

Structural implications of conserved aspartate residues located in tropomyosin's coiled-coil core

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Polar residues lying between adjacent α -helical chains of coiled-coils often contribute to coiled-coil curvature and flexibility, while more typical core hydrophobic residues anneal the chains together. In tropomyosins, ranging from smooth and skeletal muscle to cytoplasmic isoforms, a highly conserved Asp at residue 137 places negative charges within the tropomyosin coiled-coil core in a position which may affect the conformation needed for tropomyosin binding and regulatory movements on actin. Proteolytic susceptibility suggested that substituting a canonical Leu for the naturally occurring Asp at residue 137 increases inter-chain rigidity by stabilizing the tropomyosin coiled-coil. Using molecular dynamics, we now directly assess changes in coiled-coil curvature and flexibility caused by such mutants. Although the coiled-coil flexibility is modestly diminished near the residue 137 mutation site, as expected, a delocalized increase in flexibility along the overall coiled-coil is observed. Even though the average shape of the D137L tropomyosin is straighter than that of wild-type tropomyosin, it is still capable of binding actin due to this increase in flexibility. We conclude that the conserved, non-canonical Asp-137 destabilizes the local structure resulting in a local flexible region in the middle of tropomyosin that normally is important for tropomyosin steady-state equilibrium position on actin.

Introduction

Tropomyosin, the α -helical coiled-coil actin binding protein, associates superhelically along F-actin and polymerizes via end-to-end interactions to form a continuous cable-like strand. Tropomyosin mechanically stabilizes the thin filament and increases its flexural rigidity.¹ In non-muscle cells, tropomyosin also regulates the interaction of many actin binding proteins, while in muscle, tropomyosin specifically controls the cooperative binding of myosin with actin, and hence motor activity.

The α -helical coiled-coil is a common structural motif found in a wide array of structural proteins such as keratin, myosin and tropomyosin. Typically the coiled-coil is defined by the heptad amino acid repeat sequence (*abcdefg*)_n. A 3.5 residue periodicity along the supercoil axis aligns *a* and *d* position hydrophobic residues in the adjacent α -helix, thus stabilizing the interaction interface,^{2,3} while residues at the *e* and *g* positions are typically oppositely charged and flank the hydrophobic core, forming salt bridges between apposed helical chains. Right-handed α -helices in coiled-coils wrap around each other to form a quaternary structure with function related to the specific 'design' of their coiled-coil domains.⁴ In tropomyosin the superhelical coiled-coil matches the contours of the F-actin helix.^{5,6}

Interestingly, the canonical hydrophobic *a/d* seam along the heptad repeat of tropomyosin is disrupted by a highly conserved

substitution placing an aspartic acid in the *d* position at residue 137 where typically a hydrophobic residue would reside. Biochemical studies have shown a decrease in proteolytic susceptibility when the Asp at 137 was replaced with a canonical Leu (D137L). A decrease in proteolytic susceptibility may result from this stabilization of the coiled-coil interaction and potentially a reduction in the flexibility of the tropomyosin molecule.⁷

Here we used Molecular Dynamic (MD) simulations to directly evaluate the structural consequences of the D137L mutation on coiled-coil structure, curvature, and flexibility. MD predicts that introduction of the leucine residue reduces both curvature and flexibility near the site of the mutation, which results in a straighter molecule. Although the overall structure of the molecule is disrupted, we find that tropomyosin containing the D137L mutation spends a fraction of time in a structure similar to the one required for tropomyosin to be able to wrap natively around F-actin, thus explaining the ability of the mutant tropomyosin to still bind to actin.⁷

Results

Biochemical studies previously indicated that mutation of the conserved Asp at position 137 to Leu resulted in a decrease in proteolytic susceptibility, suggesting a region existed in the core of the tropomyosin molecule that is accessible to enzymatic cleavage.⁷ To

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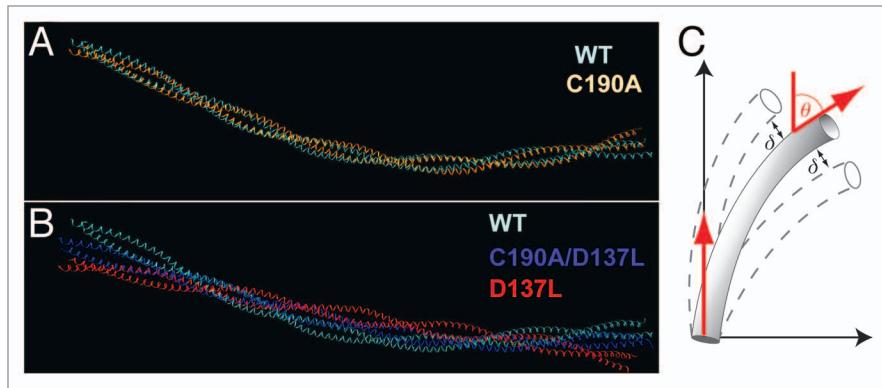


