The Structural Basis for Matrix Metalloproteinase 1 Catalyzed Collagenolysis

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Supporting Information

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Table S1. List of interchain NOEs connectivities from ^{1}H-^{15}N HSQC-NOESY spectrum of THP. Black stars (*) correspond to those experiencing significant intensity decrease by addition of FL-MMP-1. Red triangles (▲) correspond to those experiencing significant intensity decrease by addition of the isolated HPX domain.

Chain	Residue	Label	Chain	Residue	Label	Rem
1T	Q15	HA	2T	G13	H	*
1T	Q15	H	2T	P14	HD3	
1T	Q15	HE21	2T	I17	H	▲
1T	Q15	HE22	2T	I17	H	▲
1T	Q15	HE22	2T	I17	H	
1T	Q15	HE21	2T	I17	H	
1T	G16	H	2T	P14	HG3	
1T	G16	H	2T	P14	HB3	
1T	G16	H	2T	P14	HD3	
1T	A18	HA	2T	I17	H	
1T	G19	H	2T	I17	H	
1T	G19	H	2T	I17	H	
1T	R21	HA	2T	Q20	H	
1T	V23	HG1	2T	G22	H	
1T	V24	H	2T	G22	HA2	
1T	G25	H	2T	V23	HB	▲
1T	G25	H	2T	V23	HA	
1T	L26	H	2T	V24	HA	
1T	Q15	HA	3T	G13	H	
1T	I17	H	3T	Q15	HA	*
1T	I17	H	3T	G16	H	
1T	I17	HD1	3T	G16	H	
1T	I17	H	3T	G16	H	*
1T	I17	HG2	3T	G16	H	*
1T	I17	HA	3T	G16	H	*,▲
1T	G19	H	3T	I17	HB	*
1T	G19	HA2	3T	A18	H	
1T	Q20	H	3T	Q15	HE21	
1T	Q20	H	3T	Q15	HG	
1T	Q20	H	3T	Q15	HE22	▲
1T	Q20	H	3T	Q15	HE21	
1T	Q20	H	3T	Q15	HE22	
1T	Q20	H	3T	A18	HB	
1T	Q20	H	3T	A18	HA	
1T	Q20	H	3T	G19	H	*
1T	Q20	HB3	3T	G19	H	
1T	Q20	H	3T	G19	H	
1T	G22	HA3	3T	R21	H	
1T	V23	H	3T	R21	HA	
1T	V23	H	3T	R21	HB2	
1T	V23	HG2	3T	R21	H	
1T	V23	H	3T	G22	H	
1T	V23	H	3T	G22	H	
1T	V23	HG2	3T	G22	H	
1T	V23	HG1	3T	G22	H	
1T	G25	HA2	3T	V24	H	
1T	L26	H	3T	G25	H	*,▲
1T	L26	H	3T	G25	H	*

1T	L26	HB	3T	G25	H	
2T	G13	H	3T	G13	H	
2T	G13	H	3T	G13	H	
2T	G13	H	3T	P14	HD3	*
2T	Q15	H	3T	G13	HA3	
2T	Q15	H	3T	P14	HD3	
2T	Q15	HG	3T	I17	H	
2T	G16	H	3T	P14	HD3	
2T	G16	H	3T	P14	HG2	
2T	G16	H	3T	P14	HG3	
2T	A18	HB	3T	G16	H	
2T	A18	HA	3T	I17	H	
2T	G19	H	3T	I17	HB	
2T	G19	H	3T	I17	H	
2T	G19	H	3T	I17	H	
2T	R21	HA	3T	Q20	H	
2T	R21	HG3	3T	Q20	H	▲
2T	R21	HD2	3T	V23	H	*
2T	R21	HB2	3T	V23	H	*
2T	R21	HG3	3T	V23	H	*
2T	G22	HA3	3T	V23	H	
2T	V24	H	3T	G22	HA3	*
2T	G25	H	3T	V23	HB	▲

Table S2. ^{15}N R_1, R_2, and NOEs for the THP at 298 K and 700 MHz.

Chain I (**1T**)		R_1 (s^{-1})			R_2 (s^{-1})		NOE
	Exp.	Error	Calc.	Exp.	Error	Calc.	Exp.
G13	1.68	0.09	1.28	15.88	3.20	23.09	0.68
P14							
Q15	1.52	0.13	1.28	19.66	1.48	23.04	0.63
G16	1.66	0.09	1.29	20.03	1.85	23.36	0.73
I17	1.45	0.09	1.21	18.31	1.47	21.14	0.78
A18	1.48	0.13	1.23			21.41	0.57
G19	1.68	0.11	1.29	16.45	1.71	23.26	0.80
Q20	1.47	0.09	1.28	17.09	2.28	22.83	0.72
R21	1.64	0.11	1.25	18.43	2.38	22.08	0.72
G22			1.29			23.36	
V23	1.55	0.08	1.21	18.16	1.69	21.05	0.77
V24	1.51	0.10	1.28	17.37	1.97	23.09	0.78
G25	1.70	0.08	1.29	18.40	2.10	23.31	0.77
L26	1.62	0.11	1.22	17.54	2.56	21.28	0.76

Chain II (**2T**)		R_1 (s^{-1})			R_2 (s^{-1})		NOE
	Exp.	Error	Calc.	Exp.	Error	Calc.	Exp.
G13	1.57	0.13	1.27	15.77	3.13	22.83	0.69
P14							
Q15	1.62	0.09	1.28	20.12	2.47	22.99	0.72
G16	1.63	0.10	1.28	19.97	3.67	23.04	0.76
I17	1.62	0.10	1.20	18.43	2.42	20.79	0.75
A18			1.26	14.86	2.26	22.47	0.86
G19	1.54	0.08	1.29	13.89	1.56	23.26	0.77
Q20	1.55	0.10	1.12	15.16	2.79	19.96	0.71
R21	1.71	0.15	1.28	19.23	2.23	23.04	0.74
G22	1.78	0.18	1.27			22.62	
V23	1.54	0.09	1.21	16.46	1.71	21.14	0.77
V24	1.52	0.08	1.28	18.04	1.91	22.78	0.74
G25	1.77	0.13	1.27	18.16	3.21	22.68	0.78
L26	1.40	0.09	1.28	17.16	2.04	22.83	0.74

Chain III (**3T**)		R_1 (s^{-1})			R_2 (s^{-1})		NOE
	Exp.	Error	Calc.	Exp.	Error	Calc.	Exp.
G13	1.64	0.12	1.29	16.31	3.41	23.31	0.70
P14							

Q15	1.52	0.13	1.27	20.28	2.81	22.52	0.72
G16	1.85	0.14	1.26	20.77	3.20	22.17	0.61
I17	1.54	0.09	1.22	18.03	2.32	21.41	0.72
A18	1.55	0.14	1.27	14.84	1.92	22.52	0.72
G19	1.46	0.14	1.29	17.12	3.44	23.36	0.75
Q20	1.53	0.08	1.18	16.59	2.32	20.58	0.74
R21	1.81	0.09	1.29	20.25	1.60	23.26	0.73
G22	1.83	0.17	1.29	20.05	3.64	23.26	0.58
V23	1.63	0.11	1.19	17.65	1.72	20.66	0.75
V24	1.56	0.09	1.29	17.95	2.10	23.26	0.79
G25	1.73	0.10	1.26	14.72	2.19	22.32	0.72
L26	1.59	0.12	1.23	18.66	2.69	21.55	0.76

