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Challenges and New Therapeutics

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Peptide-based Drug Discovery
Challenges and New Therapeutics

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Foreword 1

As a class, macromolecules include numerous medicinal agents with high specificity, low off-target toxicity and, most importantly, unique efficacy. Peptide therapeutics represent a subsection of this broader class and include a number of indispensable life-saving medicines, with none more notable than insulin. The application of modern synthetic and analytical technology to peptides has generated a resurgence of interest in academic and commercial laboratories dedicated to the discovery and development of novel peptide-based medicines. What does it take to develop a peptide-based medicine? What are the key challenges and how are they being addressed? In which traditional therapeutic areas have peptides been most successful and in which new fields are they likely to be employed in the future? This collection of state-of-the-art reports addresses these and related questions in a holistic manner. It presents the evolution of a chemical entity to a registered medicine, presenting the seminal themes of design, synthesis, biological assessment and clinical development with associated biomarkers. This text summarizes a wealth of recent and current research projects that exemplify the attributes associated with successful research and development. Each chapter is authored by an internationally recognized opinion leader representing a cross-section of commercial and academic laboratories. The book will serve as a valuable reference volume for experienced peptide scientists, as well as an educational resource for younger scientists in training. I recommend it as a significant contribution that highlights current themes in the discovery and development of peptide-based medicine.

Richard DiMarchi
Indiana University

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Foreword 2

Since the discovery of therapeutically important hormones such as insulin and oxytocin, peptide-based drug discovery has gained significant importance. In addition to demonstrating their utility as therapeutic agents, peptides are finding increased use as molecular probes to understand biological pathways of human disease and as diagnostic tools.

As a therapeutic modality, peptides address an important gap between classical small-molecule drugs and antibodies with a rather high molecular weight. The latter agents are typically administered *via* intravenous or subcutaneous routes. The significant success of peptides in biomedicine has become possible as a result of the remarkable progress that has been made with respect to peptide design, manufacturing, improved stability, half-life prolongation and new delivery systems. Nowadays, peptides can be designed to address targets in the intracellular space, and research into the oral delivery of peptides is making significant progress.

Currently, more than 60 peptidic drugs are approved as marketed medicines and more than 350 peptide therapeutics are under clinical investigation targeting a wide variety of disease indications; oncology and metabolic disorders, but also neurological and inflammatory disorders. Remarkably, the current scope of peptide drugs is not limited to injectables, since alternative formulations and needle-free systems allowing for pulmonary, transdermal and oral delivery have either advanced to the market or are in late-stage clinical studies. Although the United States and Europe have so far been the key markets for therapeutic peptides, Asia Pacific and Latin America will offer significant opportunities in the coming years.

Today, peptide-based drug discovery is undertaken in a large number of laboratories across the world, including in large pharmaceutical companies and biotech and academic institutions. In fact, the enormous progress and opportunities in peptide therapeutics outlined above have led to the

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launching of several peptide-focused new companies in Europe, the US and Asia over the past decade—biotech or contract research or manufacturing organizations. This book attests to a promising future for the field of peptide science, which could further broaden in scope and offer new opportunities and therapeutic applications.

In *Peptide-based Drug Discovery*, well recognized experts in the field share their insight and views on many of these aspects. Central topics include the early identification of lead structures, design considerations and peptide optimization strategies with the overall goal to develop next-generation peptides as effective drugs for a variety of indications, as well as diagnostic tools and biomarkers.

I strongly applaud and recommend this book; it will serve as a valuable source of knowledge for experienced peptide and protein scientists in industry and academia, and also for the many young scientists aspiring to enter this field of research.

Michael Wagner
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Preface

Peptide therapeutics are now becoming an innovative strategy for developing new medicines. During the past four-decades, the discovery and development of peptide therapeutics has grown exponentially, with more than a thousand peptide molecules currently being studied for therapeutic indications in a variety of disease areas, including metabolic diseases, infectious disease, cancer, and neurological disorders. Most of the clinical and commercial successes of peptide therapeutics have been seen in metabolic diseases and for peptide drugs acting on extracellular targets such as G protein-coupled receptors. Recently approved peptide-based drugs such as the glucagon-like peptide-1 agonists (Byetta™, Victoza™, Trulicity™ and Tanzeum™) for diabetes are great examples of clinical and commercial successes.

The use of peptide therapeutics directed at intracellular targets such as transcription factors, kinases and intracellular receptors, which could have utility in cancer and inflammatory diseases, has been somewhat limited. This is due not only to challenges in investigating intracellular targets, target effectiveness and validation, but also challenges in discovering and developing cell-penetrating peptides and understanding protein–protein interactions. Macrocyclic peptides have the ability to disrupt intracellular protein–protein interactions—targets often considered to be "undruggable". The use of macrocyclic peptides opens up new opportunities to address a range of human diseases such as cancer and cardiovascular disease.

While much progress has been made in developing peptide therapeutics over the past several decades, we still need to better understand (1) the pharmaceutical properties required for drug-like peptides; (2) the correlation of nonclinical pharmacokinetics/pharmacodynamics that can translate to humans; (3) oral peptide delivery technologies; and (4) cost effectiveness of peptide drugs and their manufacture.

