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2 **Evaluation of Extensional and Torsional Stiffness of**
3 **Single Actin Filaments by Molecular Dynamics Analysis**

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1 **Abstract:** It is essential to investigate the mechanical behaviour of cytoskeletal actin
2 filaments in order to understand their critical role as mechanical components in various
3 cellular functional activities. These actin filaments consisting of monomeric molecules
4 function in the thermal fluctuations. Hence, it is important to understand their mechanical
5 behaviour on the microscopic scale by comparing the stiffness based on thermal fluctuations
6 with the one experimentally measured on the macroscopic scale. In this study, we perform a
7 large-scale molecular dynamics (MD) simulation for a half-turn structure of an actin filament.
8 We analyse its longitudinal and twisting Brownian motions in equilibrium and evaluated its
9 apparent extensional and torsional stiffness on the nanosecond scale. Upon increasing the
10 sampling-window durations for analysis, the apparent stiffness gradually decreases and
11 exhibits a trend to converge to a value that is close to the experimental value. This suggests
12 that by extrapolating the data obtained in the MD analysis, we can estimate the
13 experimentally determined stiffness on the microsecond to millisecond scales. For shorter
14 temporal scales, the apparent stiffness is larger than experimental values, indicating that fast,
15 local motions of the molecular structure are dominant. To quantify the local structural
16 changes within the filament on the nanosecond scale and investigate the molecular
17 mechanisms, such as the binding of the actin-regulatory proteins to the filaments, it is
18 preferable to analyse the mechanical behaviour on the nanometre and nanosecond scales using
19 MD simulation.

20 **Keywords:** Actin filament, Extensional and torsional stiffness, Thermal fluctuation,
21 Molecular dynamics simulation, Computational biomechanics, Cell mechanics

1 **Introduction**

2 Several experimental studies have successfully estimated the mechanical properties of
3 actin filaments (Kishino and Yanagida, 1988; Janmey et al., 1990), the most abundant
4 component of cytoskeleton. They play critical mechanical roles in various cellular activities,
5 such as migration, cell division and shape control (Svitkina et al., 1997; Watanabe and
6 Mitchison, 2002; Pollard and Borisy, 2003; Pollard and Berro, 2009; Adachi et al., 2009).
7 Using direct nanomanipulation techniques for single actin filaments, the 1- μm -long
8 extensional stiffness was determined to be 0.0437 ± 0.0046 [N/m] (Kojima et al., 1994). In
9 addition, visualization of rotational beads attached to actin filaments (Tsuda et al., 1996) and
10 measurements of phosphorescence anisotropy (Prochniewicz et al., 2005) made it possible to
11 evaluate the torsional stiffness per unit length of the filament, which ranges from 2.3×10^{-27}
12 [N·m²] (Prochniewicz et al., 2005) to 8.0×10^{-26} [N·m²] (Tsuda et al., 1996).

13 In these experiments, the length of the measured filaments was on the micrometre scale,
14 and the temporal scales on which the dynamic motions were observed ranged from
15 microsecond to millisecond. However, from a more microscopic viewpoint, the filaments
16 have a double-helix structure on the nanometre scale with a period of ~ 650 Å (Holmes et al.,
17 1990; Oda et al., 2009). In the physiological environment, they are exposed to thermal
18 fluctuations yielding fast, local motions of the structure on temporal scales ranging from
19 picosecond to nanosecond. In other words, vibrating motions of atoms in the molecule lead to
20 local motions of the actin subunits, which then result in longitudinal and twisting motions of
21 the filament. These microscopic dynamics of the molecular structure are the basis of

1 determining the macroscopic mechanical properties of the filaments when they are observed
2 in the longer temporal scale; however, the temporal scale, which is long enough for capturing
3 the mechanical properties comparable with the macroscopic properties obtained in the
4 experiment, has not been investigated yet.

5 To analyse the mechanical behaviour of biomolecules at the molecular structural level,
6 numerical simulations based on the molecular dynamics (MD) method have been extensively
7 used and provided us with insights into several biologically relevant problems in various
8 biomolecular systems (Wriggers and Schulten, 1997, 1999; Lu et al., 1998; Isralewitz et al.,
9 2001; Vogel and Sheetz, 2006). For the biological filaments that build up biological materials,
10 MD simulations were also applied to obtain information about their biological and physical
11 properties (Buehler and Yung, 2009). For example, the mechanical properties of amyloid
12 fibrils associated with Alzheimer's disease have been analyzed (Paparcone et al., 2010). For
13 the cytoskeletal filaments, the bottom-up nanomechanical analysis has been performed from
14 the viewpoint of multi-scale mechanics. For example, this analysis was performed for the
15 human vimentin dimer and tetramer, which constitute the intermediate filaments (Qin et al.,
16 2009b, a).

