Advancement in Development of Peptide Drugs

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**1.1 Abstract:** For the past two decades, protein-protein interactions (PPIs), which carry out a variety of essential physiological processes, have been important therapeutic targets. Since antibodies cannot permeate the cell membrane to access bigger or flat binding sites, it has been highly challenging to interfere intracellular PPIs with tiny compounds. Peptides have emerged as attractive possibilities for tackling difficult binding surfaces in recent years due to their reduced size and balance of conformational stiffness and flexibility. Deciphering and characterizing peptide–protein recognition mechanisms are thus central for the invention of peptide-based strategies to interfere with endogenous protein interactions, or improvement of the binding affinity and specificity of existing approaches. Importantly, a variety of computation-aided rational designs for peptide therapeutics have been developed, which aim to deliver comprehensive docking for peptide–protein interaction interfaces. Over 60 peptides have been approved and administrated globally in clinics. Despite this, advances in various docking models are only on the merge of making their contribution to peptide drug development. In this book chapter two things have been emphasised (i) a holistic overview of peptide drug development and the fundamental technologies utilized to date, and (ii) an updated review on key developments of computational modeling of peptide–protein interactions (PepPIs) with an aim to assist experimental biologists exploit suitable docking methods to advance peptide interfering strategies against PPIs.

**1.2 Introduction:**

Delivering drugs specifically to patient neoplasms is a major and ongoing clinical challenge.
Function-blocking monoclonal antibodies were first proposed as cancer therapies nearly four decades
ago. The large size of these molecules hindered their commercial development so that the first antibody or antibody-fragment therapies were only commercialized for cancer therapeutics and diagnostics 20 years later [1,2]. A classic development during this period, a radiolabelled peptide analog of somatostatin (SST) was used to target neuroendocrine tumors expressing the SST receptor instead of targeting the receptor with an antibody [3]. The concept of using a peptide as a targeting moiety for cancer diagnosis and treatment has since led to current peptide drug developments in both academia and pharmaceutical industries. Peptides that resemble natural peptide hormones provide therapeutic potential in addition to cancer treatments. For example, synthetic human insulin has long served as an example of therapeutic efficacy for diabetic patients [4]. Due to their unique biochemical and therapeutic properties, peptides do in fact form a distinct class of pharmacological substances when compared to small molecules like proteins and antibodies. Peptides have been created as therapeutic candidates to disrupt protein-protein interactions (PPIs) and target or inhibit intracellular molecules including receptor tyrosine kinases in addition to peptide-based natural hormone analogues [5,6]. PPIs, or protein-protein interactions, are the basis of virtually every biological operation.

These biochemical processes frequently consist of active receptors that control a number of enzymatic activities, such as ion transport, nucleic acid transcription, and numerous post-translational modifications of translated proteins, either directly or indirectly [7]. Medications that selectively bind to these receptors can either operate as agonists or antagonists, which has an impact on cellular activity later on. Since they have the ability to alter the protein interactions that lead to disease, peptides and small compounds that interfere with PPIs are in high demand as therapeutic agents in the pharmaceutical industry. Increasing data indicates that the key to their clinical success will be improved identification of targetable disease-associated PPIs and improvement of peptide medication binding properties [8]. Regrettably, both computational biologists and protein biochemists face a difficult problem in figuring out the molecular recognition mechanism and defining binding affinity for PPIs. This is largely due to the fact that tiny compounds are more effective at attaching to proteins' deep folding pockets than bigger, flat, hydrophobic binding surfaces, which are frequently seen at PPI complex interfaces [9]. Monoclonal antibodies are better at identifying such PPI interfaces, but they are unable to cross the cell membrane to find and detect intracellular targets. Peptides up to five times larger than small molecule medicines yet with balanced structural flexibility and binding affinity have garnered a lot of attention recently [10,11]. Examples of small molecule therapeutic features include lengthy in vivo stability, strong antibody-like binding affinity and low toxicity are seen in cyclic peptides [12]. Two parts of peptide drug development will be the emphasis of this book chapter: (i) fundamental technologies used in peptide drug development to date, and (ii) significant advancements in computational modelling methods for peptide-protein interactions (PepPIs). With the goal of assisting experimental biologists in utilising appropriate docking methods to enhance peptide interfering strategies against PPIs, recent topics and fundamentals in conventional docking of PPIs will also be presented.

