**Electronic Supplementary Information**

# Elucidating proline dynamics in spider dragline silk fibre using $^{2}H$-$^{13}C$ HETCOR MAS NMR

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**Materials and Methods**

*Sample Preparation*

*A. aurantia* dragline silk was collected from adult female spiders using the forciable silking method as described in previous studies.[S1] The silking process was monitored under a microscope to avoid contamination from minor ampullate silk. The U-[$^{2}H_{7}$,$^{13}C_{5}$,$^{15}N$]-Pro labeled dragline silk were obtained from *A. aurantia* spiders fed with 30-40 μl 16% (w/v) U-[$^{2}H_{7}$,$^{13}C_{5}$,$^{15}N$]-Pro (Cambridge Isotopes, Andover, MA) during every silking process. The spiders were fed with crickets once a week. The dry silk sample refers to material used without any further processing after collecting from spiders. Supercontracted (wet) silk samples were soaked in deuterium-depleted water for at least 2 hours to achieve saturation prior to NMR experiments.

*NMR Spectroscopy*

Solid-state NMR experiments were conducted on a Varian VNMRS 400 MHz spectrometer equipped with a 3.2 mm MAS probe operating in triple resonance mode ($^{1}H/^{13}C/^{2}H$) at room temperature (25 °C). Sample heating due to MAS results in an actual sample temperature of 30 °C (temperature was calibrated with lead nitrate[S2]). $^{2}H$-$^{13}C$ MAS HETCOR and $^{2}H$ $T_1$ indirect detection experiments were set up as described in a recent publication.[S3] In the $^{2}H$-$^{13}C$ HETCOR experiments, 91 kHz (2.75 μs π/2 pulse) $^{2}H$ rf field was employed and a ~2 kHz $^{2}H$ GARP decoupling was utilized during signal acquisition. The $^{13}C$ and $^{2}H$ chemical shifts were indirectly referenced to the adamantane downfield resonance at 38.56 ppm and the $D_2O$ resonance at 4.788 ppm, respectively. Experiments were performed using 10 kHz MAS spinning rate and the following additional parameters:

- 2D $^{2}H$-$^{13}C$ HETCOR CP-MAS experiment: 25 kHz sweep width in $^{13}C$ dimension, 500 kHz sweep width with 256 $t_1$ points in $^{2}H$ dimension, 40.96 ms acquisition time, 2 ms $^{2}H$-$^{13}C$ CP contact time, a recycle delay of 2.5 s and 224 scans. In order to obtain $^{2}H$-$^{13}C$ chemical shift correlation spectrum with higher resolution, another 2D $^{2}H$-$^{13}C$ HETCOR CP-MAS experiment with rotor-synchronized sampling in $^{2}H$ dimension was performed using 10 kHz sweep width with 64 $t_1$ points in $^{2}H$ dimension.

- $^{13}$C-detected $^{2}$H $T_1$ measurement though $^{2}$H-$^{13}$C HETCOR CP-MAS: A composite π pulse ($(\pi/4)_x$-$(\pi)_y$-$(\pi/2)_x$-$(\pi)_y$-$(\pi/4)_x$) is used as the $^{2}$H inversion pulse in the pulse sequence.[S3] The additional experimental parameters were 25 kHz sweep width, 40.96 ms acquisition time, 2 ms $^{2}$H-$^{13}$C CP contact time, 5 s recycle delay time and 512 scans.

- $^{2}$H solid-echo MAS experiment: 500 kHz sweep width, 40.96 ms acquisition time, a recycle delay of 3 s and 2944 scans. The rotor-synchronized delay between the two π/2 pulses was 100 μs (rotor-synchronized for 10 kHz MAS). 20 μs delay was set between the second π/2 pulse and the beginning of acquisition. The acquired FID was left shifted 46 points to the echo maximum prior to Fourier transform in order to correct spectrum phase.

- $^{2}$H one-pulse MAS experiment: 500 kHz sweep width, 40.96 ms acquisition time, a recycle delay of 4 s and 2944 scans. 20 μs delay was set between the π/2 rf pulse and the beginning of acquisition. During data process, the raw FID was left shifted 49 points to eliminate spectrum phase distortion caused by probe ringdown.

1D $^{2}$H liquid-state NMR data was collected on an Agilent Inova 500 MHz spectrometer equipped with a 5 mm Inverse Detect Broadband PFG Probe. The experiment was performed on U-[$^{2}H_7$,$^{13}C_5$,$^{15}N$]- Pro labeled silk. About 1 mg silk sample was hydrolyzed in 6 M HCl for 2 days under 108 °C, then reconstituted in 700 μl $H_2O$ and transferred to 5 mm NMR tube. Data was acquired using the lock channel. The NMR pulse sequence is a π/2 deuterium excitation pulse followed by data acquisition. Additional experiment parameters are: 1535.63 Hz sweep width, 2.049 ms acquisition time and a recycle delay of 5s.

