Supplementary Material

Recrystallization: A Method to Improve the Quality of Protein Crystals

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Supplementary Tables: X-ray data statistics of the crystals of four different proteins from the first crystallization and recrystallization.

Supplementary Figures: A comparison of four different protein crystals obtained by the first crystallization and recrystallization in resolution limit, mosaicity and R_{merge}

Supplementary Table S1. X-ray diffraction data statistics of lysozyme crystals in first crystallization selected by 5 crystals in same size

	Diffraction data statistics of lysozyme crystals in first crystallization				
	Crystal 1	Crystal 2	Crystal 3	Crystal 4	Crystal 5
Resolution range (Å)	50-1.66 (1.69-1.66)	50-1.69 (1.72-1.69)	50-2.16 (2.20-2.16)	50-2.20 (2.24-2.20)	50-2.44 (2.48-2.44)
Mosaicity (°)	0.79	0.77	0.55	0.64	1.096
$\langle I \rangle / \langle \sigma(I) \rangle$	32.73 (2.09)	37.40 (2.99)	14.37 (2.18)	11.15 (2.03)	11.08 (2.36)
Total observations	43313	43306	32200	35097	10778
Unique reflections	12735	13134	6380	6006	4234
Redundancy	3.4 (2.4)	3.3 (2.0)	5.0 (2.6)	5.8 (2.9)	2.5 (2.1)
Completeness (%)	90.8 (81.2)	94.9 (85.9)	97.6 (86.8)	95.4 (83.5)	90.9 (87.9)
R_{merge} [a] (%)	8.1 (39.1)	6.9 (43.0)	7.8 (32.6)	15.8 (48.2)	8.6 (29.0)
Space group	$P4_32_12$	$P4_32_12$	$P4_32_12$	$P4_32_12$	$P4_32_12$
Cell dimensions *a*, *b*, *c* (Å) α, β, γ (°)	*a* = 77.69 *b* = 77.69 *c* = 37.02 $\alpha = \beta = \gamma = 90$	*a* = 77.28 *b* = 77.28 *c* = 37.04 $\alpha = \beta = \gamma = 90$	*a* = 77.92 *b* = 77.92 *c* = 36.96 $\alpha = \beta = \gamma = 90$	*a* = 78.579 *b* = 78.579 *c* = 36.886 $\alpha = \beta = \gamma = 90$	*a* = 77.56 *b* = 77.56 *c* = 36.944 $\alpha = \beta = \gamma = 90$
Exposure time (min)	5				
Oscillation angle (°)	1				
Rotation range (°)	90				
Detector distance (mm)	150				
Wavelength (Å)	1.54179				

Values in parentheses are for the highest resolution shell.

[a] $R_{merge} = \sum_h \sum_i |I_{(h)} - I_{i(h)}| / \sum_h \sum_i I_{i(h)}$, where $I_{i(h)}$ and $I_{(h)}$ are the *i*th and mean measurement of reflection *h*.

Supplementary Table S2. X-ray diffraction data statistics of lysozyme crystals in recrystallization selected by 5 crystals in same size

