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SIGNIFICANCE OF PEPTIDE THERAPEUTICS IN THE MODERN MEDICINE: FROM DRUG DESIGN TO CLINICAL APPLICATIONS

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ABSTRACT

Peptide therapeutics hasactas a basis of Modern medicinal products that represents notableefficacy, precision, and safety when compared with conventional medications. This thorough research tells a lot about the extensive topic of peptide-based treatments, evaluating their development, designing, and variety of therapeutic uses. Even though addressing significant difficulties like composition extent, distribution barriers, and other important concerns about the immunological activities, progressive improvements in the routes of administration and the drug development are important to keep this field progressing. The computational techniques are also very important to explore the interactions between peptides and proteins that will thenpromote the drug development of new medications and also improve the efficacy of current therapies. The pharmacological activities of these peptide therapeutics ranges from cancer to neurological conditions. Peptides represent the leading margin of precision medicine that provides specialized treatments for complex medical issues. When the scientific attempts advances and technical capacities grow the potential of these therapeutics, it needs to be modified for healthcare.

KEYWORDS: Peptide therapeutics, drug design, formulation, delivery systems, immunogenicity, computational methods, precision medicine, and clinical applications.

1. INTRODUCTION

Peptides are the small molecules that are highly effective in targeting specific areas in the body while being safe to use. With around 140 different peptides currently being tested the scientists are exploring new ways to design them such as making them show multiple effects and combining them with other drugs. Peptides therapeutics are the prominent

medicines due to their excellent effectiveness, safety, affordability and ease of production when compared to other medicines.[1]

Researchers are also developing some methods to enhance the durability within the body. The peptides are becoming more and more important in treating various disorders like cancer, hormonal disorders, pain management, bacterial disorders, skin disorders, cardiovascular, and neurological disorders. Around40 years ago the function-blocking monoclonal antibodies were suggested as potential cancer treatments.[2] For example, synthetic human insulin has been recognized for its clinical effectiveness for people with diabetes.[3] Peptides are a different class of pharmaceutical compounds due to their unique therapeutic properties when compared to small molecules such as antibodies and proteins. Peptides have been engineered as drugs to inhibit the protein-protein interactions and intracellular molecules such as receptor tyrosine kinases. The development of this therapeutics has become a prominent field with around 20 new peptide-based clinical trials initiated annually.[4] At present, there are 400+ peptide drugs in global development with 60+ approved for clinical use in major markets like the EU, Japan, and U.S.A.[5] Protein-protein interactions are important to almost all cellular processes such as regulating activities that range from ion transportation to transcription and post-translational modifications of proteins.[6]

1.1 Development of Peptide Therapeutics in Past and Future

The introduction of Insulin was a great innovation in medical discoveries and this innovation reshaped the modern pharmaceutical industry. The sequence modifications does with each type of insulin derived drugs is listed in the Table No. 1. With the help of DNA Recombinant and Protein Purification technologies, this human insulin was replaced by animal tissues derived insulin. Insulin has been ruling market for around 90+ years. There is around 30 peptide therapeutics that has been approved just in past two decades.[7]

Table No. 1: The sequence modifications in insulin derived drugs.

Type of Insulin	Insulin Derived Drugs	Sequence Modifications
Short-acting Insulin	Insulin aspart	B 28: Asp
	Insulin glulisine	B 3: Lys B 29: Glu
	Insulin lispro	B 28: Lys B: 29: Pro
Long-acting Insulin	Insulin degludec	B 29: Lys linked with hexadecanedioic acid
	Insulin detemir	B 29: Lys linked with myristic acid
	Insulin glargin	A 21: Gly addition to 2 Arg to C-terminus of B-chain.

