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Multiscale Modelling for investigating single molecule effects on the mechanics of actin filaments

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Abstract. This work presents a preliminary multiscale computational investigation of the effects of nucleotides and cations on the mechanics of actin filaments (F-actin). At the molecular level, Molecular Dynamics (MD) simulations are employed to characterize the rearrangements of the actin monomers (G-actin) in terms of secondary structures evolution in physiological conditions. At the mesoscale level, a coarse grain (CG) procedure is adopted where each monomer is represented by means of Elastic Network Modeling (ENM) technique. At the macroscale level, actin filaments up to hundreds of nanometers are assumed as isotropic and elastic beams and characterized via Rotation Translation Block (RTB) analysis. F-actin bound to adenosine triphosphate (ATP) shows a persistence length around 5 μ m, while actin filaments bound to adenosine diphosphate (ADP) have a persistence length of about 3 µm. With magnesium bound to the high affinity binding site of G-actin, the persistence length of Factin decreases to about 2 µm only in the ADP-bound form of the filament, while the same ion has no effects, in terms of stiffness variation, on the ATP-bound form of F-actin. The molecular mechanisms behind these changes in flexibility are herein elucidated. Thus, this study allows to analyze how the local binding of cations and nucleotides on G-actin induce molecular rearrangements that transmit to the overall F-actin, characterizing shifts of mechanical properties, that can be related with physiological and pathological cellular phenomena, as cell migration and spreading. Further, this study provides the basis for upcoming investigating of network and cellular remodelling at higher length scales.

1. Introduction

The actin filament is a structural component of the cell cytoskeleton, undergoing various dynamics and mechanical transitions related with cell remodeling. Ion influx and filament treadmilling induce respectively magnesium/calcium exchanges from the high affinity binding site of the actin monomer (G-actin) and dephosphorylation of adenosine triphosphate associated with actin polymerization. These phenomena lead to a cascade of events that, starting from the molecular rearrangements of G-actin, affect the F-actin flexibility. The underlying reasons for these concurrent phenomena rely on the multiscale organization of F-actin. F-actin is a right-handed double helix built up from actin monomers (Figure 1a). Each G-actin is tightly bound to one adenosine nucleotide, ATP or ADP, and one tightly-bound metal cation, Ca²⁺ or Mg²⁺. Nucleotides and cations are freely exchangeable, depending upon the cycle of polymerization/depolymerization and the local ion concentrations. The

nucleotide is situated in the inter-domain cleft of G-actin, where it interacts, through its phosphate oxygens, with the divalent cation positioned at the bottom of the cleft (Figure 1b).



Figure 1: Double helical assessment of F-actin (a); molecular model of G-actin (b): the nucleotide binding cleft is situated in central position and contains the nucleotide (in licore representation) and the cation (in yellow, Van der Walls representation).

The exchanges of nucleotide and cation can be concomitant or take place once at a time, but both result in variations of the kinetics of polymerization for F-actin and of the affinities towards different binding proteins: in addition to these changes in the dynamic properties of F-actin variations of the mechanical properties occur.

The flexibilities of F-actin polymerized with Mg^{2+} -G-actin and Ca^{2+} -G-actin were measured using electron microscopic techniques [1]: an higher flexibility was found for Mg^{2+} -Factin (persistence length, l_p , of about 1.6 µm) than for Ca^{2+} -F-actin (l_p of about 6 µm). To obtain information about the structural changes at the basis of these changes in flexibility, limited proteolysis [2] was employed and the conformation of specific sites in the actin monomers in Ca- and Mg-F-actin was probed, revealing that, depending on whether the high-affinity site for cation is occupied by Ca^{2+} or Mg^{2+} , there are differences in the molecular structure of F-actin. In particular, upon substitution of Ca^{2+} for Mg^{2+} localized regions of G-actin undergo rearrangements, that result in variations of the intra- and intermonomer contacts. In particular, the bound cation modulates the molecular topologies of the surface loop 38-52, including the DNase-I binding loop, those of the internal segment 61-69, in proximity of the so-called sensor loop (residue 73), and of residues 364-372, corresponding to the COOH-terminal segment. Direct E.L.I.S.A. [3] revealed that the region around residue 201 undergoes a structural rearrangement induced by the type of bound cation. The positions of these loops are illustrated in Figure 2.



Figure 2: Residue loops subjected to conformational rearrangements upon nucleotide and/or cation exchanges.

