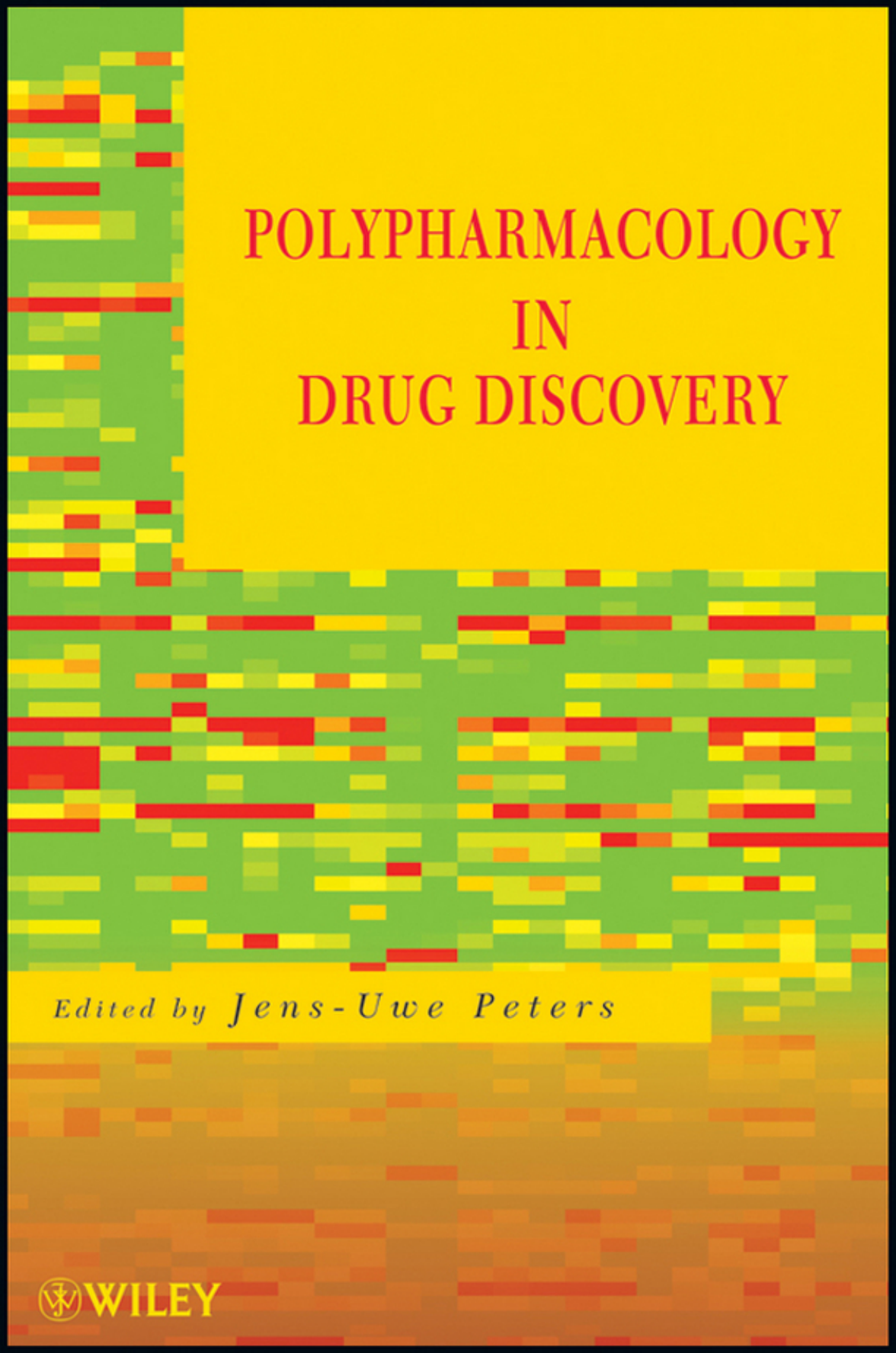
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IN
DRUG DISCOVERY
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PREFACE

Polypharmacology, the activity of compounds at multiple targets, has been gaining increasing attention since the 1990s, and is currently a hot topic in industrial drug discovery, as well as in academia. The 1990s witnessed the withdrawal of several drugs due to severe adverse effects, which led to permanent injury or deaths, with multi-billion-dollar legal damages. Some of these adverse effects have been linked to unintended interactions with specific off-targets, namely, the serotonin 5HT2B receptor for fenfluramine; the cardiac hERG channel for astemizole, terfenadine, and grepafloxacin; and the M2 receptor for rapacuronium. During this time, large screening panels were established by drug discovery and contract research organizations, which made it possible to recognize a wide range of off-target activities during the discovery process. As a consequence, more recent research has focused on identifying exquisitely selective drugs, with an expectation to avoid adverse drug reactions (ADRs), to improve compliance, and to gain a competitive advantage over less selective drugs.

