STRUCTURAL BIOLOGY

Live Session- Week 3
15 Feb, 2022

Summary

Week_3_Lecture_1: About Structural biology techniques.

1. Structural biology is the study of how biological molecules are built. Using a variety of imaging techniques, scientists view how they are assembled, how they function, and how they interact.
2. To understand the process of communication, we need to visualize molecules in the atomic level and determine proper arrangement of atoms in the system.
3. X-ray crystallography, Nuclear magnetic resonance (NMR) spectroscopy and Cryo-electron microscopy (cryo-EM) are high resolution techniques and Circular Dichroism, Fluorescence spectroscopy are low resolution techniques.
4. These techniques make it possible for researchers to determine the structure of proteins and other complex molecules.
5. X-ray crystallography uses crystalline structure which causes a beam of incident X-rays to diffract into many specific directions and then measure the angles and intensities of these diffracted beams, to produce a three-dimensional picture.
6. NMR spectroscopy is a technique that exploits the magnetic properties of certain atomic nuclei and can be used to determine the physical and chemical properties of atoms or the molecules in which they are contained.

Summary

Week_3_Lecture_2: This lecture is about protein structural biology techniques.

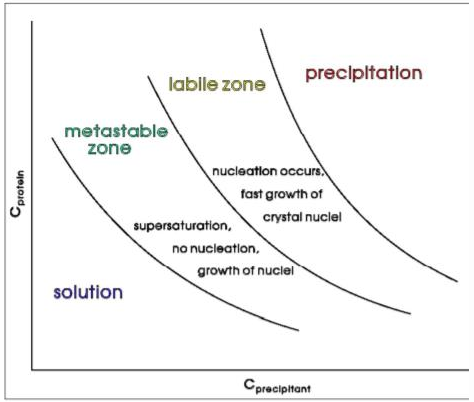
1. A **Protein crystallization** is the process of formation of a regular array of individual protein molecules stabilized by crystal contacts.
2. In the process of protein crystallization, proteins are dissolved in an aqueous environment and sample solution until they reach the supersaturated state.
3. Unlike ideal crystals, real crystals have imperfections. Any deviation from the standard lattice pattern of a crystal is an imperfection. The imperfection may be because of presence of impurity atom or a disturbed crystal structure.
4. The first step is finding , optimization and preparing the target gene. Followed by the optimization of the expression system. Next steps include choice of plasmid as cloning vector, overexpression and purification.
5. The purpose of preparative chromatography is to separate the components of a mixture for later use, and is thus a form of purification.
6. Types of chromatography :

   Affinity chromatography
   Ion exchange chromatography
   Size-exclusion chromatography
   Hydrophobic interaction chromatography

Summary

Week_3_Lecture_3: Focuses on protein crystallization.

1. The goal of crystallization is obtaining good crystals. The crystal formation means allowing the sample solution to reach the supersaturated state.
2. Before going to the crystallization, we need to check for high concentration of protein, homogeneous and stable state.
3. Crystallization has a thermodynamically closed systems, involving three main factors, which are enthalpy (ΔH), entropy (ΔS) and temperature (T).
4. ΔH relates to the chemical bonds being formed and broken upon reactions or phase changes. ΔS relates to the degree of freedom or the measurement of uncertainty.
5. Factors that affect the formation of protein crystal are: a. Purity of protein, b. Protein concentration, c. Precipitating reagent, d. Temperature, e. pH and f. Additives.
6. The nucleation range is also called the labile zone, while the growth range is known as the metastable zone.
7. For crystal nucleation to occur, the experimenter must push the protein solution into the labile zone, which is also the region where fast growth of crystal nuclei occurs.
8. The protein/precipitant mixture must approach the nucleation zone very slowly so that the developing nuclei have enough time to grow



Summary

Week_3_Lecture_4: Lecture 4 is about methods of protein crystallization.

