Solid-state NMR evidence for elastin-like β-turn structure in spider dragline silk

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Supplementary Information

MaSp1
AGQGGAGAAAAAAAAGGAGGAGRGGLGAGGAGQGYGSGLGGQGGAGGGAA
AAAAAAAGGQGGGQGGYGGLGSQGAGQGGYGAGQGGAGAAAAAAAAGGAGG
AGRGGLGAGGAGQGYGSGLGGQGGAGQGGAAAAAAAAGGQGGGQGGYGGLG
SQGAGQGGAGRGAAAAAAAAGGQGGRGGYGGLGSQGAGQGGYGAGQGGAG
AAAAAAAAGGAGEGGLGAGGAGQGYGSGLGGQGGAGQGGAAAAAAAAGGQ
GGHGGYGGLGSQGAGQGGAGRGAAAAAAAAGGQGGGQGGYGGLGSQGAGQG
GYGAGQGGAAAAAAAAAAGGAGGAGRGELGAGGAGQGYGXGLGGQGGAGQ
RGAASVAALAGGQGGQGGFGGFSSQGAGQGAYGGGAYSGQGAAASVSAASAA
ASRLSSPGAASRVSSAVTSLVSSGGPTNPAALSNTISXVVSQISE

MaSp2
PGGAGQQGPGGQGPYGPGAAAAAAAAGGYGPGAGQQGPXGAGQQGPGSQGP
GGAGQQGPGGQGPYGPGAAAAAAAVGGYGPGAGQQGPGSQGPGSGGQQGPG
GQGPYGPSAAAAAAAAGGYGPGAGQQGPGSQGPGSGGQQGPGGLGPYGPSAA
AAAAAAGGYGPGAGQQGPGSQGPGSGGQQRPGGLGPYGPSAAAAAAAAGGYG
PGAGQQGPGSQGPGSGGQQRPGGLGPYGPSAAAAAAAAGGYGPGAGQQGPGS
QAPVASAAASRLSSPQASSRVSSAVSTLVSSGPTNPAALSNAISSVVSQVSASNPG
LSGCDVLVQALLELVSALVHILGSSSIGQINYAAS

Fig. S1 The partial primary amino acid sequence for *A. aurantia* major ampullate spidroin 1 and 2 (MaSp1 and MaSp2).[1] Sequences were obtained from GenBank AAK30591 and AAK30592, respectively. Poly(Ala) and poly(Gly-Ala) motifs that are proposed to form β-sheet domains are underlined.

