Trends in peptide drug discovery

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Abstract | Since the introduction of insulin almost a century ago, more than 80 peptide drugs have reached the market for a wide range of diseases, including diabetes, cancer, osteoporosis, multiple sclerosis, HIV infection and chronic pain. In this Perspective, we summarize key trends in peptide drug discovery and development, covering the early efforts focused on human hormones, elegant medicinal chemistry and rational design strategies, peptide drugs derived from nature, and major breakthroughs in molecular biology and peptide chemistry that continue to advance the field. We emphasize lessons from earlier approaches that are still relevant today as well as emerging strategies such as integrated venomics and peptide-display libraries that create new avenues for peptide drug discovery. We also discuss the pharmaceutical landscape in which peptide drugs could be particularly valuable and analyse the challenges that need to be addressed for them to reach their full potential.

The field of peptide therapeutics started in 1922 with the first medical use of insulin — extracted from animal pancreases — which revolutionized the treatment of type 1 diabetes (FIG. 1). Four decades passed before synthetically produced peptide hormones, namely oxytocin and vasopressin, entered the clinic. Industrial groups such as those of Robert Schwyzer at Ciba and Charles Huguenin at Sandoz entered the field and increased commercial interest in peptides as therapeutics. However, at that time, synthesis of peptides by solution-phase chemistry required months to years of work, and it took the invention of solid-phase peptide synthesis (SPPS) in 1963 (REF.[1]), in combination with the development of purification methods such as high-performance liquid chromatography, to attract significant attention from the pharmaceutical industry.

Soon, the importance of peptides as key biological mediators, along with their remarkable potency, selectivity and low toxicity, was established. At the same time, however, their limitations, including low oral bioavailability, low plasma stability and short circulation time, were recognized. These developments occurred during the golden age (1970 to 1980s) of small-molecule pharmaceuticals, when approval of ~20 orally available new drugs per year was the norm. Moreover, large-scale peptide manufacturing was considered prohibitively expensive, such that only peptide hormone agonists that were effective at low doses were commercially viable. This resulted in limited interest in peptides from the pharmaceutical industry and stagnation of peptide drug development.

Nevertheless, the use of peptides as subtype-selective probes for receptor studies and their continued presence as lead compounds provided the basis for a second wave of peptide drug development beginning in the late 1980s, this time backed by venture funds and biotechnology companies. The commercial success of human insulin produced using recombinant technology (approved in 1982) as well as the synthetic gonadotropin-releasing hormones leuprolide and goserelin (approved in 1985 and 1989, respectively) confirmed the viability of the peptide drug market, and advances in drug delivery technology, formulation and synthesis encouraged increased investment and research. Consequently, the number of peptides entering clinical trials from 2000 to 2010 was nearly twice that in the 1990s[2].

At present, there are around 80 peptide drugs on the global market, and research into new peptide therapeutics continues at a steady pace, with more than 150 peptides in clinical development and another 400–600 peptides undergoing preclinical studies[3,4]. Considering the increase in investment and research efforts in the peptide field, the maturity of peptide synthesis technology, the success of biologics and the pressure for the pharmaceutical industry to maintain new drug approval rates, we anticipate that the peptide therapeutics market (FIG. 2; TABLE 1) will continue its momentum and expand over the coming years.

In this Perspective, we first outline how peptide drug discovery and development strategies have advanced, analysing the chemical, structural and mechanistic features of successful peptide drugs, as well as the key approaches for transforming peptide leads into drugs. The sections on trends below are presented approximately historically (although there are overlaps), from the era of peptide hormones through to the latest peptide-display technologies and chemical modifications, with each section progressing from the origins through to the current status and opportunities for further advances. All approved peptide drugs and diagnostic agents are listed in Supplementary Tables 1–5, which include further references. We also highlight selected examples of serendipity in BOX 1. We focus mainly on peptide therapeutics in the range of 4–100 amino acid residues and exclude shorter peptidomimetics such as angiotensin-converting enzyme (ACE) inhibitors (see REFS[5–9] for reviews), which are often similar to typical small-molecule drugs, as well as bacterium-derived macrocyclic peptide antibiotics such as vancomycin and daptomycin, for which there are other discovery trends and development challenges (see REFS[10,11] for reviews). We conclude this Perspective by discussing overarching issues and opportunities for future peptide drugs.

The era of peptide hormones

Peptide research at the beginning of the twentieth century focused largely on the role of human signalling hormones. Insulin is the classic example of the use of an endogenous hormone as a therapeutic; it was the first peptide drug to be used

clinically, and it remains by far the most commercially successful, with the market for insulin therapies showing a 10% compound annual growth rate owing to the rise in the prevalence of obesity[12]. From a chemical point of view, however, it was short peptides such as oxytocin, vasopressin, somatostatin and gonadotropin-releasing hormone (GnRH) that initiated the field of peptide drug development, and many analogues of these hormones are still in use today (Supplementary Table 1).

It became clear early on that the short metabolic half-lives (minutes) of endogenous human hormones are problematic for therapeutic development, and various medicinal chemistry approaches were used to increase their stability as well as improve other properties, such as potency, selectivity, pharmacokinetics and pharmacodynamics[13–15]. Some of the earliest attempts were established with oxytocin and vasopressin[16], for which chemical modifications such as D-amino acids[17,18], unnatural amino acids[19], amino-terminal (N-terminal) capping, deamination[20–22], extensions of N termini or carboxy termini (C termini)[23–25] and disulfide bond mimetics[26–29] were explored to increase the stability, potency and selectivity of these endogenous ligands, eventually resulting in successful drugs such as desmopressin, terlipressin, carbetocin and atosiban[30,31] (Supplementary Table 6).

More systematic truncations, as well as scans to assess the effects of replacing particular residues with alanine, N-methylated versions or D-amino acids, were performed with the peptide hormone somatostatin, which comprises 14 residues and a single disulfide bond[32–35]. Somatostatin, which inhibits the secretion of growth hormone, insulin, glucagon, gastrin, secretin and thyroid-stimulating hormone, was of little therapeutic value itself due to its short in vivo half-life of less than 3 min. These studies identified its β-turn pharmacophore, which became the lead sequence in the quest for more potent analogues with increased metabolic stability[35,36] (FIG. 3a). This led to the

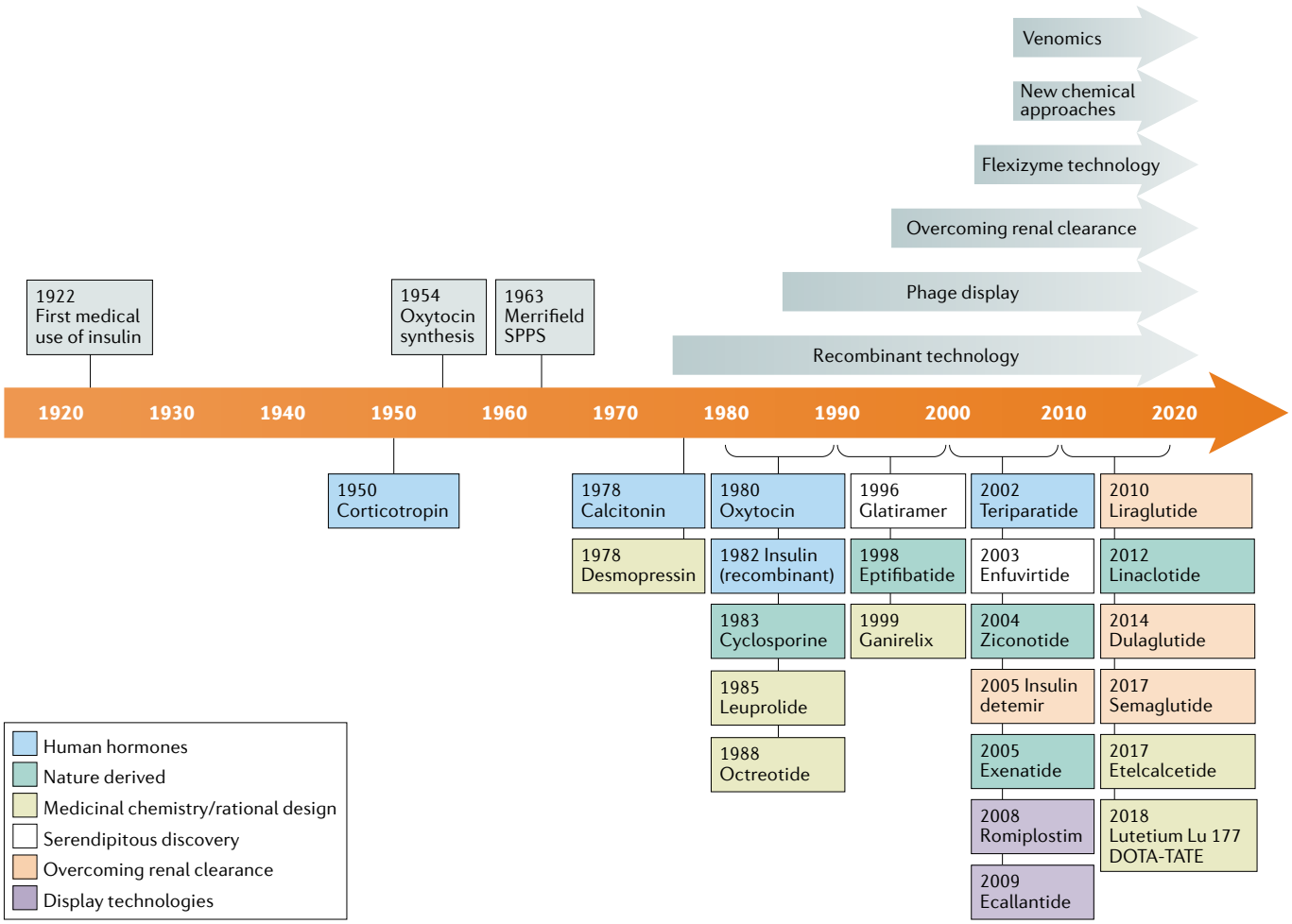


Fig. 1 | **Historical timeline of key milestones, developments and drug approvals in the peptide therapeutics field.** The first peptide drug was insulin, extracted from bovine and porcine pancreas. Polypeptides were first chemically synthesized in 1954, when Vincent du Vigneaud's group published the total synthesis of oxytocin and vasopressin (recognized with the Nobel Prize in Chemistry in 1955)[226,227]. Another leap forward was Bruce Merrifield's visionary idea to automate peptide synthesis by assembling amino acids on a solid phase, leading to the invention in 1963 of solid-phase peptide synthesis (SPPS)[1] (recognized with the Nobel Prize in Chemistry in 1984). The advent of recombinant technology in the 1980s enabled clean production of larger peptides. Subsequently, strategies to increase the molecular weight of peptides through conjugation to lipids, larger proteins and polyethylene glycol helped overcome the problem of renal clearance and increased plasma circulation times. Display technologies such as phage display now allow target-oriented discovery of peptides with more drug-like properties from vast libraries. Flexizyme technology allows the incorporation of non-proteinogenic amino acids into display libraries. Natural peptide discovery, particularly peptides from venoms, and new chemical approaches are also advancing the field. Selected peptide drugs based on advances in the areas discussed in this Perspective and their initial regulatory approval dates are highlighted in the lower part of the figure.

approval of octreotide for the treatment of acromegaly, adenomas and pancreatic, breast and prostate tumours. N- to C-terminal cyclization was used in the development of pasireotide (FIG. 3a), contributing to its long half-life of ~12 h, a vast increase compared with somatostatin.