Similarly, ATP hydrolysis leads to a softening of F-actin [4-6]. Experimental analysis of F-actin thermal fluctuations confirmed that ATP-F-actin is more rigid than ADP-F-actin with values of persistence length around 12-15 μ m and 7-10 μ m, respectively [7]. When ADP is bound, the conformation of the DB loop within the subdomain 2 of G-actin is structured as an α -helix and the nucleotide binding cleft is open. On the contrary, when ATP is bound, the DB loop is unstructured and the nucleotide binding cleft is closed [8]. Thus, a transition from a coil to an helix for the DB loop and a closure of the nucleotide binding cleft occur upon ATP hydrolysis [9, 10]. Molecular mechanisms explicable as loop rerrangments and fluctuations drive the modifications in the mechanical properties from ATP-F-actin to ADP-F-actin.

However, the sequence of molecular transitions that are induced by the combinatory effect of cation and nucleotide and that further modulate the elastic properties of F-actin remain to be elucidated. For this reason, we employ a series of simulation techniques each referring to a different length and time scale and evaluate what molecular rearrangements of G-actin induced by a nucleotide and by couples of cation/nucleotide reflect on the biomechanics of F-actin. In particular, we detect what loops of residues in G-actin undergo the highest fluctuations in presence of difference combinations of nucleotide/cation and relate them with the variations in the F-actin flexibility. We start by applying Molecular Dynamics (MD) simulations on ATP-G-actin and ADP-G-actin, then we study the same nucleotide bound monomers with bound Mg^{2+} or Ca^{2+} , exploiting all possible combinations of nucleotide/cation: ADP-Ca²⁺-, ADP-Mg²⁺-, ATP-Ca²⁺-, and ATP-Mg²⁺- Then, using the MD-refined G-actins as building units, several systems of F-actin are assembled, with lengths varying from about 50 to 500 nm. Each F-actin is further modelled as an Elastic Network (EN), where the positions of the alpha carbons of the system is taken into account and the collective motions induced from thermal fluctuations are analyzed. Finally, Normal Mode Analysis (NMA) is performed and the mechanical properties of F-actin are estimated, in terms of bending stiffness k_f (i.e. flexural rigidity), torsional rigidity, k_t , Young's modulus, E_n , and persistence length, l_p , on the basis of the identified bending, stretching and torsional modes. Specific aims of this work are to investigate: i) what inter- and intra-monomer rearrangements of ADP-G-actin and ATP-G-actin are induced by a tightly-bound Ca²⁺ and Mg²⁺; ii) how these rearrangements reflect on the overall mechanical properties of F-actin.

2. Methods

The atomic structures of ATP-G-actin and ADP-G-actin were taken from the RCSB Protein Data Bank: PDB entry 1ATN.pdb [11] and 1J6Z.pdb [8], respectively. A metal ion, Mg²⁺ or Ca²⁺, was added

at the high affinity binding site, for a total of 4 G-actin systems: Mg^{2+} -ATP-G-actin, Ca^{2+} -ATP-G-actin, Mg^{2+} -ADP-G-actin, and Ca^{2+} -ADP-G-actin. G-actin monomers were arranged in a rectangular box (of about 5nm × 8nm × 7nm) and fully solvated (Figure 3c and Figure 3d), using the Simple Point Charge (SPC) explicit water model [12, 13], neutralized by sodium ions. The total number of atoms of each system was around 26000 (about 4000 atoms for the protein and 22000 for the solvent). Periodic boundary conditions were applied and the total energy was minimized by means of 500 steps of Steepest Descent algorithm [13, 14]. Then, 150 ns equilibrium MD simulation was performed at 300 K in a NVT canonical ensemble (i.e., with a constant number *N* of atoms, constant volume *V* and constant temperature *T*). Particle Mesh Ewalds (PME) method [15] was used to model long range electrostatic interactions, with a short range cut-off set at 1.2nm and the force-field GROMOS 53a6, implemented in the code GROMACS 4 [13], was adopted [16, 17].

The analysis of the results was performed with the software packages PROCHECK [18] and VMD [19]. PROCHECK was used to check the "stereochemical quality" of G-actin at the end of the MD simulations [20, 21]. Instead, VMD was used for drawing the MD trajectories of G-actin and for the rendering of the structures. Technically, the analysis of the trajectories of G-actin was conducted in terms of Root Mean Square Deviation (RMSD) and Root Mean Square Fluctuation (RMSF).