Monomeric chain (**4M**)		R_1 (s^{-1})			R_2 (s^{-1})		**NOE**
	Exp.	Error		Exp.	Error		Exp.
G13	2.26	0.38		4.27	1.23		-0.81
P14							
Q15	2.34	0.37		4.93	1.26		-0.31
G16	2.24	0.38		5.48	1.59		-0.10
I17	2.44	0.30		4.31	0.98		-0.15
A18	2.52	0.37		4.97	1.23		-0.23
G19	2.36	0.43		4.21	1.02		-0.24
Q20	2.47	0.41		4.68	1.20		-0.19
R21	2.30	0.39		4.73	1.30		-0.19
G22	2.10	0.26		6.22	1.57		
V23	2.36	0.30		3.58	0.79		-0.10
V24	2.07	0.20		3.11	0.68		-0.19
G25	2.44	0.38		4.38	1.08		-0.35
L26	2.21	0.25		3.33	0.74		-0.29

Table S3. ^{1}H and ^{15}N Chemical Shifts for the THP (pH 7.2, 298 K).

Chemical shifts (ppm)

Chain I (**1T**)

Residue	HN	N	Hα	Hβ	Hγ	Hδ	Nε2	Hε2
G13	7.63	106.48	3.70 3.49					
P14			4.24	2.11 1.67	1.92 1.74	3.33 3.02		
Q15	8.31	121.43	4.08	1.73	2.74		111.15	7.46 6.66
G16	7.99	109.19	3.61 3.40					
I17	7.84	114.25	4.02	1.81	1.20 0.99 0.87	0.70		
A18	8.14	124.56	4.26	1.17				
G19	8.43	105.53	3.50 3.39					
Q20	8.54	120.23	3.95	1.88 1.71	2.28		112.04	7.42 6.70
R21	8.34	124.16	4.23	1.63 1.55	1.88 1.27	3.03 2.98		
G22	8.25	109.92	3.42 3.60					
V23	7.76	115.23	3.99	2.07	0.88 0.79			
V24	7.61	122.83	4.02	1.79	0.90 0.81			
G25	8.06	109.68	3.55 3.43					
L26	8.54	121.72	4.24	1.37	1.50	0.79 0.81		

Chain II (**2T**)

Residue	HN	N	Hα	Hβ	Hγ	Hδ	Nε2	Hε2
G13	7.69	106.50	3.62 3.41					
P14			4.30	2.15 1.74	1.89 1.80	3.32 3.03		
Q15	8.44	121.63	4.06	1.74	2.79		111.35	7.48 6.67
G16	8.07	108.80	3.61 3.43					
I17	8.20	116.18	3.95	1.78	1.19 1.01 0.84	0.71		
A18	8.07	125.04	4.25	1.16				
G19	8.46	106.02	3.51 3.32					
Q20	8.20	119.92	4.01	1.91 1.64	2.39		112.54	7.47 6.70
R21	8.41	124.14	4.21	1.61 1.54	1.90 1.28	3.10 2.96		
G22	8.26	109.59	3.57 3.43					
V23	8.20	117.50	3.92	2.00	0.90 0.81			
V24	7.45	123.13	3.90	1.76	0.89 0.82			
G25	7.91	108.34	3.55 3.43					
L26	8.19	120.23	4.22	1.33	1.65	0.77 0.79		

Chain III (**3T**)

Residue	HN	N	Hα	Hβ	Hγ	Hδ	Nε2	Hε2
G13	7.96	106.80	3.64 3.38					
P14			4.34	2.15 1.76	1.83 1.57	3.35 3.01		
Q15	8.37	122.14	4.27	1.71	2.85		110.98	7.47 6.85
G16	8.15	109.23	3.58 3.43					
I17	8.20	117.00	3.91	1.73	1.24 0.99 0.83	0.72		
A18	8.11	125.63	4.28	1.18				
G19	8.26	105.18	3.54 3.36					
Q20	8.21	119.27	4.05	1.94 1.64	2.34		112.67	7.48 6.71
R21	8.36	123.79	4.21	1.54 1.50	1.97 1.37	3.08 2.98		
G22	8.30	109.95	3.45 3.54					
V23	8.33	118.40	3.86	1.99	0.90 0.78			
V24	7.50	122.96	3.86	1.77	0.88 0.83			
G25	7.89	108.15	3.56 3.39					
L26	7.94	117.64	4.16	1.31	1.62	0.78 0.81		

Monomeric chain (**4M**)

Residue	HN	N	Hα	Hβ	Hγ	Hδ	Nε2	Hε2
G13	8.13	114.29	3.76					
P14			4.23	2.11 1.75	1.84	3.46		
Q15	8.41	120.16	4.18	1.82	2.23 2.17		112.17	7.37 6.69
G16	8.20	109.61	3.69					
I17	7.86	119.85	4.00	1.67	1.26 0.99 0.73	0.68		
A18	8.26	127.68	4.12	1.22				
G19	8.16	107.96	3.79 3.68					
Q20	8.00	119.67	4.16	1.82	1.82 1.95		112.64	7.35 6.69
R21	8.28	122.09	4.16	1.61 1.70	1.49	3.03		
G22	8.24	109.89	3.48 3.46					
V23	7.85	119.53	3.96	1.88	0.77 0.74			
V24	8.09	124.42	3.94	1.87	0.73 0.76			
G25	8.28	112.71	3.79 3.76					
L26	7.96	122.59	4.46	1.38	1.46	0.76 0.73		

Table S4. ^{13}C Resonance Assignments for the THP (pH 7.2, 298 K).

Chemical shifts (ppm)

Chain I (**1T**)

Residue	CO	Cα	Cβ	Cγ	Cδ
G13	170.00	45.80			
P14	176.53	62.26	32.41	27.61	49.87
Q15	176.84	56.11	28.29	33.88	180.52
G16	173.07	45.20			
I17	175.90	61.74	39.73	26.04 17.86	13.98
A18	179.84	52.46	19.76		
G19	172.84	45.18			
Q20	175.69	55.74	29.98	34.01	180.90
R21	177.59	56.91	30.33	27.55	44.15
G22	173.06	45.02			
V23	175.39	61.61	33.28	21.50 19.39	
V24	176.15	62.38	32.19	22.44 20.58	
G25	173.30	45.40			
L26	174.65	53.94	41.31	27.69	23.24 22.83

Chain II (**2T**)

Residue	CO	Cα	Cβ	Cγ	Cδ
G13	170.08	45.80			
P14	176.68	62.24	32.44	27.34	49.73
Q15	176.92	56.06	28.32	33.75	180.48
G16	172.88	45.36			
I17	175.52	61.80	39.24	26.19 17.78	13.79
A18	179.64	52.50	19.57		
G19	173.33	44.91			
Q20	175.59	55.49	30.21	34.14	180.68
R21	177.48	57.09	30.41	27.67	44.32
G22	172.95	45.21			
V23	175.00	62.06	33.16	21.84 20.34	
V24	175.27	62.29	31.98	22.30 20.42	
G25	173.40	45.13			
L26	175.10	53.76	41.23	27.17	23.00 22.51

Chain III (**3T**)

