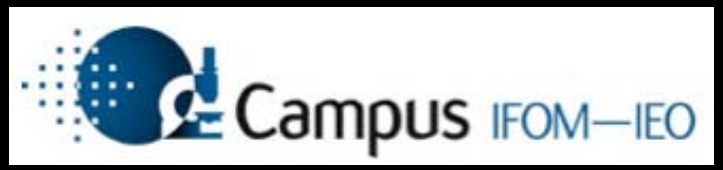
# *Struttura del DNA*
# *e nascita della Biologia Molecolare*

dott. Andrea Musacchio

Responsabile del gruppo di ricerca IEO:
Basi molecolari della segregazione cromosomica

**9 Maggio 2007**

*Che cosa è la biologia molecolare?*

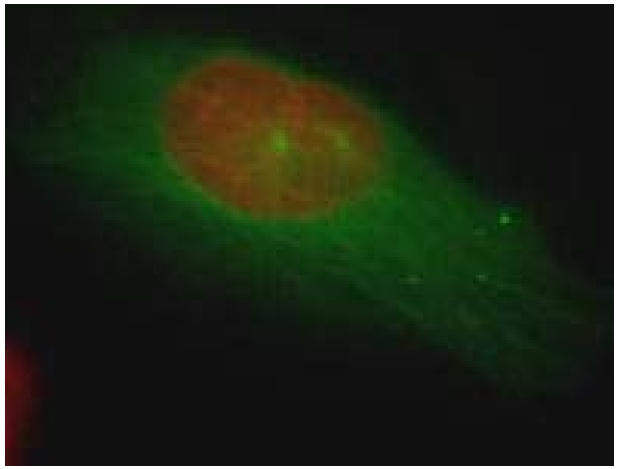
*Quale è la complessità molecolare di una cellula?*

# Fasi della mitosi

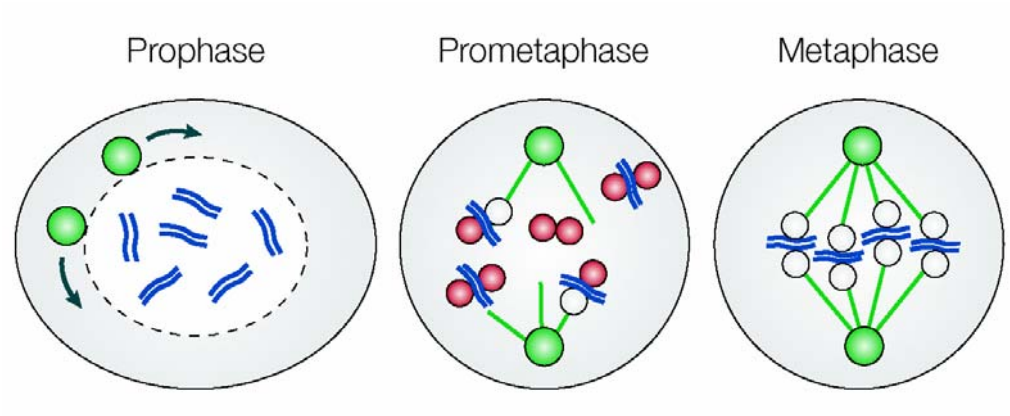
(Kops et al., Nature Rev. Cancer, 2005)

## Mitosi in fluorescenza

www

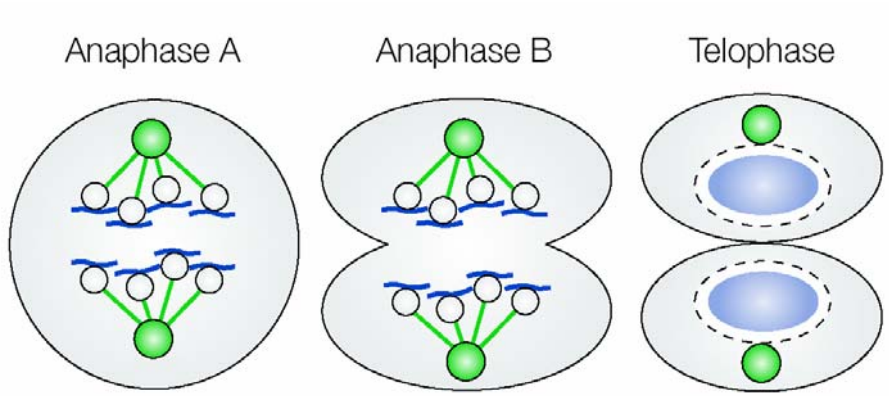


DNA Tubulin




Daniela Cimini

QuickTime™ and a
Video decompressor
are needed to see this picture.




Tubulin CENP-F

# Complessità molecolare di una cellula: un assaggio

**Pregi**

Utile schema riassuntivo

Moderata complessità (!)

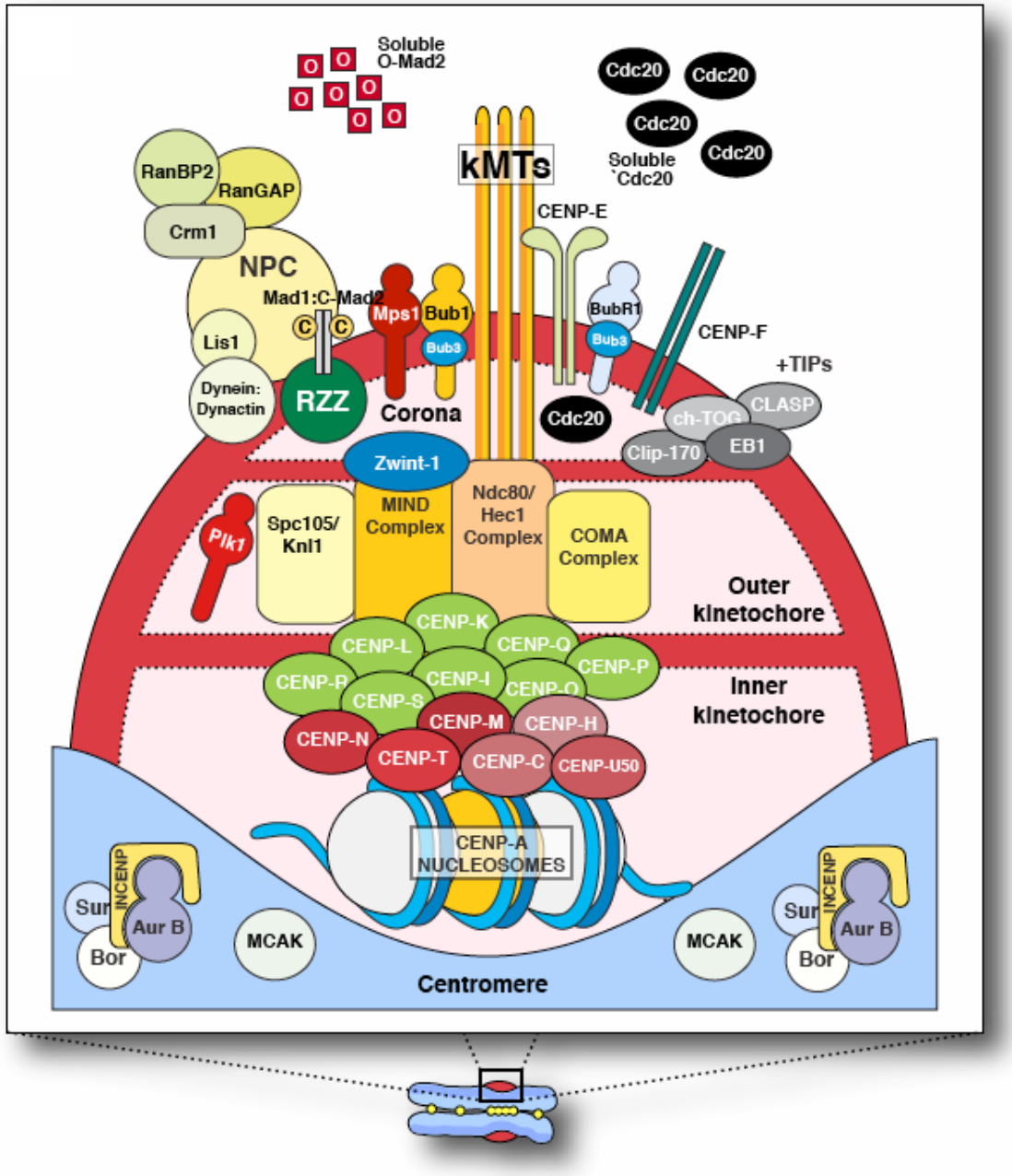
Non richiede una compresione dettagliata delle basi molecolari

**Difetti**

Dettaglio completamente perduto

Semplificazione eccessiva dei “caratteri”

Elementi dinamici assenti



## *MITOSI*

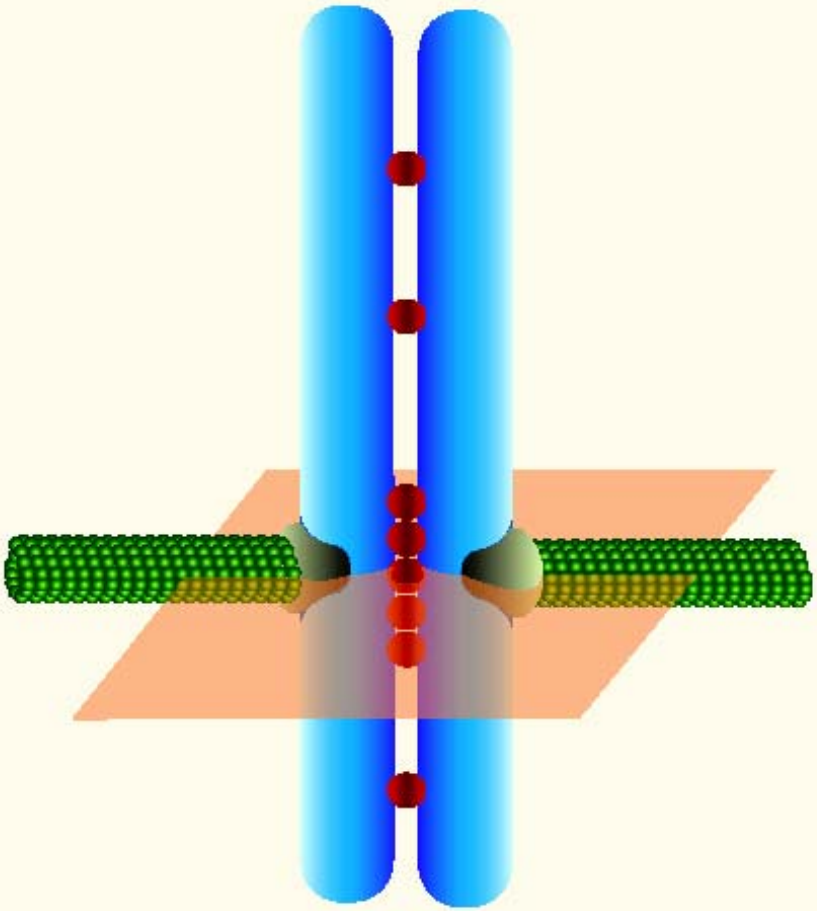
Conivolge alcune migliaia di proteine diverse
(e forse altri tipi molecolari)

La funzione di gran parte di queste proteine è regolata durante la mitosi
(modificazioni post-traduzionali, fosforilazione, ubiquitinazione, etc.)

