SUPPORTING INFORMATION

Table S1. Interatomic distances within the active-site of WT and mutant BCX proteins.

Interaction	Distances (Å)			
Active-site residues	WT[a]	Y69F[a]	Y80F[b]	Q127A[b]
Glu78 $O^{\varepsilon 2}$ – Asn35 $O^{\delta 1}$	6.4	6.9	6.6	6.8
Glu78 $O^{\varepsilon 1}$ – Gln127 $N^{\varepsilon 2}$ (Ala127 C^{β})	2.7	2.7	2.7	(4.0)
Glu78 $O^{\varepsilon 2}$ – Tyr69 O^{η} (Phe69 C^{ζ})	2.6	(3.1)	2.6	2.6
Glu78 $O^{\varepsilon 2}$ – Arg112 N^{ε}	6.2	6.3	6.2	5.7
Glu78 $O^{\varepsilon 2}$ – Glu172 $O^{\varepsilon 2}$	5.6	6.2	5.6	5.9
Glu172 $O^{\varepsilon 2}$ – Asn35 $N^{\delta 2}$	3.1	3.2	3.8	3.2
Glu172 $O^{\varepsilon 1}$ – Tyr80 O^{η} (Phe80 C^{ζ})	2.7	2.8	(4.1)	2.9
Glu172 $O^{\varepsilon 2}$ – Arg112 N^{ε}	7.1	6.9	7.2	6.9
Asn35 $N^{\delta 2}$ – Asp11 $O^{\delta 2}$	6.1	6.0	5.5	5.4
Asn35 $N^{\delta 2}$ – Arg112 N^{ε}	7.8	7.8	7.9	7.9
Water molecules				
Glu78 $O^{\varepsilon 2}$ – Wat A	2.9	3.9	2.8	3.2
Glu78 $O^{\varepsilon 1}$ – Wat B	4.4	4.5	[c]	5.3
Glu172 $O^{\varepsilon 2}$ – Wat A	3.0	3.1	2.9	2.9
Glu172 $O^{\varepsilon 2}$ – Wat B	3.8	3.7	[c]	3.1
Tyr80 O^{η} (Phe80 C^{ζ}) – Wat A	3.2	3.9	(3.9)	3.1
Tyr80 O^{η} (Phe80 C^{ζ}) – Wat B	2.8	2.9	[c]	3.1
Asn35 $O^{\delta 1}$ – Wat A	3.8	3.2	4.4	4.0
Asn35 $N^{\delta 2}$ – Wat A	4.7	4.4	5.3	4.8
Tyr69 O^{η} (Phe69 C^{ζ}) – Wat A	4.0	(4.7)	4.0	3.8
Gln127 $N^{\varepsilon 2}$ (Ala127 C^{β}) – Wat A	5.2	6.0	4.7	(4.9)
Gln127 $N^{\varepsilon 2}$ (Ala127 C^{β}) – Wat B	3.1	3.2	[c]	7.3

[a]Structural coordinates used for distance measurements were obtained from the RCSB Protein Data Bank (*31*), PDB identification number 1XNB for WT BCX (*8*) and 2BVV for Y69F BCX (*7*).

[b]Structural coordinates of Y80F and Q127A BCX have RCSB identification numbers: 1HV0 and 1HV1 respectively.

[c]Wat B was not present in the crystal structure of Y80F BCX.

Table S2. Interatomic distances within the active-site of WT BCX at different pH values.

