Heteronuclear NMR of Nucleic Acids

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

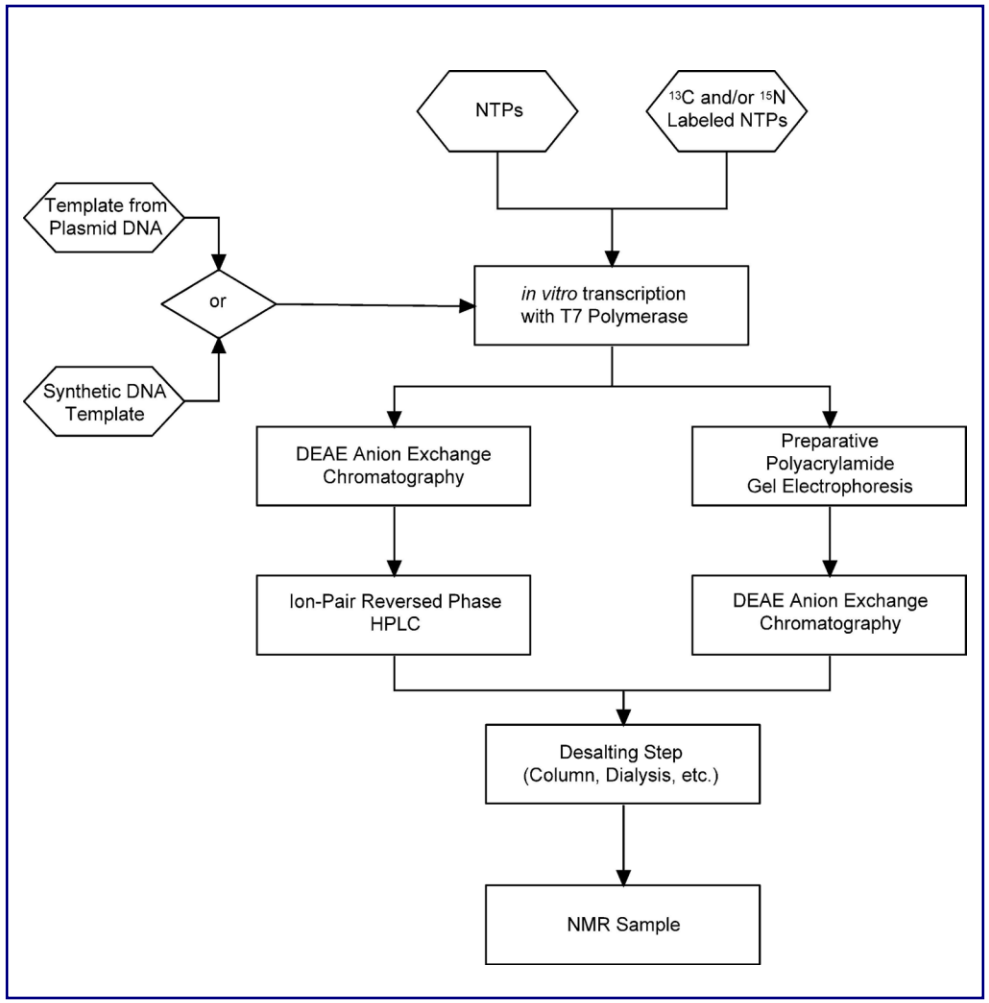
Assignment uses NOE or through-bond experiments

Traditional constraints (NOEs, J-couplings)

Novel constraints (RDCs, residual CSA)

Studies of intramolecular dynamics

RNA preparation



Labeled NTPs

Available commercially

Can be grown in cells

E. Coli
^{13}C-glucose, ^{15}N-ammonium

M. Methylotropus
^{13}C-methanol, ^{15}N-ammonia

Assignment procedure for labeled NA NOE based (I)

I. (H_2O)

Correlation of exchangeable protons with ^{15}N

2D ^{1}H-^{15}N HSQC NH imino optimized (Gua and Ura)

2D ^{1}H-^{15}N HSQC NH_2 amino optimized (Cyt, Gua, Ade)

Sequential assignment of exchangeable protons

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

imino-imino and imino-amino interactions

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

amino-imino interactions

↓

II. (H_2O)

Assignment of non-exchangeable protons with NOE connectivities to imino and amino protons

3D NOESY-HSQC (^{1}H-^{1}H-^{15}N, imino/amino ^{15}N edited NOESY)

interactions of aromatic protons with imino and amino groups

↓

Assignment procedure for labeled NA
NOE based (II)

III. (^2H_2O)

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. (^2H_2O) **Assignment of ^{31}P resonances**

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

Assignment procedure for labeled NA
Through bond correlations (I)

I. (H_2O) **Correlation of exchangeable protons with ^{15}N**

2D ^{1}H-^{15}N HSQC NH imino optimized (Gua and Ura)

2D ^{1}H-^{15}N HSQC NH_2 amino optimized (Cyt, Gua, Ade)