On the other hand, the "one drug–one target" paradigm has been increasingly challenged in recent years. Not only has it been associated with a productivity decline throughout the pharmaceutical industry; it has also been increasingly being recognized that the therapy for polygenic diseases benefits more from a polypharmacological approach, which modulates a network of disease-related targets, rather than "switching" a single target on or off. For instance, despite a long quest for selective drugs, all clinically established antipsychotics today are polypharmacological drugs, with the gold standard clozapine having nanomolar activities at more than 25 targets. Polypharmacological therapies are often superior in the prevention of drug resistance, which is a major issue in the treatment of infections and cancer. In some disease areas, such as inflammatory diseases, the parallel inhibition of redundant disease-relevant pathways may be an attractive strategy for pharmacological intervention. In many instances, the inhibition of several targets may have synergistic therapeutic effects and may thus lead to more efficacious drugs. Additionally, polypharmacological drug discovery provides an opportunity to diversify research, to obtain drug candidates with unique pharmacological profiles, and to avoid a heavy focus on single targets that are often pursued across the whole industry at the same time.

Moreover, the idea that highly selective drugs are inherently safer and better tolerated than multitargeted drugs has been questioned. Rofecoxib may be a point in case; this drug, as well as other "coxibs," was designed to more selective, and thus to be safer, than older antiinflammatory drugs such as ibuprofen. Rofecoxib was, however, found to increase the risk for cardiovascular events such as myocardial

infarction and stroke. These cardiovascular risks are believed to be the result of rofecoxib's selectivity for one cyclooxygenase isoform, and have led to the withdrawal of this multi-billion-dollar drug in 2004. Another example are the selective serotonin reuptake inhibitiors (SSRIs) and serotonin / norepinephrine reuptake inhibitiors (SNRIs); although they are considered to be very safe antidepressants, they have a high incidence of unpleasant side effects, which contribute to a high discontinuation rate. These side effects, such as disrupted sleep, sexual dysfunction, and acute nausea and anxiety, are thought to be alleviated by intervention at additional targets. Consequently, several ongoing research programs aim to combine reuptake inhibition with, for example, antagonism at certain serotonin receptors for an improved tolerability. As a more general concept, it has been argued that polypharmacological drugs can even have a safety advantage, because the modulation of target networks, without permanent, full blockade or activation of a single target, may reduce target-related adverse effects, or may lead to lower efficacious doses.

Thus, there are two sides of the polypharmacology coin: (1) unwanted off-target activities may lead to adverse drug reactions and need to be avoided, while (2) polypharmacological drugs with multiple activities across a disease-relevant target class, such as G-protein-coupled receptors (GPCRs), ion channels, or kinases represent opportunities for improved therapies, as illustrated by the approvals of asenapine (2009), dronedarone (2009), and sunitinib (2006), respectively. Both of these sides will be discussed in this book. For an easy orientation according to the reader's background and interests, the book is divided in four parts:

Part A. Unintended activities at "antitargets" are typically discovered late in the drug discovery process, and have been a reason for late-stage failures, or at least a major hurdle in late lead optimization. The first part of the book discusses concepts and tools that help to recognize, interpret, and address such "antitarget" liabilities early in the drug discovery process, and thus to reduce costly late-stage attrition. Part A opens with an introduction to the relevance of polypharmacology for the safety of drugs, illustrated with salient cases of drugs and drug candidates with off-target-related toxicity. This is followed by an insightful guide to why, when, and how to screen for off-target activities, and how to predict and mitigate potential ADRs. The avoidance of promiscuity-related molecular properties as a strategy to reduce the risk for promiscuity is discussed in the third chapter. Numerous important antitargets will be introduced in these first three chapters, with a focus on GPCR targets. Two other classes of frequently encountered antitargets, kinases and cardiac ion channels, are discussed next in dedicated chapters. Part A concludes with a chapter on data mining and pharmacological "fingerprint" profiling, which allows for the prediction of otherwise nonobvious ADRs. All this is supported by useful reference information, such as lists of off-targets associated with potential ADRs, frequently hit off-targets, and practical examples of lead optimization.

