Rapid Cyclic Peptide Development Enabled by Artificial Intelligence

Doudou Qin[1], Genwei Zhang[1], Weijia Wei, Yanwei Ding, Hongfei Dai, Nan Jing, Qingxia Zhang, Kuo Yu, Lipeng Lai[2]

[1] CO-FIRST AUTHORS, [2] CORRESPONDING AUTHOR

Corresponding author: Lipeng Lai – lipeng.lai@xtalpi.com
PepiX™ platform: pepix@xtalpi.com

Background

Cyclic peptides have emerged as an increasingly attractive therapeutic modality, occupying a distinctive space between small molecules and biologics. Their conformational rigidity, high binding affinity, and tunable physicochemical properties enable access to biological targets that are often challenging for conventional drug modalities[1,2]. Yet, despite these advantages, the discovery of high-affinity cyclic peptides against predefined targets remains intrinsically difficult. A central limitation is not simply the size of sequence space, but the low information density of each optimization cycle once cyclization introduces strong conformational coupling. In cyclic peptides, local sequence changes frequently propagate global structural consequences, making structure–activity relationships (SAR) highly non-additive and difficult to extrapolate from standard medicinal chemistry logic[2–4].

The discovery of high-affinity cyclic peptides against predefined targets remains intrinsically difficult.. What slows progress is not only how many variants can be screened, but how efficiently each round of data is converted into the next round of design choices.

As a result, traditional discovery workflows—large-scale screening followed by empirical, experience-driven refinement—often struggle to scale efficiently in rapid biotech settings. Bigger libraries may increase hit opportunities, but they do not automatically improve decision quality. What slows progress is not only how many variants can be screened, but how efficiently each round of data is converted into the next round of design choices. This is particularly relevant for cyclic peptides, where many experimental iterations can yield only incremental or low-value information. In this context, artificial intelligence (AI), when tightly integrated with high-throughput experimental technologies, offers a practical route to accelerate discovery by increasing the information extracted from each Design–Make–Test–Analyze (DMTA) cycle[3,5].

Technological Overview

This work illustrates an AI-enabled workflow for rapid, target-defined cyclic peptide development that integrates mRNA display–based library screening with AI-assisted sequence and structural optimization. Discovery begins with a predefined protein target, but the central objective is not simply to generate more candidates. Rather, it is to improve how efficiently experimental information is translated into design decisions. mRNA display provides access to ultra-large peptide libraries and supports rapid exploration of diverse sequence and cyclization architectures[6]. That experimental throughput is important, but experimental enrichment alone is often insufficient to resolve non-linear SAR or to identify which optimization paths are truly worth pursuing[3,6].

A key distinction of this workflow is that AI is not positioned as a black-box affinity predictor. Instead, it combines sequence-level modeling, structure-aware ranking, and modification prioritization to identify variants most likely to improve affinity while preserving developability[3,5]. In practical terms, AI is used to interpret emerging sequence–activity relationships from screening data, rank productive substitutions, and narrow the set of variants that need to be synthesized and tested. Its value lies in maximizing information gain per DMTA cycle rather than merely assigning a score to each sequence[3].

AI-Enabled Cyclic Peptide Discovery as a Closed DMTA Cycle

The workflow can be usefully framed as a closed DMTA cycle tailored for cyclic peptide discovery (**Figure 1**). In the **Design** phase, AI-guided prioritization defines a feasible and information-rich modification space rather than attempting exhaustive enumeration. In the **Make** phase, high-throughput platforms such as mRNA display and rapid peptide synthesis enable parallel generation of diverse cyclic architectures[6].

In the **Test** phase, quantitative assays including surface plasmon resonance (SPR), cellular uptake measurements, and in vivo studies provide readouts that are sufficiently consistent to guide iteration. In the **Analyze** phase, AI is used to interpret outcomes under non-linear SAR conditions, identifying patterns that would be difficult to capture heuristically from individual experiments alone. The practical advantage of this closed loop is not merely predictive accuracy; it is decision compression—shortening the path between one experimental round and the next[3,4].