↓

II. (H_2O) **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. (^2H_2O)

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

H_sC_sN and H_bC_bN

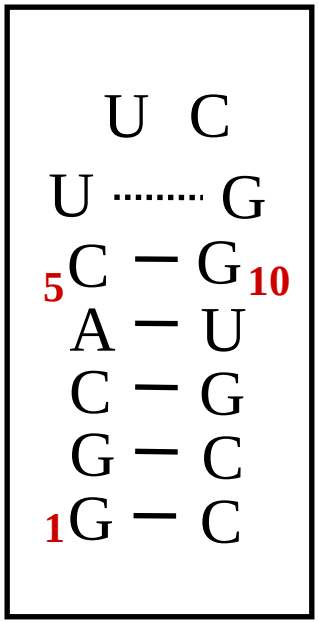
$H_sC_sNC_bH_b$ / $H_sC_sNH_b$

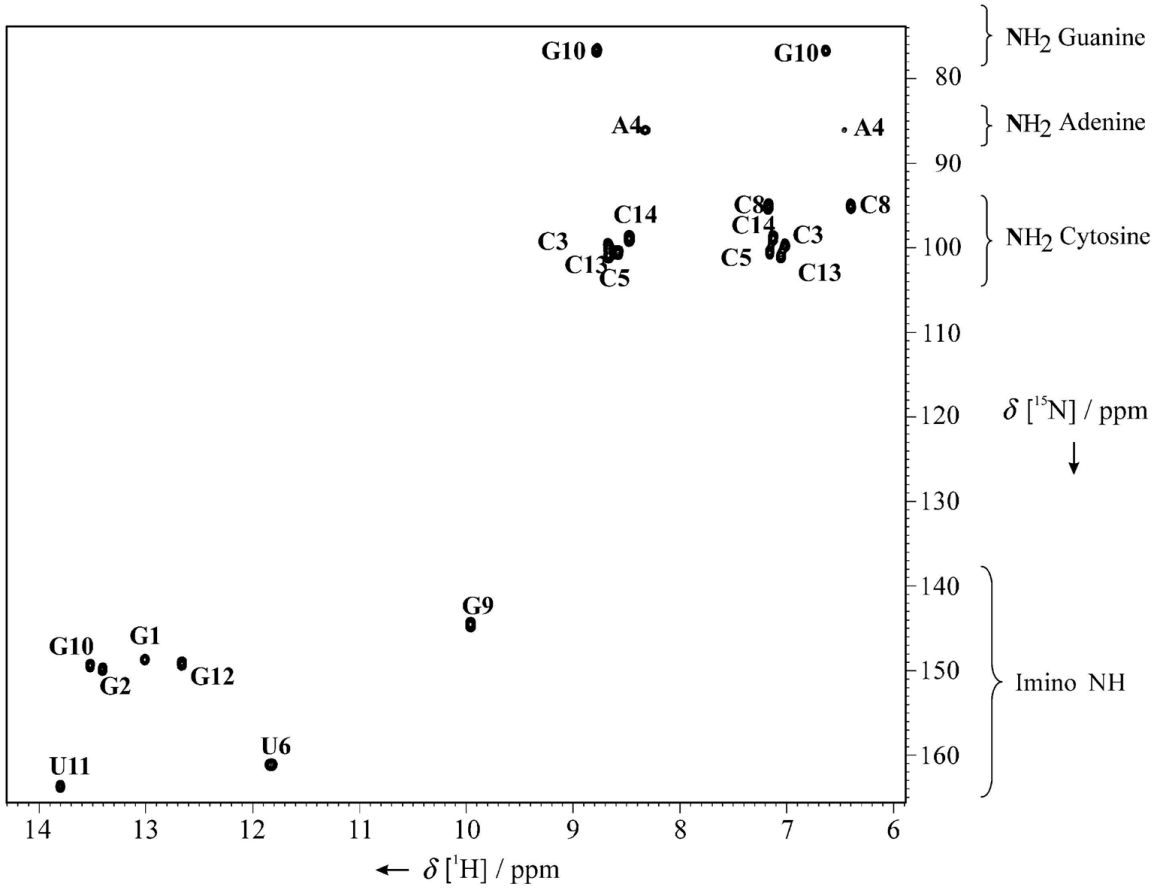
↓

IV. (^2H_2O) **Sequential assignment of ^{31}P resonances across the sugar-phosphate backbone**

HCP / PCH / PCCH-TOCSY / HPHCH

