Published in final edited form as:

Arch Biochem Biophys. 2014 June 15; 0: 68–73. doi:10.1016/j.abb.2013.09.011.

The structural dynamics of α -tropomyosin on F-actin shape the overlap complex between adjacent tropomyosin molecules

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Abstract

Coiled-coil tropomyosin, localized on actin filaments in virtually all eukaryotic cells, serves as a gatekeeper regulating access of the motor protein myosin and other actin-binding proteins onto the thin filament surface. Tropomyosin's modular pseudo-repeating pattern of approximately 39 amino acid residues is designed to allow binding of the coiled-coil to successive actin subunits along thin filaments. Even though different tropomyosin isoforms contain varying numbers of repeat modules, the pseudo-repeat length, in all cases, matches that of a single actin subunit. Thus, the seven pseudo-repeats of 42 nm long muscle tropomyosin bind to seven successive actin subunits along thin filaments, while simultaneously bending into a super-helical conformation that is preshaped to the actin filament helix. In order to form a continuous cable on thin filaments that is free of gaps, adjacent tropomyosin molecules polymerize head-to-tail by means of a short (~ 9 residue) overlap. Several laboratories have engineered peptides to mimic the N- and C-terminal tropomyosin association and to characterize the overlap structure. All overlapping domains examined show a compact N-terminal coiled-coil inserting into a partially opened C-terminal partner, where the opposing coiled-coils at the overlap junction face each other at up to $\sim 90^\circ$ twist angles. Here, Molecular Dynamics (MD) simulations were carried out to determine constraints on the formation of the tropomyosin overlap complex and to assess the amount of twisting exhibited by full-length tropomyosin when bound to actin. With the exception of the last 20 to 40 C- and N-terminal residues, we find that the average tropomyosin structure closely resembles a “canonical” model proposed in the classic work of McLachlan and Stewart, displaying perfectly symmetrical supercoil geometry matching the F-actin helix with an integral number of coiled-coil turns, a coiled-coil helical pitch of 137 Å, a superhelical pitch of 770 Å, and no localized pseudo-rotation. Over the middle 70% of tropomyosin, the average twisting of the coiled-coil deviates only by 10° .

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Supplementary data: Supplementary data associated with this article can be found, in the online version, at <http://>

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from the canonical model and the torsional freedom is very small (std. dev. of 7°). This small degree of twisting cannot yield the orthogonal N- and C-termini configuration observed experimentally. In marked contrast, considerable coiled-coil unfolding, splaying and twisting at N- and C-terminal ends is observed, providing the conformational plasticity needed for head-to-tail nexus formation.

Keywords

actin; coiled-coil; molecular dynamics; thin filaments; tropomyosin

Introduction

Actin-containing thin filaments localize to diverse sub-cellular structures involved in a wide variety of specific cellular processes (reviewed in [1]). Actin filament function is often defined by interactions with one or another set of actin-binding proteins, and these interactions are gated by distinct tropomyosin isoforms restricting or promoting binding protein access to actin [1-8]. The best known and most thoroughly investigated thin filament gatekeeper is α -tropomyosin operating in skeletal and cardiac muscles, which, under the influence of troponin and Ca^{2+} , regulates myosin-head – actin interaction on thin filaments. As a result, tropomyosin gatekeeping controls the actin-myosin cross-bridge cycle and contraction (reviewed in [9]).

Coiled-coiled muscle tropomyosin is an elongated 42 nm long modular protein composed of 7 pseudo-repeating units designed to bind to 7 successive actin subunits along thin filaments (reviewed in [10]). Tropomyosin molecules polymerize head-to-tail to form a continuous super-helical cable that wraps around the actin filaments [10,11]. The unique form-fitting association of semi-rigid tropomyosin on actin, sometimes called Gestalt-binding [12], and the azimuthal shifting movements of the coiled-coil by up to 2.5 nm over the surface of actin are well suited for the protein's gatekeeper role in muscle thin filaments [13-18]. Indeed, at low Ca^{2+} concentration during muscle relaxation, semirigid tropomyosin is trapped by Ca^{2+} -free troponin in a “blocking” B-state position which limits myosin interaction on thin filaments by obstructing myosin-binding sites on actin subunits. In contrast, at high Ca^{2+} in activated muscles, azimuthal movement of tropomyosin induced by troponin and myosin exposes the myosin-binding patches, leading to myosin motor activity on actin [13-18]. Since the tropomyosin cable behaves as a mechanically continuous material exhibiting high persistence length [19,20], the azimuthally induced shifting is cooperative and propagates over 10 to 14 neighboring actin subunits with little or no decrement over the head-to-tail overlap structure [9,17,20].

