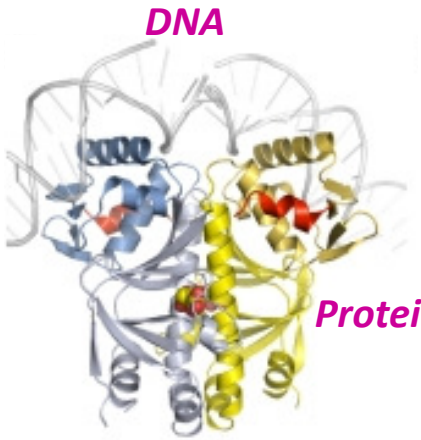

XALOC: The Macromolecular crystallography beamline at Alba
ALBA
Fernando Gil

Which is the motivation of XALOC?

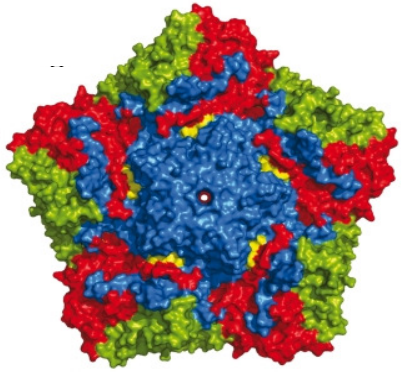
- ***Macromolecular Crystallography*** ***or*** ***X-ray crystallography*** ***is a technique for visualizing the structures of macromolecules such as proteins, viruses and nucleic acids (RNA and DNA) at atomic resolution.***

- **Crystallography is essentially a form of very high resolution microscopy.**

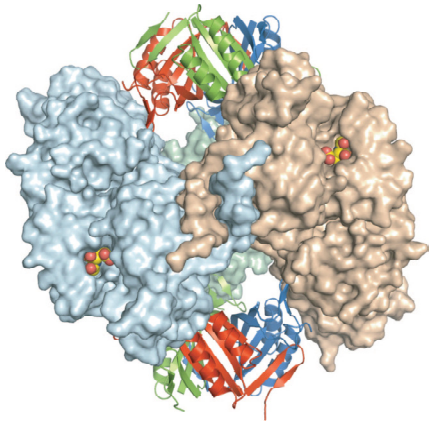
- ***XALOC is a beamline focused to obtain the molecular structure of proteins using single crystal X-ray Crystallography.***



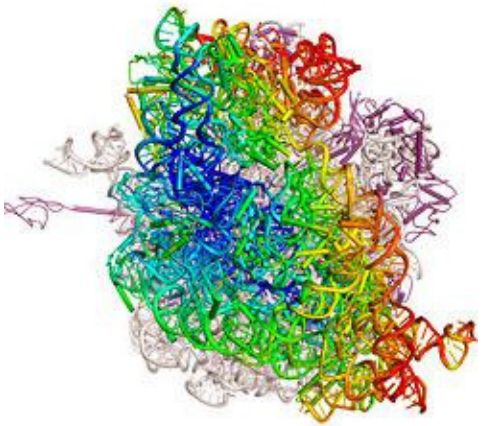
DNA-Protein complexes



Virus



Protein-Protein complexes



RNA-Protein complex (Ribosome)

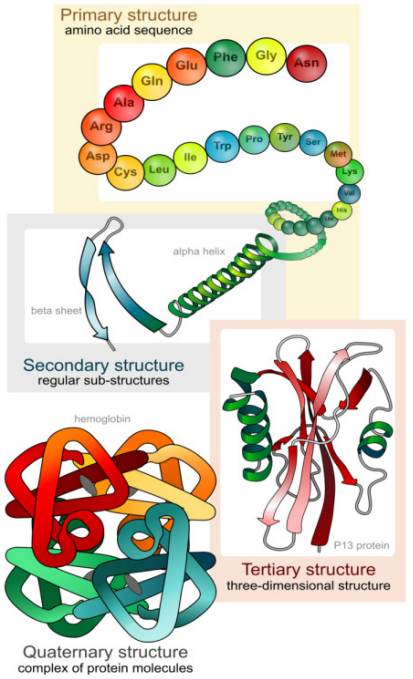
Why is important to study proteins?

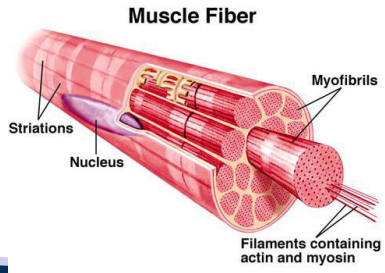
- **Proteins are large biological molecules essential for living organisms and are involved in virtually every process within cells (~ 20% weight human body).**

- **Proteins are encoded by DNA. Proteins carry out the different functions.**

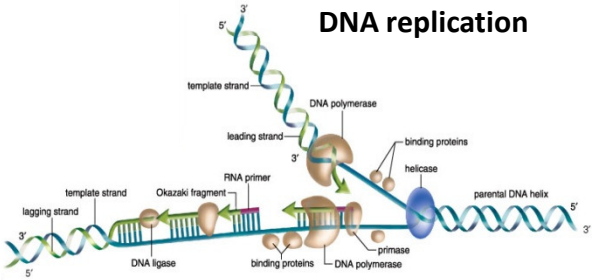
- **Polymers of amino acids. The folding of its linear sequence of amino acids give rise to the 3D-structure, which is characteristic of each protein and is closely related with its physiological activity.**

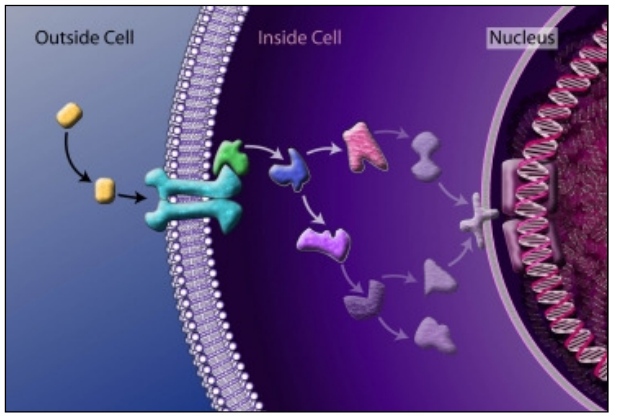
- ***Proteins are involved in a vast variety of functions:***
  - ***Transporters of essential substances, e.g. hemoglobin (oxygen).***
  - **Enzymes catalyze biochemical reactions and are vital to metabolism.**
  - ***DNA replication, e.g DNA polimerase.***
  - **Structural or mechanical functions, e.g. actin/myosin (muscle), keratin (hair).**
  - **Cell signalling.**
  - **Inmune responses, e.g. antibodies.**
  - **Cell adhesion.**
  - **Cell cycle.**





DNA replication





and for industry…

Table 1. Four classes of enzymes are generally used in detergents.

Proteases	Most widely used enzymes in the detergent industry remove protein stains such as grass, blood, egg and human sweat which have a tendency to adhere strongly to textile fibers.
Amylases	Used to remove residues of starch-based foods like potatoes, spaghetti, custards, gravies and chocolate.
Lipases	Decompose fatty material. Lipase is capable of removing fatty stains such as fats, butter, salad oil, sauces and the tough stains on collars and cuffs.
Cellulases	Modify the structure of cellulose fiber on cotton and cotton blends. When it is added to a detergent, it results in; color brightening, softening and soil removal.

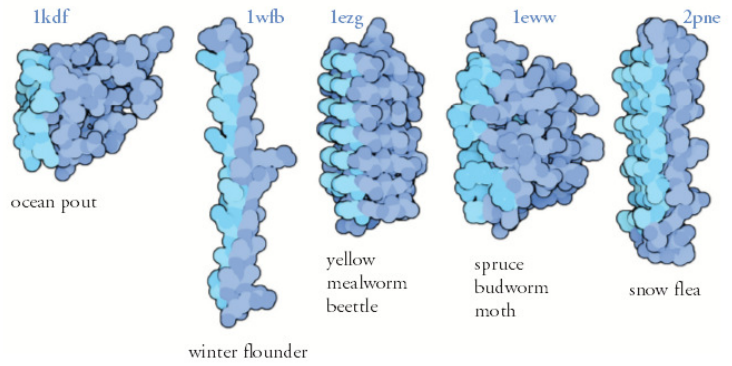
Table 2 Commercial bacterial lipases, sources, applications and their industrial suppliers. *n.s.* Not specified

