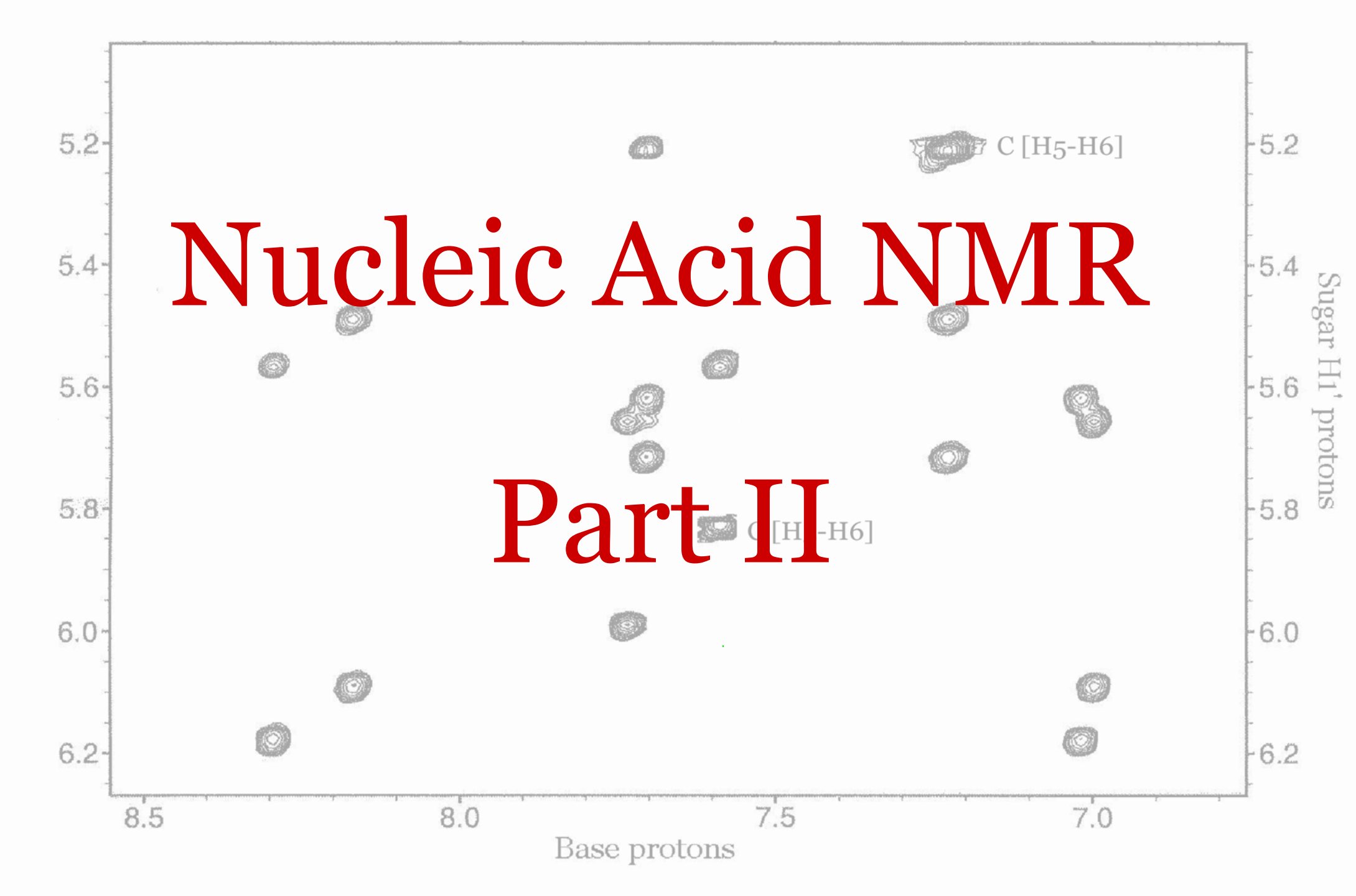
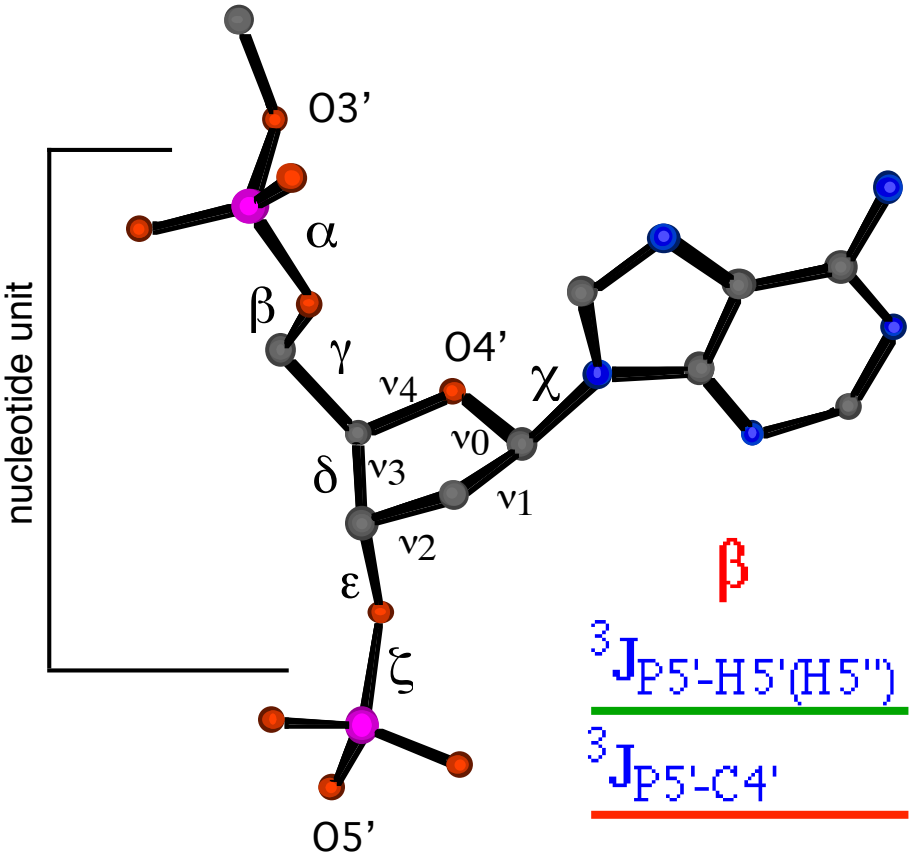


Nucleic Acid NMR

Part II



α and ζ pose problems
→ determinants of ^{31}P chem shift

ε and ζ correlate. ζ = -317-1.23 ε

β

$^{3}J_{P5'-H5'(H5'')}$

$^{3}J_{P5'-C4'}$

γ

$^{3}J_{H4'-H5'(H5'')}$

$^{3}J_{C3'-H5'(H5'')}$

ε

$^{3}J_{P3'-H3'}$

$^{3}J_{P3'-C2'}$

$^{3}J_{P3'-C4'}$

χ

$^{3}J_{H1'-C6}$ (U,C,T)

$^{3}J_{H1'-C2}$ (U,C,T)

$^{3}J_{H1'-C8}$ (A,G)

$^{3}J_{H1'-C4}$ (A,G)

Ranges	χ	α	β	γ	δ	ε	ζ
B-DNA	-119	-61	180	57	122	-187	-91
Bf-DNA	-102	-41	136	38	139	-133	-157
Af-DNA	-154	-90	-149	47	83	-175	-45

Sanger, Principles of nucleic acid Structures
Springer 1984

Σ Backbone Experiments

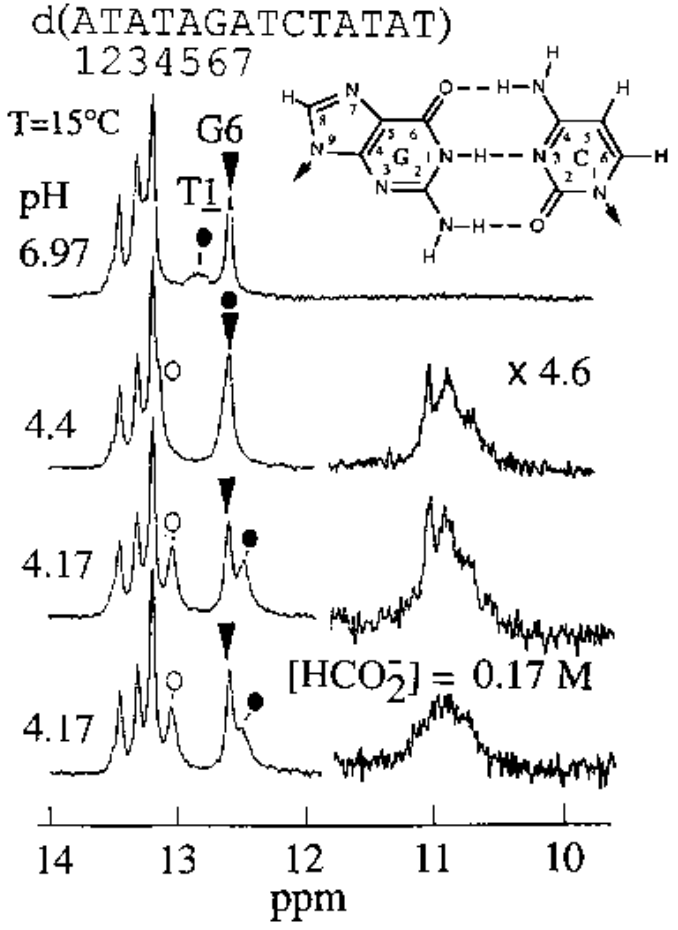
- Z. Wu, N. Tjandra, and A. Bax, Measurement of **H3'-^{31}P** dipolar couplings in a DNA oligonucleotide by constant-time NOESY difference spectroscopy, J. Biomol. NMR 19, 367-370 (2001).

- G. M. Clore, E. C. Murphy, A. M. Gronenborn, and A. Bax, Determination of three-bond **H3'-^{31}P** couplings in nucleic acids and protein-nucleic acid complexes by quantitative J correlation spectroscopy, J. Mag. Reson. 134, 164-167 (1998).

- H. Schwalbe, W. Samstag, J. W. Engels, W. Bermel, & C. Griesinger, "Determination of **^{3}J(C,P)** and **^{3}J(H,P)** Coupling Constants in Nucleotide Oligomers", J. Biomol. NMR 3, 479-486 (1993).

- BioNMR in Drug Research 2003 Editor: O. Zerbe
  Methods for the Measurement of Angle Restraints from Scalar, Dipolar Couplings and from Cross-Correlated Relaxation: Application to Biomacromolecules Chapter Author: Christian Griesinger:
  J-Resolved Constant Time Experiment for the Determination of the Phosphodiester Backbone Angles α and ζ.

Imino protons and pH



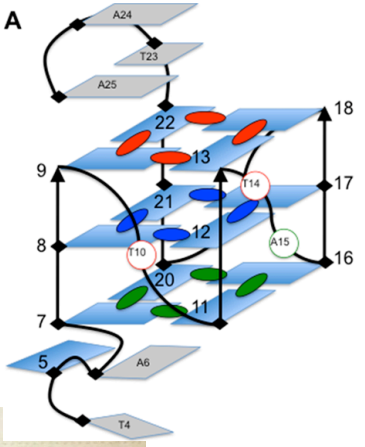
Sylvie Nonin[1,2], Jean-Louis Leroy[1] and Maurice Guéron[1,*]

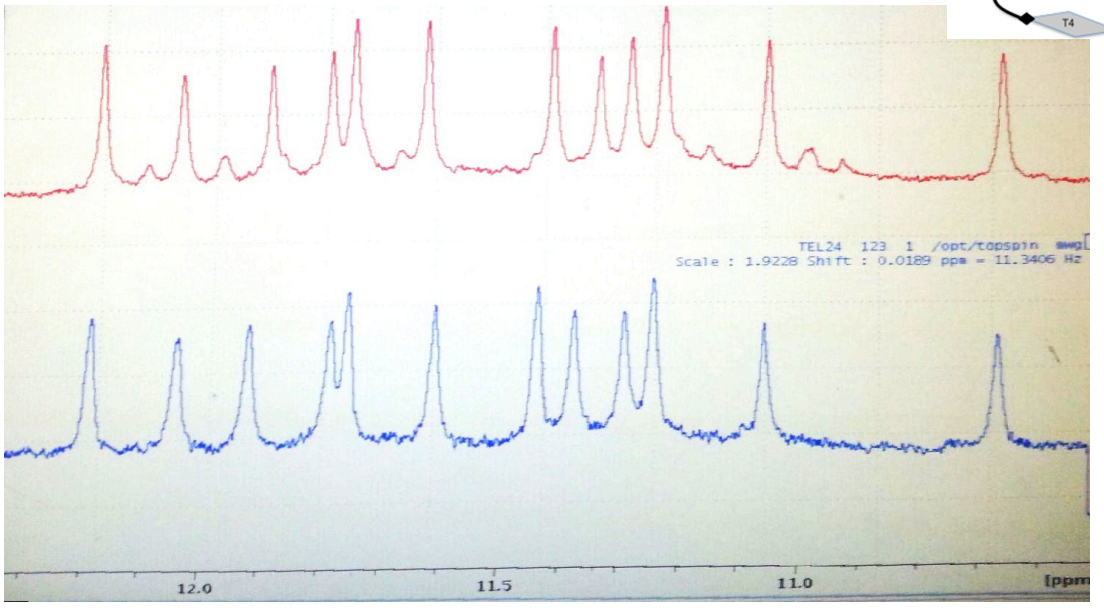
Nucleic Acids Research, 1996, Vol. 24, No. 4

→ pH may change structure
pH changes may hide or show weak base pairs

→ Buffer changes spectral properties
e.g. phosphate vs Tris buffer

→ Some DNA structures are incredibly stable

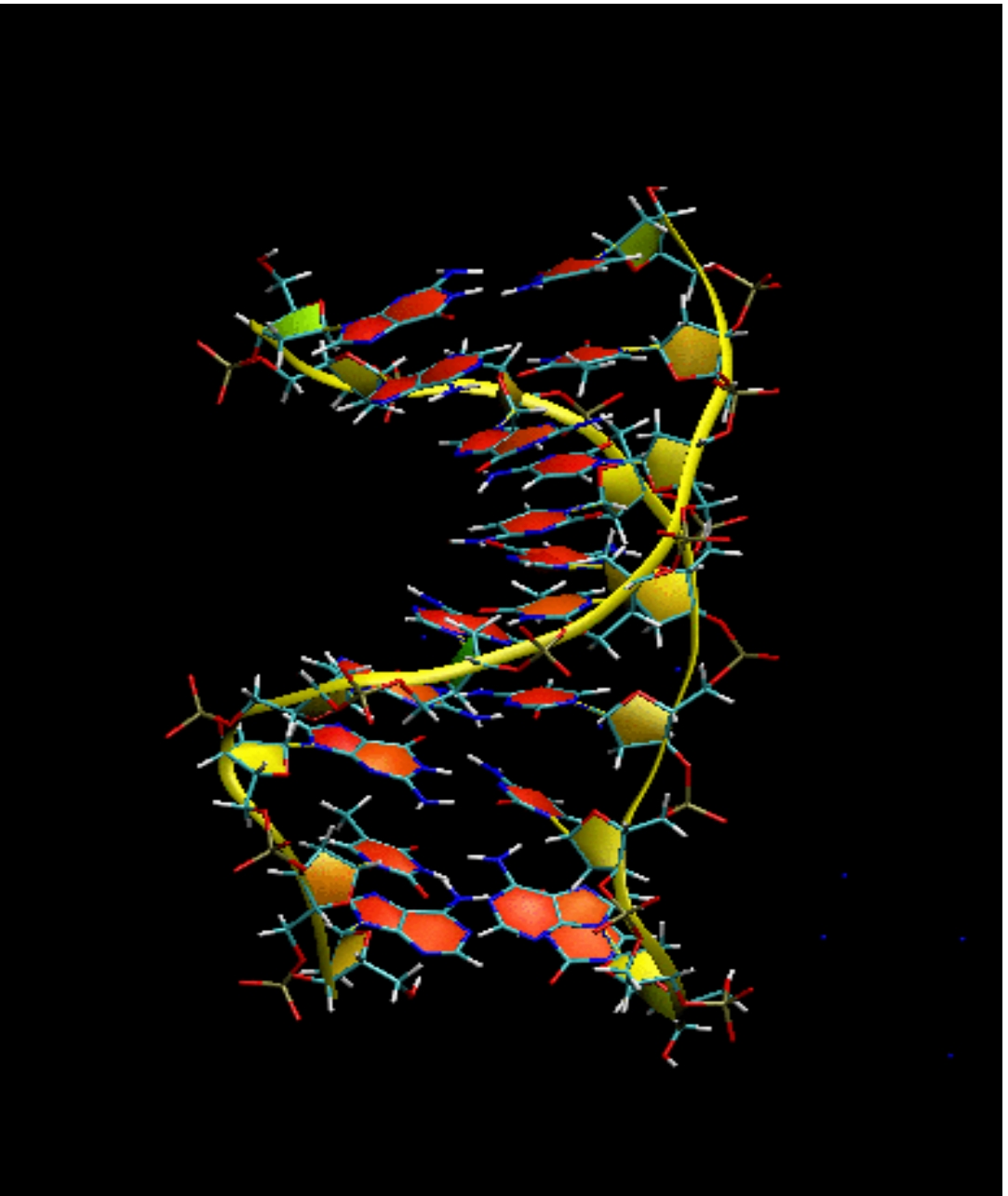


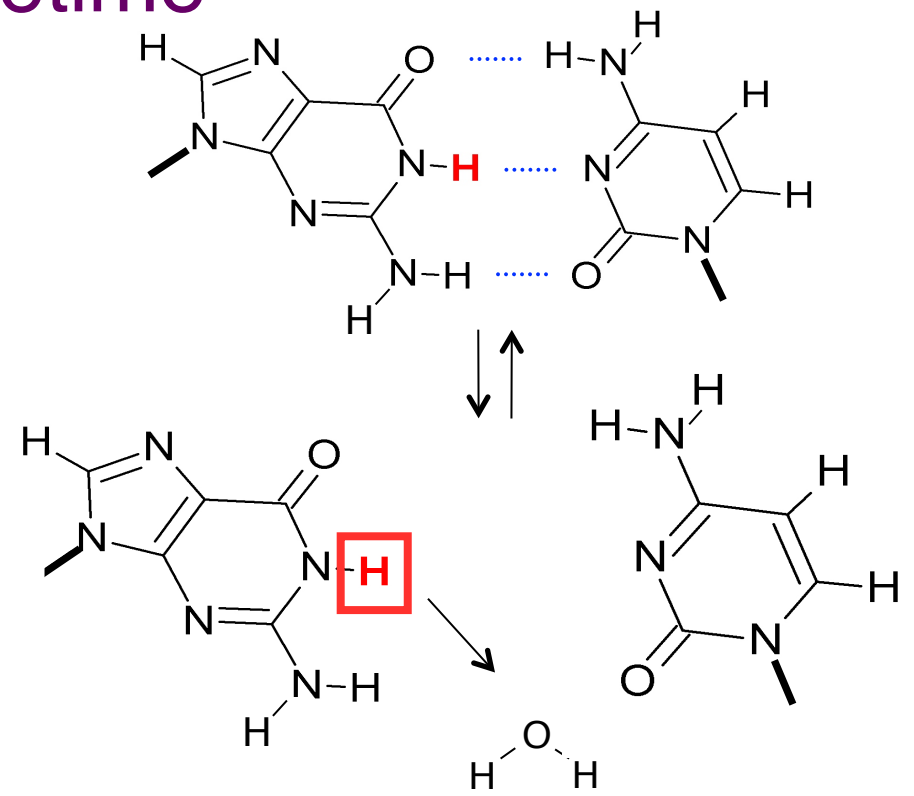


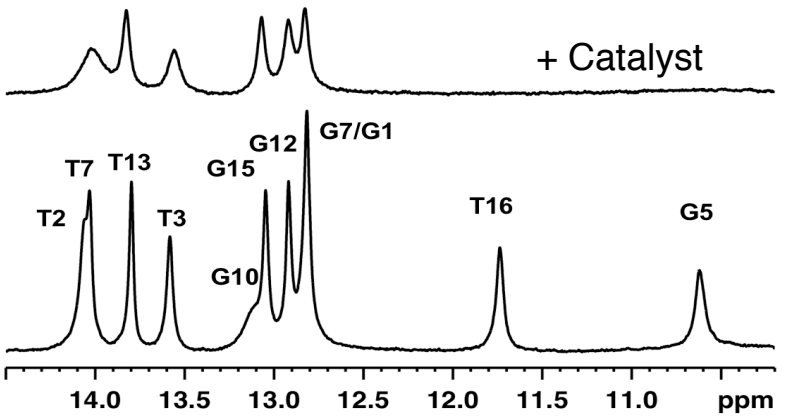
pH 7.0

pH 11.0

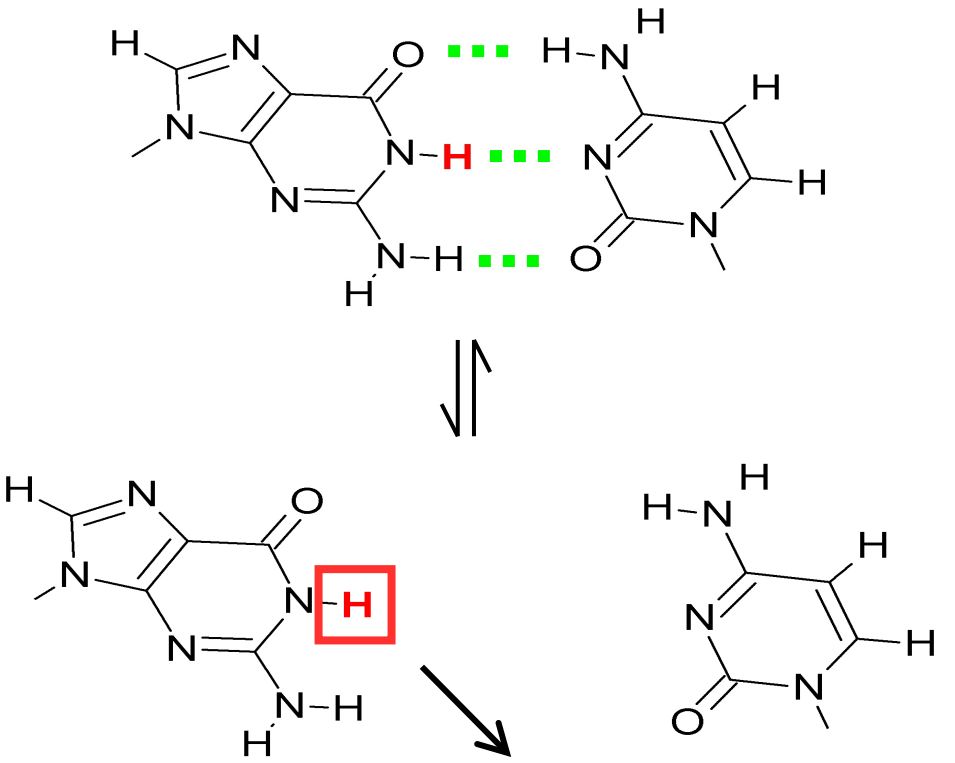
Base Pair Lifetime

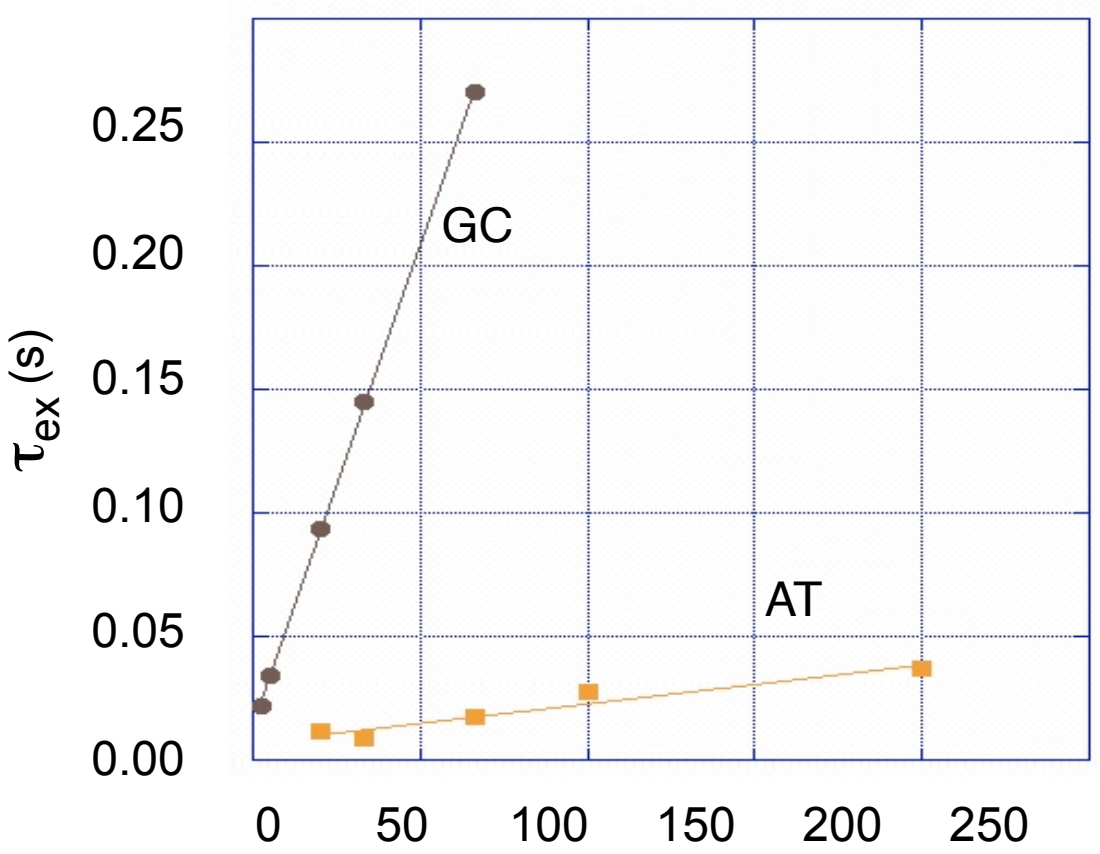






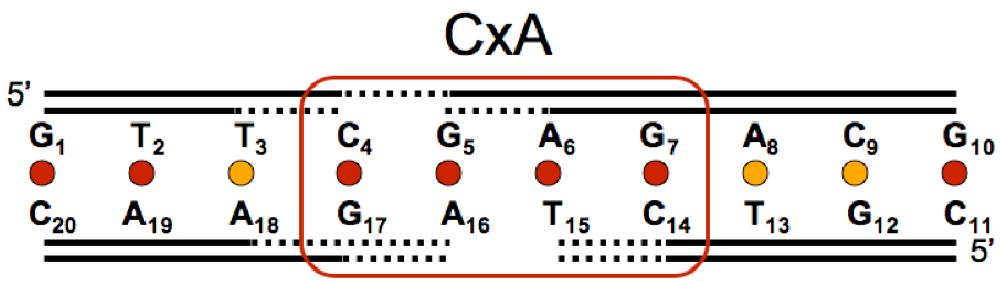
AxC base pair life times

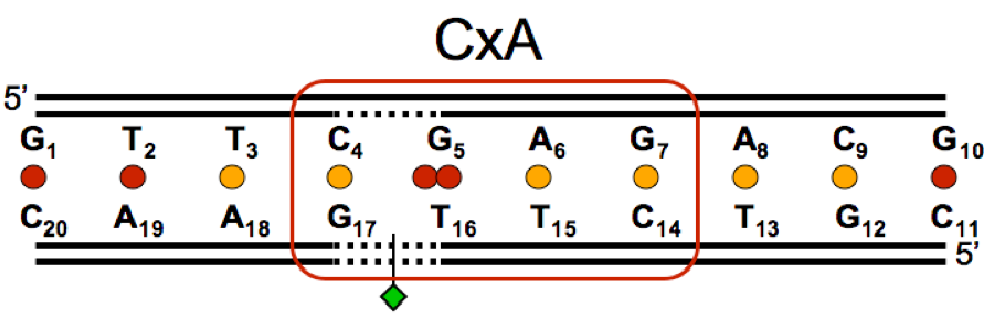


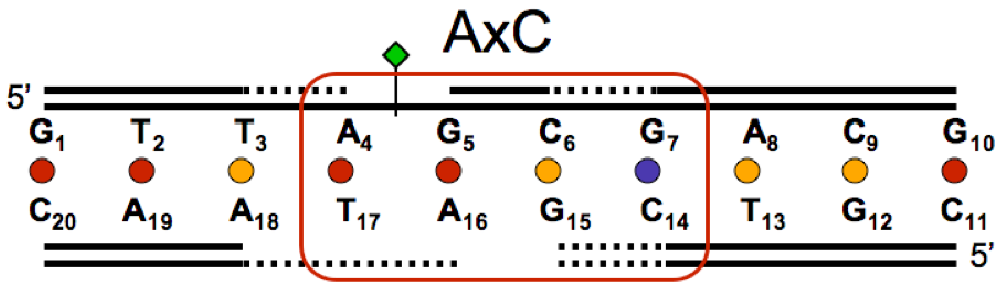


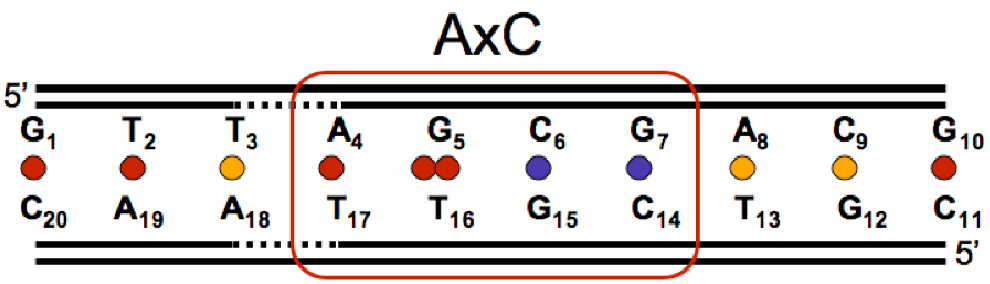
$$\tau_{ex} = \tau_{op} + \frac{1}{\alpha K_d K_{tr}[\text{Catalyst}]}$$

Mazurek et al. PNAS 2009









^{31}P backbone perturbations ◆

Base pair lifetimes

● < 5ms,

● 6 ms - 30 ms,

● 31 ms - 65 ms.