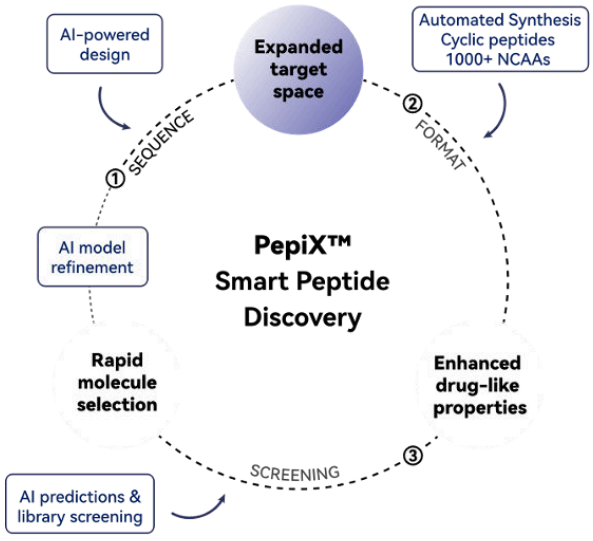

Figure 1. Closed DMTA cycle for AI-enabled cyclic peptide discovery.

Case Study 1: mRNA Display and AI-Assisted Optimization for High-Affinity Target Binders

The first case study illustrates how integrating AI-guided refinement with mRNA display screening changes optimization behavior across three distinct stages: previously reported sequence space, mRNA display–enriched hits, and AI-refined variants.

As an initial benchmark, peptides from this previously reported sequence set were evaluated by SPR and showed measurable target engagement, but with broad variability in affinity (**Table 1**). The median affinity for this group was approximately 49 nM, with the best binder reaching 4 nM, and 19% of candidates falling below the predefined activity threshold of 10 nM. Cyclic peptides identified through mRNA display screening improved both hit quality and hit rate: the median affinity shifted to 5 nM, the best binder improved to 2 nM, and the proportion of candidates below the activity threshold increased to 67%.

When AI-guided sequence and structural refinement was applied to selected mRNA display hits, the optimized variants showed a further shift toward stronger and more uniform binding. The median affinity improved by approximately 2-fold relative to the mRNA display group and 24-fold relative to the previously reported sequence set, while the best binder reached 0.6 nM. The practical importance of this result is not simply that AI found a stronger binder, but that it reduced the number of unproductive optimization paths that had to be explored experimentally.

Table 1: Representative stage-wise comparison of discovery efficiency in Case Study 1

Stage	Sequence Source	Median Affinity	Best Affinity	Below Activity Threshold
Stage 1	Previously reported sequence space	49 nM	4 nM	19%
Stage 2	mRNA display–enriched hits	5 nM	2 nM	67%
Stage 3	AI-refined variants	2 nM	0.6 nM	75%

Case Study 2: AI-Guided Discovery of Cyclic Peptides for Blood–Brain Barrier Transport

Case Study 2 explores the use of AI-enabled cyclic peptide discovery in the context of blood–brain barrier (BBB) transport, a functional challenge that extends beyond conventional target binding. Efficient delivery of macromolecules to the brain remains a major limitation for many therapeutic strategies, as most biologics and large molecules cannot cross the BBB through passive diffusion[5].

To address this challenge, cyclic peptides were identified as potential transport ligands capable of mediating receptor-driven BBB translocation. Initial hits were obtained through mRNA display screening of large peptide libraries against BBB-associated targets. These libraries contained both linear and cyclic peptide architectures, enabling exploration of diverse binding topologies. Following hit identification, AI-guided refinement was applied to improve binding performance and structural compatibility with receptor-mediated transport mechanisms.

