

Heteronuclear NMR of Nucleic Acids

In most cases, requires samples isotopically enriched by ^{13}C and ^{15}N (except for HSQC, HMQC)

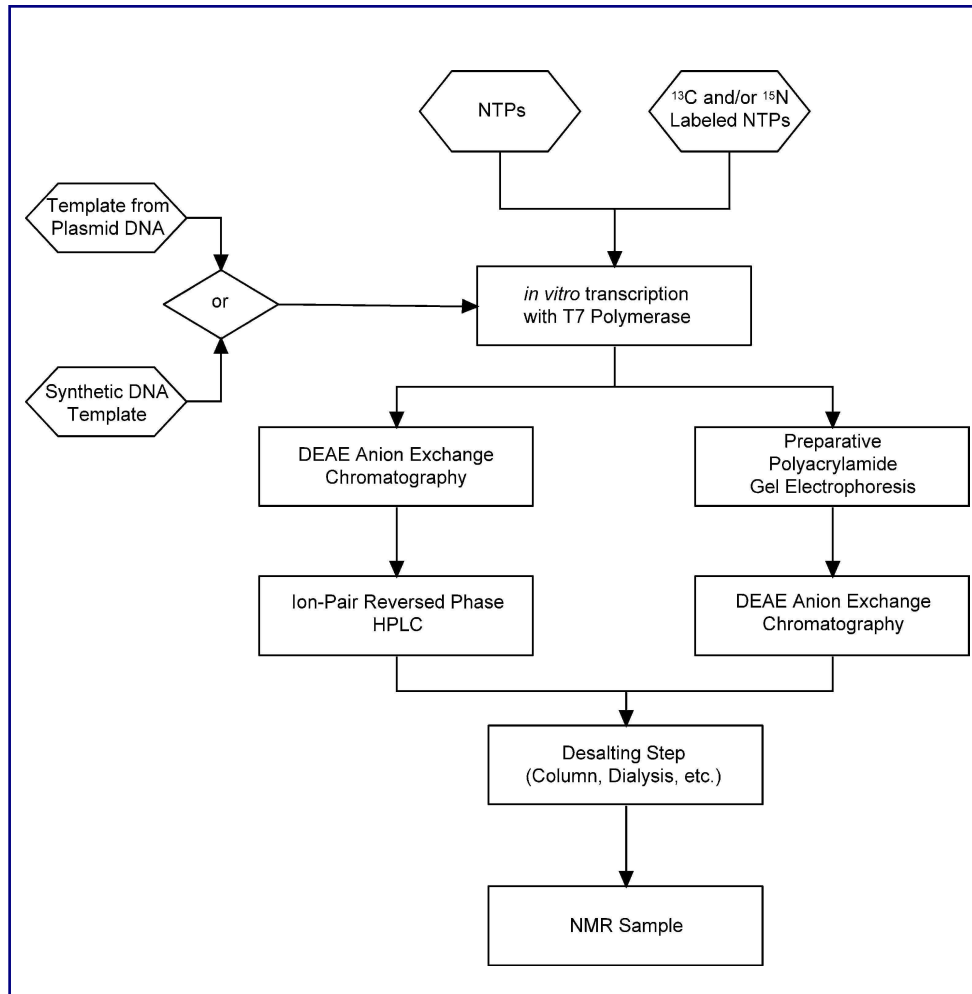
Assignment uses NOE or through-bond experiments

Traditional constraints (NOEs, J-couplings)

Novel constraints (RDCs, residual CSA)

