# CH 312 - Dynamics of biomolecular processes

Spring Semester 2025

Beat Fierz

- **Course material**:
  - Lecture notes
  - Articles

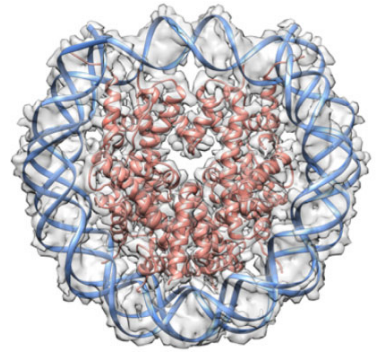
  } Moodle page

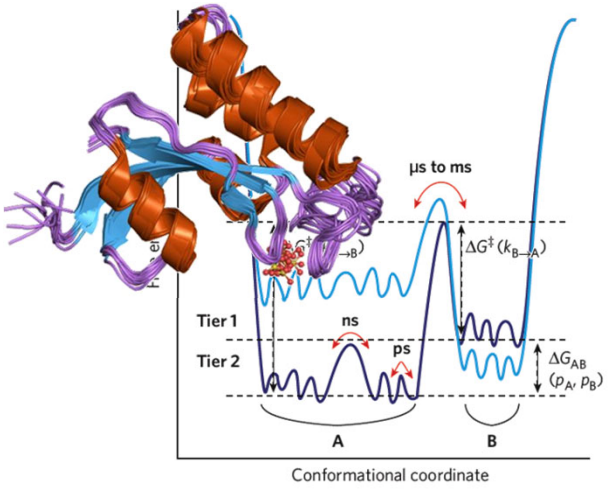
  - Text books: Physical Biology of the Cell (Phillips et al.)

- **Contact**:
  - Office: CH B3 485
  - Email: beat.fierz@epfl.ch

# Lecture plan

- **Part I:** Structural biology
  - Protein structure: Models and visualization
  - X-ray crystallography
  - Electron microscopy
- **Part II:** Dynamics of biomolecular processes
  - Protein dynamics & folding kinetics
  - Structural dynamics
  - Diffusion in 1, 2 and 3D
  - Interaction dynamics
  - Directed motion and molecular motors
- **Part III:** Discussion of selected papers





# Practical information

**Work load:**

2 credits: 2 hours lectures + 2 hours study/exercises per weeks

**Exercises**

For each lecture, there will be several questions to solve, 2 exercise sheets in total.

A completed exercise sheet has to be submitted to a deadline that will be announced.

**Paper discussions**

In the second half of the semester (starting on the 26$^{th}$ of April), we will spend 6 lectures for paper presentations (6 papers) by groups of students.

**Exam**

Written

# Papers for the discussion

1

nature structural & molecular biology

## Linkage between dynamics and catalysis in a thermophilic-mesophilic enzyme pair

Magnus Wolf-Watz, Vu Thai, Katherine Henzler-Wildman, Georgia Hadjipavlou, Elan Z Eisenmesser & Dorothee Kern

2

## Microtubule Assembly Dynamics at the Nanoscale

Henry T. Schek III,[1,4] Melissa K. Gardner,[2,4] Jun Cheng,[3] David J. Odde,[2,5,*] and Alan J. Hunt[3,5,*]
[1]European Molecular Biology Laboratory
Meyerhofstrasse 1
69117 Heidelberg
Germany
[2]Department of Biomedical Engineering
University of Minnesota
Minneapolis, Minnesota 55455
[3]Department of Biomedical Engineering
University of Michigan
Ann Arbor, Michigan 48109

stochastically between alternate periods of slow growth and rapid shortening such that individual αβ-tubulin heterodimeric subunits undergo net addition and loss, respectively [1]. This allows the microtubule cytoskeleton to rapidly reconfigure to support morphologic changes, and microtubules can probe the intracellular environment to explore possible arrangements [2]. Microtubules presumably sense the local environment by interaction with microtubule-associated proteins, and this interaction can vary spatially through, for example, kinase-phosphatase and GEF-GAP (guanine nucleotide exchange factor GTPase-activating protein)-mediated

3

## Single-molecule imaging of transcription factor binding to DNA in live mammalian cells

J Christof M Gebhardt[1,5], David M Suter[1,5], Rahul Roy[1,4], Ziqing W Zhao[1,2], Alec R Chapman[1,2], Srinjan Basu[1,3,4], Tom Maniatis[3] & X Sunney Xie[1]

4

articles

## Resolution of distinct rotational substeps by submillisecond kinetic analysis of $F_1$-ATPase

Ryohei Yasuda*†‖, Hiroyuki Noji*, Masasuke Yoshida‡*, Kazuhiko Kinosita Jr†* & Hiroyasu Itoh§*
* CREST 'Genetic Programming' Team 13, Teikyo University Biotechnology Center 3F, Nogawa 907, Miyamae-Ku, Kawasaki 216-0001, Japan
† Department of Physics, Faculty of Science and Technology, Keio University, Yokohama 223-8522, Japan
‡ Chemical Resources Laboratory, Tokyo Institute of Technology, Yokohama 226-8503, Japan
§ Tsukuba Research Laboratory, Hamamatsu Photonics KK, Tokodai, Tsukuba 300-2635, Japan

5

## Myosin V Walks Hand-Over-Hand: Single Fluorophore Imaging with 1.5-nm Localization

Ahmet Yildiz,[1] Joseph N. Forkey,[3] Sean A. McKinney,[1,2] Taekjip Ha,[1,2] Yale E. Goldman,[3] Paul R. Selvin[1,2]*

6

ARTICLES

## Dynamics of nucleosome remodelling by individual ACF complexes

Timothy R. Blosser[1,2], Janet G. Yang[3], Michael D. Stone[1,4], Geeta J. Narlikar[3] & Xiaowei Zhuang[1,4,5]

# Part I: Structural biology

- What is structural biology?

**Elucidation of the three dimensional atomic structure of biomacromolecules**

- proteins, polypeptides
- DNA
- complexes

- Methods of structural biology

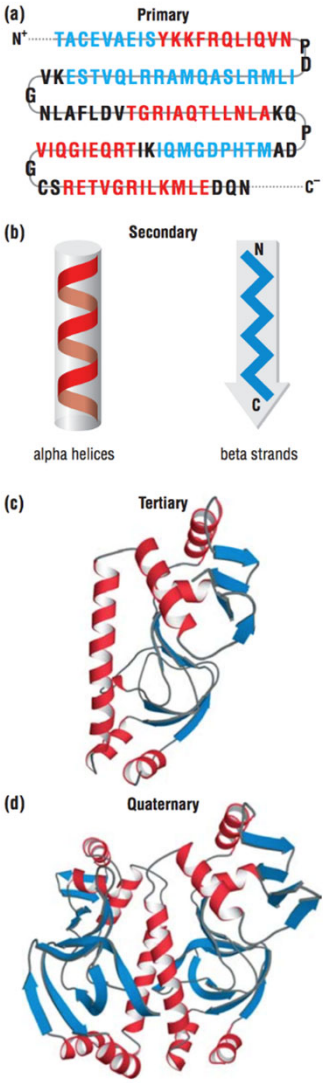
**Biochemical and biophysical methods**

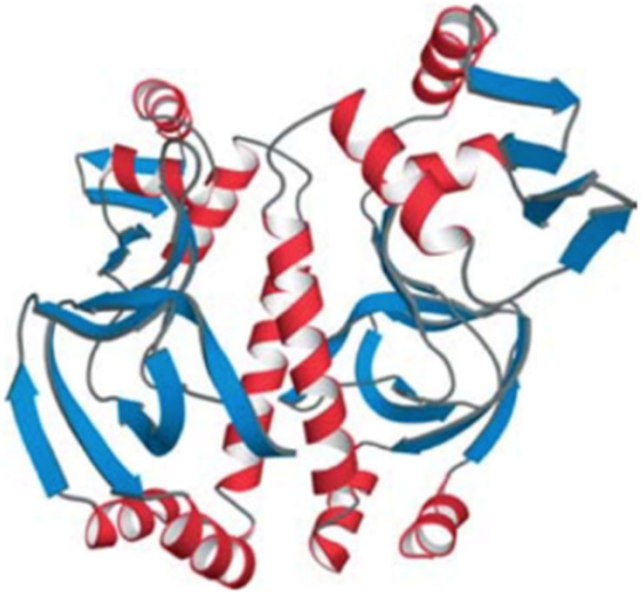
including:

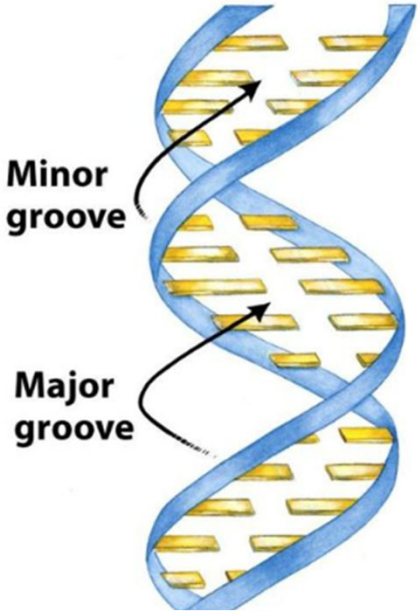
- X-ray crystallography (and other diffraction methods)
- NMR spectroscopy
- electron microscopy

- Why structural biology?

# Part I: Structural biology




*Images are from:*
*Biochemistry, "Stryer"*
*Principles of Biochemistry, "Lehninger"*
*Protein Structure and Function, Petsko & Ringe, 2004*

# Structural biology of proteins – Why ?

## Understand protein function

BET bromodomain proteins

Key transcriptional regulators
- cancer
- inflammation

BET bromodomain

*PDB 2E3K*
Umehara et al., JBC 2010

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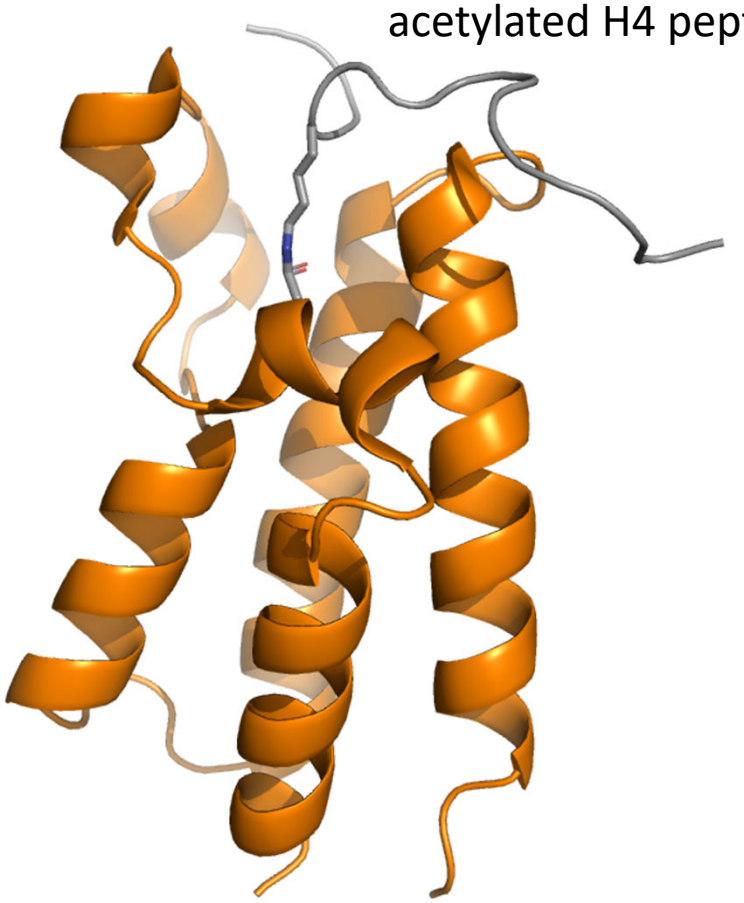
Bind to acetylated histones

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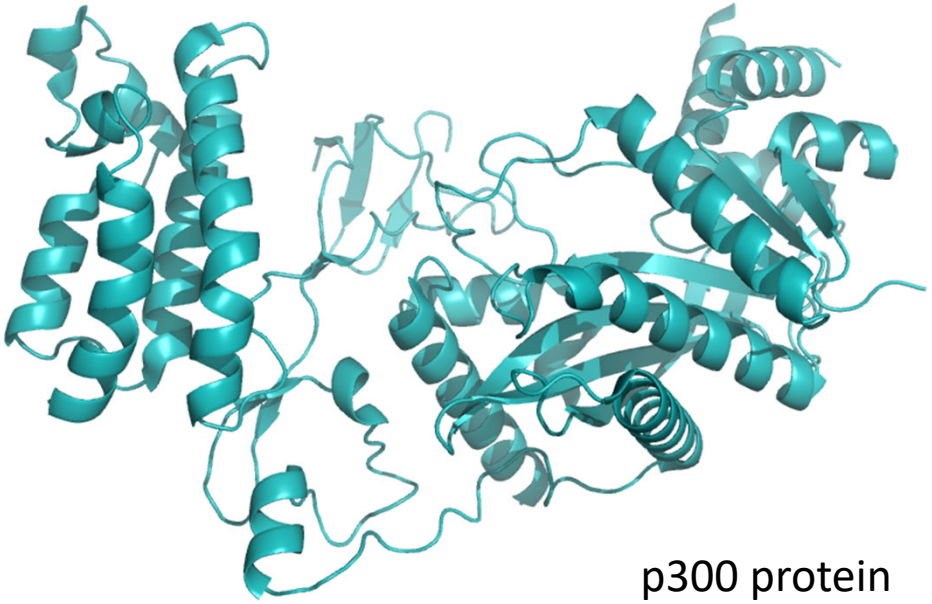

p300 protein

*PDB 8HAG*
Kikuchi et al., Nat Commun 2023

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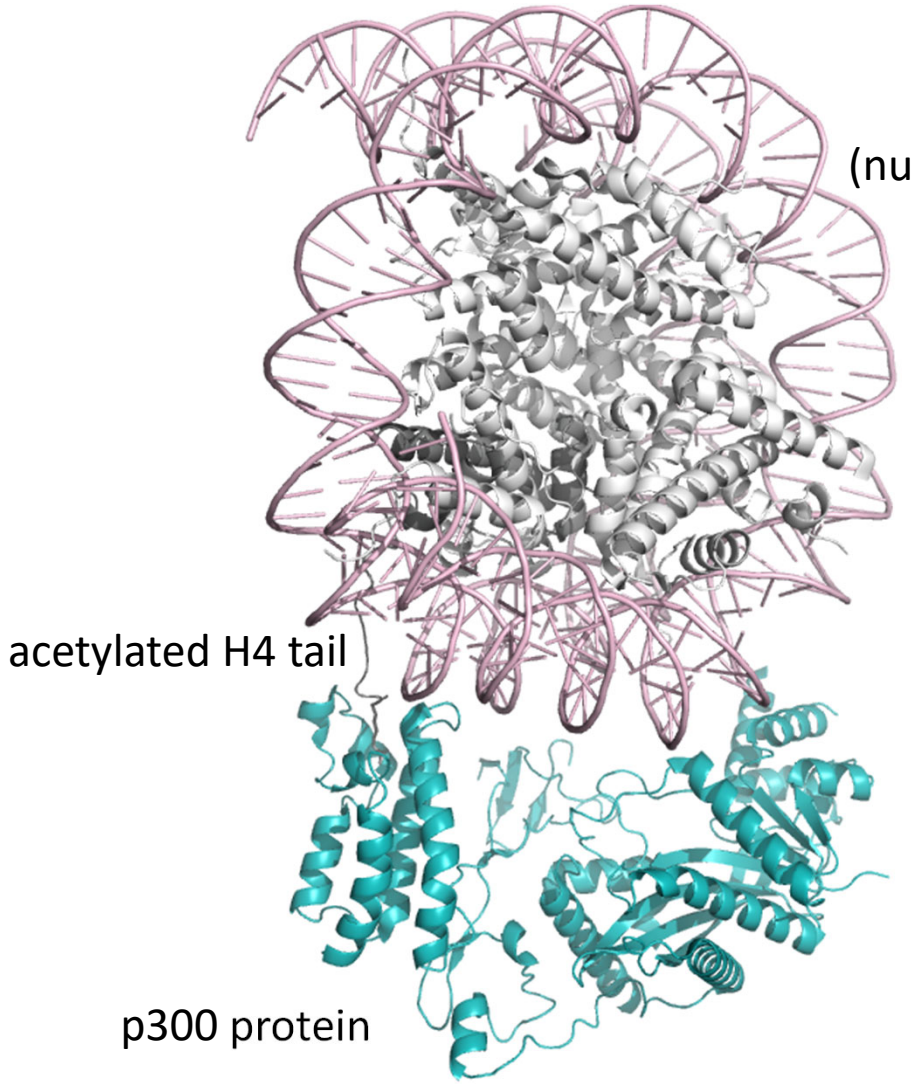



*PDB 8HAG*
Kikuchi et al., Nat Commun 2023

# Structural biology of proteins – Why ?

## Drug discovery

BET bromodomain proteins

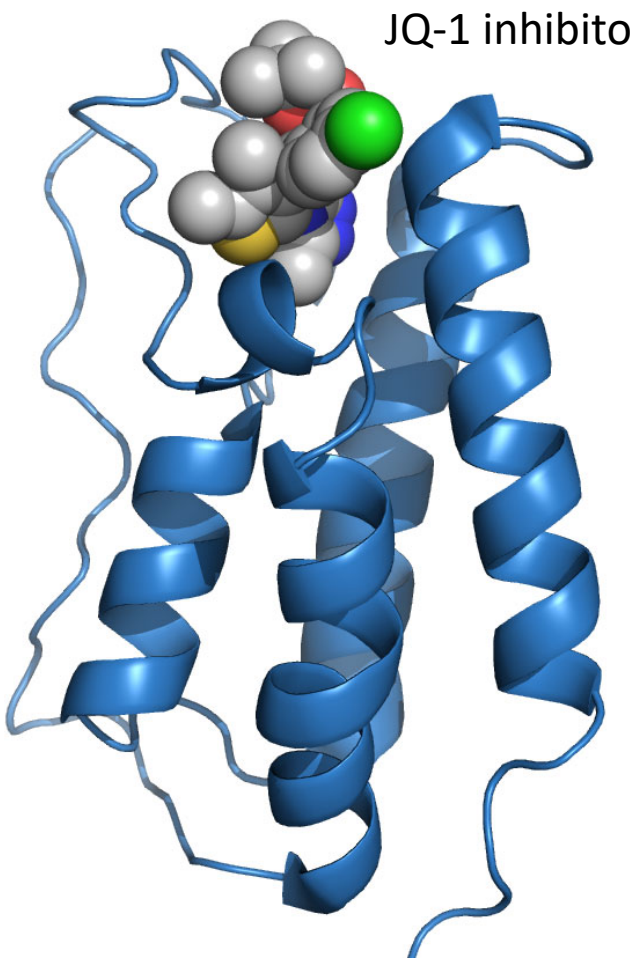
Key transcriptional regulators
- cancer
- inflammation

Bind to acetylated histones

Inhibition:
- therapy options for cancer
- inflammation

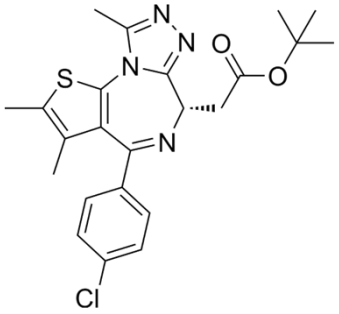
Structure-based drug design!

*PDB 3MXF*
Filipakopoulos et al., Nature 2010



BET bromodomain

JQ-1 inhibitor

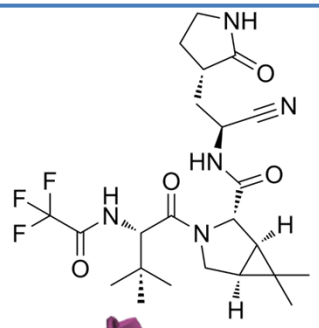


# Structural biology of proteins – Why ?

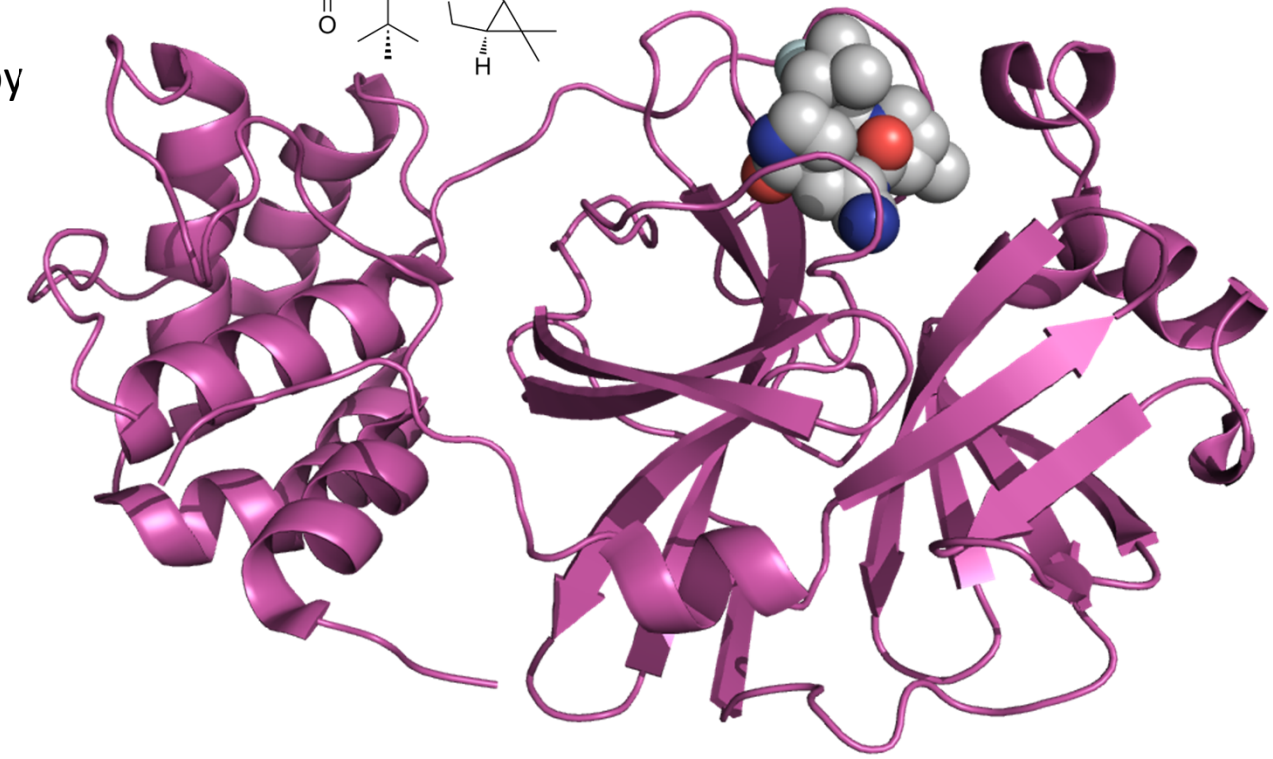
## Drug discovery

Viral proteins

Targets for antiviral therapy
- proteases
- RNA polymerases
- capsid proteins




nirmatrelvir
(Paxlovid, Pfizer)



SARS-CoV-2 main protease

*PDB 7U29*
Greasley et al., JBC 2022

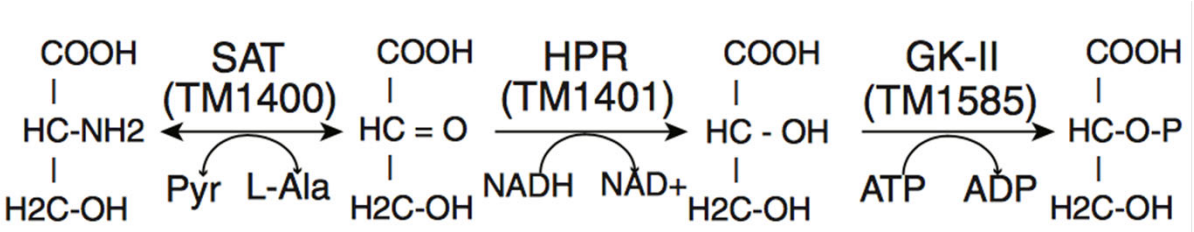
# Structural biology of proteins – Why ?

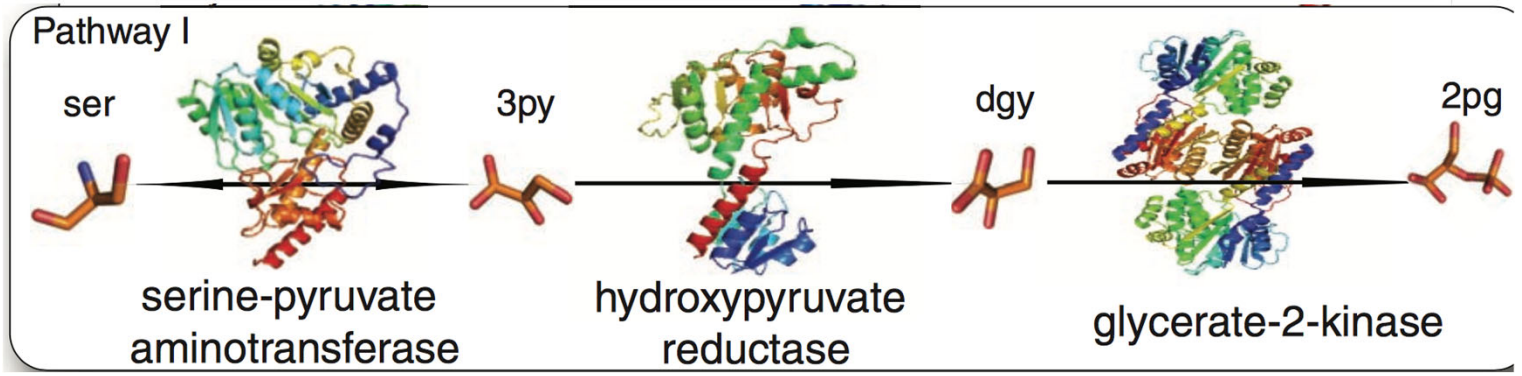
## Catalytic mechanisms

Enzymes facilitate specific chemical reactions

Metabolic pathways
- transferases
- kinases
- oxidoreductases
- dehydrogenases
- etc.....

e.g., conversion of Ser to 2PG

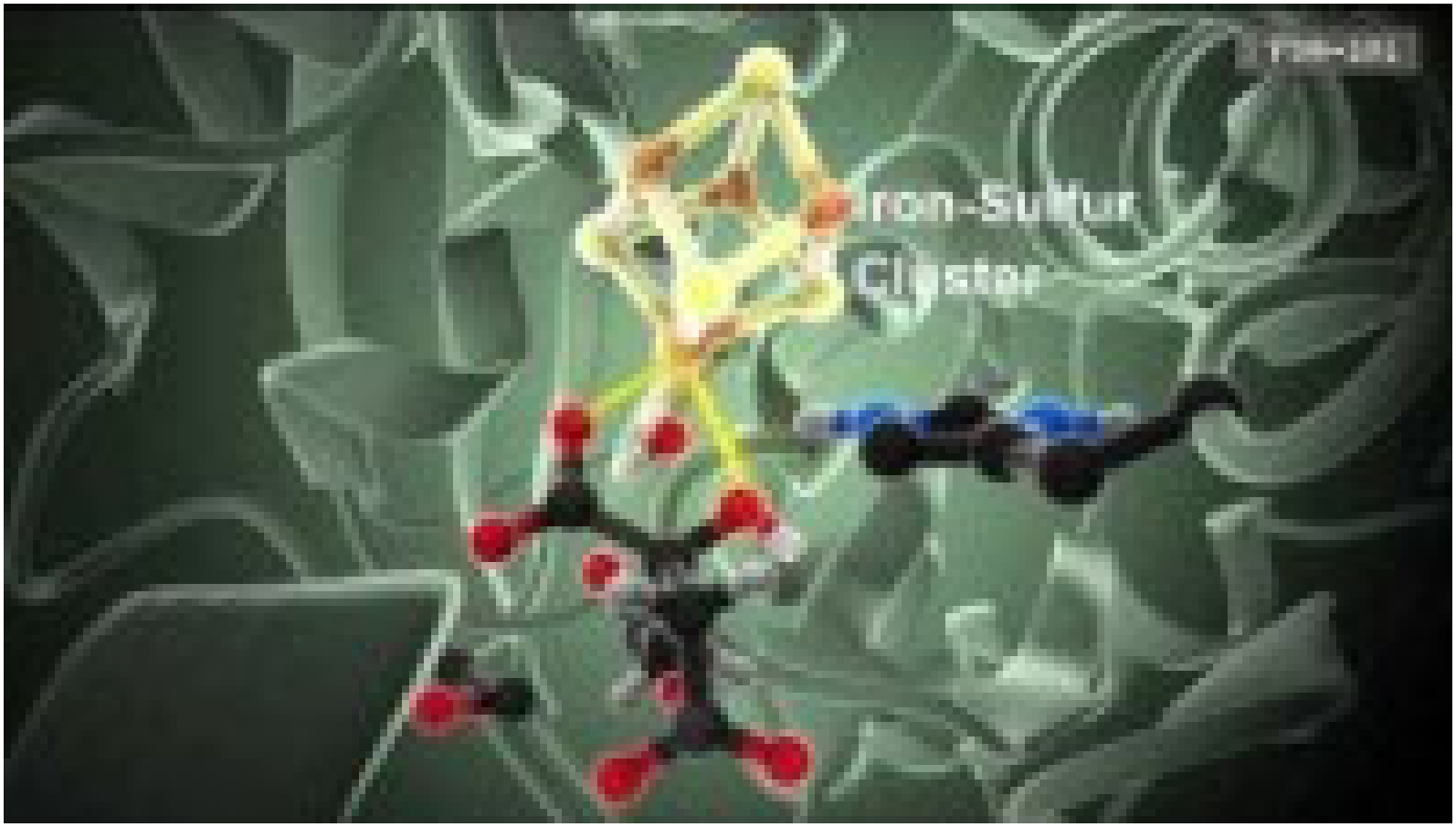



Zhang et al., Science 2009 – 3D reconstruction of the central metabolic network of *T. Maritima* – 120 str. + 358 models

# Structural biology of proteins – Why ?

## Catalytic mechanisms



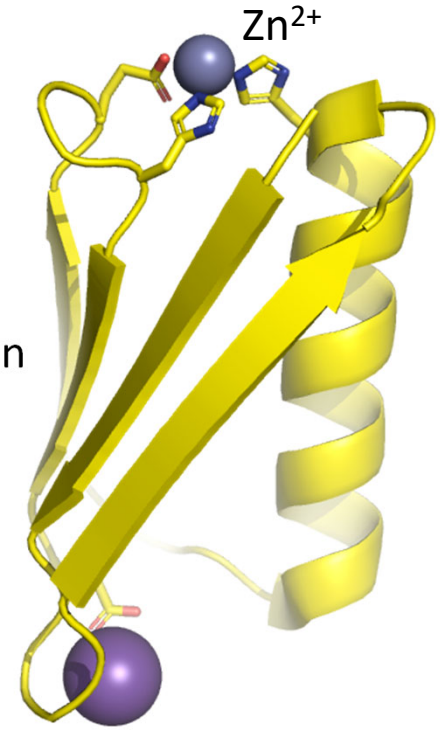

RCSBProteinDataBank - https://youtu.be/yk14dOOvwMk?t=125

# Structural biology of proteins – Why ?

## Protein engineering

Functions new to Nature

metalloprotein

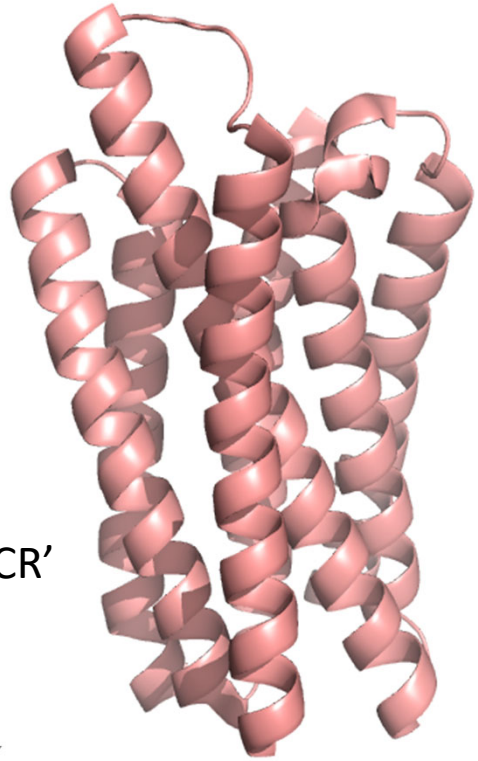



*PDB 5O94*

Bozkurt et al., JACS 2018 - Röthlisberger (ISIC_EPFL)

Improved properties (stability, solubility...)

soluble ‘GPCR’

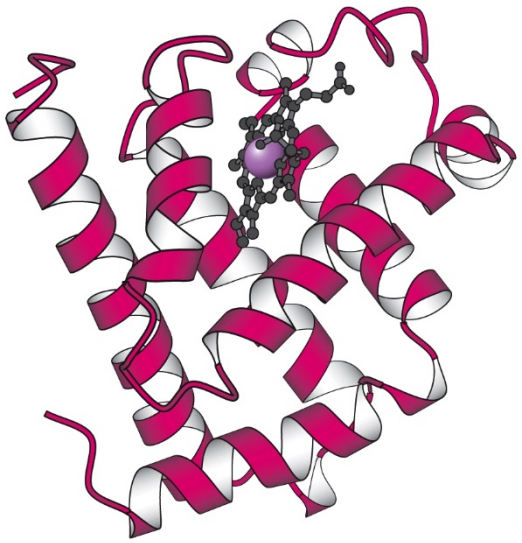


*PDB 8OYX*

Goverde et al., Nature 2024 - Correia (STI_EPFL)

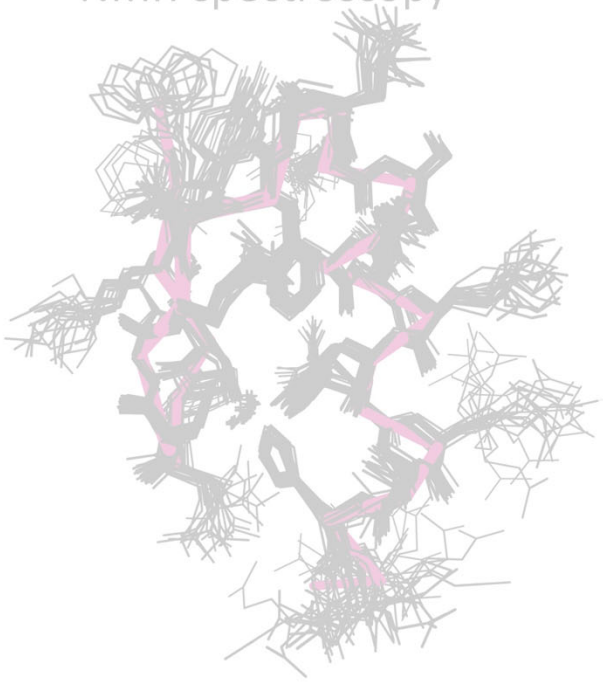
# Protein structure determination

X-ray crystallography



Crystallization required
averaged picture, no dynamics
no size limit

NMR-spectroscopy



Size limit (smaller proteins)
calculated ensemble of structures
protein solubility (µM)
Dynamics can be observed

Electron microscopy

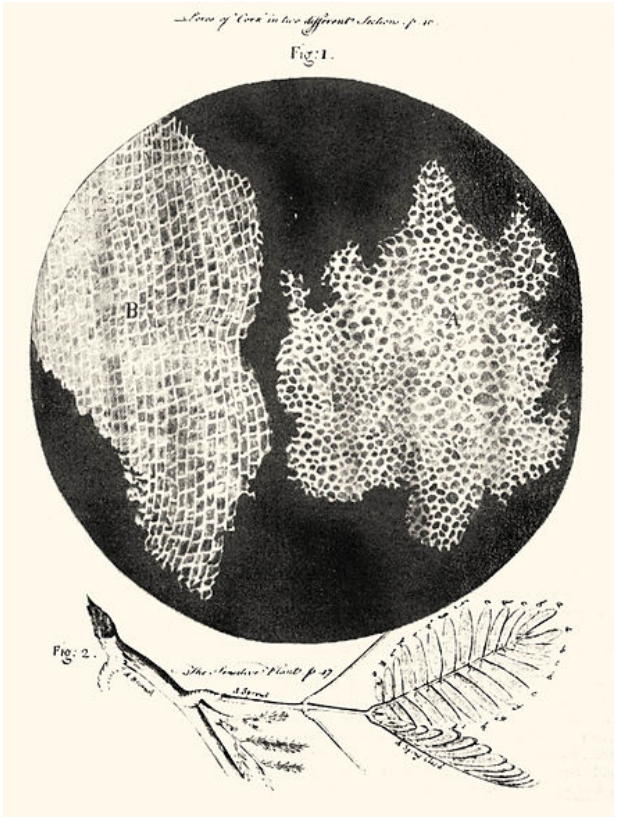


*Chua et al. NAR 2016*

No crystallization required, single molecule technique
large complexes
no size limit

# What is the structure of the molecules of life?

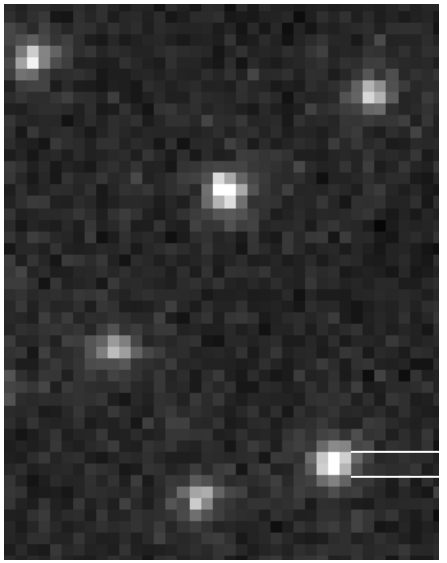
Microscope of **Antonie van Leeuwenhoek**, 1600s



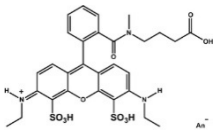

Cells in cork, by R. Hooke 1665

# Light microscopy cannot resolve individual molecules

- **Due to the wave nature of light**
- **Diffraction** of light from the edges of the objective aperture
- Broadens light into a diffraction pattern having a central disk of finite but larger size than the original point
- **Interference** : Recombination and summation of wavefronts dependent on their phase

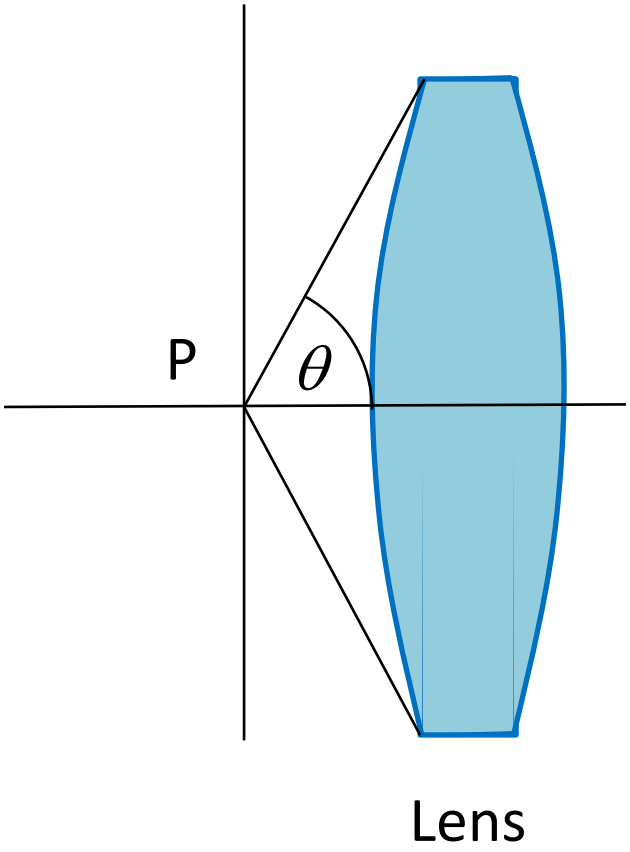



single molecules



1 nm

# The Diffraction Limit



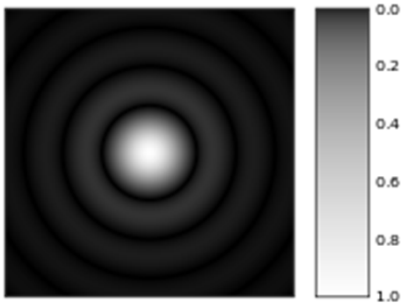

**Numerical Aperture:** $N_a = n\ sin(\theta)$

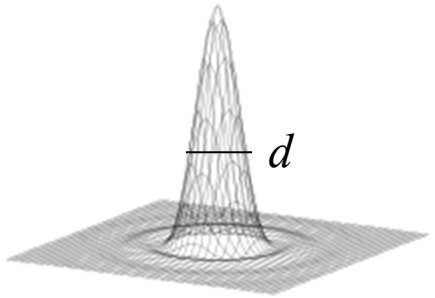
$\theta$: the maximal angle by which an objective gathers light from a sample and $n$, the refractive index of the medium above the lens (e.g. immersion oil, n = 1.51)

**Abbe diffraction limit**

the maximal obtainable resolution with a conventional lens

$$d = \frac{\lambda}{2(n\ sin\theta)}$$



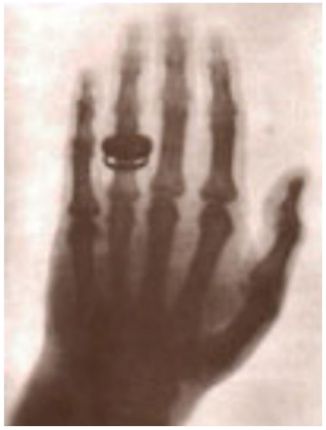




Airy disk

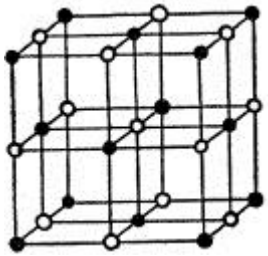
point spread function of a laser focused on a pinhole

# Radiation with smaller wavelength required

- **Wilhelm Röntgen**: Discovery of X-rays in 1895
- **J.J. Thompson**: Discovery of electrons in 1897
- **James Chadwick**: Discovery of the neutron in 1932



- 1912: **Max von Laue** suggested to use X-rays to investigate crystalline solids
- 1913: **Lawrence Bragg** determines the structure of sodium chloride

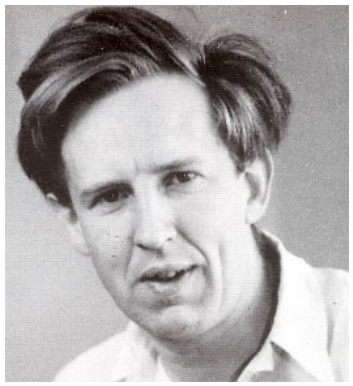


Structure of NaCl, by L. Bragg

Diamond, model by L. Bragg

# Protein structure, the beginning

- 1934: **J.D. Bernal** and **Dorothy Crowfoot (Hodgkin)** place a crystal of pepsin (small enzyme) in X-ray beam → Diffraction pattern





## X-Ray Photographs of Crystalline Pepsin

FOUR weeks ago, Dr. G. Millikan brought us some crystals of pepsin prepared by Dr. Philpot in the laboratory of Prof. The Svedberg, Uppsala. They are in the form of perfect hexagonal bipyramids up to 2 mm. in length, of axial ratio $c/a = 2{\cdot}3 \pm 0{\cdot}1$. When examined in their mother liquor, they appear moderately birefringent and positively uniaxial, showing a good interference figure. On exposure to air, however, the birefringence rapidly diminishes. X-ray photographs taken of the crystals in the usual way showed nothing but a vague blackening. This indicates complete alteration of the crystal and explains why previous workers have obtained negative results with proteins, so far as crystalline pattern is concerned[1]. W. T. Astbury has, however, shown that the altered pepsin is a protein of the chain type like myosin or keratin giving an amorphous or fibre pattern.

It was clearly necessary to avoid alteration of the crystals, and this was effected by drawing them with their mother liquor and without exposure to air into thin capillary tubes of Lindemann glass. The first photograph taken in this way showed that we were dealing with an unaltered crystal. From oscillation photographs with copper $K\alpha$-radiation, the dimensions of the unit cell were found to be $a = 67$ A., $c = 154$ A., correct to about 5 per cent. This is a minimum value as the spots on the $c$ row lines are too close for accurate measurement and the $c$ axial length is derived from the axial ratio. The dimensions of the cell may still be multiples of this. Using the density measured on fresh material[2] as 1·32 (our measurements gave 1·28), the cell molecular weight is 478,000, which is twelve times 40,000, almost exactly Svedberg's value arrived at by sedimentation in the ultracentrifuge. This agreement may however be quite fortuitous as we have found that the crystals contain about 50 per cent of water removable at room temperature. But this would still lead to a large molecular weight, with possibly fewer molecules in the unit cell.

Not only do these measurements confirm such large molecular weights but they also give considerable information as to the nature of the protein molecules and will certainly give much more when the analysis is pushed further. From the intensity of the spots near the centre, we can infer that the protein molecules are relatively dense globular bodies, perhaps joined together by valency bridges, but in any event separated by relatively large spaces which contain water. From the intensity of the more distant spots, it can be inferred that the arrangement of atoms inside the protein molecule is also of a perfectly definite kind, although without the periodicities characterising the fibrous proteins. The observations are compatible with oblate spheroidal molecules of diameters about 25 A. and 35 A., arranged in hexagonal nets, which are related to each other by a hexagonal screw-axis. With this model we may imagine degeneration to take place by the linking up of amino acid residues in such molecules to form chains as in the ring-chain polymerisation of polyoxy methylenes. Peptide chains in the ordinary sense may exist only in the more highly condensed or fibrous proteins, while the molecules of the primary soluble proteins may have their constituent parts grouped more symmetrically around a prosthetic nucleus.

At this stage, such ideas are merely speculative, but now that a crystalline protein has been made to give X-ray photographs, it is clear that we have the means of checking them and, by examining the structure of all crystalline proteins, arriving at far more detailed conclusions about protein structure than previous physical or chemical methods have been able to give.

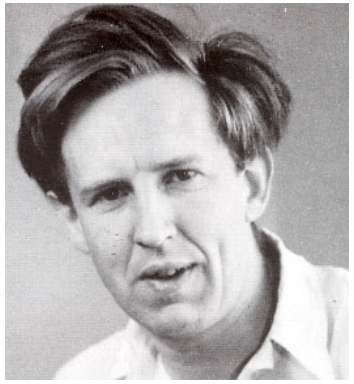
J. D. BERNAL.
D. CROWFOOT.

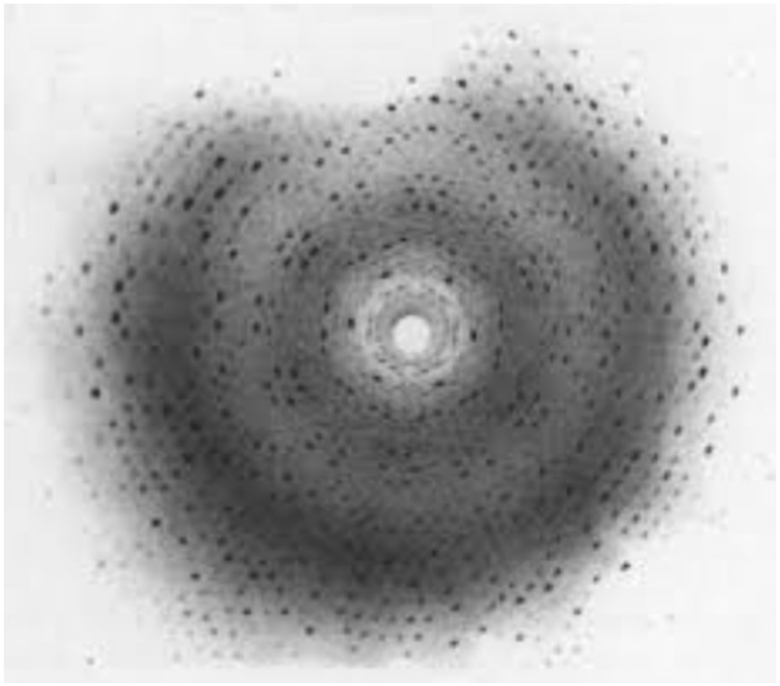
Department of Mineralogy and Petrology,
Cambridge.
May 17.

[1] G. L. Clark and K. E. Korrigan (*Phys. Rev.*, (ii), **40**, 639; 1932) describe long spacings found from crystalline insulin, but no details have been published.
[2] J. H. Northrop, *J. Gen. Physiol.*, **13**, 739; 1930.

