Supporting information for

Backbone assignment for minimal protein amounts of low structural homogeneity in the absence of deuteration

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Experimental Details:

Sample preparation:

Spectra of the SH3 domain of chicken α-spectrin were recorded using approximately 1 mg of uniformly ^{15}N,^{13}C-labelled protein, which was expressed and purified as described earlier.[1] Micro-crystallization was pursued in a buffer containing 100% H_2O as well as 75 mM $(NH_4)_2[Cu(EDTA)]$ by a pH shift from 3.5 to 7.5. The Tau protein preparation (K19 C322A variant) was obtained as published.[2] The material was then center-packed into a 1.3 mm rotor using fluorinated rubber plugs on the bottom and top of the rotor.

Solid-state NMR experiments:

All NMR experiments were carried out at 800 MHz proton Larmor frequency, 55 kHz MAS, and 35 °C effective temperature, using a 1.3 mm triple-resonance probe on a Bruker Avance III spectrometer.

The pulseprogram of the 4D (H)COCANH is shown in Figure 1A. The initial magnetization of protons is first transferred to C'_i by cross polarization. Before chemical shift evolution, the unwanted magnetization on $C\alpha_i$ is removed by a band-selective suppression module adopted from the literature[3]. The magnetization transfer between $C\alpha_i$ and C'_i was achieved by HORROR[4]. The remaining magnetization was removed as described before[3]. Subsequently, the magnetization is distributed further to N_i by Cα-N SPECIFIC-CP[5], and finally to H^N_i for detection. During the C'_i and $C\alpha_i$ evolution period, 180° selective pulses on Cα or C' are applied to refocus Cα-C' *J* couplings, and hard 180° pulse on N are applied to remove N-Cα and N-C' *J* couplings. Protons were decoupled from C and N by low-power XiX[6]. Waltz-16[7] was employed on N during proton detection. The water suppression is carried out in accordance with the MISSISSIPPI

approach[8]. The complementary pulse program, 4D (H)CACONH, was obtained by swapping the carrier frequencies of C' and Cα. The following phase cycle was employed: ϕ1=x,-x; ϕ2=4(x) , 4(-x) ; ϕ3=x,-x, x,-x,-x, x,-x, x; ϕ4=-x, x, -x, x, x, -x, x,-x; ϕ5=x, x, -x, -x; ϕrec=x,-x,-x, x,-x, x, x,-x.

Amide-to-amide correlation experiments were implemented as described for deuterated and amide back-exchanged samples previously[9]. The following phase cycle was employed: ϕ1=x,-x; ϕ2=4(x) , 4(-x) ; ϕ3=x, x,-x,-x; ϕrec=x,-x,-x, x,-x, x, x,-x.

3D (H)CBCANH was set up similarly to the previous reported pulse program[10]. The 3D HB/HA(CB/CA)NH pulseprogram was obtained from the (H)CBCANH by implementing the first indirect evolution time on H instead of Cα and Cβ. The following phase cycle was employed: ϕ1=x,-x; ϕ2=4(x) , 4(-x) ; ϕ3=x, x,-x,-x; ϕrec=x,-x,-x, x,-x, x, x,-x.

Non-uniform sampling (NUS) schemes were employed in the two 4D experiments to reduce the experimental time by covering only 5% of the complete sampling space in the indirect dimensions. In the case of Tau, 3D (H)CBCANH and (H)N(COCA)NH were also recorded with NUS covering 10% of whole sampling grid. The sampling schemes were generated by Poisson-Gap sampling[11] and the sparsely sampled NMR data processed using Iterative Soft Thresholding[12] (both provided by the istHMS software package). The contact time and pulses employed in the transfer steps are listed in Table S1.

Table S1. The parameters for each dipolar-transfer step.

Transfer steps	Contact time(μs)	Pulses
H->C'	950	H:15.5 kHz/C:42.6 kHz(80-100 % ramp on C')
H->Cα	300	H:15.5 kHz/C:46.5 kHz(80-100 % ramp on Cα)
H->Cβ/Cα	400	H:17.8kHz/C:49.2kHz(80-100 % ramp on Cβ/Cα)
H->N	400	H:18.9kHz/N:41.8kHz(80-100 % ramp on N)
N->C'	9000	N:20.2kHz/C':42.6kHz(100-80 % ramp on C')
C'->Cα	7000	26.9kHz(100-80 % ramp on Cα)
Cα->C'	7000	24.7kHz(100-80 % ramp on C')
Cβ->Cα	4000	60kHz (tangent modulated ramp, mean at 50%,on Cα)
C'->N	9000	N:23.4kHz/C':33.9kHz(100-80 % ramp on C')
Cα->N	8500	N:35kHz/Cα:20.2kHz(100-80 % ramp on Cα)
N->H^N	400	H:18.8kHz/N:42.8(100-80 % ramp on N)

