Supporting Information

Solid-state NMR at Natural Isotopic Abundance for the Determination of Conformational Polymorphism– The Case of designed β-turn peptides containing di-prolines

Jayasubba Reddy Yarava,[a,c,d,], Rajesh Sonti,[b,c,e], K. Kantharaju[b,f] S. Raghothama[c,*] and K.V. Ramanathan[c,*]

[a]Department of Physics, [b]Molcular Biophysics Unit, [c]NMR Research Center
Indian Institute of Science, Bangalore-560012, India.

Materials and Methods

The peptides Piv-^{L}Pro-ψ^{H,CH3}Pro-Leu-NHMe (Peptide – 1) and Piv-^{D}Pro-ψ^{CH3,CH3}Pro-Leu-NHMe (Peptide – 2) were synthesized using the protocol known already.[1] NMR spectra of the samples were recorded on a Bruker Avance-III 500WB NMR spectrometer operating at a proton resonance frequency of 500.17 MHz. All experiments were performed at room temperature using a Bruker 4 mm triple resonance MAS probe, operating in double resonance mode. Sample spinning speeds employed were typically 11 kHz. Carbon-13 magnetization was generated by cross polarization with a rf amplitude ramp on the ^{1}H channel with a contact time of 2.0 ms. SPINAL-64[2] heteronuclear dipolar decoupling was employed throughout with an acquisition time of 40.0 ms. A proton rf filed amplitude of 62.5 kHz was used 64 and 32 transients were co-added with a recycle delay of 5s employed for peptide-1 and 2 respectively. The assignment of all the proton, carbon and nitrogen resonances were carried out with the help of a set of 1D and 2D correlation experiments. The chemical shifts of nuclei relevant for the present study are given in Tables S1 to S3.

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* Authors for Correspondence
Current address:
d) Institut des Sciences et Ingènierie Chimiques, EcolePolytechniqueFèdèrale de Lausanne, Switzerland.
e) Biozentrum, Structural Biology, Universität Basel, Basel, Switzerland.
f) Department of Chemistry, Rani Channamma University, Belgavi, India.

2D ^{1}H (DQ) – ^{13}C (SQ) Correlation Experiment

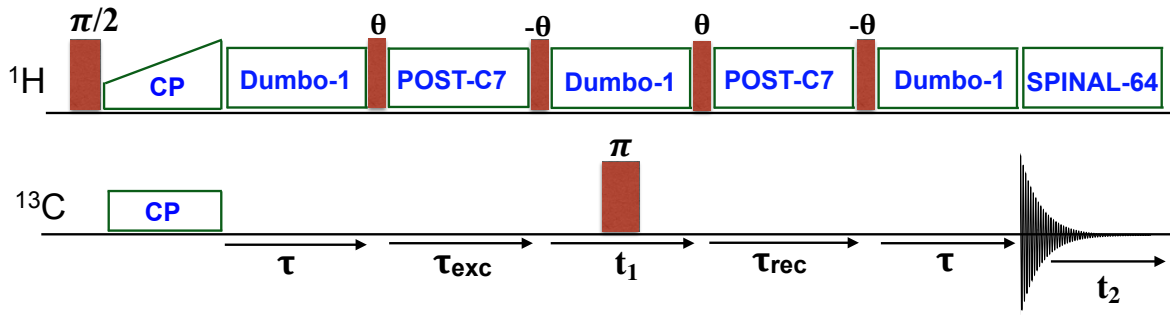

Figure S1: The pulse sequence for the two-dimensional MAS-J-^{1}H(DQ)-^{13}C-HMQC correlation experiment.

