SUPPLEMENTARY MATERIAL

Figure S1.

Mass spectra of proteins used for conformational studies. (**A-E,i**) Intact mass spectra of Elongin BC and Vif constructs used. (**A-E,ii**) Deconvoluted mass spectra of Elongin BC and Vif constructs used.

Figure S2.

HX MS titration results for all Vif:Elongin BC interactions. Panel A is the same as the data shown in Figure 3. The +7 charge state of Elongin C is shown in the presence of each Vif construct at the ratios indicated. As in Figure 3, the blue distribution represents the unbound form of Elongin C and the red distribution represents the bound form.

Figure S3.

SDS PAGE gels of (A) anion exchange and (B) size exclusion chromatography purification steps of the Elongin BC complex. The two proteins have high affinity for one another and are always found together from the moment of expression.

Figure S4.

HX MS peptide data for Elongin B and C. These data include those shown in Figure 5. All peptides that could be followed are shown. Some peptides do not have data in the presence of Vif due to interference from Vif peptic peptides.

Figure S5.

Deuterium exchange into intact Vif in the absence and presence of the Elongin BC complex. The error of determining the deuterium level was ± 2 Da.

Movie S1.

Animation (approximately 6 seconds) illustrating the deuterium incorporation for the pepsin digested Elongin BC over time. Frames are: 0, 10 s, 1 m, 5 m, and 20 m, as indicated. See also Figure 4C.