	Diffraction data statistics of lysozyme crystals in recrystallization				
	Crystal 1	Crystal 2	Crystal 3	Crystal 4	Crystal 5
Resolution range (Å)	50-1.49 (1.52-1.49)	50-1.65 (1.68-1.65)	50-1.67 (1.70-1.67)	50-1.72 (1.75-1.72)	50-1.77 (1.80-1.77)
Mosaicity (°)	0.41	0.61	0.517	0.485	0.85
$\langle I \rangle / \langle \sigma(I) \rangle$	46 (3.06)	30.46 (2.29)	31.20 (2.04)	29.85 (2.025)	23.49 (2.05)
Total observations	122877	59645	82504	52951	37289
Unique reflections	18998	14480	13915	12614	11225
Redundancy	6.5 (3.8)	4.1 (2.6)	5.9 (2.7)	4.2 (2.2)	3.3 (2.4)
Completeness (%)	97.3 (84.2)	95.1 (85.7)	99.3 (90.5)	95.5 (88.6)	95.9(87.3)
R_{merge} [a] (%)	5.8 (43.1)	4.2 (34.2)	4.3 (38.4)	5.9 (29.4)	6.1 (32.9)
Space group	$P4_32_12$	$P4_32_12$	$P4_32_12$	$P4_32_12$	$P4_32_12$
Cell dimensions *a*, *b*, *c* (Å) α, β, γ (°)	a = 78.72 b = 78.72 c = 36.92 $\alpha = \beta = \gamma = 90$	a = 78.34 b = 78.34 c = 36.915 $\alpha = \beta = \gamma = 90$	a = 78.501 b = 78.501 c = 36.926 $\alpha = \beta = \gamma = 90$	a = 78.23 b = 78.23 c = 36.93 $\alpha = \beta = \gamma = 90$	a = 78.12 b = 78.12 c = 36.99 $\alpha = \beta = \gamma = 90$
Exposure time (min)	5				
Oscillation angle (°)	1				
Rotation range (°)	90				
Detector distance (mm)	150				
Wavelength (Å)	1.54179				

Values in parentheses are for the highest resolution shell.

[a] $R_{merge} = \sum_h \sum_i |I_{(h)} - I_{i(h)}| / \sum_h \sum_i I_{i(h)}$, where $I_{i(h)}$ and $I_{(h)}$ are the *i*th and mean measurement of reflection *h*.

Supplementary Table S3. X-ray diffraction data statistics of proteinase K in first crystallization selected by 5 crystals in same size

	Diffraction data statistics of proteinase K crystals in first crystallization				
	Crystal 1	Crystal 2	Crystal 3	Crystal 4	Crystal 5
Resolution range (Å)	50-1.87 (1.90-1.87)	50-2.13 (2.17-2.13)	50-2.23 (2.27-2.23)	50-2.26 (2.30-2.26)	50-2.24 (2.28-2.24)
Mosaicity (°)	0.71	0.53	0.68	0.69	0.80
$\langle I \rangle / \langle \sigma(I) \rangle$	17.93 (2.81)	10.43 (2.03)	7.84 (2.05)	8.01 (2.11)	6.69 (2.07)
Total observations	83422	76180	40263	46075	40642
Unique reflections	18180	13778	11741	11316	11308
Redundancy	4.6 (4.1)	5.5 (3.0)	3.4 (2.3)	4.1 (3.3)	3.6 (2.9)
Completeness (%)	91.2 (90.7)	98.8 (90.2)	93.4 (88.2)	94.2 (87.4)	93.5 (88.5)
R_{merge} [a] (%)	10.0 (49.2)	12.4 (45.1)	12.4 (35.9)	11.7 (37.0)	14.2 (40.8)
Space group	$P4_32_12$	$P4_32_12$	$P4_32_12$	$P4_32_12$	$P4_32_12$
Cell dimensions *a*, *b*, *c* (Å)	a = 67.25	a = 67.72	a = 67.57	a = 67.62	a = 67.77
	b = 67.25	b = 67.72	b = 67.57	b = 67.62	b = 67.77
	c = 101.15	c = 102.23	c = 101.83	c = 101.97	c = 102.46
α, β, γ (°)	$\alpha = \beta = \gamma = 90$	$\alpha = \beta = \gamma = 90$	$\alpha = \beta = \gamma = 90$	$\alpha = \beta = \gamma = 90$	$\alpha = \beta = \gamma = 90$
Exposure time (min)	5				
Oscillation angle (°)	1				
Rotation range (°)	90				
Detector distance (mm)	150				
Wavelength (Å)	1.54179				

Values in parentheses are for the highest resolution shell.

[a] $R_{merge} = \sum_h \sum_i |I_{(h)} - I_{i(h)}| / \sum_h \sum_i I_{i(h)}$, where $I_{i(h)}$ and $I_{(h)}$ are the *i*th and mean measurement of reflection *h*.

