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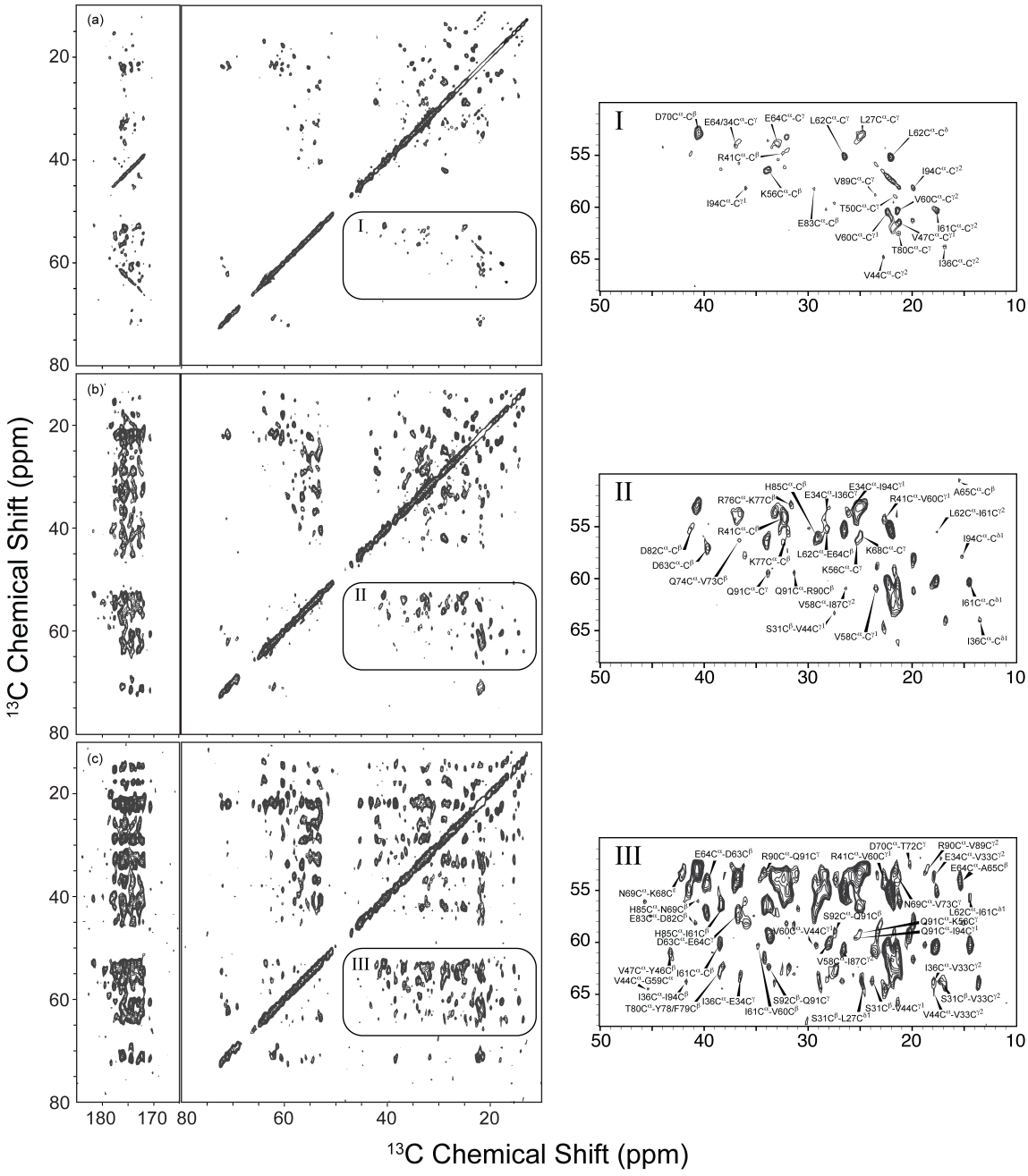
