Supplementary Table 1 Crystallization conditions for each variant

Variant	Protein concentration (mg/ml)	Concentration of ammonium sulfate (M)	pH
A	54.0	2.75	5.40
	61.5	2.75	5.40
B	80.0	2.75	5.35, 5.40, 5.45
E	51.0	2.75, 2.80, 2.85	5.35, 5.40, 5.45
	52.5	2.75, 2.80, 2.85	5.35, 5.40, 5.45
BEG	56.5	2.40, 2.45, 2.50, 2.55	5.45, 5.50
	58.1	2.50, 2.55, 2.60, 2.65	5.45, 5.50
CEF	61.8	2.55, 2.60, 2.65, 2.70, 2.75	5.45
	68.8	2.65, 2.70, 2.75	5.40, 5.45, 5.50
DEF	35.0	2.70, 2.75, 2.80, 2.85	5.40, 5.45, 5.50
	45.0	2.65, 2.70, 2.75	5.40, 5.45, 5.50
	54.0	2.70, 2.75, 2.80, 2.85	5.40, 5.45, 5.50
	64.8	2.70, 2.75, 2.80, 2.85	5.40, 5.45, 5.50
Co-crystallized DEF	45.0	2.75, 2.80, 2.85	5.40

This table shows the crystallization conditions that gave the large crystals.

Supplementary Table 2 Data collection statistics. Values in parentheses are for the outermost resolution shell.¶

Crystal	**WT-01**	**WT-02**	**Variant A**	**Variant B**
Post-crystallization treatments	No soaking / No cryo-protection	Soaking (THL§, 277 K) / Cryo-protection	Soaking (THL, 293 K) / Cryo-protection	Soaking (THL, 293 K) / Cryo-protection
X-ray source	FR-D	PF, BL-6A	PF-AR, NW12	PF-AR, NW12
Detector	R-Axis IV++	ADSC Quantum 4R	ADSC Quantum 210	ADSC Quantum 210
Distance (mm)	302.1	250	280	280
Oscillation angle (°)	1	1	1	1
Exposure time (sec)	600	45	5	12
Temperature (K)	95	95	95	95
Wavelength (Å)	1.542	1.0000	0.9788	0.9791
Space group	*C2*	*C2*	*C2*	*C2*
Unit-cell parameters				
a (Å)	142.0	133.3	122.5	129.9
b (Å)	60.6	62.6	61.2	63.0
c (Å)	61.3	61.1	61.0	61.5
β (°)	93.1	91.2	91.4	92.4
Resolution (Å)	20-4.50	20.0–3.85	19.4–3.18	19.8–3.30
Outermost shell	4.66-4.50	4.05–3.85	3.35–3.18	3.43–3.25
Observations	11752 (1206)	21859 (2951)	38225 (5558)	18829 (2756)
Unique reflections	3071 (313)	4849 (672)	7690 (1103)	6956 (1047)
Completeness (%)	97.6 (99.1)	99.1 (99.6)	99.4 (100)	87.5 (89.9)
$I / \sigma(I)$	5.8 (3.1)	19.7 (8.0)	18.0 (5.9)	10.7 (4.8)
Redundancy	3.8 (3.9)	4.5 (4.4)	5.0 (5.0)	2.7 (2.6)
R_{merge}	0.090 (0.237)	0.056 (0.183)	0.054 (0.290)	0.059 (0.193)
$R_{mrgd\text{-}F}$	-	0.097 (0.247)	0.069 (0.246)	0.094 (0.253)
Mosaicity	0.860	0.31	0.31	0.47
Overall B ($Å^2$)	164.5	61.8	73.4	98.0

¶ Statistics of the best data for each variant are shown in the table. Numbers of the total data sets for each variant are given in Table 1. Resolution limit was determined with $R_{mrgd\text{-}F}$ value (Diederichs & Karplus, 1997) less than 0.25. § THL: trehalose, GLC: glucose

Supplementary Table 2 (continued) Data collection statistics. Values in parentheses are for the outermost resolution shell.

