Supporting Information

Kim et al. 'Protein Dynamical Transition at 110 K'

1. Sample information

In this study, X-ray diffraction data sets from 6 Thaumatin crystals were used (5 crystals for the main text, 1 additional crystal for Supporting Information). Information on each crystal is as follows.

1) **Thau-0M-1**: Equilibrated to de-ionized water, pressure cryocooled at 200 MPa.
2) **Thau-0M-2**: Equilibrated to de-ionized water, pressure cryocooled at 200 MPa.
3) **Thau-0.45M**: Equilibrated to 0.45 M Sodium Potassium tartrate solution, pressure cryocooled at 200 MPa.
4) **Thau-0.9M**: Equilibrated to 0.9 M Sodium Potassium tartrate solution, pressure cryocooled at 200 MPa.
5) **Thau-control**: Equilibrated to 0.9 M Sodium Potassium tartrate solution, cryoprotected with 20 % glycerol (v/v), cryocooled at 0.1 MPa.
6) **Thau-control-noGLY**: Equilibrated to 1.5 M Sodium Potassium tartrate solution, no glycerol added, cryocooled at 0.1 MPa.

X-ray diffraction data collection and refinement statistics for each crystal can be found in Table S1-S12 at the end.

2. Interpretation of the B-factor and the self-RMS deviation profiles

The protein dynamical transition has been studied by monitoring the mean square atomic displacement ($\langle x^2 \rangle$) of protein atoms. In X-ray protein crystallography, the atomic displacement consists of three major terms (1).

$$\langle x^2 \rangle_{total} = \langle x^2 \rangle_{lattice} + \langle x^2 \rangle_{vib} + \langle x^2 \rangle_{conf}$$

The first term ($<x^2>_{lattice}$) is the contribution due to lattice disorder. It is a static disorder and typically does not change at cryogenic temperatures if a protein crystal is cryocooled at ambient pressure. However, it was observed that crystal lattice disorder can be changing during crystal warming when the crystal is cryocooled at high pressure(2). The changes in the crystal lattice disorder can be monitored by crystal mosaicity.

The second term ($<x^2>_{vib}$) is the contribution due to atomic vibrational motions and is linearly increasing when temperature increases both in the high pressure cryocooled and ambient pressure cryocooled protein crystals. The third term ($<x^2>_{conf}$) is the contribution due to the protein conformational fluctuations and reflects either the static distributions of conformational states or the dynamical motions between conformational states. Although the X-ray diffraction measurement cannot distinguish the difference between the static distribution and the dynamical motions, the temperature dependence of the third term provides the clues for the dynamical fluctuations. The nonlinear increase in the third term indicates that protein conformational fluctuations are enabled at least transiently while temperature increases.

In our study, it was observed that the B-factor profiles and the self-RMS deviation profiles from high pressure cryocooled crystals show nonlinear behavior upon warming. The crystal mosaicity profiles of high pressure cryocooled crystals (section 4 in this supporting information) indicate that the crystal lattice disorder is not a major term for the nonlinearity. As lattice disorder and atomic vibrational motions do not account for this nonlinear behavior, we concluded that the experimental results (Fig 1, Fig 2) reflect the gained dynamical fluctuations during the HDA-LDA phase transition. Our interpretation is following.

When a protein molecule is trapped in LDA ice (Thau-control), large conformational fluctuations are frozen out. Therefore, only a small linear increase in the B-factor and self-RMS deviation profiles is observed when temperature increases.

On the other hand, during the HDA to LDA ice transition, protein molecules are possibly exposed to a transient fluid environment, allowing large conformational fluctuations(2). When the transition is completed, further dynamical motions of the protein molecules may be again frozen. But this frozen state represents the final, higher temperature distribution of the conformational states right before being refrozen. This can cause a rapid increase in the B-factor and the self-RMS deviation profiles, showing nonlinearity.

It is important to note that the B-factor and the self-RMS deviation values of a cryocooled protein are not fully determined by the final ice state because there is no guarantee that the state that is frozen is in full thermal equilibrium. The approach to thermal equilibrium may be path dependent. The final conformational states of a protein after the HDA-LDA transition at a given temperature can be different from the states of a protein trapped in the LDA ice. This can explain the observed higher B-factor and self-RMS deviation values for the high-pressure cryocooled protein crystals after HDA-LDA transition (table S1-S12) compared to proteins which were warmed to the same temperature, but have been trapped in LDA ice throughout the warming process.

Note that the absolute B-factor values (table S1-S12) of a protein depend on the initial crystal quality and the way that the protein crystal is handled before X-ray diffraction measurement. Therefore, directly comparing the absolute B factor values between different crystals can be misleading. The protein dynamical transition is detected by the nonlinear changes in the B-factor profiles, which does not require the absolute values. For this reason, the initial B factor values of different thaumatin crystals are normalized to be 10 at 80 K in Fig. 1C in the main article.