Commercial lipase	Source	Supplier	Application	References
Lumafast	*Pseudomonas mendocina*	Genencor International, USA	Detergent	Jaeger et al. 1994; Jaeger and Reetz 1998
Lipomax	*P. alcaligenes*	Gist-Brocades, The Netherlands; Genencor International, USA	Detergent	Jaeger et al. 1994; Jaeger and Reetz 1998
n.s.	*P. glumae*	Unilever, The Netherlands	Detergent	Jaeger et al. 1994
n.s.	*Bacillus pumilus*	Solvay, Belgium	Detergent	Jaeger et al. 1994
Chiro CLEC-PC, Chirazyme L-1	*P. cepacia*	Altus Biologics, Manheim	Organic synthesis	Jaeger and Reetz 1998
Amano P, P-30, PS, LPL-80, LPL-200S	*P. cepacia*	Amano Pharmaceuticals, Japan	Organic synthesis	Jaeger and Reetz 1998
Lipase AH	*P. cepacia*	Amano Pharmaceuticals, Japan	Organic synthesis	Jaeger and Reetz 1998
Lipase AK, YS	*P. fluorescens*	Amano Pharmaceuticals, Japan	Organic synthesis	Jaeger and Reetz 1998
Lipase 56P	*P. fluorescens*	Biocatalysts, UK	Biotransformations, chemicals	Godfrey and West 1996
Lipase K-10	*Pseudomonas* sp.	Amano Pharmaceuticals, Japan	Organic synthesis	Jaeger and Reetz 1998
Chromobacterium viscosum lipase	*C. viscosum*	Asahi Chemical Biocatalysts	Organic synthesis	Godfrey and West 1996
Lipase 50P	*C. viscosum*	Biocatalysts, UK	Biotransformations, chemicals	Godfrey and West 1996
Lipase QL	*Alcaligenes* sp.	Meito Sankyo Co., Japan	Organic synthesis	Jaeger and Reetz 1998
Lipoprotein lipase	*Alcaligenes* sp.	Meito Sankyo Co., Japan	Research	Godfrey and West 1996
Lipase PL, QLQLL, PLC/PLG, QLC/QLG	*Alcaligenes* sp.	Meito Sankyo Co., Japan	Technical grade	Godfrey and West 1996
Alkaline lipase	*Achromobacter* sp.	Meito Sankyo Co., Japan	Research	Godfrey and West 1996
Lipase AL, ALC/ALG	*Achromobacter* sp.	Meito Sankyo Co., Japan	Technical grade	Godfrey and West 1996
Combizyme 23P (proteinase/lipase mix)	n.s.	Biocatalysts, UK	Waste treatment	Godfrey and West 1996
Combizyme 61P (proteinase/lipase mix)	n.s.	Biocatalysts, UK	Waste treatment	Godfrey and West 1996
Combizyme 209P (amylase/lipase/proteinase mix)	n.s.	Biocatalysts, UK	Waste treatment, grease disposal	Godfrey and West 1996
Greasex (lipase)	n.s.	Novo Nordisk	Leather	Godfrey and West 1996

Table 1: Application of proteases in industry

Industry	Protease	Application
Baking	Neutral protease	Dough conditioner
Beverage	Papain	Chill proofing, removal of haze in beverages
Dairy	Fungal proteases, chymosin, other proteases	Replacement of calf rennet, whey protein processing, production of enzyme modified cheese (EMC)
Detergent	Alkaline protease, subtilisin	Laundry detergents for protein stain removal
Food processing	Several proteases	Modification of protein rich material i.e., soy protein or wheat gluten
Leather	Trypsin, other proteases	Bating of leather, dehairing of skins
Meat and fish	Papain, other proteases	Meat tenderization, recovery of protein from bones and fish waste
Medicine	Trypsin	Dead tissue removal, blood clot dissolution
Photography	Several proteases	Recovery of silver from used X-ray and photographic films
Sweetner	Thermolysin	Reverse hydrolysis in aspartame synthesis

ANTIFREEZE PROTEINS



- Ice is a big problem for organisms that live in cold climates.
- Cells make specialized antifreeze proteins to protect themselves as the temperature drops.
- Antifreeze proteins have been used as a preservative in ice cream.

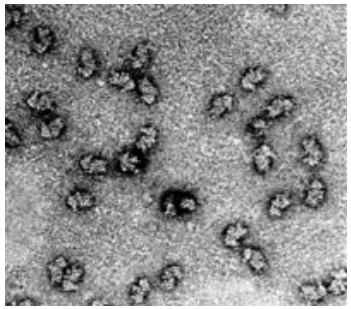
Nobel Laureates, directly or indirectly, through Crystallography

- *Crystallography* has produced the largest number of Nobel Laureates throughout history...

2012 Chemistry R.J. Lefkowitz, B.K. Kobilka *Structure and function of G-protein-coupled receptors.*
2011 Chemistry D. Shechtman Discovery of quasicrystals.
2010 Physics A. Geim and K. Novoselov Groundbreaking experiments regarding the 2D material graphene.
2009 Chemistry V. Ramakrishnan, T.A. Steitz, Ada E. Yonath *Studies of the structure and function of the ribosome*
2006 Chemistry R. Kornberg *Studies of the molecular basis of eukaryotic transcription*
2003 Chemistry R. MacKinnon *Discoveries concerning channels in cell membranes.*
1997 Chemistry P.D. Boyer, J.E. Walker, J.C. Skou *Elucidation of the enzymatic mechanism underlying the synthesis of ATP and for the first discovery of an ion-transporting enzyme, Na^+, K^+-ATPase.*
1996 Chemistry R. Curl, H. Kroto, R. Smalley Discovery of fullerenes.
1994 Physics C. Shull, N. Brockhouse Pioneering contributions to the development of neutron scattering techniques for studies of condensed matter.
1992 Physics G. Charpak Invention and development of particle detectors, in particular the multiwire proportional chamber.
1991 Physics P-G de Gennes Discovering that methods developed for studying order phenomena in simple systems can be generalized to more complex forms of matter, in particular to liquid crystals and polymers.
1988 Chemistry J. Deisenhofer, R. Huber, H. Michel *The determination of the 3D structure of a photosynthetic reaction centre.*
1985 Chemistry H. Hauptman, J. Karle Outstanding achievements in the development of direct methods for the determination of crystal structures.
1982 Chemistry A. Klug Development of crystallographic EM and structural elucidation of biologically important nucleic acid-protein complexes.
1976 Chemistry W.N. Lipscomb Studies on the structure of boranes illuminating problems of chemical bonding.
1972 Chemistry C.B. Anfinsen *Work on ribonuclease, especially concerning the connection between the amino acid sequence and the biologically active conformation.*
1964 Chemistry D. Hodgkin Determinations by X-ray techniques of the structures of important biochemical substances.
1962 Chemistry J.C. Kendrew, M. Perutz *Studies of the structures of globular proteins.*
1962 Physiology or Medicine F. Crick, J. Watson, M. Wilkins *Discoveries concerning the molecular structure of nucleic acids and its significance for information transfer in living material.*
1954 Chemistry L.C. Pauling Research into the nature of the chemical bond and its application to the elucidation of the structure of complex substances.
1946 Chemistry J.B. Sumner Discovery that enzymes can be crystallized.
1937 Physics C.J. Davisson, G. Thompson Experimental discovery of the diffraction of electrons by crystals.
1936 Chemistry P. Josephus Wilhelmus Debye Contributions to our knowledge of molecular structure through investigations on dipole moments and on the diffraction of X-rays and electrons in gases.
1929 Physics L-V de Broglie Discovery of the wave nature of electrons.
1917 Physics C. Glover Barkla Discovery of the characteristic Röntgen radiation of the elements.
1915 Physics W.H. Bragg, W.L. Bragg Services in the analysis of crystal structure by means of X-rays.
1914 Physics M. von Laue Discovery of the diffraction of X-rays by crystals.
1901 Physics W.C. Röntgen Extraordinary services rendered by the discovery of the remarkable rays subsequently named after him

Advantages of Crystallography

- There are essentially 3 techniques to obtain a protein structure:

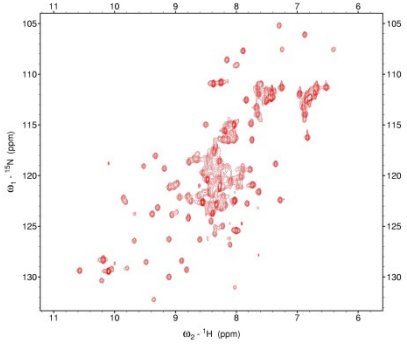


a) Electron Cryo-Microscopy:

- *Low resolution (7.5-25 Å).*
- *Good for large complexes or assemblies.*

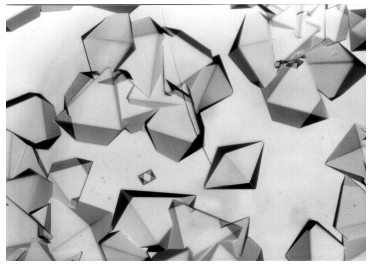
b) NMR (Nuclear Magnetic Resonance):

