Supplemental Information for

HOW SEQUENCE DIRECTS BENDING IN TROPOMYOSIN AND OTHER TWO-STRANDED ALPHA-HELICAL COILED COILS

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running title: Direction of Bends in Alpha-Helical Coiled Coils

Supplemental Figure Legends

Supplemental Figure S1 Legend: The magnitude of bends of the coiled-coil axis in many locations of tropomyosin is relatively variable between different crystal environments (see main text). Scatter plot shown here is similar to that of figure 2 in the main text (see legend) except x-axis here shows the magnitude of the bends. The overall sample standard deviation for the magnitudes of all the bends in this plot is 1.52°. The composite sample standard deviation, based on the 23 bends from eight locations that show bending in two or more crystal environments (see main text), is 0.88°, yielding a variability index of 0.58. Excluding the two largest outliers, the composite sample standard deviation is 0.75°, yielding a variability index of 0.49. (See main text for definitions.)

Supplemental Figure S2 Legend: The correlation of change in axial stagger with the magnitude of bends (especially for those directed nearly within the local plane of the dimer) in parallel homodimeric alpha-helical coiled coils suggests that change in axial stagger (such as a junction between axially staggered and in-register regions) promotes specific local bends. Both parameters are in Å. (See figure 3 and other sections of main text for definitions and for results on direction of bending.)

Supplemental Figure S3 Legend: The correlation of gap volume [1] with the magnitude of bends in parallel homodimeric alpha-helical coiled coils suggests that poorly packed cores promote local bending. Magnitude of bending is in degrees and gap volume is in $Å^3$. (See figure 4 and other

sections of main text for definitions and for results on direction of bending.) Note that the occurrence of poorly packed cores in the regions of bends appear to be consistent with an analysis of these structures with SOCKET [2]: only 42 of the 117 (15-residue-long) structures centered about the bends have the expected minimum of two pairs of well-packed type 4 knobs-into-holes interactions.

Supplemental Figure S4 Legend: Nearly all large bends (>6.5° in magnitude) in parallel homodimeric alpha-helical coiled coils are directed closer to out of the local dimeric plane than to within the local dimeric plane. (See main text)

Supplemental Tables

Supplemental Table S1: Geometry of Bends of the Coiled-Coil Axis: Conventions Used

Fully-signed Value of Direction Of Bend Angle (°)	Direction of Bending Relative to an Observer	Direction of Bending Relative to the Local Dimeric Plane
0	left	in-plane towards chain1
+45	left/up	
+90	up	out-of-plane
+135	right/up	
+180 /-180	right	in-plane towards chain2
-135	right/down	
-90	down	out-of plane
-45	left/down	

Supplemental Table S1 legend: The descriptions in the "Direction of Bending Relative to an Observer" column (left, right, up, down) are based on the observer looking from the dimer axis at residue i-n (typically i-7) towards the dimer axis at residue i while the molecule is oriented so that the chain1 axis at residue i is directly to the left of the dimer axis. Refer to Figure 1 in the main text.

Supplemental Table S2 : List of Tropomyosin Structures examined

Pdb code	Chains	Residues included	Reference
1ic2	AB	10-68	[3]
1ic2	CD	10-68	[3]
2b9c	AB	107-200	[4]
2d3e	AB	184-259	[5]
1kql	AB	none	[6]
2z5h	AB,CD,EF,GH	261-272	[7]
2z5i	AB,CD	261-272	[7]
2efs	AB	183-266	[8]
2efs	CD	183-266	[8]
2efr	AB	183-266	[8]
2efr	CD	183-266	[8]

Supplemental Table S3 : List of Non-tropomyosin Alpha-Helical Coiled Coils Examined

