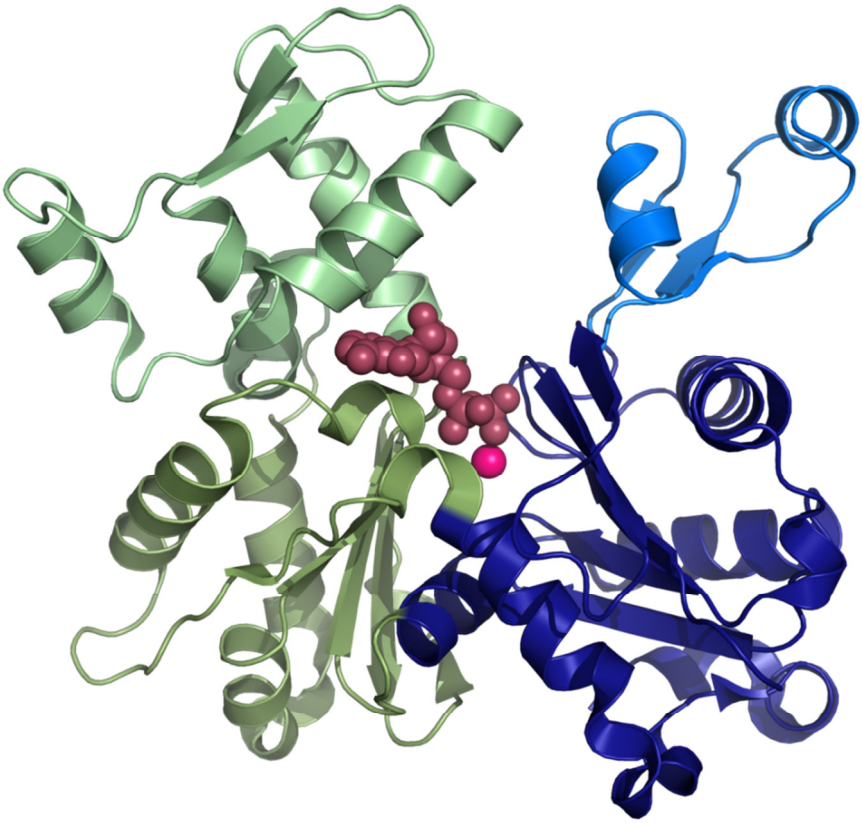
THESIS OF Ph.D. DISSERTATION

Effect of actin-binding proteins on the structure and conformational dynamics of the actin monomer



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INTRODUCTION

Actin is the most abundant protein found in all eukaryotic organism from plants to animals. The property of the actin molecule to assemble into filament in a reversible way make it suitable for playing an indispensable role in different cellular processes such as cell locomotion, morphogenesis, membrane trafficking, and cell division. To precisely fulfil its versatile function, the spatiotemporal organisations of diverse actin filament networks are controlled by a large number of actin-binding proteins. These effectors can disrupt unnecessary "old" actin structures and assist in the assembly of new actin networks with diverse architecture depending on the cellular process in which they are involved. To understand the actin-based cellular processes in details it is necessary to explore the conformational and dynamic changes occur within the actin molecule due to the action of actin-binding proteins.

Actin is an ATPase protein sharing similar three-dimensional structure with hexokinases, glycerokinases and Hsp70 proteins. Based on the first atomic structure of actin monomer it consists of two main domains termed as large and small domain. The main domains can be further divided into subdomains; the large domain is composed of subdomains 3 and 4, while the small domain is built up from subdomains 1 and 2. The ATP-binding cleft is located between the two main domains in the centre of the molecule.

The actin monomers under appropriate conditions can bind to each other in a head to tail manner forming a double-stranded helix that is termed actin filament (F-actin). The F-actin has structural polarity due to the asymmetric nature of the actin monomers. Based on the electron micrograph of actin filaments decorated with myosin one of the ends of the filament is called barbed and the other is termed pointed end.

