CHAPTER

14

The Role of Functional Groups in Drug–Receptor Interactions

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OUTLINE

Alice remained looking thoughtfully at the mushroom for a minute, trying to make out which were the two sides of it; and, as it was perfectly round, she found this a very difficult question. ***(Alice's Adventures in Wonderland, Lewis Carroll)***

I. INTRODUCTION

An understanding of noncovalent interactions in ligand–receptor complexes is essential for the appreciation of drug action mechanisms as well as for rational drug design. The purpose of this chapter is to provide an overview of the physical and chemical factors that contribute most significantly to the strength of drug–receptor interactions [1–3]. The first part consists of a physical description of the influence of electrostatic and steric match on the various types of nonbonded drug–receptor interactions. The second part provides a chemical interpretation, concentrating on the intrinsic strengths of individual functional group contributions to the affinity of drugs for their receptors. Some practical applications for the medicinal chemist will then be proposed. The concept of cooperative binding will be exposed in the conclusion in order to underline the limitations of the pure additive calculation method based on average binding energies.

II. GENERAL PRINCIPLES

When a ligand and a receptor are sufficiently close, the ligand can diffuse up to and dock into its binding site on the receptor. This may first be mediated by the long-range electrostatic interactions between the ligand and the receptor, and then strengthened by short-range hydrogen bonds and van der Waals' interactions. Binding is accompanied by conformational changes ranging from modest shifts of a few atoms to movements of whole macromolecule domains. When the ligand is a drug, its biological activity is directly related to its affinity for the receptor, that is, the stability of the drug–receptor complex. The strength of this interaction is measured by its K_d, the dissociation constant for the complex at equilibrium:

$$K_d = \frac{[\text{drug}][\text{receptor}]}{[\text{complex}]} \tag{14.1}$$

The smaller the K_d is the greater is the affinity of the drug for the receptor. This dissociation constant is related to the corresponding Gibbs free energy change, which itself is composed of an enthalpic (ΔH) and entropic contribution ($T\Delta S$) [1,4]:

$$\Delta G = -2.303\ RT \log K_d = \Delta H - T\Delta S \tag{14.2}$$

Under physiological conditions (T = 310 K) this is approximated (in kJ/mol) by

$$\Delta G = -5.85 \log K_d \tag{14.3}$$

The experimental measurement of the equilibrium constant thus provides a direct calculation of ΔG. Typically, K_d is in the range of 10^{-2} and 10^{-12} M, meaning that the affinity of a ligand toward its receptor falls into an energy interval between −10 and −70 kJ/mol in aqueous solution [5]. A drug binding with a K_d of 10^{-9} M requires, for example, $(-5.85) \times (-9) = 52.6$ kJ/mol to dissociate from the receptor.

According to Eq. 14.2, ligand–receptor interactions are characterized by enthalpy–entropy compensation in which one term favors and the other disfavors binding. While enthalpic contributions include electrostatic, hydrogen bond, and van der Waals' interactions, entropic contributions arise from several sources. On the one hand, the loss of flexibility upon binding has an important entropic cost, which is counterbalanced on the other hand by the displacement of ordered water molecules. This will be discussed in the next section along with the various types of drug–receptor interactions.

III. THE IMPORTANCE OF THE ELECTROSTATIC AND STERIC MATCH BETWEEN DRUG AND RECEPTOR

What determines K_d? In other words, how does the binding affinity relate to structural properties of a complex and its formation from separate, individually solvated species? What are the prerequisites that allow a receptor to bind a ligand tightly and selectively? On first glance, the most important requirement appears to be a good steric and electronic complementarity between receptor and ligand, usually described by van der Waals and Coulomb interactions. Although most noncovalent interactions depend to some degree on both types of complementarity, we will separate them in the following discussion into those which are primarily electrostatic and those which are primarily steric.

A. Electrostatic Interactions

Electrostatic interactions are the net result of the attractive forces between the positively charged nuclei and the negatively charged electrons of the two molecules. The attractive force between these opposite charges leads to three main bond types: charge–charge, charge–dipole, and dipole–dipole interactions. The strength of any electrostatic interaction can be calculated with Eq. 14.4, where q_i and q_j are two charges separated by a distance r_{ij} in a medium of dielectric constant ε. This equation applies equally to ionic interactions, where the charges q_i and q_j are integer values, and to polar interactions, in which the total energy is summed over the contributions calculated from the partial charges on all the individual atoms.

$$E = \frac{q_i \cdot q_j}{\varepsilon \cdot r_{ij}} \tag{14.4}$$

TABLE 14.1 Main Ionizable Groups in Proteins and Nucleic Acids [6,7]

Chemical function	Charge	pK_a
FULLY OR ALMOST FULLY IONIZED GROUPS AT pH 7.4		
Carboxyl α (terminal COOH)	−	1.8–2.4
Carboxyl β (Asp)	−	3.7
Carboxyl γ (Glu)	−	4.3
Primary phosphoryl	−	0.7–1.0
Secondary phosphoryl	−	5.9–6.0
α-Ammonium (Lys)	+	10.5
Guanidinium	+	12.5
PARTIALLY IONIZED GROUPS AT pH 7.4		
Sulfhydryl (Cys)	−	8.2
Imidazolium (His)	+	6.0
α-Ammonium (terminal peptide NH_2)	+	7.5–10.3
N Amidic (Glu)	+	0.1
N Amidic (Asp)	+	8.8
NONIONIZED GROUPS AT pH 7.4		
Phenolic hydroxyl (Tyr)	−	10.0
Heteroaromatic hydroxyl (uracyl, thymine, guanine)	−	9.2–9.8
Osidic hydroxyl	−	12.3–12.6
Amino residue (adenine, guanine, cytosine)	+	3.3–4.6

1. *Charge–Charge Interactions or Ionic Bonds*

According to Eq. 14.4, the strength between two charges is inversely proportional to the distance separating them. Since the strengths of other noncovalent bonds are even more sharply dependent on distance than that of ionic bonds, ionic attraction frequently dominates the initial long-range interactions between drugs and receptors. A simple ionic interaction basically provides a ΔG of -20 kJ/mol. It also follows from Eq. 14.4 that the strengths of ionic interactions are crucially dependent on the dielectric constant ε of the surrounding medium. Indeed, in biological systems, charges are often separated by water or other molecules and dielectric micro-environments are variable, with less shielding of charges in regions of hydrocarbon side-chains and greater shielding in regions of polar side-chains. For instance, in hydrophobic pockets, like the interior of a protein molecule, the dielectric constant is around 4, whereas in bulk-phase water the corresponding value is 80. In other environments, intermediate values are appropriate, for example, for interactions occurring near the surface of a protein, an ε value of 28 is commonly used. Charge–charge interactions between biological systems and drugs are possible insofar as ionic species are strongly present in biomacromolecules and drugs at physiological pH. Cationic environments are provided by protonation of basic groups such as the amino acid side-chains lysine, arginine, and—to a much lesser extent—histidine (Table 14.1). On the other hand, acidic groups, such as the carboxylic acid side-chains of glutamic acid and aspartic acid, are deprotonated to give anionic groups.

Concerning drugs, both cationic and anionic compounds are commonly used (protonated basic side-chain, protonated aza heterocycles, deprotonated carboxylic acids, enolic species, and acidic sulfonamides; Figure 14.1).

The antihypertensive drug captopril is an example of a molecule that participates in an ionic bond with the Lys1087 residue of the angiotensin-converting enzyme (ACE) receptor (Figure 14.2).

2. *Charge–Dipole and Dipole–Dipole Interactions*

Molecules composed of atoms of different electronegativities usually have an asymmetric distribution of electrons, which produces electronic dipoles. These dipoles within a cell or in aqueous medium can be attracted by a

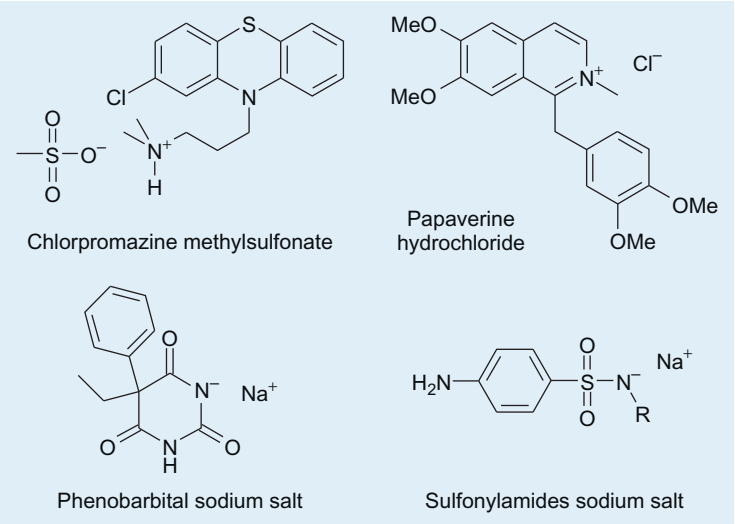


FIGURE 14.1 Cationic and anionic drugs.

