Design and Classification of Innovative Peptides for Musculoskeletal Infection Treatment using Artificial Intelligence

Bingyun Li, PhD;[1] Alexa Sowers[1,2]
[1]Department of Orthopaedics, School of Medicine, West Virginia University, Morgantown, WV 26506, United States
[2]Department of Pharmaceutical and Pharmacological Sciences, School of Pharmacy, West Virginia University, Morgantown, WV, 26506, United States

INTRODUCTION: Antimicrobial resistance (AMR) is one of the most significant global challenges of the 21st century, which is projected to cause 10 million deaths annually and a financial burden of $100 trillion dollars by 2050 if no effective solutions emerge. The development of antibiotics has declined over the past 30 years, due to concerns about rapid resistance. Antimicrobial peptides (AMPs) have emerged as a promising alternative to conventional antibiotics due to their unique mechanisms of action. However, traditional peptide design, especially when designing multi-functional peptides, is costly and time-consuming. This study explores the potential of ChatGPT in classifying and designing peptides for musculoskeletal infection treatment. It was *hypothesized* that integrating optimized prompt engineering and peptide training sequences into ChatGPT would enable accurate *in silico* design and classification of AMPs, achieving ≥0.9 accuracy.

METHODS: ChatGPT was applied to design and classify AMPs with and without functions like non-hemolytic, cell-penetrating, and/or anti-cancer properties. Prompts were optimized using prompt engineering and using in-context training sequences, which were sourced from validated databases corresponding to each of these functions. Sequences for in-context training in peptide design and classification were collected from well-known databases and selected randomly using a number generator to avoid bias. Most generations used ChatGPT-4.0, with comparisons across 3.5 and 4o versions. Peptides designed by ChatGPT were validated using established machine learning classifiers, where prediction scores >0.5 confirmed biological activity. The information included in the prompts, such as peptide length and number of training examples, were selected based on the performance of the prompt used for the model. Peptides with dual, triple, and quadruple functions were designed using prompts that combined antimicrobial activity with additional properties such as non-hemolytic (NHP), cell-penetrating (CPP), or anti-cancer (ACP) functions. Each design prompt incorporated specific combinations of these functions to generate sequences with more than one function.

RESULTS: Various prompt engineering techniques were applied to improve ChatGPT's ability to design and classify AMPs. The basic prompt produced the least accurate results, with low probablity scores (Fig. 1A and E). Informed task prompt, few-shot with rationale, and both positive and negative training sequences resulted in higher probability score and accuracy (Fig. 1A-E). High probablity scores were also achieved in designing petides with triple functions like AMP, NHP, and CPP (Fig. 1D). For classificatiom, few-shot with rationale, both positive and negative training sequences, and more recent ChatGPT version led to higher F1 scores (Fig. 1F-H). Overall, ChatGPT was successfully used to design peptides with single, dual and multi-functional properties that show high probablity and accuracy.

DISCUSSION: Traditional peptide design methods are time-consuming and rely heavily on expert knowledge. While machine learning has helped identify peptide properties, it typically requires large datasets and technical expertise to build classification tools. In contrast, ChatGPT offers an accessible alternative by accurately classifying and generating functional peptides with minimal training. Our study showed that ChatGPT can design and classify peptides such as AMPs, CPPs, NHPs, and ACPs with high accuracy and F1 scores. Unlike previous models that require fine-tuning, ChatGPT can perform well using prompt engineering, even for less common peptide functions with limited data. Importantly, we demonstrated that ChatGPT has the capability to design peptides with multiple biological functions.

CONCLUSIONS: Our findings demonstrate that prompt engineering and in-context training significantly enhance ChatGPT's ability to design and classify AMPs. The most effective strategies included the use of informed task prompts, inclusion of both positive and negative training sequences, optimal sequence lengths (20-30 amino acids), and the latest ChatGPT versions (4.0 and 4o). ChatGPT exhibited strong potential for designing dual- and multi-functional peptides, though performance declined with increasing functional complexity. Overall, these results highlight ChatGPT's utility as a powerful tool for peptide discovery to be used in musculoskeletal infection treatment.