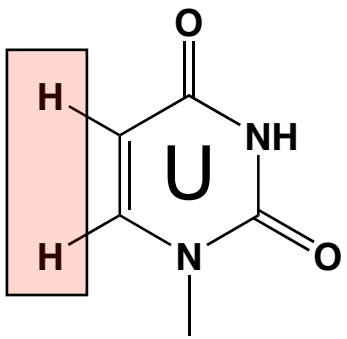
Resonance Assignment DNA/RNA (Homonuclear)

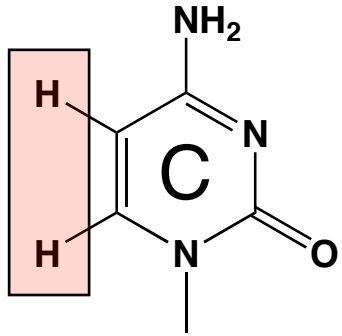
A)	Non Exchangeable Protons	
	•Aromatic Spin Systems	NOESY, DQFCOSY, TOCSY
	•Sugar Spin Systems	DQFCOSY, TOCSY
	•Sequential Assignment	NOESY, ^{31}P-^{1}H HETCOR
B)	Exchangeable Protons	1D, NOESY (11, WG, etc)
C)	Correlation of Exchangeable and Non Exchangeable Protons	NOESY (excitation sculpting)

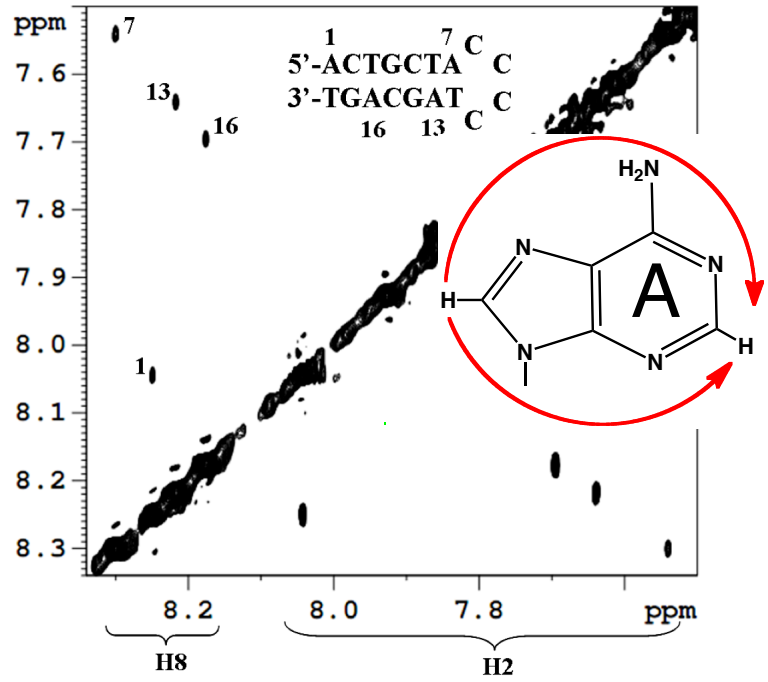
Assignment of Non Exchangeable Protons

Base and Sugar

COSY/TOCSY	C:	H5-H6
	U:	H5-H6
	T:	CH_3-H6
TOCSY	A:	H8-H2 (H2 are generally difficult to assign)
COSY/TOCSY		H1’ -H2’ (H2’’) etc

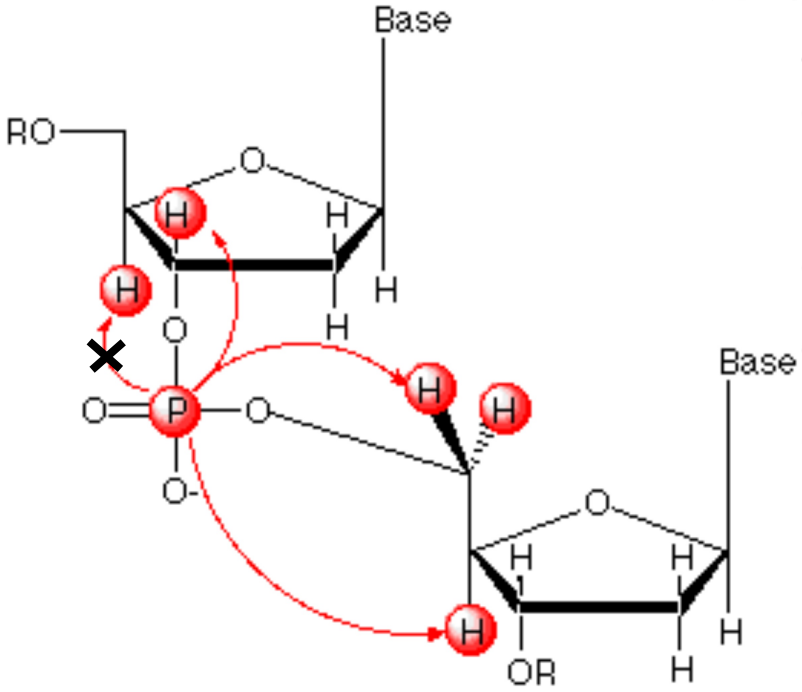


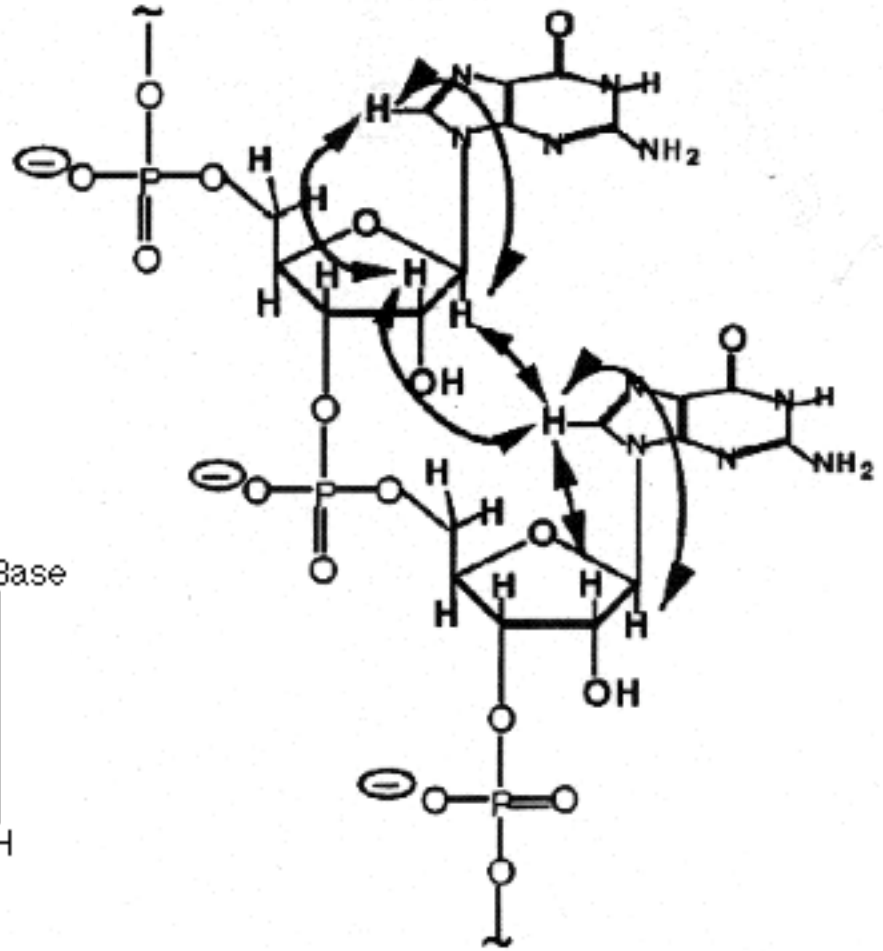




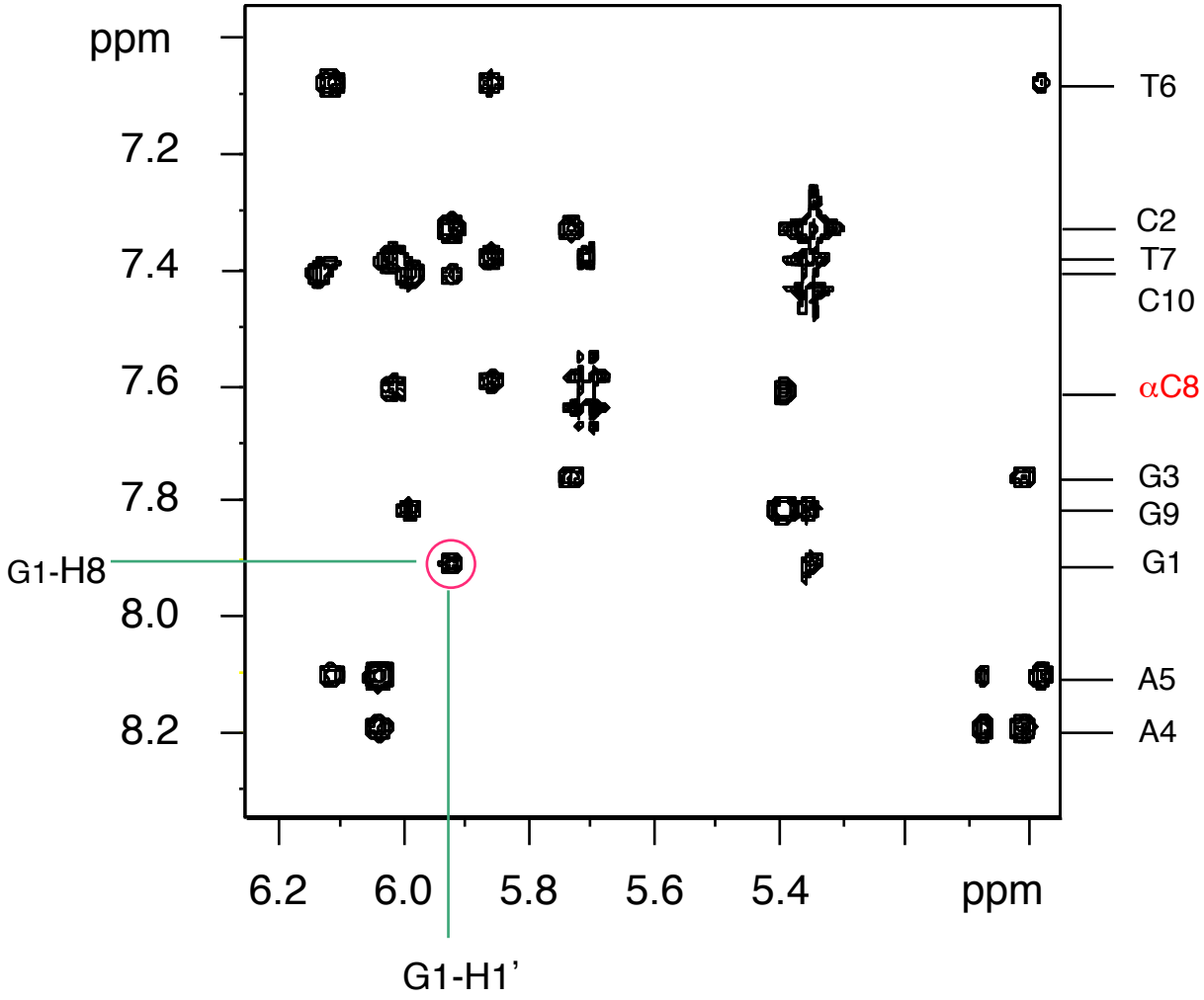
J Zhang, A Spring, M W Germann J. Am. Chem. Soc. 131 5380. (2009)

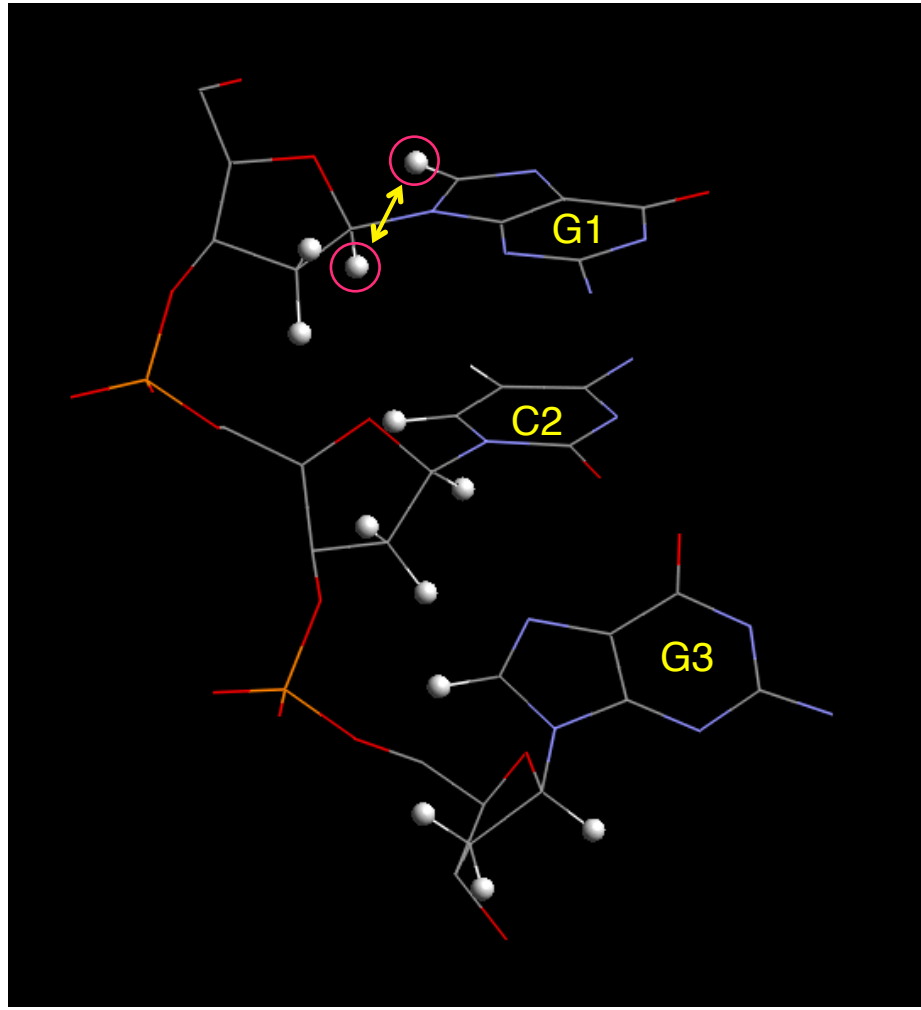
Sequential Assignment

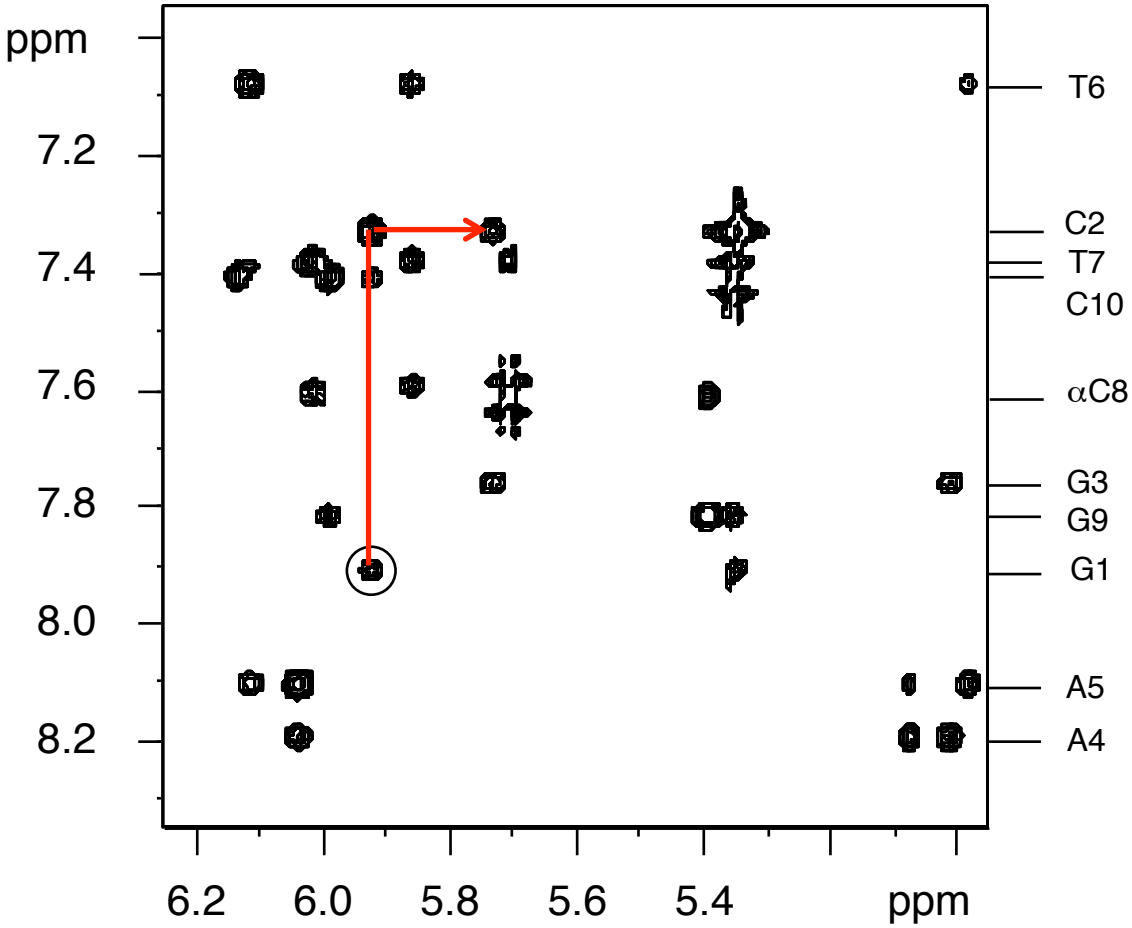


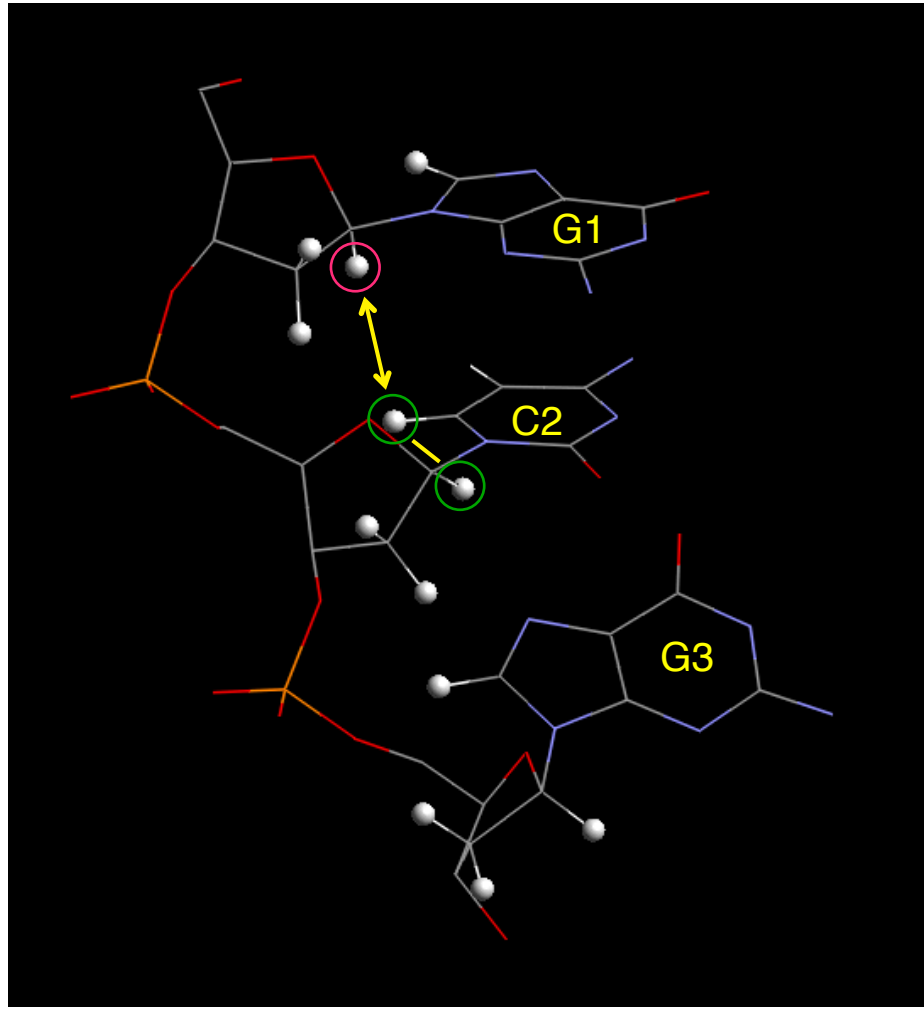


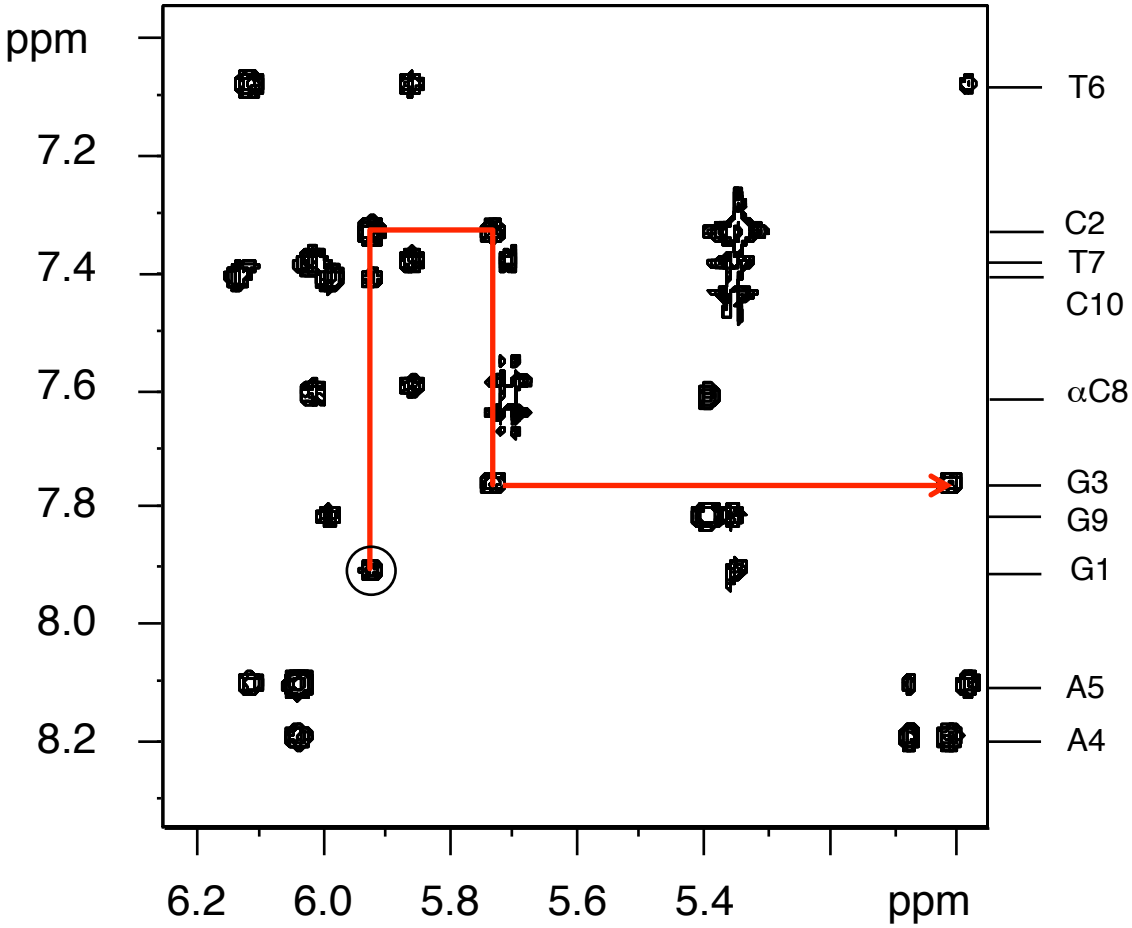
NOESY Connectivity (e.g. α C Decamer)