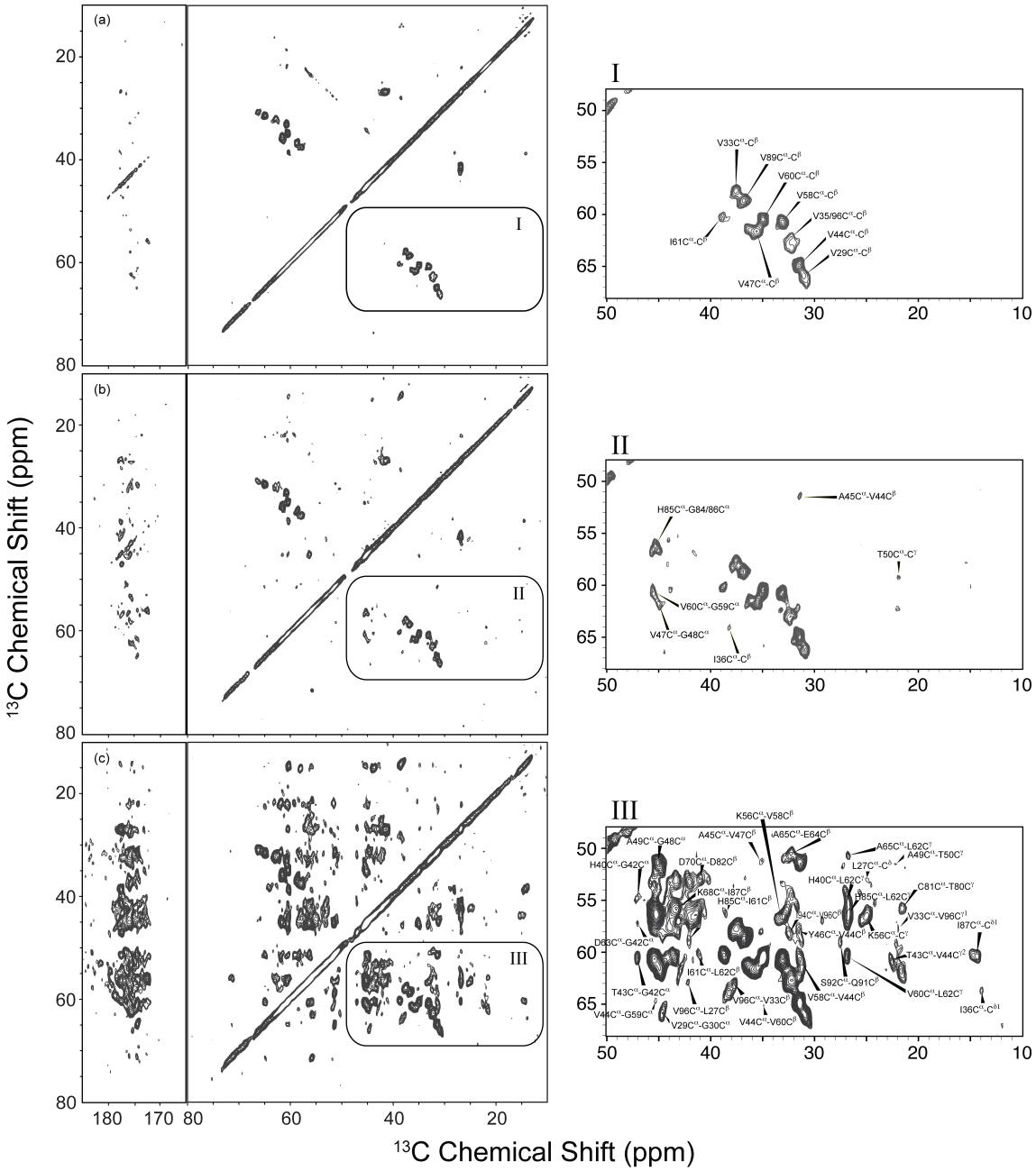
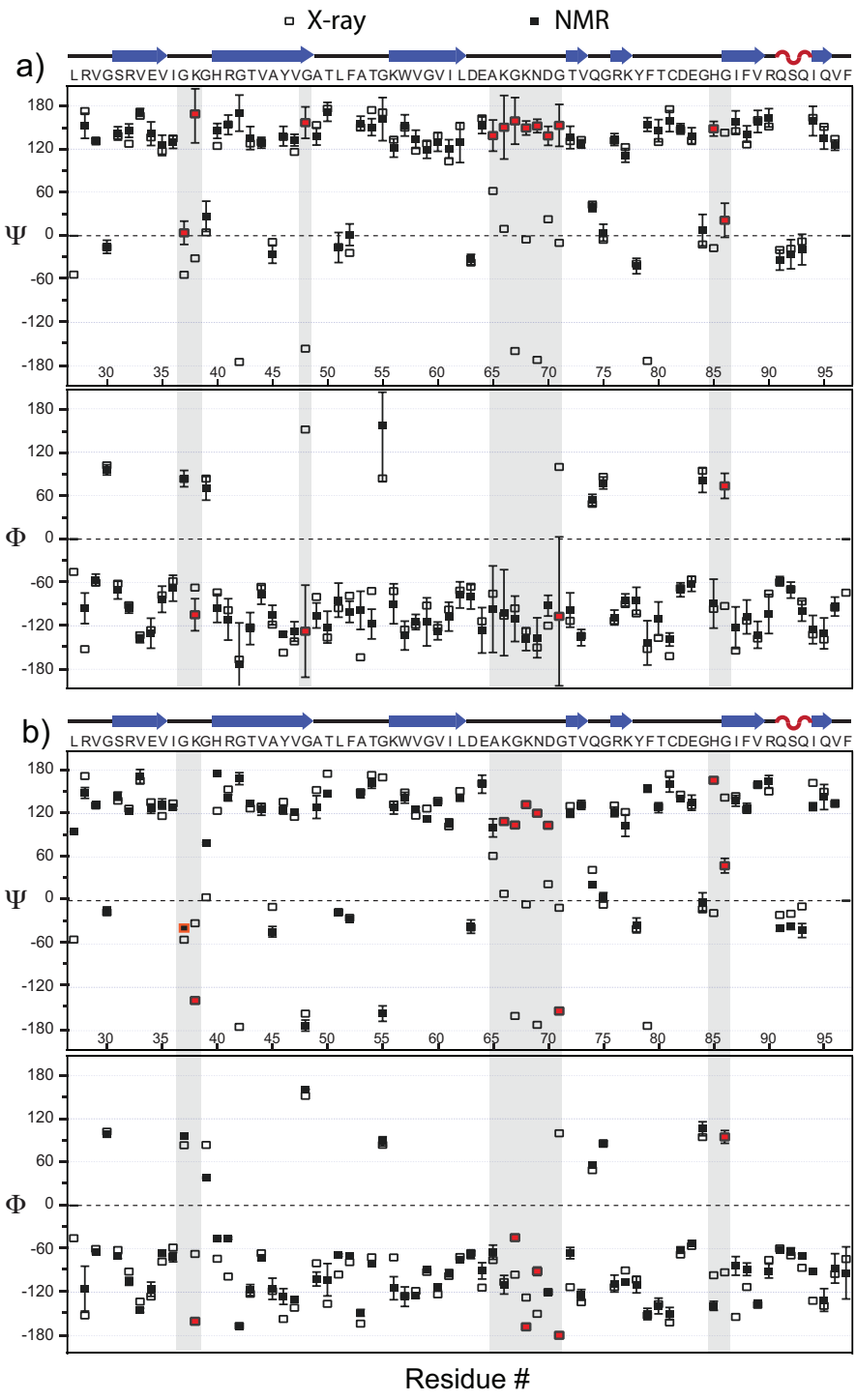