# Protein structure, the beginning

- 1934: **J.D. Bernal** and **Dorothy Crowfoot (Hodgkin)** place a crystal of pepsin (small enzyme) in X-ray beam → Diffraction pattern



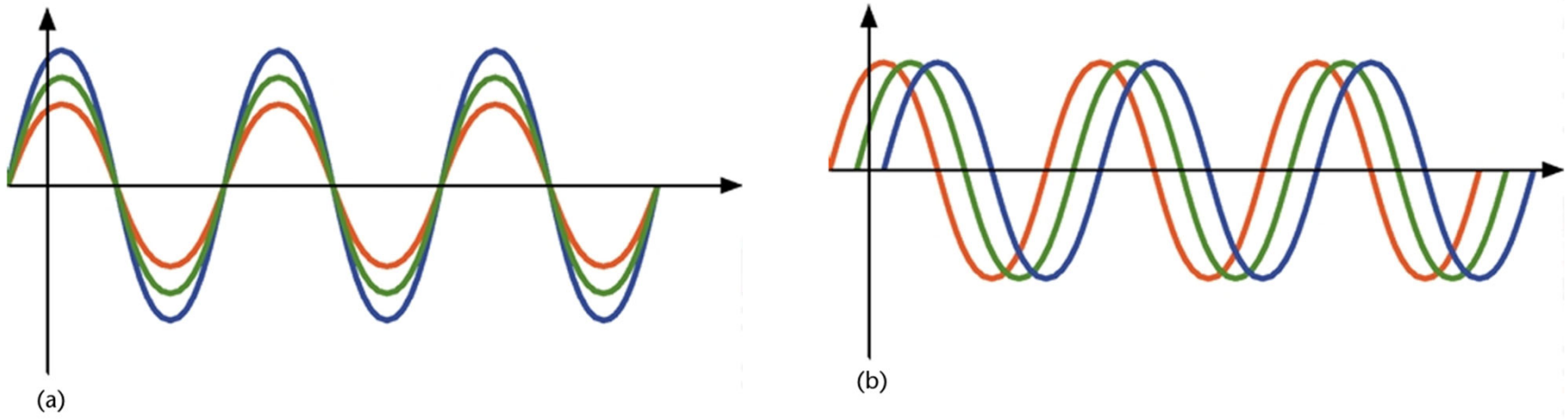


**Surprise finding**: Protein crystals have an ordered structure, are not amorphous colloids

# First structures of proteins by crystallography

- 1936: **Max Perutz** joined Bernal's laboratory to investigate the structure of hemoglobin

- Interpretation of the diffraction pattern is a major problem (the phase problem)



# First structures of proteins by crystallography

- 1936: **Max Perutz** joined Bernal's laboratory to investigate the structure of hemoglobin
- Interpretation of the diffraction pattern is a major problem (the phase problem)
- > 30 years later: structures of myoglobin (Kendrew) and hemoglobin (Perutz) solved
- 10 structures solved in 1973
- 27 structures solved in 1983
- 922 in 1993
- **>9’000 depositions in 2024**

Max Perutz John Kendrew

# Where to find experimental protein structures ?

**Protein Data Bank** :

*http://www.rcsb.org*

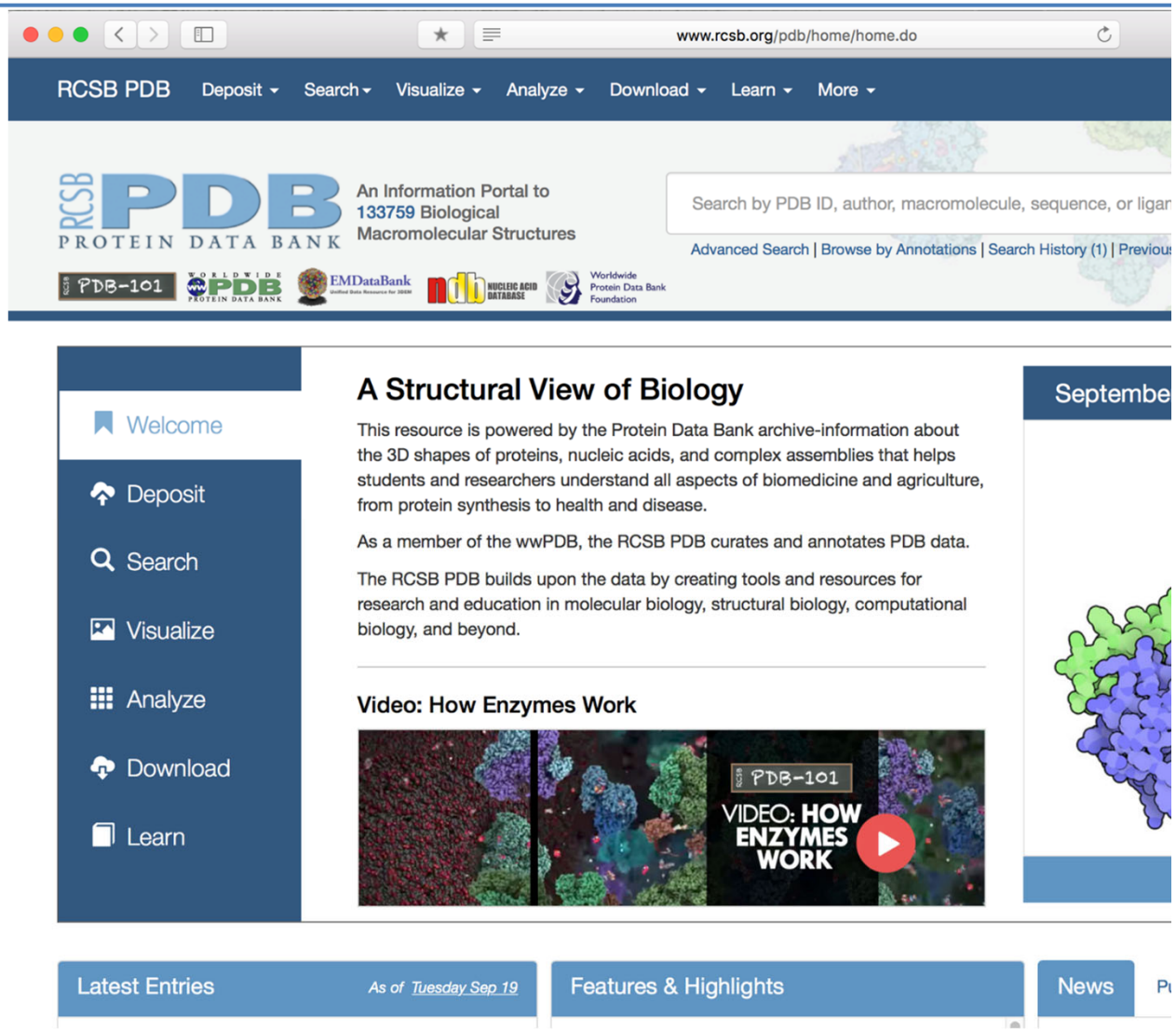
231,564 models:

- from X-ray, NMR & EM
  - ÷ annotated
  - ÷ curated

- Information

- Tools

- Useful links



# Where to find experimental protein structures ?

## Crystal structure of thrombin in complex with macrocycle T1

PDB code 8ASF

https://www.rcsb.org/structure/8ASF

## Info available

- Experimental data Snapshot
- Publication?
- Description protein
- Description small molecules
- Interaction small – protein
- Data & Validation

- Download Files

Nielsen et al., ACIE 2024 - Heinis (ISIC_EPFL)

# Analyze models with PyMOL

## Crystal structure of thrombin in complex with macrocycle T1

PDB code 8ASF

«fetch 8ASF»

- 3-Button viewing
- Display (change appearance)
- Change color, cartoon
- Display sequence, chains
- Select chain, extract object

Nielsen et al., ACIE 2024 - Heinis (ISIC_EPFL)

# Analyze models with PyMOL

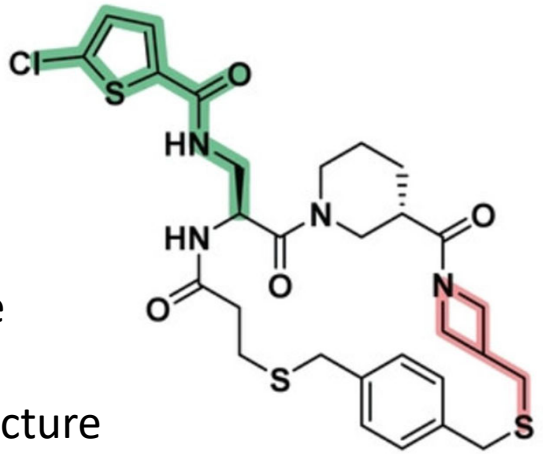
## Crystal structure of thrombin in complex with macrocycle T1

PDB code 8ASF

«fetch 8ASF»

- 3-Button viewing
- Display (change appearance)
- Change color, cartoon
- Display sequence, chains
- Select chain, extract object

- Hide waters
- Show protein as surface
- Look at macrocycle structure
- Select residues
  find Gly 216 – find H-bond
  find Tyr228 – find halogen-aromatic interaction
- Show surface potential
  action-generate-vacuum electrostatics



T1

Nielsen et al., ACIE 2024 - Heinis (ISIC_EPFL)

# 1SEP in PyMOL – Distances & angles

Geometric parameters are important to evaluate e.g. interactions or conformations

- Hide all

„**Show** => **Organics** => **Lines**

- Select with mouse the organics => (sele) => **Action** => **Origin** & **Center**
- Zoom in
- From **Wizard** menu choose **Measurements**
- Use mouse matrix to select measurement mode
    - ÷ **Distance** Click on the 2 atoms of interest
    - ÷ **Angle** Click on the 3 atoms defining the angle
    - ÷ **Dihedral** Click on the 4 atoms defining the dihedral

- Click on „**Done**“ with the measuremens

# 1SEP in PyMOL - H-bonding of $NADP^+$

**The enzyme SPR**

Put as „**cartoon**“ and color gray

**The co-factor**

**Display**=> **Sequence** & click on **NAP** to select the co-factor => (sele)

(sele) => **Show => Sticks**

**Color** => **by element**

**The polar contacts**

Use mouse in „**residue mode**“ to select co-factor

(sele) => Action=> Find=> Polar contacts => to others excluding solvent

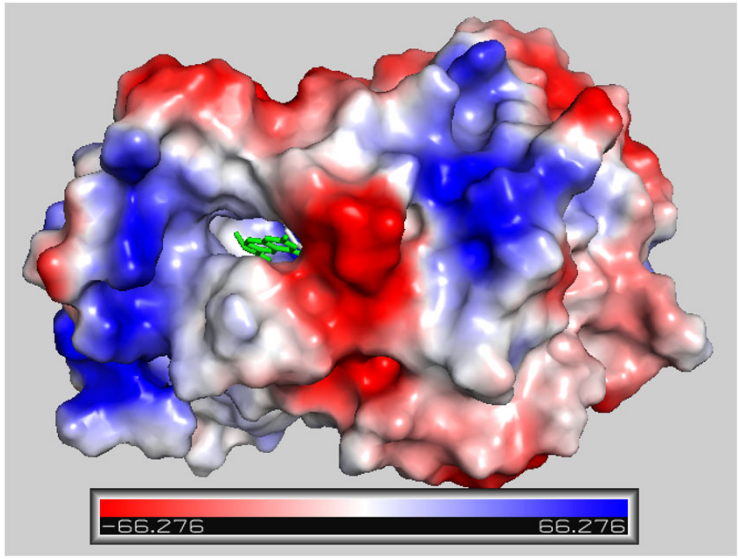
Then, for **1SEP** => **Show** => **Sticks** :: dotted lines indicate H-bonds

Click on H-bonding residues => (sele) => **Label** => **Residue**

# 1SEP in PyMOL - Electrostatic surface potential

**Mouse SPR with substrate & co-factor/substrate**

**Action** => **Generate** => **Vacuum electrostatics**




Red for negative, blue for positive

Have a look at the cofactor; what do you notice ?

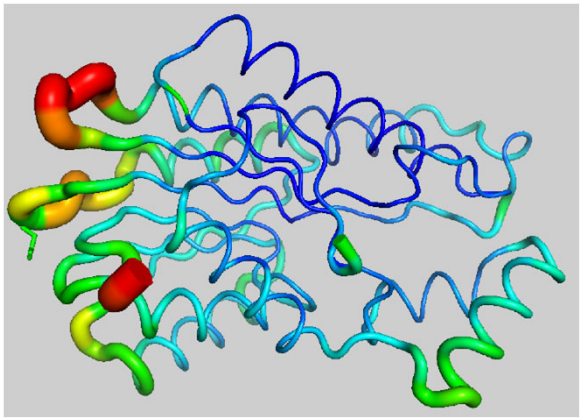
Relevant for molecular interactions

larger interfaces like protein-protein
protein-lipid membrane
protein-DNA

- Small pockets like substrate-enzyme

# 1SEP in PyMOL - The B-factor

There are 2 manners to visualize B-factors



- The fancy way

**Action** => **Preset** => **B factor putty**

- The more flexible manner

Create the structure you want & select parts for which to show the B-factors

**Color** => **Spectrum** => **B-factor**

Color scheme : low B-factor blue thin

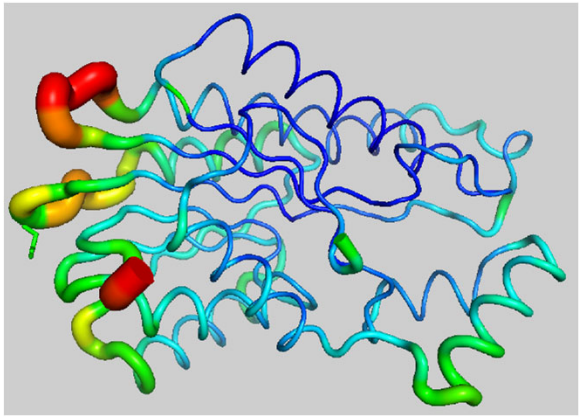
high B-factor red thick

NB : the B-factor is also called „*Temperature*“ factor

# PyMOL – Saving your screens

There are several manners to save:

As molecule => a **.pdb** file with coordinates

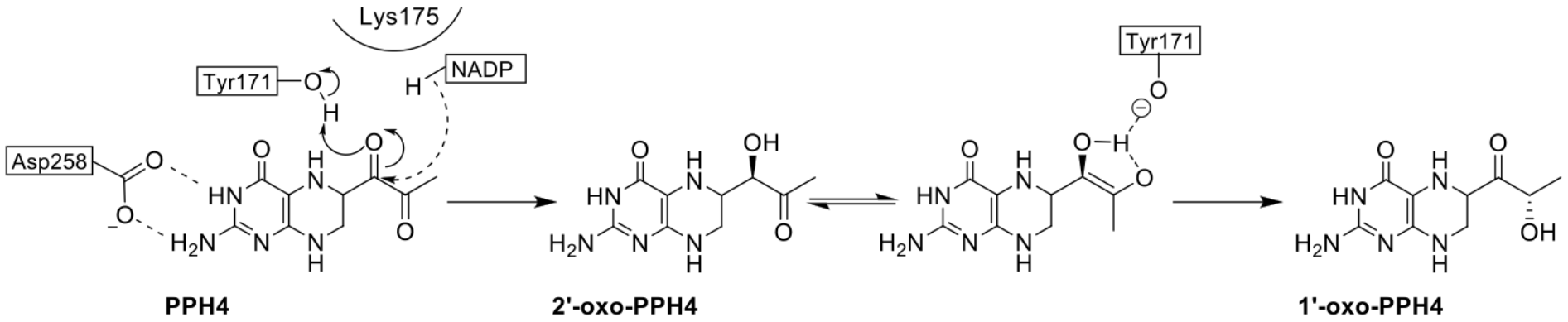


As image => **.png** or **ray-traced**

As scene => a **.pse** that rebuilds the current content of your PyMol session

# PyMOL – Exercises

Investigate the proposed catalytic mechanism:




÷ Are the proposed residues indeed close to the substrate ?

÷ What could be the role of SER_158 ?

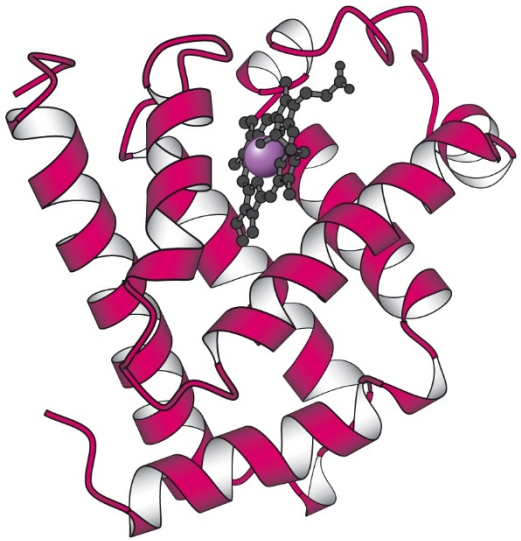
÷ How are substrate and co-factor oriented such that one could expect reduction ?

÷ „NAP“ is this the reduced or oxidized co-factor ?

=> look at distances, H-bonds, stacking, ..

# Protein structure determination

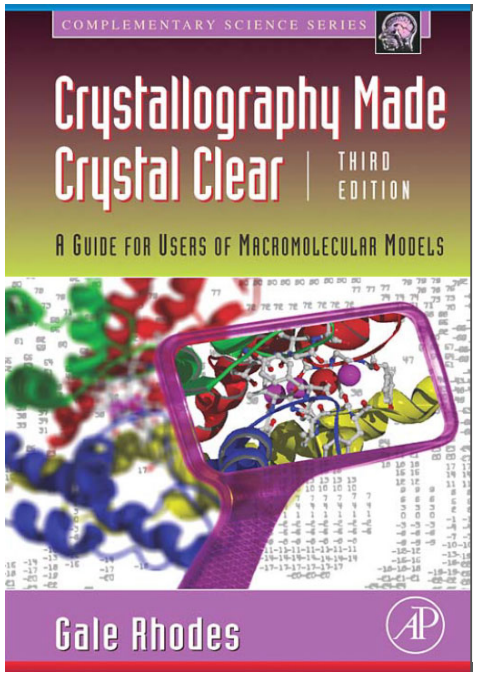
X-ray crystallography



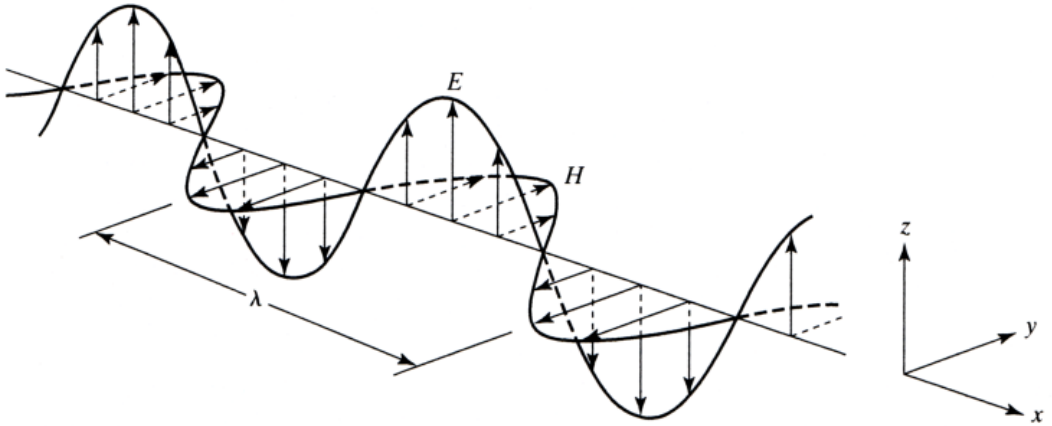
Crystallization required
averaged picture, no dynamics
no size limit

**Literature:**

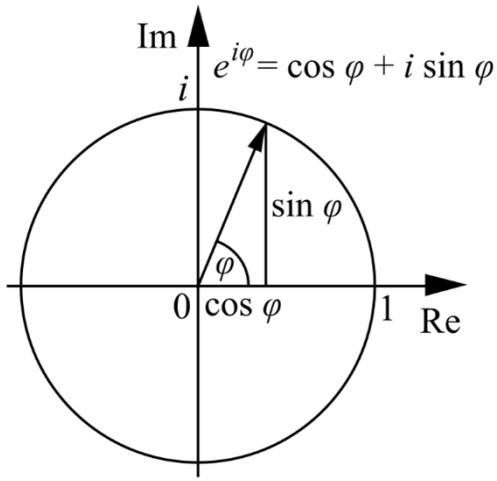
Biophysical Chemistry, Van Holde
Crystallography made crystal clear, Rhodes



# Electromagnetic waves




$$\mathbf{E} = \mathbf{E}_0 \cos 2\pi(\nu t - x/\lambda + \phi)$$
$$\mathbf{H} = \mathbf{H}_0 \cos 2\pi(\nu t - x/\lambda + \phi)$$
$$\mathbf{E} \cdot \mathbf{H} = 0$$

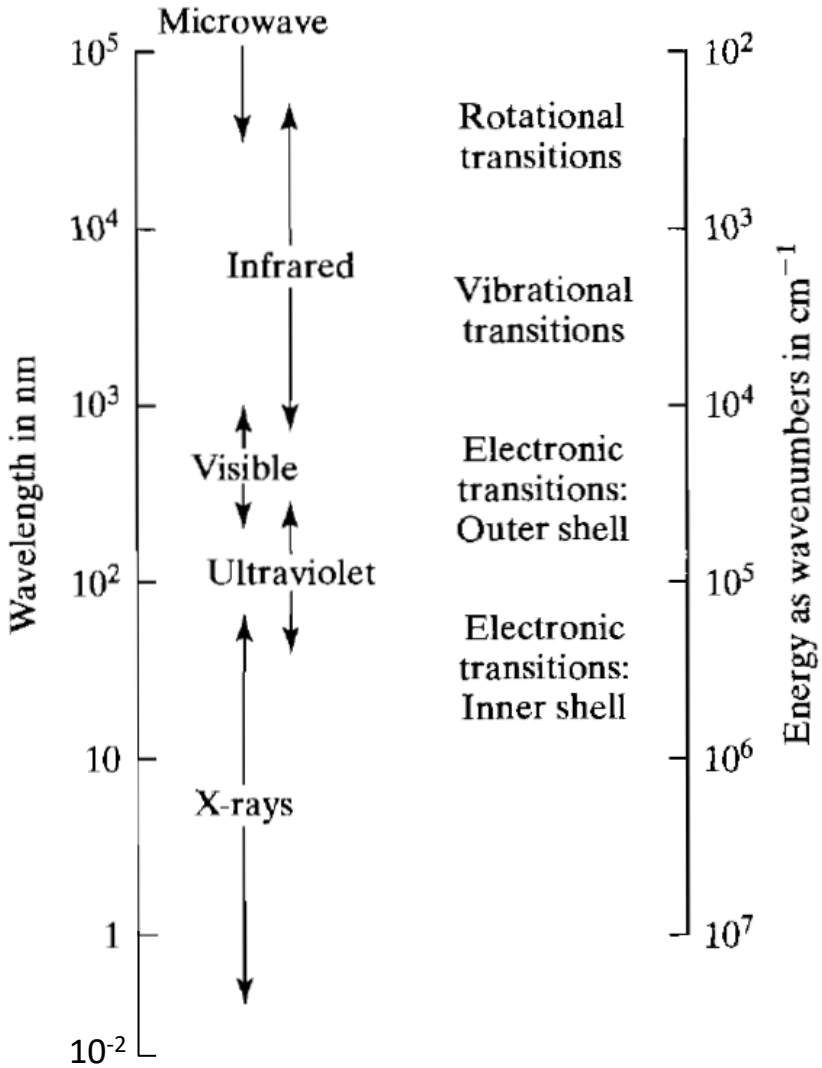



using **Euler's formula**:

$$E(x,t) = |E_0| e^{2\pi i(\nu/t - x/\lambda)} e^{2\pi i \phi}$$

amplitude wavelgth. phase

# Why X-rays?




The **resolution limit** of a spectroscopic method:

$$LR = \frac{\lambda}{2}$$

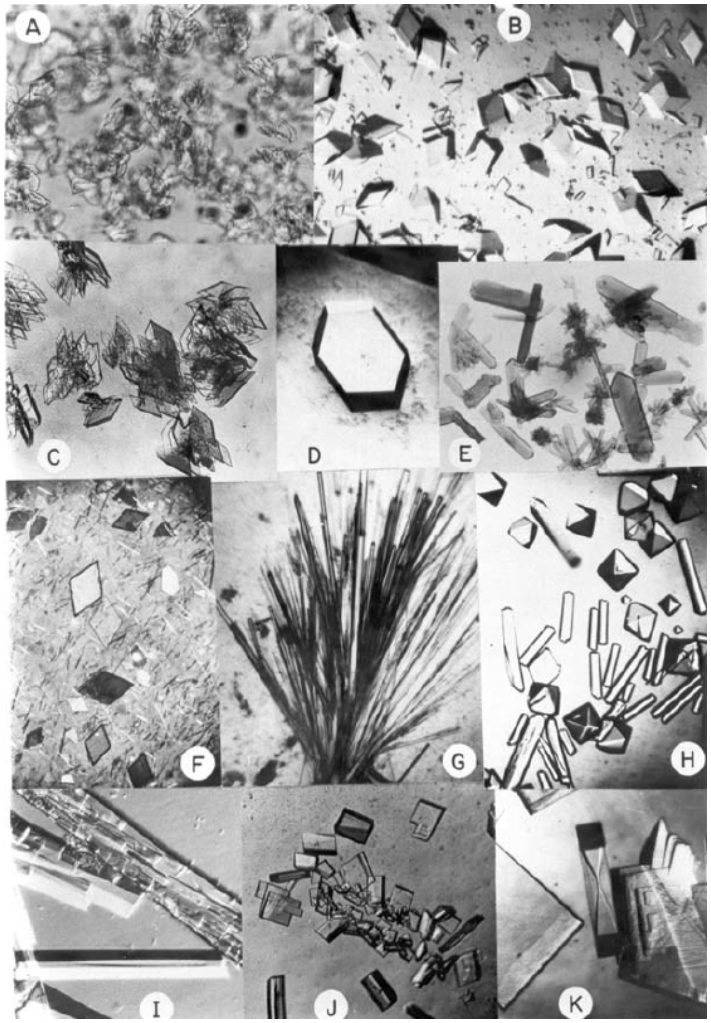
For **atomic resolution** (1 Å), X-rays are required.

**Problem:**

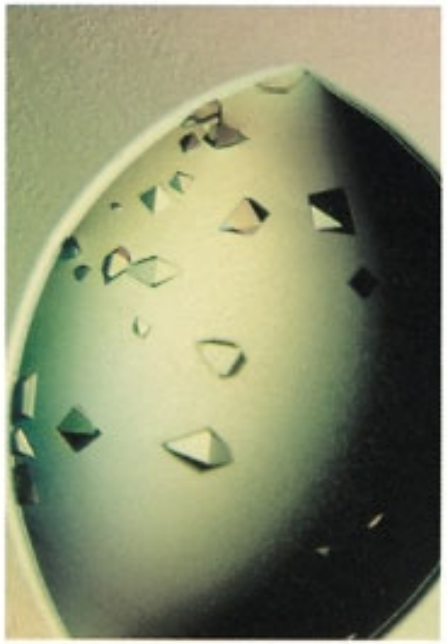
1. no lenses are available, image cannot be focused
2. single molecules are weak scatterers of X-rays, many molecules are needed for a signal

# Crystals are required!

- Scattering arises from electrons of a periodic structure (crystal)
- Wavelength of X-rays is comparable to periodic spacings of molecular structure
- A crystal is built up from many billions of small identical units or unit cells packed against each other in 3-dimensions. A unit cell may contain one or more molecules.



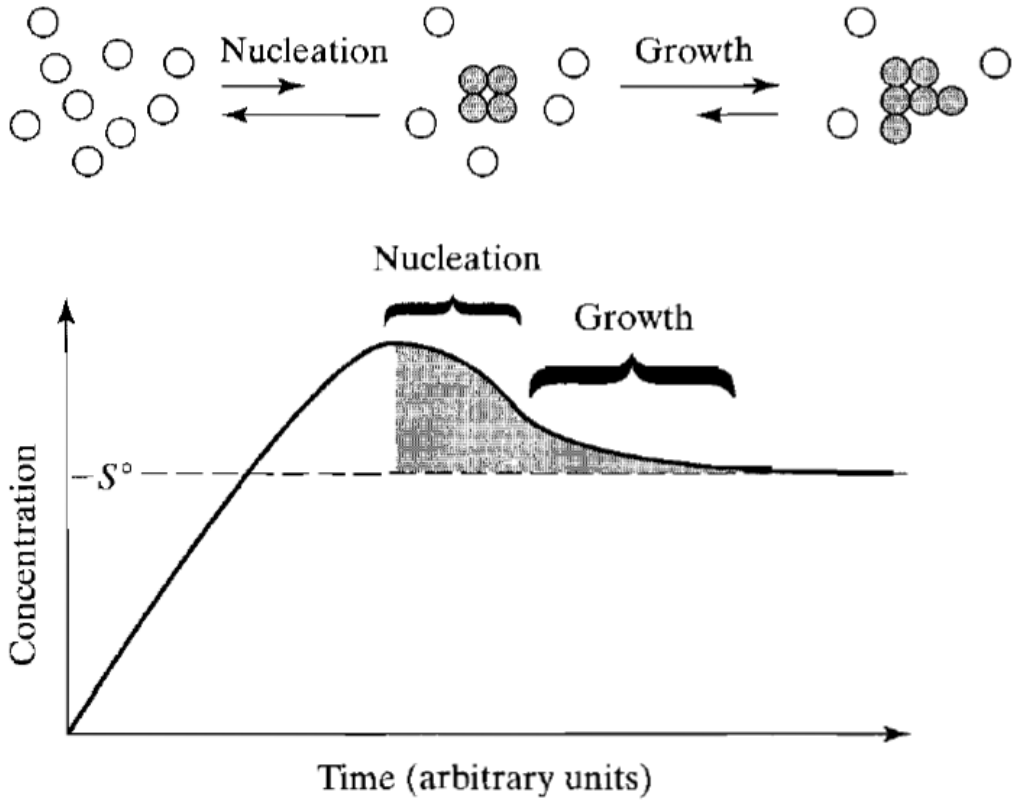
# Crystallization of proteins



## Protein crystallization:

- very high purity required
- local disorder in proteins can inhibit crystal formation
- limited proteolysis can identify unstructured regions
- mutation screen to obtain crystallizable proteins
- protein homologs from thermophilic organisms crystallize often better
- cofactors and cosolvents

# Protein crystal formation



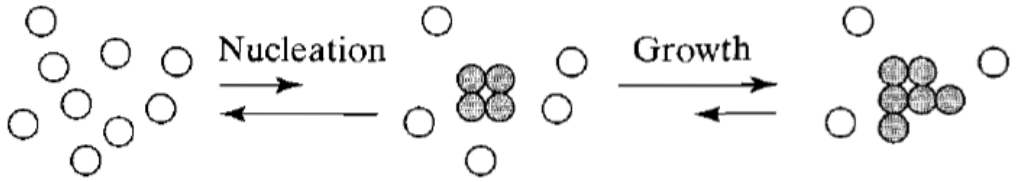

## Growth of crystals

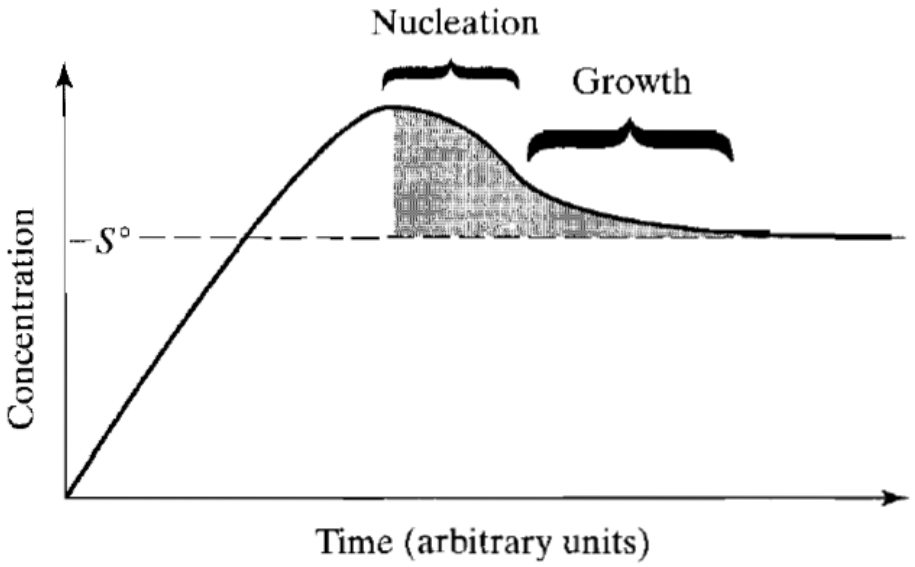
Generation of supersaturated solution (above solubility $S^0$)

Formation of small crystallization nuclei

Growth phase: Slow crystal growth by molecule addition

# Creating supersaturated conditions



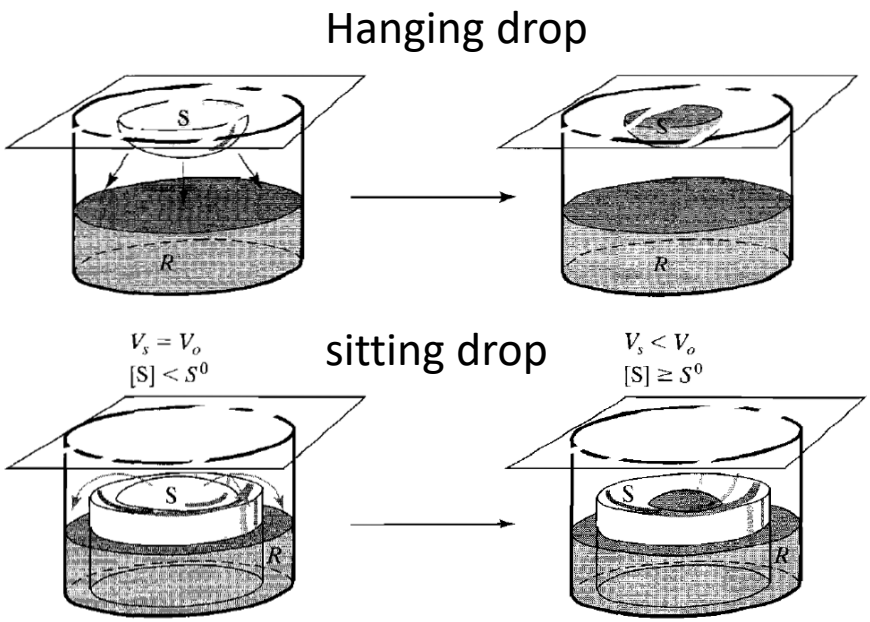




**Salts**
Na citrate
Mg2+
BaCl2
CaCl2

**Buffers**
Tris
Cacodylate

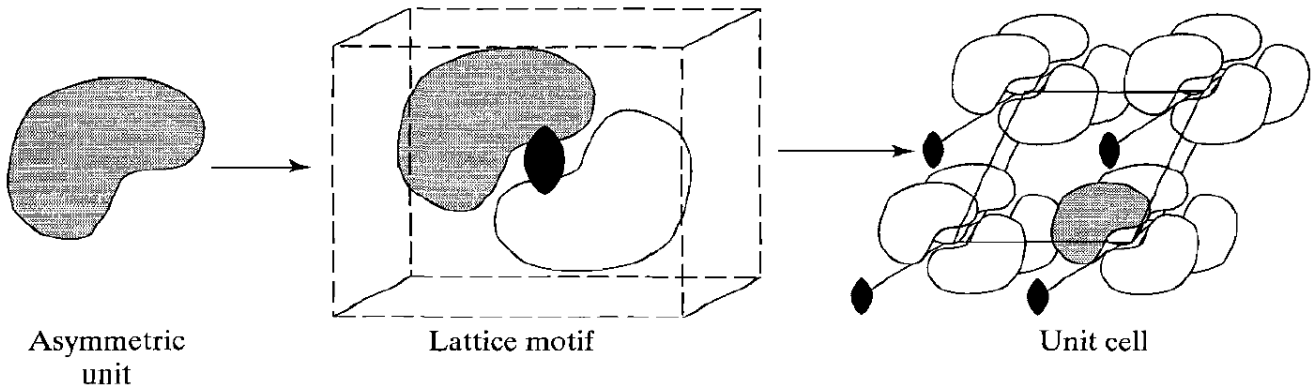
**Precipitants**
Ammonium sulfate
polyethyleneglycol
2-Methyl-2,4-pentanediol
propanol

# Controlled crystal growth




- Creation of conditions favoring crystallization by **vapor diffusion**
- drop containing protein, precipitant and buffer
- Same compounds in reservoir at higher concentration
- Equilibration of concentrations due to differences in vapor pressure between drop and reservoir
- Slow and gentle change in concentrations

# Protein crystals




- **asymmetric unit**: protein monomer
- **lattice motif**: repeating unit (e.g. dimer)
- **unit cell**: box containing the lattice points of a crystal lattice

# Crystal lattices

the 14 **Bravais lattices** in X-ray crystallography

P: primitive, lattice points only at corners

C: centered, lattice points at face centers

F: face centered: lattice points at **all** face centers

I: lattice point at center of mass

**triclinic**: all angles and edge lengths different

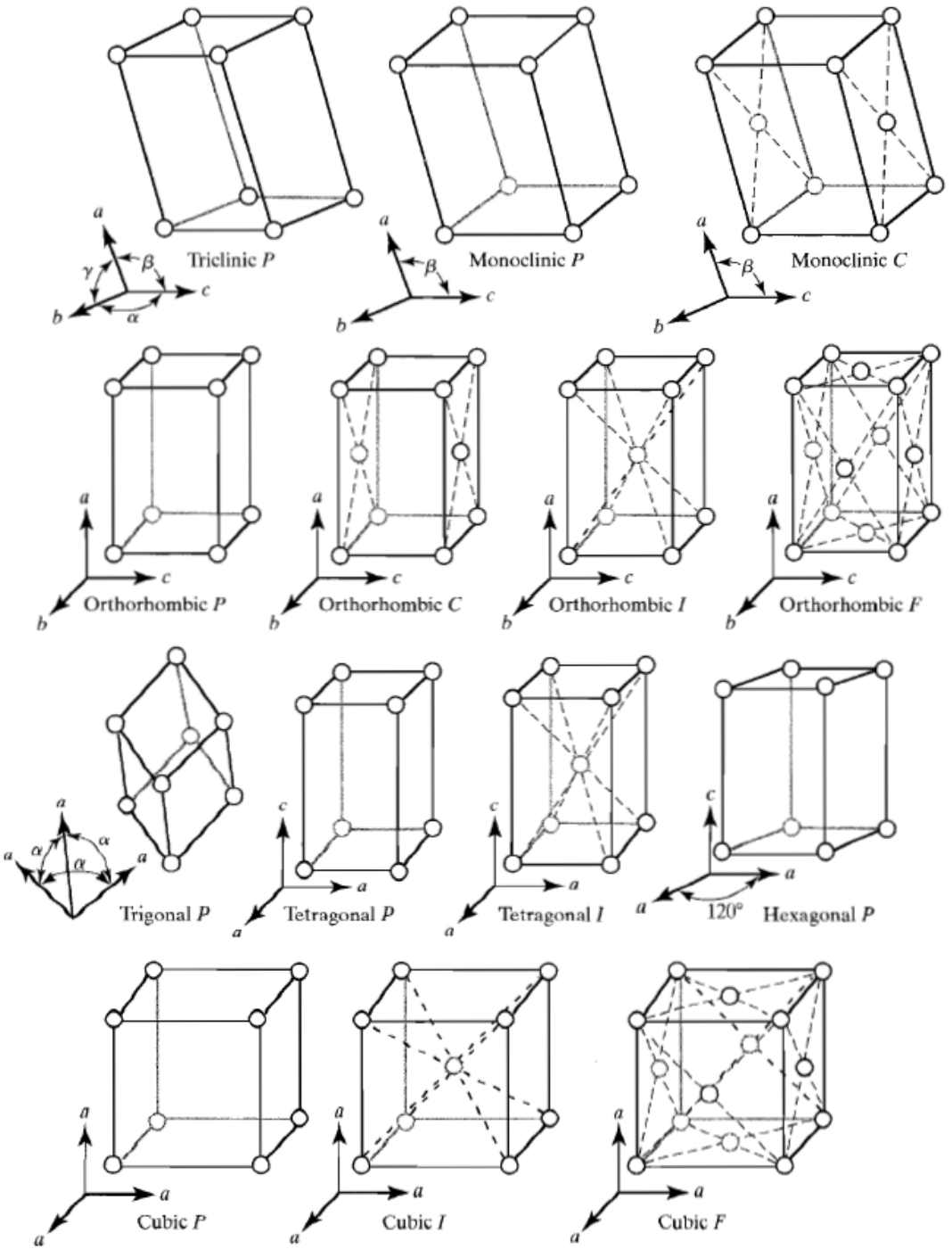
**monoclinic**: one angle is 90°

**orthorhombic**: all angles 90°

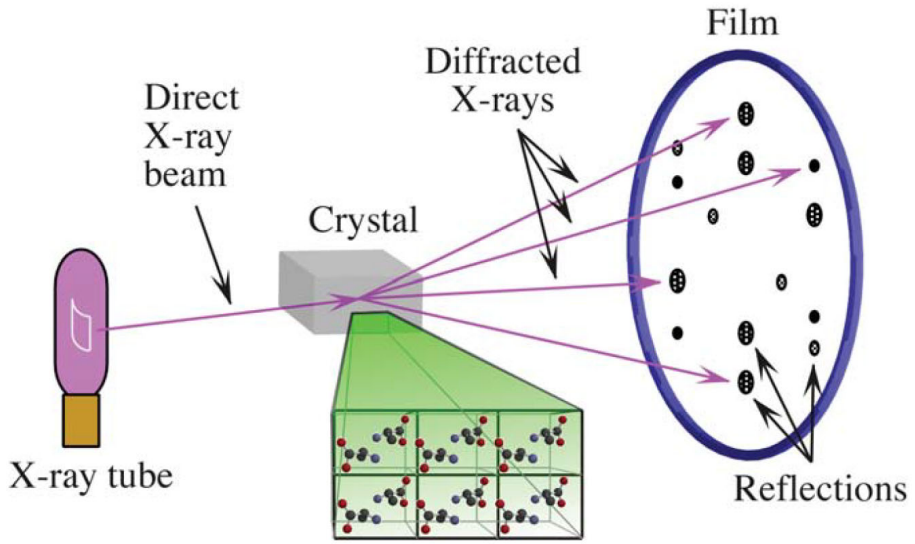
**trigonal**: all faces the same, 3 angles the same

**tetragonal, hexagonal**: 2 faces the same, angles: 90° or 120°

**cubic**: all angles 90°, all faces the same

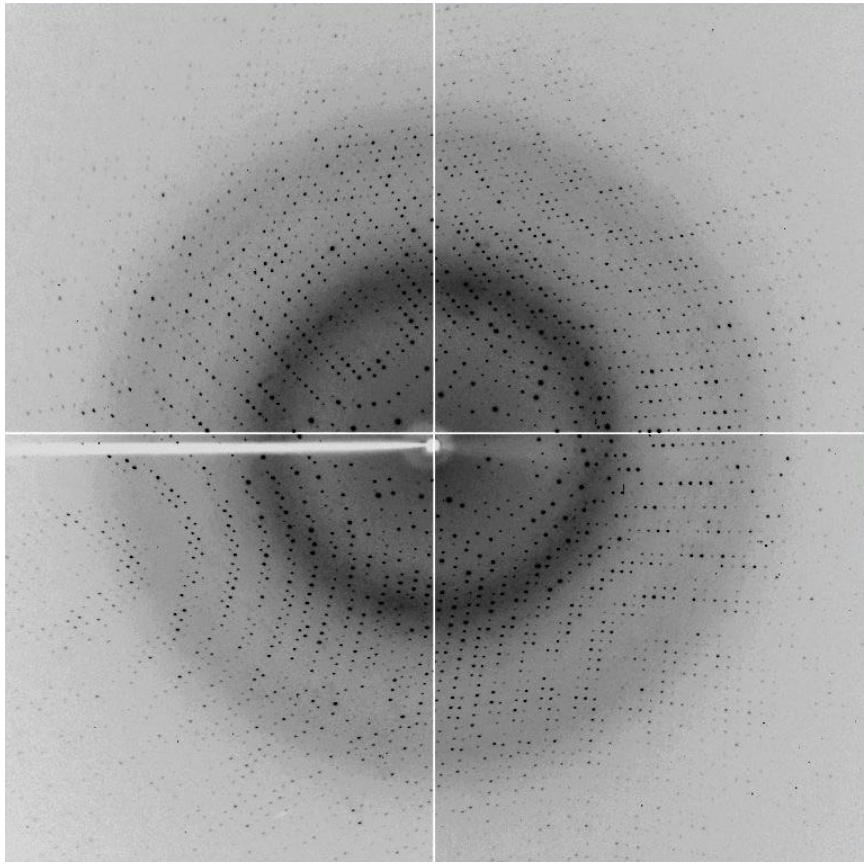


# An X-ray diffraction experiment




Crystallography made crystal clear, Gale Rhodes, 2006

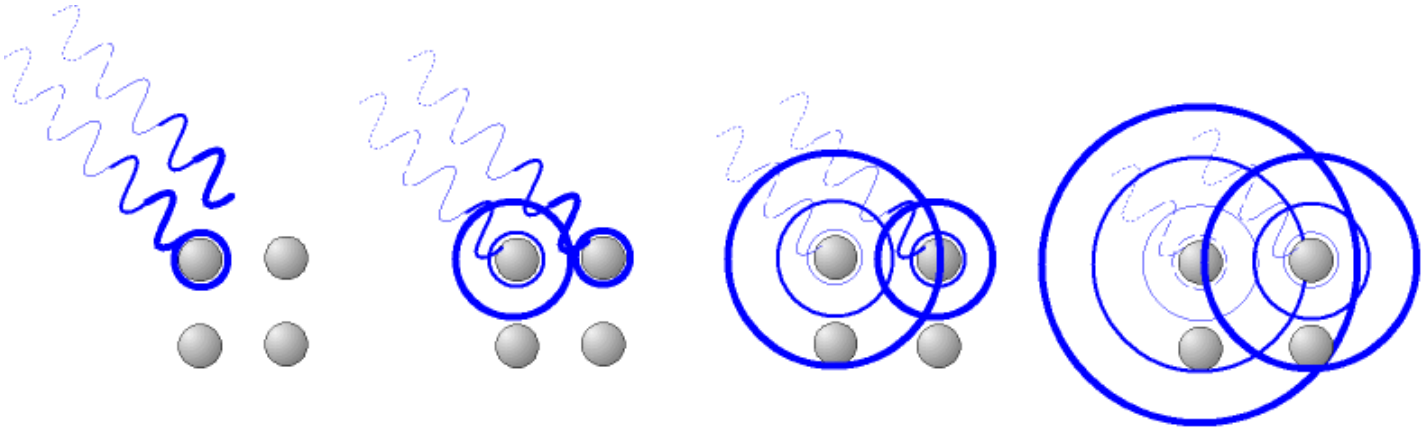
X-rays are diffracted in crystal lattice, resulting in a **diffraction pattern** (reflections) on a detector.



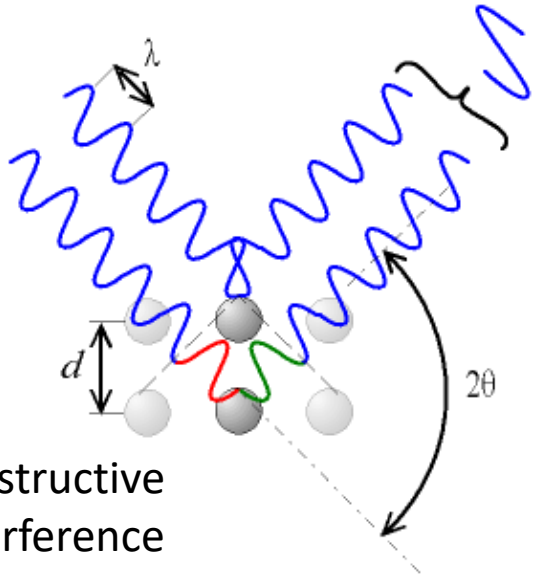
X-ray diffraction pattern from a protein crystal (glucose isomerase with 1.4 Å resolution, *https://lithium.gsu.edu/faculty/Huang/new_page_3.htm*)

# X-Ray diffraction

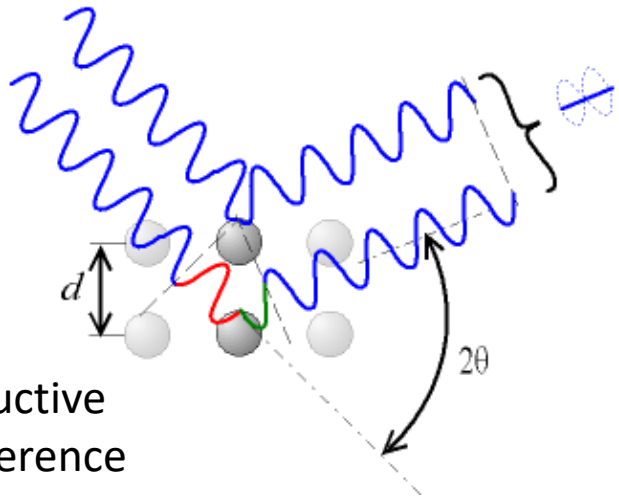
diffraction at the crystal lattice



interference at **different angles**

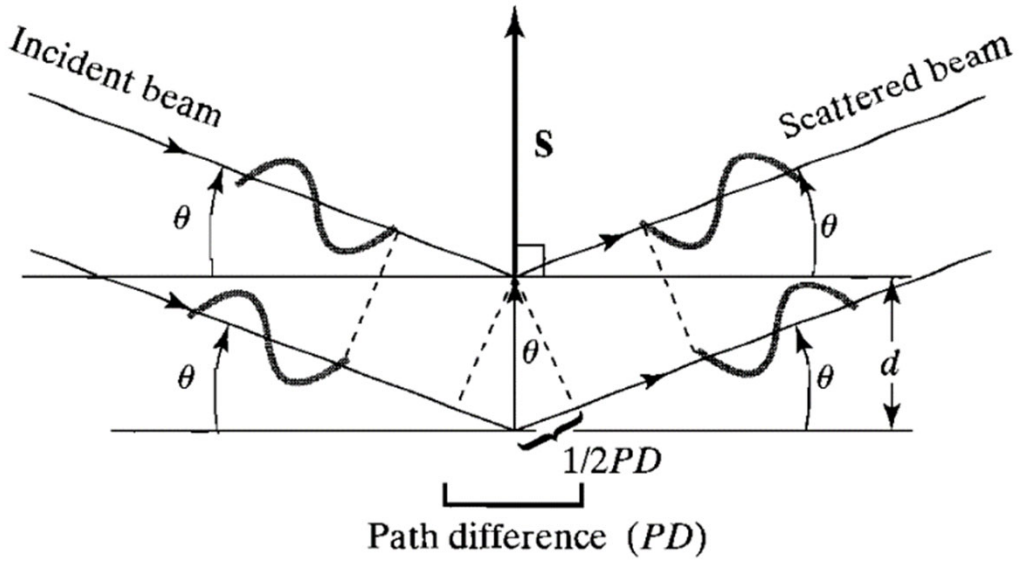



constructive interference




destructive interference

# Bragg's Law




Depending on the angle, the **path difference (PD)** is given by

$$1/2 \cdot PD = d \sin\theta$$

Constructive interference occurs, when the PD is n-fold the wavelength (n: integer)

$$PD = n\lambda$$

resulting in **Bragg's law:**

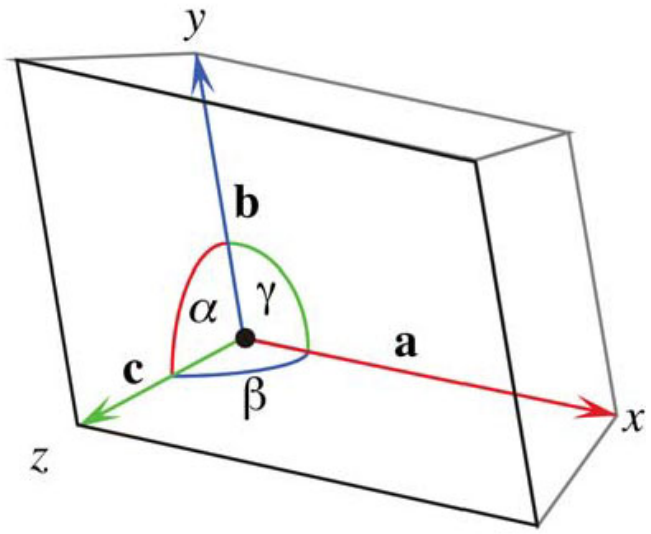
$$2d \sin\theta = n\lambda \quad or \quad \frac{2\sin\theta}{\lambda} = \frac{n}{d}$$

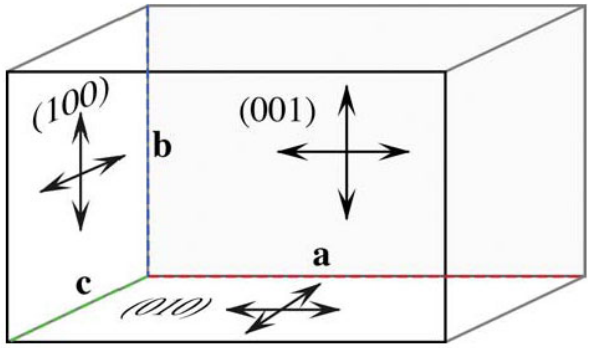
Scattering vector **S** is **orthogonal to the plane** on which the X-ray beam is scattered.

$$\frac{2\sin\theta}{\lambda} = |\mathbf{S}|$$

# Reflections on three dimensional unit cells

Unit cell with angles $\alpha,\beta,\gamma$ and edges a,b,c



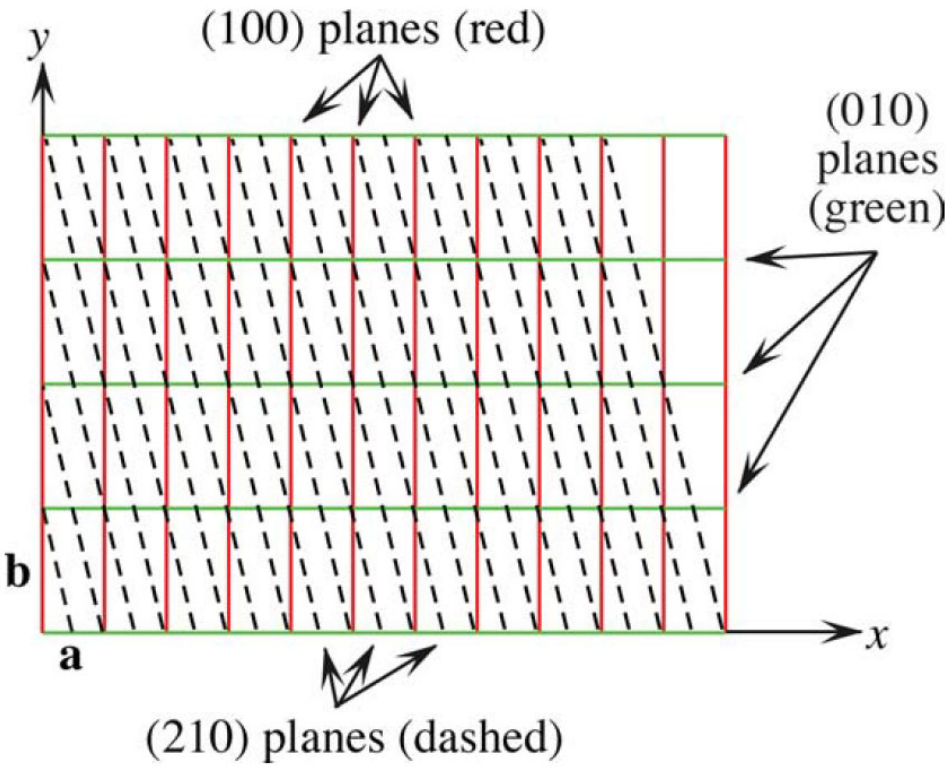

Reflections in a 3D crystal can occur on crystal planes, such as the **faces of the unit cell**

each reflection is characterized by a set of **Miller indices: h,k,l**

Index **h** gives number of planes in the set per unit cell in the x direction
**k:** planes in y direction
**l**: planes in the z direction

each face of an orthorhombic unit cell corresponds to a fundamental plane, incated by a **(hkl) triplet**

