SUPPORTING INFORMATION

Solid-State NMR – a Complementary Technique for Protein Framework Characterization

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Experimental

Sample preparation. Uniformly $^{13}C/^{15}N$-labelled RSL was produced in *E. coli* BL21 transformed with the pET25rsl plasmid and was purified by mannose-affinity chromatography.[1] D-mannose was removed from the sample by extensive dialysis against water. Protein concentrations were determined spectro-photometrically with ε_{280} = 44.46 $mM^{-1}cm^{-1}$ for the monomer. The ssNMR samples were prepared from 0.5 mL solutions containing 1 mM $^{13}C/^{15}N$-labelled RSL trimer, 20 mM potassium phosphate, 50 mM NaCl, 5 mM D-fructose, plus 0 or 10 mM $\mathbf{sclx_8}$. The pH was adjusted to 3.4 by adding HCl, which resulted in an instantaneous microcrystalline precipitate in the sample containing $\mathbf{sclx_8}$. The microcrystalline sample (9.0 mg) was packed in a Bruker 3.2 mm rotor by using a ultra-centrifugal device (courtesy of Giotto Biotech s.r.l.) with a Beckman Coulter Optima L80K floor preparative ultracentrifuge equipped with a SW32 swinging bucket at 15,000 rpm and 4 °C. The rotor was sealed with a silicon plug (courtesy of Bruker) and the microcrystals were further packed by MAS spinning at 14 kHz. The sample devoid of $\mathbf{sclx_8}$ was flash-frozen in liquid nitrogen and dried in a LIO5P lyophilizer. The freeze-dried sample (13.4 mg) was manually filled into a 3.2 mm rotor with the Bruker tools. This sample was rehydrated by multiple microlitre additions of milli-Q H_2O until the maximum resolution was achieved in the 1D $\{^1H\}^{13}C$ cross-polarization spectrum (Figure S3).

NMR spectroscopy. All of the ssNMR spectra were acquired on a Bruker Advance III spectrometer operating at 800 MHz (19 T, 201.2 MHz ^{13}C Larmor frequency) and equipped with a Bruker 3.2 mm Efree NCH probe-head. The spectra (Table S1) were recorded at 14 or 20 kHz MAS frequency and the sample temperature was kept at ~280 K. 2D ^{15}N-^{13}C NCA, NCO, ^{13}C-^{13}C DARR (mixing times 15, 50 and 100 ms) and 3D NCACX, NCOCX, CANCO, NCACB, N(CO)CACB were recorded at 14 kHz. 3D CANCOCA and 2D ^{13}C-^{13}C PDSD (mixing times 25, 50, 100, 200 and 400 ms) were recorded at 20 kHz. All the experiments were carried out using the pulse sequences reported in the literature.[2-3]

