SOLUTION STRUCTURES OF THE ACTUATOR DOMAIN OF ATP7A AND ATP7B, THE MENKES AND WILSON DISEASE PROTEINS

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Supplementary Material

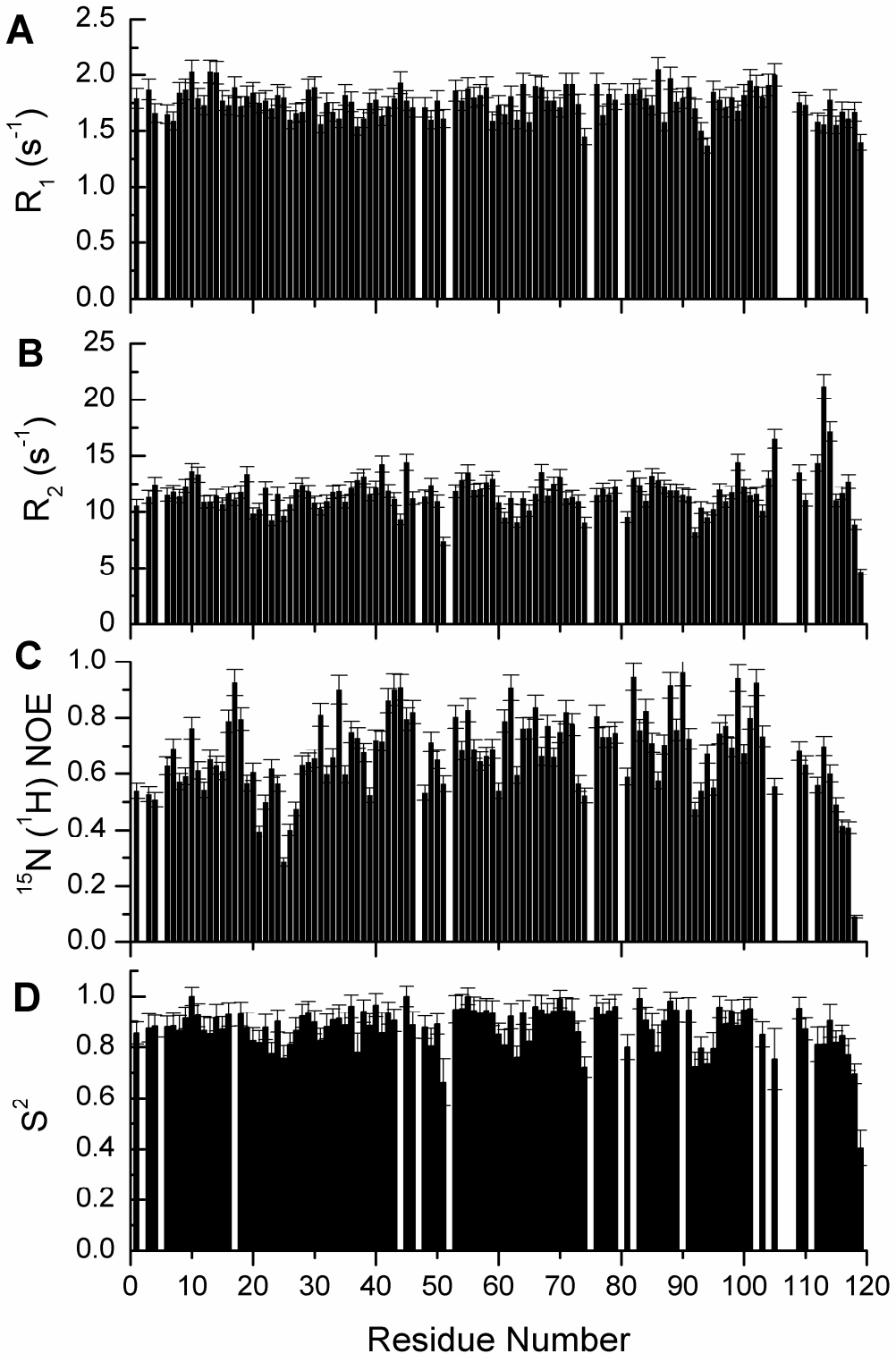

Figure S1 Backbone amide ^{15}N R_1 (A) and R_2 (B) relaxation data and $^{15}N\{^1H\}$-NOE (C) and order parameters (S^2) (D) measured at 500 MHz and 298 K.

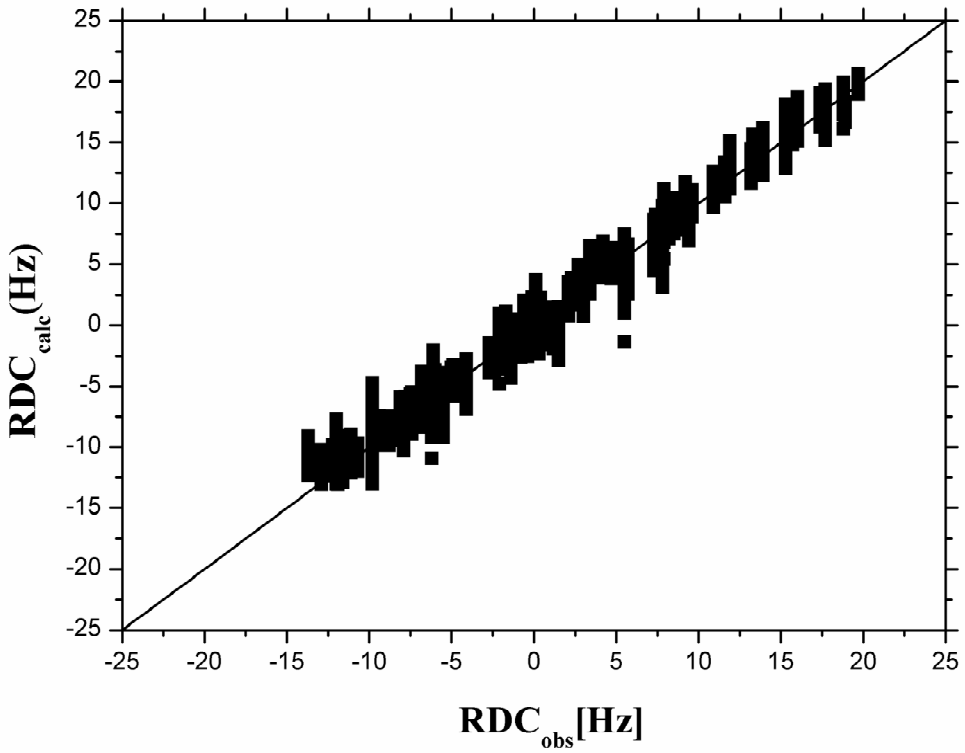

Figure S2. Observed versus calculated residual dipolar couplings in the final structure refinement with the RDC constraints. The line represents the expected correlation ($y = x$).
The RDC values of the following residues were used for structure calculations: 1,3-46,48-49,53,56-57,60-74,76-79,81-85,87-93,95-103,105-106,109,110,112-119