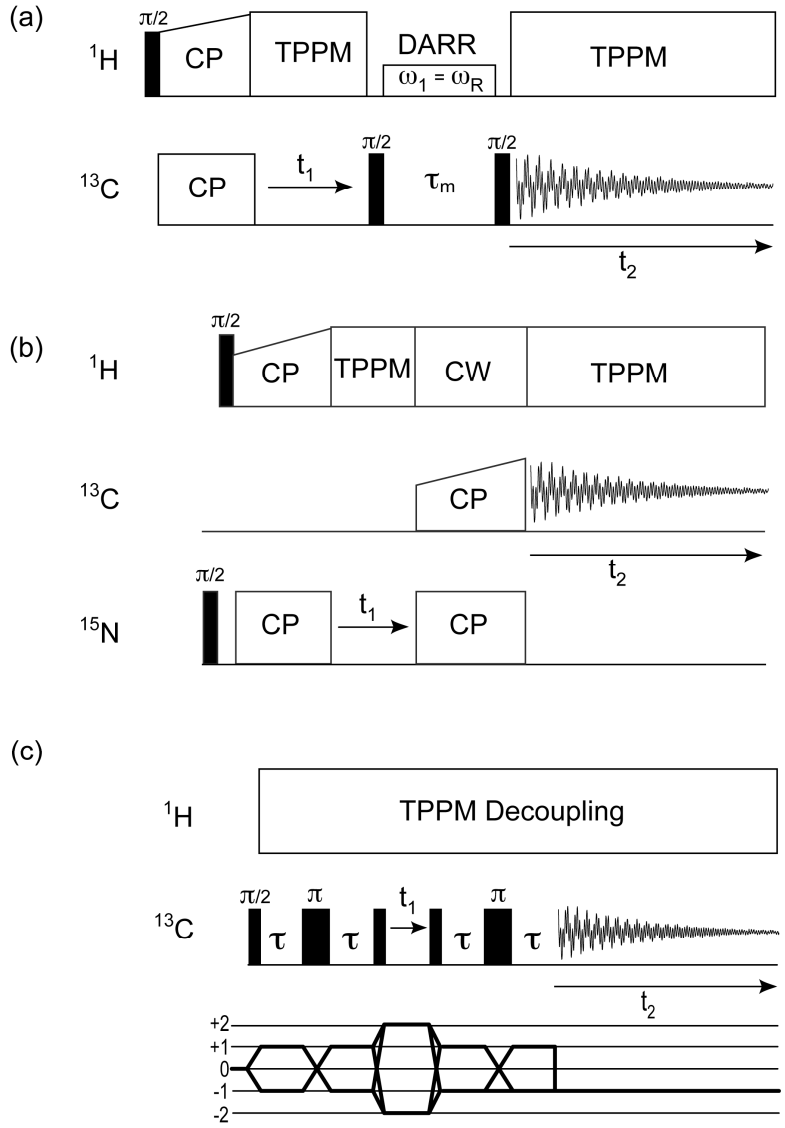

Fig. S2 The NMR pulse sequences for collecting two-dimensional (2D) (a) ^{13}C–^{13}C correlation spectra with dipolar assisted rotational resonance[2] (DARR) recoupling, (b) ^{15}N–^{13}C correlation spectra with double cross polarization[3] (DCP), and (c) refocused INADEQUATE spectra with direct ^{13}C excitation.[4] The coherence transfer pathway is shown below the pulse sequence (c) for the INADEQUATE experiment. For 2D ^{13}C–^{13}C spectra, the typical experimental parameters were a 1 ms CP contact time, 10 kHz MAS, and DARR continuous wave (CW) irradiation during the mixing period (τ_m) with a ^{1}H radio frequency (rf) field strength (ω_1) equal to the MAS rotation frequency (ω_R). The 2D ^{15}N–^{13}C correlation spectra were collected with a 2 ms initial ^{1}H→^{15}N CP step, a 1 ms ^{15}N→^{13}C CP step, and 18 kHz MAS. For the refocused INADEQUATE spectra the τ delays were set to 3.5 ms with 20 kHz MAS. Two pulse phase modulated (TPPM) ^{1}H decoupling was applied during acquisition in all experiments with a 100 kHz rf field strength. All NMR data was collected on a 400 MHz Varian VNMRS spectrometer equipped with a 3.2 mm triple resonance MAS probe.