The process of actin polymerization starts with formation of actin nuclei consisting of 2-4 actin monomers. The nucleation step of the actin polymerisation is an unfavourable process from a thermodynamic point of view due to the unstable nature of the actin dimers and trimers. However when the actin seeds are formed, the actin nuclei start to elongate with different kinetics at the two ends of the filament. The barbed end of the filament grows fast (fast-growing end or + end), while the pointed end elongates slowly (slow-growing end or - end). The elongations of actin filaments proceed until a dynamic equilibrium is established between the actin monomers and filaments. The dynamic equilibrium between monomers and

filaments maintains a continuous presence of around 0.14 μM free actin monomer in the solution. This steady-state concentration (C_{SS}) of free actin monomers is slightly above the critical concentration of the barbed end (C_{cB}=0.12 μM) and well below the critical concentration of the pointed end (C_{cP}=0.62 μM). The critical concentration refers to the concentration of available actin monomers at which the filament elongation can take place. The main consequence of the steady-state stoichiometry between actin monomers and filaments is the predominant incorporation and dissociation of actin subunits at the barbed and pointed end of the filament, respectively. The continuous flux of actin subunits from the barbed end to the pointed end is called the treadmilling (or turnover) of the actin filaments. This unidirectional growing of the actin filaments provide the pushing force against the cell membrane in actin-based cell locomotions.

The actin monomer has a very weak ATPase activity, but when actin subunits incorporate into a filament the bound-ATP is hydrolized to ADP+P_i within few seconds. Subsequently the inorganic phosphate is released with the half time of 6 minutes leading to the appearance of ADP-loaded actin subunits within the filament. The dissociating ADP-loaded actin monomers has to undergo a nucleotide exchange process before the next round of incorporation. The replacement of ADP to ATP occurs spontaneously in the living cells despite it is influenced by the most of the actin monomer binding proteins such as profilin, cofilin, cyclase associated protein and twinfilin.

The replacement of ADP for ATP is an important process, because the ADP-loaded actin monomer has weaker polymerization ability than the ATP-loaded actin. In addition, the filaments built up from ADP-loaded actin subunits are more exposed to the disassembly activity of several actin filament binding proteins (e.g. cofilin and Aip1), because these proteins preferentially interact with ADP-containing actin filament segments.

The nucleotide exchange process can be enhanced by most of the members of the profilin protein family. In one of the resolved atomic structure of profilin-actin complex shows a wide opening of the nucleotide-binding cleft. This conformation of the actin molecule can allow the fast moving of the nucleotide between the actin molecule and the solvent. The connection between the conformation of the nucleotide-binding cleft and the kinetics of the nucleotide exchange process was also supported by the atomic structure of twinfilin ADF-homology domain in complex with actin monomer. In this structure the nucleotide-binding cleft is in a closed conformation that is consistent with impaired nucleotide exchange observed in the presence of ADF-homology domain containing proteins.

ADF/COFILIN FAMILY

Members of the ADF/cofilin protein family are small (13-19 kDa) actin-binding proteins containing one (coactosin, cofilin, GMF) or two copies (e.g. twinfilin) of the characteristic ADF/cofilin (AC) domain. They form 1:1 complex with actin monomer and also able to associate to actin filaments. The main function of cofilin in the living cells is to enhance the actin filament treadmilling in the highly dynamic regions of the actin cytoskeleton system. There are several mechanisms how cofilins are able to do that. They increase the rate of actin subunit dissociation at the pointed ends of the filaments, disrupt actin structures composed of "older" actin filaments by its severing activity, and under certain circumstances stabilize actin dimers in the nucleation process of new filaments. On the other hand they inhibit the replacement of ADP for ATP on the building blocks of the disrupted actin structures that can slow down the flux of the actin monomers from the shrinking pointed end to the fast growing barbed end of the filaments.

PROFILIN FAMILY

The profilin is a small (19 kDa) actin-binding protein widely distributed in eukaryotic organisms from plants to mammals. The main function of profilin is to maintain a large pool of polymerisation competent actin in the cell cytoplasm that can be used to build up new structures as there is a demand on the cell. This function can be fulfilled by different actions of profilins. Most of the profilins facilitate the exchange of ADP for ATP on the dissociating actin subunits leading to the accumulation of a large pool of polymerization-competent actin monomer pool. The actin monomers recharged with ATP in complex with profilin are not able to form actin nuclei, but they are able to be effectively added solely to the fast-growing barbed end of the pre-existing filaments. These effects of profilin drive the ATP-recharged actin subunits toward the fast growing end of the filament.