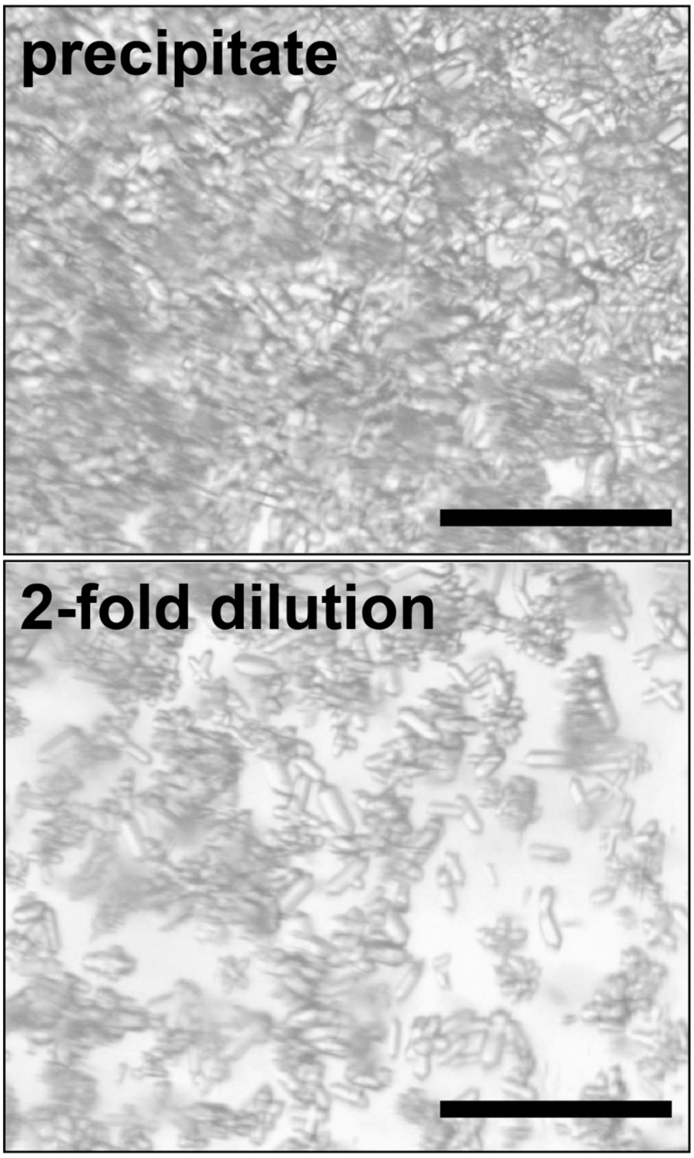

Figure S1. Microscope images of the microcrystalline precipitate formed from 1 mM $^{13}C/^{15}N$-labelled RSL, 10 mM **sclx$_8$**, 20 mM potassium phosphate, 50 mM NaCl, and 5 mM D-fructose upon adjusting the pH to 3.4. Images were acquired immediately after pH adjustment. The lower panel is the precipitate diluted 2-fold with buffer. Scale bars are 100 μm.