17 For the actin monomers (Dalhaimer et al., 2008; Pfaendtner et al., 2009) and filaments
18 (Chu and Voth, 2006, 2005; Pfaendtner et al., 2010), MD simulations have successfully
19 analyzed their structural and dynamic properties. For example, the effect of the DB loop on
20 the nucleotide-binding cleft has been investigated (Pfaendtner et al., 2010; Chu and Voth,
21 2006, 2005). Using the MD method, we can quantitatively observe the structural dynamics of

1 the actin molecules and evaluate the stiffness of actin filaments based on their thermal
2 fluctuations.

3 In this study, we quantitatively evaluate the extensional and torsional stiffness of single
4 actin filaments on the nanosecond scale by analyzing the thermal fluctuations of the
5 molecular structure using the MD simulation, and compare the results with experimental data.
6 We construct a model of the half-turn structure of an actin filament in an ionic solvent and
7 analyse its longitudinal and twisting Brownian motions in equilibrium using MD simulation.
8 We then evaluate both the extensional and torsional stiffness of the actin filament from the
9 thermal fluctuations based on the principle of equipartition of energy.

10

11 **Methods**

12 *Simulation procedures*

13 The 2.0-Å resolution X-ray crystallographic structure of actin filament was obtained from
14 the Protein Data Bank (PDB code: 1MVW) (Holmes et al., 1990; Chen et al., 2002). From an
15 original structure derived from rabbit skeletal muscle with six myosins, an actin filament
16 structure consisting of 14 actin subunits was extracted as shown in Fig. 1. Then, ADP was
17 placed into the individual actin subunits in the filament, in which the coordinates of the ADP
18 were obtained from the ADP-bound monomer structure (PDB code: 1J6Z) (Otterbein et al.,
19 2001). The actin filament structure of dimensions ($97 \times 98 \times 423 \text{ \AA}^3$) was placed into a water
20 box of dimensions ($117 \times 118 \times 473 \text{ \AA}^3$), in which we utilized the TIP3P model for water
21 molecules (Jorgensen et al., 1983). To the water box, Na^+ and Cl^- counter ions were added to

1 reach a concentration of 30 mM. The total system consisted of 496,434 atoms; 80,836 actin
2 filament atoms, 415,212 water atoms, 236 Na⁺ ions and 150 Cl⁻ ions.

3 MD simulation was performed using NAMD 2.6 (Kale et al., 1999) with the
4 CHARMM27 force field for proteins (MacKerell et al., 1998). We used periodic boundary
5 conditions for simulations in which the van der Waals interactions were calculated with a
6 cutoff of 13 Å and the electrostatic interactions were calculated using the Particle Mesh
7 Ewald method (Darden et al., 1993). A multiple time-stepping algorithm (Schlick et al., 1999)
8 was used with a 2-fs step.

9 MD simulations were performed in an NPT ensemble (pressure = 1 atm, temperature =
10 310 K), in which the pressure was controlled by the hybrid Nosé-Hoover Langevin piston
11 method (Hoover, 1985; Martyna et al., 1992) and the temperature was controlled using
12 Langevin dynamics. After relaxing the water molecules for 200 ps keeping all the protein
13 atoms fixed, a free dynamics simulation for the entire system was performed for 12 ns to
14 obtain the equilibrium structure.

15 *Evaluation of stiffness*

16 Length of the actin filament structure, $L(t)$, is defined as

$$17 \quad L(t) = z_{\text{plus}}(t) - z_{\text{minus}}(t), \quad (1)$$

18 where $z_{\text{plus}}(t)$ is the position in the z -axis of the centre of mass of G-actins, G₁₃ and G₁₄, at the
19 plus-end, and $z_{\text{minus}}(t)$ is that of the G-actins, G₁ and G₂, at the minus-end, as shown in Fig. 2
20 (left).

21 Twist angle of the actin filament structure, $\Theta(t)$, is defined as

$$\Theta(t) = \cos^{-1} \left(\frac{\mathbf{n}_{\text{plus}}(t) \cdot \mathbf{n}_{\text{minus}}(t)}{|\mathbf{n}_{\text{plus}}(t)| |\mathbf{n}_{\text{minus}}(t)|} \right), \quad (2)$$

where

$$\mathbf{n}_{\text{minus}}(t) = \mathbf{P}_{G2}(t) - \mathbf{P}_{G1}(t), \quad (3)$$

$$\mathbf{n}_{\text{plus}}(t) = \mathbf{P}_{G14}(t) - \mathbf{P}_{G13}(t), \quad (4)$$

and $\mathbf{P}_{G_i}(t)$ is the position vector of the centre of mass of G-actin G_i projected onto the x - y plane, as shown in Fig. 2 (right). For the analysis, the filament length $L(t)$ and twist angle $\Theta(t)$ are determined by aligning the longitudinal axis of the filament along the z -direction in each frame.