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This book provides a holistic story from molecules to medicine, combining the themes of design, synthesis, biomarkers, and clinical applications of peptide-based therapeutics. Within each of these areas, authors cover essential background, key challenges, and strategies for overcoming these challenges. In some instances, authors share their views on the future of peptide therapies.

Reading Chapters 1 to 18 in succession will provide a comprehensive overview on peptide therapeutics. The extensive references covered in each chapter offer additional detail on the subject matter.

The first introductory chapter describes *Renaissance in Peptide Drug Discovery: the Third Wave*, highlighting a renaissance of peptide drug discovery relative to drug design, chemical space, cell permeability, and drug delivery to tackle intracellular protein–protein interaction targets.

The next chapters discuss the *Identification and Validation of Peptide Therapeutic Targets and Indications*—their discovery from knowledge of normal and pathologic physiology, biologic assays including cell-based molecular systems, and high-content *in vivo* screens; *Peptide Biomarkers and Assay Development*—including pre-clinical applications; and *Peptide Library Technologies*—screening and deconvolution of peptide libraries, including mathematical theory and computational analyses.

These are followed by chapters covering *Peptide Lead Optimization*—strategies and tactics for designing peptide analogs, with specific examples of peptide drug candidates, including clinical studies. For example, *Macrocyclic Peptides for Intracellular Drug Targets* discussed case studies in cyclic peptide cell permeability through active transport and transporter-mediated permeability; *Structural Design for Bioactive Peptides* covers metal-complexation and terminus- and side-chain modifications and cyclization; and *ADME Properties of Peptide Therapeutics in Drug Discovery and Development* explores understanding and integrating concepts of improving subcutaneous absorption, peptide elimination, identifying areas susceptible to metabolism in the lead-optimization process, and predicting human pharmacokinetics from nonclinical data.

Subsequent chapters focus on future therapeutic areas, illustrating peptide medicinal chemistry tools and techniques. For example, *Designing an Effective Peptide Vaccine* against viral disease, allergy and autoimmune disease, cancer immunotherapy; *Peptide Therapeutics: Oncology*; *Development of Peptide-based Diagnostic and Therapeutic Agents in Oncology*; *Optimizing Peptides for Metabolic Diseases*; *Peptide Therapeutics: Neuropeptides*; *Developing Selective $Na_v1.7$ Peptide Inhibitors for Pain*; and *Stress-responsive Peptides in Insects* for wound healing and growth blocking.

Next are a set of future perspective chapters covering *Technologies for Oral Delivery of Peptides*—a comprehensive review of and strategies to increase paracellular or transcellular transport, and peptide molecules currently in pre-clinical or different stages of clinical development; *Phylomer Libraries* for peptide hits in phenotypic and target-directed screens; and the *Solid-phase Peptide Synthesis, the State of the Art: Challenges and Opportunities*, discussing green processes and integrated strategies.

The chapters are written by well-known key opinion leaders on the subject matter, from industry and academia all around the world. The goal of this book is to provide a valuable resource and reference, not only for the peptide researcher in the academic and pharmaceutical setting, but also for graduate students learning the discovery and development process as it relates to peptide-based medicines.

I would like to thank and express my gratitude to all the authors who have contributed to *Peptide-based Drug Discovery: Challenges and New Therapeutics* for their hard work in writing the chapters and sharing their expertise with a broad spectrum of readers. Thanks to the Royal Society of Chemistry project team leaders, especially Rowan Frame and Katie Morrey for their guidance and support. My special thanks to Professor David Rotella of Montclair State University for constantly encouraging me to put together this collection. I am grateful to Professor Richard DiMarchi, Linda & Jack Gill Chair in Biomolecular Sciences at Indiana University, and to Dr Michael Wagner, Head of Peptide Chemistry at Sanofi, Germany for writing a foreword and making recommendations for the book. I dedicate this book to my parents (Shravan and Kusum), and to my wife (Nisha) and children (Aaron and Nikita).

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Biography

Dr Ved Srivastava is Vice President of Peptide Chemistry at Intarcia Therapeutics. Prior to that he co-founded and was Vice President of Chemistry at Phoundry Pharmaceuticals, a peptide therapeutic discovery company that was acquired by Intarcia. Prior to Phoundry, he was the Head of Peptide Chemistry at GlaxoSmithKline. Ved spent several years in a leadership role with Amylin Pharmaceuticals, where he focused on the discovery and development of novel peptide hormones for diabetes, obesity and neuropsychiatric therapies. He has participated in the development and commercialization of Symlin™, Byetta™ and Bydureon™, first-in-class medicines for the treatment of diabetes.

Ved has more than 25 years of experience with expertise in drug discovery and development in the area of metabolic diseases, the central nervous system and inflammation, with major emphasis in peptide medicinal chemistry, chemistry manufacturing and control and peptide drug delivery. He has numerous scientific disclosures, including patents, scientific articles and invited lectures.