- *High resolution.*
- *Protein in solution.*
- *For small proteins (< 30 kDa).*
- *A ensemble of possible models are obtained.*

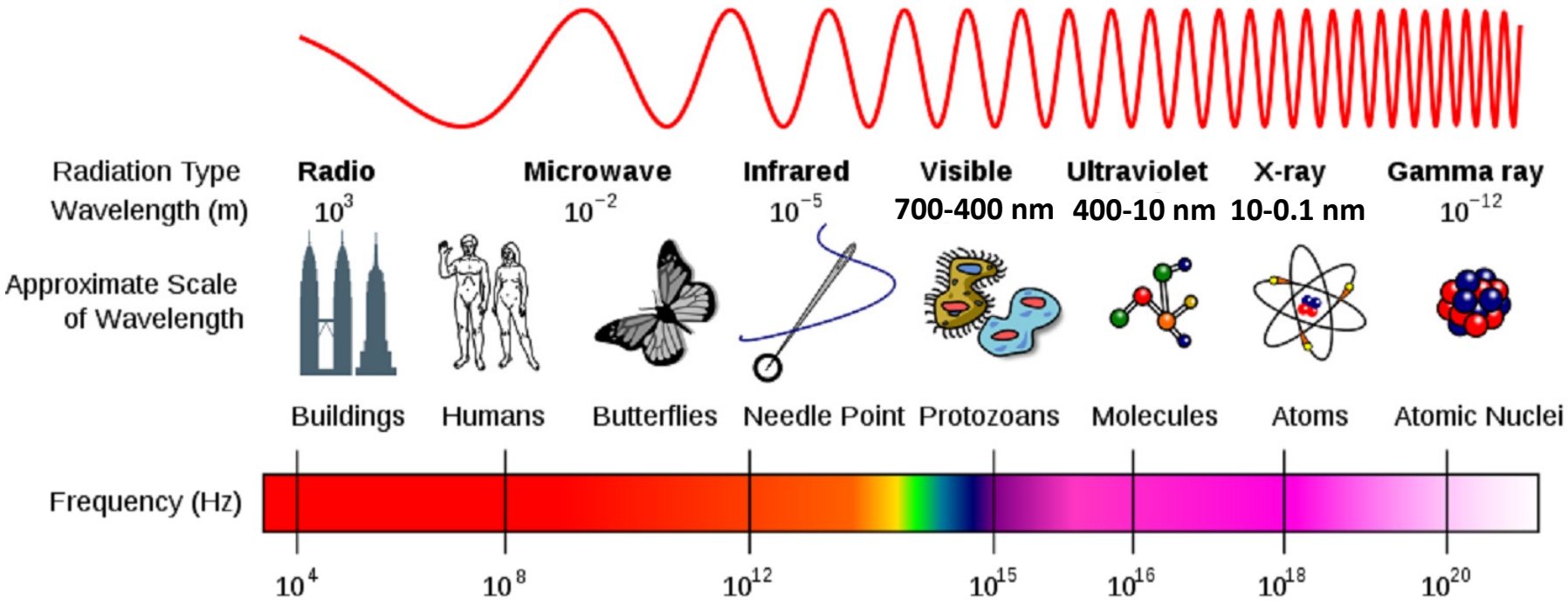


c) X-ray Crystallography:

- *High resolution.*
- *Useful for large proteins and complexes (Ribosome 1.5 MDa).*
- *Only a model is obtained.*
- *Bottleneck: Obtaining good crystals*



Why use X-rays?

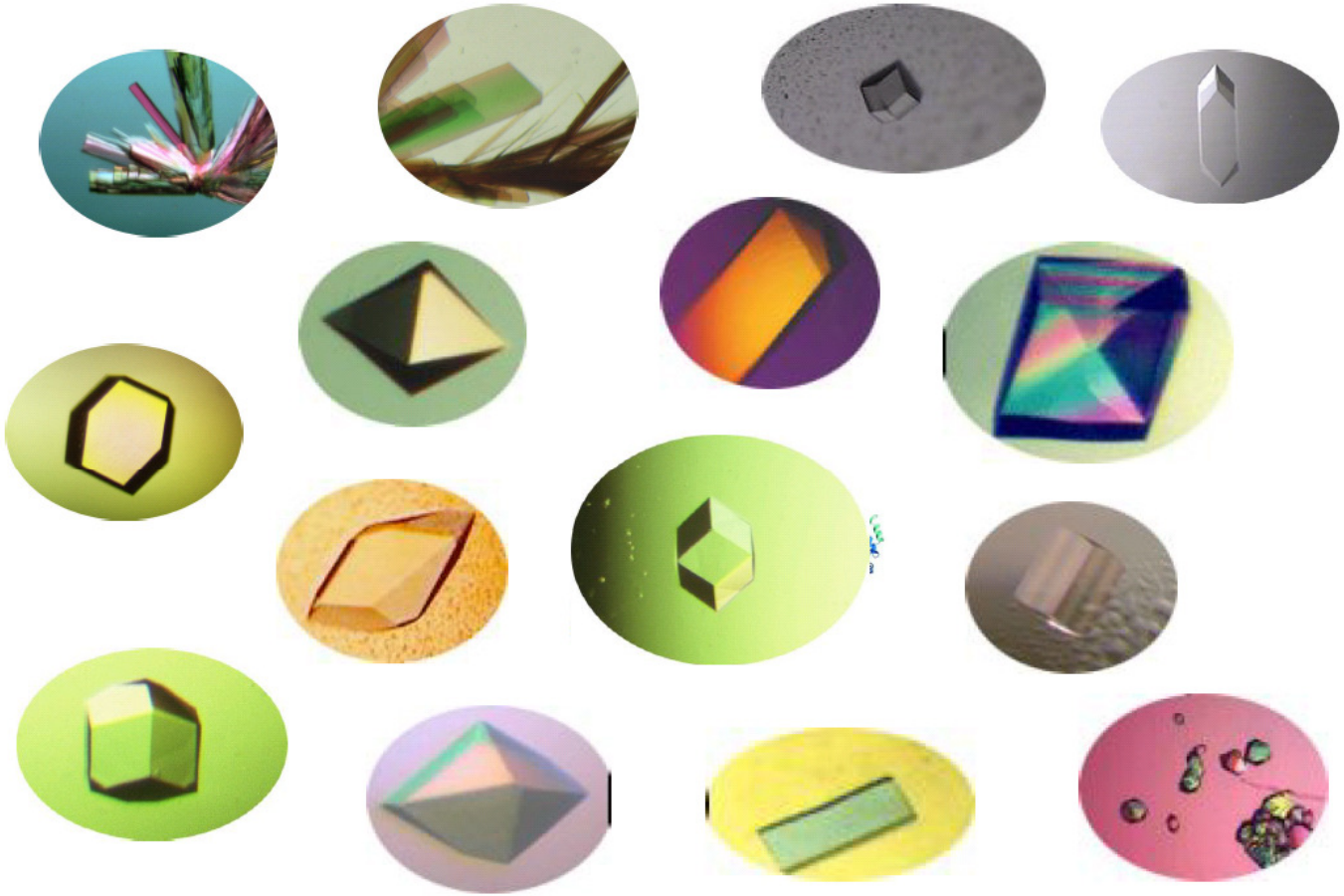


- In all forms of microscopy, the amount of detail or the resolution is limited by the wavelength of the electro-magnetic radiation used:

- ***Light microscopy → (400-700 nm) Individual cells and sub-cellular organelles.***

- ***In order to see proteins in atomic detail: λ = 0.1 nm (1 Å) → X-rays.***

Protein Crystals



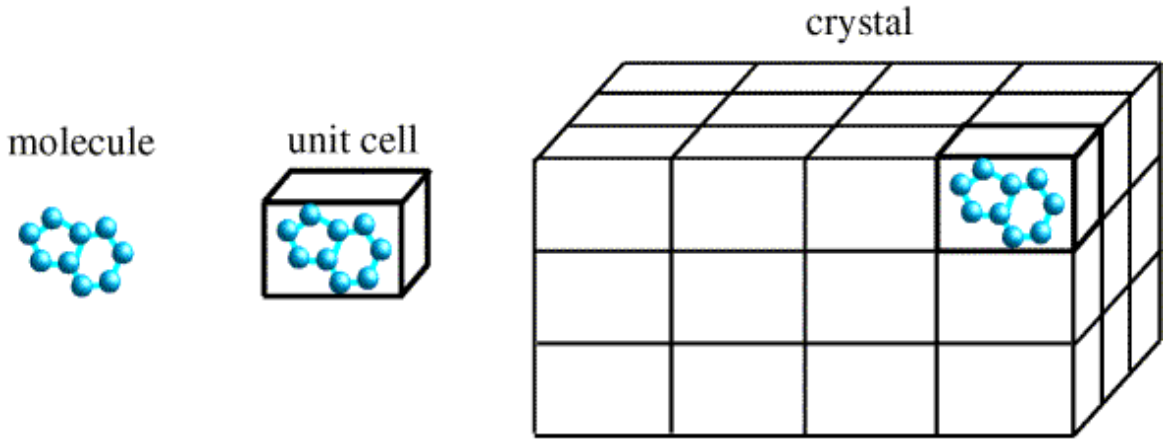
First bottleneck in structure determination

Why crystals?

