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1. Introduction

Protein-protein interactions form the basis of many cellular processes. Disruption or deregulation of these complex interactions is the main cause of a significant number of human ailments. Consequently, there is intense research effort to design inhibitors that target specific protein-protein interactions. This places intricate protein-protein interactions in the heart of the development for novel drug leads. The emergence of ‘omic’ technologies, namely genomics, transcriptomics and proteomics, has greatly accelerated our understanding of the protein-protein interaction networks leading to the discovery of a number of proteins and their interaction interface as potential drug targets.

The “druggable proteins” are targeted by commercially viable, and preferably orally bioavailable, therapeutics [1]. These drugs are usually small organic molecules that function as competitive or noncompetitive proteins inhibitors [2]. On the other hand, many “undruggable” proteins are important targets in various disease states. These proteins are considered undruggable because they lack a cavity for the small organic inhibitors to bind and they interact with their protein partners through extensive and flat surfaces. The use of protein based therapeutics expands the repertoire of “druggable proteins” by targeting those proteins that cannot be inhibited by the available small molecules [3]. Besides their improved specificity offered by their high compatibility with the target proteins, the major advantage of peptide therapeutics is their reduced immunogenicity and improved safety. On the other hand, low oral bioavailability, low protease/peptidase resistance, low cellular uptake, high rate of hepatic and renal clearance, high biodegradability and high flexibility are some limitations of peptides as therapeutics [4].

Peptide drugs take advantage of the highly specific and selective interaction between proteins. The peptide is usually based on the sequence of the binding region between the
two proteins. The linear sequences might originate from a loop within a structured domain, or from a disordered region in protein termini or between defined domains. In order to achieve desired efficacy, following delivery to the correct cellular compartment, the designed peptide needs to target the appropriate site and bind it. A perfect therapeutic agent is then a short protein sequence that will reach, bind and modulate the function of a target protein for the required amount of time and efficacy. These linear sequences are difficult to discover due to their short length and a tendency to reside in disordered regions in proteins. Increase in the available structural information on the protein – protein interactions has spurred the design of novel peptide therapeutics. Furthermore, it is now possible to screen and select high affinity peptides for these targets with the advent of peptide libraries and array techniques.

Peptide drugs may function by interacting with different targets such as proteins, lipids, nucleotides or metabolites. Particularly, there is significant research focused on antimicrobial peptides that target lipid cell membranes. This review focuses on the challenges and opportunities in the design and development of peptide based drugs that bind and inhibit some important protein targets.

2. Protein–peptide interactions

Diverse cellular events such as protein and vesicle trafficking, gene expression, DNA repair, control of the cytoskeleton and targeted protein degradation as well as signaling cascades are regulated through dynamic protein interactions [5-7]. Enhancing the efficacy of a peptide therapeutic addressing one of these processes is tightly bound to basic principles governing protein-peptide interactions. Despite their significance and estimated abundance, a large fraction of protein-peptide interactions lack detailed characterization and some questions of scientific and commercial interest remain: How does a peptide overcome the energetic cost involved in switching from an unstructured, flexible peptide to a rigid, well-defined bound structure? What is the recognition process for the binding event? What stabilizes these interactions? If a peptide binds to a protein, what is the spatial configuration and what is the strength of this interaction? If they don’t bind each other, can they be made to bind by modifications? The increasing number of resolved protein-peptide structures sheds light into the mechanistic details of binding.

2.1. Protein – Peptide structures in the PDB

The rational design of peptide drugs is stimulated by the availability of structural information on protein – protein complexes. Peptides derived from the binding region of an inhibitor protein usually serve as a starting point in the design of peptide inhibitors against the protein – protein interaction. Coordinate and distance information about the binding interface can be based on X-ray crystallography or NMR methods. Other experimental methods that identify interface residues include alanine scanning mutagenesis [8], chemical modification, mass spectrometry and phage display [9].
We have filtered the Protein Data Bank [10] for the keyword “peptide” in the structure description and with chain length between 5 to 35 amino acids and found a total of 1816 crystal structures and 307 structures determined by solution NMR in March 2012. Additionally, two electron microscopy structures of the *Escherichia coli* 70S ribosome in the presence of the leader peptide were also reported. A 2010 study clustered the complex structures determined by crystallography in the Pep-X database (http://pepx.switchlab.org) [11]. This database contains 505 nonredundant protein-peptide interface complexes. 14% of these complexes are with the Major Histocompatibility Complex, 12% of them are with thrombin and 8% are with alpha-ligand binding domain. Another nonredundant protein – peptide database of 103 structures was reported in 2010 by the Schueler-Furman group [12].