$^{13}C'/^{13}C\alpha$ band-selective suppressions were carried out by a 9.19 μs trim pulse at 12.88 kHz followed by a 120μs spin-lock at 40 kHz. Decoupling fields were set to 3.1 kHz on ^{15}N for Waltz16 and 16 kHz on ^{1}H for XiX respectively. Recycle delays were set to 500 ms. The total experimental time for each experiment is listed in Table S2. The acquisition parameters are listed in Table S3. The data sets were recorded in blocks of 1-day length. The drift of external magnetic field was compensated using the internal DSS signal between separated blocks. The data sets were processed by NMRpipe[13] and analyzed by CCPNmr.[14] Apodization of the each dimension was achieved with a squared sine bell window function shifted by 90°. For the direct H^N dimension, only data points of the first 20 ms were retained for processing.

Table S2. Experimental time for each experiment.

Spectrum	SH3 domain		Tau K19	
	Number of Scans	Total experimental time	Number of Scans	Total experimental time
4D (H)CACONH	8	19 h 12 min NUS covers 5%	64	85 h 44 min NUS covers 5%
4D (H)COCANH	8	19 h 12 min NUS covers 5%	96	114 h 44 min NUS covers 5%
3D (H)N(COCA)NH	32	25 h 28 min	624	93 h 32 min NUS covers 10%
3D (H)CBCANH	32	43 h 12 min	360	52 h 48 min NUS covers 10%
3D HBHA(CBCA)NH	24	18 h 29 min		

Table S3. Acquisition parameters.

Spectrum	Nucleus	Spectral width (Hz)		Maximum acquisition time (ms)	
		SH3 domain	Tau K19	SH3 domain	Tau K19
4D (H)CACONH	$C\alpha_i$	6028	5224	7.0	5.0
	C'_i	2411	2009	8.7	8.9
	N_{i+1}	2186	2915	19.6	12.0
	H^N_{i+1}	24038	24038	40.0	40.0
4D (H)COCANH	C'_i	2411	2009	8.7	8.9
	$C\alpha_i$	6029	5224	7.0	5.0
	N_i	2186	2915	19.6	12.0
	H^N_i	24038	24038	40.0	40.0
3D (H)N(COCA)NH	N_{i+1}	2187	2915	15.1	15.1
	N_i	2187	2915	14.6	14.7
	H^N_i	24038	24038	40.0	40.0
3D (H)CBCANH	$C\beta_i/C\alpha_i$	14069	14066	4.0	3.5
	N_i	2187	2915	14.6	15.1
	H^N_i	24038	24038	40.0	40.0
3D (H)CBCA(CO)NH	$C\beta_i/C\alpha_i$	14069		4.0	
	N_{i+1}	2187		14.6	
	H^N_i	24038		40.0	
3D HBHA(CBCA)NH	$H\beta_i/H\alpha_i$	6393		5.0	
	N_i	2187		14.6	
	H^N_i	24038		40.0	

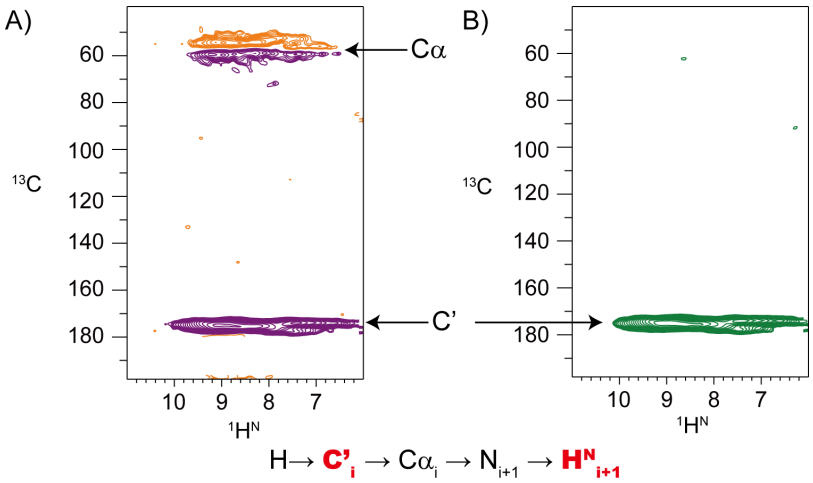

Figure S1: Band-selective suppression. A) 2D (H)CO(CAN)H spectrum without band-selective suppression. There are some artifacts in the region of Cα due to the incomplete transfer in the C'-Cα step. B) The spectrum from the same experimental setting as in A), but with band-selective suppression. Now the signals are purely from C'.

