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In silico prospecting for novel bioactive peptides from Bigeye Tuna *Thunnus obesus*

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Abstract

The bigeye tuna (Thunnus obesus), a widely consumed pelagic fish with high nutritional and economic value, is recognized as a promising source of bioactive compounds. Despite several studies on its protein hydrolysates, the identification of specific bioactive peptides (BAPs) remains limited due to the laborious and expensive nature of conventional discovery workflows. To address this, an in silico prospecting approach was employed to accelerate BAP discovery. Major tuna muscle proteins were virtually digested under simulated gastrointestinal conditions to generate peptide fragments. These fragments were then screened against established databases for predicted bioactivities, toxicity, bitterness, intestinal, and plasma stability, and novelty. After multi-step in silico screening using integrated bioinformatics tools and comparison with established peptide databases, 179 peptides (2–10 amino acids) were selected from a total of 1,311 peptides generated by simulated gastrointestinal digestion. These were further narrowed to 26 peptides with PeptideRanker scores greater than 0.5, of which 13 peptides candidates were identified as non-toxic, stable, water-soluble, and exhibiting high predicted bioactivity. Molecular docking analyses indicated favorable interactions with Angiotensin-Converting Enzyme (ACE) and Dipeptidyl peptidase-4 (DPP-IV), with IRP showing the strongest predicted binding to ACE ($p = 5.993 \times 10^{-5}$) and GCHPK exhibiting the highest affinity toward DPP-IV ($p = 9.811 \times 10^{-4}$). This computational workflow enables faster and wider identification of tuna-derived BAPs and provides an efficient strategy for nutraceutical development and targeted functional food formulation based on marine protein resources.

Keywords: Bioactive peptides, in silico prospecting, seafood, thunnus obesus.

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1. Introduction

Bioactive peptides (BAPs) are short chains of amino acids, typically 2 to 20 residues, that exert beneficial effects on human health. During the digestion of food proteins, biologically active BAPs can be released under the action of proteolytic enzymes in the gastrointestinal tract. Marine organisms represent an important and promising source of protein-based materials for the production of BAPs [1].

Tuna is a commercially valuable marine species widely distributed in the Vietnamese seas. Among the most common oceanic tuna species in Vietnam are yellowfin tuna and bigeye tuna, both characterized by high nutritional value, desirable flavor, and significant export potential. Proteins derived from bigeye tuna *(Thunnus obesus)* have been reported to generate BAPs with diverse biological functions, including antibacterial, antioxidant, anti inflammatory, angiotensin-converting enzyme (ACE) inhibitory, and anticancer activities [2, 3].

BAPs possess several advantages such as high bioactivity, target specificity, and low toxicity. However, their bioavailability and stability during gastrointestinal digestion remain major challenges for their application in food and nutraceutical industries [4]. *In silico* screening and predictive bioinformatics tools offer an efficient approach to address these limitations and facilitate the discovery of novel BAPs. Recent studies have effectively integrated *in silico* analyses with conventional experimental methods to accelerate peptide discovery, improving both accuracy and research efficiency [5]. Although several investigations have assessed the biological activity and sequence profiles of BAPs from tuna and related species using bioinformatics tools, most have focused on peptides derived from experimentally hydrolyzed fractions. Zhou L. *et al*. used bioinformatic tools such as PeptideCutter and BIOPEP to predict peptides with potential antidiabetic and antihypertensive activities derived from *Macrobrachium* protein sources [5]. Similarly, Guo X. *et al*. applied molecular docking simulations to predict the antioxidant activity of peptides obtained from tuna protein extracts [2]. Few studies have systematically predicted the potential BAPs directly obtainable from protein raw materials, which is crucial for selecting suitable sources for peptide extraction. Therefore, this study aims to explore and screen bioactive peptide candidates from the protein database

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of *T. obesus* using digestive enzyme simulation, bioinformatic analysis, biological activity prediction, structural characterization, and molecular interaction assessment.

2. Materials and Methods

2.1. Materials

Protein sequences and related information of *Thunnus obesus* were obtained from the UniProt and NCBI databases (https://www.uniprot.org/uniprotkb/, https://www.ncbi.nlm.nih.gov/refseq/; accessed October 15, 2025). Only proteins validated with high confidence from the SwissProt and NCBI RefSeq databases were selected for analysis. Detailed information on the selected sequences is presented in Table 1.