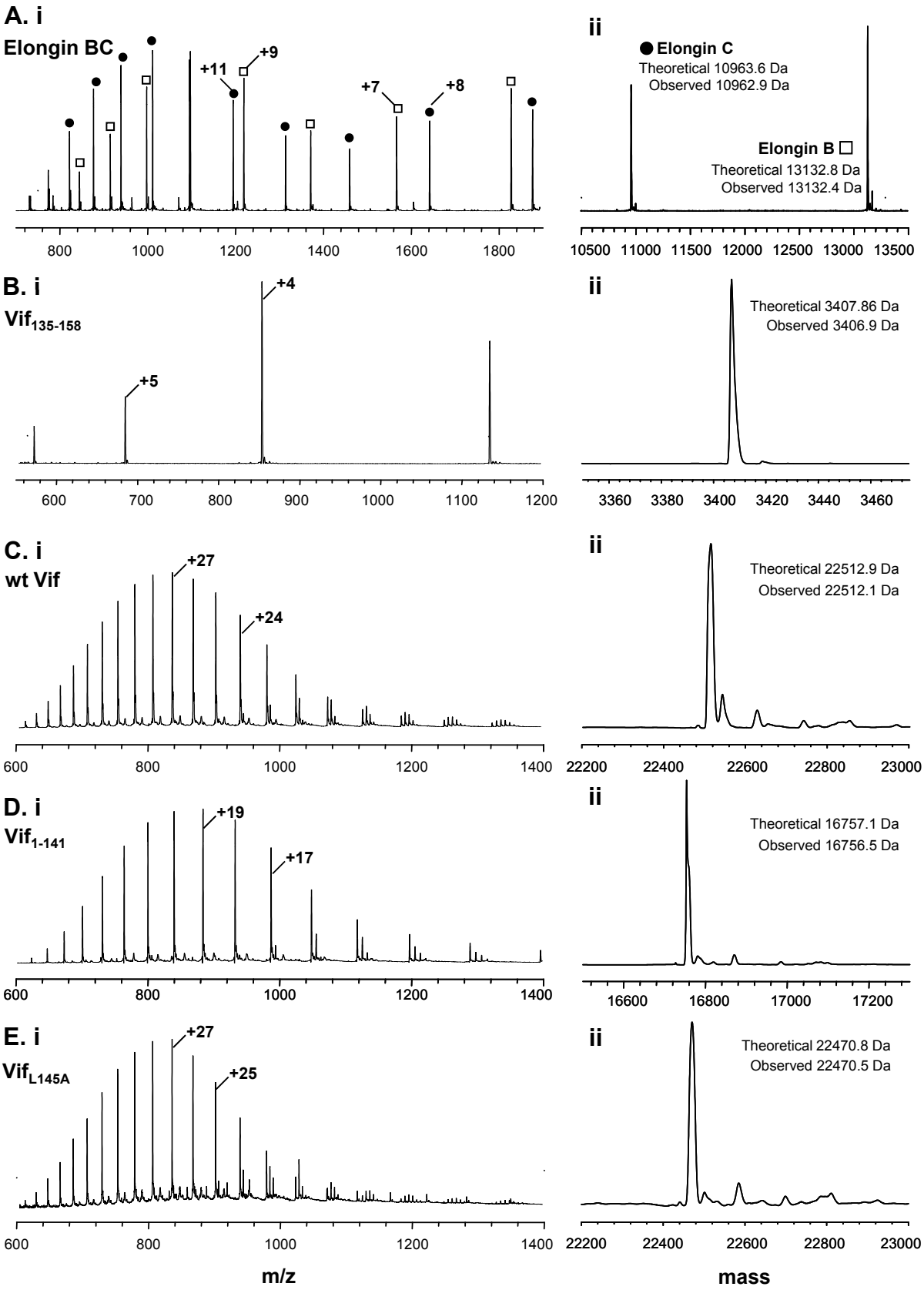

A. i
Elongin BC
+11
+9
+7
+8
800
1000
1200
1400
1600
1800
ii
Elongin C
Theoretical 10963.6 Da
Observed 10962.9 Da
Elongin B
Theoretical 13132.8 Da
Observed 13132.4 Da
10500
11000
11500
12000
12500
13000
13500
B. i
Vif135-158
+4
+5
600
700
800
900
1000
1100
1200
ii
Theoretical 3407.86 Da
Observed 3406.9 Da
3360
3380
3400
3420
3440
3460
C. i
wt Vif
+27
+24
600
800
1000
1200
1400
ii
Theoretical 22512.9 Da
Observed 22512.1 Da
22200
22400
22600
22800
23000
D. i
Vif1-141
+19
+17
600
800
1000
1200
1400
ii
Theoretical 16757.1 Da
Observed 16756.5 Da
16600
16800
17000
17200
E. i
VifL145A
+27
+25
600
800
1000
1200
1400
m/z
ii
Theoretical 22470.8 Da
Observed 22470.5 Da
22200
22400
22600
22800
23000
mass

