Protein Crystallization and x-ray structure

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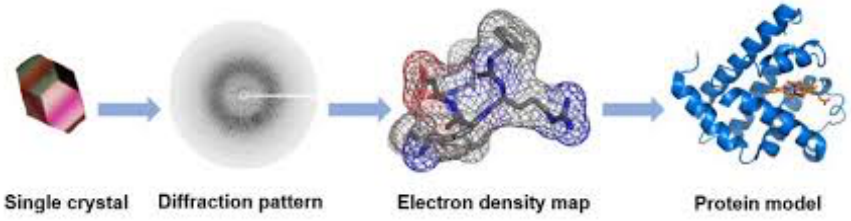
1

Protein Crystallization

- There many methods can observe the protein structure ?
- So, Why do we need to crystalize a protein?
- To observe the shape ‘’structure ’’ of the protein in the crystal.
- So, what is the phase of the crystal?
- Rigid solid.



2

How to get a good crystal structure?

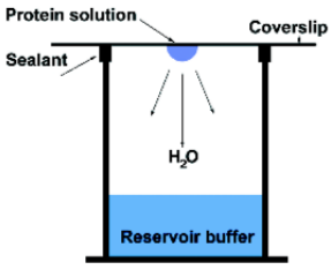
- When to get a best structure produced by X-ray of a protein?
- the best structure produced by X-ray when the molecules is well crystalized.
- What does protein crystallization require?
- a formation of large and stable crystals.
- How do we crystalize e protein molecule?
- there are two experimental methods used to form crystals from protein solutions are

1. vapour diffusion
2. equilibrium dialysis.

3

Vapour diffusion

- Vapour diffusion : it is based on 'hanging drops' containing protein solution plus 'precipitant' at a concentration insufficient to precipitate the protein.
- The drop is equilibrated against a larger reservoir of solution containing precipitant.
- after sealing the chamber equilibration leads to supersaturating concentrations that induce protein crystallization in the drop.

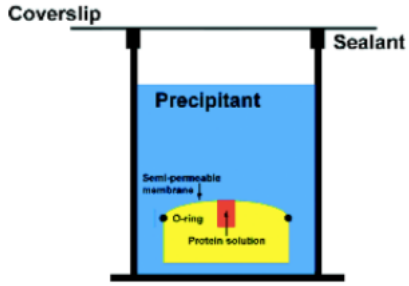


The 'hanging drop' or vapour diffusion method of protein crystallization. As little as 5 µl of concentrated solution (protein + solvent) may be suspended on the coverslip

4

equilibrium dialysis

- The equilibrium dialysis method and is used for crystallization of proteins at low and high ionic strengths.
- Small volumes of protein solution are placed in a container separated from precipitant by a semi-permeable membrane.
- Slowly the precipitant causes crystal formation within the well containing the protein solution.



Equilibrium dialysis can be achieved with many different 'designs' although the basic principle involves the separation of protein solution from the precipitant by a semipermeable membrane. Diffusion across the membrane promotes ordered crystallization

5

Optimization the conditions for the crystallization

- **Optimization means an** improvement process.
- It involves sequential and incremental changes in the chemical and physical parameters that influence **crystallization**.

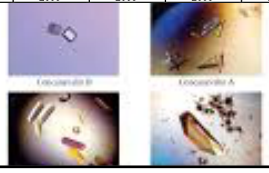
1. The The chemical parameters: pH, ionic strength and precipitant concentration.
2. The physical parameters such as temperature, sample volume and overall methodology.

a

	Initial Crystallization Condition:	Optimization Screen:
Buffer	50 mM Tris-Cl pH 7.5	50 mM Tris-Cl pH 7.5
Salt	200 mM NaCl	200 - 275 mM NaCl
Precipitant	25% PEG 2000	25-35% PEG 2000

