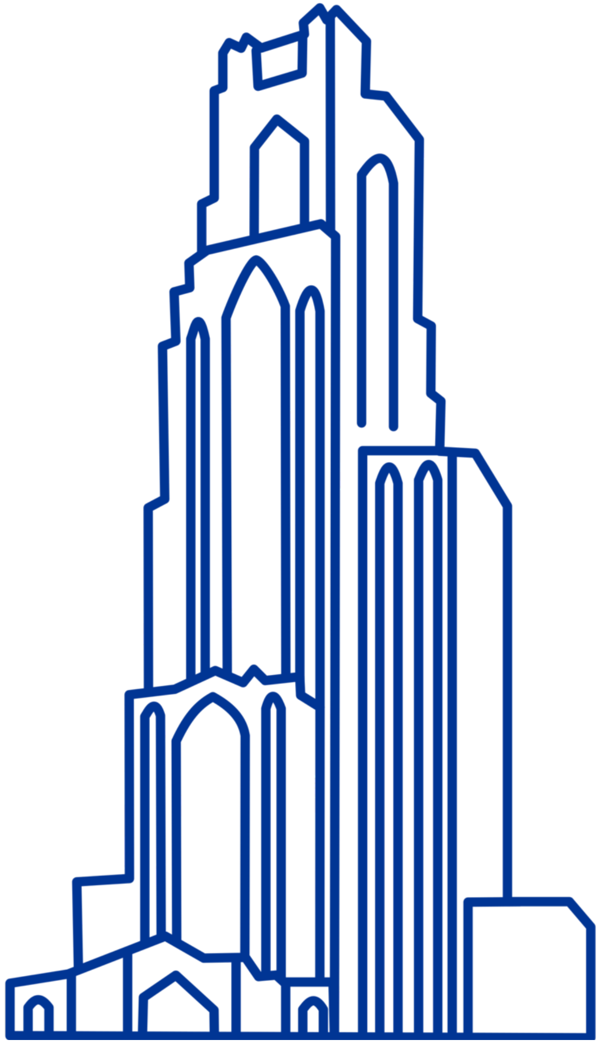
Computational Biology
(BIOSC 1540)



Lecture 09A

Structural Biology

Foundations

Mar 11, 2025

University of Pittsburgh

Announcements

Assignments

- P02A is due Mar 14
- P02B is due Mar 28
- P02C will be due Mar 28 (not yet released)

Quizzes

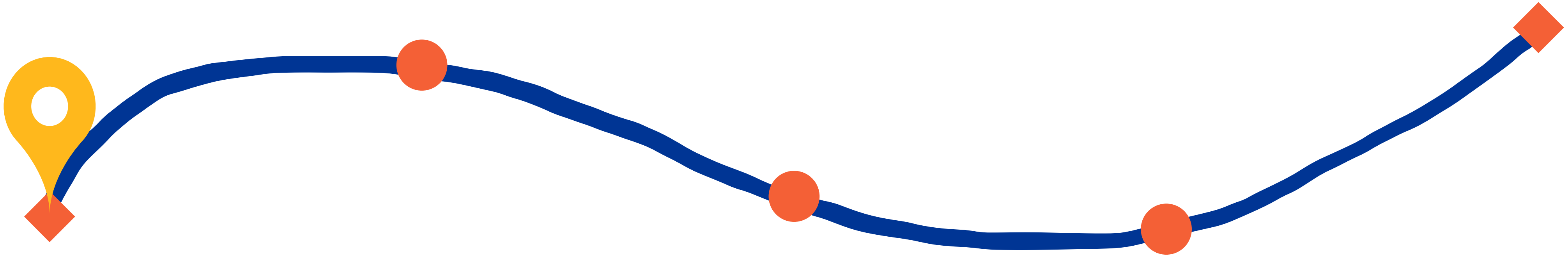
- Quiz 03 is on Mar 18 and will cover L06B to L08A

CBits

- Please fill out the Canvas discussion for CBit 08

Supplementary material is available to read; not required, but recommended

After today, you should have a better understanding of

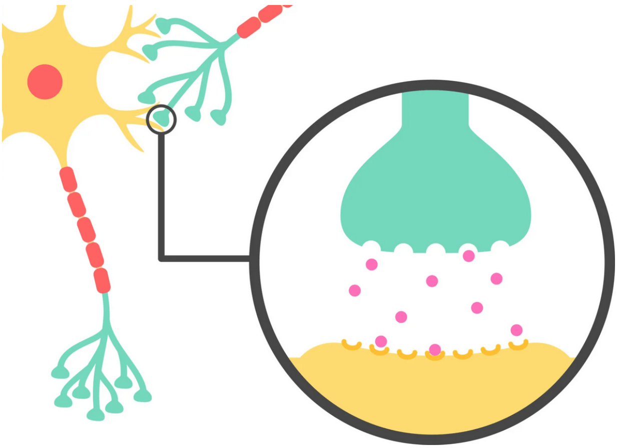


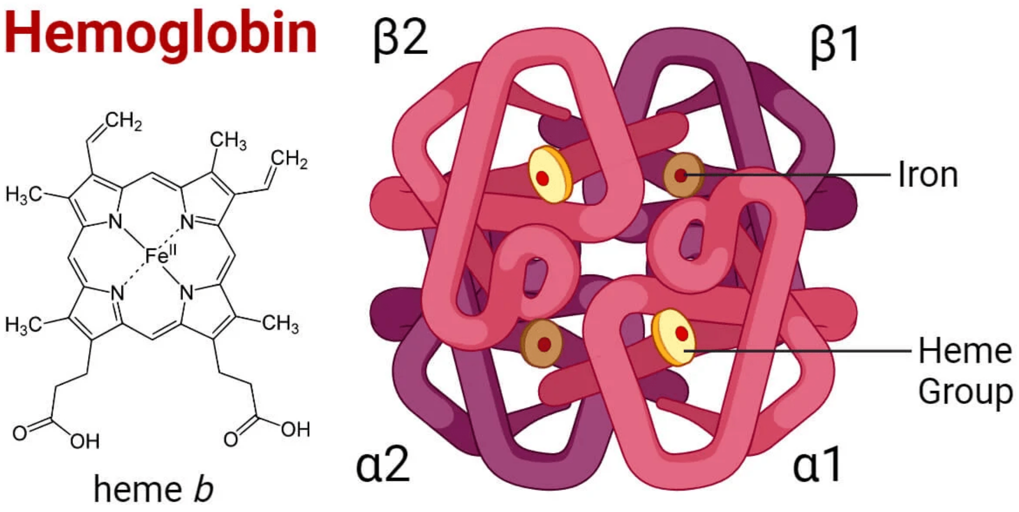
The definition and biological importance

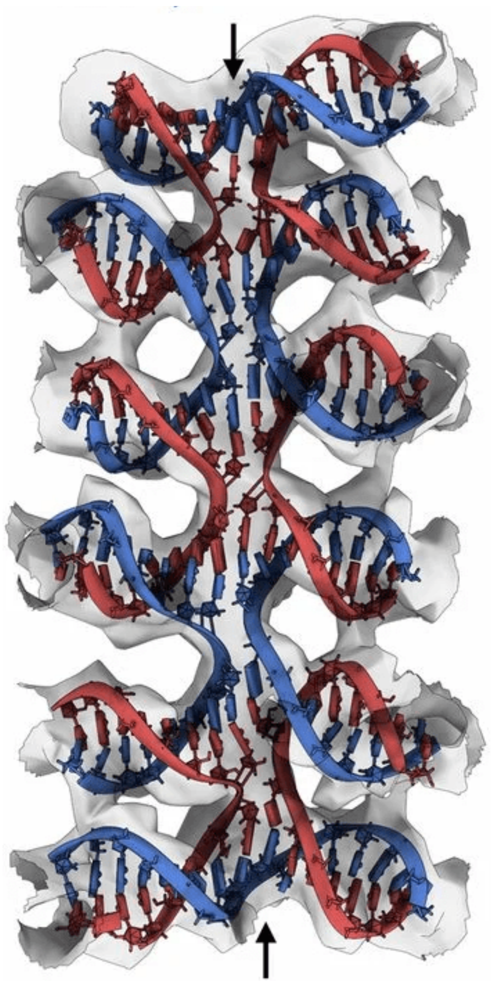
of structural biology

The atomic world of biology

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







What is structural biology?

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

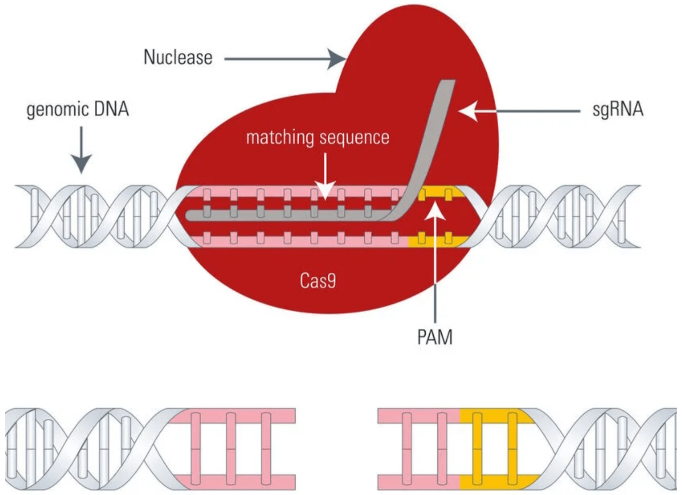
Why study structure?

- 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.

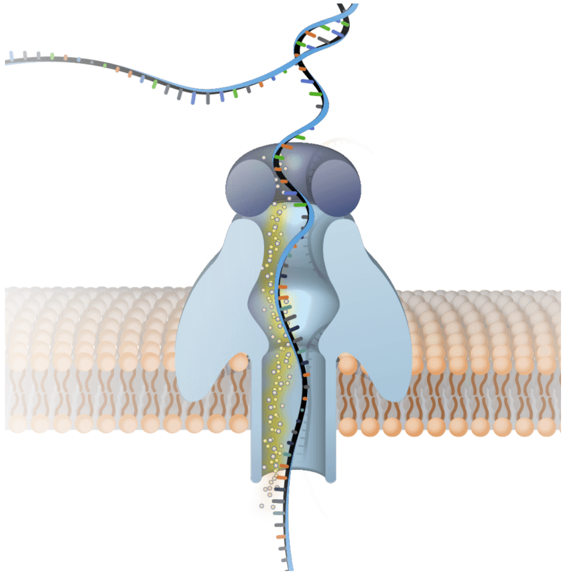
Importance of structural biology: We cannot exploit what we do not understand

Innovation and biotechnology depend on molecular understanding



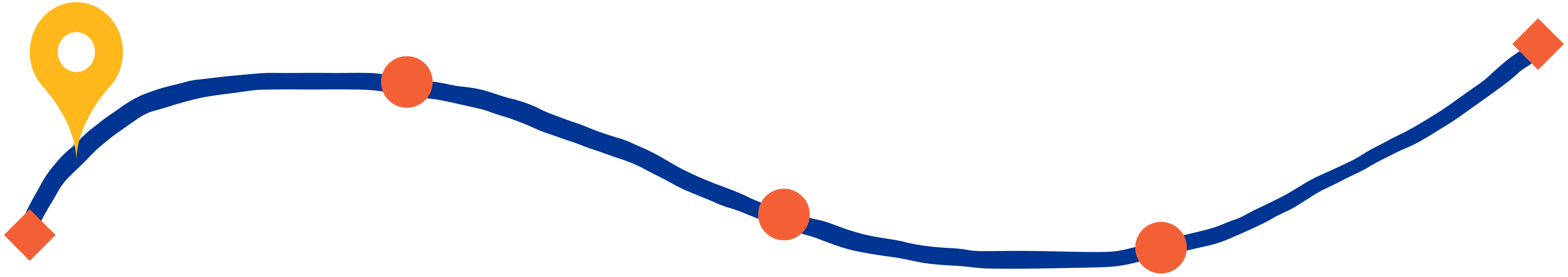
CRISPR-Cas9

COVID-19 treatments



High-throughput sequencing

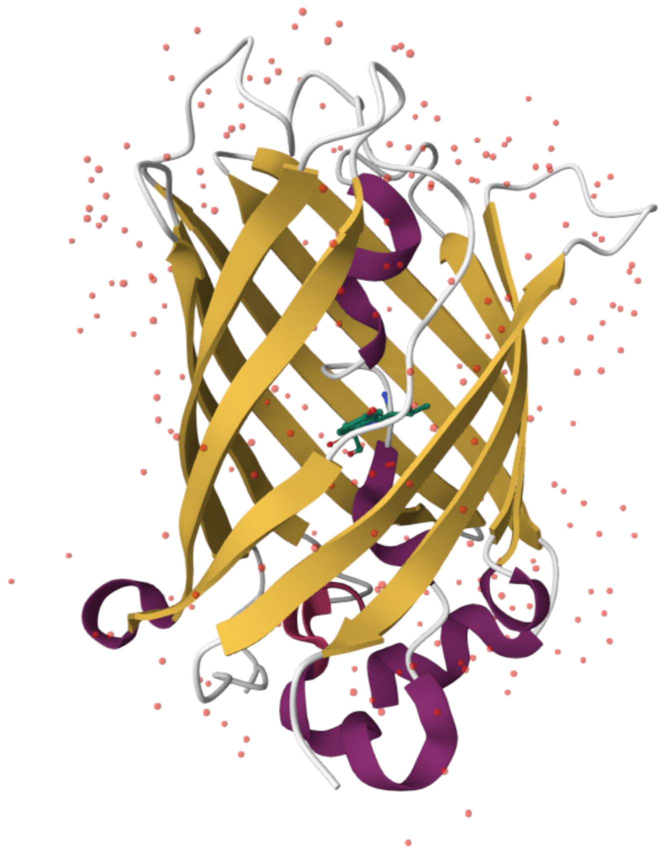
After today, you should have a better understanding of



The definition and biological importance of structural biology

Alex's research example: Engineering green fluorescent protein with Dr. Rosenbaum and Dr. Carlson

Green Fluorescent Protein (GFP) is a fluorescent protein from the jellyfish *Aequorea victoria*



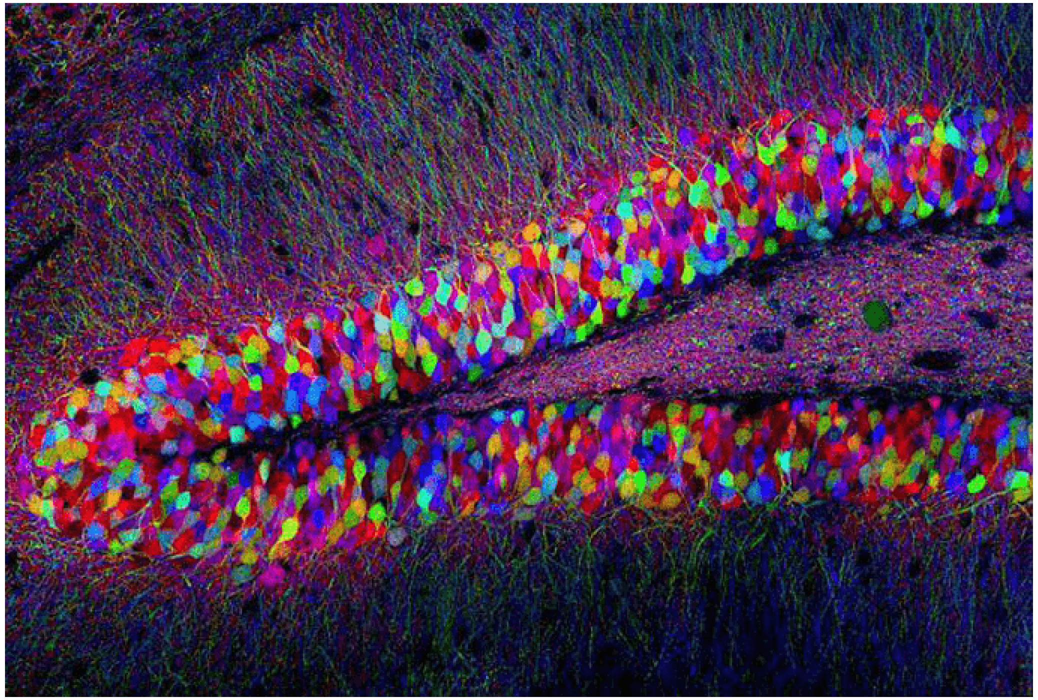
Enhanced GFP (eGFP) absorbs violet/blue light
(400 - 490 nm) and emits green light ~507 nm

We can repurpose GFP to do many things!

Track molecules by adding it as a tag

First video of cellular transfer of HIV

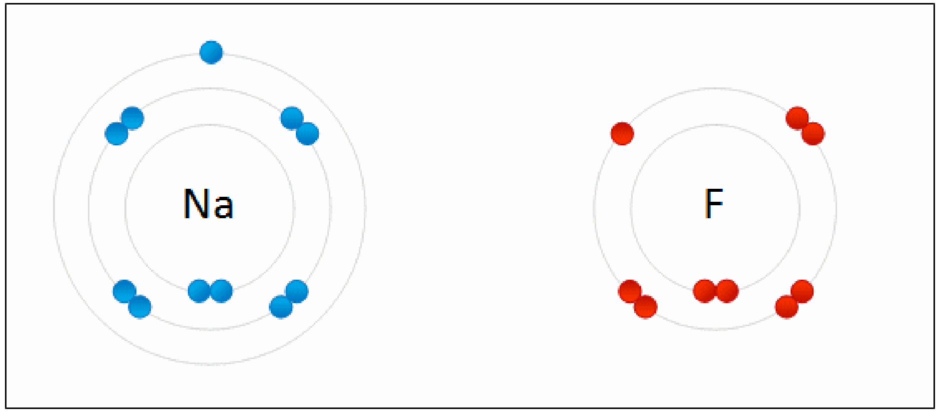
Differentiate cells with GFP variants



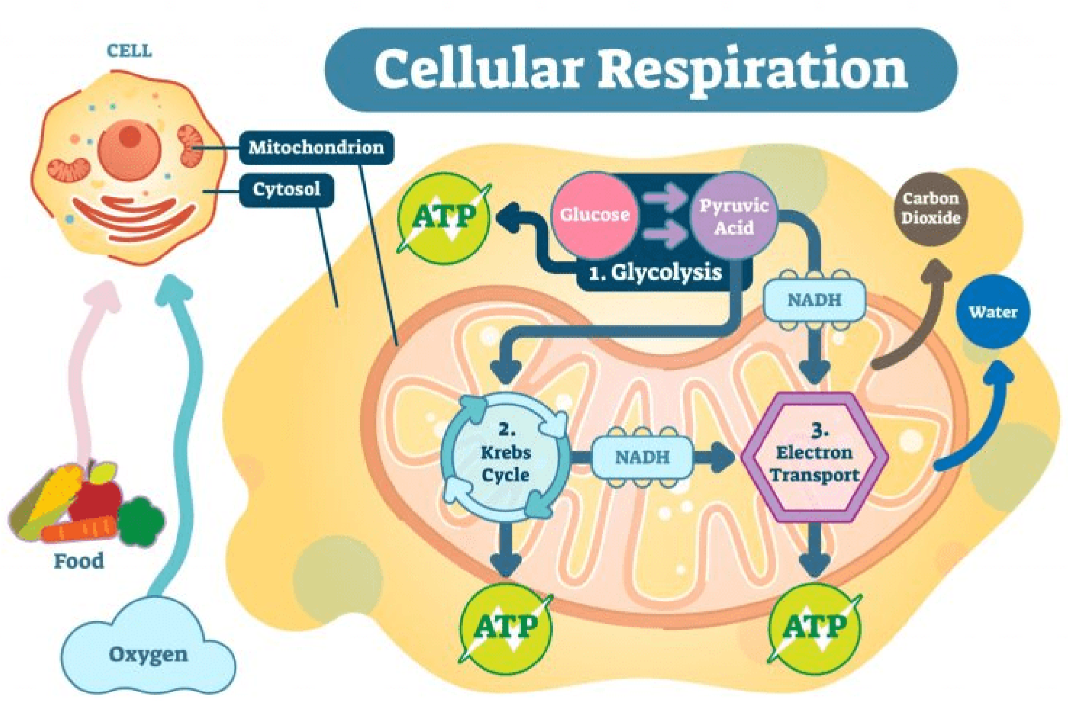
Multicolored GFPs used to map mouse brain