Ved is the editor of two other books, *Peptide 2015* (American Peptide Society) and *Comprehensive Medicinal Chemistry III, Volume 7. Biologics Medicine* (Elsevier). He is an editorial board member of *The FASEB Journal* (the Federation of American Societies for Experimental Biology); and an editorial advisory board member of the *Current Protein & Peptide Science* journal.

Ved serves in the governance and leadership team of the American Peptide Society and the American Chemical Society and other peptide societies. Ved is also an appointed member of the BIO1 Peptides and Insulins Expert Committee and the Therapeutic Peptides Expert Panel of the US Pharmacopeial Convention in partnership with the US Food and Drug Administration.

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He earned a PhD in organic chemistry from the University of Lucknow, India, and had subsequent postdoctoral appointments at the University of Georgia and the University of Colorado School of Medicine.

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CHAPTER 1

Renaissance in Peptide Drug Discovery: The Third Wave

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1.1 Peptide Science and Technology

Peptide drug discovery has evolved from highly focused efforts on specific receptors and proteases to a plethora of targets spanning receptors to enzymes and protein–protein interactions, and shattering a long-lived challenge to penetrate into cells to modulate intracellular targets in promising ways to expand "druggable" target space. Since the turn of the new millennium (2000) there is no doubt among peptide scientists that there is a genuine renaissance of peptide drug discovery. So, what has really inspired and propelled such a renaissance? Scholarly passion and intellectual perseverance have been absolutely essential, as this has been the proverbial long and winding road. And, of course, science and technology are empowering key advancements. In particular, synthetic chemistry (*e.g.*, novel amino acid building blocks and peptide secondary structure mimetics) and superdiverse phage-display, mRNA-display and DNA-encoded libraries are expanding peptide chemical space in amazing ways. Likewise, molecular genetics, structural biology and computational chemistry are continuing to play powerful roles to unveil extraordinary target space opportunities. Furthermore, our increased understanding of the complex pharmacology of disease

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mechanisms and peptide drug delivery, including cell permeability to prosecute intracellular targets, is re-defining the term "druggability". Beyond such convergent, multidisciplinary science and enabling technology, the steady growth of a pipeline of marketed and clinically investigated peptides, as well as a competitive resurgence of peptide research and development in both pharma and biotech are propelling this renaissance of the peptide therapeutic modality.

1.1.1 Past Milestones in Peptide Science and Technology

Historically, one of the earliest archetypal flags in the ground for peptide chemistry can be traced to that of Emil Fischer and the synthesis of the simplest dipeptide Gly-Gly at the very beginning of the twentieth century. In retrospect the sheer number of milestones that deserve recognition are well beyond those highlighted in this chapter (Figure 1.1), albeit they exemplify some quite significant achievements in peptide science and technology. Specifically, these include the discoveries of insulin, penicillin, oxytocin, pepstatin, thyrotropin-releasing hormone, gonadotropin-releasing hormone (GnRH), somatostatin, melanocyte-stimulating hormone (MSH), enkephalin and, more recently, tri-cystine knotted cyclotides. They include the development of potent peptide and peptidomimetic analogs thereof that have provided working models for bioactive conformations, agonist/antagonist pharmacophores and cellular receptor signaling mechanisms. Likewise, and

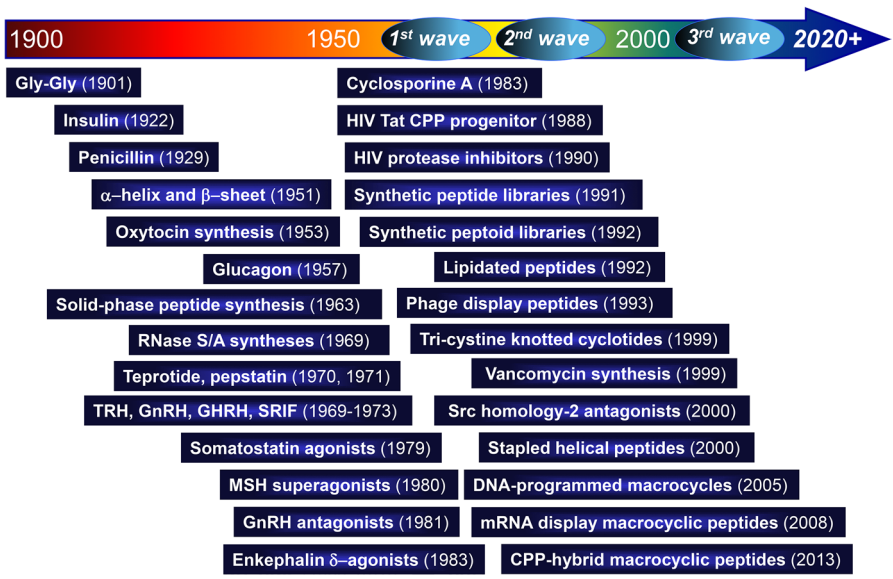

Figure 1.1 Some key milestones in peptide science and technology. Several "waves" of peptide and peptidomimetic drug discovery over the past three decades are highlighted.