The outcome of the atomistic simulations, i.e. the G-actin monomers with atomic coordinates renewed from the fluctuations at equilibrium, were used to assembly actin filaments: ATP-F-actin, ADP-F-actin, Mg^{2+} -ATP-F-actin, Mg^{2+} -ADP-F-actin, Ca^{2+} -ATP-F-actin, and Ca^{2+} -ADP-F-actin. The correct orientation of the monomers was achieved by docking G-actin on the first monomer of a validated structure of F-actin (PDB entry 3B5U.pdb [22]). Then, other G-actins were added longitudinally, each one rotated of 166° and translated of 2.75 nm, according to Holmes et al. [6, 23]. Several models of F-actin with lengths varying from about 50 nm (corresponding to 6500 aminoacids, or alpha carbons C_{α}) to about 500 nm (corresponding to 68000 C_{α}) were assembled.

The atomic structure of each filament was modelled as an elastic network (ENM) composed of nodes (in the positions of the C_{α}) and springs (harmonic potentials of 1 kcal/molA²) [24-26]. The total potential energy, E_p , was expressed in the anisotropic network model formulation [27] as follows: $E_p = \Delta \mathbf{R}^T H \Delta \mathbf{R}$ (1)

where $\Delta \mathbf{R}$ is a 3N dimensional vector describing the fluctuations $\Delta \mathbf{R}_i$ of the position vector \mathbf{R}_i with respect to the equilibrium position $(1 \le i \le N)$, where N is the total number of nodes), and \mathbf{H} is the Hessian matrix (3Nx3N).

The Hessian matrix for F-actin, even when the filament is assumed to be made only of C_{α} atoms, is dramatically large for direct diagonalization. For this reason, the C_{α} atoms corresponding to the 4 functional subdomains of G-actin were clustered into rigid blocks.

Using the Rotation Translation Block (RTB) approach [28-31], F-actin was modelled as made of rigid blocks with interactions derived from atomistic interactions. The vibrational properties of F-actin were computed performing the Normal Mode Analysis (NMA) [31]. Trajectories of the filaments were analyzed (for modes of motion identification) by employing the package VMD.

Assuming F-actin as an isotropic and elastic beam [32-34], the first mode of bending (Figure 2c), of stretching (Figure 2d) and of torsion (Figure 2e) were directly related to the bending stiffness (k_f) , stretching stiffness (k_s) and torsional stiffness (k_l) [30, 31, 35], respectively, following the same approach used in recent papers by Deriu and co-workers [31, 36]. The effect that MD-based refinements of ATP-G-actin and ADP-G-actin bound to different ions have on actin filaments was evaluated in terms of the computed mechanical properties of the corresponding F-actin.

3. Results

All G-actins, throughout the whole equilibrium MD simulations, resulted stable in aqueous environment in terms of tertiary and secondary structure, as no major unfolding took place. The root mean square deviation (RMSD) at 150 ns resulted around 0.4 nm (Figure 3a). The trend of the RMSD curves was similar for ADP- and ATP- G-actin. It is worth noticing that between 50 and 100 ns these

structures seemed to reach a stable RMSD value around 0.3 nm. However in the range of 100 ns a structural change can be observed. As shown by Figure 3b, this change corresponds to the onset of high fluctuations of residues 223-230, with a RMSF peak of 0.6 nm in the interval 90-120 ns: these fluctuations do not correspond to a secondary structure transition of the region, within this time interval.



Figure 3: RMSD of ATP-G-actin and ADP-G-actin (a); RMSF of ATP-G-actin and ADP-G-actin between 90 and 120 ns (b): enhanced are the highest peak.

In general, the RMSF values of C_{α} atoms of ATP-G-actin are higher than those of ADP-G-actin (Figure 4), although the overall average displacements, in terms of RMSD, is lower for ATP-G-actin than for ADP-G-actin (Figure 3a).

The highest values of RMSF for both nucleotide bound forms of the monomer range from 0.4 to 0.8 nm and are distributed as follows: one peak is relative to few residues of subdomain 1 (part of the N- terminal region, residues 1-7); one peak pertain to subdomain 2 (the DB loops, residues 38-52); one peak is relative to a group of residues of subdomain 4 (residues 223-230), as shown in Figure 4a. At equilibrium, RMSF values show that ATP-G-actin is more stable than ADP-G-actin (Figure 4b). Two chain regions of ADP-G-actin are characterized by RMSF values higher than 0.2 nm, i.e., residues of subdomain 1 (part of the N- terminal region, residues 1-7) and the DB loop in subdomain 2 (residues 38-52).



