Nucleic Acid NMR

Part II
α and ζ pose problems
Determinants of $^{31}$P chem shift.

ε and ζ correlate. $\zeta = -317 - 1.23 \varepsilon$

Ranges

<table>
<thead>
<tr>
<th></th>
<th>$\chi$</th>
<th>$\alpha$</th>
<th>$\beta$</th>
<th>$\gamma$</th>
<th>$\delta$</th>
<th>$\varepsilon$</th>
<th>$\zeta$</th>
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</thead>
<tbody>
<tr>
<td>B-DNA</td>
<td>-119</td>
<td>-61</td>
<td>180</td>
<td>57</td>
<td>122</td>
<td>-187</td>
<td>-91</td>
</tr>
<tr>
<td>Bf-DNA</td>
<td>-102</td>
<td>-41</td>
<td>136</td>
<td>38</td>
<td>139</td>
<td>-133</td>
<td>-157</td>
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<tr>
<td>Af-DNA</td>
<td>-154</td>
<td>-90</td>
<td>-149</td>
<td>47</td>
<td>83</td>
<td>-175</td>
<td>-45</td>
</tr>
</tbody>
</table>

Sanger, Principles of nucleic acid
Springer 1984
Backbone Experiments: CT-NOESY, CT-COSY

Σ Backbone Experiments

• Z. Wu, N. Tjandra, and A. Bax, Measurement of H3'-31P dipolar couplings in a DNA oligonucleotide by constant-time NOESY difference spectroscopy, J. Biomol. NMR 19, 367-370 (2001).


• BioNMR in Drug Research 2003 Edito: O. Zerbe
  Methods for the Measurement of Angle Restraints from Scalar, Dipolar Couplings and from Cross-Correlated Relaxation: Application to BiomacromoleculesChapter Author: Christian Griesinger: J-Resolved Constant Time Experiment for the Determination of the Phosphodiester Backbone Angles α and ζ.
Resonance Assignment DNA/RNA (Homonuclear)

A) Non Exchangeable Protons
   • Aromatic Spin Systems
     NOESY, DQFCOSY, TOCSY
   • Sugar Spin Systems
     DQFCOSY, TOCSY
   • Sequential Assignment
     NOESY, $^{31}$P-$^1$H HETCOR

B) Exchangeable Protons
   1D, NOESY (11, WG, etc)

C) Correlation of Exchangeable and Non Exchangeable Protons
   NOESY (excitation sculpting)
A) Assignment of Non Exchangeable Protons

Base and Sugar

COSY/TOCSY
C: H5-H6
U: H5-H6

TOCSY
A: H8-H2 (H2 are generally difficult to assign)

COSY/TOCSY
H1’ -H2’ (H2’’') etc

J Zhang, A Spring, M W Germann J. Am. Chem. Soc. 131 5
Resonance Assignment DNA/RNA (Homonuclear)

Sequential Assignment
NOESY Connectivity (e.g. $\alpha$ C Decamer)

G1-H1' and G1-H8 interactions are highlighted in the NOESY spectrum.

[Graph showing the NOESY connectivity and highlighted peaks and interactions]
DNA Miniduplex

5'-CATGCATG
GTACGTAC-5'

