Sequence-driven modulation of the multistage self-assembly process in lamellin-type β-peptides

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Introduction

Self-assembling peptides serve as useful building blocks of functional supramolecular materials and have gained increasing attention in nanotechnology, drug delivery, and molecular biology. Among these systems, non-natural β-peptides are particularly promising due to their high chemical and conformational diversity, increased resistance to proteolytic degradation, and ability to form stable intermolecular interactions that support higher-order assembly. The side-chain chirality pattern of β-peptides plays a crucial role in the formation of higher-order structural elements. To explore new directions in β-peptide supramolecular assembly, and inspired by the extended strand conformation of natural peptides, in which alternating side-chain positions can facilitate intermolecular interactions between neighbouring molecules, we designed a series of short, acyclic β³-peptide sequences with alternating side-chain chirality.

Results and Discussion

We previously designed [1] a charge-neutral β³-hexapeptide comprising a Leu-rich hydrophobic region together with Glu and Lys residues, in which alternating side-chain chirality favours an extended, non-helical zig-zag backbone conformation. MD simulations revealed that this amphiphilic arrangement promotes the formation of finite oligomeric bundles consisting of two nearly parallel layers held together by a hydrophobic Leu-rich core in aqueous solution (Figure 1). The resulting hydrophobic interior can host fluorescent probes and hydrophobic guest molecules.

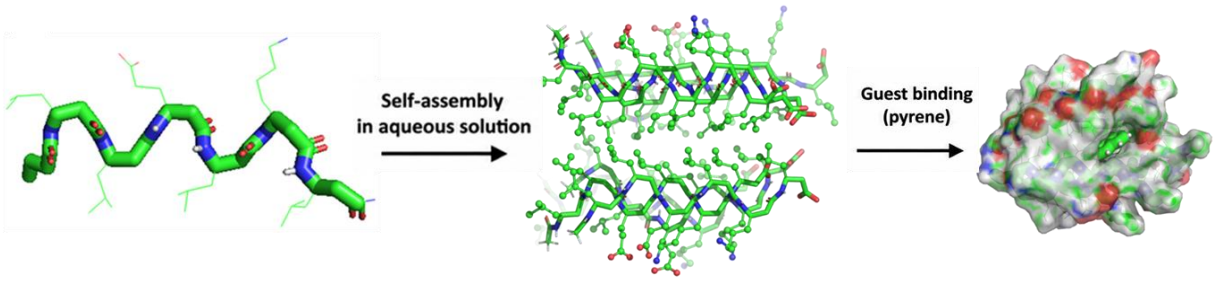


Figure 1. Self-assembly of an acyclic β³-hexapeptide with alternating side-chain chirality into nanometer-sized oligomeric bundles with a hydrophobic Leu-rich core, enabling the encapsulation of small hydrophobic guest molecules.

Based on this initial design concept, subsequent sequence modifications were used to control higher-order assembly and to develop environmentally responsive lamellar structures with membrane-active and antimicrobial properties.

Increasing the cationic character of the peptides transforms this adaptable scaffold into an environment-responsive antibacterial system [2, 3]. Some of these sequences with alternating chirality self-assemble into characteristic striped lamellae, which were termed lamellins. While the 2K and 3K sequences predominantly adopt a random-coil structure in water, inorganic phosphate (e.g., in PBS) and bacterial cell-wall components (e.g., LPS) trigger their organization into extended lamellar structures (Figure 2). Molecular dynamics simulations, electron microscopy, and bacterial assays demonstrated that this phosphate-induced self-assembly leads to the formation of ordered, striped lamellae capable of incising the cell envelope of Gram-negative bacteria, thereby exerting antibacterial activity.