1. **Critical Elements in the Creation of Bioactive Peptide Drugs:**
	1. **Background:**

Peptide medications have significantly changed the pharmaceutical industry since insulin, a 51 amino acid peptide, was first isolated and commercialised in the early 1920s [13]. The animal tissue-derived insulin product, which had been available for almost 90 years, has been superseded by human recombinant insulin thanks to advancements in DNA recombination and protein purification technology. More than 60 peptide medicines have been authorised globally in the last 20 years, with roughly 30 more peptide medicines having received approval. When the intended uses of these licenced peptide medications are broken down, it seems that the most frequently targeted illness groups are cancer and metabolic diseases. Peptide medicine sales were expected to surpass 70 billion USD in 2019 according to a global industry research on peptide treatments, which forecasted a compound annual growth rate (CAGR) of 9.1% from 2016 to 2024 [14]. Yet, the robust expansion of this industry is probably due to the anticipated rise in the prevalence of malignancies and metabolic diseases. Liraglutide (Victoza) and glucagon-like peptide 1 (GLP-1) are two of the top selling peptide medications for metabolic disorders, with combined sales of at least two billion USD annually. . Around four billion USD in sales were also attributed to well-known peptide medications including leuprolide (Lupron), gosarelin (Zoladex), and somatostatin analogues like octreotide and lanreotide.

* 1. **Addressing Peptide Medications' Internal Drawbacks:**

Contrary to synthetic peptide medications, natural polypeptides like hormones, growth factors, or neurotransmitters are known to play crucial functions in healthy physiology. Due to their in vivo instability and membrane impermeability, peptide medications have two significant limitations [15].

The blood proteolytic breakdown of peptide medicines decreases the bioavailable concentration and shortens the half-life of the drug. To keep the medicine at a concentration that is clinically effective, routine dosing can be required. The in vivo half-life of peptide medicines has been extended and proteolytic breakdown has been prevented using a variety of chemical modification techniques. The section that follows provides a guide to contemporary techniques frequently used to make peptides more resistant to proteolysis.

**2.3 Termini Protection:**

Up to 500 proteases and peptidases, including serum aminopeptidases and carboxypeptidases, have the potential to break down peptides at their N- and C-termini [16]. Different amino acid residues at the N- or C-terminal will result in variable degrees of proteolysis and breakdown, as has been well-documented. For instance, peptides rich in Pro, Glu, Ser, and Thr are more sensitive to plasma breakdown than peptides rich in Met, Ser, Ala, Thr, Val, and Gly [17]. If the N- or C-terminal sequences can be changed while retaining the necessary targeting specificity and affinity, the proteolytic degradation can be reduced and the bioavailability increased [18]. Similarly, C-terminal amidation or N-terminal acetylation can also be employed to improve in vivo stability as long as such alterations allow the medicine to operate properly [19]. Alteration using synthetic amino acid analogues could achieve the same result.

**2.4 Finding Important Residues Using Non-Chemical Techniques:**

For biologists, selecting a chemical alteration frequently necessitates working with chemists who have chemistry knowledge. However, there are a few techniques that are simple to use while still being crucial for the biological study of peptide drug design. The first step is to determine the minimal required amino acid residue(s) for peptide activity. This can be done by repeatedly trimming amino acids off a lead sequence's N- or C-terminus in order to identify the essential core peptide motif required for biological activity. Second, the contribution of each individual amino acid to the biological activity of the peptide can be assessed using a traditional screening technique termed alanine scanning [20]. Critical amino acids can be found by examining the biological functioning of a library of peptides in which specific amino acids have been replaced with alanine. Since its short, neutral side chain doesn't affect the operation of nearby side chains, alanine is utilised in place of it [21]. More contemporary scanning methods have been developed that take into account the enantiomers of amino acids as well as additional physical properties as acidity, basicity, and hydrophobicity. For the mature creation of enhanced biological activity, these scanning techniques still need to be validated by molecular biology and in silica methodologies including mutagenesis, stability, and pharmacokinetic (PK) tests. These structure-activity relationship (SAR) research will help identify the amino acids in a peptide sequence that are proteolytically labile.

**2.5 Backbone modification and synthetic amino acid substitution:**

The aforementioned amino acid scanning approaches offer helpful information for the creation of additional alterations, particularly on the side chain group of a specific residue. Although their stereochemically reversed side-chains are not recognised as protease substrates, synthetic enantiomer amino acids, for example, have been proposed to boost protease resistance [23]. Particularly, homoarginine, lysine, or ornithine can all be used as excellent substitutes for arginine [24]. The stiffness and shape of the peptide can be changed by effectively substituting one or more close analogues of each natural amino acid on the crucial sites. In order to increase proteolytic resistance, synthetic analogues of aromatic amino acids can be utilised in place of the heterocycles' -methyl groups. Activating the GLP-1, glucose-dependent insulinotropic polypeptide (GIP), and glucagon receptors all at once was achieved recently in a preclinical success using side-chain modification of a momomeric helical peptide. This triagonist peptide dramatically lowered body weight and diabetes consequences in a mouse obesity model without causing cross-reactivity at other receptors [26].

