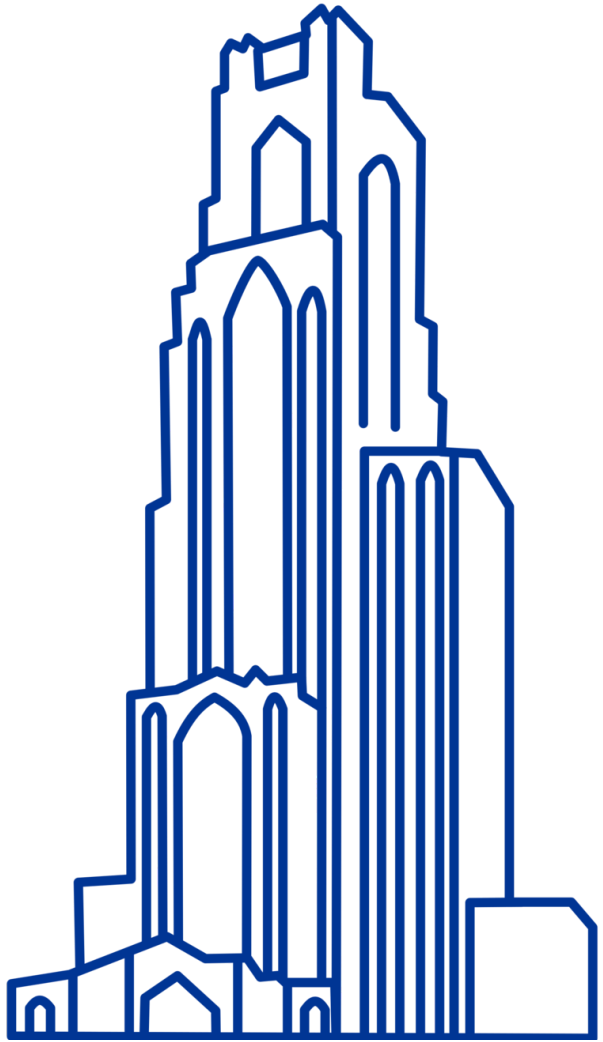


Computational Biology
(BIOSC 1540)

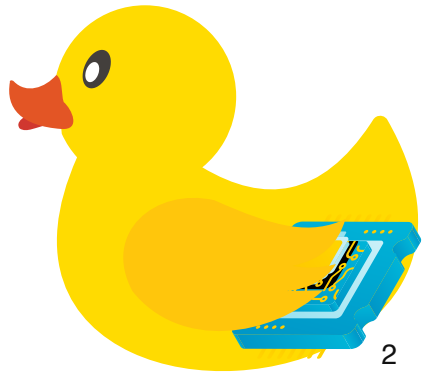
Lecture 14:
Molecular system representations

Oct 22, 2024

University of Pittsburgh

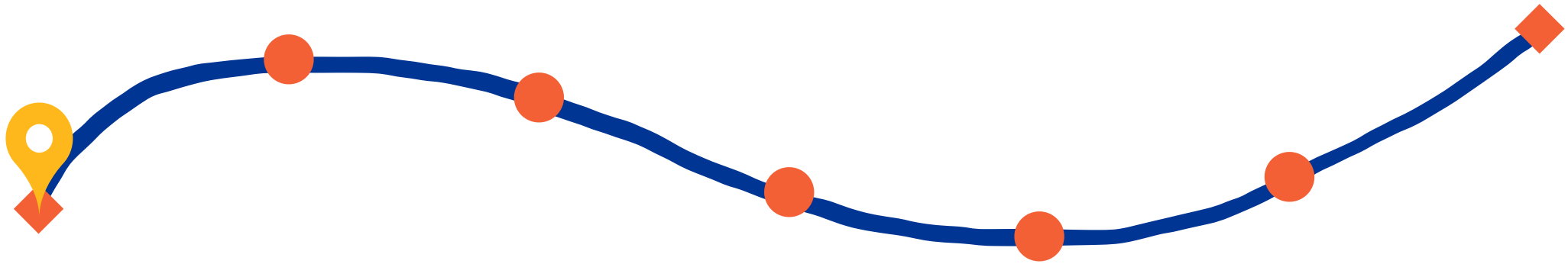
Announcements

- A05 is due Thursday by 11:59 pm
- A06 will be posted on Friday



Mol* (molstar) example

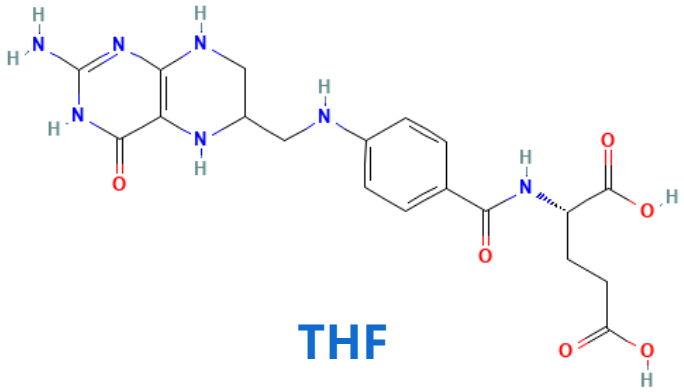
After today, you should be able to



Explain why DHFR is a promising drug target.

THF production is crucial for cellular growth

5,6,7,8-tetrahydrofolate (THF) is essential for all organisms



THF

THF is needed for

- Producing red blood cells,
- Synthesizing purines,
- Interconverting amino acids,
- Methylating tRNA,
- Generating and using formate.

Disrupting THF production has a cascading effect on essential cellular processes, primarily affecting DNA and RNA synthesis and amino acid metabolism

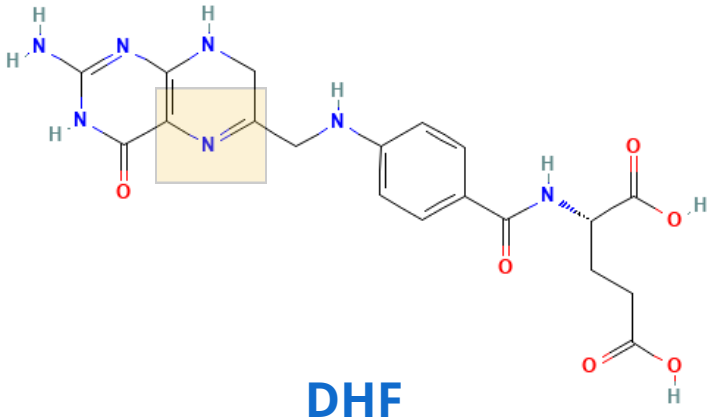
This is a useful process for drug design

Schnell et al., 2004

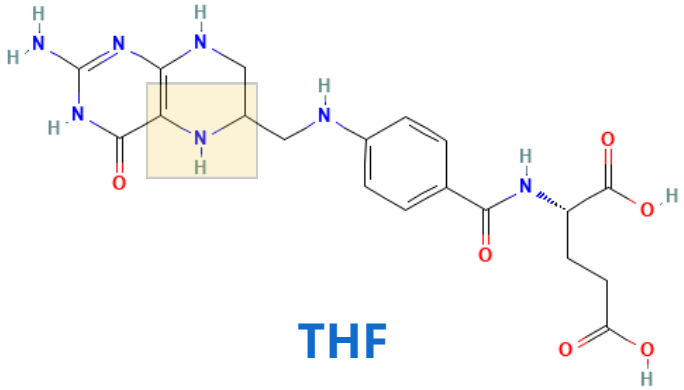
DHFR is responsible for synthesizing THF

Dihydrofolate reductase (DHFR) is a crucial enzyme that produces THF from dihydrofolate (DHF)

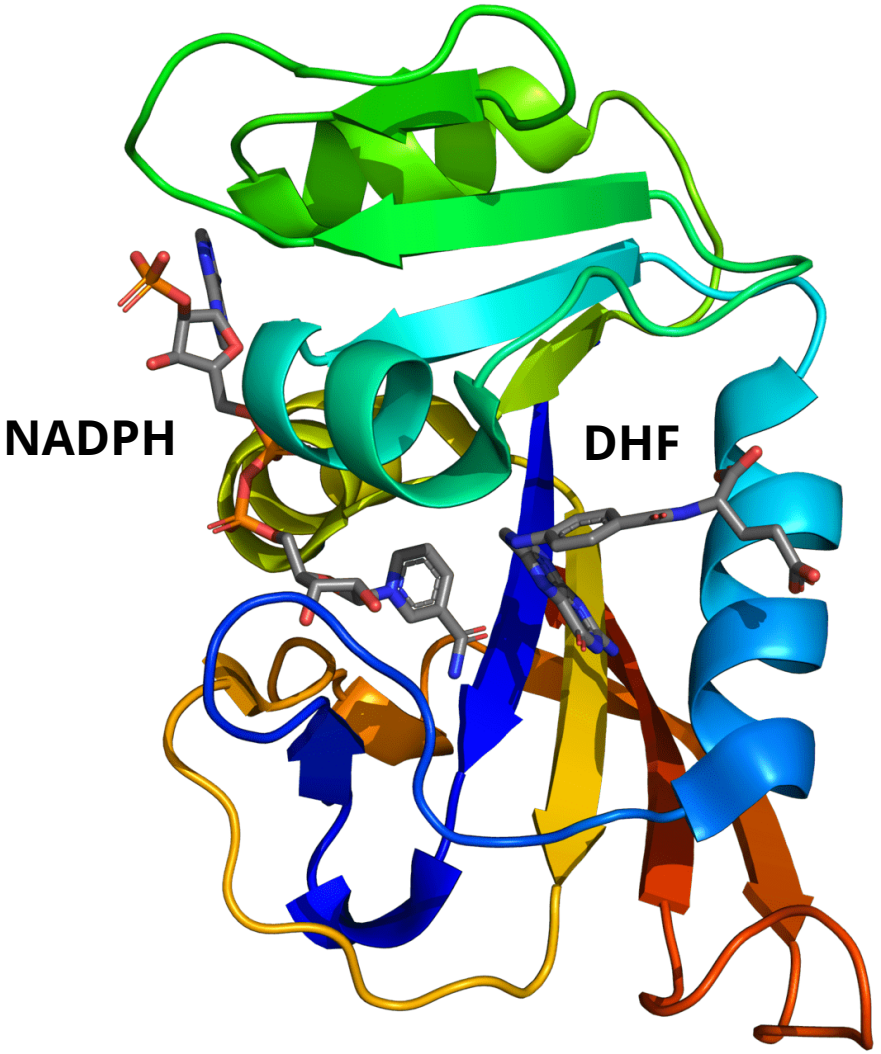
DHF + NADPH ⟶ THF + NADP(+)



DHF



THF



DHFR has been extensively studied as an antibiotic (e.g., trimethoprim) and cancer (e.g., methotrexate) target

(We will use this protein for our project)

