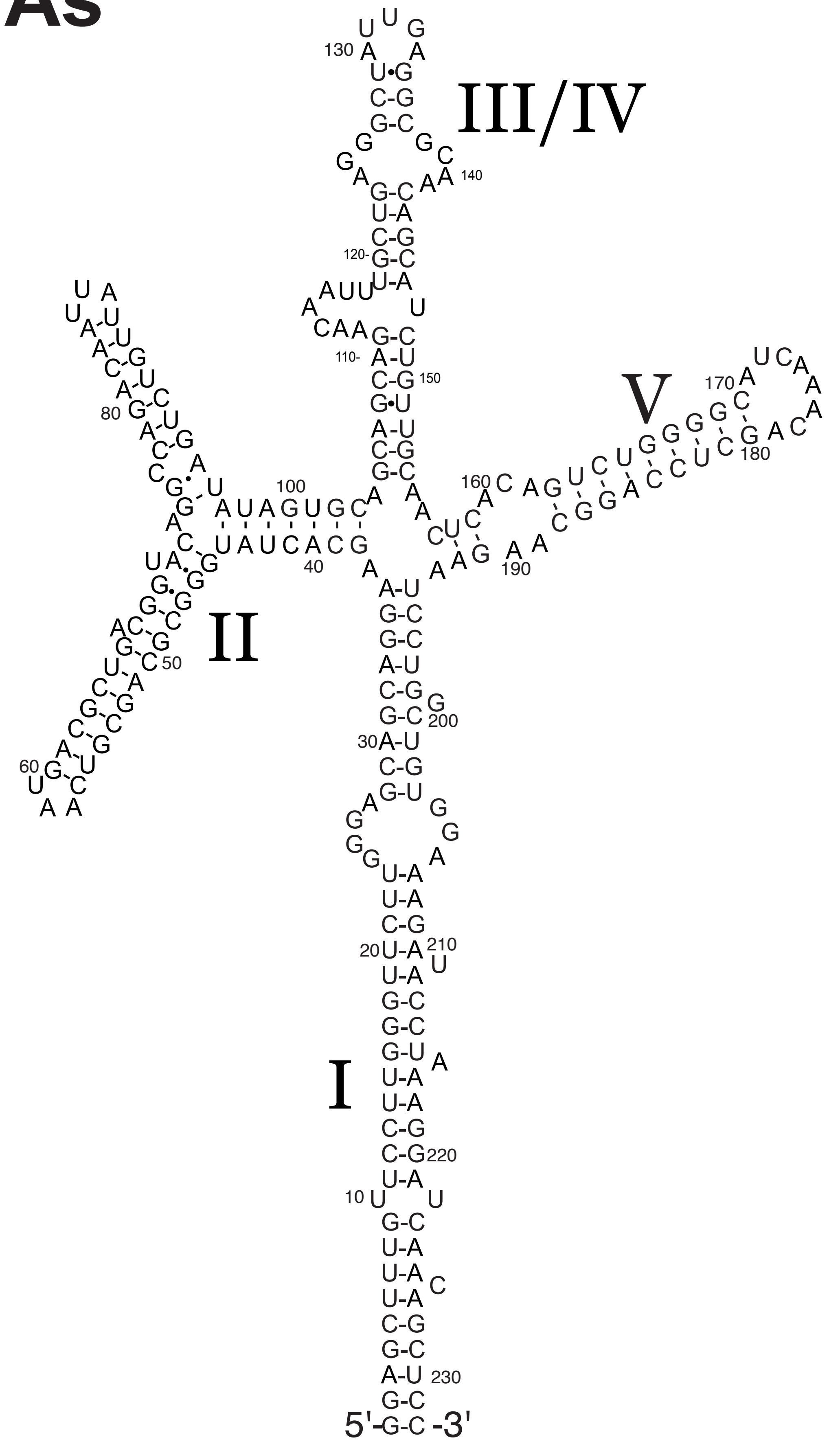
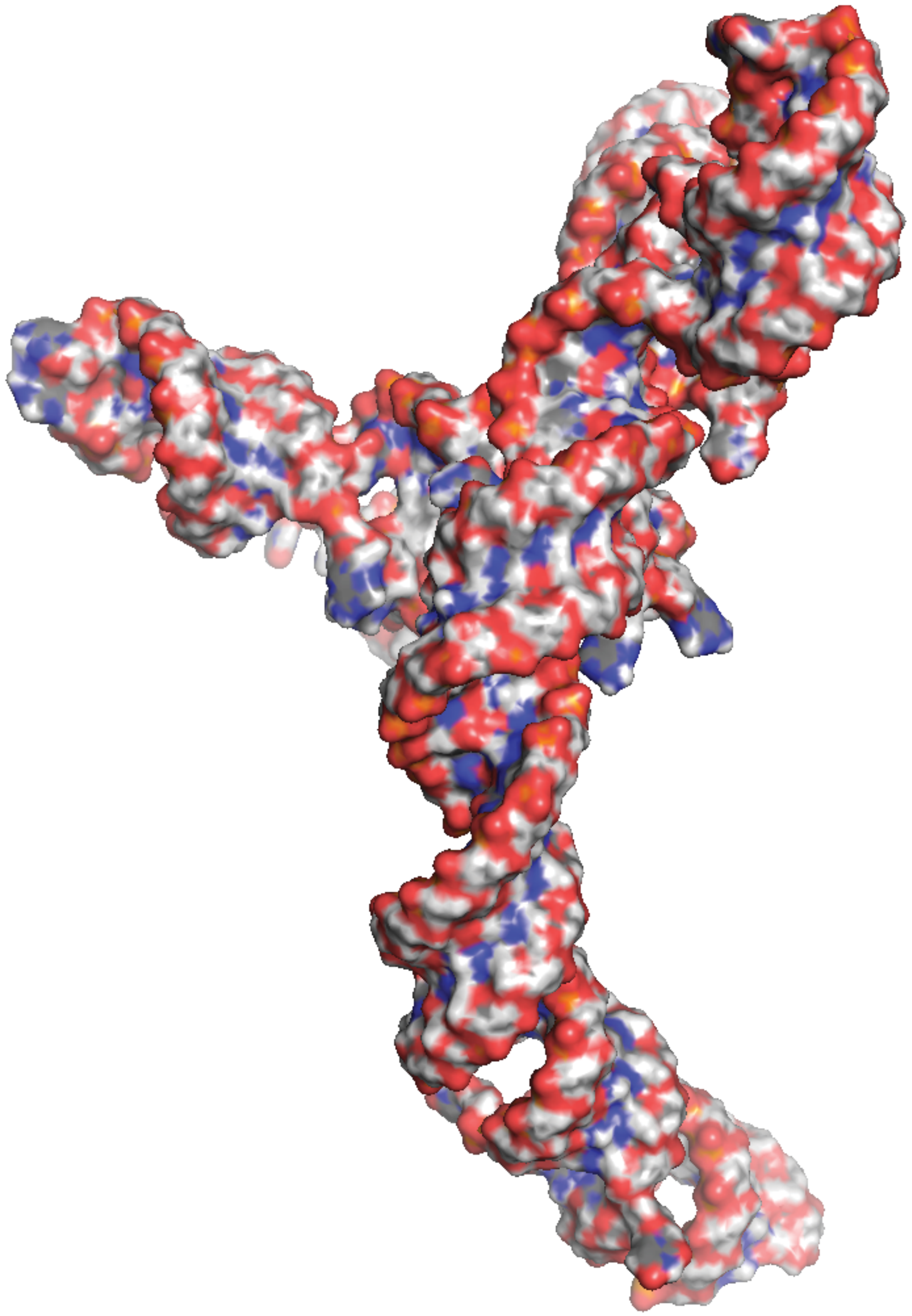
Structure calculation of large RNAs

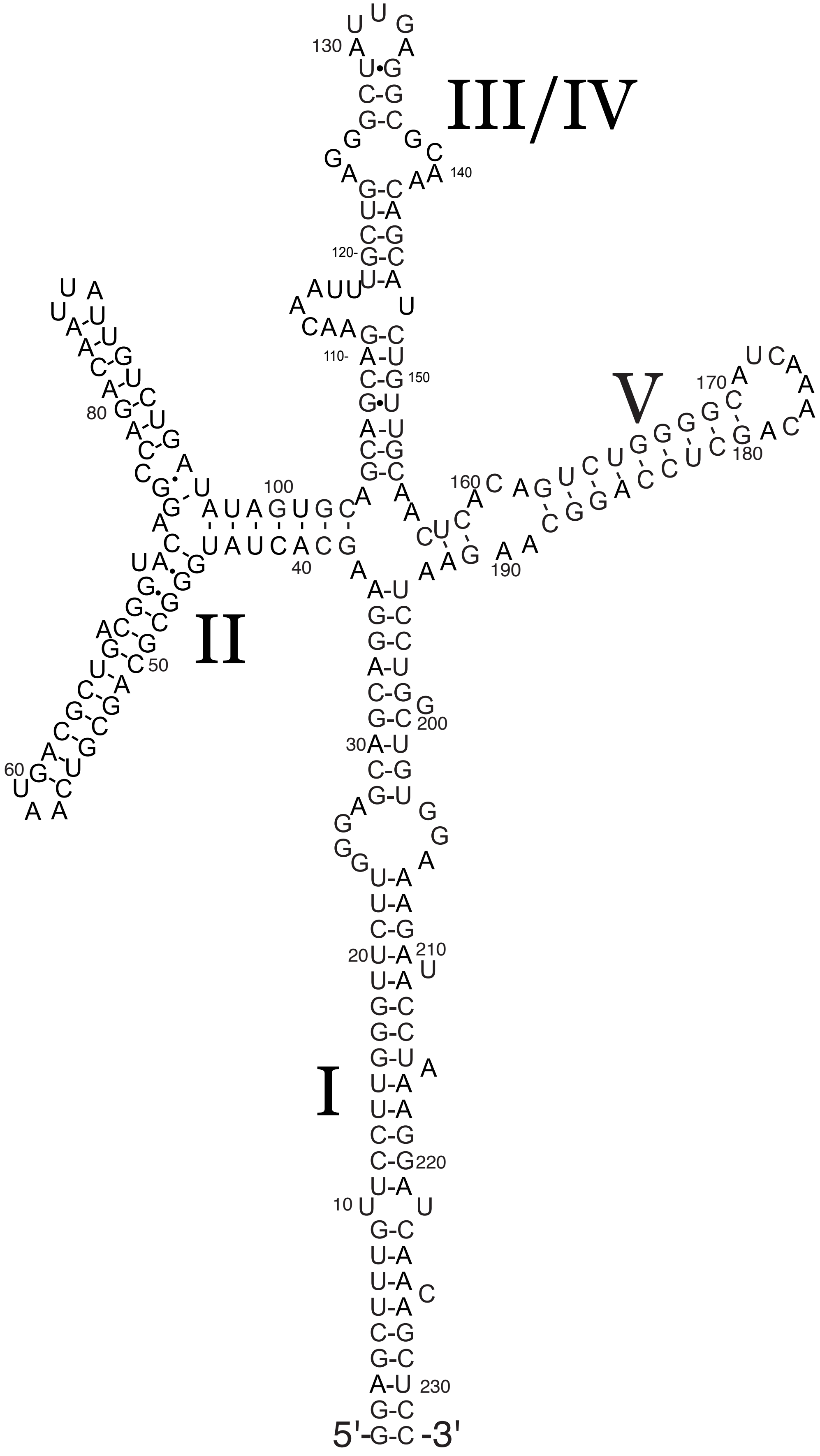
RRE is 232 nt (75 kDa)

