

SUPPORTING INFORMATION

for

Investigations of dynamic amyloid-like structures of the Wnt signalling pathway by solid-state NMR

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Materials and methods

Expression and purification of the Axin DIX domain. The human Axin 1a DIX domain (residues 778-862) was cloned into a PET24 expression vector. Axin-DIX proteins were expressed in *E. coli* Rosetta2 cells. For isotopically labelled samples, M9 medium was supplemented with ^{13}C glucose and $^{15}\text{NH}_4\text{Cl}$. Protein expression was induced in 1-l bacterial cultures at an OD_{600} of ~ 0.6 with the addition of IPTG to a final concentration of 0.5 mM. Protein expression continued at 18 °C for ~ 20 h. Bacterial pellets were resuspended in 50 mM NaH_2PO_4 (pH = 8), 1 M NaCl, 10 mM imidazole, 1 mM β -mercaptoethanol and 20 mg/L lysozyme and were subsequently lysed by sonication. Lysates were cleared by centrifugation (30 min, 4 °C, 25 000 g) and filtration (0.45 μm). Proteins were purified by nickel-affinity chromatography (Quiagen) using a batch procedure. Following elution, proteins were concentrated using an Amicon Ultra-15 10 K centrifugal filter. At high concentrations, the Axin-DIX domain self-associates and can be pelleted through ultracentrifugation and packed into a solid-state NMR MAS rotor.

Two phase system. Organic molecule extraction from the purified Axin-DIX domain was performed as has been described previously¹. To begin, 320 μl of ice-cold MeOH (containing 50 $\mu\text{g}/\text{ml}$ of BHT) was added to approximately 0.25 mg of protein, followed by the addition of 640 μl of ice-cold CHCl_3 . This mixture was then incubated on ice for 20 min with occasional vortexing before high purity water (300 μl) was added. The sample was then incubated on ice for an additional 10 min with occasional mixing. Next, the sample was centrifuged for 5 min at 2 000 g and the upper aqueous phase was removed and re-extracted by addition of 500 μl of ice-cold CHCl_3 :MeOH (2:1, v/v) as above. The upper phase was discarded and both organic phases were combined and dried under nitrogen gas. The dried phase was then resuspended in 200 μl CDCl_3 .

Magic angle spinning solid-state NMR spectroscopy. All experiments were collected on Avance III spectrometers (Bruker Biospin, Billerica, MA) operating at either 700.13 MHz or 950.20 MHz using 3.2 mm triple resonance MAS probes at a sample temperature of ~ 5 °C. For both spectrometers typical $\pi/2$ pulses were 2.5-3 μs for ^1H , 5 μs for ^{13}C , and 7 μs for ^{15}N .

Resonance assignments were obtained using a minimal set of three 3D experiments. CANCO, NCACX, and NCOCX experiments were collected on a narrow-bore Bruker Avance 700 MHz spectrometer with MAS at 13 kHz. The $^1\text{H}/\text{X}$ (where X is ^{15}N or ^{13}C) cross-polarization (CP)² contact times were 0.8-1 ms, with a constant radio frequency (rf) field of 35 and 50 kHz on nitrogen and carbon, respectively, while the proton lock field was ramped linearly around the $n = 1$ Hartmann/Hahn condition³. $^{15}\text{N}/^{13}\text{C}$ and $^{15}\text{N}/^{13}\text{CO}$ band-selective transfers⁴ were implemented with a contact time of 4-5 ms. For the $^{13}\text{CA}/^{15}\text{N}$ CP, a constant lock field of $2.5 \times \nu_r$ ($\nu_r = \omega_r/2\pi$, spinning frequency) strength was applied on ^{15}N , while the ^{13}C field was ramped linearly (10% ramp) around $1.5 \times \nu_r$. For the $^{13}\text{CO}/^{15}\text{N}$ transfer, a constant lock field of $3.5 \times \nu_r$ field strength was applied on ^{13}C , while the ^{15}N field was ramped linearly (10% ramp) around $2.5 \times \nu_r$. Continuous wave proton decoupling at 83 kHz was used during $^{15}\text{N}/^{13}\text{C}$ CP. SPINAL64⁵ decoupling optimized around 83 kHz was used during ^{15}N and ^{13}C direct and indirect chemical shift evolutions. For homonuclear ^{13}C - ^{13}C mixing, PARIS^{6,7} mixing of 30 and 50 ms for the NCACX and NCOCX experiments, respectively, were used.