Residue	CO	Cα	Cβ	Cγ	Cδ
G13	170.35	45.60			
P14	176.26	62.20	32.47	27.25	49.79
Q15	177.28	55.95	27.80	33.56	181.02
G16	172.94	45.44			
I17	175.60	61.78	39.07	26.33 17.68	13.77
A18	179.57	52.43	19.29		
G19	173.36	44.75			
Q20	175.54	55.49	30.29	34.29	180.60
R21	177.31	56.94	30.67	27.34	44.53
G22	173.07	45.39			
V23	174.94	62.23	32.89	21.60 20.63	
V24	175.48	62.54	32.21	21.97 20.34	
G25	172.64	45.20			
L26	175.23	53.76	41.10	27.33	22.66 23.06

Monomeric chain (**4M**)

Residue	CO	Cα	Cβ	Cγ	Cδ
G13	174.57	45.49			
P14	177.61	63.56	32.26	27.41	49.96
Q15	176.86	56.10	29.80	34.01	180.89
G16	174.38	45.51			
I17	176.56	61.38	38.97	27.36 17.55	13.14
A18	178.59	53.12	19.10		
G19	174.51	45.52			
Q20	176.37	56.09	29.57	34.01	180.66
R21	177.00	56.52	30.87	27.32	43.56
G22	174.08	45.39			
V23	176.54	62.46	32.94	21.02 20.83	
V24	176.70	62.64	33.00	21.37 21.28	
G25	173.80	45.17			
L26	176.02	53.20	42.04	27.14	25.17 23.53

Table S5. Upper limit distances on THP structure calculation from ^{1}H-^{15}N HSQC-NOESY spectrum.

Intra Residue							
Chain	Residue	Label	Chain	Residue	Label	Distance (Å)	Volume
1T	Q15	H	1T	Q15	HE21	5.50	8.54E+03
1T	Q15	H	1T	Q15	HE22	5.50	3.26E+03
1T	Q15	H	1T	Q15	QB	3.30	2.05E+05
1T	Q15	H	1T	Q15	QG	4.64	2.64E+04
1T	Q15	HA	1T	Q15	HE21	5.15	1.94E+04
1T	Q15	HA	1T	Q15	HE22	5.15	1.42E+04
1T	Q15	HA	1T	Q15	QE2	4.46	1.94E+04
1T	Q15	QB	1T	Q15	QE2	3.92	6.37E+04
1T	I17	H	1T	I17	HB	4.21	4.74E+04
1T	I17	H	1T	I17	HG12	4.25	4.50E+04
1T	I17	H	1T	I17	HG13	4.25	4.98E+04
1T	I17	H	1T	I17	QD1	4.52	3.09E+04
1T	I17	H	1T	I17	QG2	4.00	6.45E+04
1T	A18	H	1T	A18	QB	3.56	1.29E+05
1T	Q20	H	1T	Q20	HB2	4.05	6.01E+04
1T	Q20	H	1T	Q20	HB3	4.05	8.37E+04
1T	Q20	H	1T	Q20	HE21	5.50	1.04E+04
1T	Q20	H	1T	Q20	HE22	5.50	1.45E+03
1T	Q20	H	1T	Q20	QB	3.50	8.37E+04
1T	Q20	H	1T	Q20	QG	4.25	4.50E+04
1T	Q20	HA	1T	Q20	HE21	5.50	7.10E+03
1T	Q20	HA	1T	Q20	HE22	5.50	1.04E+04
1T	Q20	HB2	1T	Q20	HE21	5.50	1.09E+04
1T	Q20	HB2	1T	Q20	HE22	5.50	1.12E+04
1T	Q20	HB3	1T	Q20	HE21	5.50	1.08E+04
1T	Q20	HB3	1T	Q20	HE22	5.50	8.75E+03
1T	Q20	QB	1T	Q20	QE2	4.29	1.12E+04
1T	R21	H	1T	R21	HD2	5.50	5.58E+03
1T	R21	H	1T	R21	HD3	5.50	9.62E+03
1T	R21	H	1T	R21	HG2	4.82	4.74E+04
1T	R21	H	1T	R21	HG3	4.82	2.12E+04
1T	R21	H	1T	R21	QB	3.49	1.00E+05
1T	R21	H	1T	R21	QG	3.96	4.74E+04
1T	V23	H	1T	V23	QG1	3.73	9.84E+04
1T	V23	H	1T	V23	QG2	3.73	9.84E+04
1T	V24	H	1T	V24	HB	3.77	9.26E+04
1T	V24	H	1T	V24	QG1	3.71	1.25E+05
1T	V24	H	1T	V24	QG2	3.71	1.02E+05
1T	V24	H	1T	V24	QQG	3.24	1.25E+05
1T	L26	H	1T	L26	HG	4.06	5.88E+04
1T	L26	H	1T	L26	QB	3.81	8.63E+04
1T	L26	H	1T	L26	QD1	4.41	3.64E+04
1T	L26	H	1T	L26	QD2	4.41	3.61E+04
2T	Q15	H	2T	Q15	HE21	5.50	3.21E+03
2T	Q15	H	2T	Q15	HE22	5.50	2.11E+03
2T	Q15	H	2T	Q15	QB	3.54	1.35E+05
2T	Q15	H	2T	Q15	QG	4.95	1.80E+04
2T	Q15	HA	2T	Q15	HE21	5.50	1.08E+04
2T	Q15	HA	2T	Q15	HE22	5.50	3.99E+03
2T	Q15	HA	2T	Q15	QE2	4.85	1.08E+04
2T	Q15	QB	2T	Q15	HE21	4.75	2.29E+04
2T	Q15	QB	2T	Q15	HE22	4.75	2.79E+04
2T	Q15	QB	2T	Q15	QE2	4.16	2.79E+04
2T	I17	H	2T	I17	HG12	4.63	4.36E+04
2T	I17	H	2T	I17	HG13	4.63	2.68E+04

