Introduction to Protein Crystallography

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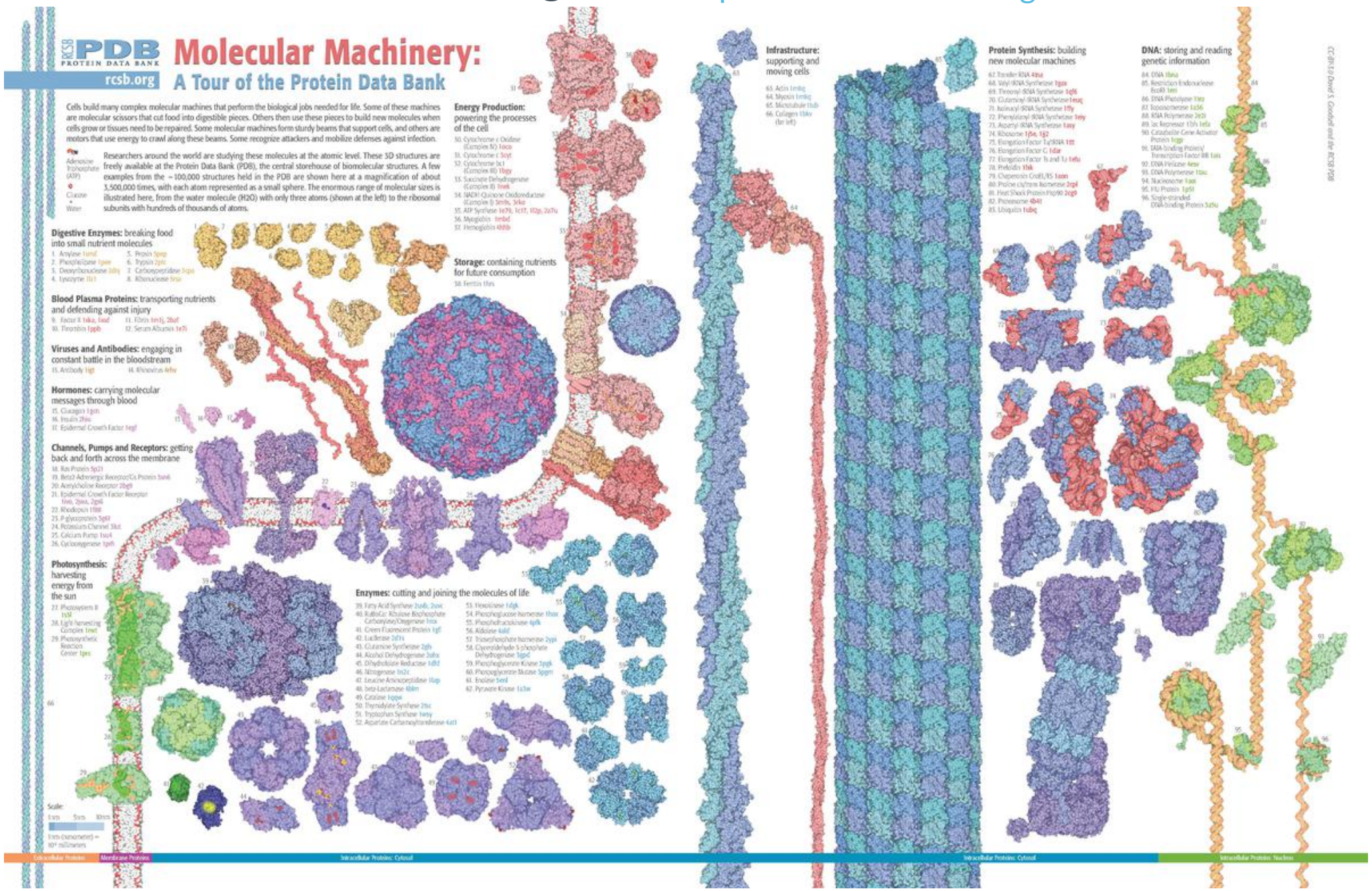


1. Crystallography and its application in Biology

> How is crystallography useful in biology?

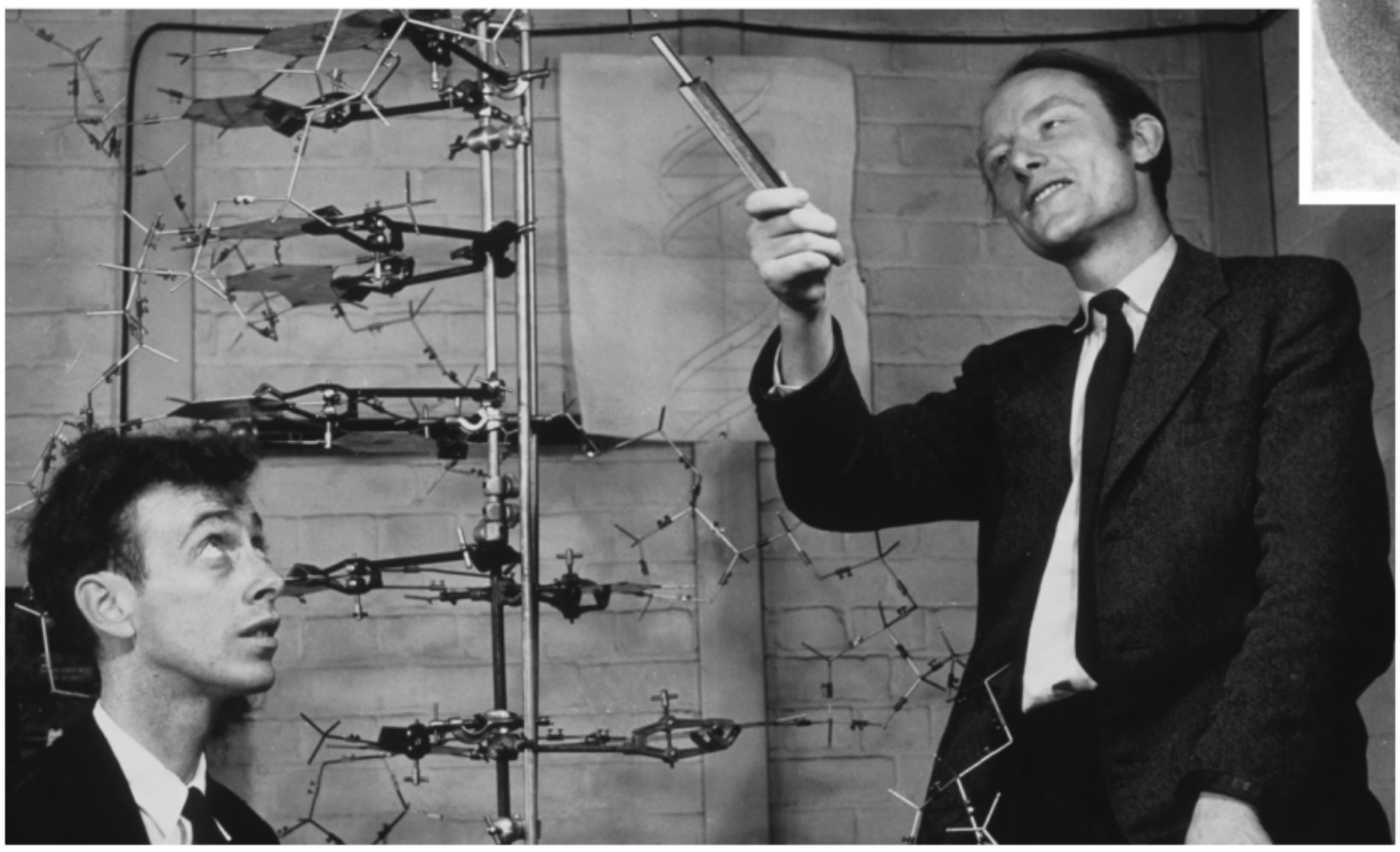
2. Practical protein X-ray crystallography

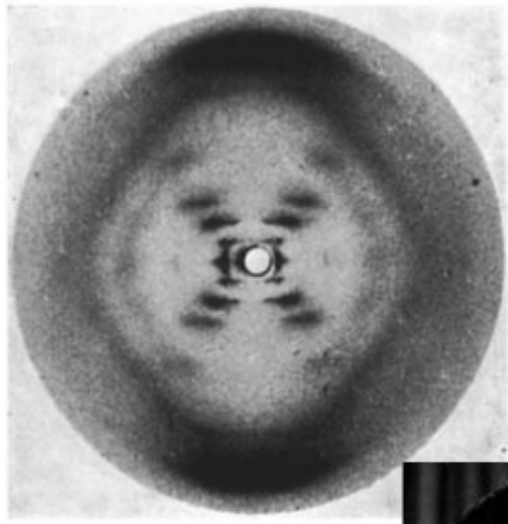
> Walk-through the individual steps of X-ray structure solution.

What is the aim of structural biology?

