Supplementary Figures and Tables

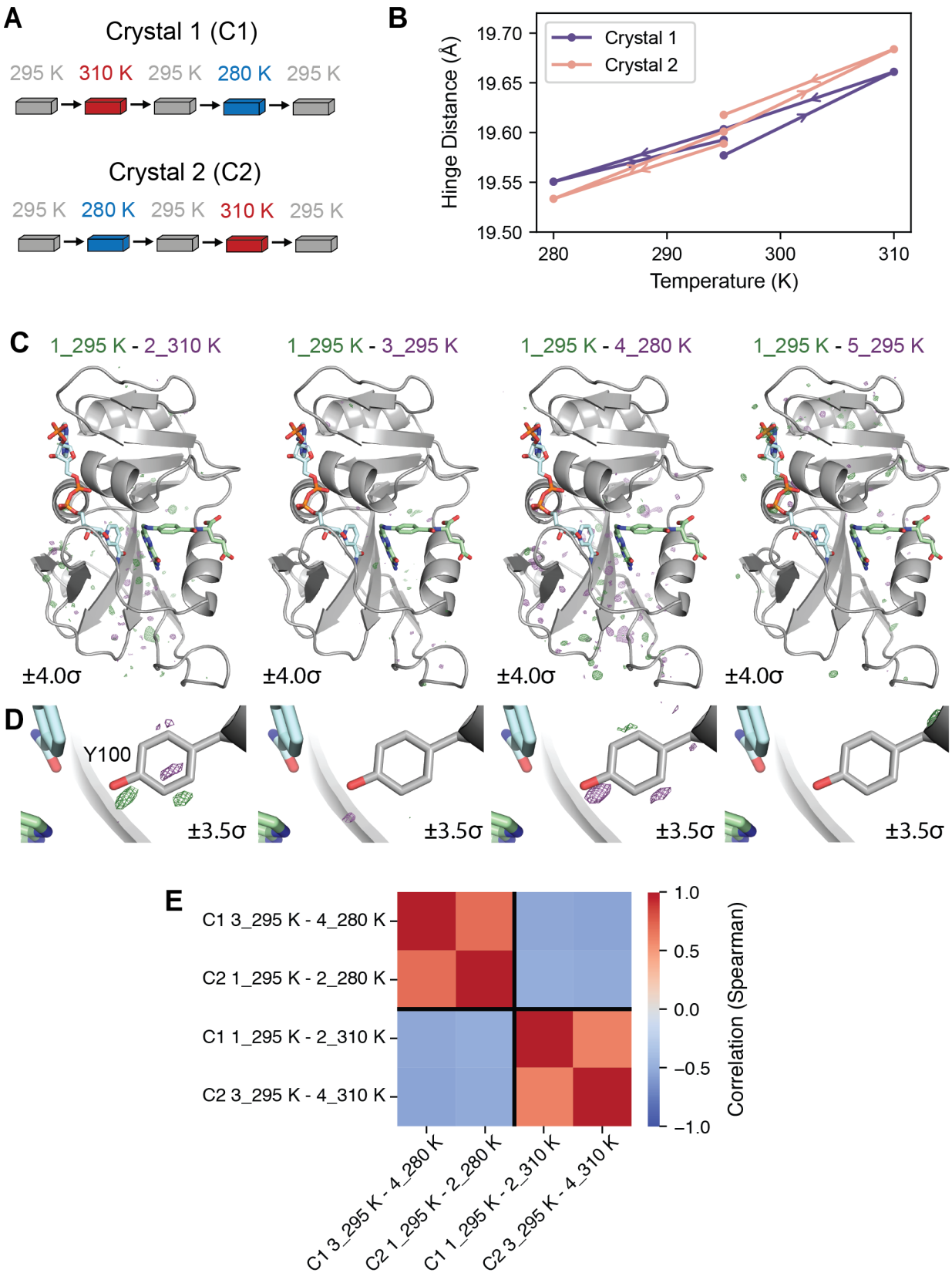

Fig. S1 *Caption on following page.*

Figure S1: **Reversibility and reproducibility of multi-temperature diffraction experiments.** (A) Schematic of single-crystal, multi-temperature diffraction experiments. (B) Plots of the refined hinge distance versus temperature for both single-crystal experiments demonstrate that the experiment is reversible. (C) Temperature-resolved difference maps between the first dataset from crystal 1 and the subsequent four datasets. More significant density peaks are observed for maps generated from datasets collected at different temperatures. (D) Zoom-in on Tyr100 in the difference maps emphasizes that observed features are temperature-dependent (carved within 2 Å of Tyr100). (E) Heatmap of the Spearman correlation coefficients between difference structure factor amplitudes computed from independent single-crystal experiments. Equivalent temperature changes yield strongly correlated difference amplitudes, while the opposite temperature changes produce strongly anti-correlated results. This demonstrates that the observed structural changes in the single-crystal, multi-temperature experiments are reproducible between independent experiments.

