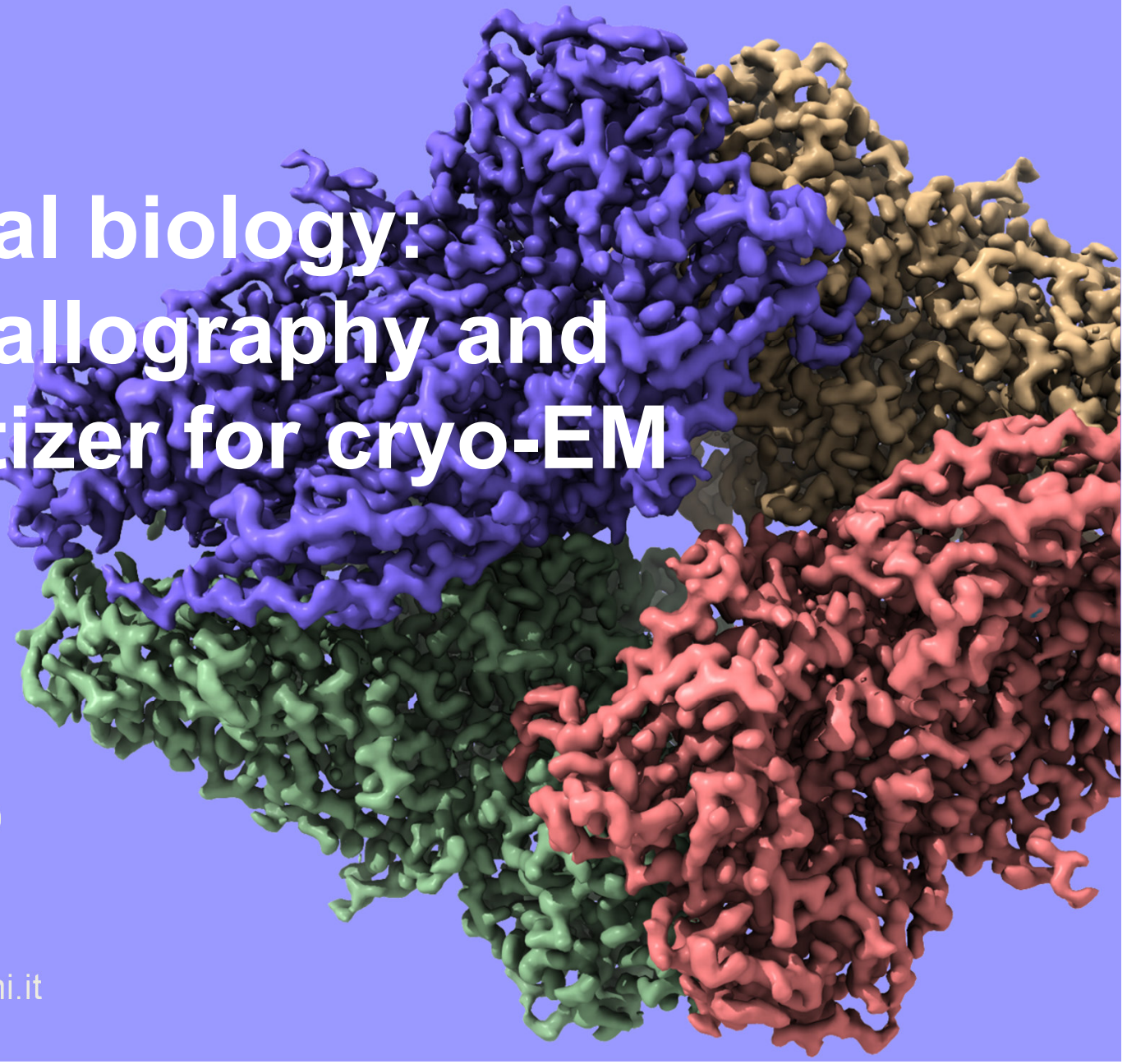
"Gilberto Vlaic" XVII School on Synchrotron Radiation:
Fundamentals, Methods and Applications
16-26 Sept. 2024, Muggia (Italy)

Structural biology: biocrystallography and an appetizer for cryo-EM



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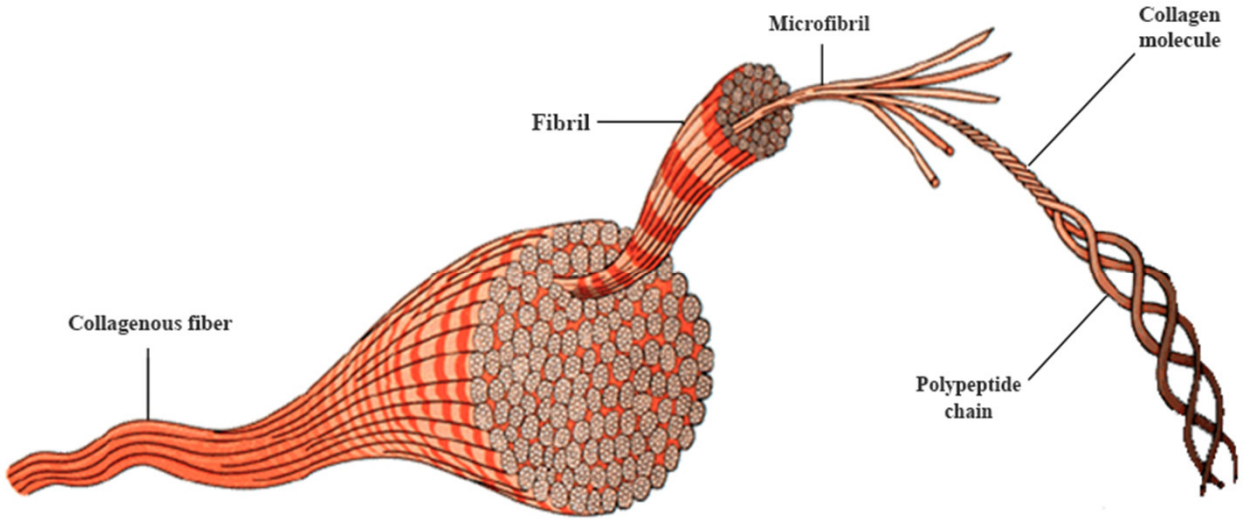
Dept. of Biosciences
University of Milan
marco.nardini@unimi.it

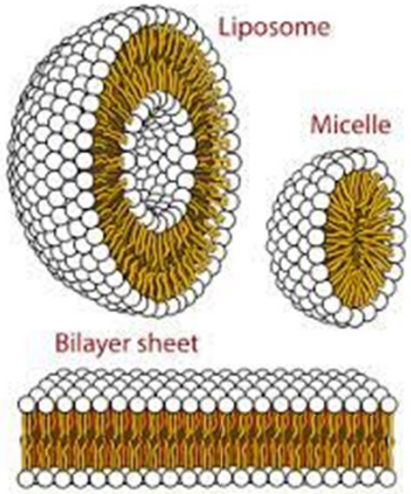
Structural biology

3D Structure ⟷ Function

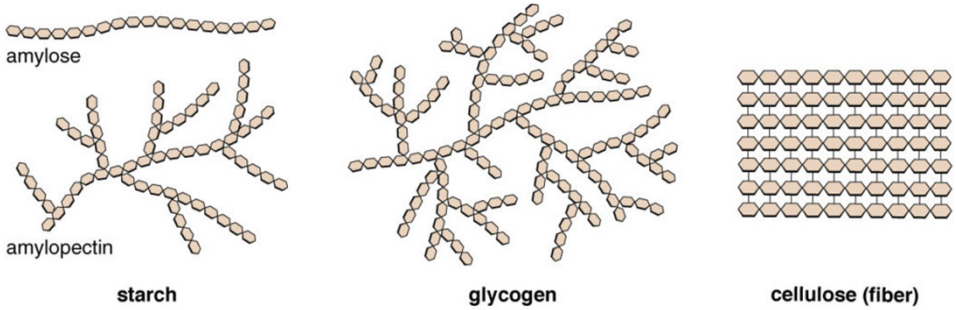


Structural biology targets

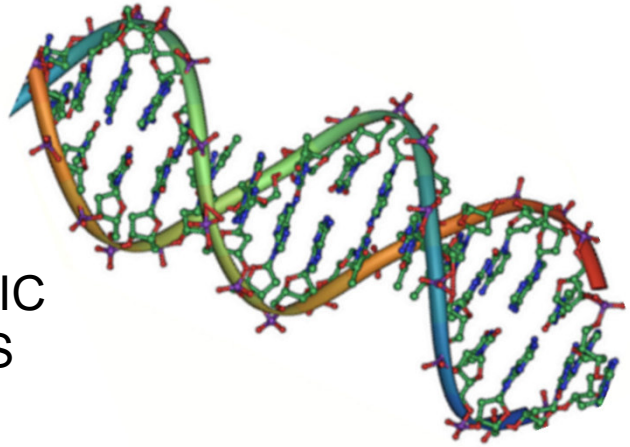
LIPIDS



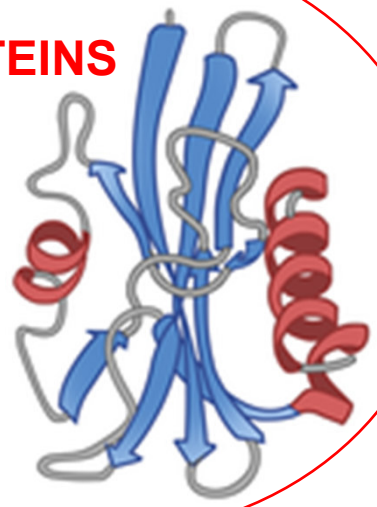
CARBOHYDRATES



NUCLEIC ACIDS

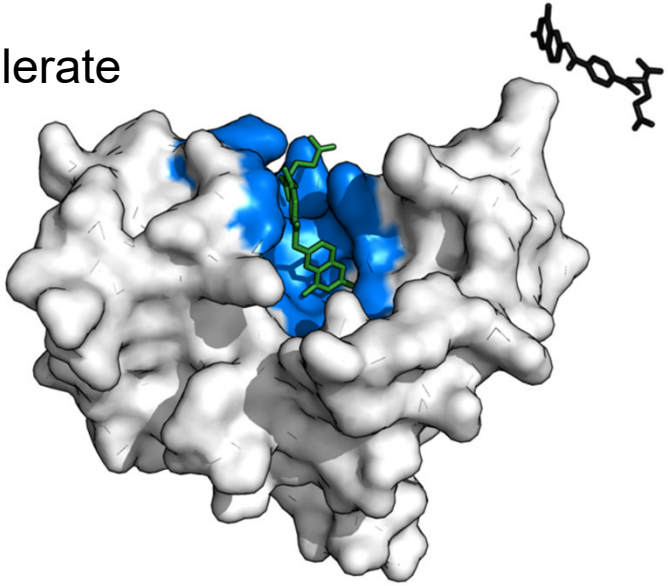


PROTEINS



Examples of protein targets

- **Catalytic reactions:** **enzymes** that catalyze/accelerate biochemical reactions, and are vital for metabolism



- Transcription factors: **protein/DNA** interaction
- Cell signaling: **receptors**
- Immune responses: **antibodies**
- Molecular transport: carriers for small molecules and/or ions (**hemoglobin**)
- **Membrane channelling:** **membrane proteins** control the flow of small molecule (i.e. ions) through cell membranes
- **Viruses**

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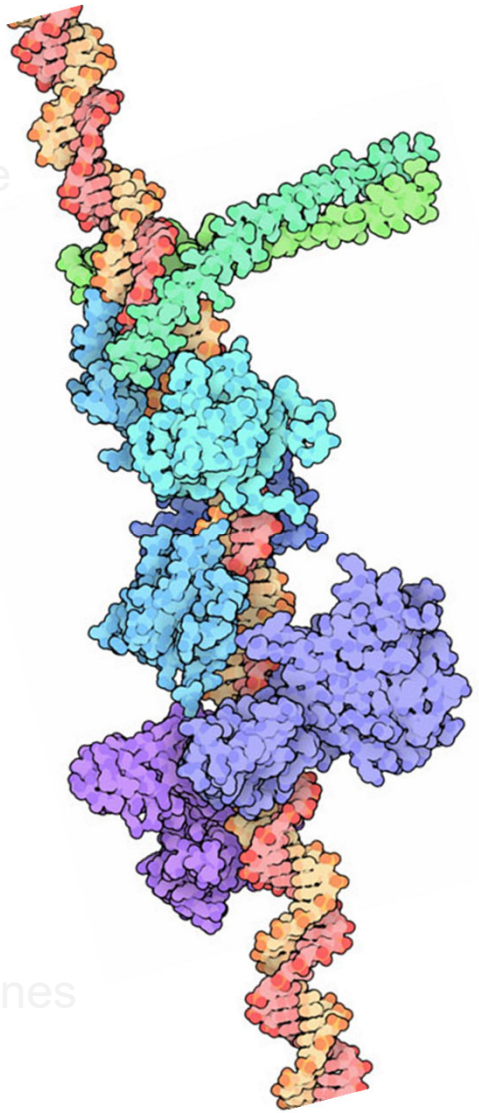

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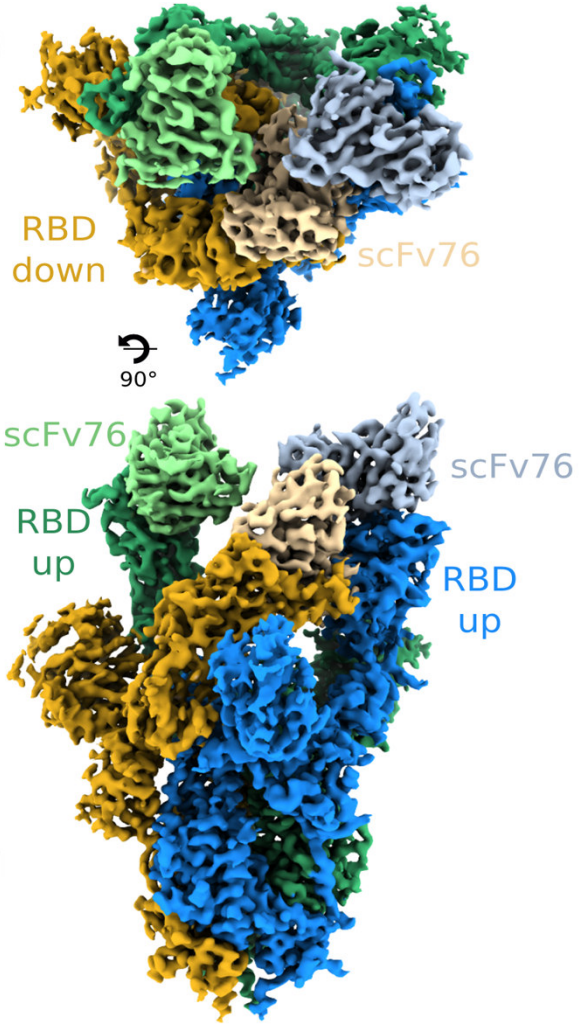

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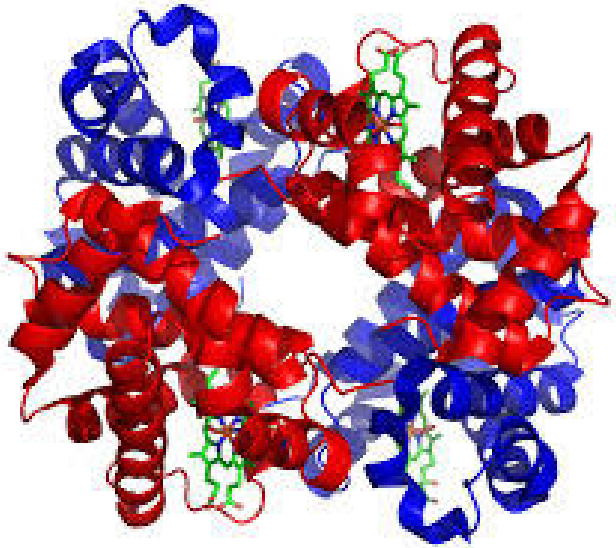

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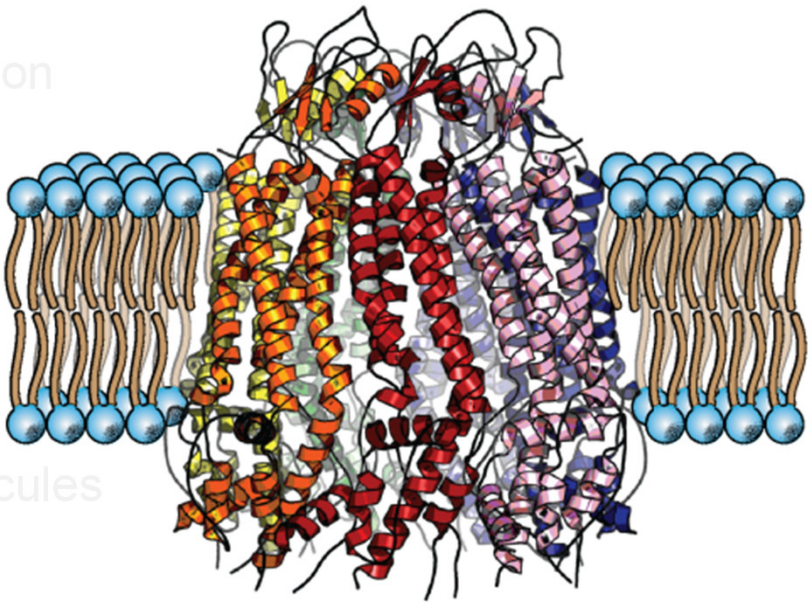

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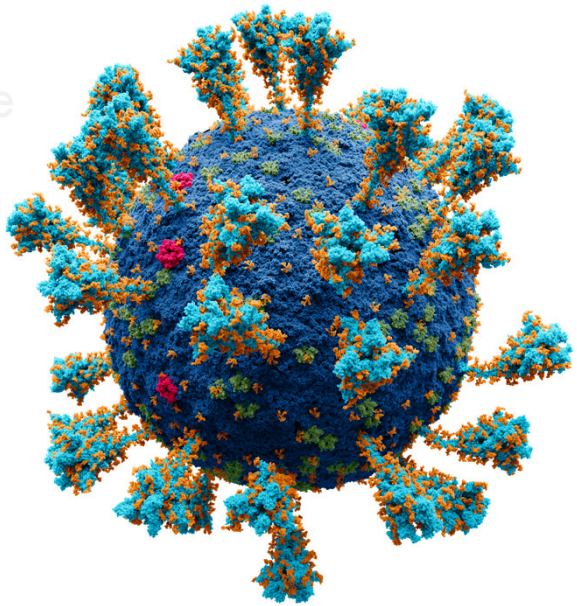
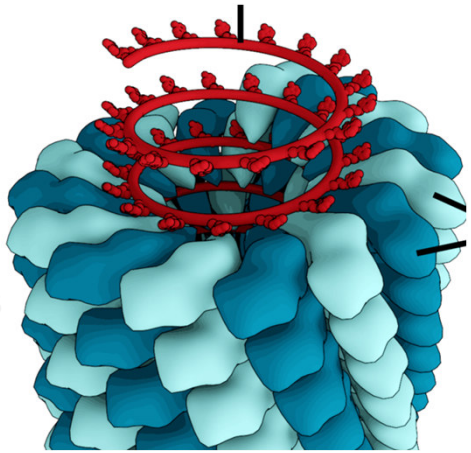
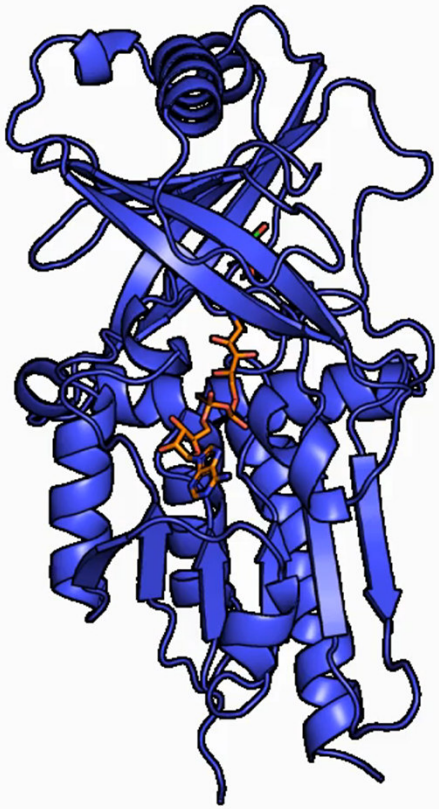




Applications of structural biology

3D structures of macromolecules allow us to understand **biological processes and interactions** at atomic resolution (i.e. how a particular macromolecule accomplishes its various functions)

- macromolecule to macromolecule interactions
- macromolecule to small molecules (substrates, cofactors, inhibitors, ions …) interactions
- structural-functional studies on enzymes
- **rational drug design** (how drug lead compounds interact with their protein targets)
- **biotech applications**



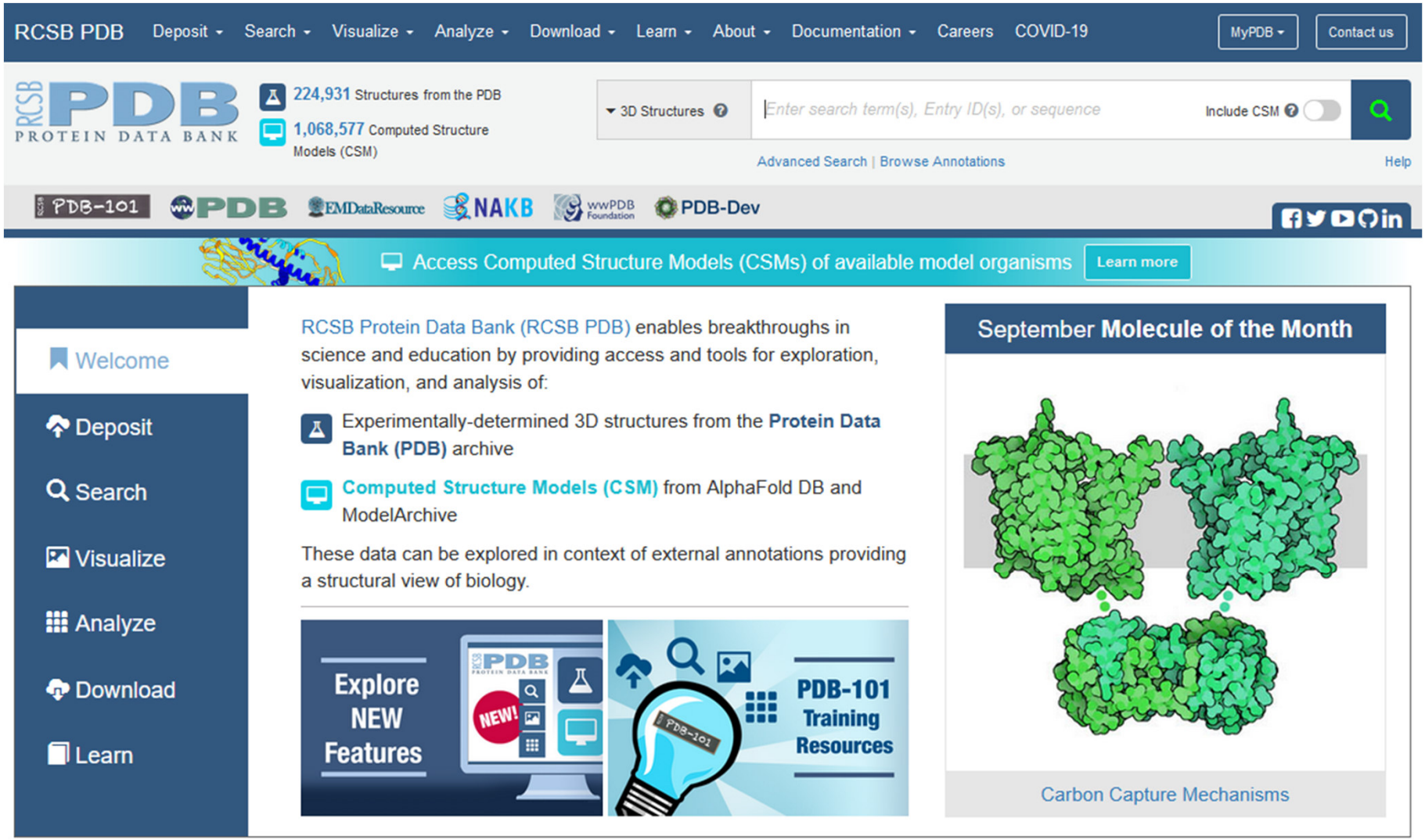
Structural biology techniques

- **Biocrystallography (or macromolecula crystallography, MX)**
- **Single particle cryo-electron microscopy (or cryo-EM)**
- **Nucleic magnetic resonance (or NMR)**
- **Small angle X-ray scattering (or SAXS)**

"INTEGRATED" Structural biology

	PROS	CONS	RESOLUTION
MX	• Any molecular weigth • Precise atomistic details	• High amount of highly pure protein • Sample in a crystalline state • Statistic structure	High (1-2 Å)
Cryo-EM	• Structure in solution • Small amount of highly pure protein • Acceptable heterogeneity level	• Target above 150 kDa • Expensive	Medium/high (2-5 Å)
NMR	• Structure in solution • Dynamic structure	• High amount of highly pure (marked) protein • Target below 35 kDa • Expensive • Very slow data processing	High (1-2 Å)
SAXS	• Structure in solution • Dynamic structure	• Highly pure and homogeneous protein • No atomistic details	Low (20 Å)

