

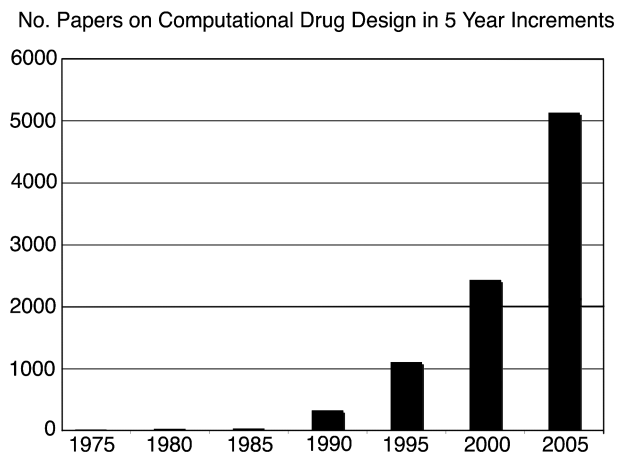
# *Preface*

The idea that disease could be cured by inhibiting a specific protein target, which was articulated by Ehrlich as early as 1909,<sup>1</sup> is at the heart of the computational drug-design methods discussed in this book. Even before any protein structures were known, mechanism-based inhibitors had been developed that very specifically inhibited a chosen enzyme targ.<sup>2,3</sup> In some cases, the structure of a target's binding site could be deduced from the binding surfaces of its ligands.<sup>4</sup> However, scientists appreciated that a general, high-affinity inhibitor would need to be highly complimentary to the shape and electrostatic properties of a target's active site, and designing such inhibitors would require atomic resolution protein structures. Thus modern methods of rational, target-directed drug design did not emerge until the late 1970's and early 1980's, when protein crystal structures could be routinely determined, and increasingly sophisticated computer graphics software allowed visual analysis of protein-ligand interactions.<sup>5-7</sup>

Searching the biomedical literature in the PubMed database with the search phrases “drug design” AND (structure OR comput\*) roughly gauges growth of this field: 12 papers were found for years 1971–1975, 323 were found for 1986–1990, and 5126 were found for 2001–2005 (see Figure 1). More impressive than the growth in number of published papers, perhaps, is the speed with which computational methods of drug design moved from being academic exercises to actually producing drugs that entered clinical trials. The history of the development of these methods, an assessment of their successes and failures, and prospects for improvement are all topics covered in this volume.

The book is divided into five sections. Section 1 includes two chapters that provide overviews of the drug-discovery field, written from the points of view of structural chemists and a medicinal chemist, respectively. Chapter 1 is an assessment of the reasons why structural and computational approaches have failed to live up to their potential for producing “designer drugs”, and the sorts of technological developments required to reliably predict compound specificity and affinity from structure. Novel methods for targeting unconventional sites in proteins are cited as some of the most promising areas for progress in drug discovery.

In Chapter 2 a scientist with over 40 years of experience in medicinal and combinatorial chemistry traces the development of drug-discovery methods



**Figure 1** The growth of impact of computational approaches to drug design is indicated by the sum on the ordinate, over each five-year interval ending in the year displayed along the abscissa. The growth follows an exponential growth that essentially doubles every five years.

from the 1960's to the present. This chapter shows how new technological breakthroughs, such as the ability to clone and express proteins, increases in computer power, or progress in protein-structure determination, have led to novel approaches for rapidly identifying new drug targets and drug leads. But the first applications of these approaches have been disappointing in terms of introducing new drugs to the clinic. Initial optimism has been tempered with the realization that complex biological issues have to be considered even in the early stages of drug discovery. Nevertheless, each of the methods has validity and, after refinements that addressed early failures, has served to facilitate drug discovery.

The themes in Section 1 are explored in greater detail in the next four Sections. Subsequent chapters in the book include in-depth discussions and examples of several of the computational methods used in four popular approaches to drug discovery: structure-based design, docking, high-throughput screening, and fragment-based design. Section 2 includes several chapters that are illustrations, descriptions, or evaluations of structure-based drug design methods. One of the earliest and most successful applications of structure-based drug design is described in Chapter 3. In the early 1990's, high-affinity, specific inhibitors of human purine nucleoside phosphorylase were designed using high-resolution crystal structures. These efforts culminated in a transition-state analog inhibitor that entered clinical trials for treatment of cutaneous T-cell lymphoma.

Balancing the success story described in Chapter 3 is the warning in Chapter 4 that not all crystal structures extracted from the Protein Data Bank are reliable enough for structure-based design, and some may actually lead the chemist astray. Water structure in particular can be inaccurate in

low-resolution X-ray crystal structures, or in nuclear magnetic resonance structures, and a well-defined water structure is critical for calculating binding free energies. Fortunately, as discussed in Chapter 5, computational simulations can be used to both predict the positions of bound waters in protein cavities and to determine the solvent density around a protein. Chapter 5 also includes a discussion of how reorganization of waters upon ligand binding contributes to the binding free energy and how well-ordered waters in the active site may enhance affinity for a ligand or the plasticity of the binding site. Useful guidelines are presented for when to include explicit waters in a target protein structure during docking.

