ADVANCEMENT IN DEVELOPMENT OF PEPTIDE DRUGS

Abstract

For the past two decades, protein-protein interactions (PPIs), which carry out a variety of essential physiological processes, have been important therapeutic targets. It has been extremely difficult to interfere with intracellular PPIs with small molecules because antibodies cannot penetrate the cell membrane to access bigger or flat binding sites. Due to their smaller size and balanced structural toughness and flexibility, peptides have recently become intriguing candidates for overcoming challenging binding surfaces. The development of peptide-based tactics to disrupt endogenous protein interactions or the enhancement of the binding affinity and specificity of current approaches depends on our understanding of and ability to manipulate peptide-protein recognition mechanisms. It is significant to note that numerous sensible designs with the aid of computing for Peptide therapies have been developed with the goal of providing thorough docking for peptide-protein interaction interfaces. Over 60 peptides have received universal approval for use in clinics. Despite this, improvements of different docking models are just beginning to affect the creation of peptide drugs. In this book chapter two things have been emphasised (i) a comprehensive analysis of the essential technologies used so far in the creation of peptide drugs, and (ii) an updated summary of significant advancements in computational modelling of peptide-protein interactions (PepPIs) with the goal of assisting experimental biologists in advancing peptide interfering tactics against PPIs by helping them use appropriate docking methods.

Keywords: Peptide drugs, Protein Interactions, GalaxyPepDock

Author

Dr. SudeshnaKar
Assistant Professor
Department of Chemistry
School of Basic and Applied Sciences
Adamas University
Kolkata, West Bengal, India

I. INTRODUCTION

A significant and ongoing therapeutic difficulty is the delivery of medications selectively to patient neoplasms. Nearly forty years ago, function-blocking monoclonal antibodies were first suggested as cancer treatments. The first antibody related therapies were only commercialised for cancer medicines and diagnostics twenty years later due to the huge size of these molecules impeding their commercial development. [1,2]. The use of a radiolabelled peptide analogue of somatostatin (SST), rather than an antibody, to target neuroendocrine tumours that express the SST receptor, was a classic advance during this time. [3]. Current peptide medication advances in academia and the pharmaceutical industry are the result of the idea of using a peptide as a targeted moiety for cancer detection and treatment. 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 effectiveness for diabetics [4]. For 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. In addition to analogues of natural hormones based on peptides, peptides have been developed as therapeutic candidates to disrupt protein-protein interactions (PPIs) and target or inhibit intracellular molecules, such as receptor tyrosine kinases. [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, the pharmaceutical sector has a significant demand for peptides and other small molecules that block PPIs. Increasing data indicates that the key to their clinical success will be improved identification of disease-associated PPIs that can be targeted and enhancements to the peptide drug binding qualities [8].Regrettably, the determination of the molecular recognition mechanism and binding affinity for PPIs is a challenging topic for computational biologists and protein biochemists alike. 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 composite interfaces [9]. Monoclonal antibodies are improved at identifying such PPI interfaces, but they are unable to locate and identify intracellular targets because they cannot cross the cell membrane. Recently, peptides with sensible structural suppleness and binding affinity those are up to five times larger than small molecule drugs have attracted a lot of attention. [10,11].Examples of small molecule therapeutic features include lengthy in vivo stability, cyclic peptides exhibit a high affinity for binding that is similar to an antibody and minimal toxicity. [12]. Two parts of peptide drug development will be the emphasis of this book chapter: (i) fundamental expertise used in peptide based drug development so far, and (ii) significant advancements in computer simulation 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.

II. CRITICAL ELEMENTS IN THE CREATION OF BIOACTIVE PEPTIDE DRUGS

1. **Background:** Peptide medications have significantly changed the pharmaceutical industry since In the early 1920s, insulin, peptide having 51 amino acids, was discovered and first made commercially available. [13]. The insulin made from animal tissue, which had been available for almost 90 years, has been superseded by human recombinant insulin thanks to technological advances in purification of protein and DNA recombination. More than 60 peptide medicines have been authorized 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 research conducted by the peptide industry globally, which forecasted a compound yearly 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.

2. **Addressing Peptide Medications' Internal Drawbacks:** Contrary to synthetic peptide medications, natural polypeptides like hormones, developmentfeatures, 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 create peptides more unaffected to proteolysis.

3. **Termini Protection:** 500 or more proteases and peptidases, including carboxypeptidases and serum aminopeptidases, have the potential to disruption peptides at their N- and C-termini [16]. Variable levels of proteolysis and breakdown will be caused by variety of amino acid residues at the N- or C-terminal, 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, Val, Ala, Thr,Ser and Gly [17]. If the C- or N-terminal sequences can be changed while retaining the necessary directing specificity and affinity, the proteolytic degradation can be reduced and the bioavailability increased [18].Similarly, to increase in vivo stability, additionally, N-terminal acetylation and C-terminal amidation can be applied, provided that doing so doesn't interfere with the drug's capacity to function. [19]. Alteration using synthetic amino acid analogues could achieve the same result.

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 biotic study of peptide drug scheme. The main step is to determine the minimal required amino acid residue(s) for peptide action. 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. Secondly, the influence of each specific amino acid to the biotic activity of the peptide can be assessed using a traditional screening technique termed alanine scanning [20]. Critical amino acids can be found by investigating the biotic functioning of a library of peptides in which specific amino acids have been exchanged with alanine. Since its short, neutral side chain doesn't affect the operation of nearby side chains, alanine is utilized in place of it [21]. More contemporary scanning methods have been established 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 stability, mutagenesis, and pharmacokinetic (PK) tests. These structure-activity relationship (SAR) research will help identify the amino acids in a peptide sequence that are proteolytically labile.

5. **Backbone Modification and Synthetic Amino Acid Changeover:** 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 renowned as protease substrates, synthetic enantiomer amino acids, for example, have been planned 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 were achieved recently in a preclinical achievement using side-chain variation of a monomeric 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 novel L-peptide can be compromised by the structural changes caused by enantiomer amino acid (D-amino acid) replacement, despite the fact that it has been a frequent strategy to guard 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 benefit of retaining the side-chain at its novelthree-dimensionalalignment, 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 β–/–D-amino acids, α–/-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 tumor suppression [6]. Such PPI-targeting techniques have enormous therapeutic potential because, despite the abundance of knowledge on disease-related PPIs in the works, peptide-based inhibitors have only recently begun to reach their full potential.

6. **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.Fruitful peptide-based directing 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 inactive uptake is one possible tactic, as is conjugating the active drug peptide to a cell-penetrating peptide (CPP) to to facilitate its active transport.Because peptide bio-therapeuticsare 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, ccSOLomics allows for the discovery of soluble motifs inside 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 sheet, coil or helix [40].