2T	I17	H	2T	I17	QD1	4.77	2.25E+04
2T	I17	H	2T	I17	QG1	3.94	4.36E+04
2T	I17	H	2T	I17	QG2	4.30	4.18E+04
2T	A18	H	2T	A18	QB	3.42	1.64E+05
2T	Q20	H	2T	Q20	HB2	4.00	6.44E+04
2T	Q20	H	2T	Q20	HB3	4.00	6.49E+04
2T	Q20	H	2T	Q20	HE21	5.50	3.53E+03
2T	Q20	H	2T	Q20	HE22	5.50	1.48E+03
2T	Q20	H	2T	Q20	QG	4.07	5.83E+04
2T	Q20	HA	2T	Q20	HE21	5.50	2.93E+03
2T	Q20	HA	2T	Q20	HE22	5.50	3.90E+02
2T	Q20	QB	2T	Q20	QE2	4.53	3.22E+03
2T	R21	H	2T	R21	HB2	3.98	6.71E+04
2T	R21	H	2T	R21	HB3	3.98	6.68E+04
2T	R21	H	2T	R21	HD2	5.50	8.37E+03
2T	R21	H	2T	R21	HD3	5.50	8.45E+03
2T	R21	H	2T	R21	QG	4.07	4.36E+04
2T	V23	H	2T	V23	HB	3.90	7.45E+04
2T	V23	H	2T	V23	QG1	3.87	8.67E+04
2T	V23	H	2T	V23	QG2	3.87	7.85E+04
2T	V24	H	2T	V24	HB	3.70	1.02E+05
2T	V24	H	2T	V24	QG1	3.67	1.09E+05
2T	V24	H	2T	V24	QG2	3.67	1.12E+05
2T	L26	H	2T	L26	HG	3.84	8.29E+04
2T	L26	H	2T	L26	QB	3.67	1.08E+05
2T	L26	H	2T	L26	QQD	3.65	1.08E+05
3T	Q15	H	3T	Q15	HE21	5.50	1.62E+04
3T	Q15	H	3T	Q15	HE22	5.50	9.02E+03
3T	Q15	H	3T	Q15	QB	3.52	1.39E+05
3T	Q15	H	3T	Q15	QE2	4.66	1.62E+04
3T	Q15	H	3T	Q15	QG	4.89	1.93E+04
3T	Q15	HA	3T	Q15	HE21	5.12	2.79E+04
3T	Q15	HA	3T	Q15	HE22	5.12	1.47E+04
3T	Q15	HA	3T	Q15	QE2	4.29	2.79E+04
3T	Q15	QB	3T	Q15	QE2	3.50	1.10E+05
3T	I17	H	3T	I17	HB	4.08	5.76E+04
3T	I17	H	3T	I17	HG12	4.29	5.33E+04
3T	I17	H	3T	I17	HG13	4.29	4.25E+04
3T	I17	H	3T	I17	QD1	4.78	2.21E+04
3T	I17	H	3T	I17	QG1	3.75	5.33E+04
3T	I17	H	3T	I17	QG2	3.92	7.31E+04
3T	A18	H	3T	A18	QB	3.47	1.51E+05
3T	Q20	H	3T	Q20	HB2	4.10	5.53E+04
3T	Q20	H	3T	Q20	HB3	4.10	9.92E+04
3T	Q20	H	3T	Q20	HE21	5.50	1.48E+03
3T	Q20	H	3T	Q20	HE22	5.50	9.55E+03
3T	Q20	H	3T	Q20	QB	3.46	9.92E+04
3T	Q20	H	3T	Q20	QG	4.13	5.29E+04
3T	Q20	HA	3T	Q20	HE21	5.50	4.80E+03
3T	Q20	HA	3T	Q20	HE22	5.50	6.24E+03
3T	Q20	HB2	3T	Q20	HE21	5.50	4.56E+03
3T	Q20	HB2	3T	Q20	HE22	5.50	6.49E+03
3T	Q20	HB3	3T	Q20	HE21	5.50	5.05E+03
3T	Q20	HB3	3T	Q20	HE22	5.50	6.88E+03
3T	R21	H	3T	R21	HB2	3.95	7.65E+04
3T	R21	H	3T	R21	HB3	3.95	6.92E+04
3T	R21	H	3T	R21	HD2	5.50	1.17E+04
3T	R21	H	3T	R21	HD3	5.50	8.79E+03
3T	R21	H	3T	R21	QD	4.82	1.17E+04
3T	R21	H	3T	R21	QG	4.03	5.26E+04
3T	V23	H	3T	V23	HB	3.94	7.11E+04
3T	V23	H	3T	V23	QG1	3.71	1.02E+05

3T	V23	H	3T	V23	QG2	3.71	1.02E+05
3T	V24	H	3T	V24	HB	3.64	1.13E+05
3T	V24	H	3T	V24	QG1	3.73	9.87E+04
3T	V24	H	3T	V24	QG2	3.73	9.88E+04
3T	L26	H	3T	L26	HG	4.00	6.45E+04
3T	L26	H	3T	L26	QB	3.75	9.44E+04
3T	L26	H	3T	L26	QD1	4.15	5.19E+04
3T	L26	H	3T	L26	QD2	4.15	5.43E+04
Sequential							
1T	G13	H	1T	P14	QD	4.29	3.73E+04
1T	P14	HA	1T	Q15	H	3.47	1.50E+05
1T	P14	QB	1T	Q15	H	3.91	4.89E+04
1T	Q15	H	1T	G16	H	4.64	2.66E+04
1T	Q15	HE21	1T	G16	H	5.11	1.49E+04
1T	Q15	HE22	1T	G16	H	5.11	1.49E+04
1T	Q15	QG	1T	G16	H	4.57	2.90E+04
1T	I17	H	1T	A18	H	5.50	9.29E+03
1T	I17	HA	1T	A18	H	3.55	1.32E+05
1T	I17	HB	1T	A18	H	4.19	4.86E+04
1T	I17	QG2	1T	A18	H	4.26	4.40E+04
1T	A18	H	1T	G19	H	4.43	3.48E+04
1T	A18	QB	1T	G19	H	4.05	5.97E+04
1T	Q20	HA	1T	R21	H	3.47	1.50E+05
1T	Q20	QG	1T	R21	H	5.06	1.58E+04
1T	R21	HA	1T	G22	H	3.55	1.31E+05
1T	R21	HB2	1T	G22	H	4.72	3.44E+04
1T	R21	HB3	1T	G22	H	4.72	2.39E+04
1T	R21	HG2	1T	G22	H	4.27	4.44E+04
1T	R21	HG3	1T	G22	H	4.27	4.38E+04
1T	R21	QB	1T	G22	H	4.07	3.44E+04
1T	R21	QD	1T	G22	H	5.05	1.35E+04
1T	G22	H	1T	V23	H	4.63	2.67E+04
1T	G22	QA	1T	V23	H	3.13	1.54E+05
1T	V23	HB	1T	V24	H	4.10	5.60E+04
1T	V24	H	1T	G25	H	4.70	2.44E+04
1T	V24	HB	1T	G25	H	4.53	3.05E+04
1T	V24	QQG	1T	G25	H	3.71	9.86E+04
1T	G25	H	1T	L26	H	5.17	1.39E+04
2T	G13	H	2T	P14	QD	4.29	3.76E+04
2T	P14	QB	2T	Q15	H	4.11	2.94E+04
2T	Q15	H	2T	G16	H	4.86	2.00E+04
2T	Q15	HE21	2T	G16	H	5.50	4.43E+03
2T	Q15	HE22	2T	G16	H	5.50	1.04E+04
2T	Q15	QG	2T	G16	H	4.57	2.91E+04
2T	I17	HA	2T	A18	H	3.30	2.04E+05
2T	I17	HB	2T	A18	H	4.23	4.64E+04
2T	I17	QG2	2T	A18	H	4.07	5.84E+04
2T	A18	QB	2T	G19	H	4.05	5.99E+04
2T	Q20	QG	2T	R21	H	4.98	1.72E+04
2T	R21	HB2	2T	G22	H	5.03	1.64E+04
2T	R21	HB3	2T	G22	H	5.03	2.47E+04
2T	R21	HG2	2T	G22	H	4.35	4.36E+04
2T	R21	HG3	2T	G22	H	4.35	3.89E+04
2T	R21	QB	2T	G22	H	4.31	2.47E+04
2T	R21	QD	2T	G22	H	5.21	1.12E+04
2T	V23	H	2T	V24	H	4.71	2.41E+04
2T	V23	HB	2T	V24	H	4.17	5.03E+04
2T	V24	H	2T	G25	H	5.11	1.49E+04
2T	V24	HB	2T	G25	H	4.55	2.99E+04