PDB i.d.	Chains	Residues Scanned
Leucine zippers		
2zta, 2ahp	AB	1-31
1zik, 1zil	AB	1-30
1pyI	AB	76-94
1zme	CD	72-98
1hwt	CD, GH	107-128
2hap	CD	107-128
1qp9	AB, CD	107-128
1gd2	EF, GH	101-139
1gu4 , 1gtw, 1h8A ,1h88 ,1hjb	AB	296-332
1hjb	DE	296-332
1io4	AB	296-331
1nwq	AC	307-340
2c9l, 2c9n	YZ	197-222
Myosin Rod		
1nkn	AB, CD	846-917
3bas	AB	840-917
3bat	AB	843-917

3bat	CD	840-917
2fxm	AB	850-961
2fxo	AB, CD	843-961
Other Coiled Coils		
1dkg	AB	39-87
1d7m	AB	243-343
1deb	AB	5-44
1joc	AB	1289-1341
1no4	AB, CD	37-71
1noh	AB, CD	37-71
1uix	AB	979-1044
1s1c	XY	950-1013
1x79	BC	555-585
1tu3	FG, HI	806-834
1T6F	AB	1-37
1uii	AB	94-150
2gzd	CD	468-487
2d7c	CD	716-741
2hv8	EF	716-741
2ocy	AB	14-162

Supplemental References

1 Reynolds C, Damerell D, Jones S. (2009). ProtorP: a protein-protein interaction analysis server. Bioinformatics 25:413-4.

2 Walshaw J, Woolfson DN. (2001). Socket: a program for identifying and analysing coiled-coil motifs within protein structures. J Mol Biol 307:1427-50.

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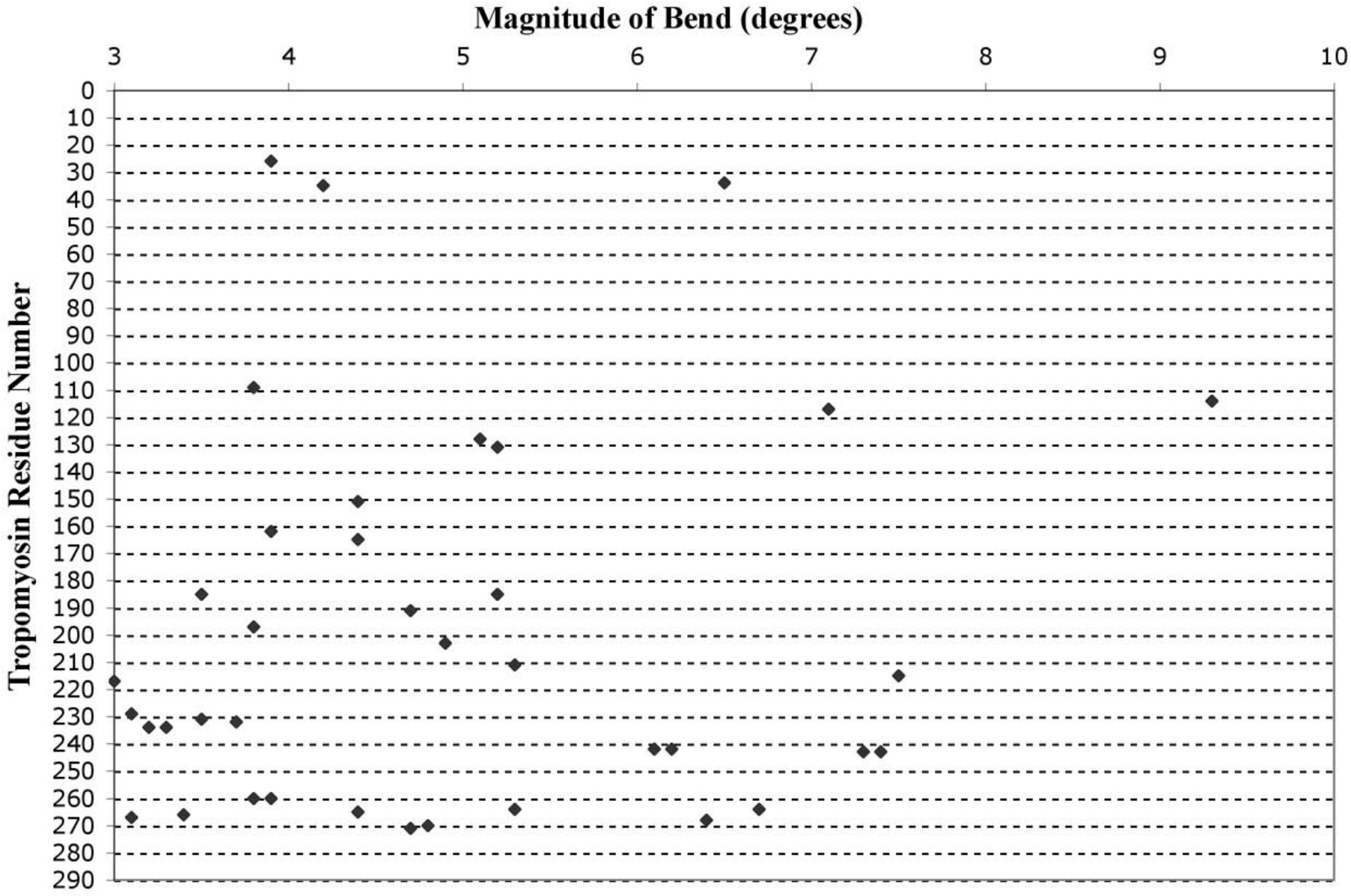
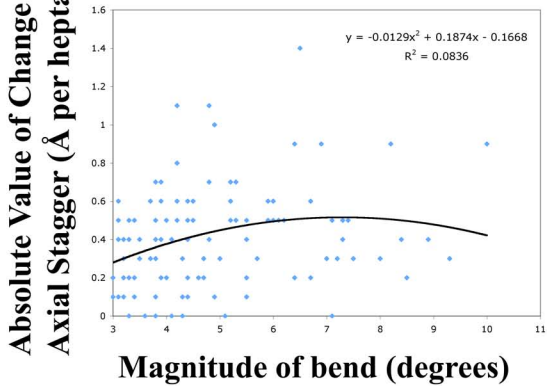
5 Nitanai Y, Minakata S, Maeda K, Oda N, Maeda Y. (2007). Crystal structures of tropomyosin: flexible coiled-coil. Adv Exp Med Biol 592:137-51.

