Machine Learning-Guided Design of Next-Generation Triple Agonist Peptide Therapeutics for Metabolic Disease

Anthony Wong BS[1], Sanskruthi Guduri BS[1], Tsungyen Chen MD[1,2], Kunal Patel MD[1,2]

1 Carle Illinois College of Medicine, 506 S Mathews Ave, Urbana, IL 61801

2 Carle Foundation Hospital, 611 W Park St, Urbana, IL 61801

Carle Illinois COLLEGE OF MEDICINE

Carle ILLINOIS

INTRODUCTION

The global burden of obesity and type 2 diabetes mellitus (T2DM) is a major public health challenge. Over 650 million adults worldwide are obese, and 537 million individuals live with diabetes. In the U.S., ~40% of adults have metabolic syndrome, which doubles cardiovascular risk and increases annual healthcare costs to $5,732 per patient compared to $3,581 for those without the condition [1].

Clinical breakthroughs:

- New Incretin Therapeutics Targeting Glucagon Receptor (GCGR), Gastric Inhibitory Peptide Receptor (GIPR), and GLP1 Receptor (GLP1R).
- Tirzepatide (dual GIPR/GLP1R agonist): HbA1c reduction up to 2.58% in SURPASS trials [2].
- Retatrutide (triple GCGR/GLP1R/GIPR agonist): 24.2% weight loss at 48 weeks (Phase 2) [3].

Multi-task convolution neural networks (CNN) [4]:
- Constrained to dual GCGR/GLP1R agonist design
- Fixed Sequence Length of 30 AAs
- Limited to standard AAs

Graph Attention Networks [5]
- Accommodates variable length peptide sequences
- Preserves molecular topology

METHODS

We developed a GAT-based framework to predict multi-receptor peptide binding and combined it with a genetic algorithm for sequence optimization. Independent validation confirmed predictive performance, while GA-driven optimization produced novel peptide candidates for experimental prioritization.

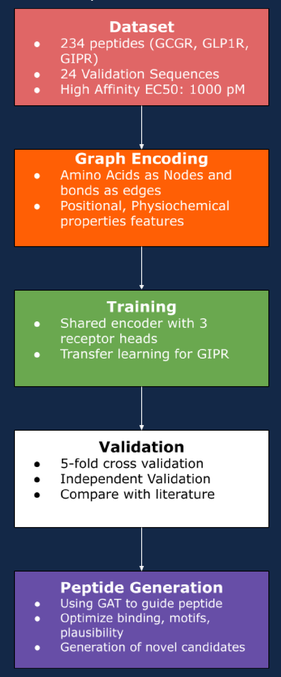

Figure 1: Data Pipeline.

RESULTS

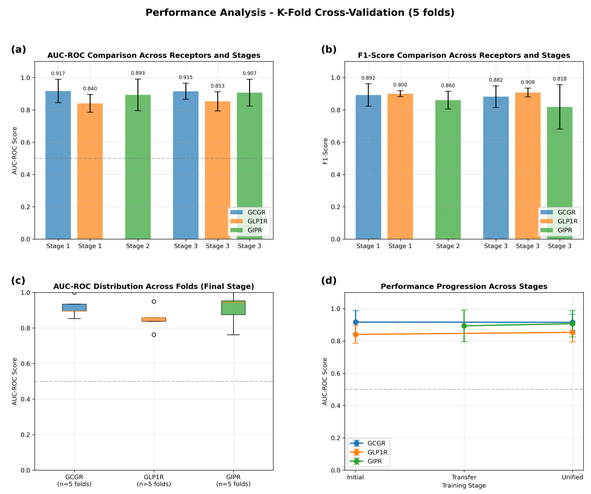

Figure 2: Performance Analysis of Graph Attention Network Using 5-Fold Cross-Validation. Transfer learning evaluation across three training stages: Stage 1 (initial GCGR+GLP1R training), Stage 2 (GIPR transfer learning), and Stage 3 (unified fine-tuning). (A) AUC-ROC scores demonstrating consistent high performance across all receptors and stages (>0.84 for all conditions). (B) F1-scores showing robust classification performance with values exceeding 0.81 across all receptor-stage combinations. (C) Box plots illustrating AUC-ROC score distributions across 5 folds for the final unified stage, with median values above 0.9 for GCGR and GIPR, and 0.85 for GLP1R. (D) Performance progression trajectories showing stable or improved AUC-ROC scores from initial to unified training stages for all three receptors. Error bars represent standard deviation across folds. Dashed horizontal line indicates random classifier performance (AUC-ROC = 0.5).

