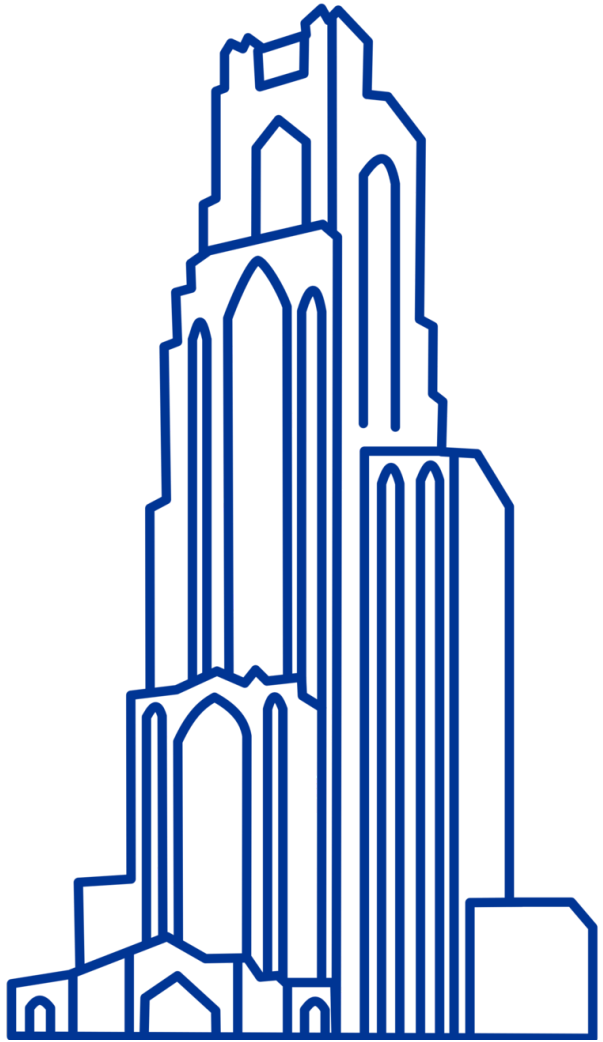


Computational Biology
(BIOSC 1540)

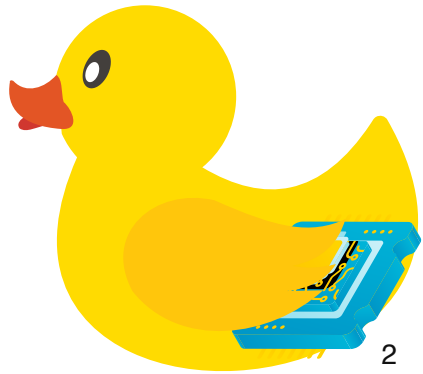
Lecture 16:
Structure-based drug design

Oct 29, 2024

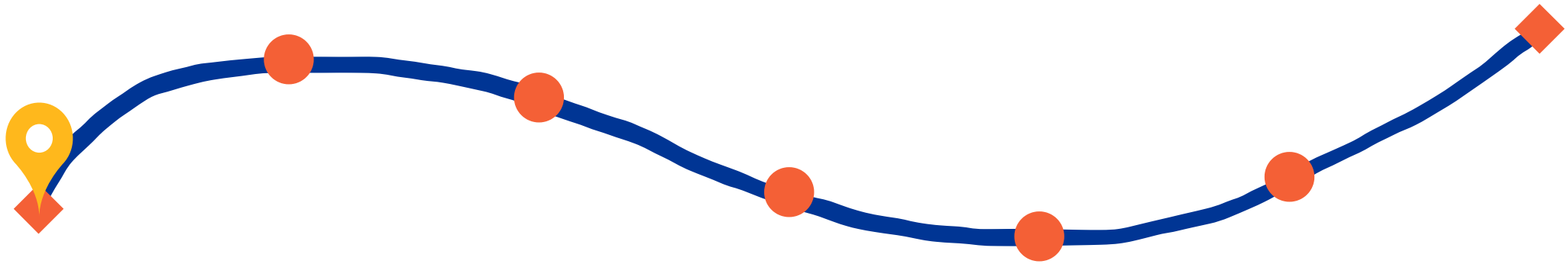
University of Pittsburgh

Announcements

- A06 is due Thursday by 11:59 pm
  - **Reminder:** There is a (soft) limit of 100 words for each question
- A07 (final assignment) will be released Friday
- No class on Nov 5 for election day
- The next exam is on Nov 14
  - We will have a review session on Nov 12
  - Request DRS accommodations if needed
- Project will be released Nov 21 and is due on Dec 10



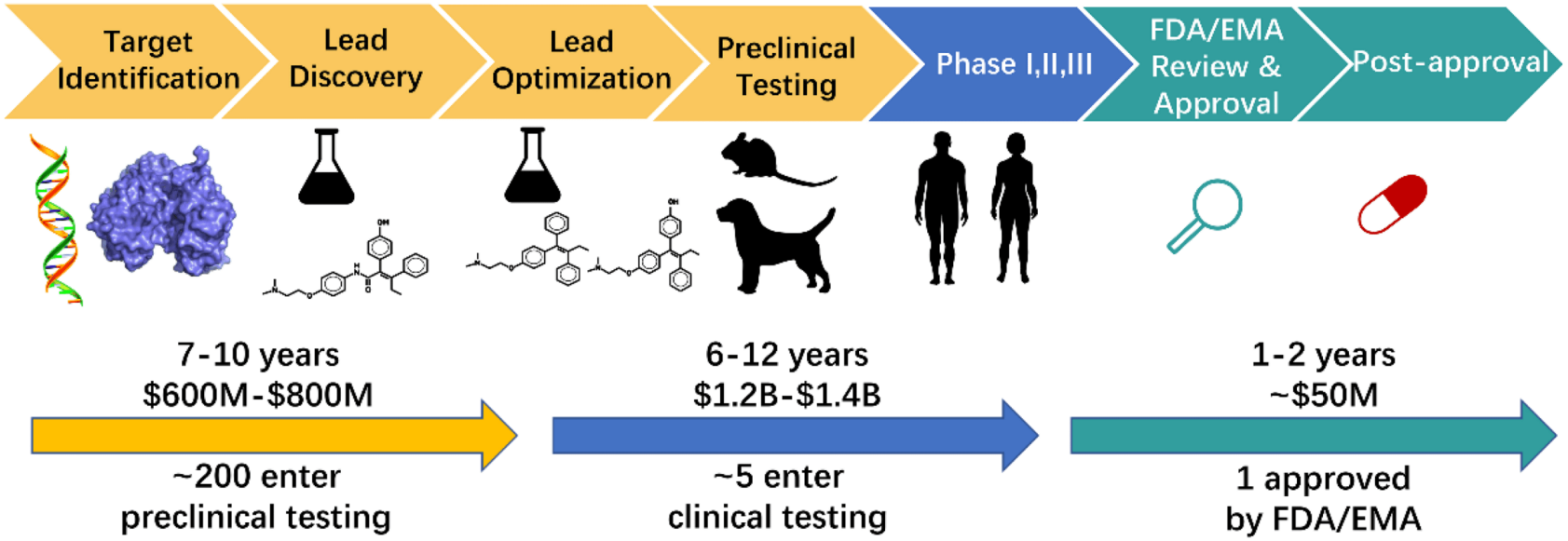
After today, you should better understand



Drug development

pipeline

Drug development is a complex, multi-stage process requiring significant time and resources



Computation is most helpful with the drug discovery stage

1. Discovery and Preclinical Research
Potential drugs are identified and tested in non-human studies

2. Clinical Trials
Testing in human subjects to assess safety and efficacy

3. Regulatory Approval
Evaluation by agencies like the FDA before the drug can be marketed

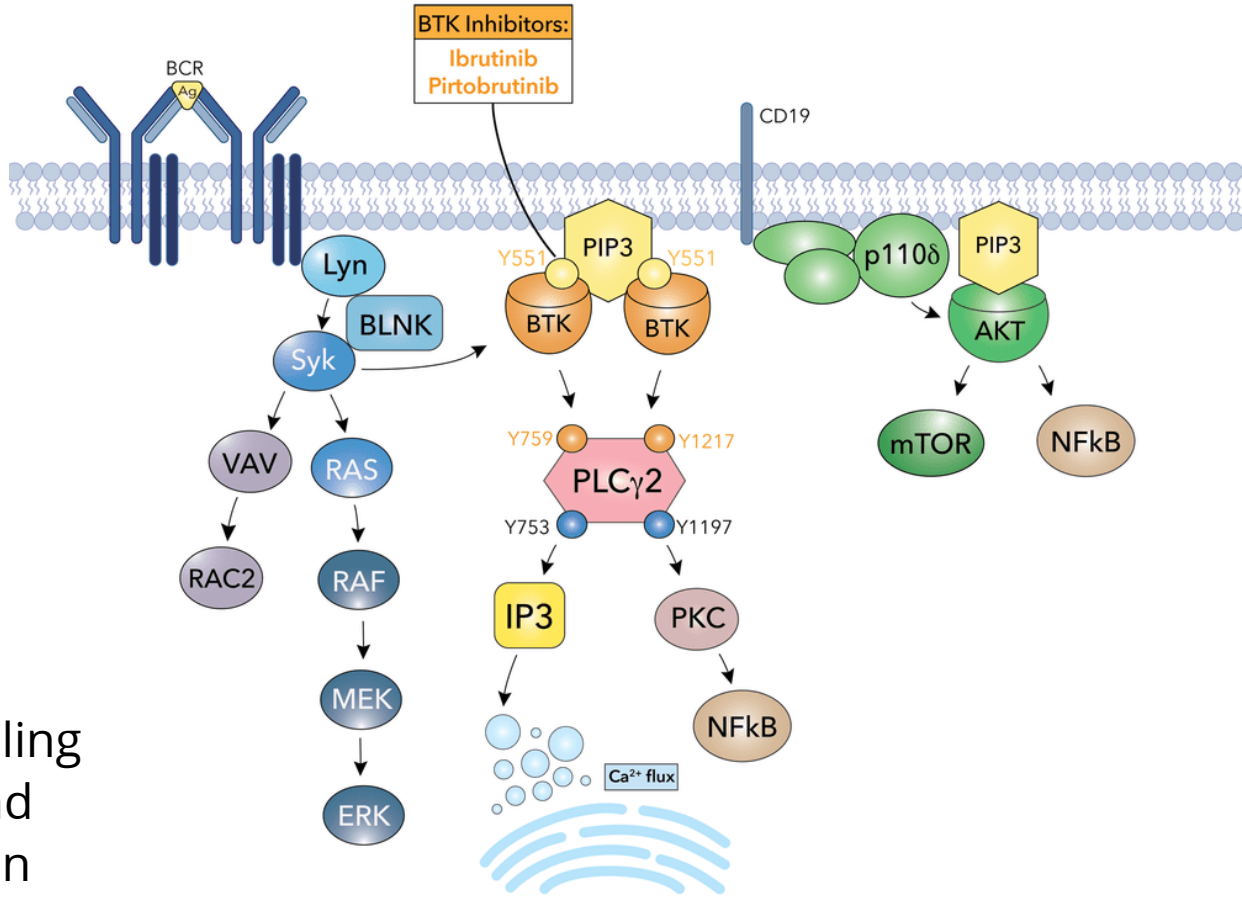
4. Post-Marketing Surveillance:
Ongoing monitoring after the drug is available to the public

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

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.