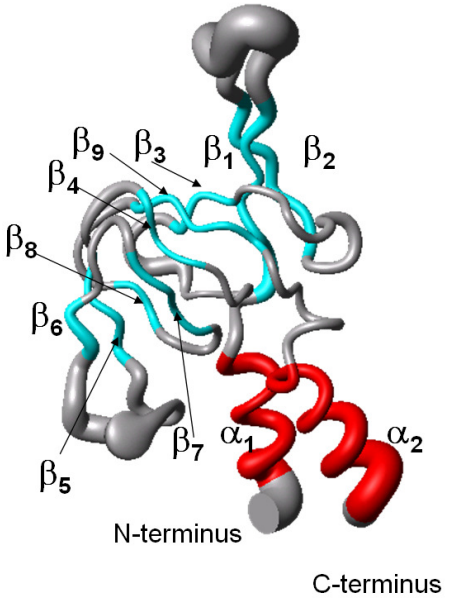

Figure S3. Solution structure of A-WLN (residues 1-119). The radius of the tube is proportional to the backbone RMSD of each residue. The secondary structure elements are shown: β-strands are shown in cyan and helices in red. The α-helices and the β-strands are labeled.

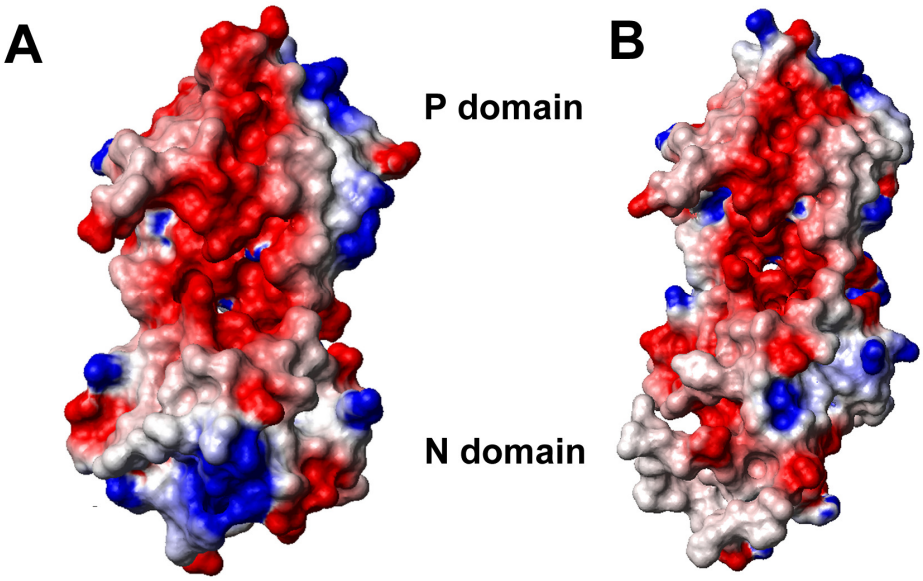

Figure S4. Electrostatic surfaces of ATP-domain of ATP7A (A) and ATP7B (B). Areas of negative, positive, and neutral electrostatic potential are depicted in red, blue, and white, respectively

Table S1. Acquisition parameters for NMR experiments performed on A-MNK and A-WLN.

Experiments [b]	Dimension of acquired data (nucleus)			Spectral width (ppm)			n[a]	Refs
	t_1	t_2	t_3	F_1	F_2	F_3		
[^{1}H-^{1}H]-NOESY	1024(^{1}H)	1024(^{1}H)		12	12		96	(1)
[^{1}H-^{1}H]-TOCSY	1024(^{1}H)	1024(^{1}H)		12	12		64	(1)
^{1}H-^{15}N-HSQC	128(^{15}N)	1024(^{1}H)		40	12		16	(2)
^{1}H-^{13}C-HSQC	256(^{13}C)	2048(^{1}H)		80	14		32	(3)
hncocacb [c]	112(^{13}C)	54(^{15}N)	1024(^{1}H)	75	36	12	40	(4)
hncacb [c]	112(^{13}C)	54(^{15}N)	1024(^{1}H)	75	36	12	32	(4)
hnco [c]	80(^{13}C)	56(^{15}N)	1024(^{1}H)	18	36	14	16	(5)
hncaco [c]	80(^{13}C)	56(^{15}N)	1024(^{1}H)	18	36	14	32	(6)
hnca [c]	88(^{13}C)	52(^{15}N)	1024(^{1}H)	33	36	14	16	(4)
hncoca [c]	88(^{13}C)	52(^{15}N)	1024(^{1}H)	33	36	14	32	(4)
^{13}C(H)CCH-TOCSY[d]	240(^{13}C)	80(^{13}C)	1024(^{1}H)	76	76	14	16	(7)
^{15}N-edited [^{1}H-^{1}H]-NOESY	240(^{1}H)	56(^{15}N)	2048(^{1}H)	15	41	15	16	(8);(3)
^{13}C-edited [^{1}H-^{1}H]-NOESY [d]	192(^{1}H)	64(^{13}C)	2048(^{1}H)	14	80	14	16	(8))
^{13}C-edited [1H-1H]-NOESY [e]	128(^{1}H)	56(^{13}C)	2048(^{1}H)	14	24	14	16	(8)
^{15}N R_1 [f]	256(^{15}N)	1024(^{1}H)		40	14		8	(9;10)
^{15}N R_2 [f]	256(^{15}N)	1024(^{1}H)		40	14		16	(9;10)
^{1}H-^{15}N NOE [f]	480(^{15}N)	1024(^{1}H)		40	14		32	(9;10)

[a] number of acquired scans. [b] All the experiments were acquired on a 500, 700 and 900 MHz spectrometer equipped with a triple resonance cryoprobe at 298 K. All the triple resonance (TXI 5-mm) probes used were equipped with Pulsed Field Gradients along the z-axis. [c] The experiments were acquired on ^{15}N ^{13}C samples. [d] The F2 carrier was at 39 ppm. [e] The F2 carrier was at 43 ppm. [f] The ^{15}N longitudinal relaxation rates, R_1, were measured using delays in the pulse sequence of 2.5, 35, 75, 125, 200, 370, 675, 1000, 2000, and 2500 ms for all samples. The ^{15}N transverse relaxation rates, R_2, were measured using the CPMG sequence. The relaxation delays used were 16.96, 33.92, 50.88, 84.81, 118.72, 152.64, 186.56, 220.48, 254.40, 271.36, 288.30 ms. The interscan delay (d1) was 3 s for R_1 and R_2 and 6 s for ^{15}N{^{1}H}-NOE respectively. All 3D and 2D spectra were processed using the standard Bruker software TOPSPIN and analyzed through CARA and XEASY programs (11-13)