1. Three steps to achieve super-saturation:
   Maximize concentration of purified protein.
   Add a precipitating reagent
   Allow vapor diffusion for protein crystallization
2. In vapor diffusion, droplets containing purified protein, buffer, and precipitant are allowed to equilibrate with a larger reservoir containing similar buffer and precipitants in higher concentrations.
3. The drop and reservoir equilibrate i.e., the precipitant and protein concentrations increase in the drop and crystal growth occurs in the drop.
4. Vapor diffusion can be performed in either hanging-drop or sitting-drop format. Both of these methods require sealing of the environment so that equilibration between the drop and reservoir can occur.
5. Hanging-drop apparatus involve a drop of protein solution placed on an inverted cover slip, which is then suspended above the reservoir. Sitting-drop crystallization apparatus place the drop on a pedestal that is separated from the reservoir.
6. Micro dialysis has a semi-permeable membrane, across which small molecules and ions can pass, while proteins and large polymers cannot cross. By allowing the system to move towards equilibrium, the system can slowly attain supersaturation, at which point protein crystals may form.

Summary

Week_3_Lecture_5: Lecture 5 is about Crystal Mounting

1. When crystals are observed, one must determine if the crystals are of the target biological macromolecule (protein) or salt (inorganic, small molecule) crystals.
   - A protein crystal typically has a high solvent content and will dehydrate when removed from the drop. Salt crystals typically do not possess large solvent channels and have very little solvent content. Allowing the drop to evaporate will destroy or change the appearance of the salt crystal.
   - A protein crystal will powder, crumble, or fall apart easily when touched with a probe. Salt crystals break apart, but they require more force .
   - A protein crystal typically has large solvent channels which will accommodate a small molecule dye. Salt crystals do not possess such solvent channels and will not absorb the molecule dye.
2. The crystal is mounted for measurements so that it may be held in the X-ray beam and rotated. Protein crystals are scooped up by a loop, then flash-frozen with liquid nitrogen. This freezing reduces the radiation damage of the X-rays.
3. The capillary or loop is mounted on a goniometer and the crystal is positioned accurately within the X-ray beam and rotated.
4. The most common type of goniometer is the "kappa goniometer", which offers three angles of rotation: the ω angle, which rotates about an axis perpendicular to the beam; the κ angle, about an axis at ~50° to the ω axis; and, finally, the φ angle about the loop/capillary axis.

Question : 1

Wavelength of X-rays?

1. 0.01 - 10 nm
2. 0.1 -1 nm
3. 1 -100 nm

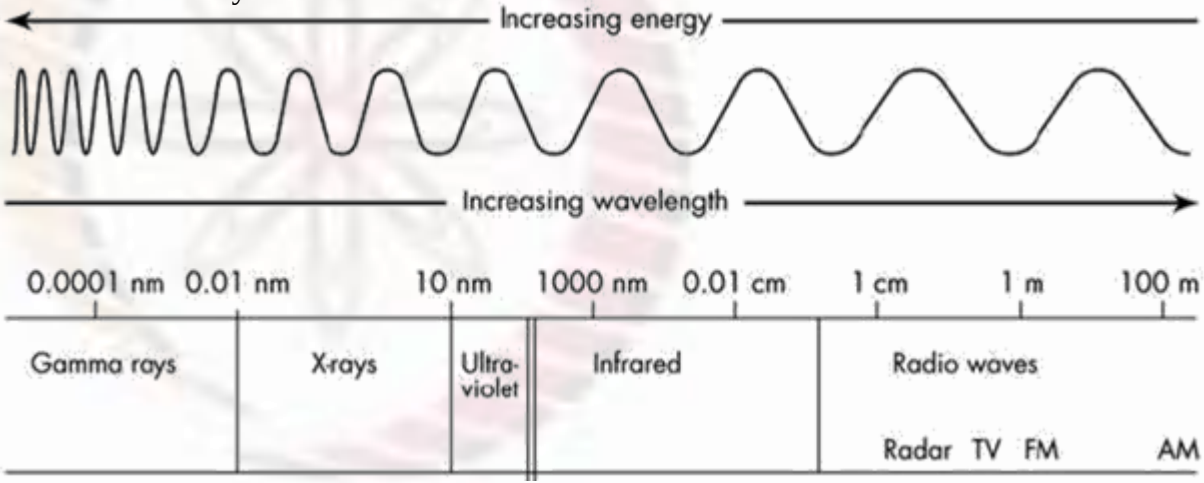
Answer the question on Google meet poll

Wavelength of X-rays?

1. 0.01 - 10 nm
2. 0.1 -1 nm
3. 1 -100 nm

Solution:

1. X-rays were discovered by Roentgen.
2. X-rays is an electro-magnetic radiation. The x-ray region lies between gamma and ultra violet rays.