3. Changes in crystal lattice disorder during protein dynamical transition

Crystal mosaicity is a measure of disorder in unit-cell alignment and reflects the lattice disorder in a protein crystal. Fig. S1A shows the crystal mosaicity profiles of 4 high pressure cryocooled crystals (Thau-0M-1, Thau-0M-2, Thau-0.45M, and Thau-0.9M) and 1 ambient pressure cryocooled crystal (Thau-control). The mosaicity profiles of high pressure cryocooled crystals show significant fluctuations compared to the profile from

Thau-control. Note that the crystal mosaicity of high-pressure cryocooled crystals show improvement (between 110 K and 140 K for Thau-0M-2/Thau-0.45M/Thau-0.9M, and between 125 K and 142 K for Thau-0M-1), indicating that the crystal lattice disorder actually decreased over the temperature range. This observation supports that the observed nonlinear behaviors in the B-factor profiles and the self-RMS profiles (Figure 1C and Figure 2 in the main text) are the consequence of the protein conformational fluctuations rather than the increased lattice disorder. Fig. S1B shows that the improvement in the crystal mosaicity above 126 K affects the B-factor profile of the high pressure cryocooled crystal, suggesting that the B-factor profile of a high pressure cryocooled crystal not only represents the protein conformational fluctuations but also the changes in the crystal lattice disorder. Note that the B-factor profile still rises above 126 K when the crystal mosaicity improves (i.e. lattice disorder decreases). This indicates that the B-factor profile mainly reflects the protein dynamical fluctuations rather than the crystal lattice disorder.

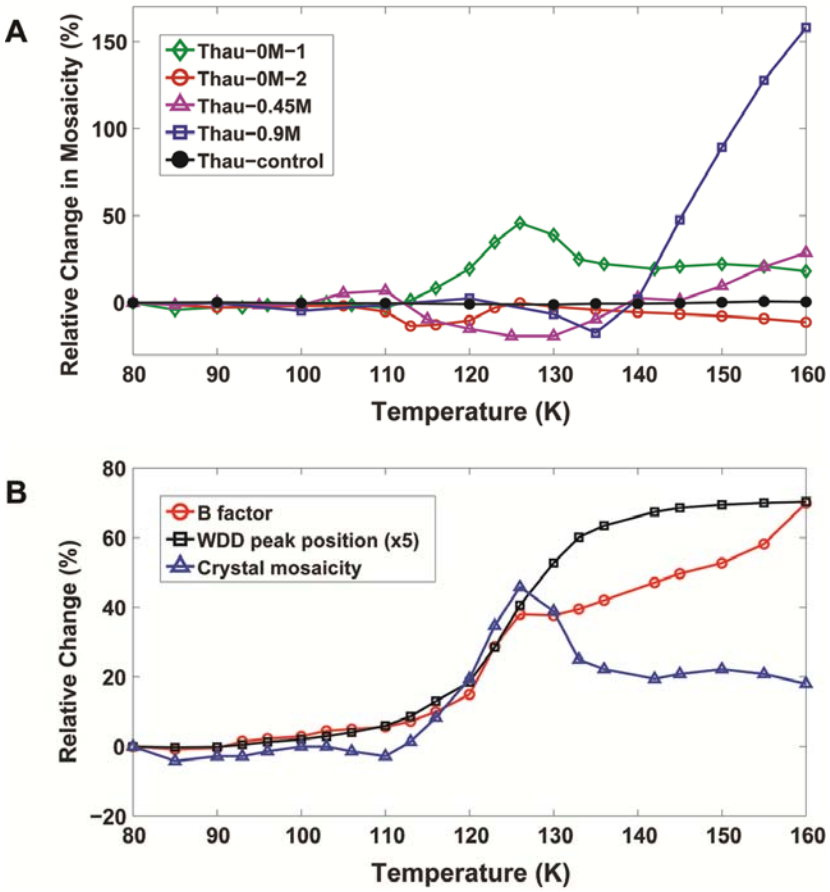

Fig. S1 Crystal mosaicity profiles of cryocooled crystals (Thau-0M-1, Thau-0M-2, Thau-0.45M, Thau-0.9M, and Thau-control) from 80 K to 160 K. (A) The high pressure cryocooled crystals show significant fluctuations in the temperature range, indicating some changes in the crystal lattice disorder via molecular rearrangement. In contrast, the ambient pressure cryocooled crystal (Thau-control) shows little fluctuations. (B) Superposition of crystal mosaicity with B factor and WDD primary peak position profiles of Thau-0M-1. Note that the B factor profile shows some deviation from the WDD peak position profile right above 126 K where the crystal mosaicity begins to fall down.

4. Effects of glycerol on cryogenic protein dynamical transition

The 4 high pressure cryocooled crystals (Thau-0M-1, Thau-0M-2, Thau-0.45M, Thau-0.9M) were cryocooled without adding glycerol. However, high quality X-ray diffraction data sets could be collected because cryocooling of protein crystals under high pressure eliminates the need for chemical cryoprotectants(3). In contrast, the ambient pressure cryocooled crystal (Thau-control) was soaked into 20 % glycerol (v/v) containing solution before cryocooling for crystal cryoprotection. To see the effect of glycerol on the protein dynamical transition at cryogenic temperature, additional Thaumatin crystal (Thau-control-noGLY) was cryocooled at ambient pressure without adding glycerol. The B factor profiles of Thau-control and Thau-control-noGLY in Fig. S2 show no features of protein dynamical transition at cryogenic temperatures, therefore eliminating the possibility that the absence of glycerol in the high pressure cryocooled crystals is the cause for the protein dynamical transition. Note that the X-ray diffraction quality of Thau-control-noGLY as shown in Table S11 is poorer (in terms of crystal mosaicity, R_{sym}, and $I/\sigma(I)$) than Thau-control (Table S9) as a consequence of crystal damage during cryocooling at ambient pressure without chemical cryoprotectants.