Redox reactions are a cornerstone of biology

Redox potentials indicate a solution's tendancy to gain or lose electrons



For example, mitochondria are highly reducing with a redox potential around -0.36 V

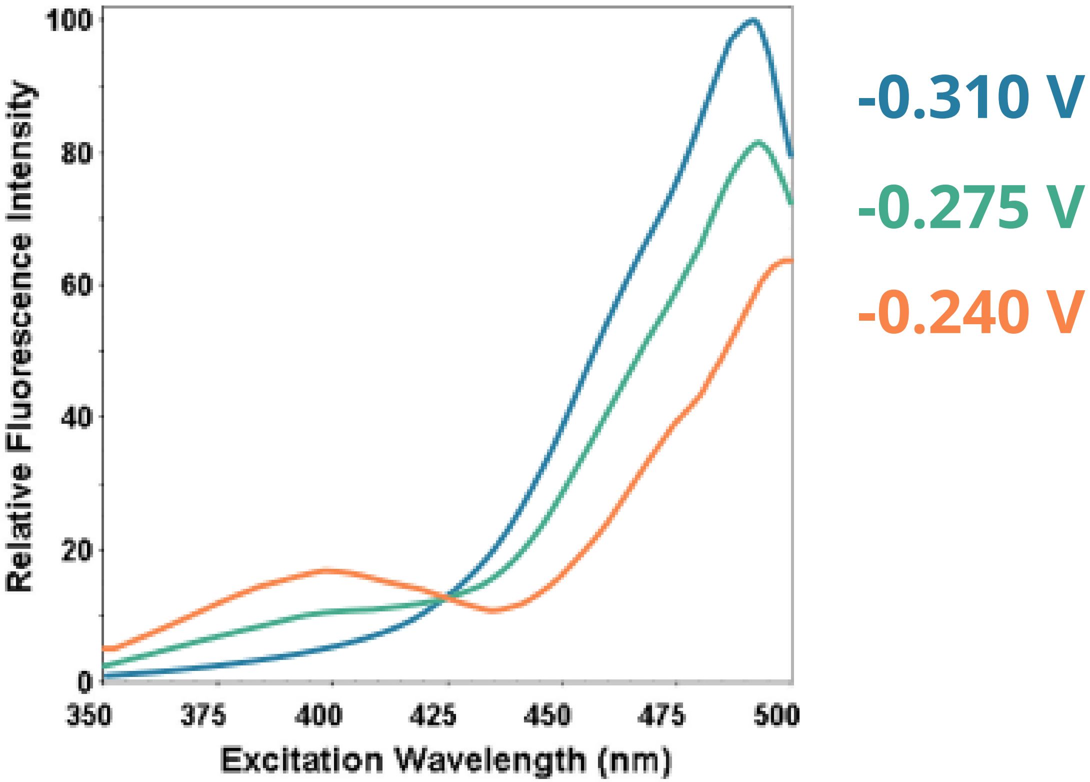


Reduction: NAD^{+} to NADH

Oxidation: NADH to NAD^{+}

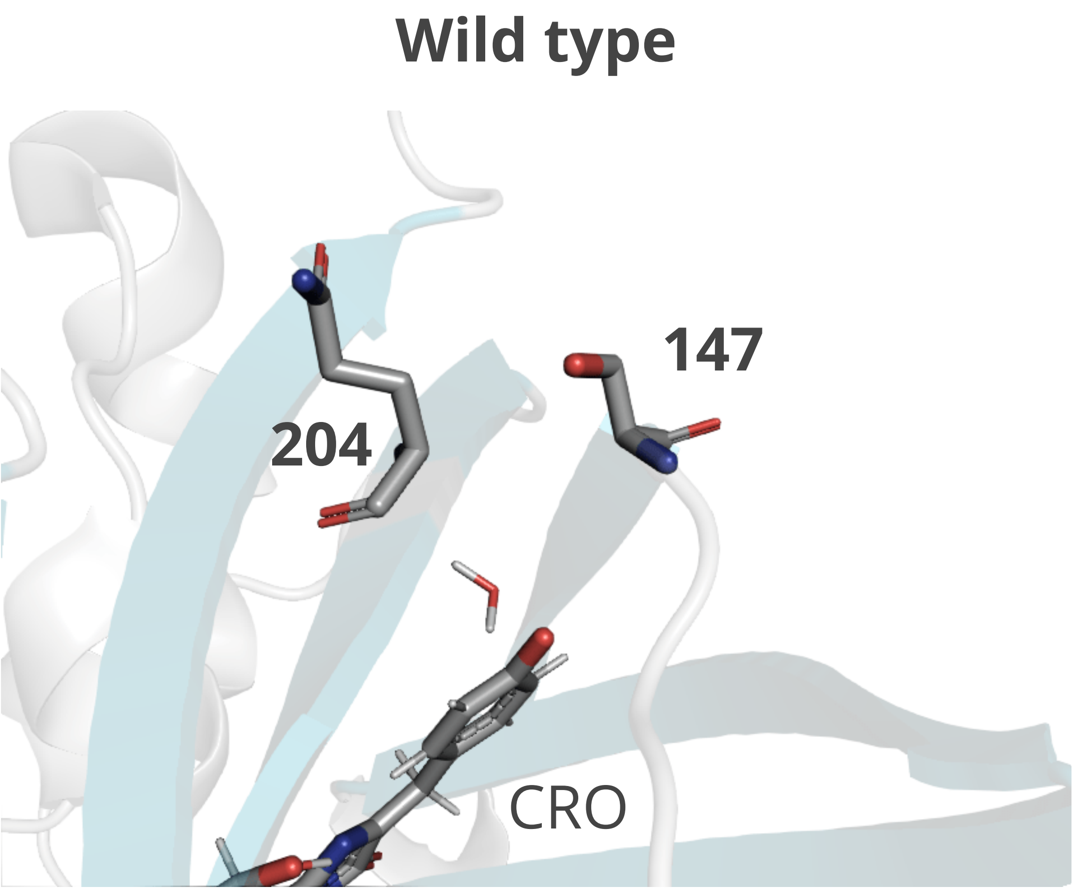
Remington and coworkers developed redox-sensitive GFPs

Fluorescence ratio after **400/488 nm** excitation correlated to redox potential of roGFP2 environment

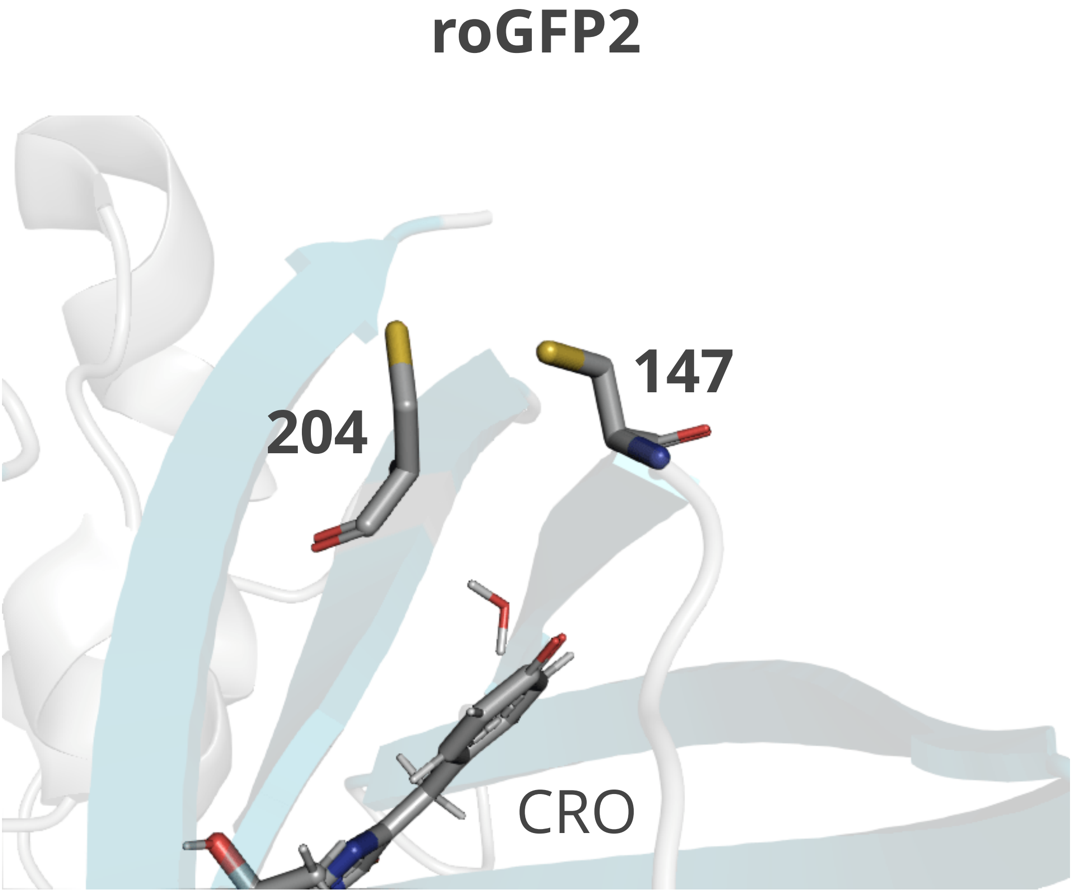


Hanson, G. T., et al. (2004). Journal of Biological Chemistry, 279(13), 13044-13053. DOI: 10.1074/jbc.M312846200

roGFP2 has S147C and Q204C mutations



PDB ID: 2Y0G



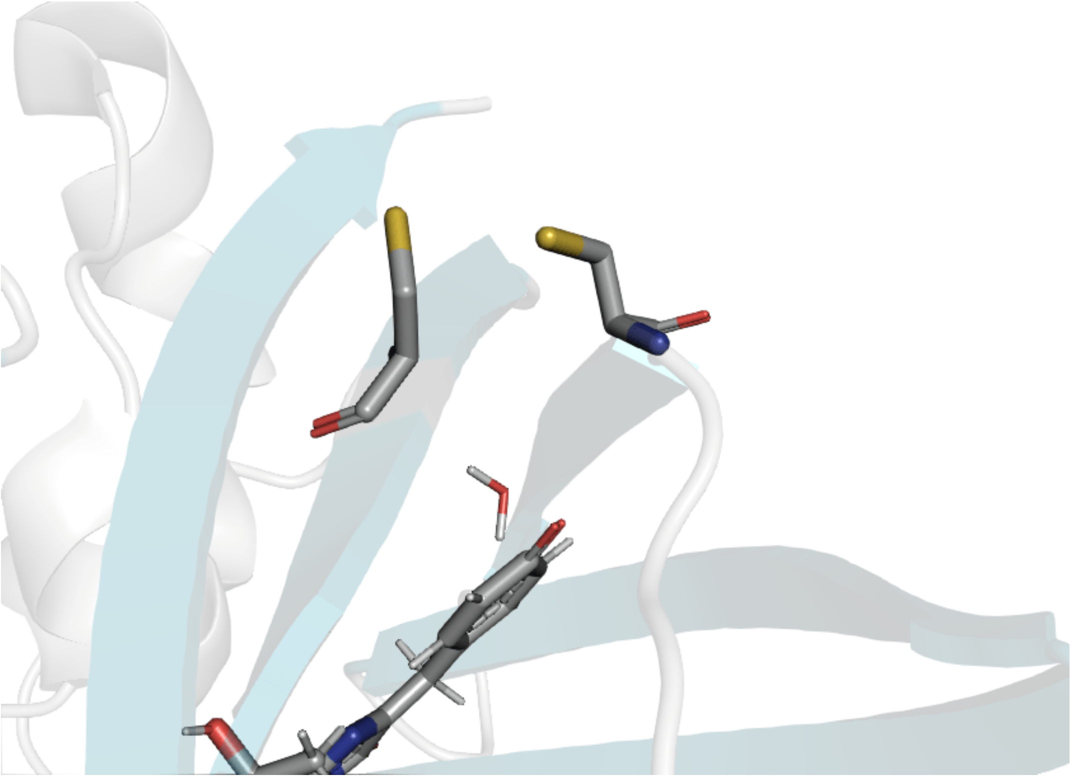
PDB ID: 1JC0

Hanson, G. T., et al. (2004). Journal of Biological Chemistry, 279(13), 13044-13053. DOI: 10.1074/jbc.M312846200

(Contains S65T "enhanced" mutation)

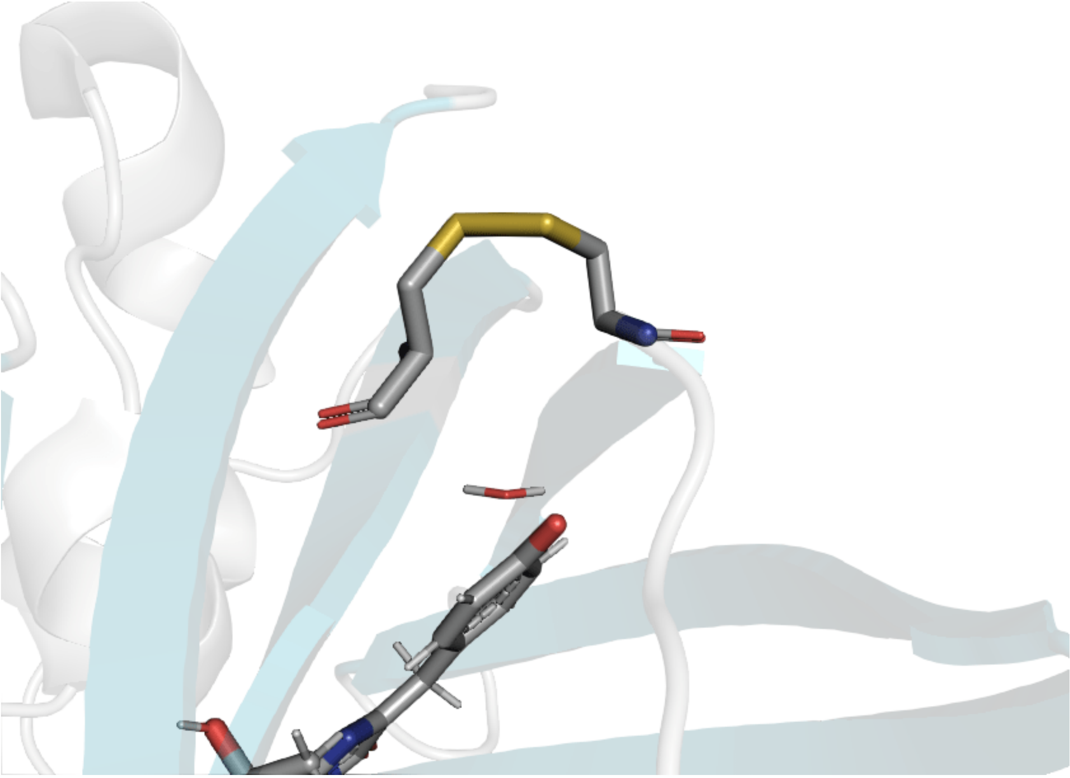
Disulfide bond formation is driven by redox potential

Reduced



PDB ID: 1JC0

Oxidized

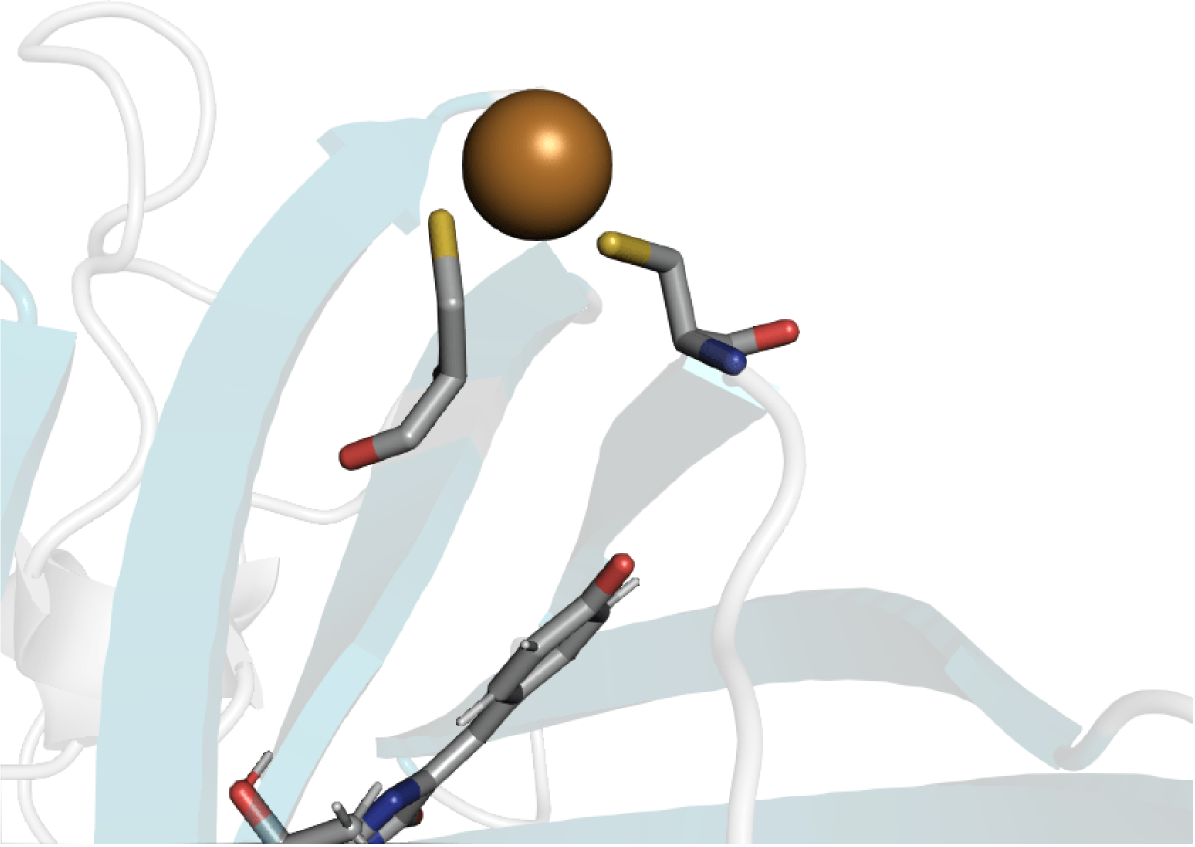


PDB ID: 1JC1

Hanson, G. T., et al. (2004). Journal of Biological Chemistry, 279(13), 13044-13053. DOI: 10.1074/jbc.M312846200

(Contains S65T "enhanced" mutation)