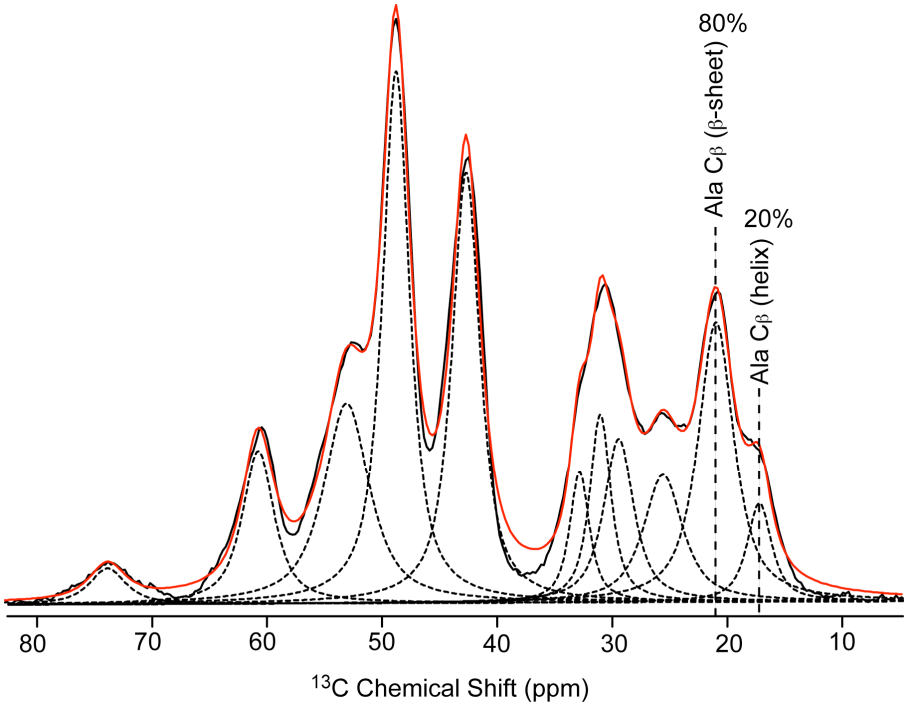

Fig. S3 The $^{1}H \rightarrow ^{13}C$ CP-MAS NMR spectrum of $^{13}C/^{15}N$-alanine labeled *A. aurantia* dragline silk was collected to confirm that poly(Ala) forms a β-sheet structure in a MaSp2-rich silk. The Ala C_β is fit to extract the fraction of Ala in a β-sheet conformation. The NMR spectrum is shown in black, the fit is dashed and the sum of the fit in red. The fit yields an Ala β-sheet component of 80 ± 5% similar to MaSp1-rich dragline silks from *N. clavipes* spiders.[4-6] It was confirmed from 2D ^{13}C-^{13}C correlation experiments that the resonance assigned to Ala C_β does not have any appreciable contributions from Leu or Val (data not shown). This is consistent with previous results on *N. clavipes* dragline silk where $^{13}C/^{15}N$-alanine labeling was found to enrich Ala, Gly, Ser and Gln with no detectable ^{13}C-enrichment observed for Leu or Val.[6]