Example: Bruton's tyrosine kinase (BTK) is a critical signaling enzyme that controls B-cell development, maturation, and activation by mediating B-cell receptor signal transduction

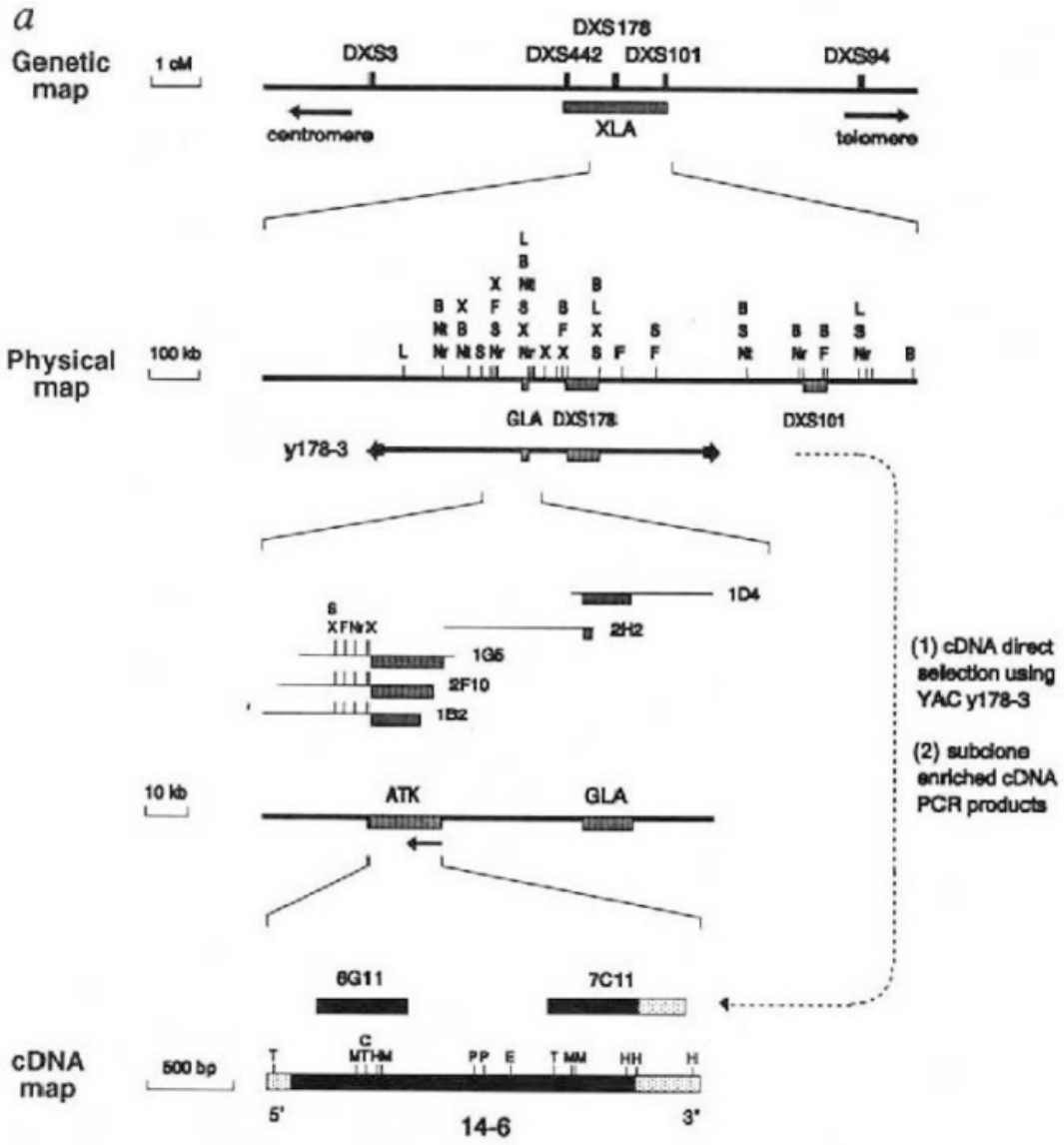
(Mohamed et al., 2009)

Target identification is accelerated with bioinformatics

BTK gene was implicated in X chromosome-linked agammaglobulinemia (XLA)

Now we can use …

Genome-wide association studies, high-throughput screening



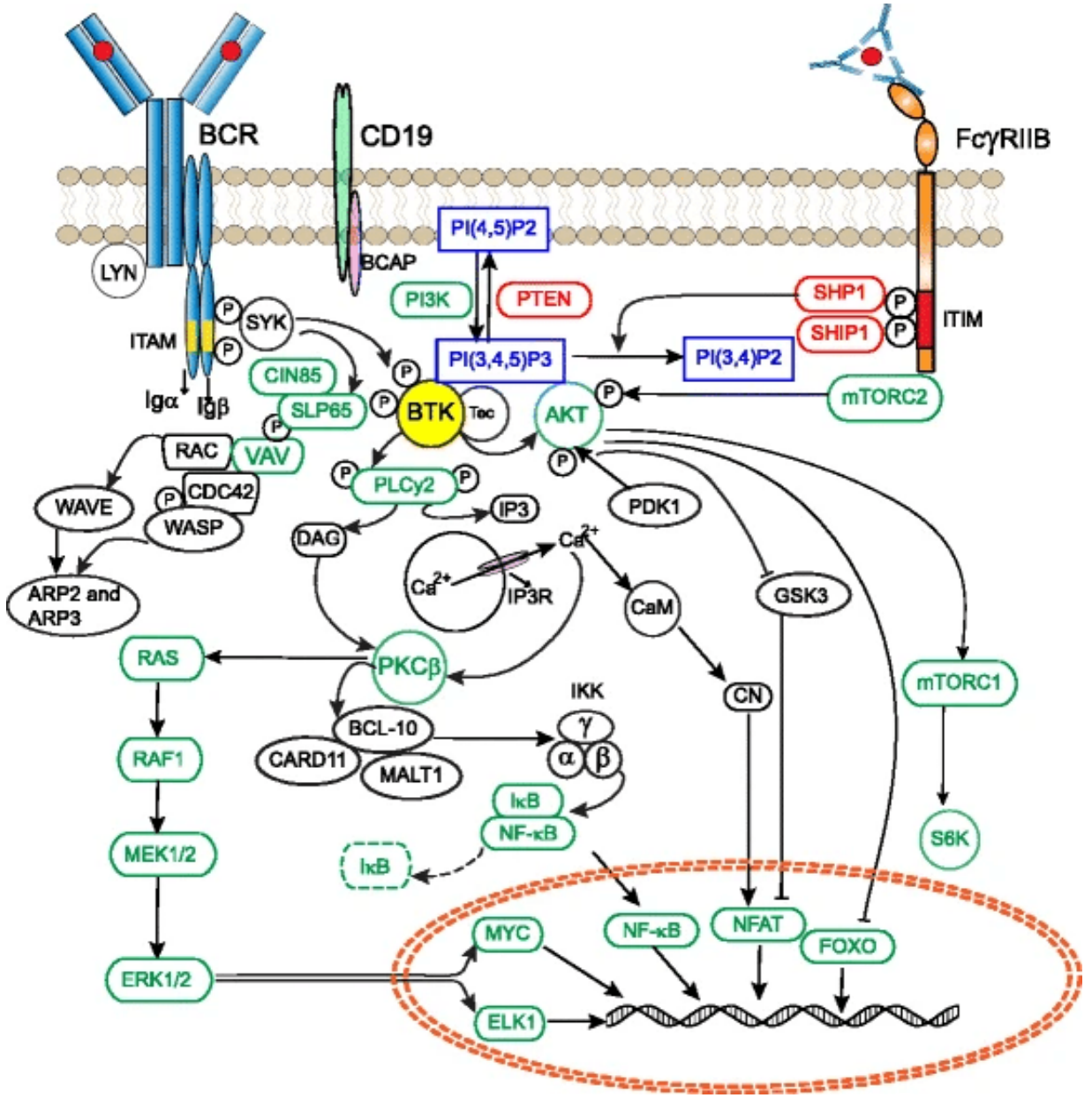
(Vetrie et al., 1993 & Tsukada et al., 1993)

Target identification is accelerated with bioinformatics

Revealed that BTK as a central hub in B-cell receptor (BCR) signaling

Now we can use …

Proteomics, transcriptomics

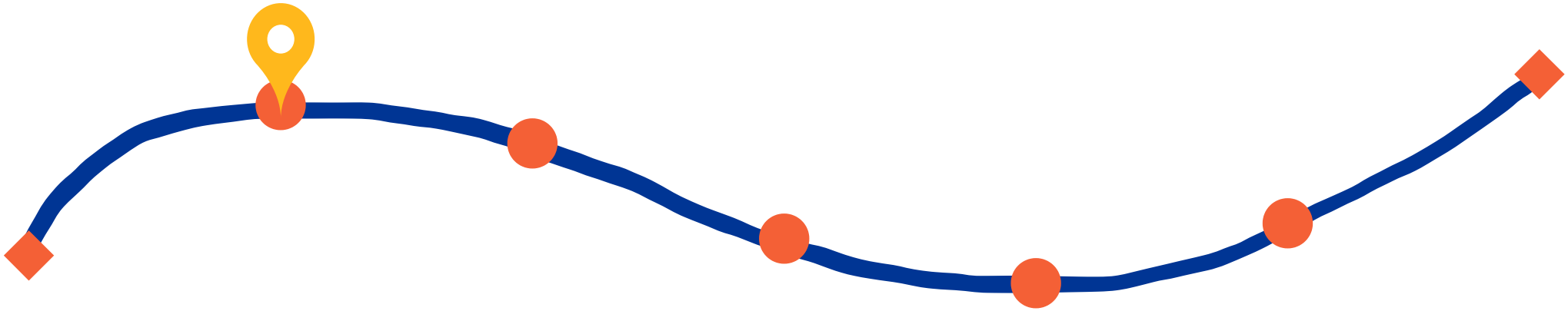


(Singh et al., 2018)