The biological function of the original L-peptide can be compromised by the structural changes caused by enantiomer amino acid (D-amino acid) substitution, despite the fact that it has been a frequent strategy to protect peptides from protease breakdown [27]. Together with D-amino acids, -methylation and N-methylation have also been applied frequently. While N-methylation has been shown to improve solubility and decrease unwanted polymerization, -methylation of amino acids has the advantage of retaining the side-chain at its original spatial orientation, which is important for helical peptides. Such side-chain functionality-modifying techniques have led to the evolution of peptide secondary structures and the production of novel peptidic compounds known as peptidomimetics. These reviews [29-33] provide additional details on the chemistry and uses of -/-D-amino acids, -/-N-methylations, or backbone-modified semicarbazide-peptides, peptoids, and peptidomimetics. Peptide cyclization can also increase peptides' protease resistance. There are several methods for producing cyclized peptides. One of them creates a peptide link between the original N- and C- termini through head-to-tail cyclization. When the amino group on lysine side chains reacts with the free C-terminus, aspartic or glutamic acid side chains, or both, an amide bond is created. As an alternative, the side chains of two cysteine pairs can react to produce a disulfide bond. These tactics can keep peptides in their bioactive shape by protecting their termini and limiting their structural flexibility [34]. For helical peptides, cyclization between side-chains has been shown to be particularly successful in enhancing conformational stability. One recent breakthrough is the cyclized peptide medicine ATSP-7041 [35]. The specific binding and inhibition of MDM2/MDMX by this side-chain cyclized -helical (stapled) peptide activates p53-dependent tumour suppression [6]. Such PPI-targeting techniques have enormous therapeutic potential because, despite the abundance of knowledge on disease-related PPIs in the literature, peptide-based inhibitors have only recently begun to reach their full potential.

**2.5 Computational Techniques to Increase Membrane Permeability and Aqueous Solubility:**

Peptides' limited capacity to penetrate cell membranes has prevented them from being used against inaccessible intracellular targets. Because to this restriction, the development of peptide therapeutics has mostly concentrated on extracellular targets. Successful peptide-based targeting of intracellular PPIs will depend on increasing membrane permeability or creating techniques that promote active intracellular absorption. Modulating the hydrophobicity and electrostatic charges to enhance passive uptake is one possible tactic, as is conjugating the active drug peptide to a cell-penetrating peptide (CPP) to promote its active transport. Because peptide biotherapeutics are more water soluble, their bioavailability is typically significantly increased because effective serum concentrations may be easily sustained. To preserve bioactivity while modulating the pI, it is possible to substitute unneeded hydrophobic amino acids with charged or polar residues, which optimises aqueous solubility mostly through experimentation [36,37]. Two SVM machine learning bioinformatic tools have recently been created to speed up this procedure [38]. In addition to providing a proteome-wide prediction, ccSOL omics allows for the discovery of soluble motifs within any given amino acid sequence [39]. Another SVM learning-based online tool, PROSO II, predicts solubility based on the main sequence's physiochemical characteristics, such as its degree of hydrophobicity and hydrophilicity, and its propensities for secondary structural forms like coil, helix, or sheet [40].

**2.6 Internal Peptide Uptake Facilitated by Membrane Proteins:**

A superfamily of transmembrane receptors called G-protein coupled receptors (GPCRs) is in charge of moving various chemicals across membranes. Although while peptides can act as GPCR ligands in their native state, very few extracellular peptides actively cross the plasma membrane. These peptides are now known as cell permeable peptides since they can pass through cell membranes (CPPs). They typically range in length from five to thirty amino acids and are very hydrophobic [41].

Research into CPPs has been vigorous with the ultimate goal of producing peptide medicines that are cell-permeable and orally accessible [42]. The molecular and structural mechanisms underpinning CPP intracellular transport remain unknown. Significant advancements in biotherapeutic peptides have been made possible thanks to CPPs' capacity to penetrate the membrane's lipid bilayer.

