J Biomol NMR (2010) 46:191–197
DOI 10.1007/s10858-009-9392-1

ARTICLE

S3EPY: a Sparky extension for determination of small scalar couplings from spin-state-selective excitation NMR experiments

Petr Novák · Lukáš Žídek · Veronika Motáčková · Petr Padrta · Alžběta Švenková · Jean-Marc Nuzillard · Libor Krásný · Vladimír Sklenář

Received: 2 October 2009 / Accepted: 16 November 2009 / Published online: 12 December 2009

Abstract S3EPY is a Python extension to the program Sparky written to facilitate the assessment of coupling constants from in-phase/antiphase and spin-state-selective excitation (S^3E) experiments. It enables the routine use of small scalar couplings by automating the coupling evaluation procedure. S3EPY provides an integrated graphical user interface to programs which outputs graphs and the table of determined couplings.

Keywords Software · Sparky · Residual dipolar couplings · NMR

Introduction

The residual dipolar couplings (RDCs) carry valuable information about long range and local fragment geometry which is particularly useful in the structure determination of nucleic acids, proteins, and their complexes. The RDC values D are defined as a difference of the peak splitting measured in the partially oriented media $J + D$ and in the isotropic sample J, where J is a scalar coupling. To avoid problems with spectral overlaps, experiments have been developed to separate peaks in two different subspectra, corresponding to α and β states. Most frequently, one-bond ^1J(CH), ^1J(NH), and ^1J(CC) scalar couplings are utilized as their large values (175–215 Hz, 85–96 Hz, 30–70 Hz, respectively) allow direct determination of the splitting by simple subtraction of measured peak positions in the α and β subspectra. Since the magnitudes of the other scalar couplings in both proteins and nucleic acids are significantly lower (^1J(NC) 8–19 Hz, ^2J(NH) 8–15 Hz, and the remaining ^2J couplings $<$ 5 Hz), and often comparable to the resonance linewidth, more sophisticated procedures are required for their accurate determination.

In an ideal case, the α and β peaks are fully separated in their respective subspectra and the differences in the peak frequencies give the correct values of the coupling constants J and D. In reality, the imperfect separation of the α and β peaks, commonly referred to as a cross-talk, is frequently observed. If the linewidth of signals is comparable to or larger than the coupling constant, the cross-talk between the α and β subspectra shifts the apparent maxima of the observed peaks (see Fig. 1). Consequently, the measured displacement of the α and β peaks in a doublet represents an inaccurate value of the coupling constant and has to be corrected. The cross-talk appears due to differences between the value of the actual couplings and the value J_0 used for the setting the delays in the spin-state-selective NMR experiment (e.g., $\sigma = 1/(8J_0)$ in Fig. 2). Two sources of the aforementioned differences can be identified. The first one is variation of actual coupling constants caused by different magnitude of RDCs and by local changes in the chemical environment influencing the values of J. The second one stems from the evolution of J-coupling during radio-frequency pulses.

P. Novák · L. Žídek (✉) · V. Motáčková · P. Padrta · V. Sklenář
National Centre for Biomolecular Research, Faculty of Science, Masaryk University, Kotlářská 2, 611 37 Brno, Czech Republic
e-mail: lzidek@chemi.muni.cz

A. Švenková · L. Krásný
Laboratory of Molecular Genetics of Bacteria, Institute of Microbiology, Academy of Sciences of the Czech Republic, Vídeňská 1083, 142 20 Prague, Czech Republic

J.-M. Nuzillard
CNRS, Institute de Chimie Moléculaire de Reims, BP 1039, 51687 REIMS Cedex 2, France

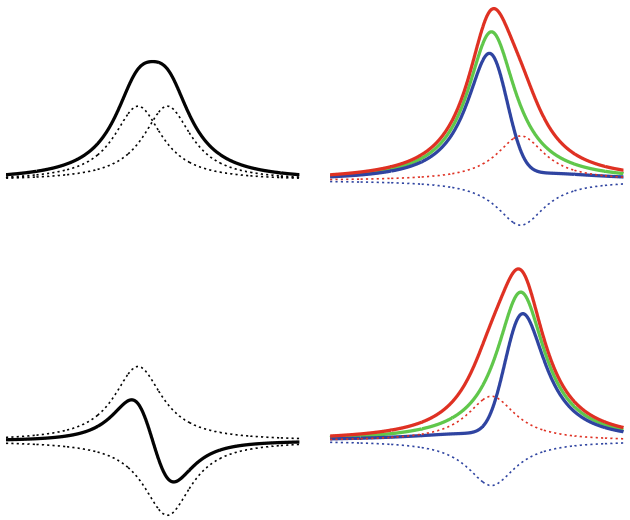

Fig. 1 Effect of the cross-talk on the apparent maxima of the separated α and β peaks. *Left*, *solid lines* represent unresolved in-phase (*top*) and antiphase (*bottom*) doublets, while *dotted lines* represent the α and β components. *Right*, *solid lines* represent the separated α and β peaks in the absence of cross-talk (*green*) and in the presence of a contribution of a positive (*red*) or negative (*blue*) peak of unwanted spin-state. The unwanted contributions are shown as *dotted* peaks of corresponding color