Protein Data Bank (https://www.rcsb.org/)



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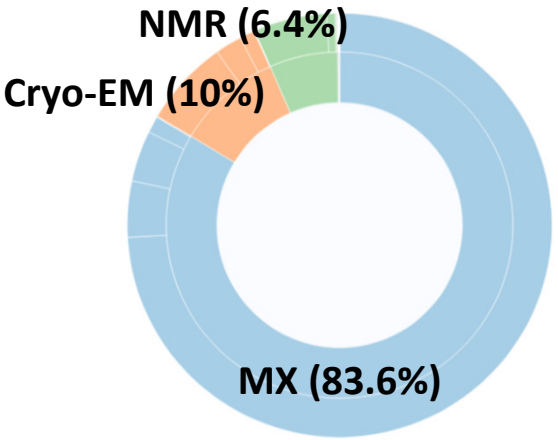
All Statistics

PDB Data Distribution by Experimental Method and Molecular Type

Copy | CSV

Molecular Type	X-ray	EM	NMR	Multiple methods	Neutron	Other	Total
Protein (only)	166,454	15,112	12,490	208	77	32	194,373
Protein/Oligosaccharide	9,618	2,576	34	8	2	0	12,238
Protein/NA	8,675	4,593	286	7	0	0	13,561
Nucleic acid (only)	2,864	137	1,505	14	3	1	4,524
Other	170	10	33	0	0	0	213
Oligosaccharide (only)	11	0	6	1	0	4	22
Total	187,792	22,428	14,354	238	82	37	224,931



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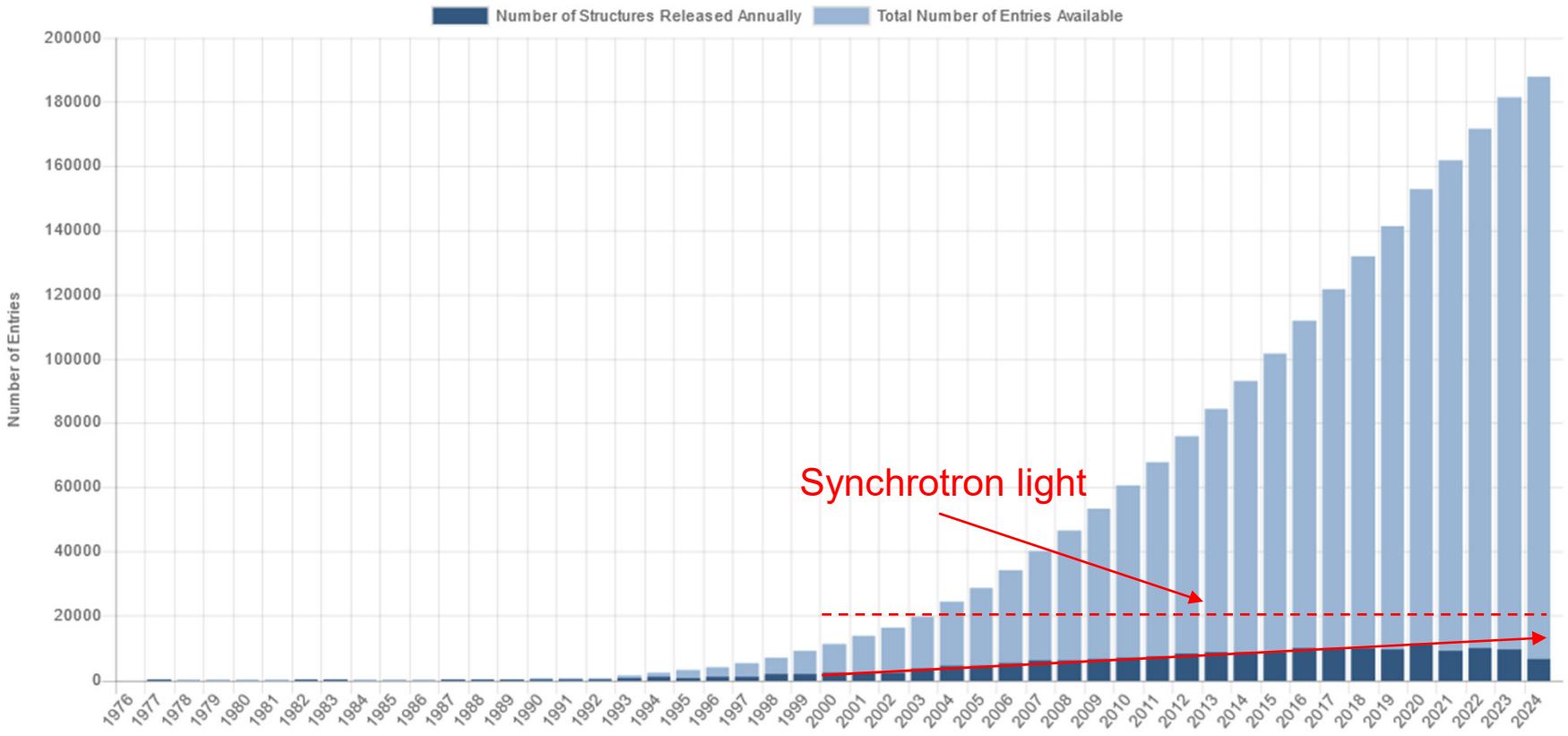
All Statistics

PDB Statistics: Growth of Structures from X-ray Crystallography Experiments Released per Year

Experimental methods such as X-ray crystallography, NMR spectroscopy, and 3D electron microscopy are used to determine the location of each atom relative to each other in the molecule.

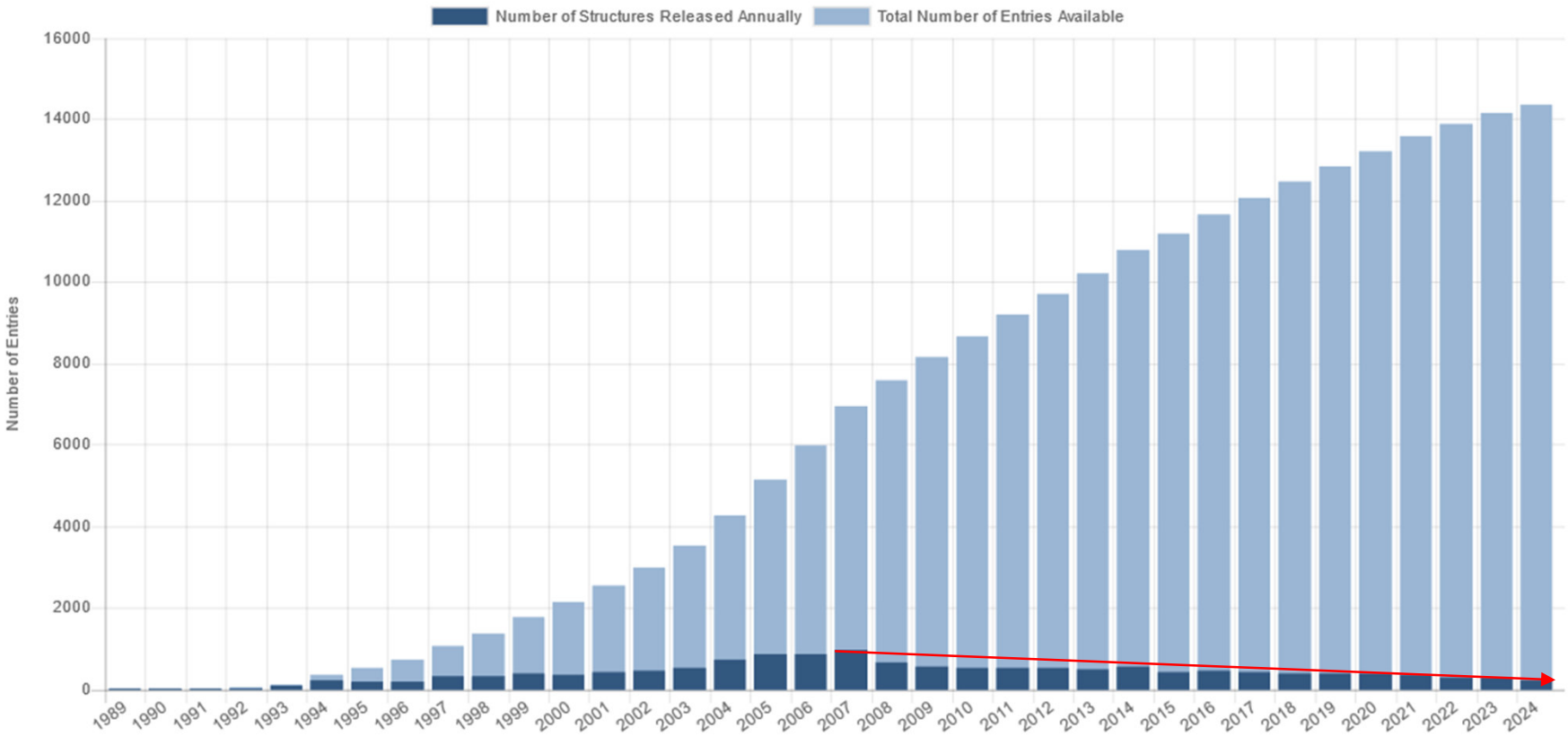


Protein Data Bank (https://www.rcsb.org/)

All Statistics

PDB Statistics: Growth of Structures from NMR Experiments Released per Year

Experimental methods such as X-ray crystallography, NMR spectroscopy, and 3D electron microscopy are used to determine the location of each atom relative to each other in the molecule.



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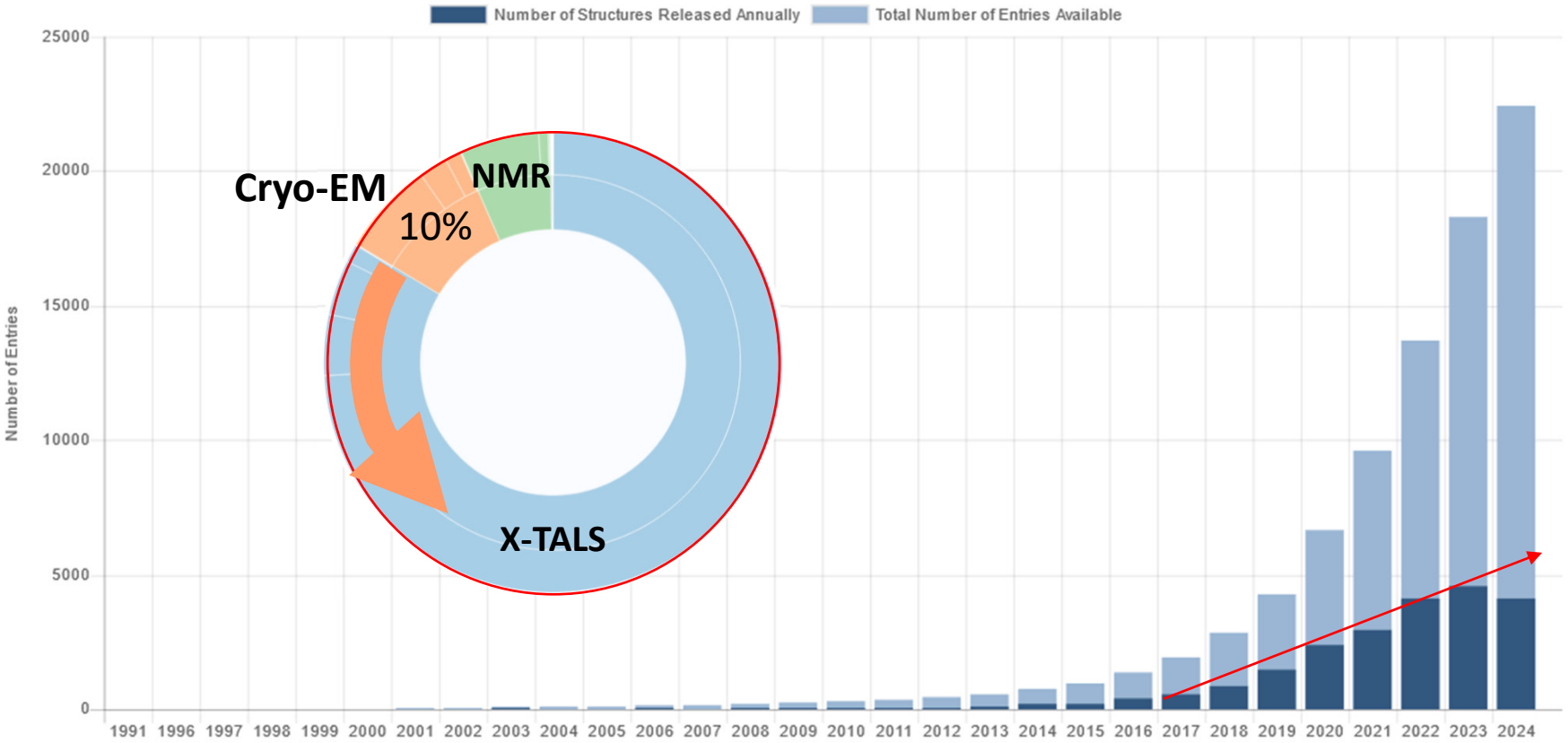
All Statistics

PDB Statistics: Growth of Structures from 3DEM Experiments Released per Year

Experimental methods such as X-ray crystallography, NMR spectroscopy, and 3D electron microscopy are used to determine the location of each atom relative to each other in the molecule.



Biocrystallography (MX)

X-ray crystallography is the science of determining the arrangement of atoms within a **crystal** from the manner in which X-rays are scattered by the crystal.

Aim:

3D structure determination of biological macromolecules at **atomic resolution** (x, y, z positions for each atom of the macromolecule), but ...

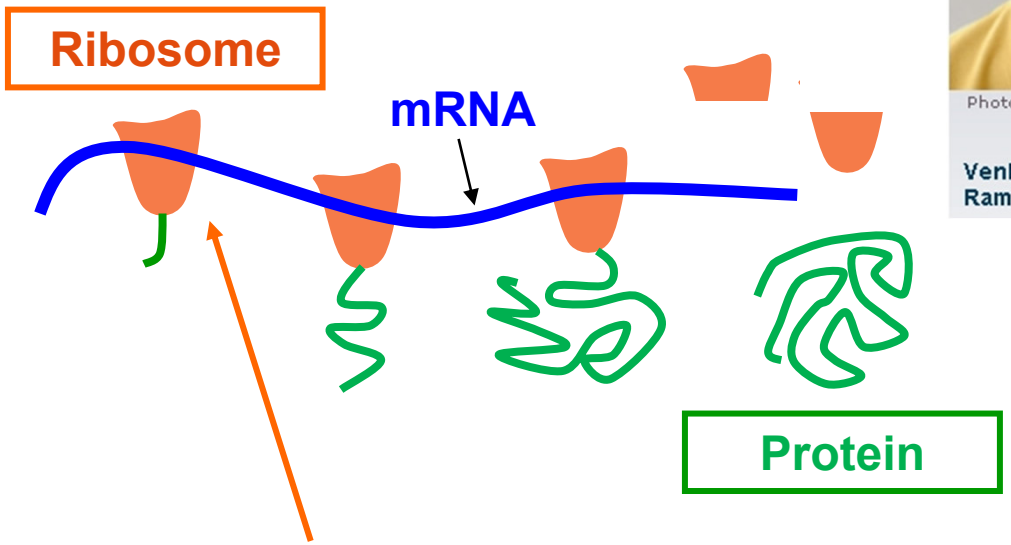
"*strictly speaking*", X-ray crystallography measures only the **density of electrons** within the crystal, from which the atomic positions can be inferred.

Object: real system (proteins, DNA, RNA, and their complexes)

- the specimen should not be damaged during the experiment (the **sample** is **X-ray sensitive**)

- the system is usually big (the smallest proteins have well **over 1000 atoms** and the largest proteins may have **between 10000 and 100000 atoms**)

Biocrystallography (MX)



The Nobel Prize in Chemistry 2009

"for studies of the structure and function of the ribosome"

Photo: MRC Laboratory of Molecular Biology

Venkatraman Ramakrishnan

Credits: Michael Marsland/Yale University

Thomas A. Steitz

Credits: Micheline Pelletier/Corbis

Ada E. Yonath

Ribosome: 19198 protein atoms, 32470 RNA atoms (**>50000 atoms**)

Biocrystallography (MX)

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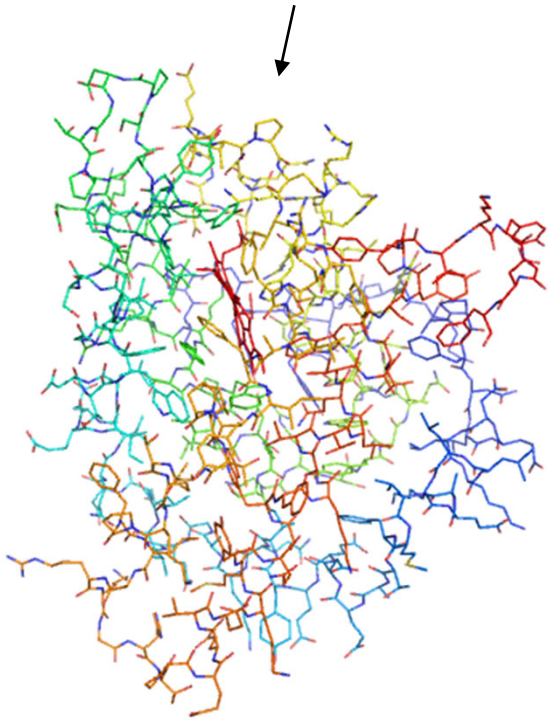
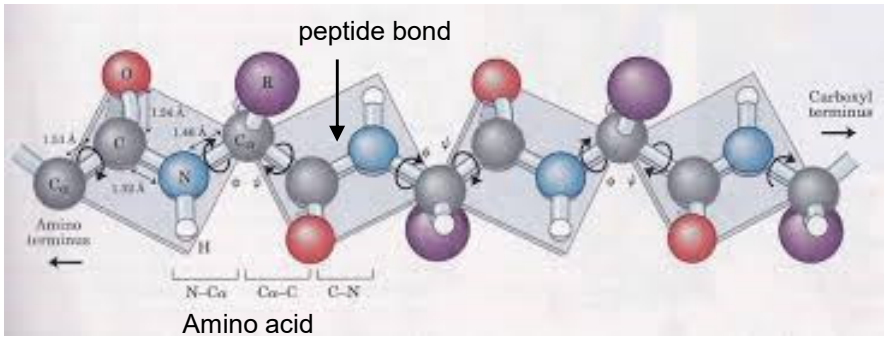
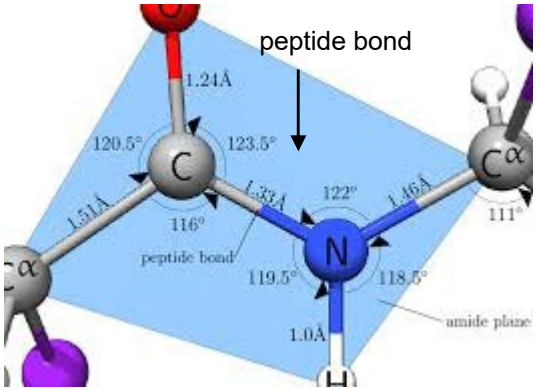
Why X-rays?

protein = polypeptide chain made by **covalently bound amino acids**





Amino acid



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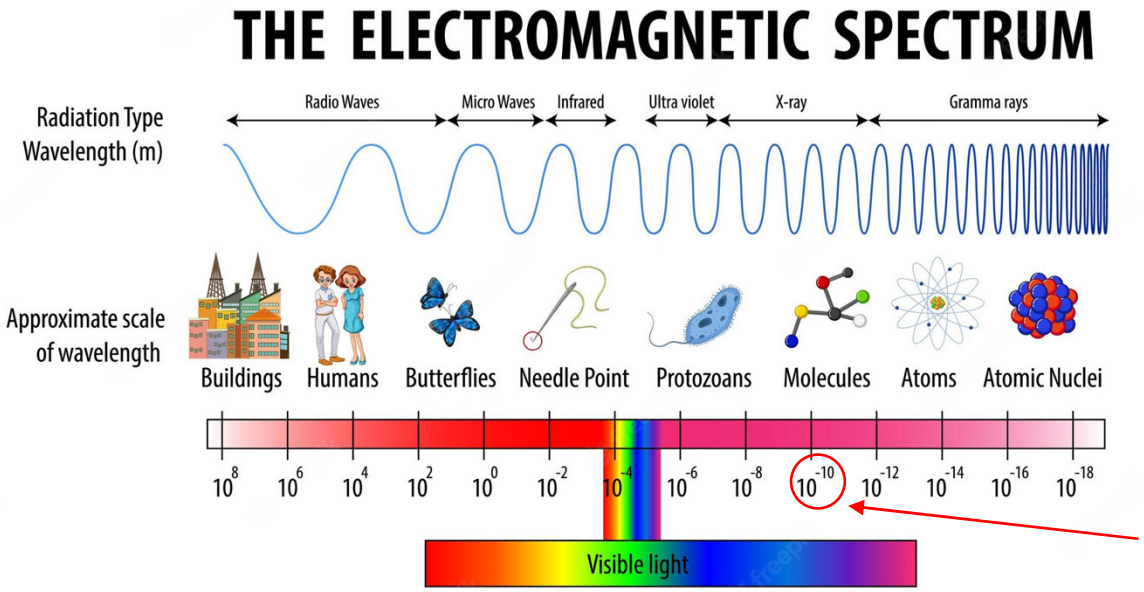
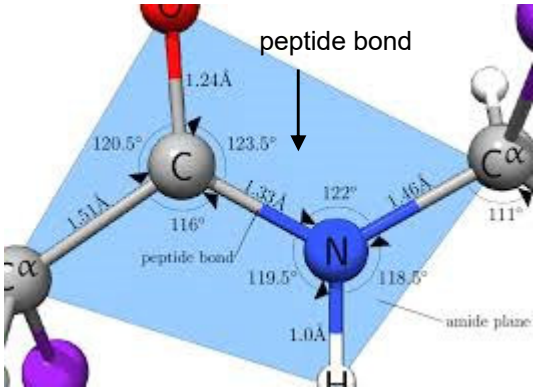
Why X-rays?

The wavelength of a X-rays is roughly 1 Å, which is on the scale of a single atom, and it allows to have **sufficient resolution to determine the atomic positions**





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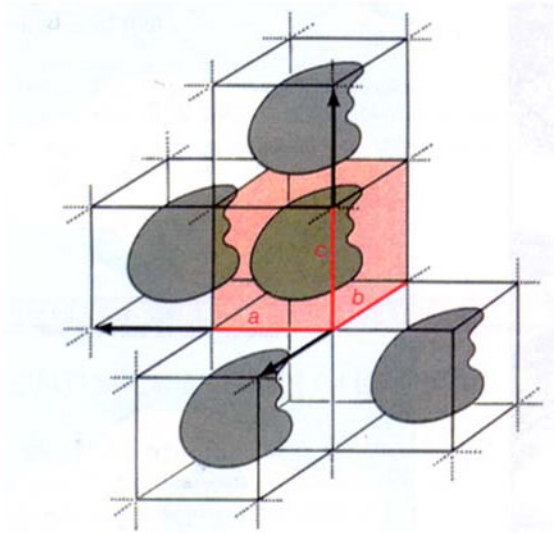
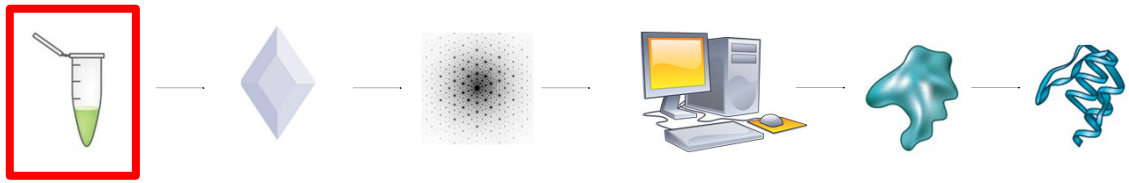
Why crystals?

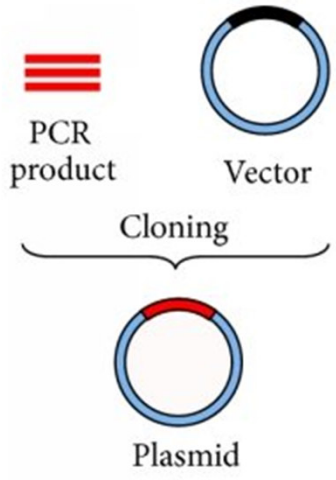
X-ray crystallography requires a crystal to amplify the signal (**10^{15}-10^{16} identical molecules**).

