Supporting Information

Protein–Protein Interfaces Probed by Methyl Labeling and Proton-Detected Solid-State NMR Spectroscopy

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Experimental Section

Sample preparation:

Three different protein samples were prepared: 1.) Isoleucine-Cδ1-methyl group-(^{1}Hδ1, ^{13}Cδ1)-u-^{15}N-labeled gp17.1; 2.) Isoleucine-(^{1}Hδ1, ^{13}Cδ1, ^{13}Cγ1, ^{13}Cα, ^{13}CO)-u-^{15}N-labeled gp17.1; and 3.) A mixed sample containing 50% Isoleucine-Cδ1-methyl group-(^{1}Hδ1, ^{13}Cδ1)-labeled gp17.1 and 50% u-^{15}N-labeled gp17.1. Protons at labile sites were 100% back-exchanged in all samples. Generally, gp17.1 protein was expressed and purified as described in Zinke et al. 2017.[1], with the exception that $^{12}C_6$,D_7-glucose was used instead of $^{13}C_6$,D_7-glucose.

For either isoleucine labeling scheme, 60 mg/L of the precursor molecules *2-Ketobutyric acid-4-^{13}C-3,3-d_2* (Sigma-Aldrich) or *2-Ketobutyric acid-$^{13}C_4$-3,3-d_2* ("assignment precursor", Sigma-Aldrich) (Supporting Information Figure S3) were added to the bacterial cultures 1 h prior to induction. Protein expression after IPTG induction was conducted for 3h. After protein purification, gp17.1 was stored at room temperature for 3 weeks to polymerize. Polymers were pelleted by ultracentrifugation at 500.000 x g for at least 10 h. From 2 L of bacterial culture, protein pellets of ~140 mg could be isolated after centrifugation for each sample.

For the mixed sample, isoleucine-Cδ1-methyl group-(^{1}Hδ1, ^{13}Cδ1)-labeled gp17.1 was expressed from 0.5 L of bacterial culture as described above but with $^{14}ND_4Cl$ instead of $^{15}ND_4Cl$ as the sole nitrogen source. ^{15}N-labeled gp17.1 was expressed from 0.5 L bacterial culture containing $^{12}C_6$,D_7-glucose and $^{15}ND_4Cl$ as the sole carbon and nitrogen sources. After purification, both gp17.1 species were united in a protein amount ratio of 1:1 before dialysis. Polymerization and ultracentrifugation were conducted as described above. A protein pellet of ~80 mg could be isolated after centrifugation. 1.9 mm rotors equipped with bottom spacers were filled with pelleted protein, a few DSS crystals for spectral referencing and temperature control[2], and 1 µL of D_2O for field locking.

Solid-state NMR spectroscopy:

Solid-state NMR spectroscopy was conducted at an external magnetic field strength according to 900 MHz ^{1}H Larmor frequency and with a 1.9 mm, four-channel (^{1}H, ^{13}C, ^{15}N, ^{2}H) probe. The magic angle spinning (MAS) frequency was set to 40 kHz for all experiments and the temperature calibrated to around +18°C by means of internally added DSS. Pulse program parameters, acquisition parameters and processing parameters for the presented experiments are summarized in Supporting Information Tables S1-S3.

The procedure for setting up the HccanH experiment for the isoleucine Cδ1 methyl assignment is not straightforward and thus explained in the following in detail: The hCγ1-CP is optimized with a ^{13}C-detected 1D experiment for maximum signal on Cγ1. Note: This experiment might require many scans due to low signal-to-noise (S/N) ratio from the limited number of isoleucines in the protein and ^{13}C detection with a 1.9 mm probe. Next, following an offset switch from Cγ1 to Cα, a DREAM pulse is further included in the pulse program, which is also optimized in a 1D ^{13}C-detected experiment. Note: The S/N of this experiment is even lower and the magnetization stemming from the DREAM transfer is negative. An hCANH 1D experiment has to be set up following the conditions described in detail in Fricke et al. 2017.[3] After successful optimization, all steps can be assembled into the final HccanH experiment (hCγ1-CP, cγ1Cα DREAM, cαN-CP and nH-CP).

