Actin & Actin-binding Proteins

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A Comparison of Actin Filament Models by Molecular Dynamics Simulation

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Actin is a major structural protein of the eukaryotic cell, involved in cell motility and the structure of the cytoskeleton. While the cellular functions of actin are well understood, the atomistic structure of the actin filament (F-actin) is still unknown. Several models of F-actin have been proposed. Here, we introduce a new actin filament model based on X-ray fiber diffraction intensities and a previous filament model. Molecular dynamics (MD) simulations of a 13 subunit repeat of the filament were carried out for this new F-actin model, the Oda 2009 model and the Holmes 2004 model in order to investigate the conformational details of the actin filament and assess their quality in terms of structural integrity. Analysis of a number of structural determents such as the protomer dihedral angle, number of hydrogen bonds or the structural deviation among the 13 protomers suggest the Holmes 2004 model to be of lower quality than the other two models. The different strengths of our new filament model and Oda model may be bundled into an improved future F-actin model.

In addition, simulations of the new filament model were carried out in the ADP and the ATP-bound states to study the differences in the hydrogen-bonding network of the nucleotide. Our simulations predict the significance of the residue Gln137 and shed light into the question why ATPase activity is only exhibited by the filamentous form of actin.

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Multiple Structural Forms of Actin in the Filamentous State

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One of the most abundant proteins in eukaryotes is actin, a ubiquitous protein that plays a role in cell dynamics like cell migration. The dynamics of actin filament treadmilling is regulated by two actin structural states: globular actin (G-actin) and filamentous actin (F-actin). While G-actin's crystal structure has been solved by several groups, F-actin's has not. Although recently it was reported that the structure for the two actins differ (Oda et al), there is still much to resolve on the matter of their dynamic structures. Here we observed the dynamics of the actin structural states under various conditions by using single-molecule FRET in combination with total internal reflection fluorescence microscopy. To conduct these experiments, we first labeled actin residues 41 and 374, having substituted Gln 41 with Cys. The new Cys 41 site along with Cys 374 were used for site-directed labeling by SH-group reactive fluorescent dyes. We found that F-actin has at least two distinct states, and that the population distribution of these states was dependent on the ionic conditions. We are currently investigating these states by performing FRET measurements for observing the long -time transition between the two states.

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Tertiary Structure Model of Wild-Type and Mutated Actin using a Novel Coarse Graining Technique to Study Aortic Aneurysms

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Recent work from Dong Guo, et al. at the UT Health science center has shown the location of many of the amino acid mutations in alpha-actin (ACTA2) that are responsible for aortic aneurysms (AA) (Gong, et al, 2007, Nat. Gen. 39(12):1488-1493). However, the exact mechanism of how this mutation leads to AAs is currently unclear. In addition, current molecular dynamics (MD) simulation techniques are too computationally expensive to be advantageous in studying the long term dynamics of larger proteins such as actin. Therefore, a recently developed technique is applied to smooth muscle cell (SMC) actin, ACTA2, to generate its Hamiltonian potential function, or Tertiary Structure Model (TSM). The TSM begins with domain selections from tertiary (and secondary) structures yielding abbreviated centers of mass and principal axis directions. Parameterization then specifies the interactions between the domains, and the potential function will come from the summation of these domain interactions. These statistical potentials describe the inter-domain interactions and allow for a better understanding of the domain rearrangements as external loads are applied to ACTA2. TSMs are developed for both the wild-type (WT) and mutated (M) ACTA2. 'Virtual' pulling experiments are simulated for the WT actin and will be verified with experimental data. Similar simulations for M ACTA2 will be performed, and the results are discussed. Insights for possible mechanisms of ACTA2's reduced functionality are drawn.

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Thermodynamic Model Study on the Modulation of Binding Affinity between Actin Filament and its Regulatory Proteins in Response to Mechanical Stresses

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Because mechanical stress plays crucial roles in controlling dynamic cellular behaviors, it is important to understand how mechanical stresses modulate the binding affinity between cytoskeletal proteins and their regulatory proteins. In this study, in order to relate stress-induced conformational changes of actin filaments (F-actin), in which mechanics has a central role in cytoskeletal structure, to the binding affinity of actin-regulatory proteins, we introduce a thermodynamic model based on Gibbs-Duhem equation. The model expresses the binding affinity as the chemical potential of the regulatory protein in F-actin, where the binding affinity increases with decreasing chemical potential and vice versa. We investigated the chemical potential as a function of mechanical stress arising from tension, bending, or twisting of F-actin using the model, and found that (1) the chemical potential decreased with increasing tensile strain; (2) the change of chemical potential was proportional not only to tensile strain but also to the volume of binding domain; (3) the bending of F-actin consequently generated gradient of chemical potential in F-actin: the chemical potential decreased on the tensile side of the bent F-actin but increased on the compressive side; (4) the twisting of F-actin induced the decrease of the chemical potential. These results give key factors for better understanding of how mechanical stresses are converted to chemical signals that modulate signaling pathways leading to adaptation of cytoskeletal structure in response to mechanical stresses.

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Effects of Molecular Crowding in Actin Polymerization

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Molecular crowding in cells alters all binding affinities. Spatial differences in cytosolic composition differentially affect crowding properties regulating attachment and release of molecules and/or molecular complexes. Actin polymerization in cell occurs in a variety of places that may be differentially affected by effects of molecular crowding. There are also specific effects exerted by different crowding agents. We found that a naturally occurring osmolyte trimethylamine *N*-oxide (TMAO) dramatically increases affinities of intrinsically disordered actin-regulatory proteins thymosin beta 4 (TB4) and thymosin beta 10 to ATP-G-actin and significantly decreases barbed end actin critical concentration A_c in presence of ATP. On the other hand, effect of TMAO on the affinity to ATP-G-actin of a globular actin regulating protein profilin is much weaker.

Interestingly, the dependence of the amount of sequestered actin in presence of TB4 is bell-shaped. The standard free energy change depends non-linearly on TMAO concentration for A_c and linearly for the affinity of TB4 to actin. This implies that spatial variation in intracellular conditions can exert complex effects on actin sequestration.

We also found that the TMAO facilitates a ternary complex formation between actin, profilin, and TB4 which may very significantly increase the amount of unpolymerized actin in presence of profilin and TB4 and slow down actin polymerization. Our results support the hypothesis that regulation of actin polymerization could be spatially regulated by modulation of the effects of natural osmolytes in different locations inside the cells. Moreover, the data suggest that a potential explanation for the perplexing question as to why these two actin-binding proteins, profilin and thymosin are well-structured and disordered, respectively. We speculate that the compactly structured profilin can impart a structural change to actin while disordered thymosin may create a mechanism for regulation of the ternary complex by crowding effects.