Novel model peptide for Atx1-like metallochaperones

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Supplementary Information

Table S1 ^{1}H NMR (500 MHz) chemical shifts (δ ppm) for **1**·H_2 in H_2O/D_2O 9:1 at 298K.

Residue	HN	Hα	Hβ	Others
GLY 1	8.66	3.66 (*pro-R*), 4.08 (*pro-S*)		
MET 2	7.91	4.64	1.91, 2.07	CH_2(γ): 2.35, 2.44; CH_3(ε): 2.00
THR 3	8.31	4.37	4.18	CH_3(γ): 1.10
CYS 4	8.18	4.53	2.93	
SER 5	8.44	4.33	3.81	
GLY 6	8.52	3.77, 4.00		
CYS 7	7.90	4.50	2.86	
SER 8	8.48	4.42	3.80	
ARG 9	7.82	4.61	1.62, 1.76	CH_2(γ): 1.58; CH_2(δ): 3.12; HN(ε): 7.08
PRO 10	-	4.25	2.21 (*pro-S*), 1.80 (*pro-R*)	CH_2(γ): 1.91 (*pro-R*), 2.09 (*pro-S*); CH_2(δ): 3.52 (*pro-S*), 3.76 (*pro-R*)

Table S2 $^3J_{HN,H\alpha}$ coupling constants (Hz) for **1**·H_2 in H_2O/D_2O 9:1 at 298K.

MET 2	THR 3	CYS 4	SER 5	CYS 7	SER 8	ARG 9
8.0	8.0	6.7	6.0	7.4	7.2	7.5

Table S3 ROESY-derived distances (Å) for **1**·H_2 in H_2O/D_2O 9:1 at 298K. d(H1,H2)$_{min}$ and d(H1,H2)$_{max}$ are the lower and upper distance limits, respectively, used for X-PLOR calculations.

H1	H2	d(H1,H2)	d(H1,H2)$_{min}$	d(H1,H2)$_{max}$
GLY 1 HN	GLY 1 HA2	2.35	2.11	2.58
GLY 1 HN	GLY 1 HA1	2.78	2.50	3.06
GLY 1 HN	PRO 10 HA	2.11	1.90	2.32
GLY 1 HN	PRO 10 HB2	3.55	3.20	3.91
GLY 1 HN	PRO 10 HB1	3.70	3.33	4.07
GLY 1 HN	MET 2 HN	2.69	2.42	2.96
MET 2 HN	MET 2 HA	2.50	2.25	2.75
MET 2 HN	GLY 1 HA1	3.26	2.93	3.58
MET 2 HN	GLY 1 HA2	3.19	2.87	3.51
MET 2 HN	PRO 10 HA	3.60	3.24	3.95
MET 2 HN	MET 2 HG*	3.17	2.85	4.19
MET 2 HN	MET 2 HB*	2.82	2.53	3.80
THR 3 HN	MET 2 HN	2.81	2.53	3.09
THR 3 HN	THR 3 HA	2.64	2.37	2.90
THR 3 HN	MET 2 HB*	3.18	2.86	4.20
THR 3 HN	THR 3 HG2*	3.48	3.13	4.23
THR 3 HG2*	THR 3 HB	2.52	2.27	3.17
THR 3 HG2*	THR 3 HA	2.75	2.47	3.42
THR 3 HN	CYS 4 HN	3.07	2.76	3.37
CYS 4 HN	CYS 4 HA	2.67	2.40	2.94
CYS 4 HN	THR 3 HA	2.34	2.10	2.57