Peptide medicine sales are expected to exceed 70 billion USD in next three years according to global market research on peptide treatments which projects a CAGR of 4.1%.[8] On the other hand, the industry's healthy expansion is probably due to the anticipated rise in the prevalence of cancer and metabolic illnesses. Sales of the top-selling peptide medications for metabolic disorders, such as GLP-1 and liraglutide exceeded 70 billion USD annually.[9] Over four billion USD in sales were also attributed to popular peptide medications, such as leuprolide, gosarelin and somatostatin analogues, octreotide and lanreotide.[10]

2. Peptide Drug Design

With the help of Peptide Drug Design, the development of peptide medications is a vivacious and diverse subject. Peptides are therapeutic agents with great specificity, efficacy, and comparatively low toxicity. These are made up of amino acids bound together by peptide bond.[11] To improve the effectiveness and pharmacokinetic characteristics of

peptide-based medicines, major developments have been made in computer modelling, high-throughput screening techniques, and some considerations. An overview of the peptide drug design emphasis the significance in the creation of cutting-edge therapies for a variety of disorders and disabilities.[12]

Some of the methods used for peptide drug design are listed below:

2.1 Computational Methods

Peptides have been used more often to target extracellular proteins than intracellular proteins because of their typically low permeability of cell membranes. To successfully target intracellular PPIs this constraint must be overcome.[13] Modifying hydrophobicity and electrostatic charges to promote passive absorption or attaching the therapeutic peptide to a CPP for active transport are two methods to improve intracellular uptake. Increased water solubility of peptide therapies enhances their bioavailability greatly and makes it easier to maintain effective serum concentrations.[14] To preserve bioactivity while adjusting the isoelectric point, hydrophobic amino acids can be substituted with charged or polar residues in an empirical process known as optimisation of solubility.[15] To speed up this optimisation process, two machine-learning bioinformatic tools have recently been developed: PROSO-II and ccSOLomics. Large-scale solubility predictions across proteomes and the identification of soluble themes within amino acid sequences are made possible by ccSOLomics. However, PROSO-II makes use of SVM learning to forecast solubility in accordance with physiochemical characteristics such as hydrophilicity, hydrophobicity, and propensities for secondary structures. These developments might expedite the development of peptide therapeutics and improve their suitability for targeting intracellular targets.[16]

2.2 HTS for New Peptide Leads

The refinement hits found in a screen of 5.7 million bicyclic peptides targeting oncogenic K-RasG12V led to the identification of the Ras-Raf bicyclic peptide inhibitor. Although the discovery of PPI-inhibitors has been accelerated by high-content combinatorial library screening, peptides with lower inhibitory efficacy may remain undetected.[17] Even still, even little interactions are important to consider since cyclization or sequence optimisation can greatly increase affinity. The discovery of weaker contacts has been strengthened by the repetitive "biopanning" enrichment process in phage display library screening; this technique was recently recognised with the Chemistry Nobel Prize.[18] Over the last three decades, phage display and recombinant DNA technologies have revolutionized the identification and refinement of lead peptides against various biological targets.[19] Although traditional biopanning rounds are prone to biases and false positives NGS analysis has mitigated these issues by offering quantitative and sensitive insights, reducing the necessity for multiple cycles. While the low cycle number demands relatively strong interactions, recent advancements have expanded the horizon of phage-displayed libraries.[20] Innovations now permit on-phage chemical modifications, including cyclization linkers, fluorophores, small molecules, and post-translational modifications like glycosylation. These modern biopanning techniques advocate for the identification of lead peptides with higher affinity and genuine bioactivity, which can then be subjected to rational sequence optimization and modifications for clinical development.[21]

2.3 Sequence-Structural Predictions

In order to calculate binding free energies and improve predictions of binding affinities between interacting proteins, a variety of computational docking strategies, in particular flexible-body docking methods, rely on structural information such as the number of hydrogen bonds, buried surface area, mutation hotspots, geometric angles, and allosteric

effects.[22] Sequence-based techniques, on the other hand, predict binding affinity by using sequence and functional data from publically accessible databases. For example, PPA-Pred created a model based on sequence characteristics that categorised protein-protein complexes according to biological roles and the proportion of binding residues for the purpose of predicting affinity.[23] Sequence-based models can be improved over time using dataset updates and machine learning approaches, even if they could provide less accurate predictions and are unable to anticipate conformational binding poses. Small experimental datasets and inadequate processing capacity impede development despite improvements in scoring functions from both methods.[24] Computationally predicted and empirically determined protein complex architectures are compared in community-wide experiments such as CAPRI to assess prediction accuracy. Among the best prediction servers are HADDOCK and ClusPro, which use rigid-body docking techniques to compute binding free energy and buried surface area with excellent accuracy based on RMSD.[25]