2.2. Method

2.2.1. Enzyme hydrolysis using ExPASy tool

In silico enzymatic hydrolysis of *T. obesus* proteins was simulated using the ExPASy PeptideCutter tool [5]. The enzymes used included pepsin (pH > 2; EC 3.4.23.1), trypsin (EC 3.4.21.4), and chymotrypsin A (EC 3.4.21.1), as these represent the major proteases in the human digestive system. The virtual digestion generated peptide fragments and single amino acids; single residues were excluded, and only peptides ranging from 2 to 20 amino acids in length were retained for further screening.

2.2.2. Screening for toxicity, stability and solubility

All peptide sequences were exported in FASTA format. The PeptideRanker tool was first used to evaluate the biological potential of each peptide, and sequences with scores greater than 0.5 were selected for toxicity assessment [5]. The ToxinPred program, which employs a Support Vector Machine (SVM)–based supervised learning algorithm (using a threshold score of 0.0), was then applied to predict non-specific cytotoxicity. Peptides predicted as toxic were excluded from further analysis [6].

The Innovagen PepCalc tool was used to estimate peptide solubility, while the PepDraw software was utilized to visualize the chemical structure and physicochemical properties of the selected peptides. Peptide stability was further evaluated using the PLifePred and HLP programs to predict their half-life in plasma and intestinal environments. Peptides with predicted half-lives greater than 800 seconds in plasma and longer than 1.0 second in the intestine were selected for subsequent bioactivity screening [7].

Table 1. List of selected *T.obesus* proteins based on UniProt and NCBI databases

No.	Protein ID	Symbol	Protein	Length
1	Q9YGK2	COLI_THUOB	Pro-opiomelanocortin (POMC) (Corticotropin-lipotropin)	222
2	Q76G09	MYG_THUOB	Myoglobin (Nitrite reductase MB)	147
3	P37204	GLHA_THUOB	Glycoprotein hormones alpha chain (GTH-alpha)	94
4	P37205	GTHB1_THUOB	Gonadotropin subunit beta-1 (GTH-I-beta)	102
5	P37206	GTHB2_THUOB	Gonadotropin subunit beta-2 (GTH-II-beta)	115
6	P80971	COX42_THUOB	Cytochrome c oxidase subunit 4 isoform 2	176
7	P80977	CX6C1_THUOB	Cytochrome c oxidase subunit 6C-1	76
8	Q36090	ATP6_THUOB	ATP synthase F(0) complex subunit a (F-ATPase protein 6)	133
9	Q6S9V8	CISY_THUOB	Citrate synthase, mitochondrial (EC 2.3.3.1)	469
10	P68957	GLUC_THUOB	Glucagon	29
11	P80972	COX5A_THUOB	Cytochrome c oxidase subunit 5A-1	20
12	P80973	COXC_THUOB	Cytochrome c oxidase subunit 5A-2	24
13	P80974	COX5B_THUOB	Cytochrome c oxidase subunit 5B	20
14	P80975	COX6A_THUOB	Cytochrome c oxidase subunit 6A	9
15	P80976	COX6B_THUOB	Cytochrome c oxidase subunit 6B	34
16	P80978	CX6C2_THUOB	Cytochrome c oxidase subunit 6C-2	15
17	P80979	COX7A_THUOB	Cytochrome c oxidase subunit 7A	15
18	P80980	COXN_THUOB	Cytochrome c oxidase subunit 7B-heart	20
19	P80981	COXM_THUOB	Cytochrome c oxidase subunit 7B-liver	20
20	P80982	COX7C_THUOB	Cytochrome c oxidase polypeptide 7C	10
21	P80983	COX8B_THUOB	Cytochrome c oxidase subunit 8B	20
22	P80984	COX8A_THUOB	Cytochrome c oxidase subunit 8A	19
23	Q9I8U0	COX41_THUOB	Cytochrome c oxidase subunit 4 isoform 1	169
24	BFE12136		Myosin heavy chain	839

