

Introduction to Crystallography

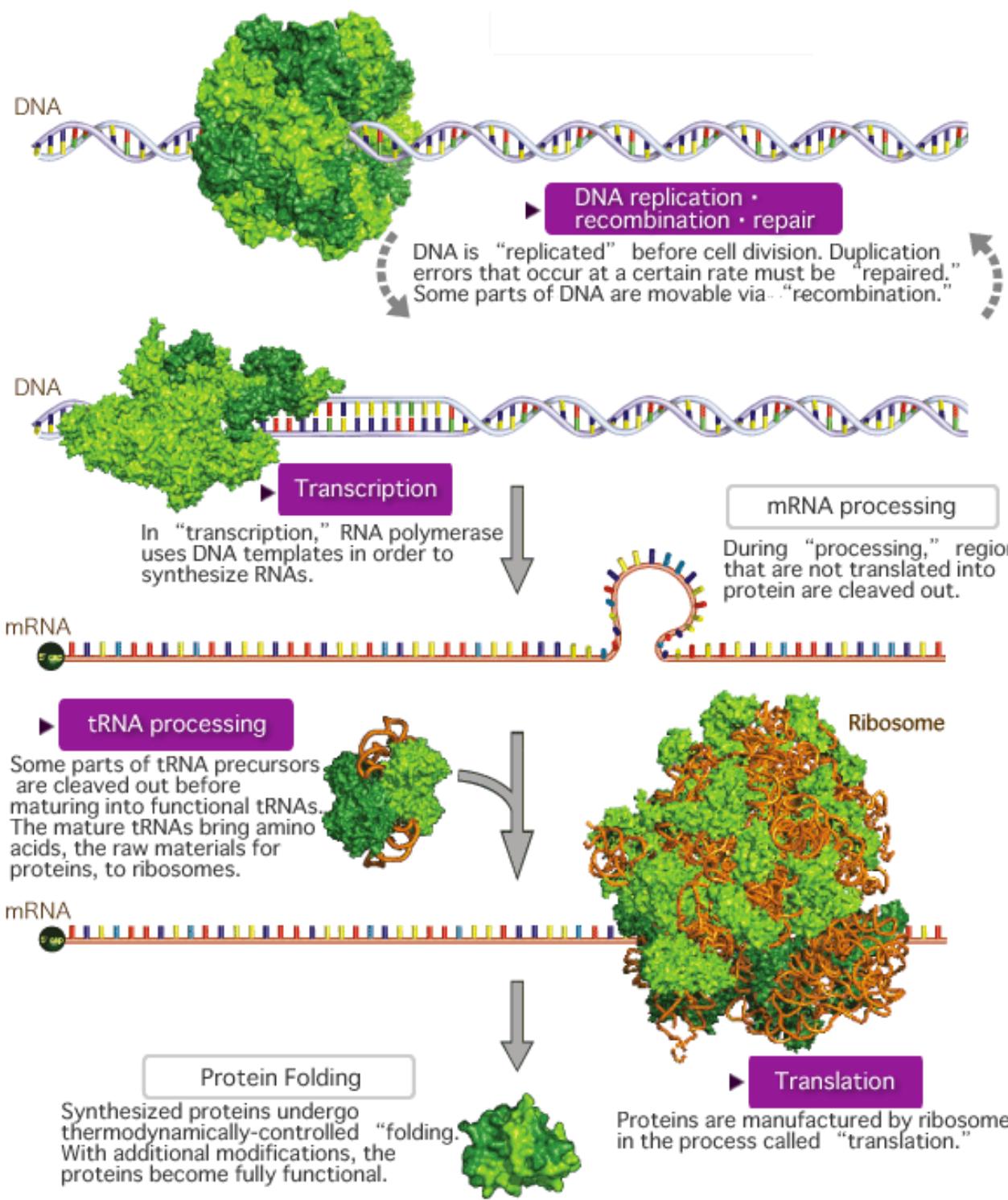
Pedro Alzari

Structural Microbiology

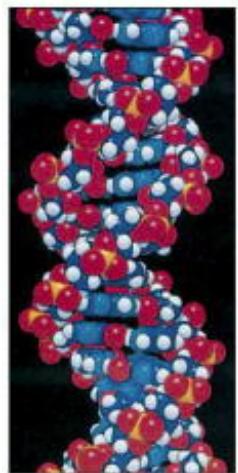


Bioinformatics and Genome Analyses Course
Institut Pasteur Tunis, Tunisia. September 18 – December 15, 2017

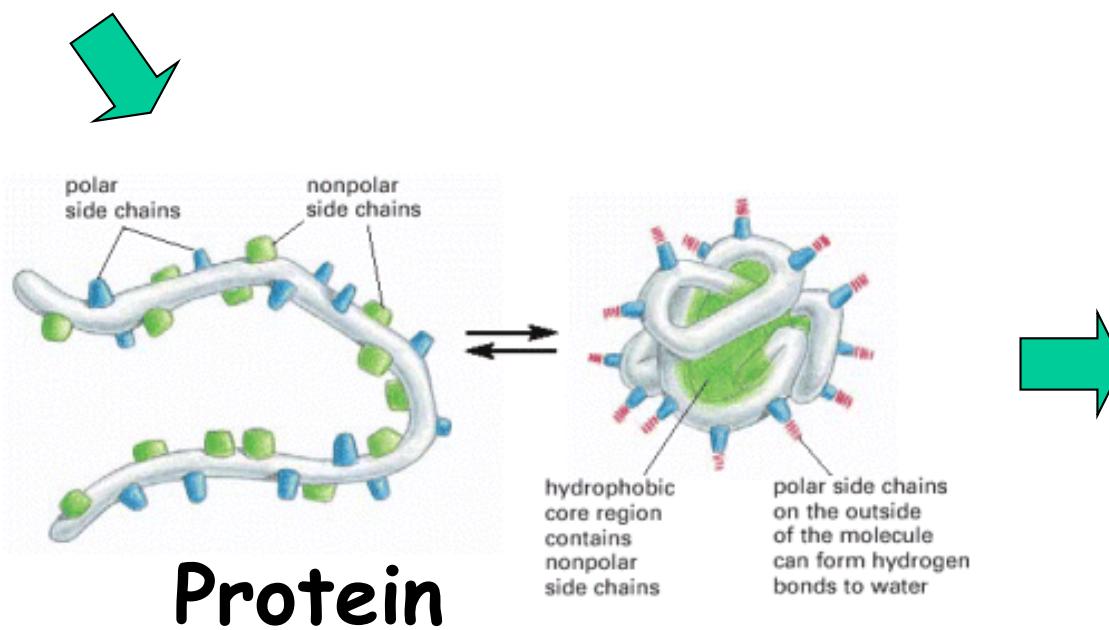
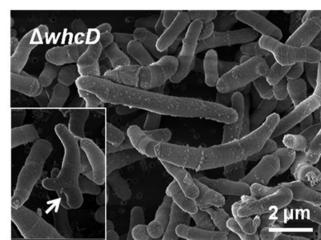
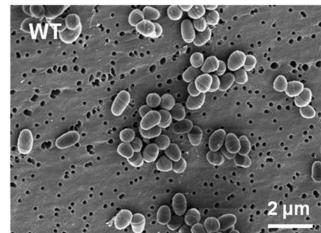
Institut Pasteur



The Central Dogma (F. Crick, 1958)



Gene
(information)



Phenotype
(observable)

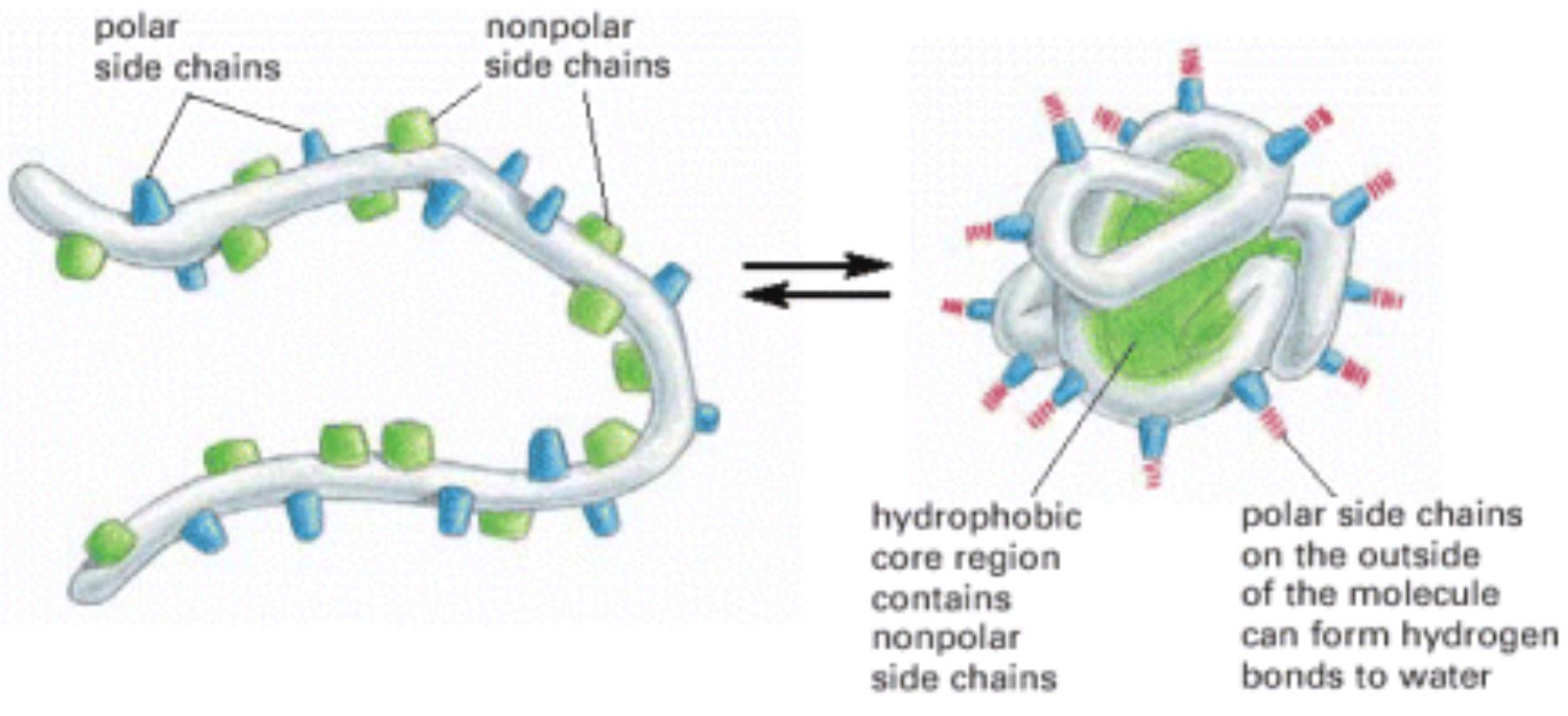


Biological
function



Biochemical
function
(interactions)

Protein folding, an unsolved problem



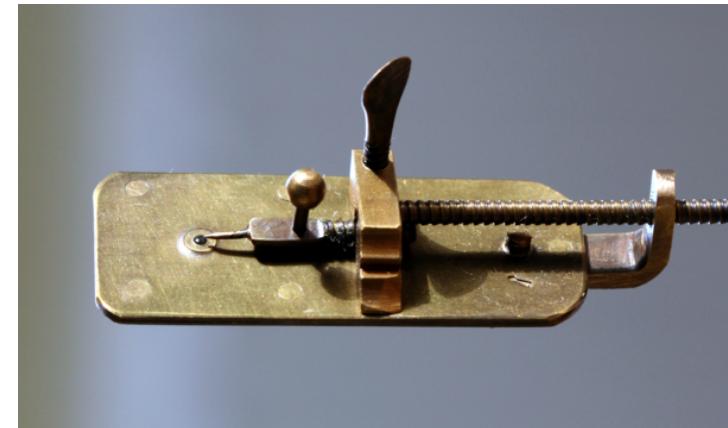
Amino acid sequence

Structure 3D

Microscopy in biology



Anton van Leeuwenhoek
(1632-1723)

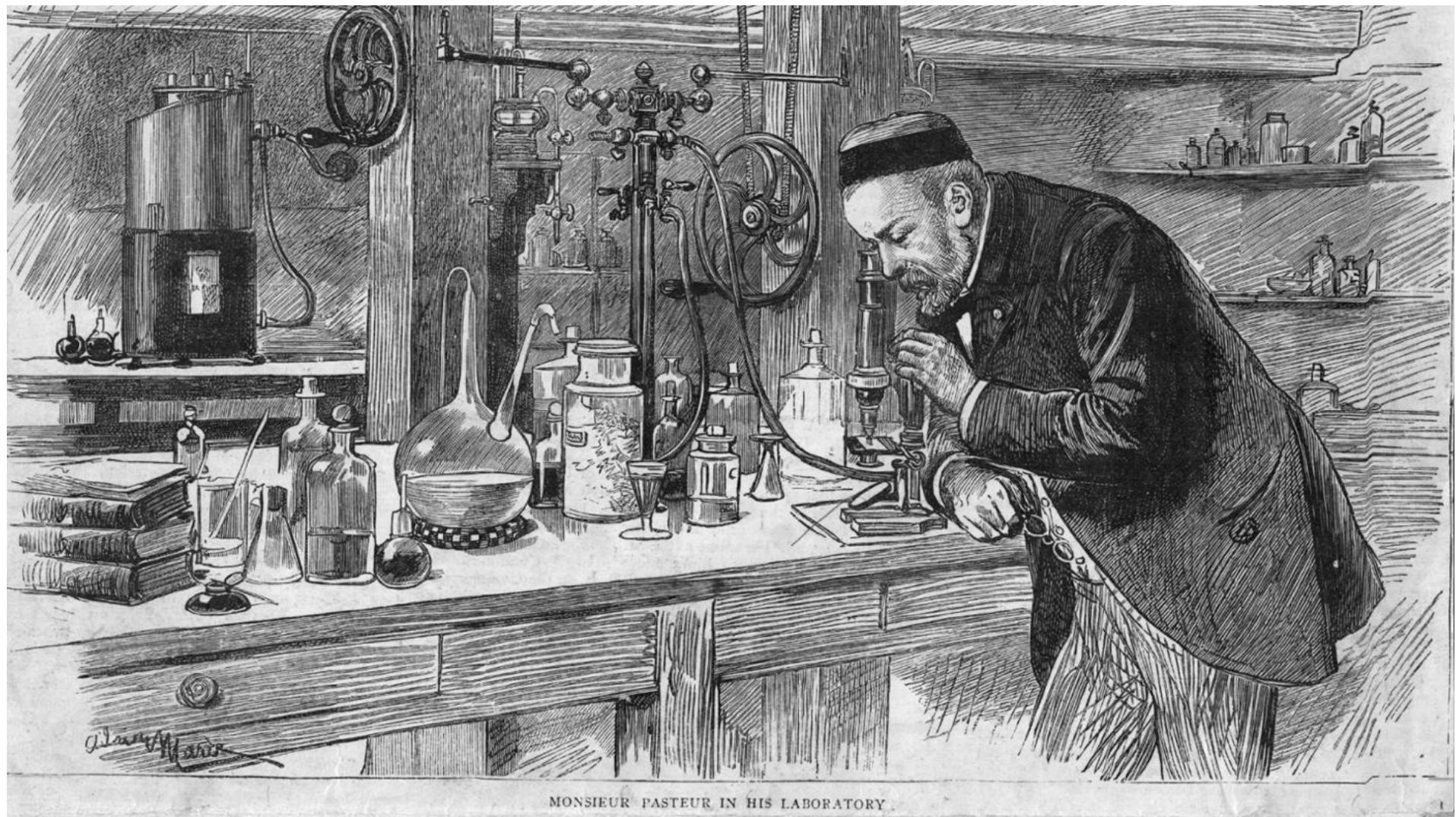


Leeuwenhoek's microscope

PLATE XXIV

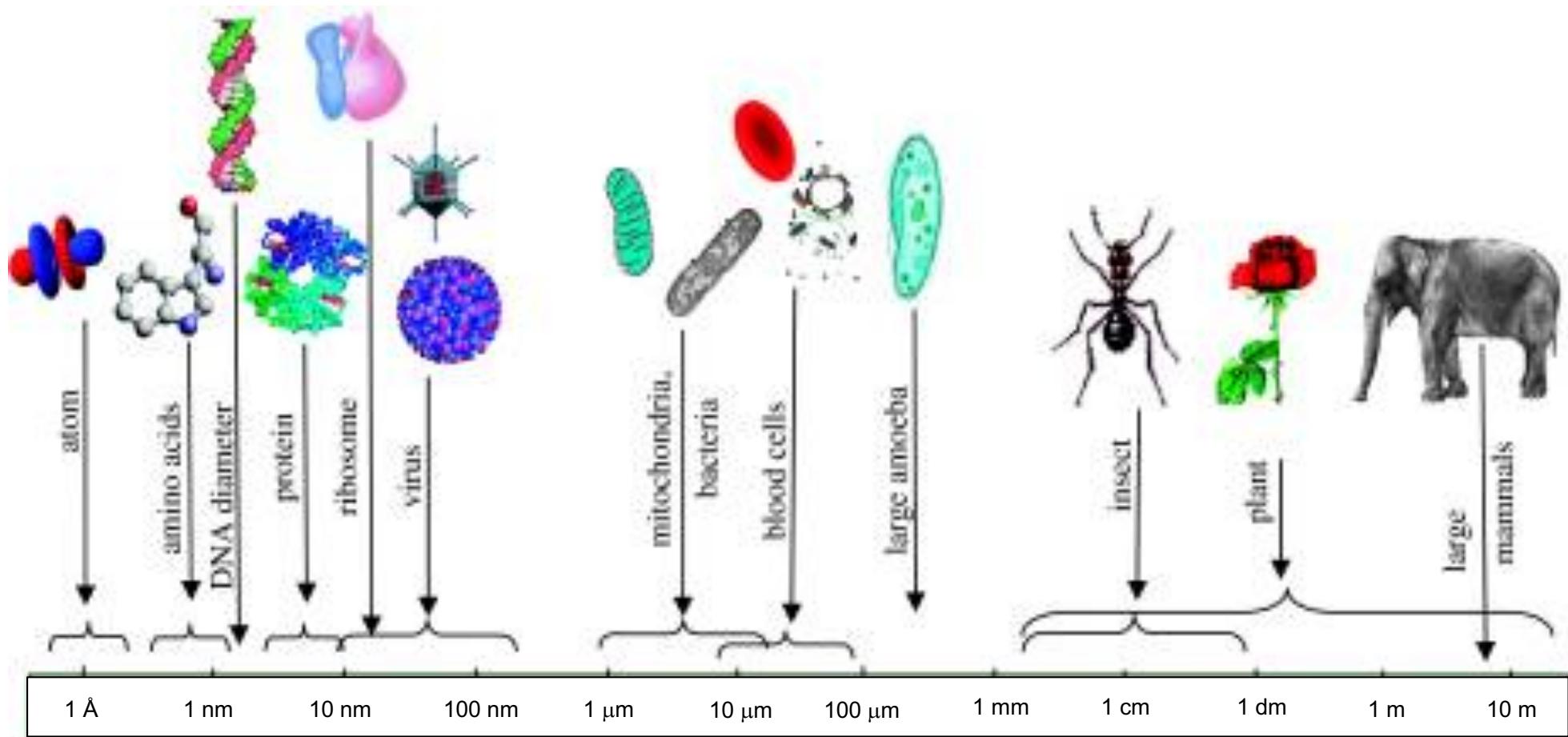


Leeuwenhoek's 'animalcules'