Analysis confirmed measurable binding interactions for optimized peptide candidates, with representative affinities in the sub-micromolar range. In addition, fluorescence imaging experiments indicated strong cellular uptake for selected peptides, suggesting efficient cell-penetrating properties (**Figure 2a**).

To evaluate functional transport *in vivo*, representative peptide candidates were assessed in mouse models following systemic administration. Brain imaging and tissue analysis demonstrated detectable peptide exposure within brain tissue at defined time points after dosing (**Figure 2b**).

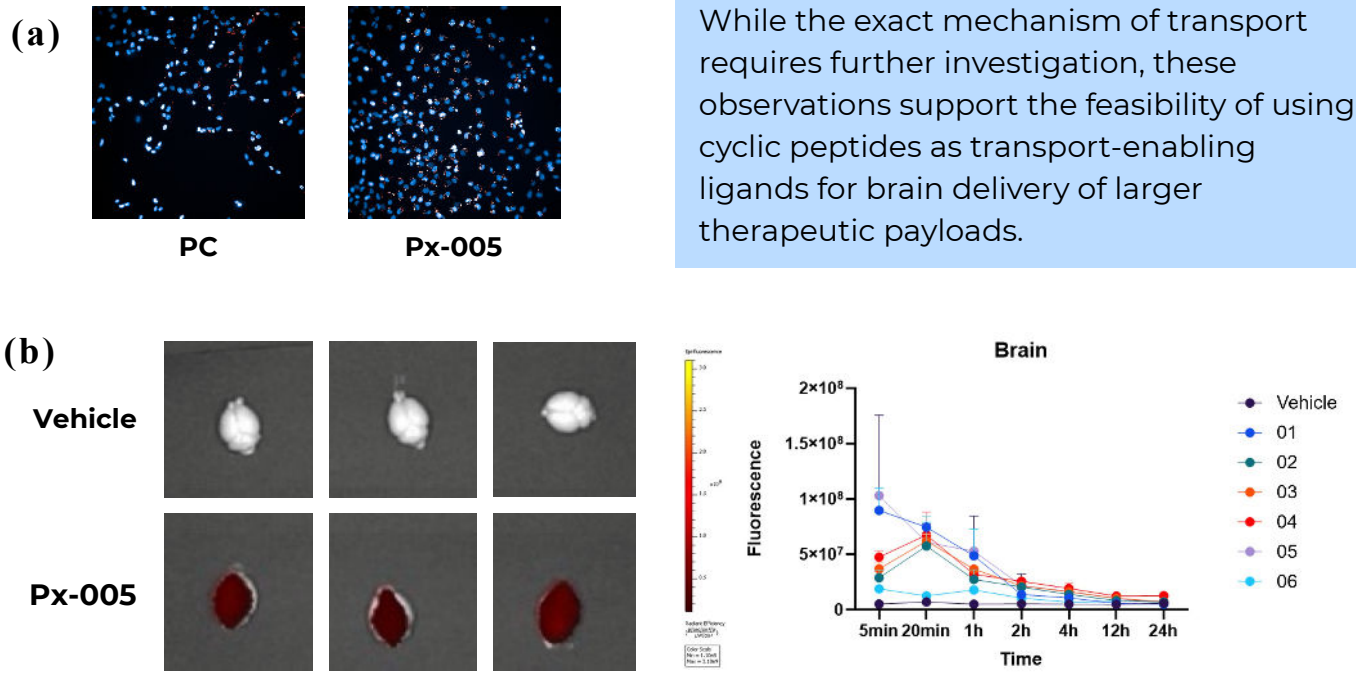

Figure 2. AI-enabled cyclic peptides supporting blood–brain barrier transport. (a) Fluorescence imaging showing cellular uptake of selected peptides. (b) *In vivo* imaging indicating peptide exposure in mouse brain.

While the exact mechanism of transport requires further investigation, these observations support the feasibility of using cyclic peptides as transport-enabling ligands for brain delivery of larger therapeutic payloads.