Table S2 ^{1}H, ^{15}N and ^{13}C resonance assignments for A-MNK at 298 K, in 50 mM phosphate, 50 mM arginine, 50 mM glutamate at pH 7.0. Proton resonances were calibrated with respect to the signal of 2,2-dimethylsilapentane-5-sulfonic acid (DSS). Nitrogen chemical shifts were referenced indirectly to the ^{1}H standard using a conversion factor derived from the ratio of NMR frequencies. Carbon resonances were calibrated using the signal of dioxane at 69.4 ppm (298 K) as secondary reference.

aa	N (HN)	CO	Cα (Hα)	Cβ (Hβ)	Others
S-1	117.3 (8.20)	174.62	59.0 (4.19)	63.2 (3.93, 3.79)	
E-2		178.32			
A-3	121.7 (8.10)	179.48	54.3 (3.92)	18.0 (1.29)	
L-4	118.7 (7.81)	177.61	57.3 (3.92)	41.2 (1.44, 1.70)	Cδ1 25.0 (0.82) Cδ2 23.9 (0.84) Cγ 26.6 (1.55)
A-5	120.1 (7.89)	180.52	54.7 (3.90)	17.6 (1.32)	
K-6	117.7 (7.73)	178.73	58.3 (3.92)	31.6 (1.75)	Cδ 28.6 (1.51) Cε 41.7 (2.77) Cγ 24.5 (1.37, 1.29)
L-7	120.3 (7.69)	178.83	57.7 (3.90)	41.9 (1.34, 1.77)	Cδ1 25.2 (0.71) Cδ2 23.0 (0.67) Cγ 26.4 (1.63)
I-8	117.5 (8.20)	178.87	64.1 (3.61)	37.7 (1.74)	Cδ1 12.8 (0.68) Cγ1 28.8 (1.49, 1.09) Cγ2 17.0 (0.77)
S-9	115.5 (7.62)	174.40	60.2 (4.22)	63.0 (3.90, 3.86)	
L-10	120.9 (7.51)	177.15	55.0 (4.08)	41.4 (1.83, 1.55)	Cδ1 23.3 (0.78) Cδ2 21.9 (0.74) Cγ 25.3 (1.84)
Q-11	117.3 (7.30)	175.78	55.9 (4.01)	28.6 (1.99, 1.94)	Cγ 33.6 (2.32, 2.20) Nε2 110.7 (7.29, 6.70)
A-12	126.7 (8.58)	176.33	51.7 (4.39)	19.6 (1.40)	
T-13	109.6 (8.55)	174.81	61.8 (4.34)	69.1 (4.29)	Cγ2 21.6 (1.09)
E-14	121.2 (7.74)	173.72	54.2 (5.08)	33.3 (1.73, 1.88)	Cγ 35.5 (2.01)
A-15	121.9 (9.08)	174.56	49.8 (4.58)	23.3 (1.06)	
T-16	119.0 (9.00)	173.53	61.5 (4.79)	68.5 (4.26)	Cγ2 20.7 (0.97)
I-17	127.3 (8.90)	176.44	59.5 (4.79)	39.0 (1.59)	Cδ1 13.4 (0.67) Cγ1 27.5 (1.44, 0.95) Cγ2 16.9 (0.78)
V-18	123.4 (8.74)	174.37	58.9 (5.00)	34.0 (1.98)	Cγ1 22.4 (0.75) Cγ2 20.6 (0.59)
T-19	114.1 (7.92)	173.10	60.6 (4.49)	70.7 (3.97)	Cγ2 20.9 (1.01)
L-20	124.5 (8.63)	176.98	53.1 (5.08)	45.5 (1.57, 1.32)	Cδ1 25.2 (0.66) Cδ2 23.4 (0.50) Cγ 26.7 (1.34)
D-21	121.5 (8.56)	178.32	51.8 (4.64)	41.2 (2.54, 3.19)	
S-22	113.0 (8.23)	174.43	60.7 (4.06)	62.5 (3.77, 3.83)	
D-23	121.8 (8.27)	174.56	54.1 (4.70)	41.2 (2.48, 2.76)	
N-24	113.2 (8.12)	172.97	54.6 (4.04)	35.7 (2.95, 2.87)	Nδ2 112.0 (6.55, 7.51)
I-25	120.8 (8.11)	175.98	59.8 (3.86)	36.0 (1.94)	Cδ1 10.9 (0.67) Cγ1 27.4 (1.27, 1.06) Cγ2 16.9 (0.72)
L-26	126.9 (8.08)	176.56	56.8 (4.10)	41.7 (1.44)	Cδ1 25.6 (0.78) Cδ2 24.9 (0.65) Cγ 27.5 (1.43)
L-27	126.9 (9.05)	176.98	55.2 (4.31)	43.2 (1.56, 1.29)	Cδ1 24.8 (0.68) Cδ2 22.5 (0.69) Cγ 26.1 (1.50)
S-28	111.3 (7.51)	171.33	57.4 (4.47)	65.1 (3.74, 3.64)	
E-29	118.7 (8.15)	174.34	55.0 (4.99)	32.7 (1.74)	Cγ 35.5 (1.87)
E-30	122.5 (8.72)	173.44	54.8 (4.56)	33.2 (1.83, 1.72)	Cγ 35.3 (2.02)
Q-31	122.4 (8.53)	175.92	54.8 (4.98)	29.8 (1.90, 1.76)	Cγ 34.2 (2.21, 2.13) Nε2 111.9 (7.68, 6.89)
V-32	120.1 (9.07)	174.37	58.8 (4.56)	36.0 (2.01)	Cγ1 21.1 (0.74) Cγ2 18.9 (0.63)
D-33	123.0 (8.15)	177.89	54.8 (4.55)	41.2 (2.72, 2.46)	
V-34	124.1 (7.79)	176.44	65.1 (3.54)	31.7 (1.58)	Cγ1 22.1 (0.82) Cγ2 20.0 (0.83)
E-35	120.2 (8.91)	176.82	58.3 (3.99)	28.5 (1.91, 1.86)	Cγ 36.3 (2.19)
L-36	117.6 (7.96)	177.87	54.5 (4.18)	41.4 (1.74, 1.50)	Cδ1 25.0 (0.88) Cδ2 22.3 (0.73) Cγ 27.3 (1.50)
V-37	121.5 (7.29)	174.24	63.6 (3.60)	31.0 (1.93)	Cγ1 23.3 (0.77) Cγ2 22.4 (0.80)
Q-38	126.3 (9.10)	175.45	53.0 (4.47)	31.4 (1.92, 1.68)	Cγ 33.5 (2.28, 2.18) Nε2 113.1 (7.39, 6.68)