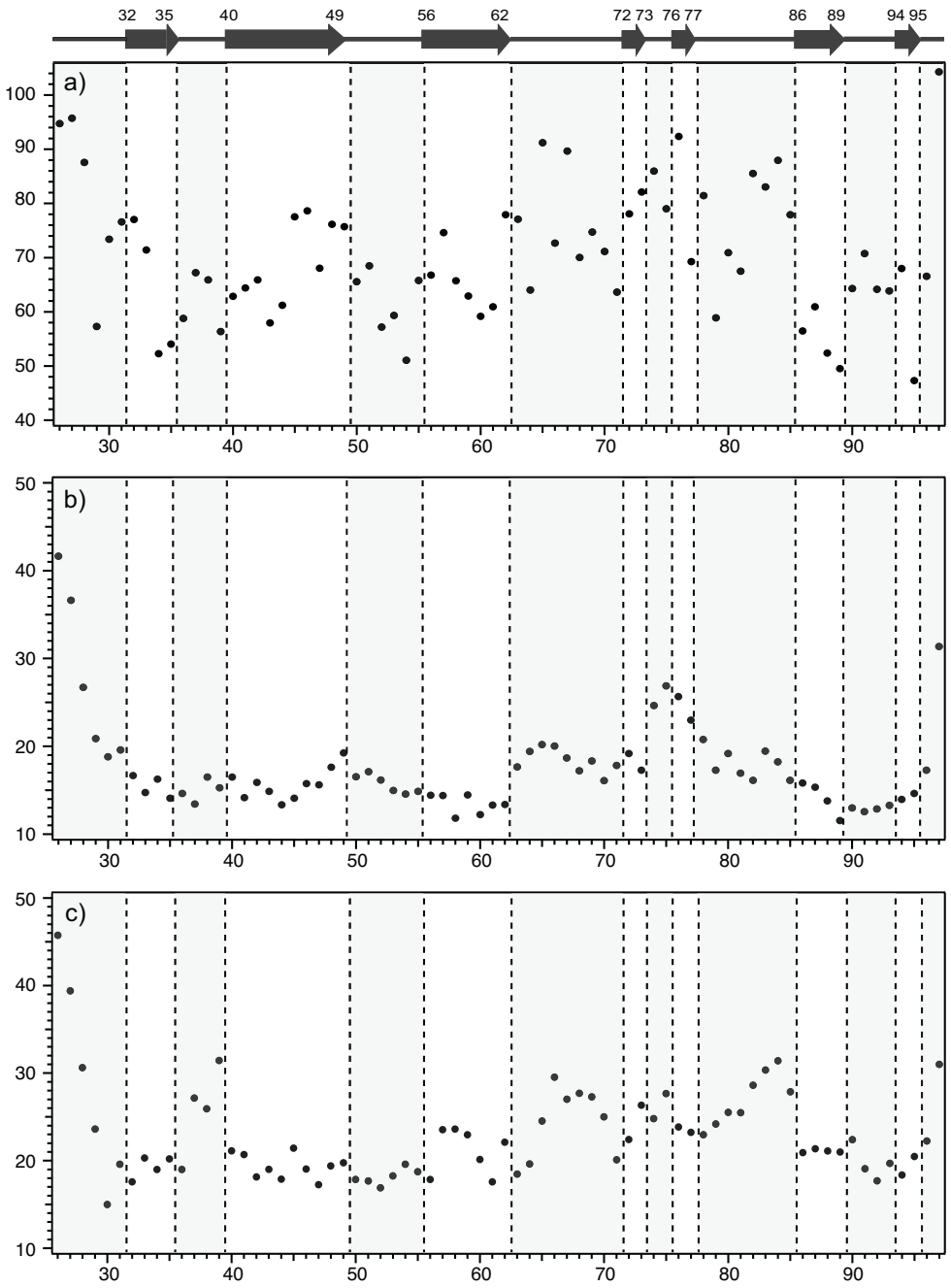
Supplementary Figure 1. Left: $R2_1^1$ spectra of 1,3-^{13}C-glycerol CAP-Gly acquired with the mixing times of a) 50 ms, b) 200 ms, c) 500 ms. Right: The expansions of each spectrum as indicated on the left, showing the resonance assignments. All spectra were acquired at 14.4 T and the MAS frequency of 10 kHz.



