Instituto de Medicina Molecular João Lobo Antunes

Master Project Proposal

Title: Amyloid nanofibrils as scaffold to support antimicrobial peptide-nanoparticle conjugates for the inhibition of bacterial growth

Synopsis:

Amyloids are fascinating molecular species. Toxicity, initially misattributed to mature amyloid fibrils, is in fact associated mostly with the smaller oligomeric precursors of the fibrils, as previously shown by us [1-3] and other. Mature fibrils are in fact quite stable, non-toxic and with predictable properties [1-3]. Amyloid peptides and proteins form a stable molecular arrangement that simultaneously combines mesoscopic scale organization with a degree of morphological heterogeneity at a larger scale that results in amyloid gels, amyloid fibrils, branched amyloids, non-branched and other appearances [2,3]. This variety of supramolecular arrangements gives these nanomaterials their potential to perform multiple functions [3]. It is also due to this set of properties that amyloids have become interesting tools for nanotechnology, alongside their intrinsic bio-compatibility, as reviewed by us [3]. This also led to an enormous interest in understanding the structure of amyloids, at the atomic level, to be able to perform a rational molecular design of new amyloids, with newly engineered chemical properties into them to enable new functions. With this in mind, we developed amyloid fibrils that are biotinylated and able to bind desired streptavidin labeled molecules, namely nanoparticles, antibodies, and other proteins. Such fibrils can be employed as a scaffold matrix, where desired particles bind and, by being in a close-knit space, increase their action synergistically.

We have also used nanoparticles combined with antimicrobial peptides (AMPs) to inhibit bacterial growth. Briefly, AMPs have been proposed as a promising alternative to conventional medicines [4-8]. Some AMPs exhibit a broad spectrum of action against bacteria, fungi, viruses and protozoa [8]. AMPs may also present other activities such as a defence (innate immune system), anti-tumour or even regenerative mechanism [8]. AMPs, often of natural origin, can significantly improve current therapy, namely because they are less likely to lead to the development of antibiotic-resistant microorganisms than conventional antibiotics [4-7], being a topic in which the host lab has already expertise [8-11]. We recently tested PaMAP19 peptide, demonstrating effective antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus* and *Klebsiella pneumoniae* at low peptide concentration, while exhibiting non-hemolytic effects, an action that is improved when star-shaped nanoparticles (NSs) are conjugated with PaMAP19.

Now, we aim to harness the synergistic potential of PaMAP19-NSs and amyloid fibrils forming a composite matrix capable to inhibit bacterial growth. The specific objectives to be accomplished are: *i)* Evaluate the antimicrobial and cytotoxic effects of the composite matrix in bacteria (*e.g.*, *E. coli*, *S. aureus*); *ii)* Determine the inhibitory behavior of the matrix on bacterial biofilms; and, *iii)* Determine the hemolytic and toxicity effects in cells.

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