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ARTICLE

Automated NMR resonance assignment strategy for RNA via the phosphodiester backbone based on high-dimensional through-bond APSY experiments

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Abstract A fast, robust and reliable strategy for automated sequential resonance assignment for uniformly [^{13}C, ^{15}N]-labeled RNA via its phosphodiester backbone is presented. It is based on a series of high-dimensional through-bond APSY experiments: a 5D HCP-CCH COSY, a 4D H1′C1′CH TOCSY for ribose resonances, a 5D HCNCH for ribose-to-base connection, a 4D H6C6C5H5 TOCSY for pyrimidine resonances, and a 4D H8C8(C)C2H2 TOCSY for adenine resonances. The utilized pulse sequences are partially novel, and optimized to enable long evolution times in all dimensions. The highly precise APSY peak lists derived with these experiments could be used directly for reliable automated resonance assignment with the FLYA algorithm. This approach resulted in 98 % assignment completeness for all ^{13}C–^{1}H, ^{15}N1/9 and ^{31}P resonances of a stem-loop with 14 nucleotides.

Keywords Nucleic acids · NMR · Projection spectroscopy · APSY · Automated assignment · FLYA · Novel sampling methods

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Introduction

The assignment of RNA resonances for detailed structural and functional studies of RNA and RNA–protein complexes by liquid state NMR can be a challenge due to the small signal dispersion of oligonucleotide resonances, particularly for the resonances of the ribose. ^{13}C labeling and heteronuclear experiments of higher dimensionality can partially resolve this problem. Still, the dimensionality and/or the resolution of conventional experiments are often not sufficient. Higher dimensionality and improved resolution can be obtained with novel sampling strategies, such as sparse sampling (Orekhov et al. 2003), the filter-diagonalization method (FDM) (Pardi et al. 2008), projection spectroscopy (Kupce and Freeman 2003), G-matrix Fourier transformation (GFT) (Kim and Szyperski 2003), and automated projection spectroscopy (APSY) (Hiller et al. 2005). Among them, APSY has proved a reliable method that leads to high-dimensional peak lists with a 10–50 times higher chemical shift precision than usually achieved with conventional experiments, thus providing a reliable basis for subsequent automated resonance assignment strategies (Narayanan et al. 2010; Krähenbühl and Wider 2012). In this work, we present an efficient method for the reliable and unambiguous automated assignment of RNA resonances with a set of high-dimensional through-bond APSY experiments with uniformly [^{13}C, ^{15}N]-labeled RNA.

For smaller RNAs or for RNAs with favorable relaxation properties we present here a strategy for the complete automated resonance assignment of all ^{13}C–^{1}H moieties of a [^{13}C, ^{15}N]-labeled RNA based on unambiguous through-bond APSY experiments. The suite of APSY experiments (illustrated in Fig. 1) includes a 5D HCP-CCH COSY, a 4D H1′C1′CH TOCSY for ribose resonances, a 5D