CYS 4 HN	THR 3 HB	3.15	2.84	3.47
CYS 4 HN	CYS 4 HB*	2.93	2.64	3.92
CYS 4 HN	MET 2 HB*	3.92	3.53	5.02
CYS 4 HN	THR 3 HG2*	3.95	3.56	4.75
CYS 4 HA	CYS 4 HB*	2.48	2.24	3.43
SER 5 HN	CYS 4 HA	2.32	2.09	2.56
SER 5 HN	SER 5 HA	2.66	2.39	2.92
SER 5 HN	SER 5 HB*	2.74	2.46	3.71
SER 5 HN	CYS 4 HB*	3.06	2.75	4.06
SER 5 HA	SER 5 HB*	2.51	2.26	3.46
GLY 6 HN	CYS 7 HN	2.71	2.44	2.98
GLY 6 HN	SER 5 HA	2.31	2.08	2.54
GLY 6 HN	GLY 6 HA*	2.48	2.23	3.43
CYS 7 HN	GLY 6 HA*	2.89	2.60	3.88
CYS 7 HN	CYS 7 HA	2.74	2.47	3.02
CYS 7 HN	CYS 7 HB*	1.94	1.74	2.83
CYS 7 HA	CYS 7 HB*	2.45	2.20	3.39
SER 8 HN	ARG 9 HN	2.76	2.49	3.04
SER 8 HN	CYS 7 HA	2.26	2.04	2.49
SER 8 HN	SER 8 HB*	2.75	2.47	3.72
SER 8 HN	CYS 7 HB*	3.35	3.01	4.38
SER 8 HA	SER 8 HB*	2.42	2.18	3.37
ARG 9 HN	ARG 9 HA	2.77	2.49	3.04
ARG 9 HN	SER 8 HA	2.42	2.18	2.66
ARG 9 HN	CYS 7 HB*	4.08	3.67	5.19
ARG 9 HN	SER 8 HB*	3.54	3.18	4.59
ARG 9 HA	PRO 10 HD1	2.04	1.84	2.25
ARG 9 HA	PRO 10 HD2	2.43	2.18	2.67
ARG 9 HE	ARG 9 HD*	2.77	2.49	3.74
ARG 9 HE	ARG 9 HG*	3.00	2.70	4.00
PRO 10 HB1	PRO 10 HA	2.30	2.07	2.53
PRO 10 HA	PRO 10 HB2	2.82	2.54	3.10
CYS 4 HN	THR 3 HA	2.34	2.10	2.57
CYS 7 HB*	MET 2 HB*	3.66	3.29	5.03
CYS 7 HN	SER 5 HA	3.82	3.44	4.20
ARG 9 HG*	MET 2 HB*	2.57	2.31	3.83
CYS 7 HB*	ARG 9 HG*	4.38	3.95	5.82
ARG 9 HN	MET 2 HB*	3.66	3.29	4.72

Table S4 ^{1}H NMR (500 MHz) chemical shifts (δ ppm) for **1**·Hg in H_2O/D_2O 9:1 at 298K.

Residue	HN	Hα	Hβ	Others
GLY 1	8.69	3.70 (*pro-R*), 4.10 (*pro-S*)		
MET 2	8.09	4.58	1.78, 2.06	CH_2(γ): 2.29, 2.37; CH_3(ε): 2.01
THR 3	7.93	4.09	4.06	CH_3(γ): 1.18
CYS 4	8.56	4.96	3.54	
SER 5	8.88	4.29	3.89	
GLY 6	8.63	3.78, 4.19		
CYS 7	7.59	4.98	3.32, 3.47	
SER 8	8.48	4.42	3.81, 3.88	
ARG 9	7.34	4.50	1.66, 1.76	CH_2(γ): 1.59; CH_2(δ): 3.18; HN(ε): 7.06
PRO 10	-	4.25	2.22 (*pro-S*), 1.85 (*pro-R*)	CH_2(γ): 1.94 (*pro-R*), 2.03 (*pro-S*); CH_2(δ): 3.54 (*pro-S*), 3.89 (*pro-R*)

Table S5 $^{3}J_{HN,H\alpha}$ coupling constants (Hz) for **1**·H_g in H_2O/D_2O 9:1 at 298K.

MET 2	THR 3	CYS 4	SER 5	CYS 7	SER 8	ARG 9
8.9	4.9	6.0	4.7	5.2	8.9	7.0

Table S6 ROESY-derived distances (Å) and distances calculated using X-PLOR for **1**·Hg in H_2O/D_2O 9:1 at 298K. $d(H1,H2)_{min}$ and $d(H1,H2)_{max}$ are the lower and upper distance limits used for X-PLOR calculations. The calculated distances are those of the lowest energy structure.