The binding of single tropomyosin molecules to actin is very weak ($K_a \sim 3 \times 10^3 \text{ M}^{-1}$) [21]; hence, in the fully assembled thin filament, tropomyosin can be easily moved from one to another position on actin without a large energy cost, for example in striated muscle when perturbed by troponin or myosin interactions [12,22,23]. At the same time, the collective effect of tropomyosin head-to-tail polymerization raises the aggregate binding of the tropomyosin cable on actin to the micromolar range without compromising the local

azimuthal shifting of tropomyosin on actin [12,21]. Thus, head-to-tail polymerization promotes the global binding of tropomyosin on actin, while the azimuthal displacement of tropomyosin required for the gatekeeping function still operates at the local level because of the intrinsic low K_a at individual actin-tropomyosin contact sites. The overall importance of the tropomyosin end linkages in sustaining thin filament stability is highlighted by observations showing that chemical modification and/or mutations that diminish end-to end tropomyosin association result in a large diminution of affinity for actin [24-27].

The residues on the surface of tropomyosin that interact with actin are highly conserved [28-30]. In contrast, those on the ends of the tropomyosin molecule, particularly near the C-terminus, vary greatly among tropomyosin isoforms [28-30]. In the case of striated and smooth muscle tropomyosins, these isoform-specific differences manifest themselves in cable stiffness differences [20]. In somatic cells where about 40 tropomyosin sub-types are found, end-domains may also specify self-recognition motifs needed to segregate thin filament classes [31,32]. Structural studies indicate that the N-terminal residues of well studied striated muscle α -tropomyosin form coiled-coils consisting of two α -helices, like the rest of the molecule. However, C-terminal residues display non-canonical features that may destabilize the tip of the coiled-coil [10]. Indeed, studies on synthetic peptides suggest that opening of the C-terminus accommodates a more closed N-terminal end to form a stable four-coiled nexus [33,34]. To date, however, no high resolution structures are available for the head-to-tail connection of full length tropomyosin on actin; thus, whether or not this type of connection occurs in muscle remains unclear.

Generally, structural models used to describe the tropomyosin coiled-coil on F-actin are completely “canonical”; i.e., their seven pseudo-repeating actin-binding interfaces are built to align and bind to seven successive actin subunits with the same superhelical symmetry as the F-actin filament and without any non-coiled-coil interruptions [19,35-37]. Such a canonical model results in the overlapping of the last 9 or 10 residues at the N- and C-termini of adjacent tropomyosin molecules, with the overlapping coiled-coils oriented parallel to each other (Fig. 1, Supplementary Information - Movie 1). In other words, the twisting angle of one coiled-coil relative to the adjacent coiled-coil is equal to zero degrees. In contrast, solution NMR and crystal structures of N- and C-terminal overlap complexes formed from model peptides have coiled-coils that are not parallel, but instead show the N-terminal coiled-coil twisted $\sim 90^\circ$ (around an axis roughly parallel to the coiled-coil axis) relative to the coiled-coil of the C-terminus [33,34]. In attempting to understand this disparity, we analyzed the twisting behavior of tropomyosin when bound to F-actin, using Molecular Dynamics simulations. These simulations were carried out as performed previously (to evaluate the flexural oscillations of F-actin bound tropomyosin) [37], at 300° K in 150 mM NaCl and explicit solvent, starting from a canonical tropomyosin structure. This *in silico* approach yields information about local and global motions of tropomyosin on actin that is not easily obtained by experimental methods. It allows us to assess whether or not the torsional flexibility of tropomyosin is sufficient to produce the 90° head-to-tail configuration seen in the overlap structures assembled from synthetic tropomyosin peptides. We find that local coiled-coil unfolding and splaying, coupled with a localized propensity for N- and C-terminal twisting, appear to satisfy requirements for generating an orthogonally twisted

