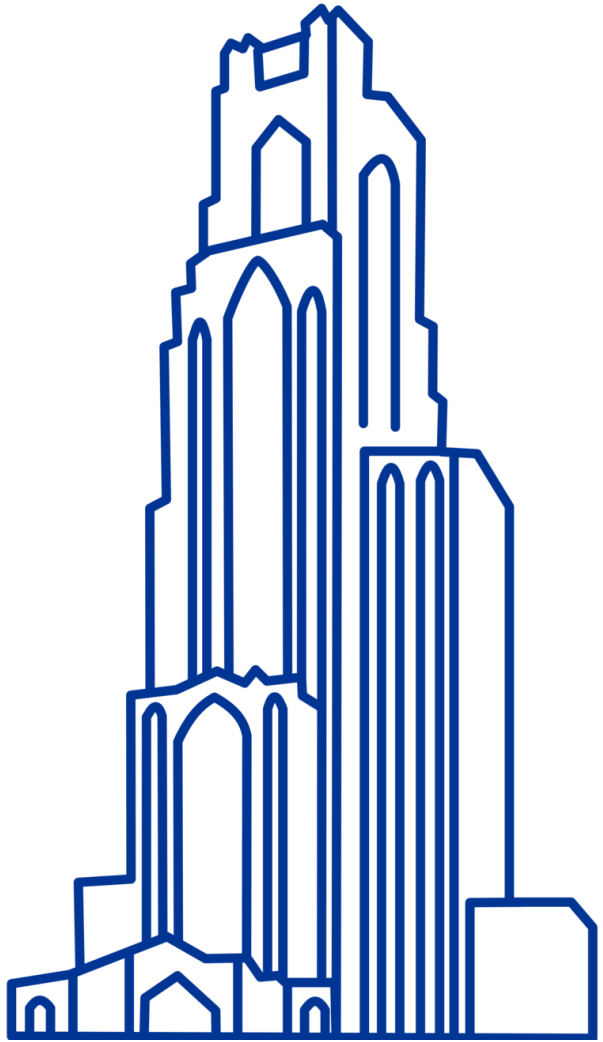


Computational Biology

(BIOSC 1540)

Lecture 11:

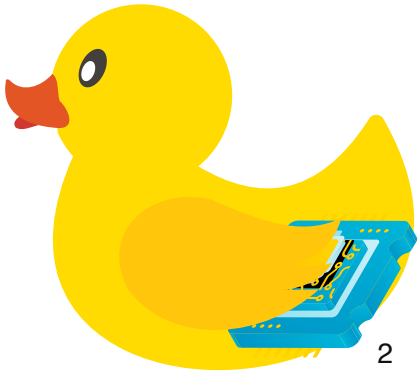
Structural biology

Oct 8, 2024

University of Pittsburgh

Announcements

- A03 and A04 will be graded this week
- A05 will be posted on Friday
- We are now entering the world of structural biology (Physical chemistry and Biochemistry)



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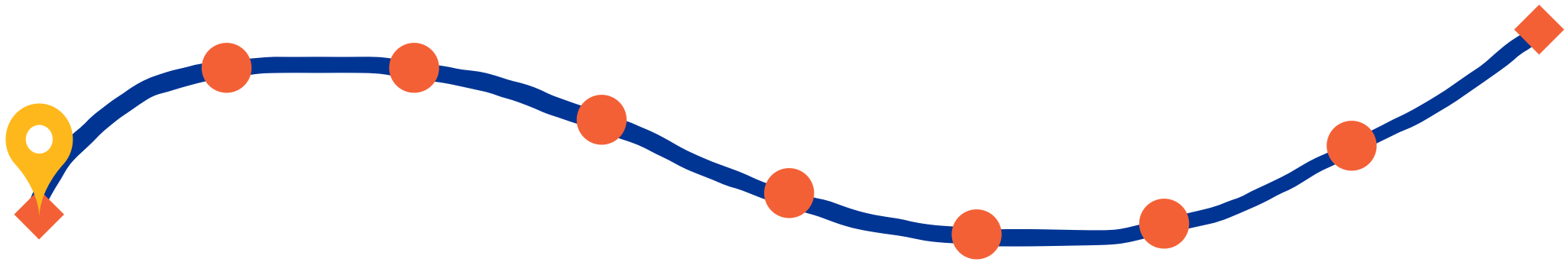
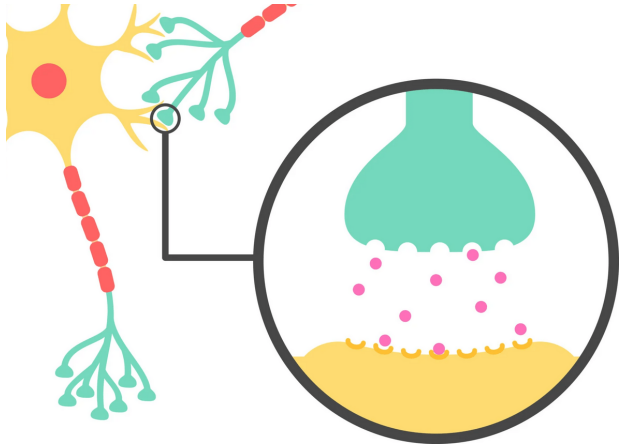


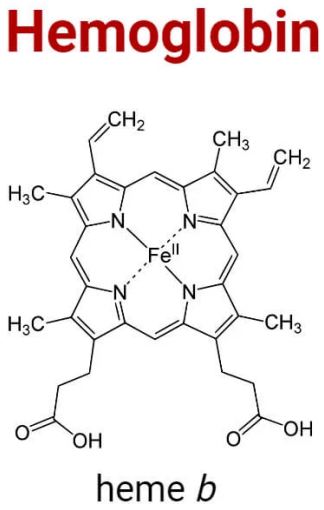
Categorize atomic interactions

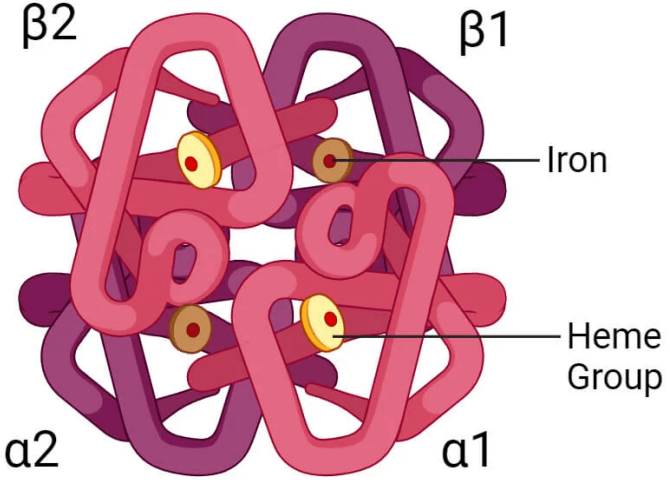
and their importance

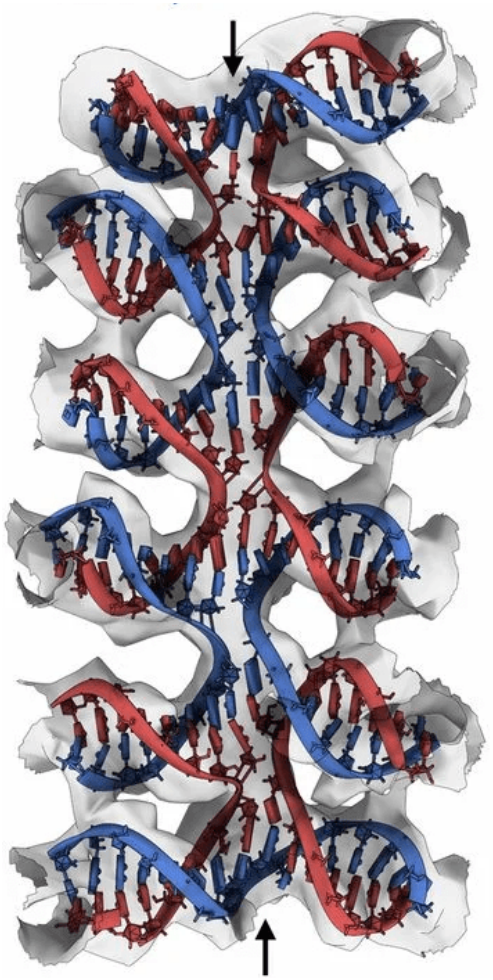
The atomic world of biology

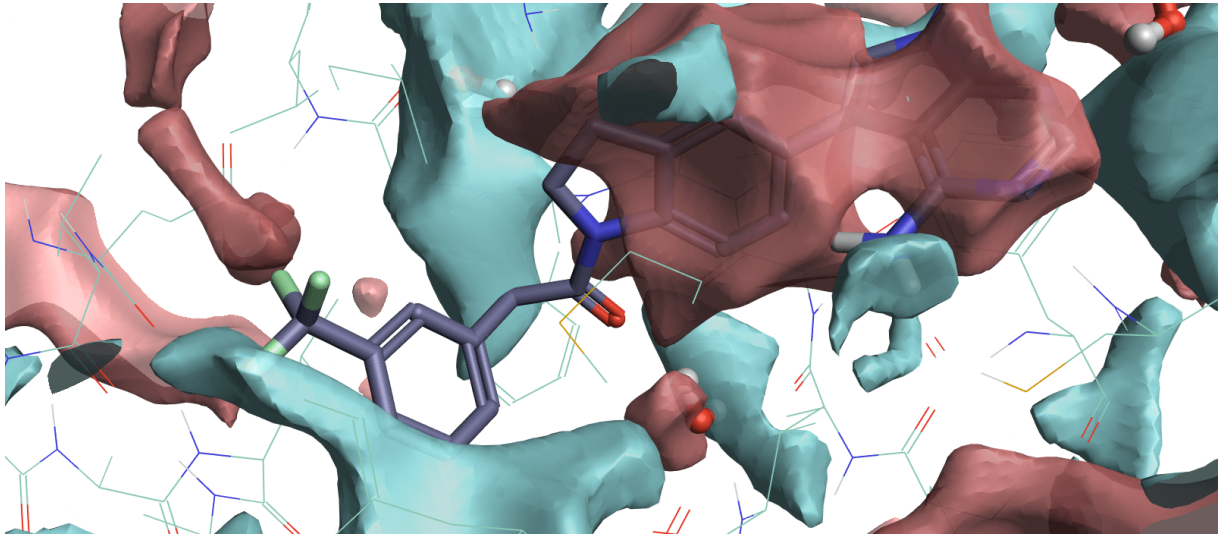
At the foundation of biological processes lie **atoms and their interactions**







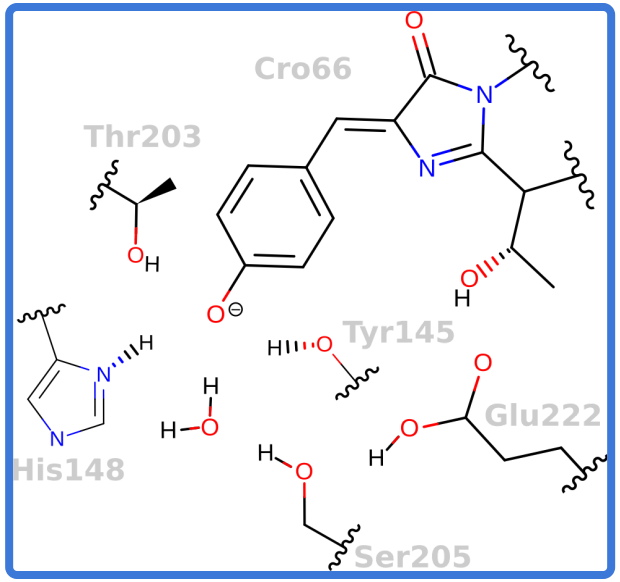




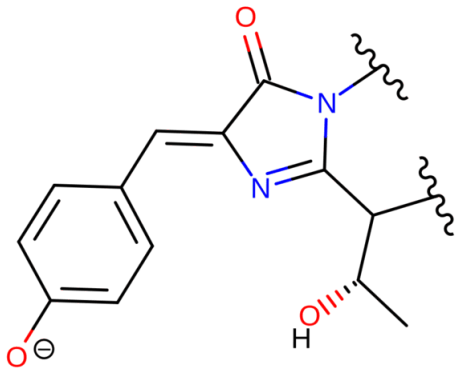
Atomistic structure determines behavior of biomolecules

Why is Green Fluorescent Protein (GFP) fluorescent,
but not the chromophore in solution?