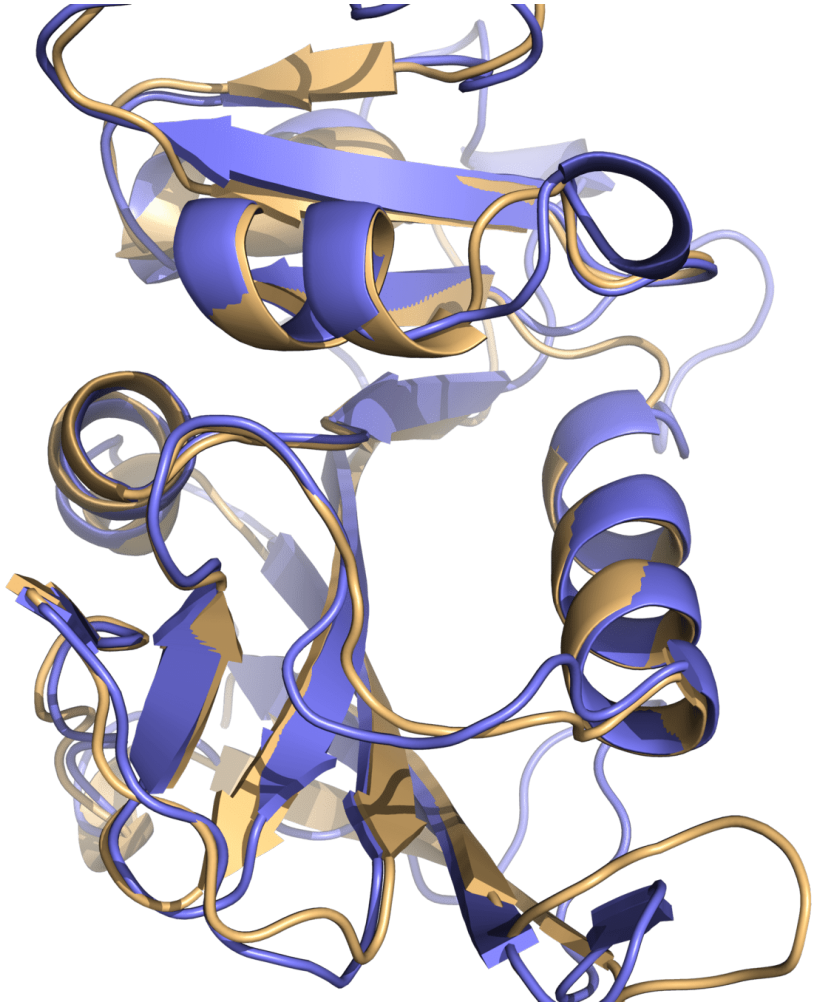
Schnell et al., 2004

DHFR conservation complicates drug design

What would happen if a patient with a bacterial infection is prescribed a drug loosely targeting DHFR

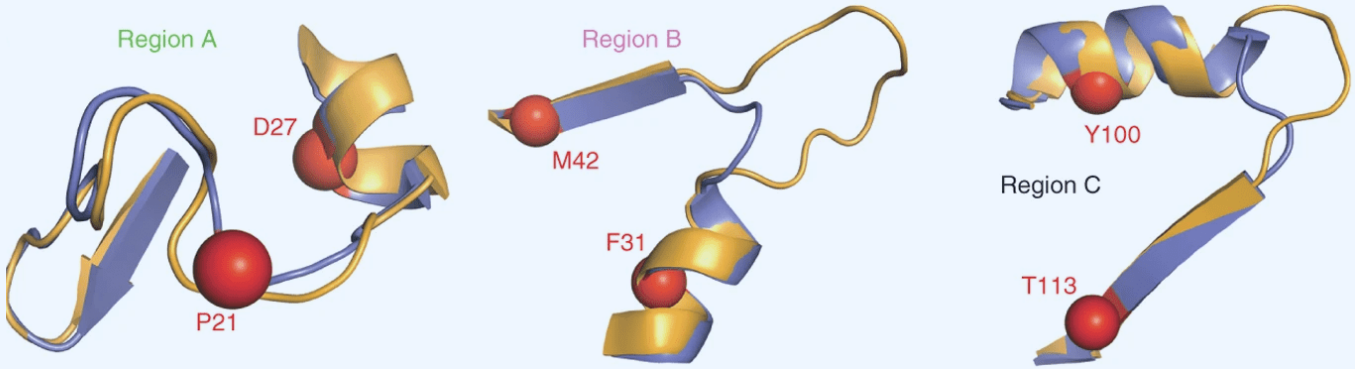
Patient could have deleterious side effects

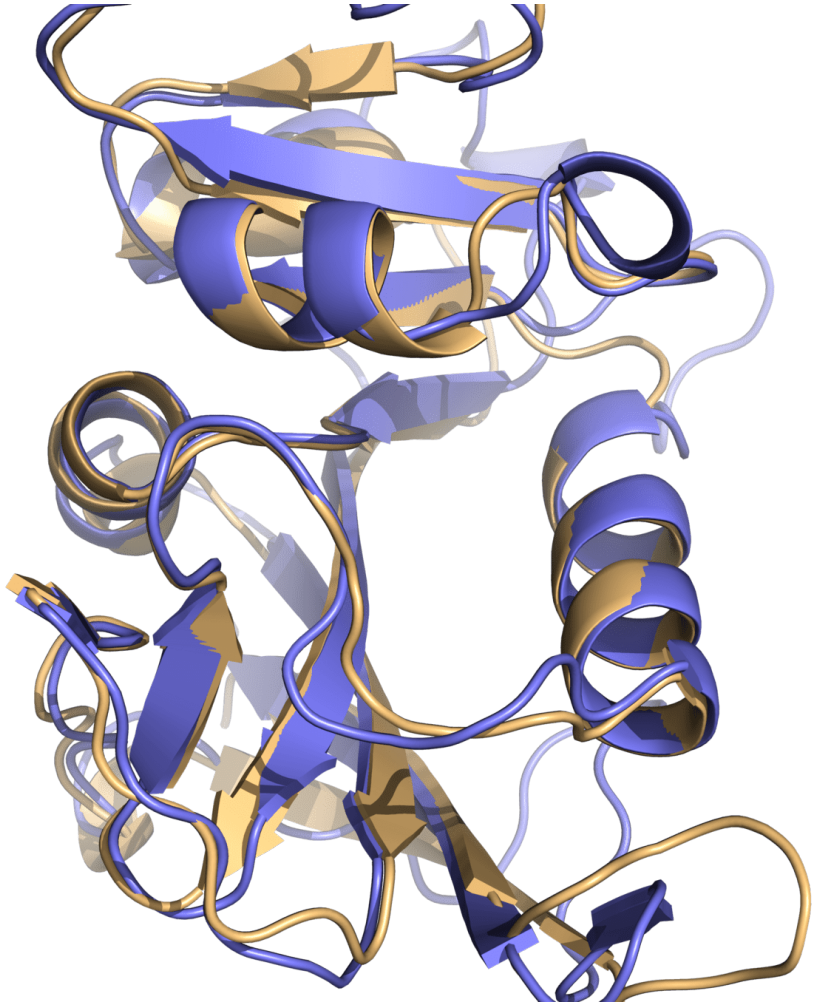
Both proteins have high structural similarity, even around the active site



DHFR conservation complicates drug design

Bacteria and humans have similar structures, but their dynamics are different





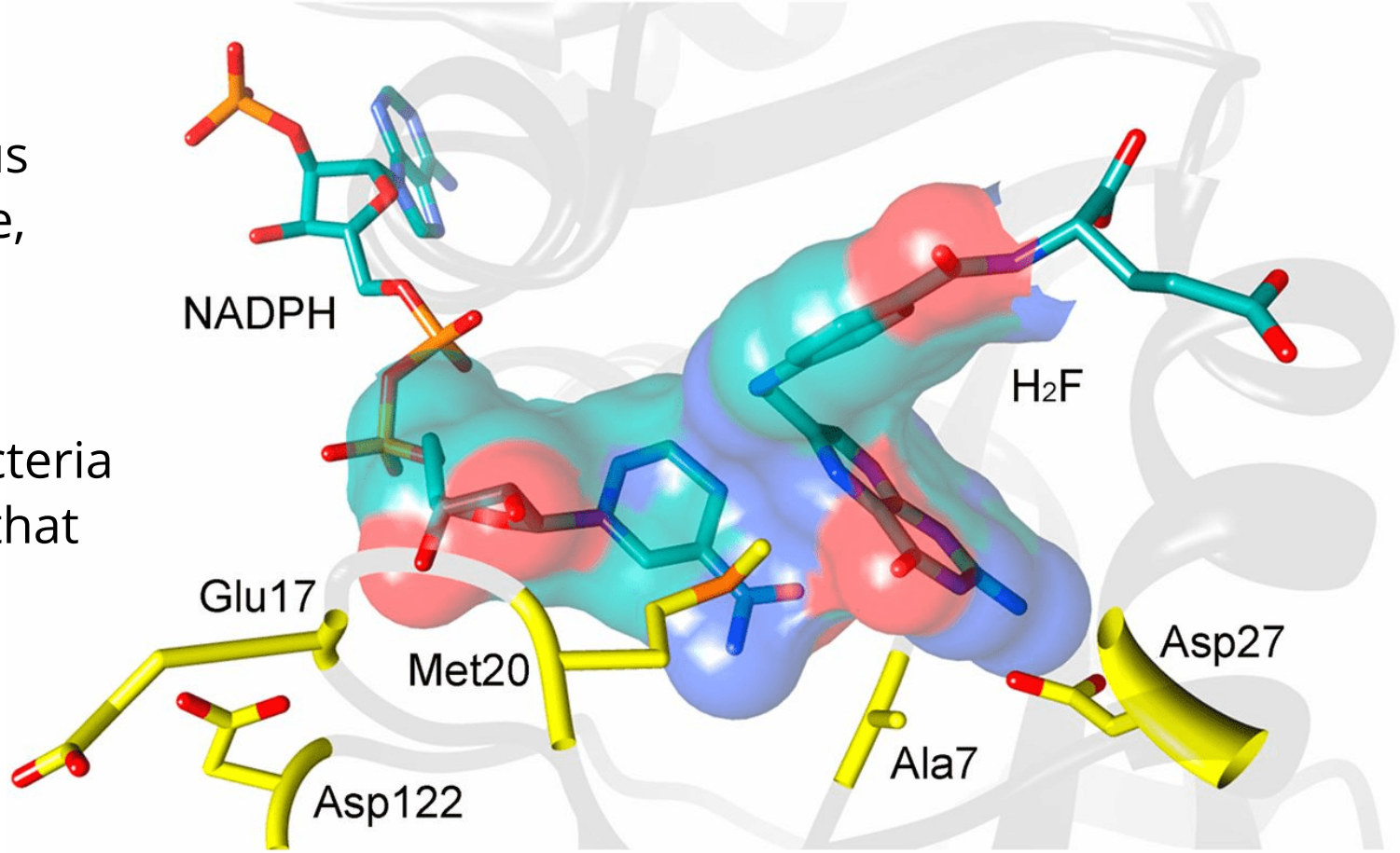
Outcome: We need to ensure drugs only bind to bacterial proteins by exploiting dynamic insights

Bhabha et al., 2013

Simulating DHFR provides insight into druggable conformations

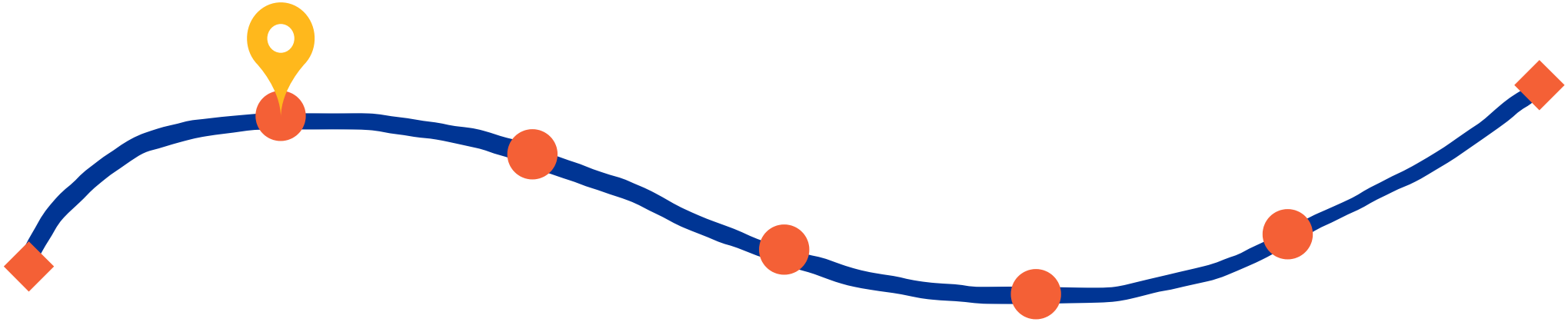
MD simulations will explore various low-energy conformations that are, hopefully, similar to reality

