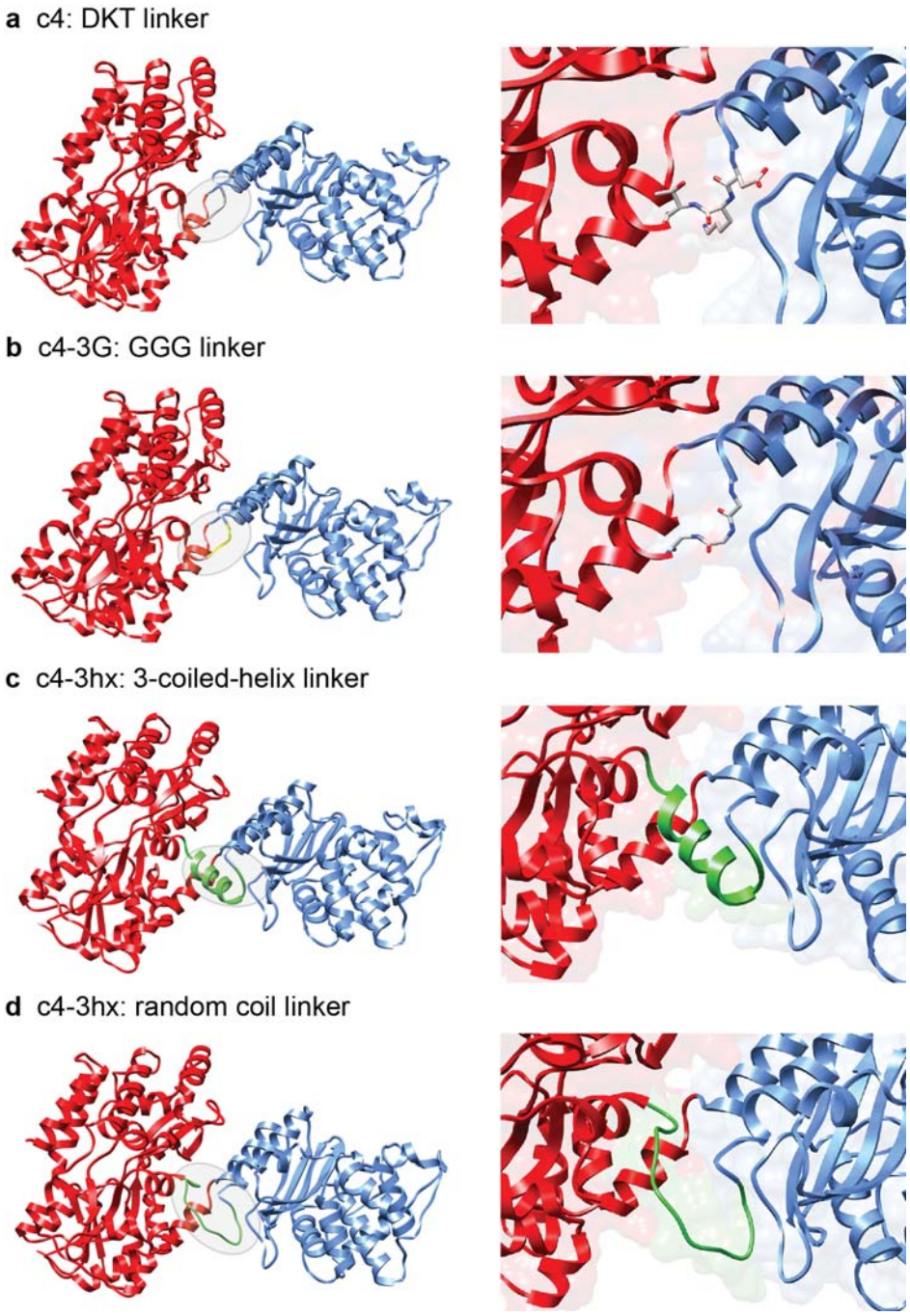
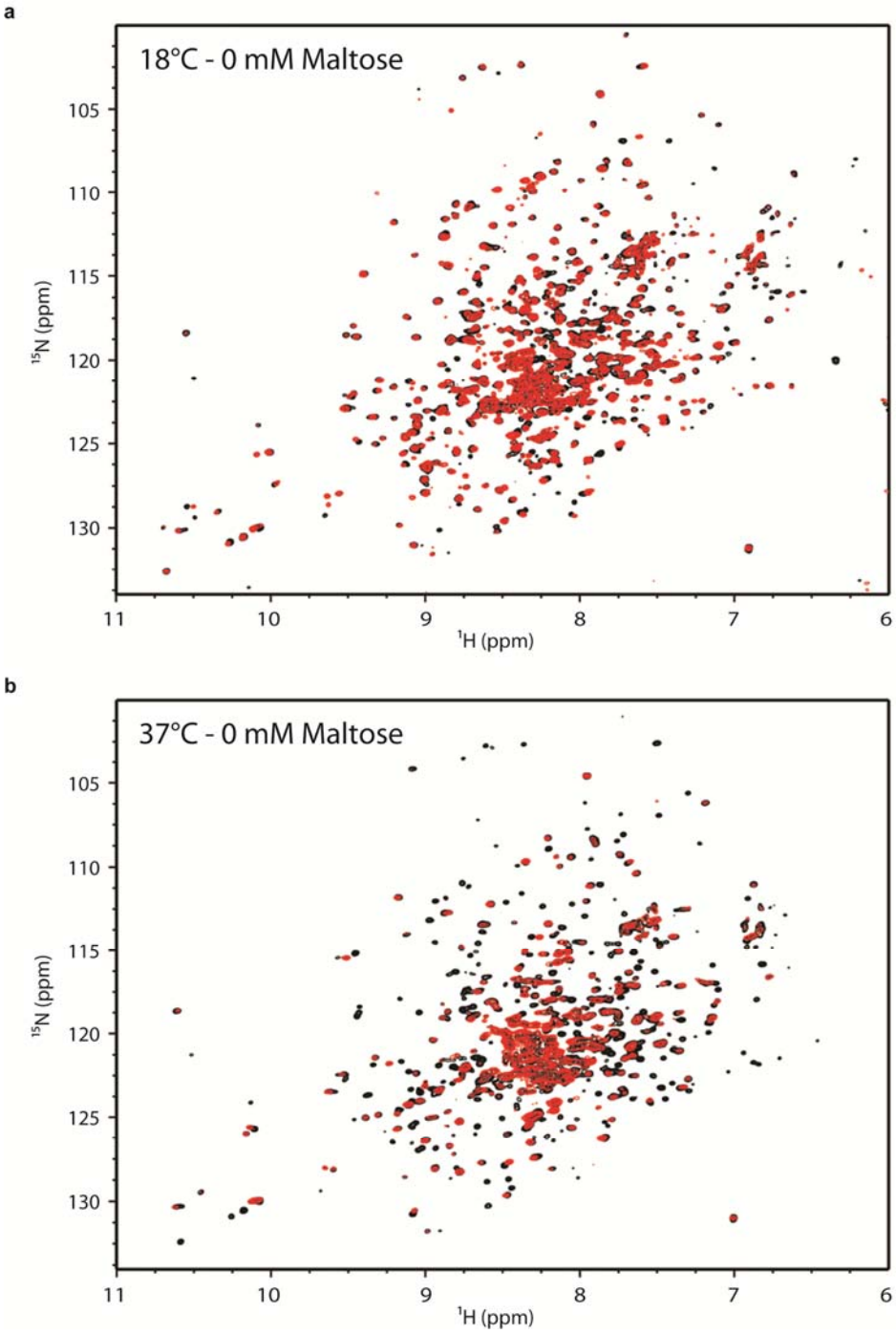