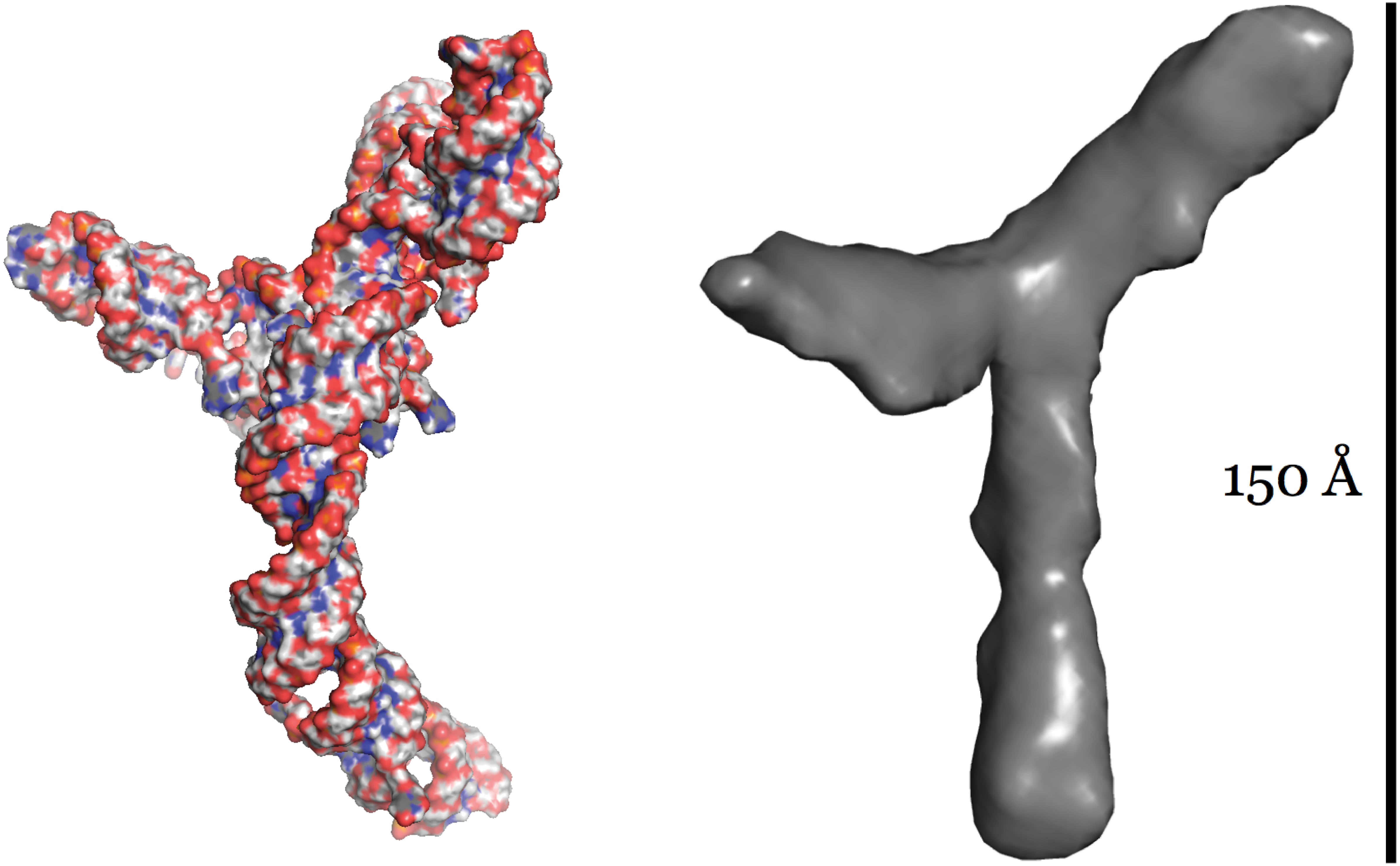
Current approach based on deuterium labelling

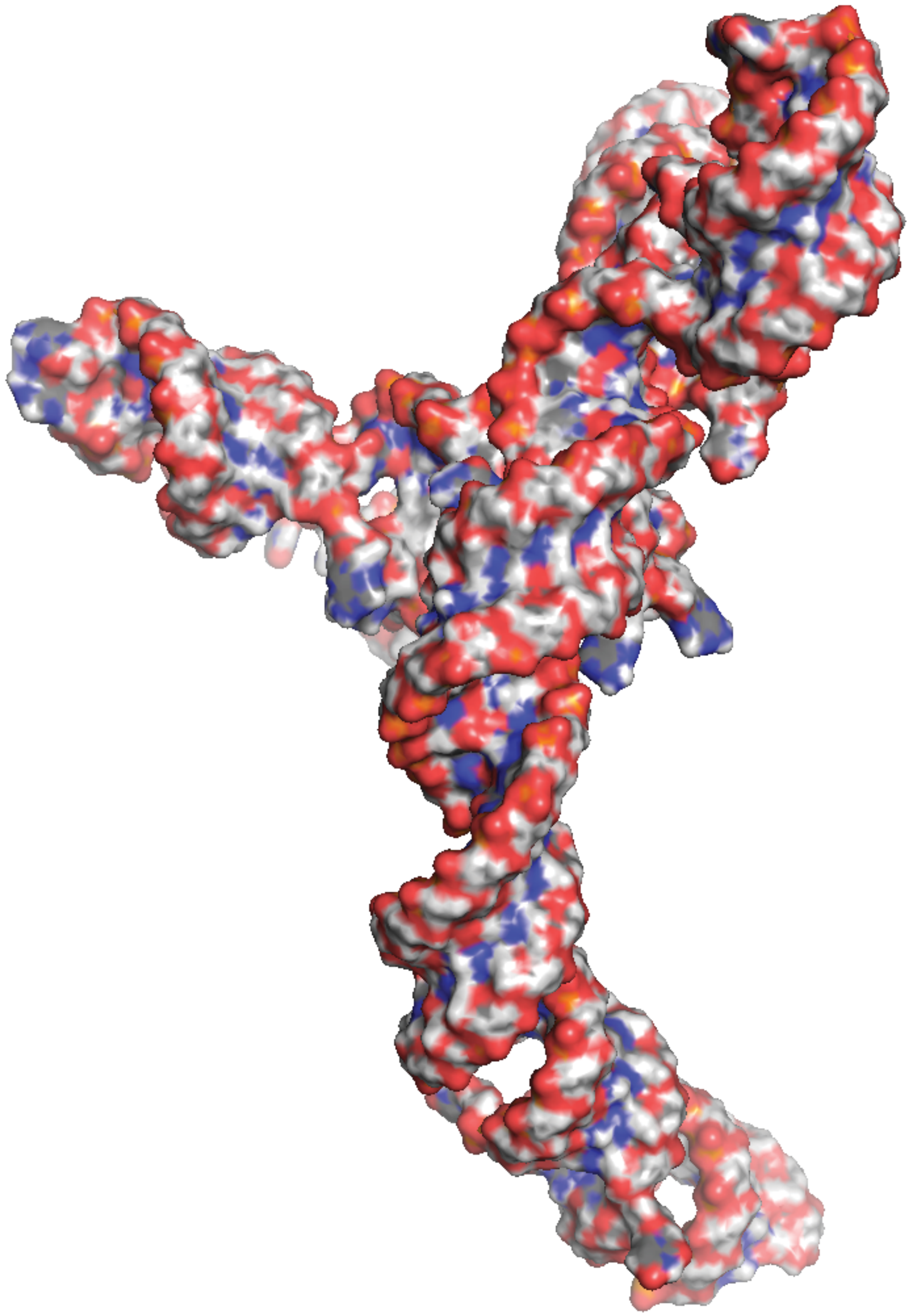
Can assign chemical shifts and NOEs throughout the molecule

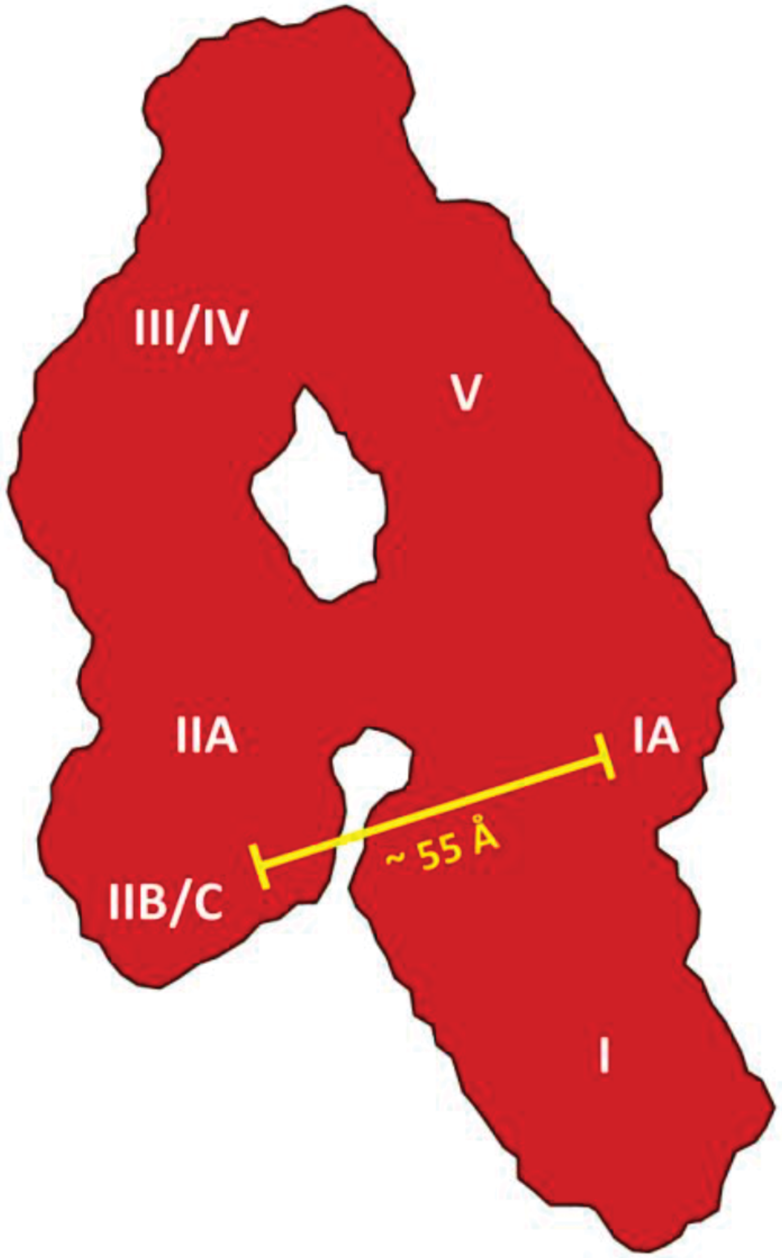





III/IV
II
V
I
5'
-3'
130
140
120
110
150
100
160
170
180
190
80
60
50
40
30
200
20
210
10
220
230

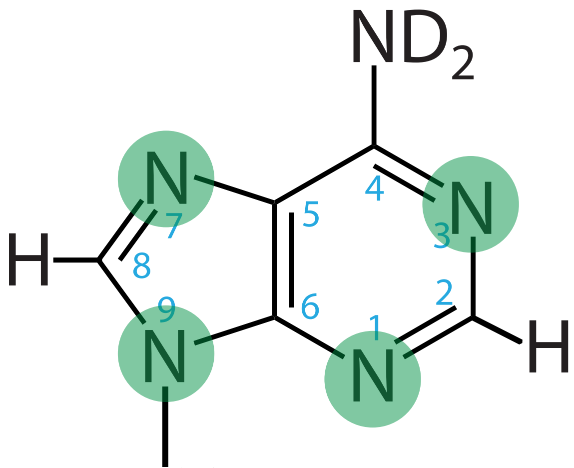

150 Å



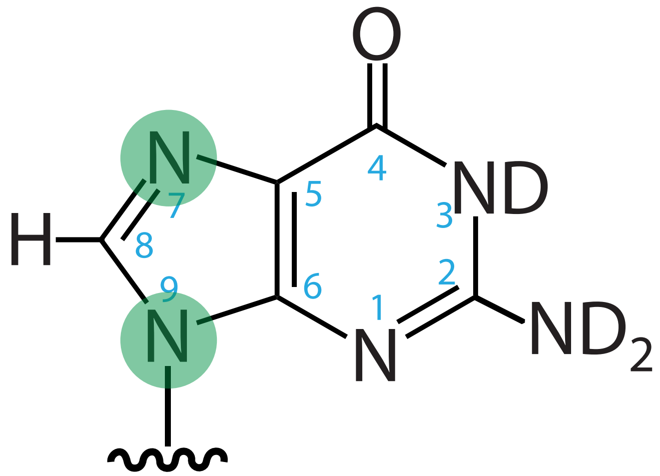


Wang & co-workers, Cell (2013)

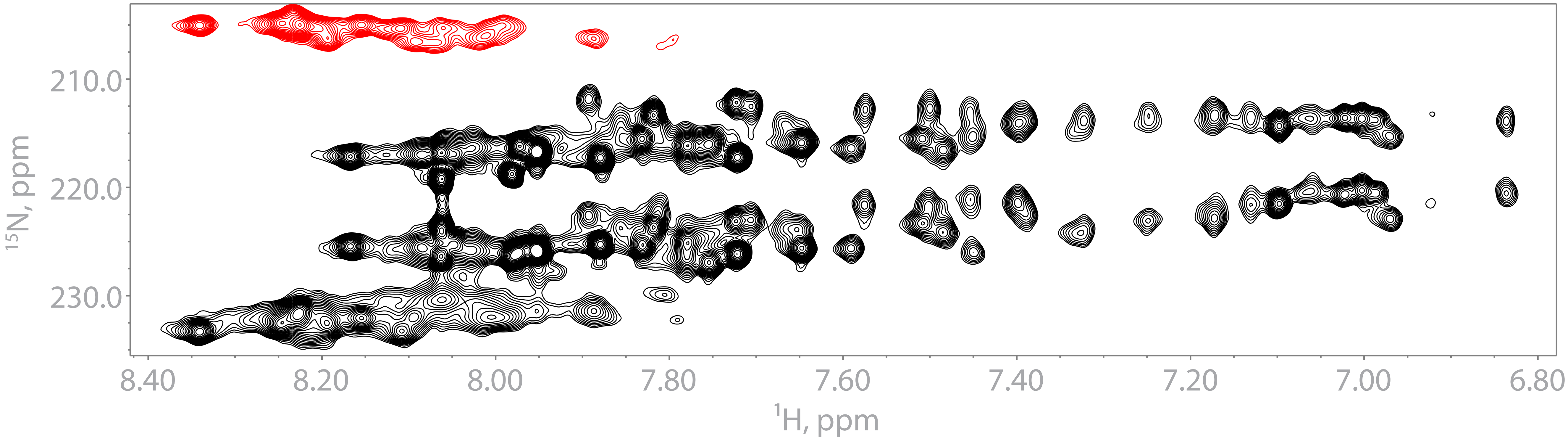
Can we get useful RDC data for large RNAs?