The observation that many tumours overexpress at least one of the five somatostatin receptor subtypes[37] not only drove development of subtype-selective analogues such as lanreotide, vapreotide and pasireotide (FIG. 3a) but also introduced peptide scintigraphy and the field of peptide receptor radionuclide therapy, where radioactive elements such as ^{111}In, ^{90}Y, ^{68}Ga or ^{99m}Tc were attached via chelating groups to selective somatostatin analogues[38]. This has resulted in approved radiopharmaceutical agents such as indium In 111 pentetreotide, technetium Tc 99m depreotide, gallium Ga 68 DOTA-TATE, gallium Ga 68 DOTA-TOC and copper Cu 64 DOTA-TATE to detect tumours. Furthermore, the recent approval of lutetium Lu 177 DOTA-TATE for the treatment of somatostatin receptor-positive gastroenteropancreatic neuroendocrine tumours has revitalized interest in the application of peptides in radionuclide therapies[39–41] (Supplementary Table 2).

The strategy of developing superagonists to desensitize and downregulate receptors to produce a therapeutic response similar to treatment with an antagonist was first successfully translated in the drug development programmes based on GnRH. Blockade of GnRH hormonal function is applied clinically in cancer treatment, delay of puberty, management of oestrogen-dependent female disorders, sex reassignment and in vitro fertilization therapy[42]. Some of these early superagonists (Supplementary Table 7) are still very successful; for example, leuprolide acetate and goserelin acetate had annual sales of US$2 billion and $813 million, respectively, in 2019 (based on company financial reports). The development of GnRH antagonists that directly arrest GnRH action followed soon after, and advances in SPPS allowed more extensive use of unnatural amino acids in antagonist design, ultimately producing successful drugs such as cetrorelix and ganirelix[43] (Supplementary Table 7).

Different peptide delivery systems were subsequently developed, including slow-release subcutaneous or intramuscular injections, as well as intranasal delivery. The most common delivery form used was that of hydrophobic depots, which increased the duration of action[44]. This eventually led to the approval of biodegradable

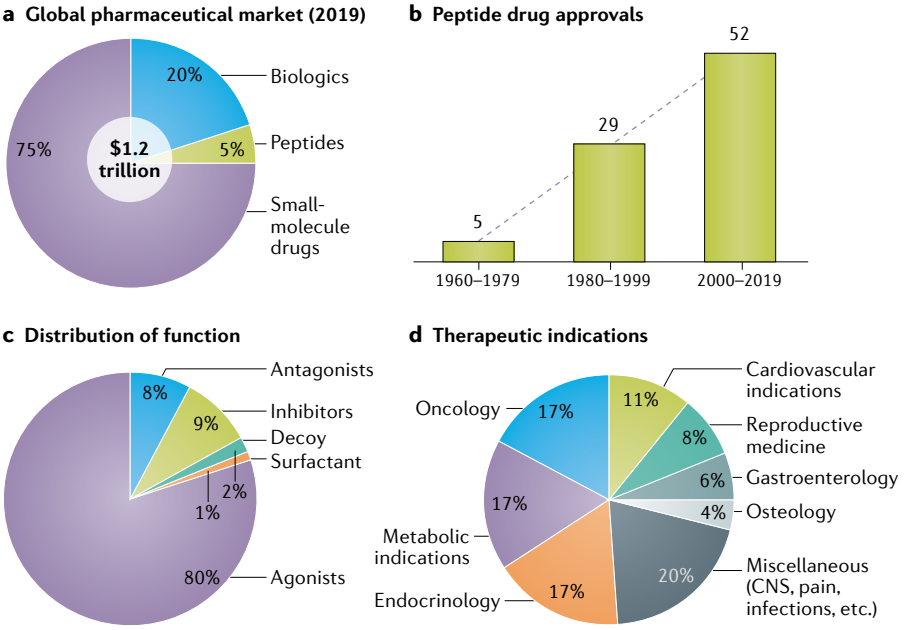


Fig. 2 | **The peptide drug market.** Peptide drugs occupy a distinct pharmaceutical space in between small-molecule drugs and biologics. **a** | They account for 5% of the global pharmaceutical market, with global sales exceeding US$50 billion in 2019. **b** | Approvals have steadily increased over the last six decades, with an average growth rate of 7.7% for the global peptide therapeutics market[228]. Insulin and analogues are responsible for ~50% of peptide drug revenue ($25 billion), followed by the glucagon-like peptide 1 (GLP1) receptor agonist dulaglutide, marketed as Trulicity for the treatment of diabetes ($4.4 billion), the GLP1 receptor agonist liraglutide, marketed as Victoza and Saxenda for the treatment of diabetes and obesity, respectively ($4.1 billion), and the synthetic gonadotropin-releasing hormone analogue leuprolide, marketed as Lupron and Eligard for the treatment of cancer ($2 billion) (TABLE 1). **c**,**d** | Of the currently approved peptide therapeutics, most are agonists (part **c**), and the most commonly targeted indications are related to endocrinology, metabolism and oncology (part **d**). CNS, central nervous system. Sources: 2019 company financial reports and global sales analysis reports.

poly(D,L-lactic-*co*-glycolic acid)/poly(lactic acid) microparticle depot formulations able to deliver stable drug concentrations with once-weekly or less frequent dosing (for example, once in 6 months for Lupron Depot for the treatment of prostate cancer)[45]. There are currently eight peptide drugs on the market that use a poly(D,L-lactic-*co*-glycolic acid) or poly(lactic acid) extended release delivery system (Supplementary Table 5)[46].

Further details on individual drug candidate development of the aforementioned drugs and additional supporting examples, such as pramlintide, an analogue of amylin (Supplementary Fig. 1), icatibant, a bradykinin receptor antagonist (Supplementary Table 8), and afamelanotide, bremelanotide and setmelanotide, analogues of α-melanocyte-stimulating hormone, can be found in Supplementary information.

The advent of recombinant technology

Despite the success of early hormone analogues, production of longer peptides was limited by the synthetic methods available, and insulin derived from pigs or cows was not ideal as it often induced allergic reactions. Therefore, selective expression of endogenous human peptides and proteins in cell culture systems was highly desirable, and the advent of recombinant technology was a milestone in peptide drug development (FIG. 1). Somatostatin was the first human peptide to be produced recombinantly[47], and in 1982, insulin, a complex heterodimer comprising a 21-residue A chain, a 30-residue B chain and three disulfide bonds (FIG. 3b), was the first licensed drug to be produced by this technology (developed by Genentech and Eli Lilly)[48]. Advances in genetic engineering soon permitted single amino acid alterations to modulate the absorption, distribution, metabolism and excretion characteristics of peptides[49,50]. This was particularly applicable to insulin, as it naturally forms inactive hexamers during long-term storage in the body, which is therapeutically undesirable. Genetic engineering allowed development of fast-acting and slow-release insulin analogues for different treatment options[51] (FIG. 3b), which now constitute a multibillion-dollar market (FIG. 2).

Table 1 | **Top peptide drugs by sales**

Generic name	Most common brand names	Companies	Indication	First approval	Sales in 2019 (US$ million)
Insulin and analogues	Ademelog, Apidra, Humulin, Humalog, Insuman, NovoMix, NovoRapid, Lantus, Levemir, Ryzodeg, Tresiba, Toujeo	Novo Nordisk, Eli Lilly, Sanofi	Diabetes	1982	25,000
Dulaglutide	Trulicity	Eli Lilly, Dainippon Sumitomo	Diabetes	2014	4,394
Liraglutide	Victoza, Saxenda	Novo Nordisk	Diabetes, obesity	2010	4,142
Leuprolide	Lupron, Eligard	AbbVie, Astellas, Takeda	Cancer	1985	2,022
Semaglutide	Ozempic, Rybelsus	Novo Nordisk	Diabetes, obesity	2017, 2019	1,694
Octreotide	Sandostatin	Novartis	Cancer	1988	1,585
Glatiramer	Copaxone, Glatopa	Teva, Sandoz	MS	1996	1,531
Teriparatide	Forteo	Eli Lilly	Osteoporosis	2002	1,405
Cyclosporine	Restasis	Allergan	Immune diseases, organ transplants	1983	1,189
Lanreotide	Somatuline	Ipsen	Acromegaly	2007	1,124
Carfilzomib	Kyprolis	Amgen	Multiple myeloma	2012	1,044
ACTH	Acthar	Mallinckrodt	IS, MS	1950	953
Linaclotide	Linzess, Constella	Allergan, Astellas Pharma	IBS-C	2012	877
Romiplostim	Nplate, Romiplate	Amgen, Kyowa Kirin	Chronic ITP	2008	841
Goserelin	Zoladex	AstraZeneca	Cancer	1989	813
Etelcalcetide	Parsabiv	Amgen, Ono Pharma	Hyperparathyroidism	2017	693
Exenatide	Byetta, Bydureon	AstraZeneca	Diabetes	2005	659
Teduglutide	Gattex, Revestive	Takeda	Short bowel syndrome	2012	555
Vasopressin	Vasostrict	Endo Pharma	CDI	NR	532

Sources: 2019 company financial reports, https://www.fda.gov, and global sales analysis reports. ACTH, adrenocorticotropic hormone; CDI, central diabetes insipidus; IBS-C, irritable bowel syndrome with constipation; IS, infantile spasms; ITP, immune thrombocytopenia; MS, multiple sclerosis; NR, not recorded.

