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Supporting information for article:

Probing ligand binding of endothiapepsin by `temperature-resolved' macromolecular crystallography

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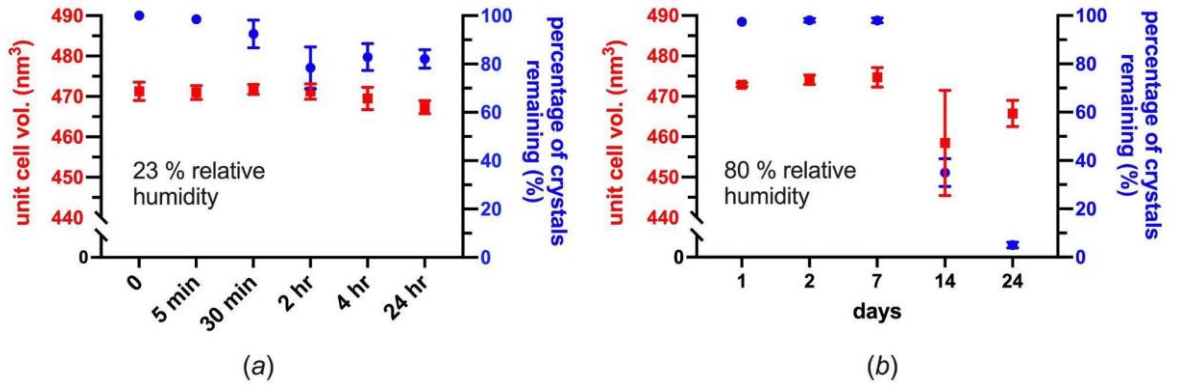


Figure S1. Insulin crystal lifetime when left at 23% and 80% relative humidity. Hundreds of 30 µm insulin crystals were loaded onto 3 µm COC (Cyclic Olefin Copolymer) supports and sealed with 13 µm COP (Cyclic Olefin Polymer). The crystals were left at 24 °C for given periods of time. **(*a*)** and **(*b*)** show how the unit cell and crystal number change when the supports were either stored at ambient room humidity 23% and 80%, respectively.

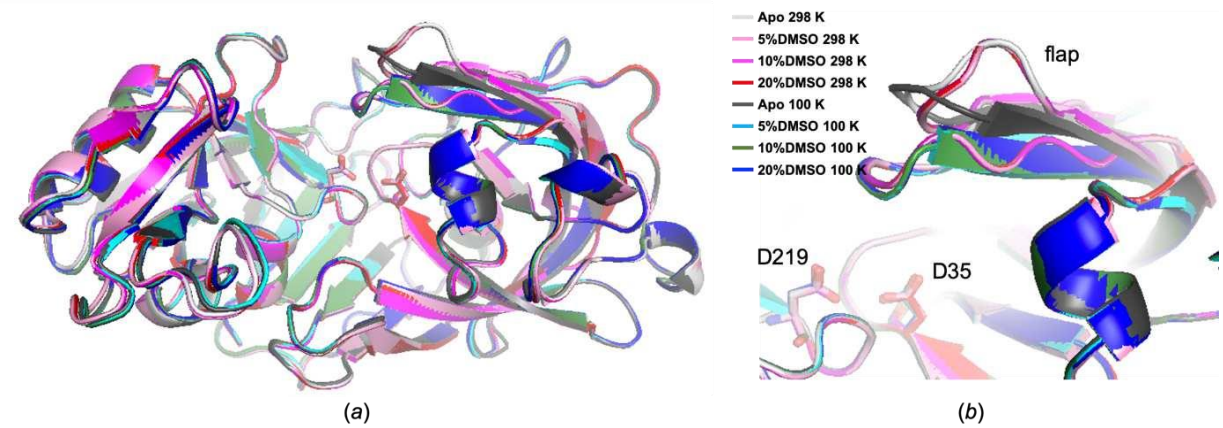


Figure S2. Comparison of the EP structures without TL00150 in a series concentration of DMSO at 100 K and 298 K. **(*a*)** Overlay of overall structures. **(*b*)** Enlarged view of the EP active site and flap domain. The EP with 5%, 10%, 20% (*v/v*) DMSO and EP apo, both at 100 K and 298 K are indicated in the panel **(*b*).** From residues 1 to 330 of the EP, the RMSD (Root Mean Square Deviation) for structures of 5%, 10%, and 20% DMSO at 298 K to apo structure at 298 K are 0.065 Å, 0.054 Å, and 0.050 Å, respectively. The RMSD for the same range of residues in structures with 5%, 10%, and 20% DMSO at 100 K to apo structure at 100K are 0.102 Å, 0.121 Å, and 0.126 Å, respectively.

