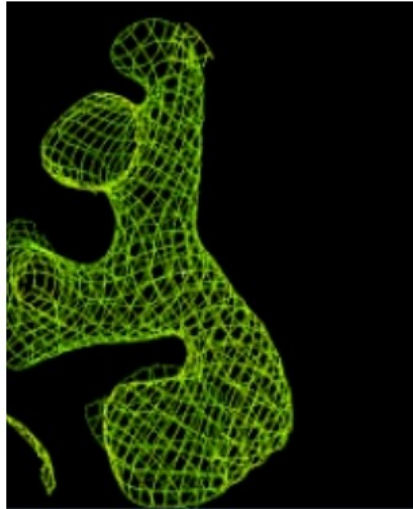
SAD and MAD require tunable X-rays → Synchrotron radiation

Workflow checkpoint: model interpretation

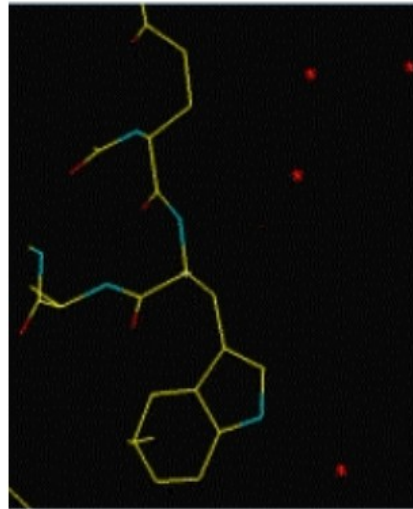


Model building and refinement are iterative processes

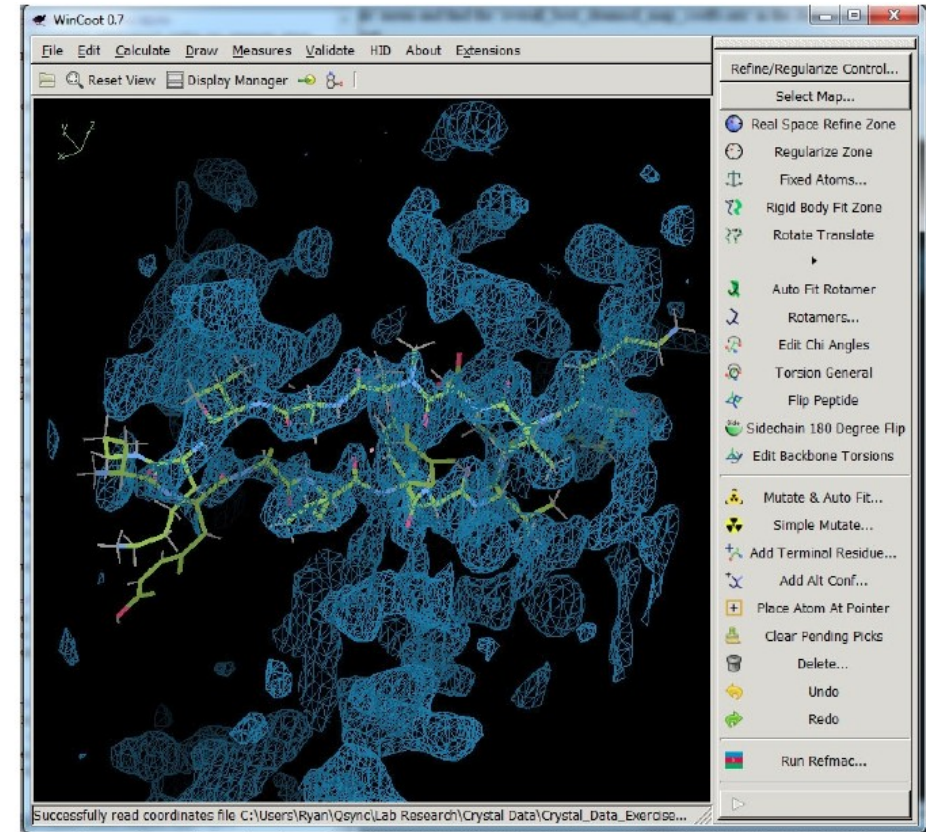
An electron-density map needs interpretation



What you see

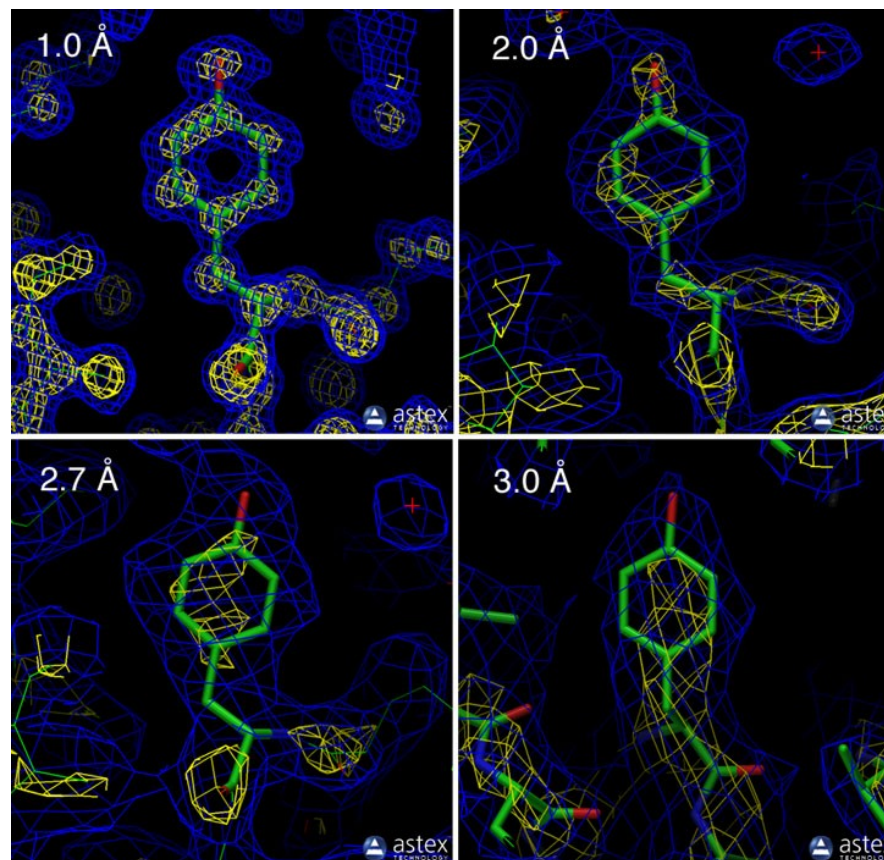


What you think



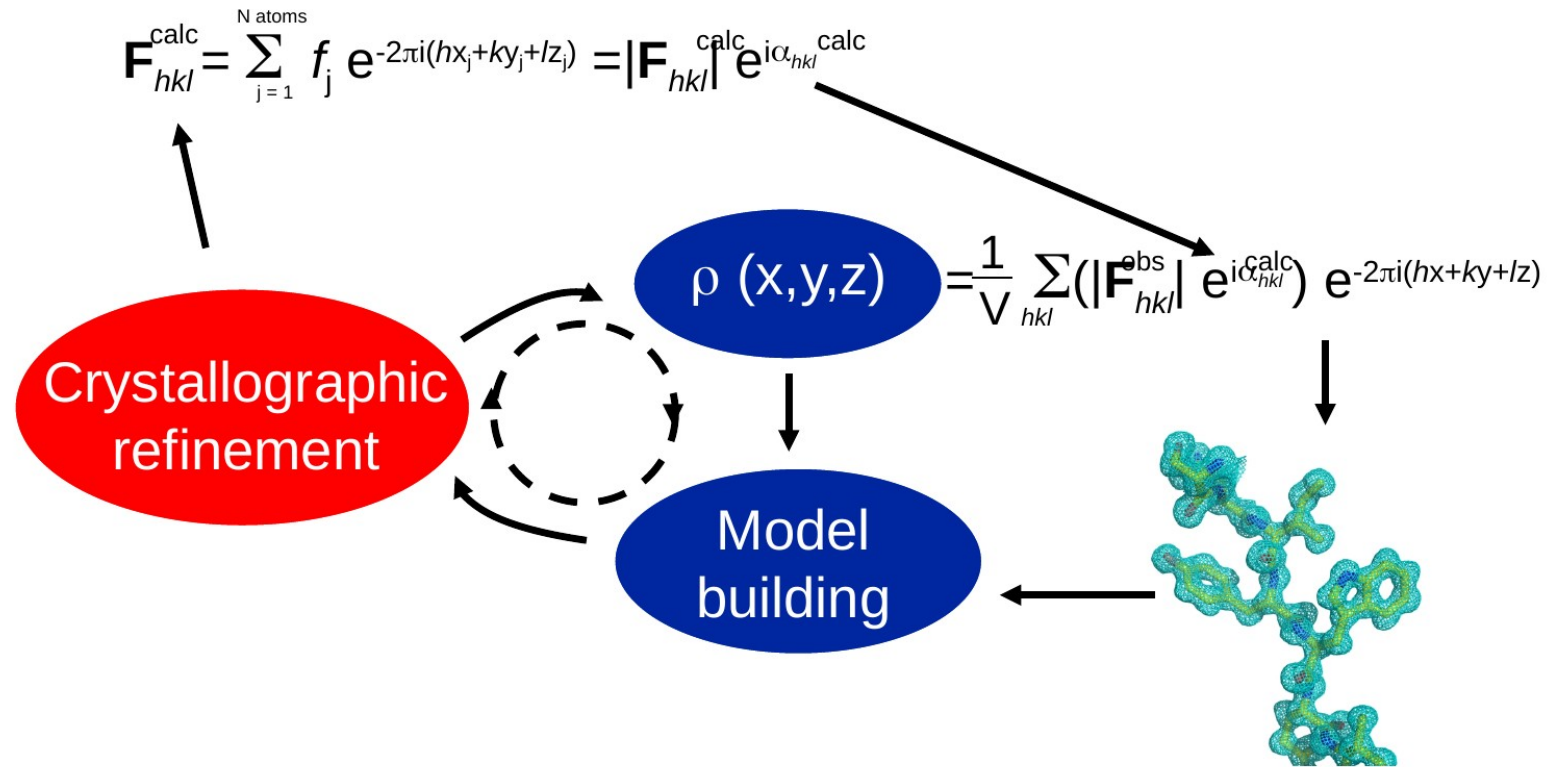
Phases and amplitudes produce a map
The map needs an atomic interpretation