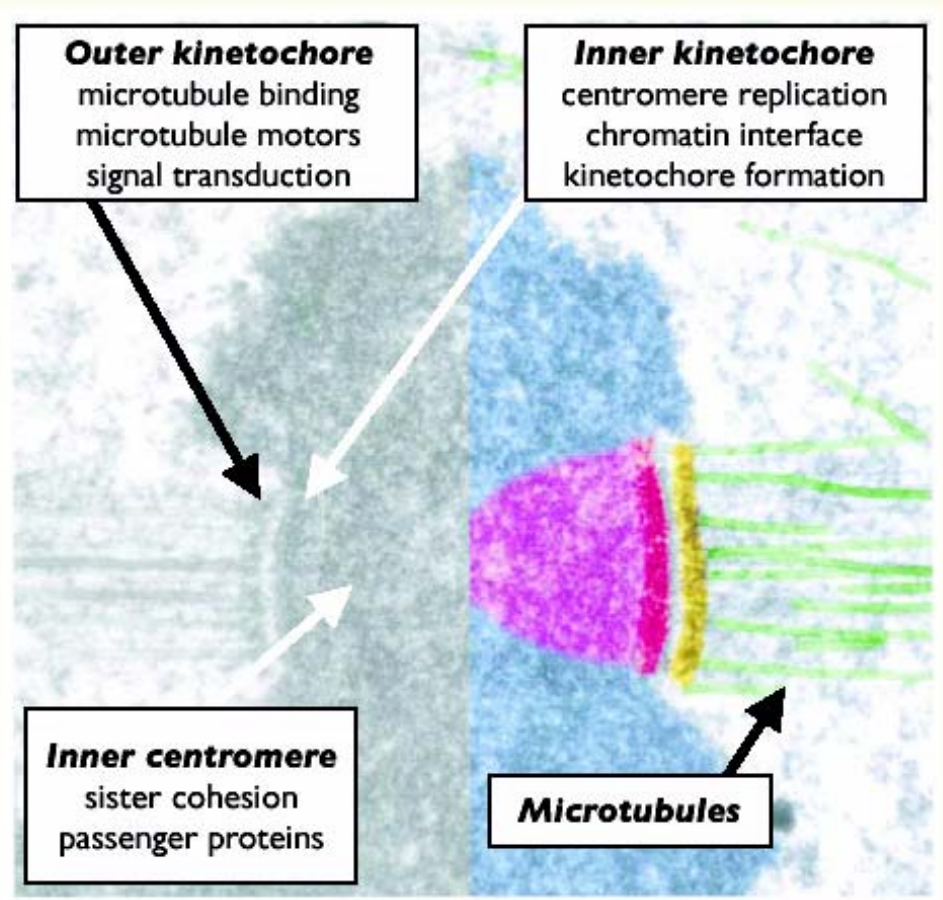
Molte di queste proteine agiscono in complessi macromolecolari

***Idealmente, vorremmo conoscere le basi molecolari della funzione di queste macromolecole***

# Esempio: cinetocori al microscopio elettronico



A section through the centromere

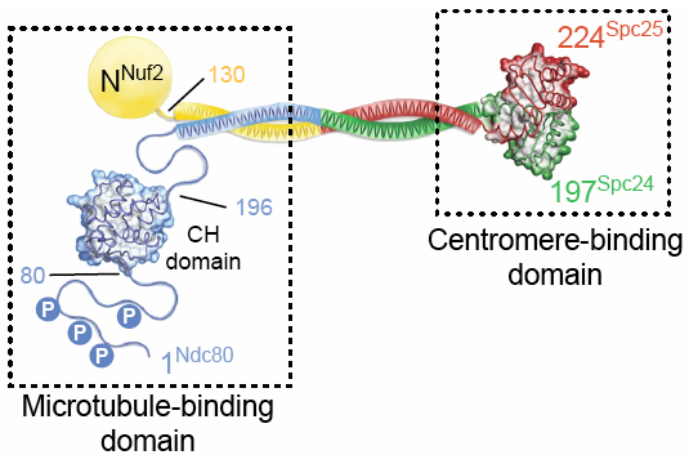


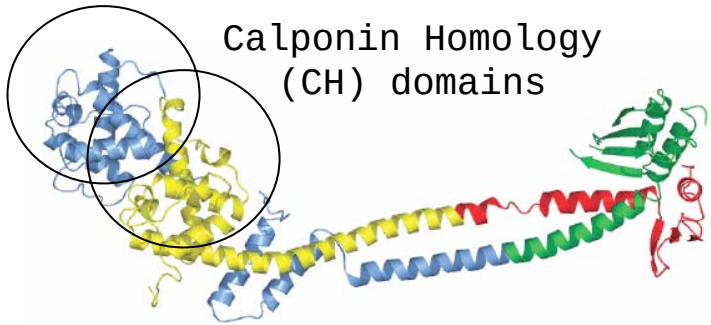

Cleveland, Mao, and Sullivand, Cell (2003)

**Dove sono le 100 e passa proteine del cinetocoro?**

# *Uso di tecniche strutturali per capire la organizzazione del cinetocoro*

*Claudio Ciferri, Sebastiano Pasqualato*

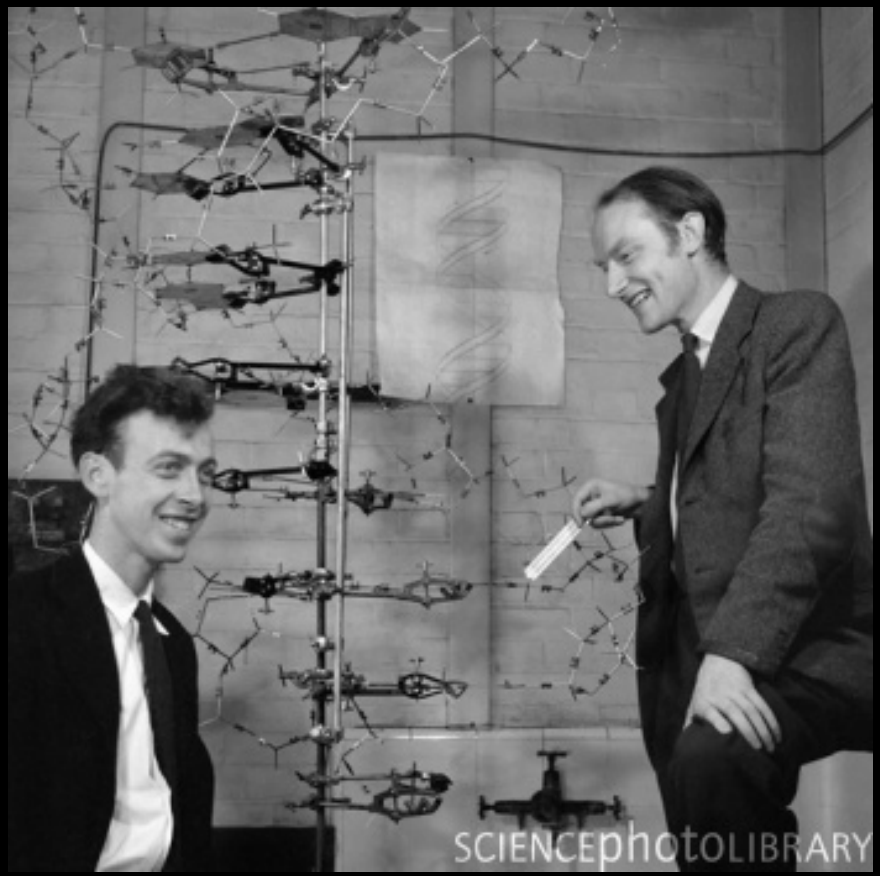


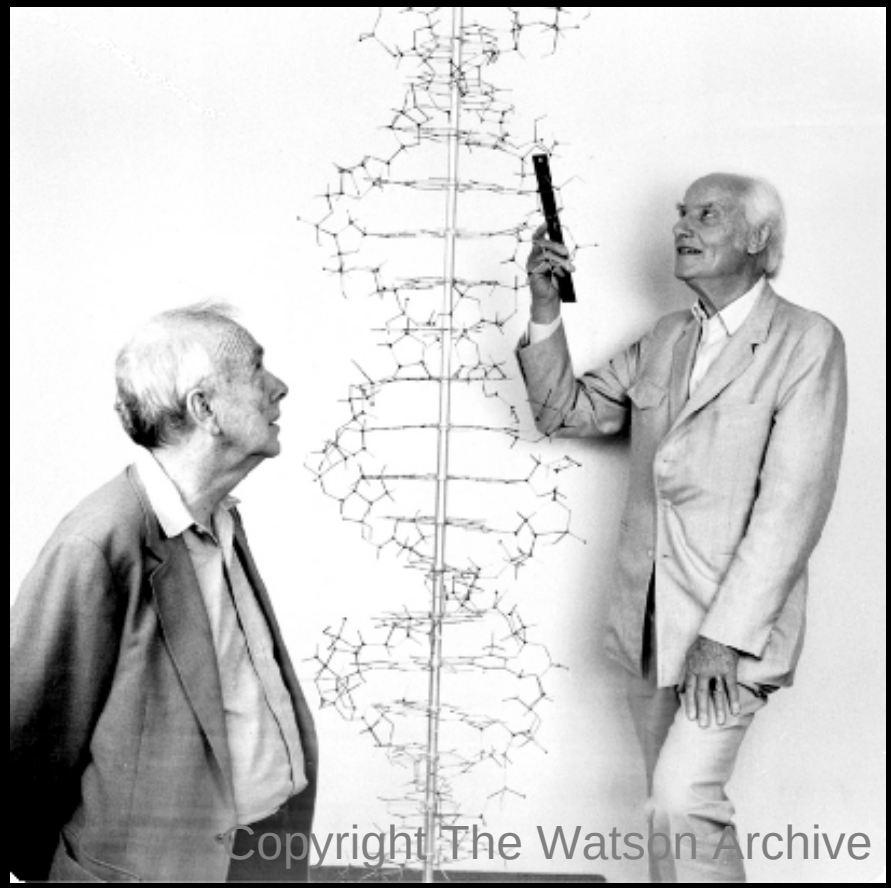




QuickTime™ and a
Sorenson Video 3 decompressor
are needed to see this picture.

*Come si acquisisce l'evidenza*
*che costituisce*
*la base della biologia molecolare?*
*Quale è il livello di dettaglio richiesto?*

# James Watson and Francis Crick
# Scoperta della struttura a doppia elica del DNA



Professor Francis Harry Compton Crick, OM FRS (8 June, 1916 – 28 July, 2004)
British physicist, molecular biologist and neuroscientist, most noted for being one of the co-discoverers of the structure of the DNA molecule in 1953. He, James D. Watson, and Maurice Wilkins were jointly awarded the 1962 Nobel Prize for Physiology or Medicine for their discoveries concerning the molecular structure of nucleic acids and its significance for information transfer in living material

National Institutes of Health (USA)

James D. Watson and Francis H. C. Crick
"Molecular Structure of Nucleic Acids: A Structure for Deoxyribose Nucleic Acid" (1953)



equipment, and to Dr. G. E. R. Deacon and the captain and officers of R.R.S. *Discovery II* for their part in making the observations.