The peptide binding site is usually a large and shallow pocket on the protein surface and it does not change its conformation upon peptide binding. In addition, hydrogen bonds with the peptide backbone and interactions with hot spot residues provide the enthalpic contribution to protein – peptide recognition. The protein – peptide interface is enriched in Leu and Ile as well as aromatic residues. The protein – peptide interface was shown to resemble the core of the protein, with more hydrophobic residues than the protein surface and with the structural motifs found in protein folds [12, 13].

### 2.2. Protein interaction domains in peptide recognition

It has become apparent that a significant number of protein interactions are commonly formed between conserved protein recognition domains and short linear peptide motifs, often less than 10 amino acids in length [14-17]. Members of a given protein domain family usually recognize a consensus motif but they may recognize different variations of this motif and they may possess unique binding specificities [17-24].

Peptides can interact with globular protein domains in very diverse ways. These include binding of a peptide onto a protein domain by forming an additional beta-sheet, binding to clefts in extended beta or proline type II helical conformations or adoption of a helical conformation. For example, SH2 and phosphotyrosine-binding (PTB) domains recognize phosphotyrosine motifs [6, 25-27], while polyProline helices are recognized by SH3, WW and EVH1 domains [14, 28, 29] (Figure 1). 14-3-3 proteins, FHA and WD40 domains recognize phosphothreonine/serine-containing elements [30]; bromo and chromo domains recognize acetylated or methylated lysine [31, 32]; VHL proteins recognize hydroxyproline motifs [33]. On the other hand, short amino acid motifs at the carboxyl termini of target proteins, such as ion channels, are important for recognition by PDZ domains [34].

Design of peptide based inhibitors against proteins with such modules is hampered by the similarity between the recognized peptide sequences. However the structural information available clarifies many ambiguities regarding protein-peptide interactions. The specificity and selectivity of the protein modules in the cell suggest the presence of a mechanism whereby a selective peptide drug can be designed that interferes with the binding of protein domains to their respective partners.
3. Identification/development of peptide ligand drugs

Figure 2 illustrates the primary steps involved in the design and development of peptide ligand drugs. The initial step in peptide drug design is the identification of the protein target. This is usually a protein that is implicated in a disease state. If possible (and/or available), the interaction partners of the target protein are also determined. Information from structure-activity relationship studies is then used for rational design. Structural information of the protein – protein interface is fundamental for rational drug design. If there is no information about the interacting partner, combinatorial approaches, such as phage display, peptide arrays or peptide aptamers, should be used to screen tight binding peptide sequences. Rational design may follow combinatorial approaches to design a peptide sequence with improved specificity and higher affinity. Once a tight binding potential peptide sequence is identified, the peptide is usually modified to enhance stability, uptake and delivery. These may include alteration of amino acids to nonnatural amino acids, cyclization of the peptide or constraining the peptide so that it forms an alpha helix. This modified peptide is a peptidomimetic, which has the properties of the peptide with respect to binding mechanism but also has higher stability and uptake potential than a natural peptide ligand. After \textit{in vitro} tests of the modified peptide, \textit{in vivo} tests and clinical trials are performed. Peptide may undergo further modifications during these tests. The ones that pass clinical trials are then marketed.

3.1. Rational design

Increase in the availability of crystallographic structures of protein complexes has conveyed valuable information for rational drug design efforts [36, 37]. Given a known (or predicted) protein – protein complex structure, inhibitors that target the interface between the two...
proteins can interfere with this interaction. Design of peptides based on the interface has also been an area of intense research [38, 39].

Figure 2. Schematic diagram of drug design (Target protein is beta-lactamase (PDB code: 1zg4, [35]), photo of the patient in hospital by Randy Glasbergen (www.glasbergen.com, Copyright 2002).