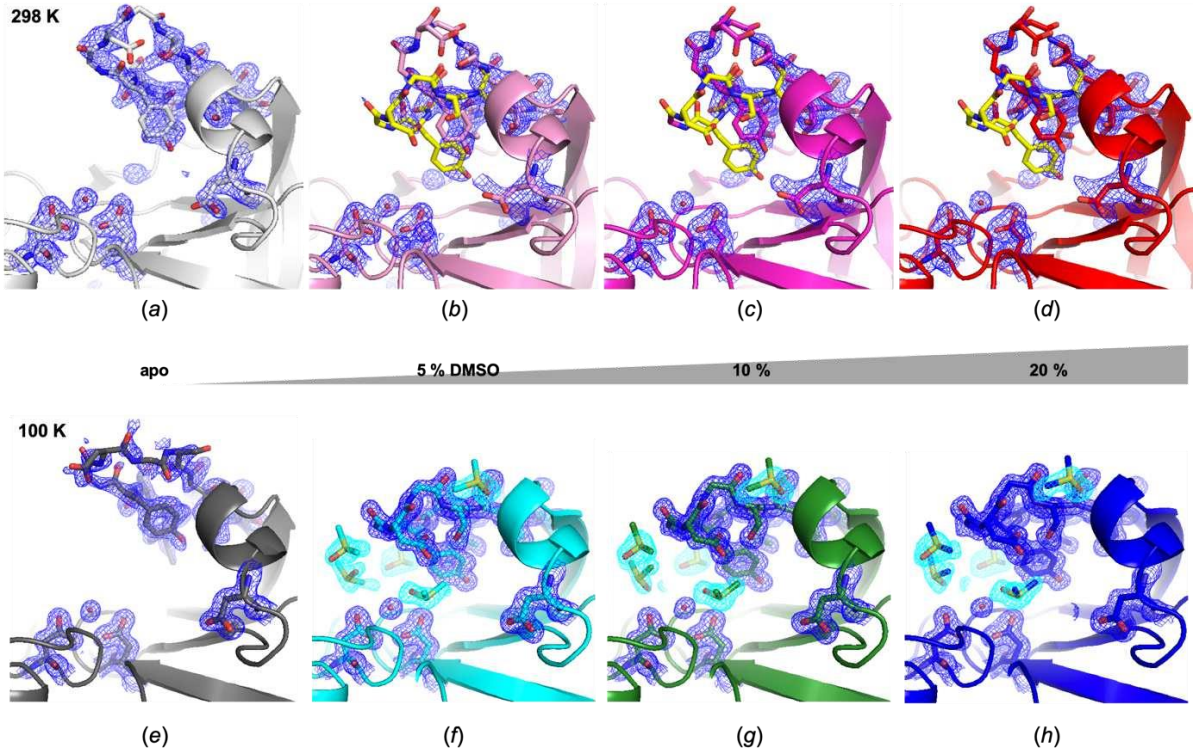


Figure S3. $2F_o$-F_c electron density maps of the EP structures without TL00150 with an increasing concentration of DMSO at 100 K and 298 K. The $2F_o$-F_c electron density maps contoured at 1σ around the active site and flap domain (residues S76 to S86) of EP structures are shown. Maps of the EP apo, EP structure with 5% (v/v) DMSO, with 10% (v/v) DMSO and with 20% (v/v) DMSO at 298 K are shown in **(*a*)**, **(*b*)**, **(*c*)** and **(*d*)**, respectively. Maps of the EP apo, EP structure with 5%, 10% and 20% (v/v) DMSO at 100 K are shown in **(*e*)**, **(*f*)**, **(*g*)** and **(*h*)**, respectively. The color code for the structures is the same as in Fig. S2. The alternative conformations of RT EP structures are highlighted in yellow. The $2F_o$-F_c meshes are shown in blue and contoured to 1σ for the flap domain, D35, D119 and D219 and water molecules. The $2F_o$-F_c meshes for the DMSO molecules are colored in cyan.

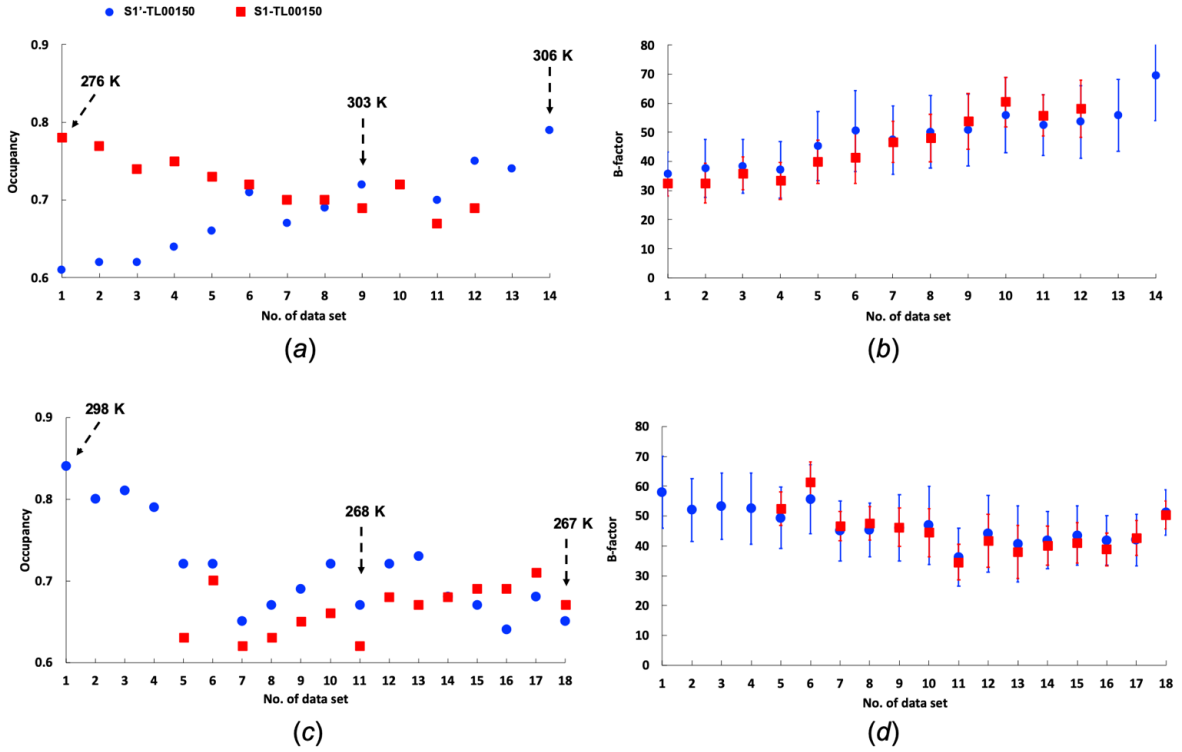


Figure S4. Comparison of the occupancy and B-factors of TL00150 in the EP structures. **(*a*)** Occupancy and (***b***) average B-factors of TL00150 for the temperatureramp-up experiment**. (*c*)** Occupancy and (***d***) average B-factors of TL00150 for the temperature ramp-down experiment**.** The temperatures of 276 K, 303 K, and 306 K are indicated in **(*a*)** and 298 K, 268 K and 267 K are indicated in **(*c*)**. The temperatures discussed here were measured from the sample by infrared camera (details describedin §2.3).

