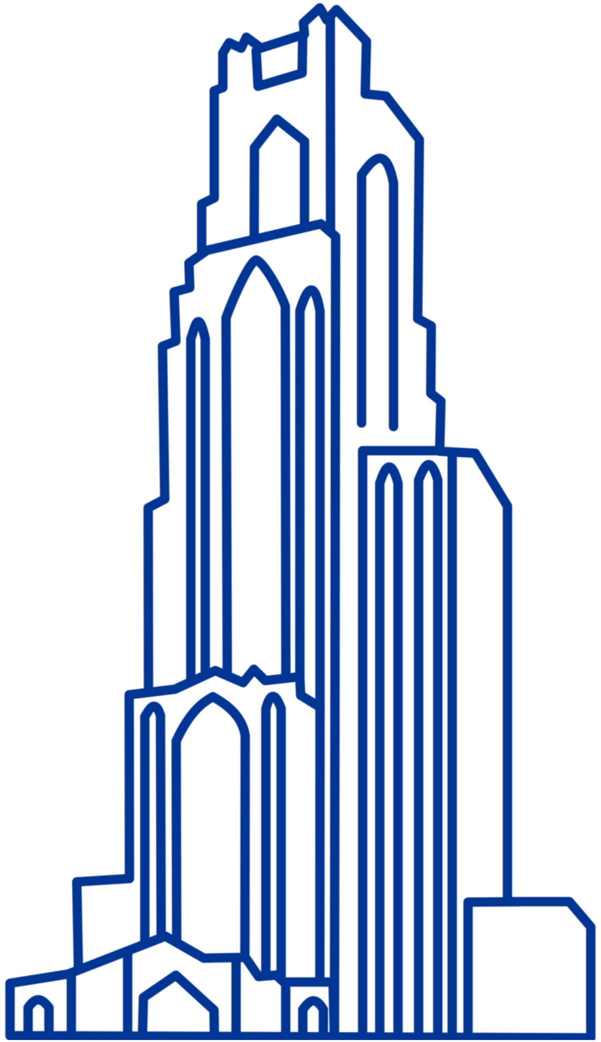


Computational Biology

(BIOSC 1540)

Lecture 12A

Docking

Foundations

Apr 1, 2025

University of Pittsburgh

Announcements

Assignments

- P03A is extended to Apr 8
- P04A will be our last assignment

Quizzes

- Quiz 04 will be on Apr 8 and cover L09A to L12A

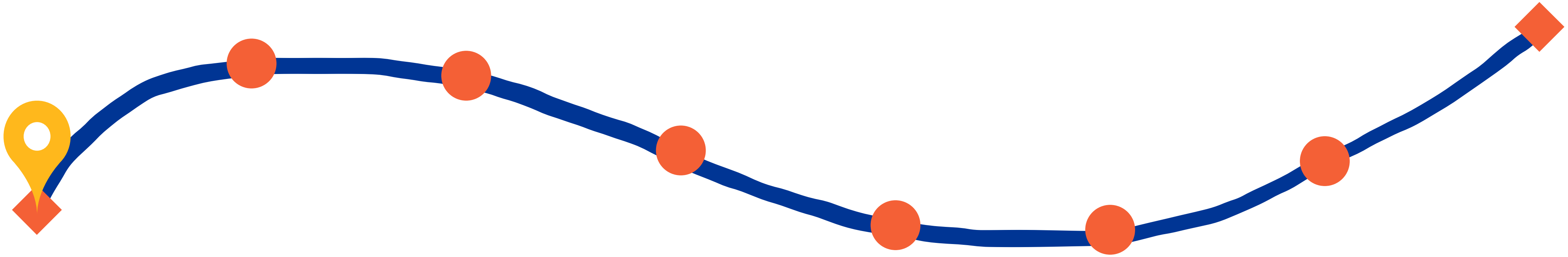
Final exam

- The final exam is on **Monday, Apr 28, at 4:00 pm in 244 Cathedral of Learning**

OMETs

- If the response rate is 80% or higher, I will drop your lowest assignment

After today, you should have a better understanding of



Drug targets

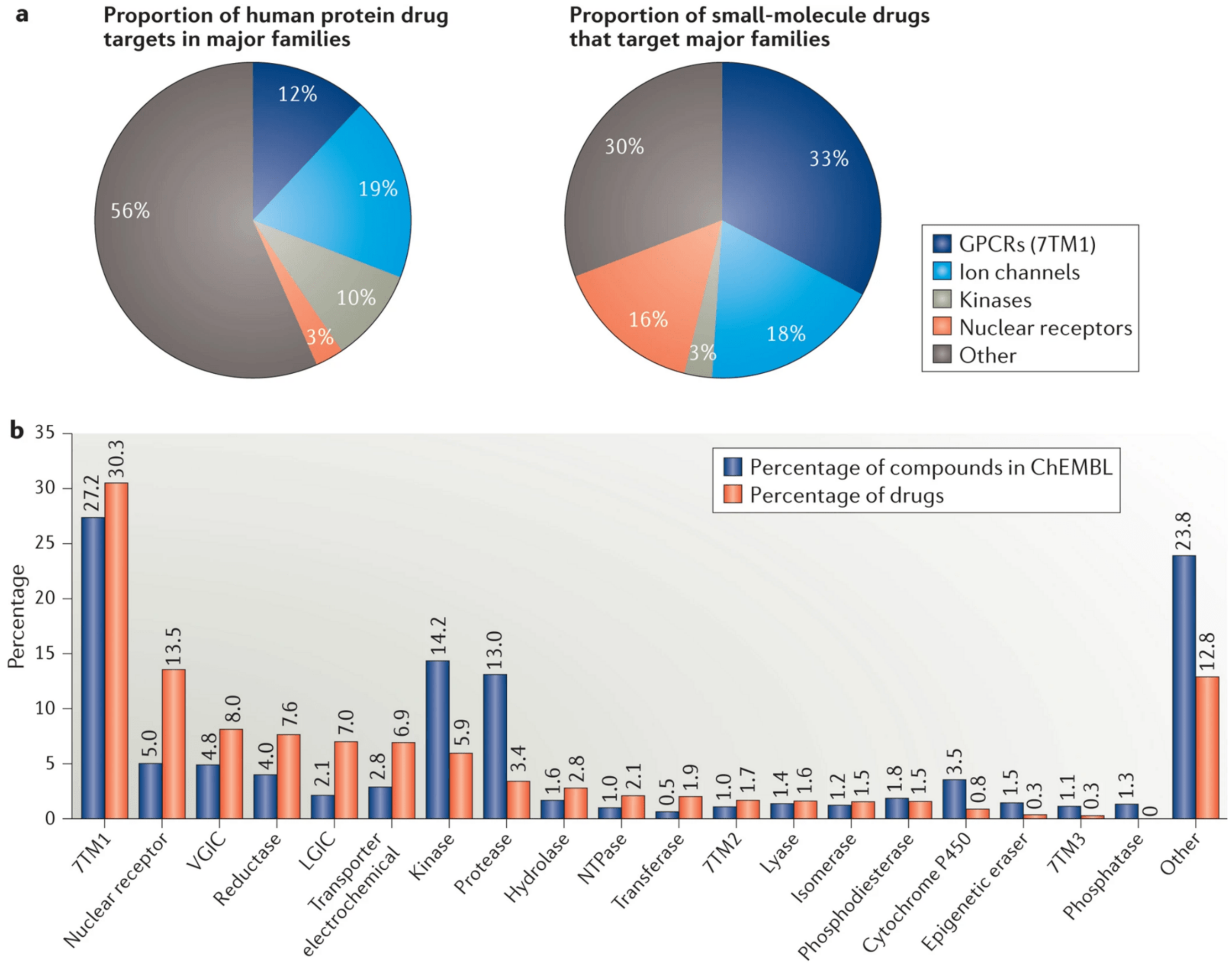
Proteins

Proteins are the primary targets of most small-molecule drugs

Over **90% of FDA-approved drugs act on proteins**—enzymes, receptors, ion channels, and transporters.

These proteins play key roles **in signaling, metabolism, immune response, and other vital functions.**

Modulating protein activity with small molecules allows us to influence biological pathways precisely.



Nature Reviews | Drug Discovery

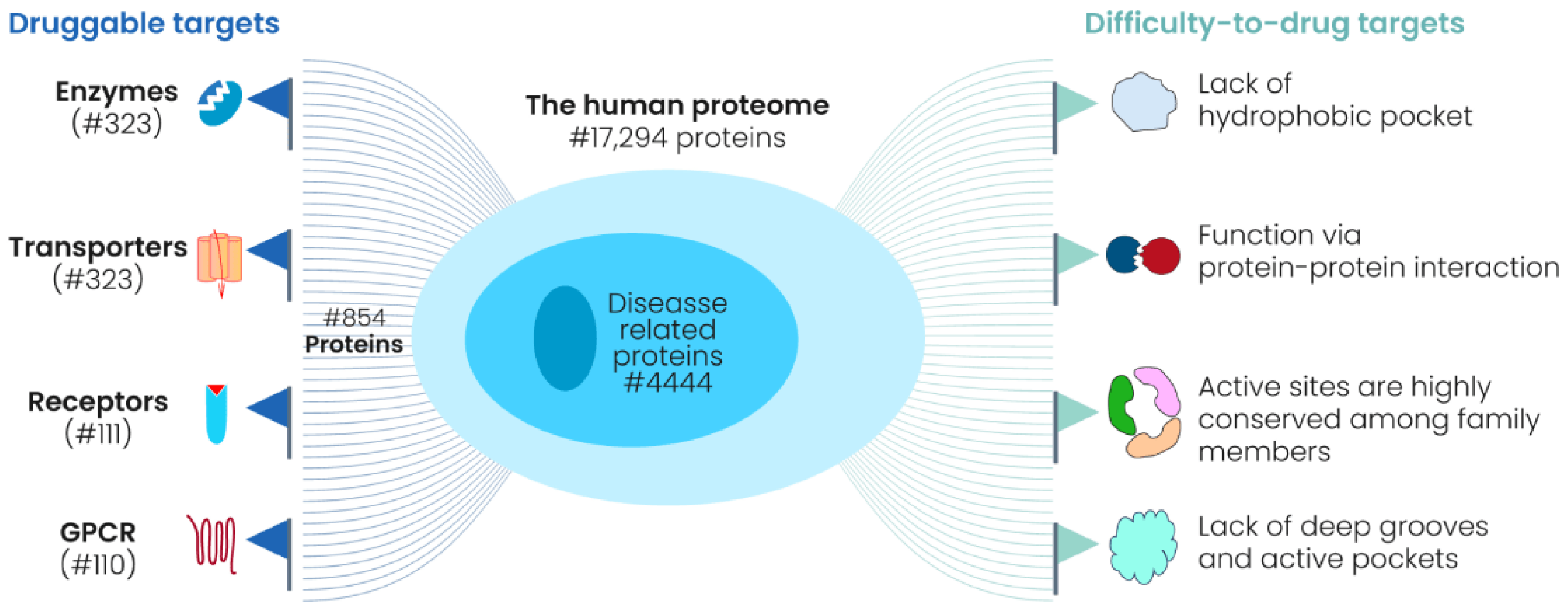
A protein is a good drug target when it is causally linked to disease

Not every protein is "druggable"—target selection must be biologically and chemically justified.

Genomics, proteomics, and phenotypic screens help identify candidate targets.

Criteria for Selecting a Protein Target

- **Disease Relevance:** The protein plays a critical role in the disease mechanism.
- **Druggability:** The target has a structure that allows it to bind with drug-like molecules.
- **Specificity:** Targeting the protein minimizes effects on healthy cells, reducing side effects.

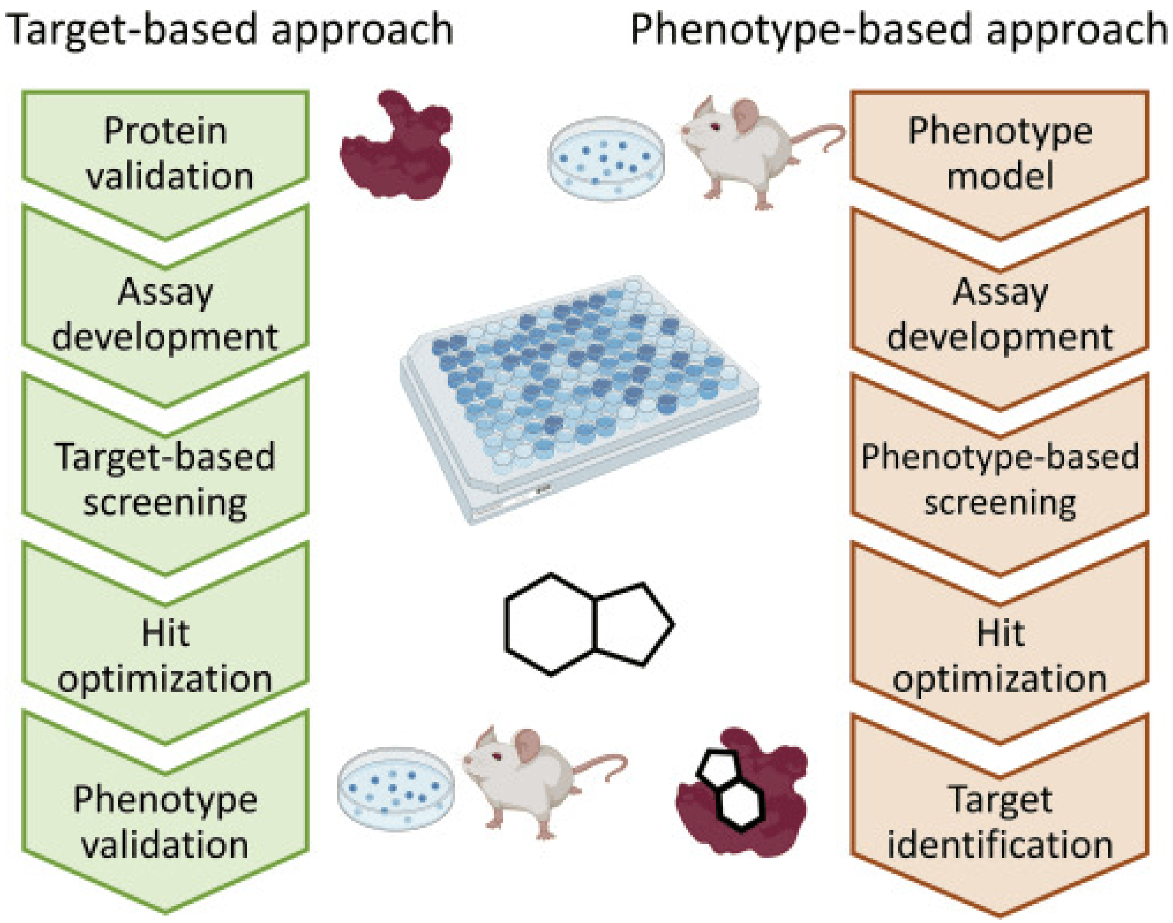

Druggable targets
Enzymes (#323)
Transporters (#323)
Receptors (#111)
GPCR (#110)
#854 Proteins
The human proteome #17,294 proteins
Diseasse related proteins #4444
Difficulty-to-drug targets
Lack of hydrophobic pocket
Function via protein-protein interaction
Active sites are highly conserved among family members
Lack of deep grooves and active pockets

Target-centric drug discovery begins with the biology, not the molecule

In **target-centric** approaches, researchers start with a well-understood protein and search for molecules that modulate its activity.

This contrasts with **phenotypic screening**, which starts with observed effects and works backward to find the target.

Target-centric methods allow rational design, structure-based modeling, and docking campaigns.