configuration at the head-to-tail nexus. Despite these symmetry-breaking features of the tropomyosin termini, the intervening stretches (representing 70% of tropomyosin's length) remain essentially canonical during MD and display insufficient torsional variance themselves to account for an appreciable end-to-end twisting of the molecule.

Results and Discussion

The canonical model of coiled-coil tropomyosin

The canonical tropomyosin structure used here as a reference structure for comparison was built by Lorenz et al. [36] and refined by Li et al. [19,37]. It takes on the symmetry of the actin helix at a 39 Å radius with successive tropomyosin pseudo-repeat segments in register and paired with consecutive actin subunits when bound along F-actin. The coiled-coil pseudo-rotation and the axial translation on F-actin were chosen to optimize electrostatic interactions with actin subunits and to be consistent with tropomyosin densities identified in EM reconstructions [37]. Its side chains are positioned as in respective crystal structures [19]. The coiled-coil pitch (called the z-repeat in [36]) of this canonical tropomyosin is 136.9 Å (Table 1), which yields an integral number of half-turns of the coiled-coil (in this case six) to span the ~410 Å distance on F-actin (i.e., tropomyosin's full contour length of 420 Å minus the 10 Å for the head-to-tail overlap segment [35,36]). The integral number of coiled-coil half-turns characterizing the canonical model implies that the relative twist angle between the coiled-coil of the C-terminus and the coiled-coil of the N-terminus is zero, and each terminal α -helix completely overlaps with the α -helix of the adjacent tropomyosin molecule (Fig. 1, Supplementary Information Movie 1). In this canonical tropomyosin, the twist at equivalent points along successive tropomyosin molecules in a growing polymer will be the same and “in phase” with respect to each thin filament actin-subunit. Thus, such a configuration allows the formation of identical contacts between tropomyosin pseudo-repeats with actin binding sites along the entire filament [35,36].

MD simulations of F-actin-bound tropomyosin

Since in the current study a single tropomyosin on F-actin was simulated, it can be considered to mimic the behavior of the first tropomyosin molecule bound to an F-actin filament, prior to head-to-tail polymerization with neighboring tropomyosins. MD simulations are most meaningful if successive snapshots (i.e. the trajectory) converge, meaning that the relevant conformational space has been explored. Here, the translational, flexural and twisting parameters of tropomyosin on F-actin all were within relatively constant limits about an average value following the initial 4 ns of the simulation. The variance of each of these parameters then remained relatively constant over the rest of the 30 ns MD simulation. Inspection of the RMSD values of tropomyosin conformations during the MD run shows that deviations from the mean conformation are small (~2.5 Å), demonstrating this convergence.

The average structure of F-actin-bound tropomyosin during MD

The actin-bound tropomyosin retained its superhelical shape at every time point during the simulation. This is different from the behavior of unbound tropomyosin, which was shown to adopt a superhelical-like shape but only on average [9,37]. When the corresponding

average structure of actin-bound tropomyosin is built (from all the frames of the MD), the value of the coiled-coil pitch is 138 Å (Table 1), close to the 134 Å of an experimentally determined tropomyosin structure obtained from high resolution cryo-EM of actin-tropomyosin [38]. In the latter, atomic structures of various tropomyosin fragments were flexibly fit into 8 Å resolution reconstructions. These values are also close to the coiled-coil pitch of the canonical tropomyosin (137 Å) and to that of the standard leucine zipper coiled-coil (134.5 Å) [39] (see Table 1).