The periodicity of the electron density is used to diffract the X-rays with manageable measurement error



MX: expression and protein purification

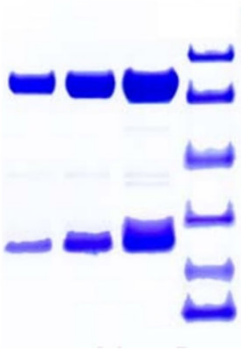




Construct design and cloning
(full-length, domains, mutants, …)

Heterologous
expression systems:
Bacteria
Yeast
Insect cells
Mammalian cells

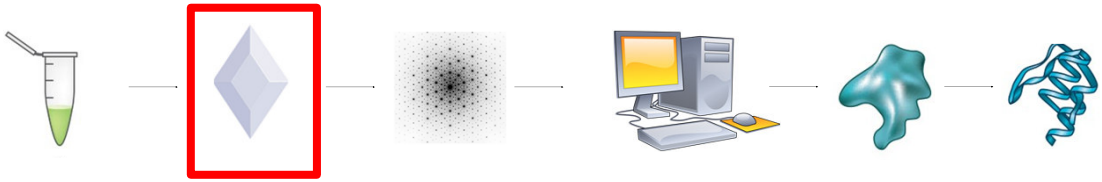
Purification:
affinity resins, tags, ..

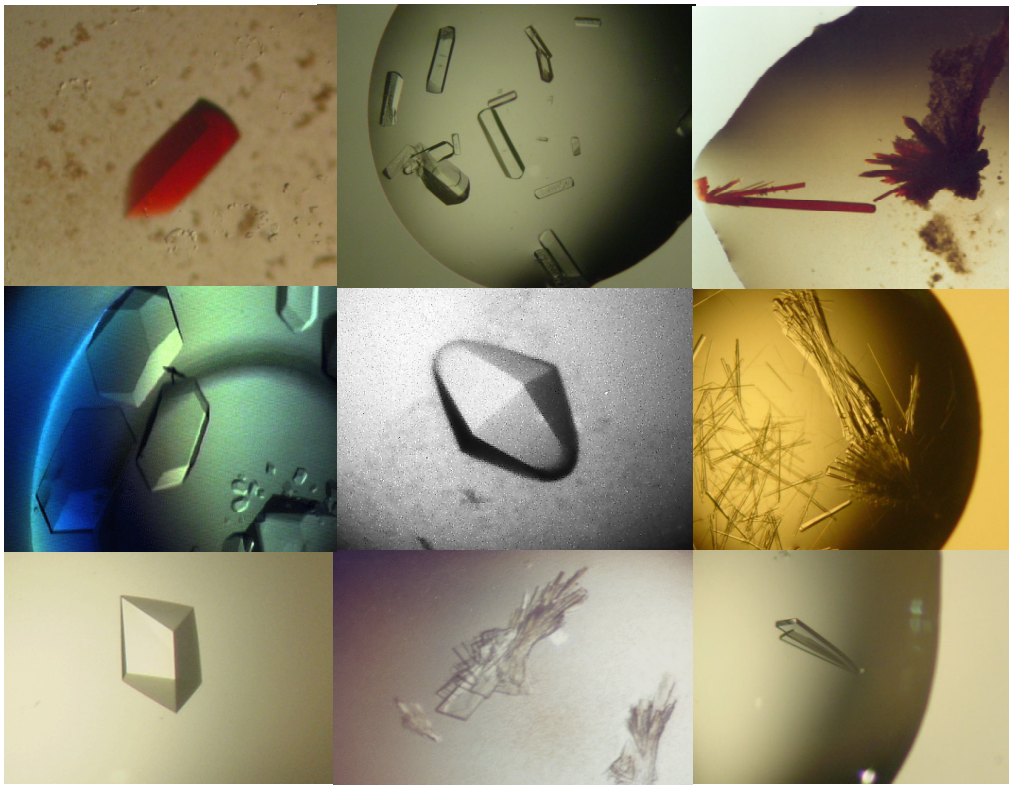


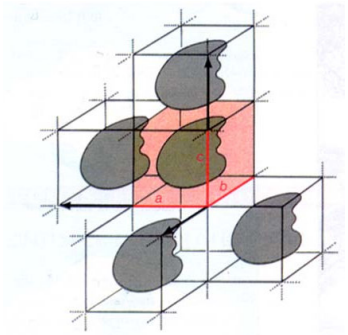
Analysis:
SDS-Page
electrophoresis

mgs of pure protein
needed

MX: crystallization

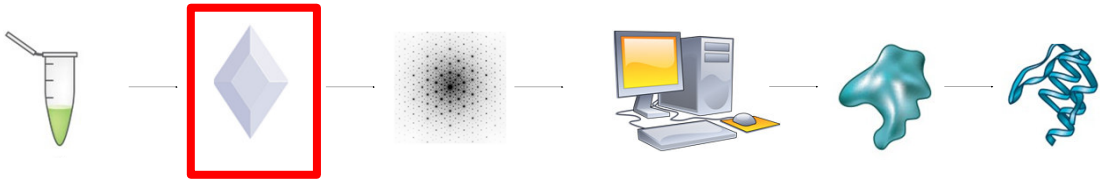






- volume ≤ 0.1 mm^3
- crystal lattice periodicity > 100 Å
- solvent content 30% - 80% v/v
- non-covalent (weak) interactions (surface amino acids)
- mechanic fragility ($E_{stab.}$ < 10 kcal/mol, less than protein folding energy)

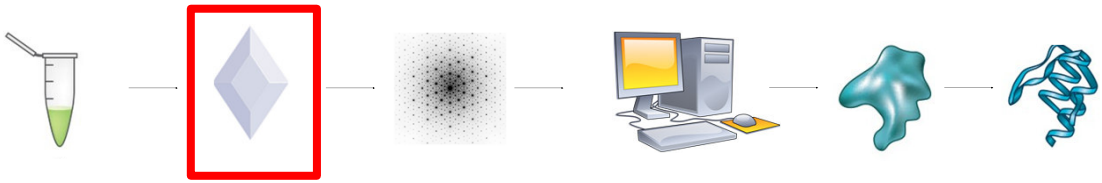
MX: crystallization



How to crystallize a protein:

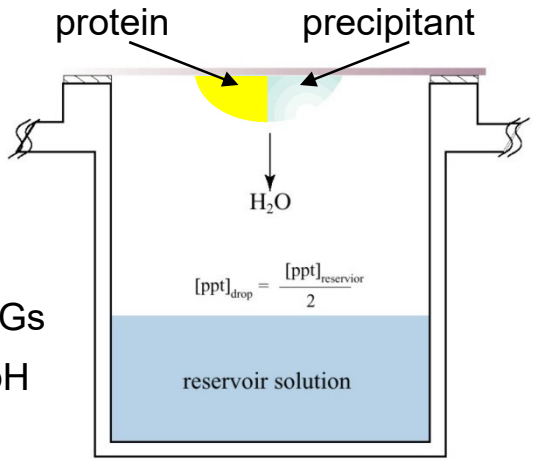
the "bottleneck" of the procedure !!!

MX: crystallization

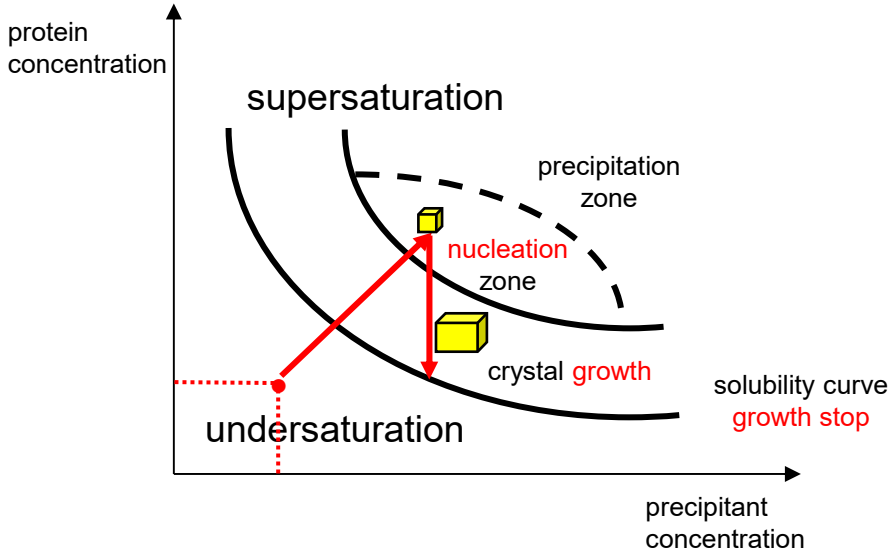


- protein crystallization is mainly **a trial-and-error procedure** in which the **protein is slowly precipitated** from its solution (to avoid formation of useless dust or amorphous gel).

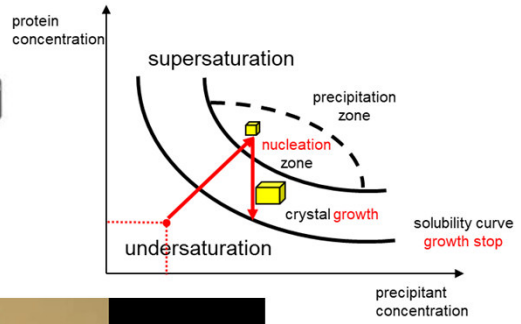
- crystal growth in solution is a multiparameter process involving three basic steps: **nucleation** (possibly having only 100 molecules), **growth**, and **cessation of growth**.

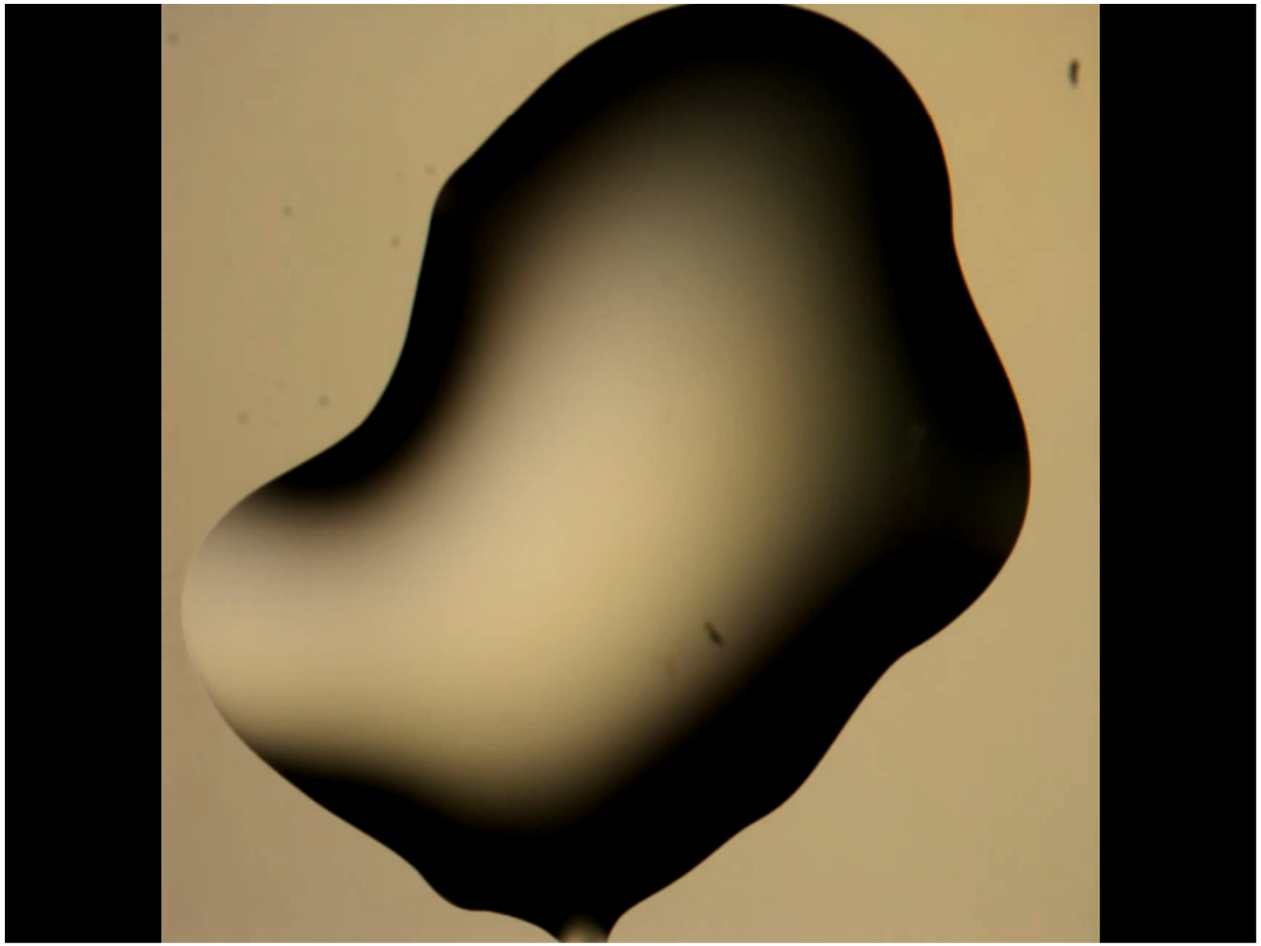


- salt, PEGs
- buffer, pH
- ions

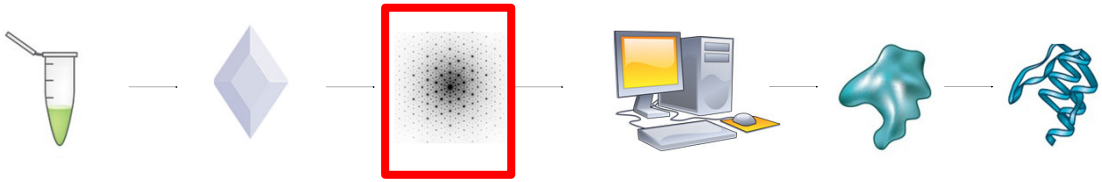


MX: crystallization (live)





MX: X-ray diffraction



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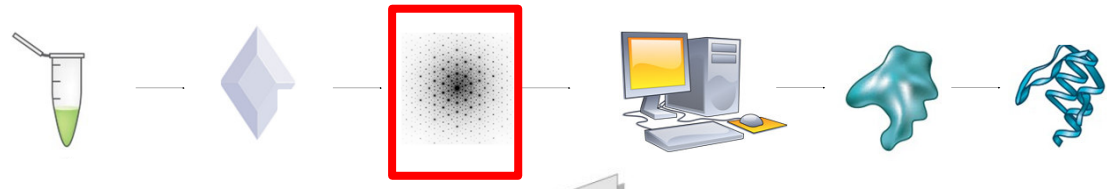
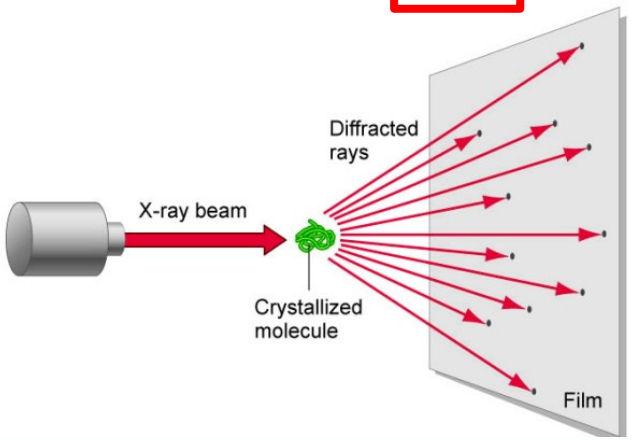
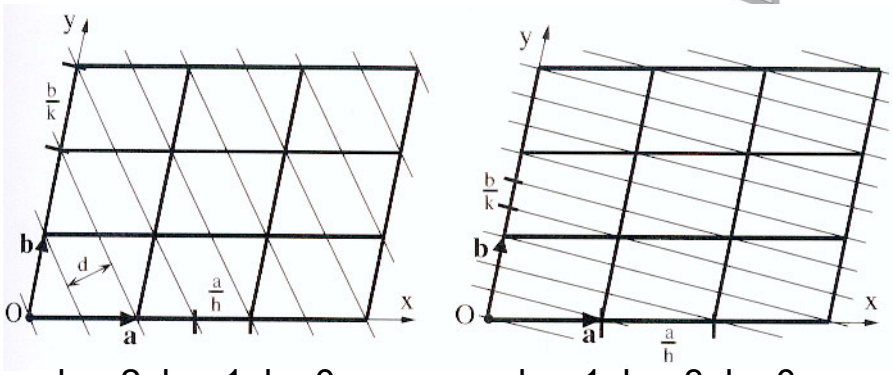
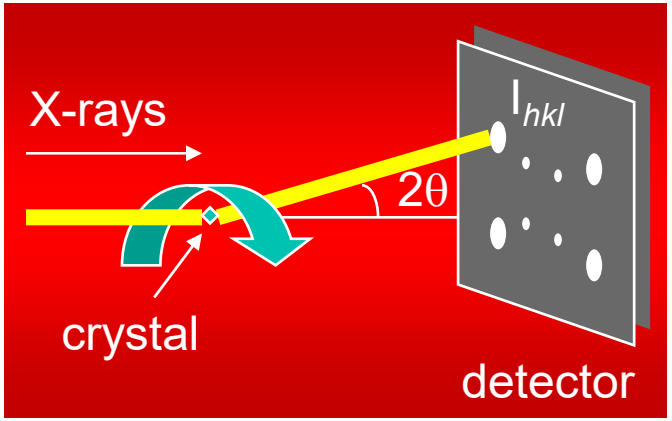


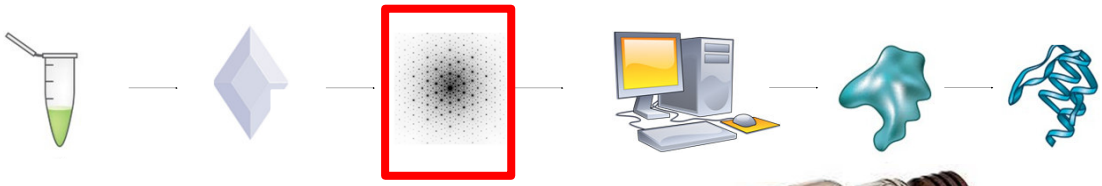


$2d_{hkl} \sin\theta = n\lambda$ **(Bragg's equation)**





MX: X-ray diffraction



X-ray tube

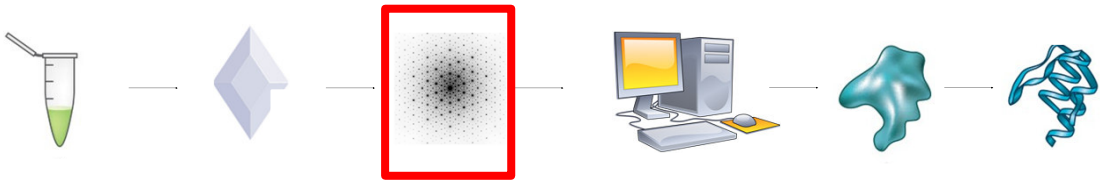
X-ray sources

Synchrotron radiation

- High brightness
- High collimation
- Broad energy spectrum

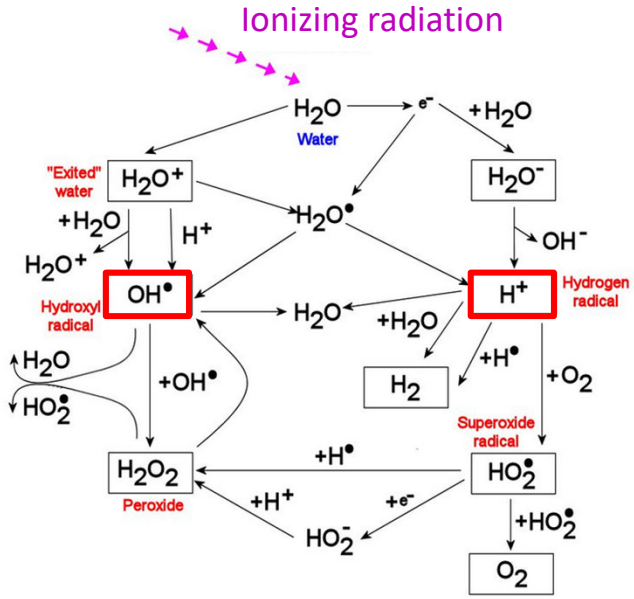
- X-rays selected out of a relatively wide range of wavelengths to optimise the experiment around the sample properties (i.e. λ tuned to exploit the absorption properties of heavier chemical elements naturally present or added to the crystal) → **«anomalous scattering»**
- **cryo-cooling** of the sample is mandatory

MX: Radiation damage

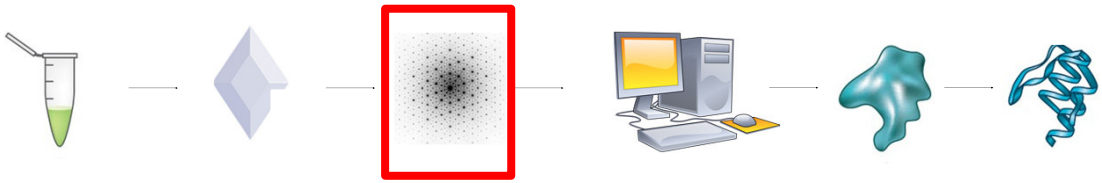


The energy range of X-rays used for diffraction (6 - 15 keV) is a severely ionizing radiation

⇒ formation of reactive **radicals** in the sample (water radiolysis), which rapidly destroy any protein crystal, particularly at dose rates experienced at synchrotrons

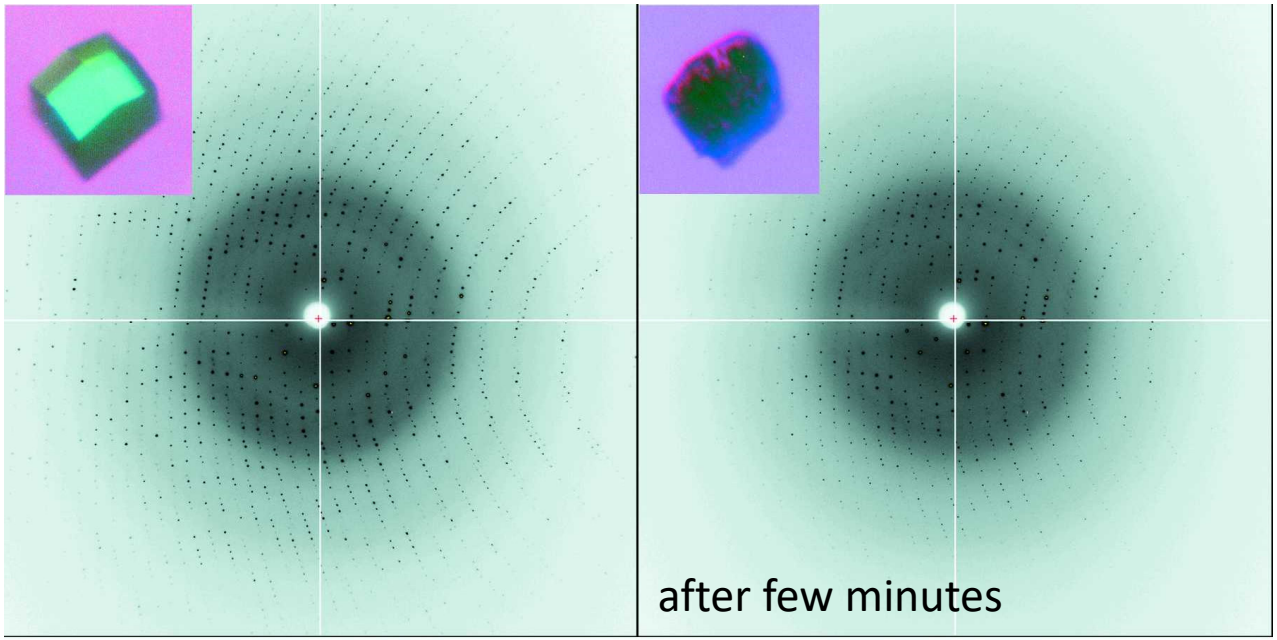


MX: Radiation damage

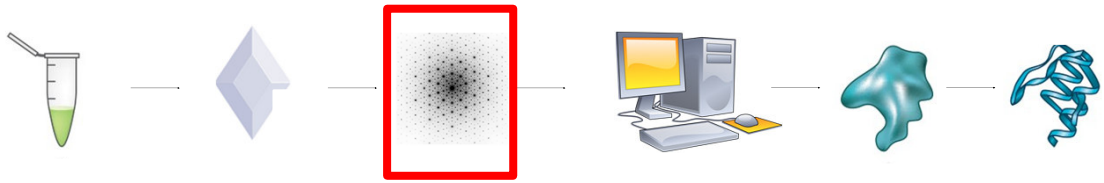


The energy range of X-rays used for diffraction (6 - 15 keV) is a severely ionizing radiation

$\Rightarrow$ formation of reactive **radicals** in the sample (water radiolysis), which rapidly destroy any protein crystal, particularly at dose rates experienced at synchrotrons