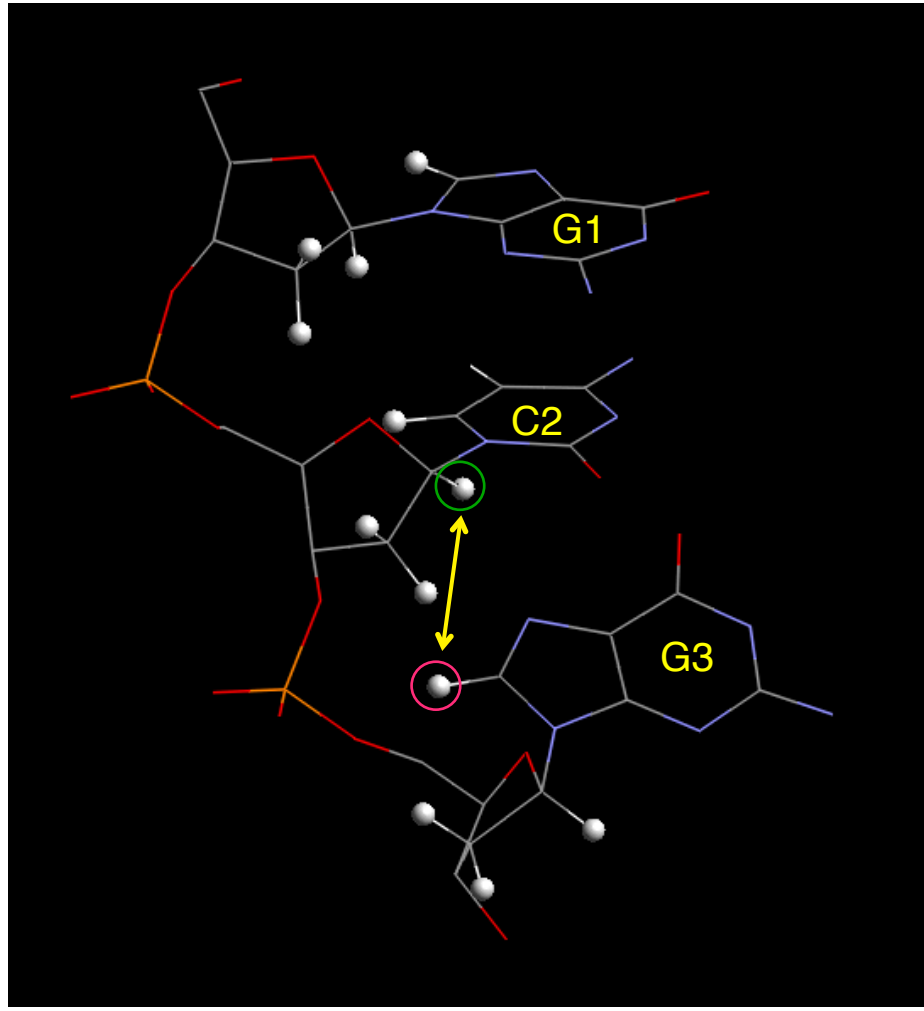





ppm
7.2
7.4
7.6
7.8
8.0
8.2
6.2
6.0
5.8
5.6
5.4
ppm
T6
C2
T7
C10
αC8
G3
G9
G1
A5
A4

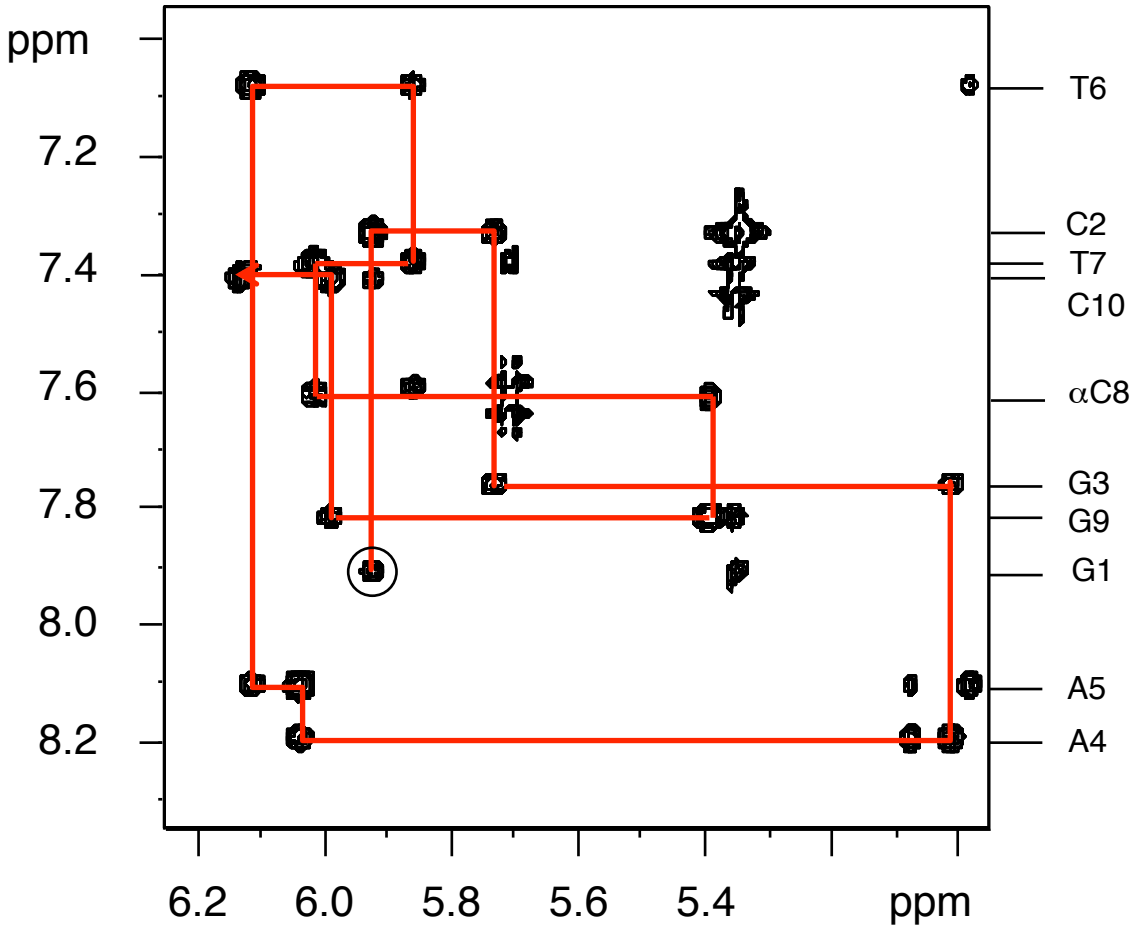

G1
C2
G3

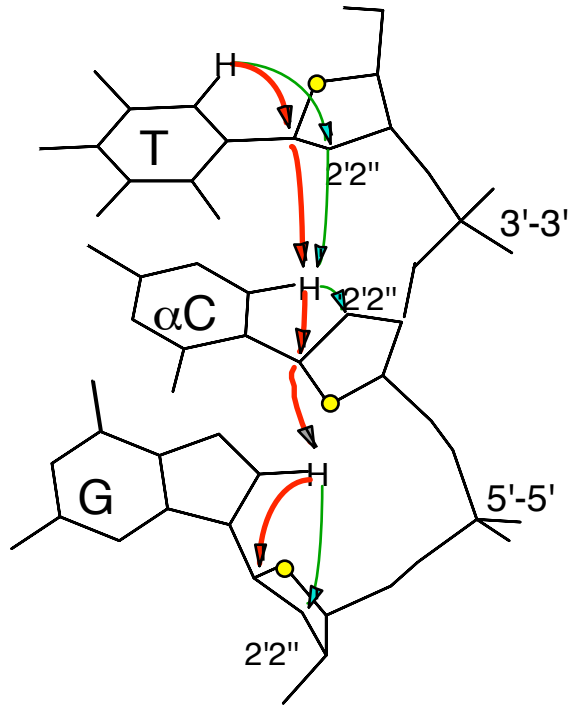

ppm
7.2
7.4
7.6
7.8
8.0
8.2
6.2
6.0
5.8
5.6
5.4
ppm
T6
C2
T7
C10
αC8
G3
G9
G1
A5
A4


G1
C2
G3

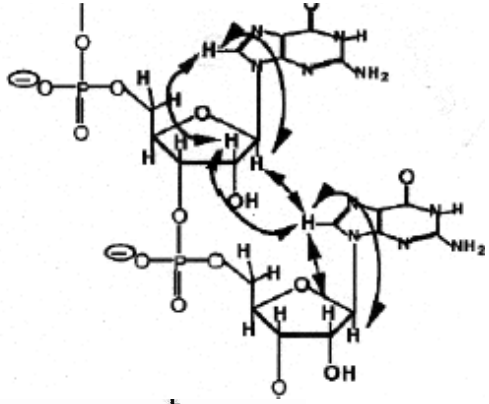
alphaC


5'-G C G A A T T αC G C
C G αC T T A A G C G-5'


ppm
7.2
7.4
7.6
7.8
8.0
8.2
6.2
6.0
5.8
5.6
5.4
ppm
T6
C2
T7
C10
αC8
G3
G9
G1
A5
A4

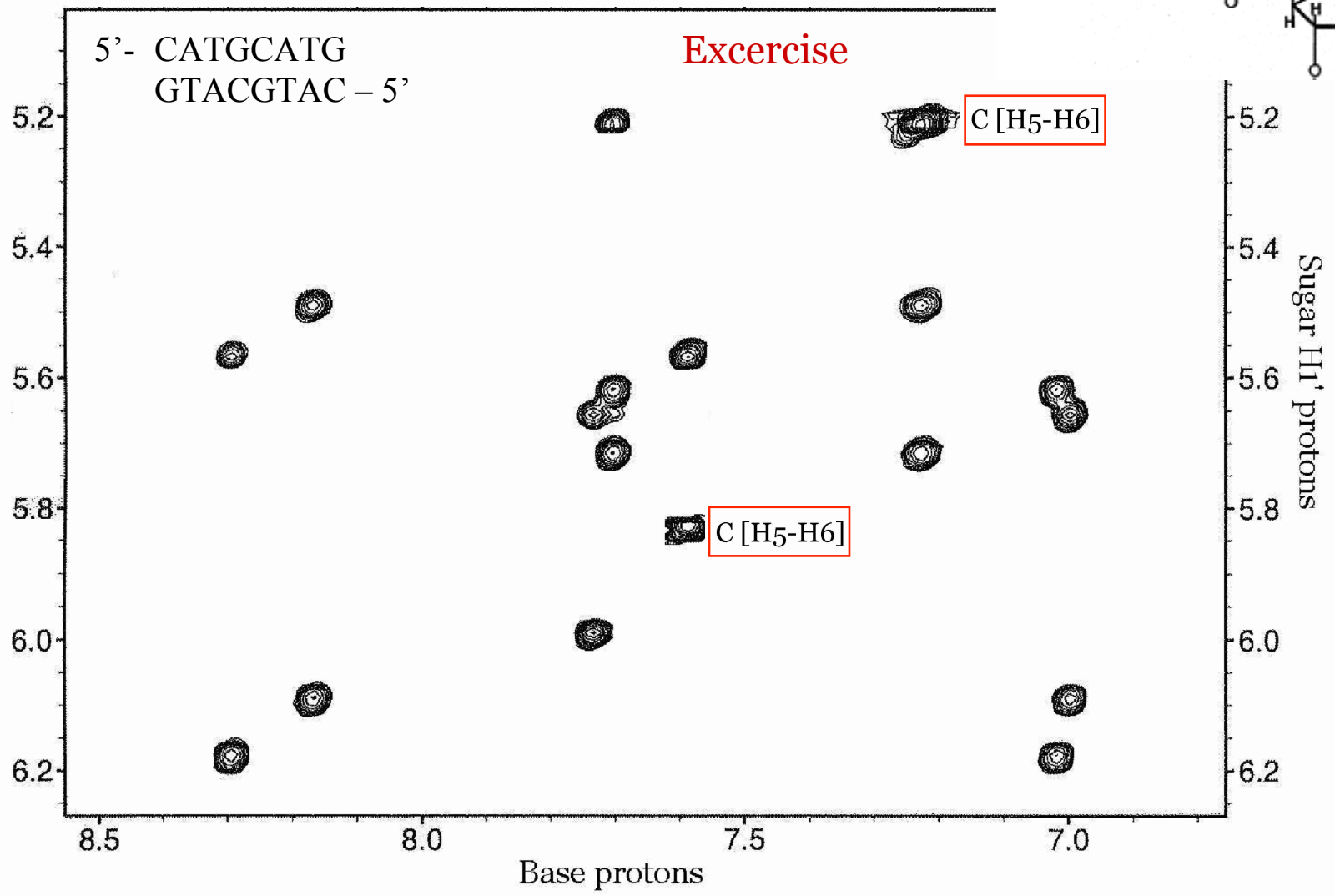

T
H
2'2"
3'-3'
αC
H
2'2"
G
H
5'-5'
2'2"

DNA Miniduplex

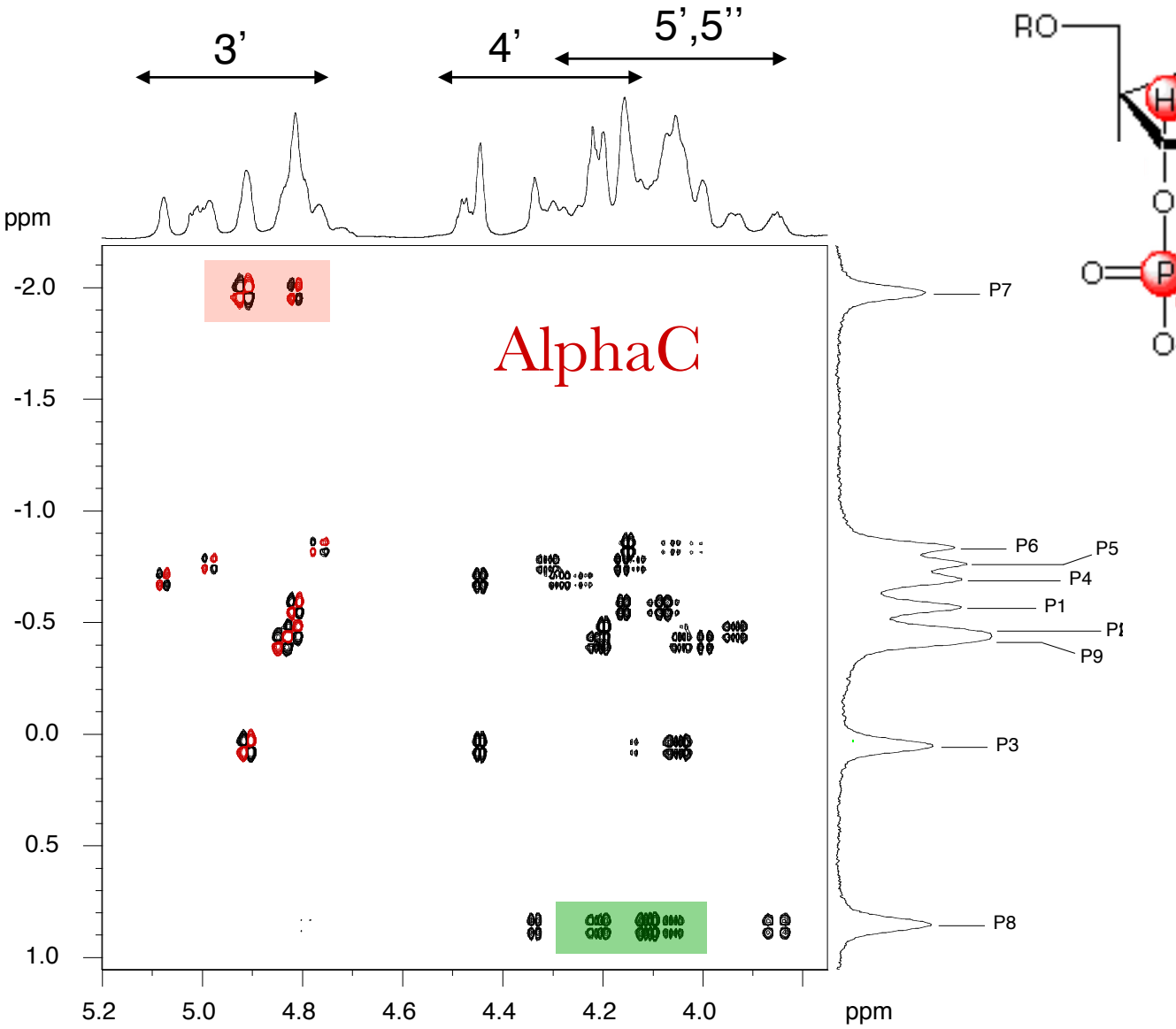


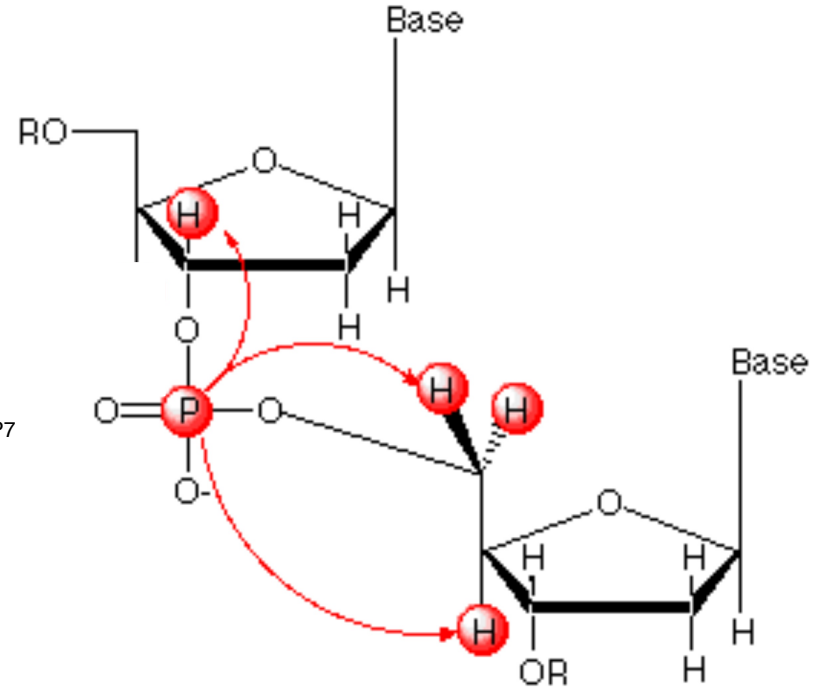
5’- CATGCATG
GTACGTAC – 5’

Excercise



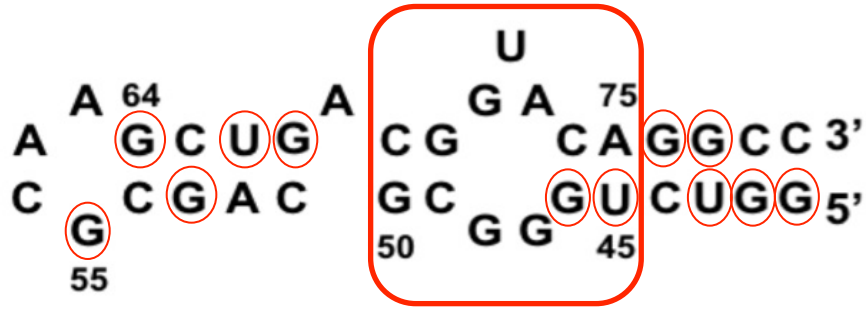
^{31}P NMR

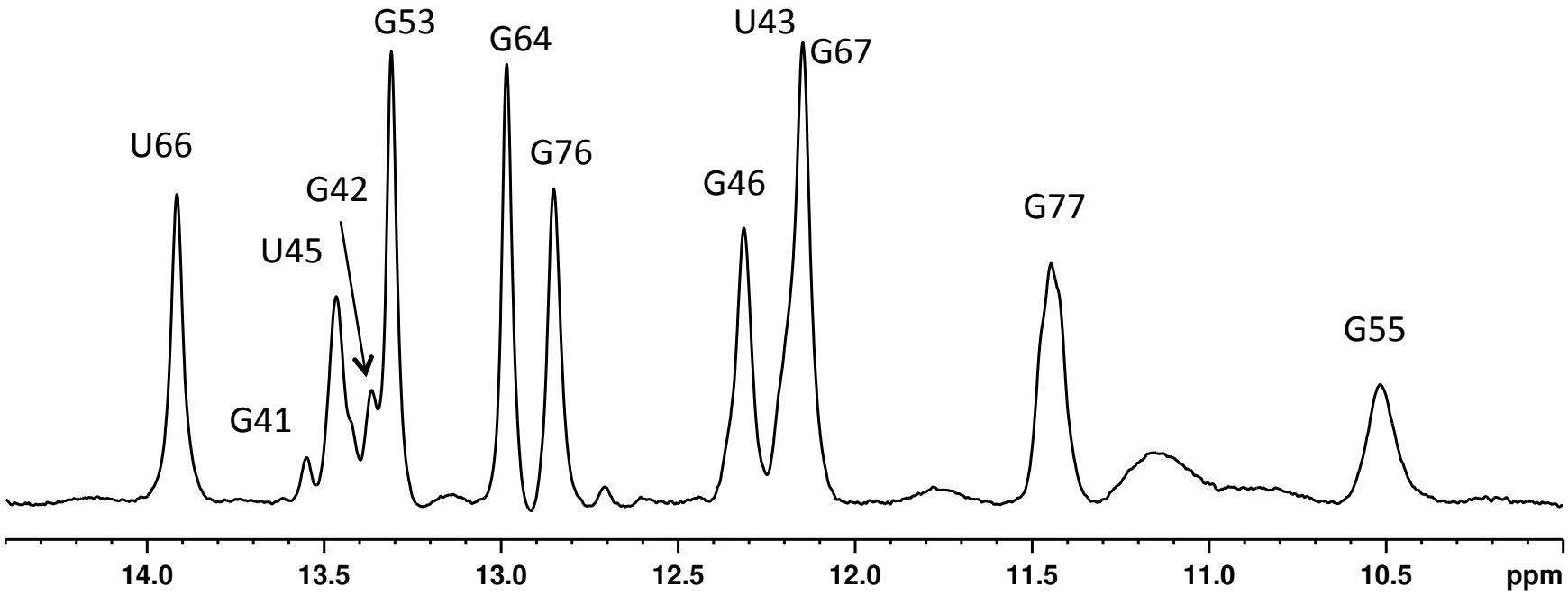

3'
4'
5',5''
ppm
-2.0
-1.5
-1.0
-0.5
0.0
0.5
1.0
AlphaC
P7
P6
P5
P4
P1
P9
P3
P8
5.2
5.0
4.8
4.6
4.4
4.2
4.0
ppm