Adenine



Guanine

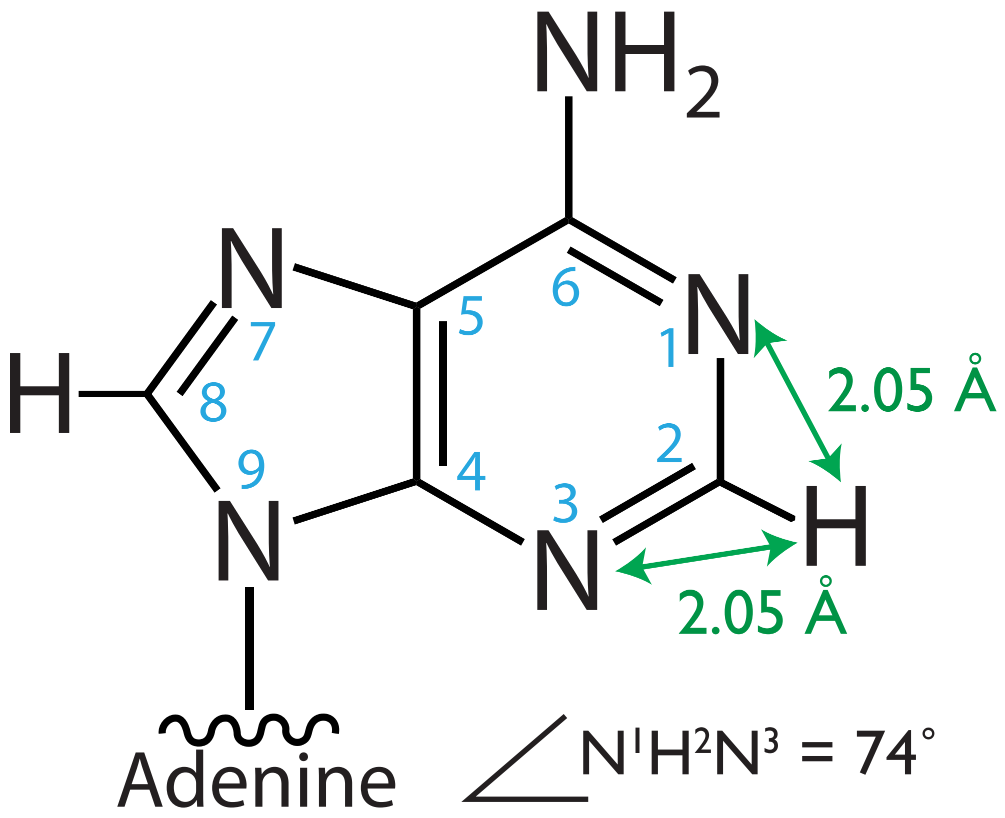


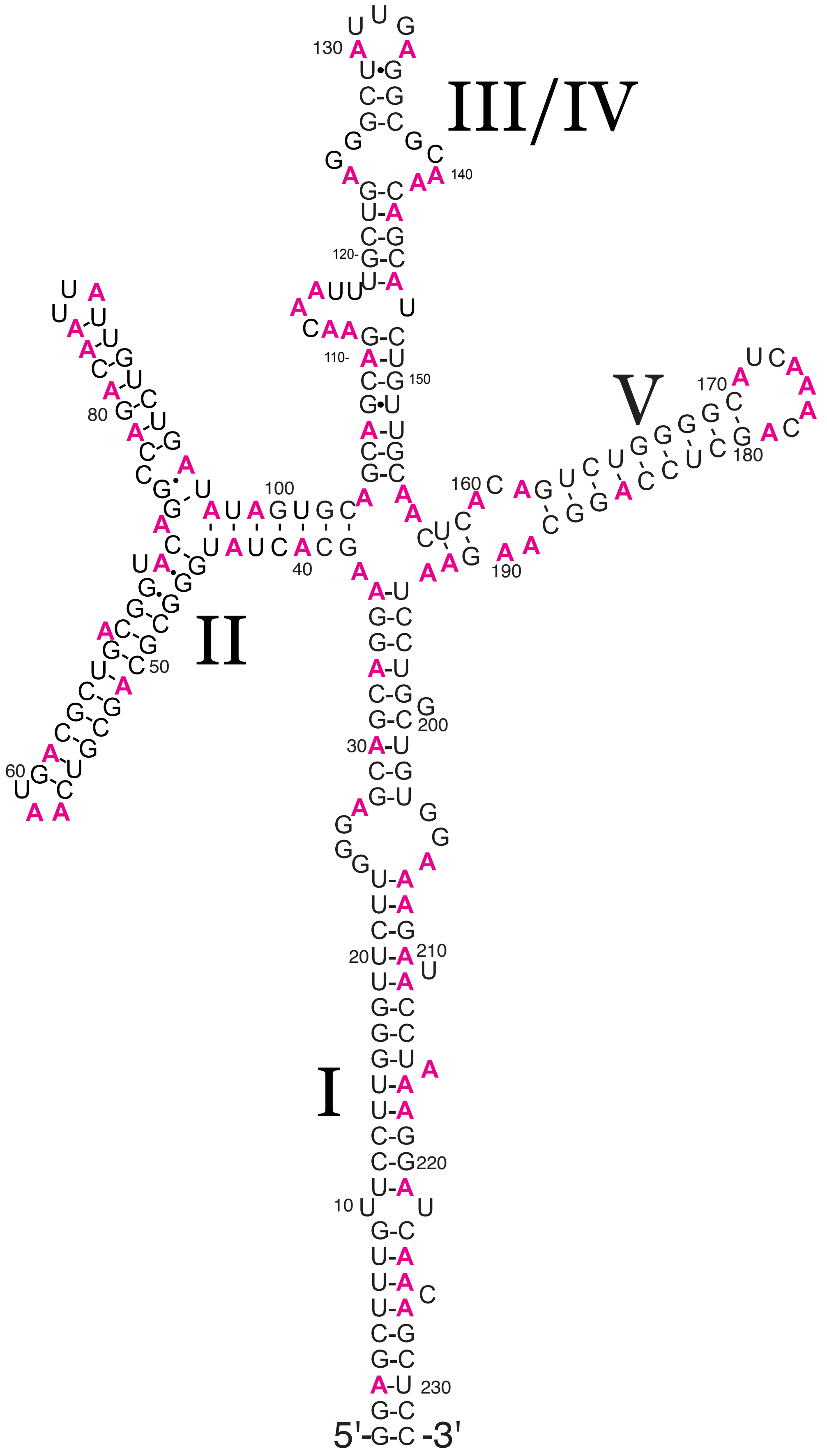
Can we get useful RDC data for large RNAs?

63 well-dispersed Adenosines

D^{max} approx 2.8 kHz

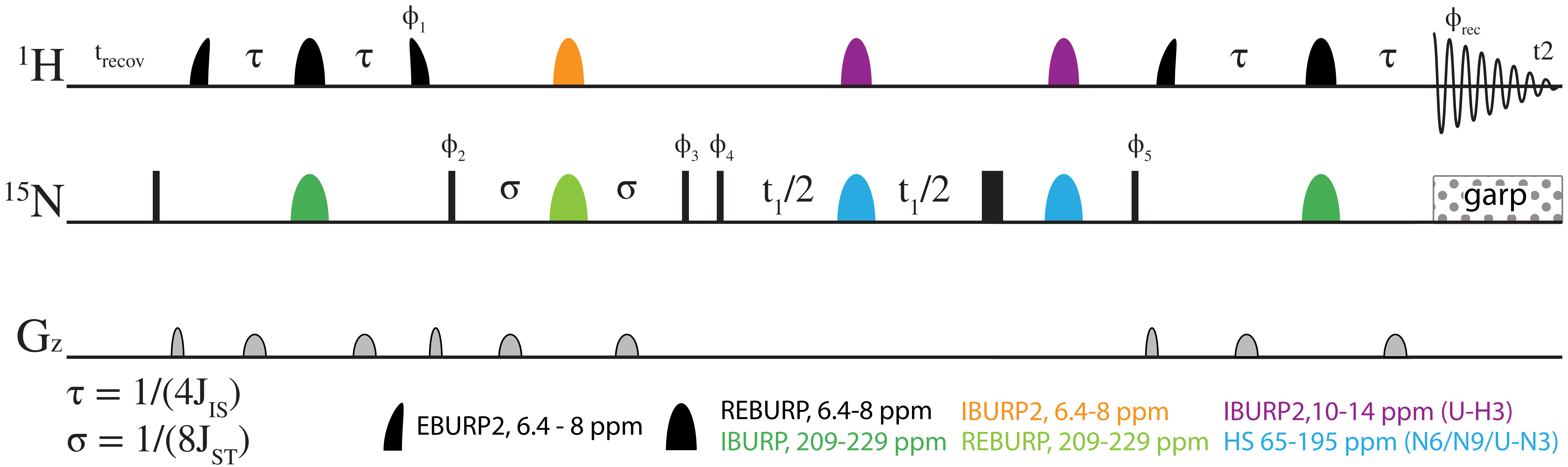
Low proton density - perhaps 5-10 Hz RDC feasible?



