Structural basis for the identification of short novel peptides as potential GLP1R agonists using in silico approaches

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Abstract

Glucagon-like peptide-1 receptor (GLP-1R) agonists are effective anti-diabetic drugs that improve energy balance by increasing glucose-dependent insulin production. Currently numerous GLP-1 peptides are marketed and few small molecular agonists are in clinical trial stage. GLP-1 is a 31 amino acid peptide and the marketed peptides are even larger or of similar length residue peptides. This prompted our research group to identify novel short peptides mimicking the N-terminus portion of native GLP-1 peptide as this portion residues have proven role in stabilizing the active conformation to trigger intracellular signalling of GLP-1 receptor. In this work, all the nine residues starting from His7 to Asp15 has been selected as the template and single point mutation of the nine amino acid peptide has been done and a total of 181 peptides are designed. Protein peptide docking analysis of these designed peptides gave us thirty-seven potential peptides with good binding affinity followed by hydration site analysis eventually leading us to seven promising peptides that can be further optimized to mimic the long 31 residue GLP-1 peptide. In fact, a hybrid peptide “WGYLQRNDD” was designed taking all the vital residues from each position from 7 to 15. Also, the hydration site map analysis suggested the role of hydration sites in designing the GLP-1receptor peptide agonists. This work highlights a strategy of peptide designing followed by screening coupled with chemical exploration of residues along with crystal water molecules that could be used to generate novel agonists for other receptors with peptide ligands.

1) Introduction

Diabetes is a chronic, metabolic disorder characterized by increased blood glucose levels that causes serious damage to the heart, blood vessels, eyes, kidneys, and nerves over a period of time. The most common is Type 2 diabetes, usually in adults, which occurs due to insulin resistance or insufficient insulin production **(Shoback D et al. 2011).** Type 1 diabetes is a chronic condition in which insulin production is lowered or stopped by the pancreas **(Chiang J et al. 2014).** Access to affordable treatment, including insulin, is very critical for diabetic people for their survival **(Chiang J et al. 2014).** According to IDF (International Diabetes Federation), diabetes is one of the leading global challenges to the health and well-being of mankind. The current data suggests that approximately 537 million adults (20–79 years) are living with diabetes and this number is expected to reach 643 million by 2030. Diabetes has caused 6.7 million deaths in 2021, i.e., one in every five seconds. An expenditure of around 966 billion USD has been reported so far over the last 15 years (IDF Diabetes Atlas 2021). The prevalence of Type 2 diabetes is rising steadily worldwide and along with it comes various macro and microvascular complications **(**Cade WT 2008**).** The macrovascular complications include cardiovascular disease, myocardial infarction, cerebrovascular disease, peripheral artery disease; whereas the microvascular complications include diabetic retinopathy, neuropathy, and nephropathy **(**Cade WT 2008**).** Numerous therapeutic agents are available in the market but many patients fail to achieve their target values for glucose concentration in blood. The development of multiple new therapies has widened the options for patients to achieve their desired blood glucose level and cure other related complications. Incretins are gut-derived hormones,

principally GLP-1 and glucose-dependent insulinotropic peptide (GIP), secreted at low glucose levels in the fasting state **(**Drucker DJ et al. 2010**).**

Glucagon-like peptide-1 is a gut-derived peptide hormone that prevents hypoglycemia by inducing insulin production in a glucose-dependent manner **(**Nauck M A et al. 2002**).** GLP-1 has gained prominence since its discovery in the early 1980s **(**Bell G I et al. 1983; Lund P K et al. 1982**)** due to its unique anti-diabetic action **(Perry T** et al. **2003)** and pleiotropic effects in multiple organs **(Drucker D** et al. **2006, Brubaker P** et al. **2004**, Nauck M A et al. 1993, Wettergren A et al. 1993, Flint A et al. 1998, Zander M et al. 2002, Moreno C et al. 2002, During M J et al. 2003, Bose A K et al. 2005,). Glucagon-like peptide-1 is a 31 amino acid peptide hormone derived from the tissue-specific posttranslational modification of the proglucagon peptide. It is produced and secreted by L-cells of the pancreas and neurons of the solitary tract of the brainstem with food consumption. Proteolytic cleavage and amidation of GLP-1 (1–37) result in two shortened and physiologically active equipotent peptides, GLP-1 (7–36) amide and GLP-1 (1–37) amide (7–37). The active GLP-1 is made up of two α-helices with amino acids 13 to 20 and 24 to 35 positions connected by a linker region **(Drucker DJ et al. 2018).** The GLP-1 peptide agonist binds to its receptor; this leads to a chain of reactions involving dissociation of γ, β, and α subunits from the heterotrimer G-protein. The α subunit and attached GTP then attach to Adenylyl cyclase leading to its activation. When glucose is ingested, it produces ATP, which causes cAMP stimulation, which in turn activates Protein Kinase A. When PKA is activated, K^+ ions are ejected and Na^+ ions are influxed. In addition, cAMP activates the CREB protein, which initiates the transcription and translation of proinsulin into insulin. Also, membrane depolarization leads to Ca^{+2} ions influx that leads to increased insulin secretion, causing homeostasis of blood glucose level **(Verspohl E et al. 2009).**

There are many approved GLP-1 agonist marketed till now that includes Exenatide (contain 39 amino acids and having 53 percent similarity to native GLP-1), Liraglutide (contain 31 amino acids with 97 percent homology to native GLP-1), Dulaglutide (contain 31*2 amino acids with 90 percent homology with native GLP-1), etc. (Yu M et al. 2018). (See Table 1)

Table 1
Representing the marketed GLP-1 agonists. (Nauck MA et al. 2021

GLP-1 AGONIST	Brand name	Administration	Company
Exenatide	Byetta	Twice daily	AstraZeneca
Lixisenatide	Adlyxin	Once daily	Sanofi
Liraglutide	Victoza	Once daily	Novo Nordisk
Dulaglutide	Trulicity	Once weekly	Eli Lilly and Company
Albiglutide	Tanzeum	Once weekly	GlaxoSmithKline
Semaglutide	Ozempic	Once weekly	Novo Nordisk

These peptides are all between 30 and 64 amino acids long. As a result, an approach for designing shorter peptides can be investigated. Short peptides are now widely regarded as a viable scaffold for a variety of medicinal and diagnostic applications (Apostolopoulos V et al. 2021). When compared to their larger counterparts, short peptides have numerous advantages, including chemical diversity, ease of modification, increased bioactivity, absorption, accessibility, finely tuned functionalization, and cost-effective synthesis on a small and large scale (Apostolopoulos V et al. 2021, Soudy R et al. 2019). Research groups like Hashemi et al. 2021, Dhanda et al. 2017 employed computational methods to develop peptides for therapeutic targets. The goal of this research is to design potential short peptides for GLP-1 receptor that would resemble the N-terminal portion of GLP-1 peptide having similar binding characteristics.

2) Methodology

2.1 Design of short peptides

The 31 amino acid natural GLP-1 peptide was trimmed to 9 amino acid residues (7–16) and utilized to design small peptides. Each of the 9 amino acid residues was replaced with the remaining 19 amino acids to create a library of short peptides. All the 171 short peptides were designed using the “Build biopolymer from sequence” wizard of the BioLuminate module of the Schrödinger package (**Schrödinger Release 2022-4: BioLuminate**). The input type for Biopolymer was chosen as “peptide” followed by sequence as the input and select “alpha-helix structure” as the shape for the peptides to be designed.

