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Supporting Material

Title: Vinculin activation is necessary for complete talin binding

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Supplementary Table

Table S1. Nudging Force Enhances the Probability of Binding.

	0 pN	5 pN	12.5 pN	15 pN
Auto-Inhibited Vinculin				
VBS Drift Away from D1	4 trials			0 trials
VBS Link to D1 Surface	8 trials			12 trials
VBS Insert into D1	0 trials			0 trials
Activated Vinculin				
VBS Drift Away from D1	0 trials	0 trials	0 trials	0 trials
VBS Link to D1 Surface	13 trials	3 trials	3 trials	1 trial
VBS Insert into D1	2 trials	1 trial	1 trial	3 trials

Table S1. The binding of VBS to vinculin is simulated with a number of different parameters. Initially, VBS is simulated binding to vinculin – in both an activated and an auto-inhibited conformation – with no external nudging force applied. In the subsequent simulations a nudging force of varying magnitude is applied to VBS, briefly, at the start of each simulation. The nudging forces accelerate VBS towards vinculin in order to enhance VBS insertion into domain 1. The results show that the nudging force does indeed enhance the probability of VBS binding to D1. VBS is however unable to bind D1 of vinculin in its auto-inhibited conformation even with the application of the initial nudging force.

Supplementary Figure Legends

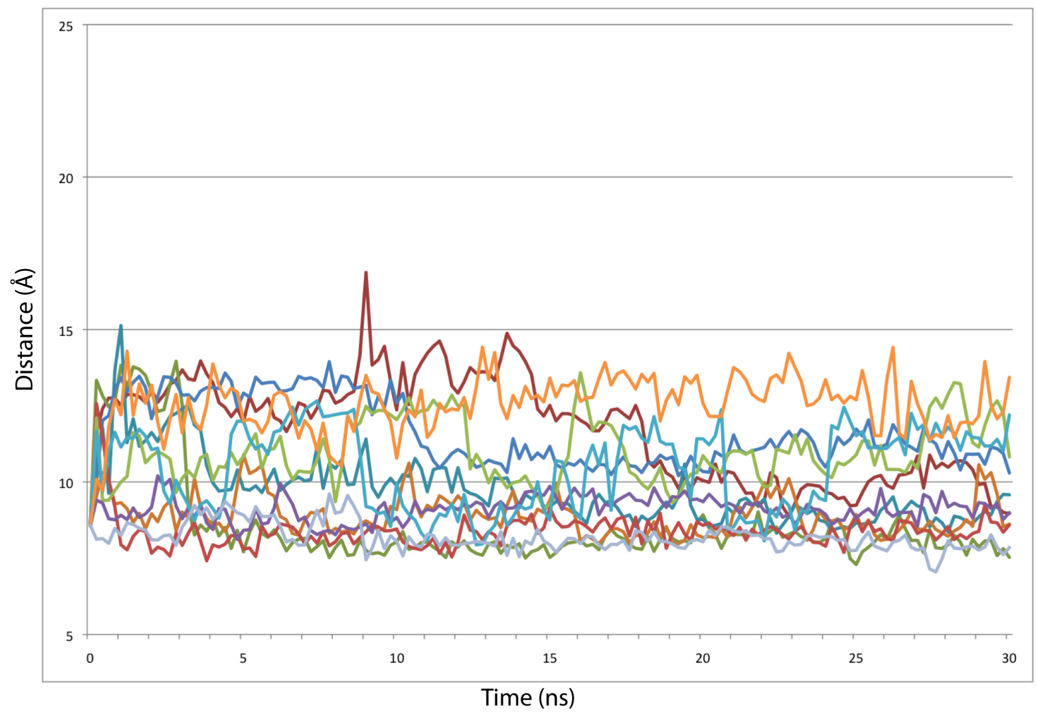
Figure S1. Simulation of VBS interacting with auto-inhibited vinculin shows VBS cannot insert into D1. (A) Distance between D1 helices near upper-VBS throughout the 30ns of simulation shown for 11 trails of VBS interacting with auto-inhibited vinculin. In none of these 11 trials did the separation increase passed the 20 Å needed to allow VBS insertion. (B) Distance between D1 helices near lower-VBS throughout the simulation for 11 trials with auto-inhibited vinculin. In none of the simulations did the helices consistently separate beyond 20 Å. (C) Closeness of upper-VBS to helix 4 of D1. Full insertion and binding is consistent with a separation of less than 13 Å. Data from all 12 trials shown. In none of the simulations with auto-inhibited vinculin did upper-VBS get close enough to helix 4 to allow for insertion. (D) Separation between lower-VBS and helix 4 of D1 shown for all 12 trials with auto-inhibited vinculin. In most of the trials lower-VBS did not get close enough to helix 4 of D1 to allow insertion. In the few trials that lower-VBS did get within 13 Å of helix 4, the lack of separation between helices near lower-VBS prevented VBS insertion. (E-F) Separation between helix 1 and helix 2 of D1 near upper-VBS and lower-VBS of one particular trail with auto-inhibited vinculin. In this trial the helices near lower-VBS separate for 10ns allowing for lower-VBS to insert into D1. However, the proximity of Vt to D1 prevents upper-VBS from separating, and furthermore, the clashes between D1 helix 1 and Vt residues reduces the D1 helical separation near lower-VBS after 10ns of simulation. The reduced separation between D1 helices near lower-VBS kicks VBS out of D1 and prevents further reinsertion.