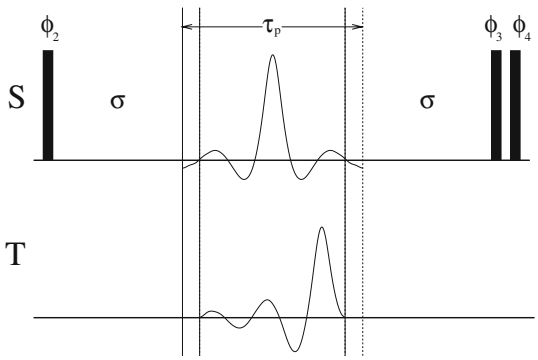

Fig. 2 S^3E element. *Bars* indicate hard π/2 pulses. Waves represents soft shaped pulses (2.5 ms Reburp and 2.0 ms IBurp2 applied on nuclei S and T, respectively). The subspectra are recorded with phase cycling $\phi_2 = 2(x + \pi/4); \phi_3 = x, y; \phi_4 = x, y;$ and $\phi_2 = x + \pi/4, x + 5\pi/4; \phi_3 = x, y; \phi_4 = -x, -y$. The delay is $\sigma = 1/(8J(ST))$

In-phase/antiphase (IPAP) and spin-state-selective excitation (S^3E) approaches are commonly utilized to separate α and β peaks.

IPAP experiment is a simple procedure for separating in-phase and antiphase components to the distinct subspectra (Ottiger et al. 1998; Andersson et al. 1998). Their sum and difference then yields subspectra with the α and β peaks of the *J*-coupled doublet separated. The IPAP requires overall evolution periods matching 1/(2*J*) and is typically used for the measurements of larger one-bond couplings where relaxation losses due to the *J*-evolution are not severe and where the imperfect peak separation causes errors only if the cross-talk peaks overlap with main signals of another residue.

For experiments based on very small scalar couplings, the pulse sequences with the S^3E element are employed as shorter evolution periods matching 1/(4*J*) bring substantial sensitivity advantage compared to sequences with the 1/(2*J*) evolution period. The pulse sequences utilizing the S^3E element are described in great detail in the literature (Meissner et al. 1997a, b). Hence only the basic principles of the S^3E element will be briefly summarized here. Considering the IS[T] experiment (Wang and Bax 1995), the S^3E element (see Fig. 2) using an appropriate phase cycling selectively excites α or β resonances of the doublets prior to an evolution period of a multidimensional experiment and allows the *J*(ST) coupling between nuclei S and T to evolve. Such an editing provides the typical E.COSY signal pattern with the individual peaks of the doublet displaced by the value of ^1J(ST) and ^2J(IT) in F_1 and F_2 dimension, respectively (see Fig. 3). Due to the comparable magnitudes of the determined couplings and linewidths of the observed signals, the cross-talk between the α and β subspectra has to be corrected. A remedy of the incorrect reading of coupling constants can be achieved using two complementary procedures. The first, a time-consuming one, is based on the measurement of a series of spectra with varying evolution delays and a subsequent interpolation of the observed displacements. The second, a more efficient approach, employs a mathematical post-processing procedure in which a linear combination of the α and β subspectra with different weighting coefficients is performed (Sørensen et al. 1999). The latter method does

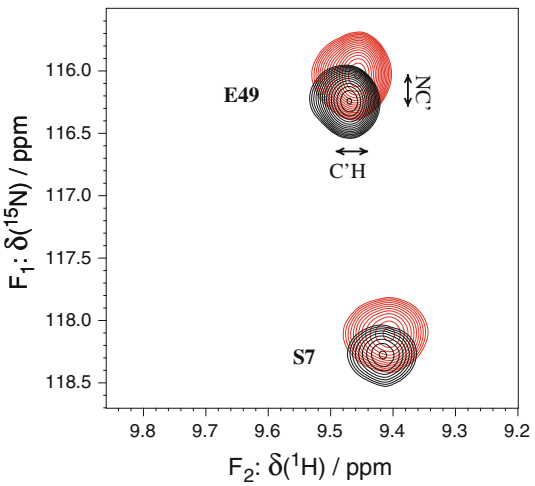

Fig. 3 Zoomed region of S^3E HN[C] spectra of N-terminal domain of RNA polymerase δ subunit from *Bacillus subtilis* for residues S7 and E49. The actual displacements based on ^1J(NC′) and ^2J(C′H) couplings are marked by arrows for the residue E49. Subspectra, with the separated α and β peaks colored in *black* and *red*, respectively, are overlaid