2.2 Protein and peptide preparation

The protein structure of the GLP-1 receptor with PDB ID 6X18 was obtained from the protein data bank (https://www.rcsb.org). The obtained receptor and designed peptides were prepared using the Protein Preparation Wizard utility in Maestro (**Schrödinger Release 2022-1: Protein Preparation Wizard**). The structure preparation involved adding hydrogen atoms, optimizing the hydrogen bonds, removing atomic clashes, adding formal charges to the hetero groups, and then optimizing it at neutral pH. An Optimized Potential for Liquid Simulations-4 (OPLS-4) force field was used to minimize the prepared structures (Lu C et al. 2021). The assignment of rotameric states for peptide residues with polar hydrogen atoms, such as Ser, Thr, Tyr, Asn, and Gln, as well as the orientations of water molecules, is a critical step in this preparation in order to maximize the hydrogen bonding network in the protein structure. The tautomeric/ionization states of His residues were also analyzed using the same criteria, and the PROPKA algorithm is used to predict the protonation states of ionizable residues (Bas DC et al. 2008). In addition to protein preparation, the tautomeric and ionization states of the peptide must be determined, and for peptides containing His residues, docking numerous copies of the same peptide with distinct tautomeric states may be necessary. Finally, the peptide termini were capped by ACE (acetyl group) and NMA (N-methyl amide)

2.3 Protein peptide docking

Piper was used to tightly dock two proteins or a peptide to a protein. It is part of the Schrödinger Suite, which runs the BioLuminate program's Protein-Protein Docking panel. Piper samples the relative orientation of the input peptide (in a fixed conformation) to the protein, which requires either (a) knowledge of the peptide structure from experimental techniques such as NMR or X-ray crystallography (and only if the peptide can be reasonably described by a single conformer) or (b) docking of multiple pre-calculated peptide conformations and aggregating the results. Piper detects and ranks probable docked protein poses in two stages: conformational sampling and structural grouping. For the first phase, Piper employs a fast FFT (Fast Fourier Transform) technique, allowing it to evaluate a large number of postures in a computationally efficient manner, reducing the required compute time (on a single processor) from days to hours. To generate and evaluate relative positions, a basic atomistic energy function is used, which efficiently differentiates potentially acceptable poses from those that are highly unlikely. 70,000 postures are typically examined, with the top 1000 scoring poses being saved for the following phase. In the second step, the 1000 poses are clustered based on the structure. The member with the closest neighbors is then picked as the cluster's representative from each cluster. In this program, we provided a produced protein and peptide as input and then used the Schrödinger BioLuminate module to search for the 30 best complexes out of 70,000 (the default numbers) potential protein-protein configurations without limitations (Kozakov D et al. 2010, **Shen Y et al.2007)**.

2.4 WaterMap

WaterMap was calculated using the prepared protein 6X18 using the default settings. For the WaterMap calculations presented here, the protein was truncated to a region within 15 Å of the ligand and the resulting system was solvated in a TIP4P water box extending at least 5 Å in all directions. A restraint was applied to the protein heavy atoms and the system was relaxed with an initial minimization followed by a short molecular dynamics simulation heating the system from 10 K to 300 K. The binding site was then filled with additional water molecules using the solvate_pocket stage in WaterMap, which consists of 100,000 steps of grand canonical Monte Carlo (GCMC) simulation to populate the binding site with a realistic number of water molecules. A final pre-production simulation of 120 ps was run at 300 K. The production simulation was run for 2 ns at 300 K in the NTP ensemble. (**Schrödinger Release 2022-1: WaterMap**).

3) Results And Discussion

3.1 Structural analysis of native GLP-1 peptide

According to the literature study (Adelhorst K et al. 1994, Hareter A et al. 1997), His7, Gly10, Phe12, Thr13, Asp15, Phe28, and Ile29 are important for receptor binding, while His7, Gly10, Asp15, and Phe28 are important for receptor activation. These findings suggest that GLP-1's N-terminal portion is involved in receptor activation and engagement, whereas the C-terminal area is only involved in receptor binding (Fig. 2). As a result, it was more reasonable to construct short peptides by selecting critical N-terminal

residues. We chose nine residues from His7 to Asp15, and five of the nine residues are crucial for receptor activation and binding.

3.2 Structure Analysis of GLP-1 receptor

GLP-1 receptor belongs to the G protein-coupled receptors Class B glucagon receptor family (Brubaker P et al. 2002). GLP1R is made up of two domains: an extracellular (ECD) domain that binds to GLP-1's C-terminal helix (Underwood C R et al. 2010), and a transmembrane (TMD) domain (Song G et al. 2017) that connects to GLP-1's N-terminal area (Wootten D et al. 2016a, Wootten, D et al. 2016b). (Fig. 3).

Numerous protein structures are available in the protein data bank (Berman H M et al. 2000) and these structures are either in complex with a ligand or in apo form. There are some crystal structures available for the extracellular domain of the GLP-1 receptor and the whole structure of the receptor is also available in the protein data bank. Table 2 enlists the complete structures available for GLP-1 with their PDB IDs, resolution, ligand complexed, and the method used to determine the structure.

Table 2
List of some Protein structures available for GLP-1 receptor complex in Protein Data Bank (Berman H M et al. 2000

Sr. No	PDB ID	Resolution (Å)	Ligand	Method
1	6X1A	2.50	PF-06882961 (small molecule)	EM
2	6X18	2.10	GLP-1 peptide	EM
3	6X19	2.10	CHU-128 (small molecule)	EM
4	6B3J	3.30	Exenidin peptide	EM
5	5NX2	3.70	Truncated peptide	XRD
6	6VCB	3.30	GLP-1 peptide and positive allosteric modulator	EM
7	5VEW	2.70	PF-06372222 (small molecule)	XRD
8	5VEX	3.00	NNC0640 (small molecule)	XRD
9	6XOX	3.10	LY3502970 (small molecule)	EM
10	6ORV (NA)	3.00	TT-OAD2 (small molecule)	EM

3.3 Protein peptide docking

To confirm the active pose for the suggested short peptides, the wild-type short peptide was docked on 6X18 and then overlaid on a co-crystallized GLP-1 native peptide. Furthermore, the docking analysis of the wild-type short peptide provided multiple reference points that aided in the selection of the best-mutated peptides from a total of 171 generated short peptides. The PIPER pose energy and PIPER pose score for the wild-type peptide was − 588.378 kcal/mol and − 170.024 kcal/mol respectively. It was

observed that His7 formed H-bond with Gln234, interacted with Arg310 via water mediated H-bond (HOH103) and formed π- π stacking with Trp306. Similarly, all the interactions ae shown in Fig. 5. **Tyr 152, Arg 190, and Tyr 241** have been marked to be important in activating the GLP-1 receptor at the peptide binding site and stabilizing the peptide inside the pocket by keeping the active receptor conformation (**Lie et al. 2018**, Wootten D et al. 2016, Wootten D et al. 2016). The peptides for the following investigation were chosen based on criteria such as a) docked pose, b) important interactions, and PIPER pose score. The following conclusions were drawn after a thorough examination of individual peptide residues.