AIMS OF THE THESIS

The main goal of the work presented in this thesis was to reveal the conformation changes in the actin molecule accompanying the modified nucleotide exchange process due to the action of cofilin and profilin. Most of these two actin monomer binding proteins have opposite effect on the nucleotide exchange process and presumably on the conformation of the nucleotide-binding cleft as well. In addition to get information about the conformation of nucleotide-binding cleft we atempted to elucidate the contribution of the conformational dynamics of the small domain of actin to the nucleotide exchange process.

The following questions were addressed:

1.) How does yeast cofilin or profilin affect the nucleotide exchange on the α-skeletal actin monomer?

2.) What type of conformational change is induced in the nucleotide binding cleft of ATP-loaded actin monomers due to the binding of yeast cofilin or profilin?

3.) How does yeast cofilin or profilin change the structure and conformational dynamics of the small domain of the actin monomer?

4.) What relationship exists between the conformational state of the nucleotide binding cleft of actin and the heat stability of the actin monomer?

EXPERIMENTAL AND PREPARATIVE PROCEDURES

PROTEIN PREPARATION

Aceton-dried muscle powder was obtained from rabbit skeletal muscle according to Feuer and colleagues. The G-actin was prepared from the aceton-dried muscle powder as described by Spudich and Watt. GST-tagged yeast cofilin and His-tagged profilin were expressed in BL21 (DE3) E. coli strain. Cofilin was collected from the cell lysate by using Glutathione-agarose beads and further purified by applying seize exclusion chromatography. Profilin was collected from the cell lysate by using Nickel-NTA beads and further purified by applying seize exclusion chromatography.

NUCLEOTIDE EXCHANGE EXPERIMENT

The kinetics of nucleotide exchange process on actin monomers labelled with a fluorescent ATP analogue (ε-ATP) was investigated by using Applied Photophysics SX.18MV-R Stopped-Flow instrument. The dissociation of ε-ATP was initiated by mixing the actin sample in the absence or presence of actin-binding proteins (cofilin or profilin) with a great excess of non-fluorescent ATP (1 mM). The ε-ATP has higher fluorescence intensity when it is bound to actin, therefore the kinetics of nucleotide exchange can be followed by recording the drop in the fluorescence intensity of ε-ATP in time after mixing the ε-ATP labelled actin with ATP. The observed rate constants characterizing the kinetics of the nucleotide exchange were obtained by fitting exponential functions on the fluorescence curves.

FLUORESCENCE QUENCHING OF ε-ATP LABELLED ACTIN

The fluorescence intensity of ε-ATP bound to actin was quenched by acrylamide (neutral quencher) in the absence and presence of actin-binding proteins. The fluorescence intensity was recorded in a Perkin-Elmer LS50B spectrofluorometer equipped with a thermostated cuvette holder. The Stern-Volmer plot of the intensity in the function of the acrylamide concentration (Q) was analysed by using a modified form of the Stern-Volmer equation:

$$\frac{F_o}{F} = \left(\sum_{i=1}^{n} \frac{\alpha_i}{\left(1 + K_{SV\_S_i}[Q]\right)\left(1 + K_{SV\_D_i}[Q]\right)} \right)^{-1}$$

where F_o and F are the fluorescence intensity in the absence and presence of the quencher, respectively. The K_{SV_Si} and K_{SV_Di} are the static and dynamic Stern-Volmer constant of the i^{th} fluorophore population represented with the fraction of α_i, respectively.