Figure 4: RMSF of ATP-G-actina and ADP-G-actin computed from the beginning of the simulations (a); RMSF of the same monomers at the equilibrium (b).

By comparing the monomers bound to the cation, ATP-G-actin bound to Ca^{2+} was slightly more stable than ATP-G-actin bound to Mg^{2+} , with lower average values of RMSD (Figure 5a). On the contrary, ADP-G-actin bound to Mg^{2+} showed lower average values of RMSD than ATP-G-actin bound to Ca^{2+} (Figure 5b) throughout the overall simulation time.



Figure 5: RMSD of Ca²⁺-ATP-G-actin and Mg²⁺-ATP-G-actin (a); RMSD of Ca²⁺-ADP-G-actin and Mg²⁺-ADP-G-actin (b).

Time [ns]

50

100

150

0

By computing the Root Mean Square Fluctuations (RMSF) of the 4 cation containing monomers, similar trends can be observed: higher fluctuations were shown for Mg^{2+} -ATP-G-actin than for Ca^{2+} -ATP-G-actin (Figure 6a), while the fluctuations were generally higher for Ca^{2+} -ADP-G-actin than for Mg^{2+} -ADP-G-actin (Figure 6b). In particular, 6 groups of residues presented values of RMSF higher than 0.4 nm: residues 38-52, 61-69, 200-208, 223-230, 230-247 and 262-274.



Figure 6: RMSF of Ca²⁺-ATP-G-actin and Mg²⁺-ATP-G-actin (a); RMSF of Ca²⁺-ADP-G-actin and Mg²⁺-ADP-G-actin (b).

In order to investigate the effect of Ca^{2+} on G-actin, the RMSF of the Ca^{2+} bound forms of ATP-Gactin and ADP-G-actin was computed: 2 peaks resulted higher for ADP- than for ATP- G-actin (Figure 6a), namely residues 38-52 and 223-230, with values of RMSF around 0.8 nm. A similar analysis was carried out for the case of Mg^{2+} bound forms of G-actin: 5 peaks resulted slightly higher for ATP-G-actin in comparison with ADP-G-actin (Figure 6b), namely residues 61-69, 200-208, 223-230, 230-247, and 262-274, with RMSF between 0.6 and 0.7 nm.



Figure 7: RMSF of Ca²⁺-ATP-G-actin and Ca²⁺-ADP-G-actin (a); RMSF of Mg²⁺-ATP-G-actin and Mg²⁺-ADP-G-actin (b).

These results obtained at the lowest scale allowed us to relate the molecular rearrangements to the mechanical properties of the actin filament. The average frequencies of vibration of ATP-F-actin (ADP-F-actin) range from about 0.25 cm⁻¹ (0.22 cm⁻¹) in the case of the first bending mode, up to about 1.99 cm⁻¹ (1.68 cm⁻¹) and 4.45 cm⁻¹ (4.10 cm⁻¹) for the first torsion and stretching modes, respectively. Independent of the nucleotide-bond, the computed values of k_f , k_s , and k_t of F-actin filaments result to be length free. The average values of elastic stiffness obtained for ATP-F-actin are: $k_f = 1.89\pm0.08 \ 10^{-26} \ \text{Nm}^2$; $k_s = 7.33\pm3.57 \ 10^{-2} \ \text{Nm}^{-1}$; $k_t = 4.11\pm0.05 \ 10^{-27} \ \text{Nm}^2$. The average elastic stiffness values obtained for ADP-F-actin are: $k_f = 1.45\pm0.07 \ 10^{-26} \ \text{Nm}^2$; $k_s = 6.\ 23\pm0.03 \ 10^{-2} \ \text{Nm}^{-1}$; $k_t = 2.92\pm0.04 \ 10^{-27} \ \text{Nm}^2$.

Under the assumption of homogeneous, isotropic beam, the Young's modulus E_n (coincident with the bending modulus E_b) is found to be not dependent on the chain length, with values of about 0.4-0.5 GPa for ATP-F-actin and of about 0.6-0.7 for ADP-F-actin (Figure 8).

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F-actin length [nm]

Figure 8: Young's modulus computed from the 1st modes of bending (a) and from the 1st modes of stretching (b) as functions of different lengths for the systems of F-actin assembled after equilibrium MD of ATP-G-actin and ADP-G-actin.