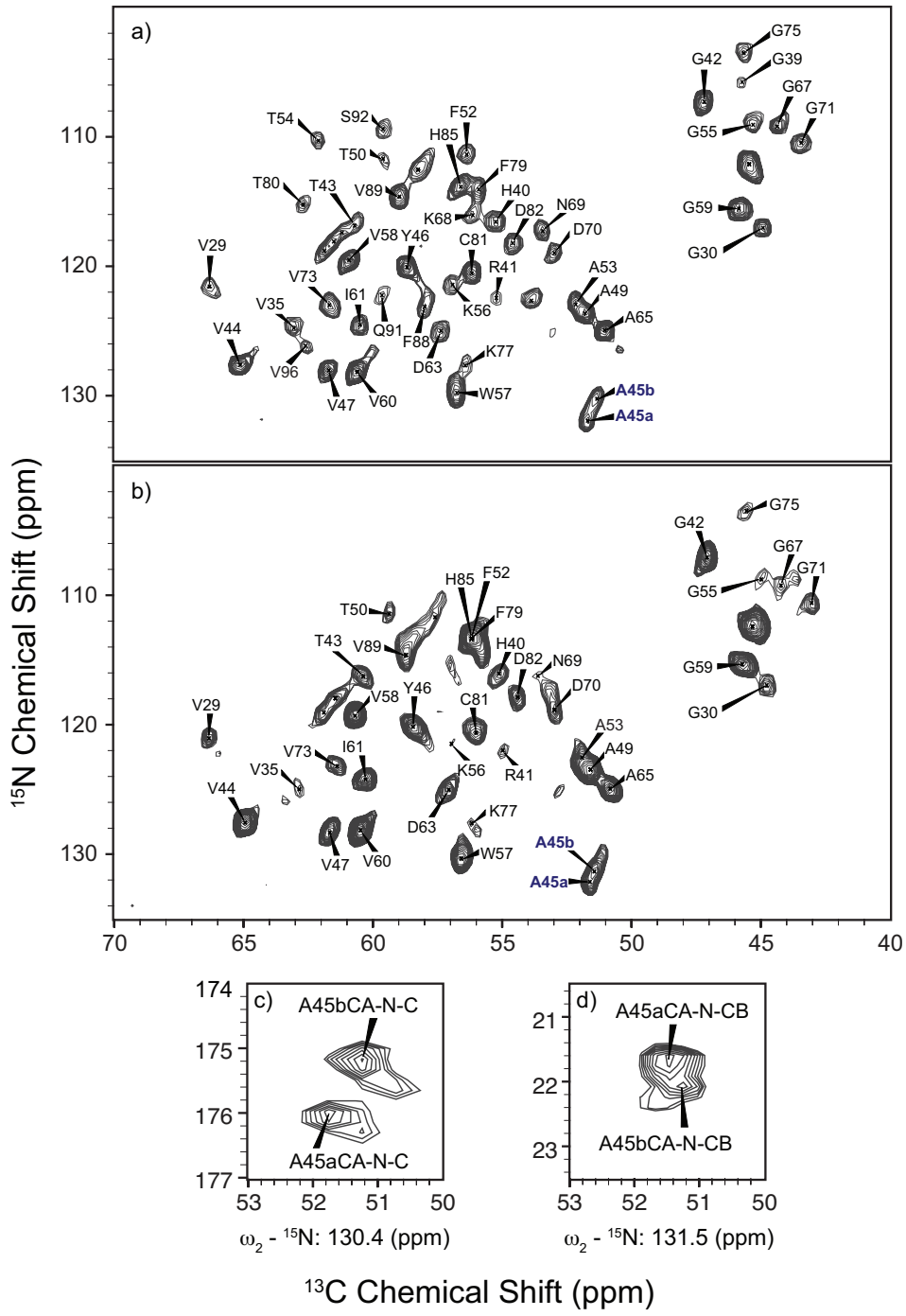
Supplementary Figure 2. Left: $R2_1^1$ spectra of 1,3-^{13}C-glycerol CAP-Gly acquired with the mixing times of a) 50 ms, b) 200 ms, c) 500 ms. Right: The expansions of each spectrum as indicated on the left, showing the resonance assignments. All spectra were acquired at 14.4 T and the MAS frequency of 10 kHz. To make the resonance assignments for the 200 ms spectra of 1,3-^{13}C-glycerol and 2-^{13}C-glycerol CAP-Gly, we first copied the intra-residue assignments from the 50 ms spectra of both samples onto the corresponding 200 ms spectra. To assign the peaks not present in 50 ms spectra, we then applied the following considerations. (i) All possible assignments should agree with the peak positions to within ± 0.30 ppm. (ii) The atoms with high ^{13}C label content exhibit high cross peak intensity, and these peaks were assigned in priority. All the assignments satisfying (i) and (ii) for each peak were subsequently treated as possible distance restraints. After finishing the assignment for the 200 ms spectra, we copied the peaks assigned in the spectrum of each sample to the 500 ms spectrum corresponding to the same sample. Then, the above assignment strategy was implemented again.