Supplementary Table S4. X-ray diffraction data statistics of proteinase K in recrystallization selected by 5 crystals in same size

	Diffraction data statistics of proteinase K crystals in recrystallization				
	Crystal 1	Crystal 2	Crystal 3	Crystal 4	Crystal 5
Resolution range (Å)	50-1.77 (1.80-1.77)	50-1.78 (1.81-1.78)	50-1.85 (1.88-1.85)	50-1.90 (1.93-1.90)	50-2.09 (2.13-2.09)
Mosaicity (°)	0.43	0.38	0.43	0.44	0.56
$\langle I \rangle / \langle \sigma(I) \rangle$	19.94 (2.05)	20.9 (2.19)	15.55 (2.01)	13.94 (2.08)	10.01 (2.025)
Total observations	146657	331858	133103	121708	71777
Unique reflections	23472	23543	20833	19373	13640
Redundancy	6.2 (3.8)	14.1 (5.9)	6.4 (4.2)	6.3 (3.2)	5.3 (2.8)
Completeness (%)	98.9 (98.6)	99.7 (96.4)	99.1 (91.3)	99.6 (94.1)	92.5 (85.3)
R_{merge} [a] (%)	8.3 (43.5)	10.5 (43.9)	8.9 (47.9)	10.1 (43.6)	11.9 (40.2)
Space group	$P4_32_12$	$P4_32_12$	$P4_32_12$	$P4_32_12$	$P4_32_12$
Cell dimensions *a*, *b*, *c* (Å)	*a* = 67.6	*a* = 67.81	*a* = 67.71	*a* = 67.87	*a* = 67.67
	b = 67.6	*b* = 67.81	*b* = 67.71	*b* = 67.87	*b* = 67.67
	c = 101.78	*c* = 102.24	*c* = 102.16	*c* = 102.35	*c* = 102.03
α, β, γ (°)	$\alpha = \beta = \gamma = 90$	$\alpha = \beta = \gamma = 90$	$\alpha = \beta = \gamma = 90$	$\alpha = \beta = \gamma = 90$	$\alpha = \beta = \gamma = 90$
Exposure time (min)	5				
Oscillation angle (°)	1				
Rotation range (°)	90				
Detector distance (mm)	150				
Wavelength (Å)	1.54179				

Values in parentheses are for the highest resolution shell.

[a] $R_{merge} = \sum_h \sum_i |I_{(h)} - I_{i(h)}| / \sum_h \sum_i I_{i(h)}$, where $I_{i(h)}$ and $I_{(h)}$ are the *i*th and mean measurement of reflection *h*.

Supplementary Table S5. X-ray diffraction data statistics of thaumatin in first crystallization selected by 5 crystals in same size

	Diffraction data statistics of thaumatin crystals in first crystallization				
	Crystal 1	Crystal 2	Crystal 3	Crystal 4	Crystal 5
Resolution range (Å)	50-2.34 (2.38-2.34)	50-3.05 (3.10-3.05)	50-3.23 (3.28-3.23)	50-3.42 (3.48-3.42)	50-3.53 (3.59-3.53)
Mosaicity (°)	0.86	0.91	0.72	1.06	1.17
$\langle I \rangle / \langle \sigma(I) \rangle$	8.94 (2.11)	4.72 (2.09)	7.88 (2.00)	5.30 (2.11)	4.66 (2.02)
Total observations	34054	15109	22573	10283	8842
Unique reflections	8832	4517	4415	3550	3132
Redundancy	3.9 (3.1)	3.3 (2.5)	5.1 (3.5)	2.9 (2.2)	2.8 (2.0)
Completeness (%)	96.7 (93.5)	93.0 (89.8)	98.0 (91.3)	92.4 (84.0)	87.4 (80.8)
R_{merge} [a] (%)	11.8 (34.2)	17.0 (26.9)	21.7 (31.0)	22.7 (30.0)	19.4 (30.4)
Space group	$P4_12_12$	$P4_12_12$	$P4_12_12$	$P4_12_12$	$P4_12_12$
Cell dimensions *a*, *b*, *c* (Å) α, β, γ (°)	$a = 57.76$ $b = 57.76$ $c = 150.5$ $\alpha = \beta = \gamma = 90$	$a = 57.76$ $b = 57.76$ $c = 150.61$ $\alpha = \beta = \gamma = 90$	$a = 57.65$ $b = 57.65$ $c = 150.93$ $\alpha = \beta = \gamma = 90$	$a = 57.52$ $b = 57.52$ $c = 150.64$ $\alpha = \beta = \gamma = 90$	$a = 57.95$ $b = 57.95$ $c = 150.98$ $\alpha = \beta = \gamma = 90$
Exposure time (min)	10				
Oscillation angle (°)	1				
Rotation range (°)	120				
Detector distance (mm)	200				
Wavelength (Å)	1.54179				

