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Peptide-Based Drug Design

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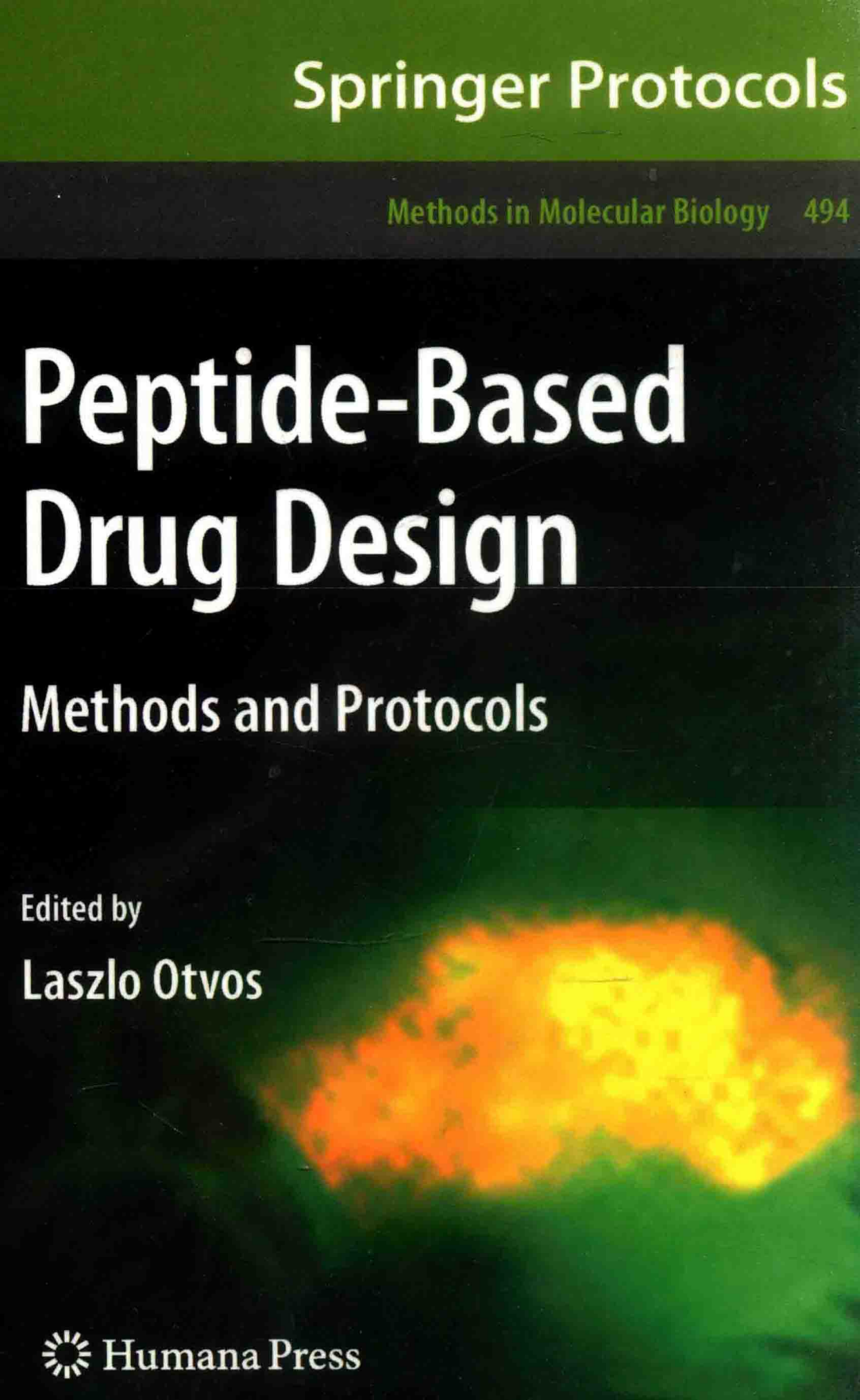

Edited by

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METHODS IN MOLECULAR BIOLOGY™

Peptide-Based Drug Design

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Peptide-Based Drug Design

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Preface

Natural products chemistry books usually open with a chapter on amino acids, peptides, and proteins. Peptides represent the interaction site between bioactive proteins. Together with peptide hormones, these molecules promise to be the starting points of drug development. Indeed, with the fast advances of peptide synthetic techniques, injectable peptide drugs obtained regulartory approval. Currently, peptide/protein drugs contstitute more than 10% of the ethical drug market. However, they are orally not available and expensive to mass produce. Thus, in the late 1980s, pharmaceutical companies quickly abandoned peptide research and focused on small molecules with more advantageous in vivo stability and pharmacokinetic properties. At the turn of the new century, the unexpected toxicity and cross-reactivity of small molecule drugs turned investors back to peptides, with their high specificity and, in most cases, low toxicity profile. The 15 years in exile was not completely useless; we all learned how to modify peptides to be competitive with small molecules in the drug development arena. Peptide synthesis technology further developed both as a research tool to produce biopolymers containing more than 100 natural or non-natural amino acid residues and a variable industrial production opportunity. Fast computers and improved algorithms brought structure-based peptide drug design to our desks. Understanding peptide metabolism, delivery, and clearance provided tools not only to increase the half-life in biological media, but also targeting to tissues and cell compartments unavailable earlier.