# Reflective planes and h,k,l indices in 2D



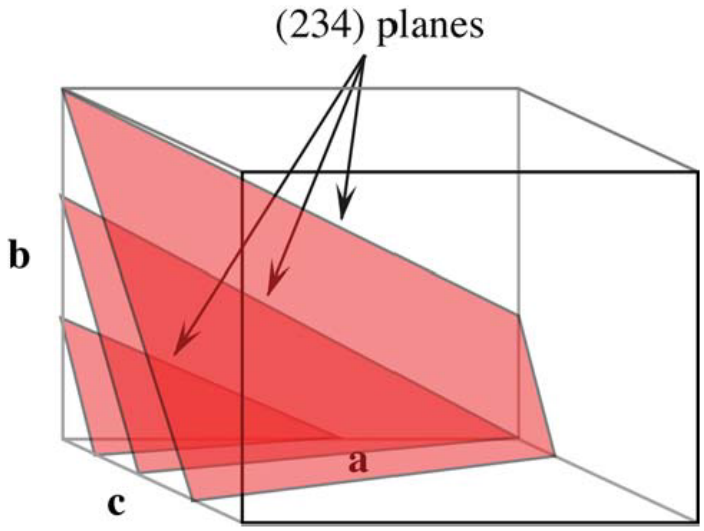

**Miller indices (h,k,l)**

**2D view:**

many different parallel crystal planes can be determined

- orthogonal to x-y plane, orthogonal to x-axis → (1,0,0) plane
- orthogonal to x-y plane, orthogonal to y-axis → (0,1,0) plane
- orthogonal to x-y plane, cuts face a into 2 fractions, b into 1 fraction → (2,1,0) plane

# Conditions for diffraction: Bragg's Law




intersection of (234) planes with an orthorhombic unit cell

reformulation of Bragg's law for reflection on planes indictated by h,k,l indices:

**Bragg's law:**

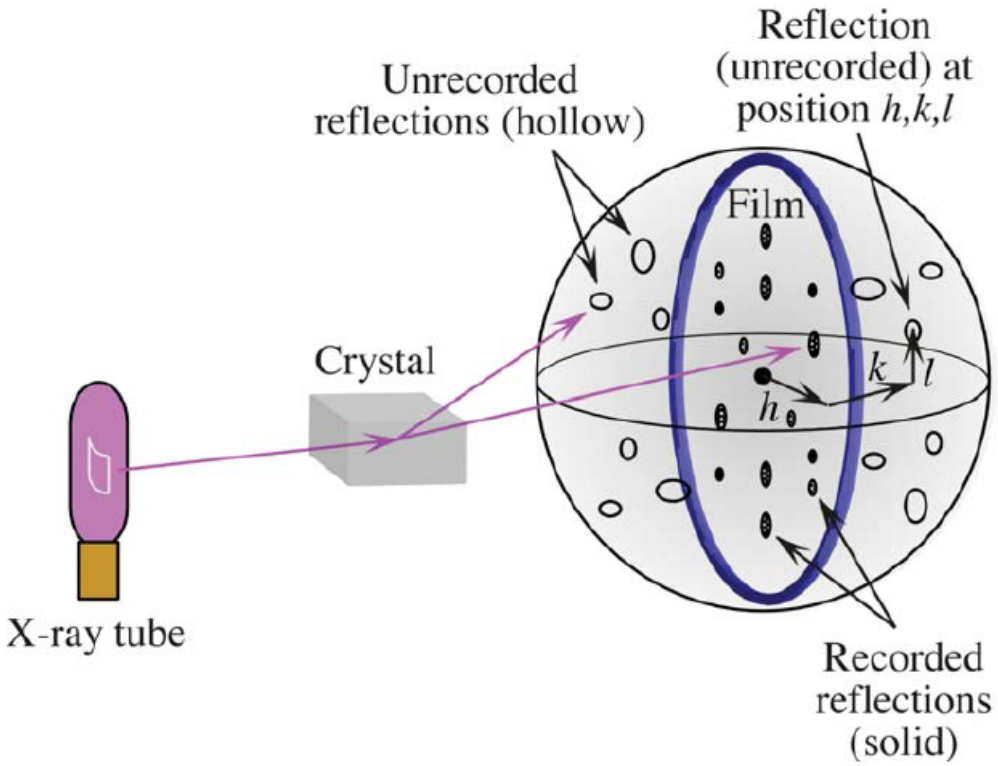
$$2d_{h,k,l}\sin\theta = n\lambda$$

relation of h,k,l with unit cell parameters:

for orthorhombic unit cells with length a, b, c

$$2\sin\theta = n\lambda\left(\frac{h^2}{a^2}+\frac{k^2}{b^2}+\frac{l^2}{c^2}\right)^{1/2}$$

# Diffraction patterns at the detector




**Crystallographic data collection:**

each reflection corresponds to the Miller indices h,k,l

**filled dots**: reflections recorderd at a particular crystal orientation

**open dots**: reflections that could be detected at different crystal orientations

**which reflections are visible for a given crystal and illumination condition?**

# Geometrical construction of reciprocal lattice

**Reciprocal lattice**

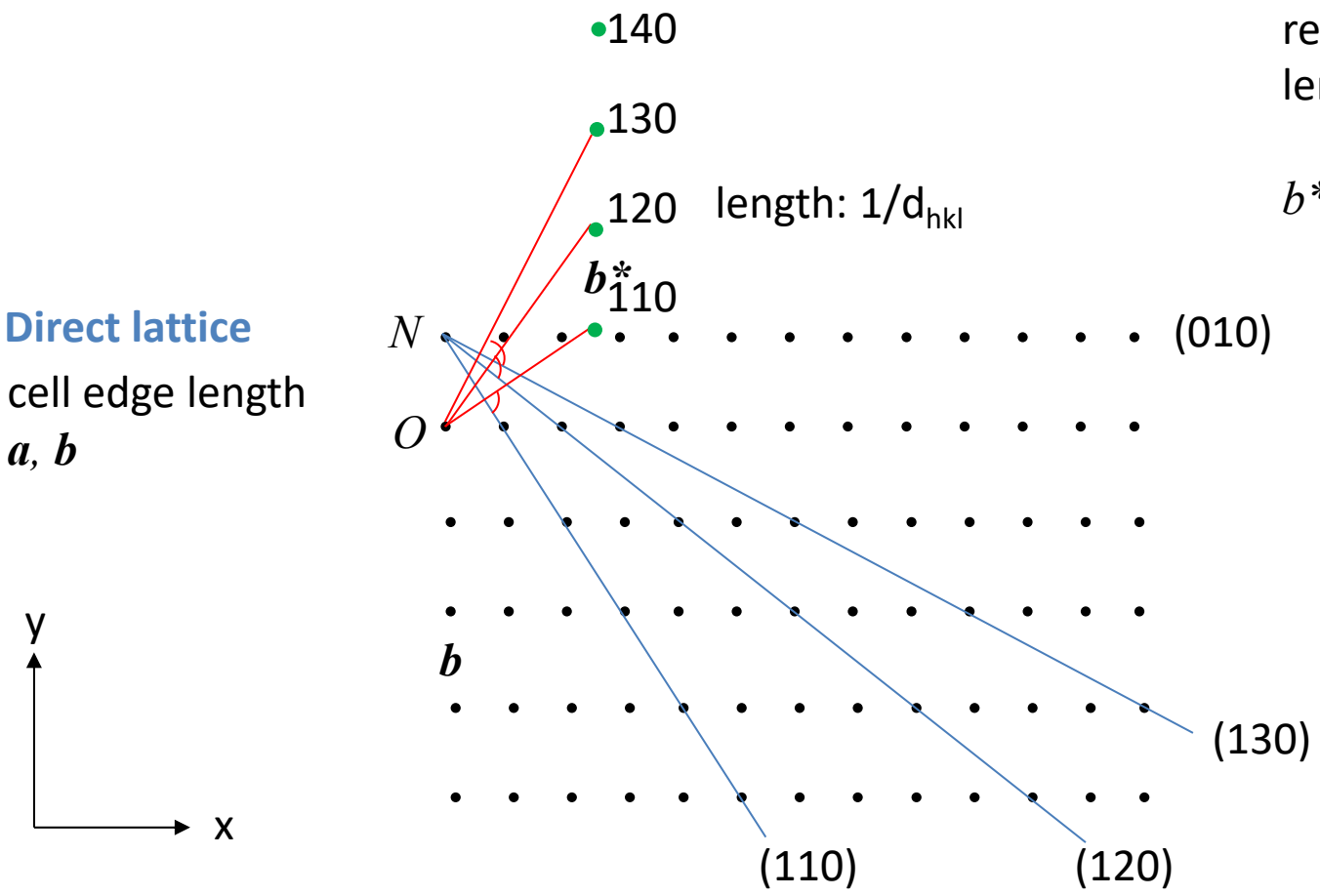
reciprocal lattice length, $\boldsymbol{b}^*$

$b^* = 1/b$

**Direct lattice**

cell edge length

$\boldsymbol{a}$, $\boldsymbol{b}$

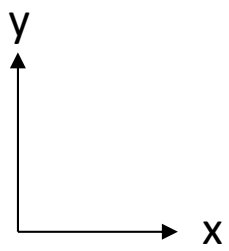


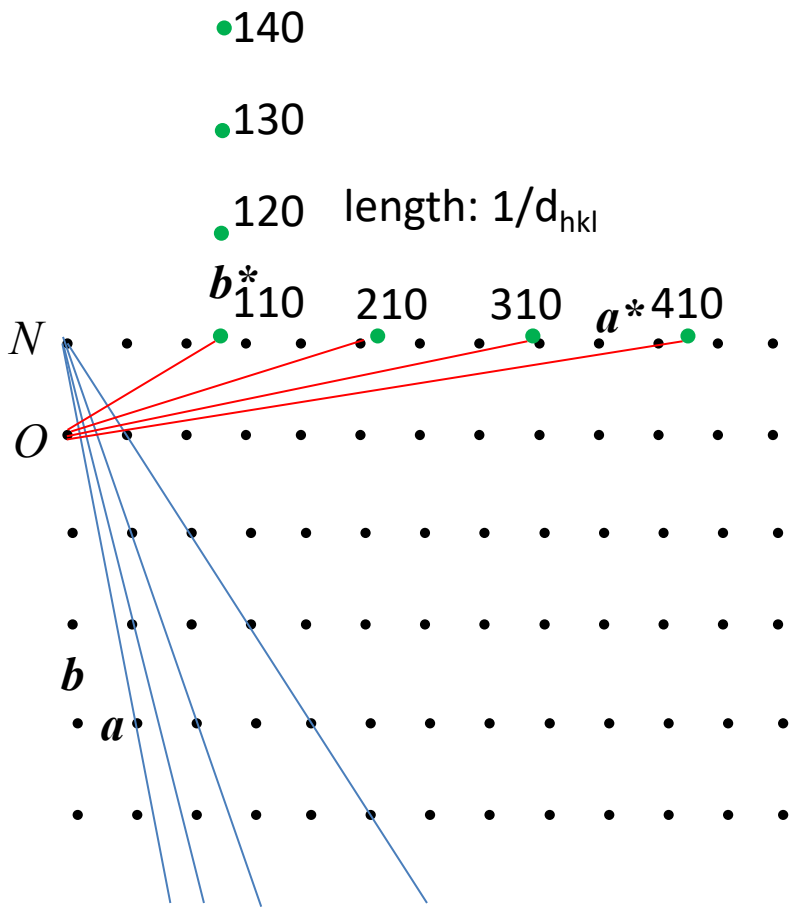
# Geometrical construction of reciprocal lattice

**Direct lattice**

cell edge length

***a***, ***b***




**Reciprocal lattice**

reciprocal lattice length, ***b***, **a****

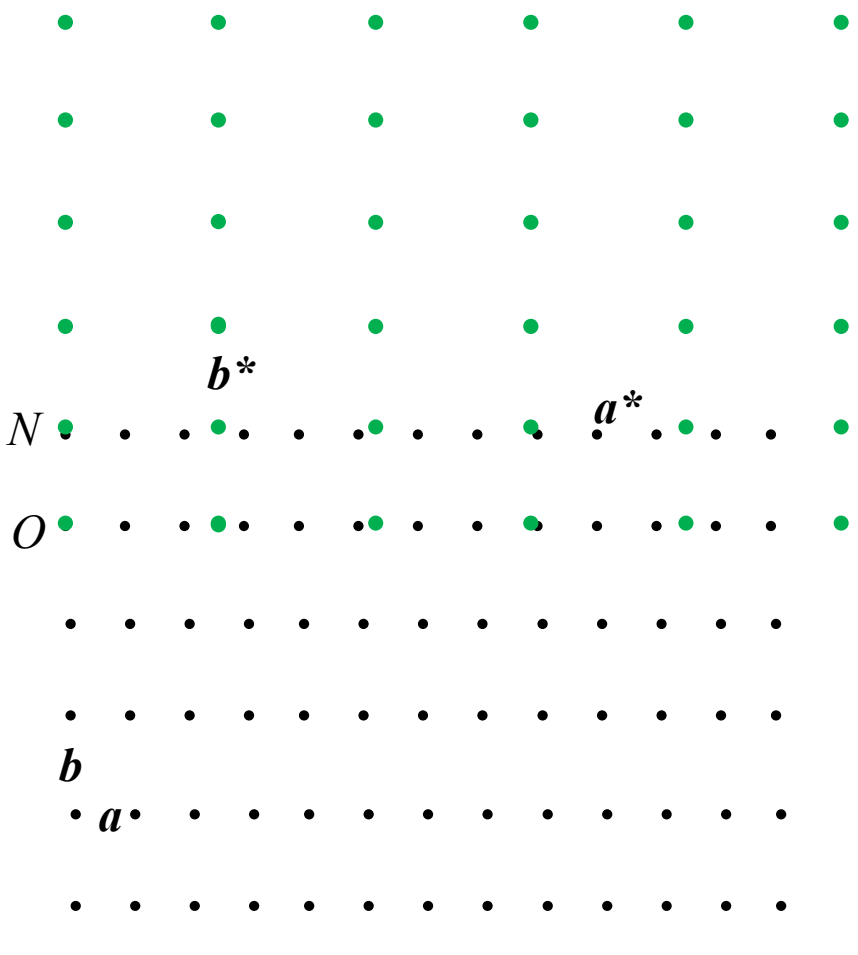
$a^* = 1/a$

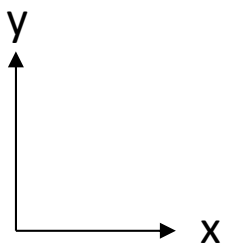
$b^* = 1/b$

# Geometrical construction of reciprocal lattice

**Direct lattice**

cell edge length
***a***, ***b***



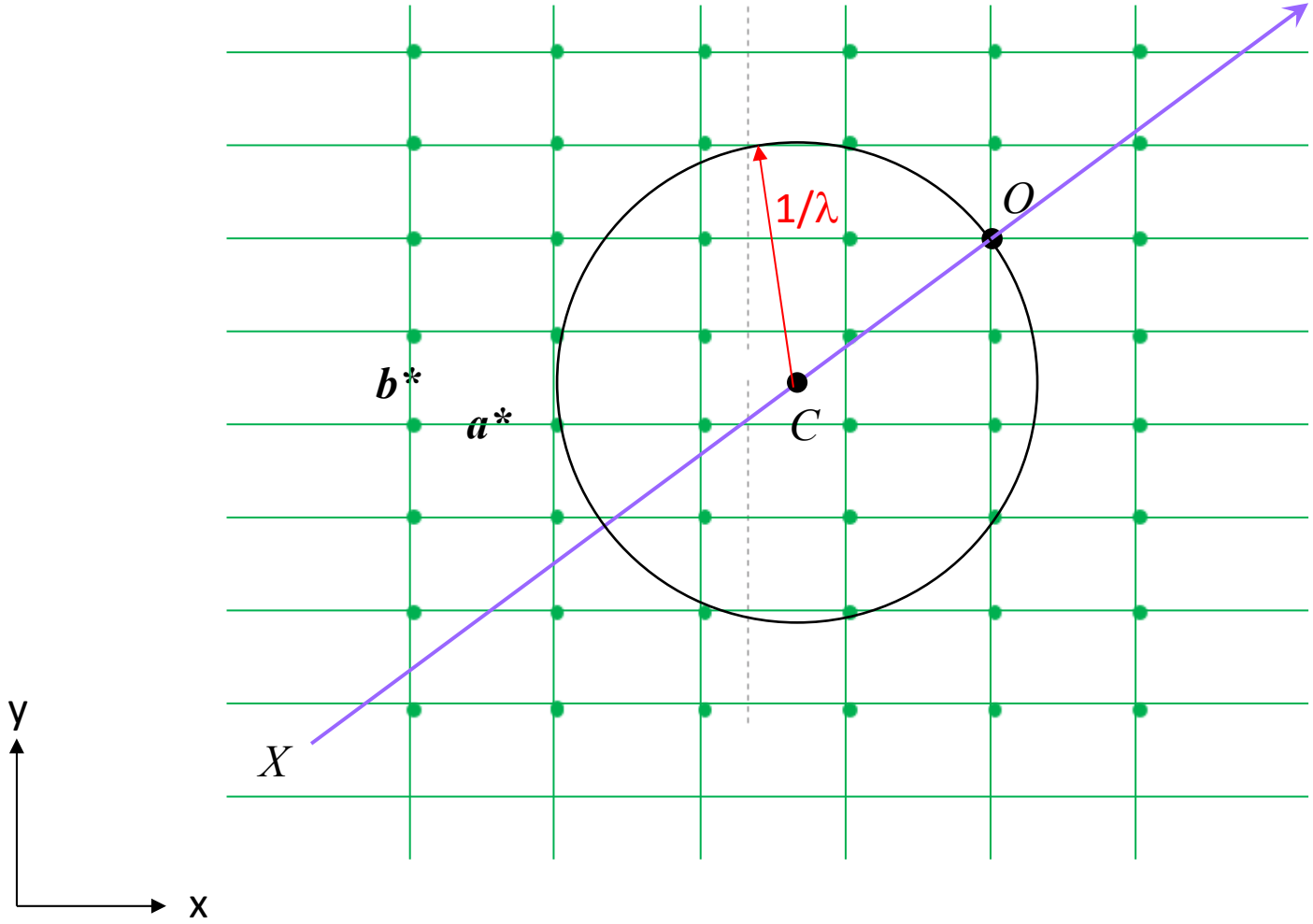

**Reciprocal lattice**

reciprocal lattice length, ***b**, a****

$a^* = 1/a$
$b^* = 1/b$

geometrically linked to the crystal
if crystal rotates, reciprocal lattice rotates also

# Bragg's Law in reciprocal space

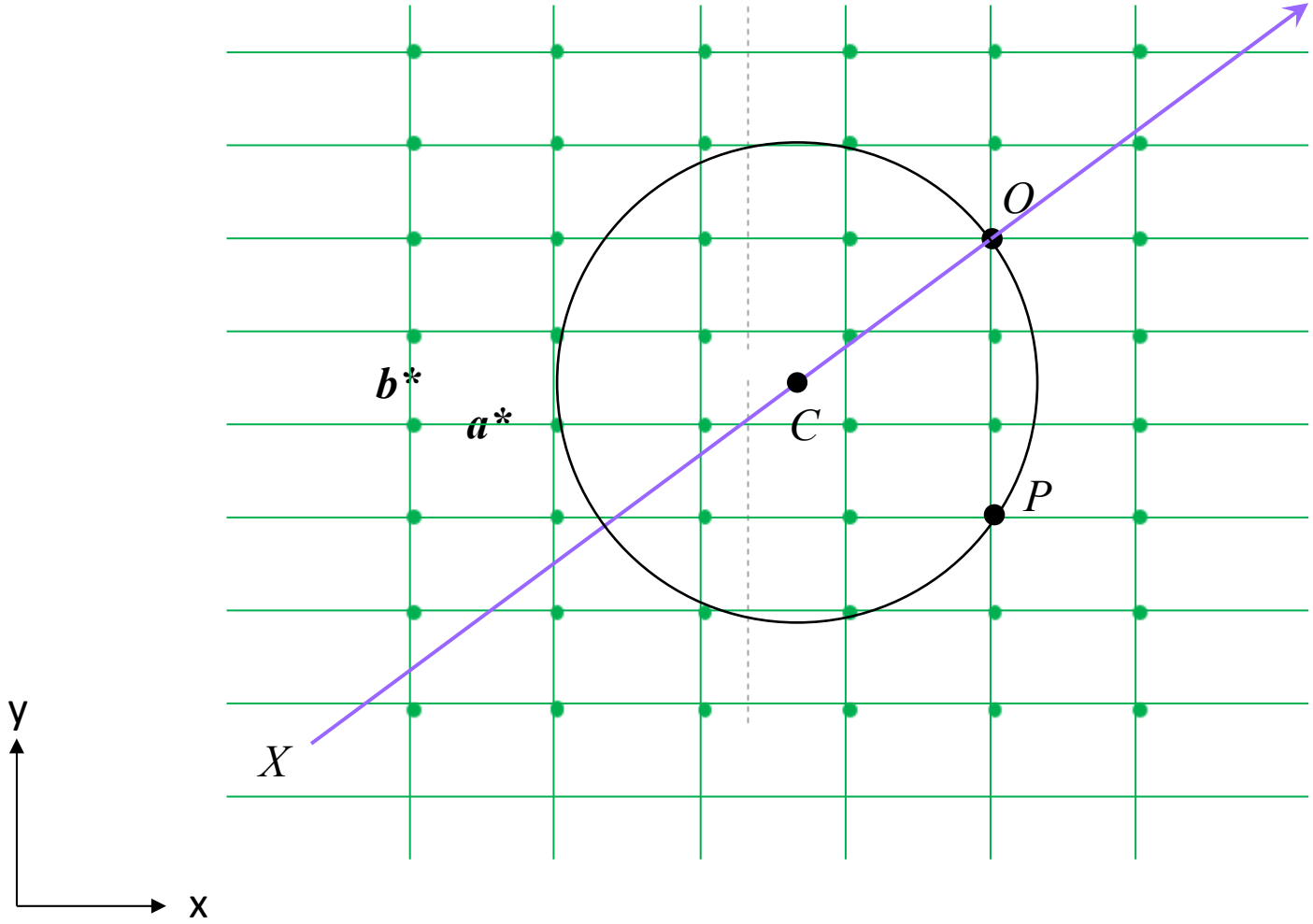



*O*: origin (both a point on the real and reciprocal lattice)

Beam of X-rays

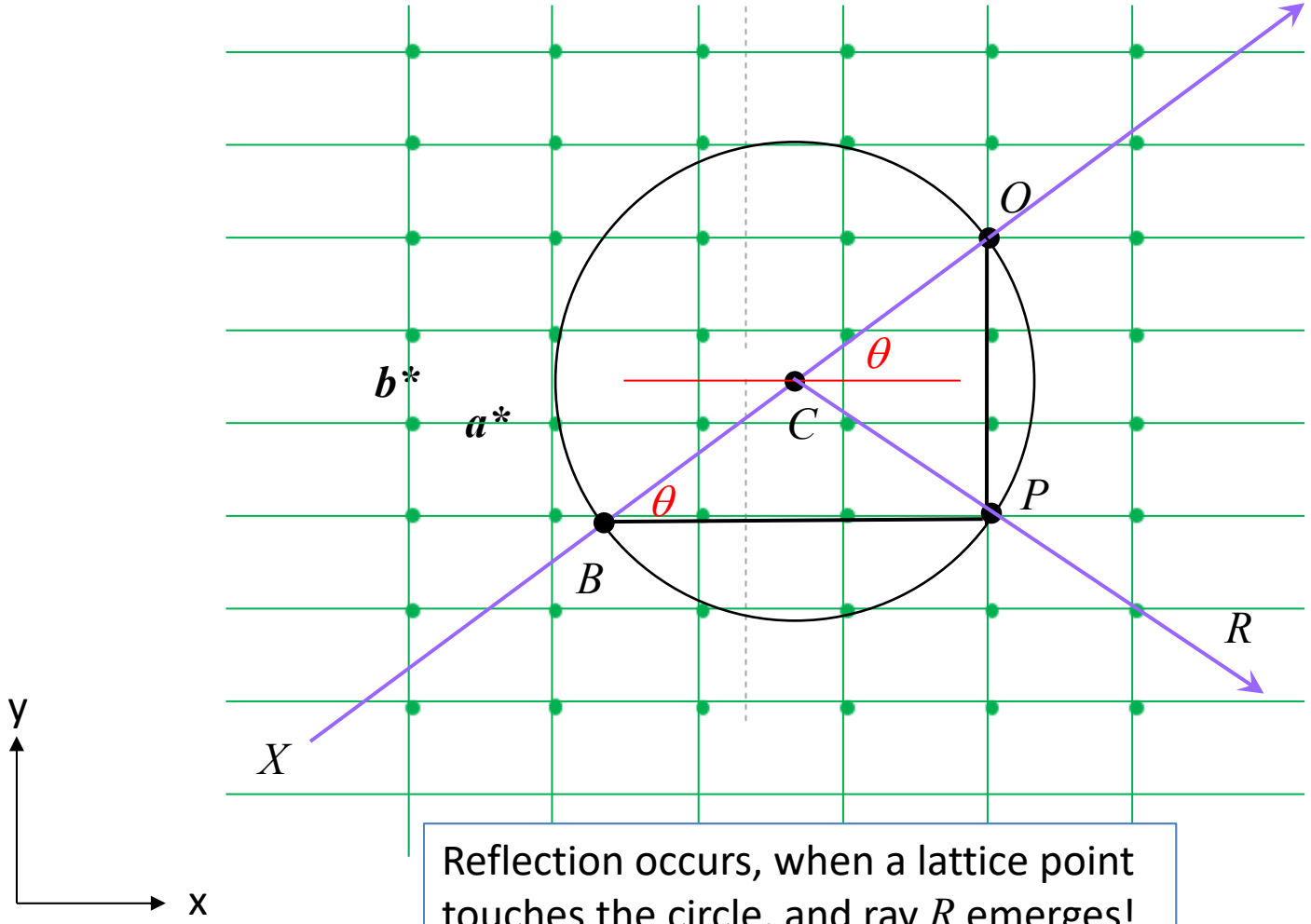
wavelength in reciprocal space: 1/λ

# Bragg's Law in reciprocal space




Lattice point *P* with indices *hkl* is in contact with circle

# Bragg's Law in reciprocal space




Reflection occurs, when a lattice point touches the circle, and ray $R$ emerges!

Bragg's Law

$$2d\sin\theta = n\lambda$$

$$\sin\theta = \frac{OP}{OB} = \frac{OP}{2/\lambda}$$

$$2\frac{1}{OP}\sin\theta = \lambda$$

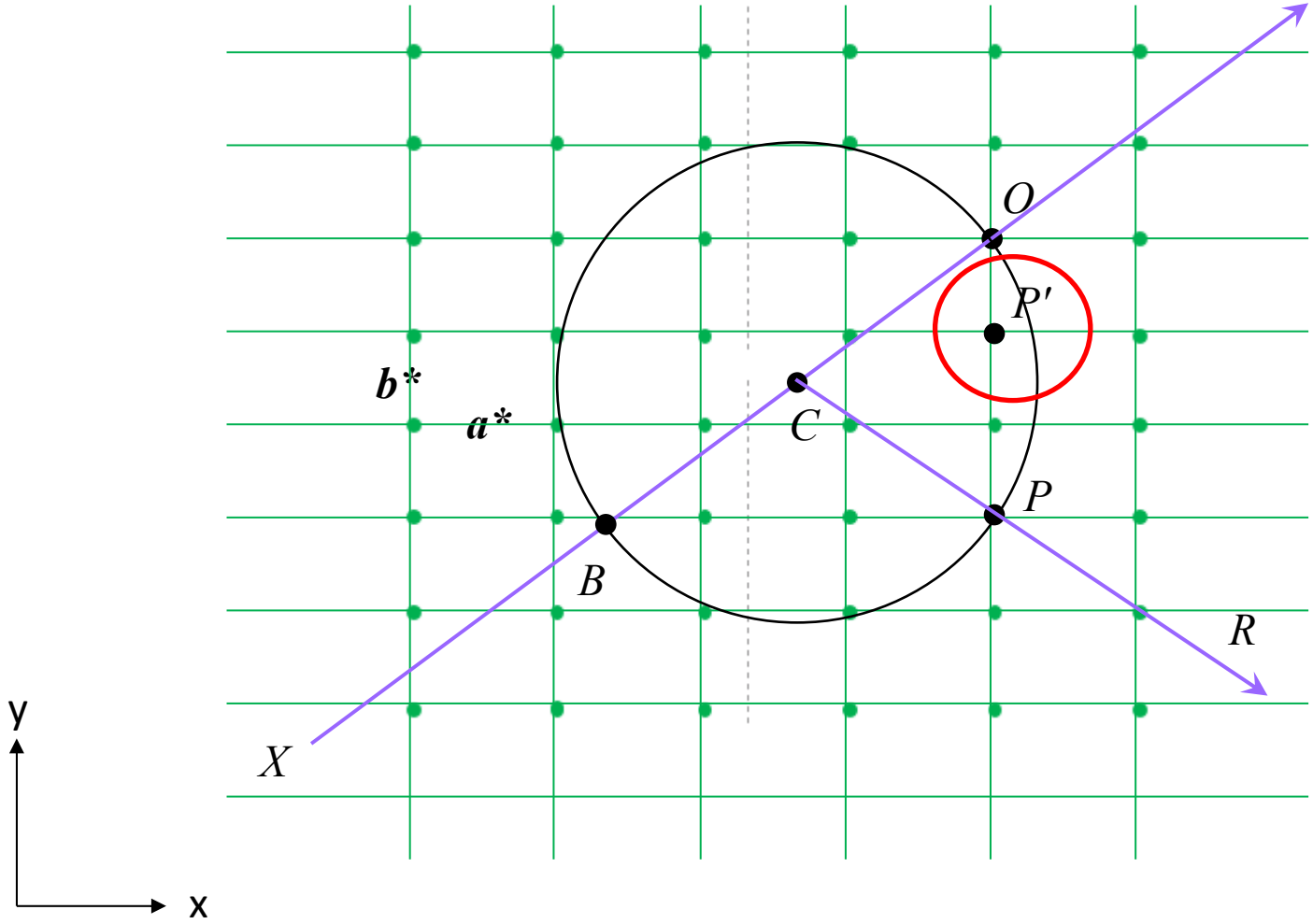
and, by definition

$$OP = \frac{1}{d_{hkl}}$$

because P is a point on the reciprocal lattice

$$2d_{hkl}\sin\theta = \lambda$$

# Bragg's Law in reciprocal space



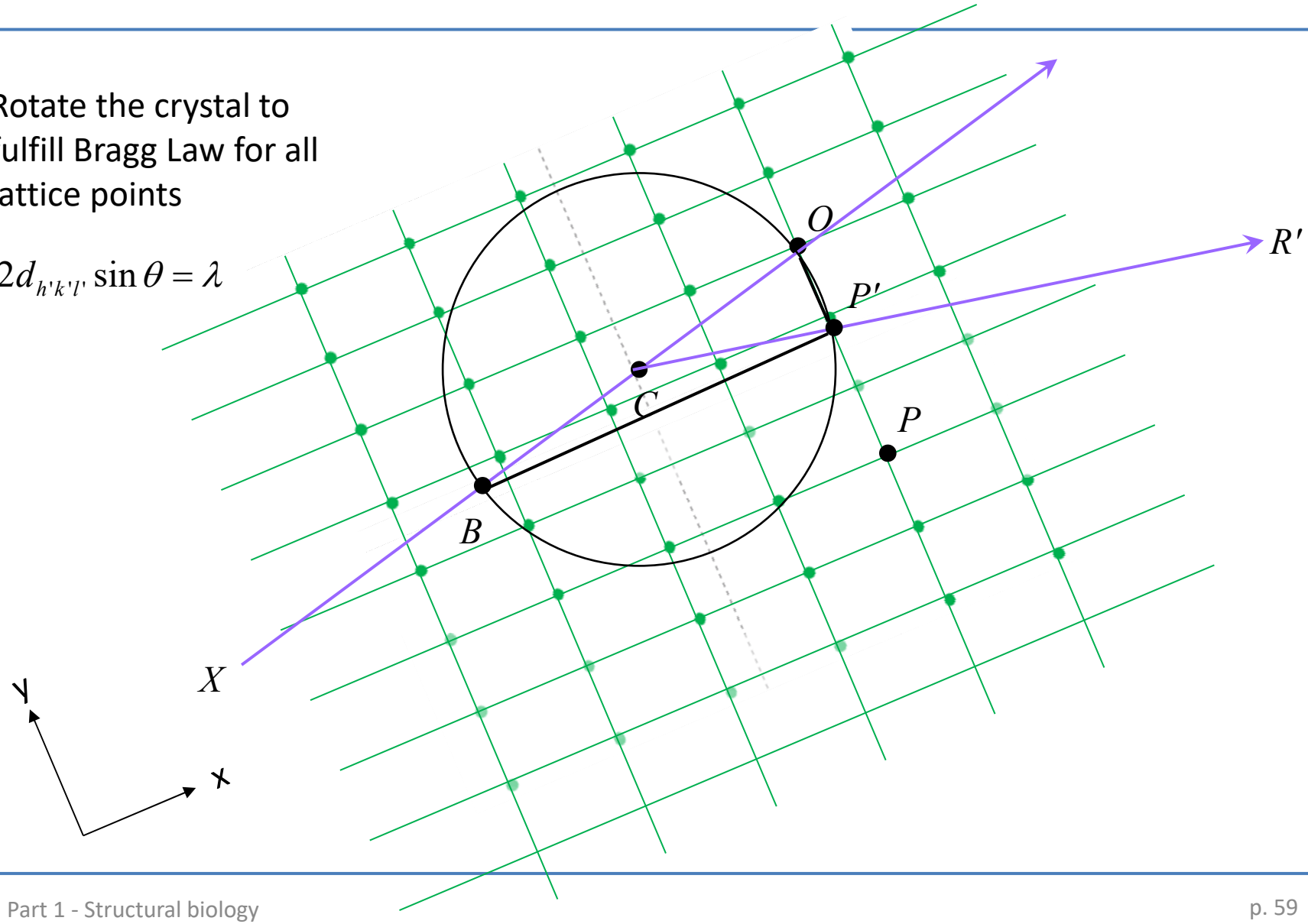

**Quiz:**

what about lattice point $P'$ with $h'k'l'$

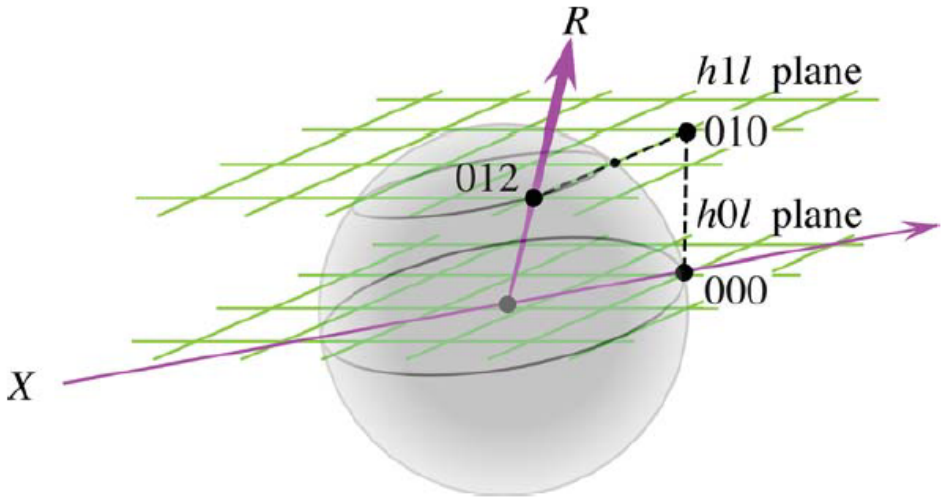
# Bragg's Law in reciprocal space

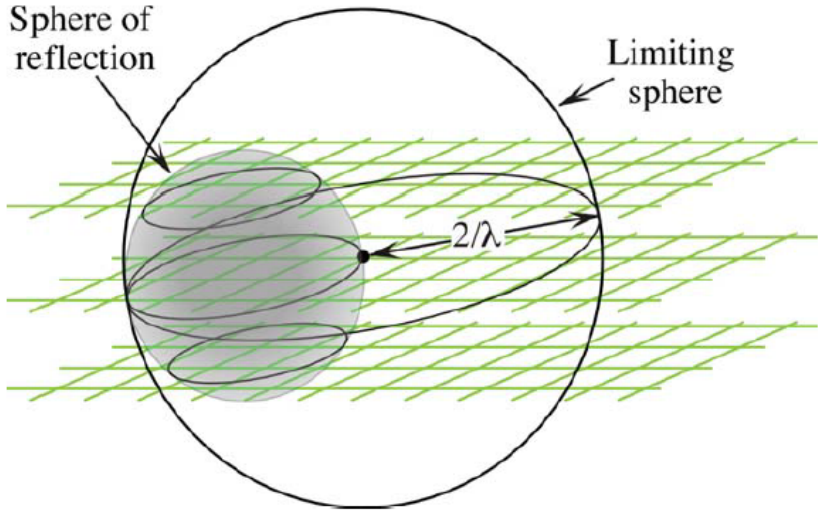
Rotate the crystal to fulfill Bragg Law for all lattice points

$$2d_{h'k'l'}\sin\theta = \lambda$$



# In 3D: Ewald sphere of reflection



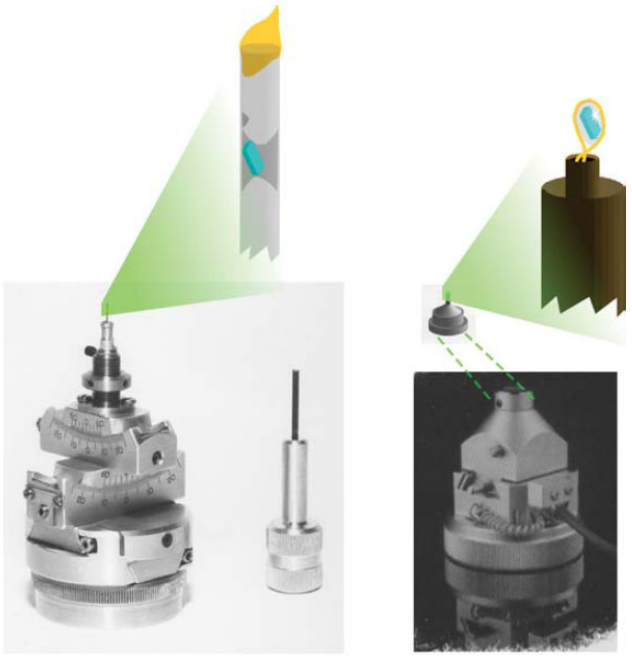

Sphere with radius 1/λ : A reflection is produced for all points in the reciprocal lattice when in contact with **Ewald sphere**.

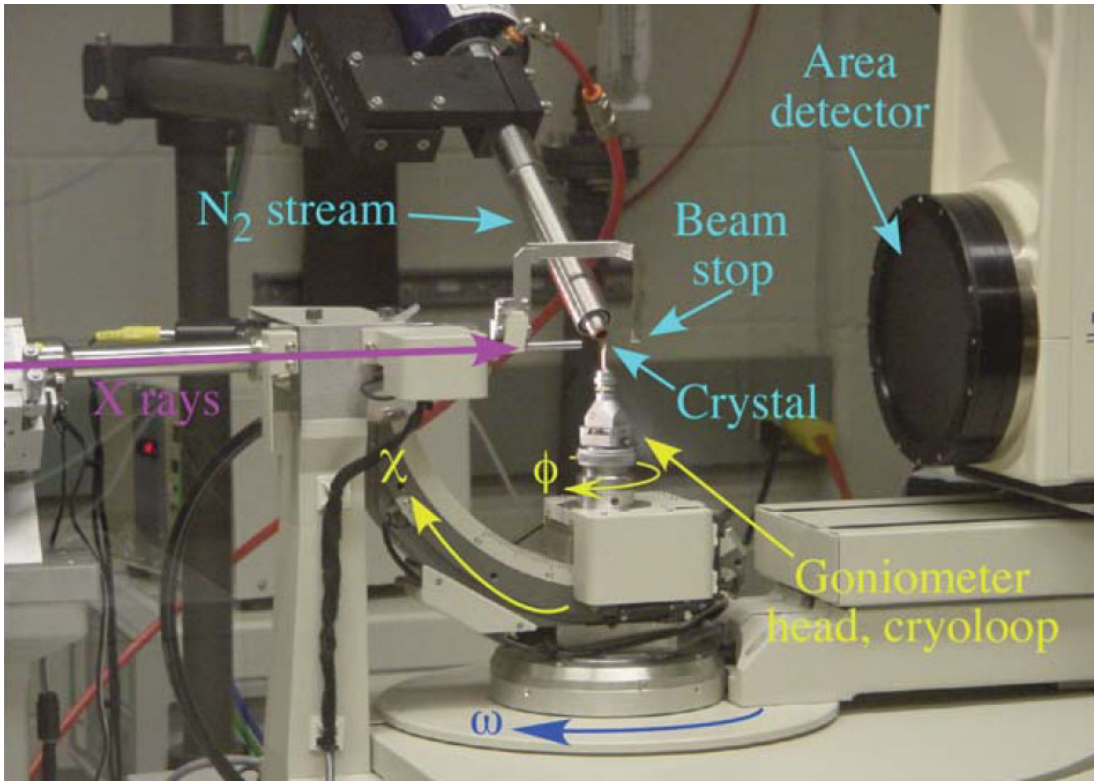
When crystal is rotated, all points eventually come into contact and the reflections are recorded

Maximal number of measurable reflections is determined by the limiting sphere and the unit cell geometry

$$N = \frac{(4\pi/3)\cdot(2/\lambda)^3}{V_{\text{recip}}}.$$

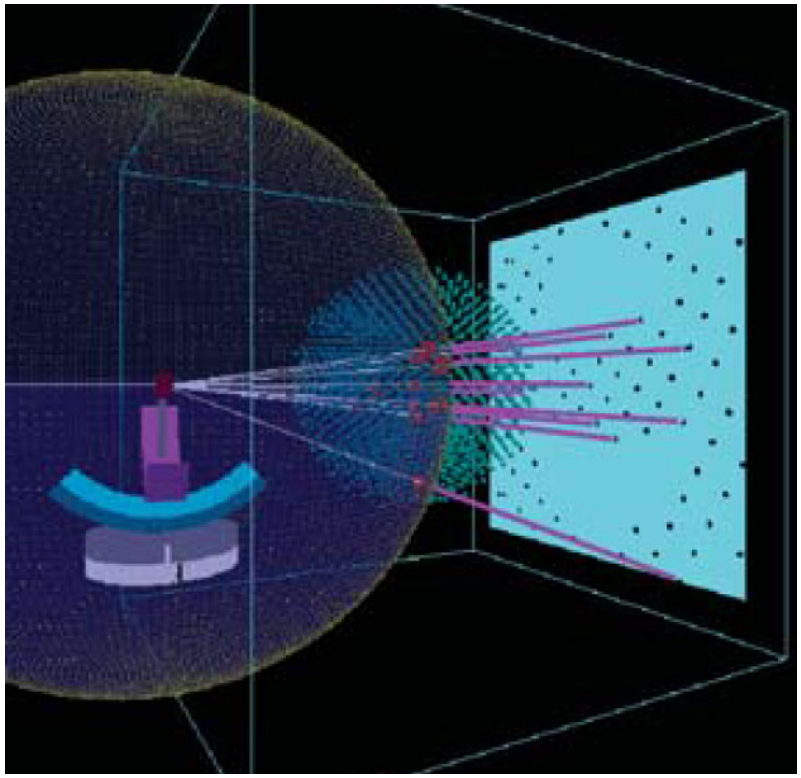
# Crystal mounting in a goniometer



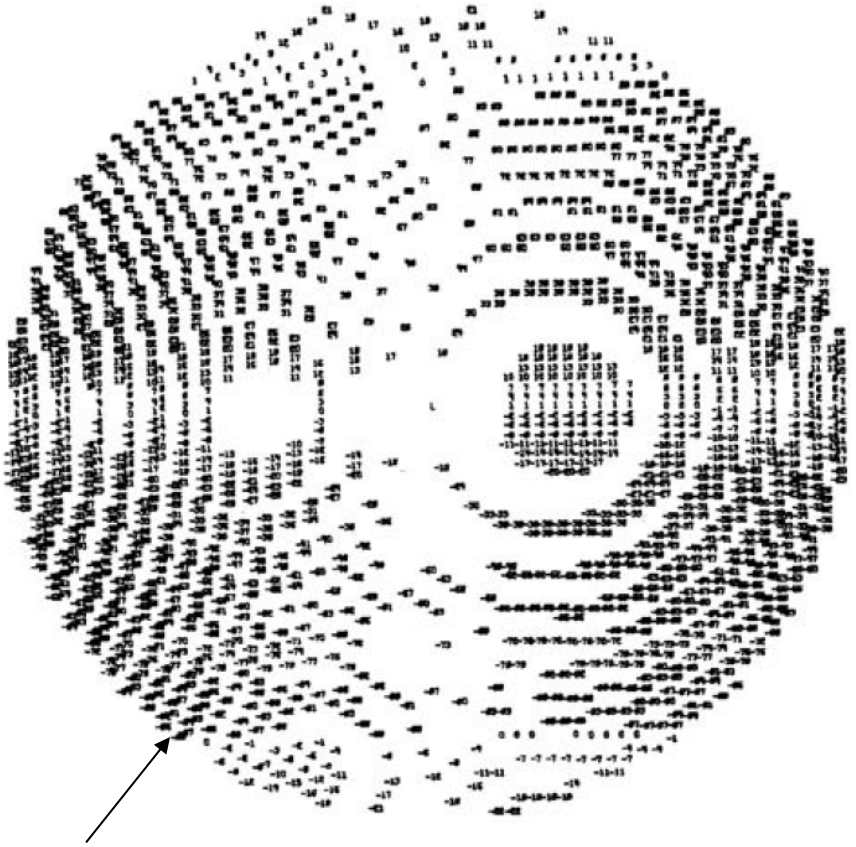



Mount for crystals in a goniometer with capillary or cryoloop

# Recoring reflections by rotation or oscillation of a crystal



Rotation by a few degrees brings many lattice points in contact with the Ewald sphere, produces reflections

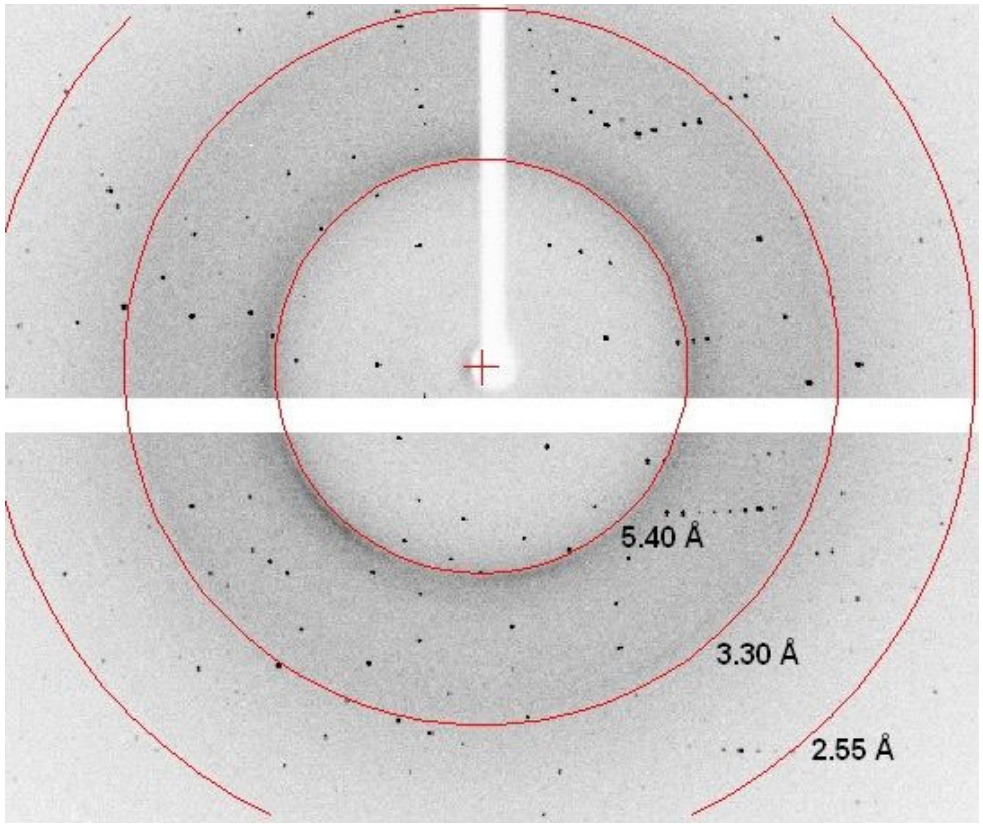


each reflection is indicated with its $hkl$ indices

# Movie to illustrate

- https://www.youtube.com/watch?v=cthNdOpWJbQ

# Resolution




The maximal resolution depends on the maximal angle under which reflections are still recorded

Diffraction image from a lysozyme crystal (0.1 mm x 0.1 mm x 0.2 mm)
(https://www.rigaku.com/en/products/protein/platemate)

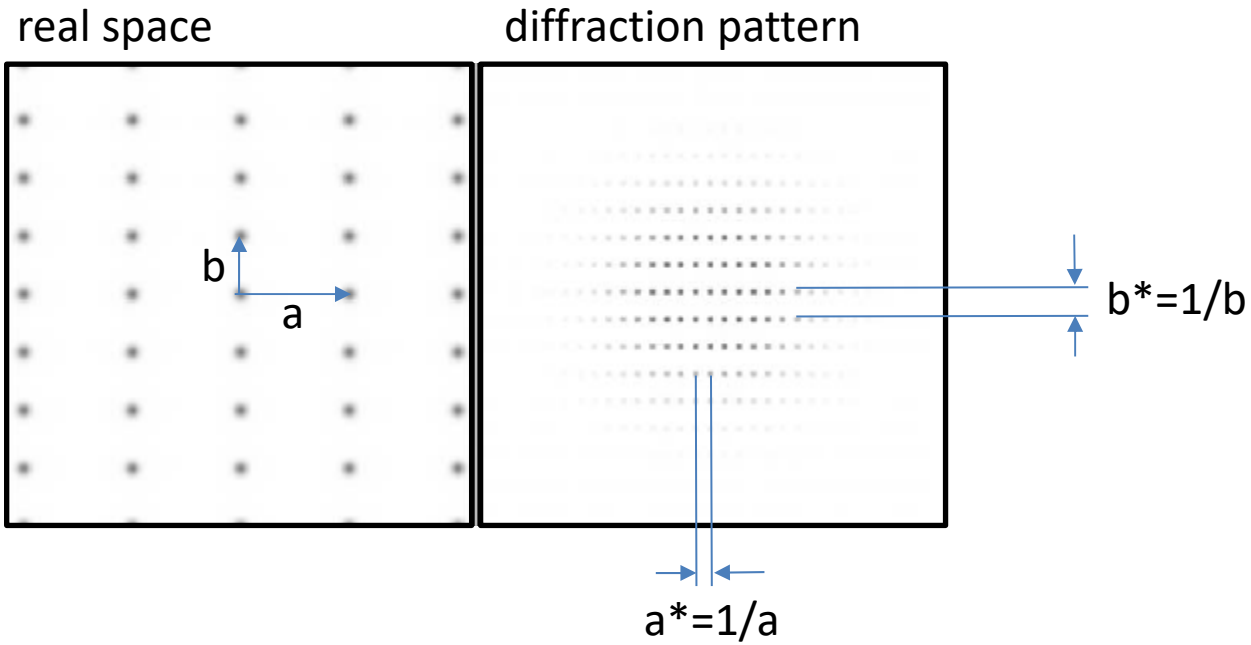
# Quiz

You have mounted a crystal of a protein at a distance of 135 mm from the detection screen.