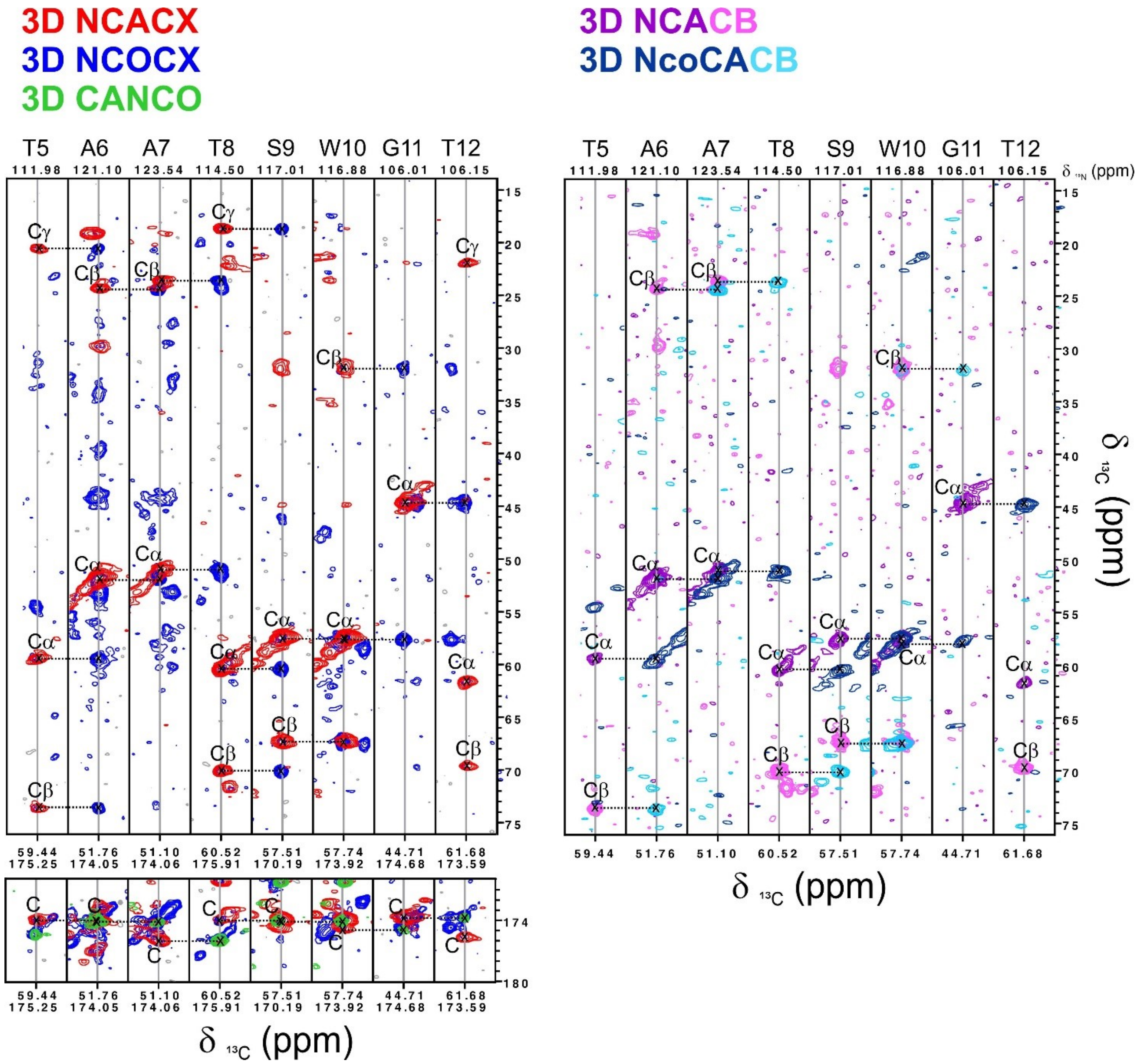

Figure S2. Representative resonance assignment of microcrystalline RSL – **sclx$_8$** as per sequential backbone walk for residues 5 to 12.

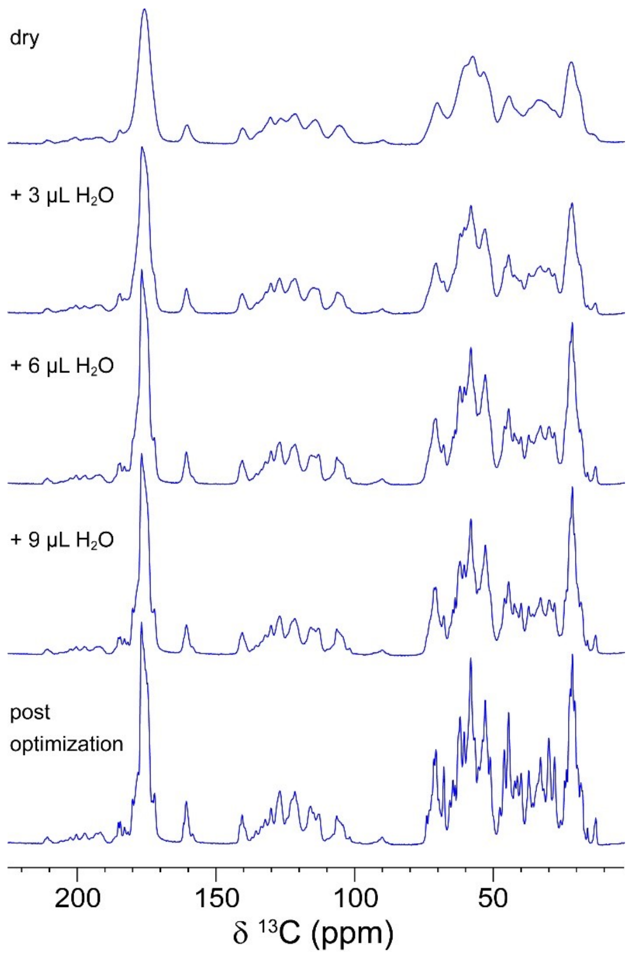

Figure S3. 1D {^1H}^{13}C CP ssNMR spectra of freeze-dried RSL with increasing volumes of Milli-Q H_2O.

