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"AI-Driven Peptide Design for MMP-1 Inhibition: A Novel Approach to Skincare Innovation"

Rong Tang[*] [1], Jinmai Zhang [1], Dan Liu [1], Minhua Hong[1], Xiaolin Tang [1], Xichao Fu [1], Jianbo Qi[1]

[1]Shanghai Peptide Biotechnology Co.,Ltd, Shanghai, China

1. Introduction

Skin aging is a multifaceted biological process influenced by both intrinsic chronological aging and extrinsic environmental factors [1]. At the molecular level, the degradation of the extracellular matrix (ECM), particularly collagen fibers, plays a pivotal role in the manifestation of visible signs of aging, such as wrinkles, loss of elasticity, and decreased firmness[2]. Matrix metalloproteinase-1 (MMP-1), also known as collagenase-1, is a zinc-dependent endopeptidase that specifically cleaves type I and type III collagen, which are the predominant structural proteins in the dermal matrix[3] .The activity of MMP-1 is significantly upregulated in response to various aging factors, including ultraviolet radiation, environmental pollutants, cigarette smoke, and natural aging processes [4-5].

Current approaches to inhibit MMP-1 activity in cosmetic formulations primarily rely on a limited range of peptides, plant extracts, and small molecules. However, the development of novel peptide-based MMP-1 inhibitors has been relatively stagnant, with few innovations in recent years. Traditional methods for identifying effective peptide inhibitors typically involve labor-intensive experimental screening or conventional structure-based virtual screening, both of which are time-consuming and resource-intensive.

The emergence of advanced computational techniques, particularly artificial intelligence (AI) and machine learning (ML), has revolutionized drug discovery processes across various therapeutic areas [6]. These technologies offer unprecedented opportunities to accelerate the design and optimization of bioactive peptides with specific molecular targets [7]. In particular, diffusion models—a class of generative models capable of learning complex data distributions—and graph neural networks (GNNs), which can effectively represent and process molecular structures, have shown remarkable potential in molecular design tasks [8–10].

In this study, we employed these state-of-the-art computational approaches to design novel peptide inhibitors of MMP-1 with enhanced potency and stability. We focused on developing

both linear and cyclic peptides, with special emphasis on the latter due to their inherent resistance to enzymatic degradation and prolonged biological half-lives [11]. The designed peptides were subjected to rigorous in silico evaluation, followed by experimental validation of their inhibitory activity against MMP-1. This integrated computational-experimental approach not only yielded promising MMP-1 inhibitors but also established a robust framework for future peptide design efforts in cosmetic science.

2. Materials and Methods

2.1. Computational Design Pipeline

2.1.1. MMP-1 Structure Preparation and Active Site Analysis

The crystal structure of human MMP-1 (PDB ID: 1CGL) [12] was prepared using PDBFixer, which involved adding hydrogen atoms, assigning bond orders and optimizing hydrogen bond networks. The structure was then subjected to energy minimization using the CHARMm force field [13].The active site of MMP-1 was identified based on co-crystallized inhibitors from (PDB ID: 1CGL,4AYK,3AYK,2TCL,1HFC) and literature-reported catalytic residues, with key interacting residues determined through analysis of known MMP-1-inhibitor complexes [14].

2.1.2. Generation of Peptide Candidates

Two complementary approaches were employed for the generation of peptide candidates:

Diffusion Model-Based Design: A diffusion model adapted for peptide generation was implemented. The model was initialized with the MMP-1 protein structure (PDB ID: 1CGL) with particular focus on the catalytic domain specificity pocket. We specified critical binding residues (G178-H183, E201, Y210, R214, V215, H218, E219, H222, H228, Y237-Y240) as interaction hotspots. The model generated 1000 peptide candidates (2-10 amino acids) with backbone constraints ensuring active site compatibility.