Table 2. *In silico* tools and databases used in the study

Bioinformatics tools	Purpose	URL	Quote
Peptide Cutter ExpASy	Simulation of enzymatic hydrolysis	https://web.expasy.org/peptide_cutter/	[5]
ToxinPred	Prediction of peptide toxicity	https://webs.iiitd.edu.in/raghava/toxinpred/multiple_test.php/	[6]
Peptide Ranker	Ranking of peptide bioactivity potential	http://distilldeep.ucd.ie/PeptideRanker/	[5]
PepCalc	Determination of peptide solubility	https://pepcalc.com/	[8]
PepDraw	Describe the chemical structure and physical and chemical properties of peptides	https://pepdraw.com/	[9]
PLifePred	Evaluation of peptide stability in blood	https://webs.iiitd.edu.in/raghava/plifepred/	[7]
HPL	Evaluation of peptide stability in the intestinal environment	http://crdd.osdd.net/raghava/hlp/interactive.htm/	[7]
AHTpin	Prediction of antihypertensive peptides	http://crdd.osdd.net/raghava/ahtpin/	[10]
AnOxPePred - 1.0	Prediction of antioxidant peptides	https://services.healthtech.dtu.dk/services/AnOxPePred-1.0/	[11]
PreAIP	Prediction of anti-inflammatory peptides	http://kurata14.bio.kyutech.ac.jp/PreAIP/	[12]
CAMP-R4	Prediction of antimicrobial peptides	http://www.camp.bicnirrh.res.in/predict/	[13]
Il2pred	Predicted interleukin-2-inducing peptide	https://webs.iiitd.edu.in/raghava/il2pred/	[14]
Il4pred	Predicted interleukin-4-inducing peptide	https://webs.iiitd.edu.in/raghava/il4pred/multi_submitfreq_S.php?ran=19248	[14]
PepSite2	Peptide-protein binding interaction model	http://pepsite2.russelllab.org/	[15]
BIOPEP-UVM	Comprehensive BAP database	https://biochemia.uwm.edu.pl/en/biopep-uwm-2	[16]
AHTpDB	Antihypertensive peptide database	http://crdd.osdd.net/raghava/ahtpdb	[10]
DBAASP v3.0	Antimicrobial peptide database	https://dbaasp.org	[13]

2.2.3. Prediction and evaluation of biological activity of peptides

All peptide sequences identified as non-toxic, non-allergenic, and stable were subjected to *in silico* prediction of biological activities, including antihypertensive, anti-inflammatory, antibacterial, and antioxidant properties (Table 2). The predicted bioactive sequences were subsequently compared with established peptide databases BIOPEP-UWM, PepBank, and to determine whether these peptides had been previously identified or reported in earlier studies.

Furthermore, the Pepsite2 program was employed to predict molecular interactions and potential binding sites between candidate BAPs and two target enzymes: the human angiotensin-converting enzyme (ACE; PDB ID: 1O8A) and dipeptidyl peptidase-4 (DPP-IV; PDB ID: 2ONC). The docking analysis was conducted following the computational procedure described by Mohd Salim and Gan [17].

3. Results and Discussion

3.1. Simulation of Hydrolysis Process in the Digestive Tract

In the human digestive tract, proteins are hydrolyzed by different enzymes located in various organs. For instance, pepsin is responsible for protein digestion in the stomach, where it is activated from its zymogen precursor under acidic conditions and subsequently hydrolyzes proteins into a mixture of polypeptides, oligopeptides, and free amino acids. Further digestion occurs in the duodenum through the action of trypsin and chymotrypsin. Trypsin cleaves peptide bonds at the carboxyl side of arginine and lysine, while chymotrypsin targets the carbonyl groups of tyrosine, tryptophan, and phenylalanine [17].

In this study, *in silico* enzymatic hydrolysis of 24 *T. obesus* proteins was simulated using the ExPASy PeptideCutter tool with the digestive enzymes pepsin (pH > 2), trypsin, and chymotrypsin (Table 2). The simulation generated a total of 1,311 peptide fragments.

After removing single amino acids and duplicate sequences, 179 unique peptides ranging from 2 to 21 amino acids in length were retained. Similar approaches have been reported in previous studies. For example, Lafarga *et al.* (2014) utilized databases and tools such as ProtParam, BLAST, ExPASy PeptideCutter, and BIOPEP to identify peptides exhibiting DPP-IV and ACE inhibitory activity in beef and pork proteins [18]. Dziuba and Dziuba (2014) simulated the hydrolysis of milk proteins using 28 different enzymes and successfully identified novel peptides with potential antibacterial activity [19]. It is important to note, however, that the ExPASy PeptideCutter assumes proteins exist in an unfolded linear form, which may overestimate the accessibility of enzymatic cleavage sites. Consequently, under actual hydrolysis conditions, fewer peptides may be generated compared to *in silico* predictions.

