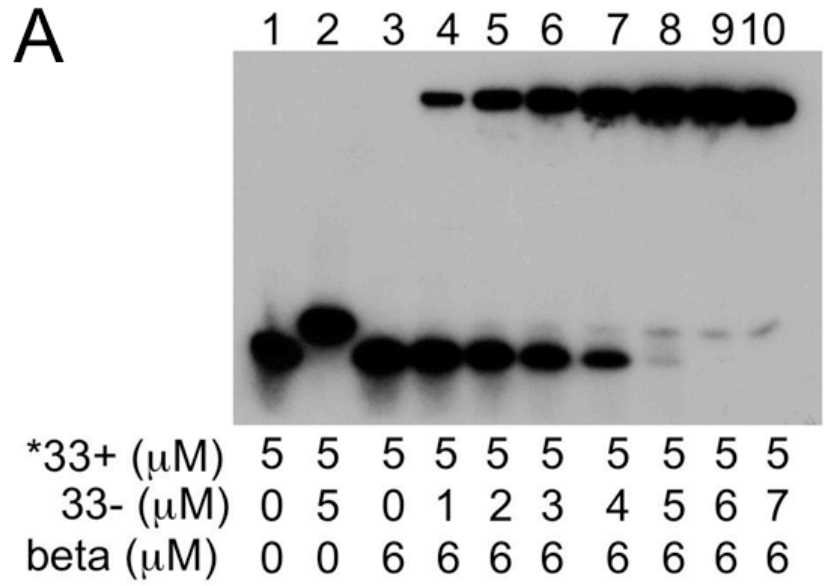
Figure 5. DNA binding of N-terminal fragments of β protein containing lysine to alanine mutations. (A) The binding of full length or N-terminal fragments of β protein to sequentially added complementary 33-mer oligonucleotides was carried out as described in Figure S1. The band at the top of the gel shows each protein in complex with the 33-mer duplex product of annealing, whereas the band at the bottom corresponds to unbound 33-mer duplex. Notice that the two fragments of β protein ($\beta^{1\text{-}188}$ and $\beta^{1\text{-}177}$) bind to DNA about as well as full length β protein (residues 1-261), and that the lysine to alanine mutants of $\beta^{1\text{-}188}$ bind less well, with the degree of impairment following the trend K172A > K36A > K69A > K11A. The K253A mutation within full-length β protein (at the far right) does not significantly impair DNA binding. (B) Binding of full length β protein and $\beta^{1\text{-}177}$ fragment to a single 33-mer ssDNA oligonucleotide (33+) was measured using a nitrocellulose filter binding assay (22), as described in Experimental Procedures. Notice that the $\beta^{1\text{-}177}$ fragment binds to the 33+ oligonucleotide with considerably higher stability than full-length β protein.

Figure S1. Binding of β protein to sequentially added complementary 33-mer oligonucleotides. (A) Formation of the β protein-DNA complex. 5 μM of ^{32}P 5'-labeled 33-mer (*33+) was incubated with β protein for 10 minutes prior to addition of the complementary 33-mer (33-), and the reactions were equilibrated for 30 minutes at 37 °C and run on a native 15% polyacrylamide gel. Notice that β protein does not bind to 33+ alone (lane 3), but as the complementary oligonucleotide (33-) is titrated into the mixture, the β protein-DNA complex is observed as the shifted band at the top of the gel. (B) Biotin modification of β protein disrupts DNA-complex formation. The indicated concentration of NHS-biotin reagent was incubated for 30 minutes at room temperature with 6 μM β protein either prior to (lanes 4-7) or subsequent to (lane 3) formation of the DNA complex by the sequential addition of complementary 33-mer oligonucleotides. Biotin-modification reactions were quenched by adding 3 mM lysine, and resolved on a 15% native polyacrylamide gel. Notice that addition of NHS-biotin to β protein disrupts subsequent formation of the DNA complex (lanes 4-7), but does not dissociate pre-formed β protein-DNA complex (lane 3).

Figure S2. Fragments of β protein produced by limited proteolysis in the presence and absence of DNA. Digestions of β protein or the β protein-DNA complex for 30 minutes with (A) trypsin, (B) chymotrypsin, (C) subtilisin, and (D) thermolysin, as shown in Figure 1b, were analyzed by LC-MS. For each major peak on the gel, the mass measured by MS, the mass calculated from the sequence, and the corresponding residues of β protein are shown.