Analytical size-exclusion chromatography:

ΔC-7 and ΔC-14 gp17.1 mutants were expressed in 0.5 L M9 medium containing $^{15}ND_4Cl$ and $^{13}C_6$,D_7-glucose as the sole carbon and nitrogen sources, and purified as described previously. Storing the protein samples for 3 weeks at room temperature did not result in the protein solution undergoing a transition into a gel-like state (as observed for polymerizing gp17.1 at these concentrations usually). 200 µl of the protein samples (at a protein concentration of ~5 mg/mL) were directly loaded onto a Superdex

Increase 200 10/300 GL (GE Healthcare) column and equilibrated in the dialysis buffer (20 mM sodium phosphate, 500 mM sodium chloride, 1 mM EDTA, pH 7.4) using an Äkta pure 25 system (GE Healthcare). For molecular weight calibration, a Protein Standard Mix 15-600 kDa from Sigma-Aldrich was used.

Solution NMR spectroscopy:

Further 500 µl of these protein solutions were supplemented with 50 µl D_2O for field locking and used for solution NMR spectroscopy. Liquid-state NMR spectroscopy was conducted at an external magnetic field strength according to 750 MHz ^1H Larmor frequency and a temperature of 25 °C**.** The protein correlation times were approximated by a method introduced by Anglister et al (via the amide proton T_2 values).[4]

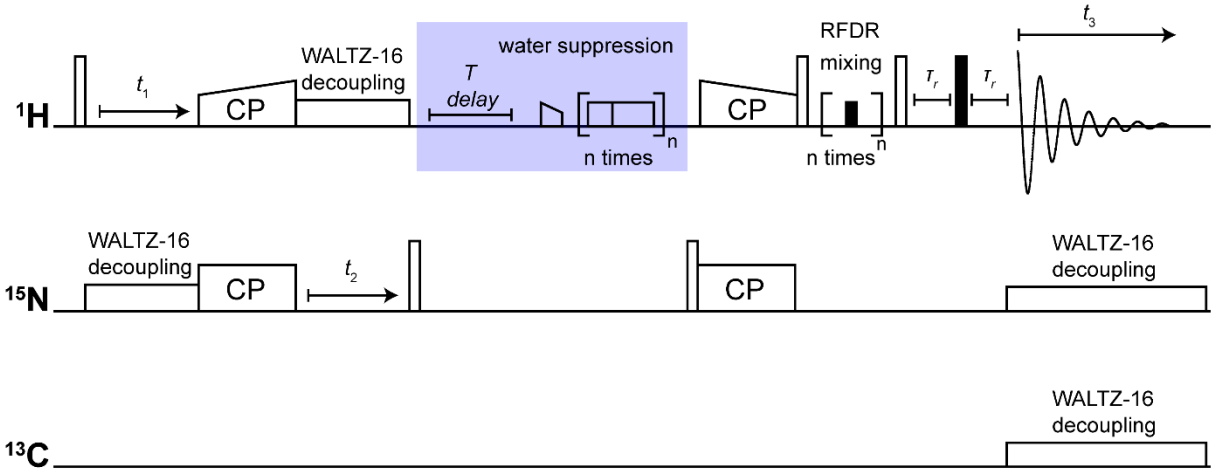

Figure S1. Scheme of the 3D HNhH pulse program. White rectangles represent 90° pulses and black rectangles 180° pulses, unless stated otherwise. The blue box represents water suppression.

