

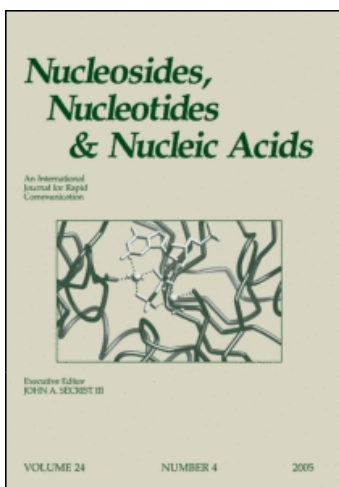
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Nucleosides, Nucleotides and Nucleic Acids

Publication details, including instructions for authors and subscription information:

<http://www.informaworld.com/smpp/title~content=t713597286>

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

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To cite this Article Földesi, A. , Maltseva, T. V. and Chattopadhyaya, J.(1999) 'The Application of “The Uppsala NMR-Window” Concept for Conformational Analysis of Large DNA & RNA by High-Field NMR Spectroscopy', *Nucleosides, Nucleotides and Nucleic Acids*, 18: 6, 1599 – 1600

To link to this Article: DOI: 10.1080/07328319908044795

URL: <http://dx.doi.org/10.1080/07328319908044795>

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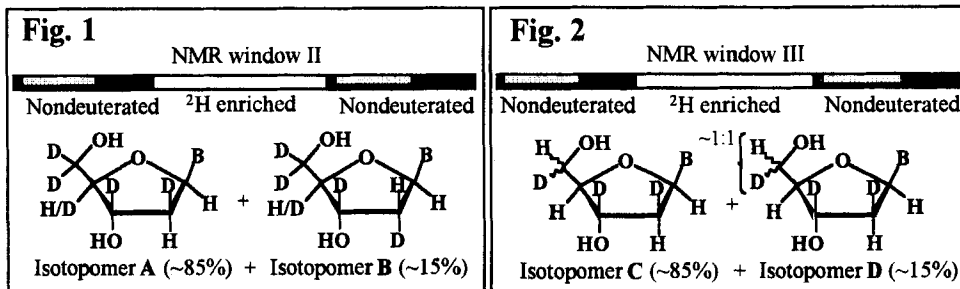
ABSTRACT: A summary of "the Uppsala NMR-window" concept (see refs. 1-9) developed for the high-field NMR studies of the solution structure of biologically functional DNAs and RNAs is presented.

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 % ²H at C2', 2'', C3' and C5', ~85 atom % ²H at C4' and ~20 atom % ²H at C1') helped indeed in the full assignment of the chemical shifts and to extract NOE volumes accurately³. Protons vicinal to a deuteron had ~2-3 fold increase of T₁ and ~20 fold increase of T₂. The letter allowed to filter away protons with shorter T₂ using a number of different NMR experiments⁴. 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 RNA⁷, 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" concept⁸ deuterated nucleoside blocks (Fig.1 A and B) give the structural information needed: their use in oligo-DNAs⁸ 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" concept⁹: 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 moiety and to determine the backbone conformation.

Acknowledgements: Authors thank Swedish Board for Technical Development (NUTEK), Swedish Natural Science Research Council (NFR) and Swedish Research Council for Engineering Sciences (TFR) for financial support.

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