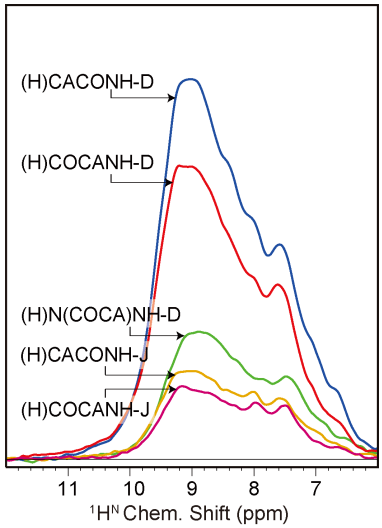

Figure S2: Sensitivity comparison of *J*-coupling-based and dipolar-coupling-based (H)CCNH experiments for fully protonated SH3 domain at 55.5 kHz MAS. The numbers of scans are 256 in all experiments. Here the first FID of each spectrum was plotted. –D and –J denotes dipolar and scalar-based experiments with respect to ^{13}C-^{13}C transfers. The green spectrum depicts the dipolar-based amide-to-amide correlation for comparision.

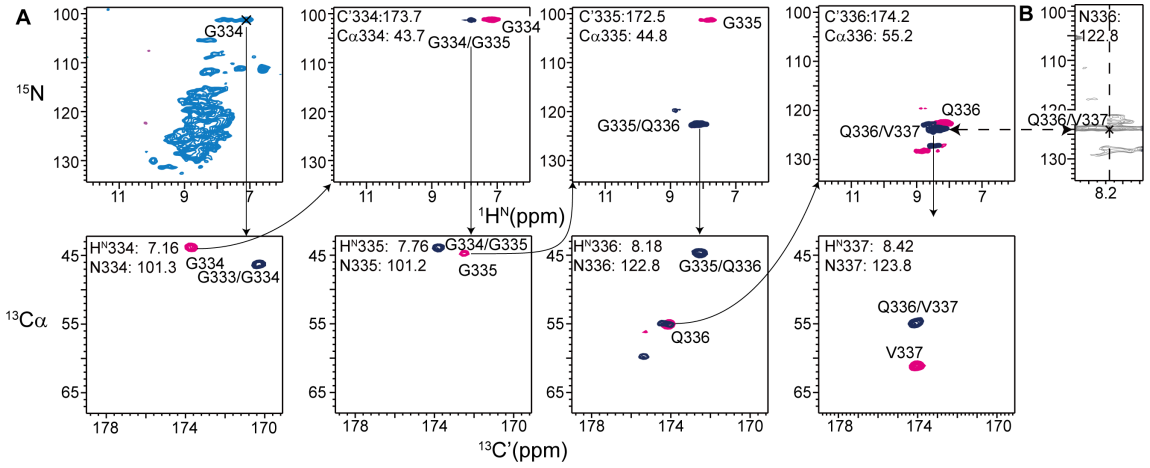

Figure S3: Backbone assignment of G334 to V337 in Tau K19 PHF. A) Sequential backbone walk starting from G334 to V337 based on 4D (H)COCANH (in magenta) and 4D (H)CACONH (in blue). The strip from (H)N(COCA)NH at the position defined by the amide group of Q336 confirms the N chemical-shift value of (next residue) V337.