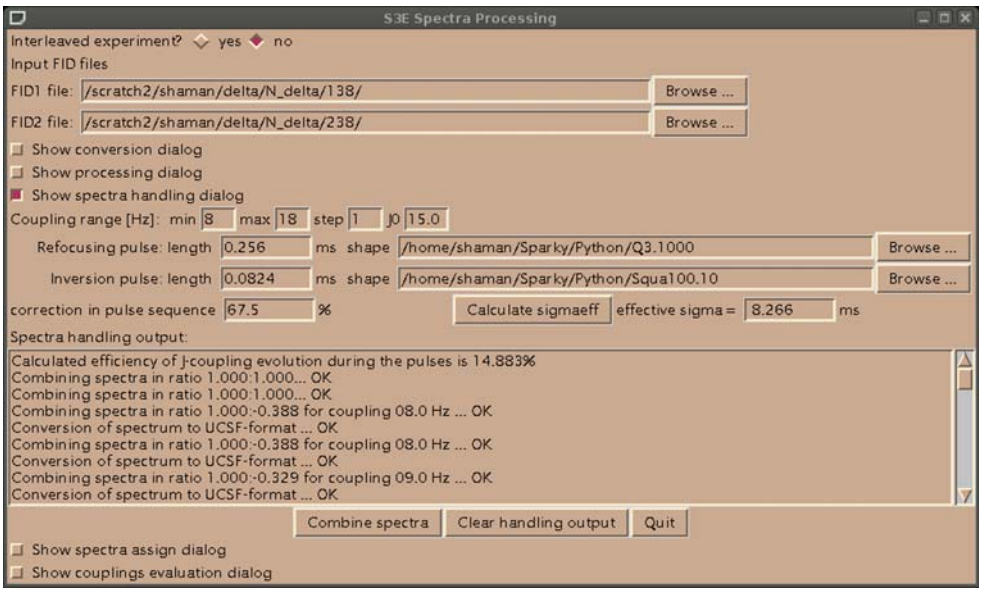

Fig. 4 The S3EPY graphical user interface. The program has an integrated interface to the NMRPipe program suite for the conversion and processing of the spectra, to program Sigma for calculation of coupling evolution efficiency, to the correction procedures done by S3EPY, to Sparky for the peak assignment and position measurement, and to gnuplot for fitting couplings and generating graphical output. An example of the user interface to the Sigma program and correction procedures is shown

not require additional measurements but finding appropriate coefficients manually is tedious, time-consuming and susceptible to errors.

Here we present S3EPY, a Python extension to the program Sparky, which facilitates the evaluation of coupling constants and makes a routine use of the S^3E experiments possible. S3EPY runs on all operating systems on which Sparky (T.D.Goddard and D.G.Kneller, UCSF, San Francisco, CA), NMRPipe (Delaglio et al. 1995), and gnuplot (Williams et al. 2007) can be installed.

Materials and methods

Samples

Two samples were used in this study in order to test applicability of the S3EPY extension to both protein and nucleic acid spectra.

The nucleic acid sample contained a uniformly ^{13}C, ^{15}N-labeled DNA d(GCGAAGC) hairpin (purchased from Silantes GmbH, München, Germany) diluted to 0.5 mM concentration in phosphate buffer, pH 6.7, including 10% of D_2O, with or without 20 mg/ml Pf1 phage for partially aligned or isotropic sample, respectively. The splitting of the HDO deuterium line in the partially aligned sample was 20 Hz. The S^3E spectra of the DNA hairpin were recorded at 303 K on a Bruker AVANCE 500 MHz spectrometer equipped with a triple resonance TXI (^1H, ^{13}C, ^{15}N) z-gradient probe.

The protein sample consisted of 0.7 mM uniformly ^{13}C, ^{15}N-labeled N-terminal domain of RNA polymerase δ subunit from *Bacillus subtilis* in 10 mM NaCl, 10% D_2O, 0.05% NaN_3, 20 mM phosphate buffer, pH 6.6. The anisotropic sample was aligned with 21 mg/ml Pf1 phage (HDO splitting of 21 Hz). IPAP and S^3E spectra were obtained at 303 K on a 600 MHz Bruker Avance spectrometer equipped with a triple resonance TCI (^1H, ^{13}C, ^{15}N) z-gradient cryoprobe.

Data evaluation

The S3EPY extension is an integrated tool for analyses of IPAP and S^3E data. The analyses are done in the following manners.

