Supplementary Material

High Resolution Observed in 800 MHz DNP Spectra of Extremely Rigid Type III Secretion Needles

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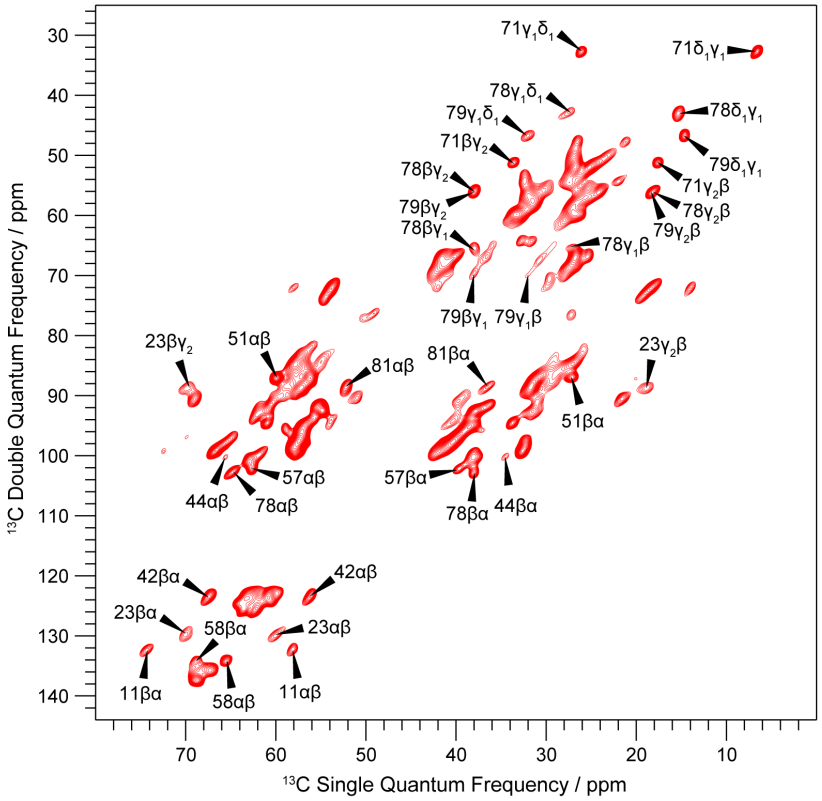

Fig. S1 ^{13}C-^{13}C SPC-5 2D NMR spectrum of MxiH needles at 800 MHz, 95 K and 8 kHz MAS under MW irradiation. Some of the peaks are assigned. Note that the indirect dimension is a double quantum dimension.

Preparation of the MxiH DNP Sample

The DNP sample was produced by preparing a 14 mM AMUPol solution in a 70/30 (v/v) glycerol-d_8/D_2O mixture. To a protein pellet consisting of uniformly ^{13}C- and ^{15}N-labeled MxiH needles roughly the same volume of this matrix was added. Subsequently, the substances were thoroughly mixed using a vortexer. The mixture was then filled into a 3.2 mm sapphire rotor which was closed with a zirconia cap (Fig. S2). Protonated water was not added to the matrix as the pellet still contained residual water buffer from the protein expression and purification.

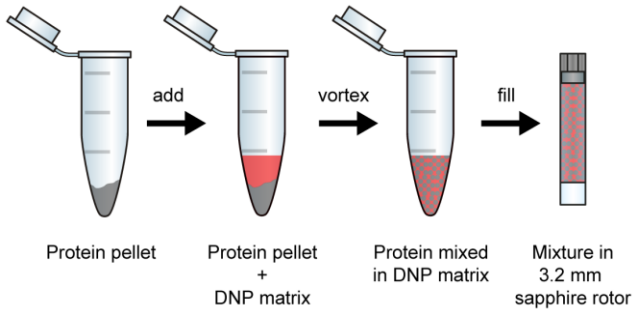

Fig. S2 Illustration of the production process of the MxiH DNP sample.