Figure S3. Overview of the mass spectrometric analysis of the biotinylation of β protein. (A) The full scan chromatograph of the trypsin digest of biotinylated β protein. Peptide fragments were separated on a C_{18} column and detected by an ion trap mass spectrometer. (B) The mass spectrum of the 38.2 min peak from A shows the co-elution of two prominent peptides. The mass spectrometer automatically performs MS/MS scans on the five most abundant ions. (C) The MS/MS spectrum of the ion with m/z 888. SEQUEST search revealed it to be a doubly charged peak of fragment 16-31 ($[16\text{-}31]^{2+}$). Similarly, the ions of m/z 1774 and 592 were assigned as a singly and triply charged peak of fragment 16-31, respectively. (D) The MS/MS spectrum of the ion with m/z of 1015. "SEQUEST" search revealed it is a doubly charged peak of fragment 162-177 with lysine 172 biotinylated. The ion with m/z of 677 in panel B is the triply charged peak of the same peptide. (E) The extract ion chromatograph of ion 888 from the full scan spectrum in panel A. The peak area is calculated and used for quantification. (F) The extract ion chromatograph of peak 1015 from the full scan spectrum in panel A.


A
1 2 3 4 5 6 7 8 9 10
*33+ (μM) 5 5 5 5 5 5 5 5 5 5
33- (μM) 0 5 0 1 2 3 4 5 6 7
beta (μM) 0 0 6 6 6 6 6 6 6 6

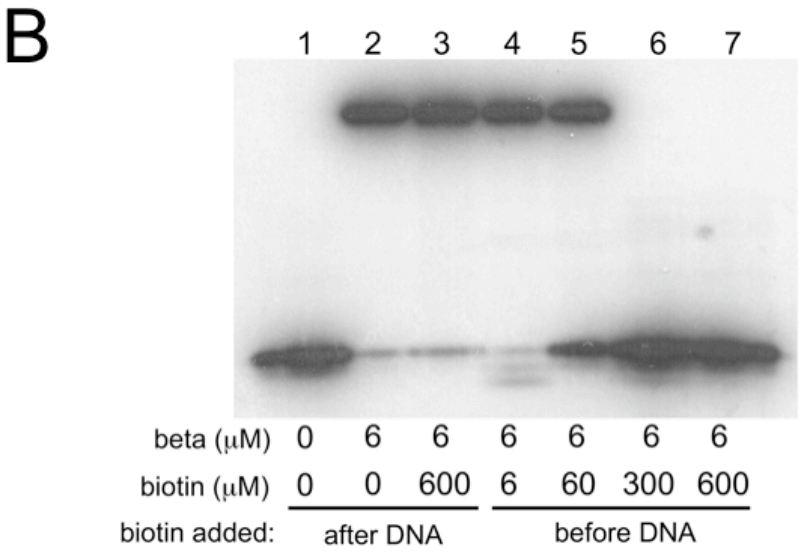

B
1 2 3 4 5 6 7
beta (μM) 0 6 6 6 6 6 6
biotin (μM) 0 0 600 6 60 300 600
biotin added: after DNA before DNA

Figure S1

(A) Trypsin						
	β protein			β protein +DNA		
Band	Meas	Calc	Resid	Meas	Calc	Resid
A				29,199	29,202	1-253
B				26,714	26,716	1-230
C				20,526	20,528	1-177
D				18,731	18,732	16-177
E	15,543	15,544	1-134	15,542	15,544	1-134

(B) Chymotrypsin						
	β prote in			β protein +DNA		
Band	Meas	Calc	Resid	Meas	Calc	Resid
A	21,318	21,319	1-184	21,318	21,319	1-184
B				20,246	20,249	9-184
C				19,878	19,879	13-184
D				17,318	17,319	36-184
E	15,157	15,159	1-131			

(C) Subtilisin						
	β protein			β protein +DNA		
Band	Meas	Calc	Resid	Meas	Calc	Resid
A	21,489	21,491	1-186			
B	21,318	21,319	1-184			
C				21,084	21,085	1-182
D				20,527	20,528	1-177
E				19,644	19,645	13-182
F				18,885	18,886	20-182
G	15,542	15,544	1-134			

(D) Thermolysin						
	β protein			β prot ein +DNA		
Band	Meas	Calc	Resid	Meas	Calc	Resid
A	29,727	29,729	1-258	29,731	29,729	1-258
B	22,401	22,402	1-193			
C	21,419	21,420	1-185			
D				20,640	20,641	1-178
E	20,526	20,528	1-177	20,526	20,528	1-177
F				19,570	19,571	8-177
G				19,199	19,202	12-177
H	16,013	16,016	1-138			
I	15,010	15,012	1-130			