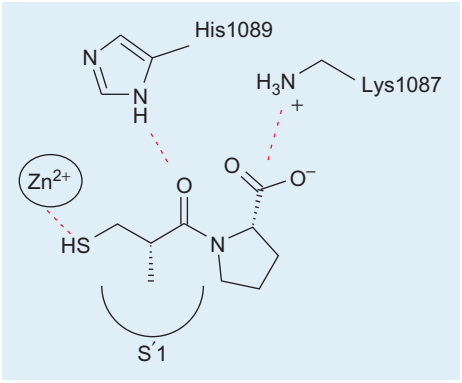


FIGURE 14.2 Captopril as example of a charge–charge interaction with the ACE receptor.

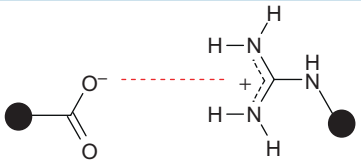
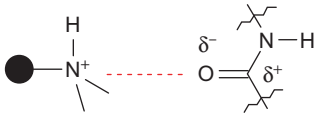
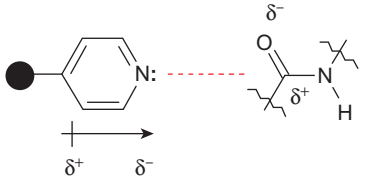
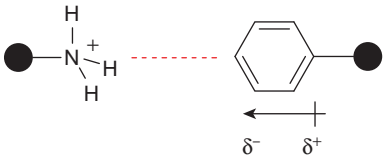
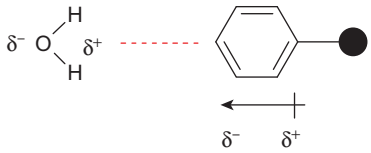
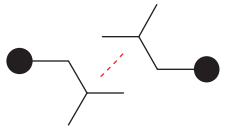
close-by ion, establishing so-called charge–dipole interactions. A permanent dipole can also interact with another permanent dipole, leading to a dipole–dipole interaction. In a recent review, Diederich et al listed these orthogonal multipolar interactions, among which are C–X···C=O (X=halogen), C=O···CO, C≡N···C=O, S=O···C=O, C–OH···C=O, and H_2O···C=O [8]. Because the charge of a dipole is less than that of an ion, charge–dipole and dipole–dipole interactions are weaker than ionic bonds. They are nevertheless key contributors to the overall strengths of drug–receptor interactions, since they occur in any molecule in which electronegativity differences between atoms result in significant bond, group, or molecular dipole moments. The key differences between ionic and dipolar interactions relate to their dependence on distance and orientation (Table 14.2).

Indeed, while steric effects are of little importance in ionic interactions, stricter geometric requirements apply to dipolar interactions, which may be either attractive or repulsive, depending on the orientation of the dipole moments. In most cases, these interactions provide a ΔG of -5 to -30 kJ/mol. The insomnia drug zafirlukast gives an illustration of dipolar interactions (Figure 14.3).

3. *Inductive Interactions*

The electric field generated by a charged molecule or a molecule with a permanent dipole can induce a dipole in a second molecule that is located nearby in space. The strength of the interaction depends on the dipole moment of the first molecule and the polarizability of the second. That means when an electron-donating molecule (or group) comes into contact with an electron-withdrawing molecule (or group), the donor may transfer some of its charge to the acceptor, forming a charge–transfer complex. In the case of an intramolecular

TABLE 14.2 Types of Noncovalent Interactions

Type of interaction	Energy and dependency on distance	Example
Charge–charge: Longest-range force, nondirectional	ΔG of −20 to −40 kJ/mol1/r	
Charge–dipole: Depends on orientation of dipole	ΔG of −12 to −20 kJ/mol1/r^2	
Dipole–dipole: Depends on mutual orientation of dipoles	ΔG of −4 to −12 kJ/mol1/r^3	
Charge-induced dipole: Depends on polarizability of molecule in which dipole is induced	ΔG of −2 to −10 kJ/mol1/r^4	
Dipole-induced dipole: Depends on polarizability of molecule in which dipole is induced	ΔG of −2 kJ/mol1/r^6	
Dispersion: Involves mutual synchronization of fluctuating charges	ΔG of −2 to −4 kJ/mol1/r^6	
Hydrogen bond: Charge attraction 1 partial covalent bond	ΔG of −4 to −30 kJ/mol	

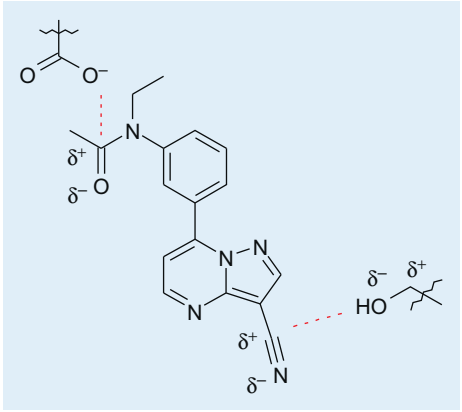


FIGURE 14.3 Zaleplon as example of ion–dipole and dipole–dipole interactions.

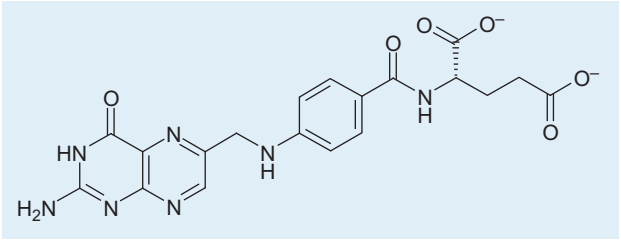


FIGURE 14.4 Folate structure.

TABLE 14.3 Potential Hydrogen Bond Donor and Acceptor Groups Classified According to Their Strength of Interaction [12]

	Donor[a]	Acceptor
Very strong	N^+H_3, X^+–H, F–H	CO_2^-, O^-, N^-, F^-
Strong	O–H, N–H, Hal–H	O = C, O–H, N, S = C, F–H, Hal^-
Weak	C–H, S–H, P–H, M–H	C = C, Hal–C, π, S–H, M, Hal–M, Hal–H, Se

[a]*X is any atom, Hal is any of the lighter halogens, and M is a transition metal.*

redistribution of charge, this will be referred to as an induced polarization, whereas a redistribution of charge between two molecules is described as a charge–transfer interaction. In either case, the resulting interactions are always attractive and strongly dependent on the distance separating the two molecules, as well as on the difference between the ionization potential of the donor and the electron affinity of the acceptor. Donor groups contain π-electrons, such as alkenes, alkynes, and aromatic moieties with electron-donating substituents, or groups presenting nonbonded electrons pairs (O, N, S). Acceptor groups contain electron-deficient π-orbitals, such as alkenes, alkynes, and aromatic moieties having electron-withdrawing substituents, and weakly acidic protons. Groups on receptors acting as electron donors are, for instance, the aromatic ring of tyrosine or the carboxylate group of aspartate. Cystein is an example of an electron-withdrawing group, whereas histidine, tryptophan, and asparagine are both electron donors and acceptors. In general, this type of interaction can contribute as much as 2–3 kJ/mol.

An interesting example of the importance of inductive interactions is the calculation by Bajorath et al on the binding of folate (Figure 14.4) and dihydrofolate to dihydrofolate reductase. This revealed a shift in net charge equivalent to half an electron from the pteridine ring to the glutamate moiety on binding to the enzyme, with the major change in density being focused on the bonds that are catalytically reduced [9].

4. *Hydrogen Bonds*

Hydrogen bonds are specific, short-range, and directional nonbonded interactions. They occur between a hydrogen atom bound covalently to an electronegative atom (usually N, S, or O) and an additional electronegative atom (Table 14.3). Distances of 2.5–3.2 Å between hydrogen-bond donor X and Y and X–H···Y angles of 130–180° are typically found [10]. Their strength is optimal when the three concerned atoms are aligned and when the H donor tends to point directly at the acceptor electron pair. As a result of its electrostatic nature, the strength of a hydrogen bond depends also on its microscopic environment and on the local dielectric constant ε of the surrounding medium (Coulombic interaction energy is proportional to ε^{-1}). Therefore, buried hydrogen bonds are regarded as more important for protein–ligand interactions than those formed in solvent-exposed regions [11]. The free energy for hydrogen bonding can be between −4 and −30 kJ/mol, but usually is in the range of −12 to −20 kJ/mol. Binding affinities increase by about one order of magnitude per hydrogen bond.