Base
RO
O
H
H
H
O
O
P
O
O-
H
H
Base
O
H
H
H
OR
H
H

B) Exchangeable Protons

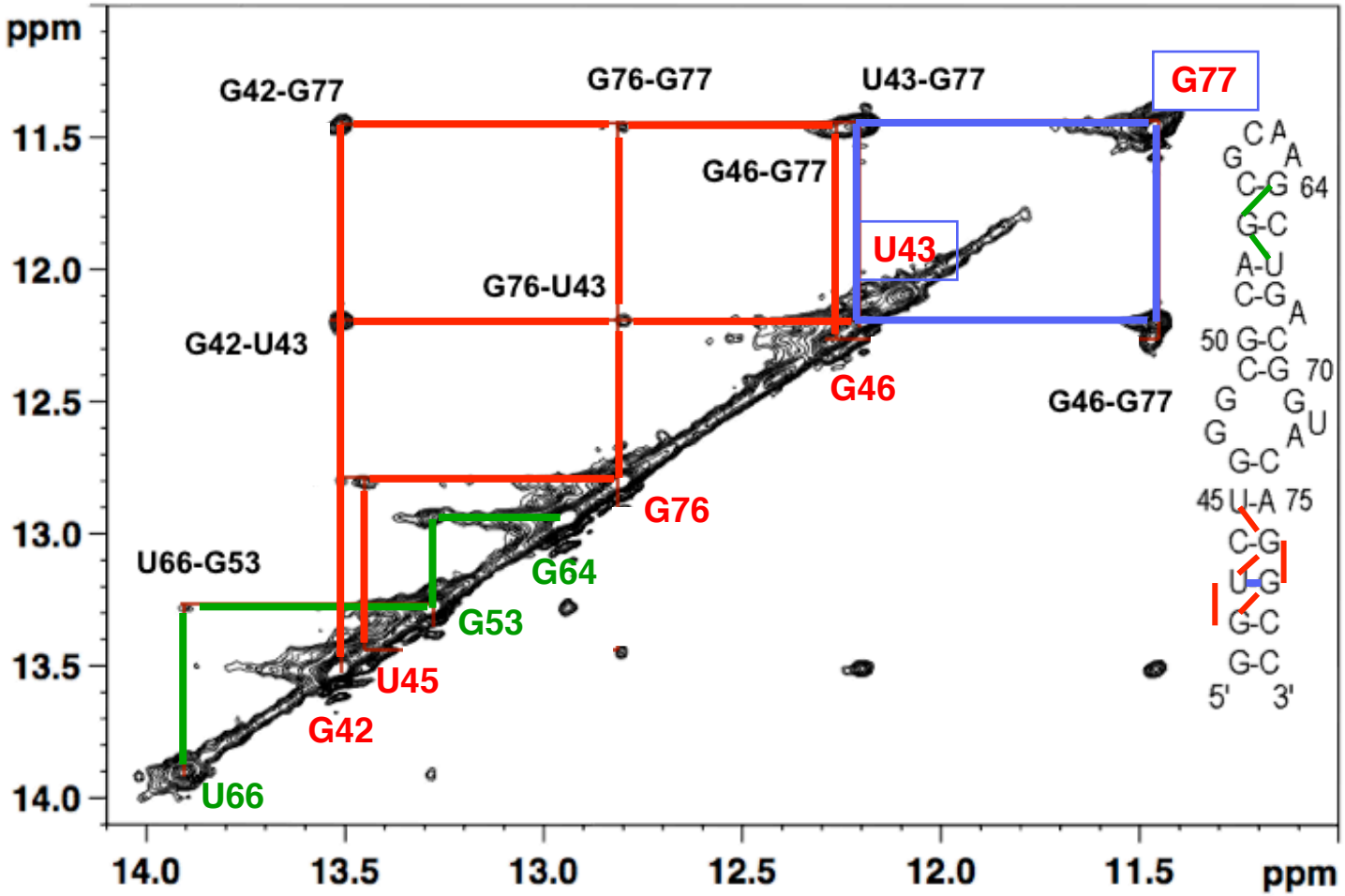
1D Imino Proton Spectrum



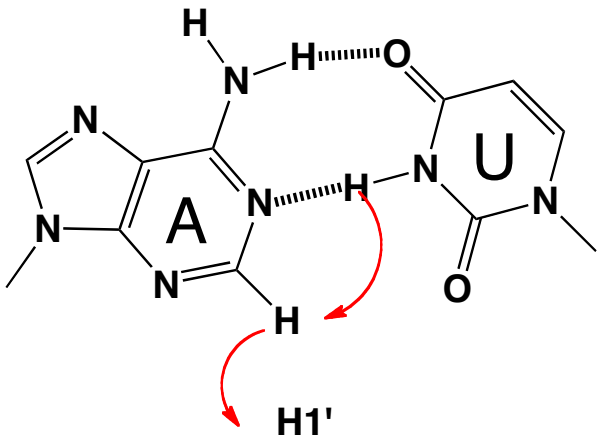


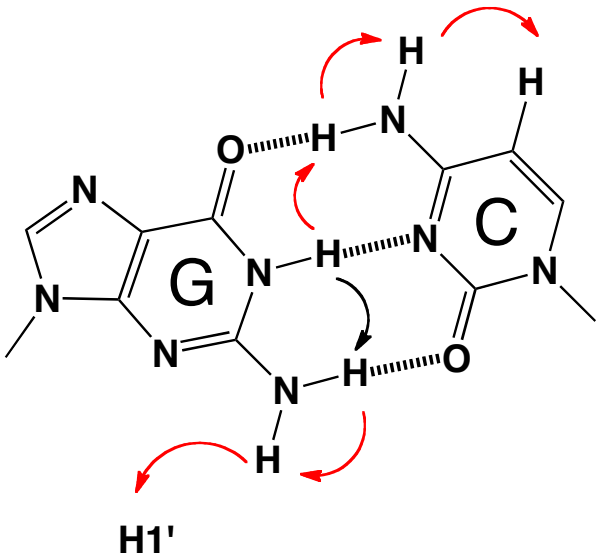
B) Exchangeable Protons

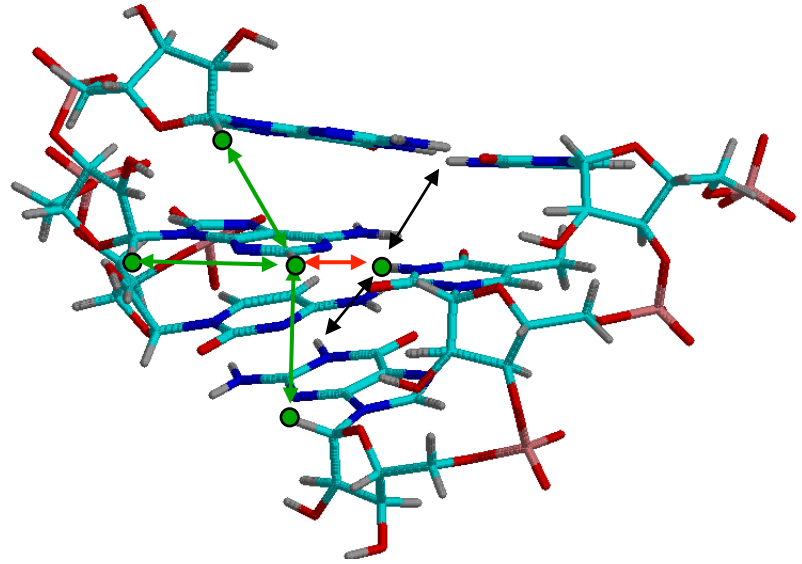
NOESY Imino Proton Region



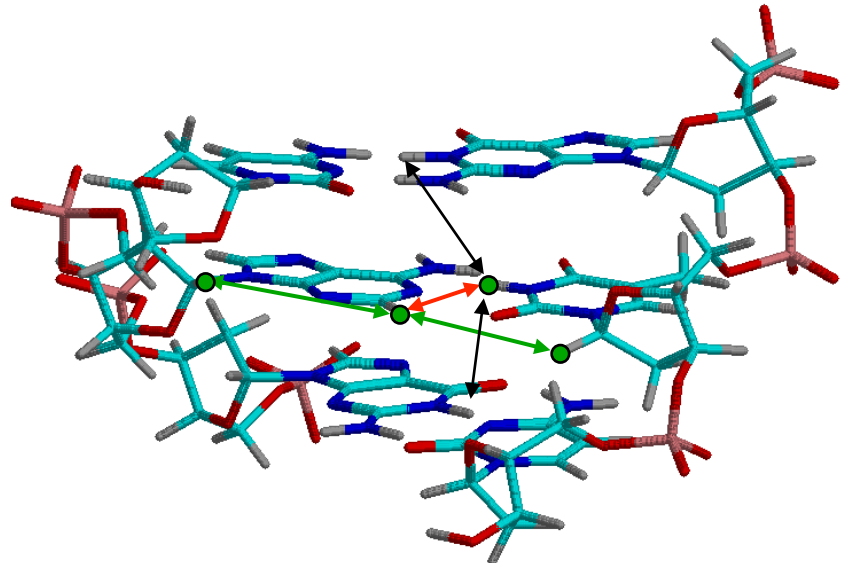
C) Correlation between exchangeable and non-exchangeable protons







RNA



DNA

Heteronuclear Methods

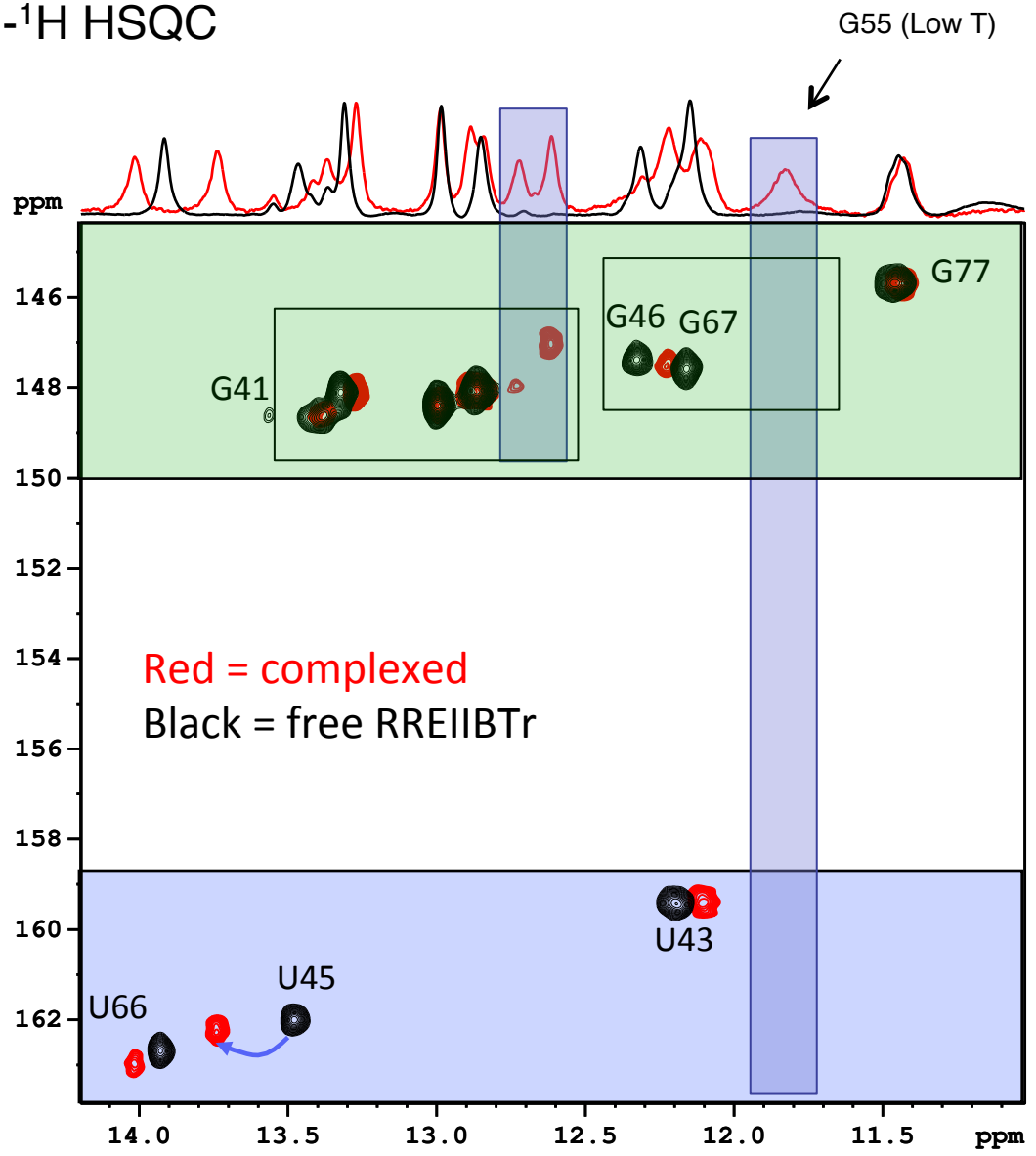
Resonance Assignment of RNA/DNA by Heteronuclear NMR
^{13}C and ^{15}N correlations

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	2/3D
	• Base-Sugar	HCN, H(CNC)H, H(CN)H	2/3D
	• Sequential	^{13}C Edited NOESY-HSQC	3/4D
		PH, P(C)H, HCP	2/3D
C)	Correlation of Exchangeable and Non Exchangeable Protons	A, C, G, U, T- specific	2D
		^{13}C Edited NOESY-HSQC	3/4D
D)	Base Pairing	NN COSY	

Natural abundance

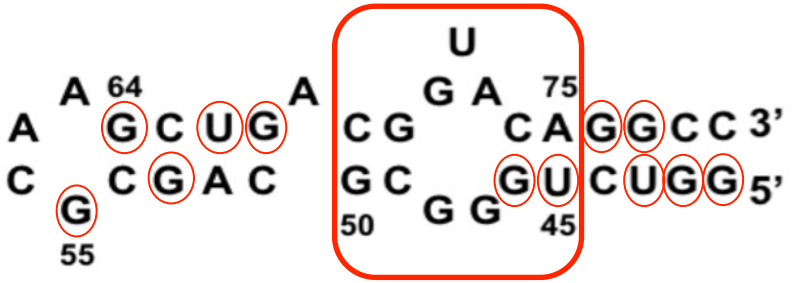
A) Exchangeable Protons

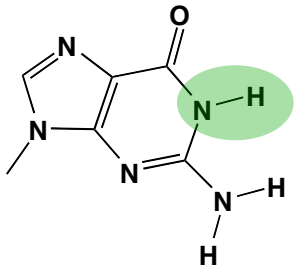
^{15}N-^{1}H HSQC

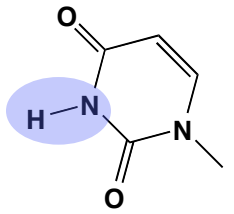


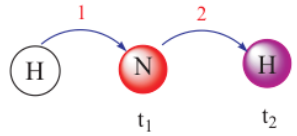
G's

U's



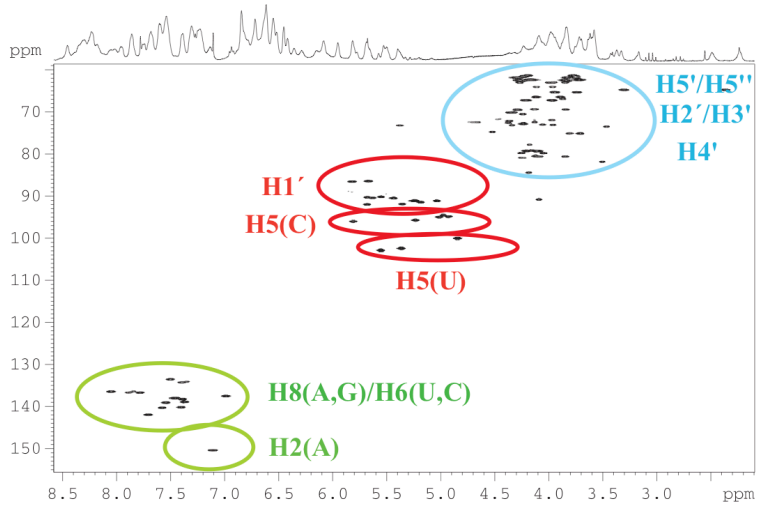


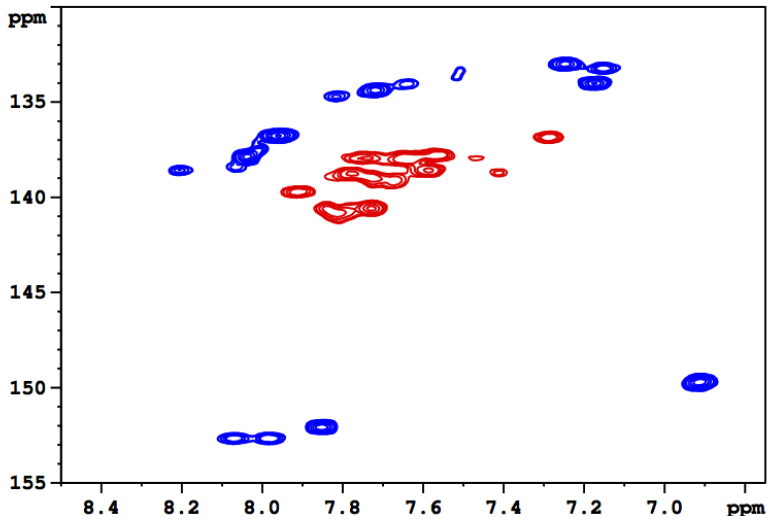


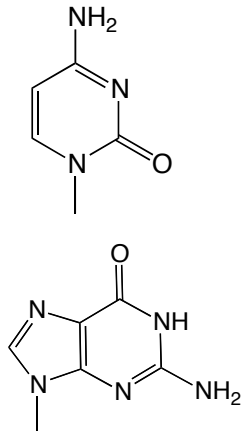


B) Non-exchangeable protons: CT-HSQC/HMQC

Use Constant time experiments (CC couplings in F1 !)





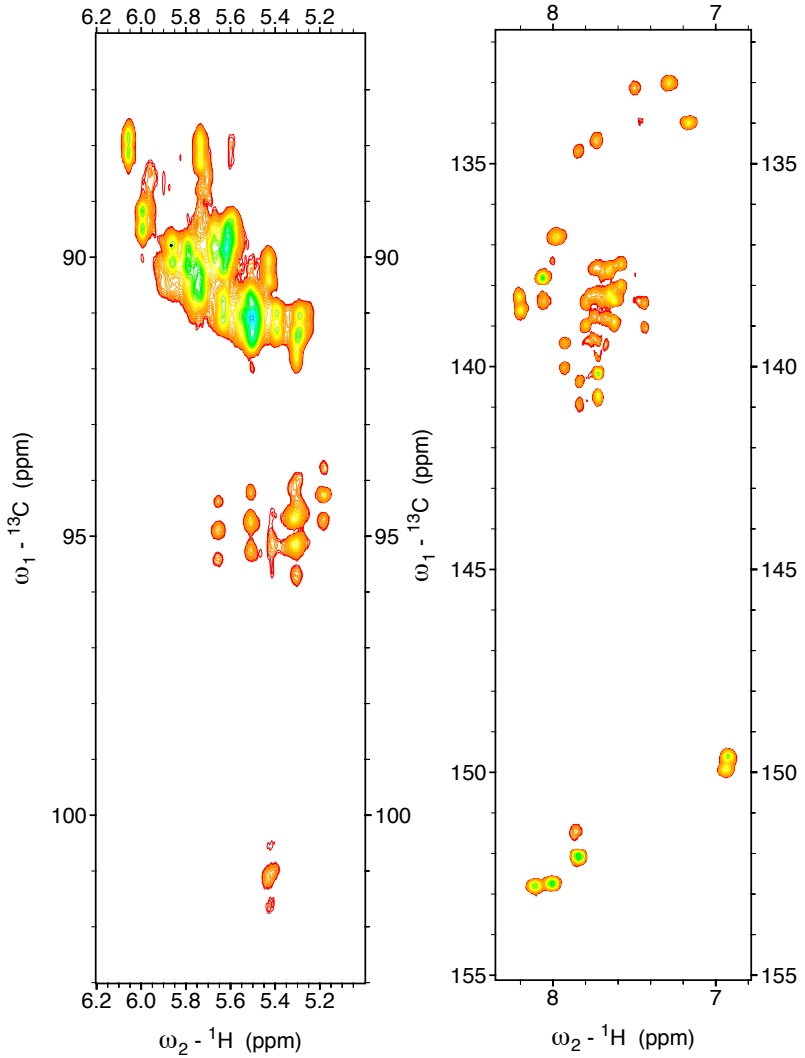


CH even #C

C8,C2,C5(pyr)
2',3',4'

CH odd #C

C6,C1',C5'



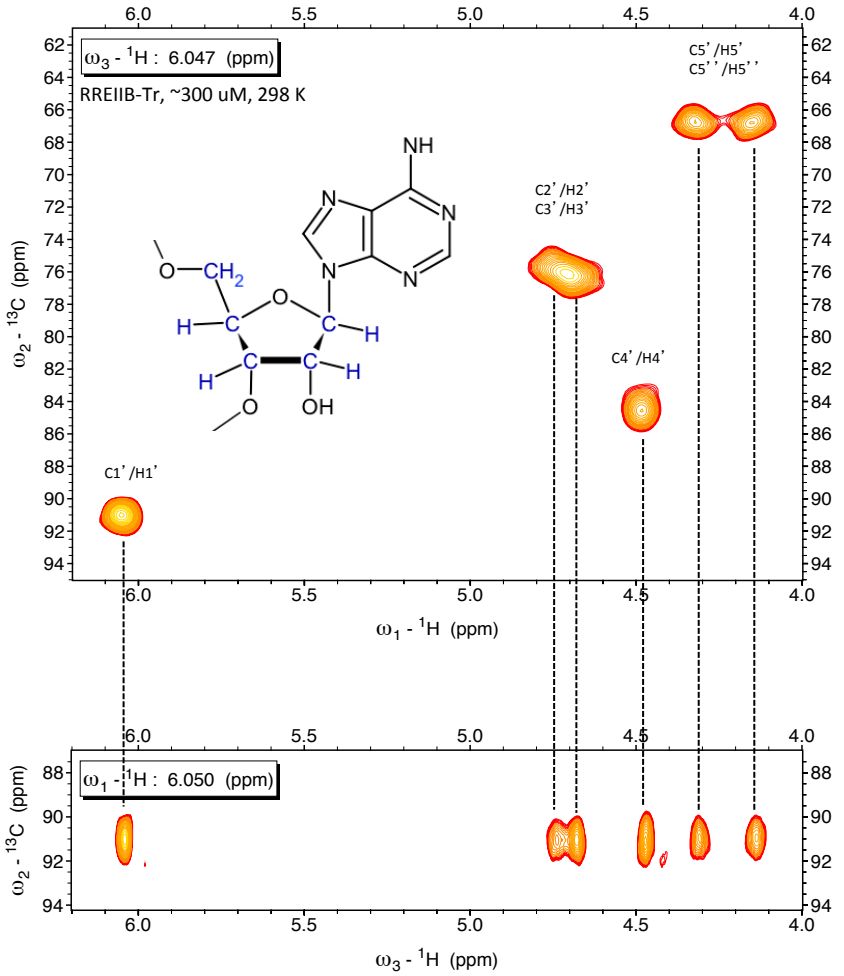
B) Non-exchangeable protons: HCCH-Type Experiments

HCCH COSY
HCCH TOCSY

$^{1}H \rightarrow {}^{13}C \rightarrow\rightarrow {}^{13}C \rightarrow {}^{1}H$

INEPT COSY RELAY TOCSY INEPT

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

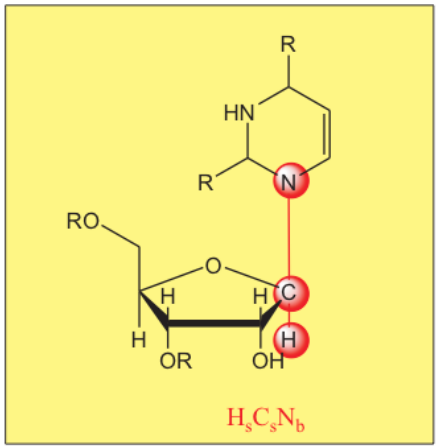


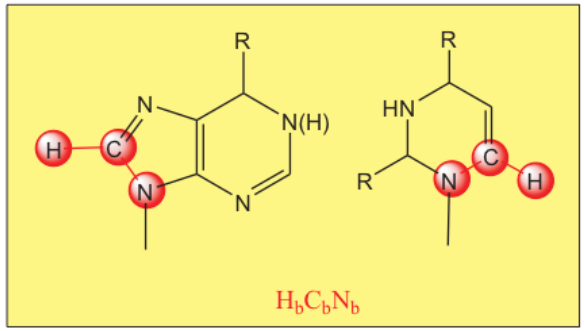
F3 x F2: Correlate each of its own sugar 1H's to the ^{13}C of a specific ^{1}H. (HCCH TOCSY)

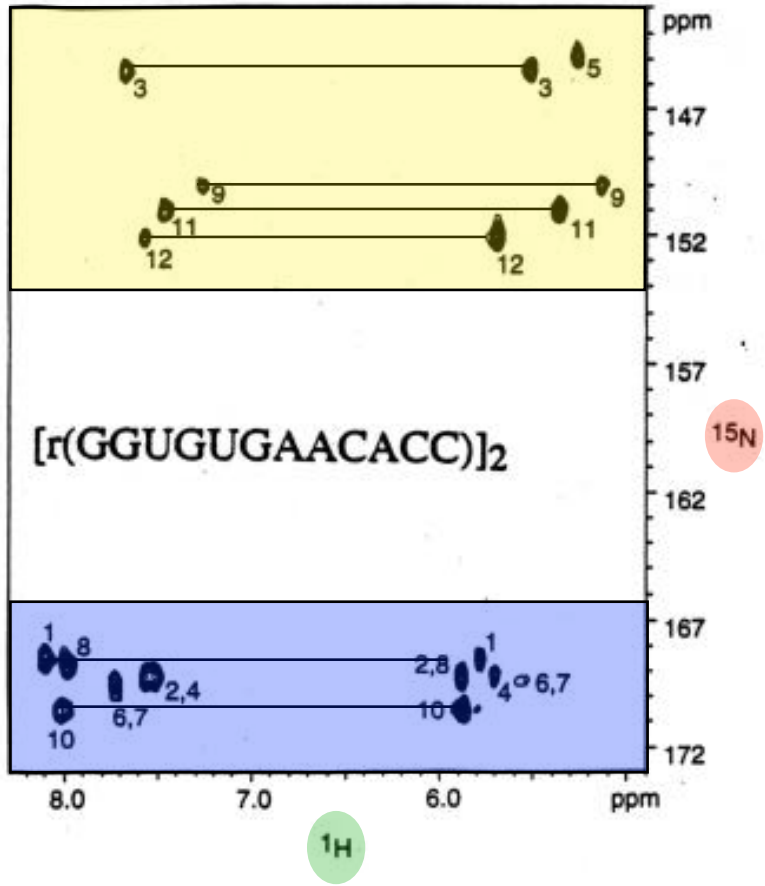
B) Non-exchangeable protons: HCN

$^{1}H \rightarrow {}^{13}C \rightarrow {}^{15}N(F1) \rightarrow {}^{13}C \rightarrow {}^{1}H(F2)$

Allows for unambiguous correlations between ^{1}H of ribose and H_6/H_8 of base



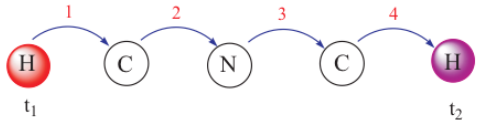


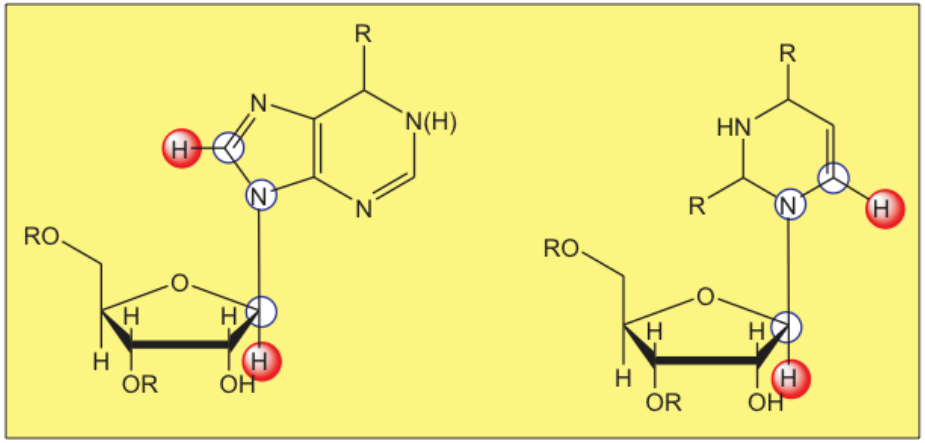


PL

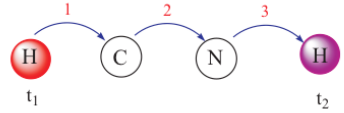
B) Non-exchangeable protons: H(CNC)H & H(CN)H

