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Modular Approach to Enhance the Bioactivity of Peptides

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Introduction

Bioactive peptides (BPs) are peptides with hormonal or pharmacological properties. The source of BPs can be from natural peptides of endogenic origin, or it can be synthesized in the lab based on rational design or screening. Herein we demonstrate how a BP of interest can be modified to a highly effective research tool as well as therapeutic lead with minimal modifications that can be done in many labs or ordered for a reasonable price [1,2]. Phosphatase and tensin homolog induced kinase 1 (Pink1) is serine/threonine kinase. Pink1 is related to mitochondrial dynamics, and it was demonstrated to regulate mitochondrial homeostasis [3]. We developed a linear peptide that targets Pink1. Based on this peptide we engineered peptide-based targeted peptidomimetics (modified peptides), biomolecular probes, with various properties.

Results and Discussion

As a proof of concept, a linear peptide (aka cargo) was developed to target Pink1 by rational design method (Figure 1). A library of peptides was synthesized to address three goals: to give the peptide 'drug-like' properties, to add an element that will allow a crosslinking experiment to determine the identity of the docking domain in the target protein, lastly to conjugate florescence dye to the peptide that will be used in molecular imaging in H9c2 cells and evaluate the interaction between the peptide and protein of interest. For better solubility and cell penetrating trait a TAT sequence was added to all synthesized peptides (Figure 2).

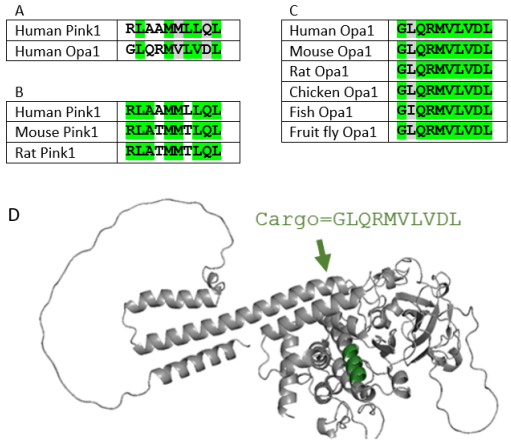


Fig. 1. Rational design of a peptide that target Pink1. Sequence alignment identifies a short sequence of homology between Opa1 and Pink1 (A), which is conserved in evolution (B,C). The Opa1 sequence (light green) is exposed in the C-lobe region of Pink1 (Alphafold predicted model of human Pink1: Q9BXM7) (D). PyMol (Schrodinger LLC) was used to generate the figure.

Peptide Name	Sequence
CVP-198	CH_3-CO-GLQRMVLVDL-K-GG-YGRKKRRQRRR-NH_2
CVP-199	CO-$(CH_2)_2$-CO-NH) GLQRMVLVDL-K-GG-YGRKKRRQRRR-NH_2
CVP-200	CO-$(CH_2)_3$-CO-NH) GLQRMVLVDL-K-GG-YGRKKRRQRRR-NH_2
CVP-201	CO-$(CH_2)_3$-CO_2H GLQRMVLVDL-K-GG-YGRKKRRQRRR-NH_2
CVP-202	CO-$(CH_2)_2$-CO_2H GLQRMVLVDL-K-GG-YGRKKRRQRRR-NH_2
CVP-205	CH_3-CO-C-GLQRMVLVDL-K-GG-YGRKKRRQRRR-NH_2
CVP-206	BDP) CH_3-CO-C-GLQRMVLVDL-K-GG-YGRKKRRQRRR-NH_2

Fig. 2. Schematic structure of the developed library. The cargo in green, addition to the cargo for enabling cyclization/crosslinking/dye conjugation in red, TAT and a linker in blue, cyclazation/precyclyzation/dye in black . BDP= Borondipyrromethene.

Table 1. Summary of the corresponding in vitro K_D values of the peptides binding to Pink1.