Base protons

Sugar H1' protons

C [H5-H6]
\textbf{\textsuperscript{31}P NMR}

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1. \textbf{AlphaC}

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**Diagram Notes:**

- **3'**
- **4'**
- **5', 5''**

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**Program Code:**

```
;mwgcropr, AMX version
;X-H correlation. H-detected
;Sklenar et al., 1986, FEBS, 208, 94-98
;===================================================
;**X-H correlation. H-detected**
;Sklenar et al., 1986, FEBS, 208, 94-98
;===================================================

d12=20u
p2=p1*2

1 ze
d11 dhi
2 d11
3 d12
p2 ph0
d2
l0 to 3 times l1
d3
(p3 ph2):d
d0
(p1 ph1) (p3 ph1):d
go=2 ph31
d11 wr #0 if #0 id0 ip2 zd
l0 to 3 times td1
do
exit

ph0=0
ph1=0
ph2=0 0 2 2
ph31=2 2 0 0

;>>>>>>>>>>>>>>>DELAYS
;d0 = 3us
;d2 = 50ms
;d3 = 3us
;d11= 30 msec

;>>>>>>>>>>>>>>>PULSES
;p1 = 90 deg proton pulse h1 = 1
;p2 = 180 deg proton pulse h1 = 1
;p3 = 90 deg X pulse

;>>>>>>>>>>>>>>>>LOOP-COUNTER
;l1 = loop counter for presaturaton

;L1*d2 = relaxation delay (L1=40, d2=50ms >>2s)
;>>>>>>>>>>>>>>>>COMMENTS

;xd=pw = 0, nd0 = 2, in0 = 1/(2*SW)
;ns = 4*n, ds = 4, MC2= TPPI
;-----------------------END of PROGRAM---------------
```
B) Exchangeable Protons

1D Imino Proton Spectrum
Assignment of Exchangeable Protons
Correlation between exchangeable and non-exchangeable protons
# Heteronuclear Methods

Resonance Assignment of RNA/DNA by Heteronuclear NMR 
$^{13}$C and $^{15}$N correlations

## A) Exchangeable Protons

- $^{15}$N-$^1$H HSQC 
- $^{15}$N edited NOESY HSQC (3D)

## B) Non Exchangeable Protons

- **Base/Sugar**
  - $^{13}$C-$^1$H HSQC
  - HCCH -TOCSY HCCH-COSY 
  - HCN, H(CNC)H, H(CNH)

- **Base-Sugar**

- **Sequential**
  - $^{13}$C Edited NOESY-HSQC 
  - PH, P(C)H, HCP 

## C) Correlation of Exchangeable and Non Exchangeable Protons

- A, C, G, U, T- specific 
- $^{13}$C Edited NOESY-HSQC 

## D) Base Pairing
$^{15}$N Chemical Shifts in RNA: ($^1$H, $^{15}$N) HSQC Spectrum
Non-exchangeable protons: **CT-HSQC/HMQC**

Use **Constant time experiments** (CC couplings in F1)
Non-exchangeable protons: HCCH-Type Experiment

HCCH COSY
HCCH TOCSY

\[ 1^H \rightarrow 13^C \rightarrow 13^C \rightarrow 1^H \]

INEPT COSY INEPT
RELAY TOCSY

F1 x F2: correlate a specific sugar \(^1H\) to its own sugar \(^1H\)'s and their respective \(^13C\)'s.

F3 x F2: Correlate each of its own sugar \(^1H\)'s to the \(^13C\) of a specific \(^1H\)
Assignments of Non-Exchangeable Protons:
2D ($^1$H, $^{15}$N) HCN

Allows for unambiguous correlations between $^1$H of ribose and H$_6$/H$_8$ of base
Correlation of Exchangeable and Non-Exchangeable Protons: G-specific H(NC)-TOCSY(C)H
Correlation of Exchangeable and Non-Exchangeable Protons: A-specific (H)N(C)-TOCSY(C)H
Correlation of Exchangeable and Non-Exchangeable Protons:
U-specific H(NCCC)H
Correlation of Exchangeable and Non-Exchangeable Protons:
C-specific H(NCCC)H
Direct Observation of Hydrogen Bonds by $^2J_{NN}$ Couplings: Watson-Crick Base-Pairs

$J_{NN} = 6.3$ Hz

$J_{NN} = 6.7$ Hz

Observation of Hydrogen Bonds by $^{2}J_{NN}$ Couplings: Non-Watson-Crick Base-Pairs