Part B. The productivity of the drug discovery industry as a whole has declined since the 1990s, with only ~20 new chemical entities per year reaching the market, despite ever-increasing research budgets. New discovery paradigms are therefore necessary to deviate from established research strategies, and to address those unmet medical needs that do not succumb to the ubiquitous "small molecule–single target protein" approach. Multitargeted, or polypharmacological, drug discovery may present opportunities where conventional approaches have been failing, especially for the treatment of diseases with multiple pathogenic factors, and diseases where resistance poses an important problem. Part B highlights four such disease areas: psychiatric diseases, cancer, bacterial infections, and epilepsy. Although the introductory chapter is dedicated to psychiatric drugs, the concepts discussed are instructive and generally applicable. In contrast to the antischizophrenia drugs, which were originally discovered serendipitously without knowledge of their polypharmacological nature, more recently approved kinase inhibitors for the treatment of cancer were developed with the knowledge of their polypharmacology, or were even deliberately designed to be polypharmacological, as outlined in the second chapter. The third chapter shows that most clinically established antibiotics rely on the inhibition of several targets, or targets encoded by multiple genes. In contrast, targets encoded by single genes, such as those obtained from bacterial genomes, have not led to novel antibacterials, or are associated with rapid resistance mutations. The fourth chapter shows how antiepilepsy drugs often enhance or inhibit multiple ligand- or voltage-gated ion channels, and proposes a strategy for the discovery of novel, multitargeted antiepilesy therapies. The final chapter of the "opportunity" part of the book is not related to a disease area, but rather highlights an approach to lead finding that exploits the polypharmacology of existing drugs: the selective optimization of side activities (SOSA). This approach has been historically very successful, but has been neglected in more recent years in favor of high-throughput screening. The chapter shows how this concept can be revived and complemented with modern *in silico* methods. As Sir James Black states: "The most fruitful basis for the discovery of a new drug is to start with an old drug."

Part C. Most of today's "multitargeted" drug discovery programs seem to have originated from the serendipitous discovery of dual, and sometimes multiple, ligands of often related disease-relevant targets. Apart from such obvious opportunities, multitargeted drug discovery is often perceived as not very feasible by industrial researchers, because of the more difficult lead finding, and the increased complexity of lead optimization. To illustrate how multitargeted drug discovery can be put into practice, a number of selected approaches are presented in Part C. The first chapter discusses how starting points for multitargeted drug discovery programs may be obtained either by screening or rational framework combination, and how such compounds can be optimized. This is illustrated with many examples. The *in silico* approaches for multitarget screening can be employed to find multitargeted

drugs or repurposing opportunities for existing drugs. This is discussed in the second chapter, with a focus on the 2010 NIH Director's Pioneer Award–winning CANDO method. This chapter provides also a detailed introduction to *in silico* screening in general, and will certainly attract the interest of *in silico* experts and nonspecialists alike. The third chapter introduces an intriguing method of high-throughput *in vivo* screening, in which a large number of behavioral readouts are automatically recognized, processed, and compared with a database of behavioral signatures of central nervous system (CNS)-active drugs. Such methods may constitute a modern version of the physiologically based screening paradigm that was the mainstay of the golden era of drug discovery. Many other possibilities are perhaps more obvious and do not warrant a detailed discussion. For instance, the mining of proprietary high-throughput screening (HTS) or safety panel data, or commercial or public pharmacological databases may be a rich source of multitargeted leads as starting points for discovery projects. Also, the "anticonvulsants" chapter of Part B proposes a generally applicable screening strategy, where novel multi-targeted leads are sought to mimic the profile of successful drugs. Finally, Part C is rounded off with an introduction to multicomponent therapeutics, and shows how combinations of drugs can be selectively synergistic for therapeutic versus adverse effects, because the drug targets of the components are expressed together only in the tissue that is responsible for the therapeutic effect.