Knowing conformations unique to bacteria allow us to design a small molecule that competitively inhibits DHFR



Bhabha et al., 2013

After today, you should be able to



Select and prepare a protein structure

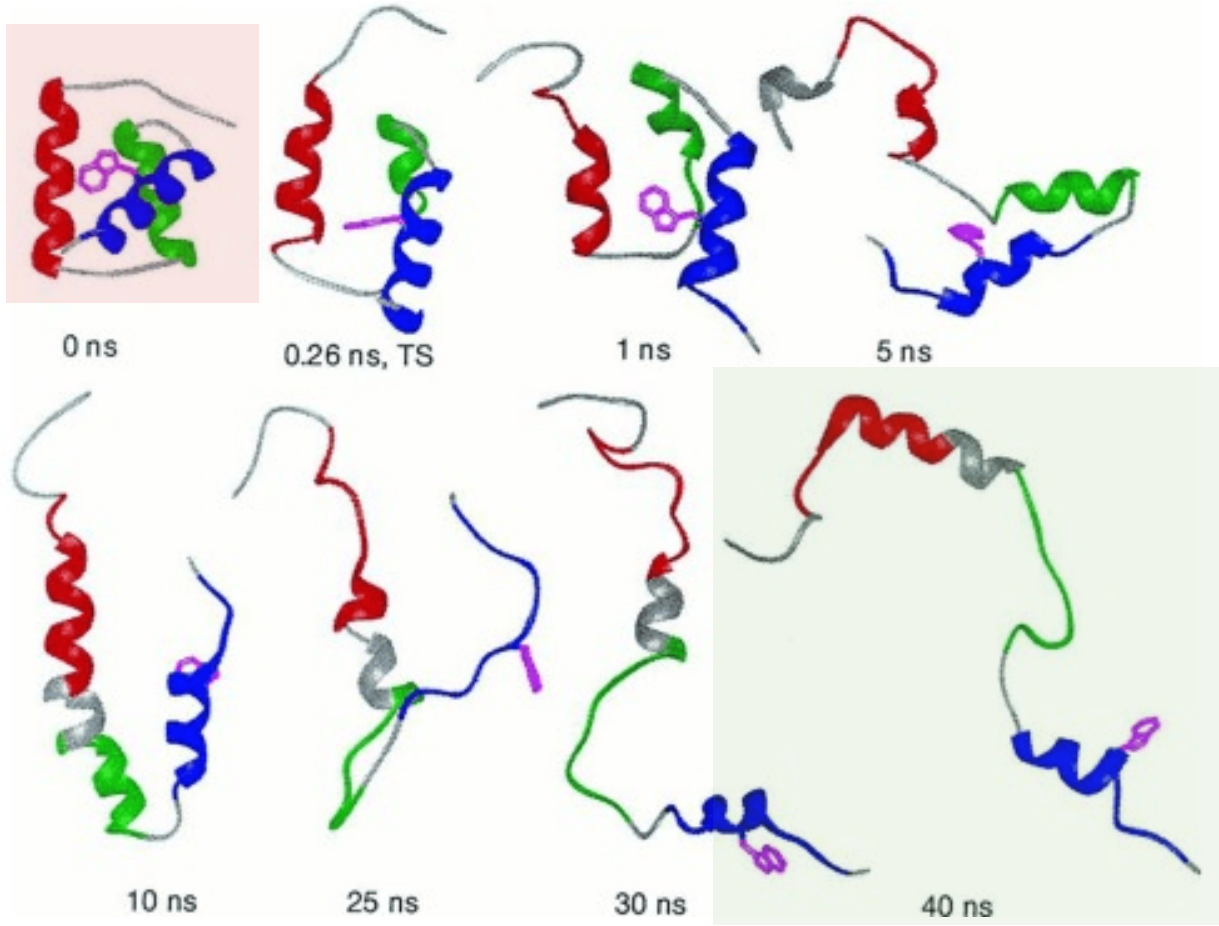
for molecular simulations.

We need a structure before starting any molecular simulation

If our starting structure is very far away from our desired equilibrium, our simulations will take longer

- Low-quality experimental structures
- Inaccurate computational predictions
- High-energy conformations
- Missing or incorrect cofactors

For example, we would have to wait for the protein to fold to study it's dynamics



We can obtain starting structures from experimental databases

Experimental structures offer the best option for their accuracy

PDB contains experimentally determined structures for thousands of proteins

General resolution preference:
X-ray, Cryo-EM, NMR

Not all structures in the PDB are equally suitable for simulations

Resolution

The resolution of a structure refers to how well the atomic positions are determined

Tip: A resolution below 2.0 Å is generally preferred for high-quality simulations.

Completeness

Flexible loops or disordered regions are often missing from the structure

Functional state

Proteins can exist in different functional conformations: active vs. inactive state, bound to ligands or unbound

B-factors

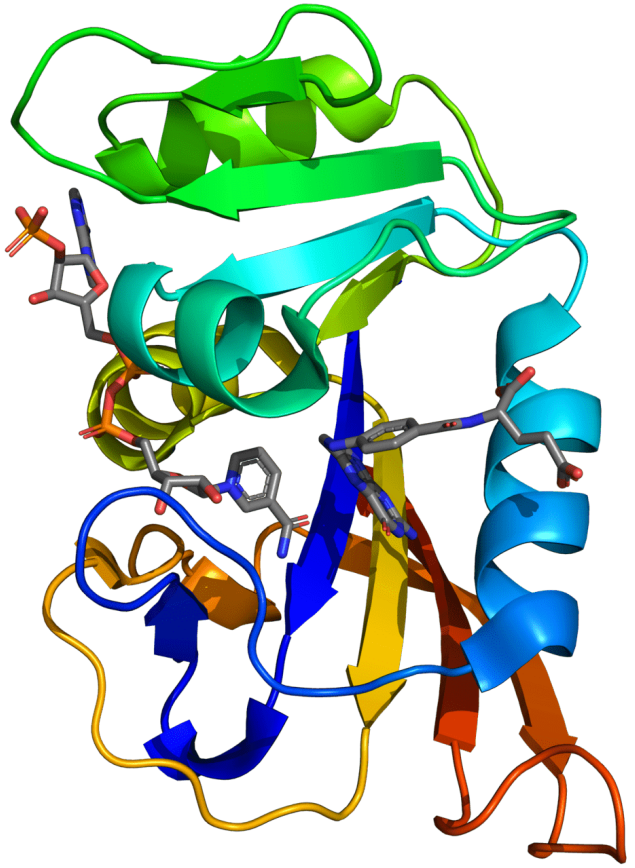
Higher B-factors suggest more uncertainty in atom positions, which might make that part of the structure less reliable

Not all structures in the PDB are equally suitable for simulations

Here are some example structural characteristics with the best value in **bold**

Factor	7D4L	4NX6	4KJK	4NX7
Resolution (Å)	1.60	1.35	1.35	**1.15**
Temperature	**298**	**298**	**298**	100
R-free	0.196	0.190	**0.166**	0.170
Clashscore	**2**	5	8	12
Ramachandran outliers	**0**	**0**	**0**	**0**
Rotamer outliers	**1**	2	**1**	5

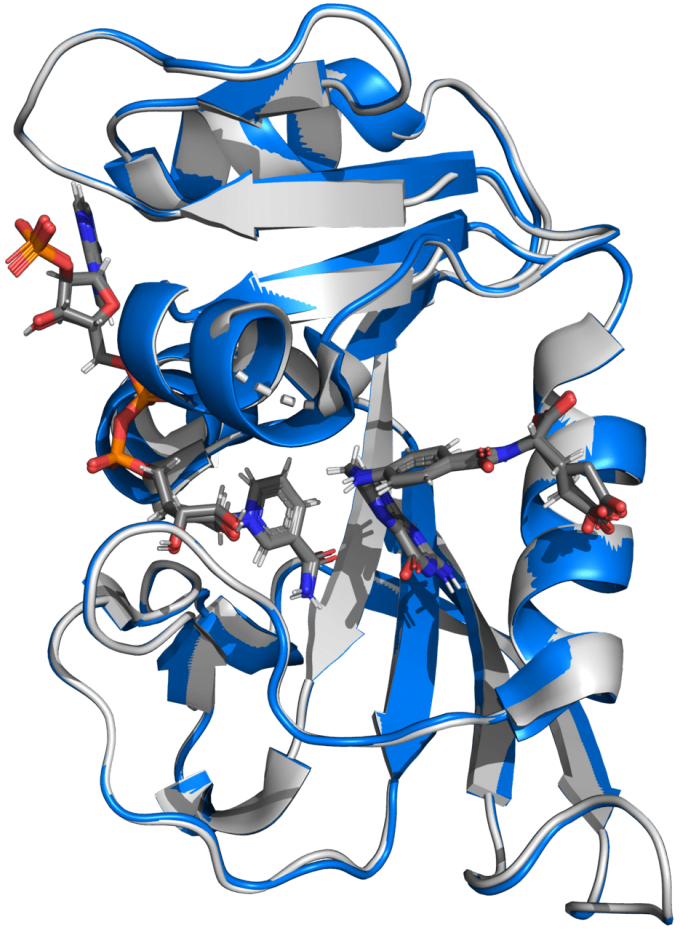
Resolution and R-free are comparable, and few clashes are highly desirable



7D4L is a good choice

Reasonable structures will likely provide similar results

Factor	7D4L	4KJK
Resolution	1.60	1.35
Temperature	**298**	**298**
R-free	0.196	**0.166**
Clashscore	**2**	8
Ramachandran outliers	**0**	**0**
Rotamer outliers	**1**	**1**



Blue: 7D4L

Grey: 4KJK

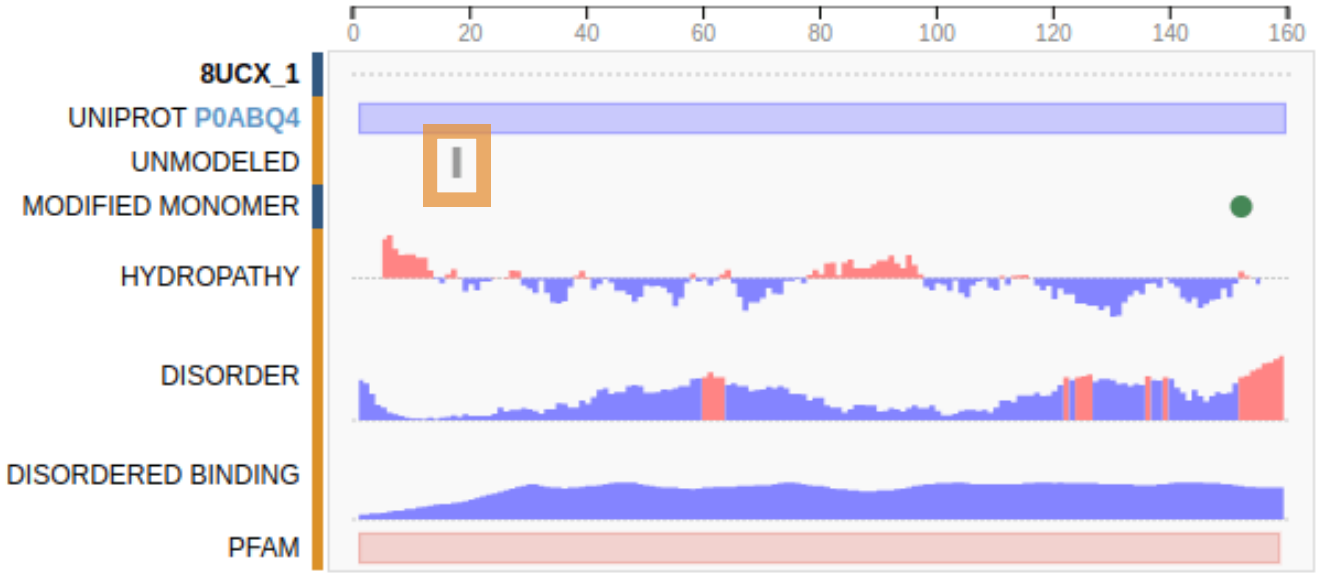
Either structure would provide comparable results if simulation protocols are appropriate