Interaction	Distances (Å)		
Active-site residues	pH 7.5[a]	"pH 5.5" [b]	"pH 4.0" [b]
Glu78 $O^{\varepsilon 2}$ – Asn35 $O^{\delta 1}$	6.4	6.6	8.0
Glu78 $O^{\varepsilon 1}$ – Gln127 $N^{\varepsilon 2}$	2.7	2.7	2.7
Glu78 $O^{\varepsilon 2}$ – Tyr69 O^{η}	2.6	2.5	2.9
Glu78 $O^{\varepsilon 2}$ – Arg112 N^{ε}	6.2	6.2	6.6
Glu78 $O^{\varepsilon 2}$ – Glu172 $O^{\varepsilon 2}$	5.6	5.5	5.8
Glu172 $O^{\varepsilon 2}$ – Asn35 $N^{\delta 2}$	3.1	3.3	3.9
Glu172 $O^{\varepsilon 1}$ – Tyr80 O^{η}	2.7	3.0	3.4
Glu172 $O^{\varepsilon 2}$ – Arg112 N^{ε}	7.1	7.0	7.2
Asn35 $N^{\delta 2}$ – Asp11 $O^{\delta 2}$	6.1	6.0	5.9
Asn35 $N^{\delta 2}$ – Arg112 N^{ε}	7.8	7.8	7.9
Water molecules			
Glu172 $O^{\varepsilon 2}$ – Wat A	3.0	2.8	5.4
Glu172 $O^{\varepsilon 2}$ – Wat B	3.8	3.7	2.9
Tyr80 O^{η} – Wat A	3.2	3.3	4.3
Tyr80 O^{η} – Wat B	2.8	2.9	2.9
Asn35 $O^{\delta 1}$ – Wat A	3.8	3.6	5.7
Asn35 $N^{\delta 2}$ – Wat A	4.7	4.5	6.6
Glu78 $O^{\varepsilon 2}$ – Wat A	2.9	3.1	3.5
Glu78 $O^{\varepsilon 1}$ – Wat B	4.4	4.5	2.6
Tyr69 O^{η} – Wat A	4.0	4.0	5.6
Gln127 $N^{\varepsilon 2}$ – Wat A	5.2	5.2	3.8
Gln127 $N^{\varepsilon 2}$ – Wat B	3.1	3.2	3.8

[a]Structural coordinates used for distance measurements were obtained from the RCSB Protein Data Bank (*31*), PDB identification number 1XNB for WT BCX at pH 7.5 (*8*).

[b]pH values of the buffer into which protein was transferred for four hours after crystallization at pH 7.5.