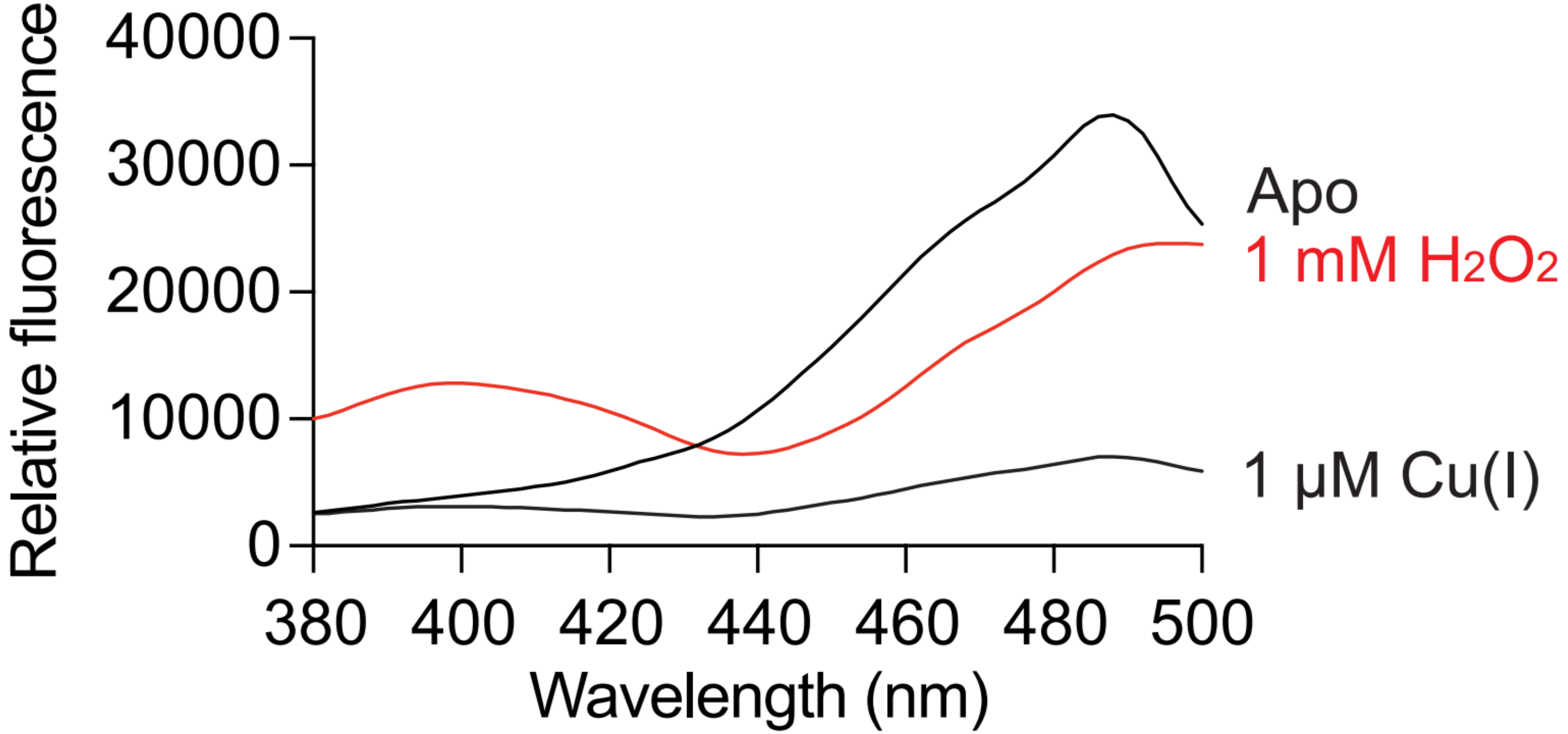
Cysteines can also bind metals

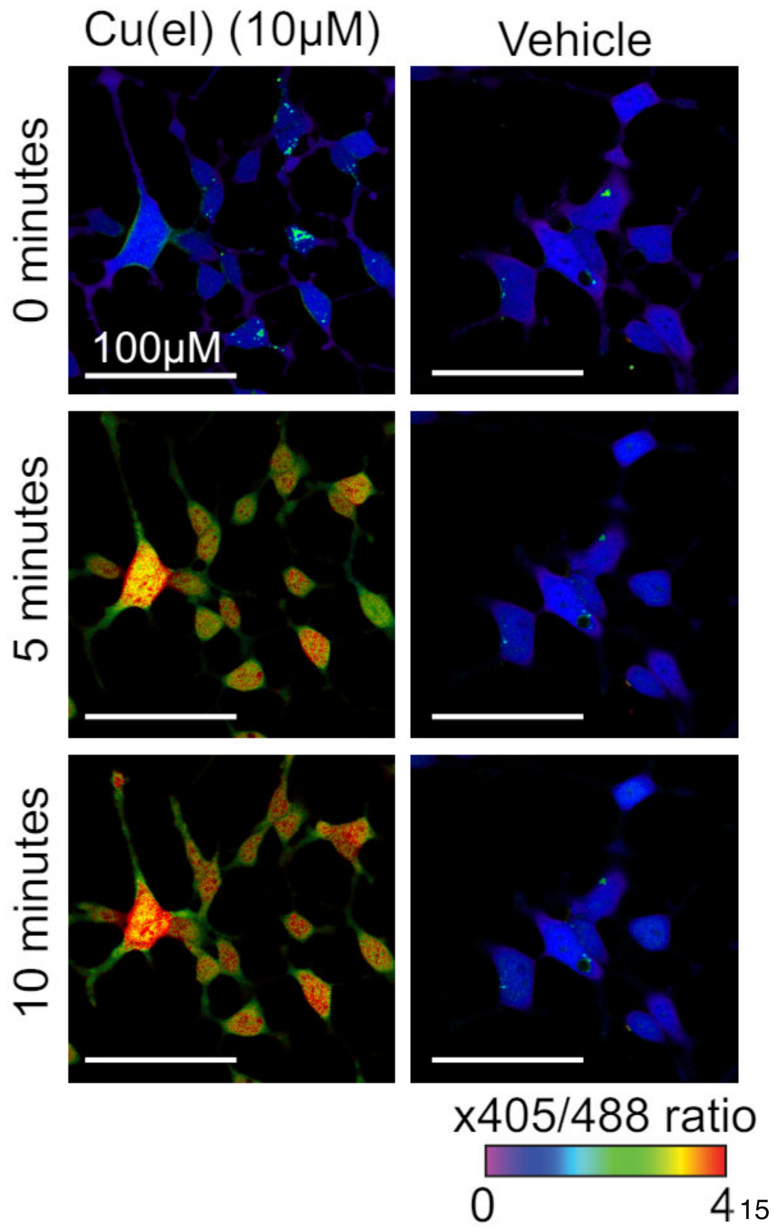


roGFP2 can bind Cu(I) to CYS147 and CYS204

Cu(I) sensing GFP

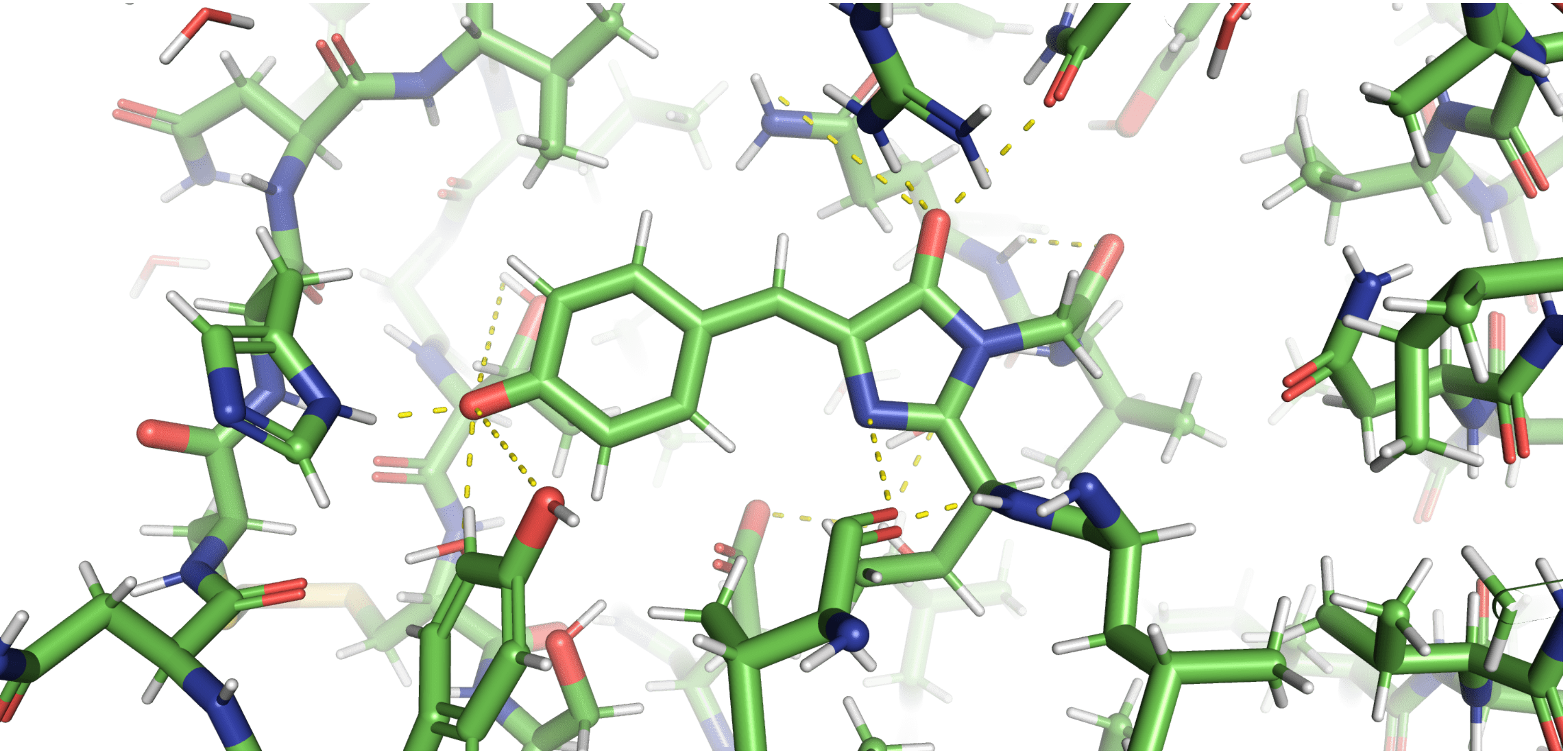
roGFP2 will also change fluorescence in a different way when copper is present

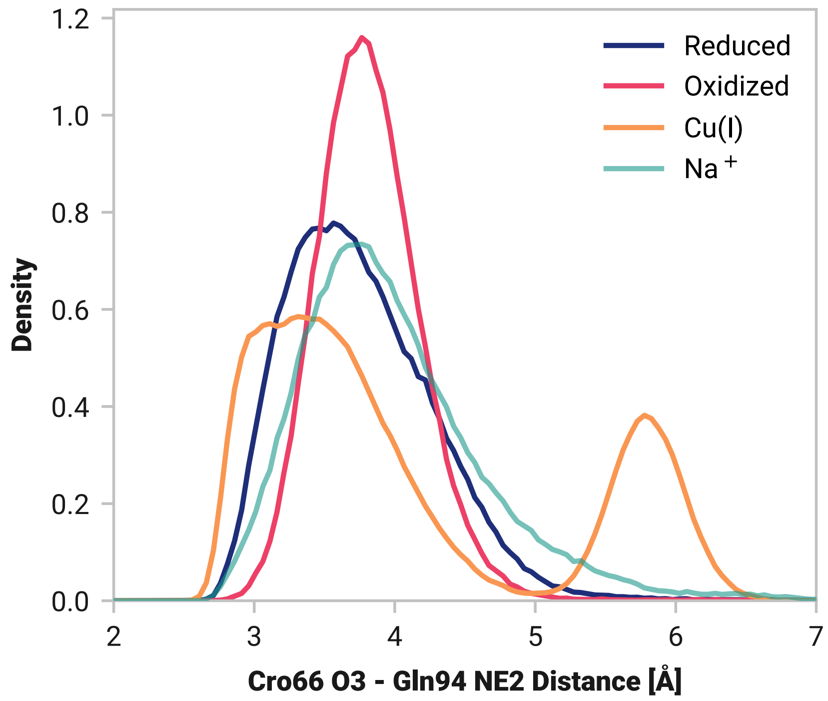




Computational question: How does Cu(I) binding quench roGPF2 florescence?

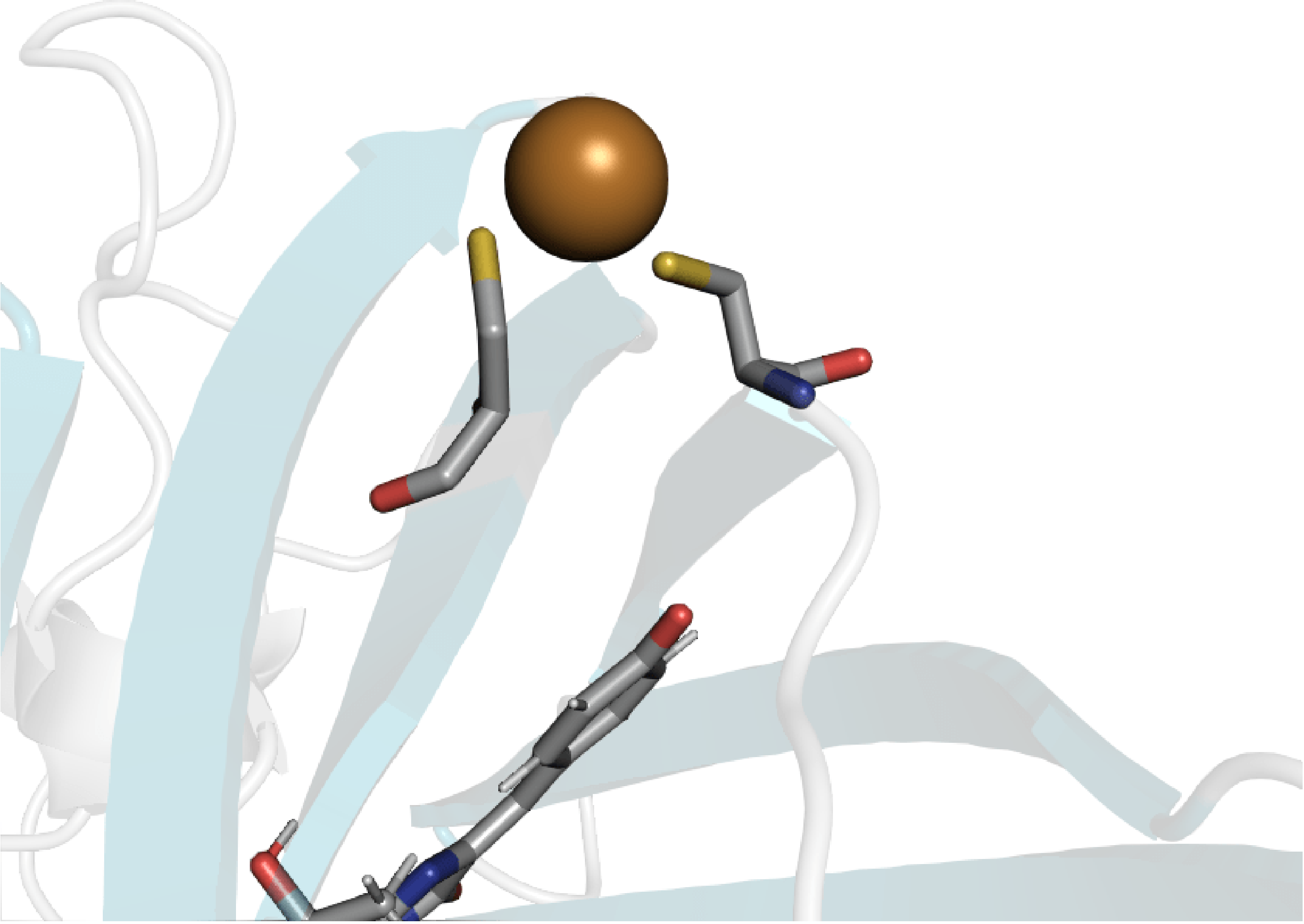
Cu(I) binding to Cys147 and Cys204 disrupts the chromophore's hydrogen bonding network





When the chromophore has increased flexability, it will de-excite through vibrations instead of emitting photons

Application: We can tailor the position of these cysteines to bind other heavy metals like lead, arsenic, etc.



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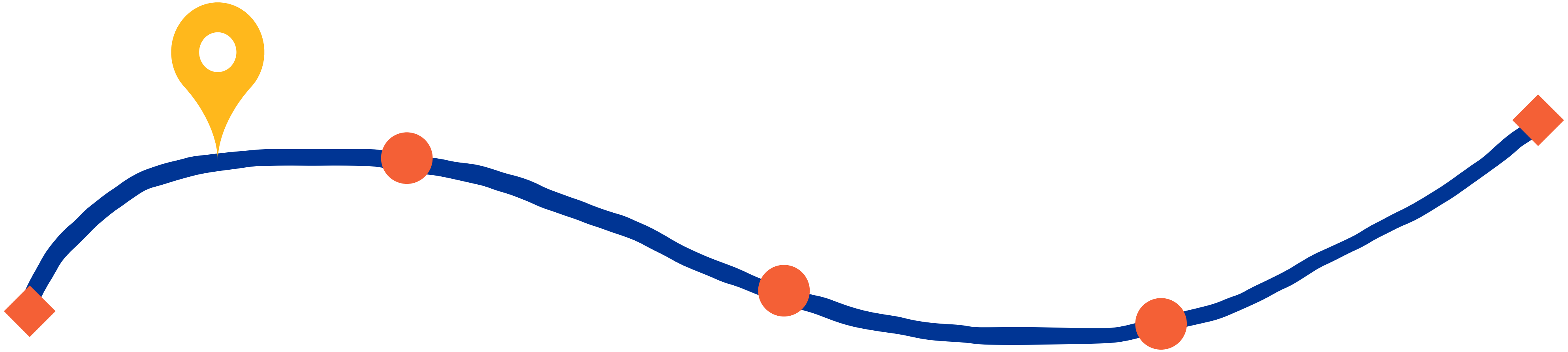


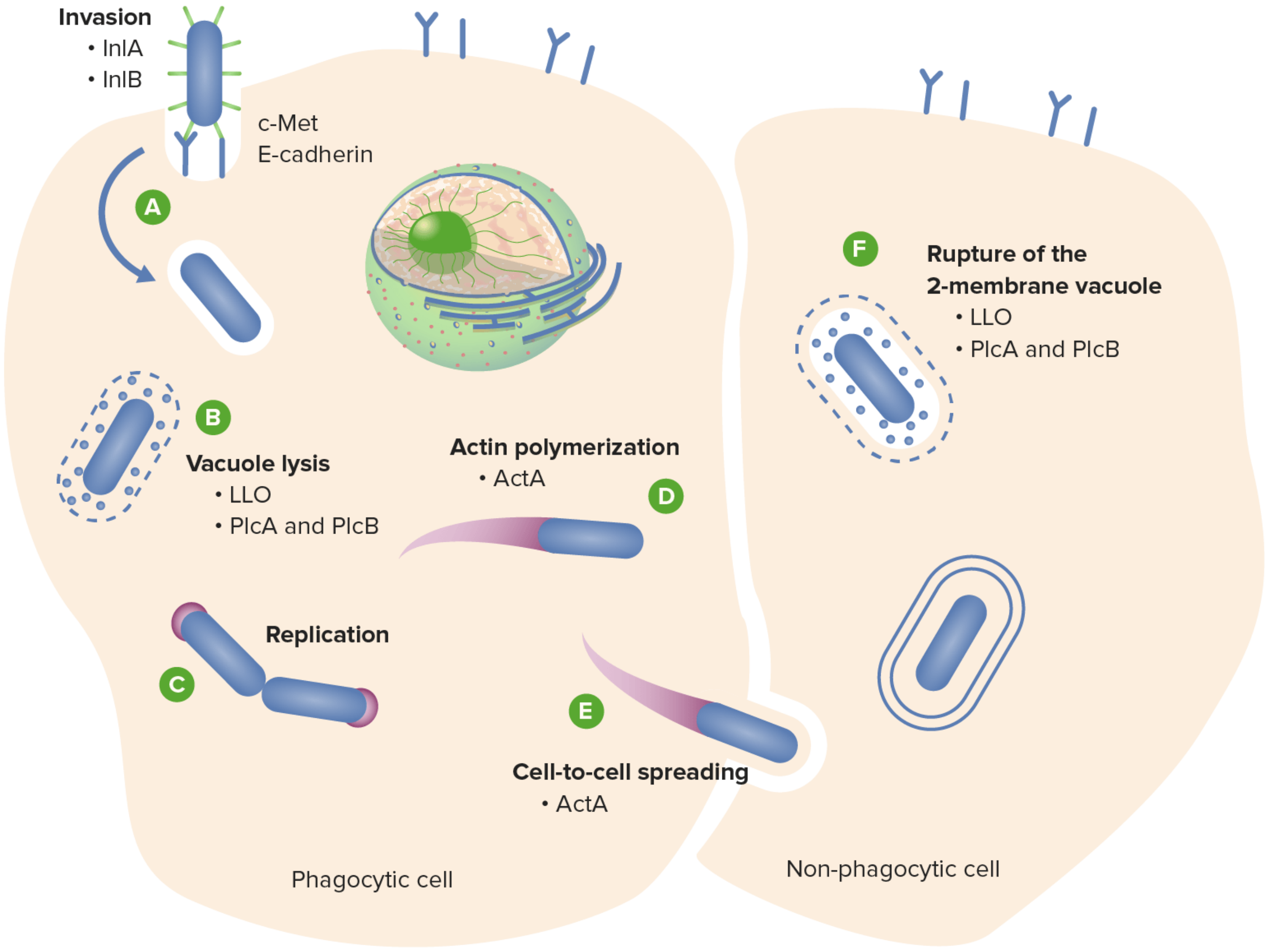
The definition and biological importance
of structural biology