MX: Radiation damage

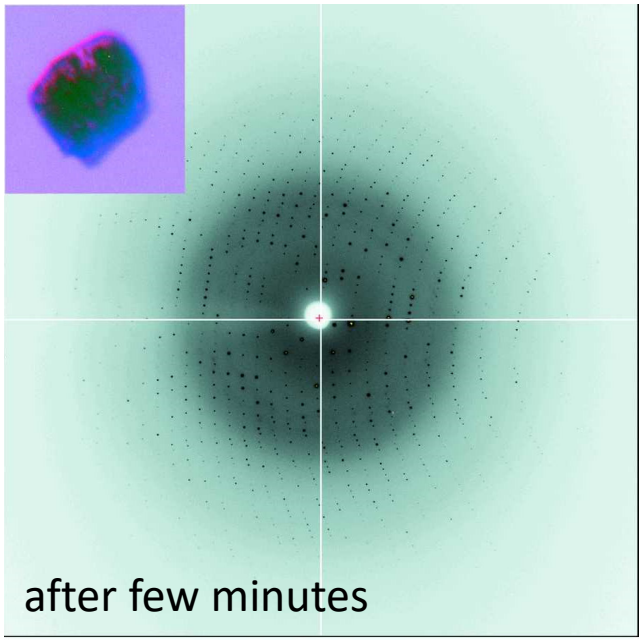


The energy range of X-rays used for diffraction (6 - 15 keV) is a severely ionizing radiation

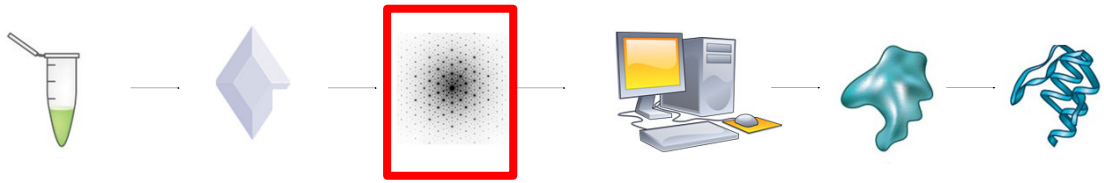
$\Rightarrow$ formation of reactive **radicals** in the sample (water radiolysis), which rapidly destroy any protein crystal, particularly at dose rates experienced at synchrotrons

Symptoms:

- increase in unit cell parameters
- decrease in crystallographic symmetry
- decrease of intensity and resolution
- site-specific damages (disulphide bond breakage, decarboxylation of acidic residues, reduction of metal centers, …)



MX: Cryo-crystallography at the Synchrotron



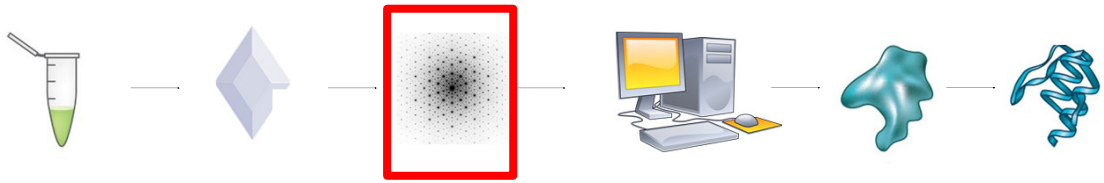
- an efficient way to suppress radiation damage by slowing down the kinetics of the radical reactions is **cryogenic cooling**

⇒ **flash-cooling** crystals to liquid nitrogen temperatures, either in cold nitrogen gas streams or directly into liquid nitrogen

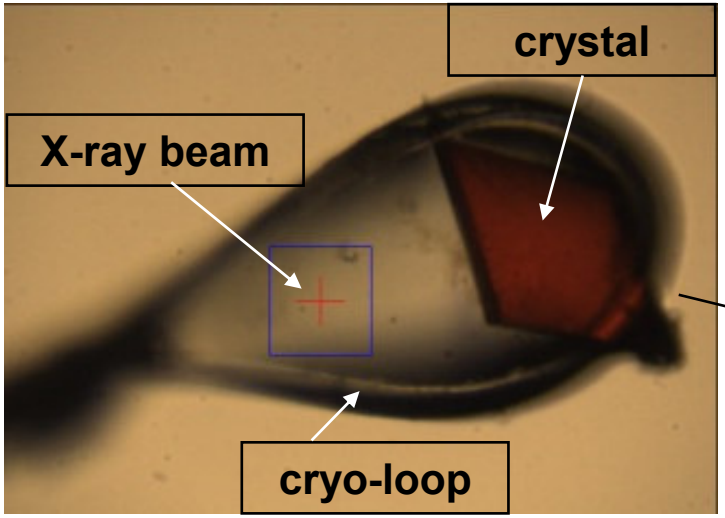
- to prevent the formation of crystalline ice during flash-cooling of the crystals, **cryoprotectants** are necessary

⇒ ethylene glycol (the anti-freeze in automobile radiators), glycerol, higher alcohols, … etc

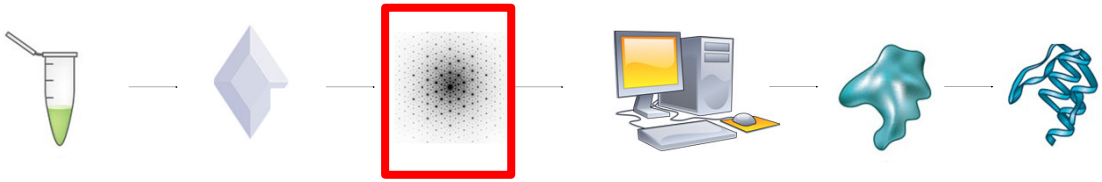
MX: Cryo-crystallography at the Synchrotron



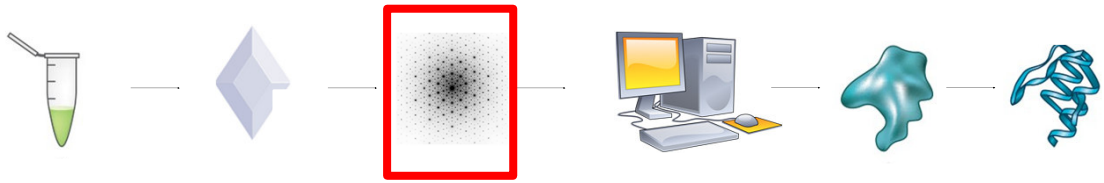
- the crystal is removed from the crystallization drop using **cryo-loops** and briefly dipped into a cryoprotectant before being immersed into liquid nitrogen



MX: Cryo-crystallography at the Synchrotron



MX: Cryo-crystallography at the Synchrotron



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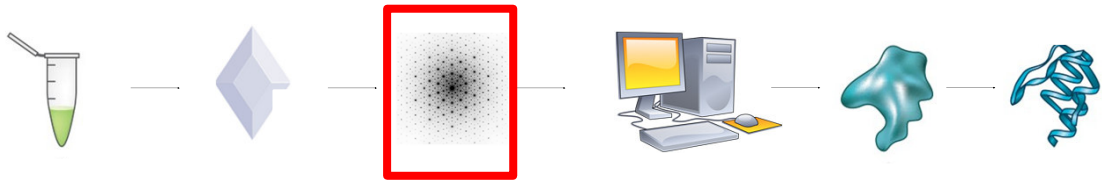
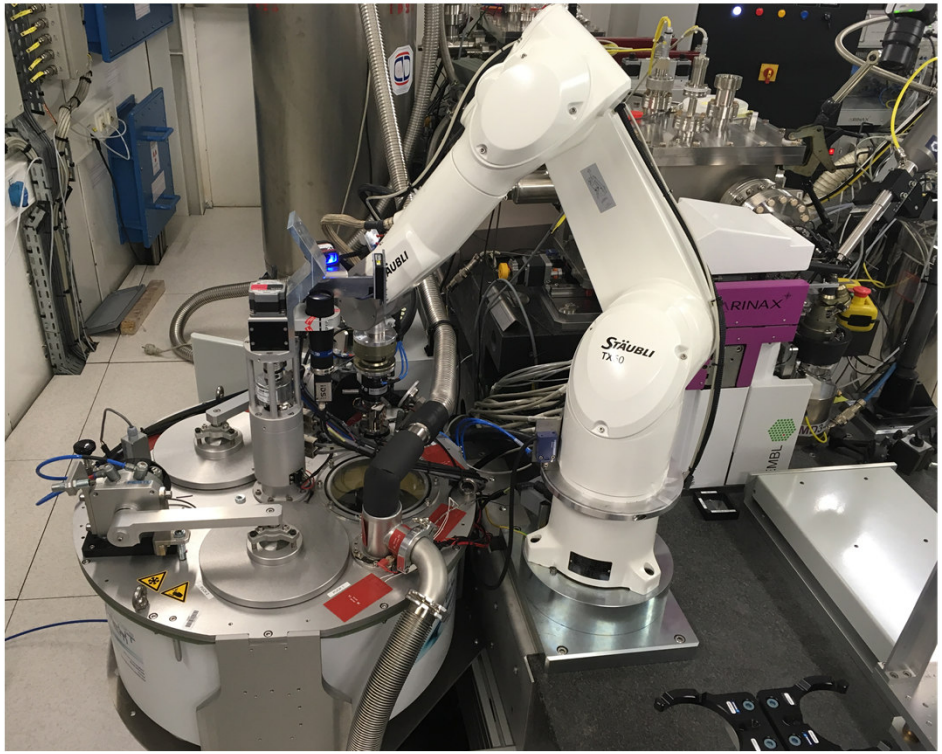
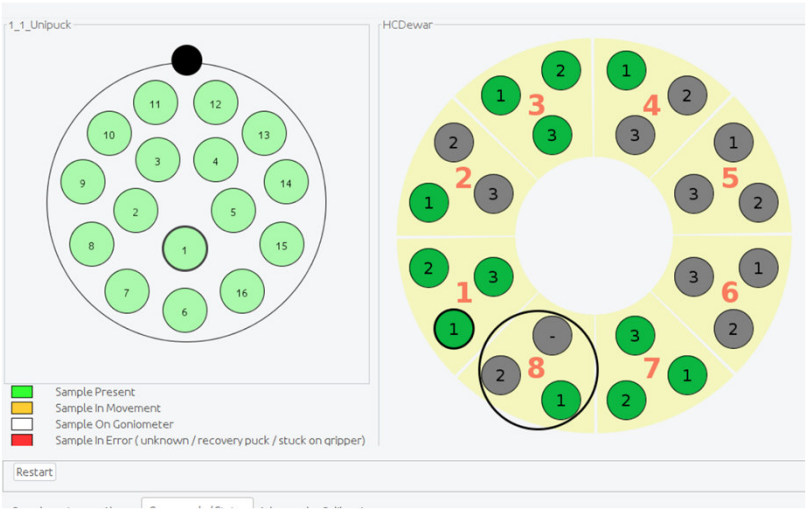
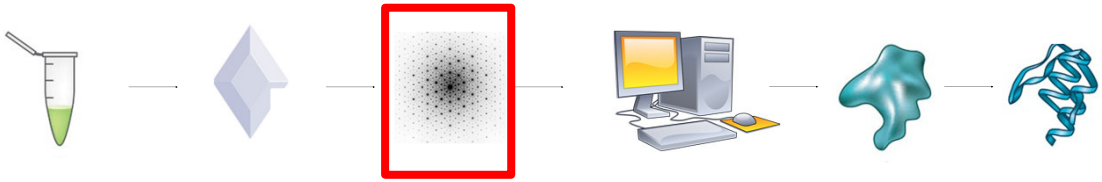


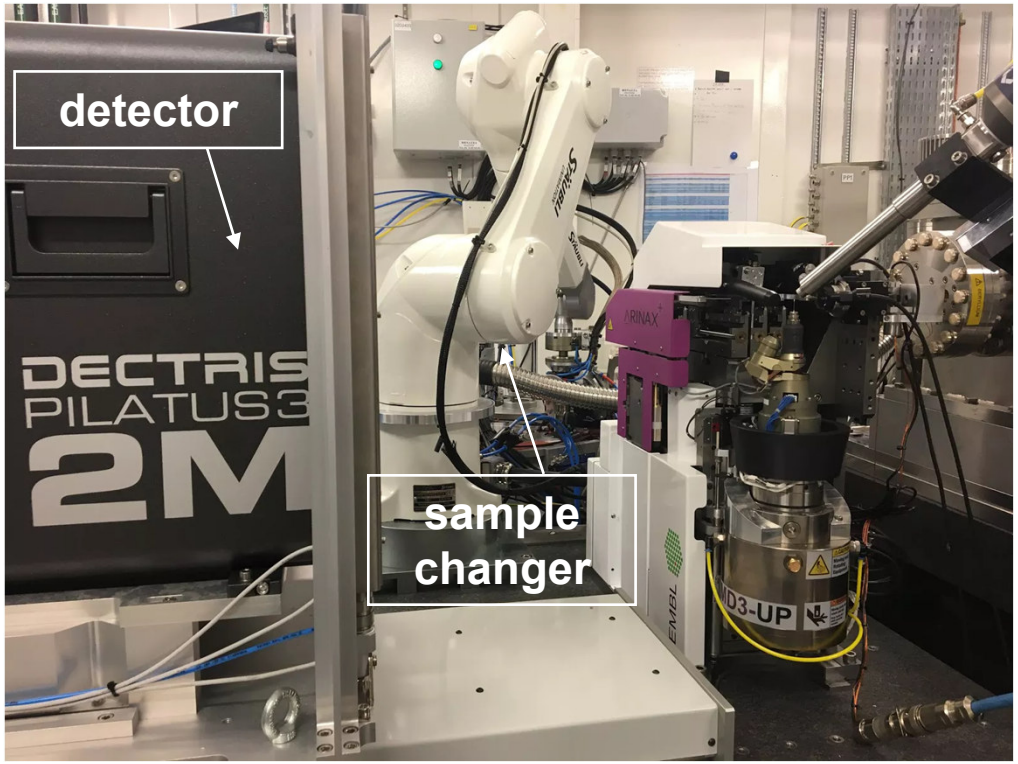




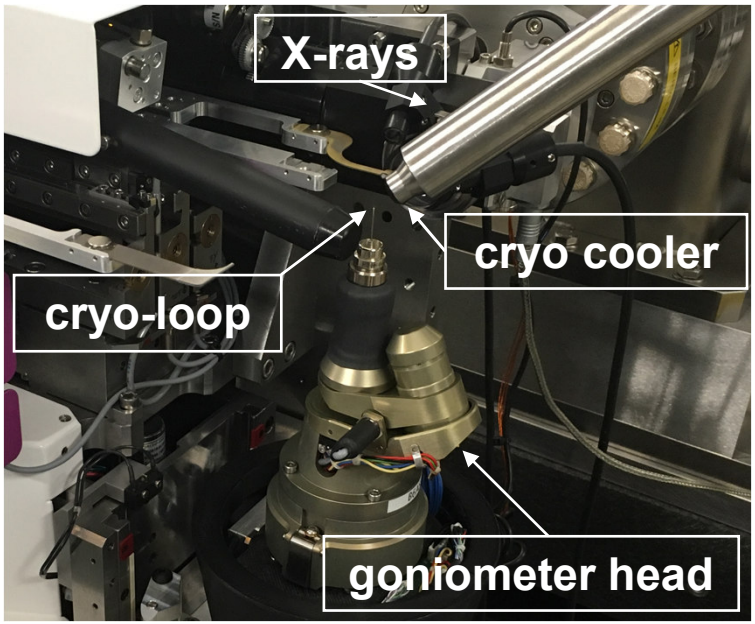
- Sample changer robot mounts the crystals in the goniometer

MX: Cryo-crystallography at the Synchrotron

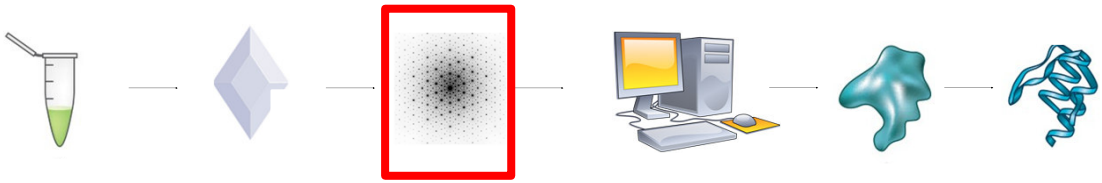


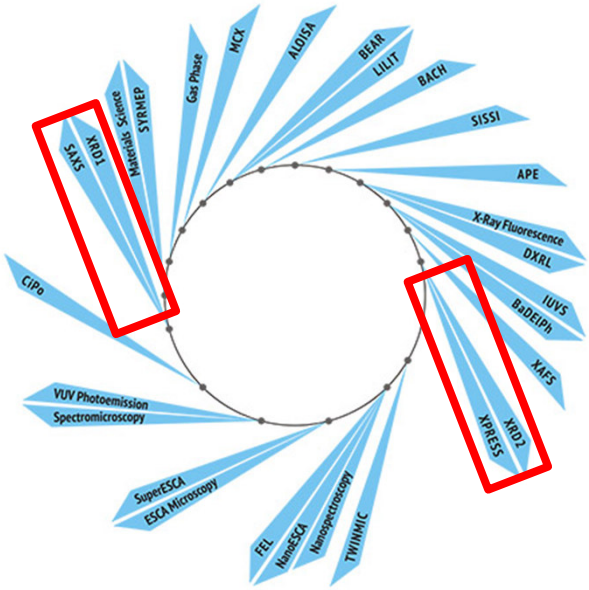


MX beamline
(ESRF ID-23-2)

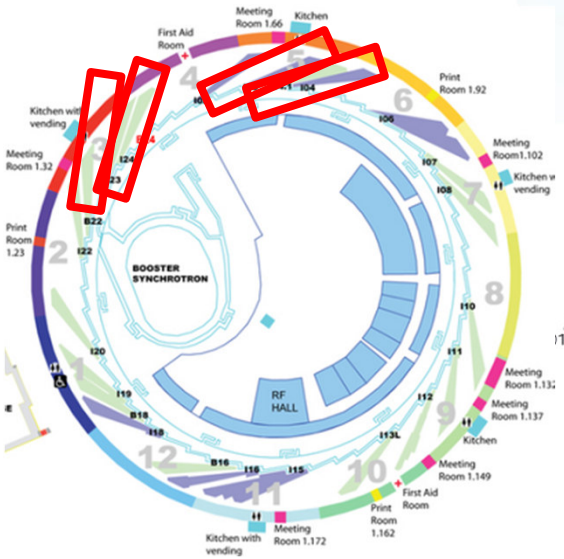


MX: Cryo-crystallography at the Synchrotron

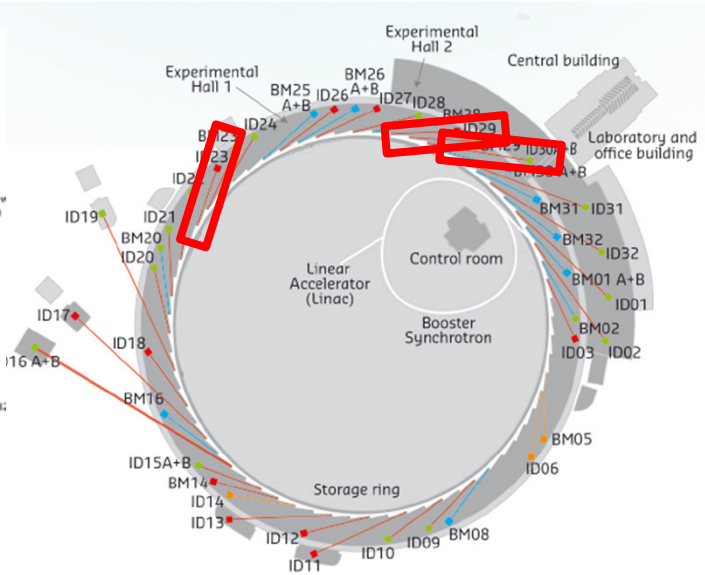




Elettra (Trieste)
XRD1, XRD2

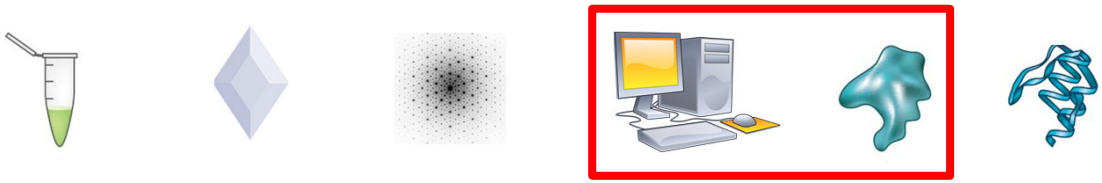


Diamond (Didcot)
I03, I04, I04-1, I23, I24



ESRF (Grenoble)
ID23-1, ID23-2, ID29,
ID30A-1, ID30A-3, ID30B

MX: The "phase problem"

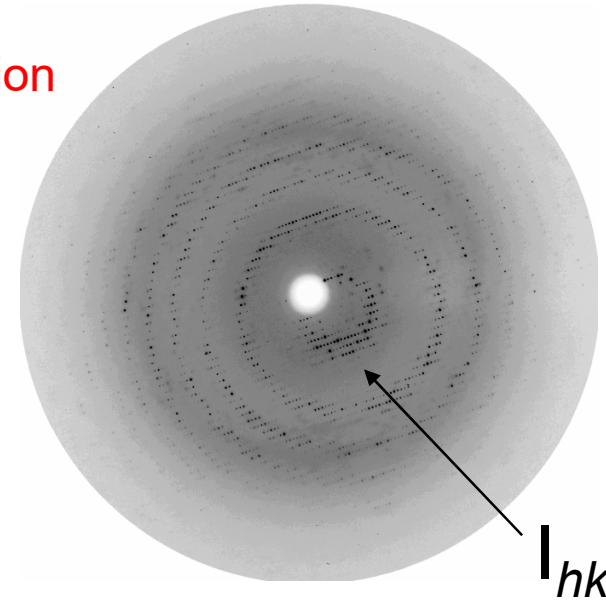


REAL SPACE — FT — RECIPROCAL SPACE

crystal structure — diffraction (structure factors)

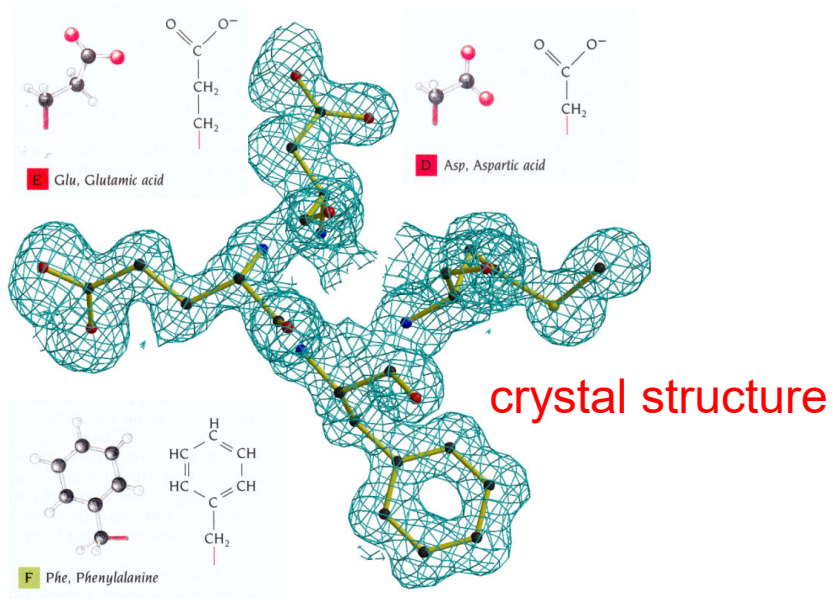
FT^{-1}

diffraction



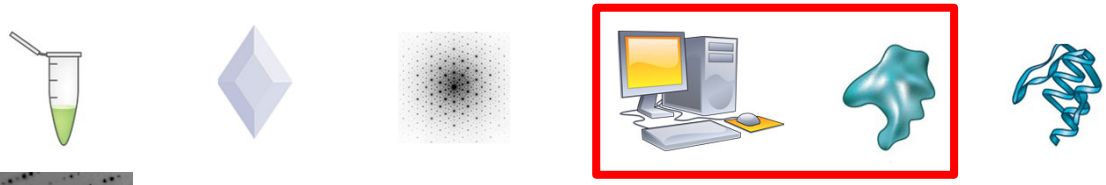
I_{hkl}

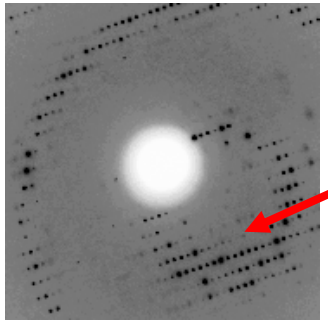
?



crystal structure

MX: The "phase problem"



