SUPPORTING INFORMATION

Circular Permutation of *Bacillus circulans* Xylanase: A Kinetic and Structural Study

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Supplemental Table S1: Primers used for cloning "designed" Bcx permutants and for generation of the *cpBcx_NsiI* gene

Permutant	Primer pair	All primer sequences in 5' → 3' direction	Combination [a]
cpG102^{G1}	G102_fwd G102_rev	GATCTCGAGTTAATCGCTTTTGACTGTGCC ACTAGATCCATGGGTGGTACCTATGACATCTAC	LinkG1_fwd LinkG1_rev
cpA123^{G1}	A123_fwd A123_rev	GATCTCGAGTTAATCGCCATCGATGGAAG ACTAGATCCATGGCCACCTTTACTCAGTATTGGAG	LinkG1_fwd LinkG1_rev
cpG139^{G1}	G139_fwd G139_rev	GATCTCGAGTTAAGTCGGCCGCTTAGATTG ACTAGATCCATGGGTTCGAACGCCACCATTAC	LinkG1_fwd LinkG1_rev
	LinkG1_fwd LinkG1_rev	GTGCTAGCTCCCCACACTGTAACGTTGG CAGTGTGGGGAGCTAGCACAGATTACTGGC	
cpA123^{G0}	LinkG0_fwd LinkG0_rev	GTGCTAGCCCACACTGTAACGTTGG CAGTGTGGGCTAGCACAGATTACTGGC	A123_fwd A123_rev
cpA123^{G2}	LinkG2_fwd LinkG2_rev	GTGCTAGCTCCTCCCCACACTGTAACGTTGG CAGTGTGGGGAGGAGCTAGCACAGATTACTGGC	A123_fwd A123_rev
cpBcx_*Nsi*I	NsiI-for NsiI-rev	TGAATGCATGGAAATCCCACGGTATGAAC TCCATGCATTCACGTGATTGGTGAACG	BcxG2-for BcxG2-rev
	BcxG2-for BcxG2-rev	CCTTCCATCGATGGCGATGCCACCTTTACTCAGTATTG ATCGCCATCGATGGAAGGTGCGTTG	

[a] Permutants were generated with four-primer PCR technology using the primer pair in column 3 together with the primers listed in column 4.

Supplemental Table S2: Sequences of "designed" circular permutants [a]

Permutant	Sequence
cpG102^{G1}	MGGTYDIYTTTRYNAPSIDGDRTTFTQYWSVRQSKRPTGSNATITFTNHVNAWKSHGMNLGSNWAYQVMATEGYQSSGSSNVTVWGASTDYWQNWTDGGGIVNAVNGSGGNYSVNWSNTGNFVVGKGWTTGSPFRTINYNAGVWAPNGNGYLTLYGWTRSPLIEYYVVDSWGTYRPTGTYKGTVKSD
cpG139^{G1}	MGSNATITFTNHVNAWKSHGMNLGSNWAYQVMATEGYQSSGSSNVTVWGASTDYWQNWTDGGGIVNAVNGSGGNYSVNWSNTGNFVVGKGWTTGSPFRTINYNAGVWAPNGNGYLTLYGWTRSPLIEYYVVDSWGTYRPTGTYKGTVKSDGGTYDIYTTTRYNAPSIDGDRTTFTQYWSVRQSKRPT
cpA123^{G0}	MATFTQYWSVRQSKRPTGSNATITFTNHVNAWKSHGMNLGSNWAYQVMATEGYQSSGSSNVTVWASTDYWQNWTDGGGIVNAVNGSGGNYSVNWSNTGNFVVGKGWTTGSPFRTINYNAGVWAPNGNGYLTLYGWTRSPLIEYYVVDSWGTYRPTGTYKGTVKSDGGTYDIYTTTRYNAPSIDGD_
cpA123^{G1}	MATFTQYWSVRQSKRPTGSNATITFTNHVNAWKSHGMNLGSNWAYQVMATEGYQSSGSSNVTVWGASTDYWQNWTDGGGIVNAVNGSGGNYSVNWSNTGNFVVGKGWTTGSPFRTINYNAGVWAPNGNGYLTLYGWTRSPLIEYYVVDSWGTYRPTGTYKGTVKSDGGTYDIYTTTRYNAPSIDGD_
cpA123^{G2}	MATFTQYWSVRQSKRPTGSNATITFTNHVNAWKSHGMNLGSNWAYQVMATEGYQSSGSSNVTVWGGASTDYWQNWTDGGGIVNAVNGSGGNYSVNWSNTGNFVVGKGWTTGSPFRTINYNAGVWAPNGNGYLTLYGWTRSPLIEYYVVDSWGTYRPTGTYKGTVKSDGGTYDIYTTTRYNAPSIDGD_

[a] The wild type terminal Al and W185 are highlighted in magenta, and the linker glycines in green. Due to the cloning strategy, cpA123^{G0}, cpA123^{G1}, and cpA123^{G2} have the ΔArg122 deletion (underscore) and the T123A mutation, identified in yellow. The N-terminal methionines (grey) may be removed post-translationally by methionine aminopeptidase, as is the case for the wild type enzyme. Wild type numbering is applied.

