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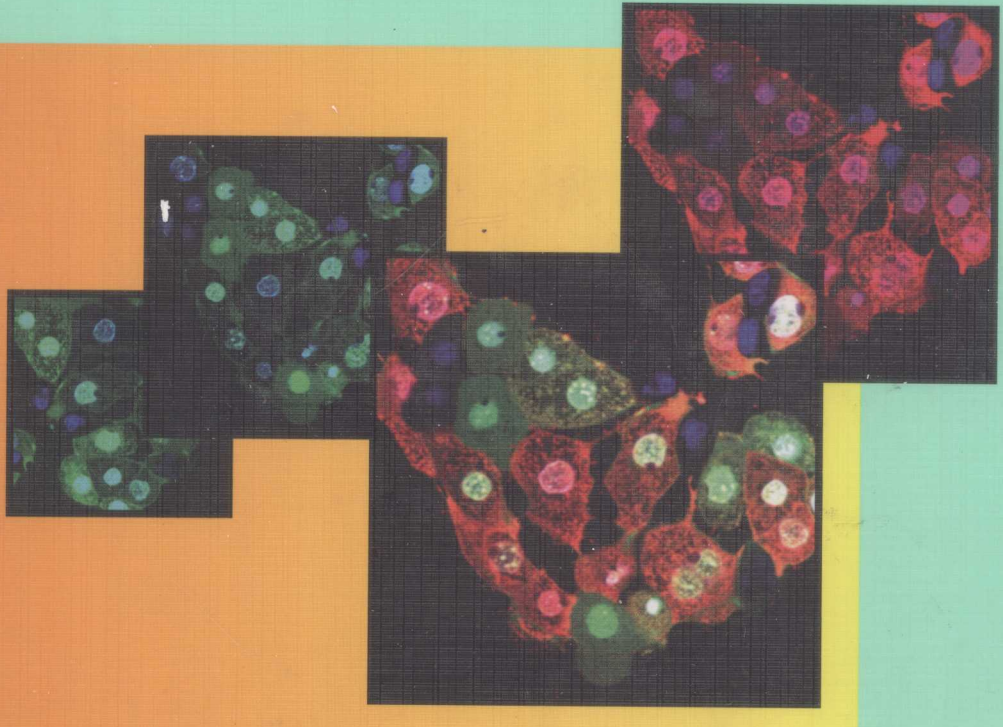
Biological, Physicochemical, and Computational Strategies



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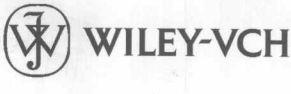

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Preface

The two Symposia organized at the University of Lausanne in March 1995 and 2000 met with considerable success, and the two resulting books [1][2] continue to receive frequent citation. In March 2004, *LogP2004 – The Third Lipophilicity Symposium* was organized at the Swiss Federal Institute of Technology (ETH) in Zurich as a logical sequel. Its theme (*Physicochemical and Biological Profiling in Drug Research*) is of the greatest current significance in drug research. A total of 26 invited lectures and 94 free communications were presented, most of the latter being also submitted for inclusion in the attached CD-ROM. The book with its 28 chapters and the CD-Rom form the Proceedings of the Symposium.

Informatics and robotics are the workhorses of a technological revolution in drug research. On them are based combinatorial chemistry which yields compounds by the many thousands, and high-throughput bioassays which screen them for bioactivity. The results are avalanches of hits which invade the databases like swarms of locusts. But far from being a plague, these innumerable hits become a blessing if properly screened for 'drug-ability', *i.e.*, for 'drug-like' properties such as good pharmacokinetic (PK) behavior. Pharmacokinetic profiling of bioactive compounds has thus become a *sine qua non* condition for cherry-picking the most-promising hits. Just as important, but less visible, are the structure–property and structure–ADME relationships which emerge from PK profiling and provide useful feedback when designing new synthetic series.