Studies of intramolecular dynamics

Sample preparation



Labeled NTPs

Available commercially

Can be grown in cells

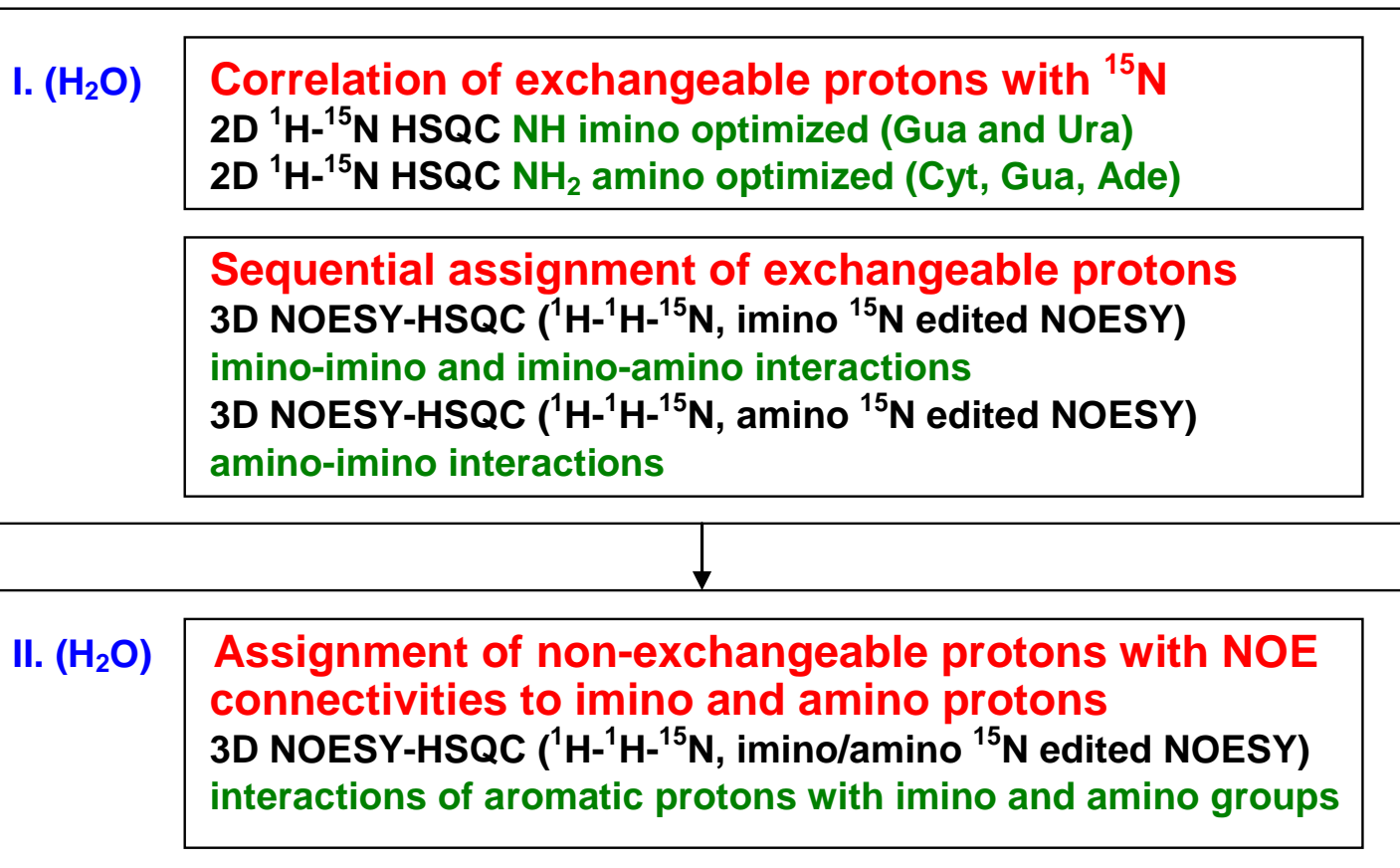
E. Coli

¹³C-glucose, ¹⁵N-ammonium

M. Methylotronus

¹³C-methanol, ¹⁵N-ammonia

Assignment procedure for labeled NA NOE based (I)



Assignment procedure for labeled NA NOE based (II)

III. ($^2\text{H}_2\text{O}$) **Identification of hydrogen and carbon atoms in sugars**

2D ^1H - ^{13}C CT-HSQC identification of H-C pairs

3D HCCH-COSY identification of neighboring C-H groups

3D HCCH-RELAY H1'-C2'/C3' correlation

3D HCCH-TOCSY H1'-C2'/C3'/C4'/C5' correlation

Identification of hydrogen and carbon atoms in bases

2D ^1H - ^{13}C CT-HSQC identification of H-C pairs

2D/3D HCCH-COSY H5-H6 and C5-C6 correlations in pyrimidines

Sequential assignment

3D NOESY-HSQC (^1H - ^1H - ^{13}C), H6/8-H1', H6/8-H2' correlations

IV. ($^2\text{H}_2\text{O}$) **Assignment of ^{31}P resonances**

^1H - ^{31}P HETCOR/heteroTOCSY

Assignment procedure for labeled NA

Through bond correlations (I)

I. (H₂O) **Correlation of exchangeable protons with ¹⁵N**
2D ¹H-¹⁵N HSQC NH imino optimized (Gua and Ura)
2D ¹H-¹⁵N HSQC NH₂ amino optimized (Cyt, Gua, Ade)



II. (H₂O) **Correlation of imino and amino protons with non-exchangeable base protons**
HCCNH-TOCSY / HNCCH-TOCSY



Assignment procedure for labeled NA Through bond correlations (II)

III. ($^2\text{H}_2\text{O}$)

Correlation of non-exchangeable protons with ^{13}C

2D ^1H - ^{13}C CT-HSQC identification of H-C pairs
3D HCCH-COSY identification of neighboring C-H groups
3D HCCH-TOCSY H1'-C2'/C3'/C4'/C5' correlation