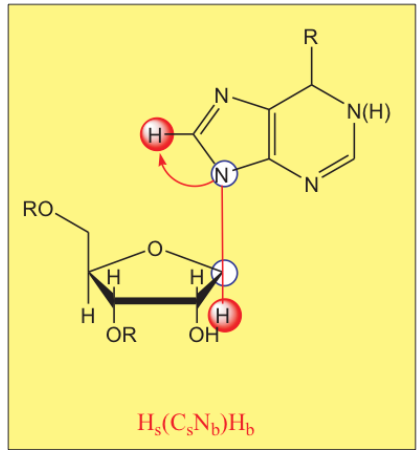
H(CNC)H





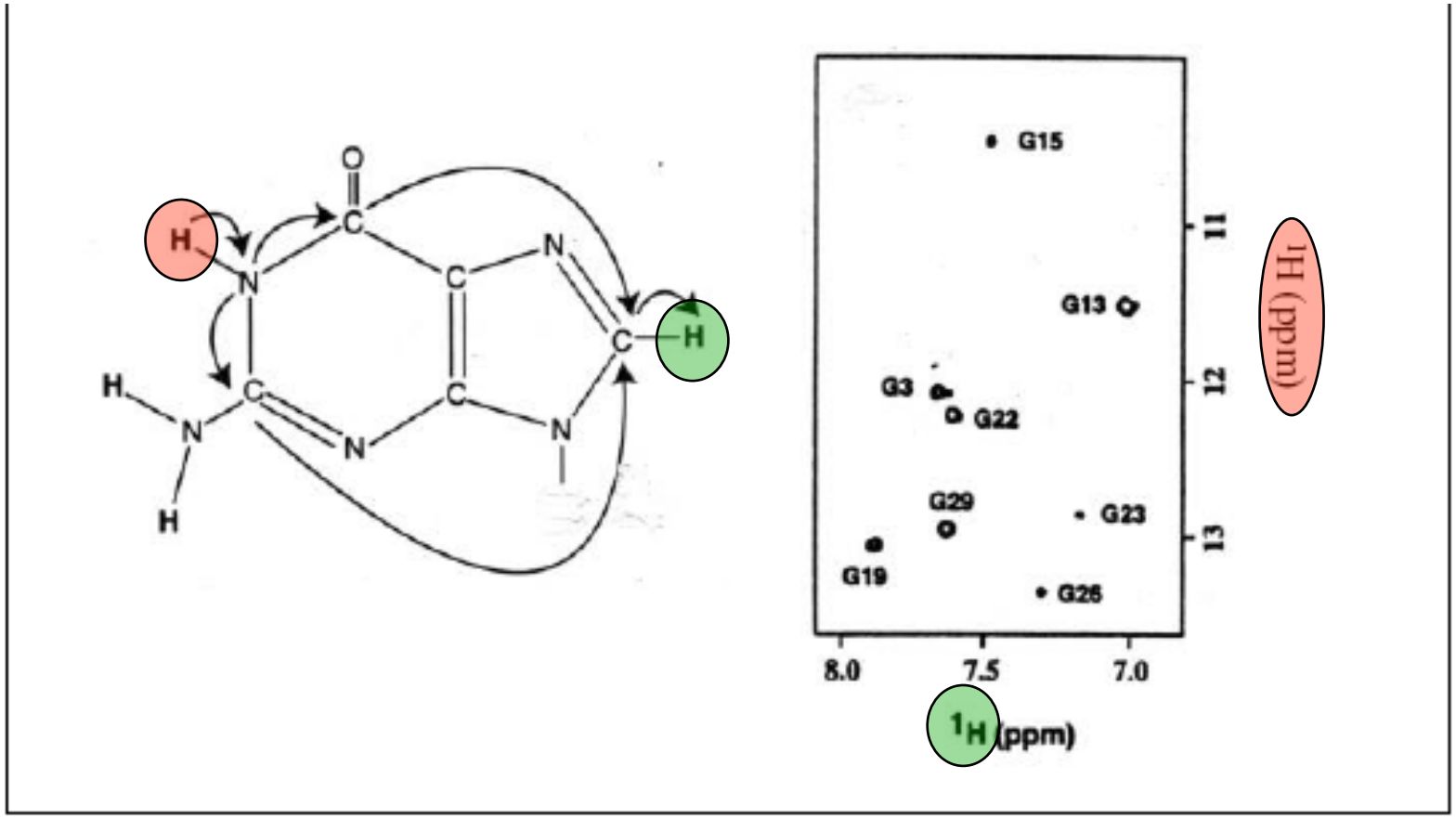
H(CN)H





C) Correlation of Non-exchangeable and exchangeable ^{1}H

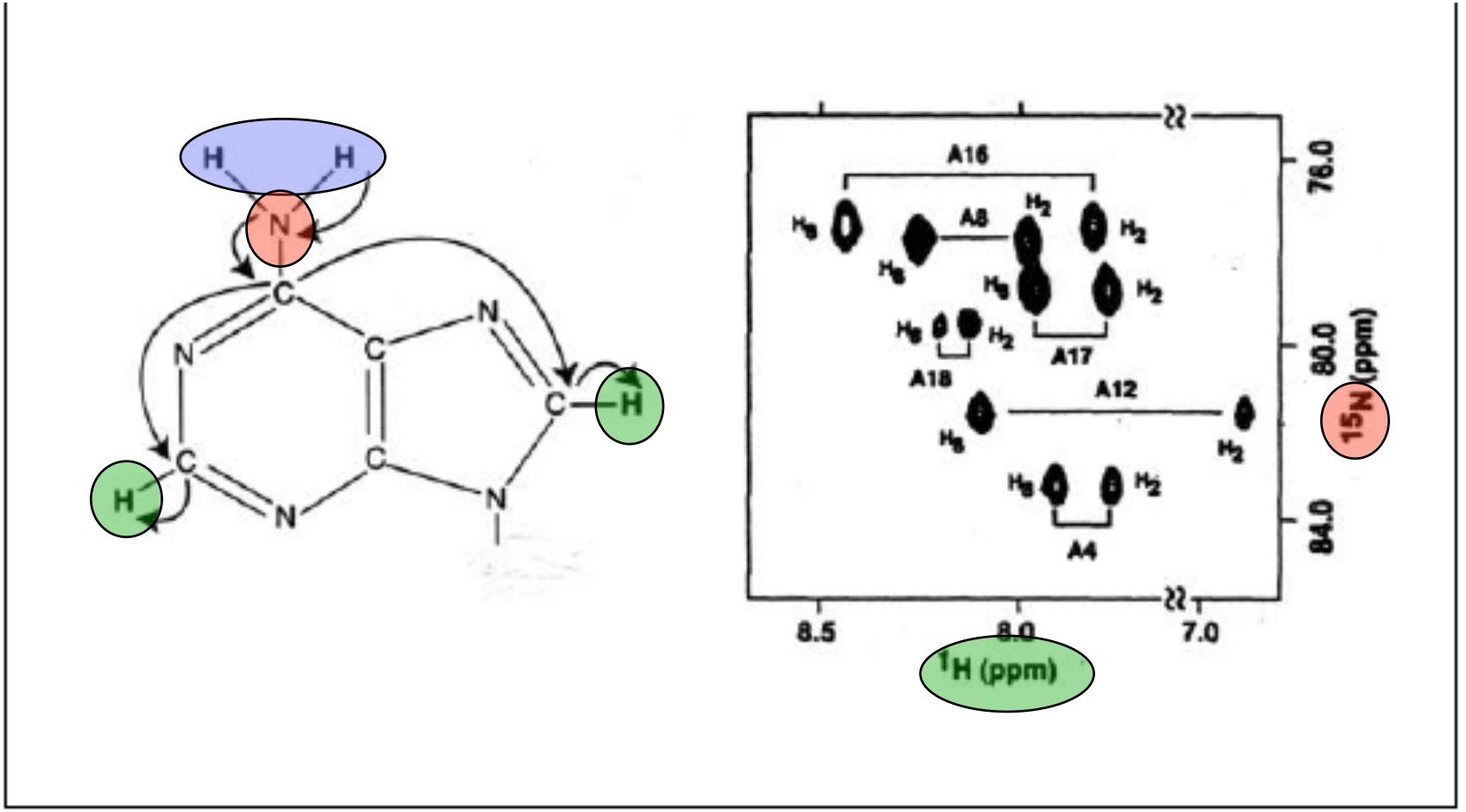
G-specific H(NC)-TOCSY(C)H



PL

C) Correlation of Non-exchangeable and exchangeable ^{1}H

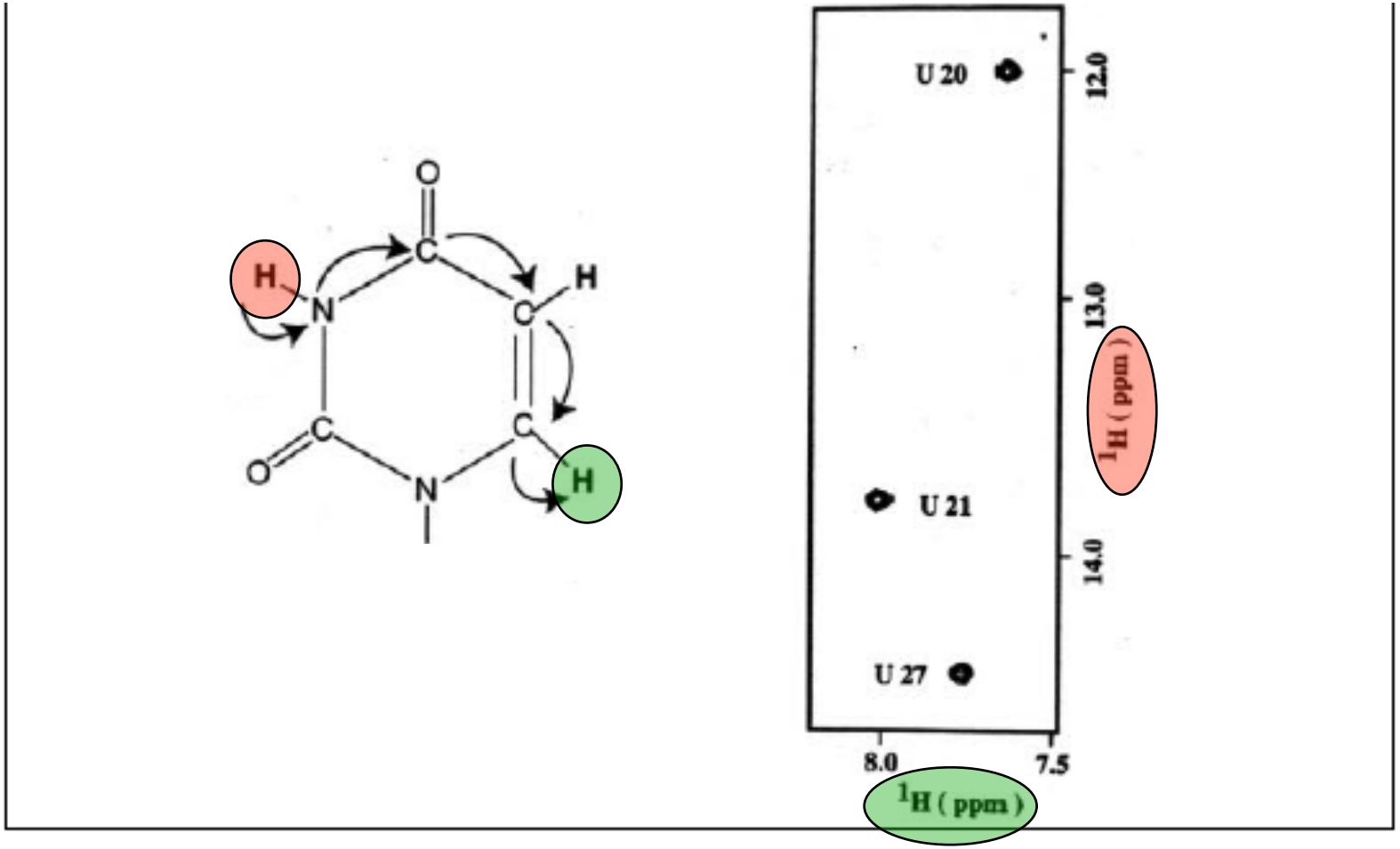
A-specific (H)N(C)-TOCSY(C)H



PL

C) Correlation of Non-exchangeable and exchangeable ^{1}H

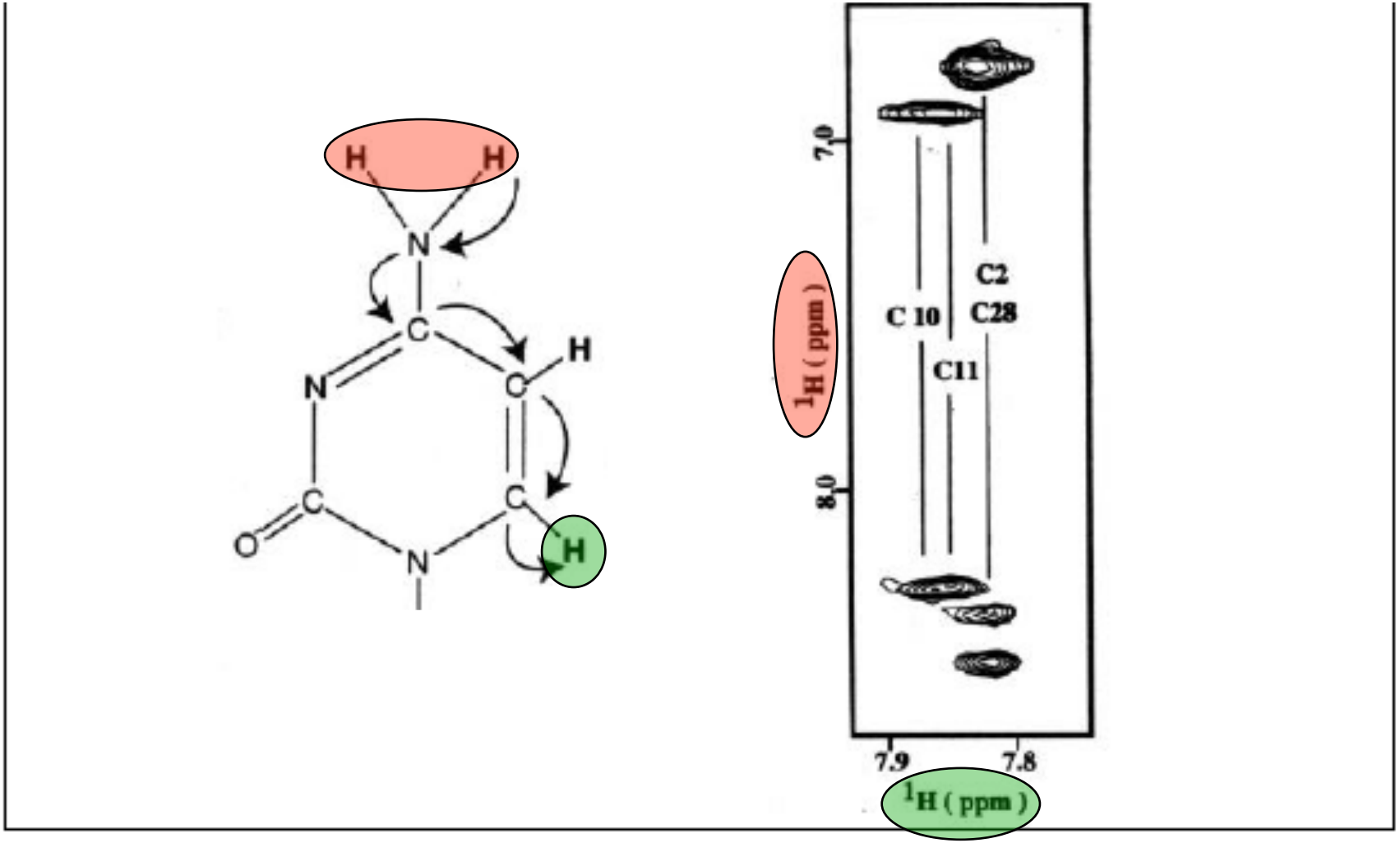
U-specific H(NCCC)H



PL

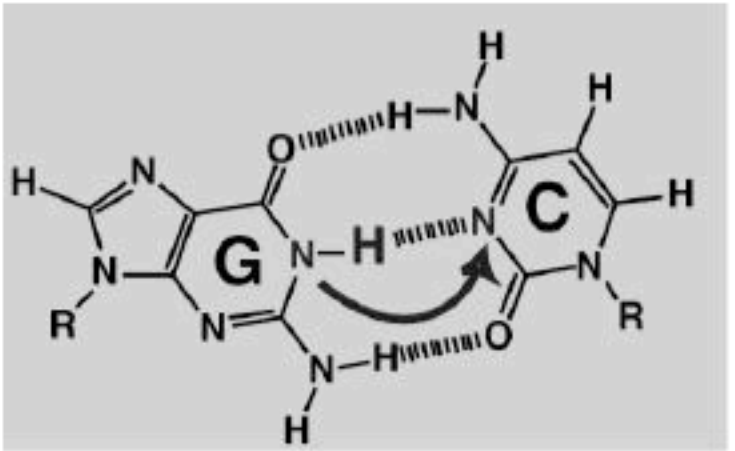
C) Correlation of Non-exchangeable and exchangeable ^{1}H

C-specific H(NCCC)H

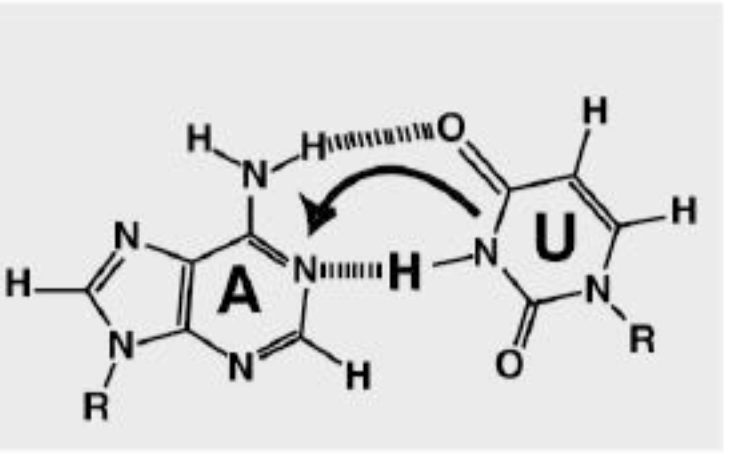


PL

D) Direct Observation of Hydrogen Bonding by $^{2}J_{NN}$ Couplings



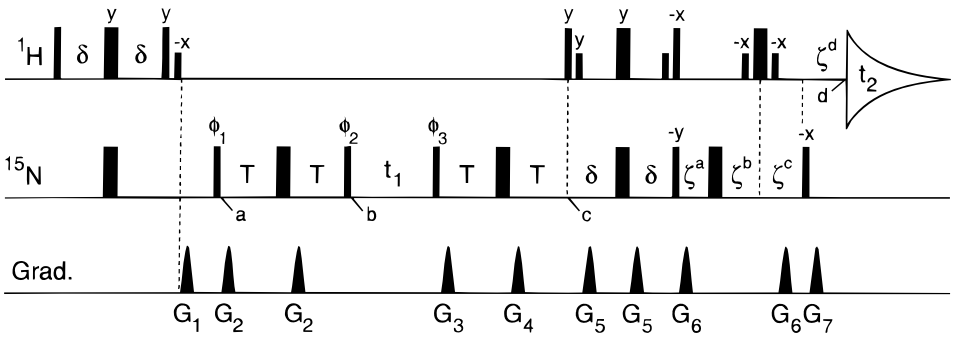
$J_{NN} = 6.3$ Hz

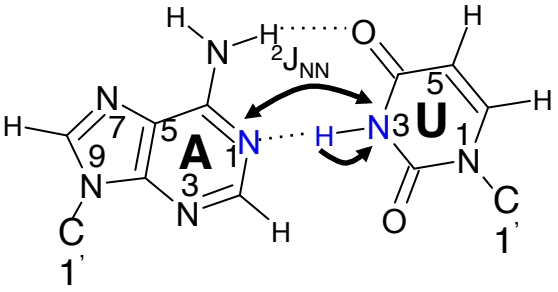


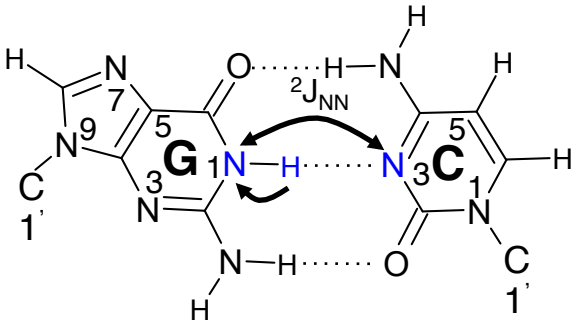
$J_{NN} = 6.7$ Hz

Dingley, J. A. and Grzesiek, S. *J. Am. Chem. Soc.* 120; 8293-8297 (1998)

D) Scalar Coupling Across H Bonds: HNN-COSY

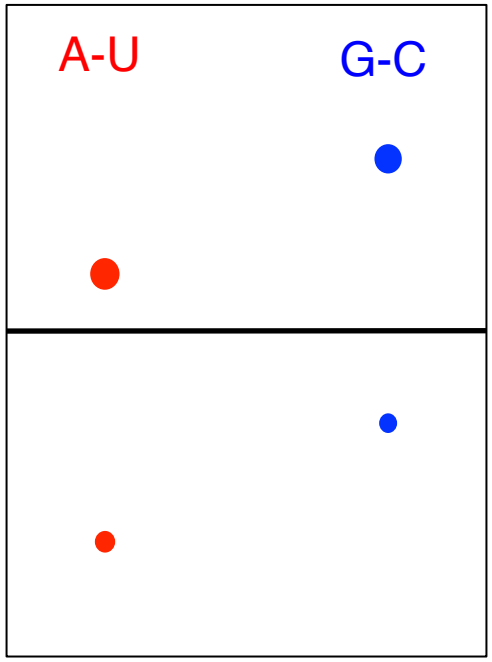






^{15}N of imino donor
G's 140 – 150 ppm
U's 155 – 170 ppm

^{15}N of b.p. acceptor
C's 190 – 205 ppm
A's 215 - 230 ppm

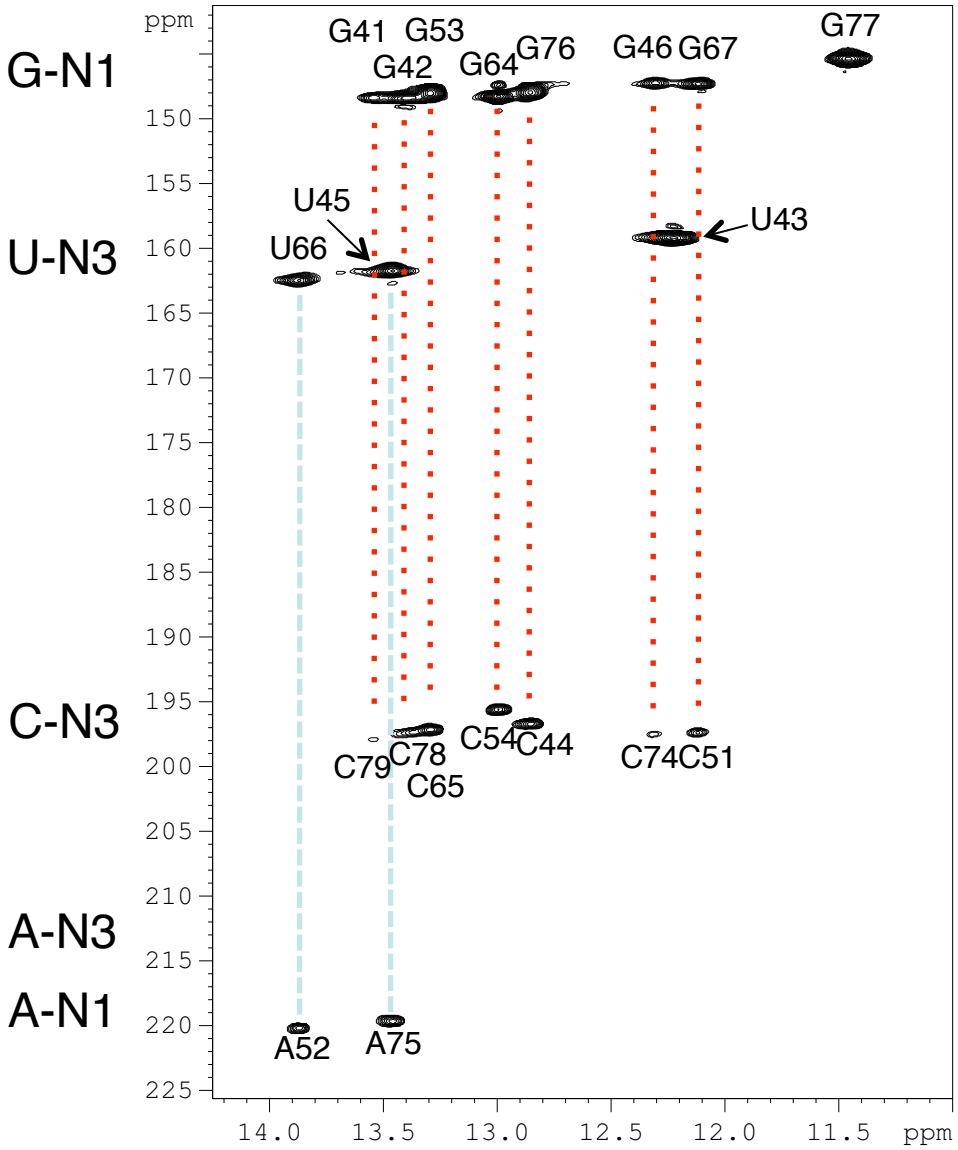


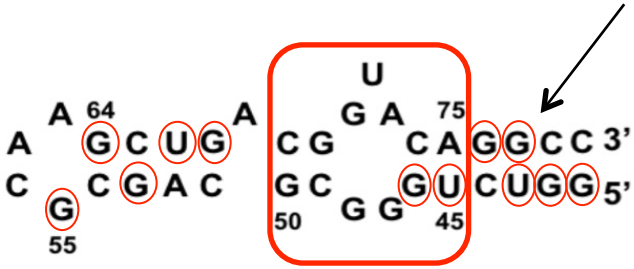
Imino ^{1}H (10 – 15 ppm)

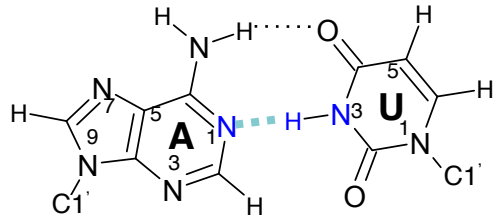
- $J_{NN} \approx 7$ Hz; $^1J_{NH} \approx 90$ Hz
- Unambiguous assignment of GN1 – CN3 and UN3 to AN1
- Quantitative determination of $^2J_{NN}$
  $|^2J_{NN}| = \text{atan}[(-I_{Na}/I_{Nd})^{1/2}]/(\pi T)$

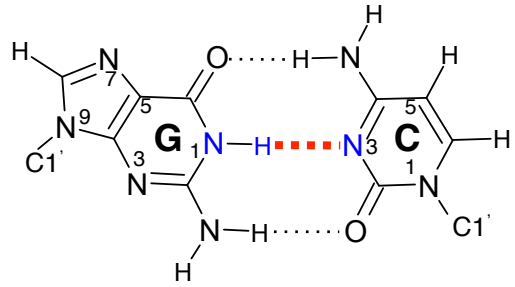
Dingley, A.J. & Grzesiek, S., *J. Am. Chem. Soc.*, **1998**, 120 (33), 8293 – 7.