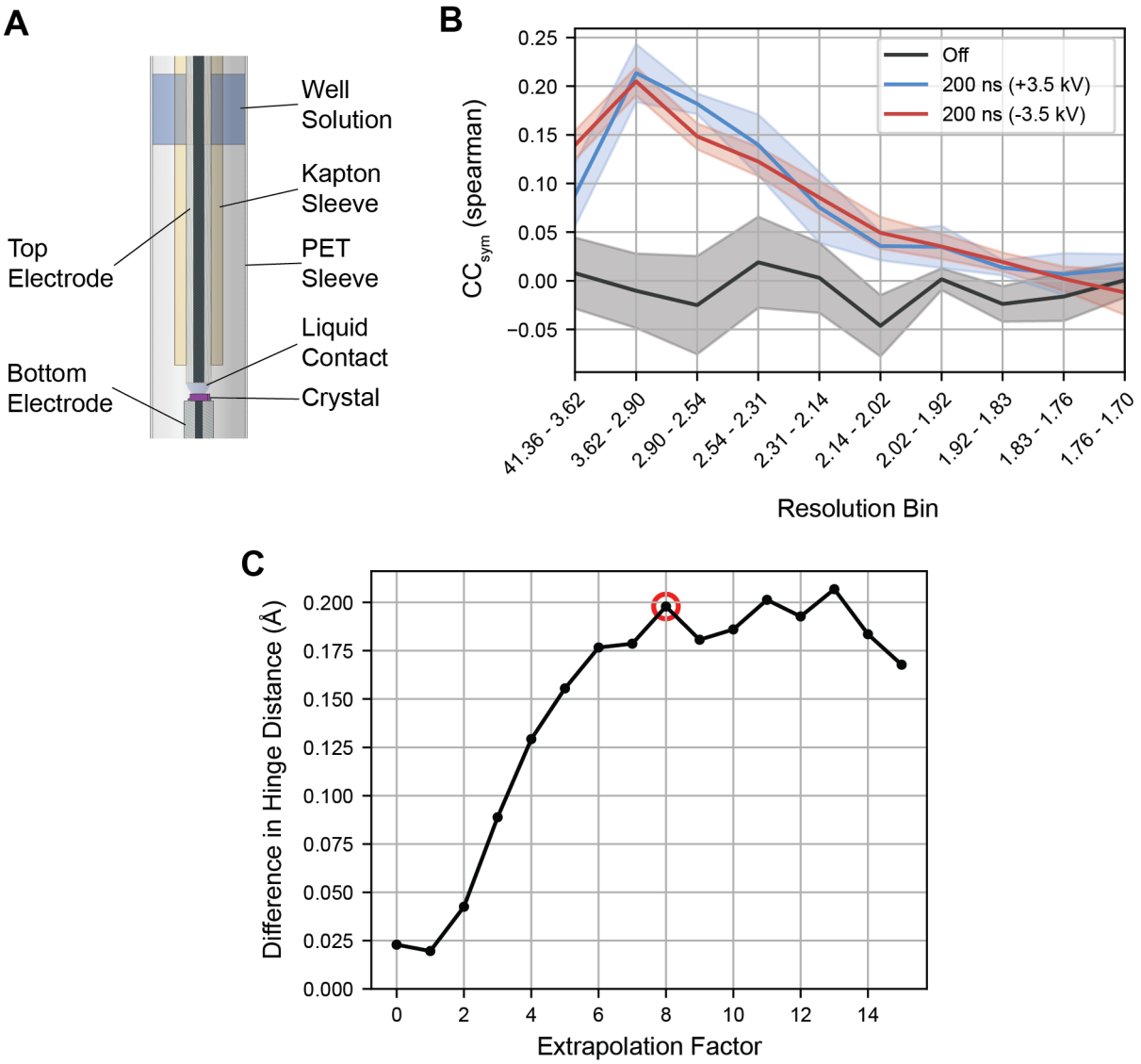

Fig. S2 Experimental apparatus and analysis for electric-field-stimulated X-ray diffraction of *ec*DHFR. (A) Diagram of the revised experimental apparatus for EF-X. Liquid contact is made within a band of well solution that is osmotically matched to the crystal, ensuring a high humidity environment for the duration of the experiment. (B) Plot of CC_{sym} versus resolution bin. CC_{sym} is an indicator of the reproducibility of observed symmetry breaking during an EF-X experiment. The 95% confidence interval from 5 random partitions of the diffraction images is shown. For the 'Off' dataset in which the symmetry operation is preserved, no significant correlation between half-datasets is expected because differences for symmetry-related observations should only reflect experimental error. The positive correlations for differences measured during the high-voltage pulses indicates significant electric-field-dependent symmetry breaking.(C) Plot of the refined difference in hinge distance between the two copies of DHFR in the $P2_1$ ASU as a function of extrapolation factor. With an extrapolation factor of zero, the data is equivalent to 'Off' structure factor amplitudes processed in the reduced-symmetry spacegroup. The difference in hinge distance increases linearly with extrapolation factor until a value of 8 and plateaus at a difference of approximately 0.2 Å. The extrapolation factor chosen for ESF refinement of the excited state is indicated with a red circle.