Figure S1

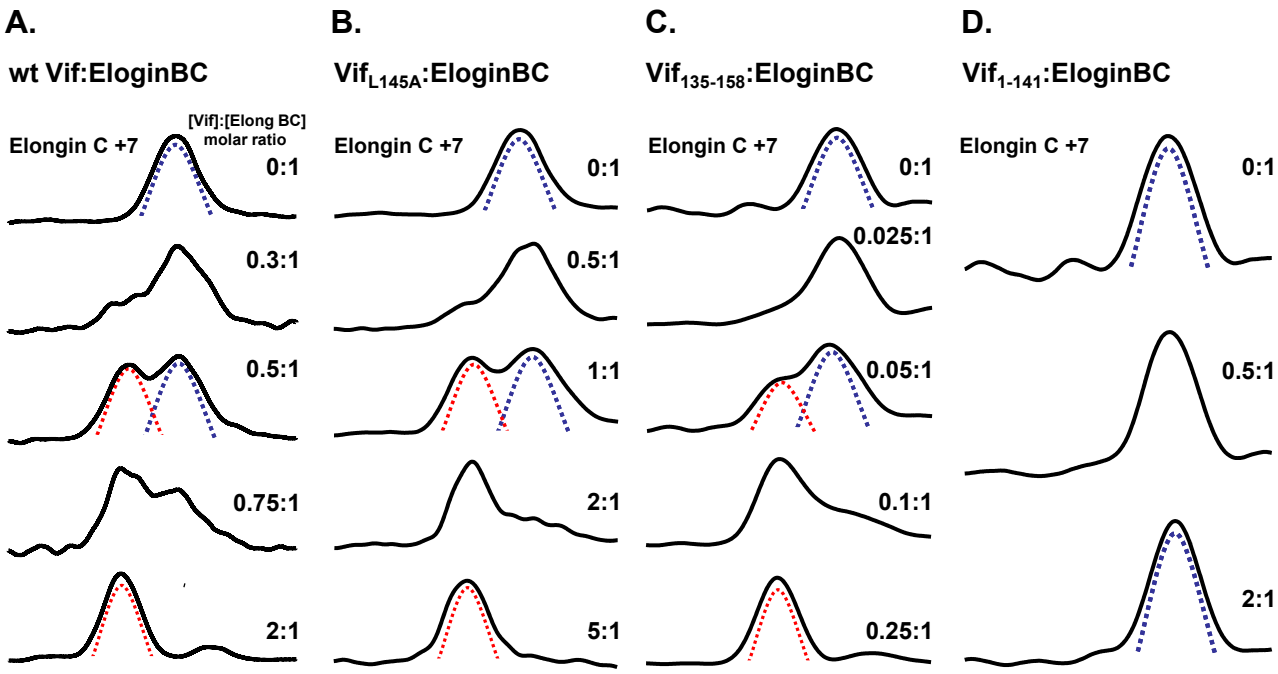
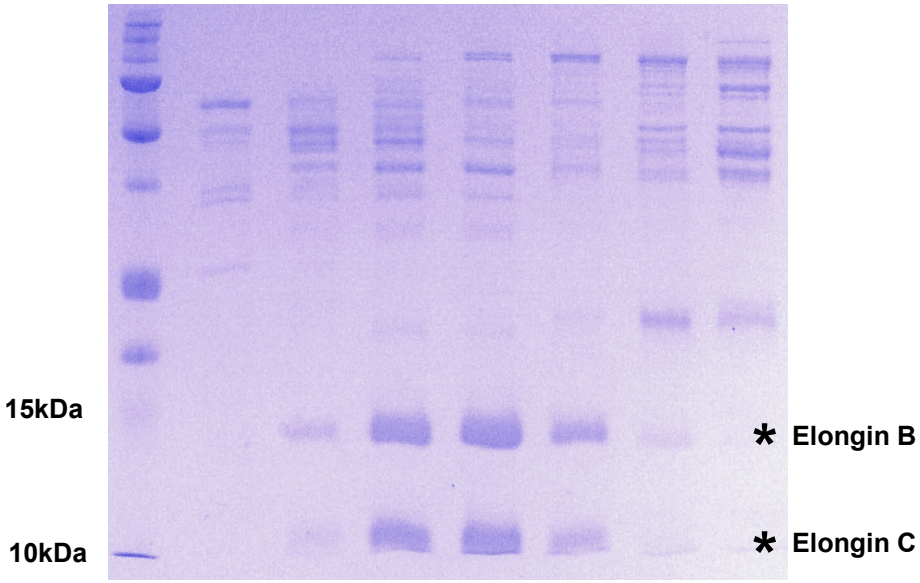

A.
wt Vif:EloginBC
[Vif]:[Elong BC]
molar ratio
Elongin C +7
0:1
0.3:1
0.5:1
0.75:1
2:1
B.
Vif$_{L145A}$:EloginBC
Elongin C +7
0:1
0.5:1
1:1
2:1
5:1
C.
Vif$_{135-158}$:EloginBC
Elongin C +7
0:1
0.025:1
0.05:1
0.1:1
0.25:1
D.
Vif$_{1-141}$:EloginBC
Elongin C +7
0:1
0.5:1
2:1