Using the law of equipartition of energy, the extensional spring constant $k_{\text{ext}}^{\Delta t}(t)$ and torsional spring constant $k_{\text{tor}}^{\Delta t}(t)$ of the filament can be estimated from the variances of the filament length $L(t)$ and twist angle $\Theta(t)$ during the sampling-window duration Δt , as

$$\frac{1}{2} k_{\text{ext}}^{\Delta t}(t) \langle (L(t) - \langle L(t) \rangle_{\Delta t})^2 \rangle_{\Delta t} = \frac{1}{2} k_B T, \quad (5)$$

$$\frac{1}{2} k_{\text{tor}}^{\Delta t}(t) \langle (\Theta(t) - \langle \Theta(t) \rangle_{\Delta t})^2 \rangle_{\Delta t} = \frac{1}{2} k_B T, \quad (6)$$

where k_B is the Boltzmann constant, T is the absolute temperature and $\langle \rangle_{\Delta t}$ indicates the average over a time $\left(t - \frac{\Delta t}{2} \leq t < t + \frac{\Delta t}{2} \right)$. In this study, the spring constants $k_{\text{ext}}^{\Delta t}(t)$ and

$k_{\text{tor}}^{\Delta t}(t)$ of the filament for $\Delta t = 0.5, 1.0, 2.0, 4.0, 8.0$ and 16.0 ns were evaluated based on the MD simulations.

In this study, we evaluate the stiffness by using the conventional units, as used in previous experimental studies (Kojima et al., 1994; Prochniewicz et al., 2005). Extensional stiffness

1 has been calculated as the value per 1- μm length, and torsional stiffness as the value per unit
 2 length. From the spring constants $k_{\text{ext}}^{\Delta t}(t)$ and $k_{\text{tor}}^{\Delta t}(t)$, the 1- μm -long apparent extensional
 3 stiffness, $K_{\text{ext}}^{\Delta t}(t)$, is given by

$$4 \quad K_{\text{ext}}^{\Delta t}(t) = \frac{\langle L(t) \rangle_{\Delta t}}{1\mu\text{m}} k_{\text{ext}}^{\Delta t}(t), \quad (7)$$

5 and the apparent torsional stiffness, $K_{\text{tor}}^{\Delta t}(t)$, per unit length of filament is given by

$$6 \quad K_{\text{tor}}^{\Delta t}(t) = \langle L(t) \rangle_{\Delta t} k_{\text{tor}}^{\Delta t}(t). \quad (8)$$

7

8 **Results**

9 *Changes in filament length and twist angle*

10 For the 25-ns equilibrium simulation, we monitored the longitudinal and twisting thermal
 11 fluctuations of the actin filament, which are shown in Fig. 3. In this figure, the changes in
 12 filament length $L(t)$ and twist angle $\Theta(t)$ are plotted at intervals of 1 ps. The average value of
 13 the filament length $\langle L(t) \rangle_{25\text{ ns}}$ over the 25-ns period is 326.6 Å, and its standard deviation,
 14 $\sqrt{\langle \Delta L^2(t) \rangle_{12\text{ ns}}} = \sqrt{\langle (L(t) - \langle L(t) \rangle_{12\text{ ns}})^2 \rangle_{12\text{ ns}}}$, is 0.6 Å. Using these values, we estimate
 15 the average extensional strain of the filament driven by thermal energy to be ~0.2 %. Similarly,
 16 the average value of the twist angle $\langle \Theta(t) \rangle_{25\text{ ns}}$ over a 25-ns period is 182.2 deg, and its
 17 standard deviation, $\sqrt{\langle \Delta \Theta^2(t) \rangle_{25\text{ ns}}} = \sqrt{\langle (\Theta(t) - \langle \Theta(t) \rangle_{25\text{ ns}})^2 \rangle_{25\text{ ns}}}$, is 6.6 deg. Thus, we
 18 estimate the twist angle per unit length of the filament driven by thermal energy to be ~0.02
 19 deg/Å. These structural fluctuations driven by thermal energy characterize the stiffness of the
 20 actin filament and determine its mechanical behaviour.