Two further key examples of peptide hormones for which recombinant approaches have been harnessed are calcitonin and parathyroid hormone (PTH). Calcitonin, a 32-residue peptide hormone, was discovered in 1961 as a substance that lowers blood calcium levels in dogs[52–54]. It was subsequently shown to potently inhibit bone resorption, and salmon calcitonin, which differs from human calcitonin by 16 residues and is 40–50-fold more potent, was approved for the treatment of hypercalcaemia and postmenopausal osteoporosis in 1978. Salmon calcitonin was initially extracted from the thyroid-like glands of salmon, but a synthetic version was approved in 1978 and a recombinant version was approved in 2005. PTH, an 84-residue hormone discovered in 1925 (REF.[55]), opposes the effects of calcitonin. It acts on PTH 1 and PTH 2 receptors and increases blood calcium levels largely by enhancing the release of calcium from the reservoir in bones. Prolonged elevation of PTH levels will deplete bone stores, but intermittent exposure activates osteoblasts (the cells responsible for creating bone) rather than osteoclasts. The first therapeutic based on PTH to reach the market was teriparatide, a recombinant form of the first 34 residues of human PTH (the bioactive portion of the hormone) that was approved for the treatment of osteoporosis in 2002. Teriparatide was the first agent that promotes bone formation (rather than inhibiting bone resorption) to be approved for the treatment of osteoporosis. More recently, a recombinant full-length PTH therapy to control low blood calcium concentration in patients with hypoparathyroidism[56] was approved in 2015, and abaloparatide, a synthetic analogue of PTH-related protein (PTHrP) with 41% identity to teriparatide[57], was approved for the treatment of osteoporosis in 2017.

Recombinant technology also provided a reliable and facile alternative to synthetic peptide production, which further accelerated peptide research and led to drug approvals for recombinantly produced glucagon, carperitide, lepirudin, nesiritide, mecasermin, desirudin and romiplostim, with the majority being used for the treatment of cardiovascular conditions and haematological disorders (Supplementary Table 1). Details of another example of a recombinant peptide drug, teduglutide, a glucagon-like peptide 2 (GLP2) analogue, are provided in Supplementary information.

In recent years, there has been an increased focus on technologies for expanding the genetic code to produce peptides and proteins containing non-canonical amino acids[58–60]. Furthermore, the combination of genetic code expansion with display technologies (discussed further later) enables incorporation of N^{α}-methylated amino acids[61,62], D-amino acids[63–65], β-amino acids[66,67] and thioamides[68] into peptides and the creation of peptoids[69] and various macrocycles that can be screened in large display libraries against therapeutic targets[70–72]. Such technologies are likely to be increasingly used for the discovery and production of larger and more complex peptide drugs.

Peptide drugs based on natural products

Over the years, increasing numbers of peptides from bacteria, fungi, plants and animals have been characterized that often have better therapeutic properties than their human counterparts. These include higher selectivity, potency and in vivo stability, which are especially important for therapeutic development. Since many receptors in the animal kingdom are very similar to their human counterparts, these

vast natural sources provide novel bioactive peptides that can be mined for therapeutic development. Supplementary Table 3 lists approved peptide therapeutics derived from natural products. Two early key examples are the immunosuppressive drug cyclosporine isolated from fungi and the thrombin inhibitor bivalirudin isolated from saliva of the medicinal leech.

Cyclosporines were discovered in 1970 at Sandoz during a project that attempted to identify new antifungal agents[73,74]. Although the crude extracts of two new strains of *fungi imperfecti* had only low antifungal activity, their toxicity was unusually low, which prompted further screening. Observation of immunosuppressive effects ultimately led to the isolation and structural characterization of cyclosporine, a neutral, hydrophobic, cyclic 11-residue peptide that has some peculiar features. Its high degree of N-methylation and its cyclic structure make it resistant to proteolytic degradation, and its hydrophobicity and conformational flexibility due to its intramolecular hydrogen bonding network render it orally bioavailable[75] (FIG. 4a). Cyclosporine was approved as an immunosuppressive drug in 1983, and it remains an inspiring example of the value of natural product discovery as well as a reminder that peptides and peptide mimetics can be orally bioavailable.

Medicinal leeches were a focus of research in the late eighteenth century due to the anticoagulant properties of their saliva, but it took nearly a century until hirudin, the peptide responsible for this action, was isolated and its structure was determined[76]. Hirudin, a 65-residue peptide with a compact N-terminal domain stabilized by three disulfide bonds and a flexible C-terminal domain, is a potent thrombin inhibitor. A recombinant form, lepirudin, with two modifications (replacement of Leu with Ile at position 1 and removal of Tyr sulfonation at position 63) was approved in 1998 as an anticoagulant. It was followed in 2002 by the first 'hirulogue', bivalirudin, a 20-residue fragment of hirudin that contains the active site of thrombin inhibitor D-Phe-Pro-Arg linked via a Pro-$(Gly)_4$ linker to a dodecapeptide analogue of the C terminus of hirudin[77]. Thrombin can slowly cleave the N-terminal inhibitor off at the Arg-Pro site of the linker, which leads, in contrast to hirudin, to beneficial reactivation of thrombin's active site and subsequent haemostasis (the half-life of bivalirudin is 20–30 min).

Continuous advances in synthetic methodology permitted the synthesis of larger and more protein-like peptides that featured higher selectivity, potency and in vivo stability due to a more rigid secondary structure[78–85]. Evolutionarily optimized venoms have become particularly attractive for such discovery efforts, providing millions of unique and highly potent bioactive venom peptides that target a wide range of proteins, including ion channels, enzymes, G-protein-coupled receptors (GPCRs) and transporters[86–90] (see BOX 2). Two examples of approved venom-derived peptide drugs are exenatide, a GLP1 receptor agonist from the venom of the Gila monster (*Heloderma suspectum*), a venomous lizard, and ziconotide, a peptidic inhibitor of voltage-gated calcium (Ca_V) channels from the venom of a predatory marine cone snail. Both drugs are identical to the native venom peptide.

Exenatide became the first of a highly successful class of drugs that act as agonists of the GLP1 receptor with its approval for the treatment of type 2 diabetes in 2005. GLP1 belongs to a group of gastrointestinal hormones called 'incretins' that cause an increase in insulin secretion after food consumption, even before blood glucose levels become elevated[91]. Its biologically active form GLP1(7–37) also inhibits glucagon secretion, appetite and food intake. Considerable efforts were made to develop GLP1(7–37) into a drug after it was found that intravenous infusion has dramatic effects on insulin secretion and blood glucose in patients with type 2 diabetes[92]. However, GLP1(7–37) is rapidly broken down by dipeptidyl peptidase 4 (DPP4) and undergoes renal clearance within 1–2 min (REF.[91]). Although replacement of Ala at position 2 with other short-side-chain amino acids protected the molecule against enzymatic degradation, fast renal clearance still rendered it unsuitable for therapy[91]. In the early 1990s, a 39-residue peptide

Box 1 | **Peptide drugs based on serendipitous discoveries**

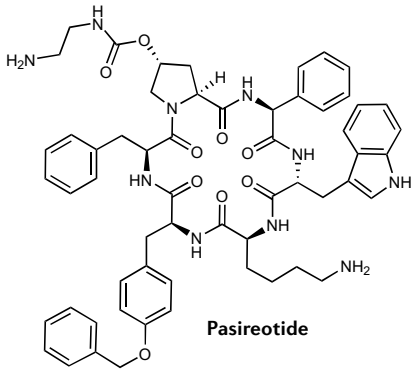
While medicinal chemistry and structure–activity relationship studies were the core concepts in early peptide drug development, it was serendipity coupled with outstanding cross-disciplinary science that played a major role in development of the peptide drug blockbuster glatiramer, which introduced the important concept of decoys for immunomodulators. This concept also found an important application in development of the HIV-entry inhibitor enfuvirtide. The discovery of both of these peptide drugs is described here.

Glatiramer (formerly known as copolymer 1 or Cop-1) is not a single peptide but is a synthetic polymer of four amino acids, Ala, Lys, Glu and Tyr, in a molar ratio of 4.2, 3.4, 1.1 and 1.0, respectively[229,230]. Given that the sequences can differ from one peptide to another, the composition and molar ratios are fixed. The copolymers range from 40 to 90 residues in length and the molecular mass ranges from 4.7 to 10 kDa (REF.[230]). Glatiramer was initially synthesized to resemble the structure of myelin basic protein. The purpose was to study the interaction of myelin proteins with lipids capable of inducing experimental autoimmune encephalomyelitis, which has many parallels to the human disease multiple sclerosis[230]. However, rather than inducing the disease, it protected against acute and chronic relapsing experimental autoimmune encephalomyelitis[230]. This discovery prompted further study and eventually led to its market approval in 1996 for the treatment of multiple sclerosis[231]. The exact mechanism is still not completely understood, but its resemblance to myelin basic protein suggests that it acts by inducing a decoy response, diverting the autoimmune response away from myelin[232,233]. Although sales declined from US$3.8 billion in 2017 to $1.5 billion in 2019 due to the approval of generic versions, glatiramer is still one of the most successful peptide drugs.

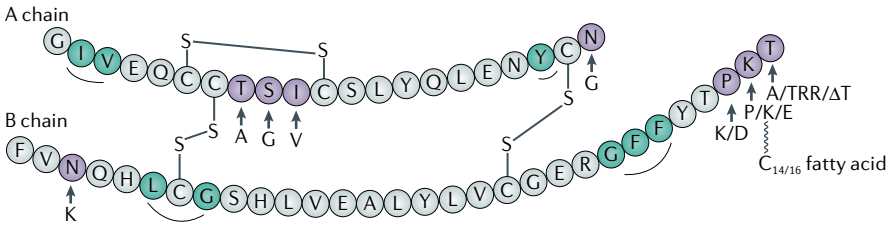
Another example of serendipity in peptide drug discovery that had important implications for the field was the development of enfuvirtide for the treatment of HIV infection. The discovery of the HIV life cycle in the 1980s led to the identification of a variety of HIV treatment targets[234]. A set of peptides was derived from HIV glycoprotein gp41 for an epitope mapping experiment to find an effective HIV vaccine. However, these peptides turned out to exhibit antiviral effects when incubated with human T cells. Additional studies of the fusion process and understanding of how the envelope glycoproteins interact showed that these peptides act as decoys, inhibiting HIV-1 entry into cells. Enfuvirtide (formerly known as T-20 or DP-178) was finally selected out of a pool of similar peptides due to its above-average antiviral efficacy[235]. It is a linear 36-mer that corresponds to residues 643–678 of the α-helical carboxy-terminal region of gp160; it consists of only L-amino acids with an acetylated amino terminus and a carboxy-terminal amide[235]. With a serum half-life of 3.8 h, it is administered by subcutaneous injection twice daily. This requirement for frequent administration resulted in an unprecedented scale of peptide production[194,236]. Large-scale production of enfuvirtide involves 106 steps (in comparison to an average of 8–12 steps for a small-molecule drug), and enfuvirtide is to date the most complex synthetic peptide manufactured at such a scale (more than 3 metric tons per year in 2004). Achieving such complexity and scale at good manufacturing practice level involved innovative engineering, method development and optimizations, processes that have been beneficial for improving the capabilities and increasing the efficiency of large-scale production of peptide therapeutics.