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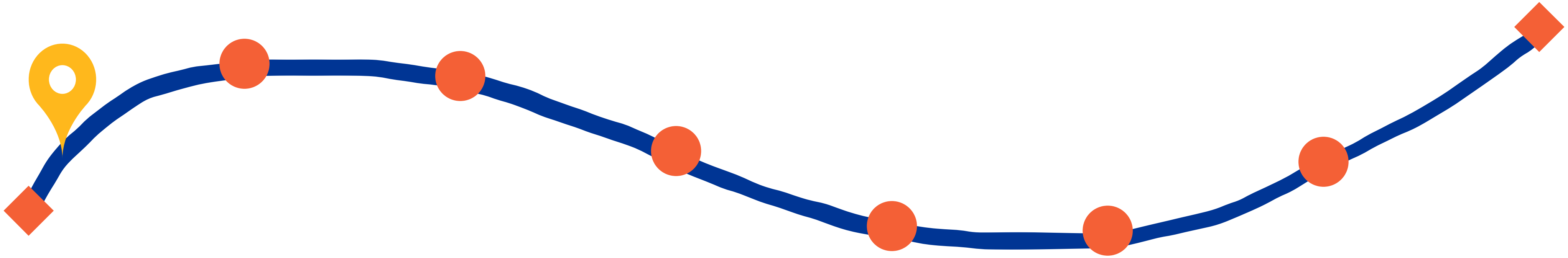


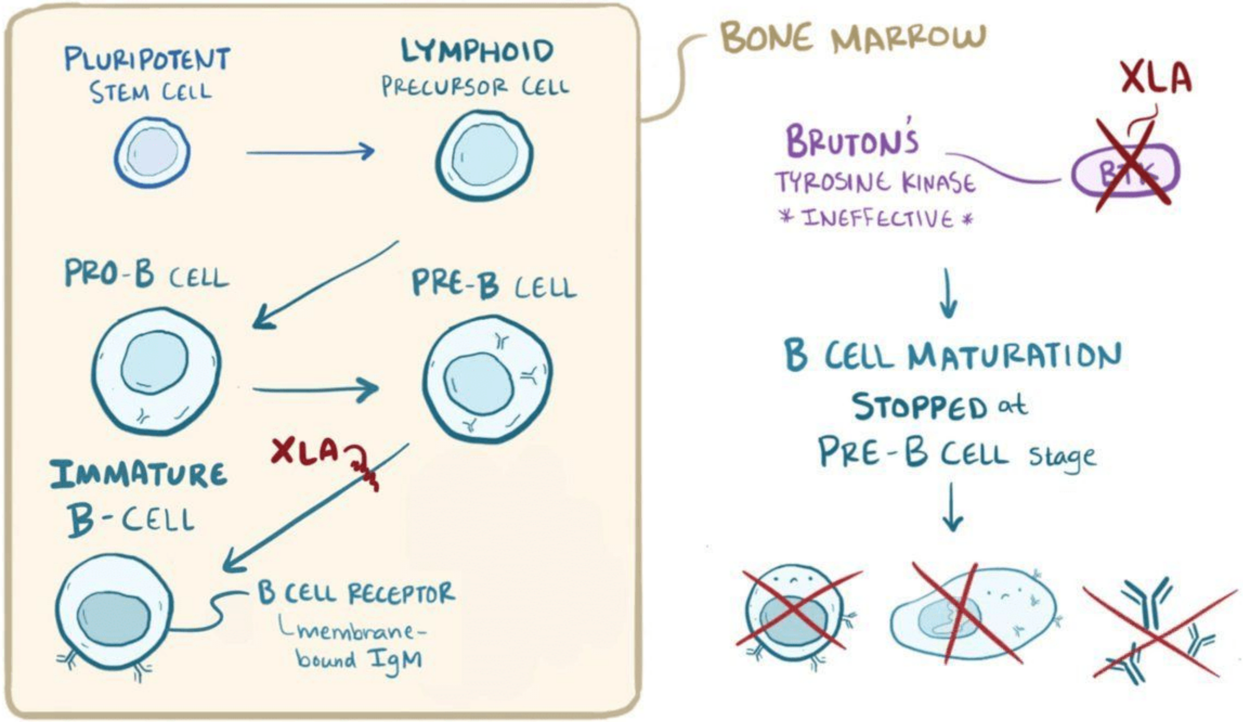
Drug targets

Bruton’s tyrosine kinase (BTK)

Identifying the right protein target is crucial for developing effective and safe drugs

Proteins regulate nearly all cellular processes and drugs can inhibit or activate proteins to correct disease states

Example: Bruton's tyrosine kinase (BTK) is a critical signaling enzyme that causes XLA, a genetic disorder marked by a severe lack of mature B cells that leads to immunodeficiency.



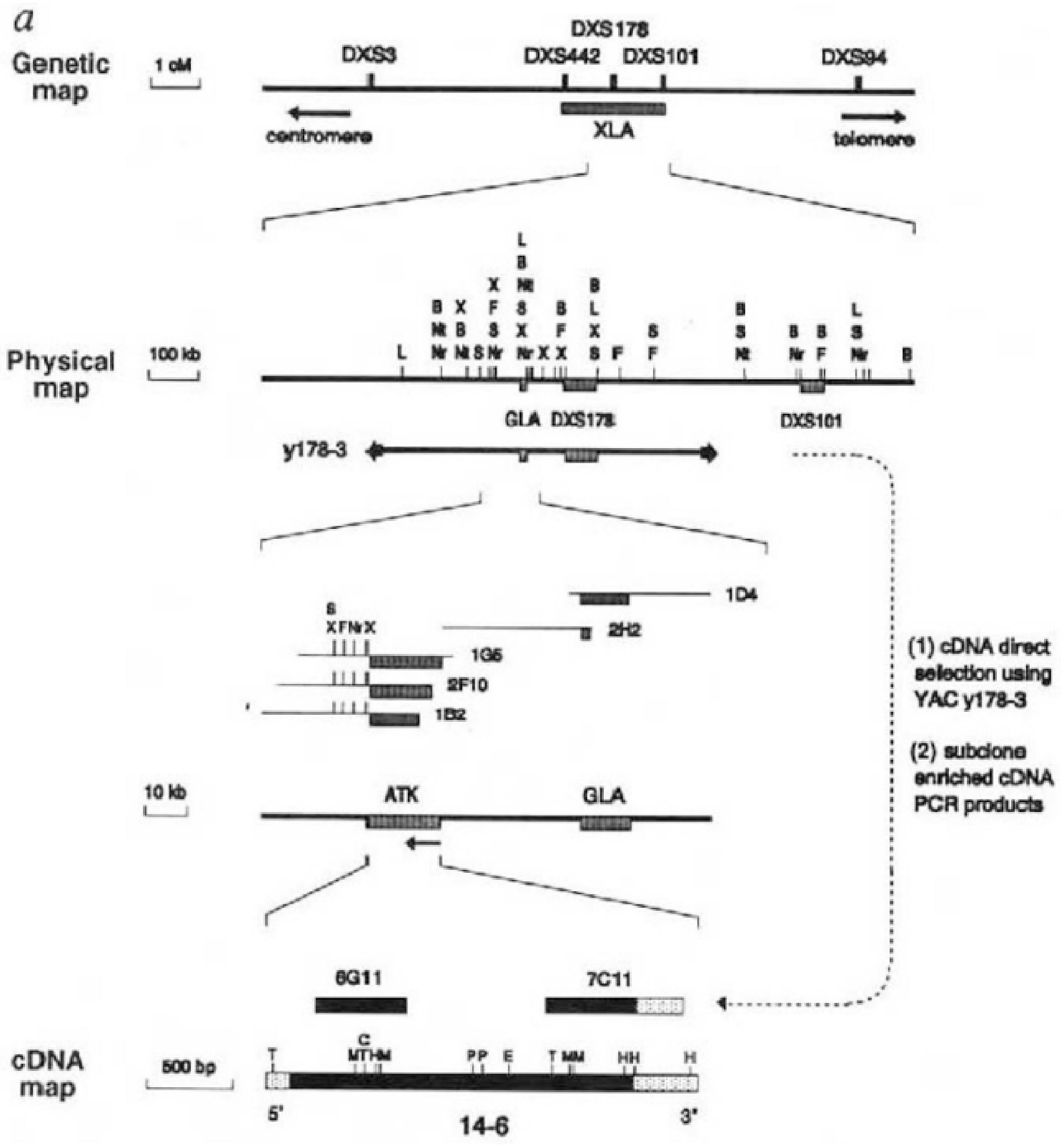
(Mohamed et al., 2009)

Positional and sequence-based analyses identified BTK as the XLA gene

Family-based genotyping of affected males revealed tight linkage between the disease and genetic markers.

Bioinformatic comparison of the novel gene’s sequence to known kinases identified conserved domains with non-receptor tyrosine kinases.

In silico analysis of XLA patient sequences showed missense mutations affecting critical residues (e.g., Lys430 in the ATP-binding site).



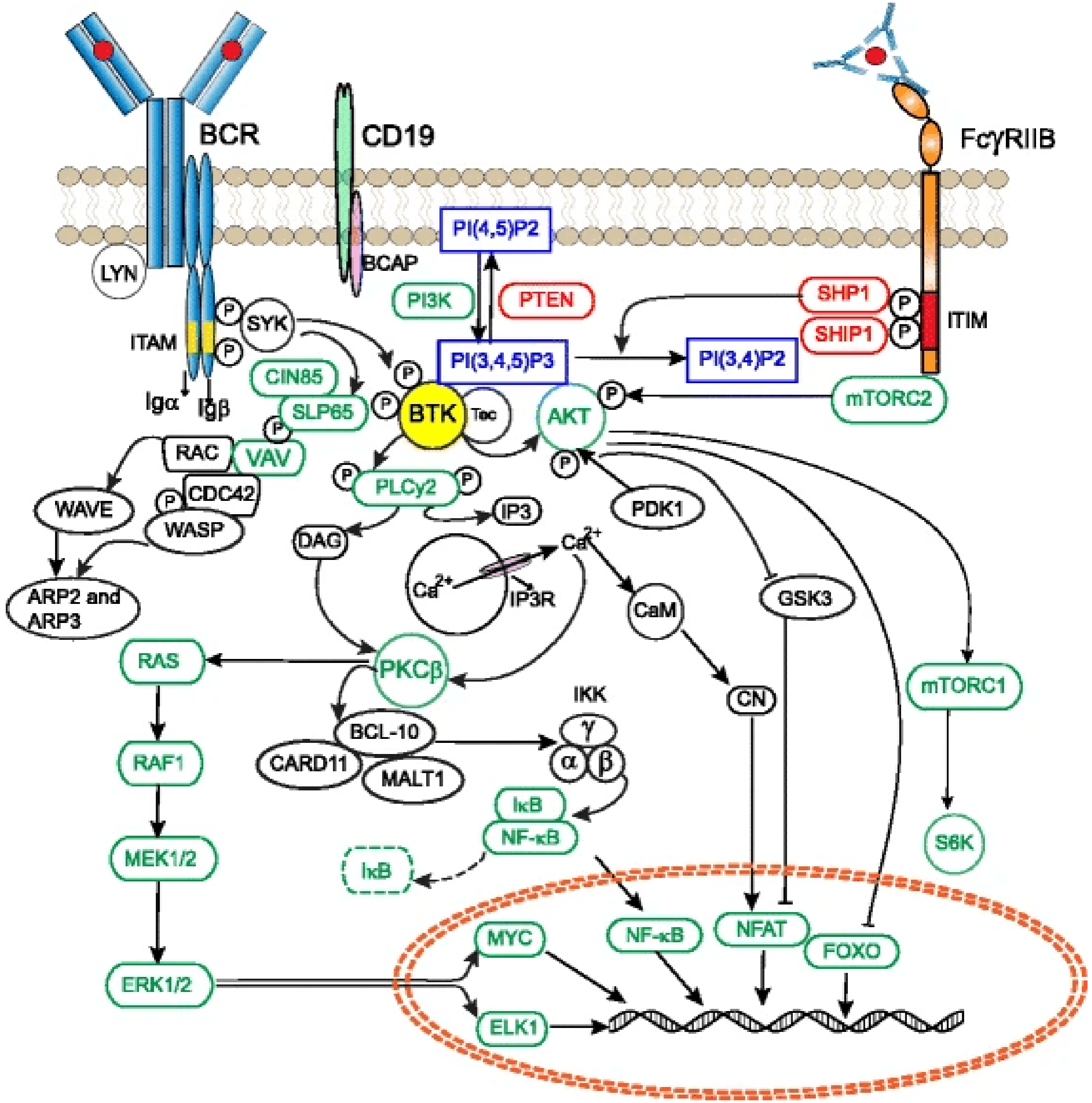
(Vetrie et al., 1993 & Tsukada et al., 1993)

Network-based analyses revealed BTK as a central hub in BCR signaling

Tyrosine phosphorylation datasets and known B-cell signaling proteins placed BTK at a convergence point of multiple BCR-related pathways

Comparative genomics shows high conservation of BTK's domains across vertebrates that are overrepresented among central signaling hubs.

(Aokl et el., 1994; Weers et al., 1994; Saouaf et al., 1994)



(Singh et al., 2018)

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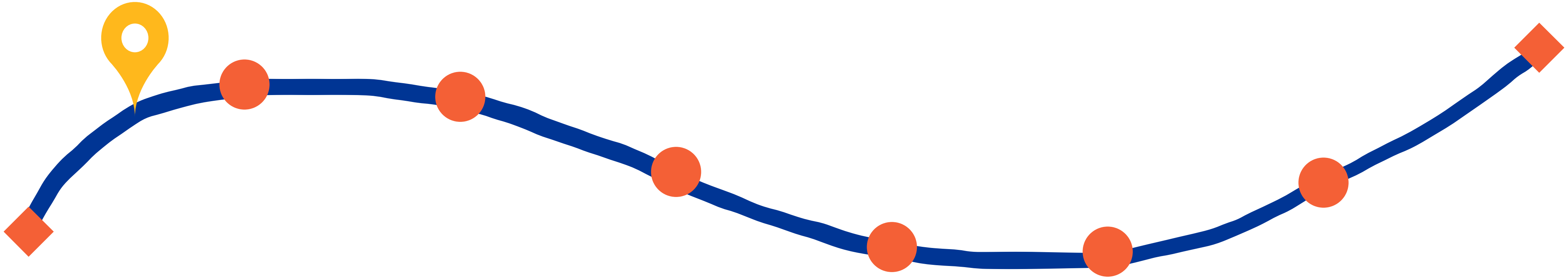
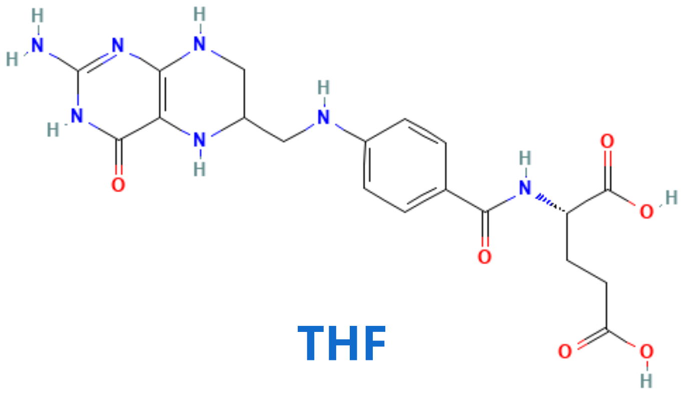


Drug targets

Dihydrofolate Reductase (DHFR)

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

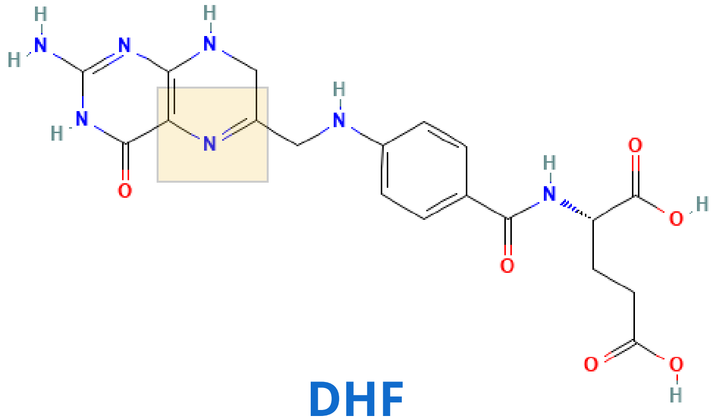
This is a useful process for drug design

Schnell et al., 2004

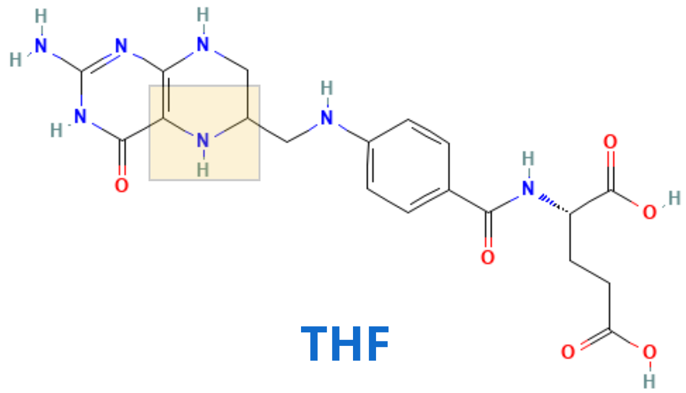
DHFR is responsible for synthesizing THF