This case illustrates an important distinction from affinity-focused discovery. For BBB transport, binding affinity alone is not sufficient to predict functional performance. Instead, molecular size, structural rigidity, receptor engagement, and systemic pharmacokinetics jointly determine delivery efficiency. The integration of AI-guided design with high-throughput experimental screening provides a practical way to navigate these competing requirements within a closed DMTA cycle.

Conclusion

Taken together, these examples show that rapid cyclic peptide discovery is not simply a matter of running larger libraries or generating more screening data. The more consequential shift comes from embedding AI within a closed DMTA cycle so that each round produces more useful design information. In Case Study 1, that translated into quantifiable gains in median affinity, reduced affinity dispersion, fewer low-performing outliers, and a measurable reduction in low-value optimization rounds. In Case Study 2, the same framework was extended to a more complex biological objective, demonstrating that the platform can move beyond binder discovery toward functionally relevant transport behavior at the blood–brain barrier.

From a platform perspective, the differentiated value lies in the combination of experimental throughput, flexible screening modalities, and an AI layer designed to improve decision quality rather than merely rank candidates. More broadly, this work suggests that the future of cyclic peptide discovery will be shaped not by library scale alone, but by tighter integration between computational design, experimental validation, and learning velocity across the DMTA cycle[1,7]. Platforms that can reliably reduce unproductive iterations or compress discovery to the same endpoint in fewer experimental cycles may define the next generation of peptide drug hunting.

References

(1). You, S.; McIntyre, G.; Passioura, T. The Coming of Age of Cyclic Peptide Drugs: An Update on Discovery Technologies. Expert Opin. Drug Discov. 2024, 19 (8), 961–973. https://doi.org/10.1080/17460441.2024.2367024.

(2). Ji, X.; Nielsen, A. L.; Heinis, C. Cyclic Peptides for Drug Development. Angew. Chem. Int. Ed. 2024, 63(3), e202308251. https://doi.org/10.1002/anie.202308251.

(3). Goles, M.; Daza, A.; Cabas-Mora, G.; Sarmiento-Varón, L.; Sepúlveda-Yañez, J.; Anvari-Kazemabad, H.; Davari, M. D.; Uribe-Paredes, R.; Olivera-Nappa, Á.; Navarrete, M. A.; Medina-Ortiz, D. Peptide-Based Drug Discovery through Artificial Intelligence: Towards an Autonomous Design of Therapeutic Peptides. Brief. Bioinform. 2024, 25 (4), bbae275. https://doi.org/10.1093/bib/bbae275.

(4). Nissan, N.; Allen, M. C.; Sabatino, D.; Biggar, K. K. Future Perspective: Harnessing the Power of Artificial Intelligence in the Generation of New Peptide Drugs. Biomolecules 2024, 14 (10), 1303. https://doi.org/10.3390/biom14101303.

(5). Haqqani, A. S.; Bélanger, K.; Stanimirovic, D. B. Receptor-Mediated Transcytosis for Brain Delivery of Therapeutics: Receptor Classes and Criteria. Front. Drug Deliv. 2024, 4, 1360302. https://doi.org/10.3389/fddev.2024.1360302.

(6). Wilson, D. S.; Keefe, A. D.; Szostak, J. W. The Use of mRNA Display to Select High-Affinity Protein-Binding Peptides. Proc. Natl. Acad. Sci. 2001, 98 (7), 3750–3755. https://doi.org/10.1073/pnas.061028198.

(7). Merz, M. L.; Habeshian, S.; Li, B.; David, J.-A. G. L.; Nielsen, A. L.; Ji, X.; Il Khwildy, K.; Duany Benitez, M. M.; Phothirath, P.; Heinis, C. De Novo Development of Small Cyclic Peptides That Are Orally Bioavailable. Nat. Chem. Biol. 2024, 20 (5), 624–633. https://doi.org/10.1038/s41589-023-01496-y.