My colleagues frequently come to me and ask: "I have this exciting peptide sequence (real or virtual). It produces miraculous biological results in vitro (or so I hope). What should I do with it? What can I do to make it work in vivo, attrack investor's interest, or just grant funding more readily available for developmental projects than basic science?" These questions prompted us to assemble a handbook that offers a selection of research and production tools suitable for transforming a promising protein fragment or stand-alone bioactive peptide to a pharmaceutically acceptable composition. The chapter subjects include peptide-based drug development, from the early stages of computer-aided design and target identification through sequence modifications to satisfy pharmacologists until the actual production of difficult sequences and in vivo imaging. As with all contemporary and somewhat complicated chemistry projects, the devil is in the details. The format of the *Methods in Molecular Biology* series allows the contributing authors to provide sufficiently detailed information to reproduce the

protocols or make the technology available for other projects calling for similar solutions.

The current buzzwords, chemical biology, somewhat degrade peptide chemistry to a service of biology. With the current book, we demonstrate that peptide-based drug design is an independent science that is well and on the rise. Our ultimate satisfaction will arrive when the first molecules, helped to conception or manufacture from the ideas set forth in this volume, gain regulatory approval and market share as stand-alone or combination medicines.

Laszlo Otvos

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Contents

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Peptide-Based Drug Design: Here and Now

Laszlo Otvos, Jr.

Summary

After many years of stagnation, peptide therapeutics once again became the focus of innovative drug development efforts backed up by venture funds and biotechnology companies. Designer peptide drugs overcome the unattractive pharmacological properties of native peptides and protein fragments and frequently feature nonnatural amino acid or backbone replacements, cyclic or multimeric structures, or peptidic or nonpeptidic delivery modules. With their high specificity and low toxicity profile, biologicals offer viable alternatives to small molecule therapeutics. The development of peptide drugs requires specific considerations of this family of biopolymers. Historically, peptide vaccines to viral infections and antibacterial peptides led the way in clinical development, but recently many other diseases have been targeted, including the big sellers AIDS, cancer, and Alzheimer's disease. This book gives practical advice to the most important steps in peptide-based drug development such as isolation, purification, characterization, interaction with targets, structural analysis, stability studies, assessment of biodistribution and pharmacological parameters, sequence modifications, and high throughput screening. This brief overview provides historical background for each of the listed techniques and diseases.

Key Words: Backbone; design; nonnatural amino acids; pharmacology; recognition; stability; toxicity.

The vast majority of current designer drugs target biopolymers or biopolymer interactions. There is no surprise here: the more we know about complex biochemical pathways, the larger the number of potential target proteins, nucleic acids, or lipidic structures that surface and offer themselves for agonist or antagonist development. In many cases a protein fragment, either part of the unprocessed protein or a cleaved piece, can be identified that serves as a ligand or the target itself. Naturally occurring peptide drugs are just the beginning. With the

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advance of computer power and molecular interaction databases, ligands can be designed for those protein fragments where nature has not yet succeeded but the models show validated structural features. With their high specificity and low toxicity profile, peptide-based drugs should be the first choice as contemporary therapeutics.

Unfortunately, until very recently, large pharmaceutical companies and biotech investors saw a more promising business opportunity in making one cent per dose of a small molecule—frequently generic—drug than a hundred dollars per dose of a groundbreaking biological therapeutic. This trend was the consequence of large amounts of money invested in the 1980s in peptide drugs only to learn that natural peptides lack pharmacological properties, such as serum resistance, tissue penetration, oral bioavailability, and delayed elimination—all needed for a fast and painless drug-development process. In addition, large-scale peptide manufacturing was considered prohibitively expensive, and only peptide hormones, given in low doses, could attract commercial interest. However, the rising number of and publicity about side effects seen for small molecule blockbusters (e.g., cancer chemotherapeutics or COX-2 inhibitors) together with innovative synthetic strategies and low monomer prices suddenly opened closed doors and liberated closed minds for peptide-based drug development.

