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Filaments Under Force: A Computational Molecular-Scale Investigation of Type IV Pili From Multiple Organisms

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Type IV pili (T4P) are biopolymers comprised of many protein subunits called pilin. These pilin subunits are not covalently bonded to one another, however remarkably T4P filaments are very strong and flexible. T4P emanate from the surface of prokaryotic cells and are utilized for many functions, including biofilm formation, surface adhesion, motility, and infection. The recent cryo-EM based structures for T4P from *Escherichia coli, Neisseria meningitidis, Pseudomonas aeruginosa, and Neisseria gonorrhoeae* have provided unprecedented insights into the structures of these filaments. However, although the structures of T4P are known, the dynamics of these filaments at the molecular scale at equilibrium and under tensile forces is not well characterized. In this work we provide an overview of our research into these various T4P filaments and their constituent pilin monomers under force. Specifically we carried out steered molecular dynamics simulations using a multiscale approach encompassing all-atom simulations and two levels of coarse-grained simulation. We have analyzed the changes in secondary structure of pilin subunits, global changes in filament architecture, and calculated the Young's modulus of each of the different T4P filaments. By drawing comparisons between all of these filament systems, we are able to obtain a broader picture of T4P dynamics than experimental structures alone can provide. In particular, we observe elongation of the alpha helix region of pilin subunits in each of these systems, which has been previously attributed to T4P flexibility and strength.

View Abstract

modification of hydrophobins to add new functionalities or influence their self-assembly.

1414-Pos
Understanding the Packing of Amyloidogenic Peptide Segments using Electronic Structure Calculations
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It has been proposed that antiparallel amyloid fibrils are more likely to fold into potentially toxic, transient morphologies as they are typically less stable than parallel fibrils. Recent studies of some amyloidogenic protein segments revealed that crystallize as antiparallel out-of-register β-strands, unlike most amyloid fibrils that fold into an in-register β-sheet packing. Therefore, it has been suggested that such arrangement may be responsible for the cytotoxicity in amyloid diseases. Interestingly, crystal structures of the amyloidogenic peptide segments NFGAILS and FGAILSS showed antiparallel zippers assembled into out-of-register and in-register sheets, respectively, even though they differ in only one amino acid at the termini. In this work, we use density functional theory (DFT) to elucidate the molecular mechanism driving their different crystallization patterns by investigating the differences in energetics and structure between them. Our results primarily show that the electrostatic potential (ESP) at the termini in the out-of-register peptide is significantly larger than in the in-register system. These findings may help to understand the underlying molecular phenomena dictating the toxicity of amyloid fibrils.

1415-Pos
Effect of Residue Substitutions on the Hydrophobic Core of the C-Terminus of the Prion Protein
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Prion diseases are fatal neurodegenerative diseases that affect mammals, including humans. The hallmark of prion diseases is the prion protein: The misfolded and aggregated form of the prion protein PrPSc binds to the cellular form of the prion protein PrPC and induces the conformational conversion. Although PrPC gene polymorphisms correlate with varying degrees of susceptibility of the host to develop prion diseases, how residue substitutions modulate PrPC structural dynamics towards pathological misfolding is unknown. Deciphering such mechanism will identify potential druggable hot spots to stabilize PrPC and prevent pathological conversion. Our structural bioinformatics study focuses on the effect of residue substitutions on the stability of the hydrophobic core of the C-terminal of PrPC. We will discuss our results in light of the correlation between structural domains, increased stability in the hydrophobic core and degree of susceptibility of the species to developing prion diseases.