with respect to intracellular targets, the natural product macrocyclic peptide cyclosporine A, HIV Tat (a progenitor of the first cell-penetrating peptides), synthetic peptidomimetic HIV protease inhibitors, designed non-peptide Src homology-2 antagonists and macrocyclic α-helical proteomimetic antagonists of MDM2/X collectively illustrate the scope of both past and current peptide drug discovery approaches to overcome the challenge of cell permeability. Lastly, some key past and emerging disruptive innovations that have leveraged the power of molecular biophysics and molecular diversity to enable peptide drug discovery include X-ray crystallography (*i.e.*, identification of canonical secondary structures such as the α-helix and β-sheet), solid-phase peptide synthesis, synthetic peptide/peptoid libraries, phage-display peptide (monocyclic/bicyclic) libraries and mRNA-displayed macrocyclic peptide libraries. In the case of macrocyclic peptides, there is no doubt that the impact of stapled helical peptides and non-helical macrocyclic peptides having varying size and inclusion of *N*-methyl amino acids, D-amino acids and other unique amino acid building blocks is both driving the generation of novel lead molecules and expanding druggable target space. Lastly, varying chemical modification of clinically investigated peptides that exemplify improvement in pharmacokinetic (half-life) properties have been achieved (see later), and include lipidation, pegylation and, more recently, macrocyclization (*e.g.*, amphipathic α-helical stapled peptides).

Since the 1980s, several hundred peptide and peptidomimetic candidates have advanced into clinical trials for a wide range of therapeutic indications,[1–3] including endocrine, metabolic, cardiovascular, cancer, immune and central nervous system diseases, and more than 50 such agents have been approved by the United States Food and Drug Administration (Table 1.1).

Table 1.1 Some peptide and peptidomimetic drugs approved by the United States Food and Drug Administration (FDA) or European Medicines Agency (EMA).

Trade name (INN)	Therapeutic target	Primary use	Approval
DDAVP (desmopressin)	Vasopressin receptor[a]	Diabetes insipidus	1978 (FDA)
Sandimmune (cyclosporine)	Cyclophilin/calcineurin[b]	Immunotherapy	1983 (FDA)
Lupron (leuprorelin)	GnRH receptor[a]	Oncology	1985 (FDA)
Zoladex (goserelin)	GnRH receptor[a]	Oncology	1989 (FDA)
Invirase (saquinavir)	HIV-1 protease[b]	Infectious disease	1996 (FDA)
Copaxone (glatiramer)	T-cell function[a]	Allergy, immunology	1996 (FDA)
Crixivan (indinavir)	HIV-1 protease[b]	Infectious disease	1996 (FDA)
Viracept (nelfinavir)	HIV-1 protease[b]	Infectious disease	1997 (FDA)
GlucaGen (recombinant glucagon)	Glucagon receptor[a]	Metabolic	1998 (FDA)
Integrilin (eptifibatide)	Integrin receptor[a]	Cardiovascular	1998 (FDA)
Sandostatin (octreotide)	Somatostatin receptor[a]	Acromegly	1998 (FDA)
Angiomax (bivalirudin)	Thrombin[a]	Hematology	2000 (FDA)
Agenerase (amprenavir)	HIV-1 protease[b]	Infectious disease	1999 (FDA)

(*continued*)

Table 1.1 (*continued*)

