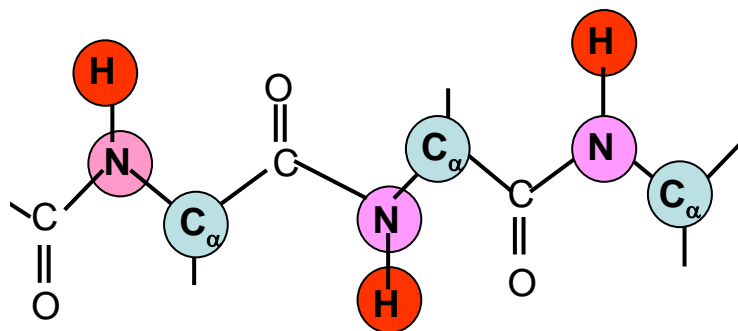


Rapid data acquisition of 3D triple resonance experiments via projection reconstruction NMR (PR-NMR)

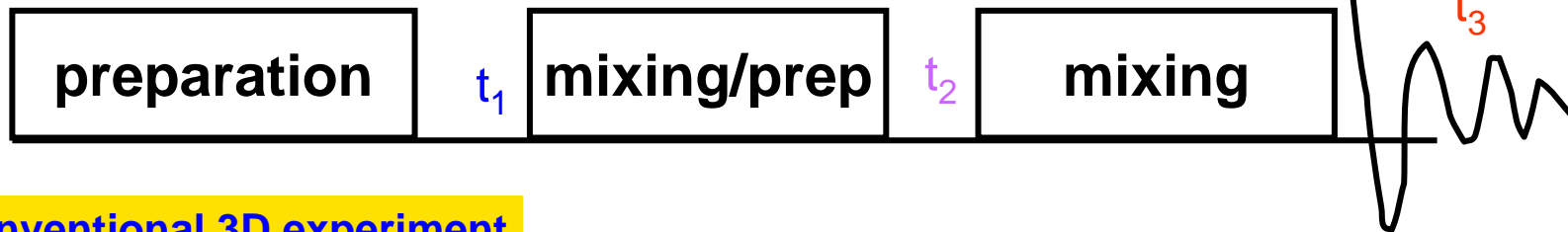
by

Wen-Jin Wu

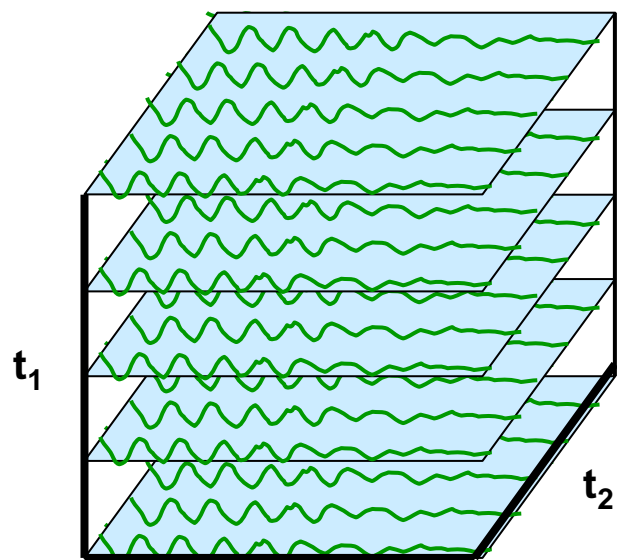
High-Field NMR Center
National Research Program for Genomic Medicine
Institute of Biomedical Sciences
Academia Sinica



3D HNCA:
 Three frequencies, ^1H , ^{13}C and ^{15}N are to be recorded.



