Nucleic Acids

NMR Spectroscopy

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NMR of Nucleic Acids 1

1) NMR Spectroscopy for Structural Studies

2) Primary Structure of DNA and RNA

3) Resonance Assignment of DNA/RNA by Homonuclear NMR

A) $^1$H Chemical shifts
B) Assignment of exchangeable
C) Assignment of non-exchangeable proton
D) Typical NOEs in helical structures
E) Correlation between non-exchangeable and exchangeable protons
NMR Spectroscopy is an Important Method for Structural Studies of Nucleic Acids:

PDB Holding, March 2, 2010

<table>
<thead>
<tr>
<th>Technique</th>
<th>Molecule</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Proteins</td>
<td>Nucleic Acids</td>
<td>Protein/Nucleic Acid Complexes</td>
<td>Other</td>
<td></td>
</tr>
<tr>
<td>X-ray Diffraction</td>
<td>51’485</td>
<td>1’193</td>
<td>2’372</td>
<td>17</td>
<td>55’067</td>
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<tr>
<td>NMR</td>
<td>7’219</td>
<td>894</td>
<td>153</td>
<td>7</td>
<td>8’273</td>
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<tr>
<td>Other 1)</td>
<td>322</td>
<td>23</td>
<td>78</td>
<td>14</td>
<td>436</td>
</tr>
<tr>
<td>total</td>
<td>59’026</td>
<td>2’109</td>
<td>2’603</td>
<td>38</td>
<td>63’776</td>
</tr>
</tbody>
</table>

1) EM, Hybrid, other

http://www.rcsb.org/pdb
Nucleic Acids are Polymers of Nucleotides

Phosphate

Pentose

Base
Common Pyrimidine Bases

Cytosine (RNA)

Uracil

Thymine (DNA)

Numbering
Common Purine Bases

Adenine

Guanine

Numbering
Alternate Bases & Modifications (small selection):

- Inosine
  Base: Hypoxanthine

- Xanthosine

- 2Amino Adenosine

- O6 Me Guanosine

- 7 deaza Adenosine

- Nebularine

- 6 Dimethyl aminopurine

- 2 Aminopurine

- 5Me Cytosine
HETERO BASE PAIRS

Hoogsteen

Watson-Crick

Reverse Watson-Crick

Nucleic acids: structures, properties, and functions (2000) By Victor A. Bloomfield, Donald M. Crothers, Ignacio Tinoco
HOMO BASE PAIRS

Nucleic acids: structures, properties, and functions (2000) By Victor A. Bloomfield, Donald M. Crothers, Ignacio Tinoco

TT(I)

TT(II)

AA(II)

GG(II)

AA(I)

GG(I)
Structure Determination:

I) Assignment
II) Local Analysis
   • glycosidic torsion angle, sugar puckering, backbone conformation, base pairing
III) Global Analysis
   • sequential, inter strand/cross strand, dipolar coupling

Nucleic Acids have few protons.....
   • NOE accuracy
     > account for spin diffusion
   • Backbone may be difficult to fully characterize
     > especially α and ζ.
   • Dipolar couplings
Chemical shift ranges in nucleic acids
<table>
<thead>
<tr>
<th>DNA</th>
<th>RNA</th>
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<tr>
<td>H1' 5–6</td>
<td>H1' 5–6</td>
</tr>
<tr>
<td>H2' 2.3–2.9(A,G) 1.7–2.3(T,C)</td>
<td>H2' 4.4–5.0</td>
</tr>
<tr>
<td>H2'' 2.4–3.1(A,G) 2.1–2.7(T,C)</td>
<td>H3' 4.4–5.2</td>
</tr>
<tr>
<td>H3' 4.4–5.2</td>
<td>H4' 3.8–4.3</td>
</tr>
<tr>
<td>H4' 3.8–4.3</td>
<td>H5' 3.8–4.3</td>
</tr>
<tr>
<td>H5' 3.8–4.3</td>
<td>H5'' 3.8–4.3</td>
</tr>
<tr>
<td>H5'' 3.8–4.3</td>
<td></td>
</tr>
<tr>
<td>C1' 83–89</td>
<td>C1' 87–94</td>
</tr>
<tr>
<td>C2' 35–38</td>
<td>C2' 70–78</td>
</tr>
<tr>
<td>C3' 70–78</td>
<td>C3' 70–78</td>
</tr>
<tr>
<td>C4' 82–86</td>
<td>C4' 82–86</td>
</tr>
<tr>
<td>C5' 63–68</td>
<td>C5' 63–68</td>
</tr>
</tbody>
</table>