1 *Changes in extensional and torsional stiffness*

2 Based on Eqs. (5) – (8), we estimated the extensional stiffness $K_{\text{ext}}^{\Delta t}(t)$ and the torsional
3 stiffness $K_{\text{tor}}^{\Delta t}(t)$ of the actin filament from the variances of the filament length and the twist
4 angle. Figures 4A and 4B show the change over time in both the variance of the filament
5 length $\langle \Delta L^2(t) \rangle_{\Delta t}$ and the extensional stiffness $K_{\text{ext}}^{\Delta t}(t)$, plotted at intervals of 1 ps, determined
6 for each sampling-window duration Δt (= 0.5, 1.0, 2.0, 4.0, 8.0 and 16.0 ns). For the longer
7 sampling-window durations Δt , the extensional stiffness tends to decrease with increasing
8 sampling-window duration Δt . Thus, on the shorter time scales, the filaments appear to exhibit
9 much larger stiffness than that measured experimentally. To show the dependence of the
10 apparent stiffness on the sampling-window duration Δt , the distribution of the extensional
11 stiffness $K_{\text{ext}}^{\Delta t}(t)$ is plotted against the sampling-window duration Δt , as shown in Fig. 4C.
12 Similarly, the dynamics of the variances of the twist angle $\langle \Delta \Theta^2(t) \rangle_{\Delta t}$, the torsional stiffness
13 $K_{\text{tor}}^{\Delta t}(t)$ and the distribution of torsional stiffness exhibit a tendency similar to that found for
14 the extensional stiffness, as shown in Fig. 5.

15 As plotted by solid circles in Figs. 4C and 5C, the average values of both extensional and
16 torsional stiffness gradually decrease with increasing sampling-window durations Δt and
17 exhibit a trend to converge close to values that are comparable to the experimental data
18 (Kojima et al., 1994; Prochniewicz et al., 2005) indicated by dashed lines. The increase in
19 apparent stiffness for shorter sampling-window durations is mainly due to a limitation in
20 capturing the structural fluctuations of the entire filament. In other words, the fluctuations of
21 the entire filament can be observed through the longer sampling-window durations, whereas

1 only instantaneous fluctuations of local parts such as the actin subunits and subdomains can
2 be observed through the shorter sampling-window durations, resulting in the larger apparent
3 stiffness. The extensional stiffness estimated for the longest sampling-window duration $\Delta t =$
4 16.0 [ns] in the MD simulation, $K_{\text{ext}}^{\Delta t=16.0\text{ns}} = 0.035 \pm 0.004$ [N/m], is in good agreement with
5 the experimentally measured values, $K_{\text{ext}}^{\text{exp}} = 0.0437 \pm 0.0046$ [N/m] (Kojima et al., 1994).
6 The torsional stiffness for $\Delta t = 16.0$ [ns], $K_{\text{tor}}^{\Delta t=16.0\text{ns}} = (1.1 \pm 0.1) \times 10^{-26}$ [N·m²/radian], is
7 comparable but slightly larger than the experimental value, $K_{\text{tor}}^{\text{exp}} = (0.23 \pm 0.10) \times 10^{-26}$
8 [N·m²/radian] (Prochniewicz et al., 2005), which was determined on the microsecond scale.
9 Consequently, these results suggest that by extrapolating the data obtained in the MD analysis
10 to longer time scale, we can adequately estimate the filament stiffness observable in the
11 experiments.

12 To investigate the effects of free ends of the filament, we extracted trajectory data from
13 the central part of the filament consisting of 10 actin subunits without the end monomers G₁,
14 G₂, G₁₃ and G₁₄, and evaluated the stiffness of the central part. As a result, although the
15 extensional and torsional stiffness of the central part exhibited slightly larger values than
16 those of the entire filament containing free ends, both stiffnesses are comparable to the
17 experimental values, as shown in Figs. 4C and 5C.

18

19 **Discussion**

20 In this study, we evaluated the extensional and torsional stiffnesses of the actin filament
21 on the basis of thermal fluctuations, in nanosecond scales. The results demonstrate that with

1 increasing analyzed sampling-window duration, the apparent stiffness evaluated on the
2 nanosecond scale gradually decreases and exhibits a trend to converge to a value that is close
3 to the experimental value. That is, we determined the length of a temporal scale sufficient for
4 capturing the mechanical properties comparable with the macroscopic properties evaluated in
5 the experiments.

6 The estimated extensional stiffness of the half-turn filament of $\sim 326.6 \text{ \AA}$ for the longest
7 sampling-window duration $\Delta t = 16.0 \text{ [ns]}$ is in good agreement with the experimentally
8 measured stiffness of the micrometre-long filaments. This result indicates that the half-turn
9 filament could be regarded as a basic unit whose extensional stiffness adequately represents
10 the stiffness of long filaments. The torsional stiffness of the half-turn filament is comparable
11 but slightly larger than the stiffness of the micrometre-long filaments. This indicates that the
12 sampling-window duration $\Delta t = 16.0 \text{ [ns]}$ is slightly shorter than that required for evaluating
13 the torsional stiffness of long filaments, in which it is necessary to extrapolate the data to a
14 longer time scale. These results may lead to state that the mechanical properties on the
15 macroscopic scale could be adequately captured over the different spatiotemporal scales by
16 examining the mechanical behaviour of the half-turn filament structure, indicating the validity
17 of thermal fluctuation analyses of the actin filament using MD simulations.