The two-dimensional MAS-J-^{1}H (DQ)-^{13}C-HMQC pulse sequence[3] used for peptide-2 is shown in **Figure S1**. The phase modulated decoupling scheme DUMBO-1[4] homonuclear decoupling with rf filed amplitude of 100 kHz was applied in the τ and t_1 periods. The t_1 increment was set to 32 µs corresponding to one basic DUMBO-1 cycle. The τ period was set to 2.0 ms. The POST-C7[5] recoupling was used for exciting DQ coherences and also for reconversion periods with the MAS of 11 kHz. 77 kHz rf power was used for the POST-C7 sequence. Two basic POST-C7 blocks were used with duration of 52 µs long for the DQ excitation and reconversion. 448 scans were used for each of 188 TD points with recycle delay of 4.2 s between scans. Resulting total experimental time was 98.2 h. Spectra were acquired in the State-TPPI method.[6]

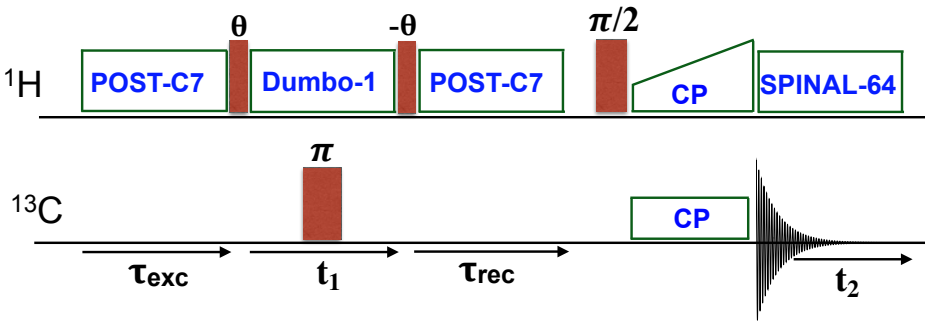

Figure S2: Pulse sequence for the two-dimensional ^{1}H(DQ)-^{13}C-HETCOR experiment.

For peptide-1, the 2D ^{1}H DQ-^{13}C HETCOR experiment[7] was employed which gave better S/N. The DUMBO-1 homonuclear dipolar decoupling was used for the t_1 period. The CP contact time was set to 100 µs. For each of 256 t_1 increments 64 transients were co-added with a recycle delay of 4s, resulting total experimental time of 18.2h. All the other parameters were same as for peptide-2.

Assignments:

Peptide-1, Piv-^{L}Pro- ψ^{H,CH3}Pro-Leu-NHMe

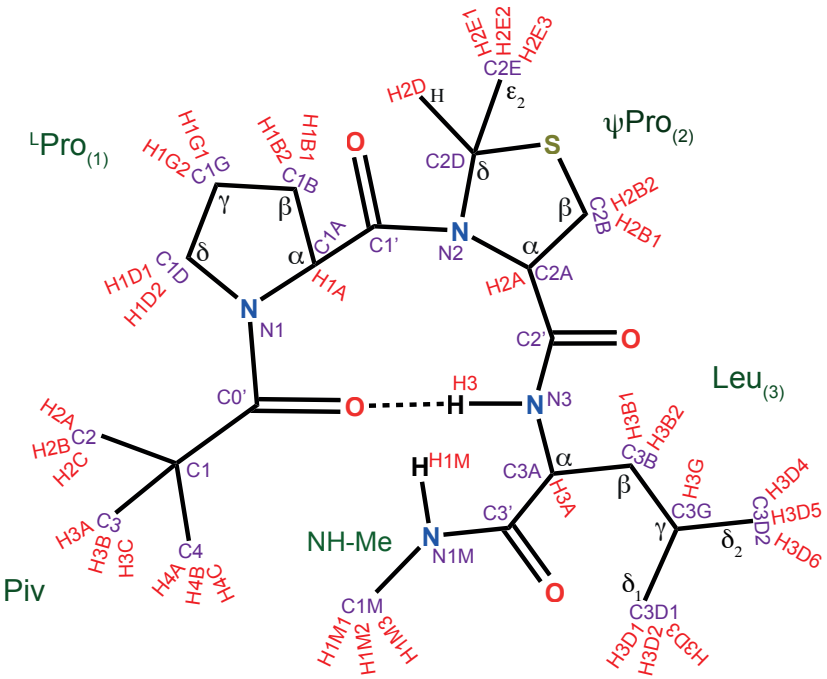

Table-S1: ^{1}H and ^{13}C chemical shifts of nuclei across the peptide bond in the diproline segment of Peptide -1