7. **Internal Peptide Uptake Facilitated by Membrane Proteins:** A superfamily of transmembrane receptors called G-protein attached receptors (GPCRs) is in charge of moving various chemicals across membranes. 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 final goal of producing peptide medicines that are cell-permeable and orally accessible [42]. Unknown are the chemical and structural processes underlying the intracellular transit of CPP. The ability of CPPs to penetrate the lipid bilayer of the membrane has enabled significant advances in biotherapeutic peptides. Due to their extremely amphipathic and cationic features,

antimicrobial peptides (AMPs), for example, have been able to penetrate cell membranes through controlling immunological responses [43]. CPPs have also been employed as targeting moieties by conjugation to transport cargos such as small molecules, peptides, proteins, or antibodies that would otherwise be membrane-impermeable [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 operators to forecast and improve their investigational designs for CPPs. Using machine learning-based models, CPPpred web servers like CPPpred-RF and KELM-CPPpred enable the extrapolation and construction of CPPs from anenquiry input protein arrangement [46–48]. Physiochemical characteristics including hydrophobicity, steric hindrance, amphipathicity, molecular and weight chargeare used by CellPPD, another free website, to predict permeability [49,50].Although physiochemical analyses are lacking, CPPpred-RF and KELM-CPPpred use specific databanks to predict CPP uptake efficiency and strong 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 peptide solubility and cell penetrating peptide prediction techniques [52]

Method	Learning Machine Model	Input Length (aa)	Input Format	Multiple Entry	Database	Web Server	Refs
				Peptide Solubility			
ccSOL omics	Super vector machine (SVM)	–	FASTA	Yes (up to 10^6)	Target Track (non-redundant) (http://sbkb.org/tt/)	http://s.tartaglialab.com/static_files/ shared/tutorial_ccsol_omics.html	[39]
PROSO II	Super vector machine (SVM)	21 to 2000	FASTA	Yes (up to 50)	Target Track (http://sbkb.org/tt/)	http://mbiljj45.bio.med.uni-muenchen. de:8888/prosoII/prosoII.seam	[40]
				Cell-Penetrating Peptides			
CPPpred	Artificial neural networks (ANN)	5 to 30	FASTA	Yes	CPPsite	http://bioware.ucd.ie/cpppred	[47]
CPPpred-RF	Random forest (RF)	–	FASTA	Yes	CPP924 and CPPsite3	http://server.malab.cn/CPPred-RF	[46]
KELM-CPPpred	Kernel extreme learning model (KELM)	5 to 30	FASTA	Yes	Curated 408 CPP/non-CPP	http://sairam.people.iitgn.ac.in/KELM-CPPpred.html	[48]
CellPPD	Super vector machine (SVM)	–	FASTA	Yes	CPPsite1,2,3	http://crdd.osdd.net/raghava/cellppd/ multi_pep.php	[50]
CPPsite 2.0	–	–	FASTA	Yes	1855 uniquely curated	http://crdd.osdd.net/raghava/cppsite/	[51]

As was already said, cyclic peptides outperform linear peptides in terms of structural stability and proteolytic resistance. The development of cyclic peptide medicines that is cell-permeable to disrupt PPIs has received a great deal of attention. Short CPP motifs that are ordinarily cell impermeable have been strategically connected to cyclic peptides to permit their intracellular uptake. The development of drugs made on bicyclic peptides 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]. By severing the oncogenic Ras-Raf connection, a bicyclic peptide inhibitor severely reduced MEK/ATK signalling and induced death in lung cancer cells [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].

8. **High-Throughput Screening (HTS) for New Peptide Leads:**In reality, the Ras-Raf bicyclic peptide inhibitor was discovered through optimisation of hits from a screen of 5.7 million bicyclic peptides for interaction with oncogenic K-RasG12V. 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 desirability, such minor interactions shouldn't necessarily be discounted. In phage presentation library screening, the subsequent rounds of "biopanning" enrichment can enhance the revealing 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 necessary may increase the likelihood of false positives, dropouts, and selection bias [58], despite making it easier to detect weaker interactions. These issues have significantly lessened since phase display investigations started using next-generation sequencing (NGS) analyses.NGS is quantitative and sensitive enough to decrease the number of biopanning cycles necessary to discover increased interactions, hence reducing the bias caused by multi-cycle screening. 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.

III. PROTEIN–PROTEIN AND PEPTIDES INTERACTIONS

PPIs are well-known potential therapeutic targets because dysregulated protein interaction networks underlie a wide spectrum of illnesses. 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 $Å^2$), compared to the tiny molecules' binding pocket size (300–1000 $Å^2$), 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. IPs have a substantial advantage over tiny molecules in terms of targeting PPIs due to the presence of amino acid residues that can interact with other residues at protein-protein interfaces [9]. IPs

as biotherapeuticsare gaining more attention thanks to recent developments in methods to address the inherent drawbacks of peptide medicines, such as their poor solubility, stability 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.

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 amino acid containg 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].IPs with -helical structures that attach to protein interacting surfaces have shown particularly promising interaction-blocking efficacy due to their high stability and protease resistance [71]. EZH2/PRC2, -catenin/Wnt, Bax/Bcl-xLandMDM2/p53interactions are only a few of the oncogenic protein interactions that α–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 ability of peptide drugs to treat disease to specifically alter disease-causing protein interactions [72,73]

2. **PPI Determination Using Experimental and Computational Techniques:** Protein-protein interactions have been experimentally determined using a variety of biophysical techniques, including X-ray crystallography, isothermal titration calorimetry, surface plasma resonance, radio-ligand binding, bio-layer interferometry, spectrophotometric assays, NMR spectroscopy and fluorescence spectroscopy. The experimental data generated by these techniques have increased our understanding of how secondary and tertiary protein structure and interaction kinetics influence downstream biological events. These methods are used to research one particular PPI at a time, however they are frequently time-consuming. Whereas protein crystal X-ray diffraction is unquestionably a very effective structural investigation technique that can define structure down to the level of individual atoms, it faces significant methodological difficulties. Numerous proteins either don't crystallise well or only as tiny protein fields.

    Despite the fact that each protein in a complex crystallises separately, 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 progression of producing precise forecasts of protein structure, contact affinitiesandsurface charge computational docking approaches have been created.

3. **Computational Docking Techniques:** Since some docking approaches can be completed in the order of minutes, computational PPI docking has quickly and effectively supplied statistics 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]. Over the years, a broad variety of distinct docking programs have been developed due to the availability and complexity of many scoring characteristics from the most adaptable docking systems. 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].

4. **Predictions based on structures or on sequences:** A number of computational docking approaches, 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, in order to calculate the binding free energies and produce more accurate predictions on the binding affinities between the interacting proteins [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 multiplexes according to their biotic roles and the proportion of binding residues, PPA-Pred, for instance, created a model based on sequence features to predict binding affinities [77].Updated datasets in experimental and functional scaffolds can enhance sequence-based representations, 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 deficiency of high computational influence and high-quality, higherinvestigational datasets. The best-performing attendants were ranked based on extrapolationaccurateness in the CAPRI community experiment, which paralleled computationally anticipated protein compositeconstructions with experimentally proven structures [79]. Based on ClusPro,root mean square deviation (RMSD) and HADDOCK 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].