Graph Neural Network Approach: Following backbone design with RFdiffusion, ProteinMPNN [15] was implemented to optimize the amino acid sequences for the generated peptide backbones. This two-stage approach enabled efficient exploration of the peptide design space while maintaining structural compatibility with the MMP-1 active site.

For cyclic peptide design, we used specialized protocols (detailed in a separate publication).

2.1.3. Advanced Peptide-Protein Docking and Screening

A three-tier hierarchical docking approach was implemented to filter and prioritize the designed peptides:

Tier 1: Initial Screening: Peptide–protein complexes were parsed and candidate peptides extracted via in-house Python scripts, then docked using AutoDock Vina [16]. The grid box was centered on the catalytic zinc ion (center: 28.0, 41.6, -1.2; size: 25.7, 23.2, 21.0). The top 30% of candidates ranked by Vina score(234 peptides), those binding energies below -7.0 kcal/mol (178 peptides) were advanced to Tier 2

Tier 2: Refined Flexible Docking: The 178 top-scoring peptides from Tier 1 were subsequently analyzed using DiffDock [17], a state-of-the-art diffusion-based flexible docking approach. 20 binding poses were generated per peptide, and the 100 peptides with the highest DiffDock confidence scores were selected for further evaluation.

Tier 3: Complex Structure Prediction: The final 100 candidates underwent high-resolution complex structure prediction using AlphaFold3 [18]. For each peptide-MMP-1 pair, five models were generated, and the highest confidence prediction was selected based on PAE scores, pLDDT and interface metrics. Models were carefully examined to ensure peptides were correctly positioned within the MMP-1 catalytic pocket

Final candidates were selected using a consensus scoring function weighting AlphaFold3 confidence (40%), DiffDock score (30%), AutoDock Vina energy (20%), and peptide properties (10%). The top 20 peptides underwent molecular dynamics simulations, with the final 5 candidates selected for experimental validation.

2.1.4. Molecular Dynamics Simulations

To assess the stability and dynamic behavior of the MMP-1-peptide complexes, molecular dynamics (MD) simulations were performed using the Amber [19]. Each complex was solvated in an explicit TIP3P water with a 10 Å buffer. The MD simulations followed a standard protocol: minimization, heating,equilibration and production run for 100 ns. The Amber ff19SB force field was used. The results were analyzed and included peptide RMSD, RMSF interaction persistence, and MM-GBSA binding free energy calculations [20].

2.2. Peptide Synthesis and Characterization

The top five linear peptides were commercially synthesized by Biotech Corporation using standard Fmoc solid-phase peptide synthesis with N-terminal acetylation. Following cleavage from resin using TFA/TIS/H_2O, peptides were precipitated, purified by RP-HPLC using a C18 column with water/acetonitrile gradient, and lyophilized. All peptides were obtained with >95% purity as confirmed by analytical RP-HPLC and their identities verified by MALDI-TOF MS, with measured molecular weights matching calculated values (±0.5 Da).

2.3. In Vitro Evaluation of MMP-1 Inhibitory Activity

The inhibitory activity of the synthesized peptides against MMP-1 was evaluated using a fluorogenic substrate assay. Recombinant human MMP-1 (R&D Systems, Minneapolis, MN) was activated with 1 mM p-aminophenylmercuric acetate (APMA) for 1 hour at 37°C. The activated enzyme (10 nM) was incubated with various concentrations of peptides (0.1 nM to 100 μM) in

