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ARTICLE

# $^{1}H$, $^{13}C$ and $^{15}N$ resonance assignment of WHEP domains of human glutamyl-prolyl tRNA synthetase

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**Abstract** Human bifunctional glutamyl-prolyl tRNA synthetase (EPRS) contains three WHEP domains (R123) linking two catalytic domains. These three WHEP domains have been shown to be involved in protein–protein and protein–nucleic acid interactions. In translational control of gene expression, R12 repeats is known to interact with 3′UTR element in mRNAs of inflammatory gene for translational control mechanisms. While, R23 repeats interacts with NSAP1, which inhibits mRNA binding. Here we present the NMR chemical shift assignments for R12 (128 amino acids) as a 14 kDa recombinant protein and whole WHEP domains R123 (208 amino acids) as a 21 kDa recombinant protein. 97 % of backbone and 98 % of side-chain assignments have been completed in R12 analysis and 93 and 92 % of backbone and side-chain, respectively, assignments have been completed in R123 analysis based upon triple-resonance experiments.

**Keywords** EPRS · Glutamyl-prolyl tRNA synthetase · WHEP domains · Helix-turn-helix

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## Biological context

Aminoacyl tRNA synthetases (ARSs) catalyze the attachment of specific amino acids to their cognate tRNAs during protein biosynthesis. Although these enzymes are diversified in the structure and functions in all living organisms, their catalytic activity is conserved (Mirande 1991). In vertebrates, 20 cytoplasmic ARSs activities are contained in 19 proteins; one, the bifunctional EPRS, express two enzyme activities in a single polypeptide chain. Also in vertebrates, nine ARSs activities in eight enzymes (including EPRS) reside in the cytosolic tRNA multisynthetase complex (MSC) of unknown function (Rho et al. 1999). Except the enzymatic core, eukaryotic ARSs acquired appended domains to the N or C terminus such as 1Bγ, EMAP II-like domain and WHEP domain (Shiba 2002). WHEP domains were found in WRS, HRS, EPRS (for which the domains is named), GRS and MRS.

The human EPRS WHEP domains are very interesting because of their structural character. First of all, they present as tandem repeats while most of others are single copy formed a helix-turn-helix structure. And they are located between the two catalytic domains while others existed in the N- or C-termini of the core domain. In spite of their structural uniqueness, the roles of WHEP domains were not clearly verified.

Recently, Paul L. Fox group reported that human EPRS WHEP domains direct noncanonical function of EPRS in

translational control of gene expression in interferon-γ-activated monocytic cells (Jia et al. 2008). According to their report, EPRS R12 repeats directs high-affinity binding to interferon-γ-activated Inhibitor of translation (GAIT) element-bearing mRNA while overlapping, R23 repeats bind to the NSAP1 which inhibits mRNA binding for the sequence of regulation of translational control. It shows EPRS WHEP domains are critical determinant and providing a negative regulatory function that permits delayed activation of the GAIT complex which may suppresses expression of multiple IFN-γ-inducible inflammatory transcript, and thus may have an important regulatory role in the resolution of chronic inflammation (Parth and Paul 2007).

Previously we published the single copy of the WHEP domain R1 structure and R123 backbone chemical shift assignment at pH 5 (25 °C) (Jeong et al. 2000). Based on previous data, R12 assignment was completed at the same condition. However condition of whole WHEP domains had to be changed to pH 6.8 (10 °C) because of incomplete assignment of R2 repeat caused by linebroadening and peak overlap in whole WHEP domains at pH 5 (25 °C). Herein we report resonance assignment of R12 at pH 5 and R123 at pH 6.8 which will be critical for determining the structure of RNA–protein complex or protein–protein complex and understanding its mechanism.