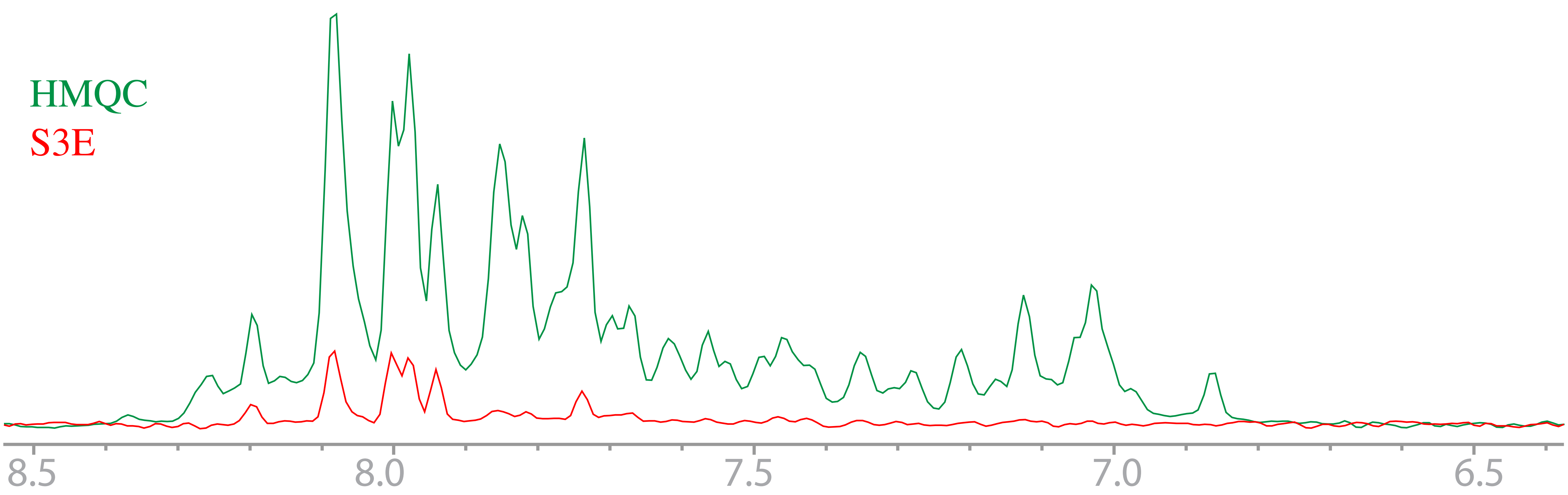


S3E

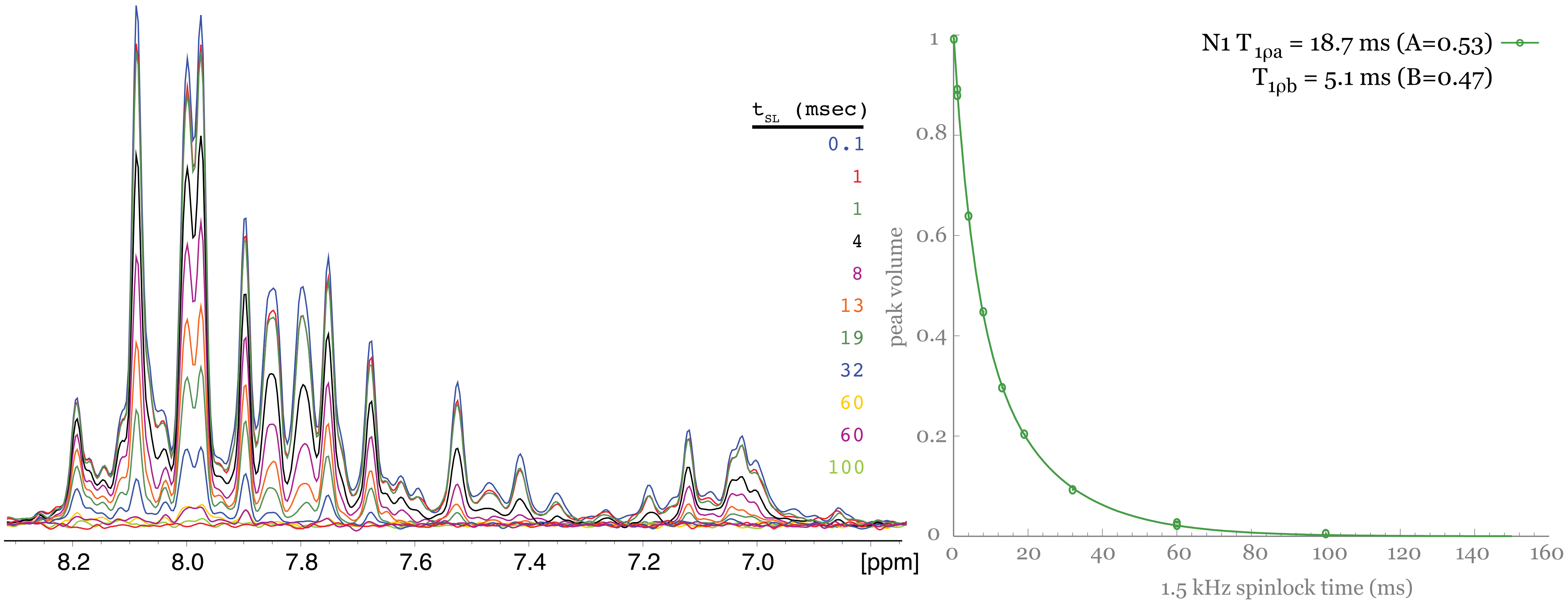
After Zídek, Wu, Feigon and Sklenár, J Biomol NMR. 2001 Oct;21(2):153-60
with I=H2, S=N1/N3, T=H2, X=U-H3, U-N3, A-N6, A-N9



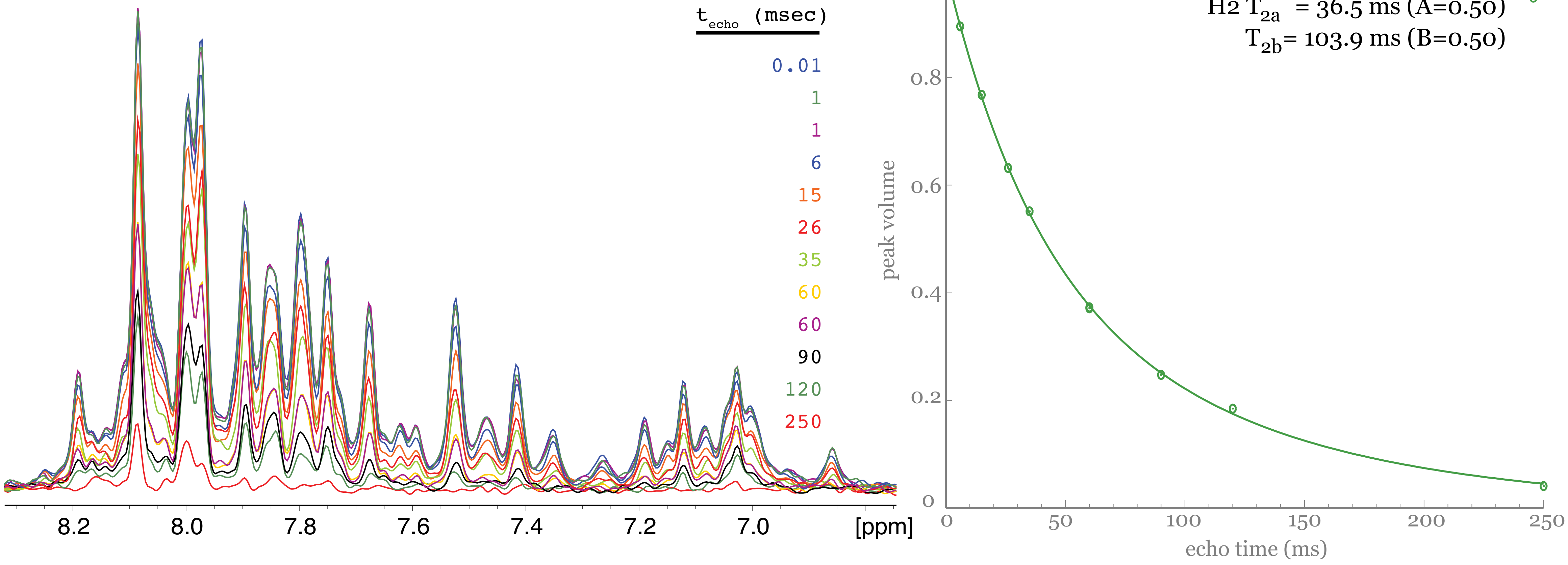
$$\tau = 1/(4J_{IS})$$
$$\sigma = 1/(8J_{ST})$$