3. They are shorter than ultra violet rays and longer than gamma rays.

Final answer: **0.01 – 10 nm**

Question : 2

Which property of X-rays is critical to their use in X-ray diffraction?

1. Scattering by electrons
2. Go right through the most of matter
3. Made of photons

Answer the question on Google meet poll

Which property of X-rays is critical to their use in X-ray diffraction?

1. Scattering by electrons
2. Go right through the most of matter
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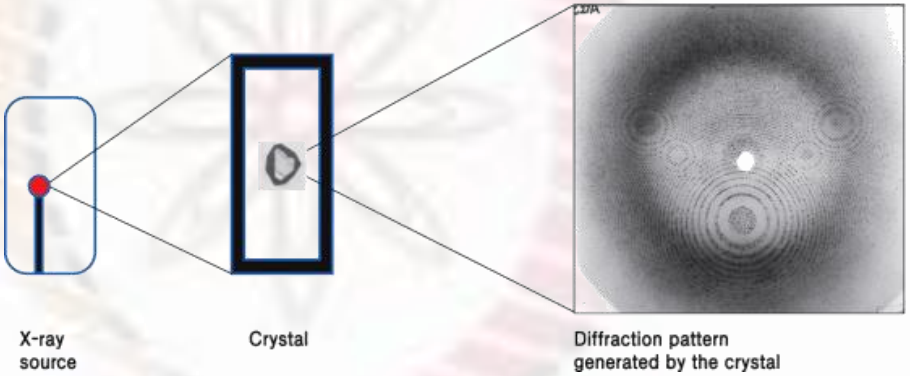
Solution:

1. X- rays have wavelengths similar to the size of atoms, so they are useful to explore within crystals.
2. Since X-rays have a smaller wavelength than visible light, they have higher energy.



3. With their higher energy, X-rays can penetrate matter more easily than visible light.

Final answer: **Go right through the most of matter**

Question : 3

Which of these are the smallest things scientists can see when using X-rays?

1. Atoms
2. Cells
3. Molecules

Answer the question on Google meet poll

Question : 4

Dimerization of Histidine Kinase takes place in?

1. Periplasmic domain
2. Transmembrane domain
3. Cytosolic domain
4. All of the above

Answer the question on Google meet poll

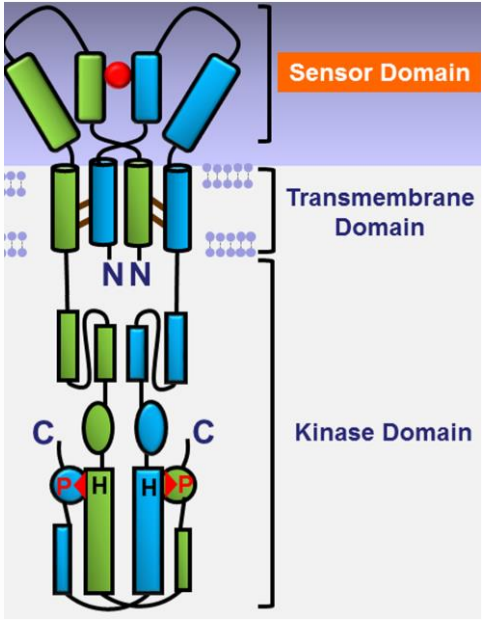
Dimerization of Histidine Kinase takes place in?

1. Periplasmic domain
2. Transmembrane domain
3. Cytosolic domain
4. All of the above

Solution:

1. An Histidine Kinase has a short N terminal cytoplasmic portion connected to an transmembrane α helix.
2. A second transmembrane α helix connects the extracellular domain to the C-terminal cytoplasmic catalytic domain.
3. The cytoplasmic domain tends to have high sequence homology and contains several well-known motifs.
4. The dimeric unit is held together by a four-helix bundle, formed when the C-terminal segments of the α helices on each subunit interact in an antiparallel manner with both α helices.
5. The stability of the dimer is aided by several interactions at the interface of each monomer such as hydrophobic interactions at conserved hydrophobic residues as well as two hydrogen bonds.

Final answer: **Cytosolic domain**



Question : 5

Histidine Kinase catalyses the transfer of the phosphate group to the specific residue of the response regulator. What is the name of that residue?