His7

Histidine is a polar residue with a cationic side chain, and Hareter et al. 1997 has discussed the importance of the imidazole moiety in receptor activation and binding. Several intriguing observations were made when His 7 was replaced with 19 different amino acids. The ‘oxygen atom’ of the carboxyl group for Cys7, Gly7, Asn7, Ser7, and Val7 interacted with Tyr152 via H-bond, 'oxyanion' and ‘oxygen' atoms of Asp7 formed salt-bridge and H-bond with Arg190, Benzene ring of Phe7 interacted with Arg190 via -cation interaction, carboxyl group ‘oxygen’ atom of Ile7, Gln7 interacted with Arg190 via the H-bond, NH_3^+ group of Lys7 interacted with Phe369 and Ala368 through π-cation and H-bond interaction respectively, guanidine group of Arg7 attracted a couple of interactions that include the H-bond and salt bridge with Glu364 and Glu387, imidazole ring, ‘oxygen’ atom of the carboxyl group, and NH group of Trp7 interacted with Tyr148, Arg190, and Glu387 via π-π and H-bond interactions respectively, benzene ring of Tyr7 interacted with Glu387 through π-cation interaction and the hydroxyl group of Tyr7 formed H-bond with Val370. All of these findings suggested that the aromatic ring, carboxyl group, and NH group are critical for the receptor's interaction with important amino acids. As a result, thirteen of the nineteen amino acids interacted with receptor residues, emphasizing the importance of the peptide's initial position and its potential to attract multiple connections. Because His7 contains all of these crucial characteristics, mimicking this amino acid group with others is challenging. Among all the nineteen peptides, Trp7 substituted peptide showed the highest PIPER pose energy of -669.755 kcal/mol and PIPER pose score of -224.9 kcal/mol. Therefore, interaction analysis, as well as the pose analysis and PIPER pose scores, indicated that Phe7, Trp7, Tyr7, Asp7, and Arg7 may be substituted for His7. Biological assays, on the other hand, will be necessary to back up the theory. (Supplementary Fig. 1 (a-s) and Supplementary Table 1). Figure 6 shows the 2D interaction diagram for “W^7AEGTFTSD” peptide docked on glp-1 receptor.

Ala8

Alanine is an aliphatic amino acid with a non-polar side chain. In the wild-type peptide, this amino acid had no interaction. However, when we replaced Ala with other amino acids, we found that 'oxygen' atom of Asp8's carboxyl group showed H-bond interaction with Gln234, amine group of Gln8 formed H-bond with Tyr152, guanidine group of Arg8 interacted with Leu388 by forming H-bond, ‘oxyanion’ and ‘oxygen’ atoms of Glu8 showed H-bond and salt bridge formation with Tyr152 and Arg190 respectively, NH_3^+

group of Lys8 interacted with Glu364, Glu 387, and Thr391 via H-bond and salt bridge with Glu364, Hydroxyl group of Ser8 and Thr8 interacted with Thr298 and Gln234 respectively via H-bond formation, benzene ring of Tyr8 interacted with Trp284 through π- π interaction. Eight of the nineteen amino acids were shown to be involved in receptor interactions. Tyr8 contains an aromatic ring and the polar side chain showed the highest PIPER pose energy of -640.496 kcal/mol and PIPER pose score of -170.024. Gly8 did not demonstrate interaction with the receptor, but the peptide interacted with the receptor through all important residues such as Arg190 and Tyr152. In summary, Asp8, Glu8, Ser8, Lys8, and Tyr8 demonstrated improved contacts, poses, and PIPER scores, suggesting that these changes might be employed to test their activity in biological experiments. (Supplementary Fig. 2 (a-s) and Supplementary Table 1). Figure 7 shows the 2D interaction diagram for "HY^{8}EGTFTSD" peptide docked on glp-1 receptor.

Glu9

Glutamate is a polar residue with an anionic side chain and in the wild-type peptide, it attracts H-bond and salt bridge interaction with Lys383 of the receptor. The substitution of Glu9 with other residues showed H- bond interaction of Ala9 backbone NH group with Glu387, guanidine group Arg9 interacting with Ala368 via h-bond, H-bond formation by SH group of Cys9 interacting with Glu387, 'oxygen' atom of the carbonyl group of Gln9, Asn9 interacts with Arg190 via H-bond, NH_3^+ group of Lys9 attracted interactions like H-bond and salt bridge with Glu364 and H-bond with Thr391, main-chain NH group of Ser9 interacted with Glu387 through H-bond, Hydroxyl group of Thr9 interacted with Thr391 through H-bond. 'oxygen' atom of the carboxyl group of Asp9 interacted with Tyr152 and Arg190 via H-bond and salt bridge formation. In one case Tyr9 did not participate in interaction but the peptide obtained all the essential interactions required for receptor binding and showed the highest PIPER pose energy and score of -663.079 kcal/mol and – 267.396 kcal/mol respectively. Here, ten out of nineteen residues interacted with the receptor but Ala9, Asp9, and Tyr9 might be better for potentially replacing polar side amino acid Glu9. (Supplementary Fig. 3 (a-s) and Supplementary Table 1). Figure 8 shows the 2D interaction diagram for "HAY^{9}GTFTSD" peptide docked on glp-1 receptor.

Gly10

Glycine contains an aliphatic side chain and Gly10 did not show any interaction for the wild-type peptide. oxyanion' atom of Glu10 formed π-cation interaction with Lys383, Asn10 amine group formed H-bond with Glu387, guanidine group of Arg10 interacted with Tyr152 via H-bond, 'oxyanion' and 'oxygen' atoms of Asp10 formed salt-bridge and H-bond with Lys197. SH group of Cys10 formed H-bond with Glu387, imidazole ring of His10 formed π- π interaction with Trp306, the amine group of Gln10 formed H-bond with Glu387, the hydroxyl group of Thr10 and Ser10 formed H-bond with Asp372 and Glu387 respectively, benzene ring and imidazole ring of Trp10 formed π- π interaction and H-bond with Trp306 and Trp297, the hydroxyl group of Tyr10 interacted with Arg 190 by forming H-bond. Therefore, eleven out of nineteen residues interacted in place of Gly10. As an outliner, Trp10 and Leu10 itself did not interact with the receptor but the peptide itself nicely interacted with the vital residues. Based on all the vital interactions, poses, and PIPER scores; peptides with Arg10, His10, Tyr10 can potentially replace Gly10.

(Supplementary Fig. 4 (a-s) and Supplementary Table 1). Figure 9 shows the 2D interaction diagram for "HAER^{10}TFTSD" peptide docked on glp-1 receptor.

Thr11,13

Threonine is a polar residue with a neutral side chain. The substitution study of Thr11 gave us the following observations. The backbone carbonyl group of Asp11 interacted with Lys197 via H-bond, the carboxyl group oxygen atom of Asn11 formed H-bond interaction with Arg380, the amine group of Gln11 interacted with Asp372 through H-bond, the backbone carbonyl group of Cys11 formed H-bond with Lys197, 'oxyanion' and 'oxygen' atoms of Glu11 showed H-bond and salt bridge formation with Arg380 and Lys383, imidazole ring and N atom of His11 showed π-π interaction and H-bond with Tyr148 and Tyr152 respectively. It was seen that out of nineteen residues only six made interaction with the receptor. (Supplementary Fig. 5) Similarly, the second Threonine of the peptide was substituted by other remaining amino acids but this time the position changed to 13 and the following were the observations. The guanidine group of Arg13 interacted with Gln140 via H-bond, Asn13 carbonyl group formed H-bond with Arg380, 'oxyanion' atom of Asp13 formed a salt bridge with Arg380, the 'oxyanion' and 'oxygen' atoms of Glu13 showed H-bond and salt bridge formation with Arg380, the benzene ring of Trp13 formed π-cation interaction with Lys197. Hence, Glu11, Gln11, and His11 could potentially replace Thr11 whereas, Asn13 and Asp13 could possibly mimic Thr13. Also, it was found that Gly13, Ala13, and Gln13 did not interact on their own but did bind to the receptor in a fashion that they acquired interactions with all the vital residues like Tyr152, Arg190, and Tyr241. (Supplementary Fig. 5 (a-s) ,7 (a-p) and Supplementary Table 1). Figure 10 shows the 2D interaction diagram for "HAEGQ^{11}FTSD" peptide docked on glp-1 receptor.