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

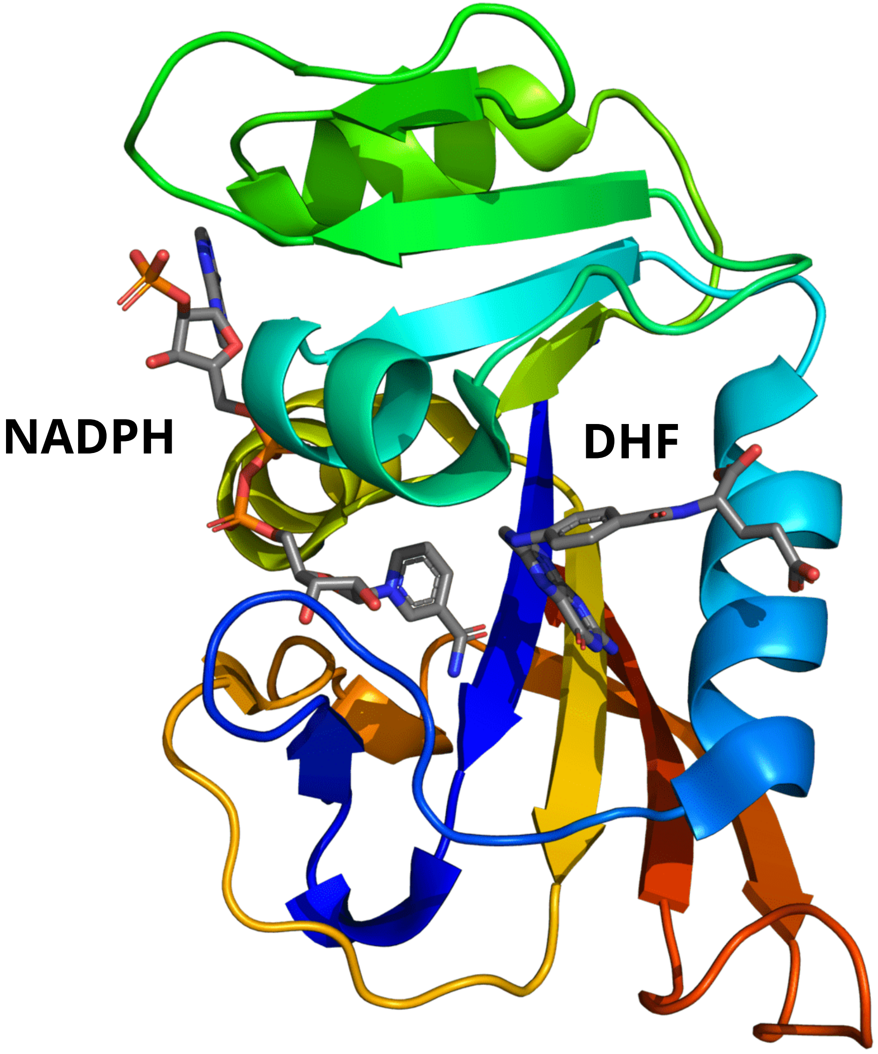
DHF + NADPH ⟶ THF + NADP(+)



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

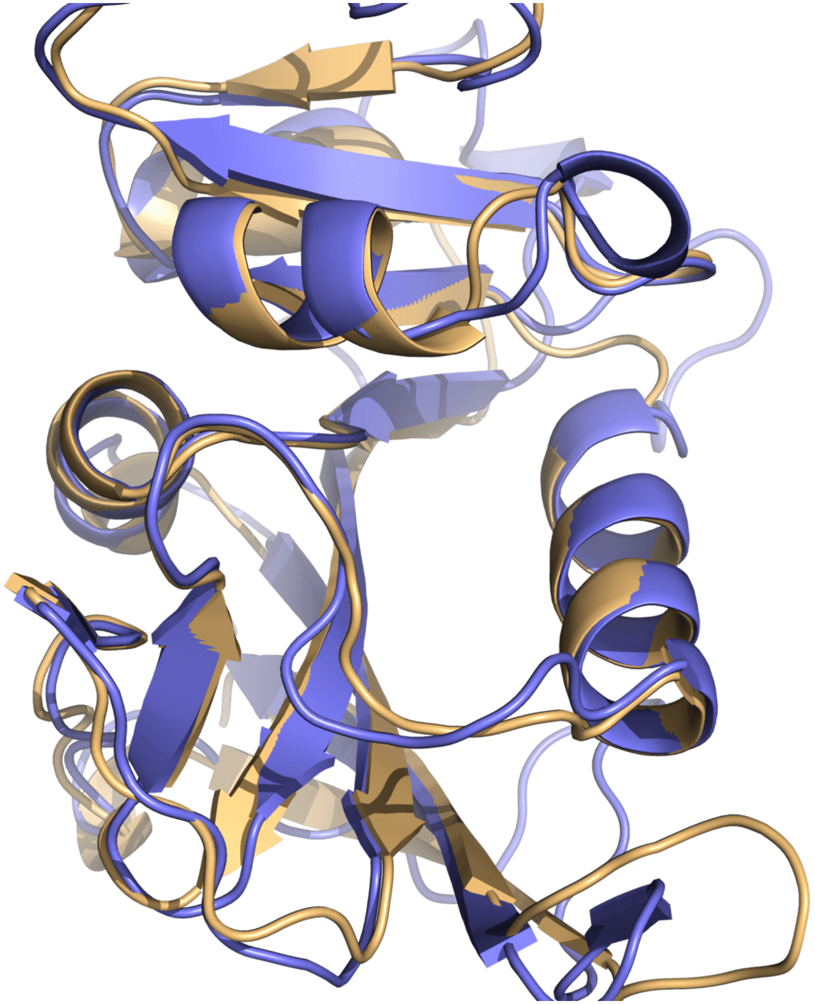
What would happen if a patient with a bacterial infection is prescribed a drug loosely targeting DHFR?

Patient could have deleterious side effects

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

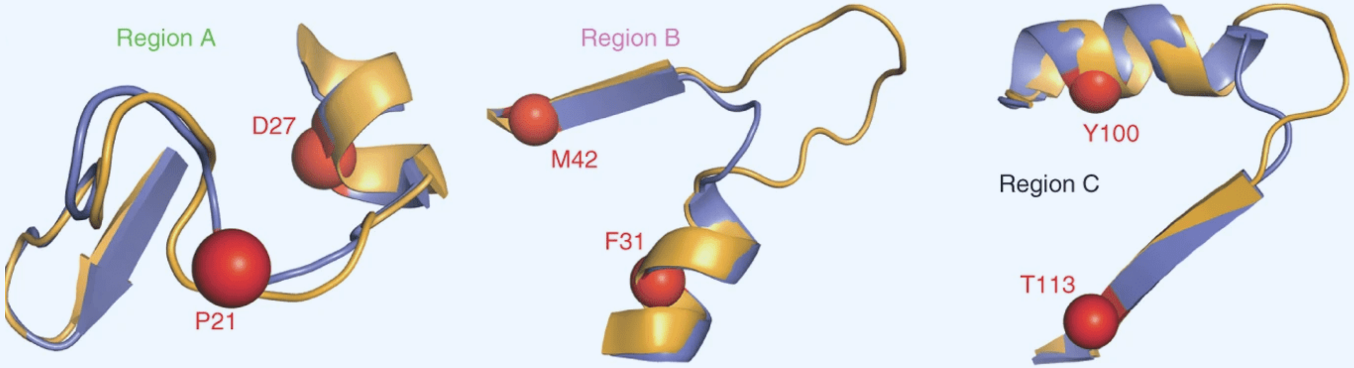
```
                                Region A        Region B
E. coli   ---MISLIAALAVDRVIGMENAMPWN-LPADLAWFKRNTLN-------KPVIMGRHTWESIG
Human     MVGSLNCIVAVSQNMGIGKNGDLPWPPLRNEFRYFQRMTTTSSVEGKQNLVIMGKKTWFSIP

E. coli   ---RPLPGRKNIILSSQPG--TDDRVTWVKSVDEAIAACG------DVPEIMVIGGGRVYEQ
Human     EKNRPLKGRINLVLSRELKEPPQGAHFLSRSLDDALKLTEQPELANKVDMVWIVGGSSVYKE
              Region C
E. coli   FL--PKAQKLYLTHIDAEVEGDTHFPDYEPDDWESVFS---EFHDADAQNSHSYCFEILERR-
Human     AMNHPGHLKLFVTRIMQDFESDTFFPEIDLEKYKLLPEYPGVLSDVQEEKGIKYKFEVYEKND
```

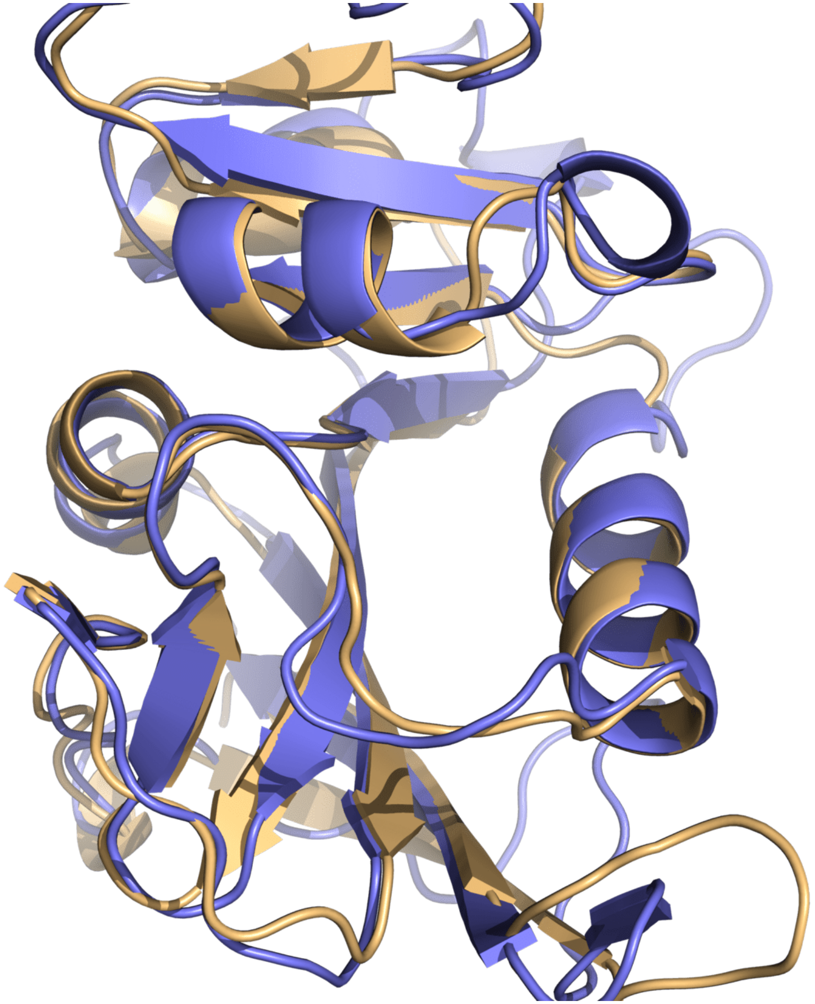


DHFR conservation complicates drug design

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

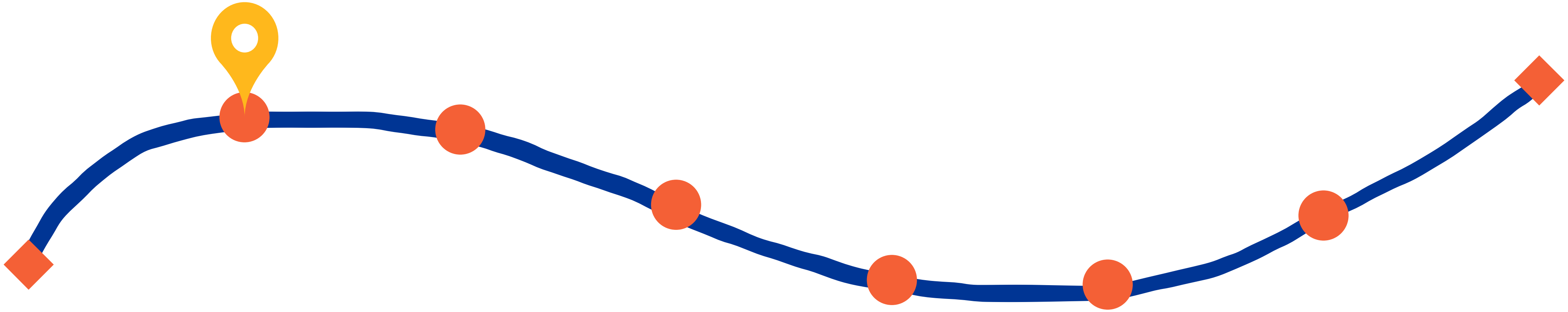


Outcome: We need to ensure drugs only bind to bacterial proteins by exploiting dynamic insights



Bhabha et al., 2013

After today, you should have a better understanding of



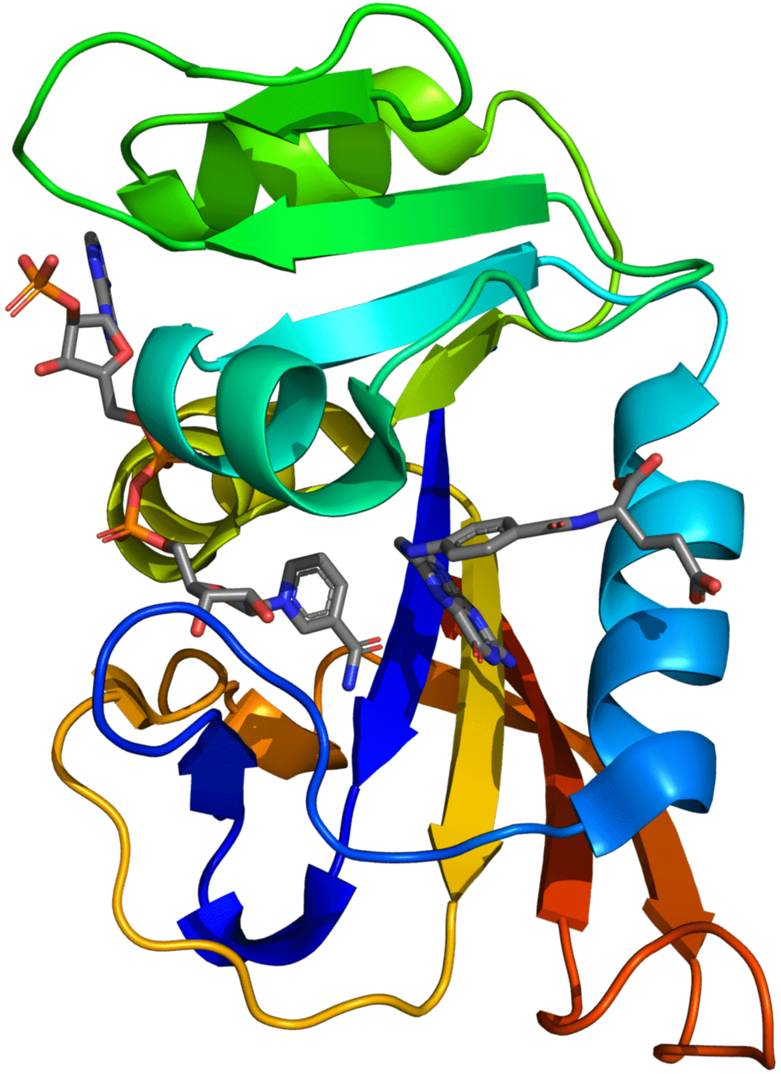
Structure-based drug design

We can modulate protein's function by binding small molecules to specific sites

A **protein's activity can be altered by binding a small molecule**—often called a ligand—to a functional site on its surface.

This interaction can **inhibit, activate, or subtly reshape the protein's behavior**.

These small molecules act like "molecular switches" that control protein action without altering the underlying gene.