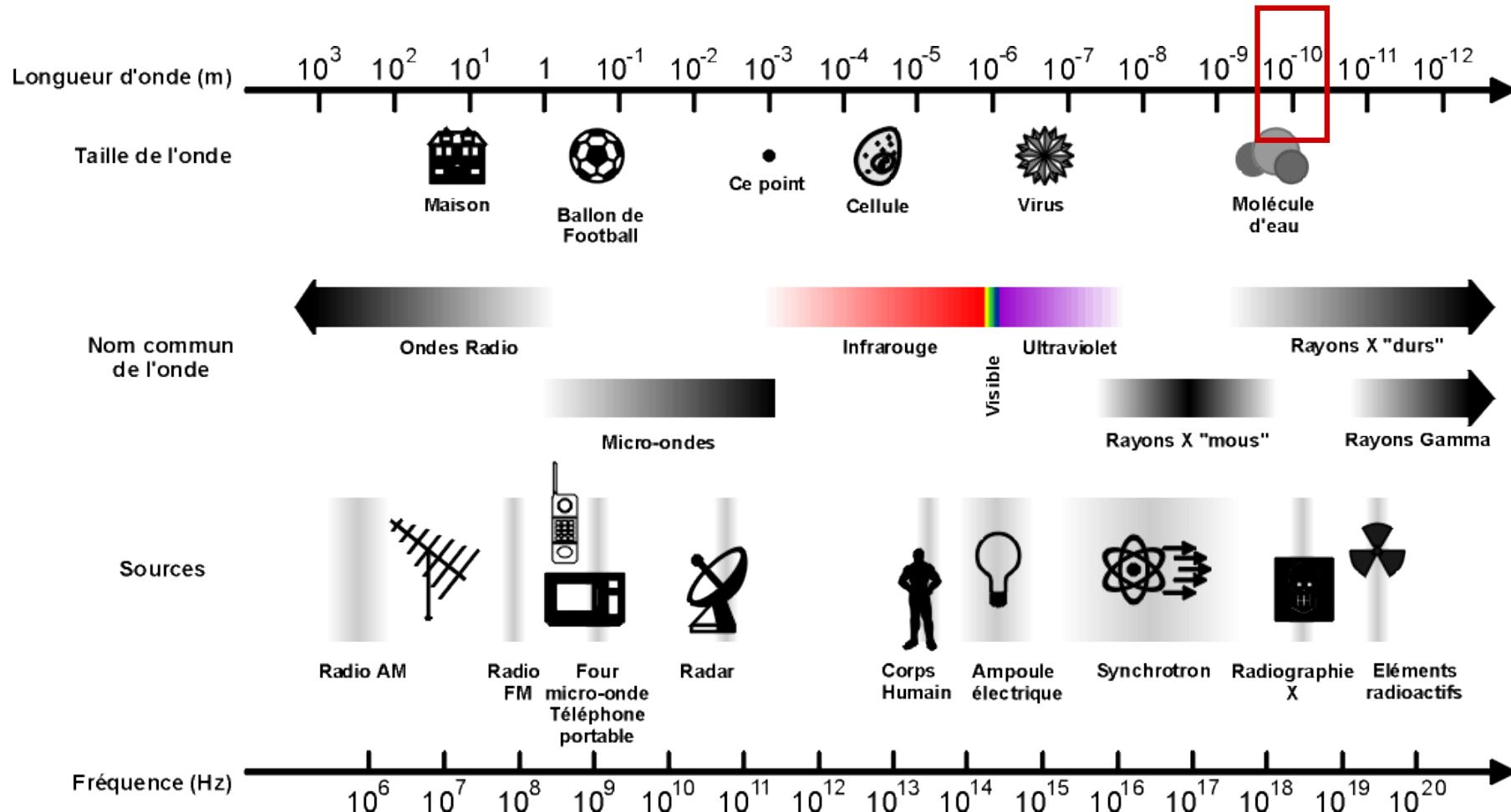


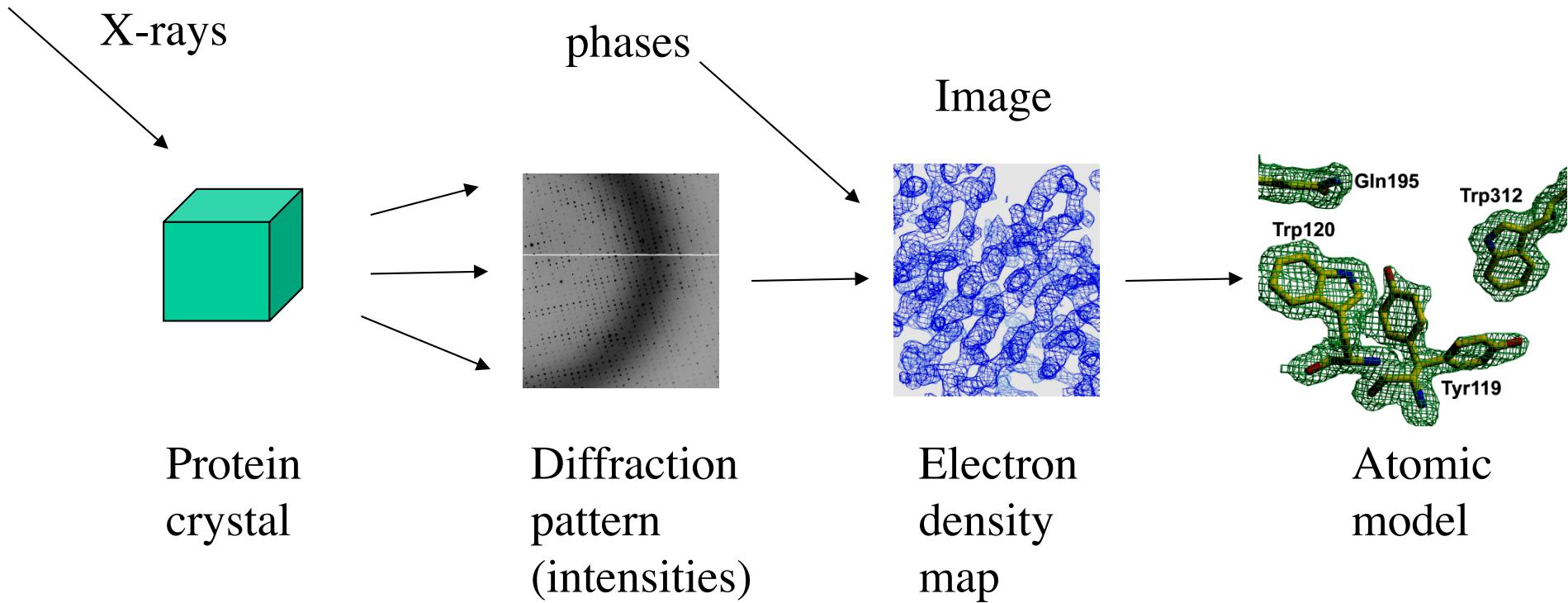
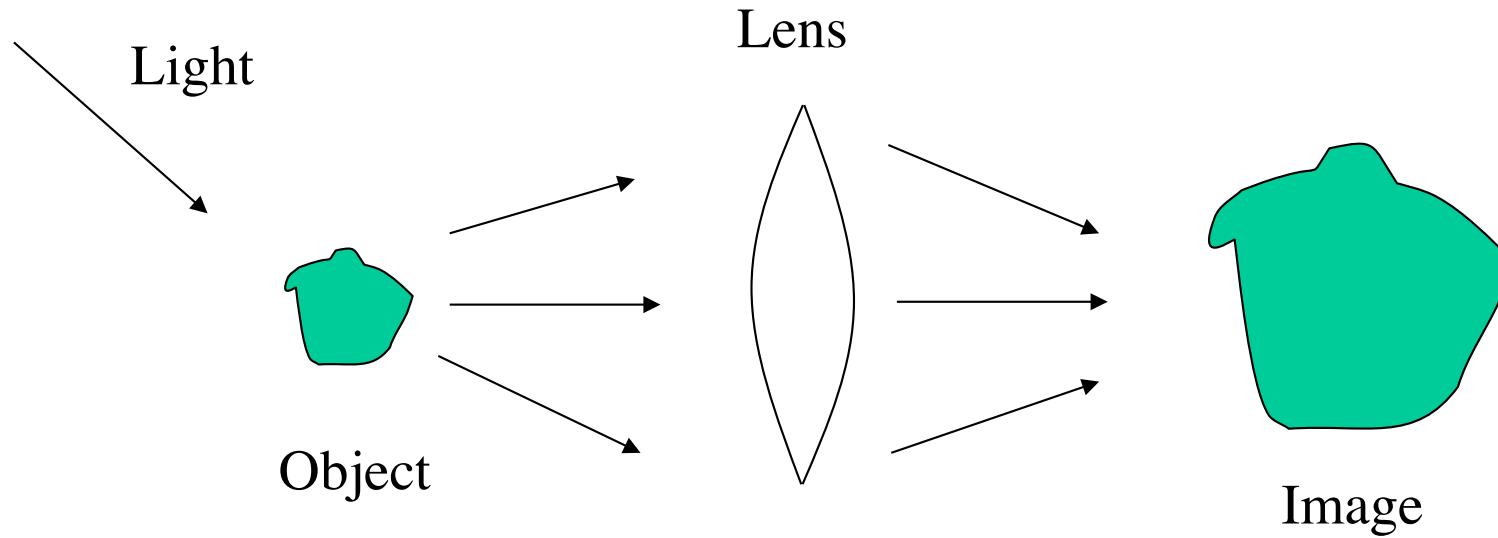
MONSIEUR PASTEUR IN HIS LABORATORY

Size and scale



$$1\text{ \AA} = 0.1\text{ nm}$$





Light

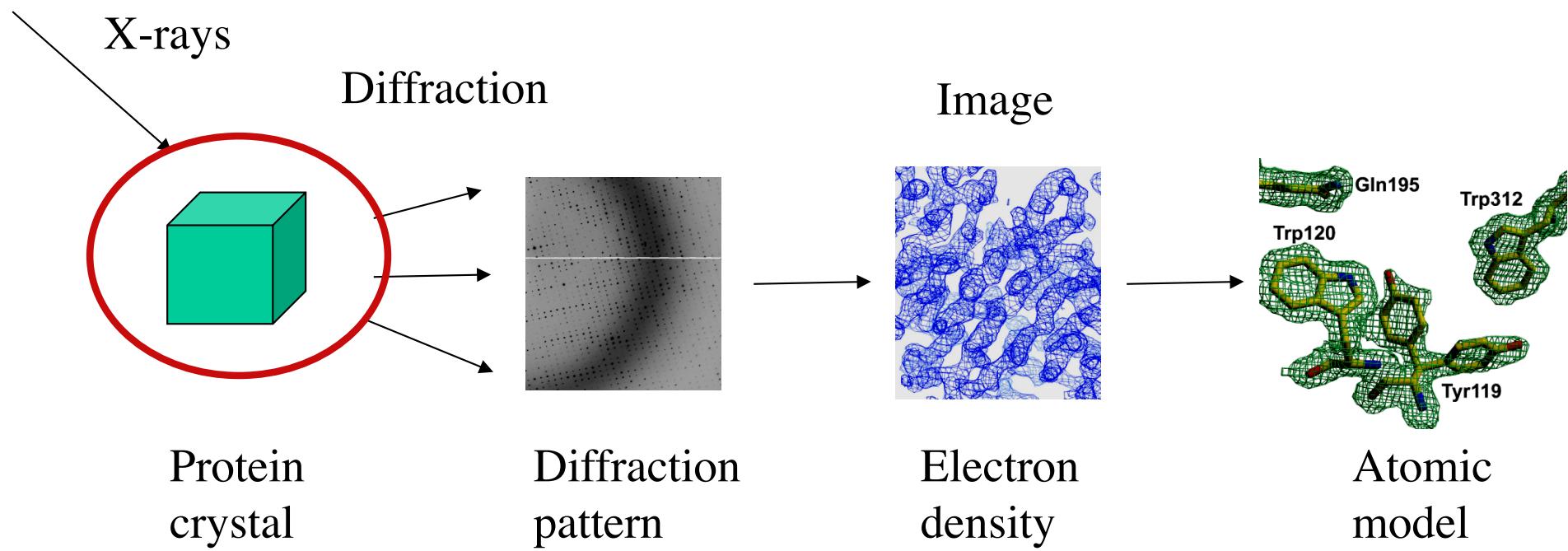
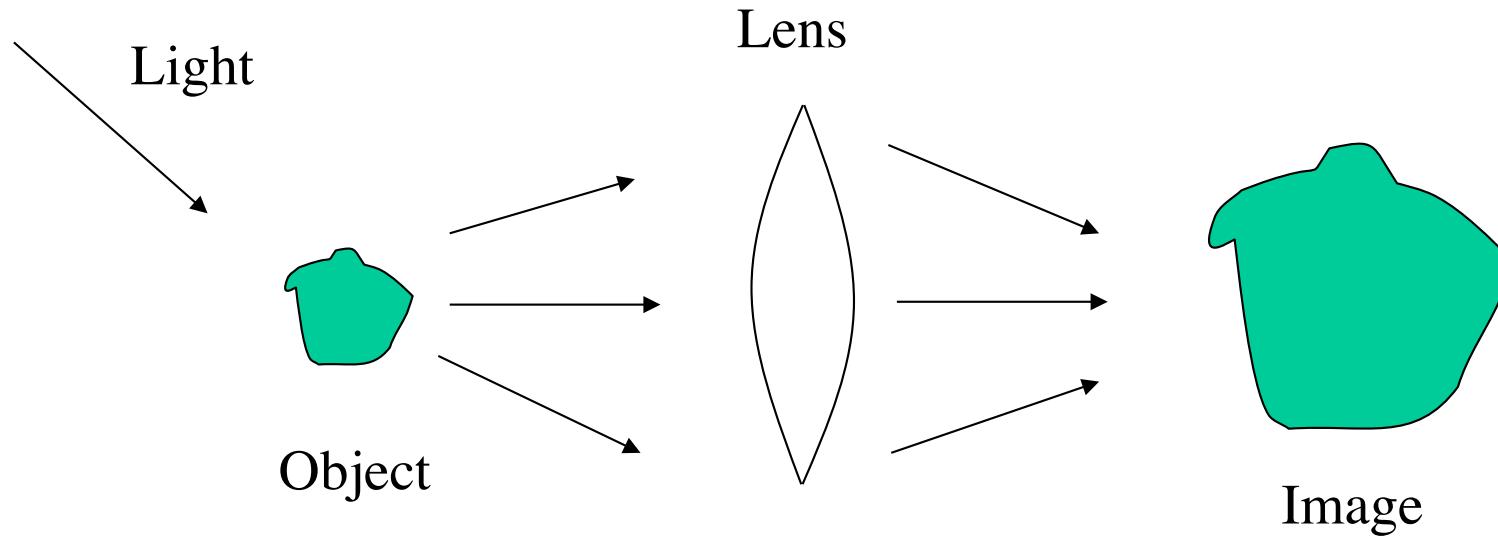
Typical interatomic distances: ~1 Å