X-rays from a copper anode ($\lambda$ = 1.54 Å) produce clearly discernable reflections giving at a distance of 81 mm from the detector center.

→ Estimate the achieved resolution

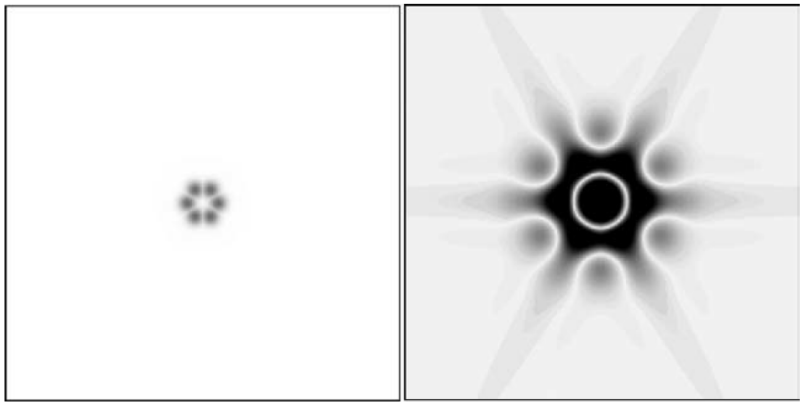
# The reciprocal lattice



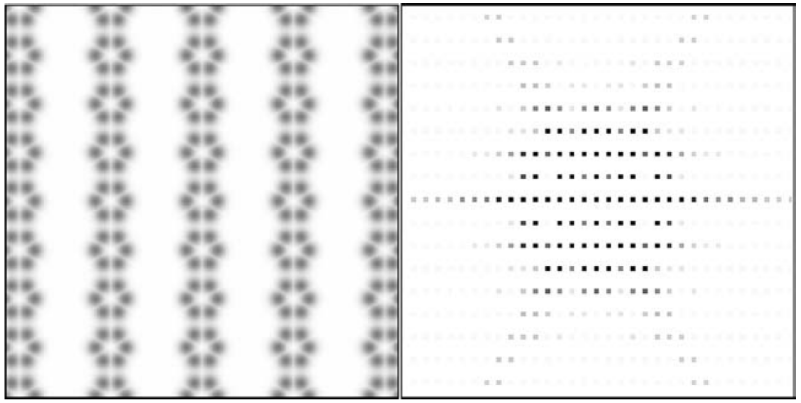

The real space and the diffraction pattern are related by **Fourier transformation**.

In an orthorombic crystal, distances in diffraction space are the inverse of distances in real space.

# Diffraction of a lattice containing complex objects



diffraction pattern (FT)
of a hexagon

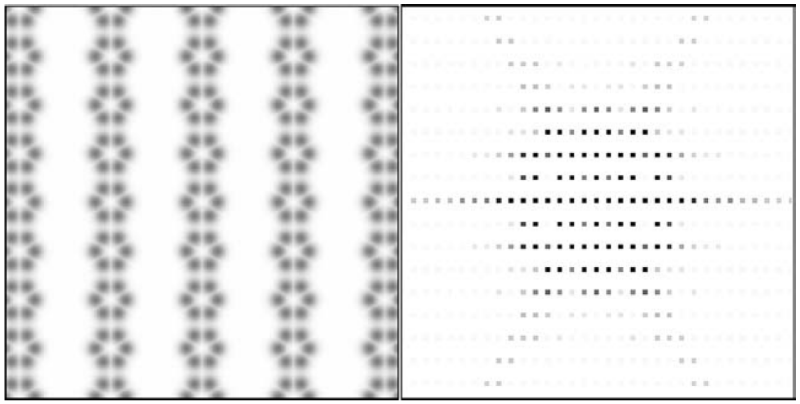


diffraction pattern (FT) of
"crystal" of hexagons

The spacing of the reflections
arises from the crystal form

the intensities of the reflections
is determined by the structure
of the "molecule"

# The molecular scattering factor



$$E(x,t) = \left|E_0\right| e^{2\pi i(\mathrm{v/t - x/\lambda})}\, \mathrm{e}^{2\pi \mathrm{i}\phi}$$

For each atom-type, a scattering efficiency dependent on the electron number is defined: the **atomic scattering factor** $f$

The **structure factor**, or molecular scattering factor **F** is then a function of all

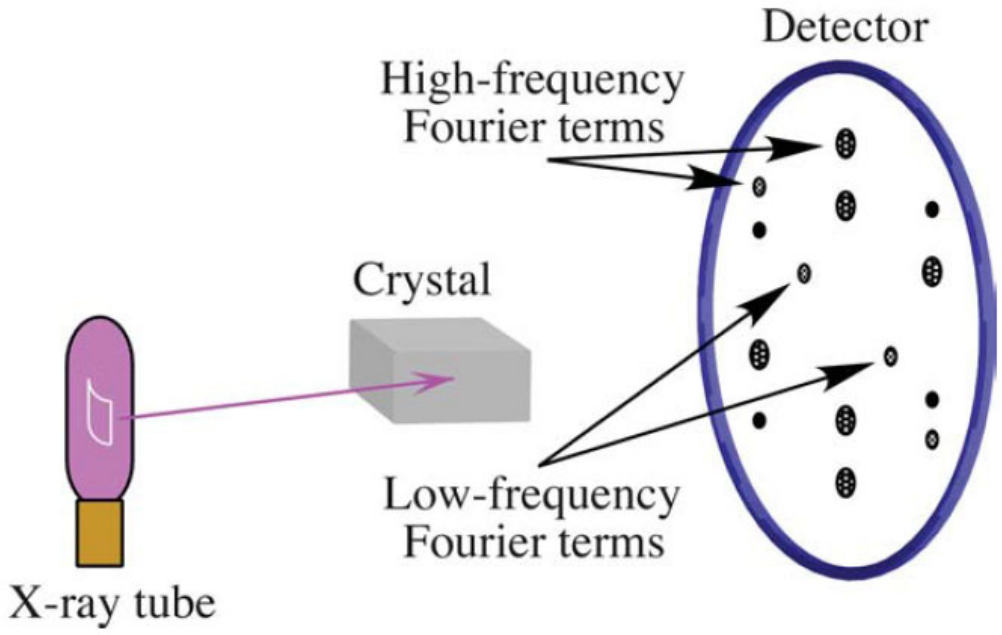
$$\mathbf{F}(hkl) = \sum_{j=1}^{N} f_j e^{2\pi i \mathbf{S}\mathbf{r}_j}$$

Or, using a continous electron density:

$$\mathbf{F}(hkl) = \int\int\int V\,\rho(\mathbf{r})\,\mathrm{e}^{2\pi i \mathbf{S}\mathbf{r}}\,dxdydz$$

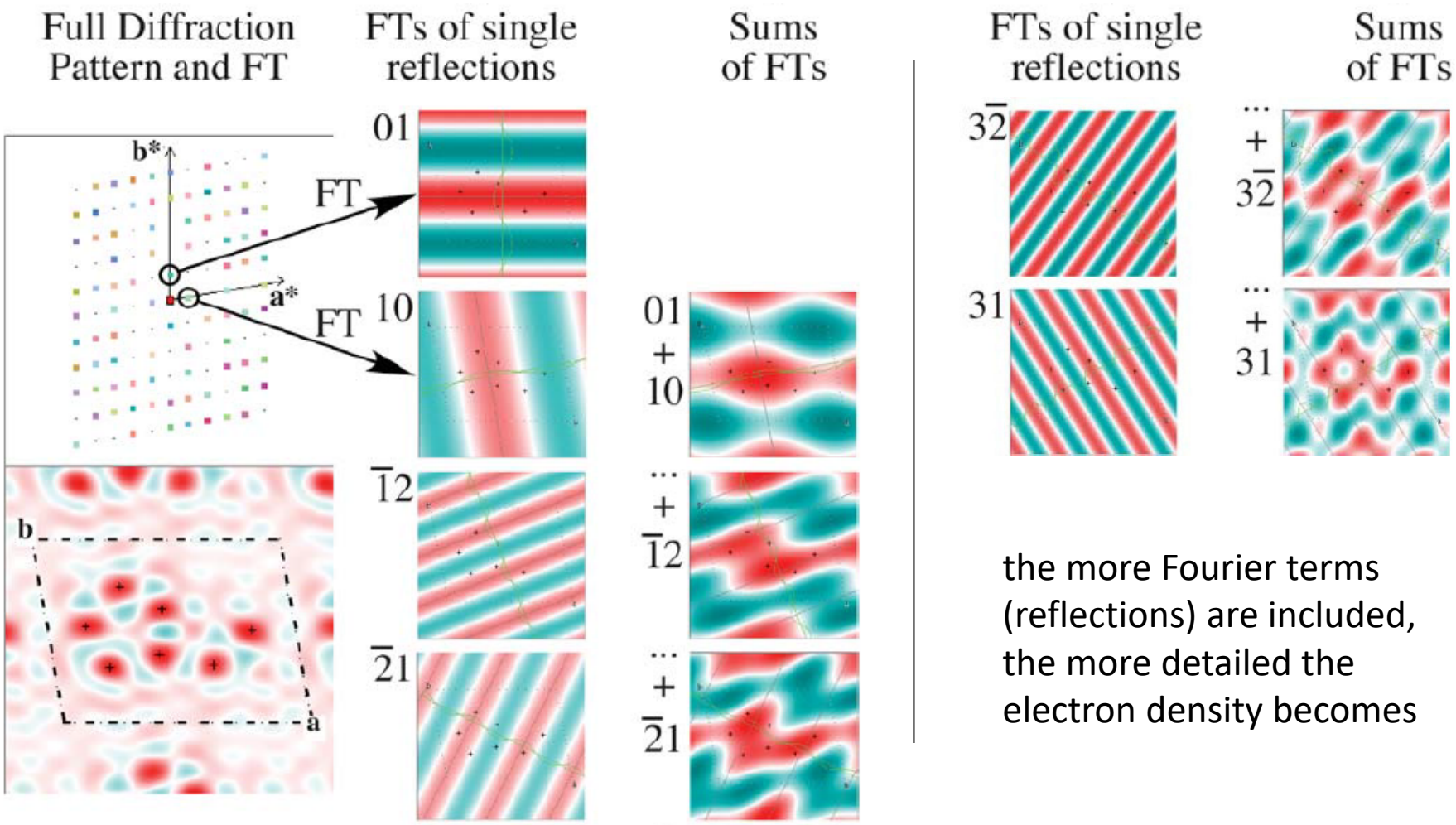
This form ncorresponds to **a <u>Fourier transform</u> of the electron density**

# Each reflection is a Fourier term, defining the structure




Reflections at high angle (high *hkl* values) are high frequency Fourier terms, containing detailed information

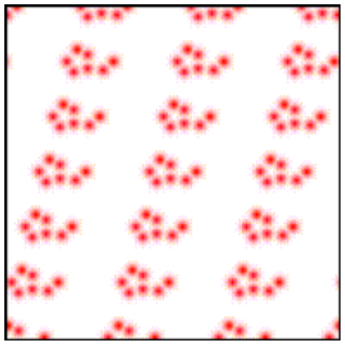
# Meaning of structure factor Fourier terms

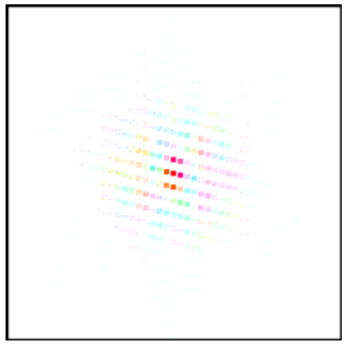



the more Fourier terms (reflections) are included, the more detailed the electron density becomes

# How to calculate structure from diffraction pattern?

crystal
and its FT





convolution of the molecule and the lattice

Back-calculation of the molecular structure:

$$\rho(\mathbf{r}) = \frac{1}{V}\int dV e^{-2\pi i \mathbf{S}\mathbf{r}}\mathbf{F}(\mathbf{S})$$

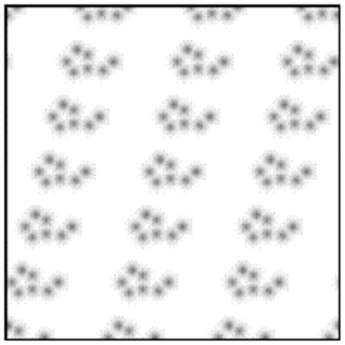
inverted Fourier transform yields electron density

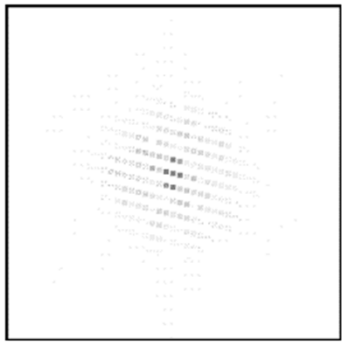
$$\rho(\mathbf{r}) = \frac{1}{NV}\sum_h\sum_k\sum_l \mathbf{F}(\mathbf{S})e^{-2\pi i \mathbf{S}\mathbf{r}}$$

discrete spots (Miller indices)

# How to calculate structure from diffraction pattern?

crystal
and its FT





convolution of the molecule and the lattice

**observed:**
$I(\mathbf{S}) = |\mathbf{F}(\mathbf{S})|^2$

Back-calculation of the molecular structure:

$$\rho(\mathbf{r}) = \frac{1}{V}\int dV e^{-2\pi i \mathbf{S}\mathbf{r}}\mathbf{F}(\mathbf{S})$$

inverted Fourier transform yields electron density

$$\rho(\mathbf{r}) = \frac{1}{NV}\sum_h\sum_k\sum_l \mathbf{F}(\mathbf{S})e^{-2\pi i \mathbf{S}\mathbf{r}}$$

discrete spots (Miller indices)

$$\rho(\mathbf{r}) = \frac{1}{NV}\sum_h\sum_k\sum_l |\mathbf{F}(\mathbf{S})| e^{-2\pi i \mathbf{S}\mathbf{r}} \cdot e^{-i\alpha_{hkl}}$$

phase information factured out

cannot be measured

# Information in the Phases vs. Intensities

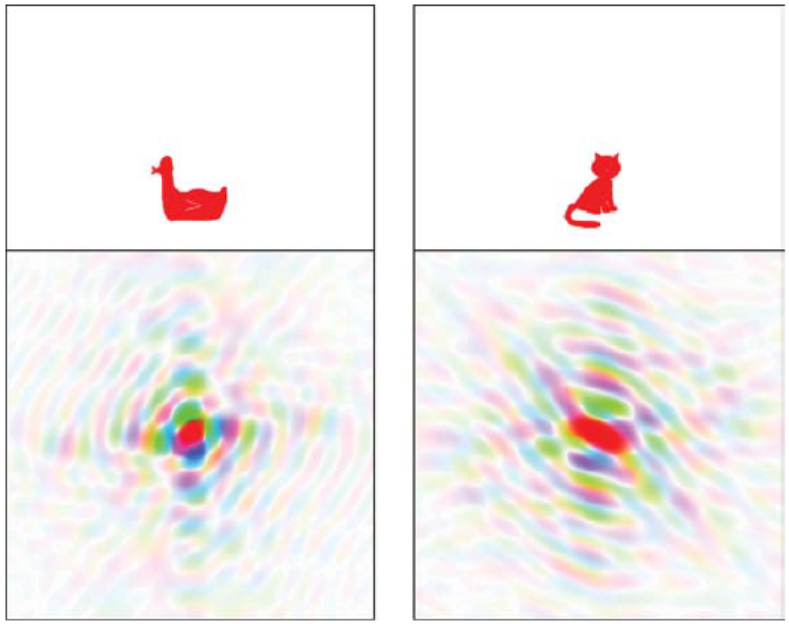
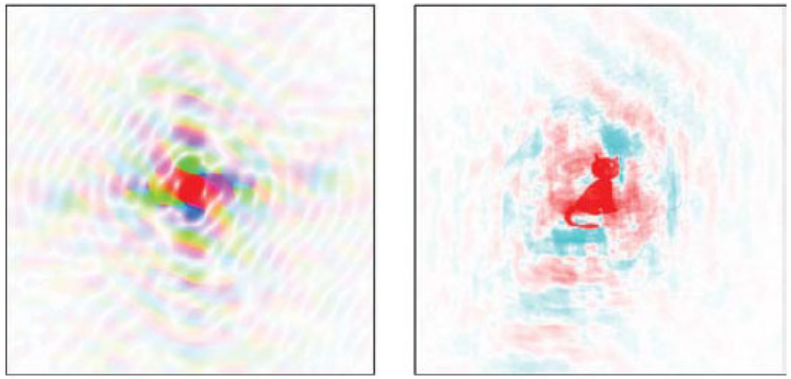


**image with corresponding diffraction images**

phase information color-coded

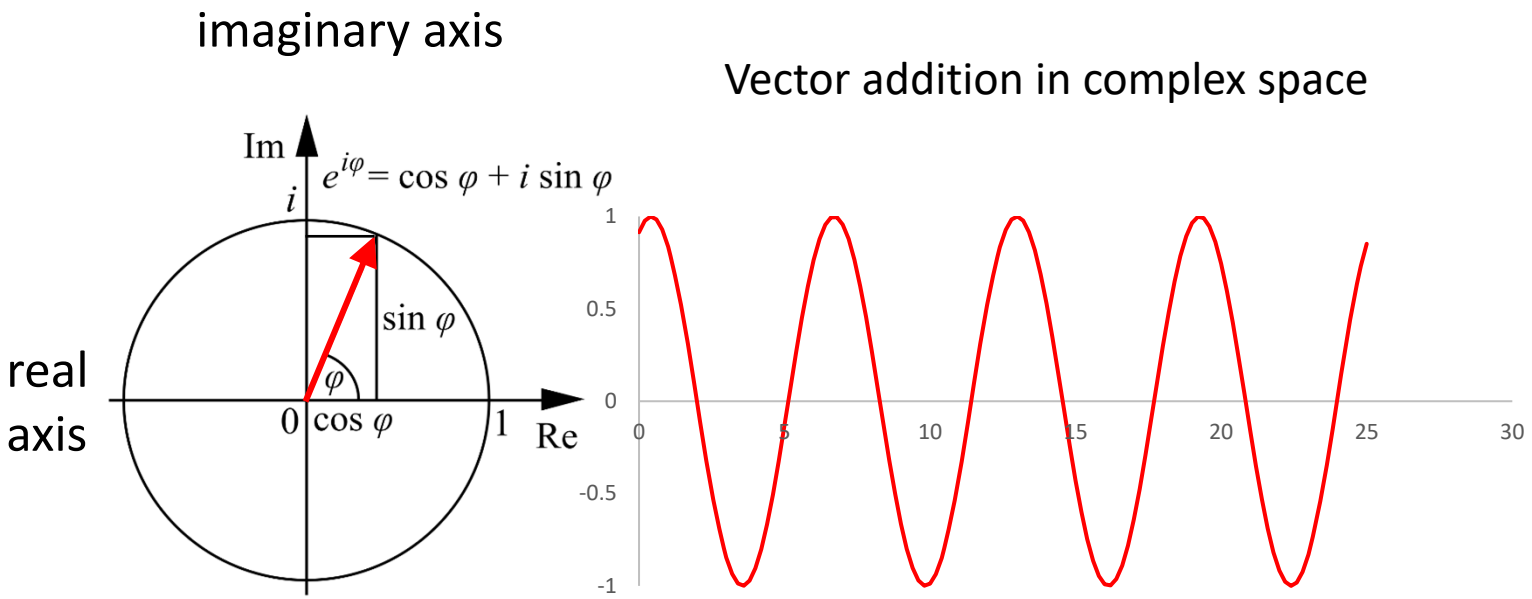
**diffraction pattern** **backtransform**



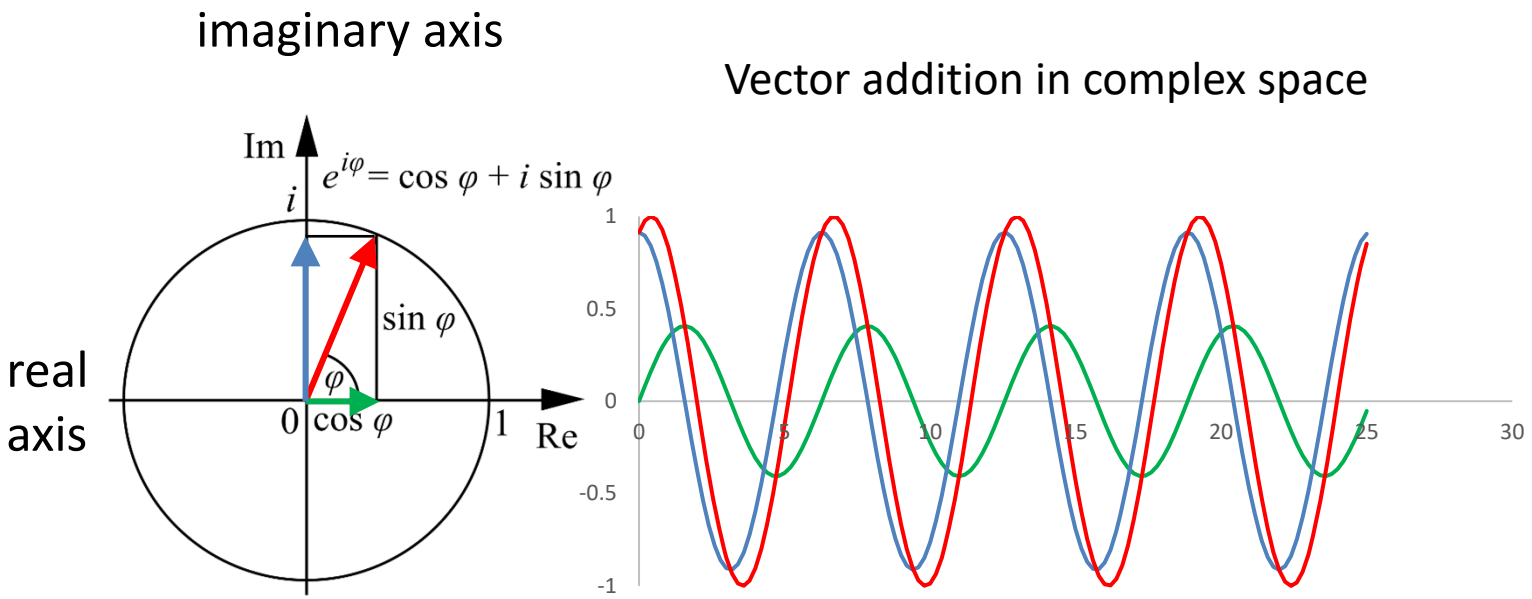
intensities: duck

phases: cat

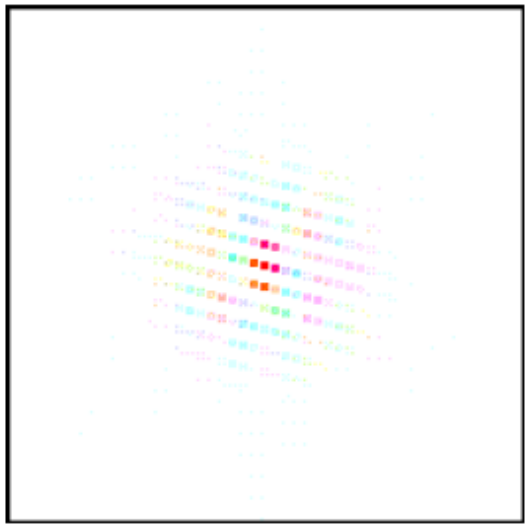
# Structure factors as complex vectors



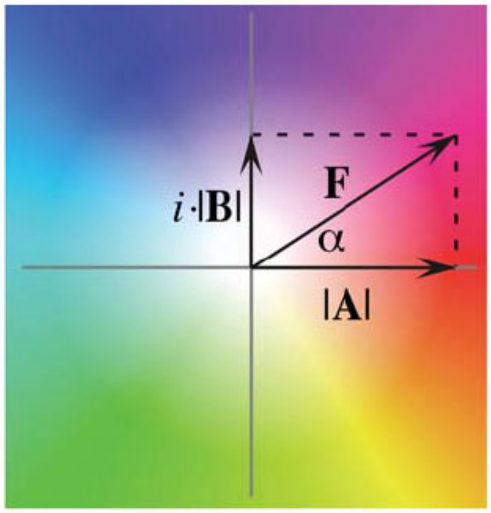
# Structure factors as complex vectors



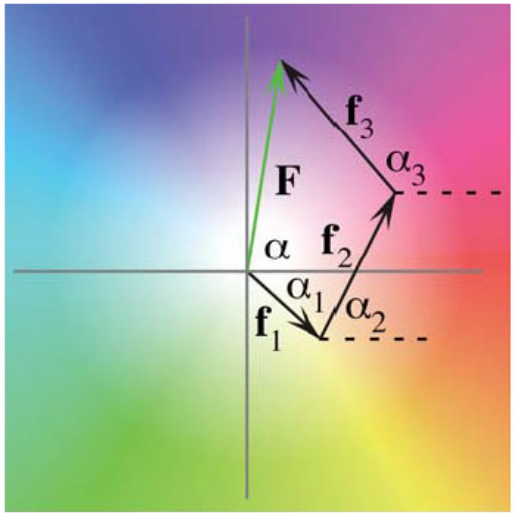
# The diffractive contributions of each atom to the structure factor F



Diffraction pattern:
Color indicates phase



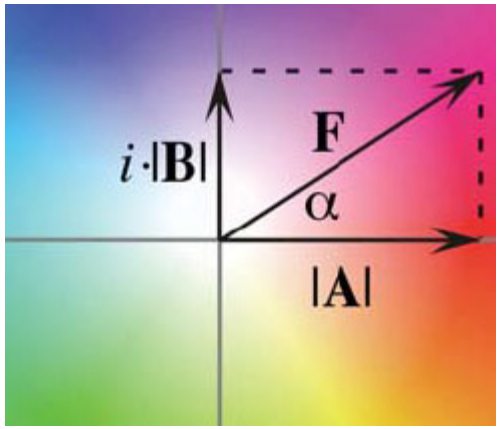

each structure factor is a complex vector




the molecular structure factor **F** is the sum of atomic structure factors **$f_1$, $f_2$, $f_3$,** ....

sum of atomic structure factors

# Connection to electron density




$$\cos\alpha = \frac{|\mathbf{A}|}{|\mathbf{F}|} \qquad \sin\alpha = \frac{|\mathbf{B}|}{|\mathbf{F}|}$$

$$\mathbf{F} = |\mathbf{A}| + i|\mathbf{B}| = |\mathbf{F}| \cdot (\cos\alpha + i\sin\alpha)$$

with Euler's formula

$$\mathbf{F} = |\mathbf{F}| \cdot e^{i\alpha}$$

$$\rho(x,y,z) = \frac{1}{V}\sum_h\sum_k\sum_l \mathbf{F}_{hlk} e^{-2\pi i(hx+ky+lz)}$$

$$\rho(x,y,z) = \frac{1}{V}\sum_h\sum_k\sum_l |\mathbf{F}_{hkl}| e^{-2\pi i(hx+ky+lz)} \cdot e^{-i\alpha_{hkl}}$$

substitute $\alpha_{hkl} = 2\pi\alpha'_{hlk}$

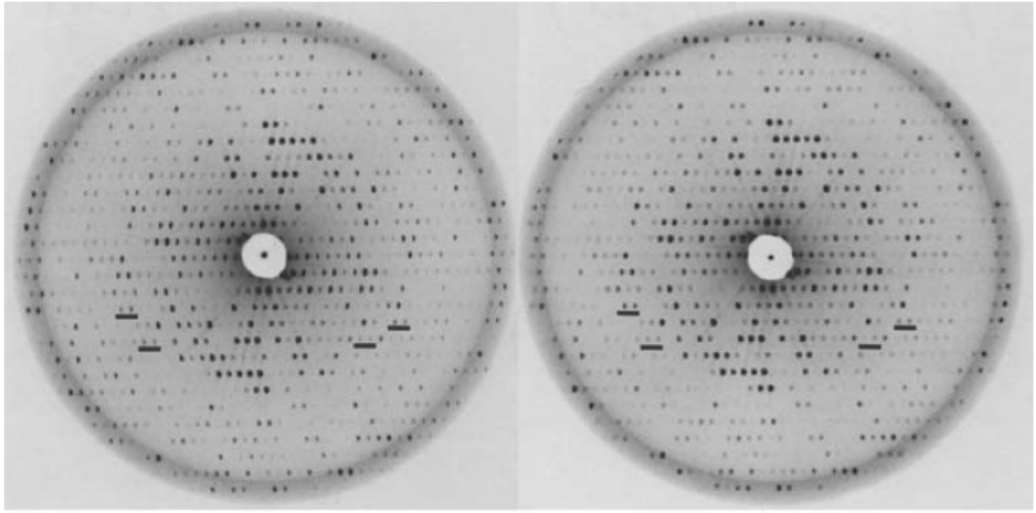
Solution by vector operation

$$\rho(x,y,z) = \frac{1}{V}\sum_h\sum_k\sum_l |\mathbf{F}_{hkl}| e^{-2\pi i(hx+ky+lz-\alpha'_{hkl})}$$

# Approaches to solve the phase problem

## Isomorphous replacement

- produce derivative crystal incorporating heavy metals
- those produce clearly identifiable diffraction spots
- using **Patterson method** the positions of the heavy metals is identified, phases calculated
- used to iteratively determine phases



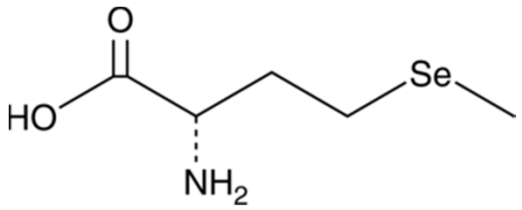
wt heavy atom

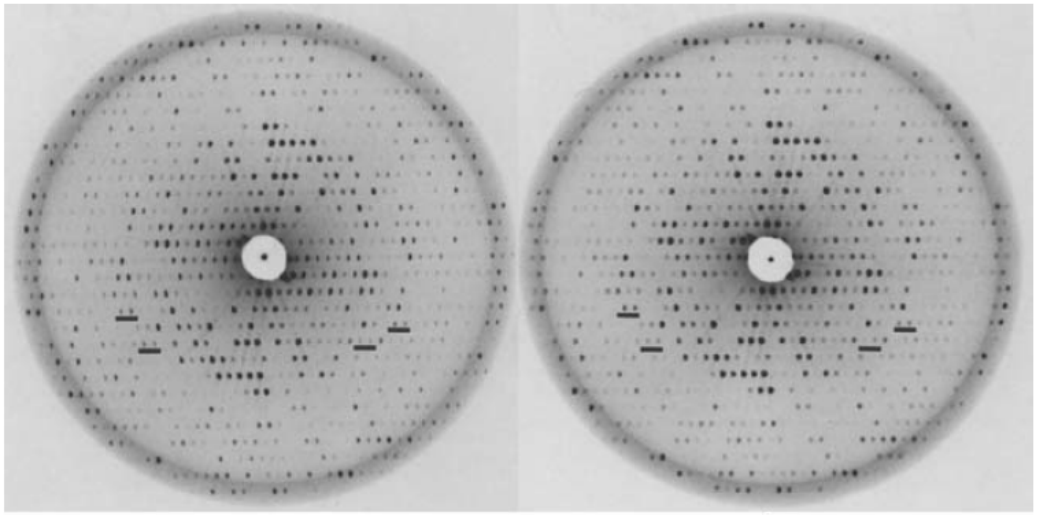
# Methods to obtain derivatives

## Soak crystal in heavy metal solutions

- $Hg^{2+}$ ions bind to thiol groups.
- Uranyl salts ($UO_2$ + $NO_3$) bind between carboxyl groups in Asp and Glu
- Pb bind to Cys
- $PtCl_4^{2-}$ (ion) bind to His

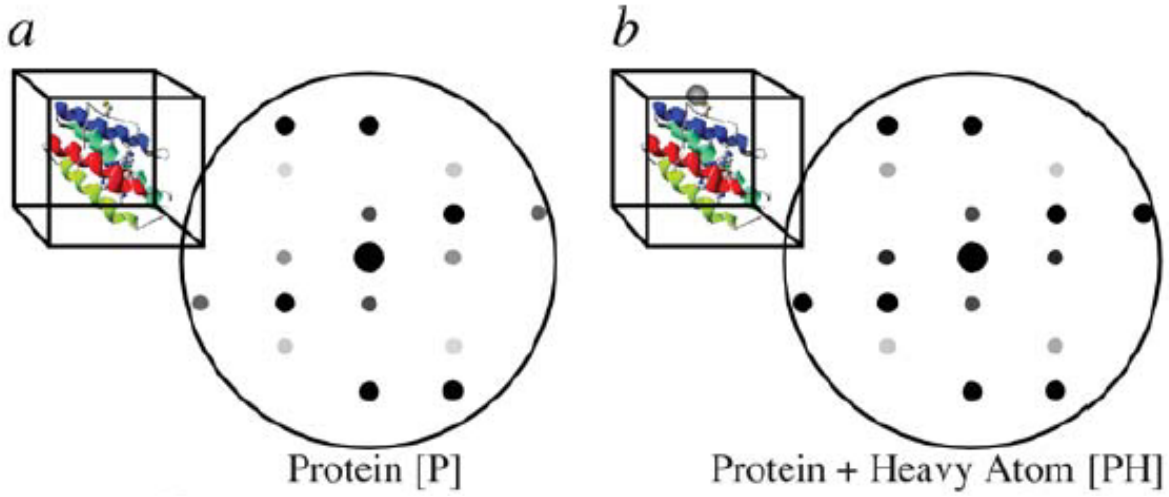
## Express protein with selenomethionine



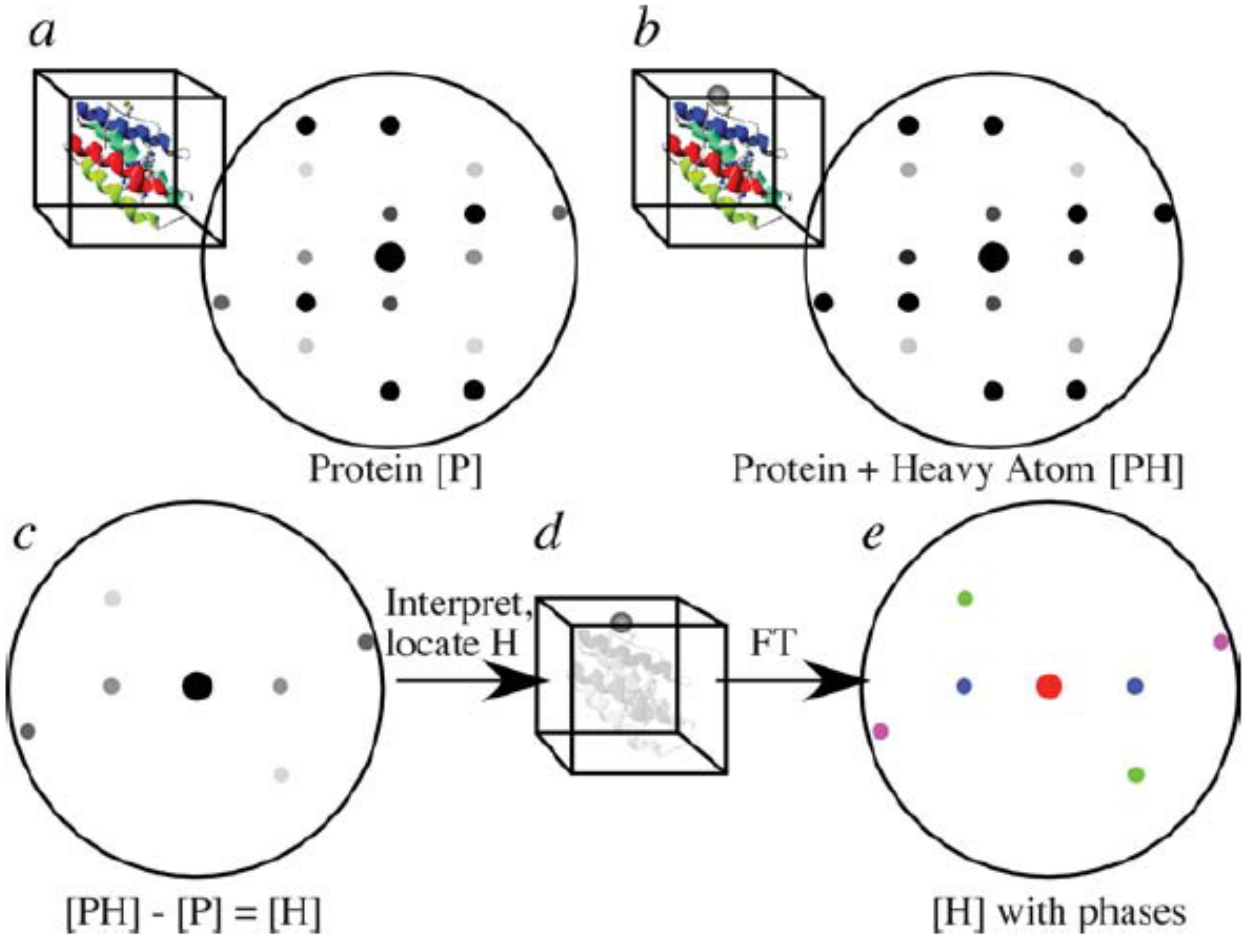



wt heavy atom

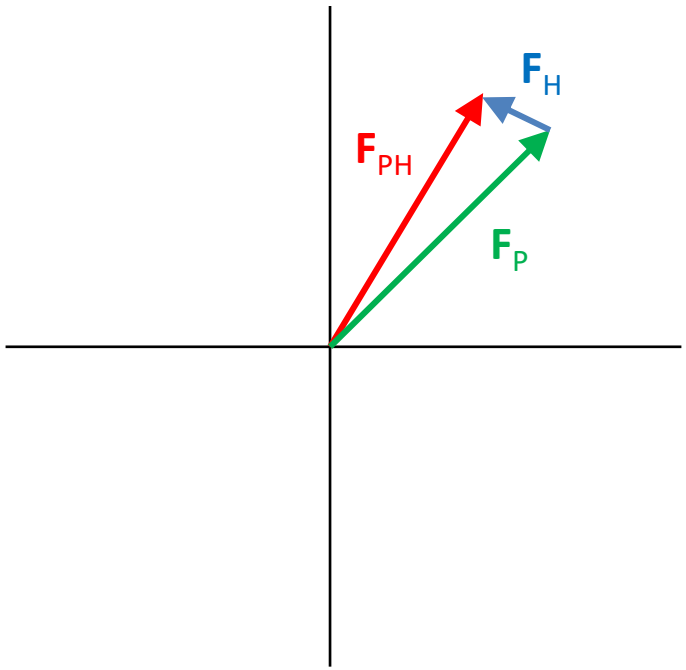
# Isomorphous replacement: Strategy



# Isomorphous replacement: Strategy



# Obtaining the phases by isomorphous replacement




Consider a reflection measured in the protein (P) and the heavy metal derivative (PH).

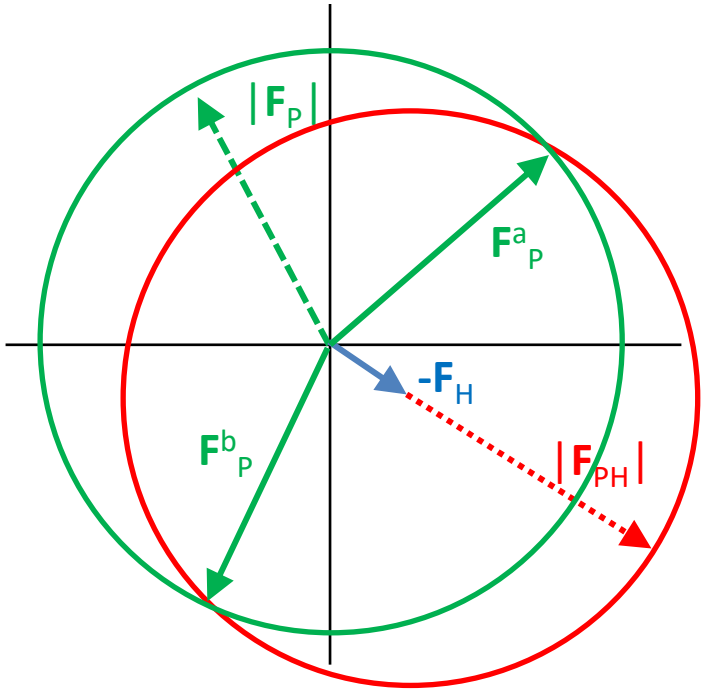
$$\mathbf{F}_{PH} = \mathbf{F}_H + \mathbf{F}_P$$

$$\mathbf{F}_P = \mathbf{F}_{PH} - \mathbf{F}_H$$

$|\mathbf{F}_{PH}|$, $|\mathbf{F}_P|$ — known from measurements (intensities)

$\mathbf{F}_H$ can be determined (incl. phase)
→ we will discuss this in a minute

# Solving the phase problem by vector addition




$$\mathbf{F}_P = \mathbf{F}_{PH} - \mathbf{F}_H$$

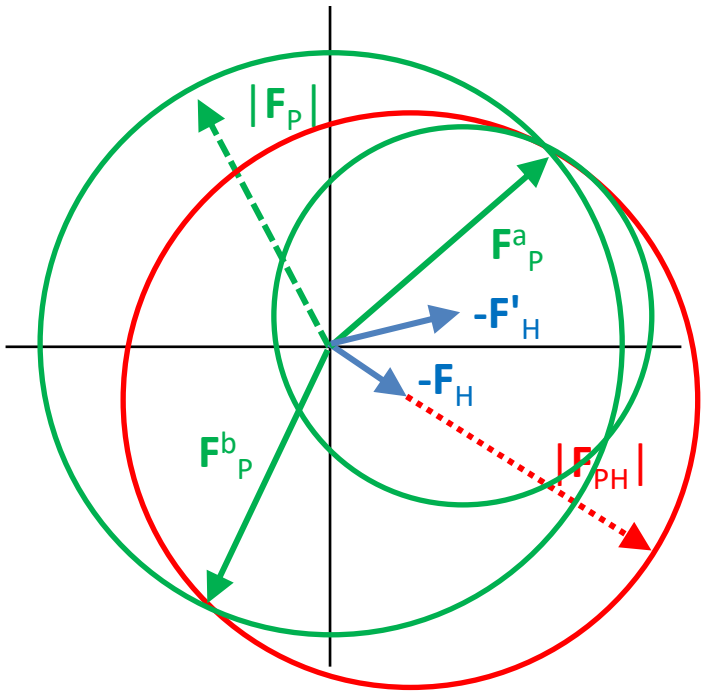
draw a circle at tip $\mathbf{F}_H$
with radius $|\mathbf{F}_{PH}|$

of all points on this circle satisfy the equation above

draw a circle around origin with length $|\mathbf{F}_P|$

Intersection points: one of two **Solutions**

# How to discriminate between solutions a and b?

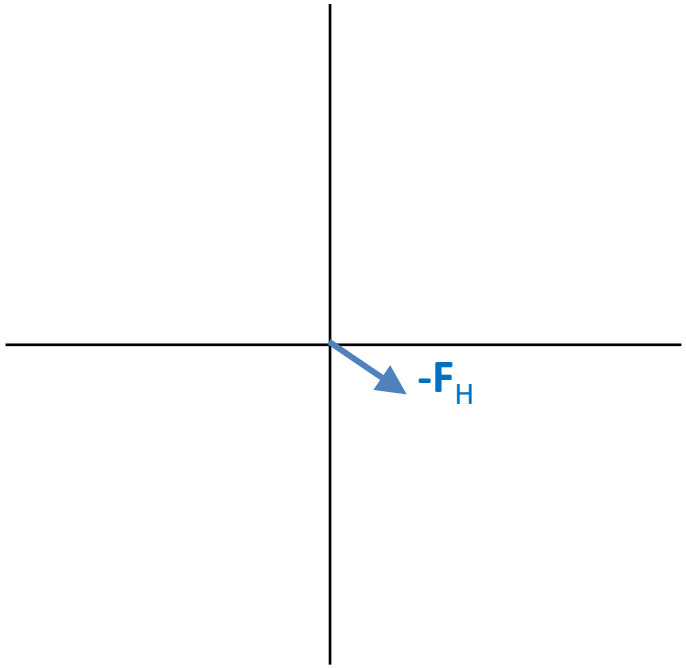



**Solution: Multiple Isomorphous Replacement (MIR)**

Prepare two heavy metal derivatives

- The heavy metal atoms have to bind to different places
- This will result in different $\mathbf{F}_H$ allowing clear determination of phases

# Problem: Locating the heavy metal atoms in the unit cell




The method depends on the ability to **locate the heavy metal atoms in the unit cell**

Tool: The **Patterson function** $P(u,v,w)$

Patternson function is a variation of the Fourier sum for $\rho(x,y,z)$

$$P(u,v,w) = \frac{1}{V}\sum_h\sum_k\sum_l |\mathbf{F}_{hkl}|^2 e^{-2\pi i(hu+kv+lw)}$$

The coordinates $u,v,w$ locate a point in a **Patterson map**, does not contain phase information (square of the structure factor!)

Thus it can be directly constructed.