Figure S2. Simulation of VBS interacting with an activated vinculin conformation shows that VBS can insert into D1 after removal of the proximity between D1 and Vt. (A) Distance between D1 helices near upper-VBS in 13 of the trials with an activated vinculin conformation. In these 13 trials vinculin cannot fully insert into D1 because helix 1 and helix 2 near upper-VBS fail to separate passed 20 Å in the 30 ns of simulation. (B) Distance between helix 1 and helix 2 of D1 near lower-VBS for simulation with an activated vinculin conformation. By the end of the 30ns simulations most of the trails show significant separation between helix 1 and helix 2 near lower-VBS. This increased separation allows for local insertion of lower-VBS into D1. (C) The distance between upper-VBS and helix 4 of D1 is traced throughout the simulation of 13 trials of VBS interacting with D1 of an activated vinculin conformation. In these 13 trials upper-VBS fails to insert into D1 and the distances do not decrease bellow 13 Å. (D) The distance between lower-VBS and helix 4 of D1. In most of these trials the distance between VBS and helix 4 of D1 and lower-VBS decreases to bellow 13 Å indicating lower-VBS inserts into D1 in most of these trials. (E) Separation between D1 helices near upper-VBS in 2 trials that showed complete insertion of VBS into D1 of activated vinculin. In both trails after 30 ns of simulation helix 1 and helix 2 of D1 are nearly 20 Å apart. This separation is sufficient to allow upper-VBS to insert into D1. (F) Separation between D1 helices near lower-VBS in 2 trials showing complete insertion of VBS into D1 of an activated vinculin conformation. In both trials the lower-VBS helices separate early in simulation allowing for VBS to insert itself into lower-VBS first then into upper-VBS. (G-H) Separation between VBS and helix 4 of D1 in two simulations with an activated vinculin conformation showing complete insertion; lower-VBS inserts into D1 early and soon after upper-VBS inserts into D1.

Figure S1

A

Helical Separation Near Upper-VBS



B

Helical Separation Near Lower-VBS

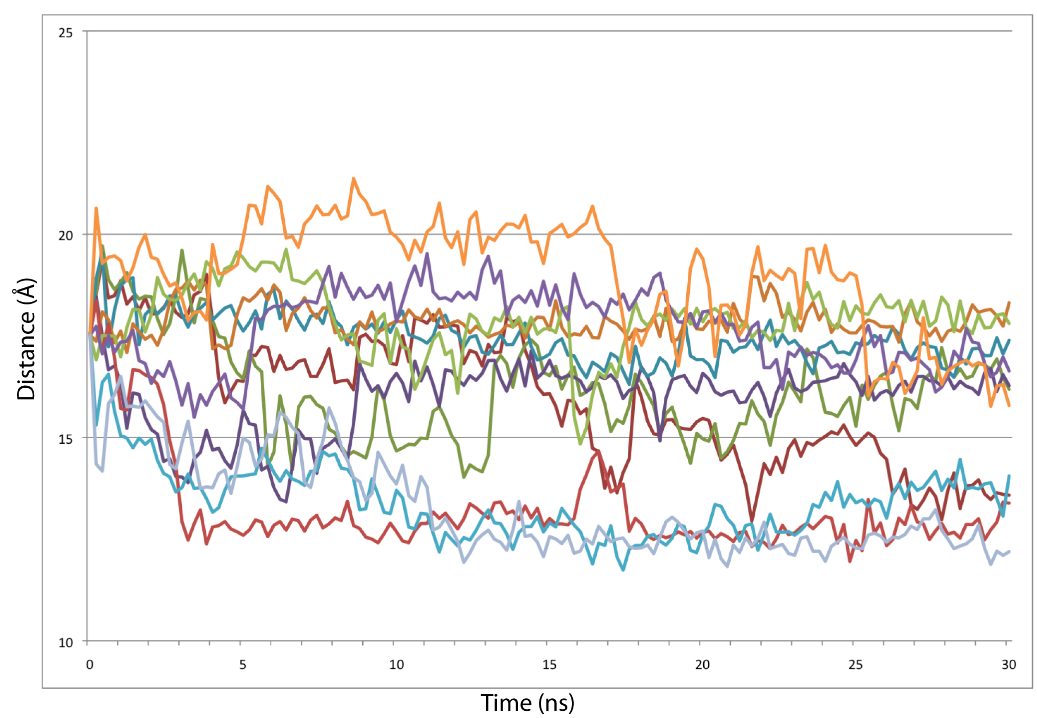
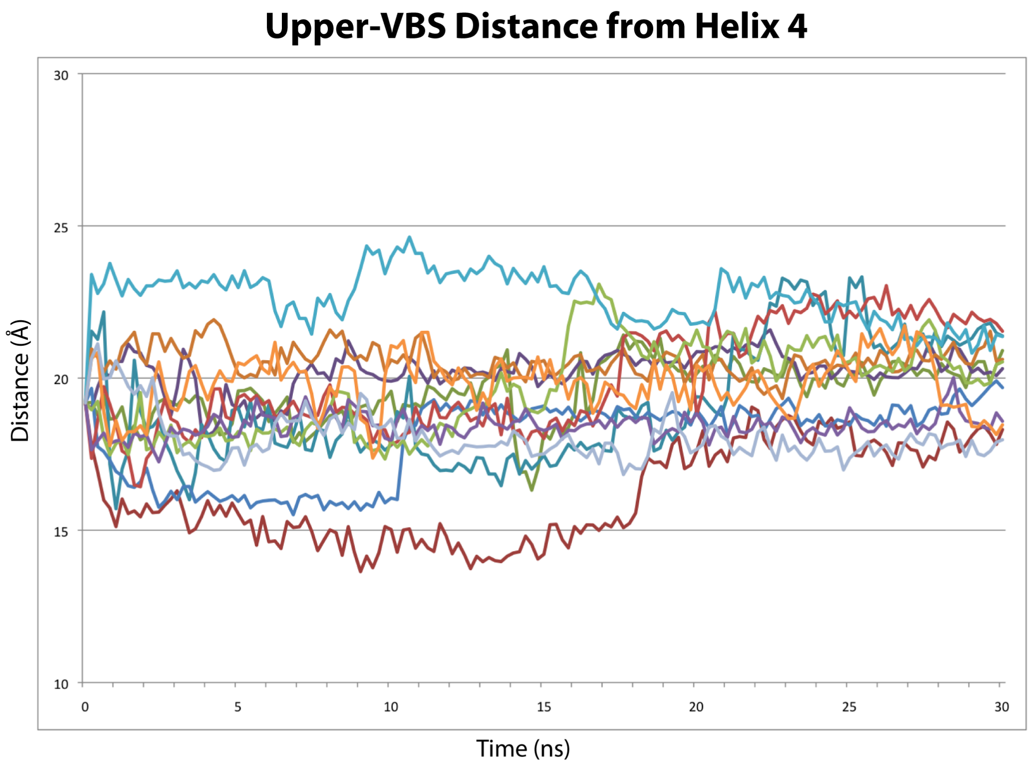