Fluorescent

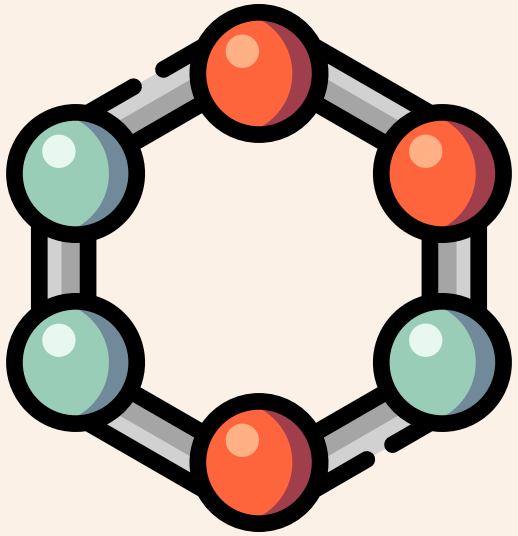


Not Fluorescent

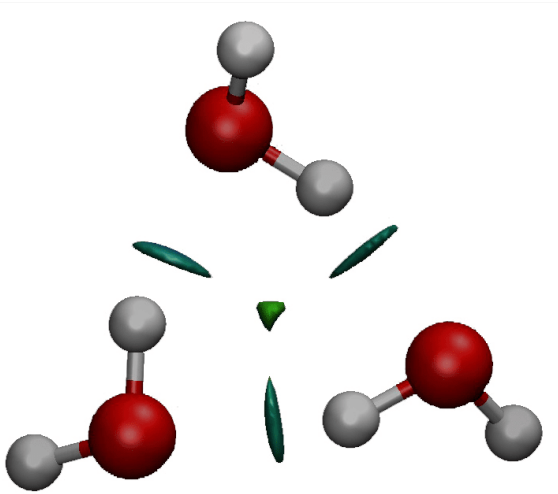


GFP keeps the chromophore planar and facilitates an excited-state proton transfer

Two types of atomistic interactions



Covalent

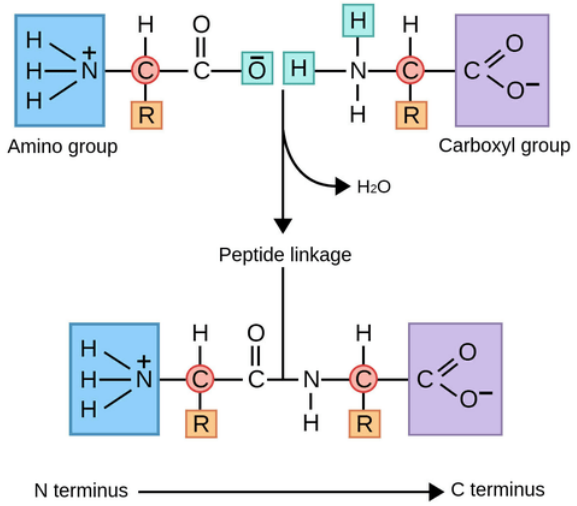


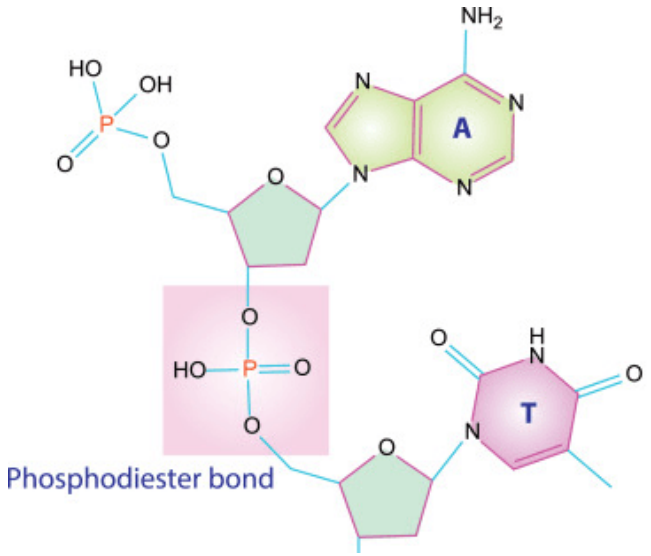
Noncovalent

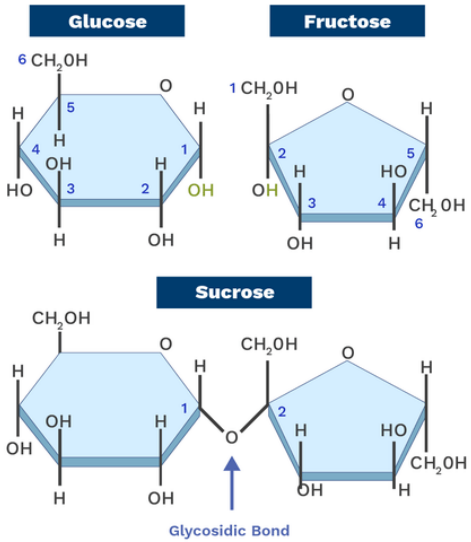
Covalent bonds: The framework of biomolecules

Covalent bonds are formed when atoms **share pairs of electrons** that holds molecules together

- **Peptide bonds** covalently link amino acids into polypeptide chains
- **Phosphodiester bonds** form the sugar-phosphate backbone of DNA and RNA
- **Glycosidic bonds** join monosaccharides to form complex sugars







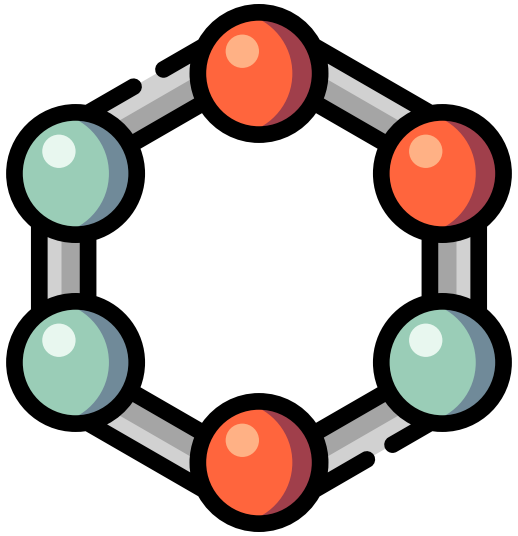
Relevant characteristics of covalent bonds

Strength and stability: Covalent bonds provide the necessary stability for complex biological structures

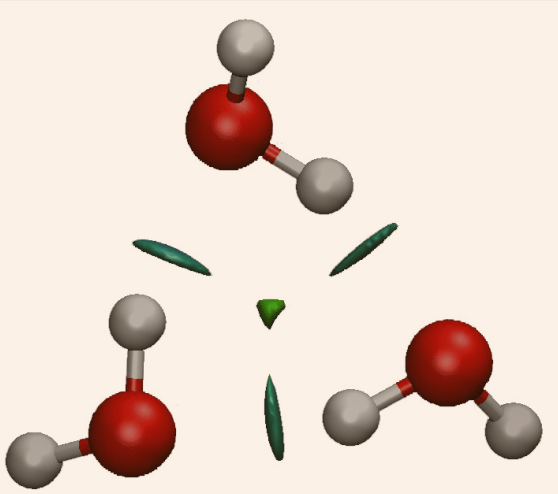
Directionality: Covalent bonds limit the specific angles and orientations leading to the 3D shapes of biomolecules

- **Single Bonds**: Allow rotation, contributing to molecular flexibility
- **Double/Triple Bonds**: Restrict rotation, affecting the rigidity and function of molecules

Two types of atomistic interactions



Covalent



Noncovalent

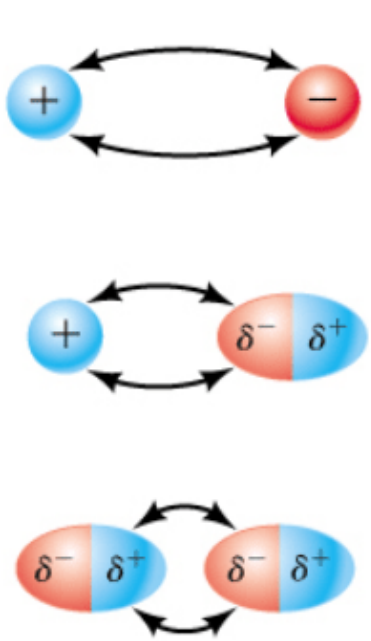
Noncovalent Forces: The Dynamic Glue

Noncovalent interactions are **weaker than covalent** bonds and involve **electrostatics**

(a) Charge–charge

(b) Charge–dipole

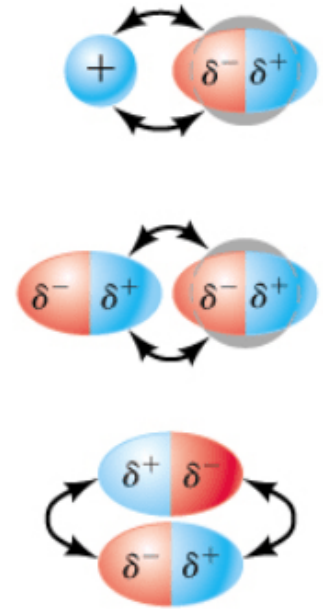
(c) Dipole–dipole



(d) Charge–induced dipole

(e) Dipole–induced dipole

(f) Dispersion (van der Waals)



We will cover this in a later lecture

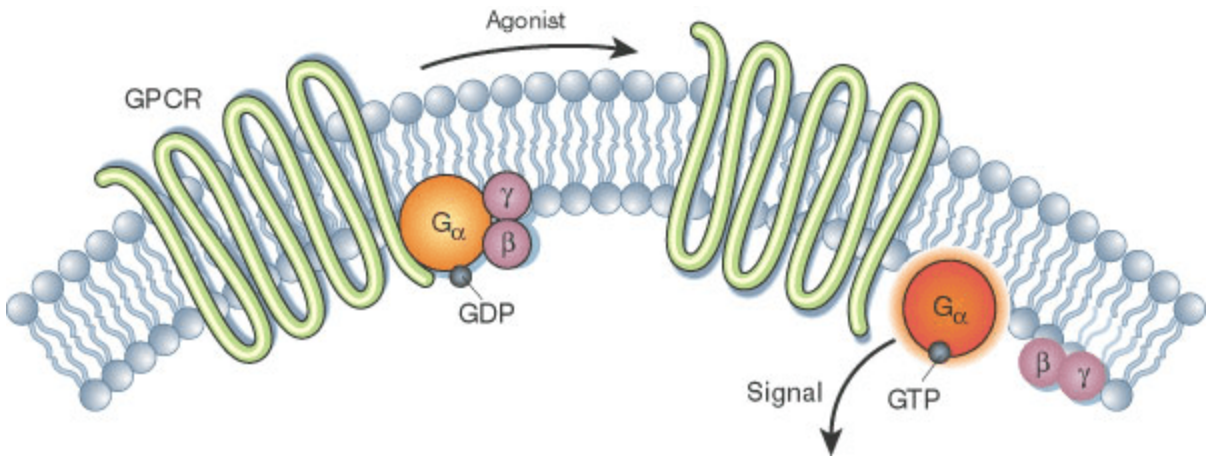
Noncovalent interactions drive most of biology

Molecular recognition

- Enzyme-Substrate Binding
- Antigen-Antibody Interactions

Macromolecular structure

- Membrane Formation
- Protein-Protein Interactions
- Base pairing in DNA and RNA
- Protein folding



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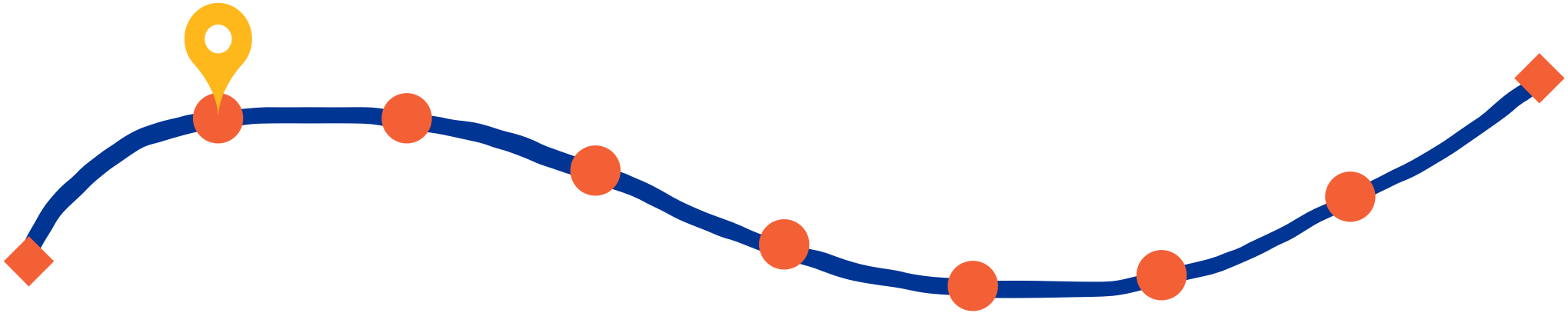
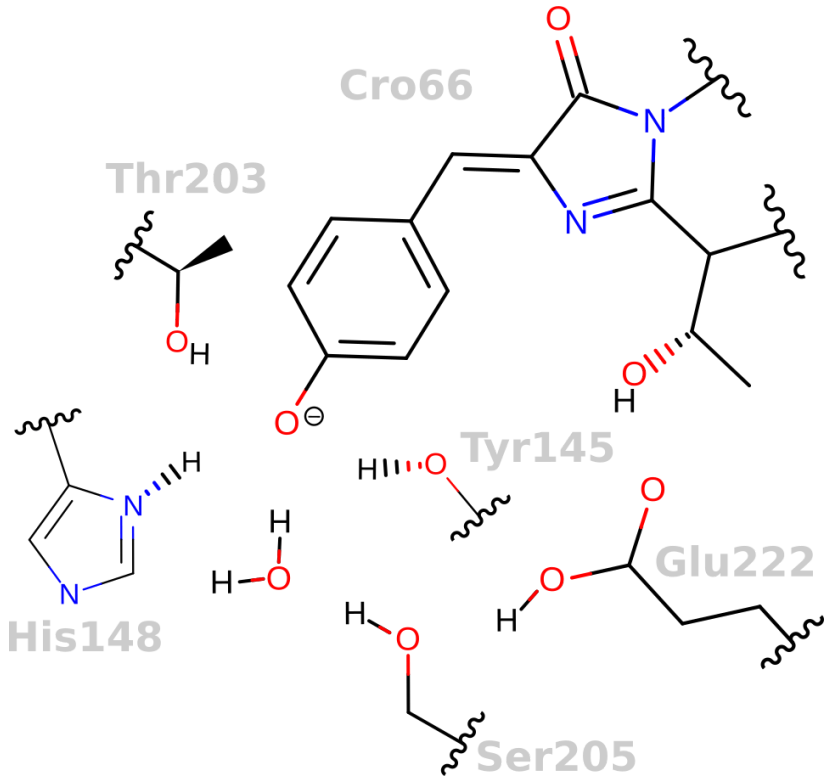


What is structural biology

and why is it important?