Knowledge-based methods of predicting binding modes are reviewed in Chapter 6. An explosion in the number of publicly available structures of ligand-bound macromolecules has provided an opportunity to statistically analyze binding preferences of atom types or of chemical groups. Incorporation of these preferences into “binding propensity surfaces” has been a useful way of identifying binding hot spots in proteins. Knowledge-based scoring functions have also been used for virtual screening.

Finally, a novel strategy for designing robust drugs, not subject to drug resistance, is discussed in Chapter 7. This strategy grew out of analysis of numerous crystal structures of substrate-bound and inhibitor-bound HIV-protease.

Section 3 focuses on docking algorithms and their use in drug discovery. Chapter 8 is a critical assessment of the success rates of docking methods in binding-mode prediction, virtual screening, and prediction of potencies. The bottom line of this analysis is that docking algorithms are now quite successful in predicting binding modes, but identification of the most promising hits often fails because of inadequate scoring functions. Protocols for unbiased evaluations of docking algorithms and scoring functions are proposed, and address a critical need in the field.

The various roles of docking in drug discovery are illustrated in Chapter 9. Here, a scenario is presented for employing docking methods during each stage of drug discovery, starting with target identification and ending with lead-compound optimization. The appropriate protocols and scoring functions for each stage are described.

Docking algorithms are becoming increasingly sophisticated. Many recent applications have taken into account flexibility in both ligands and receptors. Chapter 10 is a discussion of the types of protein conformational changes that most often occur upon ligand binding, and the protocols that take these movements into account during docking and screening. Chapter 11 provides several examples of how using a flexible receptor was critical for correctly identifying inhibitors.

Section 4 includes several chapters on library screening. High-throughput screening of chemical libraries is a widely accepted approach to drug discovery. However, as noted in Chapter 2, early applications of this approach yielded a large number of hits that, for various reasons, could never have been developed into drugs. The importance of constructing chemically diverse libraries

whose members have drug-like properties is emphasized throughout Section 4. Chapter 12 is a summary of several methods used to predict whether a compound will have drug-like properties. Computational methods are being developed to predict not only a compound's physicochemical properties, such as solubility and lipophilicity, but also its metabolism and hERG channel blockade activity.

Using libraries of drug-like molecules for high-throughput screening does not guarantee that hits will be useful drug leads. Inhibitors discovered by screening often include a number of promiscuous inhibitors, which by many measures are "drug like", but which actually inhibit by forming large aggregates in the presence of the target protein. The fascinating story of how the phenomenon of inhibition by aggregation was discovered and characterized, and a suggested counter-screen for aggregating inhibitors, are the subjects of Chapter 13.

Virtual (*in silico*) ligand screening is a cost-effective way of extracting a subset of likely inhibitors from a large database of compounds. Some of the ways docking is used for virtual screening are described in Section 3. Chapter 14 contains detailed accounts of how one docking program, Autodock, has been used in an iterative screening approach to screen diverse libraries and rank the hits. The iterative screening strategy is compared to other screening strategies, and new directions in virtual ligand screening are described.

One of the major challenges in *in silico* screening is ranking sets of candidate drugs according to binding affinity. Chapter 15 is a review of how binding free energies can be rigorously calculated from first principles and used to rank compounds. While great progress has been made in calculating the electrostatic components of the binding free energy, entropies of binding and the effects of changes to water structure in the active site are not yet adequately accounted for in binding free-energy calculations.

Section 5 includes three chapters on use of fragment-based methods in drug design. These methods combine high throughput screens of low molecular weight compounds with iterative structure-based drug design. Initial hits from screening are generally nonspecific, weak binders, but they can often be elaborated into specific, high-affinity inhibitors without increasing their size beyond the ideal molecular weight range for drugs. A comprehensive review of the common fragment-based approaches is found in Chapter 16, while Chapter 17 is focused on one specific approach, Tethering. Tethering is a fragment-based method that is especially useful for discovering inhibitors that bind to traditionally difficult target sites on receptors. Libraries of disulfide-containing compounds are screened for members that bind specifically near a cysteine at the target site, and subsequently form a disulfide link to that cysteine. Applications of Tethering to five protein targets are described.

One of the most explored classes of drug targets is the class of protein kinases. Therefore it is fitting to conclude the book with a discussion of drug development against these proteins. Chapter 18 is a summary of successful kinase inhibitors in the clinic today, including their binding modes and inhibition mechanisms. A challenge for treating complex diseases involving

kinase-signaling pathways is to optimize kinase inhibitors for inhibiting a specific subset of the kinome. As described in this chapter, fragment-based methods are well suited to discovering selective inhibitors because small compounds can bind to nonconserved pockets, and can also induce conformational changes in order to access nonconserved residues. The chapter ends with a description of a comprehensive approach to kinase drug discovery involving high-throughput fragment-based screening, high-throughput crystallography, bioassays against the whole set of human kinases, and ultimately, bioinformatics to guide the design of inhibitors with the desired inhibition profiles.

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