FLUORESCENCE RESONANCE ENERGY TRANSFER (FRET)

FRET experiments were carried out with a Perkin-Elmer LS50B spectrofluorometer equipped with a thermostated cuvette holder. The energy transfer efficiency (E) was calculated between Cys374 (located in the subdomain 1) labelled with the donor fluorophore (IAEDANS) and Lys61 (located in subdomain 2) labelled with the acceptor (FITC) according to the following equation:

$$E = \left[1 - \left(\frac{F_{DA}}{F_D} \cdot \frac{c_D}{c_{DA}}\right)\right] / \beta$$

where F_{DA} and F_D are the fluorescence intensity of donor in the presence and absence of the acceptor, respectively. The β is the labelling ratio of the acceptor, c_{DA} and c_D are the concentrations of the donor molecule in the presence and absence of the acceptor, respectively.

The flexibility of the protein matrix between the labelled positions was estimated by calculating the flexibility factor (f') in the function of the temperature (from 5 ºC to 35 ºC) by applying the following equation:

$$f' = E/F_{DA}$$

ANISOTROPY DECAY MEASUREMENTS

Time-resolved fluorescence anisotropy measurements were carried out by using a Horiba Jobin-Yvon Nanolog spectrofluorometer operating in time-resolved single photon counting

mode. The fluorescence emission of the samples was collected in parallel (I_{VV}) and perpendicular (I_{VH}) polarization in respect to the vertically polarized excitation beam. The rotational correlation times (θ) characterizing the rotational diffusion of the reporter molecules and protein complexes were resolved from the parallel and perpendicular fluorescence decay curves according to the following equations:

$$I_{VH}(t) = G\int_{-\infty}^{t} IRF_{VH}(t')\frac{1}{3}\sum_{i=1}^{n}\alpha_i\, e^{-\frac{t-t'}{\tau_i}}\left[1+2(R_{INF}+\sum_{j=1}^{m}\beta_j e^{-\frac{t-t'}{\theta_j}})\right]dt'$$

$$I_{VV}(t) = G\int_{-\infty}^{t} IRF_{VV}(t')\frac{1}{3}\sum_{i=1}^{n}\alpha_i\, e^{-\frac{t-t'}{\tau_i}}\left[1+2(R_{INF}+\sum_{j=1}^{m}\beta_j e^{-\frac{t-t'}{\theta_j}})\right]dt'$$

where θ_j and β_j rotational correlation time of the j^{th} component and the contribution of that to the anisotropy decay process, respectively. G is the ratio of the sensitivity of the detection system for the vertically and horizontally polarised light (geometry factor), R_{INF} is the residual anisotropy, τ_i and α_i are the lifetime and amplitude of the i^{th} fluorescence decay component, respectively.

The spatial restriction of the reporter molecules' motion was characterised by half-cone angle (θ_o) of their motion based on the following equation:

$$\frac{r_G}{r_o} = \left[\frac{1}{2}cos\theta_0(1+cos\theta_o)\right]^2$$

where r_G and r_o are the fractional amplitude of the global motion of the protein, and the time zero anisotropy, respectively.

DIFFERENTIAL SCANNING CALORIMETRY (DSC)

DSC experiments were carried out with a Setaram Micro DSC III calorimeter. The heatflow-temperature curve of the samples was recorded between 0 °C and 100 °C, under 0.7 atm pressure at a scanning rate of 0.3K/min. The samples were heated twice in order to reveal the nature of the heat denaturation of the samples (reversible or irreversible). The melting temperature (T_m) of the proteins and the full width at half maximum (FWHM) were determined by fitting Gaussians on the heat transition curves. The enthalpy change (ΔH) was calculated by taking the integral of the area under the heatflow-time denaturation curve.

RESULTS AND DISCUSSION

EFFECT OF COFILIN AND PROFILIN ON THE NUCLEOTIDE EXCHANGE PROCESS

In order to test how yeast cofilin and profilin are able to modify the nucleotide exchange process, transient kinetic assays were performed on fluorescent ATP analogue labelled actin monomers. The nucleotide exchange experiments showed that the cofilin and profilin have opposite effect on the kinetics of replacement of nucleotides. In our experiment setup the observed rate constant for the nucleotide replacement was 0.012 s^{-1} in the absence of actin-binding proteins. In the presence of yeast cofilin the observed rate constant decreased to 0.002 s^{-1} while yeast profilin increased that to the value of 0.75 s^{-1}. Based on these results yeast cofilin slows down and yeast profilin enhances the nucleotide exchange process as the typical members of the cofilin and profilin protein family.