b

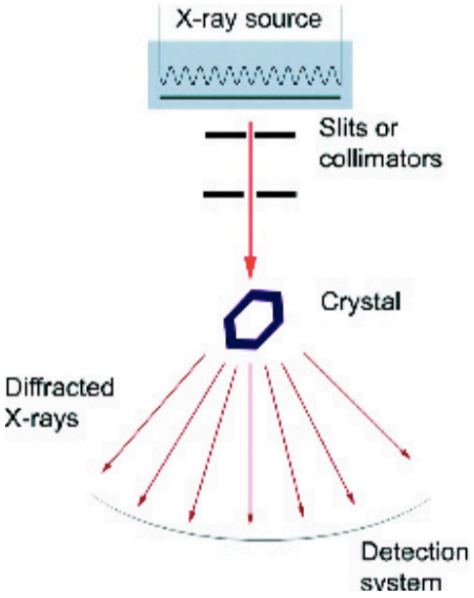
	1	2	3	4	5	6
A	50 mM Tris-Cl pH 7.5, 200 mM NaCl, 25% PEG 2000	50 mM Tris-Cl pH 7.5, 200 mM NaCl, 27% PEG 2000	50 mM Tris-Cl pH 7.5, 200 mM NaCl, 29% PEG 2000	50 mM Tris-Cl pH 7.5, 200 mM NaCl, 31% PEG 2000	50 mM Tris-Cl pH 7.5, 200 mM NaCl, 33% PEG 2000	50 mM Tris-Cl pH 7.5, 200 mM NaCl, 35% PEG 2000
B	50 mM Tris-Cl pH 7.5, 225 mM NaCl, 25% PEG 2000	50 mM Tris-Cl pH 7.5, 225 mM NaCl, 27% PEG 2000	50 mM Tris-Cl pH 7.5, 225 mM NaCl, 29% PEG 2000	50 mM Tris-Cl pH 7.5, 225 mM NaCl, 31% PEG 2000	50 mM Tris-Cl pH 7.5, 225 mM NaCl, 33% PEG 2000	50 mM Tris-Cl pH 7.5, 225 mM NaCl, 35% PEG 2000
C	50 mM Tris-Cl pH 7.5, 250 mM NaCl, 25% PEG 2000	50 mM Tris-Cl pH 7.5, 250 mM NaCl, 27% PEG 2000	50 mM Tris-Cl pH 7.5, 250 mM NaCl, 29% PEG 2000	50 mM Tris-Cl pH 7.5, 250 mM NaCl, 31% PEG 2000	50 mM Tris-Cl pH 7.5, 250 mM NaCl, 33% PEG 2000	50 mM Tris-Cl pH 7.5, 250 mM NaCl, 35% PEG 2000
D	50 mM Tris-Cl pH 7.5, 275 mM NaCl, 25% PEG 2000	50 mM Tris-Cl pH 7.5, 275 mM NaCl, 27% PEG 2000	50 mM Tris-Cl pH 7.5, 275 mM NaCl, 29% PEG 2000	50 mM Tris-Cl pH 7.5, 275 mM NaCl, 31% PEG 2000	50 mM Tris-Cl pH 7.5, 275 mM NaCl, 33% PEG 2000	50 mM Tris-Cl pH 7.5, 275 mM NaCl, 35% PEG 2000



6

X-ray method

- Once we get the crystal, How can we see the shape ‘’structure ’’ of the protein in a crystal?
- By applying an X- ray source incident on a crystal located close to a detector. This leads to reflect the X-rays by series of atomic planes.



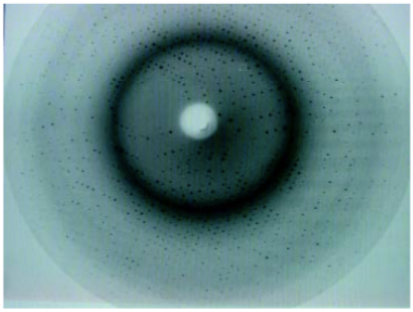
7

Bragg’s law

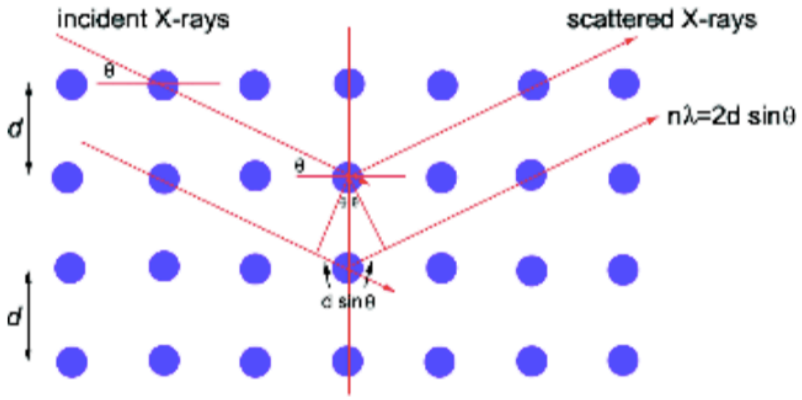
- Who has developed the x-ray method?
- Bragg.
- What does it explain ?
- It explains the the angles measurements when an incident X-ray beam is diffraction by a atoms of the molecules which leads to scatter the beam by the crystal lattice.
- What is the Bragg’s equation?
- $n\lambda = 2d \sin \theta$

8

The diffraction of the X-ray in the lattice



Protein diffraction patterns

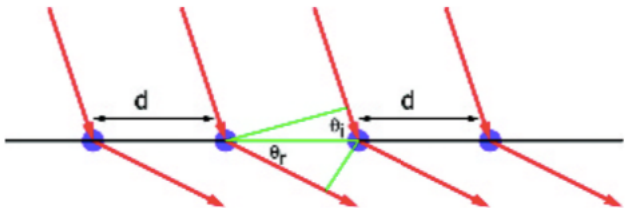


X-rays scattered by a crystal lattice

9

Laue equation

- Laue arranged the eq: $d\cos\theta_i - d\cos\theta_r = n\lambda$ (where n=1,2,3,...) to calculate three dimensions 3D.