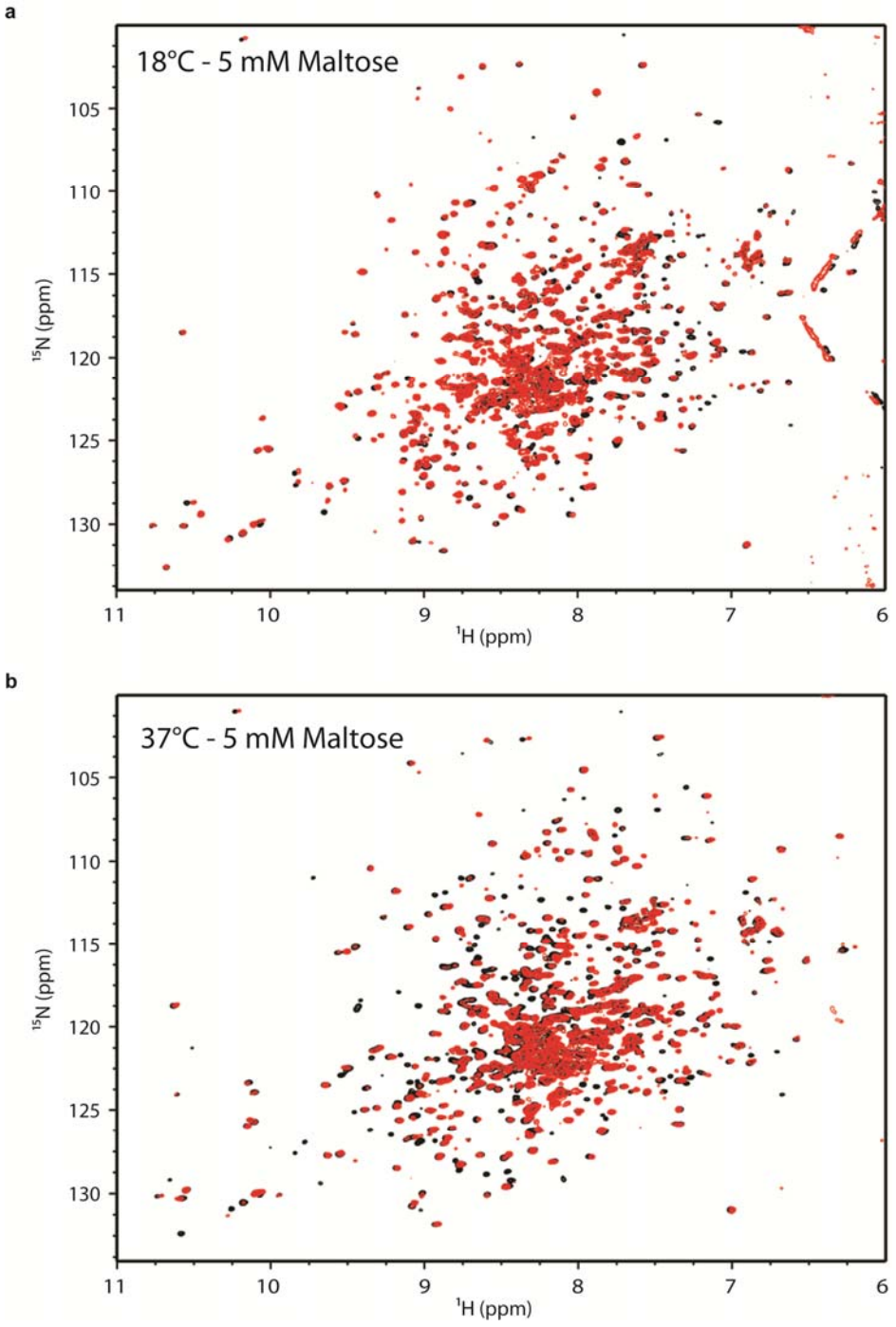