Resolution changes what we can claim



High resolution makes your life easier

The model-building and refinement cycle



Improved model, revised phases, a new map

Repeat until further changes no longer improve the supported model

Refinement combines data and chemistry

Crystallographic refinement

Iterative process of model correction to minimize:

$$C = Q + kU$$

$$Q = \sum_{hkl} w_{hkl} (|F_{hkl,o}| - |F_{hkl,c}|)^2$$

$$U = \sum_j^{hkl} \frac{1}{2} k_{b,j} (b_{j,c} - b_{j,o})^2 + \sum_j \frac{1}{2} k_{t,j} (t_{j,c} - t_{j,o})^2 + \sum_j k_f [1 + \cos(nf_j - d)] + \sum_{ij} [(Ar^{-12})_{ij} + (Br^{-6})_{ij}]$$

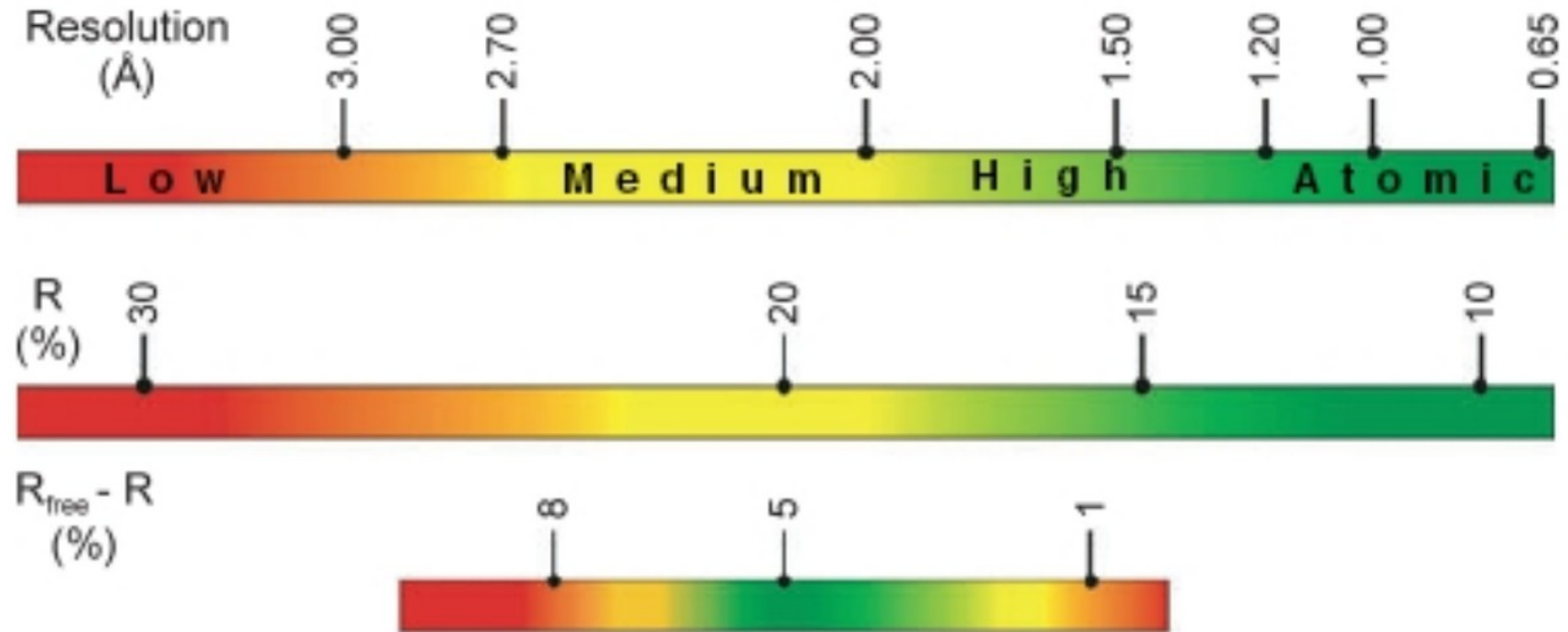
Diagram illustrating the components of the refinement function U:

- bond lengths** (indicated by an upward arrow pointing to $b_{j,c} - b_{j,o}$)
- bond angles** (indicated by a downward arrow pointing to $t_{j,c} - t_{j,o}$)
- torsion angles** (indicated by an upward arrow pointing to $nf_j - d$)
- van der Waals** (indicated by an upward arrow pointing to $(Ar^{-12})_{ij} + (Br^{-6})_{ij}$)

Diffraction data constrain the fit

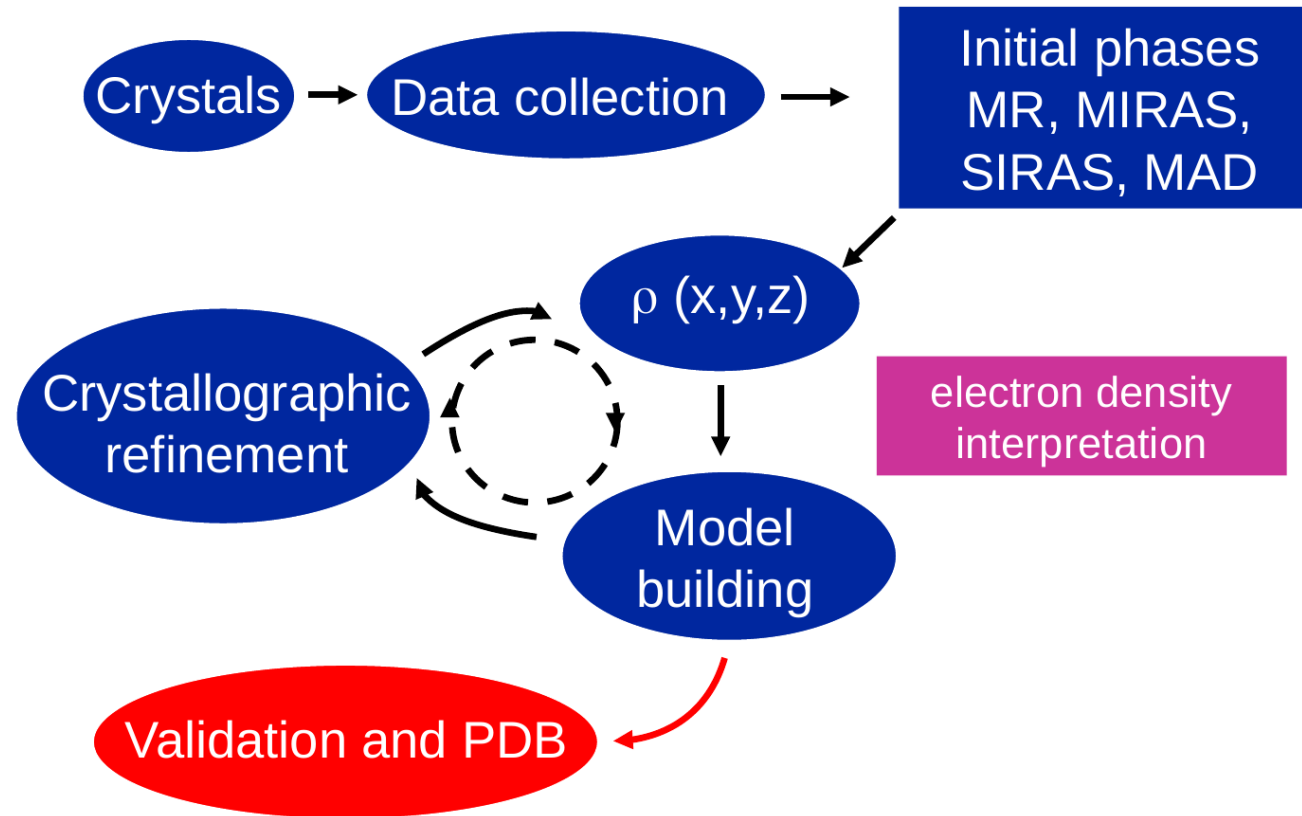
Chemical restraints stabilise an underdetermined model