SIGNIFICANCE: There is a shortage of antimicrobial agents as antibiotic resistance keeps occurring, and traditional peptide design is costly and time-consuming. ChatGPT may offer a better option to design AMPs for managing musculoskeletal injuries and diseases.

ACKNOWLEDGEMENTS: The BridgesDH NRT Fellowship funded by NSF Award # 2125872, the NIH T32 Systems Toxicology (SyTOX) fellowship (NIH T32-E S032920) and Engage WVU Giving Circle fellowship are acknowledged.

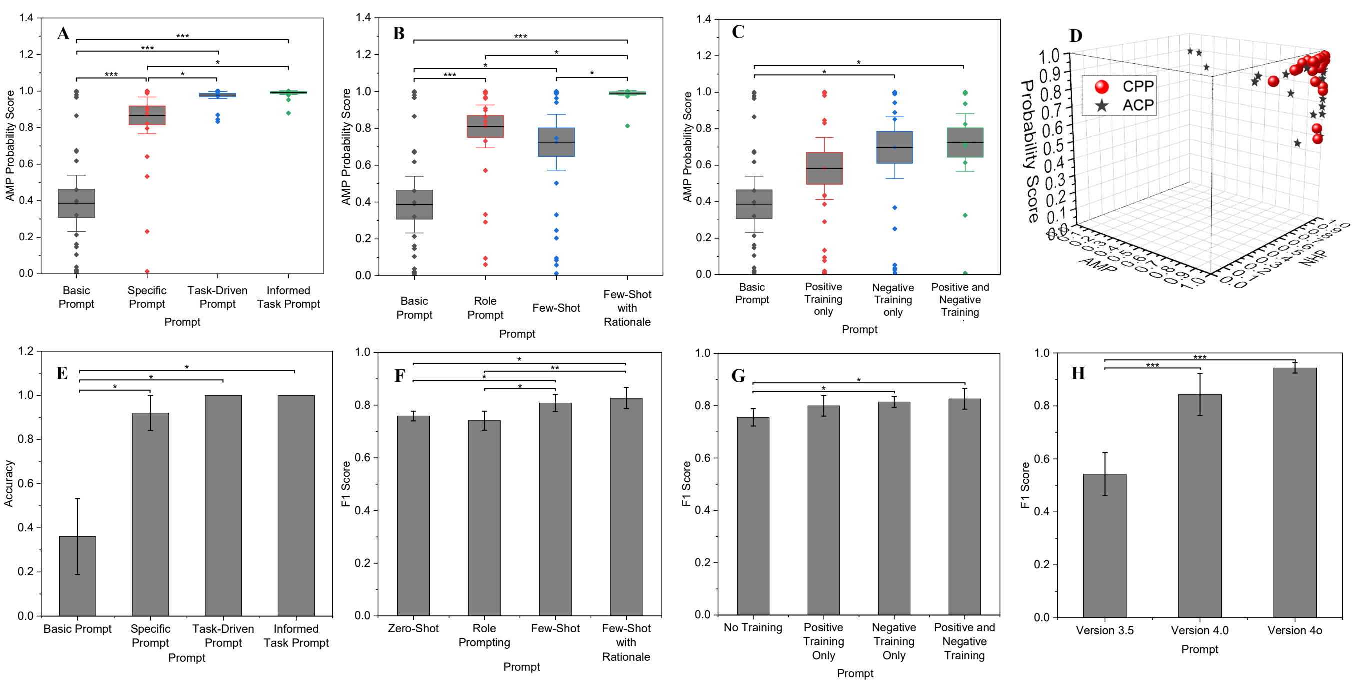

Fig. 1. Prompt optimization for designing and F1 scores for classifying AMPs using ChatGPT. (A-D) Distribution of probability scores and (E) Accuracy of designing AMPs based. (F-H) F1 scores for classifying AMPs. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.