Above: the three-dimensional structure of HIV protease complexed with a small molecule designed by scientists at Dupont-Merck to bind to and inhibit the enzyme. The two monomers of the enzyme are shown in pink and green. The carbon atoms of the small-molecule inhibitor are colored purple, with the corresponding structure shown in the lower left.
Lectures 17-18: The molecular basis of drug-protein binding:

HIV protease inhibitors

1. Drug development and its impact on HIV-infected patients
2. Energetic dissection of a small molecule binding to a protein
   a. Enthalpy changes upon binding
   b. Entropy changes upon binding
3. Case studies of saquinavir and ritonavir, two small-molecule HIV protease inhibitors
   a. Fill hydrophobic pockets with hydrophobic groups
   b. Provide complementary hydrogen bond donors and acceptors
   c. Mimic the transition state of a reaction
   d. Maximize the rigidity of the drug
   e. Displace bound water molecules

Lecture Readings

Required: Lecture Notes
McMurray p. 808-810, 640-642

In the last section of the course, we learned the energetic factors underlying reaction thermodynamics and reaction kinetics. We examined at a molecular level how enzymes such as HIV protease can manipulate some of these energetic factors to accelerate a chemical reaction. We also studied some of the interactions between HIV protease and its substrate, and learned that these interactions collectively determine HIV protease’s substrate specificity.

In the next section of the course, we will study in greater detail the interactions between small molecules and proteins. We will see how an integrated understanding of these interactions has made possible the design of drugs that block the action of a specific enzyme such as HIV protease.

Drugs have had a relatively recent but profound impact on human life. In 1900 only a small fraction of the drugs currently available were known. No drugs existed then to treat any infectious disease, and life expectancy in the U.S. was only 46 years for a man and 48 years for a woman. Today there are thousands of drugs aimed at treating the vast majority of known human diseases. In large part due to the development of many successful drugs over the past century, the life expectancy of U.S. men and women is now greater than 74 and 79 years, respectively. While macromolecular therapeutics such as the protein insulin, various protein antibodies, and “antisense” (mRNA target-complementary) nucleic acids have begun to play an increasingly important role in the treatment of certain diseases, the vast majority of drugs are still small molecules. How can small molecules exert such potent and diverse effects on biological systems?
Impact of Anti-HIV Drugs

• 1990s: anti-HIV drugs transform HIV infection from a short death sentence to a chronic (but very serious) illness

• 13 FDA-approved drugs inhibit HIV reverse transcriptase; 9 drugs inhibit HIV protease (first approved December, 1995)

• Mortality rate of U.S. patients with advanced AIDS:
  • 29% per year in 1995
  • 9% per year in mid-1997

• 1997-2003: Death rate from AIDS in Europe falls 80%

• Gains primarily attributed to combination therapy involving HIV protease inhibitors + other antiretroviral agents

1. Drug development and its impact on HIV-infected patients

The development of anti-HIV drugs in the 1990s has had a particularly profound effect on HIV-positive patients who have access to these therapies. As of early 2006 there are 13 FDA-approved drugs that inhibit HIV reverse transcriptase, and nine FDA-approved drugs that inhibit HIV protease, the most recent major target of anti-HIV drugs. The first HIV protease inhibitors were approved in 1995 and very quickly began to improve the longevity of HIV patients. For example, by mid-1997, the mortality rate of U.S. patients with advanced AIDS (defined by an unusually low count of T-cells per milliliter of blood) dropped more than three-fold compared with the mortality rate in 1995. In Europe, the death rate from 1997-2003 dropped 80%. Both dramatic gains are attributed primarily to combination therapies in which HIV protease inhibitors were administered together with other anti-HIV drugs.
Drug Development is Very Difficult

• Total cost to develop a drug = ~$1 billion + ~10-15 years

Despite such profound benefits of successful drugs, it is important to realize that drug design remains an extremely difficult endeavor. Even though we will describe some of the most successful recently developed drugs, keep in mind that each success typically requires ten to fifteen years and $500,000,000 to $1,000,000,000 of research spending to achieve. The massive amount of effort and expense necessary to develop a drug is largely due to our inability to accurately predict the chemical and biological properties of a proposed small molecule. As a result, for every successful small molecule discovered to have a perfect balance of desirable drug-like properties, researchers typically have to synthesize and test thousands of variants that turned out to possess unacceptable properties. In other words, drug “design” today is still largely driven by educated guesses refined through trial and error. Nevertheless, the principles described in this lecture remain a foundation of modern drug development.
Successful Drugs Must Satisfy Many Chemical and Biological Requirements