Values in parentheses are for the highest resolution shell.

[a] $R_{merge} = \sum_h \sum_i |I_{(h)} - I_{i(h)}| / \sum_h \sum_i I_{i(h)}$, where $I_{i(h)}$ and $I_{(h)}$ are the *i*th and mean measurement of reflection *h*.

Supplementary Table S6. X-ray diffraction data statistics of thaumatin in recrystallization selected by 5 crystals in same size

	Diffraction data statistics of thaumatin crystals in recrystallization				
	Crystal 1	Crystal 2	Crystal 3	Crystal 4	Crystal 5
Resolution range (Å)	50-2.14 (2.18-2.14)	50-2.27 (2.31-2.27)	50-2.51 (2.55-2.51)	50-3.17 (3.22-3.17)	50-3.43 (3.49-3.43)
Mosaicity (°)	0.41	0.38	0.84	1.01	0.57
$\langle I \rangle / \langle \sigma(I) \rangle$	17.90 (3.31)	13.48 (2.24)	13.7 (2.03)	6.80 (2.01)	5.83 (2.15)
Total observation	125923	61641	34223	5421	19818
Unique reflections	14885	11977	8102	2964	3725
Redundancy	8.5 (6.0)	5.1 (3.5)	4.2 (3.4)	1.8 (1.5)	5.4 (3.0)
Completeness (%)	99.8 (99.6)	99.4 (95.7)	86.3 (83.9)	86.0 (80.5)	97.5 (92.4)
R_{merge} [a] (%)	14.3 (44.4)	63.9 (92.1)	64.8 (69.6)	44.5 (35.1)	23.8 (43.2)
Space group	$P4_12_12$	$P4_12_12$	$P4_12_12$	$P4_12_12$	$P4_12_12$
Cell dimensions *a*, *b*, *c* (Å) α, β, γ (°)	$a = 57.92$ $b = 57.92$ $c = 150.48$ $\alpha = \beta = \gamma = 90$	$a = 57.65$ $b = 57.65$ $c = 151.06$ $\alpha = \beta = \gamma = 90$	$a = 58.55$ $b = 58.55$ $c = 152.81$ $\alpha = \beta = \gamma = 90$	$a = 52.83$ $b = 52.83$ $c = 152.51$ $\alpha = \beta = \gamma = 90$	$a = 58.01$ $b = 58.01$ $c = 150.72$ $\alpha = \beta = \gamma = 90$
Exposure time (min)	10				
Oscillation angle (°)	1				
Rotation range (°)	120				
Detector distance (mm)	200				
Wavelength (Å)	1.54179				

Values in parentheses are for the highest resolution shell.

[a] $R_{merge} = \sum_h \sum_i |I_{(h)} - I_{i(h)}| / \sum_h \sum_i I_{i(h)}$, where $I_{i(h)}$ and $I_{(h)}$ are the *i*th and mean measurement of reflection *h*.