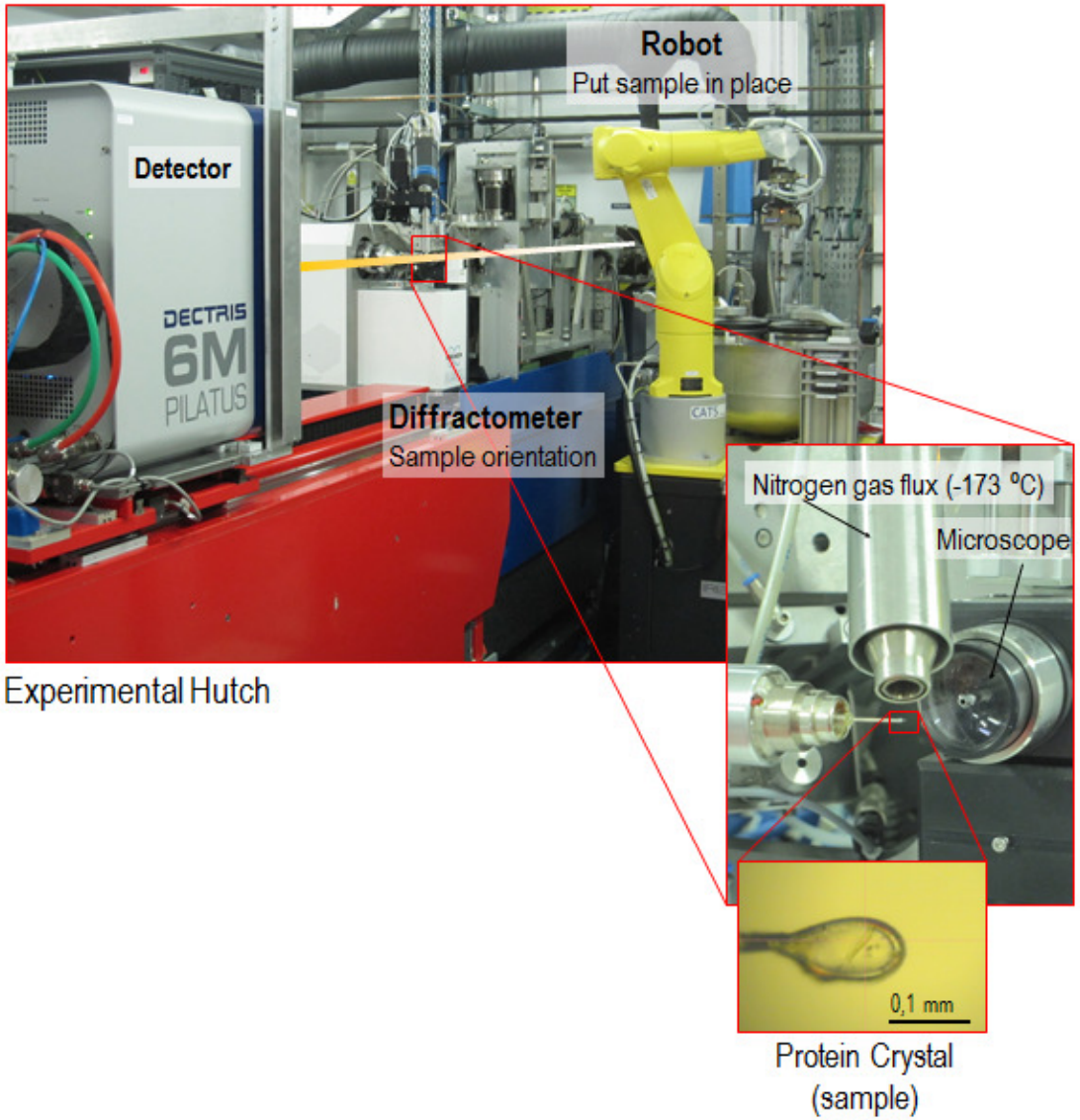
- ***X-ray scattering from a single molecule would be incredibly weak and extremely difficult to detect above the noise level.***

- ***A crystal arranges huge numbers of molecules in the same orientation, so that scattered waves can add up in phase and raise the signal to a measurable level.***

- ***A crystal acts as an amplifier.***

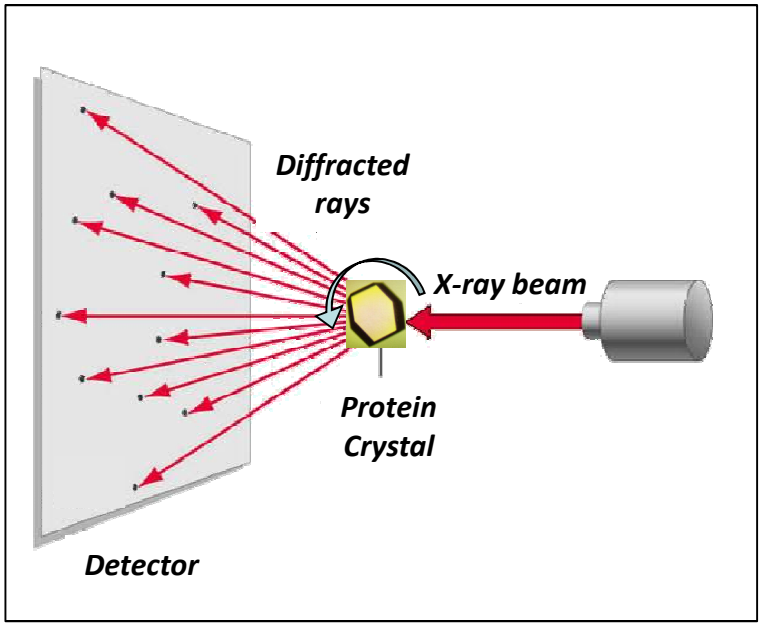


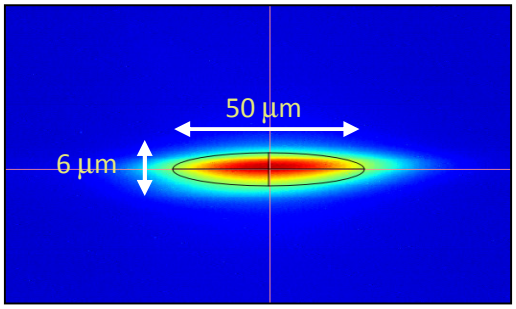
Experimental set-up



Experimental Hutch

Protein Crystal
(sample)