## Methods and experiments

### Protein preparation

The EPRS linker domain R123 was subcloned into an expression vector pET28a and R12 was subcloned into a pET15b. An uniformly $^{15}$N-labeled protein was prepared from cells grown in M9 minimal medium containing 1.0 g of $^{15}NH_4CL$/L and 2.0 g of glucose/L, and glucose was substituted by $^{13}$C-glucose for the simultaneous isotope labeling of $^{13}$C and $^{15}$N. The transformed cells were cultured at 37 °C and induced with 0.4 mM IPTG (isopropyl β-D-1-thiogalactopyranoside) at logarithmic phase of growth for 3 h. The harvested cell paste was disrupted by an ultrasonicator (Branson) in a lysis buffer (50 mM sodium phosphate, pH 7.4, and 300 mM NaCl). The protein was purified by an affinity chromatography with nickel-nitrilotriacetic acid-agarose column (Qiagen). The polyhistidine taq was removed by thrombin digestion for 9 h, and purified by superdax200 gel filtration column. Final purification was done by reversed-phase chromatography using a C8 Vydac column. Purified protein was lyophilized and store at −20 °C. NMR samples were prepared by dissolving about 2 mM protein in 0.3 ml of either 90 % $H_2O$/10 %$^2H_2O$ and the pH was adjusted to 5.0 (R12) and 6.8 (R123) with concentrated $NaO^2H$. Purified protein was verified by N-terminal amino acid sequence at Korea Basic Science Institute (KBSI) in Seoul.

### NMR spectroscopy

In the case of R12 repeats, NMR experiments were carried out using a Varian 900-MHz NMR spectrometer (at the Advanced Analysis Center of KIST) equipped with cyroprobes at 25 °C. The $^{15}$N-labeled and $^{15}$N/$^{13}$C-labeled R12 protein samples were prepared in 90 % $H_2O$/10 %$^2H_2O$ and the pH was adjusted to 5.0 (glass electrode) with concentrated $NaO^2H$ (pH 5) to a final sample volume of approximately 300 μL and a final concentration of 2 mM. The 2D and 3D NMR experiments included $^1$H–$^{15}$N HSQC, $^1$H–$^{13}$C HSQC (John et al. 1993), HNCA (Ikura et al. 1990b), HNCO (Ikura et al. 1990a), HNCACB (Ikura et al. 1990a), CBCA(CO)NH (Grzesiek and Bax 1992), HCCH-TOCSY (Bax et al. 1990; Kay et al. 1993), CCH-TOCY (Bax et al. 1990), $^{15}$N-,$^{13}$C-edited NOESY (Pascal et al. 1994) were acquired for assignment of the backbone and side-chain. All NMR data were processed using the software NMRpipe (Delaglio et al. 1995) and analyzed using the software CCPNMR (Vranken et al. 2005).

In the case of R123 repeats, NMR experiments were also carried out using a Varian 900-MHz NMR spectrometer equipped with cryoprobes at 10 °C. The $^{15}$N-labeled and $^{15}$N/$^{13}$C-labeled R123 protein samples were prepared in NMR buffer (pH 6.8) to a final sample volume of approximately 300 μL and a final concentration of 2 mM. The 2D and 3D NMR experiments are acquired and processed as R12 experiments.

## Extent of assignments and data deposition

The secondary structure estimated by chemical shift index (Wishart and Sykes 1994), shows that each repeat has a helix-turn-helix structural motif and connected by the unstructured loop between them (Jeong et al. 2000). Based on previously published data, chemical shift assignment of R12 repeats was done at the same condition. 97 and 98 % of backbone and side-chain, respectively, assignments have been completed based upon triple-resonance experiments. The good peak dispersion in $^1$H–$^{15}$N HSQC shown in Fig. 1 facilitated the successful assignment of all backbone amides and all side-chain amides.