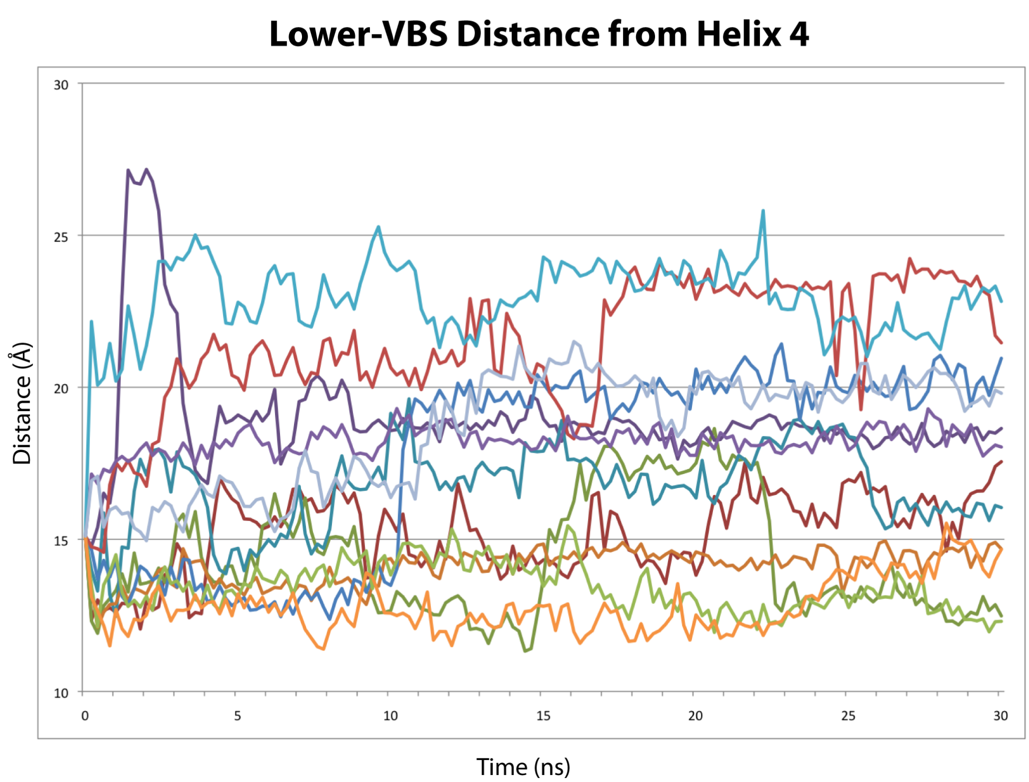
Figure S1

C



Upper-VBS Distance from Helix 4
30
25
20
15
10
Distance (Å)
0
5
10
15
20
25
30
Time (ns)