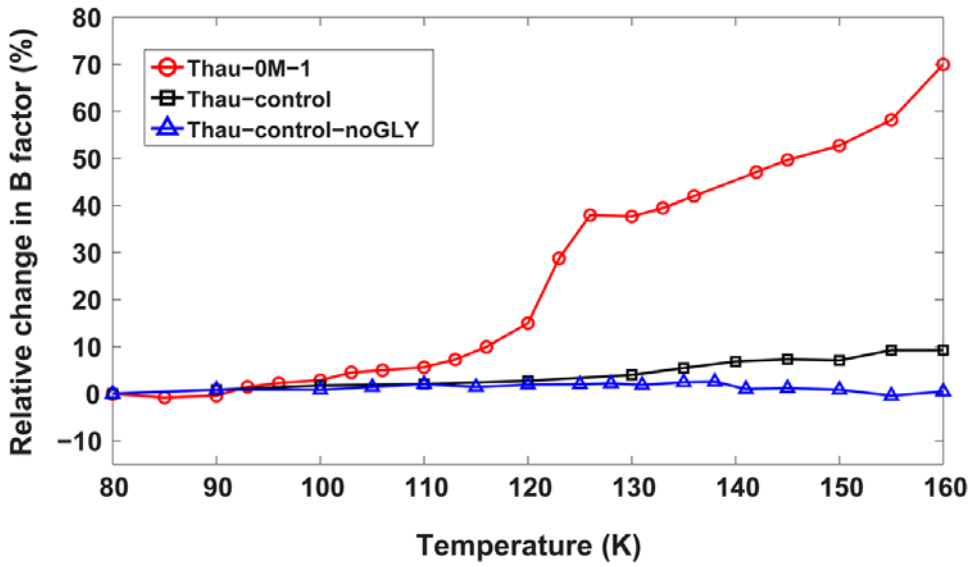

Fig. S2 The B factor profiles of Thau-control and Thau-control-noGLY along with the profile of Thau-0M-1 as a reference. The Thau-0M-1 profile shows nonlinearity above 110 K, indicative of protein dynamical transition. In contrast, no clear nonlinearity is observed from Thau-control and Thau-control-noGLY. This result suggests that the absence of glycerol in the high pressure cryocooled crystal is not responsible for the observed protein dynamical transition.

Reference

1. Ringe D, Petsko GA (1986) Study of protein dynamics by x-ray diffraction. *Methods Enzymol* 131:389-433.
2. Kim CU, Barstow B, Tate MW, Gruner SM (2009) Evidence for liquid water during the high-density to low-density amorphous ice transition. *Proc Natl Acad Sci USA* 106:4596-4600.
3. Kim CU, Kapfer R, Gruner SM (2005) High pressure cooling of protein crystals without cryoprotectants. *Acta Crystallogr D* 61:881-890.

Table S1. Data collection statistics of Thau-0M-1

Temp (K)	Unit-cell dimensions (Å)		Resolution (Å)	Mosaicity (°)	R_{sym} [a]	I/σ(I)	Completeness (%)	Redundancy
	a=b	c						
80	57.36	149.02	30-2.0 (2.03-2.0)	0.360	0.053 (0.081)	36.2 (17.1)	99.4 (99.8)	6.9 (5.6)
85	57.37	149.06	30-2.0 (2.03-2.0)	0.345	0.045 (0.076)	41.4 (18.3)	99.3 (99.8)	6.9 (5.6)
90	57.39	149.11	30-2.0 (2.03-2.0)	0.350	0.049 (0.079)	37.9 (17.4)	99.3 (99.9)	6.9 (5.6)
93	57.41	149.15	30-2.0 (2.03-2.0)	0.350	0.047 (0.078)	40.3 (18.1)	99.3 (99.9)	6.9 (5.6)
96	57.43	149.18	30-2.0 (2.03-2.0)	0.355	0.046 (0.077)	41.1 (18.0)	99.3 (99.9)	6.9 (5.6)
100	57.45	149.24	30-2.0 (2.03-2.0)	0.360	0.046 (0.079)	41.7 (18.3)	99.3 (99.9)	6.9 (5.6)
103	57.47	149.29	30-2.0 (2.03-2.0)	0.360	0.045 (0.077)	41.3 (18.2)	99.3 (99.9)	6.9 (5.6)
106	57.50	149.36	30-2.0 (2.03-2.0)	0.355	0.046 (0.082)	41.7 (17.6)	99.3 (100)	6.9 (5.6)
110	57.54	149.46	30-2.0 (2.03-2.0)	0.350	0.046 (0.082)	41.4 (17.5)	99.3 (99.9)	6.9 (5.6)
113	57.59	149.56	30-2.0 (2.03-2.0)	0.365	0.046 (0.084)	40.1 (16.5)	99.3 (99.9)	6.9 (5.6)
116	57.65	149.68	30-2.0 (2.03-2.0)	0.390	0.047 (0.086)	39.9 (15.6)	99.3 (99.7)	6.9 (5.5)
120	57.74	149.87	30-2.0 (2.03-2.0)	0.430	0.049 (0.098)	39.2 (14.6)	99.2 (99.8)	6.9 (5.7)
123	57.86	150.12	30-2.0 (2.03-2.0)	0.485	0.051 (0.111)	37.1 (12.8)	99.2 (99.9)	6.9 (5.7)
126	58.05	150.41	30-2.0 (2.03-2.0)	0.525	0.053 (0.120)	36.2 (11.3)	99.2 (99.9)	6.9 (5.8)
130	58.26	150.73	30-2.0 (2.03-2.0)	0.500	0.053 (0.119)	34.9 (12.4)	99.1 (98.5)	6.9 (5.9)
133	58.39	150.93	30-2.0 (2.03-2.0)	0.450	0.050 (0.108)	37.6 (13.9)	99.1 (98.4)	7.0 (5.8)
136	58.46	150.98	30-2.0 (2.03-2.0)	0.440	0.050 (0.108)	37.3 (12.9)	99.1 (98.9)	6.9 (5.7)
142	58.56	151.03	30-2.0 (2.03-2.0)	0.430	0.051 (0.111)	37.5 (12.7)	99.1 (99.1)	7.0 (5.7)
145	58.61	151.03	30-2.0 (2.03-2.0)	0.435	0.051 (0.118)	35.8 (11.6)	99.1 (99.4)	7.0 (5.6)
150	58.66	151.03	30-2.0 (2.03-2.0)	0.440	0.052 (0.122)	36.6 (11.6)	99.1 (99.6)	6.9 (5.6)
155	58.72	151.02	30-2.0 (2.03-2.0)	0.435	0.053 (0.129)	36.6 (10.7)	99.1 (99.6)	7.0 (5.6)
160	58.78	151.01	30-2.0 (2.03-2.0)	0.425	0.052 (0.123)	36.9 (11.0)	99.1 (99.6)	6.9 (5.6)