Trade name (INN)	Therapeutic target	Primary use	Approval
Cetrotide (cetrorelix)	GnRH receptor[a]	Endocrinology	2000 (FDA)
Trelstar (triptorelin)	GnRH receptor[a]	Oncology	2000 (FDA)
Natrecor (nesiritide)	Natriuretic peptide receptor[a]	Cardiovascular	2001 (FDA)
Byetta (exenatide)	Glucagon-like peptide-1 receptor[a]	Metabolic	2002 (FDA)
Forteo (teriparatide)	Parathyroid hormone receptor[a]	Metabolic	2002 (FDA)
Neulasta (pegfilgrastim)	G-CSF receptor[a]	Oncology	2002 (FDA)
Reyataz (atazanavir)	HIV-1 protease[b]	Infectious disease	2003 (FDA)
Cubicin (daptomycin)	Bacterial cell membrane[c]	Antibacterial	2003 (FDA)
Fuzeon (enfuvirtide)	gp41 of HIV fusion complex[a]	Infectious disease	2003 (FDA)
Plenaxis (abarelix)	GnRH receptor[a]	Oncology	2003 (FDA)
Velcade (bortezomib)	26S proteasome[b]	Oncology	2003 (FDA)
Prialt (ziconotide)	*N*-type calcium channel[a]	Central nervous system	2004 (FDA)
Symlin (pramlintide)	Amylin receptor[a]	Metabolic	2005 (FDA)
Vantas (histrelin)	GnRH receptor[a]	Oncology	2005 (FDA)
Prezista (darunavir)	HIV-1 protease[b]	Infectious disease	2006 (FDA)
Somatuline (lanreotide)	Somatostatin receptor[a]	Endocrinology	2007 (FDA)
Firmagon (degarelix)	GnRH receptor[a]	Oncology	2009 (FDA)
Victoza (liraglutide)	Glucagon-like peptide-1 receptor[a]	Metabolic	2010 (FDA)
Linzess (linaclotide)	Guanylate cyclase receptor[a]	Gastrointestinal	2012 (FDA)
Signifor (pasireotide)	Somatostatin receptor[a]	Cushing's disease	2012 (FDA)
Gattex (teduglutide)	Glucagon-like peptide-2 receptor[a]	Gastrointestinal	2012 (FDA)
Kyprolis (carfilzomib)	Proteasome[b]	Oncology	2012 (FDA)
Scenesse (afamelanotide)	Melanocortin-1 receptor[a]	Skin pigmentation	2014 (EMA)
Afrezza (inhaled insulin)	Insulin receptor[a]	Diabetes	2014 (FDA)
Saxenda (liraglutide)	Glucagon-like peptide-1 receptor[a]	Metabolic	2014 (FDA)
Trulicity (dulaglutide)	Glucagon-like peptide-1 receptor[a]	Metabolic	2014 (FDA)
Ninlaro (ixazomib)	Proteasome[b]	Oncology	2015 (FDA)
Pabal (carbetocin)	Oxytocin receptor[a]	Obstetrics	2015 (EMA)
Natpara (parathyroid hormone)	Parathyroid hormone receptor[a]	Hypocalcemia	2015 (FDA)
Toujeo (insulin glargine)	Insulin receptor[a]	Diabetes	2015 (FDA)
Tresiba (insulin degludec)	Insulin receptor[a]	Diabetes	2015 (FDA)
Adlyxin (lixisenatide)	Glucagon-like peptide-1 receptor[a]	Metabolic	2016 (FDA)
Zepatier (grazoprevir)	HCV protease[b]	Infectious disease	2016 (FDA)

[a]Extracellular/receptor therapeutic targets.
[b]intracellular therapeutic targets.
[c]antibiotic peptides that disruptive bacterial membranes.

Several of these drugs have achieved major commercial success, including Lupron, Zoladex, Sandostatin, Byetta and Forteo. Unquestionably, a strong understanding of the structure–activity relationships of such peptides at their specific targets and translation to *in vivo* preclinical disease models has been critical for their drug development. Likewise, overcoming challenges such as the metabolic instability (owing to rapid degradation by proteolytic enzymes) and generally poor pharmacokinetic properties of peptides has been strategic for their optimization relative to *in vivo* efficacy and route of administration.

1.1.2 Hierarchical Strategies to Transform Native Peptides into Drug Candidates

Peptide oral bioavailability remains elusive, although what was once thought to be the exceptional case of cyclosporine A is changing as a result of a deeper analysis of macrocyclic peptides to understand the relationship of the structural and conformational impact of backbone modifications, ring size and side-chain lipophilicity to passive transport (see later). Consequently, a majority of marketed peptide therapeutics leverage subcutaneous and injectable routes of administration. Such modified peptides and peptidomimetics exemplify classic hierarchical strategies[4–20] to achieve an effective combination of high affinity to target and proteolytic stability, including (i) backbone amide *N*-alkylation; (ii) backbone amide replacement with non-hydrolyzable surrogates; (iii) amino acid Cα-stereoinversion and/or Cα-alkylation; (iv) β-amino acids; (v) cyclic α-/β-amino acids; (vi) dipeptide replacements that mimic canonical secondary structural motifs such as α-helix, β-strand/β-sheet or β-/γ-turns; and (vii) macrocyclization designed to stabilize α-helical, β-strand/β-sheet, β-/γ-turns and/or other conformationally restricted peptide/peptidomimetic chemotypes (*e.g.*, monocyclic or multicyclic). A few examples of the pioneering and contemporary chemistry that has contributed to modified peptides and peptidomimetics are described below.

For receptor-targeted peptide therapeutics, long-lasting exposure levels *in vivo* have been achieved using varying approaches,[21–27] including both chemical conjugation (*e.g.*, fatty acids, polyethylene glycol, antibodies and related recombinant proteins and serum albumin) and sustained-release formulations applicable to parenteral routes of administration (*e.g.*, subcutaneous). Specific examples include the palmitoyl-modified glucagon-like peptide-1 (GLP-1) agonist liraglutide,[28] a pegylated GLP-1 agonist/glucagon antagonist hybrid peptide,[29] the pegylated granulocyte colony-stimulating factor drug pegfilgrastim[30] and a human Fc domain–thrombopoietin peptide agonist conjugate romiplostim.[31] Although absolute exposure levels, in terms of both time and concentration, are case-specific, the relatively short half-lives (typically a few minutes) of most endogenous (native) peptides provide an opportunity to advance viable drugs with substantially improved metabolic and pharmacokinetic properties.