Absorption, distribution, metabolism, and excretion (ADME) are the focus of this book. Since the previous Symposium in 2000, many advances have been made both in methods and concepts, and many impressive successes have been reported. Schematically, they can be categorized as biological, physicochemical, or computational strategies. Recently, a synergistic use of these various strategies has emerged under the *in combo* roof, *i.e.*, combined and multidisciplinarity approaches whose potential goes well beyond that of the individual methodologies.

The book is structured according to these three strategies, themselves framed by two introductory and two concluding chapters. In the first introductory chapter, *David Triggle* takes a bird-eye view of scientific and societal issues in drug research. *Han van de Waterbeemd* then narrows the focus by taking a global look at property-based lead optimization.

Part II is dedicated to major biological strategies in ADME profiling. The role and significance of membranes receive an in-depth treatment in *Ole G. Mouritsen*'s chapter. The state of the art in cell cultures as absorption models is examined next by *Artursson* and *Matsson*. The two

following chapters present an expert's view on the tools used in early metabolic screening (*Walther* and co-workers), and on the assessment of a candidate's capacity to induce drug-metabolizing enzymes (*Meyer et al.*). The significance of uptake and efflux transporters is gaining an ever increasing recognition in drug research and is expertly reviewed here by *Kusuhara* and *Sugiyama*. The newly recognized role of plasma protein binding in drug discovery is given convincing treatment in the next chapter by *Fessey et al. Part II* concludes with an insightful theoretical chapter on *in vivo* pharmacokinetic profiling.

Part III covers recent advances in the physicochemical strategies used to predict the ADME profile of bioactive compounds. Automated parallel synthesis is considered first, with a marked emphasis on drug-like features. The connected properties of ionization and lipophilicity continue to occupy a significant portion of the scene, as insightfully discussed by *Caron* and *Ermondi*. A novel HPLC technology to assess lipophilicity is presented by *Lombardo et al.*, while the role of lipid bilayers as permeation barriers receives an apt recognition in the next chapter. The PAMPA technology has progressed markedly since the last symposium, as explained by *Avdeef*. Interestingly, PAMPA permeation is correlated with some lipophilicity parameters, as demonstrated by *Comer* and co-workers. Predicting the intestinal solubility of poorly soluble drugs is a challenge faced head-on by *Dressman* and her co-workers in the next chapter. *Part III* then ends with two chapters covering properties, which until recently were in the province of late drug development and are now receiving earlier attention, namely chemical stability (*Kerns* and *Di*) and solid-state properties (*Giron*).

Part IV is devoted to many recent advances in the computational strategies that are having such a major impact on early predictions of physicochemical and pharmacokinetic profiles. This part opens with an overview by *Mannhold*, who offers a systematic overview of traditional and recent methods to calculate lipophilicity. In the next chapter, *Vistoli et al.* present and illustrate a concept, whose significance in structure–activity relationships remains to be better understood and recognized, namely the property space of molecules. Predicting the interactions between bioactive compounds and drug-metabolizing enzymes is a topic of the highest importance in drug discovery, and no less than four chapters are devoted to it. The computation of pharmacophores to predict biotransformation is exemplified by *Cruciani et al.*, and by *Clement* and *Güner*. *Barbosa* and her co-workers show how enzyme inhibition can be predicted based on large databases. *Judson* then presents an expert system created and developed to offer global predictions of drug metabolism. A higher order of biological complexity is covered in the chapter by *Lavé*

et al., who discuss physiologically based pharmacokinetic models. The last chapter in *Part IV* is a dense and demanding presentation of a very powerful in-house network of expert systems to process the flood of data generated during biopharmaceutical profiling.

The two concluding chapters open the reader's horizon by addressing two essential issues in PK profiling. First, *Borchardt* summarizes his views on the education of, and the communication among, scientists in drug discovery. *Cautreels et al.* then offer provocative ideas on the present and future significance of ADMET profiling in industrial drug research.

The mission of ADME profiling is to increase the clinical relevance of drug design, and to eliminate as soon as possible compounds with unfavorable physicochemical properties, pharmacokinetic profiles or toxicity. The objective of this book is to show how modern drug research achieves this mission. International authorities and practicing experts from academia and industry have been generous with their time and offer state-of-the-art presentations of concepts, methods, and technologies now in use or development in drug research.