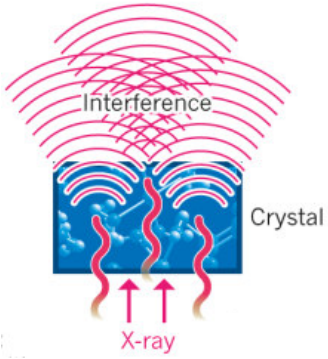


$$I_{hkl} \propto I_0 \frac{V_{xtal}}{V_{Cell}^2} |\vec{F}_{hkl}|^2$$

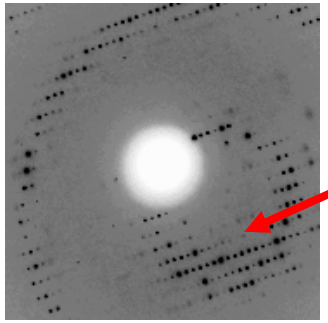
atomic scattering factors

$$\vec{F}_{hkl} = \sum_{j=1}^{N\ atoms} f_j \exp\left[2\pi i\,(hx_j + ky_j + lz_j)\right] = |\vec{F}_{hkl}| \exp\left(i\alpha_{hkl}\right)$$

structure factors — module — phase



MX: The "phase problem"



$$I_{hkl} \propto I_0 \frac{V_{xtal}}{V_{Cell}^2} |\vec{F}_{hkl}|^2$$

atomic property

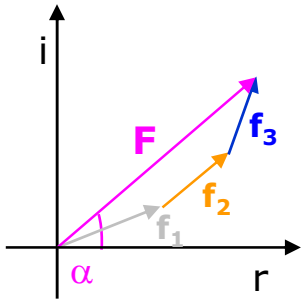
structural property (position)

$$\vec{F}_{hkl} = \sum_{j=1}^{N\ atoms} f_j \exp\left[2\pi i\,(hx_j + ky_j + lz_j)\right] = |\vec{F}_{hkl}| \exp\left(i\alpha_{hkl}\right)$$

structure factors

module

phase



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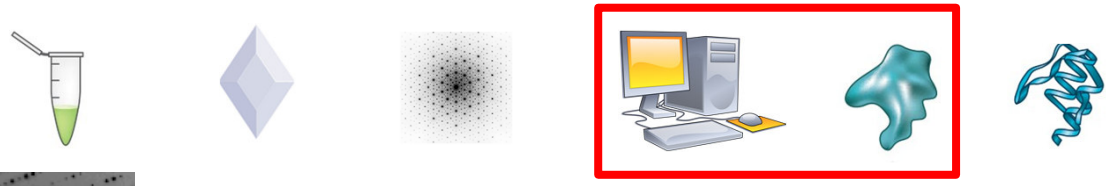
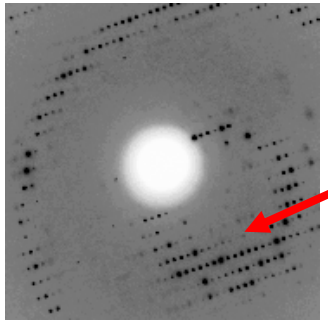




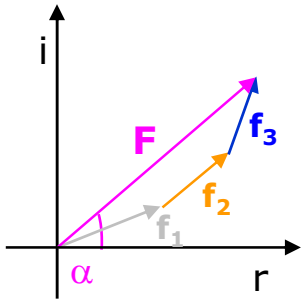
$$I_{hkl} \propto I_0 \frac{V_{xtal}}{V_{Cell}^2} |\vec{F}_{hkl}|^2$$

atomic property

structural property (position)

$$\vec{F}_{hkl} = \sum_{j=1}^{N\ atoms} f_j \exp\left[2\pi i\,(hx_j + ky_j + lz_j)\right] = |\vec{F}_{hkl}| \exp\left(i\alpha_{hkl}\right)$$

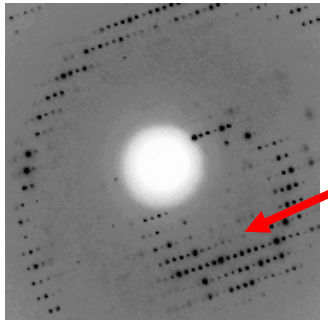
structure factors — module — phase



$$\vec{F}_{hkl} = \int_{Vcell} \rho(x,y,z) \exp\left[2\pi i(hx+ky+lz)\right] dV \qquad \text{(FT)}$$

electron density

MX: The "phase problem"



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atomic property

structural property (position)

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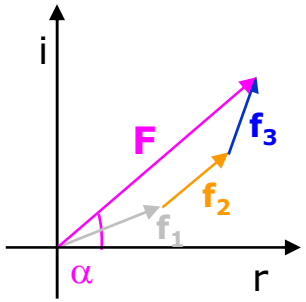
structure factors — module — phase

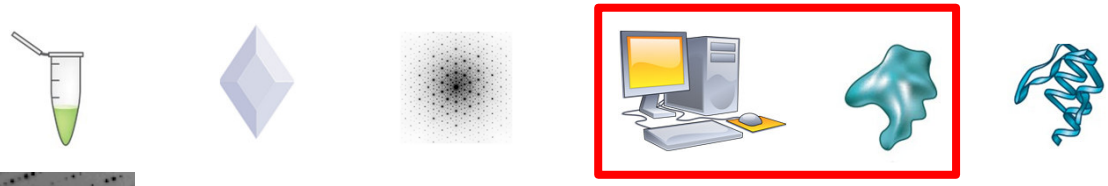


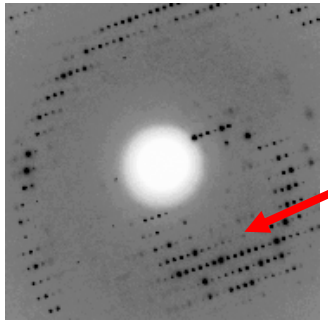
$$\vec{F}_{hkl} = \int_{Vcell} \rho(x,y,z) \exp\left[2\pi i(hx+ky+lz)\right] dV \qquad (FT)$$

electron density

$$\rho(x,y,z) = \frac{1}{V}\sum_{hkl} |\vec{F}_{hkl}| \exp\left(i\alpha_{hkl}\right) \exp\left[-2\pi i(hx+ky+lz)\right] \qquad (FT^{-1})$$

MX: The "phase problem"





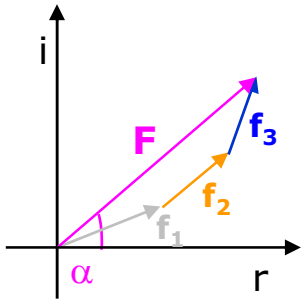
$$I_{hkl} \propto I_0 \frac{V_{xtal}}{V_{Cell}^2} |\vec{F}_{hkl}|^2$$

atomic property

structural property (position)

$$\vec{F}_{hkl} = \sum_{j=1}^{N\ atoms} f_j \exp\left[2\pi i\,(hx_j + ky_j + lz_j)\right] = |\vec{F}_{hkl}| \exp\left(i\alpha_{hkl}\right)$$

structure factors — module — phase



$$\vec{F}_{hkl} = \int_{Vcell} \rho(x,y,z) \exp\left[2\pi i(hx+ky+lz)\right] dV \qquad (FT)$$

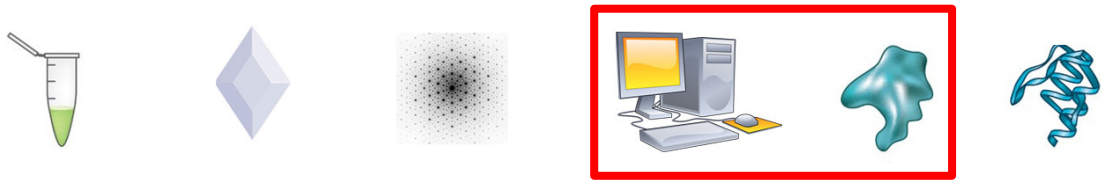
electron density

not measured

PARADOX

$$\rho(x,y,z) = \frac{1}{V} \sum_{hkl} |\vec{F}_{hkl}| \exp\left(i\alpha_{hkl}\right) \exp\left[-2\pi i(hx+ky+lz)\right] \qquad (FT^{-1})$$

MX: The "phase problem"



in X-ray crystallography, there are several ways to recover the lost phases:

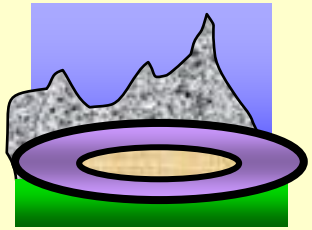
- **Molecular Replacement** (**MR**) **method**
  (synchrotron radiation not required)

- **Heavy atom method**

  - **Multiple Isomorphous Replacement** (**MIR**)
    (synchrotron radiation not required)

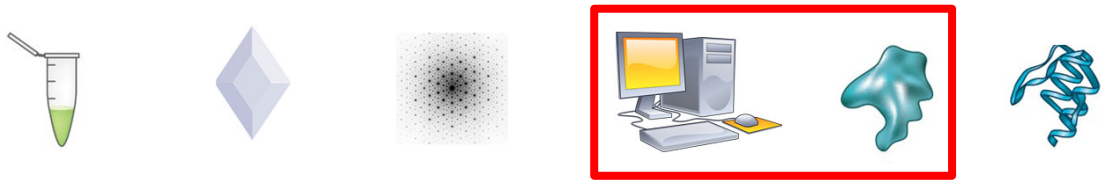
  - **Single (or Multiple) Isomorphous Replacement with anomalous scattering** (**SIRAS** or **MIRAS**)
    (synchrotron radiation required)

  - **Multiple wavelength Anomalous Diffraction** (**MAD**)
    (synchrotron radiation required)

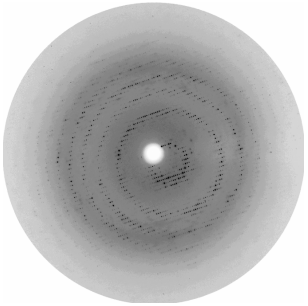


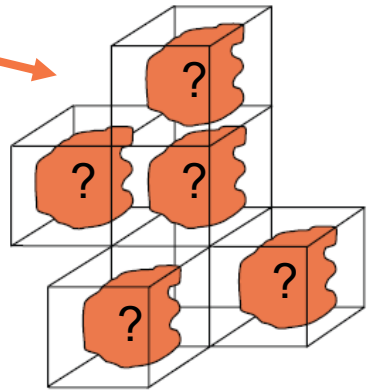
Synchrotron

MX: Molecular replacement



target protein (structrure ?)
("real" crystal)



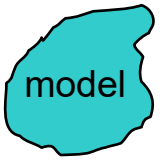


X-ray data

$$I^{obs}_{hkl} \propto |F^{obs}_{hkl}|^2$$

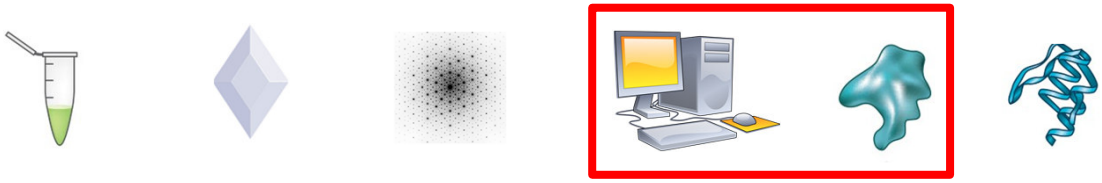
PDB PROTEIN DATA BANK

3D structure (model) with >30%
amino acids sequence identity

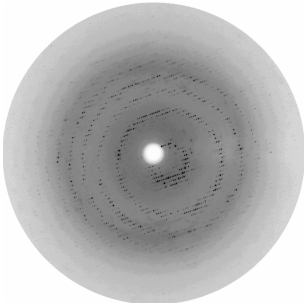


known coordinates

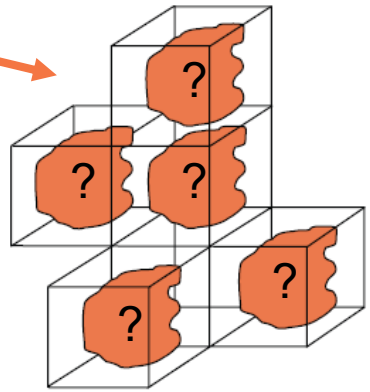
MX: Molecular replacement

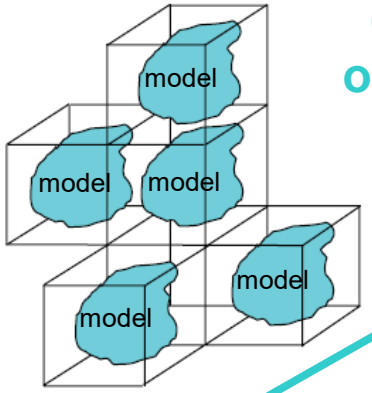


target protein (structrure ?)
("real" crystal)



X-ray data





"model" crystal
(same symmetry
of the real crystal)

$\rightarrow (x_j, y_j, z_j)$
for every atom
in the crystal

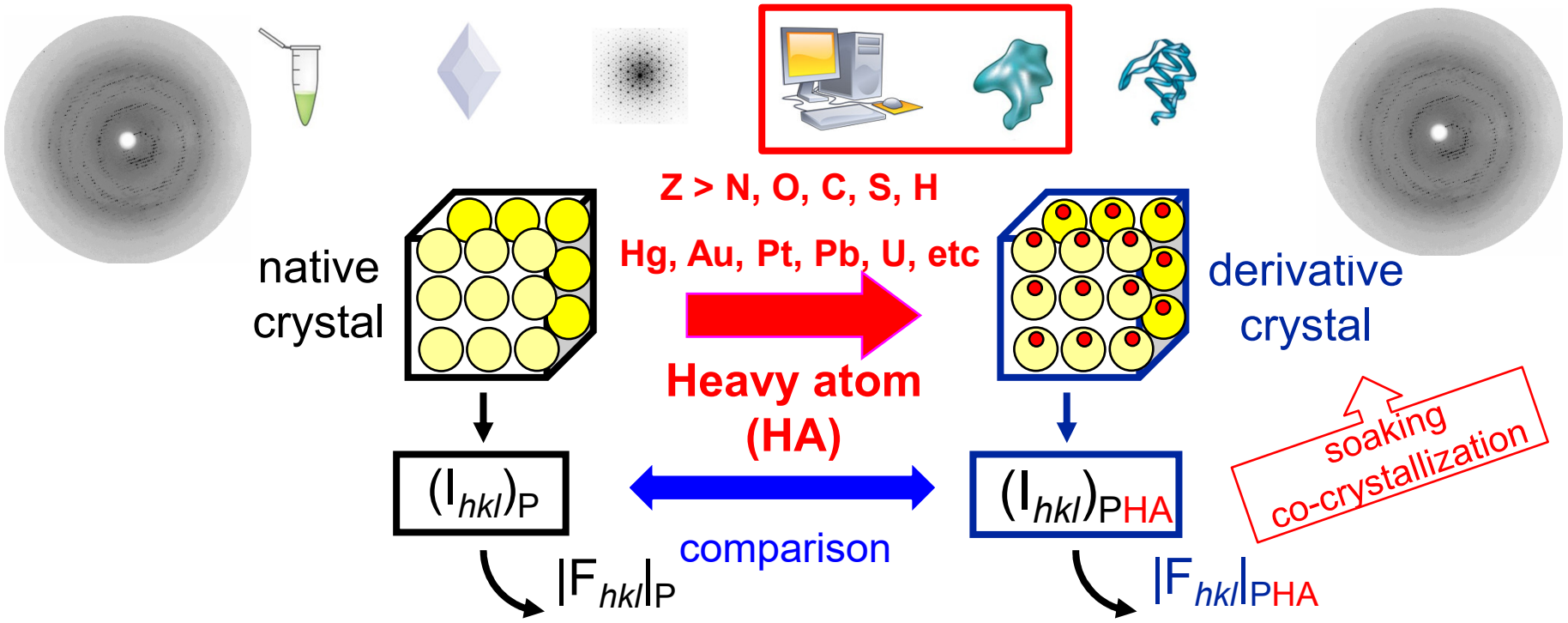
$$I^{obs}_{hkl} \propto |F^{obs}_{hkl}|^2$$

$$F^{calc}_{hkl} = \sum_{j=1}^{N\ atoms} f_j \exp\left[2\pi i\,(hx_j + ky_j + lz_j)\right] = |F^{calc}_{hkl}| \exp\left(i\alpha^{calc}_{hkl}\right)$$

hybrid electron density

$$\rho(x,y,z) = \frac{1}{V} \sum_{hkl} \left(|F^{obs}_{hkl}|\, e^{i\alpha^{calc}_{hkl}} \right) e^{-2\pi i(hx+ky+lz)}$$

MX: Heavy atoms

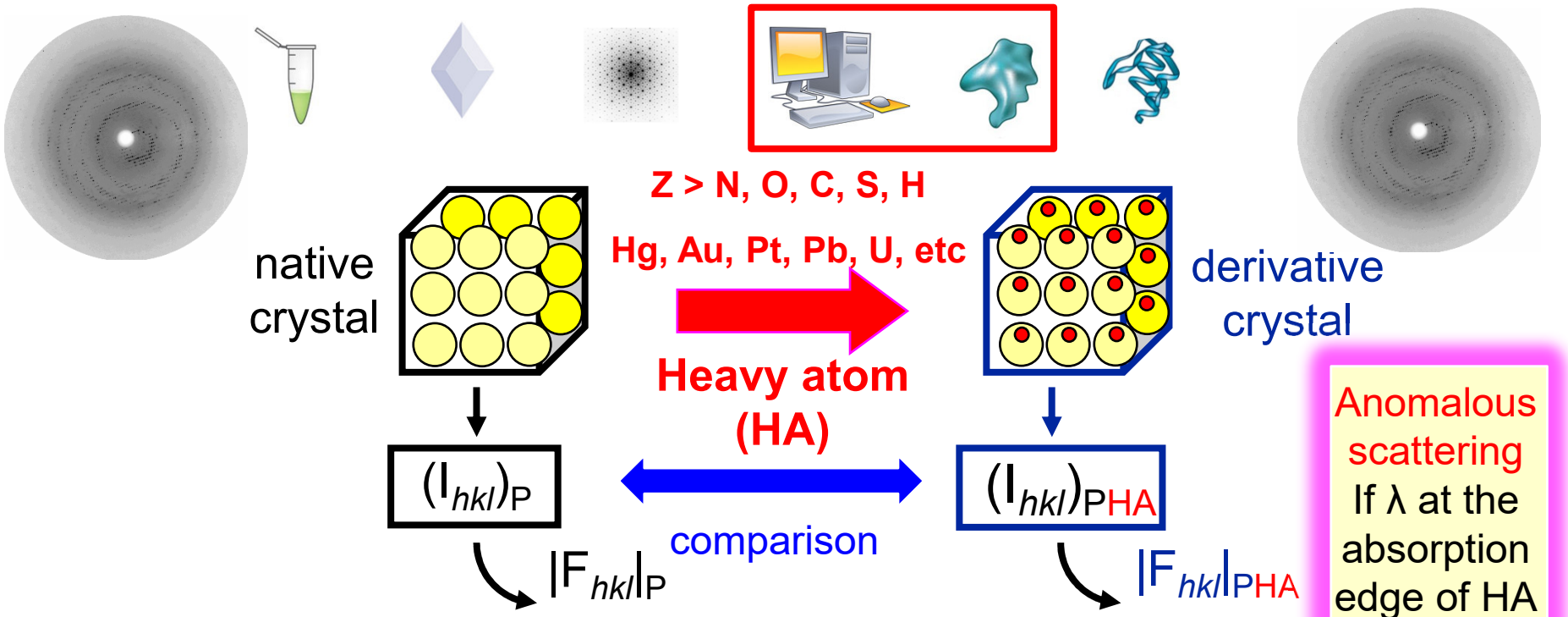


identification of the heavy atom positions within the unit cell

calculation of approximate initial phases α_P (for every hkl)

$$\rho(x,y,z) = \frac{1}{V}\sum_{hkl} \left(|\mathbf{F}_{hkl}|_P \, e^{i\alpha_{hkl}^{approx}} \right) e^{-2\pi i(hx+ky+lz)}$$

MX: Heavy atoms



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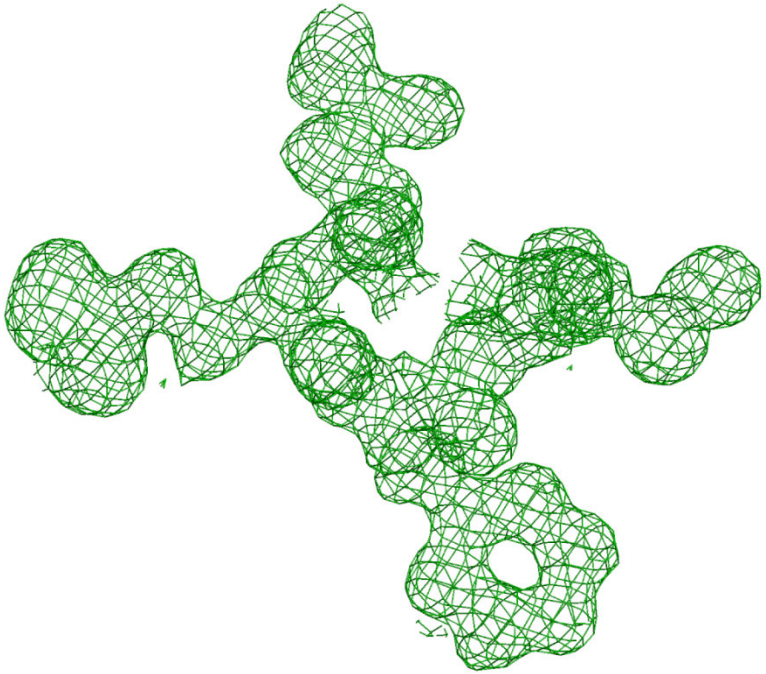
MX: Model building & refinement

Crystals → Data collection → Initial phases: Heavy atoms, MR

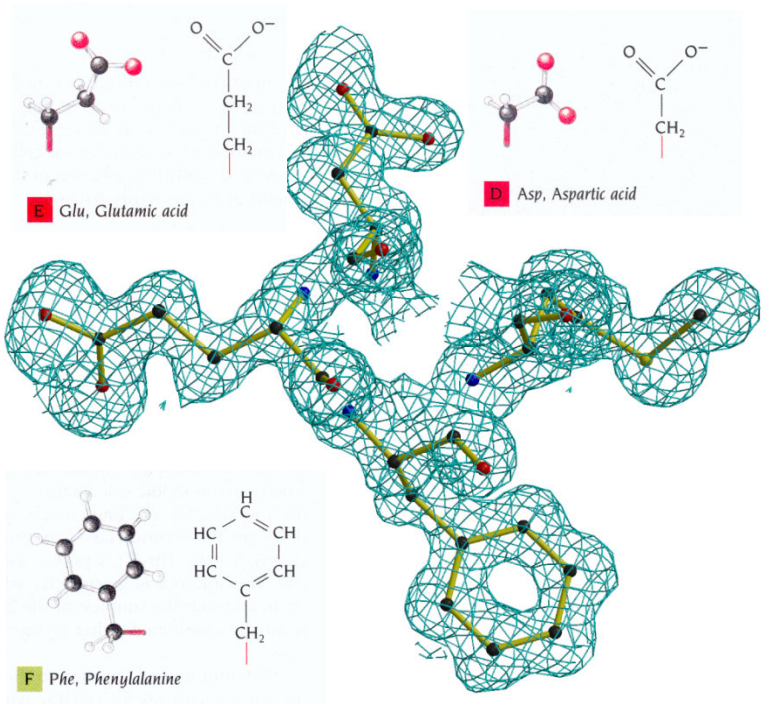


ρ (x,y,z)

electron density interpretation

MX: Model building & refinement

Crystals → Data collection → Initial phases: Heavy atoms, MR



ρ (x,y,z) → Model building

electron density interpretation

MX: Model building & refinement

Crystals → Data collection → Initial phases: Heavy atoms, MR

ρ (x,y,z)

Crystallographic refinement

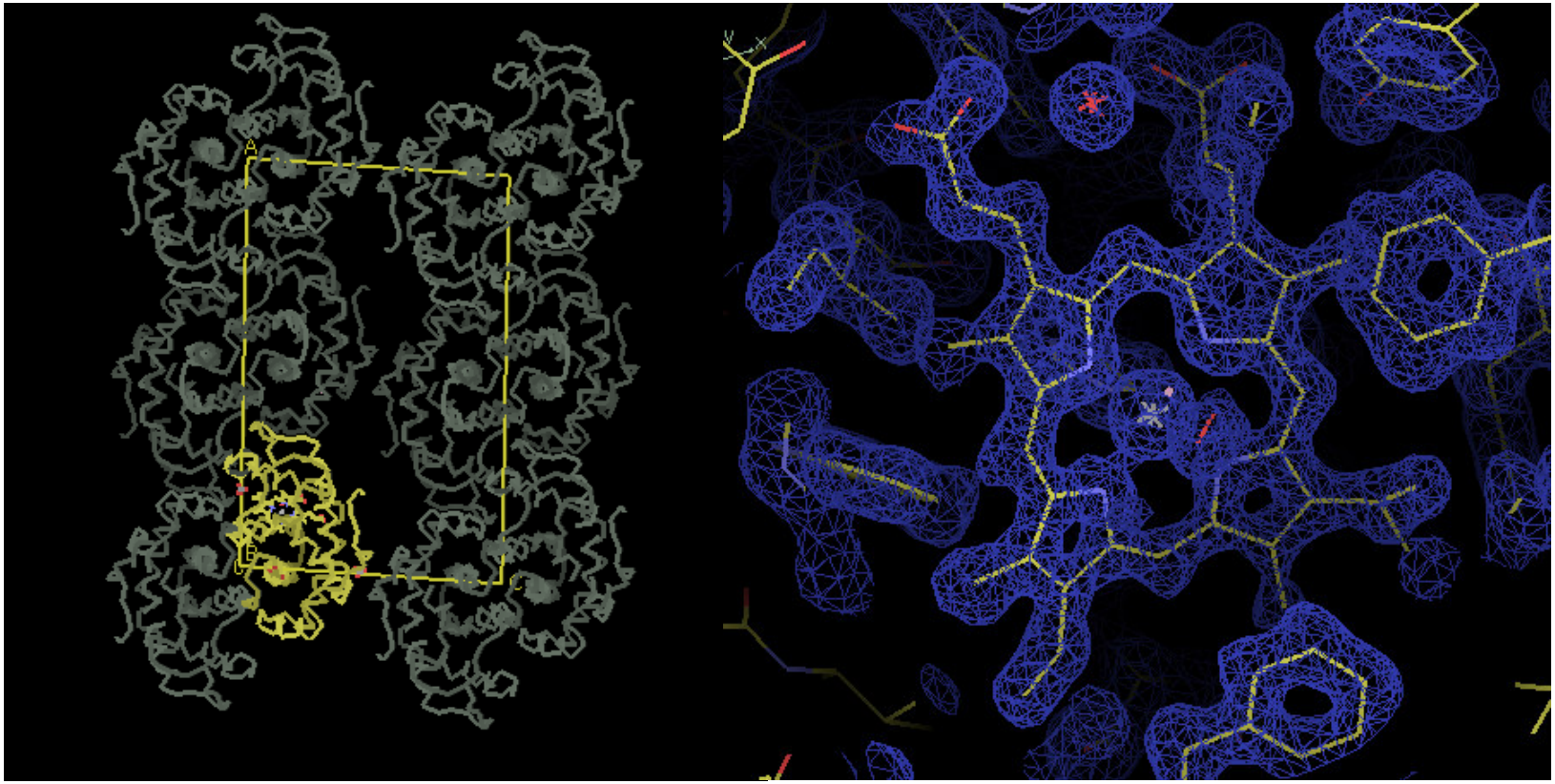
Model building

electron density interpretation

$$\left|\vec{F}_{hkl}\right|^{obs} \leftrightarrow \left|\vec{F}_{hkl}\right|^{calc}$$