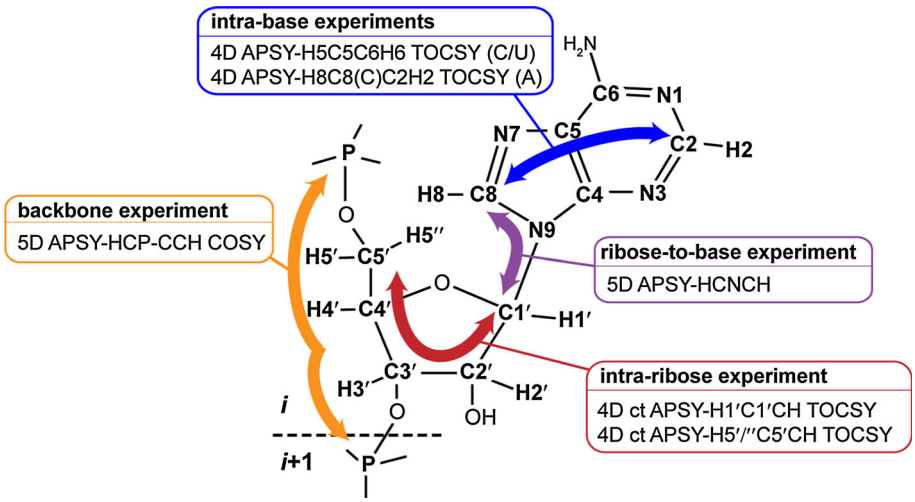

Fig. 1 Illustration of a suite of through-bond APSY experiments that allow unambiguous sequence-specific assignment of the resonances of all ^{13}C–^{1}H moieties, ^{31}P and $^{15}N1/9$ nuclei of RNA via the phosphodiester backbone. The intra-ribose correlations are denoted with a *red arrow*, and are achieved with two selective 4D APSY-HCCH TOCSY experiments that start on the CH5′/H5″ group, or on the CH1′ group, respectively, and include a constant-time evolution period in the second ^{13}C evolution period (Supplementary Material p. S9). The 4D APSY-H1′C1′CH experiment is already sufficient for complete assignment; the second experiment has been used for additional verification of the assignment results, particularly concerning the H5′/H5″ assignments. The previously published 5D APSY-HCNCH experiment (Krähenbühl et al. 2012) yields the ribose-to-base correlations from CH1′ to CH6/8 (pyrimidines/purines), which is illustrated with a *purple arrow*. CH6 or CH8 serve as an anchor for the correlation of the remaining ^{13}C–^{1}H groups in the bases using two APSY experiments: the CH2 group in adenosine (A) is connected to the CH8 group with a 4D APSY-H8C8(C)C2H2 TOCSY experiment (Supplementary Material p. S9), as is denoted with a *blue arrow*; a 4D APSY-H6C6C5H5 TOCSY experiment connects the CH5 group in pyrimidines (C/U) with the CH6 group (Supplementary Material p. S5). These APSY experiments provide complete intra-nucleotide connections. The sequential inter-nucleotide connection is performed via the phosphodiester backbone with a 5D APSY-HCP-CCH COSY experiment (Supplementary Material p. S7). All nuclei of relevance for the experiments are labeled with their IUPAC-conform names (IUPAC-IUB 1983)

HCNCH for ribose-to-base correlations (Krähenbühl et al. 2012), a 4D H6C6C5H5 TOCSY for pyrimidine resonances, and a 4D H8C8(C)C2H2 TOCSY for adenine resonances. With the exception of the ribose-to-base and the intra-pyrimidine experiment, these high-dimensional pulse sequences are novel. The APSY strategy is exemplified with a stem-loop RNA with 14 nucleotides, for which it yielded automated sequence-specific assignments for 98 % of all ^{13}C–^{1}H, ^{31}P, and $^{15}N1/9$ resonances. Based on high-dimensional APSY experiments, peak lists of very high precision and accuracy were obtained, which provided an ideal input for the automated assignment algorithm FLYA (Schmidt and Güntert 2012) implemented in CYANA (Güntert 2003; Lopez-Mendez and Güntert 2006).

Materials and methods

NMR sample

The nucleic acid used in this work was the well-studied 14mer cUUCGg tetraloop hairpin RNA (referred to as SL14 in this work) (Nozinovic et al. 2010). The uniformly [^{13}C, ^{15}N]-labeled RNA was purchased from Silantes (Silantes GmbH, Munich, Germany). Its sequence is indicated in Fig. 2. The sample for NMR spectroscopy contained ~0.7 mM RNA in 20 mM $KHPO_4$ at pH 6.4, 0.4 mM [^{2}H]-labeled EDTA and 100 % D_2O.

The 5D APSY-HCP-CCH COSY experiment

The HCP-CCH COSY experiment correlates the phosphate resonances of the nucleic acid backbone with the ribose resonances of the same and the previous nucleotide. It serves to sequentially connect resonances via the ^{31}P frequencies to achieve sequence-specific resonance assignment. Experiments with the same magnetization transfer pathway were published as a 3D HCP-CCH COSY (Marino et al. 1995; Ramachandran et al. 1996; Tate et al. 1995), which provided frequencies for ^{31}P and for the ^{13}C–^{1}H group on which the magnetization transfer ends. We modified the experiment to a 5D pulse sequence by including a constant-time evolution of the first ^{13}C dimension during the second ^{13}C–^{31}P transfer period, and a semi-constant-time evolution on the proton where the magnetization transfer starts. The pulse sequence for the 5D APSY-HCP-CCH COSY is provided in the Supplementary Material p. S7.