> The particular field which excites my interest is the division between the living and the non-living, as typified by, say, proteins, viruses, bacteria and the structure of chromosomes. The eventual goal, which is somewhat remote, is the description of these activities in terms of their structure, i.e. the spatial distribution of their constituent atoms, in so far as this may prove possible. This might be called the chemical physics of biology.
>
> -Francis Crick, 1947

Watson and Crick propose the structure of DNA

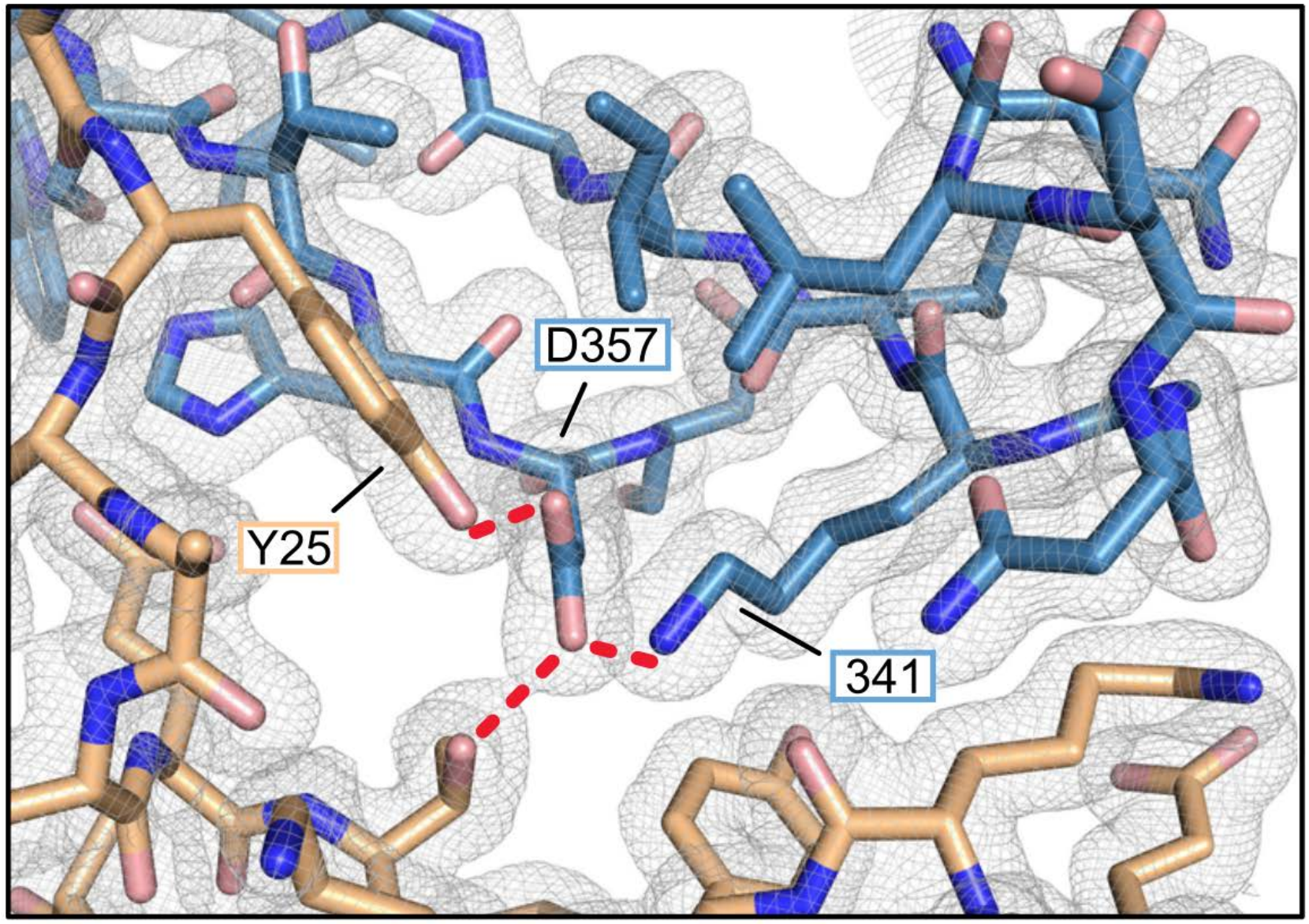




Watson and Crick present their model of DNA (left), which they deducted from fiber diffraction data of Rosalind Franklin (right). Understanding the structure of DNA laid the foundation for molecular biology as we know it. [1]

1. Horace Freeland Judson, Eighth Day of Creation

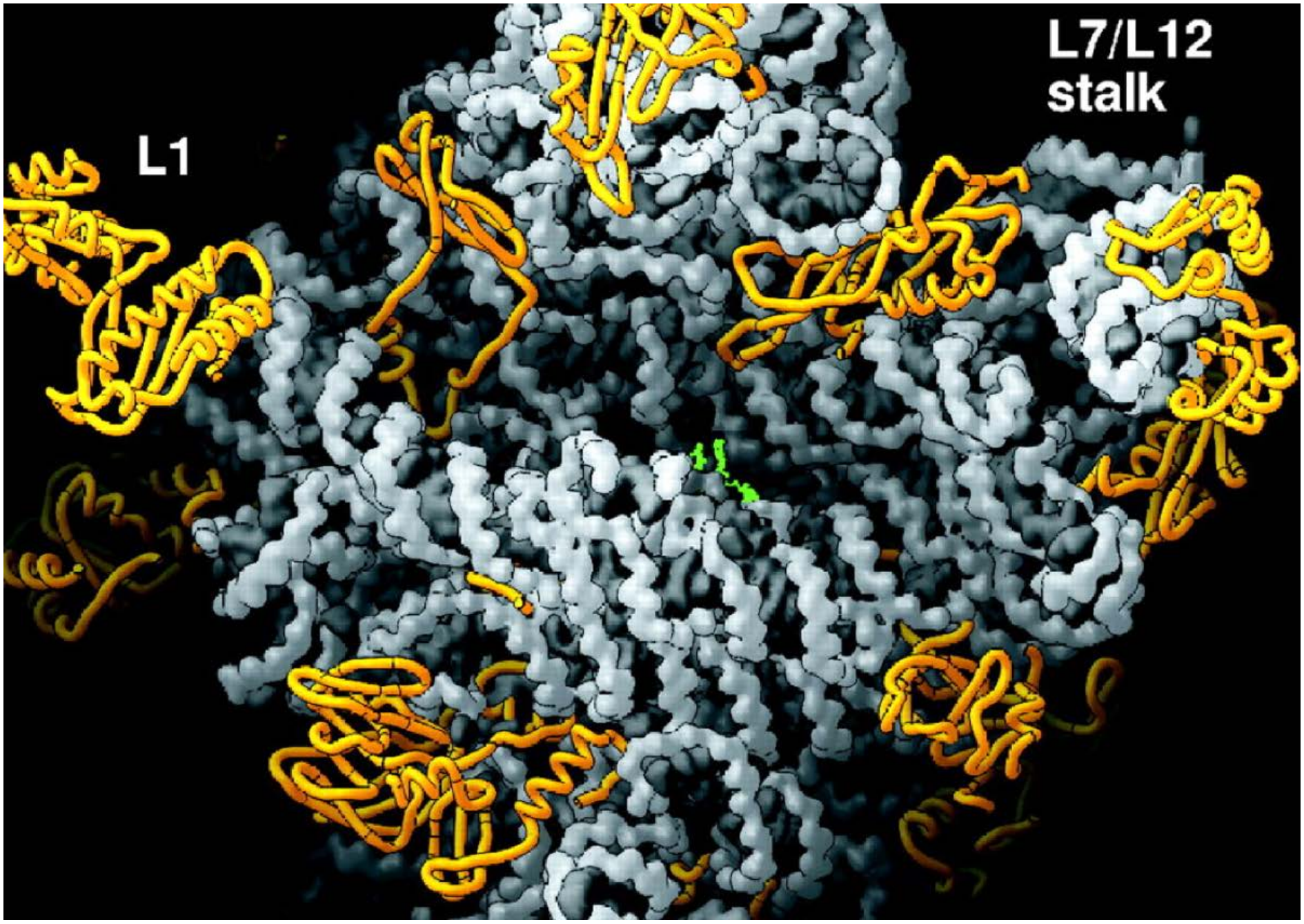
Why are crystal structures so compelling?



Electron density with atomic model of the interaction interface between chromatin remodeler Mit1 and HP1 protein Chp2 [1].

1. Leopold, K et al. (2019) Transcriptional gene silencing requires dedicated interaction between HP1 protein Chp2 and chromatin remodeler Mit1. Genes Dev. 33 565-577

The ribosome is a ribozyme



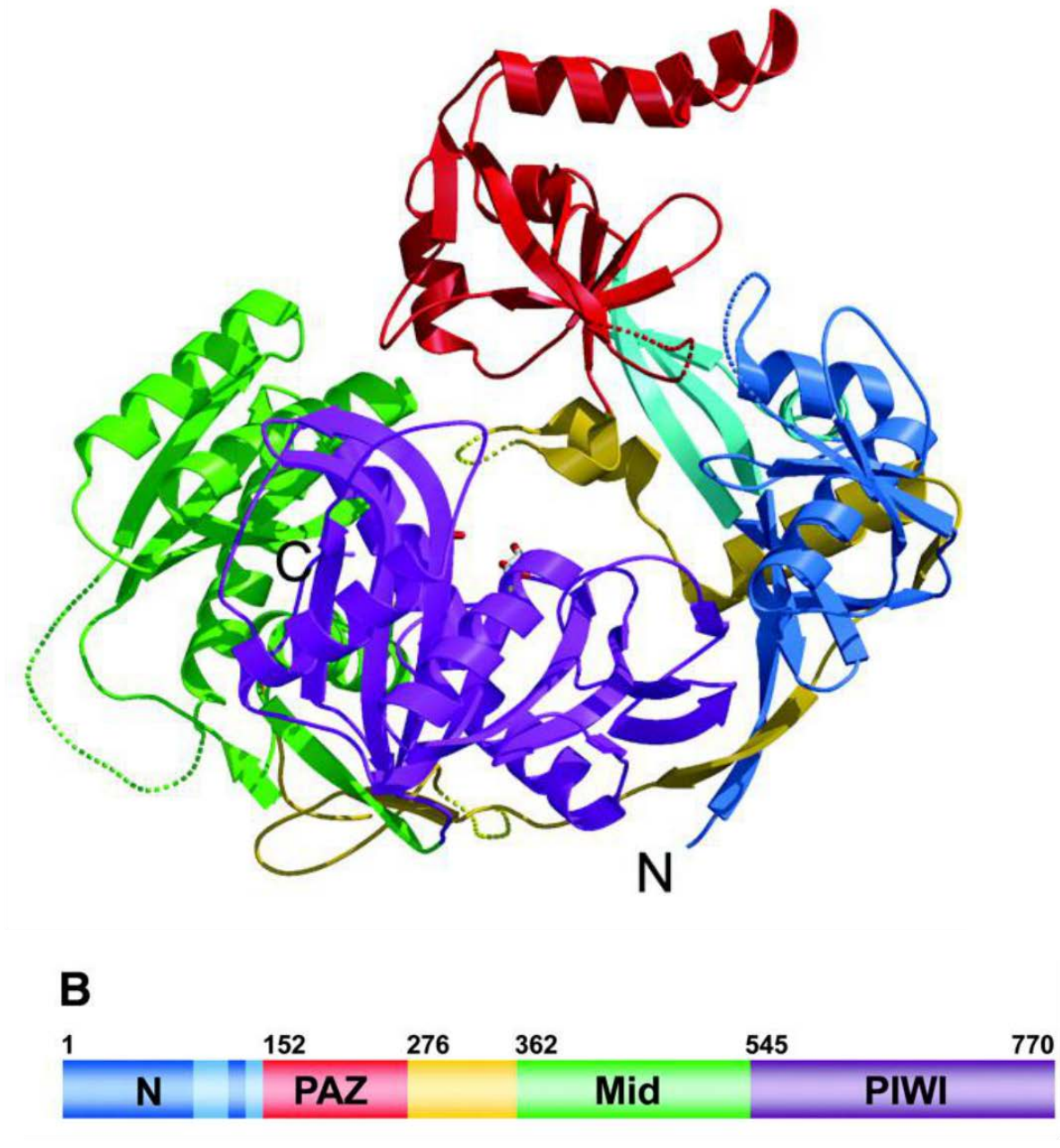
Crystal structure of the large ribosomal subunit revealed that the peptidyl transfer center contains no protein.[1]