Docking of small organic molecules to protein targets has shown good progress with the advent of docking, virtual screening and pharmacophore building algorithms [40]. However, the prediction of the complex structure between a peptide ligand and its protein partner is not easy due to the flexible nature of peptides. The solution structure and the bound structure of peptides are usually different, with the peptide adopting its bound conformation only in the presence of the protein [41]. In addition, protein – peptide docking studies are further complicated by the absence of a cavity for peptide binding, because protein – peptide interaction sites are usually shallow pockets on the surface [12]. Several algorithms have been proposed for protein – flexible peptide docking. Three recent algorithms are the molecular dynamics based Dynadock [42], the Monte Carlo based FlexPepDock [43] and PepCrawler, which uses the protein – protein interaction interface structure and the Rapidly-exploring Random Trees approach [39].

With the advent of high-throughput technologies, rational drug design led to the development of combinatorial chemistry to provide diverse libraries and arrays for drug discovery [44]. Combined with the screening of libraries and arrays against target proteins,
rational drug design is a powerful tool for discovering novel pharmacologically active small peptide leads. These drug leads can further be engineered for the development of future generations of novel therapeutics.

3.2. Peptide phage display

There are a number of display technologies (phage, ribosome, mRNA, bacterial, etc.) to select peptides for defined proteins targets. In this review, the discussion of display technologies will be restricted to phage display, the most widely utilized display method.

Phage display technique is based on displaying peptides on the surface of a bacteriophage by expressing the peptides as fusions to capsid proteins [45]. Using either lytic or filamentous phage or phagemid vectors, various phage-displayed libraries have been designed but the most common systems are based on filamentous phages in which peptides are fused to coat proteins. The choice of the coat protein is an important factor in modulating the display valency of the fusion protein on the phage particle which can vary between less than one and several thousand copies per virion on average [44]. The fact that a large number of virions occupy a small volume makes it possible to express billions of peptides on phage particles for constructing libraries of the required diversity. In such libraries, each phage displays a unique random peptide. In cases where the peptides are critically big to disrupt the integrity of the capsid at high copies, they can be constrained by cyclization through incorporating pairs of cysteine residues forming intramolecular disulfide bonds [44, 45]. Affinity purification is often used to screen phage displaying peptides of interest. Several rounds of screening might be necessary in order to isolate target specific binders. Finally, the tight binding peptides are identified by rapid sequence analysis [37, 45]. Unlike rational design, screening phage displayed libraries for bioactive ligands requires no prior knowledge of the target structure [44].

Mirror image phage display is an elegant approach to obtain peptide ligands in the D-conformation which are resistant to gut and serum proteases. In principle, the selection is carried out against a target protein synthesized in the D-amino acid configuration (the mirror image of the original target) using a phage library of peptides in the naturally occurring L-conformation. For reasons of symmetry, the mirror images of these phage-displayed peptides interact with the target protein of the natural handedness [46].

3.3. Peptide arrays

Systematically arranged peptides on a solid support, peptide arrays, show great promise in screening lead drugs [47]. Peptide arrays synthesized on cellulose membranes are very versatile and their preparation is very rapid and cost-effective [48]. Peptide arrays are primarily classified based on the method used for assembly of peptides on the surface of the solid support. The in situ peptide array has peptides directly synthesized on the solid surface. In contrast, spotting peptide array relies on immobilization of presynthesized peptides onto a suitably derivatized solid surface [47, 49].
The two techniques used *in situ* peptide synthesis are the photolithographic synthesis (light-directed parallel chemical synthesis) and the SPOT synthesis. The former approach, first reported by Fodor et al [50], uses photolabile protecting groups to simultaneously synthesize thousands of spots, each with a unique peptide sequence. Improvements to this work have been reported by McGall et al [51], Pellois et al [52] and Li et al [53, 54]. In the SPOT technique, first reported by Frank [55, 56], peptides are synthesized by sequential spotting of small volumes of activated amino acids to a porous membrane. Advances have made rapid synthesis of a large number of peptides possible [57]. The advantage of *in situ* technique is that it avoids conventional synthesis of each peptide sequence found on the array.