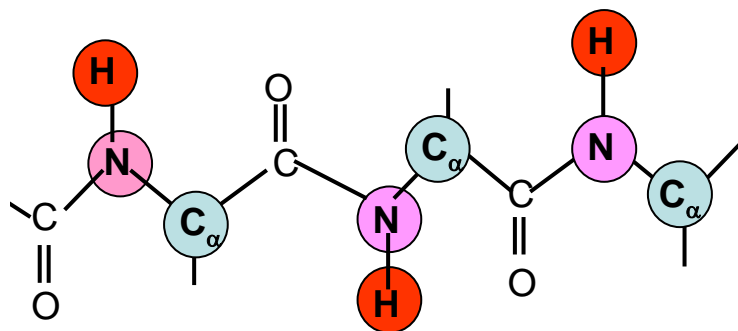
Conventional 3D experiment



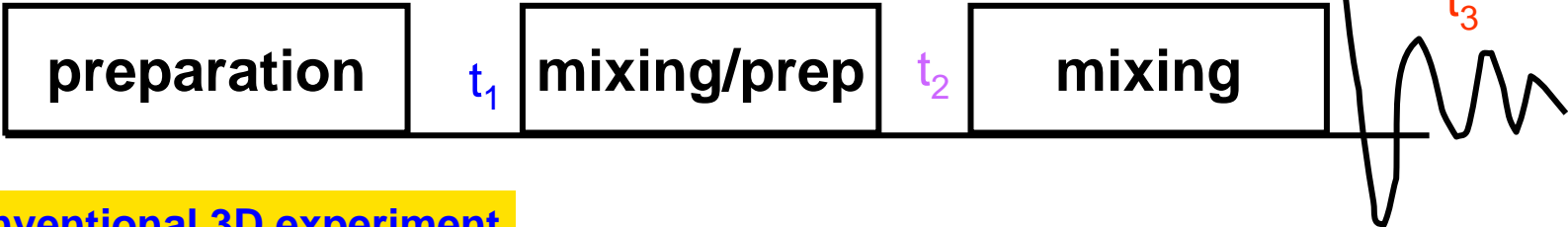
A 3D HNCA experiment:
 $n_1=48$ for ^{15}N , $n_2=80$ for ^{13}C .

48 2D ^1H - ^{13}C planes needed to be collected:
time consuming !

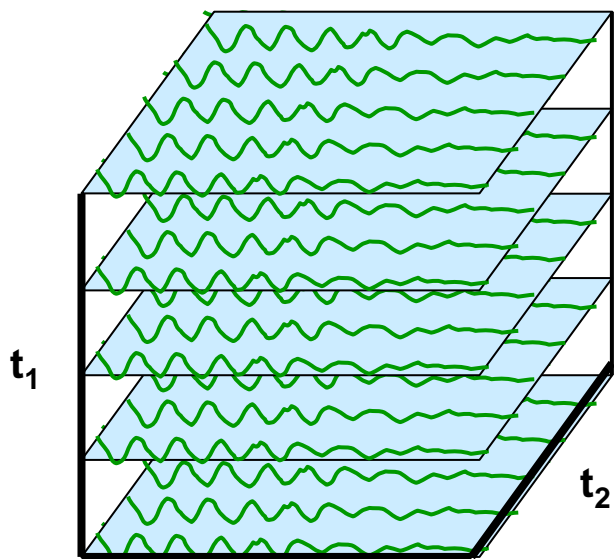
t_1 and t_2 are incremented
 independently



3D HNCA:
 Three frequencies, ^1H , ^{13}C and ^{15}N are to be recorded.

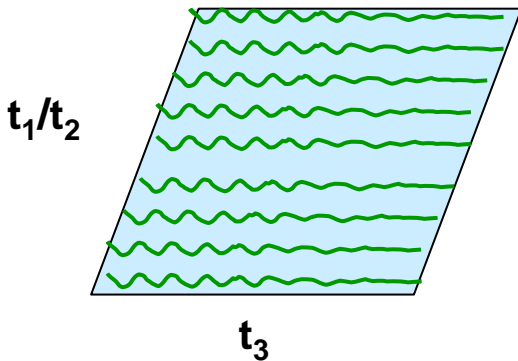


Conventional 3D experiment



t_1 and t_2 are incremented **independently**

Reduced dimensionality experiment

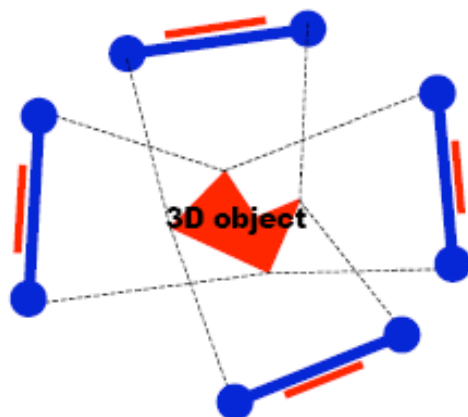


t_1 and t_2 are incremented **simultaneously**

Reduced Dimensionality Techniques ...