3. Innovations and Computational Methods for PPIs

Unlike protein-protein interactions, the accuracy of PepPIs is often limited by the availability of structural information, either for the target protein alone or in complex with a ligand.[26] The key features of these docking features are conformational sampling, binding energy scoring and many more as listed in Table No. 2. Due to the scarcity of protein co-structures, researchers frequently turn to databases like the PDB to identify sequence-binding motifs for peptide design. Another valuable resource is PepX, housing over 500 experimentally studied peptide interactions with high-resolution structures, facilitating user-defined peptide template inputs.[27] Analyses of mutation hotspots at protein-peptide interfaces indicate that peptides typically containing 6-11 amino acids often establish 2-3 critical contacts with the target protein. PepPI studies, albeit similar to protein-protein interaction modelling, are more complicated because of the possibility of structural alterations brought about by flexible side-chains and backbones in peptides.[28] Short peptides up to around 15 residues often form easier-to-predict α-helix or β-sheet structures, while backbone rearrangements make it harder to forecast longer peptide structures. Moreover, taking the target protein's conformational flexibility into account complicates the prediction of peptide structural complexity.[29] These issues have led to the development of current computer models for more dependable peptide medication designs against PPIs. We will go over these models and emphasise their salient characteristics in this part; Table 2 gives a summary.

Table No. 2: The key features and type of docking of various docking methods.

Sr. No.	Methods	Key Features	Type of Docking	References
1.	Dyna Dock	OPMD with a core potential. Faster conformation samplings. Full flexibility of target protein and the peptide.	Local Docking	[30]
2.	Clus Pro Pepti Dock	Clustering by structure scoring and CAPRI peptide. FFT-based docking method. Motif-based prediction for peptide conformation.	Global Docking	[31]
3.	HADDOCK Peptide Docking	Binding free energy scoring. Fully flexible for interacting residues of peptide and protein. User-defined residues at binding pocket.	Local Docking	[32]
4.	Pepsite2.0	Coarse-grained peptide orientation by spatial position-specific scoring matrix (S-PSSM). Generates low-resolution model of peptide. Identifies most peptide-binding site in seconds.	Global Docking	[33]
5.	Pep Crawler	Full flexible structures. Motion planning based samplings. RRT Algorithm	Local Docking	[34]

6.	Galaxy Pep Dock	Energy-based model optimization and scoring. Superior accuracy using PeptiDB datasets than other servers. Use similarity search (known template structures) as scaffolds for prediction.	Template-based Docking	[35]
7.	Rosetta Flex Pep Dock	High quality conformational sampling. Hotspot Residue. Monte-Carlo based optimization.	Local Docking	[36]

3.1 Computational Docking Strategies

Drug design has been completely transformed by computational PPI docking, which provides quick atomic-level insights. Some approaches can finish calculations in a matter of minutes.[37] A popular approach is rigid-body docking, which optimises the chemical and geometric orientation fit of the two interacting proteins by treating them as totally rigid entities. This strategy is demonstrated by Z-DOCK, which produces precise PPI predictions when backed by suitable scoring systems. Nonetheless, the abundance of scoring criteria in flexible docking techniques has resulted in the creation of several docking programmes across time.[38] Interestingly, ATTRACT is a strong PPI prediction server that provides extensive toolkits with several score criteria, but it has a higher learning curve for users.[39]

3.2 Docking Methods

In terms of the interaction complex, the presence of structural scaffolds is critical to the effective docking of a PepPI. The development of more reliable docking and refinement techniques for precisely predicting PepPIs has been greatly aided by the notable increase in the number of peptide-protein structures stored in the PDB.[40] These docking systems may be broadly divided into two types according on the amount of structural information that is supplied as inputs: local docking and global docking.[41]