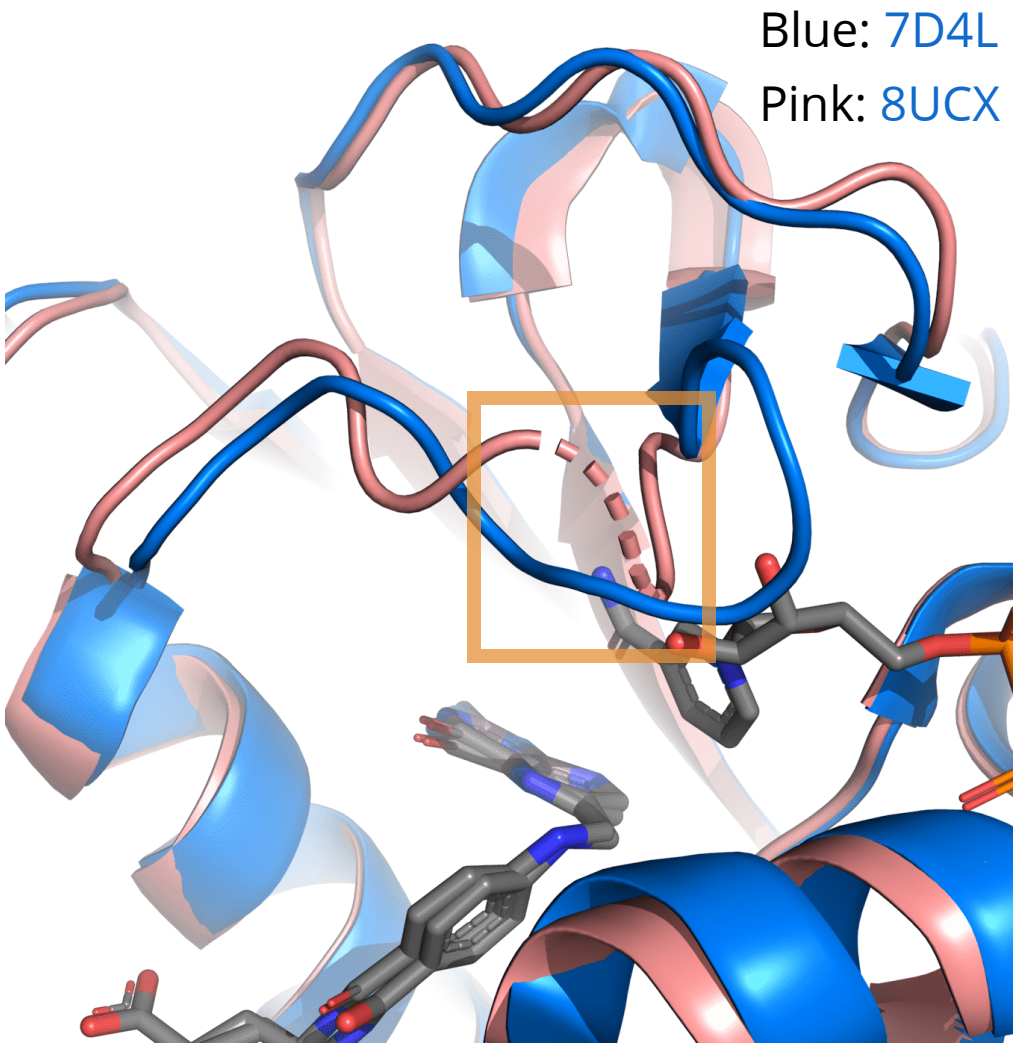
Alpha carbon RMSD is 0.141 (indicating high similarity)

Simulations cannot have missing residues

It’s essential to fix chain breaks and missing loops before simulation

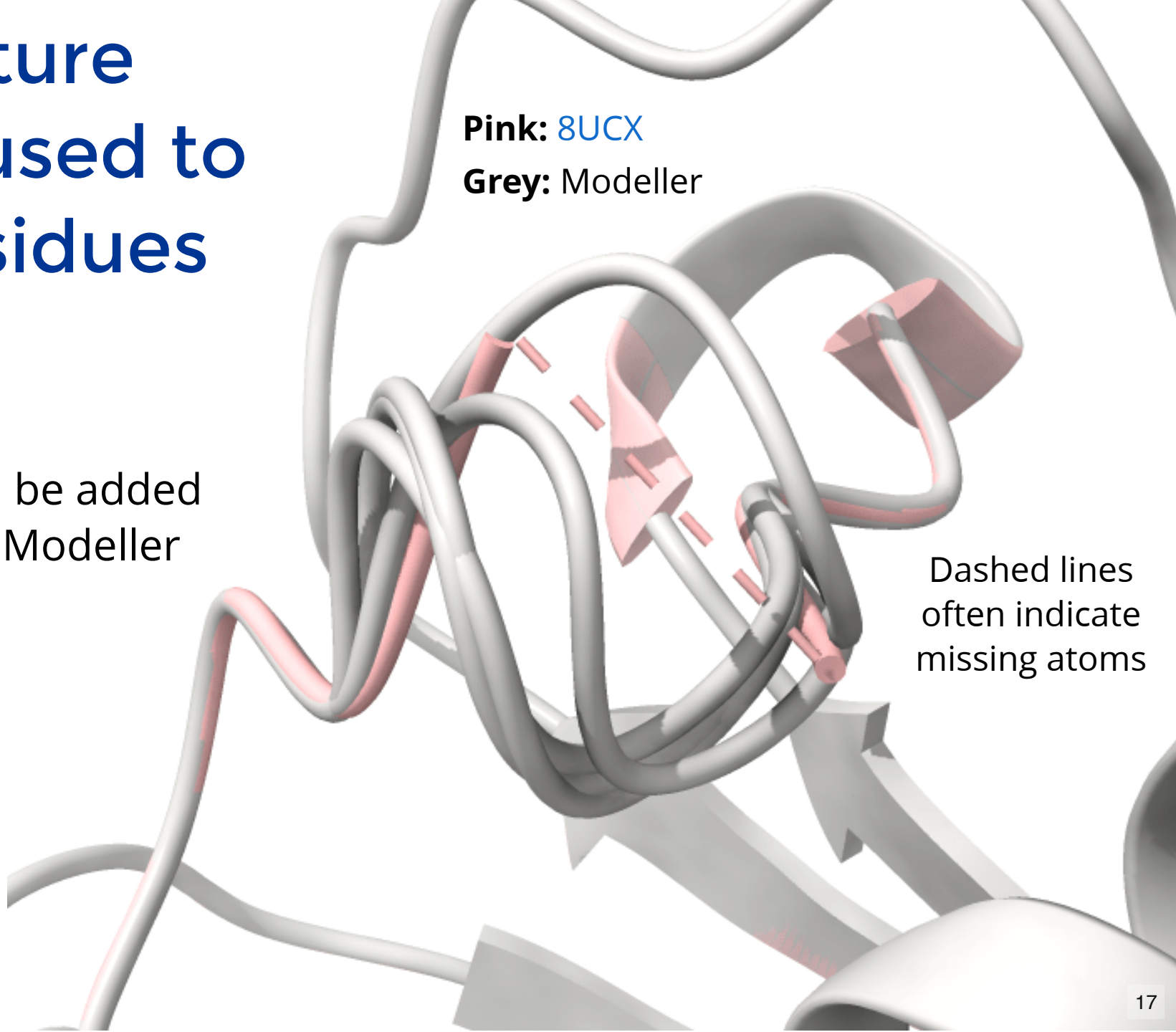
8UCX is missing residues 17 and 18





Protein structure predictions are used to add missing residues

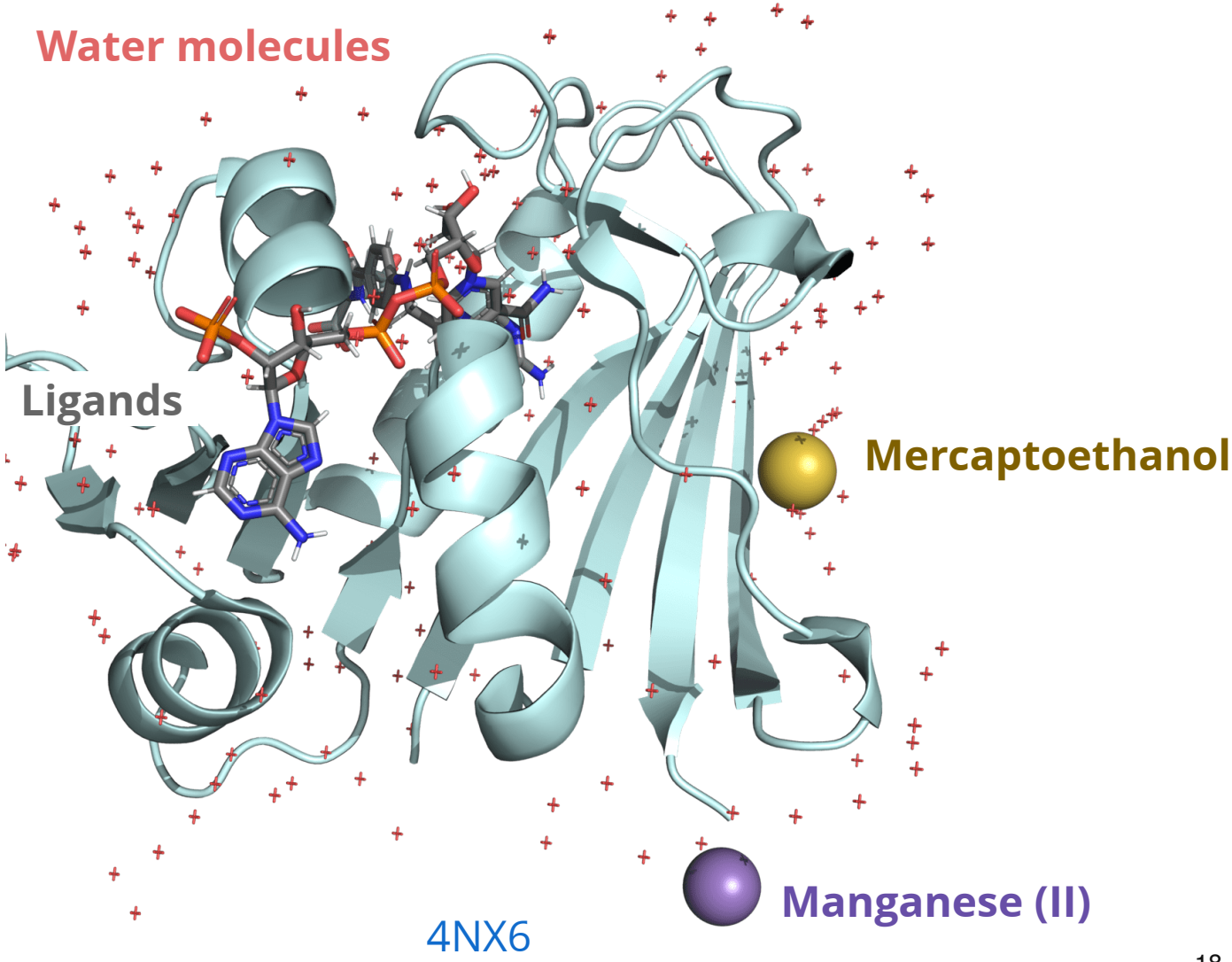
Missing atoms or residues can be added using modeling software like Modeller



