Molecular Dynamics Investigations of Actin Mutations and their Role in Disease

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In Short

- *ACTB* and *ACTG1* encode the cytoskeletal isoforms of actin
- Cytoskeletal actin participates in essential processes requiring the generation of intracellular chemomechanical force and translocation of cargo
- Cytoskeletal actin dynamics and functions are finely regulated involving over one hundred interaction partners
- Mutations in *ACTB* and *ACTG1* cause syndromic autosomal-dominant disorders
- Molecular Dynamics simulations are used to scan the conformational space of mutant actin with the aim to reveal underlying disease mechanisms

Actin is one of the most abundant proteins in eukaryotic cells. Over a hundred interaction partners of actin are known to date that regulate its dynamics and functions, including the interaction with other actin molecules in the formation of filaments. Due to its ability to polymerize and form various higher order structures, ranging from single filaments, to highly organized bundles and networks, it performs a large variety of functions. Actin supports cellular processes including endocytosis, intracellular transport, cytokinesis, migration and the definition of cell shape. The structure of actin plays a crucial role in these interactions ([1], figure 1). Consequently, it is not surprising that even small structural changes induced by missense mutations can perturb these interactions and act as disease triggers. A multitude of diseases caused by point mutations in the six human actin isoforms has been reported [2]. Mutations in the two cytoplasmic genes, *ACTB* and *ACTG1*, show strong diversity in terms of resulting disease phenotypes (for examples see figure 1) [2–5]. Pathological conditions are frequently linked to changes in filament nucleation, elongation or turnover that are caused by structural changes affecting actin–actin or actin–actin binding protein interactions, nucleotide binding or ATP hydrolysis. Certain effects are the result of specific mutations that lead to a particular disease phenotype. In some cases, the location of the affected amino acids suggests a common disease mechanism. The molecular basis of a shared molecular disease mechanism is not so obvious when different mutations cause the same effect e.g. do not allow for correct folding, completely disrupt an interaction, or change the stability of the protein [5,6].

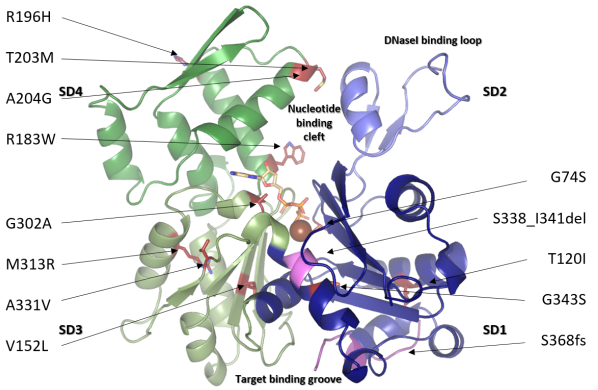

***Figure 1:** Structure of human cytoplasmic actin (PDB-ID: 2btf). Selected mutations causing actinopathies are depicted: point mutations in red, the helical turn of the deletion mutant S338_I341del in pink and the Alphafold predicted structural difference of the S368fs mutant purple.*

Several of the actinopathy-causing mutations in the two human cytoplasmic actin isoforms have been studied extensively *in vitro* and partially *in vivo* [2]. Our approach is based on combining experimental results obtained from the biochemical and biophysical characterization of mutant β-actin and γ-actin with long-time MD-simulations for the elucidation of parameters that describe the conformational behavior of actin monomers with respect to protein-stability and actin-interactions. Such a detailed analysis helps us to elucidate the extent to which mutations that bring about similar functional consequences are related to the observed disease phenotypes. Moreover, this approach will allow us to make predictions on the impact of mutations for which patient-derived cells from biopsies are currently not available.

In a first step we have identified key mutations with varying effects and potential outcomes that effect the structure and dynamical behavior of globular, i.e. monomeric actin (figure 1). The actin monomer consists of two subunits that are inherently twisted relative to one another and that are connected by a nucleotide binding cleft. Previous studies have

shown that actin undergoes several key movements, including opening and closing of the nucleotide binding cleft and flattening of the subunits during polymerization which induces the ATP hydrolysis activity of actin. Our results show a large variability of effects that the mutations have. Among other things, they can strongly reduce the opening and closing movement of the nucleotide binding cleft, which is essential for nucleotide exchange. They alter the intrinsic flexibility of actin, or parts of it and render it difficult to adopt specific conformations or lead to free fluctuations of the phosphate binding loops. They can also hinder the formation of important post-translational modifications, which then causes further effects. Mutations located inside the protein can also alter the surface structure. Each of the mutations we have studied has a specific spectrum of effects at this monomeric level, which must be considered individually.

The altered conformational landscape and dynamic behavior we observe in the actin monomer are, however, only relevant insofar that they effect the interaction with actin binding proteins and thereby the intrinsic function of actin. To get a better understanding of the effects we observe in cells, we need more information about the interaction of actin within the filament, but also with actin binding proteins that bind filamentous, or globular, actin and thus regulate actin function or carry out their function. For this, it is absolutely necessary to translate the changes we see in monomeric actin to a higher structural level, i.e., the filament, and to understand the effects that these mutations have there. This mainly affects two classes of mutations: First, those in which the mutation leads to a change in dynamics that affects the actin-actin interaction, e.g. polymerization, depolymerization, or filament stability. On the other hand, there are the mutations that affect the binding of the monomers in the filament to each other. These are either located directly in the actin-actin interaction region, or they are mutations inside the molecule that change the surface structure in the actin-actin interaction region. Detailed analysis of these interactions and mutation-related changes is important to elucidate disease mechanisms on a molecular level. Therefore, we would like to focus on this in the next project part, and look at the effects that the mutations have on the stability and fluctuation of the filament, as well as the interaction of the actin molecules with each other. This is the next important step to understand the development of the diseases and their severity.

WWW

https://www.mhh.de/bpc

More Information

[1] R. Dominguez, K. C. Holmes, *Annual Review of Biophysics* **40(1):169-86**,2011 Jun 9, doi: 10.1146/annurev-biophys-042910-155359.

[2] F. Parker, T. G. Baboolal, M. Peckham, *International Journal of Molecular Sciences* **Vol. 21** MDPI AG, 2020, doi:10.3390/ijms2109337.

[3] S. Cuvertino, H. M. Stuart, K. E. Chandler, N. A. Roberts, R. Armstrong, L. Bernardini, et al., *American Journal of Human Genetics* **101(6):1021-33**, 2017 Dec 7, doi: 10.1016/j.ajhg.2017.11.006.

[4] T. Yates, C. Turner, H. Firth, J. Berg & D. Pilz, *Clinical Genetics* **92:3-9**, 2017, doi: 10.1111/cge.12864.

[5] S. L. Latham, N. Ehmke, P. Y. A. Reinke, M. H. Taft, D. Eicke, T. Reindl, et al., *Nature Communications*, **9(1)**, 2018 Dec 1, doi: 10.1038/s41467-018-06713-0.

[6] N. Hundt, M. Preller, O. Swolski, A. Ang, H. Mannherz, D. J. Manstein & M. Müller, *FEBS Journal* **281(23):5279-91**, 2014, doi: 10.1111/febs.13068.

Project Partners

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