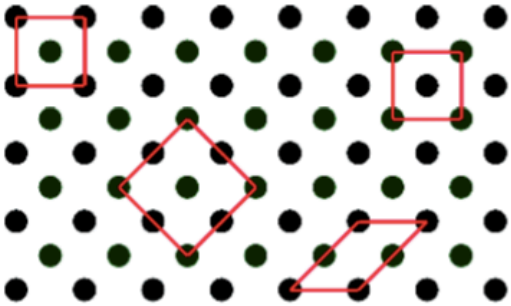


The Laue equations

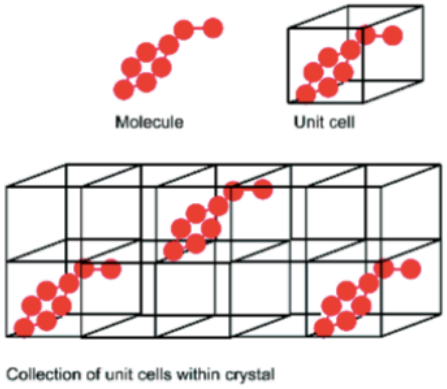
10

What are the possible unit cells in the 2D and 3D lattices?

- The unit cell, the basic building block of a crystal, is repeated infinitely in three dimensions.



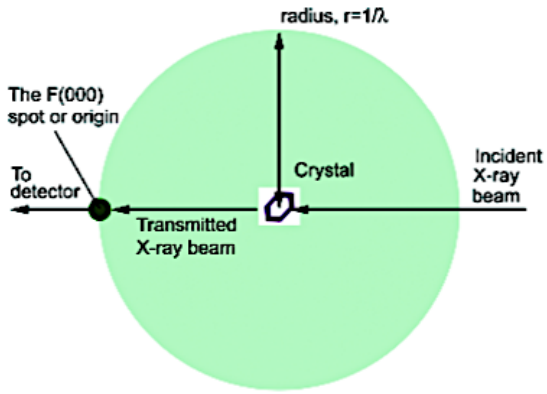
Possible unit cells in a two-dimensional lattice

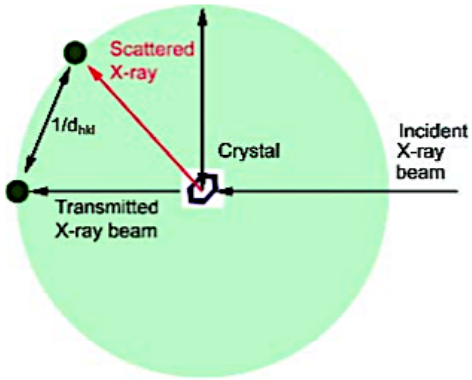


A unit cell for a simple molecule

11

Describe the scattering of X-ray by atom within a crystal?

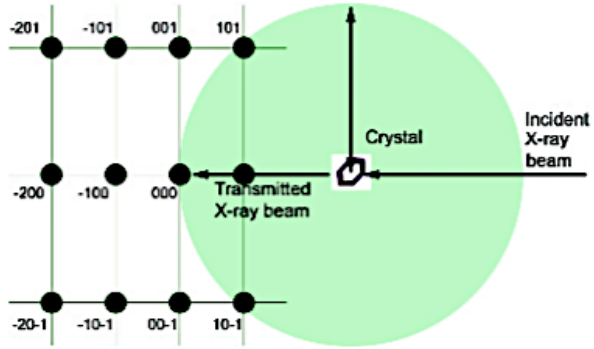




Scattering of X-rays by atoms within a crystal

12

With rotation !

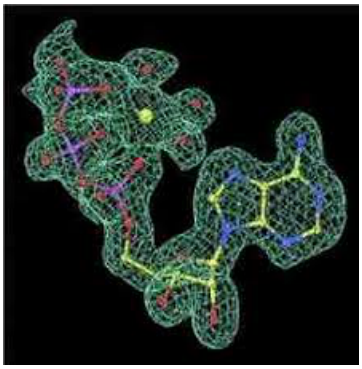


Rotation of the crystal brings more planes (collections of atoms) i

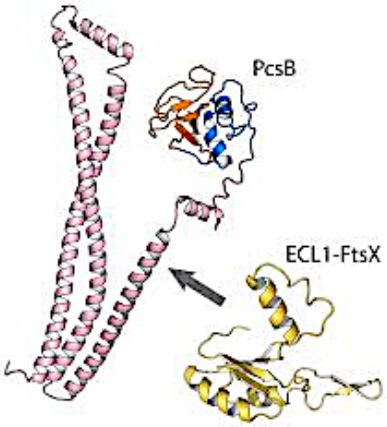
13

Proceeding data:
From electron density map to a protein modeling and structure.

- Once the data of the x-ray diffraction is collected, different software are used to process the data for protein molding.



Electron density map



3D structure

14

What is the main purpose of solving the 3D structure?

- What is the main purpose of solving 3D structure of protein?

1. **To study protein structure features.**
2. **For drug design.**
3. **To study biomolecules interactions.**

15

Biomolecule interactions

- **How can we study the protein-protein interaction via crystallization?**
- The protein in the crystal **shows** number of bonds that **can** form with another **protein** through intermolecular interactions.
- So, these interactions depend on electron densities of molecules and the **protein** side chains that change as a function of pH.

16