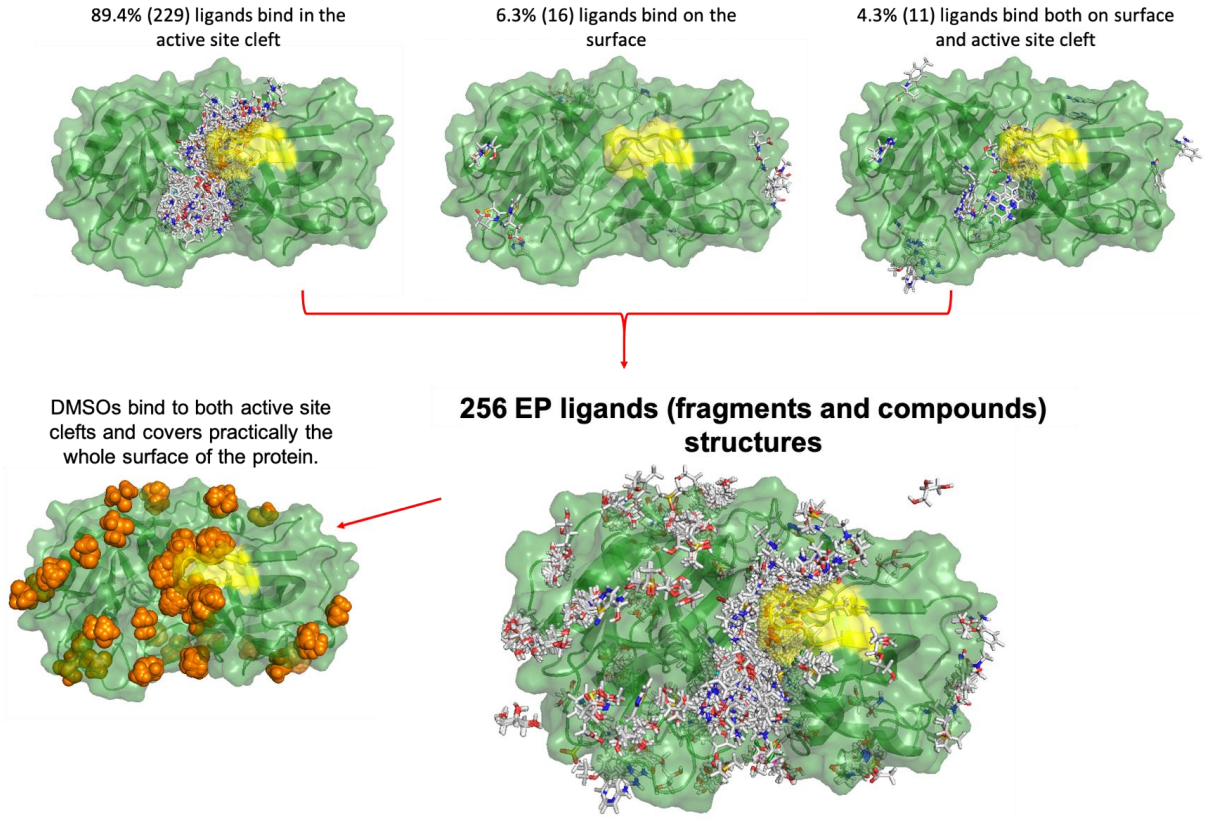


Figure S5. Comparison of 256 EP ligand structures. The 256 structures downloaded from the PDB (ref. Table S6) were aligned and classified. The EP structures are shown as surface representation with green color and flap domain is highlighted in yellow. The ligands are shown in stick representation. The DMSOs are highlighted in sphere representation with orange color. Depicted ligand hydrogens were added using the Schrödinger Preparation Wizard (Schrödinger Release 2021-1: Maestro, Schrödinger, LLC, New York, NY, 2021) without energy minimization to enable pose comparisons using Shape Screening with typed atoms.

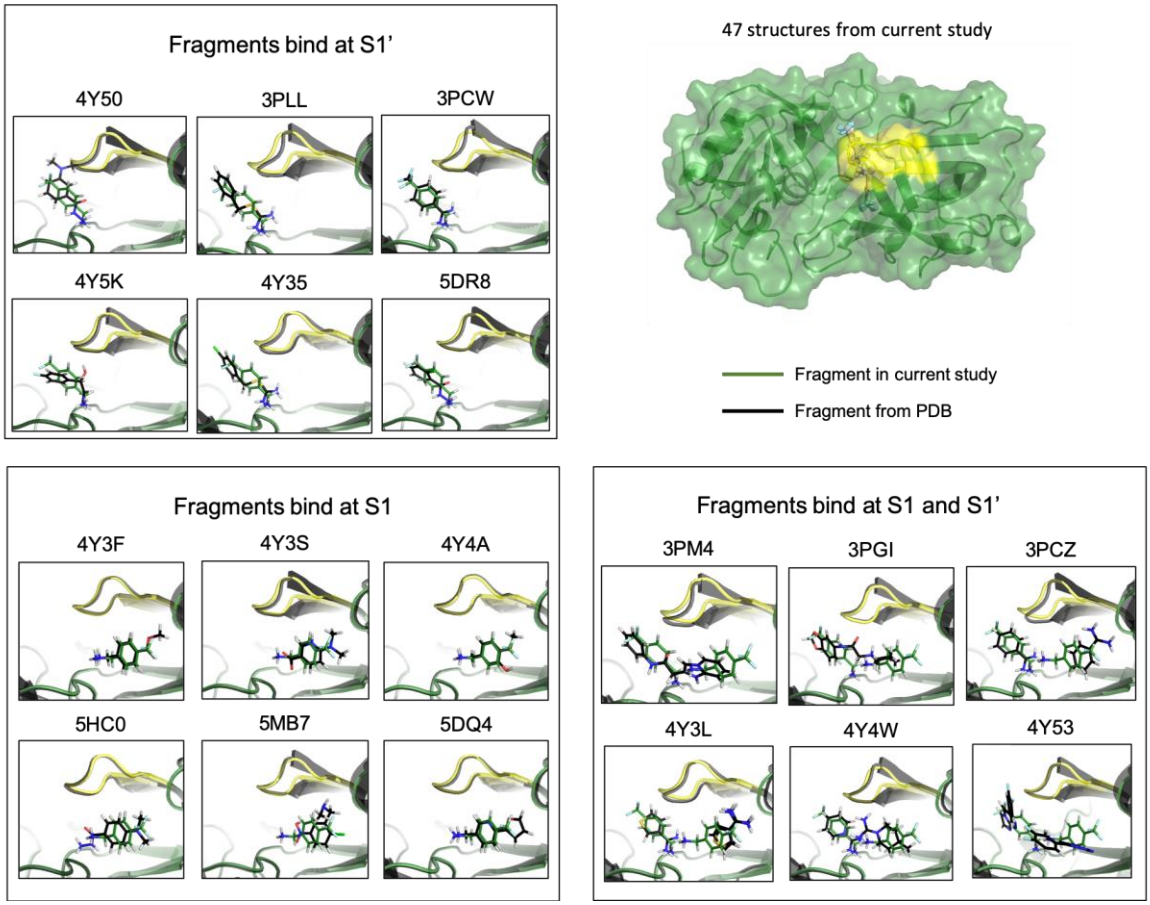
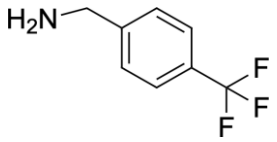


Figure S6. Comparison of the fragments from temperature-resolved structures with fragments with similar binding modes taken from the PDB. The enlarged view of the EP active site is shown. The 6 structures were chosen from 256 structures for comparison of S1', S1 and both S1' and S1 binding modes. For TL00150 and other PDB fragments, the fragments are shown in stick representation with green and black color, respectively. In the figures, the PDB codes are shown. Depicted ligand hydrogens were added using the Schrödinger Preparation Wizard (Schrödinger Release 2021-1: Maestro, Schrödinger, LLC, New York, NY, 2021) without energy minimization to enable pose comparisons using Shape Screening with typed atoms.