In case of IPAP experiments, the in-phase and antiphase subspectra are converted to the NMRPipe format, processed, added and subtracted to provide spectra with separate α and β peaks. The IPAP extension is invoked with the 'ia' accelerator in Sparky. Using the graphical interface, the user specifies paths to files with in-phase and antiphase FIDs. The script for conversion of the FIDs to NMRPipe format is supplied by the user, imported from a file, or automatically created. The subspectra are processed using the script assembled via the section of the graphical interface for the processing of the spectra and are automatically added and subtracted to obtain the subspectra with the α and β peaks separated. The user sets an approximate displacement of the peaks used for an assignment to the peaks in α and β subspectra and identifies the dimension in which the coupling is measured. In the next step, the user loads the decoupled spectrum with the assignment and selects peaks for the analysis. The extension then automatically assigns α and β spectra according to the peaks selected in the decoupled spectrum, measures the displacements of corresponding peaks, and prints out a table of the determined couplings.

The spectra obtained in S^3E experiments are analyzed using the S3EPY extension which provides an integrated

graphical user interface (Fig. 4) to all steps (*vide infra*). In this way, the user is able to control the work-flow of the whole coupling evaluation process from one place. After the program Sparky is started, S3EPY extension is invoked with the 'se' accelerator, and user specifies paths to the α and β FIDs, which will be converted to the NMRPipe format. The spectra are converted into the NMRPipe format and processed. The conversion script can be either manually written to a S3EPY conversion widget, imported from an external file, or automatically created. The NMRPipe processing script is configured by the user using the section of the graphical interface for spectra processing.

In the next step, the error stemming from the missetting of the experimental delay σ (Fig. 2) due to the *J*-coupling evolution during the simultaneous pulses of the duration τ_p as defined in Fig. 2 is corrected. The correction takes into account the efficiency of the coupling evolution for a given pair of pulses. The efficiency is simulated by the program Sigma distributed along with the S3EPY. The lengths and the text files with the shape definitions of the pulses used in the S^3E element, together with the value of correction already introduced in the pulse sequence, are supplied via the S3EPY Sigma interface and subsequently the effective delay of *J*-coupling evolution σ_{eff} is calculated (Fig. 4). Alternatively, the efficiency can be experimentally calibrated employing the simple spin-echo experiment on a suitable sample with the known scalar coupling constant.

Using the effective value of the delay σ_{eff}, the error originating from the non-uniformity of the measured couplings is corrected by the linear combination of the α and β subspectra with varying weighting coefficients calculated in the following step. User defines the range and the step of the interpolation (Fig. 4). The spectra with the α and β peaks for the linear combination are obtained by automatically adding and subtracting the processed subspectra in ratio 1:1 with the addNMR routine from the NMRPipe program suite (Fig. 3). The S3EPY extension then generates α series of spectra by linear combination of the α and β subspectra in the ratio $1{:}\tan((\pi/4)(J_k / J_o^* - 1))$ where J_k is the guess value of ${}^nJ(\text{ST})$, varied in the course of interpolation, and $J_o^* = 1/(8\sigma_{\text{eff}})$ is the corrected value J_0 used for setting the delays in the experiment (Sørensen et al. 1997). Analogically, the β series of the linear combination of the β and α subspectra is automatically generated. Examples of subspectra obtained with varying weighting coefficients are presented in Fig. 5. A distortion of the α peak by a presence of the unwanted β component is clearly visible in the left panel in the first row, obtained with the lowest weighting coefficient. The second row represents combination close to the optimal setting, while the uncorrected spectra are displayed in the third row. The last row shows the spectra obtained with the highest weighting coefficient.