IV. ADVANCES IN PEPTIDE-PROTEIN INTERACTIONS AND COMPUTATIONAL TECHNIQUES

Similar to protein-protein interactions, PepPI prediction accuracy has frequently been constrained by the fundamental information (either a single structure of the target protein or the combination it is in with the ligand) that is available for a pharmacological goal. Although protein co-structures are rare, several research use data from structural databanks like the

Protein Data Bank (PDB) to determine peptide designs using sequence-binding motifs [83]. Additionalcatalog, 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 segment will cover recent computational representations 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 the docking approach for peptide-protein interactions [52]

Methods	Key Features	Model Quality #	Web Server	Refs
	Local Docking			
DynaDock	▪ Combined Optimized Potential Molecular dynamics (OPMD) with a soft-core potential ▪ Faster conformational sampling ▪ Smoothened van der Waals and Coulomb energy potentials ▪ Full flexibility of peptide and target protein	Near-native	Not available to public	[92]
Rosetta FlexPepDock	▪ Monte Carlo-based optimization ▪ High-quality conformational sampling ▪ Hotspot residue (side-chain) modeling ▪ Receptor flexibility (side-chains to full structure) ▪ Rosetta energy function based clustering and scoring	Sub-angstrom *	http://flexpepdock.furmanlab.cs.huji.ac.il or http://www.rosettacommons.org/software	[93]
PepCrawler	▪ Rapidly-exploring Random Tree (RRT) algorithm ▪ Motion-planning based sampling ▪ Ranking by automated energy funnel analysis (clustering-based) ▪ Fully flexible peptide structures	Near-native *	http://bioinfo3d.cs.tau.ac.il/PepCrawler	[95]
Rosetta FlexPepDock *ab initio*	▪ Ab initio modeling based on Rosetta fragment library ▪ Simultaneous docking and de-novo folding of peptides ▪ Peptide secondary structure option ▪ No information for peptide conformation required	Near-native to Sub-angstrom §	http://www.rosettacommons.org/software	[96]
HADDOCK peptide docking	▪ Modeling from ensemble of three canonical secondary structures (α-helix, extended or polyproline-II helix) ▪ User-defined residues at binding pocket ▪ Binding free energy based scoring ▪ Fully flexible for interacting residues of peptide and protein	Near-native *	http://haddock.science.uu.nl/services/HADDOCK2.2/	[79,97]

Table 2 continued

PepSite 2.0	■ Identifies most peptide-binding site in seconds ■ Generates low-resolution model of peptide ■ Coarse-grained peptide orientation by spatial position-specific scoring matrix (S-PSSM)	Medium [†]	http://pepsite2.russelllab.org	[102]
Global Docking				
ClusPro PeptiDock	■ Fast Fourier Transform (FFT)-based docking method ■ Motif-based prediction for peptide conformation ■ Clustering by structure scoring and CAPRI peptide docking criteria	Near-native to Sub-angstrom [§]	https://peptidock.cluspro.org/	[81,104]
pepATTRACT	■ Peptide structure prediction by threading sequence onto the three peptide conformations (as HADDOCK peptide docking) ■ Rigid-body peptide docking within binding pocket ■ Suitable for large-scale in silico protein–peptide docking ■ Clustering based on ATTRACT scores ■ Optional flexible docking for interacting residues	Near-native to Sub-angstrom [§]	http://bioserv.rpbs.univ-paris-diderot.fr/services/pepATTRACT/	[105]
HPEPDOCK	■ Hierarchical algorithm ■ Ensemble peptide conformation by MODPEP ■ Blind global peptide docking ■ Higher success rate and lower processing time for both global and local docking	Near-native to Sub-angstrom [§]	http://huanglab.phys.hust.edu.cn/hpepdock/	[109]

Methods	Key Features	Model Quality [#]	Web Server	Refs
Template-based				
GalaxyPepDock	■ Use similarity search (known template structures) as scaffolds for prediction ■ Energy-based model optimization and scoring ■ Superior accuracy using PeptiDB datasets than other servers	Medium (ligand); Near-native (interface)	http://galaxy.seoklab.org/pepdock	[110]
SPRINT-Str	■ Predict residues at peptide–protein binding interface ■ Use SVM with optimized parameters ■ Capability to distinguish binding sites of peptide from DNA, RNA and carbohydrate	N/A	http://sparks-lab.org/server/SPRINT-Str	[111]
PBRpredict-Suite	■ Predict interacting residues based on peptide-binding domain (PDB) from template sequences in NCBI database ■ Integrated six machine learning algorithms (model stacking) ■ Proteome-wide prediction feasibility	N/A	http://cs.uno.edu/~{}tamjid/Software/PBRpredict/pbrpredict-suite.zip	[112]
PepComposer	■ Motif similarity search to defined binding interfaces from monomeric protein databases PepX (http://pepx.switchlab.org) ■ Monte carlo-implemented PyRosetta ■ User-defined options for binding site residues or chain selection	Near-native	https://cassandra.med.uniroma1.it/pepcomposer/webserver/pepcomposer.php	[113]

[#]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.

1. **Initial Peptide Scaffolds Selected:** We first want to explain recent developments during choosing the first peptide scaffolds, which also show crucial characters in the development of peptide drugs, before going into current computational methods for PepPIs. From natural proteins, selection of many well-characterized naturally occurring peptides had been shown that they retained their original activities, such as structural scaffolds or the capacity to recognize target molecules. For instance, repeated Arg-Gly-Asp (RGD) designs were originally discovered.We primarily want to explain recent developments in the choice of initial peptide frameworks, which also show crucial

characters 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 $\alpha-/\beta-$ 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 tetrapeptideSer-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 AutodockVina aided in the choosing of SLRP.

2. **Peptide-Protein Interactions in Docking:** 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.

3. **Methods of Local and Global Docking:** Local docking is the technique that is most typically used to determine a potential binding posture for a peptide at a user-defined binding site in a resolved structure of its target receptor. 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 Rosetta FlexPepDock, DynaRock and PepCrawler. For the purposes of determining receptor side-chain flexibility and conformational sampling, DynaDock uses soft-core possible in combination with molecular dynamics [93].

The van der Waals and Coulomb energy potentials were smoothed in this protocol, resulting in faster conformational sampling of the peptide-protein complex as the soft-core potential eventually converged to a physical potential as the simulation progressed. 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].This refinement approach creates a conformation tree for the peptide-protein complex by automatically clustering the generated models using local shape analysis of the energy funnel.Nevertheless, information on backbone conformation is not readily available for every query peptide. Techniques for sampling that allow for the acquisition of a nearly native peptide form become essential prior to carrying out local docking. For instance, the Rosetta FlexPepDockab initio process combines local docking and ab initio peptide folding to put the query peptide into a user-defined binding site from any arbitrary backbone conformation [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, native docking for small peptides with less than five amino acids has been carried out using a variety of small molecule docking techniques, including Surflex, Gold and AutoDockVina [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. **Comprehensive Docking Techniques:** In contrast to native docking, which just explorations for the peptide-binding posture, comprehensive docking approaches additionally look for the target protein's peptide-binding site. As binding locations are unknown beforehand, global docking is typically the preferred method. Using a three-dimensionallocation specific recording matrix (PSSM), the PepSite technique was developed to find probable binding positions with an estimated position for each residue [103,104]. Nonetheless, because of the different levels of peptide flexible-body docking, backbone/side-chain flexibilityare 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 ATTRACT (pepATTRACT) and ClusPro (ClusProPeptiDock) 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 methods, such as AnchorDock, PeptiMap, and CABS-Dock, also provide automatic docking simulation using a number of algorithms, such as small molecule binding adaptation, in-solvent simulation, flexibility of the query peptide at predicted binding proximity, and so on [107-109].Other recently discovered approaches, such HPEPDOCK, obtained considerably higher success rates and shorter simulation periods than pepATTRACT by using a collection of peptide conformations for blind general docking. [106,110]. These outcomes are in addition to the incredibly precise forecasts provided by PIPER-FlexPepDock.