Phe12

Phenylalanine is a polar amino acid with an aromatic side chain. The Guanidine group of Arg12 interacts to Tyr148 by forming H-bond, the SH group of Cys12 interacts with Thr298 via H-bond, NH_3^+ group of Lys12 formed H-bond and salt bridge with Tyr148 and Asp198 respectively, the hydroxyl group of Ser12 and Thr12 interacted with Thr298 and Trp306 through H-bond respectively. This meant that only five residues were successful in interacting with the receptor. Residues like Arg12, Cys12, Lys12, and Thr12 can be used for replacement. (Supplementary Fig. 6 (a-s) and Supplementary Table 1). Figure 11 shows the 2D interaction diagram for "HAEGTR^{12}TSD" peptide docked on glp-1 receptor.

Ser14

Serine is a polar residue with an uncharged side chain containing a hydroxyl group. The guanidine group of Arg14 interacted with Glu292 by forming a salt-bridge, 'oxyanion' and 'oxygen' atoms of Asp14 formed a salt-bridge and H-bond with Arg380, N atoms in imidazole the ring of His14 formed two H-bond with Asp372 and Lys 383, benzene ring of Trp14 formed π cation interaction with Arg376, the hydroxyl group of Tyr14 interacted with Arg190 via H-bond formation. Five residues play role in interacting with the receptor, whereas, residues like Arg14 and Asp14 can only be used to replace Ser14. (Supplementary Fig. 8 (a-s) and Supplementary Table 1). Figure 12 shows the 2D interaction diagram for "HAEGTFTD^{14}D" peptide docked on glp-1 receptor.

Asp15

Aspartic acid is a polar residue with an anionic side chain. (a) Guanidine group of Arg15 interacted with Tyr205 and formed π cation interaction, (b) Carbonyl 'oxygen' atom and NH2 group of Asn15 formed H-bond with Lys197 and Ser193 respectively, (c) The carbonyl group of Gln15 formed H-bond with Arg380, (d) The 'oxyanion' and 'oxygen' atoms of Glu15 formed a salt-bridge and H-bond with Arg190 and H-bond with Tyr152, (e) The hydroxyl group of Tyr15 interacted with Tyr152 via H-bond, (f) The benzimidazole ring of Trp15 formed two π cation interactions with Arg190 and also made π-π stacking with Tyr152. Therefore, Arg15, Asn15, Tyr15, and Trp15 can be further studied. (Supplementary Fig. 9 (a-s) and Supplementary Table 1). Figure 13 shows the 2D interaction diagram for "HAEGTFTSW^{15}" peptide docked on glp-1 receptor.

If we summarise this docking study, it is found that many residues can potentially mimic the wild-type residue provided they have similar properties to attract the vital interactions. Below we have shown some of the residues that could potentially mimic the wild-type residues and maintain the interactions nearby. (See Fig. 14) The docking poses of 171 designed peptides was thoroughly analyzed and based on peptide poses, scores, and interactions gave us 37 peptides that can potentially be used for further studies. Out of 37 peptides, five were outliers as they did not show interaction through the mutated residue but as a peptide, they seemed to be quite promising by attracting numerous vital interactions with the receptor. The wild-type 9 residue short peptide contains three non-polar residues namely Ala8, Gly10, and Phe12, and six polar residues His7, Glu9, Thr11, Thr12, Ser14, Asp15. It was observed that positions 7, 9, 12, 15 attracted the greatest number of interactions amongst all 171 designed peptides. Hence, it is quite evident that the role of these positions and the amino acids are essential for receptor binding. Our analysis came up with some potential short peptides as shown in Fig. 13. If we consider polar His7 and Glu9, then both polar and non-polar residues have been able to acquire interactions but the presence of aromatic ring and guanidine group at 7th position and carboxylic side chain at 9th position were vital for the interactions. When Phe12 was replaced by arginine the guanidine group again showed interactions with receptor amino acids. Here, Phe12 being a hydrophobic residue was seen to be replaced by hydrophilic residue giving us an interesting quest to the flexibility of this position as far as the nature of the surrounding residues of the receptor. The polar Asp15 being the most consistent interacting residues of the peptide was found to be conserving the interactions when replaced by Trp15 as it interacted with one of the biologically important residue Tyr241.

3.4 Hydration sites role in binding affinity

The WaterMap calculations aided in the discovery of localised hydration hotspots around the binding cavity of the GLP-1 receptor using thermodynamic energetic parameters like enthalpy (ΔH), entropy (−TΔS), and differential binding energy (ΔΔG). The overlapping hydration sites on ligand functional groups could be classified as displaceable ($\Delta\Delta G \gg 0$ and $\Delta H \gg 0$), replaceable ($\Delta H \ll 0$ and yet $\Delta\Delta G \gg 0$ or $\cong 0$), and stable ($\Delta\Delta G \ll 0$) water molecules from the perspective of drug design based on differential binding energy ΔΔG (Cappel D et al. 2017). In comparison to bulk solvent, the entropy of a hydration site

is always unfavourable near the protein (Pearlstein R A et al. 2010). Since the displacement of high-energy hydration sites from the protein binding site is a driving source of binding affinity, a detailed thermodynamic characterization of hydration sites (HSs) is vital for drug design (**Beuming T et al.2009**). WaterMap calculations were performed using the prepared protein structures 6X18 in this study. Figure illustrates the protein's water map which was produced. For this study, only hydration sites that are within 5 Å of ligand binding sites were analyzed. Hydrophobic areas are recognised as displaceable hydration sites with both ΔΔG and ΔH > > 0 kcal/mol, which can be favourably displaced with sufficient hydrophobic residues of the peptide. It should be emphasised that the displacement of such hydration sites has a significant impact on the peptide's binding. Analysis of the water map of the 6X18 binding site showed ten unstable waters in the vicinity of the peptide, which are displaced by His7, Phe12 and Ser13 side chains. This indicates the role of these residues in increasing the binding affinity of the peptide. The enthalpy (ΔH), entropy (-TΔS) and free energy (ΔΔG) for the ten water molecules, HS36, HS88, HS114, HS119, HS189, HS194, HS224, HS288 and HS315, are given in Table 3.

Table 3
Thermodynamic Properties of Computationally Predicted and Selected Hydration Sites for 6X18

Hydration Site	ΔH (kcal/mol)	-TΔS (kcal/mol)	ΔΔG (kcal/mol)
36	1.10	2.81	3.91
88	1.33	1.96	3.29
114	2.82	1.52	4.34
119	1.83	1.50	3.83
189	1.51	1.23	2.74
194	3.34	1.17	4.51
196	4.08	1.14	5.22
224	3.59	0.92	4.51
288	3.46	0.88	4.34
315	3.34	0.75	4.09

When the wild type peptide was overlapped on the waterMap hydration sites it was quite evident that His7 displaced three Hydration sites HS36, HS114 and HS119, where as Phe12 displaced HS194 and Ser13 displaced HS315. (See Fig. 14) There were other five hydration sites as well, which where near to other residues of the peptide as shown in Figure. The analysis of the environment of hydration sites showed that they were surrounded by neutral residues like Tyr152, 148, hydrophilic residues like Arg 380, 190, and hydrophobic residues like Trp 306. There are also waters with ΔΔG < < 0, which are difficult to displace or replace but can make water-bridged interactions with proteins. The water molecules like HOH513, HOH518, HOH105, HOH102 were observed to be near unstable hydration sites indicating the

importance of these water molecules when mediated interaction with peptides and amino acid of target protein. Also, the most stable hydration sites with $\Delta G << 0$ (shown as bright green balls in Fig. 14) are a part of the conserved regions of the protein and should be avoided while designing molecules. In 6X18 there is a region in the binding site which can be further explored using the thermodynamic properties of the hydration sites for designing novel peptides. There are a few more hydration sites that can be considered for replacement as per the given thermodynamic parameters. It was observed that many of the seven screened peptides had significant overlap with the reference ligand, and a few of them showed a more significant binding affinity in terms of the calculated parameters.