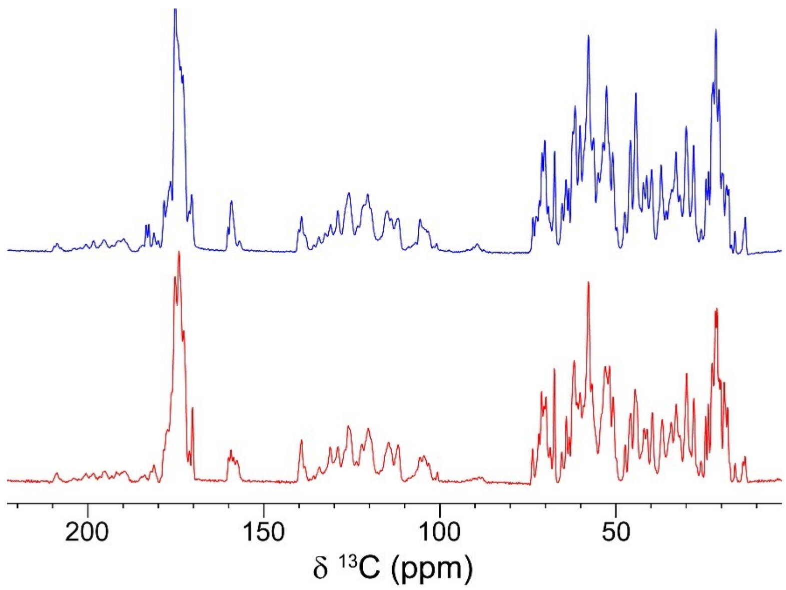

Figure S4. 1D {^{1}H}^{13}C CP ssNMR spectra of rehydrated freeze-dried RSL (blue) and microcrystalline RSL – $\mathbf{sclx_8}$ (red).

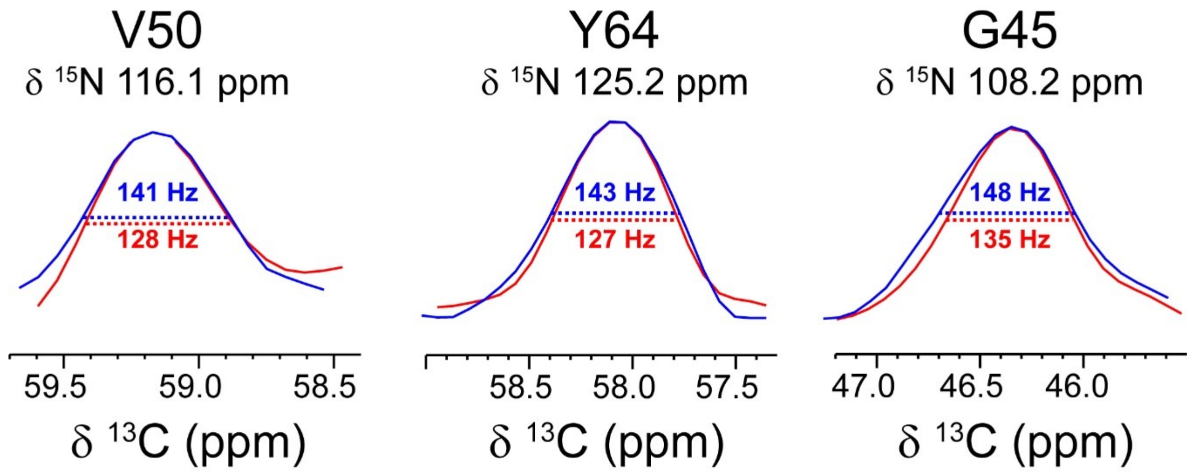

Figure S5. 1D projections along the ^{13}C dimension from representative signals in the 2D ^{15}N-^{13}C NCA spectra of rehydrated freeze-dried RSL (blue) and microcrystalline RSL – **sclx$_8$** (red).