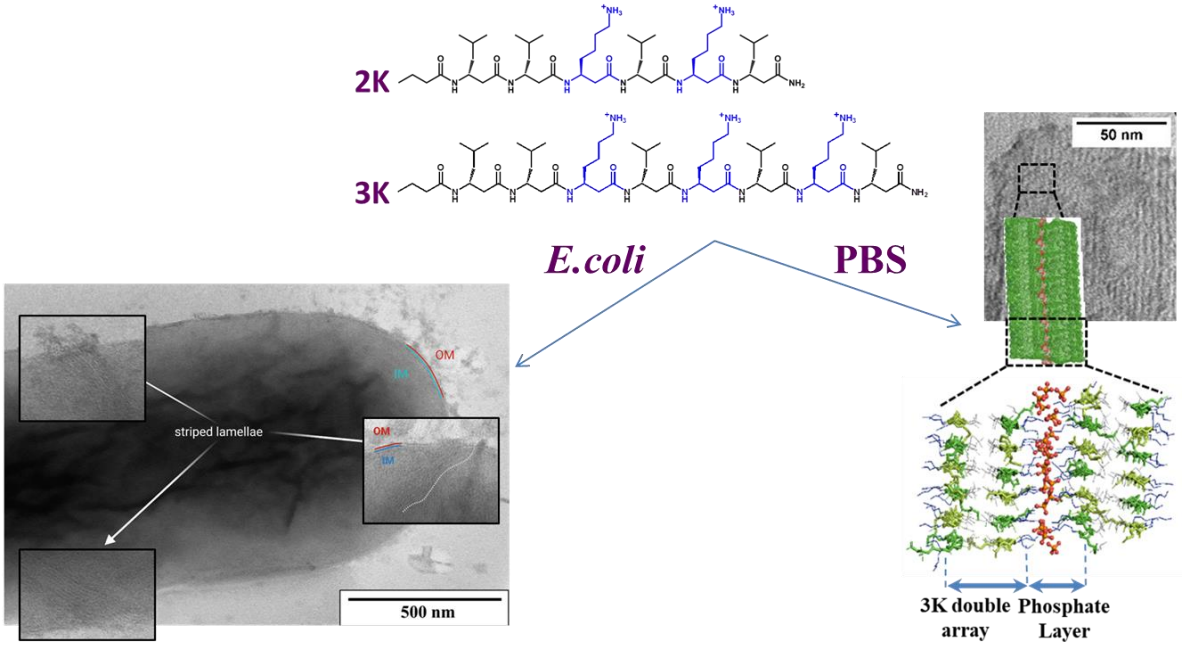


Figure 2. Environment-responsive self-assembly of cationic lamellin-2K and lamellin-3K in the presence of PBS and E.coli.

To gain a more comprehensive understanding of β-peptide assembly behaviour, we investigated systems with an overall neutral charge, in which supramolecular organization could be modulated in a controlled manner. We systematically replaced [4] β^3 -homoleucines (Leu) with β^3 -homoalanines (Ala) and changed the hydrophobic N-terminal butyryl protecting group (But) to the more water-soluble acetyl group (Ac) (Figure 3) to examine how changes in hydrophobic core formation influence the assembly process and the resulting morphology. MD simulations confirmed that all investigated peptides predominantly adopt a zig-zag conformation, facilitating their association into sandwich-type oligomers. Peptides containing an N-terminal acetyl group and homoleucine at the second position preferentially formed twisted structures, whereas the combination of an N-terminal butyryl group and homoalanine favoured more ordered assemblies with reduced internal twisting. Based on the combined experimental (cryo-EM, NS-TEM and molecular biophysics data) and

computational (MD simulations) observations five distinct structural stages of self-assembly process were identified (Figure 3), from small oligomers, twisted strands to extended flat lamellae, which can assemble to lamellar bundles, and finally to macroscopic lamellar clusters.

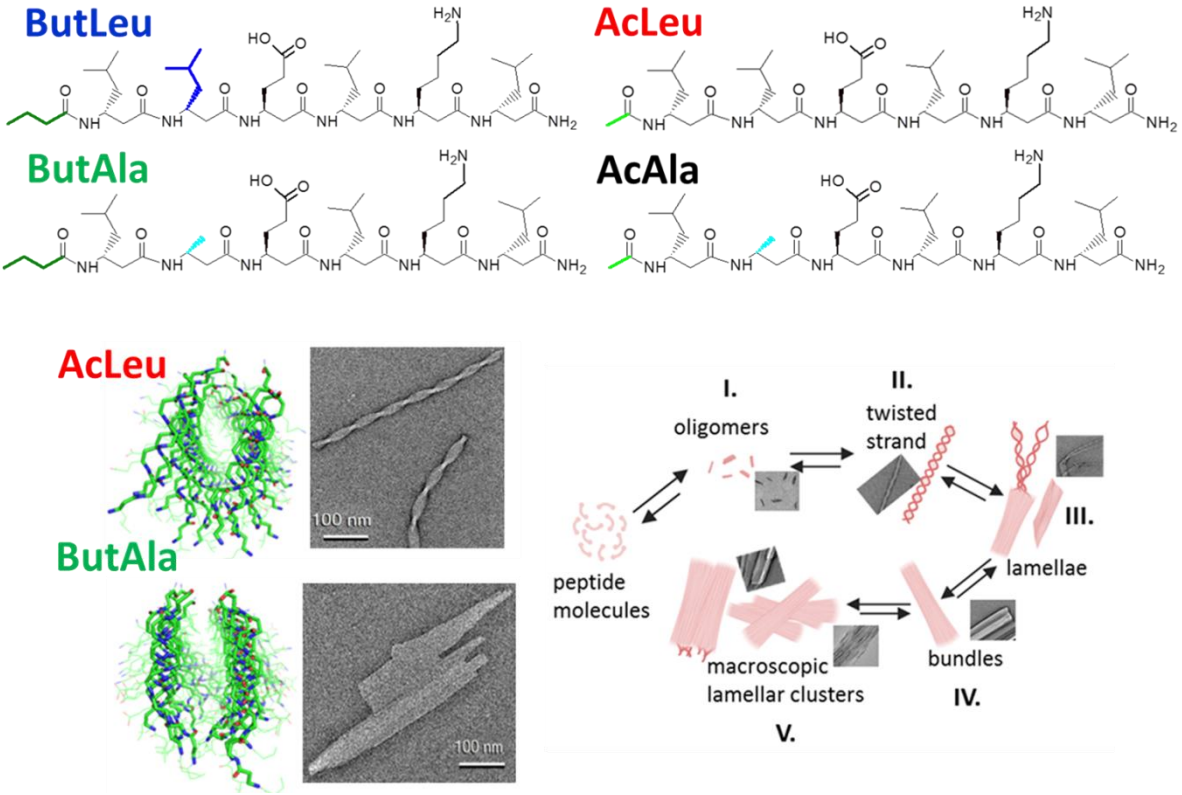


Figure 3. Selected chemical structures of β³-hexapeptides with representative twisting and packing of the formed morphologies in view of EM images and MD simulations for the two extremes, AcLeu and ButAla. Schematic representation of assembly formation with the five identified stages based on morphological investigations.

Acknowledgements

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