Although their strength is weaker than ionic or covalent bonds, they are in general the predominant contribution to the specificity of molecular recognition [13,14]. They help also to determine the conformation and folding ways of numerous macromolecules.

The double helical structure of DNA, for example, is due largely to hydrogen bonding between the base pairs, which link one complementary strand to the other and enable replication (Figure 14.5). Hydrogen bonds are also

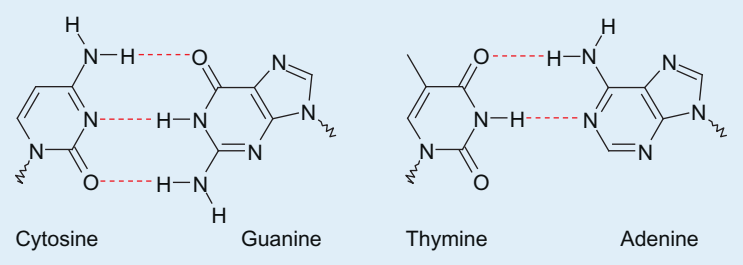


FIGURE 14.5 Hydrogen bonds between DNA base pairs.

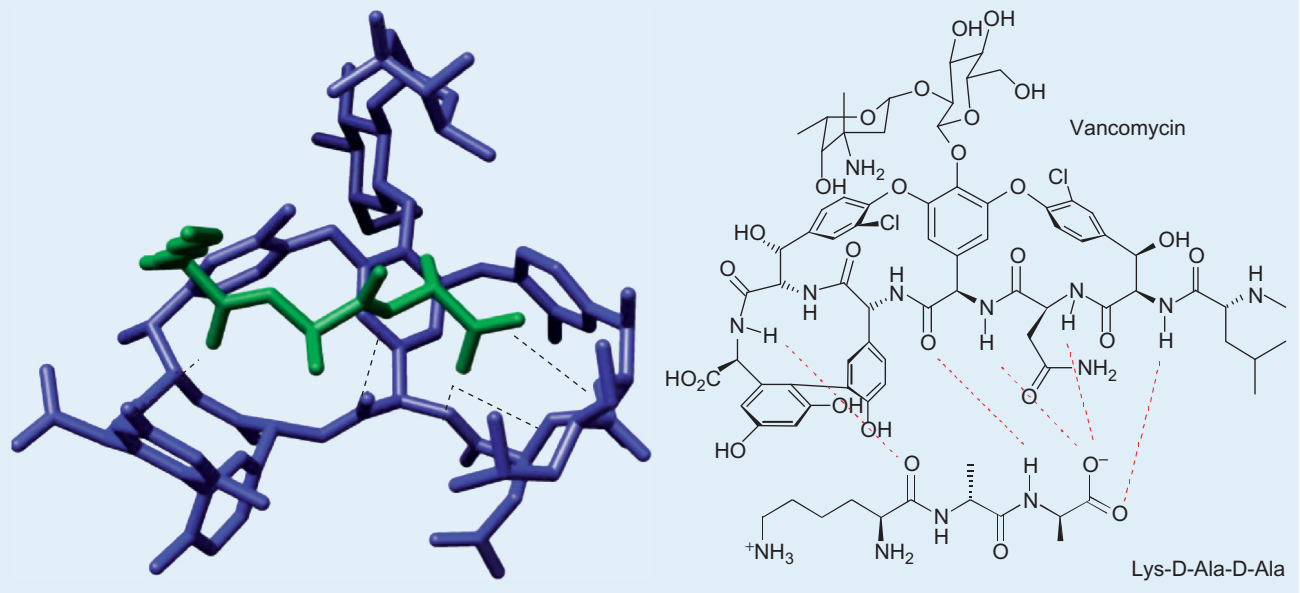


FIGURE 14.6 Crystal structure of a short peptide L-Lys-D-Ala-D-Ala (bacterial cell wall precursor [in green]) bound to the antibiotic vancomycin (in blue) through five hydrogen bonds [15].

essential to maintaining the structural integrity of α-helix and β-sheet conformations of peptides and proteins. By causing the macromolecules to fold into a specific shape, the hydrogen bonds contribute to the apparition of their biochemical functions.

In drug design, hydrogen bonds are exploited to obtain specificity, which is achieved through favorable, short-range, directionally specific interactions and the fact that ligand-receptor arrangements that leave bonding capacity unsatisfied are disfavored [15a]. The number of hydrogen bonds in a drug molecule may be limited by requirements on polarity for absorption and permeation. The Lipinski rule-of-five, for example, suggests that compounds with more than five hydrogen-bond donors or more than ten hydrogen bonds acceptors are likely to have poor absorption or permeation characteristics.

Among the numerous examples of drug−receptor interactions through hydrogen bonds, the antibiotic vancomycin is especially interesting because it binds selectively with peptides having a terminal D-Ala-D-Ala moiety in a bacterial cell through five hydrogen bonds (Figure 14.6). Vancomycin is lethal to the bacteria, since once it has bound to these particular peptides they are unable to be used to construct the bacteria's cell wall.

5. *Cation−π Interactions*

Such interaction occurs between a cation and the large, permanent quadrupole moment of an aromatic ring. The interaction energy depends on both the nature of the π-system and the nature of the cation. The importance of cation-π interactions were first recognized by Ma and Dougherty [16]. In fact, cation-π interactions play a key role in molecular recognition in biological receptors. They have been considered in such diverse systems as acetylcholine receptors (nicotinic, muscarinic, and Ach esterase), K^+ channels, the cyclize enzymes of steroid biosynthesis, and enzymes that catalyze methylation reactions involving S-adenosylmethionine [17]. A remarkable

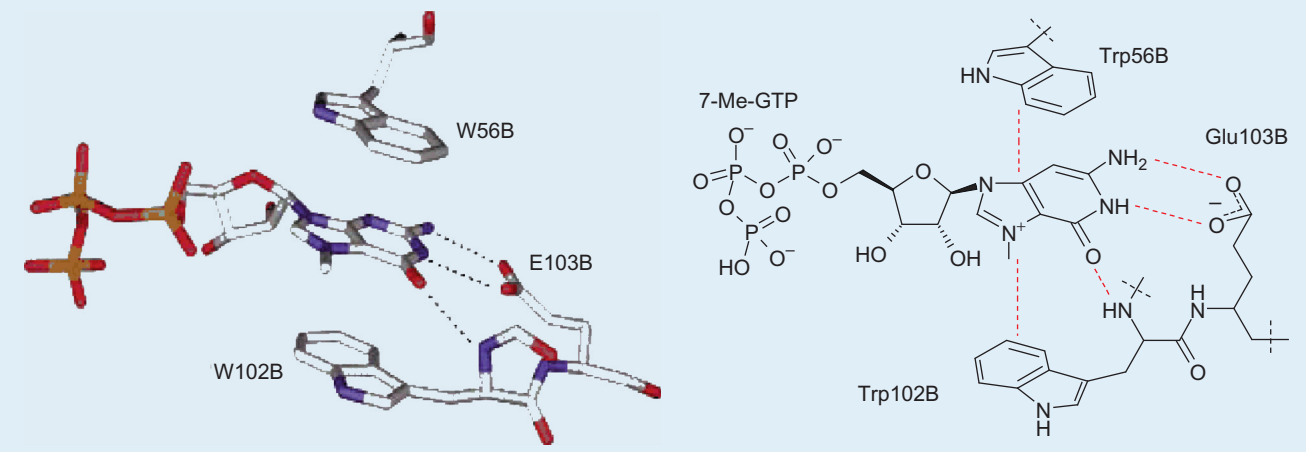


FIGURE 14.7 Partial view of the X-ray crystal structure (PDB code: 1L8B) of the messenger RNA 5′-cap-binding protein eIF4E bound to 7-methyl-GTP, which shows the sandwiching of the cationic nucleobase between the side chains of Trp102B and Trp56B.