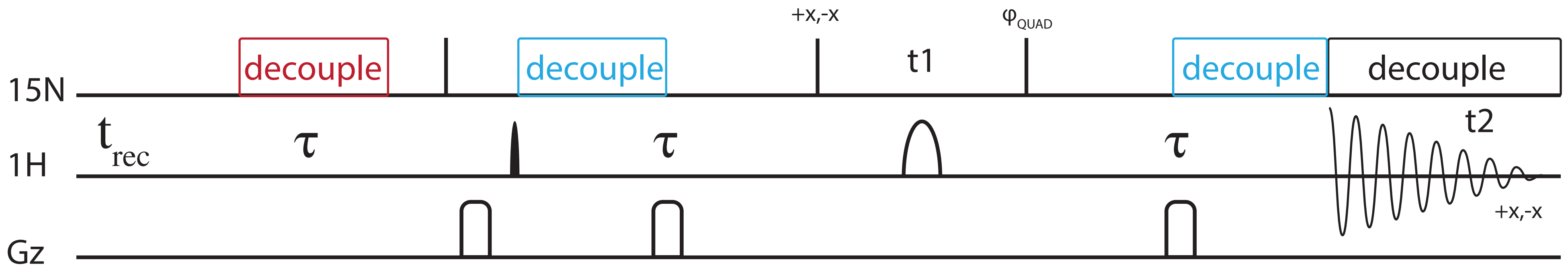
Rapid ^{15}N relaxation to blame



Adenosine H2 relaxes slowly



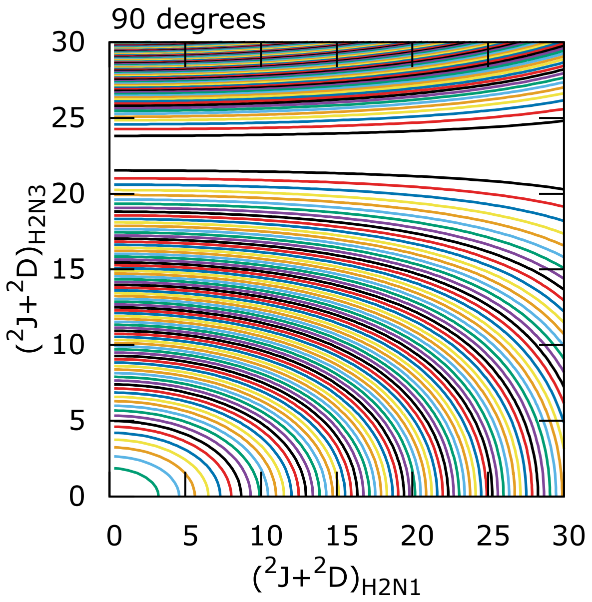
Quantitative J correlation



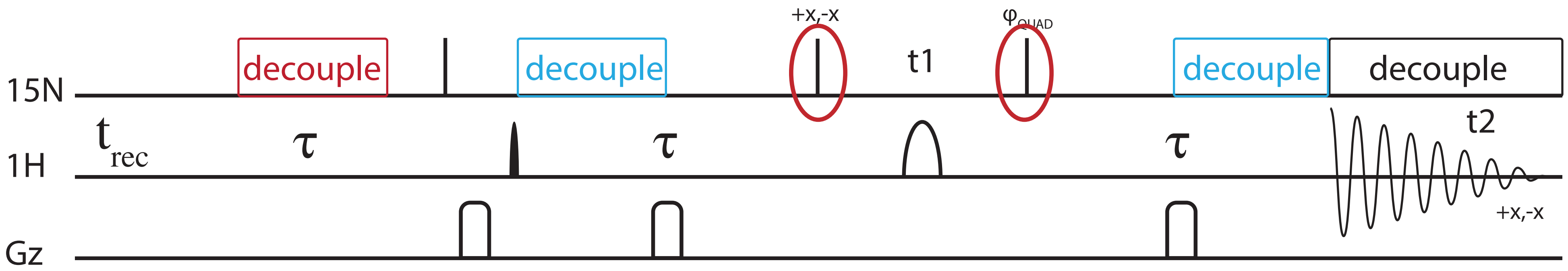
Large dependence on passive spin

Can fit if both signals resolved

Can use selective / semi-selective pulse



Quantitative J correlation

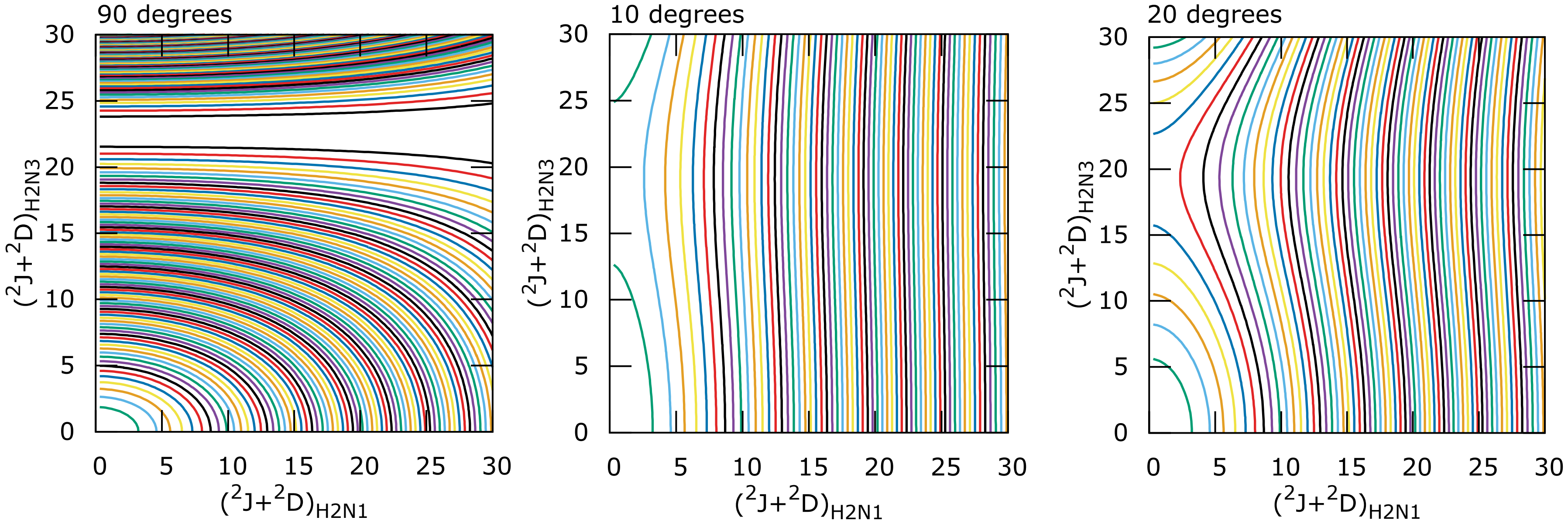


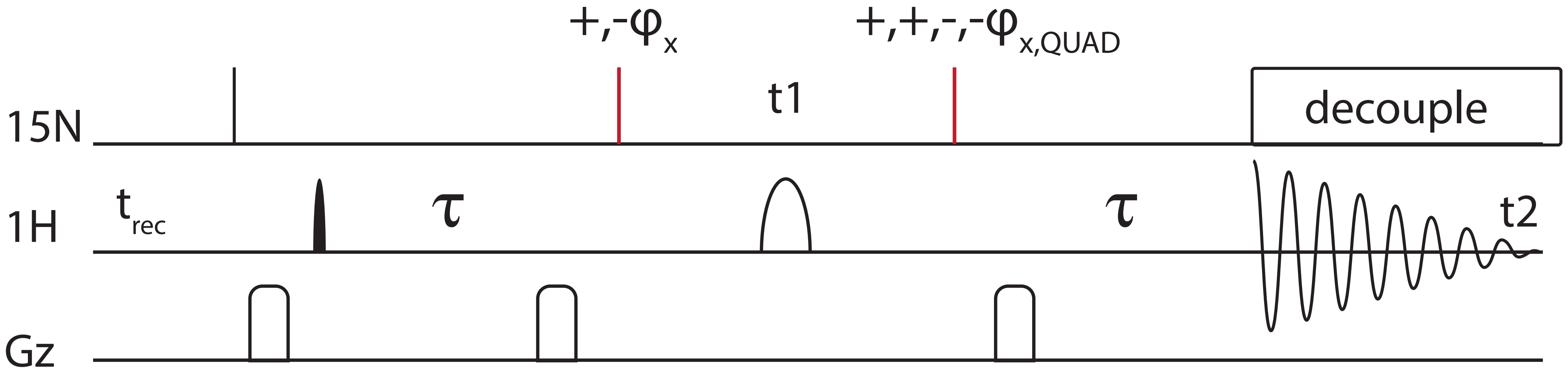
Large dependence on passive spin

Can fit if both signals resolved

Can use selective / semi-selective pulse

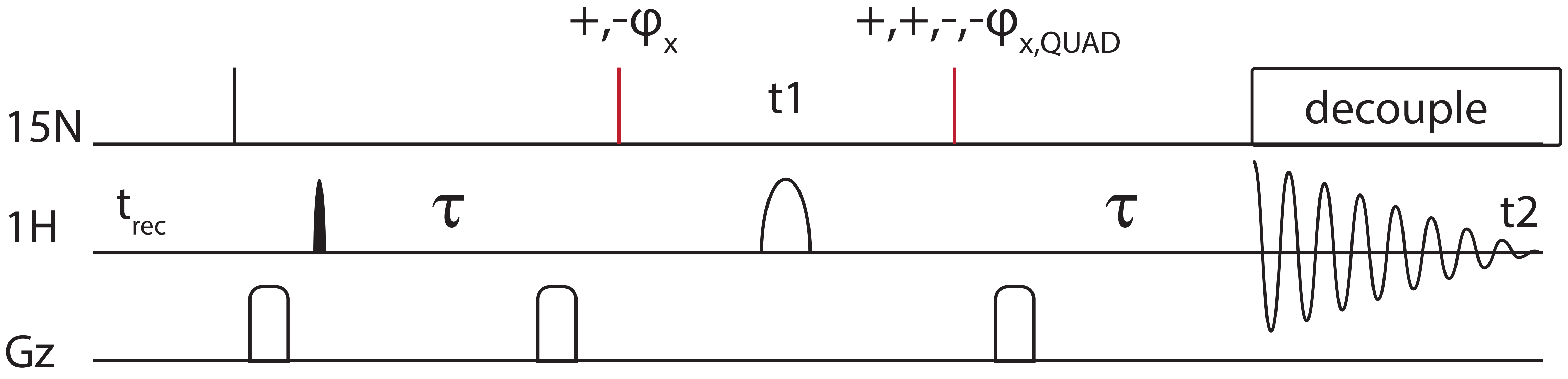
Can use small flip angle ^{15}N pulses





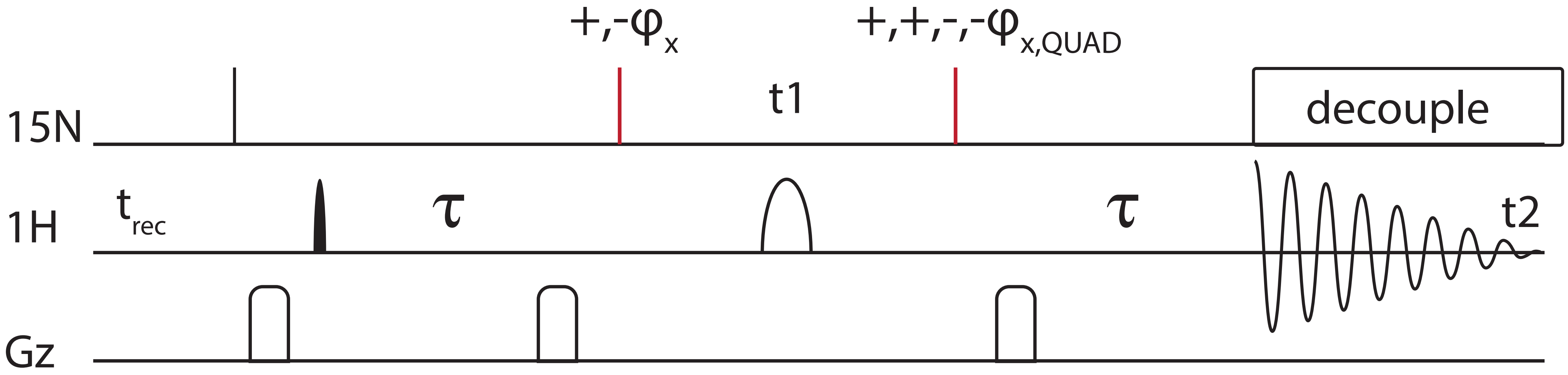
$$\sin^2(\phi)\left\{\cos(\Omega_S t_1)\sin^2(\pi J_{IS}\tau)\left[\,\cos^2(\pi J_{IT}\tau)+\,\sin^2(\pi J_{IT}\tau)\cos^2(\phi)\,\right]\right.$$

$$\left.+\,\cos(\Omega_T t_1)\sin^2(\pi J_{IT}\tau)\left[\,\cos^2(\pi J_{IS}\tau)+\,\sin^2(\pi J_{IS}\tau)\cos^2(\phi)\,\right]\right\}$$

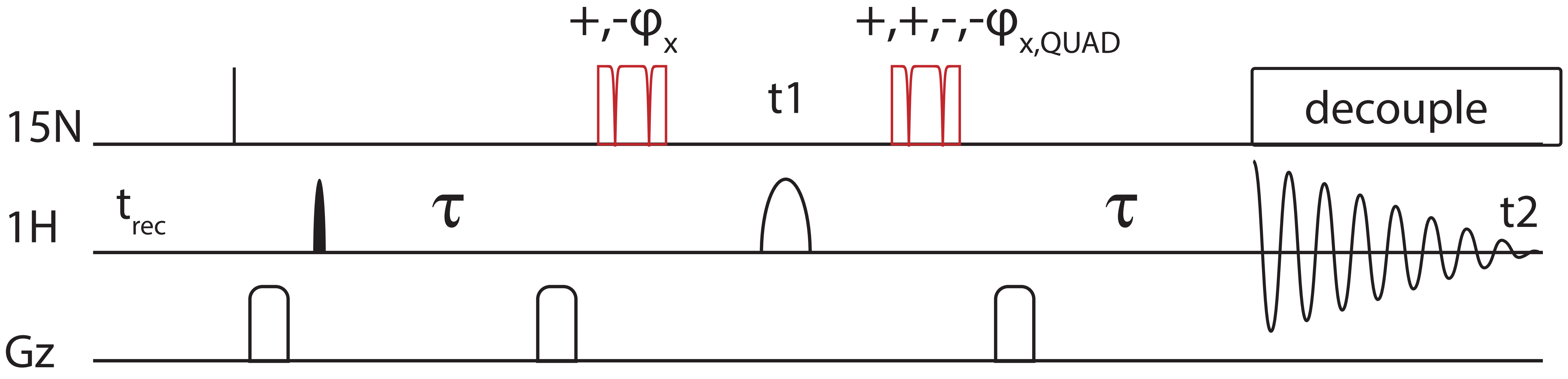


$$\sin^2(\phi)\left\{\cos(\Omega_S t_1)\sin^2(\pi J_{IS}\tau)\left[\cos^2(\pi J_{IT}\tau)+\sin^2(\pi J_{IT}\tau)\cos^2(\phi)\right]\right.$$

$$\left.+\cos(\Omega_T t_1)\sin^2(\pi J_{IT}\tau)\left[\cos^2(\pi J_{IS}\tau)+\sin^2(\pi J_{IS}\tau)\cos^2(\phi)\right]\right\}$$



$$\sin^2(\phi)\left\{\cos(\Omega_S t_1)\sin^2(\pi J_{IS}\tau)\left[\cos^2(\pi J_{IT}\tau)+\sin^2(\pi J_{IT}\tau)\cos^2(\phi)\right]\right.$$
$$\left.+\cos(\Omega_T t_1)\sin^2(\pi J_{IT}\tau)\left[\cos^2(\pi J_{IS}\tau)+\sin^2(\pi J_{IS}\tau)\cos^2(\phi)\right]\right\}$$

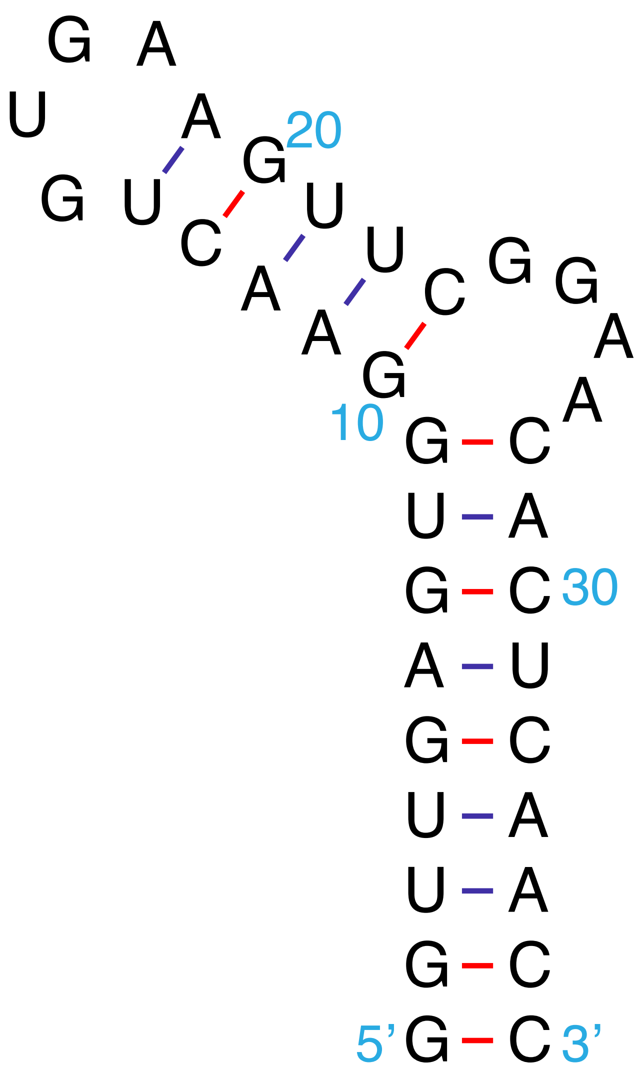


$$\sin^2(\phi)\left\{\cos(\Omega_S t_1)\sin^2(\pi J_{IS}\tau)\left[\cos^2(\pi J_{IT}\tau)+\sin^2(\pi J_{IT}\tau)\cos^2(\phi)\right] + \cos(\Omega_T t_1)\sin^2(\pi J_{IT}\tau)\left[\cos^2(\pi J_{IS}\tau)+\sin^2(\pi J_{IS}\tau)\cos^2(\phi)\right]\right\}$$