Identification of hydrogen and carbon atoms in bases

2D ^1H - ^{13}C CT-HSQC identification of H-C pairs
2D/3D HCCH-COSY H5-H6 and C5-C6 correlations in pyrimidines
HCCH-TOCSY / ^1H - ^{13}C HMBC H2-H8 correlations in Ade

Sugar-base correlations

$\text{H}_s\text{C}_s\text{N}$ and $\text{H}_b\text{C}_b\text{N}$
 $\text{H}_s\text{C}_s\text{NC}_b\text{H}_b$ / $\text{H}_s\text{C}_s\text{NH}_b$

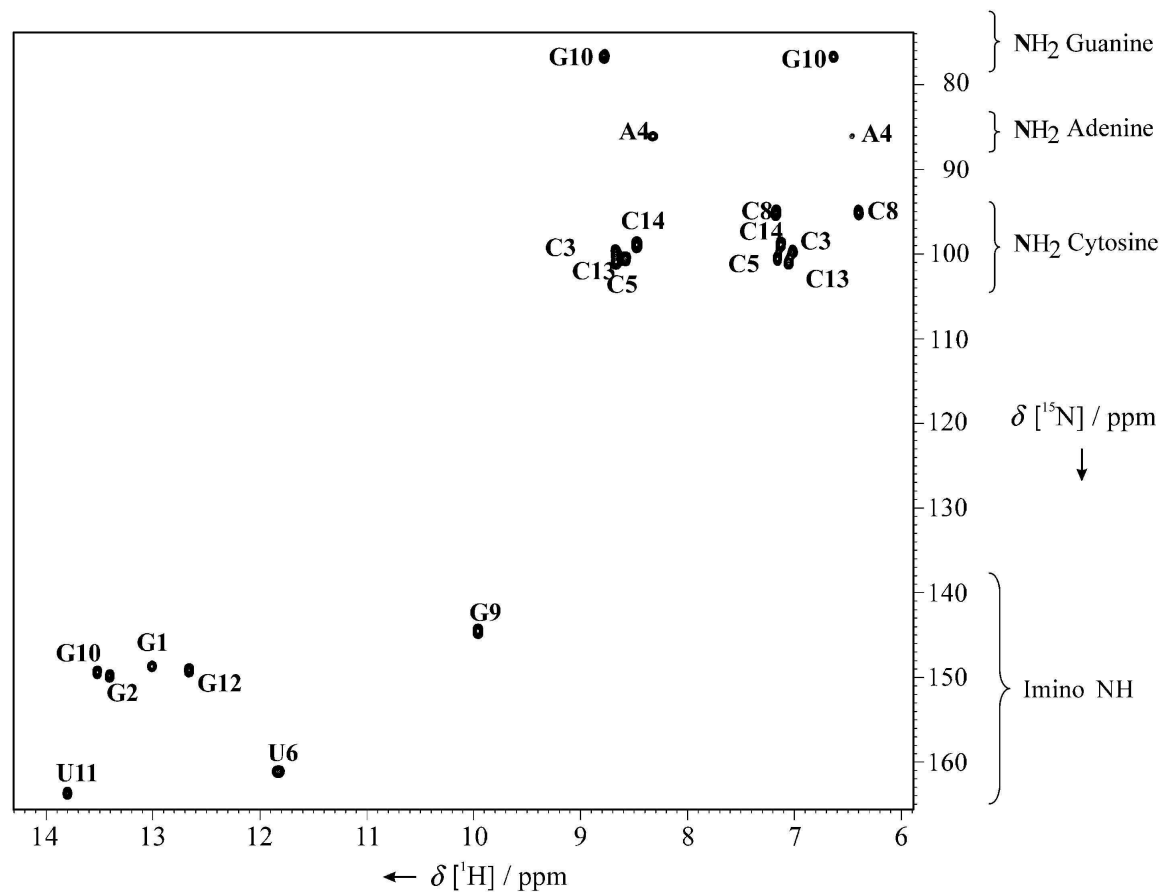
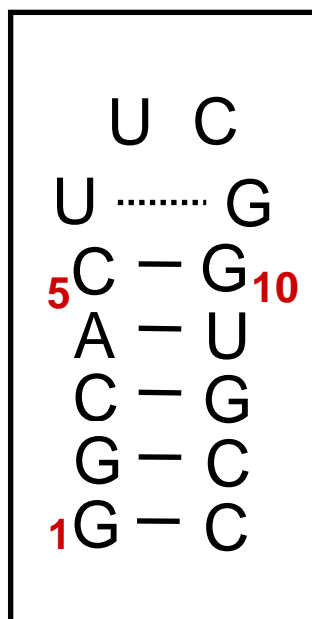