a Somatostatin drug and diagnostic agent development

Compound	Drug name	N terminus	Sequence	Half-life	Year
Somatostatin			AGCKNFFWKTFTSC	3 min	1973
Vale compound			CFwKTC	ND	1979
Vapreotide	Sanvar		fCYwKVCW	0.5 h	1987
Octreotide	Sandostatin		fCFwKTCT-ol	2 h	1982
Indium In 111 pentetreotide	Octreoscan	^{111}In–DTPA–	fCFwKTCT-ol	2 h	1991
Lanreotide	Somatuline		D-Naph-CYwKVCT	1 h	1988
Pasireotide	Signifor		See structure below	12 h	2012
Lutetium Lu 177 DOTA-TATE	Lutathera	^{177}Lu–DOTA–	fCYwKTCT	3.5 h	2013
Gallium Ga 68 DOTA-TATE	Netspot	^{68}Ga–DOTA–	fCYwKTCT	68 min	2016
Gallium Ga 68 DOTA-TOC	SomaKit TOC	^{68}Ga–DOTA–	fCYwKTCT-ol	68 min	2019
Copper Cu 64 DOTA-TATE	Detectnet	^{64}Cu–DOTA–	fCYwKTCT	12.7 h	2020



b Insulin drug development



Analogue	A chain	B chain	Approval
Porcine		T30A	1966
Bovine	T8A, I10V	T30A	1966
Sheep	T8A, S9G, I10V	T30A	NA
Human			1982
Lispro		P28K, K29P	1996
Aspart		P28D	2000
Glargine	N21G	+31R, +32R	2000
Glulisine		N3K, K29E	2004
Detemir		K29K (C_{14} fatty acid)	2005
Degludec		ΔT30, K29E (C_{16} fatty acid)	2015

Fig. 3 | **Selected examples of therapies based on peptide hormones. a** | Somatostatin drug and diagnostic agent development. Systematic structure–activity relationship studies identified somatostatin's pharmacophore (FWKT, blue) and the shortest active mimetic (Vale compound), which became the lead sequence in the quest for more potent and stable analogues. Amino-terminal (N-terminal) D-Phe (f) and a carboxy-terminal (C-terminal) amino alcohol (T-ol) extension were introduced to mask enzymatic recognition sites, increasing activity and stability (shown in salmon pink and green, respectively). This led to the approval of octreotide for the treatment of acromegaly and various cancers. Further subtype-selective analogues, such as lanreotide and vapreotide, have been developed for oncology applications, including peptides with radioactive elements such as ^{111}In, ^{90}Y, ^{68}Ga, ^{64}Cu or ^{99m}Tc attached via chelating groups to the N terminus for peptide scintigraphy, targeted radiotherapy and positron-emission tomography of tumours. N- to C-terminal cyclization increased peptide half-life significantly and led to the approval of pasireotide for the treatment of Cushing disease. Cys residues that form a disulfide bond are shown in orange. The half-lives listed are in vivo/elimination half-lives, except for gallium Ga 68 DOTA-TATE, gallium Ga 68 DOTA-TOC and copper Cu 64 DOTA-TATE, for which the radionuclide half-lives are given. **b** | Insulin drug development. A schematic structure of insulin highlighting the chemical modifications used in drug analogues is shown at the top. The purple residues and the table highlight point modifications in insulin drug analogues, with fast-acting analogues on a blue background and long-acting analogues on an olive background. Green and underlined residues are crucial for receptor binding, demonstrating why it is difficult to develop a small-molecule drug that can directly activate the insulin receptor. The first drug to use single amino acid alterations was fast-acting insulin lispro, in which the C-terminal Lys and Pro residues of the B chain were swapped, preventing formation of inactive hexamers without affecting activity. Other fast-acting analogues followed: insulin aspart in 2000 and insulin glulisine in 2004. Long-acting analogues such as insulin glargine were produced by adding two positively charged Arg residues to the C terminus of the B chain, which increased the isoelectric point. This rendered the analogue less soluble at physiological pH, resulting in slower release from the injection site. Addition of fatty acids produced further long-acting analogues — insulin detemir and insulin degludec — where the fatty acid delays the release from the injection site and reduces renal clearance due to binding to serum albumin. NA, not applicable; ND, not determined.

called 'exendin 4' with 53% identity to GLP1(7–37) was isolated from venom of the Gila monster. It was found to be a full agonist of the GLP1 receptor[93,94] (FIG. 4b) with stability against DPP4 degradation and lower renal clearance in humans (5–7 h)[91,93,95], supporting its development as a drug (named 'exenatide' and administered twice daily by subcutaneous injection) for type 2 diabetes. Several other GLP1 agonists (as well as a longer-acting formulation of exenatide[96]) have since been approved for the treatment of this disease, including liraglutide, albiglutide, dulaglutide, lixisenatide and semaglutide, with some also gaining approval for the treatment of obesity. Advances in this major peptide drug class also illustrate other trends that are discussed further below.

Initial studies on ziconotide date back to the 1980s, when the potential therapeutic applications of the rigid and protein-like peptides from cone snail venom were first explored[90]. These conotoxins are small disulfide-rich peptides, generally 10–40 residues in length, that target various ion channels, GPCRs and transporters with high potency and selectivity. Ziconotide is a 25-residue peptide derived from *Conus magus*

with three disulfide bonds that stabilize and orient the short β-sheets in a unique three-dimensional arrangement[97]. Its defined topology and compact structure enable this conotoxin to selectively inhibit $Ca_V2.2$ channels. Although hundreds of analogues were studied for their pharmacological properties, it was the naturally occurring peptide that was finally approved by the FDA in 2004 for the treatment of severe chronic pain. Notably, this compound is about 1,000 times more potent than morphine without the problems related to addiction. A major downside, however, is that its administration requires an implanted pump for infusion into the cerebrospinal fluid because the target population of $Ca_V2.2$ channels is located in central spinal cord neurons.

There are millions of neurologically active venom peptides remaining to be explored, with spiders, scorpions and cone snails providing some of the richest chemical diversity. These venom libraries remain largely untapped, in part because of the slow pace of the traditional discovery approach based on bioactivity-guided fractionation, in which crude venom fractions are screened against known targets, followed by further purification and sequencing to elucidate the sequence of a hit. However, various technological advances are now forming the basis of a powerful approach termed 'integrated venomics', which combines collated sequence data from venom gland or venom duct transcriptomes with proteomic data obtained from crude venom via liquid chromatography–tandem mass spectrometry in a rapid and cost-effective manner[98,99] (FIG. 5). With this method, it is possible to obtain thousands of novel sequences that can be produced by SPPS or recombinant methods in bacteria, yeast and insect cells[100]. Whereas most of these native disulfide-rich peptides fold into their bioactive isomer, new synthetic approaches use selenocysteine-directed folding to facilitate synthetic library design and efficient folding of modified peptides for structure–activity relationship (SAR) studies[101,102]. Numerous high-throughput screening platforms (such as FLIPR, Cell Lux, FDSS 6000, AlphaScreen and Tango) can be used to screen these peptides against a large panel of validated targets for therapy, with ion channels being a target class for which venom peptides could be particularly promising, given that identifying modulators with very high selectivity is often needed in ion channel drug discovery[98,103] (BOX 3).

Further examples of approved peptides derived from natural products — eptifibatide, romidepsin, carfilzomib and linaclotide (Supplementary Fig. 2) — are provided in Supplementary information. Looking to the future, genome mining of microorganisms[104] as well as other organisms[105–109] and new methods to grow previously uncultured bacteria or promote their production of novel secondary metabolites, such as cultivation in their natural environment or the use of specific growth factors, will lead to the discovery of novel therapeutic peptide leads, as recently exemplified by the discovery of the antimicrobial peptide teixobactin[110–112]. Cationic host defence peptides are another interesting class of natural products that encompass not just direct microbicidal properties but also interactions with the host immune system to fend off infections[113]. This peptide class is produced by vertebrates, invertebrates, plants and fungi, mostly as the first line of defence against microbial pathogens. Although the primary application of these peptides is treatment of infections, for which several candidates are in clinical trials, their immunomodulatory functions are also being pursued for the treatment of chronic inflammatory disorders and cancer, as adjuvants for vaccine formulations and for wound healing[113].

Strategies to overcome renal clearance

Unmodified peptides are rapidly cleared (within minutes) from plasma, and it was realized early in the history of peptide drug development that enzymatic protection alone was not the solution. On the basis of the understanding of the size-dependent mechanism of renal clearance[114–116], strategies to increase the molecular weight of peptides emerged, such as lipidation, conjugation to larger proteins and pegylation, which substantially