R123 assignment was started at the same condition, however chemical shift assignment of R2 repeat could not be completed at pH 5 (25 °C) in R123. Because R2 is edged with unstructured loop on both sides. Besides each repeat has very similar helix-turn-helix structure, so that all the same positioning atom of each repeat are located in the

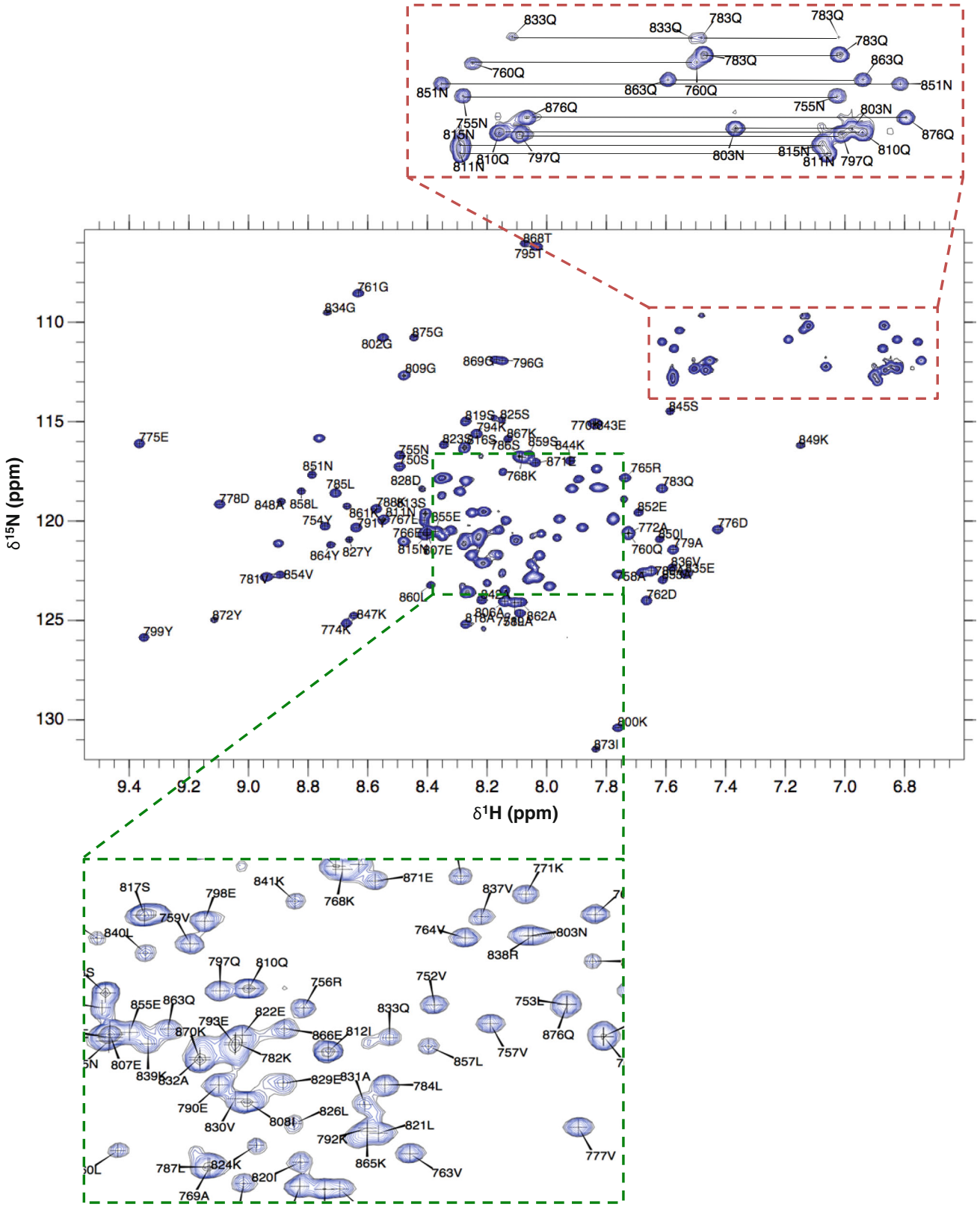


**Fig. 1** $^{1}$H–$^{15}$N HSQC spectrum of EPRS R12 repeats (2 mM in 90 % $H_2O$/10 % $^{2}H_2O$, pH 5.0, 25 °C) acquired at 900 MHz equipped with cryoprobes. All cross peaks are assigned and labelled sequence-specifically. Asn and Gln side-chain amide resonances are connected by *horizontal lines*