Unwanted components like ligands or non-essential ions should be removed

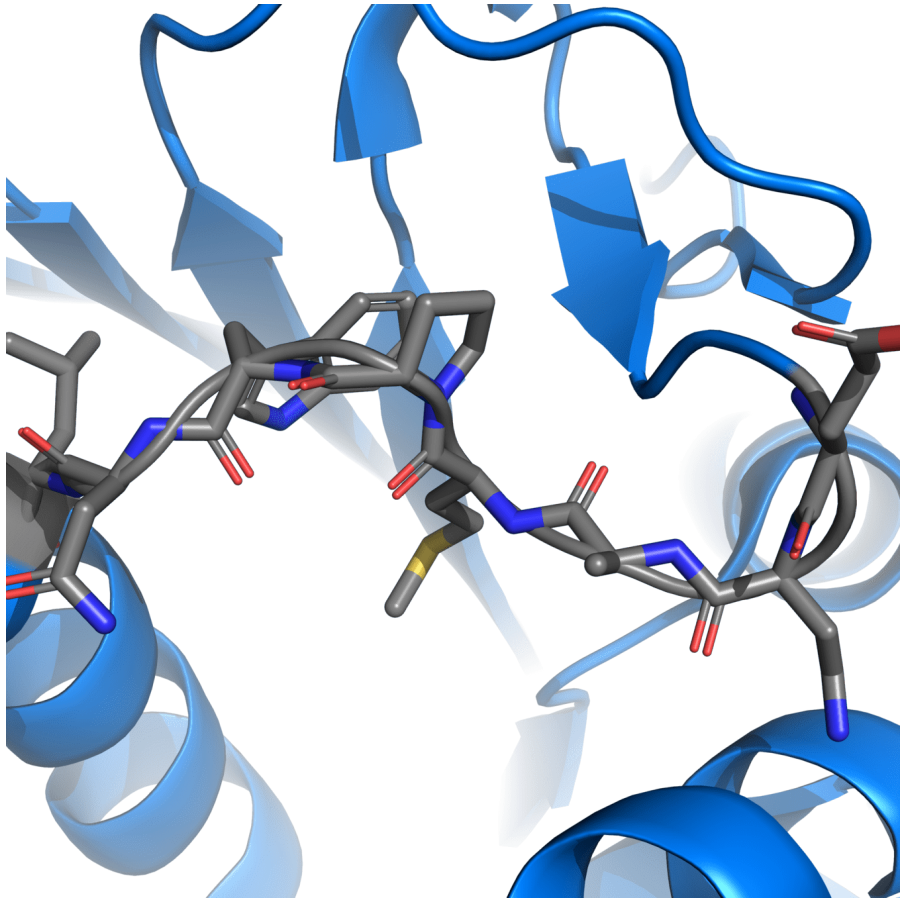
Many PDB structures contain ligands, ions, or crystallization agents that are not physiologically relevant

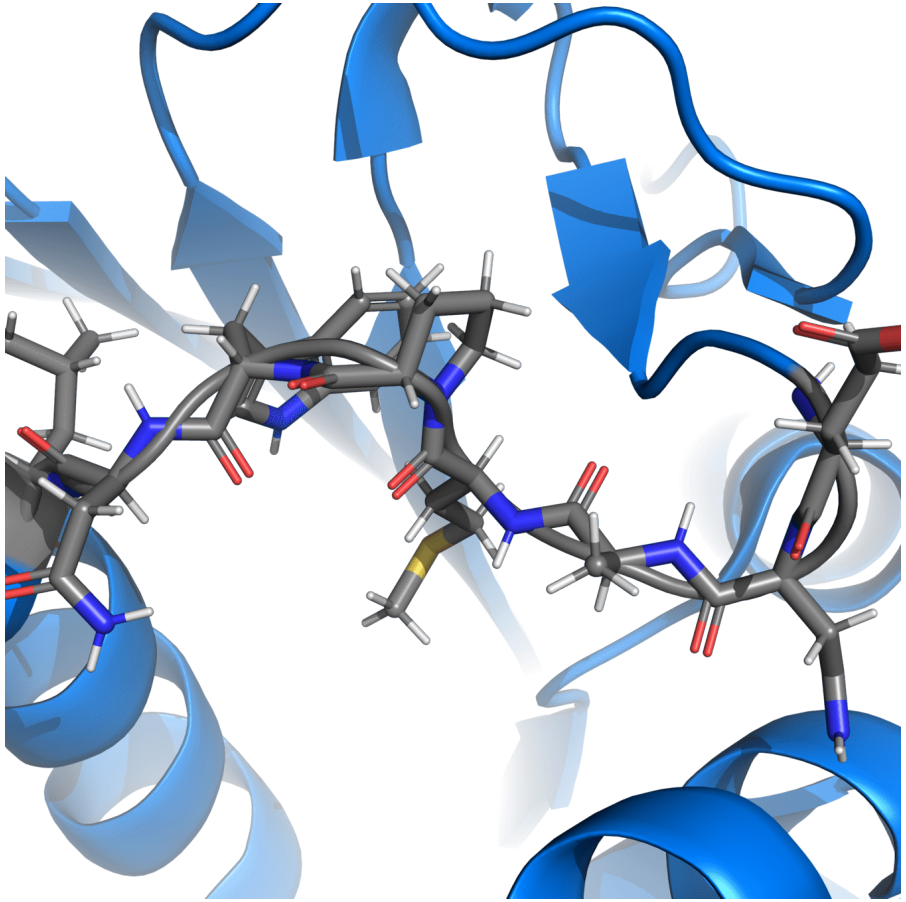
These can distort the protein's behavior in a simulated biological environment if not removed



Correct protonation states are essential for accurate simulations

Experimental structures often cannot resolve hydrogens, so we need to add them ourselves





pH-sensitive residues

Protonation states of amino acids affect the charge distribution, which influences electrostatic interactions during the simulation

Histidine (His, H): pKa ~6.0

Protonation switching around pH 6 - 7

Cysteine (Cys, C): pKa ~8.3

Could form disulfide bonds in oxidizing environments

Aspartic Acid (Asp, D): pKa: ~3.9

Affects interactions like salt bridges and hydrogen bonds

Lysine (Lys, K): pKa: ~10.5

Can form ionic bonds with negatively charged residues

Glutamic Acid (Glu, E): pKa: ~4.2

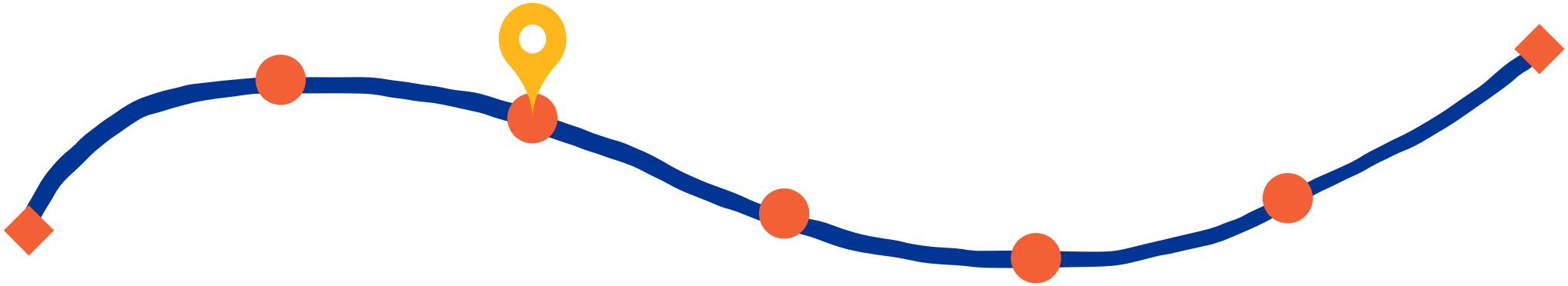
Glu's protonation state affects electrostatic interactions.

Tyrosine (Tyr, Y): pKa: ~10.1

Hydrogen bonding and in enzyme active sites

We now have a fully prepared protein

After today, you should be able to

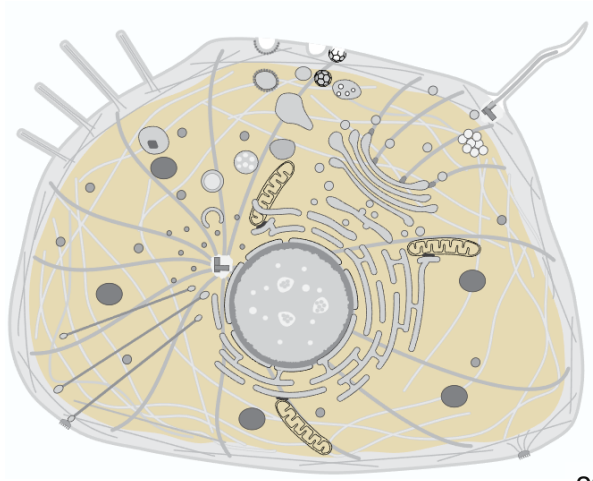


Explain the importance of approximating molecular environments.

DHFR is localized in the cytoplasm, which contains a multitude of chemical species

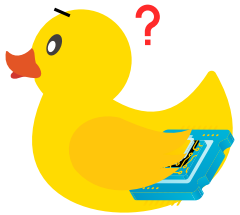
Ions	Potassium, Sodium, Calcium, Magnesium, Iron, Zinc, Copper, Manganese, Phosphate, Chloride, Bicarbonate, Sulfate, Citrate, ATP, ADP, AMP, . . .
Molecules	Glucose, pyruvate, lactate, amino acids, fatty acids, nucleotides, NADH, FADH, citrate, oxaloacetate, biotin, riboflavin, coenzyme A, ubiquinone, . . .
Proteins	Glycolytic enzymes, TCA cycle enzymes, DNA/RNA polymerases, kinases, phosphatases, G-proteins, heat shock proteins, molecular motors, transcription factors, transcription regulators, ribosomes, proteasomes, . . .
Organelles	Mitochondria, endoplasmic reticulum, golgi apparatus, lysosomes, peroxisomes, vacuoles, endosomes, ribosomes, centrosomes, . . .
Cytoskeleton	Actin, profilin, cofilin, myosin, keratins, vimentin, neurofilaments, tubulin, . . .
Membranes	Phospholipid bilayer with embedded proteins, cholesterol, clycoproteins, glycolipids, . . .

and more



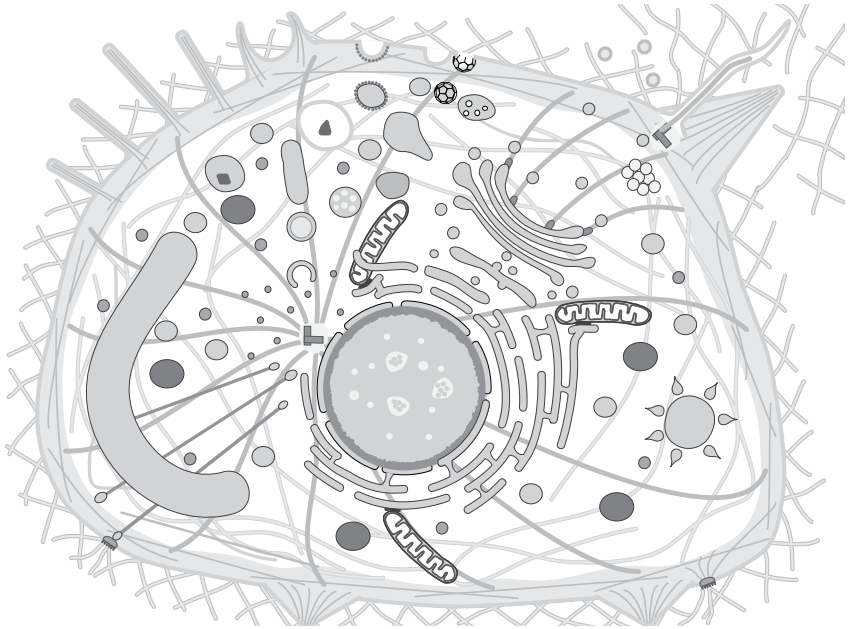
Simulations should accurately represent reality

What biological or chemical components are crucial for modeling the dynamics of a protein in the cytosol?