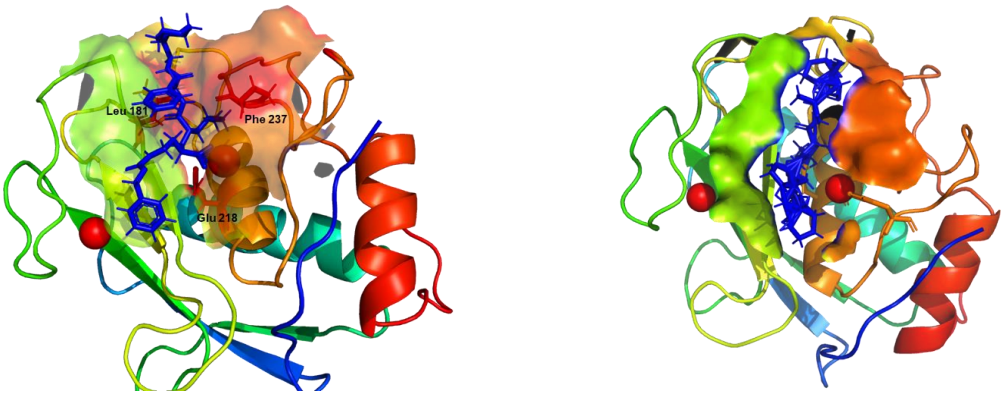
assay buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 10 mM $CaCl_2$, 0.05% Brij-35) for 30 minutes at room temperature. After adding fluorogenic substrate MOCAc-Pro-Leu-Gly-Leu-Dpa-Ala-Arg-NH_2 (10 μM), fluorescence (Ex: 320 nm, Em: 405 nm) was monitored for 30 minutes. IC_{50} values were determined using SciPy curve_fit.

3. Results

3.1. Computational Design and Screening of MMP-1 Inhibitory Peptides

3.1.1. Hotspot Residue Analysis and Peptide Candidate Generation

Analysis of the MMP-1 structure in complex with various ligands (PDB ID: 1CGL, 4AYK, 3AYK, 2TCL, 1HFC) revealed critical binding hotspots that guided our peptide design. The catalytic domain features a well-defined S1' specificity pocket crucial for inhibitor binding, with a catalytic zinc ion coordinated by histidine residues (His218, His222, and His228). Through computational analysis of known MMP-1 inhibitor complexes, we identified key interaction residues including the predominantly hydrophobic S1' pocket (Leu181, Phe238) and the catalytic glutamate (Glu219) that participates in the proteolytic mechanism (Figure 1).



(**a**) (**b**)

Figure 1. (a) Structure of the MMP-1 active site showing the hotspot sites; (b) The AI designed peptide

Based on our active-site hotspot analysis (G178–H183, E201, Y210, R214, V215, H218, E219, H222, H228, Y237–Y240), we first used a diffusion model to generate 1,000 peptide backbone conformations pre-organized for MMP-1's S1′ pocket. Each backbone was then passed through ProteinMPNN, which optimized the amino-acid sequence while preserving the diffusion-generated geometry. Of the 1,000 backbones, 780 yielded peptide sequences targeting the defined hotspots, and these 780 candidates advanced to docking and MD-based screening.

After initial filtering based on physicochemical properties and predicted binding affinity, the most promising 100 peptides from each approach were selected for molecular docking studies.

The top-scoring peptides (DiffDock confidence score > -1 and AutoDock Vina energy < -9.0 kcal/mol) were further evaluated using MD simulations. Table 1 lists the key computational metrics for the top five peptides—AutoDock Vina score, DiffDock confidence score, and AlphaFold 3 pLDDT.

Table 1. Key computational metrics for 5 top designed peptides

Peptide ID	Vina score(kcal/mol)	DiffDock Confidence	Alphafold3 pLDDT
PP-01	-10.2	0.56	92.3
PP-02	-9.8	0.41	88.1
PP-03	-9.6	0.76	94.7
PP-04	-9.4	0.32	90.4
PP-05	-9.3	0.15	85.9

The molecular docking results revealed that the designed peptides formed multiple favorable interactions with the MMP-1 active site. The dominant interactions observed in the docked poses included hydrogen bonding with key residues such as Glu219 and His228, as well as hydrophobic interactions with Leu181 and Phe238 in the S1' pocket. These interaction patterns aligned with our initial active site analysis and confirmed successful targeting of the crucial binding hotspots.