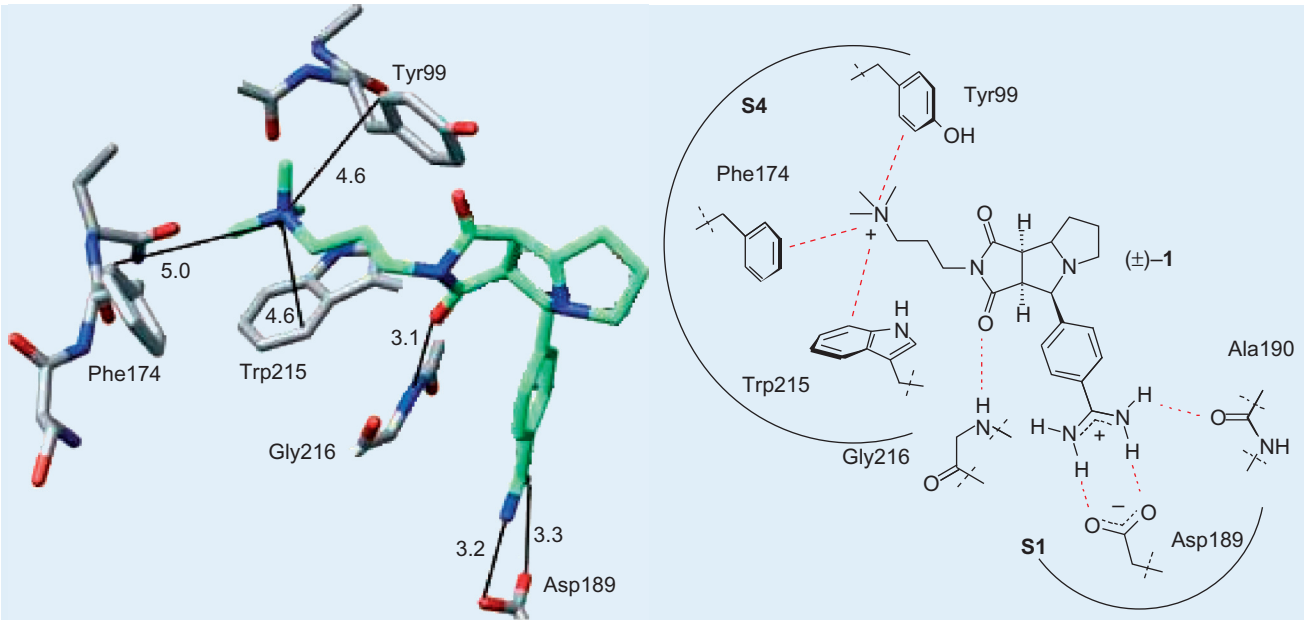


FIGURE 14.8 Schematic representation of the complex between factor Xa and the tricyclic inhibitor (±) − 1.

example is given by the strong complexation of 7-methyl-GTP (dissociation constant $K_d \approx 1.1 \times 10^8\,M^{-1}$, $\Delta G = -45\,kJ/mol)^{-1}$ by a messenger RNA 5′-cap-binding protein, the eukaryotic translation initiation factor eIF4E. The cationic nucleobase in this complex is sandwiched at van der Waals distance (c. 3.5 Å) between two tryptophan side-chains (Figure 14.7) [18].

The study of the factor Xa, a serine protease from the blood coagulation cascade, has led Diederich et al. [19] to observe that the aromatic box formed by the side chains of Phe174, Tyr99, and Trp215 in the S4-pocket is a very effective onium binding site. By comparing the affinity of the quaternary ammonium ion (±)-**1** to its tert-butyl analog, the free enthalpy increment for cation-π interactions in this box was determined as $\Delta G = -2.8 \times 4.18\,kJ/mol$ (Figure 14.8).

6. *Arene–Arene Interactions [17,20]*

Despite their weak and poorly directional character, π−π interactions have been recognized to play an important role in molecular recognition. Burley and Petsko demonstrated in a study involving thirty-four proteins that,

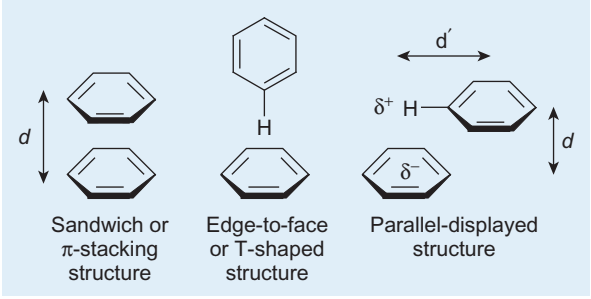


FIGURE 14.9 Proposed lowest energy structures of the benzene dimer. *d*: distance between planes, d′: lateral offset.

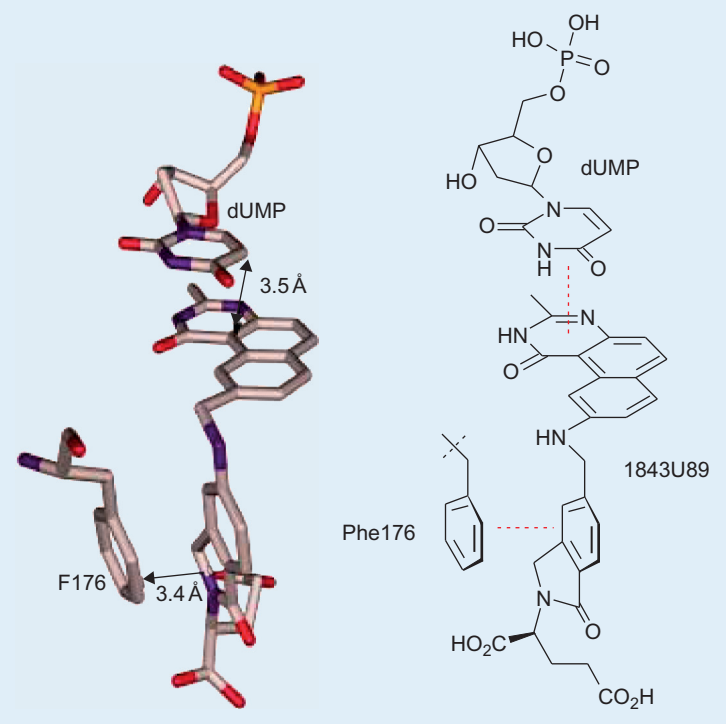


FIGURE 14.10 Heterocyclic π stacking between dUMP and the anticancer drug 1843U89 bound at the active site of thymidylate synthase (PDB code: 1TSD).

on average, 60 percent of aromatic side-chains (Phe, Trp, Tyr) are involved in aromatic–aromatic interactions [21]. It is now recognized that London dispersion interactions are the major source of stabilization energy between two aromatic molecules; however, the electrostatic component associated with the quadrupole moment of the aromatic ring is an influential factor in determining the geometry of the interaction. Assessment of binding interactions in aqueous solution is complicated by additional hydrophobic effects leading to apolar complexation or to intramolecular hydrophobic collapse.

Three lowest energy arrangements are commonly involved in π–π interactions (Figure 14.9), among which the T-shaped structure is the predominant one. The highest edge-to-face attraction is observed when an electron-attracting substituent renders the interacting H atom more acidic (higher positive partial charge) and when an electron-donating substituent increases the basicity (π-electron density) of the interacting π system. The other important arrangement of aromatic rings besides the edge-to-face contact is the parallel alignment. In this case two aromatic partners—one bearing strong electron-donor and the other strong electron-acceptor groups—form parallel stacking complexes in solution, with the geometry largely determined by molecular orbital interactions (charge–transfer complexes). The term "polar/π" interaction was introduced to emphasize the importance of the electrostatic term in π-stacking. Since attractive electrostatic interactions between atoms of opposite partial charges often overcome the repulsion between close-shell π clouds, π–π stacking interactions are also abundant between heterocyclic π systems. A nice example is provided by the ternary complex of the anticancer drug 1843U89 and dUMP formed at the active site of thymidylate synthase (Figure 14.10) [22].

B. Steric Interactions

As the lock-and-key model suggests, shape complementarity is very important for ligand–receptor binding and specificity. While electrostatic interactions are the dominant interactions involving polar molecules, there are also strong interactions between nonpolar molecules, particularly at short intermolecular distances.

1. *Dispersion Forces*

Van der Waals or London dispersion forces are the universal forces responsible for attractive interactions between nonpolar molecules. The occurrence of these short-range interactions is due to the fact that any atom will, at any given instant, be likely to possess a finite dipole moment as a result of the movement of electrons around the nuclei. When molecules are approaching each other, the temporary dipoles of one molecule induce opposite dipoles in the other approaching molecules, thus resulting in a net attractive force. Although the individual interactions between pairs of atoms are relatively weak (about 2 kJ/mol), the total contribution to binding from dispersion forces can be very significant if there is a close fit between drug and receptor. The quality of the steric match is thus the dominant factor in nonpolar interactions.

