Development of Heteronuclear-Detected NMR-Experiments for Characterization of NH- and NH_2-Groups in RNA

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With significant improvements in cryogenic probes and the availability of high magnetic fields, the direct detection of heteronuclei in NMR spectroscopy of biomolecules experienced a renaissance. The technological advances have made low-γ nuclei detection schemes feasible even at relatively low sample concentrations of around 0.5 mM. Heteronuclear-detected experiments are especially beneficial in nucleic acids due to the inherent low proton density and its limited chemical shift dispersion. Here, we show a set of ^{15}N- and ^{13}C-detected NMR experiments that enable the characterization of imino- and amino-groups in RNA, even allowing their detection at sites that are unobservable in proton-detected experiments due to unfavorable molecular dynamics.

We apply ^{15}N-detected HSQC[1] and TROSY experiments to RNA and develop an improved ^{15}N-detected BEST-TROSY[2]. For the ^{15}N-detected BEST-TROSY experiment, we demonstrate application to five RNAs ranging in size from 14 nt to 329 nt and investigate the effect of molecular size on resolution, sensitivity and relaxation behavior in comparison to the ^{1}H-detected BEST-TROSY experiment. We observe a significantly faster increase in line width with molecular size for the signals in the ^{1}H-dimensions. Further, we analyze the effect of base pair type, chemical exchange and conformational equilibria on ^{15}N and ^{1}H signal line widths. We can show that imino protons in AU base pairs exhibit smaller line widths compared to GC base pairs in both, the ^{1}H- and the ^{15}N-dimension.

Despite their structural and functional relevance, NMR signals originating from amino groups are difficult to detect as their proton-resonances usually exhibit large line widths. Especially for guanosines and adenosines very few signals of the amino-groups can be observed. The reason for the line broadening is the restricted rotation around the C-NH_2 bond. As the rates of rotation are matching the difference in chemical shift of the amino-group's protons, their NMR signals are in intermediate exchange and consequently broadened beyond detectability. Switching to the fast exchange regime through evolution of double quantum magnetization enables indirect detection of the amino-group's protons. The applicability of such an approach was recently demonstrated for arginine residues in proteins[3].

We here present two NMR-experiments allowing identification and structural characterization of amino-groups in RNA. We introduce a ^{13}C-detected C(N)H-HDQC experiment which evolves double quantum proton magnetization in the indirect dimension and therefore enables the detection of sharp signals for all amino resonances independent of their rotational frequency (Figure 1 D). When compared to the conventional ^{15}N-HSQC-experiment (Figure 1 A) the advantage of the DQ experiment can be directly seen in the increased number of detectable resonances for adenosines and guanosines.

Further, a ^{13}C-detected 'amino'-NOESY experiment (Figure 2) was developed where NOE contacts stemming from the NH_2-groups can be observed which are not detectable by conventional ^{1}H-based NOESY experiments.

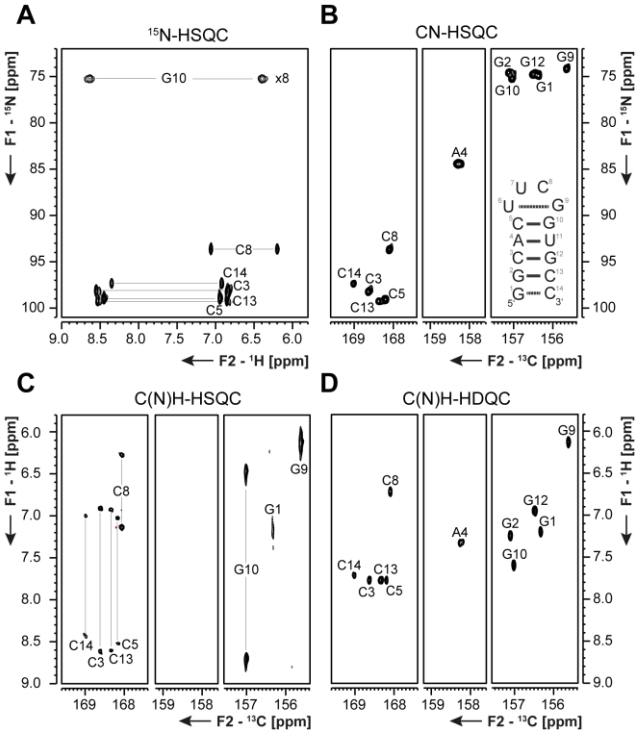

Figure 1 A) ^{15}N-HSQC, B) CN-HSQC, C) C(N)H-HSQC and D) C(N)H-HDQC spectra of the amino-region if the 14 nt RNA with UUCG tetraloop.

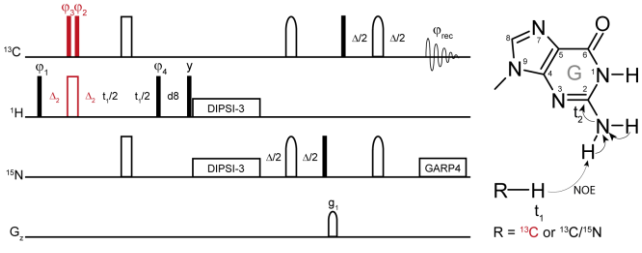

Figure 2 Pulse scheme of the 'amino'-NOESY experiment with optional ^{13}C-filter.

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