To identify BAPs from the resulting fragments, peptide sequences were analyzed using the PeptideRanker database. Among the 179 peptides, 27 sequences with scores greater than 0.5 were considered potentially bioactive and subsequently screened for toxicity and solubility (Table 3). Similar studies have used PeptideRanker effectively to identify active sequences. For instance, Ngoh *et al* [20] identified five peptides with scores above 0.8 from 511 candidates and confirmed their α-amylase inhibitory activity.

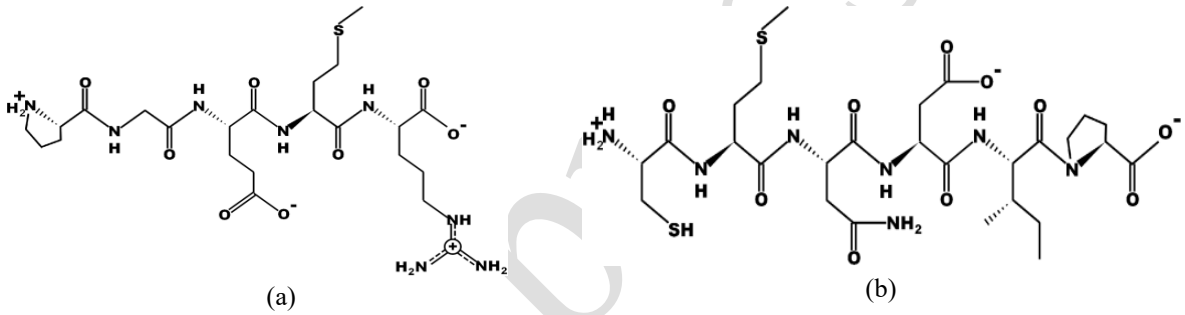
Among the selected peptides, there were five dipeptides, ten tripeptides, and twelve peptides ranging from four to seven amino acids in length. In general, most of the predicted BAPs were short, typically comprising 2–7 residues. Short-chain peptides are known to be easily absorbed through the intestinal microvilli and transported to target organs. BAPs can enter the bloodstream via four main pathways: (1) PepT1-mediated transport, (2) paracellular diffusion through tight junctions, (3) vesicle-mediated transcellular transport, and (4) passive transcellular diffusion. Xu *et al.* (2019) [21]. demonstrated that peptides within this size range could permeate Caco-2 cell monolayers via these mechanisms, exhibiting high permeability coefficients comparable to smaller peptides. Furthermore, due to their simple structures, these peptides can easily access the active sites of target proteins such as ACE and DPP-IV

Table 3. Peptide screening based on toxicity and solubility

No.	Peptide Sequence	Molecular mass	Prediction of toxicity		Hydrophilicity	Solubility
			SVM Score	Forecast		
1	LW	317.41	-0.79	Non-Toxin	-2.60	Poor
2	IPF	375.50	-0.83	Non-Toxin	-1.43	Poor
3	NWP	415.48	-0.77	Non-Toxin	-1.07	Poor
4	MCPVW	634.87	-0.75	Non-Toxin	-1.44	Poor
5	GSW	348.39	-0.84	Non-Toxin	-1.03	Poor
6	QCMGCC	643.88	0.72	Toxin	-0.68	Poor
7	LR	287.38	-0.80	Non-Toxin	0.60	Good
8	GMD	321.38	-0.81	Non-Toxin	0.57	Good
9	VPSCPSF	735.93	-0.68	Non-Toxin	-0.63	Poor
10	VYPPRPR	884.13	-0.79	Non-Toxin	0.31	Good
11	IRP	384.51	-0.80	Non-Toxin	0.40	Good
12	QSF	380.43	-0.80	Non-Toxin	-0.67	Poor
13	STHPHF	724.85	-0.76	Non-Toxin	-0.60	Poor
14	GMK	334.47	-0.80	Non-Toxin	0.57	Good
15	IGR	344.45	-0.82	Non-Toxin	0.40	Good
16	SGR	318.36	-0.72	Non-Toxin	1.10	Good
17	DATF	452.50	-0.86	Non-Toxin	-0.10	Good
18	GGMR	419.55	-0.87	Non-Toxin	0.42	Good
19	LFPK	503.69	-0.80	Non-Toxin	-0.32	Good
20	PAY	349.41	-0.81	Non-Toxin	-0.93	Poor
21	MLDMR	664.90	-1.18	Non-Toxin	0.32	Good
22	GCHPK	540.70	-0.21	Non-Toxin	0.30	Good
23	PGEMR	588.74	-1.06	Non-Toxin	0.94	Good
24	GH	212.23	-0.80	Non-Toxin	-0.25	Poor
25	GLASS	294.37	-0.80	Non-Toxin	-2.05	Poor
26	CMNDIP	691.89	-0.61	Non-Toxin	-0.15	Good
27	IG	188.25	-0.80	Non-Toxin	-0.90	Poor