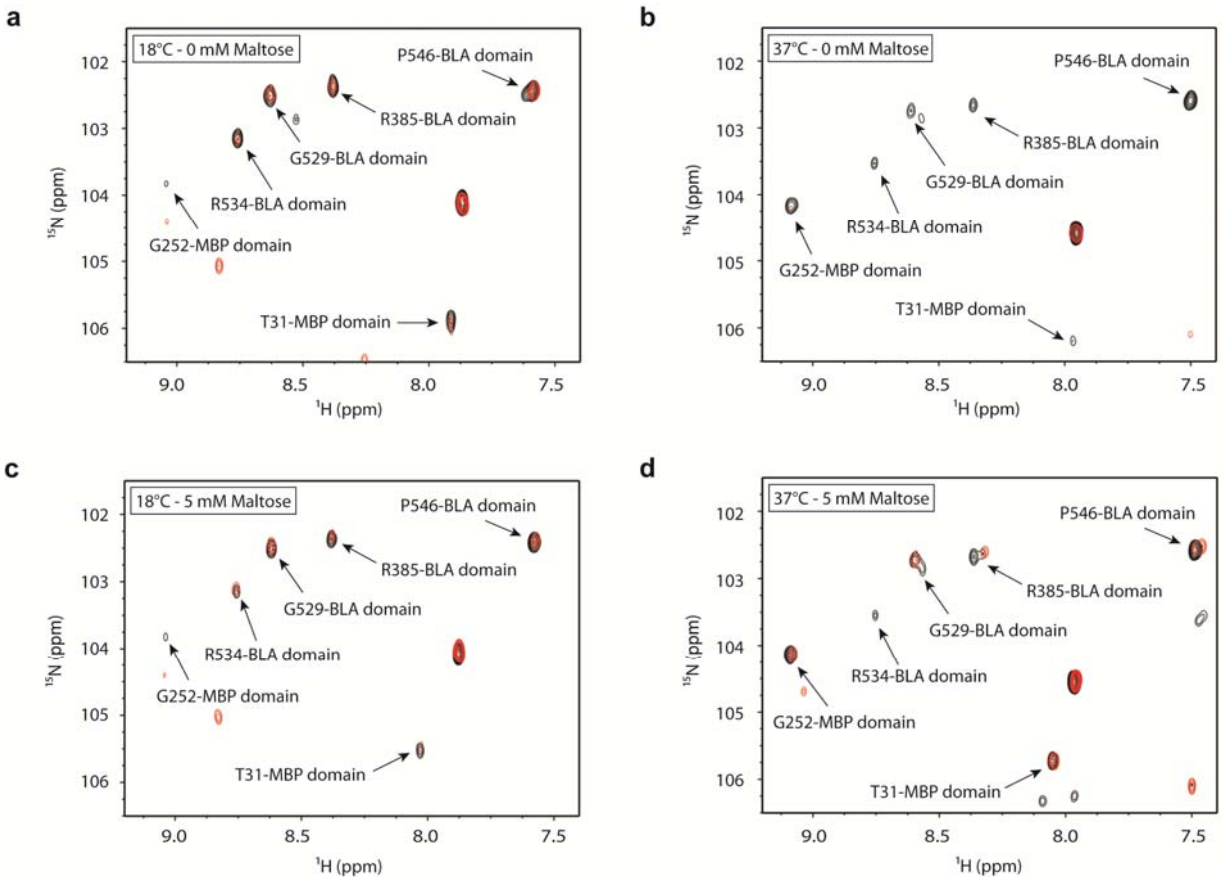
Supplementary Figure 1. Structure models of the c4 variant proteins based on the structures of maltose binding protein (MBP) in red and TEM-1 β-lactamase (BLA) in blue. (**a**) c4 model and the close-up view of DKT linker with the side-chains. (**b**) c4-3G with GGG linker (yellow) and the close-up view. (**c**) c4-3hx in acidic condition. The linker, $(EAQA)_3$, is in α-helical conformation and is shown in green. (**d**) c4-3hx in basic condition. The linker, $(EAQA)_3$, is in a random coil form and is shown in green.