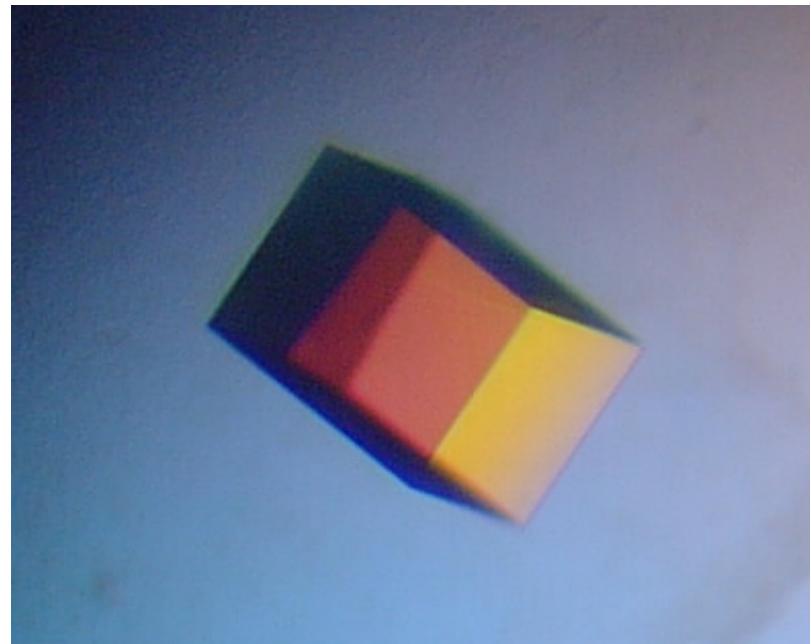
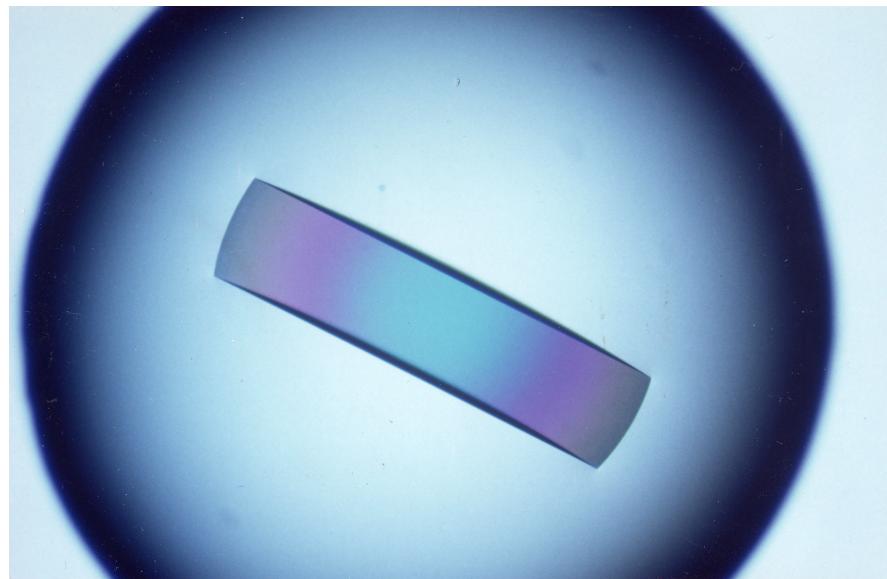
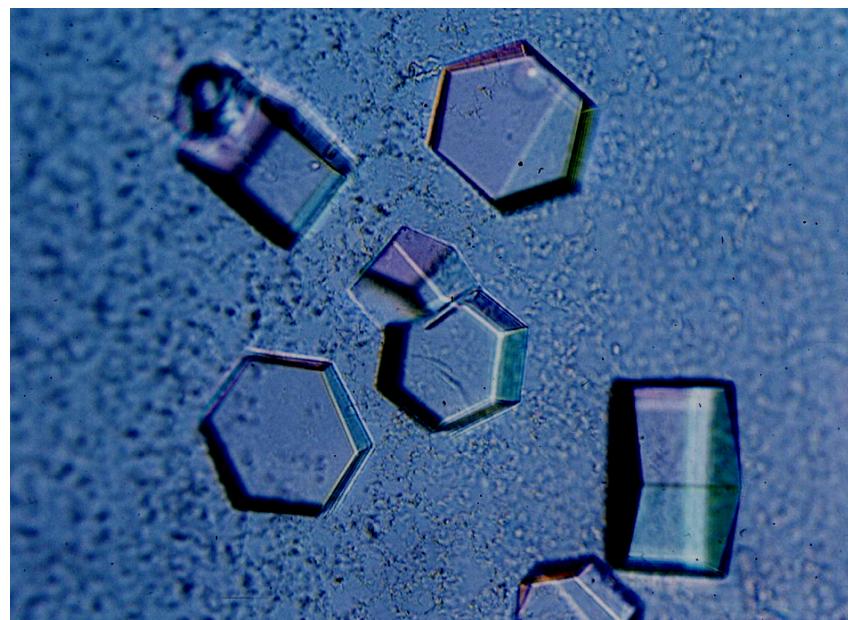
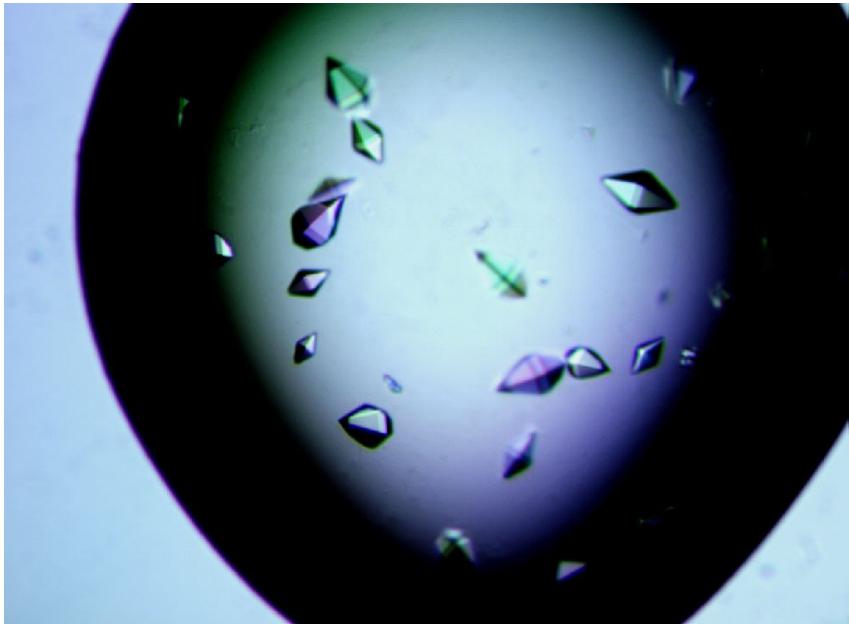
Electromagnetic radiation ($\lambda=1 \text{ \AA}$) => X-rays

Other sources of radiation: electrons, neutrons

Synchrotron (rayons X)



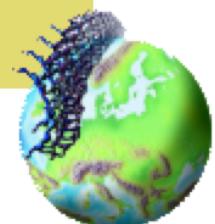






X-TB

Structural and functional genomics
of *Mycobacterium tuberculosis*

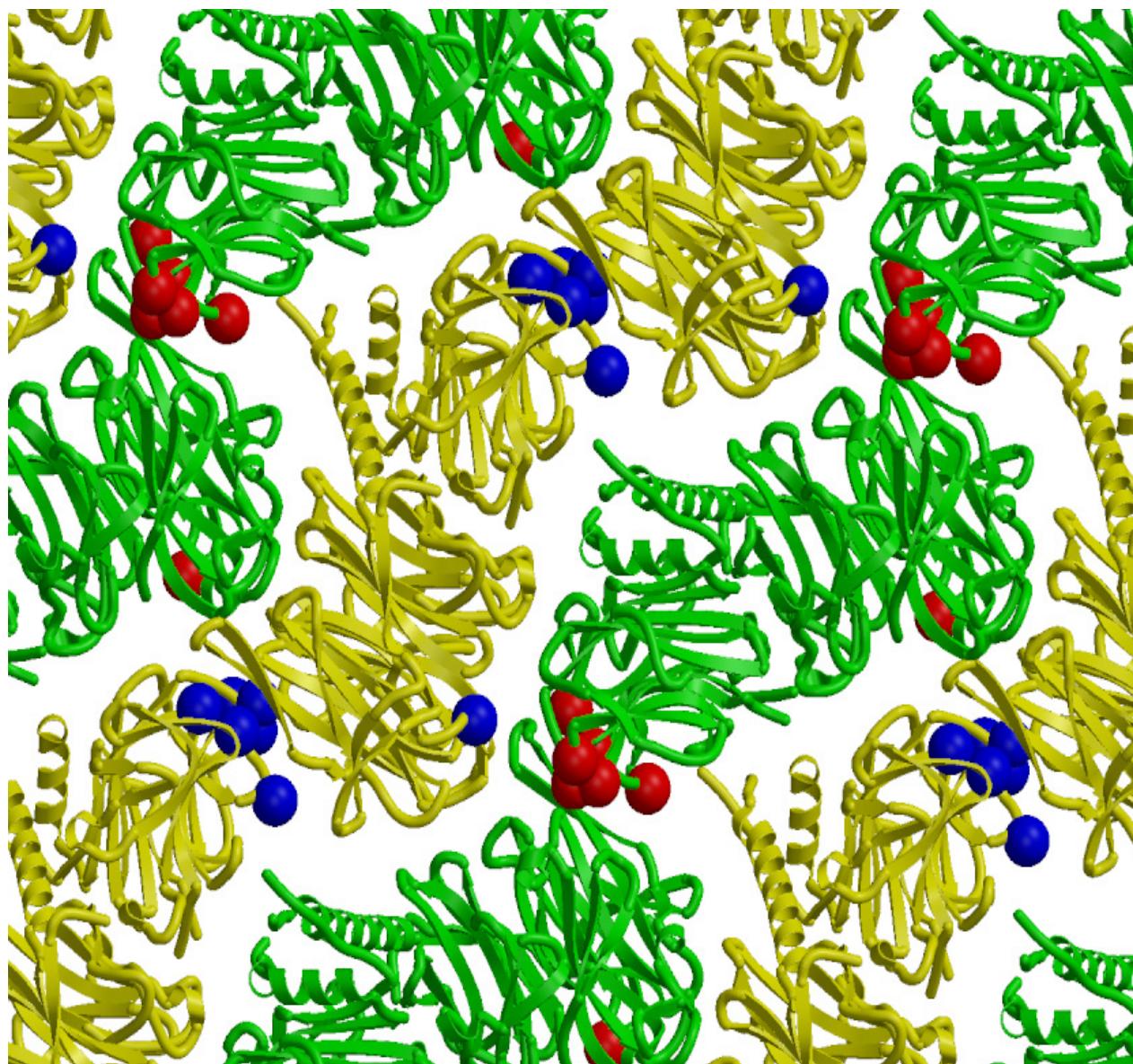


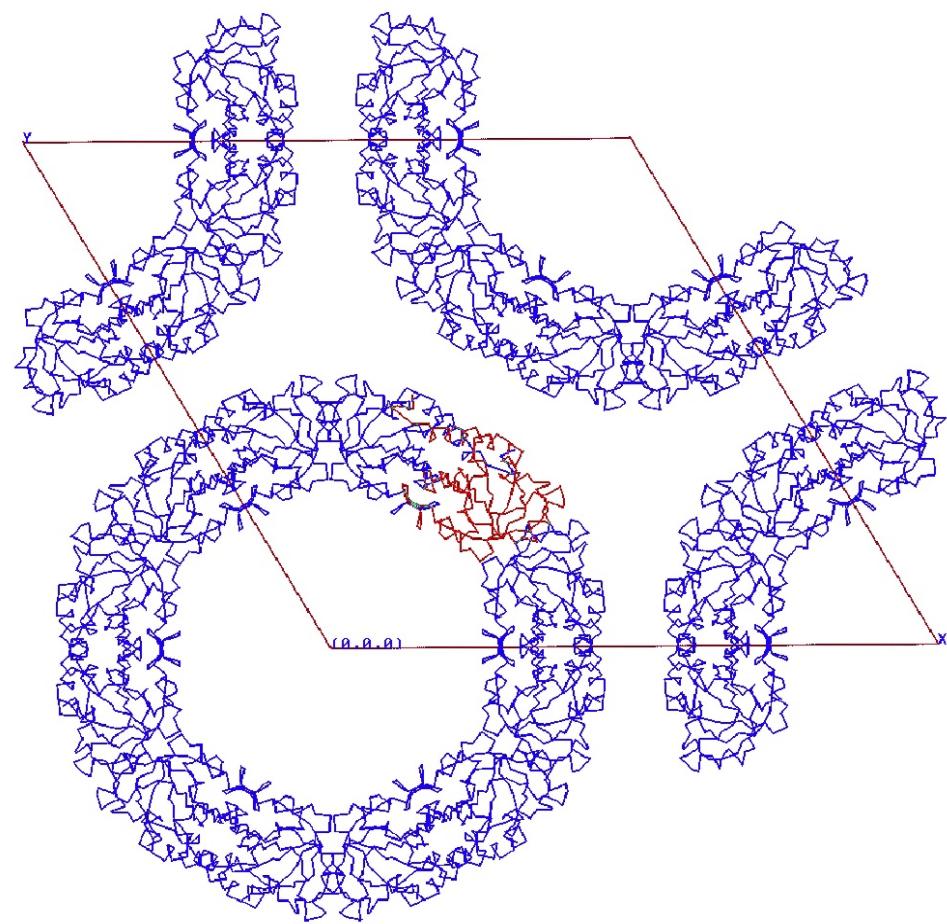
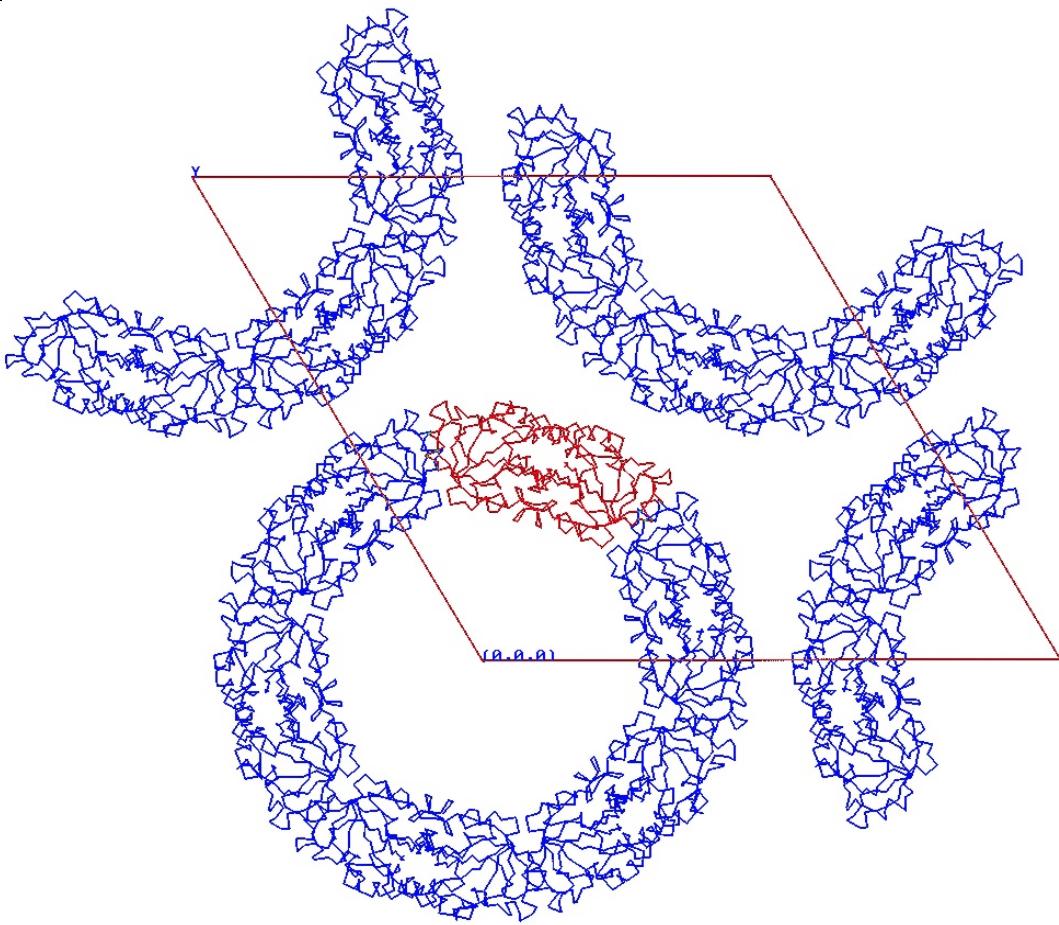
Protein crystals

Periodic array of molecules (spatial order)

Distribution of X-ray energy into a very large number of molecules (image averaging!)

High fraction (~50%) of solvent (crystals can be seen as concentrated solutions)





Crystal growth

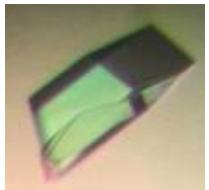
Requires large quantities of pure material
(various mgs)

Production of recombinant proteins (cloning,
overexpression, solubilization, purification)

Crystallization: empirical (necessary to try
several conditions), not always work !