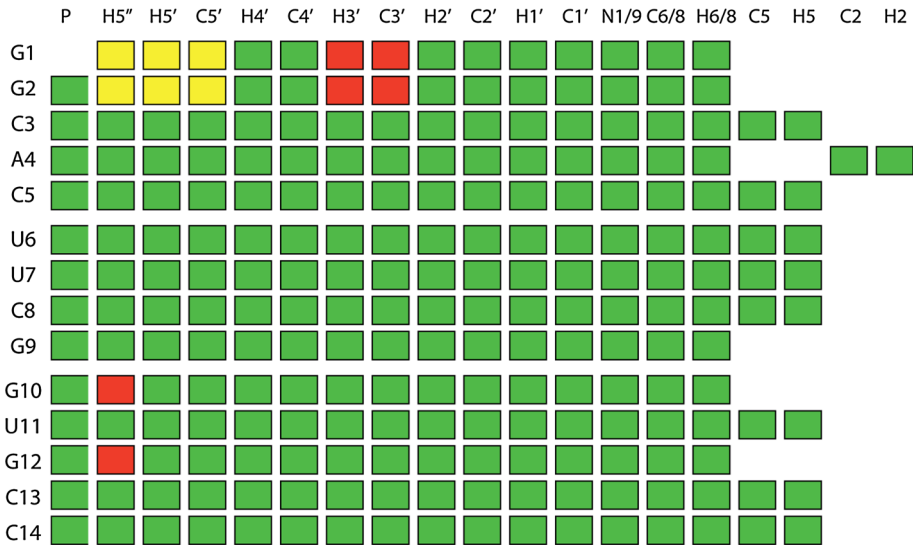

Fig. 2 Graphical representation of the correctness and completeness of the automated assignment strategy with high-dimensional APSY experiments as applied with SL14. The *rows* represent the nucleotides and are labeled with the nucleoside type and the residue number. The *columns* represent the corresponding nuclei types. Resonances marked with *green fields* are correctly assigned. *Red fields* signify that the resonances assigned to these nuclei were incorrect. *Yellow fields* signify that these resonances were not present in any spectra including the conventional 2D ^{1}H–^{13}C HSQC spectrum possibly due to small biochemical deviations of the SL14 RNA in our hands compared to the one used in previous studies (Nozinovic et al. 2010; Fürtig et al. 2004). Resonances H3′–C3′ of G1 and G2 were both assigned to the H2′–C2′ group of the same residues; the H5″ resonances of G10 and G12 were assigned to the same values as their H5′ chemical shifts. The overall assignment completeness is 98 %

TOCSY experiment for ribose resonances

These experiments are based on conventional HCCH TOCSY sequences. A general version of this experiment correlating each ribose ^{13}C–^{1}H group with all others of the same nucleotide was discarded for the following reasons: the two ^{13}C evolution periods are limited to 4–8 ms evolution time due to homonuclear couplings, the ^{13}C–^{1}H transfer time is a compromise between the time required for CH or CH_2 groups, respectively, and the high number of signals, 36, for each residue. The resulting spectra are prone to overlap even for a small RNA and for resolution-optimized methods such as APSY. The situation can be improved significantly with a selective H1′–C1′ excitation in the initial ^{1}H–^{13}C transfer, which reduces the number of signals per residue to 6, and slightly increases the sensitivity. Further, in this 4D APSY-H1′C1′CH experiment the C1′ evolution period can be extended by decoupling of C2′. An additional improvement of the resolution was achieved by implementing a constant-time evolution period for the second ^{13}C dimension, which increases the resolution by a factor of four, however, at the cost of reducing the sensitivity. The same considerations are also valid for a complementary HCCH TOCSY experiment that starts selectively on H5′/″–C5′. A detailed description and pulse schemes of the finally utilized pulse sequences can be found in the Supplementary Material p. S9.