[1] Young, F. B., Gerrard, H., and Jevons, W., *Phil. Mag.*, **40**, 149 (1920).
[2] Longuet-Higgins, M. S., *Mon. Not. Roy. Astro. Soc., Geophys. Supp.*, **5**, 285 (1949).
[3] Von Arx, W. S., Woods Hole Papers in Phys. Oceanog. Meteor., **11** (3) (1950).
[4] Ekman, V. W., *Arkiv. Mat. Astron. Fysik. (Stockholm)*, **2** (11) (1905).

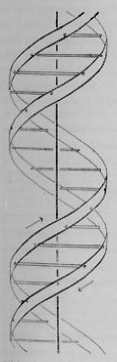

# MOLECULAR STRUCTURE OF NUCLEIC ACIDS

## A Structure for Deoxyribose Nucleic Acid

We wish to suggest a structure for the salt of deoxyribose nucleic acid (D.N.A.). This structure has novel features which are of considerable biological interest.

A structure for nucleic acid has already been proposed by Pauling and Corey[1]. They kindly made their manuscript available to us in advance of publication. Their model consists of three intertwined chains, with the phosphates near the fibre axis, and the bases on the outside. In our opinion, this structure is unsatisfactory for two reasons: (1) We believe that the material which gives the X-ray diagrams is the salt, not the free acid. Without the acidic hydrogen atoms it is not clear what forces would hold the structure together, especially as the negatively charged phosphates near the axis will repel each other. (2) Some of the van der Waals distances appear to be too small.

Another three-chain structure has also been suggested by Fraser (in the press). In his model the phosphates are on the outside and the bases on the inside, linked together by hydrogen bonds. This structure as described is rather ill-defined, and for this reason we shall not comment on it.



This figure is purely diagrammatic. The two ribbons symbolize the two phosphate–sugar chains, and the horizontal rods the pairs of bases holding the chains together. The vertical line marks the fibre axis

We wish to put forward a radically different structure for the salt of deoxyribose nucleic acid. This structure has two helical chains each coiled round the same axis (see diagram). We have made the usual chemical assumptions, namely, that each chain consists of phosphate diester groups joining β-D-deoxyribofuranose residues with 3′,5′ linkages. The two chains (but not their bases) are related by a dyad perpendicular to the fibre axis. Both chains follow right-handed helices, but owing to the dyad the sequences of the atoms in the two chains run in opposite directions. Each chain loosely resembles Furberg's[2] model No. 1; that is, the bases are on the inside of the helix and the phosphates on the outside. The configuration of the sugar and the atoms near it is close to Furberg's 'standard configuration', the sugar being roughly perpendicular to the attached base. There is a residue on each chain every 3·4 A. in the *z*-direction. We have assumed an angle of 36° between adjacent residues in the same chain, so that the structure repeats after 10 residues on each chain, that is, after 34 A. The distance of a phosphorus atom from the fibre axis is 10 A. As the phosphates are on the outside, cations have easy access to them.

The structure is an open one, and its water content is rather high. At lower water contents we would expect the bases to tilt so that the structure could become more compact.

The novel feature of the structure is the manner in which the two chains are held together by the purine and pyrimidine bases. The planes of the bases are perpendicular to the fibre axis. They are joined together in pairs, a single base from one chain being hydrogen-bonded to a single base from the other chain, so that the two lie side by side with identical *z*-co-ordinates. One of the pair must be a purine and the other a pyrimidine for bonding to occur. The hydrogen bonds are made as follows: purine position 1 to pyrimidine position 1; purine position 6 to pyrimidine position 6.

If it is assumed that the bases only occur in the structure in the most plausible tautomeric forms (that is, with the keto rather than the enol configurations) it is found that only specific pairs of bases can bond together. These pairs are: adenine (purine) with thymine (pyrimidine), and guanine (purine) with cytosine (pyrimidine).

In other words, if an adenine forms one member of a pair, on either chain, then on these assumptions the other member must be thymine; similarly for guanine and cytosine. The sequence of bases on a single chain does not appear to be restricted in any way. However, if only specific pairs of bases can be formed, it follows that if the sequence of bases on one chain is given, then the sequence on the other chain is automatically determined.

It has been found experimentally[3,4] that the ratio of the amounts of adenine to thymine, and the ratio of guanine to cytosine, are always very close to unity for deoxyribose nucleic acid.

It is probably impossible to build this structure with a ribose sugar in place of the deoxyribose, as the extra oxygen atom would make too close a van der Waals contact.

The previously published X-ray data[5,6] on deoxyribose nucleic acid are insufficient for a rigorous test of our structure. So far as we can tell, it is roughly compatible with the experimental data, but it must be regarded as unproved until it has been checked against more exact results. Some of these are given in the following communications. We were not aware of the details of the results presented there when we devised our structure, which rests mainly though not entirely on published experimental data and stereochemical arguments.

It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material.

Full details of the structure, including the conditions assumed in building it, together with a set of co-ordinates for the atoms, will be published elsewhere.

We are much indebted to Dr. Jerry Donohue for constant advice and criticism, especially on interatomic distances. We have also been stimulated by a knowledge of the general nature of the unpublished experimental results and ideas of Dr. M. H. F. Wilkins, Dr. R. E. Franklin and their co-workers at






**Inizio della biologia molecolare**

"It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material"

A-T
T-A
C-G
G-C

# Biologia molecolare

## L’appaiamento delle basi è la base dell’eredità

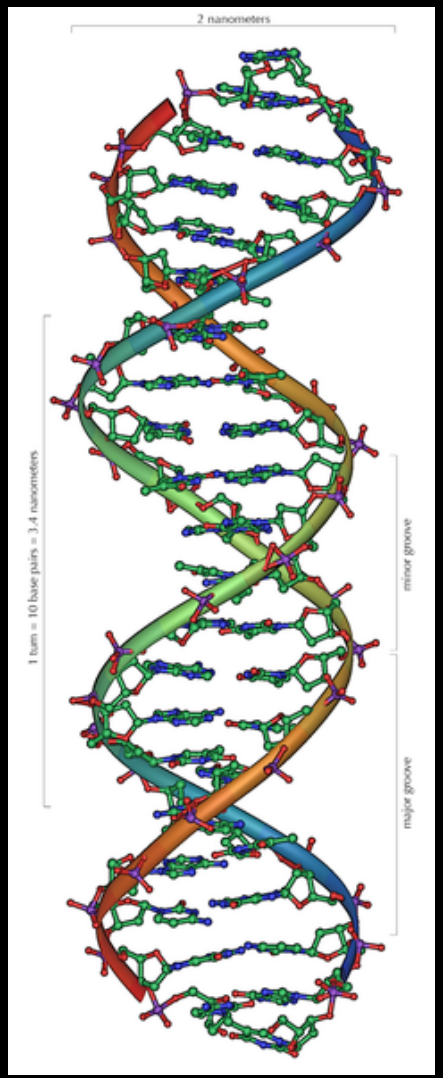
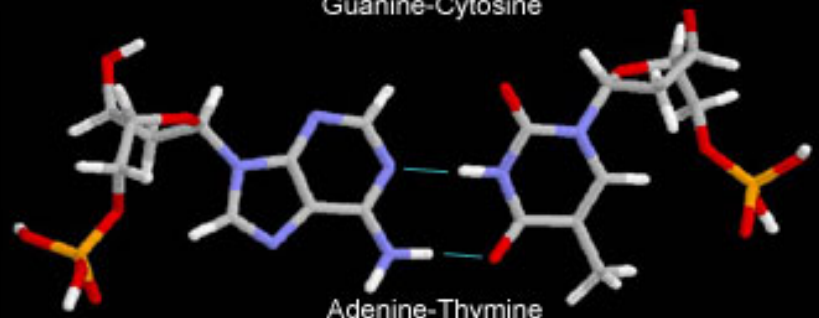



Figure B-6: Base Pairing

The chemical structure of each base allows it to match up with another base. The 3D models provide a nice simulation of the shape-dependent base pairing. The actual chemical structures of the bases are shown below, with the bonds drawn in blue.


Guanine-Cytosine



Adenine-Thymine

***Che cosa è la biologia molecolare?***

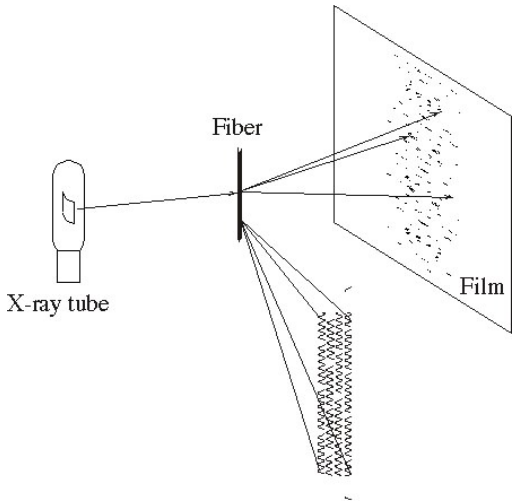
La biologia molecolare è un approccio allo studio della biologia che aspira a descrivere le basi fisico-chimiche della funzione biologica, desrivendo il macchinario molecolare ad essa sottesa con il maggior dettaglio possibile. In questo senso la biologia molecolare è eminentemente riduzionista

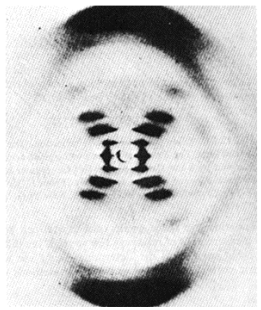
***Come si acquisisce l'evidenza? Con quale livello di dettaglio la si acquisisce?***

***Con quale tecnica Watson e Crick risalirono alla struttura del DNA?***

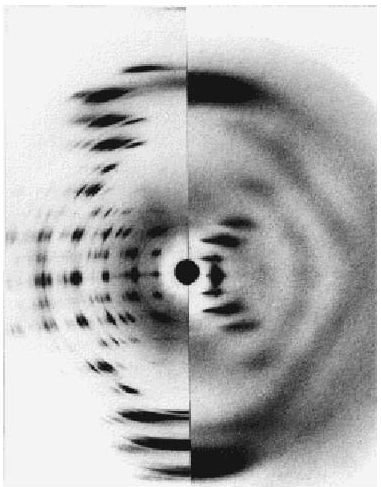
Risposta?