1416-Pos
Investigating Atomic Level Structure in Pyriform Silk Proteins
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Spider silks are biomaterials used for many diverse adaptations by spiders, with mechanical properties comparable to Kevlar and high strength steel. Orb-weaver spiders create up to seven distinct types of silk, including pyriform silk. Pyriform silk is a major constituent of spider attachment discs, which connect web silks to each other and also to disparate materials using glue-coated fibres. Although filling a critical role in web formation, prior to our work neither the structural nor mechanical properties of pyriform silk had been widely investigated. Based on the central pyriform silk repetitive domain from *Argiope argentata*, we successfully engineered recombinant pyriform silk-based proteins. Using these proteins, we showed that, in contrast to mechanical extremes of strength at the expense of extensibility or vice versa that are seen in most silks, recombinant pyriform silk is both strong and extensible. To understand the structure-function relationships for this distinctive class of silk, we are performing solution and fibre-state structural studies. Our initial studies have implied distinct regions of order and disorder in the repetitive unit based on the observation of differing degrees of NMR spectroscopy chemical shift dispersion that correlate to the sign of heteronuclear ^{1}H-^{15}N nuclear Overhauser effect enhancements, consistent with a sequence that has segregated regions of disordered and ordered tertiary structuring. Through backbone and side chain chemical shift assignment, we expanded on this, with current data suggesting a central 5-6 helix bundle with long disordered linkers at each end of the bundle that shows no structural perturbation upon the addition of extra repeat blocks, indicating that this protein is likely to behave in a ''beads-on-a-string'' manner. Current investigation into structural transitions upon fibrillogenesis have shown an anticipated shift from predominantly α-helix in solution to β-sheet in fibres, with further studies ongoing.

1417-Pos
Deciphering the Conformational Dynamics of *Escherichia coli* Type IV Pilus
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Type IV pili (T4P) are important surface fibers of many bacteria, including the human pathogen Enterohemorrhagic *Escherichia coli* (EHEC). They are long flexible filaments, capable of rapid extension and retraction. T4P promote biofilm formation, host cell adherence and invasion, motility, DNA uptake and virulence. The structure of the periplasmic domain of EHEC T4P subunit PpdD has been recently determined by Nuclear Magnetic Resonance (NMR) spectroscopy in our group. This structure, combined with cryo-EM density map of the EHEC pilus at 8Å resolution, resulted in an atomistic model of the T4P filament[1]. We performed extensive all-atom molecular dynamics (MD) simulations to study the wild-type and mutant T4P, in order to characterize their dynamic properties. The MD simulations revealed a set of key residues involved in the interactions between individual subunits, whose functional role was confirmed by the analysis of mutants. Moreover, our NMR analysis revealed putative calcium (Ca^{2+}) binding residues in pilin monomer. Therefore, to test its binding to assembled pili, we performed additional all-atom MD simulations in the presence of Ca^{2+} and identified a non-canonical calcium binding pocket formed at the interface between three pilin subunits. We characterized the residues interacting with calcium as potential pocket for drug design, and showed their importance for T4P assembly and stability. Finally, we studied the effects of other ions by performing MD simulations of T4P in the presence of sodium (Na^{+}), manganese (Mn^{2+}) or magnesium (Mg^{2+}). Despite stable binding of ions with a positive charge of two during MD simulations, only calcium and manganese increased the overall stability of pili *in vitro*. This study will allow us to identify the molecular mechanisms behind the conformational dynamics of T4P, and to develop strategies for interfering with its function.
[1]Bardiaux et al., Structure 27.7 (2019).

1418-Pos
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Type IV pili (T4P) are biopolymers comprised of many protein subunits called pilin. These pilin subunits are not covalently bonded to one another, however remarkably T4P filaments are very strong and flexible. T4P emanate from the surface of prokaryotic cells and are utilized for many functions, including biofilm formation, surface adhesion, motility, and infection. The recent cryo-EM based structures for T4P from *Escherichia coli, Neisseria meningitidis, Pseudomonas aeruginosa, and Neisseria gonorrhoeae* have provided unprecedented insights into the structures of these filaments. However, although the structures of T4P are known, the dynamics of these filaments at the molecular scale at equilibrium and under tensile forces is not well characterized. In this work we provide an overview of our research into these various T4P filaments and their constituent pilin monomers under force. Specifically we carried out steered molecular dynamics simulations using a multiscale approach encompassing all-atom simulations and two levels of coarse-grained simulation. We have analyzed the changes in secondary structure of pilin subunits, global changes in filament architecture, and calculated the Young's modulus of each of the different T4P filaments. By drawing comparisons between all of these filament systems, we are able to obtain a broader picture of T4P dynamics than experimental structures alone can provide. In particular, we observe elongation of the alpha helix region of pilin subunits in each of these systems, which has been previously attributed to T4P flexibility and strength.