H1	H2	$d(H1,H2)$	$d(H1,H2)_{min}$	$d(H1,H2)_{max}$	Calculated
GLY 1 HN	MET 2 HN	2.78	2.50	3.05	2.86
GLY 1 HN	THR 3 HN	3.77	3.39	4.15	3.34
GLY 1 HN	PRO 10 HA	2.12	1.91	2.33	2.19
GLY 1 HN	GLY 1 HA1	2.74	2.46	3.01	2.98
GLY 1 HN	GLY 1 HA2	2.30	2.07	2.53	2.44
GLY 1 HN	PRO 10 HB1	3.83	3.45	4.21	3.95
GLY 1 HN	PRO 10 HB2	3.42	3.08	3.77	3.51
GLY 1 HN	THR 3 HG2*	5.11	4.60	6.02	6.02
MET 2 HN	THR 3 HN	2.46	2.21	2.71	2.68
MET 2 HN	MET 2 HA	2.88	2.59	3.17	2.99
MET 2 HN	PRO 10 HA	3.55	3.20	3.91	3.69
MET 2 HN	GLY 1 HA1	2.93	2.63	3.22	2.92
MET 2 HN	GLY 1 HA2	3.14	2.82	3.45	3.52
MET 2 HN	MET 2 HG*	3.01	2.71	4.01	3.99
MET 2 HN	MET 2 HB*	2.87	2.59	3.86	3.11
MET 2 HN	THR 3 HG2*	5.25	4.73	6.18	6.21
MET 2 HA	MET 2 HG*	3.02	2.71	4.02	2.91
MET 2 HA	MET 2 HB*	2.72	2.45	3.70	2.84
THR 3 HN	CYS 4 HN	3.23	2.90	3.55	4.03
THR 3 HN	ARG 9 HN	4.41	3.97	4.86	5.04
THR 3 HN	MET 2 HA	2.69	2.42	2.96	2.82
THR 3 HN	PRO 10 HA	4.00	3.60	4.40	4.27
THR 3 HN	THR 3 HA	2.65	2.38	2.91	2.85
THR 3 HN	THR 3 HB	2.69	2.42	2.96	2.85
THR 3 HN	MET 2 HB*	3.41	3.07	4.45	4.33
THR 3 HN	THR 3 HG2*	3.50	3.15	4.26	4.46
THR 3 HA	THR 3 HG2*	2.82	2.54	3.51	2.84
CYS 4 HN	CYS 7 HN	3.05	2.75	3.36	3.44
CYS 4 HN	CYS 4 HA	2.86	2.57	3.14	2.97
CYS 4 HN	THR 3 HA	2.25	2.03	2.48	2.09
CYS 4 HN	THR 3 HB	3.50	3.20	3.80	4.15
CYS 4 HN	CYS 4 HB*	3.55	3.20	4.61	3.42
CYS 4 HN	MET 2 HB*	3.89	3.50	4.98	5.12
CYS 4 HN	THR 3 HG2*	3.43	3.09	4.18	4.27
CYS 4 HA	CYS 4 HB*	2.58	2.32	3.54	2.47
CYS 4 HA	THR 3 HG2*	4.24	3.81	5.06	5.34
SER 5 HN	GLY 6 HN	3.15	2.84	3.47	2.81
SER 5 HN	CYS 4 HN	4.46	4.02	4.91	4.44
SER 5 HN	CYS 4 HA	2.43	2.18	2.67	2.77
SER 5 HN	SER 5 HA	2.76	2.48	3.03	2.92
SER 5 HN	SER 5 HB*	2.70	2.43	3.67	2.63
SER 5 HN	CYS 4 HB*	2.63	2.37	3.60	3.09
SER 5 HA	SER 5 HB*	2.42	2.18	3.36	2.84
GLY 6 HN	CYS 7 HN	2.56	2.31	2.82	2.13
GLY 6 HN	CYS 4 HA	3.96	3.57	4.36	4.29
GLY 6 HN	SER 5 HA	2.78	2.50	3.06	3.16
GLY 6 HN	GLY 6 HA*	2.49	2.24	3.44	2.72
GLY 6 HN	SER 5 HB*	3.32	2.98	4.35	4.05
GLY 6 HN	CYS 4 HB*	4.44	4.00	5.58	4.13
CYS 7 HN	SER 8 HN	3.61	3.25	3.97	4.32
CYS 7 HN	CYS 7 HA	2.71	2.44	2.98	2.92
CYS 7 HN	SER 5 HA	3.56	3.21	3.92	4.05
CYS 7 HN	GLY 6 HA*	3.17	2.85	4.18	3.34
CYS 7 HN	CYS 7 HB1	3.18	2.86	3.49	3.27
CYS 7 HN	CYS 7 HB2	3.57	3.21	3.93	3.99
CYS 7 HN	CYS 4 HB*	5.16	4.64	6.38	5.26