Antimicrobial peptides (AMPs), for instance, have been able to penetrate cell membranes via regulating immune responses due to their highly amphipathic and cationic properties [43]. In order to transport cargos such tiny molecules, peptides, proteins, or antibodies that would otherwise be membrane-impermeable, CPPs have also been used as targeting moieties by conjugation [44]. The successful cytosolic transport of normally membrane-impermeable peptides to target PPIs was shown to be facilitated by covalent attachment of an HIV TAT peptide or, more recently, an amphipathic cyclic peptide [45]. Several potent bioinformatic tools are available that enable users to forecast and improve their experimental designs for CPPs. Using machine learning-based models, CPPpred web servers like CPPpred-RF and KELM-CPPpred enable the prediction and construction of CPPs from a query input protein sequence [46–48]. Physiochemical characteristics including hydrophobicity, amphipathicity, steric hindrance, charge, and molecular weight are used by CellPPD, another free website, to predict permeability [49,50]. Although physiochemical analyses are lacking, CPPpred-RF and KELM-CPPpred use specific databases to predict CPP uptake efficiency and robust CPP/non-CPP, respectively. Currently, 1855 distinct empirically certified CPPs with their secondary and tertiary structures can be found in the repository CPPsite 2.0. This offers a useful tool to help web-lab researchers create more effective CPPs before labor- and time-intensive experiments [51]. Several online tools for peptide solubility analysis, prediction, and CPP design are summarised in Table 1.

**Table 1:** Overview of prediction methods for peptide solubility and cell penetrating peptides [52]



As was already said, cyclic peptides outperform linear peptides in terms of structural stability and proteolytic resistance. The development of cell-permeable cyclic peptide medicines to disrupt PPIs has received a great deal of attention. To enable their intracellular uptake, short CPP motifs have been strategically coupled to cyclic peptides that are normally cell impermeable. The development of bicyclic peptide medicines with one membrane-crossing CPP moiety and one cyclic peptide PPI inhibitor has used this delivery method more extensively while maintaining target selectivity and affinity [53]. A bicyclic peptide inhibitor dramatically hindered MEK/ATK signalling and caused apoptosis in lung cancer cells by blocking the oncogenic Ras-Raf connection [54]. Although CPPs by themselves are not immunogenic, CPPs that have been conjugated with bioactive peptides might occasionally cause an immune reaction, which may limit their ability to be used against specific targets [55].

**2.7 High-Throughput Screening (HTS) for New Peptide Leads:**

In reality, optimisation of hits from a screen of 5.7 million bicyclic peptides for interaction with oncogenic K-RasG12V led to the identification of the Ras-Raf bicyclic peptide inhibitor.

The quick identification of PPI inhibitors has been made possible by high content combinatorial library screening, yet peptides with less potent inhibitory action might not be picked up as well. While sequence modification or cyclization may be able to significantly increase affinity, such minor interactions shouldn't necessarily be discounted. In phage display library screening, the subsequent rounds of "biopanning" enrichment can enhance the detection of weaker connections. In fact, the Nobel Prize for Chemistry was recently given in recognition of the significance of this method during the past three decades [56,57]. Phage display and recombinant DNA technologies have made it easier to find and improve novel lead peptides throughout time that are effective against a variety of biological targets.

In the original method, affinity enrichment and expansion cycles were performed in succession before enriched phages were identified. The high number of biopanning rounds required can lead to selection bias, dropouts, and enrichment of false positives [58], despite making it easier to detect weaker interactions. The recent use of next generation sequencing (NGS) analysis of phase display experiments has greatly decreased these problems. To reduce the bias brought on by multi-cycle screening, NGS is quantitative and sensitive enough to reduce the number of biopanning cycles required to find enhanced interactions. Nonetheless, the low cycle number necessitates that interactions bind quite firmly [58]. Phage-displayed libraries have hitherto been confined by the requirement to use only linear display of naturally occurring, unmodified amino acids. The inclusion of chemical entities like cyclization linkers, fluorophores, small compounds, or post-translational modifications like glycosylation, as well as other ways for on-phage chemical alterations, have lately helped to overcome this constraint. [60,61]. These developments in contemporary biopanning methods lend credence to the idea that lead peptides with higher affinity and real bioactivity might be found and then rationally optimised in terms of sequence and alterations for use in clinical trials.

**3. Peptides and Protein–Protein Interactions:**

Because that deregulated protein interaction networks underlie a wide range of diseases, PPIs are well-known prospective therapeutic targets. There are thought to be at least 140,000 pairwise PPIs in the human interactome [62]. Pathogenic PPIs have been the target of numerous attempts to modify downstream signalling events via peptide innovations. With such modulation the huge area of most bigger PPI interfaces (about 1500–3000 Å2), compared to the tiny molecules' binding pocket size (300–1000 Å2), has made it challenging to bind small molecules, however [63]. The majority of the time, small compounds do not bind to target proteins over an area sufficient to block the interaction surface [64]. As was already said, peptides are considerably better candidates for PPI inhibition than small compounds due to their unique physiochemical properties, particularly their long and flexible backbones.