# Properties of a Patterson Map

## Electron density function

$$\rho(x,y,z) = \frac{1}{V}\sum_h\sum_k\sum_l \mathbf{F}_{hlk} e^{-2\pi i(hx+ky+lz)}$$

requires structure factor (intensities & phases)

shows **3D atom positions** atoms in space

for N atoms -> N densities

## Patterson map

$$P(u,v,w) = \frac{1}{V}\sum_h\sum_k\sum_l \left|\mathbf{F}_{hkl}\right|^2 e^{-2\pi i(hu+kv+lw)}$$

requires square of structure factor (intensities)

shows **length of inter-atom vectors** in space

for N atoms -> $N^2$ densities

For a protein: uninterpretable!
for only heavy metals -> can be interpreted

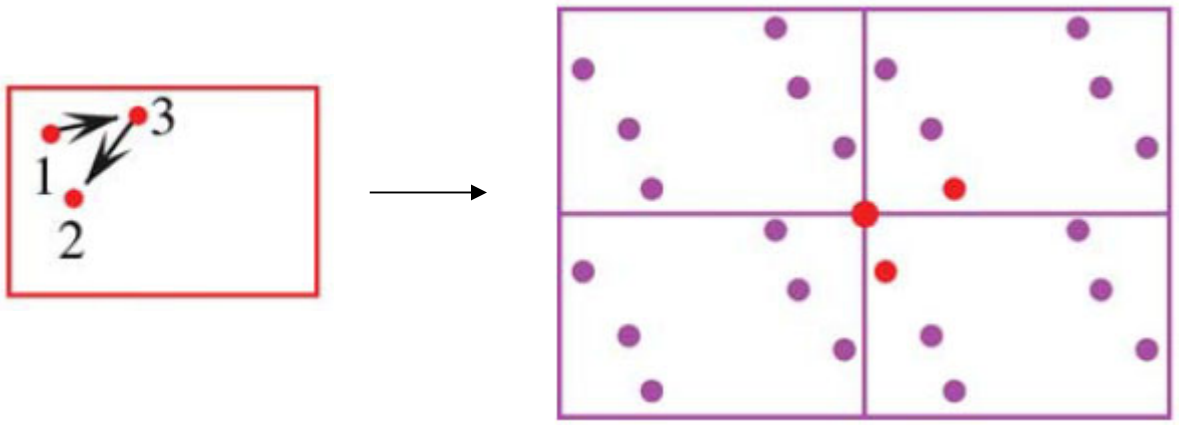
# Locating the heavy metal atoms in the unit cell

Construction of the difference **Patterson function** that reflects the heavy metals alone

$$(\Delta \mathbf{F})^2 = \left(|\mathbf{F}_{PH}| - |\mathbf{F}_P|\right)^2$$

$$\Delta P(u,v,w) = \frac{1}{V}\sum_h\sum_k\sum_l \Delta \mathbf{F}_{hkl}{}^2 e^{-2\pi i(hu+kv+lw)}$$

Sum of simple sine and cosine terms
no phases, can simply be calculated

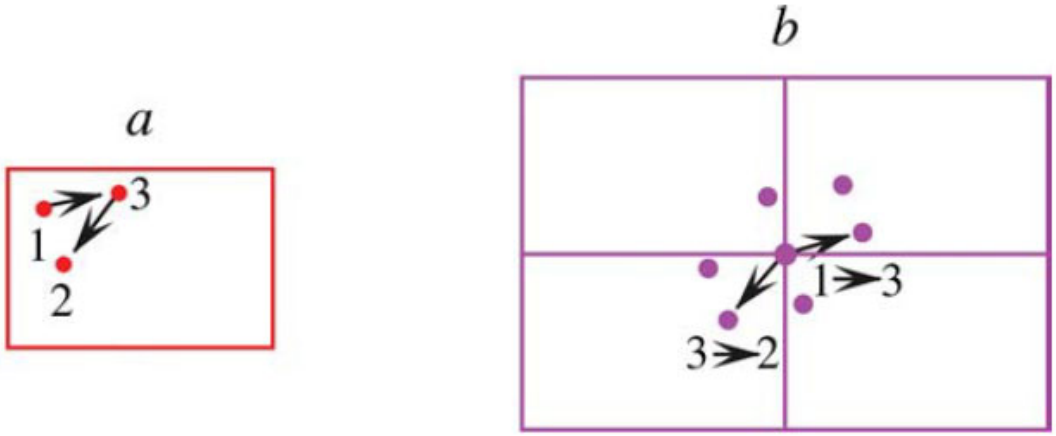



**Patterson map:**

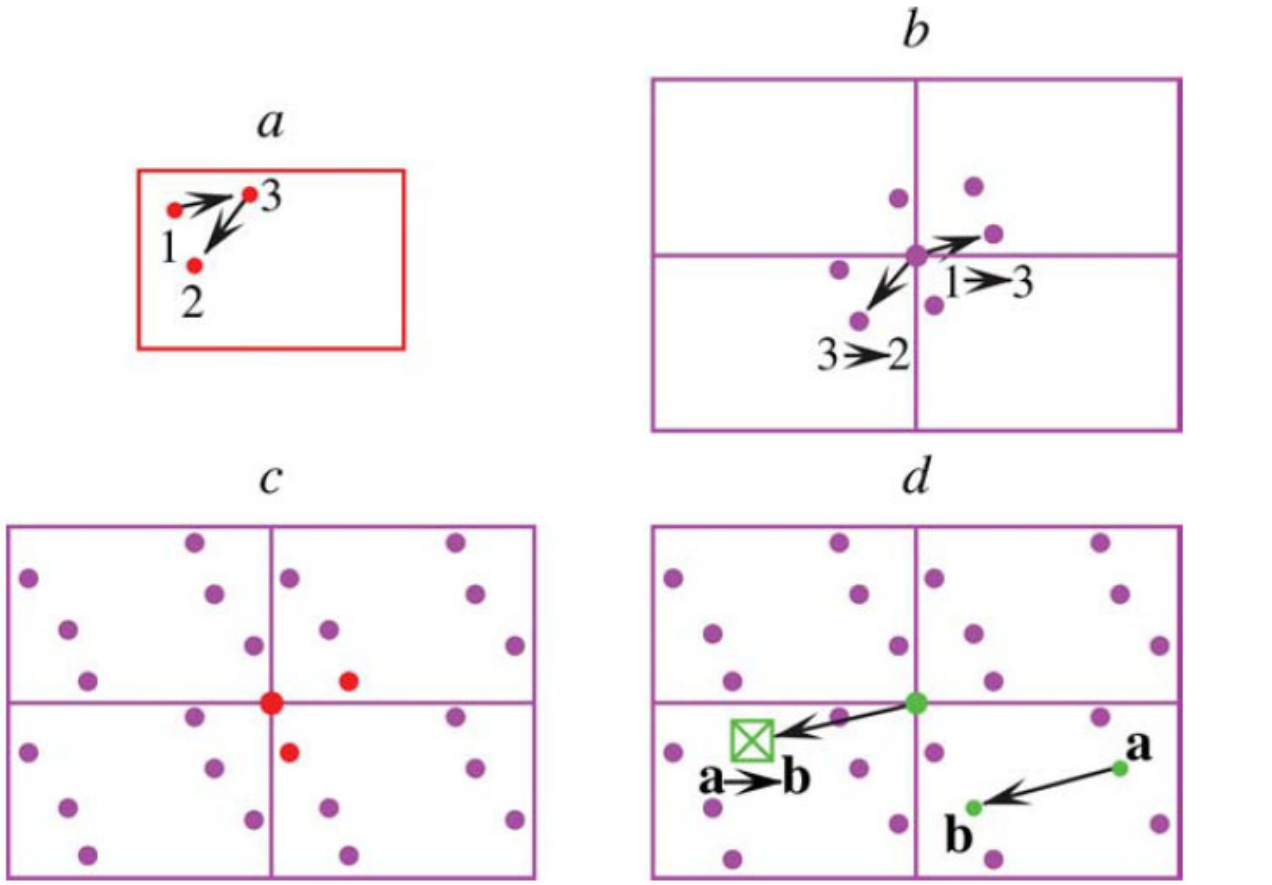
for N atoms, shows $N^2$ peaks

Peaks indicate vectors between all atoms

# Constructing and solving a Patterson Map



# Constructing and solving a Patterson Map



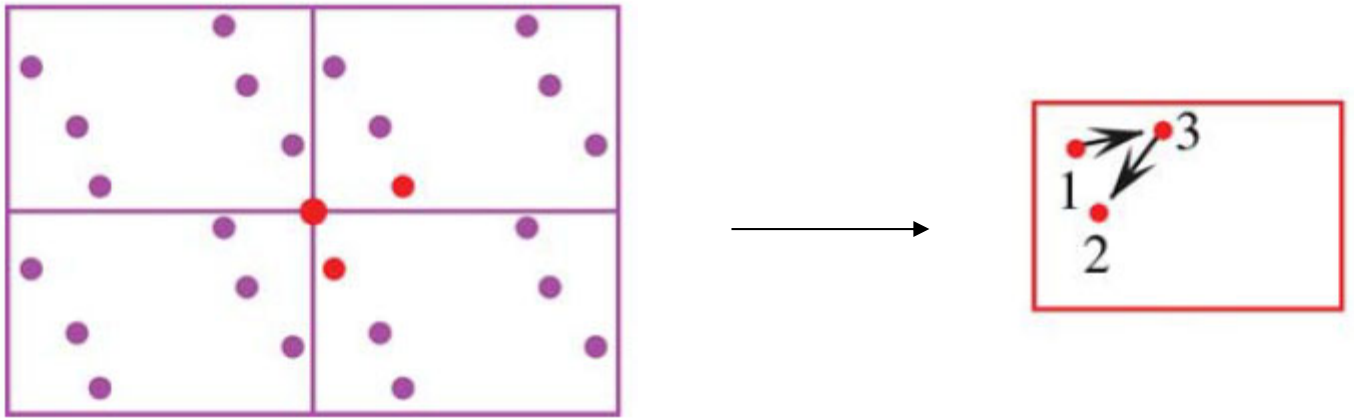

all distances have to be satisfied
a → b: no real distance

For few atoms, the Patterson map can be solved by computer

As we are only looking at heavy metal derivatives, low number of atoms

→ **solvable problem**
→ **yields 3D position of heavy atoms in unit cell**

# The Patterson map yields the distribution of the heavy atoms in the unit cell

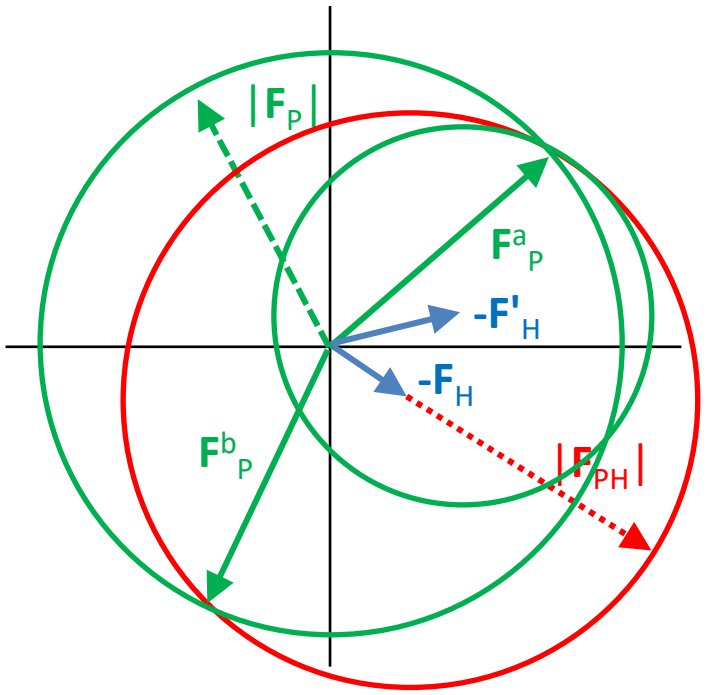



Placement of **heavy metal atoms in unit cell**

→ calculation of structure factor $\mathbf{F}_H$
→ phases for $\mathbf{F}_H$ are known
→ using vector addition, phases for $\mathbf{F}_P$ can be calculated

Computer algorithms can solve Patterson Maps for many 10s of atoms (> 100)

# Using the obtained phases to determine individual phases of $F_{hkl}$




- Only a percentage of phases are determined
- Several heavy metal derivatives are required
- Initial model is produced with a subset of reflections
- Iterative refinement process results in high resolution structure

# Other methods: Anomalous scattering

Synchrotron in Grenoble

selectable wavelengths
~ 0.6 – 2 Å

Employs the effect, that heavy attoms can **absorb** and **scatter** X-rays

Using X-ray sources of variable wavelength, crystals can be irradiated close to the **absorption edge** of a heavy atom

Absorption results in reemission of X-rays with **altered phase**

There exists again a **Vector solution** revealing the phase

Multiwavelength experiments speed up process: **Muliwavelength anomalous dispersion (MAD)**: State of the art

# Molecular replacement

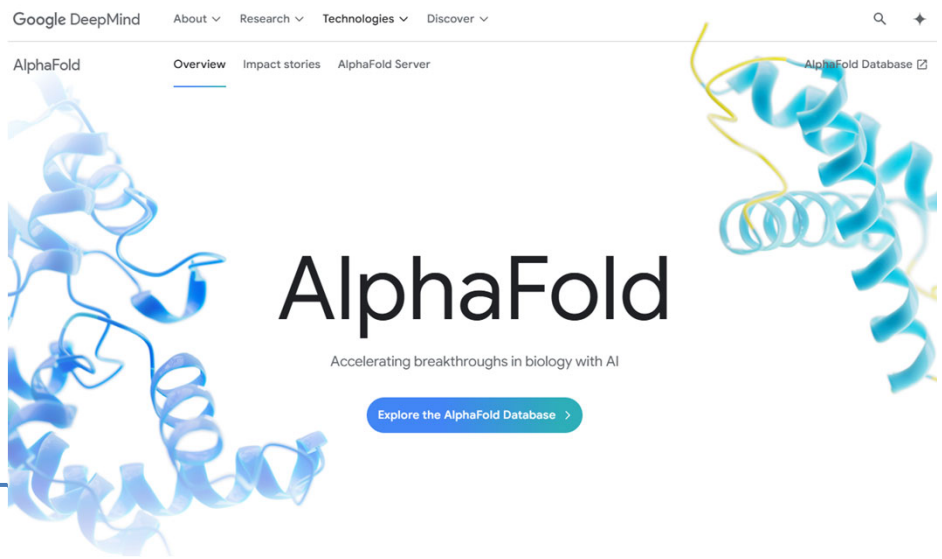
Ubiquitin

SUMO

**Phases can be estimated** if a sufficiently related structure is known

E.g. of a homologous protein from a different organism

or, a structurally related protein

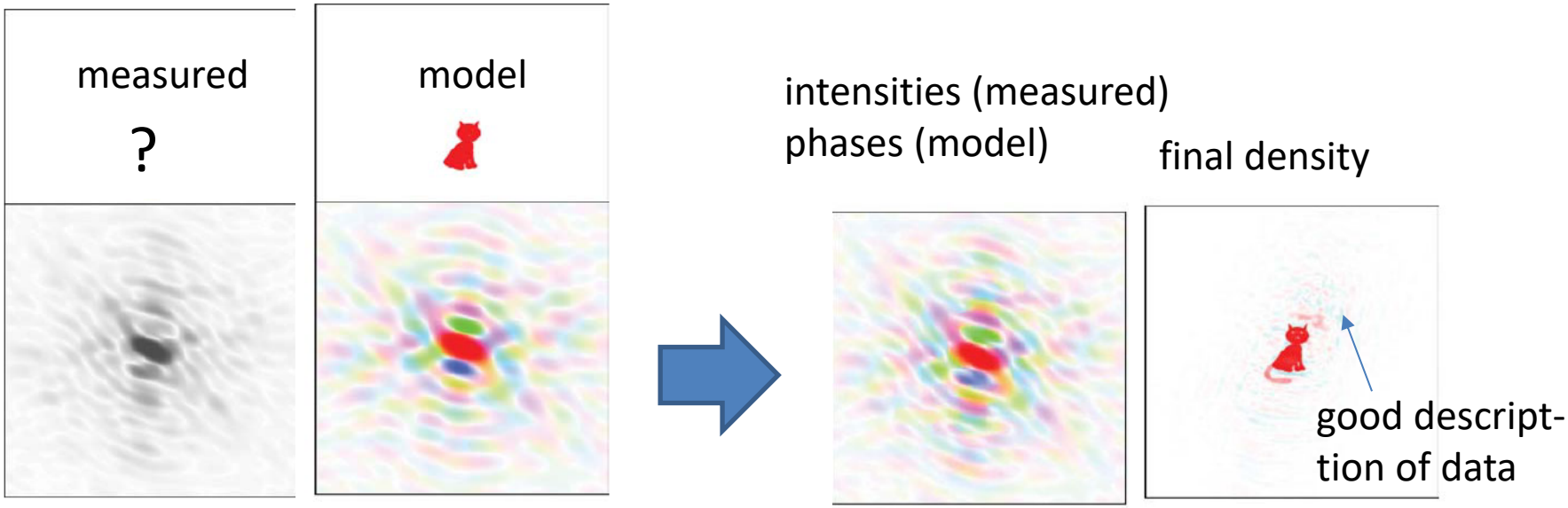


# Molecular replacement

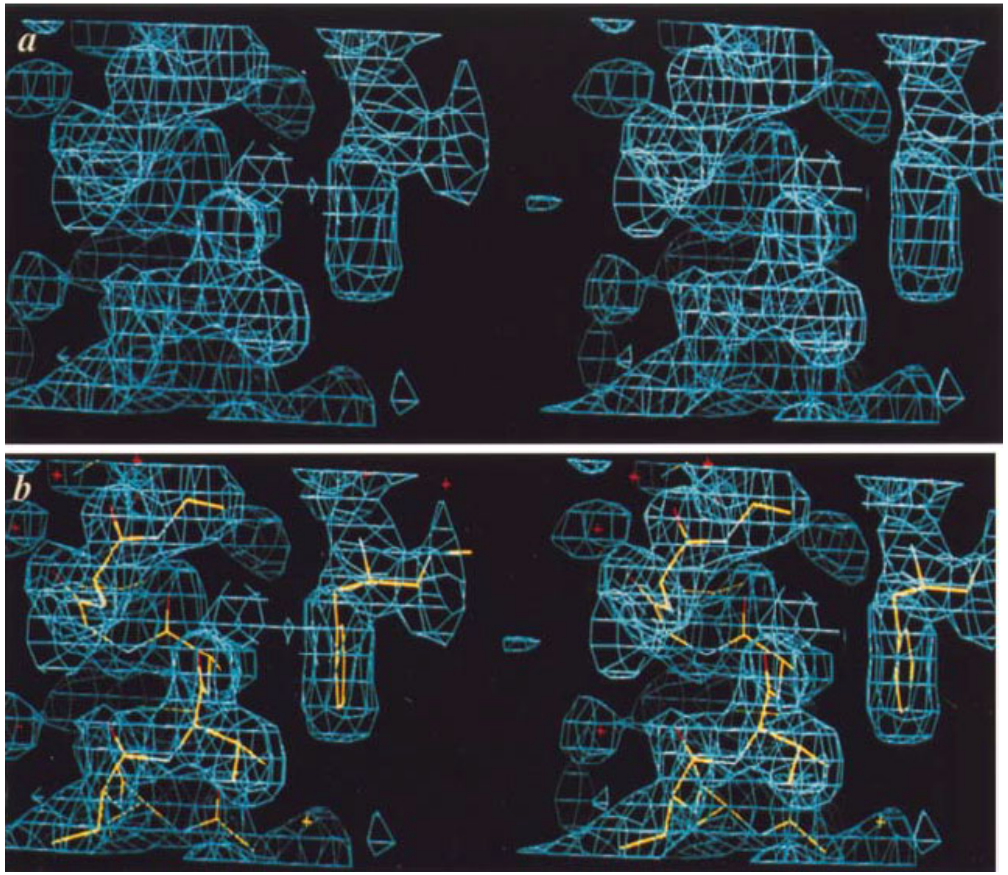
## Molecular replacement

- use of a homologous structure
- calculate phases from model
- compare to experiment and adjust structure
- repeat until good fit is obtained

$$\rho(x,y,z) = \frac{1}{V}\sum_h\sum_k\sum_l |\mathbf{F}_{hkl}|\, e^{-2\pi i(hx+ky+lz-\alpha_{hkl}^{\text{model}})}$$



# Electron density maps




electron density map

chemical structure of the molecule **is modeled** into this map to obtain the 3D structure of the molecule.

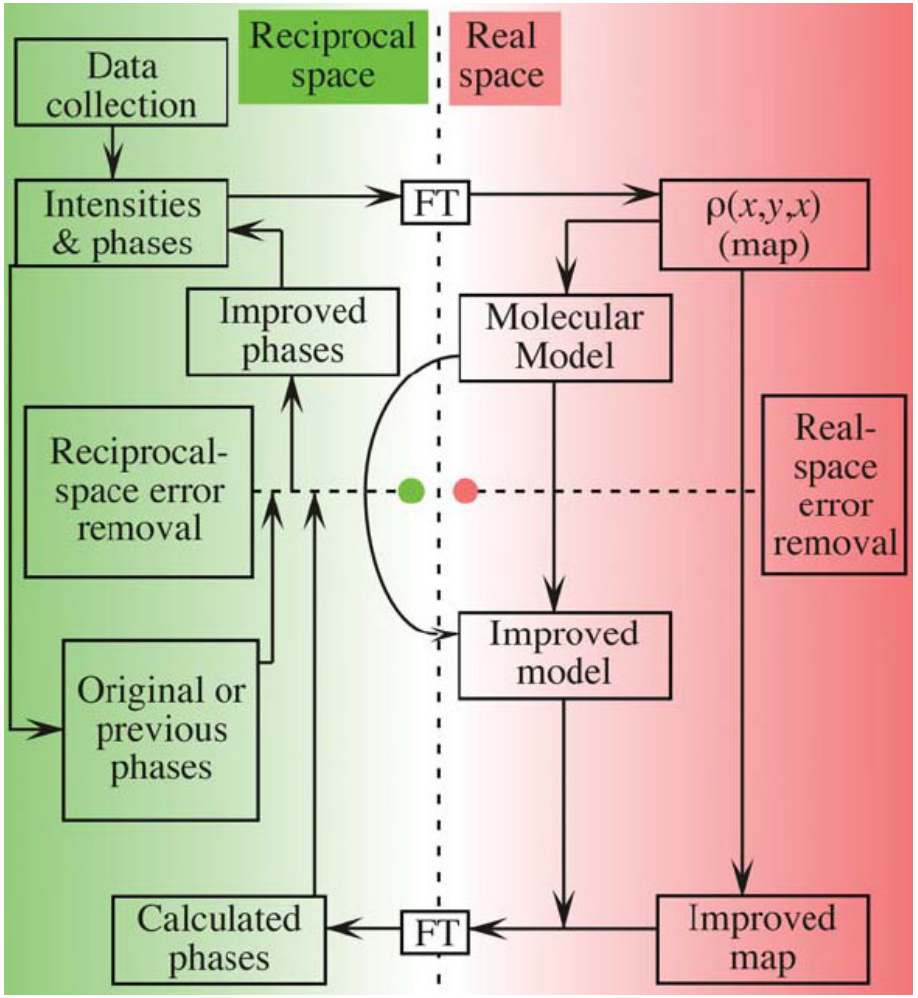
# Refinement of the structure

The quality of the structure solution is judged by the **R-factor**:

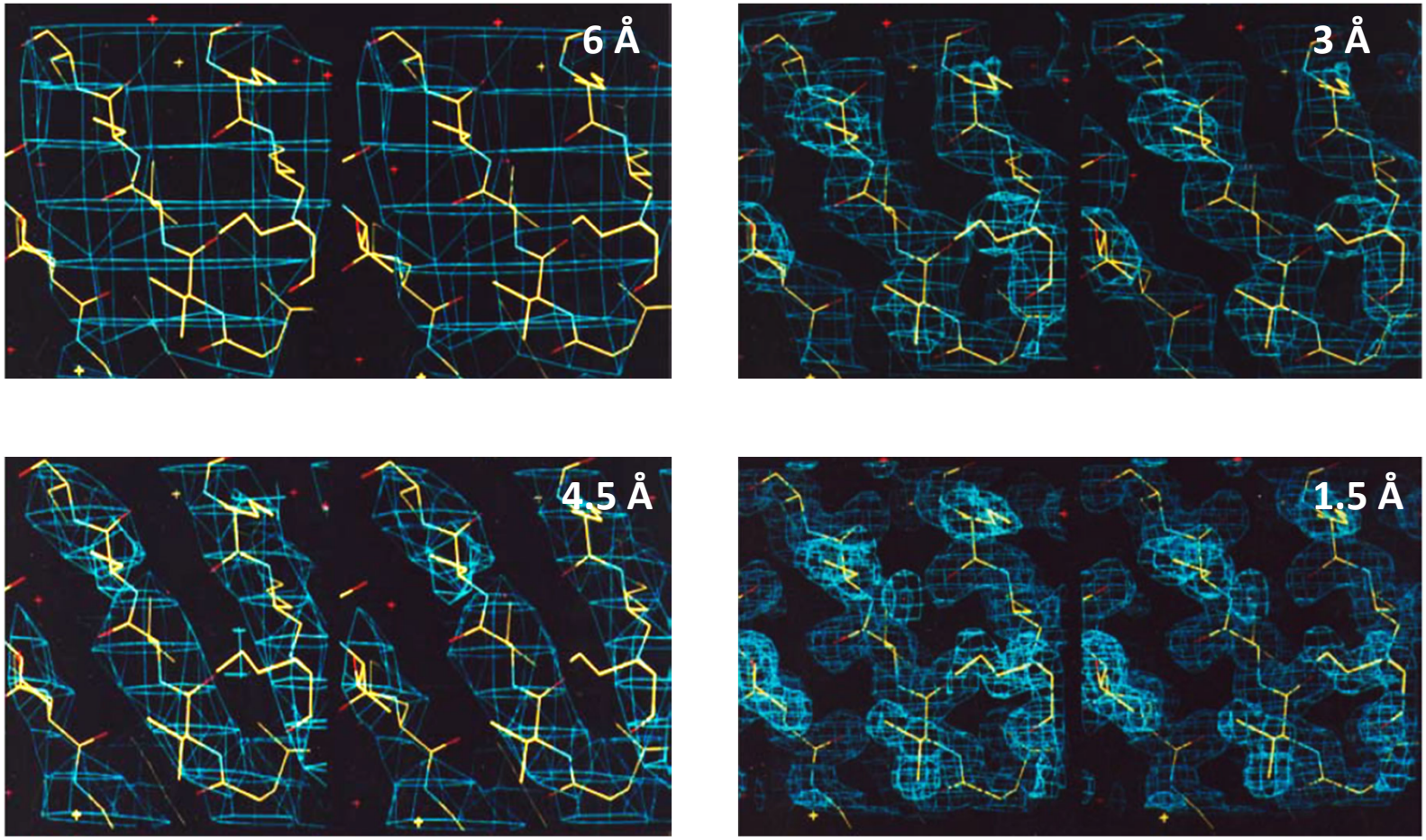
→ difference between calculated and measured structure factor

→ R < 20% is considered a good fit

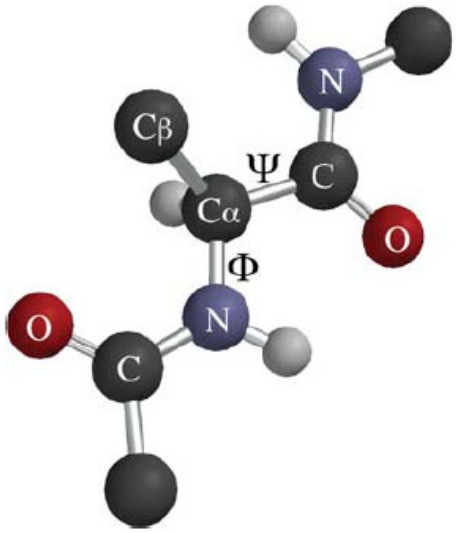
$$R = \frac{\sum \left| |\mathbf{F}_{obs}| - |\mathbf{F}_{calc}| \right|}{\sum |\mathbf{F}_{obs}|}$$

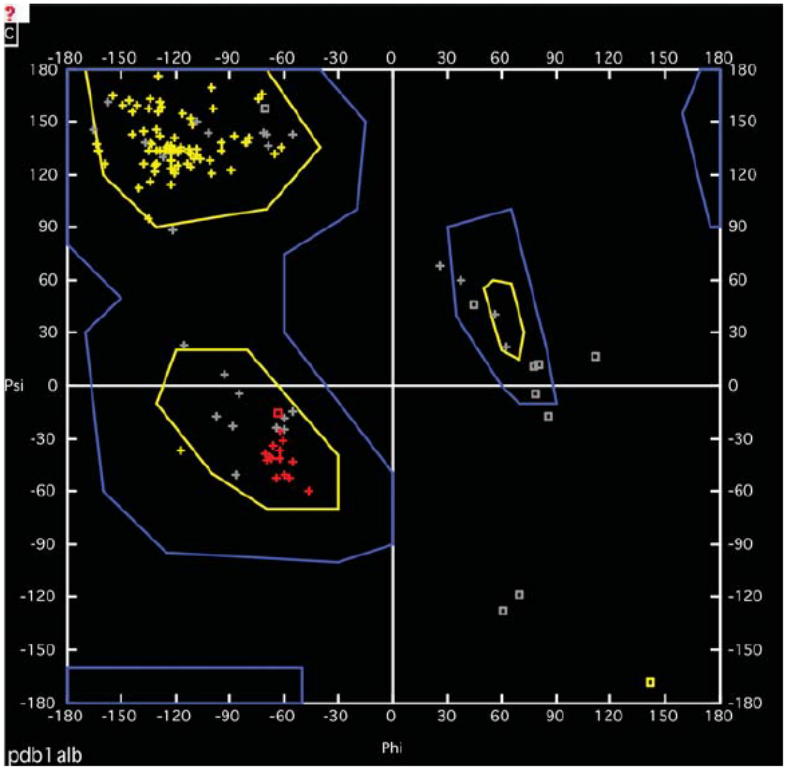


# Electron density map at different resolution



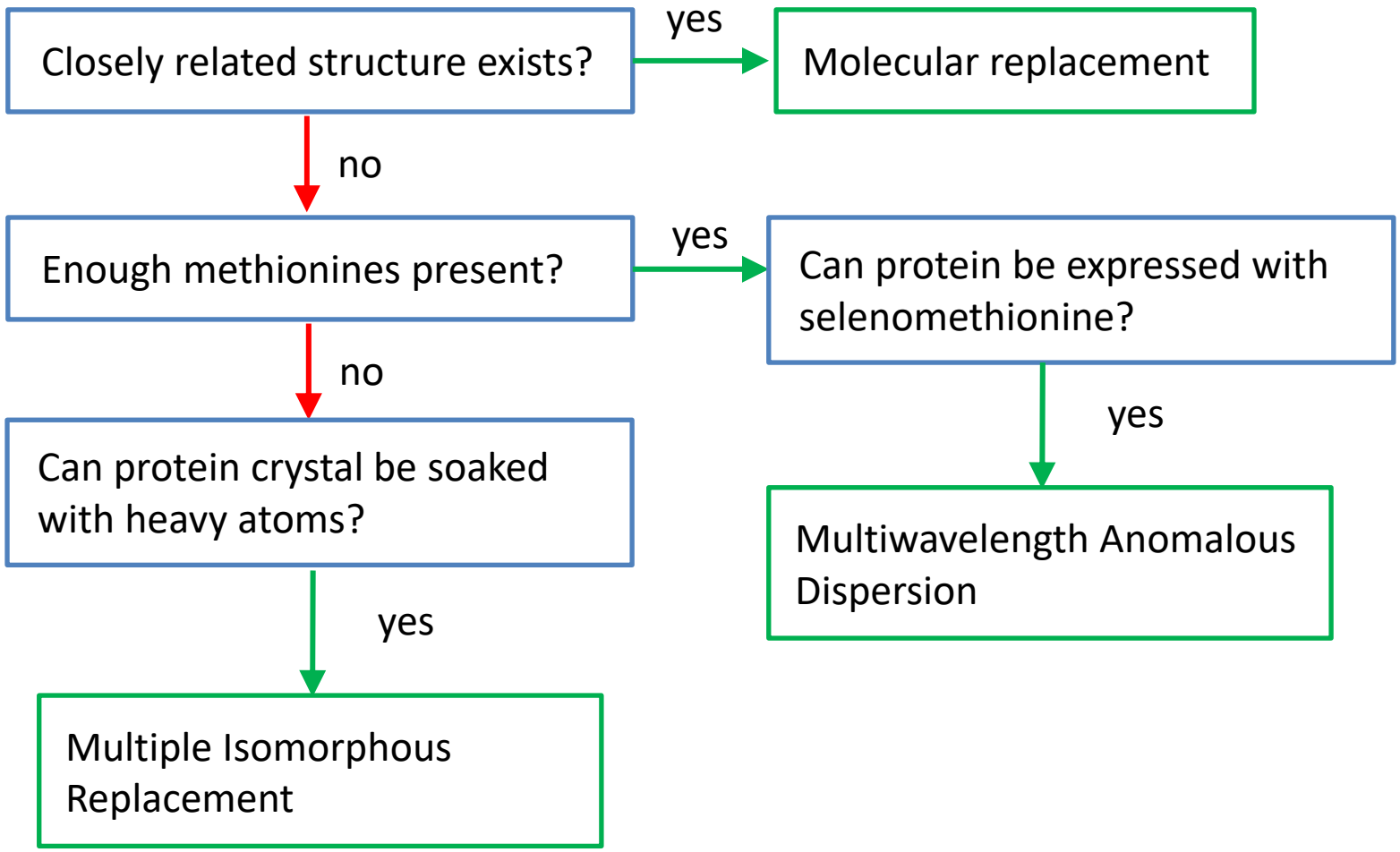
# Judging the model



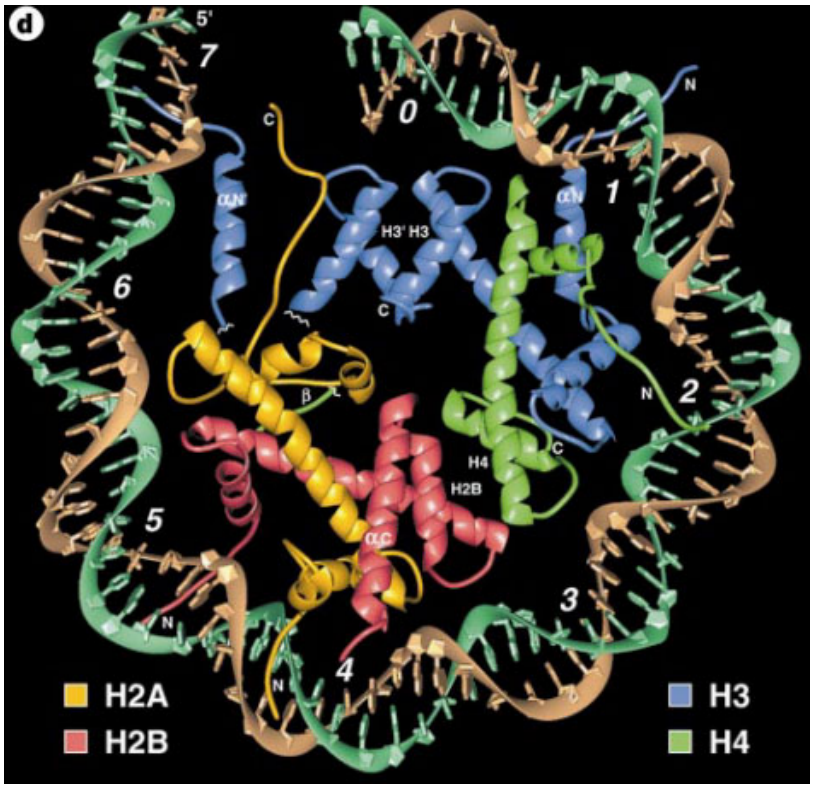




- Ramachandran maps
- Disordered regions
- Unexplained density
- Crystal packing

# Decision process



# Final structure




Luger, K., Richmond, T., et al.
**Nature**, 1997

**Table 1 Crystallographic analysis**

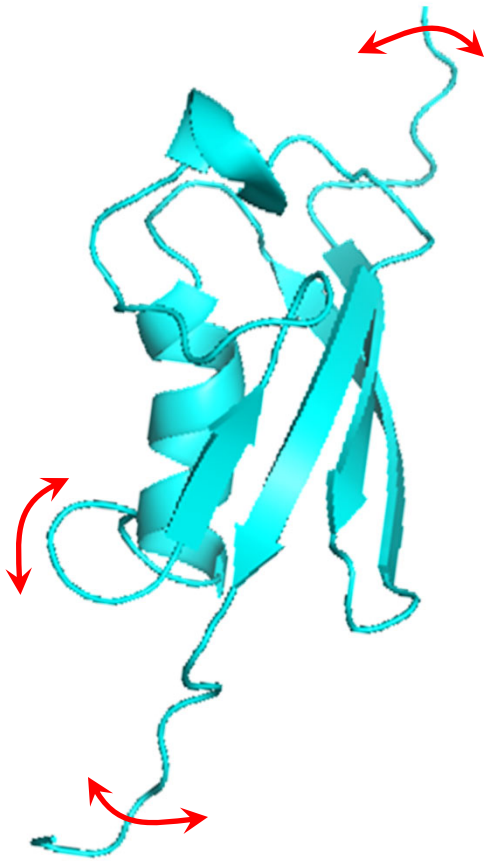
| Diffraction data | Native | 1 × TAMM | 2 × TAMM | 4 × $CH_3HgNO_3$ |
|---|---|---|---|---|
| Resolution | 25–2.8 Å | 25–2.8 Å | 25–2.8 Å | 25–2.8 Å |
| Observations | 297, 397 | 235, 751 | 229, 513 | 186, 451 |
| Unique *hkl* | 52, 432 | 52, 345 | 52, 372 | 50, 986 |
| Completion (%) | 0.99 | 0.98 | 0.98 | 0.96 |
| $R_{merge}$* | 0.056 | 0.064 | 0.068 | 0.056 |
| $R_{merge}$* 3.0–2.8 Å | 0.132 | 0.135 | 0.127 | 0.129 |
| **MIR analysis** | | | | |
| Heavy-atom sites | | H3 C110 | H4 T73 | H3 E133, H4 S47 |
| Phasing power† (MLPHARE) | | 0.84 | 0.92 | 0.97 |
| Cullis *R*-factor‡ | | 0.89 | 0.89 | 0.85 |
| **Refinement statistics** | | | | |
| Observations ($F > 3 \times \sigma$), working/test | | | 43,638/2,639 | |
| *R*-factor§/free *R*-factor (%) | | | 22.4/30.2 | |
| Component | Number of atoms | R.m.s.d. bond length | R.m.s.d. bond angle | Mean *B*-factor |
| Protein | 6,387 | 0.010 Å | 1.92° | 40.4 |
| DNA | 5,980 | 0.006 Å | 1.13° | 80.2 |
| Total | 12,367 | 0.009 Å | 1.31° | 59.7 |



**SUMMARY:**

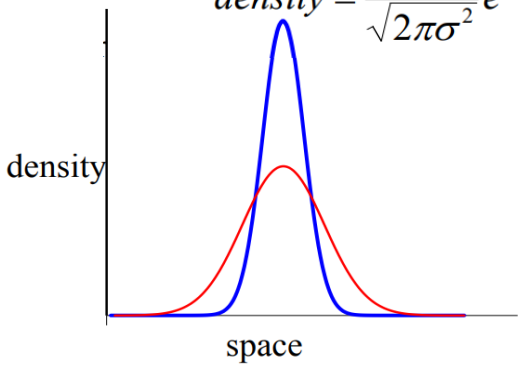
X-Ray structures are not actual images of the molecule but **models** that describe the experimental data while including chemical, structural and thermodynamic information.

# Atomic motion: Crystallographic B-factors



- Atomic motion, e.g. oscillation of atoms at termini
- Broadening of electron density at this site

$$density = \frac{1}{\sqrt{2\pi\sigma^2}} e^{-(x-\mu)^2/2\sigma^2}$$

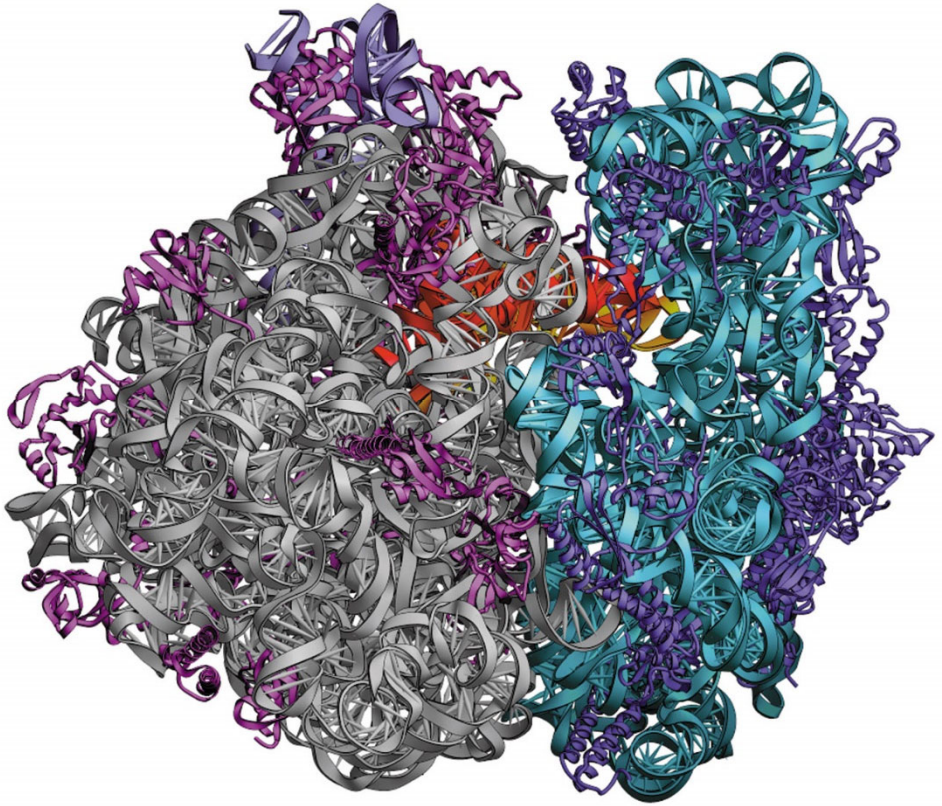



- $B_j$ is a measure of the motion of each atom j

$$\mathbf{F}_c = G \cdot \sum_j n_j f_j e^{2\pi i(hx_j+ky_j+lz_j)} \cdot e^{-B_j[(\sin\theta)/\lambda]^2}.$$

# What can we learn from X-ray diffraction of proteins (biopolymers)?

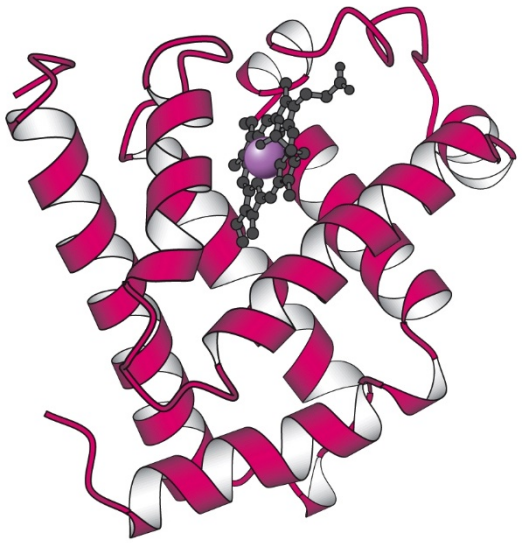
- Average position of each atom of the protein in 3D space with up to 1 Å precision = **3D structure of protein**
- 3D crystals are required
- No direct information about mobility within protein
- Most of the presently known 3D structures were obtained by X-ray diffraction



Ribosome X-ray structure

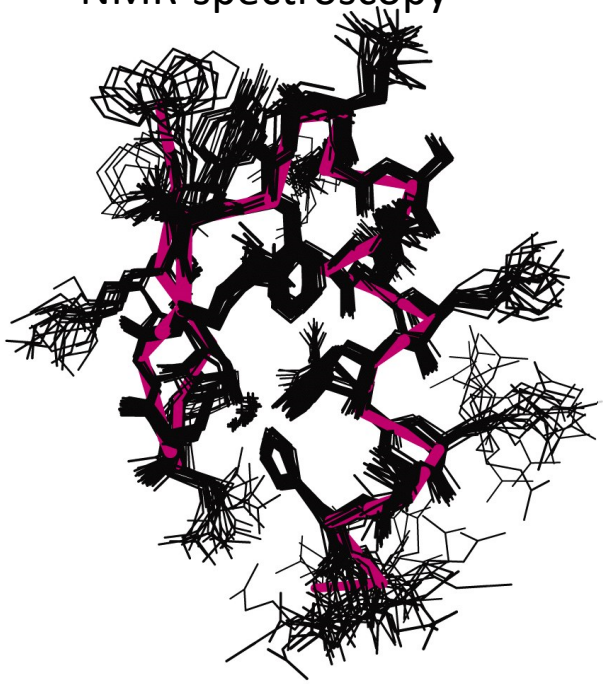
# Protein structure determination

X-ray crystallography



Crystallization required
averaged picture, no dynamics
no size limit

NMR-spectroscopy



Size limit (smaller proteins)
calculated ensemble of structures
protein solubility (μM)
Dynamics can be observed

Electron microscopy



*Chua et al. NAR 2016*

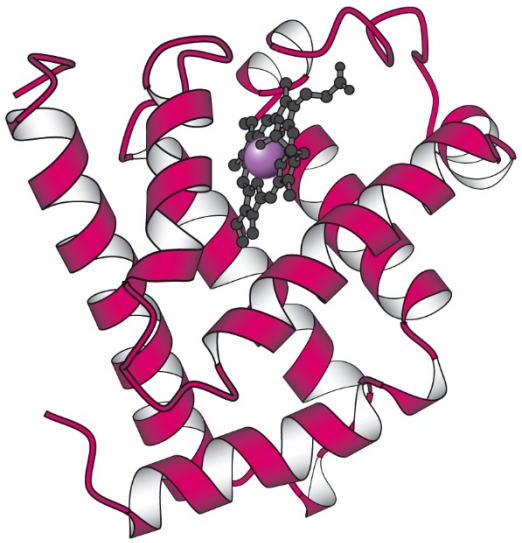
No crystallization required, single molecule technique
large complexes
no size limit

# Nucleic magnetic resonance

- 1946: **Edward Purcell**, **Felix Bloch** independently detected protons resonanting with rf radiation in magnetic field.
- In comparison very low energy method
- 1953: **Albert Overhauser** describes dipolar coupling between spins (basis for NOE)
- 1957: First published protein NMR spectrum (Kirkwood)
- 1960s: **Richard Ernst** develops Fourier transform NMR
- 1985: Protein sequence assignement done by Kurt Wüthrich

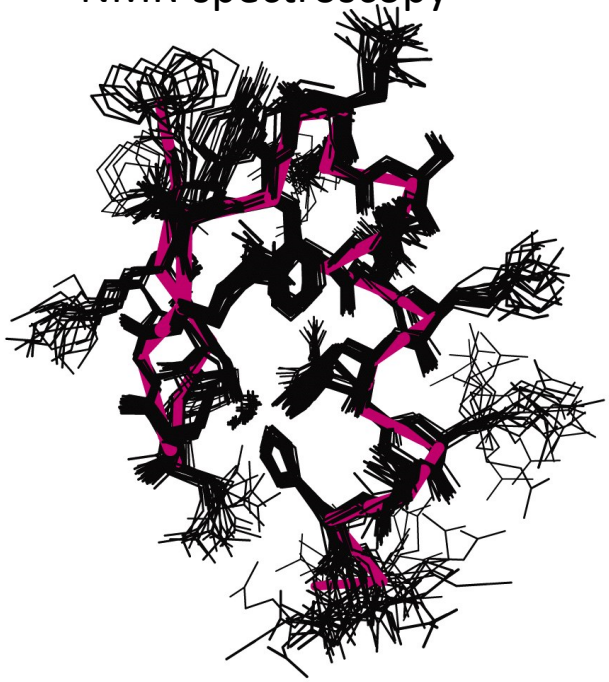
# Protein structure determination

X-ray crystallography



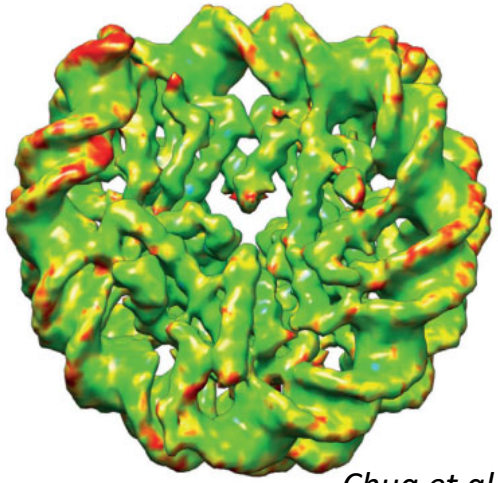
Crystallization required
averaged picture, no dynamics
no size limit

NMR-spectroscopy



Size limit (smaller proteins)
calculated ensemble of structures
protein solubility (μM)
Dynamics can be observed

Electron microscopy

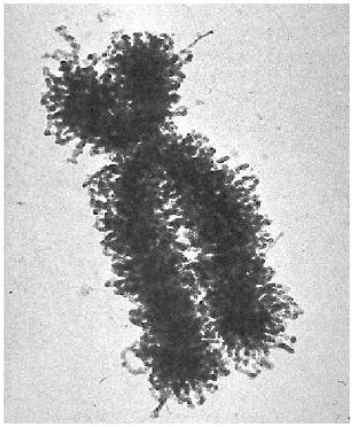


*Chua et al. NAR 2016*

No crystallization required, single molecule technique
large complexes
no size limit

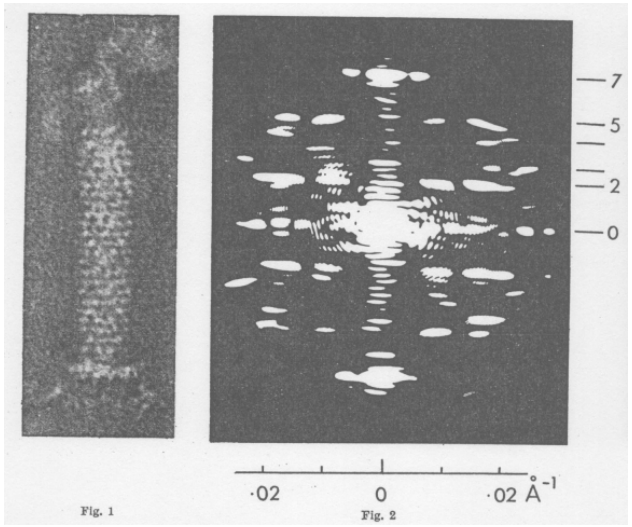
# Electron microsopy – short history

- **Ernst Ruska**: Developed the electron microscope in the 1930's
- e- are charged: two EM coils act as lenses
- 1940's: first commercial EM
- negative staining using heavy metals

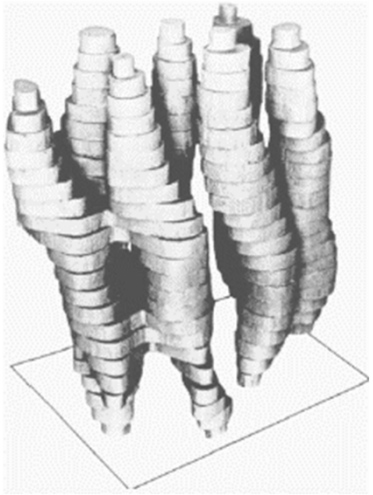


# EM of biomacromolecules

- 1968: **David De Rosier** and **Aaron Klug** use image reconstruction to determine 3D structure of T4 bacteriophage
- 1975: **Richard Henderson** and **Nigel Unwin** – 3D structure of bacteriorhodopsin
- **Ken Taylor** and **Robert Glaeser**, **Jacques Dubochet** (UNIL): CryoEM




*De Rosier & Klug, Nature 1968*



*Henderson & Unwin, Nature 1975*

# Today: CryoEM Revolution

nature

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NEWS · 10 FEBRUARY 2020

## Revolutionary cryo-EM is taking over structural biology

The number of protein structures being determined by cryo-electron microscopy is growing at an explosive rate.

Ewen Callaway


A cryo-electron microscope at the Laboratory of Molecular Biology in Cambridge, UK. Credit: MRC Laboratory of Molecular Biology

PDF version

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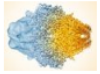
Cryo-electron microscopy wins chemistry Nobel



SUBJECTS

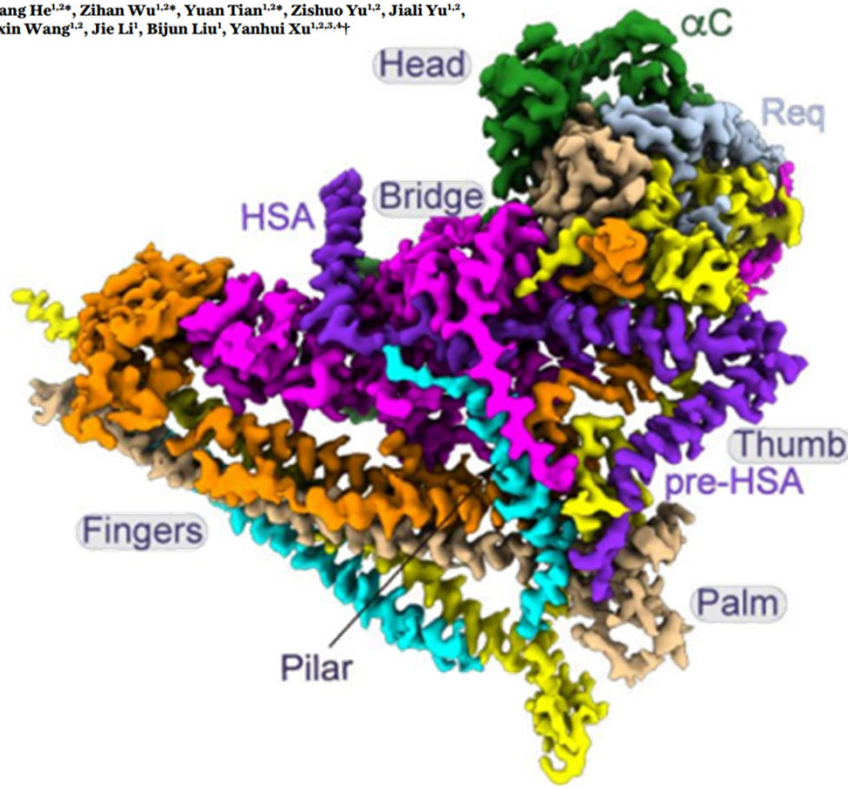
Microscopy Structural biology

Science

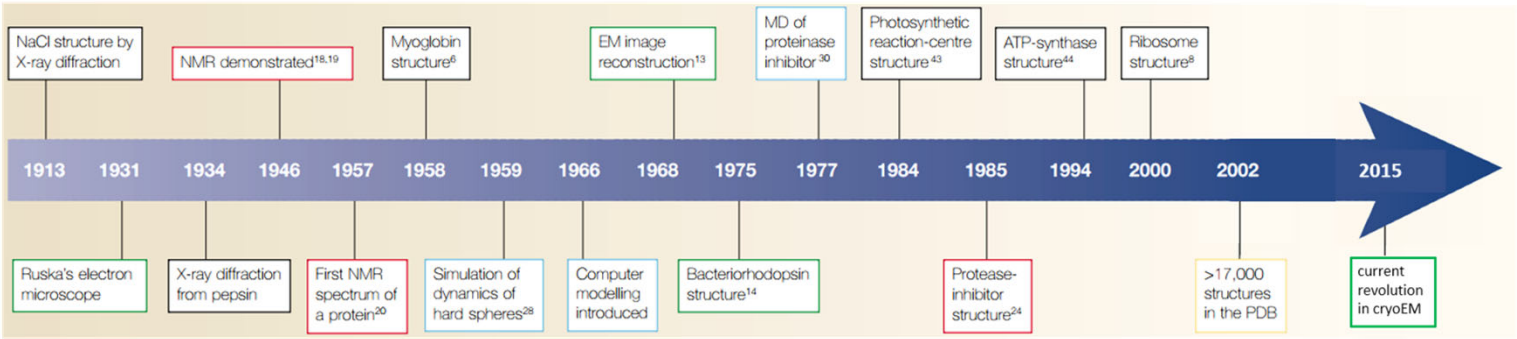
Cite as: S. He *et al.*, *Science* 10.1126/science.aaz9761 (2020).