We must balance computational feasibility with biological realism

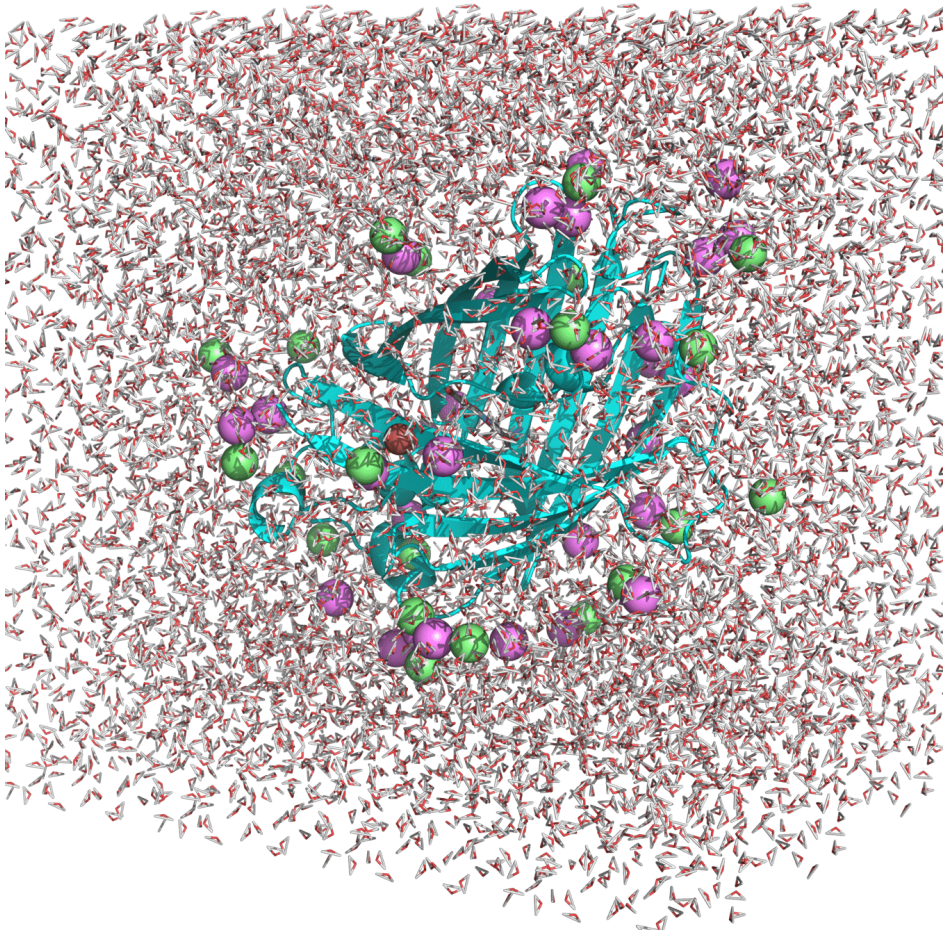
- Protein of interest (already prepared)
- Water molecular at the appropriate temperature (310 K) and pressure (1 atm)
- Cations (Na+ or K+) and anions (Cl-) at an ionic strength of 150 millimolar
- Any cofactors (e.g., NADPH and Folate for DHFR)

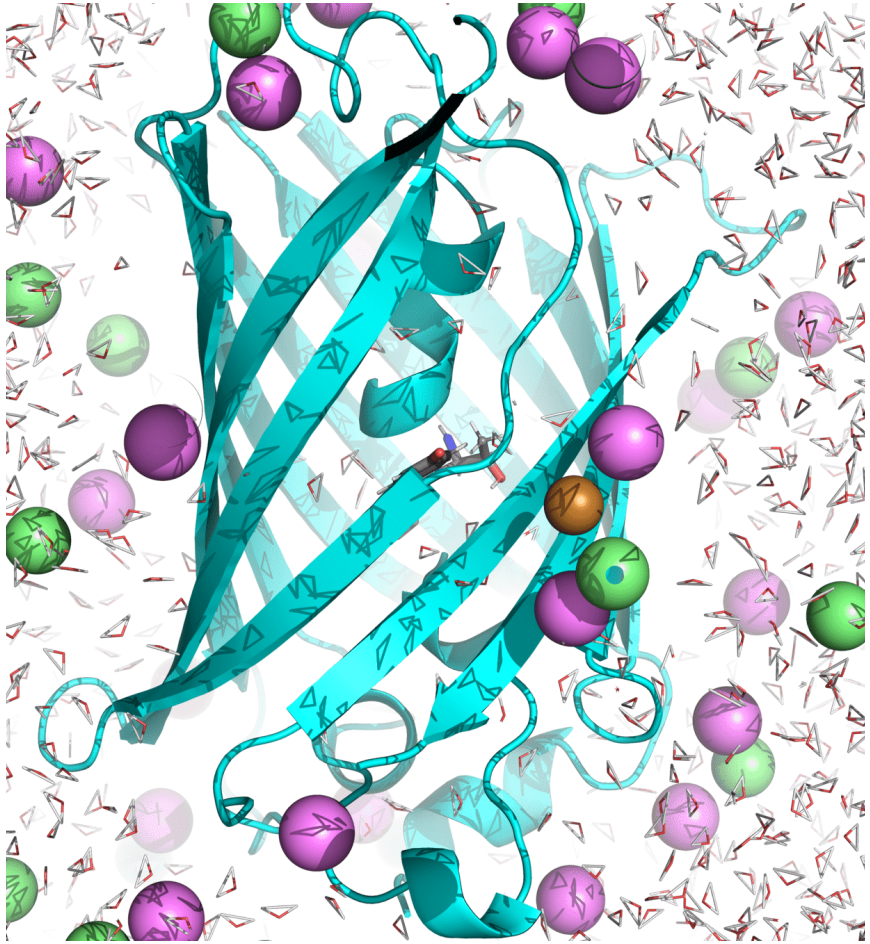


Animal cell

Example of system: roGPF2

Starting structure for simulating Cu(I) binding to Cys147 and 204 in roGFP2 with Na+ and Cl- counterions





(Actually used in my research.)

After today, you should be able to

Describe periodic boundary conditions

and their role in MD simulations.

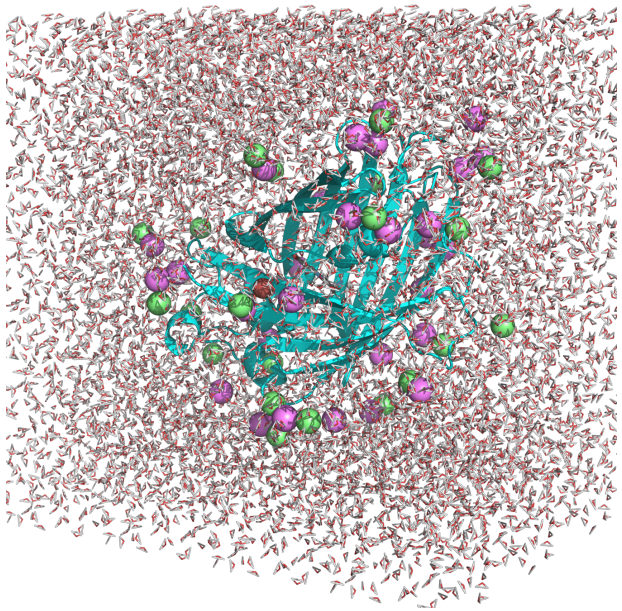
Realistic systems do not have walls

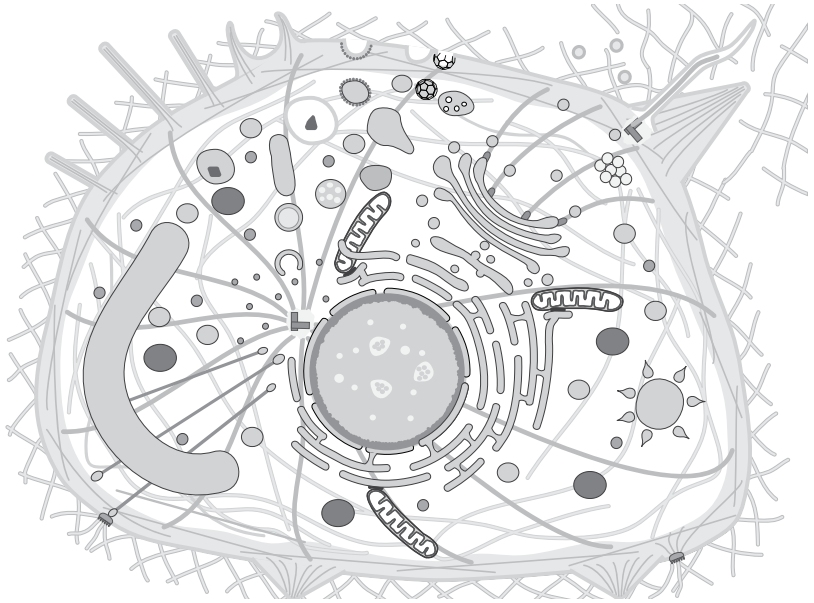
For this simulation, we would have to apply a force to keep the molecules in this box

Water molecules and proteins would bounce off these walls in an unphysical manner (i.e., edge effects)