Interfering peptides (IPs), which can attach to the deeper grooves or clefts on an interacting face and obstruct that surface, are peptides that interfere with PPIs. The existence of amino acid residues that can interact with other residues at protein-protein interfaces gives IPs a significant advantage over small compounds in terms of targeting PPIs [9]. IPs as biotherapeutics are gaining more attention thanks to recent developments in methods to address the inherent drawbacks of peptide medicines, such as their poor stability, solubility, and bioavailability. In this section, we looked at some encouraging developments in IP creation versus PPIs as well as typical methods for validating and improving IPs as efficient biotherapeutics.

**3.1 Positive Advances in Interfering Peptides:**

The basis of both healthy and pathological cell biology and physiology are protein-protein interactions. A number of diseases, such as infection, long-term inflammation, neurodegeneration, cancer, and cardiovascular disease, among others, are fueled by abnormal protein-protein interactions.

As a result, protein interaction surfaces provide for intriguing therapeutic targets, and as was previously said, peptides excel over small compounds in this regard. Clinical research is currently being done on several potential IPs. A 28-mer peptide medication that prevents the ubiquitin-ligase MDM2 from binding to its target p53 can stabilise p53 and reduce tumour growth by preventing MDM2-dependent p53 ubiquitination [65,66]. By preventing the interaction between CXCR4 and its ligand CXCL12, the 17-mer peptide medication CTCE-9908 is able to prevent CXCR4 activation in tumour cells. A phase I trial is being conducted with CTCE-9908 [67,68]. In order to inhibit JNK-driven inflammation, a peptide medication (XG-102, Brimapitide) based on the N-terminal c-Jun sequence competes with natural c-Jun for interaction with JNK. A phase III trial for brimapitide is ongoing [69, 70]. Due to their high stability and protease resistance, IPs with -helical structures that bind to protein interacting surfaces have demonstrated particularly promising interaction-blocking efficacy. [71]. EZH2/PRC2, MDM2/p53, -catenin/Wnt, and Bax/Bcl-xL interactions are only a few of the oncogenic protein interactions that -helical peptides have been shown to effectively target in the literature. These structurally altered peptide medications, also known as peptimimetics, are intended to disrupt the broad and flat surfaces of the targets to which they are directed. These instances show the therapeutic potential of peptide medicines to specifically alter pathogenic protein interactions. [72,73]

**3.2 PPI Determination Using Experimental and Computational Techniques:**

A variety of biophysical methods, including X-ray crystallography, NMR spectroscopy, surface plasma resonance, bio-layer interferometry, isothermal titration calorimetry, radio-ligand binding, spectrophotometric assays, and fluorescence spectroscopy, have been used to experimentally determine protein-protein interactions. Our understanding of how secondary and tertiary protein structure and interaction kinetics affect downstream biological events has improved as a result of the experimental data produced by these techniques. These methods are used to research one particular PPI at a time, however they are frequently time-consuming. While protein crystal X-ray diffraction is unquestionably a very effective structural analysis technique that can define structure down to the level of individual atoms, it faces significant technical difficulties. Many proteins either don't crystallise well or only as tiny protein domains.

Even if each protein in a complex crystallises on its own, co-crystallization can be particularly difficult. Although NMR spectroscopy may produce complicated protein structures, it has a lower resolution than X-ray diffraction. While optical or calorimetric methods can offer details about an interaction's energy, affinity, and disassociation characteristics, they cannot, like NMR or X-ray diffraction, pinpoint a specific interaction surface. Wet-lab experimental procedures' technical difficulties and inadequate scalability have made the development of dependable computational methods necessary. In order to speed up the process of producing precise predictions of protein structure, surface charge, and contact affinities, computational docking approaches have been created.

**3.2.1 Computational Docking Techniques:**

Since some docking approaches can be completed in the order of minutes, computational PPI docking has quickly and effectively supplied information for drug development at the atomic level. This is possible with rigid-body docking techniques, which optimise the chemical and geometric orientation fit by treating two interacting proteins as being perfectly rigid in the calculation. When suitable scoring scaffolds are offered, the rigid-body protein docking tool Z-DOCK typically produces accurate predictions of PPI [74]. The availability and complexity of multiple scoring parameters from the most flexible docking methods have led to the development of a wide range of different docking programmes throughout the years. For example, ATTRACT is a well-known PPI prediction service with robust toolkits that cover a variety of scoring factors, but it is less user-friendly [75].