2. *Short-Range Repulsive Forces*

The short-range repulsive forces resulting from the overlap of the electron clouds of any two molecules increase exponentially with decreasing internuclear separation. The balance between these repulsive interactions and the dispersion forces thus determines both the minimum and the most favorable nonbonded separation between any pair of atoms. The equilibrium distance can be determined from crystal data, and is equivalent to the sum of the van der Waals radii of the two interacting atoms. For nonpolar molecules, this balance between the attractive dispersion forces and the short-range repulsive forces is generally defined in terms of the Buckingham (6-exp) potential given in Eq. 14.5 or the alternative Lennard–Jones 6–12 potential given in Eq. 14.6.

$$E = \frac{Ae^{-Br}}{r^d} - \frac{C}{r^6} \tag{14.5}$$

$$E = \frac{A^r}{r^{12}} - \frac{C}{r^6} \tag{14.6}$$

3. *Conformational Energy*

While intramolecular interactions within the drug molecule are the primary factor in determining the lowest energy conformation of the unbound drug, intermolecular interactions with the receptor also have a significant effect on conformation. If the bound conformation of a flexible molecule is also its lowest energy conformation, there is no conformational energy cost involved in binding. If, on the other hand, the optimal interaction between drug and receptor requires a higher-energy conformation, this energy difference will reduce the apparent strength of the interaction between the two molecules.

C. Enthalpy–Entropy Compensation

Consider the formation of a specific noncovalent bond (e.g., $\mathbf{A}\cdots\mathbf{B}$ for the transformation $\mathbf{A}+\mathbf{B}\rightarrow\mathbf{A}\cdots\mathbf{B}$). An increase in its strength (which corresponds to an increasing negative contribution to ΔH and a more favorable binding process) will be accompanied by an increasing restriction in the relative motion of **A** and **B** in $\mathbf{A}\cdots\mathbf{B}$ (which corresponds to a negative contribution to ΔS, and so is unfavorable to binding). This opposing interplay between enthalpy and entropy is known as "enthalpy–entropy compensation" and is a fundamental property of noncovalent interactions [23]. An enhancement of intermolecular binding is accompanied by a loss in degrees of freedom of mobility and vice versa. The two effects can be traded off against each other because the strength of noncovalent bonds at room temperature is comparable to the thermal energies that oppose them. The enthalpy–entropy compensation is of particular importance for the prediction of receptor–ligand interactions. Whereas the individual enthalpic and entropic contributions can vary over large ranges, the total change in free enthalpy is frequently close to zero. As a consequence, small relative errors in the prediction of ΔH and ΔS can have significant influence on ΔG. This concept is less important in the study of covalent bonds, which are typically too strong to be effectively opposed by thermal motions at room temperature.

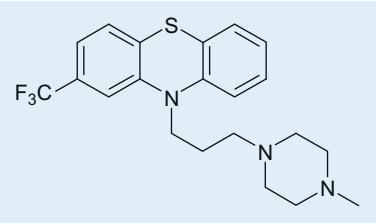


FIGURE 14.11 Trifluoperazine, a ligand of Ca^{2+}-calmodulin that induces an adaptation of the protein binding pocket.

1. *Hydrophobic Interactions*

The hydrophobic effect is that nonpolar molecules tend to self-associate in the presence of aqueous solution. This short-range attractive interaction is due to both enthalpic and entropic effects. It describes the energetic preference of nonpolar molecular surfaces to interact with other nonpolar molecular surfaces, and thereby to displace water molecules from the interacting surfaces. When a nonpolar molecule is surrounded by water, stronger than normal water−water interactions are formed around the solute molecule to compensate for the weaker interactions between solute and water [24]. This results in an increasingly ordered arrangement of water molecules around the solute and thus a negative entropy of dissolution. The decrease in entropy is roughly proportional to the nonpolar surface area of the molecule. The association of two such nonpolar molecules in water reduces the total nonpolar surface area exposed to the solvent, thus reducing the amount of structured water, and therefore providing a favorable entropy of association. The enthalpic contribution to hydrophobic interactions is due to the water molecules occupying lipophilic binding sites, which are consequently unable to form hydrogen bonds with the receptor. Their release from the hydrophobic pocket lets them form strong hydrogen bonds with the bulk water. As for van der Waals forces, hydrophobic interactions are individually weak (0.1 to 0.2 kJ/mol for every square angstrom of solvent-accessible hydrocarbon surface) [25], but the total contribution of hydrophobic bonds to drug−receptor interactions is substantial. Similarly, the overall strength of the hydrophobic interaction between two molecules is highly dependent on the quality of the steric match between the two molecules. If this is not sufficiently close to squeeze all of the solvent from the interface, a substantial entropy penalty must be paid for each of the trapped water molecules. Hydrophobic interactions are also regarded as the main driving force for conformational changes of the receptor upon ligand binding. This induced fit can be viewed as a "collapse" of the receptor about the ligand [26]. As an extreme case, the binding of trifluoroperazine (Figure 14.11) to Ca^{2+} calmodulin induces a conformational change of the protein from an extended to a compact form [27].

2. *Translational and Rotational Entropy*

The transformation of two mobile molecules into one mobile complex results in the loss of translational and rotational entropy. Indeed, by binding to its receptor, a drug molecule is losing three translational and three rotational degrees of freedom, which are replaced by six vibrational degrees of freedom in the complex [28]. The resulting entropy change is dependent on the relative tightness of the complex that is formed. For a typical ligand−protein interaction, the estimated change in free energy resulting from the loss of entropy on binding (at 310 K) ranges from 12 kJ/mol for a very weak interaction to 60 kJ/mol for a tightly bound complex [29].

3. *Conformational Entropy*

In the case of flexible drug molecules, a further entropy loss is due to the conformational restriction that accompanies binding. Based on the observed entropy changes accompanying cyclization reactions, the extent of this entropy loss is estimated [30] at 5−6 kJ/mol per internal rotation, although the actual figure again depends on the overall strength of the interaction between the drug and the receptor. In the case of rigid analogs, no such loss of conformational entropy occurs on binding. Provided that they offer a good steric and electrostatic match to the receptor, rigid analogs should therefore have a free energy advantage relative to more flexible drugs. To optimize entropic contributions, compounds are usually designed to be relatively rigid with few rotatable bonds. Conformational flexibility is, however, important in biomolecular binding processes. A recent conformational analysis of drug-like ligands binding to proteins shows that many ligands do not bind in a minimum energy conformation. Energetically unfavorable conformational rearrangements can be tolerated in some cases without penalizing the tightness of binding [31]. On the other hand, small-scale motions including bond stretching, bond angle bending, and dihedral angle variations are able to reduce slightly the receptor affinity for its ligand. The

timescale of these motions is around 10^{-12} seconds and the amplitude is less than 1 Å. For enzyme catalysis, for example, movement of less than 1 Å can alter catalytic rates by several orders of magnitude.

IV. THE STRENGTHS OF FUNCTIONAL GROUP CONTRIBUTIONS TO DRUG–RECEPTOR INTERACTIONS

The total free energy of interaction between a drug and its receptor provides a measure of the strength of the association between the two molecules but tells us little or nothing about the overall quality of their match. Does the observed binding reflect a composite of interactions between every part of the drug and its receptor, or is it a case of one or two strong interactions contributing sufficient energy to disguise an otherwise mediocre fit? Is the observed increase in interaction energy resulting from the addition of a new functional group consistent with what might have been anticipated? To answer these questions, we need some means of estimating the individual functional group contributions to drug–receptor interactions.

A. Measuring Functional Group Contributions

When cooperativity is ignored, contributions of ΔG values to the total free energies of binding may be added together. Approaches based on functional group additives (Eq. 14.7) or the additivity of free enthalpy components (Eq. 14.8) have frequently been applied to understand and predict protein–ligand interactions [32]. Pioneering studies in this field were performed by Andrews et al. [31] and Lau and Pettitt [33].

$$\Delta G = \Delta G_{\mathrm{Me}} + \Delta G_{\mathrm{OH}} + \Delta G_{\mathrm{Ph}}(\mathrm{Ph}) + \cdots \tag{14.7}$$

$$\Delta G = \Delta G_{H\text{-bridge}} + \Delta G_{\mathrm{solvation}} + \Delta G_{\mathrm{conformation}} + \cdots T\Delta S \tag{14.8}$$

In order to have a brief overview of the methods used to predict the free energy of binding of a ligand to its receptor, we will describe and discuss some of them here.