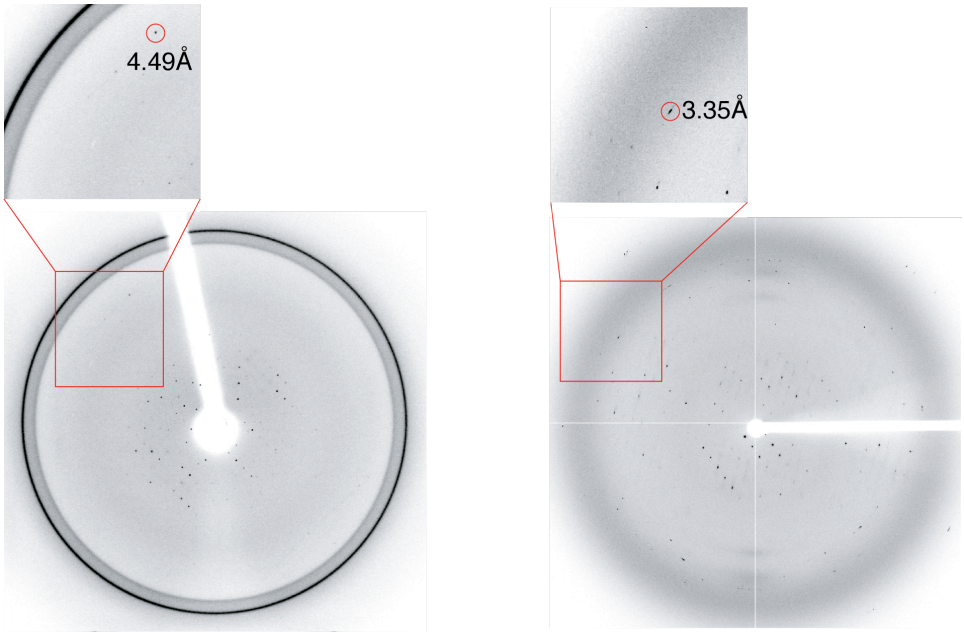
Crystal	**Variant E**	**Variant BEG**	**Variant CEF**	**Variant DEF-01**
Post-crystallization treatments	Soaking (THL[§], 293 K) / Cryo-protection	Soaking (THL, 293 K) / Cryo-protection	Soaking (THL, 293 K)/ Cryo-protection	No soaking / No cryo-protection
X-ray source	PF-AR, NW12	PF-AR, NW12	PF, BL-18B	PF, BL-6A
Detector	ADSC Quantum 210	ADSC Quantum 210	ADSC Quantum 4R	ADSC Quantum 4R
Distance (mm)	280	280	220	300
Oscillation angle (°)	1	1	1	1
Exposure time (sec)	10	10	60	30
Temperature (K)	95	95	95	95
Wavelength (Å)	0.9791	0.9787	1.1500	0.9780
Space group	*C2*	*C2*	*C2*	*C2*
Unit-cell parameters				
a (Å)	123.0	121.7	122.8	134.8
b (Å)	63.1	60.9	63.6	60.2
c (Å)	61.0	61.5	60.9	61.5
β (°)	90.7	92.0	89.1	94.9
Resolution (Å)	19.6–3.01	19.6–3.22	15.0–5.70	19.4–4.19
Outermost shell	3.17–3.01	3.39-3.22	5.96–5.70	4.42–4.19
Observations	46415 (6703)	36318 (5102)	6642 (849)	18161 (2733)
Unique reflections	9359 (1336)	7364 (1025)	2613 (326)	3689 (547)
Completeness (%)	99.4 (100)	99.4 (99.9)	96.9 (99.7)	98.8 (99.8)
I / $\sigma(I)$	21.3 (7.6)	13.5 (6.2)	12.6 (7.2)	12.5 (6.6)
Redundancy	5.0 (5.0)	4.9 (5.0)	2.5 (2.6)	4.9 (5.0)
R_{merge}	0.048 (0.210)	0.076 (0.225)	0.061 (0.128)	0.075 (0.221)
$R_{mrgd\text{-}F}$	0.057 (0.254)	0.084 (0.254)	0.094 (0.223)	0.128 (0.258)
Mosaicity	0.365	0.311	0.476	0.807
Overall B (Å^2)	66.4	68.3	74.5	72.3

§ THL: trehalose, GLC: glucose

Table 2 (continued) Data collection statistics. Values in parentheses are for the outermost resolution shell.