2T	V24	QG1	2T	G25	H	4.07	7.49E+04
2T	V24	QG2	2T	G25	H	4.07	5.77E+04
2T	V24	QQG	2T	G25	H	3.54	7.49E+04
2T	G25	H	2T	L26	H	4.27	4.37E+04
3T	G13	H	3T	P14	QD	4.45	2.98E+04
3T	P14	QB	3T	Q15	H	4.05	3.49E+04
3T	Q15	QB	3T	G16	H	4.35	3.90E+04
3T	Q15	QE2	3T	G16	H	4.18	3.80E+04
3T	Q15	QG	3T	G16	H	4.56	2.95E+04
3T	I17	HA	3T	A18	H	3.41	1.68E+05
3T	I17	HB	3T	A18	H	4.10	5.53E+04
3T	I17	QG2	3T	A18	H	4.20	4.80E+04
3T	I17	QG2	3T	G19	H	5.50	5.71E+03
3T	A18	QB	3T	G19	H	4.12	5.41E+04
3T	Q20	HA	3T	R21	H	3.54	1.34E+05
3T	Q20	QG	3T	R21	H	4.88	1.96E+04
3T	R21	HD2	3T	G22	H	5.46	9.96E+03
3T	R21	HD3	3T	G22	H	5.46	1.04E+04
3T	R21	HG2	3T	G22	H	4.29	4.68E+04
3T	R21	HG3	3T	G22	H	4.29	4.26E+04
3T	R21	QB	3T	G22	H	4.20	4.31E+04
3T	G22	QA	3T	V23	H	3.10	1.70E+05
3T	V23	H	3T	V24	H	4.94	1.83E+04
3T	V23	HB	3T	V24	H	4.13	5.31E+04
3T	V24	H	3T	G25	H	4.89	1.92E+04
3T	V24	HB	3T	G25	H	4.56	2.94E+04
3T	V24	QG1	3T	G25	H	4.18	6.31E+04
3T	V24	QG2	3T	G25	H	4.18	4.93E+04
3T	V24	QQG	3T	G25	H	3.64	6.31E+04
Interchain							
1T	Q15	H	2T	G13	QA	4.40	1.95E+04
1T	Q15	H	2T	G13	HA2	5.01	1.68E+04
1T	Q15	H	2T	G13	HA3	5.01	1.95E+04
1T	Q15	HA	2T	G13	H	5.50	7.07E+03
1T	Q15	H	2T	P14	QD	4.85	1.76E+04
1T	Q15	HE21	2T	I17	H	5.11	1.48E+04
1T	Q15	HE22	2T	I17	H	5.11	2.51E+04
1T	Q15	QE2	2T	I17	H	4.33	2.51E+04
1T	Q15	QG	2T	I17	H	4.74	2.34E+04
1T	G16	H	2T	P14	QG	3.89	6.81E+04
1T	G16	H	2T	P14	QD	4.67	2.23E+04
1T	A18	H	2T	G16	HA2	5.50	7.09E+03
1T	A18	H	2T	G16	HA3	5.50	8.08E+03
1T	A18	HA	2T	I17	H	4.67	2.56E+04
1T	G19	H	2T	I17	HB	5.21	1.32E+04
1T	G19	H	2T	I17	HA	5.31	1.19E+04
1T	G19	H	2T	I17	QG2	5.34	1.14E+04
1T	G19	H	2T	I17	H	5.50	7.06E+03
1T	R21	H	2T	G19	QA	5.03	1.38E+04
1T	R21	HA	2T	Q20	H	4.45	3.39E+04
1T	R21	QD	2T	Q20	H	5.32	9.82E+03
1T	G22	H	2T	Q20	QG	5.42	1.04E+04
1T	V23	QQG	2T	G22	H	4.61	2.62E+04
1T	V24	H	2T	G22	QA	4.97	1.50E+04
1T	G25	H	2T	V23	HA	3.66	1.10E+05
1T	G25	H	2T	V23	HB	4.73	2.36E+04
1T	L26	H	2T	V24	HA	4.56	2.92E+04
1T	Q15	H	3T	G13	H	5.43	1.03E+04
1T	Q15	HA	3T	G13	H	5.22	1.30E+04

1T	G16	HA2	3T	Q15	H	5.50	9.53E+04
1T	G16	HA3	3T	Q15	H	5.50	1.49E+04
1T	G16	QA	3T	Q15	H	4.70	1.49E+04
1T	I17	H	3T	Q15	HA	4.70	2.46E+04
1T	I17	H	3T	Q15	H	5.50	8.37E+03
1T	I17	H	3T	Q15	QG	5.50	9.51E+03
1T	I17	HG12	3T	Q15	H	5.50	5.89E+03
1T	I17	HG13	3T	Q15	H	5.50	9.44E+03
1T	I17	QD1	3T	Q15	HE22	4.39	3.70E+04
1T	I17	QD1	3T	Q15	QE2	3.63	7.91E+04
1T	I17	QD1	3T	Q15	HE21	4.39	7.91E+04
1T	I17	QD1	3T	Q15	H	5.50	4.32E+03
1T	I17	QG1	3T	Q15	QE2	4.02	3.67E+04
1T	I17	H	3T	G16	H	5.38	1.09E+04
1T	I17	QG1	3T	G16	H	4.99	1.47E+04
1T	I17	QG2	3T	G16	H	5.12	1.47E+04
1T	G19	H	3T	Q15	QG	5.33	1.15E+04
1T	G19	QA	3T	Q15	QE2	4.65	1.92E+04
1T	G19	HA2	3T	A18	H	5.50	8.48E+03
1T	G19	HA3	3T	A18	H	5.50	8.03E+03
1T	Q20	H	3T	Q15	QE2	4.46	1.89E+04
1T	Q20	H	3T	Q15	QG	4.58	2.86E+04
1T	Q20	H	3T	Q15	HE22	5.12	1.47E+04
1T	Q20	H	3T	Q15	HE21	5.12	1.89E+04
1T	Q20	HA	3T	Q15	QE2	5.30	9.99E+03
1T	Q20	QG	3T	Q15	QE2	4.16	4.58E+04
1T	Q20	H	3T	I17	QG2	5.50	6.24E+03
1T	Q20	H	3T	A18	HA	4.13	5.36E+04
1T	Q20	H	3T	A18	QB	4.53	3.06E+04
1T	Q20	H	3T	G19	H	4.37	3.77E+04
1T	Q20	HA	3T	G19	H	5.27	1.24E+04
1T	Q20	HB2	3T	G19	H	5.50	8.61E+03
1T	Q20	HB3	3T	G19	H	5.50	8.92E+03
1T	Q20	QG	3T	G19	H	5.50	6.76E+03
1T	G22	QA	3T	R21	H	4.91	1.62E+04
1T	V23	H	3T	G19	H	5.50	6.11E+03
1T	V23	QQG	3T	Q20	H	5.44	6.13E+03
1T	V23	H	3T	R21	HA	4.14	5.22E+04
1T	V23	H	3T	R21	QB	4.65	2.27E+04
1T	V23	H	3T	R21	H	5.50	4.81E+03
1T	V23	QQG	3T	R21	H	5.07	1.46E+04
1T	V23	H	3T	G22	H	5.07	1.56E+04
1T	V23	QQG	3T	G22	H	4.32	3.92E+04
1T	G25	QA	3T	V24	H	4.76	1.96E+04
1T	L26	H	3T	R21	HD3	5.50	9.18E+03
1T	L26	H	3T	R21	HD2	5.50	9.38E+03
1T	L26	QB	3T	V24	H	5.50	6.57E+03
1T	L26	H	3T	G25	H	5.20	1.34E+04
1T	L26	HA	3T	G25	H	5.17	1.39E+04
1T	L26	QB	3T	G25	H	4.69	2.49E+04
2T	G13	H	3T	G13	H	4.77	2.24E+04
2T	Q15	H	3T	G13	QA	4.48	1.84E+04
2T	Q15	H	3T	G13	HA3	5.14	1.84E+04
2T	Q15	H	3T	G13	HA2	5.14	1.44E+04
2T	Q15	H	3T	P14	QD	5.21	1.12E+04
2T	Q15	QG	3T	I17	H	4.68	2.52E+04
2T	G16	H	3T	P14	QG	4.01	5.70E+04
2T	G16	H	3T	P14	QD	4.92	1.60E+04
2T	A18	H	3T	G16	HA3	5.50	8.69E+03
2T	A18	H	3T	G16	HA2	5.50	8.57E+03
2T	A18	HA	3T	I17	H	4.26	4.43E+04
2T	G19	H	3T	I17	H	4.31	4.12E+04