For $\varphi_1=\pi/4$, $\varphi_2=\pi/2$:

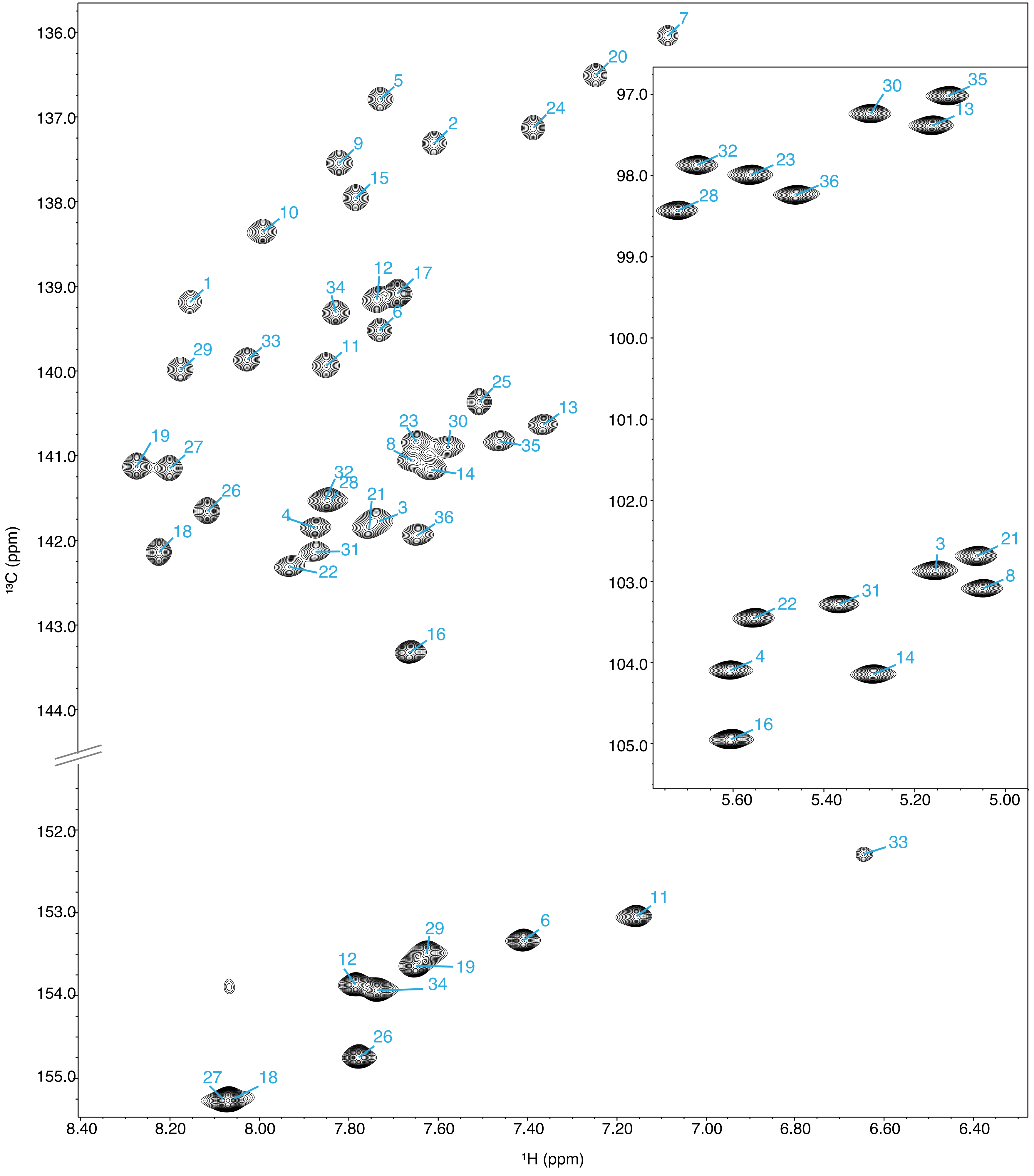
$$J_3 = \frac{\mathrm{atan}(\mathrm{sqrt}(4Q_{N1}-2))}{\pi\tau} \qquad J_1 = \frac{\mathrm{atan}(\mathrm{sqrt}(4Q_{N3}-2))}{\pi\tau}$$