5. **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 order of the query peptide and/or objective protein through template scaffolds that are known to exist [79]. Because of the sudden growth in the

quantity of peptide-protein assemblies deposited in PBD, which have considerably sped up developments and strategies in simulation algorithms, template-based docking has lately been recognised as a different class in peptide-protein docking. A well-known server called GalaxyPepDock carries out similarity-based docking by looking for models with the highest levels of similarity and developing models utilising energy optimisation to enable more precise calculations on structural suppleness between interacting complexes [111]. During CAPRI blind prediction studies, GalaxyPepDock showed better prediction outcomes than other servers employing PeptiDB datasets.PBRpredict, a different a docking strategy based on templates and machine learning, 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 popular online tool for peptide-protein computational design, incorporated a machine learning technique (Monte Carlo) for a fully automatic computational peptide design. It was demonstrated that this method accurately predicted well-known PepPIs at rates that were quite repeatable [114].

V. 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. Significant strides in computational structure prediction as well as the growth of chemical modifications accessible to improve stability, affinity, and specificity have also made it conceivable. Publicly available computational binding prediction methods have led to the development of more powerful, logical, and unique peptide drug designs. 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 although recent improvements in the computational modelling of peptide-protein and protein-protein structures. For example, 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 small-angle X-ray scattering (SAXS), cryo-electromicroscopyand 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 investigational data into algorithmic constraints that can be used as a docking choice [80,98]. In reality, experimental biologists find that these docking techniques are quite useful for confirming the suggested binding mechanism. Finally, recording had also been very difficult because several 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 method that combines energy-based recording with additional techniques like co-evolutionary information, mutagenesis, sequence- or structural-clustering role can produce exact peptide-protein docking results that are more resembling of inherent models [79,81,116].Number of studies has been compiled that are pertinent to the creation of peptide drugs from the domains of chemistry, biology 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 advance cycle that spans the range of subjects covered is shown in a modular form in Figure 1.The generalised principles and work-flow stress that neither the chemical, biological, nor computational method is required for increased peptide drug innovation and improvement, even though this figure might not include all contemporary skill employed in peptide drug development up to the present time. 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 prefabricated overview of a typical peptide drug advance cycle that addresses the range of subjects covered. The generalised principles and workf-low highlight that neither the chemical, biological nor computational technique 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.
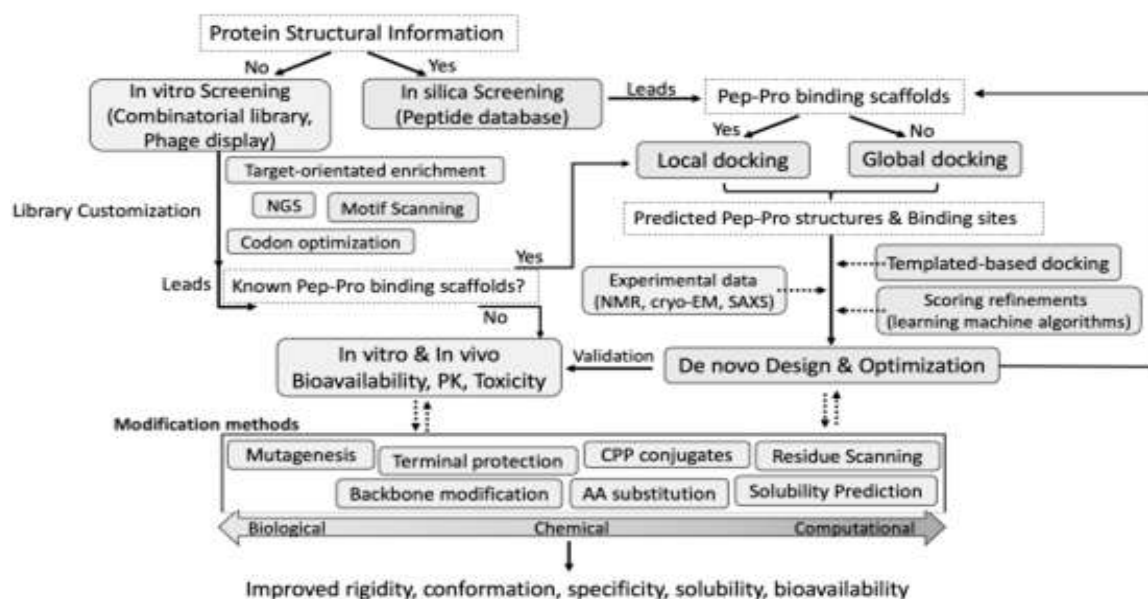

Figure 1: Shows the Peptide Drug Advance Cycle in a Modular Format.Green boxes denote computational techniques; gold, biological techniques; and grey, typical methods of peptide bioactivity improvement. The adjustment techniques that are comparatively more chemical, biological or computational are shown 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]

REFERENCES

[1]   Press, O.W.; Eary, J.F.; Appelbaum, F.R.; Martin, P.J.; Nelp, W.B.; Glenn, S.; Fisher, D.R.; Porter, B.; Matthews, D.C.; Gooley, T.; et al. Phase II trial of 131I-B1 (anti-CD20) antibody therapy with autologousstem cell transplantation for relapsed B cell lymphomas. Lancet 1995, 346, 336–340.
[2]   Goldenberg, D.M.; Deland, F.; Kim, E.; Bennett, S.; Primus, F.J.; van Nagell, J.R., Jr.; Estes, N.; DeSimone, P.; Rayburn, P. Use of radiolabeled antibodies to carcinoembryonic antigen for the detection and localization of diverse cancers by external photoscanning. N. Engl. J. Med. 1978, 298, 1384–1386.

[3]   Krenning, E.P.; Bakker, W.H.; Breeman, W.A.; Koper, J.W.; Kooij, P.P.; Ausema, L.; Lameris, J.S.; Reubi, J.C.; Lamberts, S.W. Localisation of endocrine-related tumours with radioiodinated analogue of somatostatin. Lancet 1989, 1, 242–244.

[4]   Banting, F.G.; Best, C.H.; Collip, J.B.; Campbell, W.R.; Fletcher, A.A. Pancreatic Extracts in the Treatment of Diabetes Mellitus. Can. Med. Assoc. J. 1922, 12, 141–146.