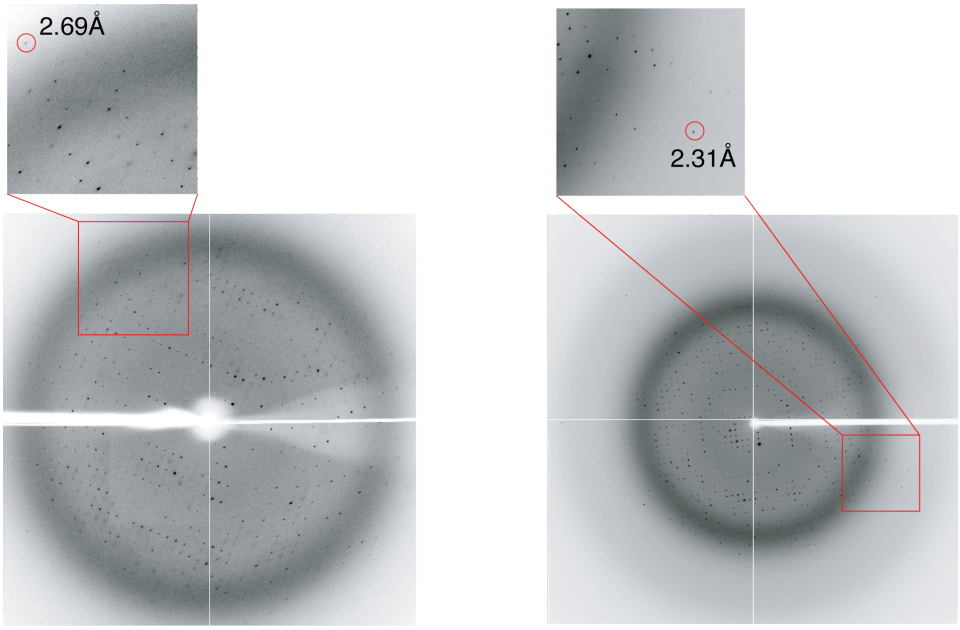
Crystal	**Variant DEF-02**	**Variant DEF-03**	**Variant DEF-04 (Co-crystallized with trehalose)**	**Variant DEF-05**†
Post-crystallization treatments	Soaking (THL§, 293 K) / Cryo-protection	Soaking (THL, 277 K) / Cryo-protection	No soaking / Cryo-protection	Soaking (GLC§, 277K) / Cryo-protection
X-ray source	PF-AR, NW12A	PF, BL-6A	PF-AR, NW12	PF, BL-5A
Detector	ADSC Quantum 210	ADSC Quantum 4R	ADSC Quantum 210	ADSC Quantum 315
Distance (mm)	240	160	190	400
Oscillation angle (°)	1	0.3	1	1
Exposure time (sec)	10	30	9	7
Temperature (K)	95	95	95	95
Wavelength (Å)	0.9790	1.0000	0.9792	0.9780
Space group	$P2_1$	$P2_1$	*C2*	*C2*
Unit-cell parameters				
a (Å)	61.1	60.9	117.2	128.7
b (Å)	64.6	64.4	63.6	60.6
c (Å)	124.9	124.3	61.2	61.6
β (°)	90.9	90.9	90.2	93.1
Resolution (Å)	19.8–2.70	19.7–2.67	19.7–2.95	20.0–3.44
Outermost shell	2.84–2.70	2.81–2.67	3.10-2.95	3.63–3.44
Observations	133113 (18898)	84761 (12186)	46463 (6505)	31470 (4787)
Unique reflections	26660 (3707)	26514 (3800)	9497 (1305)	6399 (954)
Completeness (%)	98.7 (99.1)	95.9 (97.8)	98.7 (99.8)	99.2 (100)
I / $\sigma(I)$	24.2 (8.2)	22.4 (7.2)	19.8 (10.0)	13.5 (5.9)
Redundancy	5.0 (5.1)	3.2 (3.2)	4.9 (5.0)	4.9 (5.0)
R_{merge}	0.038 (0.164)	0.038 (0.177)	0.048 (0.111)	0.067 (0.261)
$R_{mrgd\text{-}F}$	0.050 (0.246)	0.067 (0.252)	0.064 (0.255)	0.088 (0.257)
Mosaicity	0.153	0.189	0.317	0.397
Overall B ($Å^2$)	46.4	34.6	54.2	70.0

§ THL: trehalose, GLC: glucose, † Cryo-protection was performed with the artificial mother liquor containing 30 %(w/v) glucose.



