**Journal of Science and Technology – Engineering and Technology for Sustainable Development**
*Volume 36, Issue 5, November 2026, 001–008*

# *In silico* Prospecting for Novel Bioactive Peptides from Bigeye Tuna *Thunnus obesus*

***Trung Nguyen Ha[1,2], Cuong Pham Kien[1,*], Son Chu Ky[2]***
[1]*Institute of Materials, Biology and Environment, Academy of Military Science and Technology, Ha Noi, Vietnam*
[2]*School of Chemistry and Life Sciences, Hanoi University of Science and Technology, Ha Noi, Vietnam*
[*]*Corresponding author email: phamkiencuong83@gmail.com*

**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 peptide 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*.

## 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*. [5] used bioinformatic tools such as PeptideCutter and BIOPEP to predict peptides with potential antidiabetic and antihypertensive activities derived from *Macrobrachium* protein sources. Similarly, Guo X. *et al*. [2] applied molecular docking simulations to predict the antioxidant activity of peptides obtained from tuna protein extracts. 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

p-ISSN 3093-3242
e-ISSN 3093-3579
https://doi.org/10.51316/jst.195.etsd.2026.36.5.1
Received: Nov 11, 2025; Revised: Jan 14, 2026;
Accepted: Jan 21, 2026; Online: Jun 18, 2026

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.* [18] 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. Dziuba and Dziuba (2014) [19] simulated the hydrolysis of milk proteins using 28 different enzymes and successfully identified novel peptides with potential antibacterial activity. 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.

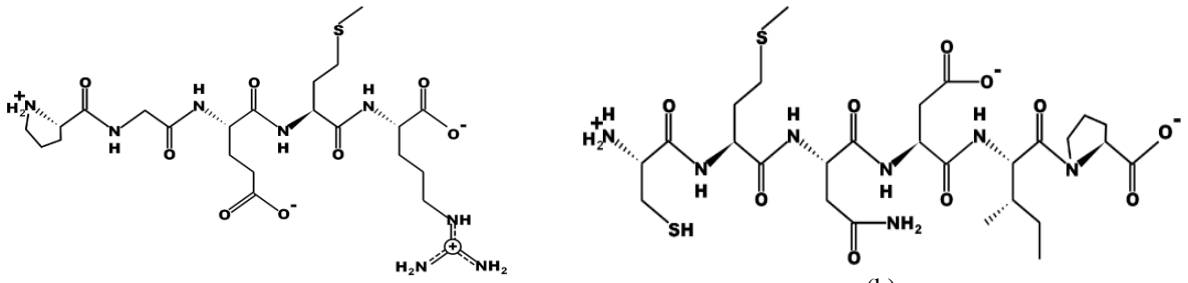
Among the selected peptides, there were five dipeptides, ten tripeptides, and twelve peptides ranging from four to seven amino acids in length. In general, most of the predicted BAPs were short, typically comprising 2–7 residues. Short-chain peptides are known to be easily absorbed through the intestinal 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 [2]. 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 r esults 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 ($5.993 \times 10^{-5}$), 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 |

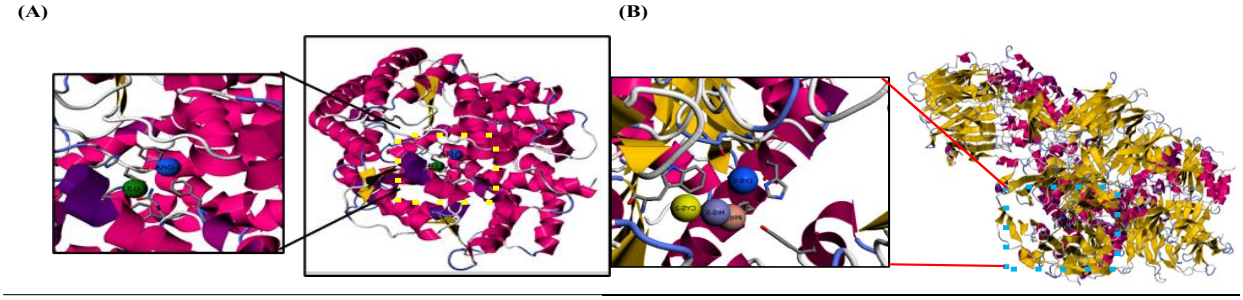



Fig. 2. Predicted interactions of the (A) LR peptide with angiotensin-converting enzyme (PDB ID: 1OA8) and (B) GCHPK peptide with dipeptidyl peptidase IV (PDB ID: 2ONC) using the PepSite2 tool