3.3 Local and Global Docking Methods

The most common approach in peptide-protein interaction research is local docking, which finds possible positions for a peptide to attach inside a user-specified binding region on the solved structure of the target receptor.[42] Many techniques are quite good at creating structural similarities within 1 to 2 angstroms RMSD of the experimental peptide conformation, which is an excellent way to improve the quality of the original model at atomic resolution.[43] Popular techniques include PepCrawler, Rosetta FlexPepDock, and DynaRock, which all use different strategies to identify peptide-binding sites. By gradually bringing the soft-core potential closer to the physical potential, DynaDock uses Molecular Dynamics in conjunction with a soft-core potential to optimise materials and accelerate conformational sampling.[44] Based on Monte Carlo methods, Rosetta FlexPepDock offers excellent conformational sampling for well-defined binding motifs containing hotspot residues. PepCrawler optimises peptide structural poses at binding sites using RRT algorithmic robotics motion planning. The generated models are then automatically clustered based on local shape analysis of the energy funnel.[45]

However, not all query peptides possess available backbone conformation information, necessitating sampling methods to acquire near-native peptide conformations before local docking.[46] For instance, Rosetta FlexPepDockab initio combines ab initio peptide folding with local docking by placing the query peptide into a user-defined binding site, even from arbitrary backbone conformations. Alternatively, the HADDOCK method explores local docking without prior backbone information, using canonical secondary structure ensembles constrained to defined binding sites.[47] Furthermore, small molecule docking methods like Gold, Surflex, and AutoDockVina have been applied for local

docking of short peptides, although sub-optimal results have prompted innovative approaches like DINC 2.0, which docks peptide fragments to overcome limitations in modeling accuracy.[48]

4. Template-Based Docking Method

The known structures that already developed are used as template scaffolds in template-based docking techniques, sometimes referred to as comparative docking strategies. To create a model of the interaction complex, they thread the target protein's or query peptide's sequence onto these templates.[49] Because of the quick accumulation of peptide-protein structures in the PDB which has greatly sped the development of simulation algorithms and design methodologies, this approach has arisen as a new category in peptide-protein docking. A well-known server called GalaxyPepDock uses similarity-based docking to improve predictions of structural flexibility within interacting complexes.[50] It does this by finding templates that have the highest similarity and building models through energy optimisation.[51]

Using PeptiDB datasets from the CAPRI, GalaxyPepDock significantly outperformed other servers in blind prediction trials. Recently, peptide-protein binding residues and sites have been reliably and consistently predicted using SPRINT-Str, a machine learning-based technique that makes use of experimental structural data.[52] In order to robustly predict interacting residues in peptide-binding domains from target protein sequences, PBRpredict, an alternative technique, combines template-based and machine learning approaches.[53] Models learned from peptide-binding residues across diverse domain types are used in this model. Methods for predicting PepPIs are increasingly using computational machine learning techniques to optimise grouping and scoring, similar to those used in CPP prediction services.[54] PepComposer, a freely available web application for computational peptide-protein design, incorporates a machine learning method into Pyrosetta to provide completely automated computational peptide design with verified and repeatable predictions of well-known PepPIs.[55]

5. Clinical Applications of Peptides

Peptides are now known to be adaptable therapeutic agents with potential uses in a variety of medical fields. They provide focused treatment for a broad range of illnesses and conditions.[56] The main therapeutic areas of peptide therapeutics are the cancer therapy, endocrine diseases, antimicrobial therapies, neurological problems, and immunomodulation.[57] Peptides are extremely specific and versatile, which makes them useful in precision medicine. They may be used to precisely target cancer cell receptors, regulate hormone imbalances, and fight infectious organisms. Research is still running strong, opening up new therapeutic options in developing domains, spurring innovation and improving patient results.[58,59]

- **Peptides in Cancer Therapy**

The developments in immunotherapy and biomarker identification are changing the face of cancer treatment. With the FDA's approval of Sipuleucel-T the first cancer vaccine to target castration-resistant prostate cancer, a breakthrough in the treatment of prostate cancer was achieved. The vaccine works by boosting the patient's immune system to fight cancer cells.[60] The peptide drugs for both diagnosis and treatment of the cancer are listed in the Table No. 3 and Table No. 4. Clinical trials are investigating the effectiveness of peptide vaccines in the meanwhile, with the goal of inducing an immune response against certain antigens in prostate cancer cells.[61] Monoclonal antibodies that target the HER-2 protein, which is overexpressed in a considerable number of patients, such as trastuzumab and ado-

trastuzumabemtansine, have revolutionised the treatment of breast cancer. Peptide vaccines are being researched as possible therapies for breast cancer, particularly in instances when the disease is resistant to chemotherapy.[62]