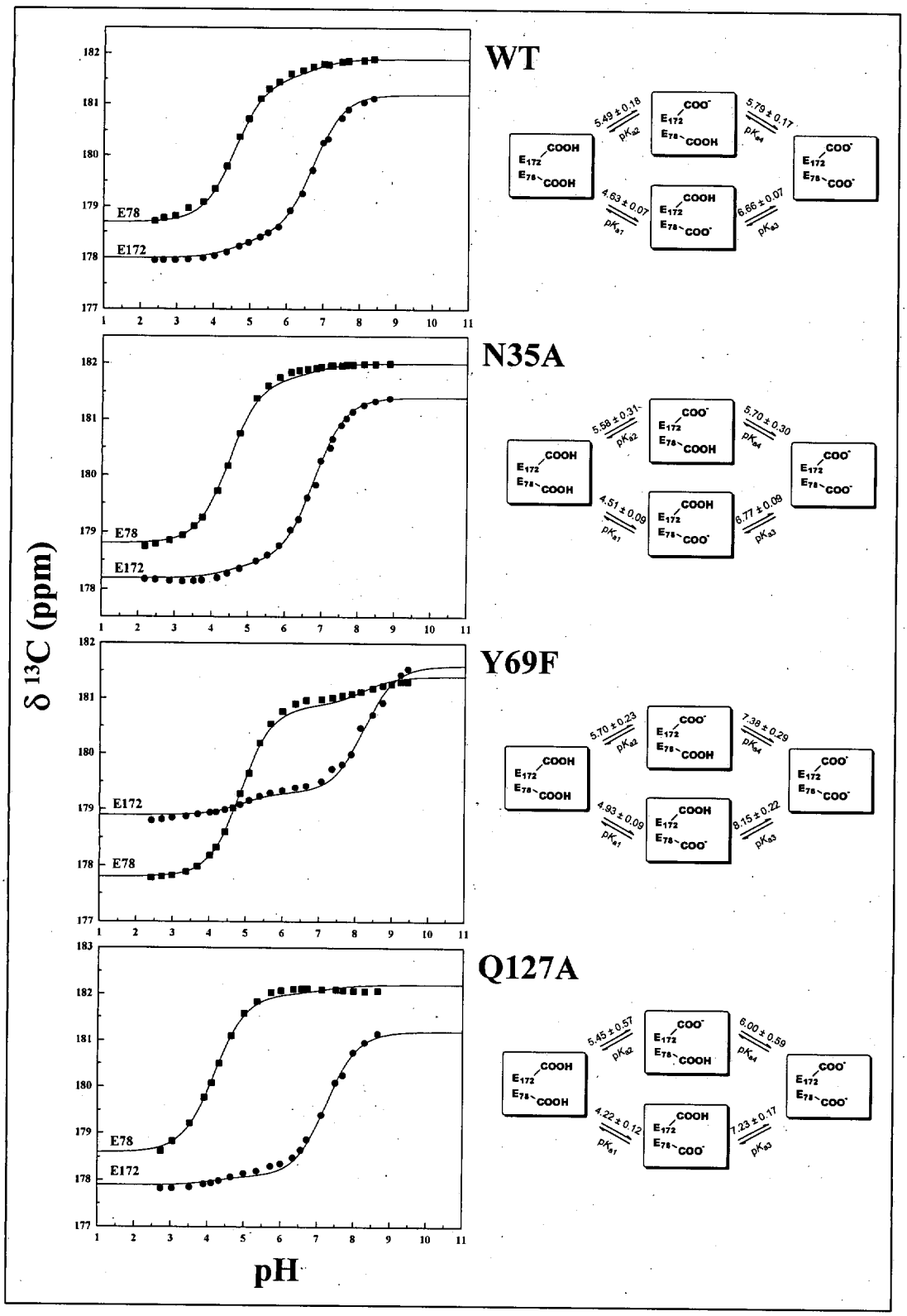
Table S3. Protein-saccharide distances within the active-sites of WT-2FXb[a] and E172C-Xb[a] BCX.

Interaction	Distances (Å)	
	WT-2FXb	E172C-Xb
Tyr69 O^{η} – proximal xylose O5	3.0	4.0
Tyr69 O^{η} – distal xylose O2	2.8	2.9
Arg112 N^{ε} – proximal xylose O2 (F2)	3.2	3.0
Arg112 $N^{\eta 2}$ – proximal xylose O3	3.3	3.2
Gln127 $N^{\varepsilon 2}$ – proximal xylose O2 (F2)	3.5	3.7
Tyr80 O^{η} – proximal xylose O5	3.6	4.6
Asn35 $O^{\delta 1}$ – proximal xylose O5	4.5	5.3
Glu78 $O^{\varepsilon 1}$ – proximal xylose O2 (F2)	2.9	3.4