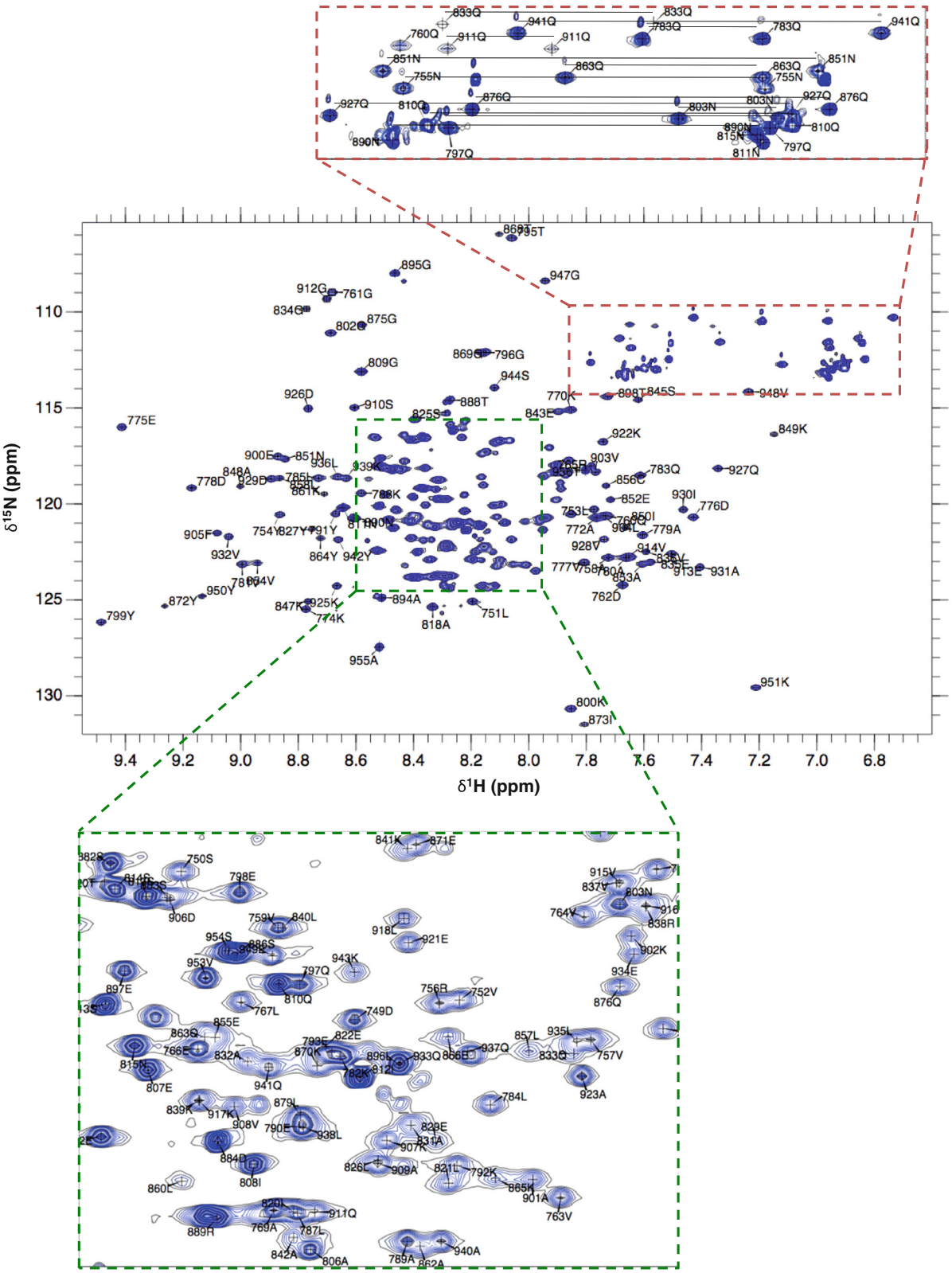


**Fig. 2** $^{1}H$–$^{15}N$ HSQC spectrum of EPRS WHEP domains R123 (2 mM in in 90 % $H_2O$/10 %$^{2}H_2O$, pH 6.8, 10 °C) acquired at 900 MHz equipped with cryoprobes. All cross peaks are assigned and labelled sequence-specifically. Asn and Gln side-chain amide resonances are connected by *horizontal lines*

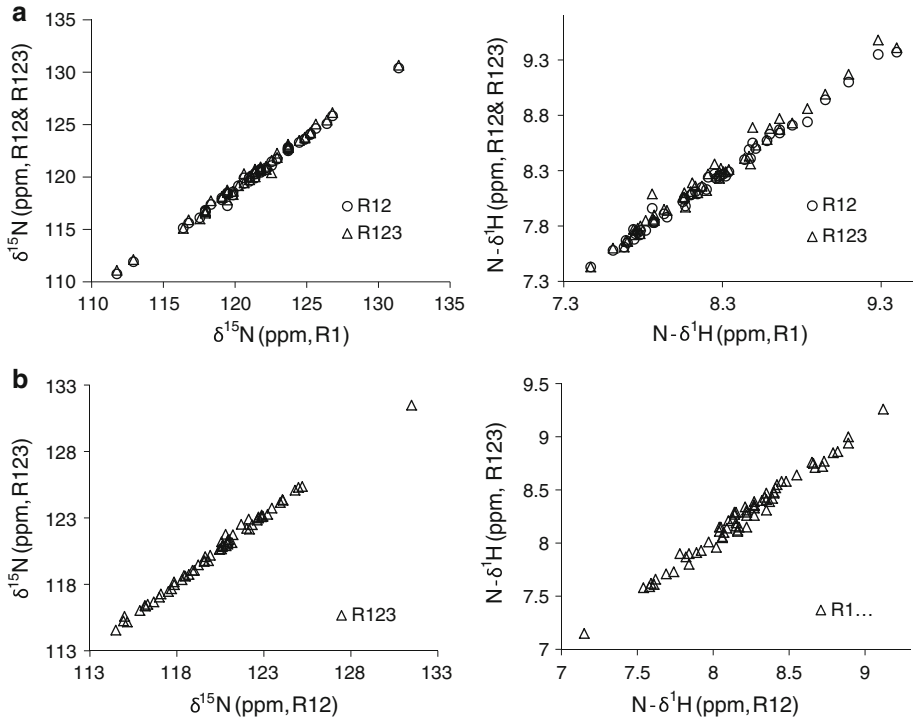


**Fig. 3** Backbone chemical shifts comparisons. **a** $^{15}$N and amide $^{1}$H chemical shifts comparisons between previously published R1 and R12/R123. **b** $^{15}$N and amide $^{1}$H chemical shifts of R2 comparisons between R12 and R123

similar atomic circumstances. Many of R2 chemical shifts didn't appear in 2 mM R123 sample and even a few ambiguous peaks, linebroadening and overlapping, are impossible to define. So we decided to change the temperature and also we did pH titration to find best condition to be able to assign whole WHEP domains. Finally pH 6.8 at 10 °C condition was decided. 92 % of triple resonance assignment of EPRS WHEP domains was completed including 93 % of the backbone atoms and all of the Ha, Hb, Ca and Cb resonances. The assignment of all backbone resonances (amide protons, amide nitrogens, alpha carbons, alpha protons and carbonyl carbons) corresponding to the 208 backbone amide resonances observed in the pH 6.8 2D $^{1}$H–$^{15}$N HSQC spectrum (Fig. 2). Assignments were further confirmed by $^{15}$N-,$^{13}$C-edited NOESY. Backbone chemical shifts comparison between published R1 and whole WHEP domains was shown in Fig. 3a. Also backbone chemical shifts of R2 were analyzed by comparison between R12 and R123 (Fig. 3b). The comparison results show that chemical environments of each repeat are very similar. The chemical shifts of $^{1}$H, $^{13}$C and $^{15}$N resonances have been deposited in the BMRB database under the accession number 19598 (R123) and 19599 (R12).