1.2 Peptide Target Space and Druggability

1.2.1 G Protein-Coupled Receptors: Class A and Class B

Indubitably, the greatest impact of peptide drug discovery so far has been that focused on receptor target space, especially the G protein-coupled receptor (GPCR) group (*e.g.*, class A and B GPCRs), which has been determined using human genome sequencing to be one of the largest protein families[32–34] (Figure 1.2), and this has translated to the first wave of peptide therapeutics.[9,11,14,19,35] Pioneering studies on class A GPCR peptides may be traced to oxytocin, vasopressin, α-MSH, GnRH, somatotropin-release inhibiting factor (somatostatin) and the opioid peptides (*e.g.*, enkephalin, β-endorphin, dynorphin) during the 1970s–1990s. Likewise, but more recently, a second wave of peptide drug discovery has successfully extended to class B GPCRs as exemplified by glucagon-like peptide-1 (GLP)-1, islet amyloid polypeptide (amylin), GLP-2 and parathyroid hormone.

Importantly, these early class A and B GPCR peptide agonist/antagonist structure–activity studies provided insight to understand the intrinsic peptide conformational properties as well as predictive 3D-pharmacophore models.[14,19,35] Unfortunately, such pioneering studies were not empowered by high-resolution X-ray crystallographic structures of class A and B GPCRs until more recently.[36,37] Instead, a systematic analysis of peptide structure–activity relationships (*e.g.*, analog modifications by D-amino acids, *N*-alkyl-amino acids, Cα-alkyl-amino acids and/or macrocyclization) as well as biophysical characterization (*e.g.*, nuclear magnetic resonance (NMR) spectroscopy and circular dichroism) revealed that β-turn and α-helix secondary structures often correlated with GPCR molecular recognition for

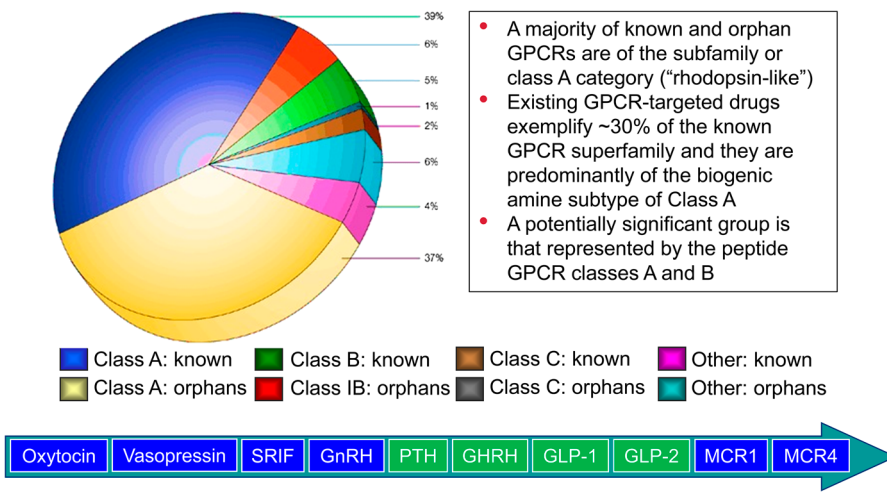

Figure 1.2 Human genome mapping of the G protein-coupled receptor (GPCR) superfamily.

such peptides (Figure 1.3). As exemplified by Scenesse, Lupron and Sandostatin, incorporation of a D-amino acid regiospecificially within their peptide sequences has been conceptualized to stabilize β-turn conformations of their respective class A GPCR agonist pharmacophores.[38–40] In contrast, Byetta, Forteo and Gattex share relatively high propensities for α-helix conformations that have been correlated with their molecular recognition of and binding to and activation of their respective class B GPCRs.[41–43]

1.2.1.1 Melanocortin Receptor Agonists/Antagonists

As a personal reflection on the first wave of peptide drug discovery, my first scientific foray at the University of Arizona contributed to the discovery of class A GPCR superagonist peptides for the melanocortin receptor MC1R[38,44–46], namely Scenesse™ (Figure 1.3) and cyclo[Cys^4, Cys^{10}]α-MSH (Figure 1.4). Indisputably, these two molecules have inspired the design of numerous linear and macrocyclic α-MSH peptide analogs,[47,48] including the recent clinical development macrocyclic α-MSH peptide analog setmelanotide (Figure 1.4) from Rhythm Pharmaceuticals.[49] It is noteworthy that the D-Phe^7 modification and macrocyclization about the central pharmacophore tetrapeptide within these peptide agonists has been consistent with stabilizing a predicted β-turn (Figure 1.4). Furthermore, the second-generation macrocyclic α-MSH superagonist Ac-cyclo[Nle^4, Asp^5, D-Phe^7, Lys^{10}]α-$MSH_{4\text{-}10}$-NH_2 [46] and the structurally related α MSH antagonist analog Ac-cyclo[Nle^4, Asp^5, D-Nal(2′)^7, Lys^{10}]α-$MSH_{4\text{-}10}$-NH_2 [50] (Figure 1.4) from the University of Arizona

Figure 1.3 Amino acid sequences of several known G protein-coupled receptor (GPCR)-targeted marketed peptide drugs that further show β-turn and α-helix substructural motifs proposed for their bioactive conformations.