Part D. The last part is a collection of various instructive case studies. Most of the chapters in this part are dedicated to the discovery of polypharmacological or specifically multitargeted drugs, ranging from the highly promiscuous anticancer drug sunitinib and antipsychotics over broad-spectrum antidepressants to the dual-acting drug candidate licofelone for inflammatory diseases. The achievement of (reasonable) selectivity in the kinase field, and activity across mutant targets, is discussed in the imatinib case study. An untypical type of dual activity is displayed by the experimental drug PA1103, which is able to inhibit the polymerization of heme, as well as to alkylate heme, both validated concepts for the treatment of malaria. The penultimate chapter discusses multitargeted approaches to the treatment of Alzheimer's disease, and specific clinical and preclinical compounds. A final chapter is dedicated to the (off-target) activities of established drugs at carbonic anhydrases; this chapter illustrates the potential of target discovery, drug repurposing and the discovery of new drugs based on the polypharmacology of existing pharmaceuticals. Throughout Part D, many authors express their personal views on the future of polypharmacological drug discovery. These views, ranging from slight skepticism to enthusiasm, may provide the reader with a balanced and realistic impression of the promises and challenges of polypharmacological drug discovery.

This book is intended as a practical guide for drug hunters to successfully navigate around the dangers of promiscuous ligands and targets, as well as a source of inspiration for new polypharmacological drug discovery projects. However, this

collection of reviews, opinions, and case studies is by no means an exhaustive treatment of all possible facets of polypharmacology. For instance, drug–drug interactions are not treated; although formally a polypharmacology issue, this is usually regarded as DMPK-related, and several excellent books cover this important topic. Similarly, pleiotropic effects through interaction with single targets, the achievement of selectivity across related targets or within target classes, or the contribution of active metabolites to the pharmacological spectra of drugs may be regarded as "polypharmacology topics," but were considered to be beyond scope of this text.

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Note: Color versions of select figures are available at `ftp://ftp.wiley.com/public/sci_tech_med/polypharmacology`.

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INTRODUCTION

The Case for Polypharmacology

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Should a drug be selective or promiscuous? The conventional goal of medicinal chemistry is to explore the structure–activity landscape to optimize the selectivity of a compound for a chosen drug target, over all others. Increasing the selectivity of a drug for one target over all others can lead to a reduction in safety liabilities. Designing drugs toward single-target profiles has been the dominant philosophy in drug design since the concept of *chemoreceptors* merged with molecular biology. Indeed, understanding the so-called off-target activities of a drug by prediction and experiment, and minimizing these is an important aspect of exploiting knowledge of a drug's promiscuity. More recent analysis of the physicochemical properties of failed and successful drug candidates have highlighted the relationships between target promiscuity, toxicity, and lipophilicity [1,2]. Indeed, promiscuous drugs are often labeled "dirty" drugs [3]. However, since the 2000s the assumption of the desirability of single drug target mechanisms has began to be questioned [4–6]. In certain circumstances, it may be advantageous for a drug to act on multiple drug targets, deliberately and specifically rather than be too selective.

This book explores the many different aspects of the concept of polypharmacology. *Polypharmacology* can be defined as the modulation of several drug targets to achieve a desired therapeutic effect. Polypharmacology stands in contrast to the dominant paradigm in current drug discovery of selectively targeting a single type of drug target. Recently there has been a growing interest in the concept of polypharmacology. Before proceeding to summarize the arguments for the importance of perturbing multiple drug targets, let's consider the concept of a drug target more generally. The concept of a drug target is as fundamental to modern pharmacology as the concept of the gene is to molecular biology. The transformation of the concept of the gene from units of phenotypic inheritance to individual protein-coding units coincides with the emergence of the concept of specific chemoreceptors as the targets for drugs. The concept of the gene transformed from the Mendalian unit of phenotypic inheritance [7] to units of protein coding, culminating in the Beadle–Tatum formation of

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the "one gene, one enzyme" model of 1941 [8], later modified to "one gene, one polypeptide," with each gene responsible for producing a singe protein [9]. In parallel, the of the chemoreceptor concept was proposed by both Clark and Ehlrich in the first decade of the twentieth century [10], yet the theory of receptor-mediated drug interactions did not gain wide acceptance until Ahlquist demonstrated the differential action of adrenaline on two distinct receptor populations, in 1948.