Therefore, 37 selected peptides were analysed it was observed that 22 peptides have water mediated interactions. Based on vital residue docking interactions, water mediated H-bond interactions and hydration site superimposition we finalised 7 peptides that could be tested experimentally to check their activity (see Fig. 15).

Also, from the seven selected peptides, we designed a peptide with 9 amino acids taking one promising residue from each position (indicated in red Fig. 15). This molecule was docked and superimposed on the waterMap 6X18 to indicate the position hydration sites of the protein in the vicinity of the peptide. (See Fig. 16)

Conclusion

We intend to find whether the short peptides will be sufficient to address the required essential contacts when compared to the larger peptides. The analysis of this research aims at providing a clue to investigate peptide optimization and development. From our study it was evident that position 7 for Histidine, position 12 for Phenylalanine, and position 15 for Aspartic acid were extremely vital. Position 7 of neutral amino acid was found to extremely vital for interacting with the catalytic residues Tyr152, Arg190 and Tyr241. When replaced by hydrophilic residue like Asp and Arg, hydrophobic residues like Phe and Trp, and neutral residue like Tyr was seen favourable too. However, mutation at position 12 and 15 as such did not attract the wanted interactions. Therefore, from our study an ensemble of short nanopeptides are designed and showcased its binding affinity towards GLP-1 receptor using docking studies as well as WaterMap study. Our study may also open a new direction towards peptidomimetics and foldamer strategy for GLP-1 agonists. Also, the hybrid peptide was designed to check the effectiveness of multiple position mutated peptide similar to native GLP-1 peptide. The hybrid peptide displaced hydration sites like HS36, HS114, HS119 by Trp1, HS194 by Tyr3, HS315 by Leu4 and HS288 by Arg6. Hence, this peptide showed its ability to displace unstable hydration sites indicating the affinity of these substitution in receptor binding. (See Fig. 16)

Declarations

Acknowledgements

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Compliance of Ethical Standards

NA

Conflict of interest

The author hereby declares that they have no conflict of interest.

Ethical Approval

This article does not contain any studies with human participants or animals performed by any of the authors.

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Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Author Contributions

All authors contributed to the study conception and design. Ketan Ghosh performed material preparation, data collection and analysis, whereas M.Elizabeth Sobhia gave the idea about the study and reviewed the research and the manuscript. All authors read and approved the final manuscript.”

Data Availability

The datasets generated during and/or analysed during the current study are available in supplementary materials, and if any other data is required by the readers it shall be available from the corresponding author on reasonable request.

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Figures

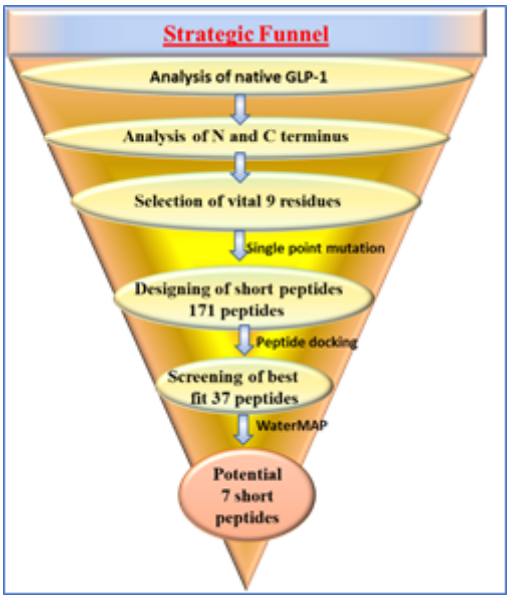

Figure 1

Strategic funnel of the methodology

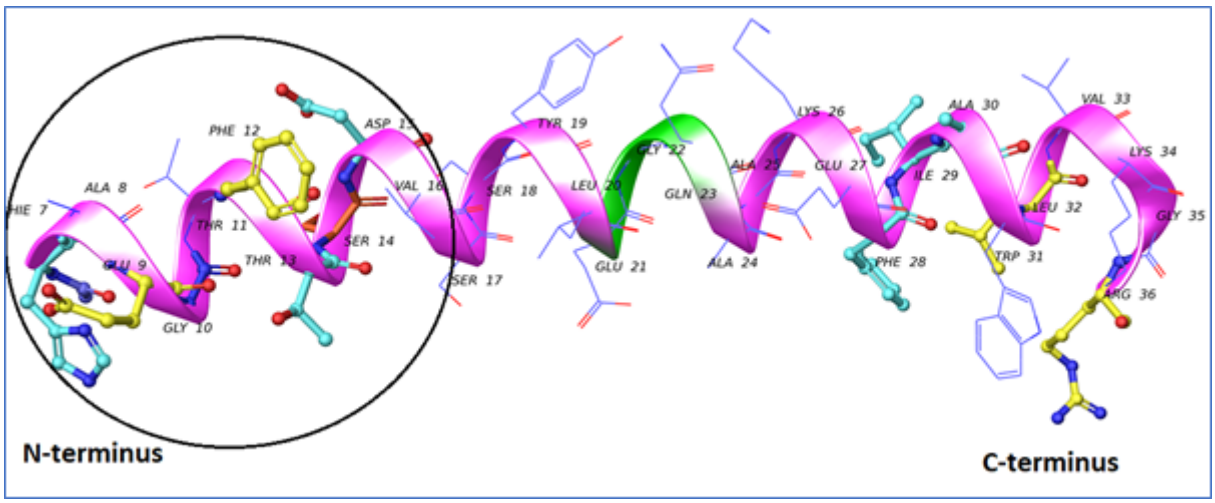

Figure 2

3D structure of the native GLP-1 PEPTIDE (7-36). Encircled part is the 9 amino acid peptide (His7 to Asp15).

Legend: Sky blue color indicates residue important for receptor activation and binding; yellow color indicates residues important for receptor interaction and green ribbon color indicates the linker region of native peptide.

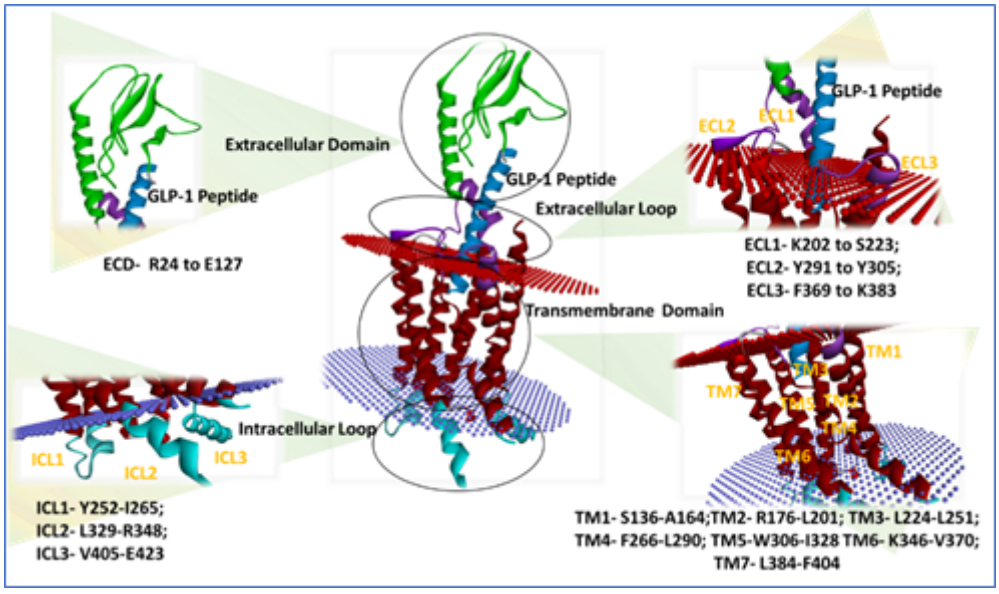

Figure 3

3D representation of GLP-1 receptor showing Extracellular domain (ECD), Intracellular domain (ICD), and Transmembrane domain (TMD)