Supplementary Table S7. X-ray diffraction data statistics of catalase in first crystallization selected by 3 crystals in same size

	Diffraction data statistics of catalase crystals in first crystallization		
	Crystal 1	Crystal 2	Crystal 3
Resolution range (Å)	50-5.21 (5.30-5.21)	50-6.56 (6.66-6.56)	50-7.13 (7.25-7.13)
Mosaicity (°)	0.77	0.83	0.94
$\langle I \rangle / \langle \sigma(I) \rangle$	4.01 (2.01)	4.32 (2.03)	3.84 (2.06)
Total observations	68547	59648	47312
Unique reflections	19589	19239	16890
Redundancy	3.5 (3.1)	3.1 (2.7)	2.8 (2.4)
Completeness (%)	93.2 (90.2)	89.3 (90.50)	86 (89.5)
R_{merge} [a] (%)	23.7 (36.5)	26.8 (46.9)	22.8 (32.0)
Space group	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
Cell dimensions *a*, *b*, *c* (Å) α, β, γ (°)	a = 85.36 b = 139.68 c = 227.65 $\alpha = \beta = \gamma = 90$	a = 84.56 b = 139.78 c = 227.45 $\alpha = \beta = \gamma = 90$	a = 84.74 b = 139.96 c = 227.53 $\alpha = \beta = \gamma = 90$
Exposure time (min)	10		
Oscillation angle (°)	1		
Rotation range (°)	120		
Detector distance (mm)	300		
Wavelength (Å)	1.54179		

Values in parentheses are for the highest resolution shell.

[a] $R_{merge} = \sum_h \sum_i |I_{(h)} - I_{i(h)}| / \sum_h \sum_i I_{i(h)}$, where $I_{i(h)}$ and $I_{(h)}$ are the *i*th and mean measurement of reflection *h*.

Supplementary Table S8. X-ray diffraction data statistics of catalase in recrystallization selected by 3 crystals in same size

	Diffraction data statistics of thaumatin crystals in recrystallization		
	Crystal 1	Crystal 2	Crystal 3
Resolution range (Å)	50-4.16 (4.23-4.16)	50-4.24 (4.31-4.24)	50-6.31 (6.41-6.31)
Mosaicity (°)	0.51	0.60	0.68
$<I>/<\sigma(I)>$	4.12 (2.05)	4.03 (2.00)	4.06 (2.05)
Total observations	73893	58927	66342
Unique reflections	20240	18220	19540
Redundancy	3.7 (3.2)	3.2 (3.1)	3.4 (3.1)
Completeness (%)	99.3 (97.1)	87.4 (89.6)	91.5 (90.2)
R_{merge} [a] (%)	25.9 (44.4)	28.2 (48.4)	23.1 (31.0)
Space group	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
Cell dimensions *a*, *b*, *c* (Å) α, β, γ (°)	*a* = 86.19 *b* = 139.8 *c* = 228.26 $\alpha = \beta = \gamma = 90$	*a* = 83.43 *b* = 140.51 *c* = 226.84 $\alpha = \beta = \gamma = 90$	*a* = 85.03 *b* = 139.86 *c* = 227.43 $\alpha = \beta = \gamma = 90$
Exposure time (min)	10		
Oscillation angle (°)	1		
Rotation range (°)	120		
Detector distance (mm)	300		
Wavelength (Å)	1.54179		

Values in parentheses are for the highest resolution shell.

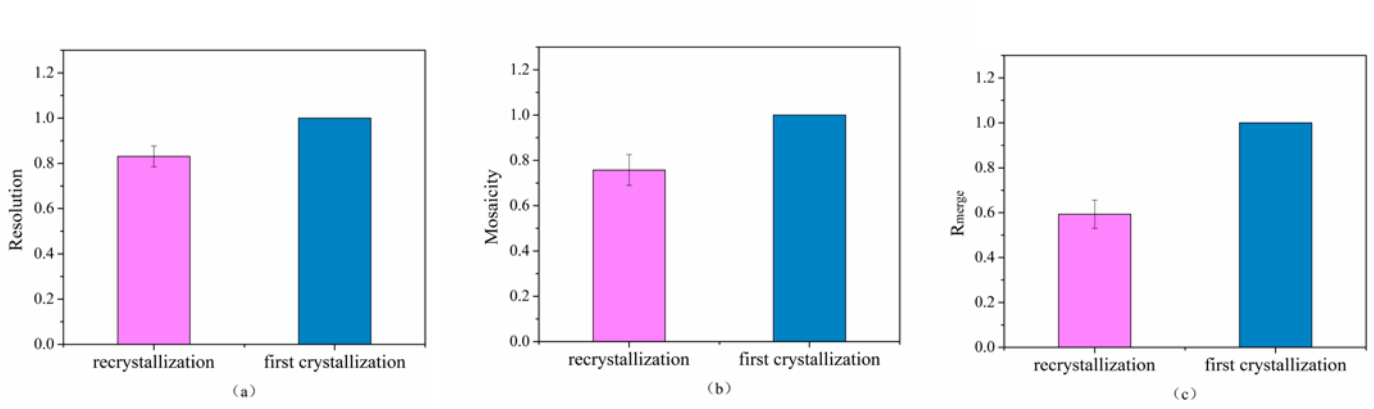
[a] $R_{merge} = \sum_h \sum_i |I_{(h)} - I_{i(h)}| / \sum_h \sum_i I_{i(h)}$, where $I_{i(h)}$ and $I_{(h)}$ are the *i*th and mean measurement of reflection *h*.