Figure 1. Average tropomyosin conformations from MD simulations. (A) Average structures for the wild-type tropomyosin sequence (light blue) and the control C190A mutant sequence (gold). Note the overall curvature of the C190A tropomyosins matches closely to wild-type tropomyosin, which closely matches the long-pitch helical repeat of actin.⁸ (B) Average structures for the D137L (red) and D137L/C190A (blue) mutant tropomyosin sequences. Overall, the average structure of the mutant tropomyosin deviates from wild-type and control average conformations resulting in a straighter molecule. (C) Tropomyosin curvature is assessed via the mean end-to-end bending angle, θ , which is calculated by comparing the tangent angles of the central coiled-coil axis of the N and C-terminal 15 residues (red arrows). Since tropomyosin is a curved molecule, flexibility is determined by examining δ , the angular deflection of the rod from its averaged curved shape.

Table 1. Tropomyosin curvature and flexibility.

	Global Properties		Local Properties	
	End-to-end angle θ (°)	Flexibility δ (°)	Curvature θ (°/Å)	Flexibility δ (°)
Wild-type	36.8	24.3	0.26 ± 0.03	4.49 ± 0.26
C190A	36.1	28.6	0.22 ± 0.02	4.50 ± 0.30
D137L	2.63	32.7	0.09 ± 0.04**	4.09 ± 0.38**
C190A/D137L	24.75	44.9	0.07 ± 0.02**	4.13 ± 0.39*

End-to-end angle, θ , and flexibility, δ , for each tropomyosin are reported (Fig. 1C). Local curvature and flexibility for the region surrounding the D137L represent the mean \pm S.D. for residues 131-143, * p < 0.05, ** p < 0.01 compared to wild-type.

assess the structural importance of tropomyosin residue 137, we studied both the single mutation, D137L, as well as the double mutant, D137L/C190A, using MD simulations. D137L/C190A and C190A tropomyosin were chosen for study to be consistent with previous *in vitro* work,⁷ which utilized the C190A mutations to avoid potential complications due to possible disulfide cross-linking at residue Cys 190. The effects of the Leu substitution were compared with those of both C190A mutants and a wild-type control, which retains the native Cys at position 190. We find that substitution of Cys at position 190 with Ala has only minor delocalized effects on the overall structure of the tropomyosin. However, substitution of Asp 137 with Leu resulted in a generalized straightening of the molecule, which is stiffer in the region surrounding the mutation but globally more flexible.

Average Structure

MD simulations were begun by using mutant tropomyosin reference structures in the superhelical coiled-coil conformation, thus

adopting the tropomyosin shape first proposed by Lorenz and Holmes⁵ and confirmed by Li et al.⁸ The supercoil fits to the long-pitch F-actin helix, as it is presumed to be when bound to muscle thin filaments.⁶ During the simulation, the coiled-coiled structures of the wild-type, D137L/C190A, D137L, and C190A tropomyosins remained completely intact during their respective 30 ns runs (the double mutant was run twice for 30 ns with no apparent distinctions between runs). For example, no unfolding of the α -helices, lengthwise separation of the two adjoining helices, or fraying at their ends was observed.

The bending of tropomyosin observed during the simulation always was gradual and continuous, without signs of localized kinking or jointed areas (Fig. 1A, Supplementary Movies), in agreement with rotary shadowed electron microscopic images of purified tropomyosin molecules lying on mica.⁹ Although there are no distinct kinks or pivot points observed in the mutant molecule, the overall tropomyosin shape is altered by the mutations. While the end-end bending angle, θ , of the wild-type and C190A tropomyosin molecules agree with that of the F-actin bound model, the D137L mutant tropomyosin molecules were observed to be less bent (Table 1; Fig. 1A and B): The mean end-to-end bending angle during MD for the C190A/D137L double mutant and the D137L single mutant (24.75° and 2.63°, respectively), is considerably lower than that of C190A control and wild-type tropomyosin (36.1° and 36.8°, respectively). The difference in end-to-end bending angle gives the impression that the mutant molecules are much less flexible than the wild-type and C190A controls. However, the end-to-end bending angle also provides a measure of tropomyosin shape and thus the reduction likely reflects an overall straightening of the mutant molecule.