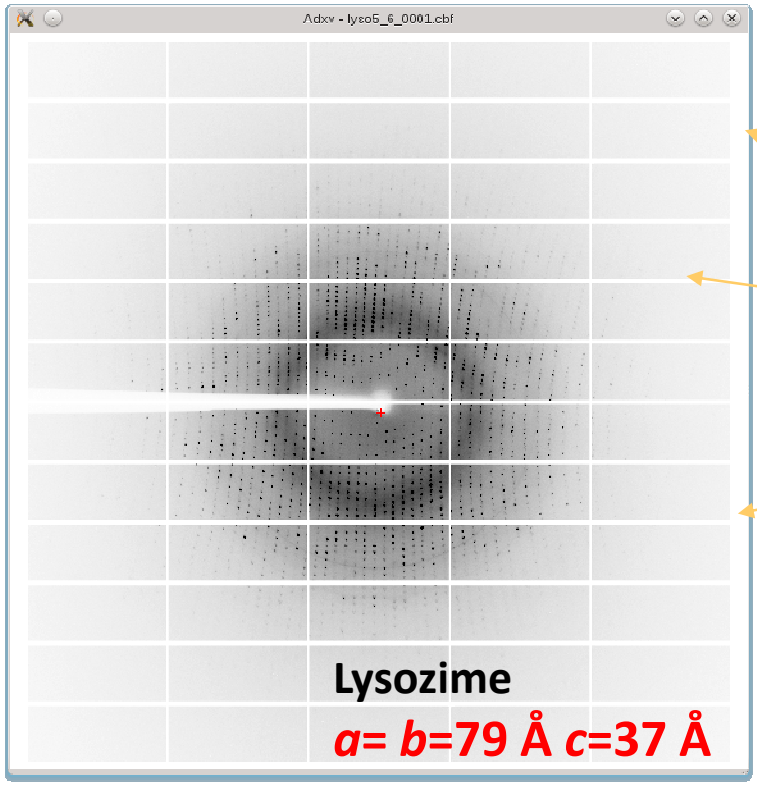


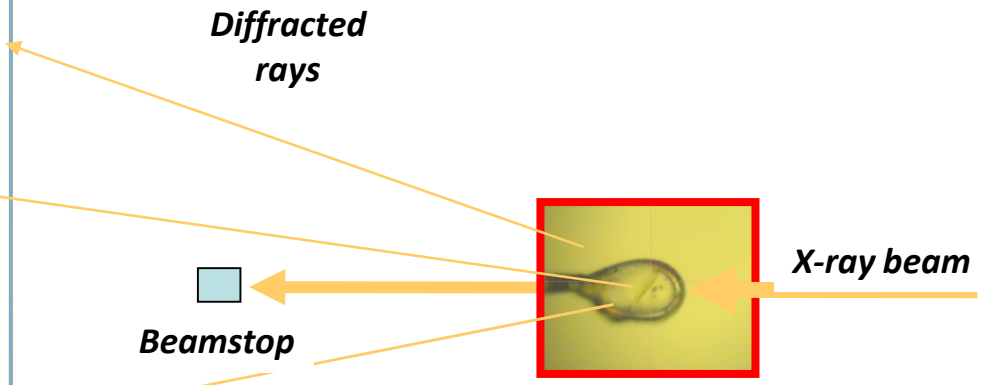


Beam at sample

Data collection

Diffraction patern

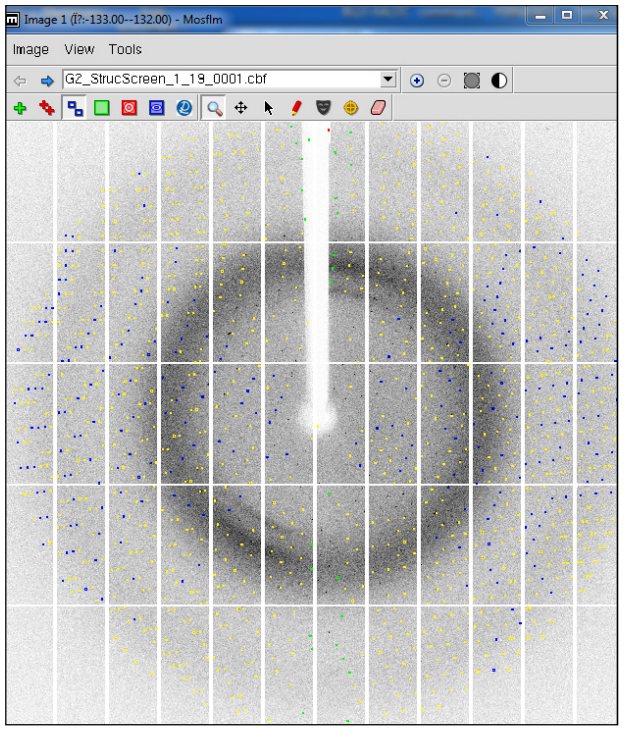


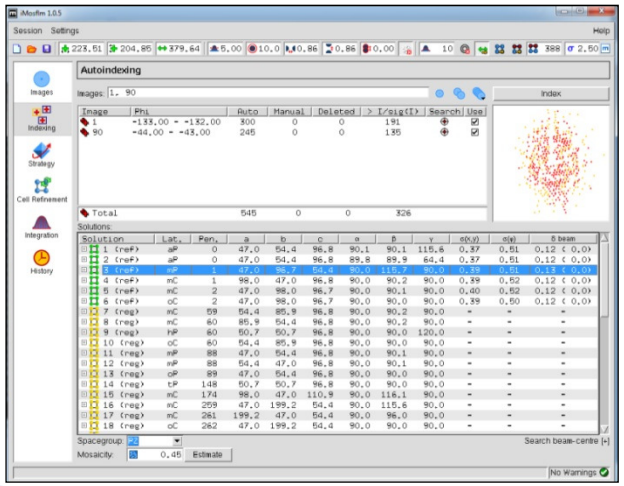


- *The data of a diffraction experiment is a collection of images (100- >1000) rotated a constant angle* **(rotation angle and number of images depend on the space group and orientation of the crystal)**

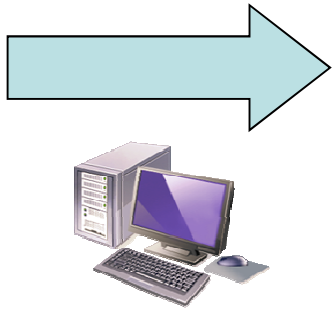
- ***XALOC: THE FAST READ-OUT OF THE PILATUS DETECTOR ALLOWS TO COLLECT A COMPLETE DATASET IN FEW MINUTES (~3-5 MIN/DATASET).***

Data processing





- $P4_32_12$
- *a*= *b*=79 Å *c*=37 Å $\alpha=\beta=\gamma=90°$
- Orientation matrix



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INDE   -22   11   21 FOBS=      59.2      0.0 SIGMA=      14.82 TEST=     0
INDE   -22   12    1 FOBS=      67.4      0.0 SIGMA=      15.61 TEST=     0
INDE   -22   12    2 FOBS=      41.4      0.0 SIGMA=      17.83 TEST=     0
INDE   -22   12    3 FOBS=      68.9      0.0 SIGMA=      16.91 TEST=     0
INDE   -22   12    4 FOBS=     231.5      0.0 SIGMA=      20.04 TEST=     0
INDE   -22   12    5 FOBS=      57.3      0.0 SIGMA=      14.24 TEST=     0
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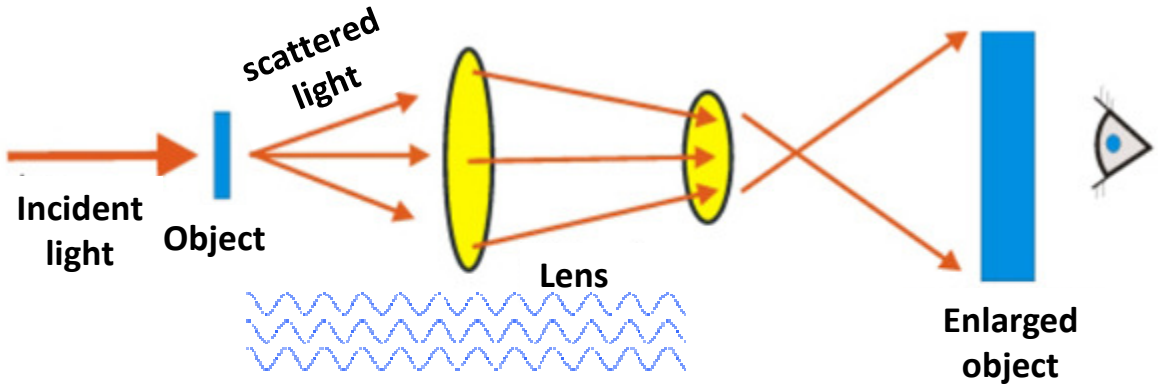
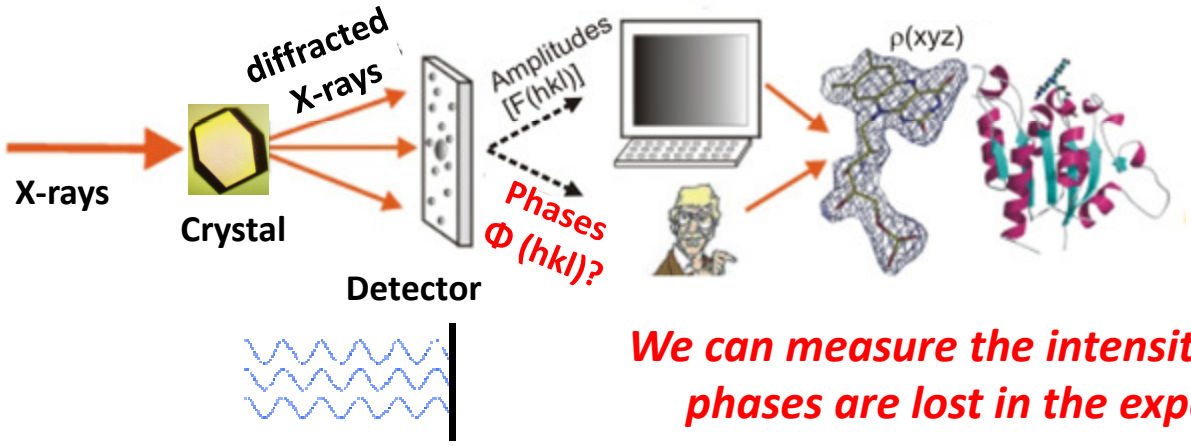
RESOLUTION LIMIT	NUMBER OF REFLECTIONS OBSERVED	UNIQUE	POSSIBLE	COMPLETENESS OF DATA	R-FACTOR observed	R-FACTOR expected	COMPARED	I/SIGMA
3.76	7563	1351	1359	99.4%	2.0%	2.4%	7550	64.62
2.67	13848	2277	2282	99.8%	2.4%	2.5%	13841	61.47
2.18	18104	2888	2895	99.8%	3.2%	3.0%	18092	50.06
1.89	21215	3388	3390	99.9%	4.1%	3.9%	21202	37.67
1.69	24546	3795	3797	99.9%	6.8%	6.8%	24531	23.78
1.54	25959	4193	4193	100.0%	11.0%	11.2%	25949	15.11
1.43	27002	4511	4514	99.9%	10.3%	10.5%	27790	9.10
1.34	28455	4862	4864	100.0%	30.8%	34.2%	28442	5.23
1.26	28810	5124	5154	99.4%	45.2%	52.1%	28746	3.41
total	196302	32389	32448	99.8%	3.8%	4.0%	196143	22.77