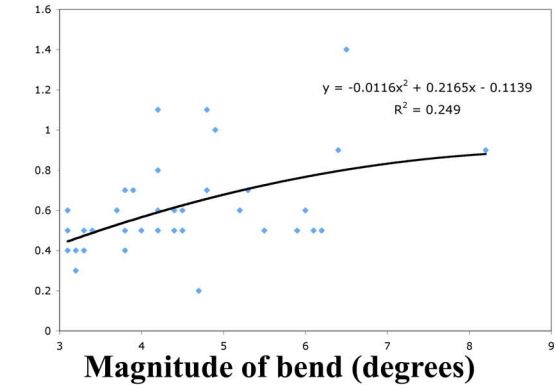
6 Li Y, Mui S, Brown JH, Strand J, Reshetnikova L, Tobacman LS, Cohen C. (2002). The crystal structure of the C-terminal fragment of striated-muscle alpha-tropomyosin reveals a key troponin T recognition site. Proc. Natl Acad. Sci. USA 99:7378-83.

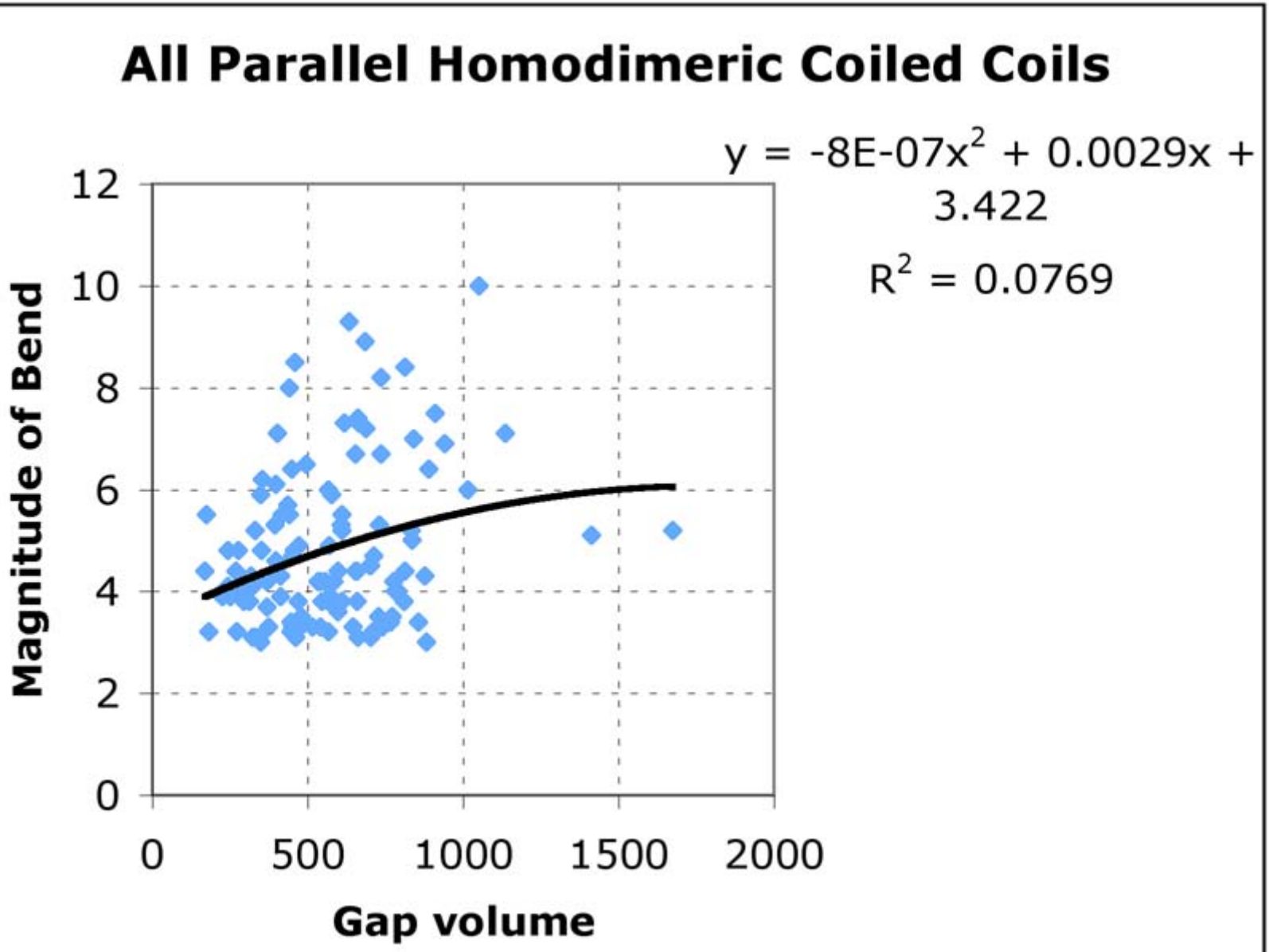
7 Murakami K, Stewart M, Nozawa K, Tomii K, Kudou N, Igarashi N, Shirakihara Y, Wakatsuki S, Yasunaga T, Wakabayashi T. (2008). Structural basis for tropomyosin overlap in thin (actin) filaments and the generation of a molecular swivel by troponin-T. Proc Natl Acad Sci U S A 105:7200-5.