Since tropomyosin is a curved molecule, the proper gauge of tropomyosin flexibility is the average deviation from the average structure, δ , during the simulation (Fig. 1C). As can be seen in Table 1, both the C190A and the D137L single mutations modestly increased flexibility (δ = 28.6° and δ = 32.7° for C190A and D137L respectively). The increased flexibility conferred by the two mutations appears to be more than additive with the double mutant yielding a much increased flexibility, δ = 44.9° (Table 1). Therefore the smaller θ reflects an overall straightening of the molecule since the flexibility increases.

Anisotropic Bending

As we have previously shown,⁸ wild-type tropomyosin does not uniformly sample all bending directions. Instead, as can be seen

in Figure 2 where the C-terminal position of tropomyosin is tracked during MD (while the N-terminus orientation is fixed at the origin) the wild-type tropomyosin molecule bends anisotropically and primarily samples positions in quadrants 3 and 4 (in the left planar slices). Thus while the wild-type tropomyosin molecule flexes it always comes back to the starting molecule conformation, i.e., where the superhelical shape of isolated tropomyosin matches its superhelical binding shape on F-actin. In contrast, the D137L and D137L/C190A mutants spend less time near the Lorenz-Holmes conformation (Square, Fig. 2) and are on average straighter (Fig. 1). However, they sample a larger amount of conformational space and are thus more flexible (Table 1). Importantly, the D137L mutants are able to occasionally visit the shape of the Lorenz-Holmes conformation (Fig. 2C and 2D).

Local Structure

As shown previously, the greatest degree of local curvature is observed in the middle portion of wild-type tropomyosin near residue 137; i.e., there is a modest local bend in the surrounds of this residue.⁸ We therefore assessed the effects of the D137L mutation on the local area proximal to the mutation (Fig. 3). Namely, we examined the local curvature of the averaged structures (a probe of intrinsic bending) and the local deviations from the average structures (a probe of local flexibility). As can be seen in Figure 3A, there is a high degree of local curvature in the residues immediately preceding Asp 137 for the wild-type tropomyosin. This curvature was diminished in the mutant tropomyosins, indicating that the effects of substitution in the tropomyosin sequence can be both short- and long-ranged. Here, substitution of Asp with Leu at position 137 caused an overall reduction in curvature in the surrounding region (Fig. 3B; Table 1). While the local curvature decreases near the site of the mutation (Fig. 3B) other regions also behave differently from the wild-type sequence (Fig. 3A). Some regions displayed an increase in curvature while others displayed a decrease resulting in an average structure that is less curved than wild-type (Fig. 1).

Local flexibility, namely the variance in curvature, is also affected by mutation of residue 137 to Leu. Changes were observed throughout the length of the molecule (Fig. 3C), suggesting some long-range effects of the mutation on supercoiling. In the region immediately surrounding the 137 mutation site MD predicts that the coiled-coil flexibility would be modestly diminished (Fig. 3D; Table 1).

The effects of the Leu substitution on the packing of the coiled-coil can be seen in Figure 4. In wild-type tropomyosin containing Asp 137, a “cavity” is introduced between the tropomyosin helices as a result of the electrostatic repulsion between the two side chains. The typical “knobs-into-holes” packing expected with a canonical hydrophobic side chain is noted after

