

Supporting information for

Towards automatic protein backbone assignment using proton-detected 4D solid-state NMR data

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Solid-state NMR experiments

In the (H)COCA(CO)NH experiment, the initial magnetization on H^{N_i} is transferred to CO_{i-1} by cross polarization and then further distributed to CA_{i-1} employing HORROR (Nielsen et al. 1994). The remaining magnetization on CO is removed by a ^{13}C spin-lock applied on the CA band, matching the rotary resonance condition on CO as described previously (Chevelkov et al. 2014). Subsequently, the magnetization is transferred back to CO_{i-1} using HORROR, then to N_i by CO–N SPECIFIC-CP (Baldus et al. 1998) and finally back to H^{N_i} for detection. At times where the transverse magnetization is on CO_{i-1} , CA_{i-1} and N_i , respectively, chemical shift evolution periods are inserted. The decoupling was implemented as in the complementary (H)CACO(CA)NH experiment (see main text).

In both 4D experiments, the initial 1H to CA/CO transfer employed ramped cross-polarization with a contact time of 3.5 ms. The CA to CO transfer (and reverse transfer) was achieved by HORROR (Nielsen et al. 1994) with a contact time of 8.7 ms. The contact time of SPECIFIC-CP (Baldus et al. 1998) for CO/CA to N transfer was set to 9 ms. The ^{13}C band-selective suppression was set up as described before (Chevelkov et al. 2014). Waltz-16 decoupling on N was carried out with an RF strength of 2.77 kHz. iBurp shaped pulses (Geen and Freeman 1991) of 650 μs length were used as 180° selective pulses on CA or CO. The number of scans was set to 32, with an inter-scan delay of 2 s. The total experimental time of one 4D experiment was 5.5 days when 20% points of the uniform sampling scheme were recorded. The sparse sampling scheme was generated using the “nussampler” routine from the mddNMR software package (Orekhov et al. 2003) with T_2 values of 10 ms, 3.5 ms and 15 ms for CO, CA and N dimensions, respectively. The data sets were recorded in blocks of 1 day length. The drift of the external magnetic field was compensated using the internal DSS signal between separate blocks.

Sparingly sampled datasets were then processed by the mddNMR software (Orekhov et al. 2003) and NMRpipe (Delaglio et al. 1995), and analyzed by CcpNmr (Vranken et al. 2005). CA and CO dimensions were processed using exponential window functions with a line broadening of 25 Hz. Apodization of the N dimension was achieved with a squared sine bell window function shifted by 60° . For the direct H^N dimension, only data points from the first 25 ms were retained. In the direct dimension, a squared sine bell window function shifted by 90° was used.

The software MARS was used to aid in the search of sequential connectivities (Jung and Zweckstetter 2004). The chemical shifts were grouped into spin systems containing six chemical shifts values: H^{N_i} , N_i , CA_i , CO_i , CA_{i-1} and CO_{i-1} . CA and CO chemical shifts were used to obtain connections between H^N -N anchor points.

	CO	CA	N	HN
Spectral width (kHz)	1.9	4.5	1.0*	16
Acquisition time (ms)	8.67	3.53	14.8	40**
Sampled time points	816, i.e. 20% of the full sampling cube: 17(CO)*16(CA)*15(N)=4080			639**

Table S1. Experimental acquisition parameters. *The spectral width of N was intentionally reduced. ** Only data points from the first 25 ms were used.