Figure S2

A. Elongin BC anion exchange fractions



B. Elongin BC size exclusion fractions

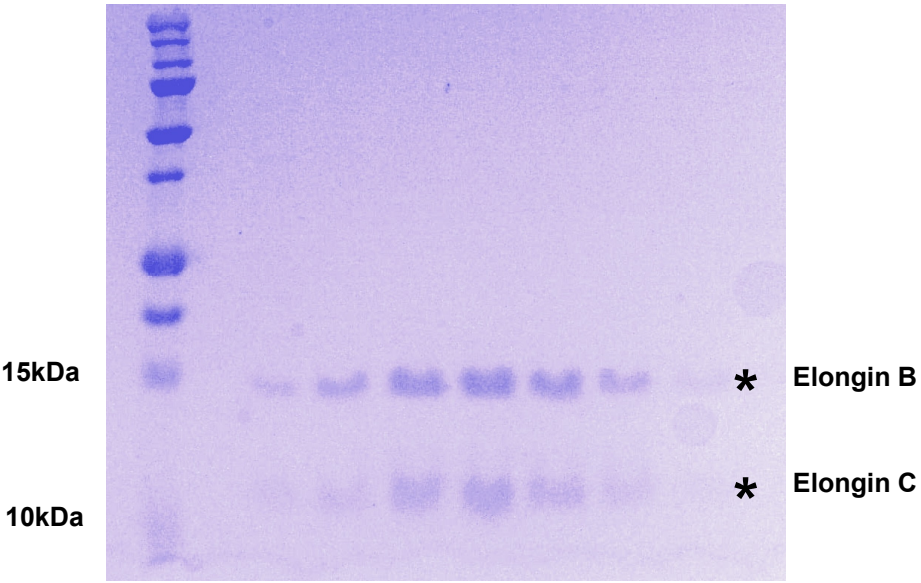
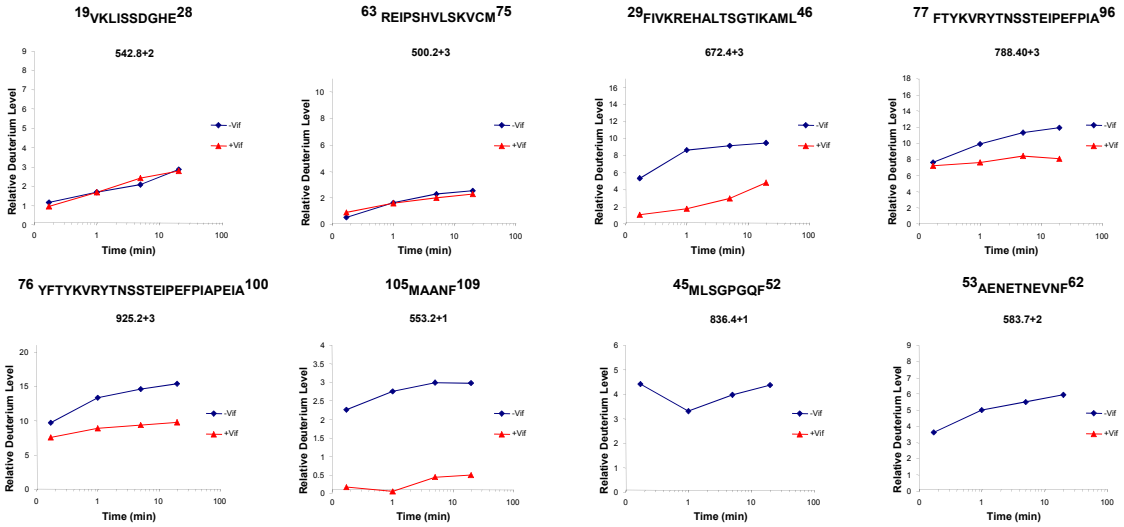