18 For shorter sampling-window durations ($\Delta t < 8.0 \text{ [ns]}$), the apparent extensional and
19 torsional stiffness of the filament are larger than the experimentally measured values,
20 indicating that, in the shorter durations, only a part of the filament fluctuations is observed.
21 That is, the shorter sampling-window duration cannot capture the mechanical properties of the

1 entire filament adequately. Despite this, the result obtained in the shorter duration will be
2 essential, for example, when we consider the binding process of actin-regulatory proteins to
3 filaments, because the structural changes in the filament during the binding process are local
4 events ranging from the amino-acid to subunit scales. These local structural changes in the
5 binding process are measured only on adequately short temporal scales. Therefore, to
6 investigate the binding process of actin-regulatory proteins to filament, it is important to
7 observe the local structural changes of actin filaments on the short temporal scales.

8 Some proteins binding to the filaments (Isenberg et al., 1980; Pollard and Cooper, 1986)
9 are known as important regulators that influence the global reorganization of the actin
10 cytoskeleton (Naruse and Sokabe, 1993; Sato et al., 2000; Neidlinger-Wilke et al., 2001; Sato
11 et al., 2005; Yamamoto et al., 2006) controlled by interactions among mechanical and
12 biochemical factors (Theriot and Mitchison, 1991; Arber et al., 1998; Hayakawa et al., 2008;
13 Okeyo et al., 2009). In the dynamic cytoskeletal reorganization process, the binding of
14 actin-regulatory proteins to the filaments causes molecular-level structural changes in the
15 double-helix structure. One of the important actin-regulatory proteins present in all eukaryotic
16 cells is cofilin, which is known as the actin depolymerizing factor. It binds along the length of
17 the filament, twisting a little more tightly (McGough et al., 1997), and increasing the torsional
18 flexibility (Prochniewicz et al., 2005). Therefore, the MD analysis allows us to measure the
19 local structural changes and elucidates the underlying molecular mechanisms of the binding
20 process.

21 We evaluated the stiffness by using the actin filament model obtained by Holmes et al.

1 (1990). Recently, Oda et al. (2009) have obtained the refined filamentous actin structure. The
2 Oda filament model has been constructed with a similar helical arrangement as the Holmes
3 model but with a different actin subunit structure. MD simulations of the actin filaments
4 conducted by Pfaendtner et al. (2010) on the basis of the structure models by Holmes (1990)
5 and Oda (2009) revealed that two different initial structures did not converge to the same
6 structure within the MD simulation time. This result implies that the initial structure is critical
7 for the biomolecular functions such as molecular interactions in the nucleotide binding cleft
8 (Pfaendtner et al., 2010). Contrary to this situation, macroscopic mechanical functions, such
9 as the persistence length of the filament, agrees well with the experimental values without a
10 significant difference between the Oda and Holmes filament models (Pfaendtner et al., 2010).
11 This result suggests that the structural difference between the two models may not result in a
12 significant difference in the macroscopic mechanical properties of the filaments such as
13 extensional and torsional stiffness estimated in this study on the basis of the MD simulation.
14 Importantly, Pfaendtner et al. (2010) revealed that the conformation of the DB loop is a more
15 significant determinant of the persistence length. Therefore, the effects of the DB loop on the
16 filament extensional and torsional stiffness need to be analyzed in detail for the next step.

17 To compare the mechanical properties of the actin filament evaluated in this study with
18 those of other biomolecules, Young's modulus of the actin filament was roughly estimated.
19 Assuming the filament to be a homogeneous rod with a circular cross section, the Young's
20 modulus is determined as $E = k_{\text{ext}}L/A$, where A is an equivalent cross-sectional area of the
21 filament. The equivalent cross-sectional area A is determined as $\sim 25 \text{ nm}^2$ from the average

1 contour of the filament cross section based on the filament atomic model (Holmes et al.,
2 1990), if the area in the contour is filled homogeneously (Kojima et al., 1994). In this study,
3 Young's modulus E is estimated as $1.4 \times 10^9 \text{ N/m}^2$, which is on the same order as other
4 proteins such as vimentin ($5.4 \times 10^8 \text{ N/m}^2$) (Qin et al., 2009b), tubulin ($2.2 \times 10^9 \text{ N/m}^2$) (Sept
5 and MacKintosh, 2010) and tropocollagen ($7.0 \times 10^9 \text{ N/m}^2$) (Uzel and Buehler, 2009).