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

With a protein target in hand, we can now identify potential drug candidates

After today, you should better understand



Role of structure-based

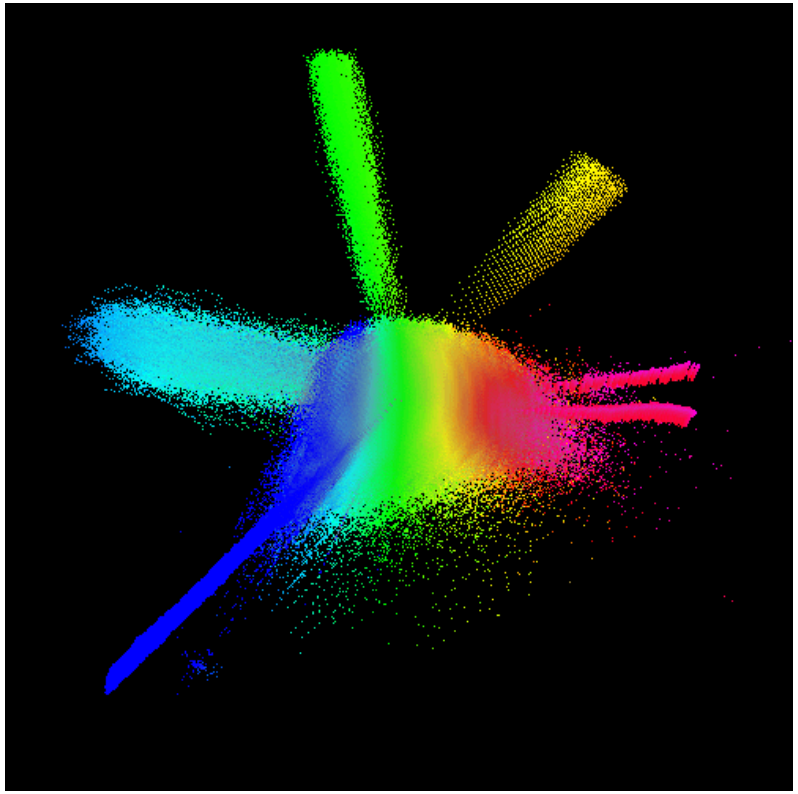
drug design

Chemical space contains an astronomical number of possible compounds to explore

Effective drugs must bind to the target protein with sufficient affinity and specificity

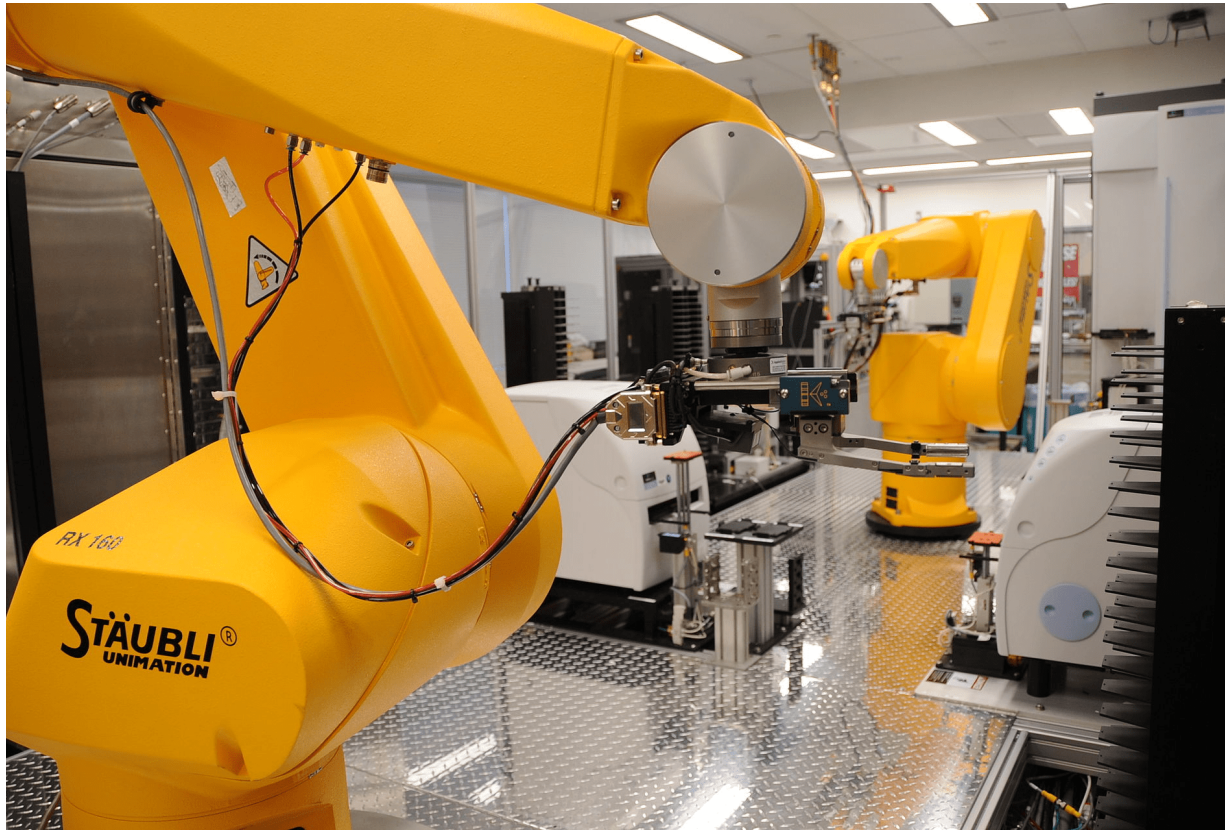
Estimated to be between 10^{60} to 10^{200} possible small organic molecules

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

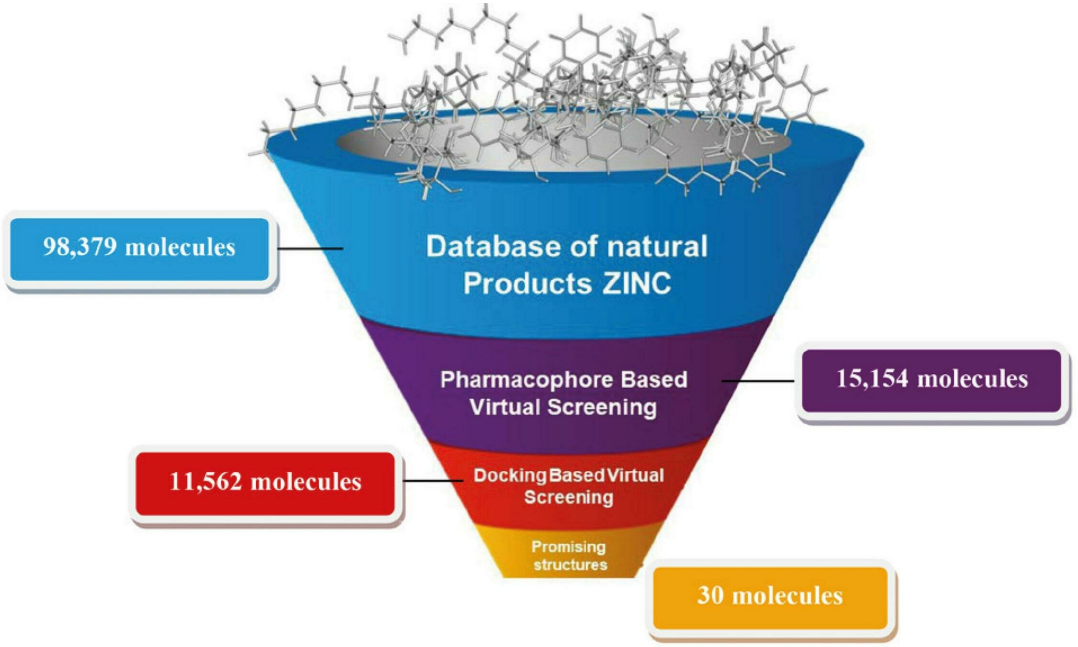


Virtual screening evaluates vast libraries to identify potential leads efficiently

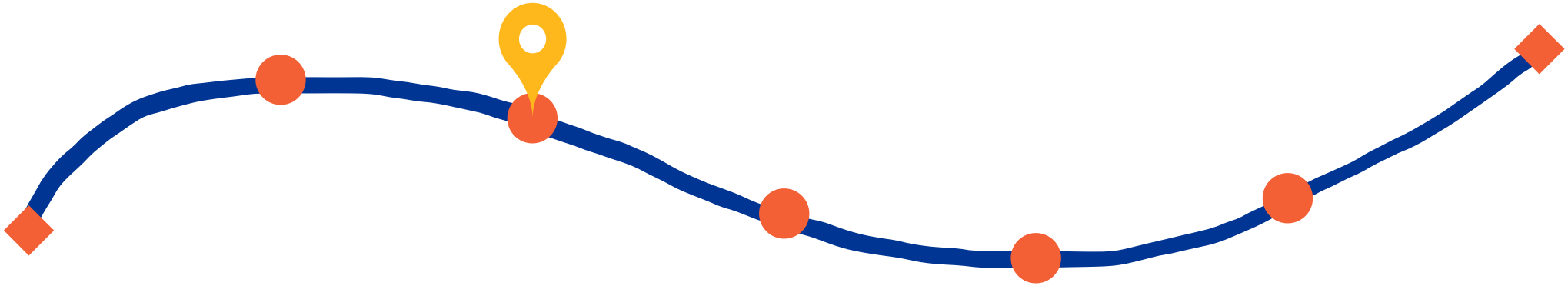
Experimental assays are still expensive, and limited to commercially available compounds