Supplemental Table S3: Sequences of circular permutants from the random screen [a]

Permutant	Sequence
Bcx_WT	ASTDYWQNWTDGGGIVNAVNGSGGNYSVNWSNTGNFVVGKGWTTGSPFRTINYNAG VWAPNGNGYLTLYGWTRSPLIEYYVVDSWGTYRPTGTYKGTVKSDGGTYDIYTTTRY NAPSIDGDRTTFTQYWSVRQSKRPTGSNATITFTNHVNAWKSHGMNLGSNWAYQVMA TEGYQSSGSSNVTVW
cpWT	ASTDYWQNWTDGGGIVNAVNGSGGNYSVNWSNTGNFVVGKGWTTGSPFRTINYNAG VWAPNGNGYLTLYGWTRSPLIEYYVVDSWGTYRPTGTYKGTVKSDGGTYDIYTTTRY NAPSIDGD_ATFTQYWSVRQSKRPTGSNATITFTNHVNAWKSHGMNLGSNWAYQVMA TEGYQSSGSSNVTVW
cpA1^{G2}	MKYLLPTAAAGLLLLAAQPAMAASTDYWQNWTDGGGIVNAVNGSGGNYSVNWSNTG NFVVGKGWTTGSPFRTINYNAGVWAPNGNGYLTLYGWTRSPLIEYYVVDSWGTYRPT GTYKGTVKSDGGTYDIYTTTRYNAPSIDGD_ATFTQYWSVRQSKRPTGSNATITFTNHV NAWKSHGMNLGSNWAYQVMATEGYQSSGSSNVTVCN **(ΔW185-GLink2)**
cpS2^{G2}	MKYLLPTAAAGLLLLAAQPAMASTDYWQNWTDGGGIVNAVNGSGGNYSVNWSNTGN FVVGKGWTTGSPFRTINYNAGVWAPNGNGYLTLYGWTRSPLIEYYVVDSWGTYRPTG TYKGTVKSDGGTYDIYTTTRYNAPSIDGD_ATFTQYWSVRQSKRPTGSNATITFTNHVN AWKSHGMNLGSNWAYQVMATEGYQSSGSSNVTVWGGAS
cpT3^{G2}	MKYLLPTAAAGLLLLAAQPAMATDYWQNWTDGGGIVNAVNGSGGNYSVNWSNTGNF VVGKGWTTGSPFRTINYNAGVWAPNGNGYLTLYGWTRSPLIEYYVVDSWGTYRPTGT YKGTVKSDGGTYDIYTTTRYNAPSIDGD_ATFTQYWSVRQSKRPTGSNATITFTNHVNA WKSHGMNLGSNWAYQVMATEGYQSSGSSNVTVWGGASTN
cpW6^{G2}	MKYLLPTAAAGLLLLAAQPAMAWQNWTDGGGIVNAVNGSGGNYSVNWSNTGNFVV GKGWTTGSPFRTINYNAGVWAPNGNGYLTLYGWTRSPLIEYYVVDSWGTYRPTGTYK GTVKSDGGTYDIYTTTRYNAPSIDGD_ATFTQYWSVRQSKRPTGSNATITFTNHVNAWK SHGMNLGSNWAYQVMATEGYQSSGSSNVTVWGGASTDYWHN
cpT10^{G2}	MKYLLPTAAAGLLLLAAQPAMATDGGGIVNAVNGSGGNYSVNWSNTGNFVVGKGWT TGSPFRTINYNAGVWAPNGNGYLTLYGWTRSPLIEYYVVDSWGTYRPTGTYKGTVKSD GGTYDIYTTTRYNAPSIDGD_ATFTQYWSVRQSKRPTGSNATITFTNHVNAWKSHGMNL GSNWAYQVMATEGYQSSGSSNVTVWGGASTDYWQNWTN
cpD11^{G2}	MKYLLPTAAAGLLLLAAQPAMADGGGIVNAVNGSGGNYSVNWSNTGNFVVGKGWTT GSPFRTINYNAGVWAPNGNGYLTLYGWTRSPLIEYYVVDSWGTYRPTGTYKGTVKSDG GTYDIYTTTRYNAPSIDGD_ATFTQYWSVRQSKRPTGSNATITFTNHVNAWKSHGMNLG SNWAYQVMATEGYQSSGSSNVTVWGGASTDYWQNWTN
cpG13^{G2}	MKYLLPTAAAGLLLLAAQPAMAGGIVNAVNGSGGNYSVNWSNTGNFVVGKGWTTGS PFRTINYNAGVWAPNGNGYLTLYGWTRSPLIEYYVVDSWGTYRPTGTYKGTVKSDGGT YDIYTTTRYNAPSIDGD_ATFTQYWSVRQSKRPTGSNATITFTNHVNAWKSHGMNLGSN WAYQVMATEGYQSSGSSNVTVWGGASTDYWQNWTN **(ΔD11-G12)**
cpA18^{G2}	MKYLLPTAAAGLLLLAAQPAMAAVNGSGGNYSVNWSNTGNFVVGKGWTTGSPFRTIN YNAGVWAPNGNGYLTLYGWTRSPLIEYYVVDSWGTYRPTGTYKGTVKSDGGTYDIYT TTRYNAPSIDGD_ATFTQYWSVRQSKRPTGSNATITFTNHVNAWKSHGMNLGSNWAYQ VMATEGYQSSGSSNVTVWGGASTDYWQNWTN **(ΔD11-GN17)**

