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# Letter to the Editor: $^{1}H$, $^{13}C$, and $^{15}N$ resonance assignments and secondary structure of the PWI domain from SRm160 using Reduced Dimensionality NMR

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## Biological context

SRm160 (the SR-related nuclear matrix associated protein of 160 kDa) belongs to a large group of pre-mRNA processing proteins which contain one or more domains rich in alternating serine/arginine residues (RS domains). SRm160 functions as a coactivator of both constitutive and exon-enhancer dependant splicing (Blencowe et al., 1998; Eldridge et al., 1999), and may play a role in the communication between splicing and 3′-end processing machinery (McCracken et al., 2002).

Here we report the nearly complete $^{1}H$, $^{13}C$ and $^{15}N$ resonance assignments and secondary structure of a 12.5 kDa polypeptide containing the PWI motif of SRm160, of which the biological function is currently not known. The PWI motif, named after a highly conserved PWI tri-peptide at its N-terminal end, is highly conserved in homologs of SRm160 and other splicing or splicing-related proteins (Blencowe and Ouzounis, 1999). The assignments will serve as the basis for calculating the domain's three-dimensional solution structure, which will help elucidate its possible role in pre-mRNA processing. Assignment of the resonances was greatly facilitated by use of reduced dimensionality (RD) NMR methods.

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## Methods and experiments

A fragment of human SRm160 encoding amino acids 27-134, which includes the PWI motif, was cloned into the pET-15b expression vector (Novagen) and expressed in *E. coli* BL21-Gold (DE3) cells (Stratagene). $^{15}N$, $^{13}C$-labeled samples were synthesized in cells grown in standard M9 minimal media containing $^{15}NH_4Cl$ and $^{13}C$-glucose and purified to homogeneity using $Ni^{2+}$-affinity chromatography. Samples were prepared in 25 mM phosphate buffer (pH = 7.0), 300 mM NaCl, 1 mM DTT, 10%$D_2O$/90%$H_2O$, to final protein concentrations between 1.3 and 1.6 mM.

NMR experiments were recorded at 25 °C on a Varian INOVA 600MHz spectrometer. Resolution of the spectra was increased using linear prediction in both the $^{15}N$ and $^{13}C$ dimensions. For data processing and analysis, the NMRPipe program package (Delaglio et al., 1995), and the SPSCAN (Glaser and Wüthrich) and XEASY (Bartels et al., 1995) programs were used.

Assignment of the protein backbone and side chain atoms was primarily accomplished using RD experiments with short acquisition times (Szyperski et al., 1998, submitted). 3D HNNCAHA [acquisition time: 7.5 h], HACA(CO)NHN [5.2 h], $H^{\alpha/\beta}C^{\alpha/\beta}$(CO)NHN [9.9 h], and conventional HNCACB [7.9 h] experiments were used to assign the backbone chemical shifts. Aliphatic side chain

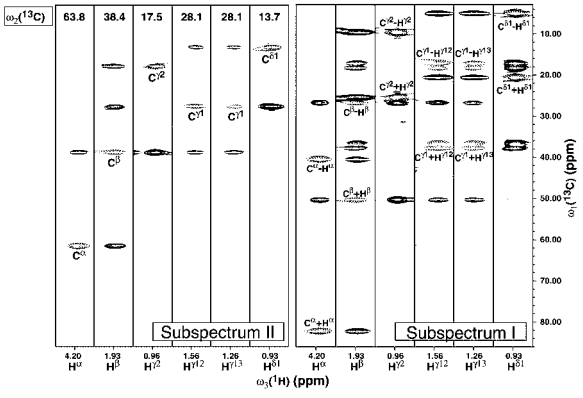


*Figure 1.* Contour plot of [$\omega_1$($^{13}$C), $\omega_3$($^1$H)]-strips taken from the two subspectra of the 3D HCCH-COSY experiment. Strips were taken at the sidechain $^{13}$C chemical shifts of Ile 116 in $\omega_2$($^{13}$C) (indicated at top), and are centered about the corresponding $^1$H chemical shifts in $\omega_3$($^1$H) (indicated at the bottom). Subspectrum II contains the central peaks, while Subspectrum I contains the RD NMR peak pairs, which are centered about the peaks in Subspectrum II. The observed correlations, indicated by solid lines, enabled identification of the spin system. Chemical shifts are relative to 2,2-dimethyl-2-silapentane-5-sulfonate.

chemical shifts were assigned predominantly using the 3D HCCH-COSY [8.9 h], and aided partially by heteronuclear resolved NOESY. Aromatic $H^\delta$ protons were linked to aliphatic $H^\beta/C^\beta$ resonances using 2D $H^\beta C^\beta(C^\gamma C^\delta)$HD [6.0 h], and the other aromatic chemical shifts were then obtained from 2D $^1$H-TOCSY-HCH-COSY [7.0 h]. The total acquisition time for the entire set of RD experiments used was 44.5 h.

RD NMR experiments facilitated resonance assignment by resolving chemical shift degeneracies and providing additional correlations not observed in conventional 3D NMR experiments. Figure 1 shows signals observed for Ile116 in the two subspectra obtained from 3D HCCH-COSY, which was recorded with acquisition of central peaks (Szyperski et al., 1998). The resonances in subspectrum II are derived from $^{13}$C magnetization, and provide the same information as the conventional (H)CCH-COSY. Subspectrum I is derived from $^1$H magnetization, and exhibits pairs of peaks (doublets) centered about each $^{13}$C frequency for a given $CH^n$ moiety. As these pairs encode the chemical shifts of the associated hydrogens, the two 3D subspectra contain the same information as 4D HCCH-COSY. Consequently, the doublets may resolve degenerate carbon chemical shifts. For example, the two doublets associated with $C^{\gamma 1}$ in subspectrum I indicate that the two methylene hydrogen chemical shifts are not degenerate. Moreover, as the PWI domain of SRm160 is predominately α-helical, $C^\alpha$ chemical shift degeneracy would have impeded sequential resonance assignment. Using RD NMR techniques, splitting of the $C^\alpha$ signals by $H^\alpha$ chemical shifts resolved most of these degeneracies.

## Extent of assignments and data deposition

The assignments of the PWI motif are virtually complete. With the exception of residual histidine tag amino acids at the N-terminus, the backbone is completely assigned and every backbone amide resonance is accounted for in the [$^{15}$N,$^1$H]-HSQC. The assignment of backbone $H^\alpha$ and $C^\alpha$ resonances is complete, and 98% of all side chain resonances are assigned. Secondary structure determination based on $C^\alpha/C^\beta$, $H^N$, and $H^\alpha$ chemical shifts reveals four helical regions which include amino acids 45-60, 65-74, 81-91, and 94-113. Chemical shifts of the PWI motif are deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under accession number 5162.

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