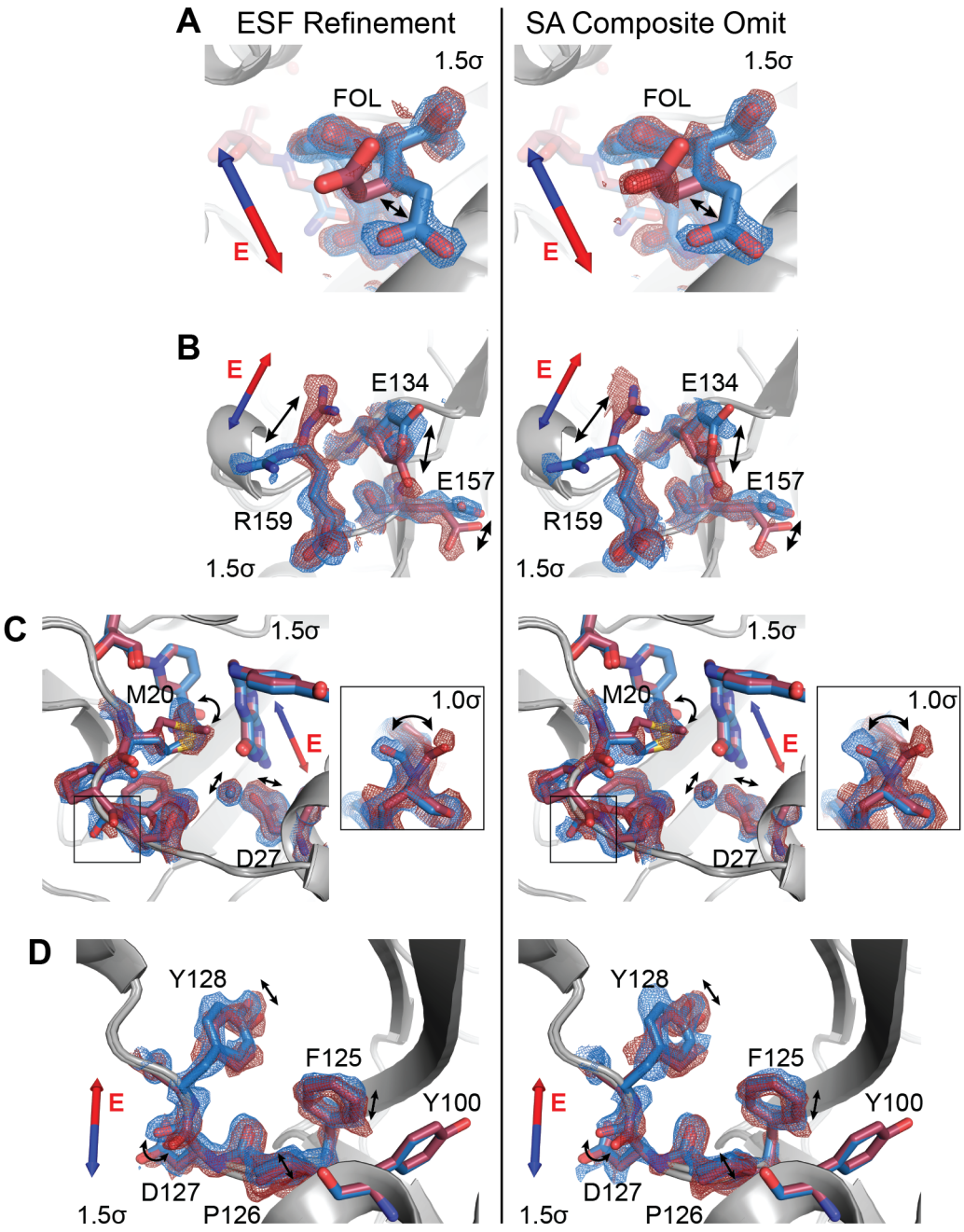

Fig. S3 Composite omit maps validate modeling of EF-X excited state. (A) to (D) Comparison of $2mF_o - DF_c$ maps from ESF refinement (left column) and corresponding simulated annealing (SA) composite omit maps (right column). Superposed models and maps from both protein molecules of the excited-state ASU highlight electric-field induced structural changes. Blue and red arrows depict electric field vector for the blue and red models, respectively, and maps are contoured at 1.5σ and carved within 1.5 Å of shown atoms. (A) Carboxylate sidechain of folate and (B) charged sidechains near the C-terminus demonstrate electric-field-dependent structural changes consistent with the formal charges of the residues. (C) Active site residues and Pro21 backbone carbonyl (inset; contoured at 1.0σ) differs between protein molecules. (D) Conformational changes among residues 125 to 128. The similarity between the electron density maps from ESF refinement and the SA composite omit maps indicates that the observed structural differences between the molecules of the excited-state ASU are not the result of modeling bias.

