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Finite Element and Statistical Methods Applied to Actin Bundles and Networks under Load

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Actin binding proteins (ABPs) organize F-actin into ensembles_generally bundles or networks_thereby affecting actin cytoskeletal function and dynamics. They also participate in binding F-actin to the cell membrane. Some ABPs, e.g. α-actinin, fascin, bind F-actin into parallel bundles whose inter-filament spacing, which depends upon the ABP length and distance between its actin-binding domains, may or may not allow F-actin to interact with other proteins, e.g. myosin. Other, generally longer, ABPs, e.g. filamins, bind F-actin into compliant, albeit tightly-entangled, orthogonal networks imbued with gel-like mechanical properties, as a result of the 2D-rotational flexibility of the V-shaped ABP hinges.

As the actin structure in non-muscle cells is complex and dynamic, a couple of features have been made to build the 3D model: determination of the representative model space that contains enough number of actin filaments to model the typical actin-actin interactions over larger scales; identification of the average effect of actin crosslinking in the model space, including the possible links and number of actin filaments in the neighborhood with respect to actin length, length distribution, and number density; construction of the fundamental elements that repeat in the crosslinked structures and maintain the physical constraints of bundles and networks; application of Bell's model to back out the passive force and bond lifetime of actin structure under load.

Myosin mini-filaments work together with actin filaments in non-muscle cells during cell migration and division. Instead of providing passive load bearing like ABPs, myosin filaments walk along F-actins to generate contraction forces. This model is being integrated with the presence of myosin mini-filaments and the relative interactions, into a larger model for the production and transmission of biochemically-mediated intracellular forces, and actin cytoskeleton dynamics in whole cells.

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Different Types Of Polymorphisms Within The F-actin And ParM Filaments

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Cytoskeletal proteins form dynamic networks in eukaryotic cells which are vital for chromosome segregation, movement of cellular organelles, motility and morphology. All three types of cytoskeletal elements found in eukaryotic cell - actins, tubulins, and intermediate filaments - are also present in bacteria. MreB and ParM are prokaryotic actin homologs that have little sequence identity with eukaryotic actin, but have a very similar fold. MreB maintains cell shape, while ParM is involved in plasmid segregation. Significant effort has been devoted to building an atomic model of the actin filament, but the structure of the ParM filament has been controversial.

We used electron cryo-microscopy and the IHRSR helical reconstruction approach to compare the structures of actin and ParM filaments We show that F-actin, in addition to variable twist, possess a significant structural disorder and exists in multiple structural states. ParM filaments are even more heterogeneous. In addition to variability in twist, which is greater than that observed in F-actin, they have a variable axial rise. ParM filaments also have structural heterogeneity which arises from opening of the ATP-binding cleft. The subunit within the ParM filament, with a non-hydrolyzable ATP analog bound, can exist in both open and closed conformations. This variability in the opening of the cleft introduces a variability in the interface between the protomers in the ParM filament. Altogether, our results show that F-actin and ParM form very different filaments, and these filaments possess quite different types of structural heterogeneity. This is consistent with a lack of conservation between actin and ParM in the regions involved in the subunit-subunit interface within the filament. We conclude that both actin and ParM filaments are not uniform in their structure, and thus can not be described by a single atomic model.

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The Effect of Actin-Binding Proteins on the Dynamics of Monomeric Actin

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In eukaryotic cells, actin, which is the main component of the microfilament network, can be found in monomer (globular) and polymer (filament) form as well. The balance between these two forms is maintained by the assistance of the intracellular actin-binding proteins. Cofilin and profilin are small actin-binding proteins that can be found in nearly all eukaryotic cells. Cofilin can induce the disaggregation of the microfilament system by splitting the actin filaments and increasing their depolymerisation. Profilin has an opposite effect by increasing the rate of polymerisation at the plus end of the actin filaments. Cofilin and profilin are able to modify the rate of nucleotide exchange on G-actin via closing and opening the nucleotide binding cleft.

Fluorescence resonance energy transfer (FRET) measurements were completed to answer the question of what kind of dynamic and conformational changes can be identified behind the functions of these actin-binding proteins. Fluorescent donor and acceptor molecules were attached to the actin monomers at Lys61 and Cys374. The possible conformational actions and the flexibility of the protein matrix between the probes were investigated by temperature dependent FRET experiments. The distance didn't change significantly between the labeled residues, while the protein matrix became more rigid after the binding of cofilin and profilin as well.

With the help of the applied measurements it is possible to demonstrate that although the cofilin and profilin have an opposite effect on the conformation of the nucleotide binding cleft, both proteins modify the flexibility between the subdomain I and II in the same direction. These results can demonstrate that the small domain on actin behaves as a rigid unit during the opening and closing of the nucleotide binding pocket in the presence of profilin and cofilin as well.

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Computational Analysis of the Interaction Between Paired CH-Domains - Implications for their F-actin Bound Conformations

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Paired calponin-homology (CH)-domains form the actin-binding domains (ABD) of an important family of cytoskeletal proteins, including utrophin, dystrophin, fimbrin, alpha-actinin, plectin, and spectrin. While the crystal structures of various ABDs exhibit extensive inter-CH interactions and display a compact conformation, the functional conformation of F-actin-bound ABDs is still unresolved. Studies suggest that upon binding to F-actin the compact conformation observed in crystal structures persists; others suggest that the CHs separate and the ABD becomes extended. To resolve this, we calculated the energy of inter-CH interfaces by computational alanine scanning ($\Delta\Delta G_{binding}$ = 32.9kcal/mol) and computed the energy (6.7kcal/mol) of the utrophin-F-actin interaction based on the 13uM Kd (Moores, 2000). This sets a lower limit for the $\Delta\Delta G_{binding}$ for the open CH model of the utrophin-F-actin interaction at 39.6kcal/mol. Without ABD-actin crystal structures, we cannot compare the interfaces to see if there is enough energy to bind the ABD while disassociating the CH domains. Instead, we computed the minimum energy density of a utrophin-F-actin interface necessary to open the CH domains and compared this value with other known actin interfaces. We divided the area (610Å^2 obtained from the closed fimbrin-fitted model [Galkin 2008]) by the core binding energy (39.6kcal/mol) to get 15.6Å^2/kcal/mol; an energy density that is statistically significantly different ($p=0.0491$) than the mean value for other actin-binding proteins (26.7Å^2/kcals/mol), including DNase-I, gelsolin, profilin, and DBP. This seems to contraindicate a model of an ABD extended into solution because the actin interface proposed to disassociate the CH domains would be unusually energy dense with respect to other known actin binding interfaces and if this solvent exposed model is an intermediate on the path to extended ABD binding, this would preclude the formation of an extended interface on actin.

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Molecular Dynamics Simulation Reveals The Role Of Cross-linkers In Semi-flexible Filament Assembly

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Hierarchically ordered protein networks are formed in solution by the self-assembly of Factin, a semi-flexible biopolymer, in the presence of cross-linkers. We model the assembly of a 3D branching network of filament bundles using a coarse-grained molecular dynamics simulation, finding excellent agreement with experimental observations. We examine the role of cross-linker density on network morphology and filament curvature and observe a clear evolution from a loose single filament network to a network of bundles generated by cross-linker-induced branch points.