TOCSY experiments for base resonances

For correlations in pyrimidines, a 4D APSY-H6C6C5H5 experiment was used with the magnetization transfer pathway H6–C6–(CC-TOCSY)–C5–H5 (Supplementary Material p. S5). The correlation of the H8–C8 group with the H2–C2 group in adenine was obtained with a 4D APSY-H8C8(CC-TOCSY)C2H2 experiment. The experiment was not needed for the assignment of the SL14 resonances since SL14 contains only one adenosine residue and the 2D H8–H2 and C8–H2 spectra, along with the C2–H2 correlation from the ^{1}H–^{13}C HSQC spectrum, were sufficient for unambiguous connection. The description of the pulse sequence is nevertheless included in the Supplementary Material p. S9, since it is useful for A-rich RNA.

Data acquisition and processing

The experiments were performed on a 500 MHz spectrometer equipped with a cryogenic ^{1}H/^{13}C/^{15}N triple resonance probe, and on a 600 MHz spectrometer equipped with a cryogenic ^{1}H/^{13}C/^{15}N/^{31}P quadruple resonance probe, both of them being Bruker Avance III spectrometers equipped with *z*-gradient accessory. All experiments were measured at 25 °C. The 5D APSY-HCP-CCH COSY experiment was acquired as 28 projections within 14 h. The spectral widths were set to 1,680, 486, 4,226, 4,226,

and 7,764 Hz, and the maximal acquisition times in the projection spectra to 8.0, 11.2, 19.7, 6.0, and 102.5 ms for $^{1}H_1$, ^{31}P, $^{13}C_1$, $^{13}C_2$, and $^{1}H_2$ (aq), respectively. The 4D APSY-H1′C1′CH TOCSY experiment was acquired as 41 projections within 12 h. The spectral widths were set to 1,800, 1,082, 5,282, and 7,764 Hz, and the maximal acquisition times in the projection spectra to 35.0, 50.0, 22.1, and 131.9 ms for $^{1}H1'$, $^{13}C1'$, ^{13}C, and ^{1}H (aq), respectively. The 5D APSY-HCNCH ribose-to-base experiment was acquired as 16 projections within 1.5 h. The spectral widths were set to 1,700, 1,132, 1,774, 1,384, and 8,503 Hz, and the maximal acquisition times in the projection spectra to 34.1, 33.6, 33.8, 16.6, and 120.5 ms for $^{1}H1'$, $^{13}C1'$, $^{15}N1/9$, $^{13}C6/8$, and $^{1}H6/8$ (aq), respectively. The 4D APSY-H6C6C5H5 TOCSY experiment was acquired as 25 projections within 3.4 h. The spectral widths were set to 660, 755, 1,509, and 7,764 Hz, and the maximal acquisition times in the projection spectra to 63.6, 49.0, 49.7, and 131.9 ms for $^{1}H6$, $^{13}C6$, $^{13}C5$, and $^{1}H5$ (aq), respectively. More information about the setup of the APSY experiments is provided in the Supplementary Material p. S2 and detailed descriptions of experimental parameters can be found in the Supplementary Material on pages S5, S6, S7, and S9.

The experiments were first set up conventionally as high-dimensional data sets with the spectrometer software Topspin 3.1 (Bruker, Karlsruhe, Germany). The 2D projection experiments were then set up from these parent data sets with the Bruker software "manageapsy", which is available as part of the Topspin software free of charge. The same program can also be used for automated serial processing, peak picking, and GAPRO analysis (Hiller et al. 2005), resulting in high-dimensional peak lists. More detailed descriptions of the procedures for measurement and processing of the experiments are provided in the Appendix of the Supplementary Material p. S15.