Rwork and Rfree



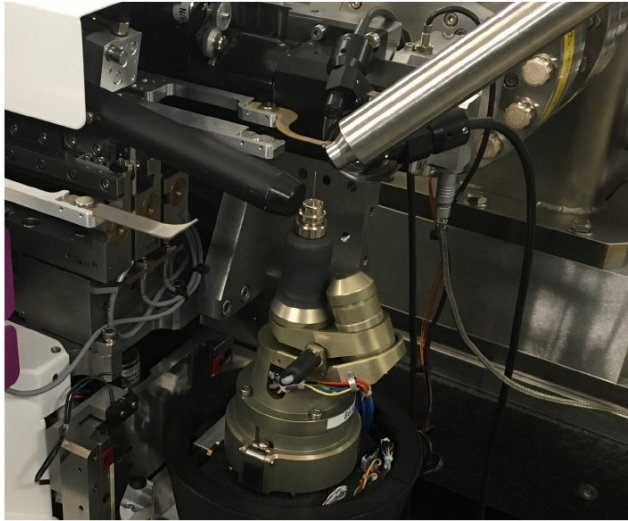
Rwork measures the working-set fit
Rfree checks reflections withheld from refinement
Illustrative guide, not universal acceptance limits

Deposition makes the model inspectable



Deposit the atomic model and experimental data
Validation makes strengths and limitations visible

Why synchrotrons matter for protein crystallography



High flux and small beams for small or weak crystals

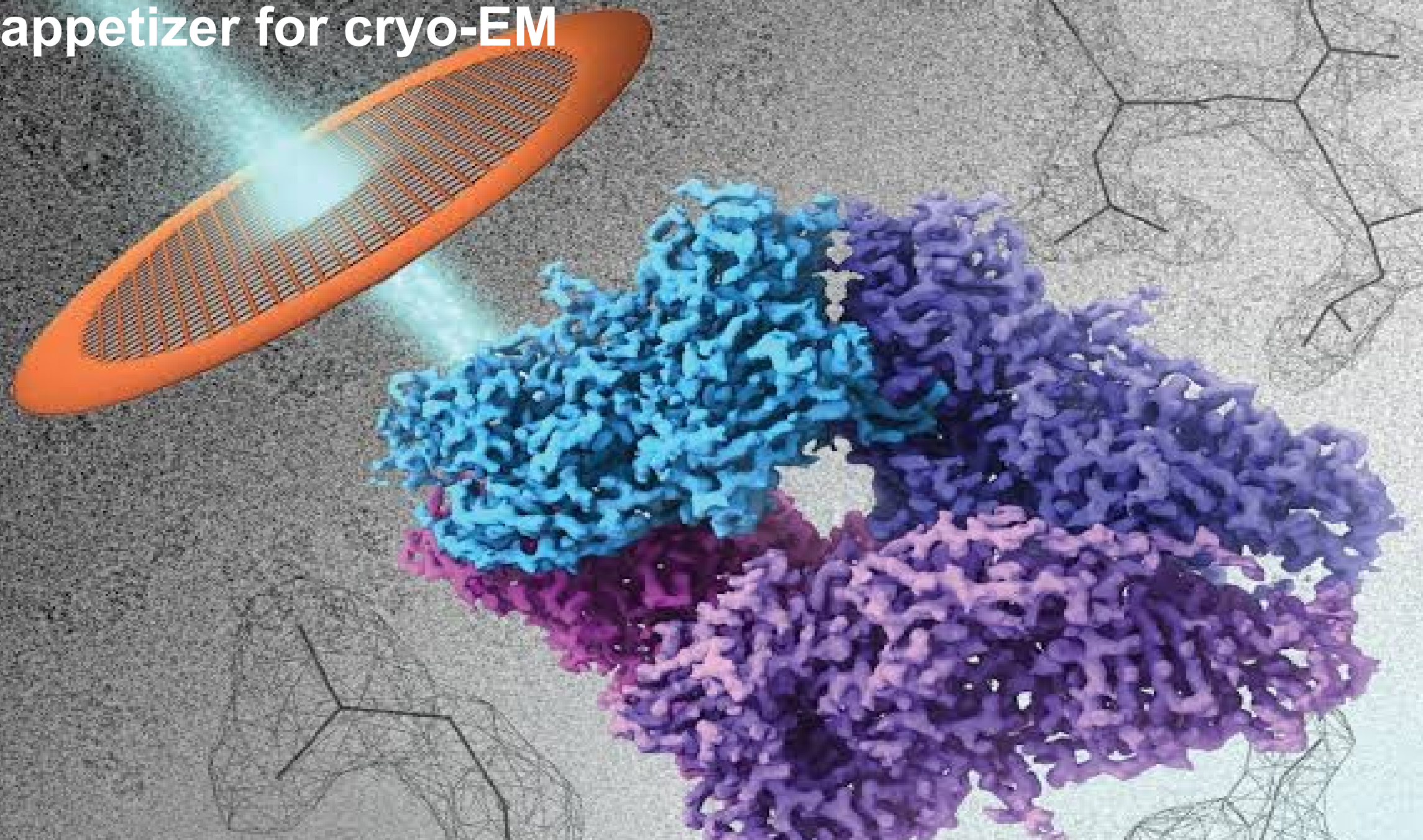
Automation and high intensity enable collection of diffraction data from many crystals

Low divergence for closely spaced reflections

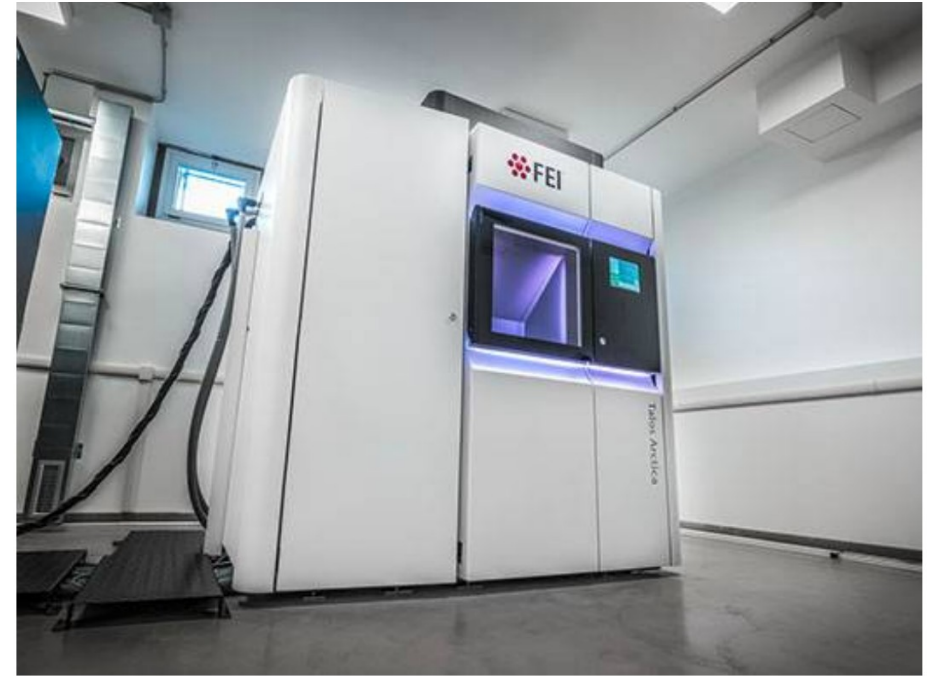
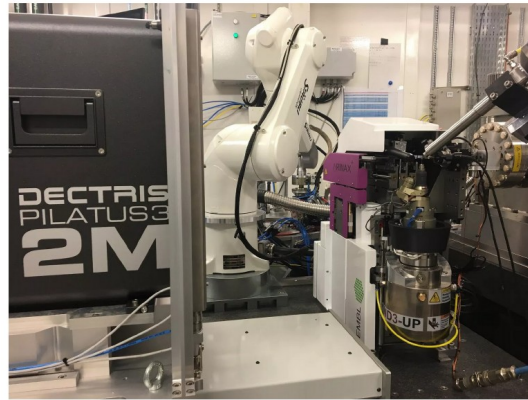
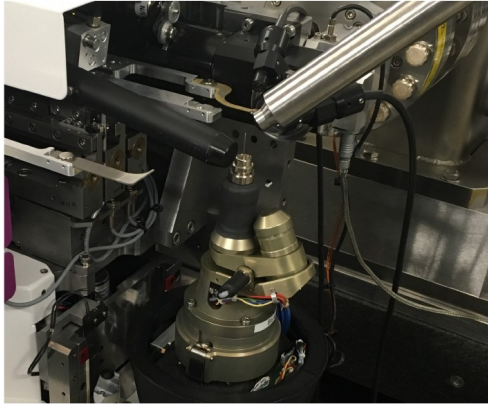
Tunable wavelength for phasing experiments