Peptides that target underglycosylated MUC-1 protein, including the FITC-labeled peptide EPPT1, are the focus of colorectal cancer research. Serum C-peptide levels are linked to an increased risk of adenoma, indicating their potential use as diagnostic biomarkers for colorectal cancer.[63] Peptides are promising diagnostic indicators for lung cancer that may be used to distinguish NSCLC patients from controls. These biomarkers provide opportunities for prognostic evaluation and early identification. Similarly, uMMP-2 and uTIMP-1 are suggested diagnostic indicators for pancreatic cancerPeptide vaccines, which provide tailored immune responses against cancer cells, have the potential to cure pancreatic cancer as well. Current clinical trials are being conducted to assess their effectiveness in enhancing the quality of life and survival rates of patients with advanced pancreatic cancer.[64]

Table No. 3: Peptides used for Cancer Diagnosis.

Peptide for Cancer Diagnosis			
Type of Cancer	**Peptide**	**Year**	**References**
Breast Cancer	HER-2	2014	(65)
	MUC-1	2011	(66)
Colorectal Cancer	CPAA-783-EPPT1	2012	(66)
	HNP1-3	2006	(67)
	Serum-C-peptide	2014	(68)
Lung Cancer	11 Novel Peptides	2014	(69)
	C-peptide (Serum)	2014	(70)
	HCBP-1	2014	(71)
Prostate Cancer	EN-2	2013	(72)
	UCP-2	2013	(73)
Pancreatic Cancer	MIC-1/GDF15	2014	(74)
	RGS6	2014	(75)

Table No. 4: Peptides used for Cancer Treatment.

Peptide for Cancer Treatment			
Type of Cancer	**Peptide**	**Year**	**References**
Breast Cancer	E75	2014	(76)
	ErbB-2	2014	(77)
	p5	2014	(78)
Colorectal Cancer	ANP	2012	(79)
	F-56	2014	(80)
	TCP-1	201072	(81)
Lung Cancer	BPP	2014	(82)
	LFC-31	2014	(83)
Prostate Cancer	PAP-114-128	2014	(84)
Pancreatic Cancer	GV-1001	2014	(85)
	KIF-20A	2014	(86)
	WT-1	2014	(87)

- **Peptide Hormones and Endocrine Disorders**

The development of oral peptide treatments has mostly focused on endocrine problems with the goal of overcoming the drawbacks associated with parenteral delivery.[89] Oral administration has been developed for a number of hormone analogues, including calcitonin, insulin, somatostatin, PTH, thyroid hormone-releasing hormone, uroguanylin, and GLP1.[90] Notably, octreotide, an octapeptidesomatostatin analogue, has been developed as an injectable treatment for endocrine tumours and acromegaly having a high affinity for SSTR2 and a moderate affinity for SSTR5. Nevertheless,

problems including poor absorption and gastrointestinal tract degradation make it difficult to achieve efficient oral administration.[91]

- **Antimicrobial Peptides**

The fish farms produce about half of the world's fish consumed, aquaculture has grown exponentially in the last several decades to become the fastest-growing food production industry. However, as a result of extensive aquaculture operations, disease outbreaks have emerged.[92] These outbreaks are mostly caused by bacteria or viruses, and they have significantly increased production losses and mortalities. Although immunisation is frequently used to prevent illness in some fish species, especially salmonids, effective vaccines against other fish species and diseases are still undiscovered.[93] As a result, antibiotics are widely used by fish producers for both preventative and therapeutic purposes. However, the overuse of antibiotics in animal agriculture has accelerated the rise of microbes resistant to antibiotics, which poses a major threat to public health.[94]

Furthermore, antibiotics cannot cure viral infections; this emphasises the urgent need for alternative therapies. AMPs have shown great promise for the aquaculture sector because of their antibacterial qualities, immunomodulatory functions, and decreased risk of bacterial resistance emerging. Fish have an amazing variety of AMPs, including as fish-specific piscidins, cathelicidins, hepcidins, and beta-defensins. Hepcidin, formerly known as LEAP-1 in mammals, is an essential component in the regulation of iron metabolism because it inhibits ferroportin, an iron exporter, post-translationally.[95] Hepcidin has been proven in several studies to be active against a variety of infections in vitro. It has also been established that treating fish in vivo with hepcidin increases fish survival and lowers viral or bacterial burdens.[96]