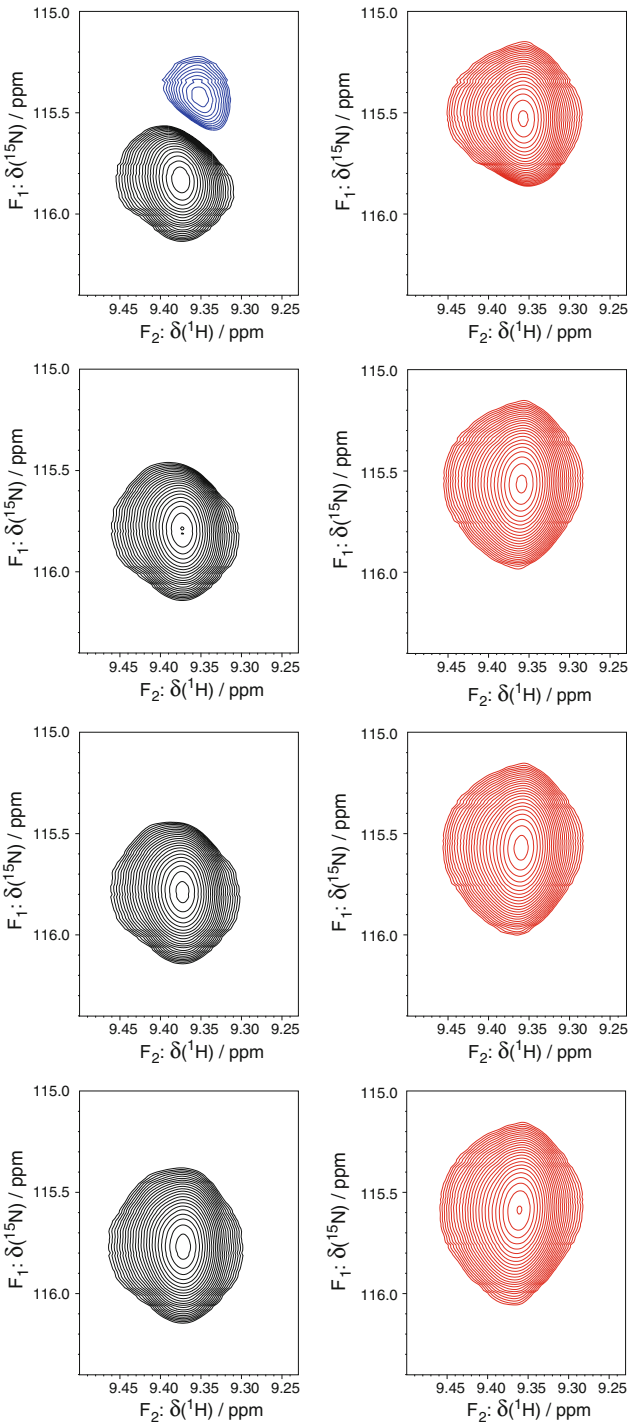

Fig. 5 Examples of various linear combinations of α and β subspectra of S^3E HN[C] experiment recorded on N-terminal domain of RNA polymerase δ subunit from *Bacillus subtilis* for residue E49. Subspectra with the separated α and β peaks are shown in *left* and *right* panels, respectively. *Negative peaks* are colored in *blue*. The displayed subspectra were obtained by combinations with the following coefficients (starting from *top*): −0.388, −0.058, −0.006, and 0.151, calculated for the guess values 8, 14, 15, and 18 Hz, respectively. The optimized value of the $J(\text{NC}')$ for residue E49 is 14.01 Hz

An assigned spectrum is loaded and the F_1, F_2 coordinates of peaks intended for analysis are selected and copied to the first spectrum of the α series by the user. The S3EPY software then copies the assignment of the selected peaks to the rest of the spectra in the α and β series. Although the displacement of the peaks due to the measured couplings is not too large, it may sometimes cause the misplacement of the copied peak coordinates. The program is designed to enable the user to check every step of the coupling determination procedure and to look for the misplacement of the automatically copied peak coordinates.

In the last part of the evaluation procedure, the program picks the assigned peaks, measures the displacements of corresponding α and β peaks, and creates sets of input data and the input script for plotting and fitting using the program gnuplot. Gnuplot automatically plots the dependencies of the measured displacements of peaks in the indirect dimension on J_k, fits them to a second order polynomial, and evaluates the intersection of the fitted curve with the diagonal providing the correct value of the coupling constant in the indirect dimension. The corrected value of the coupling in the direct dimension is automatically obtained from the plots of measured displacements of α and β peaks in the course of linear combination of the spectra shown in Fig. 6. A list of the corrected coupling constants is obtained as a text output.

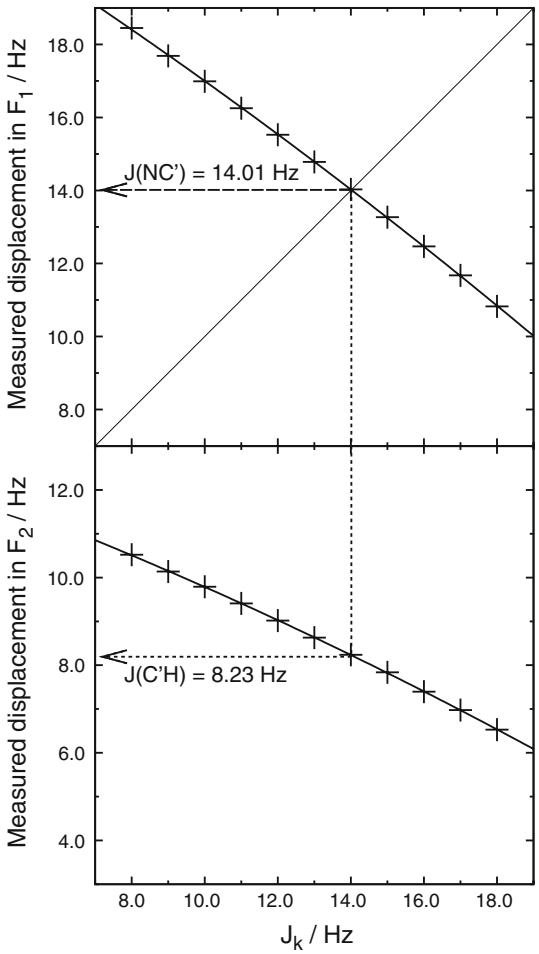

Fig. 6 Correction for missetting the σ delay in the S^3E experiment. The correct value of the coupling in the F_1 domain is determined as an intersection of the diagonal and the second order polynomial fitted to the measured displacement of the peak in the indirect dimension for varying J_k (*dashed line*). The value of the coupling in F_2 domain is found by projecting the same J_k onto the second order polynomial fitting the peak displacement in the direct dimension (*dotted line*)