IV. ($^2\text{H}_2\text{O}$)

Sequential assignment of ^{31}P resonances across the sugar-phosphate backbone

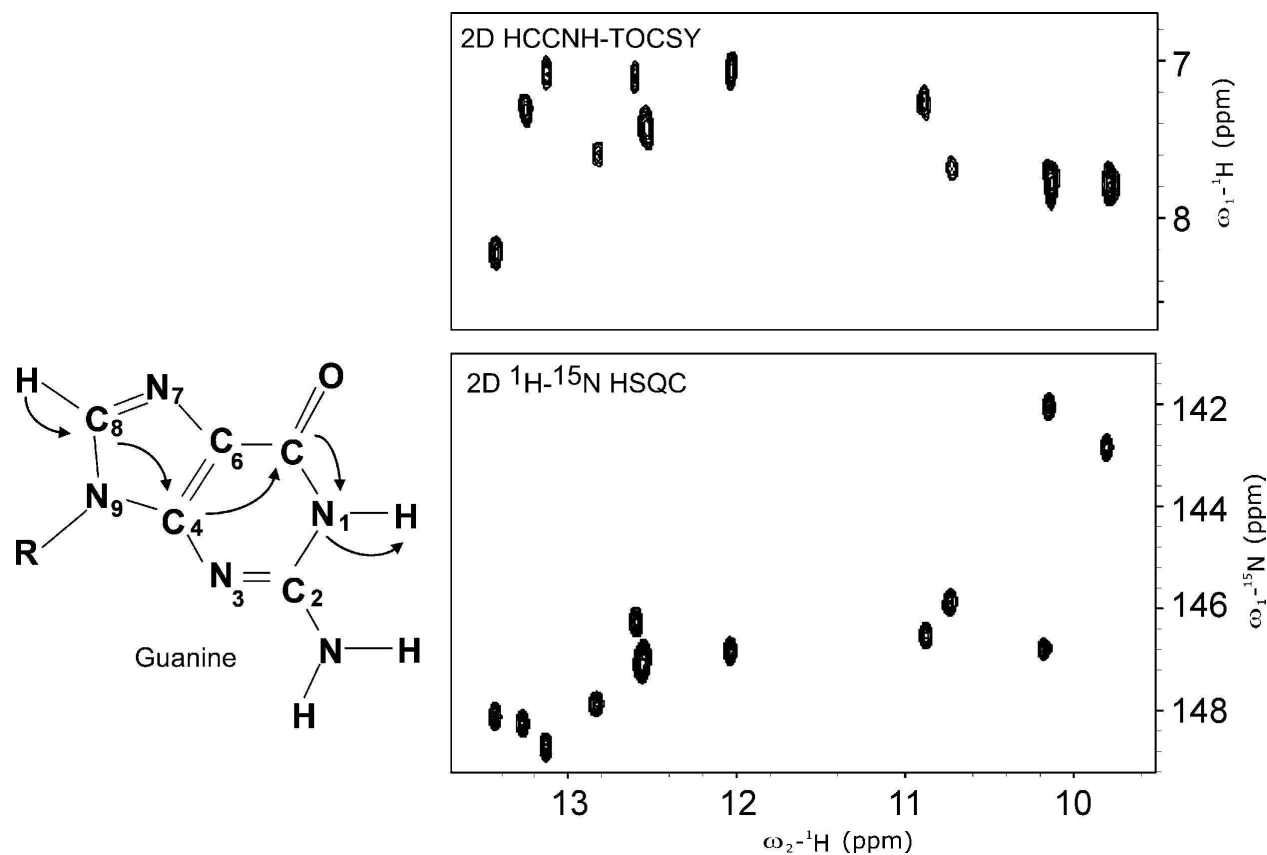
HCP / PCH / PCCH-TOCSY / HPHCH

Correlation of exchangeable protons with ^{15}N



Gradient sensitivity enhanced HSQC
 Kay, Keifer, Saarinen, JACS 1992.

Correlation of exchangeable and non-exchangeable protons



HCCNH-TOCSY, Fiala et al. JACS 1996, Sklenar et al. J. Biomol. NMR 1996.