As a first approximation, the free energy of binding can be defined in terms of the binding energies for the individual functional groups that make up the drug molecule according to Eq. 14.9.

$$\Delta G = T\Delta S_{t,r} + n_r E_r = \Sigma n_{\mathrm{x}} E_{\mathrm{x}} \tag{14.9}$$

where $T\Delta S_{t,r}$ is the loss of overall translational and rotational entropy associated with binding of the drug molecule, n_r is the number of internal degrees of conformational freedom lost on binding the drug molecule, and E_r is the energy equivalent of the entropy loss associated with the loss of each degree of conformational freedom on receptor binding.

1. *Intrinsic Binding Energy*

The final term in Eq. 14.9 is the sum of the binding energies E_{x} associated with each functional group X, of which there are n_{x} present in the drug. In the ideal case, when the specified functional group is aligned optimally and without strain with the corresponding functional group in the receptor, E_{x} is called the "intrinsic binding energy" [34]. In other cases, the term "apparent binding energy" is used.

It should be noted that each binding energy E_{x} is actually a combination of the various enthalpic and entropic interactions outlined above. These include the enthalpy of interaction between the functional group and its corresponding binding site on the receptor, the enthalpy changes associated with the removal of water of hydration from the functional group and its target site and the subsequent formation of bonds between the displaced water molecules, and the corresponding entropy terms associated with the displacement and subsequent bonding of water molecules (Figure 14.12).

It is apparent that these intrinsic binding energies may be regarded, at least approximately, as properties of the functional group that should be relatively independent of the groups to which the particular functional group is attached. Such intrinsic binding potentials might thus reasonably be used in an additive manner to provide an overall estimate of the drug–receptor interaction.

2. *Anchor Principle*

It follows from Eq. 14.9 that the binding energy E_{x}, due to the interaction between the receptor and a specific functional group X, can be estimated by comparing the binding energies for pairs of compounds that differ only

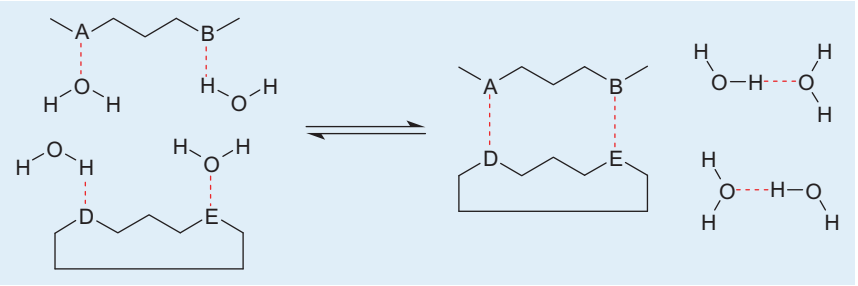


FIGURE 14.12 Complex formation between a ligand (containing polar functionalities **A** and **B**) and a receptor (containing polar functionalities **D** and **E**) with exchange of four water molecules to the bulk solvent.

in the presence or absence of the specific functional group. This approach was first applied by Page [29] who referred to it as the "anchor principle." It is based on the premise that the difference in binding of a drug molecule with or without the particular functional group is due to factors associated solely with that group, that is, the binding energy E_x plus any degrees of conformational freedom lost specifically as a result of binding of group X. Other degrees of conformational freedom lost on binding and the loss of overall rotational and translational entropy associated with the remainder of the drug molecule (the anchor) are assumed to be unaffected by the presence or absence of X.

Similarly, the impact of a single amino acid substitution in the active site of an enzyme on transition-state stabilization, as determined by the change in either catalytic efficiency or inhibitor binding, provides a measure of the relative binding energy of the two side-chains.

Clearly, the magnitude of the binding energies obtained using the anchor principle will vary widely with the quality of the interaction. If the functional groups are not properly aligned, as might reasonably be expected in many mutant proteins, a small or even repulsive interaction may result. Alternatively, the strength of the additional bond may be offset by a reduction in the strengths of the existing bonds. Under these circumstances, the anchor principle will lead to an underestimation of the true bond strength.

3. *Average Binding Energy*

An alternative to the pair-by-pair approach inherent in the anchor principle was developed by Andrews et al. [31], who sought to average the contributions of individual functional groups to the observed binding energies of 200 ligand–protein interactions in aqueous solution. For this purpose, the average loss of overall rotational and translational entropy accompanying drug–receptor binding, $T\Delta S_{t,r}$ in Eq. 14.9, was estimated at 58.5 kJ/mol at 310 K. Regression analysis against n_r (obtained by counting the number of degrees of conformational freedom in each of the 200 ligand structures) and n_x (the number of occurrences of each functional group X in each of the 200 ligand structures) as the independent variables was then used to obtain average values of the binding energies associated with each functional group and for the loss of entropy associated with each degree of conformational freedom.

The results of this analysis showed that the loss of entropy associated with each internal rotation ΔG_r on receptor binding is equivalent to a reduction in the free energy of binding by average of 3 kJ/mol.

The corresponding binding energies obtained by the averaging process were: C (sp^2 or sp^3), 3 kJ/mol; O, S, N, or halogen, 5 kJ/mol; OH and C=O, 10 and 14 kJ/mol, respectively; and CO_2^-, OPO_3^{2-}, and N^+, 34, 42, and 48 kJ/mol, respectively. Once again, it should be stressed that these are not intrinsic binding energies in the sense defined above. This would be the case only if each functional group in each drug in the series was optimally aligned with a corresponding functional group in the receptor. In fact, since every functional group of every drug was included in the analysis, the calculated values are averages of apparent binding energies, including those for some groups that may not interact with the receptor at all [35]. The calculated averages are thus almost certainly smaller than the corresponding intrinsic binding energies, although they follow expected trends in that charged groups lead to stronger interactions than polar groups, which in turn are stronger than nonpolar groups such as sp^2 or sp^3 carbons.

The apparent contributions of some functional groups and/or bond types to overall binding energies derived from the various studies reviewed above are summarized in Table 14.4. Also included are corresponding values used or suggested for the overall loss of rotational and translational entropy, $T\Delta S_{t,r}$, and the loss of conformational entropy resulting from restriction of free rotation, ΔG_r. These first attempts at a semiquantification of drug–receptor interactions in terms of costs and benefits were later refined by Williams et al. [36,37], who applied an additional term to account for the hydrophobic effect. They suggested that the magnitude of this effect

TABLE 14.4 Functional Group Contributions to Drug–Receptor Interactions (kJ/mol)

	Technique employed to determine interaction energy		
Functional group type	**Anchor principle**	**Site-directed mutagenesis**	**Average energy**
Nonpolar (per carbon atom)	12–14	1–3	3–6
H–bonding (uncharged)	16	2–6	5–14
H–bonding (charge-assisted)	20–42	15–19	
Charged (carboxyl, amine)	18–28	12–25	34–48
$T\Delta S_{t,r}$	12–60		58.5
ΔG_r (internal rotation)	5–6		3

TABLE 14.5 Average Values for the Parameters of Eq. (14.10) [25]

Parameter	**Physical process**	**Value (kJ/mol)**
$\Delta G_{t,r}$	Energy cost of bimolecular association	+5.4
ΔG_r	Energy cost of restriction of an internal rotor	+1.4
ΔG_h	Benefit of the hydrophobic effect (per $Å^2$ of buried hydrocarbon)	−0.17 ($Å^2$)
ΔG_p	Benefit of making a neutral hydrogen bond of ideal geometry	−4.7
ΔG_{ionic}	Benefit of making an ionic hydrogen bond of ideal geometry	−8.3

is proportional to the surface area of the hydrocarbon that is removed from exposure to water upon formation of the complex. It can be estimated in terms of ΔG_h per unit of area A of hydrocarbon buried, which can readily be measured with the aid of computer graphics. It leads to the following equation:

$$\Delta G = \Delta G_{t,r} + n\Delta G_r + A\Delta G_h + \Sigma\Delta G_p \tag{14.10}$$

ΔG is the observed free energy of binding, $\sum\Delta G_p$ is the sum of the free energies of binding for all the polar interactions made in the binding site, and the other terms are as defined above. Böhm "trained" a variant of Eq. 14.10 with a set of forty-five interactions of experimentally known binding constants from the association of ligands of small molecular weight with proteins through sets of known interactions [38]. He divided the original ΔG_p values into two groups: those involving ionic interactions (ΔG_{ionic}) and those involving hydrogen bonds formed between neutral entities (the term ΔG_p was retained). Since the modified form of the equation has only five types of ΔG contributions and the forty-five binding sites involve different combinations of these five types of ΔG contributions, average values for them can be obtained. These values (Table 14.5) have proven very useful in the pharmaceutical industry.