Correlation of exchangeable protons with ^{15}N



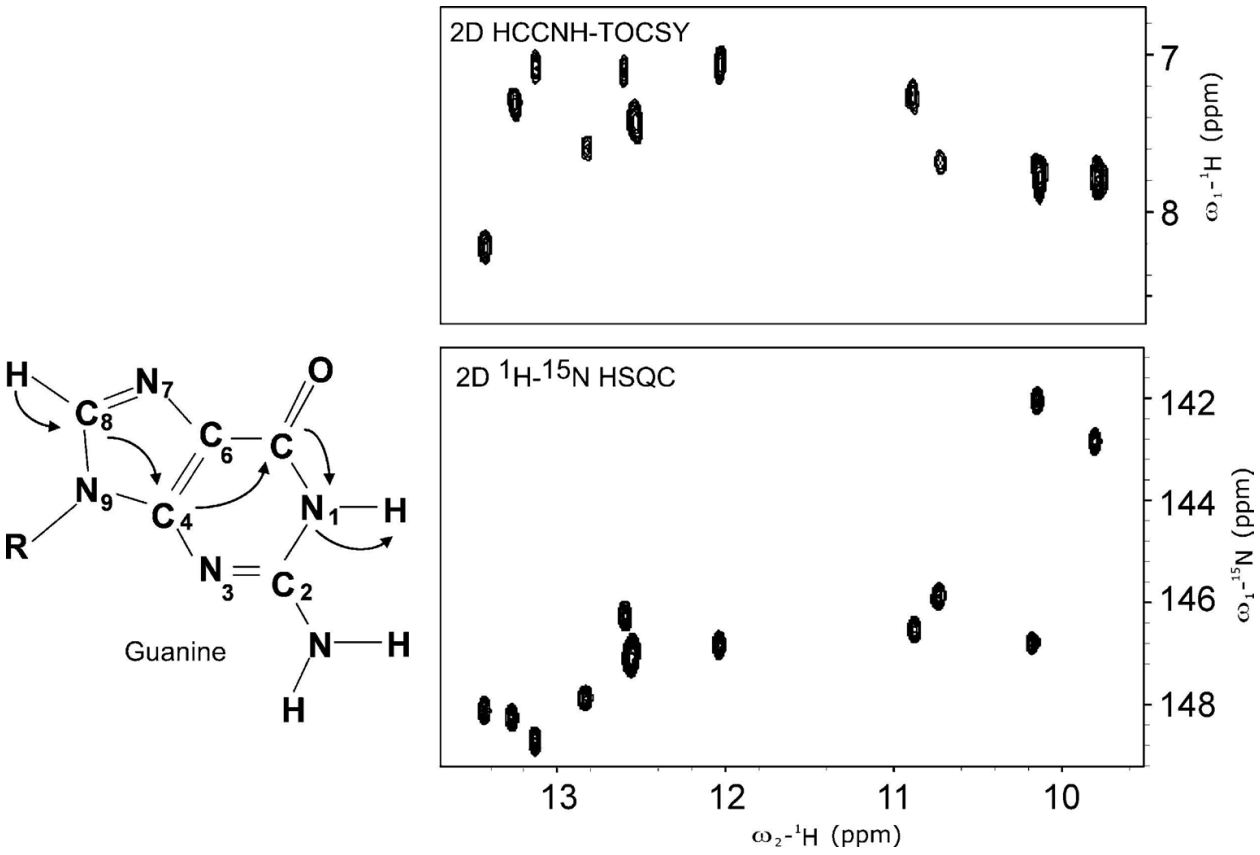


Gradient sensitivity-enhanced HSQC
Kay, Keifer, Saarinen, JACS 1992.

Assignment of imino protons in loops

Correlation of exchangeable and non-exchangeable protons

Labeled samples – HCCNH-TOCSY

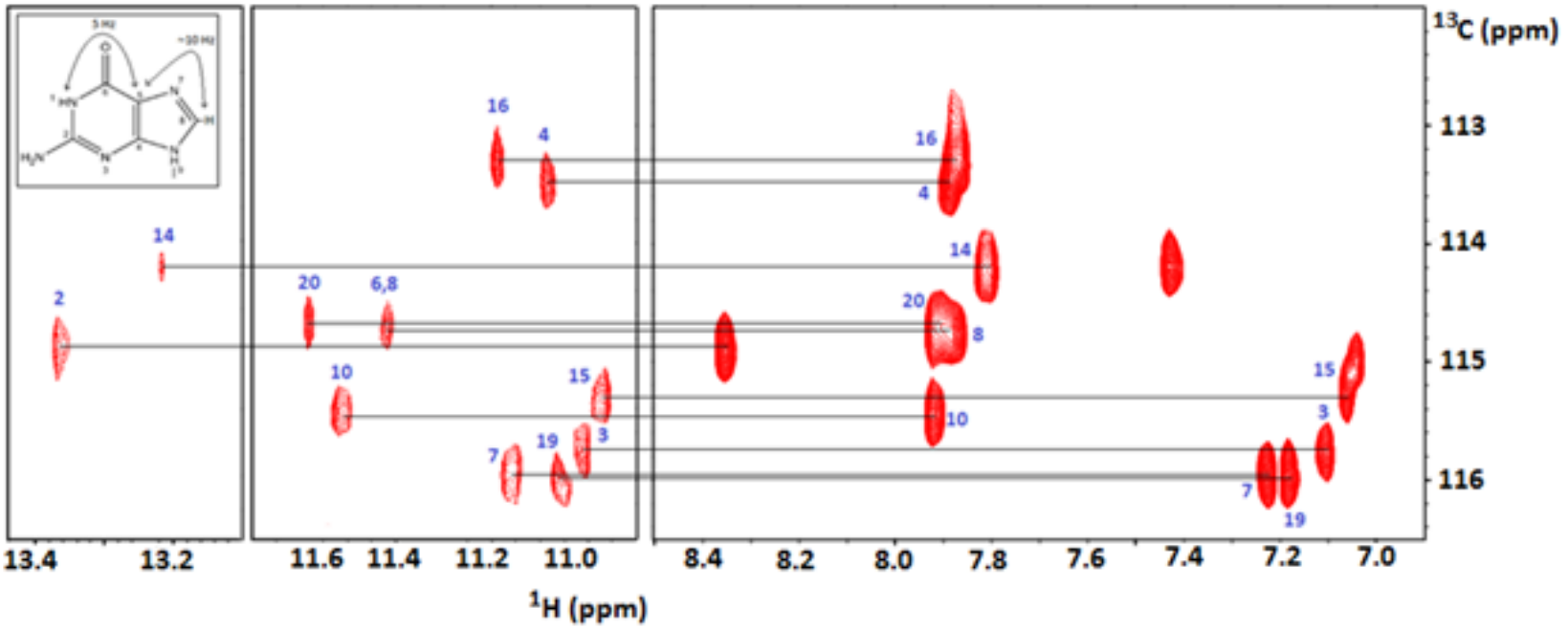


Fiala et al. JACS 1996, Sklenar et al. J. Biomol. NMR 1996.