Table S1. Fragment specification

Fragment name	SMILES description	Chemical structure*
TL00150 [4(trifluoromethyl)benzylamine]	NCc1ccc(C(F)(F)F)cc1	

*Chemical structure was generated with ChemDraw direct (https://chemdrawdirect.perkinelmer.cloud/js/sample/index.html#)

Table S2. Statistic of EP-TL00150 structures at RT of the serial DMSO concentration

	5%DMSO-TL00150	8%DMSO-TL00150	10%DMSO-TL00150	15%DMSO-TL00150	20%DMSO-TL00150	40%DMSO-TL00150
PDB code	**7QM3**	**7QM4**	**7QM5**	**7QM6**	**7QM7**	**7QM8**
Resolution (Å)	37.36 - 1.79 (1.85 - 1.79)	37.39 - 1.79 (1.85 - 1.79)	35.27 - 1.79 (1.85 - 1.79)	37.37 - 1.81 (1.88 - 1.81)	37.37 - 1.79 (1.85 - 1.79)	37.33 - 1.79 (1.85 - 1.79)
Space group	$P12_11$	$P12_11$	$P12_11$	$P12_11$	$P12_11$	$P12_11$
Unit-cell parameters *a,b,c* (Å)	45.87 73.91 53.01	45.95 74.07 53.32	45.91 74.04 53.36	45.98 74.09 53.45	46.00 74.04 53.55	45.96 74.32 53.75
β (°)	109.29	109.49	109.64	109.72	109.79	110.09
Unique reflections	31104 (3061)	31299 (3022)	31279 (3066)	30425 (2980)	31619 (3072)	31552 (3116)
Multiplicity	6.93 (6.57)	6.94 (6.56)	6.99 (6.67)	7.02 (6.72)	6.93 (6.57)	1.05 (6.67)
Completeness (%)	98.57 (97.61)	98.37 (95.00)	98.46 (96.69)	98.71 (97.67)	98.96 (96.21)	98.43 (97.16)
Mean *I/σ(I)*	10.30 (1.09)	13.16 (1.44)	19.86 (4.08)	13.07 (1.26)	9.40 (0.68)	23.18 (4.28)
Wilson B-factor	29.47	28.96	24.36	30.84	31.41	22.10
R_{meas}	0.12 (1.39)	0.19 (1.12)	0.06 (0.41)	0.10 (1.38)	0.14 (2.28)	0.05 (0.41)
CC1/2	0.99 (0.65)	0.99 (0.73)	0.99 (0.94)	0.99 (0.67)	0.99 (0.37)	0.99 (0.95)
ISa	16.88	24.73	28.95	25.96	19.10	40.09
Reflections used in refinement	31089 (3061)	31296 (3022)	31276 (3066)	30420 (2978)	31576 (3069)	31548 (3114)
Reflections used for R_{free}	1555 (153)	1563 (152)	1564 (154)	1520 (149)	1579 (153)	1577 (156)
R_{work}/R_{free}	0.19/0.22	0.16/0.19	0.15/0.17	0.15/0.19	0.20/0.23	0.14/0.17
No. of atoms	2530	2530	2569	2578	2614	2608
macromolecules	2384	2384	2379	2379	2379	2379
ligands	32	32	32	52	44	36
solvent	114	114	158	147	191	193
Protein residues	330	330	330	330	330	330
RMS(bonds) (Å)	0.008	0.003	0.004	0.005	0.003	0.006
RMS(angles) (°)	0.96	0.61	0.72	0.78	0.59	0.83
Ramachandran favored (%)	99.09	99.09	99.09	99.09	99.09	98.78
Ramachandran allowed (%)	0.91	0.91	0.91	0.91	0.91	1.22
Ramachandran outliers (%)	0.00	0.00	0.00	0.00	0.00	0.00
Clashscore	1.28	1.70	1.28	1.27	0.64	0.85
Average B-factor (Å^2)	34.57	34.19	29.67	33.14	35.05	25.99
B-factor macromolecules (Å^2)	34.28	33.55	28.60	32.08	33.97	24.81
B-factor ligands (Å^2)	48.24	59.52	60.43	57.82	50.25	46.25
B-factor solvent (Å^2)	36.86	40.45	39.49	41.68	44.99	36.76

*Statistics for the highest-resolution shell are shown in parentheses.
**Data processing statistics are reported with Friedel pairs merged.

Table S3. Statistic of EP apo structures, EP structures without TL00150 with an increasing concentration of DMSO and EP structure with TL00150 at both 298 K and100 K