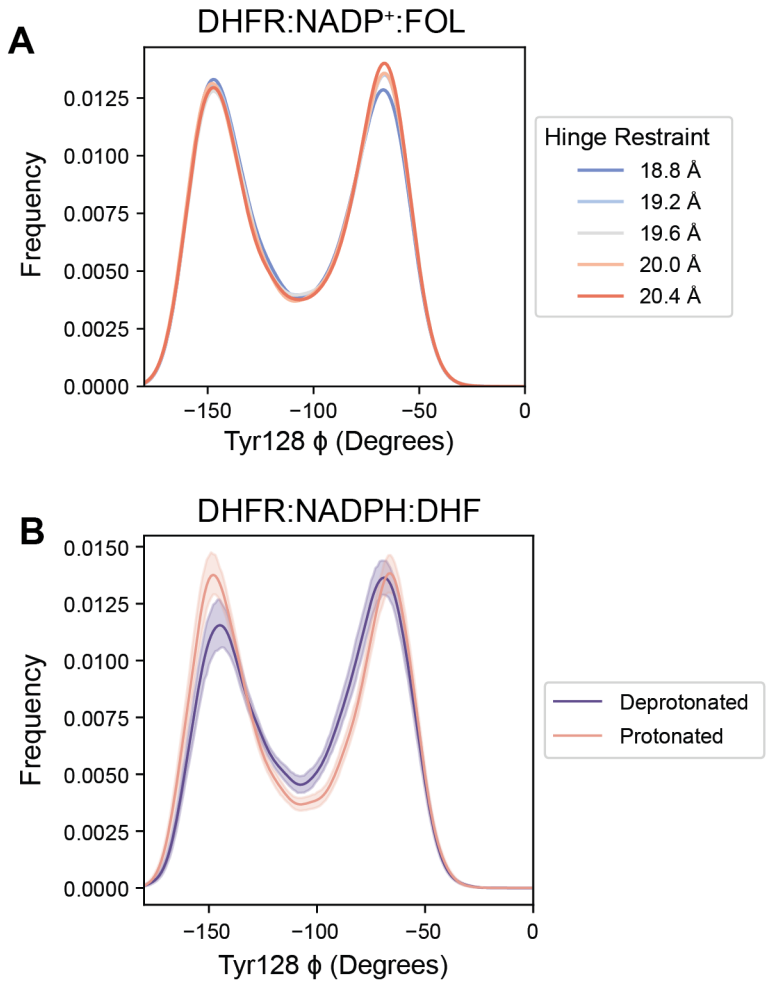

Fig. S4 Tyr128 backbone conformations in MD simulations. (A) Kernel density estimates of the Tyr128-ϕ dihedral from MD simulations at each imposed hinge distance restraint. The Tyr128-ϕ dihedral does not exhibit a monotonic relationship as a function of hinge distance. (B) Kernel density estimates of the Tyr128-ϕ dihedral from MD simulations of the reactive ternary complex (95% confidence interval is shown). The Tyr128-ϕ dihedral distribution is altered by substrate protonation.

Table S1 Summary statistics for DHFR:$NADP^+$:MFOL complex

PDB ID	8DAI
Temperature	285 K
Data Collection[1]	
Wavelength (Å)	0.9537
Spacegroup	$P2_12_12_1$
Cell dimensions (Å)	
a, b, c	34.25, 45.36, 98.85
Total observations	2,736,784
Unique observations	105,471
Resolution (Å)	49.42 - 1.14 (1.16 - 1.14)
Multiplicity	25.9 (14.4)
Completeness (%)	97.2 (73.0)
Mean I/σ_I	11.9 (0.4)
R_{pim}	0.028 (0.980)
$CC_{1/2}$	0.999 (0.326)
Refinement[2]	
R_{work} (%)	12.68
R_{free} (%)	16.00
R.M.S. Deviations	
Bonds (Å)	0.013
Angles (°)	1.357
Wilson B ($Å^2$)	15.57
Mean B factor ($Å^2$)	
Total	22.97
Macromolecules	21.07
Ligands	21.71
Water	39.71
Clashscore	2.23
Ramachandran	
Favored (%)	98.70
Allowed (%)	1.30
Outliers (%)	0.00

[1] Reported by *dials.scale* in *DIALS* [44]
[2] Reported by *PHENIX* [50]

Table S2 Summary statistics for datasets at 270 K

Crystal	1	2	3	4
PDB ID	5SSS	5SST	5SSU	5SSV
		Data Collection[1]		
Wavelength (Å)	0.8265	0.8265	0.8265	0.8265
Spacegroup	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
Cell dimensions (Å)				
a	34.11	34.08	34.10	34.12
b	45.34	45.29	45.18	45.26
c	99.11	99.00	99.09	99.06
Total observations	2,999,634	3,330,004	3,101,071	3,366,693
Unique observations	107,967	128,870	109,637	125,784
Resolution (Å)	49.56 - 1.14 (1.16 - 1.14)	33.41 - 1.07 (1.09 - 1.07)	32.24 - 1.12 (1.14 - 1.12)	45.26 - 1.08 (1.10 - 1.08)
Multiplicity	27.8 (28.1)	25.8 (27.6)	28.3 (29.5)	26.8 (27.7)
Completeness (%)	99.8 (99.9)	98.7 (96.6)	96.3 (93.6)	98.8 (96.5)
Mean I/σ_I	13.7 (0.7)	22.5 (1.5)	18.6 (0.5)	19.7 (0.4)
R_{pim}	0.077 (2.382)	0.101 (2.200)	0.134 (2.876)	0.077 (3.610)
$CC_{1/2}$	0.999 (0.400)	0.999 (0.551)	0.999 (0.415)	0.999 (0.309)
		Refinement[2]		
R_{work} (%)	14.70	13.00	13.93	13.98
R_{free} (%)	16.74	14.82	16.89	17.38
R.M.S. Deviations				
Bonds (Å)	0.010	0.007	0.008	0.012
Angles (°)	1.085	1.018	1.027	1.237
Wilson B ($Å^2$)	16.04	15.50	15.85	15.12
Mean B factor ($Å^2$)				
Total	21.71	21.28	22.90	21.95
Macromolecules	20.11	19.64	21.16	20.29
Ligands	19.14	18.73	20.27	19.27
Water	37.28	36.80	39.62	38.19
Clashscore	1.57	1.27	0.94	1.57
Ramachandran				
Favored (%)	99.35	99.35	99.35	99.35
Allowed (%)	0.65	0.65	0.65	0.65
Outliers (%)	0.00	0.00	0.00	0.00