Assignment of imino protons in loops

Correlation of exchangeable and non-exchangeable protons

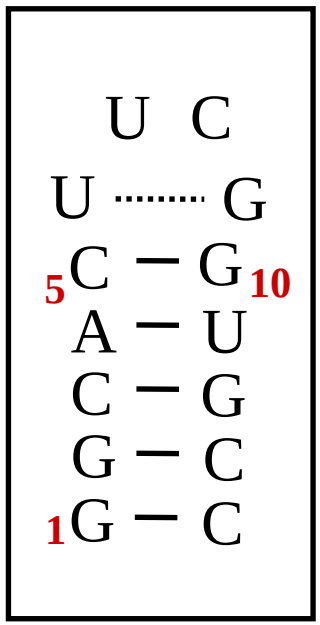
Unabeled samples – JRHMBC

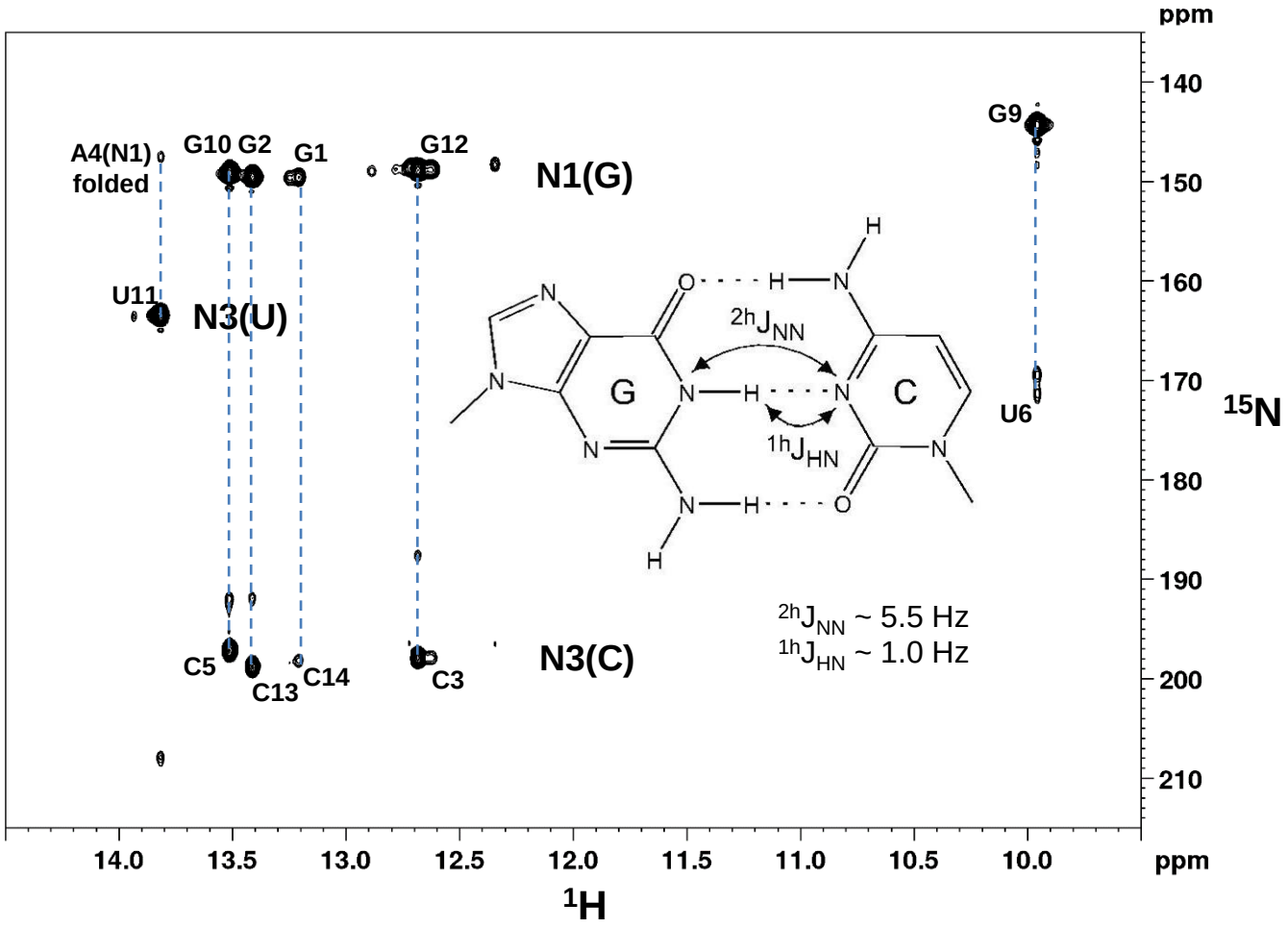


"Green Monkey" 5'-CGGGCGGGCGCGAGGGAGGGC-3' (21 nt)

Phan, Journal of Biomolecular NMR, 16: 175–178, 2000.