The phase problem



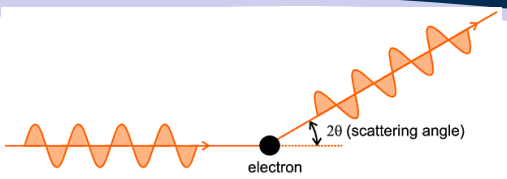


We can measure the intensities, but the phases are lost in the experiment.

Amplitudes are related to intensities: $I(hkl) = |F(hkl)|^2$

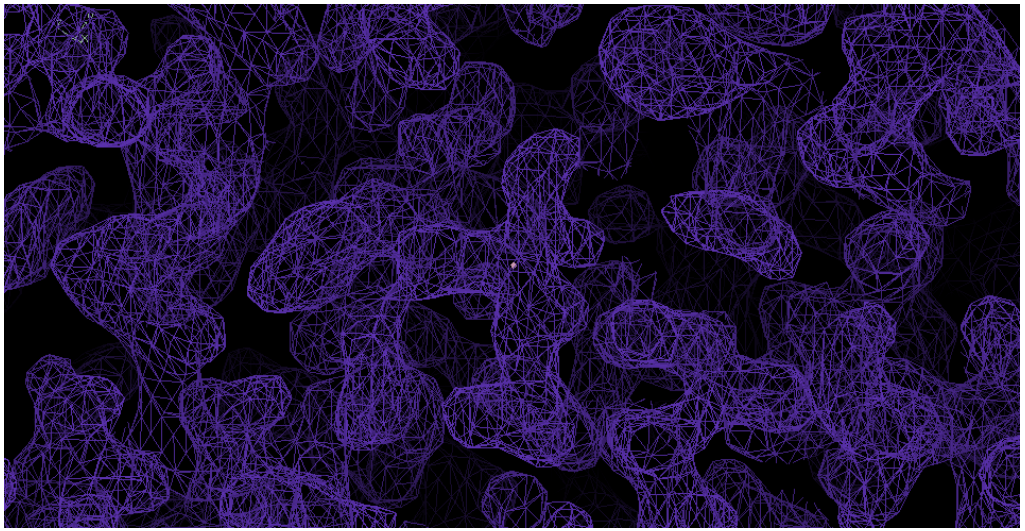
$$\textit{ELECTRON DENSITY} = (1/V) \Sigma\Sigma\Sigma \overbrace{|F_o(hkl)|}^{\textit{AMPLITUDES}} \cos 2\pi (hx + ky + lz - \overbrace{\phi_c(hkl)}^{\textit{PHASES???}})$$

Electron density map



• X-rays are scattered by the electron clouds of atoms.

• Electron-density (ED) map: "a representation of the variation in the local concentration of electrons throughout the crystal".



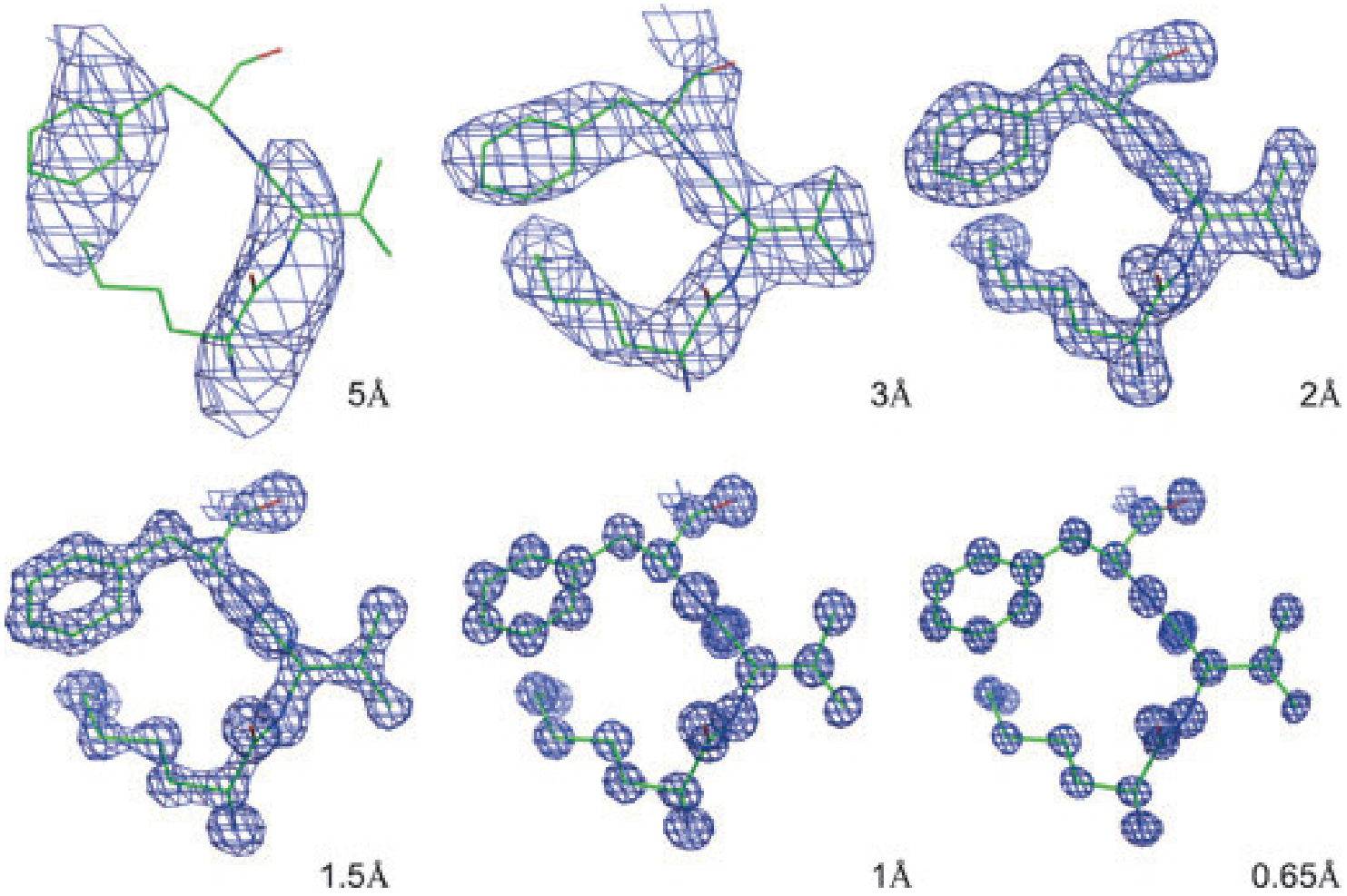
• ED maps represent the primary result of crystallographic experiments. It is constructed by a summation of waves of known phase, amplitude and frequency using Fourier transform.

$$\textbf{\textit{ELECTRON DENSITY}} = (1/V)\ \Sigma\Sigma\Sigma\ \overbrace{|F_o(hkl)|}^{\textbf{\textit{AMPLITUDES}}}\ \cos 2\pi\ (hx + ky + lz - \overbrace{\phi_c(hkl)}^{\textbf{\textit{PHASES}}})$$