Table 4. Predicted biological activities of peptides from *T. obesus*

No.	Peptides	Antibacterial			Antihypertensive		Antioxidant (FRS score)	Anti-inflammatory		Immunoregulation	
		Forecast	AMP score	SVM score	Forecast	pIC_{50}		Score	Forecast	Induction with IL-2	Induction with IL-4
1	LR	+	0.96		+	3.13	0.389	0.253	-	+	+
2	GMD	+	1.00		+	4.73	0.406	0.281	-	+	+
3	IRP	-	0.00		+	5.34	0.418	0.274	-	+	+
4	GMK	+	0.75		+	4.52	0.395	0.275	-	+	+
5	IGR	-	0.00		+	4.47	0.405	0.291	-	+	+
6	SGR	-	0.00		+	4.52	0.397	0.248	-	+	+
7	DATF	+	1.00	-1.16	-		0.349	0.299	-	+	+
8	GGMR	-	0.18	0.07	+		0.442	0.349	Short	+	+
9	LFPK	-	0.05	1.07	+		0.418	0.330	-	+	+
10	MLDMR	+	1.00	-1.46	-		0.359	0.385	Short	+	+
11	GCHPK	-	0.02	1.87	+		0.564	0.436	Medium	+	+
12	PGEMR	+	0.90	-0.01	-		0.415	0.410	Medium	+	-
13	CMNDIP	+	0.94	-0.25	-		0.369	0.339	-	+	+



(a) (b)

Fig. 1. Chemical structures of some peptides (PGEMR and CMNDIP) (a) Structure of PGEMR and (b) Structure of CMNDIP

Toxicity screening using the ToxinPred tool identified one potentially toxic peptide (sequence QCMGCC), which was excluded from further analysis. Solubility prediction using Innovagen's PepCalc tool revealed that 13 peptides possessed good water solubility. Since solubility strongly influences peptide bioavailability, these 13 peptides were selected for subsequent evaluation.

Peptide stability was then assessed using the PLifePred software, which predicted half-lives in plasma ranging from 831.31 to 854.81 seconds. These values are consistent with naturally occurring food-derived peptides, typically ranging from 800 to 900 seconds, as reported by Gülseren and Vahapoglu in a study analyzing 3,074 peptides from 12 food sources [22]. Further intestinal stability analysis using HLP software indicated that four peptides - MLDMR, CMNDIP, GCHPK, and PGEMR - exhibited relatively high intestinal stability, with predicted half-lives exceeding 1.0 second. However, since the HLP algorithm only evaluates peptides containing five or more residues, all 13 peptides were retained for subsequent bioactivity prediction analyses.

3.2. Prediction of Biological Activity of the Obtained Peptide Sequence.

From the 179 potential peptide sequences, 13 peptides exhibiting non-toxicity, good water solubility, and stability in both plasma and intestinal environments were selected for *in silico* bioactivity prediction (Table 4). Peptides were considered potentially bioactive when their predicted scores exceeded the defined thresholds of each respective computational model.

All 13 peptides demonstrated the potential to exhibit one or more biological activities, including antibacterial (7 peptides), antihypertensive (9 peptides), antioxidant (5 peptides), anti-inflammatory (4 peptides), as well as immunomodulatory functions. The immunomodulatory activity was indicated through their predicted ability to induce interleukin-2 (13 peptides) and interleukin-4 (12 peptides). These peptides were further characterized for their physicochemical properties (Table 5) and structural features using the PepDraw database.