Alex's research example: *Listeria monocytogenes* with Dr. Cahoon

Dr. Cahoon is studying how *Listeria monocytogenes (Lm)* infects cells

Lm is a gram-positive bacteria responsible for listeriosis, a foodborne illness

A key step in the Lm life cycle is escaping vacuoles and continue infecting



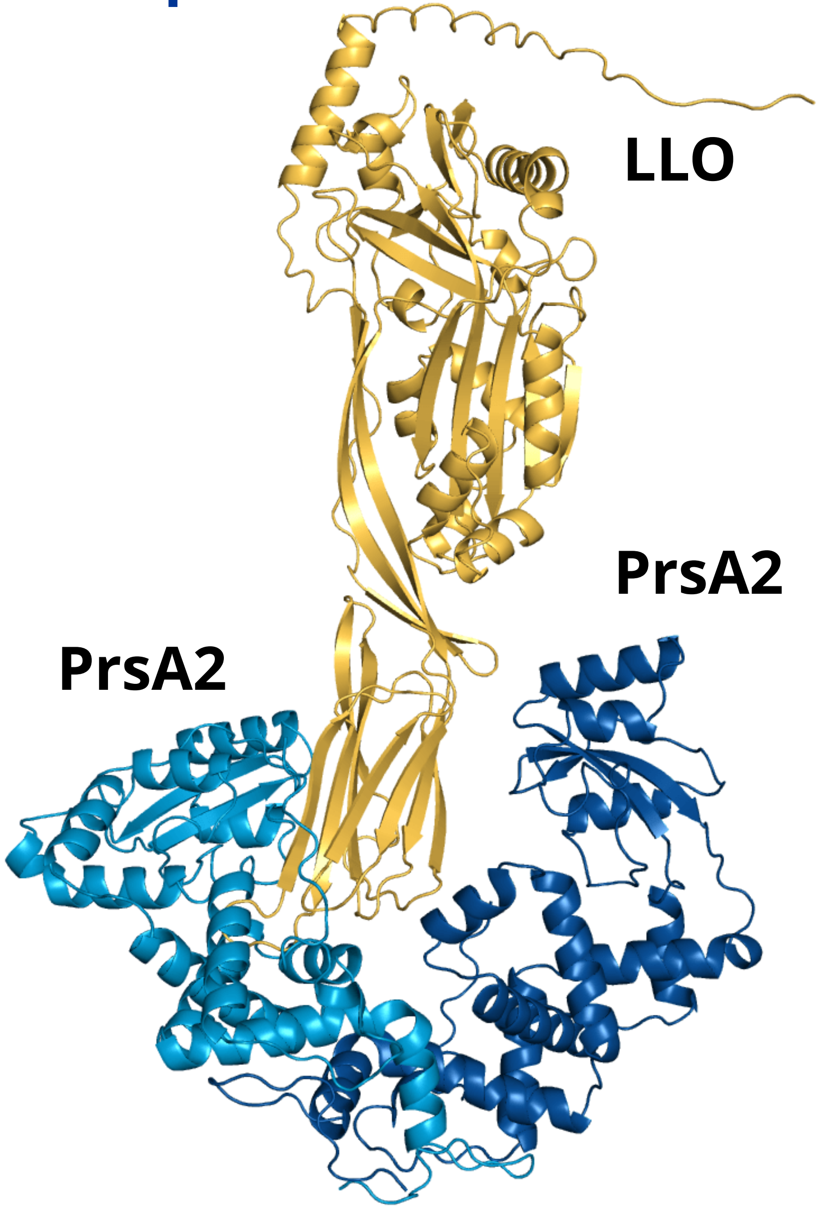
Agbavor, C.; et al. DOI: 10.1128/mbio.00743-24

Lm secretes listeriolysin O (LLO) which forms pores in vacuoles allowing it to escape

Lm secretes a pore-forming, cholesterol-dependent toxin called **listeriolysin O (LLO)** to escape vacuoles and infect cells

The Cahoon lab (alongside several collaborators) demonstrated that **PrsA2** (a chaperone) regulates LLO activity through a pH-dependent mechanism

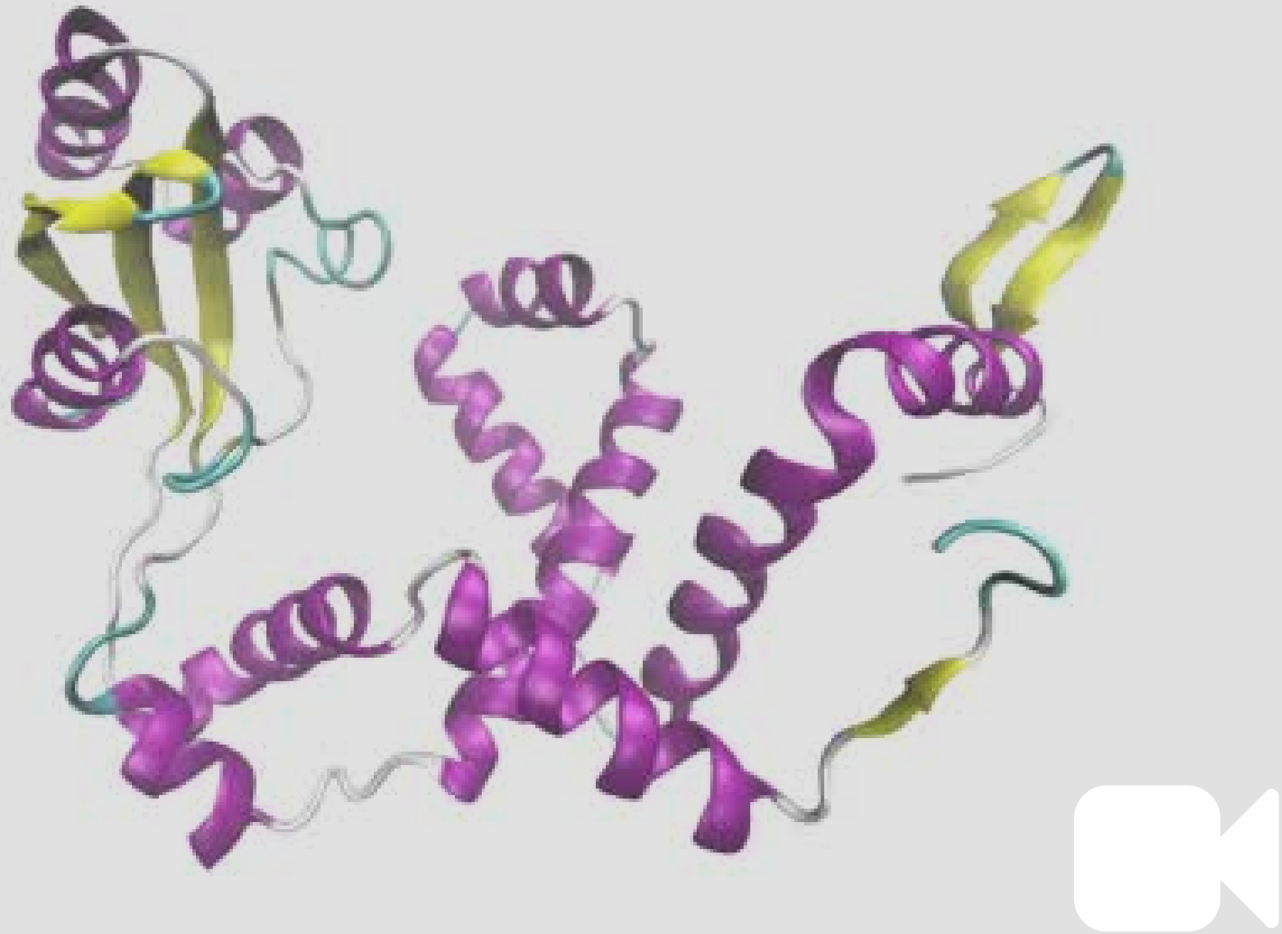
At **pH 7**, PrsA2 remains bound to LLO, preventing it from forming pores. At **pH 5**, PrsA2 releases LLO to escape acidic vacuoles



Agbavor, C.; et al. DOI: 10.1128/mbio.00743-24

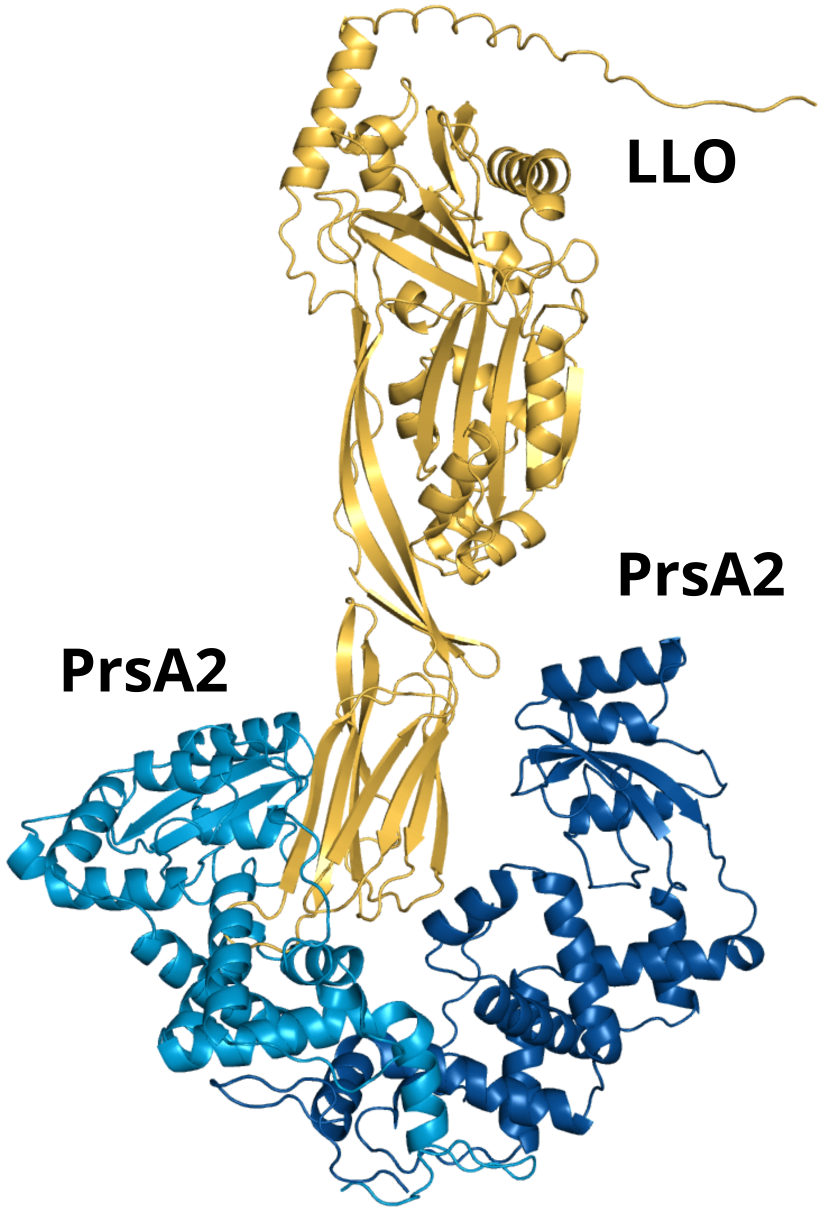
What is our computational question?

Are PrsA2-LLO interactions destabilized in acidic (i.e., pH 5) environments? If so, how?



This is a new project, so we do not know yet!

Application: Once we understand this interaction, we can design a new antibiotic for gram positive bacteria



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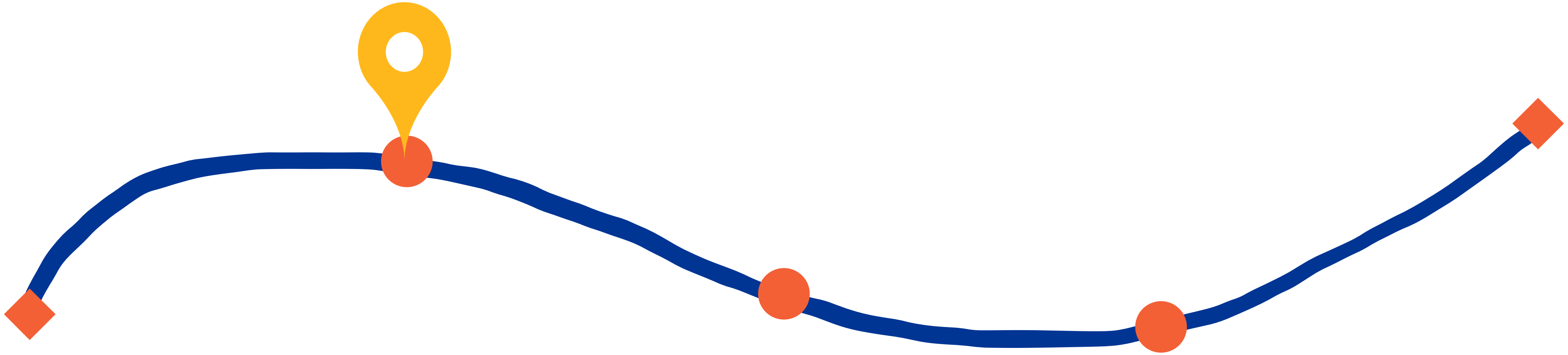
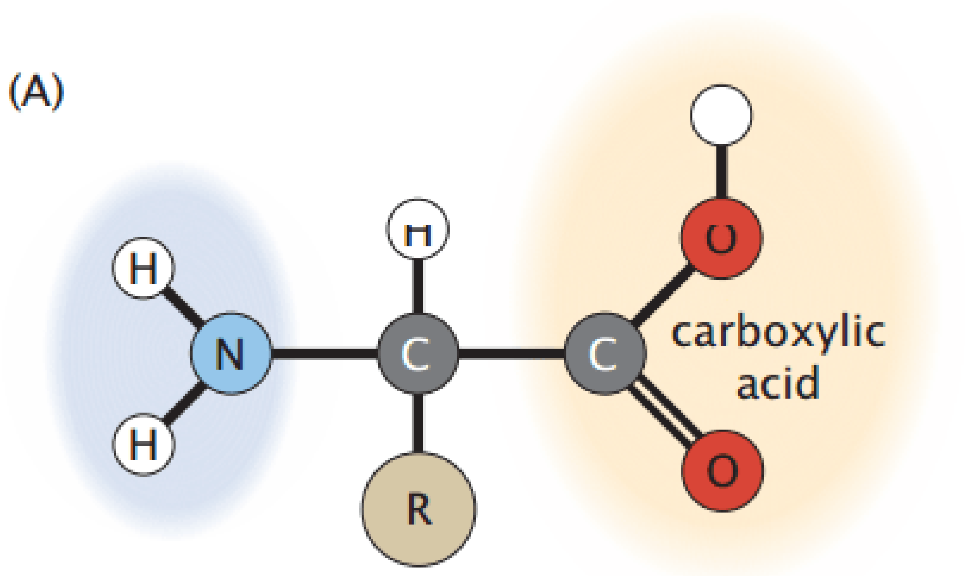


Basic principles of protein structure

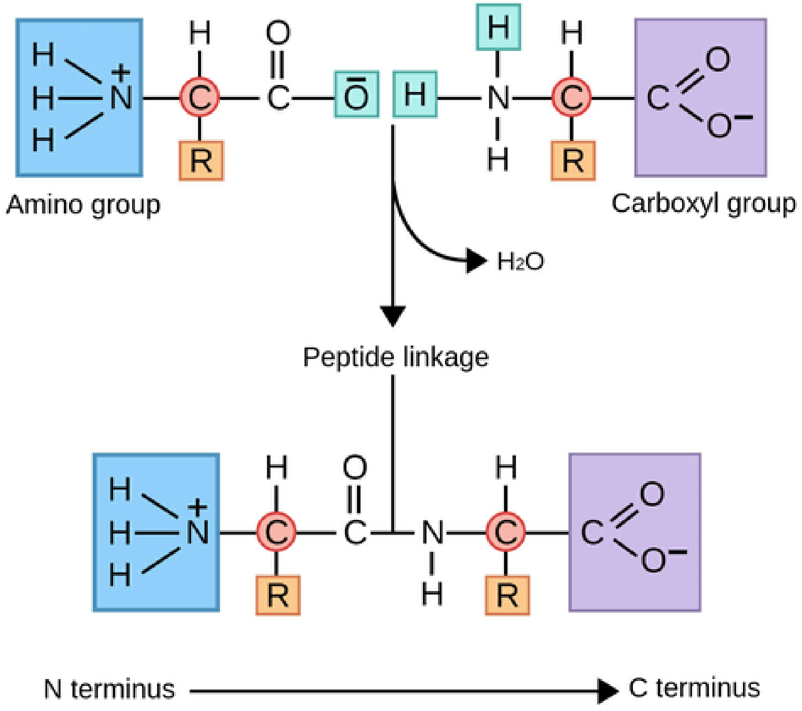
Amino acids are the fundamental building blocks of proteins

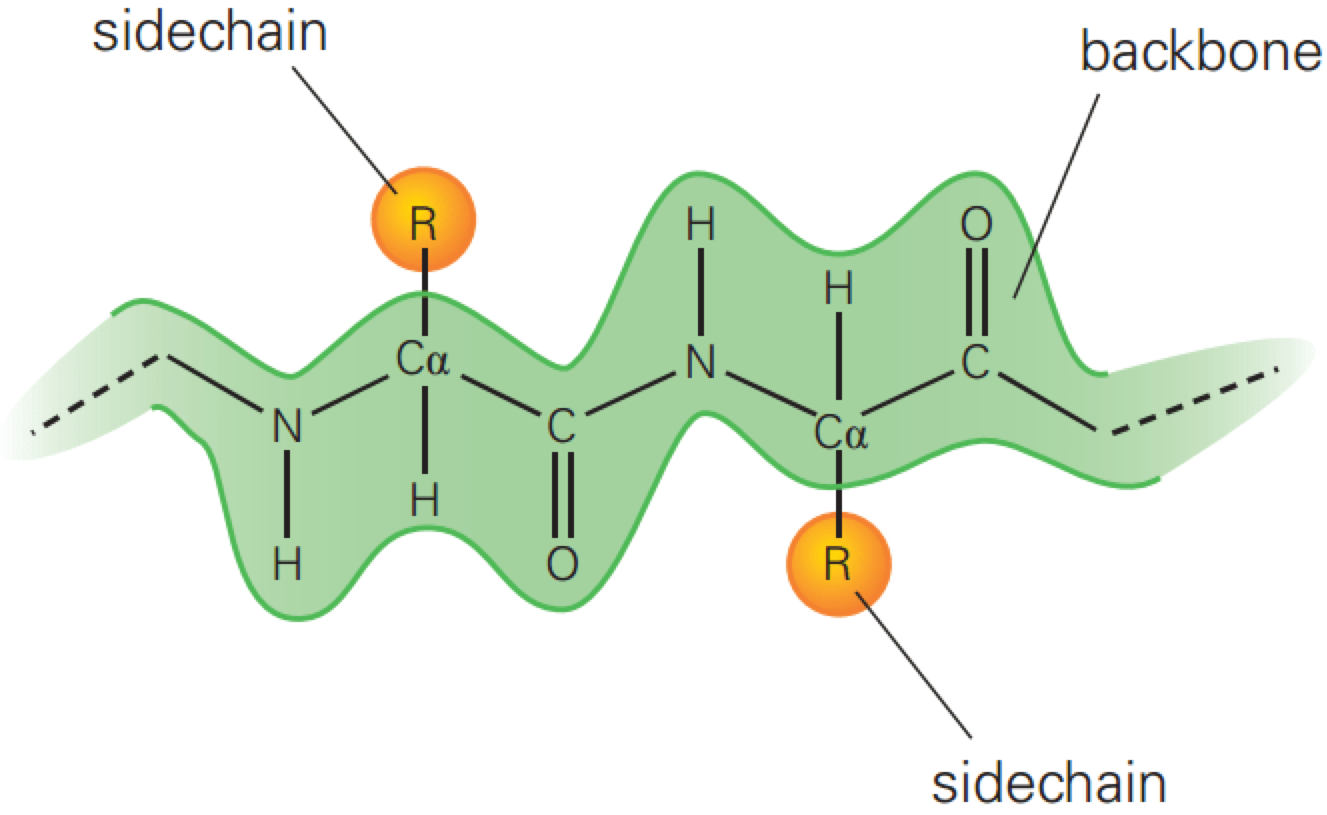
All proteins are composed of smaller molecules called **amino acids**, which are linked together in specific sequences.