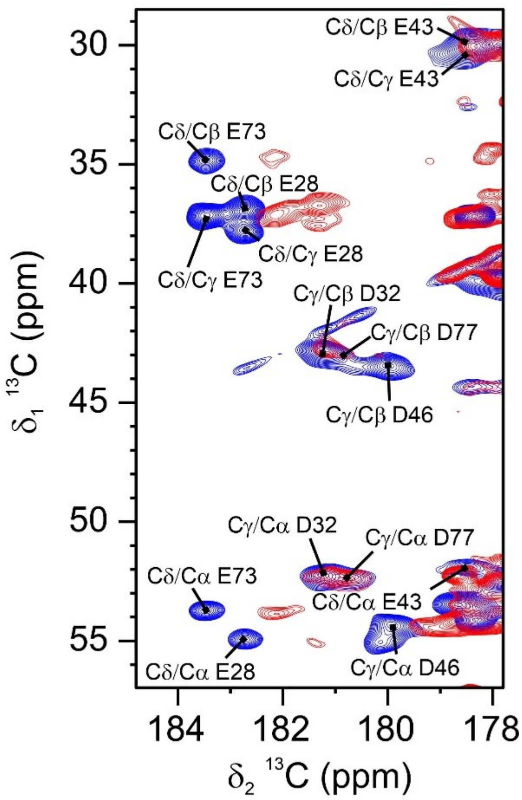

Figure S6. Superposed spectral region corresponding to Asp and Glu carboxylate side chains in 2D ^{13}C-^{13}C DARR spectra (50 ms mixing time) of microcrystalline RSL – **sclx$_8$** (red) and rehydrated freeze-dried RSL (blue).

Table S1. Parameters used for the acquisition of 2D and 3D ssNMR spectra.

Experiment	**2D NCA**	**2D NCO**	**2D DARR/PDSD**
TD points	3072 (f2) x 192 (f1)	3072 (f2) x 192 (f1)	4096(f2) x 1024 (f1)
Number of scans	64	64	40
D1 (sec)	1.5	1.5	1.5
Transfer 1	HN CP	HN CP	HC CP
field [kHz]	49(H)35(N)	49(H)35(N)	78(H)50(C)
shape if applicable	100-70 ramp on H	100-70 ramp on H	70-100 ramp on H
time [ms]	1.5	1.5	2
Transfer 2	NCA DCP	NCO DCP	Mixing (ms)
field [kHz]	80(H)35(N)21(C)	80(H)35(N)49(C)	
shape if applicable	tacn80 on C	tacn80 on C	
time [ms]	6	5	At 14 kHz: 15, 50, 100; at 20 kHz: 25, 50, 100, 200, 400

Experiment	**3D NCACX**	**3D NCOCX**	**3D CANCO**	**3D NCACB**
TD points	3072(f3) x 80(f2) x 64(f1)	3072(f3) x 40(f2) x 64(f1)	3072(f3) x 40(f2) x 64(f1)	2048(f3) x 80(f2) x 72(f1)
Number of scans	32	32	32	16
D1 (sec)	1.5	1.5	1.5	1.5
Transfer 1	HN CP	HN CP	HCA CP	HN CP
field [kHz]	49(H)35(N)	49(H)35(N)	78(H)50(C)	49(H)35(N)
shape if applicable	100-70 ramp on H	100-70 ramp on H	70-100 ramp on H	100-70 ramp on H
time [ms]	1.5-2	1.5-2	1	1.5-2
Transfer 2	NCA DCP	NCO DCP	CAN DCP	NCA DCP
field [kHz]	80(H)35(N)21(C)	80(H)35(N)49(C)	80(H)35(N)21(C)	80(H)35(N)21(C)
shape if applicable	tacn80 on C	tacn80 on C	tacn80 on C	tacn80 on C
time [ms]	6	5	6	6
Transfer 3	CACX		NCO DCP	CACB
field [kHz]			80(H)35(N)49(C)	7
shape if applicable			tacn80 on C	dream
time [ms]	15 and 50	50	5	
Transfer 4				
Time [ms]				