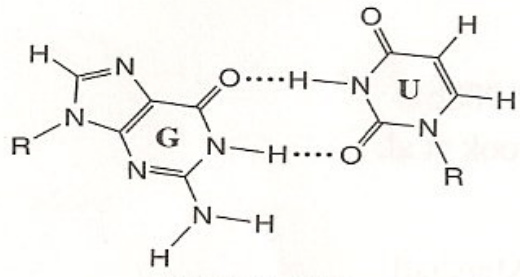
HNN-COSY of Free RREIIB-Tr (300 μM)







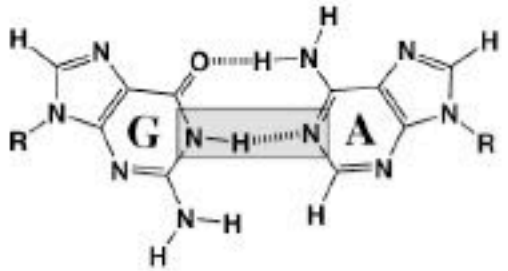




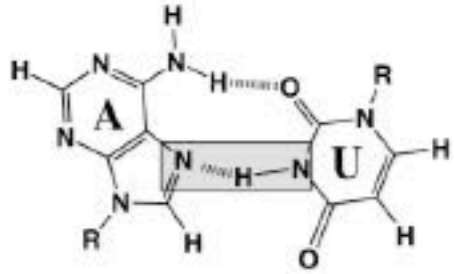
Spring et al. unpublished

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

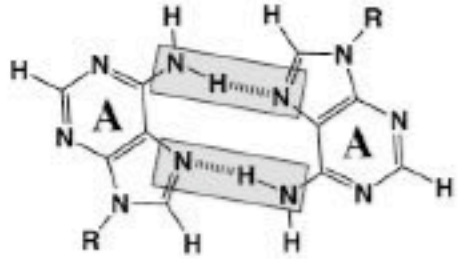
Imino-hydrogen-bonded GA (JNN = 5 Hz)



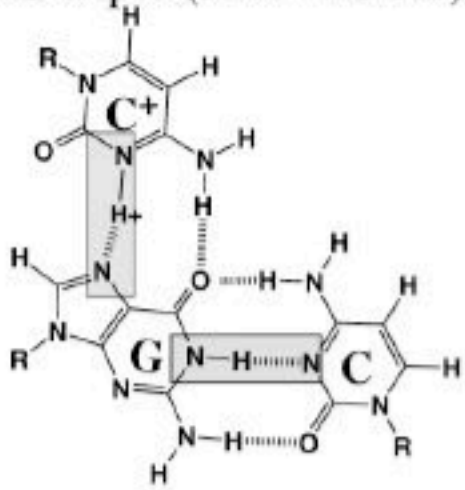
Reversed Hooogsteen AU (JNN = 5.5 Hz)



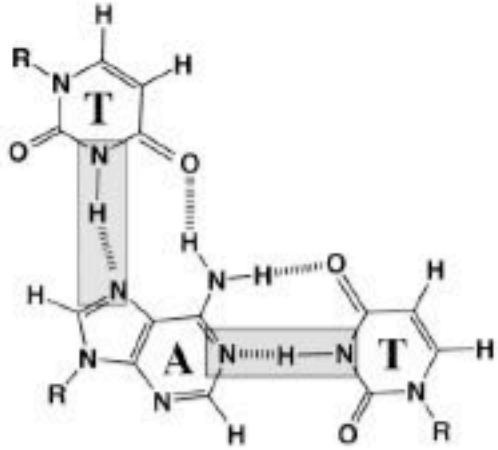
AA mismatch (JNN = 2.5 Hz)



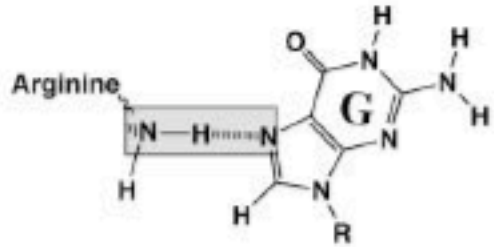
Hoogsteen GC in GC+G triple (JNN =10 Hz)



Hoogsteeen TA TAT Triple (JNN = 6.6 Hz)



Arginine-RNA (JNN = 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.....

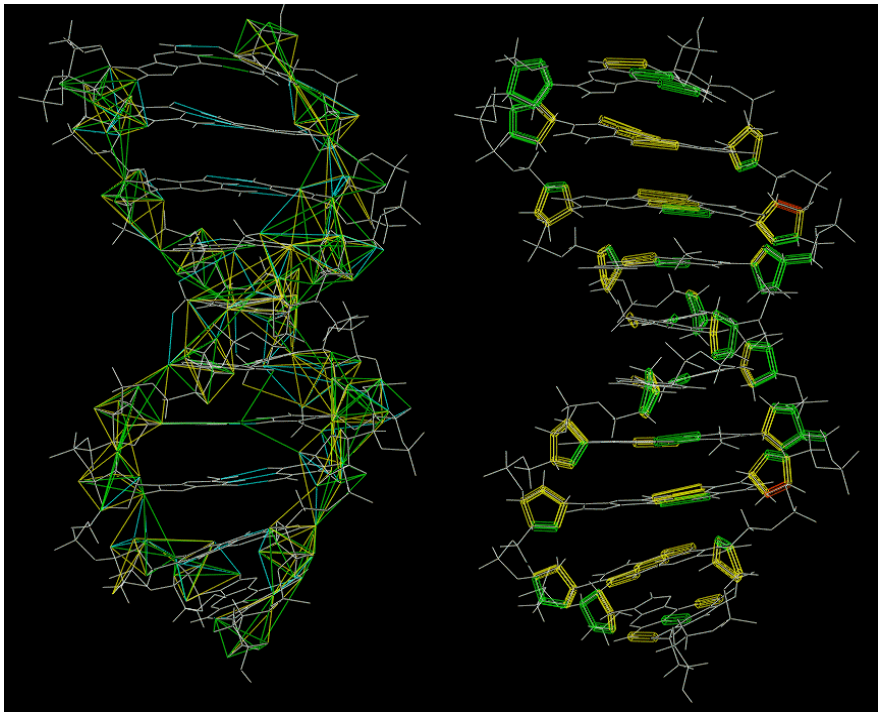
- NOE accuracy
  - > account for spin diffusion
- Backbone may be difficult to fully characterize
- Dipolar couplings

What do we know?

•Distance, Torsion, H-Bond constraints, Orientation

What do we want?

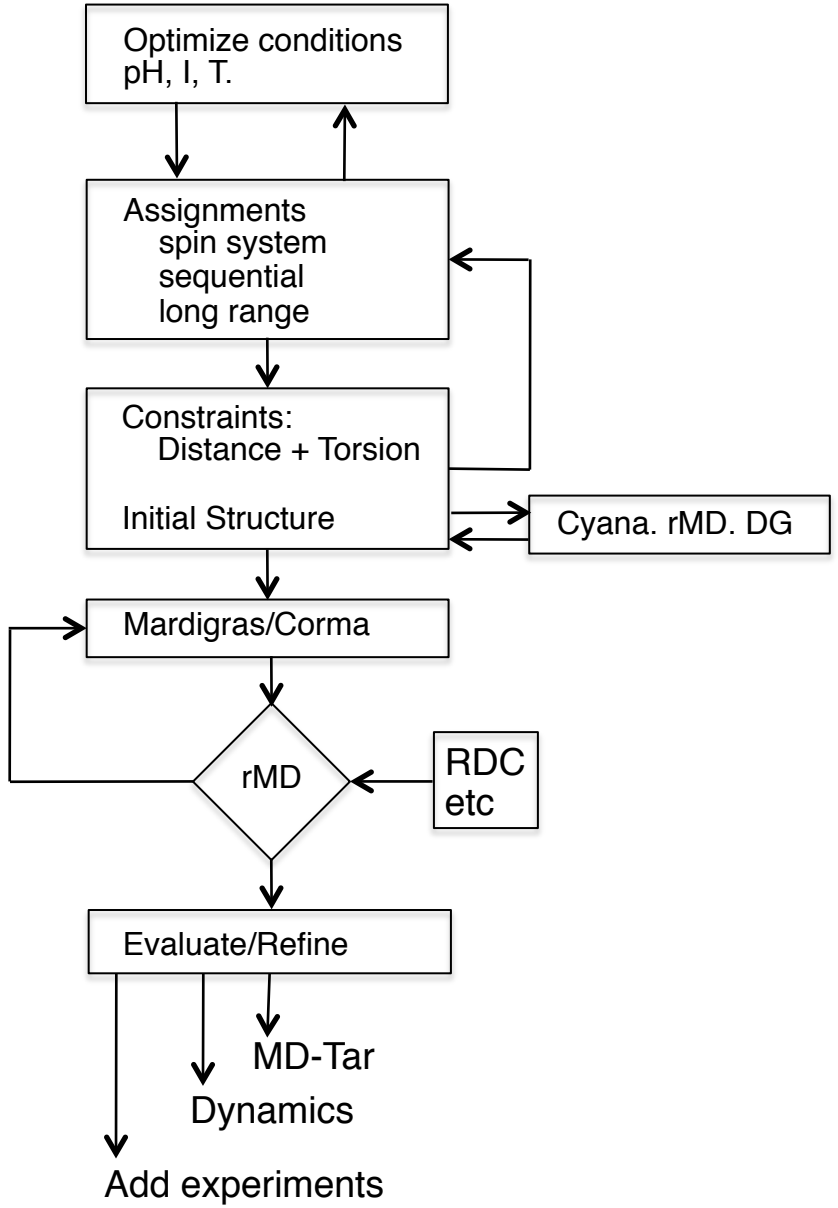
•Low energy structures in agreement with NMR

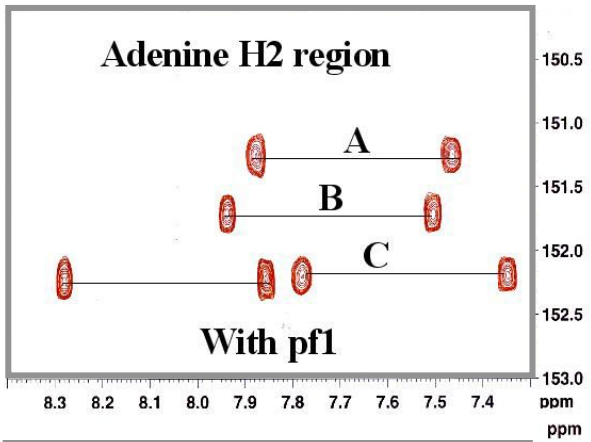


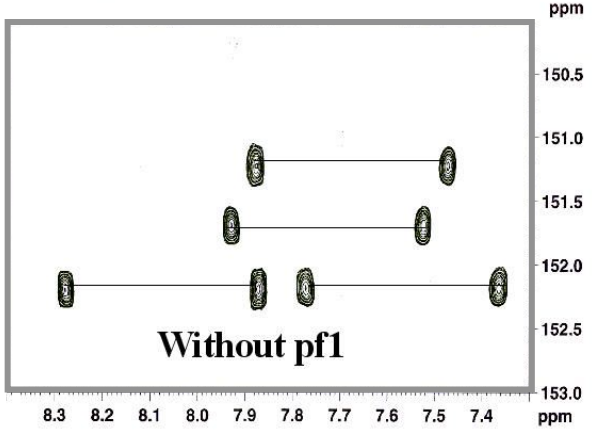
Relaxation Matrix method:

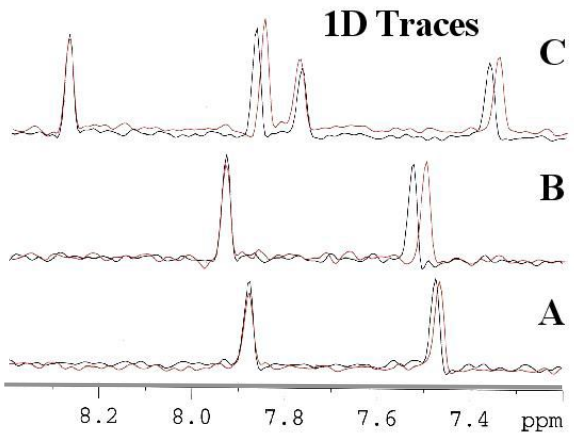
use of longer mixing times

(need initial structure, dynamics!)



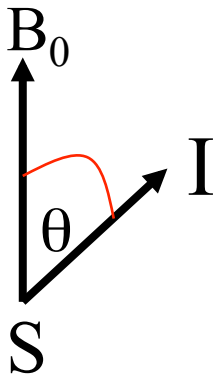






Dipolar couplings

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

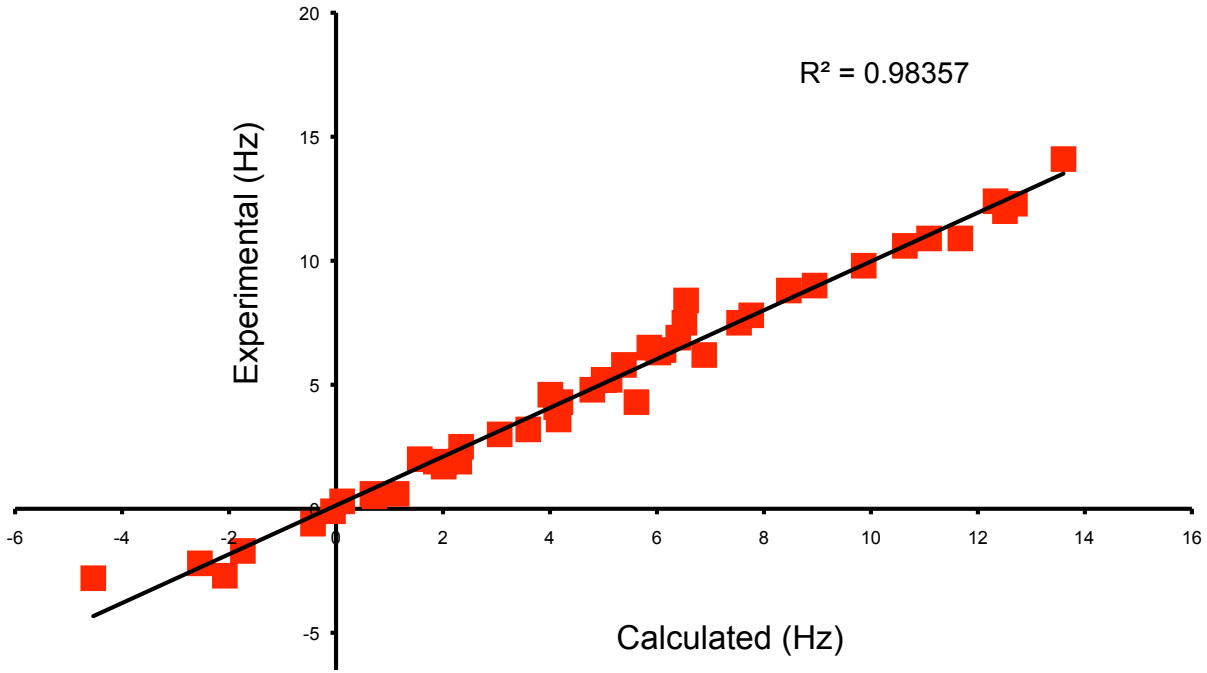


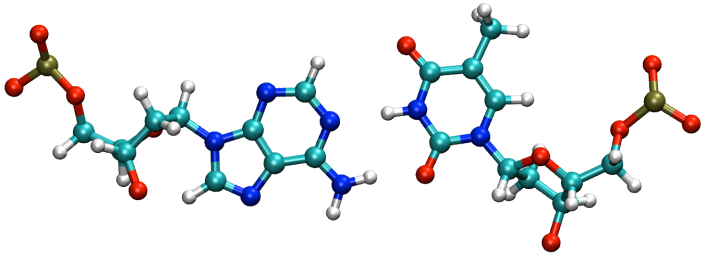
$$D^{IS} = D^{IS}_{\max}\left\langle \frac{1}{2}(3\cos^2\theta - 1)\right\rangle$$

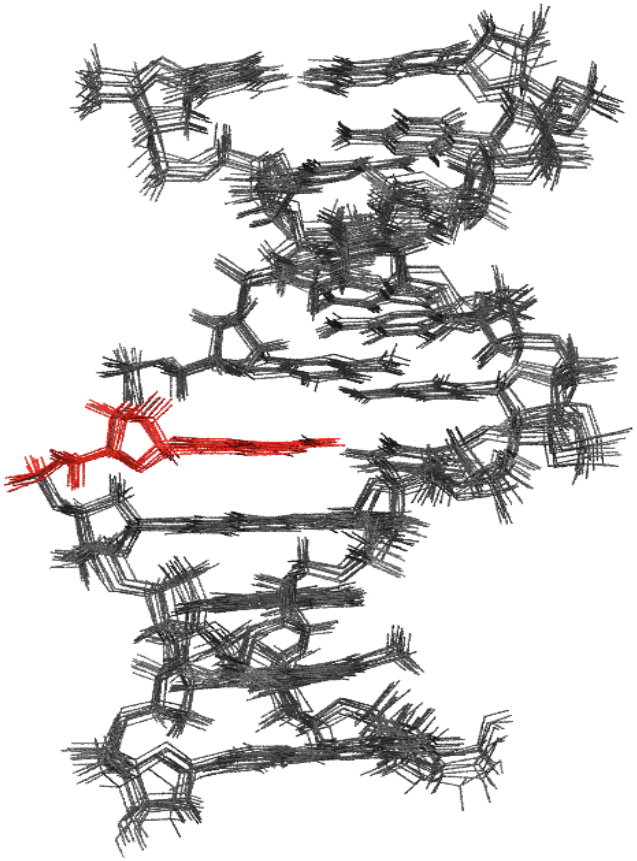
$$D^{IS}_{\max} = -\frac{\mu_0\gamma_I\gamma_S\hbar}{4\pi^2 r^3_{IS}}$$

Sp borano modified DNA / RNA hybrid residual dipolar splittings

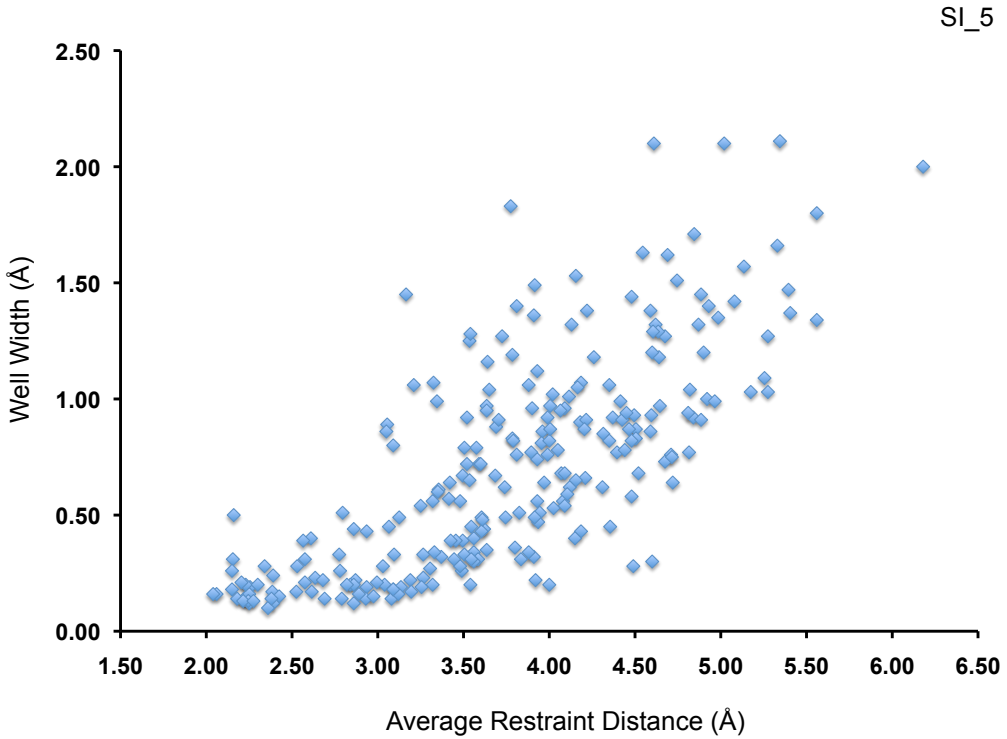
First atom			Last atom			Calc.	Exp.	Deviation	penalty
C1'	DA5	1 -- H1'	DA5	1:		-0.308	-0.700	0.392	0.154
C1'	DT	2 -- H1'	DT	2:		7.435	7.400	0.035	0.001
C1'	DG	3 -- H1'	DG	3:		-0.788	-0.900	0.112	0.012
C1'	DG	4 -- H1'	DG	4:		-5.398	-5.500	0.102	0.010







rMD with RDC
R.M.S.D. 0.63



Parameter	CαAG	Force Constant (k)[a]
Quantitative Distance Restraints (RANDMARDI)		
non exchangeable (total)	246	30
intra residue	154	30
inter residue (sequential)	92	30
inter residue (cross strand)	1	30
average well width (Å)	0.70 (stdev 0.46)	
exchangeable (total)	27	30
average well width (Å)	3.0	
Endocyclic Torsion Angle Restraints		
deoxyribose (pseudo rotation analysis)	95	50
average well width | r2- r3| / N	30	
Watson Crick Restraints		
distance	25	25
flat angle	25	10
Backbone Torsion Angle Restraints		
DNA / RNA hybrid broad rsts	68	50
well width $\alpha\ \beta\ \gamma\ \zeta$ (deg)	60, 80, 60, 65	
ε (C_T NOESY) (deg)	18	50
average well width	varries 20 -50 depending on # of data points available	
Residual Dipolar Coupling		
total RDC restraints	46	
base (C6, C8, C2, C5)	24	1.0 (dwt)
sugar (C1')	12	1.0 (dwt)
sugar (C3')	10	1.0 (dwt)
Total Restraints	550	
total restraints / residue	27.5	

CORMA R^x Values

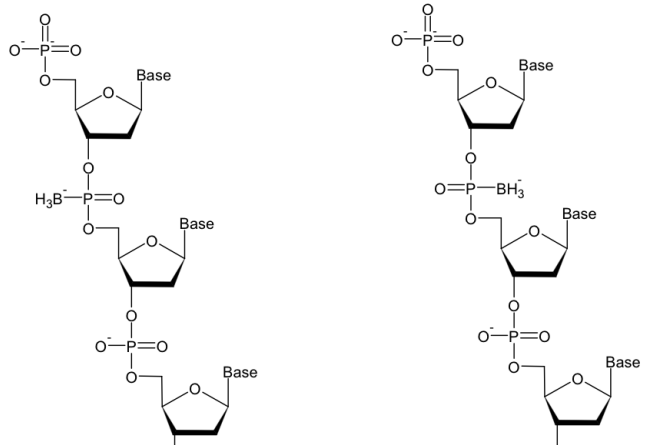
T_M (ms)	R^X (number of unique cross-peaks)		
	Intra	Inter	Total
75	4.73 (93)	6.55 (44)	5.25 (134)
125	4.13 (143)	5.61 (77)	4.62 (220)
250	3.81 (136)	5.19 (83)	4.29 (291)

Final Amber Parameters	
Total Distance Penalty (kcal/mol)	55.4
Total Angle Penalty (kcal)/(mol)	0.24
Total Torsion Angle Penalty (kcal)/(mol)	4.6
Residual Dipolar Coupling (RDC) Allignment Constraint	4.9
Bundle of 10 Final Structures	
Heavy Atom R.M.S.D.	0.63

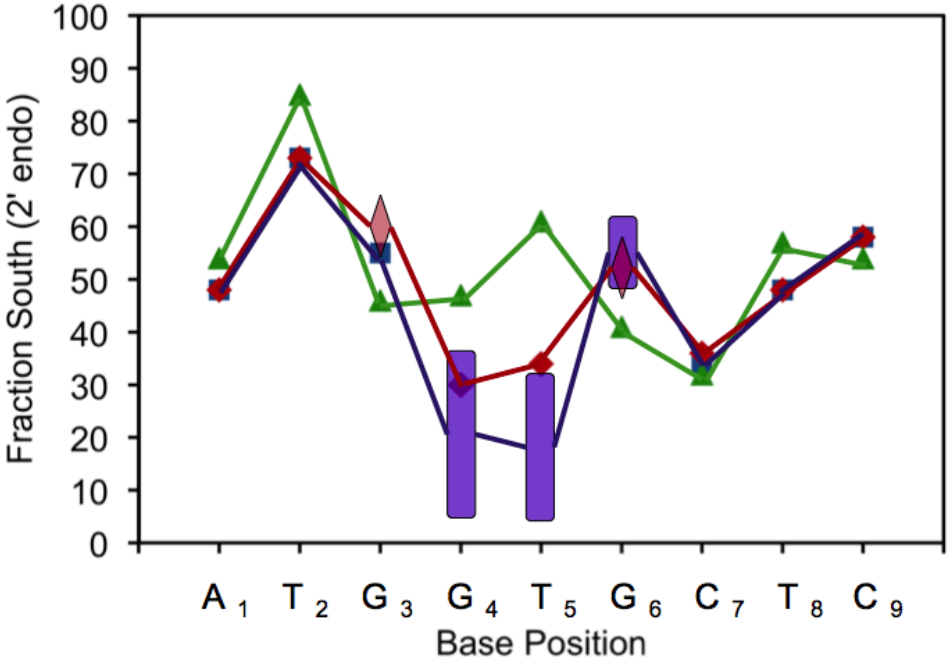
[a] kcal/(mol x unit of violation)

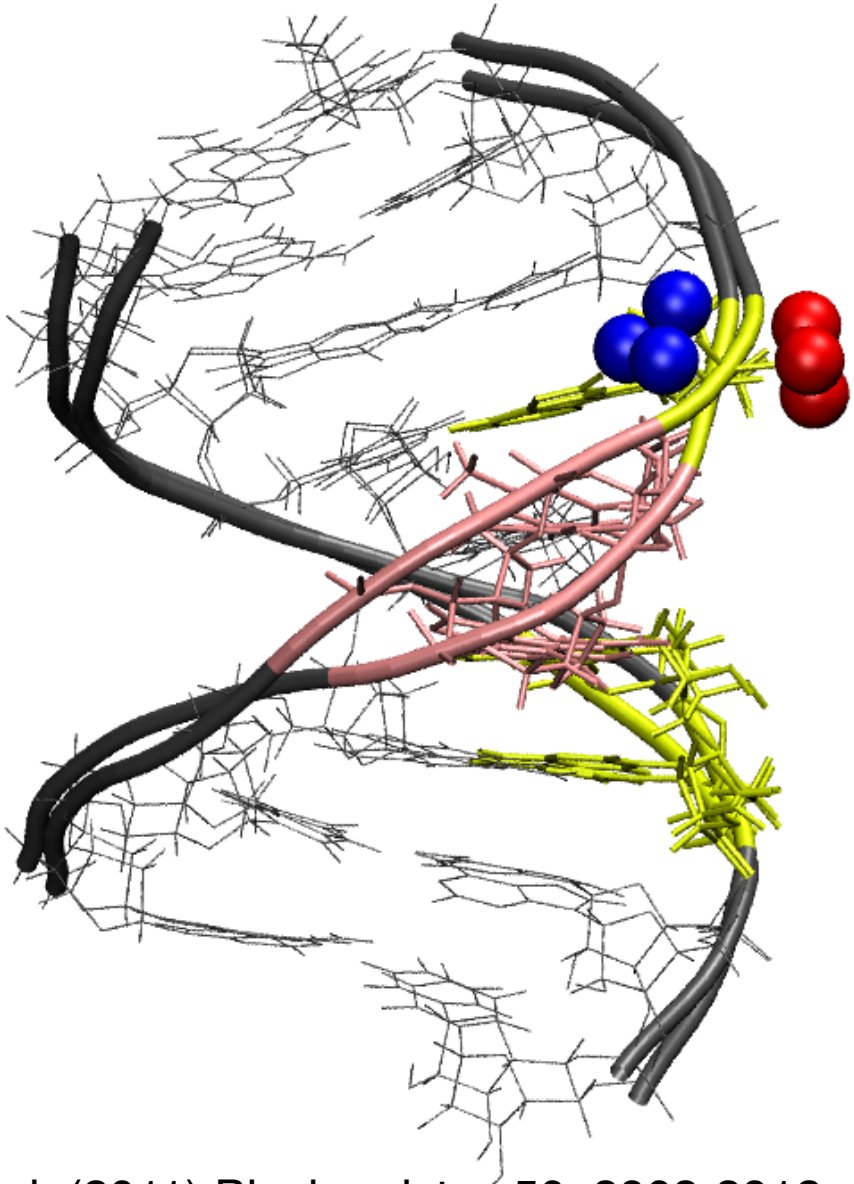
Johnson et al: “DNA sequence context conceals α anomeric lesions.” J. Mol. Biol. (2012) 416, 425-437.