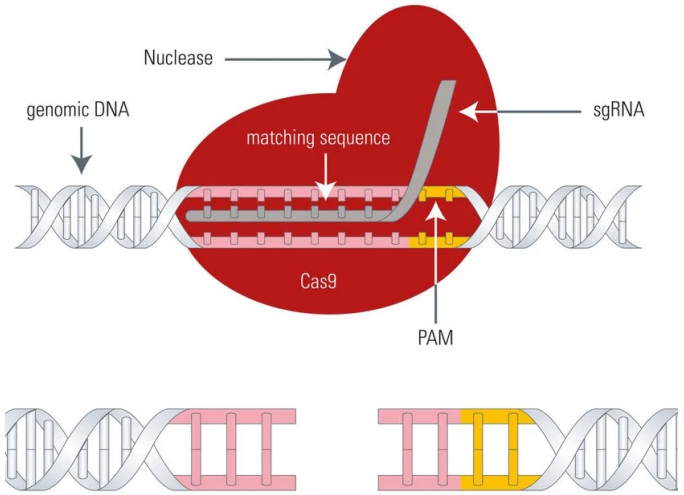
What is the value of atomistic insight?



The precise arrangement of atoms determines how molecules fold, bind, and perform biological tasks

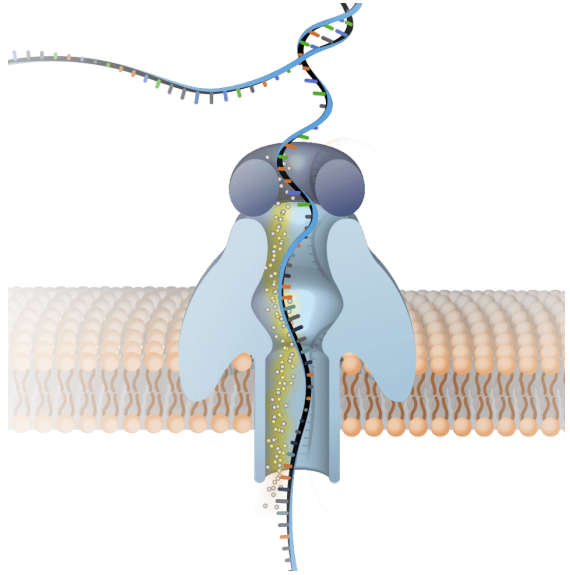
We cannot exploit what we do not understand

Innovation and biotechnology depend on molecular understanding



CRISPR-Cas9

COVID-19 treatments



High-throughput sequencing

What is structural biology?

Structural biology determines the 3D shapes of biological macromolecules and how these shapes relate to function

Why study structure?

- Proteins and nucleic acids adopt specific shapes crucial for their biological roles.
- **Example:** The shape of an enzyme's active site determines how it binds substrates and catalyzes reactions.

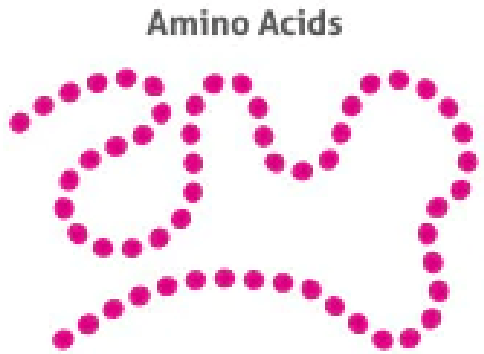
Primary Goal: To understand how molecular machines in cells work by deciphering their atomic arrangements.

Primary structure

The **primary structure** of a protein is the linear sequence of amino acids, held together by covalent peptide bonds

The primary structure is crucial because it dictates how the protein will fold into higher-order structures

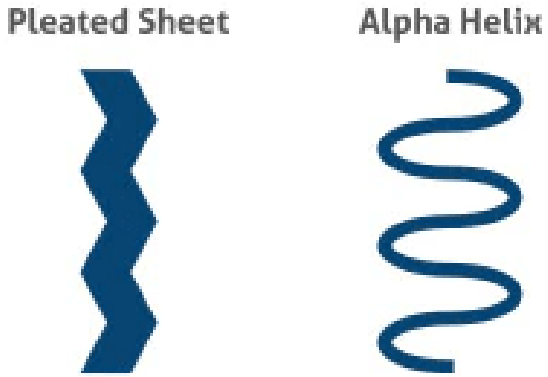
The primary structure alone does not reveal the protein's functional form or activity



While the primary sequence is critical, the folding process may also depend on cellular factors (e.g., chaperones)

Secondary structure

The **secondary structure** refers to local conformations of the polypeptide chain, stabilized primarily by hydrogen bonds



These structural motifs are critical for certain functions

- For example, DNA-binding domains often contain alpha-helices

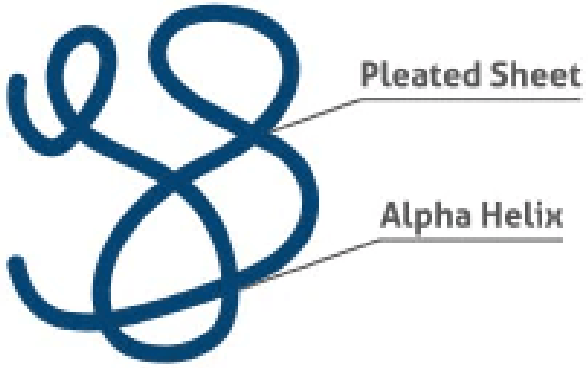
Secondary structures can undergo local fluctuations—alpha helices can unwind, and beta-sheets can twist—adding to functional flexibility

While alpha-helices and beta-sheets dominate, other structural motifs (e.g., 310 helices) are less common and sometimes overlooked

Tertiary Structure

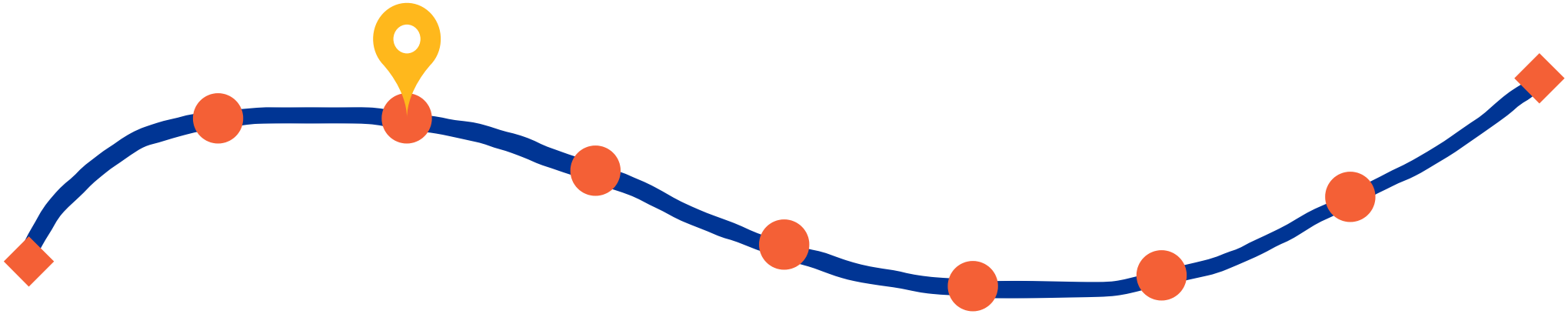
The **tertiary structure** refers to the complete 3D shape of a single polypeptide chain

Predicting how a sequence folds into its tertiary structure is complex, even with knowledge of secondary structures



Tertiary structures reveal active sites or binding pockets where catalysis or molecular interactions occur

After today, you should be able to



Explain the fundamentals of electron density

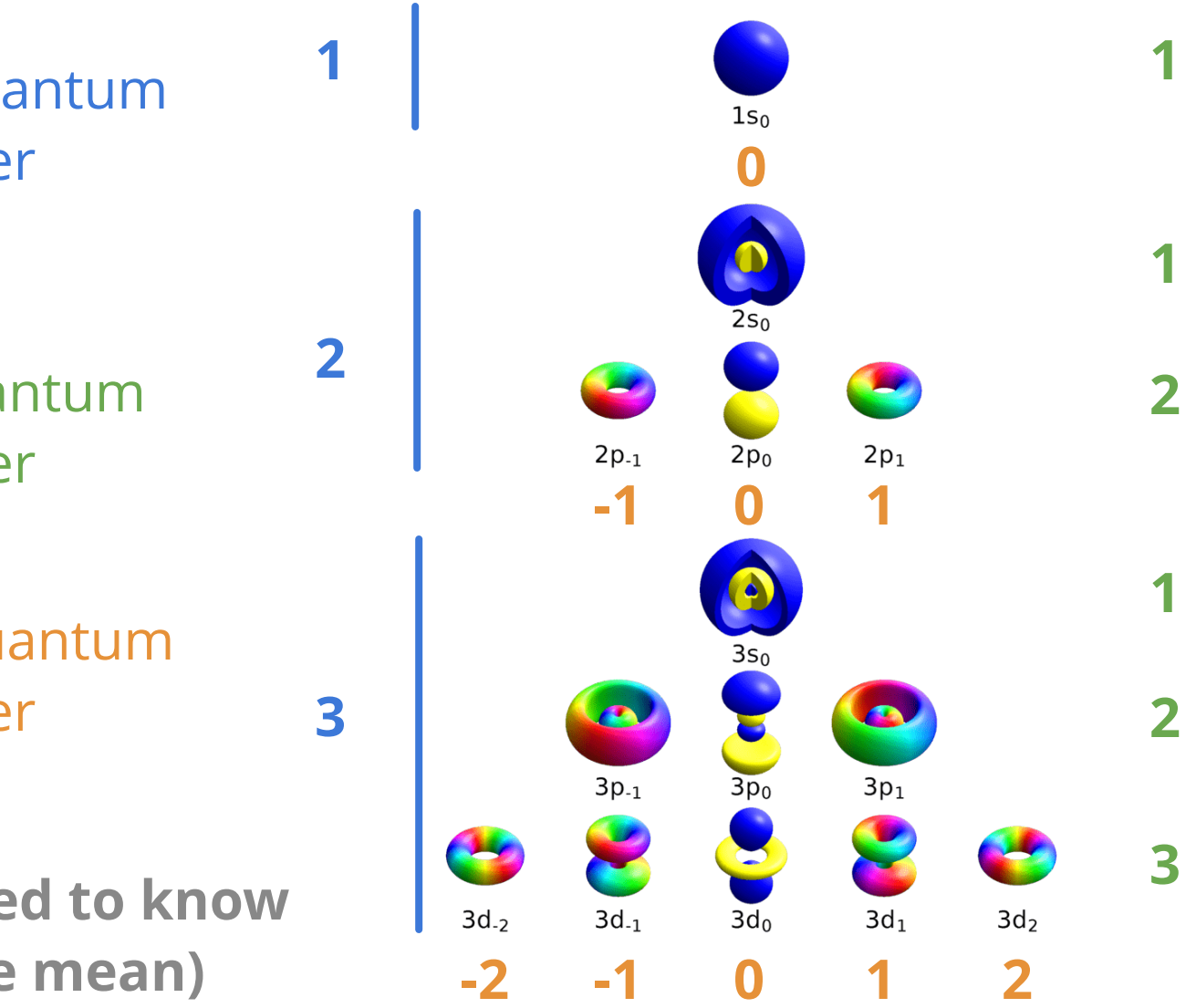
Particle behaviors are determined by quantum numbers