1) Potency (affinity)

\[ K_{\text{eq}} = K_a = 1/K_d \]

2) Specificity (toxicity, immunogenicity)

3) Bioavailability

4) Biostability

5) Economics

An effective drug must simultaneously satisfy many complex chemical and biological requirements. First, the drug must be able to bind and to inhibit potently its target (typically a protein)—that is, protein-drug binding must take place with a very large value of \( K_{\text{eq}} (= K_a, \text{the } \text{"association constant"}) \) for the binding equilibrium shown above. In addition, the drug must not bind strongly to a significant number of non-target proteins. For example, binding to beneficial proteins in human cells frequently leads to undesired side effects or toxicity. The specificity of a drug candidate—its ability to bind uniquely to the desired target in the cell—is therefore a crucial property of a successful drug.

In addition to potency and specificity, there are several other requirements of successful drugs. A drug must be bioavailable. Bioavailability commonly refers to either of two important properties. Cellular bioavailability reflects the ease with which a drug reaches the physical location of its target once taken by the patient. In the cases where the target is located inside cells, the drug must readily diffuse across, or be actively transported across, the cell membrane. Oral bioavailability reflects how much drug reaches its target if the drug is taken orally. A drug must possess at least some cellular bioavailability to be effective unless its target protein is naturally exposed to the blood. Drugs that are not orally bioavailable can still be effective but must be injected, a practice that is much less convenient and easy to self-administer than swallowing a pill.

Once present in the body, an effective drug must be stable for the right amount of time. If it is degraded too quickly, for example by enzymes in the liver, then it can fall below effective levels in the body or can generate toxic byproducts. Conversely, drugs that are cleared from the body too slowly can also have their undesired side effects magnified.

Finally, there are economic issues which are important considerations in drug development even though these issues frequent spark ethical debates. Drug companies that choose to treat diseases that are very rare or that afflict only populations that cannot afford the drug typically face an uphill battle to survive in the highly competitive and risky pharmaceutical industry. Sometimes a highly effective drug cannot be produced in a commercially viable way. Achieving an optimal balance of ethical and practical considerations remains an important challenge in industrial drug development.
Lectures 17-18a: The molecular basis of drug-protein binding: HIV protease inhibitors

1. Drug development and its impact on HIV-infected patients
2. Energetic dissection of a small molecule binding to a protein
   a. Enthalpy changes upon binding
   b. Entropy changes upon binding
3. Case studies of saquinavir and ritonavir, two small-molecule HIV protease inhibitors
   a. Fill hydrophobic pockets with hydrophobic groups
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   d. Maximize the rigidity of the drug
   e. Displace bound water molecules
Enthalpy Changes (ΔH) Involving Water Upon Drug-Protein Binding

- Interactions with water can play crucial roles in binding!

2. Energetic dissection of a small molecule binding to a protein

Although potent binding of a drug to its target protein is not the only criterion of an effective drug, it is arguably the most basic and essential feature of all drugs and continues to be the focus of enormous research in the life sciences. To achieve a molecular-level understanding of the changes that take place when a small molecule binds to a protein requires keeping track of the primary changes in enthalpy and entropy that result from binding. Recall that enthalpy changes arise from the formation or loss of interactions between groups (such as hydrogen bonding or ionic interactions), while entropy changes arise from changes in the degree of disorder in molecules.