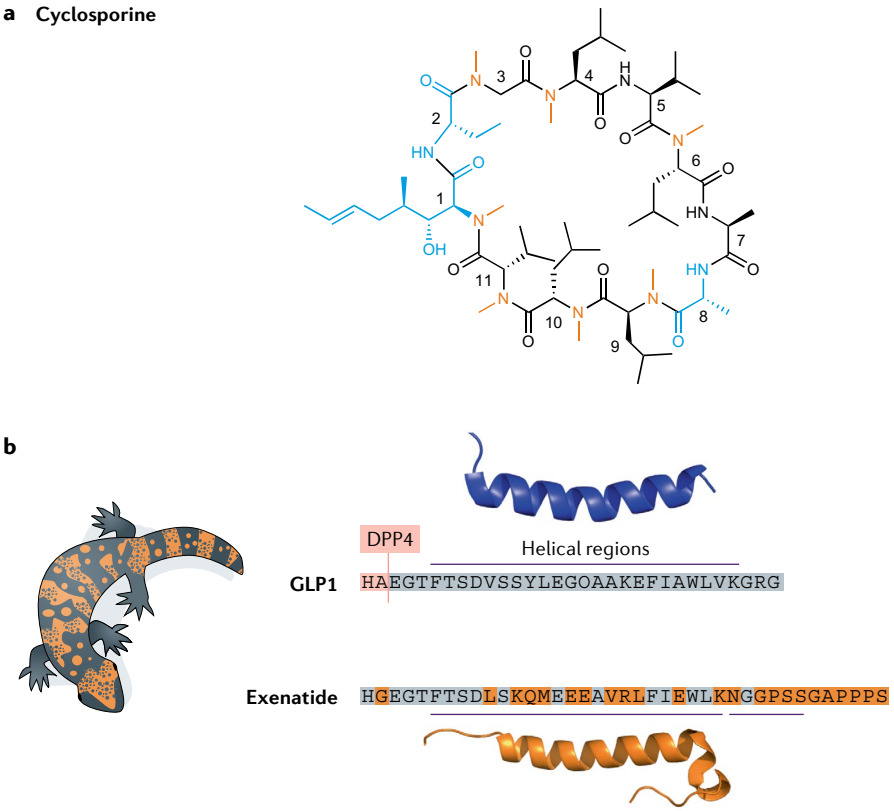


Fig. 4 | **Selected examples of therapies based on natural product peptides. a** | Chemical structure of the immunosuppressant cyclosporine, initially isolated from a fungus. Cyclosporine can be classified as a peptide mimetic, considering that 7 of the 11 peptide bonds are N-methylated (orange); it contains two unusual amino acids (3-hydroxy-4-methyl-2-methylamino-6-octonoic acid at position 1 and aminobutyric acid at position 2, blue) and one D-amino acid (D-Ala at position 8, blue). These modifications translate into outstanding stability and oral bioavailability. **b** | Sequence alignment and three-dimensional structures of human glucagon-like peptide 1 (GLP1) and the natural product exenatide (53% sequence identity) isolated from the saliva of the Gila monster (*Heloderma suspectum*). GLP1 is rapidly broken down by dipeptidyl peptidase 4 (DPP4) and undergoes renal clearance within 1–2 min. By contrast, exenatide is stable to DPP4 degradation and remains in circulation for 5–7 h. The alteration from G16 in GLP1 to E16 in exendin 4 reduces flexibility and further stabilizes its helical conformation, which might be one of the reasons for its increased stability.

increased plasma circulation times[117,118]. Steric hindrance by these size-increasing moieties also protects against proteolytic degradation[117,118].

Lipid conjugation moieties that have been used in approved peptide therapies include a $C_{14/16/18}$ fatty acid in insulin detemir, insulin degludec (FIG. 3b), liraglutide and semaglutide, resulting in longer-acting analogues that allow administration once daily, or once weekly in the case of semaglutide. The covalently attached fatty acid binds to serum albumin, leading to reduced degradation and elimination of the peptides, significantly improving their pharmacokinetics[117,119,120]. The use of fatty acids has additional benefits, including delayed release from the injection site and reduction of immunogenic responses. A similar approach was applied in the development of tesamorelin, an analogue of human growth hormone-releasing hormone (GHRH) that stimulates the synthesis and release of endogenous growth hormone. Tesamorelin was approved in 2010 to treat HIV-related lipodystrophy[121]. This synthetically produced 44-residue sequence of GHRH has a hexenoyl moiety at the N-terminal Tyr residue, which protects against DPP4 degradation[122].

Selective conjugation to serum albumin or immunoglobulin — two plasma proteins with an unusually long circulation time (weeks instead of days) — is another strategy used to exceed the molecular weight cut-off for glomerular filtration, thereby prolonging the duration of action. This strategy was used for dulaglutide and albiglutide to extend the half-life and thereby allow once-weekly injections[117,123,124]. Dulaglutide consists of two identical, truncated and modified GLP1 analogues, each covalently linked by a flexible peptide to a modified human immunoglobulin G4 heavy chain fragment (Fc), which are joined through two disulfide bonds[125]. Albiglutide consists of two tandem copies of a truncated and modified GLP1 analogue fused to human albumin[125,126]. The GLP1 point modifications and protein linkages also protect the GLP1(7–37) segments from DPP4 degradation.

Finally, in the past two decades, pegylation, in which polyethylene glycol (PEG) chains are attached to peptides or proteins, has gained momentum, with multiple FDA-approved pegylated proteins (such as PEG–bovine adenosine deaminase and PEG–α-interferon) and some pegylated peptides in clinical trials[118,126,127]. For example, the complement C3 inhibitor pegcetacoplan, a pegylated compstatin analogue, is in clinical trials for the treatment of paroxysmal nocturnal haemoglobinuria, geographic atrophy, and C3 glomerulopathy[128,129]. A pegylated form of exenatide is in phase II trials for the treatment of Parkinson disease and type 2 diabetes, while a pegylated analogue of the gut hormone oxyntomodulin is in phase I trials for the treatment of obesity[130]. Oxyntomodulin is an agonist of both GLP1 and glucagon receptors and is a natural appetite suppressant.

Display technologies

Display systems — which include phage display, yeast display, mRNA display, ribosome display and DNA display — play an important role in today's state-of-the-art peptide drug discovery (FIG. 5). They create a link between phenotype (peptide) and genotype (DNA or RNA coding sequence) to enable affinity (or more rarely activity) selection at the peptide level, with subsequent recovery and amplification of the genetic material (through PCR or infection of host cells by phage virions). Phage display, the oldest technique[131], can produce libraries comprising up to 10^{10} unique peptides. In vitro display systems (in particular mRNA display and ribosome display) have the advantage that there is no need for host cell transfection, allowing the creation of more diverse libraries (10^{13}–10^{15} peptides)[70,132].

The first approved peptide therapy discovered using display technology was peginesatide, an erythropoiesis-stimulating agent[133–135]. Phage display libraries were screened to isolate small peptides that bind to and activate the receptor for the cytokine erythropoietin[136], leading to the identification of a synthetic dimeric peptide comprising two identical 21-residue chains each with a single disulfide bond[137]. The peptides have no homology to erythropoietin, yet their effects appear to be identical to those induced by the natural ligand[136,138]. Covalent linkage of the dimeric peptide (~5 kDa) to a single Lys-branched bis(methoxypoly(ethylene glycol)) PEG chain (~40 kDa) to improve its pharmacokinetic characteristics resulted in peginesatide[137], which was approved by the FDA in 2012 for the treatment of anaemia associated with chronic kidney disease in adult patients receiving dialysis. However, in 2013, postmarketing reports of serious hypersensitivity reactions, including potentially life-threatening anaphylaxis[135], led to peginesatide being withdrawn from the market.

Phage display also led to other approved peptide drugs (that is, romiplostim[139] and ecallantide[140], see Supplementary information), but it has generally found greater applications in the development of monoclonal antibody therapeutics than peptide drugs[141], in part because peptides from phage display often have poor pharmaceutical properties. More drug-like properties, such as peptidase resistance,

Box 2 | Animal venoms as a rich source for peptide drug leads

Venom acquisition transforms predator–prey interactions from physical encounters to chemical battles, thereby enabling venomous animals to both prey on and defend themselves against much larger animals. Venom acquisition is such a transformative event in animal evolution that about 15% of all extant species are venomous[88].

Although there are some exceptions (for example, reptiles and some ant subfamilies), the dominant components of most venoms, such as those from centipedes, cone snails, sea anemones, scorpions, spiders and most venomous insects, are disulfide-rich peptides. The disulfide bonds provide a rigid scaffold upon which pharmacophores can be displayed within the intercystine loops. These loops are typically highly permissive to mutation, so during millions of years of evolution there has been massive pharmacological diversification within each venom-peptide family. This permissiveness to mutation makes these disulfide-rich peptides ideal for engineering new functions[237].

The most widely recruited disulfide scaffold in animal venoms is the three-disulfide inhibitor cystine knot (ICK or knottin), in which two of the disulfide bridges and the intervening sections of the peptide backbone form a closed loop that is pierced by the third disulfide to form a pseudoknot[238]. The ICK may be the most ubiquitous disulfide-rich scaffold in the natural world as it is found in viruses, fungi and animals, and cyclized versions (cyclotides) are present in plants[239]. Although cystine knots are not true knots in a mathematical sense, they nevertheless provide knottins with exceptional stability and resistance to proteases, making them excellent drug leads. The analgesic drug ziconotide is an ICK peptide derived from a venomous cone snail[97]. Amgen[240], Janssen[241], MedImmune[242] and Merck[243] have all developed ICK peptides from spider venom as leads for analgesics that target the $Na_V1.7$ voltage-gated sodium channel. Venom-derived ICK peptides are also being developed as drug leads for the treatment of epilepsy, hypokalaemic periodic paralysis and stroke, and they are providing templates for engineering of novel diagnostic agents and therapeutics[237].

cell membrane permeation ability and oral bioavailability, can be achieved by macrocyclization and the introduction of non-proteinogenic amino acids (see earlier). A natural example of a macrocyclic peptide is cyclosporine, but recent technological advances now allow the synthesis and high-throughput screening of such peptides. For example, split-intein circular ligation of peptides and proteins (SICLOPPS) can be used to generate macrocyclic peptides inside cells and phenotypically screen cells for them; however, it is limited to proteinogenic amino acids and has a maximum library size of ~10^6 (REF.[142]). Phage display can also be used to identify macrocyclic peptides; for example, the Bicycle screening platform is a relatively new approach that identifies bicyclic peptides using phage display[143,144]. Bicyclic peptides are conformationally more constrained than monocyclic peptides and therefore bind with higher affinity and are typically more resistant to proteolytic degradation, especially if this property is selected for[145]. Using this platform, a bicyclic peptide inhibitor of coagulation factor XII has been developed[146], and a modified version of the peptide has shown promise in preclinical work as a thrombosis treatment[147]. However, as for SICLOPPS, the Bicycle platform is generally limited to proteinogenic amino acids. An elegant strategy that could overcome this is mirror-image phage display to identify L-peptides that bind potently to chemically synthesized all-D drug targets[148–151]. This, in turn, delivers blueprints for metabolically ultrastable all-D-peptide drugs that — combined with chemical modifications to evade renal clearance or formulations that improve oral uptake — have the potential to become a game-changer for peptide drug development.

Techniques using mRNA display can also be used to screen large libraries of macrocyclic peptides (more than 10^{12}) including non-proteinogenic amino acids (as well as D-amino acids), due to the use of in vitro translation[71]. For example, RaPID (random non-standard peptide integrated discovery) uses 'flexizymes' (catalytic RNAs) that can charge almost any tRNA with a wide variety of non-proteinogenic amino acids[72,152]. Such techniques may be useful for targeting proteins that have proved intractable to targeting with small molecules and are increasingly being used in the pharmaceutical industry. For example, the company PeptiDream has commercialized this technology and provided multiple therapeutic leads to the pharmaceutical industry. Although no peptides developed by PeptiDream have yet entered late-stage clinical trials, one peptide (zilucoplan), developed using similar technology (Ra Pharmaceuticals mRNA display platform)[153], is currently in phase III trials for the treatment of generalized myasthenia gravis (a neuromuscular disease). Zilucoplan was identified from a library of macrocyclic peptides using complement component C5 as a target. It inhibits cleavage of C5 into C5a and C5b, thereby preventing formation of the terminal complement complex, which can damage and destroy the postsynaptic membrane, disrupt ionic channel conductance and impair neuromuscular transmission[154]. Overall, display technologies along with natural product discoveries are likely to be a major source of next-generation peptide drugs (FIG. 5).