Cristallisation

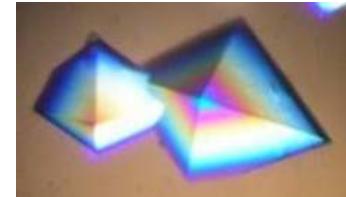
- L'étape limitante est l'obtention de cristaux de la macromolécule
- La connaissance de la physico-chimie des macromolécules n'est pas suffisante pour établir une méthode rationnelle, la cristallisation reste très empirique
- Une fois les cristaux obtenus, ils doivent être optimisés afin d'atteindre la résolution la plus haute possible pour résoudre la structure avec la plus grande précision



23 % PEG 4K
pH = 4.6
200 mM KCl
8 mg/ml

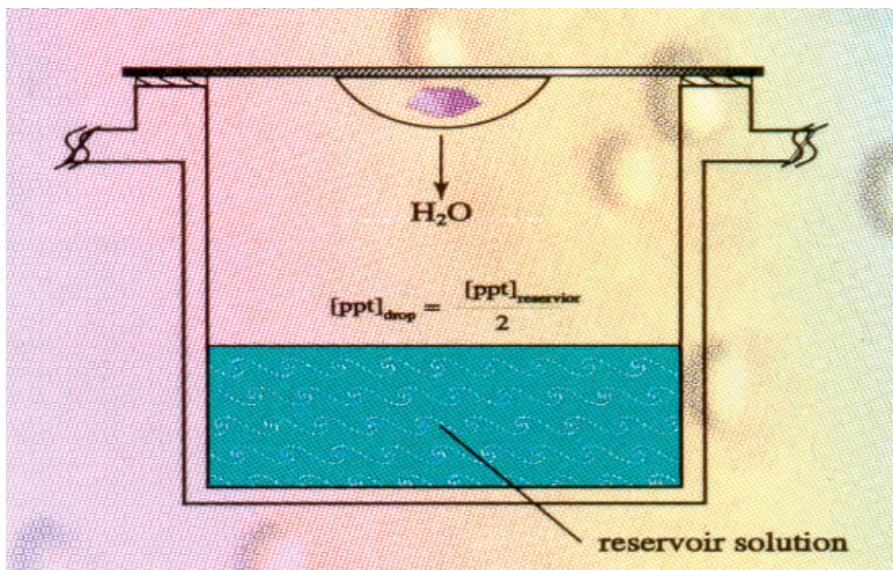


28 % MPD
pH = 8.5
150 mM NaCl
12 mg/ml

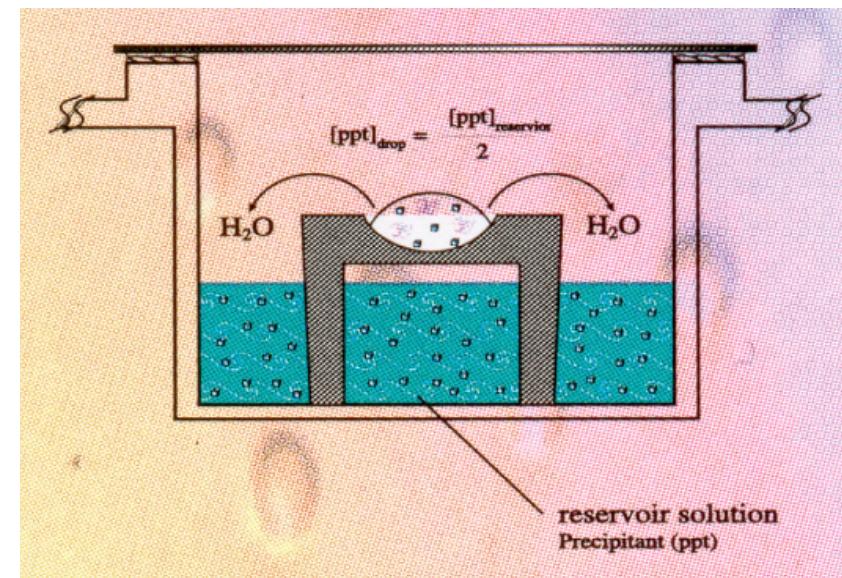


Cristallisation

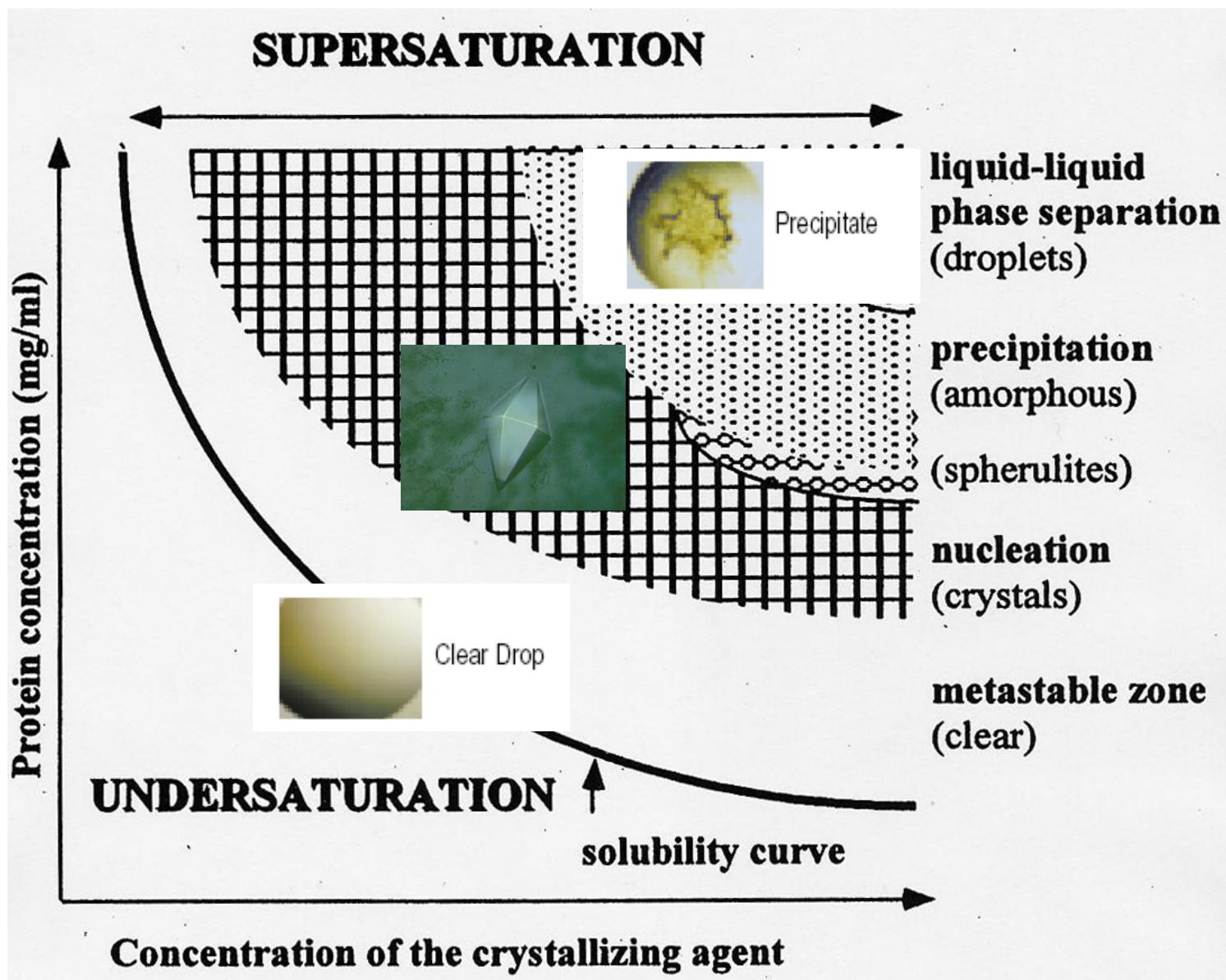
Goutte suspendue



Goutte assise



Diffusion jusqu'à l'équilibre thermodynamique



Paramètres physico-chimiques importants dans la cristallisation des macromolécules biologiques

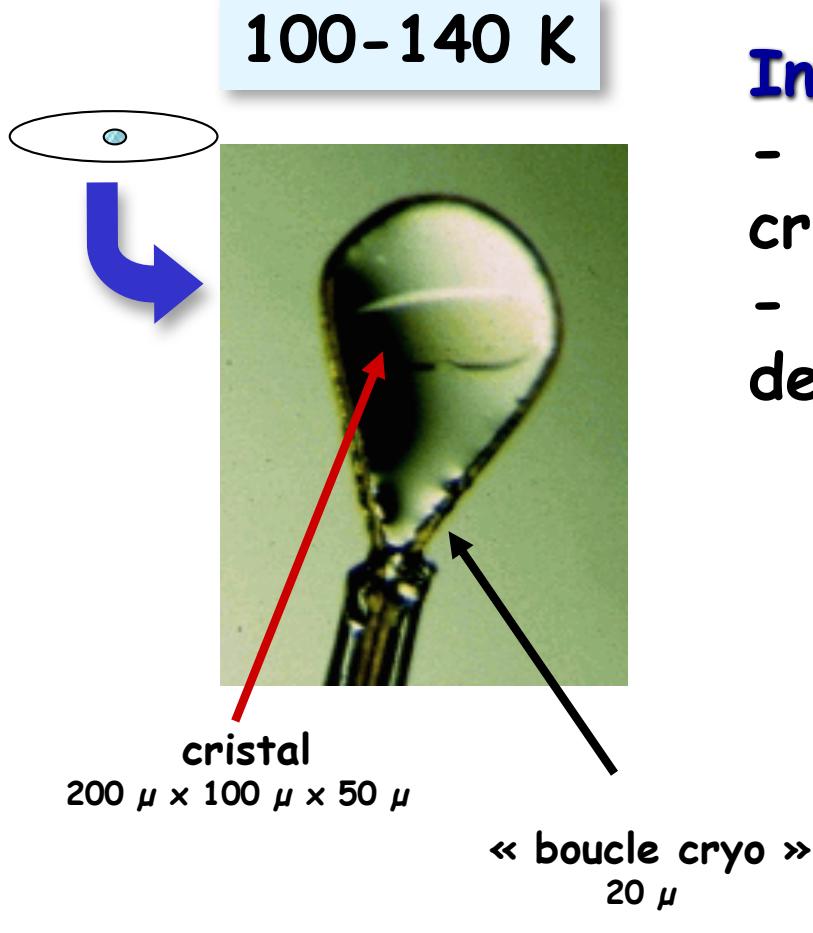
Precipitants used in macromolecular crystallization.

Salts	Volatile organic solvents	Polymers	Nonvolatile organic solvents
Ammonium sulfate	Ethanol	Polyethylene glycol 1000, 3350, 6000, 8000, 20000	2-Methyl-2,4-pentanediol (MPD)
Ammonium phosphate	Propanol	Jeffamine T, Jeffamine M	2,5-Hexanediol
Lithium sulfate	Isopropanol	Polyethylene glycol monomethyl ester	1,3-Propanediol
Lithium chloride	Dioxane	Polyethylene glycol monostearate	Polyethylene glycol 400
Sodium citrate	Acetone	Polyeneamine	Jeffamine 400
Ammonium citrate	Isobutanol		
Sodium phosphate	<i>n</i> -Butanol		
Sodium chloride	<i>tert</i> -Butanol		
Potassium chloride	Acetonitrile		
Sodium acetate	Dimethyl sulfoxide		
Ammonium acetate	1,3-Butyrolactone		
Magnesium sulfate			
Magnesium chloride			
Calcium chloride			
Sodium formate			
Sodium tartrate			
Cadmium sulfate			
Sodium succinate			
Sodium malonate			

Factors affecting crystallization.

Physical	Chemical	Biochemical
<ol style="list-style-type: none"> 1. Temperature/temperature variation 2. Surfaces/heterogeneous nucleants 3. Methodology/approach to equilibrium 4. Mother-liquor volume 5. Geometry of chamber or capillary 6. Gravity 7. Pressure 8. Time 9. Vibrations/sound/mechanical perturbations 10. Electrostatic/magnetic fields 11. Dielectric properties of the medium 12. Viscosity of the medium 13. Rate of equilibration 	<ol style="list-style-type: none"> 1. pH 2. Precipitant type 3. Final precipitant concentration 4. Ionic strength 5. Cation type and concentration 6. Anion type and concentration 7. Degree of supersaturation 8. Reductive/oxidative environment 9. Concentration of the macromolecule 10. Metal ions 11. Initial precipitant concentration 12. Cross-linkers/polyions 13. Detergents/surfactants/amphophiles 14. Non-macromolecular impurities 15. Chaotropes 	<ol style="list-style-type: none"> 1. Purity of the macromolecule/nature of impurities 2. Ligands, inhibitors, effectors 3. Aggregation state of the macromolecule 4. Post-translational modifications 5. Source of macromolecule 6. Proteolysis/hydrolysis 7. Chemical modifications 8. Genetic modifications 9. Inherent symmetry of the macromolecule 10. Degree of denaturation 11. Isoelectric point 12. Unstructured regions 13. His tags, purification tags 14. α-Helix content 15. Conformational states 16. Thermal stability 17. Allowable pH range 18. History of the sample

Cryo-cristallographie

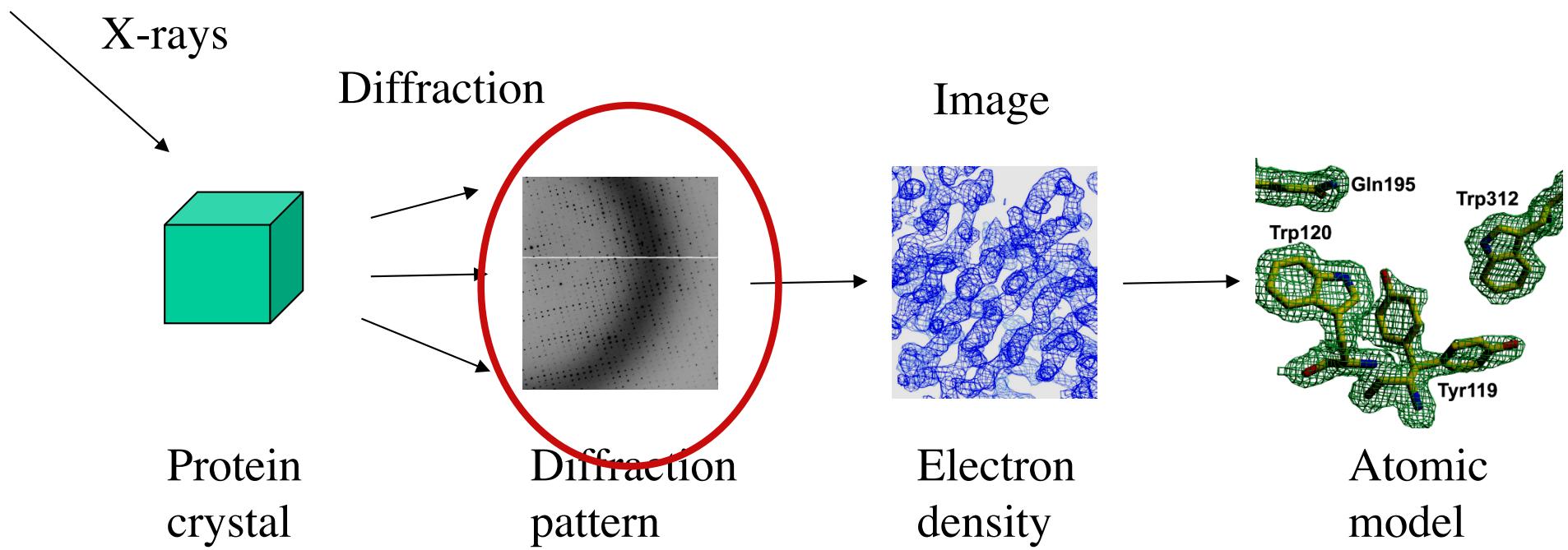
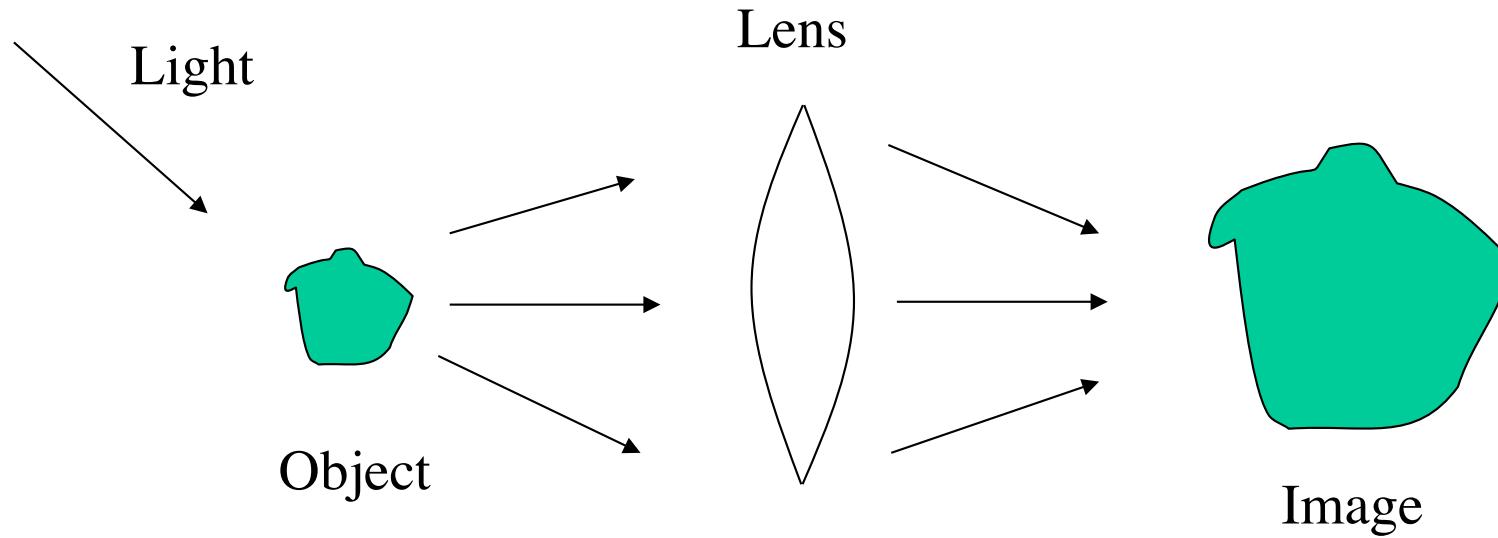