The spotting array technique is preferable when small numbers of peptides are needed in the array or when the peptides will be used to prepare large numbers of identical arrays. There are currently a variety of methods for slide derivatization and immobilization of peptides to the surface [49]. In any application, chemical surfaces should allow efficient immobilization using the appropriately chosen functional groups present in a peptide. Additionally, the protocol to introduce the functional group, the tag, to the peptide should not be tedious.

### 3.4. Peptide aptamers

Peptide aptamers are most commonly used as disrupters of protein–protein interactions *in vivo*. They are combinatorial protein molecules (Figure 3), which consist of a variable peptide loop attached at both ends to an inert, constant scaffold protein [58-60]. The scaffold should preferable be small, composed of a single chain, and with a highly stable structure [61]. The choice between different scaffolds such as thioredoxin A (TrxA) [62], staphylococcus nuclease [63], human stefin A [64], and green fluorescent protein [65] is made by taking into account the intended use of the peptide [59]. Scaffold structures restrict the conformation of the peptide such that the loop can only adopt a discrete shape from the conformational space available to it. The variable loop of the aptamer, as constrained on both ends, offers greater specificity and higher affinity for their target surfaces as compared to the free peptide [66]. The higher affinity is a result of lower entropic cost due to binding of a constrained peptide compared with the entropic cost of constraining the peptide upon binding.

![Figure 3. Peptide aptamer, a) unfolded form, b) folded form. Black region shows the scaffold protein and red loop shows the variable peptide.](image-url)
Once the scaffold has been chosen, the three basic principles followed in aptamer design are (i) generation of a pool of peptides commonly using combinatorial approaches, (ii) selection to find best candidates, and (iii) amplification by expression in bacterial cells, such as *Escherichia coli* [59].

4. Toward a peptide drug with better bioavailability and stability

Peptides are gaining increasing attention as drug leads over small molecule drugs featured by their high affinity and specificity to interact with their targets together with their low toxicity profiles [67]. Unfortunately, the major limitations encountered in stability and delivery, overshadow their remarkable success. To be competitive and profitable, the lead peptide, which is usually designed based on the protein – protein interface, needs to be improved for better cell membrane permeability and stability and ADME (Absorption, Distribution, Metabolism, Excretion) properties [4, 68, 69]. Initial attempts were mainly focused on the improvement of existing peptide leads but the need for peptides with better physicochemical properties and pharmacokinetic profiles eventually has given rise to the field of peptidomimetics, the development of small peptide like compounds with the ability to mimic the structure or action of the raw peptide. The engineering of the desired property carries the new small molecule beyond the capabilities of the raw peptide lead [70].

4.1. Improvement of half life and stability

Peptides can display half lives as short as a few minutes, which is usually too short deliver sufficient drug amounts to target tissues. Consequently, many peptide drugs with exciting pharmacological activities prove to be ineffective in vivo. The short half life, which renders the peptide ineffective, is primarily due to its fast renal clearance, connected to its hydrophilic property and small size, and its poor metabolic stability and biodegradability as a result of enzymatic degradation by proteolytic enzymes (proteases and peptidases) of the blood, liver, and kidney. Hence different strategies for targeted modifications of peptide drugs in order to prolong their plasma half lives are highly demanded to improve drugs’ pharmacokinetic profiles, [67, 71].

In performing a modification to a peptide drug to protect it from proteolytic cleavage, each peptide drug should be considered as a separate entity since, based on the sequence, each is a target for a different group of enzymes. This makes the detailed knowledge of proteases, their tissue localization and cleavage specificity very essential [71]. Only then can modifications on a particular drug be imposed to improve its susceptibility towards proteolytic enzymes targeting it. Protease resistance can be conferred by substituting the natural amino acids by unnatural amino acids (D-), an N-methyl-alpha-amino acid, or a beta-amino acid. The amide bond between two amino acids may be replaced. The N- or C-termini may be blocked or carbohydrate chains can be added. N-terminus may be esterified or pegylated. In addition, controlled release parenteral delivery, mucosal delivery and transdermal delivery have emerged as alternative strategies to oral delivery which exposes the peptide to stomach acid [4].
Constraining a peptide from an unstructured ensemble of many configurations to a fixed conformation serves two purposes: the conformational heterogeneity of the peptide in the unbound form is reduced, hence reducing the entropic cost associated with binding and more importantly the protease resistance of the peptide is enhanced [72]. Some example strategies of constraining the peptide are to “staple” or crosslink the peptide to assume an alpha helical shape [72] (Figure 4A), to cyclize the beta hairpin form [73] (Figure 4B), or to change the backbone such that it is nonrotatable [74]. Another similar modification mimics the structure of plant derived cyclotides, which contain a cyclic cysteine knot [75] (Figure 4C).