A protein *in vivo* or *in vitro* will have plenty of space to move around

We could make the box very large, but this would dramatically increase the cost



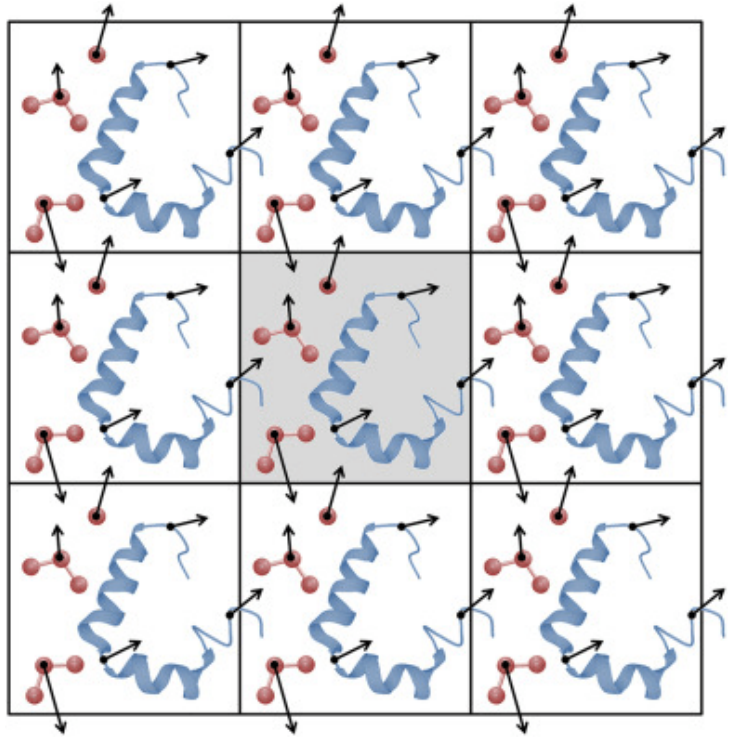


Periodic boundary conditions (PBC) is how we solve this issue

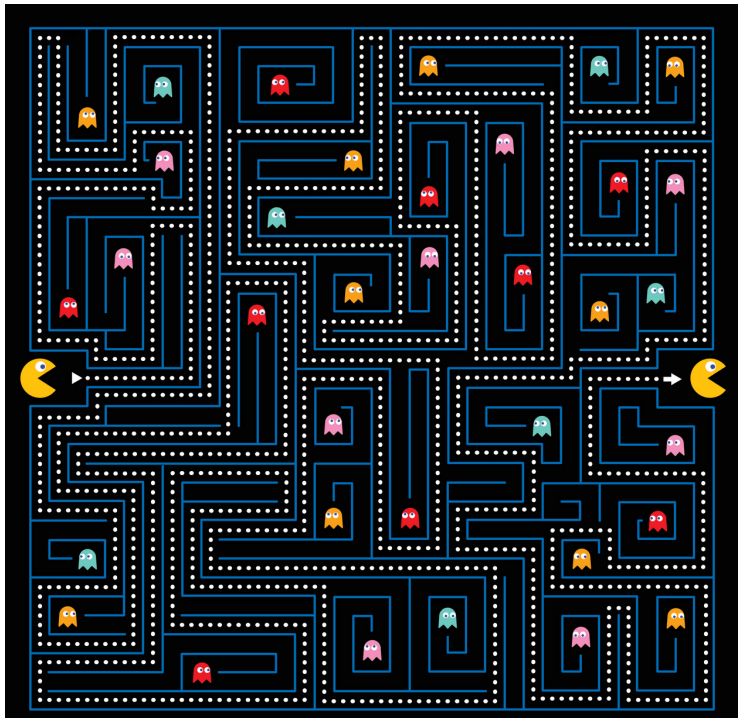
PBC simulate infinite systems from a finite box

We (virually) place exact copies of our system in all directions

Atoms that cross the box edge reappear on the other side; thus, do not have edge effects



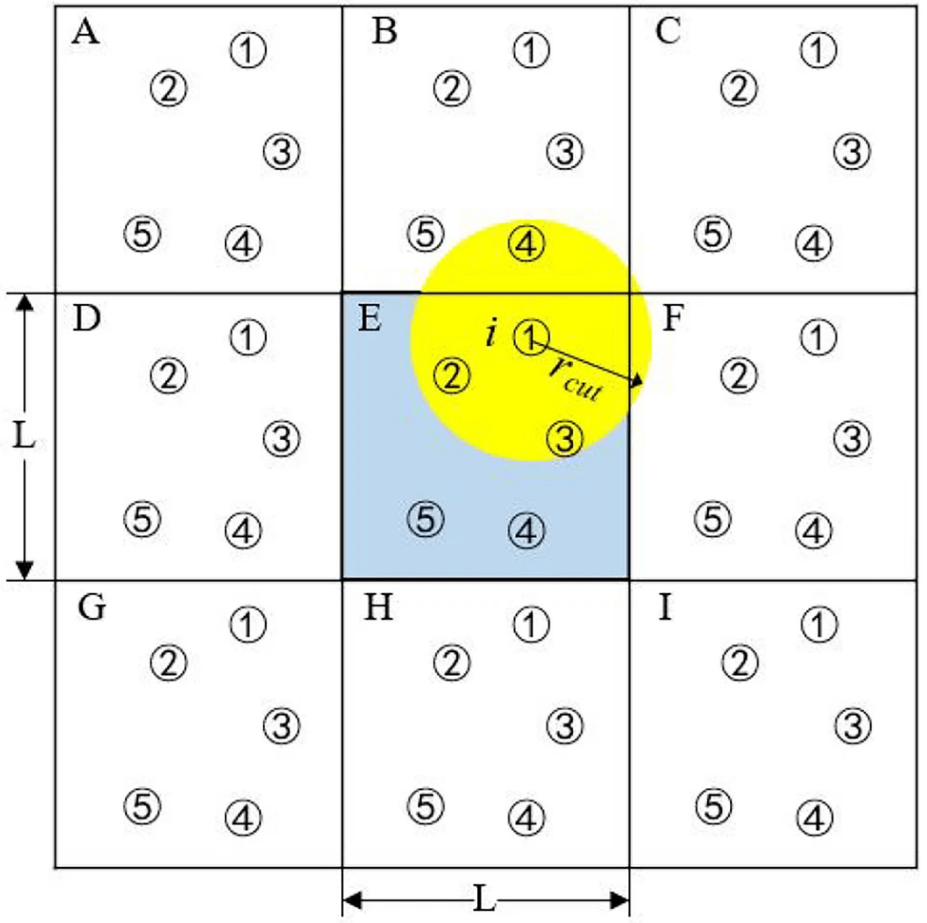
Think PackMan: If he crosses the right side of the map, he reappears on the left



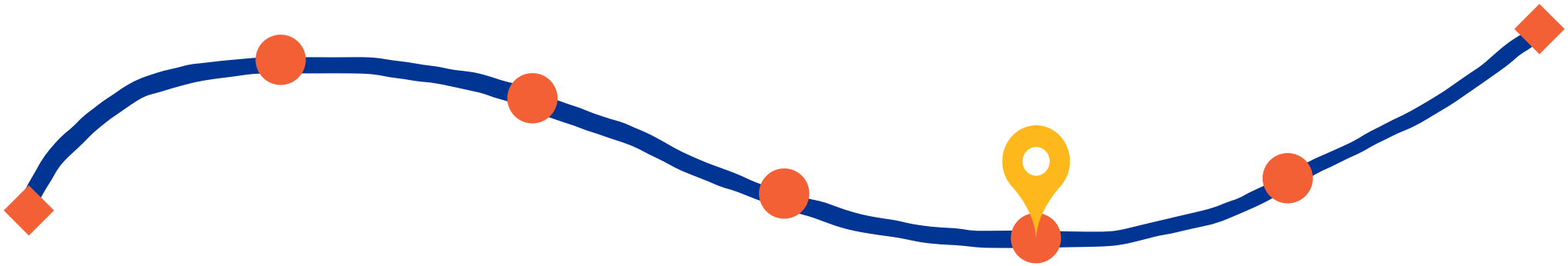
The minimum image convention ensure correct interaction

Image atoms in adjacent boxes are used to calculate interactions across the boundaries

The **minimum image convention (MIC)** ensures that an atom in the primary box only interacts with the closest image of another atom



After today, you should be able to



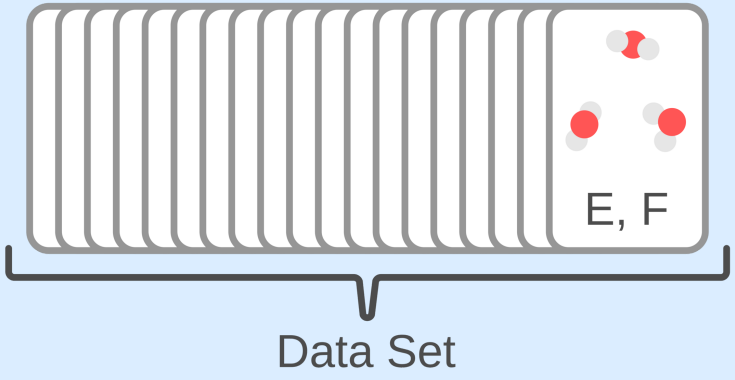
Explain the role of force field selection and topology generation.

We now have a fully prepared system, now we prepare our simulation

Force fields are parameterized to reproduce quantum chemical and experimental data

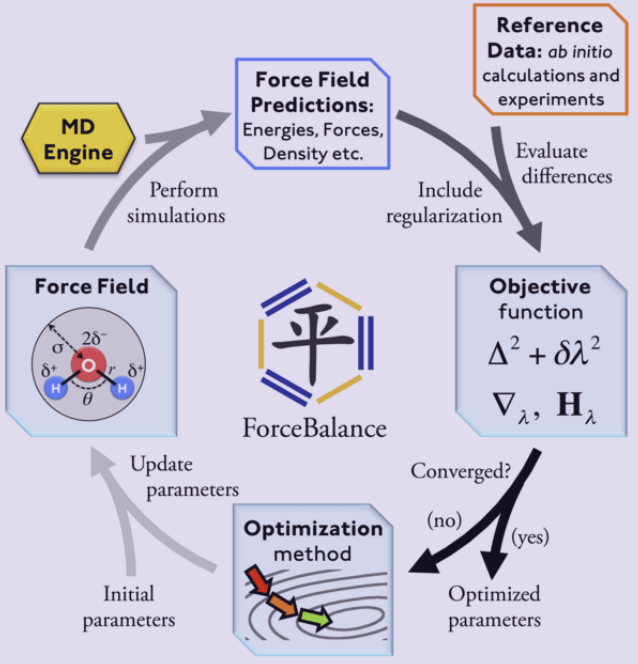
1. Generate structures and use quantum chemistry to compute energy and forces

2. Optimize force field parameters until they reproduce the quantum chemistry dataset



3. Run MD simulations and predict experimental data (e.g., NMR, Raman spectroscopy, solvation energies, etc.)

4. Continue to optimize force field parameters to minimizing quantum chemistry and simulation prediction errors



Force fields are dependent on fitting data and simulation setup

Force fields are not inherently compatible with each other

Example: **Simulating a DNA-binding protein**

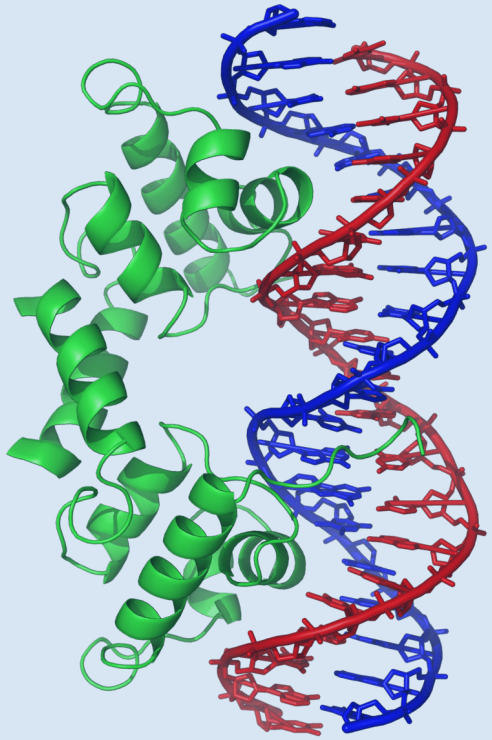
Suppose my **protein force field** was fit to:

- Membrane proteins
- Proteins and RNA

Suppose my **DNA force field** was fit to:

- Single-stranded DNA
- Protein binding with a different type of force field

Simulations would be unreliable because the force fields are incompatible with each other