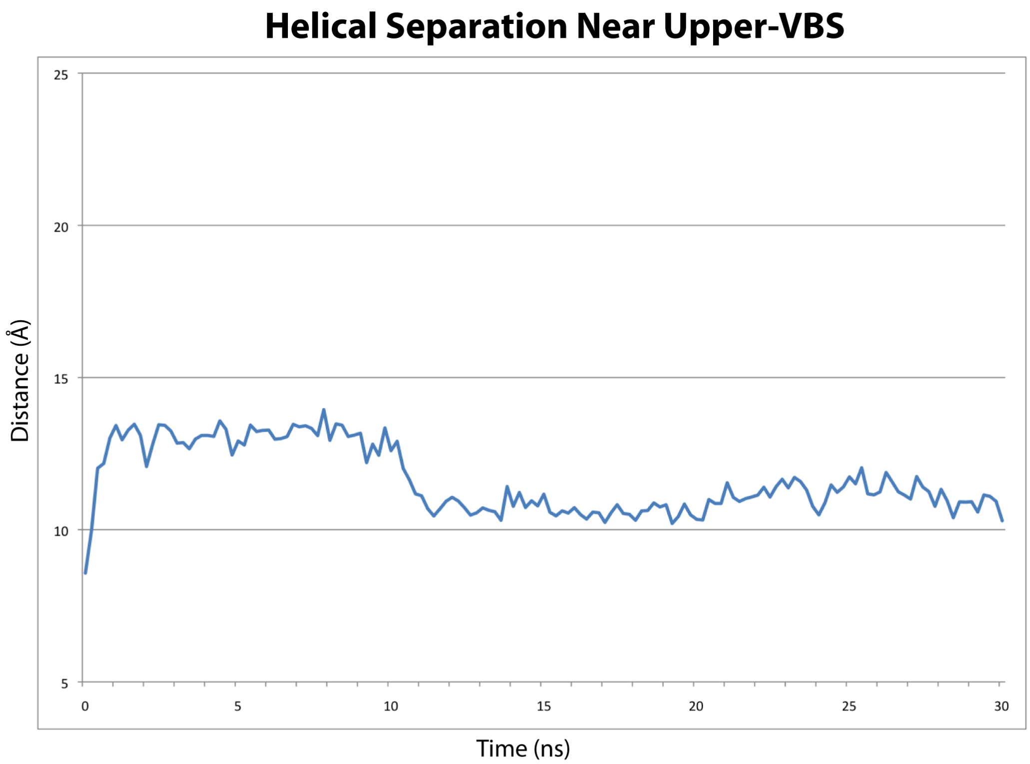
D



Lower-VBS Distance from Helix 4
30
25
20
15
10
Distance (Å)
0
5
10
15
20
25
30
Time (ns)