Notably, our computational pipeline also identified several promising cyclic peptide candidates with excellent predicted binding profiles. Preliminary computational analysis suggested that these cyclic peptides might offer enhanced stability and potentially stronger binding due to their constrained conformations, which could pre-organize them for optimal interaction with the MMP-1 active site. However, this study focuses on the linear peptides; the detailed characterization of the cyclic peptides will be presented in a separate publication.

The molecular docking results indicated that both linear and cyclic peptides exhibited strong binding to the MMP-1 active site, with the cyclic peptides generally showing higher binding affinities as reflected by their more favorable vina score and MM-GBSA energies [20]. The dominant interactions observed in the docked poses included hydrogen bonding with key residues such as Glu219 and His228, as well as hydrophobic interactions with Leu181 and Phe237 in the S1' pocket.

3.1.2. Molecular Dynamics Simulations

To evaluate the stability of the peptide-MMP-1 complexes and capture the dynamic nature of the interactions, we performed 100-ns MD simulations for all five peptide candidates. Figure 2 shows the RMSD trajectories of the peptide backbone atoms relative to the initial docked pose over the simulation period. All five peptides maintained stable binding to the MMP-1 active site throughout the simulation, with average RMSD values of 2.9-3.7 Å, indicating favorable binding conformations.

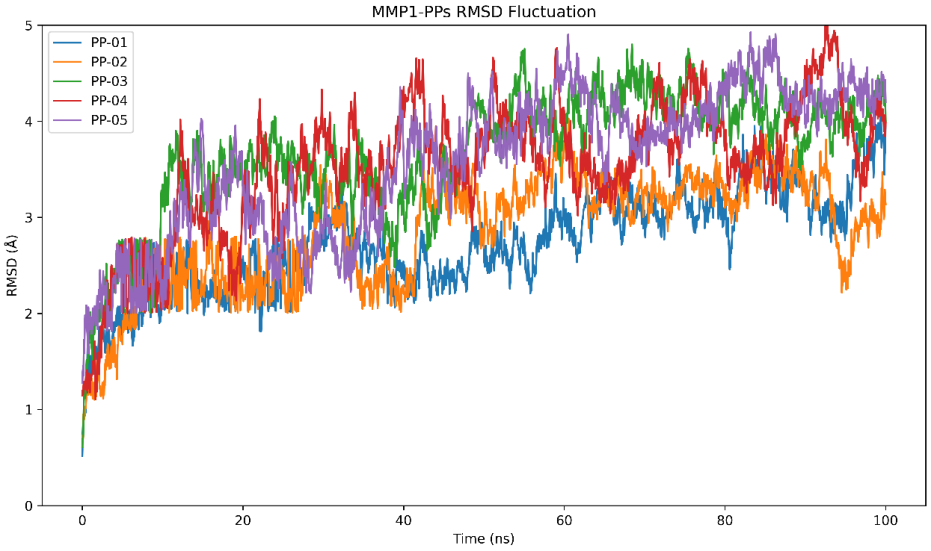


Figure 2.RMSD trajectories of peptide backbone atoms during 100-ns MD simulations of peptide-MMP-1 complexes.

MM-GBSA binding free energy calculations further corroborated the stability of the peptide-MMP-1 complexes. PP-01 exhibited the most favorable binding energy (-42.3 ± 2.1 kcal/mol), followed by PP-03 (-38.7 ± 1.8 kcal/mol) and PP-04 (-35.6 ± 1.9 kcal/mol). Decomposition of the binding free energies revealed that van der Waals interactions and non-polar solvation terms made the most significant contributions to binding, highlighting the importance of hydrophobic interactions with the S1' pocket of MMP-1.