# *Diffrazione dei raggi X da una fibra*



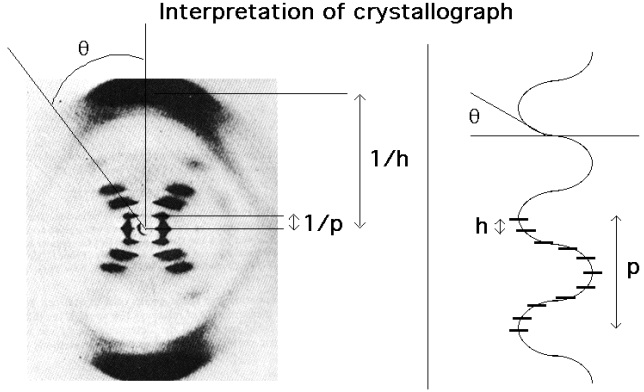



X-ray diffraction pattern from B form of DNA



A-DNA B-DNA

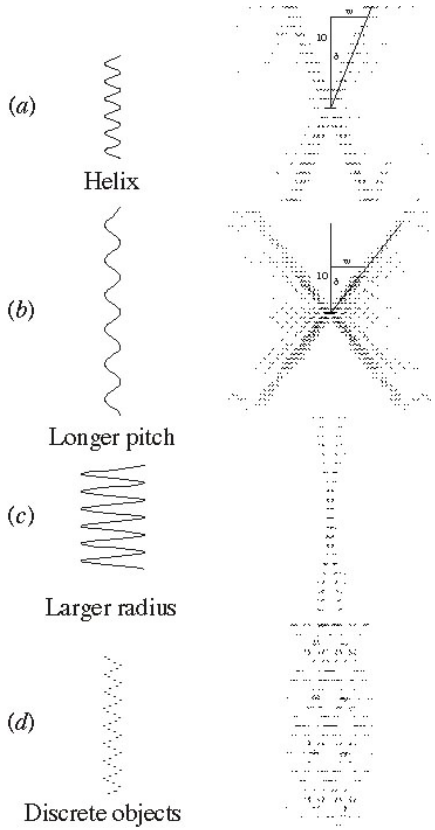
Interpretation of crystallograph




θ - tilt of helix (angle from perpendicular to long axis)

h = 3.4 Å (Distance between bases)

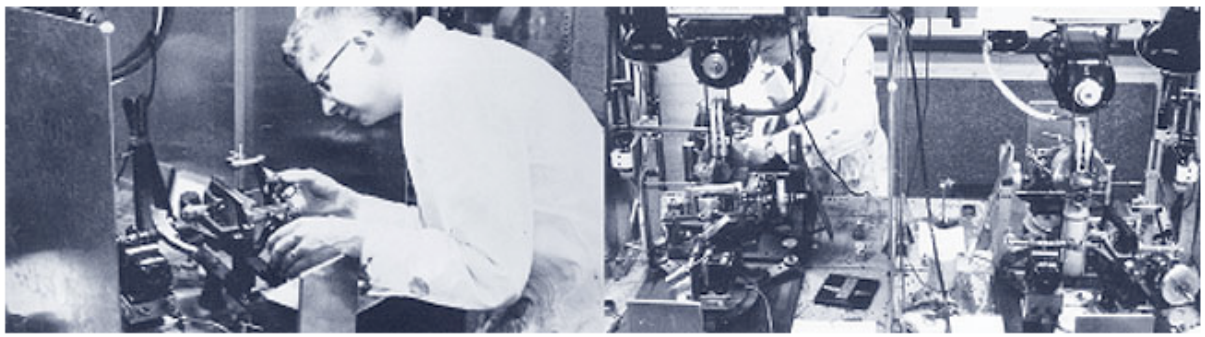
p = 34 Å (Distance for one complete turn of helix; Repeat unit of the helix)



# Nascita del Laboratorio di Biologia Molecolare di Cambridge (UK)
# http://www2.mrc-lmb.cam.ac.uk/origins.html



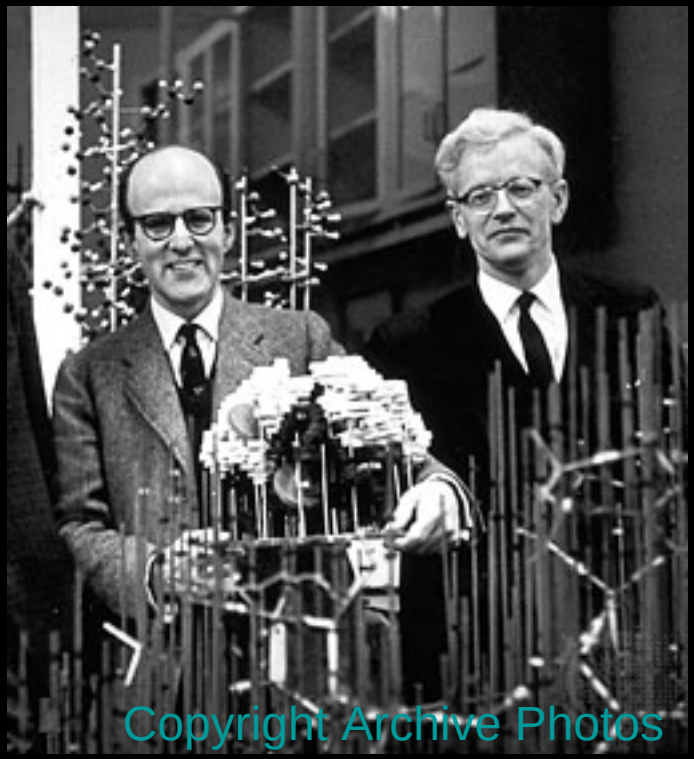
## A BRIEF HISTORY OF THE LAB



In 1947 the Medical Research Council set up a Unit for "Research on the Molecular Structure of Biological Systems" to enable Max Perutz and John Kendrew to develop their work using X-ray diffraction to study proteins. At that time, the application of X-ray diffraction to determine the architecture of proteins and nucleic acids was a formidable undertaking, because these molecules were larger by orders of magnitude than the sugars and vitamins whose atomic structures were then known.

The first success came in 1953 when Jim Watson and Francis Crick, using data from Rosalind Franklin's X-ray diffraction work in London, proposed the double helical structure of DNA. 1953 was also the year when Perutz found a way of deciphering the X-ray diffraction patterns from crystalline proteins using heavy atom derivatives. This method later allowed Kendrew, Perutz and their collaborators to solve the structures of myoglobin and haemoglobin to atomic resolution, and pioneered the way for the elucidation of many thousands of different protein structures to date. At about

# Nascita del Laboratorio Europeo di Biologia Molecolare di Heidelberg (Germania, 1965 circa)

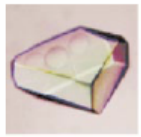




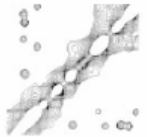
The European Molecular Biology Laboratory was the idea of prominent scientists such as the American physicist and molecular biologist Leo Szilárd and Nobel Prize winners James D. Watson and John C. Kendrew.

Their goal was to create a CERN-like supranational research centre to redress the balance in the strongly US-dominated field of molecular biology.

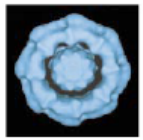

**David Phillips “The structure of lysozyme” The Royal Institution, London, 1965**



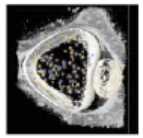
| X-ray crystallography |
|---|
| Subunit structure |
| Subunit shape |
| Subunit–subunit contact |
| Subunit proximity |
| Subunit stoichiometry |
| Assembly symmetry |
| Assembly shape |
| Assembly structure |



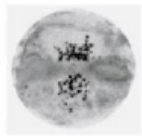
| NMR spectroscopy |
|---|
| Subunit structure |
| Subunit shape |
| Subunit–subunit contact |
| Subunit proximity |
| Subunit stoichiometry |
| Assembly symmetry |
| Assembly shape |
| Assembly structure |



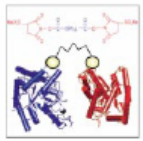
| 2D and single-particle electron microscopy |
|---|
| Subunit shape |
| Subunit–subunit contact |
| Subunit proximity |
| Assembly symmetry |
| Assembly shape |



| Electron tomography |
|---|
| Subunit shape |
| Subunit–subunit contact |
| Subunit proximity |
| Assembly symmetry |
| Assembly shape |

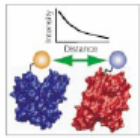


| Immuno-electron microscopy |
|---|
| Subunit proximity |
| Assembly symmetry |

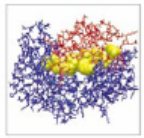


| Chemical cross-linking |
|---|
| Subunit structure |
| Subunit–subunit contact |
| Subunit proximity |

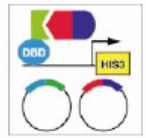
| Affinity purification mass spectroscopy |
|---|
| Subunit–subunit contact |
| Subunit proximity |



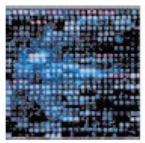

| FRET |
|---|
| Subunit–subunit contact |
| Subunit proximity |



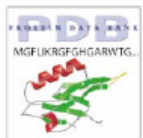
| Site-directed mutagenesis |
|---|
| Subunit–subunit contact |



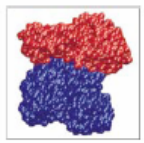

| Yeast two-hybrid system |
|---|
| Subunit–subunit contact |
| Subunit proximity |



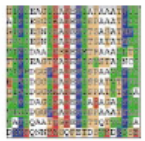
| Gene/protein arrays |
|---|
| Subunit–subunit contact |
| Subunit proximity |