CONFORMATION OF THE NUCLEOTIDE-BINDING CLEFT

The possible conformation changes leading to the modified nucleotide exchange rates was investigated by using fluorescence quenching method. The ATP bound to actin was replaced by a fluorescent ATP analogue (ε-ATP) and the accessibility of that was explored by using acrylamide as a neutral quencher. The quenching process was complex due to the presence of free and actin-bound ε-ATP, therefore the data obtained in the steady-state fluorescence quenching experiments were analysed by a modified from of the Stern-Volmer equation.

The time-resolved fluorescence quenching experiments revealed that the free and the actin-bound ε-ATP can be quenched solely in dynamic and static quenching processes, respectively. Furthermore in these assays it was pointed out that neither cofilin nor profilin change the fluorescence lifetime of the actin-bound ε-ATP suggesting that these proteins do not alter the hydrogen bonding network connecting the nucleotide to the actin molecule.

The Stern-Volmer constant characterizing the accessibility of the ε-ATP located in the nucleotide-binding cleft was 0.24 M^{-1}. This value was two orders of magnitude smaller than the K_{SV} obtained for the free ε-ATP suggesting the strong shielding of the nucleotide by the surrounding protein matrix. This finding is consistent with the atomic structures of actin monomer showing the nucleotide deeply embedded in the centre of the molecule. The cofilin decreased the K_{SV} to 0.034 M^{-1} indicating a less accessible environment around the fluorophore. In contrast, profilin increased the value of the K_{SV} to 3.5 M^{-1} implying on a more

accessible environment around the nucleotide. These results indicate a closed and an open conformation of the nucleotide-binding cleft in the cofilin-actin and profilin-actin complexes, respectively.

EFFECT OF COFILIN AND PROFILIN ON THE CONFORMATION AND DYNAMICS OF THE SMALL DOMAIN OF ACTIN

In order to explore how the orientation of the subdomain 2 of actin relative to the rest of the molecule contribute to the widening of the nucleotide-binding cleft temperature-dependent FRET assays were employed. In the experimental setup the donor and acceptor fluorophores were attached to Cys374 (subdomain 1) and Lys61 (subdomain 2), respectively. According to the results of the measurements neither cofilin nor profilin alter the energy transfer efficiency between the labelled positions, consequently the distance between Cys374 and Lys61 is unaffected by the presence of both actin-binding proteins in the investigated temperature range. Nevertheless the tendency of the flexibility parameter in the function of the temperature was steeper in the lack of cofilin or profilin reflecting a less flexible protein structure between the labelled positions in the cofilin- and profilin-actin complex. These results suggest that the highly mobile subdomain 2 of actin does not contribute to the closing and opening of the nucleotide-binding cleft.

EFFECT OF COFILIN AND PROFILIN ON THE DYNAMICS OF THE DONOR AND ACCEPTOR FLUOROPHORES

In order to decide whether the cofilin and profilin cause a change in the dynamics of the donor and acceptor fluorophores, that can contribute to the observed decline in the flexibility of the small domain of actin, fluorescence anisotropy decay measurements were implemented. The anisotropy decay of the donor (IAEDANS) and acceptor (FITC) labelled actin monomer in the absence and presence of actin-binding proteins were recorded from 5 $^{\circ}$C to 35 $^{\circ}$C. Two rotational correlation times were resolved explicitly from the anisotropy decay curves. One of that was a slow and the other one was a fast rotational correlation time, which were attributed to the global tumbling of the actin monomer and to the local wobbling of the attached fluorophore, respectively. The fast rotational time reflects the dynamics of the fluorophore attached to the surface of a protein.