Correlation across the hydrogen bond
HNN-COSY experiment



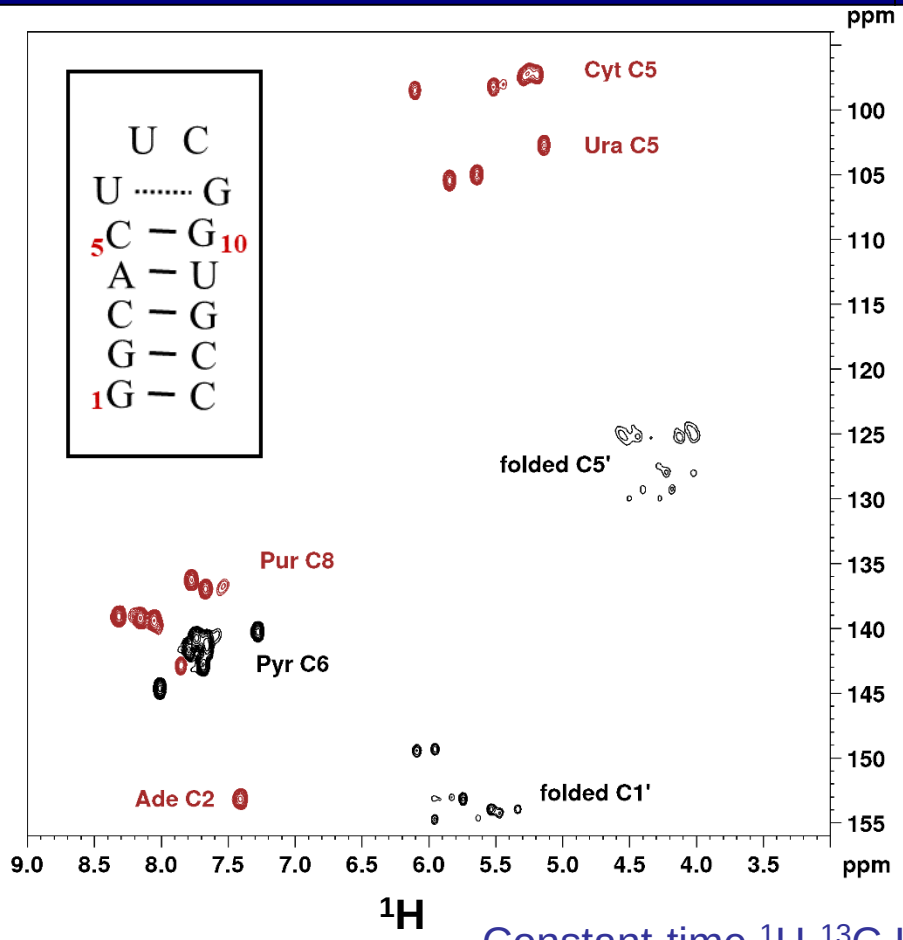


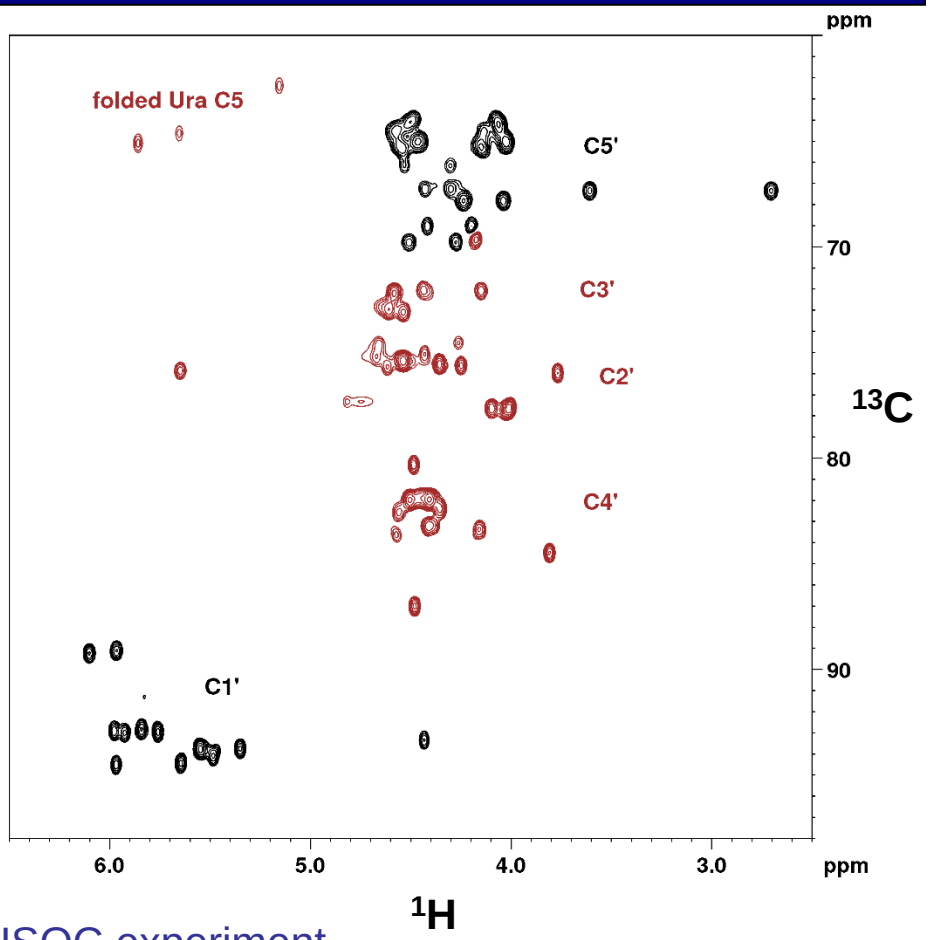
Dingley and Grzesiek, JACS 1998

Identification of hydrogen and carbon atoms in bases and sugars

base

sugar





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