6 In experiments to measure the stiffness, the actin filaments are usually slightly tensioned
7 (Kishino and Yanagida, 1988) and labelled with fluorescence markers conjugate with
8 phalloidin. Phalloidin is known as a structural stabilizer for actin filaments; therefore, its
9 binding may result in an increase in the filament stiffness (Chu and Voth, 2006). In contrast,
10 MD analyses evaluate the stiffness under ideal conditions without artifacts such as tension and
11 the binding of markers. Therefore, we expect that the method proposed in this study of
12 estimating the filament stiffness with MD analysis will be applied over a wide range to
13 evaluate other filamentous proteins. Furthermore, the steered MD simulations, which enable
14 us to control positions and velocities of some specified atoms by applying external steering
15 forces, provide useful information about the extensional and torsional behaviour of proteins
16 such as α -actinin rod (Golji et al., 2009), intermediate filaments (Qin et al., 2009b) and
17 collagen (Gautieri et al., 2008). Thus, by using the SMD method, we will be able to
18 quantitatively observe the structural dynamics of the actin molecules.

19 To understand the dynamic reorganization of actin filaments, various coarse-grained (CG)
20 modelling and simulation methods have been developed, such as the Brownian dynamics
21 model (Sept et al., 1999; Chu and Voth, 2005; Shimada et al., 2009), and continuum model,

1 (Ming et al., 2003; Yamaoka and Adachi, 2007, 2010). Based on CG simulations, we can
2 analyse the behaviour of actin filaments on larger spatiotemporal scales in comparison to the
3 MD analysis. For example, normal mode analysis (Tirion and Benavraham, 1993) was
4 applied to investigate the structural properties of the filaments combined with the substructure
5 synthesis method (Ming et al., 2003) applied for the actin filament. In CG and continuum
6 simulations, the stiffness in particular, is a fundamental parameter in filament dynamics,
7 because the mechanical behaviour of actin filaments and their interactions with actin
8 regulatory proteins strongly depend on the mechanical properties of the filament, such as
9 extensional and torsional stiffness. Therefore, MD analysis, by which the dynamics can be
10 evaluated over a wide range of time scales, gives us critical information for quantitative CG,
11 continuum modelling and simulation for multiscale analysis of actin filament dynamics. The
12 analysis for larger-scale structures in a longer temporal scale will provide challenging
13 opportunities for better understanding of the mechanical behaviour of the actin filaments as
14 they interact with the other filaments and binding proteins that produce network dynamics
15 with higher-order hierarchical structures. Therefore, large-scale simulations will provide
16 further insight into more complicated phenomena that cannot be easily approached by the
17 current experiments.

18

19 **Conflict of Interest**

20 None.

21

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8

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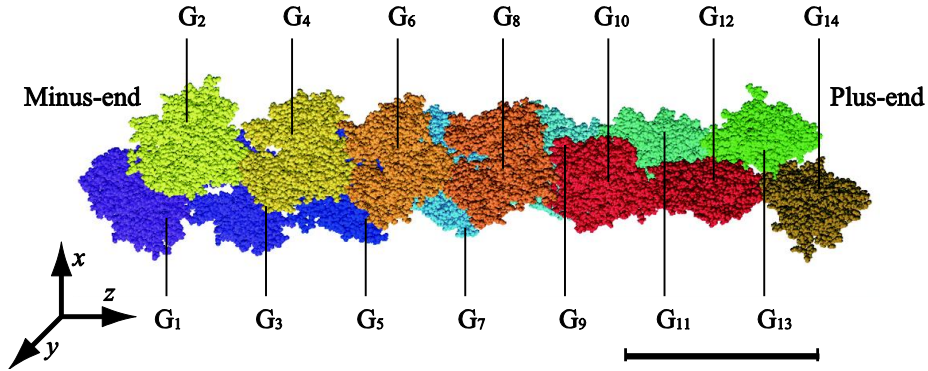
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1 **Figure Legends**

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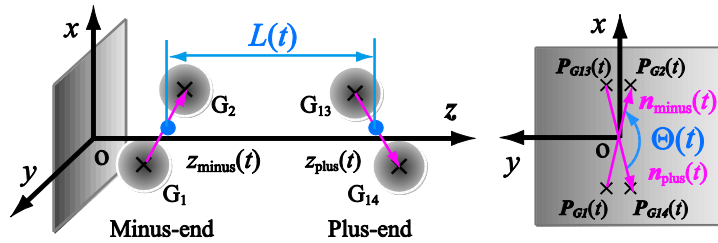
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5 **Figure 1:** Double-helix structure of an actin filament consisting of 14 actin subunits. The
 6 total protein size is 80,836 atoms. The Cartesian coordinate axis z is along the filament axis,
 7 and the orthogonal axes are x and y . Each actin subunit is numbered from the minus-end as G_1 ,
 8 G_2, \dots, G_{14} . The size of the scale bar is 100\AA .

