Supporting Information

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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-C\(\delta\)1-methyl group-(\(^1\)H\(\delta\)1, \(^{13}\)C\(\delta\)1)-u-\(^{15}\)N-labeled gp17.1; 2.) Isoleucine-(\(^1\)H\(\delta\)1, \(^{13}\)C\(\delta\)1, \(^{13}\)C\(\gamma\)1, \(^{13}\)C\(\alpha\), \(^{13}\)CO)-u-\(^{15}\)N-labeled gp17.1; and 3.) A mixed sample containing 50% Isoleucine-C\(\delta\)1-methyl group-(\(^1\)H\(\delta\)1, \(^{13}\)C\(\delta\)1)-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, 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-C\(\delta\)1-methyl group-(\(^1\)H\(\delta\)1, \(^{13}\)C\(\delta\)1)-labeled gp17.1 was expressed from 0.5 L of bacterial culture as described above but with \(^{14}\)ND\(_4\)Cl instead of \(^{15}\)ND\(_4\)Cl 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\(_4\)Cl 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 \(\mu\)L of D\(_2\)O for field locking.
Solid-state NMR spectroscopy:

Solid-state NMR spectroscopy was conducted at an external magnetic field strength according to 900 MHz 1H Larmor frequency and with a 1.9 mm, four-channel (1H, 13C, 15N, 2H) 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 13C-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 13C 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 13C-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 15ND4Cl and 13C6,D7-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$_2$O 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 $^1$H 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 Cβ1 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 Cβ1 methyl groups ("Assignment precursor").
Figure S4. Unambiguous (based on chemical shifts; cutoffs $^{15}$N $\sim$0.15 ppm, HN $\sim$0.05 ppm, H$^\delta 1$ $\sim$0.03 ppm) long-distance restraints visualized in a residue-residue plot. The horizontal axis represents the NH-groups, the vertical axis the isoleucine-C$^\delta 1$ 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 B₀ field corresponding to 900 MHz ¹H Larmor frequency.

| Parameter                      | Value                                                                 |
|--------------------------------|----------------------------------------------------------------------|
| **Experiment**                 | **2D hCH**                                                           | **3D HNNH**                                                        | **2D HccanH**                                                      |
| Recycle delay                  | 1 s                                                                 | 1.15 s                                                             | 1 s                                                                |
| 90° initial ¹H 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                                                            |
| ¹H evolution time              |                                                                      |                                                                    |                                                                    |
| WALTZ r.f. power               | 3.4 kHz (¹N)                                                        | 13.8 kHz (¹3C)                                                     |                                                                    |
| WALTZ pulse duration           | 60 µs                                                               | 60 µs                                                              |                                                                    |
| WALTZ carrier position         | 117.7 ppm                                                           |                                                                    |                                                                    |
| ¹H-¹5N CP step                 |                                                                      |                                                                    |                                                                    |
| ¹H r.f. power                  | 81 kHz                                                              |                                                                    |                                                                    |
| ¹H carrier position            | 8.5 ppm                                                             |                                                                    |                                                                    |
| ¹5N r.f. power                 | 29.6 kHz                                                            |                                                                    |                                                                    |
| ¹5N carrier position           | 117.7 ppm                                                           |                                                                    |                                                                    |
| Ramp shape                     | Ramp 80-100% on ¹H                                                  |                                                                    |                                                                    |
| Duration                       | 1400 µs                                                             |                                                                    |                                                                    |
| ¹5N evolution time             |                                                                      |                                                                    |                                                                    |
| WALTZ r.f. power               | 9.5 kHz (¹H)                                                       |                                                                    |                                                                    |
| WALTZ pulse duration           | 40 µs                                                               |                                                                    |                                                                    |
| WALTZ carrier position         | 8.5 ppm                                                             |                                                                    |                                                                    |
| ¹H-¹3CX CP step                |                                                                      |                                                                    |                                                                    |
| ¹H r.f. power                  | 52.5 kHz                                                            |                                                                    |                                                                    |
| ¹H carrier position            | 0.4 ppm                                                             |                                                                    |                                                                    |
| ¹3CX r.f. power                | 10.2 kHz                                                            |                                                                    |                                                                    |
| ¹3CX carrier position          | 60.3 ppm                                                            |                                                                    |                                                                    |
| Ramp shape                     | Ramp 80-100% on ¹H                                                  |                                                                    |                                                                    |
| Duration                       | 6 ms                                                                |                                                                    |                                                                    |
| ¹3CX evolution time            |                                                                      |                                                                    |                                                                    |
| WALTZ r.f. power               | 2.8 kHz (¹H)                                                       |                                                                    |                                                                    |
| WALTZ pulse duration           | 40 µs                                                               |                                                                    |                                                                    |
| WALTZ carrier position         | 0.4 ppm                                                             |                                                                    |                                                                    |
| ¹H-¹5 CG1 CP step              |                                                                      |                                                                    |                                                                    |
| ¹H r.f. power                  | 52.5 kHz                                                            |                                                                    |                                                                    |
| ¹H carrier position            | 0.4 ppm                                                             |                                                                    |                                                                    |
| ¹5 CG1 r.f. power              | 10.2 kHz                                                            |                                                                    |                                                                    |
| ¹5 CG1 carrier position        | 28.4 ppm                                                            |                                                                    |                                                                    |
| Ramp shape                     | Ramp 80-100% on ¹H                                                  |                                                                    |                                                                    |
| Duration                       | 1 ms                                                                |                                                                    |                                                                    |
| ¹5 CG1-¹5 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                                                            |                                                                    |                                                                    |
| ¹5 CA-¹5 N CP step             |                                                                      |                                                                    |                                                                    |
| ¹5 CA r.f. power               | 26.2 kHz                                                            |                                                                    |                                                                    |
| ¹5 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      |                                                                      |                                                                    |                                                                    |
Table S2. Acquisition parameters for 2D and 3D spectra. The highest dimension is always the direct dimension.