Correlation across the hydrogen bond HNN-COSY experiment

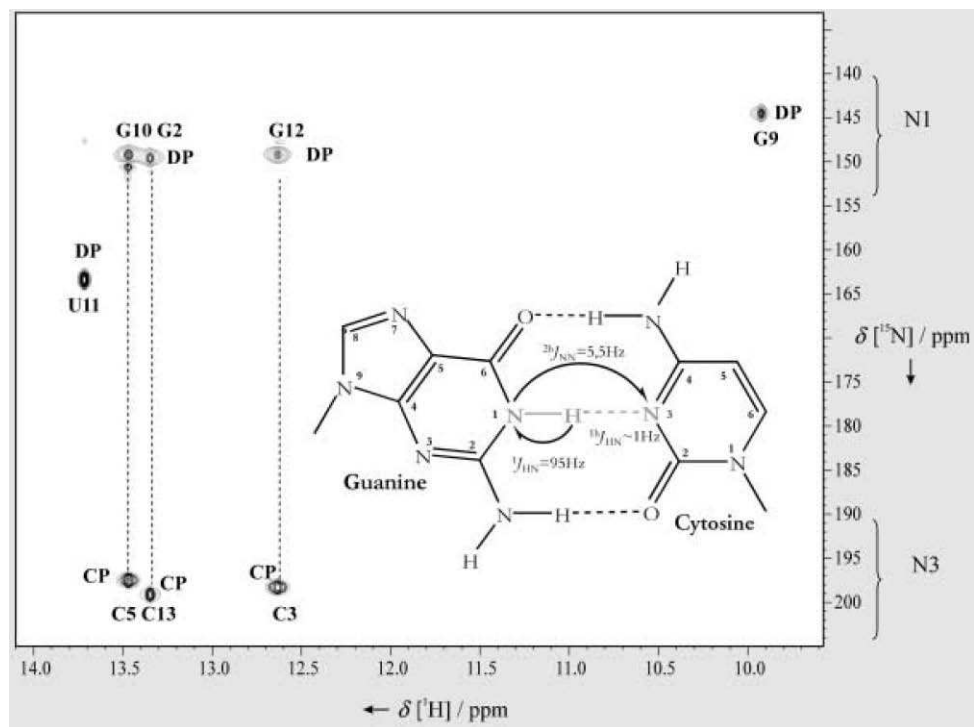
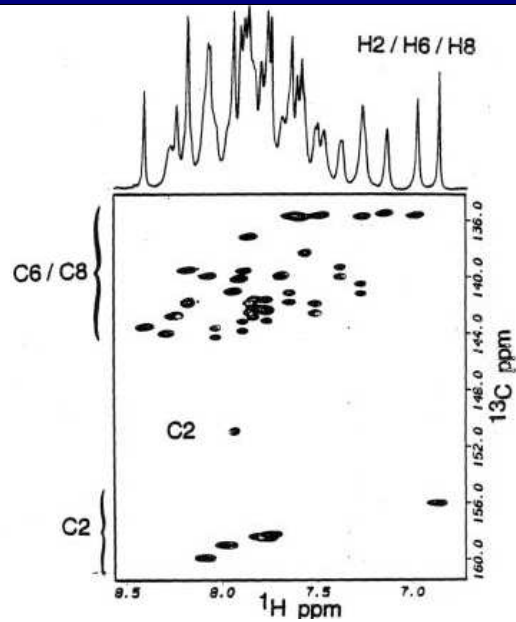


Figure 11. HNN-COSY experiment at 700 MHz and 298 K. On the right side, a Watson-Crick G:C base pair is depicted. The coupling constants are annotated. In this experiment, N1 of guanine can be correlated with the quaternary nitrogen atom of the cytosine residue.

