The chemical transformation of substrate to product almost always involves the formation of a sequential series of intermediate chemical species along the reaction pathway. Paramount in this reaction pathway is the formation of a short-lived, high energy species referred to as the transition state. To facilitate this sequential process of intermediate species formation, the ligand binding pocket(s) of enzymes must undergo specific conformational changes that induce strains at correct locations and align molecular orbitals to augment the chemical reactivity of the appropriate functionalities on the substrate molecule(s), at defined moments during the reaction cycle. The basis of mechanistic enzymology includes understanding the chemical nature of the various intermediate species formed, and their interactions with those elements of the enzyme binding pocket that facilitate chemical transformations. When these studies are coupled with structural biology methods, such as X-ray crystallography and multi-dimensional nuclear magnetic resonance (NMR) spectroscopy, a rich understanding of the structure–activity relationships (SAR) that attend enzyme catalysis can be obtained. What is germane to the present discussion is that this structural and mechanistic understanding can be exploited to discover and design small molecule inhibitors—mimicking key structural features of reaction intermediates—that form high-affinity interactions with specific conformational states of the ligand binding pocket of the target enzyme. In this chapter, we describe the application of mechanistic and structural enzymology to drug discovery efforts with an emphasis on the evolution of structural changes that attend catalysis and the exploitation of these various conformational forms for high-affinity inhibitor development.

11.2 MODES OF INHIBITOR INTERACTION WITH ENZYMES

The simplest enzyme-catalyzed reaction that one can envisage is that of a single substrate (S) being converted by the enzyme (E) to a single product (P). This reaction can be summarized by the following equation:

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_{cat}} E + P \tag{11.1}$$

As summarized by Equation 11.1, enzyme and substrate combine to form a reversible initial encounter complex (ES) that is governed by a forward rate constant for association (k_1) and a reverse rate constant for dissociation (k_2). The equilibrium dissociation constant for the ES complex is given the symbol K_S and is mathematically equivalent to the ratio of the rate constants k_2/k_1. Subsequent to initial complex formation, a series of chemical steps ensue that are collectively quantified by the cumulative rate constant k_{cat}. Thus, k_{cat} is not a microscopic rate constant, but rather summarizes all of the intermediate states that must be formed during the chemical transformation of substrate to product (see Section 11.3 for more details on the individual intermediate steps that may contribute to k_{cat}).

Three modes of inhibitor interaction with an enzyme target can be defined, based on their effects on the catalytic steps summarized in Equation 11.1. Competitive inhibitors bind to the free enzyme in a manner that blocks the binding of substrate so that they increase the apparent value of K_S, but have no effect on the apparent value of k_{cat}. Noncompetitive inhibitors can bind to both the free enzyme and to the ES complex (or intermediate species that follow formation of the ES complex). Such inhibitors can have some effect on the value of K_S but show the greatest effect on k_{cat}, as they inhibit by blocking catalytic steps subsequent to substrate binding. Finally, uncompetitive inhibitors have no affinity for the free enzyme and only bind subsequent to formation of the ES complex. These inhibitors decrease the apparent value of K_S (i.e., increasing the apparent affinity of the enzyme for substrate) and also decrease the apparent value of k_{cat} (i.e., diminishing the ability of the enzyme to catalyze chemical steps subsequent to substrate binding). Among drugs in current clinical use, one finds multiple examples of each of these three modalities of enzyme inhibition.