PDB deposition

MX: Model building & refinement

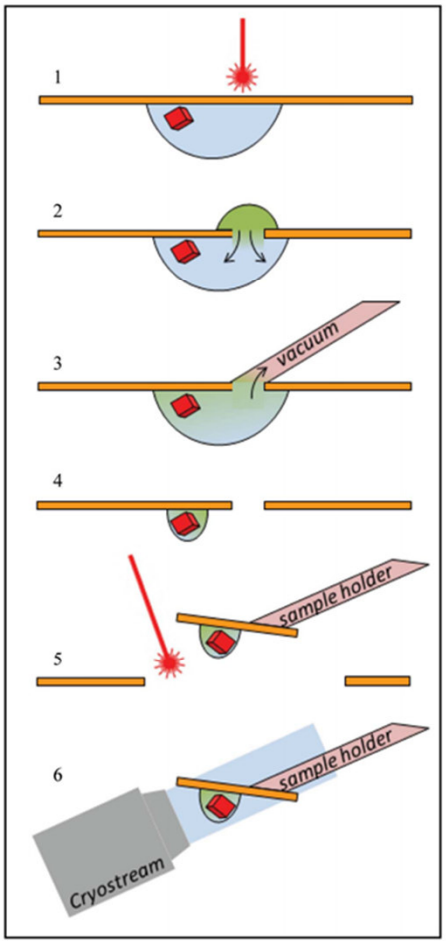


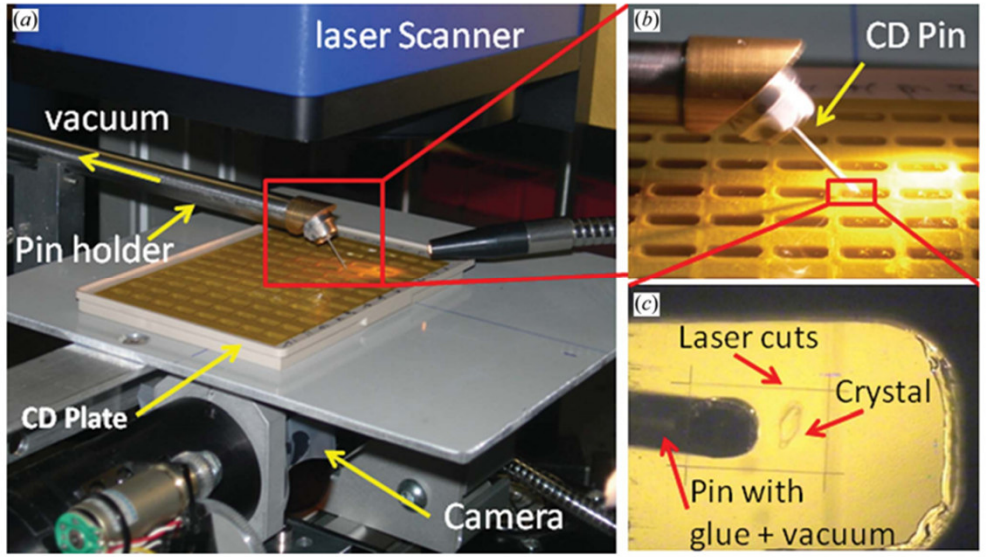
crystal packing

electron density & atomic model

MX: Fragment screening at the synchrotron

- CrystalDirect





MASSIF
Automated Data Collection

Software
CRIMS & ISPyB

CRIMS© CRystallization Information Management System developped by EMBL Grenoble

ISPyB Information System for Protein crystallography Beamlines

Pipedream: AutoProc → Molrep → Buster → Rhofit → Buster

Cryo-EM: The "resolution revolution"

Science

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HOME > SCIENCE > VOL. 343, NO. 6178 > THE RESOLUTION REVOLUTION

PERSPECTIVE | BIOCHEMISTRY

The Resolution Revolution

Advances in detector technology and image processing are yielding high-resolution electron cryo-microscopy structures of biomolecules. [Also see Report by Amunts *et al.*]

WERNER KÜHLBRANDT Authors Info & Affiliations

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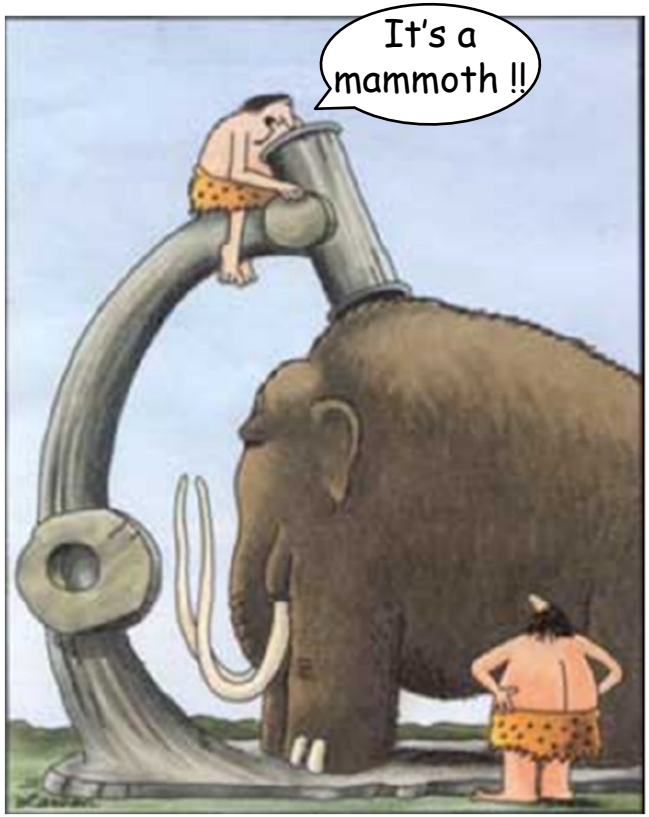
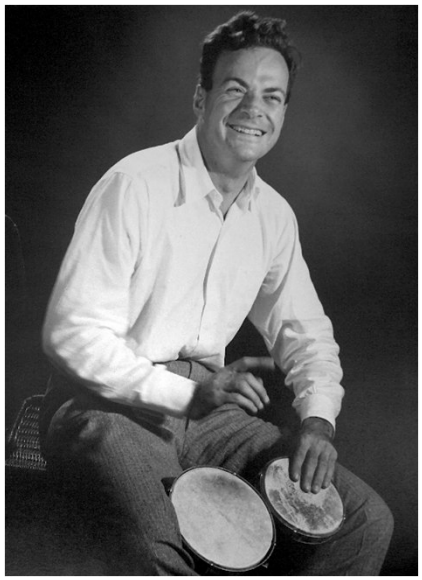
Cryo-EM: The "resolution revolution"

Early Microscopy



Unfortunately, the present [electron] microscope sees at a scale which is just a bit too crude.

Make the **microscope one hundred times more powerful**, and many problems of biology would be made very much easier.



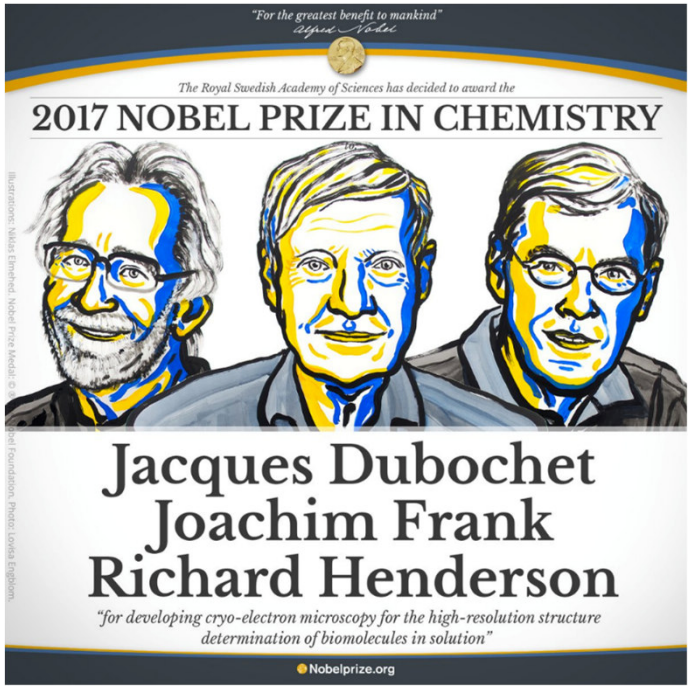
Richard P. Feynman

There's plenty of room at the bottom

(1959)

Cryo-EM: The "resolution revolution"

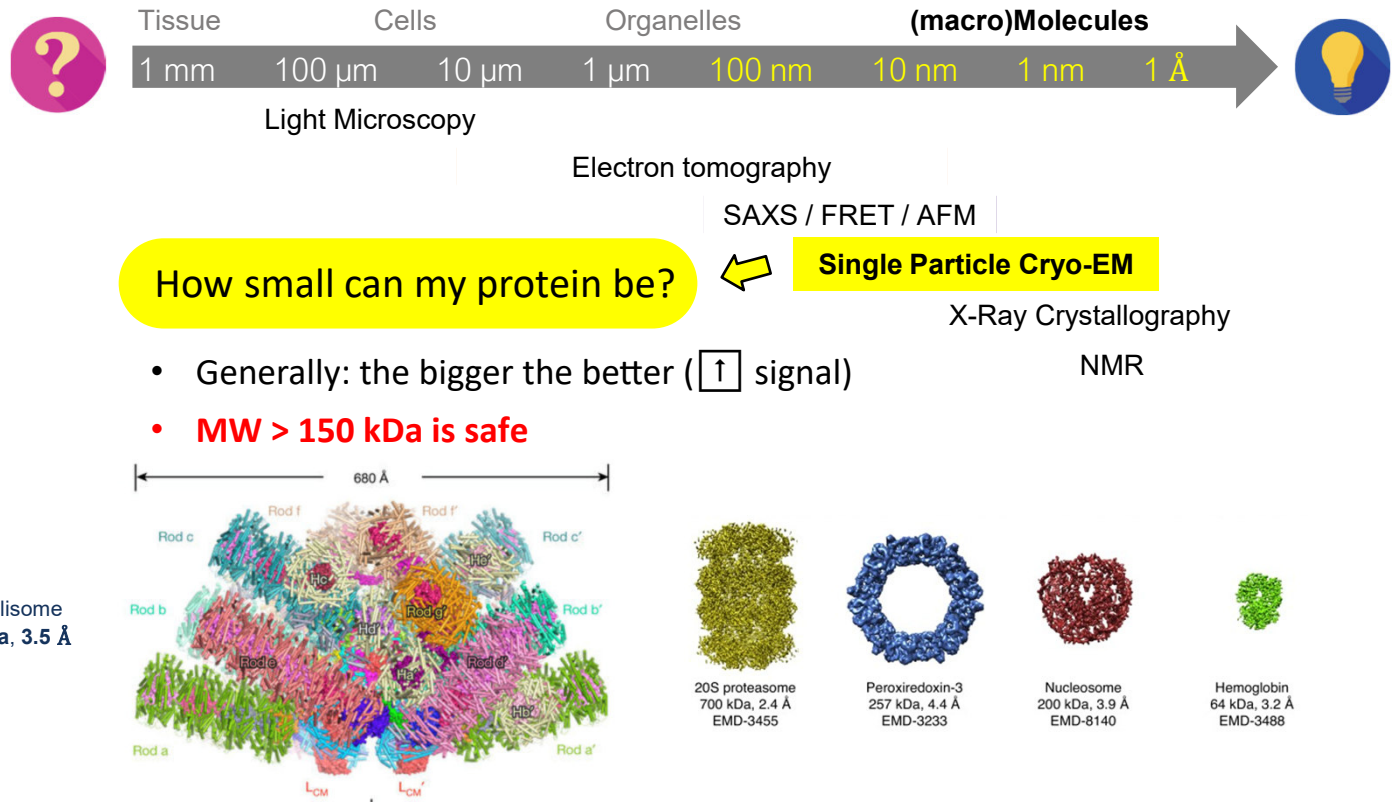
Modern Cryo-Electron Microscopy



...for developing cryo-electron microscopy for the high-resolution structure determination of biomolecules in solution...

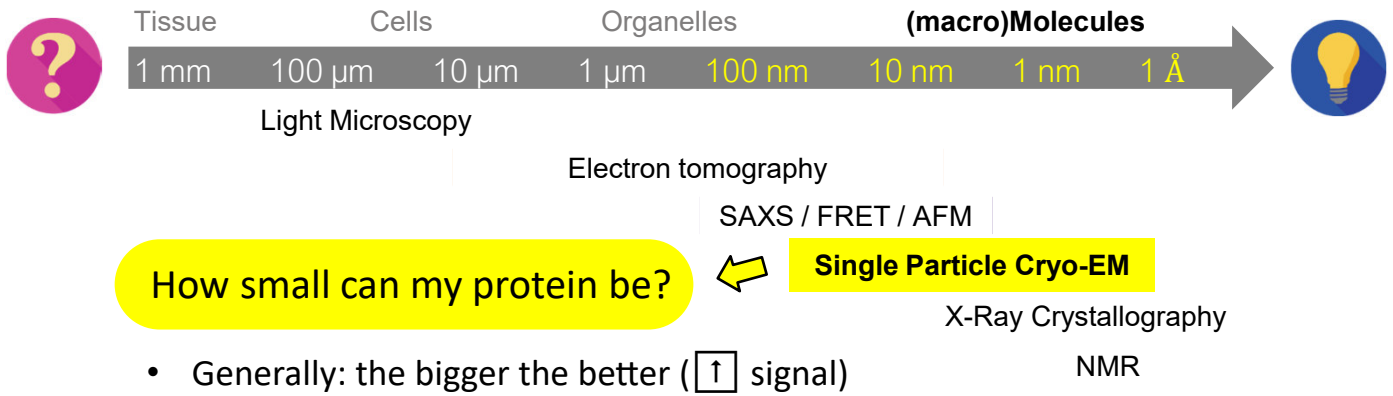
Cryo-EM: applications & limitations

For each biologically relevant question there is a biologically relevant resolution:



Cryo-EM: applications & limitations

For each biologically relevant question there is a biologically relevant resolution:



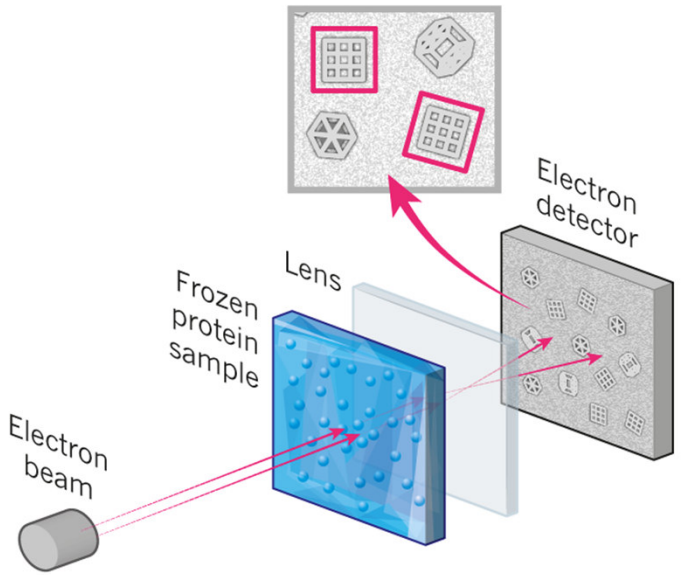
- Generally: the bigger the better (↑ signal)
- **MW > 150 kDa is safe**

- What is the structure of the complex?
- What is its stoichiometry/composition?
- How many conformations does it assume?
- Is the co-factor/substrate affecting its structure?
- …

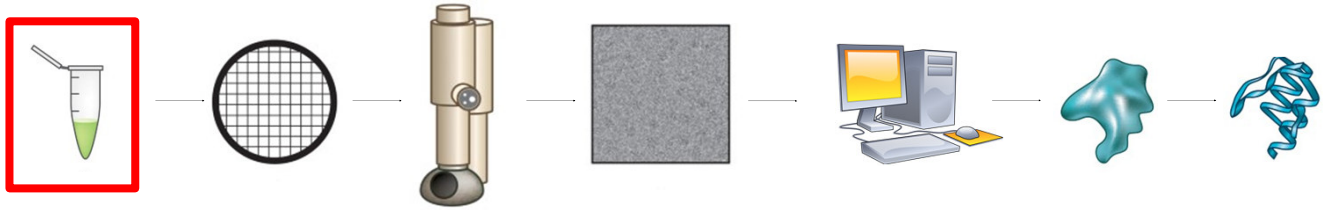
Cryo-EM: applications & limitations

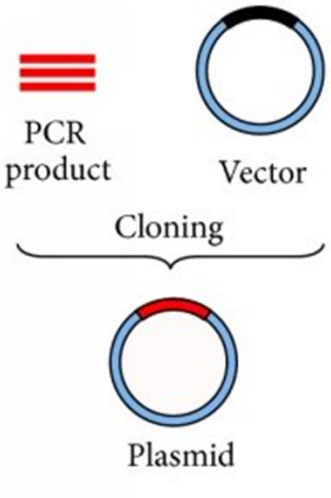
Cryo-Electron Microscopy applies Transmission Electron Microscopy (**TEM**) to study the structure of protein at cryogenic temperatures

- low concentrations of sample (few µg)
- It does not require protein crystals
- proteins are in their native status (no fixing agents or lattice constrains)
- from one sample multiple structures (conformers)
- It is fast! From the eppendorf to the 3-D structure in 24-48h



Cryo-EM: the sample

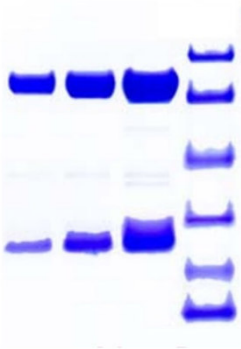




Construct design and cloning
(full-length, domains, mutants, …)

Heterologous
expression systems:
Bacteria
Yeast
Insect cells
Mammalian cells

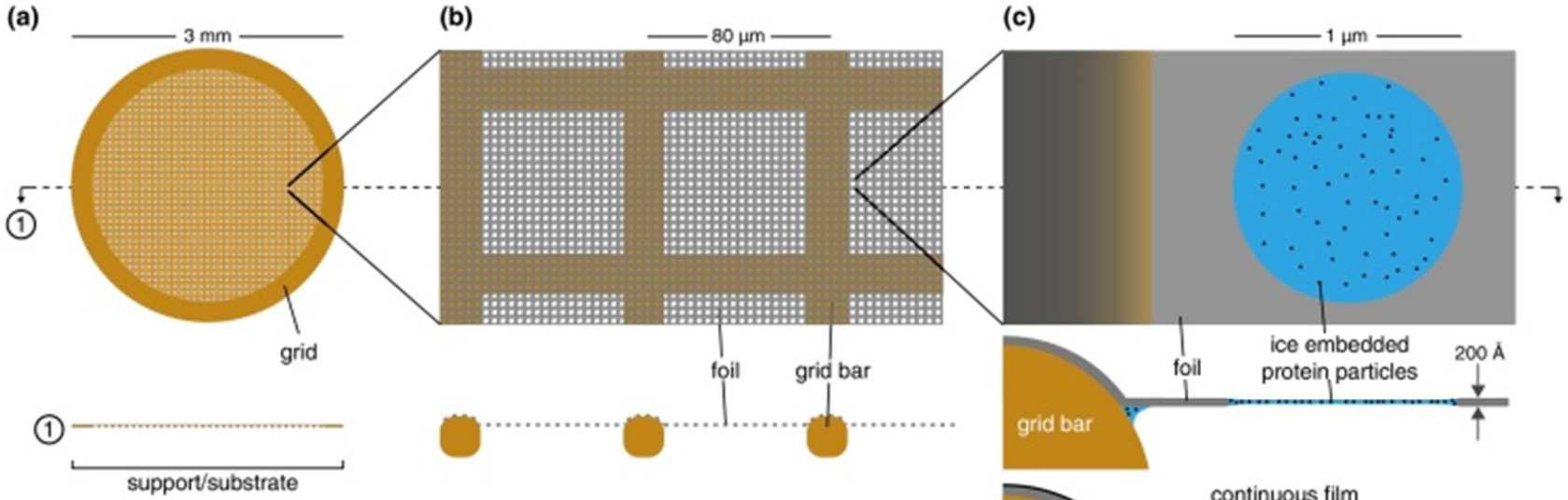
Purification:
affinity resins, tags, ..