The super-helical path displayed by the central five pseudo-repeats of the average MD structure, thus, superimpose very well onto the canonical model, as do the individual corresponding α -helices (Fig. 2). These results are consistent with previous observations that the modular repeats of tropomyosin are patterned to link to binding sites on successive actin subunits [23].

Despite the canonical symmetry displayed by the mid-piece of tropomyosin during MD on F-actin, significant deviations from this symmetry are observed over the last 30 to 40 residues at the C- and N-terminal ends of the molecule. Along these stretches, the average MD structure is seen to progressively twist and gradually bend away from the canonical superhelix (Fig. 2). There are no obvious hot-spots observed during MD akin to a universal joint pin-pointing large scale localized twisting or untwisting of the coiled-coil or isolated kinks to account for the coiled-coil bending. Relative to the canonical model, the average MD structure gradually twists counterclockwise by about 30° at the C-terminus and clockwise by 20° one at the N-terminus (viewing tropomyosin with its N-terminus pointing up, and actin with the filament pointed end facing up). The corresponding bending at the N-terminus (~20°) is oriented toward the extreme outer domain of actin, while at the C-terminus bending (~20°) is in the opposite direction toward the inner domain of actin and also away from the actin surface by 8 to 10 Å. In addition, an average of 4 but up to 8 of the C-terminal α -helical residues unfold, as occurs in the NMR structure of the C-terminus [40].

The average rise per amino acid along each coiled-coil α -helix during MD (1.51 Å) is the same as that measured for the canonical tropomyosin model and the 3D-EM structure [38], as is the coiled-coil rise (1.477 Å vs. 1.474 Å). Thus, the total contour lengths of tropomyosin in the MD, in the canonical reference and in the EM structure are all the same. In fact, a distribution histogram shows that there is little variance in tropomyosin length during the MD (Supplementary Item Fig. 1a), indicating that tropomyosin is not extensible when it is on F-actin. The potential of mean force (PMF, i.e., the free energy potential) derived from this histogram can be fitted well by a harmonic function (see Supplementary Item Fig.1b). This allows calculation of the corresponding elongation force constant k_{elong} (0.973 kcal/mol/Å², equal to 67.6 pN/Å). This longitudinal stiffness of tropomyosin on F-actin means that there is little variability likely in the 10 Å length of the head-to-tail overlap (± 1 Å), if no local unfolding of the α -helices occurs.

Coiled-coil twisting of tropomyosin bound to F-actin

With the exception of the molecule's ends, the coiled-coil twist of tropomyosin on average during MD is consistent with a near canonical conformation. However, transient and local twisting (or untwisting) at successive points along tropomyosin may be in the same direction

and therefore cumulative, or instead may cancel out. Therefore, local and overall coiled-coil twisting was determined by using the program TWISTER, in which the determination of the coiled-coil twist is insensitive to confounding effects due to coiled-coil bending [41]. The angular twist between each tropomyosin residue and its neighbors for each snapshot during MD was compared to the 3.91° twist per residue of the canonical model. The difference between these two values gives the magnitude of tropomyosin twisting motion away from the canonical reference model at any point along tropomyosin and at any time during MD, and these values can be summed to determine the cumulative twist over any length segment (Table 2, Fig. 3). Note that the average value for residues over the entire tropomyosin mid-piece (residues 41-240) is almost identical during MD to that of the static canonical model (-3.86° vs. -3.91° , see Table 2), meaning that the average twisting is comparable. In contrast, the values for N- and C-terminal fragments differ and are lower in magnitude (-3.41° , -3.09°), indicating a localized untwisting relative to the canonical model. This untwisting of the two termini leads to a cumulative end-to-end difference in twist of 56° for the MD structure relative to the canonical model (Fig. 3a), dominated by the localized untwisting of 18° (Fig. 3b) and 26° (Fig. 3d) at N- and C-terminal segments, with only a small contribution from the tropomyosin mid-piece (Fig. 3c). Note that end-to-end deviations can reach as much as $\sim 94^\circ$. The deviations from the canonical twist over the last 40 amino acids at C- and N-termini presumably reflects a relaxation from the constraints imposed by actin-binding at the molecule's end residues. Untwisting along the 200 residue mid-piece of tropomyosin was 10° , i.e. about six times smaller per unit length than that at the ends. The distribution histogram and corresponding PMF plot for this mid-segment are shown in Supplementary Figs. 1c and 1d. Using a harmonic fit, the twisting force constant k_{twist} is $0.015 \text{ kcal/mol/degree}^2$ for the middle segment. This torsional stiffness of tropomyosin means that there is little variability likely in the middle stretch of tropomyosin ($\pm 7 \text{ \AA}$) and that tropomyosin cannot easily twist when bound to F-actin.