cpG21^{G2}	MKYLLPTAAAGLLLLAAQPAMAGSGGNYSVNWSNTGNFVVGKGWTTGSPFRTINYNAGVWAPNGNGYLTLYGWTRSPLIEYYVVDSWGTYRPTGTYKGTVKSDGGTYDIYTTTRYNAPSIDGD_ATFTQYWSVRQSKRPTGSNATITFTNHVNAWKSHGMNLGSNWAYQVMATEGYQSSGSSNVTVWGGASTDYWQNWTDGGGIVNVTN **(ΔA18-N20)**
cpG23^{G2}	MKYLLPTAAAGLLLLAAQPAMAGGNYSVNWSNTGNFVVGKGWTTGSPFRTINYNAGVWAPNGNGYLTLYGWTRSPLIEYYVVDSWGTYRPTGTYKGTVKSDGGTYDIYTTTRYNAPSIDGD_ATFTQYWSVRQSKRPTGSNATITFTNHVNAWKSHGMNLGSNWAYQVMATEGYQSSGSSNVTVWGGASTDYWQNWTDGGGIVNAVNGS
cpS27^{G2}	MKYLLPTAAAGLLLLAAQPAMASVNWSNTGNFVVGKGWTTGSPFRTINYNAGVWAPNGNGYLTLYGWTRSPLIEYYVVDSWGTYRPTGTYKGTVKSDGGTYDIYTTTRYNAPSIDGD_ATFTQYWSVRQSKRPTGSNATITFTNHVNAWKSHGMNLGSNWAYQVMATEGYQSSGSSNVTVWGGASTDYWQNWTDGGGIVNAVNGSGGNYSVNWSITN
cpS31^{G2}	MKYLLPTAAAGLLLLAAQPAMASNTGNFVVGKGWTTGSPFRTINYNAGVWAPNGNGYLTLYGWTRSPLIEYYVVDSWGTYRPTGTYKGTVKSDGGTYDIYTTTRYNAPSIDGD_ATFTQYWSVRQSKRPTGSNATITFTNHVNAWKSHGMNLGSNWAYQVMATEGYQSSGSSNVTVWGGASTDYWQNWTDGGGIVNAVNGSGGNYSVNWS
cpG34 ^{G2}	MKYLLPTAAAGLLLLAAQPAMAGNFVVGKGWTTGSPFRTINYNAGVWAPNGNGYLTLYGWTRSPLIEYYVVDSWGTYRPTGTYKGTVKSDGGTYDIYTTTRYNAPSIDGD_ATFTQYWSVRQSKRPTGSNATITFTNHVNAWKSHGMNLGSNWAYQVMATEGYQSSGSSNVTVWGGASTDYWQNWTDGGGIVNAVNGSGGNYSVNWSNTGITN
cpN35^{G2}	MKYLLPTAAAGLLLLAAQPAMANFVVGKGWTTGSPFRTINYNAGVWAPNGNGYLTLYGWTRSPLIEYYVVDSWGTYRPTGTYKGTVKSDGGTYDIYTTTRYNAPSIDGD_ATFTQYWSVRQSKRPTGSNATITFTNHVNAWKSHGMNLGSNWAYQVMATEGYQSSGSSNVTVWGGASTDYWQNWTDGGGIVNAVNGSGGNYSVNWSNITN **(ΔT33-G34)**
cpN35^{G2,}	MNFVVGKGWTTGSPFRTINYNAGVWAPNGNGYLTLYGWTRSPLIEYYVVDSWGTYRPTGTYKGTVKSDGGTYDIYTTTRYNAPSIDGD_ATFTQYWSVRQSKRPTGSNATITFTNHVNAWKSHGMNLGSNWAYQVMATEGYQSSGSSNVTVWGGASTDYWQNWTDGGGIVNAVNGSGGNYSVNWSNTG
cpG56^{G2}	MKYLLPTAAAGLLLLAAQPAMAGVWAPNGNGYLTLYGWTRSPLIEYYVVDSWGTYRPTGTYKGTVKSDGGTYDIYTTTRYNAPSIDGD_ATFTQYWSVRQSKRPTGSNATITFTNHVNAWKSHGMNLGSNWAYQVMATEGYQSSGSSNVTVWGGASTDYWQNWTDGGGIVNAVNGSGGNYSVNWSNTGNFVVGKGWTTGSPFRTINYNAN
cpP60^{G2}	MKYLLPTAAAGLLLLAAQPAMAPNGNGYLTLYGWTRSPLIEYYVVDSWGTYRPTGTYKGTVKSDGGTYDIYTTTRYNAPSIDGD_ATFTQYWSVRQSKRPTGSNATITFTNHVNAWKSHGMNLGSNWAYQVMATEGYQSSGSSNVTVWGGASTDYWQNWTDGGGIVNAVNGSGGNYSVNWSNTGNFVVGKGWTTGSPFRTINYNAGVWAP
cpR73^{G2}	MKYLLPTAAAGLLLLAAQPAMARSPLIEYYVVDSWGTYRPTGTYKGTVKSDGGTYDIYTTTRYNAPSIDGD_ATFTQYWSVRQSKRPTGSNATITFTNHVNAWKSHGMNLGSNWAYQVMATEGYQSSGSSNVTVWGGASTDYWQNWTDGGGIVNAVNGSGGNYSVNWSNTGNFVVGKGWTTGSPFRTINYNAGVWAPNGNGYLTLYGWTN
cpE78^{G2}	MKYLLPTAAAGLLLLAAQPAMAEYYVVDSWGTYRPTGTYKGTVKSDGGTYDIYTTTRYNAPSIDGD_ATFTQYWSVRQSKRPTGSNATITFTNHVNAWKSHGMNLGSNWAYQVMATEGYQSSGSSNVTVWGGASTDYWQNWTDGGGIVNAVNGSGGNYSVNWSNTGNFVVGKGWTTGSPFRTINYNAGVWAPNGNGYLTLYGWTRSPLITN