Each amino acid contains a **central carbon (alpha carbon)** bonded to an **amino group** (NH_2), a **carboxyl group** (COOH), a hydrogen atom, and a variable **side chain** known as the R-group.



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



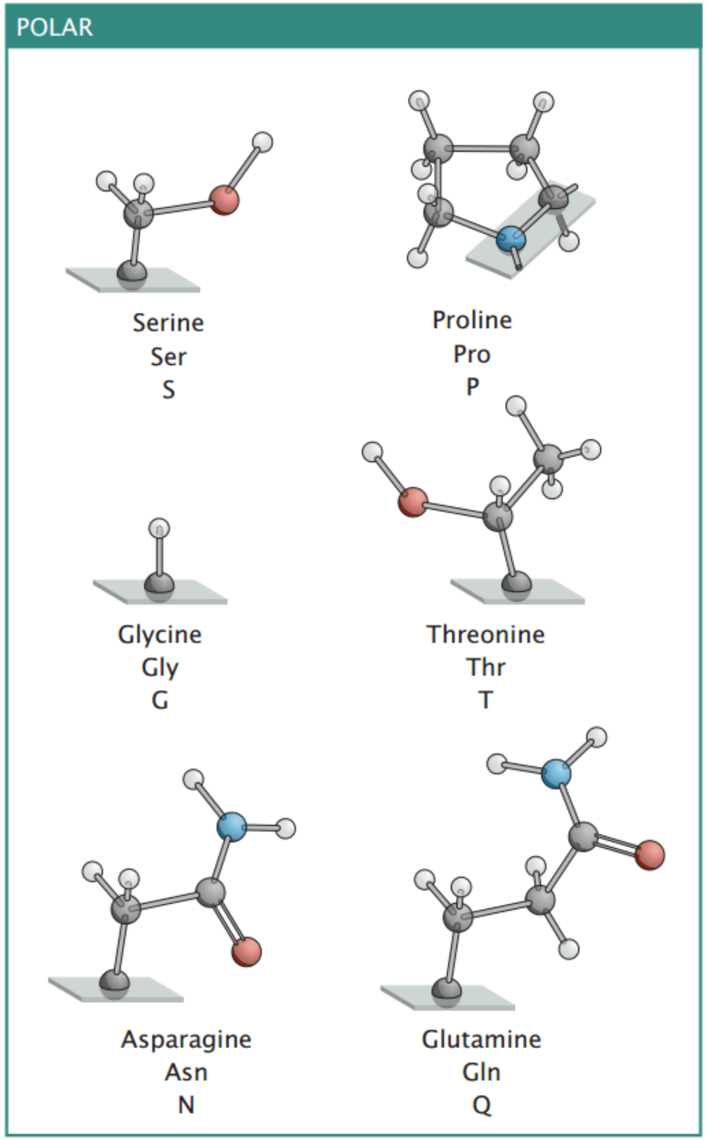


Polar amino acids enable interactions with water and other polar molecules

Polar amino acids have side chains that can **form hydrogen bonds**, making them hydrophilic

Polar amino acids contribute to **protein solubility** and help **stabilize** secondary and tertiary structures through hydrogen bonding.

Many polar amino acids are involved in enzymatic activity, facilitating catalytic reactions by stabilizing transition states or **interacting with substrates**.



You will not be tested on your amino acid abbreviations

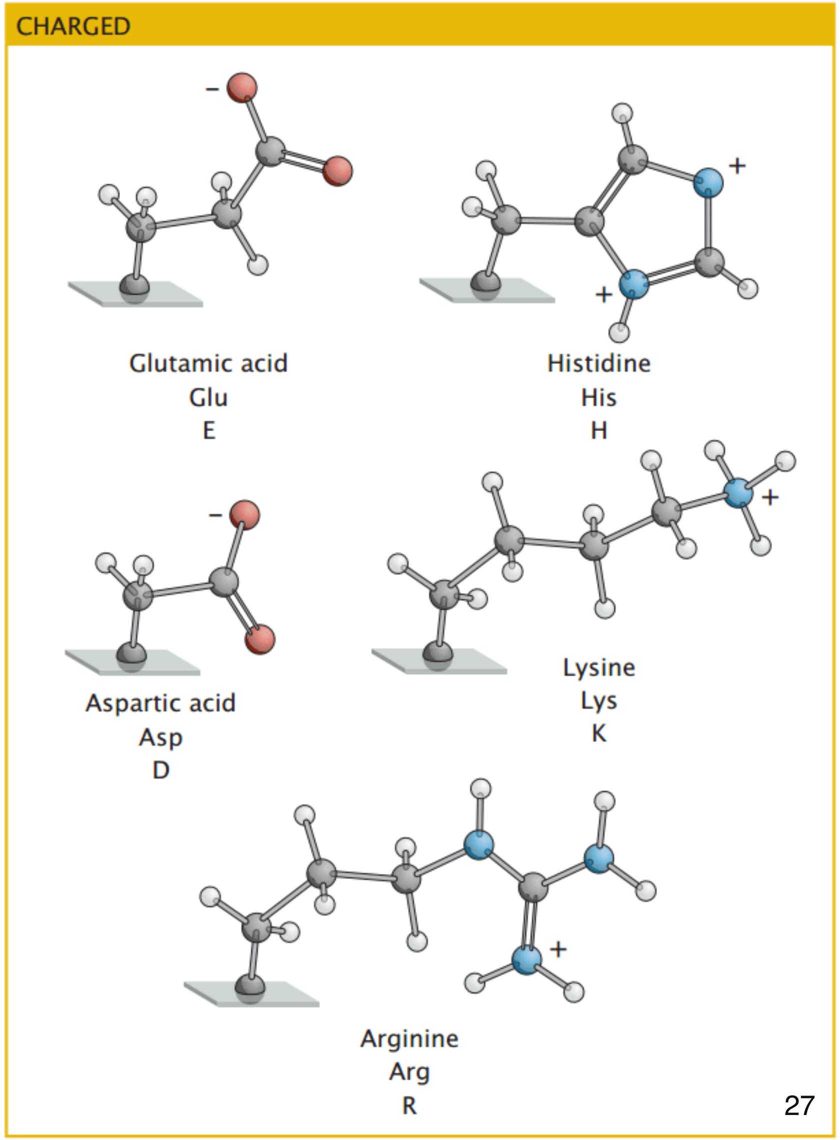
Charged amino acids play key roles in protein stability and interactions

Acidic amino acids carry negative charges and participate in ionic interactions that stabilize protein structures

Basic amino acids carry positive charges and frequently interact with negatively charged molecules like DNA and phospholipids.

Charged amino acids contribute to protein folding by forming salt bridges, which enhance stability.

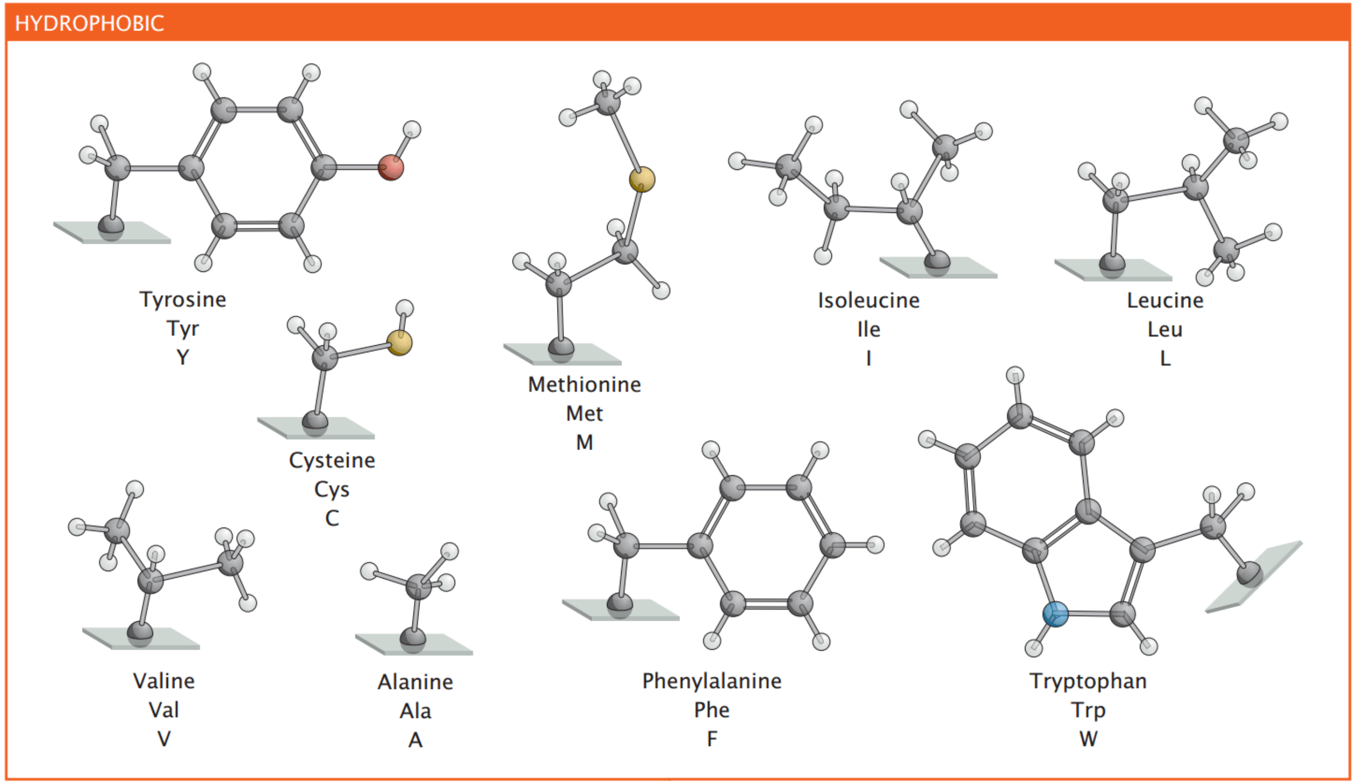
The cellular environment's **pH can influence these amino acids' charge state**, affecting protein conformation and function.



Nonpolar amino acids drive protein folding and membrane interactions

These amino acids are often found in the **interior of globular proteins**, stabilizing protein structure by minimizing exposure to water

Aromatic nonpolar amino acids participate in **stacking interactions**, influencing protein stability and ligand binding



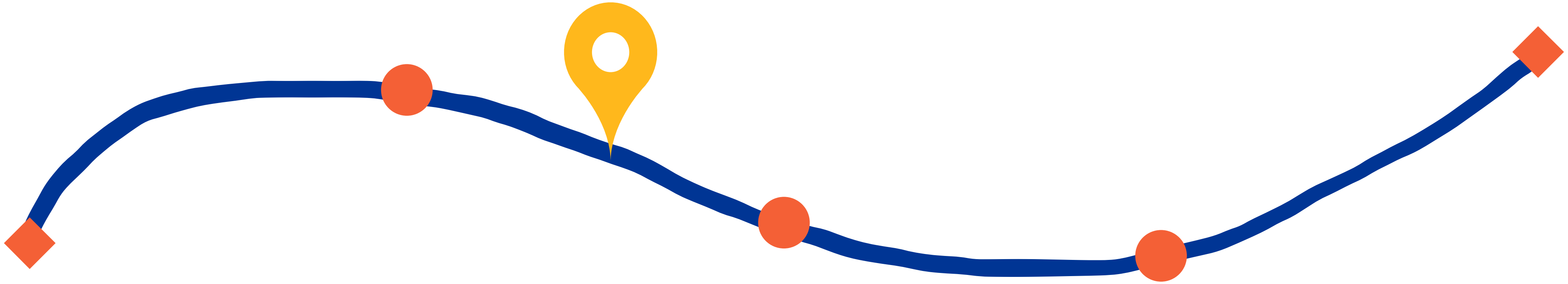
The primary structure of a protein determines its final shape and function

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

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)

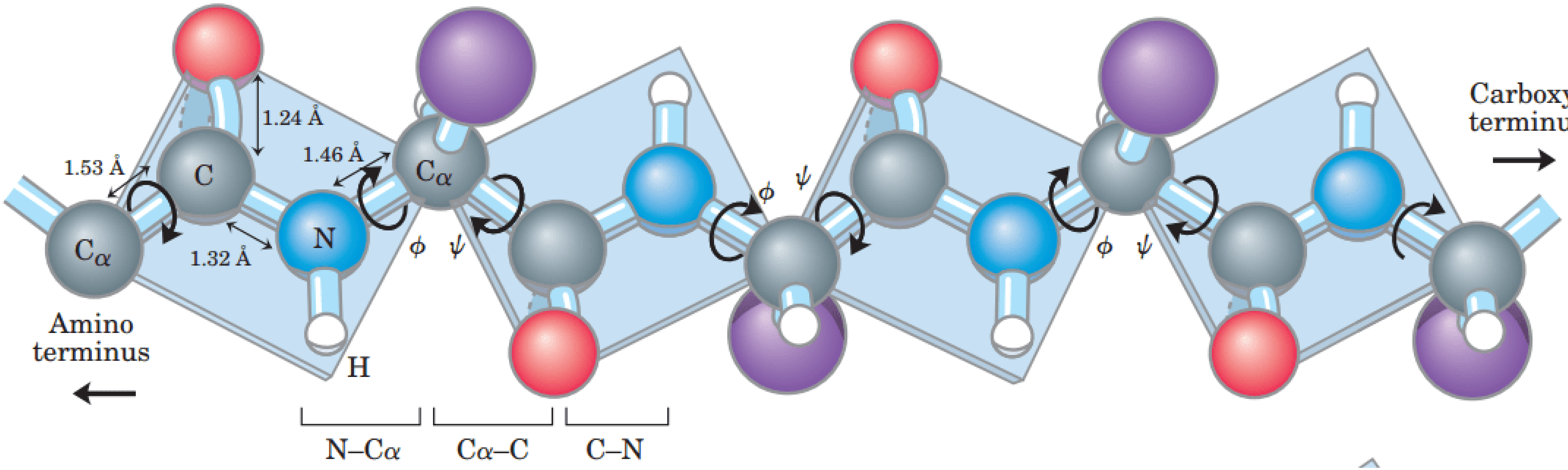
After today, you should have a better understanding of



Basic principles of protein structure

Phi (Φ) and Psi (Ψ) angles determine protein backbone flexibility and folding

Proteins are flexible due to **rotation around specific backbone bonds**: the phi (Φ) and psi (Ψ) angles.

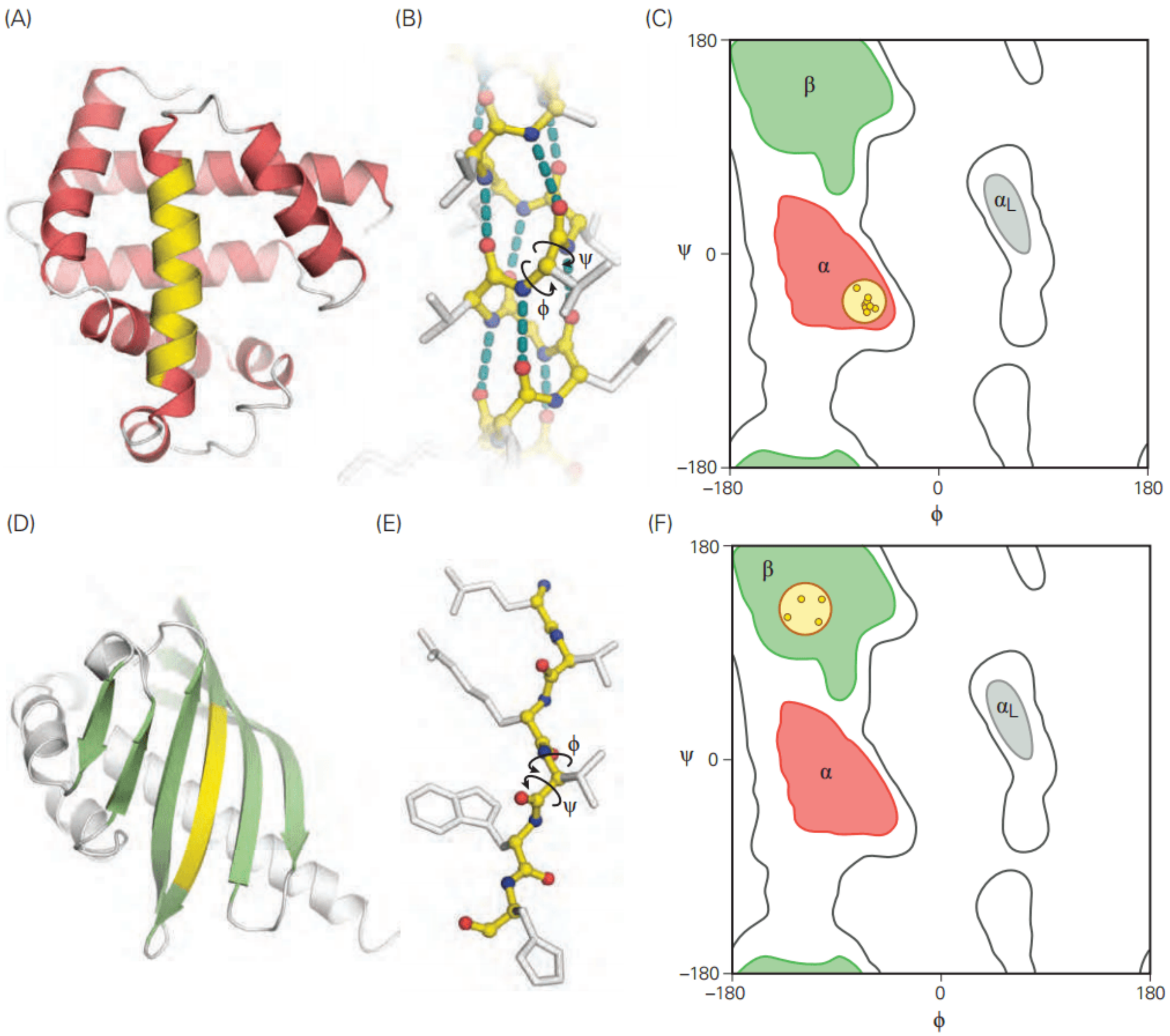


Not all angle combinations are allowed due to **steric hindrance**—this is visualized in a **Ramachandran plot**, which maps permitted conformations.