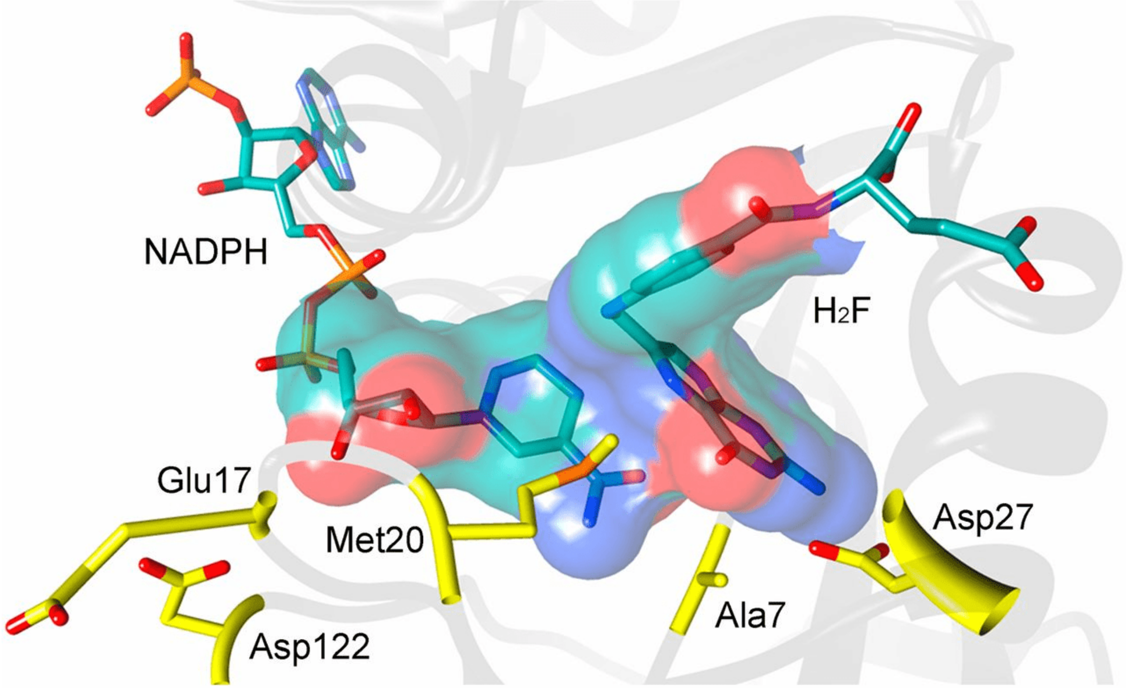


Binding affinity and specificity determine a molecule's effectiveness as a modulator

Not every small molecule that binds a protein is useful—**effective modulation depends on how tightly and selectively it binds**.

High-affinity binding ensures that a drug is effective at low doses, while
specificity minimizes off-target effects.

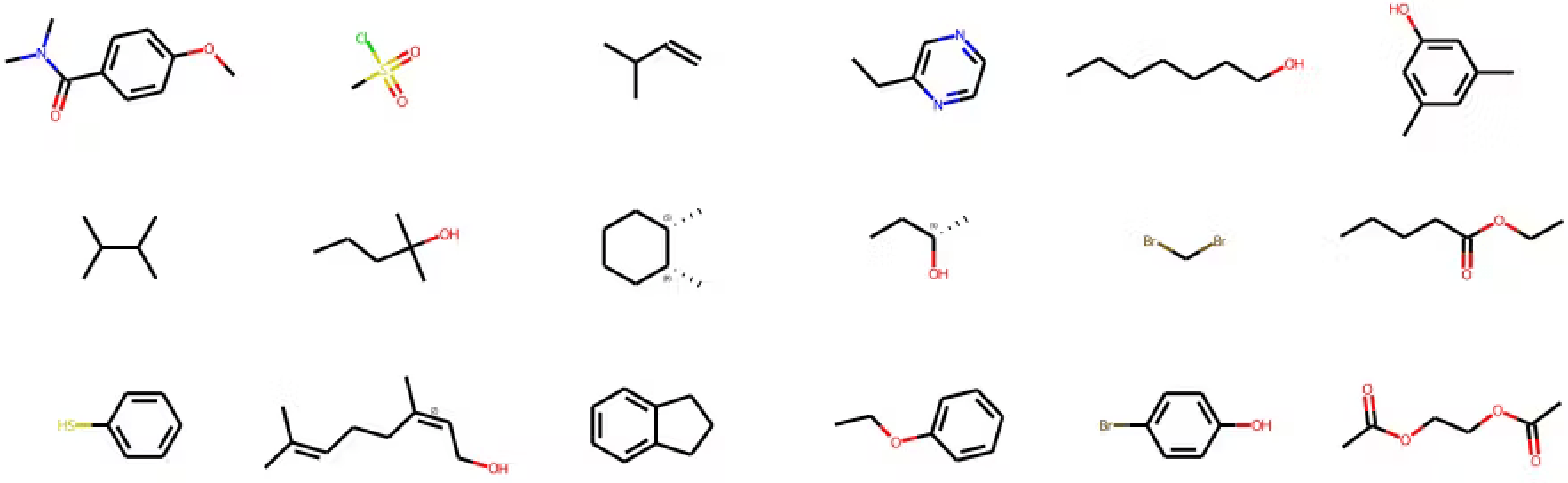
These properties can often be **optimized** through structure-based design and screening campaigns.



Critical ligand interactions in DHFR

Chemical space contains an astronomical number of possible compounds to explore

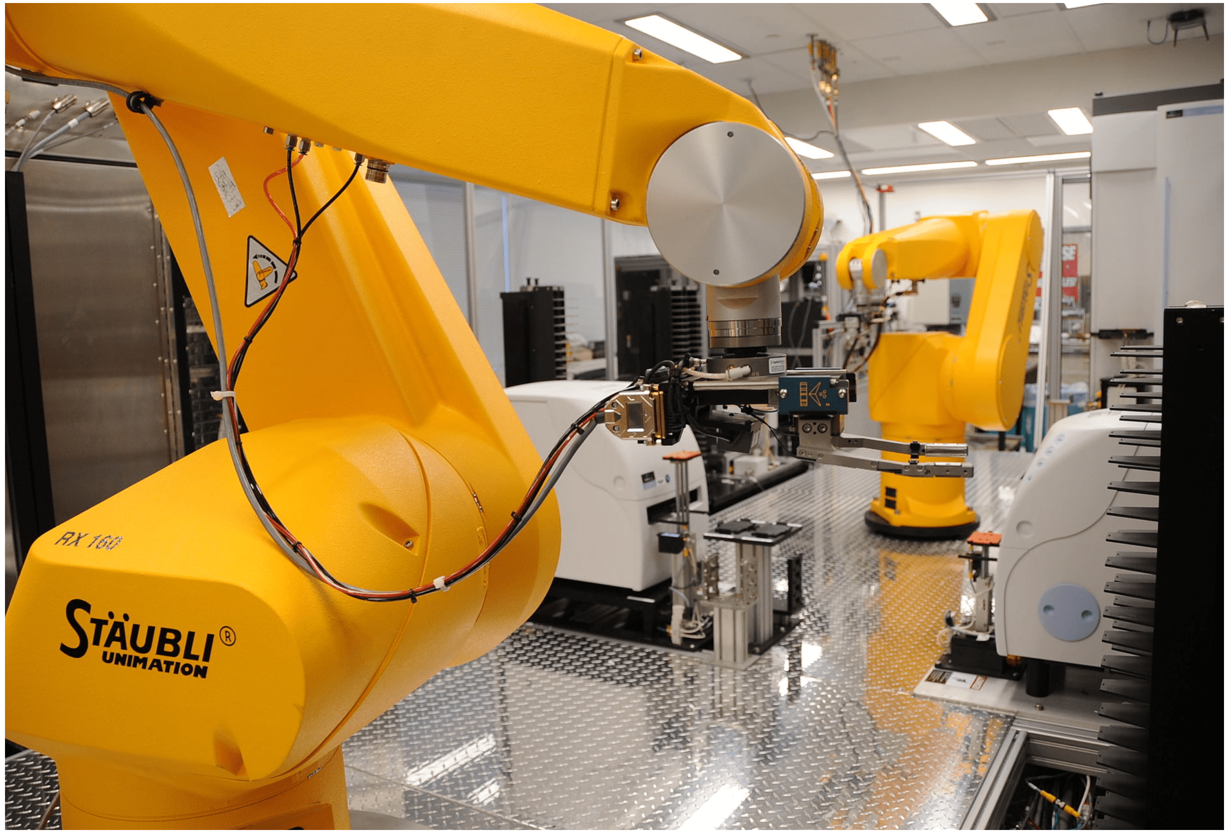
Estimated to be between 10^{60} to 10^{200} possible small organic molecules



We need methods to navigate chemical space and identify promising leads accurately and efficiently

High-throughput screening (HTS) allows testing of thousands of compounds against the target protein

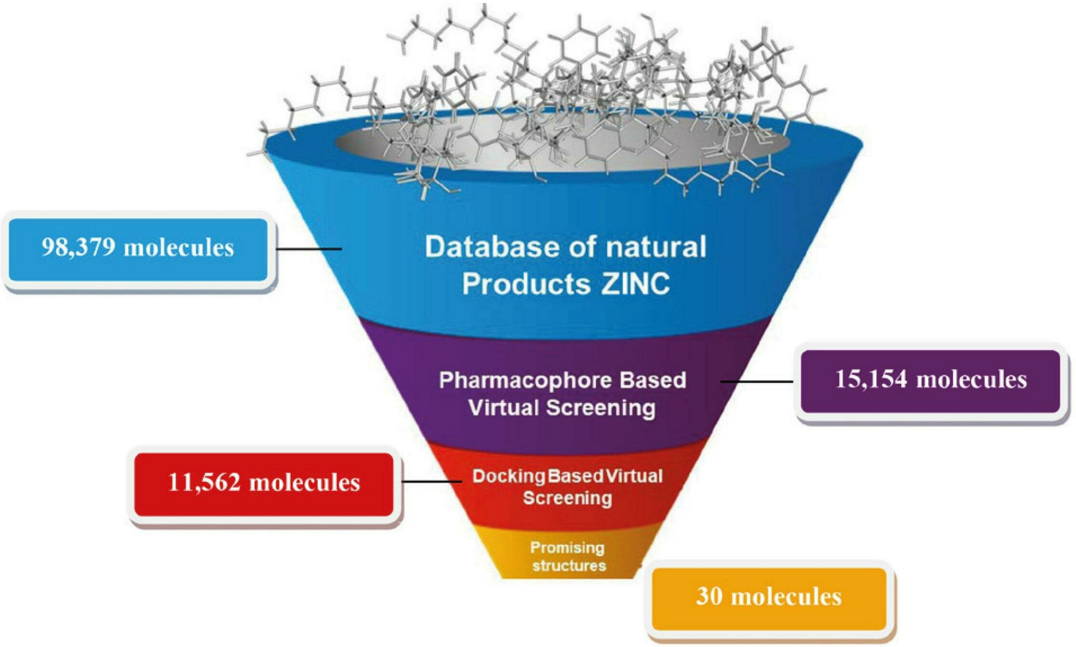
- **Library Preparation:** Collection of diverse compounds
- **Assay Development:** Design of biological assays to measure compound activity against the target
- **Screening:** Compounds are tested in miniaturized assays
- **Data Analysis:** Identification of "hits" that show desired activity



Virtual screening evaluates vast libraries to identify potential leads efficiently

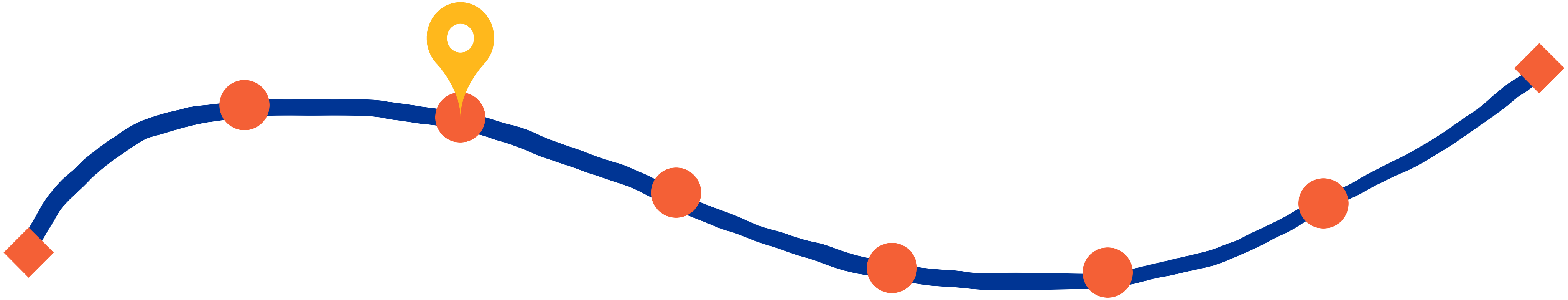
Experimental assays are still expensive, and limited to commercially available compounds

Instead, we can use **computational methods** to predict which compounds we should experimental validate


98,379 molecules
Database of natural Products ZINC
Pharmacophore Based Virtual Screening
15,154 molecules
11,562 molecules
Docking Based Virtual Screening
Promising structures
30 molecules

Can screen millions to billions of compounds *in silico,* thereby dramatically **expanding our search space**

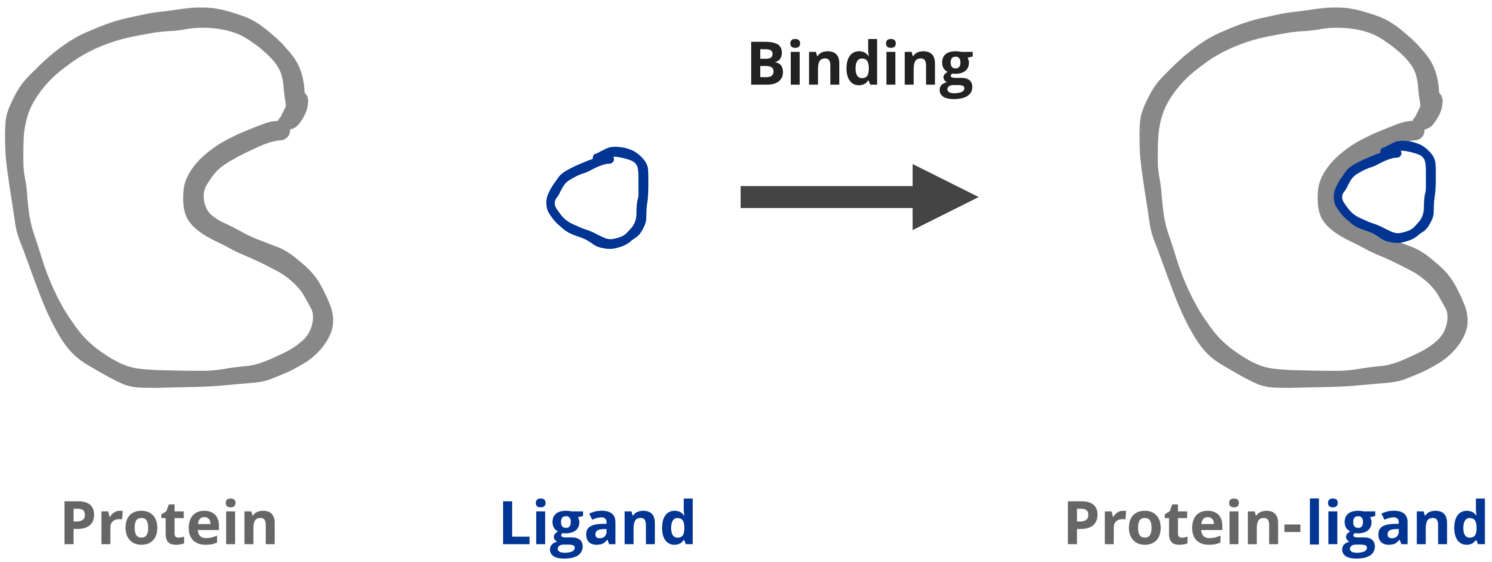
After today, you should have a better understanding of



Thermodynamics of binding

Selective binding to a protein is governed by thermodynamics (and kinetics)

Binding occurs when a compound/ligand interacts specifically with a protein



We can model this as a reversible protein-ligand binding

$$P + L \leftrightharpoons PL$$

Gibbs free energy combines enthalpy and entropy

$$\Delta G_{bind} = \Delta H_{bind} - T\Delta S_{bind}$$

Enthalpy

$$\Delta H_{bind}$$

Accounts for energetic interactions

Entropy

$$\Delta S_{bind}$$

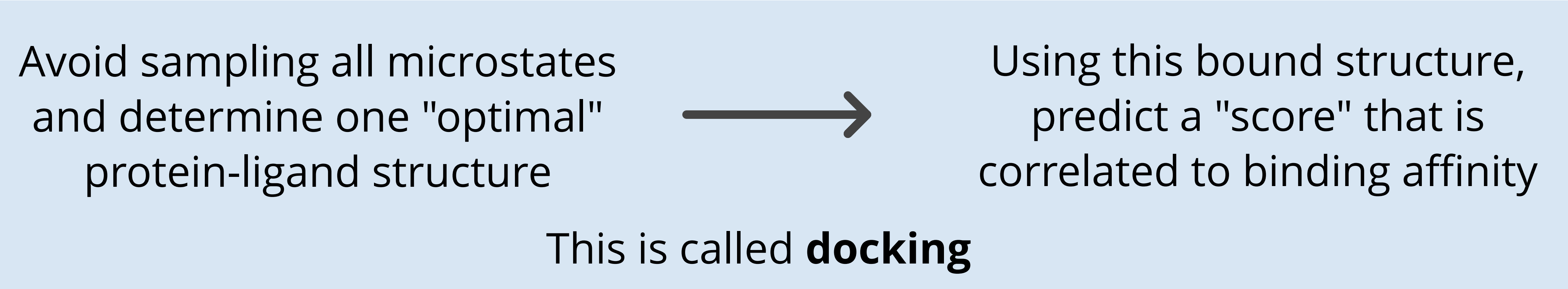
How much conformational flexibility changes

By predicting the free energy of binding, we can identify small molecules with high affinity to our drug target

Accurate and efficient binding predictions are essential

Objective: Directly predict binding affinity from protein and ligand structures with high accuracy and minimal computational resources.