## Structure of nucleosome-bound human BAF complex

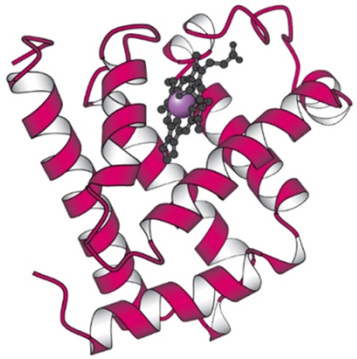
Shuang He[1,2*], Zihan Wu[1,2*], Yuan Tian[1,2*], Zishuo Yu[1,2], Jiali Yu[1,2], Xinxin Wang[1,2], Jie Li[1], Bijun Liu[1], Yanhui Xu[1,2,3,4†]



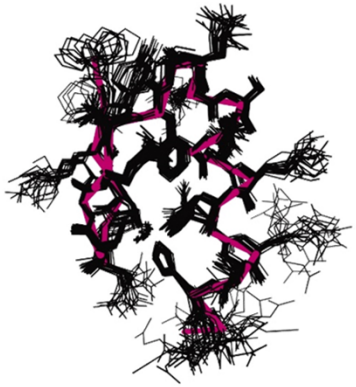
# History of structural biology



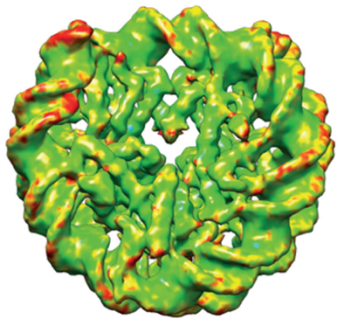

X-ray crystallography

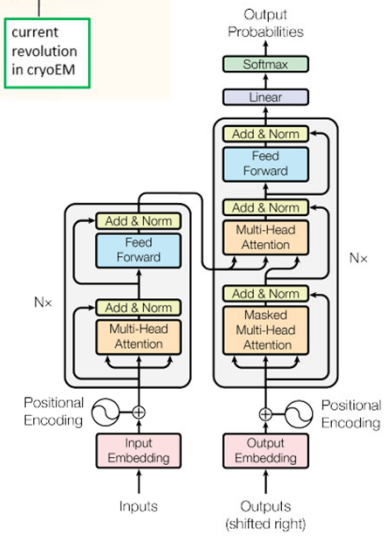


NMR-spectroscopy



Electron microscopy






AI methods

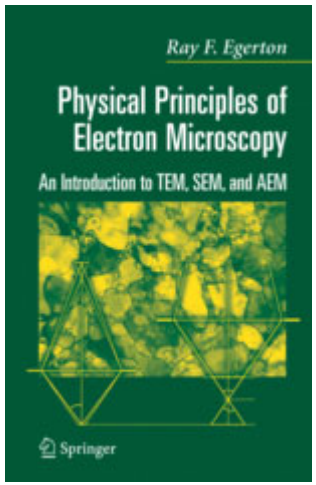
EPFL SCHOOL OF BASIC SCIENCES - SB | INSTITUTE OF CHEMICAL SCIENCES AND ENGINEERING - ISIC
LABORATORY IN BIOPHYSICAL CHEMISTY OF MACROMOLECULES – LCBM

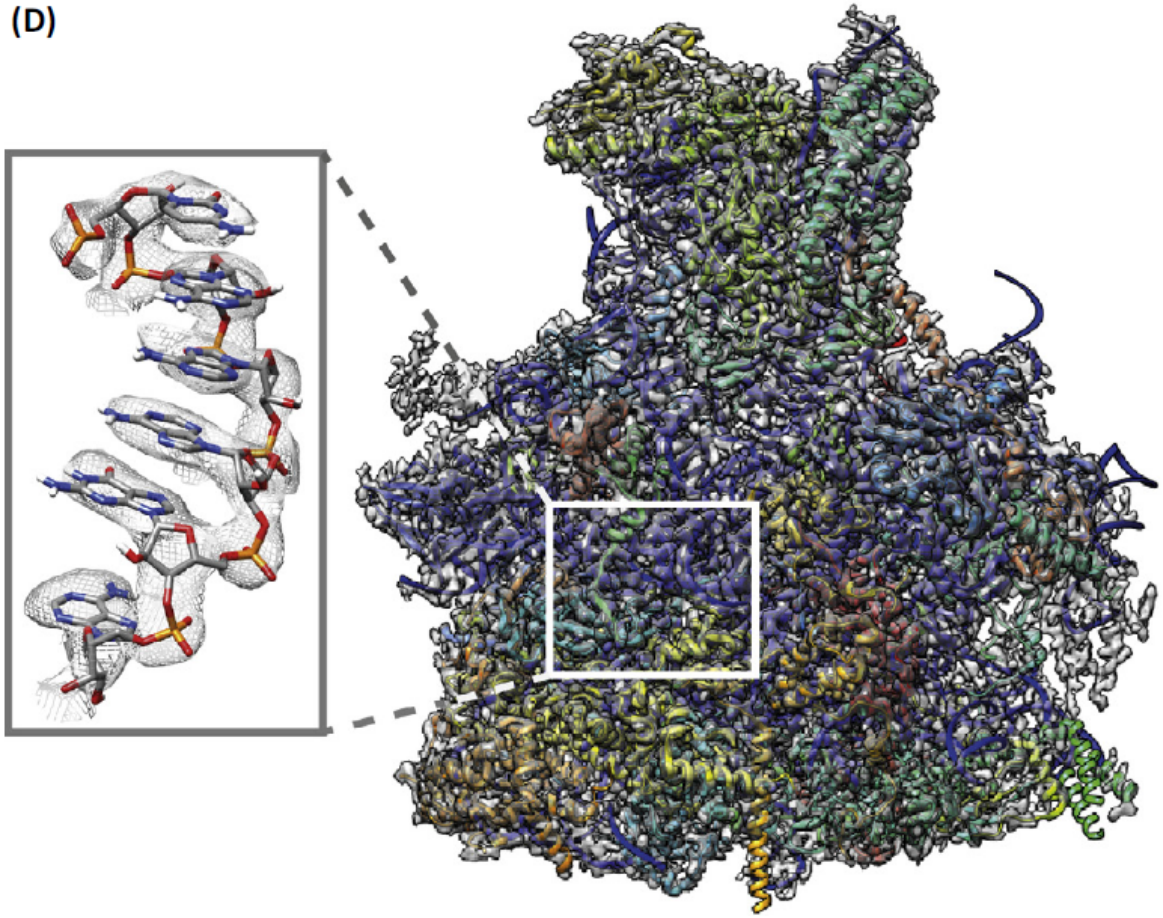
# CH-312- Dynamics of biomolecular processes

Spring Semester 2024

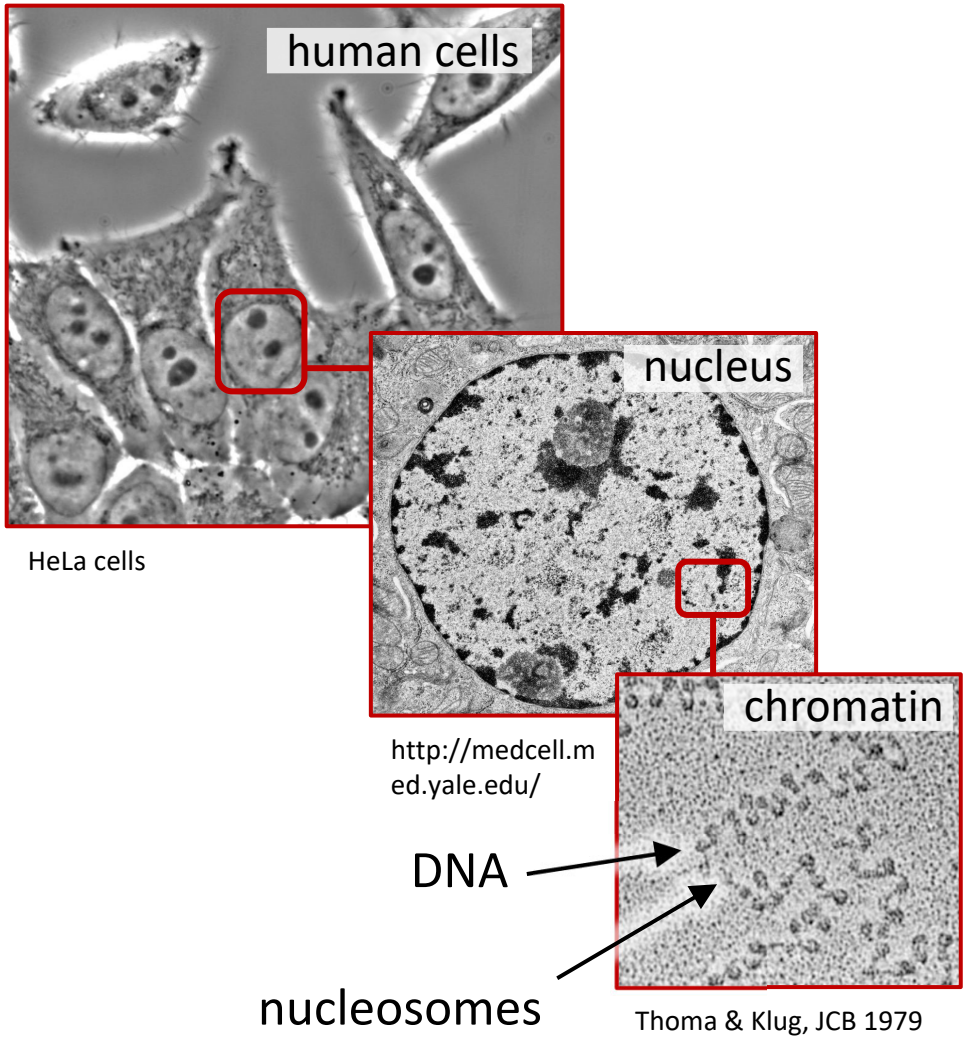
Beat Fierz

## CryoEM



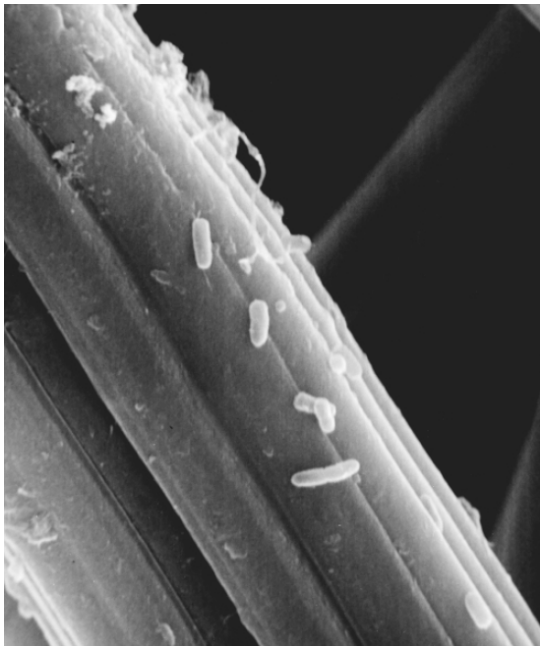
# Electron microscopy allows a view into cells




HeLa cells

http://medcell.med.yale.edu/

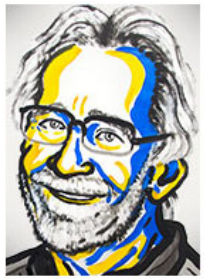
Thoma & Klug, JCB 1979



Scanning electron microscope

Bozzola et al. Enzyclopedia of Life Sciences 2002

# A revolution in structural biology



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Jacques Dubochet
Prize share: 1/3

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Joachim Frank
Prize share: 1/3

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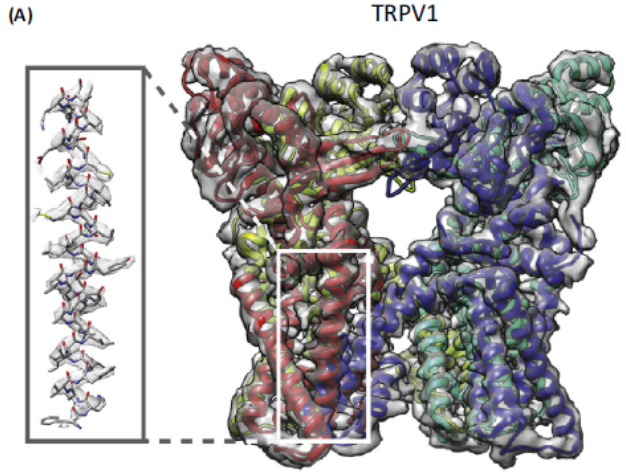
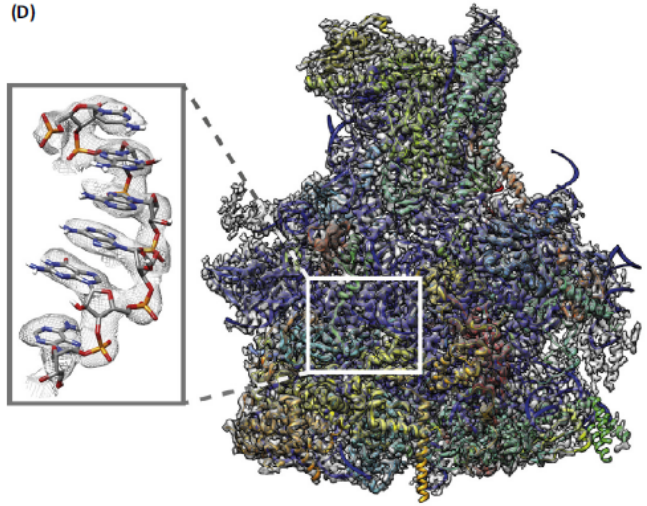
Richard Henderson
Prize share: 1/3

FEI Titan Krios
300 kV

Tecnai F20 Cryo

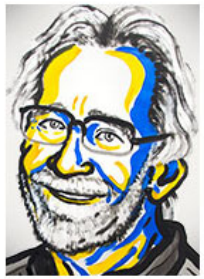



Mitoribo

- Cryo EM (Nobel 2017)
- Vitrification of water
- Direct electron detectors
- Advanced image processing

# … well represented in the Lausanne area



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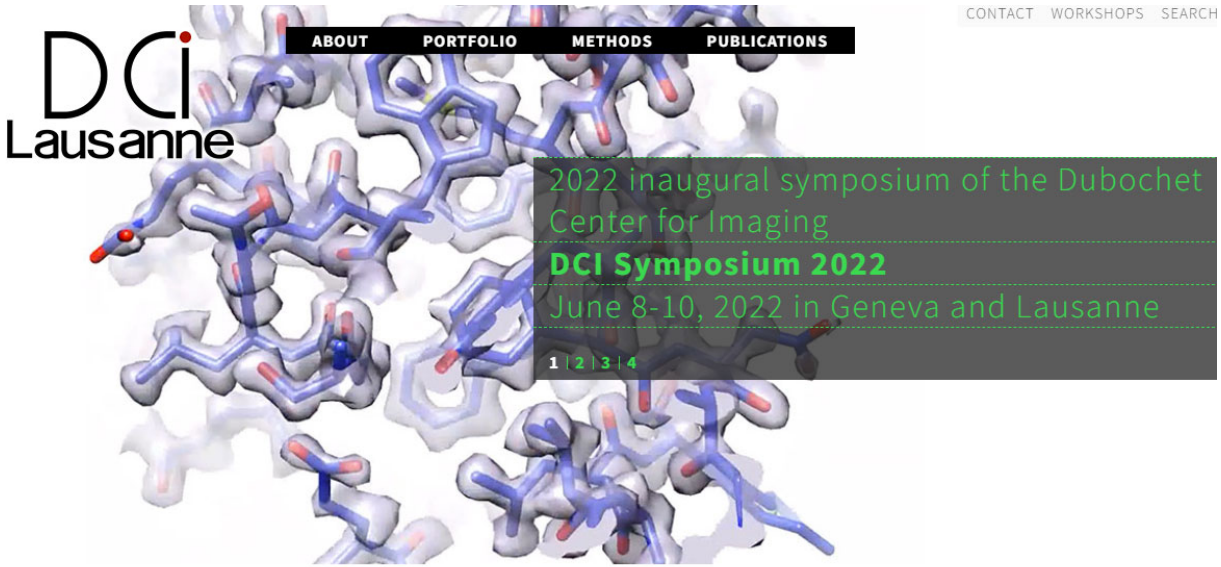
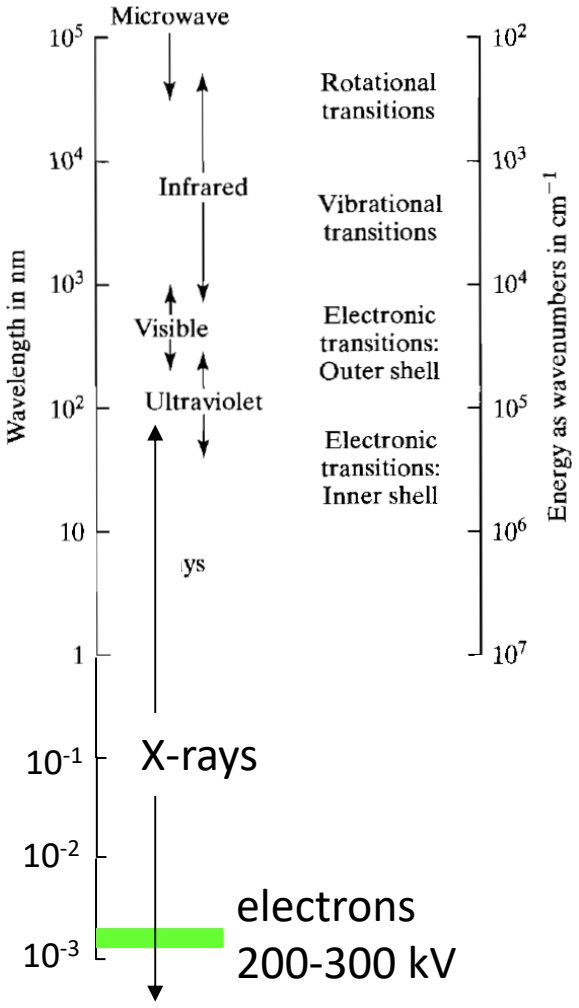



# Why electron microsopy?




De Broglie wavelength of an electron

$$\lambda = \frac{h}{p} = \frac{h}{mv}$$ momentum $p$

Energy of accelerated electrons

$$E = eU = \frac{m_0 v^2}{2}$$ U: acceleration voltage

Wavelength

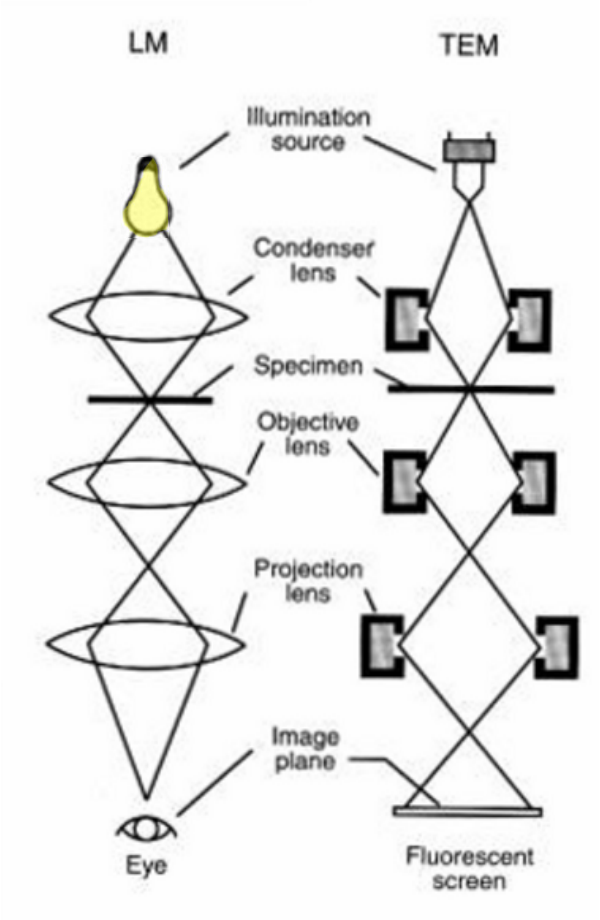
$$p = m_0 v = \sqrt{2 m_0 e U} \qquad \lambda = h \Big/ \sqrt{2 m_0 e U}$$

including relativistic effects with high acceleration voltages

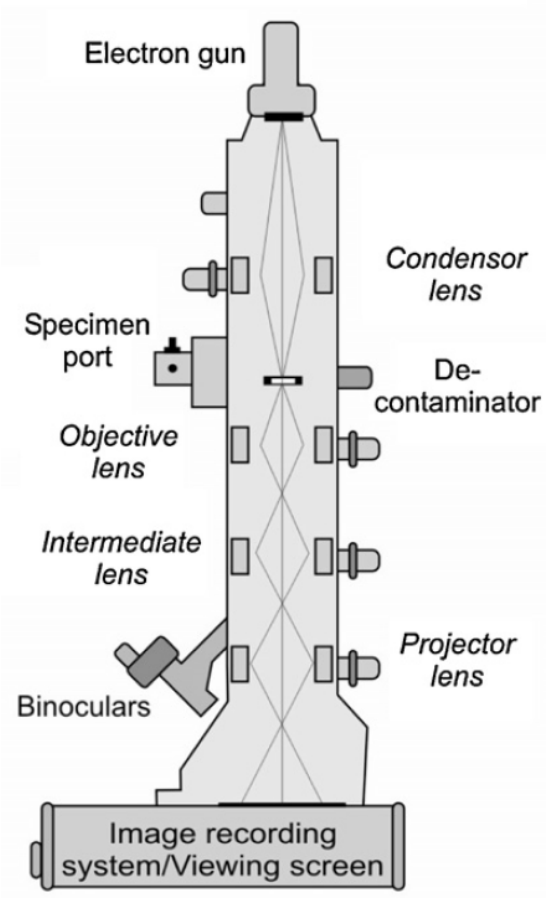
$$\lambda = h \Big/ \sqrt{2 m_0 e U (1 + eU / 2 m_0 c^2)}$$

for U = 200 kV, λ = 2.51 pm

# Function of a transmission electron microscope



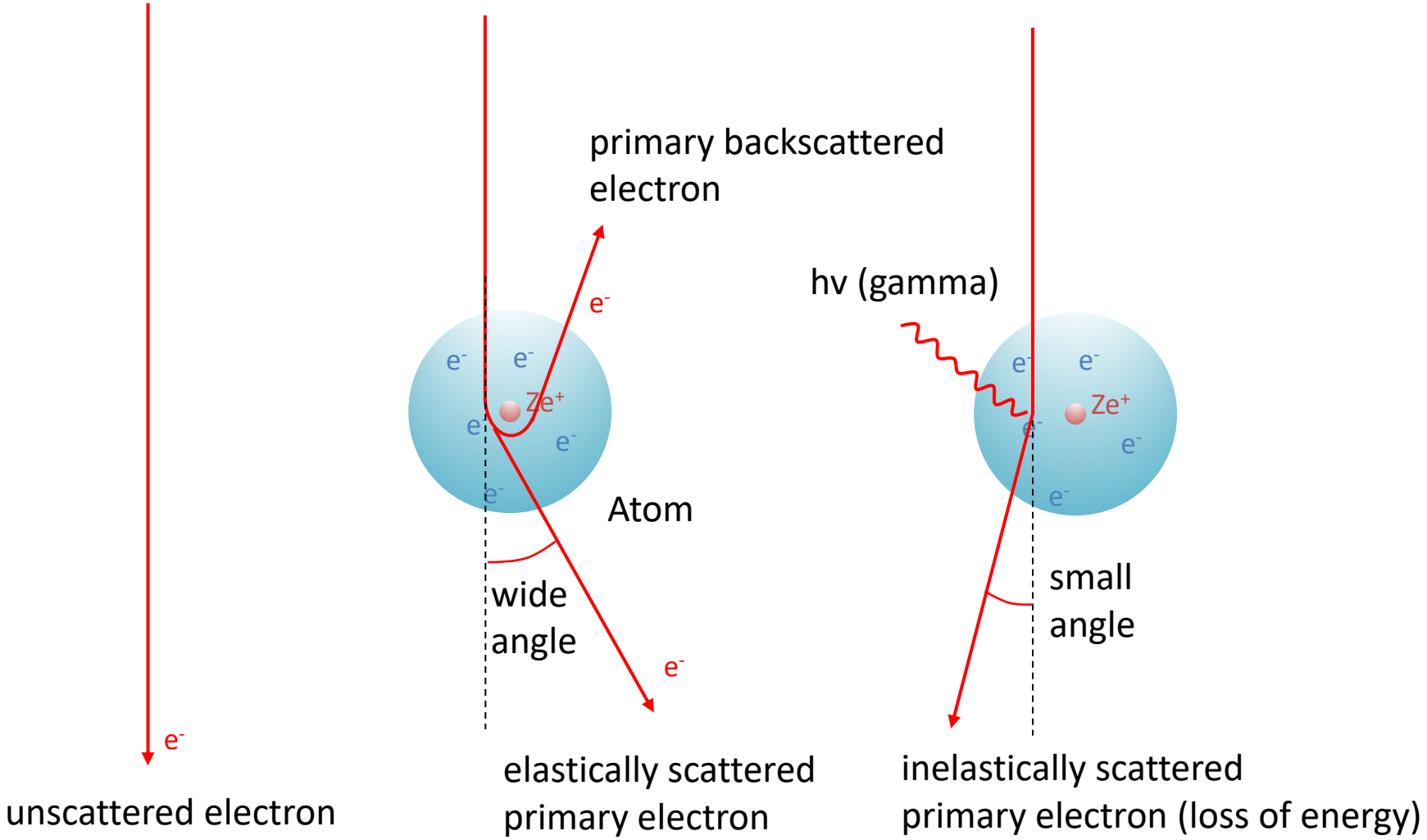

Light vs. electron microscope



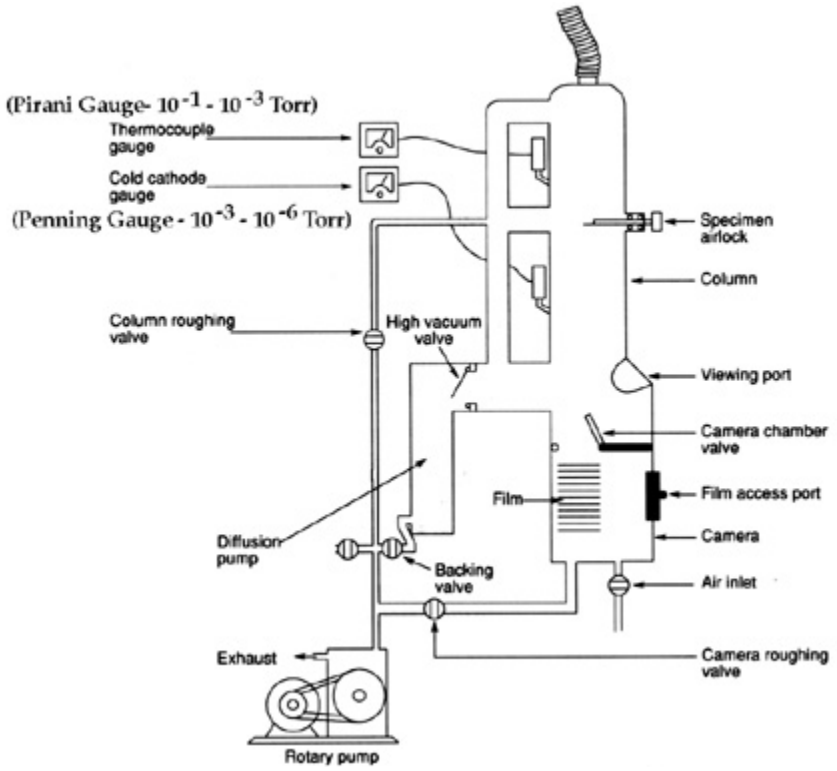

General scheme of a **TEM**

Kuntsche et al. Int J Pharmaceut. 2011

# Electrons scattering by an atom



# TEMs are operated under high vacuum




http://www1.udel.edu/biology/Wags/b617/tem/tem15.gif

TEM is evacuated to low pressure, e.g. $10^{-4}$ Pa (some parts even lower, e.g. electron gun $10^{-7}$ to $10^{-9}$ Pa)

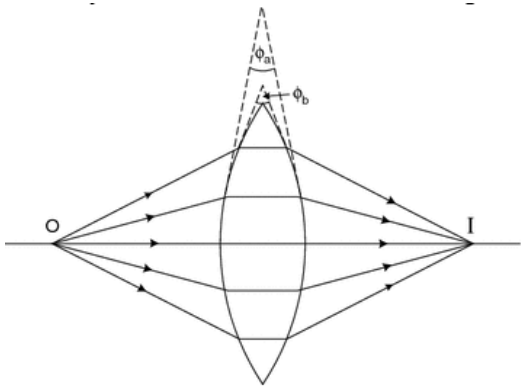
- allows high voltage for electron acceleration

Why is high vacuum necessary?

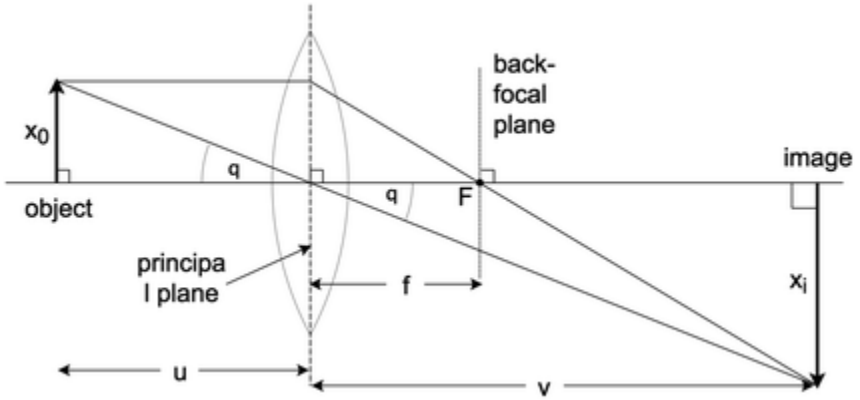
Vacuum system consists of:
- low/roughing vacuum (rotary pump)
- diffusion pump
- turbo-molecular pump for high vacuum

# Electron optics in TEM

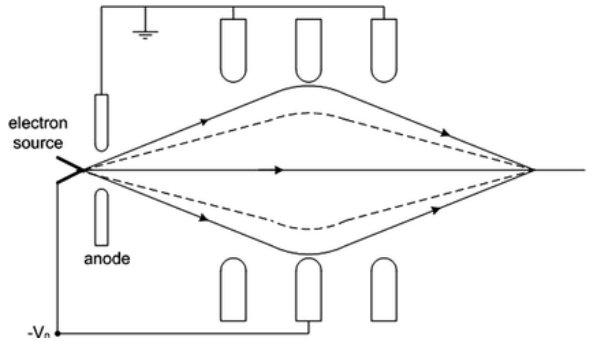
Optical imaging




optical lens, refracts light (refractive index) allowing focusing of beams




Electron imaging

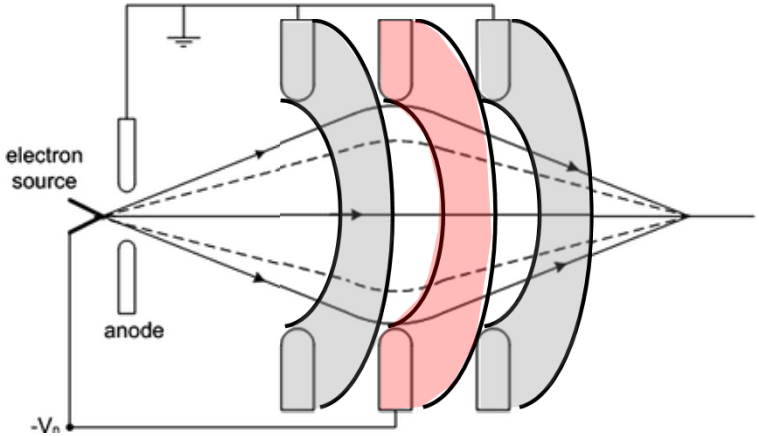



No material can be used to refract electrons (scattering)

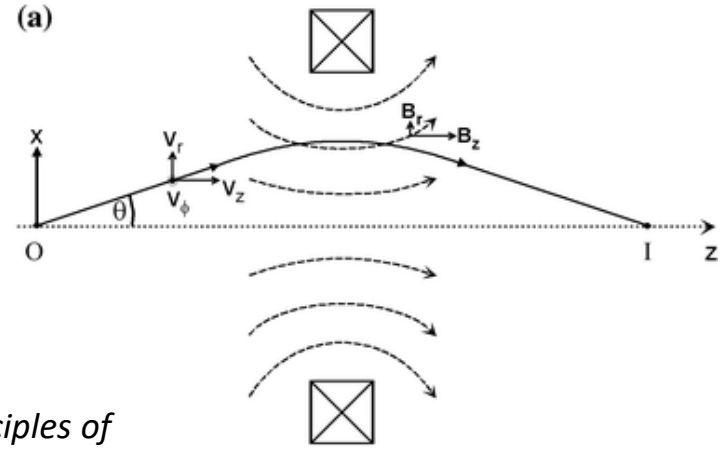
Electrons carry a **charge**

→ Electron lenses function either applying an **electric field**, or a **magnetic field**

# Electron lenses: Electrostatic & magnetic lenses




**Electric field** between electrodes (annular) are focused by repellent negative charge of the central lens (red)

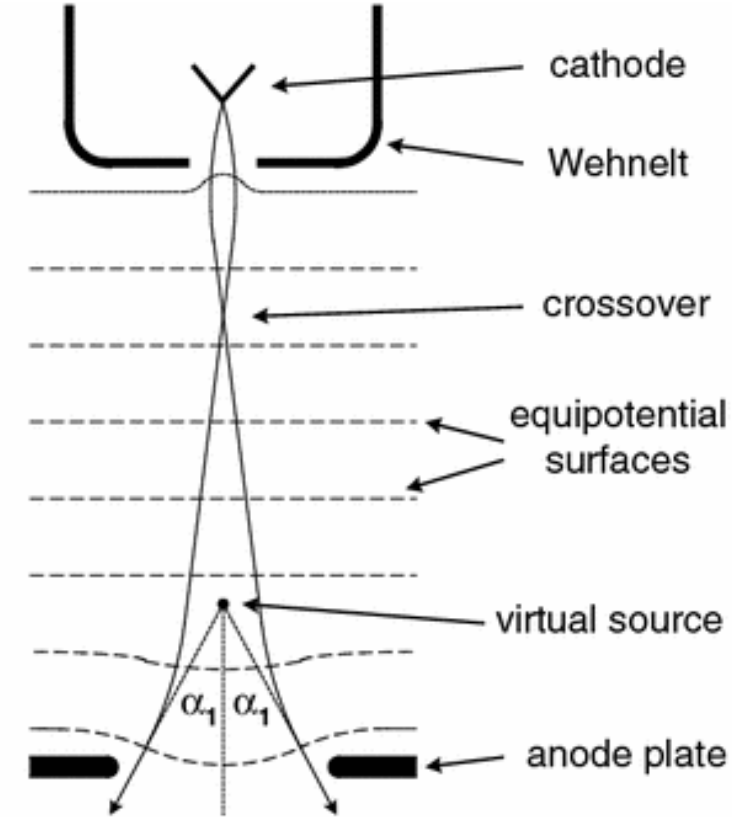



**Magnetic field** deflects electrons

Force induced on electrons induces spiral path (image rotation)

*Physical principles of electron microscopy, Egerton*

# Electron acceleration




**Thermionic electron gun:**
Current heats tungsten filament to 2700 K, electrons are ejected into surrounding high vacuum

acceleration between Wehnelt (cathode) and anode plate

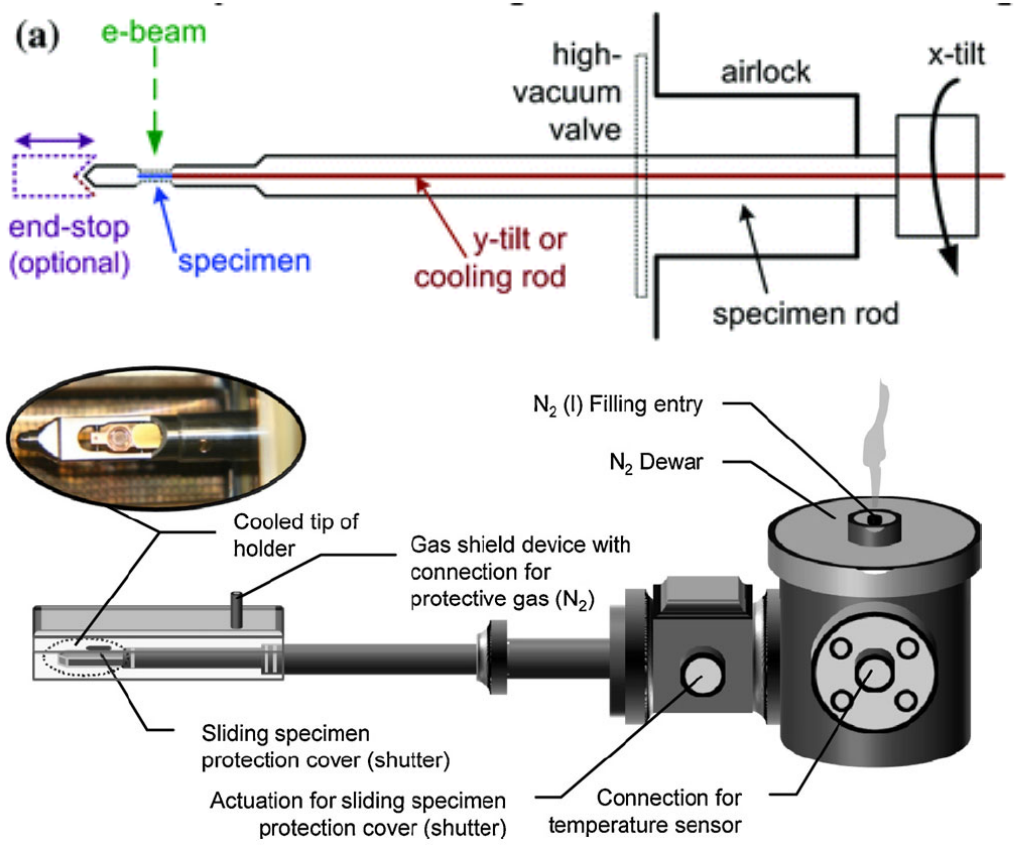
Curvature of equipotential surfaces across hole in Wehnelt and anode plate serve as electromagnetic lenses

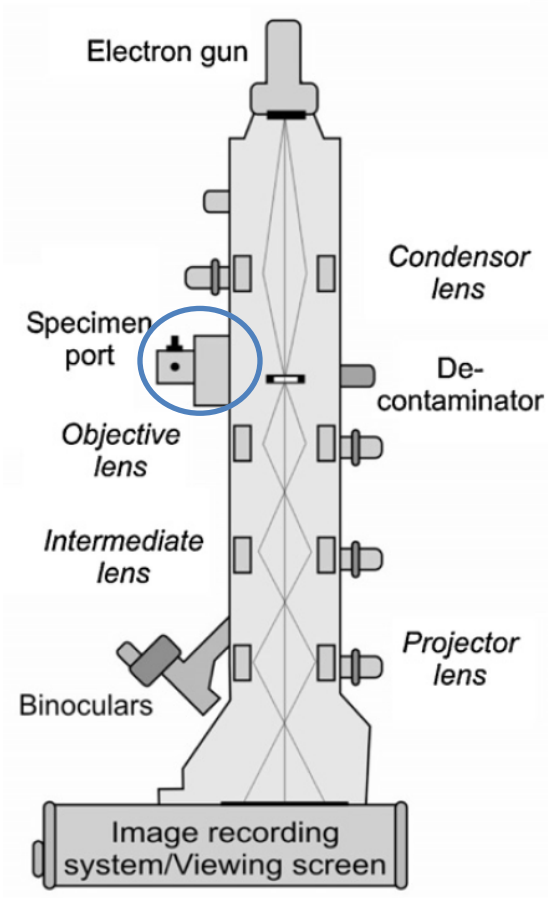
The exiting beam is divergent

Electrons are accelerated to **70-80% light speed**

→ relativistic effects!

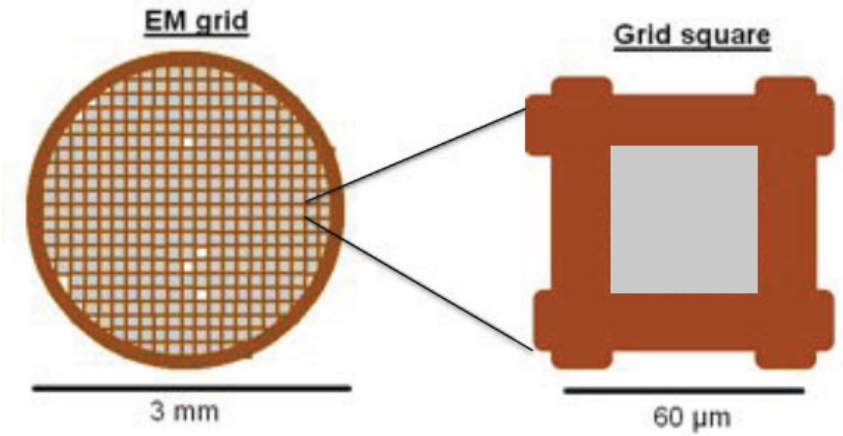
# Sample stage for TEM




biological samples are frozen to withstand high vacuum

# Sample preparation in EM

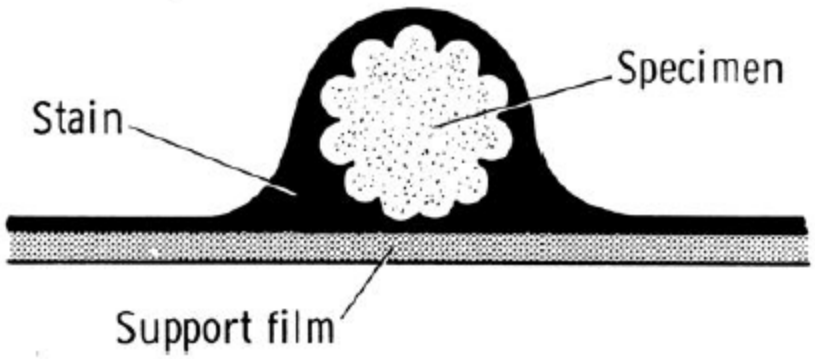


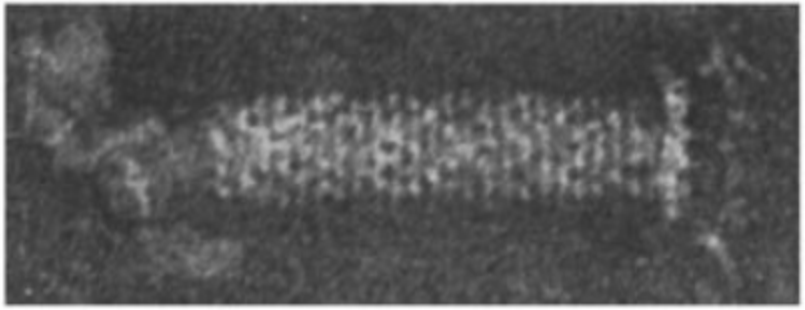

Mounting of samples on a standard EM grid.

Copper grid, coated with thin carbon film (10-30 nm)

- Contrast in **light microscopy** arises from light absorption in the sample
- In **electron microscopy**, electrons are scattered:
  - Scattering from heavy elements:
    - Negative staining
    - Metal shadowing
  - Phase contrast from light defocusing
    - Important for cryo EM of unstained samples

# Negative staining of samples



Biological sample is dried

**Negative staining** uses heavy metal salts (e.g. uranyl acetate, phosphotunstic acid)

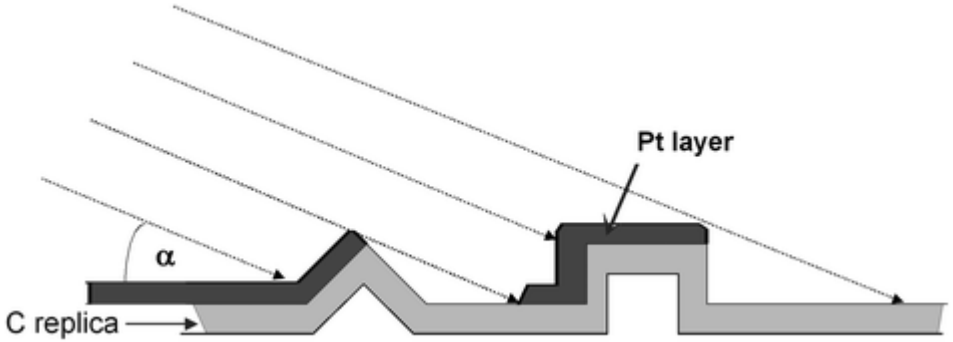
Hard "cast" is formed around biological specimen

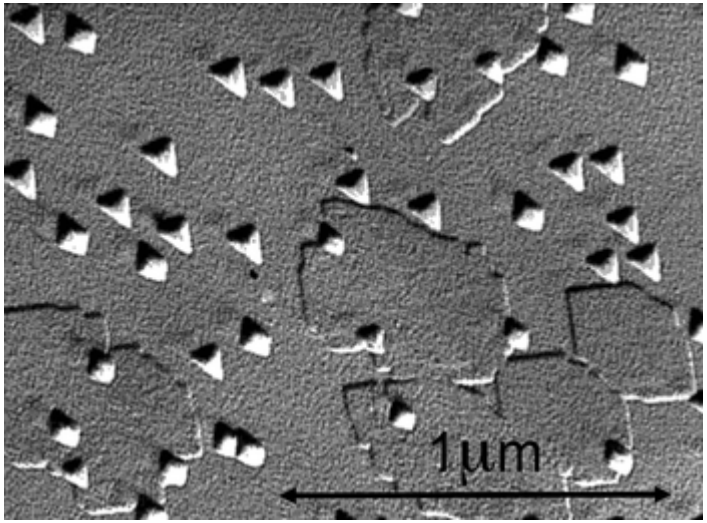
Protects sample and scatters electrons

Molecules are light, with dark edges

Resolution low (15Å)

# Metal shadowing



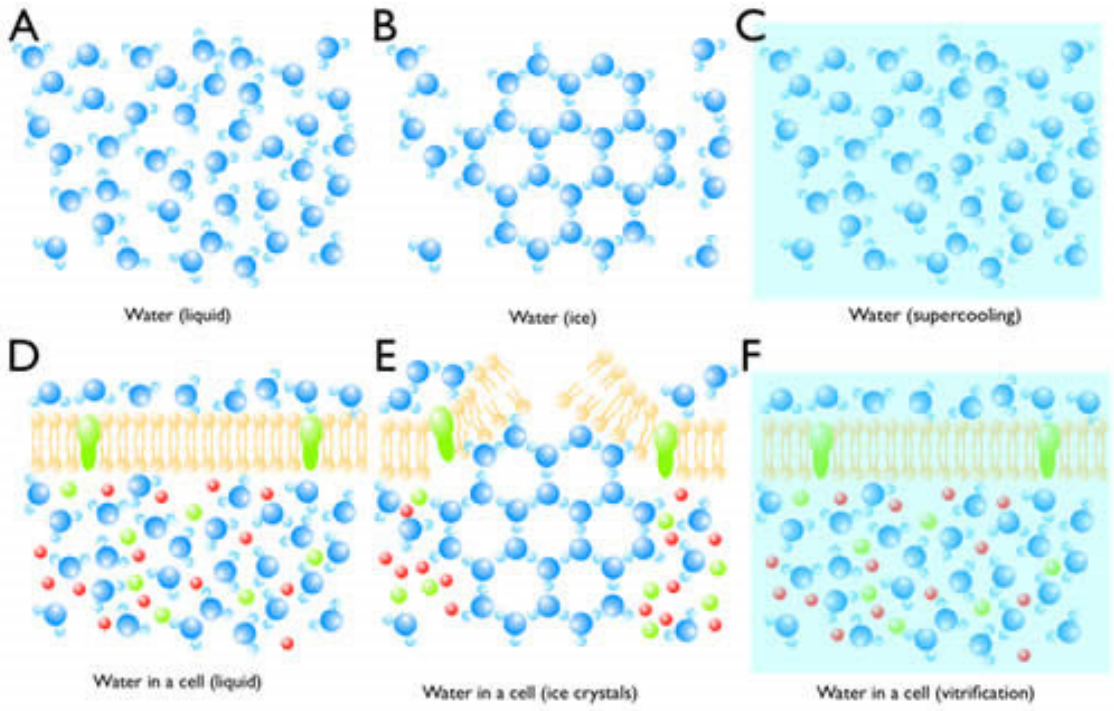

Deposition of a metal film under angle

results in dark "shadow" of sample

very high contrast and direct information on topology of sample

resolution low

# Frozen hydrated samples: Vitrification of water




## Slow freezing:

- formation of hexagonal ice

What are the problems of crystalline ice?