## 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.

## References

[1] F. Shahidi and A. Saeid, Bioactivity of marine-derived peptides and proteins: A review, Marine Drugs, vol. 23, iss. 4, Apr. 2025, Art. no. 157 https://doi.org/10.3390/md23040157

[2] X. Guo, J. Liu, C. Wang, Z. Wen, and B. Zheng, The antioxidant mechanism of peptides extracted from tuna protein revealed using a molecular docking simulation, Antioxidants, vol. 13, iss. 2, Jan. 2024, Art. no. 166. https://doi.org/10.3390/antiox13020166

[3] J-Y. Je, Z-J. Qian, S-H. Lee, H-G Byun, and S-K. Kim, Purification and antioxidant properties of bigeye tuna *(Thunnus obesus)* dark muscle peptide on free radical-mediated oxidative systems, Journal of Medicinal Food, vol. 11, iss. 4, pp. 629–637, Dec. 2008. https://doi.org/10.1089/jmf.2007.0114

[4] Y. Jiang, J. Sun, J. Chandrapala, M. Majzoobi, C. Brennan, X-A. Zeng, and B. Sun, Recent progress of food-derived bioactive peptides: Extraction, purification, function, and encapsulation, Food Frontiers, vol. 5, iss. 3, pp. 1240–1264, Mar. 2024. https://doi.org/10.1002/fft2.383

[5] L. Zhou, R. L. Mendez, and J. Y. Kwon, In silico prospecting for novel bioactive peptides from seafoods: A case study on pacific oyster *(Crassostrea gigas)*, Molecules, vol. 28, iss. 2, Jan. 2023, Art. no. 651. https://doi.org/10.3390/molecules28020651

[6] S. Gupta, P. Kapoor, K. Chaudhary, A. Gautan, R. Kumar, Open Source Drug Discovery Consortium, and G. P. S. Raghava, *In silico* approach for predicting toxicity of peptides and proteins, PLoS One, vol. 8, iss. 9, Sep. 2013, Art. no. e73957. https://doi.org/10.1371/journal.pone.0073957

[7] D. Mathur, S. Singh, A. Mehta, P. Agrawal, and G. P. S. Raghava, *In silico* approaches for predicting the half-life of natural and modified peptides in blood, PLoS One, vol. 13, iss. 6, Jun. 2018, Art. no. e0196829. https://doi.org/10.1371/journal.pone.0196829

[8] S. Lear and S. L. Cobb, Pep-Calc.com: a set of web utilities for the calculation of peptide and peptoid properties and automatic mass spectral peak assignment, Journal of Computer-Aided Molecular Design, vol. 30, pp. 271–277, Feb. 2016. https://doi.org/10.1007/s10822-016-9902-7

[9] F. Zhou, D. Li, Y. Hou, Z. Cong, K. Li, X. Gu, and G. Xiao, Exploration of hypoglycemic peptides from porcine collagen based on network pharmacology and molecular docking, PLoS One, vol. 19, iss. 3, Mar. 2024, Art. no. e0298674. http://doi.org/10.1371/journal.pone.0298674

[10] R. Kumar, K. Chaudhary, J. S. Chauhan, G. Nagpal, R. Kumar, M. Sharma, and G. P. S. Raghava, An *in silico* platform for predicting, screening and designing of antihypertensive peptides, Sciencetific Reports, vol. 5, Jul. 2015, Art. no. 12512. https://doi.org/10.1038/srep12512