Fourier Transform requires both structure factors and phases

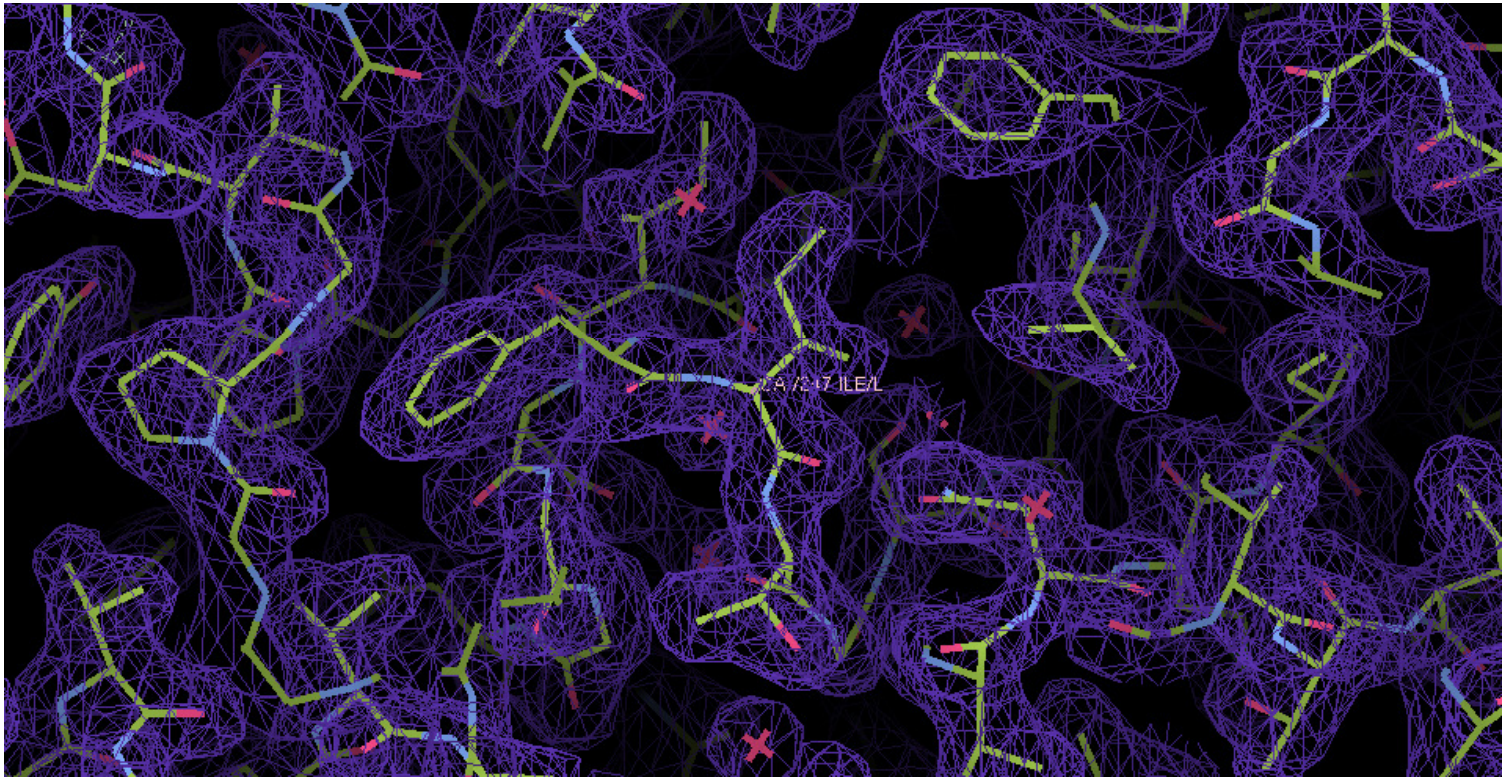
Resolution of the electron density maps

• The clarity and interpretability of ED maps, even those based on accurate phases, depend on the resolution of the diffraction data

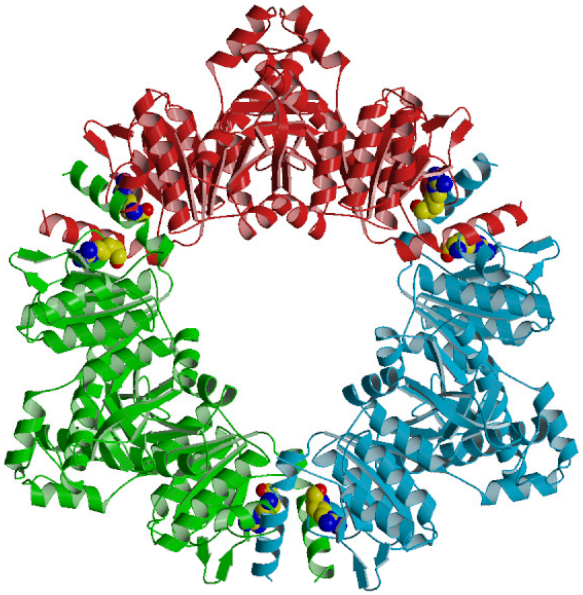


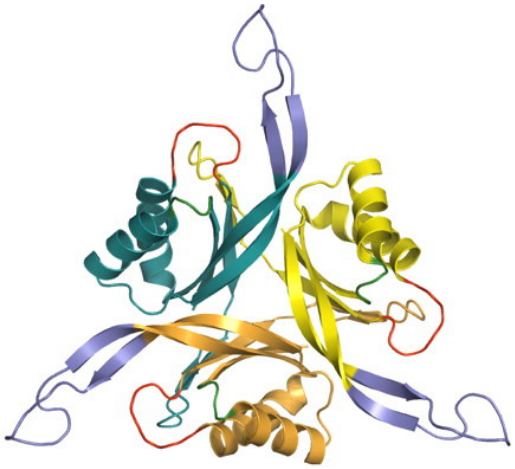
Model building/Refinement

- Once the first experimental ED map is obtained (SAD, MAD,..), the atomic model is fitted inside the map.



Final model





In 1960's -70's it took years to solve a macromolecular structure.

In 1980's it came down to months.

In 1990's to weeks.

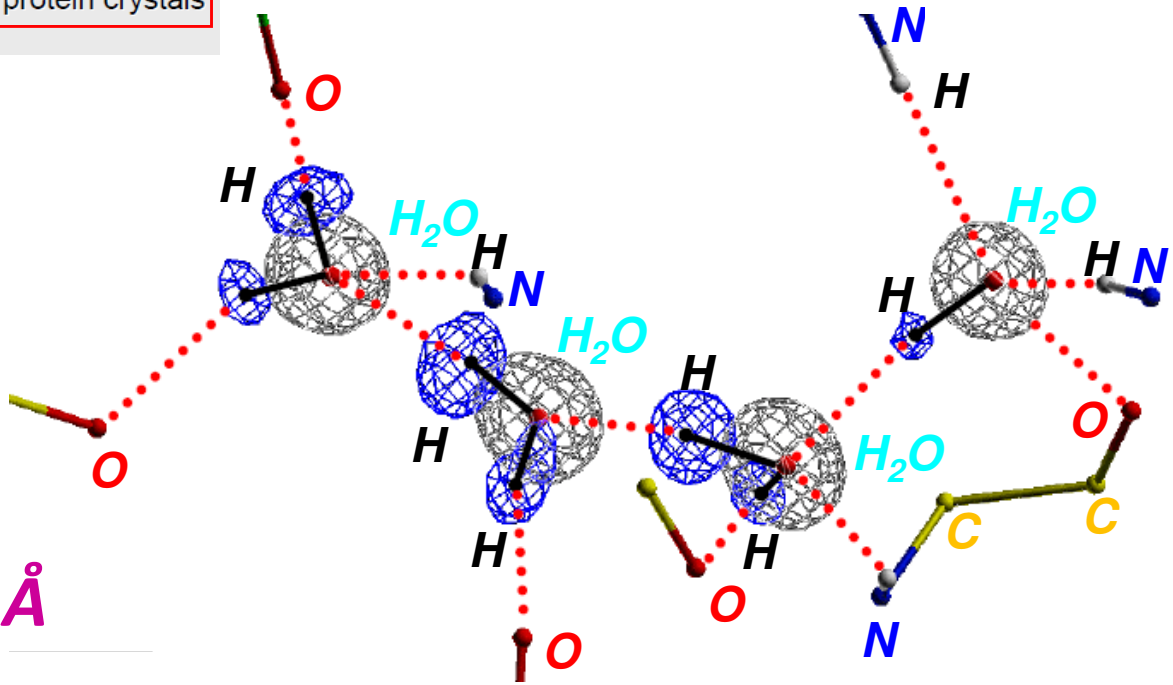
In 2000's it is only days or hours.

What information is provided by a 3D structure?