1. Aspartic Acid
2. Lysine
3. Histidine

Answer the question on Google meet poll

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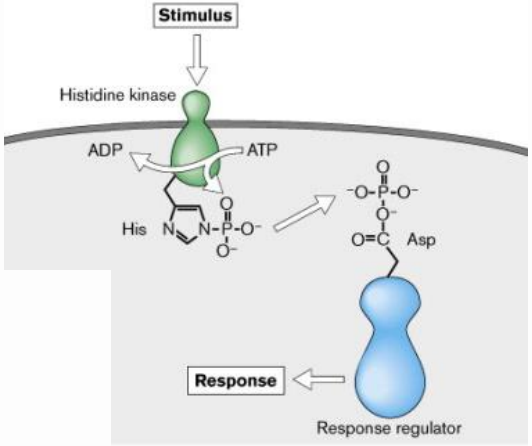

Solution:

A **two-component regulatory system** serves as a basic stimulus-response coupling mechanism to allow organisms to sense and respond to changes in many different environmental conditions.

Two-component systems consist of a membrane-bound histidine kinase that senses a specific environmental stimulus and a corresponding response regulator that mediates the cellular response.

Two-component systems perform signal transduction through the phosphorylation of a response regulator (RR) by a histidine kinase (HK).

Upon detecting a particular stimulus in the extracellular environment, the HK performs an autophosphorylation reaction, transferring a phosphoryl group from adenosine triphosphate (ATP) to a specific histidine residue.

The kinase domain is responsible for the autophosphorylation of the histidine with ATP, the phosphor transfer from the kinase to an aspartate of the response regulator.

Final Answer **: Aspartic Acid**

Question : 6

Which of the following is not a highly specific biological interaction of Affinity Chromatography?

1. Cation-Anion
2. Antigen-Antibody
3. Enzyme-Substrate
4. Receptor-Ligand

Answer the question on Google meet poll

Which of the following is not a highly specific biological interaction of Affinity Chromatography?

1. Cation-Anion
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3. Enzyme-Substrate
4. Receptor-Ligand

Solution:

Affinity chromatography is a method of separating a biomolecule from a mixture, based on a highly specific macromolecular binding interaction between the biomolecule and another substance.

In affinity chromatography experiment, the ligand is attached to a solid matrix and forming stable covalent bonds i.e molecules that bind to the ligand will remain associated with the stationary phase.

A wash buffer was applied to remove non-specific biomolecules by disrupting their weaker interactions with the stationary phase.

Target biomolecules may then be removed by applying a so-called elution buffer, which disrupts interactions between the bound target biomolecules and the ligand. The target molecule is thus recovered in the eluting solution.

Affinity chromatography does not require the molecular weight, charge, hydrophobicity, or other physical properties of the analyte of interest to be known

➢ Final Answer **: Cation-Anion**

Question : 7

Crystal formation requires?

1. Nucleation
2. Growth
3. Nucleation and Growth

Answer the question on Google meet poll

Crystal formation requires?

1. Nucleation
2. Growth
3. Nucleation and Growth

Solution:

- The Crystal formation requires two steps: nucleation and growth.
- Nucleation is the initiation step for crystallization. At the nucleation phase, protein molecules in solution come together as aggregates to form a stable solid nucleus.
- As the nucleus forms, the crystal grows bigger and bigger by molecules attaching to this stable nucleus.
- The nucleation step is critical for crystal formation since it is the first-order phase transition of samples moving from having a high degree of freedom to obtaining an ordered state (aqueous to solid).
- For the nucleation step to succeed, the manipulation of crystallization parameters is essential.
- The approach behind getting a protein to crystallize is to yield a lower solubility of the targeted protein in solution.
- Once the solubility limit is exceeded and crystals are present, crystallization is accomplished.

Final Answer :**Nucleation and Growth**

Question : 8

In vapor diffusion method, which of the following is not a constituent of the protein droplet?

1. Purified protein
2. Buffer
3. Precipitant
4. Denaturing agent

Solution: In vapor diffusion method, droplets containing ***purified protein***, ***buffer***, and ***precipitant*** are allowed to equilibrate with a larger reservoir containing similar buffers and precipitants in higher concentrations.

Answer the question on Google meet poll

Question : 9

Which of the following is true for a protein crystal?

1. Proteins remains functional
2. Protein remains non-functional
3. Protein denatured
4. All of the above

Answer the question on Google meet poll

Question :10

Irregularity in protein crystal is due to?

1. Translational disorder
2. Rotational disorder
3. Both 1 and 2

Answer the question on Google meet poll