We can carefully simplify our modeling to improve speed with (hopefully) minimal impact to accuracy



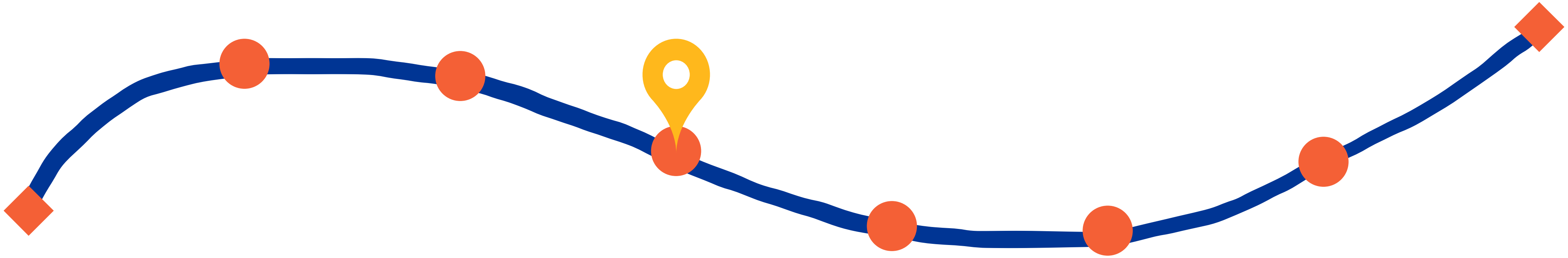
Avoid sampling all microstates and determine one "optimal" protein-ligand structure

Using this bound structure, predict a "score" that is correlated to binding affinity

This is called **docking**

Docking simplifies the binding free energy prediction problem to enhance speed

After today, you should have a better understanding of



Identifying relevant protein conformations

Accurate, reproducible docking requires a relevant protein conformation

Docking still considers the protein structure, but we only select one

Significance of Protein Conformation in Docking

- Protein-ligand interactions are highly dependent on the protein's 3D structure.
- Using an rare protein conformation can lead to inaccurate docking results.

Sources of Protein Conformational Data

Experimental Methods

X-ray Crystallography: Provides high-resolution structures but may miss dynamic conformations.

NMR Spectroscopy: Captures ensembles of conformations but is limited to smaller proteins.

Computational Techniques

- **Molecular Dynamics (MD) Simulations**: Explore the conformational space over time.
- **Normal Mode Analysis (NMA)**: Identifies collective motions in proteins.
- **Ensemble Generation Methods**: Generate multiple protein conformations for docking.

Will discuss these in L14

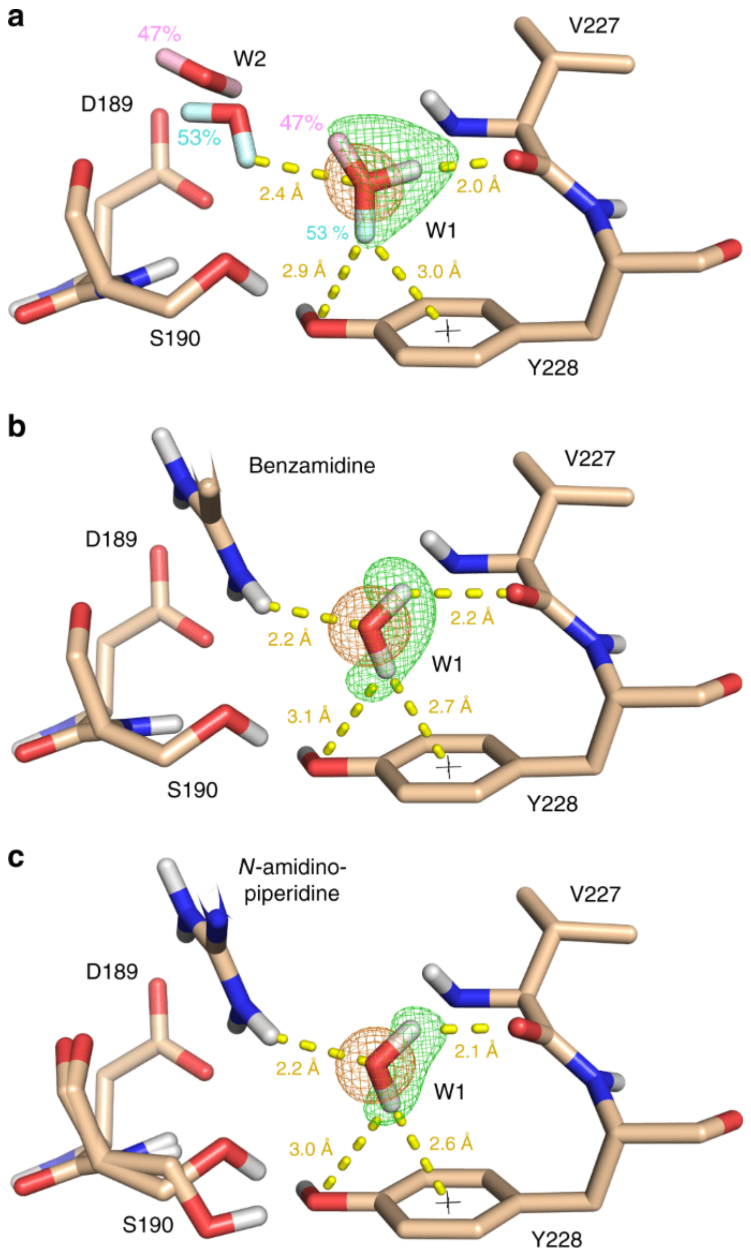
Importance of Water Molecules

Role in Binding: Structured water molecules can mediate interactions between the protein and ligand.

Inclusion Criteria: Retain water molecules that are conserved across multiple crystal structures.

Handling Water in Docking

- Some docking programs allow explicit water molecules in the binding site.
- Alternatively, consider their effect implicitly in scoring functions.



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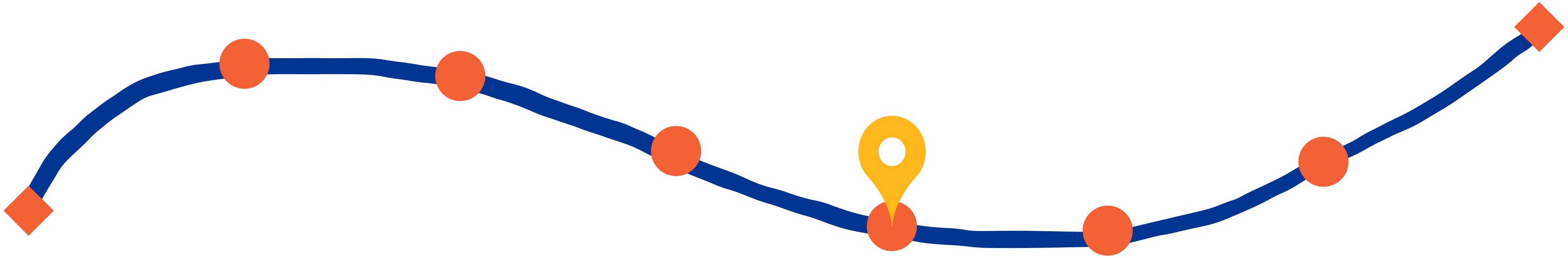


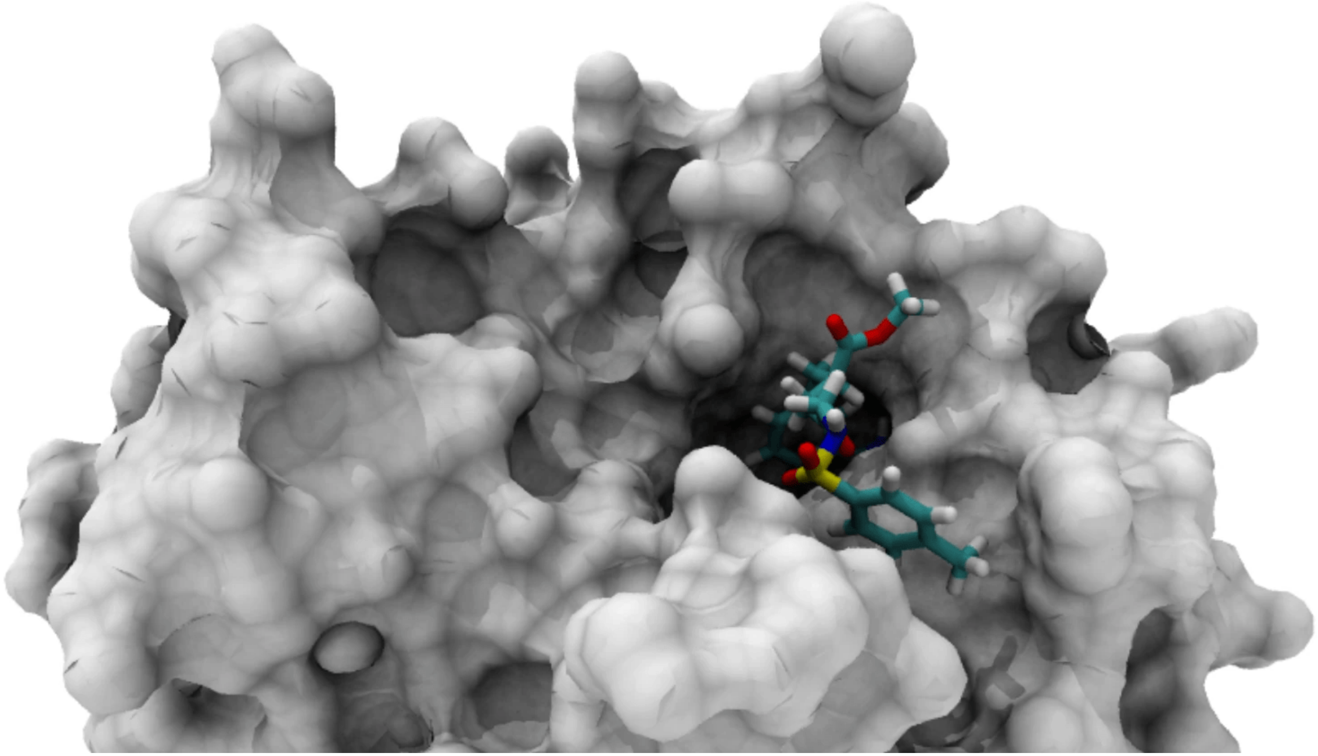
Binding pockets

Types

Accurate Binding Pocket Detection is Crucial for Docking

The binding pocket is the specific region where a ligand interacts with a protein

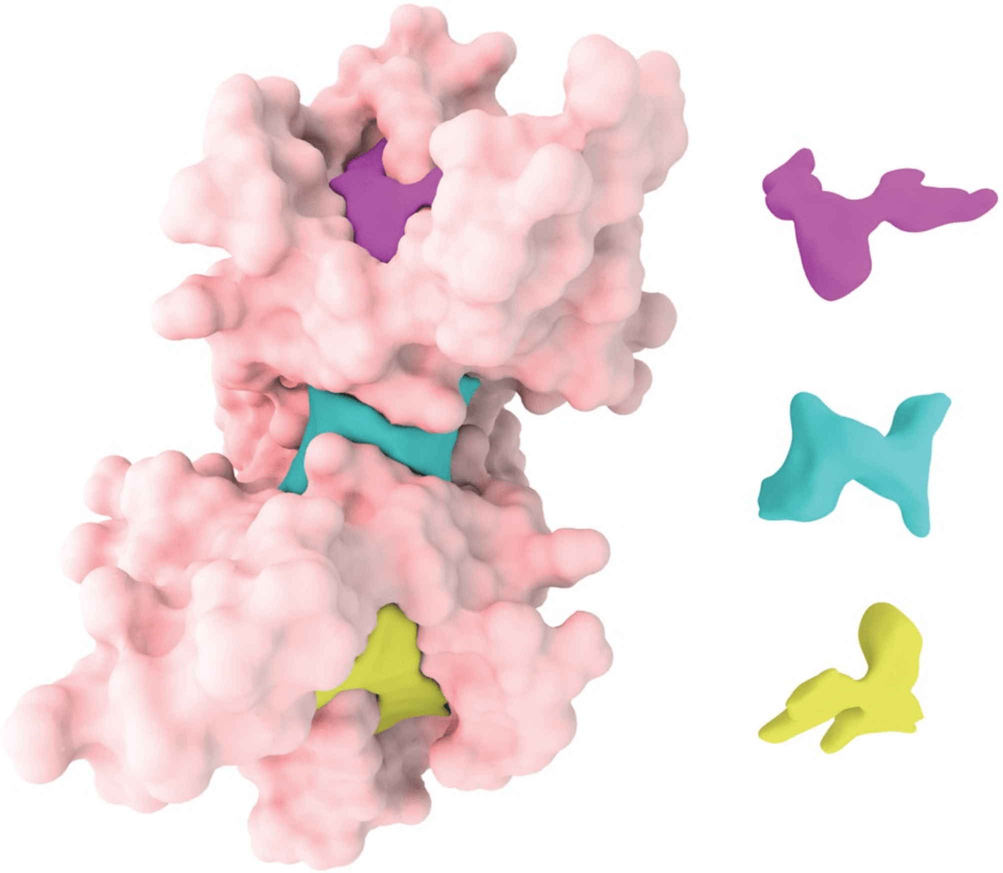
Accurate identification of binding pockets is essential for successful docking and virtual screening.



Understanding Protein Surface Topography

Binding Pocket: A cavity that can accommodate a ligand.

Active Site: The functional region where biochemical reactions occur (often a binding pocket in enzymes).



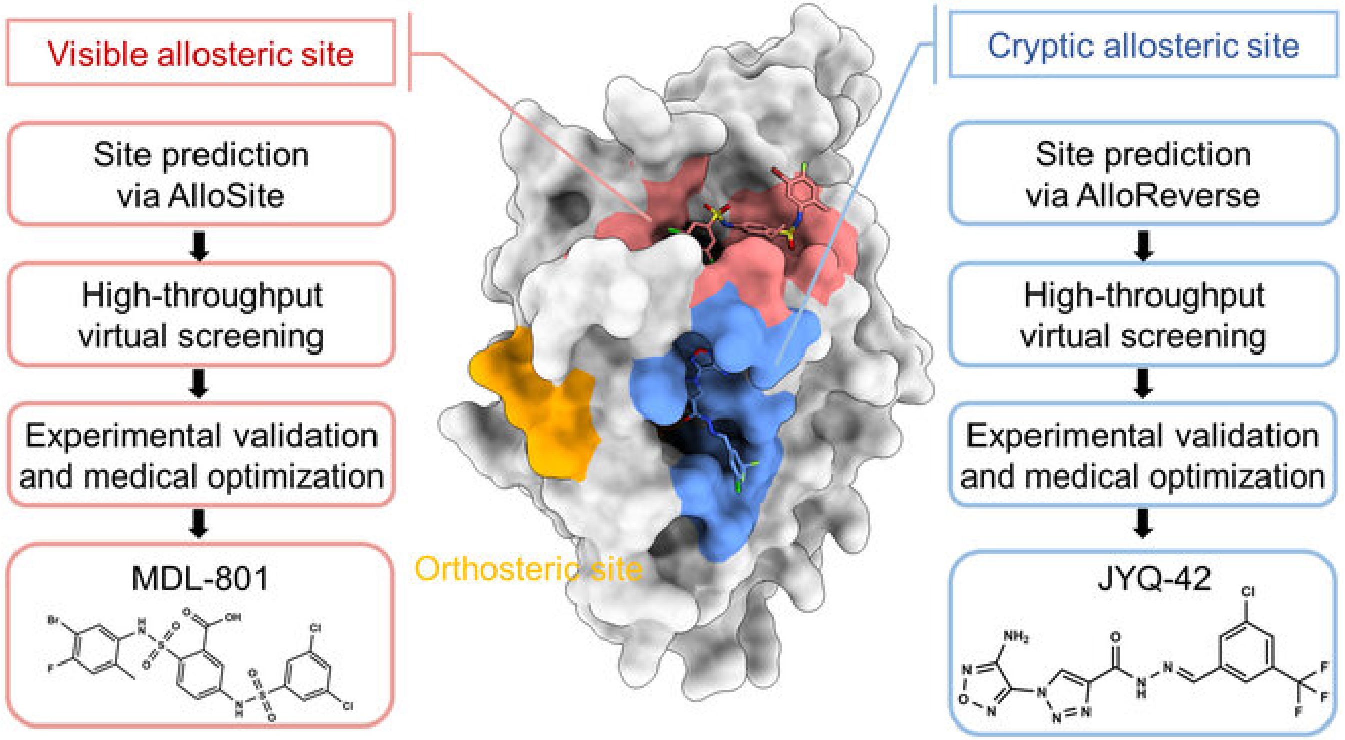
Protein Surface Characteristics

- **Convex Regions**: Typically inaccessible to ligands.
- **Concave Regions (Cavities)**: Potential binding sites.