	Resolution	Features Observed
Low	5.0 Å	Overall shape of the molecule
	3.5 Å	Cα trace
	3.0 Å	Side chains
Medium	2.7 Å	Carbonyl O atoms (bulges) First chance to see water molecules
	2.5 Å	Side chains well resolved, Peptide bond plane resolved
High	1.4 Å	Holes in aromatic rings Anisotropic B-factors possible
	1.2 Å	True atomic resolution
	1.0 Å	First chance to see H atoms
	0.6 Å	Current limit for best protein crystals

Atomic resolution

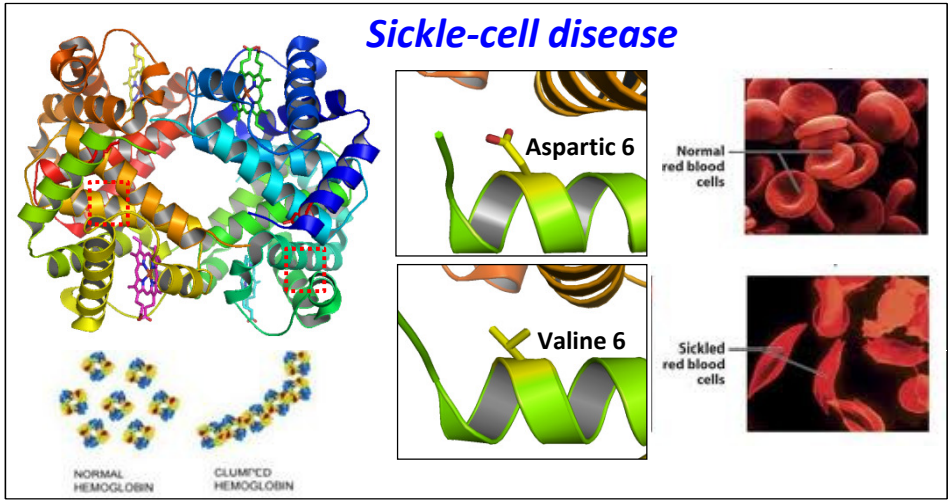
- **It enables us to visualize protein structures at the atomic level and enhances our understanding of protein function.**

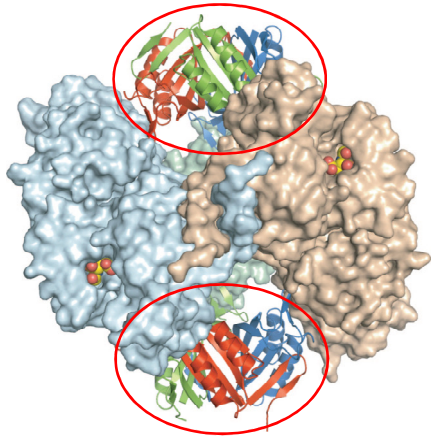


0.86 Å

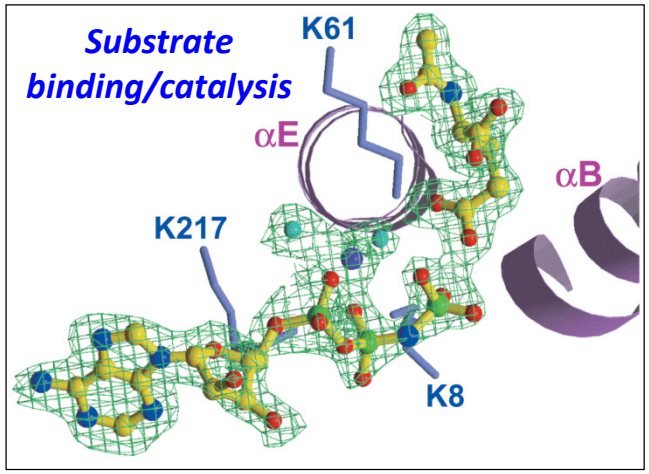
What information is provided by a 3D structure?

- ***3D structure** allows us to understand biological processes at the most basic level: which molecules interact, how they interact, how enzymes catalyse reactions. In some cases, it can allow us to understand disease at an atomic level.*

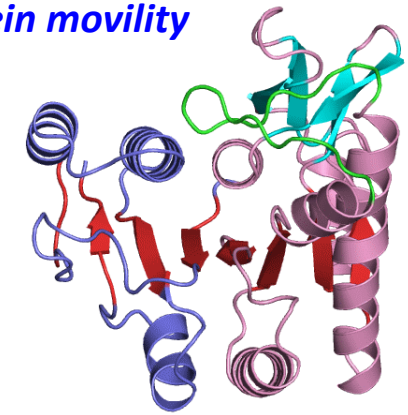


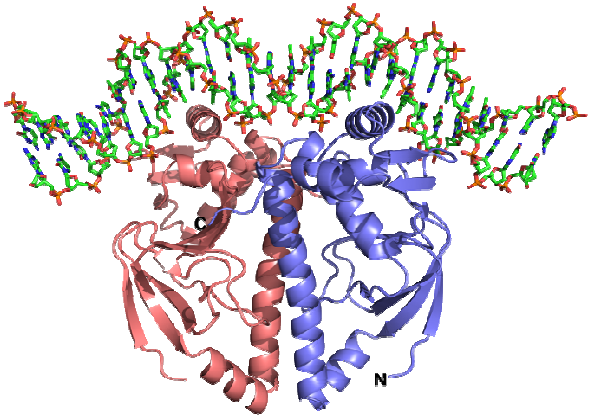


Protein-Protein interactions



Protein movility

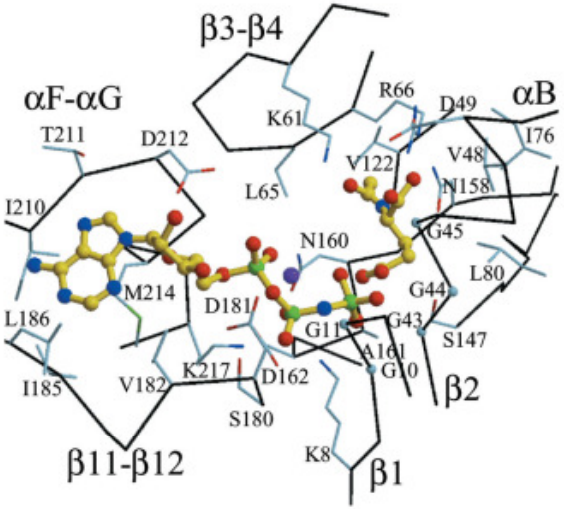


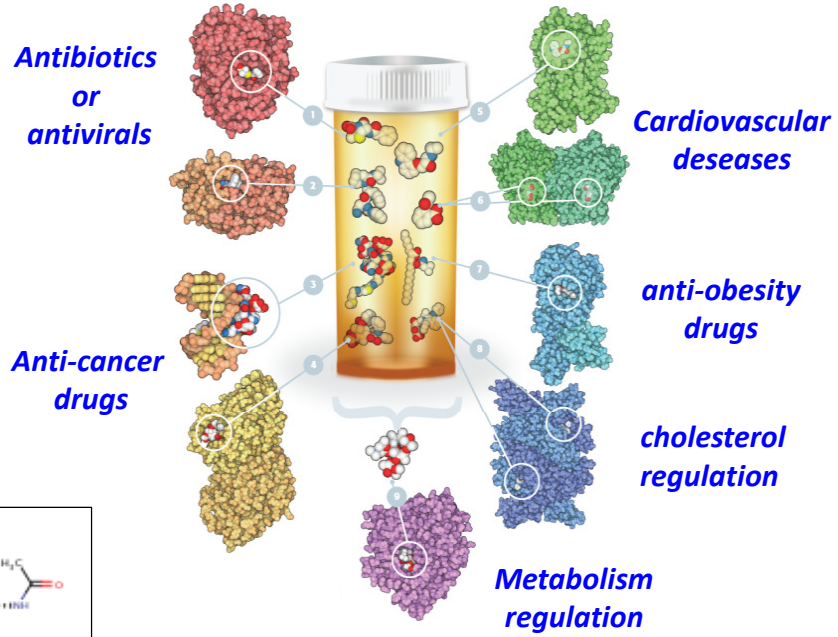


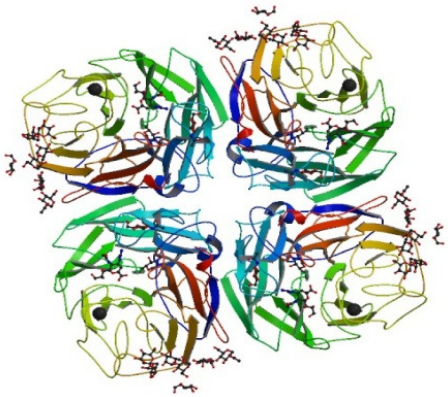
DNA-Protein interactions

Applications of the protein structure

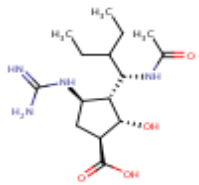
- ***Discovery of novel therapeutics using a structure-based approach (rationally based drug design): Identifies opportunities to block or modify molecular interactions.***



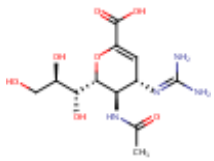




Influenza virus Neuraminidase



Tamiflu



Relenza

The anti-influenza drug Relenza is the world's first structure-based anti-viral drug obtained using this methodology

The XALOC Team

Engineering

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Daniel Fulla	
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Laura Campos	Administration

Thank you for your attention