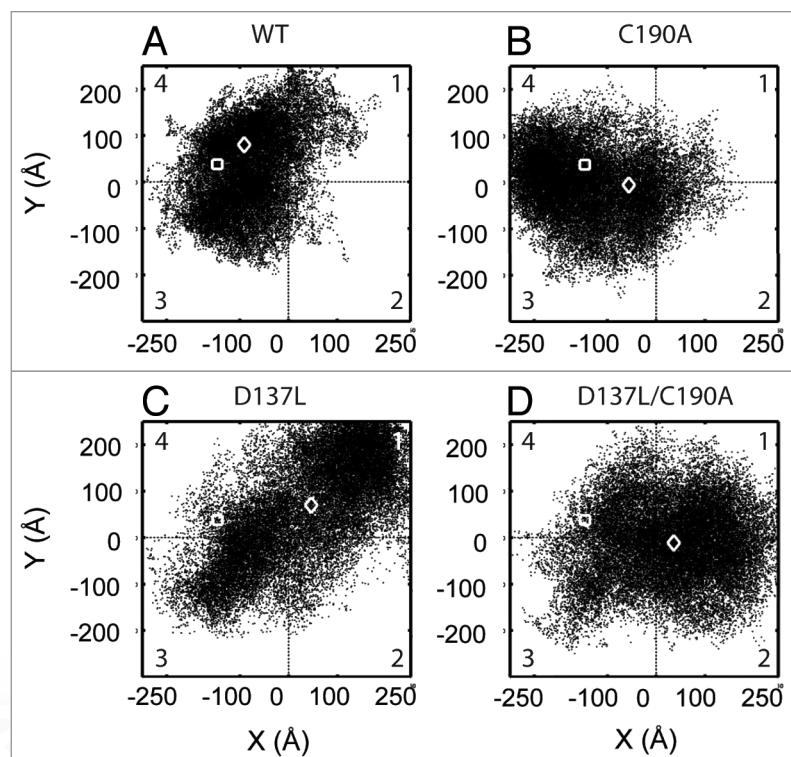


Figure 2. C-terminal variation plots. (A–D) x,y coordinates of the tropomyosin C-terminus are plotted for each frame of the simulation after superposing the first 15 residues at the N-terminus, orienting them along the z-axis (out of page), and aligning them at the origin. Each point represents the projection of the position of the C-terminal residue for each conformer observed during the MD simulation. The average C-terminal position of the MD structures for each tropomyosin is indicated in their respective panels (open diamond) for comparison with that of the Lorenz-Holmes model (open square). Wild-type (A) and C190A (B) tropomyosins display the characteristic anisotropic bending with the C-terminus frequently in quadrants 3 and 4. On the other hand the straighter D137L (C) and D137L/C190A (D) spend much more time in quadrants 1 and 2.

substitution with the leucines (Fig. 4B). The Leu side chains at position 137 within the hydrophobic core result in a straighter molecule in the region surrounding the mutation (Fig. 4C), in accord with the straight shape of a canonical coiled-coil.

Discussion

A charged aspartic acid at residue 137 is a highly conserved substitution in the *d* position of the canonical coiled-coil hydrophobic core of tropomyosin. The substitution stems from the constitutive exon 4 and is found in both low molecular weight and high molecular weight tropomyosins.¹⁰ Here we used molecular dynamics simulations to characterize the structural consequences of this unique Asp insertion in tropomyosins. The principal finding of this study is that Asp at position 137 is critically important for maintaining an average tropomyosin structure that matches the superhelical shape of its actin binding site. Substitution of Asp137 with a more typical Leu hydrophobic residue causes an overall straightening and a local stiffening of the region surrounding the mutation. This could be thought to reduce the ability of

