P-1

# Simple and fast preparation of RNA samples for NMR spectroscopy: Use of DNAzyme and Sequence-specific Affinity column chromatography

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Understanding the molecular mechanism of RNA recognition by protein or ligand requires knowledge on the three-dimensional structures of RNA and RNA-protein or RNA-ligand complexes. Chemical synthesis on large scale is possible only for short RNAs. For longer RNAs, enzymatic synthesis using T7 bacteriophage RNA polymerase was used for NMR spectroscopy and X-ray crystallography. This method allows the synthesis of longer RNAs and has an additional advantage that isotope labeling is possible. Although *in vitro* enzymatic synthesis is much more efficient and significantly more cost-effective for NMR studies, it requires a purine-rich sequence at 5'-end of RNA to obtain high yield. In addition, the run-off transcription results in heterogeneity at the 3-terminus. RNA purification requires single nucleotide resolution to separate transcripts of the correct length (N) from abortive (N-1) or add-on (N+1, N+2) transcripts, which are, in most cases, present in comparable amounts. Usually, polyacrylamide gel electrophoresis (PAGE) is used to resolve the correct RNA transcripts followed by ethanol precipitation and dialysis. However PAGE purification of RNA is tedious and time consuming along with cost ineffective.

To overcome these problems in high-throughput RNA synthesis, we devised transacting DNAzyme and sequence-specific affinity column chromatography. The affinity column chromatography is used to separate abortive transcripts from correct length and add-on transcripts. We added tag sequences at the 3'-end of RNA. The tagged RNA was picked out by the affinity column where the complementary DNA sequence is attached to. The 3'-end tag was removed by sequence specific cleavage using trans-acting DNAzyme. The desired RNA that is free from the 3'-heterogeneity could be obtained by use of the sequence-specific trans-acting DNAzyme. Moreover, to increase the transcription yields, leader sequences were added to the 5'-end and site specifically cleaved by DNAzyme. The methods described above made the purification steps simple and fast.