Binding pockets are classified by location, accessibility, and regulatory function

Orthosteric sites are the natural binding sites of endogenous ligands or substrates

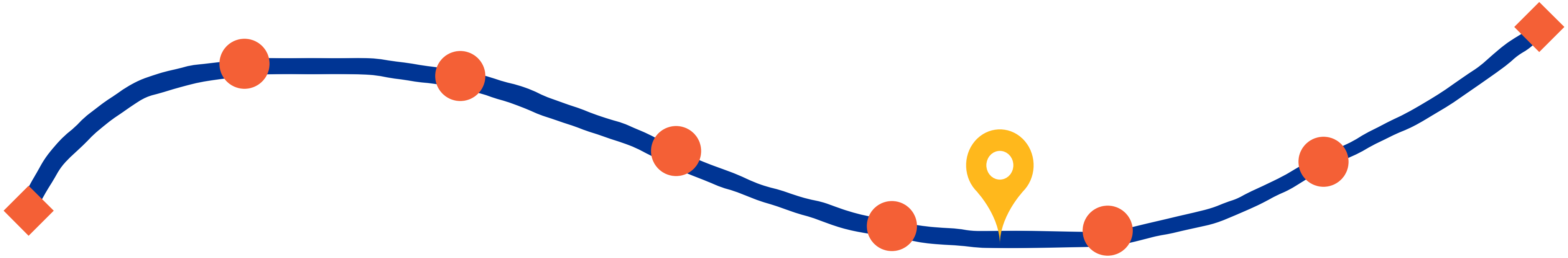
Allosteric sites are spatially distinct from the orthosteric site and modulate protein activity indirectly.



Visible allosteric site
Site prediction via AlloSite
High-throughput virtual screening
Experimental validation and medical optimization
MDL-801
Br
F
OH
Cl
Cryptic allosteric site
Site prediction via AlloReverse
High-throughput virtual screening
Experimental validation and medical optimization
JYQ-42
NH2
Cl
F
Orthosteric site

Cryptic Sites: Binding pockets not apparent in the unbound protein structure but form upon ligand binding or conformational change.

After today, you should have a better understanding of

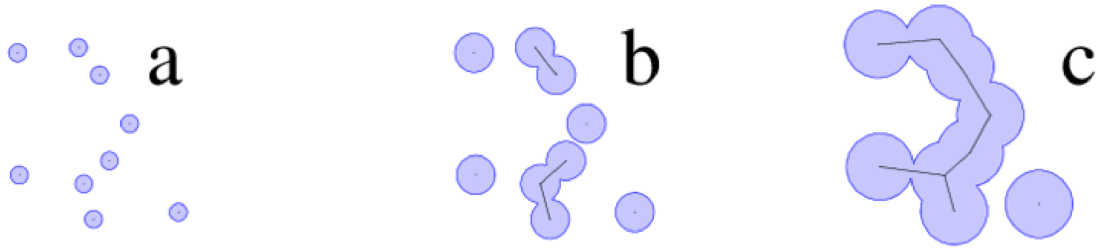


Binding pockets

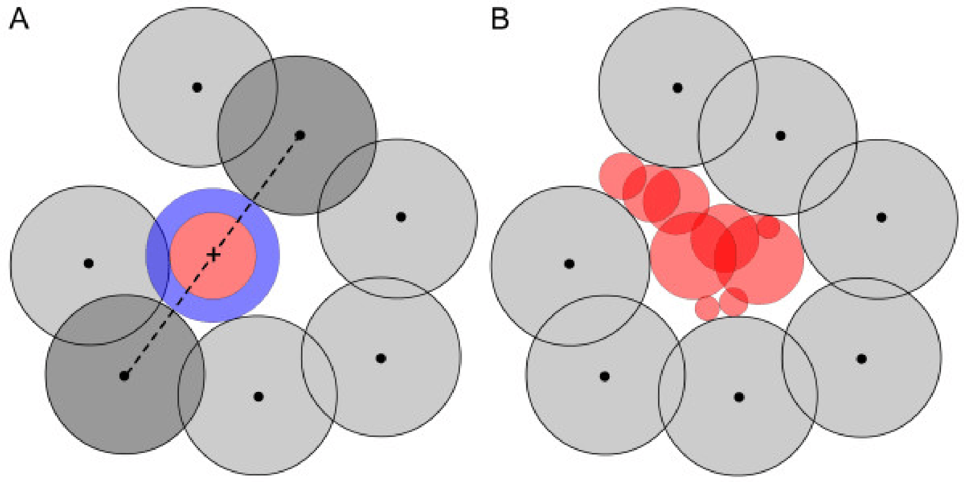
Detection

Alpha shapes detect pockets by reconstructing molecular surface topology

Alpha shapes extend the idea of a convex hull to capture the "shape" of a protein surface with cavities and tunnels.



Think of shrinking a sphere around atom centers—**small alpha values allow more detailed surface features to be resolved**.



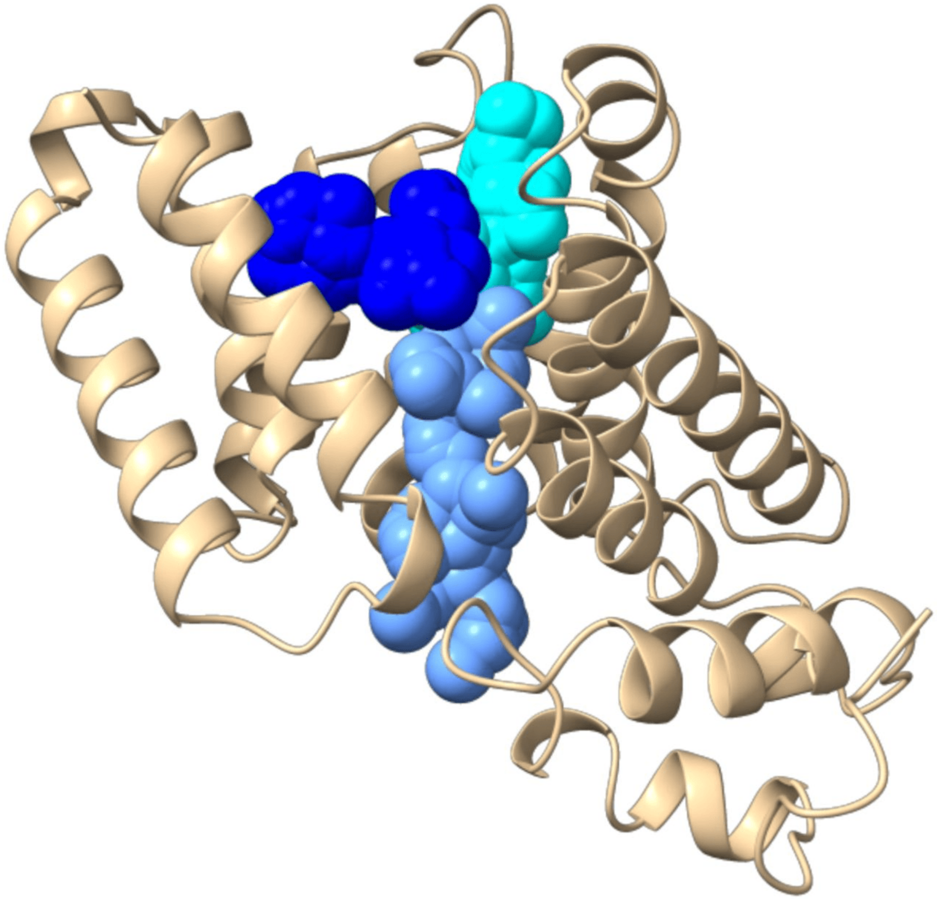
Cavities enclosed by the alpha shape can be interpreted as binding pockets.

Alpha shape–based methods reveal pockets as topological voids

Proteins are modeled as a union of spheres (atoms), and the alpha shape filters through those to reveal cavities.

The algorithm identifies **pocket volume**, **enclosure**, and **surface accessibility**—critical for ligand fit.

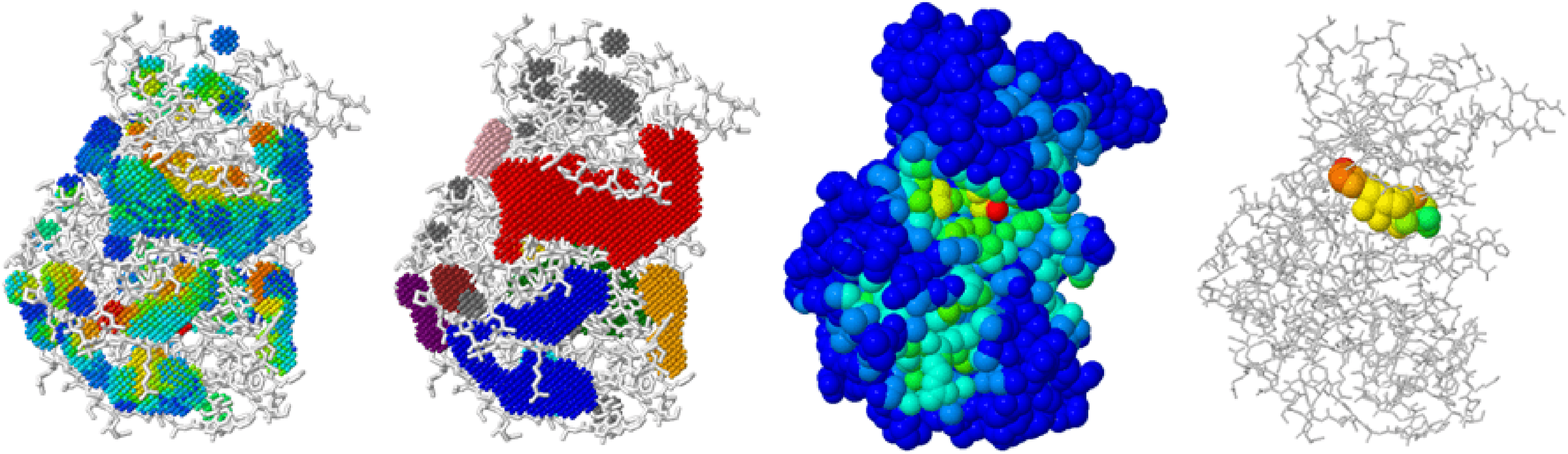
These pockets are purely **geometry-based**, independent of electrostatics or residue type.



Grid-based methods map the protein onto a 3D lattice to identify cavities

The protein is embedded in a 3D grid, and each voxel is labeled as **protein**, **solvent**, or **cavity**.

Grid points near the surface but not occupied by protein atoms are clustered into potential pockets.



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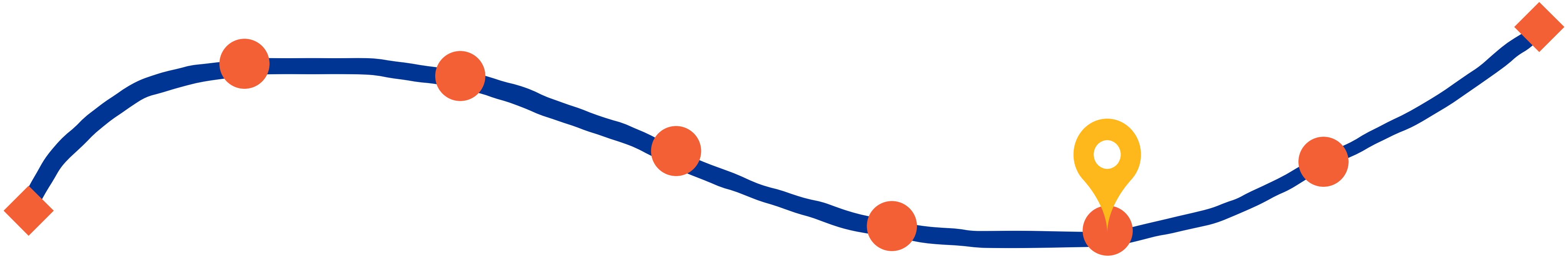
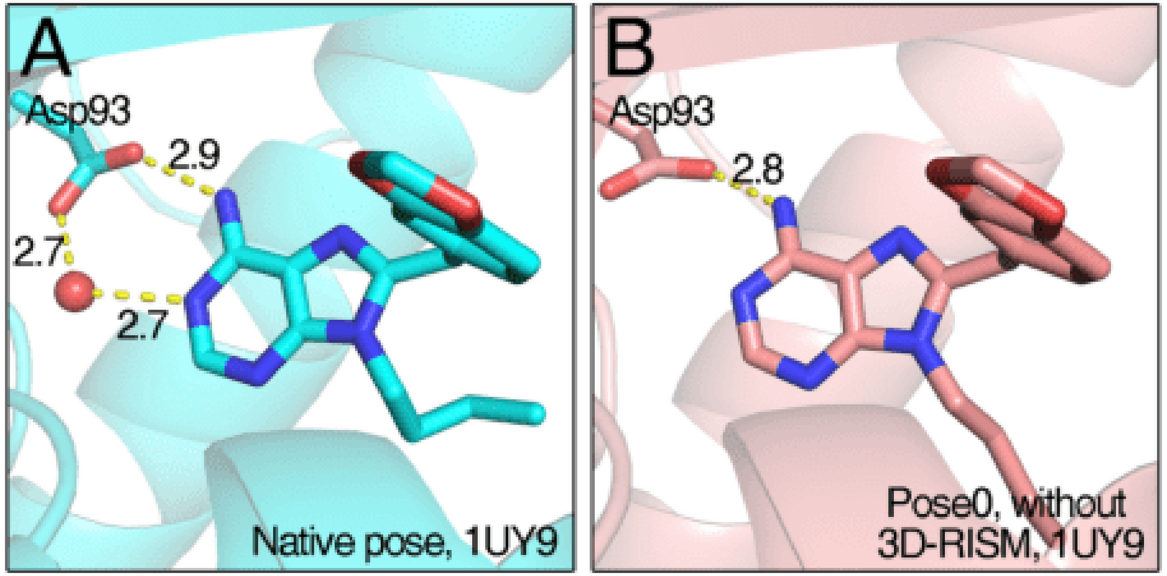


Scoring functions

Scoring functions estimate how well a ligand binds to a protein

Scoring functions assign a numerical value to a protein-ligand pose, reflecting its favorability.

The best pose maximizes favorable interactions (e.g., hydrogen bonds, hydrophobic packing) and minimizes clashes or strain.

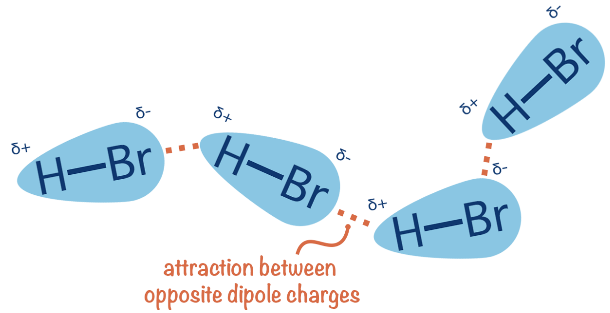


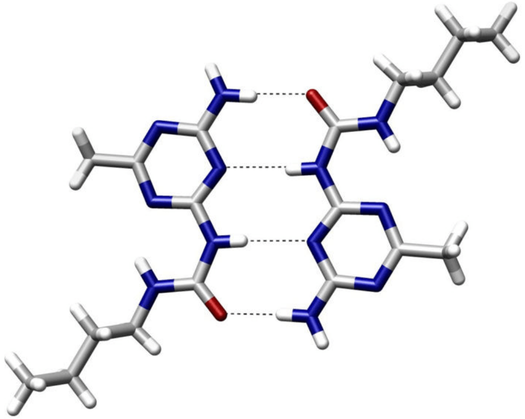
Scoring functions attempt to approximate this balance using simplified models.