Principle quantum number

Orbital quantum number

Magnetic quantum number

(You don't need to know what these mean)



An electron at (*n*, *l*, *m*) will have a specific energy level and characteristics

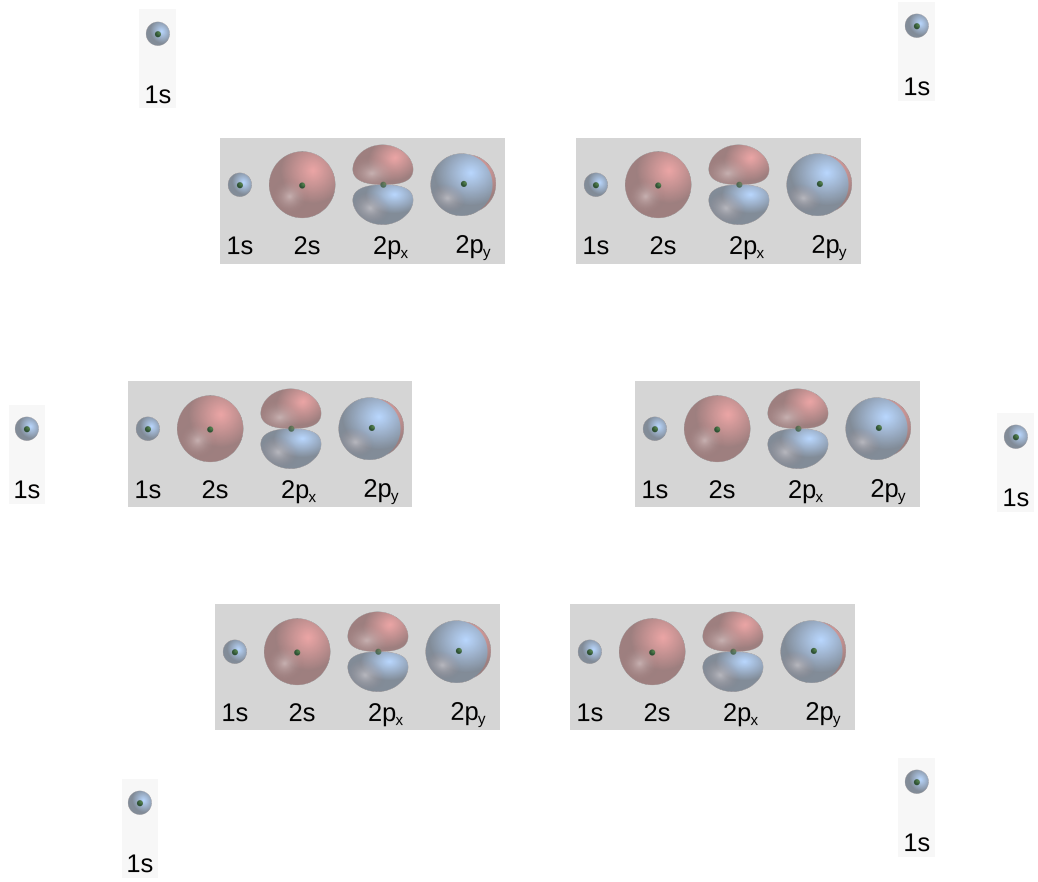
Each atom contributes electrons to the molecule

Benzene has . . .

Six carbon atoms with $1s^2\ 2s^2\ 2p^2$

Six hydrogen atoms with 1s

located at the center of each atom's position

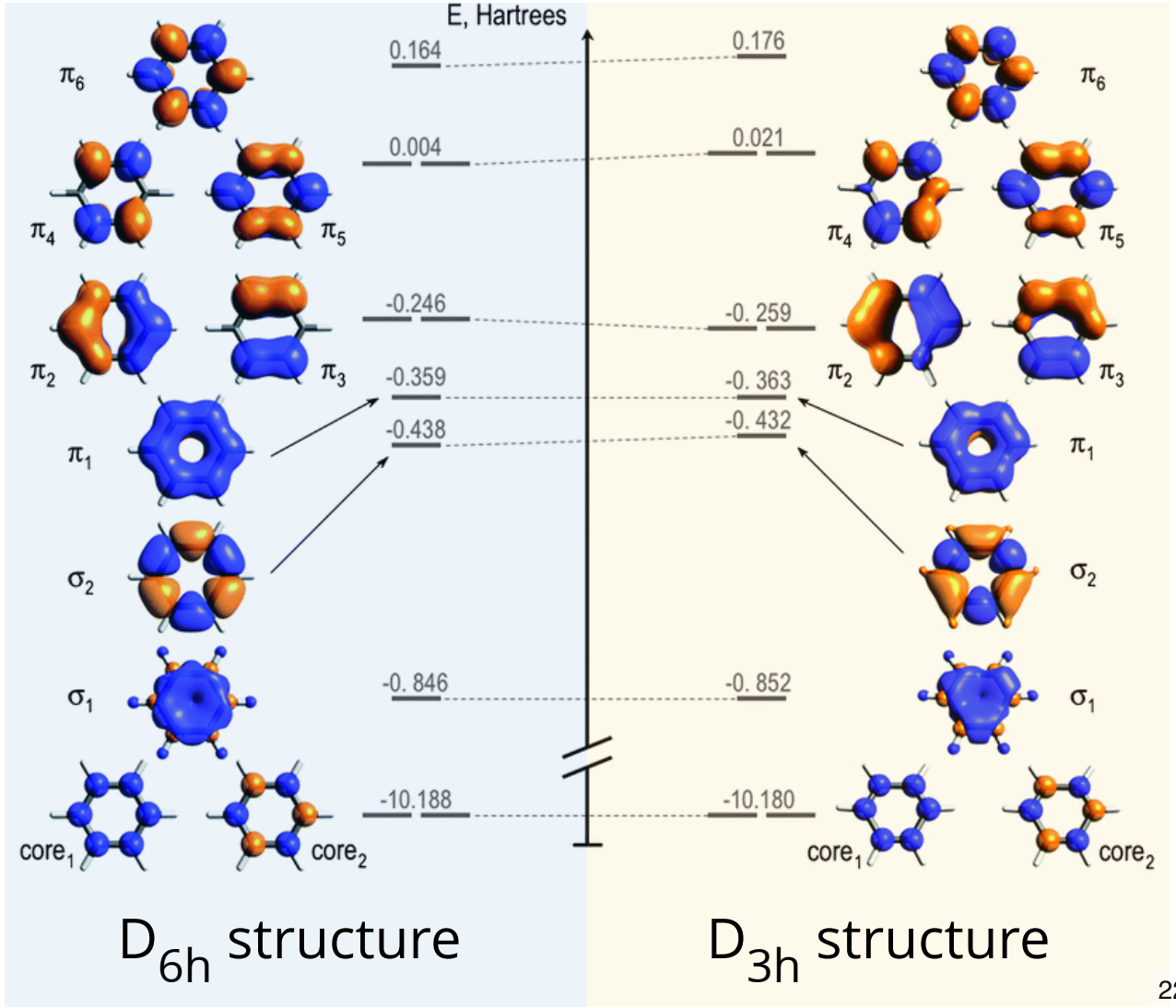


Electrons "mix" into molecular orbitals to a specific energy level

These molecular orbitals determine behavior

Particles (e.g., electrons and photons) can interact with these molecular orbials

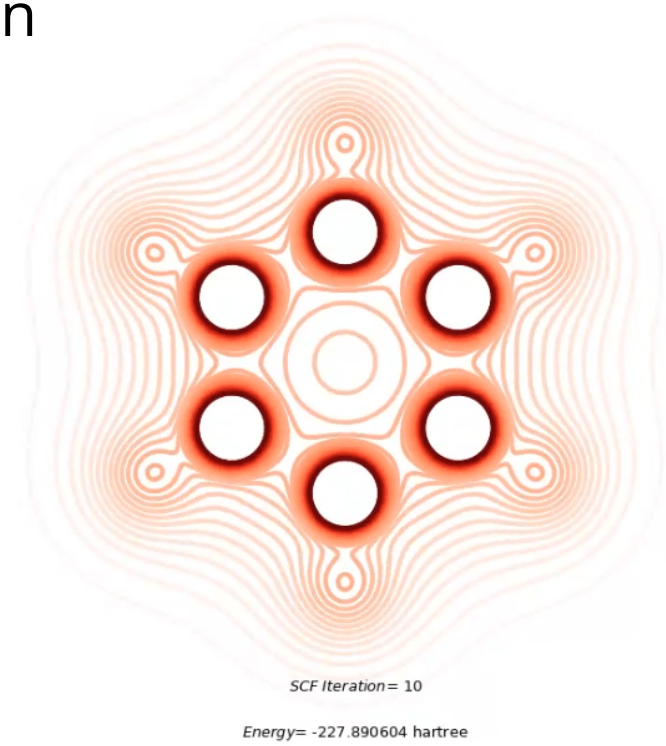
Changing the positions (or symmetry) change molecular orbitals



Result: An electron density distribution unique to that structure

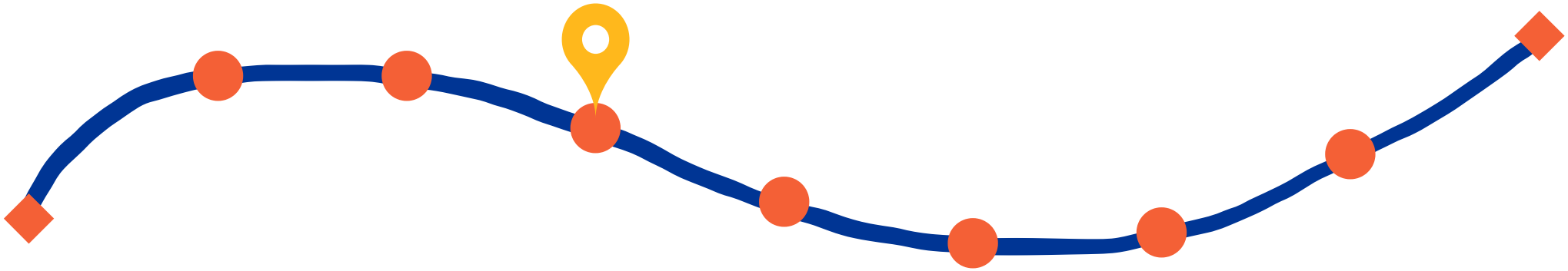
All experimental techniques are based on **probes interacting with molecule's electron density** to reveal structural information

- **X-ray Crystallography**: How a crystal of molecules diffracts X-rays
- **NMR Spectroscopy**: How atomic nuclei interact with magnetic fields and radiofrequency pulses
- **Cryo-Electron Microscopy**: How molecules scatter electron beams



Electron density of benzene

After today, you should be able to



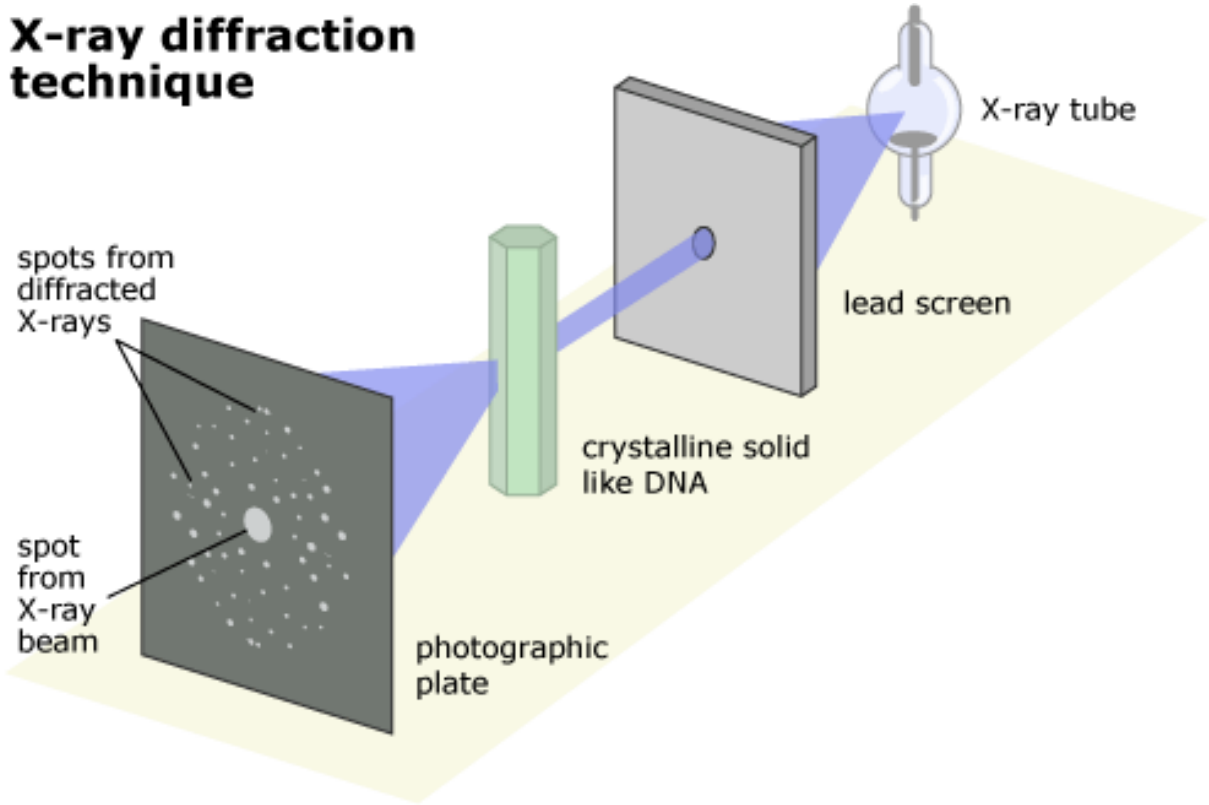
Communicate the basics of X-ray crystallization

