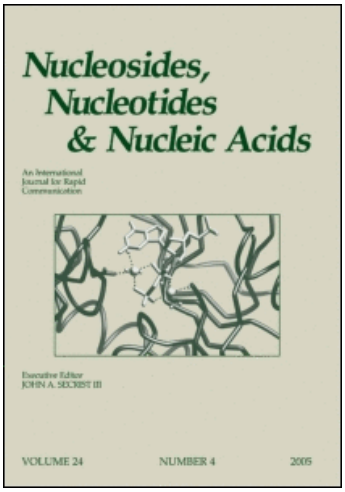

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The Application of "The Uppsala NMR-Window" Concept for Conformational Analysis of Large DNA & RNA by High-Field NMR Spectroscopy

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THE APPLICATION OF "THE UPPSALA NMR-WINDOW" CONCEPT FOR CONFORMATIONAL ANALYSIS OF LARGE DNA & RNA BY HIGH-FIELD NMR SPECTROSCOPY

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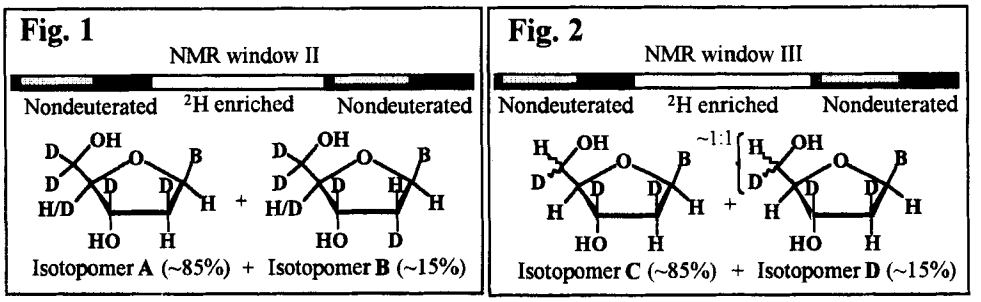
ABSTRACT: A summary of "the Uppsala NMR-window" concept (see refs. 1-9) developed for the high-field NMR studies of the solution structure of biologically functional DNAs and RNAs is presented.

The most serious problems faced in the NMR structure determination of large DNA or RNA are the *overlap of resonances, line-broadening* and *spin-diffusion.* For overcoming these difficulties, we have been progressively developing a non-uniform deuterium labelling technique[1,2] (the "Uppsala NMR-window" concept).

(A) The "NMR-window I" concept[1,2]: Site-specifica deuteration of a 20-mer DNA using 1',2',2",3',4',5',5"-d_7-2'-deoxyribonucleoside blocks (>97 atom % ^{2}H at C2', 2", C3' and C5', ~85 atom % ^{2}H at C4' and ~20 atom % ^{2}H at C1') helped indeed in the full assignment of the chemical shifts and to extract NOE volumes accurately[3]. Protons vicinal to a deuteron had ~2-3 fold increase of T_1 and ~20 fold increase of T_2. The letter allowed to filter away protons with shorter T_2 using a number of different NMR experiments[4]. When 1',2',3',4',5',5"-d_6-ribonucleoside blocks were site-specifically incorporated into a 21mer RNA hairpin loop[5,6] as well as into a 31mer stem-internal loop-stem-hairpin loop RNA[7], the number of NMR constraints increased owing to unambiguous assignments and decrease of the overlap of resonances, thereby helping to solve their 3D structures.

(B) In **the "NMR-window II" concept**[8] deuterated nucleoside blocks (Fig.1 **A** and **B**) give the structural information needed: their use in oligo-DNAs[8] enabled the collection of nOes of reduced spin-diffusion from a HAL-NOESY experiment (including H1'-H4', H4'-H2" nOe). The diastereoselective deuterium incorporation at C2' also helped in obtaining $^3J_{H1',H2'}$ and $^3J_{H1',H2"}$.

(C) The "NMR-window III" concept[9]: In order to increase the number of available NMR constraints, we refined our concept ("NMR-window III, Fig. 2). further



by applying deuterated building blocks of types **C** and **D**, having an isotopomeric mixture at the C-2' and C-5'. The uniform incorporation of these blocks into the Dickerson-Drew dodecamer allowed the extraction of the H5'/H5"-H2", H4'-H2", H4'-H5'/H5", H1'-H5', H1'-H5", H1'$_i$-H5'$_{i+1}$, H1'$_i$-H5"$_{i+1}$, H1'-H4', H1'$_i$-H4'$_{i+1}$ and arom-H5'/H5" nOe volumes with reduced spin-diffusion as well as the $^3J_{H1',H2'}$, $^3J_{H1',H2"}$, $^3J_{H4',H5'}$ and $^3J_{H4',H5"}$ homonuclear and $^3J_{P,H4'}$, $^3J_{P,H5'}$ and $^3J_{P,H5"}$ heteronuclear coupling constants for the elucidation of the conformation of the sugar mioiety and to determine the backbone conformation.

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