R-39	120.2 (8.51)	177.15	58.1 (3.37)	28.9 (1.51)	Cδ 43.3 (3.07, 3.02) Cγ 27.3 (1.53, 1.20)
G-40	113.9 (8.97)	173.72	44.7 (4.25, 3.37)		
D-41	121.7 (7.82)	174.24	55.4 (4.41)	41.2 (2.70, 2.32)	
I-42	121.1 (8.81)	174.72	58.5 (4.90)	35.2 (2.04)	Cδ1 10.7 (0.65) Cγ1 26.8 (1.43, 1.26) Cγ2 18.4 (0.58)
I-43	125.5 (9.12)	174.91	56.8 (5.23)	40.6 (1.59)	Cδ1 14.1 (0.65) Cγ1 27.7 (1.16) Cγ2 18.7 (0.76)
K-44	126.2 (8.27)	175.21	54.0 (5.06)	35.7 (1.36)	Cδ 29.5 (1.35) Cε 41.2 (2.61, 2.54) Cγ 24.2 (1.09)
V-45	129.2 (8.85)	174.50	60.9 (4.24)	33.2 (1.40)	Cγ1 21.0 (0.64) Cγ2 19.7 (-0.03)
V-46	119.3 (7.64)	176.67	59.8 (4.47)	29.2 (2.35)	Cγ1 21.4 (0.82) Cγ2 16.5 (0.66)
P-47		177.09	64.7 (3.70)	31.6 (2.17)	Cγ 30.5 (1.67)
G-48	115.2 (8.26)	174.17	45.2 (4.05, 3.69)		
G-49	107.8 (8.25)	171.38	44.4 (3.94, 3.36)		
K-50	119.3 (8.14)	177.64	54.1 (4.84)	33.6 (1.54)	Cδ 29.0 (1.55) Cε 41.6 (2.85) Cγ 24.6 (1.37, 1.26)
F-51	122.1 (8.29)	175.74	57.2 (4.27)	38.3 (3.13, 2.85)	
P-52		174.24	63.7 (4.30)	31.8 (2.17, 1.83)	Cδ 50.5 (3.72, 3.52) Cγ 26.9 (1.95, 1.89)
V-53	107.1 (7.00)	173.20	58.0 (4.44)	34.5 (2.62)	Cγ2 17.8 (0.44)
D-54	118.0 (7.65)	177.29	52.9 (5.09)	40.8 (2.85, 2.69)	
G-55	107.4 (8.23)	170.91	46.7 (4.86, 3.87)		
R-56	119.2 (8.21)	175.75	52.9 (5.08)	33.3 (1.49)	Cδ 42.7 (3.02) Cγ 26.9 (1.33, 1.28)
V-57	125.3 (9.15)	172.92	63.7 (3.77)	31.3 (2.16)	Cγ1 22.6 (0.53) Cγ2 22.7 (0.56)
I-58	125.1 (9.29)	174.43	60.9 (4.42)	39.2 (1.74)	Cδ1 13.4 (0.66) Cγ1 26.8 (1.19, 0.79) Cγ2 17.5 (0.77)
E-59	121.5 (7.49)	173.27	56.3 (4.30)	33.6 (1.87, 1.70)	Cγ 35.7 (2.14, 2.10)
G-60	114.1 (8.28)	169.88	44.0 (3.61, 4.62)		
H-61	112.4 (7.32)	174.82	54.4 (5.09)	32.4 (3.02, 2.84)	
S-62	114.3 (8.24)	173.25	55.8 (4.50)	64.7 (3.70, 3.25)	
M-63	121.9 (8.46)	174.98	53.2 (5.20)	32.8 (1.63, 2.04)	Cε 16.3 (1.85) Cγ 31.7 (2.50, 2.60)
V-64	118.8 (8.96)	174.31	60.9 (4.78)	35.5 (1.79)	Cγ1 23.8 (1.01)Cγ2 22.1 (0.97)
D-65	124.6 (9.01)	176.76	52.8 (4.79)	41.3 (2.97, 2.54)	
E-66	128.4 (9.14)	177.02	55.1 (4.24)	29.6 (1.84)	Cγ 37.0 (2.23)
S-67	119.2 (8.51)	176.95	62.6 (4.01)	70.7 (3.70, 3.42)	
L-68	122.7 (8.26)	175.86	56.9 (4.00)	41.1 (1.57, 1.34)	Cδ1 25.1 (0.78) Cδ2 23.3 (0.73) Cγ 26.8 (1.55)
I-69	114.6 (7.63)	176.44	61.7 (3.95)	38.9 (1.60)	Cδ1 12.7 (0.64) Cγ1 27.5 (1.21, 0.97) Cγ2 17.7 (0.71)
T-70	106.6 (8.15)	176.33	61.2 (4.24)	70.8 (4.31)	Cγ2 21.0 (1.03)
G-71	109.8 (7.63)	173.61	45.0 (4.08, 3.65)		
E-72	122.4 (7.50)	175.60	56.0 (4.04)	28.9 (1.78, 1.67)	Cγ 35.0 (2.18, 2.14)
A-73	128.9 (8.37)	178.77	54.1 (3.92)	19.3 (1.30)	
M-74	117.8 (8.15)	174.36	52.6 (4.73)	31.8 (2.61, 2.46)	Cγ 31.3 (1.93)
P-75		175.11	62.4 (4.50)	32.1 (1.91)	Cδ 50.3 (3.84, 3.55) Cγ 27.4 (1.91)
V-76	121.7 (9.12)	175.67	60.8 (4.10)	34.4 (1.81)	Cγ1 21.4 (0.84) Cγ2 20.1 (0.88)
A-77	129.8 (8.66)	177.41	52.9 (4.79)	19.0 (1.29)	
K-78	120.3 (8.85)	174.25	52.6 (4.67)	34.5 (1.69, 1.28)	Cδ 28.7 (1.63) Cε 41.3 (2.71) Cγ 25.5 (0.99, 1.27)
K-79	121.4 (8.37)	172.17	53.3 (4.99)	32.7 (1.63)	Cδ 29.1 (1.54) Cε 41.8 (2.80) Cγ 22.3 (1.16, 1.04)
P-80		176.77	64.5 (3.69)	30.5 (2.16)	Cδ 55.9 (4.13) Cγ 30.9 (1.86, 1.60)
G-81	114.0 (9.03)	175.14	44.6 (4.49, 3.44)		
S-82	118.0 (8.82)	172.75	60.0 (4.49)	64.6 (4.05, 3.67)	
T-83	116.8 (8.81)	173.81	61.1 (4.94)	69.5 (4.09)	Cγ2 21.5 (1.02)
V-84	115.4 (8.59)	175.10	58.0 (4.42)	34.0 (0.57)	Cγ1 21.2 (0.06) Cγ2 18.4 (0.24)
I-85	118.1 (8.88)	175.79	58.1 (4.64)	40.4 (1.64)	Cδ1 13.6 (0.60) Cγ1 26.6 (1.24) Cγ2 17.4 (0.70)
A-86	131.3 (9.69)	176.66	52.9 (3.74)	16.8 (1.09)	