Experiment	**3D N(CO)CACB**	**3D CANCOCA (at 20 kHz)**
TD points	2048(f3) x 80(f2) x 72(f1)	2048(f3) x 64(f2) x 72(f1)
Number of scans	64	128 (NUS 31%)
D1 (sec)	1.5	1.5
Transfer 1	HN CP	HCA CP
field [kHz]	49(H)35(N)	90(H)50(C)
shape if applicable	100-70 ramp on H	70-100 ramp on H
time [ms]	1.5-2	1
Transfer 2	NCO DCP	CAN DCP
field [kHz]	80(H)35(N)49(C)	80(H)12(N)8(C)
shape if applicable	tacn80 on C	tacn80 on C
time [ms]	5	6
Transfer 3	CACB	NCO DCP
field [kHz]	7	80(H)12(N)8(C)
shape if applicable	dream	tacn80 on C
time [ms]		1
Transfer 4		COCA mixing
Time [ms]		20

Table S2. ^{15}N/^{13}C chemical shifts of microcrystalline RSL – **sclx$_8$**. The spectra were collected at 800 MHz (^{1}H Larmor frequency), at ∽280 K and MAS of 14 kHz.

Residue #	Residue Name	N	C⍰	C⍰	C	C⍰		C⍰	C⍰
1	SER								
2	SER								
3	VAL								
4	GLN								
5	THR	111.98	59.44	73.67	174.05	20.61			
6	ALA	121.10	51.76	24.44	174.06				
7	ALA	123.54	51.10	23.67	175.91				
8	THR	114.50	60.52	70.11	170.19	18.74			
9	SER	117.01	57.51	67.37	173.92				
10	TRP	116.88	57.74	32.02	174.68	111.58		126.94	
11	GLY	106.01	44.71		173.59				
12	THR	106.15	61.68	69.76	175.77	22.03			
13	VAL	124.50	67.96	29.89	175.31	22.94	20.67		
14	PRO	132.95	62.25	32.79	175.38	27.80		50.85	
15	SER	110.43	58.88	63.94	173.37				
16	ILE	118.82	59.96	42.50	177.01	27.96	18.03	13.87	
17	ARG	128.69	53.16	33.82	173.58	27.60		43.07	
18	VAL	121.25	60.76	34.27	176.14	20.24	19.2		
19	TYR	132.56	56.79	41.02	174.86				
20	THR	117.67	61.65	71.07	172.76	21.17			
21	ALA	131.31	50.70	19.39	176.28				
22	ASN	122.49	52.42	41.17	175.42				
23	ASN	126.11	54.13	37.17	175.30	178.58			
24	GLY	103.84	45.71		175.57				
25	LYS	123.02	54.85	36.71	175.33	35.52		33.08	44.12
26	ILE	124.23	59.31	39.44	175.47	27.91	20.29	16.06	
27	THR	115.19	59.89	71.79	173.32	22.39			
28	GLU	121.65	54.43	37.14	175.05				
29	ARG	127.76	53.31	34.29	175.51	27.67		43.81	
30	CYS	119.26	56.25	30.33	171.21				
31	TRP	121.61	56.36	29.89	175.33	113.68		126.12	
32	ASP	126.71	52.31	43.06	174.58				
33	GLY	102.44	45.79		174.28				
34	LYS								
35	GLY	107.29	44.15		173.18				
36	TRP	122.73	57.23	30.13	177.43	113.81		126.33	
37	TYR	119.37	56.00	41.13	174.44	120.69		130.83	
38	THR	121.57	63.87	69.23	175.19	22.81			
39	GLY	116.22	44.34		174.27				
40	ALA	121.81	52.99	19.17	178.14				
41	PHE	119.05	60.15	41.69	174.83				
42	ASN	128.67	52.77	39.62	173.84	177.20			
43	GLU	121.18	51.63	29.88	172.84	30.28		176.99	
44	PRO	132.84	62.31	32.72	176.92	20.44		49.78	
45	GLY	108.39	46.19		170.31				
46	ASP	115.98	53.02	36.27	175.20				
47	ASN	118.32	52.74	39.68	172.76				
48	VAL	125.92	59.08	36.61	172.76	23.82	17.8		