[1] Reported by *dials.scale* in *DIALS* [44]
[2] Reported by *PHENIX* [50]

Table S3 Summary statistics for datasets at 280 K

Crystal	1	2	3	4	5
PDB ID	7FPL	7FPM	7FPN	7FPO	7FPP
			Data Collection[1]		
Wavelength (Å)	0.8265	0.8265	0.8265	0.8265	0.8265
Spacegroup	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
Cell dimensions (Å)					
a	34.12	34.16	34.14	34.18	34.20
b	45.50	45.51	45.44	45.55	45.47
c	99.05	99.08	99.04	99.08	99.09
Total observations	2,800,998	3,839,113	3,322,423	3484869	3,946,244
Unique observations	98,434	142,620	141,821	135,454	134,271
Resolution (Å)	32.26 - 1.17 (1.19 - 1.17)	45.51 - 1.04 (1.06 - 1.04)	45.44 - 1.04 (1.06 - 1.04)	45.55 - 1.06 (1.08 - 1.06)	32.33 - 1.03 (1.05 - 1.03)
Multiplicity	28.4 (28.4)	26.9 (20.5)	23.4 (18.5)	25.7 (25.4)	29.4 (28.1)
Completeness (%)	97.8 (96.7)	99.5 (96.4)	99.2 (93.1)	99.9 (98.4)	90.6 (59.8)
Mean I/σ_I	18.0 (0.7)	19.6 (0.4)	19.8 (0.4)	18.4 (0.5)	28.7 (0.5)
R_{pim}	0.054 (1.586)	0.061 (2.520)	0.212 (4.479)	0.106 (2.364)	0.018 (1.327)
$CC_{1/2}$	0.999 (0.343)	0.999 (0.309)	0.998 (0.388)	0.999 (0.336)	0.999 (0.324)
			Refinement[2]		
R_{work} (%)	14.39	12.91	13.55	14.74	12.51
R_{free} (%)	16.53	15.31	15.84	17.07	14.90
R.M.S. Deviations					
Bonds (Å)	0.007	0.010	0.008	0.009	0.010
Angles (°)	0.970	1.156	1.031	1.133	1.154
Wilson B ($Å^2$)	16.14	14.76	15.35	15.41	14.82
Mean B factor ($Å^2$)					
Total	21.99	21.06	21.84	22.07	21.36
Macromolecules	20.38	19.34	20.12	20.39	19.66
Ligands	19.42	18.54	19.03	19.30	18.60
Water	37.56	37.65	38.53	38.45	37.89
Clashscore	1.89	1.26	1.57	1.89	1.57
Ramachandran					
Favored (%)	99.35	99.35	99.35	99.35	99.35
Allowed (%)	0.65	0.65	0.65	0.65	0.65
Outliers (%)	0.00	0.00	0.00	0.00	0.00

[1] Reported by *dials.scale* in *DIALS* [44]
[2] Reported by *PHENIX* [50]

Table S4 Summary statistics for datasets at 290 K

Crystal	1	2	3	4	5
PDB ID	7FPR	7FPS	7FPT	7FPU	7FPV
			Data Collection[1]		
Wavelength (Å)	0.8265	0.8265	0.8265	0.8265	0.8265
Spacegroup	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
Cell dimensions (Å)					
a	34.18	34.19	34.19	34.18	34.20
b	45.49	45.56	45.59	45.60	45.56
c	99.10	99.05	99.05	99.07	99.04
Total observations	3,627,079	2,149,046	3,640,008	3,834,528	3,765,323
Unique observations	125,984	80,476	123,429	132,517	142,372
Resolution (Å)	32.31 - 1.07 (1.09 - 1.07)	49.53 - 1.26 (1.28 - 1.26)	32.32 - 1.07 (1.09 - 1.07)	32.31 - 1.05 (1.07 - 1.05)	41.39 - 1.04 (1.06 - 1.04)
Multiplicity	28.8 (29.4)	26.7 (23.3)	29.5 (30.2)	28.9 (27.8)	26.4 (20.7)
Completeness (%)	95.6 (92.8)	99.8 (98.4)	93.5 (90.4)	94.8 (80.5)	99.2 (95.4)
Mean I/σ_I	23.7 (0.6)	18.6 (1.1)	25.8 (0.8)	24.5 (0.6)	27.8 (0.5)
R_{pim}	0.111 (2.284)	0.105 (1.704)	0.028 (1.401)	0.027 (1.713)	0.031 (1.101)
$CC_{1/2}$	0.999 (0.312)	0.999 (0.357)	0.999 (0.555)	0.999 (0.349)	0.999 (0.364)
			Refinement[2]		
R_{work} (%)	12.37	13.17	11.84	12.25	12.60
R_{free} (%)	14.62	16.65	14.22	14.72	14.50
R.M.S. Deviations					
Bonds (Å)	0.008	0.009	0.009	0.009	0.007
Angles (°)	1.035	1.083	1.144	1.124	1.025
Wilson B ($Å^2$)	14.98	15.94	15.00	15.29	14.11
Mean B factor ($Å^2$)					
Total	23.05	22.82	21.68	21.81	21.80
Macromolecules	21.04	20.95	19.93	20.10	19.84
Ligands	19.85	20.08	18.66	18.81	18.35
Water	42.52	40.85	38.81	38.55	41.08
Clashscore	1.26	2.20	1.57	1.57	1.89
Ramachandran					
Favored (%)	99.35	99.35	99.35	99.35	99.35
Allowed (%)	0.65	0.65	0.65	0.65	0.65
Outliers (%)	0.00	0.00	0.00	0.00	0.00