2T	G19	H	3T	I17	QG2	5.23	1.30E+04
2T	G19	H	3T	I17	HA	5.28	1.22E+04
2T	G19	H	3T	I17	HB	5.40	1.07E+04
2T	R21	H	3T	G19	QA	5.11	1.27E+04
2T	R21	HA	3T	Q20	H	4.31	4.10E+04
2T	R21	QD	3T	Q20	H	5.34	7.46E+03
2T	R21	QG	3T	V23	H	4.69	2.17E+04
2T	V24	H	3T	G22	QA	4.87	1.72E+04
2T	G25	H	3T	V23	HB	5.02	1.65E+04
2T	L26	H	3T	L26	H	4.93	1.84E+04

NOE Statistics:				Distances		
Intra residual	125	40.7%		3 Å < dist < 4 Å	58	18.9%
Sequential	79	25.7%		4 Å < dist < 5 Å	139	45.3%
Interchain	103	33.6%		5 Å < dist < 6 Å	110	35.8%
Total	307			Total	307	

Table S6. Torsion angles used for THP structure calculation.

Residue	ϕ		ψ	
	from	to	from	to
^{1T}I17	−170.0	−60.0	110.0	170.0
^{2T}I17	−170.0	−60.0	110.0	170.0
^{3T}I17	−170.0	−60.0	110.0	170.0
^{1T}Q20	−170.0	−60.0	110.0	170.0
^{2T}Q20	−170.0	−60.0	110.0	170.0
^{3T}Q20	−170.0	−60.0	110.0	170.0
^{1T}R21	−170.0	−60.0	110.0	170.0
^{2T}R21	−170.0	−60.0	110.0	170.0
^{3T}R21	−170.0	−60.0	110.0	170.0
^{1T}V23	−170.0	−60.0	110.0	170.0
^{2T}V23	−170.0	−60.0	110.0	170.0
^{3T}V23	−170.0	−60.0	110.0	170.0
^{1T}V24	−170.0	−60.0	110.0	170.0
^{2T}V24	−170.0	−60.0	110.0	170.0
^{1T}L26	−170.0	−60.0	110.0	170.0
^{2T}L26	−170.0	−60.0	110.0	170.0
^{3T}L26	−170.0	−60.0	110.0	170.0

34 dihedral angles selected from 17 residues.

Table S7. Upper limit distances for THP *N*- and *C*-terminal residues.

Residue	1		Residue	2		Distance (Å)
1T	G13	H	2T	P12	HA	5.30
1T	G13	HA3	2T	P12	HA	5.30
1T	G13	HA3	3T	P12	HA	5.30
1T	P14	HB2	3T	P12	HB3	5.30
1T	P14	HD2	3T	P12	HG3	5.30
1T	P27	HA	2T	L26	H	5.30
1T	P27	HA	2T	L26	HD23	5.30
1T	P27	HA	3T	G25	H	5.30
1T	P27	HA	3T	G25	HA3	5.30
1T	P27	HB3	2T	L26	HG	5.30
1T	P27	HB3	2T	L26	HD21	5.30
1T	P27	HG2	2T	L26	HD21	5.30
1T	P27	HG3	2T	L26	H	5.30
1T	G28	H	3T	G25	H	5.30
1T	G28	H	3T	G25	HA3	5.30
1T	G28	HA2	3T	G25	H	5.30
1T	G28	HA3	3T	G25	HA3	5.30
2T	P12	HA	3T	P11	HD2	5.30
2T	P12	HB3	3T	P11	HB2	5.30
2T	P12	HB3	3T	P11	HG2	5.30
2T	P12	HB3	3T	P11	HD2	5.30
2T	P12	HD3	3T	P11	HD2	5.30
2T	G13	H	3T	P12	HA	5.30
2T	G13	HA3	3T	P12	HA	5.30
2T	P27	HA	3T	G25	HA3	5.30
2T	P27	HA	3T	L26	H	5.30
2T	P27	HA	3T	L26	HB2	5.30
2T	P27	HA	3T	L26	HD23	5.30
2T	P27	HB2	3T	L26	HB2	5.30
2T	P27	HB3	3T	L26	H	5.30
2T	P27	HB3	3T	L26	HG	5.30
2T	P27	HG3	3T	L26	HD23	5.30
2T	P27	HD3	3T	L26	HD23	5.30
2T	G13	H	3T	P11	O	2.40
1T	G28	H	2T	L26	O	2.40

33 interchain distances selected.
+ 2 interchain hydrogen bonds.

Table S8. Torsion angles used for THP *N*- and *C*-terminal residues.

Residue	ϕ		ψ	
	from	to	from	to
^{1T}G13	−170.0	−60.0	110.0	170.0
^{1T}P14			110.0	170.0
^{1T}P27			110.0	170.0
^{1T}G28	−170.0	−60.0	110.0	170.0
^{2T}P12			110.0	170.0
^{2T}G13	−170.0	−60.0	110.0	170.0
^{2T}P27			110.0	170.0
^{3T}P11			110.0	170.0
^{3T}P12			110.0	170.0
^{3T}G25	−170.0	−60.0	110.0	170.0

14 dihedral angles selected from 10 residues.

Table S9. Statistical analysis of the NMR structures of the THP (pH 7.2, 298 K).

Restraints used for calculation:	
Total number of NOE restraints	307
(Medium range: from (i,i+2) to (i,i+5))	
Intra-residue	125
Sequential	78
Medium range	1
Long range	103
Dihedral angle restraints	34
Phi	17
Psi	17
Average number of restraints per residue	8
RMS violations per constraint (structure averaged)[a]	
Intra-residue	0.0276 ± 0.0068 Å
Sequential	0.0220 ± 0.0058 Å
Long range	0.0486 ± 0.0063 Å
Total:	0.0354 ± 0.0036 Å
Average no. of violations > 0.3 Å:	0.1667 ± 0.3727
Average no. of violations 0.1 << 0.3 Å:	12.2667 ± 2.7681
Average backbone RMSD to mean	0.84 ± 0.26 Å
Average heavy atom RMSD to mean	1.32 ± 0.27 Å
Ramachandran plot:	
Percentage of residues present in core regions	78.3%
Percentage of residues present in allowed regions	20.0%
Percentage of residues present in generously allowed regions	1.2%
Percentage of residues present in disallowed regions	0.6%

[a] The data are calculated over the 30 conformers representing the NMR structure. The mean value and the standard deviation are given.
[b] The RMSD to the mean coordinates of 30 conformers was calculated for the atom of residues included between 13 and 26 on the three chains.