The fast correlation time for the donor-labelled actin monomer decreased from 4.83 ± 0.96 ns to 2.06 ± 0.07 ns as the temperature was increased from 5 $^{\circ}$C to 35 $^{\circ}$C. In the

presence of actin-binding proteins the fast rotational correlation time changed similarly to the situation when actin was alone in the function of the temperature. The fast rotational correlation time decreased from 4.03 ns to 1.92 ns and from 3.22 ns to 2.17 ns in the presence of cofilin and profilin, respectively. Based on these results the dynamics of the fluorophore attached to the Cys374 of actin is not affected by the presence of cofilin and profilin on the applied temperature range. Subsequently it can be ruled out that the increased rigidity of the small domain of actin in the presence of cofilin and profilin is the consequence of the decreased dynamics of the fluorophore attached to Cys374 of actin.

For the acceptor-labelled actin monomer the resolved fast rotational correlation time decreased from 1.48 ns to 1.24 ns as the temperature was raised from 5 $^{\circ}$C to 35 $^{\circ}$C. In the case of cofilin-actin complex the fast correlation time decreased from 1.47 ns to 1.38 ns while for the profilin-actin complex this value changed from 1.4 ns to 1.22 ns in the function of the temperature. According to these results none of the actin-binding proteins changes the dynamics of the acceptor fluorophore. This observation excludes that the decrease in the flexibility of the small domain of actin in the presence of cofilin or profilin is the consequence of the less dynamics of the acceptor fluorophore attached to Lys61.

EFFECT OF COFILIN AND PROFILIN ON THE PROTEIN STRUCTURE AROUND THE REPORTER MOLECULES

To get information about the structure of the actin around the labelled positions the spatial restriction of the fluorophores' motions were characterized by the half-cone angle in the temperature range of 5 $^{\circ}$C to 35 $^{\circ}$C. The amplitude of the global rotational diffusion of the actin and the time-zero anisotropy of the system were used to calculate the half-cone angle.

The half-cone angle for the rotational diffusion of IAEDANS attached to the Cys374 of actin increased from 14.52° to 32.02° as the temperature was raised from 5 $^{\circ}$C to 35 $^{\circ}$C. The temperature induced increase in the half-cone angle of the rotational motion of IAEDANS suggests that the protein structure around the labelled position becomes less compact upon elevating the temperature. The change in the half-cone angle of the fluorophore's rotational motion was not influenced significantly by the presence of actin-binding proteins. In the case of the cofilin-actin complex the θ_o changed from 15.87° to 33.65° while in the presence of profilin the θ_o increased from 14.83° to 28.3° as the temperature was elevated. According to these results neither cofilin nor profilin affect the compactness of the actin structure around the Cys374 on the applied temperature range.

The half-cone angle of the rotational diffusion of FITC located on Lys61 of actin increased from 28.59° to 34.43° as the temperature was raised from 5 °C to 35 °C. Based on this result the degree of orientational constrain of the rotational motion of FITC decreases in the function of temperature that implies the development of a less compact structure around the Lys-61 as the temperature is elevated. The change in the half-cone angle when actin was in complex with either cofilin or profilin was very similar to the case when actin was alone. As the temperature was increased the half-cone angle increased from 26.73° to 32.06° and from 27.69° to 32.68° in the presence of cofilin and profilin, respectively. Based on these results neither cofilin nor profilin cause a change in the structure of the actin molecule in the close vicinity of the Lys61 on the applied temperature range.

EFFECT OF COFILIN AND PROFILIN ON THE HEAT DENATURATION OF ACTIN MONOMER.

Recently Levitsky and his colleagues anticipated a connection between the conformation of the nucleotide-binding cleft and the heat stability of the actin molecule. According to this suggestion the wider nucleotide-binding cleft results in a less resistant actin structure against the heat denaturation. Therefore to get further support to the existence of the closed and open conformation of the nucleotide-binding cleft differential scanning calorimetry assays were performed. The cofilin increased the melting temperature of actin monomer from 55.5 ºC to 59.2 ºC suggesting a more resistant protein structure of actin against heat denaturation. In contrast, profilin decreased the melting temperature of actin monomer 55.5 ºC to 47.6 ºC indicating a decreased heat stability of the actin molecule. These results further confirmed the concept of the closed and open nucleotide-binding cleft in the presence of cofilin and profilin, respectively.