The book would not exist were it not for help given by the members of our Scientific and Advisory Board, the technical organizers of the Symposium, the graduate students of the Pharmacy Department at the ETH, and various institutions and sponsors. Editing this volume has been a challenge and a memorable experience. We hope that our readers will find it a source of information, knowledge, and inspiration.

The Editors

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Contents

Part I. Setting the Scene

Pharmaceutical Research: For What, for Whom? Science and Social Policies

by **David J. Triggle**

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1. Introduction

'Now that the liability to, and danger of disease are to a large extent circumscribed – the effects of chemotherapeutics are directed as far as possible to fill up the gaps left in this ring.' *Paul Ehrlich*, 1913

To deny that advances in health delivery and research, including therapeutic medicines during the past sixty years, have not been of significant benefit to mankind is to deny reality. Equally, few will be prepared to deny that the future will be one of at least equal promise. Children will be born with their genes profiled, 'personalized' medicines will be a reality, gene and stem cell therapies will be mature disciplines with major implications for the degenerative disorders of an aging world. This new world will be one of artificial cells and machines, many specifically created *de novo* with an expanded genetic code and that will execute unique tasks ranging from the site- and disease-specific delivery of drugs, genes, and gene repair instructions to neuronal- and DNA-based computers. These advances will have been made possible by a remarkable several generations of scientific research, culminating in the reading of multiple genomes, including the human genome.

The promise of *Ehrlich*, written ironically enough on the eve of World War 1, remains unfulfilled. Indeed, the world now faces challenges at least as large as those that existed at the turn of the 20th century. Two thirds of the world – the 'poor world' – still lives without adequate education, food, health care, sanitation and water, whilst the 'rich world' follows policies that largely ensure the continuation of this division, despite the spectacular advances in science and technology over the past one hundred years. Nowhere have these advances been as dramatic, spectacular or promising

as in medicine and the pharmaceutical sciences, yet nowhere is there greater inequity of application, distribution, or benefits.

Indeed, in many important respects, the material gap between the rich and the poor worlds has increased rather than decreased. Some 11 million children die every year from starvation and other largely preventable diseases, almost 2 billion people live on less than one dollar a day, some 1.5 billion people routinely lack clean drinking water and sanitation, and malaria and other tropical diseases affect almost one billion people and account for some 5 million annual deaths. And this year, worldwide deaths from AIDS reach 3 million. The United Nations Human Development Report for 2003 notes that the 1990s, far from being a decade of progress, have actually seen remarkable reversals: 54 out of 175 countries are poorer in 2001 than in 1991; in 14 out of 175 countries, more children are dying before the age of five; in 21 out of 175 countries, more people are starving; and in 12 out of 175 countries, fewer children are being educated [1]. The gap between the rich and the poor worlds has actually increased in several areas of the world. Science has delivered for the rich world, but party and politics have blinded our eyes and have limited the participation of the poor world. Progress will not be possible until we break their cycle of poor health driving poverty: this is not likely to occur in the present Washington-driven 'free market Darwinism' model of economic development. Indeed, in the United States, where this policy is most slavishly advocated and followed, there has been a remarkable increase in income inequality between the richest and the poorest segments together with a considerable weakening of the social infrastructure of the country [2–6]. Such market-driven ideologies provide little or no incentive for the development of drugs for the diseases of the poor world, and alternative models must be adopted [7].

The challenges for the poor world in the 21st century are many. In particular, the absence of an adequate scientific and educational infrastructure confers an enormous disadvantage in an environment dominated increasingly by trade and intellectual property imbalance. The ongoing efforts to impose the existing standards of patent and copyright protection on the poor world are, in fact, likely to exacerbate the cycle of poor health and poverty. The selfish discussions over the past several years on making AIDS and other drugs available to the poor world provide adequate, and offensive, testimony to this point. Furthermore, the increasing enclosure of the scientific commons, to which aim universities that have always been the major contributor to this commons are now enthusiastic partners, will only exacerbate the problems of the poor world by diminishing their access to scientific and technological knowledge.