[5]   Birk, A.V.; Liu, S.; Soong, Y.; Mills, W.; Singh, P.; Warren, J.D.; Seshan, S.V.; Pardee, J.D.; Szeto, H.H. The mitochondrial-targeted compound SS-31 re-energizes ischemic mitochondria by interacting with cardiolipin. J. Am. Soc. Nephrol. 2013, 24, 1250–1261.

[6]   Chang, Y.S.; Graves, B.; Guerlavais, V.; Tovar, C.; Packman, K.; To, K.H.; Olson, K.A.; Kesavan, K.; Gangurde, P.; Mukherjee, A.; et al. Stapled alpha-helical peptide drug development: A potent dual inhibitor of MDM2 and MDMX for p53-dependent cancer therapy. Proc. Natl. Acad. Sci. USA 2013, 110, 3445–3454.

[7]   Yan, Z.; Wang, J. Specificity quantification of biomolecular recognition and its implication for drug discovery. Sci. Rep. 2012, 2, 309.

[8]   Thomas, D. A Big Year for Novel Drugs Approvals; Biotechnology Innovation Organization: Washington, DC, USA, 2013.

[9]   Wells, J.A.; McClendon, C.L. Reaching for high-hanging fruit in drug discovery at protein-protein interfaces. Nature 2007, 450, 1001–1009.

[10]   Hewitt, W.M.; Leung, S.S.; Pye, C.R.; Ponkey, A.R.; Bednarek, M.; Jacobson, M.P.; Lokey, R.S. Cell-permeable cyclic peptides from synthetic libraries inspired by natural products. J. Am. Chem. Soc. 2015, 137, 715–721.

[11]   Driggers, E.M.; Hale, S.P.; Lee, J.; Terrett, N.K. The exploration of macrocycles for drug discovery-an underexploited structural class. Nat. Rev. Drug. Discov. 2008, 7, 608–624.

[12]   Matsson, P.; Doak, B.C.; Over, B.; Kihlberg, J. Cell permeability beyond the rule of 5. Adv. Drug. Deliv. Rev. 2016, 101, 42–61.

[13]   Bliss, M. Banting's, Best's, and Collip's accounts of the discovery of insulin. Bull. Hist. Med. 1982, 56, 554–568.

[14]   Research, T.M. Global Industry Analysis, Size, Share, Growth, Trends and Forecast. Pept. Mark. 2016, 2016–2024.

[15]   Fosgerau, K.; Hoffmann, T. Peptide therapeutics: Current status and future directions. Drug Discov. Today 2015, 20, 122–128.

[16]   Puente, X.S.; Gutierrez-Fernandez, A.; Ordonez, G.R.; Hillier, L.W.; Lopez-Otin, C. Comparative genomic analysis of human and chimpanzee proteases. Genomics 2005, 86, 638–647. [CrossRef]

[17]   Werle, M.; Bernkop-Schnurch, A. Strategies to improve plasma half life time of peptide and protein drugs. Amino Acids 2006, 30, 351–367.

[18]   Jambunathan, K.; Galande, A.K. Design of a serum stability tag for bioactive peptides. Protein Pept.Lett.2014, 21, 32–38.

[19]   Di, L. Strategic approaches to optimizing peptide ADME properties. AAPS. J. 2015, 17, 134–143.

[20]   Weiss, G.A.; Watanabe, C.K.; Zhong, A.; Goddard, A.; Sidhu, S.S. Rapid mapping of protein functional epitopes by combinatorial alanine scanning. Proc. Natl. Acad. Sci. USA 2000, 97, 8950–8954.

[21]   Morrison, K.L.; Weiss, G.A. Combinatorial alanine-scanning. Curr.Opin. Chem. Biol. 2001, 5, 302–307.

[22]   Eustache, S.; Leprince, J.; Tuffery, P. Progress with peptide scanning to study structure-activity relationships: The implications for drug discovery. Expert.Opin.Drug Discov. 2016, 11, 771–784. 23. Weinstock, M.T.; Francis, J.N.; Redman, J.S.; Kay, M.S. Protease-resistant peptide design-empowering nature's fragile warriors against HIV. Biopolymers 2012, 98, 431–442.

[23]   Wisniewski, K.; Galyean, R.; Tariga, H.; Alagarsamy, S.; Croston, G.; Heitzmann, J.; Kohan, A.; Wisniewska, H.; Laporte, R.; Riviere, P.J.; et al. New, potent, selective, and short-acting peptidic V1a receptor agonists.J. Med. Chem. 2011, 54, 4388–4398.

[24]   Frey, V.; Viaud, J.; Subra, G.; Cauquil, N.; Guichou, J.F.; Casara, P.; Grassy, G.; Chavanieu, A. Structure-activity relationships of Bak derived peptides: Affinity and specificity modulations by amino acid replacement. Eur. J. Med. Chem. 2008, 43, 966–972.

[25]   Finan, B.; Yang, B.; Ottaway, N.; Smiley, D.L.; Ma, T.; Clemmensen, C.; Chabenne, J.; Zhang, L.; Habegger, K.M.; Fischer, K.; et al. A rationally designed monomeric peptide triagonist corrects obesity and diabetes in rodents. Nat. Med. 2015, 21, 27–36.

[26]   Werner, H.M.; Cabalteja, C.C.; Horne, W.S. Peptide Backbone Composition and Protease Susceptibility: Impact of Modification Type.; Position, and Tandem Substitution. ChemBioChem 2016, 17, 712–718.

[27]   Liskamp, R.M.; Rijkers, D.T.; Kruijtzer, J.A.; Kemmink, J. Peptides and proteins as a continuing exciting source of inspiration for peptidomimetics. ChemBioChem 2011, 12, 1626–1653. [

[28] Cabrele, C.; Martinek, T.A.; Reiser, O.; Berlicki, L. Peptides containing beta-amino acid patterns: Challenges and successes in medicinal chemistry. J. Med. Chem. 2014, 57, 9718–9739.

[29] Chatterjee, J.; Rechenmacher, F.; Kessler, H. N-methylation of peptides and proteins: An. important element for modulating biological functions. Angew. Chem. Int. Ed. Engl. 2013, 52, 254–269.

[30] Perez, J.J. Designing Peptidomimetics. Curr. Top Med. Chem. 2018, 18, 566–590.

[31] Chingle, R.; Proulx, C.; Lubell, W.D. Azapeptide Synthesis Methods for Expanding Side-Chain Diversity for Biomedical Applications. ACC Chem. Res. 2017, 50, 1541–1556.

[32] Pelay-Gimeno, M.; Glas, A.; Koch, O.; Grossmann, T.N. Structure-Based Design of Inhibitors of Protein-Protein Interactions: Mimicking Peptide Binding Epitopes. Angew. Chem. Int. Ed. Engl. 2015, 54, 8896–8927.