Expert support and shared access

An appetizer for cryo-EM



Why photon facilities host electron microscopes

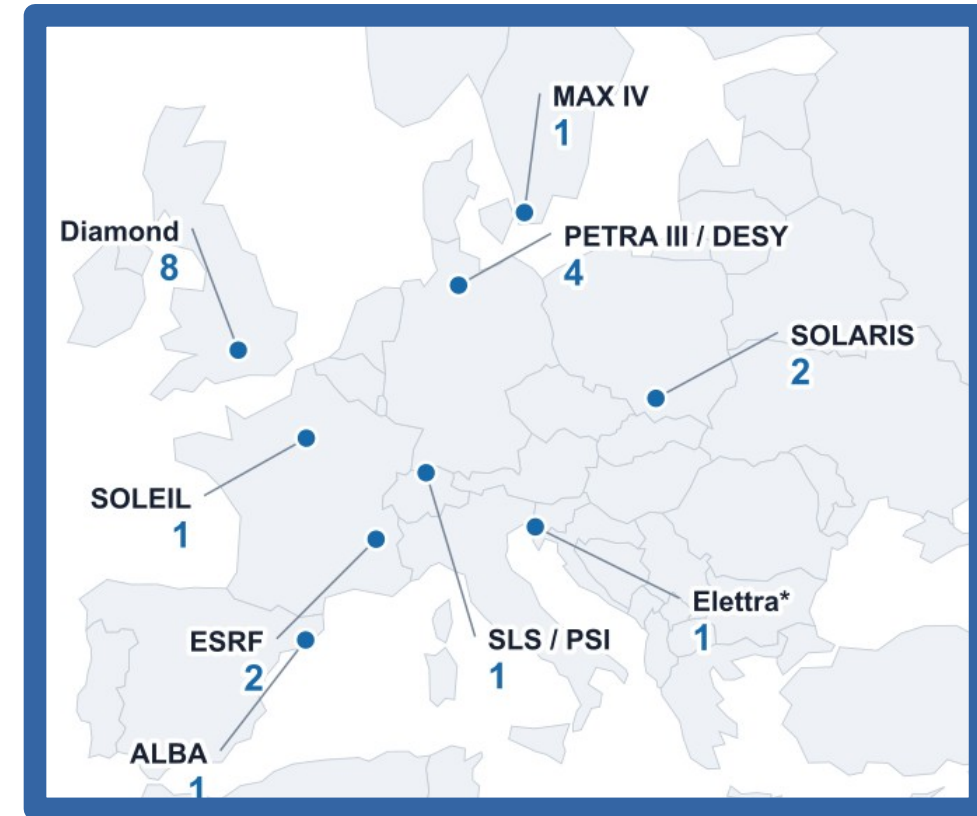
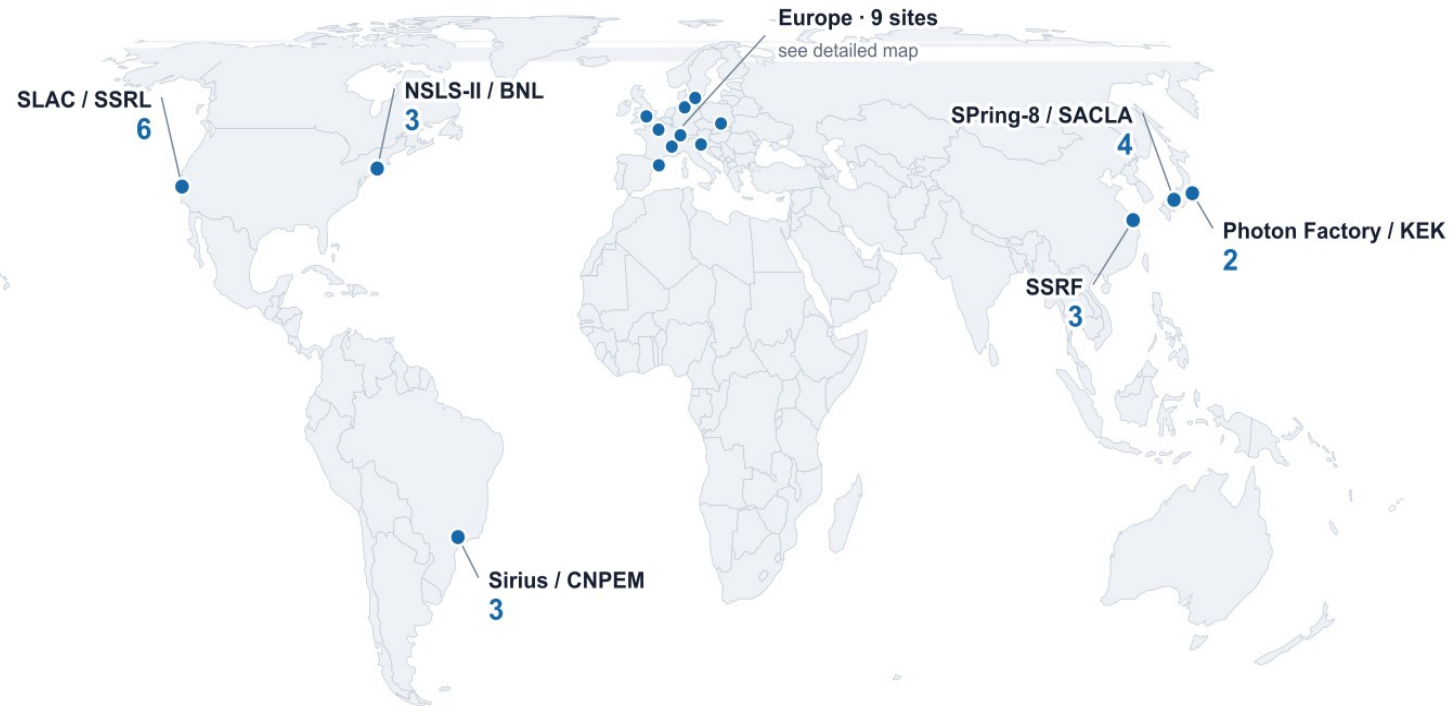


Established MX user community

Shared cryo-EM infrastructure

Expert staff, stable environments, preparation and computing

Cryo-EM across synchrotron campuses

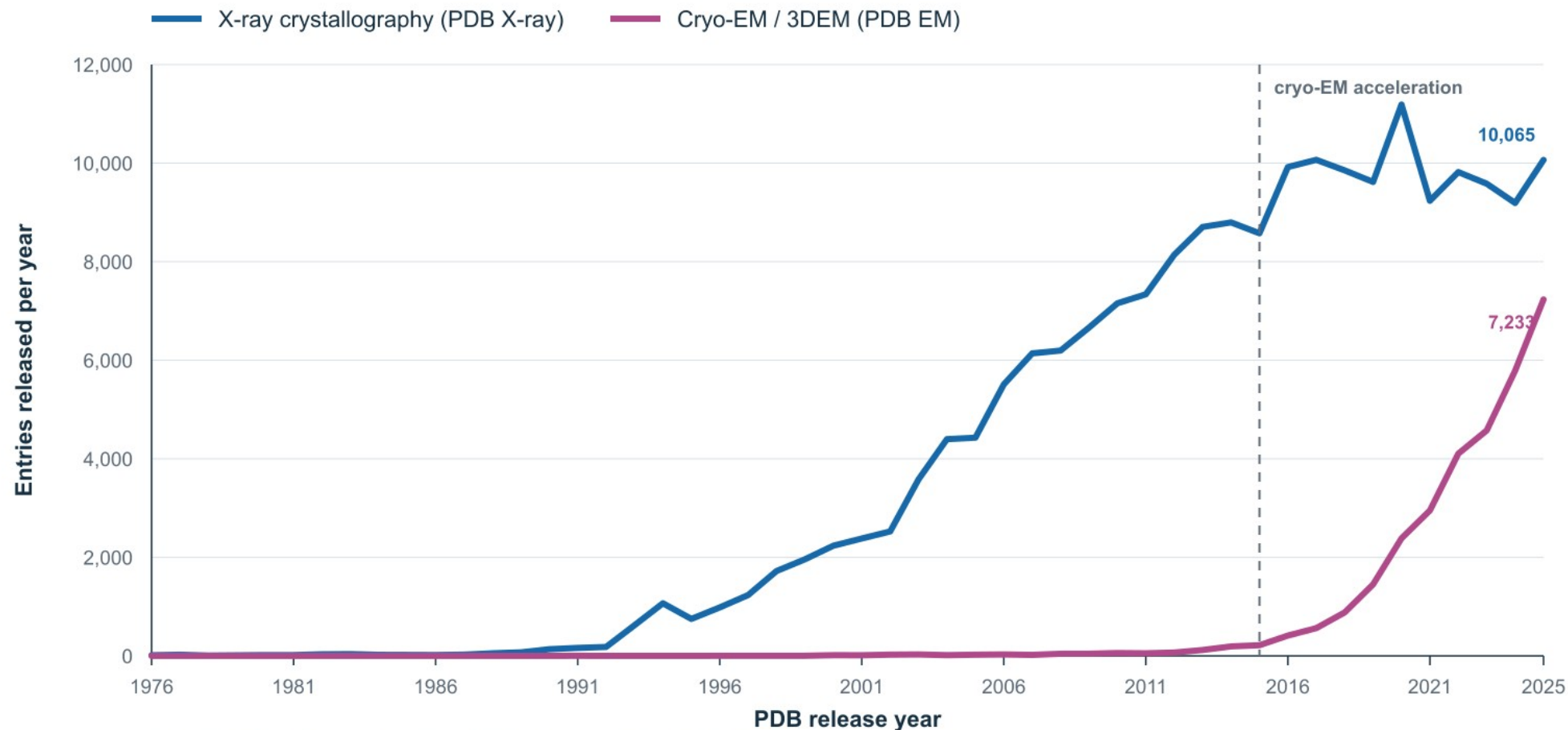


Selected campuses. Numbers count biological TEMs, including screening instruments.