The computed persistence length of the filament is found to be of about 4 μ m (Figure 9a). From our study, F-actin bound to ATP results to be slightly stiffer than F-actin bound to ADP, independent of the length of the filament (Figure 9b).



Figure 9: All-atom representation of F-actin (a); Persistence length as a function of different lengths for ATP-Factin and ADP-F-actin (b).

Also in the cases of the cation bound filaments, the computed values of k_f , k_s , and k_t showed to be length free. The average values of elastic stiffness's obtained for Mg²⁺-ATP-F-actin were: $k_f =$ $1.55\pm0.09\ 10^{-26}\ \text{Nm}^2$; $k_s = 6.45\pm0.04\ 10^{-2}\ \text{Nm}^{-1}$; and $k_t = 3.26\pm0.04\ 10^{-27}\ \text{Nm}^2$. The average values of elastic stiffness's obtained from Mg²⁺-ADP-F-actin were: $k_f = 1.15\pm0.02\ 10^{-26}\ \text{Nm}^2$; $k_s = 5.14\pm0.03\ 10^{-2}\ \text{Nm}^{-1}$; and $k_t = 2.11\pm0.41\ 10^{-27}\ \text{Nm}^2$. In the case of Ca²⁺-ATP-F-actin, the following average values were found: $k_f = 1.55\pm0.96\ 10^{-26}\ \text{Nm}^2$; $k_s = 6.45\pm0.04\ 10^{-2}\ \text{Nm}^{-1}$; $k_t = 3.26\pm0.38\ 10^{-27}\ \text{Nm}^2$. For Ca²⁺-ADP-F-actin, the average values of elastic stiffness's resulted: $k_f = 1.38\pm0.11\ 10^{-26}\ \text{Nm}^2$; $k_s =$ $7.09\pm0.05\ 10^{-2}\ \text{Nm}^{-1}$; $k_t = 2.93\pm0.69\ 10^{-27}\ \text{Nm}^2$.

Under the assumption of a homogeneous and isotropic beam, the Young's modulus E_n (coincident with the bending modulus E_b) resulted independent from the chain length, with a value from 0.3 GPa to 0.6 GPa (Figure 10a) and a persistence length of roughly 3 µm (Figure 10b).





Figure 10: Bending modulus as a function of the filament length for the 4 nucleotide/cation systems of G-actin (a); persistence length as a function of the filament length for the 4 nucleotide/cation systems of G-actin (b).

4. Discussion

This work represents a systematic investigation of the effects of different combinations of a nucleotide and a cation on the biomechanics of F-actin. The model employed for the analysis of the effects of ADP/Ca²⁺, ADP/Mg²⁺, ATP/Ca²⁺ and ATP/Mg²⁺ on the mechanics of F-actin is able to maintain the entire number of residues of the building G-actins and to reproduce the intramonomer flexibilities, as explained in [30]. This modeling strategy makes full use of a computational technique, without introducing experimental parameters. Moreover, the direct comparison with cation free systems (ATP-G-actin and ADP-G-actin) allows isolate single contributions from nucleotide and cations and elucidate what molecular reasons are at the basis of changes on flexibility for both cation bound and only nucleotide bound F-actin systems.

With reference to the local rearrangements of G-actin, Mg^{2+} -G-actin resulted slightly more stable than Ca^{2+} -G-actin, as demonstrated by the lower RMSD (Figure 5) values. This result can be related with the fact that the ionic conditions within living cells favor the binding of G-actin to Mg^{2+} rather than Ca^{2+} [37]. However, at the end of our simulation, Mg^{2+} -ATP-G-actin presents higher values of RMSD than Ca^{2+} -ATP-G-actin, thus resulting more instable. The driving force that makes Mg^{2+} -ATP-G-actin faster in polymerizing with respect to Ca^{2+} -ATP-G-actin [2] might be due to such instability: the monomer might need to find a more stable configuration given by the assembly into a filament. Other than the higher RMSD for Mg^{2+} -ATP-G-actin than Ca^{2+} -ATP-G-actin (Figure 6a): residues 38-52, 61-69, 200-208, 223-230, 230-247 and 262-274. Some of these residues are involved in longitudinal contacts among nearby monomers of the same strand in the double helical assessment of F-actin, while others are involved in lateral interactions among nearby monomers of opposite strands.