	apo-298K	5%DMSO-298K	10%DMSO-298K	20%DMSO-298K	apo-100K	5%DMSO-100K	10%DMSO-100K	20%DMSO-100K	10% DMSO-TL00150-100K
PDB code	**7QLY**	**7QLZ**	**7QM0**	**7QM1**	**7QLU**	**7QLV**	**7QLW**	**7QLX**	**7QLT**
Resolution (Å)	37.37 - 1.79 (1.85 - 1.79)	35.29 - 1.79 (1.85 - 1.79)	43.26 - 1.79 (1.86 - 1.79)	35.41 - 1.79 (1.85 - 1.79)	35.13 - 1.41 (1.46 - 1.41)	35.15 - 1.41 (1.46 - 1.41)	35.13 - 1.41 (1.46 - 1.41)	35.12 - 1.39 (1.44 - 1.39)	34.96 - 1.39 (1.44 - 1.39)
Space group	P_12_1	P_12_1	P_12_1	P_12_1	P_12_1	P_12_1	P_12_1	P_12_1	P_12_1
Unit-cell parameters *a,b,c* (Å)	45.97 74.17 53.54	45.97 74.12 53.37	45.98 74.23 53.64	45.96 74.26 53.65	45.68 72.46 53.38	45.53 73.51 53.29	45.47 73.69 53.28	45.45 73.72 53.29	45.33 73.47 52.99
Unit-cell parameters β (°)	109.78	109.56	109.81	109.90	110.35	110.20	110.08	110.07	109.9
Unique reflections	31529 (3095)	31333 (2855)	31331 (2906)	31452 (3087)	60448 (5856)	63364 (6284)	62760 (6161)	65999 (6555)	65469 (6412)
Multiplicity	6.84 (6.52)	6.94 (6.48)	7.04 (6.70)	7.00 (6.63)	6.86 (6.91)	6.77 (6.63)	6.89 (6.91)	6.61 (6.52)	6.8 (6.53)
Completeness (%)	98.70 (97.30)	98.37 (89.92)	98.14 (91.04)	98.32 (97.14)	96.20 (93.26)	99.87 (99.37)	98.71 (97.13)	99.29 (99.08)	99.76 (98.13)
Mean $I/\sigma(I)$	7.58 (0.97)	6.55 (0.32)	12.02 (0.73)	10.33 (0.32)	11.10 (1.06)	15.48 (1.34)	20.03 (1.79)	19.86 (3.78)	21.99 (2.35)
Wilson B-factor	30.41	33.53	37.36	34.17	17.15	17.28	17.08	13.09	15.41
R_{meas}	0.16 (2.10)	0.18 (4.04)	0.10 (1.87)	0.13 (3.53)	0.14 (1.88)	0.08 (1.55)	0.06 (1.19)	0.06 (0.52)	0.06 (0.87)
$CC_{1/2}$	0.99 (0.42)	0.99 (0.14)	0.99 (0.40)	0.99 (0.17)	0.99 (0.60)	0.99 (0.73)	0.99 (0.81)	0.99 (0.94)	0.99 (0.88)
ISa	8.01	11.41	32.58	26.13	14.23	21.31	28.43	21.65	29.23
Reflections used in refinement	31514 (3094)	31324 (2855)	31325 (2906)	31451 (3087)	60426 (5856)	63331 (6270)	62738 (6151)	65862 (6548)	65442 (6399)
Reflections used for R_{free}	1576 (155)	1564 (140)	1566 (146)	1572 (154)	3021 (293)	3166 (314)	3137 (309)	3296 (327)	3272 (320)
R_{work}/R_{free}	0.19/0.22	0.18/0.20	0.16/0.19	0.16/0.19	0.20/0.23	0.19/0.22	0.17/0.19	0.20/0.23	0.18/0.20
No. of atoms	2564	2573	2585	2585	2998	2982	2980	2977	2938
macromolecules	2376	2448	2448	2448	2379	2382	2382	2382	2385
ligands	0	0	0	0	12	36	36	36	48
solvent	188	125	137	137	607	564	562	559	505
Protein residues	330	330	330	330	330	330	330	330	330
RMS(bonds) (Å)	0.002	0.003	0.003	0.002	0.007	0.012	0.010	0.007	0.009
RMS(angles) (°)	0.61	0.64	0.64	0.62	0.96	1.27	1.13	0.98	1.09
Ramachandran favored (%)	99.39	98.48	98.78	98.78	97.87	98.78	98.48	98.48	98.78
Ramachandran allowed (%)	0.61	1.52	1.22	1.22	2.13	1.22	1.52	1.52	1.22
Ramachandran outliers (%)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Clashscore	1.08	1.69	1.05	1.26	1.07	1.27	2.33	1.91	1.05
Average B-factor (Å^2)	34.51	39.24	38.34	38.34	20.65	19.89	19.77	16.72	19.51
B-factor macromolecules (Å^2)	33.89	39.03	38.06	38.00	18.03	16.91	16.51	13.81	16.63
B-factor ligands (Å^2)	-	-	-	-	32.83	35.70	35.40	29.38	30.52
B-factor solvent (Å^2)	42.42	43.30	43.32	44.35	30.66	31.45	32.59	28.31	32.06

*Statistics for the highest-resolution shell are shown in parentheses.