cpG92^{G2}	MKYLLPTAAAGLLLLAAQPAMAGTYKGTVKSDGGTYDIYTTTRYNAPSIDGD_ATFTQYWSVRQSKRPTGSNATITFTNHVNAWKSHGMNLGSNWAYQVMATEGYQSSGSSNVTVWGGASTDYWQNWTDGGGIVNAVNGSGGNYSVNWSNTGNFVVGKGWTTGSPFRTINYNAGVWAPNGNGYLTLYGWTRSPLIEYYVVDSWGTYRPTN
cpY94^{G2} (1)	MKYLLPTAAAGLLLLAAQPAMAYKGTVKSDGGTYDIYTTTRYNAPSIDGD_ATFTQYWSVRQSKRPTGSNATITFTNHVNAWKSHGMNLGSNWAYQVMATEGYQSSGSSNVTVWGGASTDYWQNWTDGGGIVNAVNGSGGNYSVNWSNTGNFVVGKGWTTGSPFRTINYNAGVWAPNGNGYLTLYGWTRSPLIEYYVVDSWGTYRPN **(ΔT91-T93)**
cpY94^{G2} (2)	MKYLLPTAAAGLLLLAAQPAMAYKGTVKSDGGTYDIYTTTRYNAPSIDGD_ATFTQYWSVRQSKRPTGSNATITFTNHVNAWKSHGMNLGSNWAYQVMATEGYQSSGSSNVTVWGGASTDYWQNWTDGGGIVNAVNGSGGNYSVNWSNTGNFVVGKGWTTGSPFRTINYNAGVWAPNGNGYLTLYGWTRSPLIEYYVVDSWGTYRPTGTYITN
cpY94^{G2},	MYKGTVKSDGGTYDIYTTTRYNAPSIDGD_ATFTQYWSVRQSKRPTGSNATITFTNHVNAWKSHGMNLGSNWAYQVMATEGYQSSGSSNVTVWGGASTDYWQNWTDGGGIVNAVNGSGGNYSVNWSNTGNFVVGKGWTTGSPFRTINYNAGVWAPNGNGYLTLYGWTRSPLIEYYVVDSWGTYRPTGT
cpG96^{G2}	MKYLLPTAAAGLLLLAAQPAMAGTVKSDGGTYDIYTTTRYNAPSIDGD_ATFTQYWSVRQSKRPTGSNATITFTNHVNAWKSHGMNLGSNWAYQVMATEGYQSSGSSNVTVWGGASTDYWQNWTDGGGIVNAVNGSGGNYSVNWSNTGNFVVGKGWTTGSPFRTINYNAGVWAPNGNGYLTLYGWTRSPLIEYYVVDSWGTYRPTGTYITN **(ΔK95)**
cpV98^{G2}	MKYLLPTAAAGLLLLAAQPAMAVKSDGGTYDIYTTTRYNAPSIDGD_ATFTQYWSVRQSKRPTGSNATITFTNHVNAWKSHGMNLGSNWAYQVMATEGYQSSGSSNVTVWGGASTDYWQNWTDGGGIVNAVNGSGGNYSVNWSNTGNFVVGKGWTTGSPFRTINYNAGVWAPNGNGYLTLYGWTRSPLIEYYVVDSWGTYRPTGT **(ΔY94-T97)**
cpD119^{G2}	MKYLLPTAAAGLLLLAAQPAMADGD_ATFTQYWSVRQSKRPTGSNATITFTNHVNAWKSHGMNLGSNWAYQVMATEGYQSSGSSNVTVWGGASTDYWQNWTDGGGIVNAVNGSGGNYSVNWSNTGNFVVGKGWTTGSPFRTINYNAGVWAPNGNGYLTLYGWTRSPLIEYYVVDSWGTYRPTGTYKGTVKSDGGTYDIYTTTRYNAPSIDN
cpQ127^{G2}	MKYLLPTAAAGLLLLAAQPAMAQYWSVRQSKRPTGSNATITFTNHVNAWKSHGMNLGSNWAYQVMATEGYQSSGSSNVTVWGGASTDYWQNWTDGGGIVNAVNGSGGNYSVNWSNTGNFVVGKGWTTGSPFRTINYNAGVWAPNGNGYLTLYGWTRSPLIEYYVVDSWGTYRPTGTYKGTVKSDGGTYDIYN (ΔT109-R126)
cpK135^{G2}	MKYLLPTAAAGLLLLAAQPAMAKRPTGSNATITFTNHVNAWKSHGMNLGSNWAYQVMATEGYQSSGSSNVTVWGGASTDYWQNWTDGGGIVNAVNGSGGNYSVNWSNTGNFVVGKGWTTGSPFRTINYNAGVWAPNGNGYLTLYGWTRSPLIEYYVVDSWGTYRPTGTYKGTVKSDGGTYDIYTTTRYNAPSIDGD_ATFTQYWSVRQSKLTN
cpG139^{G2}	MKYLLPTAAAGLLLLAAQPAMAGSNATITFTNHVNAWKSHGMNLGSNWAYQVMATEGYQSSGSSNVTVWGGASTDYWQNWTDGGGIVNAVNGSGGNYSVNWSNTGNFVVGKGWTTGSPFRTINYNAGVWAPNGNGYLTLYGWTRSPLIEYYVVDSWGTYRPTGTYKGTVKSDGGTYDIYTTTRYNAPSIDGD_ATFTQYWSVRQSKRPT
cpS162^{G2}	MKYLLPTAAAGLLLLAAQPAMASNWAYQVMATEGYQSSGSSNVTVWGGASTDYWQNWTDGGGIVNAVNGSGGNYSVNWSNTGNFVVGKGWTTGSPFRTINYNAGVWAPNGNGYLTLYGWTRSPLIEYYVVDSWGTYRPTGTYKGTVKSDGGTYDIYTTTRYNAPSIDGD_ATFTQYWSVRQSKRPTGSNATITFTNHVNAWKSHGMNLG