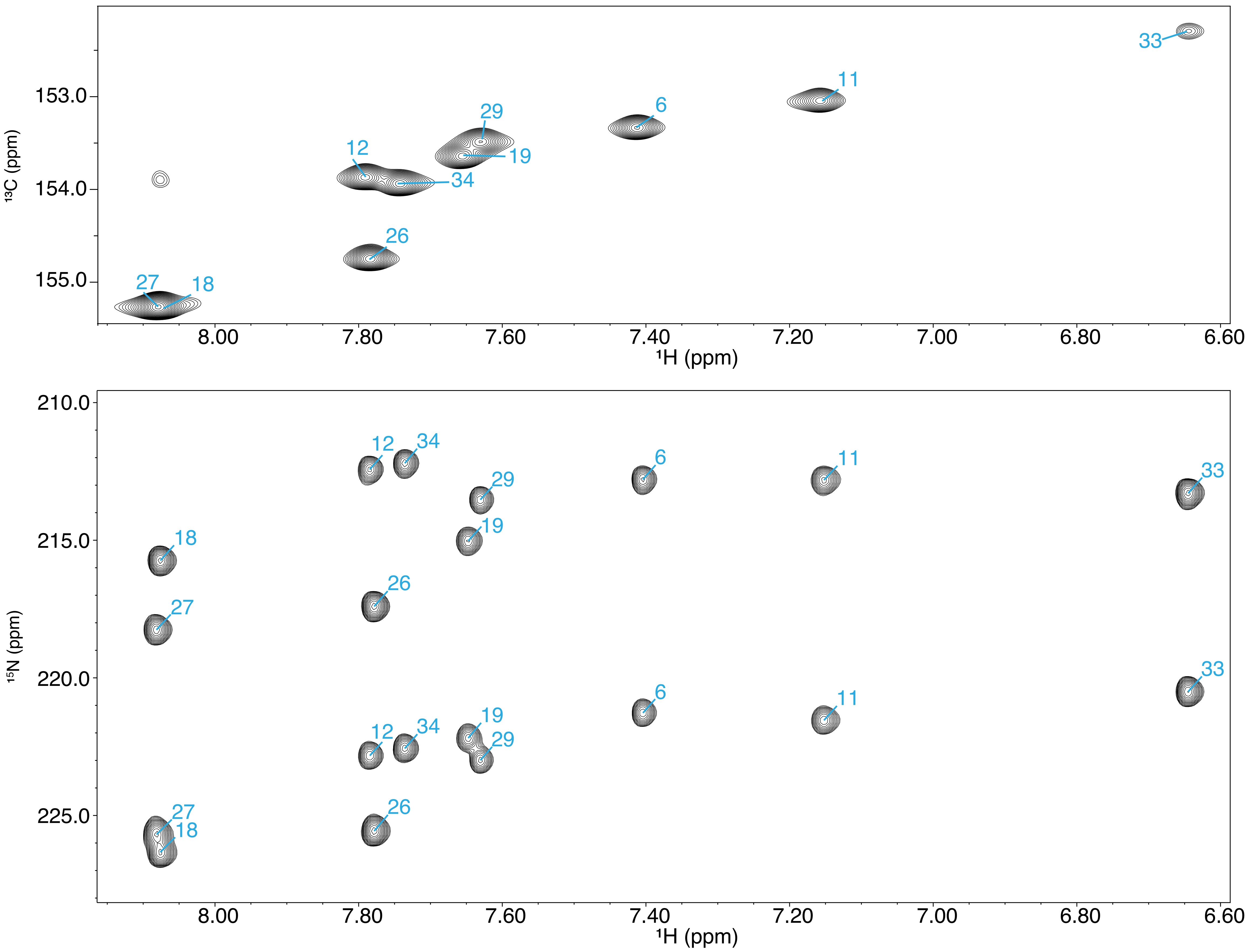
Model system

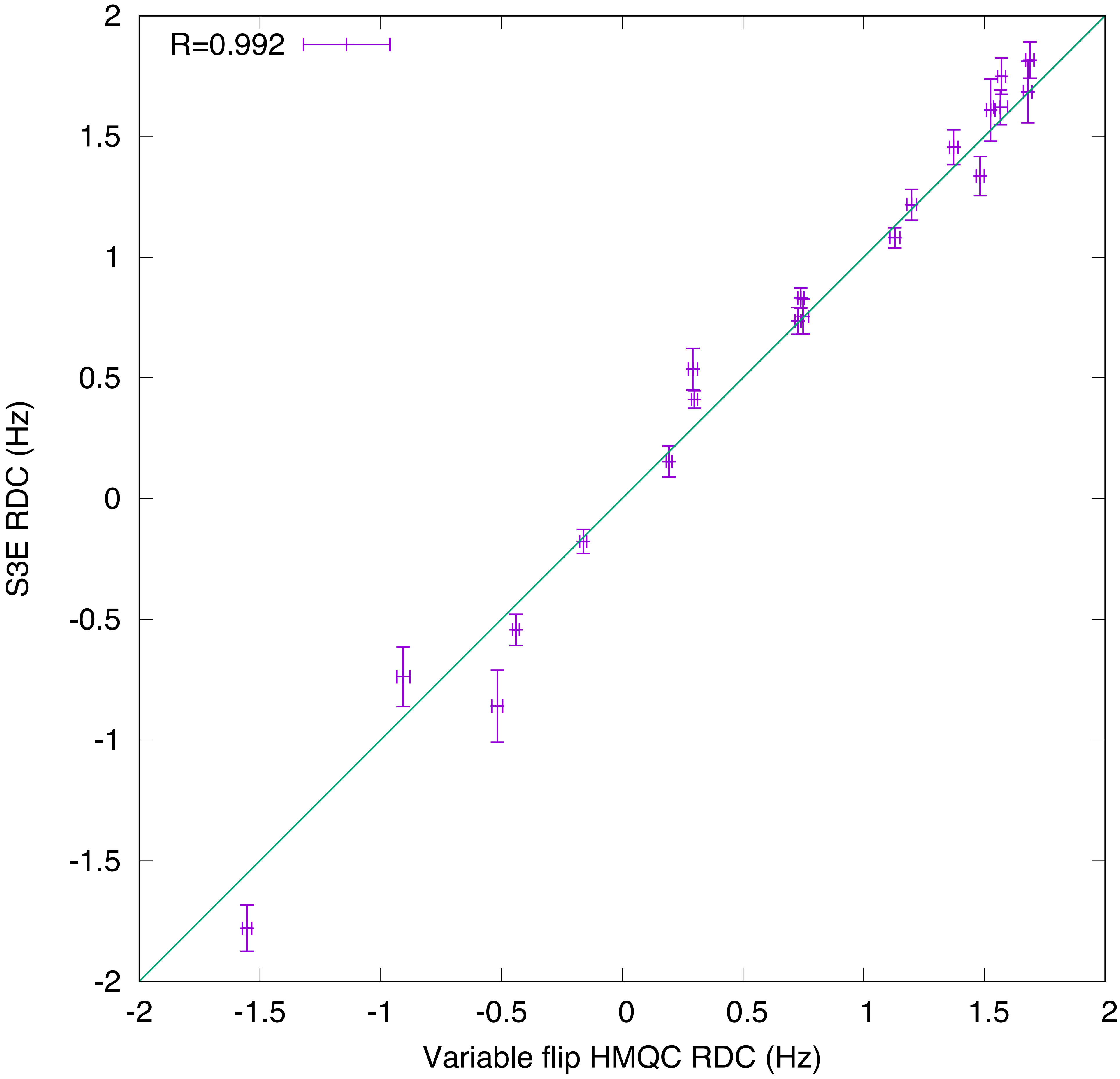


Derived from MMLV 5'-L SLC

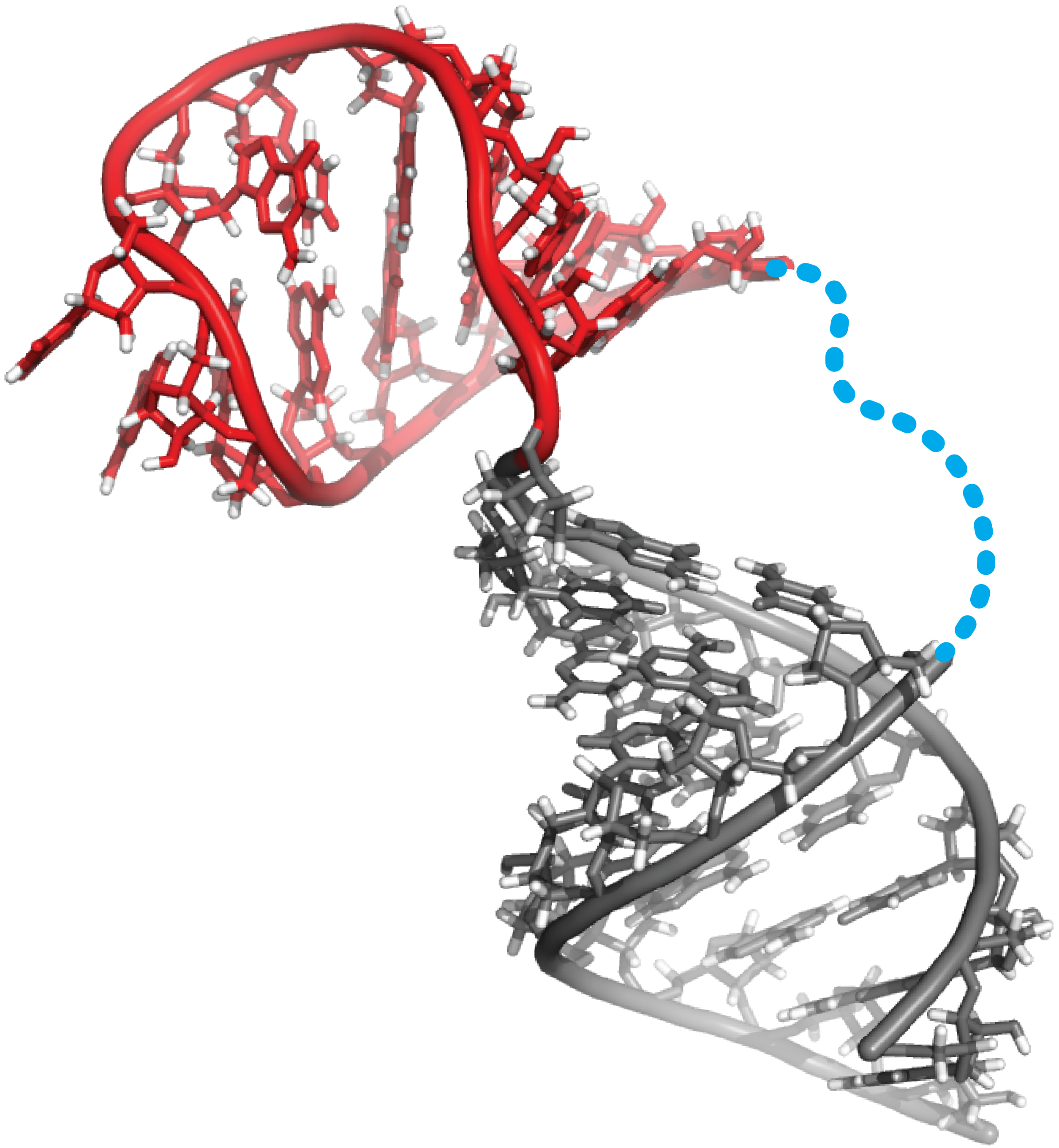
36 nt, 10 Adenosines



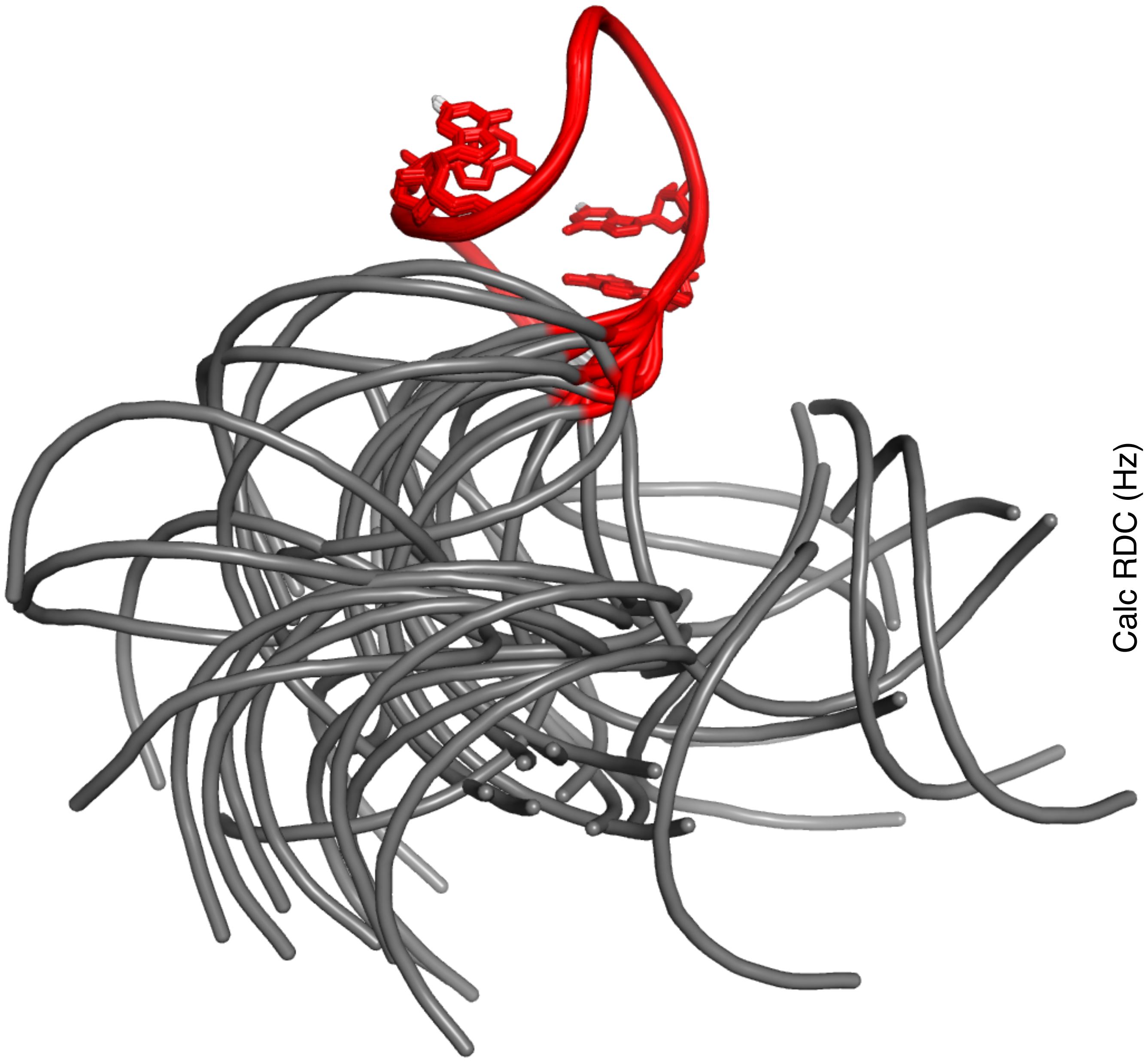

33
11
6
29
19
12
34
26
27
18
153.0
154.0
155.0
13C (ppm)
8.00
7.80
7.60
7.40
7.20
7.00
6.80
6.60
1H (ppm)
12
34
6
11
33
29
19
18
26
27
6
11
33
19
12
34
29
27
18
26
210.0
215.0
220.0
225.0
15N (ppm)
8.00
7.80
7.60
7.40
7.20
7.00
6.80
6.60
1H (ppm)

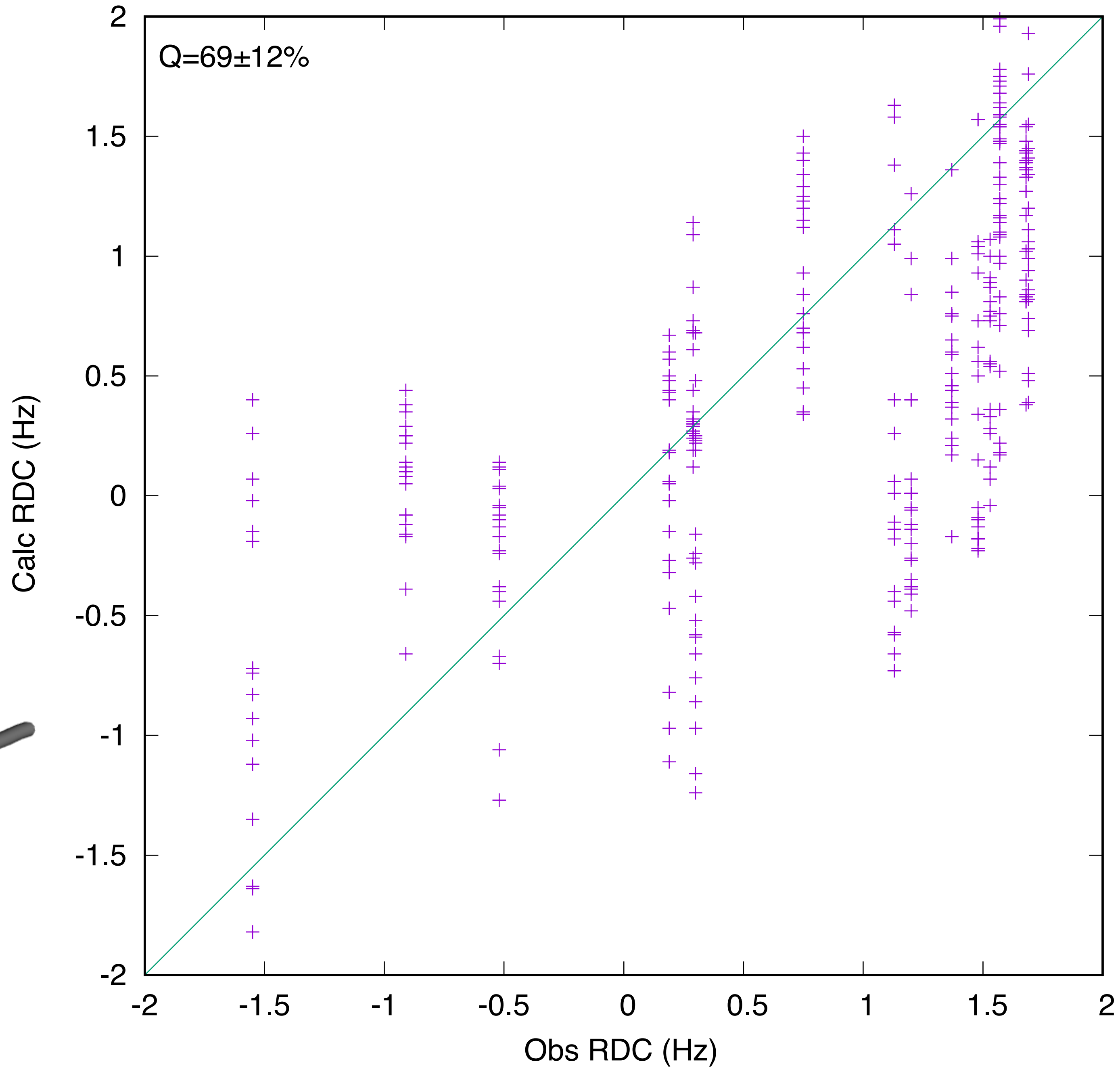

R=0.992
S3E RDC (Hz)
Variable flip HMQC RDC (Hz)
2
1.5
1
0.5
0
-0.5
-1
-1.5
-2
-2
-1.5
-1
-0.5
0
0.5
1
1.5
2