*$^{2}$H NMR line shape simulation*

Deuterium MAS quadrupole line shapes were simulated using SPINEVOLUTION 3.4.4 software packages.[S4] All simulations were conducted with the ASG powder orientation calculation scheme. For proline in dry silk, the MAS line shapes were calculated using a $CD_2$ group undergoing fast two-site reorientations with $C_Q$=170 kHz and η=0.[S5] Simulations were performed for various two-site reorientation angles to find the best one matching with experimental data for the different $CD_2$ groups on the proline sidechain. A deuterium three-

site reorientation model was used to simulate the microsecond proline backbone motion occurring in supercontracted (wet) silk. The averaged EFG principle axis system was set to reorient between (θ, 0), (θ, 120) and (θ, 240). Simulations were performed with various θ and reorientation rates and compared with experimental data to find the best match.

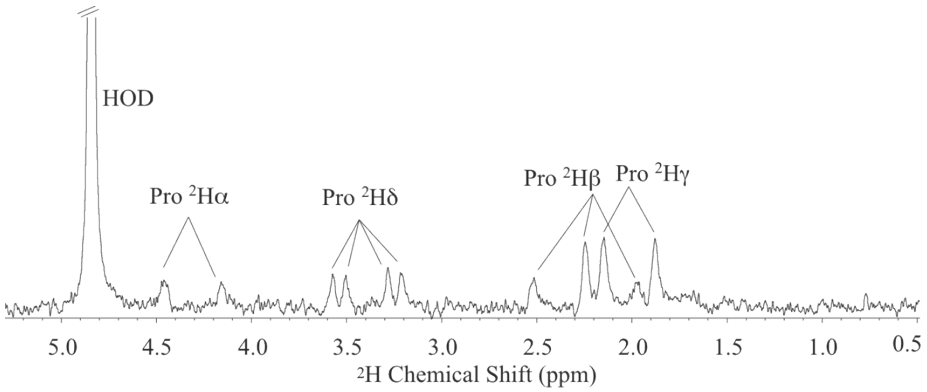


**Fig. S1** Liquid-state $^{2}H$ spectrum of hydrolyzed U-[$^{2}H_{7}$,$^{13}C_{5}$,$^{15}N$]-Pro labeled *A. aurantia* dragline silk. Silk collected from spiders fed with U-[$^{2}H_{7}$,$^{13}C_{5}$,$^{15}N$]- Pro aqueous solution. One-bond $^{2}H$-$^{13}C$ *J*-splitting peak patterns are observed for Pro α, β, γ and δ groups. The corresponding $J_{^{2}H\text{-}^{13}C}$ constant is 23.4 Hz, 20.9 Hz, 20.6 Hz and 22.6 Hz for α, β, γ and δ group, respectively. The *J*-splitting effect (5.5 Hz) caused by $^{15}N$ is also observed for $^{2}H$δ. These *J*-splitting peak patterns indicate that Pro is selectively labeled by $^{2}H$-$^{13}C$ pairs at the different sites and no additional deuterium labeling occurred for any other amino acid to any appreciable extent.