cpN163^{G2}	MKYLLPTAAAGLLLLAAQPAMANWAYQVMATEGYQSSGSSNVTVWGGASTDYWQNWTDGGGIVNAVNGSGGNYSVNWSNTGNFVVGKGWTTGSPFRTINYNAGVWAPNGNGYLTLYGWTRSPLIEYYVVDSWGTYRPTGTYKGTVKSDGGTYDIYTTTRYNAPSIDGD_ATFTQYWSVRQSKRPTGSNATITFTNHVNAWKSHGMNLTN **(ΔG161-G162)**
cpY166^{G2}	MKYLLPTAAAGLLLLAAQPAMAYQVMATEGYQSSGSSNVTVWGGASTDYWQNWTDGGGIVNAVNGSGGNYSVNWSNTGNFVVGKGWTTGSPFRTINYNAGVWAPNGNGYLTLYGWTRSPLIEYYVVDSWGTYRPTGTYKGTVKSDGGTYDIYTTTRYNAPSIDGD_ATFTQYWSVRQSKRPTGSNATITFTNHVNAWKSHGMNLGSNWAYHN
cpY174^{G2}	MKYLLPTAAAGLLLLAAQPAMAYQSSGSSNVTVWGGASTDYWQNWTDGGGIVNAVNGSGGNYSVNWSNTGNFVVGKGWTTGSPFRTINYNAGVWAPNGNGYLTLYGWTRSPLIEYYVVDSWGTYRPTGTYKGTVKSDGGTYDIYTTTRYNAPSIDGD_ATFTQYWSVRQSKRPTGSNATITFTNHVNAWKSHGMNLGSNWAYQVMATEGYQ
cpY174$^{G2'}$	MYQSSGSSNVTVWGGASTDYWQNWTDGGGIVNAVNGSGGNYSVNWSNTGNFVVGKGWTTGSPFRTINYNAGVWAPNGNGYLTLYGWTRSPLIEYYVVDSWGTYRPTGTYKGTVKSDGGTYDIYTTTRYNAPSIDGD_ATFTQYWSVRQSKRPTGSNATITFTNHVNAWKSHGMNLGSNWAYQVMATEG
cpG178^{G2}	MKYLLPTAAAGLLLLAAQPAMAGSSNVTVWGGASTDYWQNWTDGGGIVNAVNGSGGNYSVNWSNTGNFVVGKGWTTGSPFRTINYNAGVWAPNGNGYLTLYGWTRSPLIEYYVVDSWGTYRPTGTYKGTVKSDGGTYDIYTTTRYNAPSIDGD_ATFTQYWSVRQSKRPTGSNATITFTNHVNAWKSHGMNLGSNWAYQVMATEGYQSSGSSN
cpN181^{G2}	MKYLLPTAAAGLLLLAAQPAMANVTVWGGASTDYWQNWTDGGGIVNAVNGSGGNYSVNWSNTGNFVVGKGWTTGSPFRTINYNAGVWAPNGNGYLTLYGWTRSPLIEYYVVDSWGTYRPTGTYKGTVKSDGGTYDIYTTTRYNAPSIDGD_ATFTQYWSVRQSKRPTGSNATITFTNHVNAWKSHGMNLGSNWAYQVMATEGYQSS **(ΔG178-S180)**
cpW185^{G2}	MKYLLPTAAAGLLLLAAQPAMAWGGASTDYWQNWTDGGGIVNAVNGSGGNYSVNWSNTGNFVVGKGWTTGSPFRTINYNAGVWAPNGNGYLTLYGWTRSPLIEYYVVDSWGTYRPTGTYKGTVKSDGGTYDIYTTTRYNAPSIDGD_ATFTQYWSVRQSKRPTGSNATITFTNHVNAWKSHGMNLGSNWAYQVMATEGYQSSGSSNVTVW
cpLink2^{G2}	MKYLLPTAAAGLLLLAAQPAMAGASTDYWQNWTDGGGIVNAVNGSGGNYSVNWSNTGNFVVGKGWTTGSPFRTINYNAGVWAPNGNGYLTLYGWTRSPLIEYYVVDSWGTYRPTGTYKGTVKSDGGTYDIYTTTRYNAPSIDGD_ATFTQYWSVRQSKRPTGSNATITFTNHVNAWKSHGMNLGSNWAYQVMATEGYQSSGSSNVTVLTN **(ΔW185-GLink1)**