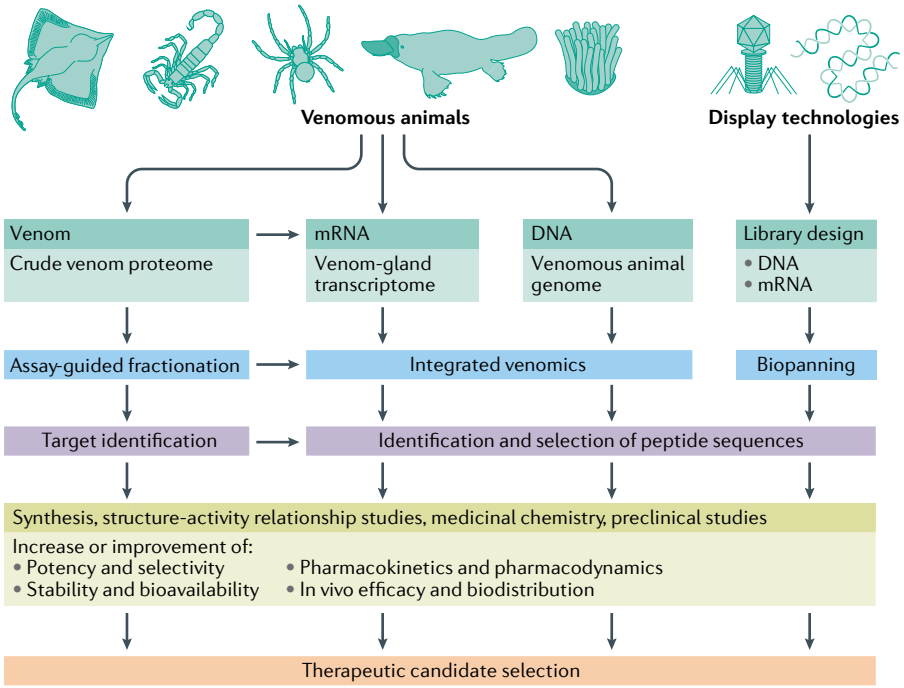


Fig. 5 | **Peptide drug discovery strategies.** Integrated venomics and display technologies are two key technologies for therapeutic lead discovery. Integrated venomics utilizes bioinformatics to analyse genomic and transcriptomic data of venomous animals along with proteomic data obtained from crude venom samples. This approach can identify a large number of venom peptide sequences, which can then be produced synthetically or recombinantly and can be screened against therapeutic targets. Display technologies can produce vast peptide libraries (10^{10}–10^{15}) that are panned against therapeutic targets. This process usually yields high-affinity target binders after a few rounds of selection. Medicinal chemistry strategies are then used to improve the drug-like properties of these leads.

New chemical approaches

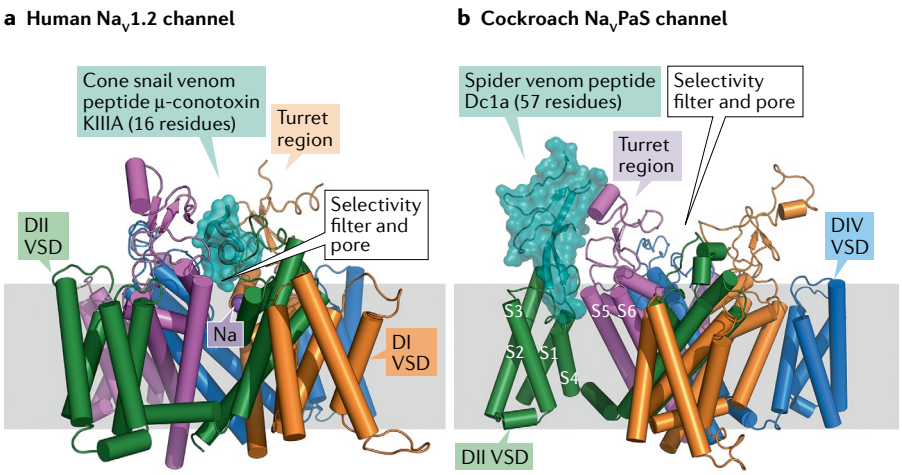
Medicinal chemistry strategies in peptide drug development have traditionally been relatively simple (yet effective) and largely focused on increasing the metabolic and circulation half-life of already very good (in terms of potency and selectivity) therapeutic peptide leads. We expect this trend to continue with very specific modifications at identified cleavage sites, including N-terminal acetylation, N-methylation, the use of D-amino acids or unnatural amino acids and amide bond mimetics (for example, thioamides, peptoids and β-amino acids) (FIG. 6). N- to C-terminal cyclization and disulfide-bond mimetics (particularly thioether bonds) will be increasingly used in the design of peptide drugs to tune metabolic stability and bioavailability[155–158].

Multiple highly stable cyclic cystine-knot scaffolds have been identified that allow grafting of therapeutically relevant pharmacophores into their loops, and it will be interesting to see how such grafted cyclic peptides fare in the clinic[159]. Advances in bioinformatics will facilitate the design of such grafts as well as the design of peptidomimetics that replicate secondary structure elements of peptides and proteins. Current in silico design, however, still faces challenges, including the sheer complexity of folding, the chirality of peptides, the lack of high-resolution structures of receptor–peptide complexes, subtle conformational changes involved in ligand binding and the presence of large surface areas instead of binding pockets. More complex chemistries could eventually appear in clinical

Box 3 | **Ion channels as promising targets for peptide drugs**

Ion channels are a diverse class of pore-forming membrane proteins that gate ions across cell membranes to initiate action potentials and signalling cascades. They regulate many physiological functions, and their accessible location in the cell membrane and diverse biological roles have made them the third most common drug target after GPCRs and kinases[244]. They comprise numerous structural classes[245] that are divided into multiple subtypes (see Supplementary Fig. 3): for example, there are nine subtypes of voltage-gated sodium (Na_V) channels ($Na_V1.1$–$Na_V1.9$) that have distinct anatomical locations and functions[246,247]. Individual subtypes often have distinct (patho)physiological functions (for example, $Na_V1.7$ is an analgesic target, whereas $Na_V1.4$ regulates skeletal muscle contractility), and therefore subtype selectivity is crucial for therapeutic targeting of this class of proteins.

Given their critical role in the nervous system of all animals, it is not surprising that voltage-gated ion channels are a major target class for venomous animals, and that venom peptides modulate the activity of these channels via diverse molecular mechanisms. These channels consist of a central pore surrounded by four voltage-sensor domains (VSDs) that enable the channel to respond to changes in membrane potential. Na_V channels comprise four homologous but non-identical domains denoted DI–DIV (coloured orange, green, magenta and blue, respectively, in the figure). Some venom peptides are simple pore blockers (antagonists) that sterically prevent ion flow, such as the 16-residue μ-conotoxin KIIIA from a venomous cone snail that occupies the extracellular vestibule of the human $Na_V1.2$ channel and occludes the channel pore [248] (see the figure, part **a**). In contrast, venom peptides known as gating modifiers modulate channel gating (that is, cycling between the open, closed and inactivated states) by interacting with one or more of the VSDs. An example is the 57-residue spider venom peptide Dc1a, which extensively interacts with both VSDII and the pore domain to stabilize an open conformation of the insect Na_VPaS channel, thereby acting as a channel agonist[249] (see the figure, part **b**). Both pore-blocker and gating-modifier peptides make extensive, multifocal contacts with ion channels, imparting high affinity and selectivity compared with small-molecule modulators. Venom peptides can also indirectly modulate ion channels, as seen with $GABA_B$ receptor-mediated inhibition of $Ca_V2.2$/$Ca_V2.3$ and potentiation of the channels GIRK1 and GIRK2 with conotoxin Vc1.1 from cone snail venom (see Supplementary information and Supplementary Fig. 4).



drug candidates, driven mainly by new platform technologies (for example, from display/automated assembly/combinatorial/bacterial expression technologies developed to improve barrier crossing and increase metabolic stability)[70–72,132,142,144,145,148,152,160,161].

Another approach that has received much attention and financial support is the concept of stapled peptides[162–164]. In this approach, the binding motifs of therapeutic proteins are stabilized within short peptides through rational positioning of covalent crosslinks (staples). In particular, α-helical stabilization has been pursued by multiple biotechnology companies to increase cell penetration and target affinity, while simultaneously decreasing proteolytic degradation. So far, two stapled peptides, both developed by Aileron Therapeutics, have entered clinical trials. ALRN-6924 is designed to reactivate p53 in tumours by inhibiting its major negative regulators MDM2 and MDMX, and it is currently in phase II trials[165,166]. ALRN-5281 is designed to activate the cell-surface receptor GHRH to increase growth hormone release in people with rare endocrine disorders and has completed phase I trials.

As highlighted earlier, conjugation to improve the therapeutic properties of a peptide lead is another concept that is increasingly pursued in peptide drug development, ranging from pegylation or protein conjugation as half-life extension strategies and cytotoxic payloads for cancer therapy to multivalent display, dual pharmacophores, targeted delivery vectors or the use of imaging tags for future theranostics. Recent advances in enzymatic ligation of chemically synthesized peptide fragments (chemoenzymatic peptide synthesis) facilitate this trend and allow cost-effective synthesis of complex peptides and peptide conjugates. Chemoenzymatic peptide synthesis uses ligases such as sortase, butelase, peptiligase or omniligase-1 for efficient tandem ligation, peptide modifications and cyclization[167]. Their cost-effectiveness and large-scale compatibility have been demonstrated with gram-scale exenatide production, resulting in a cost reduction of 50% compared with full SPPS[168]. The use of fully automated and scalable flow peptide synthesis is another technological development that shows promise for accelerated synthesis and SAR studies[169].

Advances in delivery

Most peptide drugs are delivered by injection, which comes with several drawbacks, including poor patient adherence, accidental injuries, risk of infection, improper use and biohazardous needle waste. The development of alternatives to needle-based injection has therefore been a high priority. Here we outline some of the most recent developments and refer the reader to a review that discusses these strategies in more detail[44].

Various pump technologies offer alternatives to injection. Implantable pumps for delivering insulin have been around since the 1980s, but investment in this technology dwindled in the first decade of the twenty-first century. There are some signs of a comeback; for example, with the development of microtechnology-based implantable pumps, which have more precise control over delivery[170]. However, implantation of pumps is invasive and they require refilling.