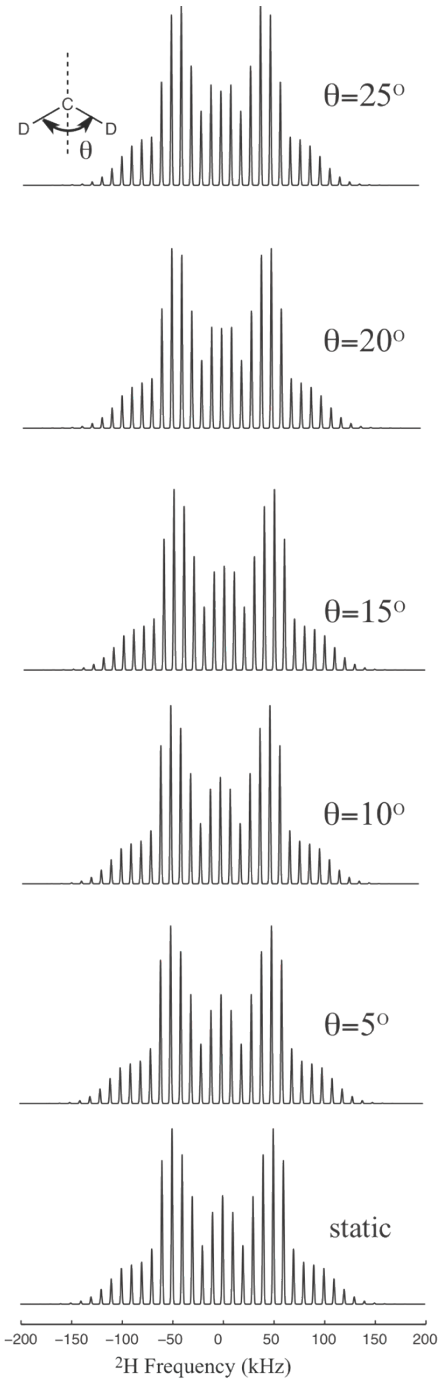


**Fig. S2** Simulated $^2$H quadrupole line shapes for deuterium undergoing fast reorientation between two sites separated by θ. Simulations were conducted using SPINEVOLUTION software[S4] with $C_Q$=170 kHz and η=0[S5]. In the current paper, Pro $^2$H molecular motions were proposed by comparing experimental deuterium line shapes with these simulations.

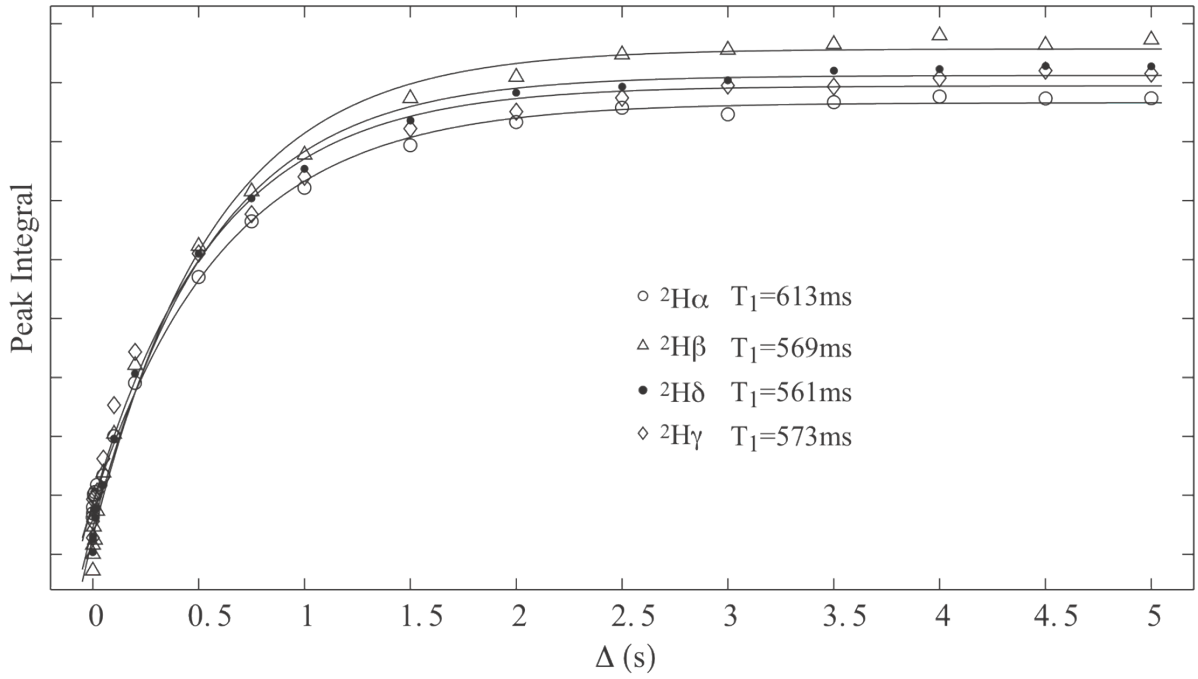


**Fig. S3** $^{13}$C-detected Pro $^2$H $T_1$ inversion recovery curves for U-[$^2H_7$,$^{13}C_5$,$^{15}N$]-Pro labeled *A. aurantia* dragline silk. The $T_1$ for each deuterium of Pro is shown in the figure.

**Calculating molecular motional rate using $^2H$ $T_1$**

The quadrupolar interaction is the dominant spin relaxation mechanism for deuterium in solids. When the interaction is reduced by molecular motion of the considered group, a specific mathematical relation can be developed between deuterium $T_1$ and the molecular motional rate. In present study, $^2H$ $T_1$s were used to calculate molecular motional rate for Pro deuterium that undergo reorientation between two sites separated by an angle (Θ). The calculation process is as follows.