1. Ban, N et al. (2000) The complete atomic structure of the large ribosomal subunit at 2.4 A resolution. Science 289 905-20

Translation in the "groove"

- Video from the Ramakrishnan lab: https://www.youtube.com/watch?v=1j_T47G37NE

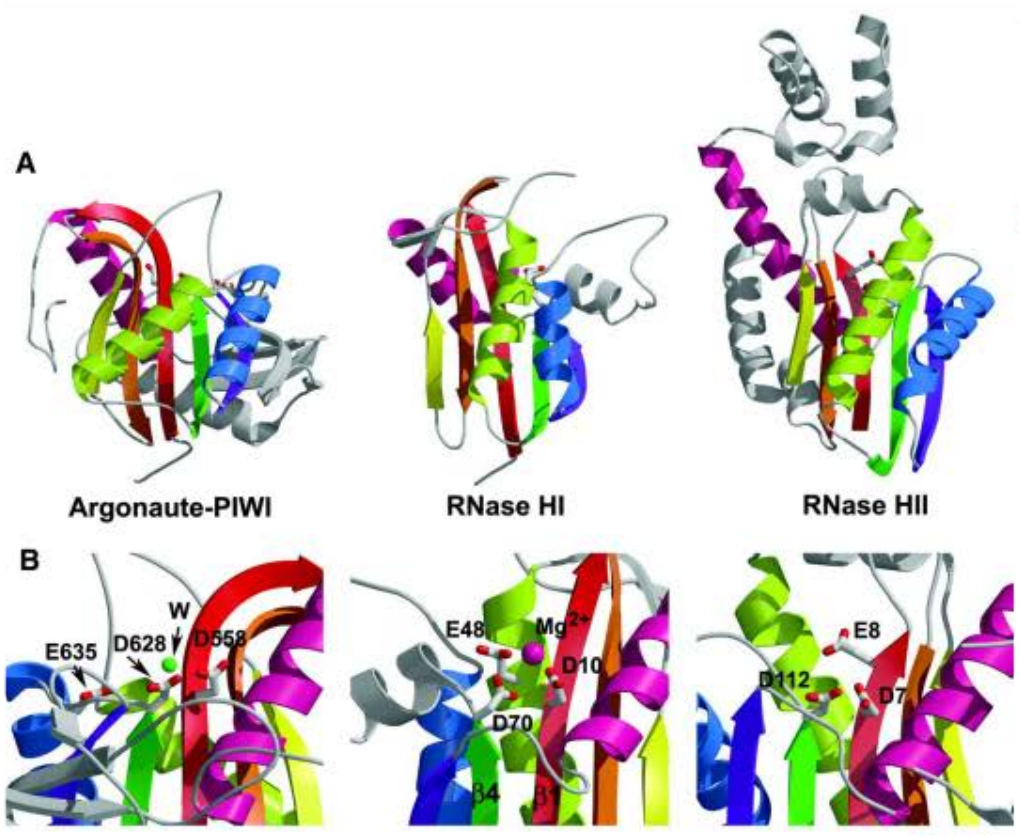
The crystal structure of Argonaute

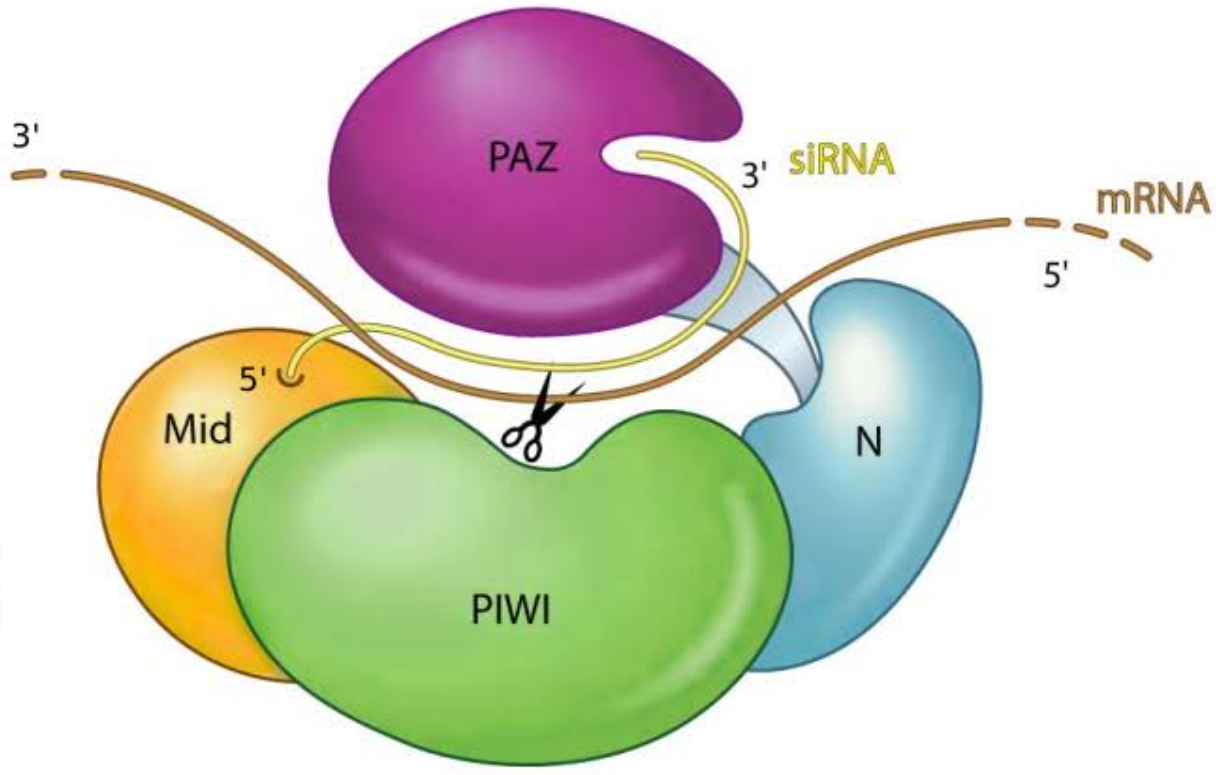


Structure of the argonaute protein from the archea Pyrococcus furiousus [1].

1. Song, JJ et al. (2004) Crystal structure of Argonaute and its implications for RISC slicer activity. Science 305 1434-7

The crystal structure identified argonaute as the 'Slicer'



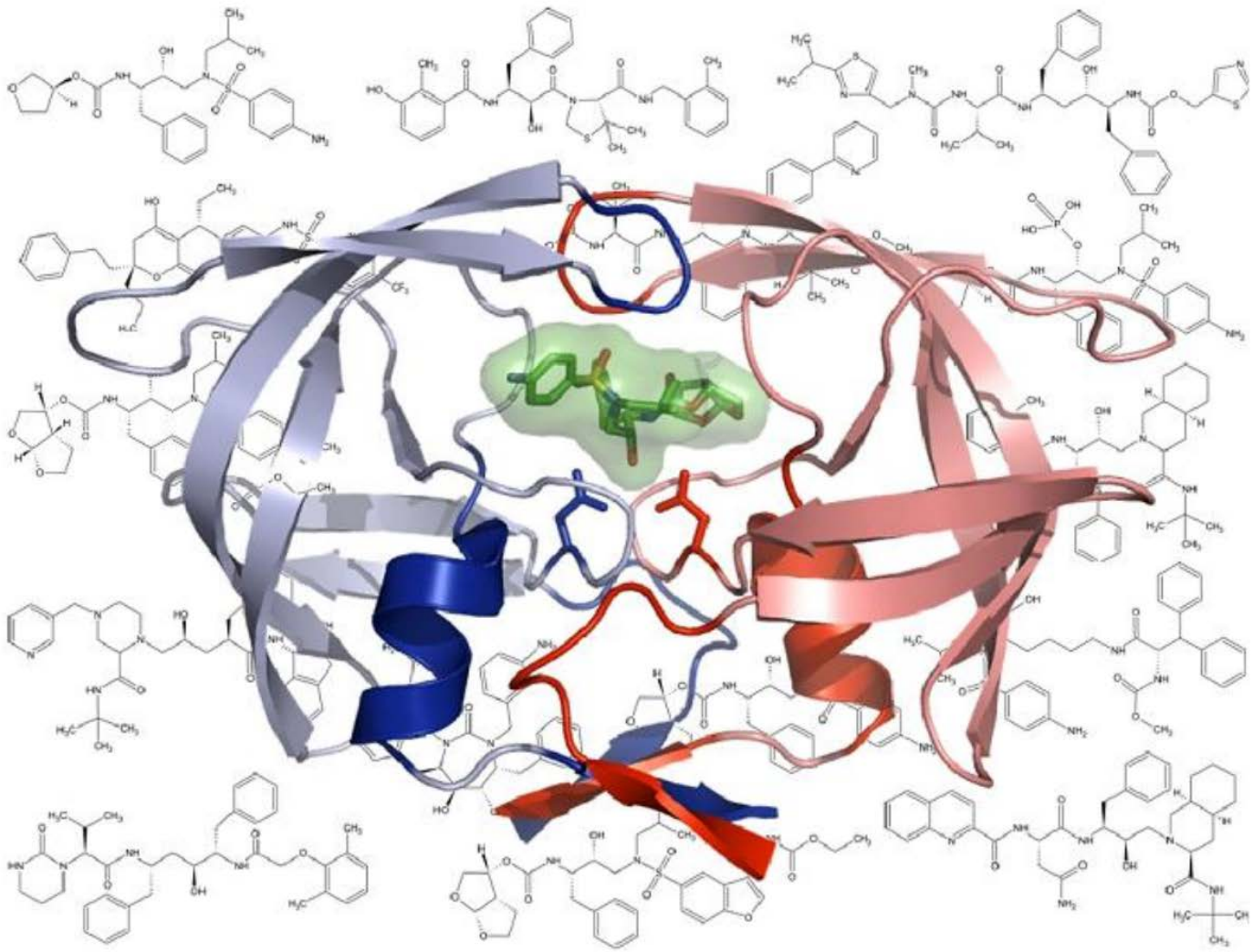


Comparison of the argonaute PIWI domain revealed similarity to RNase HI enzymes [1][2].

