Presentation 10:
Viral RNA: NMR, structure and function
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The NMR structure and function of the 65 nucleotide epsilon RNA signals (and fragments) of the human and avian hepatitis B viruses will be discussed. The conserved epsilon signal is crucial for viral replication. Reverse transcription of the pregenomic RNA is started by binding of the viral reverse transcriptase to epsilon apical loop and bulge and subsequent synthesis of a 4 nt DNA primer from a bulge in epsilon. Because the 65 nt epsilon is large for NMR structural studies, novel strategies have applied and will be highlighted. For instance, *in vitro* labeling strategies, including deuteration and multiple segmental labeling will be discussed. In addition, the usage of chemical shift prediction and prediction of residual dipolar couplings in structure calculation and validation will be discussed. It will be shown that residual dipolar couplings in RNA or DNA in Pf1 medium can reliably predicted from the phosphate charge distribution and thus from the shape of the RNA/DNA via simple analytical expressions derived from the Debye-Huckel expression for the electrostatic potential. This prediction of RDC turned out to important for the structure derivation of the human 65 nt epsilon RNA. Finally, the remarkable differences in stability between human and avian epsilon might at least in part explain why interspecies HBV infection does not occur.

Presentation 11 (Promoted Poster P626):
Complementary Segmental Labeling of Large RNAs: Economic Preparation and Simplified NMR Spectra for Measurement of More RDCs
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Knowledge of the 3D structure of large, biologically important RNAs at the atomic level assists in revealing their functions. NMR is a powerful method to study small RNA structures in solution. However, difficulties arise while studying larger RNAs due to the intrinsic nature of RNA molecules: the relatively low proton density compared to a protein of similar size and the often extended conformation, compared to more compact protein fold. Along with more conventional distance and torsion angle restraints, angular restraints derived from residual dipolar couplings (RDC) can help better define the local and global shape of a larger RNA molecule and therefore it is crucial to obtain as many RDC restraints as possible. However, with large RNAs, increasing resonance overlap severely limits the number of obtainable RDC restraints.

In order to simplify NMR spectra of large RNAs to a greater extent, thereby allowing the extraction of additional RDC information, we have established a time and cost-effective methodology for the preparation of RNAs having both ^{13}C-only and ^{15}N-only labelled segments. Using a single plasmid, the ^{13}C-only or ^{15}N-only 5'- and 3'- RNA fragments are transcribed with two cis-acting hammerhead ribozymes, which excise the two desired RNA sequences from the primary transcript with appropriate ends for subsequent combinatorial ligation with T4 RNA ligase. We applied this methodology to a 25 kDa RNA, the brain cytoplasmic 1 (BC1) RNA. In this example, we prepared two individual RNA samples containing both ^{13}C-only and ^{15}N-only labelled segments. The simplified NMR spectra revealed previously severely overlapped peaks in the original spectrum of the uniformly ^{13}C, ^{15}N-labelled RNA construct and therefore allowed us to obtain a considerably larger number of RDC restraints (34 for the imino and 95 for the aromatic protons versus 24 and 45, respectively). We will demonstrate how this approach greatly benefits the high-resolution structure determination of large RNAs.