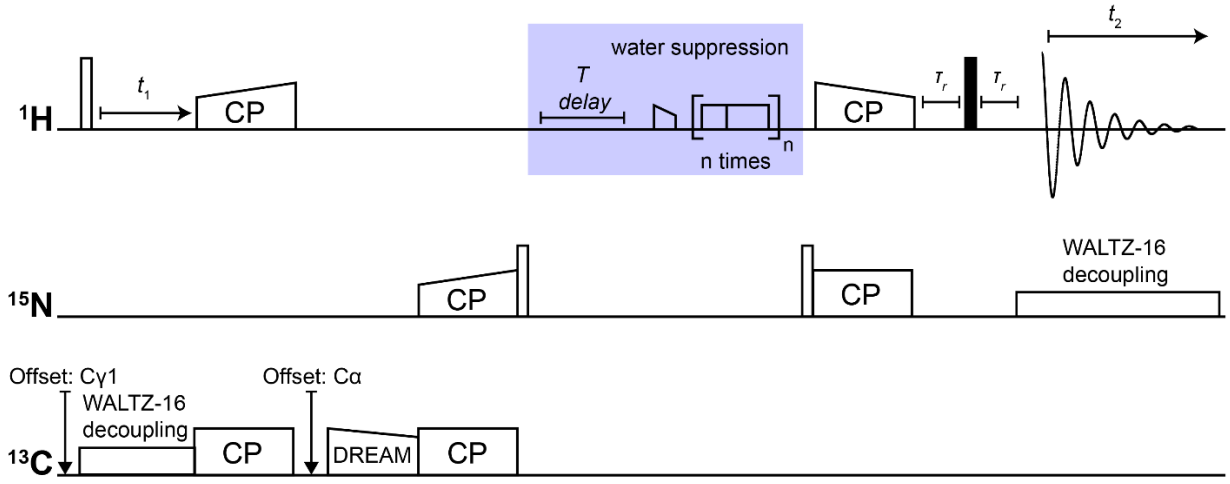

Figure S2. Scheme of the 2D HccanH pulse program. White rectangles represent 90° pulses and black rectangles 180° pulses, unless stated otherwise. The blue box represents water suppression.

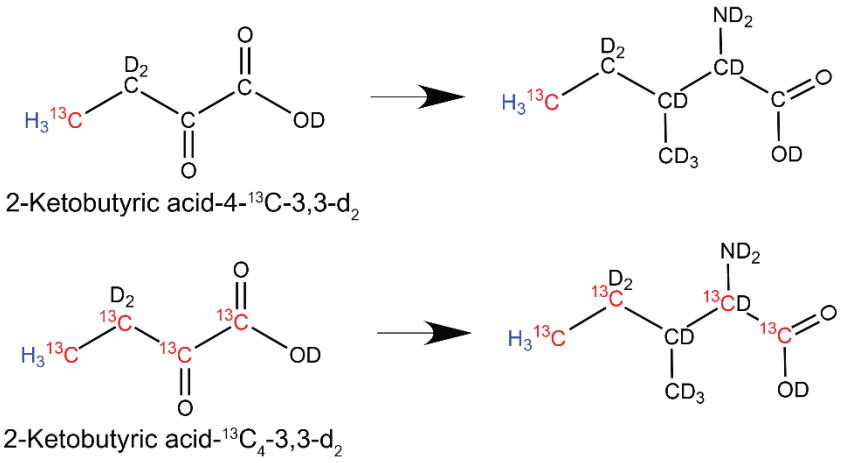

Figure S3. Isoleucine precursors. 2-Ketobutyric acid-4-^{13}C-3,3-d_2 is used to selectively label the Cδ1 methyl group of isoleucines for the collection of long-distance restraints. 2-Ketobutyric acid-$^{13}C_4$-3,3-d_2 introduces multiple ^{13}C labels into the isoleucine side chain allowing for the assignment of the Cδ1 methyl groups ("Assignment precursor").