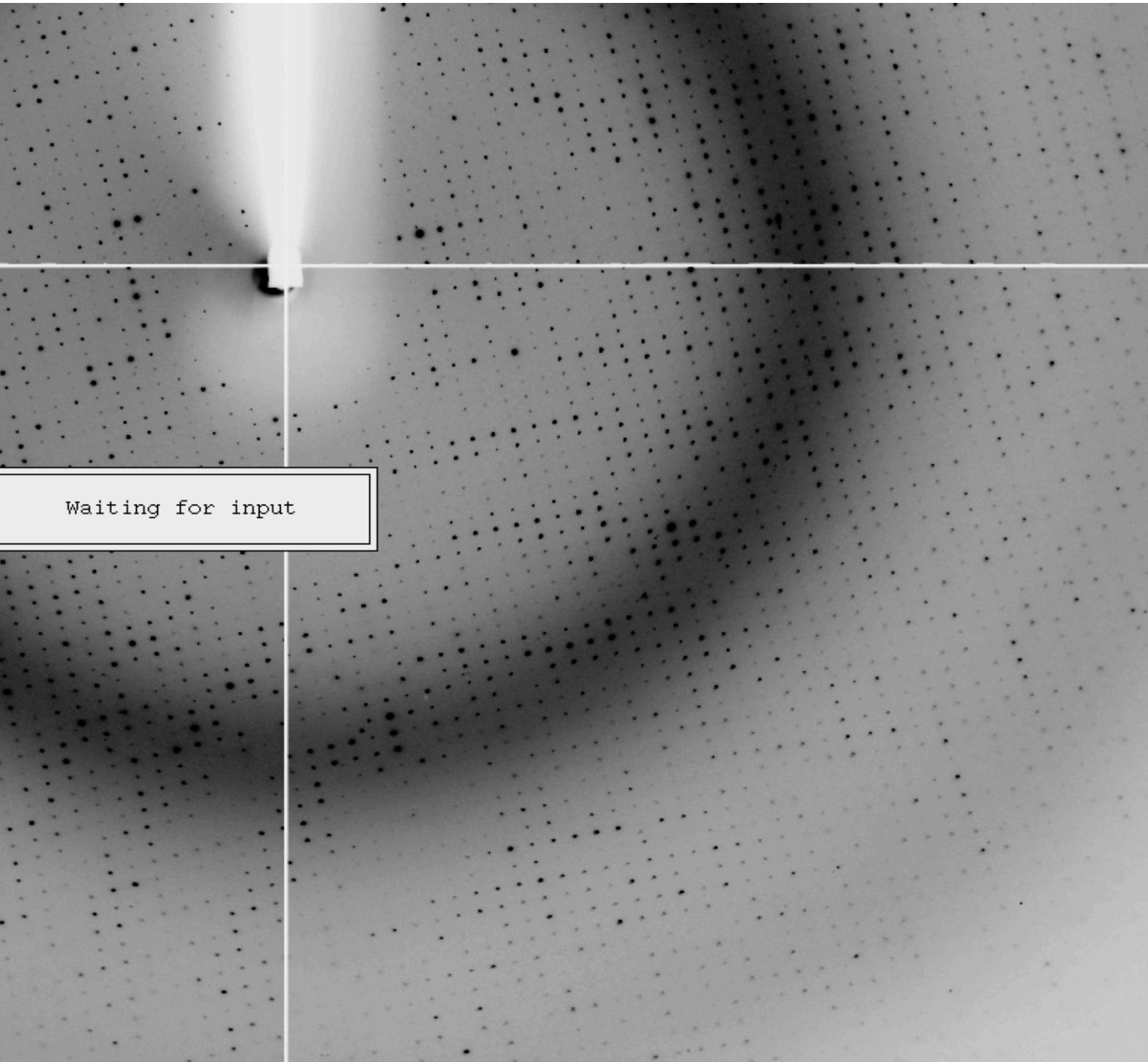
Intérêts de la technique

- augmentation de la durée de vie du cristal
- conservation de la qualité initiale de diffraction

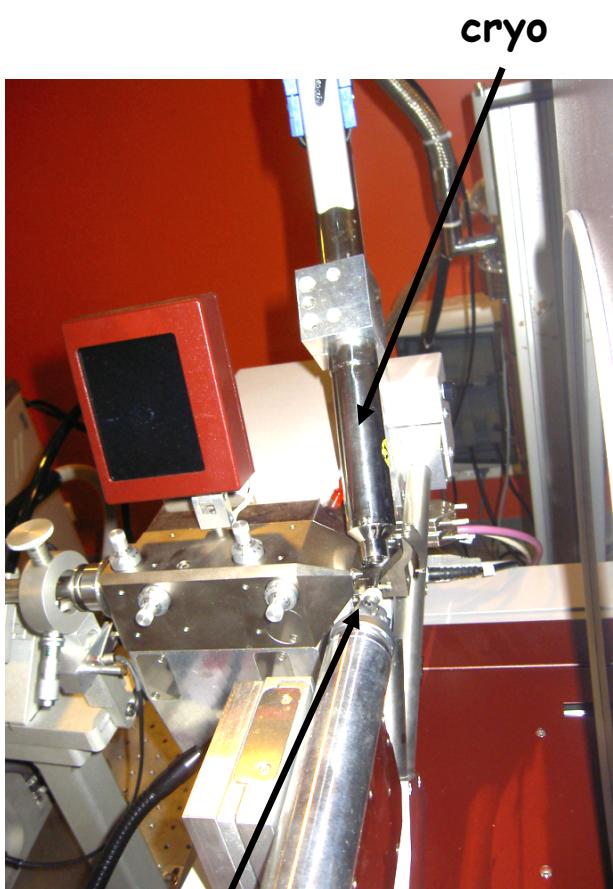
Congélation rapide du cristal dans l'azote liquide en présence de cryoprotectant:

- glycérol
- éthylène glycol
- MPD !



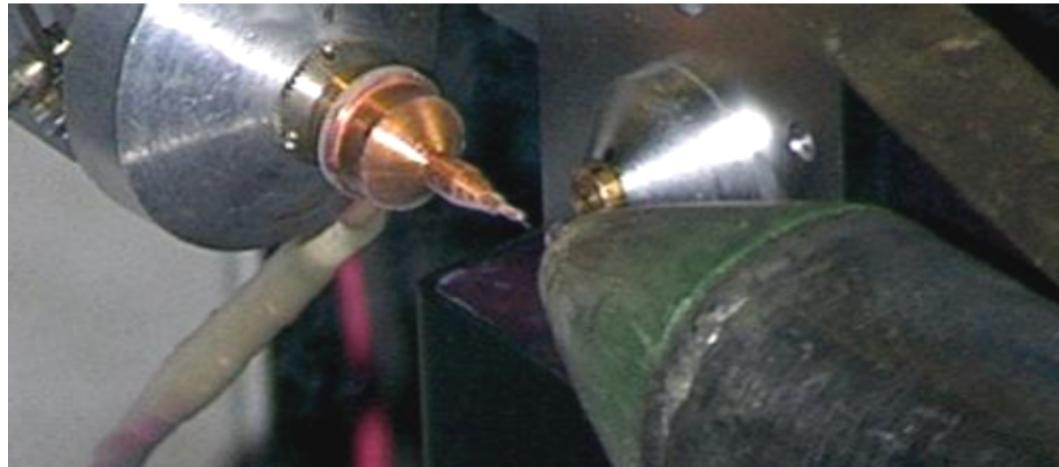


Enregistrement de données de diffraction



tête goniométrique

La boucle contenant le cristal est monté sur une tête goniométrique sous un flux d'azote (100 k) et placé dans un faisceau de rayons X
2 types de sources
- générateur à anode tournante (laboratoire)
- ligne de lumière d'un synchrotron

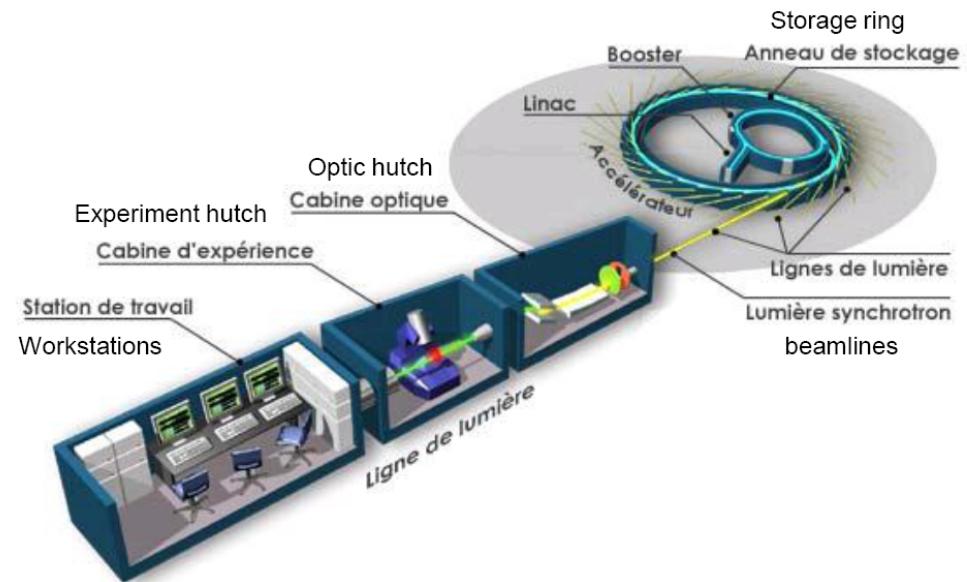


Synchrotron SOLEIL (Paris-Saclay)



SOLEIL SACLAY

Faisceau de rayons X d'une ligne synchrotron : intensité et brillance élevées



Sous l'effet de l'accélération occasionnée par une courbure de leur trajectoire, les électrons génèrent une onde électromagnétique, le rayonnement synchrotron

X-rays

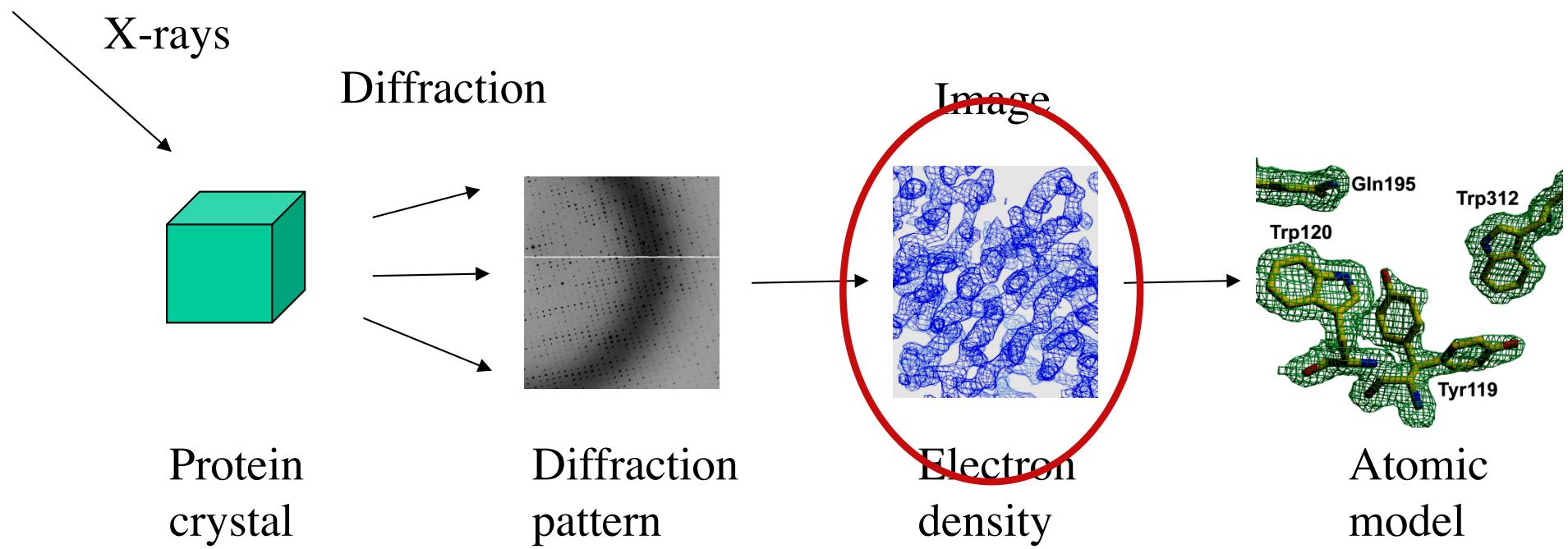
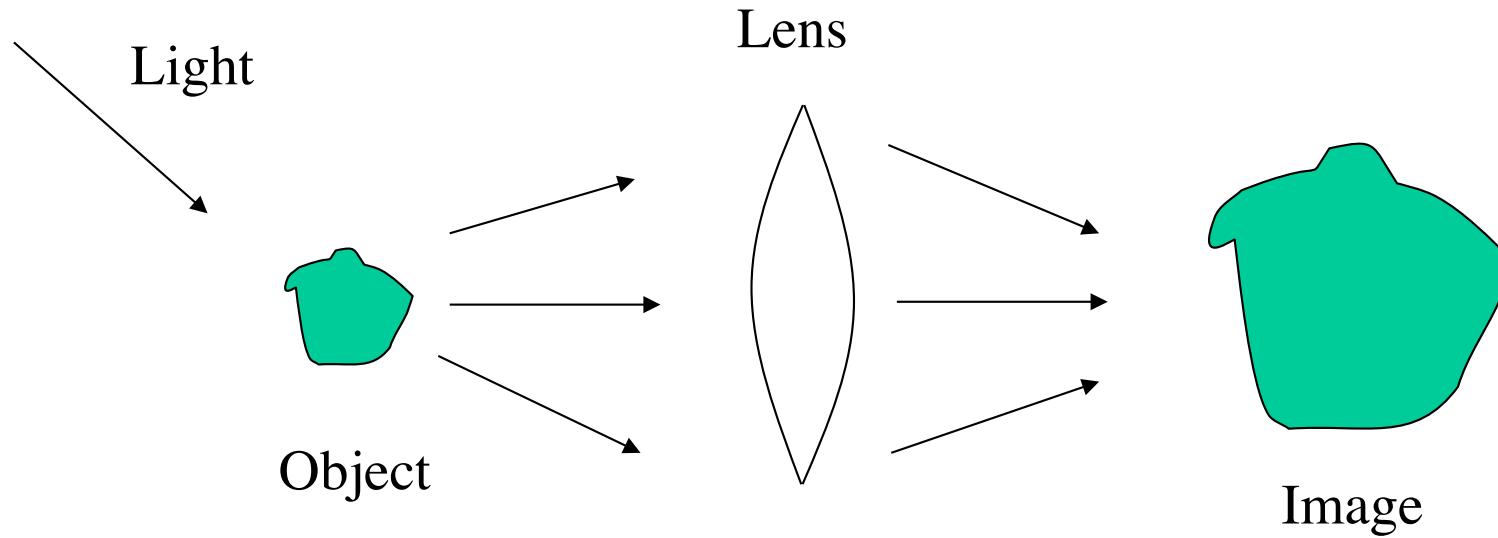
Generators:

- sealed tube
- rotating anode
- synchrotron

Detectors:

- film
- multi-wire
- image plate
- CCD (charged coupled device)

Cryo-cooling (liquid nitrogen temperature)



The phase problem

Structure factors have module and phase, but only the module can be experimentally measured.