- **Peptides in Neurological Disorders**

The discovery of the ADNP protein, known as Activity Dependent Neuroprotective was an initiate to the discoveries of neurological action of Peptides therapeutics. Protein has greatly expanded our knowledge of neurodevelopmental processes and how they relate to neurological illnesses.[97] ADNP is a key player in embryogenesis, controlling the expression of more than 400 genes and interacting with chromatin remodelling complexes through factors such as VIP.[98] Analyses on mouse embryos lacking in ADNP have shown the critical function that this protein plays in the closure of the neural tube and the development of the brain, demonstrating that it affects more than only the central nervous system.[99] Autism spectrum diseases, schizophrenia, and Alzheimer's disease have all been related to ADNP dysregulation. By using mechanisms like microtubule stabilisation and anti-inflammatory effects, derived peptides such as NAP demonstrate neuroprotective properties and hold promise for symptom relief in a variety of neurological conditions, such as ALS, Parkinson's disease, and neurodevelopmental disorders like schizophrenia and foetal alcohol syndrome.[100]

- **Immunomodulatory Peptides**

A variety of activities, such as antioxidative, antihypertensive, and immuno-modulatory qualities, may be found in plants. Commonly consumed plants, such as wheat, rice, maize, and soybean, have been the subject of substantial research about their potential health benefits[101] Amidst the COVID-19 epidemic, traditional medicinal plants like as amaranth, quinoa, and chia are seeing a resurgence of attention due to their immuno-modulatory peptides.[102] Peptide-based vaccinations are becoming more and more popular, despite ongoing difficulties with standardisation and

effectiveness. Plant-derived chemicals are being investigated as possible adjuvants and bioreactors for affordable edible vaccines.[103]

Vaxin-PAD and other similar tools are useful in the development of vaccines against different viruses, but care must be taken to avoid unintended consequences on signal transduction and cytokine generation. Utilising peptides generated from plants, particularly wheat, may be able to cure autoimmune disorders like celiac disease, while newer types such as cyclotides and cystine knot peptides may be able to treat diseases like multiple sclerosis.[104] Nevertheless, addressing noted in vivo adverse effects is necessary to convert these peptides into effective therapeutics. If mechanisms of immuno-modulatory activity and testing reliability are better understood, then expanding research into food-derived bioactive peptides from plants, including novel species and using food waste for peptide extraction, offers opportunities for sustainable food production and the discovery of new therapeutic agents.[105]

CONCLUSION

In conclusion, the peptide treatments have advanced remarkably and offer a plethora of prospects in the realm of modern medicine. The complex relationships that occur between medication formulation, delivery methods, and design demonstrate that how dynamic this area is. With the help use of these cutting-edge technologiesand computational methodologieswe canexplore the novel ideas for peptide based medicines that can be used to treat a wide range of disorders like cancers and neurological disorders. Peptides provide specialised methods that canhelp in patient care from these treatments for neurological conditions to targeted cancer treatment. Peptide treatments are used to take the lead as the era of personalised these medicines, transforming the healthcare with their efficacy, precision and therapeutic accuracy.

Abbreviations

1. **PPIs:** Protein-Protein Interactions.
2. **USD:** United States Dollar.
3. **CAGR:** Compound Annual Growth Rate.
4. **GLP-1:** Glucagon-Like Peptide 1.
5. **USA:** United States of America.
6. **EU:** Europe.
7. **SVM:** Support-vector machine.
8. **HTS:** High-throughput screening.
9. **NGS:** Next-generation sequencing.
10. **HADDOCK:** High-Affinity DNA-binding Domain.
11. **PPA-Pred:** Protein-Protein Association Prediction.
12. **CAPRI:** Critical Assessment of Predicted Interactions.
13. **ELISA:** Enzyme-linked immunosorbent assay.
14. **PDB:** Protein Data Bank.
15. **PDC:** Peptide-Drug Conjugate.
16. **siRNA:** Small Interfering RNA.

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