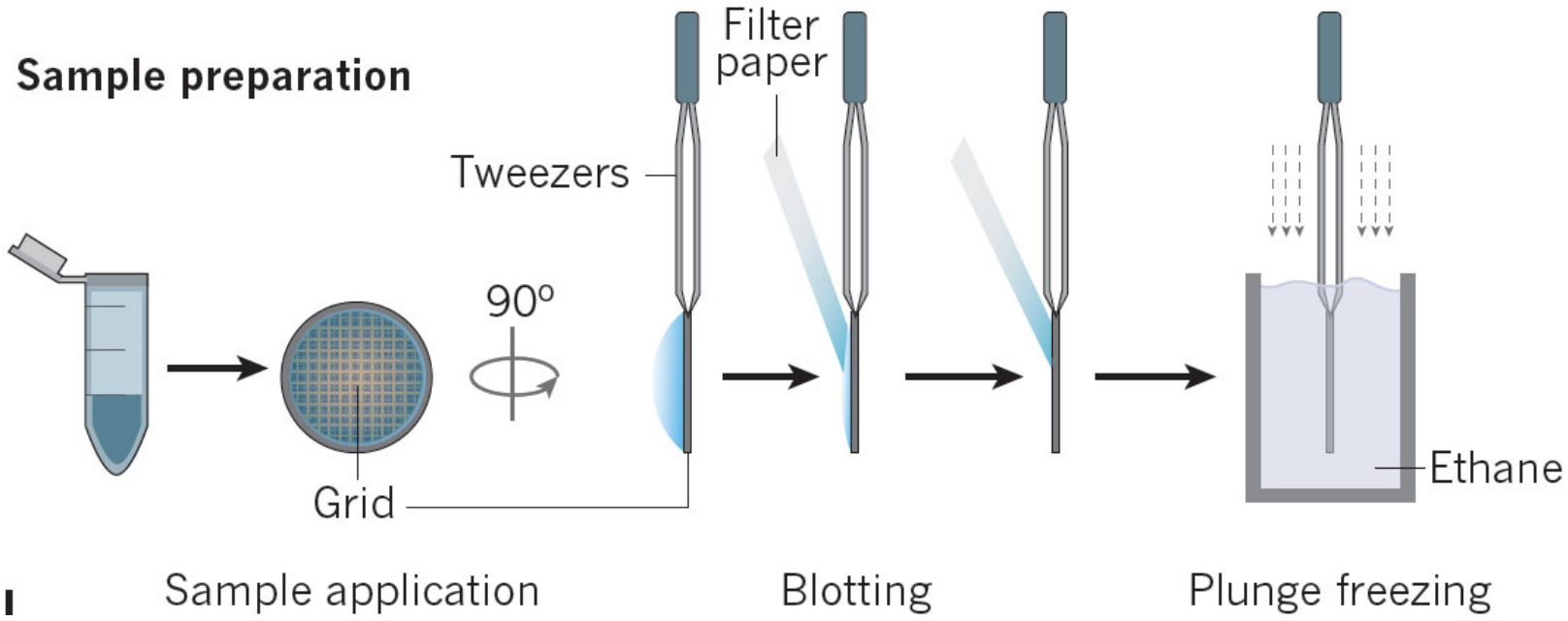
## Very rapid freezing:

- formation of amorphous ice

What are advantages of amorphous ice?

http://advanced-microscopy.utah.edu/education/electron-micro/

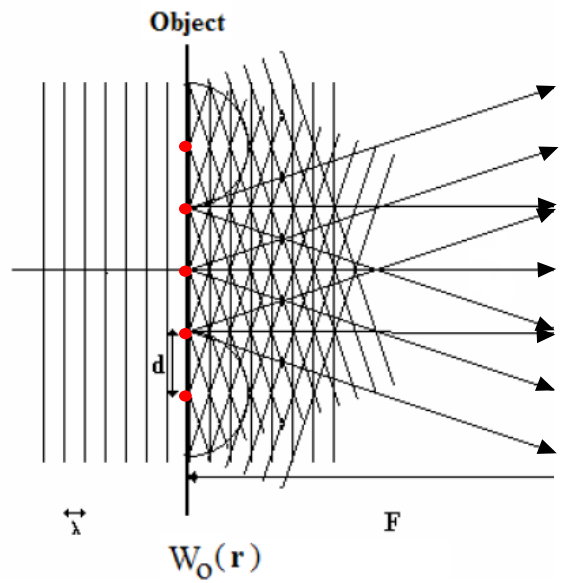
# Vitrification of sample




*Fernandez-Leiro & Scheres Nature 2016*

Plunge-freezing into supercooled ethane (-196$^{O}$ C)
**Cooling rate of $10^5$ $^{O}$C/sec**

# Imaging system




Object

sample
e.g. periodic structure

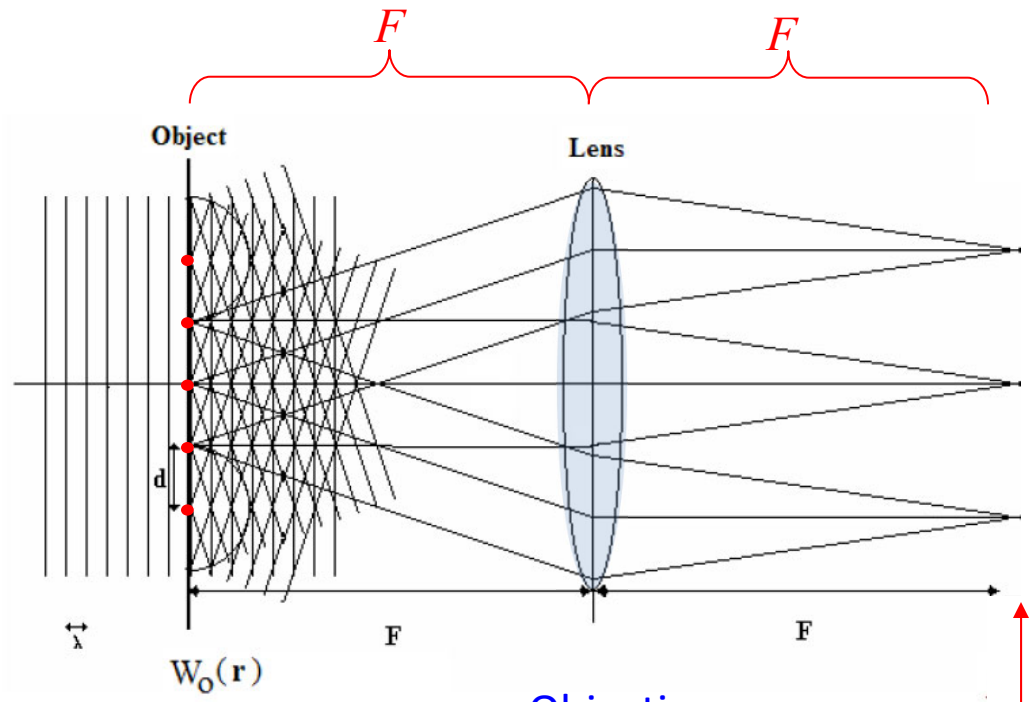
Electrons are scattered on sample

**Diffraction pattern** forms at sufficiently large distance from the object ("infinity")

(see x-ray crystallography)

http://www.singleparticles.org/methodology/MvH_Phase_Contrast.pdf

# Imaging system: Objective lenses




Object

Objective lens

back focal plane

sample
e.g. periodic structure

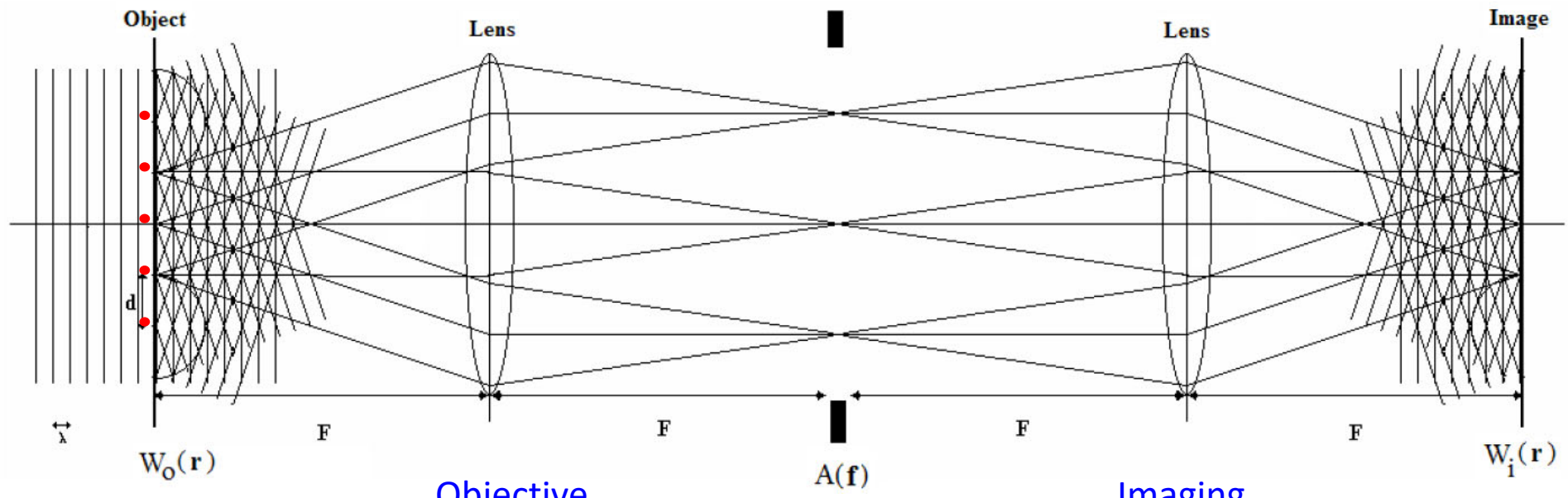
**Objective lens:**

focuses the emerging plane waves on the "backfocal plane"

→ diffraction pattern, **Fourier space**

http://www.singleparticles.org/methodology/MvH_Phase_Contrast.pdf

# Imaging system: Objective lenses




Object

Objective lens

back focal plane

Imaging lens

Image

sample
e.g. periodic structure

http://www.singleparticles.org/methodology/MvH_Phase_Contrast.pdf

**resolution limit (light):**

$$d_{\min} = \lambda / \sin(\alpha_{\max})$$

$$d_{\min} = \lambda / \left(2 \cdot \mathrm{n} \cdot \sin(\alpha_{\max})\right) = \lambda / \left(2 \cdot NA\right)$$

**However in EM:**
contrast very low

# Contrast transfer: Amplitude contrast

## Amplitude contrast

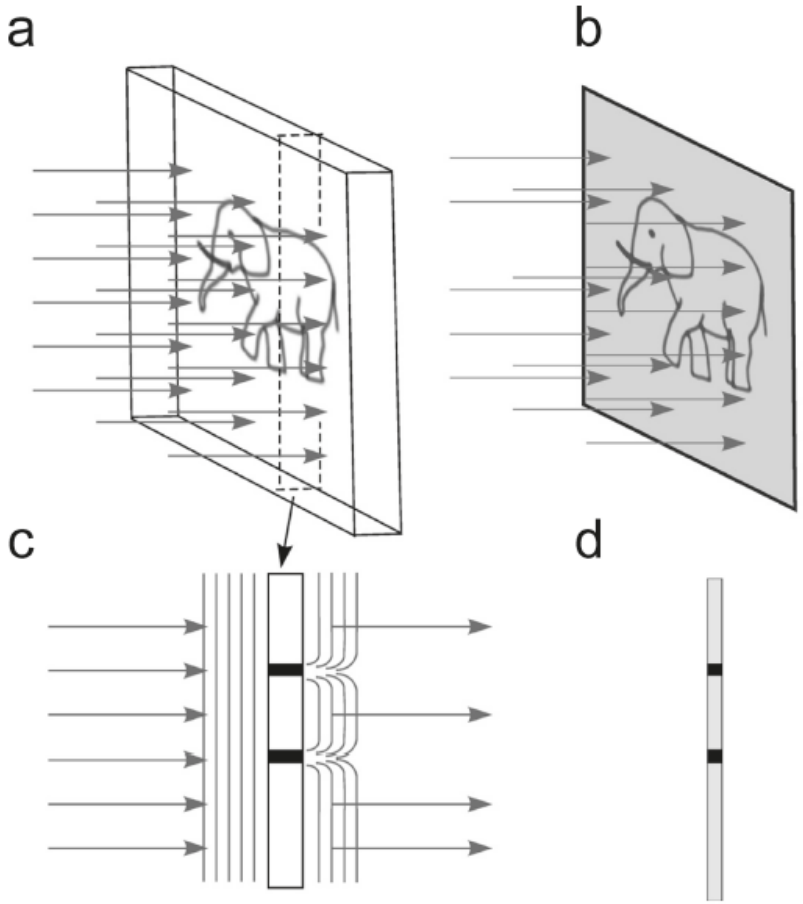
Intensity variations due to variations in specimen transmission

$$\mathrm{Cont_{im}} = \frac{\rho_{\max} - \rho_{\min}}{\overline{\rho}}$$

however, few electrons are actually absorbed by biological specimen!

(contain only H, O, N and C)

*Chem. Rev. 2011, Orlova & Saibil*



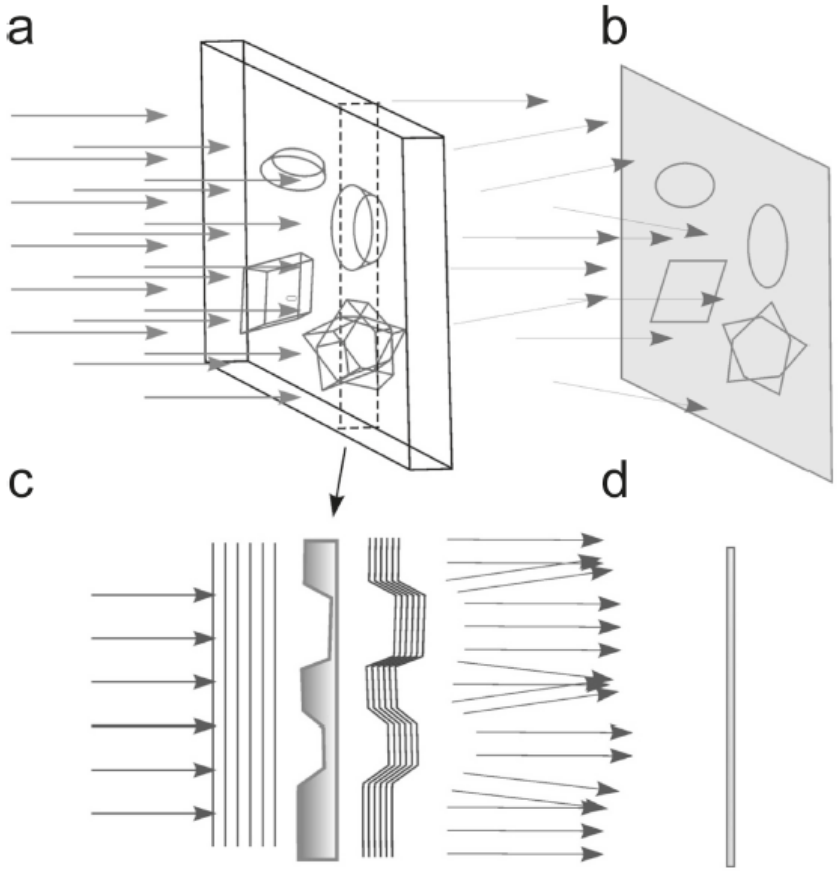
# Contrast transfer: Phase contrast

## Phase contrast

Interaction with material changes pathlength of electrons through material

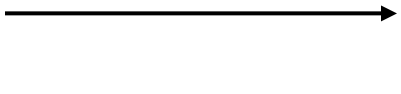
→ phase shifts

→ intereference: Phase contrast

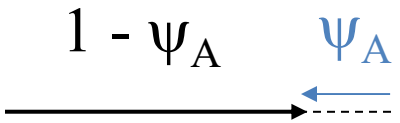



*Chem. Rev. 2011, Orlova & Saibil*

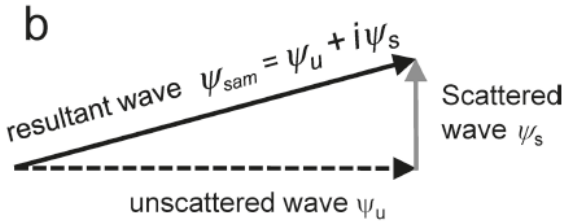
# The phase problem in electron microscopy



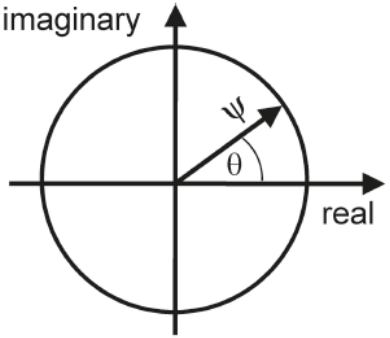
**incident beam**
plane wave described by unit vector.




**Amplitude contrast**
energy absorption




**Phase contrast**
Phase object changes the relative phase of the incident wave

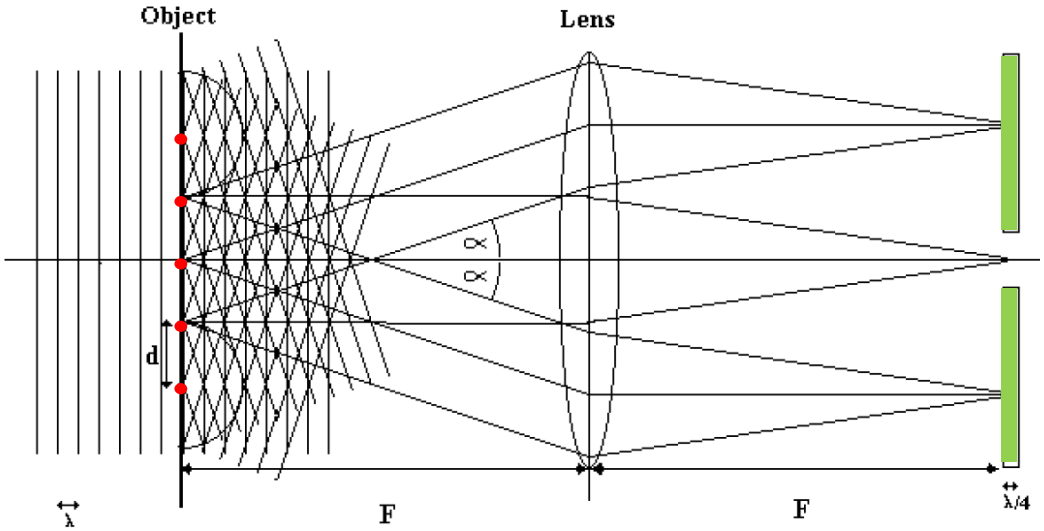



$$O(\mathbf{r}) = \psi_A(\mathbf{r}) \cdot e^{-i\psi_{Ph}(\mathbf{r})}$$ Phase object

amp. variation — phase variation

$$I(\mathbf{r}) = O(\mathbf{r})^2 = \psi_A(\mathbf{r})^2$$ Observed

# Phase contrast microscopy

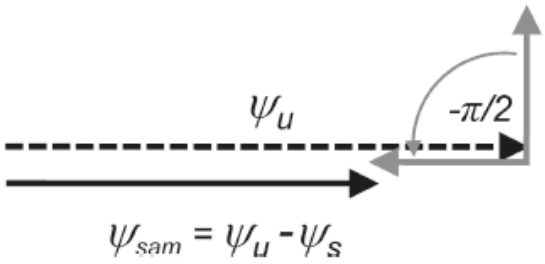



Object

Objective lens

back focal plane

phase plate changes phase of diffracted beams by λ/4

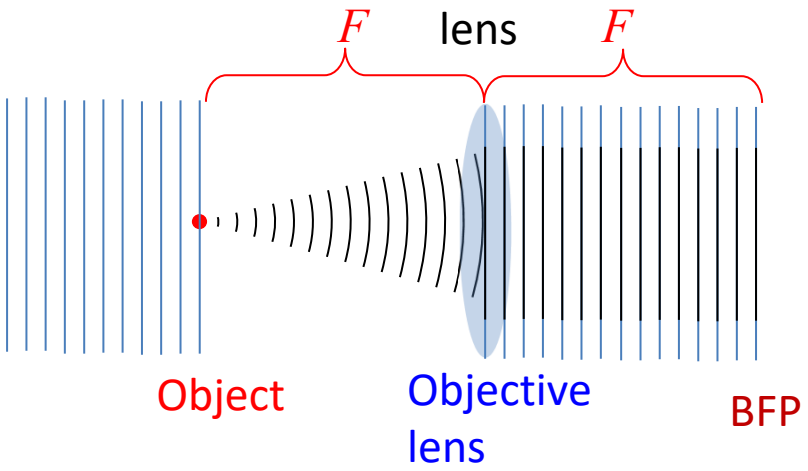



(λ = 2π)

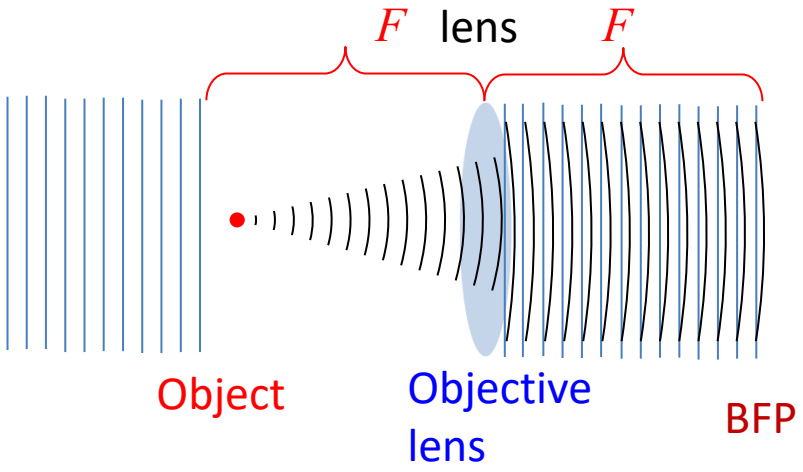
contrast enhancement

http://www.singleparticles.org/methodology/MvH_Phase_Contrast.pdf

# EM: Phase contrast by defocusing

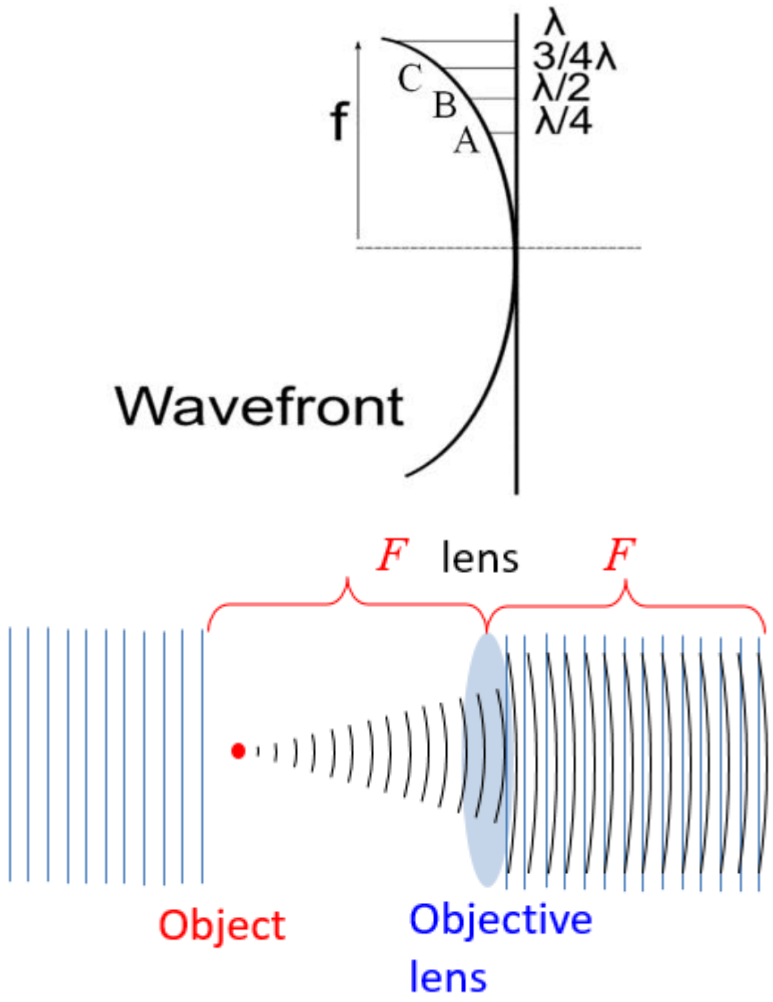



**focus**, no contrast

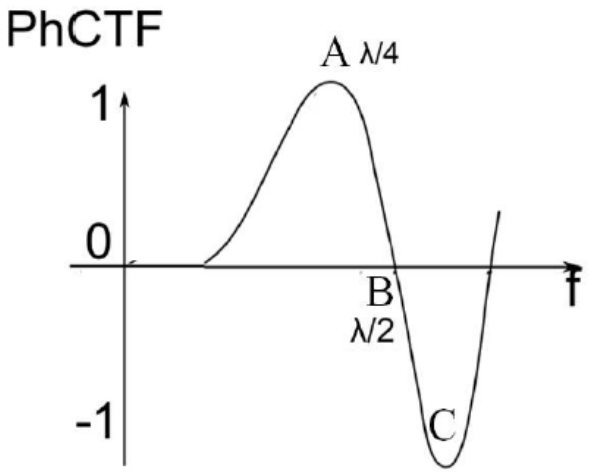



**underfocus (or overfocus)**
phase contrast due to interference

# EM: Phase contrast by defocusing

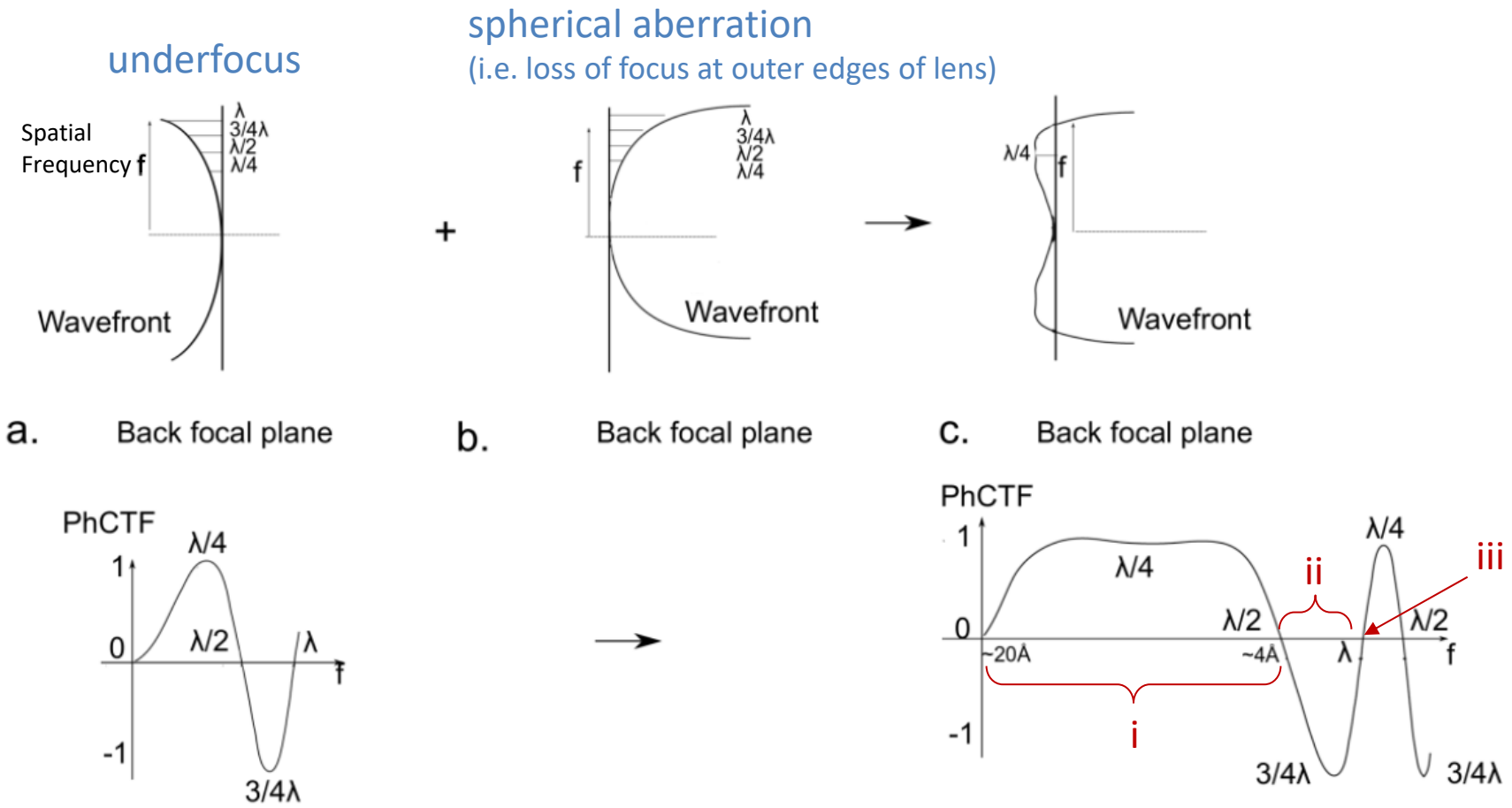



## Phase contrast transfer function




**underfocus (or overfocus)**
phase contrast due to interference

# EM: Defocusing + Aberrations

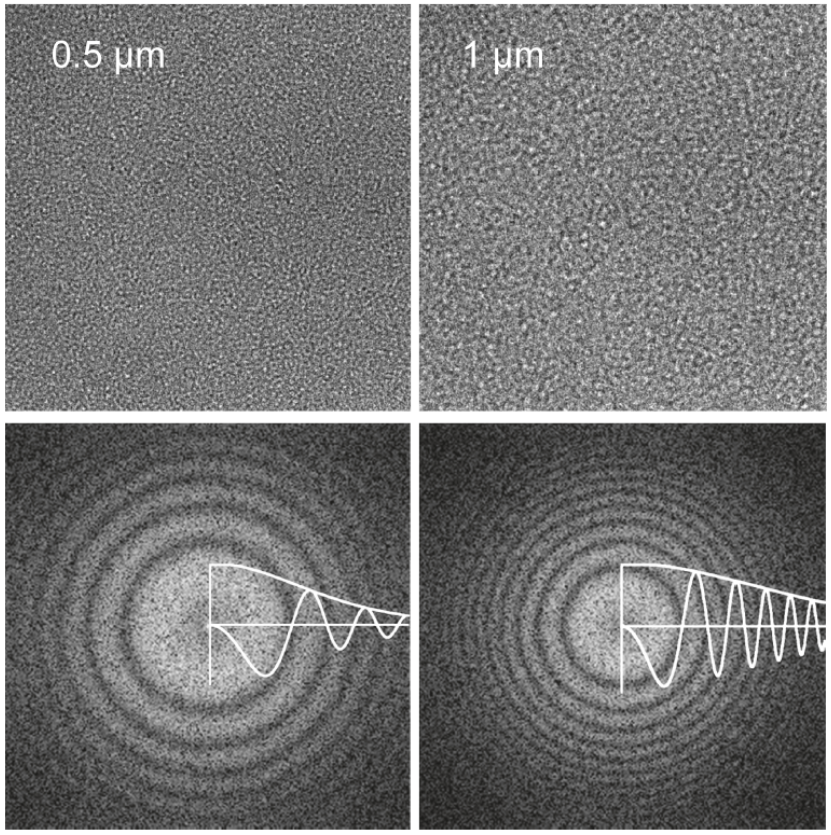



http://www.singleparticles.org/methodology/MvH_Phase_Contrast.pdf

What is the effect on the image in regions i, ii, iii ?

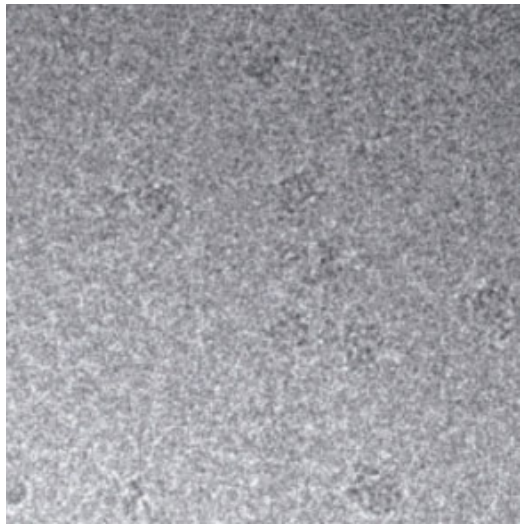
# EM: Phase contrast by defocusing

defocus




*Chem. Rev. 2011,*
*Orlova & Saibil*

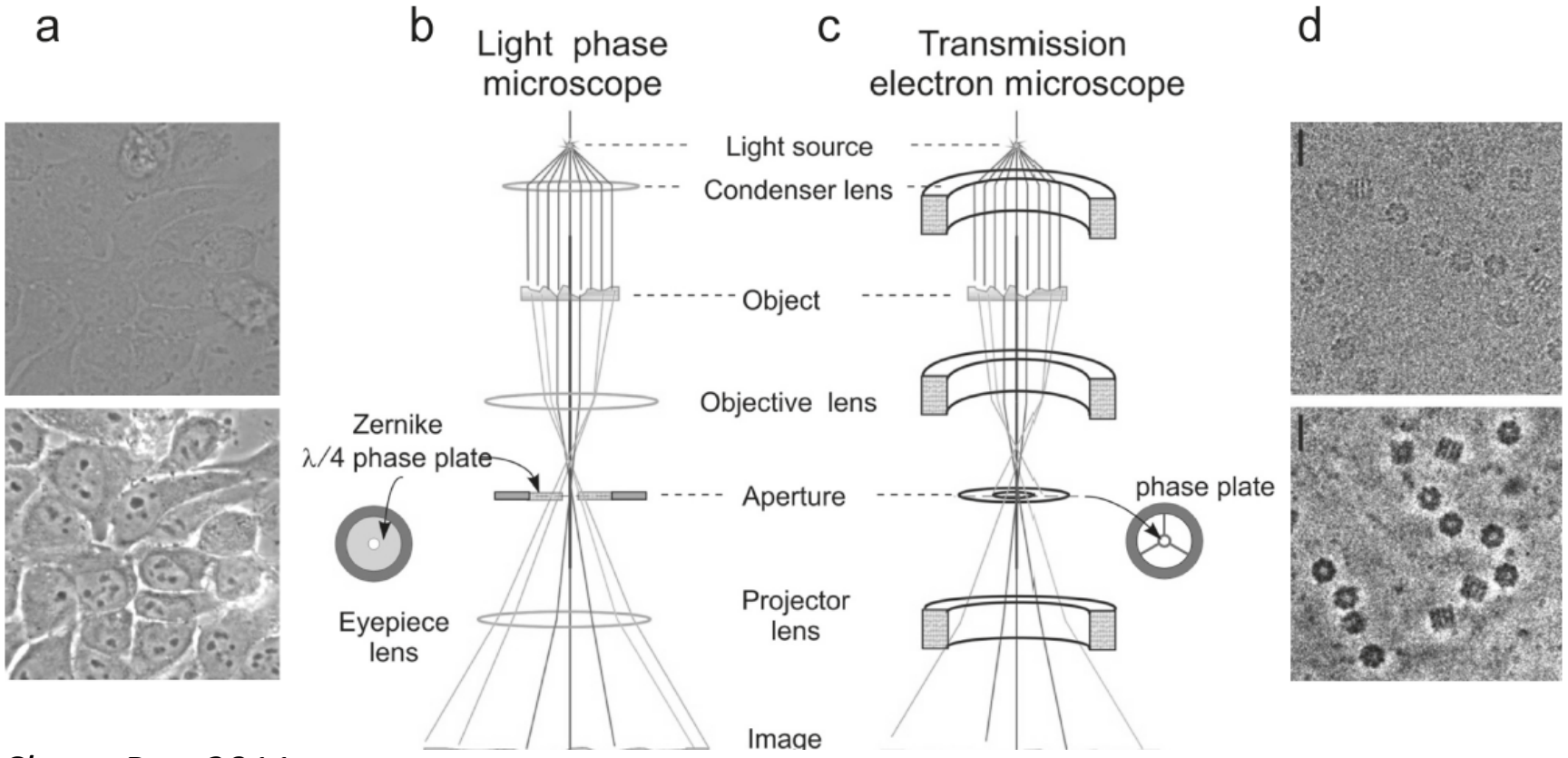
Images of **carbon films**
- their diffraction patterns
- Thon rings
- corresponding CTF curves



biological specimens

doi:10.1073/pnas.0800867105

# Use of phase plates in cryoEM




*Chem. Rev. 2011, Orlova & Saibil*

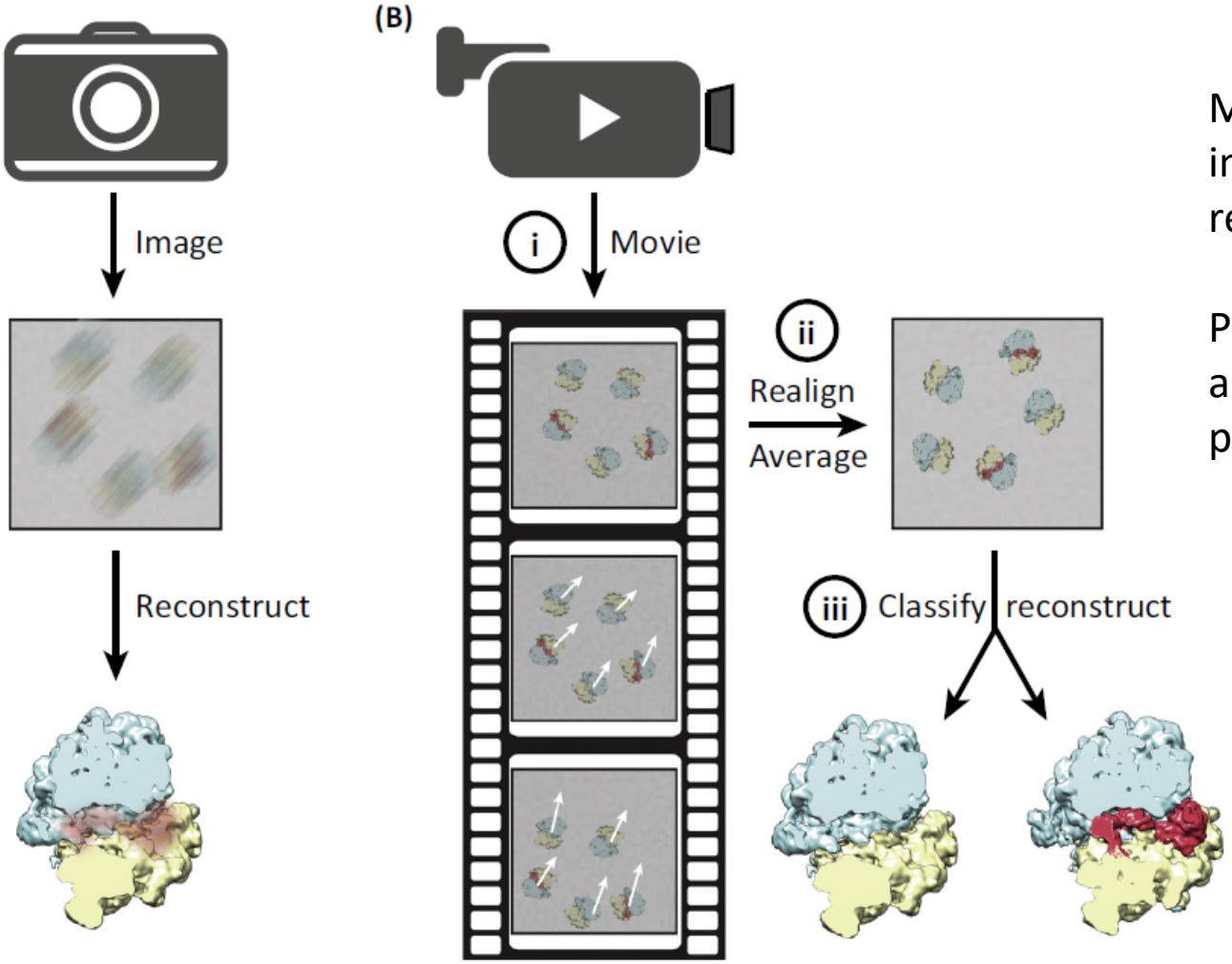
# Electron detection: Cameras


Gatan K2 direct electron detection camera

## Direct electron detection cameras:

- enables rapid electron detection with high detection efficiency
- movies at high frame rates can be recorded
- this allows alignment of sample images, to correct for electron beam induced motions

# Motion correction from Cryo EM images

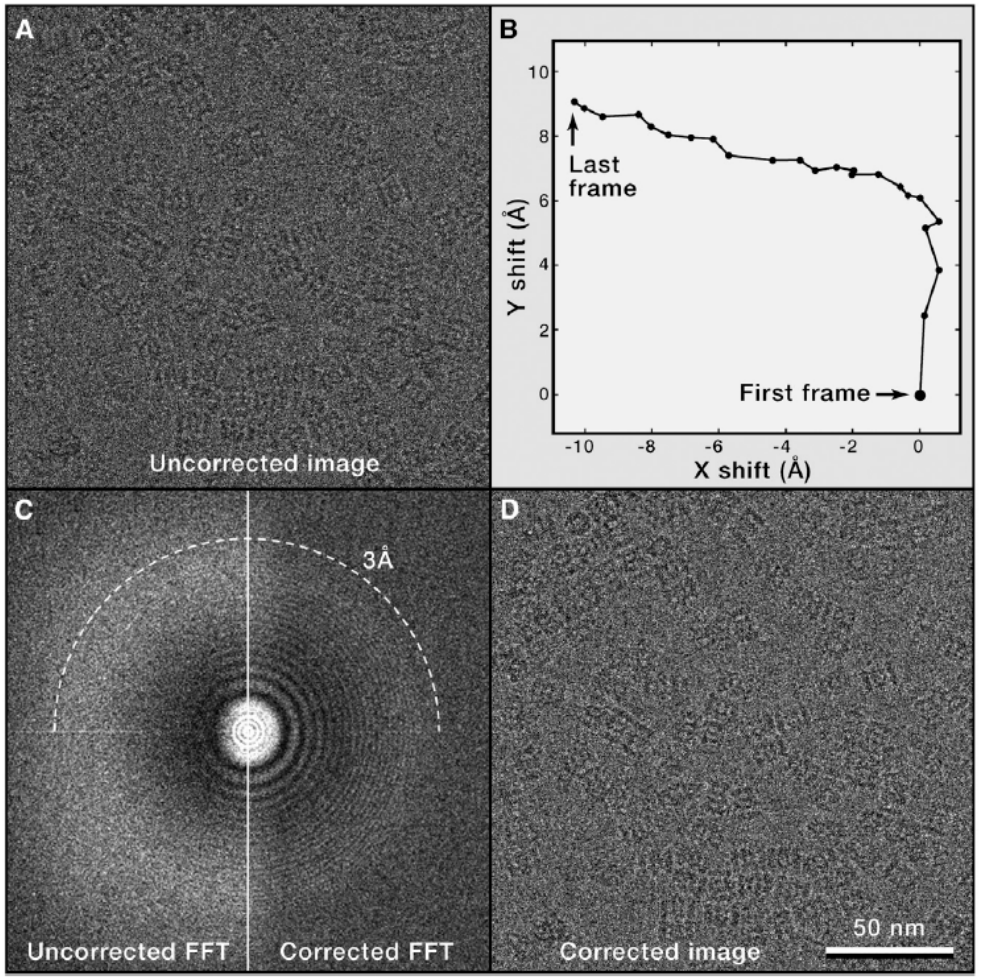



Motion correction improves resolution

Powerful algorithms enable particle averaging

Bai et al. TIBS 2015

# Motion correction from Cryo EM images



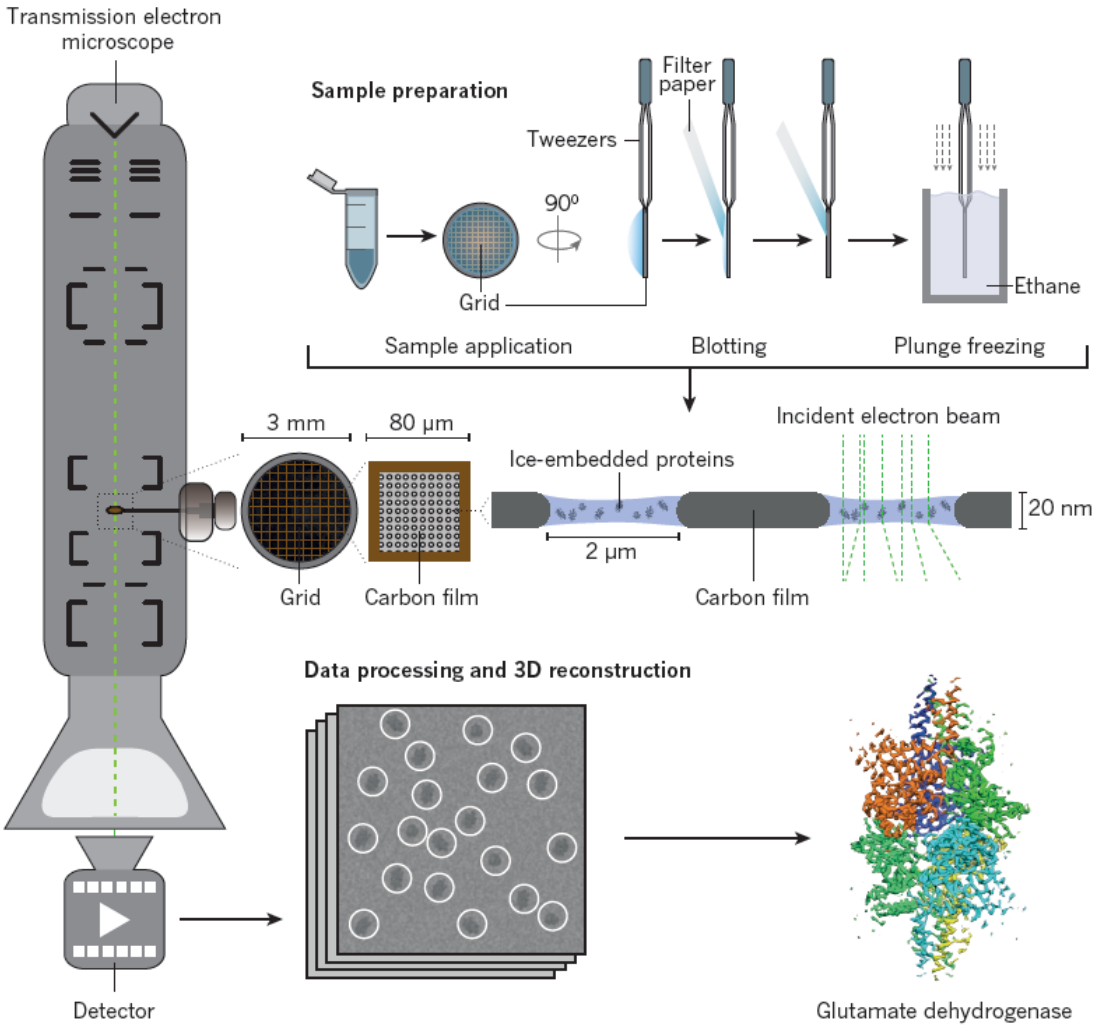

cryoTEM image of proteasomes

Sum or raw movie frames shows lower resolution due to heat induced sample drift

Frame-by-frame correction improves resolution (as seen in the power spectrum)

*Cheng et al., Cell 2015*

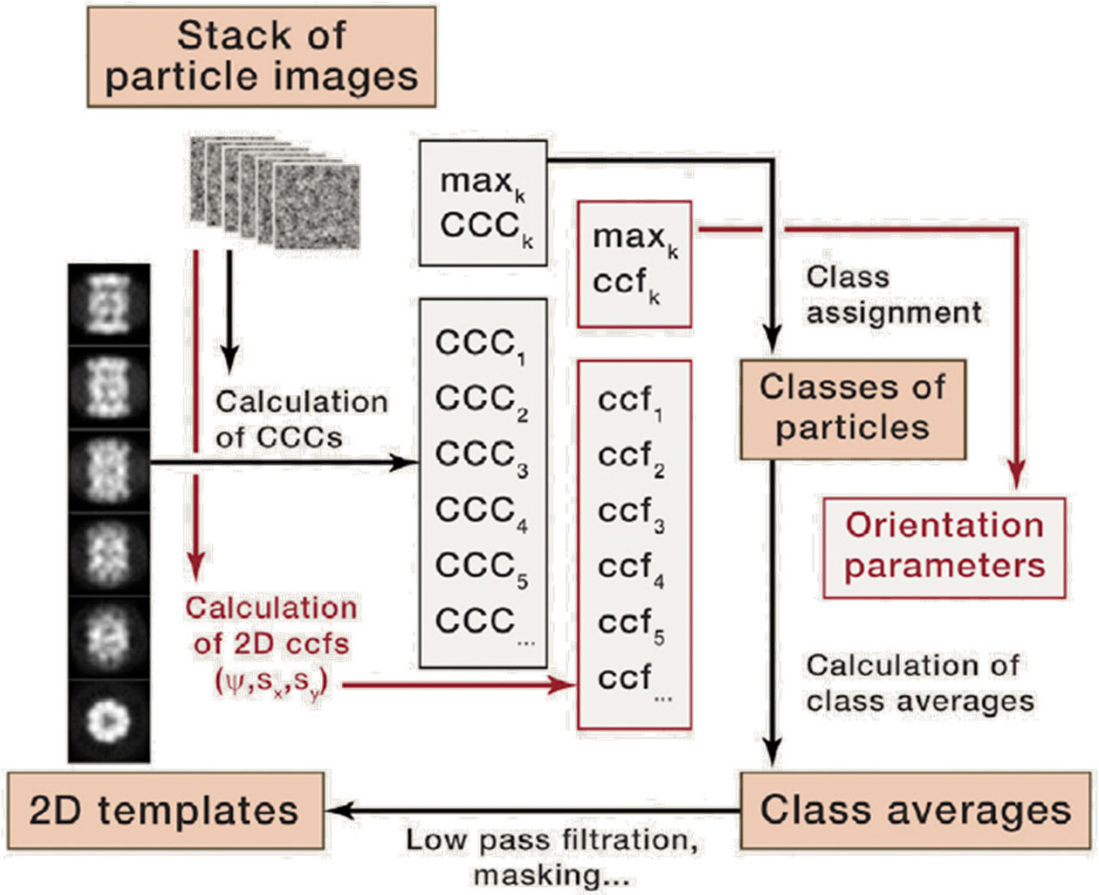
# Structural biology using cryo TEM




- Sample vitrification
- Imaging of time series of individual protein particles
- Particles are randomly oriented in ice
- Computer processing of images
- Particle classification due to orientation and structural state
- averaging of individual particles
- Strucure reconstruction

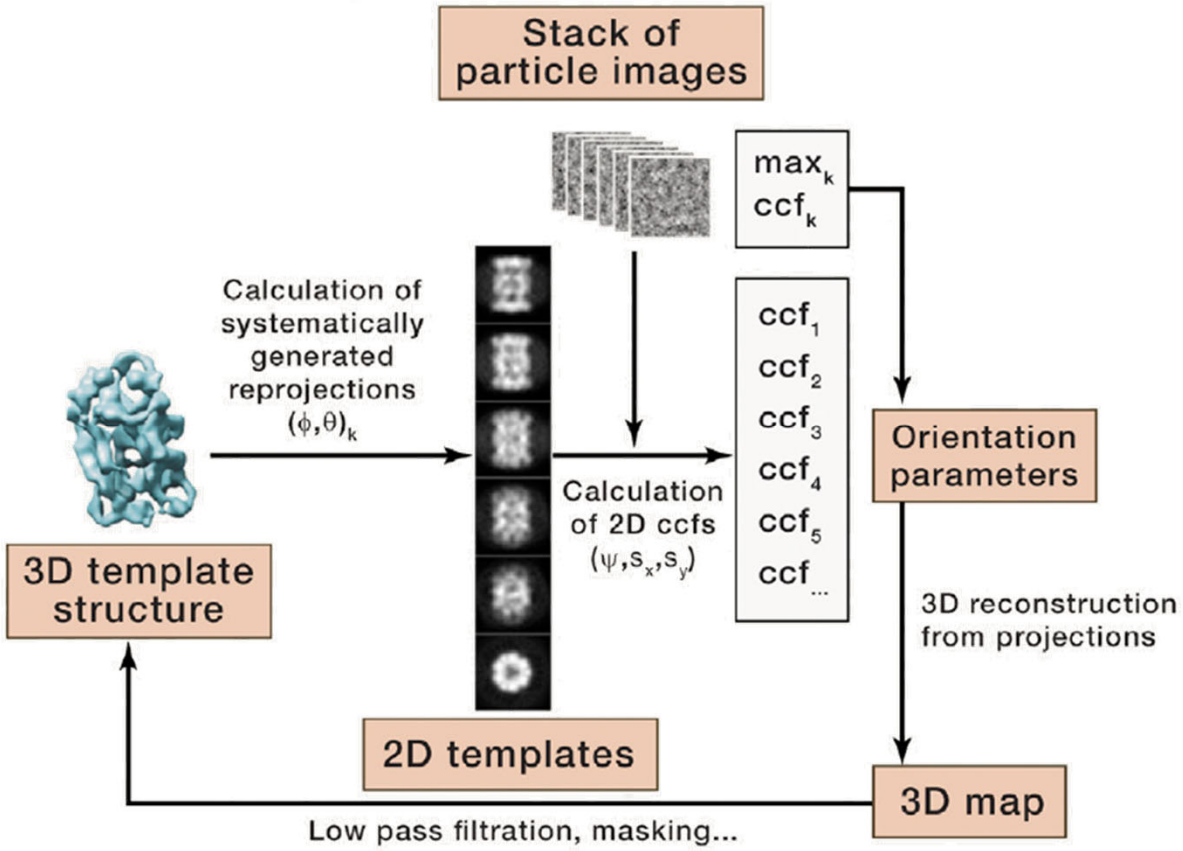
*Fernandez-Leiro & Scheres Nature 2016*

# 2D particle averaging in single-particle cryoEM




- Particles are picked to define initial views of a protein
- Selected particles are combined into stacks
- Automated alignment of different 2D views of particles
- Large datasets are automatically processed
- Iterative procedure

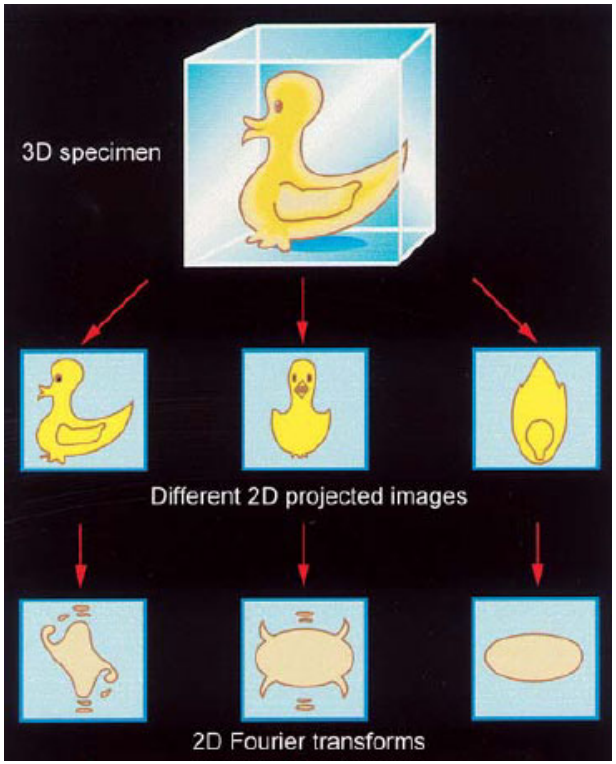
# Ab initio 3D structure calculation

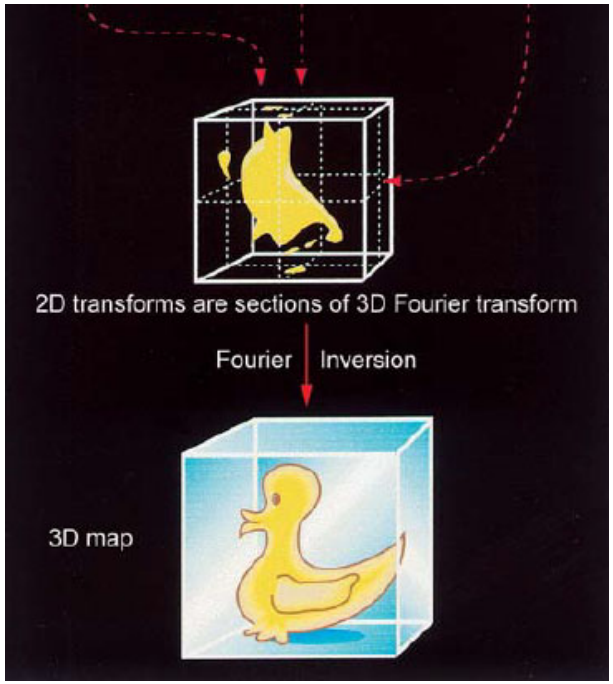



- Use image classes showing the particles from different orientation
- either from randomly oriented particles
- or from tilt-series (sample orientation is systematically changed in multiple exposures)
- From known angles, the 2D images are employed for 3D reconstruction

# 3D reconstruction via Fourier transforms

orientation 1 orientation 2 orientation 3

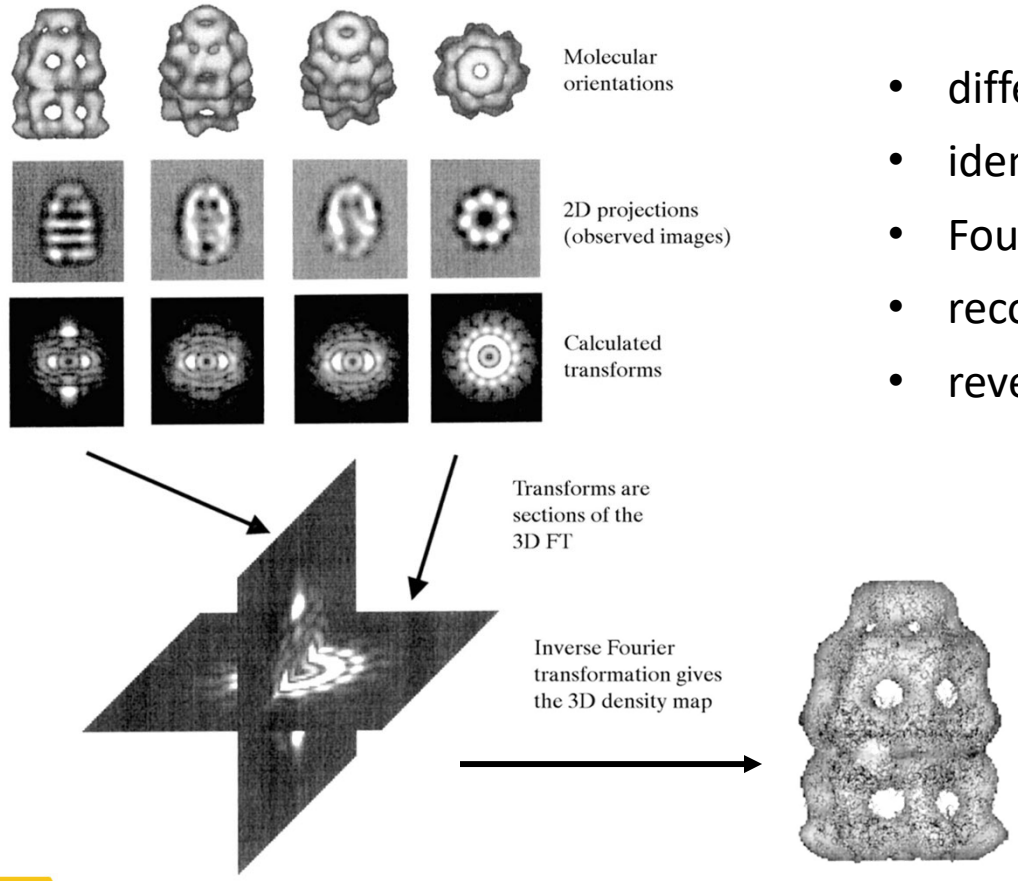



*Baker and Henderlein, 2012*

orientation 1 orientation 2 orientation 3

# 3D reconstruction via Fourier transforms



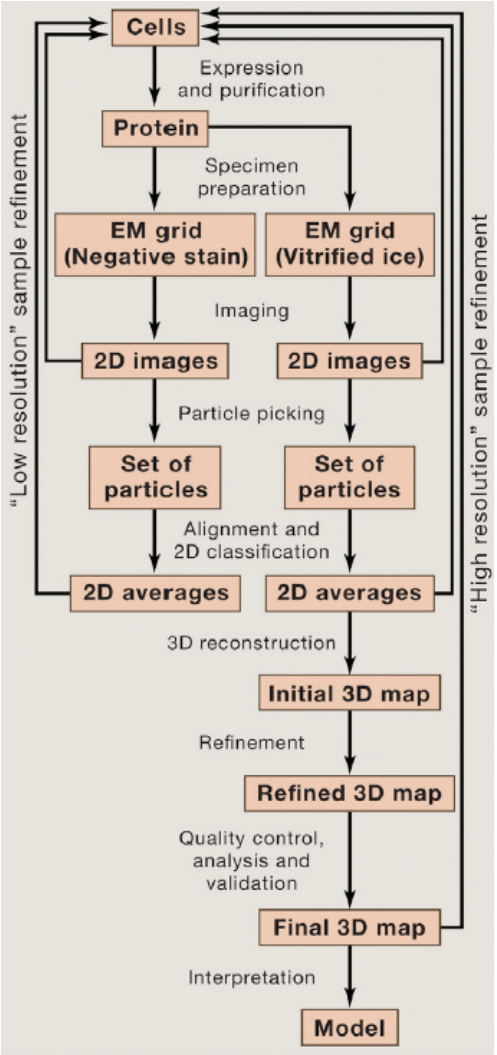

- different orientations
- identifications of the orientations
- Fourier transforms
- reconstruct 3D FT
- reverse 3D FT transform

Sometimes, proteins align with the ice surface. Why is this a problem?
How could this be circumvented?