- Imino-hydrogen-bonded GA ($J_{NN} = 5$ Hz)
- Reversed Hoogsteen AU ($J_{NN} = 5.5$ Hz)
- AA mismatch ($J_{NN} = 2.5$ Hz)
- Hoogsteen GC in GC+G triple ($J_{NN} = 10$ Hz)
- Hoogsteenen TA TAT Triple ($J_{NN} = 6.6$ Hz)
- Arginine-RNA ($J_{NN} = 6.0$ Hz)
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
   • Dipolar couplings
What do we know?
• Distance, Torsion, H-Bond constraints

What do we want?
• Low energy structures

Methods
• Distance Geometry
• Simulated annealing, rMD
• Torsion angle dynamics (DYANA)
• Mardigras/IRMA/Morass
Dipolar Couplings… is the kink real?

- Dipolar couplings add to J coupling.
- They show up as a field or alignment media dependence of the coupling.
- If the overall orientation of the molecule is known the orientation of the vectors can be determined.

\[
D_{\text{max}}^{IS} = -\frac{\mu_0 \gamma_I \gamma_S h}{4\pi^2 r_{IS}^3}
\]

\[
D^{IS} = D_{\text{max}}^{IS} \left\langle \frac{1}{2} \left(3\cos^2 \theta - 1\right) \right\rangle
\]
RMSD (all atoms) 0.66

C3'  DG5    1 --  H3'
C4'  DT      2 --  H4'
C6    DT      2 --  H6
C1'  DC      4 --  H1'
C1'  ADA     5 --  H1'
C4'  ADA     5 --  H4'
C2    ADA     5 --  H2
C4'  DC      6 --  H4'
C8    DA      8 --  H8
C1'  DC      9 --  H1'
C3'  DC      9 --  H3'
C6    DC      9 --  H6
C1'  DG3     10 -- H1'
C4'  DG3     10 -- H4'
C1'  DC5     11 -- H1'
C4'  DC5     11 -- H4'
C1'  DT      13 -- H1'
C6    DT      13 -- H6
C4'  DC      14 -- H4'
C6    DC      14 -- H6
C8    DG      15 -- H8
C1'  DT      16 -- H1'
C1'  DG      17 -- H1'
C3'  DC3     20 -- H3'
C4'  DC3     20 -- H4'
### General references, NMR techniques, sample preparation, analysis


### NMR structure determination: DNA DNA/RNA, pseudorotation analysis, dynamics. See also referenced quoted in the listed papers


Bax, A., Lerner, L. "MEASUREMENT OF H-1-H-1 COUPLING-CONSTANTS IN DNA FRAGMENTS BY 2D NMR." J Magn Reson. 79 429 - 438, 1988..


### Multinuclear experiments, DNA/RNA


P Schmieder, J H Ippel, H van den Elst, G A van der Marel, J H van Boom, C Altona, z Kessler (1992) Heteronuclear NMR of DNA with the heteronucleus in natural amino facilitated assignment and extraction of coupling constants. Nucleic Acids Res. 2 4751.


Trantirek L., Steff R., Masse J.E., Feigon J. and Sklenar V. (2002)"Determination of the torsion angles in uniformly 13C-labeled nucleic acids from vicinal coupling const 3J(C2)/H1 and 3J(C6)/H1" J. Biomol. NMR., 23(1):1-12

Szyperski, T., Ono, A., Fernández, C., Iwai, H., Tate, S., Wüthrich, K. and Kainosho N Measurement of 3J(C2)/P Scalar Couplings in a 17 kDa Protein Complex with 13 Labeled DNA Distinguishes the B I and BII Phosphate Conformations of the DNA Chem. Soc. 119, 9901-990


C. Richter, B. Reif, K. Wörner, S. Quant, J. W. Engels, C. Griesinger, and H. Schwalbe *New Experiment for the Measurement of 3J(C,P) Coupling Constants including and 3J(C4)/P,i+1) coupling constants in Oligonucleotides* J. Biomol. NMR 12, 2