Removed helix:helix NOEs and relaxed geometry

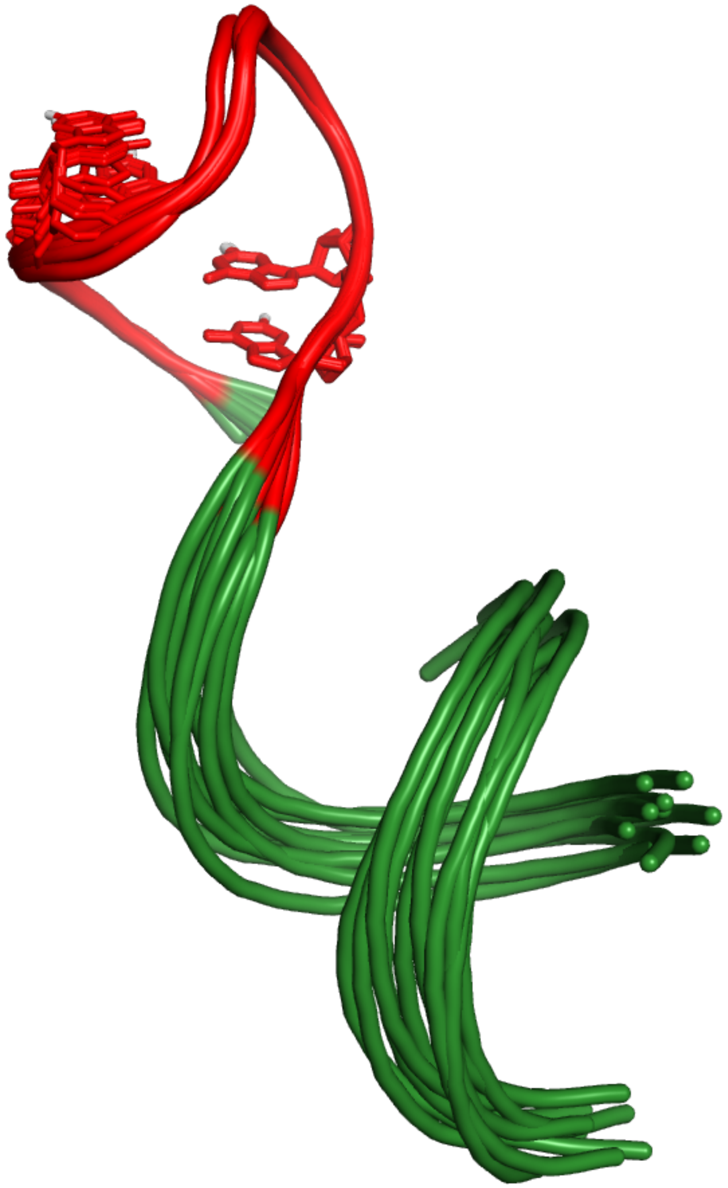


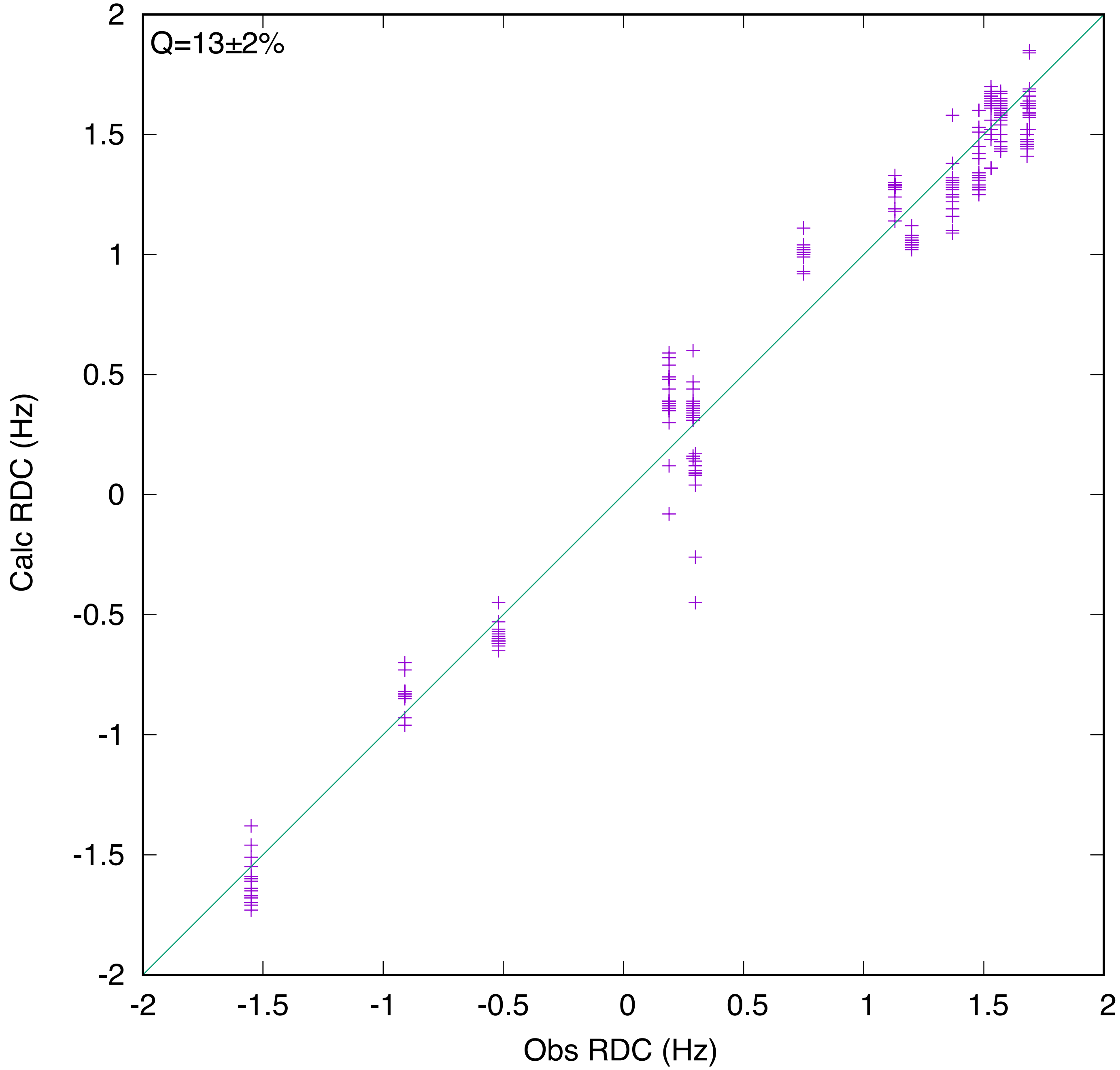
No RDCs



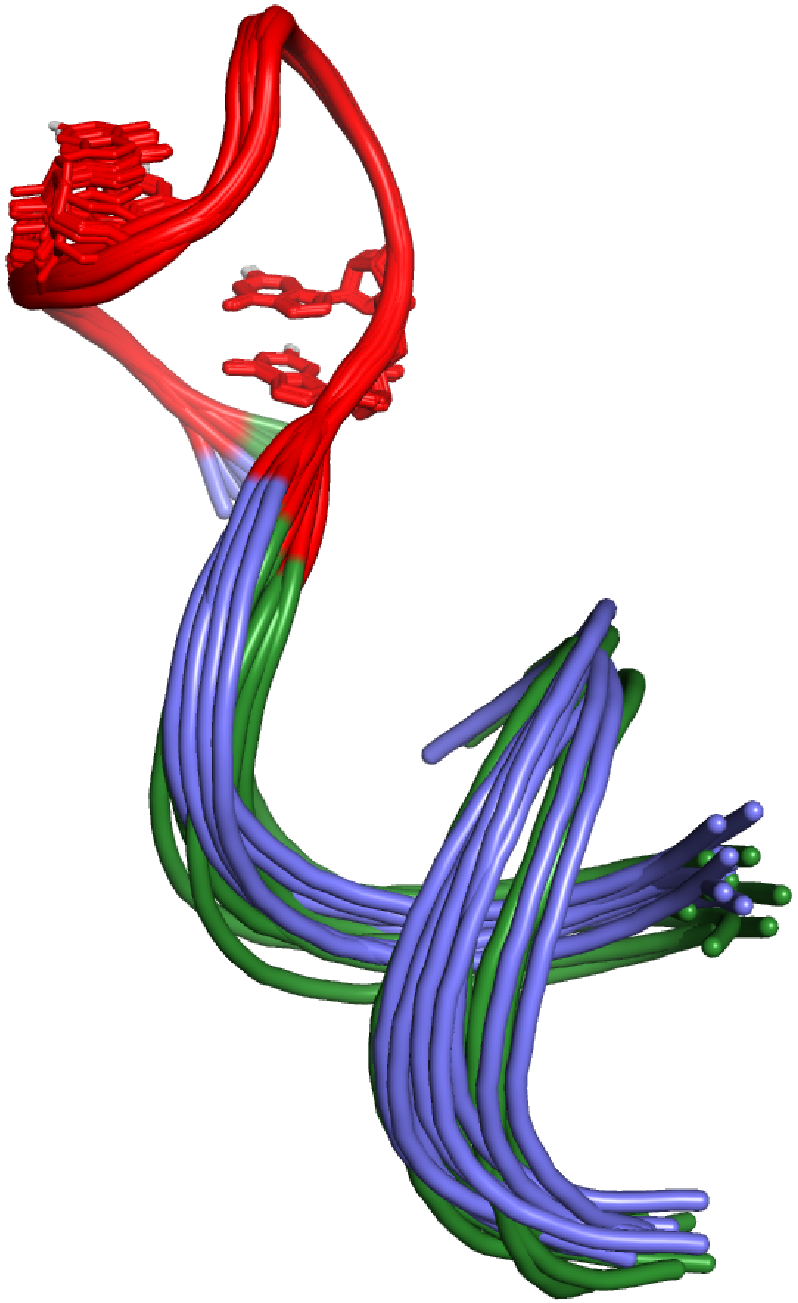


Incorporate ^{13}C RDCs only

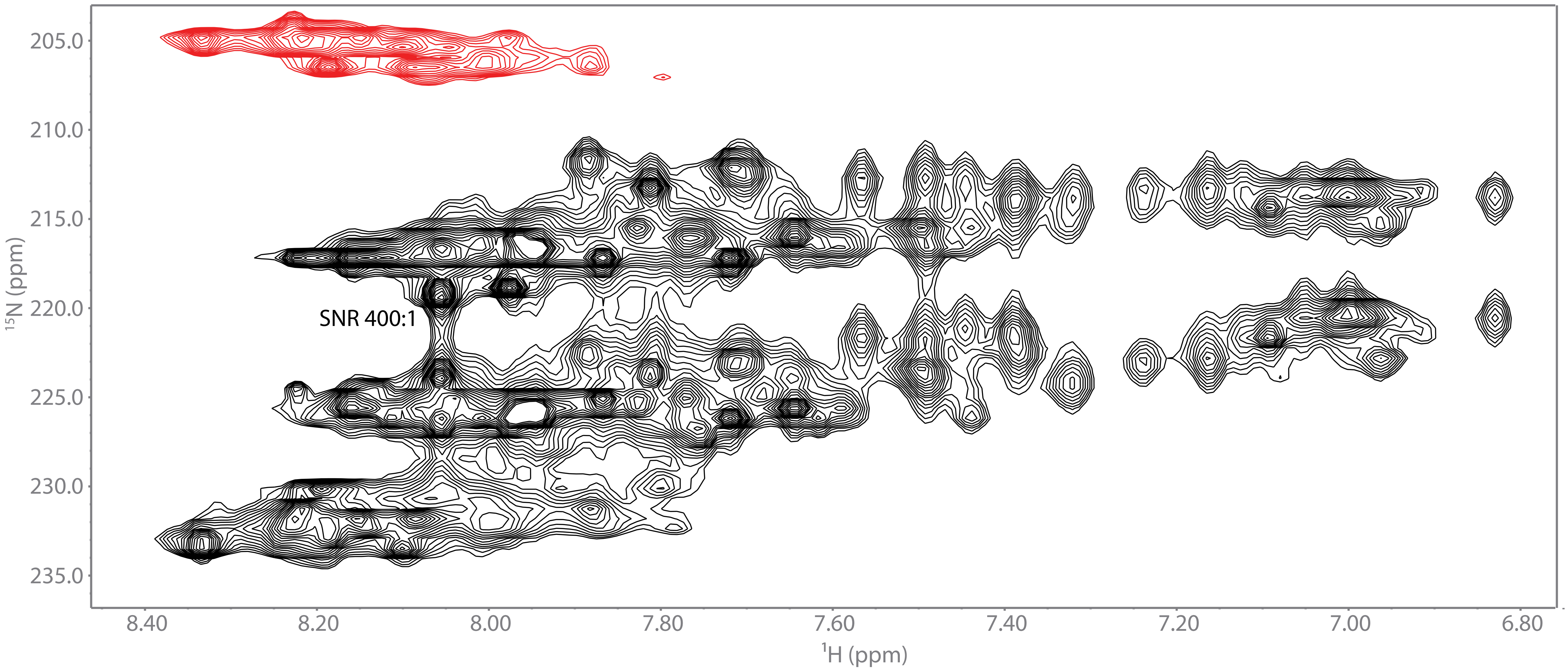




Incorporate ^{15}N RDCs only



Application to 232 nt RRE



Summary

Described an experiment to determine H2-N1/N3 RDCs with high sensitivity in large RNAs

These RDCs sufficient to define inter-helical orientation in a 36 nt model system

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The rest of the lab

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NIH National Institutes of Health

The Center for HIV RNA Studies (CRNA)