Automated resonance assignment

The unmodified APSY peak lists were used for automated sequence-specific resonance assignment with the recently published FLYA algorithm (Schmidt and Güntert 2012). Magnetization transfer pathways of high-dimensional experiments used in this study were defined in a small additional library, which is given in the Supplementary Material p. S3. The program Chess2FLYA (Aeschbacher et al. 2013) delivered narrow chemical shift ranges for the 1′/2/5/6/8 ^{13}C–^{1}H stem-region of SL14 based on its predicted secondary structure. These narrow chemical shift ranges applied for about 20 % of all signals. For the other resonances, more general statistical values, which have recently been thoroughly reassessed and calibrated (Aeschbacher et al. 2012), were used for approximate predictions. The input parameters were in general identical to the ones used for the automated assignment strategy for unlabeled RNA (Aeschbacher et al. 2013); the matching thresholds were set to 0.015/0.15/0.15/0.04 ppm for $^{1}H/^{13}C/^{15}N/^{31}P$. 50 independent assignment runs were performed, from which FLYA calculated a consensus assignment. Additional information about the use of FLYA is provided in the Supplementary Material p. S2.

Analysis of the results

The high-dimensional APSY peak lists were analyzed with MATLAB (R2010b, The MathWorks, Natick, MA, USA) for their completeness and matching precision with respect to each other. The completeness was analyzed by comparing the correlation peak lists in all dimensions with the manual assignment. Their matching precision was evaluated by overlaying the plots of the two dimensions in which the respective peak lists should be connected. When, e.g., the H1′–C1′ dimensions of the 5D HCNCH ribose-to-base peak list (H1′–C1′–N1/9–C6/8–H/8) are plotted in the same graph as the H1′–C1′ dimensions of the 4D H1′C1′CH TOCSY intra-ribose peak list, it can directly be evaluated whether these values are precise enough that the two peak lists can be connected unambiguously. The results from the automated assignment were evaluated in three steps. First, in order to give information about the reliability of the results, FLYA classifies the assignments into two categories, 'strong' and 'weak'. 'Strong' means that an assignment is consistent (within the corresponding chemical shift tolerance) over at least 80 % of the 50 independent runs of the algorithm. 'Strong' assignments are in general significantly more reliable than 'weak' ones (Schmidt and Güntert 2012). 'Weak' assignments need to be reviewed carefully, but are often still correct. Second, the FLYA assignment was compared with the conventional resonance assignment of SL14 (Fürtig et al. 2004) deposited in the BMRB database (www.bmrb.wisc.edu, entry 5,507). The ^{1}H and ^{13}C chemical shifts of individual ^{13}C–^{1}H moieties were regarded as 2D peaks, and the two corresponding assignments assumed identical when they lay within a threshold ellipse with half-axes of 0.4 and 0.12 ppm for the ^{13}C and ^{1}H dimensions, respectively. Thirdly, for APSY assignments that were classified as 'weak' by FLYA, or which disagreed with the manual assignment, confirmation was sought by re-evaluating with MATLAB the high-dimensional correlations in the APSY peak lists that led to the corresponding assignment, as described at the beginning of this section. E.g., if the assignment deposited in the BMRB did not agree with the APSY assignment for C5′, H5′ and H5″, but agreed for all other resonances of the same residue including the H1′ and C1′ resonances, the 4D H1′C1′CH peak list was evaluated: if it contains two very

precise and reliable 4D correlation peaks from these H1′–C1′ resonances to the H5′–C5′ and the H5″–C5′ resonances, the APSY assignment can be considered correct. Independent additional confirmation can be sought by verifying that the 4D correlations in the 4D H5′/H5″C5′CH peak list correspond to the assignment that was proposed by FLYA.

Results

A series of high-dimensional APSY experiments optimized for high resolution is presented, which significantly reduce one of the main obstacles for the automated assignment of RNA resonances: signal overlap. Due to the high resolution in the projection spectra (example spectra are presented in the Supplementary Material on p. S13), the high dimensionality of the APSY experiments, and statistical averaging effects, the chemical shift precision in the APSY peak lists is often a factor of 10–50 times higher than for conventional experiments (see Supplementary Material p. S4). The high dimensionality allows intra-nucleotide connections of the peak lists via two dimensions (^{1}H–^{13}C), which is not possible with conventional 2D and 3D experiments.