[33] Kluskens, L.D.; Nelemans, S.A.; Rink, R.; de Vries, L.; Meter-Arkema, A.; Wang, Y.; Walther, T.; Kuipers, A.; Moll, G.N.; Haas, M. Angiotensin-(1-7) with thioether bridge: An. angiotensin-converting enzyme-resistant, potent angiotensin-(1-7) analog. J. Pharm. Exp. 2009, 328, 849–854.

[34] Decoene, K.W.; Vannecke, W.; Passioura, T.; Suga, H.; Madder, A. Pyrrole-Mediated Peptide Cyclization Identified through Genetically Reprogrammed Peptide Synthesis. Biomedicines 2018, 6, 99.

[35] Wu, S.J.; Luo, J.; O'Neil, K.T.; Kang, J.; Lacy, E.R.; Canziani, G.; Baker, A.; Huang, M.; Tang, Q.M.; Raju, T.S.; et al. Structure-based engineering of a monoclonal antibody for improved solubility. Protein Eng. Des. Sel. 2010, 23, 643–651.

[36] Mant, C.T.; Kovacs, J.M.; Kim, H.M.; Pollock, D.D.; Hodges, R.S. Intrinsic amino acid side-chain hydrophilicity/hydrophobicity coefficients determined by reversed-phase high-performance liquid chromatography of model peptides: Comparison with other hydrophilicity/hydrophobicity scales. Biopolymers 2009, 92, 573–595.

[37] Cortes, C.; Vapnik, V. Support-Vector Networks. Mach. Learn. 1995, 20, 273–297.

[38] Agostini, F.; Cirillo, D.; Livi, C.M.; Ponti, R.D.; Tartaglia, G.G. cc SOL omics: A webserver for solubility prediction of endogenous and heterologous expression in Escherichia coli. Bioinformatics 2014, 30, 2975–2977.

[39] Smialowski, P.; Doose, G.; Torkler, P.; Kaufmann, S.; Frishman, D. PROSO II-a new method for protein solubility prediction. FEBS J. 2012, 279, 2192–2200.

[40] Derakhshankhah, H.; Jafari, S. Cell penetrating peptides: A concise review with emphasis on biomedical applications. Biomed. Pharm. 2018, 108, 1090–1096.

[41] Copolovici, D.M.; Langel, K.; Eriste, E.; Langel, U. Cell-penetrating peptides: Design, synthesis,

[42] and applications. ACS Nano. 2014, 8, 1972–1994.

[43] Mahlapuu, M.; Hakansson, J.; Ringstad, L.; Bjorn, C. Antimicrobial Peptides: An. Emerging Category of Therapeutic Agents. Front Cell Infect. Microbiol.2016, 6, 194.

[44] Qian, Z.; LaRochelle, J.R.; Jiang, B.; Lian, W.; Hard, R.L.; Selner, N.G.; Luechapanichkul, R.; Barrios, A.M.; Pei, D. Early endosomal escape of a cyclic cell-penetrating peptide allows effective cytosolic cargo delivery. Biochemistry 2014, 53, 4034–4046.

[45] Qian, Z.; Martyna, A.; Hard, R.L.; Wang, J.; Appiah-Kubi, G.; Coss, C.; Phelps, M.A.; Rossman, J.S.;

[46] Pei, D. Discovery and Mechanism of Highly Efficient Cyclic Cell-Penetrating Peptides. Biochemistry 2016, 55, 2601–2612.

[47] Wei, L.; Xing, P.; Su, R.; Shi, G.; Ma, Z.S.; Zou, Q. CPPred-RF: A Sequence-based Predictor for IdentifyingCell-Penetrating Peptides and Their Uptake Efficiency. J. Proteome. Res. 2017, 16, 2044–2053.

[48] Holton, T.A.; Pollastri, G.; Shields, D.C.; Mooney, C. CPPpred: Prediction of cell penetrating peptides. Bioinformatics 2013, 29, 3094–3096.

[49] Pandey, P.; Patel, V.; George, N.V.; Mallajosyula, S.S. KELM-CPPpred: Kernel Extreme Learning Machine Based Prediction Model. for Cell-Penetrating Peptides. J. Proteome. Res. 2018, 17, 3214–3222.

[50] Eiriksdottir, E.; Konate, K.; Langel, U.; Divita, G.; Deshayes, S. Secondary structure of cell-penetrating peptides controls membrane interaction and insertion. Biochim.Biophys.Acta 2010, 1798, 1119–1128.

[51] Gautam, A.; Chaudhary, K.; Kumar, R.; Sharma, A.; Kapoor, P.; Tyagi, A. Raghava GPS, consortium Osdd. In silico approaches for designing highly effective cell penetrating peptides.J. Transl. Med. 2013, 11, 74.

[52] Agrawal, P.; Bhalla, S.; Usmani, S.S.; Singh, S.; Chaudhary, K.; Raghava, G.P.; Gautam, A. CPPsite 2.0: A repository of experimentally validated cell-penetrating peptides. Nucleic Acids Res. 2016, 44, 1098–1103.

[53] Lee, A. C.; Harris, J. L.; Khanna, K. K.; Hong, A Comprehensive Review on Current Advances in Peptide Drug Development and Design. J.-H.Int. J. Mol. Sci. 2019, 20, 2383.

[54]   Lian, W.; Jiang, B.; Qian, Z.; Pei, D. Cell-permeable bicyclic peptide inhibitors against intracellular proteins. J. Am. Chem. Soc. 2014, 136, 9830–9833.

[55]   Trinh, T.B.; Upadhyaya, P.; Qian, Z.; Pei, D. Discovery of a Direct Ras Inhibitor by Screening a Combinatorial Library of Cell-Permeable Bicyclic Peptides. ACS Comb. Sci. 2016, 18, 75–85.

[56]   Carter, E.; Lau, C.Y.; Tosh, D.; Ward, S.G.; Mrsny, R.J. Cell penetrating peptides fail to induce an innate immune response in epithelial cells in vitro: Implications for continued therapeutic use. Eur. J. Pharm. Biopharm. 2013, 85, 12–19.

[57]   Smith, G.P. Filamentous fusion phage: Novel expression vectors that display cloned antigens on the virionsurface. Science 1985, 228, 1315–1317.

[58]   McCafferty, J.; Griffiths, A.D.; Winter, G.; Chiswell, D.J. Phage antibodies: Filamentous phage displaying antibody variable domains. Nature 1990, 348, 552–554.

[59]   Omidfar, K.; Daneshpour, M. Advances in phage display technology for drug discovery. Expert.Opin.Drug Discov. 2015, 10, 651–669.

[60]   Matochko, W.L.; Derda, R. Next-generation sequencing of phage-displayed peptide libraries.

[61]   Methods Mol. Biol. 2015, 1248, 249–266.

[62]   Ng, S.; Derda, R. Phage-displayed macrocyclicglycopeptide libraries. Org. Biomol. Chem. 2016, 14, 5539–5545.

[63]   Heinis, C.; Winter, G. Encoded libraries of chemically modified peptides. Curr.Opin. Chem. Biol. 2015, 26, 89–98.