Fundamentals of X-ray Crystallography

Probe: Photon (carrier of electromagnetic radiation)

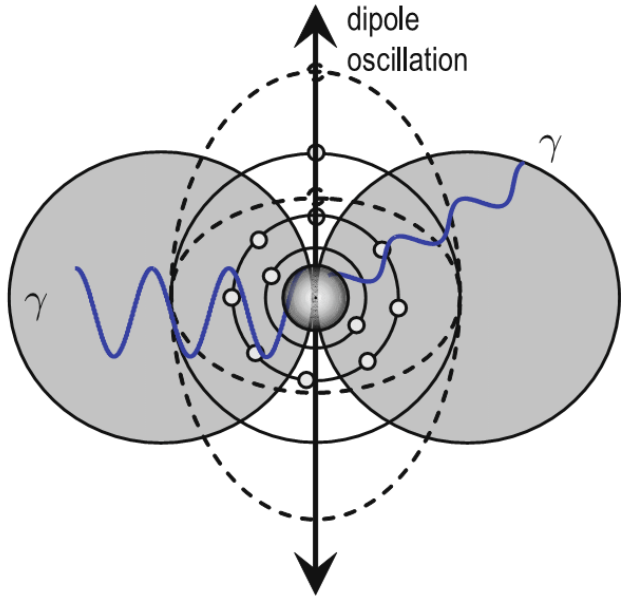
Basic Principle: Photons scatter when they interact with atoms

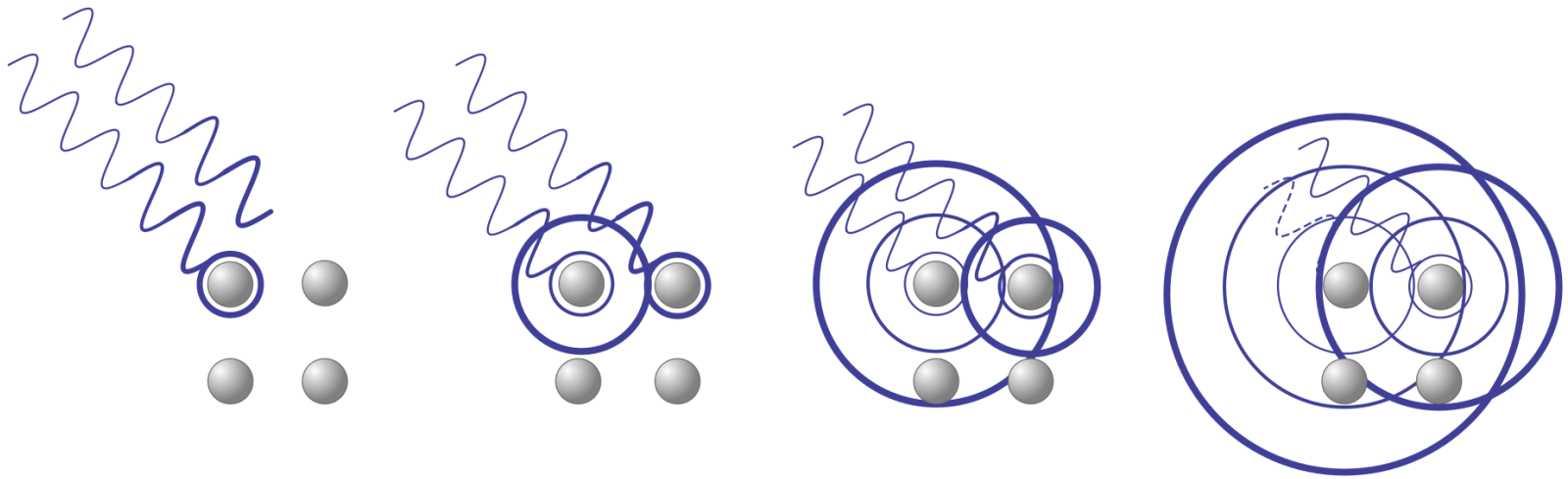
The scattered X-rays form a **diffraction pattern** unique to the crystal



X-rays undergo elastic scattering by electrons

1. Incident photon induces an oscillating dipole by distorting the electron density (Rayleigh)
2. An oscillating dipole acts as an electromagnetic source and re-emits photons at the same wavelength in all directions

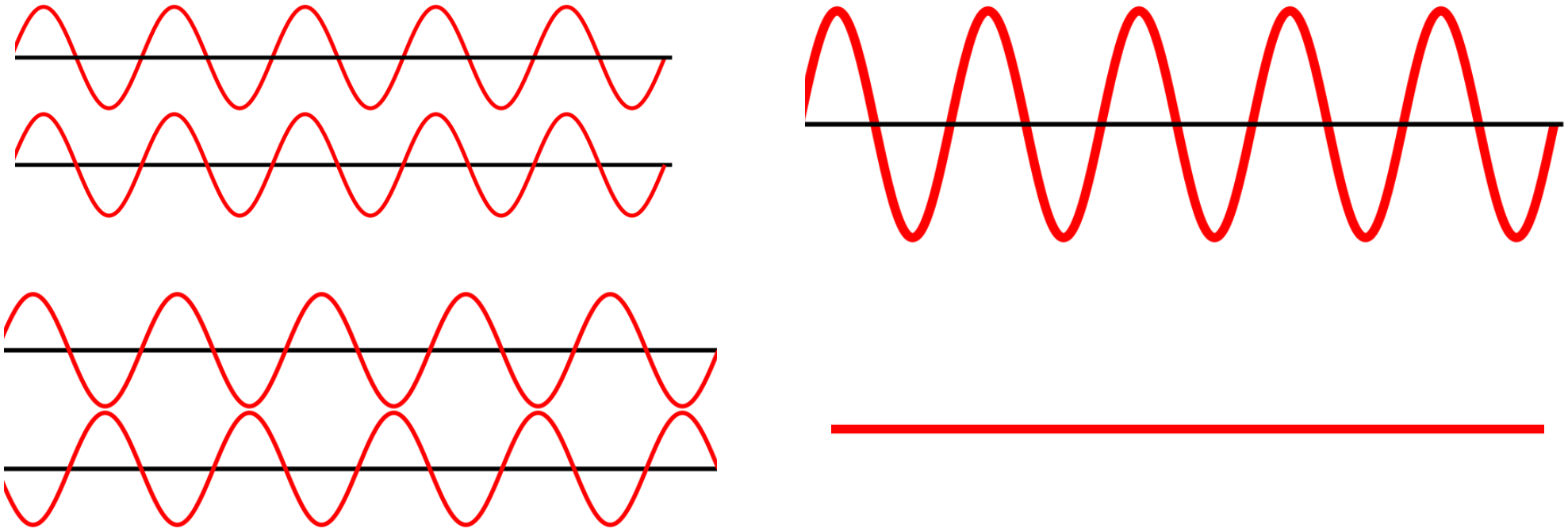




What happens when two waves overlap?

Constructive interference is needed to amplify signal for detectors

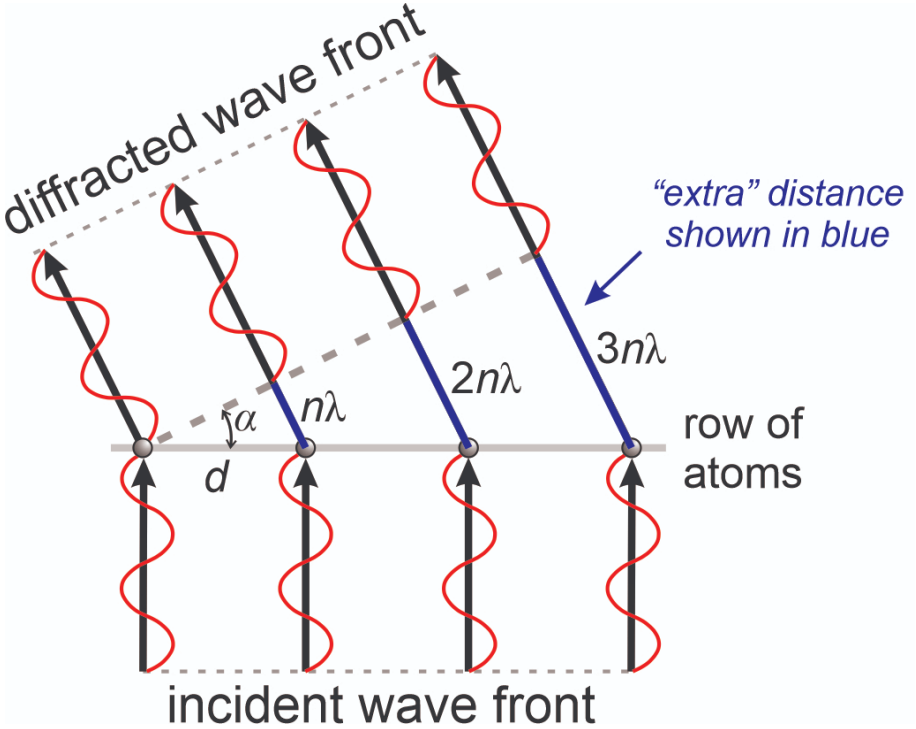
If wavelengths are similar and in phase, they constructively interfere

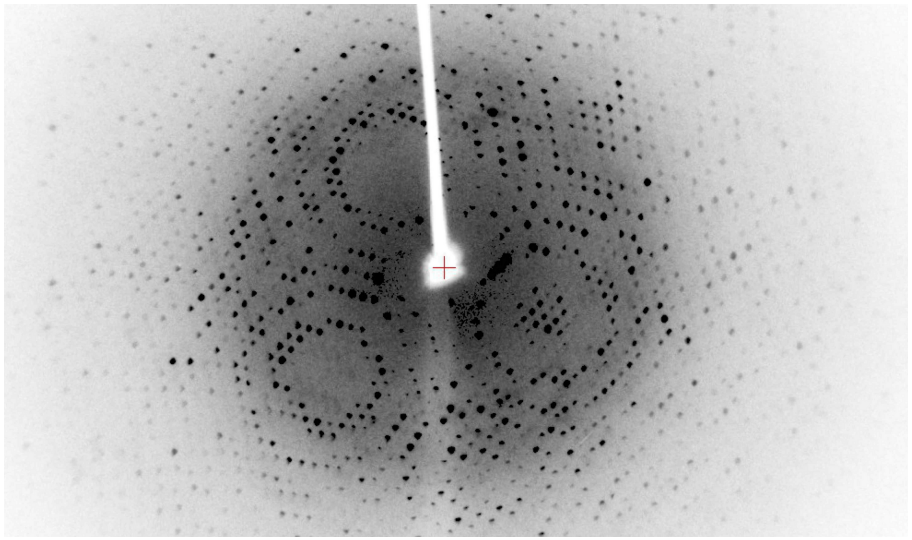


If waves are out of phase, they deconstructively interfere

Constructive interference leads to distinct patterns

If wavelengths are similar and in phase, they constructively interfere and form spots based on atom type and distance