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

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



After today, you should better understand

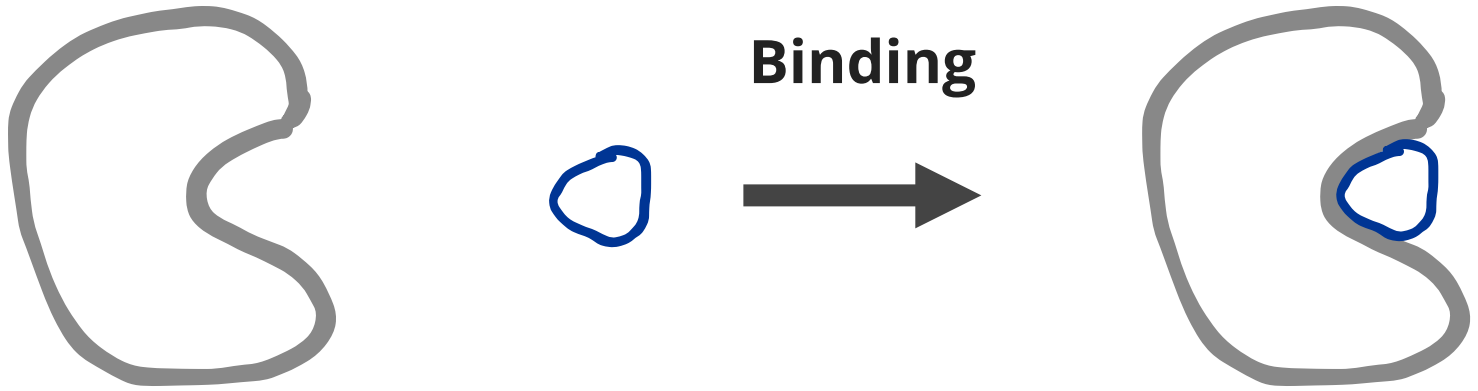


Thermodynamics of

binding

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

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



Protein **Ligand** **Protein-ligand**

We can model this as a reversible protein-ligand binding

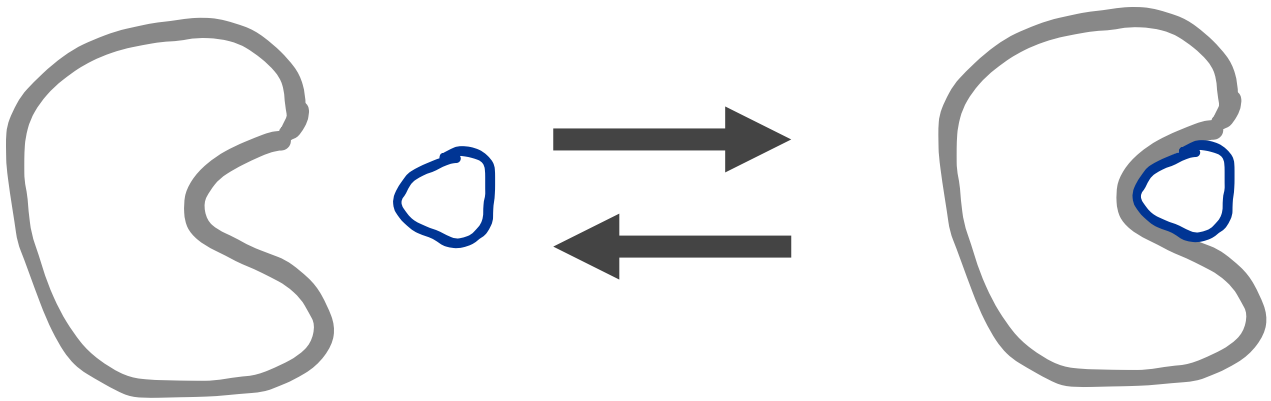
$$P + L \leftrightharpoons PL$$

Binding affinity is determined by the Gibbs free energy change

The change in free energy when a ligand binds to a protein

$$\Delta G_{bind} = G_{PL} - G_P - G_L$$

Determines binding process spontaneity



Gibbs free energy combines enthalpy and entropy

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

Enthalpy

ΔH_{bind}

Accounts for energetic interactions

Entropy

ΔS_{bind}

How much conformational flexibility changes

Note: Simulations capture free energy directly instead of treating enthalpy and entropy separately

After today, you should better understand

Enthalpic contributions

to binding

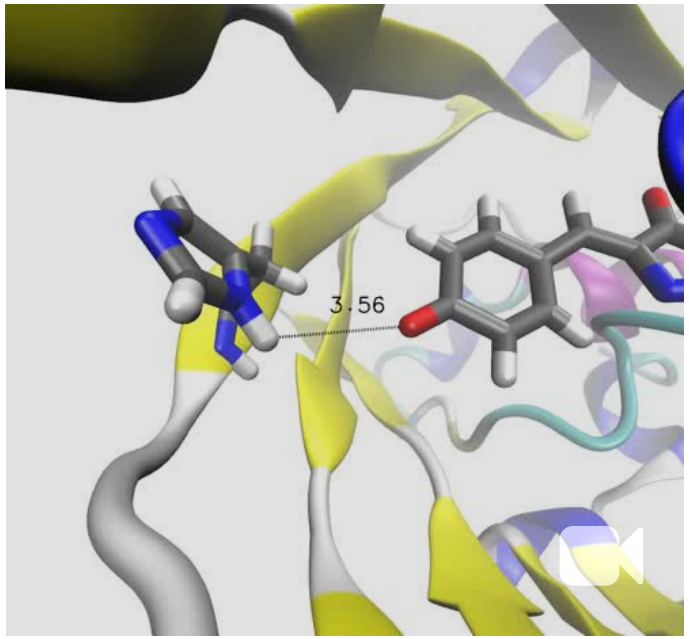
Enthalpy accounts for noncovalent interactions

Ensemble differences in noncovalent interactions provide binding enthalpy

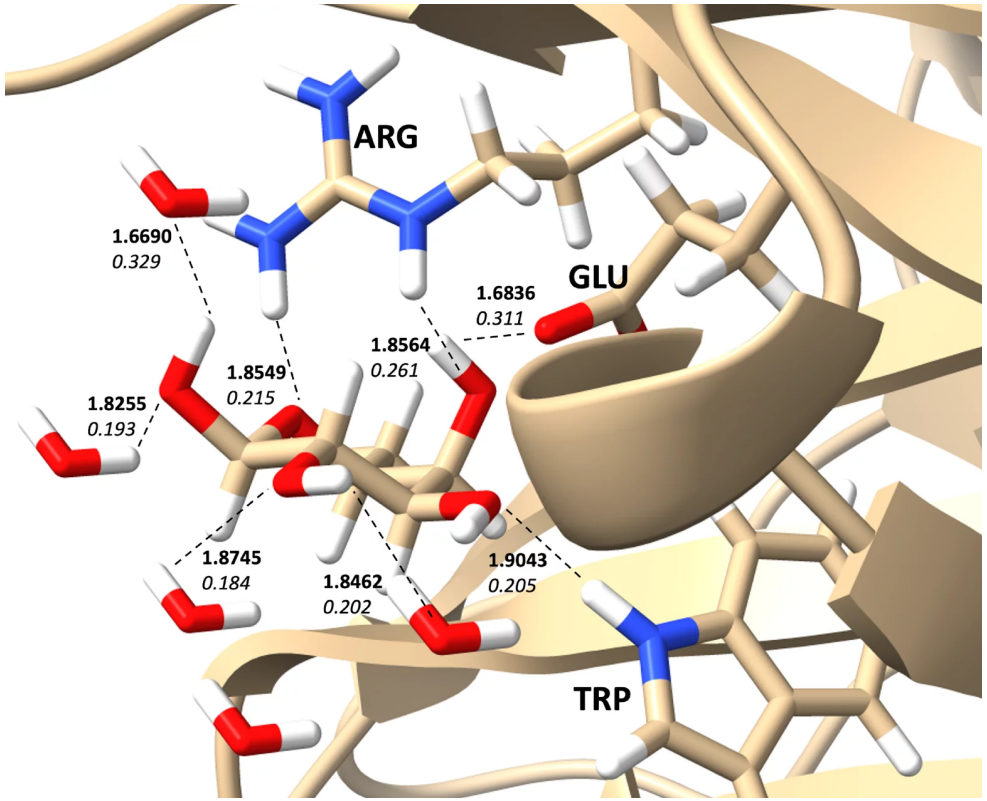
$$\Delta H_{bind} = \langle H_{PL} \rangle - \langle H_P \rangle - \langle H_L \rangle$$

$\langle \cdots \rangle$

Ensemble average



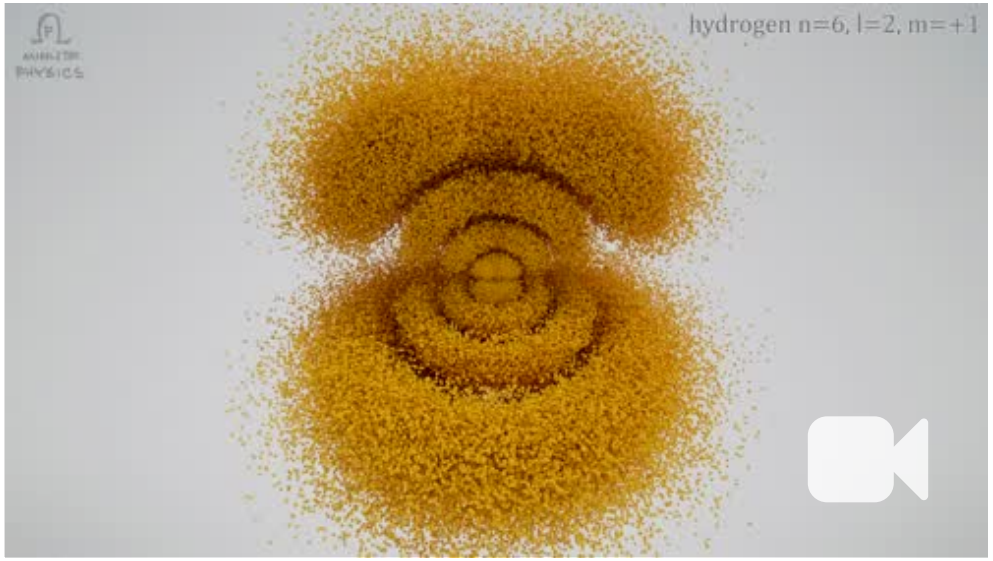
Noncovalent interactions: Electrostatics, hydrogen bonds, dipoles, π-π stacking, etc.