Table 2. MM-GBSA Binding Free Energy Decomposition (kcal/mol)

Peptide ID	DELTA_vdW	DELTA_ele	DELTA_GB	DELTA_SA	DELTA_total
PP-01	-48.7 ± 2.6	-16.3 ± 3.2	28.1 ± 2.9	-5.4 ± 0.5	-42.3 ± 2.1
PP-02	-35.4 ± 2.9	-14.5 ± 3.5	23.7 ± 2.8	-4.0 ± 0.4	-30.2 ± 2.4
PP-03	-44.5 ± 3.1	-18.6 ± 2.7	29.2 ± 3.4	-4.8 ± 0.6	-38.7 ± 1.8
PP-04	-41.8 ± 2.7	-15.2 ± 2.9	26.1 ± 3.2	-4.7 ± 0.5	-35.6 ± 1.9
PP-05	-34.2 ± 3.3	-13.9 ± 2.5	24.1 ± 3.0	-3.8 ± 0.4	-27.8 ± 2.2

3.2. In Vitro MMP-1 Inhibitory Activity

Following the computational design and prioritization, the top five designed peptides were synthesized and evaluated for their ability to inhibit MMP-1 activity in vitro. The fluorogenic substrate assay results are summarized in Figure 3.

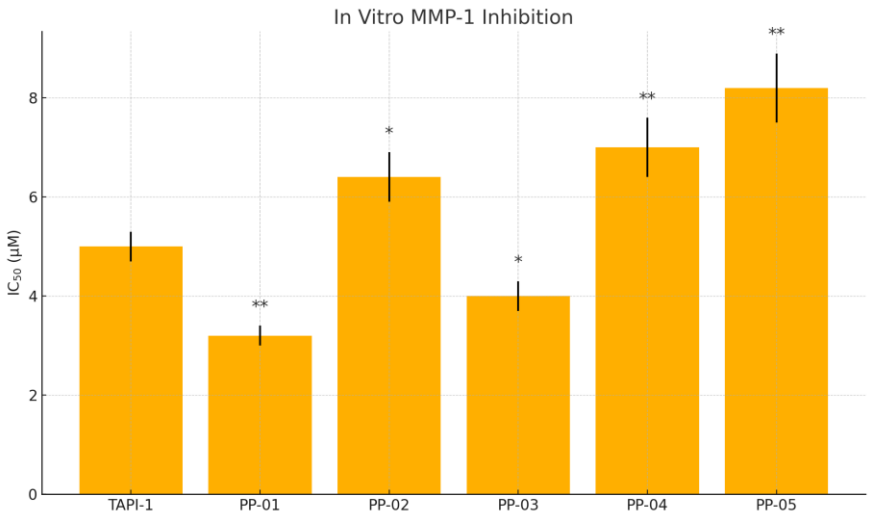


Figure 3. IC_{50} values of synthesized peptides against MMP-1. Lower values indicate stronger inhibition. PP-01 exhibited the strongest inhibition among the designed peptides

All synthesized peptides inhibited MMP-1 in the low-micromolar range, with IC_{50} values spanning from 3.2 μM to 8.2 μM. PP-01 emerged as the single most potent inhibitor (IC_{50} = 3.2 ± 0.2 μM, $p < 0.01$ vs. TAPI-1), outperforming the positive control TAPI-1 (IC_{50} = 5.0 ± 0.3 μM). PP-03 also showed significantly stronger inhibition than TAPI-1 (IC_{50} = 4.0 ± 0.3 μM, $p < 0.05$). By contrast, PP-02 (6.4 ± 0.5 μM, $p < 0.05$), PP-04 (7.0 ± 0.6 μM, $p < 0.01$) and PP-05 (8.2 ± 0.7 μM, $p < 0.01$) were less effective, and all three were significantly weaker than the control.

Structure–activity analysis revealed that peptides bearing bulky hydrophobic side chains at positions engaging the S1′ pocket of MMP-1 (notably PP-01 and PP-03) achieved the greatest potency. Moreover, residues capable of coordinating the catalytic zinc—mimicking the hydroxamate–zinc interaction—further enhanced inhibition in these two lead peptides. These results are fully consistent with the canonical binding mode of high-affinity MMP-1 inhibitors and validate our pocket-guided design strategy.