Peptide Name	K_D (μM)	Notes
CVP-198	130.47 ± 0.01	Linear peptide
CVP-199	58.31 ± 0.02	Cyclic peptide
CVP-200	138.72 ± 0.30	Cyclic peptide
CVP-201	125.89 ± 0.02	Precyclic peptide
CVP-202	143.09 ± 0.04	Precyclic peptide

Five peptides, CVP-198 - CVP-202, were tested by field effect biosensing to determine their binding to Pink1. CVP-199 a cyclic peptide showed the best K_D values. Cyclization may allow better binding of a peptide to the target protein (Table 1).

CVP-198 and CVP-199 were tested in a comparative experiment to find whether the cyclization gives protection against proteolytic conditions. By HPLC analysis it was showed that CVP-199 the cyclic peptide was more resistant to proteolytic conditions in the presence of trypsin compared to CVP-198 the linear peptide (Figure 3).

CVP-198 was tested in a crosslinking experiment with two kinds of crosslinking agents bis(sulfosuccinimidyl)suberate (BS3) and 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methyl-morpholinium chloride (DMTMM). The target protein was Pink1. The analyses were done by LC/MS. Eight sequences of a crosslink between the Pink1 sequence and the lysine next to the cargo in peptide CVP-198 using a BS3 crosslinker were found. Using DMTMM crosslinker, two peptide pairs reporting a crosslink between the protein and the peptide were identified. An overlap was made between the computerized model of the protein folding and docking of the peptide and the results of the crosslinking found in the LC/MS analysis (Figure 4). This finding confirms the suitability of the peptide for its docking site according to the rational design and gives structural information about the protein at the binding site. CVP-205 was prepared to allow conjugation to the fluorescent dye, boron-dipyrromethene (BDP). H9c2 rat cardio myoblast cell line was treated with CVP-206 (CVP-205 with fluorescent dye, boron-dipyrromethene (BDP)) in two conditions - without stress and with oxidative stress by hydrogen peroxide (H_2O_2) that induced cellular apoptosis. The cells were analyzed by confocal microscope. H_2O_2 treatment enhanced the localization of Pink1 to the mitochondria surface and the colocalization of Pink1 with the peptide, CVP-206, whereas this enhancement was significantly reduced in control cells (Figure 5). It can be concluded that the peptide reaches the target protein Pink1 in the cells.

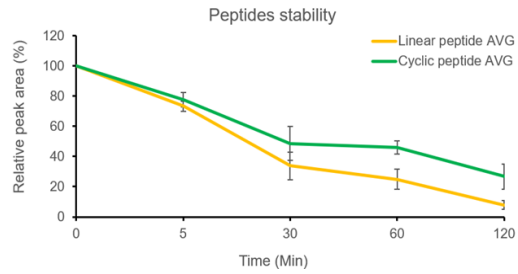
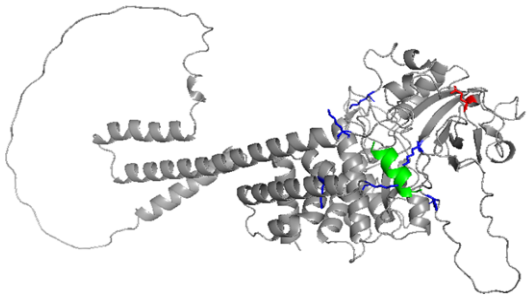


Fig. 3. Stability studies of the linear peptide, CVP-198, and the bioactive backbone cyclic peptide, CVP-199. The stability of the peptides was measured independently under tryptic degradation at 37 ℃ (by trypsin degradation of the peptide) and was determined by HPLC analysis (n=3). The starting time point (0 min, 100%) represents the peptide at the beginning of each experiment. Liner peptide (CVP-198) at 37 ℃ with trypsin (yellow), and cyclic peptide (CVP-199) at 37 ℃ with trypsin (green), were analyzed after 0 min, 5 min, 30 min, 60 min, and 120 min. Separation was performed by HPLC at 214 nm. Peptide amounts were calculated relative to the quantities determined at time point zero.