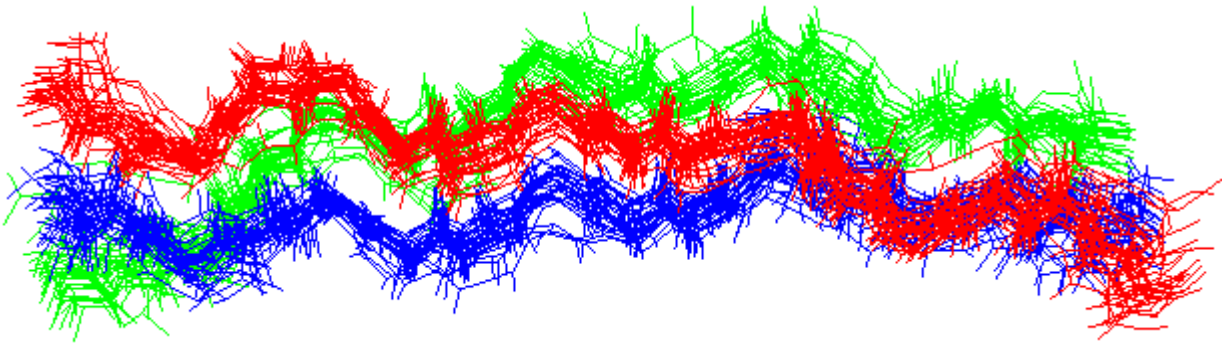


Figure S1. Solution structure of the α1(I)772-786 THP. The three chains forming the THP are 1T (green), 2T (blue), and 3T (red).

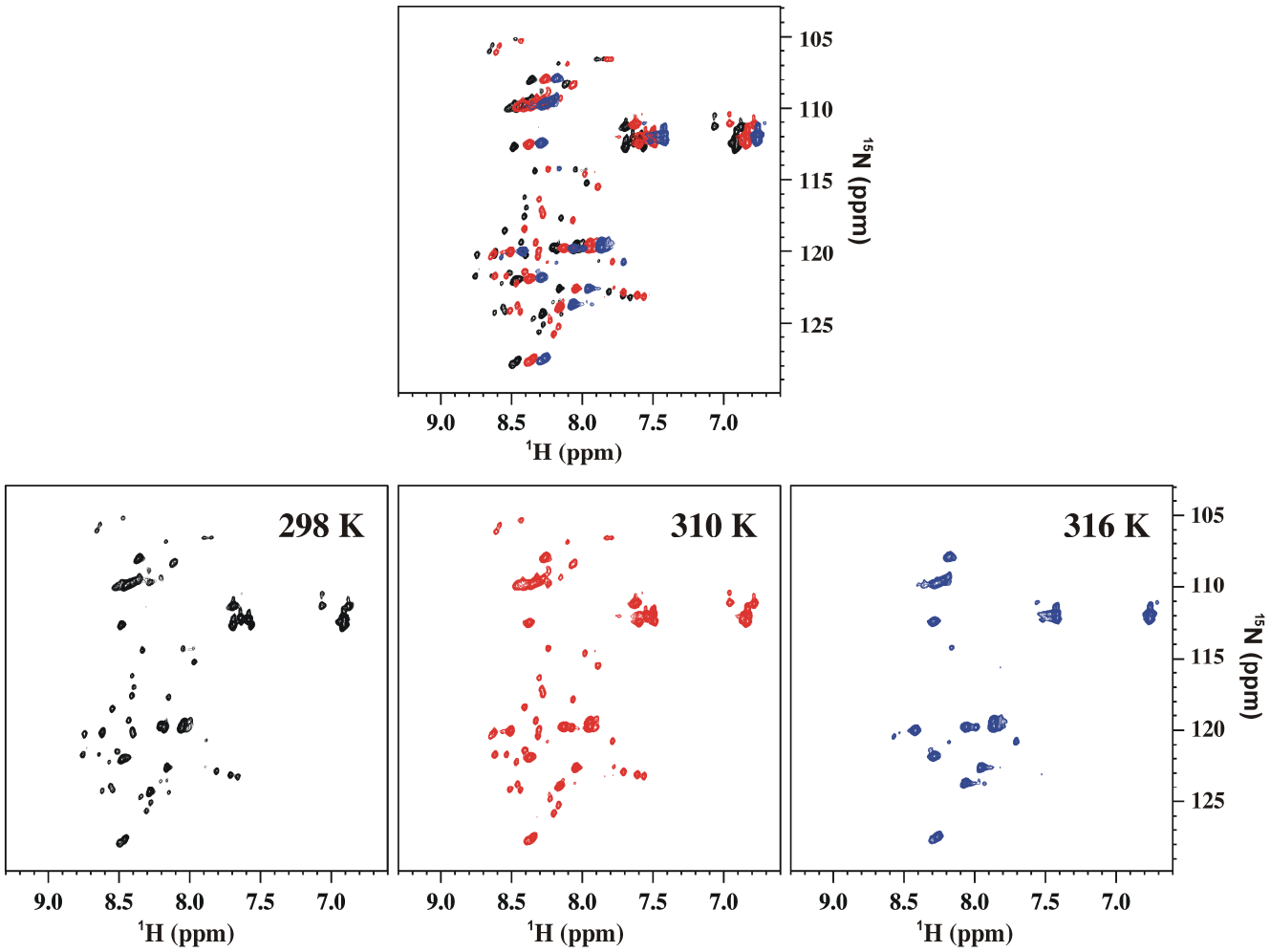


Figure S2. ^{1}H-^{15}N HSQC spectra of the THP at 298 K (black peaks), 310 K (red peaks), and 316 K (blue peaks). At 310 K all of the amide peaks of the THP are shifted but still intense. At 316 K the intensity of the THP cross-peaks are drastically reduced with only the resonances corresponding to the isolated chain remaining visible and intense.