- Mechanism and function of proteins can be deduced by comparison of their structures against the existing repository of all solved structures.
- X-ray structures provide valuable hypotheses that can be rigorously tested by mutational analyses.

1. Song, JJ et al. (2004) Crystal structure of Argonaute and its implications for RISC slicer activity. Science 305 1434-7
2. Liu, J et al. (2004) Argonaute2 is the catalytic engine of mammalian RNAi. Science 305 1437-41

Structural biology aids drug design



One of the great success stories of rational drug design: The HIV protease. [1]

- Drugs can be rationally designed and optimized based on protein crystal structures.
- Co-crystal structures of drug molecules or fragments are important guides in drug development [2].

1. Pokorná, J et al. (2009) Current and Novel Inhibitors of HIV Protease. Viruses 1 1209-39
2. The Billion Dollar Molecule, Simon & Schuster, 2013 (ebook), ISBN:9781439126813

1. Crystallography and its application in Biology

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Recombinant protein production

Proteins are often produced in organisms that grow rapidly, cheaply and in large quantities:

- E. coli: simple, cheap and rapid. Great when it works, but in many cases E. coli is not able to fold the proteins or modify in the required manner for activity and structural work.

- Yeasts: Pichia pastoris or S. cerevisiae are often used for expression, in particular secretion of extracellular proteins.
- Insect cells: Spodopter frugiperda (Sf9) cells can be infected by a virus called baculovirus that drives high expression levels of heterologous proteins. This works well for many eukaryotic proteins.
- HEK-293 human cells: These cells can produce relatively large quantities of proteins and are particularly suitable for proteins that need mammalian-specific post-translational modifications.

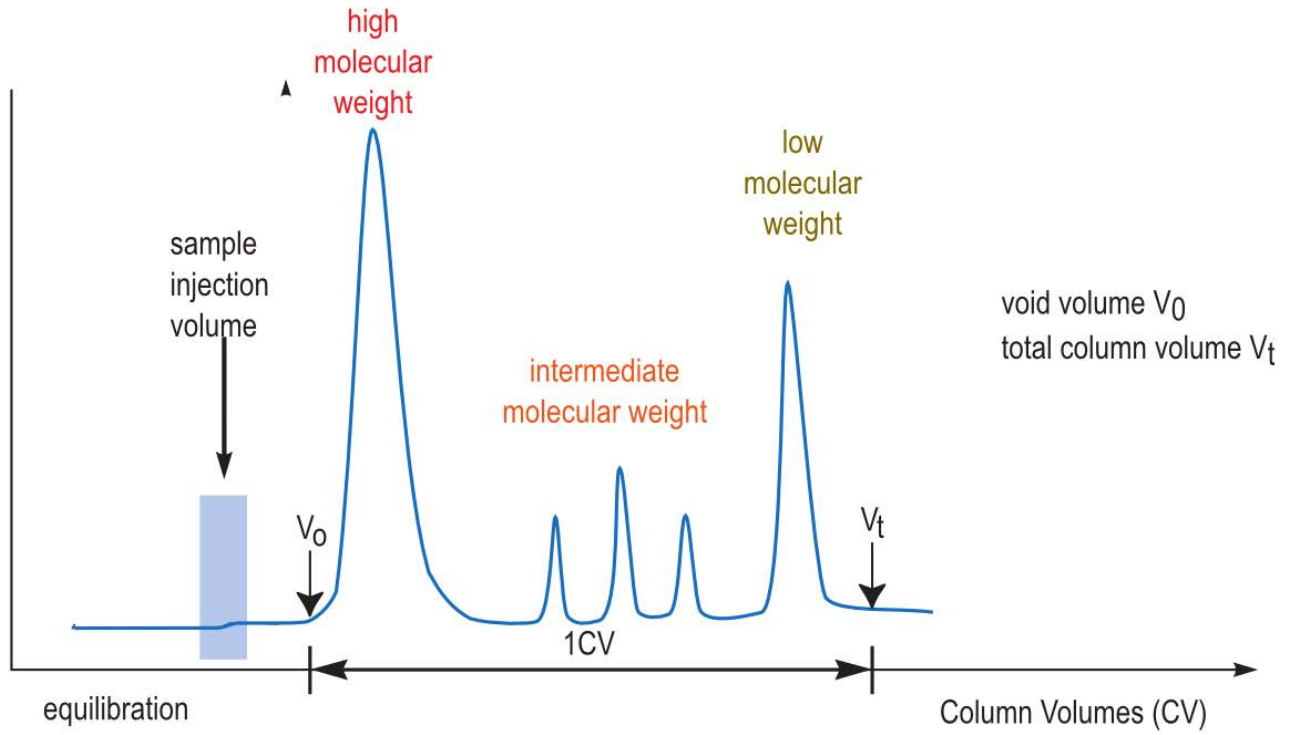
Gel filtration is an indispensable tool for protein quality control

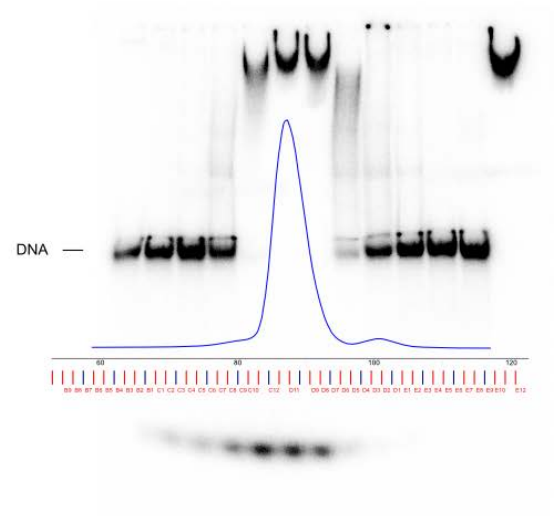
Gel filtration, also known as size exclusion chromatography (SEC) is a very important tool:

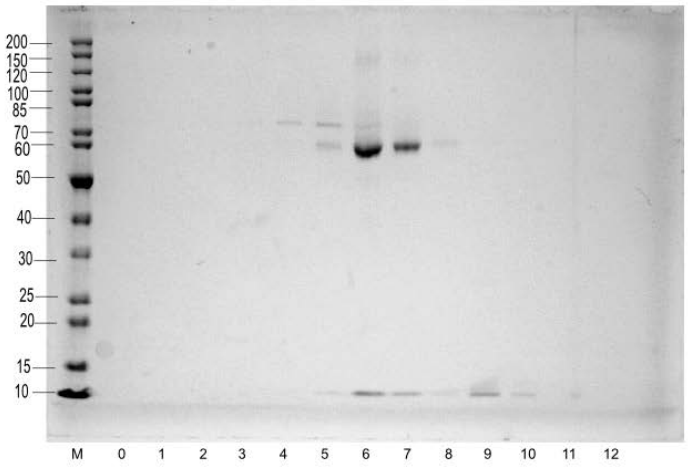
- simple and fast
- the number of peaks provide information on the structural purity (dispersity)
- the position of a peak provides an idea of the molecular weight

Gel filtration can be coupled to the various detection systems:

- **UV absorbance**
- **SDS PAGE**
- mass spectrometry
- multi-angle light scattering