Enthalpy changes upon binding

The equilibrium of a small molecule binding to a protein in water is shown above. The water molecules in the equilibrium are noted explicitly to emphasize the important point that both the small molecule and the protein binding pocket must lose interactions with water (a process called desolvation) during the process of forming a complex with each other. These interactions between protein and water, or between substrate and water, imply that to achieve potent binding it is not sufficient for the small molecule and the protein to be able to form favorable interactions. Rather, these interactions (plus any favorable gains in entropy, as will be discussed below) must be more favorable than the lost enthalpic interactions with water and losses in entropy (see below) in order to tip the energetic balance to favor binding.
Drug-Protein Binding
Enthalpy (H) Balance Sheet

- Loss of some protein-water interactions: $\Delta H_{P-W} > 0^*$
- Loss of some drug-water interactions: $\Delta H_{D-W} > 0^*$
  * These losses are minimized when the drug and protein binding pocket are more hydrophobic
- Gain of some drug-protein interactions: $\Delta H_{D-P} < 0^{**}$
  - Van der Waals  •  Hydrogen bonding  •  Ionic bonding
  ** These gains are maximized when D & P are complementary
- Gain of water-water interactions: $\Delta H_{W-W} < 0$

Using our understanding of molecular interactions such as hydrogen bonding, electrostatic interactions, and Van der Waals interactions that we’ve already presented on several occasions during this course, we are ready to construct a “balance sheet” of the changes in enthalpy that take place upon drug-protein binding.

As described in the last slide, some of the interactions between the protein and water, and between the drug and water, are lost upon binding of the protein and drug as binding displaces some of the water molecules that previously surrounded the drug binding pocket in the protein and that previously surrounded the part of the drug involved in binding. As a result, we can predict that $\Delta H_{P-W} > 0$ and $\Delta H_{D-W} > 0$, where P-W refers to protein-water interactions and D-W refers to drug-water interactions. These unfavorable changes in enthalpy are minimized when the drug or protein’s drug-binding pocket are hydrophobic and therefore form only minimal interactions with water molecules.

Drug-protein binding also results in some favorable enthalpy changes, in the form of new Van der Waals, electrostatic, and hydrogen-bonding interactions made between the drug and the protein that are not present before binding ($\Delta H_{D-P} < 0$). Finally, the water molecules that previously interacted with the drug or protein but that are now released into the surrounding aqueous solution will form new hydrogen bonds with other water molecules ($\Delta H_{W-W} < 0$).
Loss of Entropy Upon Binding

• Two freely rotating and translating molecules upon binding form one complex
• Both the protein and drug often become more rigid upon binding, leading to additional entropy loss

Entropy changes upon binding

The changes in entropy that take place upon formation of a drug-protein are also crucial to the overall potency of binding. The drug and protein molecules as a collective whole lose a significant amount of freedom to move in three-dimensional space and to rotate. These two forms of freedom are called translational and rotational entropy. The large loss of translational and rotational entropy is a consequence of converting two independently translating and rotating molecules into one complex. As a result, $\Delta S_p < 0$ and $\Delta S_D < 0$.

An additional source of lost entropy of the drug and protein molecules upon binding is more subtle, but also important. Scientists have observed that both the small molecule and the protein tend to become more rigid upon binding one another. This rigidification represents the loss of conformational freedom and therefore causes additional decreases in the entropy of the drug and the protein ($\Delta S_p < 0$ and $\Delta S_D < 0$).
Releasing Water Molecules into “Bulk Solvent” is Entropically Favorable

• Recall: the increase in entropy as water molecules are released into “bulk solvent” is the basis of the hydrophobic effect

These unfavorable changes in entropy can be offset by favorable increases in the entropy of water molecules. Binding of the small molecule and protein causes previously ordered water molecules to be released into the rest of the aqueous solvent (also called "bulk solvent"). Because bulk solvent is highly disordered, the entropy of these water molecules increases ($\Delta S_w > 0$) once the water-exposed surface area of the hydrophobic protein and small-molecule groups decreases. The resulting changes in energy are the basis of the hydrophobic effect described earlier.
Drug-Protein Binding
Entropy (S) Balance Sheet

• Protein loses translational and rotational entropy: $\Delta S_P < 0$
• Drug loses translational and rotational entropy: $\Delta S_D < 0$
• Protein and drug rigidity increases: $\Delta S_D < 0^*\text{, } \Delta S_P < 0^*$
  
  * To minimize this loss, pre-rigidify the drug

• Bound water gains entropy when released: $\Delta S_W > 0^{**}$
  
  ** To maximize this gain, design the drug to displace bound water molecules wherever possible

These decreases and increases in entropy are collectively summarized here and imply two fruitful strategies for the design of effective drugs. The loss of entropy that arises from the rigidification of the drug upon binding is minimized if the drug is already rigid. Therefore, scientists often attempt to minimize the number of available conformations of a drug by designing its structure to be as rigid as possible, so long as this conformation is compatible with binding to the protein target. You can think of this strategy as “pre-paying” the entropic cost of ordering the drug into an optimal binding conformation before the drug is administered.