| Protein structure prediction |
|---|
| Subunit structure |
| Subunit shape |



| Computational docking |
|---|
| Subunit–subunit contact |

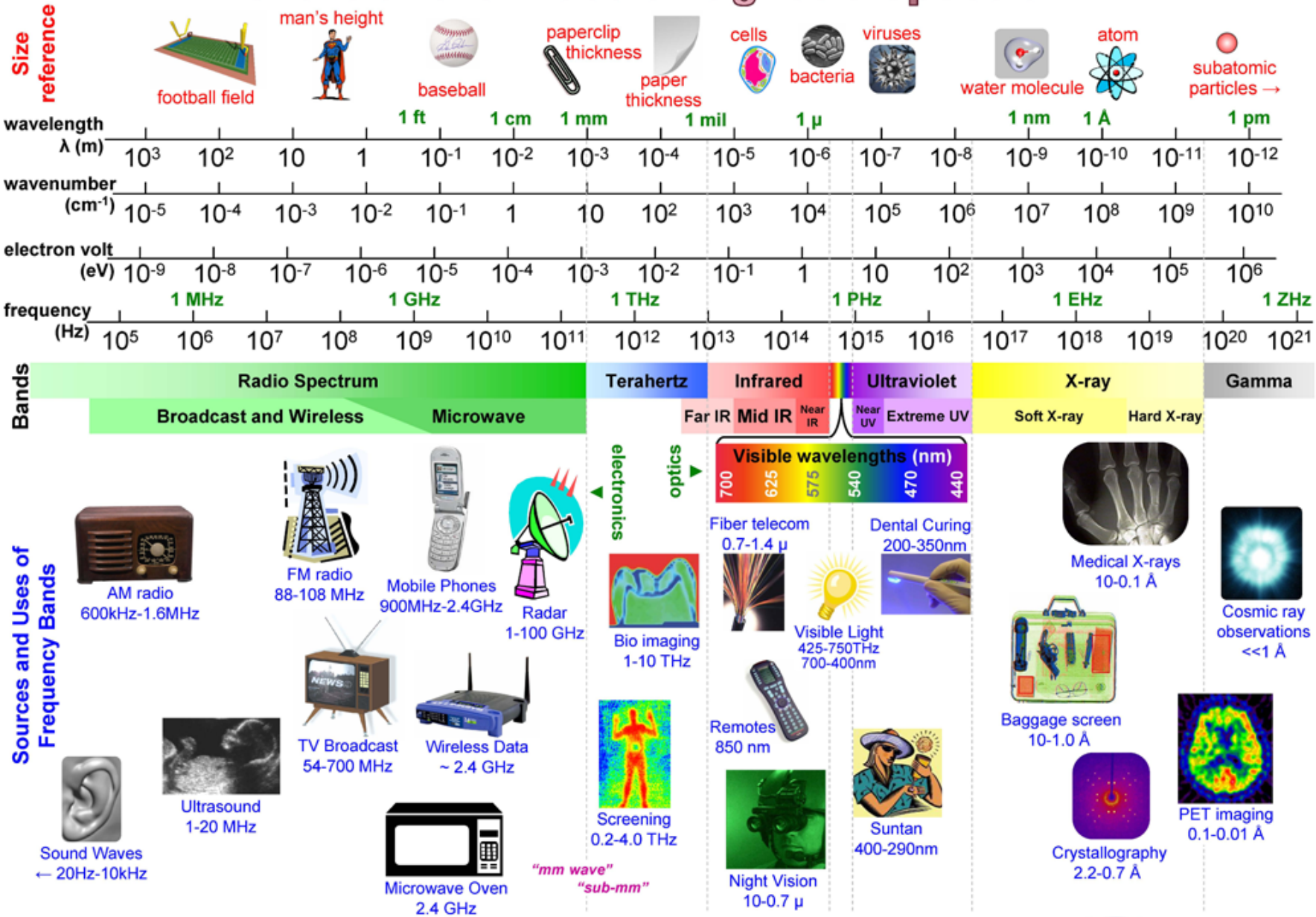


| Bioinformatics |
|---|
| Subunit–subunit contact |

**Figure 4** Experimental and theoretical methods that can provide information about a macromolecular assembly structure. The annotations below each of the panels list the aspects of an assembly that might be obtained by the corresponding method. Subunit and assembly structure indicate an atomic or near-atomic resolution at 3 Å or better. Subunit and assembly shape indicate the density or surface envelope at a low resolution of worse than 3 Å. Subunit–subunit contact indicates knowledge about protein pairs that are in contact with each other, and in some cases about the face that is involved in the contact. Subunit proximity indicates whether two proteins are close to each other relative to the size of the assembly, but not necessarily in direct contact. Subunit stoichiometry indicates the number of subunits of a given type that occur in the assembly. Assembly symmetry indicates the symmetry of the arrangement of the subunits in the assembly. Grey boxes indicate extreme difficulty in obtaining the corresponding information by a given method.

W. Baumeister and collegues

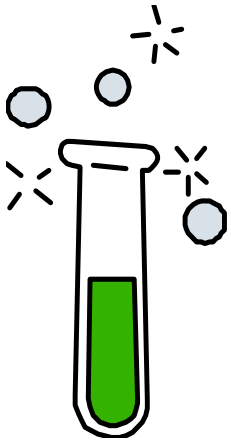
# Chart of the Electromagnetic Spectrum





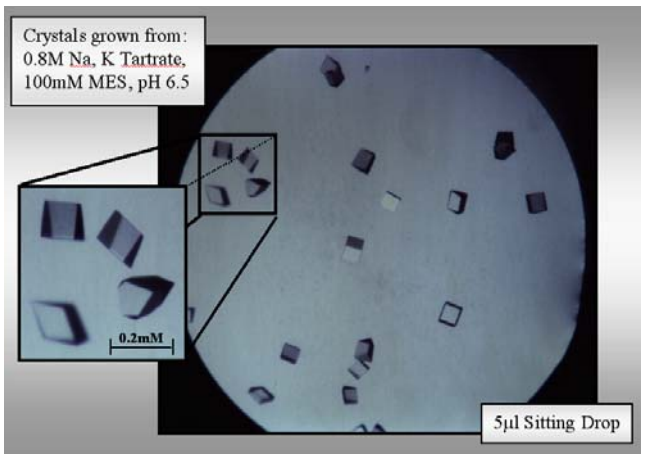

$\lambda = 3\times10^{8}/\text{freq} = 1/(\text{wn}*100) = 1.24\times10^{-6}/\text{eV}$

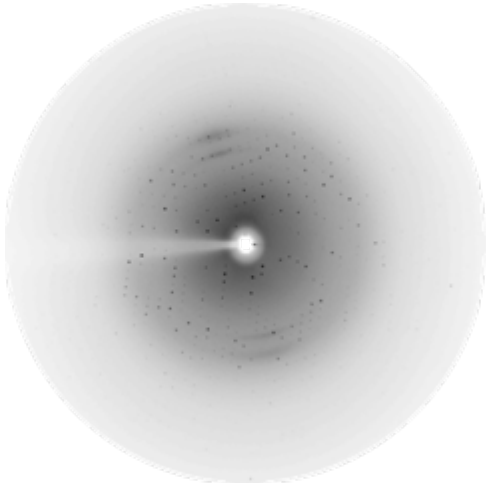
# Processi nella cristallografia macromolecolare



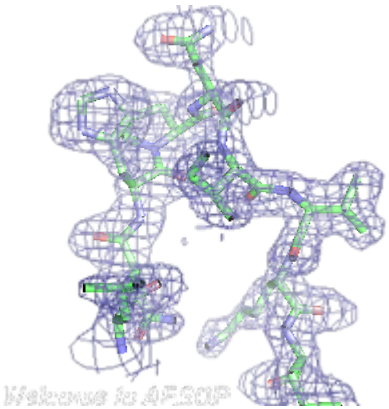
Purificazione

Cristallizzazione

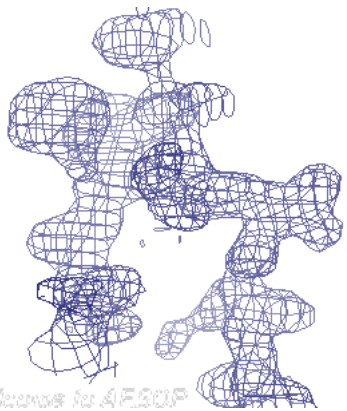


Collezione dei dati



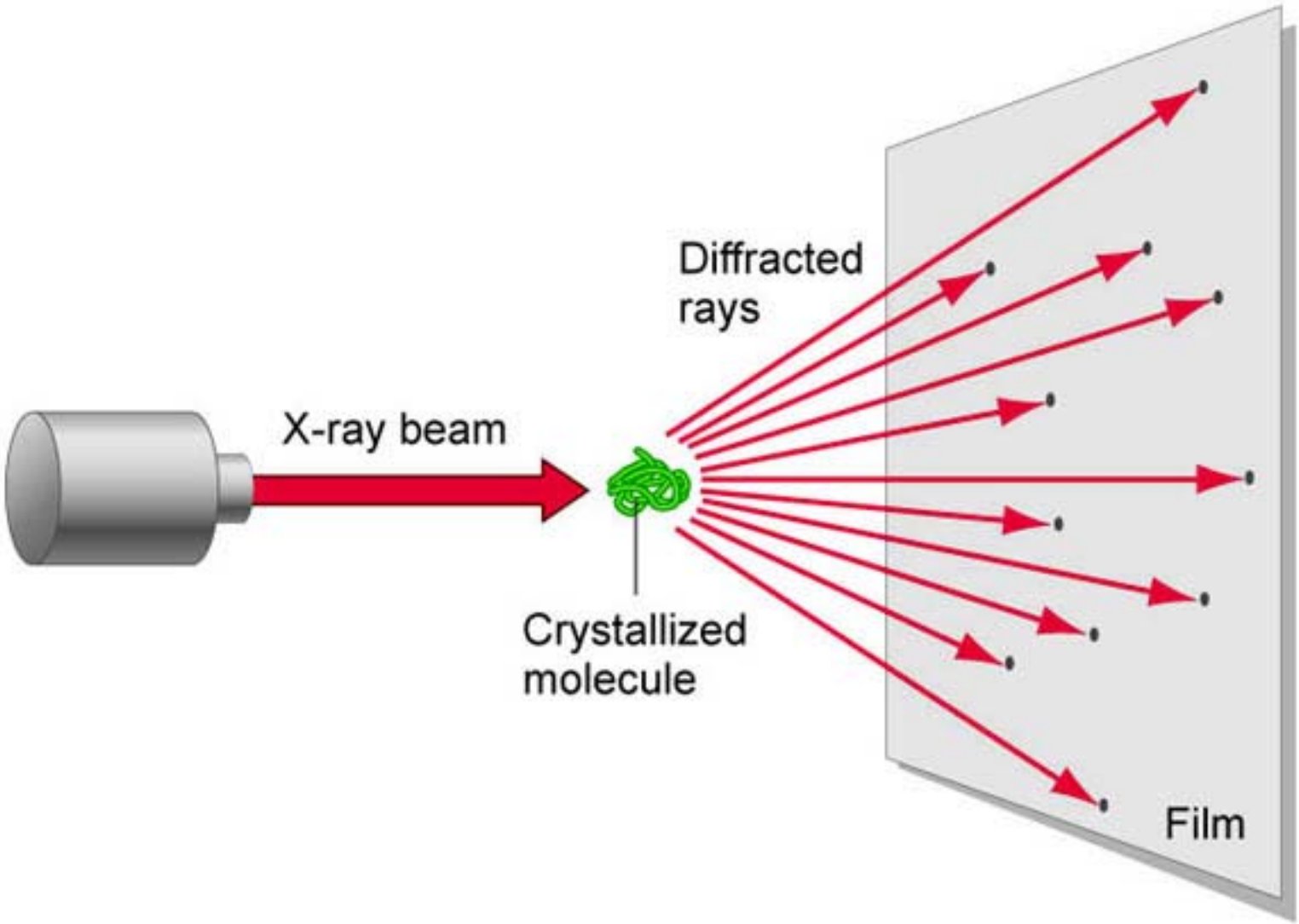
Interpretazione della mappa



Calcolo della densità elettronica

Da 10 giorni a 10 anni

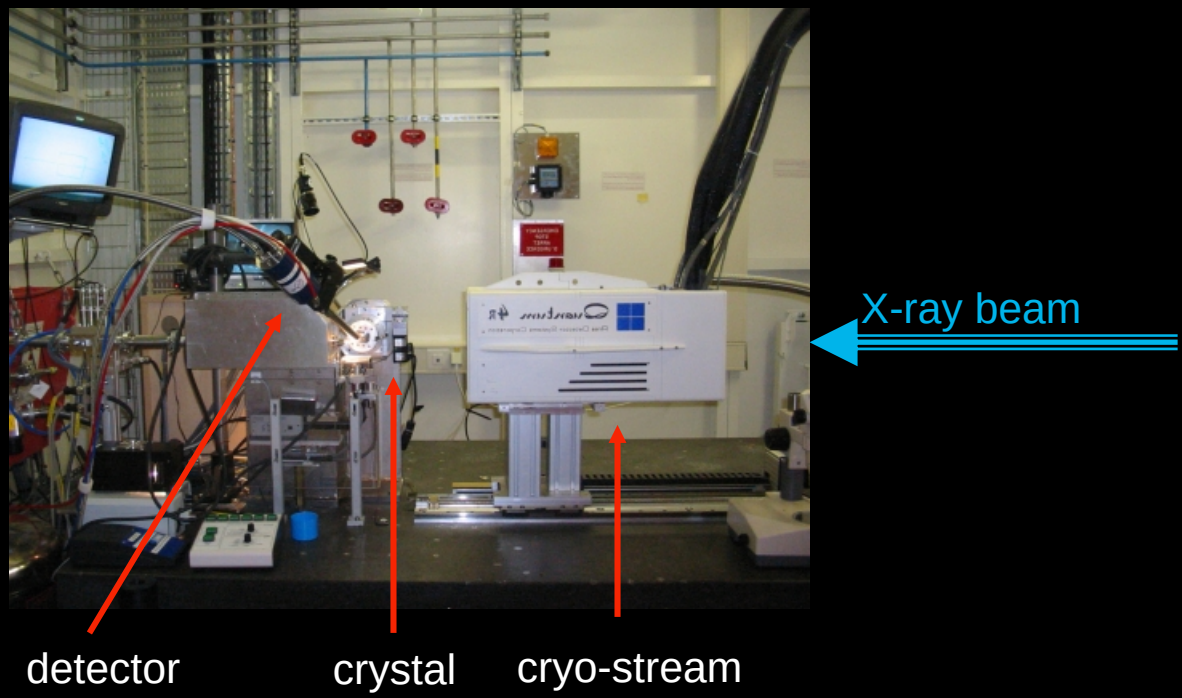
test



# X-ray diffraction experiment

Why X-ray: radiation with wavelength approximately the size of detail to be observed (atomic bonds are ~ 1.5 Å in length ) must be used.