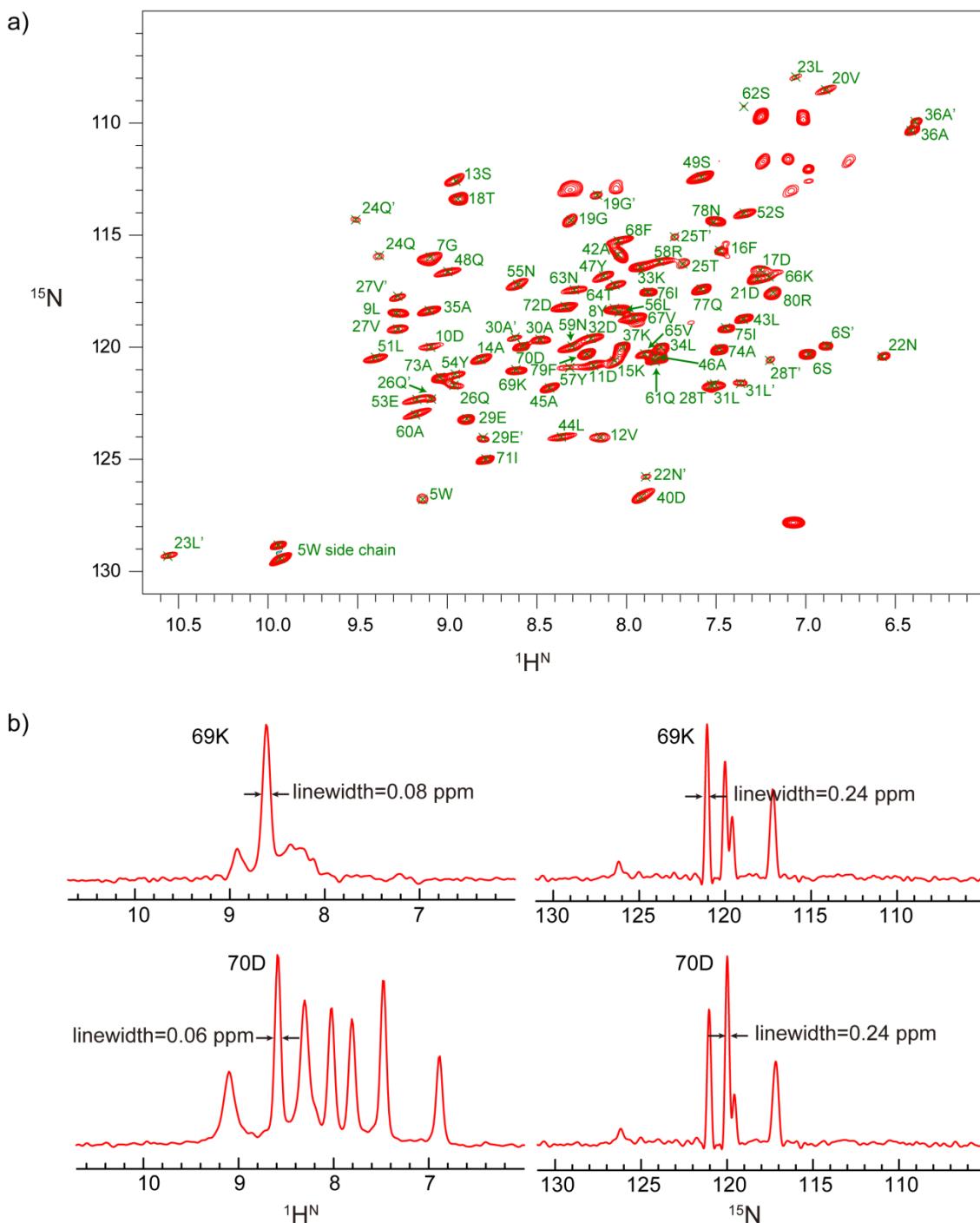


Figure S1. a) Two-dimensional (H)NH correlation spectrum of a perdeuterated PrgI sample with 20% reprotonation on exchangeable sites. The spectrum was recorded on a 600 MHz spectrometer and a spin rate of 25 kHz. The prime signs mark signals from a second conformation. b) Traces of peaks K69 and D70 along proton and nitrogen dimensions. Line widths (FWHH) are indicated.

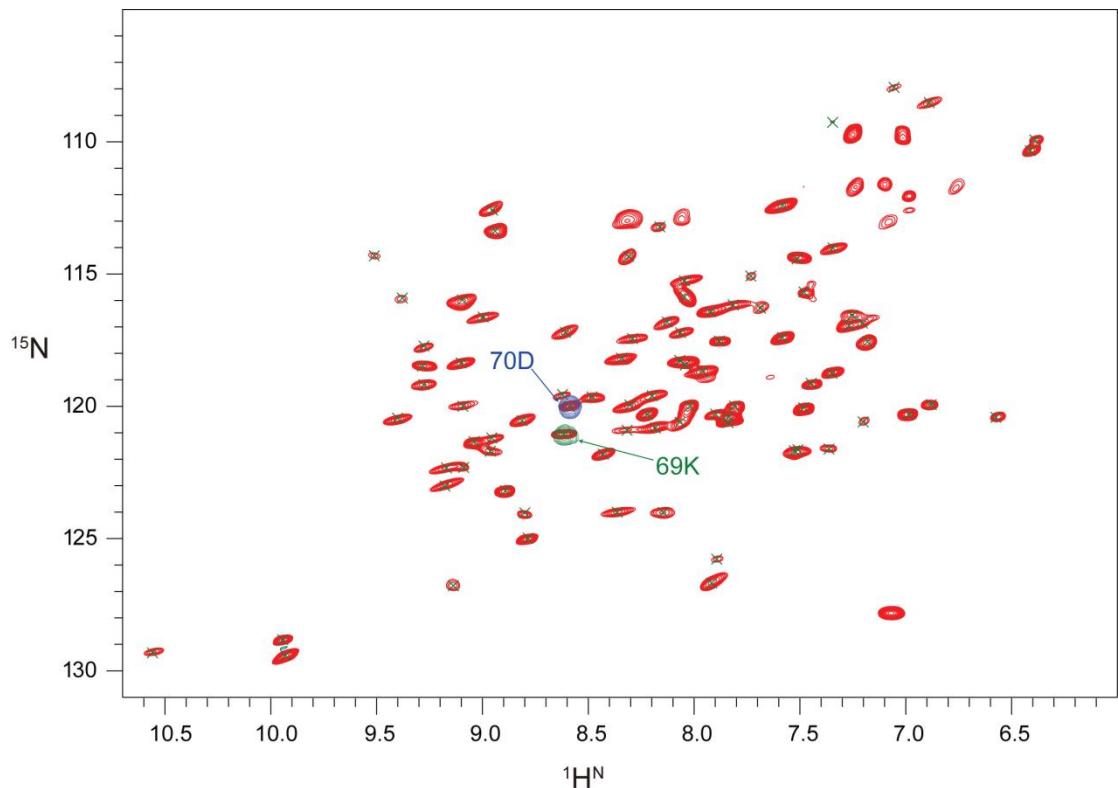


Figure S2. Overlay of two-dimensional (¹H)NH correlation spectrum with ¹H^N-N planes from 4D spectra defined by the CA and CO chemical shifts of residue K69. The blue peak is ¹H^N70(CA69CO69) from the (¹H)COCA(CO)NH spectrum and the green peak is ¹H^N69(CA69CO69) from the (¹H)CACO(CA)NH spectrum.

Supporting References

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