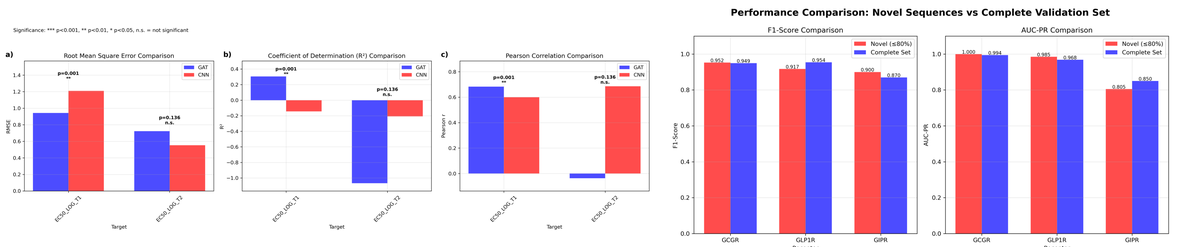

Figure 3: Comparative Performance Analysis of Graph Attention Networks versus Ensemble Multi-task Convolutional Neural Networks. Performance metrics comparing GAT (blue) and CNN ensemble (red) models across EC50 prediction targets. (A) Root mean square error (RMSE) comparison showing significantly lower prediction error for GAT on EC50_LOG_T1 (p=0.001) with comparable performance on EC50_LOG_T2 (p=0.136, n.s.). (B) Coefficient of determination (R^2) comparison demonstrating superior explained variance for GAT on EC50_LOG_T1 (p<0.001) with equivalent performance on EC50_LOG_T2 (p=0.136, n.s.). (C) Pearson correlation coefficients indicating stronger linear relationships for GAT predictions on EC50_LOG_T1 (p<0.001) and comparable correlations on EC50_LOG_T2 (p=0.136, n.s.). Statistical significance determined by paired t-tests: ***p<0.001, **p<0.01, *p<0.05, n.s. = not significant.

Figure 4: GAT Model Performance Comparison Between Novel Sequences and Complete Validation Set. Performance evaluation comparing novel sequences with ≤80% similarity to training data (red) versus the complete validation dataset (blue). (A) F1-score comparison demonstrating comparable performance for GCGR and GLP1R, with novel sequences showing superior performance for GIPR (0.900 vs 0.870). (B) Area under the precision-recall curve (AUC-PR) comparison revealing excellent performance across all receptors, with novel sequences achieving perfect discrimination for GCGR (AUC-PR = 1.000) and maintaining high performance for GLP1R (0.985) and GIPR (0.805).