Cryo-EM is reshaping the structural-method landscape

PDB structure growth: X-ray crystallography and cryo-EM

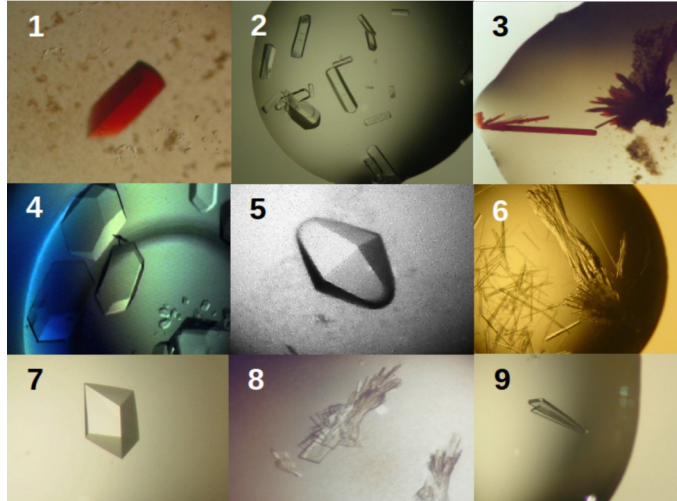
Annual structures released by experimental method · complete PDB timeline, 1976–2025



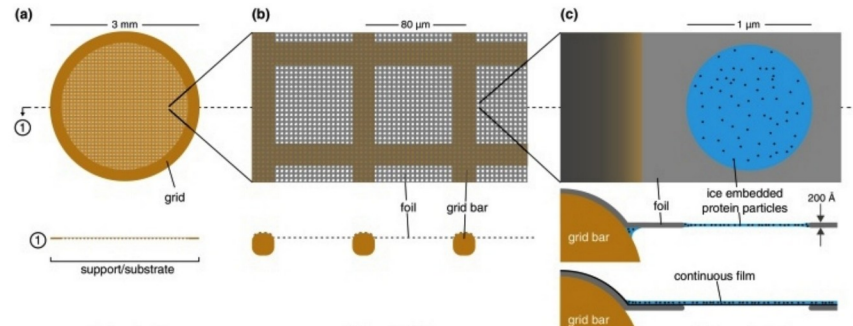
Source: RCSB PDB Statistics, Experimental Method, accessed 23 Sep 2026. 2026 is incomplete and omitted.

"EM" is the archive category for electron microscopy/3DEM and is used here as the cryo-EM proxy.