**3.2.2 Sequence- or structural-based predictions:**

In order to calculate the binding free energies and produce more precise predictions on the binding affinities between the interacting proteins, a number of computational docking strategies, particularly flexible-body docking methods, require structural information, such as the number of hydrogen bonds, buried surface area, mutation hotspots, geometric angles, and allosteric effects [76]. In contrast, sequence-based techniques provide estimates of binding affinity based on the sequence and functional data in several publically accessible databases. By categorising protein-protein complexes according to their biological roles and the proportion of binding residues, PPA-Pred, for instance, created a model based on sequence features to predict binding affinities [77]. Sequence-based models can be improved with dataset updates in experimental and functional scaffolds, albeit providing less certain predictions on binding affinity and the inability to anticipate conformational binding poses. In fact, learning machines are also used by sequence-based techniques to increase their prediction confidence over time [78]. Despite considerable advancements in both scoring systems from the two methodologies, the field has not made as much progress as it may have due to a lack of high computational power and high-quality, larger experimental datasets. The best-performing servers were ranked based on prediction accuracy in the CAPRI community experiment, which compared computationally predicted protein complex structures with experimentally proven structures [79]. Based on root mean square deviation (RMSD), HADDOCK and ClusPro are ranked as the top prediction servers for rigid-body docking algorithms that deliver binding free energy and buried surface area with the highest degree of confidence [80-82].

**4. Advances in Peptide-Protein Interactions and Computational Techniques:**

Similar to protein-protein interactions, PepPI prediction accuracy has frequently been constrained by the structural information (either a single target protein structure or complex structure with ligand) that is available for a pharmacological target. Although protein co-structures are rare, several research use data from structural databases like the Protein Data Bank (PDB) to determine sequence-binding motifs for peptide designs. [83]. Another database, PepX, has high-resolution structures for more than 500 empirically studied peptide interactions and simple inputs for user-defined peptide templates [84]. 6-11 amino acid long peptides often comprise 2-3 residues that make crucial contacts with the target protein, according to silica mutation hotspot investigations of protein-peptide interfaces. PepPI analyses can be quite complex because of the various structural changes that could result from flexible side-chains and backbones inside a peptide, despite their apparent similarity to modelling protein-protein interactions [85,86]. Longer peptides, with more than 15 residues, typically form more complex -sheet or -helix structures, making it more challenging to anticipate their structures. If the flexibility of the target protein conformation is taken into account, the complexity of peptide structure prediction further rises [87]. This section will cover recent computational models that have been created to address these issues and enable the development of more effective peptide medication designs against PPIs. Table 2 offers an overview of selected PepPI prediction algorithms and concise explanations of their important characteristics mentioned in this section.

**Table 2:** Summary for peptide-protein interactions docking method [52]



Table 2 continued.

# RMSD of experimental structural data to peptide backbone. Medium: 2 to 5 angstroms; Near-native: 1 to 2 angstroms; Sub-angstrom: less than 1 angstrom. PeptiDB dataset was tested. 405 known protein-peptide complexes with unbound receptor model in a customised dataset. On specific PeptiDB subsets.

**4.1 Selection of Initial Peptide Scaffolds:**

We first want to explain recent developments in the selection of initial peptide scaffolds, which also play crucial roles in the development of peptide drugs, before going into current computational methods for PepPIs. From natural proteins, a number of well-characterized naturally occurring peptides had been chosen, and it had been shown that they retained their original activities, such as structural scaffolds or the capacity to recognise target molecules. For instance, repeated Arg-Gly-Asp (RGD) motifs were originally discovered. We first want to explain recent developments in the selection of initial peptide scaffolds, which also play crucial roles in the development of peptide drugs, before going into current computational methods for PepPIs. From natural proteins, a number of well-characterized naturally occurring peptides had been chosen, and it had been shown that they retained their original activities, such as structural scaffolds or the capacity to recognise target molecules. For example, repeated Arg-Gly-Asp (RGD) motifs were initially derived from the fibronectin cell attachment domain, which binds to receptor proteins that are membrane-bound and triggers cellular growth, differentiation, adhesion, and migration [88]. A capacity for RGD peptides' ability to imitate the actions of their parent protein has made them an attractive tool for structural and functional investigations of proteins as well as therapeutic PPI interferences. The discovery of microtubule-binding peptides is another intriguing advance. Microtubules are hollow tubular protein assemblies made up of intracellular -/- tubulin dimers. They have important implications for nanodevices since they play a role in a variety of eukaryotic cell processes, including the development of tumours. Widespread interest has been shown in peptide-modulated nanodevice-encapsulating medicines that target intracellular tubulins in a variety of formulations, including peptide-conjugating liposomes or peptide-drug assemblies to exert synergistic anti-cancer effects [89,90].