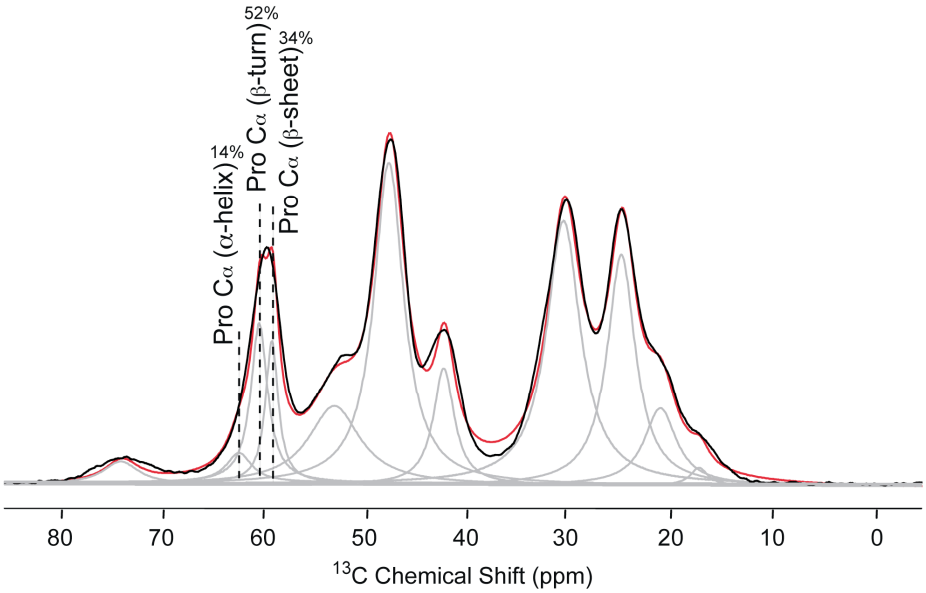

Fig. S4 The $^{1}H \rightarrow ^{13}C$ CP-MAS NMR spectrum of $^{13}C/^{15}N$-proline labeled *A. aurantia* dragline silk collected with a 1 ms contact time was fit to extract the fraction of Pro in α-helical, β-turn and β-sheet conformations from the Pro C_α resonance. The Pro C_α chemical shift ranges for different secondary structures were obtained from empirical shielding surface plots.[7] The secondary chemical shift ranges and corresponding torsion angles from the shielding surface are +1.5 ± 1.5 ppm (ϕ = -20 to -135° / ψ = -90 to 20°), -0.5 ± 1.0 ppm (ϕ = -30 to -150° / ψ = 90 to 180°), and -1.75 ± 0.75 (ϕ = -90 to -180° / ψ = 90 to 180°) for α-helix, β-turn and β-sheet, respectively. The NMR spectrum is shown in black, the fit in gray and the sum of the fit in red. This fit estimates that greater than 50% of the Pro exhibit chemical shifts that correlate to β-turn torsion angles.