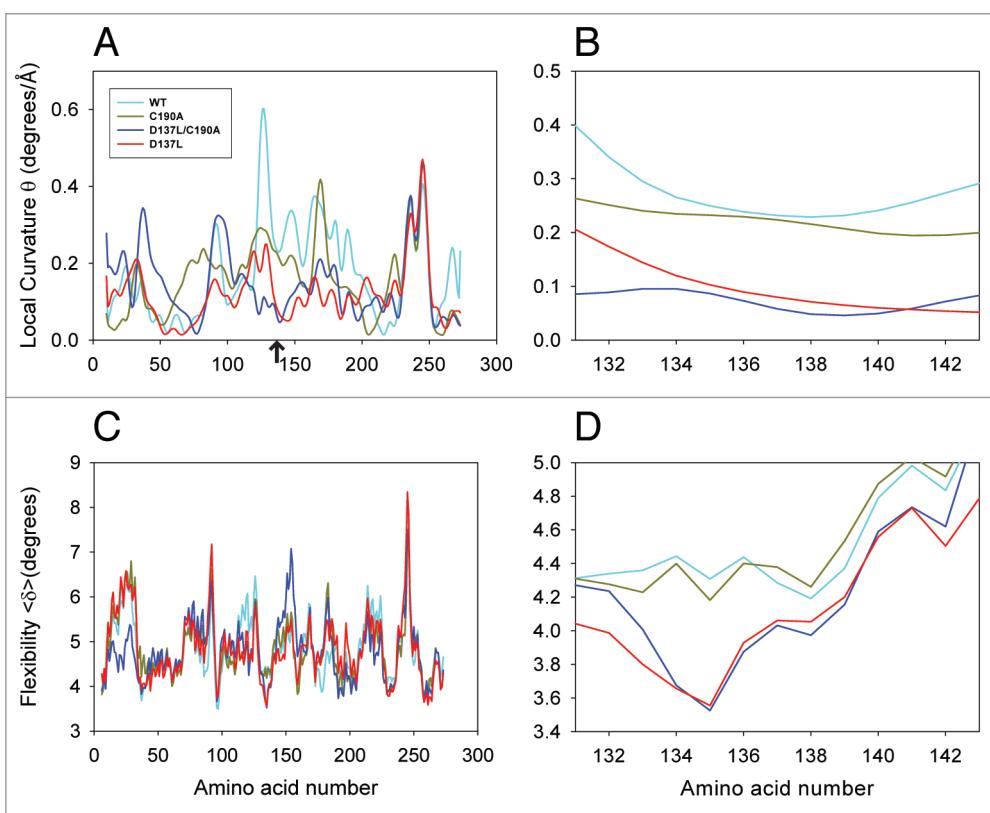


Figure 3. Local changes in tropomyosin curvature and flexibility induced by the D137L mutation. (A and B) The local curvature of the average structure. Local flexibility (C and D) is determined as the time average of the local deviations from the average structure. Local curvature and flexibility for the region immediately surrounding residue 137 (arrow) are shown in panels b and d respectively. Wild type is shown in cyan, while the C190A control, C190A/D137L and D137L mutant tropomyosins are shown in gold, blue and red respectively.

the D137L mutant to bind to actin. However, the overall flexibility of the D137L mutants increases significantly compared with the wild-type tropomyosin. The mutant molecules still can spend a fraction of time in a conformation near to that competent for actin binding, hence explaining the experimentally shown ability of the mutant molecules to retain binding affinity for actin.⁷

In close proximity to Asp137, Glu139 and Glu142 are among the few amino acids on tropomyosin involved in binding to basic amino acids on F-actin.^{11,12} Thus the role of these negatively charged residues differ: Glu 139 and Glu 142 are responsible for fine tuning the binding precision of tropomyosin to actin while the curvature conferred by Asp 137 facilitates the overall binding association of tropomyosin and F-actin.¹³ Numerous mutations in tropomyosin have been reported to be associated with various cardiomyopathies and other muscle disorders. We note that none of these are at residue 137, highlighting the essential nature of this conserved feature of the protein's architecture; mutation at Asp137 presumably would be lethal. Moreover, some degree of conformational flexibility at this site might be needed to allow accurate movement of tropomyosin on actin between regulatory states of the thin filament.

The region surrounding Asp 137 has been shown to be particularly susceptible to proteolysis. Arg 133, has been shown

to be a preferential site for trypsin cleavage,¹⁴ and V8 protease cleaves at Glu-131.¹⁵ Substitution of Asp 137 with Leu reduces proteolytic susceptibility suggesting that the D137 could destabilize or otherwise expose the surrounding region of tropomyosin. Since the proteolytic susceptibility is related to the fraction of time that the molecule is in the cleavable state and the susceptibility to proteolysis is diminished with the Leu substitution, it was proposed that Asp 137 is responsible for conformational fluctuation in this region. This is consistent with the reduced "cavity" at position 137 observed when comparing the wild-type and the mutant MD simulations (Fig. 4A and B).

In the myosin rod domain, introduction of an aspartic acid residue into the hydrophobic core at the α position of the coiled-coil produced dramatic localized bending points that correlated with the site of the mutation.¹⁶ To a degree our observation of modestly increased flexibility in tropomyosin with Asp-137 inserting into the d -position of

the hydrophobic ridge parallels this observation; however, the present MD simulations indicate a gradual curvature with no distinct pivot points or kinks for both mutant and wild-type tropomyosin molecules (Fig. 1; Movies 1–3). The distinct structural geometry¹⁷ and the amino acid selection at the α and d positions of coiled-coils¹⁸ likely accounts for distinct effects of substitution with a charged side chain at the respective positions in the two proteins.

Several other non-canonical residues are present in the tropomyosin heptad repeat. For example, Gly 126 in the g position might perturb coiled-coil structure by disrupting α -helical structure and inter-chain salt bridges. Recently it was shown that, similar to the Leu substitution at Asp 137, mutation of Gly 126 to Ala or Arg also dramatically reduced proteolytic susceptibility at Arg-133 in both smooth and skeletal muscle tropomyosin,¹⁹ suggesting that both D137 and G126 destabilize the middle region of the tropomyosin molecule, consistent with the decreased stiffness seen in the region between residues 130 and 140 of wild-type tropomyosin (Fig. 3).