Analysis:
SDS-Page
electrophoresis

µgs of pure protein
needed

Cryo-EM: the grid



Grid materials

Copper	**Gold**
Nickel	CuRh
Titanium	Molybdenum
Silicon	Aluminum
	Tungsten

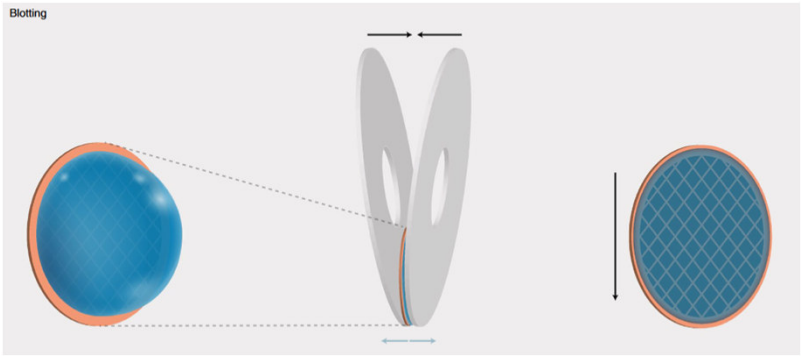
Foil materials

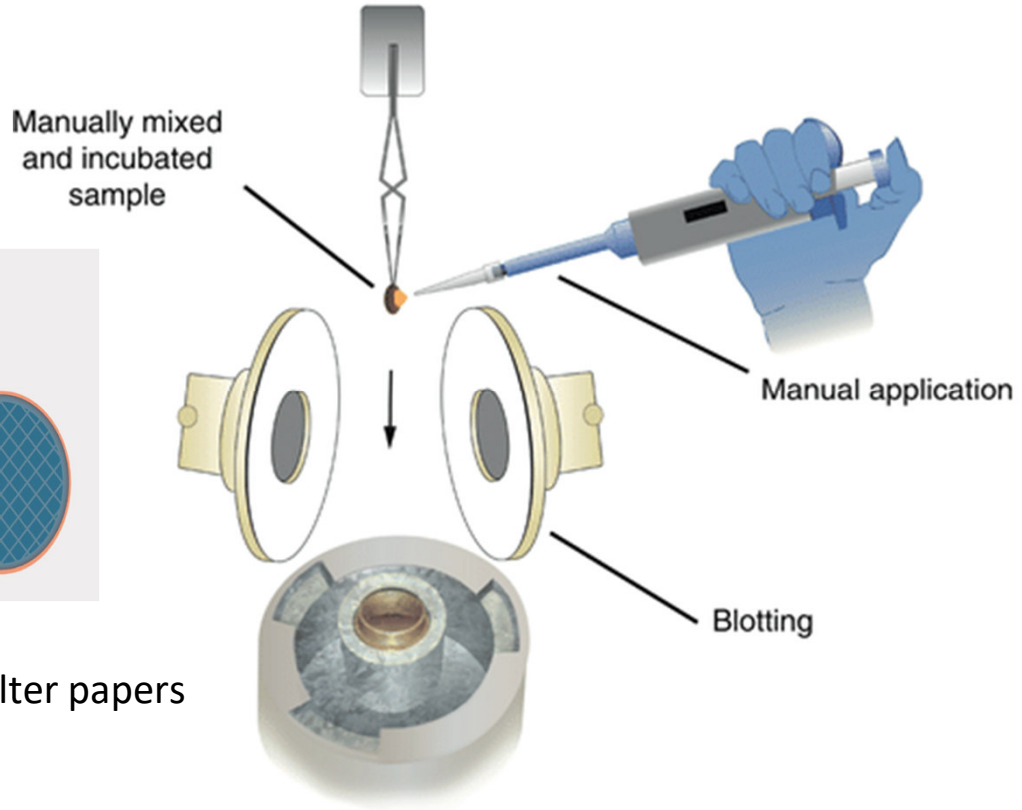
Amorphous carbon
Gold
TiSi SiN
SiO_2 SiC

Film materials

Amorphous carbon
Graphene
Graphene oxide
TiSi

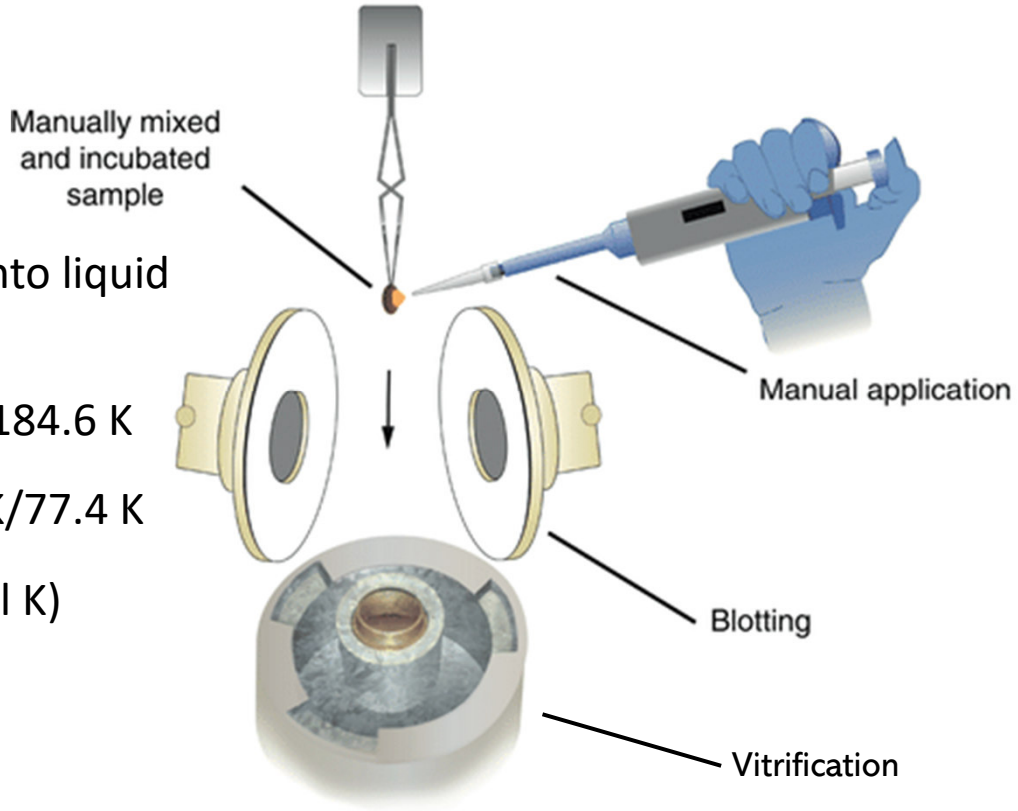
Cryo-EM: blotting and vitrification





Angular (not homogeneous) blotting with filter papers

Cryo-EM: blotting and vitrification

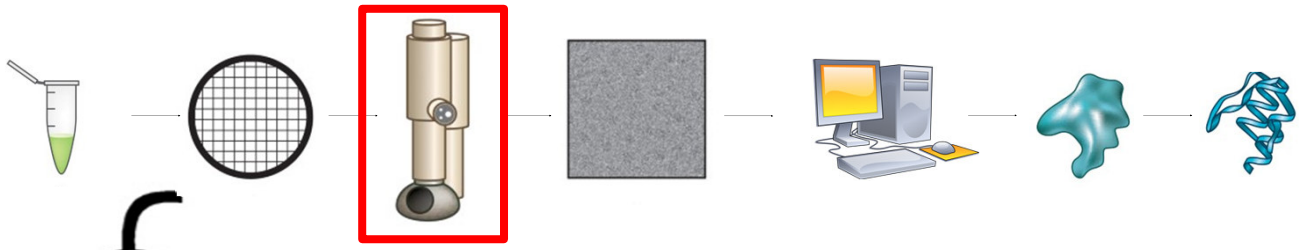


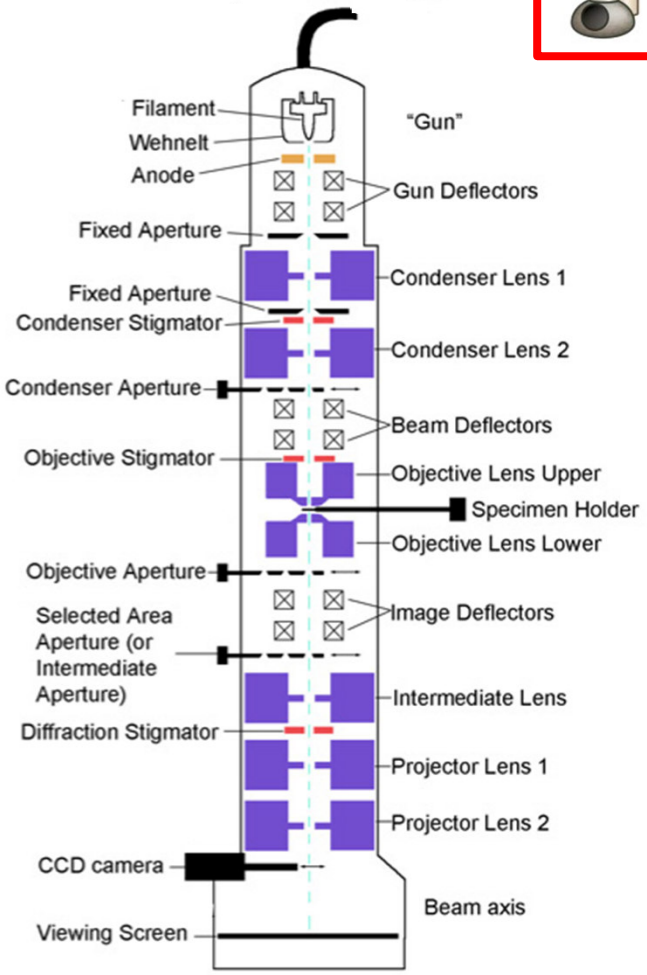
- The sample sample is plunged freeze into liquid ethane
- Ethane melting/boiling point is 90.4 K/184.6 K

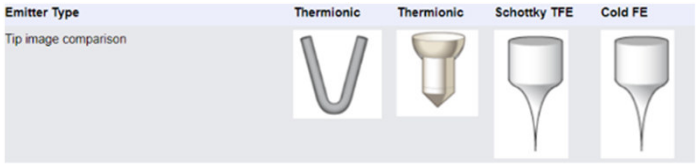
  Nitrogen melting/boiling point is 63.2 K/77.4 K
- Liquid ethane heat capacity (68.5 J/ mol K)

→ T drops faster than 10^5-10^6 K/s

Cryo-EM: the microscope "anatomy"



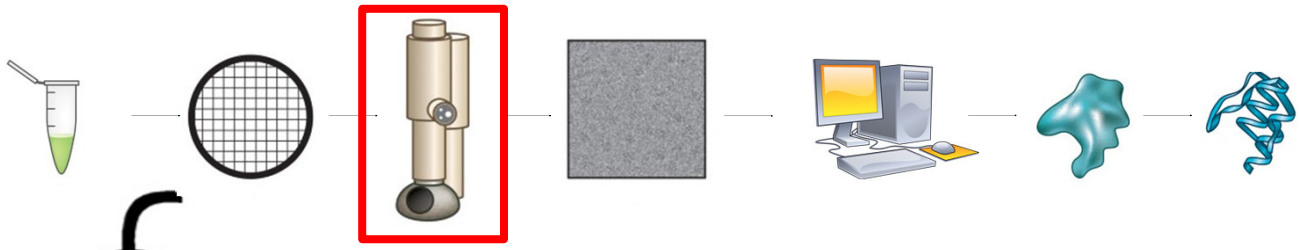


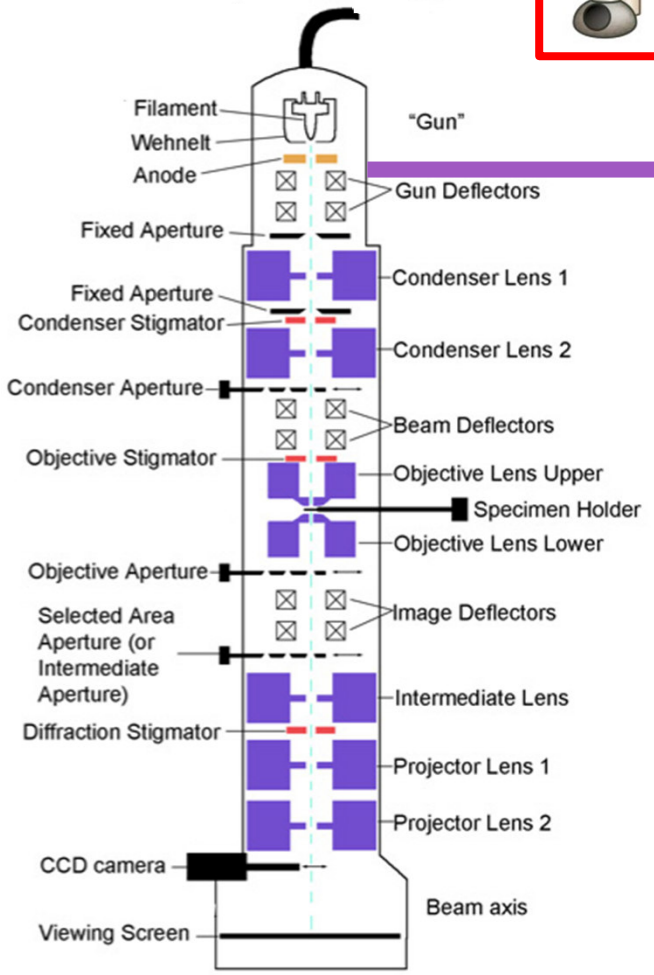


WHY ELECTRONS?

- Electrons are charged particles.
- Electron wavelength at 103 - 104 KeV is smaller than the average atomic dimension.
- They strongly interact with matter: electrons interact with the Coulomb potential of the atoms (nuclei and electron clouds).
- The atomic scattering amplitude for electrons is 103 – 104 times larger than the corresponding ones for X-rays and neutrons.
- Electron trajectories can be easily deflected by electro-magnetic fields (electron lenses)

Cryo-EM: the microscope "anatomy"

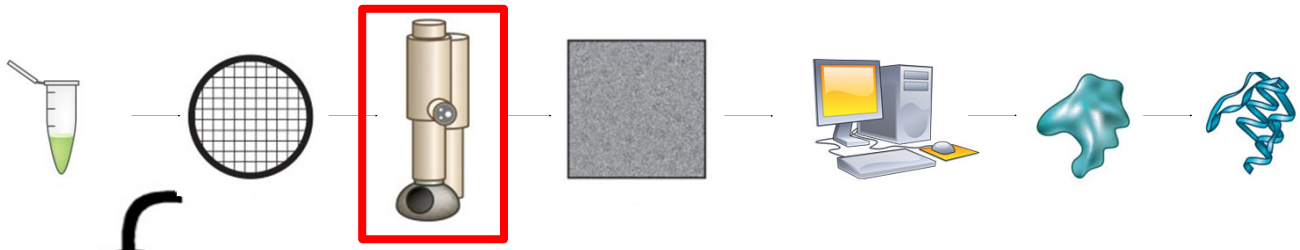


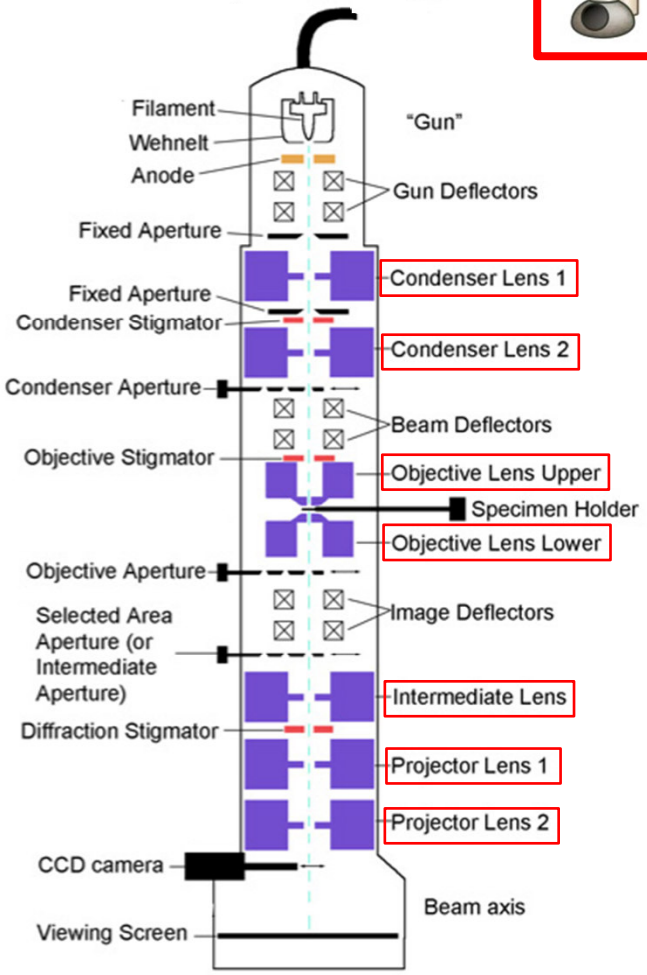


Accelerating voltage:

Accelerating voltage (kV)	Non-relativistic wavelength (nm)	Relativistic wavelength (nm)	Velocity ($\times 10^8$ m/s)
100	0.00386	0.00370	1.644
120	0.00352	0.00335	1.759
200	0.00273	0.00251	2.086
300	0.00223	0.00197	2.330
400	0.00193	0.00164	2.484
1000	0.00122	0.00087	2.823

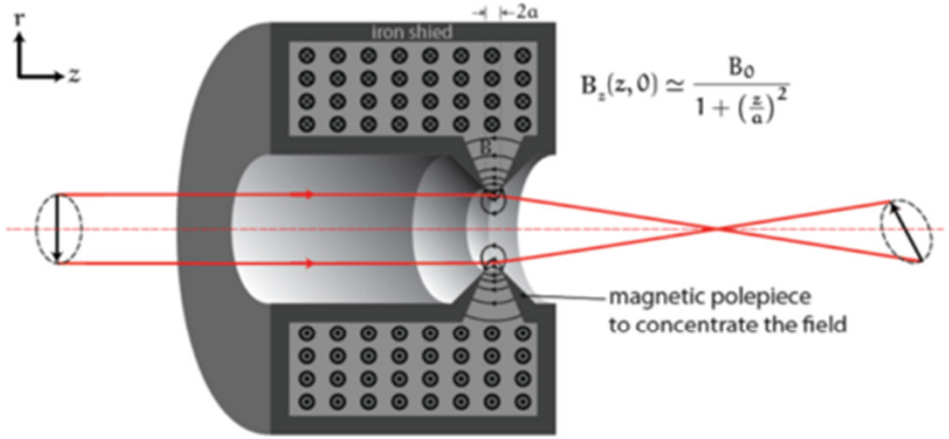
Cryo-EM: the microscope "anatomy"



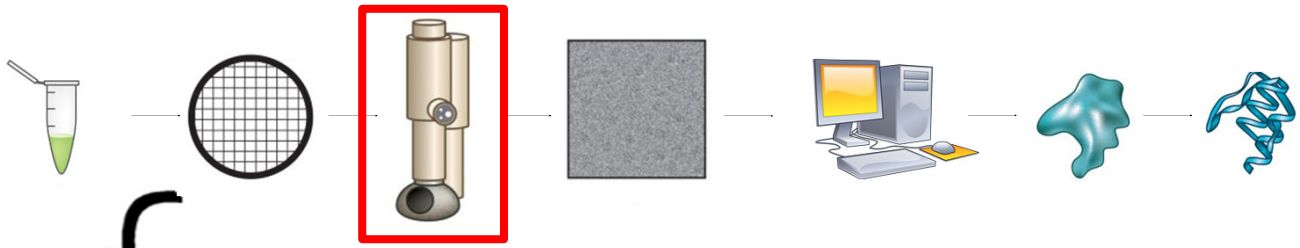


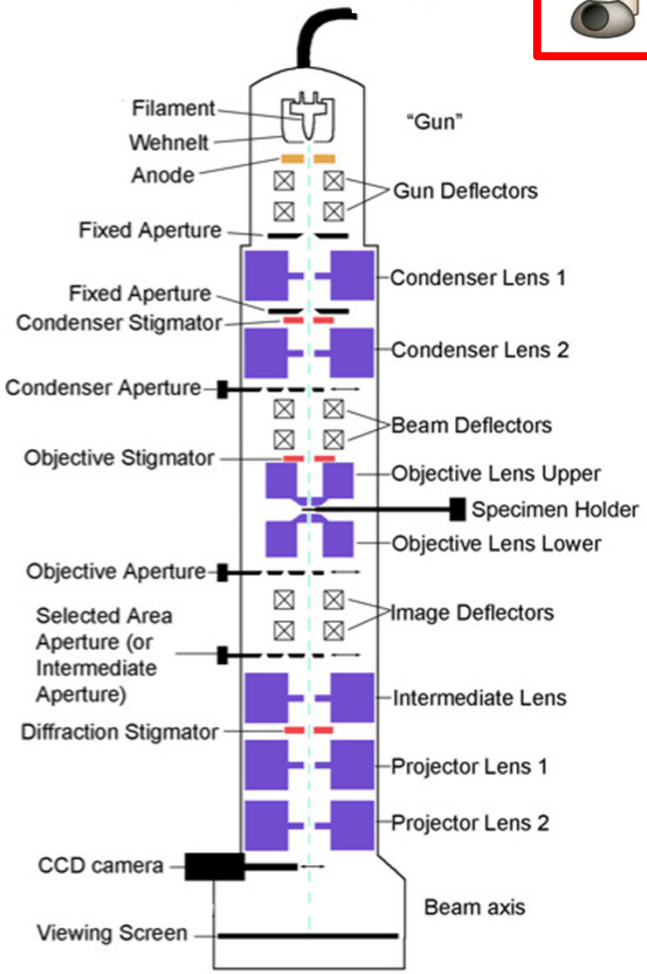
A current through a coiled wire creates a magnetic field that bends the e- trajectory

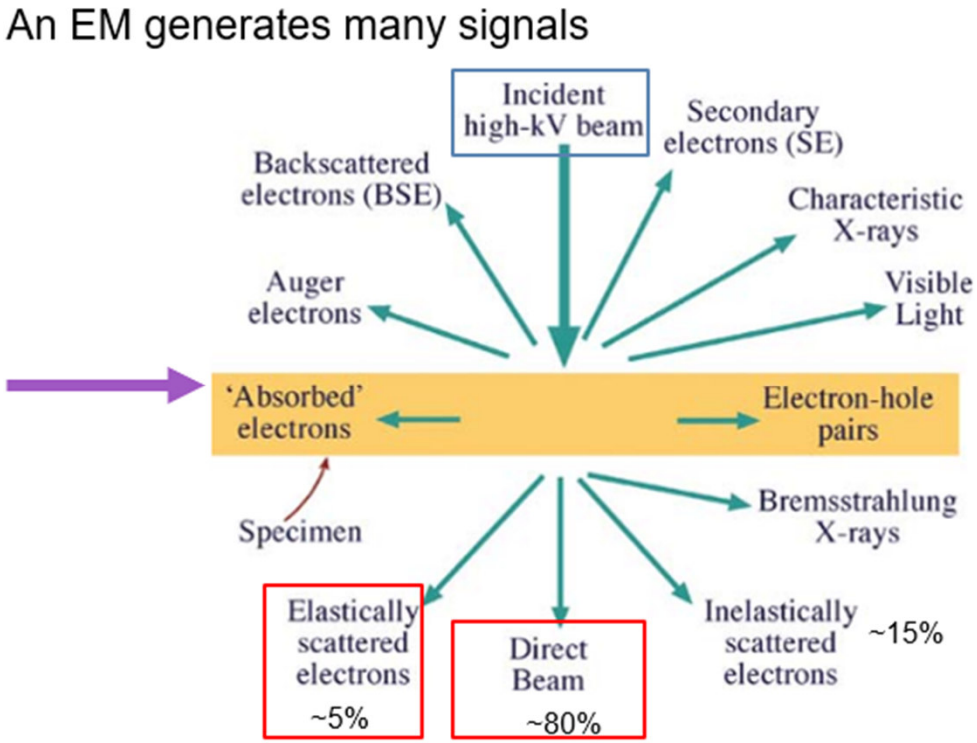
The helical trajectory flips the image at the focal point



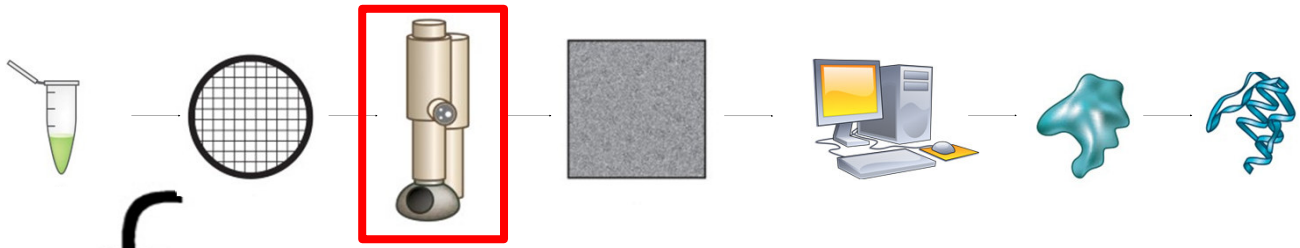
Cryo-EM: the microscope "anatomy"

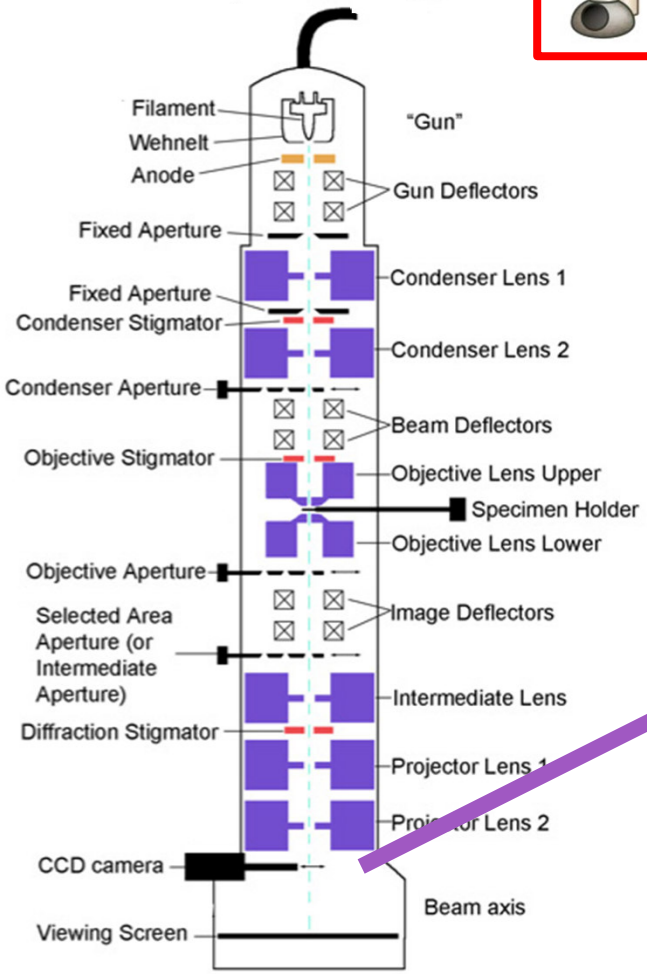




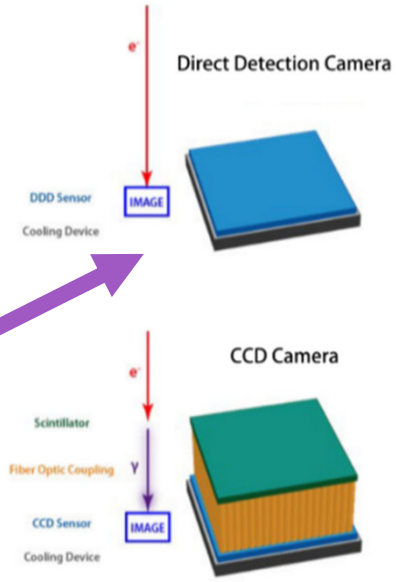


Cryo-EM: the microscope "anatomy"