Figure S2

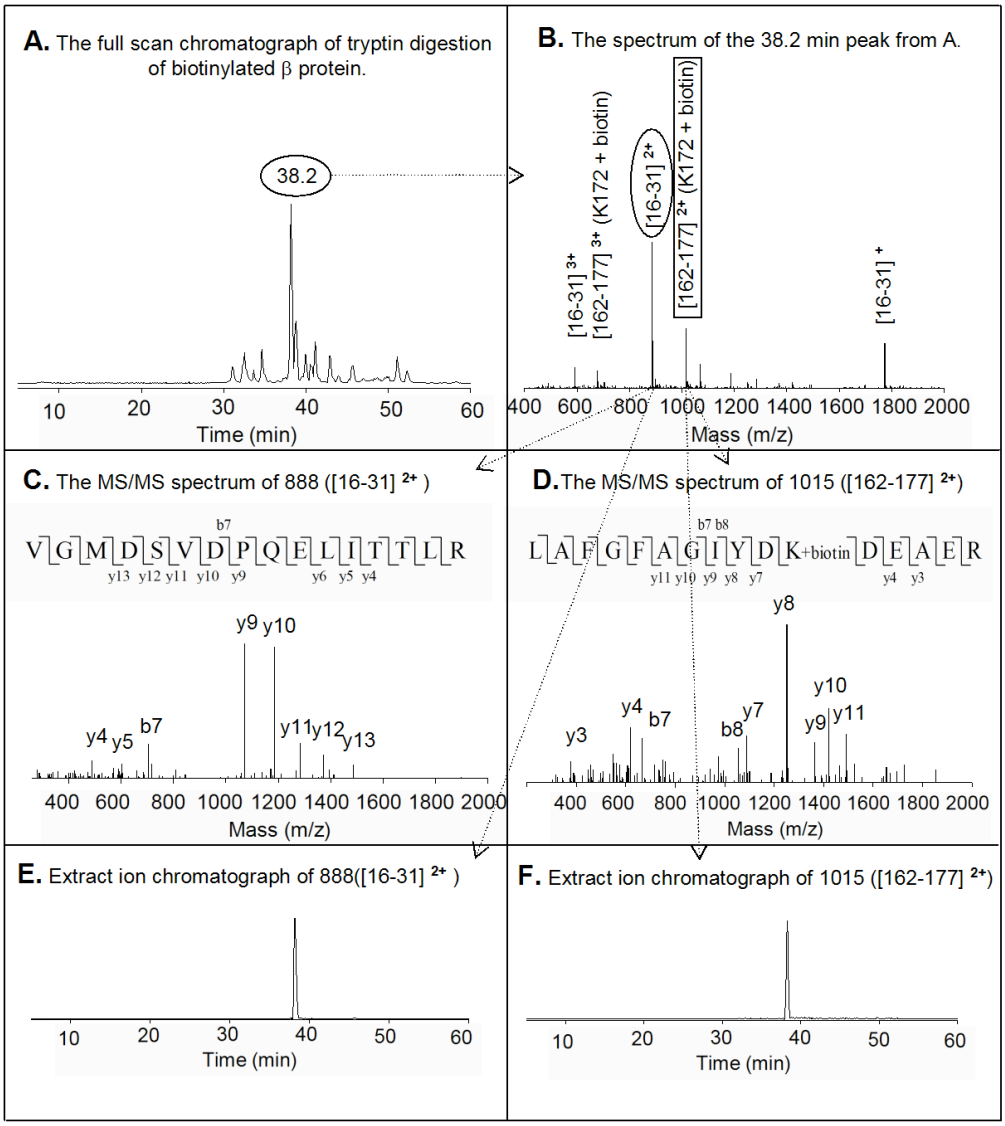

A. The full scan chromatograph of tryptin digestion of biotinylated β protein.
38.2
Time (min)
B. The spectrum of the 38.2 min peak from A.
[16-31] 3+ [162-177] 3+ (K172 + biotin)
[16-31] 2+
[162-177] 2+ (K172 + biotin)
[16-31] +
Mass (m/z)
C. The MS/MS spectrum of 888 ([16-31] 2+)
V G M D S V D P Q E L I T T L R
Mass (m/z)
D. The MS/MS spectrum of 1015 ([162-177] 2+)
L A F G F A G I Y D K+biotin D E A E R
Mass (m/z)
E. Extract ion chromatograph of 888([16-31] 2+)
Time (min)
F. Extract ion chromatograph of 1015 ([162-177] 2+)
Time (min)

Figure S3