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## References

Bax A, Clore GM, Gronenborn AM (1990) 1H–1H correlation via isotropic mixing of 13C magnetization, a new three-dimensional approach for assigning 1H and 13C spectra of 13C-enriched proteins. J Magn Reson 88:425

Delaglio F, Grzesiek S, Vuister GW, Zhu G, Pfeifer J, Bax A (1995) NMRPipe: a multidimensional spectral processing system based on UNIX pipes. J Biomol NMR 6:277–293

Grzesiek S, Bax A (1992) Correlating backbone amide and side-chain resonances in larger proteins by multiple relayed triple resonance NMR. J Am Chem Soc 114:6291

Ikura M, Kay LE, Bax A (1990a) A novel approach for sequential assignment of 1H, 13C, and 15 N spectra of proteins: heteronuclear triple-resonance three-dimensional NMR spectroscopy. Application to calmodulin. Biochemistry 29:4659

Ikura M, Kay LE, Bax A (1990b) A novel approach for sequential assignment of proton, carbon-13, and nitrogen-15 spectra of larger proteins: heteronuclear triple-resonance three-dimensional NMR spectroscopy. Application to calmodulin. Biochemistry 29:4659

Jeong EJ, Hwang GS, Kim KH, Kim MJ, Kim SH, Kim KS (2000) Structural analysis of multifunctional peptide motifs in human bifunctional tRNA synthetase: identification of RNA-binding residues and functional implications of tandem repeats. Biochemistry 39:15775

Jia Jei, Abul A, Partho SR, Paul LF (2008) WHEP domains direct noncanocical function of glutamyl-prolyl tRNA synthetase in translational control of gene expression. Mol Cell 29(6):679

John BK, Plant D, Hurd RE (1993) Improved proton-detected heteronuclear correlation using gradient-enhanced z and zz filters. J Magn Reson, Ser A 101:113

Kay LE, Xu GY, Singer AU, Muhandiram DR, Formankay JD (1993) A gradient-enhanced HCCH-TOCSY experiment for recording

side-chain 1H and 13C correlations in H2O samples of proteins. J Magn Reson, Ser B 101:333

Mirande M (1991) A component of the multisynthetase complex is a multifunctional aminoacyl-tRNA synthetase. Prog Nucleic Acid Res Mol Biol 40:95

Parth SR, Paul LF (2007) A post-transcriptional pathway represses monocyte VEGF-A expression and angiogenic activity. EMBO J 26:3360

Pascal SM, Muhandriam DR, Yamazaki T, Formankay JD, Kay LE (1994) Simultaneous acquisition of $^{15}N/^{13}C$-edited NOE spectra of proteins dissolved in $H_2O$. J Magn Reson, Ser B 103:197

Rho SB, Kim MJ, Lee JS, Seol W, Motegi H, Kim S, Shiba K (1999) Genetic dissection of protein–protein interactions in multi-tRNA synthetase complex. Proc Natl Acad Sci 96:4488

Shiba K (2002) Intron positions delineate the evolutionary path of a perva- sively appended peptide in five human aminoacyl-tRNA synthetases. J Mol Evol 55:727

Vranken WF, Boucher W, Stevens TJ, Fogh RH, Pajon A, Llinas M, Ulrich EL, Markley JL, Ionides J, Laue ED (2005) The CCPN data model for NMR spectroscopy: development of a software pipeline. Proteins 59:687–696

Wishart DS, Sykes BD (1994) The 13C chemical shift index. A simple method for the identification of protein secondary structure using 13C chemical shift data. J Biomol NMR 4:171–180