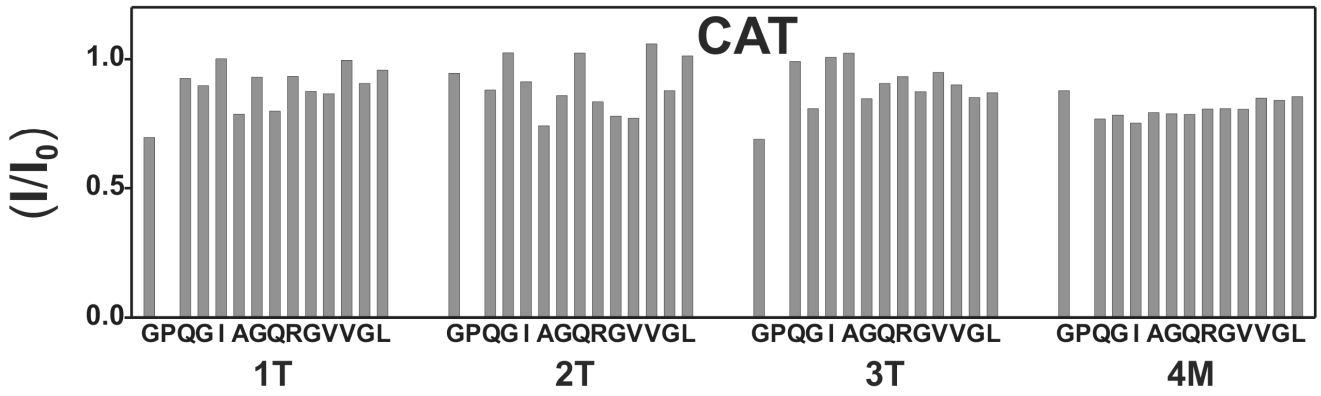
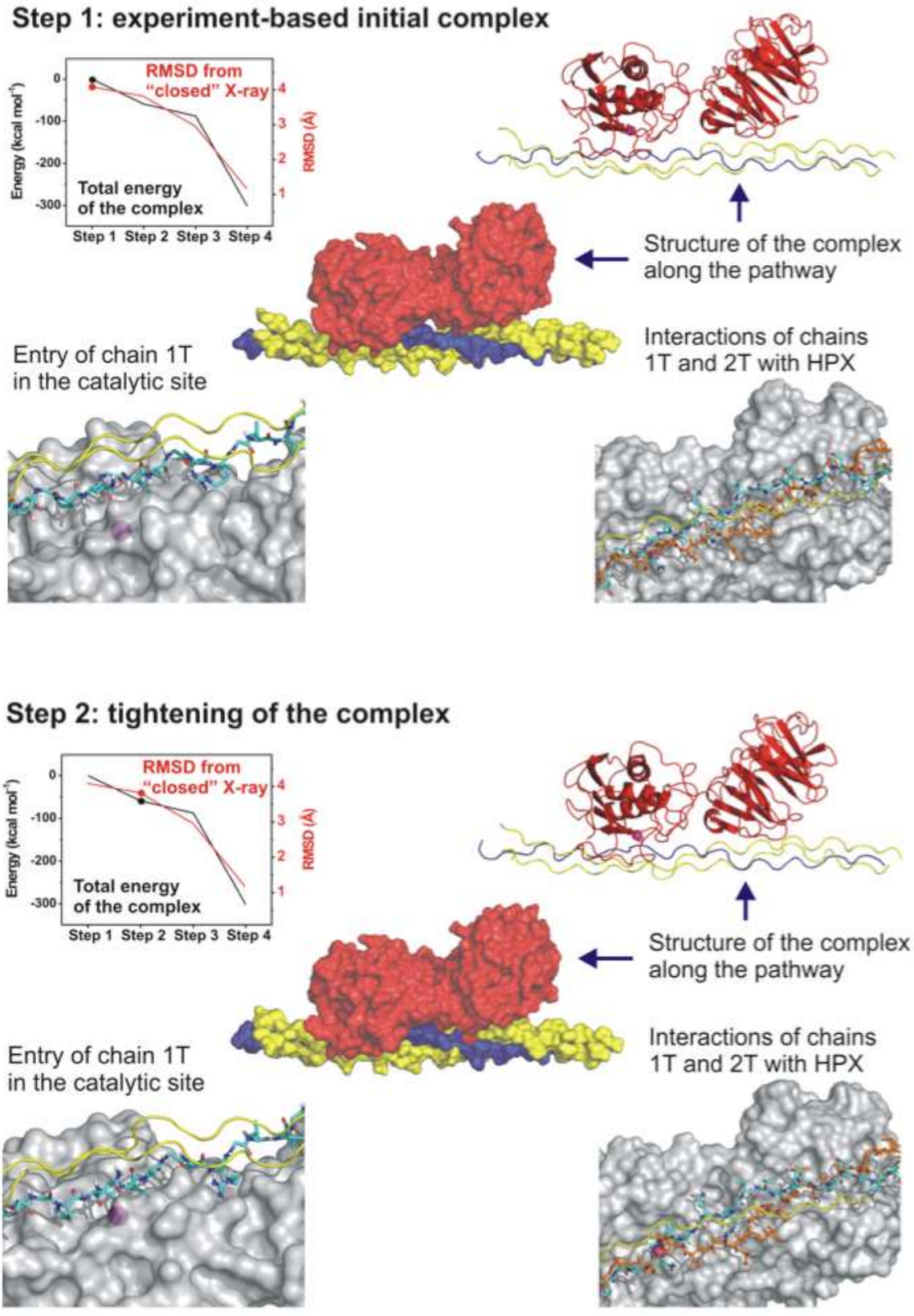
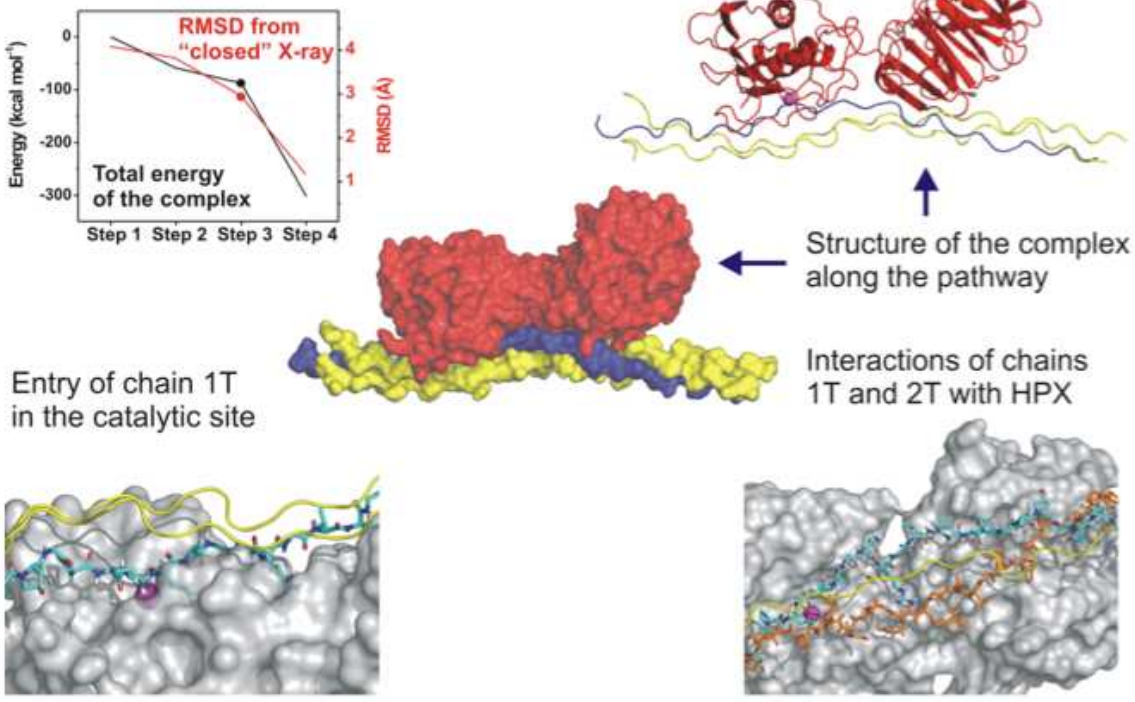


Figure S3. Intensity changes per residue observed at 310 K in the $^{13}C,^{15}N$ THP upon the addition of unlabeled CAT (0.2 eq). Greater preference for 4M is indicated by the greater overall decrease in signal intensity.

Figure S4. Energetics of collagenolysis.



Step 3: FL-MMP rewinding and THP distortion



Step 4: FL-MMP closing (X-ray) and THP unwinding