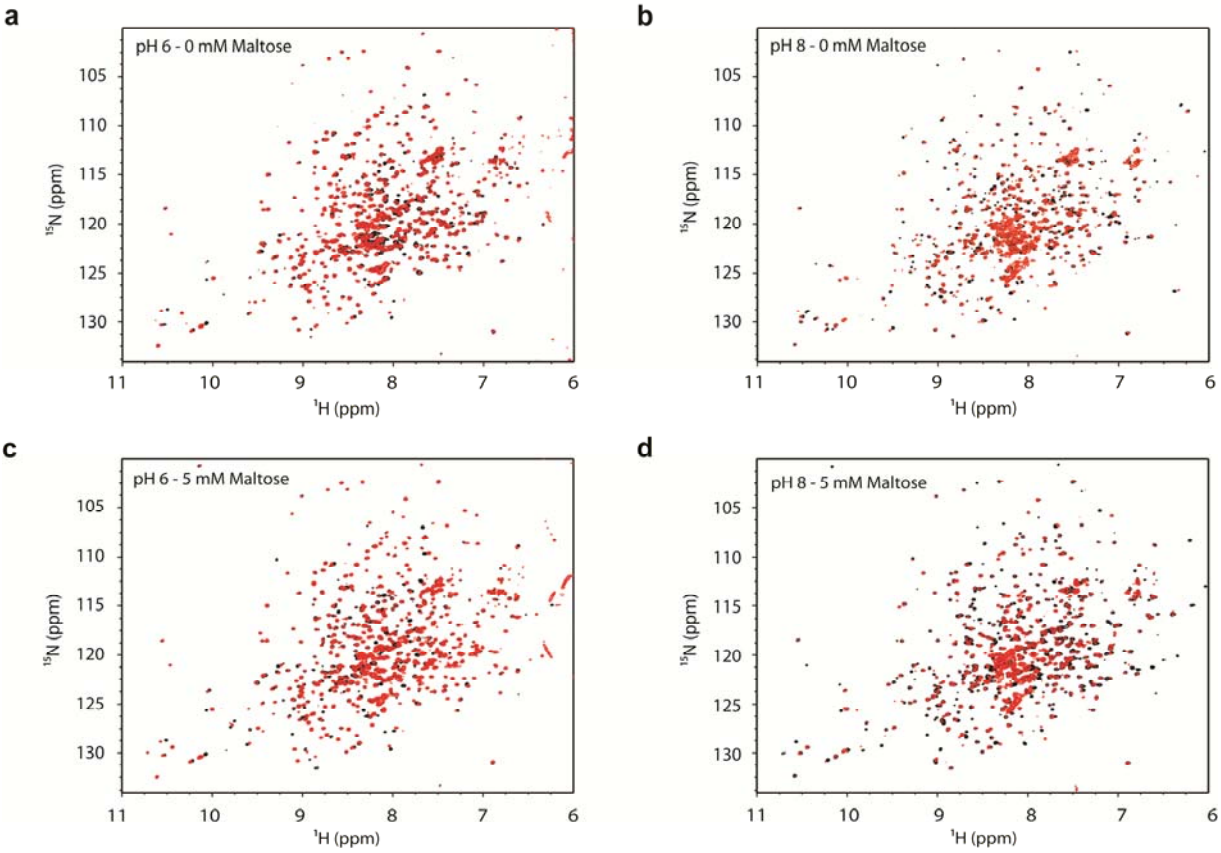
Supplementary Figure 2. The overlays of ^{15}N-TROSY-HSQC spectra of c4 and c4-3G at 18°C and 37°C in the absence of maltose. The black spectrum was acquired from c4, and the red spectrum was acquired from c4-3G at (**a**) 18°C and (**b**) 37°C.