Coiled-coil splaying

A crystal structure of the thirty C-terminal tropomyosin residues was previously observed to present coiled-coil splaying beginning near residue 262 [42]. The splaying is possibly promoted by non-canonical core residues (Glu263 and Tyr267) along the tropomyosin hydrophobic stripe at “d” and “a” positions usually occupied by leucine, isoleucine or valine residues [42]. Dramatic splaying, however, is not obvious during the MD, in which coiled-coiled features are maintained (Table 3), except (as mentioned above) for the tendency for the last 4 to 8 C-terminal residues to unfold. Note that the large non-canonical amino acids Glu263 and Tyr267 are associated with a local widening of the coil-coil radius, which largely persists over the C-terminal 40 residues measured. The modestly larger coiled-coil radius at the C-terminus over that in the rest of the molecule (Table 3) suggests a possible bias towards C-terminal splaying, when intermolecular contacts are made. Given the increased torsional freedom at the ends of tropomyosin (see above) coupled with potential helical unfolding and coiled-coil splaying at the C-terminus, it is likely that a population of conformers is well disposed to form a 4-stranded intermolecular nexus.

Terminal domain geometry is special

Despite the perfect 7:1 stoichiometry of actin and tropomyosin, each tropomyosin molecule actually interacts with eight actin monomers along filaments because tropomyosin is “half-staggered” relative to successive actin subunits [36]. Instead of starting at the very beginning of the barbed end of actin₁ and then stopping at the tip of pointed end of actin₇, C- and N-terminal tropomyosin domains span roughly one half of an actin subunit each (i.e. tropomyosin covers 6 successive full actin subunits and 2 half subunits). Thus, during filament assembly, the ends of tropomyosin may enjoy greater freedom to undergo torsional and flexural movement than the rest of the molecule, where binding interactions with actin subunits may be restrictive.

The conformational plasticity at tropomyosin C- and N-terminal ends, that is needed to promote head-to-tail interactions, appears to be an intrinsic property of the tropomyosin coiled-coil ends [10,11]. Remarkably, even experimentally truncated tropomyosins make non-native head-to-tail (and tail-to-tail) intermolecular contacts [28,43]. In fact, C-terminal sequence diversity among tropomyosin isoforms suggests that phylogenetically preserved sequence specificity is not a hallmark of the overlap structure [28-31].

Conclusions – All’s well that ends well

Our *in silico* analysis of tropomyosin conformational behavior shows that the canonical symmetry proposed by McLachlan and Stewart [35] and Lorenz et al. [36] is an accurate depiction of tropomyosin bound onto actin filaments. Hence, successive tropomyosin molecules and their constituent pseudo-repeat modules will be “in phase” relative to the underlying actin subunit periodicity, facilitating formation of periodic contacts between the pseudo-repeats and corresponding actin subunits. Our study also shows that torsional flexibility along the entire tropomyosin length does not appear to be an obligatory mechanical feature of the tropomyosin coiled-coil needed for end-to-end tropomyosin contact formation. Rather, we find that tropomyosin coiled-coil untwisting together with coiled-coil splaying and unraveling is associated with the “loose” C- and N-terminal ends. This can account for any number of head-to-tail orientations, thus facilitating intermolecular four-chain nexus formation.