A different structural experiment



Protein crystal + X-rays



Particles in ice + electrons

Different techniques, “the same” structural information

A different structural experiment



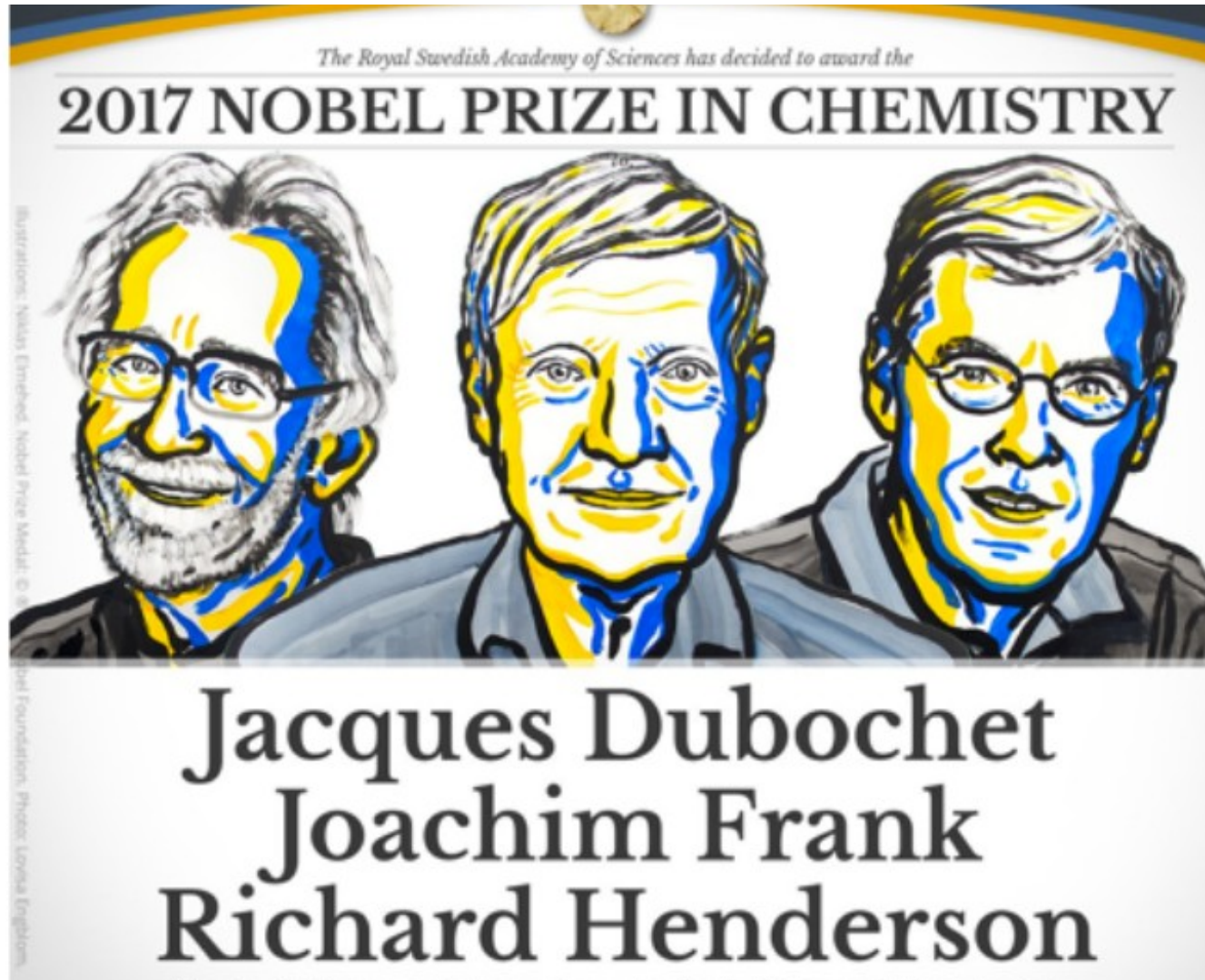
Protein crystal + X-rays



Particles in ice + electrons

Different techniques, “the same” structural information

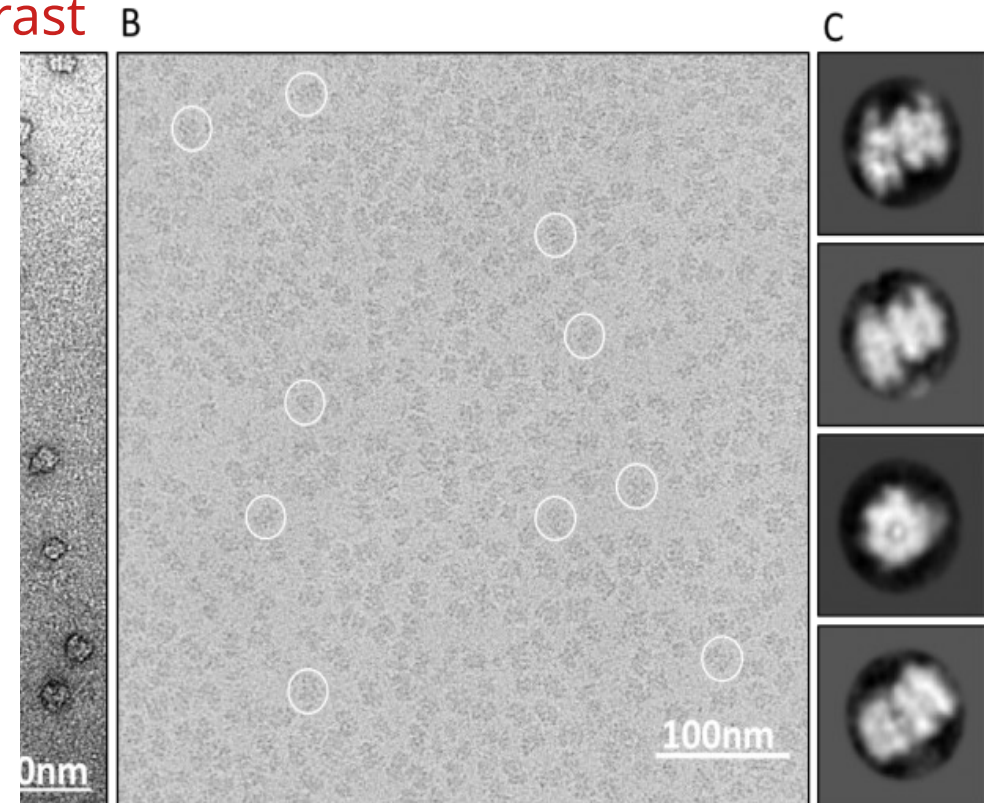
An appetizer for cryo-EM



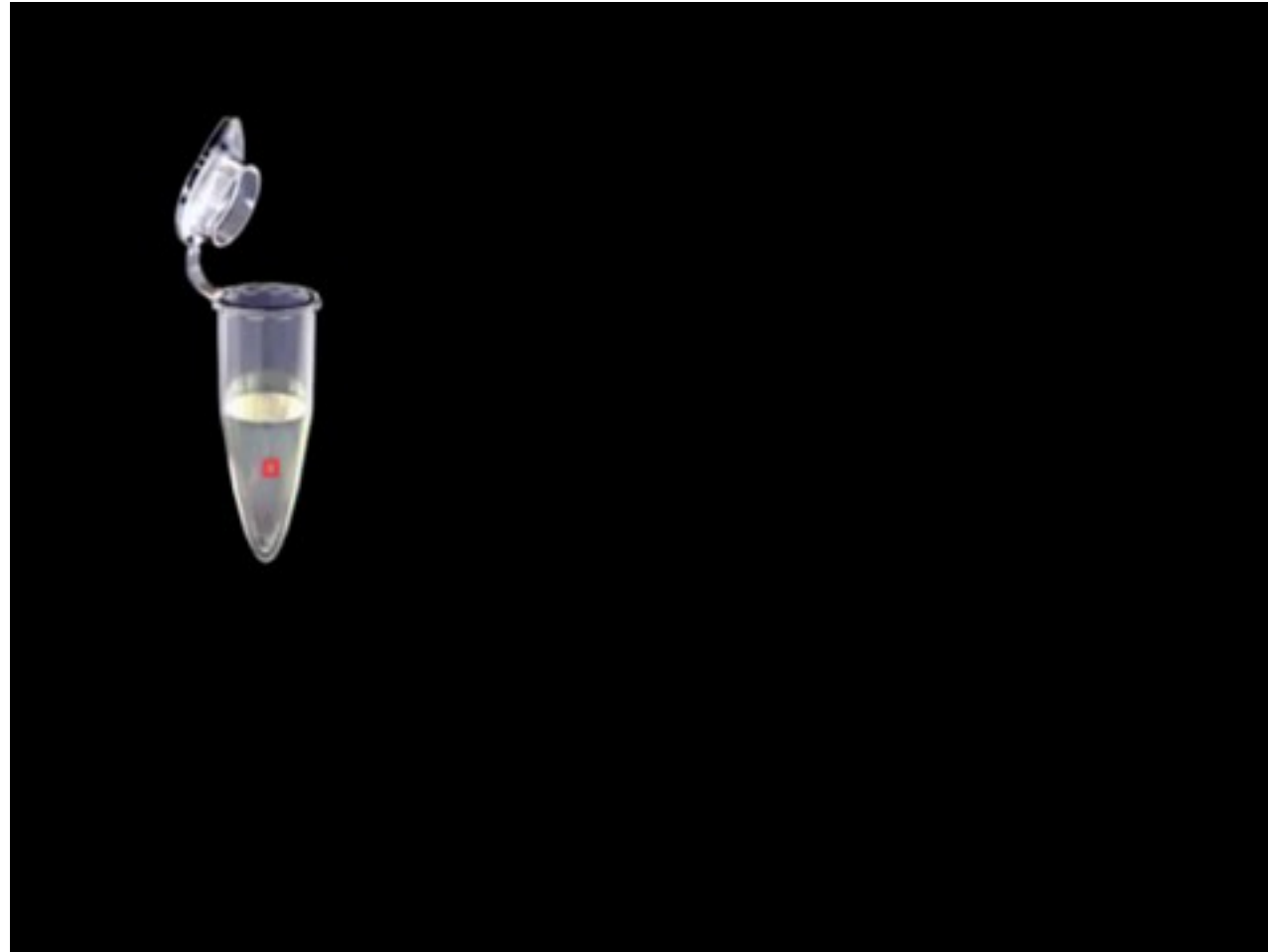
Cryo-EM most important property

Cryo-EM is a low contrast imaging technique

- TEM CHALLENGES ON BIO-SAMPLES
- Sample in solution → Incompatibility of TEM (**vacuum**)
- Sensitivity to Radiation damage → Low dose → **Low contrast**
- Atomic composition → C,O,N,H,S vs O,H → **Low contrast**
- cryo-EM
- Vitrification technology
- Million particles → Better algorithms
- Better microscopes
- Better detectors