For Pro deuterium which undergoes two sites reorientation,[S6]

$$1/T_1 = (\omega_Q^2/8)A_1\{\tau/(1+\omega^2\tau^2)[B_4 - (0.75B_1 - B_2)\cos 2\varphi] + \tau/(1+4\omega^2\tau^2)(4B_5 - 4B_2\cos 2\varphi)\}$$

where,

$$A_1 = \sin^2(2\Theta)$$
$$B_1 = \sin^2(2\theta)$$
$$B_2 = \sin^4(\theta)$$
$$B_4 = \cos^2(\theta) + \cos^2(2\theta)$$
$$B_5 = \sin^2(\theta) + 0.25\sin^2(2\theta)$$

Θ=12.5°, 17.5° and 7.5° for Pro $^2H\beta$, $^2H\gamma$ and $^2H\delta$, respectively, according to the $^2H$ line shapes determined in the current work. $\tau=(2k)^{-1}$ and k is the two sites reorientation rate. Pro $^2H$ quadrupolar patterns are independent of φ because the quadrupolar asymmetry parameter $\eta=0$[S5] ((θ,φ) are the polar angles that define the orientation of the external filed in the crystal-fixed coordinate system). Thus, for a specific θ, one would find,

$$1/T_1 = \frac{\int_0^{2\pi} (\omega_Q^2/8)A_1\{\tau/(1+\omega^2\tau^2)[B_4 - (0.75B_1 - B_2)\cos 2\varphi] + \tau/(1+4\omega^2\tau^2)(4B_5 - 4B_2\cos 2\varphi)\}d\varphi}{\int_0^{2\pi} 2\pi d\varphi}$$
$$= (\omega_Q^2/8)A_1[\tau/(1+\omega^2\tau^2)B_4 + \tau/(1+4\omega^2\tau^2)4B_5]$$

Using the experimentally measured $T_1$, the two sites reorientation rate can be calculated using the above equation for the case of a specific θ. In the current work, for each deuterium on Pro side chain, the two sites reorientation rate is considered as the average of the rates of two cases - θ=0 and θ=2π.

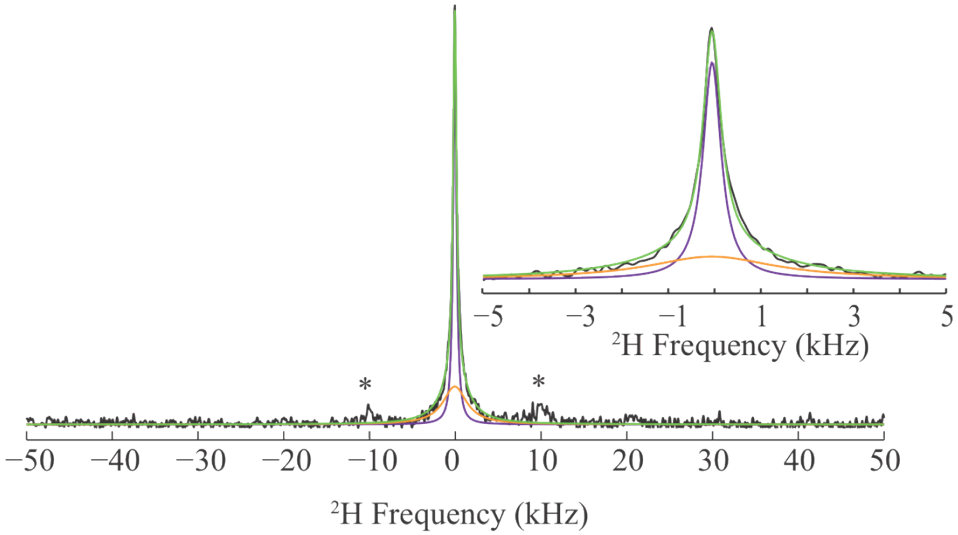


**Fig. S4** $^{2}H$ one-pulse spectrum (black) and its fit (green) for wet, supercontracted U-[$^{2}H_{7}$,$^{13}C_{5}$,$^{15}N$]-Pro labeled *A. aurantia* dragline silk. Two components were extracted from the fit – a broad peak (orange) and a narrow peak (purple). The asterisks mark the weak spinning sidebands. The fitting process is the same as that shown in Fig. 3 in the current article and the result is similar because the number of left shifts required to eliminate probe ring down were on the same time scale as the solid-echo delays (see Materials and Methods, above). The central peak is extremely broadened where the signal intensity is smaller than half maximum. The peak can not be fit with a single peak having Lorentzian, Gaussian or a combined line shape. This implies the existence of the broad component. The peak can be fit well to a combination of a broad (FWHM = 3.8 kHz) and narrow component (FWHM = 400 Hz) similar to the solid-echo data in the paper, Fig. 3. The fittings were performed using DMFit software to extract the narrow and broad component.[S7]

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