Some RNA assignment methods based on through-bond experiments for sequential inter-nucleotide connections rely on matching ^{31}P resonance frequencies. The ^{31}P resonances suffer from very poor signal dispersion (most resonances are within a range of 1 ppm), and from broad peaks due to chemical shift anisotropy (CSA) relaxation that increases with higher magnetic fields. Even the new APSY 5D experiment cannot resolve all ^{31}P resonances, but significantly improves the situation when compared to conventional 3D HCP or 3D HCP-CCH COSY experiments. If a set of cross peaks is resolved in the 5D space of the APSY-HCP-CCH COSY experiment, the precision of the ^{31}P chemical shift value can be enhanced by measuring high numbers of projections, so that ^{31}P resonances as close as 0.007 ppm can be distinguished. In contrast to conventional 3D experiments the 5D H(C)PCCH peak list derived from the 5D APSY-HCP-CCH COSY experiment contains up to three correlations from each ribose to the ^{31}P nuclei of the same or preceding residue, and hence several potential connections to the 4D H1′C1′CH peak list. The 5D H(C)PCCH peak list of SL14 contained correlations to all ^{31}P from both neighboring nucleotides, with the exception of the connection from U6 and G10 to their own ^{31}P, and from G1 to the ^{31}P of G2. The latter was due to the heterogeneity at the 5′-end, whereas the U6 and G10 links were missing due to small scalar coupling constants.

The elaborate geometric analysis with the algorithm GAPRO (Hiller et al. 2005) of the peaks picked in the 2D projection spectra led to the following peak lists: 200 5D H(C)PCCH peaks (up to $20 \times 13 = 260$ expected), 124 4D H1′C1′CH peaks ($6 \times 14 = 84$ expected), 144 4D H5′C5′CH peaks ($8 \times 14 = 112$ expected), 22 5D HCNCH (14 expected), 12 4D H6C6C5H5 peaks (8 expected), and 2 4D H8C8C2H2 peaks (1 expected). The back-projection of these peaks to the 2D projection spectra, and MATLAB plots of the correlations, confirmed that the peak lists were free of spectral artifacts: they corresponded to measured 2D projection peaks in all projections. All peaks that were contained in the APSY peak list in addition to the expected SL14 peaks belonged thus to real RNA peaks that originated from—for RNA samples not uncommon—impurities that derived from heterogeneity at the 5′-end. These peak lists were directly used as input for the automated assignment with the FLYA algorithm. FLYA proposed an assignment for all of the 227 expected ^{1}H, ^{13}C, ^{15}N and ^{31}P resonances of SL14 (without impurities) that are within the scope of the present suite of APSY experiments. 217 of these assignments were classified as strong.

The measurement time of 31 h for the APSY data set for SL14 was a consequence of the minimal number of required scans and of projection spectra per APSY experiment, which resulted in an excess of sensitivity for this 14-nucleotide RNA. E.g., the 5D APSY-HCNCH experiment that was acquired within 1.5 h for SL14 led within the same measurement time and conditions to a peak list with the same accuracy, precision and completeness for a stem-loop with 23 nucleotides (Krähenbühl et al. 2012).

Discussion and conclusions

When comparing the full APSY assignment with the BMRB version (Fig. 2), deviations were found for the following 25 ribose resonances: H3′ and C3′ in G1, G2, and U7; H4′ and C4′ in C13; H5″ in G10 and G12; H5′, H5″, and C5′ in G1, G2, C3, U6, and U11. A closer look at these assignments showed indeed that 6 of the APSY/FLYA assignments were incorrect: the H5″ chemical shifts of G10 and G12 were identical to the one of H5′, and the H3′–C3′ moieties of G1 and G2 were assigned to the same values as their H2′–C2′ moieties. All other APSY/FLYA assignments proved unambiguously correct: the 4D H1′C1′CH correlations connected clearly and precisely the—in the BMRB and the APSY assignment identical—H1′–C1′ resonances to the resonances in question, and all cross peaks of the complementary 4D APSY-H5′/″C5′CH TOCSY experiment confirmed the FLYA assignments. Two potential sources for the observed differences are differences in the NMR samples, and a significantly higher precision in the APSY peak lists, which allowed unambiguous connections that were inaccessible with