Alternative approaches to delivering drugs across the skin without injection have included needle-free injection; for example, liquid jet injectors[171]. However, pain and bleeding at the site of injection can occur, and there is variability in the amount of drug delivered, in part due to variation in skin properties among patients. Other approaches include those that aim to temporarily disrupt the skin structure, such as the use of skin-penetrating peptides[172], whereas others use ultrasound or electric

fields to increase skin permeability[173]. Microneedle technologies for the delivery of insulin[174] or abaloparatide[175] are in phase II and phase III trials, respectively.

The pulmonary route is another potential delivery pathway, but it is accompanied by safety issues and limited delivery efficiency. Inhalable insulin (Pfizer's Exubera) received FDA approval in 2006 but was discontinued in 2007 owing in part to safety concerns and high costs. With the development of Afrezza (approved by the FDA in 2014), MannKind hopes to revive interest in inhalable insulin, but safety concerns remain, with side effects including cough or throat irritation[176]. Nasal delivery is another potential route, with desmopressin available as a nasal spray. However, as with the pulmonary route, the impact of long-term delivery on sensitive mucosa remains a concern. Therefore, these routes may be more suited for occasional use; for example, glucagon is available as a dry nasal spray to treat severe hypoglycaemia reactions[177].

Much effort has been invested in developing strategies to enable oral delivery of peptide drugs, a route that is limited by enzymatic digestion of peptides in the gastrointestinal tract and their limited permeation across the intestinal epithelium (see REFS[178,179] for recent reviews). One strategy to overcome these barriers for which there has been notable clinical success is co-formulation with permeation enhancers. In 2019, semaglutide co-formulated with the permeation enhancer sodium *N*-[8-(2-hydroxybenzoyl)amino] caprylate (SNAC) for once-daily oral administration was approved for the treatment of type 2 diabetes on the basis of a phase III programme involving eight clinical trials and several thousand patients (reviewed in REF.[178]). SNAC is a small fatty acid derivative that promotes absorption of semaglutide across the gastric mucosa via effects on transcellular pathways[180,181]. An orally administered octreotide product in which the peptide is encapsulated with medium-chain free fatty acids and the permeation enhancer sodium caprylate is in phase III trials[182], and an orally administered insulin candidate (ORMD-0801) including a permeation enhancer, soybean trypsin inhibitor and a chelator is in phase II trials[183]. Other strategies that continue to be explored include pH modulation, mucus-penetrating agents, enzyme inhibition, hydrogels, intestinal patches, enteric-coated capsules and devices[178]. For example, devices that use either drug-loaded microneedles or milliposts to deliver peptides into intestinal tissue recently showed promise in ex vivo human and in vivo animal studies[184,185]. Although these developments are important and promising, we are still far from achieving true oral bioavailability, which is generally defined as more than 20% of the orally administered compound observed in systemic circulation[156,186].

Finally, given the many intracellular protein drug targets that may require a substantial surface area for binding or inhibition of protein–protein interactions, it is not surprising that chemical strategies to deliver peptides into cells have been actively pursued for many years. Although most peptides have low membrane permeation ability, several peptides with translocation capacities, known as cell-penetrating peptides (CPPs), have been discovered. These peptides, typically comprising 5–30 residues, can cross membranes via energy-dependent endocytosis or energy-independent direct penetration, although the mechanisms have not been fully elucidated[187,188]. CPPs can be used to transport various cargoes, including other peptides, proteins, nanoparticles, DNAs, small RNAs and small drugs, into cells. One of the most clinically advanced and intriguing CPP–drug constructs is AM-111/D-JNKI-1/XG-102, which is a 31-residue-long D-*retro-inverso* construct of the cell-penetrating Tat moiety linked to a JUN N-terminal kinase peptide inhibitor. This construct has been investigated for the treatment of sudden sensorineural hearing loss (phase III) and intraocular inflammation causing pain in patients undergoing cataract surgery[189]. Another intriguing construct is nerinetide, which has recently completed phase III trials for the treatment of acute ischaemic stroke[190]. Nerinetide is composed of Tat linked to the C terminus of NR2B9c, a nine-residue inhibitor of the intracellular interaction between postsynaptic density protein 95 (PSD95) and NMDA (*N*-methyl-D-aspartate) receptors in brain neurons. In this instance, Tat is used to deliver intravenously administered nerinetide across the blood–brain barrier and into neurons. There are now numerous CPP-conjugated therapeutics in preclinical and clinical development[191], and we expect multiple CPP constructs to be approved in the coming years.

Outlook for peptide drug development

The preceding examples illustrate some of the directions in which the peptide therapeutics field has evolved over the last six decades, and they provide insight into viable strategies and future directions. It is clear that the field has come a long way in overcoming key challenges, yet there remains much room for improvement and new developments.

Advances in synthetic methodology, recombinant production and automation

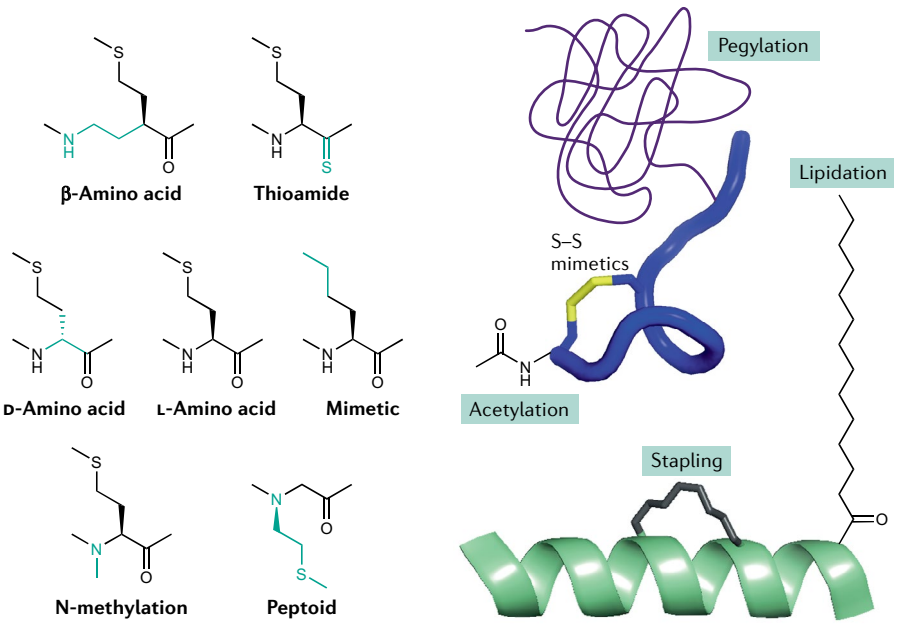


Fig. 6 | **Medicinal chemistry strategies for peptide drugs.** Overview of well-validated chemical modifications used in peptide drug development to increase metabolic stability and bioavailability. Approaches include replacement of selected amino acids by D-amino acids, β-amino acids, thioamide or side chain mimetics, N-methylation and N-terminal acetylation, peptoid design, disulfide bond mimetics, pegylation, lipidation and stapling to stabilize α-helices.

will continue to reduce manufacturing costs of structurally complex peptides. Although synthetic peptide drugs might never be cheaper to manufacture than their small-molecule counterparts, it is likely that the overall research and development costs of peptide drugs will be lower than those of their small-molecule counterparts due to their intrinsic synthetic feasibility (which enables rapid and extensive SAR studies for lead optimization) and lower likelihood of off-target effects (which could lead to higher success rates in clinical trials). Recent peptide blockbusters such as the GLP1 receptor agonists liraglutide and dulaglutide have contributed to the growing industry interest in this drug class and its acceptance by physicians and patients. Advances in peptide drug delivery technologies and peptide receptor radionuclide therapy have further fuelled this momentum.

One aspect that needs to be clearly understood, however, is that peptides occupy a specific biophysical and pharmacological space, and concepts that work for small molecules or biologics might not apply to peptide drug development. Despite advances in delivery technology, formulation techniques and medicinal chemistry, 90% of all peptide drugs are delivered by injection, and lack of oral bioavailability remains the major limiting barrier in peptide drug development. Furthermore, it is difficult to build a strong case for peptides targeting central nervous system (CNS) disorders considering that most peptides struggle to pass the gut barrier. This is unfortunate, as peptides would be ideal candidates to treat CNS disorders due to their superior selectivity and endogenous role as central neurotransmitters. Although exceptions exist, such as chlorotoxin (which targets glioblastomas[192]) and nerinetide (which targets brain neurons in ischaemic stroke[190]), this barrier will persist until new, robust and broadly applicable peptide technologies emerge that overcome this delivery problem.

Unfulfilled revenue expectation is another reason why peptide drug development is often not prioritized. For example, ziconotide, a first-in-class drug for the treatment of severe neuropathic pain, which is an area of high unmet need, had unexpected side effects (dizziness, nausea, confusion and nystagmus)[193], and the US rights were sold to Azur Pharma for only $14.6 million. Enfuvirtide for the treatment of HIV infection (BOX 1) had a decline in sales due to a combination of resistance development and cheaper and orally available small-molecule drugs receiving market approval. This raises an important point: not only is the lack of oral bioavailability an obstacle (enfuvirtide needs to be injected twice daily and ziconotide is administered intrathecally via an implanted pump) but production costs can pose problems for clinical translation. Even though the multiton synthetic production of the 36-mer enfuvirtide was an important step towards commercial viability of peptide therapeutics, the large-scale synthetic production costs for a 5,000-Da peptide will still exceed those of a typical 500-Da small-molecule drug by 10–100-fold[194,195]. Nevertheless, it is important to realize that production costs make up only ~3–5% of the total research and development and distribution costs for a new drug, and technological advances in recombinant production of peptide therapeutics that also include unnatural amino acids and modifications could play an important role in addressing this limitation.

Despite these well-known barriers, there is a growing market for peptide drugs, and the question drug developers need to ask is as follows: in which therapeutic space might peptides hold advantages over small-molecule drugs? Insulin illustrates this concept perfectly, highlighting how a peptide drug can successfully fill a particular niche in the drug market — despite some of its disadvantages, all attempts to find small-molecule replacements have failed. In the remainder of this section, we discuss the characteristics of a good peptide drug and the types of targets and indications for which they are most likely to be successful; a summary is provided in BOX 4.