However, the concept of "one gene, one protein" was extended to "one gene, one protein, one disease" and become a major intellectual assumption behind target-based drug discovery [11]. This in part is linked to the discovery of the role of individual genes in Mendalian inherited disorders, such as Linus Pauling's 1949 discovery of the single-protein cause of sickle cell anemia [12]. The extended concept of "one gene, one protein, one disease" became a powerful assumption for molecular target-driven drug discovery and development [11,13]. However the recognition that complex traits are not the result of one gene but of several interacting genes has been long been recognized, as far back as Bridge's work on sex in *Drosophila melanogaster* in the early 1920s. Furthermore, as the concept of complex traits beyond single-gene phenotypes goes back to the foundation of molecular biology, so to, can the roots of polypharmacology be argued to extend back to Ehrlich's extension of the concept of chemoreceptors to include "polyceptors, with multiple binding sites" [10].

Large-scale functional genomics studies, in a variety of model organisms, have revealed that under laboratory conditions the vast majority of single-gene knockouts by themselves exhibit little or no effect on phenotype, with approximately 19% of genes being essential across a number of model organisms [14–16]. In addition to the 19% lethality rate, systematic genomewide homozygous gene deletion experiments in yeast reveal only 15% of knockouts resulting in a fitness defect, under ideal conditions [17]. A project intended to delete each of the druggable genes [18] in the mouse genome and profile each knockout across a battery of phenotypic assays has revealed that a proportion as low as 10% of knockouts demonstrate phenotypes that may be of value for drug target validation [14,19–21]. Phenotype robustness can be understood in terms of redundant functions and alternative compensatory signaling routes that enable individual nodes to be bypassed [22].

The robustness of individual gene perturbation is also revealed by metabolic flux analysis, where modulation of single components in a pathway rarely results in large changes in metabolic flux and therefore phenotype [13,23,24]. Greater phenotype perturbation is observed in systems where two or more gene products are modulated. The emergent phenotype that occurs from the perturbation of multiple nodes is demonstrated by the systematic experiments on synthetic behaviors: synthetic lethality, synthetic sickness, and synthetic rescue. Systematic experiments with dual knockouts in model systems have shown that, while the deletion of two genes in isolation may show no effect, the simultaneous deletion of two genes can lead to "synthetic lethality" or "synthetic sickness" [25]. When dual knockouts are introduced, by genetic or chemical perturbations, the number of essential genes in yeast is predicted to significantly expand the 19% of genes for which singleton gene knockouts are lethal. A large-scale study by Hillenmeyer et al. demonstrates the extent of synthetic lethality when gene deletions are augmented by chemical intervention [26].

Under ideal conditions only 34% of single-gene deletion results in lethality or sickness. When the whole-genome panel of yeast single-gene knockouts was screened against a diverse, small-molecule library and assayed against a wide range of environment conditions, an additional 63% of gene knockouts showed a growth phenotype [26]. Thus 97% of genes demonstrate a fitness defeat when challenged with a small molecule under at least environment conditions. The vast majority of genes may be redundant under any one environment, but there appears to be little redundancy across a spectrum of conditions when a genetic perturbation is combined with a chemical insult. Genes that may appear dormant and dispensable under one specific set of conditions may prove essential under other stresses [27,28].

Insight into the experimental results describing phenotype robustness to perturbation can be found from understanding the role of biological networks. The architecture of networks in the robustness, degeneracy and redundancy of biological systems is fueling a challenge to the dominant assumption of single-target drug discovery [5,29–32]. Network analysis of biological pathways and interactions has revealed that much of the robustness of biological systems can derive from the structure of the network [33,34]. The scale-free nature of many biological networks results in a system that is resilient against random deletion of any one node but is also critically dependent on a few highly connected hubs. Network biology analysis predicts that if, in most cases, deletion of individual nodes may have little effect on disease networks, modulating multiple proteins may be required to perturb robust phenotypes [5,33,35].

The inherent robustness of interaction networks, as an underlaying property, has profound implications for drug discovery; instead of searching for the "disease-causing gene," network biology suggests that the strategy should be to perturb the disease network [36,37]. Hellerstein has argued the true targets of drugs are not individual proteins but functionally important biochemical pathways embedded in larger biological networks [11].

These intellectual foundations challenge long-held asumptions behind single-target selection. In response to these new biological insights into the complexity, robustness, and redundancy in disease phenotype, a new approach to drug discovery, namely, polypharmacology, is emerging [3,5,6,29,30,35,38–45]. Therefore, understanding the polypharmaoclogy of a drug and its effect on biological networks and phenotype is essential if we wish to improve efficiacy but also understand toxicity [3].

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