conventional methods. The first point was the main problem for residues G1 and G2 due to 5′-end heterogeneity, which had the effect that some of their resonances (CH1′/2′/5′/5″) were weaker and/or shifted. It seems that the second point was valid for C13 (H4′–C4′), U7 (H3′–C3′), as well as C3, U6 and U11 (H5′/″–C5′), since all of these resonances could be resolved with the high-dimensional APSY experiments, but were very close to other resonances; e.g., H4′–C4′ of C13 was within a range of 0.02/0.12 ppm (12/18 Hz on a 600 MHz spectrometer) for ^{1}H/^{13}C with the 4′ frequencies of N5 and N11. These resonances were thus either not resolved with conventional 2D or 3D experiments, or could not be connected unambiguously in the sequence-specific assignment, since connectivities often can only be based on one dimension if only 2D or 3D spectra are available. Experiments with high dimensionality as presented in this work do not only have a higher probability to resolve peaks, but also allow reliably establishing the intra-residue connection by matching the resulting APSY peak lists via at least two dimensions.

Sequential inter-nucleotide connections between nucleotides are obtained from coherence transfers via ^{31}P. Since ^{13}C–^{31}P coupling constants vary depending on the sugar pucker, a connection via ^{31}P may not be obtainable for both sequential sugars. In these situations the FLYA algorithm can complement the available spectral information by including predicted chemical shift ranges into the mapping strategy, as described in the Materials and Methods and earlier work (Aeschbacher et al. 2013; Lopez-Mendez and Güntert 2006; Schmidt and Güntert 2012). APSY ideally complements FLYA in this aspect, since it can provide reliable intra-residue correlations: there are spin systems with up to 16 chemical shifts per nucleotide that can be mapped to statistical values.

The 5D APSY HCP-CCH COSY experiment was required for the full assignment of SL14. Without the 5D H(C)PCCH peak list, the assignment resulting with FLYA was ambiguous for the loop residues: the resonances of G9 and G10 as well as those of C6 and C7 were interchanged. FLYA correctly recognized them as ambiguous, and classified these assignments as weak. Residue C8 was correctly assigned, but the quality factors of its resonances were still poor, since the full spin system was in some of the 50 FLYA runs assigned to C13. Consequentially, FLYA classified also the assignments of C13 as weak. The stem residues G1–C5 and U11–C14 were fully assigned based on the precise and complete APSY peak lists and the FLYA chemical shift statistics. As discussed before, the latter seems not to be sufficient outside regular stem regions in RNA (Aeschbacher et al. 2013). Sequential information is thus often required, be it from connections via ^{31}P or e.g., from a selective 4D APSY-CHCH NOESY experiment (Krähenbühl et al., submitted to J. Biomol. NMR). Whenever the inter-nucleotide through-bond experiment is feasible, it might be preferable due to the unambiguity of the connections.

In summary, 98 % of all ^{1}H–^{13}C, ^{15}N1/9, and ^{31}P resonances of SL14 could be assigned unambiguously (Fig. 2). This automated assignment was based on a novel suite of through-bond APSY experiments that provided highly precise and accurate peak lists, which were used as input to the automated resonance assignment algorithm FLYA.

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References

Aeschbacher T, Schubert M, Allain FH (2012) A procedure to validate and correct the ^{13}C chemical shift calibration of RNA datasets. J Biomol NMR 52(2):179–190. doi:10.1007/s10858-011-9600-7

Aeschbacher T, Schmidt E, Blatter M, Maris C, Duss O, Allain FH, Güntert P, Schubert M (2013) Automated and assisted RNA resonance assignment using NMR chemical shift statistics. Nucleic Acids Res 41(18). doi:10.1093/nar/gkt665