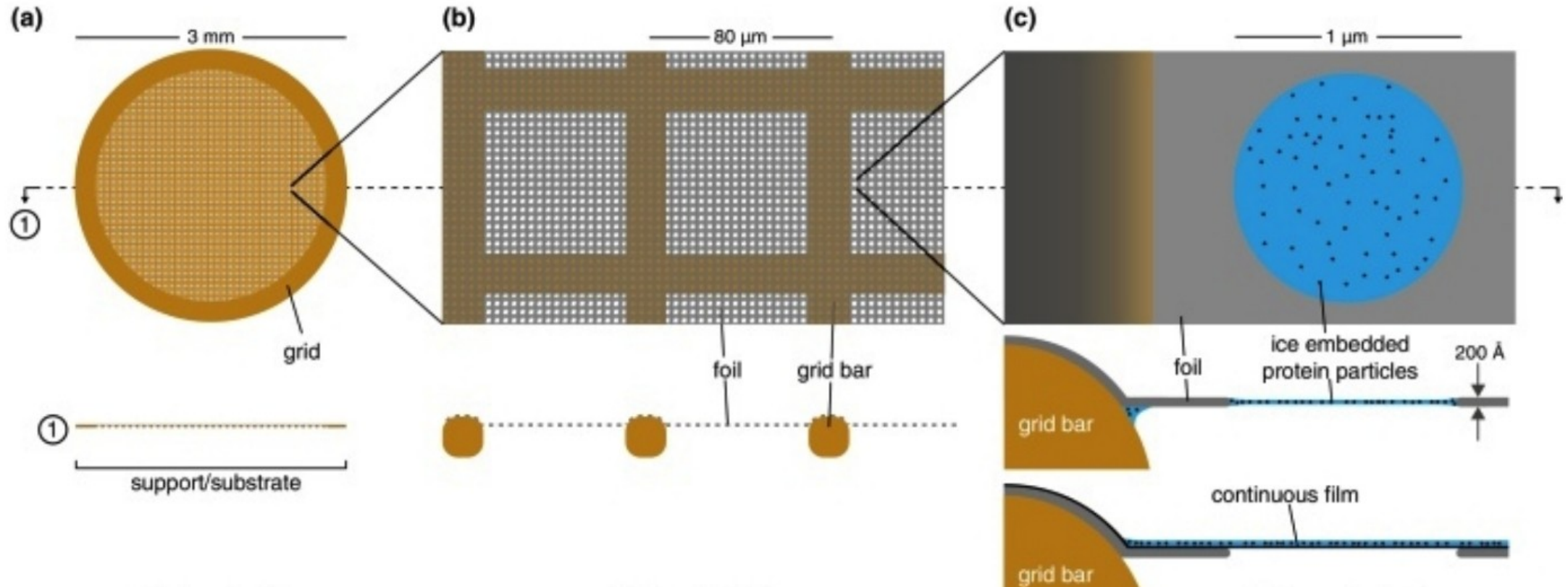
Cryo-EM in three minutes



Specimen preparation, imaging and reconstruction

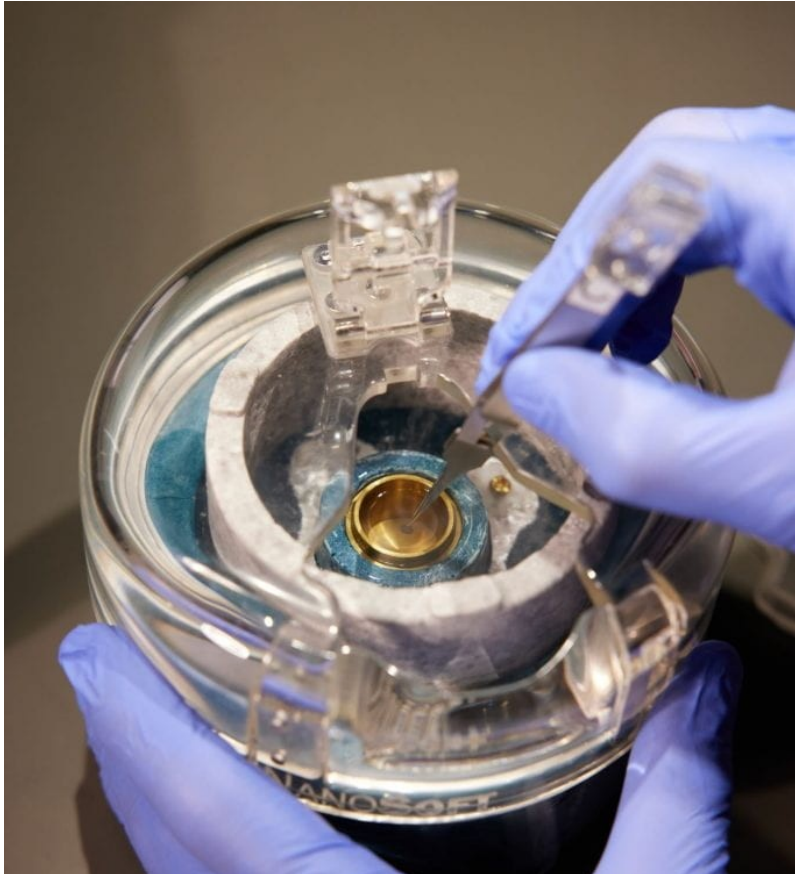
CRYO-EM IN 3 MINUTES

The cryo-EM grid



A support with small holes
Particles occupy a thin layer of vitreous ice

Sample preparation: The vitrification experiment



Vitreous ice preserves a hydrated specimen

Solid samples are compatible with TEM vacuum

Rapid plunging into liquid ethane cooled by liquid nitrogen produces vitreous ice.

Vitreous ice is electron-transparent

Ice thickness matters

The microscope (TEM)

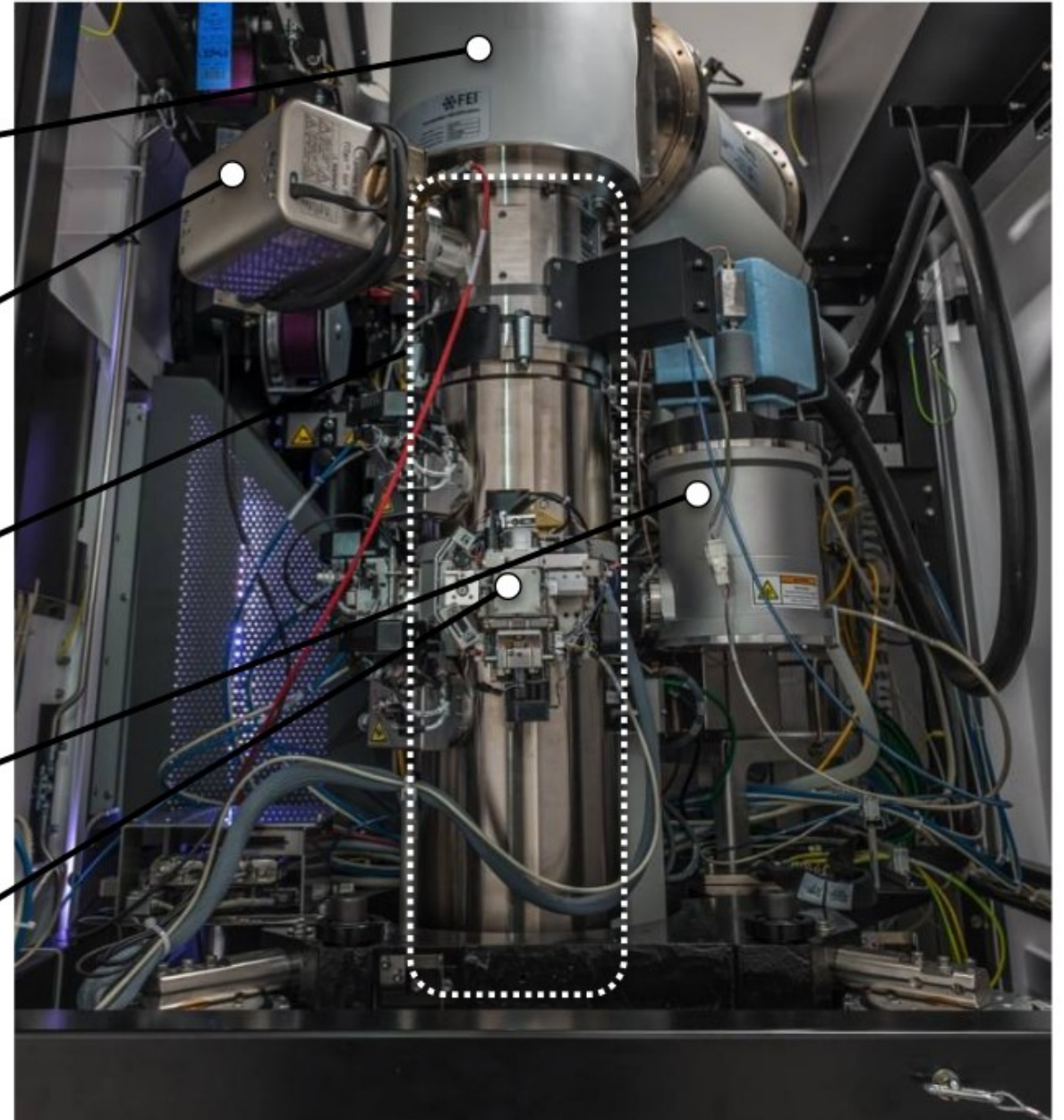
Electrons are extracted at
200'000 eV
(~70% speed of light)

Several pumps are required for
high vacuum in the column
(up to 10^{-9} Pa)

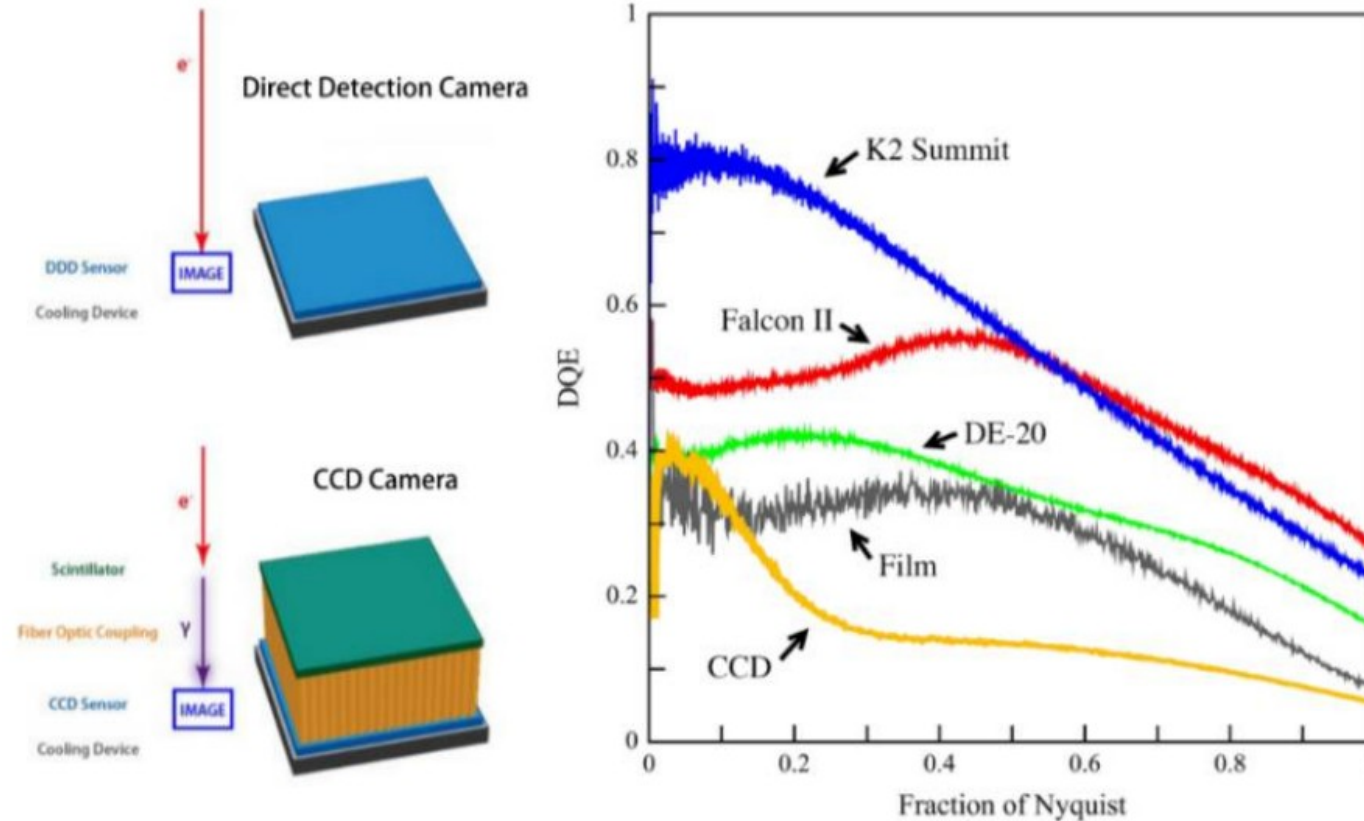
Elettromagnetic lenses to make
the electron beam coherent and
stable