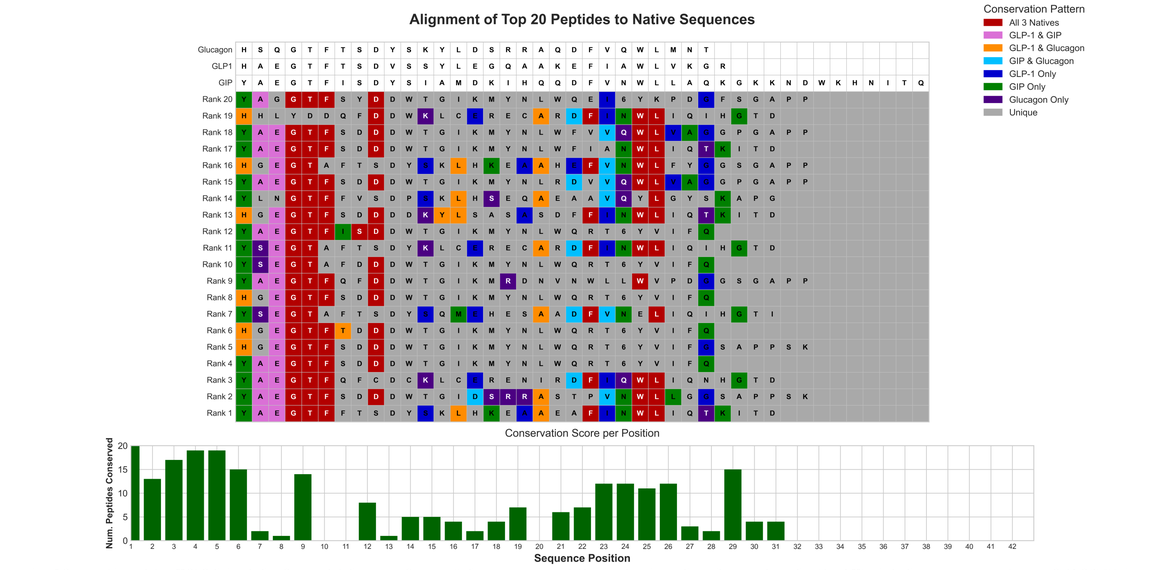

Figure 5: Sequence Alignment of Top 20 GA-Optimized Peptides Compared to Native Hormone Sequences Multiple sequence alignment of the highest-ranking genetic algorithm-generated peptides (Rank 1-20) against native hormone sequences (glucagon, GLP-1, GIP). A) Amino acid positions are colored according to sequence similarity patterns: red indicates conservation across all three native sequences, pink shows GLP-1/GIP conservation, cyan represents GIP/glucagon similarity, blue indicates GLP-1-specific residues, purple shows glucagon-specific residues, green denotes GLP-1-only conservation, and gray represents unique variations. B) Conservation Score of the top 20 rank peptides by sequence position.

DISCUSSION

Results highlight receptor-dependent modeling challenges:
- GCGR = Improved compared to models in literature
- GLP1R = Comparable to model in literature
- GIPR = data-limited but mitigated with transfer learning

Motif analysis:
The preservation of the EGTF generated sequences aligns with its known importance for incretin receptor recognition

Limitations:
Dataset may have systematic bias due to multi-laboratory origins
Experimental validation remains essential before therapeutic application.

However, The GAT-based predictor offers practical applications for peptide drug discovery.

CONCLUSION

This work presents the **first comprehensive AI framework** for rational triple agonist peptide design, demonstrating significant receptor-specific computational advantages over established methods.
The Graph Attention Network approach successfully generated optimized peptide candidates **with balanced multi-receptor binding activity**, providing a systematic pipeline to prioritize experimental validation efforts.
This computational framework offers an **accelerated pathway** for developing next-generation diabetes and obesity therapeutics while establishing a broadly applicable methodology for **multi-target drug discovery** across other therapeutic areas.

REFERENCES

1. Boudreau DM, Malone DC, Raebel MA, et al. Health care utilization and costs by metabolic syndrome risk factors. *Metab Syndr Relat Disord*. 2009;7(4):305-14.
2. Jastreboff AM, Aronne LJ, Ahmad NN, Wharton S, Connery L, Alves B, et al. Tirzepatide once weekly for the treatment of obesity. N Engl J Med. 2022;387(3):205-216.
3. Jastreboff AM, Kaplan LM, Frías JP, Wu Q, Du Y, Gurbuz S, Coskun T, Haupt A, Milicevic Z, Hartman ML; Retatrutide Phase 2 Obesity Trial Investigators. Triple-Hormone-Receptor Agonist Retatrutide for Obesity - A Phase 2 Trial. N Engl J Med. 2023 Aug 10;389(6):514-526. doi: 10.1056/NEJMoa2301972. Epub 2023 Jun 26. PMID: 37366315.
4. Puszkarska AM, Taddese B, Revell J, Davies G, Field J, Hornigold DC, et al. Machine learning designs new GCGR/GLP-1R dual agonists with enhanced biological potency. Nat Chem. 2024;16(9):1436-1444.
5. Zhang Z, Chen L, Zhong F, Wang D, Jiang J, Zhang S, Jiang H, Zheng M, Li X. Graph neural network approaches for drug-target interactions. Curr Opin Struct Biol. 2022 Apr;73:102327. doi: 10.1016/j.sbi.2021.102327. Epub 2022 Jan 21. PMID: 35074533.

ILLINOIS