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FROM DYNAMIC STRUCTURES TO FUNCTIONAL MATERIALS

Mutational Tolerance of Synthetic Nanochannel-forming Amyloid Fibrils analyzed *via* Cryogenic Electron Microscopy

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Amyloid fibrils are self-assembling protein aggregates with a highly ordered cross-β structure, often associated with diseases. Recently it has been shown that dimer inversion lock trimer (DILT) nonapeptides assemble into multi-channel amyloid fibrils with a honeycomb-like three-dimensional (3D) structure. The design rationale provides a dimerization (KVKV) and a junction interface (SQINM) to selectively produce, highly ordered multi-channel amyloid fibrils.
To probe the tolerance of this honeycomb structure against mutations, sequence variants were synthesised and fibrils assembled. Sequences “DILT2” and “DILT3” which carry an arginine substitution instead of a lysine ,at the first and third positions respectively, were chosen for further analysis.
Comprehensive structural characterisation of these mutated multi-channel amyloid fibrils (DILT2 & DILT 3) was performed via cryo-EM and subsequent helical reconstruction.
The resulting 3D electron density maps of the most common morphologies present in the sample were resolved at 1.9 Å for DILT2 and 1.78 Å for DILT3. At these resolutions, side-chain conformations within both the dimer interface and the trimeric junction interfaces are identifiable. The structures demonstrate that the peptides primarily form a morphology based on a hexagonal basic unit that extend into a regularly ordered lattice .
The hexagonal basic structure is formed by dipeptides connected by a trimeric junction interface. The structural data show that the trimeric junction, the global hexagonal lattice, and the ~5 nm continuous nanochannel pore diameter are invariant across the analysed mutants. The morphological difference between the variants is in the arrangement of the hexagonal basic units, which for DILT2 predominantly adopt a six-fold rotational symmetry, while in the case of DILT3 they primarily adopt a three-fold rotational symmetry around the fibril axis.
The obtained structural data show the robustness of the designed sequence against arginine mutations at the first and third positions and provide a basis for the rational sequence design of further sequence variants of honeycomb forming amyloidogenic peptides. Approaches could include, tuning of pore size and pore shape.