That tropomyosin is seen to be canonical over its central region when bound to F-actin is completely consistent with tropomyosin form-fitting onto the F-actin helix with a 59 Å pseudo-repeat length ($\sim 410 \text{ Å} / 7 \text{ pseudo-repeats} = 58.6 \text{ Å}$) matching the pitch (58.7 Å) of the actin fundamental helix [44]. Conspicuously, the pitch of the canonically modeled coiled-coil and the pitch of other actin-bound tropomyosin structures are all comparable (134 to 138 Å), while pitch values characterizing MD and crystal structures of isolated tropomyosin are greater (147 to 149 Å, see Table 1) [42,45,46]. This implies that a modest conformational readjustment follows tropomyosin binding to actin filaments. It follows that the 7 Å resolution Whitby-Phillips [45] solution for full-length tropomyosin (exhibiting a 147 Å coiled-coil pitch) is unlikely to be the best suited tropomyosin structure for modeling interactions with actin, as is sometimes suggested [33,47]. In fact, the Whitby-Phillips model does not display a super-helical geometry matching F-actin (see comparison with the

canonical model in Supplementary Item Movies 2, 3), unlike that of canonical MD and cryo-EM solutions.

Each tropomyosin molecule makes about 30 electrostatic contacts with the surface of F-actin, but none are present over the C-terminal 25 residues [23,37]. In our view this makes perfect sense, since strong interactions in this region would impede the local coiled-coil untwisting, chain-chain splaying, and uncoiling that are necessary for head-to-tail junction formation. Accordingly, we envision that assembly of the tropomyosin cable occurs in the following steps:

1. Those conformers of tropomyosin (with an initial pitch reaching ~ 148 Å) that most closely resemble the super-helical contours of F-actin bind to thin filaments.
2. Tropomyosin conformation once the molecule is bound on F-actin is then characterized by a canonical coiled-coil pitch of 137 Å, so that all tropomyosin pseudo-repeats align to successive actin subunits appropriately.
3. The local twist of the tropomyosin C-terminus is not constrained by actin-binding and it rotates and splays open to envelop an N-terminus of a neighboring molecule to form a four-helix bundle.
4. The process is repeated and polymerization ensues until a tropomyosin cable extends over the full length of the actin filament.

Whether or not the tropomyosin cable is initiated from one or more starting points along F-actin and how polymerization then ensues without leaving gaps along the thin filament is unclear. An exceedingly rare nucleation event may be required to initiate tropomyosin binding or polymerization, which is followed by very rapid elongation. Alternatively, tropomyosin cable formation may be a stochastic process where some growing tropomyosin polymeric chains displace others. In any case, the ends appear to justify the means.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by grants from the NIH (R37-HL036153 to W.L. and P01-HL086655 to W.L. and Kathleen G. Morgan). We thank Dr. Stefan Raunser for providing coordinates structures in reference 38.

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Manuscript Highlights

Molecular Dynamics simulations describe tropomyosin binding on actin.

During MD, tropomyosin largely matches a canonical coiled-coil model conformation.

Tropomyosin's ends twist and bend during MD, thus deviating from the canonical model.

C- and N-terminal torsional freedom allows tropomyosin head-tail nexus formation.

Figure 1.

Head-to-tail overlap of canonical tropomyosin. (a) two adjacent canonical tropomyosin coiled-coils with overlapping C- and N-termini (indicated by arrow). The N-termini and C-termini of the tropomyosin molecules are facing up and down, resp. In (b) the canonical models are fitted to a model of F-actin as in Li et al. [19,37] with actin subunits colored in green and blue on one helical actin strand, and in ochre and tan on the other; the pointed end of the F-actin filament is facing up. (c,d) enlargements of (a,b) showing the overlapping C- and N-termini. (e) actin-tropomyosin displayed in (d) now viewed end-on (from actin's barbed end with only the top surface of the actin illustrated) showing that coiled-coils in the overlap are completely parallel to each other. Figures rendered with the program CHIMERA [48].

Figure 2.