**Diagram:**
- **2'-Deoxy-β-D-Ribose**
- **β-D-Ribose**
9R-Borano DNA•RNA

5′-d(A T G G T G C T C)
(u a c c a c g a g)r-5′
<table>
<thead>
<tr>
<th>Adenine</th>
<th></th>
<th>Guanine</th>
</tr>
</thead>
<tbody>
<tr>
<td>H2 7.5-8</td>
<td>C2 152-156</td>
<td>– – C2 156</td>
</tr>
<tr>
<td>H8 7.7-8.5</td>
<td>C8 137-142</td>
<td>H8 7.5-8.3 C8 131-13</td>
</tr>
<tr>
<td>N6H 5-6/7-8</td>
<td>N6 82-84</td>
<td>N1H 12-13.6 N1 146-14</td>
</tr>
<tr>
<td>– –</td>
<td>–</td>
<td>N2H 5-6/8-9 N2 72-76</td>
</tr>
<tr>
<td>C4 149-151</td>
<td>–</td>
<td>C4 152-15</td>
</tr>
<tr>
<td>C5 119-121</td>
<td>–</td>
<td>C5 117-11</td>
</tr>
<tr>
<td>C6 157-158</td>
<td>–</td>
<td>C6 161</td>
</tr>
<tr>
<td>N1 214-216</td>
<td>–</td>
<td>N1 146-14</td>
</tr>
<tr>
<td>N3 220-226</td>
<td>–</td>
<td>N3 167</td>
</tr>
<tr>
<td>N7 224-232</td>
<td>–</td>
<td>N7 228-23</td>
</tr>
<tr>
<td>N9 166-172</td>
<td>–</td>
<td>N9 166-17</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Thymidine</th>
<th>Uridine</th>
<th>Cytidine</th>
</tr>
</thead>
<tbody>
<tr>
<td>H6 6.9-7.9 C6 137-142</td>
<td>H6 6.9-7.9 C6 137-142</td>
<td>H6 6.9-7.9 C6 136-1</td>
</tr>
<tr>
<td>Me5 1.0-1.9 Me5 15-20</td>
<td>H5 5.0-6.0 C5 102-107</td>
<td>H5 5.0-6.0 C5 94-99</td>
</tr>
<tr>
<td>N3H 13-14 N3 156</td>
<td>N3H 13-14 N3 156-162</td>
<td>– – N3 210</td>
</tr>
<tr>
<td>– – – –</td>
<td>– – –</td>
<td>N4H 6.7-7/81-8.8 N4 94-96</td>
</tr>
<tr>
<td>C2 154</td>
<td>C2 154</td>
<td>C2 159</td>
</tr>
<tr>
<td>C4 169</td>
<td>C4 169</td>
<td>C4 166-1</td>
</tr>
<tr>
<td>C5 95-112</td>
<td>C5 102-107</td>
<td>C5 94-99</td>
</tr>
<tr>
<td>N1 144</td>
<td>N1 142-146</td>
<td>N1 150-1</td>
</tr>
</tbody>
</table>
No Structure Required!

Often, depending on the question asked, a full structure determination is not required.

- Does it form a duplex?
- Which base pairs are thermo labile?
- Which base pair is which… assignment?
- Is the loop structured?
- Structure
DNA Hairpin

Thermal lability

Germann et al., Nucl. Acids Res. 1990 18: 1489-1498
“New” DNA constructs

Do the duplexes form, is there base pairing?

Does the unusual base pair form?

Do the duplexes form, is there base pairing?

Does the unusual base pair form?