[64]   Rolland, T.; Tasan, M.; Charloteaux, B.; Pevzner, S.J.; Zhong, Q.; Sahni, N.; Yi, S.; Lemmens, I.; Fontanillo, C.; Mosca, R.; et al. A proteome-scale map of the human interactome network. Cell 2014, 159, 1212–1226.

[65]   Cunningham, A.D.; Qvit, N.; Mochly-Rosen, D. Peptides and peptidomimetics as regulators of protein-protein interactions. Curr.Opin.Struct. Biol. 2017, 44, 59–66.

[66]   Petta, I.; Lievens, S.; Libert, C.; Tavernier, J.; de Bosscher, K. Modulation of Protein-Protein Interactions for the Development of Novel Therapeutics. Mol. Ther. 2016, 24, 707–718.

[67]   Warso, M.A.; Richards, J.M.; Mehta, D.; Christov, K.; Schaeffer, C.; Bressler, L.R.; Yamada, T.; Majumdar, D.; Kennedy, S.A.; Beattie, C.W.; et al. A first-in-class, first-in-human, phase I trial of p28, a non-HDM2-mediated peptide inhibitor of p53 ubiquitination in patients with advanced solid tumours. Br. J. Cancer 2013, 108, 1061–1070.

[68]   Tabernero, J.; Dirix, L.; Schoffski, P.; Cervantes, A.; Capdevila, J.; Baselga, J.; Beijsterveldt, L.V.; Winkler, H.; Kraljevic, S.; Zhuang, S.H. Phase I pharmacokinetic (PK) and pharmacodynamic(PD) study of HDM-2antagonist JNJ-26854165 in patients with advanced refractory solid tumors. J. Clin. Oncol.2009, 27, 3514.

[69]   Wong, D.; Kandagatla, P.; Korz, W.; Chinni, S.R. Targeting CXCR4 with CTCE-9908 inhibits prostate tumor metastasis. BMC Urol. 2014, 14, 12.

[70]   Huang, E.H.; Singh, B.; Cristofanilli, M.; Gelovani, J.; Wei, C.; Vincent, L.; Cook, K.R.; Lucci, A. A CXCR4 antagonist CTCE-9908 inhibits primary tumor growth and metastasis of breast cancer. J. Surg. Res. 2009, 155, 231–236.

[71]   Chiquet, C.; Aptel, F.; Creuzot-Garcher, C.; Berrod, J.P.; Kodjikian, L.; Massin, P.; Deloche, C.; Perino, J.; Kirwan, B.A.; de Brouwer, S.; et al. Postoperative Ocular Inflammation: A Single Subconjunctival Injection of XG-102 Compared to Dexamethasone Drops in a Randomized Trial. Am. J. Ophthalmol. 2017, 174, 76–84.

[72]   Borsello, T.; Clarke, P.G.; Hirt, L.; Vercelli, A.; Repici, M.; Schorderet, D.F.;Bogousslavsky, J.; Bonny, C. A peptide inhibitor of c-Jun N-terminal kinase protects against excitotoxicity and cerebral ischemia. Nat. Med. 2003, 9, 1180–1186.

[73]   Lau, Y.H.; de Andrade, P.; Wu, Y.; Spring, D.R. Peptide stapling techniques based on different macrocyclisationchemistries. Chem. Soc. Rev. 2015, 44, 91–102.

[74]   Mine, Y.; Munir, H.; Nakanishi, Y.; Sugiyama, D. Biomimetic Peptides for the Treatment of Cancer. Anticancer Res. 2016, 36, 3565–3570.

[75]   Ellert-Miklaszewska, A.; Poleszak, K.; Kaminska, B. Short peptides interfering with signaling pathways as new therapeutic tools for cancer treatment. Future Med. Chem. 2017, 9, 199–221.

[76]   Sliwoski, G.; Kothiwale, S.; Meiler, J.; Lowe, E.W., Jr. Computational methods in drug discovery. Pharm. Rev. 2014, 66, 334–395.

[77]   De Vries, S.J.; Schindler, C.E.; de Beauchene, I.C.; Zacharias, M. A web interface for easy flexible protein-protein docking with ATTRACT. Biophys. J. 2015, 108, 462–465. [CrossRef]

[78] Choi, J.M.; Serohijos, A.W.R.; Murphy, S.; Lucarelli, D.; Lofranco, L.L.; Feldman, A.; Shakhnovich, E.I. Minimalistic predictor of protein binding energy: Contribution of solvation factor to protein binding. Biophys. J. 2015, 108, 795–798.

[79] Yugandhar, K.; Gromiha, M.M. Protein-protein binding affinity prediction from amino acid sequence. Bioinformatics 2014, 30, 3583–3589.

[80] Yugandhar, K.; Gromiha, M.M. Feature selection and classification of protein-protein complexes based on their binding affinities using machine learning approaches. Proteins 2014, 82, 2088–2096.

[81] Lensink, M.F.; Velankar, S.; Wodak, S.J. Modeling protein-protein and protein-peptide complexes: CAPRI 6[th]edition. Proteins 2017, 85, 359–377.

[82] Van Zundert, G.C.P.; Rodrigues, J.; Trellet, M.; Schmitz, C.; Kastritis, P.L.;Karaca, E.; Melquiond, A.S.J.; van Dijk, M.; de Vries, S.J.; Bonvin, A. The HADDOCK2.2 Web Server: User-Friendly Integrative Modeling of Biomolecular Complexes. J. Mol. Biol. 2016, 428, 720–725.

[83] Lensink, M.F.; Velankar, S.; Kryshtafovych, A.; Huang, S.Y.; Schneidman-Duhovny, D.; Sali, A.; Segura, J.; Fernandez-Fuentes, N.; Viswanath, S.; Elber, R.; et al. Prediction of homoprotein and heteroprotein complexes by protein docking and template-based modeling: A CASP-CAPRI experiment. Proteins 2016, 84, 323–348.

[84] Comeau, S.R.; Gatchell, D.W.; Vajda, S.; Camacho, C.J. ClusPro: An automated docking and discrimination method for the prediction of protein complexes. Bioinformatics 2004, 20, 45–50.

[85] Berman, H.M.; Westbrook, J.; Feng, Z.; Gilliland, G.; Bhat, T.N.; Weissig, H.;Shindyalov, I.N.; Bourne, P.E. The Protein Data Bank. Nucleic Acids Res. 2000, 28, 235–242. [CrossRef] [PubMed]

[86] Vanhee, P.; Reumers, J.; Stricher, F.; Baeten, L.; Serrano, L.; Schymkowitz, J.; Rousseau, F. PepX: A structural database of non-redundant protein-peptide complexes. Nucleic Acids Res. 2010, 38, 545–551.

[87] London, N.; Movshovitz-Attias, D.; Schueler-Furman, O. The structural basis of peptide-protein binding strategies. Structure 2010, 18, 188–199.

[88] Clackson, T.; Wells, J.A. A hot spot of binding energy in a hormone-receptor interface. Science 1995, 267, 383–386.

[89] Buonfiglio, R.; Recanatini, M.; Masetti, M. Protein Flexibility in Drug Discovery: From Theory to Computation. ChemMedChem 2015, 10, 1141–1148.