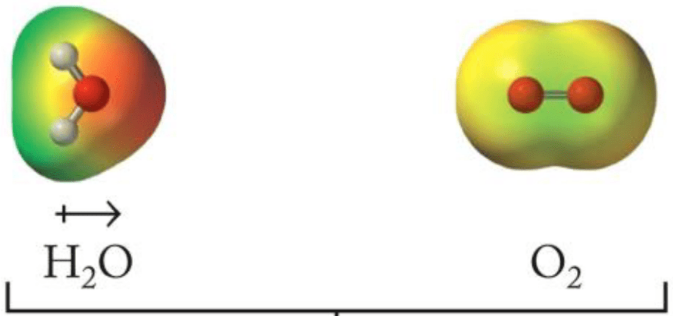
A good scoring function may rank correctly even if absolute energies are wrong.

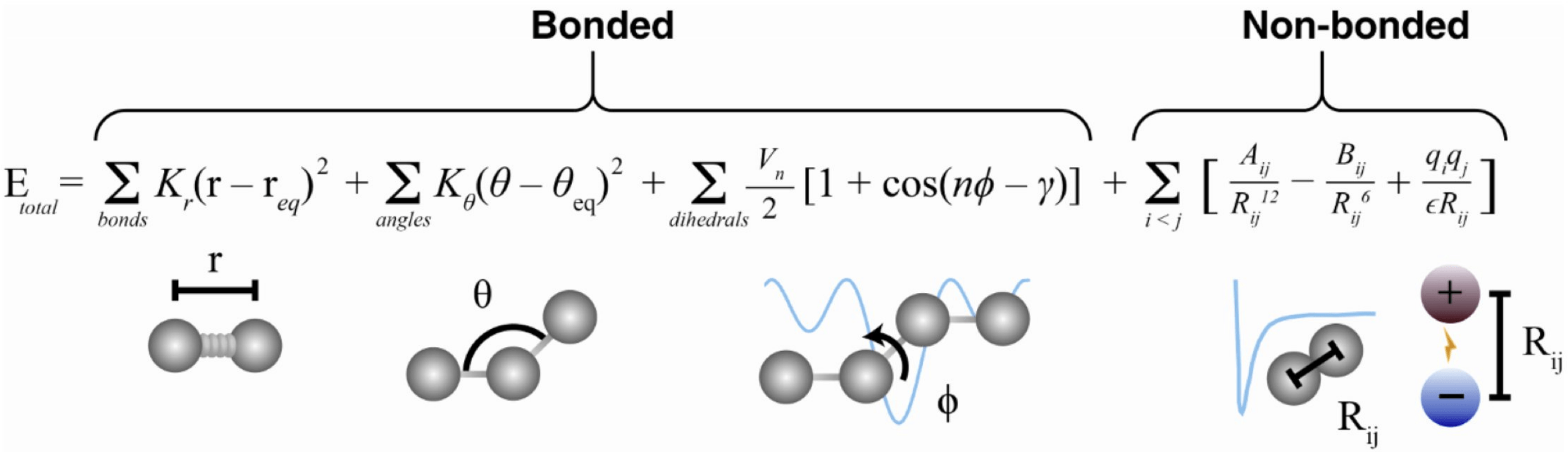
Force-field-based scoring calculates interactions from first principles

They consider van der Waals attraction/repulsion, electrostatics, and sometimes torsional strain.









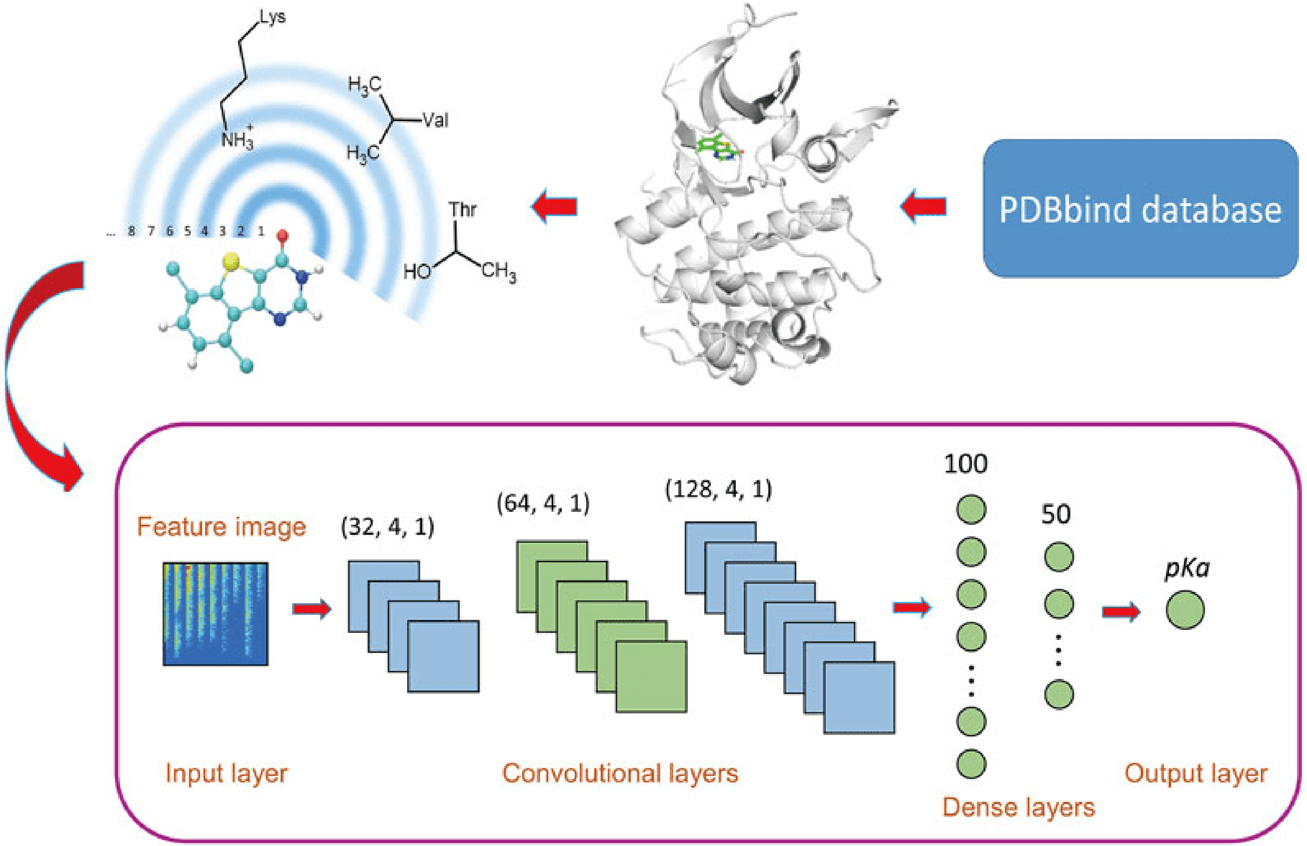
$$E_{total} = \overbrace{\sum_{bonds} K_r(\mathrm{r}-\mathrm{r}_{eq})^2 + \sum_{angles} K_\theta(\theta-\theta_{\mathrm{eq}})^2 + \sum_{dihedrals} \frac{V_n}{2}[1+\cos(n\phi-\gamma)]}^{\textbf{Bonded}} + \overbrace{\sum_{i<j}\left[\frac{A_{ij}}{R_{ij}^{12}} - \frac{B_{ij}}{R_{ij}^{6}} + \frac{q_i q_j}{\epsilon R_{ij}}\right]}^{\textbf{Non-bonded}}$$

Advances in scoring functions are driven by data and AI

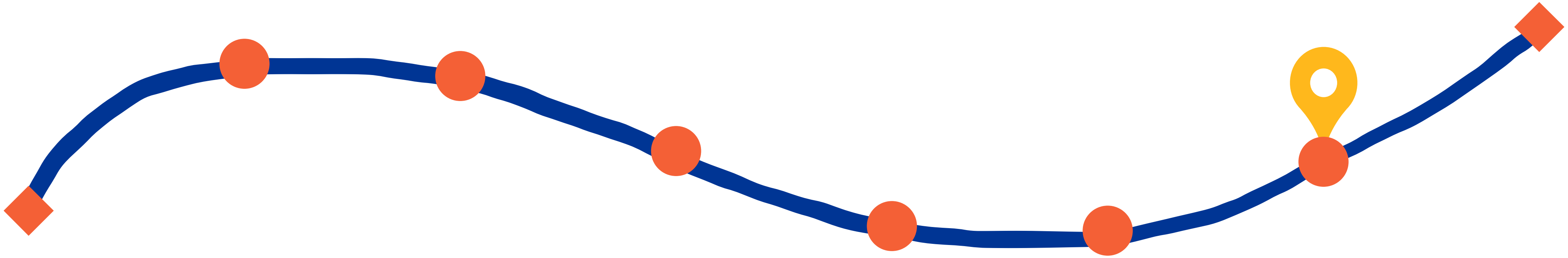
Machine learning models (e.g., RF-Score, DeepDock) are trained directly on binding data.

These models learn nonlinear relationships and use structural and chemical features.

Require large, high-quality datasets and careful validation.

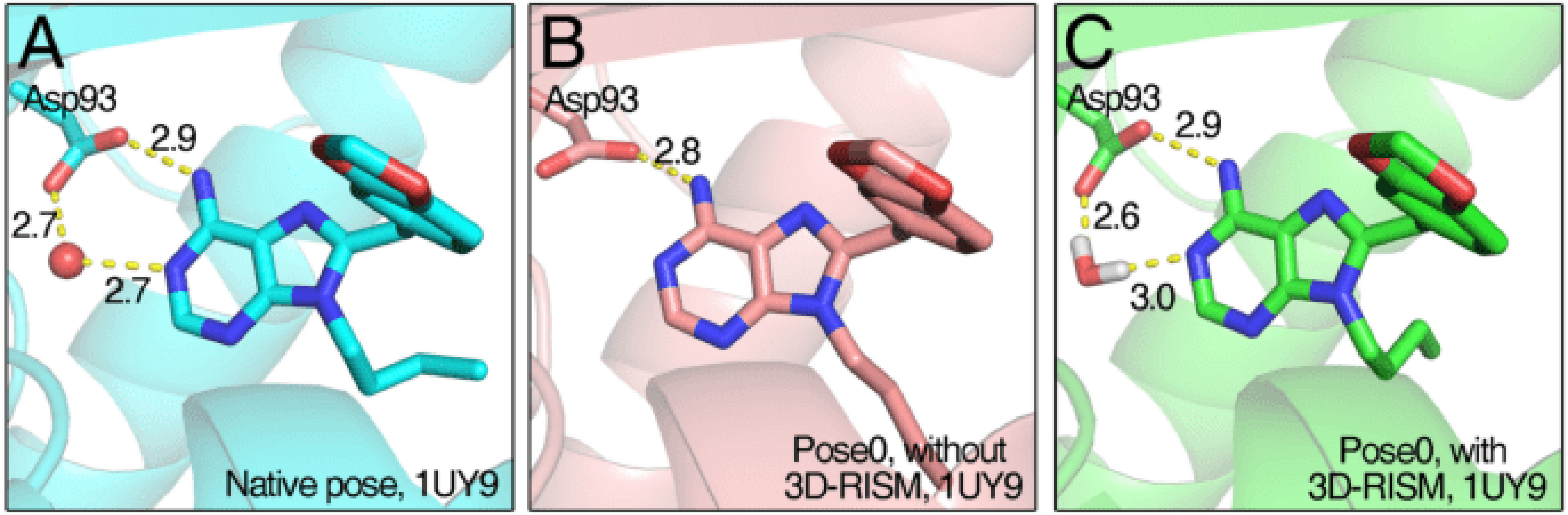


After today, you should have a better understanding of



Ligand pose optimization

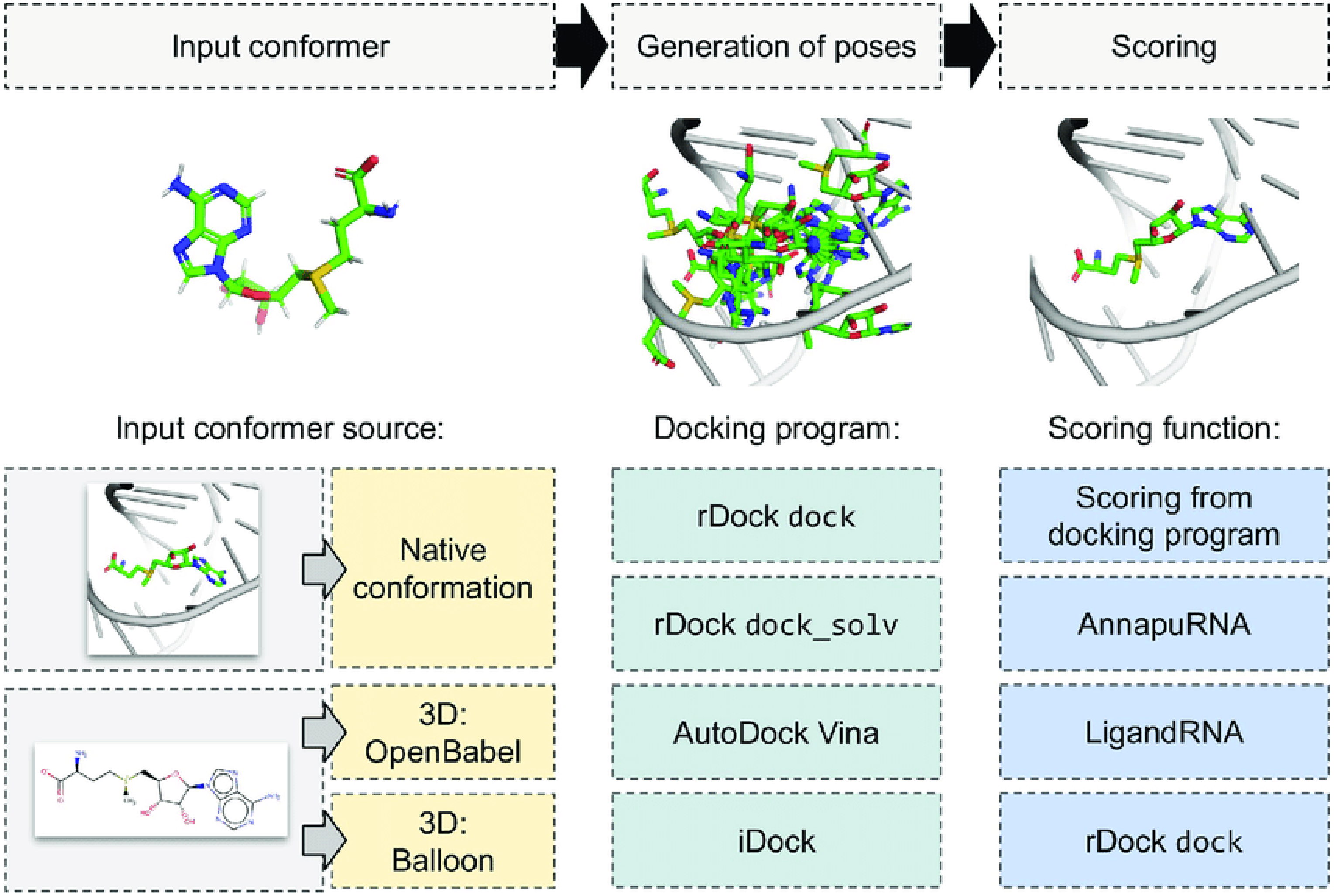
Accurate Docking Depends on Optimized Ligand Poses