Secondary structures provide local organization within proteins

Secondary structures refer to **regularly repeating local conformations** of the polypeptide backbone.

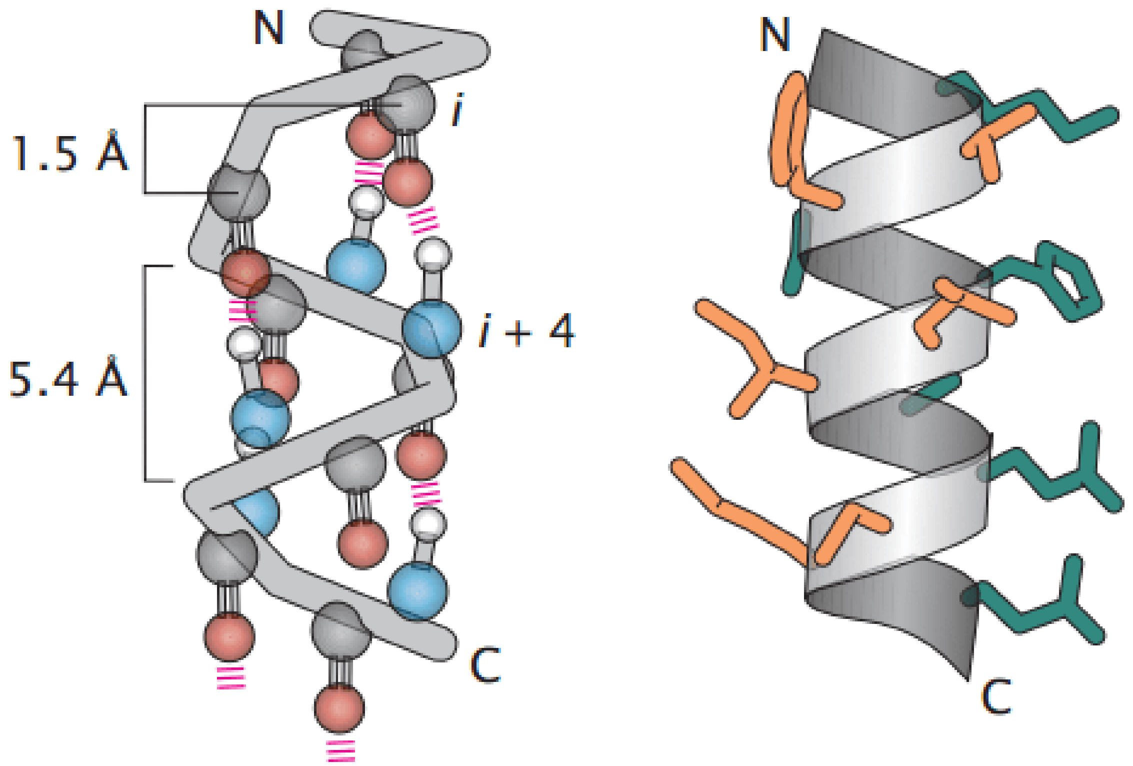
These structures help proteins achieve **compact and stable folding** while maintaining flexibility for function.



Alpha-helices are stabilized by hydrogen bonds and provide structural flexibility

An **alpha-helix** is a right-handed coil with **3.6 amino acids per turn**, stabilized by **hydrogen bonds** between the backbone carbonyl oxygen and the amide hydrogen of a residue four positions ahead.

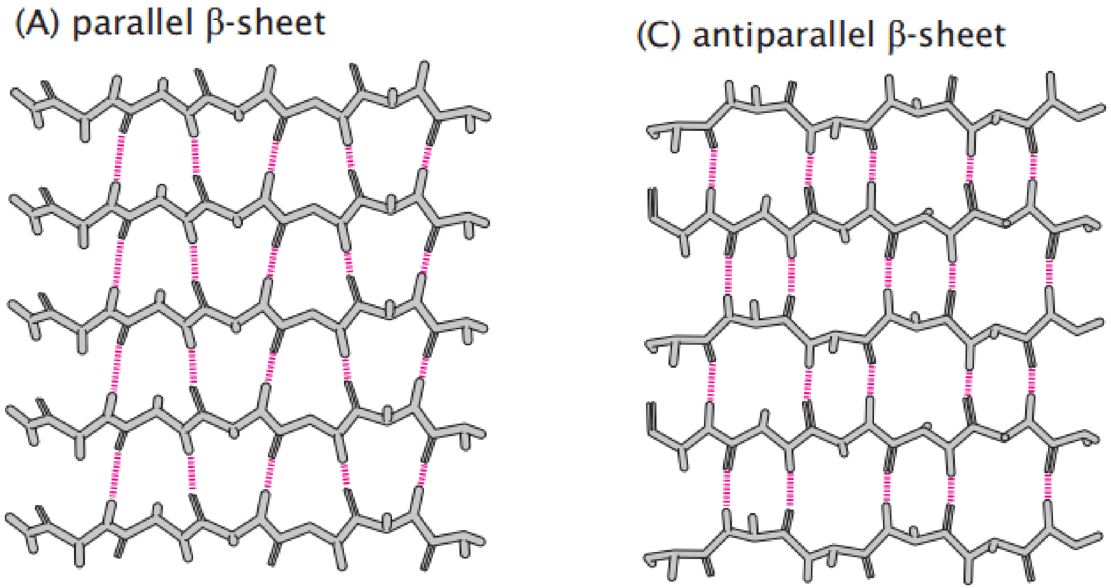
Side chains project **outward**, allowing interactions with the surrounding environment.



Beta-sheets provide strength and stability to protein structures

Beta-sheets consist of extended polypeptide strands aligned side by side, stabilized by **hydrogen bonds** between backbone atoms of adjacent strands.

Strands can be **parallel** (N-to-C direction aligned) or **antiparallel** (N-to-C in opposite directions), with antiparallel sheets being more stable.



Side chains alternate **above and below the sheet**, affecting interaction and stability.

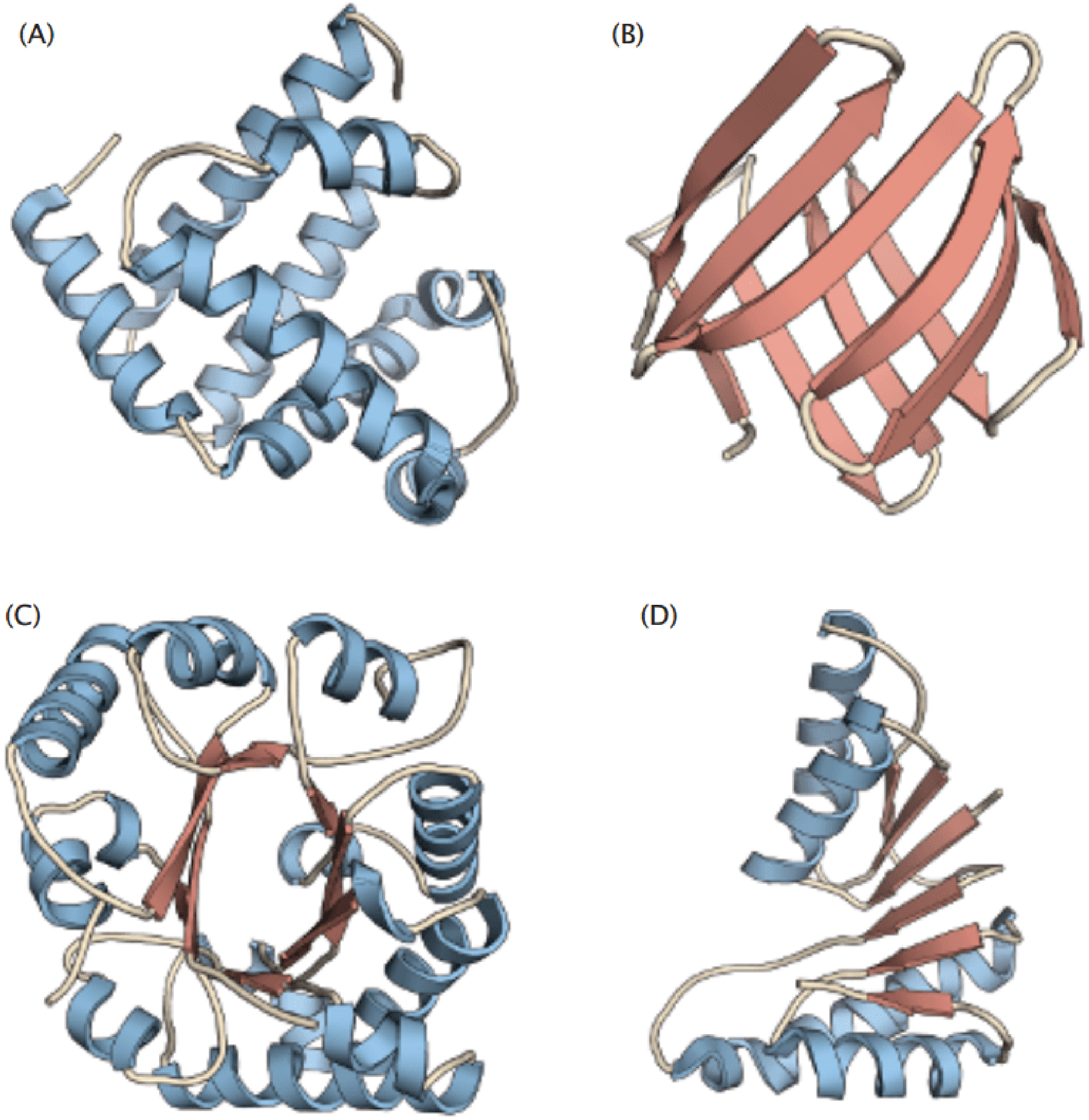
After today, you should have a better understanding of

Basic principles of protein structure

Tertiary Structure

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

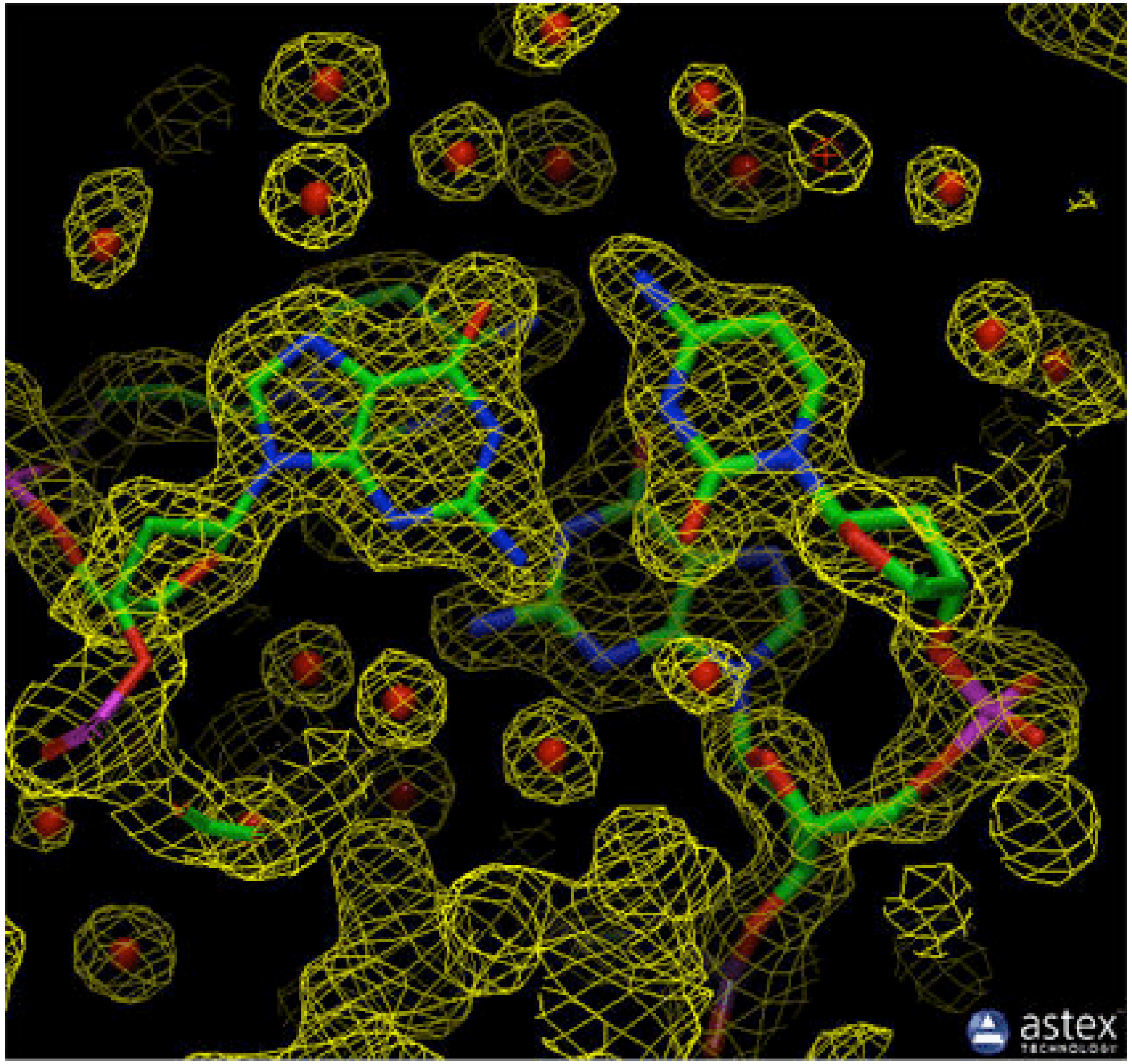
Tertiary structures reveal active sites or binding pockets where catalysis or molecular interactions occur



After today, you should have a better understanding of

X-ray crystallography and cryo-electron microscopy

How can we experimentally determine the 3D atomic structure of a protein?

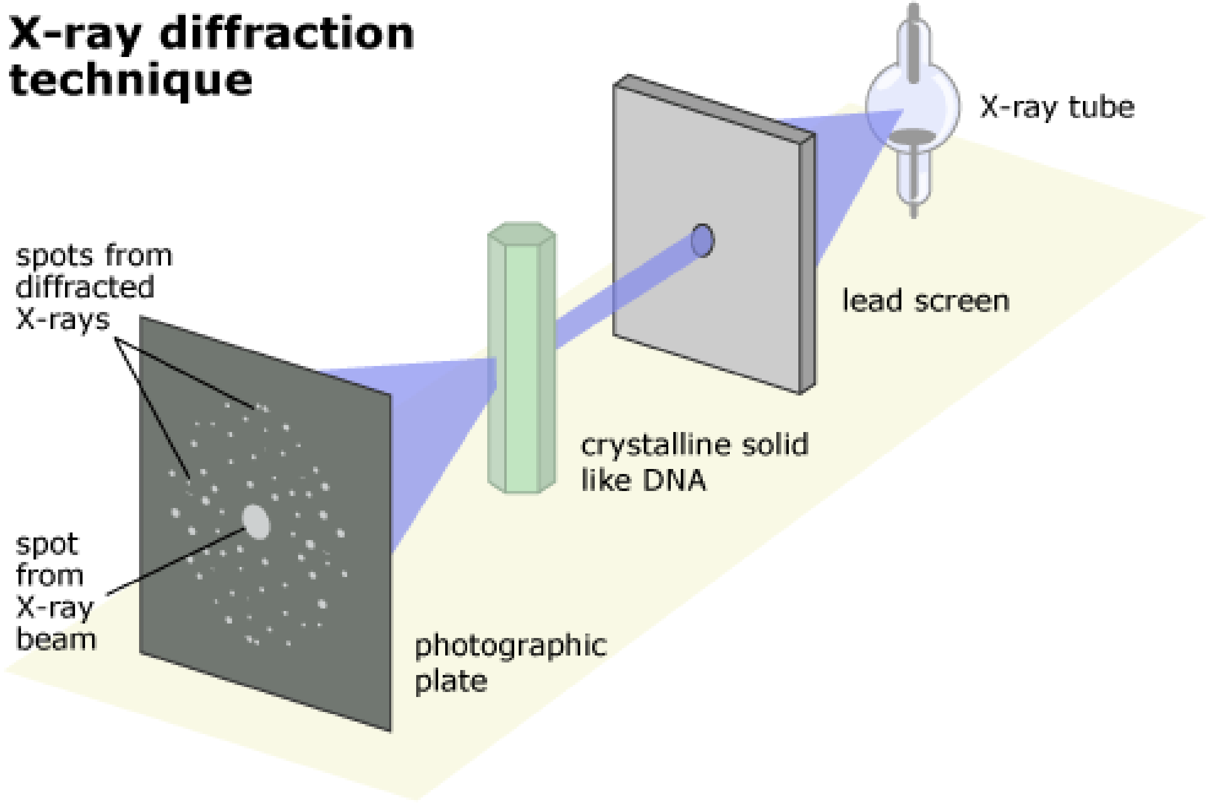


Fundamentals of X-ray Crystallography

Probe: Photon (carrier of electromagnetic radiation)

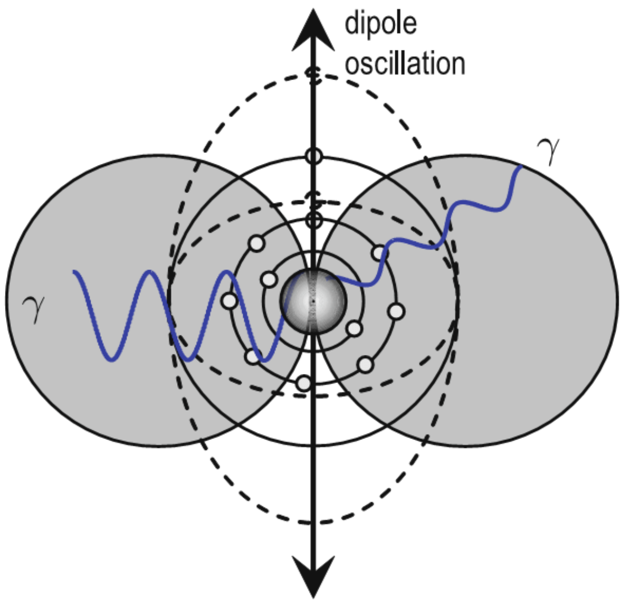
Basic Principle: Photons scatter when they interact with other particles