Differences between the canonical and MD structures of tropomyosin. (a) Tropomyosin (brown ribbon) representing the average MD structure of actin-tropomyosin fitted to a low resolution map of F-actin (yellow, from reference [22], actin pointed end facing up). The canonical model of tropomyosin (magenta) was fitted to the MD structure of tropomyosin using the CHIMERA MatchMaker program [48]. (b,c) enlargement of (a) showing respective the N-terminal (b) and the C-terminal (c) ends of the tropomyosin chains. Note that the coil-coils of the MD and canonical structures match extremely well except at the N- and C-terminal ends where they diverge from each other because of bending and twisting of the MD coiled-coil.

Figure 3.

Twisting of the tropomyosin coiled-coil during MD. The difference between the twist angle of tropomyosin measured during MD and the corresponding value for the canonical model was calculated for various segments: (a) bulk tropomyosin (residues 4 to 274), (b) N-terminus (residues 4 to 40), (c) the mid-piece of tropomyosin (residues 41 to 240), (d) C-terminus (residues 241-274). Measurements were made using the program TWISTER [41] for the particular coiled-coil segment. Note: y-axes cover different ranges.

Table 1
Coiled-coil pitch of isolated and actin-bound tropomyosin

Tropomyosin Structure Measured	Coiled-coil Pitch (Å)
Canonical model (McLachlan & Stewart, 1976)	137
Canonical model (Li <i>et al.</i> , 2010)	137
Isolated tropomyosin	
Average MD structure (Li <i>et al.</i> , 2010)	149
7 Å resolution crystal structure (Whitby & Phillips, 2000; PDB ID code 1C1G)	147
2.3 Å res. “midpiece” crystal structure (Brown <i>et al.</i> , 2005; PDB ID code 2B9C)	146
Actin-bound tropomyosin	
Average MD structure (on F-actin)*	138
Cryo-EM structure (on S1-decorated F-actin) (Behrmann <i>et al.</i> , 2012)	134
Leucine zipper	
GCN4-p1 (Lupas & Gruber, 2005; PDB ID code 2ZTA)	134.5

Coiled-coil pitch was determined using the program TWISTER [42].

* calculated for the tropomyosin mid-piece (residues 40 to 200); the value for the entire structure, including frayed ends is 140 Å.

Table 2

Coiled-coil twist.

Residues spanning the segment measured	Average twist per residue ($^{\circ}$) ¹	Cumulative segmental deviation MD vs. canonical model ($^{\circ}$) ²	Range of segmental deviations ($^{\circ}$) ³
4-274	-3.70	55.6 ± 12	16 – 94
4-40	-3.41	18.4 ± 8.1	-6 – 46
41-240	-3.86	10.5 ± 7.4	-13 – 36
241-274	-3.09	26.7 ± 8.7	-3 – 56
Canonical model	-3.91	0	0

¹The program TWISTER [42] was used to determine the coiled-coil twist along tropomyosin for each MD snapshot and these values were averaged for each residue.

²Torsional deviations of tropomyosin segments between the MD structure and the canonical model. Shown are the average values and their standard deviations.

³Minimum and maximum values observed during the MD for that segment.

Measurements were not made outside of residues 4 and 274 to avoid end effects.

Table 3
Coiled-coil radius of tropomyosin segments during MD

Residues of tropomyosin	Heptad position	Average coiled-coil radius (Å)
Glu 263	<i>d</i>	4.99 ± 0.16
Tyr 267	<i>a</i>	4.94 ± 0.18
11 - 274	<i>d</i>	4.70 ± 0.14
11 - 274	<i>a</i>	4.70 ± 0.14
11 - 232	<i>d</i>	4.68 ± 0.14
11 - 232	<i>a</i>	4.68 ± 0.14
11 - 46	<i>d</i>	4.60 ± 0.13
11 - 46	<i>a</i>	4.61 ± 0.14
235 - 274	<i>d</i>	4.83 ± 0.15
235 - 274	<i>a</i>	4.80 ± 0.19

The program TWISTER was used to determine the coiled-coil radius along tropomyosin for each MD snapshot, and these values for each particular residue or group of residues were averaged over time. Measurements were not made over the terminal 10 residues to avoid any end effects.