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14 **Figure 2:** Definition of filament length and twist angle. The filament length $L(t)$ is defined
 15 by Eq. (1) as the distance between the plus- and minus-ends of the filament (left). The twist
 16 angle $\theta(t)$ is defined by Eq. (2) as the angle between the vectors of the plus- and minus-ends
 17 (right).

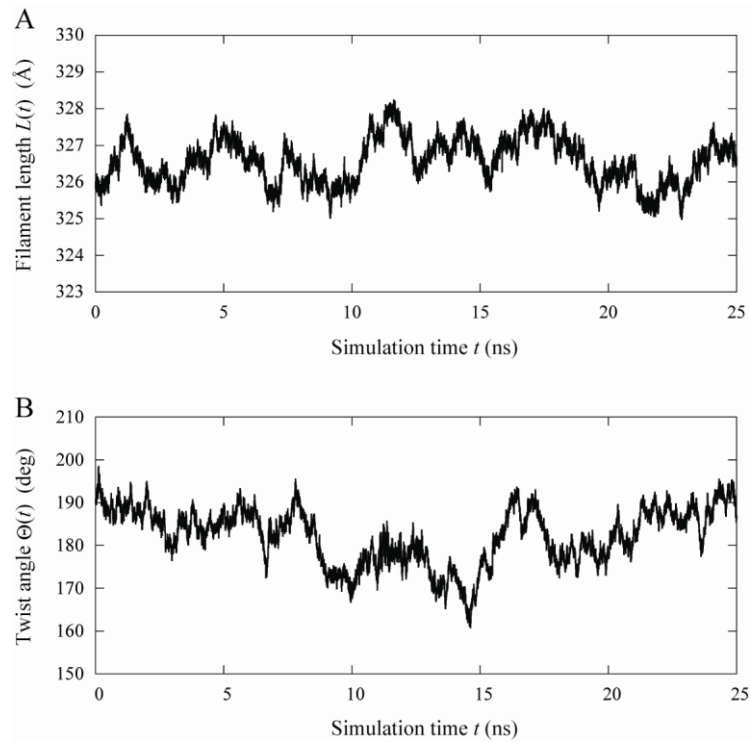
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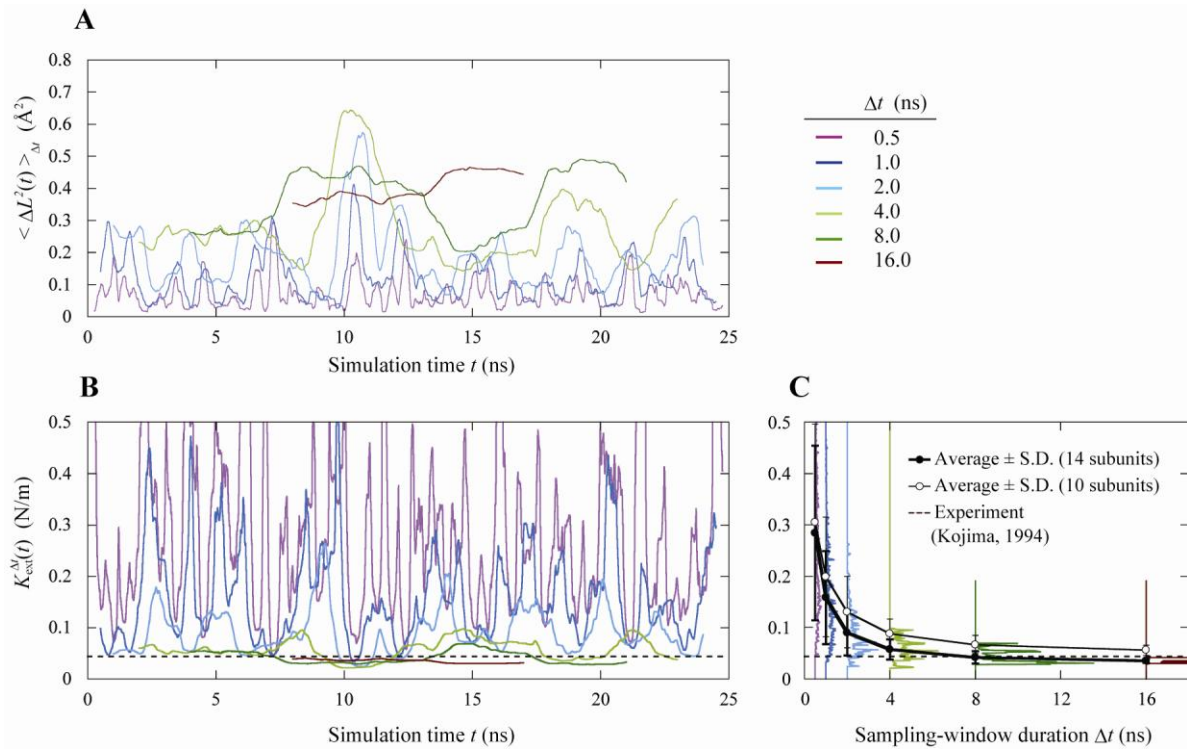
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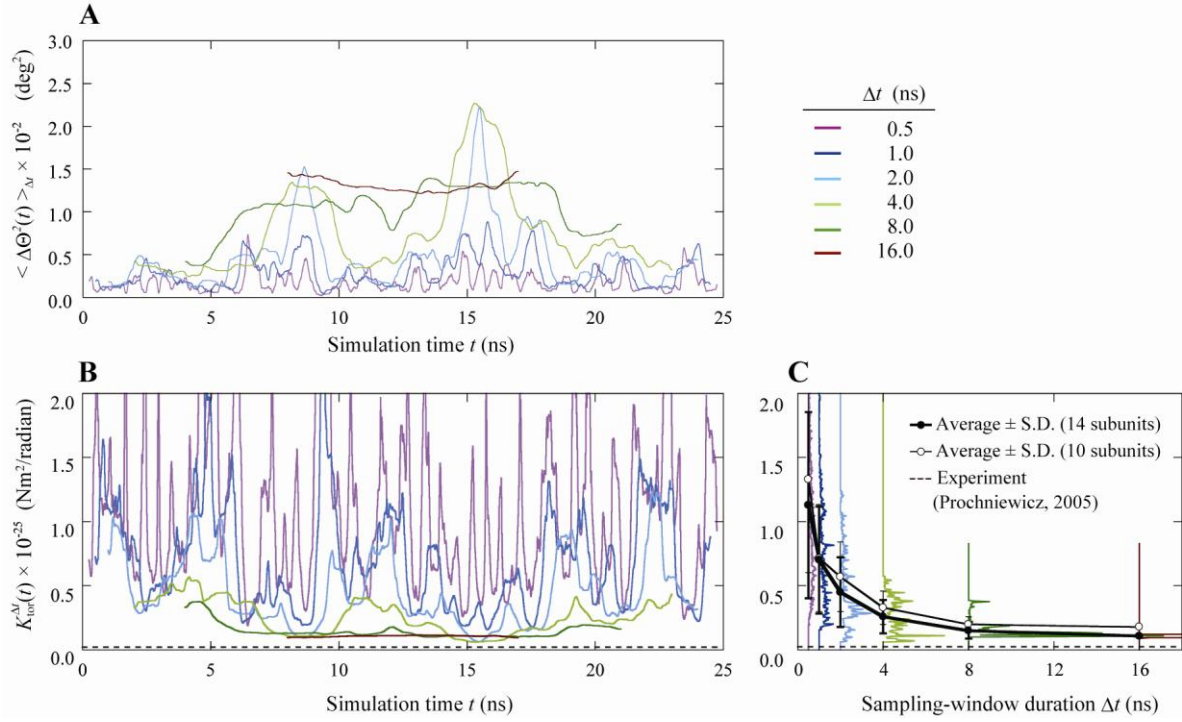
2 **Figure 3:** Structural changes during a 25-ns equilibrium subsequent to equilibrating
 3 simulation for 12 ns. (A) shows the change in filament length $L(t)$. The average filament
 4 length is 326.6 Å. (B) shows the change in twist angle $\Theta(t)$. The average twist angle is 182.2
 5 deg.

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Figure 4: Variance of filament length and extensional stiffness during equilibrium determined for each sampling-window duration Δt . The experimentally determined stiffness (Kojima et al., 1994) is represented by the dashed lines. (A) shows the change in variances of the filament length $\langle \Delta L^2(t) \rangle_{\Delta t}$. (B) shows the change in extensional stiffness $K_{\text{ext}}^{\Delta t}(t)$. (C) shows the distribution of extensional stiffness $K_{\text{ext}}^{\Delta t}(t)$.



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2 **Figure 5:** Variances of the twist angle and torsional stiffness during equilibrium determined

3 for each sampling-window duration Δt . The experimentally determined stiffness

4 (Prochniewicz et al., 2005) is represented by the dashed lines. (A) shows the change in the

5 variances of the twist angle $\langle \Delta\Theta^2(t) \rangle_{\Delta t}$. (B) shows the change in torsional stiffness $K_{\text{tor}}^{\Delta t}(t)$.

6 (C) shows the distribution of torsional stiffness $K_{\text{tor}}^{\Delta t}(t)$.