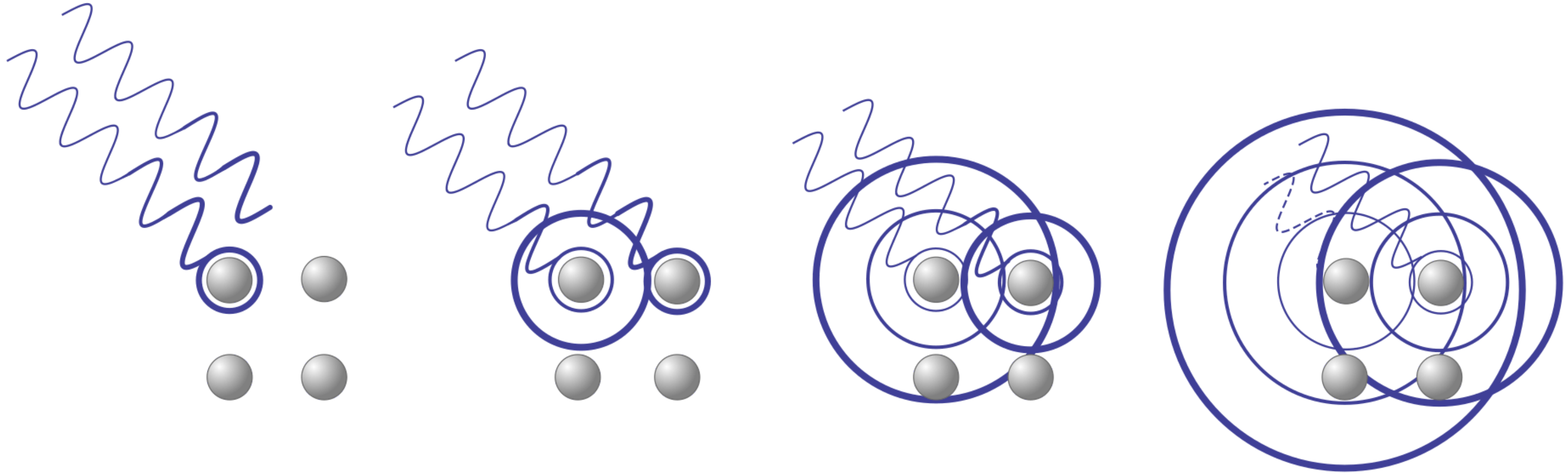
The scattered X-rays form a **diffraction pattern** unique to the crystal



X-rays undergo elastic scattering by electrons

1. An 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

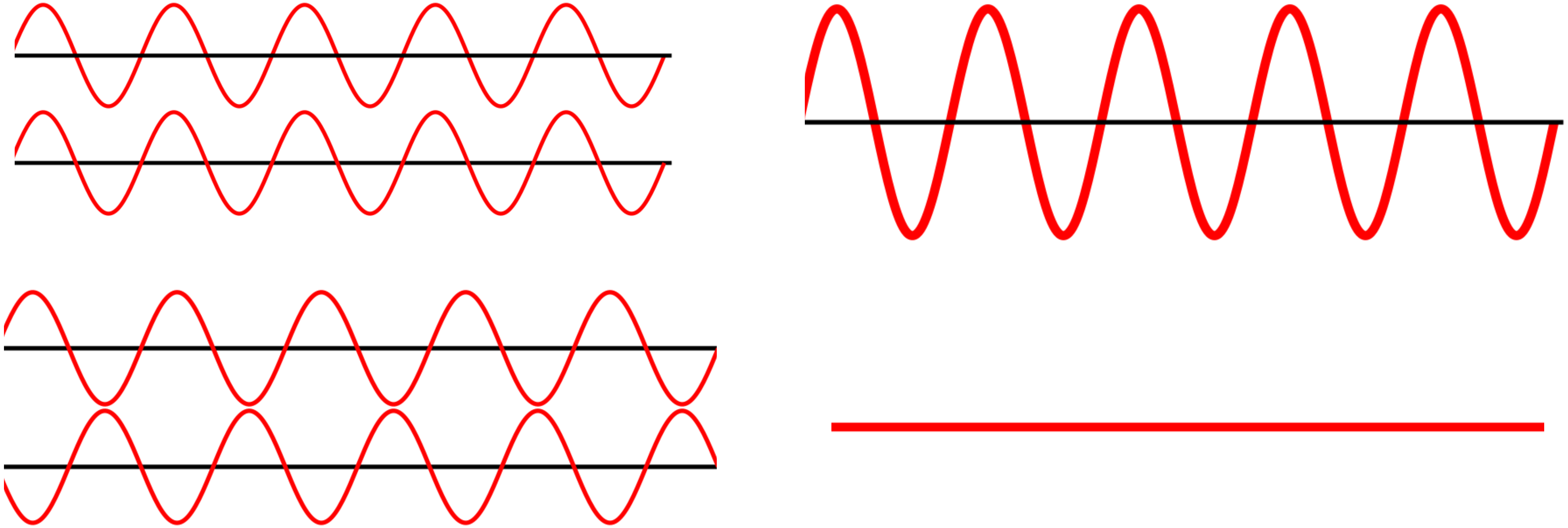




What happens when two waves overlap?

Constructive interference is needed to amplify signal for detectors

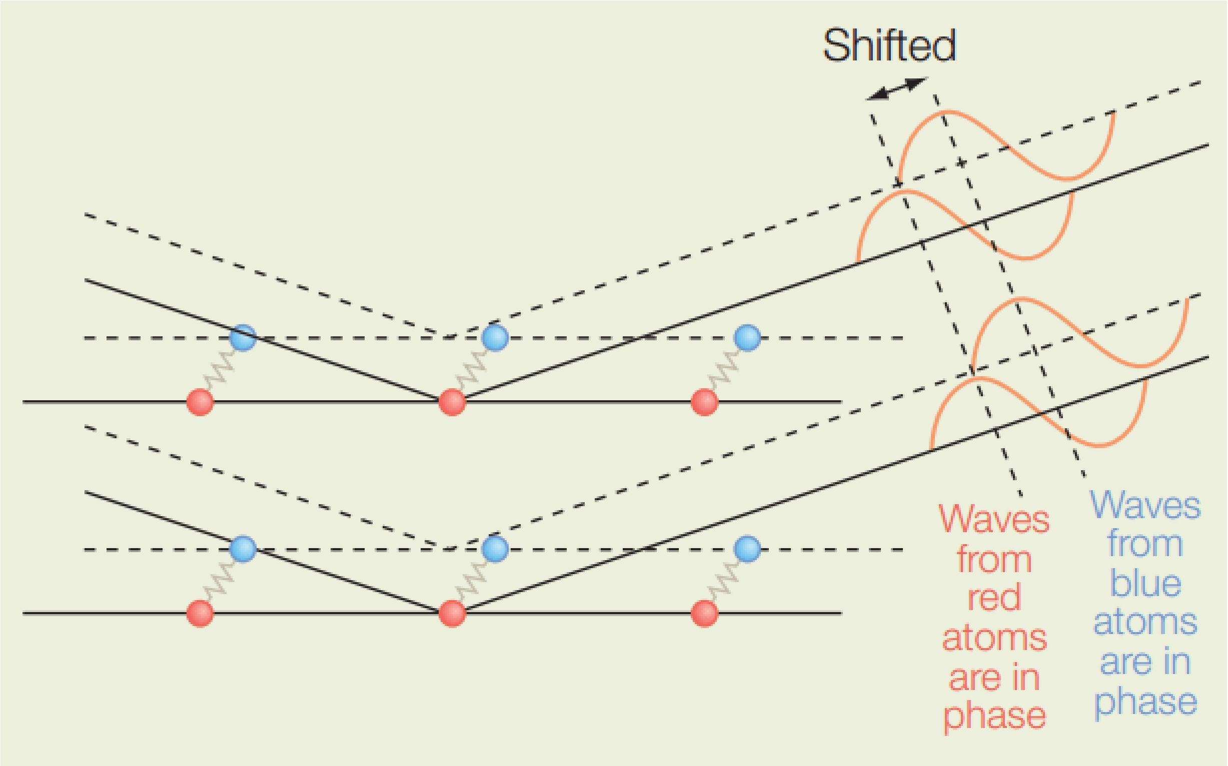
If wavelengths are similar and in phase, they constructively interfere

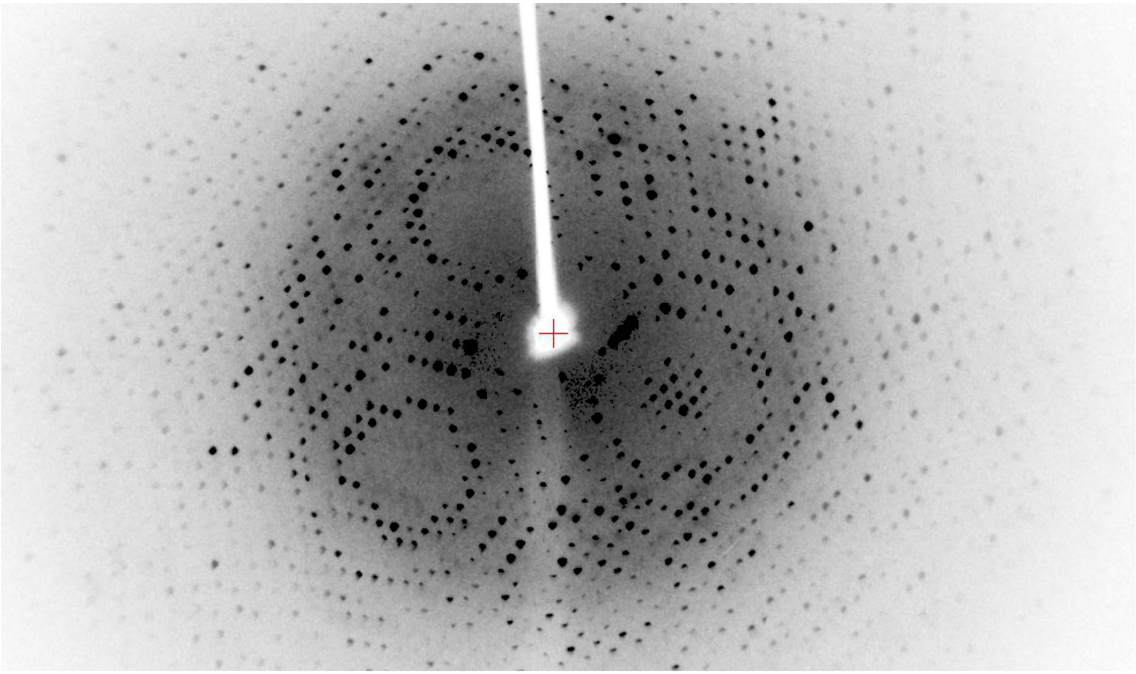


If waves are out of phase, they deconstructively interfere

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



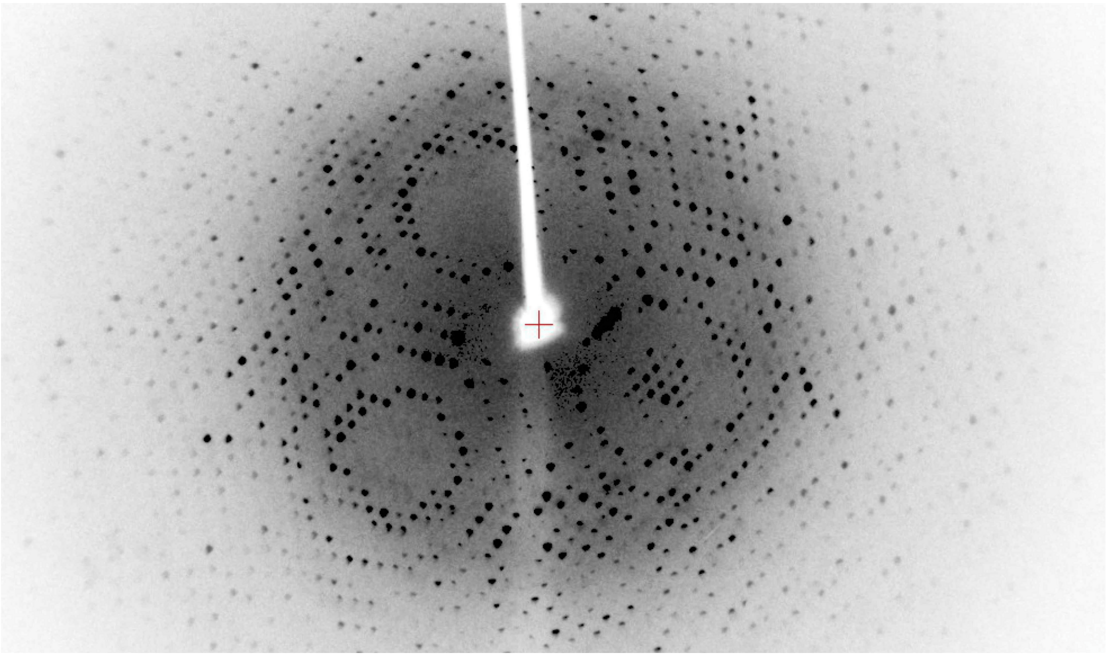


The diffraction pattern

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

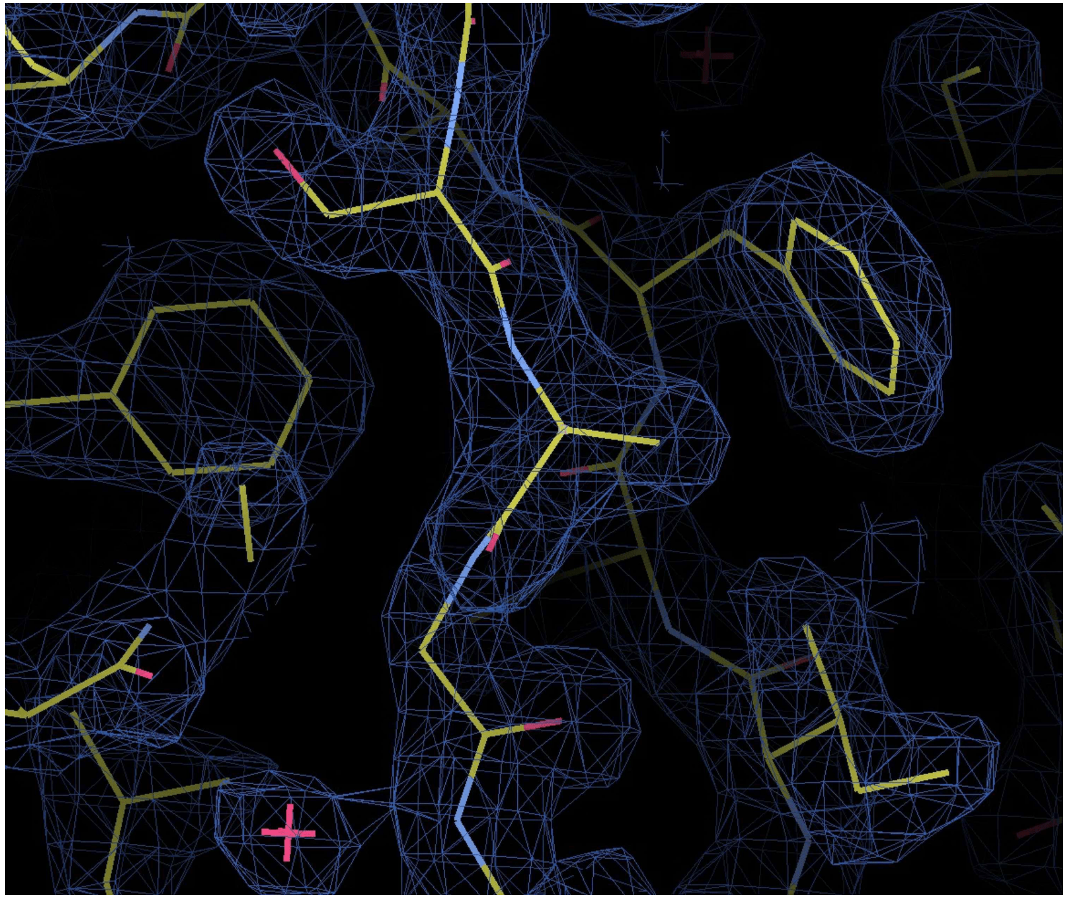
The diffraction pattern does not directly show the atomic positions, but provides the data needed to infer the electron density



Building the electron density map

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

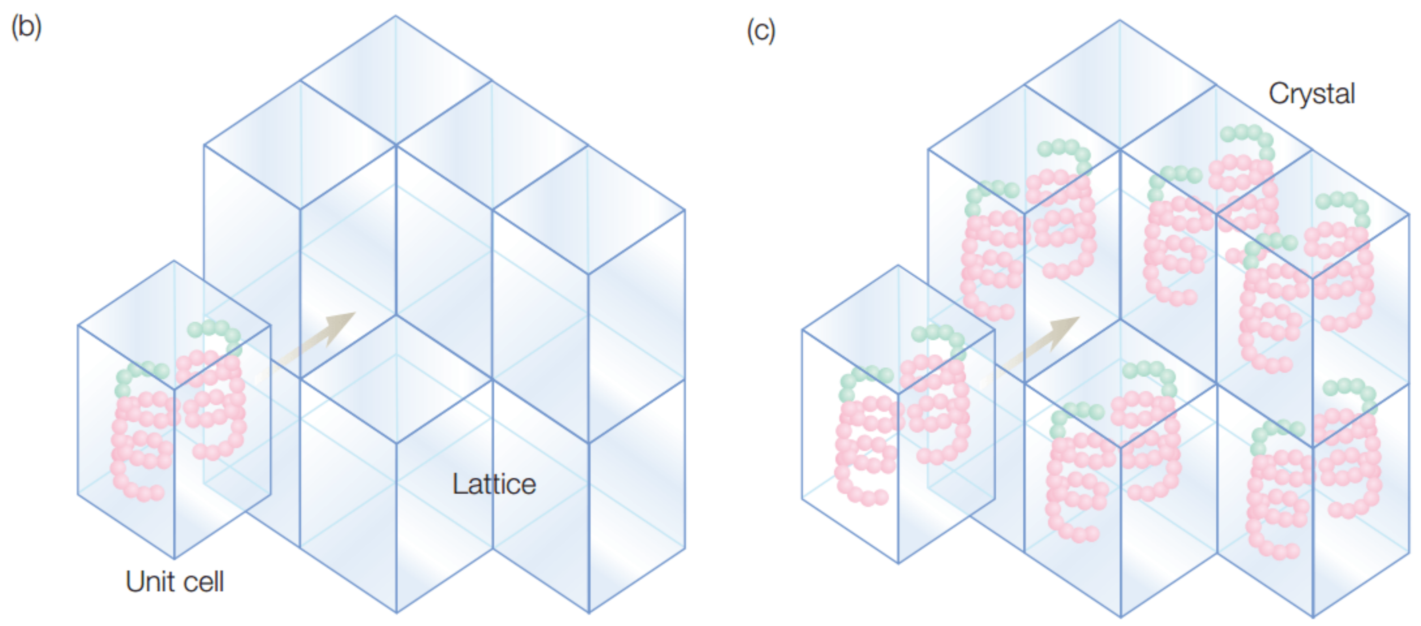
The electron density map is interpreted by fitting atomic models (e.g., amino acids for proteins) into the density