3D Fourier transform of the structure (DeRosier & Klug)

Acta Cryst D STRUCTURAL BIOLOGY

# General pipeline of structural biology: Compare to X-Ray crystallography




**Sample requirements:**
particles are monodisperse
little aggregation
low degree of heterogeneity

Iterative procedure to obtain better class averages

e.g. alteration of orientation parameters to obtain better match with reprojections calculated from models

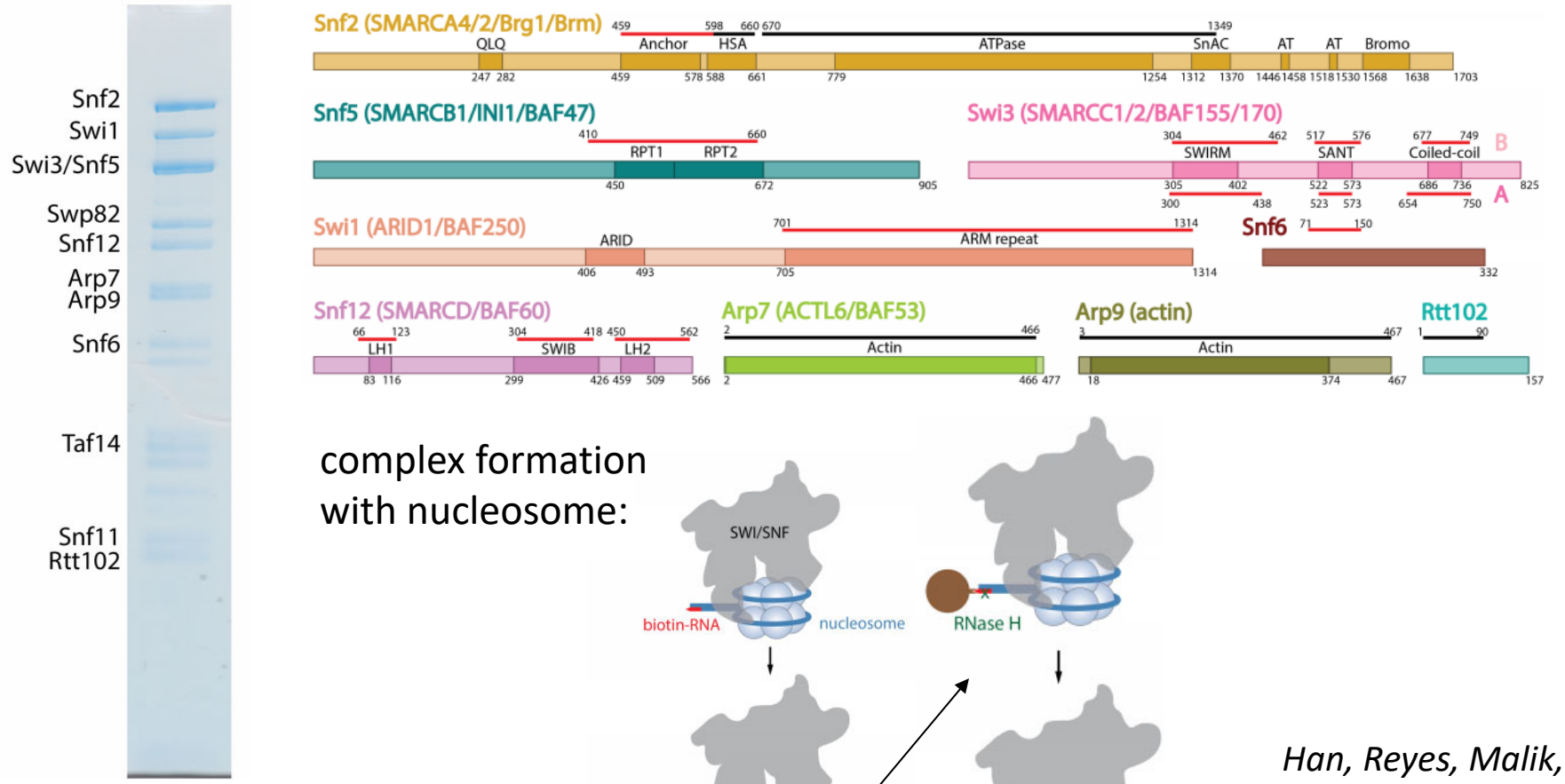
recalculation of maps after refinement to final high resolution model



*Cheng et al., Microscopy Cell 2015*

# Current example: Structure of the chromatin remodeler SWI/SNF bound to a nucleosome

protein expression

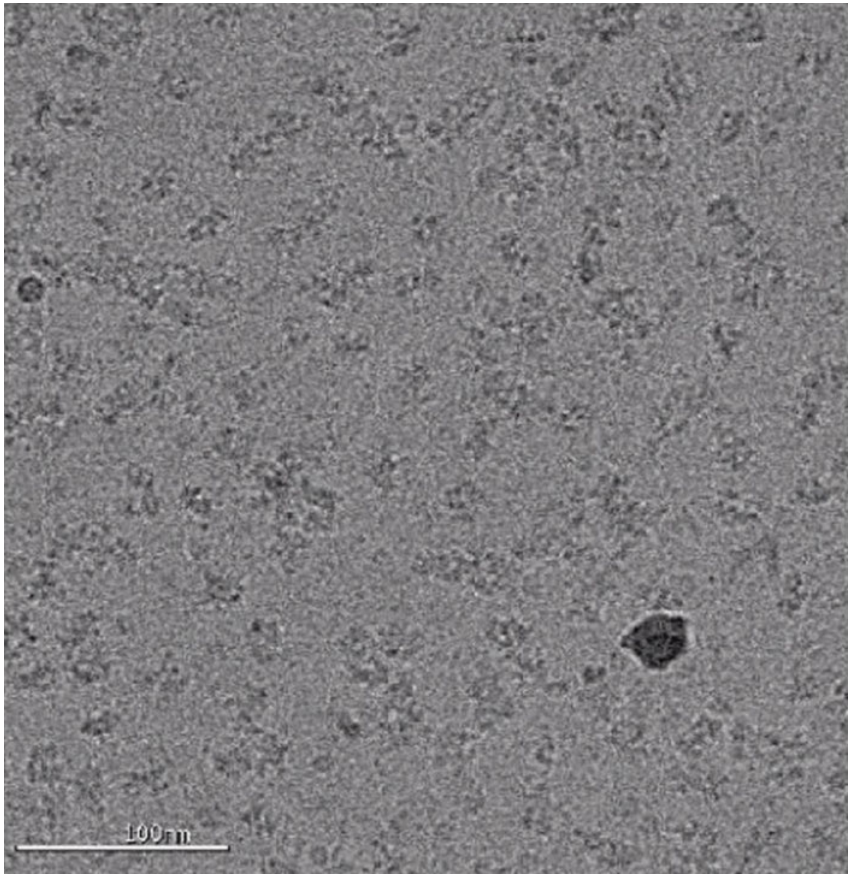


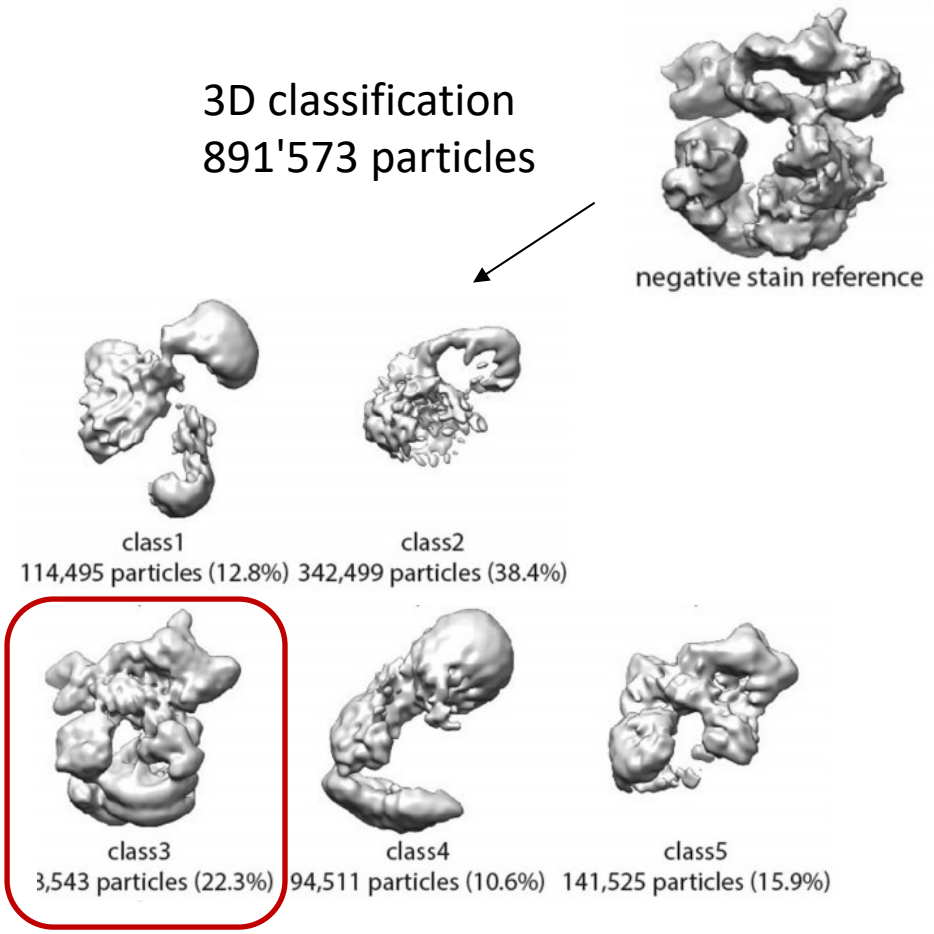

complex formation with nucleosome:




*Han, Reyes, Malik, He*
*Nature 11.3.2020*

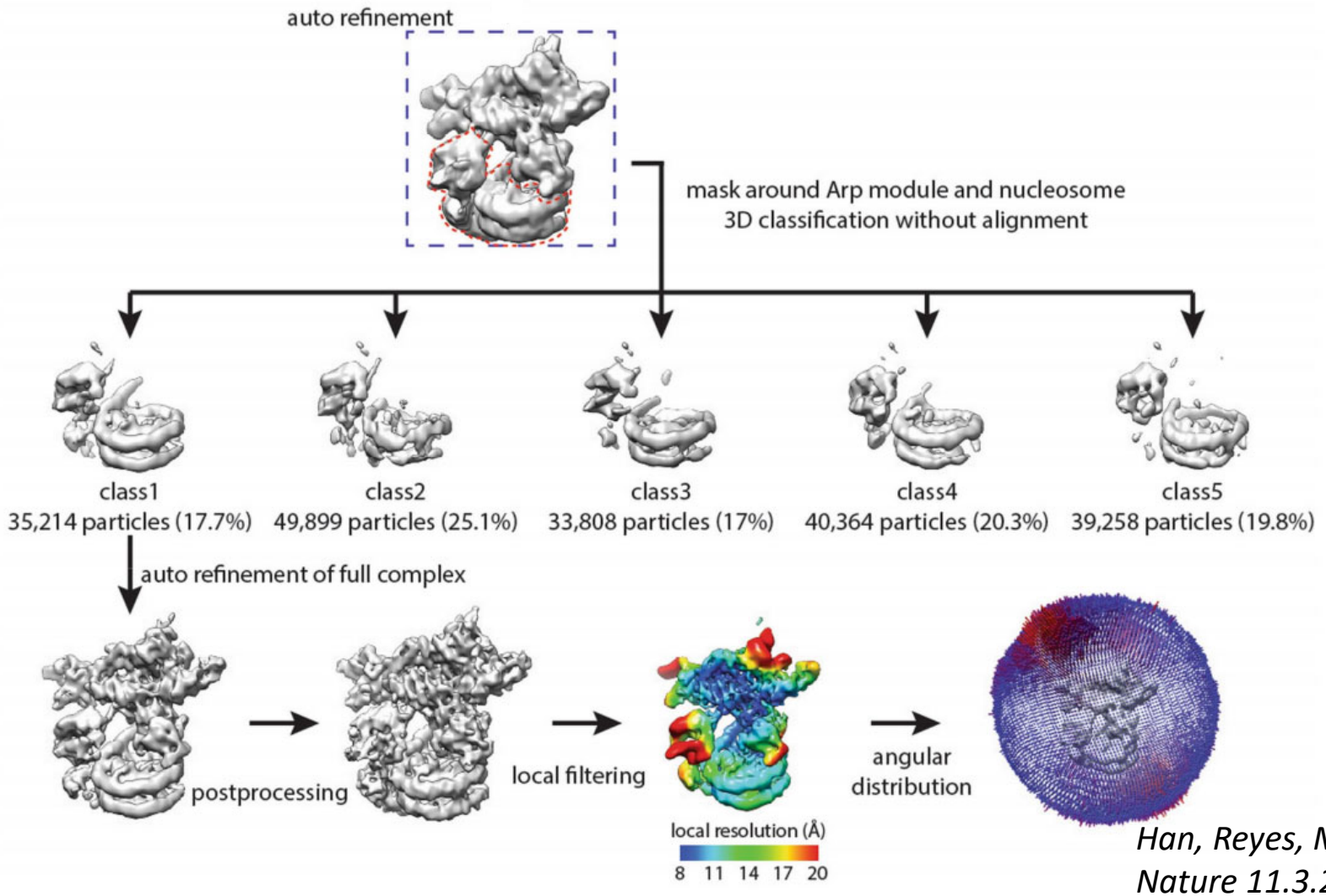
# Current example: Data treatment




*Han, Reyes, Malik, He*
*Nature 11.3.2020*

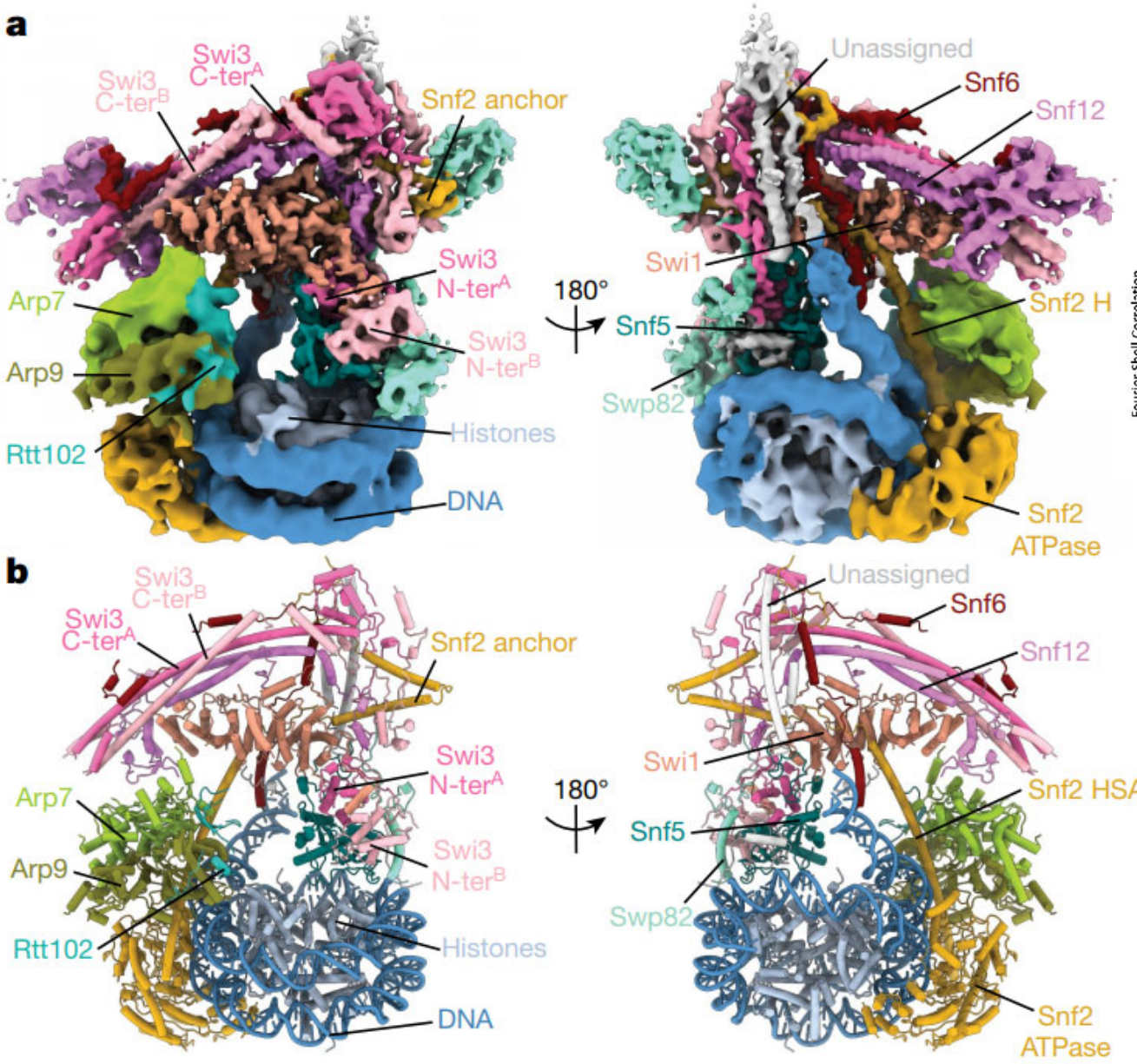
# Current example: Refinement



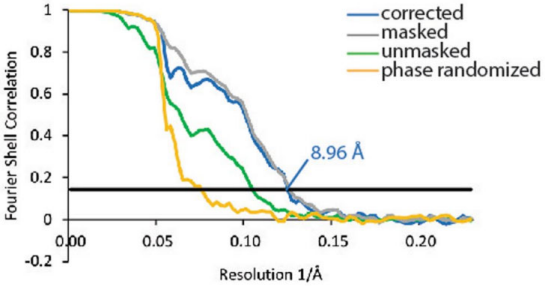

*Han, Reyes, Malik, He*
*Nature 11.3.2020*

# Final structure: SWI/SNF on nucleosome

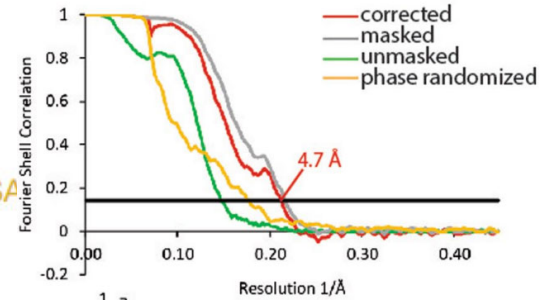
*Han, Reyes, Malik, He; Nature 11.3.2020*




**Full complex:**
resolution 8.9 A

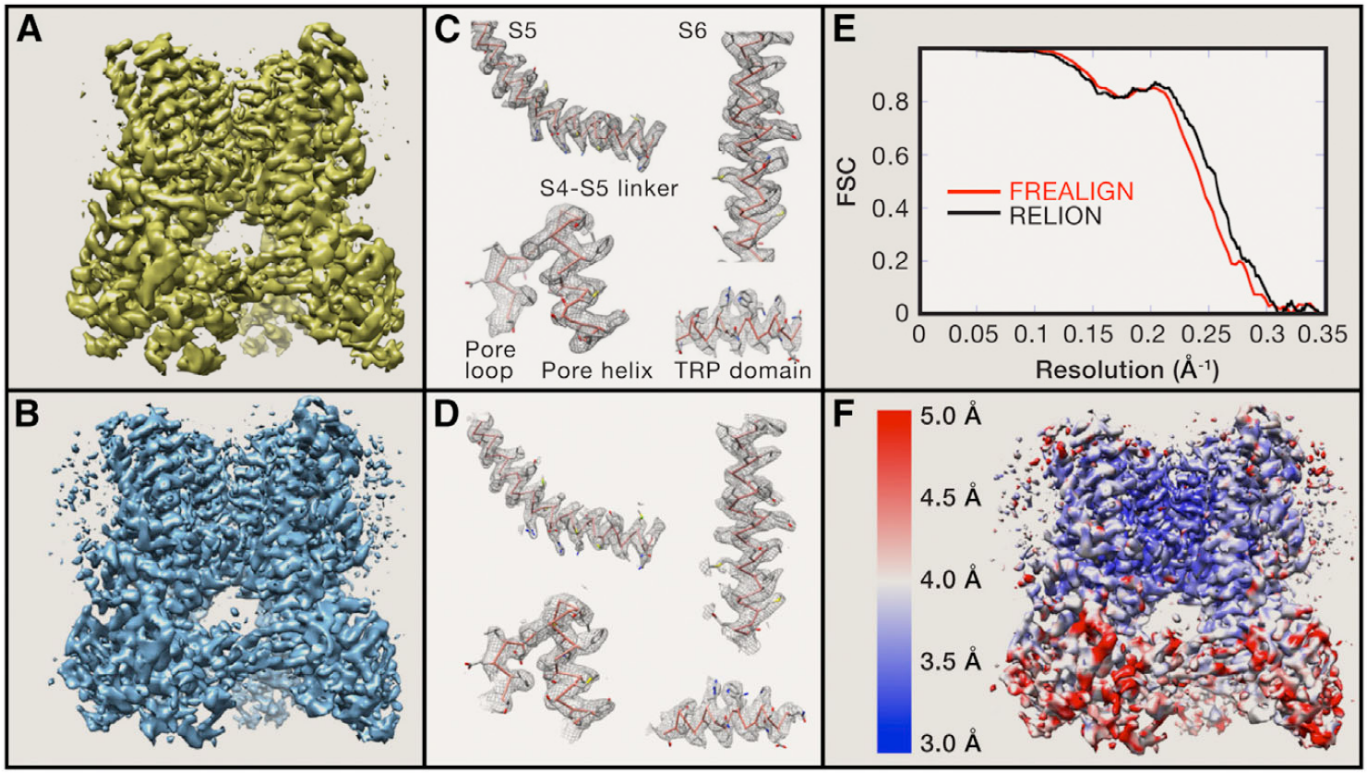



**Body of SWI/SNF:**
4.7A




→ high resolution model

# Quality assessement of structure calculation




*Cheng et al., Microscopy Cell 2015*

Independent structure calculation, i.e. using two algorithms

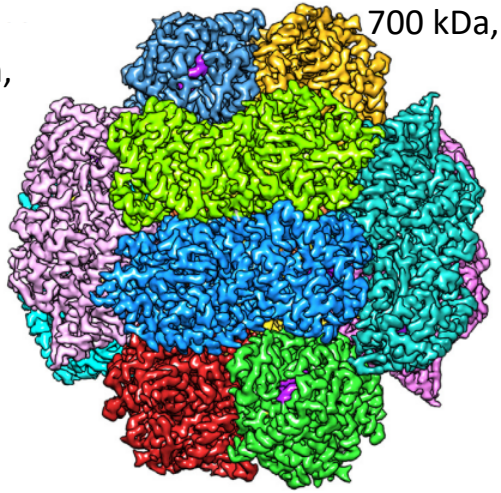
verification of molecular details

Fourier shell correlation curve provides information on S/N (spatial frequency)

Determination of resolution

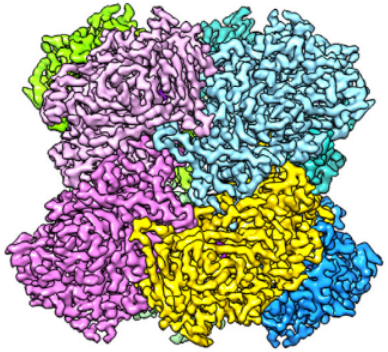
# Some recent examples from the literature

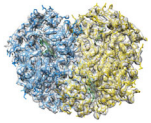
F420-reducing hydrogenase Frh, 1200 kDa 3.4A



20S proteasome, 700 kDa, 2.8A

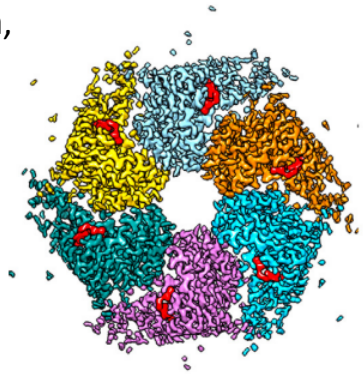
Alcohol oxidase AOX, 600 kDa, 3.4A

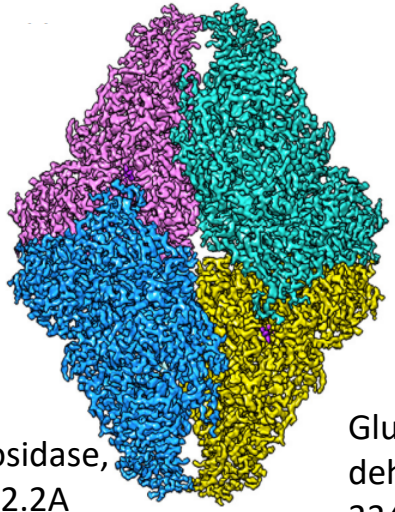




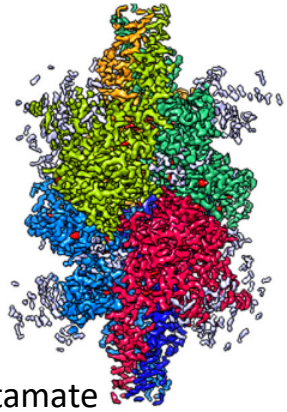
Hemoglobin, 64 kDa, 3.2A

p97, 540 kDa, 2.3A

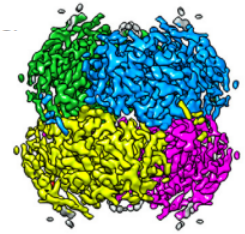




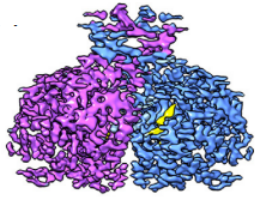
b-Galactosidase, 464 kDa, 2.2A



Glutamate dehydrogenase GDH, 334 kDa, **1.8A**



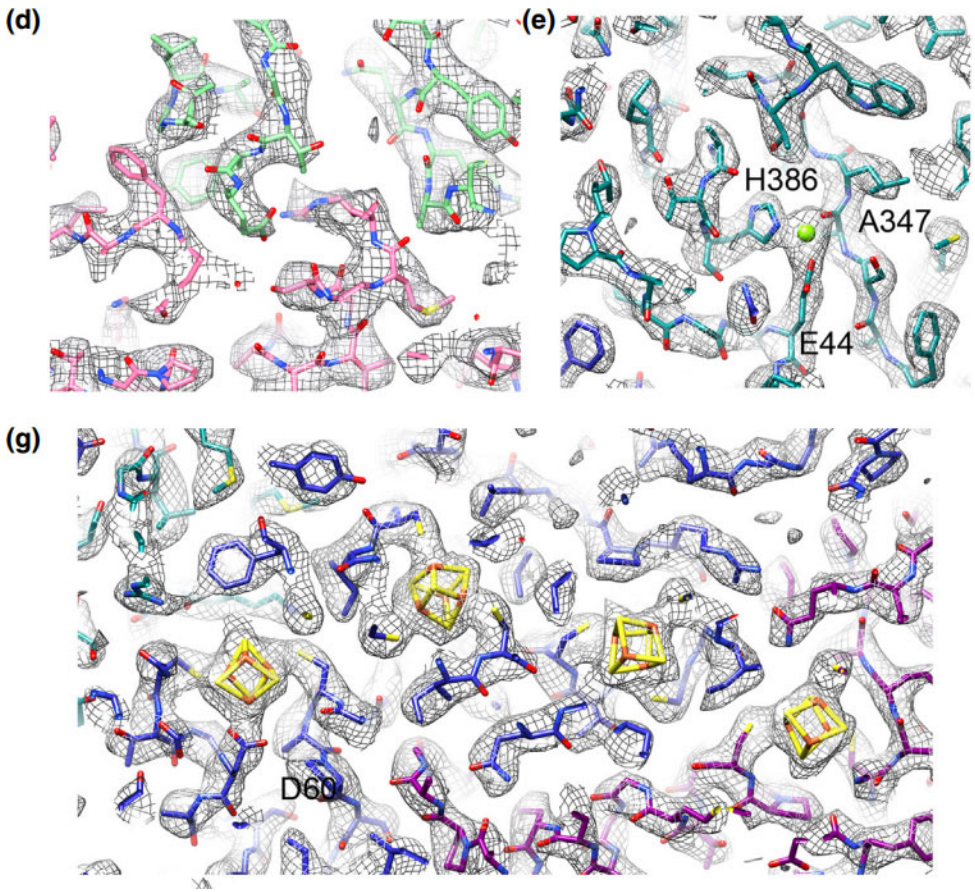
Lactate dehydrogenase LDH, 140 kDa, 2.8A



Isocitrate dehydrogenase IDH, 93 kDa, 3.8A

*Vonck & Mills*
*Curr Opin Struct Biol 2017*

# Details view of structures

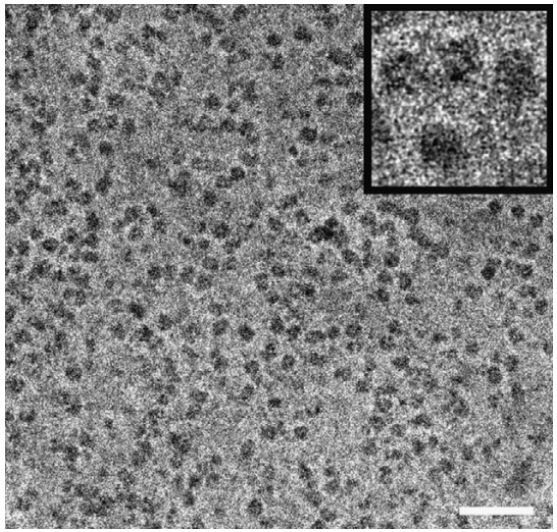


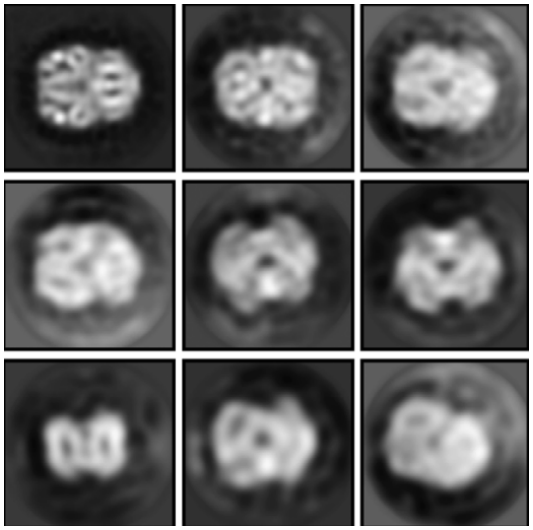

(d) **Intersubunit salt bridge** between Glu656 and Arg214 of AOX (EMD-8072).
(e) **Mg ion (green) in FrhA** is coordinated by the side chains of Glu44 and of the C-terminal His386 and the main chain oxygen of Ala347 (EMD-3518).

**Enzymes cofactors**

(g) At 3 A˚ resolution, the density for the four [4Fe4S] clusters of Frh (EMD-3518) has a tetrahedral shape. Each cluster is coordinated by four cysteine side chains (yellow)
The sulfur atoms of the clusters are visible as bumps between the ligand side chains.

# Smallest cryoEM structure: Hemoglobin 64kDa

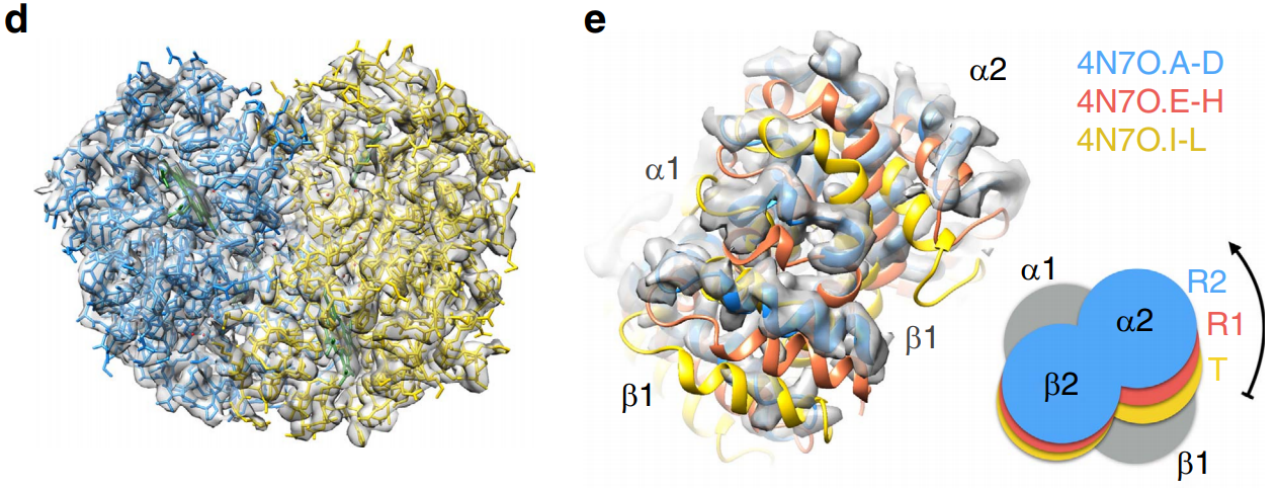




structure of human hemoglobin

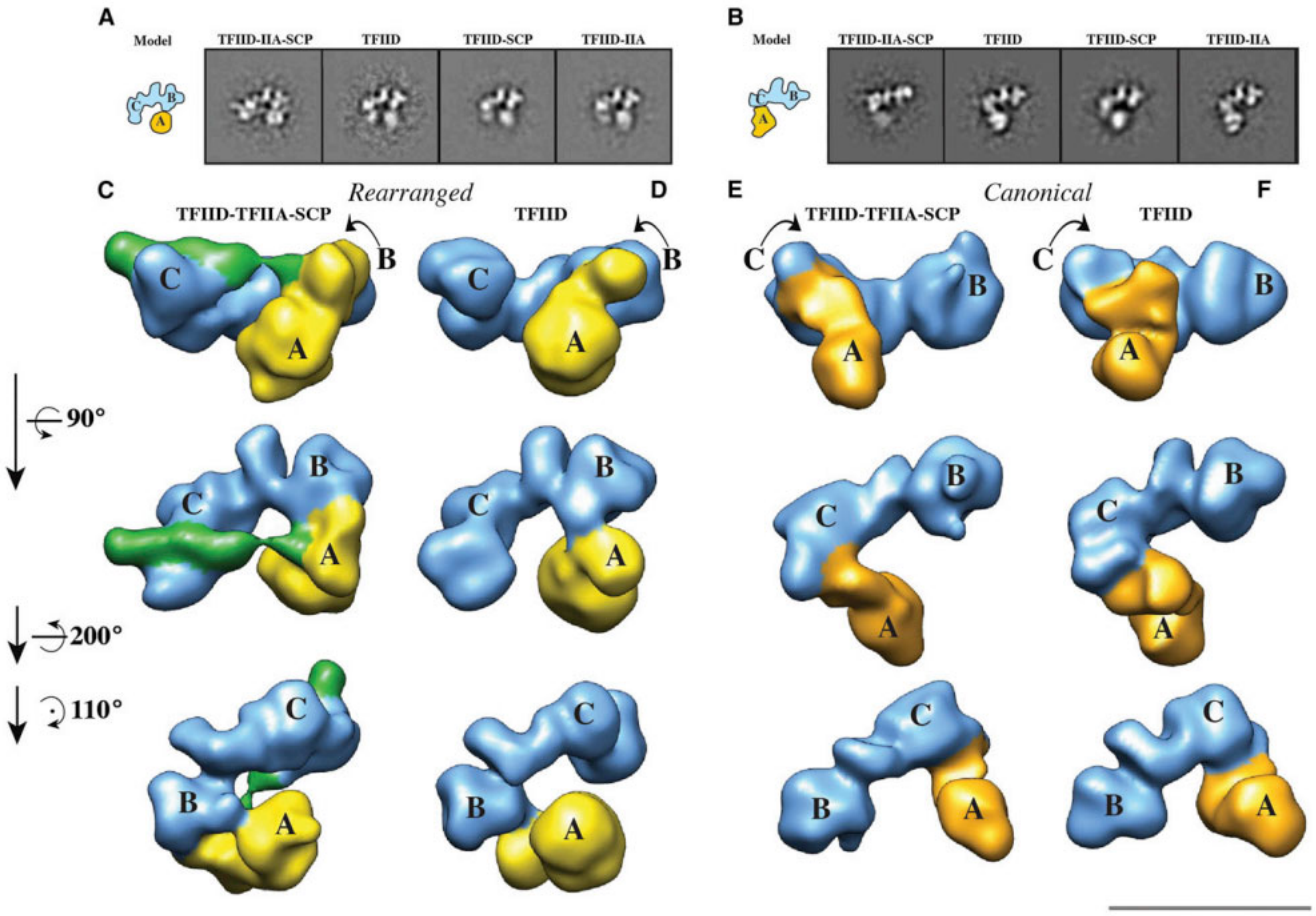
64 kDa

resolution: 3.2 Å




*Koshouei & Baumeister et al., Nature Commun 2017*

# Dynamics from Cryo EM

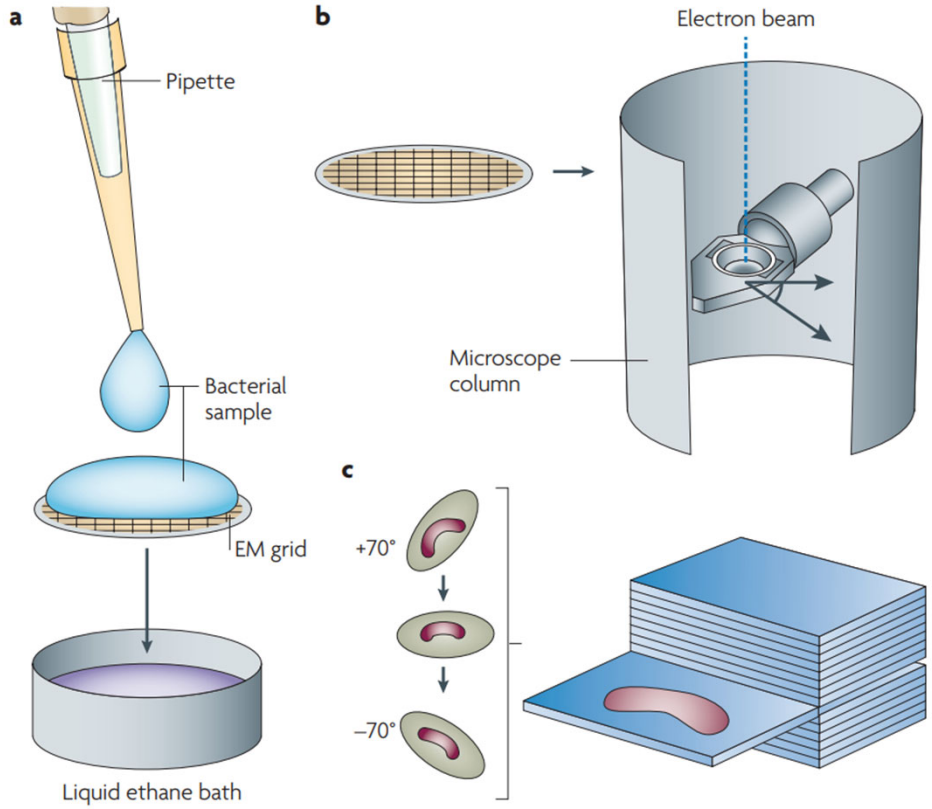



TFIID class averages show dynamic heterogeneity

Protein dynamics can be monitored

*Cianfrocco, Nogales et al., Cell 2013*

# CryoEM tomography




doi:10.1038/nrmicro2183

# CryoEM tomography

CELL NUCLEUS

## Visualizing the molecular sociology at the HeLa cell nuclear periphery

Julia Mahamid,[1]* Stefan Pfeffer,[1] Miroslava Schaffer,[1] Elizabeth Villa,[1,2] Radostin Danev,[1] Luis Kuhn Cuellar,[1] Friedrich Förster,[1] Anthony A. Hyman,[3] Jürgen M. Plitzko,[1] Wolfgang Baumeister[1]*

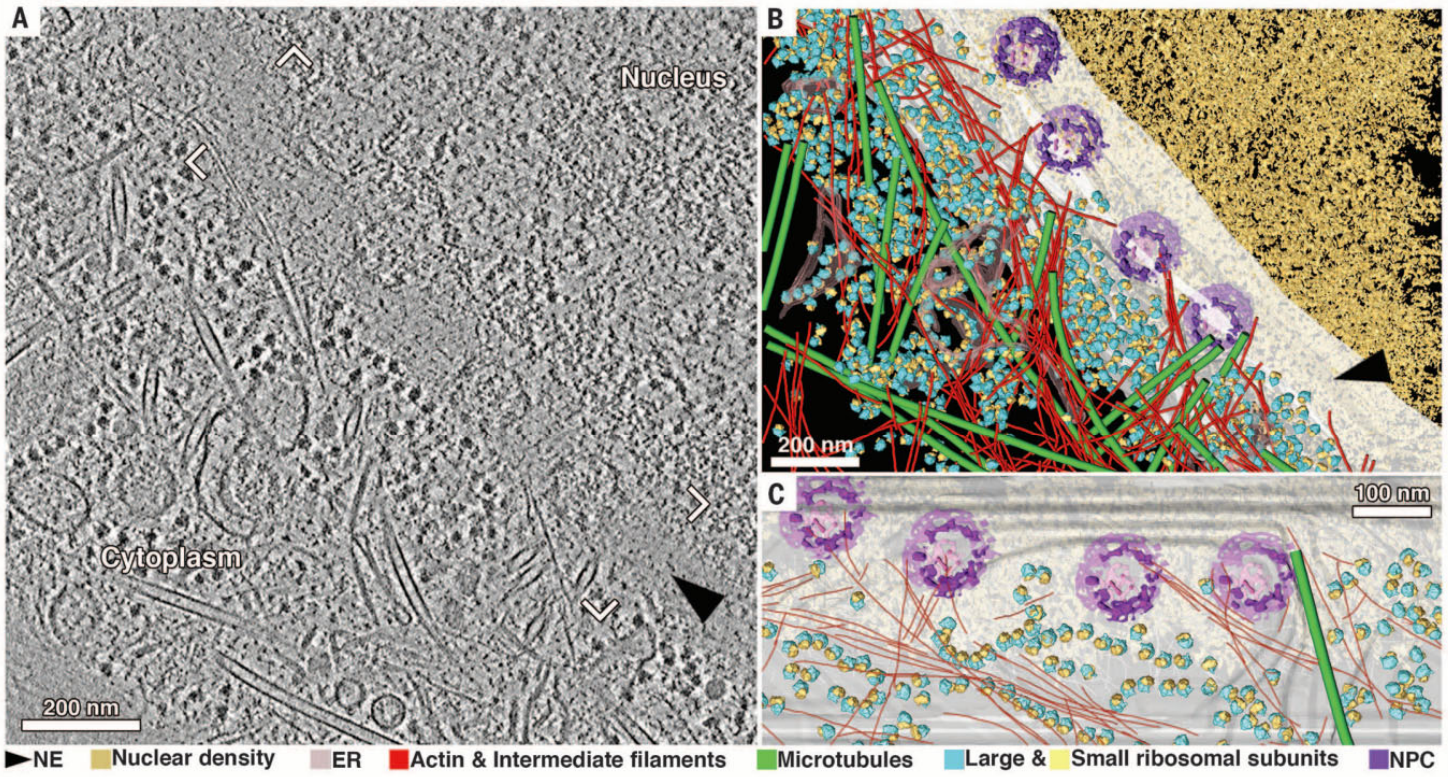


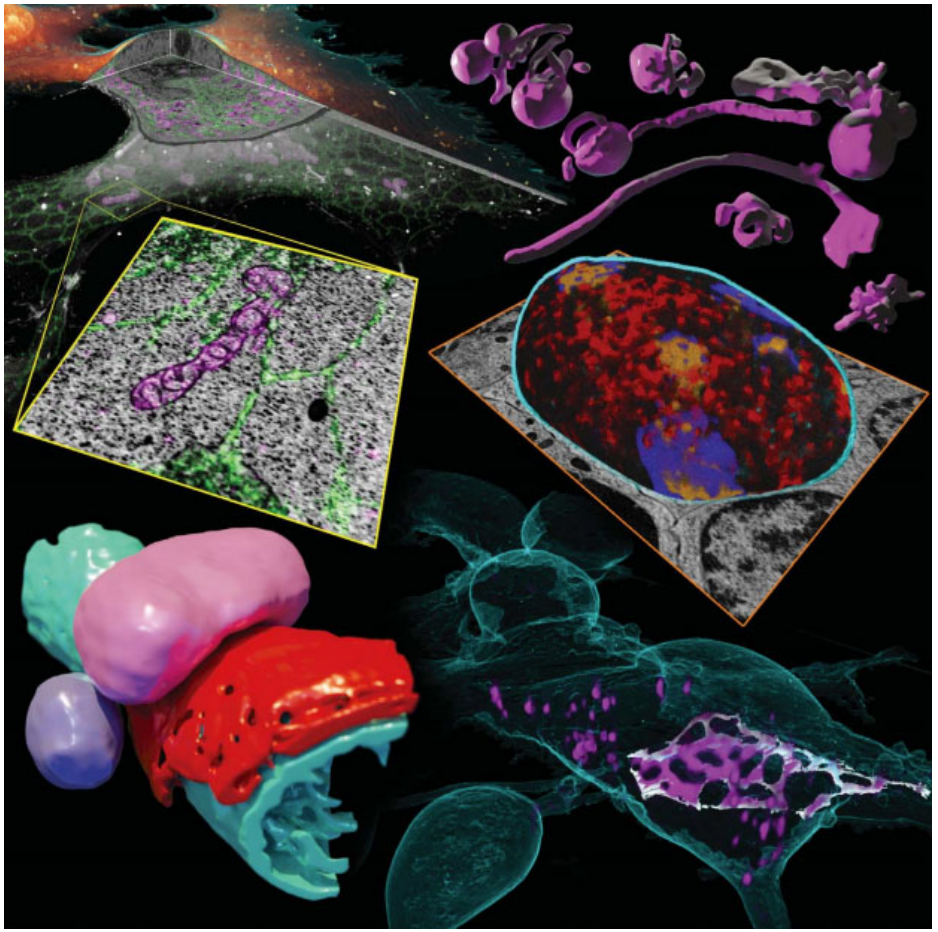

**Fig. 1. The nuclear periphery of a HeLa cell revealed by cryo-ET.** (**A**) Tomographic slice with 8.4-nm thickness of an interphase HeLa cell thinned by cryo-FIB. (**B**) Annotated view of the tomographic data. Color labels are defined for each structure in (B) and (C). (**C**) Cross-section view of the segmentation in the vicinity of the nuclear envelope [frame in (A)]. NE: nuclear envelope; ER: endoplasmic reticulum; NPC: nuclear pore complex.

# Advanced methods

ADVANCED IMAGING

## Correlative three-dimensional super-resolution and block-face electron microscopy of whole vitreously frozen cells

David P. Hoffman*, Gleb Shtengel*, C. Shan Xu, Kirby R. Campbell, Melanie Freeman, Lei Wang, Daniel E. Milkie, H. Amalia Pasolli, Nirmala Iyer, John A. Bogovic, Daniel R. Stabley, Abbas Shirinifard, Song Pang, David Peale, Kathy Schaefer, Wim Pomp, Chi-Lun Chang, Jennifer Lippincott-Schwartz, Tom Kirchhausen, David J. Solecki, Eric Betzig†, Harald F. Hess†



# Conclusion

- Cryo electron-microscopy is today one of the most important methods for structural biology

- Atomic resolution possible, due to:
  - water vitrification
  - direct electron detectors
  - advanced image processing

- CryoEM is a single-molecule method: identification of substates, dynamics, complex systems

- CryoEM tomography: Revealing structures in the cells