It is interesting to note that the average value $\Delta G_{t,r} = +5.4$ kJ/mol is remarkably small and represents only about one tenth of the maximum theoretical entropy loss corresponding to complete immobilization of the ligand. This small value presumably reflects, at least in part, the large residual motion that the drugs can exercise relative to the receptor to which they are bound. The average cost of restricting the rotation of an internal bond in the drugs (+1.4 kJ/mol) is slightly less than that found for the formation of crystals from neat liquids that contain internal rotors (2–3 kJ/mol). This finding probably reflects the fact that rotations are somewhat less restricted in these binding sites than they are in crystals. Most importantly, the application of the equation gives useful approximate binding constants in many cases.

B. The Methyl Group and Other Nonpolar Substituents

The initial application of the anchor principle described by Page [29] related to data on the selectivity of amino acid-tRNA synthetases, from which he estimated intrinsic binding energies for the methylene group in the range 12–14 kJ/mol. For example, the calculated binding energies of Eq. 14.3 for isoleucine **2** (Figure 14.13) and its

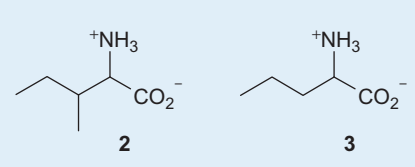


FIGURE 14.13 Isoleucine **2** and desmethyl-isoleucine **3**.

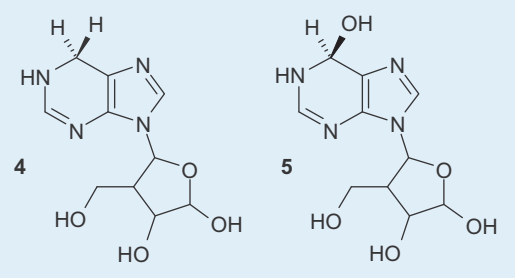


FIGURE 14.14 1,6-Dihydropurine ribonucleoside **4** and its 6-hydroxy analog **5**.

desmethyl analog **3** to isoleucyl-tRNA synthetase are 29.7 and 15.9 kJ/mol, respectively, indicating that the methyl group contributes a total of 13.8 kJ/mol to the overall interaction.

This estimate, having been derived from observations on a highly selective enzyme–substrate interaction, is probably also approaching the intrinsic limit for the binding contribution of a methyl group. Indeed, according to the calculation of Williams [36], the burial of a methyl group ($\approx$25 Å^2) contributes only -4 kJ/mol. For longer hydrocarbon side-chains the positive contribution due to dispersion forces and hydrophobic interactions tends to be offset by the loss of conformational entropy on binding. Thus, the "average" binding energy of 3 kJ/mol obtained by Andrews et al for sp^2 and sp^3 carbon groups is identical to the "average" reduction in free energy of binding estimated for the loss of conformational freedom around a single bond [31]. Clearly, this effect will be greater in saturated hydrocarbon chains than in their more conformationally constrained unsaturated or cyclic analogs.

C. The Hydroxyl Group and Other Hydrogen-bond Forming Substituents

The most extensive studies of hydroxyl group contributions to drug–receptor interactions are those of Wolfenden et al. on the contribution of hydrogen bonds formed by hydroxyl groups in transition-state analogs. In a series of thirteen examples of paired ligands with and without hydroxyl groups, they used [39] the anchor principle to determine apparent binding energies for single hydroxyl groups ranging from 20–42 kJ/mol.

Thus, in comparing the binding of 1,6-dihydropurine ribonucleoside **4** (Figure 14.14) and its 6-hydroxy derivative **5** to adenosine deaminase, they observed a difference in binding energy of 41 kJ/mol [40]. The authors suggested that the 6-hydroxyl group, which has very limited freedom of movement, is likely to be in almost ideal alignment with the active site for forming a hydrogen bond. This conjecture has been verified by the determination of the crystal structure of the inhibitory complex between adenosine deaminase and 6-hydroxy-1,6-dihydropurine ribonucleoside, which showed that the 6-hydroxyl group interacts with a zinc atom, with a protonated histidyl residue, and with an aspartic acid residue at the enzyme's active site [41].

Once again, the data from active-site mutagenesis studies are less striking, but nevertheless reveal some very substantial hydrogen-bonding interactions. In Fersht's studies [42] on tyrosyl-tRNA synthetase, for example, hydrogen bonds between this enzyme and uncharged substrate groups contributed between 2 and 6 kJ/mol toward specificity, while hydrogen bonds to charged groups contributed between 15 and 19 kJ/mol, corresponding to a factor of 1000 in specificity. These numbers are, however, higher than the average contributions determined by Böhm [38] (4.7 and 8.3 kJ/mol, respectively).

D. Acidic and Basic Substituents

Simple observations on the interactions of individual charged groups with appropriate enzymes may lead to an indication of their binding energies. The phosphate ion, for example, binds alkaline phosphatase [43] with a dissociation constant of 2.3×10^{-6} M, equivalent to a ΔG value of approximately 33 kJ/mol. Taking the most conservative estimate for the loss of rotational and translational entropy associated with this interaction (12 kJ/mol for a loosely bound complex), Eq. 14.9 then gives a lower estimate for binding of the phosphate ion of 45 kJ/mol. If the same value of $T\Delta S_{t,r}$ is applied to the binding of oxalate ion to transcarboxylase [44], for which the dissociation constant is 1.8×10^{-10} M (57 kJ/mol), Eq. 14.9 gives an apparent binding energy of 24 kJ/mol per carboxylate group after allowance for a minimal conformational entropy loss of 3 kJ/mol.

These figures are broadly consistent with the average values of Andrews et al. [31], which were in the range 34–48 kJ/mol for charged phosphate [45], amine, and carboxyl groups.

E. Practical Applications for the Medicinal Chemist

1. Assessing a Lead Compound

Summation of the average contributions of individual binding groups, including allowance for conformational, rotational, and translational entropy terms as shown in Eq. 14.9, provides a simple back-of-the-envelope calculation of the strength of binding that might be expected for a drug forming a typical interaction with a receptor. This figure, when compared to the observed affinity of the drug for the target receptor, then gives a direct indication of the actual quality of the electrostatic and steric match between the drug and the receptor.

A. BINDING IS TIGHTER THAN EXPECTED

If the observed binding of a drug to its receptor turns out to be substantially stronger than that calculated from Eq. 14.9, it is reasonable to expect that the drug structure offers a good fit to the receptor in a reasonably low-energy conformation. The structure should therefore provide an excellent starting point for the development of even more bioactive compounds.

A good example of this is biotin (Figure 14.15), which was the most extreme case of a positive deviation from the calculated "average" in the original set of 200 ligand-protein interactions studied by Andrews et al [31].

$$\begin{aligned}\Delta G_{av} &= T\Delta S_{rt} + 5E_r + 8E_{\mathrm{Csp^3}} + 2E_{\mathrm{N}} \\ &\quad + E_2 + E_{\mathrm{C{=}O}} + E_{\mathrm{COOH}} \\ &= -58.5 + 5(-3) + 8(3) + 2(5) \\ &\quad + 5 + 14 + 34 = 13.5\ \mathrm{KJ/mol}\end{aligned} \tag{14.11}$$

$$\begin{aligned}\Delta G_{\mathrm{obs}} &= -5.85 \log K_d \\ &= -5.85(-15) = 87.7\ \mathrm{KJ/mol}\end{aligned} \tag{14.12}$$

Application of Eq. 14.9 to biotin (see above) gives an average binding energy of 13.5 kJ/mol, whereas substitution into Eq. 14.3 of the experimentally observed binding constant to the protein avidin (10^{-15} mol^{-1}) gives a binding energy of 87.7 kJ/mol. The difference of almost 74 kJ/mol implies an exceptionally good fit between biotin and the structure of the protein. It has since been established that this is indeed the case, with polarization of the biotin molecule by the protein actually leading to an ionic interaction where a neutral hydrogen-bonding interaction had been assumed.

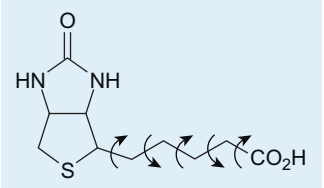


FIGURE 14.15 Structure of biotin.

B. BINDING IS LOOSER THAN EXPECTED

If the observed binding is significantly weaker than anticipated on the basis of an "average" energy calculation, the fit between the drug and the receptor is less than perfect. In some cases, this will be because the match between drug and receptor is less a matter of "hand and glove" than of "square peg and round hole," and the only realistic option for the drug designer is to start again.