Supplementary Figure 3. The overlays of ^{15}N-TROSY-HSQC spectra of c4 and c4-3G at 18°C and 37°C in the presence of maltose. The black spectrum was acquired from c4, and the red spectrum was acquired from c4-3G at (**a**) 18°C and (**b**) 37°C.



Supplementary Figure 4. A close-up view of a region in the c4 and c4-3G spectra overlay at (**a)** 18°C in the absence of maltose, (**b**) 37°C in the absence of maltose, (**c**) 18°C in the presence of 5 mM maltose, and (**d**) 37°C in the presence of 5 mM maltose. The black spectrum was acquired from c4, and the red spectrum was acquired from c4-3G.



Supplementary Figure 5. The overlays of ^{15}N-TROSY-HSQC spectra of c4 and c4-3hx in pH 6 and pH 8 in the absence and presence of maltose at 25°C. The black spectrum was acquired from c4, and the red spectrum was acquired from c4-3hx at (**a**) pH 6 in the absence of maltose, (**b**) at pH 8 in the absence of maltose, (**c**) at pH 6 in the presence of 5 mM maltose, and (**d**) pH 8 in the presence of maltose.

SUPPLEMENTARY TABLES

Supplementary Table 1. Primary sequence of fusion proteins used in this study.

Protein (linker[a])	Protein Sequence[b]
c4 (DKT)	MBP[1-316]-BLA[24-286]-DKT-MBP[319-370]-6xHis
c4-1G (GKT)	MBP[1-316]-BLA[24-286]-GKT-MBP[319-370]-6xHis
c4-2G (GGT)	MBP[1-316]-BLA[24-286]-GGT-MBP[319-370]-6xHis
c4-3G (GGG)	MBP[1-316]-BLA[24-286]-GGG-MBP[319-370]-6xHis
c4-4G (G/GGG)	MBP[1-315]-G-BLA[24-286]-GGG-MBP[319-370]-6xHis
c4-5G (GG/GGG)	MBP[1-314]-GG-BLA[24-286]-GGG-MBP[319-370]-6xHis
c4-2hx $(EAQA)_2$	MBP[1-316]-BLA[24-286]-G-$(EAQA)_2$-EG-MBP[319-370]-6xHis
c4-3hx $(EAQA)_3$	MBP[1-316]-BLA[24-286]-G-$(EAQA)_3$-EG-MBP[319-370]-6xHis
c4-4hx $(EAQA)_4$	MBP[1-316]-BLA[24-286]-G-$(EAQA)_4$-EG-MBP[319-370]-6xHis

[a] Linker residues between BLA[24-286]and MBP[319-370] and MBP[1-314] and BLA[24-286].
[b] Based on gene sequencing.