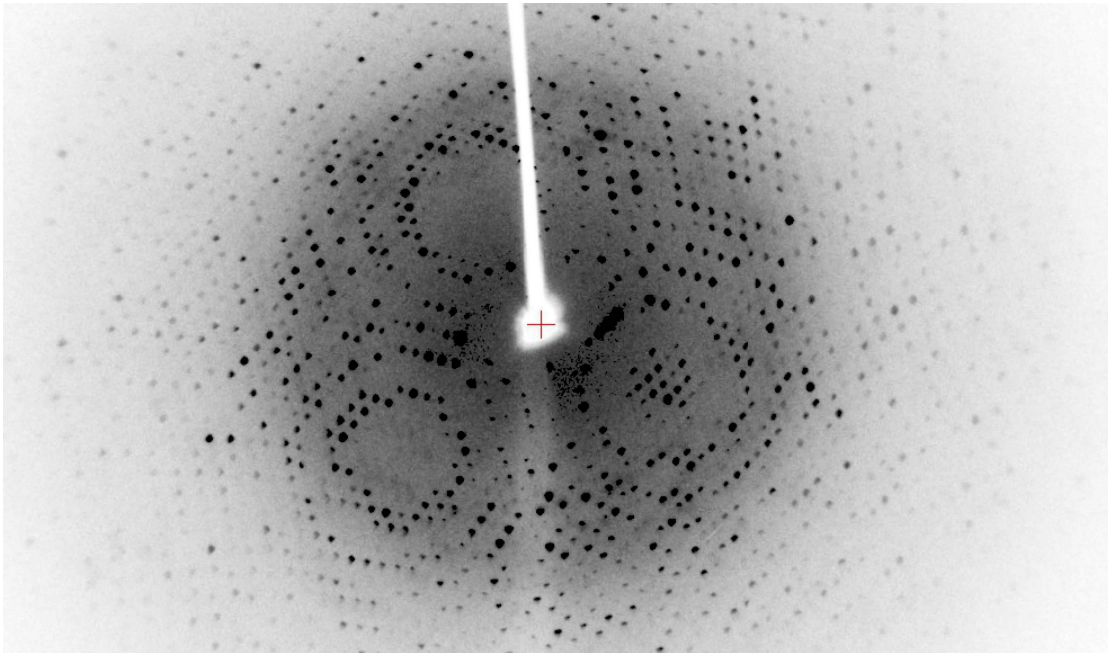


The diffraction pattern

The spots on the detector represent the **reflections** of the scattered X-rays

- **Intensity** of the spots reflects the electron density in the crystal
- **Position and angle**: The position of the spots corresponds to the geometry

The diffraction pattern does not directly show the atomic positions, but provides the data needed to infer the electron density

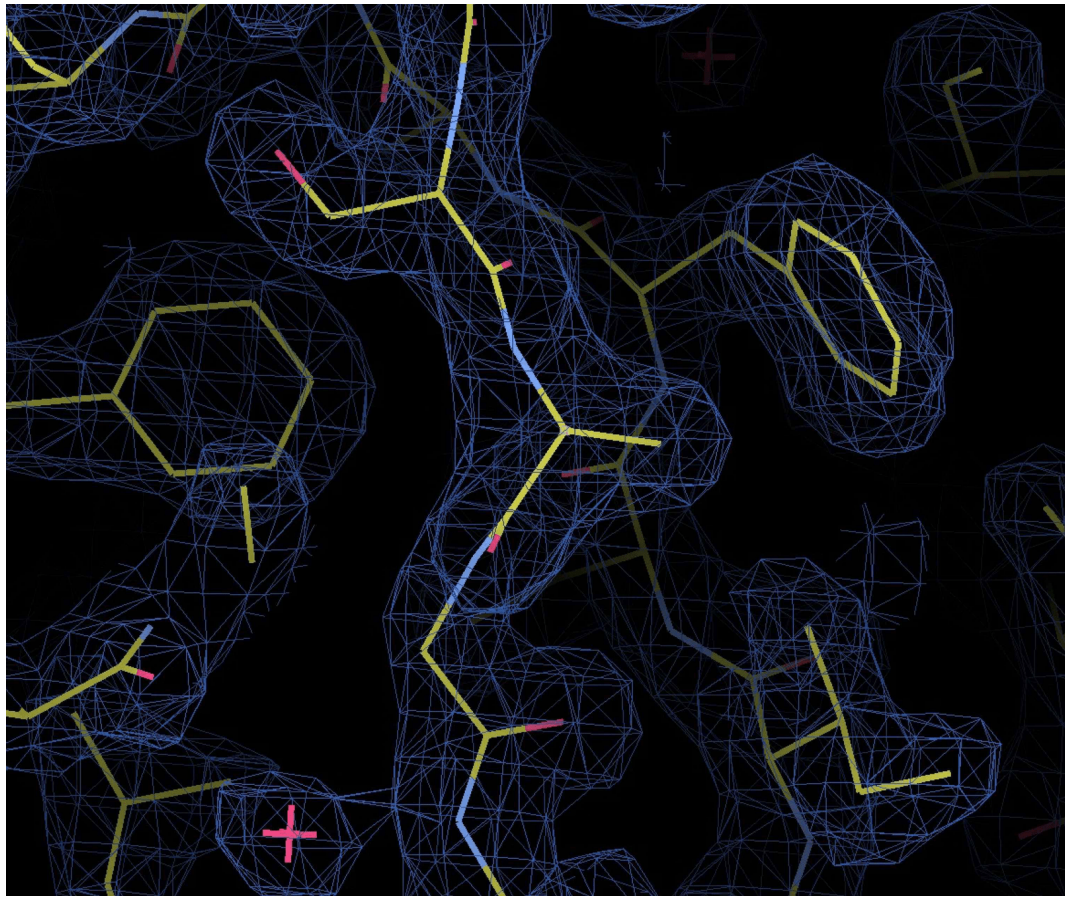


Building the electron density map

The **3D electron density map** reveals the distribution of electrons in the crystal, indicating where atoms are located

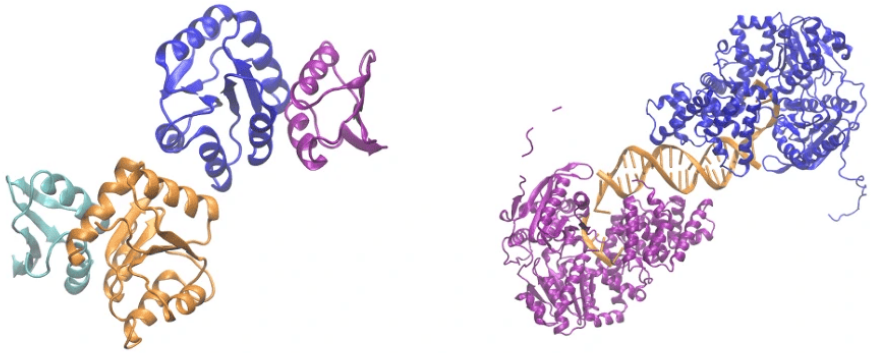
The electron density map is interpreted by fitting atomic models (e.g., amino acids for proteins) into the density

Low-resolution data make it difficult to assign atomic positions precisely, leading to uncertainty in the model



Why do we need crystals?

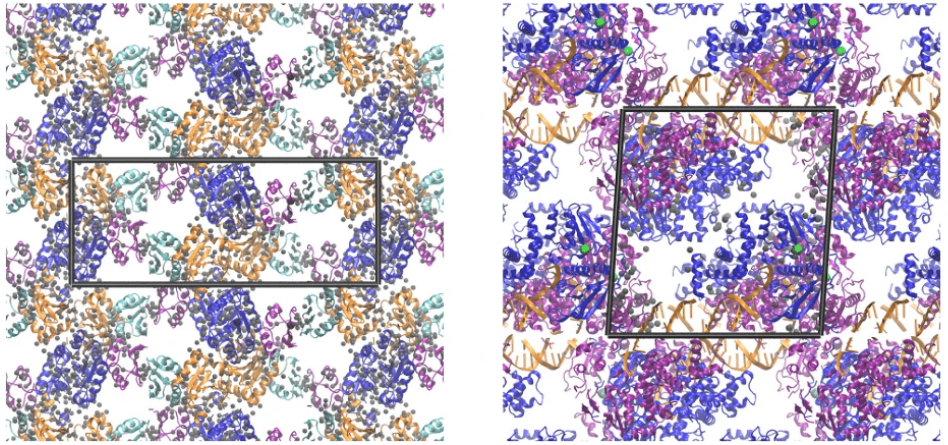
Crystals have the same repeating unit cell, which amplifies our signals



Molecules

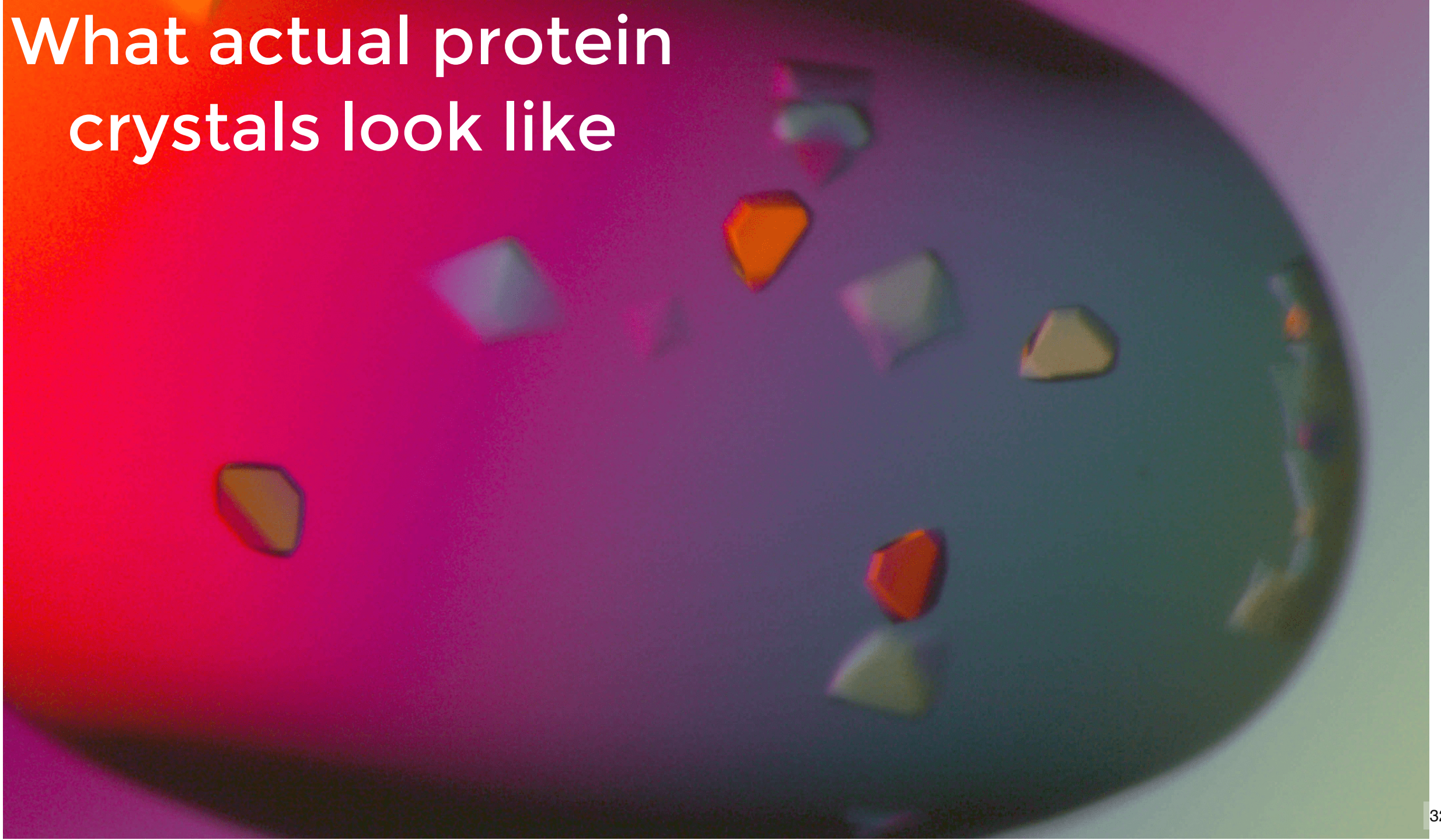
If in solution, particles would be

- Too sparse to diffract
- Moving and diffraction pattern would constantly change



Crystals

What actual protein crystals look like



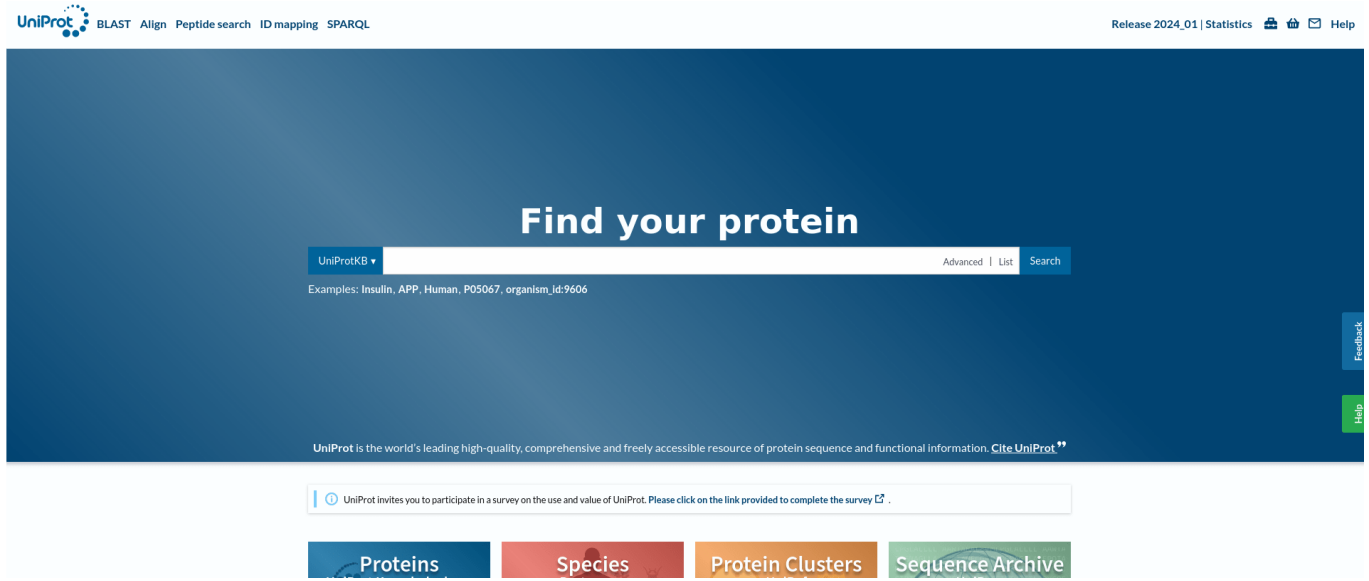
After today, you should be able to

Find and analyze protein structures

in the Protein Data Bank (PDB)

UniProt is a protein information database

www.uniprot.org



UniProt is a comprehensive database to access curated data about protein structures, functions, sequences, and annotations.