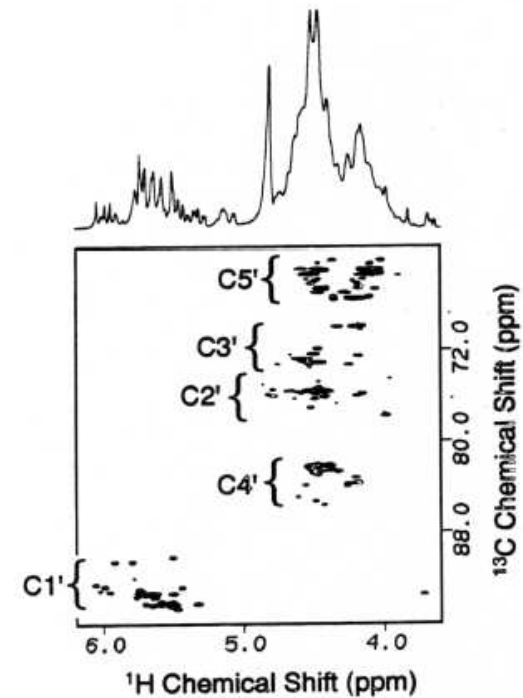
Dingley and Grzesiek, JACS 1998

Identification of hydrogen and carbon atoms in bases and sugars

base

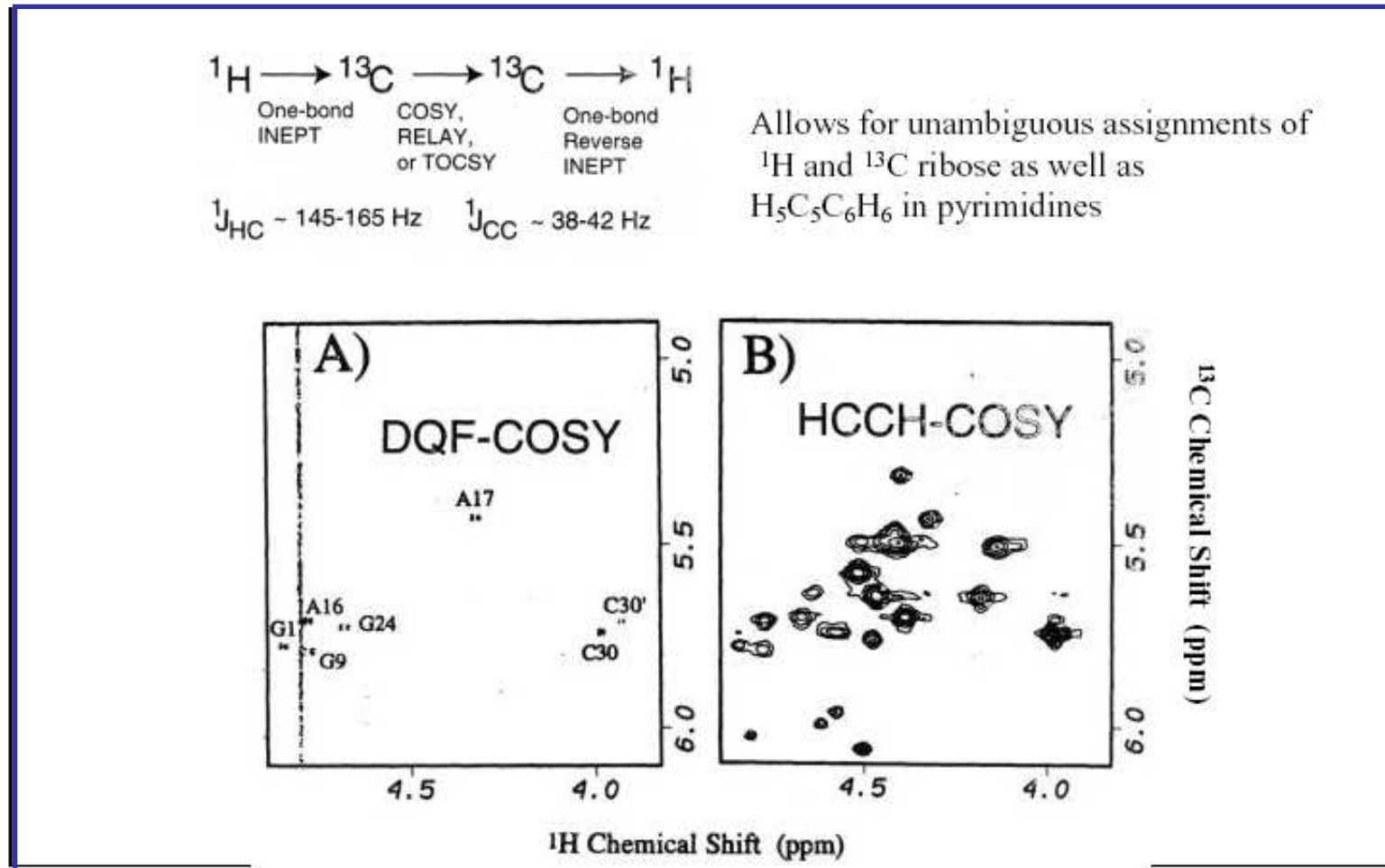


sugar

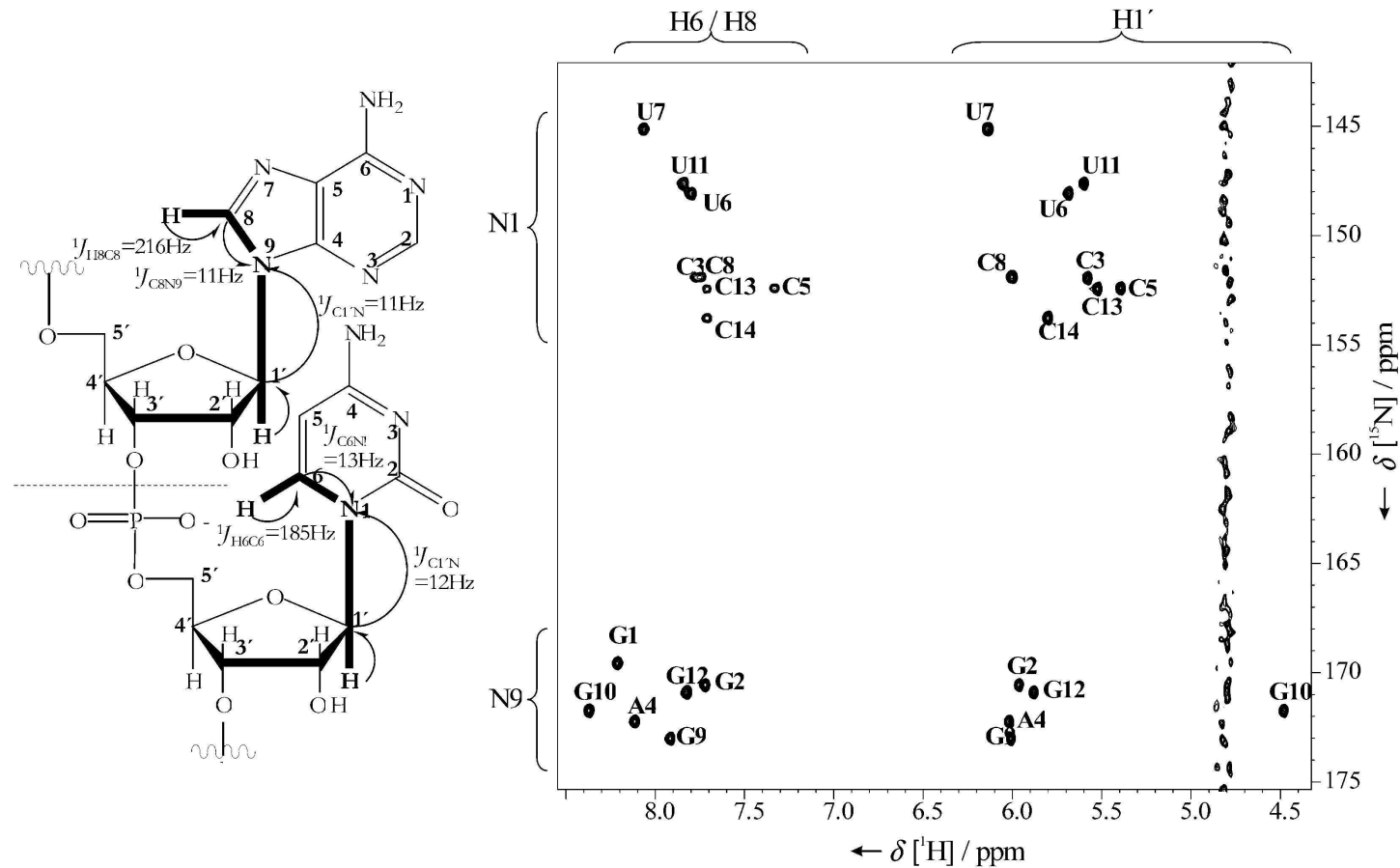