Methods to solve this problem:

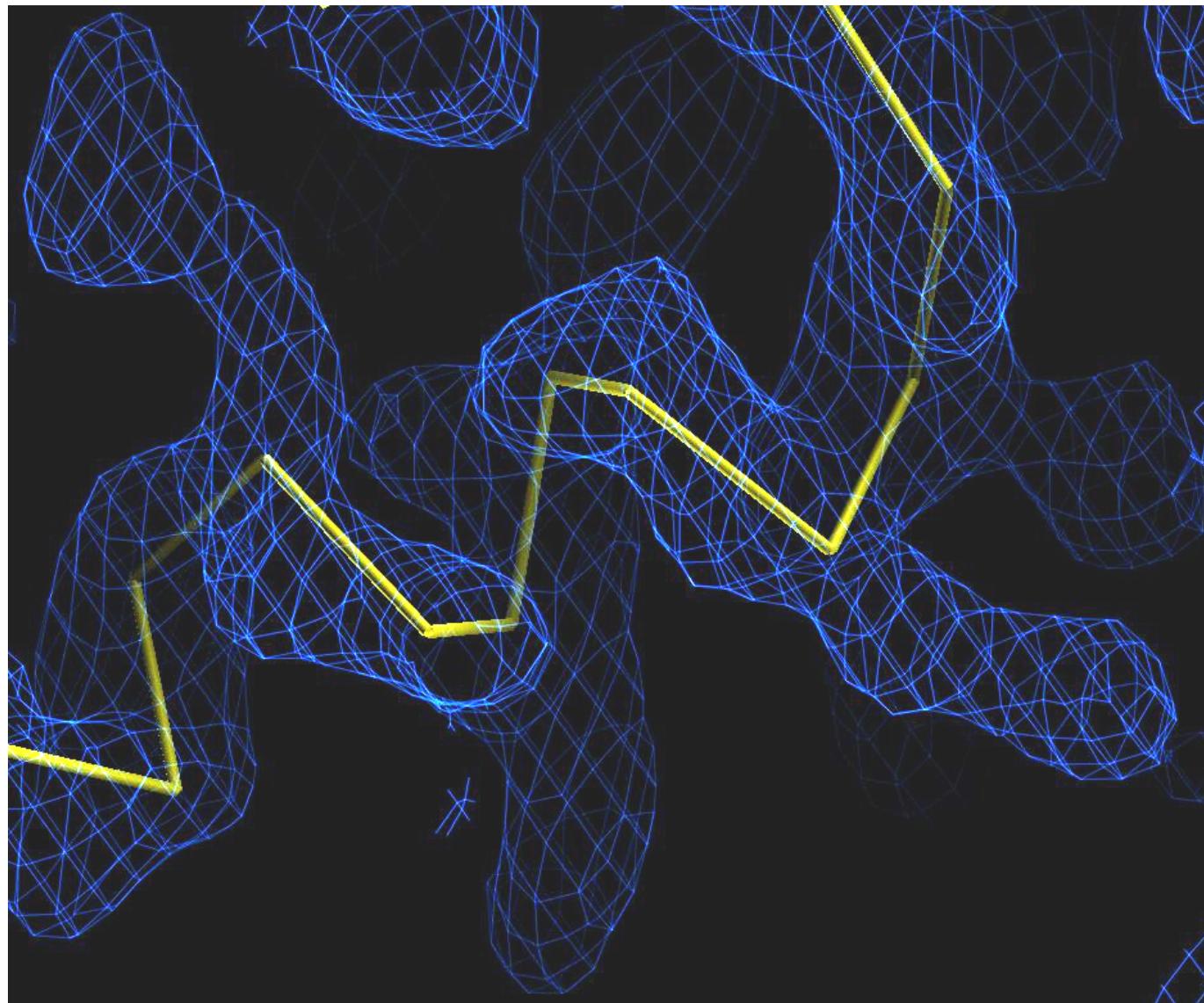
- Heavy atoms (isomorphous replacement)
- Anomalous diffraction (Se-methionine)
- Molecular replacement

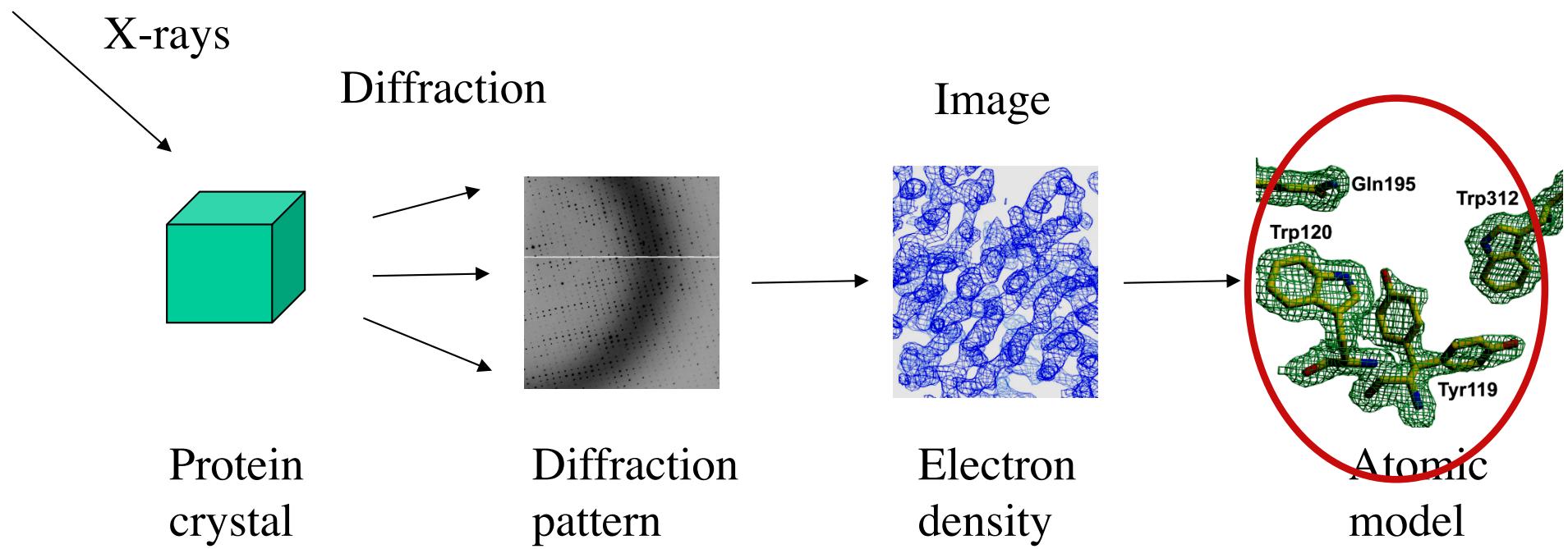
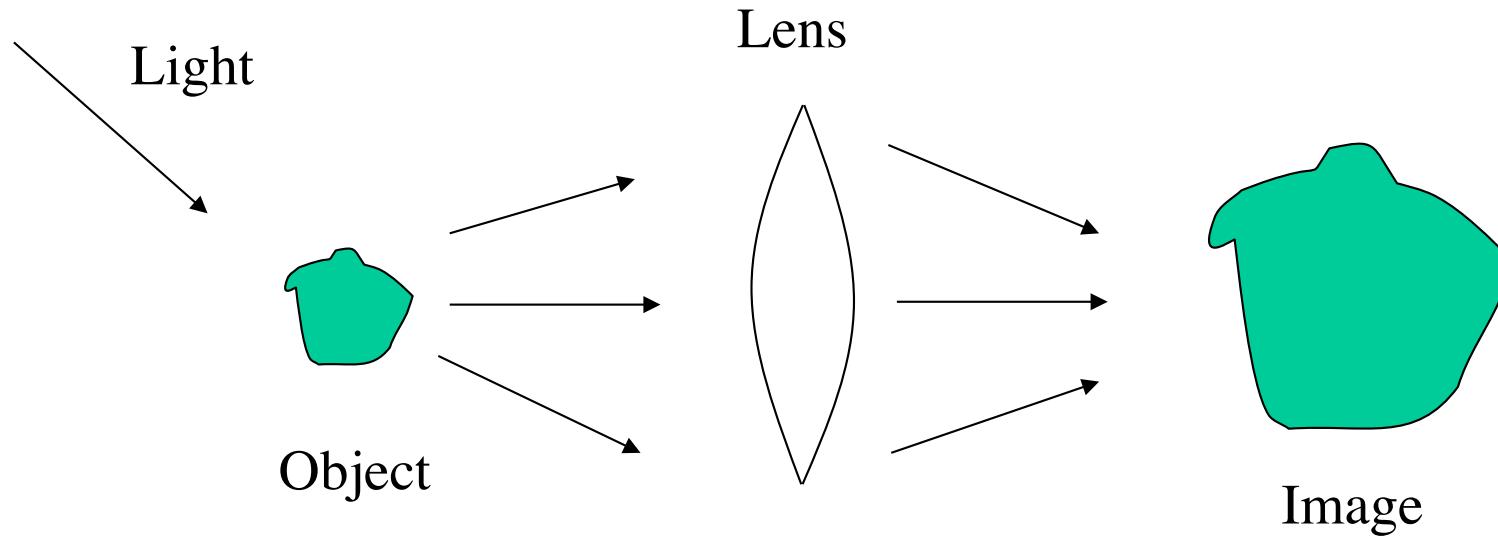
Electron density & Structure factors

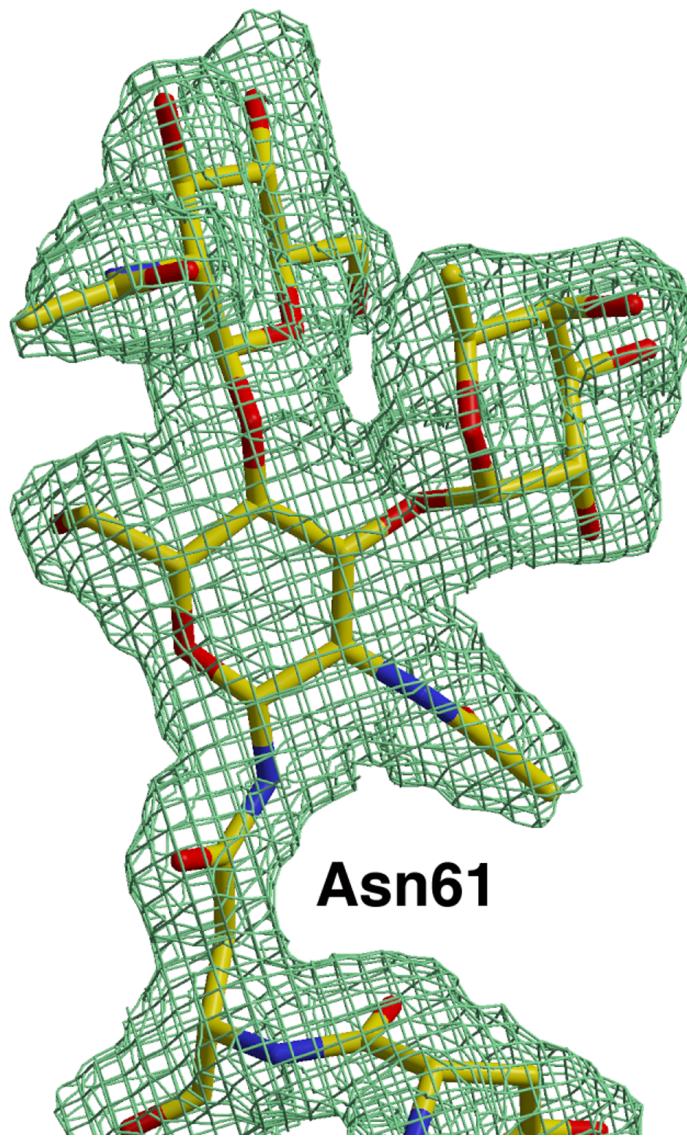
The **electron density** and the **structure factors** are related by the Fourier transform:

$$\rho(r) = \sum_H F(H) e^{2\pi i H \cdot r}$$

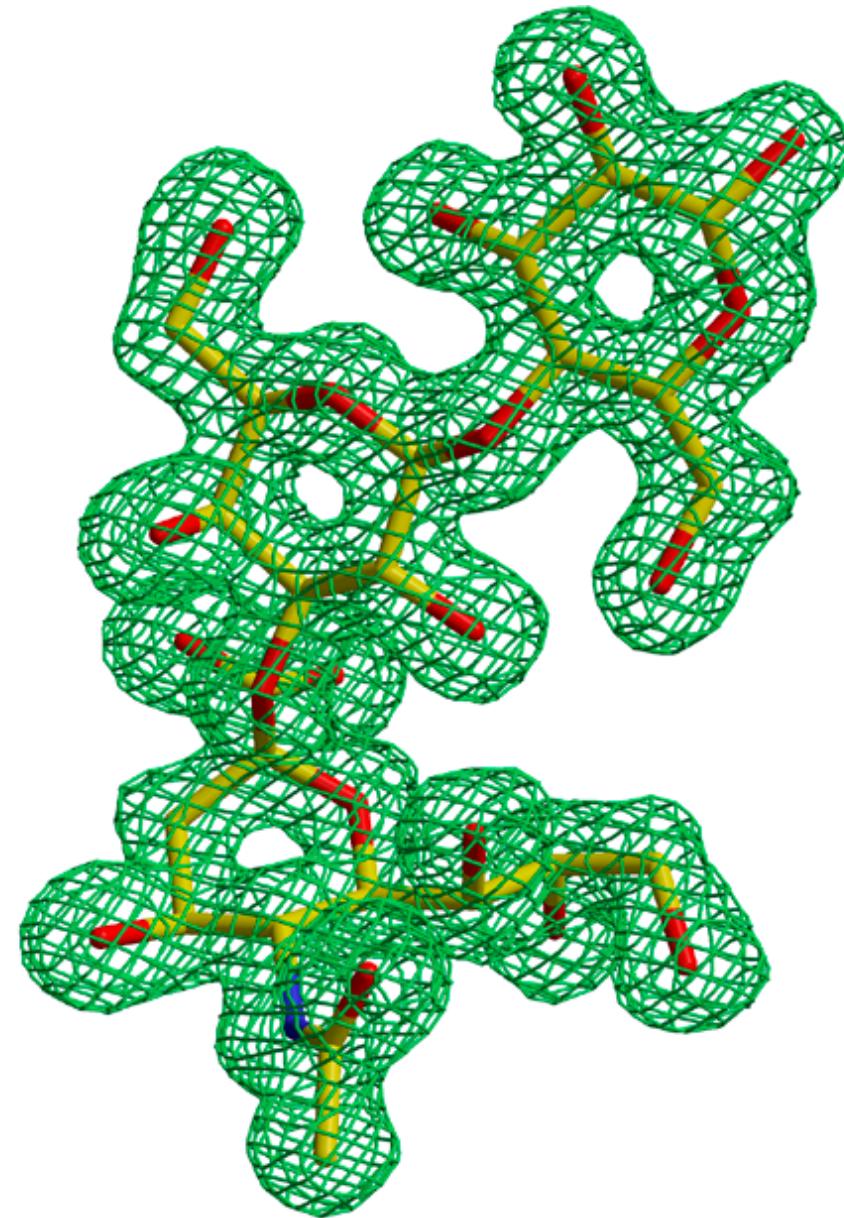
$$F(H) = \int \rho(r) e^{-2\pi i H \cdot r} dr$$



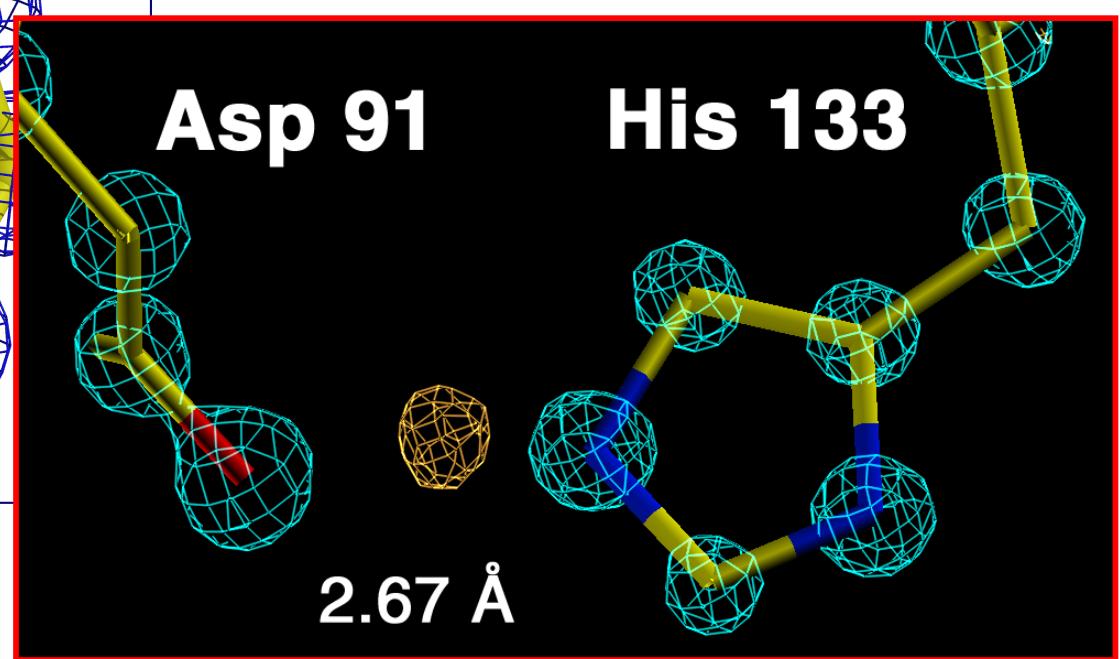
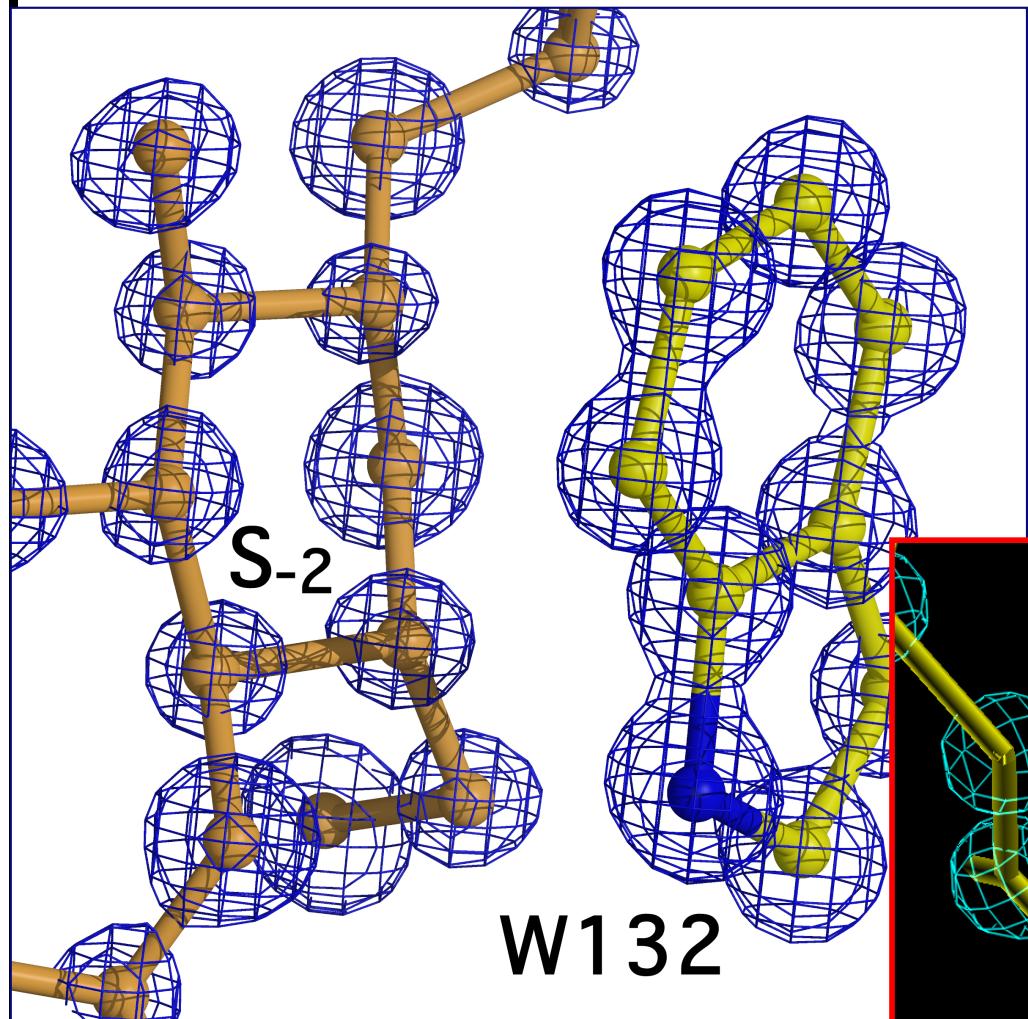