Let's find information about our project's drug target:
Dihydrofolate reductase

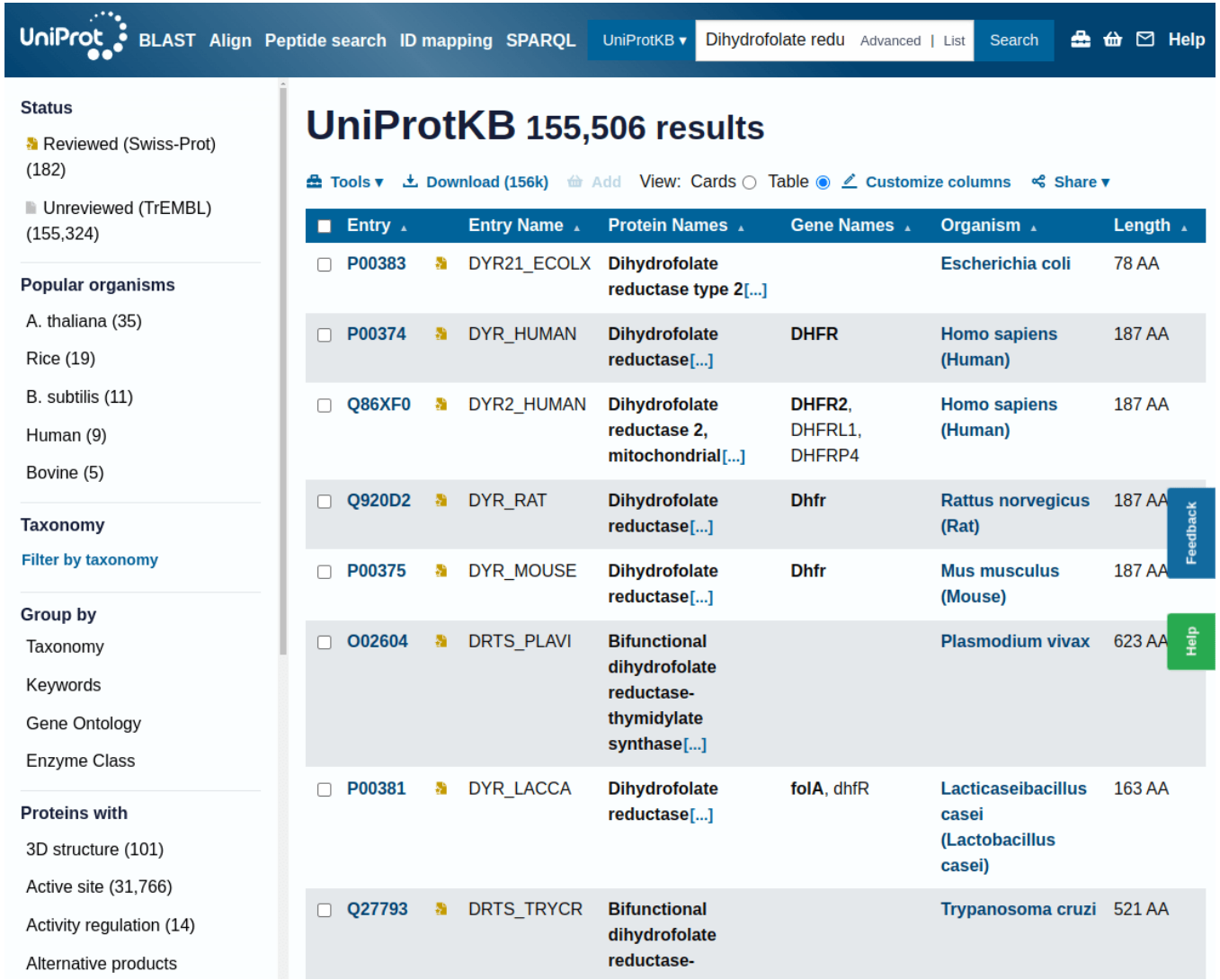
This page shows the results of a search in **UniProtKB** for a specific protein, in this case, "Dihydrofolate reductase"

On the left side, you have multiple filters to narrow your search results:

- **Reviewed (Swiss-Prot)**: Experts manually curated and verified these entries, ensuring high accuracy
- **Unreviewed (TrEMBL)**: These entries are automatically generated and have not been manually reviewed

Each row in the table represents a different protein entry

Entry ID: A unique identifier for the protein (e.g., P00383). You can click on this ID for detailed information about the protein



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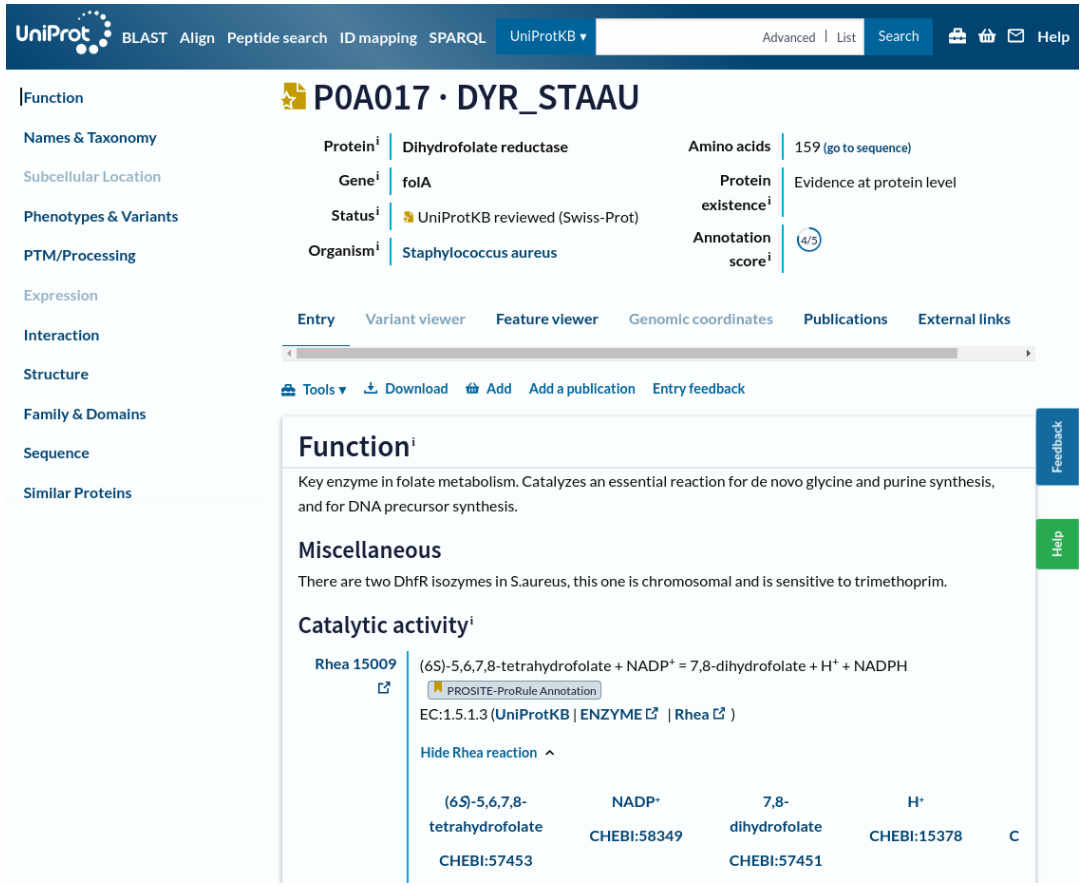

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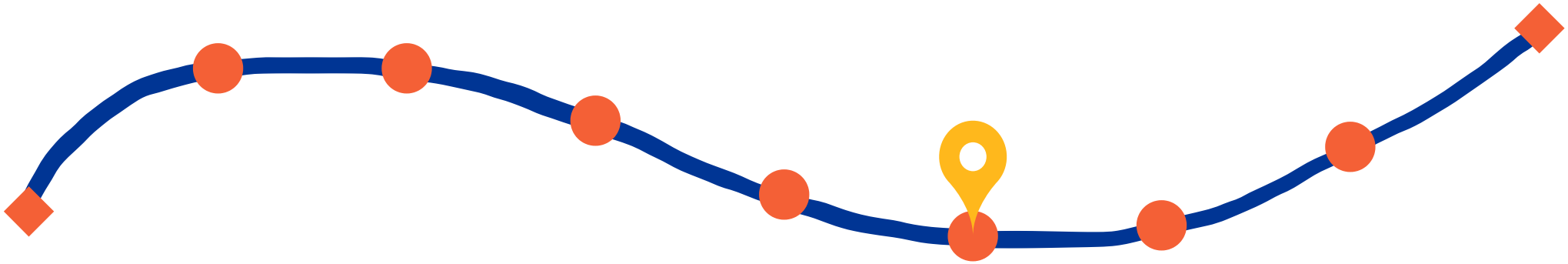
Protein Data Bank contains structures

www.rcsb.org

After today, you should be able to



Compare and contrast Cryo-EM to

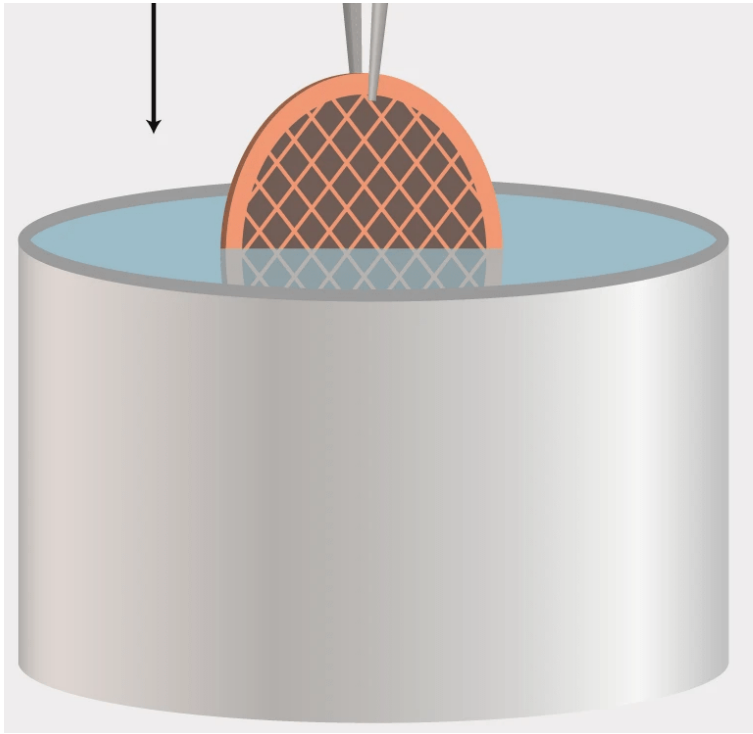
X-ray crystallization

Why Cryo-EM?

In Cryo-EM, a beam of high-energy electrons is used instead of photons

Why Electrons?