Table 5. Physicochemical properties of peptides

No.	Peptides	Length	Mass molecule	pI	Hydrophobicity (Kcal×mol $^{-1}$)	Absorption coefficient [1] ($M^{-1} \times cm^{-1}$)
1	LR	2	287,1953	11.11	8.46	0
2	GMD	3	321,0991	2.95	12.02	0
3	IRP	3	384,2479	11.12	8.73	0
4	GMK	3	334,1670	10.16	11.18	0
5	IGR	3	344,2167	11,12	9.74	0
6	SGR	3	318,1648	10.85	11.32	0
7	DATF	4	452,1901	3.05	10.58	0
8	GGMR	4	419,1946	11.13	11.34	0
9	LFPK	4	503,3099	10.14	7.88	0
10	MLDMR	5	664,3027	6.54	10.76	0
11	GCHPK	5	540,2472	9.00	14:30	0
12	PGEMR	5	588,2682	7.16	13.96	0
13	CMNDIP	6	691,2660	2.93	10.72	0

The biological activities of BAPs are largely influenced by their amino acid composition and physicochemical properties. Peptides exhibiting strong ACE-inhibitory activity commonly possess a proline residue at the C-terminus, along with hydrophobic, aromatic or positively charged ring structures, and branched-chain residues at the N-terminus. The presence of proline is also known to enhance resistance to enzymatic degradation [18]. Peptides showing potent DPP-IV inhibitory activity often contain phenylalanine (F), arginine (R), or tyrosine (Y) residues.

Anti-inflammatory peptides are typically characterized by a high content of hydrophobic residues, particularly at both termini, which enhances their inhibitory effects on nitric oxide (NO) production in vitro. Antimicrobial peptides (AMPs) generally contain hydrophobic and positively charged residues mainly lysine (K) and arginine (R) that promote electrostatic interactions with negatively charged microbial cell membranes.

Similarly, peptides with antioxidant activity frequently display a higher frequency of residues such as leucine (L), lysine (K), tyrosine (Y), and proline (P), or combinations like L, V, Y, and G, especially at their N- and C-terminal. Furthermore, peptides with lower molecular weights and shorter lengths tend to exhibit enhanced antioxidant efficacy due to their greater accessibility and ability to scavenge free radicals [23]. The peptides shortlisted in this study (Table 5) demonstrated overall consistency with the compositional and structural patterns reported in previous research.

3.3. Predicted Interactions Between Selected Peptides and ACE and DPP-IV

The results summarized in Table 6 present the *p*-values representing the interaction potential between each peptide and the angiotensin-converting enzyme (ACE; PDB ID: 1O8A), as well as the predicted binding sites. All 13 peptides were predicted to interact with the ACE molecule ($p < 0.05$). Among them, the IRP peptide exhibited the strongest interaction, with the lowest *p*-value (5.993×10^{-5}), while GGMR showed the weakest binding affinity ($p = 0.01078$).

For example, the peptide LR was predicted to bind to ACE at residues W279, Q281, F457, F460, K511, H513, Y520, and Y523 - all of which are recognized as stable amino acids in the ACE structure (Fig. 2A). Interestingly, all peptides containing arginine (R) were found to interact exclusively with stable amino acid residues [17]. Furthermore, the presence of proline (P) appeared to enhance ACE-binding affinity, as the five peptides with the lowest p-values (IRP, LFPK, GCHPK, CMNDIP, and PGEMR) all contained this residue. Proline, a unique amino acid with a cyclic structure, can introduce conformational bends within peptide chains that promote tighter binding with target proteins, thereby stabilizing the peptide - protein complex [24].

As also shown in Table 6, only six peptide sequences were predicted to exhibit significant binding interactions with the dipeptidyl peptidase-IV enzyme (DPP-IV; PDB ID: 2ONC), with *p*-values below 0.05. These peptides were GCHPK, LFPK, IGR, LR, IRP, and SGR. This reduced number of interactions compared to ACE may be attributed to the more complex tertiary structure of DPP-IV. Among the DPP-IV-binding peptides, GCHPK showed the strongest predicted interaction ($p = 0.0009811$), whereas GGMR again demonstrated the weakest binding ($p = 0.2987$), indicating limited affinity toward both ACE and DPP-IV targets.