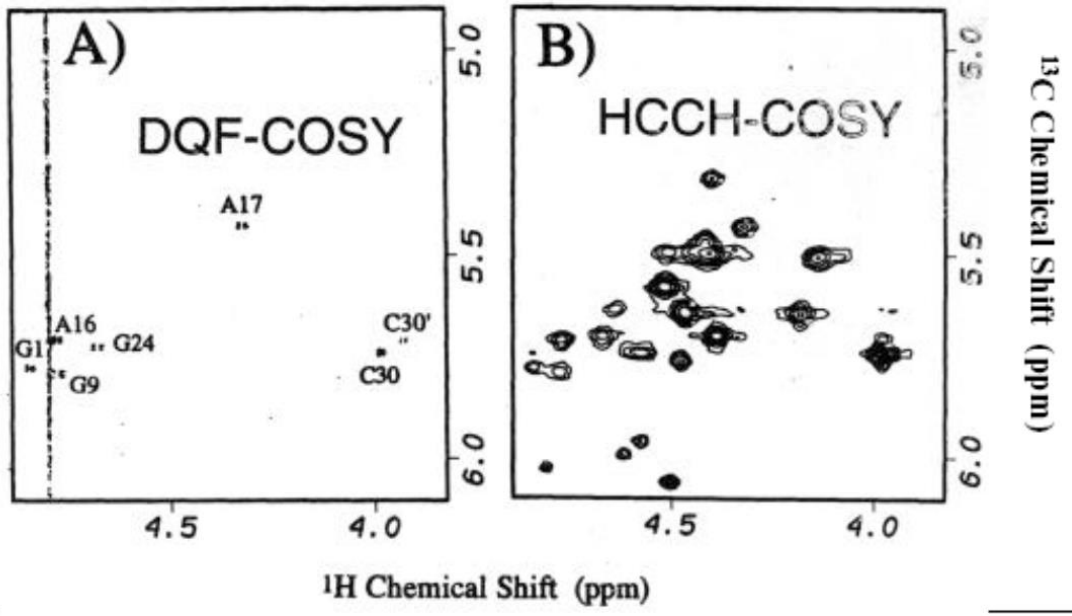
Assignment of non-exchangeable protons: HCCH-type experiments

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

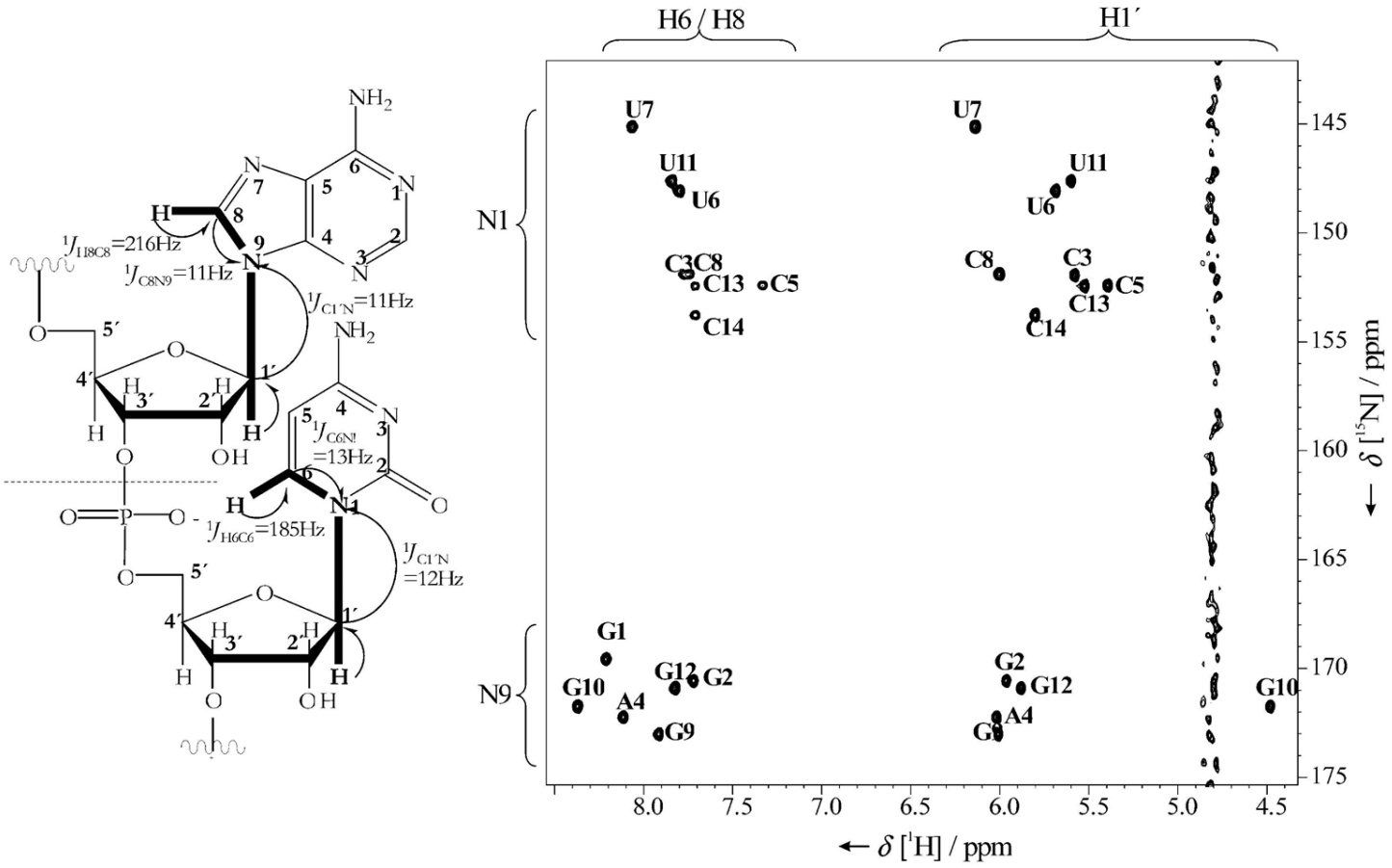
One-bond INEPT — COSY, RELAY, or TOCSY — One-bond Reverse INEPT