A recent issue of *The Economist* [8] observed, '*That the mental landscape today is almost unrecognizable from that of, say two centuries ago, is due almost entirely to the work of two groups of thinkers – scientists and economists. Add engineers to that and you have an explanation of why the physical, commercial and political landscapes have changed just as radically*'. This is true: science is mankind's greatest intellectual achievement, but its full realization will come only when it is placed fully in the service of man. We are a long way from that goal and in the absence of that achievement, particularly in the delivery of critical medicines and health services, our science will be naught for our comfort – physically or spiritually.

2. The Drug Discovery Process

'*They are ill discoverers that think there is no land, when they can see nothing but sea.*'
Francis Bacon, 1561–1626

2.1. *Overview*

The traditional process of drug discovery has been directed by target generation from observations of the biological activity of a natural product or synthetic entity on a physiological or pathological process [9]. Typically, the identification of a lead active structure was followed by iterative structural modification and biological testing. This process, essentially a 'one molecule at a time' approach relying heavily on trial and error, serendipity, scientific intuition, genius, and luck has achieved many notable therapeutic successes. Prominent examples include the development of antibiotics, β-adrenoceptor blockers, histamine H_2 receptor antagonists, ACE inhibitors, calcium blockers, and angiotensin II receptor blockers [10]. The essential mechanism of action or the structure of the underlying target not being a necessary prerequisite, the characteristics of this process are that the target is phenotypically defined and validated – blood pressure, acid secretion, smooth muscle relaxation or contraction *etc.* In contrast, the advent of genomics has led to the development of genotypically defined targets with defined structure, but frequently with undefined or only hypothetical phenotypical function.

It has been estimated that currently available drugs are directed towards some five hundred molecular targets with membrane receptors, notably G protein coupled entities, constituting almost 50% of the total. The heady promise of the genome project was that the human genome would be composed of perhaps as many as 150,000 genes generating on a

'one gene = one protein' rationale a *ca.* 300-fold increase in the number of possible drug targets. This number, together with the targets potentially realizable from bacterial and parasite genomes, was predicted to change dramatically the scale of the drug discovery enterprise. Simultaneously, the development of the new technologies of combinatorial chemistry, high-throughput screening, and informatics generated the *Viagra*-fueled 'bigger is better' model of drug development – the larger the company and the greater the throughput from chemistry and screening, the greater would be the output.

That the human genome expresses only some 30,000 genes (more than, but not dramatically so, our less complex fly, worm, and mouse relatives) means necessarily that the complexity of human organization is defined by multiple use of the same gene – splice variants, alleles in the population, post-translational modification *etc.* – and by the combinatorial diversification of regulatory and signaling pathways. From this relatively limited gene catalog, the human probably expresses in spatially and temporally limited manner as many as 100,000 proteins. A protein target may not be druggable because of its intrinsic properties or expression, but also because of a role it may play in regulatory networks other than the one of pathological interest. The elucidation of the cellular signaling network is therefore a critical component of the target validation problem [11]. Increasingly, a systems biology based approach is needed whereby an integrated approach, rather than a reductionist component analysis, is employed to understand the relationship between the overall function of a biological system and the effects of perturbations such as disease or small molecules [12]. Additionally, for a protein target to be druggable, there must be certain characteristics of the protein binding site: if one member of a gene family can bind a drug then it is assumed that other members will likely share this property. Using this approach, it has been estimated that *ca.* 10% of the proteins expressed by the human genome will fall into the druggable category. Three to four thousand targets is a far cry from the in excess of one hundred thousand targets originally claimed, although the former number may well increase as the roles of genes of previously unknown function are discovered [13].

The power of genomics to generate targets of well-defined proteins – receptors, channels, enzymes, *etc.* – to use in high-throughput screening has been extremely useful for generating 'hits' of appropriate affinity and, in a number of systems, to generate functional activity also. However, since these same systems usually, if not invariably, lack the complex signaling characteristics of 'real' cells and the integrated functional physiological properties of organ systems, they remain limited in their predictive properties and have probably served to consume very large