[1] Reported by *dials.scale* in *DIALS* [44]
[2] Reported by *PHENIX* [50]

Table S5 Summary statistics for datasets at 300 K

Crystal	1	2	3	4	5
PDB ID	7FPX	7FPY	7FPZ	7FQ0	7FQ1
			Data Collection[1]		
Wavelength (Å)	0.8265	0.8265	0.8265	0.8265	0.8265
Spacegroup	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
Cell dimensions (Å)					
a	34.14	34.14	34.09	34.23	34.24
b	45.41	45.44	45.19	45.53	45.38
c	99.04	99.00	98.89	99.14	99.23
Total observations	3,350,065	2,995,768	1,824,827	2,913,593	2,666,016
Unique observations	134,806	114,032	69,305	104,829	95,421
Resolution (Å)	49.52 - 1.06 (1.08 - 1.06)	99.00 - 1.12 (1.14 - 1.12)	49.44 - 1.32 (1.34 - 1.32)	45.53 - 1.15 (1.17 - 1.15)	32.36 - 1.18 (1.20 - 1.18)
Multiplicity	24.8 (24.7)	26.3 (26.9)	26.3 (26.8)	27.8 (28.3)	27.9 (28.4)
Completeness (%)	99.9 (98.7)	99.7 (99.7)	99.9 (99.9)	98.4 (97.7)	97.1 (96.3)
Mean I/σ_I	20.6 (0.5)	18.7 (0.6)	15.7 (0.7)	25.0 (0.5)	22.0 (0.4)
R_{pim}	0.162 (5.383)	0.128 (3.646)	0.409 (1.023)	0.038 (1.358)	0.059 (1.140)
$CC_{1/2}$	0.999 (0.346)	0.999 (0.364)	0.997 (0.443)	0.999 (0.334)	0.999 (0.312)
			Refinement[2]		
R_{work} (%)	12.90	14.11	14.34	13.28	14.19
R_{free} (%)	15.41	17.01	18.19	16.20	17.67
R.M.S. Deviations					
Bonds (Å)	0.014	0.011	0.007	0.006	0.006
Angles (°)	1.357	1.127	0.977	0.935	0.875
Wilson B ($Å^2$)	15.54	15.64	16.72	15.23	15.89
Mean B factor ($Å^2$)					
Total	22.97	22.97	23.59	24.08	23.89
Macromolecules	21.12	21.14	21.68	21.91	21.99
Ligands	19.50	19.62	20.84	20.53	20.69
Water	41.23	40.98	42.02	45.20	42.40
Clashscore	2.52	1.89	1.89	1.57	1.26
Ramachandran					
Favored (%)	99.35	99.35	99.35	99.35	99.35
Allowed (%)	0.65	0.65	0.65	0.65	0.65
Outliers (%)	0.00	0.00	0.00	0.00	0.00

[1] Reported by *dials.scale* in *DIALS* [44]
[2] Reported by *PHENIX* [50]

Table S6 Summary statistics for datasets at 310 K

Crystal	1	2	3
PDB ID	7FQ3	7FQ4	7FQ5
	Data Collection[1]		
Wavelength (Å)	0.8265	0.8265	0.8265
Spacegroup	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
Cell dimensions (Å)			
a	34.18	34.15	34.19
b	45.49	45.23	45.30
c	99.33	99.22	99.25
Total observations	1,969,232	1,829,107	1,788,359
Unique observations	73,267	67,810	65,420
Resolution (Å)	41.36 - 1.30 (1.32 - 1.30)	33.42 - 1.33 (1.36 - 1.33)	99.25 - 1.35 (1.37 - 1.35)
Multiplicity	26.9 (27.1)	27.3 (28.6)	27.3 (28.9)
Completeness (%)	99.4 (92.4)	99.1 (97.9)	99.5 (89.5)
Mean I/σ_I	15.4 (0.4)	24.5 (0.6)	21.3 (0.5)
R_{pim}	0.310 (1.596)	0.080 (0.848)	0.108 (1.048)
$CC_{1/2}$	0.997 (0.360)	0.999 (0.301)	0.999 (0.328)
	Refinement[2]		
R_{work} (%)	15.24	14.61	14.96
R_{free} (%)	18.66	18.57	18.96
R.M.S. Deviations			
Bonds (Å)	0.006	0.008	0.009
Angles (°)	1.001	1.037	1.007
Wilson B ($Å^2$)	17.48	15.77	17.55
Mean B factor ($Å^2$)			
Total	23.71	24.37	25.03
Macromolecules	21.58	22.22	22.77
Ligands	20.69	21.76	21.86
Water	44.24	44.80	46.82
Clashscore	1.89	2.20	3.15
Ramachandran			
Favored (%)	99.35	99.35	99.35
Allowed (%)	0.65	0.65	0.65
Outliers (%)	0.00	0.00	0.00

[1] Reported by *dials.scale* in *DIALS* [44]
[2] Reported by *PHENIX* [50]

