Electronic Supplementary Material (ESI) for Chemical Communications

Determining hydrogen-bond interactions in spider silk with ^{1}H-^{13}C HETCOR fast MAS solid-state NMR and DFT proton chemical shift calculations.

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Supplementary Information

Sample collection:

Major ampullate spider silk (dragline) was collected from *Nephila clavipes* spiders with the forcible silking method.[1,2] The silk was collected every other day for 1 hour under a dissection microscope to ensure only major ampullate silk was collected. During silk collection, the spiders were fed 80-100 μL of an aqueous solution of 3-^{13}C-L-alanine (Cambridge Isotopes Inc.). The alanine concentration was just below saturation (~150 mg/mL). Major ampullate spider silk was collected over the course of 1 month. Since silk samples collected during week three displayed the highest ^{13}C enrichment, they were used in the present solid-state NMR study. It should be noted that feeding 3-^{13}C-L-alanine results in ^{13}C enrichment of Gly, Ala, Gln and Ser.[2]

Solid-state NMR :

Solid-state NMR data was collected on an 800 MHz Varian VNMRS spectrometer equipped with a 1.6 mm triple resonance MAS probe configured for $^{1}H/^{13}C/^{15}N$. The NMR pulse sequence for collecting two-dimensional (2D) ^{1}H–^{13}C heteronuclear correlation (HETCOR) spectra with very fast (ν_R = 40 kHz) magic angle spinning (MAS) is shown in Fig. S1. Typical experimental parameters were a 2 μs ^{1}H π/2 pulse, a 1 ms cross polarization (CP) contact time with a ^{1}H radio frequency (rf) field strength of 125 kHz and the ^{13}C channel matched to the -1 spinning sideband (ssb) in the Hartmann-Hahn profile (^{13}C rf field strength = 85 kHz). A 15% ramp was applied on the ^{1}H channel during CP. Two pulse phase modulated (TPPM) ^{1}H decoupling was applied during acquisition in all experiments with a 150 kHz rf field strength and 12° phase shift. The sweep width in the ^{13}C dimension was 100 kHz and 128 transients were collected with a recycle delay (d1) of 5 s. The sweep width in the indirectly detected ^{1}H dimension was set to 20 kHz (rotor-synchronized) and 32

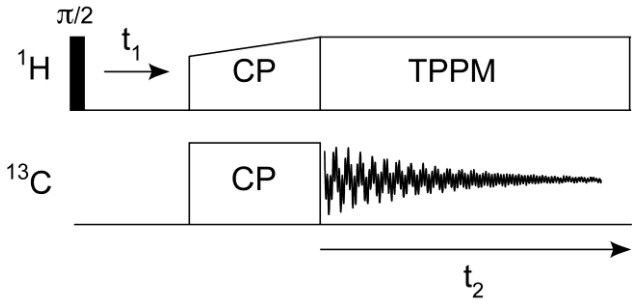

Fig. S1 The NMR pulse sequence used to collect 2D ^{1}H–^{13}C HETCOR spectra with very fast MAS.

complex t_1 points were collected with the States method. The ^{1}H and ^{13}C dimensions were indirectly referenced to TMS by setting the high ppm ^{13}C resonance and single ^{1}H resonance of adamantane to 38.56 ppm and 1.63 ppm, respectively.

DFT Proton Chemical Shift Calculations:

Proton NMR chemical shift calculations were performed using B3LYP density functional theory (DFT) and the 6-31++G(2d,2p) basis set in Gaussian09 similar to previously described approaches.[3,4] The Gly-Gly β-sheet model (Fig. S2, a) was built manually in GaussView 5. First, a single strand was constructed followed by geometry optimization in Gaussian 09 using the GIAO method with the above stated basis set. Stability was checked with the same basis set following geometry optimization. Based on the