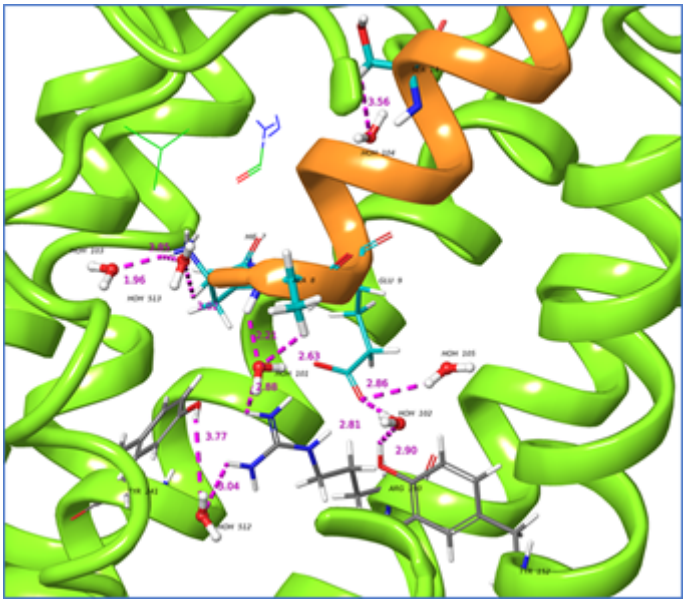

Figure 4

3-D representation of 6X18 depicting the crystal water molecules at the GLP-1 peptide binding site (Green color: GLP-1 receptor, Orange color: GLP-1 peptide, magenta color: inter atomic distance in Á)

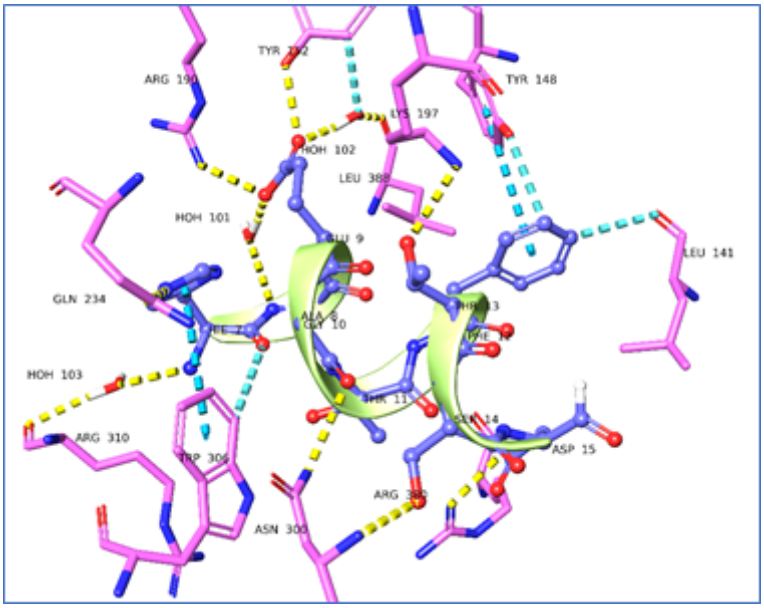

Figure 5

3D representation of molecular interactions between short nanopeptide (HAEGTFTSD) and GLP-1 receptor

Legend: yellow dots: H-bond; sky blue dots: aromatic H-bond; dark blue dots: π- π stack

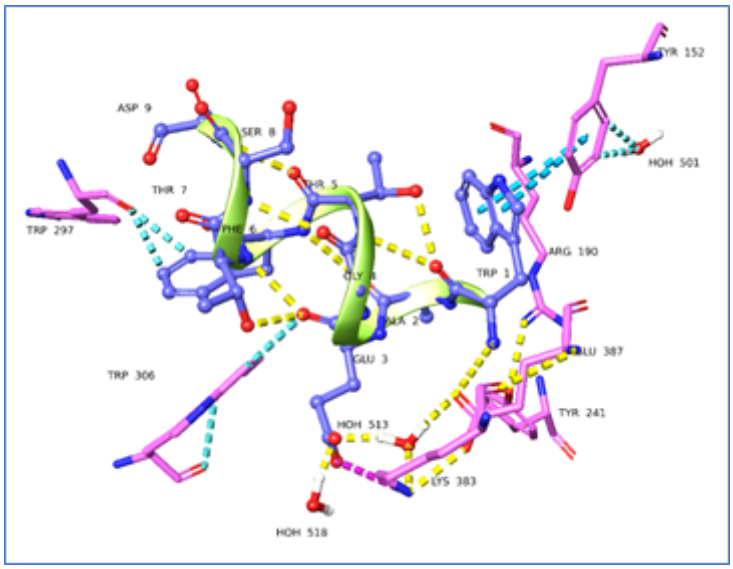

Figure 6

3D representation of molecular interactions between short mutated nanopeptide at position 7 (W^7AEGTFTSD) and GLP-1 receptor

Legend: yellow dots: H-bond; sky blue dots: aromatic H-bond; dark blue dots: π-π stack

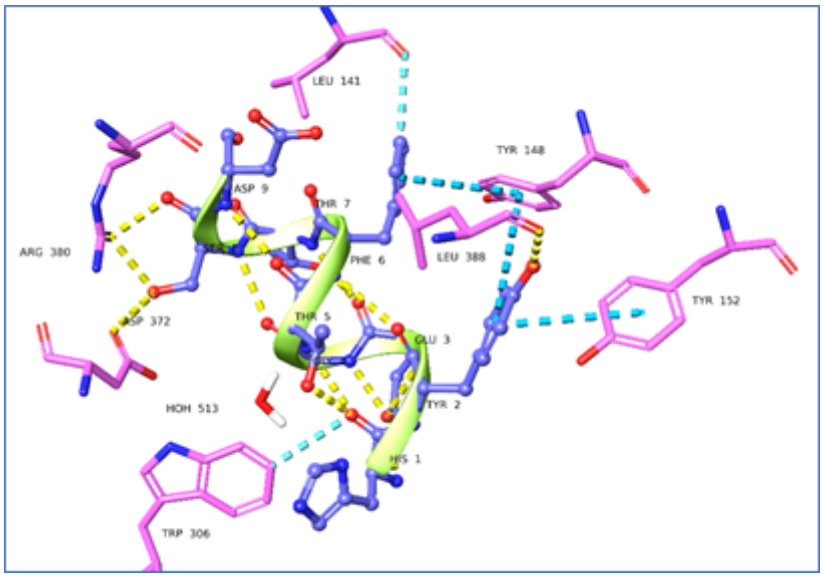

Figure 7

3D representation of molecular interactions between short mutated nanopeptide at position 8 (HY^{8}EGTFTSD) and GLP-1 receptor

Legend: yellow dots: H-bond; sky blue dots: aromatic H-bond; dark blue dots: π-π stack