- Electrons have a much shorter wavelength (~0.02 Å at 300 keV) than photons
- Light elements which scatter electrons more effectively than X-rays



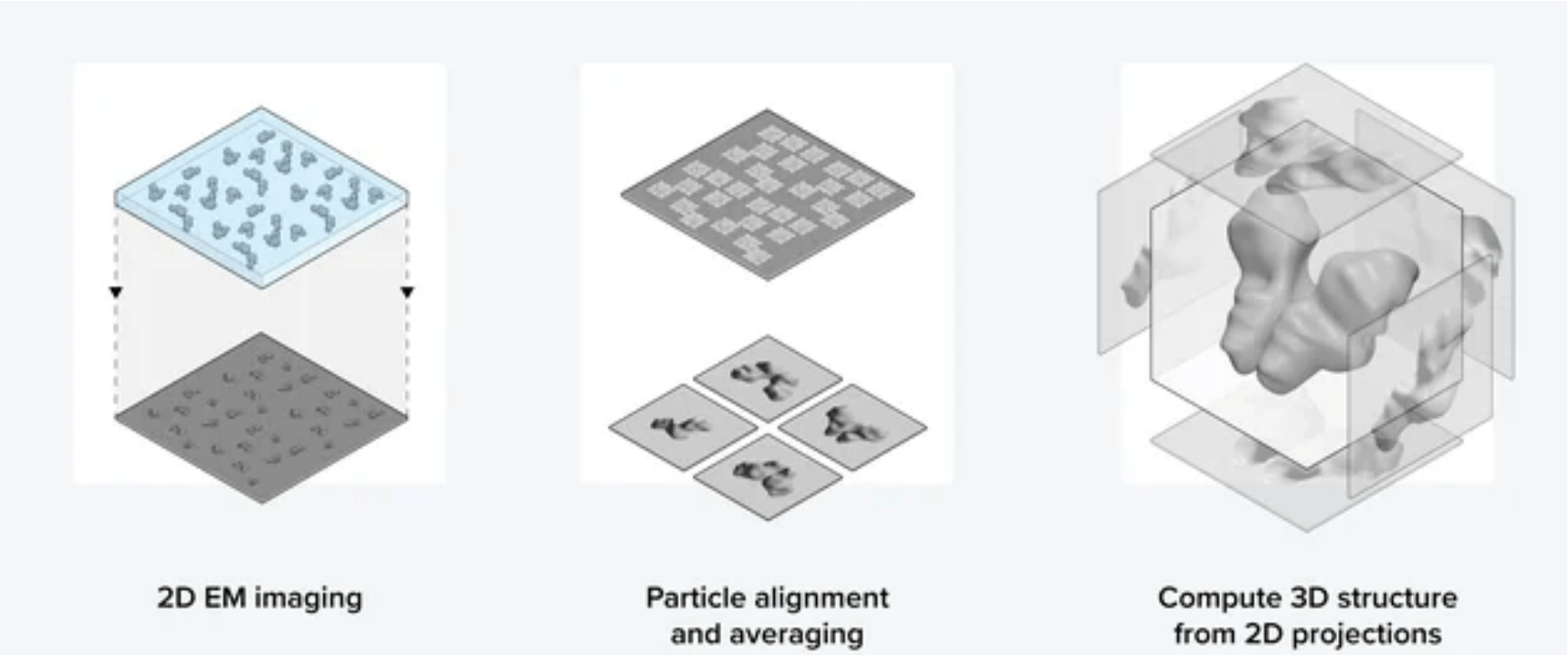
No crystals: The sample is rapidly frozen in vitreous ice to preserve its native structure

- By freezing the sample, biological molecules are imaged in their native hydrated state

Single Particle Analysis (SPA)

Single Particle Analysis is the main Cryo-EM technique used to determine the 3D structures of individual macromolecules

- Millions of images of individual particles are collected from a thin layer
- Particles are computationally aligned and classified into different orientations



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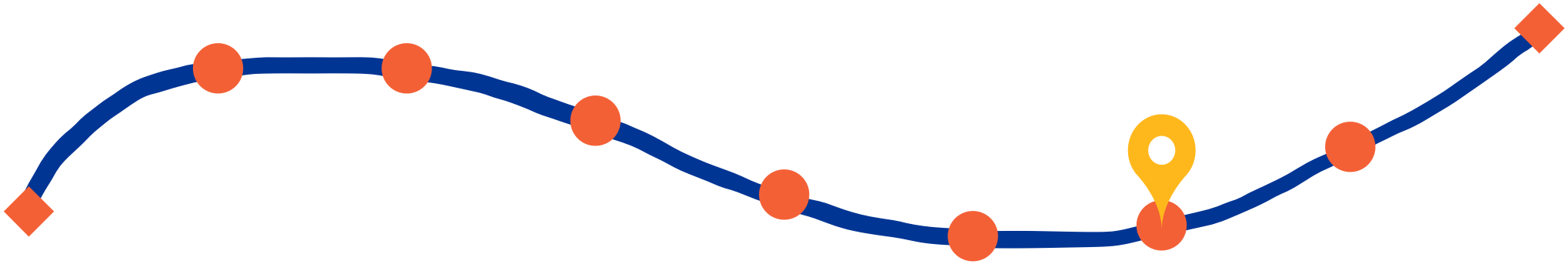


Communicate the challenges

of disorder

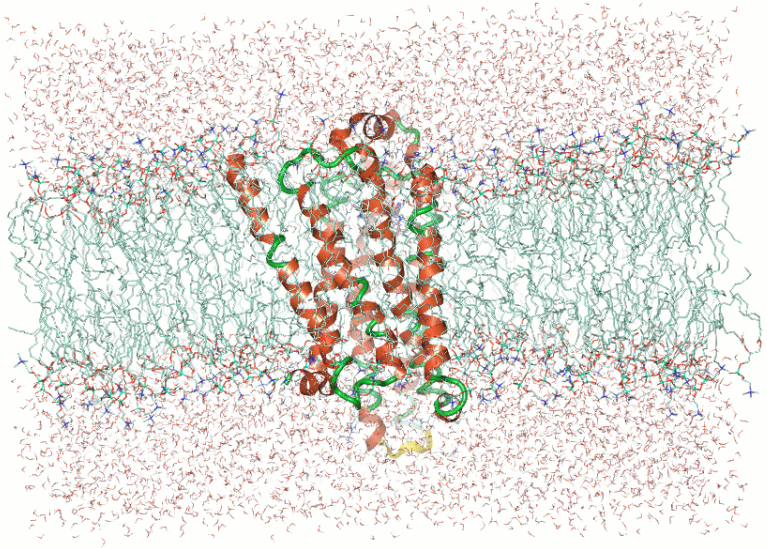
Challenge of flexibility and disorder in biomolecules

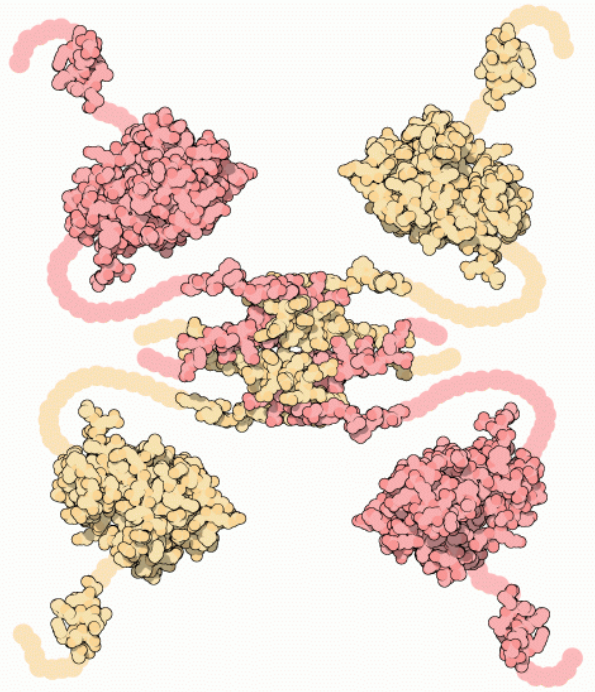
Molecules are not static

Proteins often exhibit flexibility, disordered regions, and multiple conformations

Example: The p53 tumor suppressor protein has flexible regions critical for its regulation and binding interactions

Why It Matters: Structural techniques often require ordered or stable configurations





Challenges in X-ray Crystallography

- Flexible or disordered regions do not pack into crystals well, often leading to failure in obtaining high-quality crystals.
- Even in cases where crystallization is successful, flexible or disordered regions often do not show up clearly in the electron density map.
- Crystals capture a single conformation of the molecule, often ignoring the flexibility or dynamic range.

Cryo-EM and Conformational Flexibility

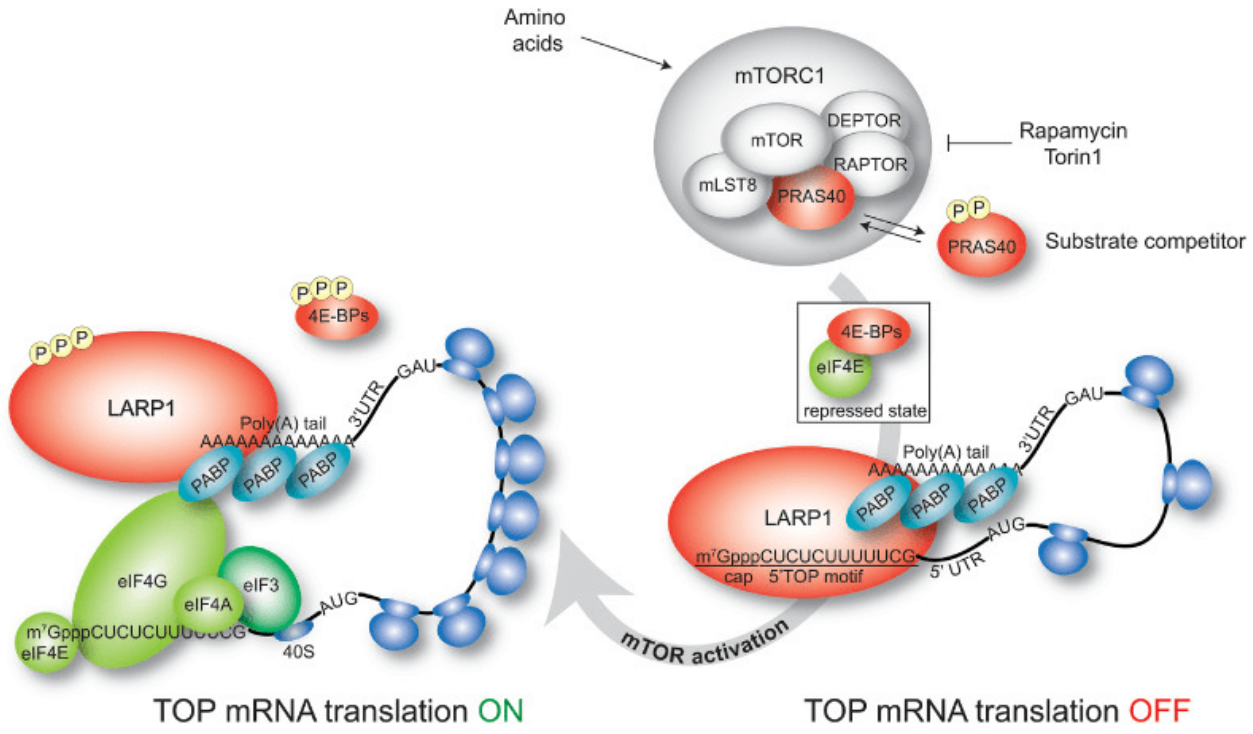
One strength of Cryo-EM is its ability to capture multiple conformational states of a molecule, providing insights into flexibility and structural heterogeneity.

Challenge: A major issue in Cryo-EM is that highly flexible or disordered molecules may appear as fuzzy or low-resolution regions in the final structure

Advanced computational techniques are required to sort out different conformations present in the Cryo-EM data

Intrinsically Disordered Proteins (IDPs)

Intrinsically disordered proteins (IDPs) or regions lack a stable 3D structure under physiological conditions but are still functional, often gaining structure upon binding to partners

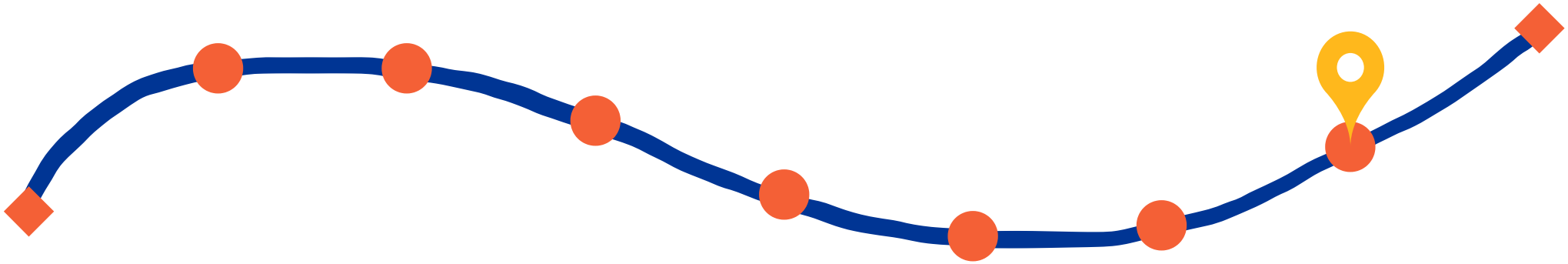


Conformational Heterogeneity and Biological Function

Many proteins function by switching between different conformations, which is essential for their activity (e.g., enzymes, transporters, and receptors).

- **Example**: G-protein coupled receptors (GPCRs) adopt different conformations when bound to different ligands, triggering different cellular responses.

After today, you should be able to



What is structural biology and why is it important?

Challenges in Experimental Structural Biology

Technical Limitations

- Difficulty in capturing dynamic and flexible regions.
- Incomplete structures due to unresolved disordered regions.

Biological Complexity

- Dynamic conformational ensembles not represented in static snapshots.

Resource Constraints

- Time-consuming and costly experiments.

Before the next class, you should

Lecture 11:
Structural biology

Lecture 12:
Protein structure prediction

Today

Thursday

- Review today's lecture