Supplementary Table 2. MIC_{Amp} of BL21 cells expressing c4 glycine variants at 18°C.

Protein	Linker residues[a]	Trial	MIC_{Amp} ($\mu g\ ml^{-1}$)[b]		
			- Maltose	+ Maltose	Ratio[c]
MBP	N/A	1	1	1	1
		2	1	1	1
		3	1	1	1
		Median	1	1	1
c4	DKT	1	4096	4096	1
		2	4096	4096	1
		3	2048	2048	1
		Median	4096	4096	1
c4-1G	GKT	1	4096	4096	1
		2	4096	4096	1
		3	2048	2048	1
		Median	4096	4096	1
c4-2G	GGT	1	4096	4096	1
		2	4096	4096	1
		3	2048	2048	1
		Median	4096	4096	1
c4-3G	GGG	1	4096	4096	1
		2	4096	4096	1
		3	2048	2048	1
		Median	4096	4096	1
c4-4G	GGG + R315G	1	4096	4096	1
		2	4096	4096	1
		3	2048	2048	1
		Median	4096	4096	1
c4-5G	GGG + R315G P316G	1	4096	4096	1
		2	4096	4096	1
		3	2048	2048	1
		Median	4096	4096	1

[a] Residues between BLA[24-286] and MBP[319-370].
[b] MIC_{Amp} was defined as the lowest concentration of ampicillin at which the OD_{600nm} was < 5 % of the OD_{600nm} in the absence of ampicillin.
[c] (With maltose) / (without maltose).

Supplementary Table 3. MIC_{Amp} of BL21 cells expressing c4 glycine variants at 37°C.

Protein	Linker residues[b]	Trial	MIC_{Amp} (μg ml^{-1})[b] - Maltose	+ Maltose	Ratio[c]
MBP	N/A	1	1	1	1
		2	1	1	1
		3	1	1	1
		Median	1	1	1
c4	DKT	1	2048	2048	1
		2	2048	2048	1
		3	1024	2048	2
		Median	2048	2048	1
c4-1G	GKT	1	2048	2048	1
		2	1024	2048	2
		3	1024	2048	2
		Median	1024	2048	2
c4-2G	GGT	1	512	2048	4
		2	512	2048	4
		3	512	2048	4
		Median	512	2048	4
c4-3G	GGG	1	128	2048	16
		2	64	1024	16
		3	64	2048	32
		Median	64	2048	16
c4-4G	GGG + R315G	1	64	2048	32
		2	32	2048	64
		3	64	2048	32
		Median	64	2048	32
c4-5G	GGG + R315G P316G	1	64	2048	32
		2	64	1024	16
		3	64	1024	16
		Median	64	1024	16

[a] Residues between BLA[24-286] and MBP[319-370].
[b] MIC_{Amp} was defined as the lowest concentration of ampicillin at which the OD_{600nm} was < 5 % of the OD_{600nm} in the absence of ampicillin.
[c] (With maltose) / (without maltose).

Supplementary Table 4. MIC_{Amp} of BL21 cells expressing c4 EAQA linker variants at 37°C.

		MIC_{Amp} ($\mu g\ ml^{-1}$)[b] when grown on media at the indicated pH[c]							
		- Maltose				+ Maltose			
Protein (Linker)[a]	Trial	6	7	7.5	8	6	7	7.5	8
c4	1	4096	2048	1024	512	4096	4096	1024	512
(DKT)	2	2048	1024	1024	512	2048	2048	1024	1024
	3	2048	1024	1024	512	2048	2048	1024	1024
	Median	2048	1024	1024	512	2048	2048	1024	1024
c4-2hx	1	4096	2048	256	256	4096	4096	1024	512
$(EAQA)_2$	2	2048	2048	256	128	4096	4096	1024	1024
	3	2048	2048	256	128	4096	4096	1024	1024
	Median	2048	2048	256	128	4096	4096	1024	1024
c4-3hx	1	2048	1024	256	32	4096	2048	1024	256
$(EAQA)_3$	2	2048	512	128	32	4096	4096	1024	512
	3	2048	512	128	64	4096	4096	1024	512
	Median	2048	512	128	32	4096	4096	1024	512
c4-4hx	1	2048	2048	512	32	4096	4096	1024	512
$(EAQA)_4$	2	1024	512	128	32	2048	2048	1024	512
	3	1024	512	128	64	2048	2048	1024	512
	Median	1024	512	128	32	2048	2048	1024	512