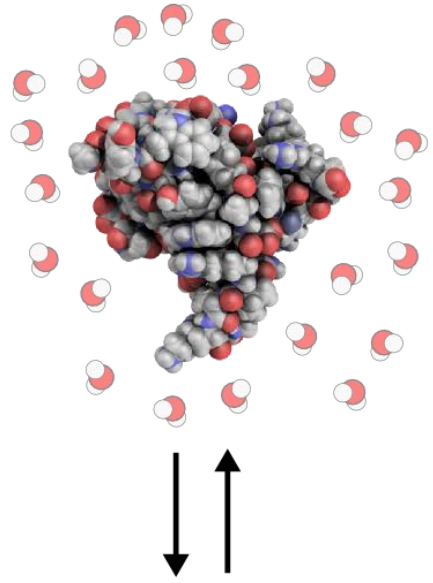


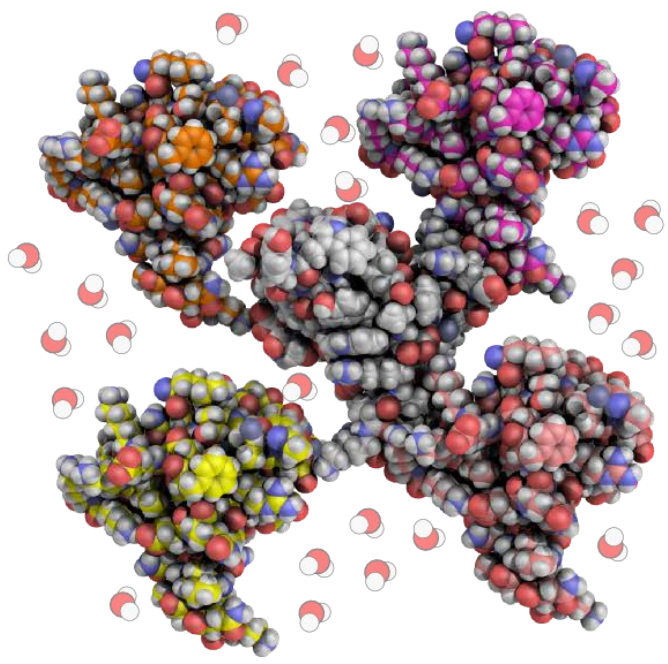


Many highly complex biological macro-molecules crystallize

Crystal formation is driven by small, weak interactions

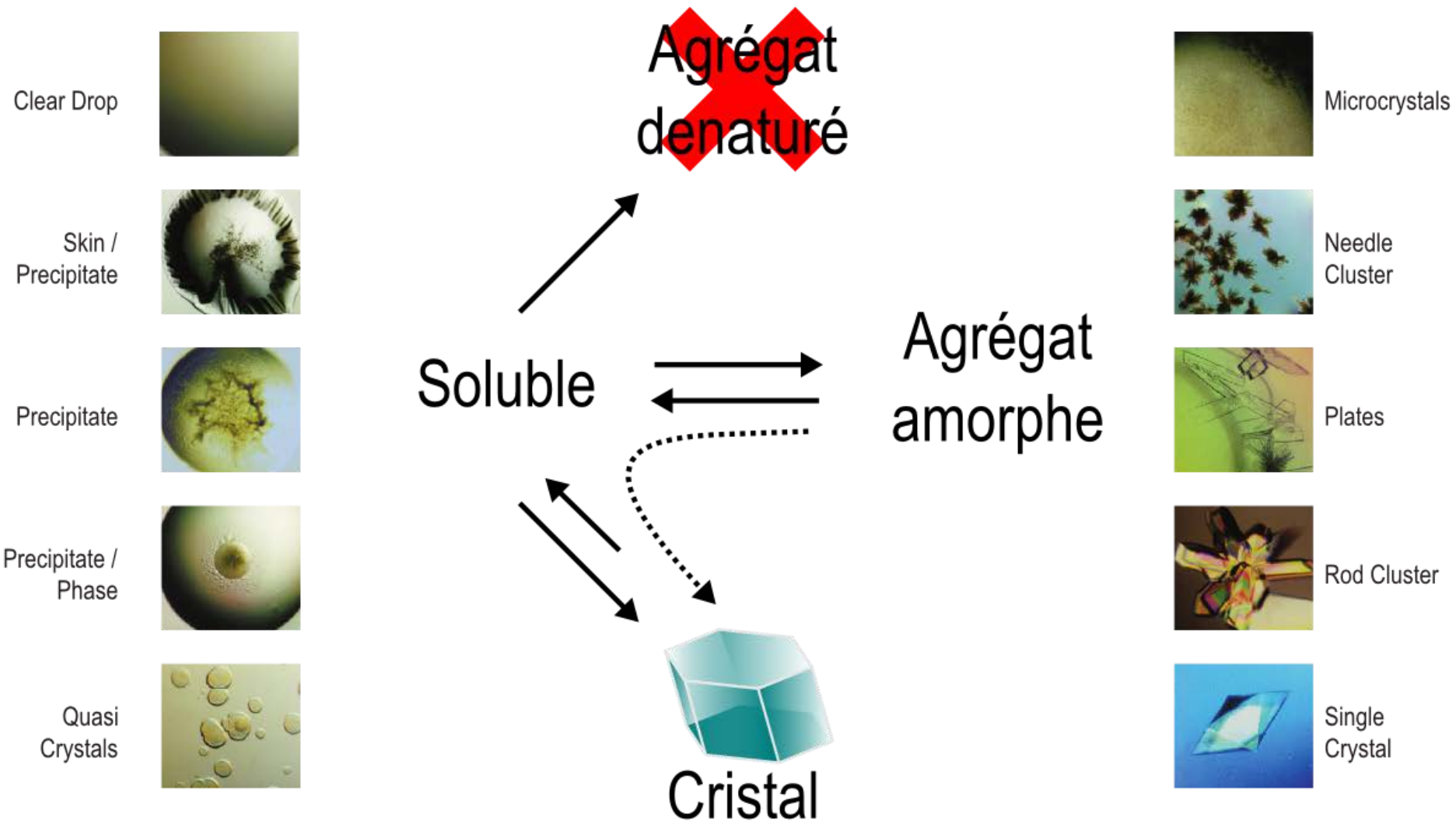
- Biological macromolecules have highly heterogeneous surfaces.
- In solution this surface interacts with water and ions. This creates a shield around the macromolecule and prevents its interaction with other macromolecules.
- Precipitants, for example polymers, salts or small organic molecules weaken the shield by competing for water and ions.
- The most popular precipitants are:
  - Polyethylene glycols (PEG) (conc. ~10-40%)
  - Ammonium sulfate, sodium chloride (conc. > 1M)
  - Alcohols like ethanol, isopropanol or 2-Méthylpentane-2,4-diol (MPD) (conc. ~10-40%)
- The art of crystallography is to find a condition where the macromolecules begin to interact weakly and in a well defined manner.
- The enemy of crystallization is aggregation which easily occurs if the condition denatures the protein or if the molecules interact in an ill-defined manner.





Chp1 chromodomain (PDB ID 3G7L)

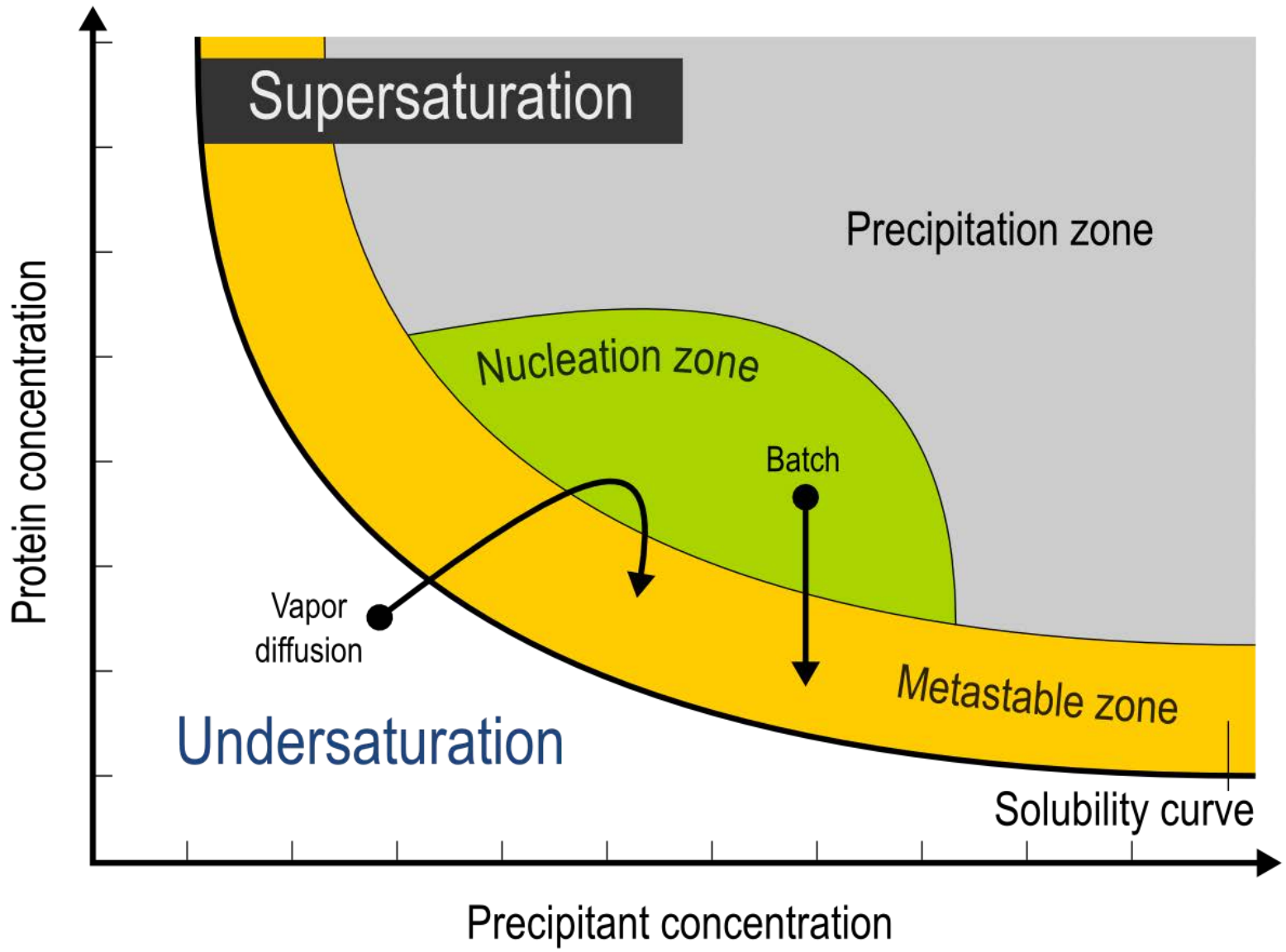
Crystallization has a chance in conditions of reversible aggregation



- A good crystallization condition has to maintain the molecule in a native state.
- Reversible aggregates that are ill defined can serve as a reservoir for crystallization
- Crystallization trials are evaluated based on the appearance of the crystallization drop.

Phase diagrams conceptualize the process of crystallization

- The phase diagram describes the solubility of a protein as a function of its own concentration versus the concentration of a preciptant. The solubility curve describes the border between under- and over-saturation.
- The first and often limiting step is the formation of a crystallization nucleus that consists of a few molecules that organize themselves in a well defined lattice. The nucleation zone is above the metastable zone and often extends into the zone of precipitation.

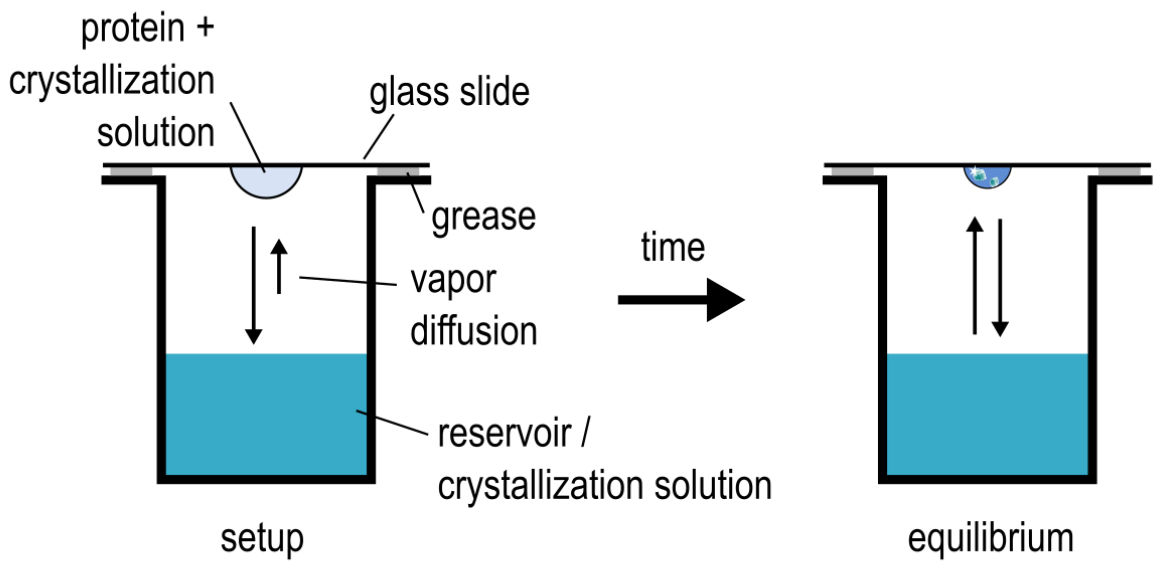


- A crystallization nucleus serves as a matrix for the integration of new molecules and drives the growth of the crystal, which in turn lowers the concentration of soluble molecules.