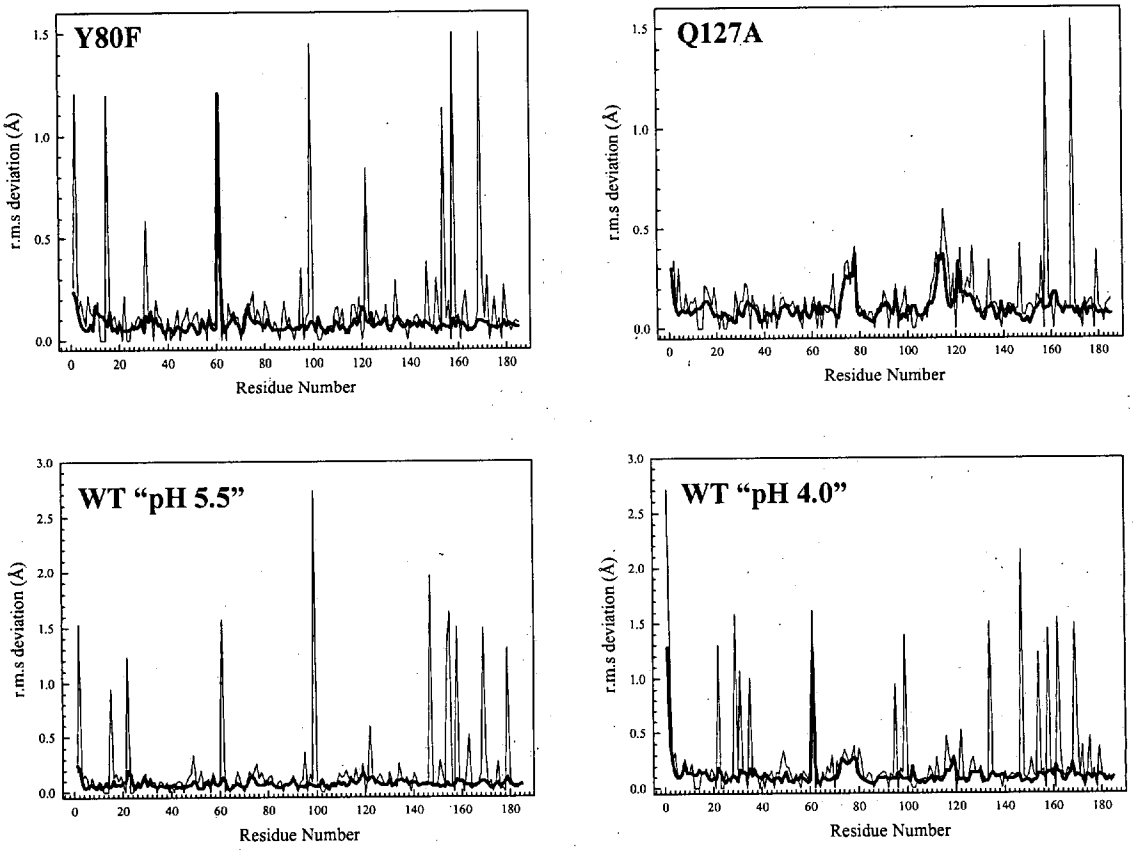
[a]Structural coordinates used for distance measurements were obtained from the RCSB Protein Data Bank (*31*) identification numbers 1BVV for WT-2FXb BCX and 1BCX for E172C-Xb (*8*).
[b]Atoms that are substituted at the 2-position in the DNP2FXb ligand are indicated in parenthesis.

Figure S1. Analysis of coupled titration behaviour of Glu78 and Glu172 in wildtype and mutant BCX proteins. ^{13}C-NMR monitored pH-titration curves for Glu78 and Glu172 were simultaneously fitted to a microscopic ionization model involving two coupled titrating groups (similar to the anaylses shown in Scheme 2). Note, the titration curves of Glu78 and Glu172 for Y80F and R112N BCX were not amenable to this type of analysis.

Figure S2. The r.m.s deviations of main chain (thick line) and side chain (thin line) heavy atoms of Y80F, Q127A, and WT BCX at apparent pH values of "5.5" and "4.0", compared to the WT species at pH 7.5. Structures were superimposed by a least-square fitting of the main chain and side chain atoms of all residues. The site of mutation was excluded from the plot of each respective mutant. A large majority of the observed side chain deviations, as well as that of the backbone at the peptide of Asn61/Gly62, are attributed to alternate conformations of surface residues. Any relevant structural deviations are discussed with the text. The main chain and side chain r.m.s deviations are respectively: 0.12 Å and 0.29 Å for Y80F BCX; 0.12 Å and 0.23 Å for Q127A BCX; 0.08 Å and 0.41 Å for WT "pH 5.5" BCX; 0.18 Å and 0.42 Å for WT "pH 4.0" BCX.



Joshi. Figure S1



Joshi - Figure S2