In other cases, simpler remedies may be followed:

1. The fit may be unsatisfactory because only part of the drug is interacting with the receptor. This situation applies particularly to large drug molecules (e.g., peptide hormones), for which selective pruning of unused parts of the structure may produce simpler compounds without loss of affinity;
2. The drug may be binding to the receptor in a comparatively high energy conformation. In this case, the design of more rigid structures that are already fixed in the desired conformation will give an increase in binding energy equivalent to the conformational energy cost of binding the more flexible analog.

2. *Assessing the Effectiveness of Substituents*

Equally simple back-of-the-envelope calculations based on Eq. 14.9 can be used to predict the increase in binding energy that might be expected upon the addition of a functional group that is optimally aligned with a corresponding group in the receptor. This figure, when compared to the observed increase in affinity, gives direct feedback on whether or not the new group is actually performing the function anticipated in the design strategy.

An interesting example of how this approach can be used to assess the validity of a drug design hypothesis is provided by the receptor-based design of sialidase inhibitors as potential anti-influenza drugs. Starting from the knowledge [46] of the structurally invariant active site of influenza A and sialidases, von Itzstein et al. [47] postulated that substitution of the 4-hydroxyl group of the nonselective sialidase inhibitor 2-deoxy-2,3-didehydro-D-acetylneuraminic acid **6** (Figure 14.16) with a positively charged substituent would fill an occupied pocket lined with anionic residues. Synthesis and testing of the 4-guanidino analog **7** revealed a reduction in K_i from 10^{-6} to 10^{-10} mole/l, equivalent to an additional binding energy of 23 kJ/mol. Although not at the upper limit of the increments in binding energy anticipated for well-aligned ionic interactions on the basis of the data in Table 14.4, this figure is certainly consistent with the design hypothesis, as is borne out by the crystal structure of the complex [47]. This shows that the guanidino lies between two target carboxyl groups in the active site of the enzyme, although only one appears to be optimally placed for a strong interaction.

F. Ligand Efficiency

The idea of ligand efficiency (LE) has recently emerged as a useful guide to optimize fragment and lead selection in the discovery process [48,49]. Preliminary work has been published by Kuntz et al. [50]. This key contribution, where affinities were examined for a variety of ligands against many different targets, showed that ΔG tends to increase little with molecular mass when the ligand contains more than c. 15 heavy atoms (HA). Later, Hopkins et al. [48] proposed to define LE as the binding free energy for a ligand divided by its number of HA:

$$\mathrm{LE} = -\Delta G/\mathrm{HA} \tag{14.13}$$

According to them, comparison of lead compounds on the basis of LE rather than the potency alone could be useful in deciding the potential for further optimization for particular hits and chemical scaffold. LE has since

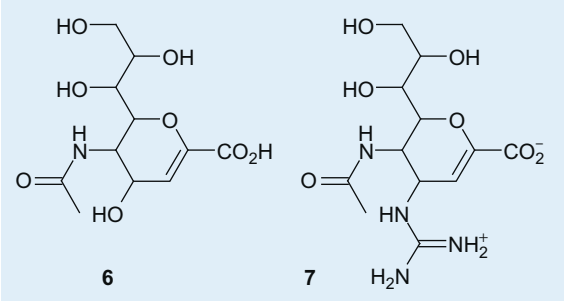


FIGURE 14.16 Sialidase inhibitors: 2-deoxy-2,3-didehydro-D-*N*-acetyl neuraminic acid **7** and its **4**-guanidino analog **8**.

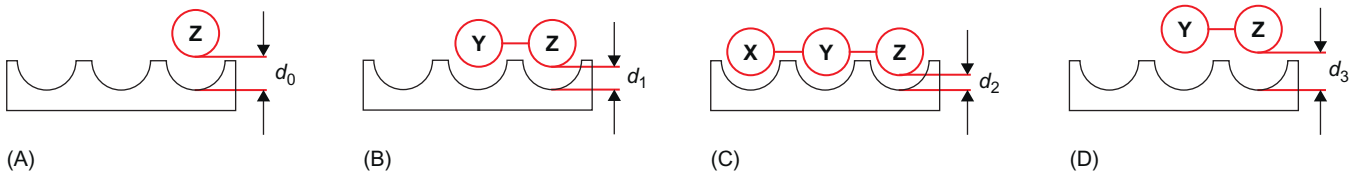


FIGURE 14.17 Schematic representation of a receptor that binds ligands **X**, **Y**, and **Z** with affinities ΔG_X, ΔG_Y, and ΔG_Z, respectively. (a) Binding of **Z** results in a structure with an intermolecular distance d_0. (b) When **Y** and **Z** are connected by a rigid, strain-free linker (**Y**–**Z**), they bind to the receptor with positive cooperativity (ΔG_{Y-Z} more negative than $\Delta G_Y + \Delta G_Z$) and there is structural tightening ($d_1 < d_0$). (c) If **X** is connected to **Y**–**Z** by a rigid, strain-free linker to form **X**–**Y**–**Z**, then further structural tightening will occur ($d_2 < d_1$), leading to a further cooperative enhancement. (d) The shorter linker between **Y** and **Z** does not allow both these binding interactions to occur with optimal geometry. **Y**–**Z** binds the receptor with negative cooperativity (ΔG_{Y-Z} more positive than $\Delta G_Y + \Delta G_Z$) and there is structural loosening ($d_3 > d_0$).

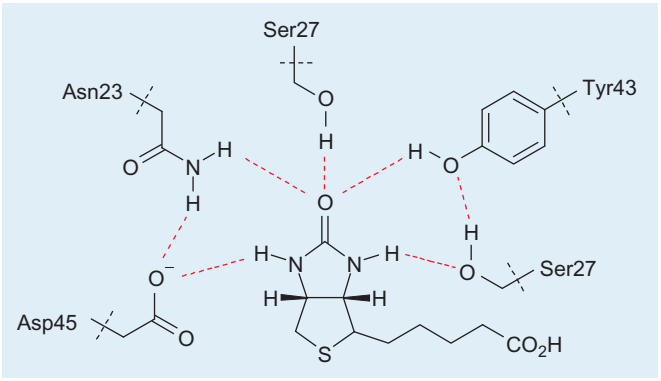


FIGURE 14.18 Schematic representation of streptavidin–biotin interactions.

become an important concept in drug discovery, partly due to the realization that large ligands have a decided disadvantage in terms of the molecular properties necessary for bioavailability [51,52]. LE is notably used when prioritizing the output from HTS or other screening strategies. It is helpful, particularly when trying to assess the relative value of fragments for follow-up in fragment-based drug design [53]. To obtain a final compound with MW $<$500 and $\approx$10 nM potency, LE needs to stay above 1.25 kJ/mol per HA [48].

V. COOPERATIVE BINDING

Equations 14.9 and 14.10 are based on the assumption that contributions to binding energies can be partitioned in term of individual interactions, and that these individual binding energies are additive and independent of each other. However, in general, it is impossible to study one binding interaction in isolation from the others at an interface. In practice, cooperativity between noncovalent interactions is observed.

Noncovalent interactions are said to interact with each other in a positively (or negatively) cooperative manner when the binding energy that is derived from their acting together is greater (or smaller) than would be derived from the sum of their acting separately. This concept, developed by Williams et al. [25,54,55], is illustrated in Figure 14.17.

The consequence of a positive cooperativity is that a structural tightening occurs in the bound state with a benefit in enthalpy and a smaller cost in entropy. By studying the unusually strong reversible binding of biotin by avidin ($K_a \approx 10^{15}$ mol/l) and streptavidin ($K_a \approx 10^{13}$ mol/l), Houk et al. [56] observed that the five hydrogen bonds of the ligand–receptor complex act cooperatively (Figure 14.18), leading to stabilization that is larger than the sum of individual hydrogen-bonding energies. The charged aspartate is the key residue that provides the driving force for cooperativity by greatly polarizing the urea of biotin. If the residue is removed, the network is disrupted.

Following the same principle, a negative cooperativity produces a decrease of the ligand–receptor interaction strength. This is illustrated by Figure 14.17d, where **Y** and **Z** are rigidly held in a conformation that does not

allow both binding interactions to occur with the preferred geometry. This situation could be induced, for example, by introducing a linker between **Y** and **Z** that is too short. The "pull" of **Y** toward its preferred binding geometry will adversely affect the binding of **Z** by forcing it away from its preferred binding geometry, and vice versa (ΔG_{Y-Z} is less negative than $\Delta G_Y + \Delta G_Z$).

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