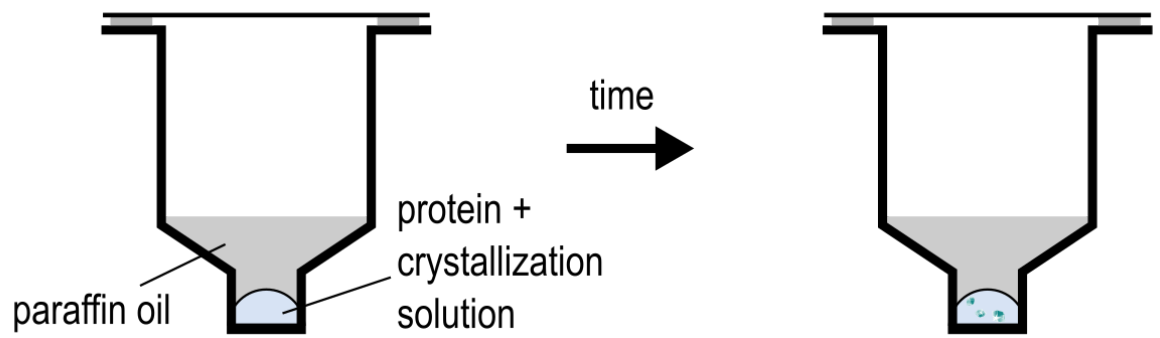
Vapor diffusion is the most popular method for crystallization but not the only one

- The method of vapor diffusion is based on the equilibration of the difference between a protein drop and a reservoir. In the ideal case the initial drop is clear and as the drop changes its size to match the reservoir concentration of precipitant it will form crystals.
- Batch techniques mix protein and crystallization solution without further changes. This method works if the initial condition falls in the zone of nucleation, which is quite often the case.

Vapor diffusion

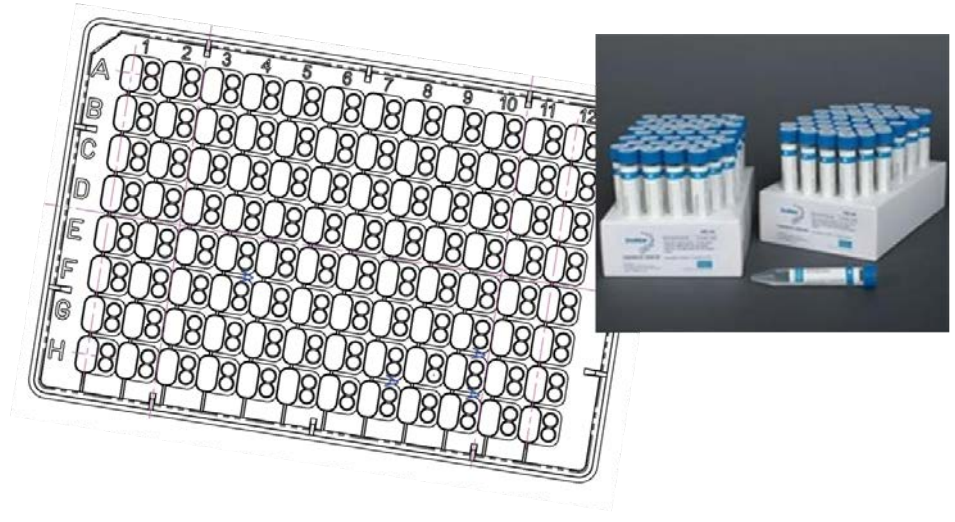


Batch / Microbatch



Crystal screening is now highly automated and miniaturized

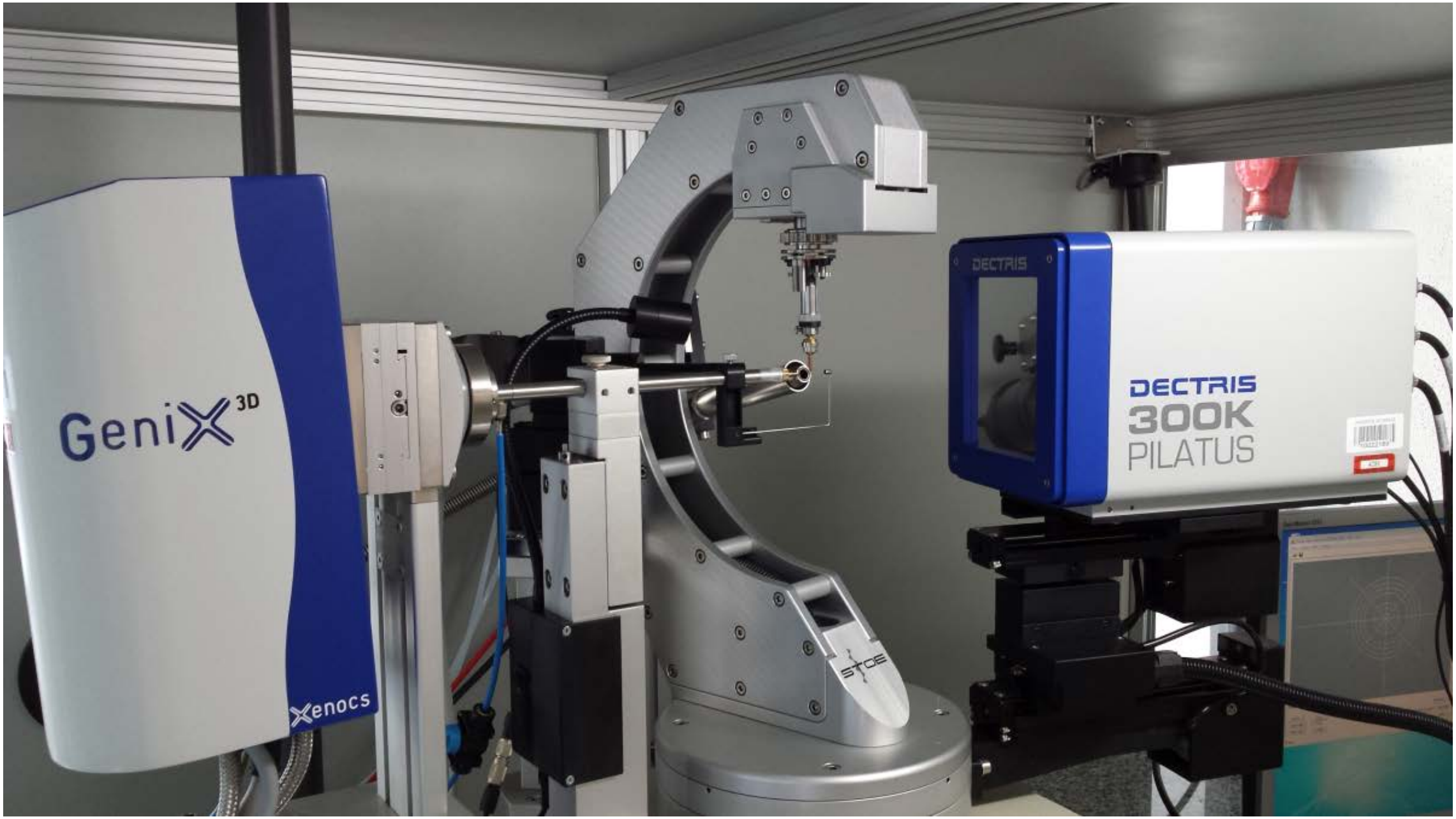
http://youtu.be/wZjLmzI4Btc



- Commercial companies sell screens in high throughput format (96-wells) that contain chemical mixtures that are known to produce protein crystals.
- Specialized drop-setting robots dispense and mix crystallization solution and protein in tiny droplets of 100 nl or less.
- Typically a thousand or more conditions are screened for the identification of a hit.
- The initial hits are refined in customized screens where precipitant, pH, salts and additives are systematically tested.