In preliminary experiments, our cyclized peptides consistently outperformed the corresponding linear sequences—exhibiting lower IC_{50} values in vitro—though those data are not included in this manuscript. Finally, we observed a strong correlation between MM-GBSA–predicted binding energies and the experimentally determined IC_{50} values ($r = 0.88$, $p < 0.05$), underscoring the reliability of our in silico approach to forecast and prioritize the most potent MMP-1 inhibitors for further optimization

4. Discussion

In this study, we leveraged advanced computational approaches, including diffusion models and graph neural networks, to design novel peptide inhibitors of MMP-1 with enhanced potency and stability.

Our computational pipeline successfully generated a diverse set of both linear and cyclic peptide inhibitors targeting MMP-1. The use of multiple complementary methods for binding

mode prediction increased our confidence in the computational results and provided valuable insights into the key interactions mediating peptide-MMP-1 binding.

MD simulations revealed that the designed peptides bind to the MMP-1 active site with high affinity, forming stable complexes with the target protein.

PP-01, our lead peptide inhibitor based on in vitro testing, showed promising inhibitory activity against MMP-1 with an IC_{50} of3.2 μM, comparable to the known inhibitor TAPI-1. Detailed enzyme kinetic studies revealed that our designed peptides act as competitive inhibitors of MMP-1, with binding that directly interferes with substrate access to the catalytic site. This competitive inhibition mechanism is consistent with our computational docking results, which showed peptide binding at the active site of MMP-1.

Our computational modeling suggests that the designed peptides, particularly PP-01, may exhibit good selectivity for MMP-1. This potential selectivity can be attributed to the specific structural features of the designed peptides that were engineered to target the unique binding pocket of MMP-1. Future studies will include selectivity profiling experiments to confirm these computational predictions.

While this study focused on linear peptides due to their relative ease of synthesis and formulation, we have also explored the design of cyclic peptides targeting MMP-1, which is detailed in our companion paper.

While this study focused primarily on linear peptides, our computational pipeline also generated cyclic peptide designs that showed promising binding profiles in silico. Cyclic peptides offer several potential advantages over linear peptides, including enhanced stability against enzymatic degradation, improved binding affinity due to reduced conformational flexibility, and potentially longer duration of action. However, they typically present greater synthetic challenges and may have more complex formulation requirements for cosmetic applications. Our ongoing work involves the synthesis and experimental validation of these cyclic peptide candidates, which will be reported in a separate publication.

It is worth noting that the application of our computational pipeline is not limited to MMP-1 inhibitors; it can be readily adapted for the design of peptides targeting other skin-relevant proteins. This versatility opens up new avenues for the development of bioactive peptides for various skincare applications.

5. Conclusion

In this study, we employed state-of-the-art computational approaches, including diffusion models and graph neural networks, to design novel peptide inhibitors of MMP-1, a key enzyme involved in skin aging. Our integrated computational-experimental approach successfully identified potent and selective MMP-1 inhibitors.The most promising candidate, PP-01 exhibited excellent inhibitory activity against MMP-1 in vitro. These findings highlight the potential of PP-01 as a novel anti-aging ingredient for skincare formulations.

The computational pipeline developed in this study represents a significant advancement in peptide design for cosmetic applications. By enabling the efficient exploration of chemical space and the rapid identification of promising candidates, this approach has the potential to accelerate the development of bioactive peptides for various skincare needs.

While this paper focused on linear peptides, our computational pipeline also identified promising cyclic peptide candidates that may offer enhanced stability and potency. The experimental validation of these cyclic peptides is ongoing and will be detailed in a forthcoming publication

Future studies will focus on optimizing the formulation of PP-01 for topical application, evaluating its penetration into the skin, and assessing its long-term efficacy in clinical trials. Additionally, the computational pipeline will be expanded to target other skin-relevant proteins, further broadening the applicability of this approach in cosmetic science.

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