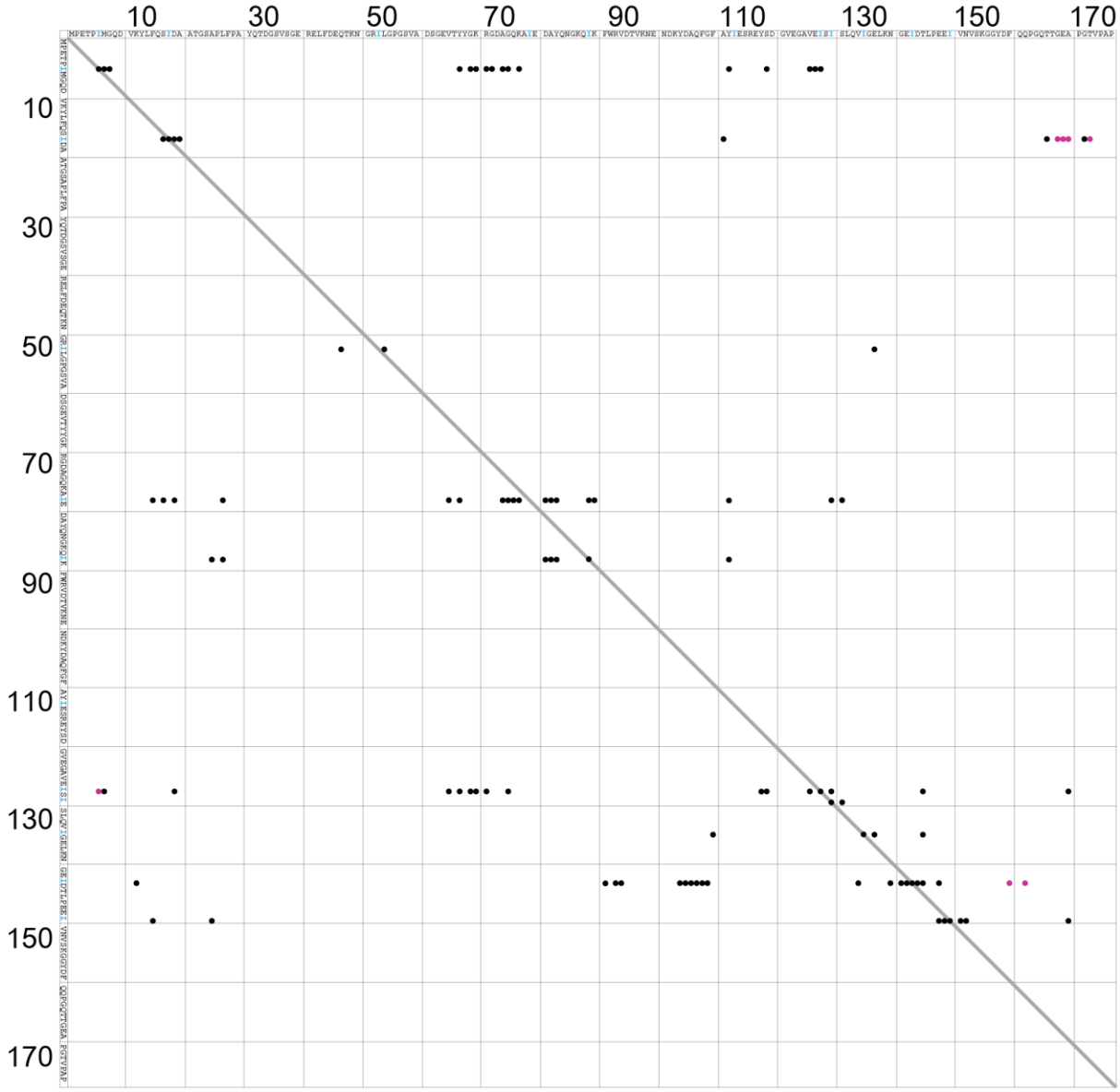

Figure S4. Unambiguous (based on chemical shifts; cutoffs ^{15}N ~0.15 ppm, HN ~0.05 ppm, Hδ1 ~0.03 ppm) long-distance restraints visualized in a residue-residue plot. The horizontal axis represents the NH-groups, the vertical axis the isoleucine-Cδ1 groups. Intermolecular restraints are highlighted in magenta.