By encasing gold nanoparticles inside microtubules, a recent groundbreaking study further proved that peptides chosen from the microtubule-associated protein Tau functionalized the inner surface of the microtubule [91]. Moreover, a tetrapeptide Ser-Leu-Arg-Pro (SLRP), another exciting finding from a peptide library, was demonstrated to disrupt microtubule activity and induce apoptosis in cancer cells [92]. It should be noted that the computed docking method Autodock Vina aided in the choosing of SLRP.

**4.2. Docking Peptide–Protein Interactions:**

The quantity of structural scaffolds provided regarding the interaction complex has been a key factor in successful docking of a PepPI's structural posture. The development of more potent docking and refinement algorithms for predicting precise PepPIs has been considerably aided by the dramatic growth in the number of peptide-protein structures made readily available in PDB. Depending on how much structural information is provided as inputs, local or global docking techniques for peptide-protein interactions are typically distinguished.

**4.3 Methods of Local and Global Docking:**

The approach that is most frequently employed to find a potential binding posture for a peptide at a user-defined binding site in a resolved structure of its target receptor is known as local docking. Many techniques can enhance the quality of the original model at atomic resolution and within 1-2 RMSD of the experimental peptide conformation. The most well-known techniques for determining peptide-binding sites include DynaRock, Rosetta FlexPepDock, and PepCrawler. For the purposes of determining receptor side-chain flexibility and conformational sampling, DynaDock uses soft-core potential in conjunction with molecular dynamics [93].

Faster conformational sampling of the peptide-protein complex was accomplished when the soft-core potential eventually converged to a physical potential as the simulation progressed because van der Waals and Coulomb energy potentials were smoothened in this protocol. A Monte Carlo-based technique called Rosetta FlexPepDock simplifies optimisation stages to produce high-quality conformational sampling for hotspot residue-containing binding motifs that have been thoroughly studied [94,95]. Rigid-body sample docking and varying levels of backbone modelling were used to test this procedure against a sizable dataset. Rapidly-exploring Random Tree (RRT), an algorithmic robotics motion planning technique, is used by PepCrawler to improve peptide structural poses at binding locations [96]. By using local shape analysis of the energy funnel to automatically cluster the resultant models, this refinement procedure constructs a conformation tree for the peptide-protein complex. Nevertheless, information on backbone conformation is not readily available for every query peptide. Prior to executing local docking, sampling techniques that enable acquisition of almost native peptide shape become crucial. For instance, the Rosetta FlexPepDock ab initio procedure places the query peptide into a user-defined binding site from any arbitrary backbone conformation, combining ab initio peptide folding with local docking [97]. A hotspot residue with a side chain can be positioned to designate the binding site, or Rosetta FlexPepDock's usual constraints for binding sites can be used. Recently, the HADDOCK approach (HADDOCK peptide docking) was employed to suggest that secondary structure might be used to localise docking without the need for prior backbone information: an ensemble of canonical conformations confined to a specific binding site, such as an extended or polyproline-II helix. [98] Moreover, local docking for short peptides with less than five amino acids has been carried out using a variety of small molecule docking techniques, including Gold, Surflex, and AutoDock Vina [99-101]. Although the findings of the near-native modelling were not ideal, an intriguing docking approach called DINC 2.0 was presented to get around the problem by docking peptide fragments [102].

**4.2.2. Global Docking Methods:**

In contrast to local docking, which just searches for the peptide-binding posture, global docking approaches also search for the peptide-binding site at the target protein. As binding locations are unknown beforehand, global docking is typically the preferred method. Using a spatial position specific scoring matrix (PSSM), the PepSite technique was developed to find potential binding sites with an estimated position for each residue [103,104]. Nonetheless, because of the different levels of peptide backbone/side-chain flexibility, flexible-body docking is particularly unsuccessful. Hence, rigid-body docking is frequently used in general peptide-protein docking protocols after input peptide conformation has been acquired. From a given query sequence, a number of global docking techniques can predict the conformation of the peptide. For threading query sequences, programmes like ClusPro (ClusPro PeptiDock) and ATTRACT (pepATTRACT) use a pre-defined motif set of template conformations. In one simulation round, the resulting peptide conformations are then rigid-body docked [105,106]. Other global docking techniques, including PeptiMap, AnchorDock, and CABS-Dock, also offer automatic simulation of docking with a variety of algorithms, including small molecule binding adaptation, in-solvent simulation, flexibility of the query peptide or target protein at predicted binding proximity [107-109]. Other recently developed methods, such as HPEPDOCK, used an ensemble of peptide conformations for blind global docking and achieved noticeably higher success rates as well as shorter simulation times than pepATTRACT [106,110]. These results are in addition to the highly accurate predictions made by PIPER-FlexPepDock.