[a] $R_{sym} = \Sigma |I - \langle I \rangle| / \Sigma \langle I \rangle$

Table S2. Refinement statistics of Thau-0M-1

Temp (K)	Unique reflections	R factor [a]	R_{free} factor [b]	B factor (Å^2) – main chain	Self-RMS deviation [c] (Å)	WDD peak position (Å) [d]
80	17513	0.166	0.225	9.24	0	3.26
85	17532	0.167	0.221	9.16	0.046	3.26
90	17549	0.165	0.222	9.20	0.044	3.26
93	17571	0.165	0.217	9.37	0.05	3.27
96	17578	0.170	0.228	9.45	0.053	3.27
100	17598	0.162	0.232	9.50	0.047	3.28
103	17606	0.167	0.240	9.65	0.053	3.28
106	17621	0.164	0.227	9.70	0.056	3.29
110	17659	0.169	0.248	9.76	0.067	3.30
113	17685	0.165	0.243	9.91	0.068	3.32
116	17883	0.166	0.234	10.16	0.080	3.35
120	17789	0.167	0.235	10.62	0.094	3.39
123	17860	0.168	0.231	11.89	0.118	3.45
126	17980	0.168	0.230	12.75	0.147	3.53
130	18118	0.168	0.224	12.72	0.174	3.61
133	18260	0.169	0.224	12.88	0.197	3.66
136	18319	0.167	0.227	13.12	0.207	3.68
142	18384	0.167	0.224	13.59	0.217	3.70
145	18415	0.168	0.219	13.83	0.219	3.71
150	18445	0.169	0.216	14.11	0.228	3.72
155	18494	0.173	0.217	14.61	0.232	3.72
160	18521	0.169	0.214	15.70	0.235	3.72

[a] R factor = Σ $|F_o| - |F_c| / \Sigma |F_{obs}|$

[b] R_{free} factor is calculated the same as R factor, except it uses 5 % of reflection data omitted from refinement

[c] Self-RMS deviation was calculated between the structure at 80 K and the structures at higher temperatures of Thau-0M-1.

[d] WDD peak position in d-spacing: $d = 2\pi/Q$, where the momentum transfer vector Q is given by $Q = 4\pi\sin(\theta)/\lambda$, where λ is the X-ray wavelength and 2θ is the angle between the incident beam and the diffracted X-rays.

Table S3. Data collection statistics of Thau-0M-2

Temp (K)	Unit-cell dimensions (Å)		Resolution (Å)	Mosaicity (°)	R_{sym} [a]	I/σ(I)	Completeness (%)	Redundancy
	a=b	c						
80	57.58	149.94	50-1.8 (1.83-1.8)	0.53	0.047 (0.120)	28.1 (8.2)	96.4 (93.4)	4.0 (3.7)
90	57.59	149.99	50-1.8 (1.83-1.8)	0.515	0.046 (0.119)	28.6 (8.5)	96.4 (93.3)	4.0 (3.7)
100	57.63	150.09	50-1.8 (1.83-1.8)	0.521	0.047 (0.119)	28.7 (8.3)	96.3 (93.2)	4.0 (3.7)
105	57.68	150.18	50-1.8 (1.83-1.8)	0.521	0.046 (0.125)	28.3 (8.2)	96.2 (92.7)	4.0 (3.7)
110	57.74	150.20	50-1.8 (1.83-1.8)	0.503	0.048 (0.126)	28.8 (8.3)	96.1 (92.4)	4.0 (3.7)
113	57.78	150.26	50-1.8 (1.83-1.8)	0.459	0.047 (0.121)	29.3 (8.6)	96.1 (92.3)	4.0 (3.7)
116	57.84	150.38	50-1.8 (1.83-1.8)	0.463	0.048 (0.122)	29.1 (8.4)	96.1 (92.1)	4.0 (3.7)
120	57.93	150.54	50-1.8 (1.83-1.8)	0.476	0.048 (0.126)	28.4 (8.2)	96.0 (92.5)	4.0 (3.7)
123	58.07	150.79	50-1.8 (1.83-1.8)	0.515	0.050 (0.139)	26.4 (7.2)	95.9 (92.5)	4.0 (3.7)
126	58.24	151.08	50-1.8 (1.83-1.8)	0.53	0.053 (0.163)	25.1 (6.0)	95.8 (92.8)	4.0 (3.7)
130	58.47	151.46	50-1.8 (1.83-1.8)	0.518	0.055 (0.171)	24.9 (5.5)	92.4 (93.1)	4.1 (3.7)
135	58.65	151.67	530-1.8 (1.83-1.8)	0.508	0.053 (0.184)	26.4 (5.5)	90.6 (92.1)	4.2 (3.7)
140	58.76	151.74	50-1.8 (1.83-1.8)	0.501	0.053 (0.201)	25.9 (5.2)	89.9 (92.1)	4.3 (3.7)
145	58.84	151.73	50-1.8 (1.83-1.8)	0.496	0.054 (0.208)	26.4 (4.8)	89.6 (91.9)	4.3 (3.7)
150	58.90	151.70	50-1.8 (1.83-1.8)	0.490	0.053 (0.220)	26.1 (4.7)	89.4 (91.7)	4.3 (3.7)
155	58.95	151.66	50-1.8 (1.83-1.8)	0.481	0.054 (0.226)	25.8 (4.2)	89.3 (91.6)	4.3 (3.7)
160	58.99	151.61	50-1.8 (1.83-1.8)	0.471	0.055 (0.247)	25.5 (4.0)	89.2 (91.4)	4.3 (3.8)

