GORILLA TO THE RESCUE – DETERMINING A NOVEL ANTIMICROBIAL FIBRIL STRUCTURE

The emergence of resistant and aggressive microbial infections calls for novel and effective drugs. Crystal structures of antimicrobial peptides revealed densely packed helical fibrils, while structure-guided mutagenesis supported the role of self-assembly in activity. The findings might facilitate the design of biomaterials with enhanced selectivity, bioavailability, and shelf-life.

Antimicrobial peptides (AMPs) are produced by many organisms as the first line of defence against pathogens and for the modulation of the immune system. They can provide new therapeutic avenues to combat severe infections, kill cancerous cells and treat autoimmune disorders. Moreover, AMPs are thought to induce less resistance compared to conventional antibiotics. Yet, their relatively low efficacy and bioavailability, and lack of chemical stability has discouraged development into therapeutic agents. Fibrillation of antimicrobial peptides into highly stable structures can provide immense stability against heat, shear force, and chemical and proteolytic degradation. This offers new possibilities for therapeutics, and for various biomedical and technological applications.

LL-37 is a mammalian cationic AMP that plays pivotal roles in the innate immune system. LL-37_{17-29} is a tentative proteolytic cleavage segment of LL-37, showing a different spectrum of antibacterial activity compared to LL-37 and other fragments, and which generates an amphipathic helix with a large hydrophobic moment. This study shows that human and gorilla LL-37_{17-29} form wide fibrils around bacterial cells (**Figure 33**). Crystal structures of helical AMPs, and of functional fibrils, are rare. Indeed, human LL-37_{17-29} was reluctant to crystallise, and crystals formed in only one out of ~1500 conditions tested. The crystals were sensitive to ice formation, and cryo-protectants had to be intensively screened. Finally, X-ray diffraction data were obtained to 1.35 Å resolution, but phases were hard to determine via molecular replacement or direct methods. In contrast, the gorilla LL-37_{17-29} homologue, having one amino acid substitution (corresponding to human F17S), formed crystals in different conditions. X-ray diffraction data were collected at beamline **ID23-2**, up to 1.1 Å resolution. This allowed phasing by molecular replacement using a polyalanine idealised helix as the search model. Phases of the human LL-37_{17-29} were then obtainable via molecular replacement using the refined structure of the gorilla LL37_{17-29} as the search model.

The human and gorilla crystal structures (PDB IDs: 6S6M and 6S6N, respectively) have similar space group and unit cell dimensions. The structures revealed densely packed helices forming an elongated hexameric arrangement featuring a nanotube along the fibril axis (**Figures 33** and **34**). The helices are packed into four-helix bundles with a hydrophobic core, while polar interactions mediate interfaces between bundles. The surface features 'zig-zagged' hydrophobic and positively charged patches, suggesting interactions and disruption of negatively charged lipid bilayers such as bacterial membranes. This overall arrangement is similar between the human and gorilla LL-37_{17-29}, differing only in the two N-terminal positions that line the central pore (**Figure 34**), with the gorilla structure showing a more occluded pore with extended side chains facing inward. Interestingly, the gorilla peptide displays weaker antibacterial activity, towards both gram positive and negative bacteria, compared to the human homologue.

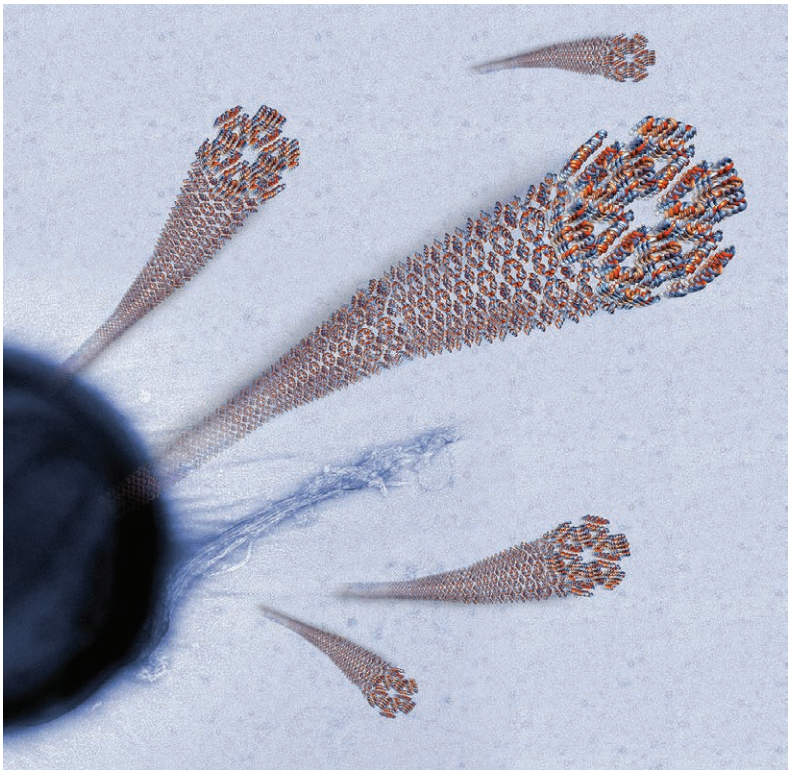

Fig. 33: A transmission electron micrograph of gorilla LL-37_{17-29} fibrils assembling on and around an *M. luteus* bacterium cell. The crystal structure of the fibrils is illustrated on the micrograph, represented as helical ribbons coloured by hydrophobicity (orange to blue indicating hydrophobic to hydrophilic residues). Credit: Sharon Amlani.