Results and discussion

S^3E and IPAP spectra were recorded on an isotropic and a weakly aligned sample of a short d(GCGAAGC) DNA hairpin (Padrta et al. 2002) and on the N-terminal domain of RNA polymerase δ subunit from *Bacillus subtilis* (Lampe et al. 1988). The couplings were obtained from ^1H–^{15}N and ^{13}C–^{13}C IPAP HSQC and from HN[H], HN[C], and HC[N] S^3E spectra.

The couplings from S^3E spectra recorded on the nucleic acid sample were determined manually and published earlier (Žídek et al. 2001). The measured scalar and dipolar coupling constants obtained automatically using the S3EPY algorithm from the same spectra were in an excellent agreement with the previously published data (Žídek et al. 2001). The differences were not larger than the round-off error (0.1 Hz) and less then the experimental error (see bellow).

While the relaxation effects were negligible for the studied spin systems (Žídek et al. 2001), corrections for the σ delay missetting and for the evolutions during the simultaneous pulses were necessary (Žídek et al. 2001). A detailed description of these effects was published by Sørensen et al. (1997). Accuracy of the measured couplings was tested by a comparison with a *J*-modulated constant-time HSQC experiment (Tjandra and Bax 1997). No systematic differences were observed (see Fig. 5 in Žídek et al. 2001). The standard deviation of the repeated measurements was approximately 0.2 Hz (Žídek et al. 2001).

The data were cross-validated (Žídek et al. 2003) and 14 couplings (82%) out of 17 couplings in nucleic bases determined using S^3E showed a good internal consistency. The remaining 3 couplings (^1D(N7H8) in A4, ^1D(*N*9*H*8) in A4, ^1D(N1C1′) in G6) were identified as inconsistent with the structure of nucleic acid bases. The fact that the same results were obtained for the manual and automatic dipolar coupling determination shows that the inconsistent couplings should be attributed to the experimental error rather then to the automation of the evaluation procedure.

The analysis of scalar and dipolar interactions in nucleic acid bases was complemented with measurements of four

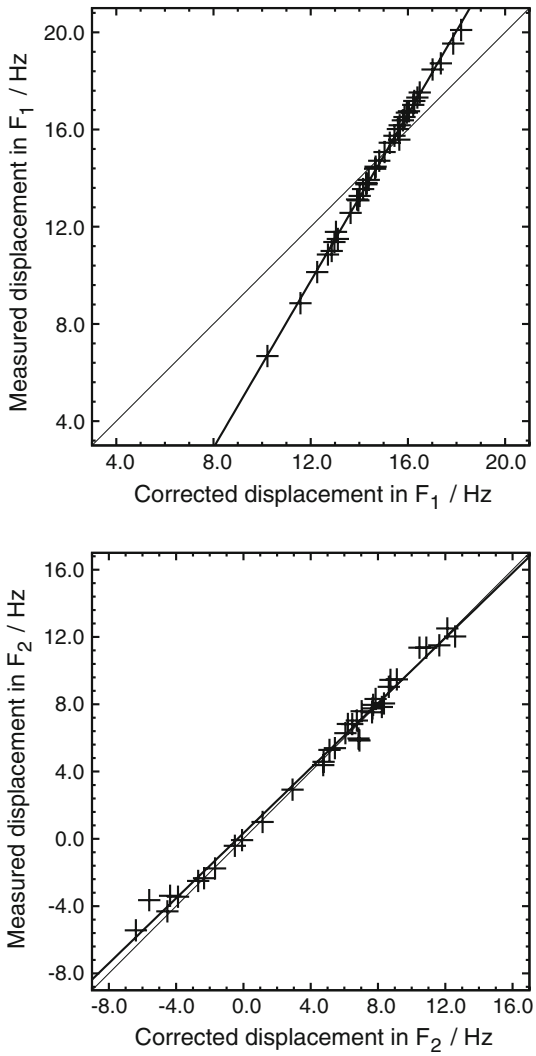

Fig. 7 Measured peak displacements in F1 (*top*, NC′ nuclear pair) and F2 (*bottom*, $C'H^N$ nuclear pair) plotted against corrected values of the displacement in F1 and F2, respectively. *Thick lines* represent linear regressions. Slopes of 1.72 (F_1) and 0.97 (F_2) and intercepts of −10.92 Hz (F_1) and 0.34 Hz (F_2) were obtained. The rms deviations of measured vs. corrected values were 0.16 and 0.50 for F_1 and F_2, respectively

