Tropomyosin: Flexibility and Regulation of the Actin Gatekeeper

Molecular Dynamics Investigations of Structure-Function Relationships in Molecular Engineered Tropomyosins

***R. Heiringhoff, E. K. Peter, D. J. Manstein**, Institute for Biophysical Chemistry, Hannover Medical School, Carl-Neuberg-Str. 1, Hannover 30625, Germany*

In Short

- Tropomyosins form cables running along the actin filament, acting as gatekeepers controlling access of actin-binding-proteins (including myosin motors)
- The stability of Tpm cables on actin surfaces is greatly affected by the formation of head-to-tail overlap complexes between neighboring Cdc8-Tpm molecules
- Structure-function relationships are examined with the help of protein engineering approaches
- Effect of post-translational modifications, local changes in charge distribution and the effect of large changes in length of individual Tpms are examined
- Steered MD and umbrella sampling are used to investigate stability of overlap region neighboring molecules

The cytoskeletal protein actin is involved in various cellular mechanisms including cell migration, cell plasticity and intracellular cargo transport that require a high degree of spatial and temporal regulation. The actin binding protein tropomyosin (Tpm) plays a central role in regulating these processes, e.g. acting as a gatekeeper for myosin motors or other actin binding proteins[1]. The majority of actin filaments inside the cell is coated by Tpm. Tpm is able to affect the length of actin filaments and its stiffness, therefore modulating the mechanical properties of the actin filament. The binding mechanism of Tpm to actin is based on a cooperative binding behavior. The affinity of a single Tpm for actin is relatively low, however Tpm is able to form highly stable cables along the actin filament. The binding behavior of Tpm is mainly orchestrated by the formation of a Tpm cable along the actin filament[2]. The mechanism of cable formation is that a single Tpm-dimer binds to the actin filament and primes it for other Tpm dimers, which form a cable starting from the initial-bound Tpm [3]. How exactly this chain elongation happens remains subject to current research just as the inherent properties of the Tpm dimer that regulate this process. A known regulatory mechanism are post-translational modifications

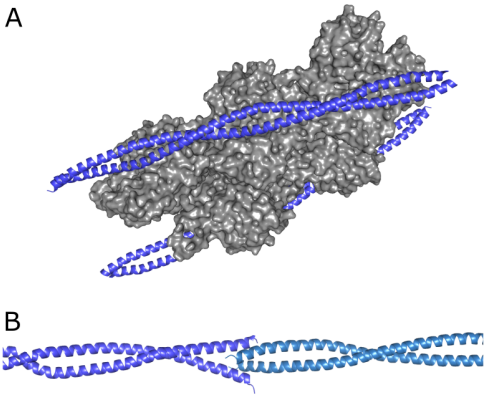

***Figure 1:** (A) Cryo-EM structure of a Tpm-coated actin filament (PDB:5JLF) (B) Tpm-overlap region*

(PTMs), like phosphorylation of serine side chains and N-terminal acetylation. Recent experimental results from our lab indicate that PTMs regulate the Tpm flexibility and have the potential to modify the energetics, the conformation and the binding kinetics of terminal contacts of Tpm dimers [4].

In the proposed project we want to expand our understanding of the regulation of Tpm by PTMs. Molecular dynamics (MD) simulation will serve as suitable method to investigate the observed changes on an atomistic level. We will investigate the effects of PTMs on Tpm itself as well as its ability to bind to actin. Recently our lab solved the crystal structure of the *S. pombe* Tpm called Cdc8p (Cdc8-Tpm). Based on the obtained structure we will perform molecular dynamics (MD) simulations to analyze protein fluctuations and determine actin binding properties by protein-protein docking. Novel *ab initio* structure prediction tools [5] allow a sequence based workflow for Tpm engineering. The workflow allows us to omit the overlap region and create a custom linker that mimics a longer Tpm, thereby creating an artificial, elongated Tpm. Combining this engineered Tpm sequences with subsequent MD simulation provides a great tool to elucidate the relationship between Tpm length and its kinetic and thermodynamical properties.

To get an in-depth understanding of the length-to-function relationship of Tpms we will compare the Cdc8-Tpm fluctuations with human Tpm fluctuations, which is nearly twice the size of Cdc8-Tpm, while both proteins have the same function.

Our main goal is to investigate the regulatory role of Tpm as part of the self-organizing actin network: How its inherent molecular properties (e.g.

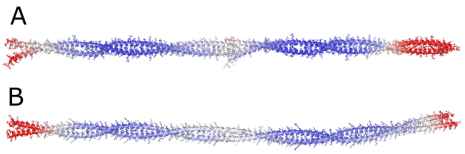

Figure 2: *Fluctuation profile of (A) two native Cdc8-Tpm molecules with intact overlap region; (B) engineered double length Cdc8-Tpm molecule; colors represent increase in fluctuation from blue to white to red;*

sequence dependent stiffness) and PTMs regulate Tpm function and therefore its binding on actin. We will focus especially on the Tpm overlap region since this region is responsible for cable elongation and target to the most abundant PTM (N-terminal acetylation). We will perform the following steps to achieve this goal:

- Structure and fluctuation analysis of Cdc8-Tpm and artificial Tpms
- Design of artificial Tpms
- Analysis of length, PTM and charge effects on the properties of the Tpm cable
- Comparison with human Tpms
- Introduce PTMs and investigate effect on protein properties
- Steered MD and umbrella sampling to investigate response to stress

WWW

https://www.mhh.de/bpc

More Information

[1] DJ. Manstein, JCM. Meiring, EC. Hardeman, PW. Gunning, *J. Muscle Res. Cell. Motil.* **41**, 11-22 (2020). doi:10.1007/s10974-019-09514-0

[2] W. Lehman, X. Li, FA. Kiani, JR. Moore, SG. Campbell, S. Fischer, MJ. Rynkiewicz *Biophys. J.* **115**, 1082-1092 (2018). doi:10.1016/j.bpj.2018.08.017

[3] I. Bareja, H. Wioland, M. Janco, PR. Nicovich, A. Jégou, G. Romet-Lemonne, J. Walsh, T. Böcking *Mol. Biol. Cell* **31**, 2452-2462 (2020). doi:10.1091/mbc.E19-10-0586

[4] T. Reindl, S. Giese, PY. Reinke, I. Chizhov, SL. Latham, DP. Mulvihill, MH. Taft, DJ. Manstein **in submission**

[5] J. Jumper *et. al. Nature* **596**, 583-589 (2021). doi:10.1038/s41586-021-03819-2

Funding

DFG project: Actin-based Force Generation in the Vascular Endothelium (Project-ID: 462266917)