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Development of AI-Designed Peptide Inhibitors Targeting the RIPK1 Allosteric Pocket

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RIPK1 (Receptor-interacting serine/threonine-protein kinase 1) plays a critical role in cell death and inflammatory pathways, and its excessive activation has been linked to various diseases. Inhibitors with high cell permeability and selectivity are essential for effective RIPK1 targeting. Although most RIPK1 inhibitors developed so far are small molecules, their restricted target specificity may lead to adverse side effects. To overcome these limitations, we developed a safe peptide inhibitors with higher specificity toward RIPK1 is essential.

RIPK1 contains a unique hydrophobic pocket located behind the ATP-binding site, where ligand binding can allosterically regulate kinase activity. To reduce the likelihood of cross-reactivity with other kinases, we selected binding site of the peptide inhibitor within this allosteric pocket. We analyzed the structure of the RIPK1 allosteric pocket from PDB data (5TX5.pdb) using PyMOL, and referred to various ligands known to bind with this region during the peptide design process.

With respect to methodology of peptide design, RFdiffusion was employed to generate 1,000 peptide backbones targeting the RIPK1 allosteric pocket, and ProteinMPNN was then used to generate five optimized sequences for each backbone. AutoDock was employed to perform docking simulations in a virtual environment and calculate binding affinity. Peptides predicted to have high permeability were further screened with AutoDock to identify those with strong affinity, and the final candidate peptide “KLP001” was selected.

The RIPK1-targeting peptide inhibitors developed in this study are expected to demonstrate superior selectivity and efficacy compared with existing small-molecule inhibitors. Furthermore, the AI-driven design strategy enabled efficient identification of high-affinity peptides, offering a significant advantage over conventional screening approaches. Future work will focus on validating the functional efficacy of these peptides through follow-up experiments, including in vitro assays.