[a] $R_{sym} = \Sigma |I - \langle I \rangle| / \Sigma \langle I \rangle$

Table S4. Refinement statistics of Thau-0M-2

Temp (K)	Unique reflections	R factor [a]	R_{free} factor [b]	B factor (Å^2) – main chain	Self-RMS deviation [c] (Å)	WDD peak position (Å) [d]
80	23420	0.159	0.216	8.34	0	3.27
90	23416	0.160	0.216	8.55	0.037	3.27
100	23439	0.162	0.211	8.52	0.043	3.28
105	23484	0.159	0.213	8.55	0.051	3.28
110	23518	0.157	0.203	8.91	0.055	3.30
113	23607	0.159	0.214	8.97	0.059	3.32
116	23676	0.158	0.202	9.02	0.065	3.34
120	23764	0.158	0.216	9.13	0.079	3.37
123	23884	0.154	0.204	9.43	0.101	3.41
126	24023	0.158	0.216	10.51	0.130	3.48
130	23407	0.160	0.209	10.90	0.171	3.55
135	23121	0.157	0.210	11.68	0.194	3.60
140	23021	0.158	0.204	12.46	0.209	3.63
145	23012	0.163	0.216	12.71	0.218	3.64
150	22999	0.163	0.209	13.20	0.229	3.64
155	23004	0.167	0.226	13.51	0.229	3.65
160	22993	0.165	0.219	14.07	0.233	3.66

[a] R factor = $\Sigma\ |F_o| - |F_c| / \Sigma\ |F_{obs}|$

[b] R_{free} factor is calculated the same as R factor, except it uses 5 % of reflection data omitted from refinement

[c] Self-RMS deviation was calculated between the structure at 80 K and the structures at higher temperatures of Thau-0M-2.

[d] WDD peak position in d-spacing: $d = 2\pi/Q$, where the momentum transfer vector Q is given by $Q = 4\pi\sin(\theta)/\lambda$, where λ is the X-ray wavelength and 2θ is the angle between the incident beam and the diffracted X-rays.

Table S5. Data collection statistics of Thau-0.45M

Temp (K)	Unit-cell dimensions (Å)		Resolution (Å)	Mosaicity (°)	R_{sym} [a]	I/σ(I)	Completeness (%)	Redundancy
	a=b	c						
80	57.26	149.39	50-1.9 (1.93-1.9)	0.365	0.051 (0.124)	29.2 (8.8)	98.2 (97.3)	4.7 (4.2)
85	57.27	149.42	50-1.9 (1.93-1.9)	0.36	0.051 (0.123)	28.6 (8.9)	98.2 (97.3)	4.7 (4.3)
90	57.27	149.45	50-1.9 (1.93-1.9)	0.365	0.052 (0.117)	28.2 (9.2)	97.9 (97.4)	4.7 (4.2)
95	57.29	149.49	50-1.9 (1.93-1.9)	0.36	0.048 (0.111)	30.5 (10.0)	98.2 (97.4)	4.7 (4.2)
100	57.31	149.55	50-1.9 (1.93-1.9)	0.36	0.046 (0.113)	31.6 (9.5)	98.1 (97.2)	4.7 (4.2)
105	57.34	149.56	50-1.9 (1.93-1.9)	0.385	0.050 (0.126)	31.4 (9.3)	98.1 (97.5)	4.7 (4.2)
110	57.38	149.63	50-1.9 (1.93-1.9)	0.39	0.047 (0.120)	31.9 (9.4)	98.2 (97.2)	4.7 (4.2)
115	57.42	149.70	50-1.9 (1.93-1.9)	0.33	0.046 (0.115)	31.7 (9.9)	98.3 (97.3)	4.7 (4.3)
120	57.48	149.83	50-1.9 (1.93-1.9)	0.31	0.046 (0.112)	31.7 (10.0)	98.3 (97.4)	4.7 (4.3)
125	57.57	150.03	50-1.9 (1.93-1.9)	0.295	0.045 (0.109)	33.8 (11.0)	98.4 (97.8)	4.7 (4.2)
130	57.69	150.27	50-1.9 (1.93-1.9)	0.295	0.046 (0.112)	34.3 (11.3)	98.4 (97.7)	4.7 (4.3)
135	57.85	150.55	50-1.9 (1.93-1.9)	0.33	0.047 (0.122)	32.3 (10.1)	98.4 (97.8)	4.7 (4.3)
140	58.01	150.82	50-1.9 (1.93-1.9)	0.375	0.050 (0.152)	31.3 (8.1)	98.4 (98.1)	4.7 (4.3)
145	58.18	151.02	50-1.9 (1.93-1.9)	0.37	0.052 (0.161)	30.0 (7.5)	98.5 (99.0)	4.7 (4.2)
150	58.30	151.12	50-1.9 (1.93-1.9)	0.4	0.055 (0.192)	28.8 (6.2)	98.6 (99.1)	4.7 (4.2)
155	58.40	151.15	50-1.9 (1.93-1.9)	0.44	0.058 (0.222)	26.8 (5.3)	98.6 (99.1)	4.7 (4.2)
160	58.48	151.14	50-1.9 (1.93-1.9)	0.47	0.061 (0.229)	25.6 (4.5)	98.6 (99.1)	4.7 (4.2)