Chemical interactions are determined by fluctuating electron densities

Molecular interactions are governed by their electron densities (Hohenberg-Kohn theorem)

This is rather difficult, so we often use conceptual frameworks to explain trends (e.g., hybridization and resonance)



Our noncovalent interactions conceptual framework:

1. Coulomb's law describes the interactions between charges

2. Molecular geometry uniquely specifies an electron density

3. Regions of increased electron density are associated with higher partial negative charges

4. Electrons are mobile and can be perturbed by external interactions

Electrostatic forces govern interactions between charged and polar regions

Charged molecules have a **net imbalance** between

- Positive charges in their nuclei
- Negative charges from their electrons

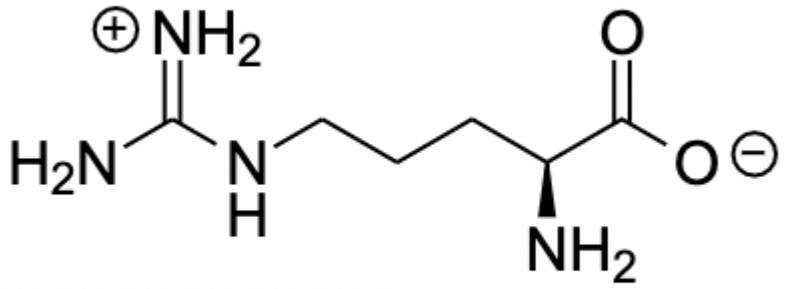
This leads to **net electrostatic attractions or repulsions** between different atoms or molecules

Role in binding

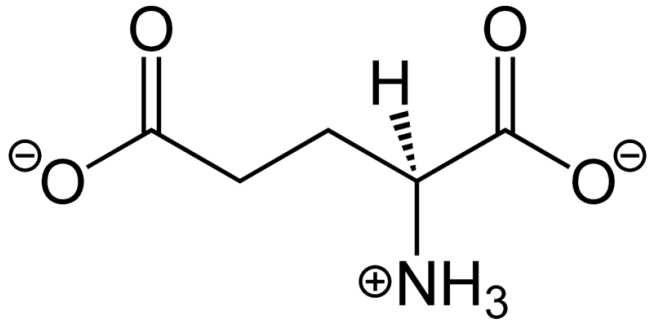
Long-Range Interaction: Can attract ligands to the binding site from a distance

Anchor Points: Often serves as key anchoring interactions in the binding site

Arginine



Glycine

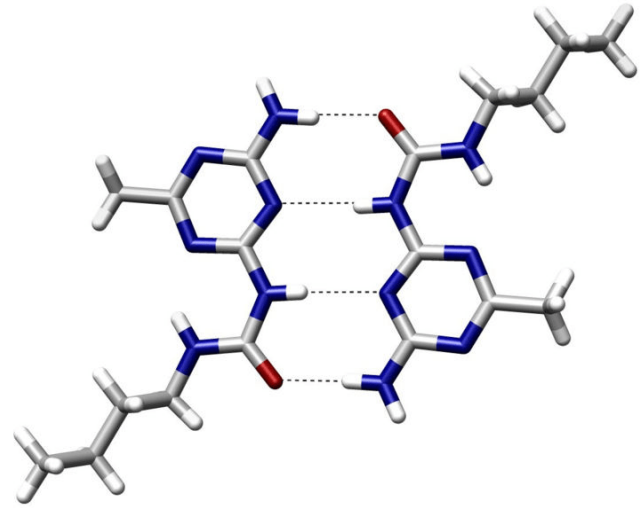


~5 to 20 kcal/mol per interaction

Hydrogen bonds are a type of electrostatic interactions

Attraction between a **(donor)** hydrogen atom covalently bonded to an electronegative atom and another **(acceptor)** electronegative atom with a lone pair

- **Common donors:** O-H, N-H groups
- **Common acceptors:** O and N atoms with lone pairs



Role in binding

Specificity: Precise orientation of the ligand

Stabilization: Moderately strong interactions

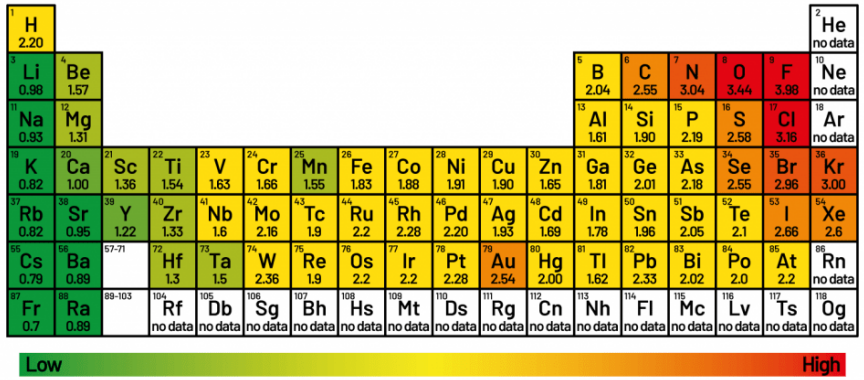
Dynamic: Allows for adaptability of ligands

Strongest when the hydrogen, donor, and acceptor atoms are colinear

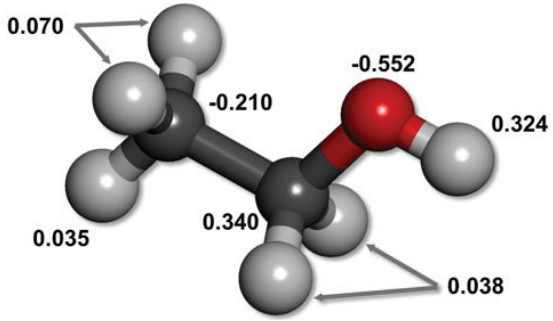
~2 to 7 kcal/mol per hydrogen bond

Uneven electron distribution creates partial charges and dipoles

Electronegativity differences lead to unequal distribution of electron density



Unequal distribution results in regions or partial positive or partial negative charges

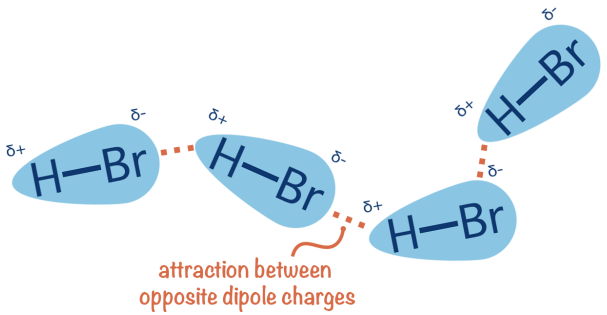


Role in binding

Directional binding: Highly directional, ensuring that the ligand aligns correctly

Flexibility: Can accommodate slight conformational changes

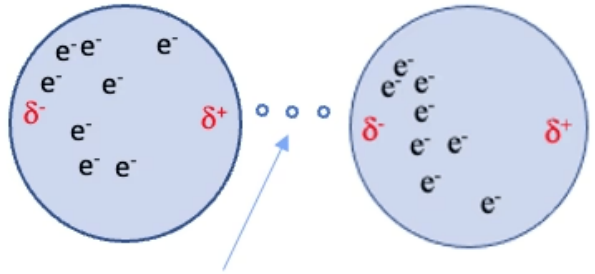
Consistent electron density spatial variation results in permanent dipoles



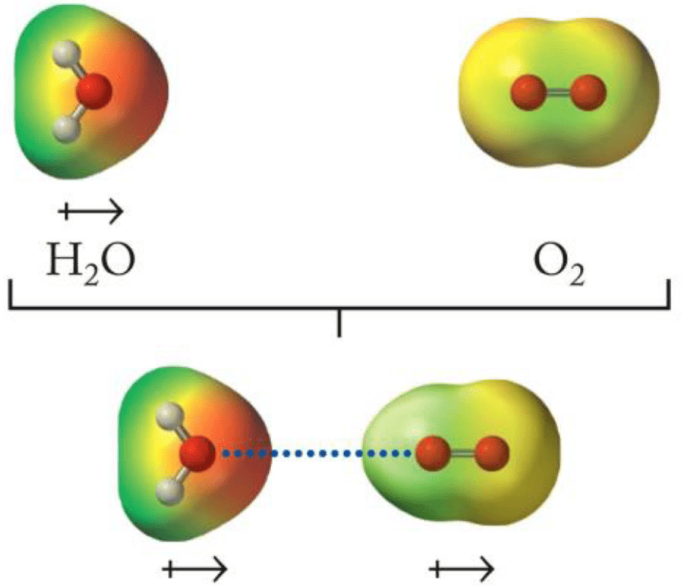
~0.01 to 1 kcal/mol per interaction

Van der Waals forces are weak, non-directional interactions

Dispersion: Electrons in molecules are constantly moving, leading to temporary uneven distributions that induce dipoles in neighboring molecules



Induction: The electric field of a polar molecule distorts the electron cloud of a nonpolar molecule, creating a temporary dipole



Role in binding

Complementary fit: Maximizes surface contact

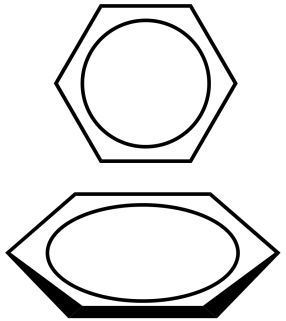
Flexibility: Allows small conformational changes

~0.4 to 4 kcal/mol per interaction

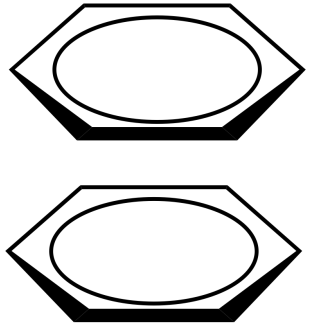
π-π interactions involve stacking of aromatic rings

Noncovalent interactions between aromatic rings due to overlap of π-electron clouds