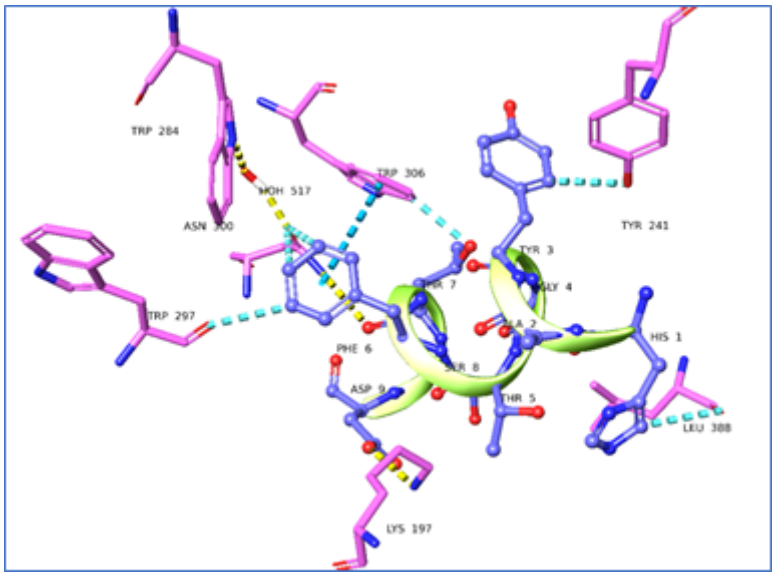

Figure 8

3D representation of molecular interactions between short mutated nanopeptide at position 9 (HAY^{9}GTFTSD) and GLP-1 receptor

Legend: yellow dots: H-bond; sky blue dots: aromatic H-bond; dark blue dots: π-π stack

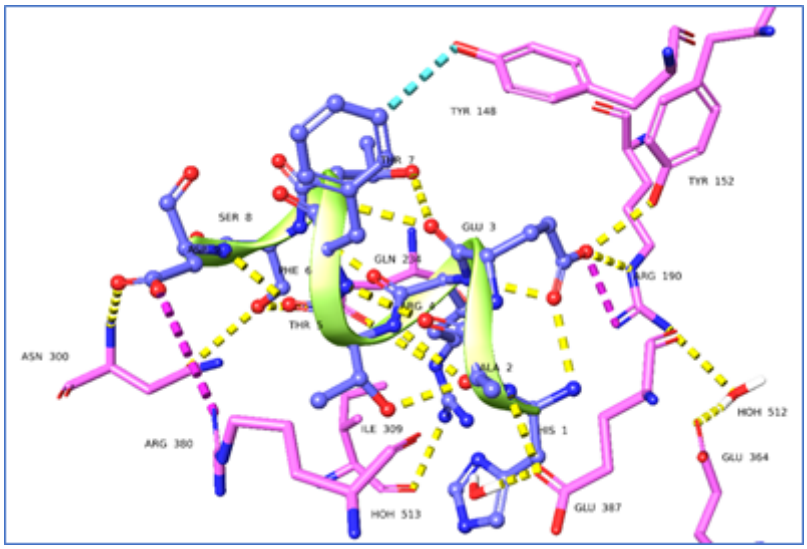

Figure 9

3D representation of molecular interactions between short mutated nanopeptide at position 10 (HAER^{10}TFTSD) and GLP-1 receptor

Legend: yellow dots: H-bond; sky blue dots: aromatic H-bond; dark blue dots: π-π stack

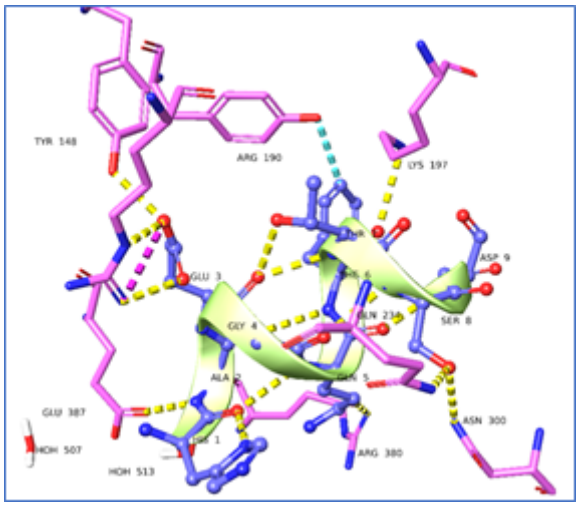

Figure 10

3D representation of molecular interactions between short mutated nanopeptide at position 11 (HAEG^{11}FTSD) GLP-1 receptor

Legend: yellow dots: H-bond; sky blue dots: aromatic H-bond; dark blue dots: π-π stack

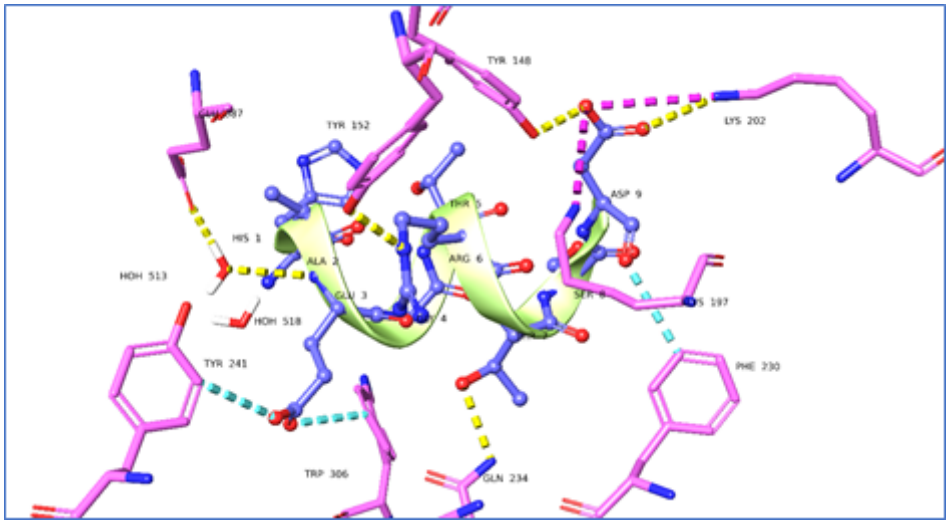

Figure 11

3D representation of molecular interactions between short mutated nanopeptide at position 12 (HAEGTR^{12}TSD) GLP-1 receptor

Legend: yellow dots: H-bond; sky blue dots: aromatic H-bond; dark blue dots: π-π stack

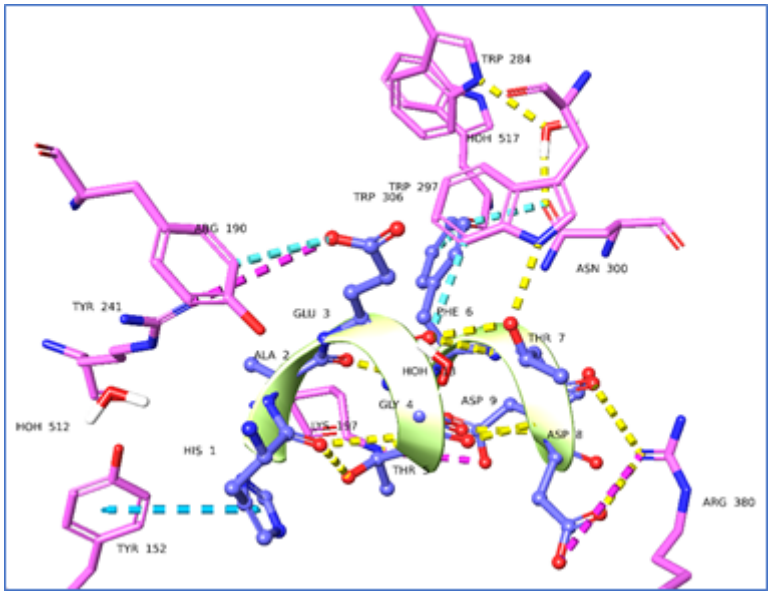

Figure 12

3D representation of molecular interactions between short mutated nanopeptide at position 14 (HAEGTFTD^{14}D) GLP-1 receptor

Legend: yellow dots: H-bond; sky blue dots: aromatic H-bond; dark blue dots: π-π stack