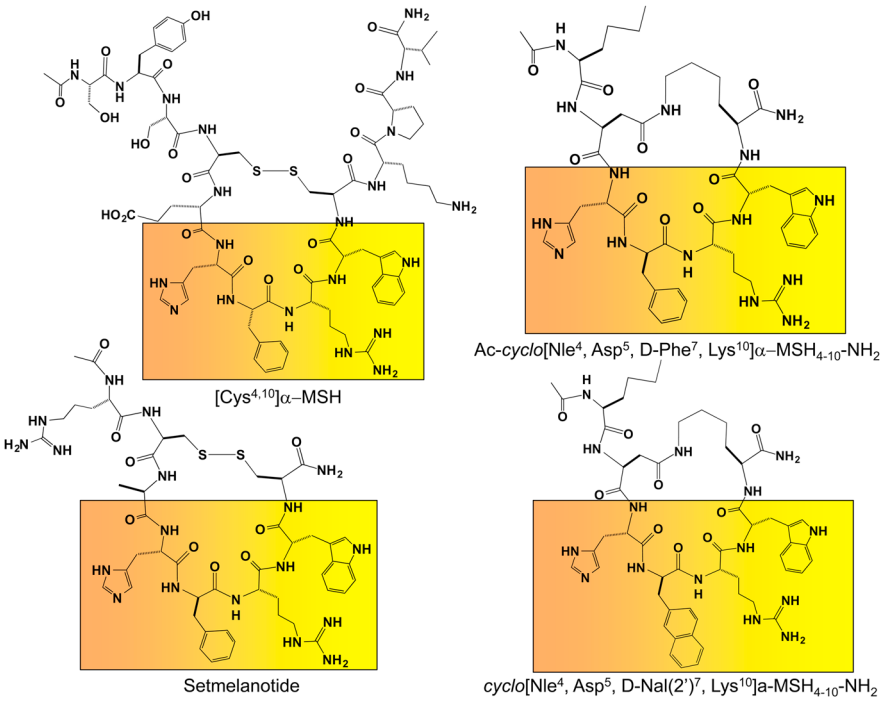

Figure 1.4 Structures of [Cys^{4,10}]α-MSH, Ac-cyclo[Nle^4, Asp^5, D-Phe^7, Lys^{10}]α-MSH$_{4\text{-}10}$-NH$_2$, setmelanotide and cyclo[Nle^4, Asp^5, D-Nal(2′)^7, Lys^{10}] α-MSH$_{4\text{-}10}$-NH$_2$ highlighting the β-turn conformations about the central His-Phe-Arg-Trp sequence of MSH.

exemplify significant benchmarks to enable the design of MSH ligands for the MC1R, MC3R, MC4R and MC5R. Most recently, MCR relationships to several key diseases (*e.g.*, energy homeostasis, inflammation and neurodegeneration) have been described[51–53] and implicate new opportunities to leverage MCR-specific peptide and non-peptide agonists/antagonists.[47,48,54]

1.2.1.2 GLP-1 Receptor Agonists/Antagonists

The development of peptide agonists for the GLP-1 receptor has been advanced in what may be the most competitive worldwide efforts focused on class B GPCRs. Such efforts have been focused on type 2 diabetes mellitus and obesity, including glucose homeostasis and regulation of gastric motility and food intake.[55–57] Currently, several GLP-1 receptor-targeted peptide agonists have reached the market and representative examples include exenatide, liraglutide, lixisenatide and semaglutide (Figure 1.5). Such GLP-1 peptide agonists illustrate regiospecific amino acid modifications to confer metabolic stability as well as the incorporation of fatty acid or other types of conjugation to enhance their *in vivo* pharmacokinetic and pharmacological

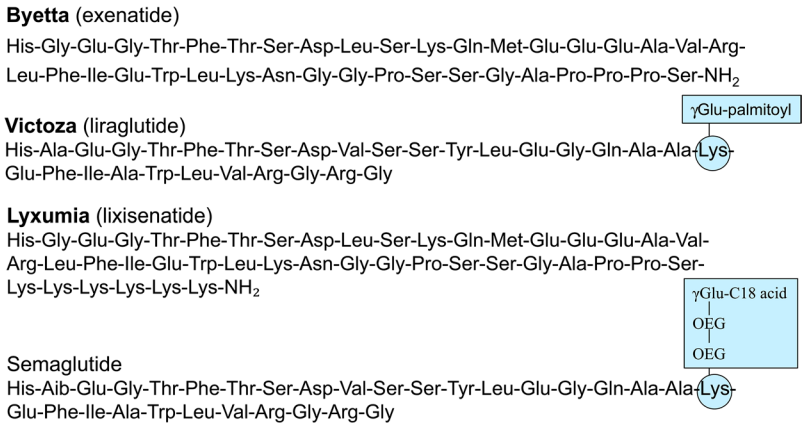

Figure 1.5 Structures of some marketed or clinically advanced (semaglutide) glucagon-like peptide (GLP)-1 agonists.