Supplementary Figure 3. (a) Backbone torsion angles Ψ and Φ derived by TALOS+, from solid-state chemical shifts of CAP-Gly, filled squares (SSNMR spectra were collected at 19.9 T) (b) Average values of the backbone torsion angles Ψ and Φ extracted from the 10 lowest-energy SSNMR structures (filled squares). The torsion angles of X-ray structure 2HQH are shown for comparison (open squares) in (a) and (b). Residues whose torsion angles are significantly different from the 2HQH X-ray structure values are shown as red filled squares. The Ψ angle for G37 in the refined SSNMR structure was reported as a violation during the structure refinement. This angle is shown as a black-filled orange square.



Supplementary Figure 4. The crystallographic B-factors for the amide nitrogen atoms plotted for each residue of CAP-Gly, as extracted from the following X-ray structures: 3E2U (a); 2HQH (b); 2HKQ (c).



Supplementary Figure 5. 14.1 T 2D and 3D MAS NMR spectra showing two conformers of A45: a) a 2D NCA spectrum of [2-^{13}C]-glycerol/U-^{15}N labeled CAP-Gly, acquired at -2 °C; b) 2D NCA spectrum of [2-^{13}C]-glycerol/U-^{15}N labeled CAP-Gly, acquired at -20 °C; c) a 2D plane of the 3D NCACX spectrum of U-^{13}C,^{15}N-labeled CAP-Gly, acquired at -20 °C, and showing the splitting of the peak corresponding to A45; d) a 2D plane of the 3D NCACB spectrum of U-^{13}C,^{15}N-labeled CAP-Gly, acquired at -20 °C, and showing the splitting of the peak corresponding to A45.

Supplementary Figure 6. The amino acid sequences for the various constructs of CAP-Gly used in the structural characterizations by solution NMR and X-ray diffraction. From top to bottom: 2COY (solution NMR, free CAP-Gly); 2HKQ (X-ray, complex of two CAP-Gly molecules with EB1 dimer); 1TXQ (X-ray, complex of two CAP-Gly molecules with EB1 dimer); 3E2U (X-ray, complex of four CAP-Gly molecules with four ZnCLIP molecules); 2HQH (X-ray, complex of four CAP-Gly molecules with four ZnCLIP molecules). The bottom sequence represents the CAP-Gly construct used in our study.

Table S1. Summary of the CAP-Gly Structure Calculation Procedure from MAS NMR-Derived Torsion Angle and Distance Restraints

Calculation Step	**1. Pass 1**	**2. Pass 2**	**3. Pass 3**	**4. Final Refinement**
Initial Structure	NA	Best output structure from (1)	Best output structure from (2)	Best output structure from (3)
Input Distance Restraints	Ambiguous Distance Restraints Table	Distance Restraints with high possibility by analyze.py	Restraints used in Pass 2 + New assigned restraints	Restraints with high possibility analyzed in Pass 3
Number of Input Distance Restraints	834	834	924	917
Type of NOE Potential	Soft	Hard	Hard	Hard
Input Dihedral Angels	All from TALOS+	All from TALOS+	All from TALOS+	All from TALOS+
Input H-bond Restraints	NA	NA	NA	H-bond Restraints from Pass 3
RMSD within ensemble (A)	2.896 (best 50 out of 500)	0.926 (best 50 out of 500)	1.016 (best 50 out of 500)	0.54 (best 10 out of 500)
RMSD w/X-ray (A)	2.45	2.02	1.82	1.70
CDIH Energy	46.86	11.05	9.34	3.60
CDIH RMSD	2.208	1.096	1.006	0.645

Table S2. Solid-state chemical shifts of free CAP-Gly prepared by controlled precipitation from PEG, recorded at the magnetic field of 19.9 T.