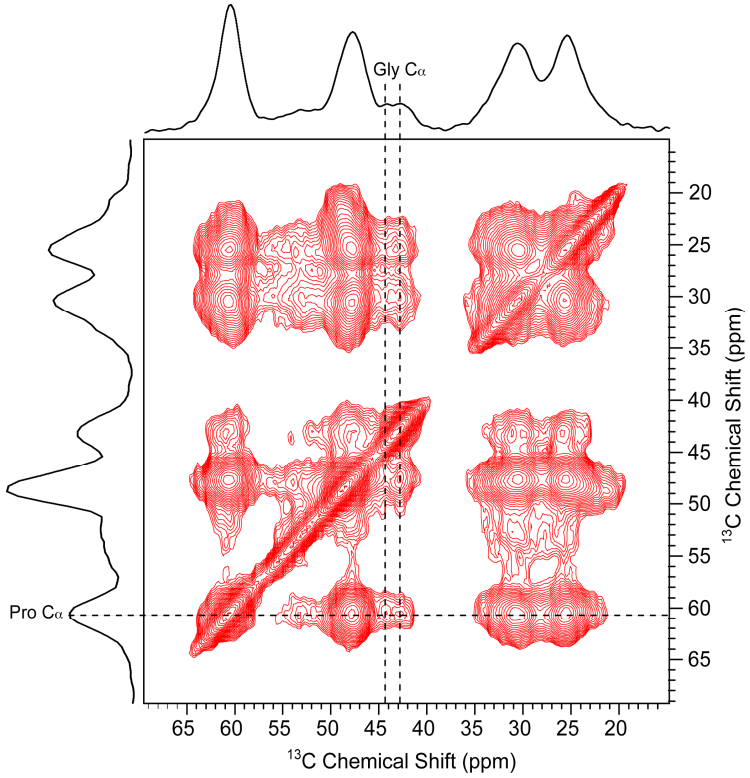

Fig. S5 The 2D ^{13}C-^{13}C correlation spectrum of $^{13}C/^{15}N$-proline labeled *A. aurantia* dragline silk collected with a 1 s DARR mixing period. With this long DARR mixing time, intermolecular correlations are detected between amino acids. A strong Pro C_α-Gly C_α correlation is observed that provides conformational information regarding Gly in Gly-Pro-Gly units. Close inspection of the contour plot and slice extracted at the Pro C_α resonance (top projection) reveals two Gly populations centered at 44.3 and 43.0 ppm. This indicates that Gly present in Gly-Pro-Gly units adopts two different conformational environments. The component centered at 44.3 is consistent with a β-sheet secondary structure[8] while, the component at 43.0 ppm exhibits a secondary shift of +0.2 ppm. This secondary shift is consistent with two regions of the shielding surface where the torsion angle ϕ = -100 to -65° and ψ = -30 to 0 or ϕ = 60 to 100° and ψ = -20 to 30°.[7] The latter set of torsion angles is consistent with the torsion angles for Gly in the i+2 position of the type II β-turn structure presented in Fig. 1c. The β-turn forming Gly-Pro-Gly motif often flanks poly(Ala) that forms a β-sheet structure (see Fig. S1, MaSp2) thus, one Gly in the Gly-Pro-Gly motif forms the β-turn while, the other is incorporated in the β-sheet domain.

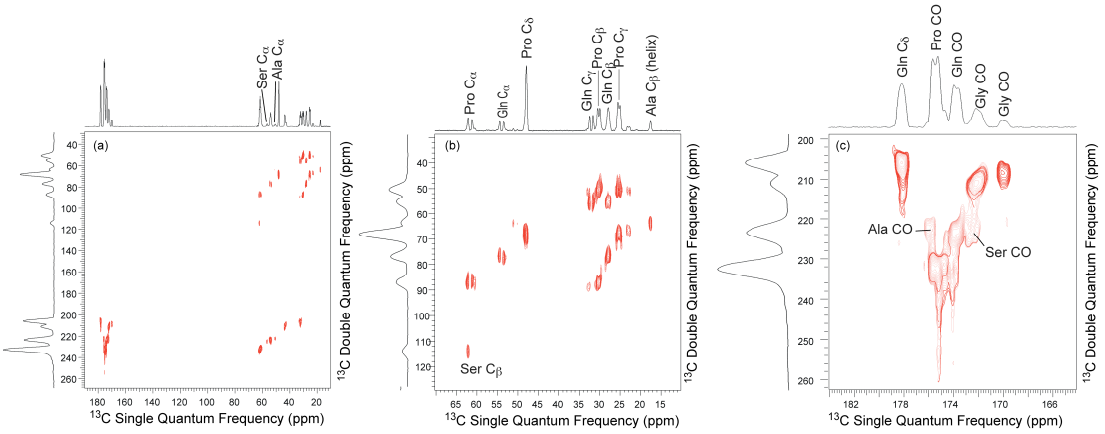

Fig. S6 Two-dimensional ^{13}C double quantum/single quantum (DQ/SQ) INADEQUATE spectrum for *A. aurantia* dragline silk plasticized with water. The (a) full spectral range, (b) up-field alkyl region and (c) down-field carbonyl region of the spectrum are shown. The spectrum was collected with direct ^{13}C excitation and 1 s recycle delay to excite mobile regions of the silk. For all other experimental details see Fig. S2. This 2D through-bond DQ/SQ correlation spectrum was used to assign the ^{13}C direct spectrum of water-wetted *A. aurantia* dragline silk.

Table S1. Proline ^{13}C chemical shifts (ppm from TMS) for *A. aurantia* dragline silk, other biopolymers with known structures, and random coil (RC) conformation[8-11]

Proline Site	*A. aurantia* (dry)	*A. aurantia* (wet)	$(Pro)_n$ 3_1-helix	$(Pro)_n$ 10_3-helix	Elastin	Collagen	VPGVG	RC
Pro C_α	60.7	61.6	58.3	58.7	60.0	58.2	60.8	61.9
Pro C_β	30.5	30.2	28.1	32.0	29.9	29.1	30.5	30.6
Pro C_γ	25.4	25.3	25.1	22.9	24.6	24.1	-	25.6
Pro C_δ	47.5	47.9	47.0	47.8	48.2	47.1	-	48.3
Pro CO	174.8	175.4	170.5	171.2	171.8	173.9	-	174.1

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