(a) Wild Type, trehalose(-)

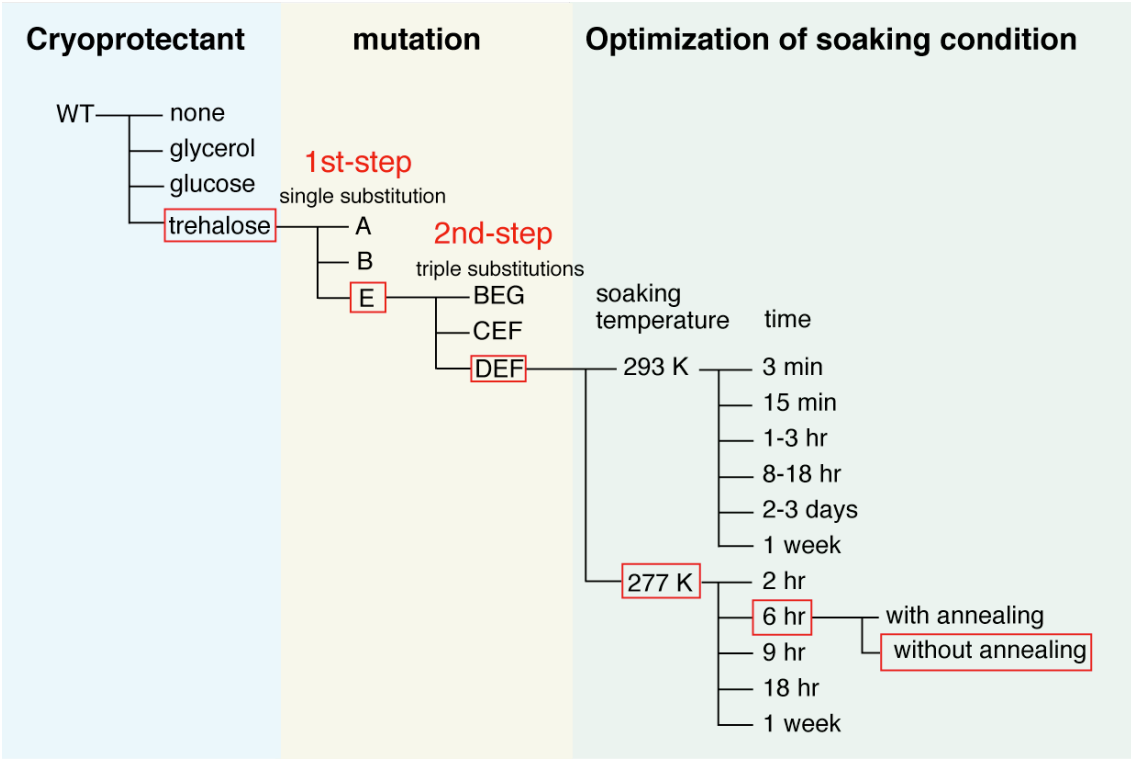
(b) Wild Type, trehalose(+)



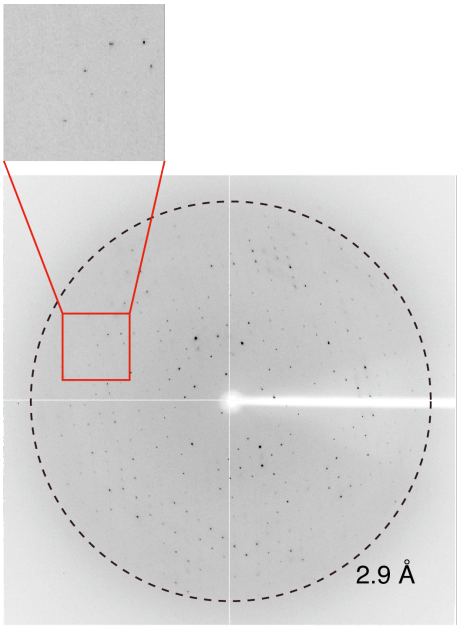
(c) Variant E, trehalose(+)