2D ^{13}C - ^{13}C experiments were collected on a narrow-bore Bruker Avance 950 MHz spectrometer equipped with a 3.2 mm triple-resonance E-Free MAS probe. To confirm and extend the assignments obtained using three dimensional spectroscopy, two dimensional ^{13}C - ^{13}C spin diffusion experiments were obtained with MAS at 14.8 kHz⁸. In order to obtain distance restraints, a CHHC experiment with H-H mixing of 250 μs was obtained, as well as additional ^{13}C - ^{13}C experiments performed with MAS of 14 kHz and long mixing times with low-powered PARIS mixing (1 s). 2D ^{13}C - ^{13}C INEPT-TOBSY⁹ experiments were performed with a TOBSY mixing time of ~ 9 ms using the $\text{P}9^1_6$ mixing sequence.

For all experiments, the carbon chemical shifts were indirectly referenced to 2,2-dimethyl-2-silapentane-5-sulfonic acid (DSS) by adjusting the position of the ^{13}C adamantane downfield peak to 40.48 ppm¹⁰. Data processing was performed using the software NMRPIPE¹¹ or Topspin 3.5 (Bruker, Karlsruhe, Germany). Noise analysis, peak picking, and assignments were performed in the CARAM¹² environment.

Sets of sequential ^{13}C - ^{13}C peaks were generated using FANDAS¹³ and sets of peaks representing atoms which were close in space were generated using a homemade script. Inter-residue ^{13}C - ^{13}C peaks in the SD and PARIS spectra were assigned based off comparison to these sets.

Solution NMR. Reference ^1H - ^{13}C HSQC NMR spectra performed on DOPE were recorded at on an Avance 600 Bruker NMR spectrometer equipped with a cryogenic triple-resonance gradient probe. Spectra were recorded with spectral widths of 12/100 ppm, centered at 4.7/40 ppm, with 1,024/300 points in $^1\text{H}/^{13}\text{C}$, respectively. ^1H - ^{13}C HSQC NMR spectra performed on the organic phase of Axin-Dix samples were recorded on an Avance 900 Bruker NMR spectrometer equipped with a triple resonance probe. Spectra were recorded with spectral widths of 20/105 ppm, centered at 4.7/50 ppm, with 1,024/128 points in $^1\text{H}/^{13}\text{C}$, respectively. All spectra were recorded with a recycle delay of 1.0 s.