[90] Takahashi, S.; Leiss, M.; Moser, M.; Ohashi, T.; Kitao, T.; Heckmann, D.; Pfeifer, A.; Kessler, H.; Takagi, J.; Erickson, H.P.; et al. The RGD motif in fibronectin is essential for development but dispensable for fibril assembly. J. Cell Biol. 2007, 178, 167–178.

[91] Mohapatra, S.; Saha, A.; Mondal, P.; Jana, B.; Ghosh, S.; Biswas, A.; Ghosh, S. Synergistic Anticancer Effect of Peptide-DocetaxelNanoassembly Targeted to Tubulin: Toward Development of Dual Warhead Containing Nanomedicine. Adv. Healthc. Mater.2017, 6.

[92] Inaba, H.; Matsuura, K. Peptide Nanomaterials Designed from Natural Supramolecular Systems.

[93] Chem. Rec. 2018.

[94] Inaba, H.; Yamamoto, T.; Kabir, A.M.R.; Kakugo, A.; Sada, K.; Matsuura, K. Molecular Encapsulation Inside Microtubules Based on Tau-Derived Peptides. Chem. Eur. J. 2018, 24, 14958–14967.

[95] Jana, B.; Mondal, P.; Saha, A.; Adak, A.; Das, G.; Mohapatra, S.; Kurkute, P.; Ghosh, S. Designed TetrapeptideInteracts with Tubulin and Microtubule. Langmuir 2018, 34, 1123–1132.

[96] Antes, I. DynaDock: A new molecular dynamics-based algorithm for protein-peptide docking including receptor flexibility. Proteins 2010, 78, 1084–1104.

[97] Raveh, B.; London, N.; Schueler-Furman, O. Sub-angstrom modeling of complexes between flexible peptides and globular proteins. Proteins 2010, 78, 2029–2040.

[98] London, N.; Raveh, B.; Cohen, E.; Fathi, G.; Schueler-Furman, O. Rosetta FlexPepDock web server–high resolution modeling of peptide-protein interactions. Nucleic Acids Res. 2011, 39, 249–253.

[99] Donsky, E.; Wolfson, H.J. PepCrawler: A fast RRT-based algorithm for high-resolution refinement and binding affinity estimation of peptide inhibitors. Bioinformatics 2011, 27, 2836–2842. Raveh, B.; London, N.; Zimmerman, L.; Schueler-Furman, O. Rosetta FlexPepDockab-initio: Simultaneous folding, docking and refinement of peptides onto their receptors. PLoS ONE 2011, 6, e18934.

[100] Trellet, M.; Melquiond, A.S.; Bonvin, A.M. A unified conformational selection and induced fit approach to protein-peptide docking.PLoS ONE 2013, 8, e58769.

[101] Verdonk, M.L.; Cole, J.C.; Hartshorn, M.J.; Murray, C.W.; Taylor, R.D. Improved protein-ligand docking using GOLD. Proteins 2003, 52, 609–623.

[102] Rentzsch, R.; Renard, B.Y. Docking small peptides remains a great challenge: An assessment using AutoDockVina. Brief. Bioinform. 2015, 16, 1045–1056.

[103] Jain, A.N. Surflex: Fully automatic flexible molecular docking using a molecular similarity-based search engine. J. Med. Chem. 2003, 46, 499–511.

[104]   Hauser, A.S.; Windshugel, B. LEADS-PEP: A Benchmark Data Set for Assessment of Peptide Docking Performance. J. Chem. Inf. Model 2016, 56, 188–200.

[105] Trabuco, L.G.; Lise, S.; Petsalaki, E.; Russell, R.B. PepSite: Prediction of peptide-binding sites from protein surfaces. Nucleic Acids Res. 2012, 40, 423–427.

[106] Petsalaki, E.; Stark, A.; Garcia-Urdiales, E.; Russell, R.B. Accurate prediction of peptide binding sites on protein surfaces. PLoSComput. Biol. 2009, 5, e1000335.

[107] Porter, K.A.; Xia, B.; Beglov, D.; Bohnuud, T.; Alam, N.; Schueler-Furman, O.;Kozakov, D. ClusProPeptiDock: Efficient global docking of peptide recognition motifs using FFT. Bioinformatics 2017, 33, 3299–3301.

[108] De Vries, S.J.; Rey, J.; Schindler, C.E.M.; Zacharias, M.; Tuffery, P.The pepATTRACT web server for blind,large-scale peptide-protein docking. Nucleic Acids Res. 2017, 45, 361–364.

[109] Lavi, A.; Ngan, C.H.; Movshovitz-Attias, D.; Bohnuud, T.; Yueh, C.; Beglov, D.; Schueler-Furman, O.; Kozakov, D. Detection of peptide-binding sites on protein surfaces: The first step toward the modeling and targeting of peptide-mediated interactions. Proteins 2013, 81, 2096–2105.

[110] Kurcinski, M.; Jamroz, M.; Blaszczyk, M.; Kolinski, A.; Kmiecik, S. CABS-dock web server for the flexible docking of peptides to proteins without prior knowledge of the binding site. Nucleic Acids Res. 2015, 43, 419–424.

[111] Ben-Shimon, A.; Niv, M.Y. AnchorDock: Blind. and Flexible Anchor-Driven Peptide Docking. Structure 2015, 23, 929–940.

[112] Zhou, P.; Jin, B.; Li, H.; Huang, S.Y. HPEPDOCK: A web server for blind peptide-protein docking based on a hierarchical algorithm. Nucleic Acids Res. 2018, 46, 443–450.

[113] Lee, H.; Heo, L.; Lee, M.S.; Seok, C. GalaxyPepDock: A protein-peptide docking tool based on interaction similarity and energy optimization. Nucleic Acids Res. 2015, 43, 431–435.

[114] Taherzadeh, G.; Zhou, Y.; Liew, A.W.; Yang, Y. Structure-based prediction of protein- peptide binding regions using Random Forest. Bioinformatics 2018, 34, 477–484.

[115] Iqbal, S.; Hoque, M.T. PBRpredict-Suite: A suite of models to predict peptide-recognition domain residues from protein sequence. Bioinformatics 2018, 34, 3289–3299.

[116] Obarska-Kosinska, A.; Iacoangeli, A.; Lepore, R.; Tramontano, A. PepComposer: Computational design of peptides binding to a given protein surface. Nucleic Acids Res. 2016, 44, 522–528.

[117] Wang, S.H.; Lee, A.C.; Chen, I.J.; Chang, N.C.; Wu, H.C.; Yu, H.M.; Chang, Y.J.; Lee, T.W.; Yu, J.C.; Yu, A.L.; et al. Structure-based optimization of GRP78-binding peptides that enhances efficacy in cancer imaging and therapy. Biomaterials 2016, 94, 31–44.

[118] Yu, J.; Andreani, J.; Ochsenbein, F.; Guerois, R. Lessons from (co-)evolution in the docking of proteins andpeptides for CAPRI Rounds 28–35. Proteins: Struct. Funct.Bioinform. 2017, 85, 378–390.