(d) Variant DEF, trehalose(+)

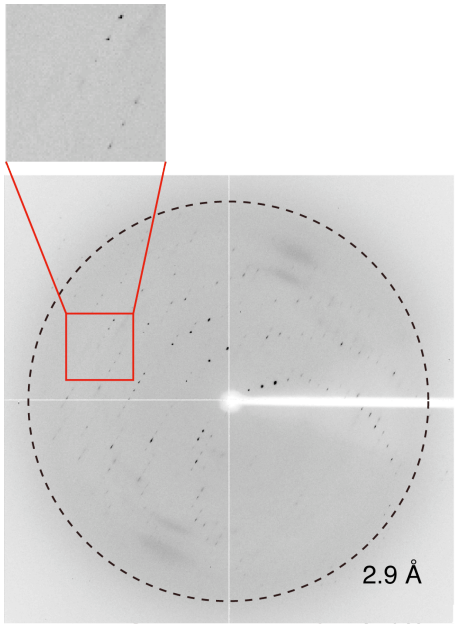
Supplementary Figure 1 Diffraction patterns of (a) the wild type crystal without cryoprotection, (b) wild type (c) variant E, and (d) variant DEF crystals soaked in the trehalose solution. The soaking conditions of the variant DEF crystal were optimized.



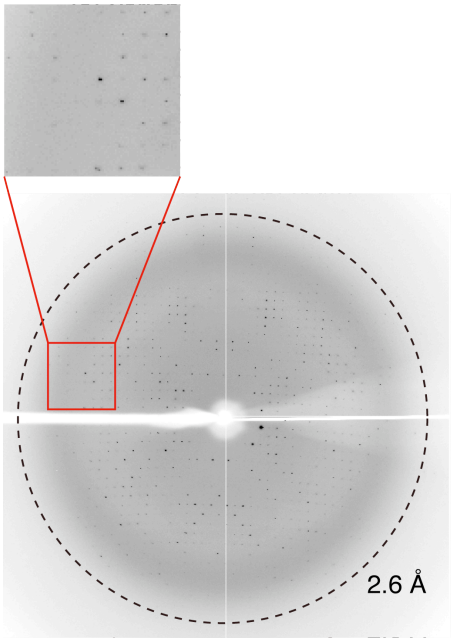
Supplementary Figure 2 Process of crystal quality improvement of TAF-IβΔC