Figure 4. Examples of constrained peptides A) Cyclotide with three disulfide bridges shown in gold [76] 2k7g B) Cyclic beta-hairpin 2ns4 [77] C) Stapled peptide with the so-called staple, or hydrocarbon link shown in gold [78] 2yja

In many cases, modification of the peptide drug significantly increases enzymatic stability, but activity loss is always an issue. Therefore it is important that improved stability counterbalances activity loss. For this reason, co-administration of peptides with inhibitors of enzymes that target the peptide might be offered as an attractive alternative tool to chemical modifications to increase peptide stability [79-81].

4.2. Enhancing uptake and delivery

An important challenge in the design and development of peptide based drugs is their size and hydrophilic character, preventing their spontaneous uptake by the cell. For peptides which target membrane receptors, delivery to the target sites may be made possible with the application of liposomes or nano- and microparticles. On the other hand, in case of peptide drugs that target intracellular proteins, intracellular delivery through the biological membrane is crucial for their efficacy. Since poor uptake and limited delivery has been an important drawback hampering the acceptance of peptide drugs in the pharmaceutical market, different approaches have been proposed to address this problem.

Sustained delivery systems based on biodegradable polymers from renewable resources such as chitosan and its derivatives, from petroleum resources such as PLGA (poly-lactic-co-glycolic acid) or PGA (polyglycolide) or blends of these have been receiving increasing attention following the nanotechnological advances applicable to peptide delivery [82-84]. As an example, progress made in the use of chitosan in peptide delivery is detailed below.
The polysaccharide based chitosan is a nontoxic linear polymer composed of β-1,4 linked D-glucosamine derived from the deacetylation of the naturally occurring polymer chitin. The biodegradable, biocompatible, bioadhesive, and permeation enhancing properties have made chitosan and its derivatives, such as N-trimethyl chitosan, outstanding polymers for delivery [85]. In addition to nasal, pulmonary, transdermal, and parenteral delivery routes using chitosan-based nano- and microparticle carriers, chitosan coated particles or pegylated chitosan particles receive particular interest for the delivery of peptides [83, 86]. Use of chitosan-based nano- and microparticles for peptide antigens based on luteinizing hormone-releasing hormone [87], peptide hormone insulin [88], glutathione [89, 90], heparin [91], and calcitonin [92] are just a few examples on the application of chitosan in peptide delivery.

Liposomes, regarded as drug delivery vehicles, are also widely used as carriers of peptides. They also enhance the local availability of peptides, protecting them from proteolytic action. Liposomes are artificially prepared microscopic vesicles composed of a lipid bilayer. The therapeutic peptide is encapsulated inside the aqueous compartment surrounded by the lipid membrane. Various types of liposome formulations have been prepared with different dimensions, composition, surface charge and structure to induce specificity and cell targeting [93, 94]. Different liposome formulations were tested for the administration of peptides such as insulin [95], calcitonin [96] and vasoactive intestinal peptide (VIP) [97].