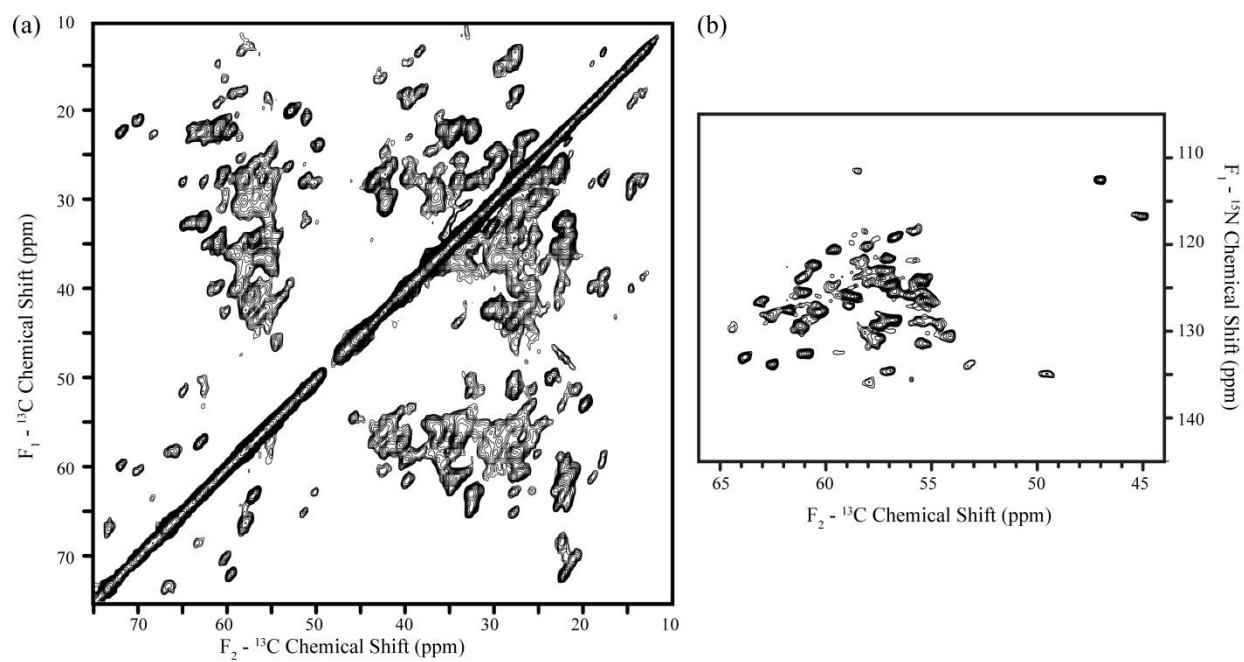


Fig. S1 (a) ^{13}C - ^{13}C PARIS (mixing time 30 ms) and (b) ^{15}N - ^{13}C spectra recorded on the isolated Axin-DIX domain

Table S1 Non-sequential peaks observed in 2D ^{13}C - ^{13}C and HCCH experiments on the Axin-DIX domain.

Atom 1			Atom 2		
2D ^{13}C - ^{13}C					
131.9	CG	F850	30.2	CD	K825
63.4	CA	V784	59.0	CA	I855
15.8	CD1	I783	14.2	CD1	I854
32.1	CB	V784	24.4	CG	K853
55.1	CA	Y788	17.5	CG2	I855
56.7	CA	E839	49.6	CD	P848
50.9	CA	A804	39.3	CB	I854
60.9	CA	V805	52.7	CA	L847
34.7	CB	V805	25.5	CG	L847
61.0	CA	V805	25.7	CG	L847
60.8	CA	I854	43.6	CB	F824
69.3	CB	T798	41.5	CB	L813
22.3	CG1	V840	19.4	CB	A845
44.7	CA	G856	33.1	CB	K825
41.8	CB	K825	17.5	CG2	I855
60.7	CA	I854	25.7	CG	L847
59.3	CA	V835	32.0	CB	V827
44.6	CA	G856	22.0	CD1	L847
44.9	CA	G856	25.7	CG	L847
59.3	CA	V835	23.7	CG	K826
69.6	CB	T798	55.2	CA	L813
62.5	CA	P848	42.0	CE	K826
39.4	CB	I855	34.0	CB	K825
38.6	CB	L847	28.6	CG1	I783
71.5	CB	T806	56.4	CA	E844
68.4	CB	T815	57.5	CA	E812
51.1	CD	P795	23.6	CB	A786
52.7	CA	A845	46.4	CA	G808
69.6	CB	T798	28.3	CB	E839
38.0	CB	I794	35.3	CB	V835
58.4	CA	L807	52.9	CA	L847
CHHC					
20.7	CB	A845	14.4	CD1	I854
71.5	CB	T806	62.7	CA	V846
59.2	CA	T806	31.7	CB	V846
71.5	CB	T806	56.4	CA	E844
43.4	CB	F824	18.2	CG2	I854
63.5	CA	V784	54.2	CA	L799
54.9	CA	Y787	50.9	CD	P795
63.9	CA	V827	59.4	CA	V835
53.9	CA	Y788	50.9	CD	P795
45.2	CA	G856	27.4	CG	P848
45.8	CB	L799	63.6	CA	V784
54.1	CA	L799	21.8	CG2	V784
49.4	CD	P848	43.4	CB	F824
39.4	CB	I855	34.0	CB	K825
43.0	CD	R841	22.3	CD2	L807
39.2	CB	I854	42.4	CB	I783
35.9	CA	V836	51.0	CB	P795

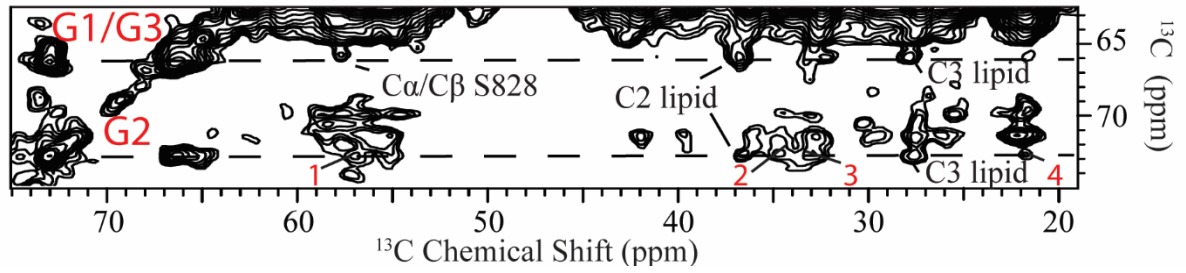


Fig. S2 Section of 2D ^{13}C - ^{13}C low-power PARIS spectrum with 1 s of mixing showing correlations between the glycerol backbone of the co-purifying lipid as well as intra-molecular lipid peaks. Possible assignments for the indicated peaks are given in **Table S2**.