Structural Basis of the RNase H1 Activity on Stereo Regular Borano Phosphonate DNA / RNA Hybrids.

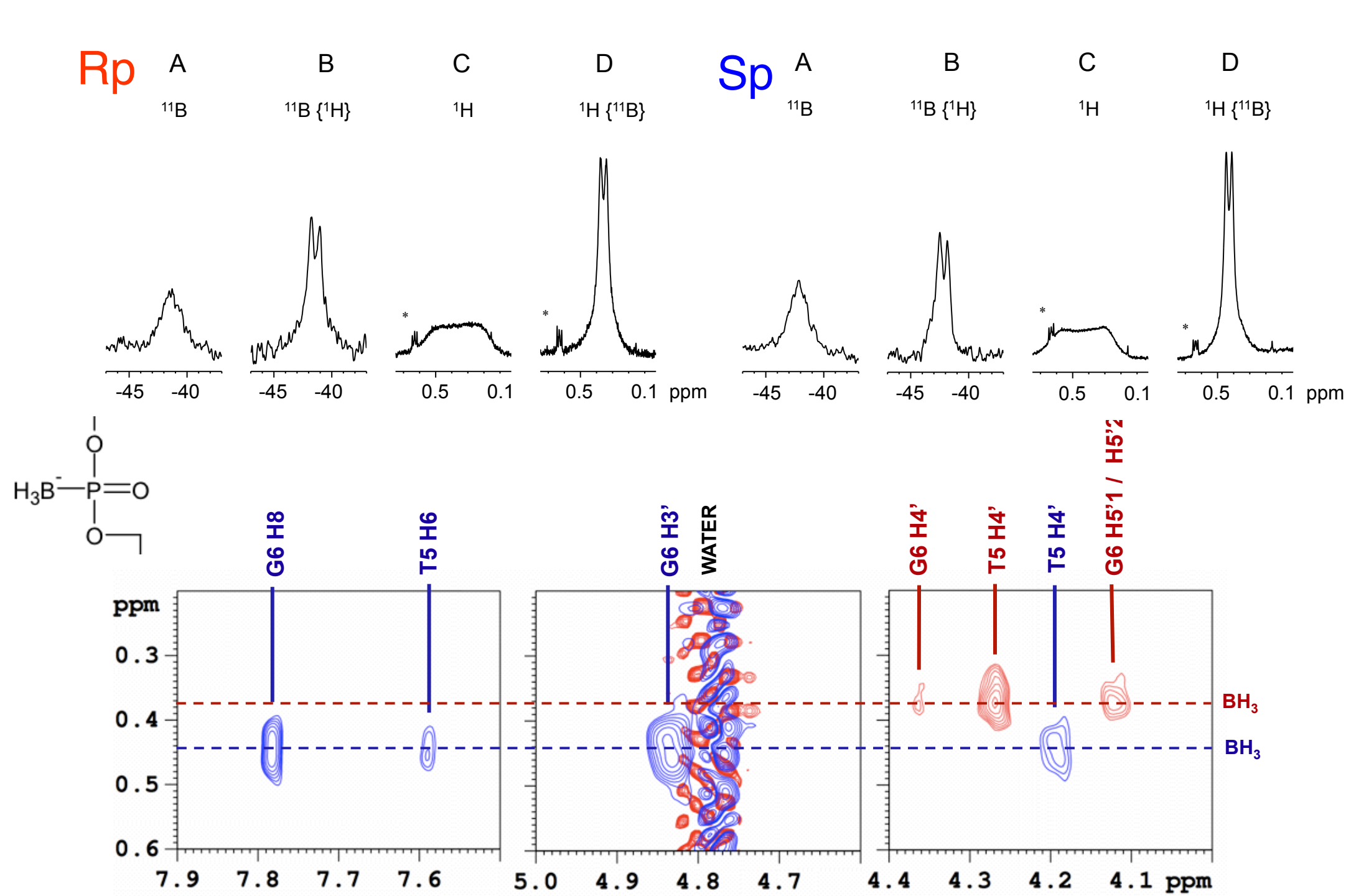


Sugar Pucker Summary

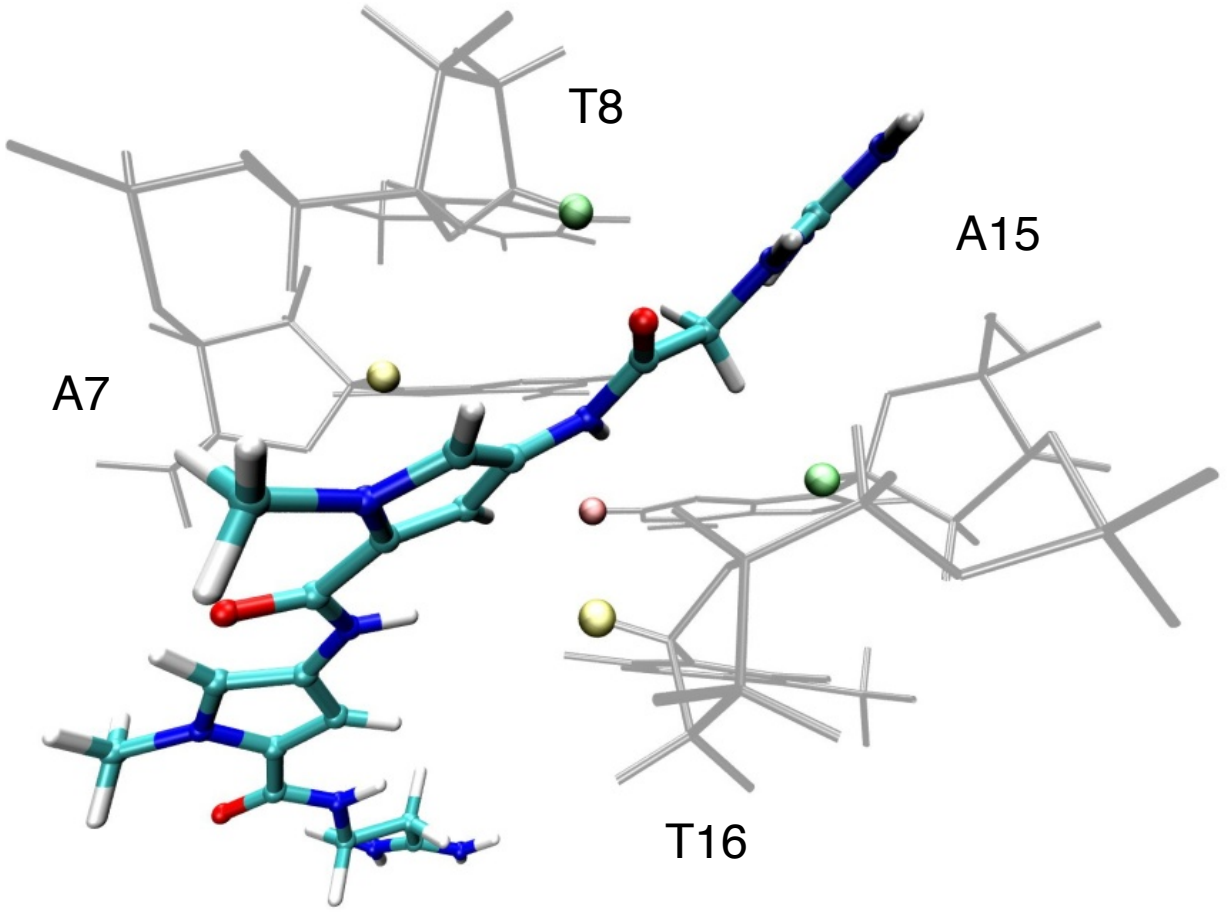


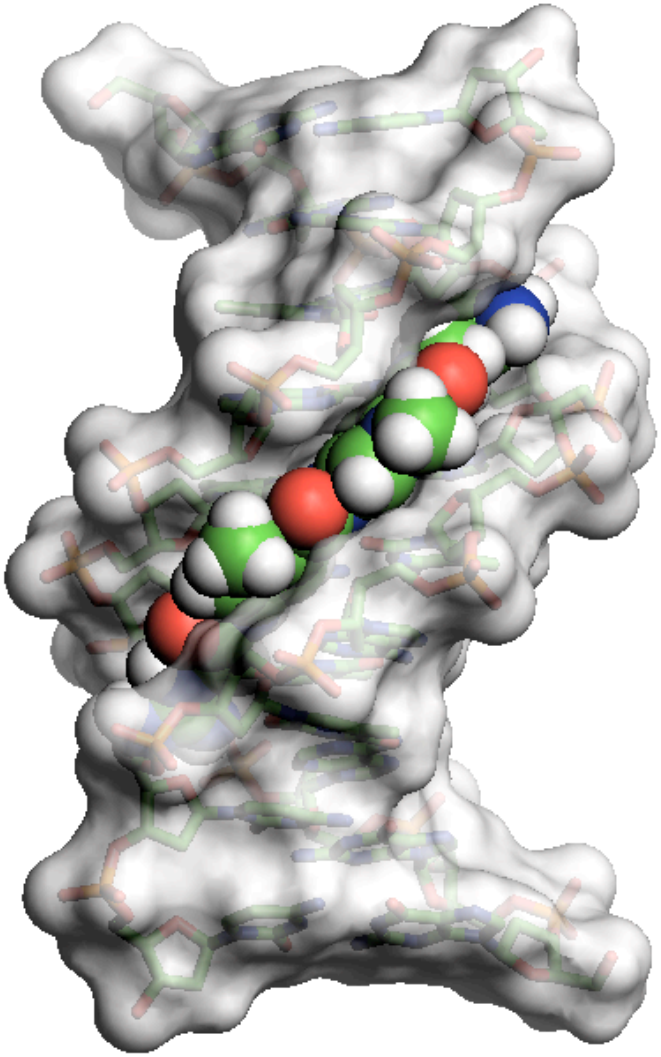


Johnson et al, (2011) Biochemistry, 50, 3903-3912


Rp
A
B
C
D
^{11}B
^{11}B {^{1}H}
^{1}H
^{1}H {^{11}B}
Sp
A
B
C
D
^{11}B
^{11}B {^{1}H}
^{1}H
^{1}H {^{11}B}
-45
-40
0.5
0.1
ppm
H_3B^-—P=O
G6 H8
T5 H6
G6 H3'
WATER
G6 H4'
T5 H4'
T5 H4'
G6 H5'1 / H5''2
ppm
0.3
0.4
0.5
0.6
7.9
7.8
7.7
7.6
5.0
4.9
4.8
4.7
4.4
4.3
4.2
4.1
ppm
BH_3
BH_3

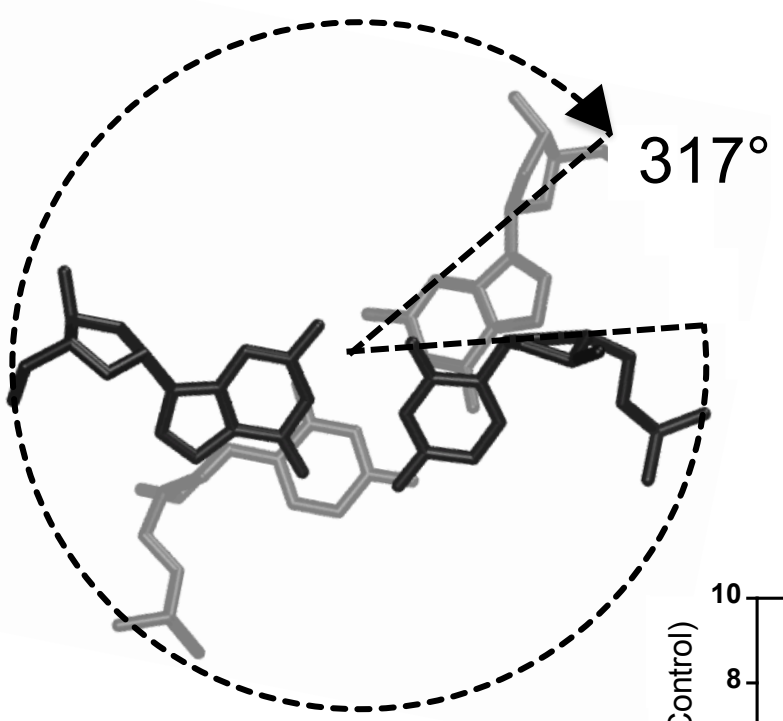
Molecular Details



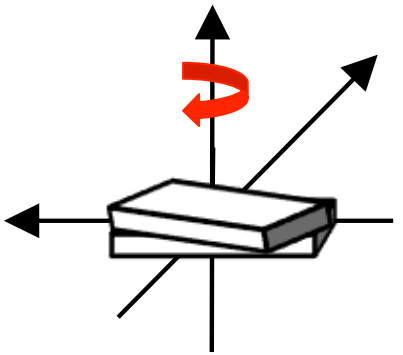


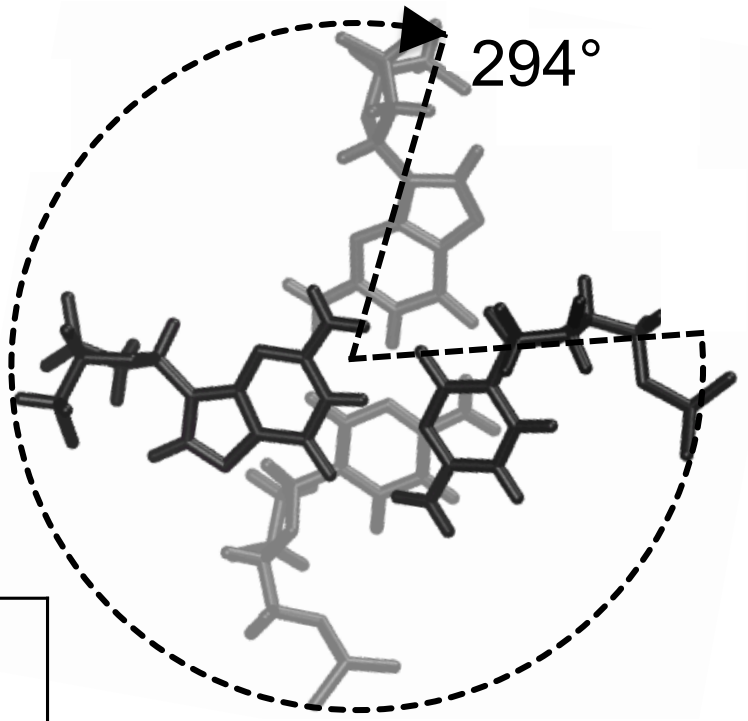
Michael Rettig, et al, 2013, ChemBioChem

Twist

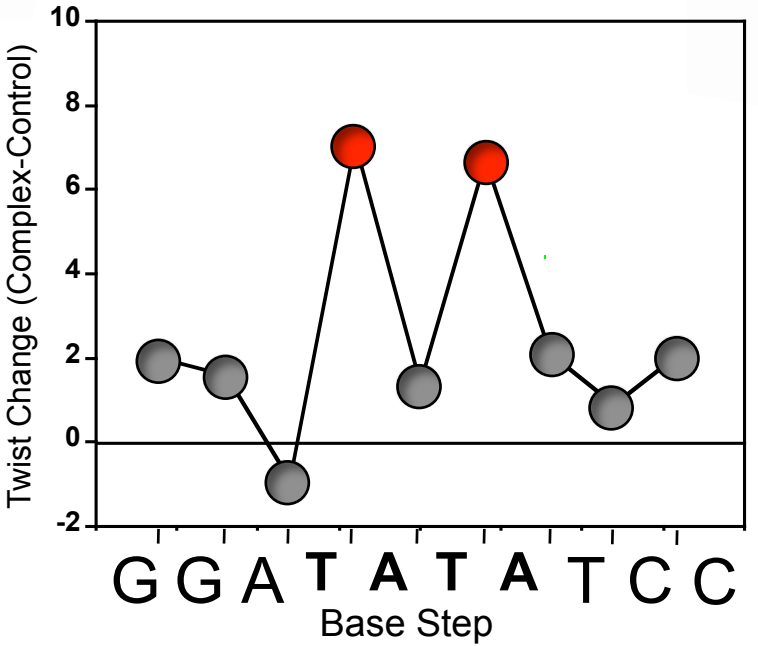


DNA-NETROPSIN



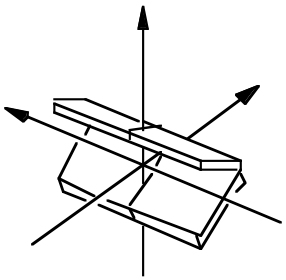


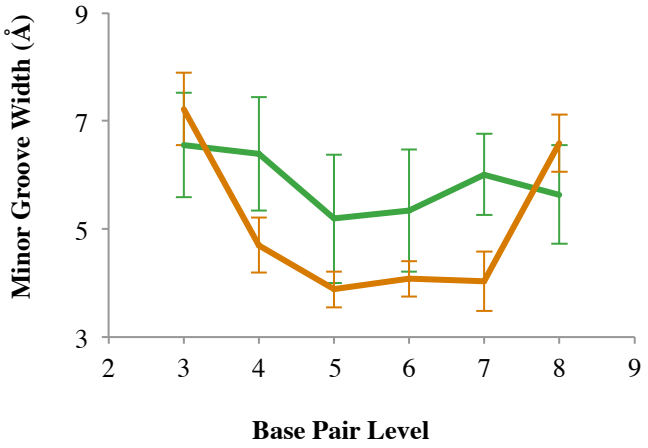
Free DNA

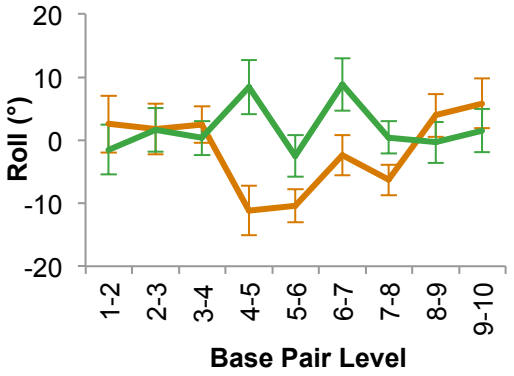


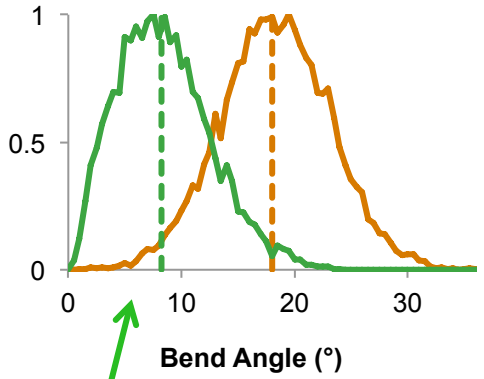
Michael Rettig, et al, 2013, ChemBioChem