[a] $R_{sym} = \Sigma |I - \langle I \rangle| / \Sigma \langle I \rangle$

Table S6. Refinement statistics of Thau-0.45M

Temp (K)	Unique reflections	R factor [a]	R_{free} factor [b]	B factor (Å^2) – main chain	Self-RMS deviation [c] (Å)	WDD peak position (Å) [d]
80	20209	0.162	0.223	9.64	0	3.12
85	20211	0.161	0.216	9.66	0.036	3.12
90	20168	0.162	0.209	9.78	0.050	3.13
95	20229	0.161	0.222	10.01	0.067	3.13
100	20252	0.158	0.222	9.96	0.069	3.14
105	20241	0.160	0.217	9.58	0.074	3.15
110	20283	0.162	0.216	10.05	0.051	3.16
115	20342	0.161	0.224	9.61	0.061	3.17
120	20409	0.162	0.208	9.69	0.064	3.18
125	20516	0.160	0.204	9.80	0.076	3.21
130	20656	0.159	0.209	9.90	0.109	3.26
135	20855	0.161	0.215	10.39	0.125	3.32
140	21028	0.162	0.208	11.64	0.147	3.40
145	21154	0.163	0.212	12.45	0.166	3.46
150	21276	0.164	0.212	13.56	0.177	3.51
155	21345	0.169	0.219	14.59	0.186	3.55
160	21421	0.174	0.215	15.54	0.194	3.56

[a] R factor = $\Sigma\ |F_o| - |F_c| / \Sigma\ |F_{obs}|$

[b] R_{free} factor is calculated the same as R factor, except it uses 5 % of reflection data omitted from refinement

[c] Self-RMS deviation was calculated between the structure at 80 K and the structures at higher temperatures of Thau-0.45M.

[d] WDD peak position in d-spacing: $d = 2\pi/Q$, where the momentum transfer vector Q is given by $Q = 4\pi\sin(\theta)/\lambda$, where λ is the X-ray wavelength and 2θ is the angle between the incident beam and the diffracted X-rays.

Table S7. Data collection statistics of Thau-0.9M

Temp (K)	Unit-cell dimensions (Å)		Resolution (Å)	Mosaicity (°)	R_{sym} [a]	I/σ(I)	Completeness (%)	Redundancy
	a=b	c						
80	57.43	149.90	30-1.9 (1.97-1.9)	0.317	0.029 (0.056)	49.2 (19.4)	98.4 (87.0)	4.4 (3.1)
90	57.46	149.98	30-1.9 (1.97-1.9)	0.316	0.041 (0.066)	35.1 (14.0)	98.3 (86.6)	4.4 (3.1)
100	57.51	150.00	30-1.9 (1.97-1.9)	0.302	0.034 (0.063)	39.9 (16.6)	98.3 (86.7)	4.3 (3.1)
110	57.60	150.08	30-1.9 (1.97-1.9)	0.313	0.030 (0.057)	48.3 (18.4)	98.2 (86.2)	4.4 (3.1)
120	57.66	150.26	30-1.9 (1.97-1.9)	0.325	0.029 (0.055)	48.2 (18.1)	98.3 (86.8)	4.4 (3.1)
130	57.82	150.53	30-1.9 (1.97-1.9)	0.296	0.028 (0.053)	49.4 (19.2)	98.2 (86.2)	4.4 (3.1)
135	57.97	150.75	30-1.9 (1.97-1.9)	0.262	0.026 (0.045)	53.1 (22.9)	98.1 (85.7)	4.4 (3.1)
140	58.08	150.88	30-1.9 (1.97-1.9)	0.323	0.027 (0.046)	50.5 (21.0)	98.1 (85.8)	4.4 (3.1)
145	58.19	150.97	30-1.9 (1.97-1.9)	0.468	0.027 (0.056)	50.2 (18.7)	97.9 (84.7)	4.4 (3.1)
150	58.29	151.02	30-1.9 (1.97-1.9)	0.600	0.030 (0.076)	45.6 (14.6)	98.0 (85.4)	4.4 (3.1)
155	58.38	151.03	30-1.9 (1.97-1.9)	0.722	0.037 (0.104)	37.8 (10.1)	98.0 (85.7)	4.4 (3.1)
160	58.45	151.00	30-1.9 (1.97-1.9)	0.818	0.046 (0.129)	31.0 (7.3)	98.0 (86.2)	4.4 (3.1)