This project presents a general approach using an identified peptide and developing various research tools as well as therapeutic leads, which can be customized to other laboratories easily [4].

A



B

Crosslinker agent	Pink1 AA number	LC/MS resulting peptides
BS3	137	S**K**PGPDPLDTRR-2
BS3	186	GCSAAVYEATMPTLPQNLEV T**K**STGLLPGR-22
BS3	266	GP**K**QLAPHPNIIR-3
BS3	319	TLFLVM**K**NYPCTLR-7
BS3	547	LTE**K**CCVETK-4
BS3	555	CCVET**K**MK-6
DMTMM	155	LJ**E**YLIGQSIGK-3

*Fig. 4. Map of the cross-link identified between the peptide CVP-198 and the Pink1 protein (AlphaFold predicted model: Q9BXM7). (**A**) Residues involved in cross-linking contacts are highlighted in blue and red stick structure on the Pink1 protein. CVP-198 peptide (shown in green cartoon structure) and hPink1 protein (shown in grey cartoon structure) (**B**) CVP-198 and Pink1 cross-linking contacts table: The positions involved Pink1 lysine residues, highlighted in the blue, and involved Pink1 glutamic acid residue, highlighted in the red. PyMoL (Schrodinger LLC) was used to generate the figure.*

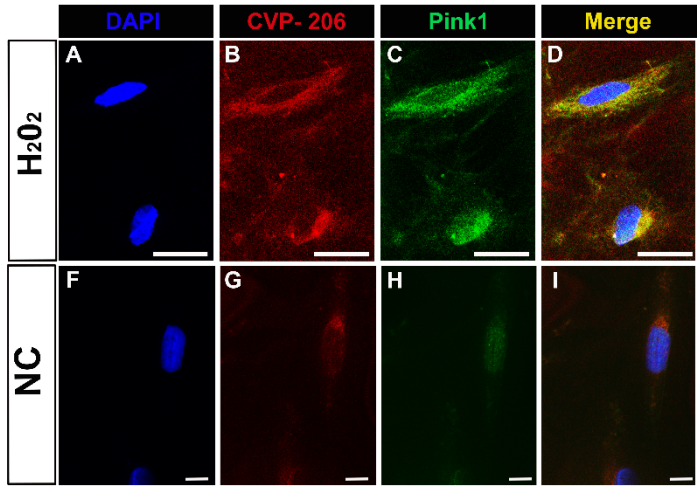


Fig. 5. Colocalization of Pink1 and CVP-206 peptide in H9c2 cells. (A-D) Immunostaining for CVP-206 (1 µM, Red) and Pink1 (Green) after H_2O_2 treatment (1.6 mM for 0.5 hour). (F-I) Immunostaining for Pink1 (Green) and CVP-206 (1 µM, Red) in H9c2 cells with no treatment, Negative control (NC). Nuclei is in blue (DAPI), scale bar indicates 20 µm for CVP-206 staining after H_2O_2, and 10 µm for Negative control (NC) (n=3). Confocal microscopy images were taken at 63x magnification for NC and H_2O_2 treated cells.

Acknowledgments

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References

1. Adhikari, S., Leissa, J.A., & Karlsson, A.J. *AIChE Journal* **66**(3), e16776 (2020), https://doi.org/10.1002/aic.16776
2. Akbarian, M., Khani, A., Eghbalpour, S., & Uversky, V.N. *International Journal of Molecular Sciences* **23**(3), 1445 (2022), https://doi.org/10.3390/ijms23031445
3. Wang, N., Zhu, P., Huang, R., Wang, C., Sun, L., Lan, B., & Gao, Y. *Life Sciences* **259**, 118247 (2020), https://doi.org/10.1016/j.lfs.2020.118247
4. Ben-Uliel, S.F., Zoabi, F.H., Slavin, M., Sibony-Benyamini, H., Kalisman, N., & Qvit, N. *International Journal of Molecular Sciences* **23**(11), 6076 (2022), https://doi.org/10.3390/ijms23116076