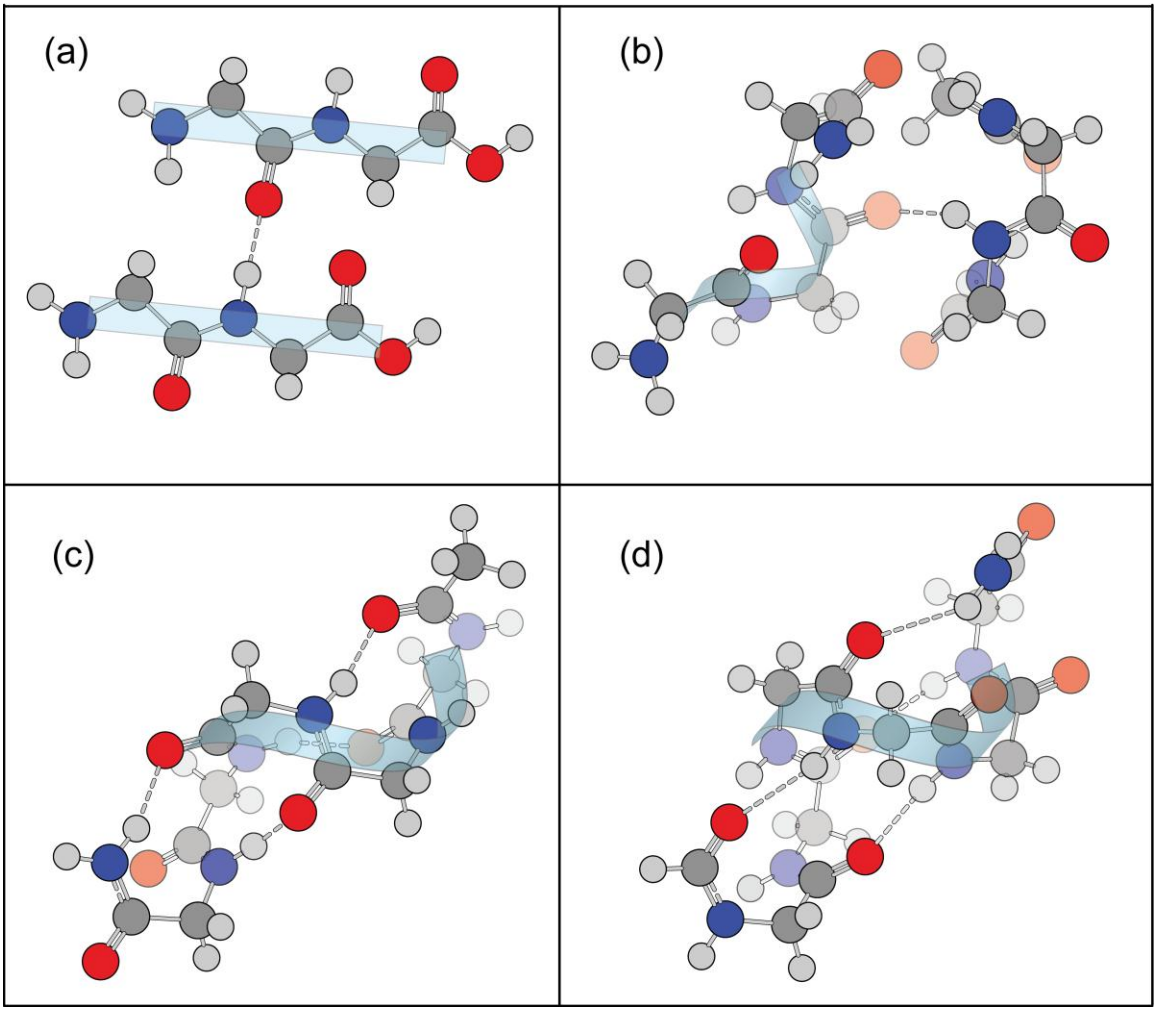

Fig. S2 The protein backbone models used for DFT proton chemical shift calculations, (a) Gly-Gly β-sheet model with inter-strand hydrogen bond, (b) 3_{10}-helical model with inter-strand hydrogen-bond, (c) 3_{10}-helical model with intra-strand hydrogen-bonding and (d) α-helix with intra-strand hydrogen-bonding.

optimized single strand structure, the Gly-Gly β-sheet model with inter-strand hydrogen-bonding was constructed by duplicating the single strand model. For the β-sheet hydrogen-bonding trend, the inter-strand NH···OC hydrogen-bond length was varied from 1.7 to 2.7 Å and the corresponding NMR chemical shifts were calculated. The calculated chemical shift was calibrated with the TMS proton magnetic shielding that is built into the Gaussian 09 database.

The 3_{10}-helix and α-helix models were obtained from the Lorieau Research Group website in the Department of Chemistry at The University of Illinois at Chicago.[5] These helical models were generated with the Hydrogen-Bond Database[6] and XPLOR-NIH.[7,8] Original helix structures were truncated to smaller helical models. Optimization and stability check were carried out in a similar fashion to the β-sheet model discussed above. For the inter-helix calculation, two small 3_{10}-helical strands measuring approximately two repeat units were constructed with one inter-helix hydrogen-bond (Fig. 2S, b). This inter-strand hydrogen-bond is similar to that first observed by Crick and Rich in the 3_1-helical structure of polyglycine II.[9] The NH···OC length was varied from 1.7 to 2.3 Å and corresponding NMR chemical shifts were calculated. For the 3_{10}-helix (Fig. 2S, c) and α-helix (Fig. 2S, d) intra-bond calculation, larger structures with approximately five repeat units were used. A two-stage geometry optimization was carried out with HF 3-21G and B3LYP 6-31G++ basis sets. NMR chemical shift calculations were then carried out as described above. The NH···OC intra-strand hydrogen-bond lengths varied between 2.0 to 2.4 Å for the 3_{10}-helix and α-helix depending on the environment. This variability is believed to occur because of the intrinsically different hydrogen-bonding sites in the two helical conformations. The amide N-H bond length remained very close to the theoretical value of 1.00 Å and varied by less than 0.3% for all structures following geometry optimization.

To illustrate the amide proton chemical shift trend as a function of hydrogen-bond distance, the calculated proton amide chemical shift data was fit to an equation of the form $\delta_{NH} = ad^{-3} + b$, where δ_{NH} is the amide proton chemical shift and d is the NH···OC hydrogen-bond distance. The calculated amide proton chemical shift data is included in Fig. 3 along with the fits and follow the expected trend.

The backbone Hα chemical shifts are known to depend on conformation and were also tabulated from the DFT proton chemical shifts calculations for the different structures. The average Hα proton chemical shift was 4.7, 4.1 and 3.7 ppm for the β-sheet, 3_{10}-helix and α-helix secondary structures. This is in agreement with experimentally determined Hα chemical shifts for various solid polypeptides with ^{1}H combined rotation and multiple pulse spectroscopy (CRAMPS) NMR where α-helical structures gave 3.9-4.0 ppm shifts while, β-sheet forms were 5.1-5.5 ppm.[10] Interestingly, results from our calculations indicate that it may be possible to distinguish between the 3_{10} and α-helix since nearly all the Hα shifts for the 3_{10}-helix were centered at 4.1 ppm with essentially no deviation and the α-helix ranged from 3.4-3.8 ppm with an average of 3.7 ppm.

Notes and references

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