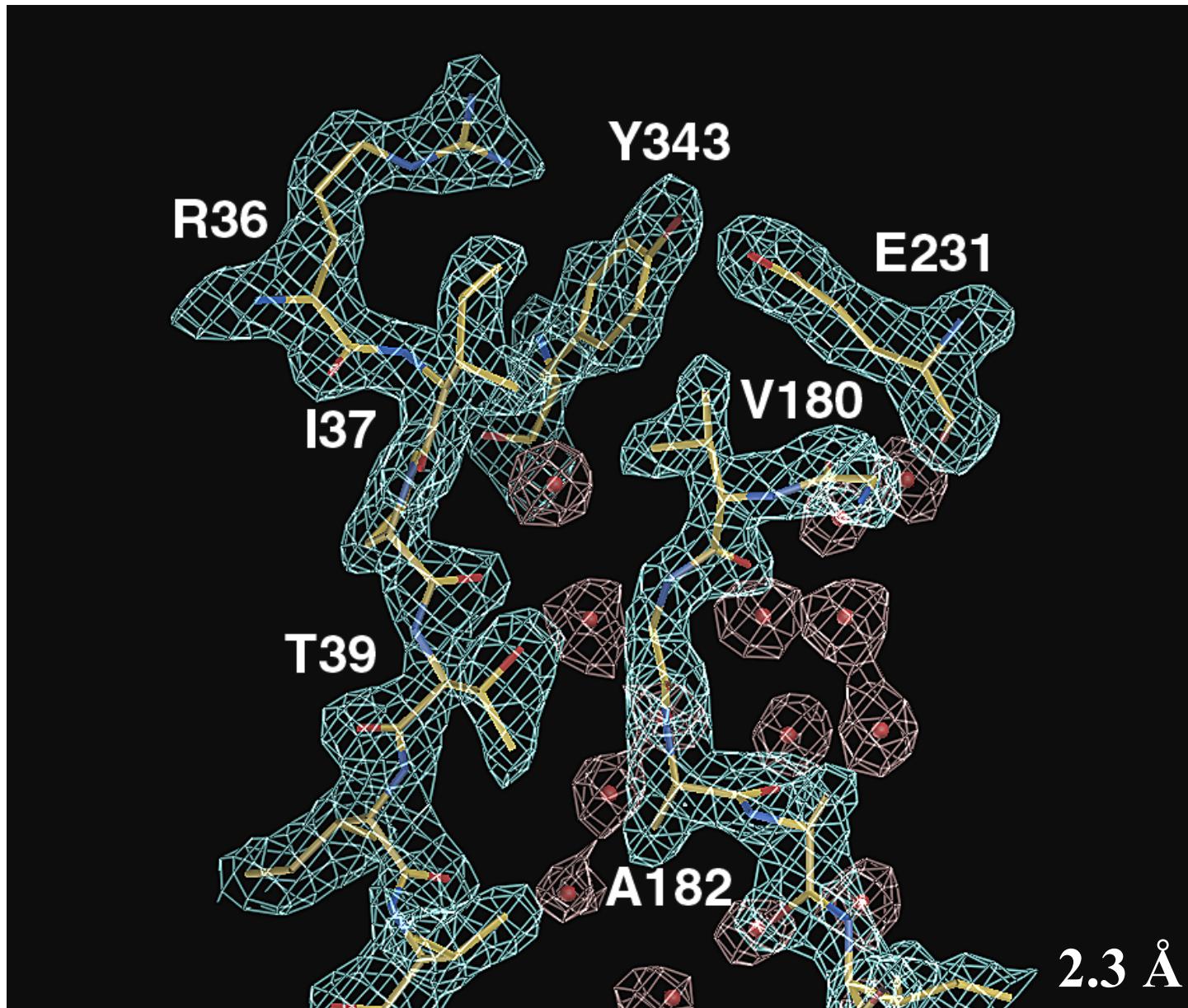
2.9 Å



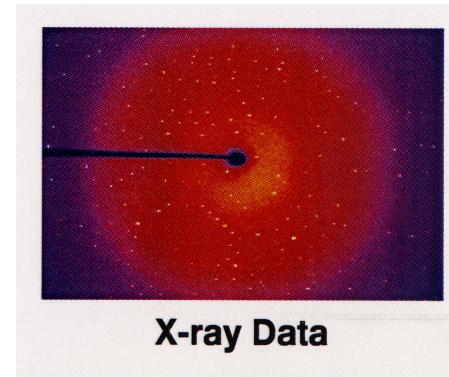
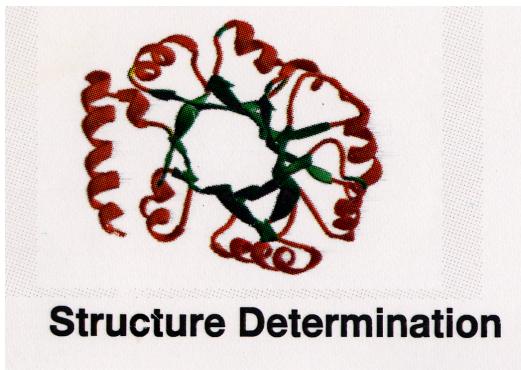
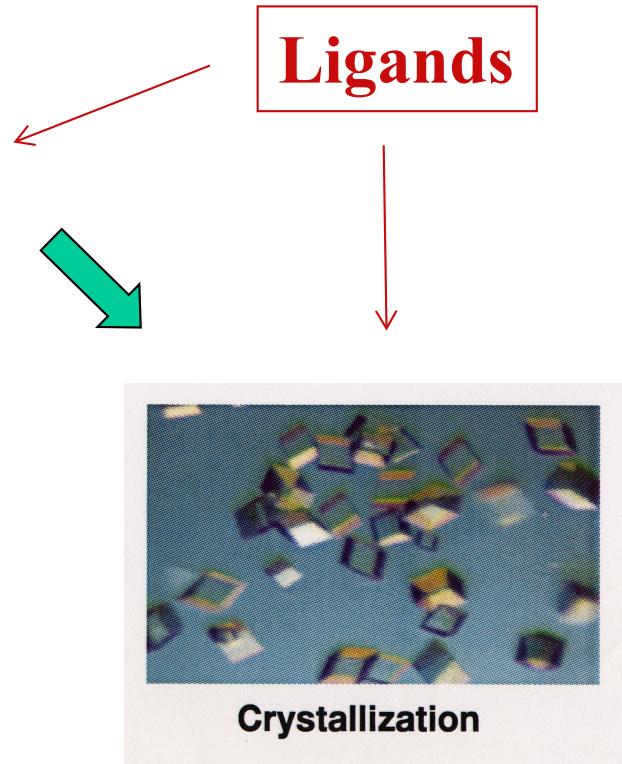
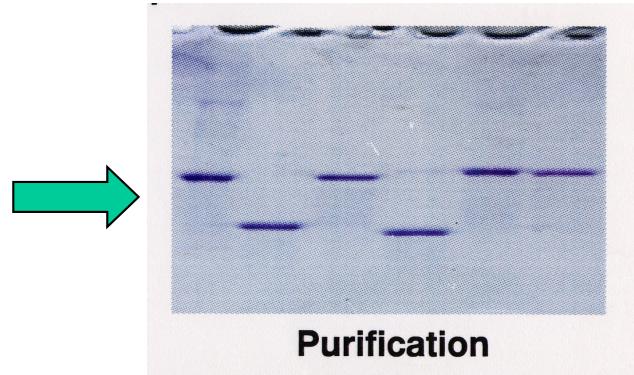
1.6 Å



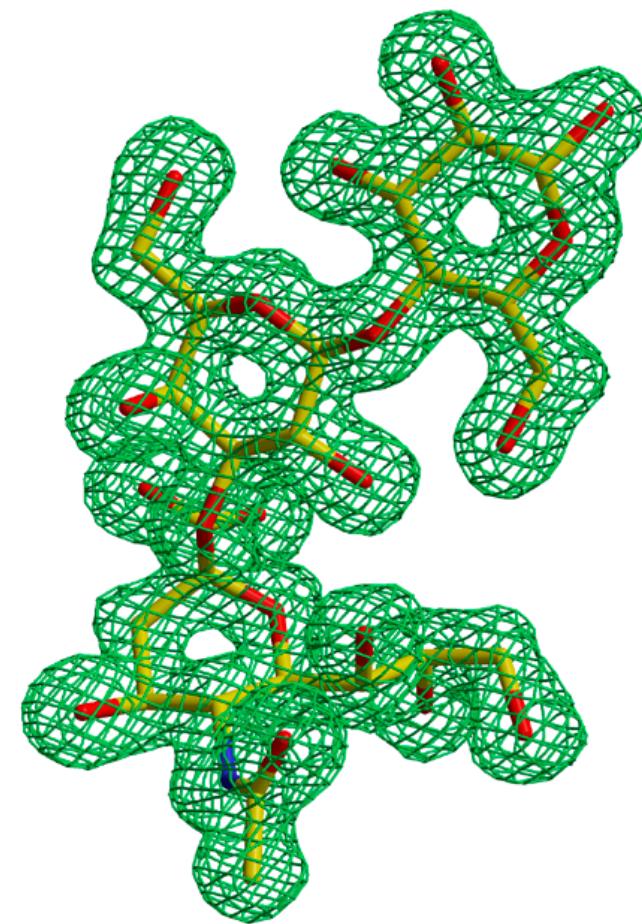
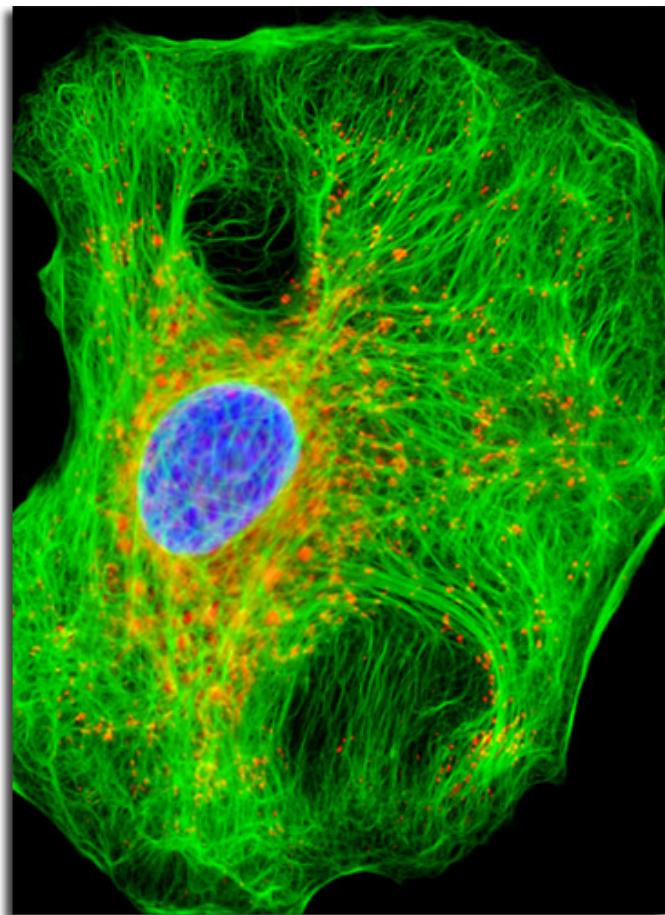
Trans-sialidase from *Trypanosoma cruzi*



The crystallographic pipeline



Cellular & Molecular Imaging

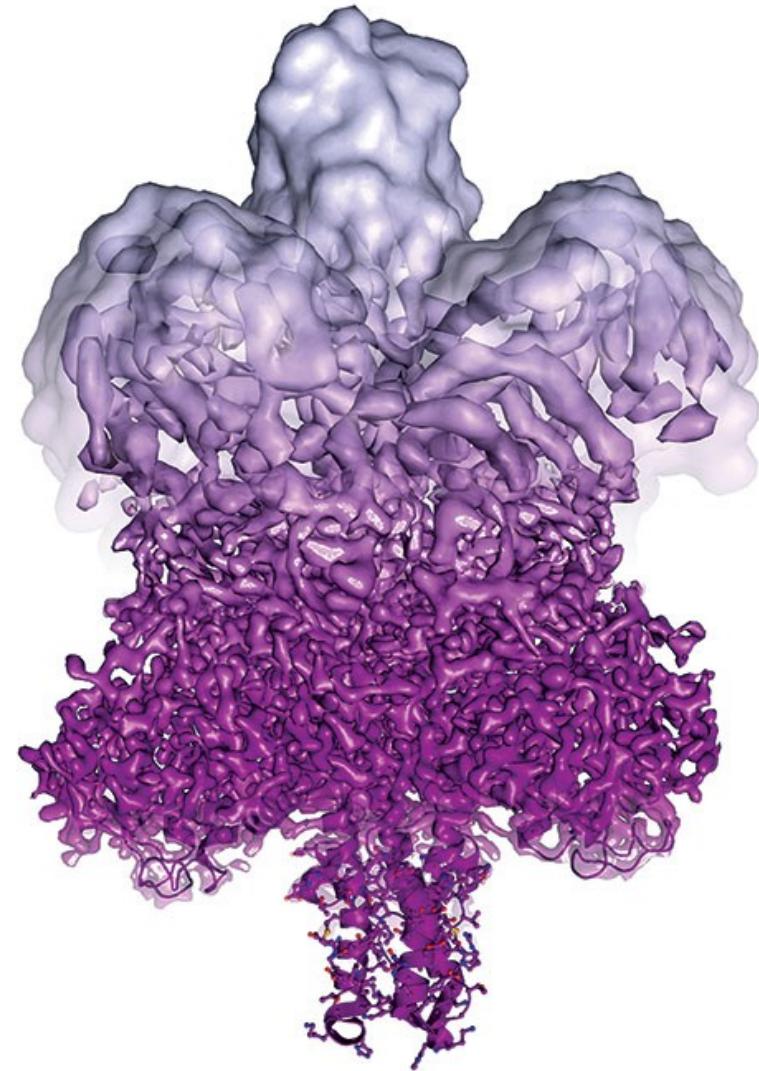


Micron scale Blind region Angstrom scale

Super-resolution microscopy,
Cryo-electron microscopy

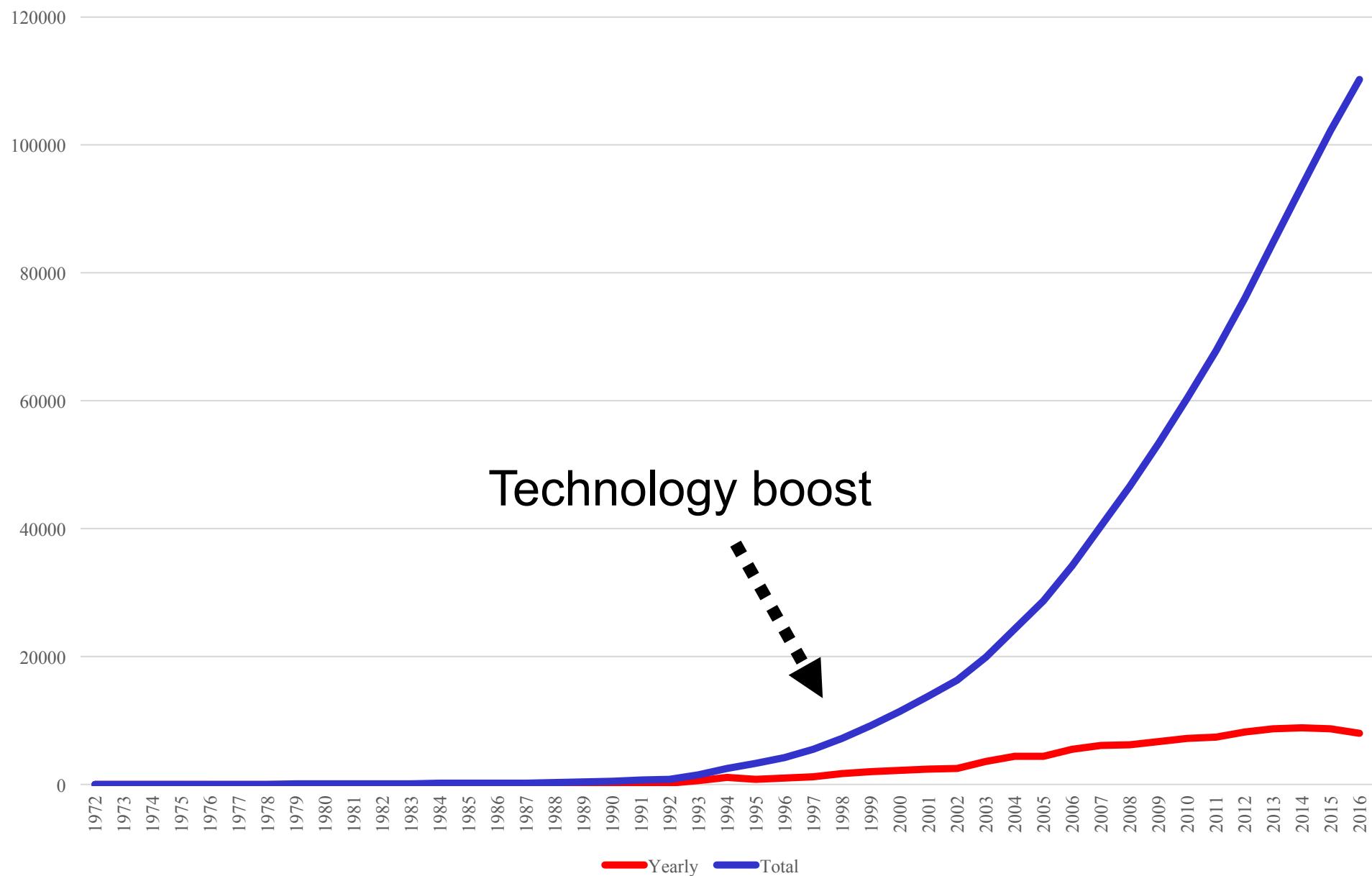
A horizontal double-headed red arrow spans the distance between the text "Micron scale" on the left and "Angstrom scale" on the right. The word "Blind region" is positioned above the center of this arrow. Below the arrow, the text "Super-resolution microscopy, Cryo-electron microscopy" is written in red.