Table S7 Summary statistics for multi-crystal, multi-temperature datasets

Temperature	270 K	280 K	290 K	300 K	310 K
PDB ID	5SSW	7FPQ	7FPW	7FQ2	7FQ6
Number of Crystals	4	5	5	5	3
			Data Collection[1]		
Wavelength (Å)	0.8265	0.8265	0.8265	0.8265	0.8265
Spacegroup	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
Cell dimensions (Å)					
a	34.10	34.16	34.19	34.14	34.18
b	45.28	45.50	45.56	45.41	45.30
c	99.08	99.08	99.05	99.04	99.25
Total observations	14,601,731	19,116,204	19,028,898	17,134,249	6,176,809
Unique observations	133,686	147,105	142,798	131,221	74,931
Resolution (Å)	49.54 - 1.06 (1.08 - 1.06)	45.50 - 1.03 (1.05 - 1.03)	49.53 - 1.04 (1.06 - 1.04)	99.04 - 1.07 (1.09 - 1.07)	99.25 - 1.29 (1.31 - 1.29)
Multiplicity	109.1 (107.6)	129.9 (87.0)	133.2 (101.9)	130.5 (128.3)	82.2 (84.7)
Completeness (%)	99.4 (96.5)	99.8 (96.3)	99.5 (96.0)	100.0 (100.0)	99.8 (94.0)
Mean I/σ_I	36.9 (1.4)	45.5 (0.7)	44.0 (0.8)	38.5 (0.8)	31.6 (0.6)
R_{pim}	0.050 (2.423)	0.045 (3.530)	0.052 (15.696)	0.170 (1.657)	0.098 (0.839)
$CC_{1/2}$	1.000 (0.446)	1.000 (0.429)	1.000 (0.398)	1.000 (0.557)	1.000 (0.326)
			Refinement[2]		
R_{work} (%)	12.34	11.83	11.55	12.13	13.96
R_{free} (%)	14.39	13.84	13.52	14.38	17.89
R.M.S. Deviations					
Bonds (Å)	0.009	0.008	0.009	0.010	0.012
Angles (°)	1.137	1.072	1.167	1.146	1.161
Wilson B ($Å^2$)	15.47	15.24	14.64	15.13	18.97
Mean B factor ($Å^2$)					
Total	21.22	21.27	21.71	23.43	25.21
Macromolecules	19.62	19.53	19.85	21.38	23.07
Ligands	18.63	18.61	18.14	19.53	22.40
Water	36.78	37.65	40.14	43.78	45.87
Clashscore	1.89	1.57	1.57	2.20	2.20
Ramachandran					
Favored (%)	99.35	99.35	99.35	99.35	99.35
Allowed (%)	0.65	0.65	0.65	0.65	0.65
Outliers (%)	0.00	0.00	0.00	0.00	0.00

[1] Reported by *dials.scale* in *DIALS* [44]
[2] Reported by *PHENIX* [50]

Table S8 Summary statistics for single-crystal, multi-temperature datasets (crystal 1)

Temperature	295 K	310 K	295 K	280 K	295 K
Pass on Crystal	1	2	3	4	5
PDB ID	7FQ7	7FQ8	7FQ9	7FQA	7FQB
			Data Collection[1]		
Wavelength (Å)	0.8265	0.8265	0.8265	0.8265	0.8265
Spacegroup	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
Cell dimensions (Å)					
a	34.26	34.29	34.27	34.20	34.22
b	45.59	45.65	45.63	45.46	45.47
c	98.96	99.03	98.97	98.99	99.02
Total observations	3,400,772	2,601,689	3,315,426	3,572,742	3,053,872
Unique observations	117,634	89,462	115,024	123,443	105,250
Resolution (Å)	32.37 - 1.10 (1.12 - 1.10)	41.46 - 1.21 (1.23 - 1.21)	41.44 - 1.11 (1.13 - 1.11)	32.32 - 1.08 (1.10 - 1.08)	32.34 - 1.14 (1.16 - 1.14)
Multiplicity	28.9 (28.8)	29.1 (29.5)	28.8 (28.6)	28.9 (29.0)	29.0 (28.0)
Completeness (%)	96.7 (95.6)	97.6 (96.6)	97.1 (96.4)	96.5 (94.5)	96.6 (96.2)
Mean I/σ_I	12.8 (0.3)	14.1 (0.4)	12.6 (0.3)	12.7 (0.3)	13.5 (0.4)
R_{pim}	0.027 (1.213)	0.037 (1.105)	0.029 (1.257)	0.025 (1.426)	0.028 (1.245)
$CC_{1/2}$	0.999 (0.324)	0.999 (0.380)	0.999 (0.354)	0.999 (0.311)	0.999 (0.332)
			Refinement[2]		
R_{work} (%)	12.80	12.64	13.13	13.11	13.36
R_{free} (%)	15.89	16.40	15.94	15.89	16.48
R.M.S. Deviations					
Bonds (Å)	0.008	0.011	0.007	0.005	0.006
Angles (°)	1.054	1.192	1.008	0.884	0.953
Wilson B ($Å^2$)	17.06	16.74	17.13	17.26	16.81
Mean B factor ($Å^2$)					
Total	22.18	23.86	23.34	21.75	22.30
Macromolecules	20.38	21.68	21.54	20.09	20.46
Ligands	18.80	19.14	19.88	18.77	18.97
Water	39.94	45.87	41.17	38.11	40.38
Clashscore	1.26	2.20	1.26	1.26	1.89
Ramachandran					
Favored (%)	99.35	99.35	99.35	99.35	99.35
Allowed (%)	0.65	0.65	0.65	0.65	0.65
Outliers (%)	0.00	0.00	0.00	0.00	0.00