There is significant discussion regarding the use of cell-penetrating peptides (CPPs) as tools to carry peptides to desired targets [37]. CPPs are usually 10-30 amino acids long and harbor a hydrophobic and a basic region. The major advantage of CPPs over antimicrobial peptides, which simply target the lipid membrane, is that CPPs help carry cargo into the cell in an energy independent manner without disrupting the cell membrane, hence can target intracellular enzymes and machinery [98, 99]. Two CPPs that have been studied in detail are the TAT peptide (GRKKRRQRRRIPQ) [100, 101] and penetratin (RQIKIWFQNRHKWKK) [102, 103]. The mechanism of uptake has been proposed to be by endosomes at low concentrations [98]. Transient pore formation, and resulting direct penetration, was also proposed as a mechanism of uptake for Tat peptide at high concentrations [104]. In direct penetration, the membrane is transiently destabilized by the interaction of the basic residues (Arg) and the negatively charged components of the cell membrane. Another CPP, TP10 (also known as transportan, AGYLGLKLKALAAALAKKIL), lacks arginine residues and has been suggested to be delivered by the endocytotic pathway or by the interaction of the positively charged Lysine residues with the membrane [105, 106]. Structure activity relationship studies on another CPP, pVEC (LLIILRRRIRKQAHAHSLK) showed that mutation of arginines to alanine did not abolish uptake, but scrambling the sequence of the peptide or mutating the first five hydrophobic residues to alanine did [107]. Conjugation of peptide drugs to CPPs is particularly relevant in the treatment of diseases which require the relevant peptide to traverse the blood-brain barrier.
5. Therapeutical peptides, present and future

5.1. Current status of peptide drugs

Although the synthesis and clinical use of the first synthetic peptide, insulin, dates back to 1920s [108, 109], it had not been possible to consider peptides as potential drugs before the introduction in 1960s by the 1984 Nobel Chemistry Prize Laureate Bruce Merrifield, of solid phase peptide synthesis, which lowered the production costs and time [110, 111]. Currently, the pharmaceutical industry and its contract manufacturers express their willingness to go into larger scale production using both solid- and solution-phase strategies. Today, there are more than 50 peptide drugs that have been approved for clinical use and the increasing number of peptides entering clinical trials now supports the notion that peptide drugs have a long and secure future. The targeted therapeutic areas of the present peptides include but are not limited to oncology, metabolic, cardiovascular and infectious diseases, all of which represent important markets. Table 1 includes a list of some of the peptide drugs that have reached high global sales (Pechon et al. development trends for peptide therapeutics, peptide therapeutics foundation, 2010 report).

Currently, most of the peptide drugs are peptide hormones (such as insulin) or peptides that mimic hormones [4]. However, the number of peptide drugs that act as enzyme inhibitors [112] or as antimicrobial peptides [113] is increasing.

5.2. Protein targets for potential peptide drugs

New peptide drugs are currently under development for a variety of protein targets. Here we focus on three major disease states, namely HIV infection, cancer and Alzheimer’s disease and discuss some of the ongoing research toward the design and development of peptide drugs against these diseases.

Primary HIV infection starts through recognition of its envelope glycoproteins (gp120 and gp41) by the CD4 receptors and CCR5 (macrophage) or CXCR4 (T cell) co-receptors on its target. Upon entry, the envelope protein undergoes a major conformational change and juxtaposes the viral and host membranes. Finally, the viral genome integrates into the host genome. The envelope proteins is the site of primary infection, therefore fusion inhibitor peptides blocking their interaction with the protein targets in the host might be regarded as potential drugs [121]. With this motive, initial efforts of the early 1990s have eventually lead to the development of the first approved anti HIV-agent originally designated DP-178, later T-20 now FUZEON or Enfuviritide, which is a synthetic peptide based on the C-terminal heptad repeat region (C-HR) sequence of HIV-1 gp41 (Table 1) [120]. Although it is highly effective in vitro, its limited use due to difficulties encountered in its administration has shown that this drug in this from is not the ultimate solution for HIV treatment. Continued research, supported with structural studies, has shown C34, also derived from the C-HR sequence of gp41, can compete with gp41 [122, 123]. T21 and N36, derived from the N-terminal heptad repeat region (N-HR) were also reported to be potent inhibitors [124, 125]. Based on the fact that peptides derived from C-HR and N-HR
regions of gp41 may serve as potent inhibitors of HIV entry, intense research effort has been made for the rational design of different inhibitors based on these sequences. This also includes modifications such as incorporation of nonnatural and D-form amino acids (C34M3, [126]), synthesis of chimeric peptides (T1249, [123]) and even construction of fatty acid C-HR based conjugates (DP, [127]). For a detailed list, refer to the review by Naider and Anglister [121]. There is also continued research that focus on the sequences of CCR5 or CXCR4 receptors to design HIV-inhibitor peptides [128].