Supplementary Table S9. The differences of data quality statistics between the first crystallization and recrystallization as compared with the models in PDB databank

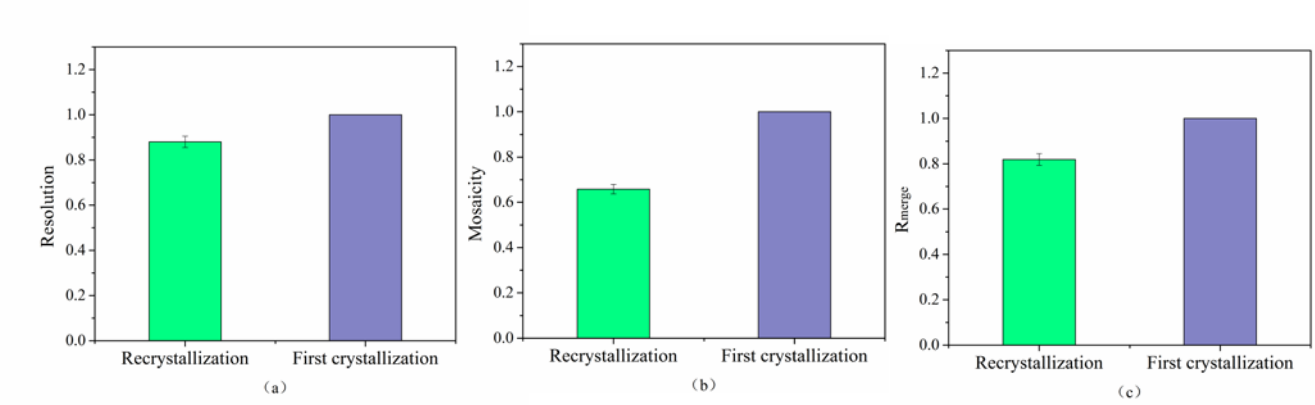
Protein	Lysozyme		Proteinase K		Thaumatin	
crystallization	first crystallization	recrystallization	first crystallization	recrystallization	first crystallization	recrystallization
Highest resolution (Å)	2.16	1.67	2.13	1.77	3.04	2.14
R_{work}	0.1824	0.1650	0.1566	0.1439	0.2083	0.1598
R_{free}	0.2434	0.1963	0.2203	0.1885	0.2655	0.2101
R factor	0.2098	0.1833	0.1705	0.1580	0.2839	0.1847
Correlation factor	0.9261	0.9564	0.9518	0.9635	0.8584	0.9441
Error in coordinates	0.2390	0.1759	0.1910	0.1569	0.6311	0.2044

Note: Phenix.refine was used to perform structure refinement and calculate R_{work} and R_{free}. SFCHECK was used to generate the statistics of the agreement between data and model, including R-factor, correlation factor and error in coordinates. The corresponding models selected for comparison are 2HU3, 1IC6, and 2VHK for lysozyme, proteinase K and thaumatin, respectively.