Local Parallel Stranded Environment is Necessary for Stable Duplex Formation

15% Native PAGE, 15 mM MgCl₂
Solvent Suppression

The presence of an intense solvent resonance necessitates an impractical high dynamic range. 110 M vs <1mM (down to 5-10 uM)
To overcome this problem several methods are currently applied:

1) Presaturation
2) Observing the FID when the water passes a null condition after a 180 degree pulse.
3) Suppression of broad lined based on their $T_2$ behavior.
4) Selectively excitation, with and without gradients
5a) Use of GRASP to select specific coherences thereby excluding the intense solvent signal. In this case the solvent signal never reaches the ADC. This allows the observation of resonances that are buried under the solvent peak.
5b) Use of GRASP to selectively dephase the solvent resonance (WATERGATE)
5c) Excitation sculpting
Presat

Presaturation field strength:
20-40 Hz corresponds to a 6-12ms 90deg pulse

Pros: Easy to set up
Excellent water suppression

Cons: Resonances under water signal! (T variation)
Labile protons not visible (some GC pairs may be)

Selective Excitation

Selective rf pulse on solvent resonance followed by a gradient pulse to dephase the water signal. This could be followed by a mild presaturation field. Selective rf pulse (1-2ms, depending on width to be zeroed usually of the gauss type. The selective rf pulse z-gradient constructs could be repeated (WET).
Jump and return

Pros: Easy to set up
    Excellent water suppression
    (with proper setup as good as presat)
    Good for broad signals!

Cons: Non uniform excitation Baseline not flat

Other sequences: 1331 etc

Watergate

Pros: Excellent water suppression
    Uniform excitation
    Baseline flat

Cons: May loose broad resonances

\[ p_1G_1 + p_2G_2 \ldots = 0 \]
Exitation Sculpting


Pros: Easy to set up
Excellent water suppression
Good for broad signals!
Uniform excitation

Cons: May lose some intensity on very broad signals

Spectra: 1.5mM DNA in Water, Nanjunda, Wilson and Germann unpublished
**Structure Determination, NMR experiments:**

<table>
<thead>
<tr>
<th>I) Assignment</th>
<th>NOESY, COSY, HSQC TOCSY.......</th>
</tr>
</thead>
<tbody>
<tr>
<td>II) Local Analysis</td>
<td></td>
</tr>
<tr>
<td>• glycosidic torsion angle</td>
<td>(NOE, COSY)</td>
</tr>
<tr>
<td>• sugar puckering</td>
<td>(COSY, COSY, NOE, +)</td>
</tr>
<tr>
<td>• backbone conformation</td>
<td>(COSY, +)</td>
</tr>
<tr>
<td>• base pairing</td>
<td>(NOE, COSY)</td>
</tr>
<tr>
<td>III) Global Analysis</td>
<td></td>
</tr>
<tr>
<td>• sequential</td>
<td>(NOE, COSY)</td>
</tr>
<tr>
<td>• inter strand/cross strand</td>
<td>(NOE, COSY)</td>
</tr>
<tr>
<td>• dipolar coupling</td>
<td>(HSQC, HSQC)</td>
</tr>
</tbody>
</table>

Black: unlabeled, **Blue: labeled** DNA or RNA
Stereospecific Assignment

Deoxyribose

Ribose

How do we determine them?

a) Rule of Thumb (5’ downfield of 5’’)
   Shugar and Remin BBRC (1972), 48, 636-642

b) Short mixing times NOESY
   dH1’H2” shorter than H1’H2’
   -> Crosspeak H1’-H2’’ > H1’H2’
Structure Determination:

I) Assignment

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Nucleic Acids have few protons.....
   • NOE accuracy
     > account for spin diffusion
   • Backbone may be difficult to fully characterize
     > especially $\alpha$ and $\zeta$.
   • Dipolar couplings
Distance information determines the glycosidic torsion angle.

How do we get distance information?

- Nuclear Overhauser effect (< 6Å)

2.5Å

3.8Å
Distance information determines the glycosidic torsion angle.