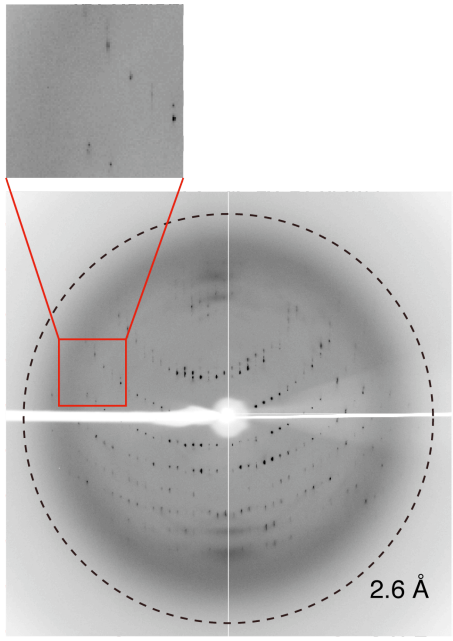
(a) Variant E, ϕ=0-1 degree



(b) Variant E, ϕ=90-91 degree

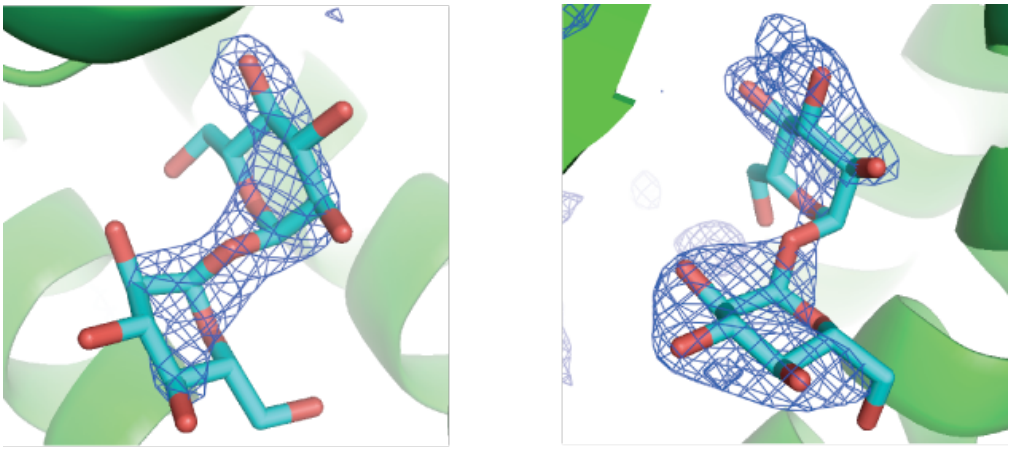


(c) Variant DEF, ϕ=0-1 degree



(d) Variant DEF, ϕ=90-91 degree

Supplementary Figure 3 Diffraction patterns of a variant E crystal (a, b) and a variant DEF crystal without cryo-condition optimization (c, d).

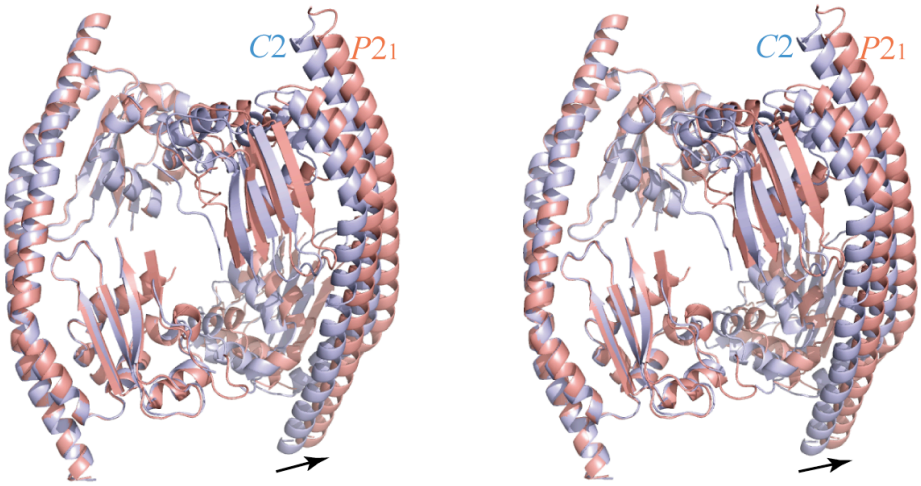


Supplementary Figure 4 The omit maps for the trehalose molecules in the co-crystal of mutant DEF. Trehalose-binding sites of A (*a*) and B (*b*) in the co-crystal of variant DEF (see Fig 2*c*). All Fo-Fc omit maps are contoured at the 3σ level. The trehalose molecules were placed on the basis of the trehalose model in the variant DEF(soaking) structure. The structure of the co-crystal of variant DEF was determined by the molecular replacement method using *CCP4* program suite (CCP4, 1994). The variant DEF(soaking) structure was used as a search model. The phases for the omit map calculation were obtained by one cycle of crystallographic refinement using the program *REFMAC5* (Murshudov *et al*., 1997). Trehalose was not included in the refined model.