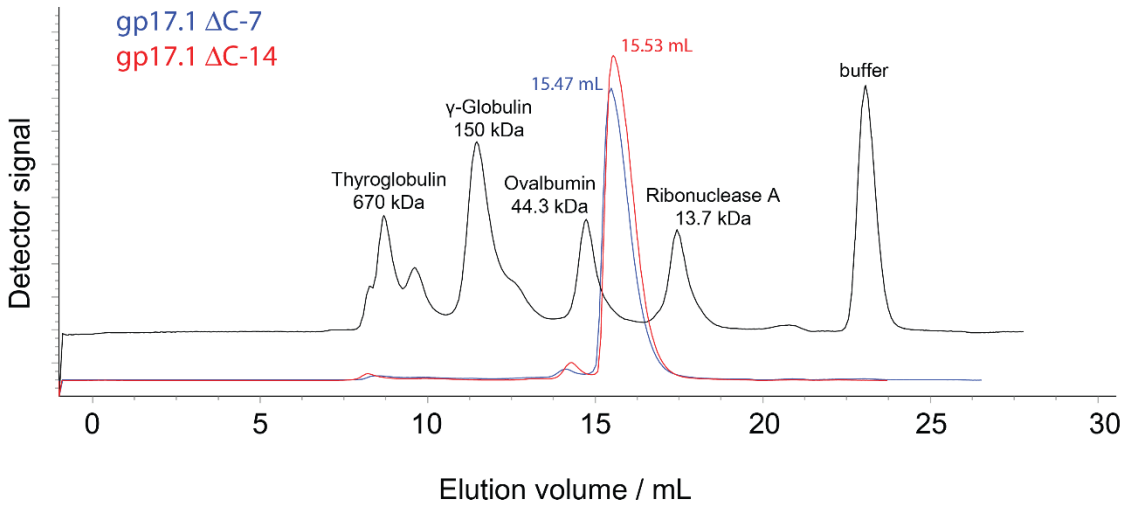

Figure S5. Size-exclusion chromatogram of ΔC-7 and ΔC-14 mutants of gp17.1. Protein samples were applied to a Superdex 200 Increase 10/300 GL column and separated with dialysis buffer (20 mM sodium phosphate, 500 mM sodium chloride, 1 mM EDTA, pH 7.4) using an Äkta system. For molecular weight calibration, a Protein Standard Mix 15-600 kDa from Sigma-Aldrich was used. The unit on the vertical axis is arbitrary.

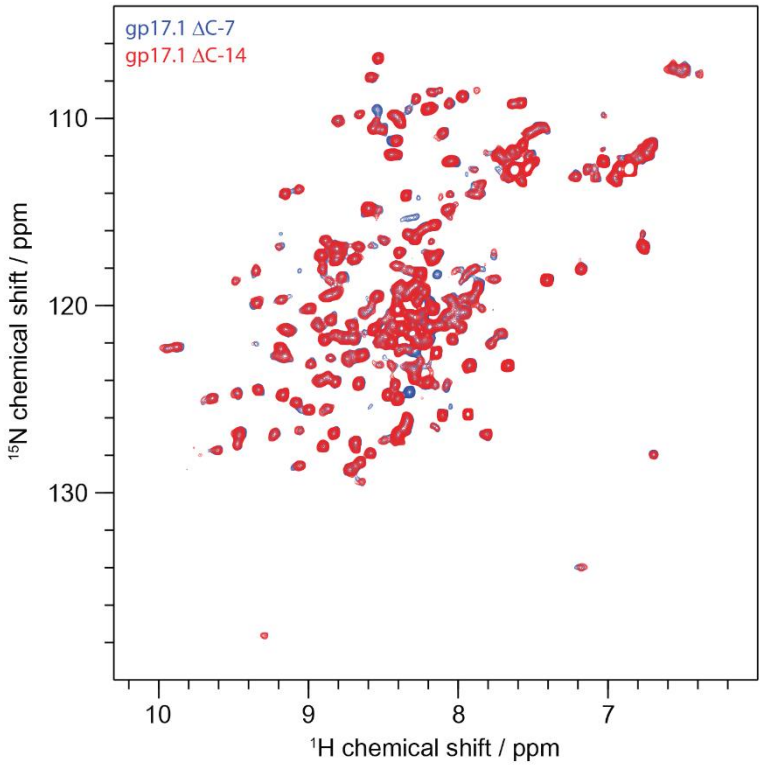

Figure S6. Solution NMR HSQC spectra of ΔC-7 and ΔC-14 mutants of gp17.1 at 25 °C and 750 MHz external magnetic field. Correlation times of ~18 ns could be approximated for both mutants.

Table S1. Pulse program parameters for all required steps of the described experiments. All experiments were conducted at a magic angle spinning rate of 40.0 kHz and an external B_0 field corresponding to 900 MHz ^{1}H Larmor frequency.