Cryo-electron microscopy

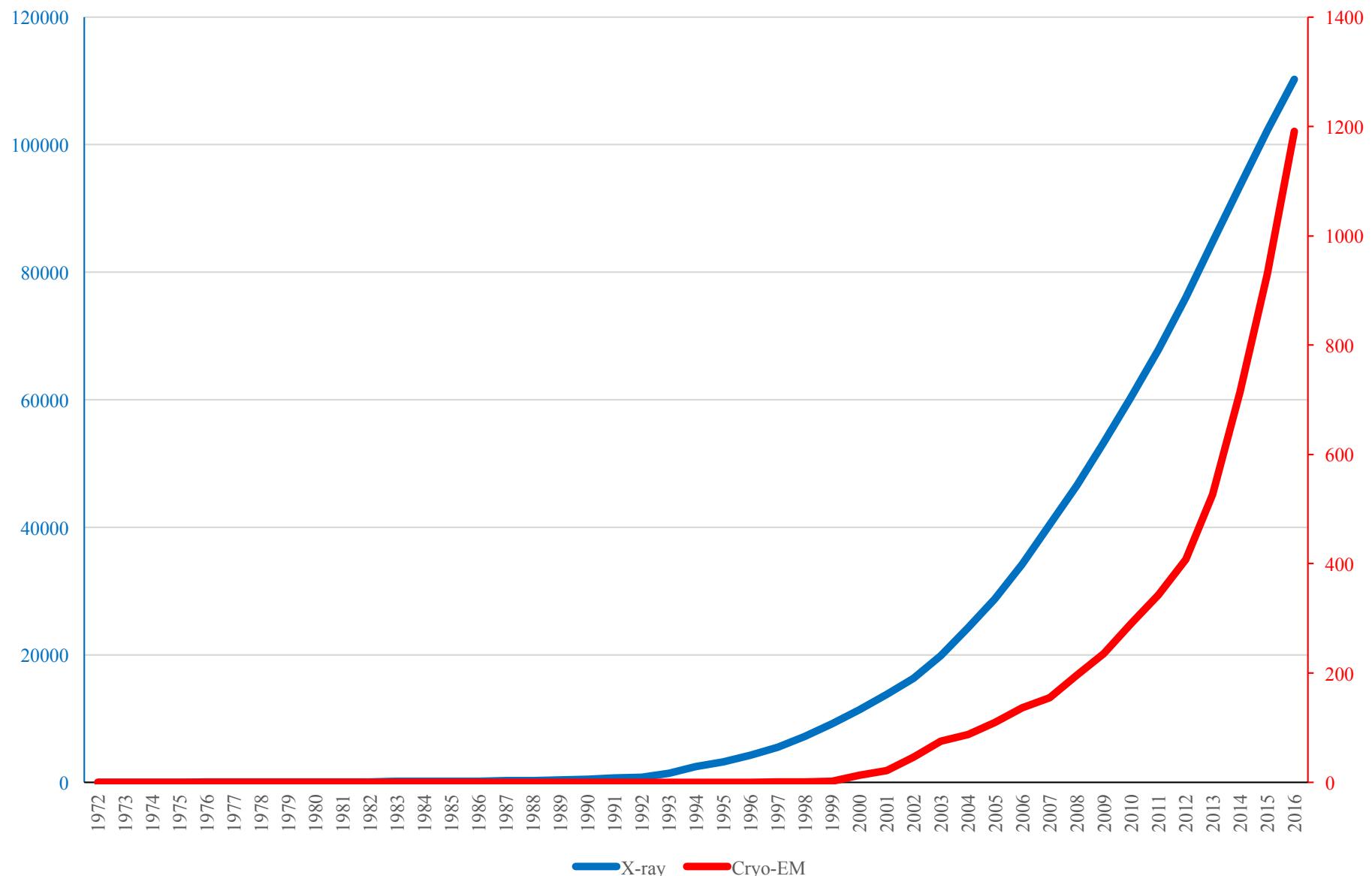


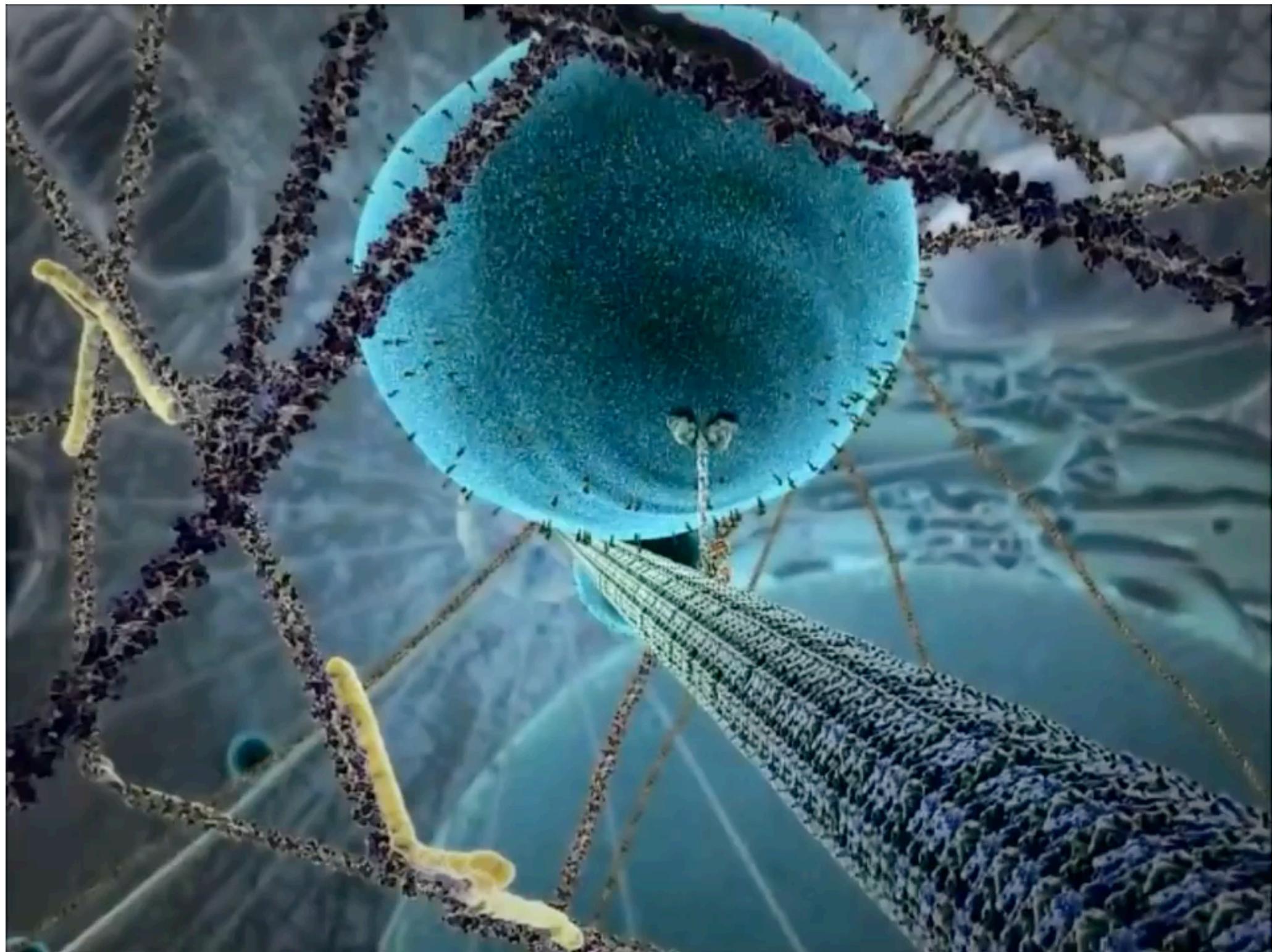
No crystals needed !

Protein Data Bank

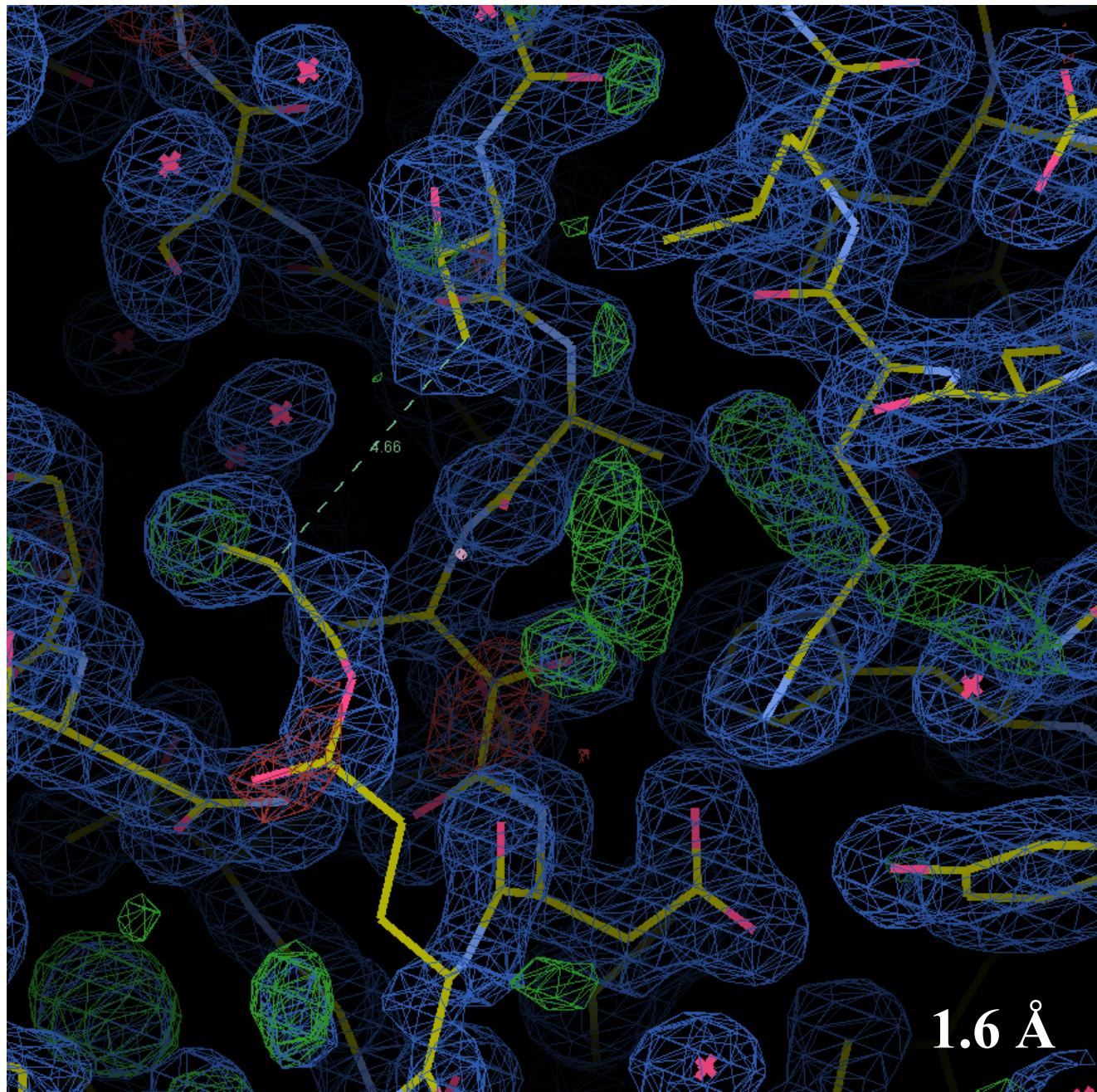


Number of PDB structures solved by X-ray and cryo-EM

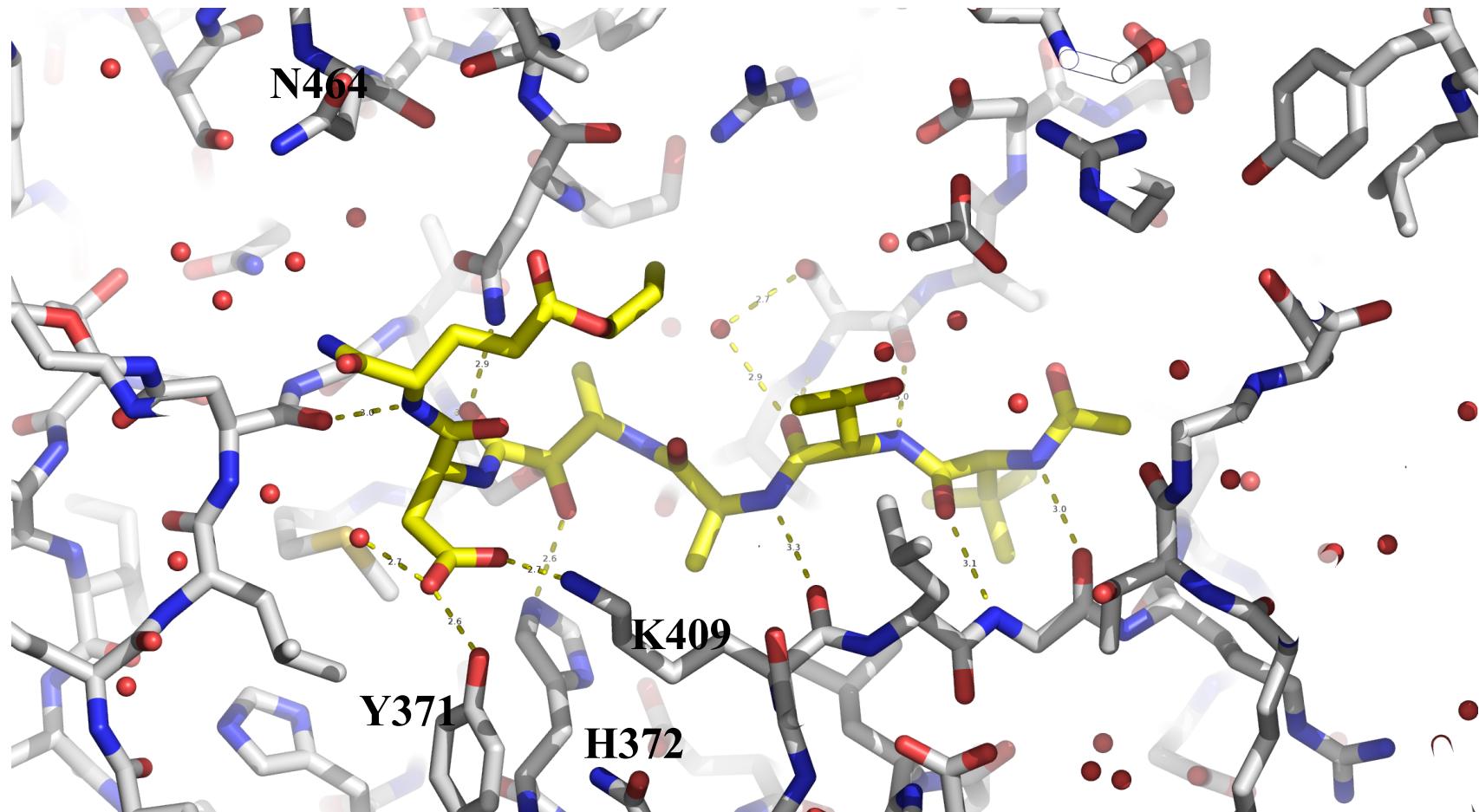




SUB1 protease from *Plasmodium falciparum*



SUB1 protease from *Plasmodium falciparum*



SUB1 protease from *Plasmodium falciparum*