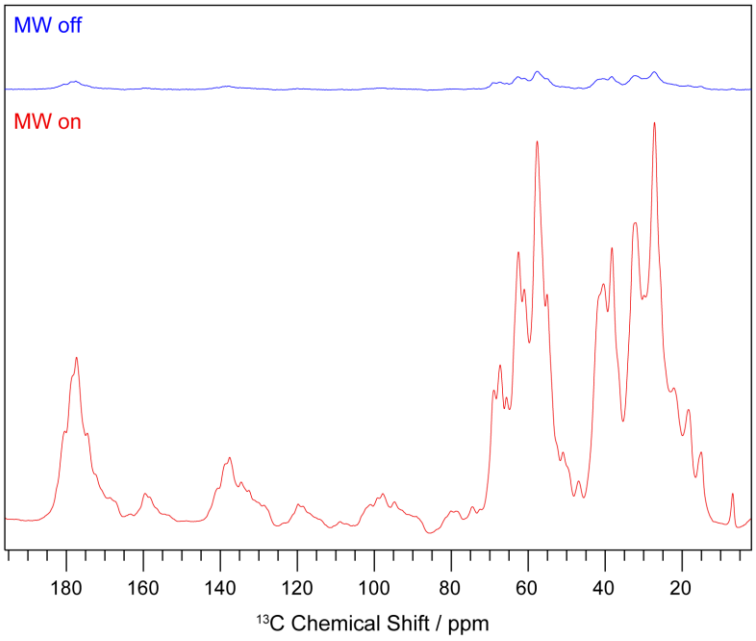

Fig. S3 Comparison of two ^{1}H-^{13}C 1D CP NMR spectra of MxiH needles at 800 MHz, 95 K and 8 kHz MAS. Under otherwise identical conditions, the blue spectrum was recorded with the microwaves (MW) turned off and the red spectrum was recorded with the microwaves turned on. The signals are about 21 times stronger in the spectrum with the microwaves turned on.

Preparation of the PrgI T3SS Needle Sample used for T_1 Measurements

Expression, purification and polymerization of wild-type PrgI needles were performed as described before (Loquet et al. 2011; Loquet et al. 2012). Isotope labeling was achieved using $^{15}NH_4Cl$ as nitrogen source and D-[2-^{13}C]-glucose as carbon source during expression. Approximately 15 mg of [2-^{13}C]-glucose-, uniformly-^{15}N-labeled protein was packed into a 3.2 mm rotor.

Solid-State NMR Experiments for Determination of T_1 times of PrgI T3SS Needles

Solid-state NMR experiments were conducted on an 800 MHz (^{1}H Larmor frequency) NMR spectrometer (Bruker Biospin, Germany) equipped with a (^{1}H, ^{13}C, ^{15}N) triple-resonance 3.2 mm probe. The sample was spun at 20 kHz. The effective sample temperature was 3±3 °C as measured by the temperature-dependent water proton resonance relative to an internal DSS reference (Boeckmann et al. 2009). Chemical shift referencing was achieved using the internal DSS reference. The longitudinal relaxation time was measured according to the pulse sequence introduced by Emsley and coworkers (Giraud et al. 2004) using a ^{15}N-^{13}CA 2D correlation spectrum to obtain residue specific information. Twelve spectra were recorded with ^{15}N relaxation delays of 0, 5, 9, 17.5, 0, 33, 0, 60, 0, 110, 110, 0 seconds. All spectra were recorded with 64 scans, except the spectra with the relaxation delay of 110 seconds, which were recorded with 16 scans each. During the direct (^{13}C) and indirect (^{15}N) isotropic chemical shift evolution periods, SPINAL-64 (Fung et al. 2000) heteronuclear decoupling was applied on the ^{1}H channel with an RF field strength of 78 kHz. WALTZ-64 (Shaka et al. 1983) decoupling with an RF field strength of 2.5 kHz was applied on the ^{15}N channel during the ^{13}C detection period to remove heteronuclear scalar couplings. A hard 180° pulse was applied on the ^{13}C channel to remove scalar couplings during the ^{15}N indirect evolution period. The ^{13}C and ^{15}N acquisition times were 22 ms and 14 ms, respectively.

References

Boeckmann A, Gardiennet C, Verel R, Hunkeler A, Loquet A, Pintacuda G, Emsley L, Meier BH, Lesage A (2009) Characterization of different water pools in solid-state NMR protein samples. J Biomol NMR 45:319-327 doi:10.1007/s10858-009-9374-3

Fung BM, Khitrin AK, Ermolaev K (2000) An improved broadband decoupling sequence for liquid crystals and solids. J Magn Reson 142:97-101 doi:10.1006/jmre.1999.1896

Giraud N, Bockmann A, Lesage A, Penin F, Blackledge M, Emsley L (2004) Site-specific backbone dynamics from a crystalline protein by solid-state NMR spectroscopy. J Am Chem Soc 126:11422-11423

Loquet A, Lv G, Giller K, Becker S, Lange A (2011) 13C spin dilution for simplified and complete solid-state NMR resonance assignment of insoluble biological assemblies. J Am Chem Soc 133:4722-4725 doi:10.1021/ja200066s

Loquet A, Sgourakis NG, Gupta R, Giller K, Riedel D, Goosmann C, Griesinger C, Kolbe M, Baker D, Becker S, Lange A (2012) Atomic model of the type III secretion system needle. Nature 486:276-279 doi:10.1038/nature11079

Shaka AJ, Keeler J, Frenkiel T, Freeman R (1983) An improved sequence for broad-band decoupling - WALTZ-16. J Magn Reson 52:335-338 doi:10.1016/0022-2364(83)90207-x