Second, the gains in entropy that are realized when previously ordered water molecules are released into bulk solvent is maximized by designing the structure of a drug to displace ordered water molecules in the free protein whenever possible.

We’ll see specific examples of how each of the conclusions from our enthalpy and entropy balance sheet can be implemented in the design of actual drugs later in this lecture.
Changes in Free Energy and Entropy
Upon Drug-Protein Binding

\[ \Delta G = \Delta H - T\Delta S \]

- \( G \) = Free energy
- \( H \) = Enthalpy (heat)
- \( T \) = Temperature in Kelvin
- \( S \) = Entropy (disorder)

In general, for \( \Delta G \) of binding to be negative (favoring binding):

Favorable enthalpic interactions (\( \Delta H_{P-D} < 0 \)) between the protein and drug and favorable changes in the entropy of water (\( \Delta S_{\text{water}} > 0 \)) must overcome...

Unfavorable entropy loss in the protein and drug (\( \Delta S_P \) and \( \Delta S_D < 0 \)), as well as the loss of enthalpic interactions between water and the protein or small molecule (\( \Delta H_{P-W} \) and \( \Delta H_{D-W} > 0 \))

Let’s put together the concepts in the last few slides to achieve a general picture of the energetic changes that take place when a small molecule and a protein bind to each other. In order for binding to take place spontaneously (\( \Delta G < 0 \)), highly favorable enthalpic interactions (\( \Delta H < 0 \)) between the protein and small molecule in the form of the favorable Van der Waals, hydrogen bonding, or electrostatic interactions described above together with any favorable change in water’s entropy must overcome the loss of favorable enthalpic interactions with water and any entropy loss in the protein and small molecule. The sum of the favorable and unfavorable energies that change upon binding determines the binding affinity of complex (recall that \( \Delta G^0 = -RT \ln(K_{eq}) \), and therefore \( K_{eq} = e^{-\Delta G^0/RT} \)). In general these changes in enthalpy and entropy have the signs shown above, but unusual examples may differ from this example.

When \( \Delta G^0 \) is highly negative, \( K_{eq} \) for a protein + small molecule forming a complex is very large, reflecting an equilibrium association constant (\( K_a \)) that strongly favors binding.

Conversely, a positive \( \Delta G^0 \) leads to a small \( K_a \), reflecting the fact that the unbound state is favored. Scientists frequently use dissociation constants (\( K_d \)) instead of \( K_a \) to describe the strength of binding. \( K_d \) is simply the equilibrium constant of the binding equilibrium written in the opposite direction (complex to the left of the arrows, and protein + small molecule to the right). Therefore, \( K_d = 1 / K_a \). Most biological binding events have \( K_d \) values in the mM to nM (10^{-3} M to 10^{-9} M) range, while most drug-protein binding events have \( K_d \) values in the µM to pM (10^{-6} M to 10^{-12} M) range.
Are you a Ron, a Harry, or a Hermione?
Breakout Question

Consider the two HIV protease inhibitors 1 and 2. Assuming that 1 and 2 bind HIV protease in a similar manner (depicted below), which of the following is most likely to be true?

A) A greater number of ordered water molecules will be released into bulk solvent when 1 binds than when 2 binds.
B) The $\Delta S$ of the water in the system upon binding of 2 will be at least as positive as the $\Delta S$ of the water molecules upon binding of 1.
C) The $\Delta H_{P-D}$ of interactions formed between 1 and HIV protease upon binding is more negative than the $\Delta H_{P-D}$ of 2 and HIV protease.
Breakout Question

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Lectures 17-18a: The molecular basis of drug-protein binding:

HIV protease inhibitors

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   c. Mimic the transition state of a reaction
   d. Maximize the rigidity of the drug
   e. Displace bound water molecules
3. Case studies of two HIV protease inhibitors: saquinavir and ritonavir