Bend → change in twist and roll collapse of minor groove

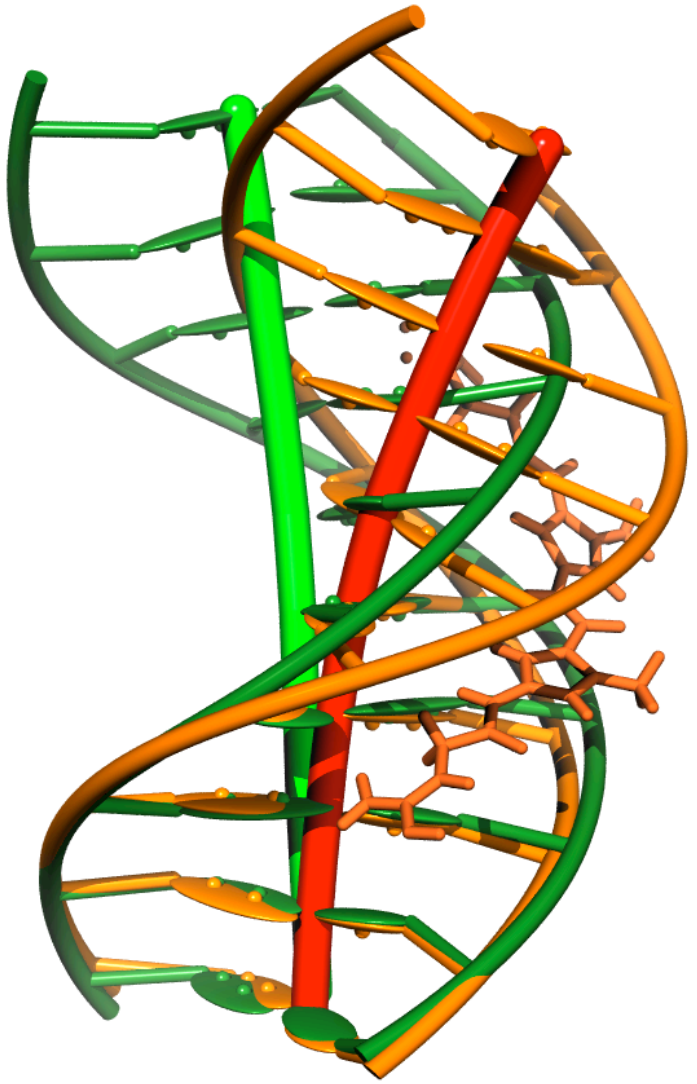






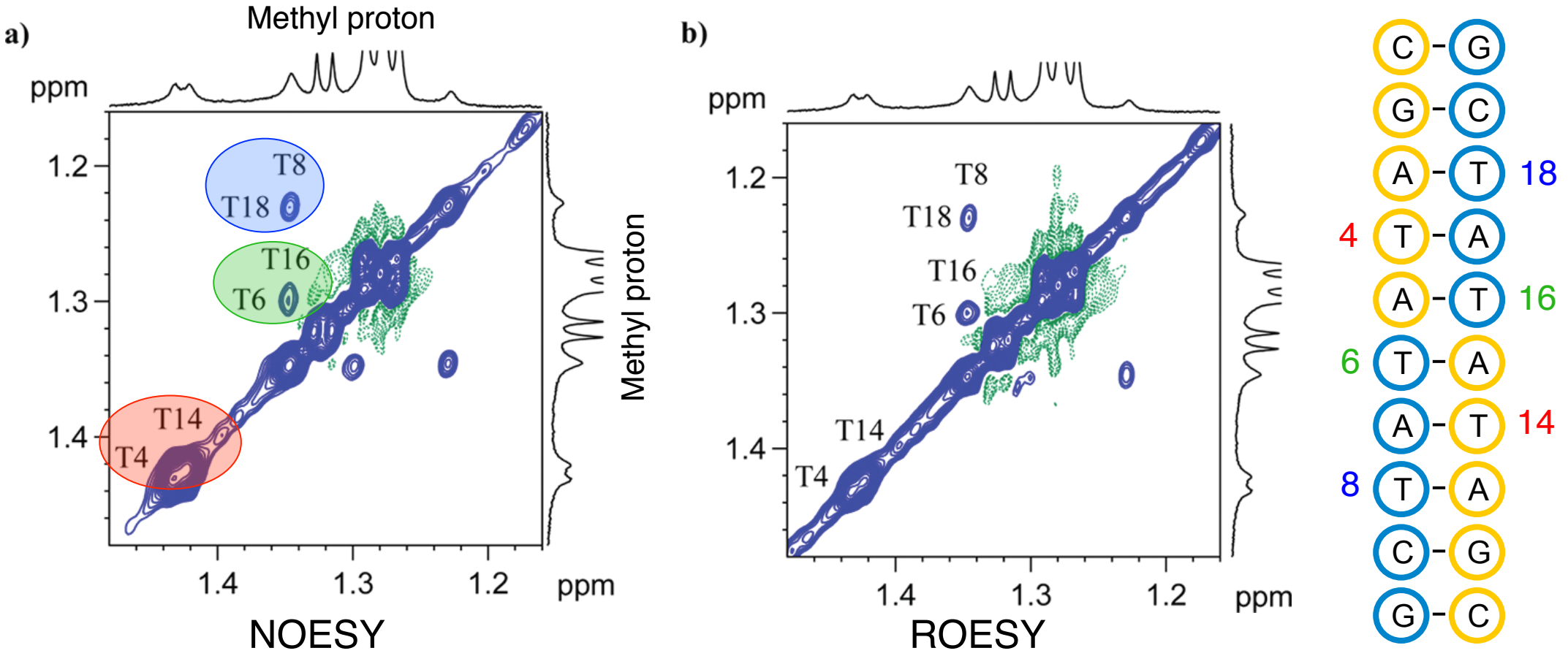


Control Bent into MAJOR groove
Complex Bent into MINOR groove



Michael Rettig, et al, 2013, ChemBioChem

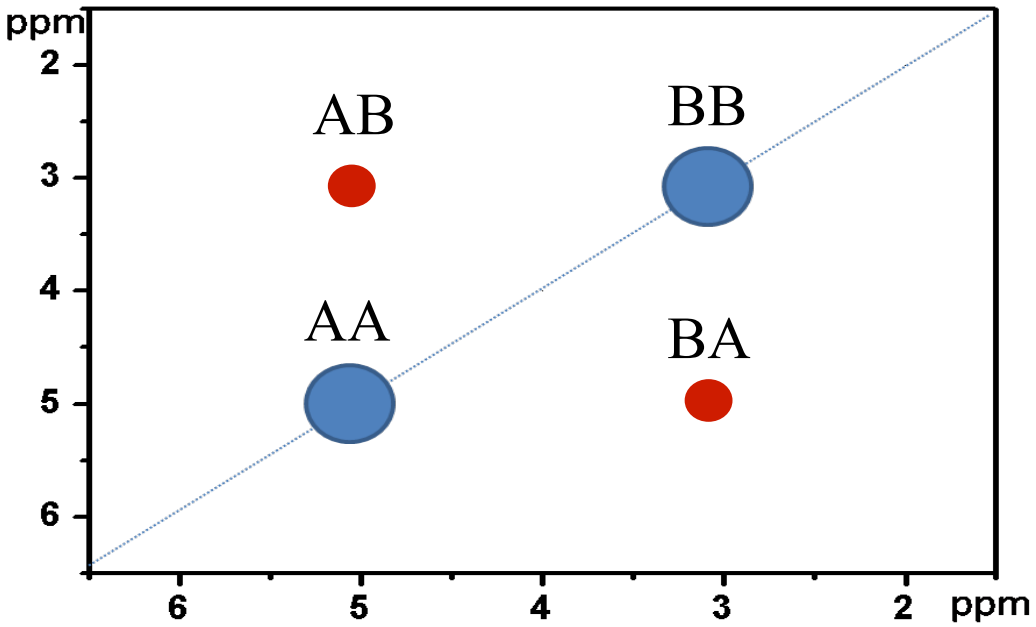
NOESY (Exchange) Peaks



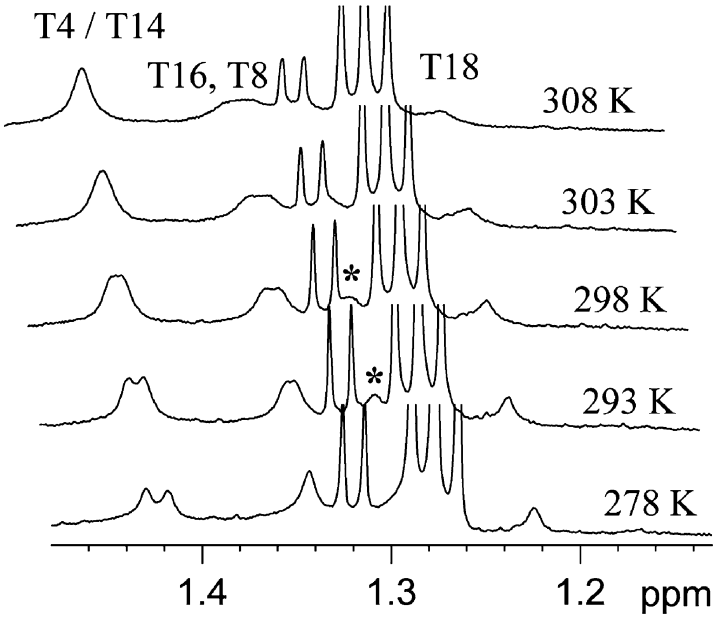
Methyl proton region of the a) 250 ms NOESY b) 150 ms ROESY spectrum of the netropsin-CG/CG complex at 283 K at 600 MHz

Michael Rettig, et al, 2012, J. Phys. Chem.

2D Exchange Spectroscopy (EXSY)
Quantifying Exchange Processes



$$k = \frac{1}{t_m} \ln \frac{r+1}{r-1} \qquad r = \frac{I_{AA} + I_{BB}}{I_{AB} + I_{BA}}$$

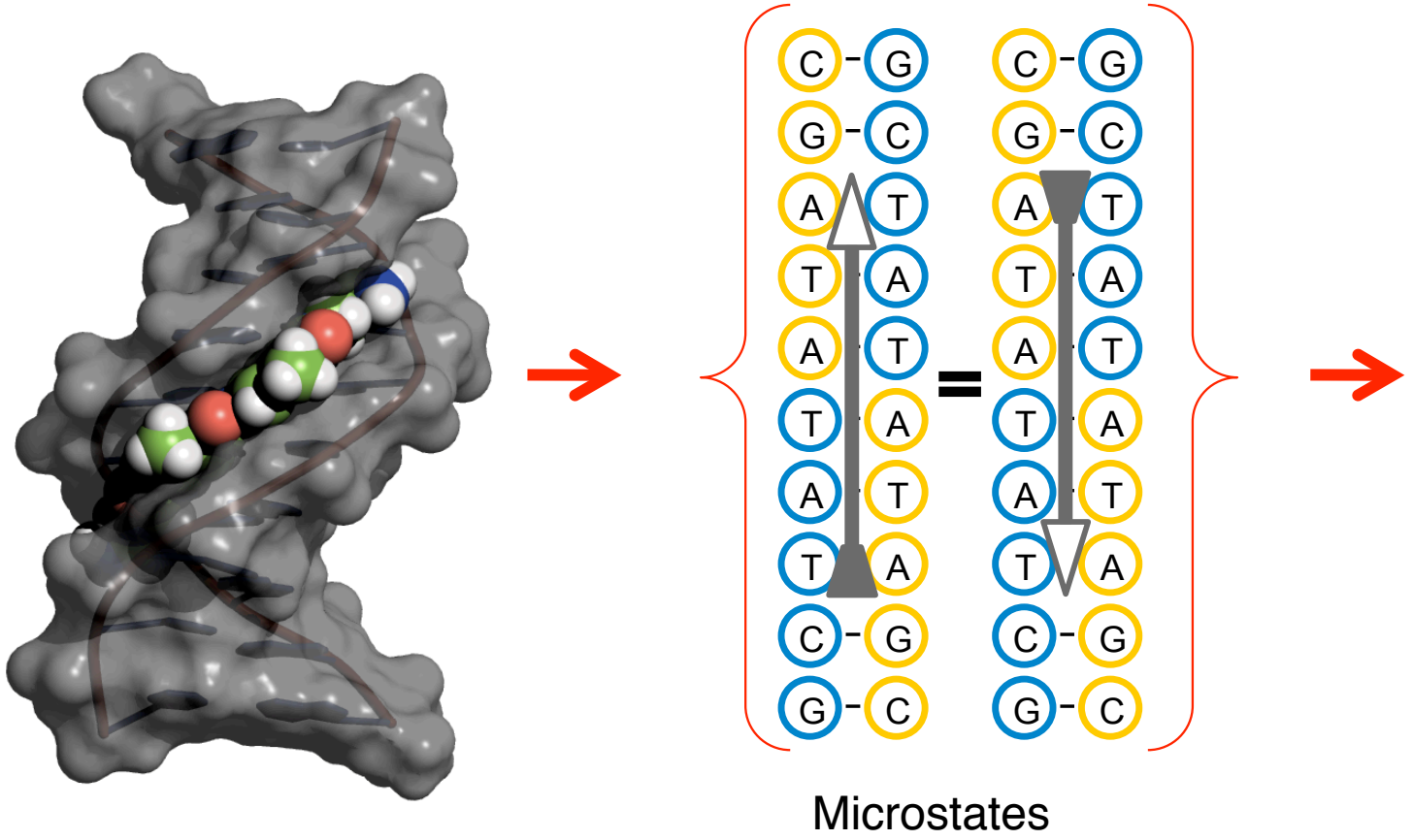


$$k_{coal} = (\pi/2^{1/2})\Delta\nu$$

Charles L. Perrin and Tammy J. Dwyer, Chem. Rev. 1990. 90, 935-967

Microscopic Rearrangement of Bound Minor Groove Binders Detected by NMR

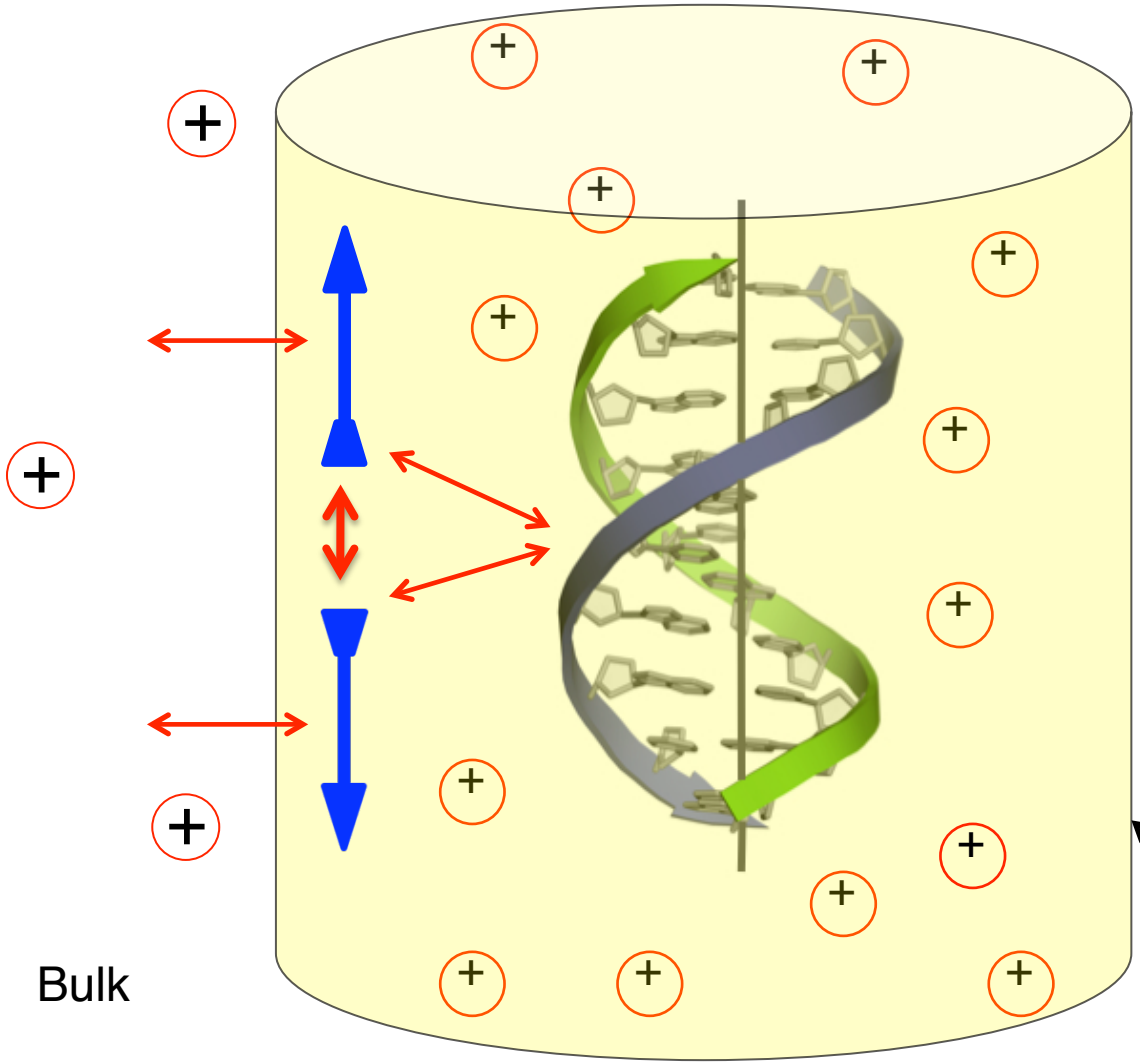
- → NMR sees exchange rates in the order of 20-60 ms at 300K
- → BUT It takes >2 s for the drug to dissociate off the DNA (SPR)



Microstates

Michael Rettig, et al, 2012, J. Phys. Chem.

Microscopic Rearrangement of Bound Minor Groove Binders Detected by NMR



→ very common

→ difficult to see depending on k

→ contributes to binding

General references, NMR techniques, sample preparation, analysis

BioNMR in Drug Research. Edited by Oliver Zerbe, 2002 Wiley Verlag

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Zidek L., Stefl R and Sklenar V. (2001) "NMR methodology for the study of nucleic acids"Curr. Opin. Struct. Biol., 11, 275-28

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

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Multinuclear experiments, DNA/RNA

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Marino, J.P, Prestegard, J.H. & Crothers, D.M. (1994) Correlation of adenine H2/H8 resonances in uniformly 13C labeled RNAs by 2d HCCH-TOCSY: a new tool for 1H assignment. *J. Am. Chem. Soc.*, 116, 2205-2206

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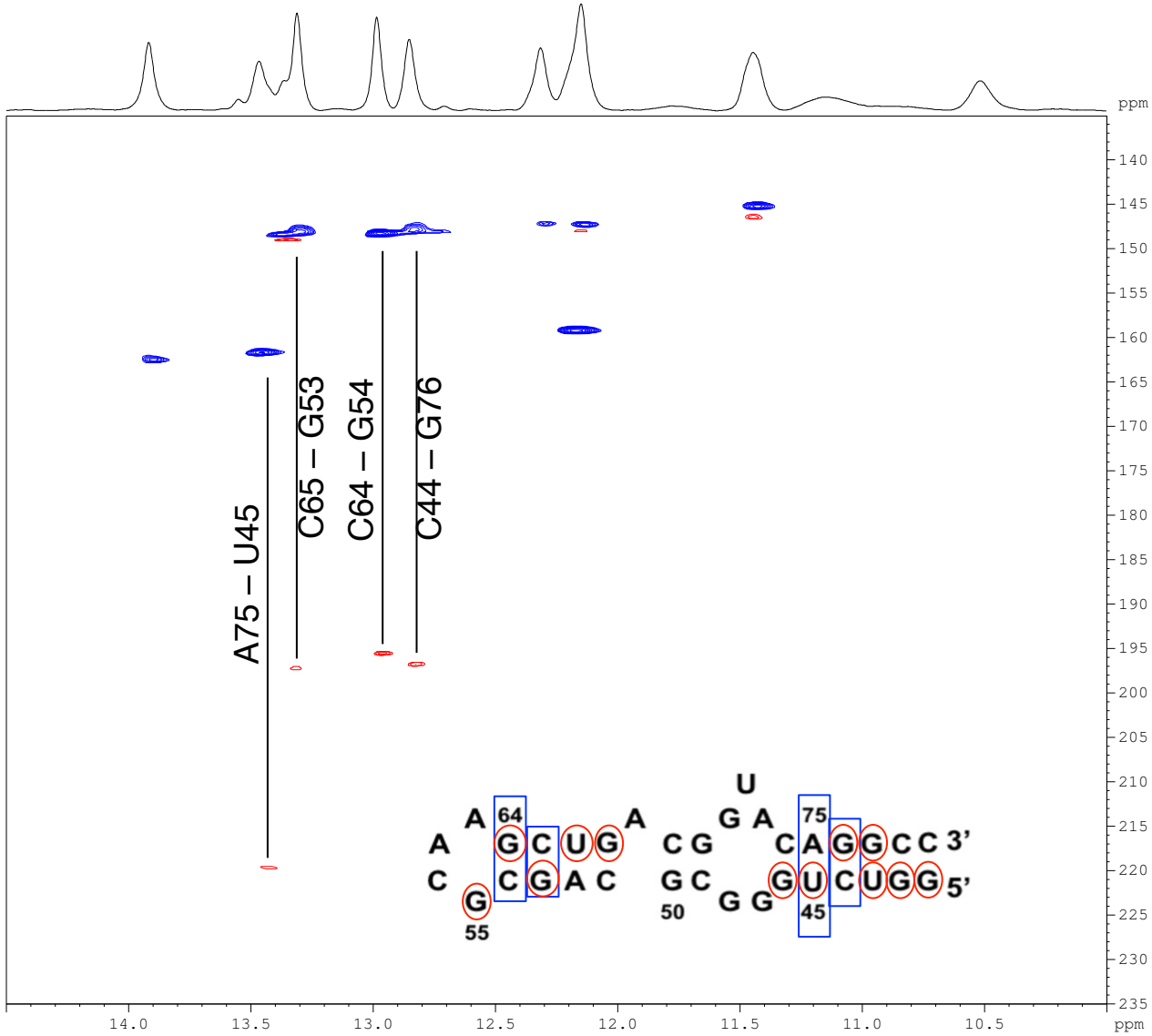
HNN-COSY of Free RREIIB-Tr (80 μM)

RREIIBTR All 13C 15N labelled Free rna, HNN-COSY, coupled, 298K

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Current Data Parameters
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PROCNO                   1

F2 - Acquisition Parameters
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PULPROG   hnncosygpphwg
TD                     2048
SOLVENT                D2O
NS                      512
DS                        8
SWH               15015.015 Hz
FIDRES             7.331550 Hz
AQ                 0.0682817 sec
RG                     29193
DW                    33.300 usec
DE                     6.00 usec
TE                   298.0 K
d0                 0.00000300 sec
D1                 0.50000000 sec
d11                0.03000000 sec
d13                0.00000400 sec
D16                0.00020000 sec
D23                0.01500000 sec
D26                0.00250000 sec
DELTA              0.00002986 sec
DELTA1             0.01495700 sec
DELTA2             0.00028800 sec
IN0                0.00008220 sec
MCREST             0.00000000 sec
MCWRK              0.00750000 sec
ST1CNT                   128

======== CHANNEL f1 ========
NUC1                     1H
P1                    11.93 usec
p2                    23.86 usec
P11                 1000.00 usec
PL0                  120.00 dB
PL1                    1.00 dB
SFO1           600.1328215 MHz
SP1                   39.47 dB
SPNAM1       Squa100.1000
SPOFF1                0.00 Hz

======== CHANNEL f3 ========
CPDPRG3                garp
NUC3                    15N
P21                   43.00 usec
p22                   86.00 usec
PCPD3                205.00 usec
PL3                   -1.45 dB
PL16                  12.12 dB
SFO3           60.8218948 MHz

====== GRADIENT CHANNEL =====
GPNAM1           SINE.100
GPNAM2           SINE.100
GPNAM3           SINE.100
GPX1                  0.00 %
GPX2                  0.00 %
GPX3                  0.00 %
GPY1                  0.00 %
GPY2                  0.00 %
GPY3                  0.00 %
GPZ1                 50.00 %
GPZ2                 60.00 %
GPZ3                 70.00 %
P16                1000.00 usec

F1 - Acquisition parameters
ND0                      2
TD                     256
SFO1           60.82189 MHz
FIDRES         23.760645 Hz
SW                100.009 ppm
FnMODE       States-TPPI

F2 - Processing parameters
SI                    2048
SF           600.1299576 MHz
WDW                  QSINE
SSB                      2
LB                    0.00 Hz
GB                       0
PC                    1.00

F1 - Processing parameters
SI                    1024
MC2          States-TPPI
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SSB                      2
LB                    0.00 Hz
GB                       0
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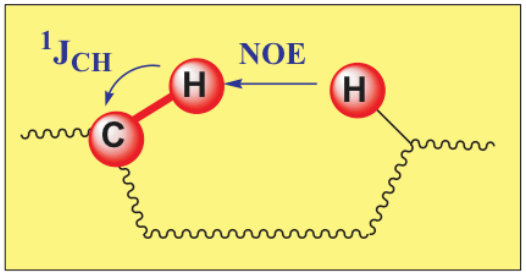
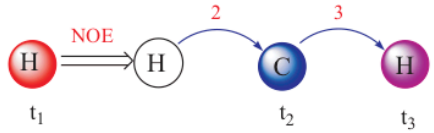
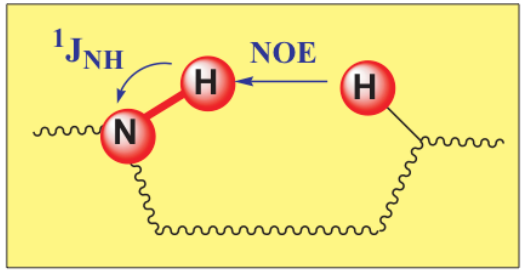
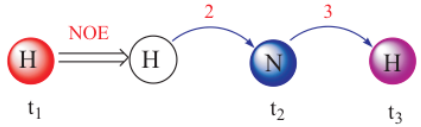
Filtered NOESY & NOESY HSQC



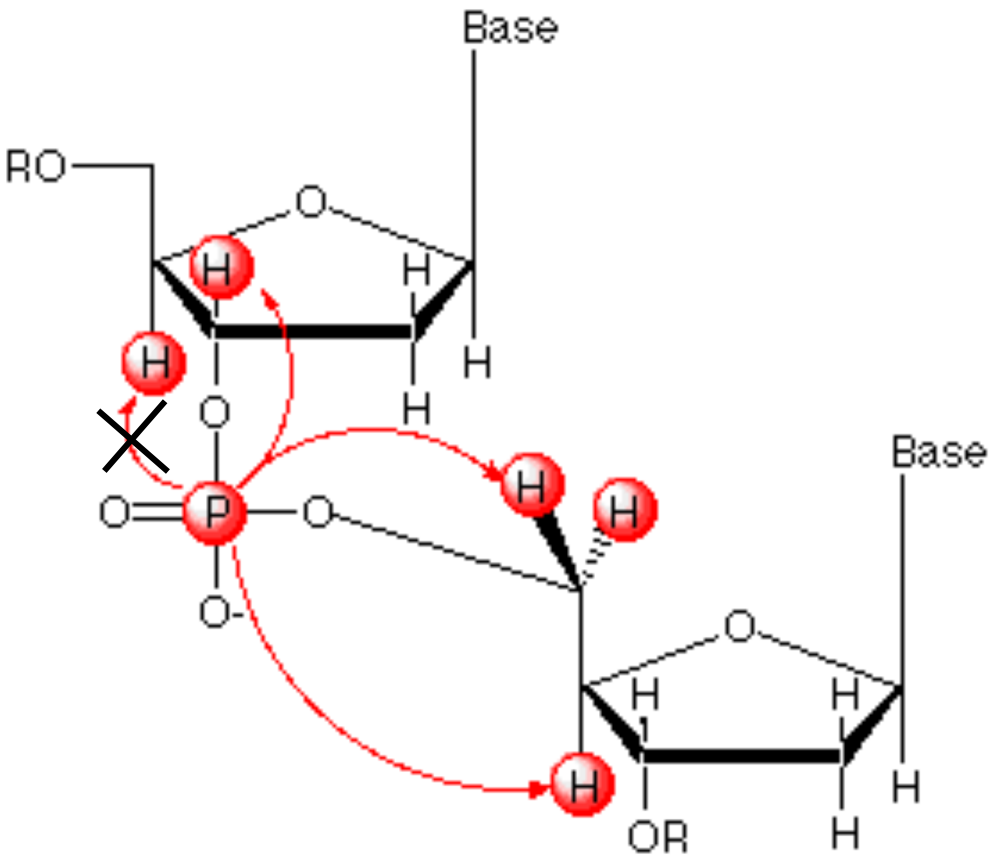




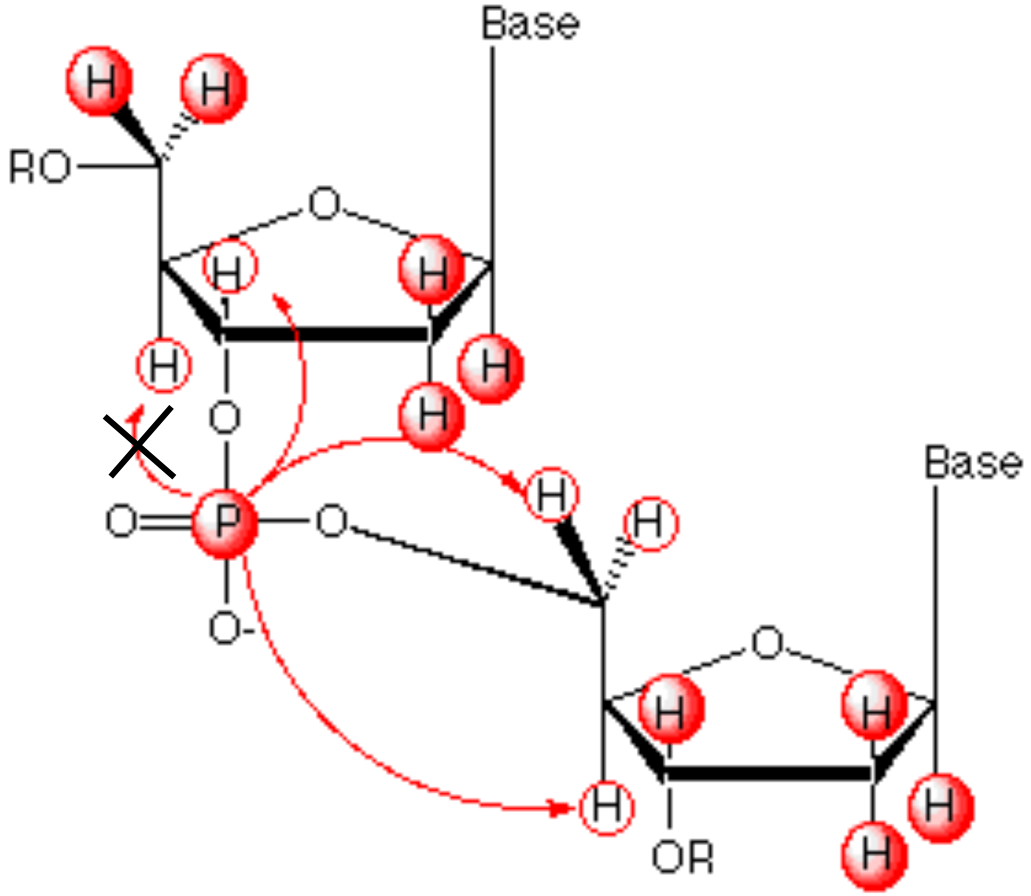


^{31}P NMR

HEHAHA



HEHAHA-TOCSY



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