In addition, most of these residues pertain specifically to subdomains 2 (residues 33-69) and 4 (residues 181-269) and are involved in the closing of the nucleotide binding cleft, upon polymerization into F-actin. At a higher scale of investigation, the different molecular structures of Ca²⁺-ATP-G-actin, Ca²⁺-ADP-G-actin, Mg²⁺-ATP-G-actin and Mg²⁺-ADP-G-actin reflect on the biomechanics of the filament. The ATP- bound forms of F-actin result stiffer than the ADP- bound forms at all lengths (Figure 8 and Figure 10). This stiffening is caused by the rearrangements of residues 38-52 and 223-230 (Figure 4a), that strengthen longitudinal and lateral interactions, respectively. In particular, high molecular rearrangements are carried out from residues 223-230, involved in lateral interactions with Cys 374 of the opposite monomer [38]. Results from our MD simulations has also confirmed the observations of Dalhaimer et al. [39] that subdomain 2 of G-actin is highly flexible regardless the nucleotide state: no secondary structure of the DNase I binding loop has formed in either cation forms of ATP-G-actin, nor ADP-G-actin during the 150 ns of MD simulations. The reason behind this tendency is the characteristics of hydrophobicity of this loop, that tend to rearrange itself to find a more stable arrangement far from the solvent. We think that, when ADP-G-actin is arranged into a filament, the DB loop is enclosed within the residues of nearby monomers and is therefore able to fold into an alpha helix, but this effect was not seen during our MD simulations of ADP-G-actin in isolation, since the DB loop was directly in contact with the polar solvent. However, Mg²⁺-ATP-Factin, if compared with the cation-free form of ATP-F-actin, thought the high rearrangements of residues 1-7 and 223-230, resulted more flexible, in agreement with the spectroscopic measurements of Yanagida et al. [40].

Further results of this study confirm that molecular rearrangements and consequent variations of the overall filament flexibility occur after hydrolysis of ATP in ADP. ADP-F-actin results more flexible than ATP-F-actin at all lengths (Figure 9), confirming that a decrease in flexibility occurs from the ATP- to the ADP- bound forms of the filament. By considering the presence of a bound cation in the ADP form of F-actin, Mg^{2+} -F-actin result more flexible than Ca^{2+} -F-actin while an equal flexibility can be seen for the ATP bound forms of the filament. The results of this study also confirm that the substitution of Ca^{2+} for Mg^{2+} in ADP-F-actin influences the surface loop 39-51, including the DNase-I binding loop, and segment 61-69 (Figure 6). Our simulations did not highlight any difference in the rearrangements of residues 364-372, corresponding to the COOH-terminal segment, between the Mg and Ca bound forms of ADP-G-actin, in contrast with previous experimental results of Strzeleckagolaszewska et al. [41]. We assume that further rearrangements of residues 364-372, not visible in our simulation, may occur upon incorporations of G-actin into F-actin. However, some of the regions that this study recognized as the most fluctuating are the same loops individuated by experimental analyses of Mejean et al. [3] and Tirion et al. [38]. Indeed, upon the substitution of Ca²⁻ for Mg²⁺, in addition to residues 39-51 and 61-69, high fluctuations were carried out from a region around residue 200, from residues 223-230, from the so-called "hydrophobic plug", among residues 230-247, and from residues 262-274. The orientation and displacements of the hydrophobic plug reduce or increases the portion of exposed surface to the neighbour monomer on the opposite strand. Taken together, these molecular rearrangements led to values of torsional rigidity for Ca²⁺-ADP-F-actin (2.93 10^{-27} Nm²) of about the same values of those of Mg²⁺-ADP-F-actin (2.11 10^{-27} Nm²). Also the flexural rigidity of Ca²⁺-ADP-F-actin (1.38 10^{-26} Nm²) was smaller than that of Mg²⁺-ADP-F-actin $(1.15 \ 10^{-26} \ \text{Nm}^2).$

The result that the flexural rigidity is sensitive to the bound metal is also in contrast with the results of Isambert *et al.* (1995), but confirms the results of Yasuda et al. [34].

Summarizing, although global analysis of the actin structures did not reveal relevant changes in conformation (RMSD) or dynamics (RMSF), the minor fluctuations of these several localized regions show a specific conformational dependence on the state of the bound cation and nucleotide and induce variations in the overall F-actin mechanics. The work points out the attention on how single molecule effect at the nanoscale level can be transferred to higher scales affecting the overall mechanics of entire actin filaments.

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