Table S2 List of potential protein assignments for peaks indicated in **Fig. S2**. Atoms which are close in space to the interface are indicated in yellow, those which are close in space to E852 are indicated in blue, and those which are close to helix $\alpha 1$ are indicated in green

1		2		3		4	
CA	D843	CB	V805	CB	K811	CG1	V835
CA	E851	CB	V800	CB	R821	CG1	V800
CA	Y796	CB	V840	CB	E859	CG2	T806
CA	S819	CG	Q809	CB	P793	CG2	V840
CA	K 860	CB	V858			CG1	V836
CA	Y822					CG2	V784
CA	E839					CG2	V827
CA	R841						
CA	K857						
CA	E844						

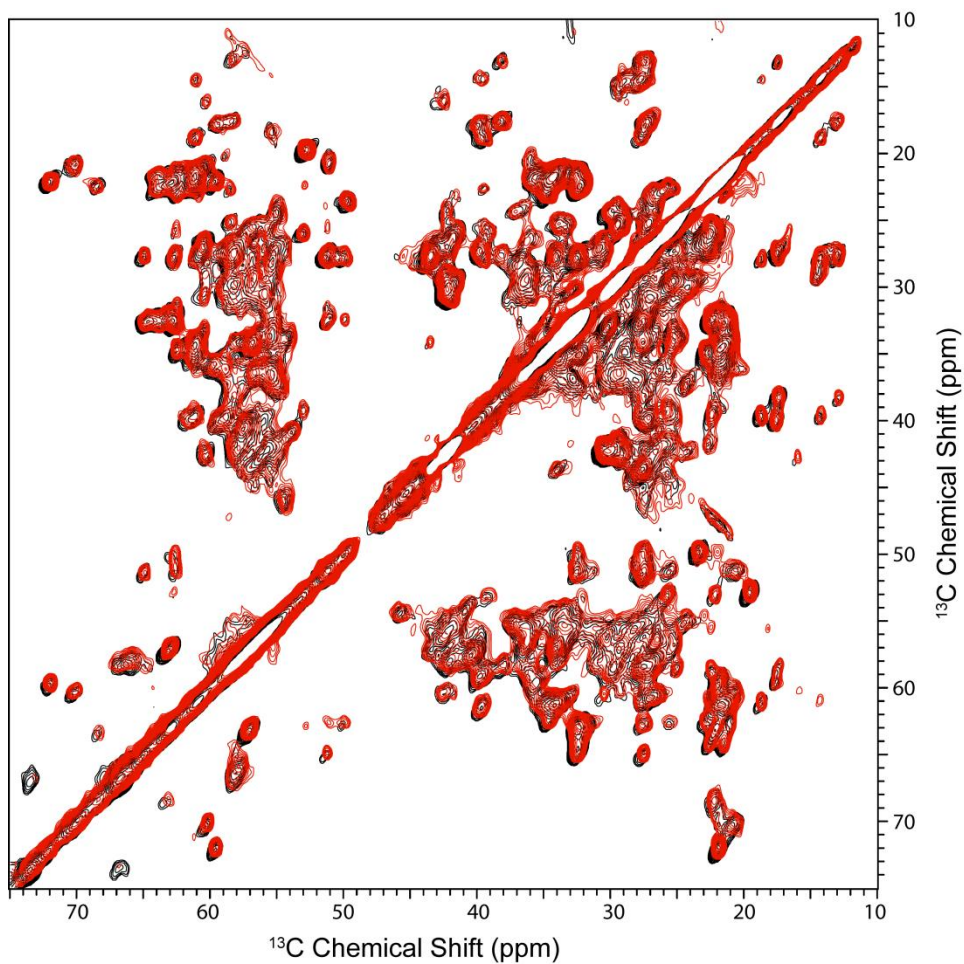


Fig. S3 2D ^{13}C - ^{13}C PARIS (mixing time 30 ms) spectra of the Axin-DIX domain collected at the beginning (black) and end of 3D experiments (red). While the glycerol backbone peak is clearly reduced in the second spectrum, no other significant perturbations to the spectra are observed.

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