Since the turn of the millennium, many biotech companies have discovered new peptides with interesting pharmacological properties, and the solid-phase peptide synthesis was optimized, allowing routine synthesis of large polypeptides and small proteins ***(1)***. In 2000 the total ethical pharmaceutical market was worth about $265 billion, with peptides and proteins, excluding vaccines, accounting for more than 10%. The number of new chemical entities (NCEs) has been almost stable for about 10 years, with around 35–40 each year, but the number of peptide and protein NCEs has been continuously increasing during recent years. The most prominent recent peptide drug is T-20 (Enfuvirtide), which blocks HIV entry into cellular CD4 ***(2)*** and can be considered the turning point of investor attitude to biotech in general and to peptide drugs in particular. This book gives practical advice for researchers who have already decided to venture into peptide-based therapeutics. The short review below takes bits and pieces of the history of how we got here and points out the theoretical considerations and practical solutions offered by the individual chapter authors.

The poster child of modern peptide drugs T-20 inhibits gp41 hexamer formation ***(3)***. With a 3.0 n*M* IC_{50} value in the HIV-mediated cell–cell fusion assay and serum half-life of 3.8 h, it has everything drug developers ask for. T-20 is a 36-mer peptide with no nonnatural amino acids except an acetylated amino-terminus. The drug is a clear success even if a yearly supply costs approximately $20,000. Another HIV drug candidate, the CCR5 transmembrane receptor ligand RANTES (68 residues), is an example of the current trends

in peptide modification. N-terminal derivatization, first with aminooxypentane, later with nonanoic acid, together with nonnatural amino acid incorporation into positions 1–3 resulted in significant increase of potency ***(4,5)*** and likely protease resistance. The N-terminus of peptides is a favored position for medicinal chemistry modifications, as the automated solid-phase peptide synthesis can be finished off by manual addition of various N-capping amino acid residues or other organic acids. While acetylation is the most common technique used to prevent aminopeptidase cleavage, incorporation of modified and difficult-to-cleave residues, such as glycoamino acids, can be equally effective ***(6)***. Chapter 11 details how to introduce sugars and glycoamino acids into synthetic peptides.

Peptides designed to work in the central nervous system (CNS) have to cross the blood-brain barrier (BBB). Glycosylation of enkephalin analogs improves both the stability of peptide drugs and their penetration across the BBB, just as increased hydrophobicity does ***(7,8)***. In addition to active transport, peptides and cationized proteins enter the brain with the help passive transport mechanisms. The major sequence features of peptides and proteins that generally penetrate through biological membranes are a concentration of positive charges (arginines, lysines) interspersed with hydrophobic residues ***(9)***. These structures can interact with both the hydrophobic protective barriers and the negatively charged cell surface lipids and can deliver peptides through cell layers, including the BBB model endothelial cell-astrocyte model ***(10)***. Similar structural features promote entry into prokaryotic and eukaryotic cells. Currently only a few peptide drugs can be administered orally, but this gap will very be soon be overcome by innovative delivery technologies ***(11)***. The peptides are either coupled to active or passive penetration enhancers or simply mixed with formulations able to pass cell and tissue layers.

This leads us to antimicrobial peptides, a land of promise not yet realized. As effectors of the innate immune system, antimicrobial peptides naturally represent the first line of defense against bacterial infections ***(12)*** and as therapeutic agents indeed made through human clinical trials ***(13)***. Their isolation from natural sources is relatively easy, but the poor pharmacological parameters of peptides are magnified for antimicrobial peptides. The low systemic stability of proline- and arginine-containing peptides, common sequence features of bacterial cell-penetrating biopolymers, requires detailed stability studies in serum. Even if the stability parameters are acceptable (as is the case with antimicrobial peptide dimers containing nonnatural amino acids in terminal positions) ***(14)***, the fast renal clearance of peptides suggests that targeting urinary tract infections offers more hope than systemic applications ***(14)***. Nevertheless, *in vivo* active antimicrobial peptides are possible to develop, and as these products are multifunctional boosters of the immune system ***(15)***, we should

see derivatives active in bacteremia models in the foreseeable future. Chapters 2 and 3 look at the intricacies of the identification and characterization of antimicrobial peptides, and Chapter 8 presents a computer-based optimization strategy to finally break into the drug marketplace. Chapter 9 investigates the mode of action of proline-rich antimicrobial peptides by using genetic approaches. Recognizing the vital importance of systemic stability studies of peptide-based drugs, Chapter 10 presents the most frequently used *in vitro* and *in vivo* stability assessment methodologies.