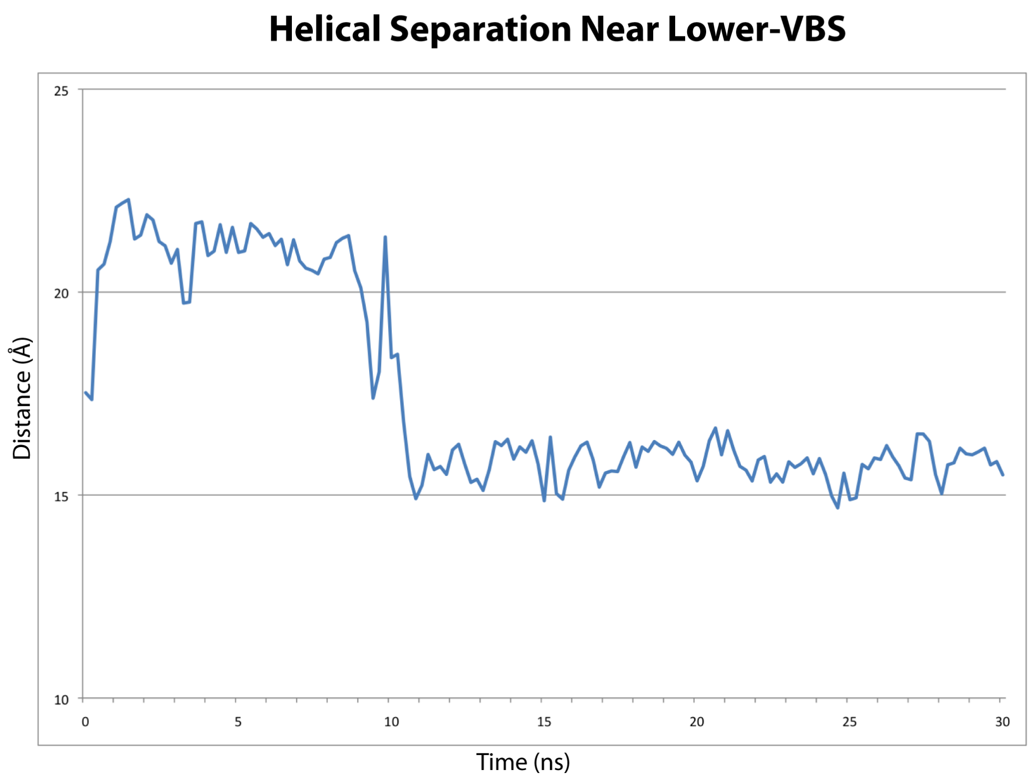
Figure S1

E



Helical Separation Near Upper-VBS
Distance (Å)
Time (ns)
25
20
15
10
5
0
5
10
15
20
25
30

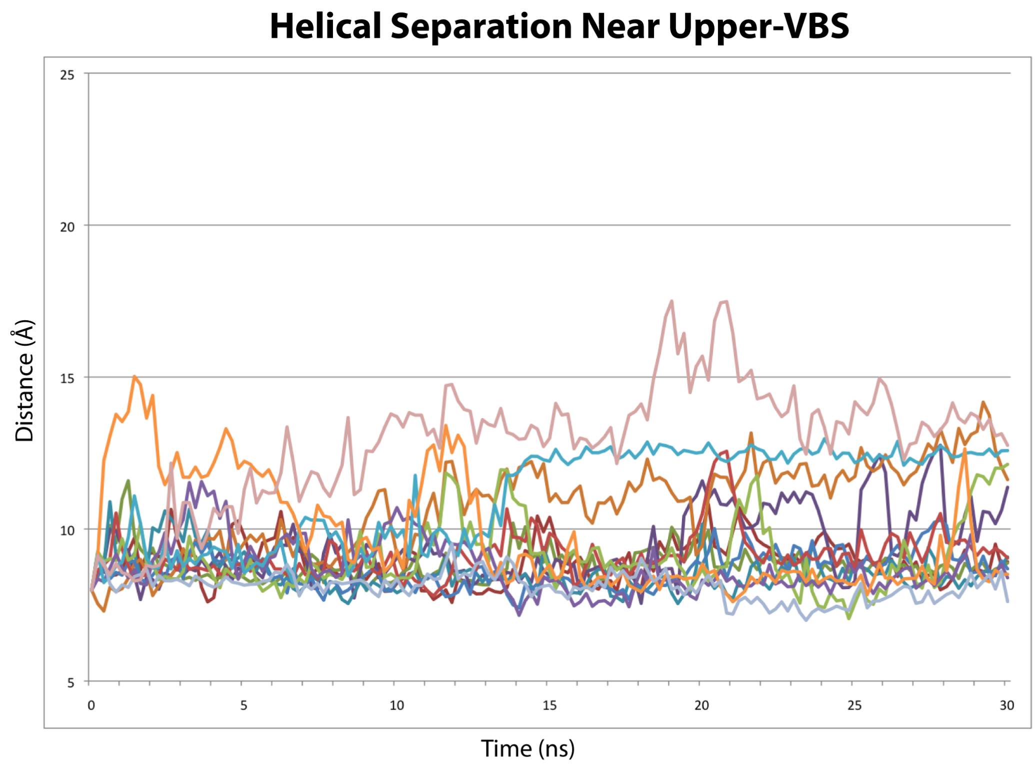
F



Helical Separation Near Lower-VBS
Distance (Å)
Time (ns)
25
20
15
10
0
5
10
15
20
25
30

Figure S2

A


Helical Separation Near Upper-VBS
Distance (Å)
25
20
15
10
5
0
5
10
15
20
25
30
Time (ns)