Figure S3

Elongin C peptic peptides



Elongin B peptic peptides

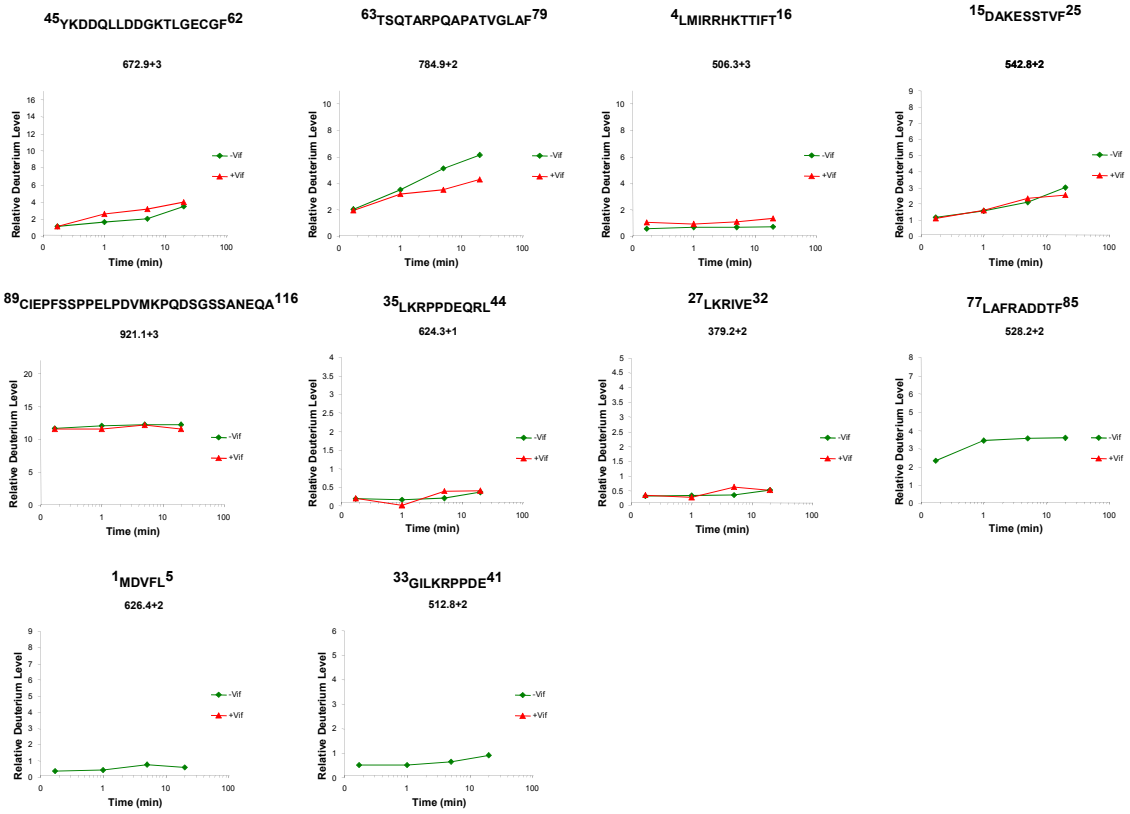


Figure S4

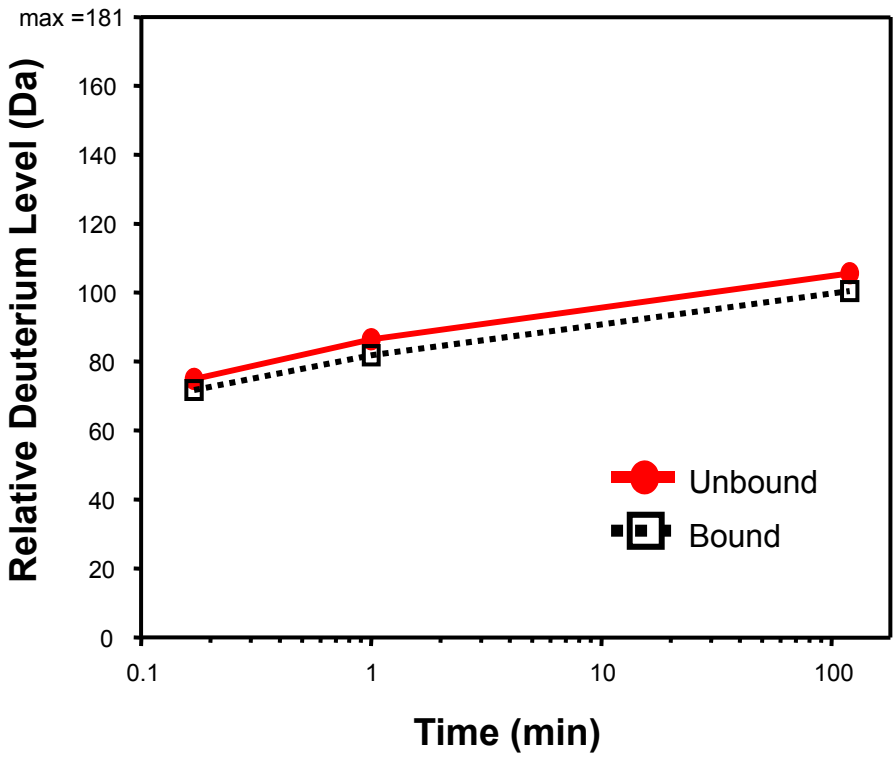

max =181
160
140
120
100
80
60
40
20
0
Relative Deuterium Level (Da)
0.1
1
10
100
Time (min)
Unbound
Bound

Figure S5