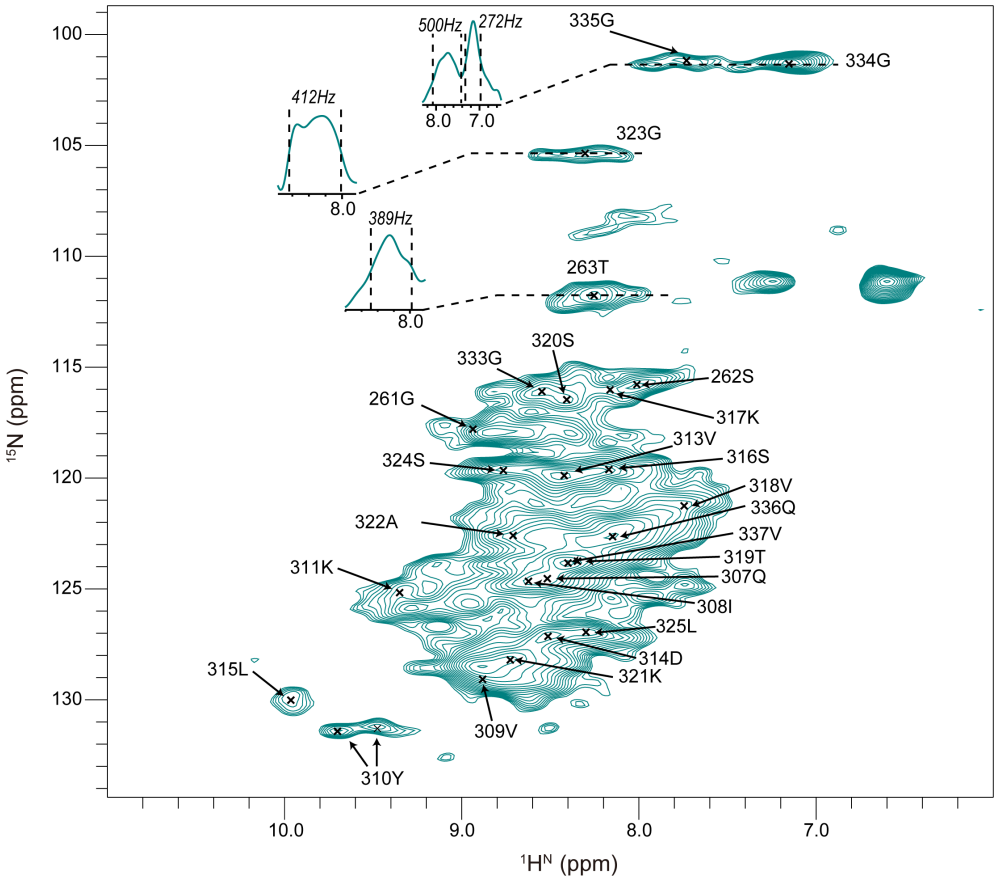

Figure S4: Assignments obtained with certainty on K19 PHF. The assigned residues are depicted on a dipolar 2D (H)NH spectrum. Representative 1D traces for a subset of residues are plotted in the spectrum to assess the large line broadening from both, inhomogeneous and homogenous contributions of the protonated fibrils. The linewidths obtained for this protein preparation at 55.5 kHz MAS amount to between 250 and 500 Hz.

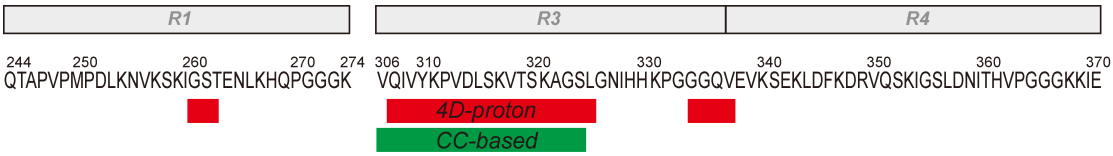

Figure S5: Extent of assignments obtained reliably in the current study. The assignments obtained using the described methodology are at least as comprehensive as the assignments obtained with confidence for K19 previously using ^{13}C-detected methods (at 850 MHz proton Larmor frequency), in spite of the low sample amount of only 1 mg in the current work (at 800 MHz ^{1}H Larmor frequency). Assignments reported previously (V306 to S324) are indicated in green ("CC-based")[2,15]. For a K19 C322A sample in the study of Ref. 2, an additional, not further characterized PGGG motif was also reported. The N and C terminus in the K19 construct are thought to be flexible at the experimental temperatures used. Using proton-detected methods ("4D-proton"), we obtained assignments for the same region (shifted by one residue) as reported previously and additional stretches N- and C-terminal (in red). For completeness, the domain structure of Tau is shown above the sequence. Repeat R2 (residues 275-305) is not present in the three-repeat 99-amino-acids K19 isoform of the NMR studies. Numbering, however, still refers to full-length Tau in all cases. For details on the K19 construct, see Ref. 2.

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