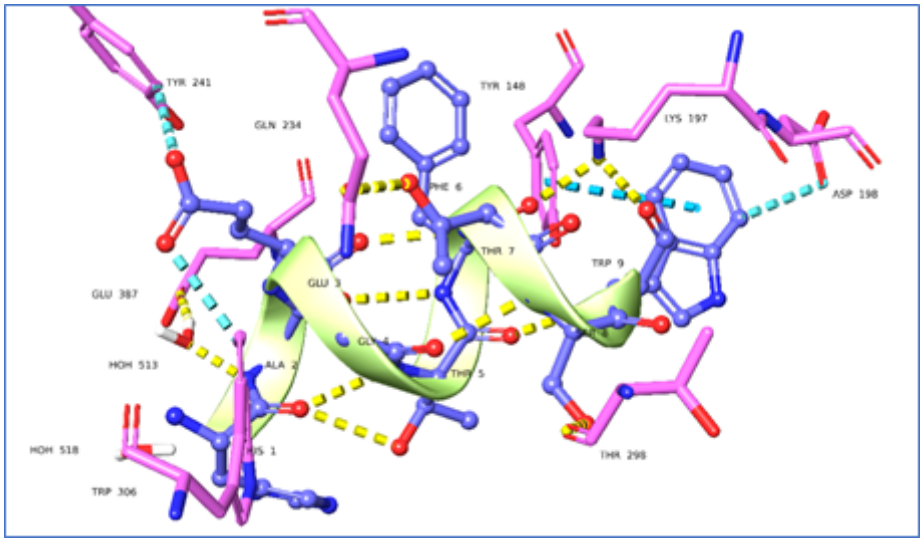

Figure 13

3D representation of molecular interactions between short mutated nanopeptide at position 15 (HAEGTFTSW^{15}) GLP-1 receptor

Legend: yellow dots: H-bond; sky blue dots: aromatic H-bond; dark blue dots: π-π stack

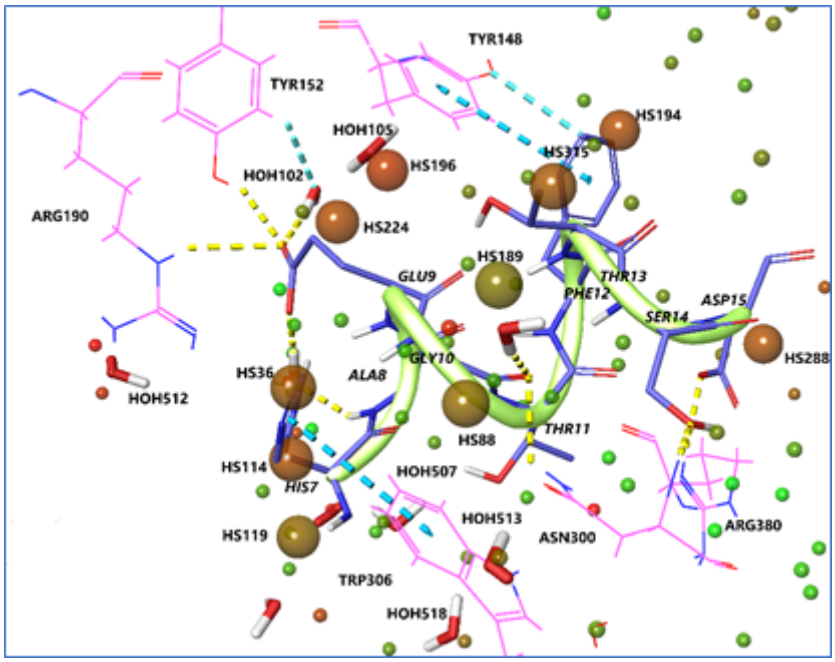

Figure 14

3D representation of molecular interactions between wild type GLP-1 nanopeptide (HAEGTFTSD) and GLP-1 receptor superimposed on WaterMAP of 6X18

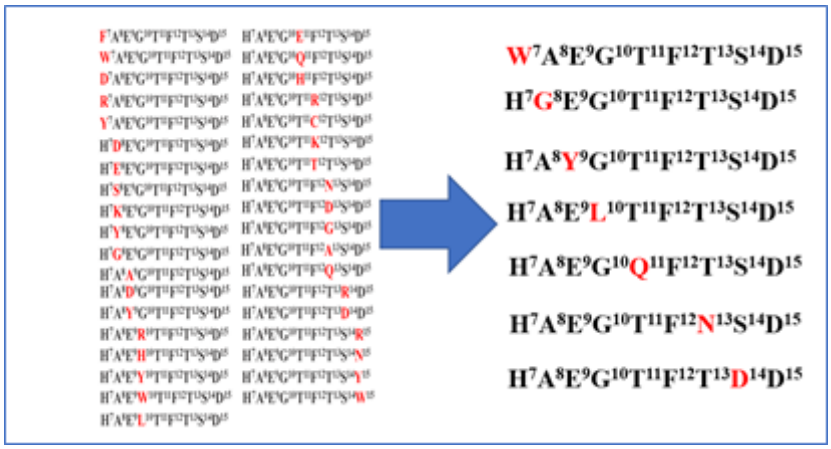

Figure 15

Representation of primary sequence of 37 designed peptides and selected 7 peptides (nine residues)

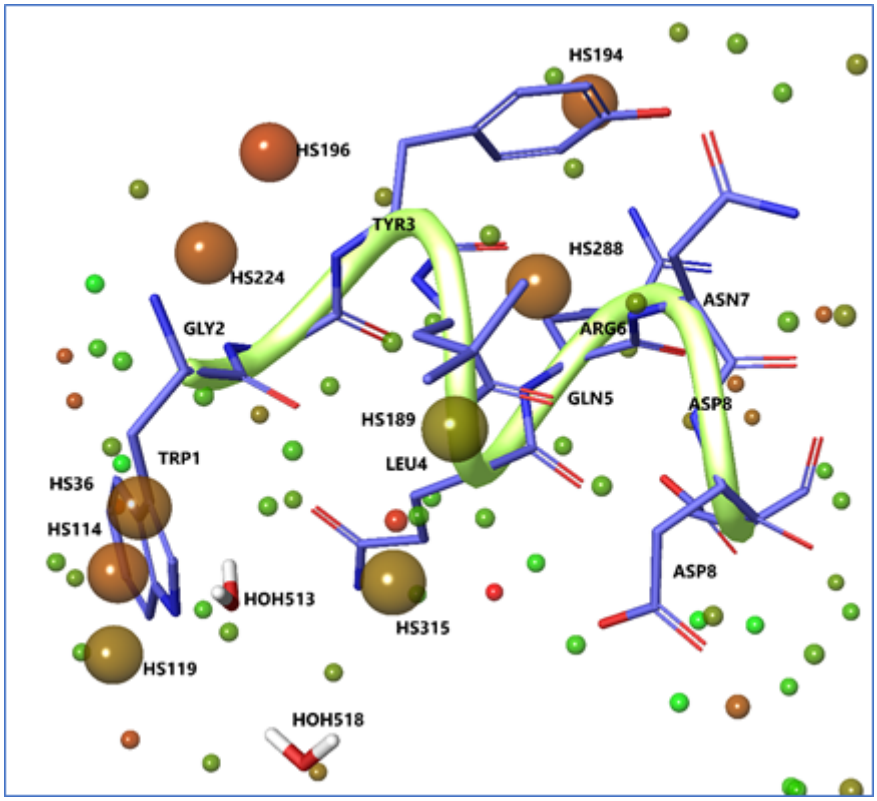

Figure 16

3D representation of hybrid peptide with multiple mutations (WGYLQRNDD)

Supplementary Files

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- SupplementaryMaterials.docx