49	SER	121.76	58.45	67.47	172.10			
50	VAL	116.30	59.02	35.27	172.53	23.68	21.35	
51	THR	118.85	60.98	69.87	170.10	18.26		
52	SER	118.86	57.66	67.45	170.15			
53	TRP	118.76	57.63	31.72	172.36	112.60		126.04
54	LEU	121.99	53.51	45.31	178.35			
55	VAL	123.03	62.44	32.38	177.55	21.35	21.15	
56	GLY	119.67	47.32		174.67			
57	SER	122.89	58.13	64.06	173.65			
58	ALA	127.50	51.74	20.11	175.33			
59	ILE	124.45	56.48	39.76	172.35	26.89	18.32	13.21
60	HIS	123.42	53.22	29.86	172.76			
61	ILE	119.05	60.25	41.69	176.66	28.01	19.07	13.31
62	ARG	129.88	53.22	32.94	172.95	27.87		43.67
63	VAL	123.70	60.23	34.14	174.21	21.43	21.26	
64	TYR	125.37	57.94	36.86	175.00	119.51		
65	ALA	132.84	50.51	22.50	176.57			
66	SER	118.67	58.01	65.33	173.42			
67	THR	121.86	62.11	71.25	174.85	21.07		
68	GLY	117.34	47.65		175.50			
69	THR	116.95	61.28	68.57	174.38	21.67		
70	THR	119.26	62.35	70.52	173.95	21.59		
71	THR	126.68	61.68	70.54	172.74	22.71		
72	THR	125.22	61.92	70.99	171.22	21.76		
73	GLU	129.06	53.74	34.75	173.32	36.97		171.2
74	TRP	129.28	55.57	33.22	176.35	111.95		125.2
75	CYS	120.77	56.97	29.81	173.58			
76	TRP	124.61	56.67	28.85	175.54			
77	ASP	126.52	52.50	42.70	174.50			
78	GLY	102.27	45.96		174.17			
79	ASN	118.72	52.50	39.70	173.73	177.68		
80	GLY	107.47	44.10		172.63			
81	TRP	121.91	57.51	29.88	178.38			
82	THR	116.76	59.99	72.05	173.24	21.48		
83	LYS	125.53	57.48	36.75	178.21			
84	GLY	116.70	44.52		174.65			
85	ALA	121.00	52.25	19.22	177.18			
86	TYR	119.90	61.20	39.90	174.90			
87	THR	114.73	58.55	72.39	173.24	22.55		
88	ALA	123.12	54.06	20.15	176.39			
89	THR							
90	ASN							

Table S3. $^{15}N/^{13}C$ chemical shifts of rehydrated freeze-dried RSL. The spectra were collected at 800 MHz (^{1}H Larmor frequency), at ∽280 K and MAS of 14 kHz.