Peptide-I Residues	Atom label	Chemical shift (ppm)
Pro$_{(1)}$ α	C1A	59.7
ψPro$_{(2)}$ α	C2A	62.2
ψPro$_{(2)}$ δ(CH)	C2D	60.4
ψPro$_{(2)}$ ε met.	C2E	27.8
Pro$_{(1)}$ α	H1A	4.6
ψPro$_{(2)}$ α	H2A	5.5
ψPro$_{(2)}$ δ (CH)	H2D	5.3
ψPro$_{(2)}$ ε met.	H2E1, E2, E3	0.7

Peptide-2, Piv-^{D}Pro-ψ ^{CH3,CH3}Pro-Leu-NHMe

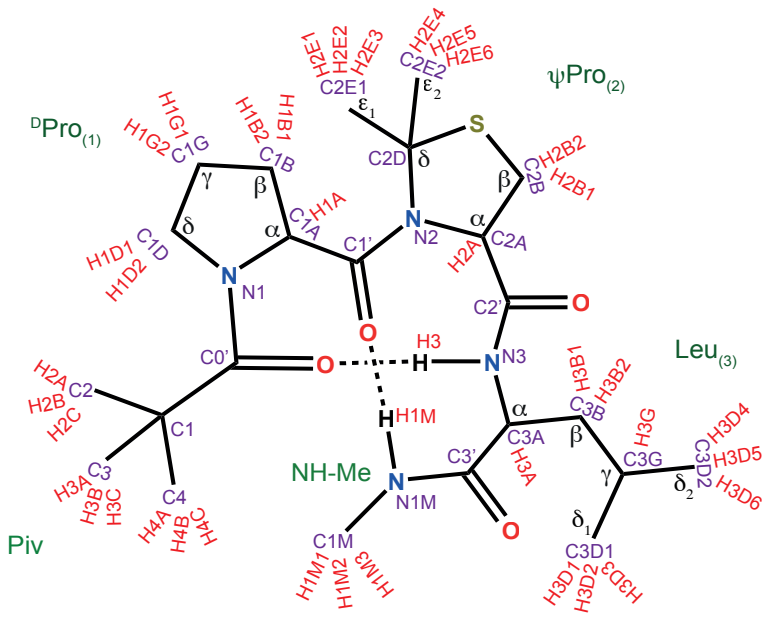

Table-S2: ^{1}H and ^{13}C chemical shifts of nuclei across the peptide bond in the diproline segment of Peptide -2

Peptide-II Residues	Atom label	Chemical shift **Mol. A , Mol. B** (ppm)
^{D}Pro$_{(1)}$ α	C1A	61.85, 61.85
ψPro$_{(2)}$ α	C2A	66.6, 66.6
ψPro$_{(2)}$ ε$_1$, ε$_2$ Met	C2E1, C2E2	28.1, 28.1
^{D}Pro$_{(1)}$ α	H1A	3.6, 3.6
ψPro$_{(2)}$ α	H2A	5.4, 6.1
ψPro$_{(2)}$ ε$_1$, ε$_2$ Met	H2E1, H2E2, H2E3, H2E4, H2E5, H2E6	0.7, 0.7

Table-S3: H$_N$ proton chemical shifts of peptide-2

Peptide-II Residues	Atom label	Chemical shift **Mol. A , Mol. B** (ppm)
Leu NH	H3	7.9, 8.3
(NH)Me	H1M	6.7, 7.9

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