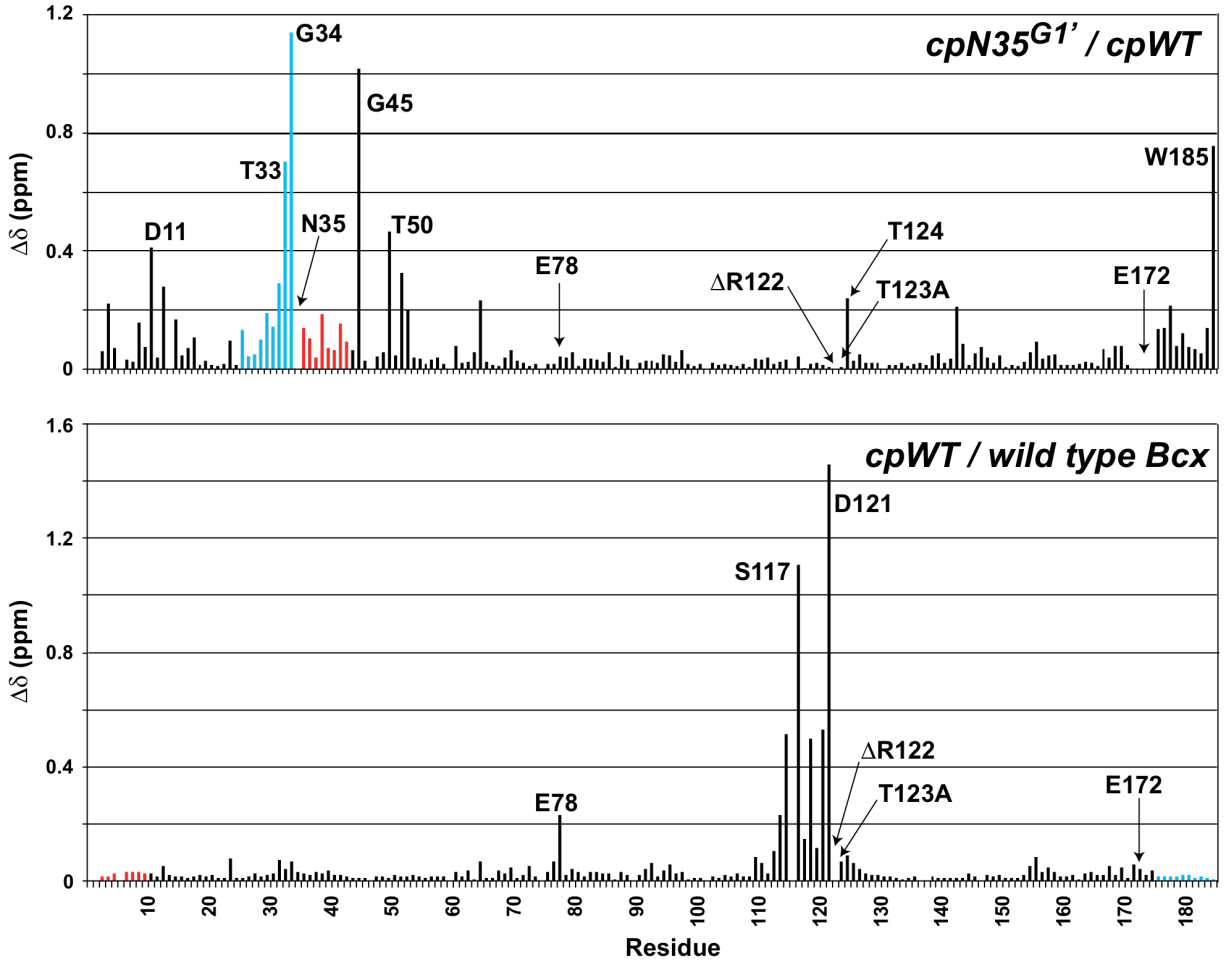
[a] The wild type terminal Alal and Trp185 are highlighted in magenta, and the two linker glycines (GLink1 and GLink2) in green. Due to the cloning strategy, the permutants also contain the ΔArg122 deletion (dash) and T123A mutation (yellow). Any additional amino acids are in cyan and missing residues are stated in brackets. The PelB signal peptide or, in the case of the sub-cloned permutations, the N-terminal methionine (grey) may be removed post-translationally. Wild type numbering is applied.