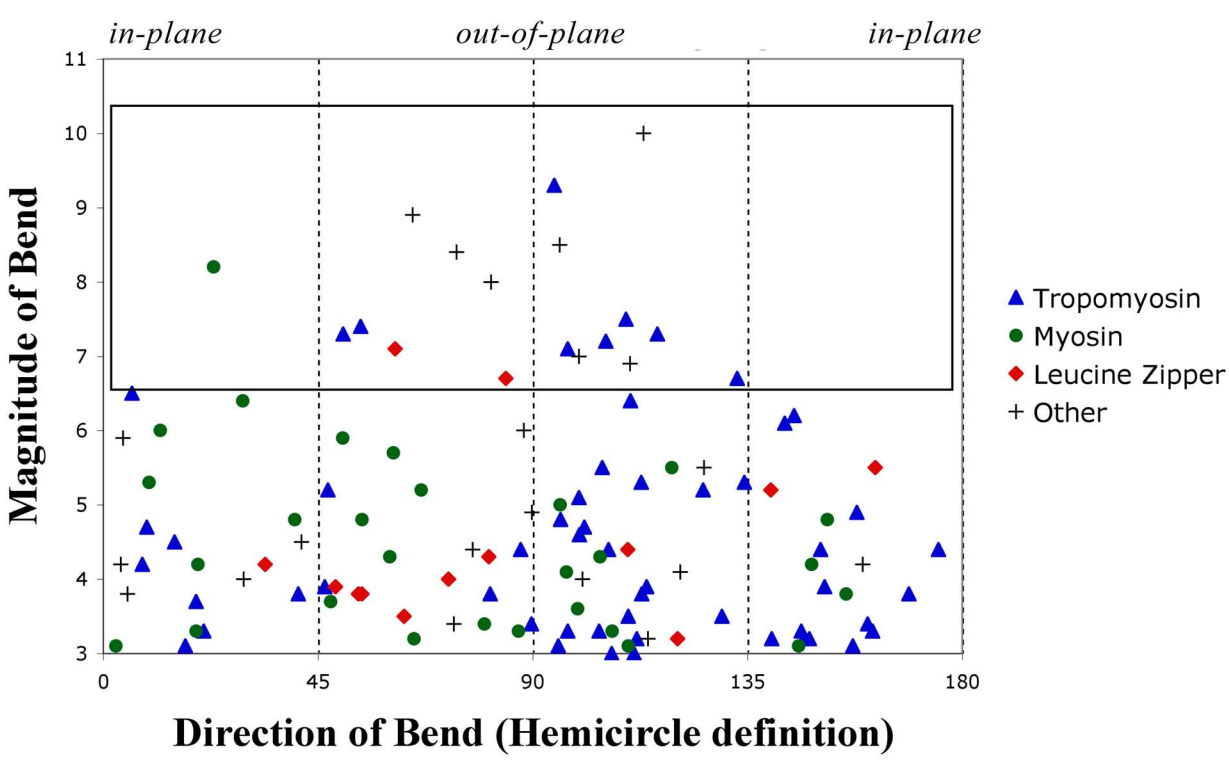
8 Minakata S, Maeda K, Oda N, Wakabayashi K, Nitanai Y, Maeda Y. (2008). Two-crystal structures of tropomyosin C-terminal fragment 176-273: exposure of the hydrophobic core to the solvent destabilizes the tropomyosin molecule. Biophys J 95:710-9.


Magnitude of Bend (degrees)
3
4
5
6
7
8
9
10
Tropomyosin Residue Number
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
200
210
220
230
240
250
260
270
280
290


Parallel Homodimer
Coiled Coils (all bends)
Absolute Value of Change in Axial Stagger (Å per heptad)
y = -0.0129x² + 0.1874x - 0.1668
R² = 0.0836
Magnitude of bend (degrees)


Parallel Homodimeric Coiled Coils
(only bends within 45 degrees of in-plane)
y = -0.0116x² + 0.2165x - 0.1139
R² = 0.249
Magnitude of bend (degrees)


All Parallel Homodimeric Coiled Coils
y = -8E-07x² + 0.0029x + 3.422
R² = 0.0769
Magnitude of Bend
12
10
8
6
4
2
0
0
500
1000
1500
2000
Gap volume


in-plane
out-of-plane
in-plane
Magnitude of Bend
Direction of Bend (Hemicircle definition)
Tropomyosin
Myosin
Leucine Zipper
Other
11
10
9
8
7
6
5
4
3
0
45
90
135
180