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Brand name</th>
<th>Target disease</th>
<th>Target protein/biological action</th>
<th>Sequence</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>glatiramer acetate</td>
<td>Copaxone, copolymer1</td>
<td>Multiple sclerosis</td>
<td>Unknown</td>
<td>Random mixture of Glu, Ala, Lys, Tyr</td>
<td>[114]</td>
</tr>
<tr>
<td>leuprolide acetate</td>
<td>Lupron</td>
<td>Prostate cancer, breast cancer</td>
<td>Binds gonadotropin-releasing hormone receptor</td>
<td>Pyr-HWSY-D-LLRP-NH2</td>
<td>[115]</td>
</tr>
<tr>
<td>goserelin acetate</td>
<td>Zoladex</td>
<td>Prostate cancer, breast cancer</td>
<td>luteinising-hormone releasing hormone analog</td>
<td>p-EHWSY-D(S(tBu)-LRP)-AzaGly-NH2,</td>
<td>[116]</td>
</tr>
<tr>
<td>octreotide acetate</td>
<td>Sandostatin</td>
<td>Acromegaly, carcinoid syndrome</td>
<td></td>
<td>H-D-F-[CFD-WKTC]-tholacetate</td>
<td>[117]</td>
</tr>
<tr>
<td>exenatide</td>
<td>Byetta</td>
<td>Type 2 diabetes mellitus</td>
<td>glucagon-like peptide 1 analog</td>
<td>HGEGFTSDL SK QMEEEAVRLFIE WLRKKLQDVH NF</td>
<td>[118]</td>
</tr>
<tr>
<td>teriparatide</td>
<td>Forteo</td>
<td>Osteoporosis</td>
<td></td>
<td>SYSEIQLMHNL GKHLSMERVE WLKKLQDVH NF</td>
<td>[119]</td>
</tr>
<tr>
<td>enfuvirtide</td>
<td>Fuzeon</td>
<td>HIV</td>
<td>Targets HIV-1 fusion machinery</td>
<td>Ac-YTLHSLIESQ QQLNQELLE LD KWASLWNW F-NH2</td>
<td>[120]</td>
</tr>
</tbody>
</table>

Table 1. Some peptide drugs that have reached high global sales
Despite current progress, there is continued challenge in developing anti-HIV peptides due to their rapid renal clearance, poor distribution, and susceptibility to peptidase degradation. Hence search for HIV fusion inhibitors has been extended to screening a wide range of different sources such as red algae Griffithsia. From the algal lectin Griffithsin, a small HIV-1 entry inhibitor of 18-residues, Grifonin-1, was derived and this peptide was found to bind the HIV surface glycoprotein gp120 and block its binding with host surface receptors [129].

Envelope glycoproteins and cell surface receptors do not constitute the only target to prevent HIV infections. The cell-surface expressed nucleolin, which is one of the major RNA binding proteins of the nucleolus and serves as a binding protein for different ligands including HIV, might be another possible target in HIV treatment. The pentameric pseudopeptide HB-19 was found to inhibit HIV infection by binding to the nucleolin and to block the attachment of virus particles to cells. Hence HB-19 represents a potential anti-HIV drug [130, 131]. Since nucleolin at the cell surface is also a binding site for a variety of ligands implicated in tumorigenesis and angiogenesis, its potential as an anticancer drug has also been evaluated [131].

Loss of apoptotic control has been implicated in many disease states ranging from cancer [132] to autoimmune diseases. Caspases, a group of proteases implicated in apoptosis and inflammatory response, are therefore an important drug target. The WEHD tetrapeptide was found to be an optimal peptide sequence for caspase inhibitors using positional scanning synthetic combinatorial library [133] and many variants of this sequence have been designed. These peptidomimetic lead compounds for caspase inhibition, such as Pralnacasan, VX-765, emricasan and NCX-1000, have been recently reviewed by MacKenzie et al. [134]. The structure of caspase-2 in complex with a pentapeptide [135] is shown in Figure 5.

Estrogen receptors have been a drug target in breast and endometrial cancers since they regulate reproduction, maintain bone density and are important for central nervous system function. Peptide inhibitors that mimic and compete with the leucine-rich pentapeptide motif (LXXLL, where X is any residue) of the ER coactivator are promising lead compounds in the design of selective peptide inhibitors [136]. One modification to these lead compounds is using a hydrocarbon link to stabilize them in the alpha-helix form, also known as a stapled peptide [78]. The structure of the estrogen receptor in complex with a staple peptide is shown in Figure 5.