G-87	106.6 (8.66)	173.34	44.8 (4.30, 3.51)		
S-88	111.5 (7.76)	172.68	57.8 (4.46)	64.5 (3.70)	
I-89	117.7 (7.94)	175.58	60.5 (4.47)	40.1 (1.82)	Cδ1 12.1 (0.74) Cγ1 27.1 (1.48) Cγ2 17.0 (0.71)
N-90	127.9 (9.11)	175.53	53.4 (4.79)	39.9 (2.64, 2.16)	Nδ2 112.9 (5.80, 7.70)
Q-91	123.2 (8.79)	176.90	57.2 (4.50)	28.2 (2.19)	Cγ 33.7 (1.85) Nε2 111.2 (6.71, 6.93)
N-92	112.8 (7.92)	174.63	55.6 (4.72)	39.5 (2.79, 2.68)	Nδ2 112.7 (7.54, 6.84)
G-93	111.5 (9.00)	171.85	44.0 (4.55, 3.97)		
S-94	117.4 (8.44)	173.98	58.8 (4.58)	63.6 (3.61)	
L-95	123.0 (8.46)	175.92	53.1 (5.08)	47.2 (1.47 , 1.17)	Cδ1 26.7 (0.68) Cδ2 22.8 (0.48) Cγ 25.5 (1.55)
L-96	118.6 (8.28)	176.76	53.2 (5.26)	43.2 (1.62 , 0.91)	Cδ1 25.4 (0.70) Cδ2 23.2 (0.70) Cγ 25.8 (0.68)
I-97	120.7 (9.51)	172.88	57.3 (4.90)	40.5 (1.58)	Cδ1 14.4 (0.61) Cγ1 26.7 (1.22) Cγ2 17.7 (0.57)
C-98	124.0 (9.28)	174.55	56.6 (4.91)	27.4 (2.58 , 2.49)	
A-99	131.3 (9.19)		52.9 (4.19)	18.9 (1.19)	
T-100	117.0 (9.52)	173.98	61.8 (4.24)	69.2 (4.29)	Cγ2 20.6 (1.06)
H-101	120.0 (7.36)	173.03	55.6 (4.72)	32.2 (2.61 , 2.90)	
V-102	116.8 (8.73)	176.05	59.0 (4.46)	34.4 (2.06)	Cγ1 21.0 (0.56) Cγ2 18.5 (0.67)
G-103	111.6 (8.64)	175.67	46.6 (3.82, 3.66)		
A-104	129.1 (8.84)	176.70	53.0 (4.11)	18.5 (1.32)	
D-105	114.7 (7.78)		54.1 (4.60)	41.7 (2.75 , 2.84)	
T-106	109.7 (7.34)		61.3 (4.09)	70.6 (4.51)	Cγ2 20.9 (1.18)
T-107		172.95	61.4 (4.90)	69.9 (4.05)	Cγ2 21.4 (1.08)
L-108	127.9 (7.87)	177.35	57.4 (3.85)	41.5 (1.47)	Cδ1 23.3 (0.78) Cδ2 25.1 (0.66) Cγ 26.8 (1.34)
S-109	114.5 (7.85)	176.58	61.5 (3.84)	63.3 (3.65)	
Q-110	120.3 (7.93)	178.12	58.3 (3.92)	28.4 (1.97 , 2.07)	Cγ 33.6 (2.34, 2.24) Nε2 112.8 (6.96, 6.63)
I-111	121.5 (7.98)	177.37	64.8 (3.63)	37.8 (1.86)	Cδ1 13.9 (0.68) Cγ1 28.7 (1.63, 0.95) Cγ2 17.1 (0.77)
V-112	119.9 (8.12)	176.83	66.2 (3.33)	31.5 (1.98)	Cγ1 22.9 (0.81) Cγ2 21.1 (0.75)
K-113	118.7 (7.54)	177.80	58.3 (4.00)	32.1 (1.74)	Cδ 28.7 (1.56) Cε 41.7 (2.86) Cγ 24.4 (1.37, 1.28)
L-114	119.1 (7.50)	179.23	57.0 (3.99)	41.8 (1.75 , 1.47)	Cδ1 25.0 (0.76) Cδ2 23.2 (0.71) Cγ 26.6 (1.63)
V-115	119.8 (7.99)	177.95	65.1 (3.63)	31.4 (2.00)	Cγ1 22.1 Hγ (0.84)
E-116	121.5 (8.48)	178.12	57.9 (3.89)	29.7 (1.92 , 2.02)	Cγ 36.3 (2.25)
E-117	118.0 (7.91)	176.18	56.9 (4.06)	29.5 (1.99 , 1.92)	Cγ 36.3 (2.36, 2.17)
A-118	123.2 (7.58)	176.57	52.1 (4.25)	18.6 (1.34)	
Q-119	123.3 (7.52)	180.56	57.3 (3.94)	29.9 (2.01 , 1.83)	Cγ 34.2 (2.27) Nε2 112.6 (7.41, 6.67)

Table S3 ^{1}H, ^{15}N and ^{13}C resonance assignments for A-WLN at 298 K, in 50 mM phosphate, 50 mM arginine, 50 mM glutamate at pH 7.0. Proton resonances were calibrated with respect to the signal of 2,2-dimethylsilapentane-5-sulfonic acid (DSS). Nitrogen chemical shifts were referenced indirectly to the ^{1}H standard using a conversion factor derived from the ratio of NMR frequencies. Carbon resonances were calibrated using the signal of dioxane at 69.4 ppm (298 K) as secondary reference.