Chemical synthesis, solution and solid-phase alike, is steadily gaining importance in manufacturing peptide drugs. In 2000 more than 40 different peptide drugs were made by chemical synthesis, with a sequence limit of 75 linear residues ***(16)***. However, with improvements in coupling reagents and fluidic transport machinery, we can expect the ceiling to soon reach 100 amino acids, the arbitrary border between peptides and proteins. The production price of peptide drugs made in large scale under good manufacturing practice (GMP) regulations hovers around $50–75 per gram, although the inclusion of nonnatural amino acids for which the synthetic monomers are not easily available can take it up to $1,000 per gram (for leuprolide made for a controlled release application). Indeed, the synthetic difficulties, sometimes precipitated in the price, or delivery difficulties limit the volume of peptide-based drug sales. At the time of its regulatory approval in late 2004, Elan's ziconotide, a synthetic conopeptide for the treatment of chronic pain, was expected to reach a sales peak at $250 million per year, well below a small molecule painkiller similarly working by calcium channel blockage, approved around the same time ***(17)***.

In addition to T-20, currently angiotensin-converting enzyme (ACE) inhibitors (zestril, enalapril) as well as peptide hormones are the major sellers, including insulin and calcitonin. However, many more peptide drugs are in the development pipeline. Among the potential drug families, clinically viable peptide-based subunit vaccines or epitope-based diagnostics were long considered possible to create. Monoclonal antibodies recognize a relatively extended stretch of protein fragments (6–20 residues), impossible to cover by small molecules ***(18)***. Likewise, binding to the major histocompatibility complex proteins for T-cell vaccines requires a 9- to 12-amino-acid stretch of continuous peptide. Peptide vaccines based on the human papillomavirus against cervical cancer and cancer of the head and the neck reached the clinical trial stage ***(19)***. As with almost all other peptide-based dugs, modern peptide vaccines are multimeric constructs often carrying additional boosters of the immune system. Chapters 14 and 15 show examples how complex peptide vaccines can be designed and manufactured. Due to their small size compared to proteins, peptides are preferred carriers of labels used for *in vivo* imaging where it is essential that the amount of label be sufficient per probe per receptor for optimal signal-to-noise ratio ***(20)***.

In Chapter 16, Joseph Backer (the inventor of site-specific labels by using Cys-tags) ***(21)*** presents imaging alternatives for peptide and protein drugs. In addition, Chapter 16 provides useful strategies to deliver polyamide-based drugs into biological systems to improve the diagnostic and therapeutic potential of these hydrophilic substances.

Alzheimer's disease (AD) is characterized by the brain accumulation of peptide and protein aggregates, i.e., the 42-mer Aβ peptide and the hyperphosphorylated τ protein ***(22)***. Synthetic peptides were essential to identify short τ fragments that are specifically recognized by AD-derived monoclonal antibodies ***(23)***. Due to the severity of the disease and the easy synthetic access to the culprit protein aggregates and their fragments, aggregation inhibitors to AD were always in the forefront of peptide drug studies. Peptidic β-sheet breakers were developed, but medicinal chemistry manipulations were needed to improve the *in vitro* and *in vivo* stability parameters ***(24)***. In general, frequently used peptide modifications include the introduction not only of nonnatural amino acid residues (or preassembly side-chain variations, if you will), but also of alterations of the peptide backbone as well as postassembly sequence modifications ***(25)***. These possibilities are too diverse to list; companies specialized in amino acid and peptide sales are well equipped to provide mimics of all 20 proteinogenic amino acid residues, monomers or oligopeptide carriers of structural determinants (β-turns, for example), or altered amide bonds. Chapter 13 presents a panel of potential synthetic routes to apply the most commonly used medicinal chemistry changes to the peptide backbone. Chapters 5 and 12 show how Aβ or τ phosphopeptides, respectively, can help the development of diagnostic or therapeutic tools in AD. It needs to be mentioned that the last two techniques are widely applicable to either additional peptide–protein interactions or non-AD phosphopeptides, for example, those dominating signal transduction research.

With the completion of the human genome sequence identification together with developments in the proteomics field, peptides are emerging as important molecules for many other small- or high-volume drug applications, including the most emotionally attractive cancer therapy ***(26)***. Several peptides with exciting preclinical results have now entered into clinical trials for the treatment of human cancers as inhibitors of oncogenic signaling pathways ***(27)***. However, the complexity of these signaling processes and the large number of potential targets involved require a rational approach for drug development, or at least the availability of a high-throughput screening technology. SOM230, a result of rational drug design based on somatostatin–receptor interactions, is a cyclic hexapeptide mimetic containing nonnatural Lys, Phe, Tyr, and Trp analogs. SOM230 shows improved receptor-binding profile and pharmacological properties compared to natural somatostatin ***(28)*** and is highly effective against pituitary adenomas in mice ***(29)***. Another example is related to a similar cyclic peptidomimetic that