Parameter	**Value**		
Experiment	**2D hCH**	**3D HNhH**	**2D HccanH**
		Recycle delay	
Recycle delay	1 s	1.15 s	1 s
		90° initial ^{1}H excitation pulse	
R.f. power	100 kHz	100 kHz	100 kHz
Duration	2.5 µs	2.5 µs	2.5 µs
Carrier position	0.4 ppm	8.5 ppm	0.4 ppm
		^{1}H evolution time	
WALTZ r.f. power		3.4 kHz (^{15}N)	13.8 kHz (^{13}C)
WALTZ pulse duration		60 µs	60 µs
WALTZ carrier position		117.7 ppm	28.4 ppm
		^{1}H-^{15}N CP step	
^{1}H r.f. power		81 kHz	
^{1}H carrier position		8.5 ppm	
^{15}N r.f. power		29.6 kHz	
^{15}N carrier position		117.7 ppm	
Ramp shape		Ramp 80-100% on ^{1}H	
Duration		1400 µs	
		^{15}N evolution time	
WALTZ r.f. power		9.5 kHz (^{1}H)	
WALTZ pulse duration		40 µs	
WALTZ carrier position		8.5 ppm	
		^{1}H-^{13}CX CP step	
^{1}H r.f. power	52.5 kHz		
^{1}H carrier position	0.4 ppm		
^{13}CX r.f. power	10.2 kHz		
^{13}CX carrier position	60.3 ppm		
Ramp shape	Ramp 80-100% on ^{1}H		
Duration	6 ms		
		^{13}CX evolution time	
WALTZ r.f. power	2.8 kHz (^{1}H)		
WALTZ pulse duration	40 µs		
WALTZ carrier position	0.4 ppm		
		^{1}H-^{13}CG1 CP step	
^{1}H r.f. power			52.5 kHz
^{1}H carrier position			0.4 ppm
^{13}CG1 r.f. power			10.2 kHz
^{13}CG1 carrier position			28.4 ppm
Ramp shape			Ramp 80-100% on ^{1}H
Duration			1 ms
		^{13}CG1-^{13}CA DREAM/HORROR transfer	
DREAM pulse r.f. power			20.2 kHz
DREAM pulse shape			Ramp 100-80%
DREAM pulse duration			15 ms
DREAM pulse carrier position			61.5 ppm
		^{13}CA-^{15}N CP step	
^{13}CA r.f. power			26.2 kHz
^{13}CA carrier position			61.5 ppm
^{15}N r.f. power			14.8 kHz
^{15}N carrier position			117.7 ppm
Ramp shape			Ramp 80-100% on ^{15}N
Duration			10.5 ms
		90° ^{15}N/^{13}C flip pulses	

R.f. power	50 kHz	35.7 kHz	35.7 kHz
Duration	5 µs	7 µs	7 µs
Carrier position	60.3 ppm	117.7 ppm	117.7 ppm
Water suppression			
T delay	44 ms	44 ms	44 ms
Spoil pulse r.f. power	43.6 kHz	43.6 kHz	43.6 kHz
Spoil pulse duration	1 ms	1 ms	1 ms
Spoil pulse shape	Ramp 100-60%	Ramp 100-60%	Ramp 100-60%
First pulse duration in train	33 ms	33 ms	33 ms
Second pulse duration in train	56 ms	56 ms	56 ms
Train r.f. power	13.8 kHz	13.8 kHz	13.8 kHz
Wat. sup. carrier position	4.9 ppm	4.9 ppm	4.9 ppm
Loops through train (n)	1	1	1
^{15}N-^{1}H CP step			
^{1}H r.f. power		81.6 kHz	79.3 kHz
^{1}H carrier position		8.5 ppm	8.5 ppm
^{15}N r.f. power		33.5 kHz	33.5 kHz
^{15}N carrier position		117.7 ppm	117.7 ppm
Ramp shape		Ramp 100-80% on ^{1}H	Ramp 100-80% on ^{1}H
Duration		900 µs	900 µs
RFDR ^{1}H-^{1}H Mixing			
RFDR r.f. power		100 kHz	
RFDR mixing time		10 ms	
RFDR pulse carrier position		4.9 ppm	
^{13}CX-^{1}H CP step			
^{1}H r.f. power	52.5 kHz		
^{1}H carrier position	0.4 ppm		
^{13}CX r.f. power	10.2 kHz		
^{13}CX carrier position	60.3 ppm		
Ramp shape	Ramp 100-80% on ^{1}H		
Duration	6 ms		
Spin echo step			
^{1}H pulse r.f. power	100 kHz	100 kHz	100 kHz
^{1}H pulse duration	5 µs	5 µs	5 µs
^{1}H pulse carrier position	8.5 ppm	8.5 ppm	8.5 ppm
Acquisition			
^{15}N WALTZ r.f. power		3.4 kHz	4.1 kHz
^{15}N WALTZ pulse duration		60 µs	60 µs
^{15}N WALTZ carrier position		117.7 ppm	117.7 ppm
^{13}C WALTZ r.f. power	9.8 kHz	5 kHz	
^{13}C WALTZ pulse duration	60 µs	60 µs	
^{13}C WALTZ carrier position	60.3 ppm	24.9 ppm	

Table S2. Acquisition parameters for 2D and 3D spectra. The highest dimension is always the direct dimension.