[1] Reported by *dials.scale* in *DIALS* [44]
[2] Reported by *PHENIX* [50]

Table S9 Summary statistics for single-crystal, multi-temperature datasets (crystal 2)

Temperature	295 K	280 K	295 K	310 K	295 K
Pass on Crystal	1	2	3	4	5
PDB ID	7FQC	7FQD	7FQE	7FQF	7FQG
			Data Collection[1]		
Wavelength (Å)	0.9795	0.9795	0.9795	0.9795	0.9795
Spacegroup	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
Cell dimensions (Å)					
a	34.26	34.20	34.25	34.30	34.28
b	45.63	45.52	45.60	45.71	45.68
c	99.03	99.06	99.09	99.12	99.04
Total observations	2,722,807	2,756,746	2,721,740	2,438,928	2,674,944
Unique observations	97,218	99,044	97,041	86,426	95,020
Resolution (Å)	49.52 - 1.18 (1.20 - 1.18)	49.53 - 1.17 (1.19 - 1.17)	49.55 - 1.18 (1.20 - 1.18)	49.56 - 1.23 (1.25 - 1.23)	49.52 - 1.19 (1.21 - 1.19)
Multiplicity	28.0 (23.7)	27.8 (21.2)	28.0 (23.8)	28.2 (28.4)	28.1 (26.5)
Completeness (%)	98.5 (94.9)	98.2 (91.2)	98.4 (94.4)	98.9 (98.2)	98.6 (95.5)
Mean I/σ_I	13.0 (0.4)	12.8 (0.4)	12.6 (0.4)	12.3 (0.3)	12.1 (0.4)
R_{pim}	0.033 (1.029)	0.031 (1.107)	0.033 (1.127)	0.044 (1.404)	0.038 (1.220)
$CC_{1/2}$	0.999 (0.359)	0.999 (0.308)	0.999 (0.308)	0.999 (0.321)	0.999 (0.333)
			Refinement[2]		
R_{work} (%)	12.74	14.09	12.88	13.06	13.13
R_{free} (%)	16.00	16.17	16.38	16.91	16.41
R.M.S. Deviations					
Bonds (Å)	0.008	0.008	0.009	0.013	0.005
Angles (°)	1.028	1.030	1.114	1.238	0.905
Wilson B ($Å^2$)	17.21	17.63	17.19	17.08	16.96
Mean B factor ($Å^2$)					
Total	22.47	21.53	22.56	25.06	22.56
Macromolecules	20.65	19.92	20.71	22.89	20.73
Ligands	19.14	18.95	19.18	20.60	19.09
Water	40.35	37.10	40.75	46.94	40.71
Clashscore	2.20	1.89	2.20	2.20	1.57
Ramachandran					
Favored (%)	99.35	99.35	99.35	99.35	99.35
Allowed (%)	0.65	0.65	0.65	0.65	0.65
Outliers (%)	0.00	0.00	0.00	0.00	0.00

[1] Reported by *dials.scale* in *DIALS* [44]
[2] Reported by *PHENIX* [50]

Table S10 Data reduction statistics for DHFR EF-X from Laue diffraction

Dataset	Off	Off (reduced sym.)	200 ns (+3.5 kV)	200 ns (-3.5 kV)
No. of Images	363	363	363	363
Spacegroup	$P2_12_12_1$	$P2_1$	$P2_1$	$P2_1$
Cell dim. (Å)				
a	34.29	34.29	34.29	34.29
b	45.53	45.53	45.53	45.53
c	99.00	99.00	99.00	99.00
Total obs.	723,372	723,372	710,019	709,472
Unique obs.	17,637	33,671	33,671	33,669
Resolution (Å)	41.36 - 1.70 (1.76 - 1.70)	41.36 - 1.70 (1.76 - 1.70)	41.36 - 1.70 (1.76 - 1.70)	49.50 - 1.70 (1.76 - 1.70)
Multiplicity	35.53 (27.40)	18.63 (14.16)	18.35 (13.73)	18.34 (13.68)
Completeness (%)	99.5 (99.4)	99.4 (99.5)	99.4 (99.5)	99.4 (99.5)
Mean F/σ_F[1]	39.38 (19.51)	28.54 (14.00)	28.85 (13.86)	28.85 (13.83)
$CC_{1/2}$[1]	0.991 (0.957)	0.987 (0.927)	0.989 (0.929)	0.988 (0.929)

[1] Statistics were computed based on output from *careless* [46]

Table S11 Refinement statistics for DHFR EF-X[1]

Dataset	Off	On
PDB ID	8G4Z	8G50
Spacegroup	$P2_12_12_1$	$P2_1$
Extrapolation factor	N/A	8
Resolution (Å)	1.70	1.70
Unique observations	17,636	33,646
Completeness	99.43	99.26
R_{work} (%)	14.71	30.37
R_{free} (%)	19.53	34.98
R.M.S. Deviations		
Bonds (Å)	0.009	0.011
Angles (°)	1.15	1.09
Mean B factor (Å^2)		
Total	8.53	5.54
Macromolecules	7.52	5.16
Ligands	7.74	4.81
Water	20.08	9.97
Clashscore	2.01	3.05
Ramachandran		
Favored (%)	99.35	99.35
Allowed (%)	0.65	0.65
Outliers (%)	0.00	0.00

[1] Reported by *PHENIX* [50]