$^1J_{HC}$ ~ 145-165 Hz $^1J_{CC}$ ~ 38-42 Hz

Allows for unambiguous assignments of ^1H and ^{13}C ribose as well as $H_5C_5C_6H_6$ in pyrimidines

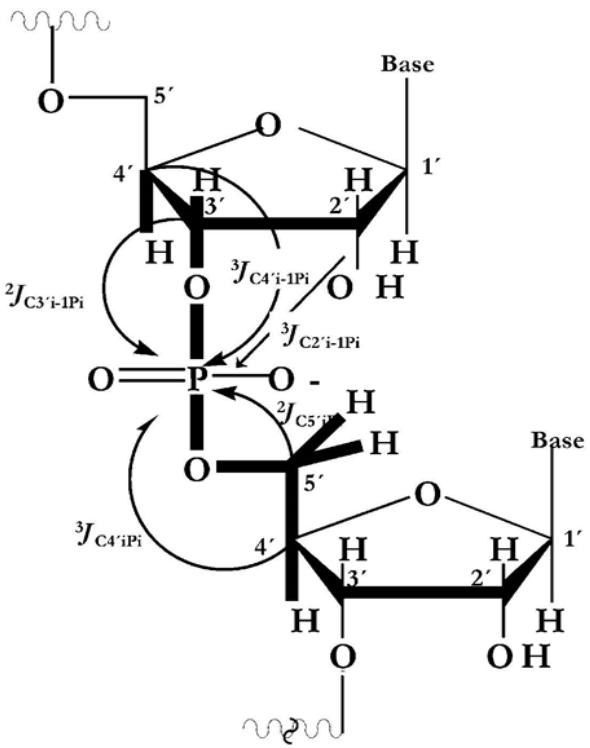


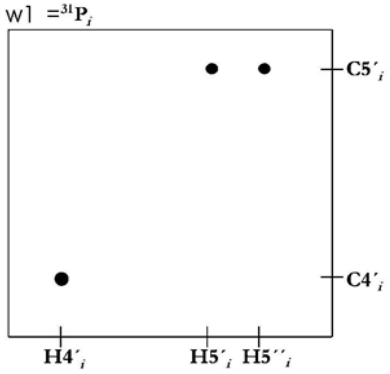
Sugar to base correlation – the HCN experiment

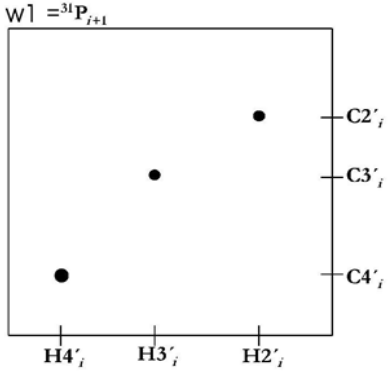


Sklenar et al., J. Biomol. NMR 1993, 1994, Fiala et al., J. Biomol. NMR 1998, 2000.