	Acquisition time / ms (number of points)					
Experiment	**F1**	**F2**	**F3**	**ns**	**Total Number of acquired points**	**Total Time**
2D hCH	10 ms (600) (^{13}C)	30 ms (1610) (^{1}H)	N/A	80	600	18 h 18 min
2D HccanH	10 ms (220) (^{1}H)	8 ms (800) (^{1}H)	N/A	360	220	1 d 6 h 35 min
3D HNhH	15 ms (104) (^{15}N)	10 ms (80) (^{1}H)	20.8 ms (1118) (^{1}H)	24	8320	3 d 14 h 59 min
3D HNhH (mixed)	15 ms (104) (^{15}N)	7.5 ms (60) (^{1}H)	20.8 ms (1118) (^{1}H)	32	6240	2 d 19 h 15 min

Table S3. Processing parameters. The highest dimension is always the direct dimension.

	Points after FT			Window function		
Experiment	**F1**	**F2**	**F3**	**F1**	**F2**	**F3**
2D hCH	2k (^{13}C)	4k (^{1}H)	N/A	sin^2, φ=60°	sin^2, φ=60°	N/A
2D HccanH	2k (^{1}H)	2k (^{1}H)	N/A	sin^2, φ=60°	sin^2, φ=60°	N/A
3D HNhH	128 (^{15}N)	128 (^{1}H)	4k (^{1}H)	sin^2, φ=60°	sin^2, φ=60°	sin^2, φ=60°
3D HNhH (mixed)	128 (^{15}N)	128 (^{1}H)	4k (^{1}H)	sin^2, φ=90°	sin^2, φ=90°	sin^2, φ=90°

Table S4. Determined chemical shift values for gp17.1 isoleucines (deposited in the BMRB: ID 27468). Chemical shifts were referenced using internal DSS and are given in ppm. For some residues, a second set of resonances was identified and is marked with grey background color. The backbone assignment for gp17.1 is deposited in the BMRB: ID 27099.

Residue		**^{1}H**	**^{15}N**	**^{13}Cα**	**^{13}Cγ1**	**^{13}Cδ1**	**^{1}Hδ1**
6	Ile	8.41	122.31	62.43	27.74	14.18	0.91
18	Ile	9.49	122.07	64.2	23.86	15.85	1.01
53	Ile	9.51	130.12	59.79	25.94	12.81	0.52
79	Ile	7.36	120.20	65.21	29.69	12.96	-0.09
89	Ile	9.22	117.81	60.37	28.85	15.16	0.97
128	Ile	9.68	129.16	59.89	27.64	14.59	1.13
128	Ile	9.46	130.90	60.02	28.19	14.59	1.10
130	Ile	8.95	121.18	57.88	24.85	14.04	0.80
135	Ile	9.01	129.06	61.46	27.99	14.15	0.58
135	Ile	9.15	130.04	63.52	28.29	14.16	0.86
143	Ile	9.81	124.78	60.18	28.28	13.39	0.36
150	Ile	7.54	116.75	62.79	28.19	14.04	0.78
150	Ile	7.54	116.75	62.66	28.10	14.04	0.72

[1] M. Zinke, P. Fricke, C. Samson, S. Hwang, J. S. Wall, S. Lange, S. Zinn-Justin, A. Lange, *Angew. Chemie Int. Ed.* **2017**, 1–6.

[2] A. Böckmann, C. Gardiennet, R. Verel, A. Hunkeler, A. Loquet, G. Pintacuda, L. Emsley, B. H. Meier, A. Lesage, *J. Biomol. NMR* **2009**, *45*, 319–327.

[3] P. Fricke, V. Chevelkov, M. Zinke, K. Giller, S. Becker, A. Lange, *Nat. Protoc.* **2017**, *12*, 764–782.

[4] J. Anglister, S. Grzesiek, H. Ren, C. B. Klee, A. Bax, *J. Biomol. NMR* **1993**, *3*, 121–126.