Residue	N	Cα	Cβ	C'	Cγ	Cγ1	Cγ2	Cδ	Cδ1	Cδ2	Cε
S19											
T20											
E21											
A22											
S23											
A24											
R25											
P26					27.77			50.89			
L27	122.69	55.47	43.51	175.81							
R28	122.39	54.01	31.22	175.67							
V29	121.61	66.35	31.16	177.62		22.95	21.62				
G30	117.22	45.23		174.61							
S31	119.06	61.84	63.83	173.27							
R32	125.45	53.17	29.53	176.27	28.03			41.27			
V33	112.33	58.11	37.80	174.33		23.18	18.23				
E34	118.46	53.82	33.60	176.09							
V35	124.99	63.16	32.06	176.18		22.18	21.14				
I36	132.31	64.23	38.38	178.30		29.15	17.12		14.11		
G37	117.58	45.66		174.61							
K38	116.97	55.80	34.73	177.59							
G39	105.71	45.82		173.86							
H40	116.73	55.26	32.21	175.51	136.07						
R41	122.83	55.23	32.60	177.59	28.66						
G42	107.51	47.25		171.07							
T43 a	116.95	60.65	71.40	174.66			22.96				
T43 b	117.30	60.99	72.14	174.68			22.47				
V44 a	127.88	65.22	31.67	176.01		24.24	23.16				
V44 b	127.56	65.01	31.71	174.73		24.19	23.13				
A45 a	131.89	51.69	22.01	176.28							
A45 b	130.60	51.56	21.97	176.38							
Y46	120.22	58.72	43.41	173.21	128.65				132.25	135.11	
V47	128.29	61.82	35.54	174.75		21.72	21.72				
G48	113.20	45.39		172.10							
A49	123.77	51.84	20.53	178.70							
T50	112.42	59.60	72.52	173.50			22.23				
L51	113.03	54.73	41.83	177.80	27.62						
F52	111.44	56.37	38.73	174.56	138.03				132.35	132.35	
A53	123.08	52.14	22.59	176.33							
T54	110.25	62.18	71.07	176.64			22.31				
G55	109.16	45.33		172.50							
K56	121.46	57.03	33.73	176.60	25.53						

W57	130.11	56.86	34.23	173.96	111.89				127.07		
V58	119.65	61.01	33.41	176.43		23.75	20.82				
G59	115.70	45.88		172.33							
V60	128.40	60.72	35.12	173.36		22.69	21.68				
I61	124.69	60.63	38.88	177.79		28.36	17.95		14.77		
L62	133.06	55.61	41.67	177.92	26.93				22.39		
D63	125.11	57.44	40.08	177.87	180.07						
E64	118.09	54.38	32.96	176.81	37.16			179.98			
A65	125.05	51.11	15.75	178.01							
K66	122.67	53.79	33.38	176.33	25.47						
G67	109.20	44.41		172.93							
K68	116.29	56.19	37.24	175.84	25.34			29.10			42.46
N69	117.30	53.43	40.82	173.42							
D70	119.00	53.13	41.00	178.13	182.03						
G71	110.99	43.47		174.38							
T72	118.00	61.61	69.35	174.69			20.22				
V73	123.29	61.71	36.43	176.84		22.20	21.52				
Q74	130.21	56.88	26.98	176.11	34.39			176.31			
G75	103.65	45.72		173.01							
R76	122.07	53.84	30.63	172.85							
K77	128.26	56.55	32.72	176.04	24.45			29.48			
Y78	127.69	60.53	39.42	175.16							
F79	114.21	56.04	38.79	172.50							
T80	115.34	62.82	71.34	172.78			21.53				
C81	120.70	56.21	32.33	172.33							
D82	118.44	54.71	41.31	176.72							
E83	121.23	58.43	29.94	177.91	36.05			183.77			
G84	112.19	45.51		174.55							
H85	113.93	56.66	29.48	174.19	132.53						
G86	112.30	45.66		174.58							
I87	118.46	60.61	43.82	172.70		27.21	19.17	14.80			
F88	122.47	58.04	42.31	175.78							
V89	114.84	59.08	37.05	175.69		23.66	19.04				
R90	117.65	53.42	31.66	179.07	25.51			42.25			
Q91	122.57	59.72	28.07	176.70	34.04			179.18			
S92	109.71	59.66	62.87	175.94							
Q93	117.98	56.76	31.42	176.09	36.65						
I94	112.97	58.43	43.63	173.85		25.02	20.18				
Q95	118.57	53.80	33.62	175.00							
V96	126.73	63.04	32.58	176.00		21.18					
F97	124.97	57.36	40.08								

Table S3. Solid-state chemical shifts of CAP-Gly in complex with EB1 prepared by controlled precipitation from PEG, recorded at the magnetic field of 19.9 T.