couplings within a peptide bond plane. Slowly relaxing carbonyl ^{13}C, employed as a passive spin in the HN[C] experiment, does not introduce any significant relaxation effect. Possible contributions of the evolution during simultaneous pulses to the cross-talk are smaller compared to the nucleic acid measurements due to a shorter length of pulses. On the other hand, relatively large magnitude of alignment achieved in our study ($A_a = 1.0 \times 10^{-3}$) represented a considerable challenge for corrections of the σ delay missetting. A large set of peaks in the protein spectrum allowed us to perform a statistical analysis of the systematic error introduced by the cross-talk. Figure 7 documents that the apparent peak displacements correlate with the corrected values but the slope of linear regression significantly deviates from unity for the indirect dimension, being 1.72. Therefore, the correction procedure implemented in the S3EPY extension is necessary to obtain reliable structural restraints.

Standard deviations of the measured peak displacements, estimated for well-resolved peaks in spectra of the aligned protein obtained by repeated measurements, were 0.52, 0.21, 0.27, and 0.10 Hz for the NH^N, $C_\alpha C'$, $C'H^N$, and NC′ spin pairs, respectively. For the studied N-terminal domain of RNA polymerase δ subunit (91 residues excluding His-tag), 41 $^1D(NH^N)$, 34 $^1D(C_\alpha C')$, 37 $^2D(C'H^N)$, and 37 $^1D(NC')$ couplings were determined from reliable peaks using S3EPY extension. Out of this set of couplings, 19 $^1D(NH^N)$, 14 $^1D(C_\alpha C')$, 18 $^2D(C'H^N)$, and 18 $^1D(NC')$ coupling constants fall into the identified secondary structure elements, therefore can be subjected to the validation based only on the known local geometry without any prior knowledge of the overall 3D structure (Žídek et al. 2003; Mesleh and Opella 2003). From this subset of peaks in the secondary structure elements, 80% dipolar couplings were found to be internally consistent with the local geometry of the secondary structure elements. It should be noted that peaks which did not pass the consistency test were not necessarily measured or determined incorrectly, as the RDC can be influenced by the dynamical behavior of the protein backbone.

Although the applicability of the IPAP and S^3E experiments for the measurements of the small scalar and residual dipolar couplings was demonstrated (Ottiger and Bax 1998; Ottiger et al. 1998; Žídek et al. 2001) only few examples of their actual utilization have been reported so far (Ottiger and Bax 1998; Permi et al. 1999; Yang et al. 1999; Padrta et al. 2002; Jansen et al. 2007). While the tools for convenient evaluation of the IPAP experiments have been implemented into the NMRPipe package (Delaglio et al. 1995), analysis of the S^3E experiments, with the necessary corrections, has not been automatized so far.

The main obstacle preventing their wider applications represents the necessary post-processing which, without automation, is very cumbersome and labor intensive. However, small residual dipolar couplings bring valuable information allowing extensive cross-validation of data measured both in the peptide bond plane and in the nucleic acid bases using the test of internal consistency (Žídek et al. 2003). Moreover, they improve local and global geometry in the 3D structure determination process. Therefore, both the quantity and the quality of the structural restraints can be improved by incorporating small RDCs. We hope that the S3EPY program extension will facilitate the utilization of small RDCs and will make their use more routine.

Conclusions

A Python extension for program Sparky automating the determination of scalar and dipolar couplings from IPAP and S^3E spectra was written. The procedure was tested both on nucleic acid and protein samples and provided precise and accurate results satisfying the cross-validation. Results are obtained in a significantly shorter time compared to manual evaluation.

Software availability

Python code of S3EPY along with the HTML documentation is freely available at http://ncbr.chemi.muni.cz/s3epy .

Acknowledgements This work was supported by the Ministry of Education of the Czech Republic [MSM0021622413, LC06030]; and the Czech Science Foundation [204/09/0583].