aa	N (HN)	CO	Cα (Hα)	Cβ (Hβ)	Others
S-1	116.6 (8.22)	176.52	59.3 (4.26)	- (3.87)	
E-2		175.08	-	-	
A-3	121.9 (8.12)	178.25	54.9 (3.90)	17.9 (1.30)	
L-4	118.2 (7.94)	179.54	58.2 (4.00)	41.7 (1.55)	Cδ1 - (0.79) Cδ2 - (0.72) Cγ 26.6 (1.57)
A-5	120.9 (7.93)	178.10	54.7 (3.94)	17.3 (1.33)	
K-6	118.4 (7.81)	180.85	58.2 (3.95)	32.4 (1.76)	Cδ - (1.55) Cε - (2.85) Cγ 24.8 (1.40 , 1.31)
L-7	120.7 (7.79)	179.26	57.9 (3.90)	41.2 (1.67, 1.46)	Cδ1 - (0.70) Cδ2 - (0.69) Cγ - (1.60)
M-8	116.2 (8.40)	179.03	57.8 (4.04)	31.9 (2.60, 1.96)	Cε 16.6 (1.99) Cγ 31.9 (2.45)
S-9	114.0 (7.66)	178.98	60.0 (4.24)	62.6 (3.90)	
L-10	120.8 (7.49)	174.90	- (4.06)	41.7 (1.78)	Cδ1 24.8 (0.77) Cδ2 - (0.72) Cγ 26.1 (1.83)
Q-11	117.0 (7.43)	177.44	56.4 (4.00)	28.9 (1.98, 2.00)	Cγ 34.1 (2.35, 2.19) Nε2 110.4 (7.32, 6.72)
A-12			51.2 (4.43)	14.7 (2.25)	
T-13				.-	
E-14	121.2 (7.88)	174.13	54.5 (4.81)		Cγ - (1.90)
A-15	122.0 (8.92)	174.89	49.7 (4.59)	22.5 (1.15)	
T-16	119.3 (9.14)	173.39	61.4 (4.71)	68.1 (4.28)	Cγ2 22.2 (0.97)
V-17	128.8 (9.00)	176.80	61.5 (4.72)	33.0 (1.81)	Cγ1 - (0.76) Cγ2 - (0.71)
V-18	122.9 (8.91)	174.08	58.4 (5.03)	34.1 (1.92)	Cγ1 22.8 (0.75) Cγ2 20.2 (0.66)
T-19	118.5 (8.35)	173.27	61.2 (4.37)	69.9 (3.70)	Cγ2 20.4 (1.06)
L-20	127.2 (8.87)	177.73	52.9 (4.91)	43.6 (1.64, 1.21)	Cδ1 25.3 (0.67) Cδ2 23.2 (0.46) Cγ 26.7 (1.40)
G-21	109.9 (8.98)	174.47	43.0 (4.38, 3.65)		
E-22	122.2 (8.66)	176.71	57.6 (3.96)	29.0 (1.88, 1.83)	Cγ 35.9 (2.19)
D-23	118.4 (8.55)	174.87	54.0 (4.21)	38.9 (2.65)	
N-24	110.3 (8.02)	172.84	54.3 (3.98)	36.0 (2.88)	Nδ2 111.9 (7.55 , 6.59)
L-25	118.3 (7.11)	177.00	53.8 (4.21)	41.9 (1.26, 1.54)	Cδ1 - (0.74) Cδ2 23.0 (0.73) Cγ 26.3 (1.34)
I-26	123.5 (8.46)	176.94	62.7 (3.84)	37.6 (1.60)	Cδ1 13.2 (0.78) Cγ1 27.7 (1.60, 0.65) Cγ2 17.3 (0.73)
I-27	125.2 (8.81)	175.75	61.2 (4.20)	38.7 (1.69)	Cδ1 13.1 (0.65) Cγ1 26.4 (0.81, 1.12) Cγ2 17.2 (0.73)
R-28	119.4 (7.38)	172.66	55.4 (4.40)	33.1 (1.65)	Cδ 42.9 (3.03) Cγ 26.9 (1.48 , 1.38)
E-29	122.0 (8.31)	174.32	54.7 (4.86)	32.6 (1.67)	Cγ 35.5 (1.88)
E-30	122.5 (8.68)	173.62	54.3 (4.56)	33.5 (1.69, 1.81)	Cγ 35.3 (2.01)
Q-31	121.7 (8.56)	175.81	54.1 (4.85)	29.4 (1.94, 1.72)	Cγ 34.1 (2.27, 2.11) Nε2 111.3 (7.77, 6.92)
V-32	125.7 (9.18)	172.75	58.1 (4.64)	35.0 (1.82)	Cγ1 20.7 (0.86) Cγ2 20.6 (0.71)
P-33		177.87	62.4 (4.49)	31.9 (1.84)	
M-34	121.0 (7.82)	178.00	57.7 (4.03)	32.0 (1.86, 2.44)	Cγ - (1.93)
E-35	116.5 (9.30)	176.37	58.2 (4.01)	28.2 (1.94)	Cγ 35.8 (2.13 , 2.18)
L-36	118.2 (8.04)	177.16	53.7 (4.32)	41.8 (1.57)	Cδ1 24.7 (0.81) Cδ2 - (0.74) Cγ 27.1 (1.43)
V-37	124.3 (7.29)	174.35	63.3 (3.64)	30.8 (1.90)	Cγ1 23.1 (0.72) Cγ2 20.9 (0.69)
Q-38	125.9 (8.93)	175.86	53.0 (4.48)	31.5 (1.92, 1.72)	Cγ 33.3 (2.28, 2.15) Nε2 113.0 (7.37, 6.68)
R-39	120.4 (8.54)	176.92	58.3 (3.29)	29.3 (1.51)	Cδ 43.1 (3.04) Cγ 27.1 (1.50, 1.23)
G-40	114.3 (8.81)	173.56	44.5 (4.38, 3.25)		
D-41	121.8 (7.90)	174.12	55.2 (4.42)	40.6 (2.71, 2.32)	