We are now ready to examine two specific case studies: the first two FDA-approved HIV protease inhibitors, saquinavir and ritonavir. As we discussed earlier, these and other HIV protease inhibitors have revolutionized the treatment of HIV-infected patients, resulting in major improvements in the quality and length of their lives. These two drugs have very different structures, even though they bind to the same protein with comparably high affinity ($K_d \approx 1 \text{ nM}$). Based on our understanding of the energetics of drug-protein binding, we can pick out five distinct chemical strategies that explain how each drug achieves potent binding to HIV protease.

a. Fill hydrophobic pockets with hydrophobic groups

As we learned above, the affinity of a small molecule for a protein is dependent on maximizing the number and quality of favorable interactions between the two molecules while minimizing the unfavorable interactions. In practice, a major goal of drug design is therefore to design hydrophobic regions of a small molecule that fill as completely as possible the hydrophobic binding pockets of a protein target, so that the maximum number of Van der Waals interactions can be made between the drug and the protein, and so that the greatest number of ordered water molecules can be released into bulk solvent upon binding.
The hydrophobic groups of saquinavir fit precisely into hydrophobic binding pockets in HIV protease.

Saquinavir contains several six-membered rings made predominantly of carbon and hydrogen atoms. A close examination of the structure of HIV protease bound to saquinavir reveals that two of these hydrophobic rings adopt a three-dimensional conformation that fits precisely into a binding pocket of HIV protease made of amino acid side chains Leu 23, Pro 81, Val 82, and Ile 84. As you know from your understanding of amino acid structure, the side chains of these four amino acids are made entirely of hydrophobic hydrocarbon groups, making this binding pocket of HIV protease truly hydrophobic.
Hydrophobic Surface Complementarity: Saquinavir and HIV Protease

As you can see from the space-filling structure, the fit between saquinavir and HIV protease is sufficiently tight in this region that there are no observed water molecules trapped between the protease and small molecule. Therefore, saquinavir maximizes the entropic benefit of releasing water molecules into bulk solvent upon binding to HIV protease (makes more positive $\Delta S_W > 0$), and also maximizes the enthalpic benefit of Van der Waals interactions between the protease’s active site and the complementary surface of the drug (makes more negative $\Delta H_{D,P} < 0$).
Hydrophobic Surface Complementarity: Saquinavir vs. the “Runner-Up” Candidate

- Removing one of the hydrophobic pocket-filling groups of saquinavir (only 4 carbons!) greatly reduces binding potency

What happens when hydrophobic surface complementarity between a small molecule and a protein is less ideal? In most cases it is extremely difficult to isolate the effect of a single hydrophobic interaction on binding. However, saquinavir presents a rare opportunity. It turns out that the “runner-up” molecule to saquinavir lacks one of the two hydrophobic six-membered rings that in saquinavir fill the hydrophobic binding pocket of HIV protease. Other than this difference, the runner-up molecule is identical to saquinavir. When scientists compared the ability of the runner-up and saquinavir to inhibit the activity of HIV protease, they found that saquinavir is about ten times more potent (has a 10-fold lower $K_i$) than the runner-up! The simple addition of a hydrophobic six-membered ring to the runner-up therefore increased the strength of the resulting molecule’s binding to HIV protease by about an order of magnitude and was one of the final breakthroughs in the development of the first FDA-approved HIV protease inhibitor. Saquinavir is therefore a striking example of how a simple change in a small molecule that improves its ability to complement a hydrophobic surface can have a profound and important effect on target protein binding.
Hydrophobic Surface Complementarity: Ritonavir and HIV Protease

Animation rendered by Brian Tse
As a second example of the principle of filling hydrophobic binding sites with hydrophobic groups on a small molecule, examine the structure of ritonavir bound to HIV protease. Ritonavir contains four hydrophobic groups: two isopropyl (–CH(CH₃)₂) groups and two phenyl (–C₆H₅) groups. When bound in the HIV protease active site, ritonavir adopts a conformation that places one of the isopropyl groups next to one of the phenyl groups.
Collectively these two hydrophobic groups precisely complement the hydrophobic surface of the protease created by the side chains of Leu 23, Pro 81, Val 82, and Ile 84. Note that these amino acids are the same four hydrophobic residues that formed the hydrophobic binding pocket for part of saquinavir, even though the structures of the small-molecule groups being bound are very different. We can conclude that there are multiple distinct small-molecule structures that are capable of adopting conformations that fit a particular hydrophobic binding pocket in a protein. In addition, the amino acids that make up an enzyme's binding pocket are capable of assuming different conformations to maximize favorable interactions with different small molecules, a principle known as *induced fit*.
HIV Protease Changes Shape Slightly When Binding Saquinavir vs. Ritonavir
HIV Protease Changes Shape Slightly When Binding Saquinavir vs. Ritonavir