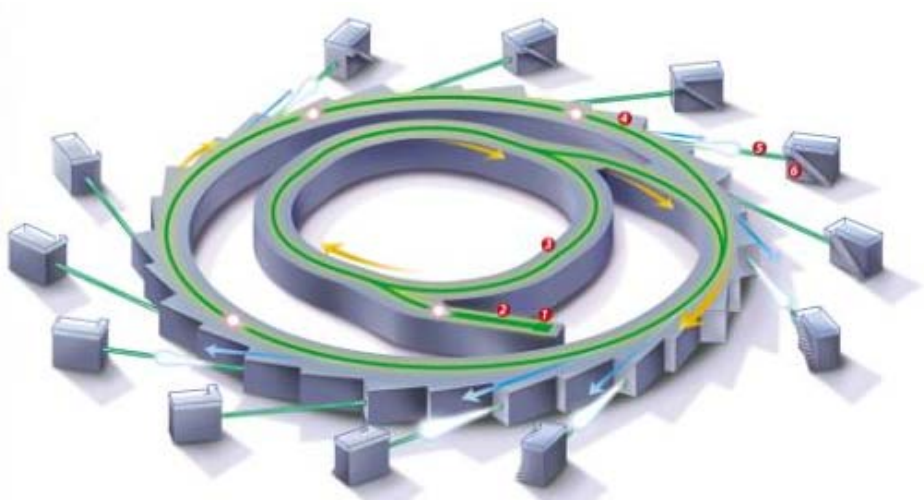
How: monochromatic X-ray beams are generated either by rotating metal anodes, or by charged particles (electrons or positrons) rotating at high speed with circular trajectories (synchrotrons).