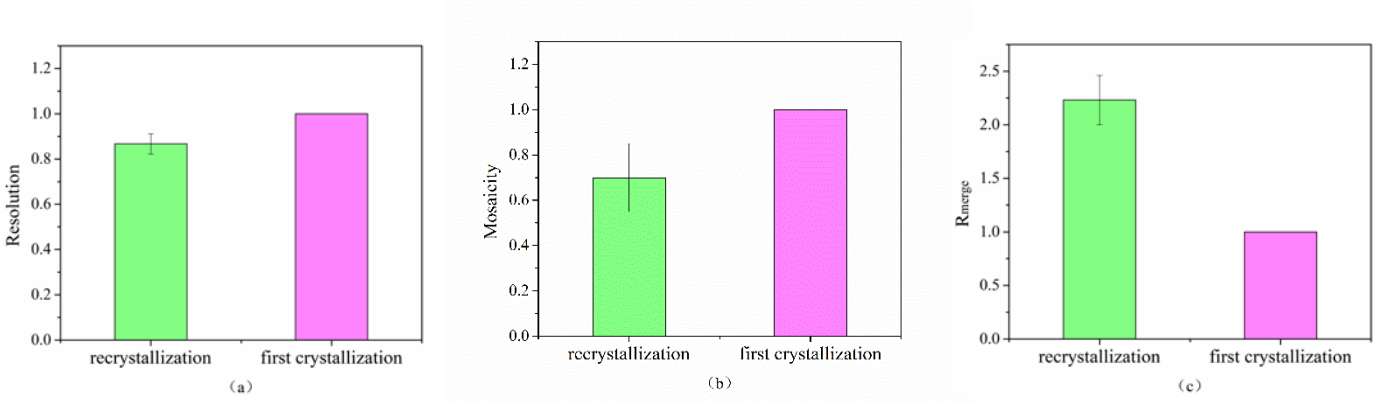
Supplementary Figure S1. A statistical analysis of the lysozyme protein of five different crystals after normalization to the values found for the first crystallization (the error bars show the standard error of the mean; n = 5). (*a*) In terms of the resolution limit of the five crystals, the results demonstrated an extremely significant difference between the two groups (n = 5, P = 0.0063, *i.e.* <0.01). The crystals obtained by recrystallization (labeled 'recrystallization' in the image) exhibited improvement in the resolution limit compared with the control. (*b*) The results indicated that the crystals obtained by recrystallization (labeled 'recrystallization' in the image) exhibited improvement in the mosaicity compared with the first crystallization. For mosaicity, the results demonstrated extremely significant difference between the two groups (n = 5, P = 0.007, *i.e.* <0.01). (*c*) The recrystallization demonstrated an improvement in R_{merge} compared with the control. Moreover, for the R_{merge}, the difference between the two groups was also extremely significant (n = 5, P = 0.0002, *i.e.* <0.01).