Residue #	Residue Name	N	Cα	Cβ	C	Cγ		Cδ	Cε
1	SER								
2	SER								
3	VAL								
4	GLN								
5	THR	112.84	59.43	73.66	174.01	20.78			
6	ALA	121.66	51.91	24.51	174.65				
7	ALA	124.33	51.29	23.79	175.66				
8	THR	114.22	60.51	70.34	170.8	18.74			
9	SER	116.52	57.44	67.45	174.38				
10	TRP	117.22	57.82	32.02	174.93	111.58		126.94	
11	GLY	106.35	44.87		173.96				
12	THR	106.24	61.68	70.14	175.71	22.27			
13	VAL	124.41	67.99	30.12	175.55	23.28	20.79		
14	PRO	132.74	62.52	32.91	175.24	28.02		51.02	
15	SER	109.93	58.91	64.17	173.55				
16	ILE	118.97	60.02	42.49	177.81	28.38	18.43	13.79	
17	ARG	128.13	53.91	34.35	174.31	27.68		44.34	
18	VAL	120.84	60.94	34.74	176.48	20.7	19.02		
19	TYR	133.06	57.13	41.2	175.21				
20	THR	116.94	61.83	71.21	173.1	21.25			
21	ALA	131.77	50.96	19.57	176.57				
22	ASN	122.15	52.83	41.35	175.42				
23	ASN	125.88	54.31	37.17	175.69	178.54			
24	GLY	104.42	45.92		175.53				
25	LYS	123.25	55.3	35.75	174.37	29.95		34.78	44.58
26	ILE	123.91	59.54	39.8	175.69	28.1	20.52	16.25	
27	THR	115.03	60.22	71.84	173.32	22.39			
28	GLU	122.44	55.05	37.09	175.76	37.94		182.96	
29	ARG	127.32	52.81	33.66	175.42	25.84		42.09	
30	CYS	119.16	56.33	30.22	171.33				
31	TRP	121.56	56.34	29.89	175.55	111.73		126.12	
32	ASP	126.73	52.39	43.06	174.65	181.46			
33	GLY	102.36	45.74		174.78				
34	LYS								
35	GLY	107.14	44.57		173.27				
36	TRP	123.13	58.06	29.98	177.92	113.81		126.69	
37	TYR	119.05	56.75	40.58	174.67	120.69		130.83	
38	THR	122.16	64.53	69.45	175.38	22.99			
39	GLY	115.81	44.56		174.36				
40	ALA	120.53	53.65	20.07	178.9				
41	PHE	117.81	60.73	41.69	175.11				
42	ASN	128.79	52.99	39.77	174.01	175.58			
43	GLU	121.42	52.12	29.88	173.1	30.28		178.72	
44	PRO	132.6	62.56	33.01	177.07	28.05		49.72	
45	GLY	108.16	46.32		170.47				
46	ASP	117.9	54.6	43.63	175.88	180.2			
47	ASN	116.88	52.63	43.74	172.91				
48	VAL	125.58	59.27	36.64	172.99	23.97	17.93		

49	SER	122.36	58.54	67.55	172.6			
50	VAL	116.46	59.3	35.8	172.65	23.68	21.94	
51	THR	118.28	61.57	70.09	170.53	18.01		
52	SER	118.29	57.87	67.45	170.78			
53	TRP	118.37	58.02	31.72	172.47	112.6		126.04
54	LEU	123.14	53.09	44.46	178.64			
55	VAL	123.03	62.57	32.38	177.58	22.73	21.29	
56	GLY	119.89	47.71		175.09			
57	SER	122.98	58.27	64.18	173.73			
58	ALA	127.27	51.91	20.55	175.46			
59	ILE	123.87	56.59	40	172.53	27.08	18.45	13.3
60	HIS	123.26	53.11	30.12	173.09			
61	ILE	119.21	60.31	41.41	177.23	28.29	19.38	13.2
62	ARG	129.9	53.45	32.97	173.15	27.91		44.04
63	VAL	123.31	60.34	34.16	174.5	21.77	21.42	
64	TYR	125.37	58	36.84	175.49	119.51		
65	ALA	132.61	50.74	22.56	176.94			
66	SER	118.54	58.28	65.34	173.75			
67	THR	121.61	62.47	71.25	174.85	21.21		
68	GLY	118.25	47.55		175.36			
69	THR	115.96	61.2	68.86	174.86	22.04		
70	THR	119.92	62.59	70.52	174.16	21.83		
71	THR	127.33	61.73	70.64	173.14	22.82		
72	THR	125.56	62.13	70.95	171.68	21.63		
73	GLU	128.7	53.88	34.96	173.57	37.25		183.7
74	TRP	129.5	55.51	33.62	176.65	111.95		125.2
75	CYS	120.59	57.47	29.55	173.68			
76	TRP	124.62	56.86	28.41	175.54			
77	ASP	126.4	52.56	43.53	174.83	180.91		
78	GLY	101.99	46.03		174.52			
79	ASN	118.35	52.7	40.06	173.95	177.23		
80	GLY	107.09	44.61		174.19			
81	TRP	123.82	58.16	29.88	178.54			
82	THR	116.49	60.29	72.55	174.45	21.64		
83	LYS	124.97	56.97	36.75	178.43			
84	GLY	114.94	44.24		174.98			
85	ALA	120.73	52.91	19.86	177.4			
86	TYR	119.05	61.77	40.14	175.35			
87	THR	115.12	58.76	72.86	173.28	22.76		
88	ALA	124.28	53.11	20.92	176.67			
89	THR							
90	ASN							

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