Constant-time ^1H - ^{13}C HSQC experiment

Assignment of non-exchangeable protons: HCCH-type experiments

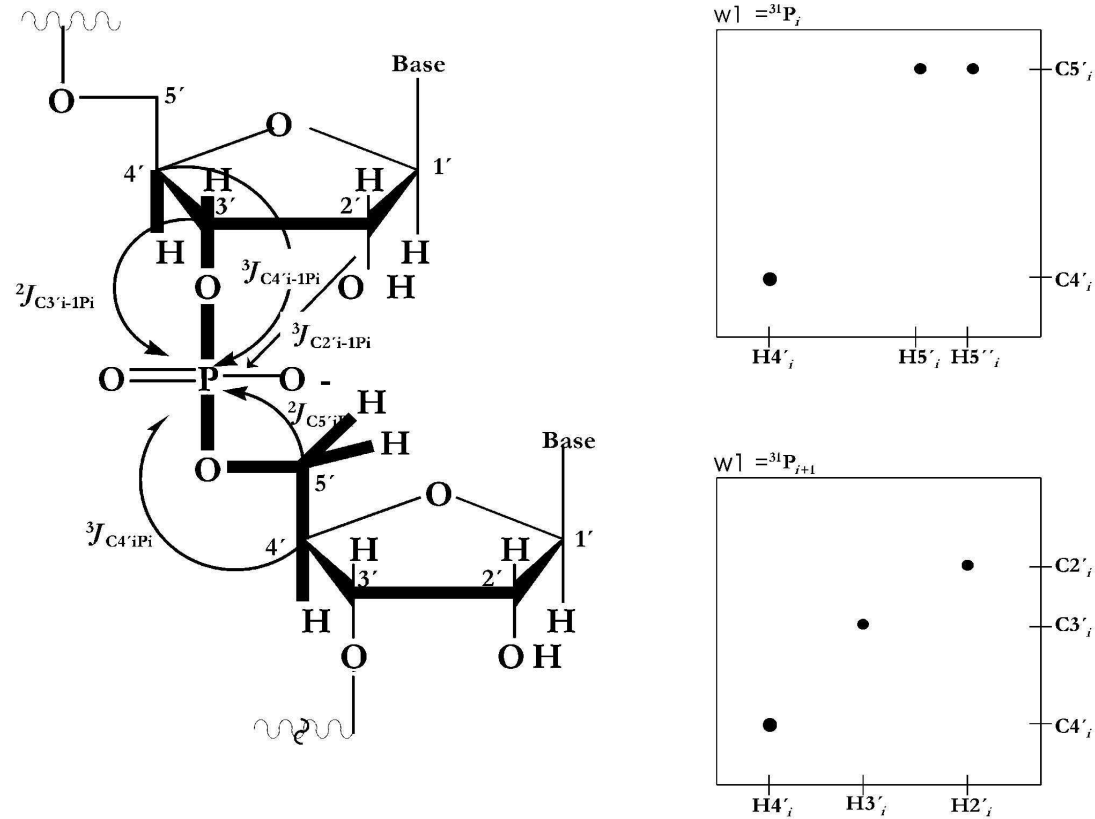


Sugar to base correlation – the HCN experiment

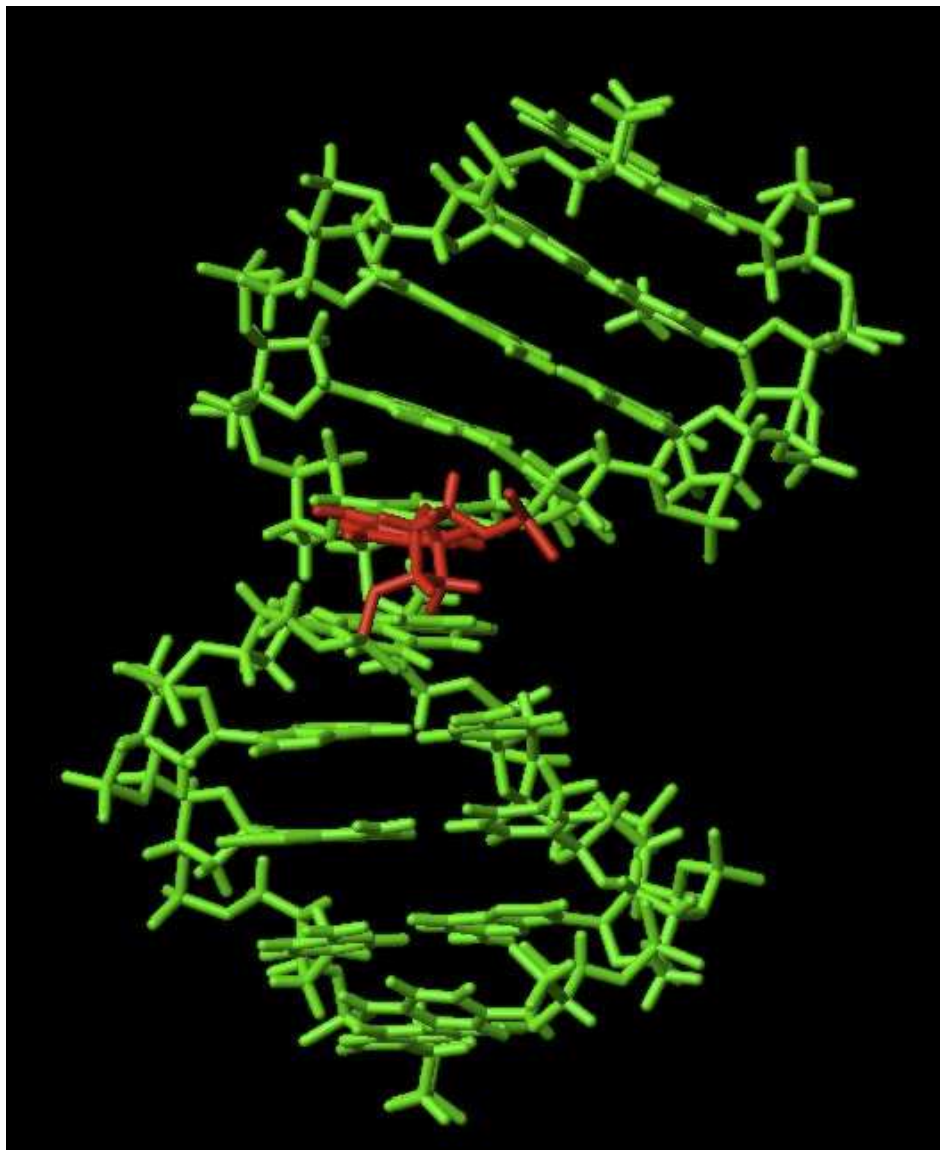


Sklenar et al., J. Biomol. NMR 1993, 1994, Fiala et al., J. Biomol. NMR 1998, 2000.

Sugar to phosphate correlation – the HCP experiment



Dipolar couplings



- Dipolar couplings add to J couplings
- 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