**Data processing statistics are reported with Friedel pairs merged.

Table S4. Statistic of EP-TL00150 structures of the temperature ramp-up

	dataset1	dataset2	dataset3	dataset4	dataset5	dataset6	dataset7	dataset8	dataset9	dataset10	dataset11	dataset12	dataset13	dataset14
PDB colde	**7QMR**	**7QMS**	**7QMT**	**7QMU**	**7QMV**	**7QMW**	**7QMX**	**7QMY**	**7QMZ**	**7QN0**	**7QN1**	**7QN2**	**7QN3**	**7QN4**
Temperature (K)	276	280	282	286	289	292	295	298	301	303	305	305	306	306
Resolution (Å)	37.26 - 1.79 (1.85 - 1.79)	35.37 - 1.79 (1.85 - 1.79)	37.29 - 1.79 (1.85 - 1.79)	37.29 - 1.79 (1.85 - 1.79)	35.37 - 1.79 (1.85 - 1.79)	35.37 - 1.79 (1.85 - 1.79)	40.24 - 1.79 (1.85 - 1.79)	37.37 - 1.79 (1.85 - 1.79)	37.39 - 1.79 (1.85 - 1.79)	37.40 - 1.79 (1.85 - 1.79)	37.41 - 1.79 (1.85 - 1.79)	37.41 - 1.79 (1.86 -1.79)	35.29 - 1.79 (1.85 - 1.79)	35.25 - 1.79 (1.85 - 1.79)
Space group	P_12_1	P_12_1	P_12_1	P_12_1	P_12_1	P_12_1	P_12_1	P_12_1	P_12_1	P_12_1	P_12_1	P_12_1	P_12_1	P_12_1
Unit-cell *a,b,c* (Å)	45.85 74.17	45.87 74.15	45.88 74.14	45.89 74.13	45.92 74.13	45.94 74.13	45.97 74.13	45.98 74.13	46.00 74.14	46.00 74.13	46.01 74.13	46.00 74.11	46.01 74.10	46.02 74.12
parameters	53.65	53.64	53.62	53.62	53.59	53.58	53.55	53.53	53.49	53.44	53.42	53.36	53.31	53.21
β (°)	109.99	109.95	109.92	109.94	109.88	109.8	109.80	109.78	109.72	109.67	109.65	109.60	109.54	109.42
Unique reflections	31420 (3101)	31415 (3106)	31420 (3096)	31436 (3097)	31445 (3101)	31442 (3096)	31465 (3096)	31444 (3068)	31445 (3066)	31443 (3079)	31438 (3080)	31391 (3083)	31376 (3060)	30983 (2690)
Multiplicity	7.04 (6.62)	7.01 (6.61)	7.01 (6.64)	7.02 (6.61)	7.00 (6.61)	7.00 (6.61)	6.99 (6.59)	6.98 (6.60)	6.99 (6.59)	6.99 (6.57)	6.98 (6.57)	6.99 (6.59)	6.99 (6.57)	7.00 (6.61)
Completeness (%)	98.58 (96.94)	98.59 (97.03)	98.56 (96.84)	98.58 (96.81)	98.60 (97.15)	98.55 (96.78)	98.56 (96.99)	98.50 (96.24)	98.51 (96.17)	98.54 (96.70)	98.58 (96.89)	98.61 (97.16)	98.54 (96.50)	97.36 (84.94)
Mean *I/σ(I)*	11.9 (1.62)	14.00 (2.33)	8.93 (0.88)	13.59 (2.39)	12.30 (1.54)	12.64 (1.54)	12.23 (1.10)	12.64 (1.33)	12.59 (1.22)	12.27 (0.95)	11.95 (0.92)	12.50 (1.13)	12.09 (0.95)	9.80 (0.28)
Wilson B-factor	23.42	22.84	24.25	22.53	25.56	26.16	29.61	28.98	30.58	32.96	33.02	31.96	32.94	40.55
R_{meas}	0.12 (0.98)	0.10 (0.70)	0.15 (1.67)	0.10 (0.70)	0.11 (1.03)	0.10 (1.07)	0.10 (1.54)	0.10 (1.27)	0.09 (1.37)	0.10 (1.73)	0.10 (1.76)	0.09 (1.37)	0.10 (1.66)	0.12 (3.88)
$CC_{1/2}$	0.99 (0.76)	0.99 (0.85)	0.99 (0.65)	0.99 (0.85)	0.99 (0.73)	0.99 (0.70)	0.99 (0.59)	0.99 (0.61)	0.99 (0.56)	0.99 (0.46)	0.99 (0.44)	0.99 (0.54)	0.99 (0.47)	0.99 (0.12)
ISa	19.47	20.01	18.72	18.37	19.63	19.60	20.37	18.62	20.11	21.27	20.25	22.20	23.20	23.28
Reflections used in refinement	31416 (3100)	31413 (3106)	31416 (3096)	31432 (3097)	31441 (3100)	31438 (3094)	31461 (3095)	31439 (3068)	31440 (3066)	31436 (3079)	31431 (3080)	31386 (3083)	31370 (3060)	30972 (2690)
Reflections used for R_{free}	1633 (148)	1639 (156)	1644 (162)	1634 (150)	1634 (151)	1633 (149)	1636 (148)	1635 (148)	1634 (147)	1636 (148)	1634 (147)	1632 (146)	1631 (144)	1614 (130)
R_{work}/R_{free}	0.17/0.20	0.17/0.19	0.16/0.19	0.16/0.19	0.17/0.19	0.16/0.18	0.17/0.20	0.16/0.19	0.16/0.18	0.17/0.19	0.17/0.19	0.16/0.19	0.16/0.19	0.16/0.20
No. of atoms	2661	2650	2638	2656	2634	2604	2589	2578	2582	2561	2552	2547	2531	2501
macromolecules	2379	2379	2379	2379	2379	2379	2379	2379	2379	2382	2379	2379	2379	2379
ligands	52	52	52	52	52	52	52	52	52	52	52	52	40	40
solvent	230	219	207	225	203	173	158	147	151	127	121	116	112	82
Protein residues	330	330	330	330	330	330	330	330	330	330	330	330	330	330
RMS(bonds) (Å)	0.006	0.003	0.006	0.006	0.003	0.003	0.005	0.014	0.005	0.005	0.005	0.016	0.015	0.010
RMS(angles) (°)	0.84	0.62	0.82	0.82	0.64	0.68	0.76	1.26	0.79	0.77	0.79	1.45	1.34	1.00
Ramachandran favored (%)	98.78	99.09	98.78	99.09	98.78	99.09	99.09	98.17	98.78	98.78	98.48	99.09	98.78	97.87
Ramachandran allowed (%)	1.22	0.91	1.22	0.91	1.22	0.91	0.91	1.83	1.22	1.22	1.52	0.91	1.22	2.13
Ramachandran outliers (%)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Clashscore	1.27	1.27	1.48	1.27	1.70	1.70	1.27	1.48	1.48	1.69	1.48	1.48	1.70	1.49
Average B-factor (Å²)	26.59	25.96	27.30	25.70	29.31	29.85	33.51	32.55	33.73	37.38	37.23	35.47	38.38	48.45
B-factor macromolecules (Å²)	25.51	24.86	26.19	24.48	28.18	28.74	32.63	31.70	32.74	36.59	36.50	34.72	37.80	48.04
B-factor ligands (Å²)	36.55	38.15	40.02	38.27	44.28	48.72	48.28	50.33	53.40	58.01	54.60	56.16	57.49	71.89
B-factor solvent (Å²)	35.45	35.00	36.90	35.72	38.73	39.31	41.96	40.03	42.43	43.90	43.98	41.55	43.90	48.77

*Statistics for the highest-resolution shell are shown in parentheses.