Residue	N	Cα	Cβ	C'	Cγ	Cγ1	Cγ2	Cδ	Cδ1	Cδ2	Cε
S19											
T20											
E21											
A22											
S23											
A24											
R25											
P26											
L27											
R28											
V29	121.54	65.67	31.57	177.00		22.66	20.74				
G30	115.29	45.31		174.26							
S31	117.56	61.27	63.97	173.35							
R32	124.91	52.97	28.81	176.68	25.09			40.86			
V33	113.02	58.13	37.68	174.32		23.39	17.95				
E34	118.16	54.01									
V35	124.44	62.79	32.49	176.76		21.97	21.13				
I36	131.85	63.97	38.47	177.55		28.94	17.23		13.91		
G37	118.07										
K38											
G39	104.59	46.39		173.87							
H40	116.89	55.58	31.58	175.63							
R41	123.03	55.47	32.46	178.16	29.01			43.96			
G42	107.30	47.34		170.84							
T43	117.53	61.04	71.95	174.39			22.18				
V44	128.15	65.46	31.61	174.48		24.56	22.72				
A45	130.49	51.32	22.42	176.62							
Y46	119.76	58.28	43.35	173.39							
V47	129.88	61.95	35.31	175.53		21.77	21.77				
G48	114.87	45.58		173.37							
A49	127.08	52.35	19.99	178.47							
T50	113.08	59.87	72.37	171.95			22.05				
L51	111.60	55.10	43.56	178.76	27.37				26.78	24.69	
F52	111.31	57.02	38.19	174.33							
A53	122.57	52.03	22.03	176.54							
T54	108.75	62.13	71.83	177.03			22.69				
G55	108.57	45.32		171.87							
K56	121.29	57.29	33.34	176.09	25.71			29.77			42.33
W57	130.68	57.05	34.04	174.11					127.18	127.18	127.18
V58	119.58	61.45	33.50	176.22		23.18	20.80				
G59	115.40	45.49		172.59							

V60	128.07	60.72	35.42	174.16		22.23	22.23				
I61	124.04	60.73	39.01	177.45		28.73	18.00		14.86		
L62	133.72	55.38	41.73	178.08	27.04				22.42	22.42	
D63	125.41	57.36	39.93	177.86	179.84						
E64	117.84	54.41	33.40	176.97	37.56			184.41			
A65	124.98	50.99	15.69	178.06							
K66	122.69	53.51	33.17	176.44	25.24			28.52			42.52
G67	109.39	44.06		173.13							
K68	117.18	56.83	36.84	175.98	25.46			29.00			41.94
N69	117.98	54.05	40.99	173.21	175.52						
D70	119.49	52.90	40.83	178.65	182.07						
G71	112.62	44.24		174.63							
T72	117.98	61.49	69.23	174.68			19.94				
V73	123.47	61.61	36.51	176.72		22.44	21.67				
Q74	130.01	56.83	26.68	176.09	34.11			180.72			
G75	103.65	45.56		173.55							
R76	122.12	54.56	31.41	172.58	28.62			42.90			
K77	128.35	56.35	32.23	175.81	24.49			29.40			41.62
Y78	128.97	61.21	39.65	175.72	130.83				133.78	133.78	117.50
F79	114.12	56.04	38.94	172.78	138.16				131.13	131.13	
T80	115.63	63.06	71.09	172.97			21.69				
C81	121.30	56.27	31.93	172.03							
D82	118.32	54.53	41.40	176.88	180.15						
E83	121.20	58.61	29.97	177.90	36.03						
G84	112.95	45.42		174.47							
H85	114.46	56.71	29.17	173.99	132.17					121.95	
G86	112.45	45.11		174.50							
I87	121.61	60.96	43.93	171.25		28.84	15.94		14.72		
F88	126.83	57.44	42.52	175.55							
V89	112.15	58.85	37.05	175.92		23.51	18.74				
R90	118.04	53.66	31.50	178.96	25.72			42.20			
Q91	122.03	59.52	27.96	176.93	33.79			179.55			
S92	110.84	59.73	62.74	175.86							
Q93	119.28	55.94	30.37	175.18	35.42						
I94	114.73	58.09	41.66	174.94		25.79	19.48				
Q95											
V96			32.96			21.57					
F97											

Table S4. Summary of the MAS NMR CAP-Gly structure quality validation.

Ramachandran Plot Summary by Procheck-NMR [1]	
Most favored regions	86.5%
Additionally allowed regions	12.3%
Generously allowed regions	1.2%
Disallowed regions	0.0%
Equivalent Resolution by Procheck-NMR [1]	
Percentage of residues in A, B, L	1.7 Å
H-bond energy standard deviation	1.9 Å
Chi-1 pooled standard deviation	1.3 Å
Chi-2 trans standard deviation	1.0 Å
Structure Z-scores by Whatif [2,3]	**Average Z-scores**
Coarse packing quality control	-2.44
Anomalous bond lengths	0.464
Planarity	Normal
Hand check (Improper dihedral RMS Z-score)	0.745
Ramachandran plot evaluation	-1.144
Anomalous bond angles	0.825
Equivalent Resolution by ResProx[4]	
Mean for the ensemble	2.411 Å
Standard Deviation	0.211 Å
Global Quality Scores by PSVS[5]	**Z-score**
Procheck[6] G-factor (phi/psi only)	-2.20
Procheck[6] G-factor (all dihedral angles)	-2.96
Verify3D[7]	-1.61
MolProbity[8] clash score	-2.77

Table S5. TALOS+ Determined Secondary Structure for Free CAP-Gly and CAP-Gly in Complex with EB1.*