[a] Residues between BLA[24-286] and MBP[319-370].
[b] MIC_{Amp} was defined as the lowest concentration of ampicillin at which the OD_{600nm} was < 5 % of the OD_{600nm} in the absence of ampicillin.
[c] Initial pH of growth media

Supplementary Table 5. Enzymatic activity of c4 and c4-3G at 18 °C and 37 °C.

		Initial Rate[a] (sec^{-1})		
Protein	Temperature	- Maltose	+ Maltose	Ratio[b]
c4	18 °C	60.2 ± 3.4	64.2 ± 0.9	1.07 ± 0.05
	37 °C	99.9 ± 1.5	103.6 ± 2.1	1.04 ± 0.04
c4-3G	18 °C	56.3 ± 2.1	58.0 ± 2.3	1.03 ± 0.04
	37 °C	44.2 ± 1.2	90.7 ± 4.6	2.05 ± 0.15

[a] Initial rate of hydrolysis of 100 μM nitrocefin at pH 7.2 at the indicated temperature in the presence or absence of 5 mM maltose. Error is the standard deviation of three measurements.
[b] (With maltose) / (without maltose).

Supplementary Table 6. Enzymatic activity of c4 and c4-3hx at pH 6 and pH 8.

		Initial Rate[a] (sec^{-1})		
Protein	pH	- Maltose	+ Maltose	Ratio[b]
c4	6.0	117.6 ± 5.4	124.0 ± 6.9	1.06 ± 0.07
	8.0	106.9 ± 4.3	120.7 ± 2.1	1.13 ± 0.03
c4-3hx	6.0	97.1 ± 1.6	104.1 ± 10.2	1.07 ± 0.11
	8.0	25.3 ± 1.9	68.9 ± 2.9	2.73 ± 0.23

[a] Initial rate of hydrolysis of 100 μM nitrocefin at 37°C at the indicated pH in the presence or absence of 5 mM maltose. Error is the standard deviation of three independent measurements.
[b] (With maltose) / (without maltose).

Supplementary Table 7. Number of identifiable peaks for c4 and c4-3G at 18 °C and 37 °C, and c4 and c4-3hx in pH 6 and pH 8 at 25 °C compared to the number of assigned residues for MBP[a] and BLA[b].

Protein	Temperature or pH[c]	Maltose	Both domains	MBP domain	BLA domain
c4	18 °C	0 mM	387 / 579 (67%)	221 / 333 (66%)	166 / 246 (68%)
		5 mM	400 / 579 (69%)	230 / 333 (69%)	170 / 246 (69%)
	37 °C	0 mM	398 / 579 (69%)	217 / 333 (65%)	181 / 246 (74%)
		5 mM	399 / 579 (63%)	222 / 333 (67%)	177 / 246 (72%)
c4-3G	18 °C	0 mM	395 / 579 (68%)	228 / 333 (69%)	167 / 246 (68%)
		5 mM	393 / 579 (68%)	225 / 333 (68%)	168 / 246 (68%)
	37 °C	0 mM	247 / 579 (43%)	143 / 333 (43%)	104 / 246 (42%)
		5 mM	382 / 579 (66%)	218 / 333 (66%)	164 / 246 (67%)
c4	pH 6	0 mM	375 / 579 (65%)	215 / 333 (65%)	160 / 246 (65%)
		5 mM	395 / 579 (68%)	224 / 333 (67%)	171 / 246 (70%)
	pH 8	0 mM	388 / 579 (67%)	228 / 333 (69%)	160 / 246 (65%)
		5 mM	376 / 579 (65%)	221 / 333 (66%)	155 / 246 (63%)
c4-3hx	pH 6	0 mM	371 / 579 (64%)	214 / 333 (64%)	157 / 246 (64%)
		5 mM	371 / 579 (64%)	213 / 333 (64%)	158 / 246 (64%)
	pH 8	0 mM	362 / 579 (63%)	214 / 333 (64%)	148 / 246 (60%)
		5 mM	363 / 579 (63%)	214 / 333 (64%)	149 / 246 (61%)

[a] Evenas et al.[1].
[b] Savard et al.[2].
[c] Where a temperature is indicated, the pH was pH 7.2. Where a pH is indicated, the temperature was 25 °C.