I-42	121.2 (8.90)	175.11	58.7 (4.89)	35.8 (2.02)	Cδ1 11.7 (0.71) Cγ1 26.8 (1.53, 1.06) Cγ2 18.7 (0.58)
V-43	123.9 (9.26)	174.67	58.2 (5.27)	34.4 (1.80)	Cγ1 21.9 (0.75) Cγ2 20.6 (0.68)
K-44	126.5 (8.53)	175.25	54.1 (5.01)	35.3 (1.39, 1.28)	Cδ 29.4 (1.35) Cε 41.1 (2.54) Cγ 24.1 (1.13)
V-45	128.8 (8.69)	174.35	60.8 (4.29)	32.5 (1.52)	Cγ1 21.5 (0.74) Cγ2 19.6 (0.07)
V-46	119.3 (7.65)	174.52	59.4 (4.48)	28.9 (2.36)	Cγ1 21.2 (0.84) Cγ2 16.5 (0.68)
P-47		177.76	64.2 (4.02)	31.2 (2.12, 1.64)	Cδ 50.6 (3.60, 3.48) Cγ 27.9 (1.97, 1.41)
G-48	114.0 (8.36)	174.18	44.8 (4.02, 3.73)		
G-49	107.4 (8.18)	171.44	43.8 (3.97, 3.36)		
K-50	118.9 (8.24)	177.61	54.3 (4.66)	32.9 (1.52)	Cδ - (1.57) Cε - (2.85) Cγ 24.2 (1.37)
F-51	123.5 (8.69)	176.68	57.3 (4.31)	37.7 (3.11, 2.75)	
P-52		173.75	63.5 (4.72)	- (1.95)	Cδ 50.4 (3.48) Cγ 27.0 (2.44)
V-53	106.7 (6.97)	173.80	57.8 (4.47)	33.9 (2.50)	Cγ1 23.0 (0.78) Cγ2 17.6 (0.38)
D-54	118.2 (7.68)	177.55	52.4 (5.22)	40.5 (2.79, 2.74)	
G-55	108.2 (8.10)	170.80	46.5 (4.70, 4.07)		
K-56	118.3 (8.05)	176.37	52.9 (5.12)	35.4 (1.45, 1.52)	Cδ - (1.45) Cε - (2.82) Cγ 24.1 (1.24, 1.16)
V-57	123.3 (9.02)	176.27	63.5 (3.75)	30.8 (2.17)	Cγ1 21.3 Hγ (0.48)
L-58	131.3 (9.51)	175.67	55.2 (4.38)	43.8 (1.47, 1.31)	Cδ1 24.8 (0.77) Cδ2 - (0.71) Cγ - (1.52)
E-59	117.3 (7.31)	173.45	55.2 (4.33)	33.5 (1.74)	Cγ 35.7 (2.08)
G-60	113.5 (8.34)	169.54	43.6 (4.58, 3.60)		
N-61	112.5 (7.50)	174.15	51.7 (5.19)	41.7 (2.62, 2.51)	Nδ2 112.8 (7.55, 6.83)
T-62	114.4 (8.69)	173.21	57.7 (4.32)	69.4 (3.95)	Cγ2 17.8 (0.93)
M-63	119.1 (8.97)	172.69	53.7 (5.25)	36.6 (1.71)	Cγ 31.9 (2.50, 2.42)
A-64	120.4 (9.07)	175.82	49.4 (5.51)	23.9 (1.23)	
D-65	120.5 (9.22)	177.39	52.5 (4.83)	40.1 (2.91, 2.61)	
E-66	128.0 (9.20)	176.83	54.9 (4.15)	29.3 (2.28)	Cγ 36.4 (1.83)
S-67	119.3 (8.40)	176.92	62.3 (3.46)	69.3 (4.01, 3.69)	
L-68	122.4 (8.35)	175.66	56.3 (4.04)	40.9 (1.58, 1.32)	Cδ1 - (0.78) Cδ2 - (0.73) Cγ 26.7 (1.57)
I-69	114.8 (7.64)	176.16	61.9 (3.97)	38.9 (1.62)	Cδ1 13.1 (0.66) Cγ1 27.8 (1.22, 1.01) Cγ2 - (0.70)
T-70	106.2 (8.21)	176.27	60.9 (4.25)	70.6 (4.32)	Cγ2 - (1.03)
G-71	109.8 (7.51)	173.56	44.7 (4.09, 3.66)		
E-72	122.5 (7.51)	175.69	55.9 (4.02)	29.0 (1.76, 1.71)	Cγ 34.8 (2.17)
A-73	129.5 (8.42)	178.78	54.2 (3.95)	19.1 (1.31)	
M-74	118.0 (8.20)	174.38	52.3 (4.74)	31.7 (2.48, 1.95)	
P-75		175.69	62.6 (4.50)	32.2 (2.44)	Cδ 50.1 (3.85 , 3.58) Cγ 27.2 (1.97)
V-76	122.9 (8.93)	176.77	61.1 (4.16)	34.5 (1.86)	Cγ1 21.3 (0.92) Cγ2 20.4 (0.94)
T-77	124.4 (8.78)	173.30	64.6 (4.28)	68.9 (4.00)	Cγ2 21.5 (1.17)
K-78	123.3 (8.51)	173.21	52.5 (4.63)	34.2 (1.55, 1.19)	Cδ - Cε 43.0 (2.72 , 2.38) Cγ 24.2 (1.15)
K-79	122.7 (8.38)	172.60	53.1 (5.12)	32.5 (1.74)	Cδ 28.7 (1.54) Cε - (2.84) Cγ 29.6 (1.23)
P-80		177.24	64.8 (3.83)	29.9 (1.99, 1.71)	
G-81	113.8 (9.15)	175.07	44.5 (4.50, 3.45)		
S-82	118.0 (8.86)	172.43	59.4 (4.47)	63.5 (4.03, 3.70)	
T-83	117.5 (8.84)	173.91	61.1 (4.99)	69.9 (4.02)	Cγ2 21.7 (1.02)
V-84	116.7 (8.74)	174.76	57.7 (4.29)	33.5 (0.66)	Cγ1 20.7 (-0.04) Cγ2 18.3 (0.26)
I-85	118.0 (8.77)	175.95	58.0 (4.61)	40.6 (1.58)	Cδ1 13.4 (0.60) Cγ1 - (1.25, 0.85) Cγ2 - (0.72)
A-86	132.5 (9.91)	177.03	52.3 (3.70)	17.1 (1.11)	
G-87	106.7 (8.81)	173.62	44.5 (4.33, 3.45)		
S-88	111.9 (7.87)	172.37	57.8 (4.49)	66.0 (4.02, 3.72)	
I-89	118.6 (7.81)	176.16	60.0 (4.38)	39.4 (1.88)	Cδ1 - (0.74) Cγ1 27.1 (1.26) Cγ2 17.1 (0.75)
N-90	126.6 (9.01)	174.58	52.8 (4.79)	39.0 (2.62, 1.71)	Nδ2 112.9 (5.97, 7.69)
A-91	129.0 (8.48)	177.09	51.3 (4.55)	18.5 (1.30)	
H-92	113.6 (7.26)	174.85	58.1 (4.51)	32.5 (3.10, 3.04)	
G-93	110.3 (8.28)	172.17	43.9 (4.45, 3.79)		
S-94	118.4 (8.45)	175.08	58.1 (4.69)	63.1 (3.63, 3.68)	