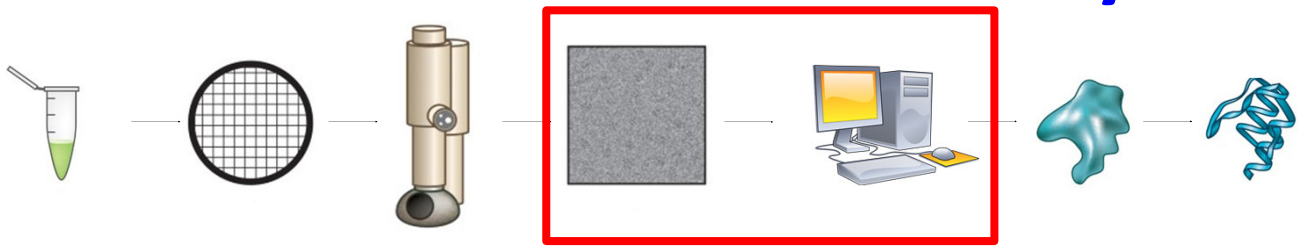




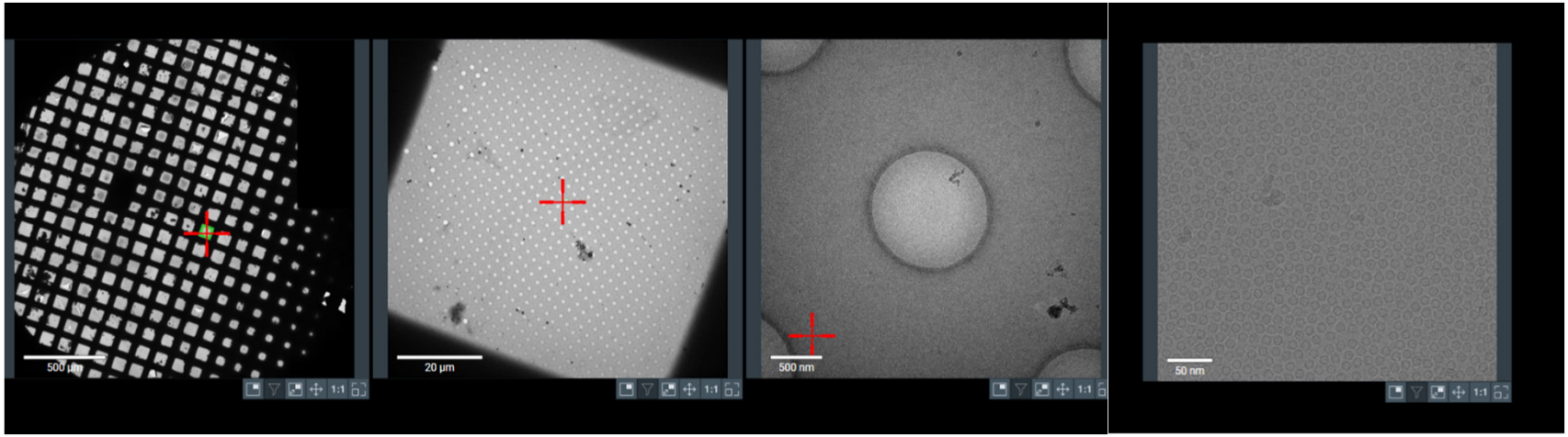
- DED has faster readout and reduced noise
- Movies instead of Images
- Electron counting mode



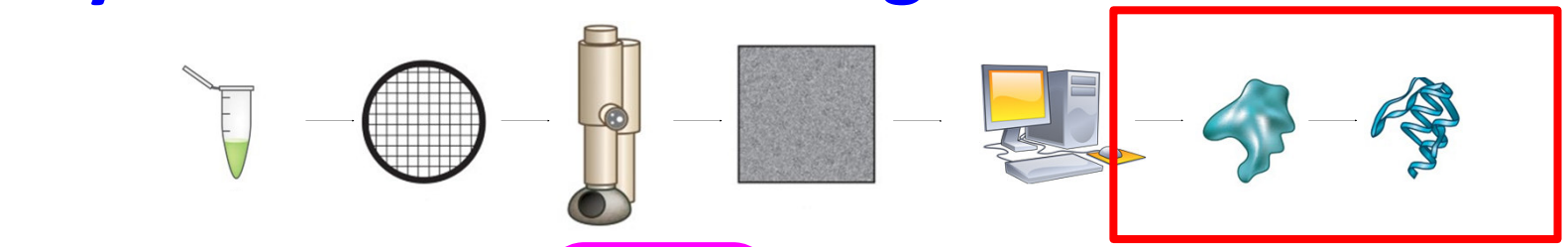
Cryo-EM: data collection and analysis

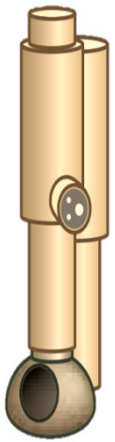


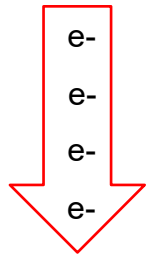
ATLAS ACQUISITION → SQUARE SELECTION → HOLE SELECTION → IMAGING

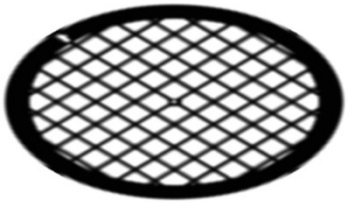


Cryo-EM: model building

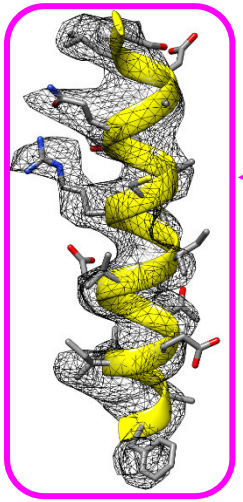


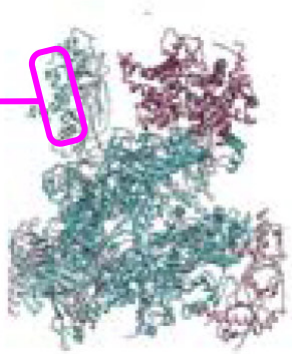






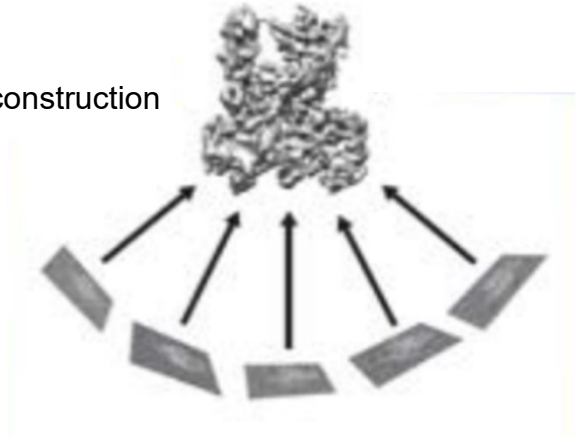
EM grid



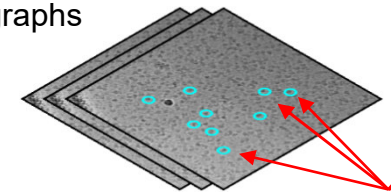


3D Model

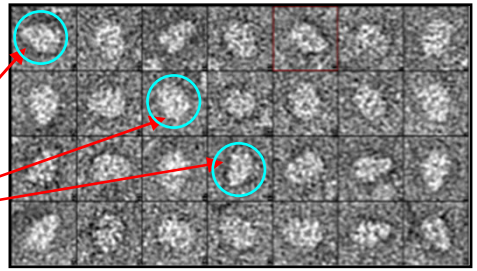
3D Reconstruction



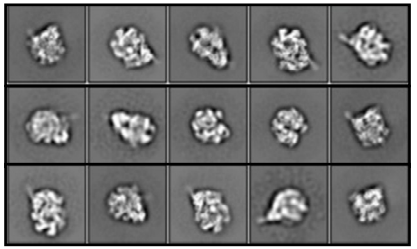
Micrographs



Particles

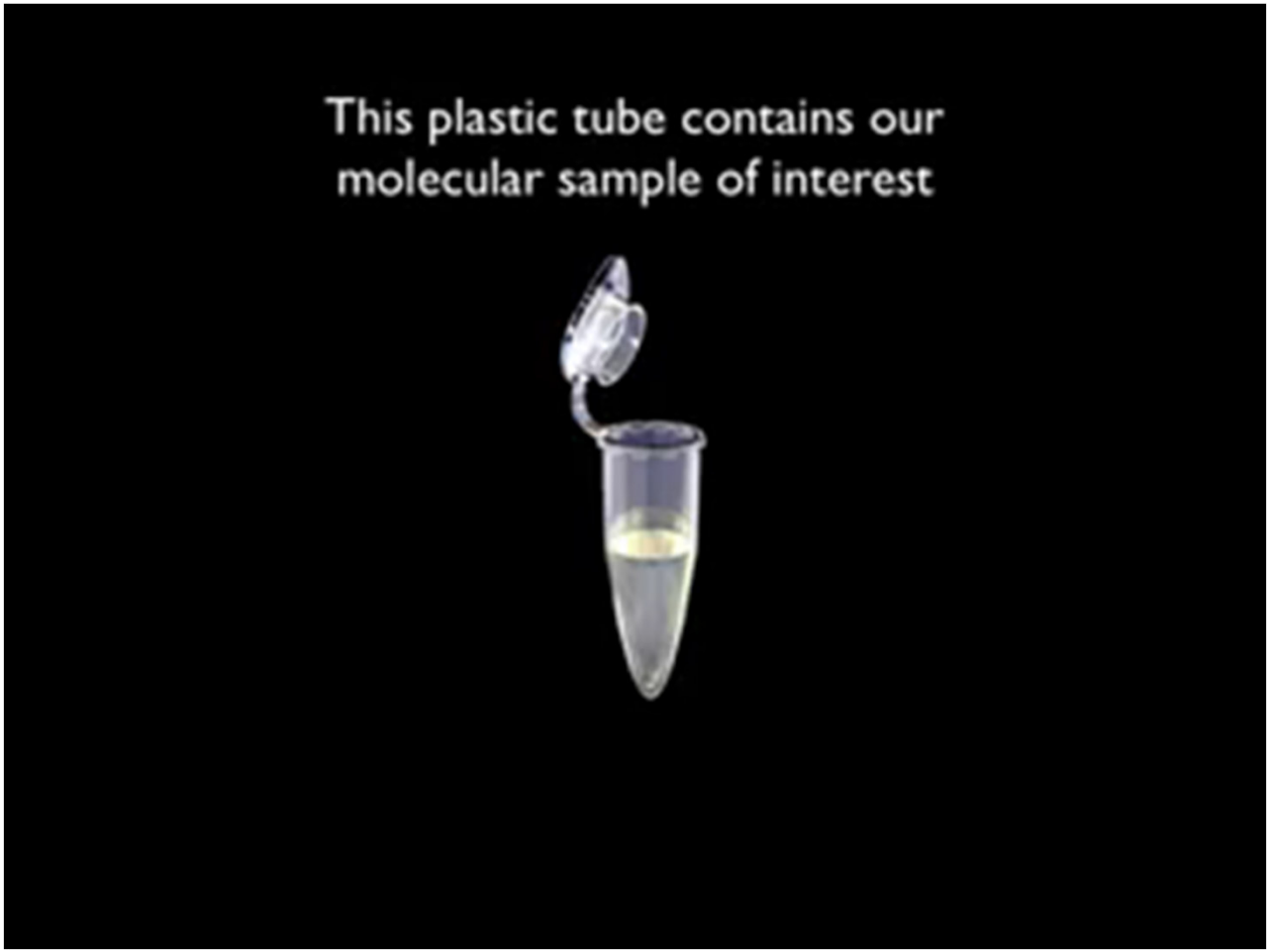


alignment & averaging



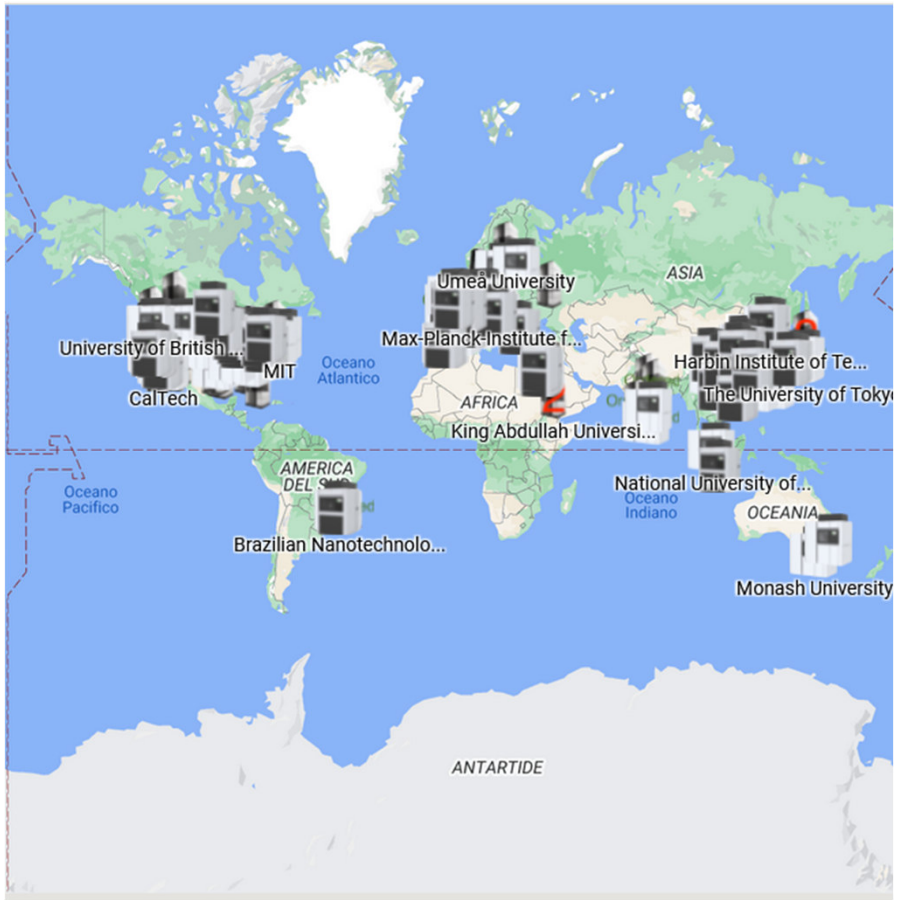
2D Classification

Cryo-EM in 3 minutes



Cryo-EM at the Synchrotron

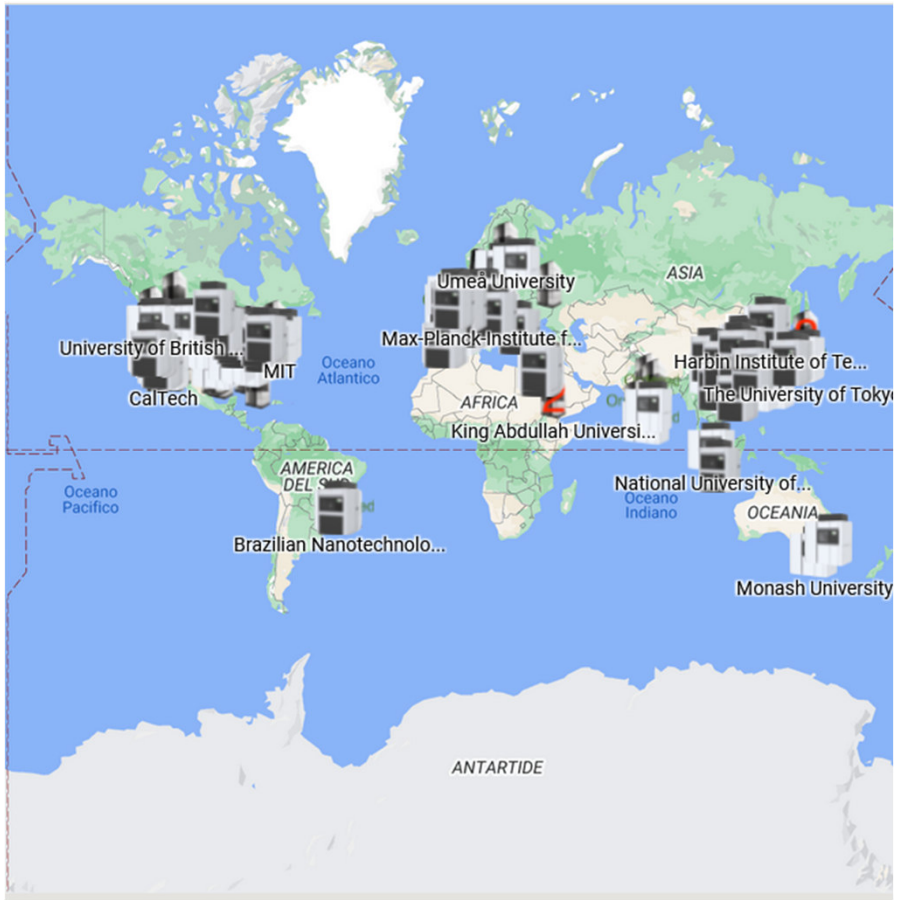
Cryo-EM microscopes (1040)



Cryo-EM research centers (1149)

Cryo-EM at the Synchrotron

Cryo-EM microscopes (1040)



Cryo-EM at the Synchrotron

- National Synchrotron Radiation Centre SOLARIS (Kraków)
- IBMB-CSIC and the ALBA Synchrotron (Barcelona)
- ESRF Grenoble - The European Synchrotron Radiation Facility
- Synchrotron SOLEIL (Paris)
- Electron Bio-Imaging Centre (eBIC) at Diamond (Didcot, Oxfordshire)
- RIKEN SPring-8 Center CryoEM Facility
- Kurchatov Institute (Moscow)

Cryo-EM: future

Cryo-Electron Microscopy Market Report: Trends, Forecast and Competitive Analysis to 2030

Report | 150 Pages | October 2023 | Region: Global | Lucintel | ID: 5910209

Cryo-Electron Microscopy Trends and Forecast

The future of the global cryo-electron microscopy market looks promising with opportunities in the life science research & academia, cancer research, omics research, pharma & biotech manufacturing, cell & gene therapy, vaccines, preclinical & clinical research, and healthcare/medical applications markets. The global cryo-electron microscopy market is expected to reach an estimated $2.72 billion by 2030 with a CAGR of 12.4% from 2024 to 2030. The major drivers for this market are increasing demand for high-resolution structural biology data, on-going technological advancements in cryo-EM instrumentation and software, and growing healthcare expenditure across the globe.

Cryo-EM @UNIMI

UNIVERSITÀ DEGLI STUDI DI MILANO
LA STATALE

FONDAZIONE ROMEO ED ENRICA INVERNIZZI

- **May 2016**: first *press release* of the newly purchased microscope TALOS Arctica

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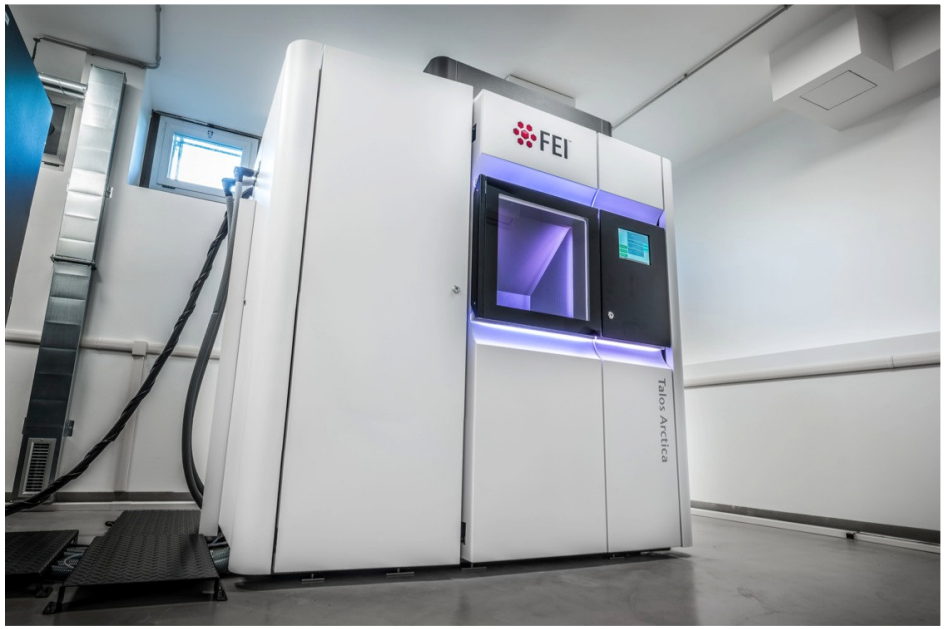
«CONSENTE DI SCRUTARE IL MONDO ALL'INTERNO DELLA CELLULA»

Il super microscopio da 3 milioni di euro alla Statale di Milano

È il primo del genere in Italia. Funziona a temperature bassissime ed è in grado di osservare singole molecole. È riuscito a «fotografare» il virus Zika

di Giovanni Caprara

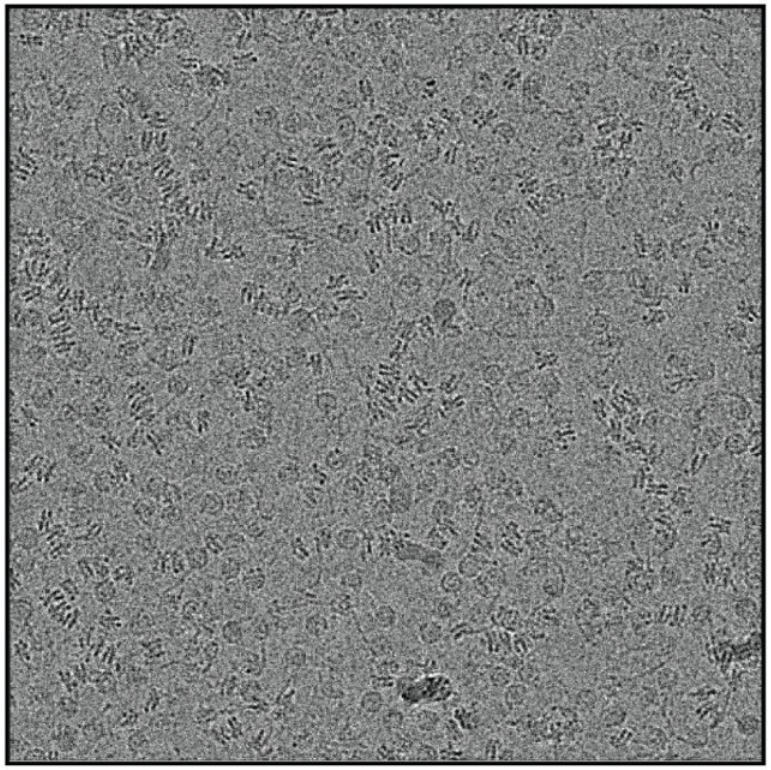


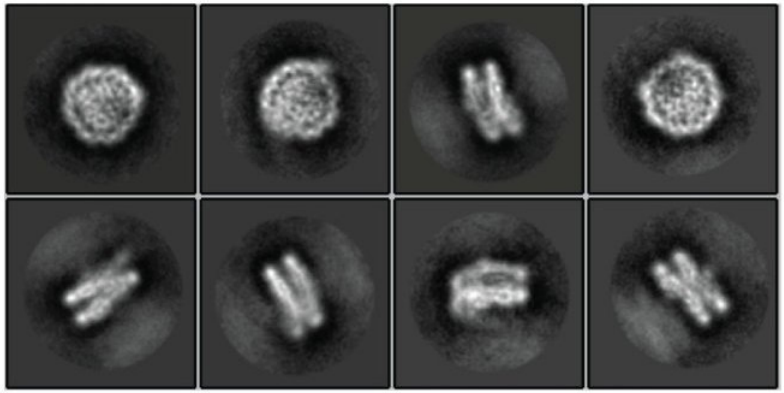
MAY 2017

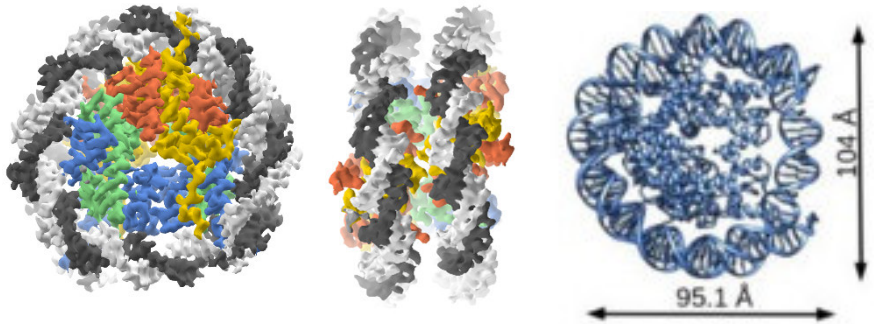
http://users.unimi.it/biolstru/em.html

http://crcpediatrico.org/index.html

Cryo-EM @UNIMI







200 kDa → 75 kDa