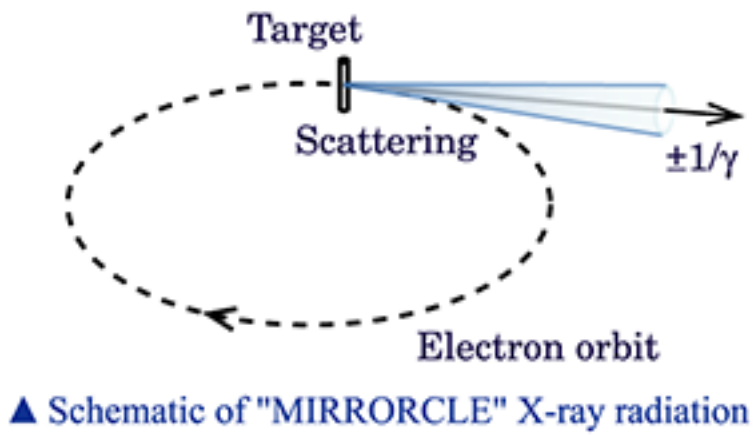


# Sincrotoni per la collezione di dati cristallografici


ESRF Grenoble

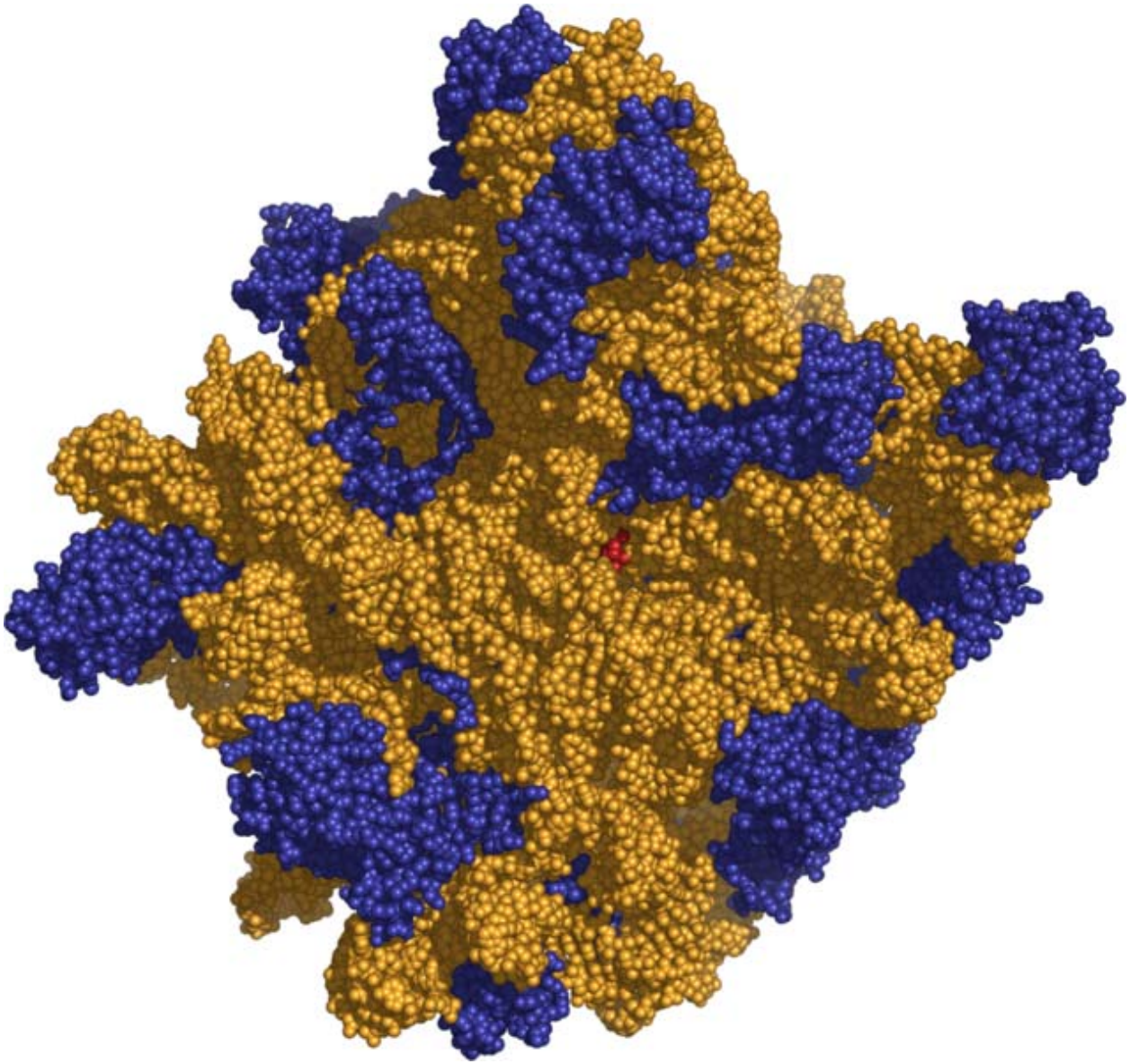

Diamond Oxford

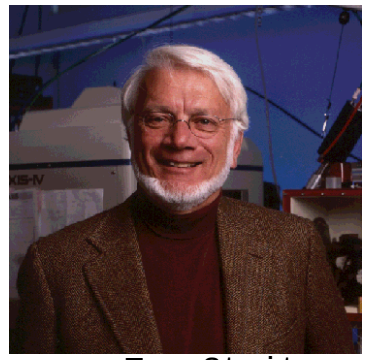





▲ Schematic of "MIRRORCLE" X-ray radiation

# *Modello atomico del ribosoma: La sconfitta della blobbologia*





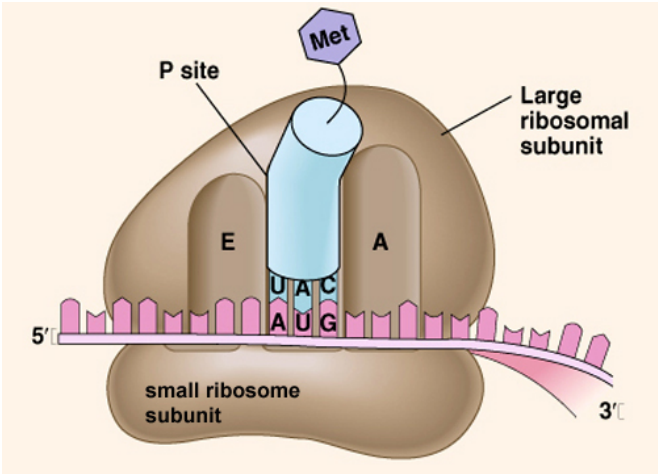
Tom Steitz

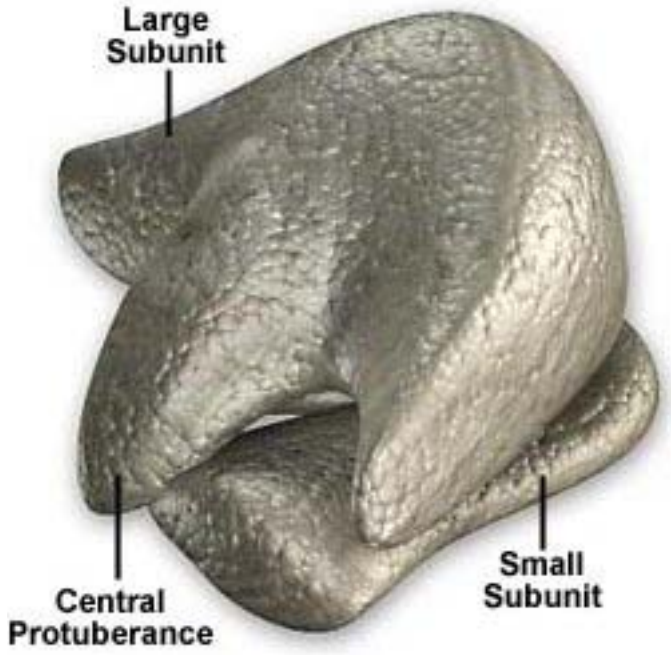
Venki Ramakrishnan

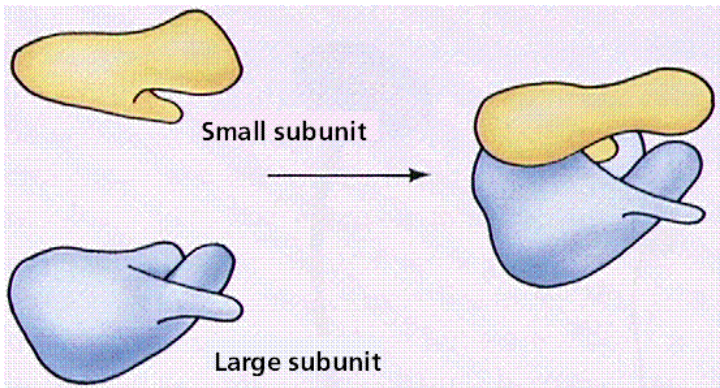
Ada Yonath

# Una macchina fatta di proteine e RNA

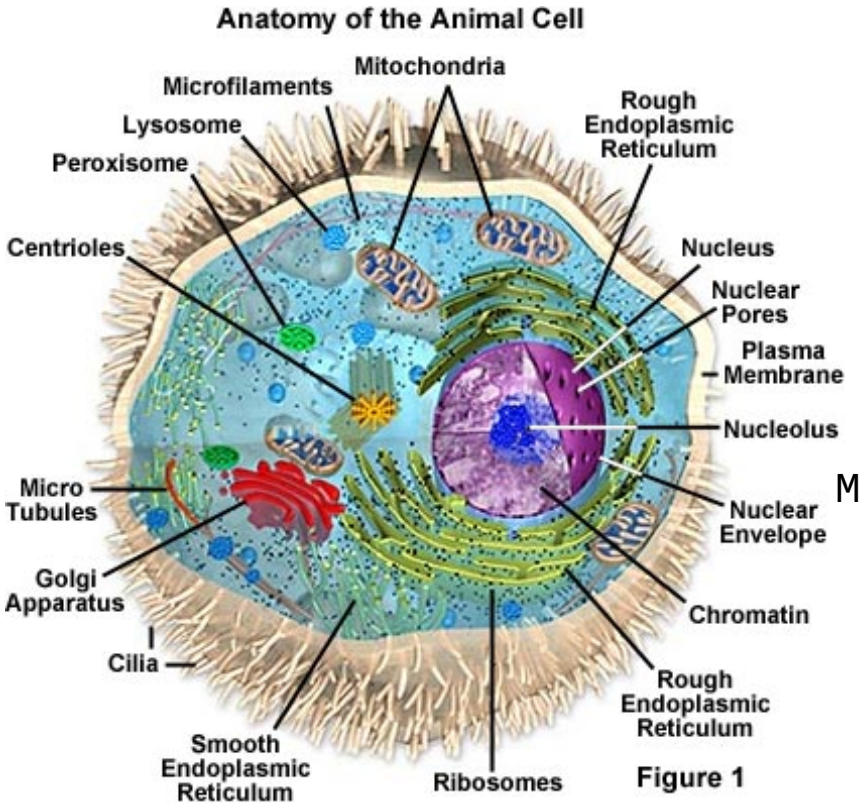
# Varie rappresentazioni blobbologiche

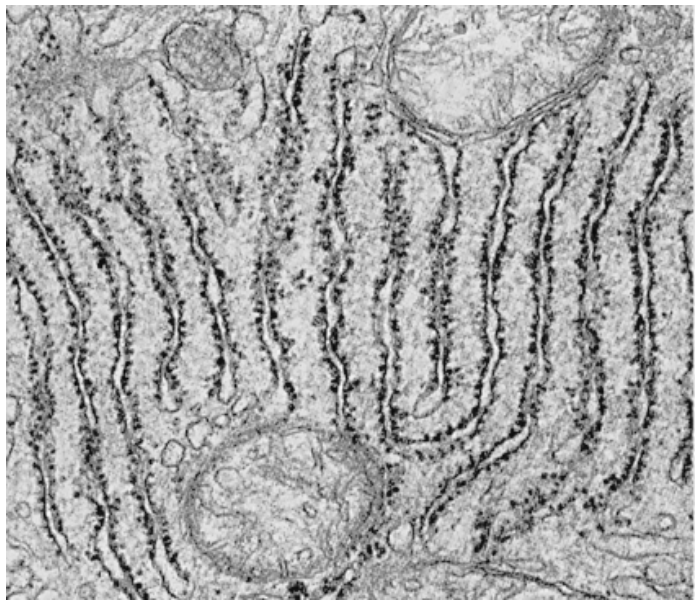


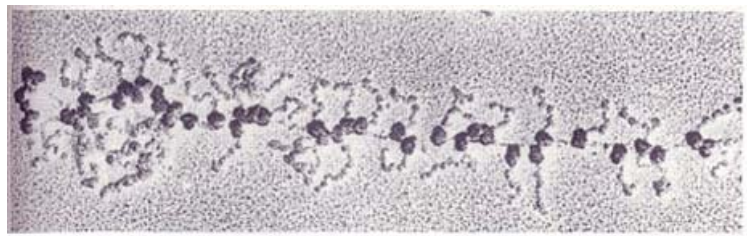



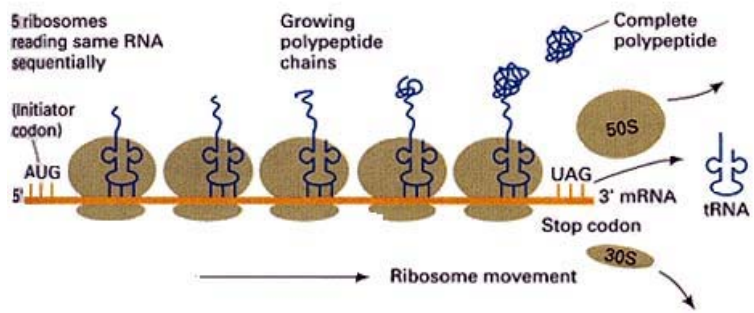

# *I ribosomi in una cellula*



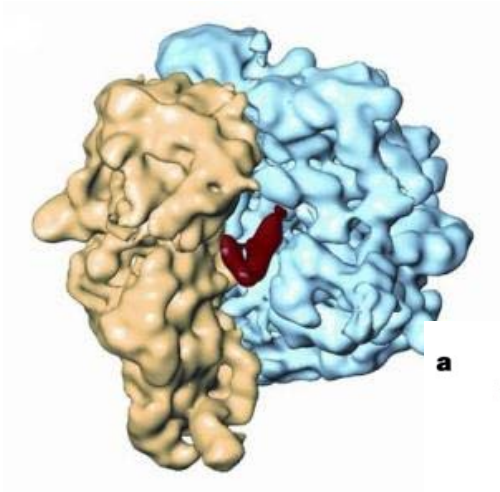



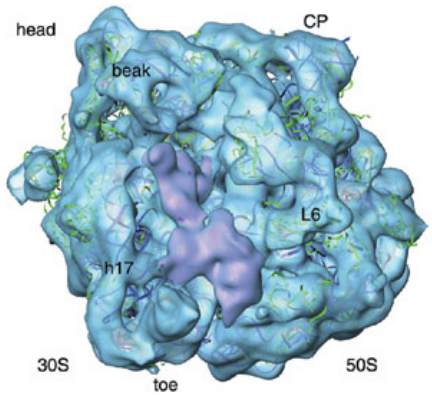
Microscopio elettronico a trasmissione

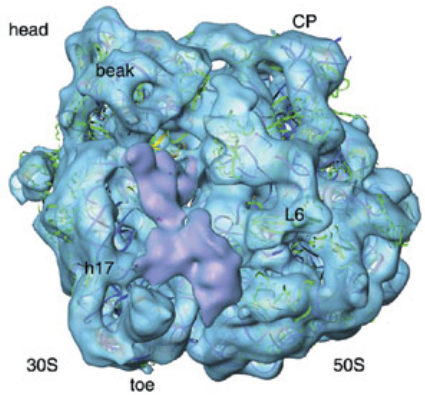


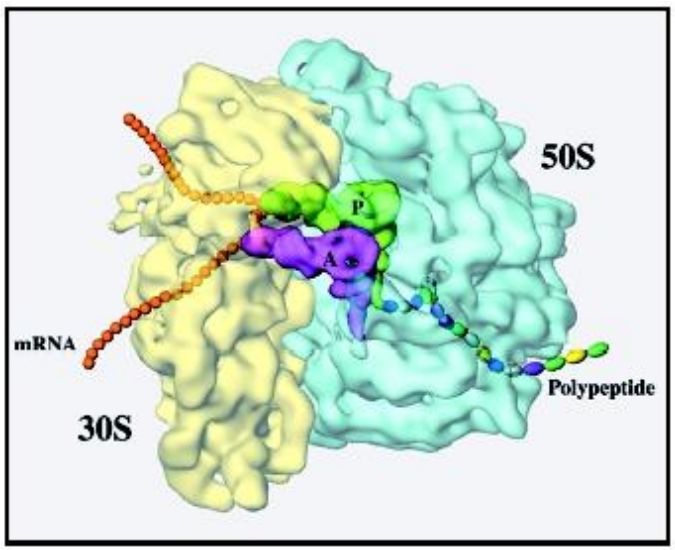


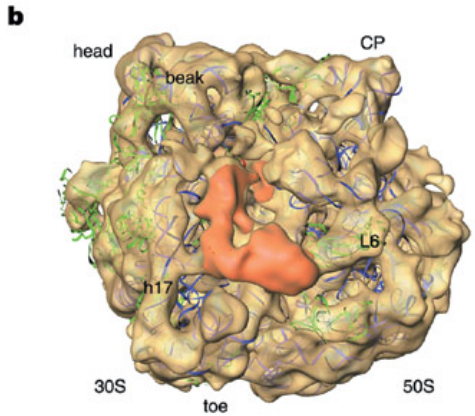
Dal lavoro dei microscopisti elettronici J. Frank e M. van Heel