Sugar to phosphate correlation – the HCP experiment

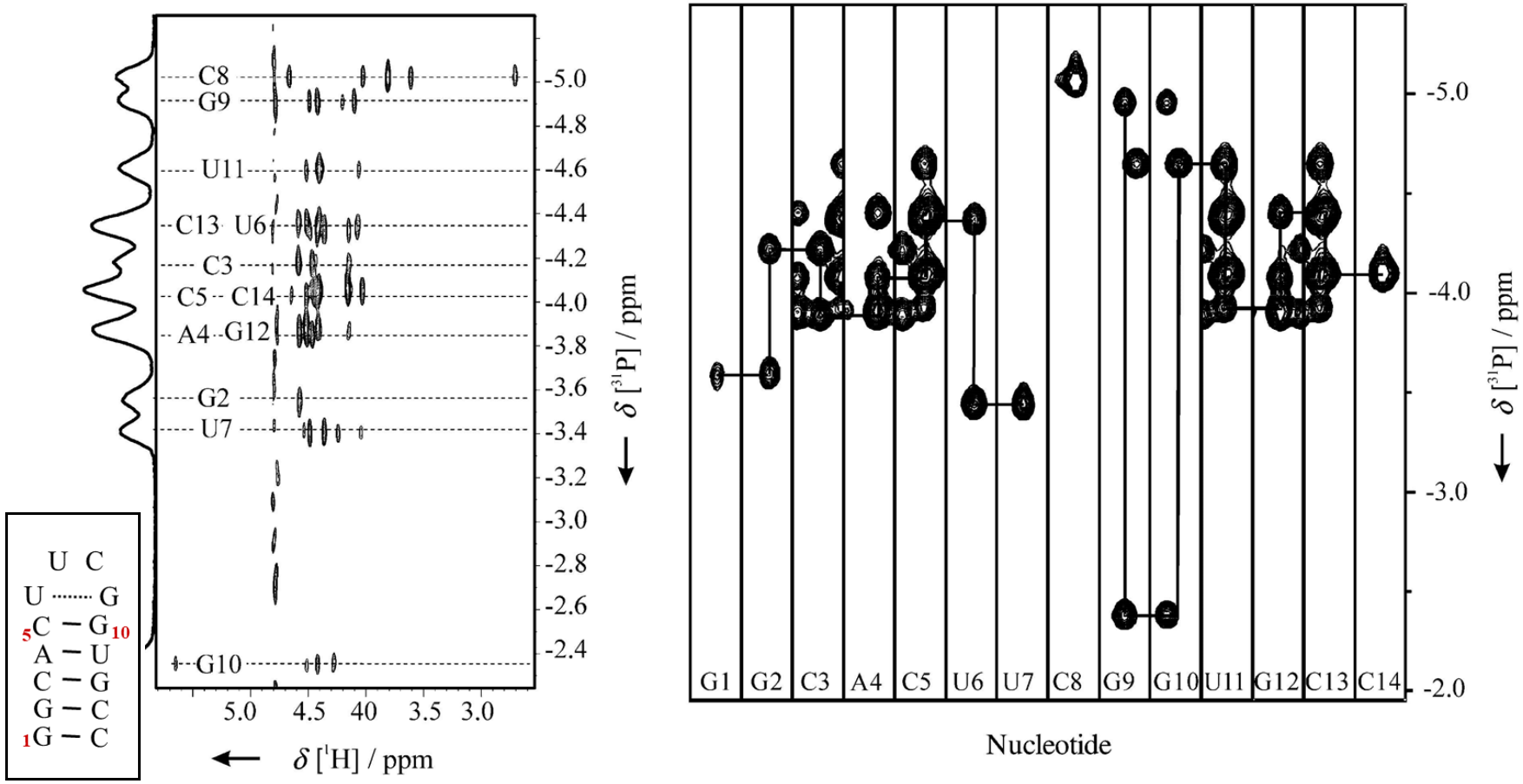




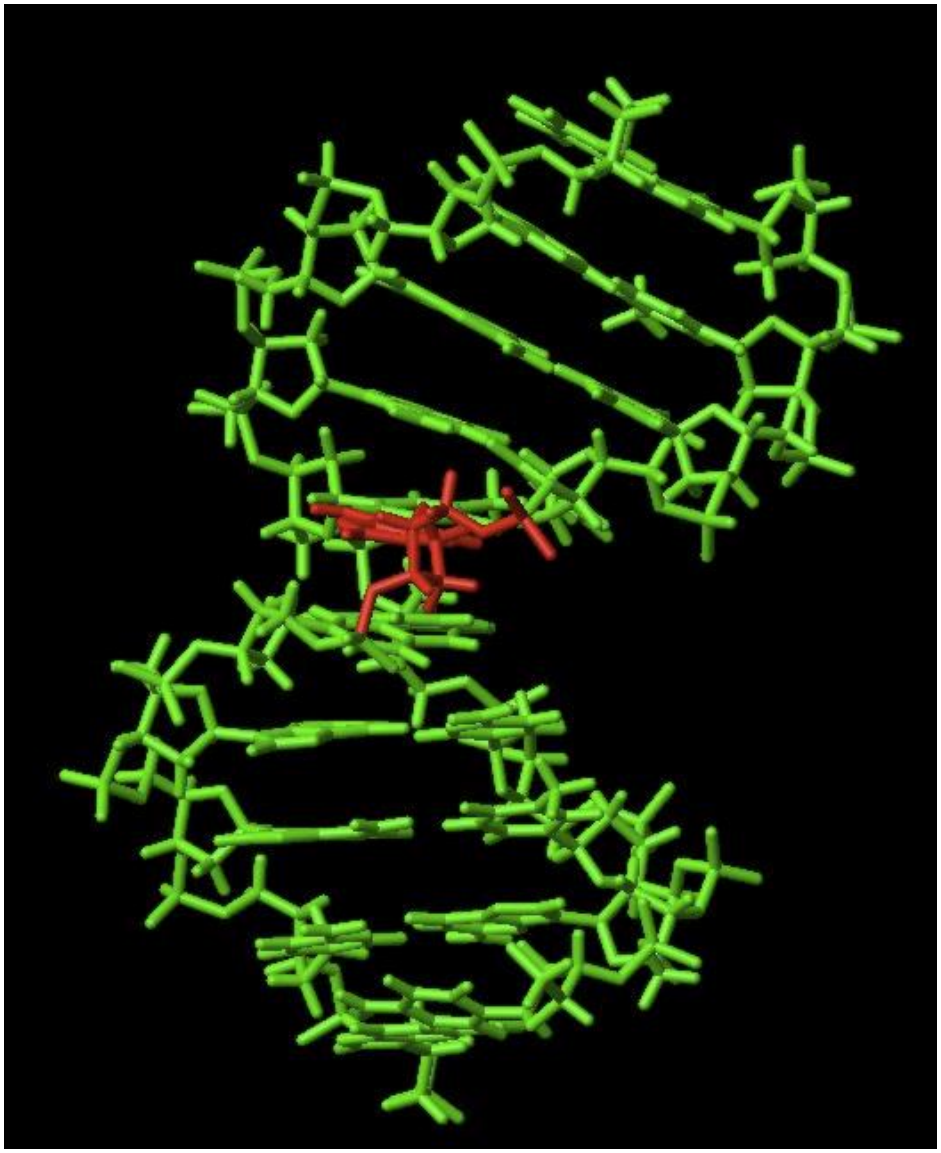


Sugar to phosphate correlation – the HCP experiment

real spectra – nt-14 RNA with UUCG loop



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