Fürtig B, Richter C, Bermel W, Schwalbe H (2004) New NMR experiments for RNA nucleobase resonance assignment and chemical shift analysis of an RNA UUCG tetraloop. J Biomol NMR 28(1):69–79

Güntert P (2003) Automated NMR protein structure calculation. Prog Nucl Magn Reson Spectrosc 43(3–4):105–125. doi:10.1016/S0079-6565(03)00021-9

Hiller S, Fiorito F, Wüthrich K, Wider G (2005) Automated projection spectroscopy (APSY). Proc Natl Acad Sci USA 102(31):10876–10881. doi:10.1073/pnas.0504818102

IUPAC-IUB (1983) Abbreviations and symbols for the description of conformations of polynucleotide chains. Recommendations 1982. IUPAC-IUB Joint Commission on Biochemical Nomenclature (JCBN). Eur J Biochem FEBS 131(1):9–15

Kim S, Szyperski T (2003) GFT NMR, a new approach to rapidly obtain precise high-dimensional NMR spectral information. J Am Chem Soc 125(5):1385–1393. doi:10.1021/ja028197d

Krähenbühl B, Wider G (2012) Automated projection spectroscopy (APSY) for the assignment of NMR resonances in biological macromolecules. Chimia 66(10):767–771

Krähenbühl B, Hofmann D, Maris C, Wider G (2012) Sugar-to-base correlation in nucleic acids with a 5D APSY-HCNCH or two 3D APSY-HCN experiments. J Biomol NMR 52(2):141–150. doi:10.1007/s10858-011-9588-z

Kupce E, Freeman R (2003) Projection-reconstruction of three-dimensional NMR spectra. J Am Chem Soc 125(46):13958–13959. doi:10.1021/ja038297z

Lopez-Mendez B, Güntert P (2006) Automated protein structure determination from NMR spectra. J Am Chem Soc 128(40):13112–13122. doi:10.1021/ja061136l

Marino JP, Schwalbe H, Anklin C, Bermel W, Crothers DM, Griesinger C (1995) Sequential correlation of anomeric ribose protons and intervening phosphorus in RNA oligonucleotides by

a ^{1}H, ^{13}C, ^{31}P triple-resonance experiment—HCP-CCH-TOCSY. J Biomol NMR 5(1):87–92

Narayanan RL, Dürr UHN, Bibow S, Biernat J, Mandelkow E, Zweckstetter M (2010) Automatic assignment of the intrinsically disordered protein Tau with 441-residues. J Am Chem Soc 132(34):11906–11907. doi:10.1021/ja105657f

Nozinovic S, Fürtig B, Jonker HRA, Richter C, Schwalbe H (2010) High-resolution NMR structure of an RNA model system: the 14-mer cUUCGg tetraloop hairpin RNA. Nucleic Acids Res 38(2):683–694. doi:10.1093/Nar/Gkp956

Orekhov VY, Ibraghimov I, Billeter M (2003) Optimizing resolution in multidimensional NMR by three-way decomposition. J Biomol NMR 27(2):165–173. doi:10.1023/A:1024944720653

Pardi A, Douglas JT, Latham MP, Armstrong GS, Bendiak B (2008) High-resolution pyrimidine- and ribose-specific 4D HCCH-COSY spectra of RNA using the filter diagonalization method. J Biomol NMR 41(4):209–219. doi:10.1007/s10858-008-9253-3

Ramachandran R, Sich C, Grüne M, Soskic V, Brown LR (1996) Sequential assignments in uniformly 13C- and 15N-labelled RNAs: The HC(N, P) and HC(N, P)-CCH-TOCSY experiments. J Biomol NMR 7(3):251–255

Schmidt E, Güntert P (2012) A new algorithm for reliable and general NMR resonance assignment. J Am Chem Soc 134(30):12817–12829. doi:10.1021/Ja305091n

Tate S, Ono A, Kainosho M (1995) Sequential backbone assignment in ^{13}C-labeled DNA by the ^{1}H, ^{13}C, ^{31}P triple-resonance experiment, HCP-CCH-COSY. J Magn Reson Ser B 106(1):89–91. doi:10.1006/jmrb.1995.1016