Freezing crystals protects them from X-ray damage

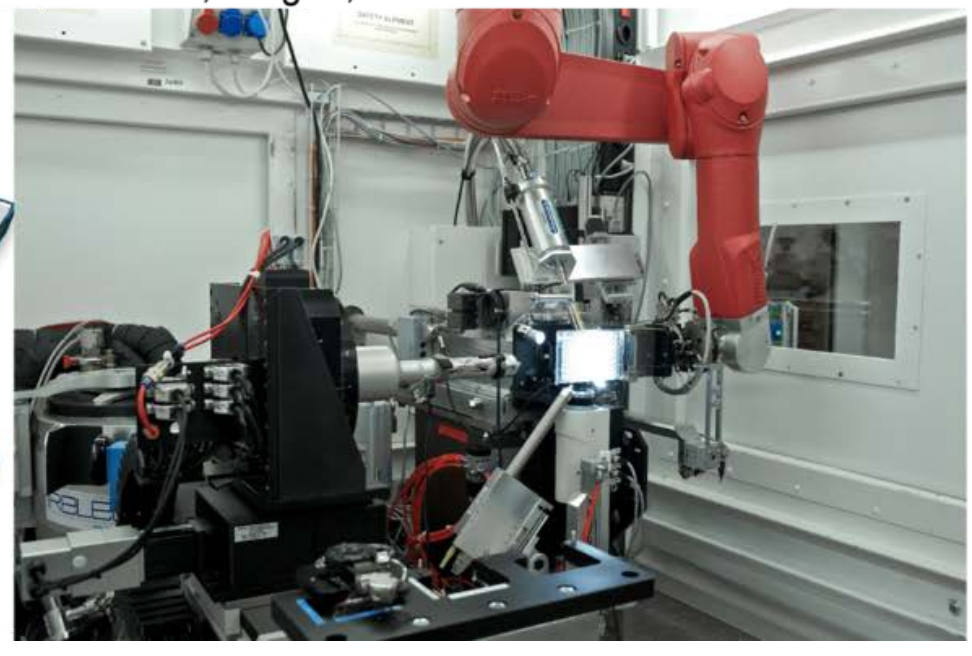
The diffractometer is the camera that records diffraction patterns



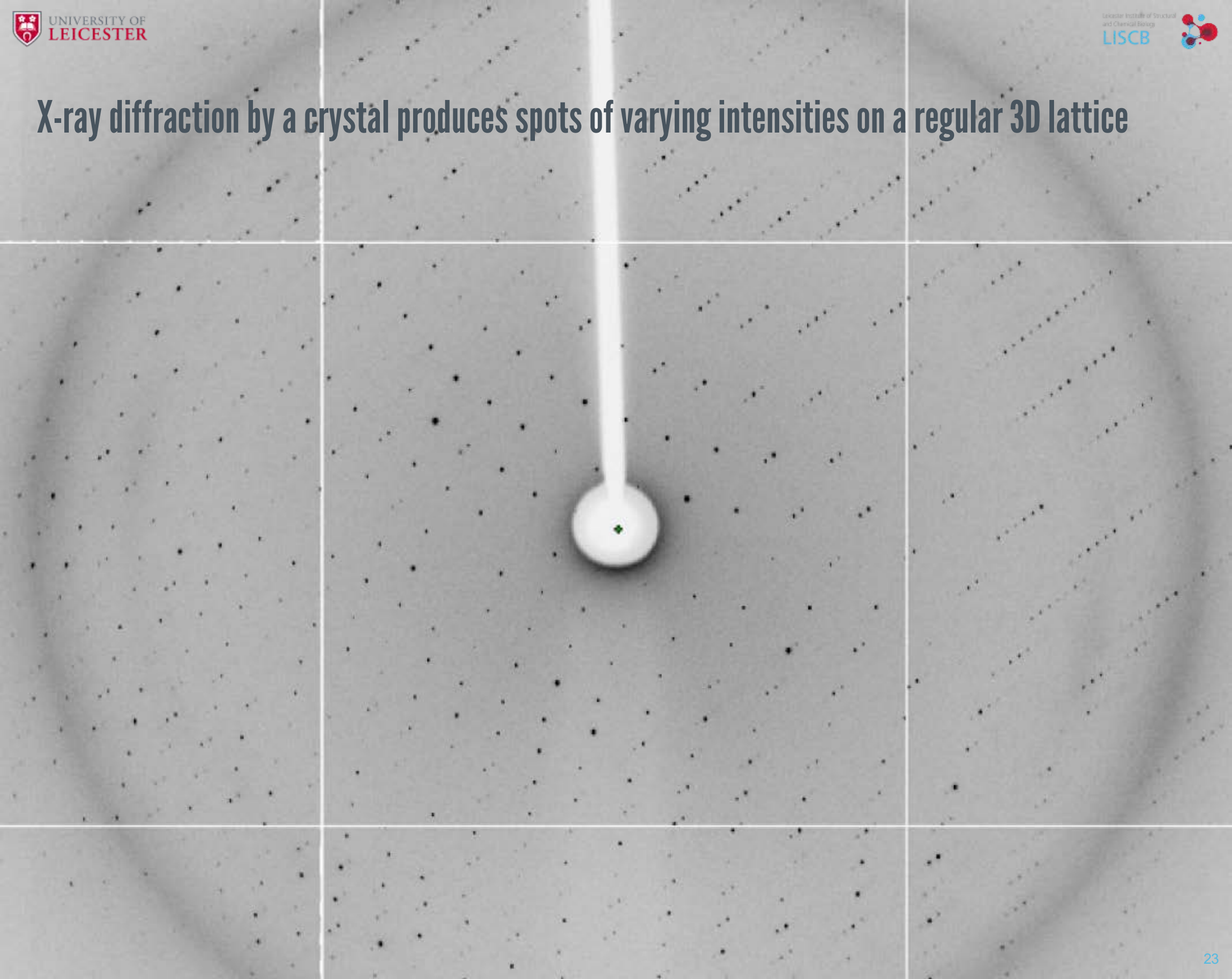
Synchrotrons are high-end radiation sources