Supplemental Table S4: Primers used for sub-cloning randomly generated Bcx permutants

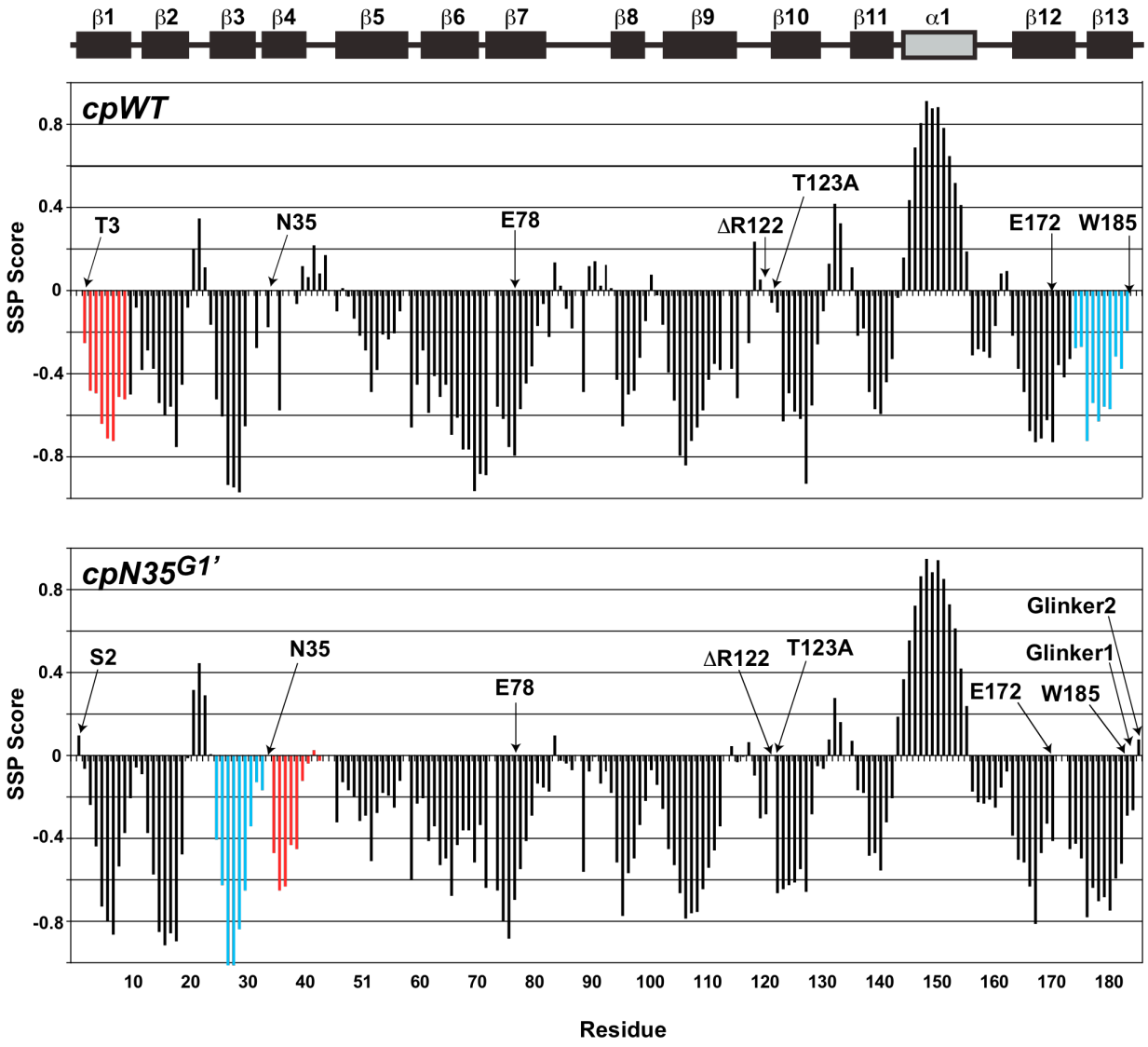
Permutant	Sequence	All primer sequences in 5' → 3' direction
cpWT	A1/W185	For: CTTCATATGGCTAGCACAGATTACTGGCAAAAC Rev: GTTAAGCTTTTACCACACTGTAACGTTGGAAGAAC
cpN35$^{G2'}$	N35/G34	For: CTTCATATGAACTTCGTAGTCGGAAAAGGTTGG Rev: GTTAAGCTTTTACCCAGTATTAGACCAATTCACGC
cpY94$^{G2'}$	Y94/T93	For: CTTCATATGTACAAAGGCACAGTCAAAAGCG Rev: GTTAAGCTTTTAGGTTCCAGTCGGACGGTACGTTC
cpY174$^{G2'}$	Y174/G173	For: CTTCATATGTACCAGAGCTCTGGTTCTTCCAAC Rev: GTTAAGCTTTTAGCCTTCGGTCGCCATTACTTGATA

Supplemental Table S5: X-ray crystallographic data collection and refinement statistics for cpA123[G1]

Data collection	
X-ray source	CuKα
Wavelength (Å)	1.541
Space group	C2
Molecules/AU	3
Unit cell (Å, deg)	a = 69.8 b = 114.9 c = 65.0 β = 113.4
Resolution (Å)	2.8
Highest shell (Å)	2.8-2.93
# Observations	39,878 (4,229)
Unique reflections	10,649 (1,147)
I/σ	10.2 (2.6)
R_{sym} (%)*	14.7 (41.2)
Completeness (%)	99.8 (99.2)
Refinement	
Resolution range (Å)	20-3
# Reflections	9441
R_{work} / R_{free} (%)*	15 / 26
# Atoms	4,303
Protein	4,263
Solvent	40
Mean B value ($Å^2$)	18.7
RMSD:	
Bond length (Å)	0.008
Bond angle (deg)	1.2
PDB accession code	3LB9