B

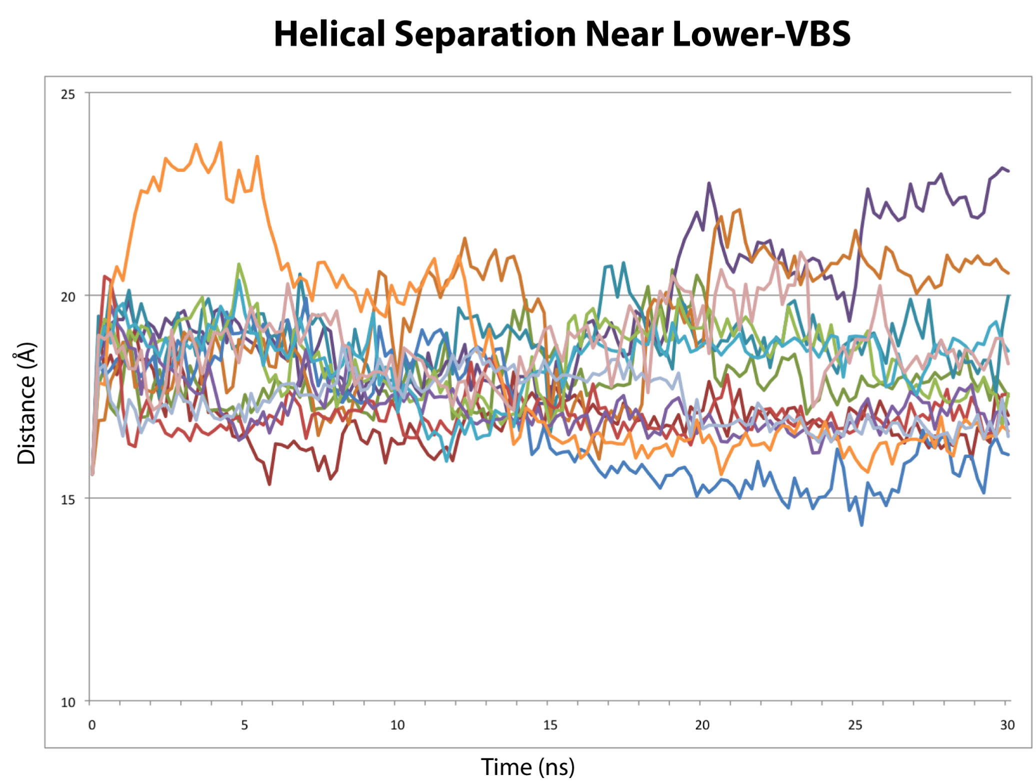
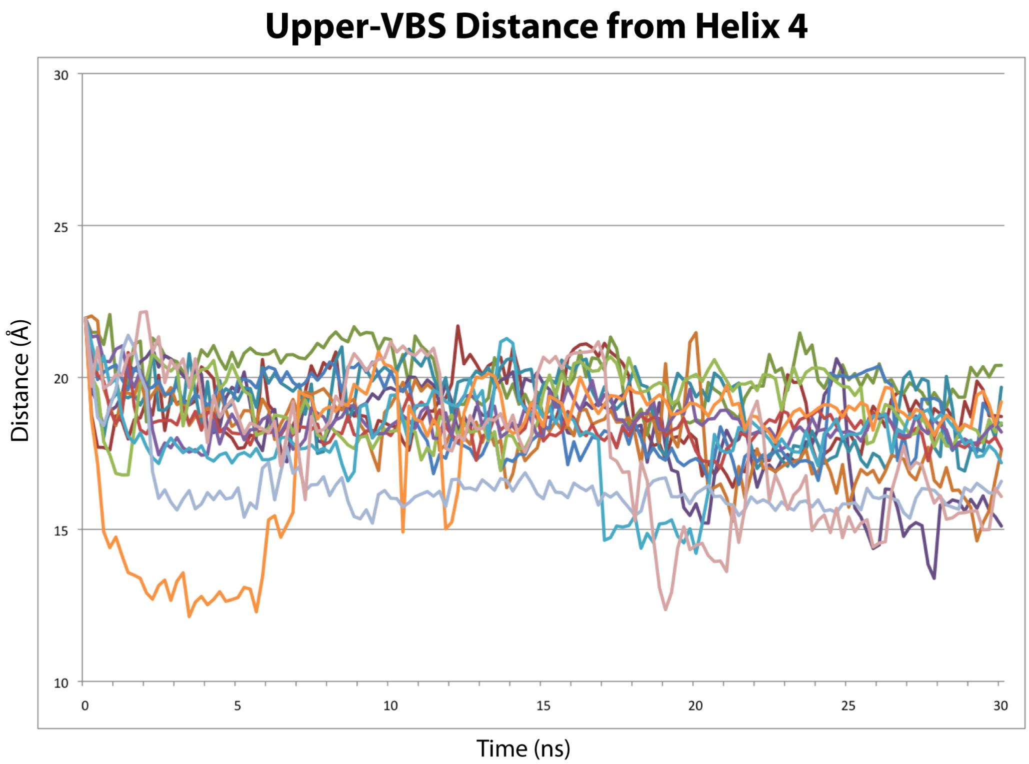

Helical Separation Near Lower-VBS
Distance (Å)
25
20
15
10
0
5
10
15
20
25
30
Time (ns)