	CAP-Gly				CAP-Gly in Complex with EB1			
	Possibility of Helix	Possibility of Strand	Possibility of Coil	Secondary Structure Context	Possibility of Helix	Possibility of Strand	Possibility of Coil	Secondary Structure Context
L27	0.333	0.333	0.333	L	NA	NA	NA	NA
R28	0.013	0.14	0.847	L	0.333	0.333	0.333	L
V29	0.029	0.058	0.913	L	0.054	0.08	0.866	L
G30	0.048	0.035	0.917	L	0.052	0.029	0.919	L
S31	0.013	0.077	0.91	L	0.019	0.076	0.906	L
R32	0	0.623	0.377	E	0	0.499	0.501	L
V33	0.005	0.893	0.102	E	0	0.755	0.245	E
E34	0.003	0.946	0.051	E	0.002	0.902	0.096	E
V35	0	0.852	0.148	E	0	0.814	0.186	E
I36	0.004	0.281	0.716	L	0.004	0.714	0.282	E
G37	0	0.113	0.887	L	NA	NA	NA	NA
K38	0.011	0.061	0.928	L	NA	NA	NA	NA
G39	0.016	0.034	0.95	L	0.02	0.043	0.937	L
H40	0.016	0.111	0.873	L	0.027	0.111	0.862	L
R41	0.001	0.44	0.56	L	0.004	0.312	0.684	L
G42	0.007	0.776	0.217	E	0.005	0.703	0.292	E
T43 a	0	0.854	0.146	E	0	0.855	0.145	E
V44 a	0	0.867	0.133	E	0	0.877	0.123	E
A45 a	0	0.924	0.076	E	0	0.937	0.063	E
Y46	0	0.951	0.049	E	0	0.96	0.04	E
V47	0	0.927	0.073	E	0	0.944	0.056	E
G48	0	0.841	0.159	E	0	0.808	0.192	E
A49	0	0.741	0.259	E	0	0.541	0.459	E
T50	0.009	0.305	0.686	L	0	0.407	0.593	L
L51	0.007	0.107	0.885	L	0.001	0.176	0.823	L
F52	0.021	0.198	0.782	L	0.051	0.17	0.779	L
A53	0	0.256	0.744	L	0.012	0.138	0.85	L
T54	0.001	0.187	0.812	L	0	0.291	0.709	L
G55	0	0.168	0.832	L	0	0.668	0.332	E
K56	0.003	0.548	0.45	E	0.003	0.847	0.15	E
W57	0	0.908	0.092	E	0	0.929	0.071	E

V58	0	0.949	0.051	E	0	0.939	0.061	E
G59	0	0.943	0.057	E	0	0.937	0.063	E
V60	0	0.957	0.043	E	0	0.956	0.044	E
I61	0	0.902	0.098	E	0	0.899	0.101	E
L62	0.051	0.267	0.682	L	0.034	0.283	0.684	L
D63	0.047	0.068	0.884	L	0.036	0.077	0.888	L
E64	0.03	0.053	0.918	L	0.026	0.057	0.917	L
A65	0.007	0.079	0.915	L	0.006	0.082	0.912	L
K66	0	0.342	0.658	L	0	0.236	0.764	L
G67	0	0.87	0.13	E	0	0.614	0.386	E
K68	0.004	0.758	0.239	E	0.003	0.576	0.421	E
N69	0.013	0.19	0.797	L	0.017	0.152	0.831	L
D70	0.008	0.065	0.926	L	0.01	0.057	0.933	L
G71	0	0.11	0.89	L	0	0.1	0.9	L
T72	0	0.535	0.465	E	0	0.479	0.521	L
V73	0	0.727	0.273	E	0	0.718	0.282	E
Q74	0.015	0.194	0.791	L	0.016	0.182	0.802	L
G75	0.004	0.068	0.928	L	0.003	0.065	0.931	L
R76	0	0.166	0.834	L	0	0.154	0.846	L
K77	0	0.42	0.58	L	0	0.388	0.612	L
Y78	0.006	0.492	0.501	L	0.008	0.542	0.45	E
F79	0.01	0.754	0.236	E	0.01	0.766	0.224	E
T80	0	0.925	0.075	E	0	0.911	0.089	E
C81	0	0.711	0.289	E	0	0.64	0.359	E
D82	0.01	0.196	0.794	L	0.01	0.179	0.811	L
E83	0.074	0.048	0.878	L	0.074	0.048	0.877	L
G84	0.046	0.016	0.938	L	0.045	0.015	0.939	L
H85	0.011	0.044	0.945	L	0.001	0.08	0.919	L
G86	0	0.242	0.758	L	0	0.557	0.443	E
I87	0	0.833	0.167	E	0.001	0.884	0.114	E
F88	0	0.965	0.035	E	0	0.972	0.028	E
V89	0	0.771	0.229	E	0	0.823	0.177	E
R90	0.027	0.157	0.816	L	0.031	0.168	0.801	L
Q91	0.431	0	0.569	L	0.55	0.005	0.445	H
S92	0.653	0.001	0.346	H	0.662	0	0.338	H
Q93	0.302	0.15	0.548	L	0.279	0.041	0.679	L

I94	0.025	0.751	0.224	E	0.036	0.307	0.657	L
Q95	0.003	0.933	0.064	E	NA	NA	NA	NA
V96	0	0.897	0.103	E				
F97	0.333	0.333	0.333	L				

* H- helix, E- strand, C- coil