Supplemental Figure S1: Amide shift perturbation plots ($\Delta\delta = [(\Delta\delta_{1H})^2 + [(0.1\Delta\delta_{15N})]^{1/2}$) of (top) cpN35$^{G2'}$ versus cpWT and (bottom) cpWT versus wild type Bcx. Chemical shift changes are localized to the sites of the old and new termini in (A) and the deletion of Arg122 and mutation of Thr123 to Ala in (B). Thus, both the permutation and deletion/mutation cause only localized structural perturbations. Wild type numbering is used, and the first/last 10 residues at the N/C-termini of cpN35$^{G2'}$ in (A) and cpWT and wild type Bcx in (B) are colored in red/blue, respectively.



Supplemental Figure S2: Secondary structural propensity (SSP) scores of (top) cpWT and (bottom) cpN35$^{G2'}$ derived from available main chain $^{13}C^{\alpha}$, $^{13}C^{\beta}$, $^{13}C'$, $^{1}H^{N}$, and ^{15}N chemical shifts are highly similar. Values approaching +1 and -1 are indicative of well defined α-helices and β-strands, respectively (*62*). Wild type numbering is used, and the first/last 10 residues at the N/C-termini of each protein are colored in red/blue. Also shown is a cartoon of the β-strands and single α-helix observed in the crystal structure of wild type Bcx, as defined by Promotif (*65*).