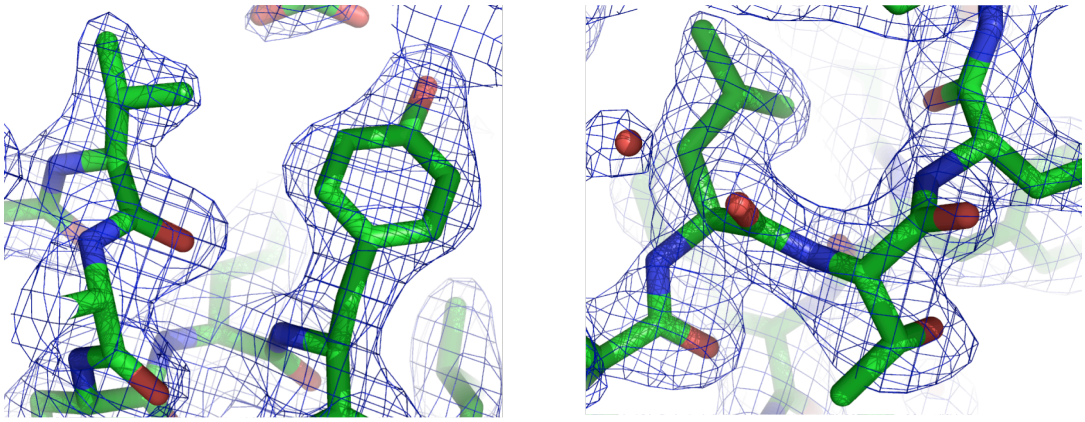
References

Collaborative Computational Project, Number 4. CCP4 suite (1994). *Acta Cryst*. D**50**, 760-763.

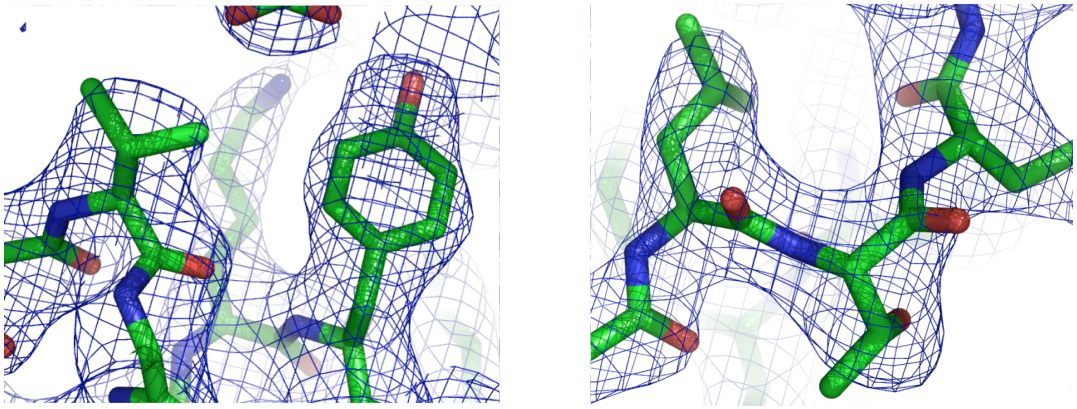
Murshudov, G. N., Vagin, A. A. & Dodson, E. J. (1997). *Acta Cryst*. D**53**, 240-255.



Supplementary Figure 5 The shift of the TAF-IβΔC molecules occurred in the space group change from *C*2 to $P2_1$ (Stereo pair). The light blue and pink molecules represent the TAF-IβΔC molecules in space groups *C*2 (variant DEF(co-crystal)) and $P2_1$ (variant DEF(soaking)), respectively. The black arrow shows the shift during the space group change from *C*2 to $P2_1$. The TAF-IβΔC dimer shifts nearly along the *a* axis of the *C*2 crystal by about 4–5 Å. The shift causes the loss of the crystallographic two-fold axis in the *C*2 crystal, resulting in the space group $P2_1$.



(a) Variant DEF (soaked crystal)



(b) Variant DEF (co-crystallized crystal)

Supplementary Figure 6 Comparison of the 2mFo-DFc map between the different treatments of variant DEF, (a) soaked crystal in trehalose and (b) co-crystallized with trehalose. The 2mFo-DFc maps are contoured at the 1.2σ level.

The structure of the co-crystal of variant DEF was determined by the molecular replacement method using *CCP4* program suite (CCP4, 1994). The variant DEF(soaking) structure was used as a search model. The phases for the 2mFo-DFc map calculation were obtained by three cycles of crystallographic refinement using the program *REFMAC5* (Murshudov *et al*., 1997). Further crystallographic refinement of the variant DEF(co-crystal) is in progress.

References

Collaborative Computational Project, Number 4. CCP4 suite (1994). *Acta Cryst*. D**50**, 760-763.

Murshudov, G. N., Vagin, A. A. & Dodson, E. J. (1997). *Acta Cryst*. D**53**, 240-255.