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



If in solution, particles would be

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

Crystal quality

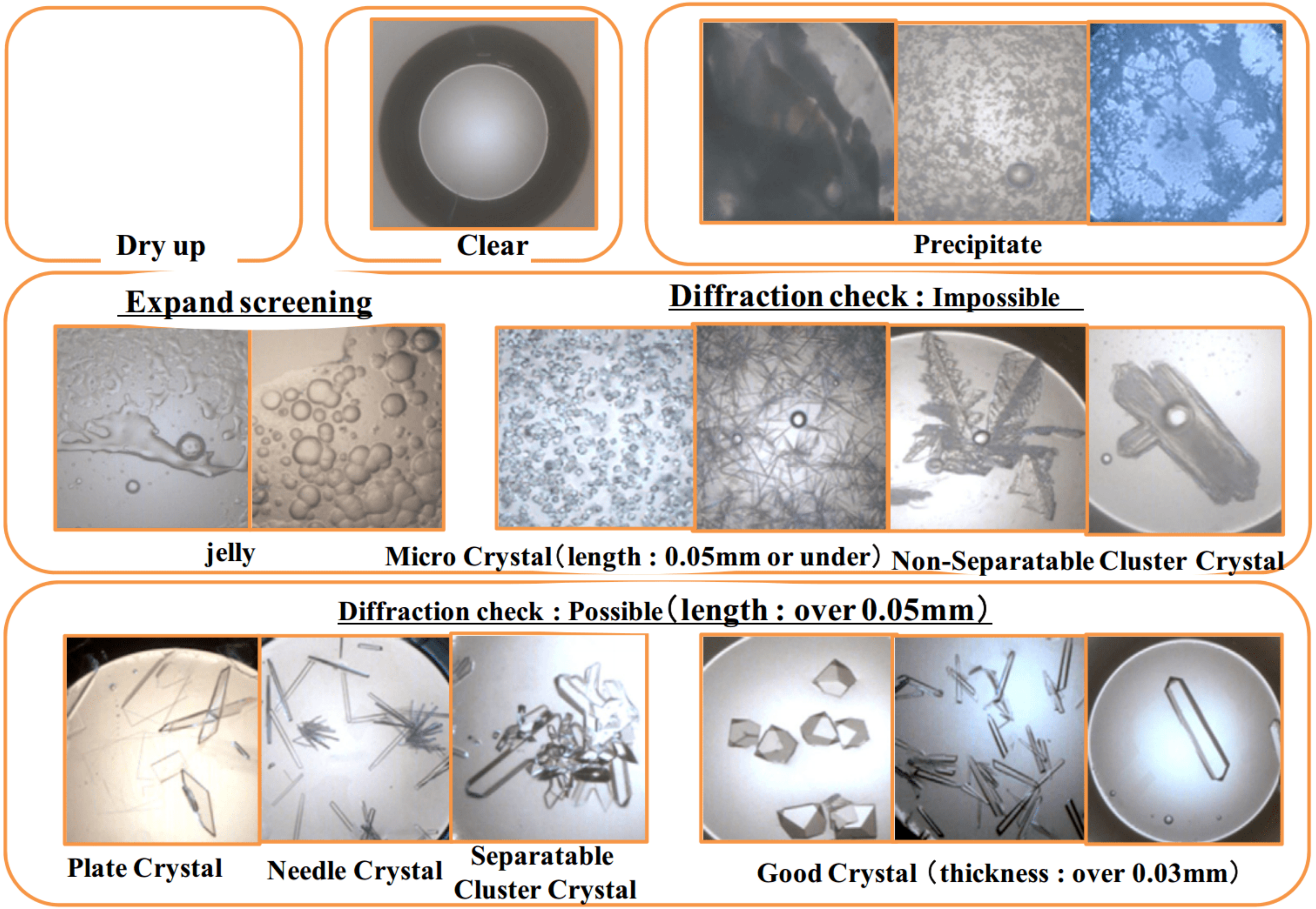
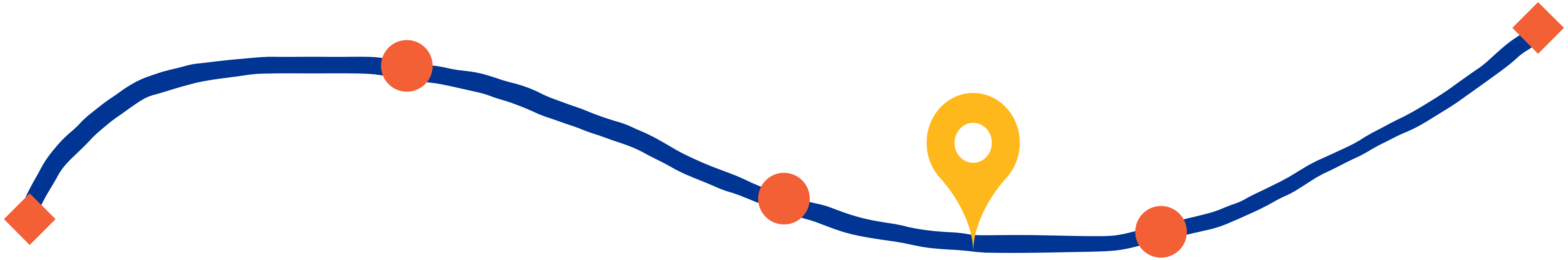


FIG. 6.3 Various types of crystal growth in crystallization process.

After today, you should have a better understanding of



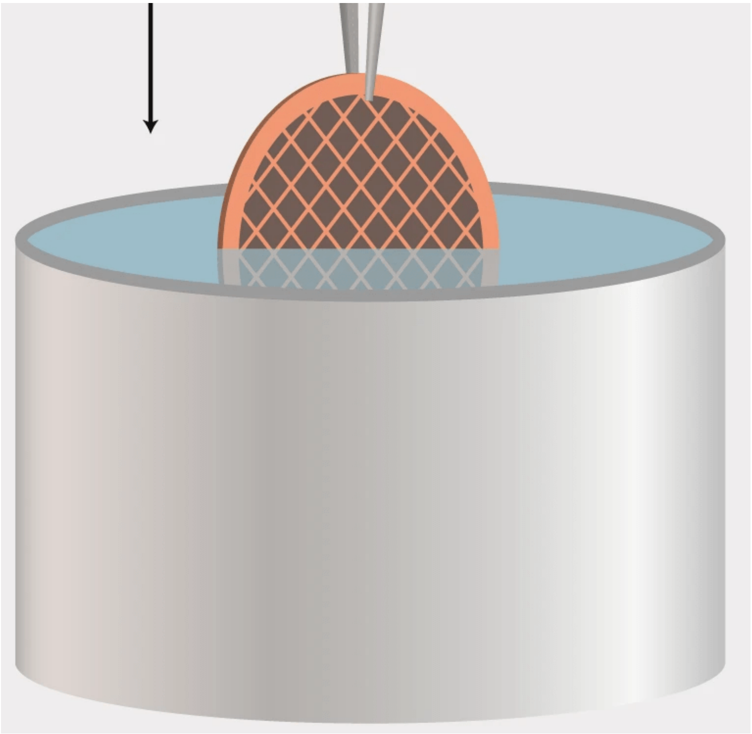
X-ray crystallography and cryo-electron microscopy

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 scatter electrons more effectively than X-rays



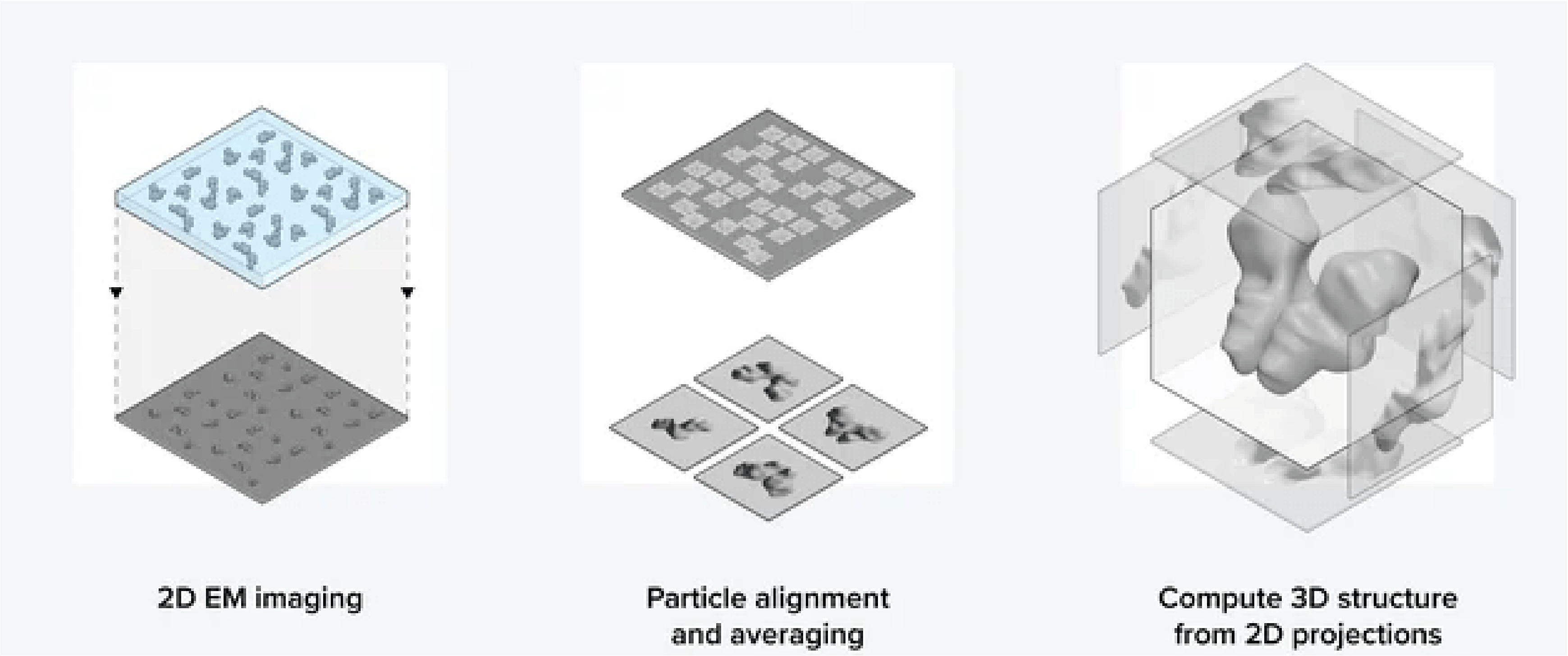
No crystals: The sample is rapidly frozen in vitreous ice to preserve its native structure

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

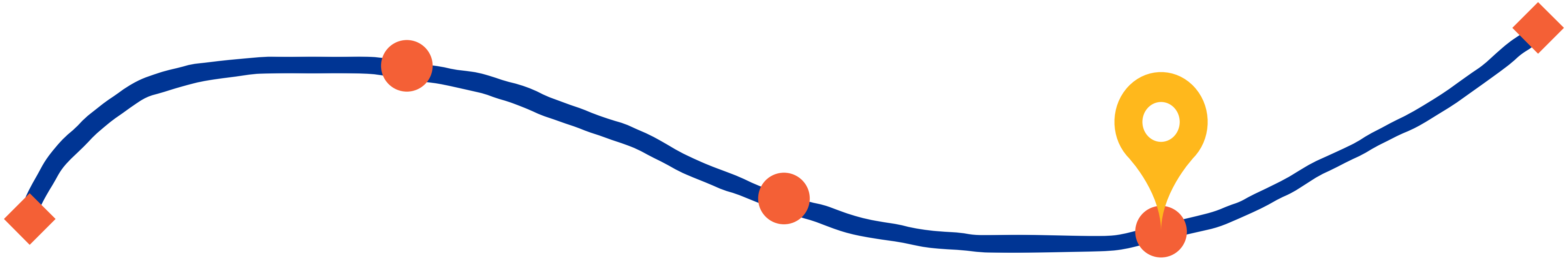
Single Particle Analysis (SPA)

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



After today, you should have a better understanding of



The challenge of protein disorder

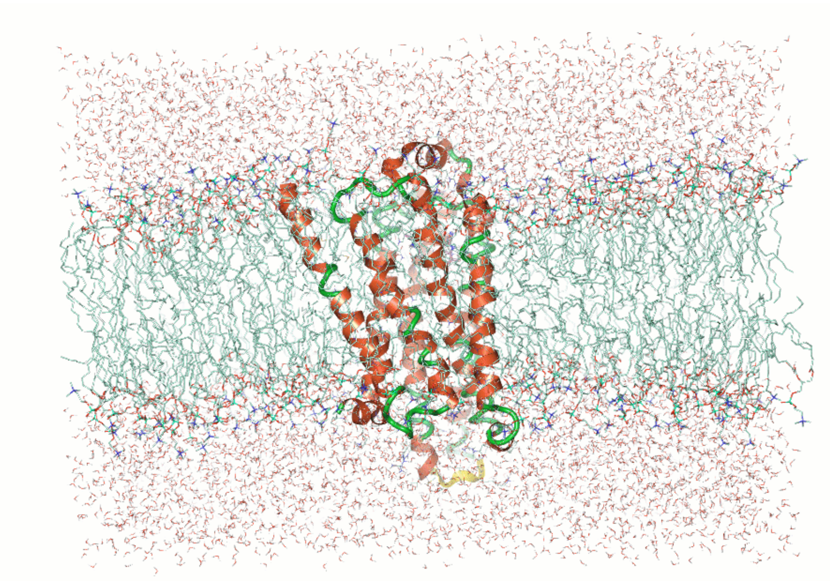
Challenge of flexibility and disorder in biomolecules

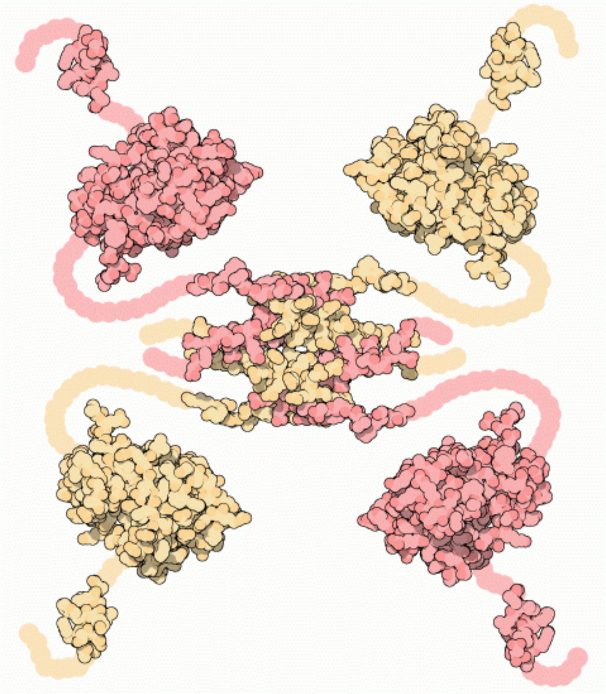
Molecules are not static

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

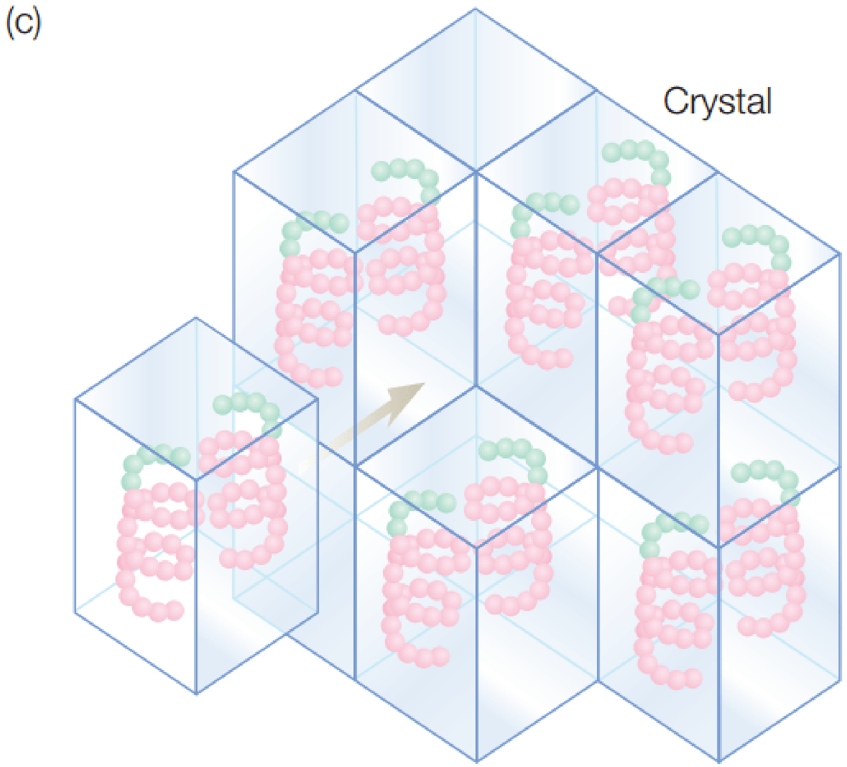
Why It Matters: Structural techniques often require ordered or stable configurations





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 molecule conformation**, often ignoring the flexibility or dynamic range.



Cryo-EM and Conformational Flexibility

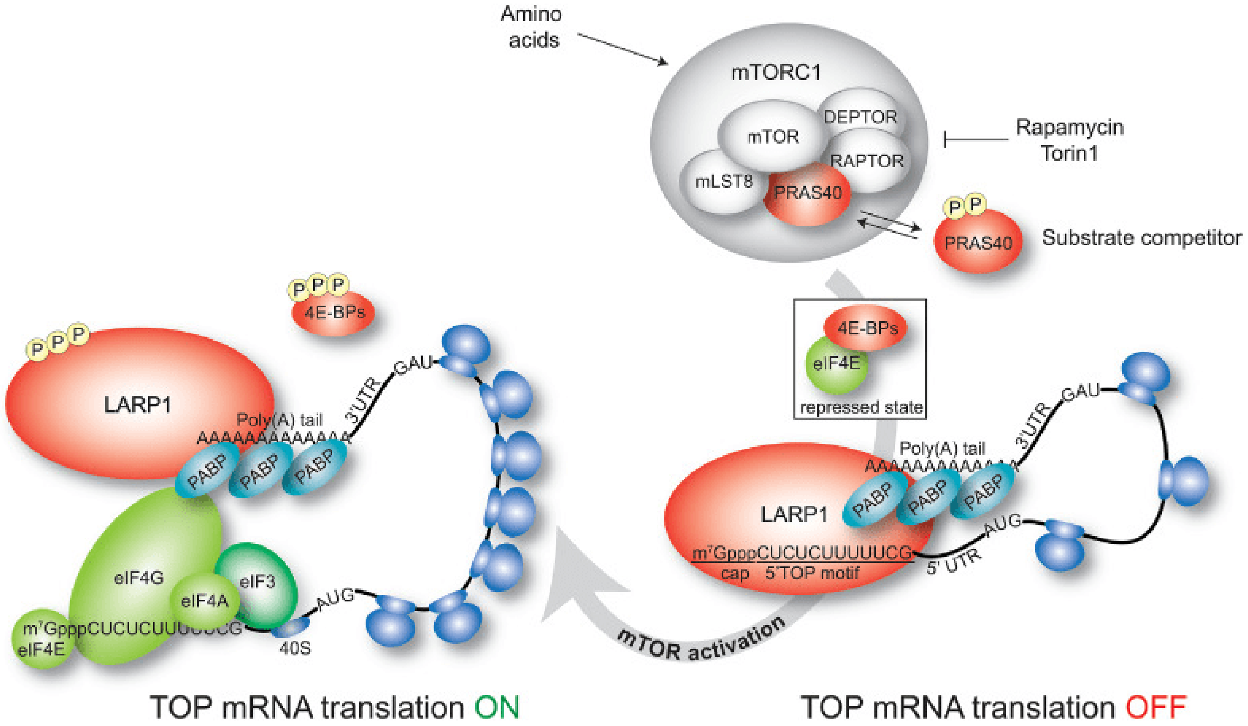
One strength of Cryo-EM is its ability to capture multiple conformational states of a molecule, providing insights into flexibility and structural heterogeneity.

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



Before the next class, you should

Lecture 09A:

Structural Biology - Foundations

Today

Lecture 09B:

Structural Biology - Methodology

Thursday

- Work on P02B
- Study for Quiz 03
- Download and install PyMOL with an educational license