SUMMARY

Based on the results of this work we state that:

- Yeast cofilin inhibits, while yeast profilin enhances the nucleotide exchange process on the actin monomer.
- The ε-ATP located in the nucleotide-binding cleft of actin is strongly shielded by the main domains of actin, because the Stern-Volmer constant characterising the accessibility of the actin-bound ε-ATP is two orders of magnitude smaller than the accessibility of the free ε-ATP.
- Neither cofilin nor profilin change the local environment of the nucleotide because the fluorescence lifetime of ε-ATP is not altered due to the presence of these actin-binding proteins.
- Cofilin closes the nucleotide-binding cleft of actin, because the accessibility of ε-ATP located in the cleft reduced in the presence of it.
- Profilin opens up the nucleotide-binding cleft of actin, because the accessibility of the ε-ATP located in the cleft increased dramatically in the presence of it.
- The opening and closing of the nucleotide-binding cleft occur through the integrated motion of the main domains, because neither cofilin nor profilin can change the orientation of the SD1 and SD2 relative to each other.
- The autonomous motion of the SD2 relative to SD1 is restricted in the presence of both actin-binding proteins, because the flexibility of the small domain is drastically reduced in the presence of either cofilin or profilin.
- The local dynamics of the reporter molecules do not contribute to the observed decrease in the flexibility of the small domain in the presence of actin-binding proteins, because neither cofilin nor profilin can change the dynamics and spatial degree of the fluorophores' motion.
- The applied actin-binding proteins have opposite effect on the heat stability of the actin monomer; cofilin increases, while profilin decreases that.
- The heat stability of the actin monomer strongly depends on the conformation of the nucleotide-binding cleft, wider the nucleotide-binding cleft less resistant the actin monomer against the heat denaturation.

PUBLICATIONS

PUBLICATIONS RELATED TO THE THESIS

The effects of ADF/cofilin and profilin on the conformation of the ATP-binding cleft of monomeric actin. *Roland Kardos, Kinga Pozsonyi, Elisa Nevalainen, Pekka Lappalainen, Miklós Nyitrai, and Gábor Hild,* Biophysical Journal, Volume 96, March 2009, Pages 2335–2343, IF: 4.39

The Effect of ADF/Cofilin and Profilin on the Dynamics of Monomeric Actin
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The other side of the coin: Functional and structural versatility of ADF/cofilins

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POSTERS RELATED TO THE THESIS

-The effect of actin-binding proteins on the dynamic properties of G-actin *Roland Kardos, Elisa Nevalainen, Pekka Lappalaine, Miklós Nyitrai and Gábor Hild,* Symposium for Graduate Students in Biology, November 2009, Pécs, Hungary.

-The effect of actin-binding proteins on the dynamic properties of monomeric actin *Roland Kardos, Mónika Tóth, Kinga Futó, Elisa Nevalainen, Pekka Lappalainen, Miklós Nyitrai and Gábor Hild,* 23rd Congress of Hungarian Biophysical Society, August 2009, Pécs, Hungary.

-The effect of actin-binding proteins on the dynamic properties of monomeric actin *Roland Kardos, Andrea Vig, Réka Dudás, Tünde Kupi, Elisa Nevalainen, Pekka Lappalainen, Miklós Nyitrai and Gábor Hild,* 23rd European Cytoskeleton Forum, FEBS/ECF Workshop, 2008, Potsdam, Germany.

-Effect of actin binding proteins on the conformation of nucleotide binding cleft of actin
Gábor Hild, Roland Kardos, Kinga Pozsonyi, Andrea Vig, Miklós Nyitrai, 37th Membrane Transport Conference, May 2007, Sümeg, Hungary.

TALKS RELATED TO THE THESIS

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-Aktinkötő fehérjék hatása az aktin nukleotidkötő zsebének konformációjára *Hild Gábor, Kardos Roland, Pozsonyi Kinga, Vig Andrea, Nyitrai Miklós,* 37. Membrán-Transzport Konferencia Sümeg 2007 Május.