When bound to actin, the D137L tropomyosin significantly increases the ATPase activity of myosin in the presence of thin filaments at maximal calcium activation.⁷ This effect can be explained by considering the consequences of the three state

model of thin filament regulation. Tropomyosin molecules are known to undergo regulatory movement azimuthally over the surface of the actin filament. In the so-called “blocked” position, tropomyosin lies over successive actin subunits on their outer domains while in the “closed” position, tropomyosin lies on the actin inner domain near the junction of the inner and outer domains.²⁰ Myosin binding results in a further movement to the “open” position. Movement between these three discrete positions on actin affect myosin binding, actomyosin ATPase, and actin filament velocity. In the absence of calcium, myosin-binding sites on actin are covered by tropomyosin constrained in the blocking position. The transition from the blocked state to the closed state is regulated by Ca^{2+} binding to troponin while full activation requires the myosin induced movement of tropomyosin from the closed position to the open state of the thin filament. This latter component is cooperative in nature and results in the rapid switching on of muscle during activation. The lack of effect on calcium sensitivity⁷ suggests that the structural alterations caused by the mutation do not affect the blocked-open equilibrium. Instead the increased activity observed for the mutation could be explained by a shift in either the open-closed equilibrium or the cooperative unit size. It seems reasonable that the introduction of a flexible region near Asp 137 in a molecule less well designed to match the F-actin helical contour could affect the ability of tropomyosin position switching to be propagated along the thin filament and decrease cooperative unit size. In contrast, the D137L mutation may disrupt the open-closed equilibrium via an altered interaction with troponin. Consistent with this notion, recent experiments that show cross-linking of tropomyosin residue 146 to the C-terminus of troponin I²¹ indicate that a direct interaction between tropomyosin and troponin I may modulate tropomyosin position. Alterations in the interaction of the D137L mutant tropomyosin with the C-terminus of troponin I could result in a perturbation of the tropomyosin with an equilibration toward an open or near-open state as has been proposed from related studies on the effect of a troponin I truncation mutant.²²

Conclusions

Tropomyosins possess a conserved Asp at amino acid position 137. Here MD simulations indicate that substitution of Asp 137 with a hydrophobic residue in the canonical *d* position of the tropomyosin coiled-coil decreases overall curvature, yet increases flexibility. The strong conservation of Asp 137 in tropomyosin isoforms suggests that this non-canonical residue, is required for proper actin binding and modulation of tropomyosin equilibrium position, and consequently is necessary for fine-tuning physiological responses.

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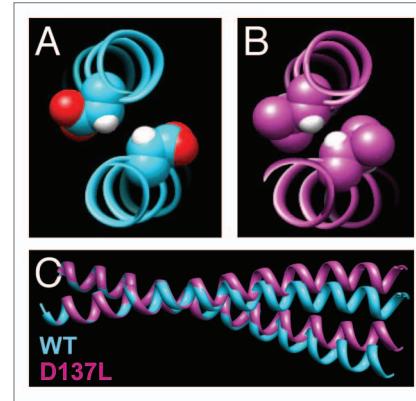


Figure 4. Comparing the local packing around position 137 (A) Packing of Asp 137 at the *d*-position residue (B) Packing of Leu 137 at the *d*-position. Note that the Leu side chains of the D137L mutant stably pack into the hydrophobic core of the α -helical coiled-coil while the Asp side chains are pushed outwards. The average structure of the region surrounding Asp 137 (residues 131–142) for the wild-type and D137L mutant tropomyosin (C) show that the D137L substitution yields a straighter molecule.

Materials and Methods

Molecular Dynamics: Molecular Dynamics simulations were run on homology models of tropomyosin “single mutants” (D137L or C190A) and a D137L/C190A “double mutant.” The models were constructed to otherwise match the structure of a previously determined atomic model of $\alpha\alpha$ -cardiac tropomyosin;⁸ in each case, the Asp137 and/or the Cys190 on each chain of the $\alpha\alpha$ -cardiac tropomyosin template was replaced with a corresponding Leu for Asp or Ala for Cys. The starting structure was first energy optimized and MD run for 25 to 50 ns at 300° K with Langevin dynamics and an implicit solvent model using the program CHARMM c33b2^{23,24} as described in reference 8. Previously developed methods^{8,25} to determine tropomyosin local curvature and flexibility along the length of tropomyosin were implemented.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Note

Supplemental material can be found at:
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