Box 4 | **Characteristics of an ideal peptide drug and peptide drug target**

Ideal peptide drug

- Potent (effective concentration for half-maximum response/half-maximum inhibitory concentration in the low nanomolar range or lower) and selective (more than 50-fold) over key off-targets.
- Agonist. Only low receptor occupancy (5–20%) is necessary for receptor activation, whereas antagonists generally must occupy more than 50% of receptors to be effective. Furthermore, antagonism can be achieved by allosteric receptor interactions, leaving ample room for competing orally available small-molecule drugs.
- Antagonist/inhibitor for targets where a large surface area provides an advantage over small-molecule drugs (for example, protein–protein interactions and ion channels).
- Stable due to rigid three-dimensional structure with secondary structural motifs stabilized by disulfide bonds, thioethers or staples; N/C termini cyclized, capped or truncated; incorporation of unnatural amino acids to remove metabolic cleavage sites.
- Replacement of methionine residues (for example, with norleucine) to avoid oxidative shelf-life problems.
- Pharmacokinetics and dynamics matched with biological action, dose regime and safety.
- Incorporation of fatty acids ($C_{14/16}$), pegylation or protein conjugation to evade renal clearance, if required. Rapid clearance can be beneficial in some cases as it leads to a desirable side effect profile.
- Compatible with a delivery route/formulation strategy that maximizes patient adherence.

Ideal peptide drug target

- Extracellular and peripheral to bypass delivery challenges.
- Multiple receptor subtypes to leverage the selectivity advantages of peptides.
- Targets that require a large surface area for a therapeutic response (for example, insulin receptor, ion channels and protein–protein interactions).

Characteristics of a good peptide drug.
One key advantage of peptides over small molecules is their larger surface area and greater chiral and structural complexity. These attributes can be exploited for drug targets that require interaction at multiple and distant sites for target activation. This is the case for insulin and is a major reason for its success: SAR studies illustrate that the N terminus and C terminus of insulin's A chain and individual residues from the B chain (~14 residues/16 Å apart) are crucial for receptor activation[196] (FIG. 3b).

Agonism versus antagonism is another consideration when peptide drugs are being developed. Peptide antagonists were slower to reach the market; this is related to the high production costs in the early days and the fact that only small quantities of agonists (5–20% receptor occupancy) are necessary for receptor activation, whereas antagonists have to compete with the native ligand and must occupy more than 50% of the receptor population to be effective[197,198]. More important, however, is that antagonism can be achieved by allosteric receptor interactions, where the larger surface area

of peptides may provide little advantage over competing small-molecule drugs. An exception is antagonists developed for targets that require drugs with a large surface area and structural complexity to achieve subtype selectivity and avoid off-target effects (for example, ion channels; BOX 3). Of note, receptor inactivation by agonist-mediated receptor desensitization leading to receptor downregulation has been successfully exploited by the class of GnRH superagonist peptide drugs.

Administration by injection and rapid clearance are often-cited weaknesses of peptide drugs; however, in many cases, this is beneficial as it allows fast onset of action and leads to a favourable side effect profile due to rapid clearance. Particularly in the treatment of diabetes, fast-acting drugs are desirable so that drug intake does not have to precede mealtimes by hours, giving people with diabetes more flexibility in their daily schedules. At the same time, insulin is cleared rapidly with a half-life of 4–6 min, providing little chance for side effects. Oxytocin, which is clinically used to induce labour, is another example where a short half-life is beneficial due to the ubiquitous presence of oxytocin and closely related vasopressin receptors that can cause complications during birth.

The three-dimensional structure of a peptide often displays evolutionarily conserved motifs, such as α-helices, β-sheets, β-turns and γ-turns that are crucial for receptor recognition, potency, selectivity and proteolytic stability[95,199]. Insulin, for example, possesses three helical regions stabilized by three disulfide bonds that align the residues important for activity[196]. Such structural and chiral complexity is often lost in small-molecule drugs, in part due to the controversial decision to abandon natural product discovery and move to combinatorial libraries in the 1980s[200]. One of the main reasons for this decision was the synthetic challenges of accessing the steric complexity of natural products in a time-effective and cost-effective manner. This is, however, not the case in peptide synthesis, where assembly of the intrinsically chiral building blocks through SPPS produces chirally and structurally complex peptides. The same synthetic method can be used to rapidly advance SAR and drug optimization studies, a task that is not so simple with complex chiral small molecules. It is this chiral complexity, large surface area and the protein-like structural features (often stabilized by disulfide bonds) that is the true power of this drug class, as these features result in the remarkable potency and selectivity that peptides are known for, and sometimes even metabolic stability.

Preferred therapeutic targets and indications for peptides. Most peptide drugs are used to treat endocrine, metabolic or cardiovascular disorders, or cancer, with metabolic disorders (particularly diabetes) and cancer accounting for the largest revenue (FIG. 2; TABLE 1). Most peptide drugs modulate peripheral extracellular targets, which is no surprise considering their difficulty in crossing cell membranes. We predict this trend to continue, with the ideal targets for peptide drugs being extracellular receptors with multiple functional subtypes[201]. For such receptors, it is difficult to develop small-molecule drugs, and therefore the better selectivity profile of peptides (driven by their larger surface area and chiral complexity) can be fully exploited. One example of such a complex receptor subtype system is the various ion channel subtypes involved in pain signalling, illustrated in BOX 3. An additional class of productive targets for peptide drugs is protein–protein interactions that are often classified as 'undruggable' due to the lack of binding pockets in either of the binding partners[202,203]. A surface area of 1,500–3,000 Å^2 must be covered for specific recognition, which is a viable task for peptides[202] but less so for small molecules.

Reaching intracellular targets, on the other hand, is still a daunting task for peptide drugs, although recent developments in cell-penetrating[187,191] and stapled[162–164] peptides are promising. Similar limitations hold for CNS targets, even though the commercial potential is attractive and the selectivity advantage of peptides is more pronounced considering the density and subtype diversity of receptors in the brain (many modulated by neuropeptides). We do not predict many new peptide drug approvals in this space until the emergence and clinical validation of a new delivery technology or a different route of administration that applies to a broad range of peptides. One potential delivery route that is being explored is intranasal delivery[204], which is strongly driven by the behavioural effects observed in human test participants given oxytocin intranasally[205,206]. Intranasally administered oxytocin had been marketed to facilitate lactation for breastfeeding mothers but it was withdrawn for commercial reasons. Initial approval, however, has enabled multiple human studies validating CNS effects induced by intranasally administered oxytocin, which is now being investigated as a potential treatment option for autism, migraine and bipolar disorders. How much oxytocin actually gets across the blood–brain barrier and the exact mechanism of action are still under debate[207,208]. A potential downside, however, is the uptake variability of intranasal administration, which could make it difficult for clinical trials to find dosing that is both effective and safe. Other emerging approaches and delivery technologies (for example, blood–brain barrier shuttles and the gut–brain axis) have been covered in recent reviews[209–214].

The fundamental role of peptides as molecular probes and diagnostic tools in pharmacological and neurological studies has led to several diagnostic agents and devices reaching the commercial market (Supplementary Table 2). Their main therapeutic application lies in oncology, where these peptides — often equipped with radiopharmaceuticals — are used for tumour detection and targeted radiotherapy[215]. Cancer, in general, has been a steady target for peptide drugs, since an average of one in five peptides entering the clinic may have application in cancer treatment. This is for a variety of reasons beyond the substantial unmet medical needs in the oncology area and the supportive regulatory pathways for new drug development to meet them. First, many peptide hormone receptors are upregulated in tumour cells, thereby forming ideal targets to differentiate them from healthy cells, and peptides (including imaging tags or drug cargo) can enter tumour cells via receptor-mediated internalization. Second, peptides can have extraordinary receptor subtype selectivity similar to that of monoclonal antibodies, but with superior tumour tissue penetration and less immunogenicity. In addition to their selectivity, peptides are also rapidly cleared, thereby further reducing the chance of off-target effects and toxicity. Third, some peptide hormones have the ability to reduce tumour growth and slow cancer progression. Finally, injection is widely accepted by patients with cancer. We therefore anticipate that this area will continue to expand, including in the field of peptide-based cancer vaccines, which is receiving considerable attention[216–219].

Another promising anatomical target that has received little attention (due to stability concerns) is the gastrointestinal tract, which is the most highly innervated organ after the brain. There are many therapeutically relevant gastrointestinal receptors as well as numerous peptides that are expressed and function in the hostile gastrointestinal environment (for example, defensins and guanylin). Additionally, disulfide-rich or

cyclic peptides (for example, cyclotides and sunflower trypsin inhibitor) can be grafted to display target specificity as well as prolonged stability in the gut[159]. The feasibility of this approach has been validated with orally administered linaclotide and plecanatide for the treatment of irritable bowel syndrome with constipation[220]. This approach should be of particular interest to the pharmaceutical community as it presents a shift to orally administered peptide drugs with a high therapeutic window (due to non-systemic exposure) without having to overcome long-standing oral bioavailability issues.

Finally, topical applications, particularly for antimicrobial, antiviral and anti-ageing peptides, are also expected to grow in the future[221]. Indeed, this may be the most promising route for antimicrobial peptides, which often fail in preclinical testing or in the discovery phase due to proteolytic degradation or toxicity in vivo[222]. Peptides are also becoming increasingly well known in the cosmetics industry, where they are marketed as anti-ageing agents. For example, a synthetic hexapeptide marketed under the name Argireline acts similarly to botulinum neurotoxins to reduce wrinkles[223]. Palmitoyl pentapeptide 4 (Matrixyl), launched in 2000, is a fragment of procollagen I and is thought to reduce wrinkles by stimulating extracellular matrix renewal in fibroblasts[224]; the palmitoyl chain improves delivery across skin. Copper peptide GHK-Cu is a naturally occurring tripeptide with high affinity for copper ions that has anti-inflammatory properties and stimulates collagen and glycosaminoglycan synthesis in skin fibroblasts[225].

Conclusions

The last 60 years has seen a steady approval of peptide therapeutics, a trend that we expect to accelerate, since biologics have removed the traditional assumption that a drug needs to be orally available to be successful. The peptide field has reached maturity in many aspects, including platforms that can reliably and rapidly produce structurally complex peptides of up to ~100 amino acids on a large scale, which enables efficient and cost-effective SAR and lead optimization studies. Emerging peptide drug discovery technologies provide a rational and powerful approach to develop potent and selective lead compounds on short timescales, and the integration of new and optimized delivery technologies with innovative chemical strategies will continue to contribute to the future growth of the peptide field.

Peptide therapeutics occupy a well-defined space in the pharmaceutical landscape, in which they can outperform small molecules and larger biologics. Understanding this space is crucial for long-term success, and decisions need to be made at an early stage as to whether a peptide lead should be moved forward into clinical trials or not, depending on the therapeutic target, its mechanism of action and how difficult it is to develop a small-molecule drug for the same target. Incorporation of internal/external peptide discovery and development programmes alongside existing small-molecule pipelines, which is currently not the norm, could be beneficial to companies, given the important role peptides play as molecular probes for bioassays as well as leads to complement and guide small-molecule drug development.

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