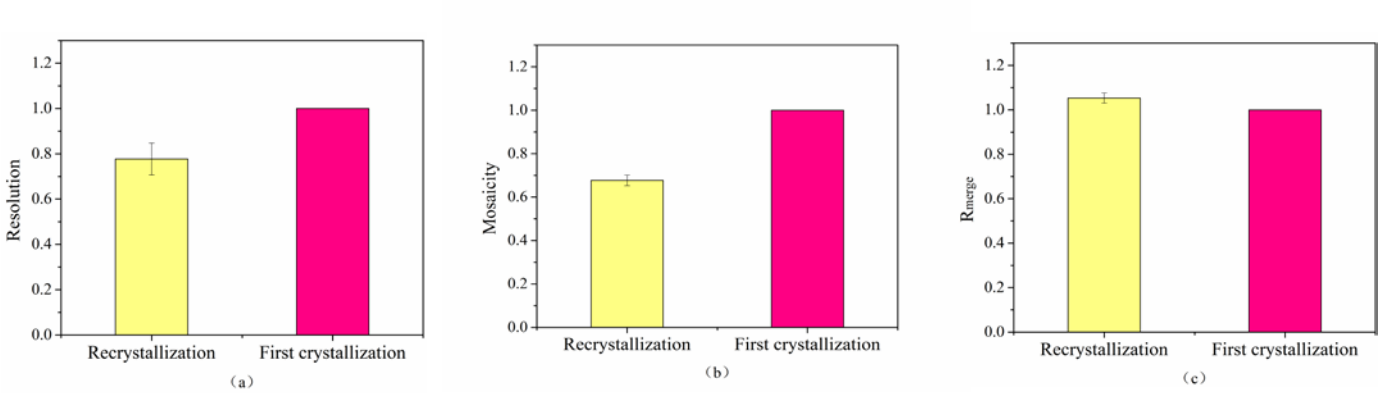
Supplementary Figure S2. A statistical analysis of the proteinase K protein of five different crystals after normalization to the values found for the first crystallization (the error bars show the standard error of the mean; n = 5). (*a*) In terms of the resolution limit of the five crystals, the results demonstrated an extremely significant difference between the two groups (n = 5, P = 0.0014, *i.e.* <0.01). The crystals obtained by recrystallization (labeled 'recrystallization' in the image) exhibited improvement in the resolution limit compared with the control. (*b*) The results indicated that the crystals obtained by recrystallization (labeled 'recrystallization' in the image) exhibited improvement in the mosaicity compared with the first crystallization. For mosaicity, the results demonstrated extremely significant difference between the two groups (n = 5, P = 2.28E-7, *i.e.* <0.01). (*c*) The recrystallization demonstrated an improvement in R_{merge} compared with the control. Moreover, for the R_{merge}, the difference between the two groups was also extremely significant (n = 5, P = 0.0001, *i.e.* <0.01).



Supplementary Figure S3. A statistical analysis of the thaumatin protein of five different crystals after normalization to the values found for the first crystallization (the error bars show the standard error of the mean; *n* = 5). (*a*) In terms of the resolution limit of the five crystals, the results demonstrated an significant difference between the two groups (*n* = 5, *P* = 0.018, *i.e.* <0.05). The crystals obtained by recrystallization (labeled 'recrystallization' in the image) exhibited improvement in the resolution limit compared with the control. (*b*) The results indicated that the crystals obtained by recrystallization (labeled 'recrystallization' in the image) exhibited improvement in the mosaicity compared with the first crystallization. For mosaicity, nevertheless, the results showed no significant difference between the two groups (*n* = 5, *P* = 0.083, *i.e.* >0.05). (*c*) The recrystallization compared with the control in R_{merge} (*n* = 5, *P* = 0.040, *i.e.* <0.05).



Supplementary Figure S4. A statistical analysis of the catalase protein of three different crystals after normalization to the values found for the first crystallization (the error bars show the standard error of the mean; *n* = 3). (*a*) In terms of the resolution limit of the three crystals, the results demonstrated an significant difference between the two groups (*n* = 3, *P* = 0.032, *i.e.* <0.05). The crystals obtained by recrystallization (labeled 'recrystallization' in the image) exhibited improvement in the resolution limit compared with the control. (*b*) The results indicated that the crystals obtained by recrystallization (labeled 'recrystallization' in the image) exhibited improvement in the mosaicity compared with the first crystallization. For mosaicity, the results demonstrated extremely significant difference between the two groups (*n* = 3, *P* = 0.0002, *i.e.* <0.01). (*c*) The recrystallization demonstrated an improvement in R_{merge} compared with the control. Nevertheless, for the R_{merge}, the difference between the two groups was not significant (*n* = 3, *P* = 0.083, *i.e.* >0.05).



Supplementary Figure S5. The purity of Lysozyme protein at the outset and after recrystallisation. SDS-PAGE was carried out to test the purity of all the proteins. Lane *M*, Protein Marker; Lane *1*, the "purchased product" which represents the sample bought from the company directly; Lane *2*, the "first crystallization" which stands for the crystal sample lyophilized by lyophilizer after the first crystallization; Lane *3*, "after the recrystallization" that represents the sample after recrystallization also lyophilized by lyophilizer. After that, these three samples were weighted and used same amount quantity for the gel. It was obvious that recrystallization purify the protein.