Liquid nitrogen dewars to
maintain low temperature
(- 195 °C)

The sample is here



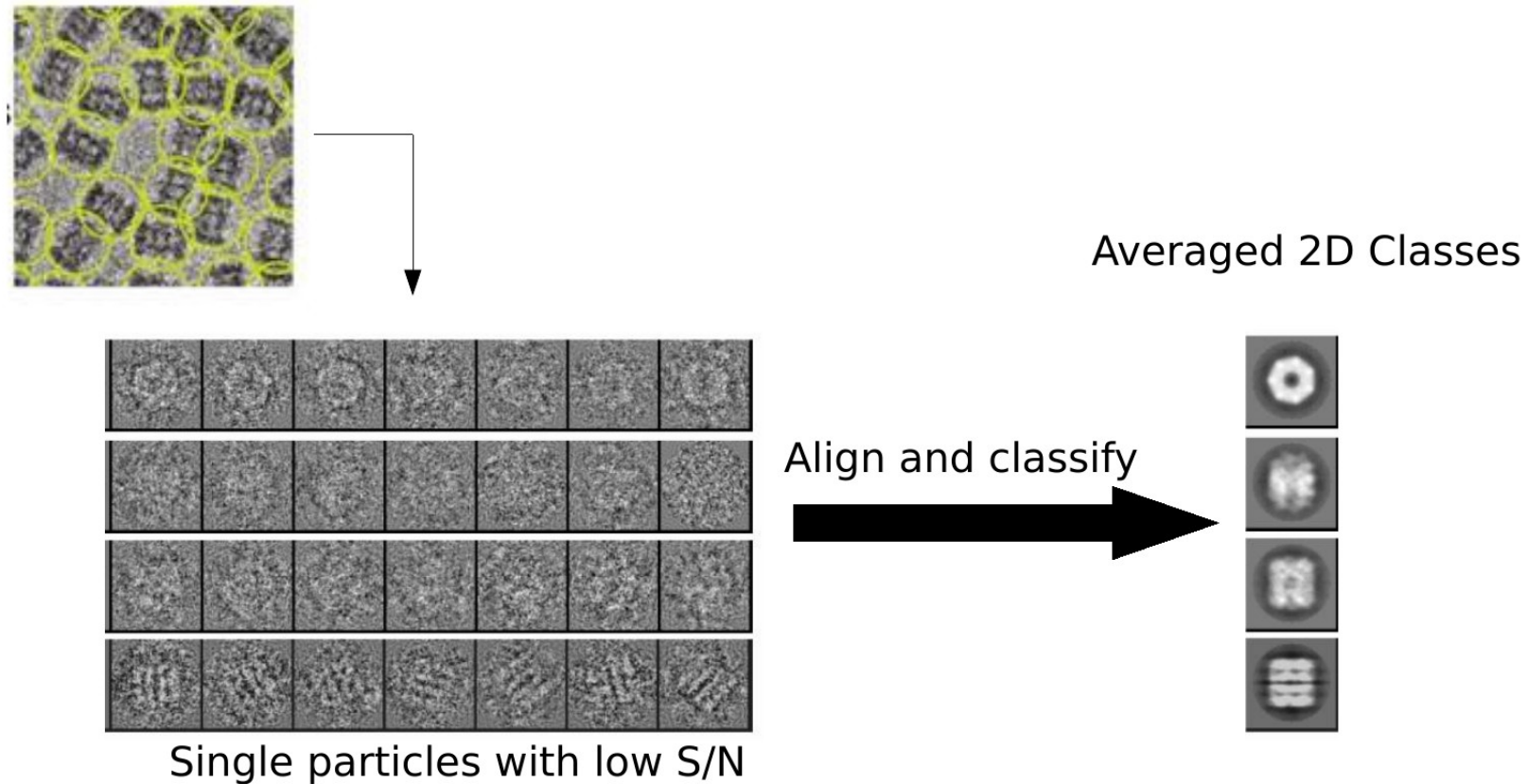
Why cryo-EM improved so dramatically



Direct electron detectors record movies

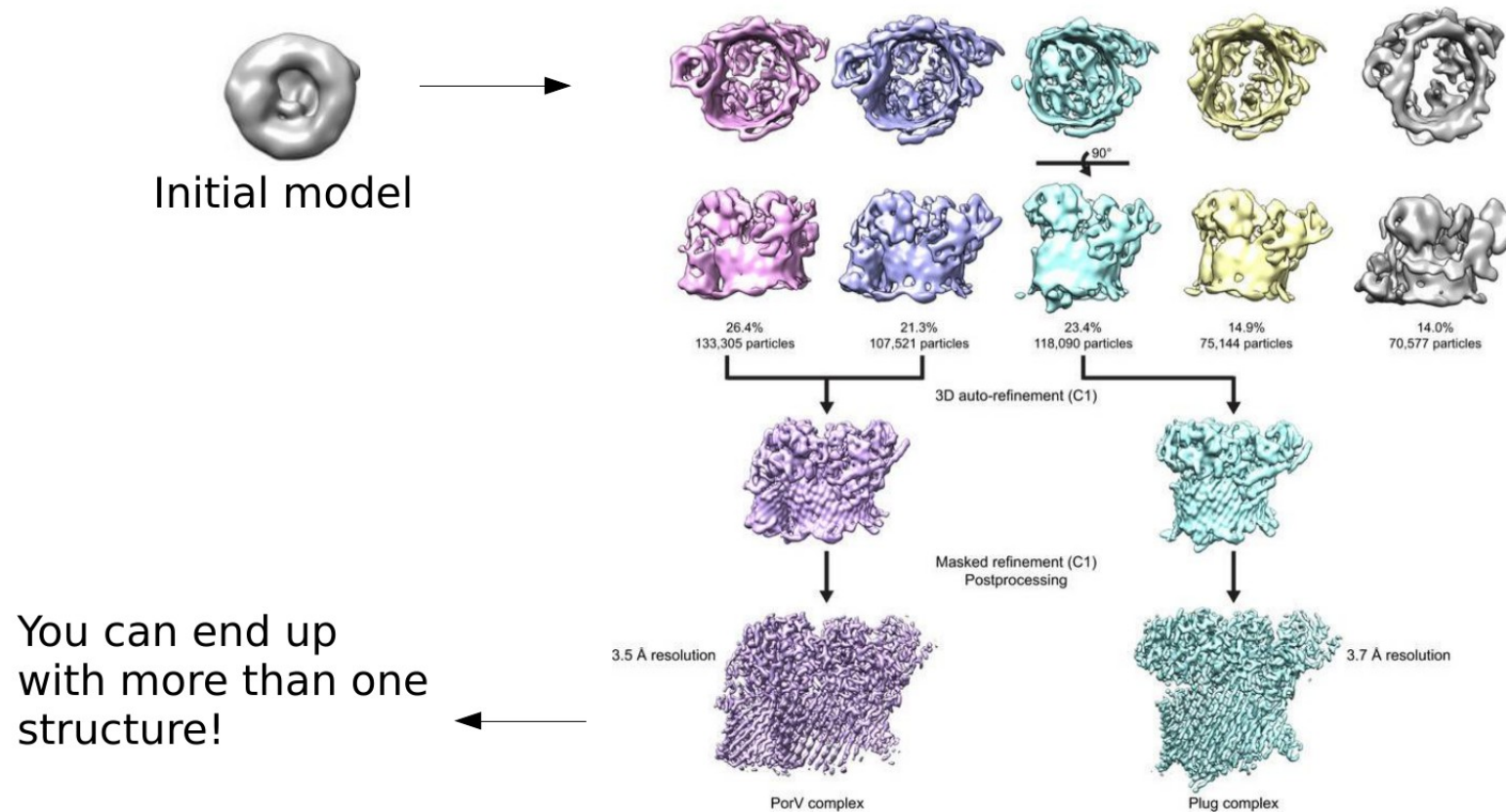
Motion correction preserves high-resolution information

Many noisy particles, clearer averages



Individual particles are noisy
Alignment and classification reveal common views

Three-dimensional reconstruction and heterogeneity



Different orientations support a 3D reconstruction
Different conformations may need separate classes

What to take home

Protein 3D structure

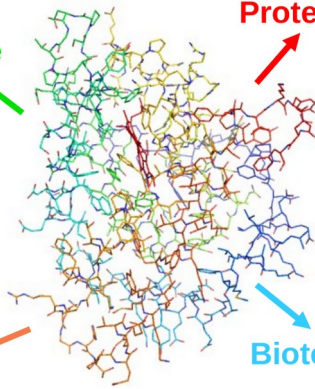
Basic science

Protein engineering

Drug design

Biotech applications

Structure ↔ Function



The protein crystal sets the X-ray experiment

Phase information turns diffraction into a map

Refinement and validation support interpretation

Cryo-EM adds an alternative structural method

Shared facilities connect the techniques