G.mellonella as an in-vivo model in therapeutic peptide research

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Gram-negative multidrug resistant (MDR) bacteria pose a significant concern in hospitals. To address this issue, we employed a chemical space guided approach to design novel antimicrobial peptides (AMP). Our discovery includes the bicyclic antimicrobial peptide (AMBP) **bp3**, which exhibits activity limited to the gram-positive *Bacillus subtilis*.[1] By conducting a chemical space nearest neighbor search using molecular fingerprinting, we identified **bp65** as a close analog of **bp3** with expanded antibacterial activity against *Pseudomonas aeruginosa*, albeit with strong hemolytic effects. To investigate the role of cyclization in antibacterial activity, we synthesized **ln65**, the linear counterpart of **bp65**. **ln65** demonstrated antibacterial activity and comparable hemolytic potential to the parent AMBP. Furthermore, for peptide optimization, we synthesized **ln69**, an heterochiral version of **ln65** with inverted lysine chirality. **ln69** displayed preserved antibacterial activity and an increased serum stability while exhibiting reduced hemolytic effects compared to its homochiral analog. The CD spectra and X-ray crystallography analysis of **bp65**, **ln65**, and the mixed-chirality AMP **ln69** revealed an α-helical conformation in a membrane-like environment. Surprisingly, this conformation usually associated with high hemolytic activity, was also observed in **ln69**.[2]

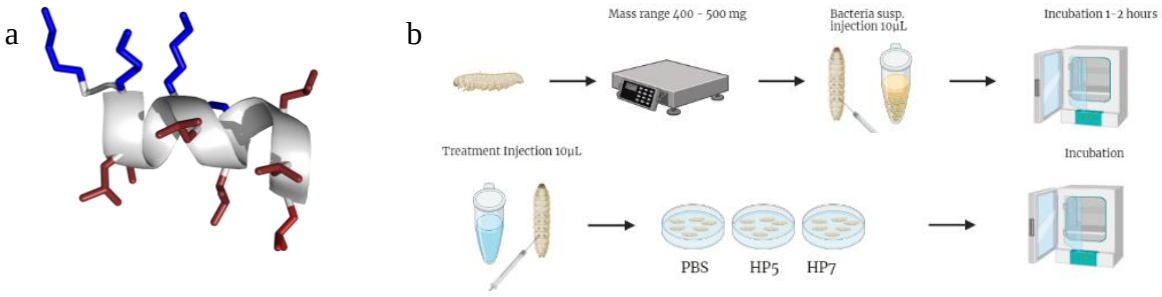

Figure 1 a) HP5 crystal structure, in blue L- and D- lysines, in red L- and D- leucines. b) G.mellonella efficacy experiment workflow.

Building on these results, we further enhanced the biological properties of **ln69** by selectively inverting the chirality of lysine and leucine residues, resulting in the development of two AMPs: **HP5** and **HP7** (Figure 1a). Both **HP5** and **HP7** exhibited α-helical secondary structure, potent activity against gram-negative bacteria, low hemolytic activity, excellent serum stability, and minimal toxicity against HEK293 cells.[3] The next step in this research aims to study the in-vivo behavior of these mixed-chirality α-helical AMPs using the *Galleria mellonella* model (Figure 1b). *G. mellonella*, an insect from the Pyralidae family, is commonly used in microbiology and therapeutics research as an in-vivo model. The main advantages of this model are its ease of handling, affordability, and physiological similarities with mammals.[4]

[1] I. Di Bonaventura, S. Baeriswyl, A. Capecchi, *Chem. Commun*., **2018**, 54, 5130-5133.
[2] S. Baeriswyl, H. Personne, I. Di Bonaventura, *RSC Chem. Biol.*, **2021**, *2*, 1608-1617.
[3] H. Personne, T. Paschoud, S. Fulgencio, J. *Med. Chem.* **2023**, 66, 11, 7570–7583.
[4] M. A. Cutuli , G. Petronio , F. Vergalito, *Virulence,* **2019**, *10*, 527-541.