[11] T. H. Olsen, B. Yesiltas, F. I. Marin, M. Pertseva, P. J. García-Moreno, S. Gregersen, M. T. Overgaard, C. Jacobsen, O. Lund, E. B. Hansen, and P. Marcatili, AnOxPePred: using deep learning for the prediction of antioxidative properties of peptides, Scientific Reports, vol. 10, Dec. 2020, Art. no. 21471. https://doi.org/10.1038/s41598-020-78319-w

[12] M. S. Khatun, M. M. Hasan, and H. Kurata, PreAIP: Computational prediction of anti-inflammatory peptides by integrating multiple complementary features, Frontiers in Genetics, vol. 10, Mar. 2019, Art. no. 129. https://doi.org/10.3389/fgene.2019.00129

[13] F. H. Waghu, R. S. Barai, P. Gurung, and S. Idicula-Thomas, CAMPR3: a database on sequences, structures and signatures of antimicrobial peptides, Nucleic Acids Research, vol. 44, iss. D1, pp. D1094–D1097, Jan. 2016. https://doi.org/10.1093/nar/gkv1051

[14] N. K. Mehta, A. Lathwal, R. Kumar, D. Kaur, and G. P. S. Raghava, *In silico* tool for predicting, designing and scanning IL-2 inducing peptides, Scientific Reports, vol. 15, Jul. 2025, Art. no. 25692. https://doi.org/10.1038/s41598-025-08388-2

[15] L. G. Trabuco, S. Lise, E. Petsalaki, and R. B. Russell, PepSite: prediction of peptide-binding sites from protein surfaces, Nucleic Acids Research, vol. 40, iss. W1, pp. W423–W427, Jul. 2012. https://doi.org/10.1093/nar/gks398

[16] P. Minkiewicz, A. Iwaniak, and M. Darewicz, BIOPEP-UWM database of bioactive peptides: Current opportunities, International Journal of Molecular Sciences, vol. 20, iss. 23, Nov. 2019, Art. no. 5978. https://doi.org/10.3390/ijms20235978

[17] M. A. S. M. Salim and C.-Y. Gan, Dual-function peptides derived from egg white ovalbumin: Bioinformatics identification with validation using in vitro assay, Journal of Functional Foods, vol. 64, Jan. 2020, Art. no. 103618. https://doi.org/10.1016/j.jff.2019.103618

[18] T. Lafarga, P. O'Connor, and M. Hayes, Identification of novel dipeptidyl peptidase-IV and angiotensin-I-converting enzyme inhibitory peptides from meat proteins using *in silico* analysis, Peptides, vol. 59, pp. 53–62, Sep. 2014. https://doi.org/10.1016/j.peptides.2014.07.005

[19] B. Dziuba and M. Dziuba, New milk protein-derived peptides with potential antimicrobial activity: An approach based on bioinformatic studies, International Journal of Molecular Sciences, vol. 15, iss. 8, pp. 14531–14545, Aug. 2014. https://doi.org/10.3390/ijms150814531

[20] Y.-Y. Ngoh and C.-Y. Gan, Identification of Pinto bean peptides with inhibitory effects on α-amylase and angiotensin converting enzyme (ACE) activities using an integrated bioinformatics-assisted approach, Food Chemistry, vol. 267, pp. 124-131, Nov. 2018. https://doi.org/10.1016/j.foodchem.2017.04.166

[21] Q. Xu, H. Hong, J. Wu, and X. Yan, Bioavailability of bioactive peptides derived from food proteins across the intestinal epithelial membrane: A review, Trends in Food Science & Technology, vol. 86, pp. 399–411, Apr. 2019. https://doi.org/10.1016/j.tifs.2019.02.050

[22] I. Gülseren and B. Vahapoglu, The stability of food bioactive peptides in blood: An overview, International Journal of Peptide Research and Therapeutics, vol. 28, Nov. 2021, Art. no. 2. https://doi.org/10.1007/s10989-021-10321-w

[23] T. Schmidt, A. J. Situ, and T. S. Ulmer, Structural and thermodynamic basis of proline-induced transmembrane complex stabilization, Scientific Reports, vol. 6, Jul. 2016, Art. no. 29809. https://doi.org/10.1038/srep29809