[a] $R_{sym} = \Sigma |I - \langle I \rangle| / \Sigma \langle I \rangle$

Table S8. Refinement statistics of Thau-0.9M

Temp (K)	Unique reflections	R factor [a]	R_{free} factor [b]	B factor (Å^2) – main chain	Self-RMS deviation [c] (Å)	WDD peak position (Å) [d]
80	20343	0.162	0.212	9.05	0	3.09
90	20347	0.163	0.215	9.42	0.045	3.10
100	20390	0.161	0.215	9.55	0.04	3.10
110	20455	0.165	0.219	9.94	0.056	3.11
120	20505	0.161	0.222	10.20	0.064	3.14
130	20646	0.162	0.223	10.05	0.089	3.18
135	20767	0.163	0.216	10.02	0.105	3.26
140	20843	0.160	0.216	10.55	0.122	3.29
145	20922	0.165	0.215	11.82	0.144	3.35
150	21003	0.164	0.226	13.38	0.152	3.42
155	21088	0.169	0.215	14.87	0.166	3.56
160	21150	0.170	0.221	16.43	0.172	3.64

[a] R factor = $\Sigma |F_o| - |F_c| / \Sigma |F_{obs}|$

[b] R_{free} factor is calculated the same as R factor, except it uses 5 % of reflection data omitted from refinement

[c] Self-RMS deviation was calculated between the structure at 80 K and the structures at higher temperatures of Thau-0.9M.

[d] WDD peak position in d-spacing: $d = 2\pi/Q$, where the momentum transfer vector Q is given by $Q = 4\pi\sin(\theta)/\lambda$, where λ is the X-ray wavelength and 2θ is the angle between the incident beam and the diffracted X-rays.

Table S9. Data collection statistics of Thau-control

Temp (K)	Unit-cell dimensions (Å)		Resolution (Å)	Mosaicity (°)	R_{sym} [a]	I/σ(I)	Completeness (%)	Redundancy
	a=b	c						
80	57.76	149.80	30-1.9 (1.97-1.9)	0.377	0.043 (0.071)	30.9 (13.2)	97.0 (92.8)	4.4 (2.8)
90	57.78	149.84	30-1.9 (1.97-1.9)	0.378	0.057 (0.077)	24.8 (11.0)	97.1 (93.2)	4.4 (2.9)
100	57.79	149.88	30-1.9 (1.97-1.9)	0.376	0.044 (0.072)	29.8 (12.8)	97.2 (93.1)	4.5 (2.9)
110	57.80	149.92	30-1.9 (1.97-1.9)	0.376	0.043 (0.070)	30.0 (12.9)	97.3 (93.5)	4.5 (2.9)
120	57.81	149.96	30-1.9 (1.97-1.9)	0.374	0.041 (0.070)	31.0 (13.2)	97.3 (93.4)	4.4 (2.9)
130	57.82	150.00	30-1.9 (1.97-1.9)	0.373	0.041 (0.071)	30.9 (13.0)	97.3 (93.4)	4.4 (2.9)
135	57.83	150.02	30-1.9 (1.97-1.9)	0.375	0.041 (0.070)	30.9 (12.9)	97.3 (93.3)	4.4 (2.9)
140	57.84	150.05	30-1.9 (1.97-1.9)	0.375	0.043 (0.073)	30.3 (12.2)	97.3 (93.1)	4.4 (2.8)
145	57.85	150.08	30-1.9 (1.97-1.9)	0.376	0.042 (0.071)	31.2 (12.9)	97.4 (93.5)	4.4 (2.9)
150	57.87	150.11	30-1.9 (1.97-1.9)	0.378	0.042 (0.071)	31.1 (12.6)	97.4 (93.4)	4.4 (2.9)
155	57.89	150.14	30-1.9 (1.97-1.9)	0.380	0.042 (0.073)	30.8 (12.4)	97.4 (93.6)	4.4 (2.9)
160	57.91	150.19	30-1.9 (1.97-1.9)	0.379	0.042 (0.075)	30.8 (12.0)	97.4 (93.5)	4.4 (2.9)

[a] $R_{sym} = \Sigma |I - \langle I \rangle| / \Sigma \langle I \rangle$

Table S10. Refinement statistics of Thau-control

Temp (K)	Unique reflections	R factor [a]	R_{free} factor [b]	B factor (Å^2) – main chain	Self-RMS deviation [c] (Å)	WDD peak position (Å) [d]
80	20434	0.152	0.201	10.27	0	3.78
90	20231	0.157	0.200	10.35	0.038	3.74
100	20249	0.152	0.202	10.45	0.038	3.73
110	20232	0.152	0.200	10.48	0.037	3.72
120	20310	0.150	0.211	10.55	0.041	3.73
130	20331	0.153	0.192	10.68	0.044	3.73
135	20334	0.152	0.196	10.83	0.044	3.74
140	20549	0.151	0.194	10.97	0.047	3.76
145	20363	0.151	0.193	11.02	0.049	3.74
150	20384	0.152	0.202	11.00	0.052	3.75
155	20394	0.155	0.201	11.22	0.055	3.76
160	20432	0.154	0.197	11.22	0.058	3.77

[a] R factor = $\Sigma |F_o| - |F_c| / \Sigma |F_{obs}|$

[b] R_{free} factor is calculated the same as R factor, except it uses 5 % of reflection data omitted from refinement

[c] Self-RMS deviation was calculated between the structure at 80 K and the structures at higher temperatures of Thau-control.