| Experiment        | F1                      | F2                      | F3                      | ns | Total Number of acquired points | Total Time       |
|-------------------|-------------------------|-------------------------|-------------------------|----|---------------------------------|------------------|
| 2D hCH            | 10 ms (600) (15N)       | 30 ms (1610) (1H)       | N/A                     | 80 | 600                             | 18 h 18 min      |
| 2D HccanH         | 10 ms (220) (1H)        | 8 ms (800) (1H)         | N/A                     | 360| 220                            | 1 d 6 h 35 min   |
| 3D HNhH           | 15 ms (104) (15N)       | 10 ms (80) (1H)         | 20.8 ms (1118) (1H)    | 24 | 8320                            | 3 d 14 h 59 min  |
| 3D HNhH (mixed)   | 15 ms (104) (15N)       | 7.5 ms (60) (1H)        | 20.8 ms (1118) (1H)    | 32 | 6240                            | 2 d 19 h 15 min  |
Table S3. Processing parameters. The highest dimension is always the direct dimension.

| Experiment  | Points after FT | Window function |
|-------------|-----------------|-----------------|
|             | F1   | F2   | F3   | F1        | F2   | F3       |
| 2D hCH      | 2k (13C) | 4k (1H) | N/A  | sin², ϕ=60° | sin², ϕ=60° | N/A     |
| 2D HccanH   | 2k (1H)   | 2k (1H) | N/A  | sin², ϕ=60° | sin², ϕ=60° | N/A     |
| 3D HNH     | 128 (15N) | 128 (1H) | 4k (1H) | sin², ϕ=60° | sin², ϕ=60° | sin², ϕ=60° |
| 3D HNH (mixed) | 128 (15N) | 128 (1H) | 4k (1H) | sin², ϕ=60° | sin², ϕ=60° | sin², ϕ=60° |

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 | ¹H   | ¹⁵N  | ¹³Cα | ¹³Cy1 | ¹³C51 | ¹H51 |
|---------|------|------|------|-------|-------|------|
| 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 |
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