Table 6. Simulation of docking with ACE and DPP-IV molecules by Pepsite2

No.	Peptides	ACE (1O8A)		DPP-IV (2ONC)	
		p-value	Expected mounting location	*p*-value	Expected mounting location
1	LR	0.003746	L1, R2	0.006993	L1, R2
2	GMD	0.002139	G1, M2, D3	0.078	G1, M2
3	IRP	5.993e-05	I1, M2, P3	0.01179	M2, P3
4	GMK	0.0009196	G1, M2, K3	0.078	G1, M2
5	IGR	0.0008181	I1, G2, R3	0.00325	I1, G2, R3
6	SGR	0.0008506	S1, G2, R3	0.0151	S1, G2, R3
7	DATF	0.001557	D1, A2, T3, F4	0.2639	A2, T3
8	GGMR	0.01078	G2, M3, R4	0.2987	G2, M3
9	LFPK	9.002e-05	L1, F2, P3, K4	0.00315	F2, P3, K4
10	MLDMR	0.002691	M1, L2, M4, R5	0.08875	M1, M4, R5
11	GCHPK	0.0001009	G1, H3, P4, K5	0.0009811	C2, H3, P4, K5
12	PGEMR	0.000298	P1, G2, M4, R5	0.2489	P1, R5
13	CMNDIP	0.000181	C1, M2, N3, I5, P6	0.08847	M2, I5, P6

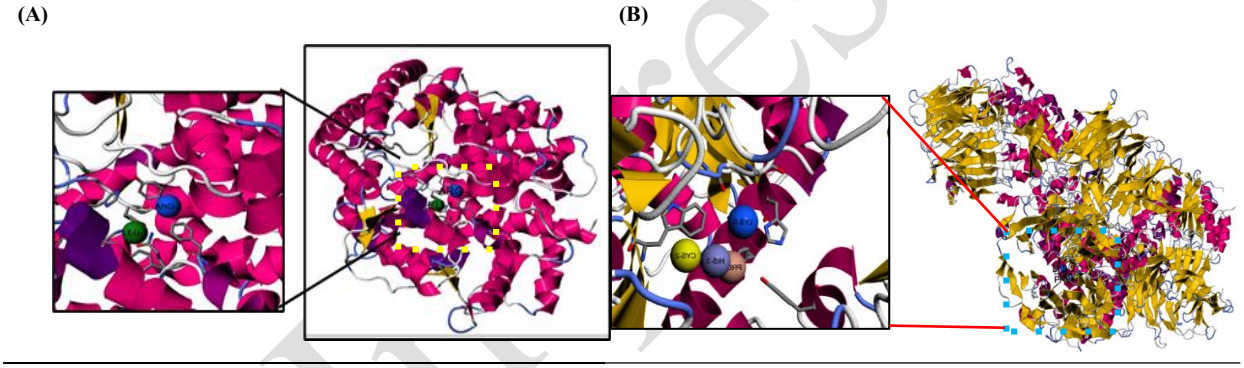


Fig. 2. Predicted interactions of the (A) LR peptide with angiotensin-converting enzyme (PDB ID: 1OA8) and (B) GCHPK peptide with dipeptidyl peptidase IV (PDB ID: 2ONC) using the PepSite2 tool

4. Conclusion

In silico methodologies are an increasingly important research trend for the identification and characterization of BAPs. In this study, a computational digestion system was employed to predict peptides potentially released from *T. obesus* proteins using simulated gastrointestinal enzymes. Following screening using multiple bioinformatics tools and comparison with established peptide databases, a total of 1,311 peptide fragments were identified. Among these, 179 sequences ranging from 2 to 10 amino acids in length were retained, and 26 peptides with PeptideRanker scores greater than 0.5 were shortlisted. Following further evaluation, 13 peptides were identified as non-toxic, stable, water-soluble, and exhibiting high potential biological activity.

Subsequently, the binding affinities of the selected peptides toward ACE and DPP-IV were assessed through molecular docking analyses. Although not all potential peptide candidates may be captured by this *in silico* approach, its effectiveness in accelerating BAP discovery was clearly demonstrated, with substantial reductions in time, labor, and experimental costs compared with conventional biochemical workflows.

Overall, the potential of *in silico* strategies as efficient tools for rapid screening and functional prediction of BAPs from marine protein sources is highlighted. Nevertheless, experimental validation using mass spectrometry and peptide sequencing is required to confirm these computational predictions. In addition, enzyme kinetics studies on ACE and DPP-IV inhibition should be conducted to further elucidate the underlying mechanisms of action.

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