[d] WDD peak position in d-spacing: $d = 2\pi/Q$, where the momentum transfer vector Q is given by $Q = 4\pi\sin(\theta)/\lambda$, where λ is the X-ray wavelength and 2θ is the angle between the incident beam and the diffracted X-rays.

Table S11. Data collection statistics of Thau-control-noGLY

Temp (K)	Unit-cell dimensions (Å)		Resolution (Å)	Mosaicity (°)	R_{sym} [a]	I/σ(I)	Completeness (%)	Redundancy
	a=b	c						
80	57.58	149.40	50-1.8 (1.83-1.8)	0.654	0.105 (0.339)	15.7 (2.6)	95.0 (97.2)	4.0 (3.5)
90	57.60	149.41	50-1.8 (1.83-1.8)	0.665	0.103 (0.347)	15.3 (2.5)	95.2 (97.2)	4.0 (3.5)
100	57.61	149.46	50-1.8 (1.83-1.8)	0.655	0.105 (0.361)	15.2 (2.3)	95.1 (95.3)	4.0 (3.4)
105	57.63	149.49	50-1.8 (1.83-1.8)	0.659	0.105 (0.360)	15.2 (2.4)	95.0 (96.4)	4.0 (3.5)
110	57.63	149.52	50-1.8 (1.83-1.8)	0.659	0.106 (0.372)	15.0 (2.1)	95.0 (95.3)	4.0 (3.5)
115	57.65	149.56	50-1.8 (1.83-1.8)	0.660	0.105 (0.362)	15.2 (2.4)	95.0 (96.9)	4.0 (3.5)
120	57.67	149.59	50-1.8 (1.83-1.8)	0.661	0.106 (0.402)	14.6 (2.0)	95.0 (95.8)	4.0 (3.4)
125	57.68	149.61	50-1.8 (1.83-1.8)	0.664	0.105 (0.371)	14.8 (2.2)	95.0 (96.9)	4.0 (3.5)
128	57.69	149.64	50-1.8 (1.83-1.8)	0.663	0.105 (0.389)	14.7 (2.1)	95.0 (95.7)	4.0 (3.5)
131	57.70	149.66	50-1.8 (1.83-1.8)	0.667	0.106 (0.402)	14.5 (2.0)	95.0 (95.7)	4.0 (3.5)
135	57.71	149.68	50-1.8 (1.83-1.8)	0.666	0.106 (0.395)	14.4 (1.9)	95.0 (96.0)	4.0 (3.5)
138	57.71	149.69	50-1.8 (1.83-1.8)	0.662	0.106 (0.397)	14.4 (1.9)	95.0 (96.1)	4.0 (3.4)
141	57.72	149.72	50-1.8 (1.83-1.8)	0.660	0.106 (0.390)	14.3 (1.9)	95.0 (96.0)	4.0 (3.4)
145	57.73	149.75	50-1.8 (1.83-1.8)	0.657	0.105 (0.401)	14.1 (1.9)	95.0 (96.0)	4.0 (3.4)
150	57.75	149.80	50-1.8 (1.83-1.8)	0.654	0.105 (0.406)	14.0 (1.9)	95.0 (96.2)	4.0-(3.5)
155	57.78	149.86	50-1.8 (1.83-1.8)	0.643	0.104 (0.400)	14.0 (1.9)	95.1 (96.2)	4.0 (3.5)
160	57.82	149.93	50-1.8 (1.83-1.8)	0.628	0.102 (0.376)	14.1 (2.0)	95.1 (96.7)	4.0 (3.5)

[a] $R_{sym} = \Sigma |I - \langle I \rangle| / \Sigma \langle I \rangle$

Table S12. Refinement statistics of Thau-control-noGLY

Temp (K)	Unique reflections	R factor [a]	R_{free} factor [b]	B factor (Å^2) – main chain	Self-RMS deviation [c] (Å)	WDD peak position (Å) [d]
80	23204	0.169	0.211	17.20	0	n/a
90	23271	0.168	0.206	17.35	0.045	n/a
100	23706	0.169	0.225	17.35	0.048	n/a
105	23246	0.169	0.209	17.45	0.047	n/a
110	23637	0.169	0.215	17.56	0.050	n/a
115	23291	0.170	0.208	17.45	0.052	n/a
120	23729	0.172	0.236	17.53	0.054	n/a
125	23304	0.173	0.220	17.54	0.057	n/a
128	23679	0.168	0.217	17.58	0.056	n/a
131	23672	0.173	0.227	17.52	0.057	n/a
135	23679	0.168	0.226	17.62	0.055	n/a
138	23767	0.168	0.224	17.64	0.063	n/a
141	23779	0.170	0.222	17.37	0.065	n/a
145	23788	0.170	0.223	17.40	0.067	n/a
150	23803	0.168	0.222	17.35	0.067	n/a
155	23824	0.169	0.222	17.13	0.071	n/a
160	23484	0.168	0.207	17.28	0.080	n/a

[a] R factor = $\Sigma\ |F_o| - |F_c| / \Sigma\ |F_{obs}|$

[b] R_{free} factor is calculated the same as R factor, except it uses 5 % of reflection data omitted from refinement

[c] Self-RMS deviation was calculated between the structure at 80 K and the structures at higher temperatures of Thau-control-noGLY.

[d] WDD peak position in d-spacing: $d = 2\pi/Q$, where the momentum transfer vector Q is given by $Q = 4\pi\sin(\theta)/\lambda$, where λ is the X-ray wavelength and 2θ is the angle between the incident beam and the diffracted X-rays.