HIV Protease + Saquinavir
Hydrogen Bonding: Saquinavir and HIV Protease

- Saquinavir complements hydrogen bond donors provided by the enzyme, enhancing favorable (negative) $\Delta H_{D-P}$

b. Provide complementary hydrogen bond donors and acceptors

In addition to filling hydrophobic binding sites of the protein with complementary hydrophobic groups from the small molecule, a second principle underlying drug design is the placement of small-molecule hydrogen bond donors and acceptors to complement the acceptors and donors presented by the protein. Installing such hydrogen bonding groups is very challenging because small differences in the placement or orientation of the hydrogen bonding groups can significantly reduce the stability of the resulting hydrogen bond. Therefore, scientists often rely on mimicking the hydrogen bond donors or acceptors that are present in the natural substrate of the target enzyme under the assumption that the enzyme has already evolved to form strong hydrogen bonds with these groups.

Both saquinavir and ritonavir contain hydrogen bond acceptors that participate in hydrogen bonds with enzyme-associated hydrogen bond donors. For example, both inhibitors contain two carbonyl (C=O) groups near their centers. These two carbonyl groups serve as hydrogen bond acceptors to the bound water molecule that is held in place by hydrogen bonds with HIV protease residues Ile 50 and Ile 50'. Two other carbonyl groups in saquinavir and one other carbonyl group in ritonavir also serve as hydrogen bond acceptors to enzyme hydrogen bond donors. The formation of drug-protein hydrogen bonds contributes to binding affinity by making more favorable (more negative) the enthalpy change upon binding ($\Delta H_{D-P} < 0$).
Hydrogen Bonding: Ritonavir and HIV Protease

- H-bonds between small molecules and proteins help to offset the penalty of giving up H-bonds to water upon binding

Just as we discussed in the case of DNA hybridization, these hydrogen bonds may not be major energetic driving forces behind the binding of small molecules to proteins because even in the unbound state these groups can make hydrogen bonds to bulk solvent water molecules. However, in the inhibitor-enzyme complex, these hydrogen bonds help to favor binding by offsetting the energetic penalty that would otherwise be incurred upon giving up hydrogen bonds to water molecules.
Recall from our recent discussion of enzyme catalysis that enzymes must bind the transition state of a chemical reaction better than they bind the substrate in order for catalysis to take place. Scientists have applied this principle to drug design by designing small molecules that mimic as closely as possible the transition state of the reaction catalyzed by the target enzyme. Of course it is impossible to mimic a transition state perfectly because, by definition, transition states are fleeting, unstable species and often contain partial charges and partially formed bonds. Nevertheless, in many cases it is possible to approximate the geometry and charge distribution of a transition state through careful design.

For example, recall the first transition state of HIV protease-catalyzed peptide bond cleavage. You will notice that this transition state resembles the tetrahedral intermediate that we previously described and is partially tetrahedral at the carbon atom that is being attacked. As you learned earlier during the lecture introducing proteins, a simple amide group is not tetrahedral but instead is planar and therefore would not bind as potently to the enzyme active site as a transition state analog containing a tetrahedral carbon atom at this position.
Virtually all HIV protease inhibitor drugs use a simple tetrahedral carbon connected to a hydroxyl group as a transition state mimic. You may wonder if an even better transition state mimic would be a tetrahedral carbon bonded to both a hydroxyl group and to an amine (–NH–) group. Indeed, such a molecule (called a hemiaminal) would more closely approximate the transition state of the HIV protease-catalyzed reaction; however, such a molecule is chemically unstable in water and therefore is not suitable for either research or drug purposes.