V-95	116.8 (8.27)	174.32	58.1 (5.15)	35.9 (1.99)	Cγ1 21.9 (0.60) Cγ2 19.6 (0.82)
L-96	118.4 (8.07)	176.36	52.4 (5.22)	43.6 (1.59, 0.99)	Cδ1 25.3 (0.70) Cδ2 22.8 (0.70) Cγ - (1.65)
I-97	121.7 (9.58)	173.42	56.5 (4.79)	39.3 (1.73)	Cδ1 13.5 (0.59) Cγ1 26.4 (1.38, 1.14) Cγ2 17.1 (0.46)
K-98	125.9 (9.13)	175.46	- (4.87)	33.2 (1.73, 1.37)	Cδ 28.9 (1.53) Cε 41.3 (2.84) Cγ 24.1 (1.13)
A-99	129.7 (9.22)	178.52	52.6 (4.20)	18.3 (1.18)	
T-100	118.3 (9.83)	173.94	61.8 (4.22)	68.7 (3.92)	Cγ2 - (1.04)
H-101	119.7 (7.41)	173.00	55.8 (4.79)	32.2 (2.90, 2.58)	
V-102	117.3 (8.78)	176.13	58.8 (4.49)	34.6 (1.99)	Cγ1 21.2 (0.58) Cγ2 18.7 (0.63)
G-103	111.9 (8.75)	175.54	46.4 (3.80, 3.59)		
N-104	124.9 (8.93)	174.15	53.9 (4.37)	37.5 (2.79, 2.68)	Nδ2 112.5 (7.51, 6.85)
D-105	116.3 (7.89)	176.21	- (4.69)	41.6 (2.77)	
T-106	109.0 (7.12)	175.14	61.0 (4.05)	70.7 (4.61)	Cγ2 21.2 (1.12)
T-107		-	-	-	
L-108	(7.95)	176.81	57.1 (3.81)	-	Cδ1 25.4 (0.67) Cδ2 23.6 (0.84) Cγ 26.5 (1.32)
A-109	119.6 (7.58)	180.47	54.6 (3.76)	- (1.31)	
Q-110	116.7 (7.87)	176.22	57.9 (3.91)	28.6 (1.54, 1.46)	Cγ - (2.28 , 2.04) Nε2 112.7 (7.43, 6.70)
I-111	121.4 (7.93)	176.46	64.7 (3.68)	- (1.85)	Cδ1 13.7 (0.67) Cγ1 - (1.65) Cγ2 16.6 (0.91)
V-112	119.1 (8.04)	176.88	66.0 (3.35)	31.1 (2.00)	Cγ1 23.0 (0.81) Cγ2 21.3 (0.77)
K-113	118.5 (7.50)	177.79	- (3.98)	31.9 (1.76)	Cδ - (1.56) Cε - (2.86) Cγ 24.2 (1.39, 1.30)
L-114	118.7 (7.49)	179.33	- (4.01)	41.3 (1.48)	Cδ1 24.8 (0.80) Cδ2 - (0.72) Cγ 26.7 (0.77)
V-115	119.7 (7.93)	177.96	64.6 (3.67)	31.1 (2.02)	Cγ1 21.8 (0.86) Cγ2 - (0.78)
E-116	121.6 (8.42)	178.05	58.0 (3.94)	31.3 (1.97)	Cγ - (2.06)
E-117	118.0 (7.95)	176.20	56.7 (4.07)	29.4 (1.94)	Cγ 36.2 (2.36)
A-118	123.3 (7.61)	176.53	51.8 (4.25)	18.5 (1.33)	
Q-119	123.5 (7.56)	176.30	57.2 (3.96)	29.8 (1.84, 2.03)	Cγ 34.1 (2.28) Nε2 112.9 (7.00, 6.67)

Table S4. Statistical analysis of the energy minimized family of conformers of A-MNK.

	A-MNK (30 Conformers)
Total number of meaningful NOE upper distance constraints [b]	1992
Intra-residue	669
Inter-residue	
Sequential (|i-j| = 1)	496
Medium-range (|i-j| < 4)	289
Long-range (|i-j > 5)	538
Total meaningful dihedral angle restraints	125
Phi	64
Psi	61
One-bond ^{1}H-^{15}N dipolar couplings	102
RMS violations per meaningful distance constraint (Å):	
Intraresidue	0.0114±0.0018
Sequential	0.0038±0.0030
Medium range	0.0076±0.0028
Long range	0.0063±0.0011
RMS violations per meaningful dihedral angle constraints (°):	
Phi	5.0844±0.7955
Psi	7.1980±0.9699
Average number of violations per conformer:	
Intraresidue	11.200 ±2.587
Sequential	2.167±1.571
Medium range	3.567±1.498
Long range	4.300±1.320
Phi	7.000±2.030
Psi	2.270±0.629
NOE violations larger than 0.3 Å	0.000
Average RMSD to the mean (Å)	
Residue range 2-20,28-118 (backbone atoms)	0.83±0.04
Residue range 2-20,28-118 (all heavy atoms)	1.10±0.13
residual CYANA Target Function ($Å^2$)	1.15±0.13
Structural analysis[c]	
% of residues in most favorable regions	87.4
% of residues in allowed regions	10.1
% of residues in generously allowed regions	1.9
% of residues in disallowed regions	0.5
WHAT IF structure Z-scores[d]	
1st generation packing quality	-1.99
2st generation packing quality	-1.42
Ramachandran plot appearance	-1.52
χ_1/χ_2 rotamer normality	-2.46
Backbone conformation	-1.35
WHAT IF RMS Z-scores	
Bond lengths	0.62
Bond angles	1.15
Omega angle restraints	1.31
Side chain planarity	1.62
Improper dihedral distribution	0.95
Inside/Outside distribution	1.10

Global quality scores [e]	
Verify3D	-3.05
ProsaII (-ve)	-1.57
Procheck (phi-psi)	-1.49
Procheck (all)2	-2.60
MolProbity Clashscore	1.30

[a] Structure calculations were performed with the program CYANA 2.1 [Güntert, P. *et. al* J.Mol.Biol. 273, 1997]. A total of 500 random conformers were subjected to 10000 steps of a simulated annealing process. Each member of the family were subjected to refinement with the AMBER 10.0 package [Case D.A. *et al* University of California: San Francisco, CA, 2008]. Values of 50 kcal mol-1 $Ã^{-2}$ and 32 kcal $mol^{-1}rad^{-2}$ were used as force constants for the NOE and torsion angle restraints, respectively. The data are calculated over the 30 conformers of the family. The mean value and the standard deviation are given.

[b] Number of meaningful constraints for each class. Backbone dihedral angle constraints were derived from ^{15}N,$^{13}C'$,$^{13}C\alpha$,$^{13}C\beta$, and Ha chemical shifts, using TALOS and added as restrains in the structure calculations as well.

[c] As it results from the Ramachandran plot analysis performed with PROCHECK.

[d] A Z-score is defined as the deviation from the average value for this indicator observed in a database of high-resolution crystal structures, expressed in units of the standard deviation of this database-derived average. Typically, Z-scores below a value of -3 are considered poor, those below -4 are considered bad.

[e] Z-score as it results from PSVS (*14*)

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