References

Andersson P, Weigelt J, Otting G (1998) Spin-state selection filters for the measurement of heteronuclear one-bond coupling constants. J Biomol NMR 12(3):435–441. doi:10.1023/A:1008239027287

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(3):277–293. doi:10.1007/BF00197809

Jansen S, Chmelík J, Žídek L, Padrta P, Novák P, Zdráhal Z, Picimbon JF, Löfstedt C, Sklenář V (2007) Structure of *Bombyx mori* chemosensory protein 1 in solution. Arch Insect Biochem Physiol 66(3):135–145. doi:10.1002/arch.20205

Lampe M, Binnie C, Schmidt R, Losick R (1988) Cloned gene encoding the delta subunit of *Bacillus subtilis* RNA polymerase. Gene 67(1):13–19. doi:10.1016/0378-1119(88)90003-0

Meissner A, Duus JØ, Sørensen OW (1997a) Integration of spin-state-selective excitation into 2D NMR correlation experiments with the heteronuclear ZQ/2Q π rotations for $^1J_{XH}$-resolved E.COSY-type measurements of heteronuclear coupling constants in proteins. J Biomol NMR 10(1):89–94. doi:10.1006/jmre.1997.1213

Meissner A, Duus JØ, Sørensen OW (1997) Spin-state-selective excitation. Application for E.COSY-type measurement of J_{HH} coupling constants. J Magn Reson 128(1):92–97. doi:10.1006/jmre.1997.1213

Mesleh MF, Opella SJ (2003) Dipolar waves as NMR maps of helices in proteins. J Magn Reson 163(2):288–299. doi:10.1016/S1090-7807(03)00119-8

Ottiger M, Bax A (1998) Determination of relative N-H^N, N-C′, C^α–C′, and C^α–H^α effective bond lengths in a protein by NMR in a dilute liquid crystalline phase. J Am Chem Soc 120(47):12334–12341. doi:10.1021/ja9826791

Ottiger M, Delaglio F, Bax A (1998) Measurement of *J* and dipolar couplings from simplified two-dimensional NMR spectra. J Magn Reson 131(2):373–378. doi:10.1006/jmre.1998.1361

Padrta P, Štefl R, Králík L, Žídek L, Sklenář V (2002) Refinement of d(GCGAAGC) hairpin structure using one- and two-bond residual dipolar couplings. J Biomol NMR 24(1):1–14. doi:10.1023/A:1020632900961

Permi P, Heikkinen S, Kilpeläinen I, Annila A (1999) Measurement of $^1J_{NC'}$ and $^2J_{H^NC'}$ couplings from spin-state-selective two-dimensional correlation spectrum. J Magn Reson 140(1):32–40. doi:10.1006/jmre.1999.1817

Sørensen MD, Meissner A, Sørensen OW (1997) Spin-state-selective coherence transfer via intermediate states of two-spin coherence in IS spin systems: Application to E.COSY-type measurement of *J* coupling constants. J Biomol NMR 10(2):181–186. doi:10.1023/A:1018323913680

Sørensen MD, Meissner A, Sørensen OW (1999) ^{13}C natural abundance S^3E and S^3CT experiments for measurement of *J* coupling constants between $^{13}C^\alpha$ or $^1H^\alpha$ and other protons in a protein. J Magn Reson 137(1):237–242. doi:10.1006/jmre.1998.1635

Tjandra N, Bax A (1997) Measurement of dipolar contributions to $^1J_{CH}$ splittings from magnetic-field dependence of *J* modulation in two-dimensional NMR spectra. J Magn Reson 124(2):512–515. doi:10.1006/jmre.1996.1088

Wang AC, Bax A (1995) Reparametrization of the Karplus relation for $^3J(H^\alpha$–N) and $^3J(H^N$–C′) in peptides from uniformly $^{13}C/^{15}N$-enriched human ubiquitin. J Am Chem Soc 117(6):1810–1813. doi:10.1021/ja00111a021

Williams T, Kelley C, Bröker HB, Campbell J, Cunningham R, Denholm D, Elber G, Fearick R, Grammes C, Hart L, Hecking L, Koenig T, Kotz D, Kubaitis E, Lang R, Lecomte T, Lehmann A, Mai A, Merritt EA, Mikulík P, Steger C, Tkacik T, der Woude JV, Woo A, Zandt JRV, Zellner J (2007) Gnuplot an interactive plotting program

Yang D, Venters RA, Mueller GA, Choy W, Kay LE (1999) TROSY-based HNCO pulse sequences for the measurement of $^1HN-^{15}N$, $^{15}N-^{13}CO$, $^1HN-^{13}CO$, $^{13}CO-^{13}C^\alpha$ and $^1HN-^{13}C^\alpha$ dipolar couplings in ^{15}N, ^{13}C, ^2H-labeled proteins. J Biomol NMR 14(4):333–343. doi:10.1023/A:1008314803561

Žídek L, Wu H, Feigon J, Sklenář V (2001) Measurement of small scalar and dipolar couplings in purine and pyrimidine bases. J Biomol NMR 21(2):153–160. doi:10.1023/A:1012435106858

Žídek L, Padrta P, Chmelík J, Sklenář V (2003) Internal consistency of NMR data obtained in partially aligned biomacromolecules. J Magn Reson 162(2):385–395. doi:10.1016/S1090-7807(03)00116-2