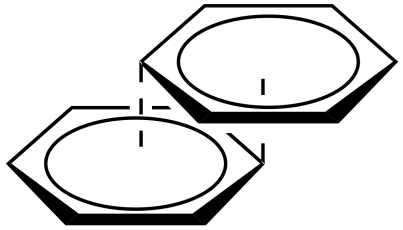
Edge-to-face



Face-to-face



Displaced



Role in binding

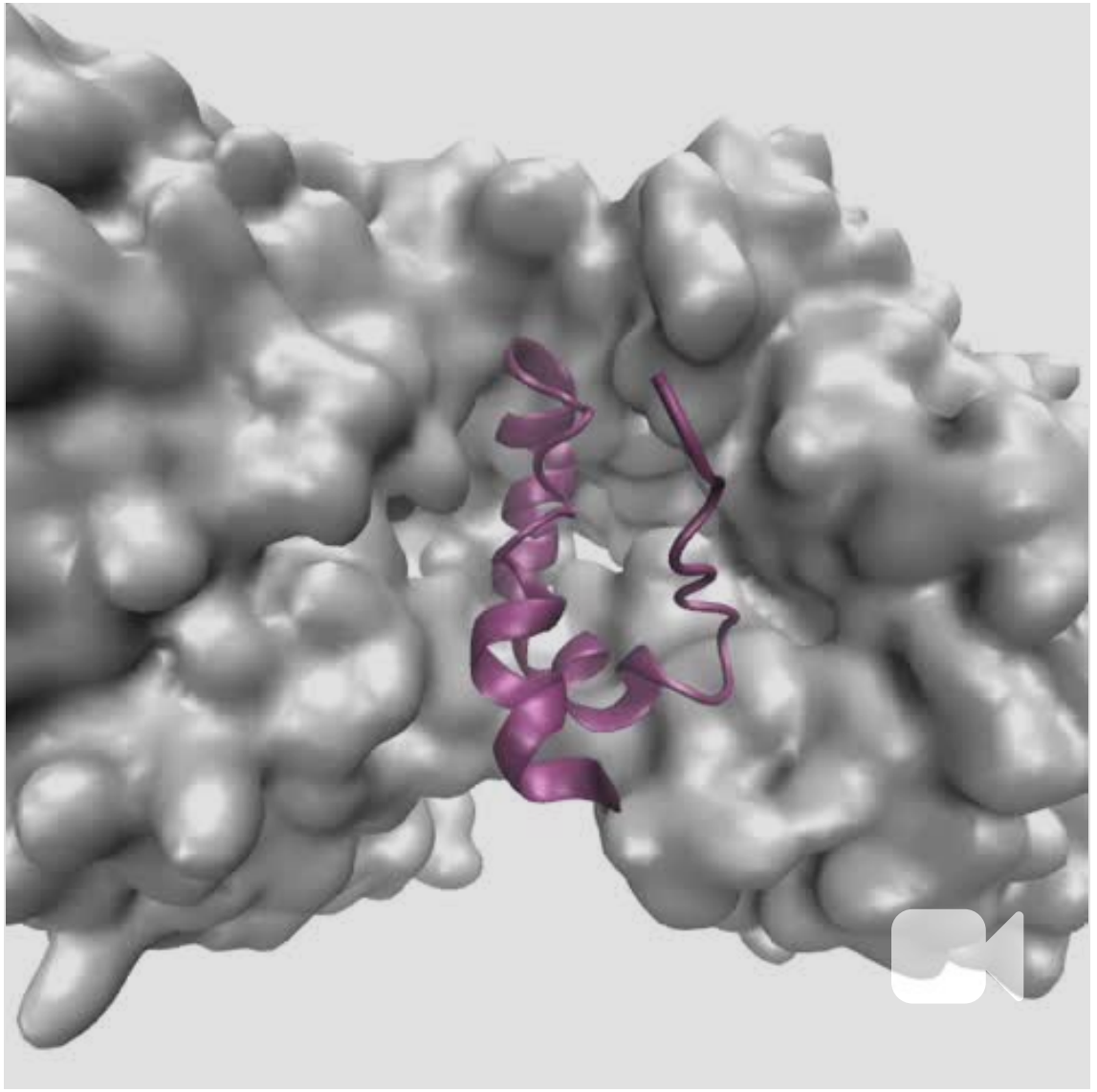
Orientation: Proper positioning of aromatics

Selectivity: Recognition of ligands

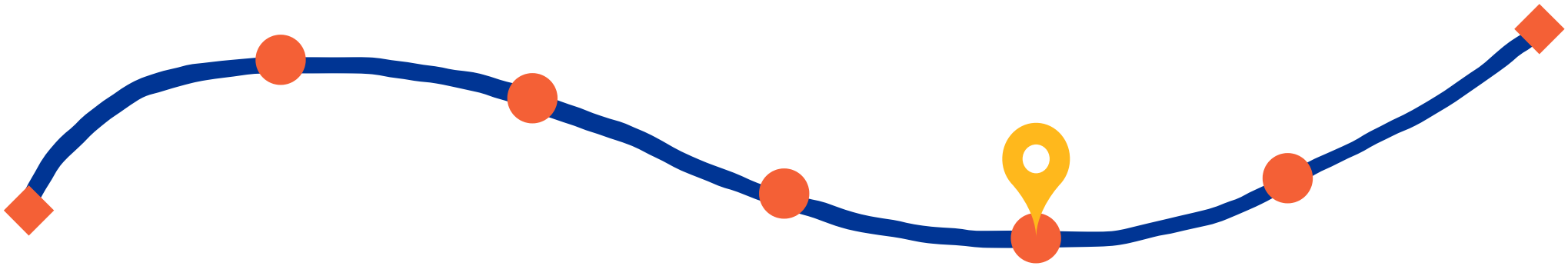
~1 to 15 kcal/mol per interaction

Summing all of these contributions during a simulation provides our ensemble average

$$\langle H_{AB} \rangle$$



After today, you should better understand



Entropic contributions

to binding

Entropy accounts for microstate diversity of a single system state

One of Alex's esoteric points: "Entropy is disorder," is a massive oversimplification that breaks down in actual practice

Entropy is formally defined as $S = k_B \ln \Omega$

Entropy is "energy dispersion"

Ω is the total number of microstates available to the system without changing the system state

Higher entropy implies greater microstate diversity

"System state" can be arbitrarily defined and compared as

- Unbound ligand vs. bound ligand
- Unfolded protein vs. folded protein
- Liquid water at 300 K vs. 500 K

Grid-based protein-ligand binding

Suppose I have a system with

- Protein receptor
- Ligands positioned on a grid

My macrostate (number of particles, temperature, and pressure) remain constant

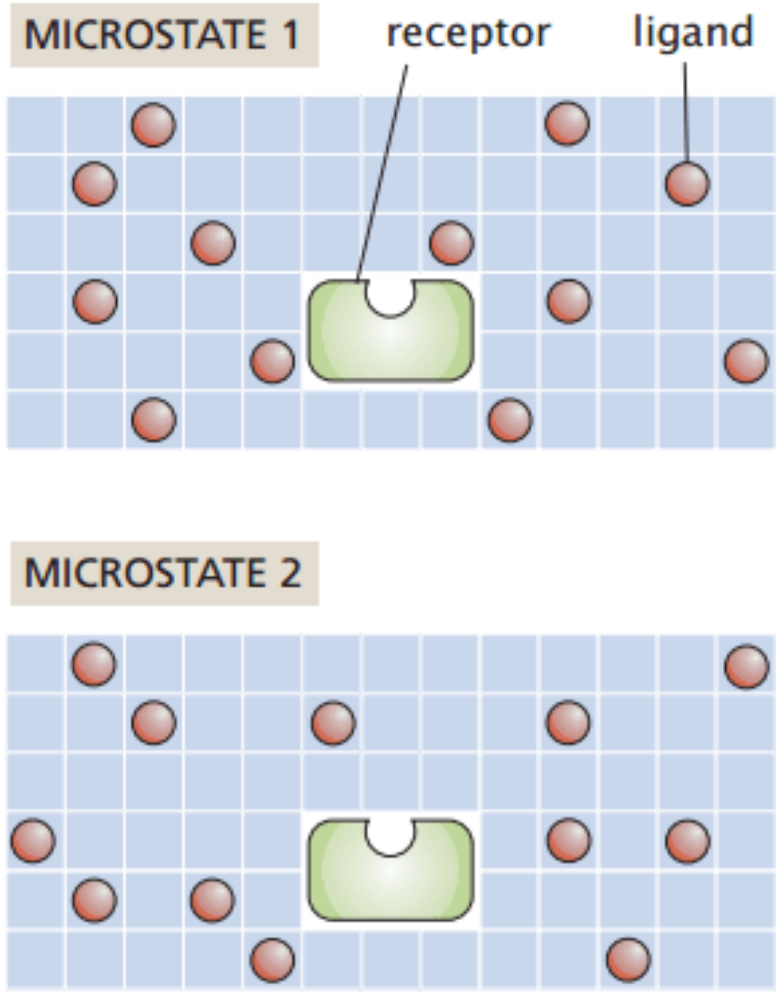
How many ways can I rearrange the ligands without binding to the receptor?

L Number of ligands

N Number of sites

$$\Omega = \frac{N!}{L!\,(N-L)!}$$

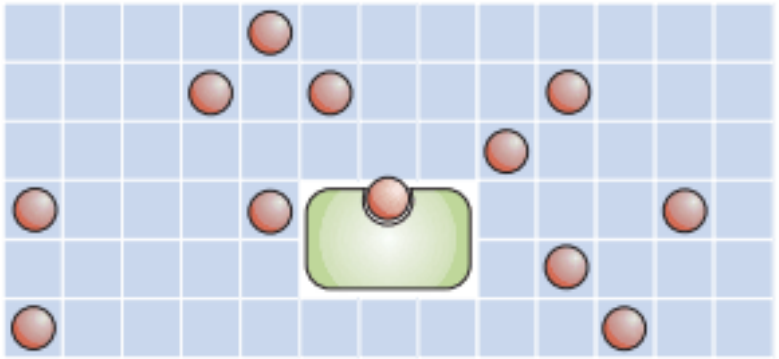
Number of ways to choose *L* grid sites out of *N* is the binomial coefficient



Grid-based protein-ligand binding

What if one ligand binds to the receptor?

$$\Omega = \frac{N!}{(L-1)!\,(N-L+1)!}$$

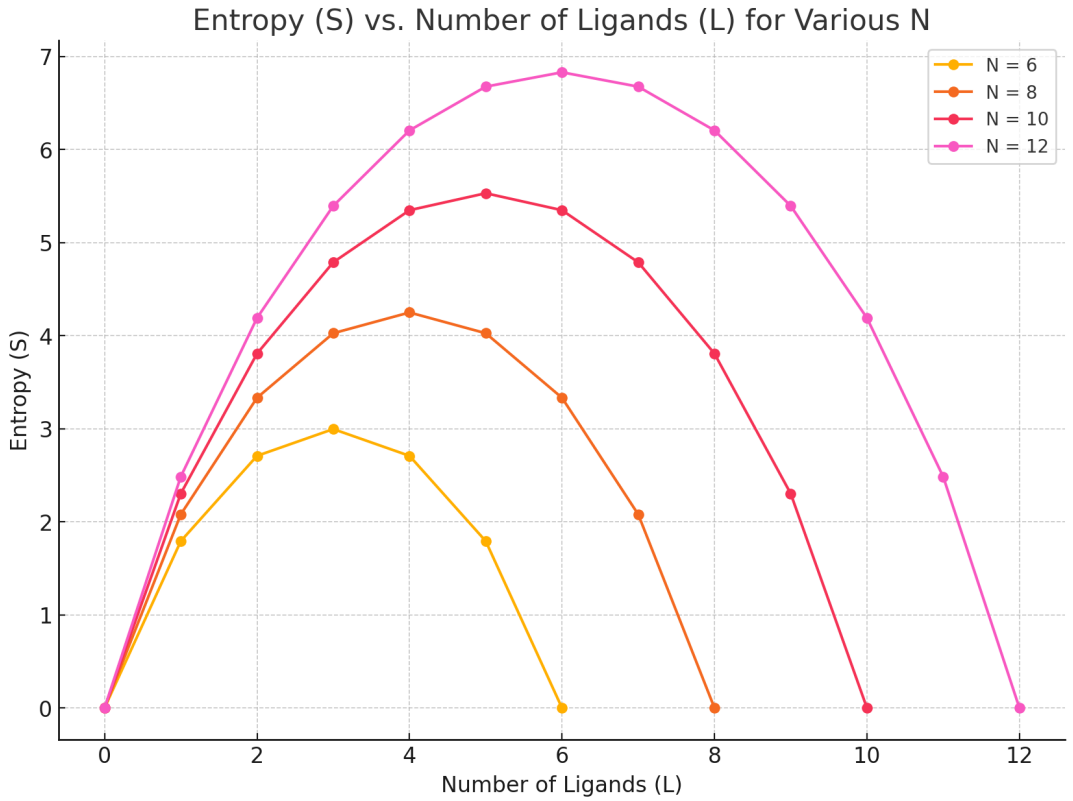


How does entropy change?