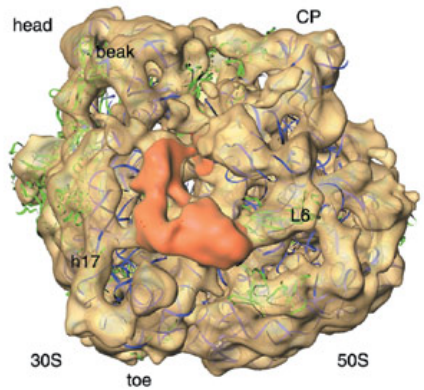


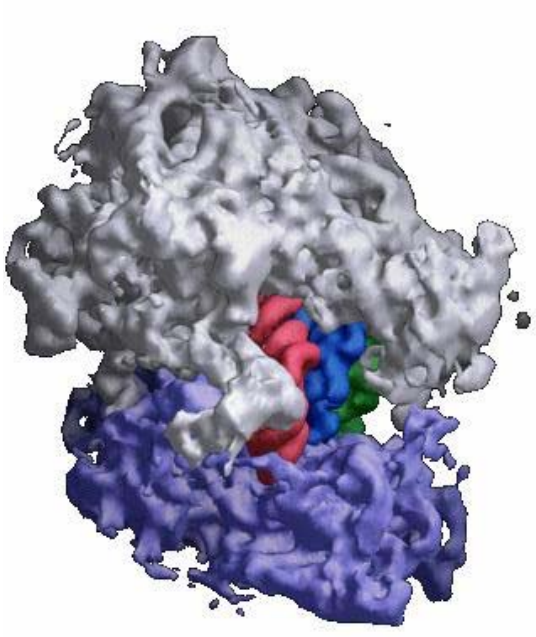



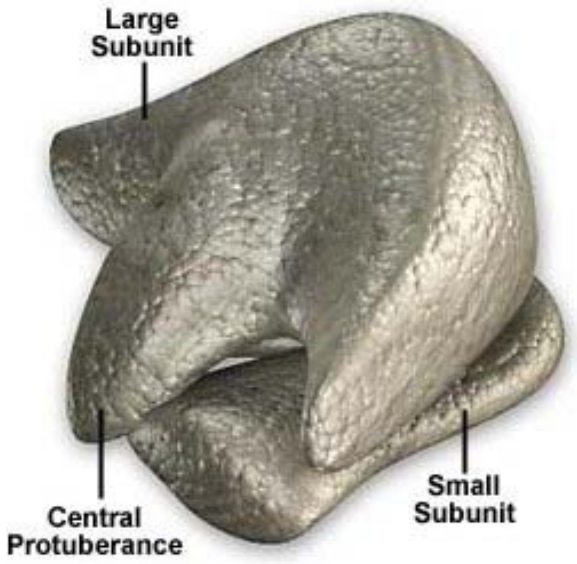



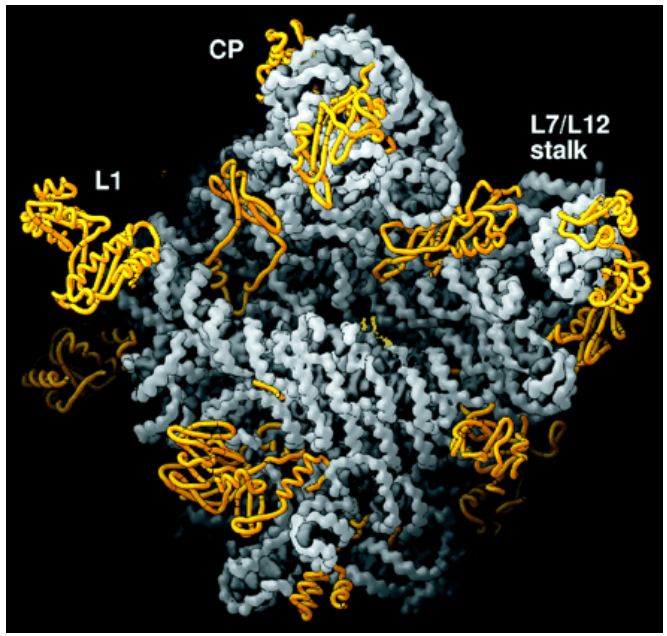



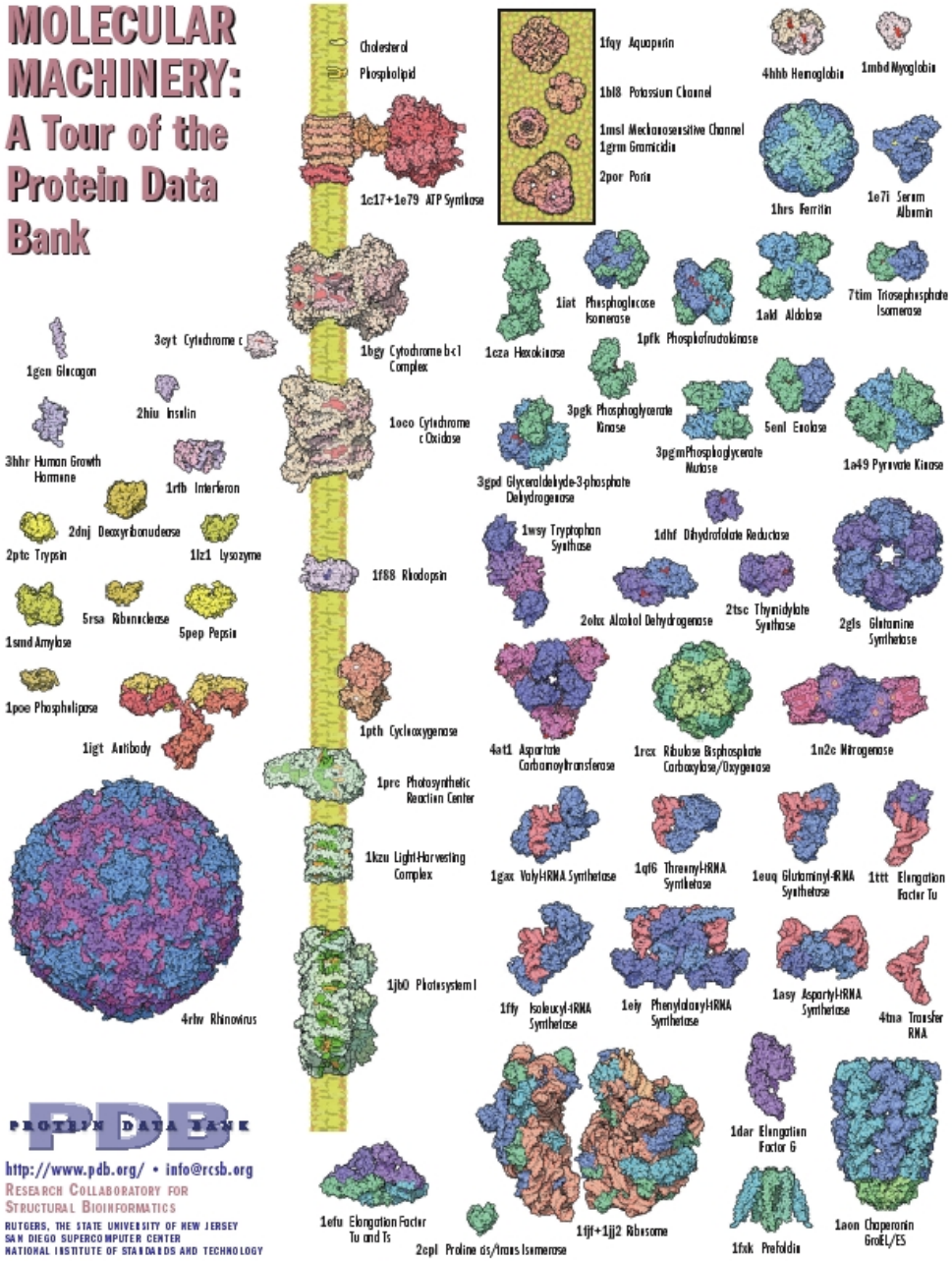
# La differenza si vede!





# MOLECULAR MACHINERY: A Tour of the Protein Data Bank




PDB
PROTEIN DATA BANK

http://www.pdb.org/ • info@rcsb.org

RESEARCH COLLABORATORY FOR STRUCTURAL BIOINFORMATICS

RUTGERS, THE STATE UNIVERSITY OF NEW JERSEY
SAN DIEGO SUPERCOMPUTER CENTER
NATIONAL INSTITUTE OF STANDARDS AND TECHNOLOGY

# i file .pdb contengono coordinate atomiche

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CRYST1   73.980   40.427   39.663  90.00  92.85  90.00
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ORIGX2      0.000000  1.000000  0.000000        0.00000
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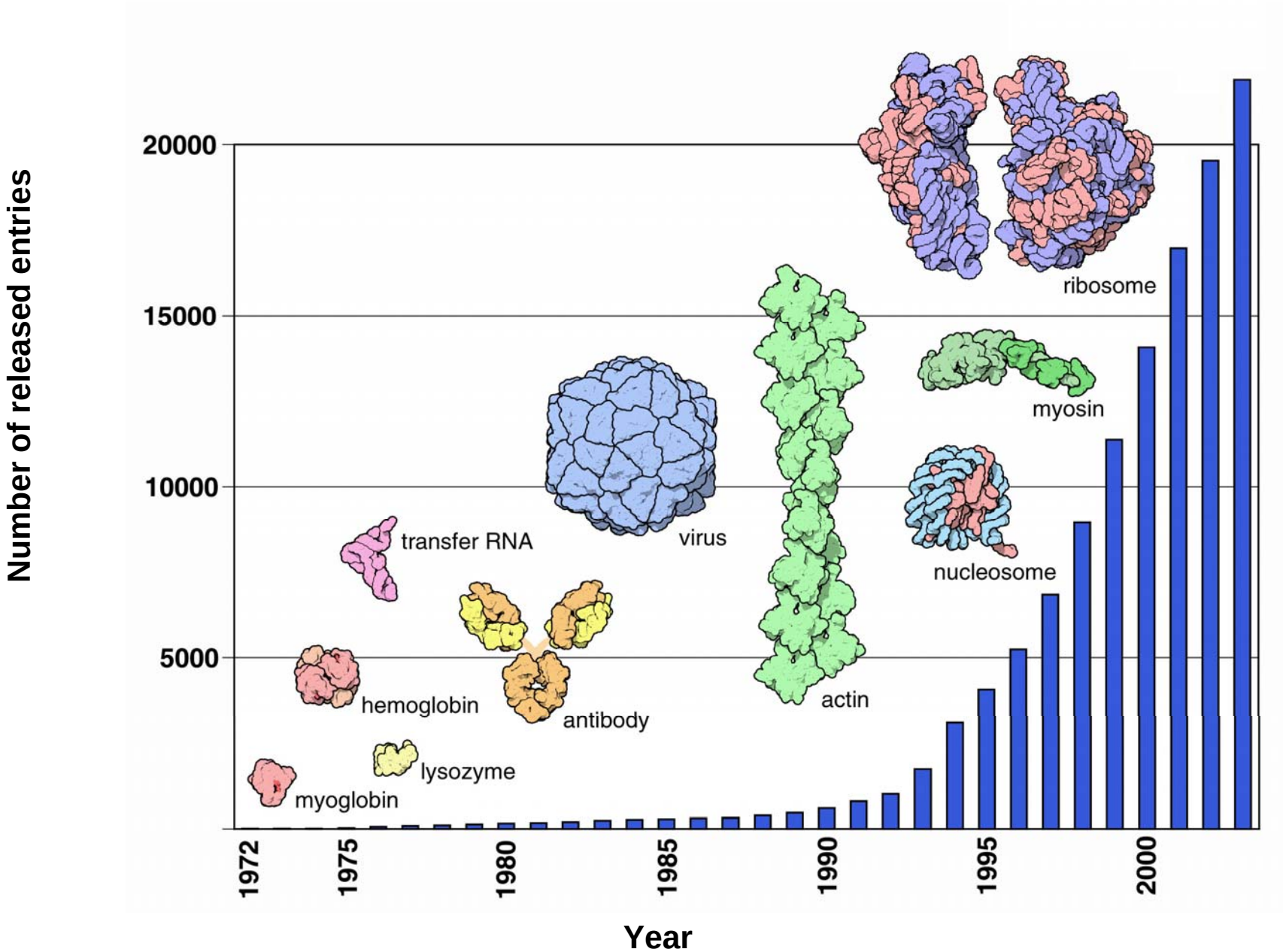
Number of released entries
20000
15000
10000
5000
1972
1975
1980
1985
1990
1995
2000
Year
ribosome
myosin
nucleosome
virus
transfer RNA
actin
hemoglobin
antibody
lysozyme
myoglobin

Struttura cristallina di Aurora B:INCENP:ZM447439

1.9 Å, R = 0.19, Rfree = 0.24, mean B = 26 Å$^2$

(Fabio Sessa, Fabrizio Villa)