**Data processing statistics are reported with Friedel pairs merged.

Table S5. Statistic of EP-TL00150 structures of the temperature ramp-down

	dataset1	dataset2	dataset3	dataset4	dataset5	dataset6	dataset7	dataset8	dataset9	dataset10	dataset11	dataset12	dataset13	dataset14	dataset15	dataset16	dataset17	dataset18
PDB code	**7QM9**	**7QMA**	**7QMB**	**7QMC**	**7QMD**	**7QME**	**7QMF**	**7QMG**	**7QMH**	**7QMI**	**7QMJ**	**7QMK**	**7QML**	**7QMM**	**7QMN**	**7QMO**	**7QMP**	**7QMQ**
Temperature (K)	298	296	293	290	287	284	281	278	276	273	271	270	268	267	266	266	267	267
Resolution (Å)	39.86 - 1.79 (1.85 - 1.79)	35.11 - 1.79 (1.85 - 1.79)	35.14 - 1.79 (1.85 - 1.79)	37.36 - 1.79 (1.85 - 1.79)	37.37 - 1.79 (1.86 - 1.79)	35.23 - 1.79 (1.86 - 1.79)	37.36 - 1.79 (1.86 - 1.79)	35.25 - 1.79 (1.86 - 1.79)	37.31 - 1.79 (1.85 - 1.79)	35.26 - 1.7 (1.86 - 1.79)	37.28 - 1.79 (1.86 - 1.79)	35.24 - 1.79 (1.86 - 1.79)	37.27 - 1.79 (1.85 - 1.79)	43.14 - 1.79 (1.85 - 1.79)	36.96 - 1.79 (1.85 - 1.79)	37.02 - 1.79 (1.85 - 1.79)	37.00 - 1.79 (1.85 - 1.79)	40.04 - 1.79 (1.86 - 1.79)
Space group	*P*121	*P*121	*P*121	*P*121	*P*121	*P*121	*P*121	*P*121	*P*121	*P*121	*P*121	*P*121	*P*121	*P*121	*P*121	*P*121	*P*121	*P*121
Unit-cell parameters *a,b,c* (Å)	45.86 73.90 52.96	45.87 73.93 53.04	45.89 73.96 53.11	45.89 73.96 53.18	45.90 74.01 53.31	45.92 74.05 53.34	45.92 74.07 53.48	45.88 74.02 53.40	45.85 73.99 53.42	45.86 74.01 53.46	45.82 73.99 53.46	45.81 73.97 53.45	45.81 73.98 53.48	45.80 73.99 53.51	45.75 73.92 53.43	45.73 73.91 53.39	45.79 74.00 53.50	45.77 73.95 53.40
Unit-cell parameters β (°)	109.19	109.22	109.25	109.32	109.42	109.37	109.54	109.52	109.57	109.59	109.64	109.52	109.63	109.65	109.70	109.72	109.7	109.6
Unique reflections	31109 (3070)	31147 (3065)	31187 (3062)	31201 (3056)	31270 (3070)	31222 (3084)	31662 (3116)	31685 (3134)	31686 (3140)	31678 (3136)	31676 (3146)	31657 (3143)	31652 (3134)	31671 (3121)	31611 (3163)	31549 (3144)	31678 (3150)	31191 (2782)
Multiplicity	6.96 (6.56)	6.94 (6.56)	6.96 (6.55)	6.93 (6.57)	6.91 (6.56)	6.94 (6.55)	6.38 (6.70)	6.83 (6.38)	6.83 (6.49)	6.83 (6.41)	6.81 (6.40)	6.81 (6.39)	6.81 (6.37)	6.72 (6.33)	6.80 (6.36)	6.79 (6.38)	6.80 (6.40)	6.82 (6.42)
Completeness (%)	98.61 (97.62)	98.59 (97.46)	98.60 (97.36)	98.57 (96.83)	98.58 (97.06)	98.68 (97.75)	99.65 (98.39)	99.80 (98.77)	99.84 (99.49)	99.85 (99.33)	99.86 (99.53)	99.85 (99.52)	99.78 (98.80)	99.74 (98.39)	99.92 (99.91)	99.86 (99.87)	99.73 (99.06)	98.63 (88.32)
Mean $I/\sigma(I)$	10.38 (0.65)	13.55 (1.29)	12.21 (1.06)	12.73 (1.15)	11.45 (0.93)	8,35 (0.47)	9.43 (1.04)	11.39 (1.16)	12.52 (1.67)	13.57 (2.37)	16.12 (3.42)	15.80 (3.26)	15.07 (3.18)	11.87 (2.07)	11.70 (1.59)	7.89 (0.92)	6.70 (0.57)	5.71 (0.17)
Wilson B-factor	33.79	30.26	30.92	31.01	31.19	33.29	28.64	30.01	27.10	25.53	22.52	22.46	22.02	23.98	25.39	26.35	28.66	35.08
R_{meas}	0.12 (2.21)	0.09 (1.35)	0.10 (1.60)	0.09 (1.51)	0.11 (1.76)	0.15 (3.27)	0.15 (1.75)	0.10 (1.49)	0.10 (1.04)	0.09 (0.70)	0.08 (0.48)	0.08 (0.50)	0.09 (0.53)	0.13 (0.83)	0.13 (0.95)	0.23 (1.69)	0.28 (2.57)	0.26 (4.78)
$CC_{1/2}$	0.99 (0.36)	0.99 (0.61)	0.99 (0.52)	0.99 (0.54)	0.99 (0.44)	0.99 (0.24)	0.99 (0.51)	0.99 (0.54)	0.99 (0.69)	0.99 (0.83)	0.99 (0.91)	0.99 (0.91)	0.99 (0.90)	0.99 (0.83)	0.99 (0.80)	0.99 (0.63)	0.99 (0.41)	0.99 (0.14)
ISa	21.03	24.99	22.31	22.23	21.23	16.29	17.12	17.65	17.92	16.77	19.94	20.02	18.42	18.71	20.66	16.70	17.72	17.65
Reflections used in refinement	31094 (3070)	31136 (3065)	31167 (3062)	31195 (3056)	31251 (3070)	31208 (3084)	31646 (3116)	31678 (3133)	31672 (3140)	31665 (3136)	31660 (3144)	31639 (3140)	31641 (3132)	31664 (3120)	31605 (3162)	31532 (3142)	31648 (3148)	31180 (2782)
Reflections used for R_{free}	1540 (160)	1541 (160)	1542 (160)	1545 (159)	1547 (160)	1545 (158)	1567 (164)	1571 (165)	1569 (165)	1569 (165)	1567 (164)	1566 (165)	1568 (166)	1568 (166)	1568 (164)	1565 (164)	1569 (168)	1552 (149)
R_{work}/R_{free}	0.20/0.23	0.19/0.21	0.20/0.22	0.19/0.22	0.19/0.21	0.19/0.23	0.19/0.22	0.19/0.22	0.18/0.22	0.18/0.21	0.18/0.20	0.16/0.19	0.17/0.19	0.17/0.20	0.17/0.20	0.19/0.22	0.21/0.25	0.21/0.24
No. of atoms	2533	2546	2543	2549	2565	2564	2596	2605	2622	2610	2664	2657	2673	2659	2652	2631	2615	2585
macromolecules	2384	2384	2384	2384	2379	2379	2379	2379	2379	2379	2379	2379	2379	2379	2379	2379	2379	2379
ligands	36	36	36	36	48	48	48	48	48	48	48	48	48	48	48	48	48	48
solvent	113	126	123	129	138	137	169	178	195	183	237	230	246	232	225	204	188	158
Protein residues	330	330	330	330	330	330	330	330	330	330	330	330	330	330	330	330	330	330
RMS(bonds) (Å)	0.006	0.003	0.003	0.005	0.002	0.007	0.005	0.006	0.005	0.004	0.004	0.006	0.004	0.004	0.004	0.002	0.005	0.003
RMS(angles) (°)	0.80	0.64	0.65	0.72	0.59	0.88	0.80	0.84	0.81	0.71	0.69	0.82	0.73	0.71	0.69	0.58	0.75	0.59
Ramachandran favored (%)	98.78	98.48	98.78	98.78	99.09	98.78	99.09	98.78	98.48	98.48	99.09	98.48	98.78	99.09	98.78	98.78	98.78	99.09
Ramachandran allowed (%)	1.22	1.52	1.22	1.22	0.91	1.22	0.91	1.22	1.52	1.52	0.91	1.52	1.22	0.91	1.22	1.22	1.22	0.91
Ramachandran outliers (%)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Clashscore	1.49	1.06	1.49	1.28	1.27	1.27	0.42	0.64	1.06	1.70	0.42	0.85	0.64	0.64	1.06	1.06	0.64	0.64
Average B-factor (Å^2)	40.28	36.57	38.05	36.29	35.54	39.97	32.29	33.37	30.53	28.63	23.66	26.02	23.61	28.36	28.56	31.20	32.14	39.96
B-factor macromolecules (Å^2)	39.84	36.01	37.40	35.73	34.91	39.47	31.41	32.52	29.40	27.61	22.32	24.51	21.99	27.12	27.38	30.36	31.43	39.32
B-factor ligands (Å^2)	56.14	51.48	53.60	52.55	49.69	55.56	46.28	46.20	45.56	46.45	37.76	45.53	40.60	42.64	43.55	41.70	43.06	50.79
B-factor solvent (Å^2)	44.50	42.98	46.12	42.06	41.45	43.28	40.72	41.39	40.58	37.24	34.22	37.57	35.99	38.23	37.77	38.54	38.37	46.29