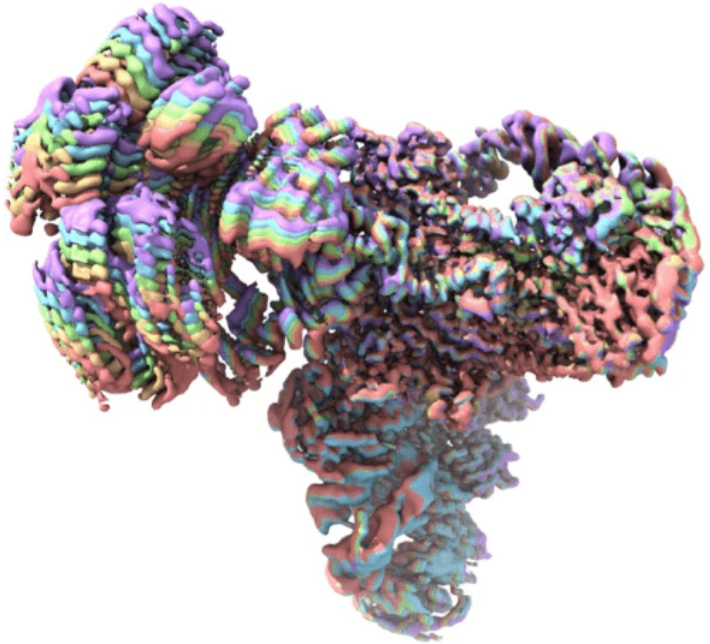
Increase	**No change**	**Decrease**

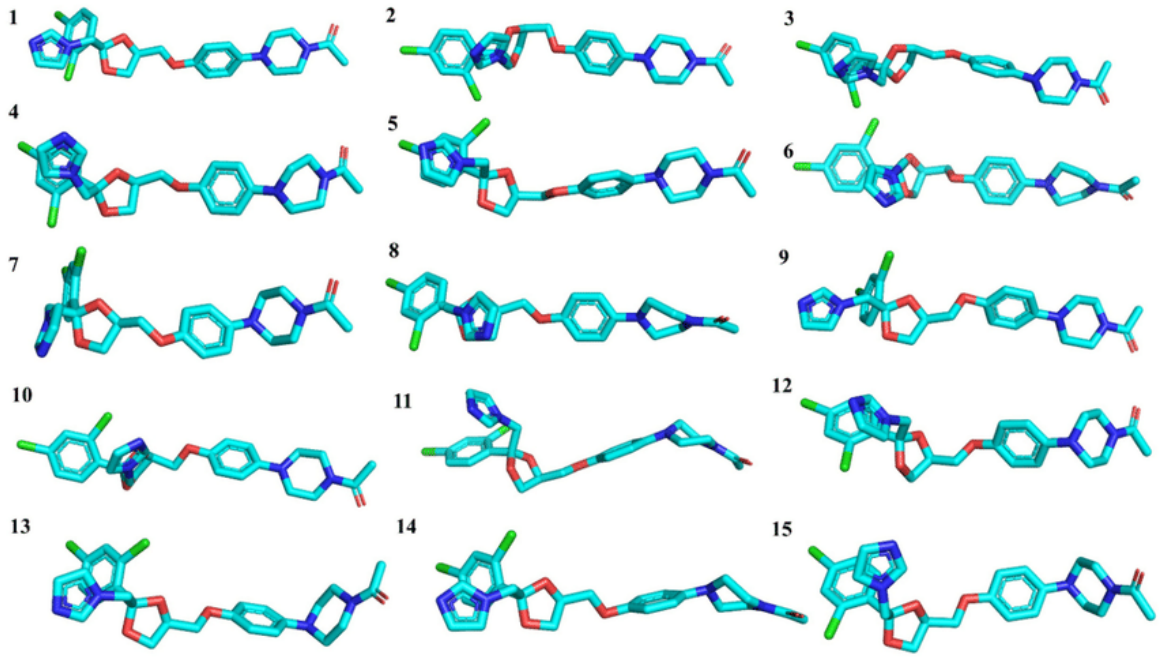
It depends on our ligand concentration!

How to interpret this: Pick a number of ligands and move to the right (L - 1), does entropy go up or down?

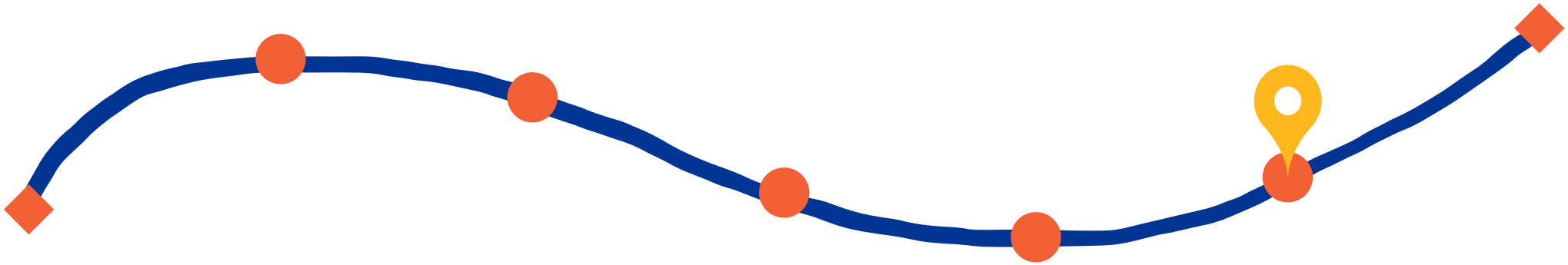


For protein-ligand binding, we need to account for how the number of accessible microstates/configurations for protein and ligand





After today, you should better understand



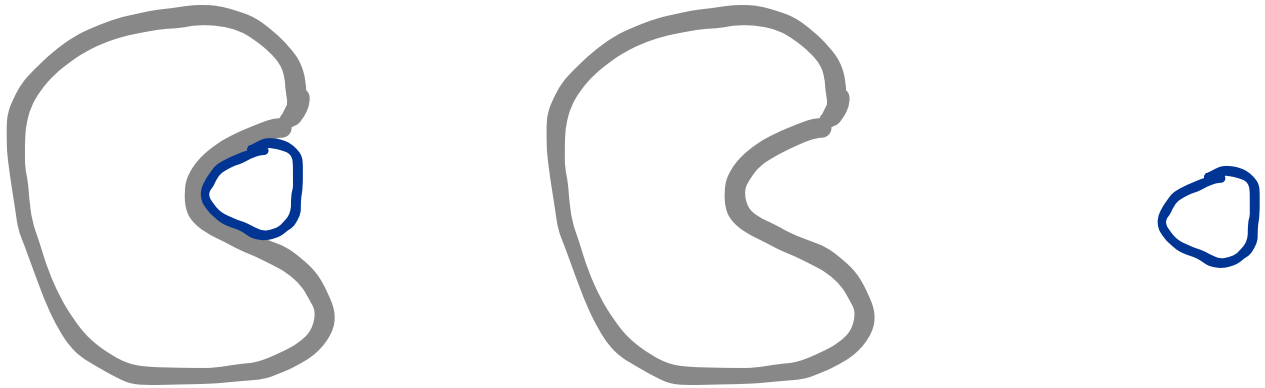
Alchemical free

energy simulations

We can now run molecular simulations of different states

We can run simulations and directly compute the **ensemble average free energy**

This is theoretically valid but not practical. Why?



$$\Delta G_{PL} = \langle G_{PL} \rangle - \langle G_P \rangle - \langle G_L \rangle$$

$$\langle G \rangle = -k_B T \ln Z$$

To compute the free energy of a "system state", we have to compute the **state's partition function, *Z***

Exact partition functions include all microstates

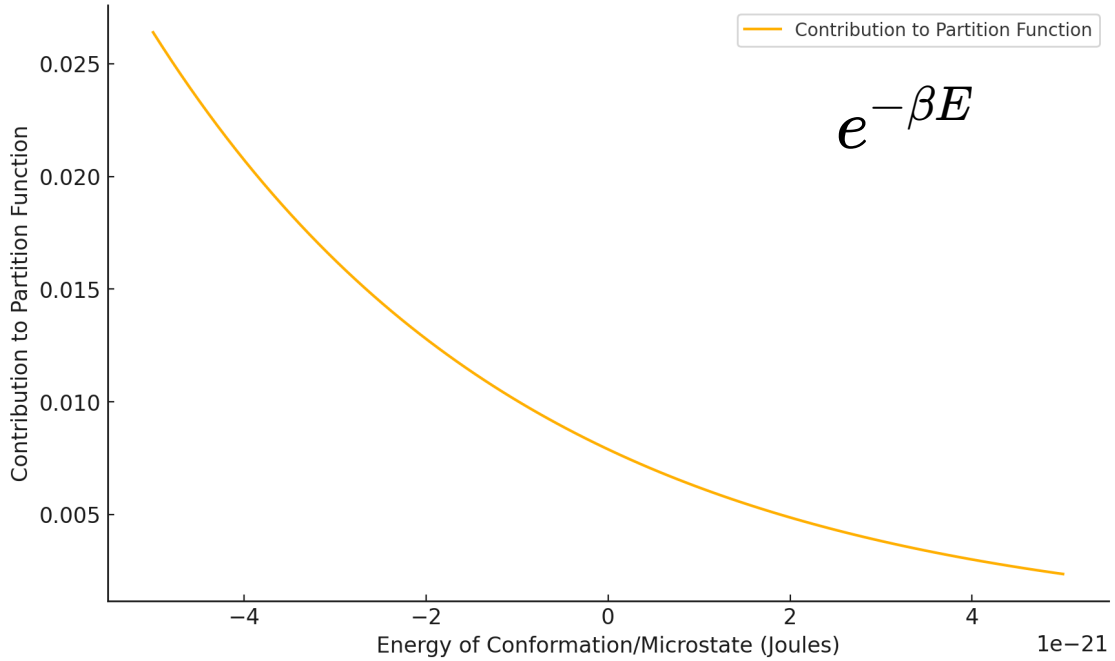
To compute the partition function of protein;
for example, we need to know the energy for