ESRF Grenoble, France

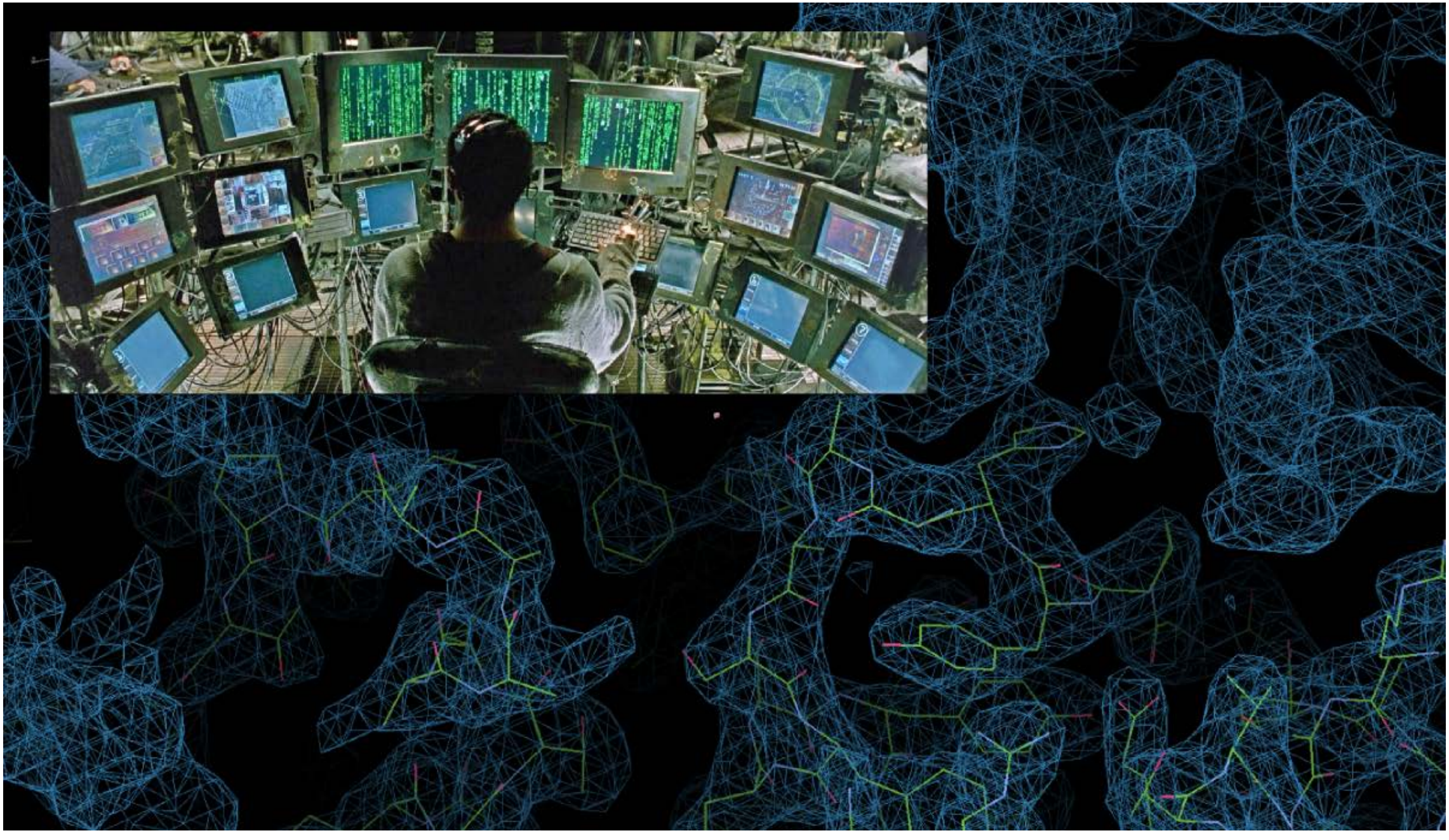
SLS, Villigen, Suisse



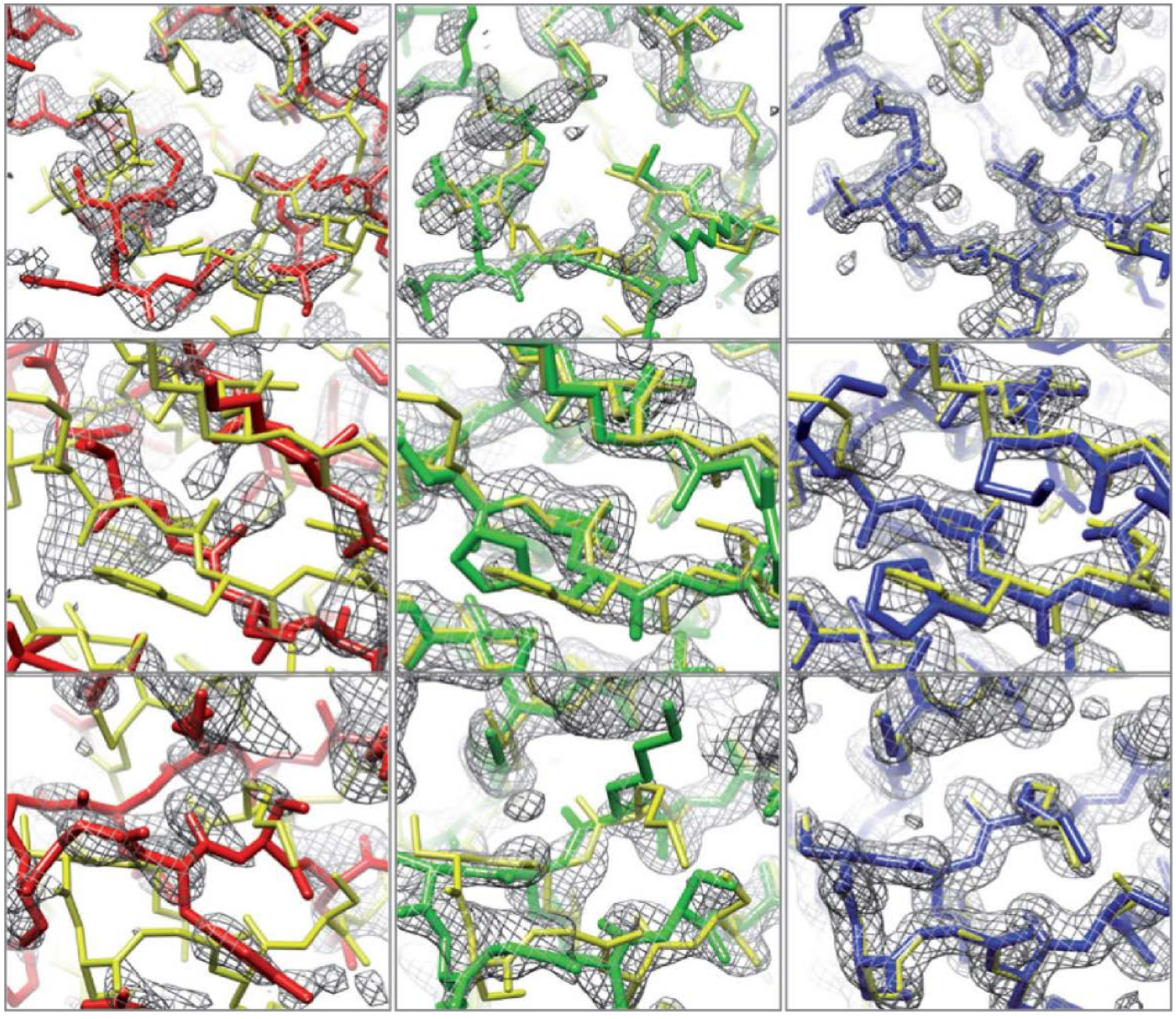
X-ray diffraction by a crystal produces spots of varying intensities on a regular 3D lattice



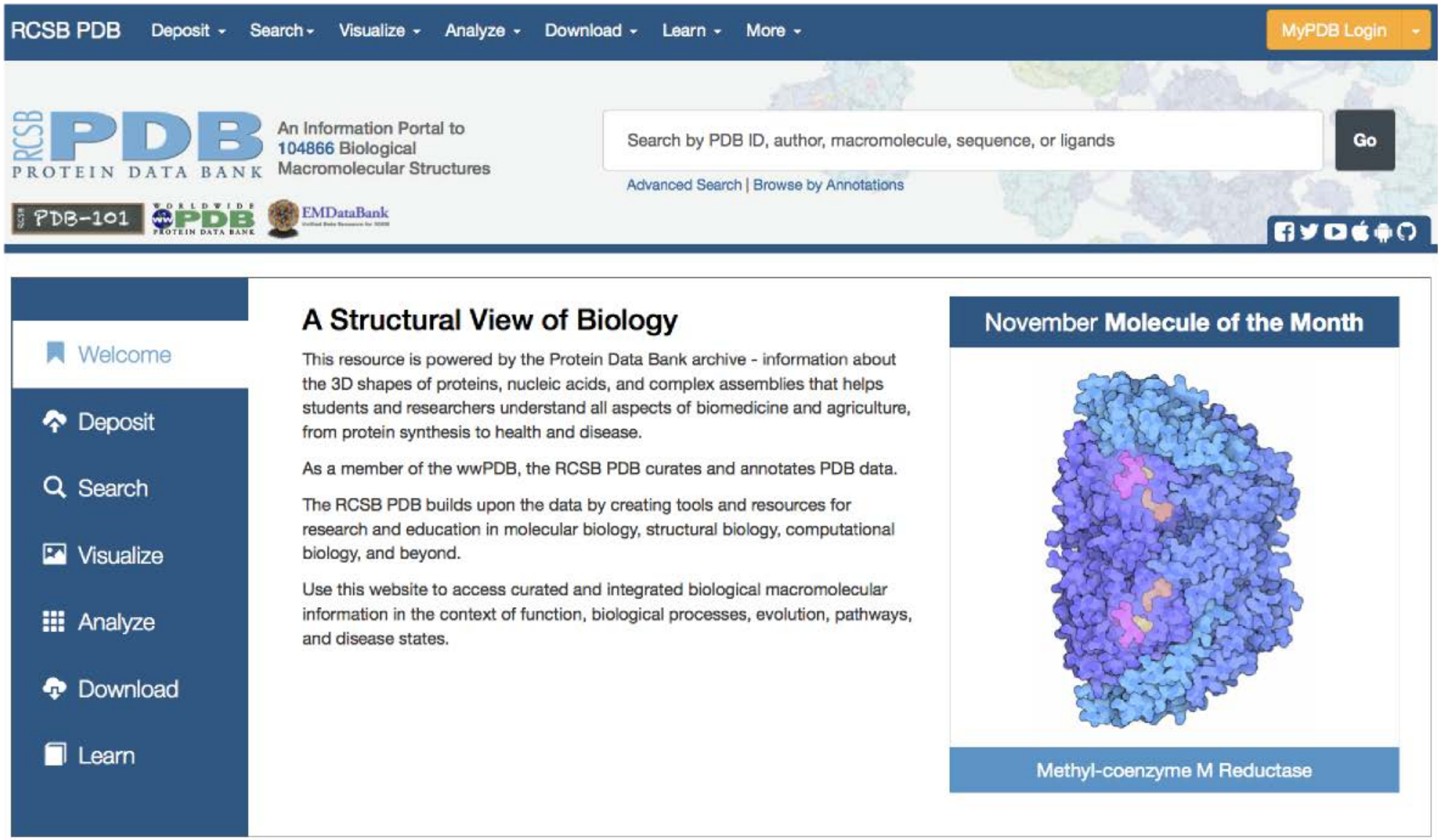
The computational analysis of the diffraction pattern produces the electron density of the molecule in the crystal



The atomic model is an interpretation of the electron density

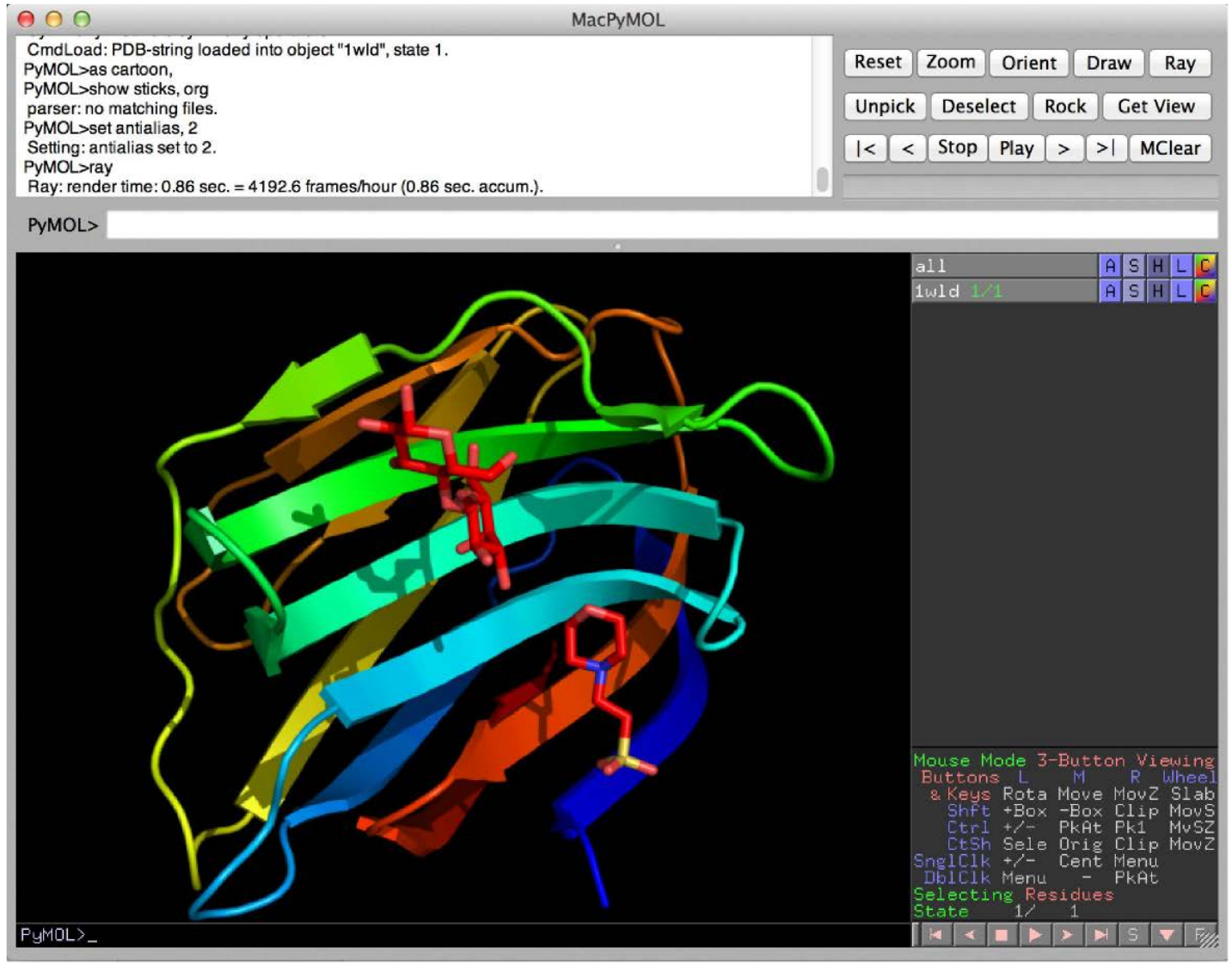


Data and models are deposited and freely accessible in the Protein Data Bank (PDB)



Everybody can analyze biological structures using specialized software

- The software "pymol" is our favorite tool for structure visualization.
- **Try it yourself**:



http://www.pymolwiki.org/index.php/Practical_Pymol_for_Beginners

- A very powerful alternative is Chimera X: https://www.cgl.ucsf.edu/chimerax/