How do we get distance information?
- Nuclear Overhauser effect (< 6 Å)

- 2.5 Å
- 3.8 Å
Sugar puckering

The five membered furanose ring is not planar. It can be puckered in an envelope form (E) with 4 atoms in a plane or it can be in a twist form. The geometry is defined by two parameters: **the pseudorotation phase angle** (**P**) and the **pucker amplitude** (**Φ**).

In general:
RNA (A type double helix) C3' endo.
DNA (B type double helix) C2' endo.

\[
\nu_j = \Phi_m \cos (P + 144 (j-2))
\]

\[\Phi_m \text{ range: } 34° - 42°\]

\[\delta = \nu_3 + 125°\]
N (Northern)

(Southern)

Ribose: $^3J_{H1'\cdash H2'} \approx 1$ Hz (Angle $\approx 90$ deg)
Deoxyribose: $^3J_{H1'\cdash H2'} \approx 1.8$ Hz

Ribose: $^3J_{H1'\cdash H2'} \approx 7.9$ Hz (Angle $\approx 170$ deg)
Deoxyribose: $^3J_{H1'\cdash H2'} \approx 10$ Hz
2’endo sugar H1’, H2’, H2'', H3’ region

3’endo sugar H1’, H2’, H2”, H3’ region

No relaxation Tdeff 900x400

-NMR-Sim sample spin system
-Deoxyribose 3’endo N conf
-Widmer and Wuthrich

proton a 5.80; H1’
proton b 2.30; H2’
proton c 2.50; H2”
proton d 4.90; H3’

couple a b 1.5
couple a c 7.7
couple b c -14.1
couple b d 7.2
couple c d 9.7
COSY

2'endo sugar

NO RELAXATION 1KX1K (Td
=======================
; NMR-Sim sample spin s
; Deoxyribose 2'endo S
;
proton a 5.80 t=0.5
proton b 2.30 t=0.5
proton c 2.50 t=0.5 ; H
proton d 4.90 t=0.5 ; H

couple a b 9.5 1'-2'
couple a c 5.8 1'-2'
couple b c -14.1

couple b d 5.5
couple c d 1.3

NO RELAXATION TDef=900
=======================
; NMR-Sim sample spin s
; Deoxyribose 3'endo N
;
proton a 5.80 t=0.5 ; H
proton b 2.30 t=0.5 ; H
proton c 2.50 t=0.5 ; H
proton d 4.90 t=0.5 ; H

couple a b 1.5 1'-2'
couple a c 7.7 1'-2'
couple b c -14.1

couple b d 7.2
couple c d 9.7

3'endo sugar
LFA- COSY

2’endo sugar H1’, H2’, H2” region

5.8 Hz = J H1’–H2”

9.5 Hz = J H1’–H2’

14.1 Hz

5.80 ppm

NMR-Sim sample spin syst
Deoxyribose 2’endo S con
proton a 5.80 t=0.5 ;H1
proton b 2.30 t=0.5 ;H2
proton c 2.50 t=0.5 ;H2”
proton d 4.90 t=0.5 ;H3’
couple a b 9.5
couple a c 5.8
couple b c -14.1
couple b d 5.5
couple c d 1.3
Sugar puckering

Usually (DNA) one observes equilibrium of the S and N forms sugar re-puckering. Unless one form greatly dominates the local analysis requires quite a few parameters: $P_N$, $P_S$, $\Phi_N$, $\Phi_S$, $f_S$

Several methods for analysis exist, graphical and the more rigorous simulation. In practice the desired outcome determines the effort to be made. Sums of the coupling constants are often easier to obtain.

$$f_S = \frac{(\sum 1' - 9.8)}{5.9}$$

See also our pure examples:
- $f_S=0$ and $\sim 1$ respectively

\[\begin{align*}
\Sigma 1' &= J_{1'2'} + J_{1'2''} \\
\Sigma 2' &= J_{1'2'} + J_{2'3'} + J_{2'2''} \\
\Sigma 2'' &= J_{1'2''} + J_{2''3'} + J_{2'2''} \\
\Sigma 3' &= J_{2'3'} + J_{2''3'} + J_{3'4'}
\end{align*}\]