Structure-guided mutagenesis suggested the important role of self-assembly in antibacterial activity. Moreover, the LL-37_{17-29} fibrils display thermostability up to 80°C and a high zeta potential compared to non-fibrillating mutants or other helical protein polymers. The findings offer a promising approach to develop durable antimicrobials as an alternative to common antibiotics for coating of medical devices, implants and stents and, potentially, as therapeutics for autoimmune disorders and as anti-cancer supportive therapy. Moreover, controlling fibrillation might provide controlled-release medications under specific triggers.

Interestingly, LL-37_{17-29} shares sequence similarity and the ability to form helical fibrils with the bacterial toxic peptide PSMα3, which forms cross-α amyloid fibrils that play a role in killing human immune cells **[1]**. This suggests a possible molecular or structural mimicry mechanism used by the bacteria to provide immune-evasive and survival strategies. The similarity also points to potential functional building blocks across kingdoms of life in the form of densely packed amphipathic helical fibrils, complementing the exciting hypotheses about short amyloid peptides serving as prebiotic information-coding molecules.

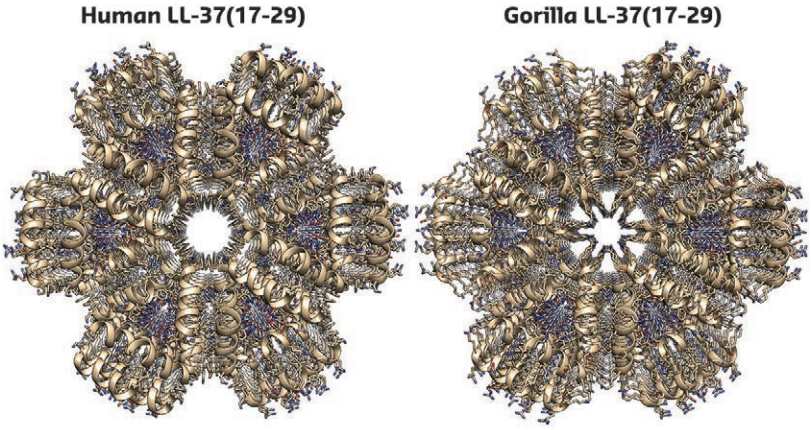

Fig. 34: Atomic structures of human and gorilla LL-37_{17-29} fibrils, with view down the fibril axis, featuring the similar hexameric architecture of densely packed helices, with a nanotube running along the fibril. The two N-terminal residues, phenylalanine (human) or serine (gorilla) at position 17, and lysine at position 18, are facing the pore. These residues show a more extended conformation in the gorilla structure, leading to a more occluded nanotube.

PRINCIPAL PUBLICATION AND AUTHORS

The Human LL-37(17-29) antimicrobial peptide reveals a functional supramolecular structure, Y. Engelberg (a) and M. Landau (a,b), *Nat. Commun.* **11**, 3894 (2020); https://doi.org/10.1038/s41467-020-17736-x.

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HOW AND WHY CERTAIN MEMBRANE PROTEINS ARE DELIVERED TO THE FUNGAL CELL WALL

The transfer of GPI-anchored substrates from the plasma membrane to the cell wall as catalysed by a unique type of glycoside hydrolases is essential for fungal life. Combined structural, computational and *in-vivo* analysis reveal this important step in fungal cell wall biogenesis, providing insights into the sorting mechanism at the outer leaflet and a structural base for the development of new, potentially side effect-free antimycotics.

Systemic infections by invasive fungal pathogens in immunocompromised patients are an increasing, yet underestimated and unsatisfactorily addressed threat to human health **[1]**. The fungal cell wall biogenesis is a promising candidate for side effect-free drug targets, as the underlying architecture and features are unique for fungi **[2]**. A key step in the synthesis and maturation of the cell wall is the transfer of a subset of glycosylphosphatidylinositol (GPI) anchored proteins from the plasma membrane into the cell wall (**Figure 35a**). In baker's yeast, the underlying transglycosylation has long been suggested to be catalysed by two homologous glycoside hydrolases of the GH76 family, Dfg5 (defective in filamentous growth) and Dcw1 (defective cell wall) **[3]**. As they are related to bacterial GH76 homologues, an α1,6-mannanase function of Dfg5 enzymes in filamentous fungi has also been suggested **[4,5]**.

The crystal structure of Dfg5 from the filamentous fungus *Chaetomium thermophilum* shows that the overall fold and the active site DD-motif is conserved among GH76 enzymes,