Forcefields are compatible by design, or are validated against experimental data

Key factors for selecting a force field

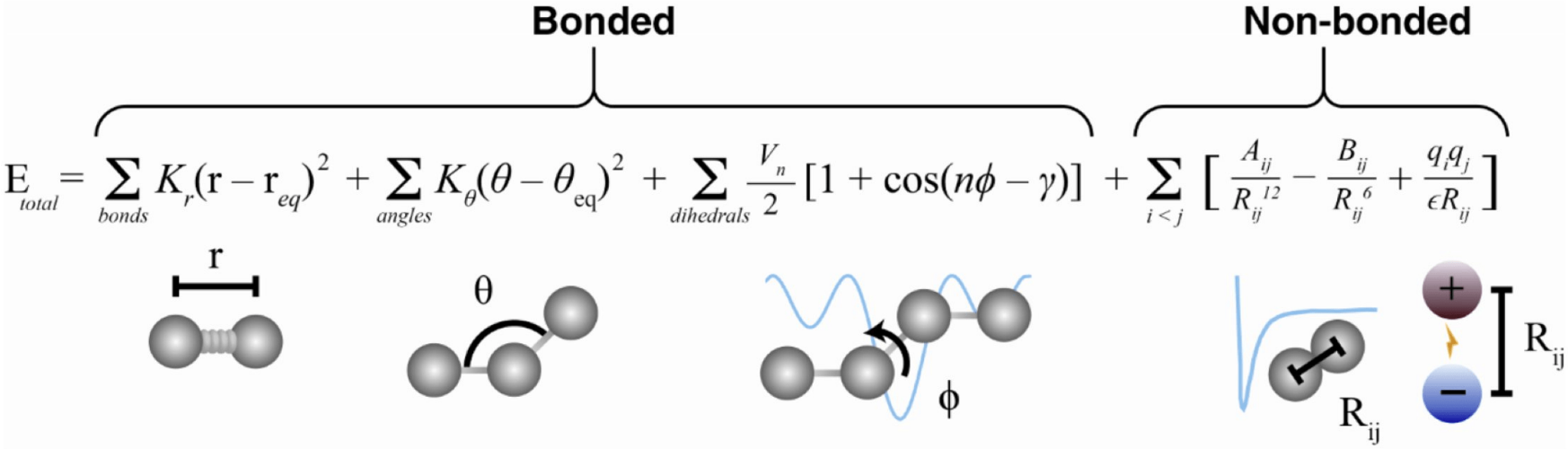
- **System type**: Different force fields are optimized for specific systems
- **Accuracy vs. speed**: High-accuracy force fields may require more computational resources
- **Compatibility**: Choose a force field based on compatibility with available topology generators and the type of molecules in your simulation.

Examples:

- **AMBER**: Best for proteins and nucleic acids, optimized for biomolecular interactions.
- **CHARMM**: Known for its extensive parameter set, suitable for complex systems including proteins, lipids, and membranes.
- **OPLS**: Optimized for small molecules, organic compounds, and polymers, with emphasis on accurate non-bonded interactions.

Topology files define the molecular structure and interactions in a simulation

A topology file contains information on atom types, bonds, angles, dihedrals, and non-bonded interactions based on the chosen force field



Essentially tells the program which force field parameters to use where

Example AMBER topology

We never actually look at these files

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CD  HD2 HD3 CE  HE2 HE3 NZ  HZ1 HZ2 HZ3 C   O   N   H   CA  HA2 HA3 C   O   N
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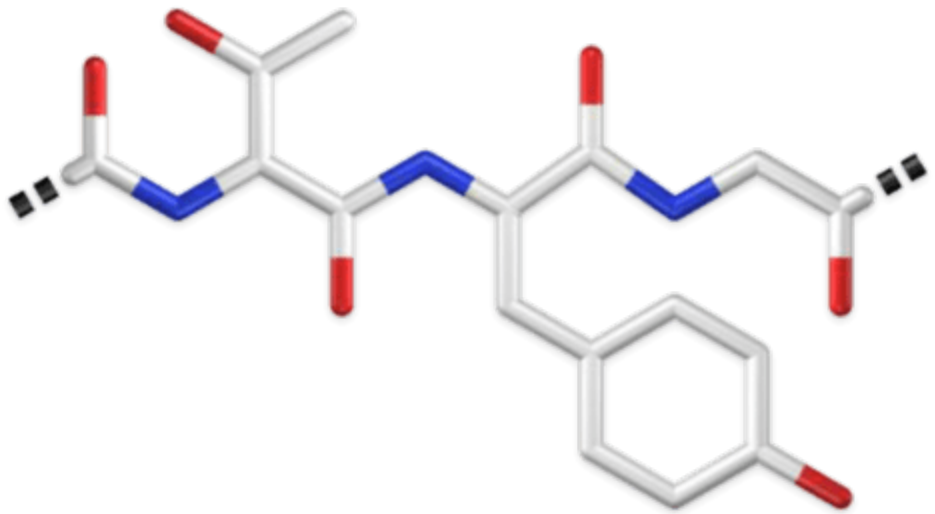
O   N   CD  HD2 HD3 CG  HG2 HG3 CB  HB2 HB3 CA  HA  C   O   N   H   CA  HA  CB
```

Complex molecules and ligands requires parameterization and careful integration

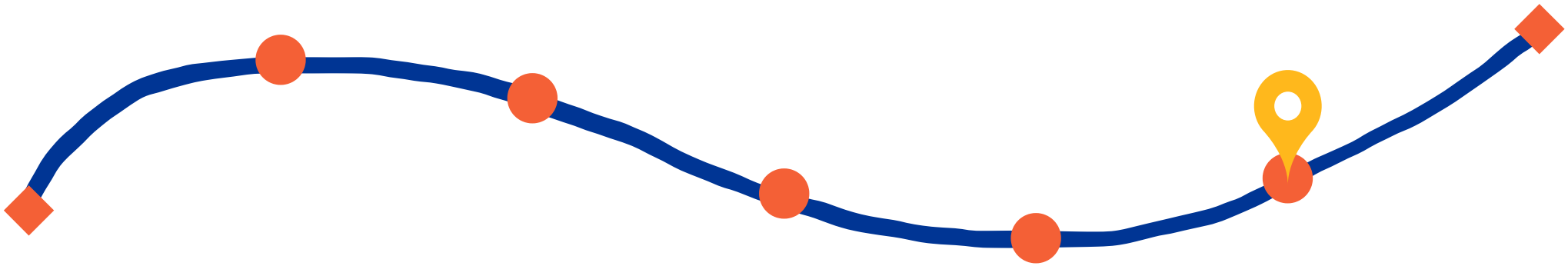
Non-standard residues or ligands are not always included in standard force field parameter sets

These require additional parameterization to ensure proper interactions in the simulation

Example: GFP chromophore



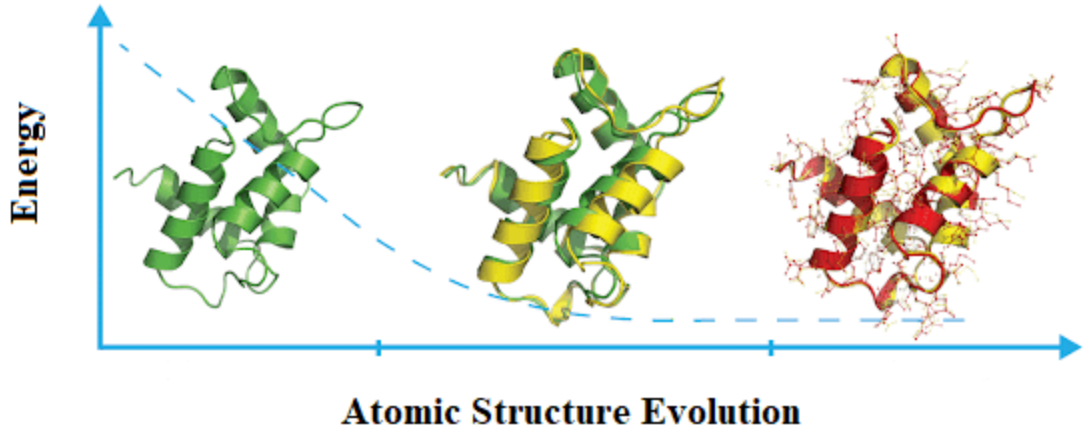
After today, you should be able to



Outline the process of energy minimization and its significance.

Energy minimization is necessary before running molecular dynamics simulations

Energy minimization adjusts the initial structure to remove unfavorable atom positions and steric clashes that could cause instability during simulations

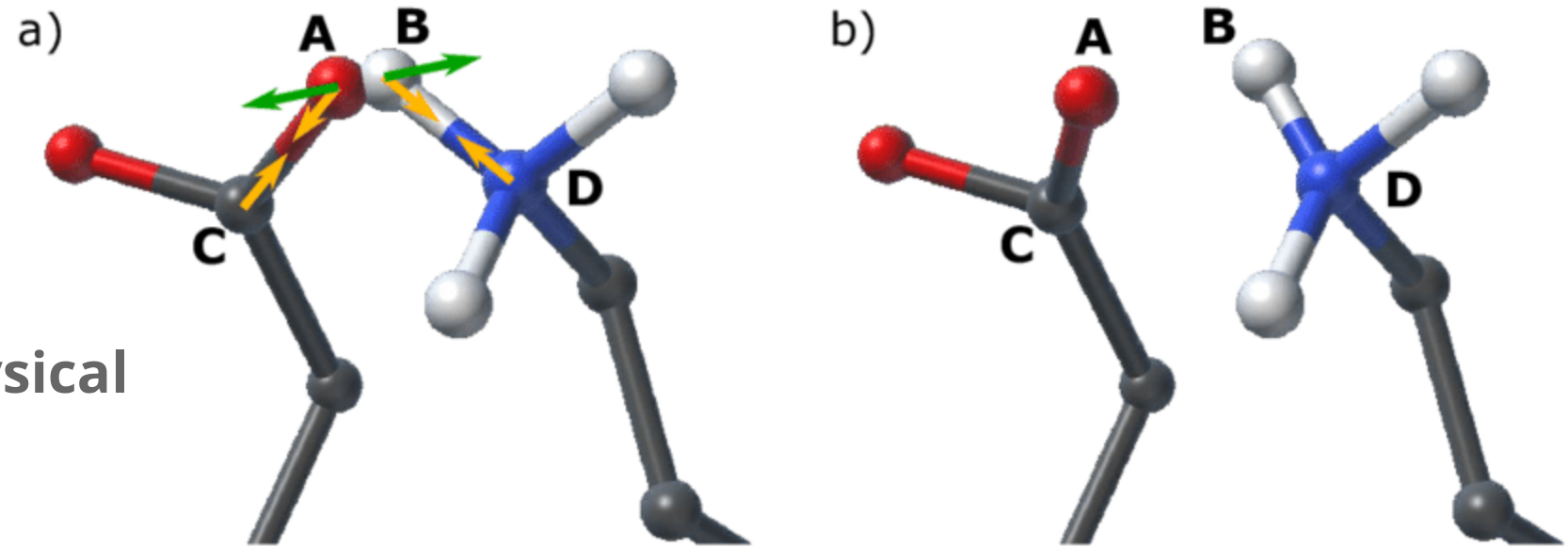


Without minimization, high-energy configurations may lead to unrealistic results or early failures in the molecular dynamics simulation

Energy minimization removes steric clashes and optimizes the initial geometry

Steric clashes occur when atoms are too close together, resulting in excessively high energy

Energy minimization gently adjusts the structure to lower the system's energy



Unphysical

Physical

Before the next class, you should

Lecture 14:
Molecular system representations

Lecture 15:
Atomistic insights

Today

Thursday

- Work on A05