properties.[58–60] Of historical significance to class B GPCRs, the first X-ray crystallographic structures of GLP-1 receptor extracellular domain complexes with GLP-1 peptide analogs have provided the 3D molecular maps and insight to their receptor binding mechanism.[61] Furthermore, the deeper biological study of GLP-1 peptides (including truncated, modified and chimeric analogs) as well as small-molecule modulators of the GLP-1 receptor activation have provided further understanding of molecular recognition and biased cellular signaling.[62] Importantly, biased cellular signaling is being found in an increasing number of GPCRs by both peptide and non-peptide agonists.[63]

Beyond GPCRs, there has been steady progress in the development of peptide modulators of growth factor and cytokine receptors, integrins and ion channels to expand the scope of receptor target space for peptide drug discovery. Noteworthy for such receptors are the more structurally complex peptide agonists and antagonists, including those having multiple disulfide bridges such as insulin, linaclotide, ziconotide and ProTx-II, and which exemplify high specificity for the insulin receptor, guanylate cyclase-C, *N*-type Ca^{2+} channel and voltage-gated Na^{1+} channel targets, respectively.[64–67]

1.2.2 Intracellular Protein–Protein Interaction Targets

A significant opportunity for the peptide drug modality is emerging relative to a third wave that is focused on modulating intracellular targets relative to stapled α-helical peptides,[68–73] structurally/conformationally diverse macrocyclic peptides inspired by cyclosporine A[74–81] and both linear and macrocyclic peptides incorporating cell-penetrating peptide motifs.[82–85] Both β-strand and α-helix secondary structures are widely found in at the

interfaces of protein–protein interactions, and have been described[86–91] with respect to comprehensive analysis of the Protein Data Bank (www.rcsb.org). Examples of β-strand protein–protein interactions include PDZ domains, PTB/PI domains, Mad2:Cdc20, NS3 protease:NS4A and PKA:Rbα.[89,90] Representative α-helical protein–protein interactions of >1600 non-redundant, unique high-resolution 3D structures derived from the Protein Data Bank[87–91] include BIM BH3:Mcl-1,[92] BAD:Bcl-Xl,[93] p53:MDM2,[94] MAML:Notch,[95] HIF-1α:p300,[96] Myc-Max,[97] eIF4G:eIF4E,[98] TIF2:GCCR,[99] Scr-1:RORγ[100] and IKKβ:NEMO[101] (Figure 1.6). As detailed later, α-helical secondary structures have inspired significant peptide drug discovery efforts to modulate such protein–protein interactions, with a specific focus on intracellular targets of therapeutic interest.

1.2.3 Exploring Peptide–Target Molecular Recognition

Peptide drug discovery leverages the typical high affinity and/or selectivity properties that endogenous peptides have for their cognate targets (*e.g.*, ≤10^{-9} M range for most GPCR-targeted peptides). We understand that the high-fidelity molecular recognition between peptides and their targets is achieved through intermolecular interactions by means of a dynamic orchestration of specific hydrophobic, electrostatic and hydrogen-bonding forces.

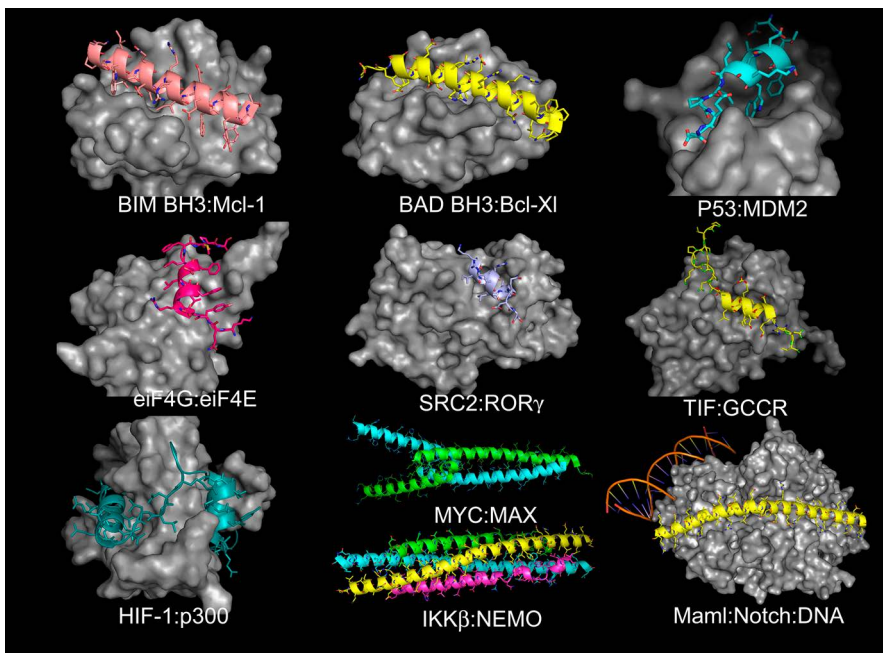

Figure 1.6 Some representative protein–protein interactions highlighting α-helical interfaces.