- All possible conformations (folded, partially folded, unfolded)
- All possible atomic positions (backbone and sidechains)
- All possible velocities of the atoms
- All possible rotational states
- All possible vibrational states

This is impossible

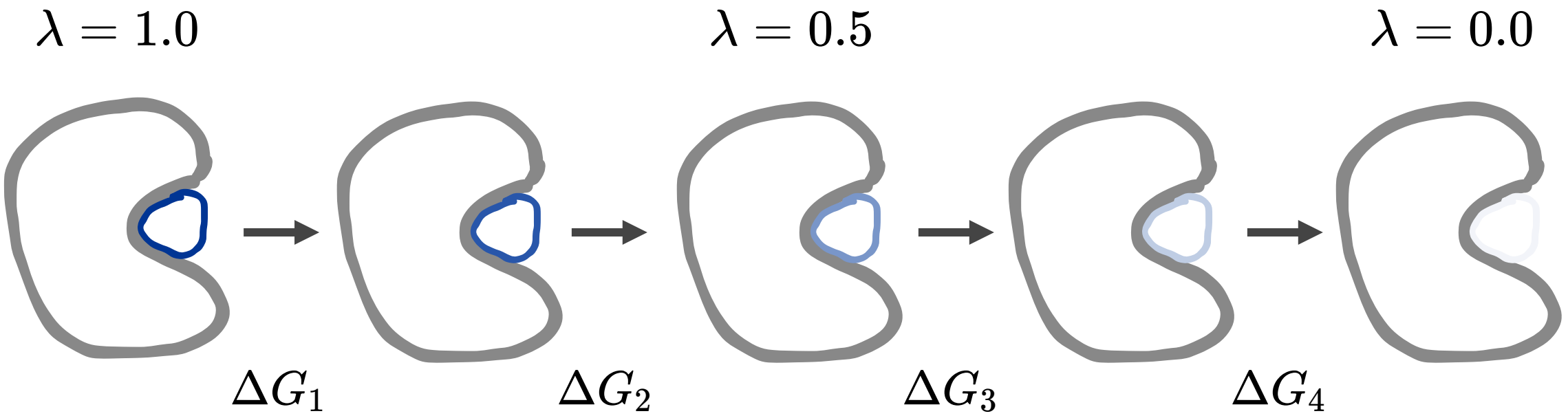
Fortunately, the **low-energy conformations contribute the most to the partition function**

Molecular simulations can sample some low-energy conformations; however, **minor errors will drastically impact absolute free energy calculation**



What if we slowly disappear the ligand?

We could use an alchemical parameter, λ, to scale noncovalent interactions between protein and ligand



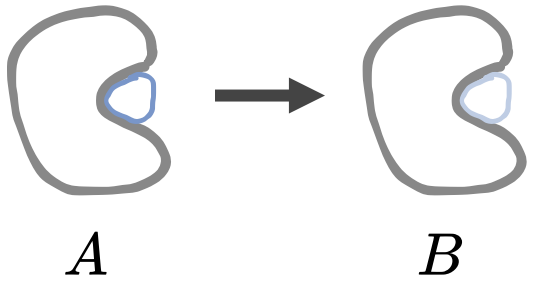
This allows us to sum relative free energies to estimate amount of energy to bind/unbind the ligand

$$\Delta G = \sum \Delta G_i$$

How does this help us?

Relative free energies are expressed as a partition function ratio

The free energy change from state *A* to *B* can be computed as

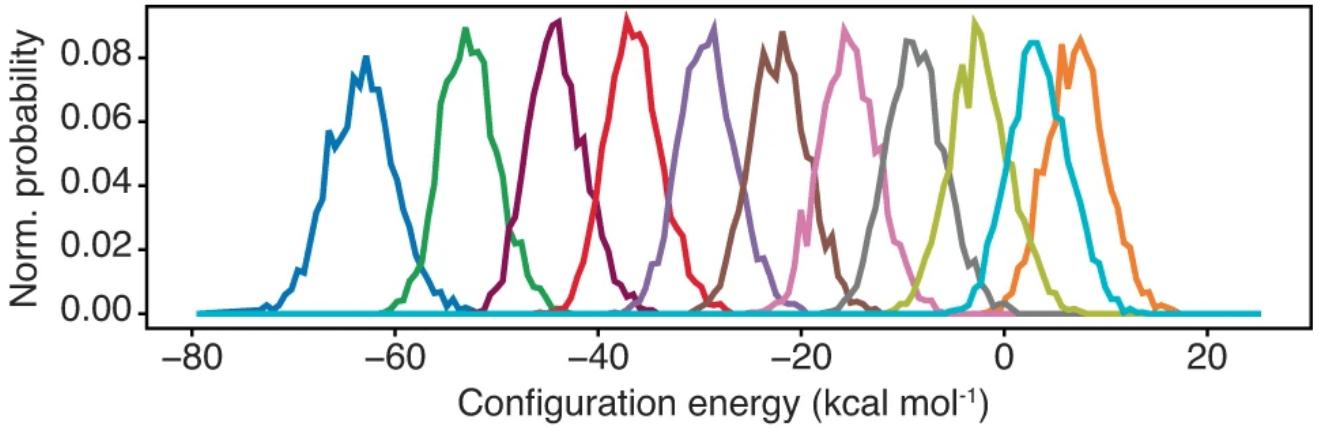


$$\Delta G = -k_B T \ln Z_B + k_B T \ln Z_A = -k_B T \ln \left(\frac{Z_B}{Z_A} \right)$$

Advantage: Partition function ratios are dominated by overlapping microstates common between states *A* and *B*

Maintaining phase space overlap ensures more reliable and converged free energy estimates

(This is conceptually similar to having a small integration step size.)

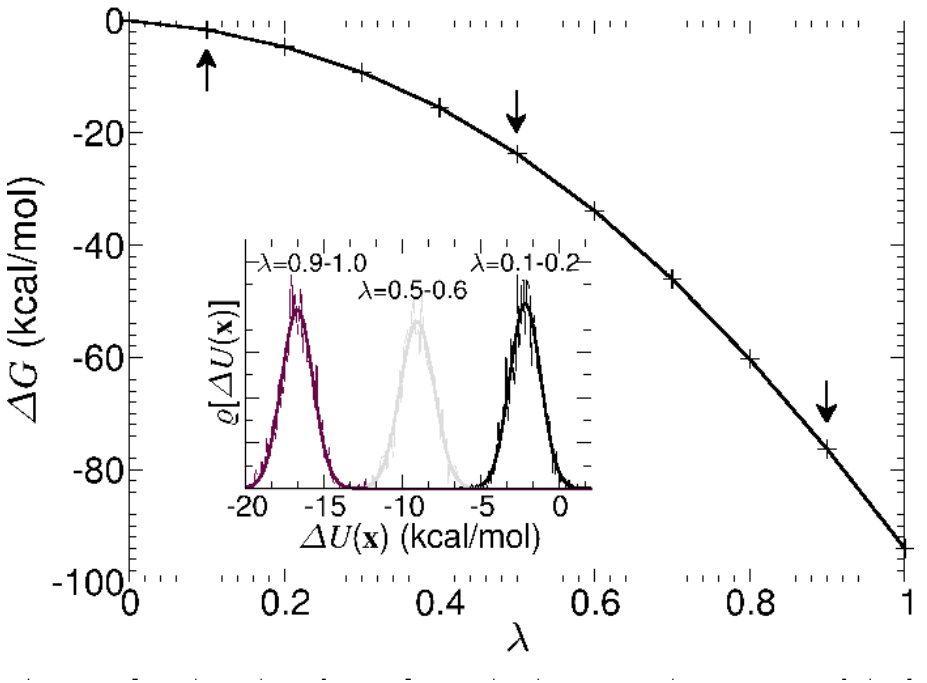


Thermodynamic integration provides a way to compute free energy differences

We can to integrate over these small free energy changes

$$\Delta G_{A\to B} = \int_0^1 \left\langle \frac{\partial U(\lambda)}{\partial \lambda} \right\rangle_\lambda d\lambda$$

We can use this to reliably calculate the free energy difference between bound and unbound states



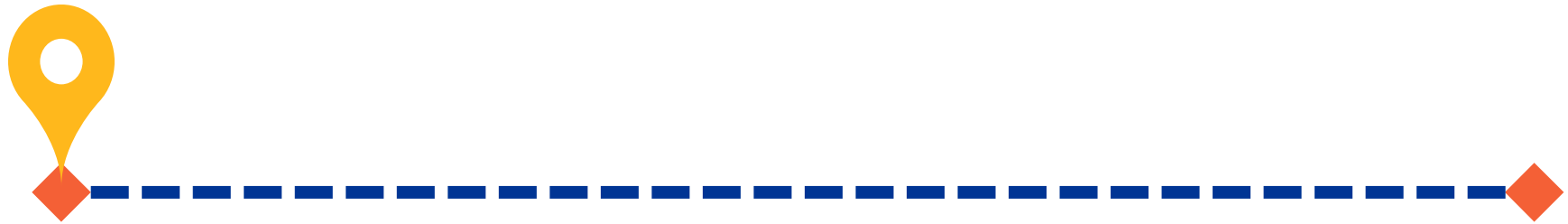
Alchemical simulations are actually very expensive

We use "docking" to more efficiently screen molecules before (if ever) doing alchemical simulations

Before the next class, you should

Lecture 16:
Structure-based drug design

Lecture 17:
Docking and virtual screening



Today

Thursday

- Work on A06