- If $f_S < 50\%$ $J_{1',2'} < J_{1',2''}$
- If $f_S \approx 0\%$ $J_{1',2'}$ very small
- If $f_S > 70\%$ $J_{1',2'} > J_{1',2''}$
# Sugar puckering

<table>
<thead>
<tr>
<th></th>
<th>control</th>
<th>alphaT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nt</td>
<td>Σ1'</td>
<td>f_s</td>
</tr>
<tr>
<td>G1</td>
<td>15.2</td>
<td>0.92</td>
</tr>
<tr>
<td>C2</td>
<td>15.1</td>
<td>0.90</td>
</tr>
<tr>
<td>G3</td>
<td>16.2</td>
<td>1.00</td>
</tr>
<tr>
<td>A4</td>
<td>16.2</td>
<td>1.00</td>
</tr>
<tr>
<td>A5</td>
<td>15.7</td>
<td>1.00</td>
</tr>
<tr>
<td>T6</td>
<td>15.1</td>
<td>0.90</td>
</tr>
<tr>
<td>T7</td>
<td>16.0</td>
<td>1.00</td>
</tr>
<tr>
<td>C8</td>
<td>15.1</td>
<td>0.90</td>
</tr>
<tr>
<td>G9</td>
<td>15.7</td>
<td>1.00</td>
</tr>
<tr>
<td>C10</td>
<td>(14)</td>
<td>(0.7)</td>
</tr>
</tbody>
</table>

**Diagram:**

**MD calculation**

Aramini, 2000, J. Biomolecular NMR, 18, 287-302
Pseurot calculations

Φ_{S,N} = 37°

P_S = 125-165

f_S = 0.4

Introduction to Cross-Correlated Relaxation

Relaxation in NMR
→ determines experimental strategies and experiments
→ dynamic and structural parameters

Mechanisms
→ Dipole -dipole
→ CSA (e.g. $^{31}$P at higher fields; proportional to $B^2$)
→ Scalar relaxation (first and second kind)
→ paramagnetic, etc

Recently it became possible to use cross correlated relaxation (CCR) to directly measure bond angles without using a calibration curve as is needed for J’s.

→ DD -DD
→ DD -CSA
Sugar Puckering from Cross-Correlated Relaxation \( \Gamma_{DD-DD} \)

\[ \Gamma_{C1'H1'-C2'H2'} = k (3 \cos^2 \theta - 1) \tau_c \]

\[ \theta = 180: \text{ for 2'endo (B form)} \]

\[ \theta = 90: \text{ for 3'endo (A form)} \]

Small and negative

Large and positive

\[ \theta_{1,2} = 121.4^\circ + 1.03 \psi_m \cos(P-144^\circ) \]

--

Sugar puckering: Summary

-Coupling constants: COSY, E.COSY, low flip angle COSY
   Homonuclear, Heteronuclear
-CT NOESY
-CSA-DD and DD-DD cross correlated data
-\(^{13}\)C chemical shifts, in favorable cases

Some references

Measurement of Deoxyribose \(^3\)JHH Scalar Couplings Reveals Protein-Binding Induced Changes in the Sugar Puckers of the DN

An efficient NMR experiment for analyzing sugar-puckering in unlabeled DNA:. Couplings via constant time NOESY.

NMR determination of sugar-puckers in nucleic acids form CSA-dipolar cross correlated relaxation,

BioNMR in Drug Research 2003 Editor(s): Oliver Zerbe (Wiley-VCH)
Methods for the Measurement of Angle Restraints from Scalar, Dipolar Couplings and from Cross-Correlated Relaxation: Application to Biomacrom-
Chapter 7 p147-178. Christian Griesinger (also for \(\alpha\) and \(\zeta\))

Summary to get J’s
   E. Cosy
   DQ/ZQ principle
   FIDS Principle
   Quant. J correlation
α and ζ pose problems
Determinants of $^{31}$P chem shift.

ε and ζ correlate. ζ = -317-1.23 ε
Backbone Experiments: CT-NOESY, CT-COSY