Supplementary Table 8. MIC_{Amp} of BL21 cells expressing c4 and c4-3hx at 25°C.

Protein	Linker residues[a]	MIC_{Amp} ($\mu g\ ml^{-1}$)[b]			
		Trial	- Maltose	+ Maltose	Ratio[c]
c4	DKT	1	1024	1024	1
		2	1024	1024	1
		3	1024	1024	1
		Median	1024	1024	1
c4-3hx	$(EAQA)_3$	1	1024	1024	1
		2	1024	1024	1
		3	1024	1024	1
		Median	1024	1024	1

[a] Residues between BLA[24-286] and MBP[319-370].
[b] MIC_{Amp} was defined as the lowest concentration of ampicillin at which the OD_{600nm} was < 5 % of the OD_{600nm} in the absence of ampicillin.
[c] (With maltose) / (without maltose).

Supplementary Table 9. Transition midpoint (T_m) of c4, c4-3G, and c4-3hx quantified by circular dichroism in the absence and presence of maltose.

			Transition Midpoint[a] (°C)	
Protein			-Maltose	+Maltose
c4	pH 7.2	T_m^1	47.7 ± 0.7	N/A
		T_m^2	64.5 ± 0.2	63.5 ± 0.3
c4-3G	pH 7.2	T_m^1	45.4 ± 1.0	N/A
		T_m^2	66.9 ± 0.5	63.9 ± 0.1
c4	pH6	T_m^1	47.9 ± 0.5	58.4 ± 0.2
		T_m^2	N/A	N/A
	pH8	T_m^1	41.7 ± 0.4	56.8 ± 0.2
		T_m^2	69.3 ± 0.5	N/A
c4-3hx	pH6	T_m^1	51.6 ± 0.1	59.8 ± 0.2
		T_m^2	N/A	N/A
	pH8	T_m^1	35.3 ± 0.3	54.4 ± 1.6
		T_m^2	N/A	N/A

[a] Error is the standard deviation calculated from three independent measurements.

Supplementary Table 10. Calorimetric parameters of c4, c4-3G, and c4-3hx quantified by differential scanning calorimetry in the temperature range 20°C to 53°C.

Protein	Calorimetric Parameters[a]			
	T_m (°C)	ΔH_{cal}[b] (kcal mol^{-1})	ΔH_{vH}[c] (kcal mol^{-1})	ΔH_{cal} / ΔH_{vH}
c4[d]	47.0 ± 0.0	195.3 ± 1.2	187.7 ± 4.0	1.04 ± 0.03
c4-3G[d]	42.5 ± 0.0	90.2 ± 6.8	100.6 ± 3.4	0.90 ± 0.09
	46.6 ± 0.2	62.3 ± 0.1	200.9 ± 4.4	0.31 ± 0.01
	48.4 ± 0.1	37.3 ± 6.4	277.7 ± 19.7	0.14 ± 0.03
c4[e]	45.1 ± 0.0	161.7 ± 1.5	142.3 ± 2.6	1.14 ± 0.03
c4-3hx[e]	35.2 ± 0.7	22.8 ± 1.6	77.1 ± 5.1	0.30 ± 0.01
	39.3 ± 0.1	59.7 ± 4.5	112.4 ± 3.1	0.53 ± 0.06
	43.3 ± 0.0	29.9 ± 0.7	175.7 ± 0.6	0.17 ± 0.01

[a] Error is the standard deviation calculated from three independent measurements.
[b] The calorimetric enthalpy at the transition midpoint.
[c] The van't Hoff enthalpy at the transition midpoint.
[d] Protein was in the sample buffer of pH 7.2.
[e] Protein was in the sample buffer of pH 8.0.

SUPPLEMENTARY REFERENCES

1. Evenas, J. et al. Ligand-induced structural changes to maltodextrin-binding protein as studied by solution NMR spectroscopy. *J Mol Biol* **309**, 961-74 (2001).
2. Savard, P.Y. & Gagne, S.M. Backbone dynamics of TEM-1 determined by NMR: evidence for a highly ordered protein. *Biochemistry* **45**, 11414-24 (2006).