As this example demonstrates, scientists must balance several often-conflicting goals when designing drug candidates. The HIV protease inhibitor with the highest possible affinity may be chemically unstable, or impossible to synthesize on an industrial scale, or toxic to patients, or cleared too quickly by the liver. Finding a molecule that simultaneously meets all of these complex criteria is a daunting challenge that requires most of the time and expense associated with drug development.
Rigid Versus Flexible Inhibitors

D. Maximize the rigidity of the drug

Earlier we mentioned that most small molecules and proteins become more rigid upon formation of the small molecule-protein complex. This increase in rigidity decreases the entropy of these molecules and is therefore unfavorable. Scientists have taken advantage of this fact by rigidifying inhibitors as much as possible, so that they have very little entropy to lose upon binding to their protein targets and therefore do not incur as great an entropic penalty as a more flexible inhibitor binding to a protein.

Once again an HIV protease inhibitor provides an excellent example. Examine the structure of the Dupont-Merck HIV protease inhibitor shown here. You’ll notice that unlike saquinavir or ritonavir, this inhibitor is not predominantly linear in structure but instead contains a large ring at its central core. Because a cyclic molecule is more rigid than a linear molecule of the same length and composition, this cyclic inhibitor suffers less entropy loss upon binding to HIV protease (its binding results in a less negative $\Delta S_D$ than the case of a floppy inhibitor). As a result, the rigid inhibitor binds to the enzyme with higher affinity (more negative $\Delta G$, larger $K_{eq}$ for binding) than would the corresponding flexible, acyclic molecule. Such a flexible variant must first adopt the conformation compatible with binding to HIV protease out of many more possibilities than in the case of a rigid inhibitor; this requirement imposes a significant entropic penalty, resulting in a more negative $\Delta S_D$. 

- Rigidity reduces entropy lost upon binding (less negative $\Delta S_D$)
e. Displace bound water molecules

Another important design feature of the Dupont-Merck inhibitor is more subtle, but also very important to its ability to bind HIV protease. Compare the observed structure of the empty HIV protease active site with that of the protease bound to the Dupont-Merck inhibitor. You’ll see that the bound water molecule we previously noted (the one held in place by hydrogen bonds to Ile 50 and Ile 50’) has been replaced by an oxygen atom from the small-molecule inhibitor upon binding. This water molecule is highly ordered in the unbound enzyme, but is released into bulk solvent (and is therefore highly disordered) in the enzyme-inhibitor complex. The release of this formerly bound water molecule is therefore highly favorable from an entropic standpoint, making more positive $\Delta S_W$. This example is thought to demonstrate that a small molecule’s ability to mimic and displace a bound water molecule from an enzyme active site can contribute significantly to its affinity for the enzyme.
Replacing a Bound Water Molecule
With a Small-Molecule Group

• The Dupont-Merck inhibitor replaces the bound water in the HIV protease active site with a carbonyl oxygen
• Releasing bound water is entropically favorable ($\Delta S_w > 0$)

A deep understanding of the nature of interactions between molecules and the ways in which these interactions influence the binding of a small molecule to a protein target is crucial to drug development. However, as we noted earlier, this understanding is only one necessary component of successful drug development. Our ability to design other essential features of drugs in addition to target binding, including low toxicity, efficacy when taken orally, resistance to being altered in the liver, and long lifetime in blood stream remains modest at best. Improving the efficiency of the drug discovery process is a key opportunity for future scientists.

In the next section of the course we will learn how HIV protease has evolved in response to the widespread use of HIV protease inhibitors as anti-AIDS therapeutics. The incessant chemical warfare between scientists and the organisms they seek to combat has been a major focus of the life sciences over the past several decades, and ensures that there will always be crucial roles for students such as yourselves to play in our efforts to apply the key principles of chemistry and biology to the treatment of disease.
Key Points: Molecular Basis of Drug Binding

• Drug development has had a major impact on society and on the lives of patients infected with HIV

• Effective drugs must meet several chemical and biological requirements, including potent binding to a target

• The combination of enthalpic and entropic changes that occur upon small molecule-protein binding ultimately determines the binding potency ($K_d$) of a drug

• HIV protease inhibitors bind favorably by (i) filling hydrophobic pockets with complementary hydrophobic groups, (ii) providing hydrogen bonding partners, (iii) mimicking the amide hydrolysis transition state, (iv) being rigid, and (v) releasing bound water molecules