Docking needs to generate diverse conformations

Search strategies

- Systematic
- Stochastic
- Empirical
- Machine learning



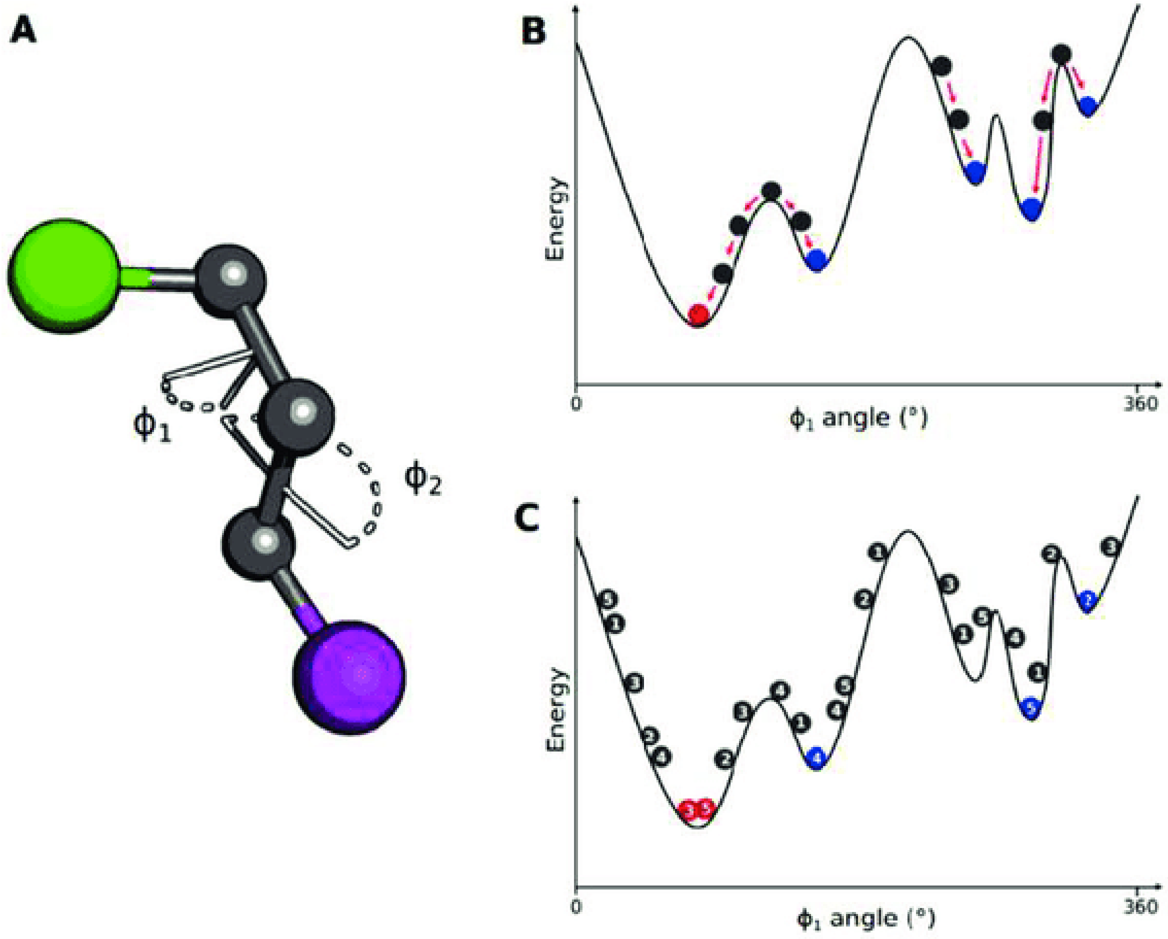
Systematic searches numerically iterate over all possible conformations

Identify important degrees of freedom

- Angles
- Dihedrals

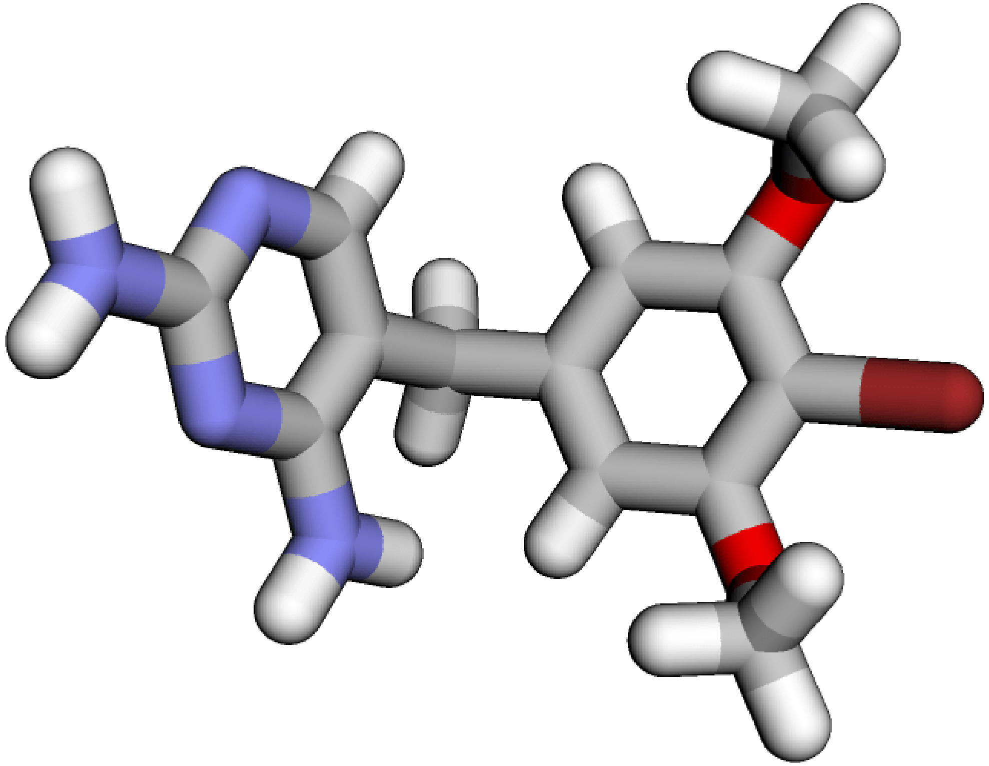
Scan along each angle with a step size of a *N* degrees

Remove structures with high strain



Systematic searches are only possible for very small molecules

How many different conformations would we have in this molecule if we scanned only dihedrals every 45 degrees?



Systematic searches are only possible for very small molecules

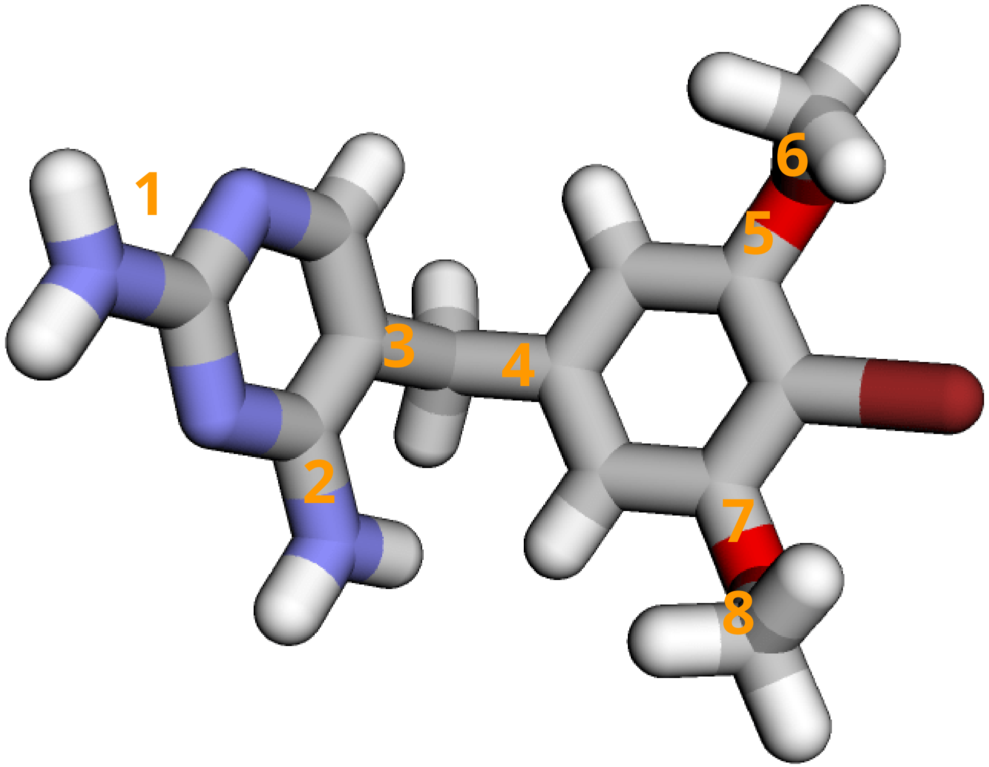
8 × 8 × 8 × 8 × 8 × 8 × 8 × 8 = 16,777,216

That's a lot of structures, and many of them will clash!

We almost never do a systematic search in practice without some precautions to combinatorics

8 dihedrals

8 angles



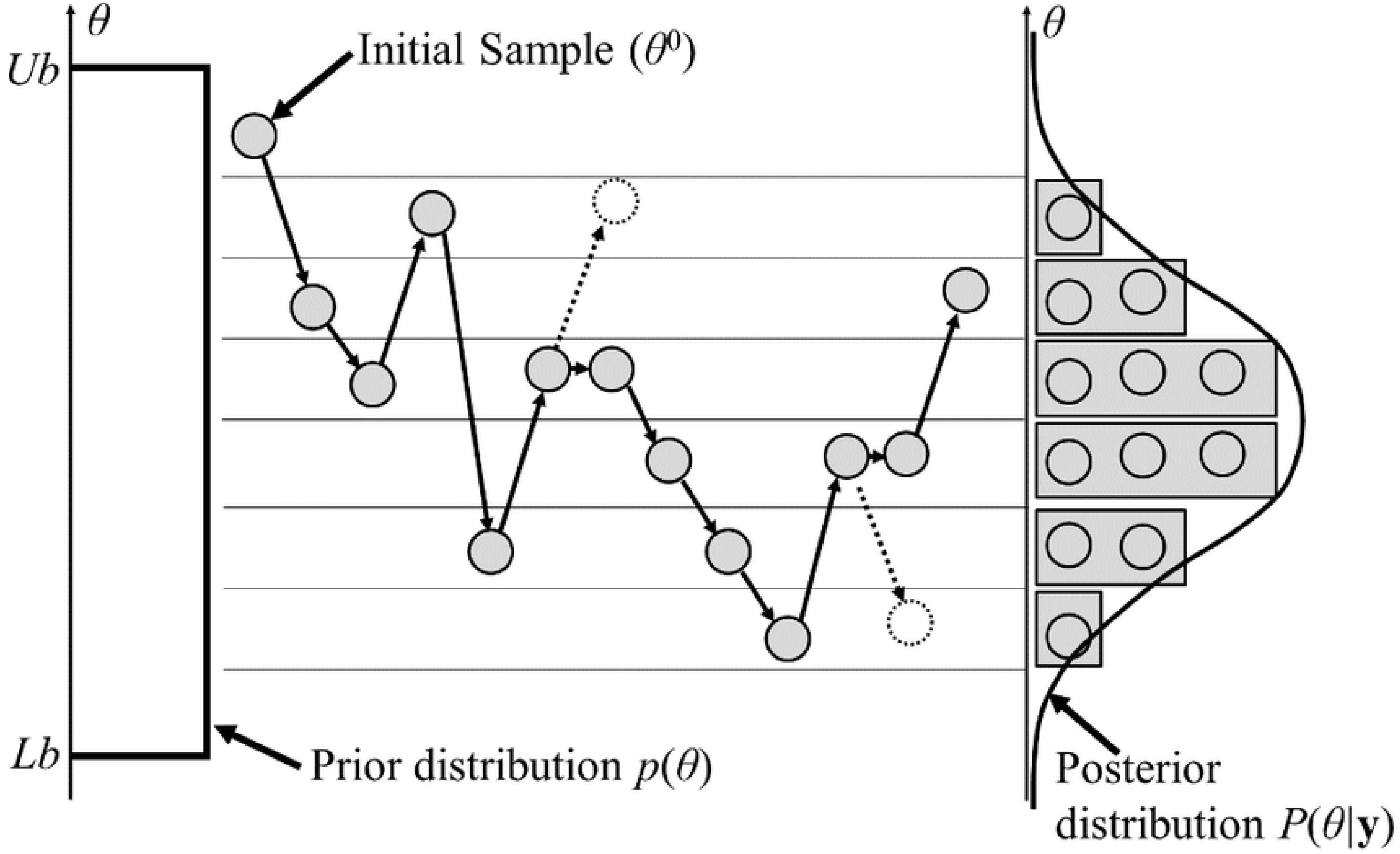
Stochastic algorithms provide better balance of sampling and cost

Monte Carlo

Steps:

- Generate conformation
- Compute energy change
- If energy change less than a random sample: make move
- Repeat

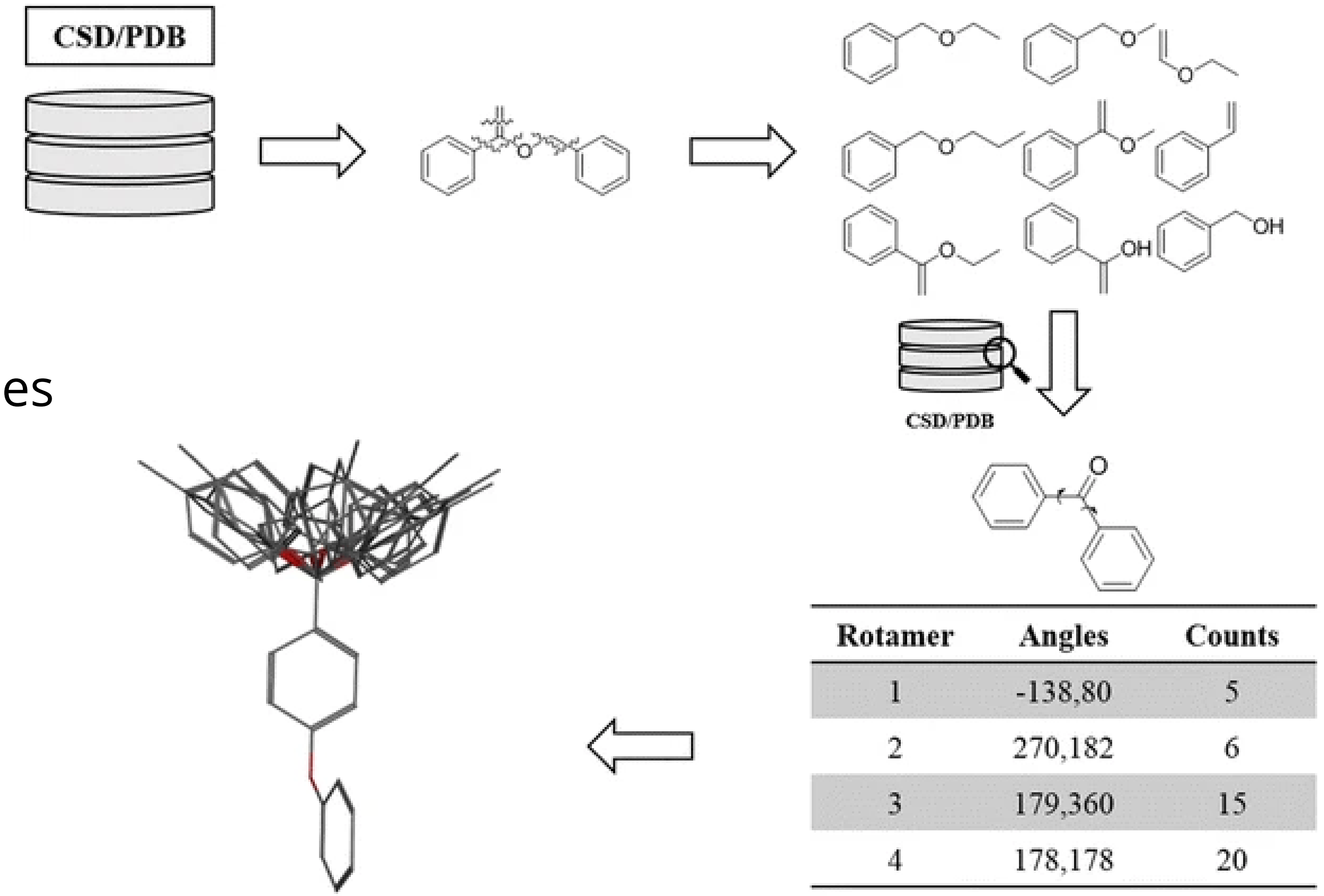
Allows us to sample efficiently



θ
Ub
Lb
Initial Sample (θ^0)
Prior distribution $p(\theta)$
θ
Posterior distribution $P(\theta|\mathbf{y})$

We also have conformer libraries

Use pre-generated libraries



Rotamer	Angles	Counts
1	-138,80	5
2	270,182	6
3	179,360	15
4	178,178	20

Before the next class, you should

Lecture 12A:

Docking - Foundations

Lecture 12B:

Docking - Methodology

Today

Thursday

- Work on P03A