Bcl-2 is another protein involved in apoptosis and it has been the target of many drug design efforts [3]. However, the shallow groove which interacts with its binding partners renders it “undruggable” and therefore peptide based drug design against Bcl-2 has emerged as a promising approach [137]. The structure of Bcl-xL in complex with a Bad peptide is shown in Figure 5.

The phosphotyrosine recognition domain, SH2, is a subunit of many kinases, which are the key players in important signal transduction events. In Src kinases, SH2 – kinase domain intramolecular interaction keeps the kinase in its downregulated form [138], while in Stat3 (signal transducer and activator of transcription 3), the SH2 domain serves as a binding site...
for downstream signaling [68]. Loss of kinase regulation or constitutive kinase activation has been implicated in cancer and autoimmune diseases. As such, SH2 is an important drug target and SH2 inhibitor design is an area of intense research. The Stat3 SH2 recognition sequence pYXXQ (where pY is the phosphotyrosine and X is any residue) was modified into a lead peptidomimetic, nanomolar affinity was attained [68]. The structure of Grb2 SH2 domain in complex with a pYXN-derivative [139] is shown in Figure 5.

The proline rich AMAP-1 protein interacts with the SH3 domain of cortactin and this complex formation is implicated in tumor invasion [140]. This interface is therefore a drug target in breast cancer invasion and metastasis. The proline rich domain of AMAP-1 was used to design a proline rich peptide [141], which was then made cell-permeable by the addition of the HIV Tat sequence [142].

![Figure 5](some of the protein targets discussed in this review and their interaction with their designed peptide ligands. Caspase (PDB code: 3r6l), estrogen receptor (2yja), Bcl-2 (1g5j) and Grb2 SH2 domain (3ov1) are shown in silver surface representation and the peptide ligand is red.)
The molecular origins of a number of neurodegenerative disorders such as Alzheimer’s, Huntington’s, Parkinson’s and Creutzfeldt-Jakob diseases have been associated with the aggregation of proteins [143, 144]. In particular, the pathological event in Alzheimer’s disease (AD) is the progressive accumulation of 42 residue β-amyloid peptides and resulting formation of insoluble β-amyloid fibrils [145]. Presently there is no cure for the treatment of AD but novel peptides that can inhibit and or reverse this abnormal conformational change are the subject of many recent reports. β-amyloid fibrillogenesis involves the conversion of α-helix/random coil to β-sheet motifs and proceeds via oligomeric and protofibrillar intermediates therefore it can be inhibited by destabilizing the β-sheet-rich β-amyloid intermediates, using β-sheet breaker peptides [146-149]. One example of a β-sheet breaker peptide, which comprises a short fragment of β-amyloid peptide (KLVFF; residues 16-20), can bind full-length β-amyloid peptide and prevent its assembly into amyloid fibrils [150]. The peptide LPFFD derived from another fragment of the β-amyloid peptide (LVFFA; residues 17-21) is another β-sheet breaker that abolishes fibril formation [146, 147]. Permanne et al further modified this peptide to extend the serum half-life and increase the blood-brain barrier permeability [151]. Rationally designed hybrid molecules composed of β-breaker elements combined with aromatic moieties (e.g. D-Trp-α-aminoisobutyric acid) have also emerged as promising leads [152]. Development of beta-sheet breaker peptides have also been reported toward diabetes 2, which is another amyloidogenic disease state [153].

6. Conclusion

As our understanding of complex biological networks increases, different proteins emerge as potential targets for peptide based drug development. Peptides are gaining increasing importance as drug leads over small molecule drugs featured by their high affinity and specificity to interact with the protein targets together with their low toxicity profiles. Many peptides that are currently administered do not meet the required criteria in cost, stability, long serum half-life and delivery. Nevertheless they serve as a source of inspiration for the development future generation peptide drugs. Evaluating the overall success, the increasing number of peptide leads going into clinical trials is a significant triumph for structural biology.

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7. References

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