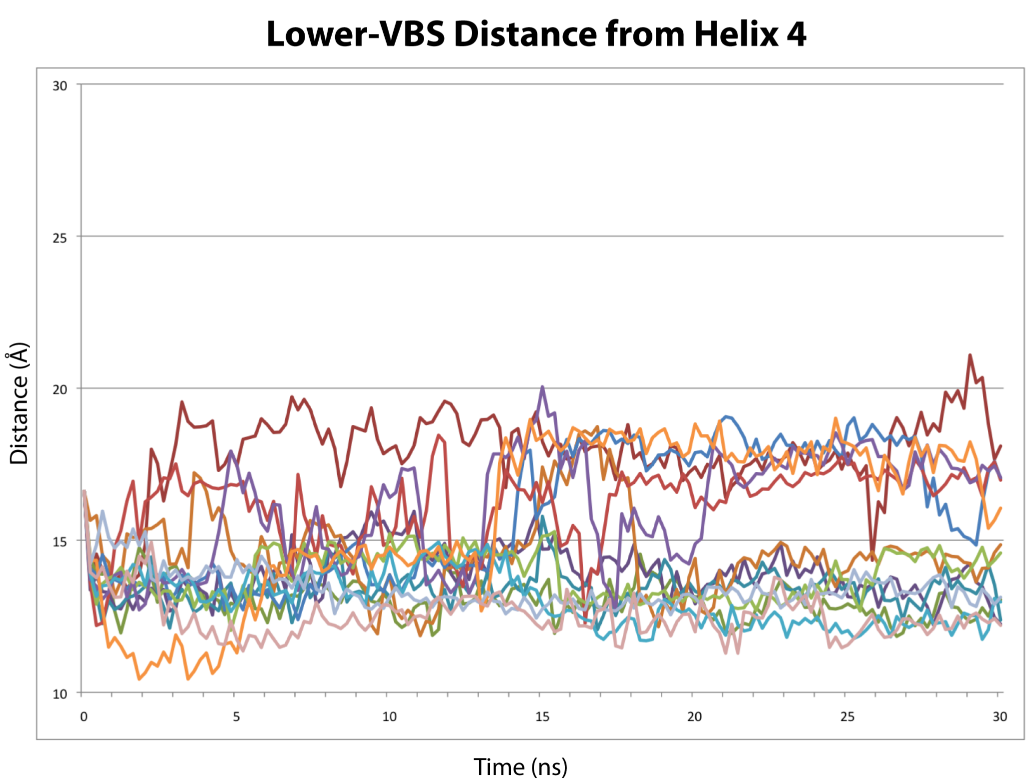
Figure S2

C



Upper-VBS Distance from Helix 4
Distance (Å)
Time (ns)
30
25
20
15
10
0
5
10
15
20
25
30

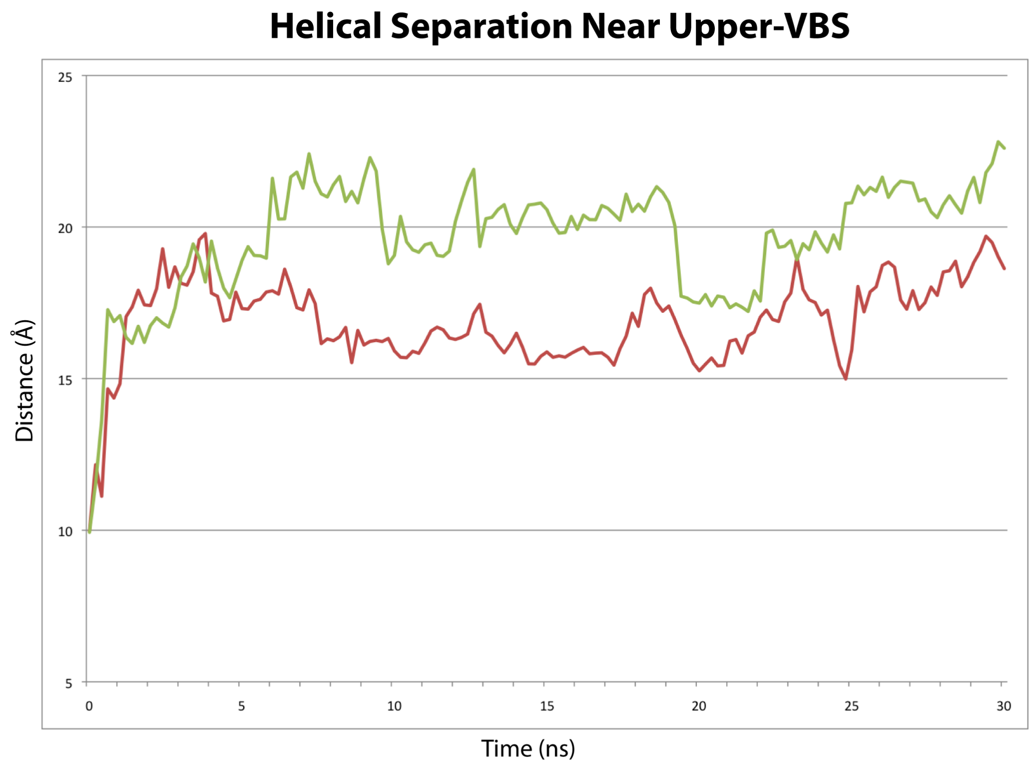
D



Lower-VBS Distance from Helix 4
Distance (Å)
Time (ns)
30
25
20
15
10
0
5
10
15
20
25
30

Figure S2

E


Helical Separation Near Upper-VBS
Distance (Å)
Time (ns)
25
20
15
10
5
0
5
10
15
20
25
30