CYS 7 HA	CYS 7 HB1	2.37	2.14	2.61	2.56
CYS 7 HA	CYS 7 HB2	2.56	2.31	2.82	2.40
CYS 7 HB2	ARG 9 HG*	3.51	3.16	4.56	4.06
SER 8 HN	ARG 9 HN	2.69	2.42	2.96	2.84
SER 8 HN	CYS 7 HA	2.28	2.05	2.51	2.55
SER 8 HN	SER 8 HB*	2.92	2.62	3.91	3.29
SER 8 HN	CYS 7 HB1	3.73	3.36	4.11	3.68
SER 8 HN	CYS 7 HB2	2.51	2.25	2.76	2.23
SER 8 HN	ARG 9 HG*	4.09	3.68	5.20	4.21
ARG 9 HN	ARG 9 HA	2.71	2.44	2.98	2.99
ARG 9 HN	THR 3 HA	3.43	3.09	3.78	3.08
ARG 9 HN	SER 8 HB*	4.18	3.76	5.30	4.43
ARG 9HN	CYS 7 HB1	4.15	3.73	4.56	4.65
ARG 9 HN	CYS 7 HB2	3.25	2.93	3.58	3.53
ARG 9 HN	THR 3 HG2*	4.84	4.36	5.73	4.55
ARG 9 HN	ARG 9 HG*	2.85	2.57	3.84	3.72
ARG 9 HN	ARG 9 HB*	2.75	2.47	3.72	2.89
ARG 9 HA	PRO 10HD1	2.10	1.89	2.31	2.31
ARG 9 HA	PRO 10 HD2	2.50	2.25	2.75	2.66
ARG 9 HA	ARG 9 HD*	2.80	2.52	3.78	3.88
ARG 9 HA	ARG 9 HG*	2.67	2.41	3.64	2.61
ARG 9 HA	ARG 9 HB*	2.66	2.39	3.62	2.84
CYS 7 HN	THR 3 HA	4.59	4.13	5.05	4.76

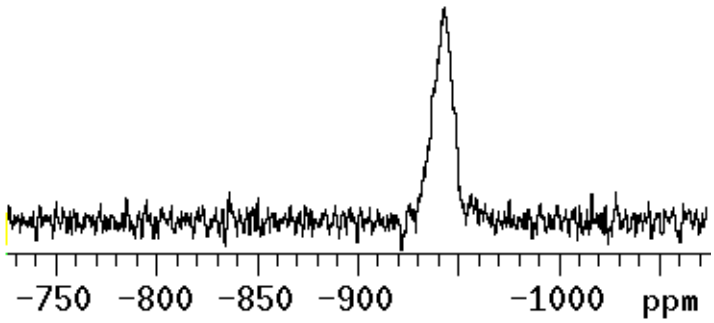

Figure S1 ^{199}Hg NMR spectrum of **1**·Hg in H_2O/D_2O 9:1 at 298K. The spectrum was recorded on a Varian Mercury Spectrometer at 71.6 MHz. The sample (**1**·Hg, 3 mM in H_2O/D_2O 9:1, pH 4.0) was prepared with 91% enriched $^{199}Hg^{2+}$. The spectrum was collected with 10^6 scans. A 50 Hz line broadening was applied during the processing (S/N = 16, $\Delta\nu_{1/2}$ = 780 Hz). Chemical shifts are referenced to $Hg(CH_3)_2$ (0 ppm).