*Statistics for the highest-resolution shell are shown in parentheses.
**Data processing statistics are reported with Friedel pairs merged.

Table S6. PDB ID and its corresponding DOI

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5RBS	10.2210/pdb5RBS/pdb
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5RC1	10.2210/pdb5RC1/pdb
5RC2	10.2210/pdb5RC2/pdb
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PDB ID	PDB DOI
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5RCB	10.2210/pdb5RCB/pdb
5RCC	10.2210/pdb5RCC/pdb
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5SAQ	10.2210/pdb5SAQ/pdb
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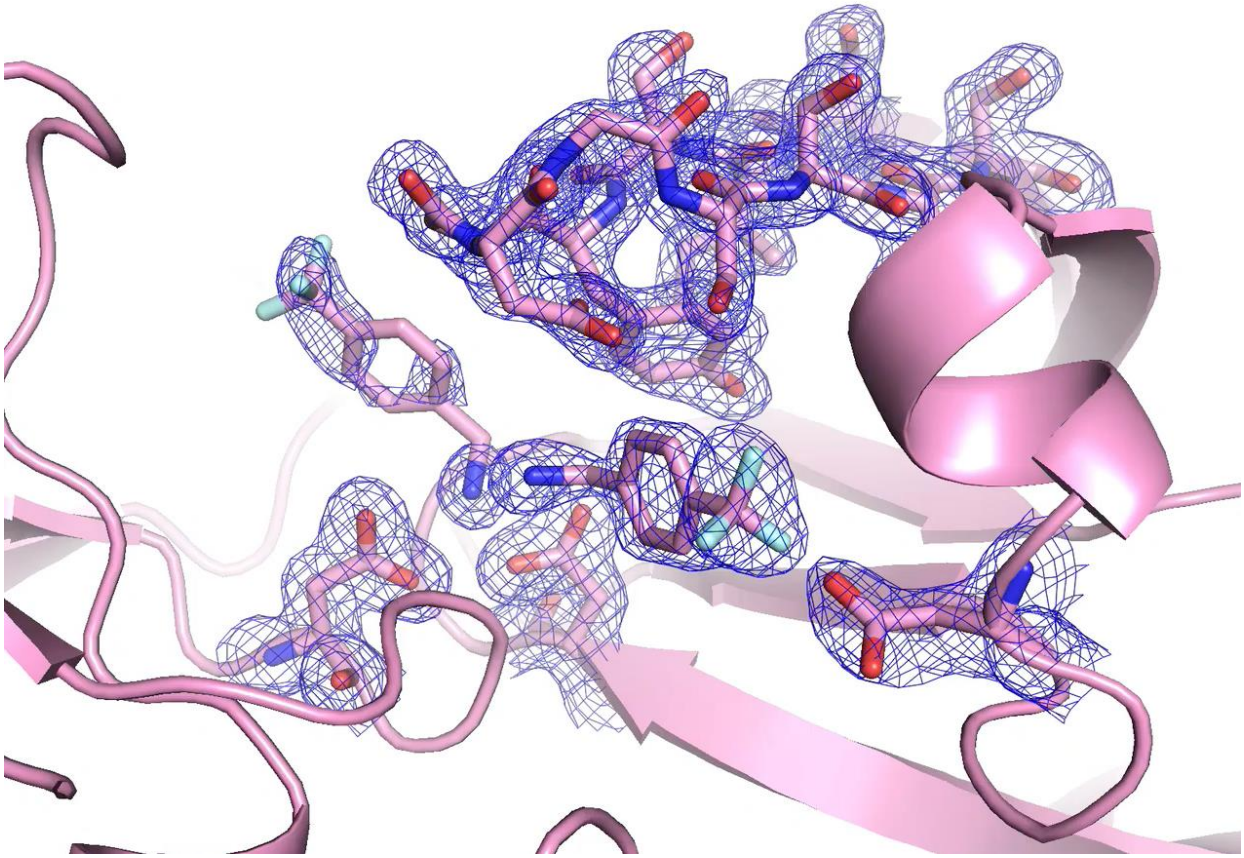
Movie S1 EP-TL00150 temperature ramp-up experiment



Movie S2 EP-TL00150 temperature ramp-down experiment