**4.2.3 Docking Technique Based on Templates:**

Comparative docking strategies are another name for template-based docking techniques. They construct a model of the interaction complex by weaving the sequence of the query peptide and/or target protein through template scaffolds that are known to exist [79]. Due to the sudden growth in the quantity of peptide-protein structures deposited in PBD, which have significantly sped up developments and designs in simulation algorithms, template-based docking has lately been recognised as a new category in peptide-protein docking. A well-known server called GalaxyPepDock carries out similarity-based docking by looking for templates with the highest levels of similarity and developing models utilising energy optimisation to enable more precise predictions on structural flexibility between interacting complexes [111]. During CAPRI blind prediction studies, GalaxyPepDock showed better prediction outcomes than other servers employing PeptiDB datasets. PBRpredict, a different template- and machine-learning-based docking technique, used models trained from peptide-binding residues of various types of domains to construct models that accurately predict interaction residues in peptide-binding domains from target protein sequences [113]. The optimisation of grouping and scoring in techniques for predicting PepPIs frequently makes use of computational machine learning algorithms, which are similar to the prediction servers for CPP. PepComposer, a widely used online tool for peptide-protein computational design, included a machine learning technique (Monte Carlo) for a fully automatic computational peptide design that was shown to predict well-known PepPIs at highly repeatable rates [114].

**5. Conclusion**

The popularity of peptides has increased, and in recent years, there have been more authorised peptide biotherapeutics. Due to their greater interfacial pocket capacity compared to small molecules, this strategy has proven to be appealing. It has also been made possible by significant advancements in computational structural prediction and the expansion of available chemical modifications to enhance stability, affinity, and specificity. The use of publicly accessible computational binding prediction tools has resulted in more potent novel peptide medication designs that are effective and logical. In a recent work, we developed a cancer-specific targeting peptide with dramatically improved in vitro, in vivo, and therapeutic efficacy [115] using both biological and computational approaches. Major difficulties persist despite recent improvements in the computational modelling of protein-protein and peptide-protein structures. For instance, it is still difficult to forecast the bound structure while simultaneously taking into account the peptide's target protein's side-chain and backbone flexibility. Second, it is very challenging to incorporate experimental data from cryo-electromicroscopy, small-angle X-ray scattering (SAXS), and high-resolution NMR spectroscopy into computational prediction software because it is frequently unclear how to interpret the data in order to obtain accurate experimental structures. It has been explored by computational servers to convert confusing experimental data into algorithmic constraints that can be used as a docking option [80,98]. In reality, experimental biologists find that these docking techniques are quite useful for confirming the suggested binding mechanism. Finally, scoring had also been very difficult because many models with lower rankings were discovered to have higher quality docking results, and vice versa. It was claimed that the majority of scoring systems relied entirely on binding energy for grouping. Recent CAPRI experiments have shown that a hybrid model selection methodology that combines energy-based scoring with additional techniques like mutagenesis, co-evolutionary information, sequence- or structural-clustering function can produce accurate peptide-protein docking results that are more resemblant of native models [79,81,116]. Number of studies has been compiled that are pertinent to the creation of peptide drugs from the domains of biology, chemistry, and computation in this review. Rapid improvements in chemical and biocomputational techniques have been prompted by rising interest in peptide biotherapeutics. A conventional peptide drug development cycle that spans the range of subjects covered is shown in a modular form in Figure 1. The generalised principles and workflow stress that neither the biological, chemical, nor computational method is required for increased peptide drug discovery and development, even though this figure might not include all contemporary technique employed in peptide drug development to date. In order to enhance experimental efforts for improved structurally based peptide drug design and discovery, it can be predicted that peptide-protein docking approaches will become more widely utilised technologies. Advances in chemical and biocomputational approaches have been made quickly as a result of growing interest in peptide biotherapeutics. Figure 1 offers a modular overview of a typical peptide drug development cycle that addresses the range of subjects covered. The generalised principles and workflow highlight that neither the biological, chemical, nor computational method is essential for increased peptide drug discovery and development, even though this figure may not include every contemporary technique employed in peptide drug development to date. We also predict that peptide-protein docking techniques will be employed more frequently as experimental work tools to help peptide drug discovery and design that is structurally based.



**Figure 1.** shows the peptide drug development cycle in a modular format. Green boxes denote computational techniques; gold, biological techniques; and grey, typical methods of peptide bioactivity improvement. The modification techniques that are comparatively more biological, chemical, or computational are shown by the blue two-headed arrow. Depending on the facts at hand, the methods indicated by white dashed boxes can be selected next. Direct links between methods are shown by solid or dashed arrows, depending. [52]

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