F

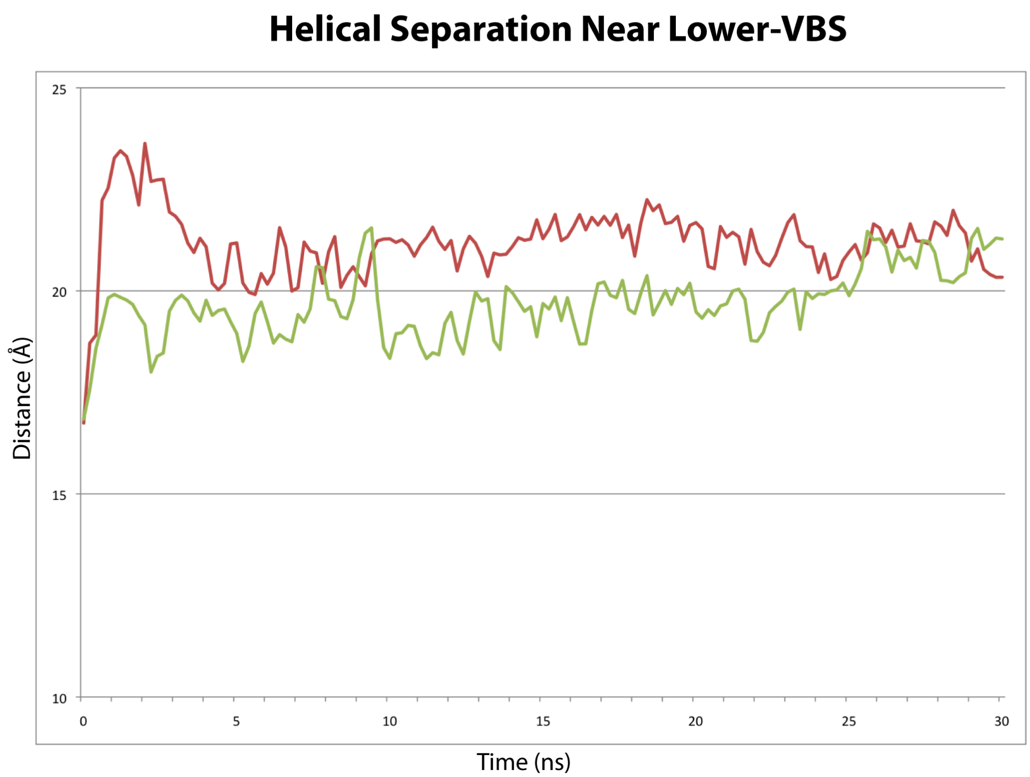
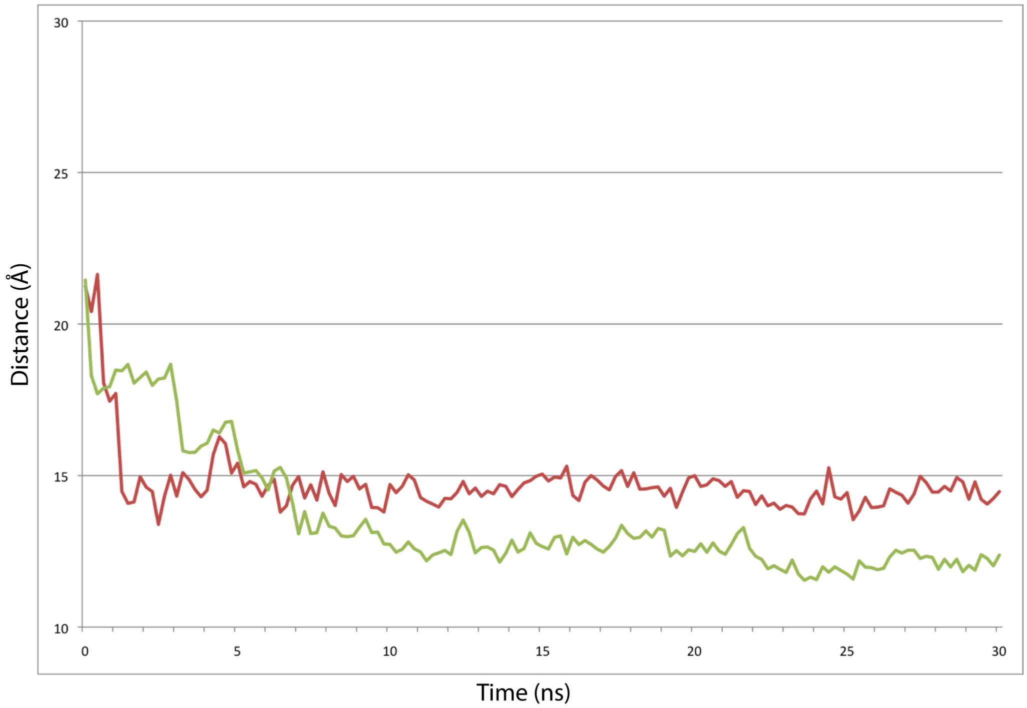

Helical Separation Near Lower-VBS
Distance (Å)
Time (ns)
25
20
15
10
0
5
10
15
20
25
30

Figure S2

G

Upper-VBS Distance from Helix 4



H

Lower-VBS Distance from Helix 4