

# Leveraging the biotechnological potential of combinatorial libraries of cyclic peptides

Chiara Bertaso PhD in Molecular and Cellular Biology, University of Milan Bertaso C.\*, Tagliani A.\*, Spolti S.\*, Akaberi D.^, Marcianò D.\*, Rosa S.º, Masiero S.\*, Pesaresi P.\*

\*Department of Biosciences, Università degli Studi di Milano, Milan, Italy, ^Department of Medical Biochemistry and Microbiology; Infection and Immunity; \*Northeastern University, Boston, MA, USA

## ABSTRACT

Cyclic peptides (CP) are a valuable class of bioactive compounds, also for their ability to selectively target proteins generally considered undruggable. CPs can modulate proteins and enzyme activity by interfering with protein-protein interactions (PPIs). In this context, combinatorial libraries are useful tools for the identification of cyclic peptide binders, due to a cheaper and easier screening pipeline, coupled with quicker isolation of hit molecules when compared with, e.g., DNA-encoded chemical libraries. In this framework, we adapted a combinatorial library of 8 amino acid-long cyclic peptides to the most widely used in vivo protein-protein interaction assay, namely the GAL4-based Yeast Two-hybrid system. Our platform (CYCLIC) uses yeast to identify peptides interacting with target proteins of interest, which are then tested for their bioactivity. Peptide cyclization, which increases CPs stability, specificity, and resistance to proteases, is achieved in vivo through the post-translational splicing activity of intein protein elements. By exploiting our libraries, we identified cyclic peptides interacting and/or inhibiting target proteins, like cell-wall associated enzymes of plant pathogens, transcription factors regulating sugar metabolism in yeast, and a viral enzyme crucial for the replication of SARS-CoV2. CYCLIC applications can be leveraged to any protein of interest, such as receptors, which are interesting targets for the cultured meat industry. Finally, we envision the production at scale of cyclic peptides employing precision fermentation and synthetic biology.





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#### **CYCLIC: A COMBINATORIAL LIBRARY OF CYCLIC**



ppl partner

<sup>A</sup>rotein target

Cyclic peptides interfere with protein-protein interactions (PPI) modulating signaling pathways in living organisms



(Intein

Intein trans-splicing

InteinN

39-nt tail 8xNNK Millions of different possible

combinations

SspIntei CP pGADT7-KanMX-SspIntein

39-nt tail

We developed a Yeast two-hybrid based Combinatorial Library of Cyclic Peptides (CYCLIC) for fast peptide isolation *in vivo* in yeast

GAL4

AD

GAL4

### **ANTIMICROBIAL PEPTIDES (AMPs)**







8xNNK

Peptide library

Nrul

#### Rosa, Tagliani et al 2023

#### **PROOF OF CONCEPT INTERFERING CYCLIC PEPTIDE MODULATING TRANSCRIPTION FACTOR ACTIVITY IN YEAST** Rosa, Tagliani et al 2023



CYCLIC is a Yeast Two-Hybrid based combinatorial library of cyclic peptides (CPs). CPs are isolated based on their ability to interact with a given target protein