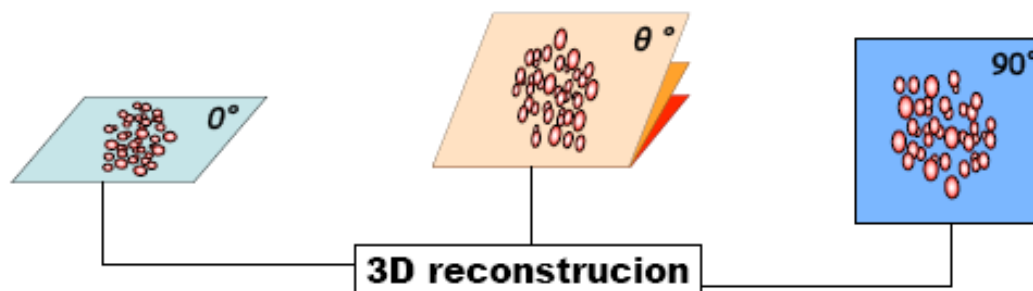
Projection Reconstruction

Simple **3D objects** can be reconstructed from **2D projections** collected at different angles

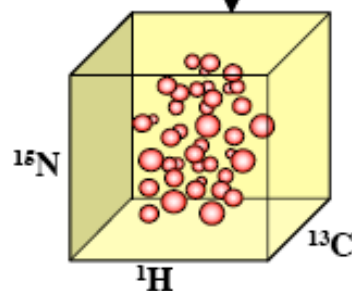


3D
reconstruction

*inverse
Radon
transformation*



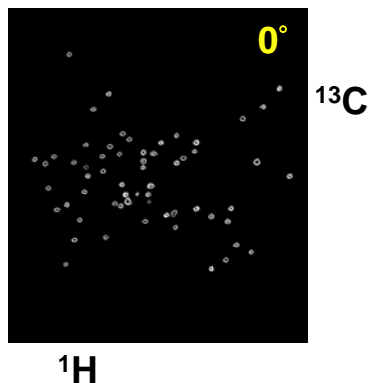
In principle, it is feasible to reconstruct a **3D spectrum** from a number of **2D tilted planes** collected at different angles.



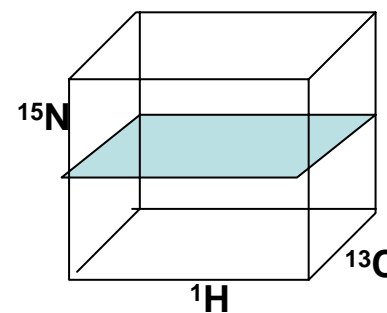
Slide from M. Tornelli
NMRFAM, UW-Madison

2D projection plane of 3D spectra \equiv tilted plane

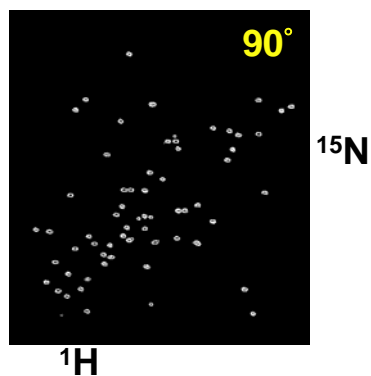
1H-13C plane
of HNCO



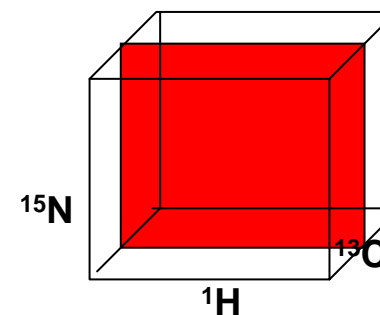
0° CH plane



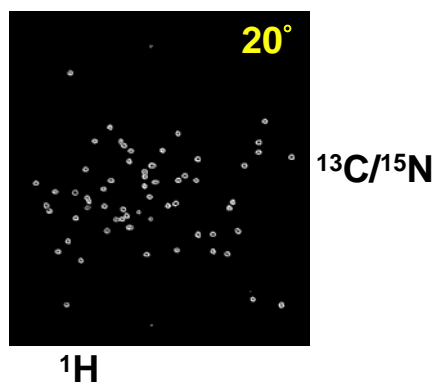
1H-15N plane
of HNCO



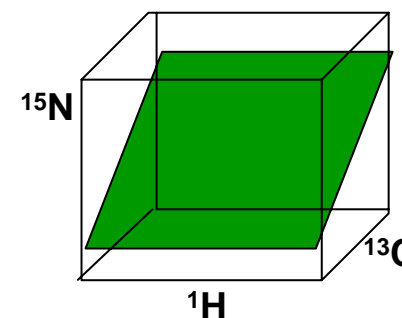
90° N-H plane



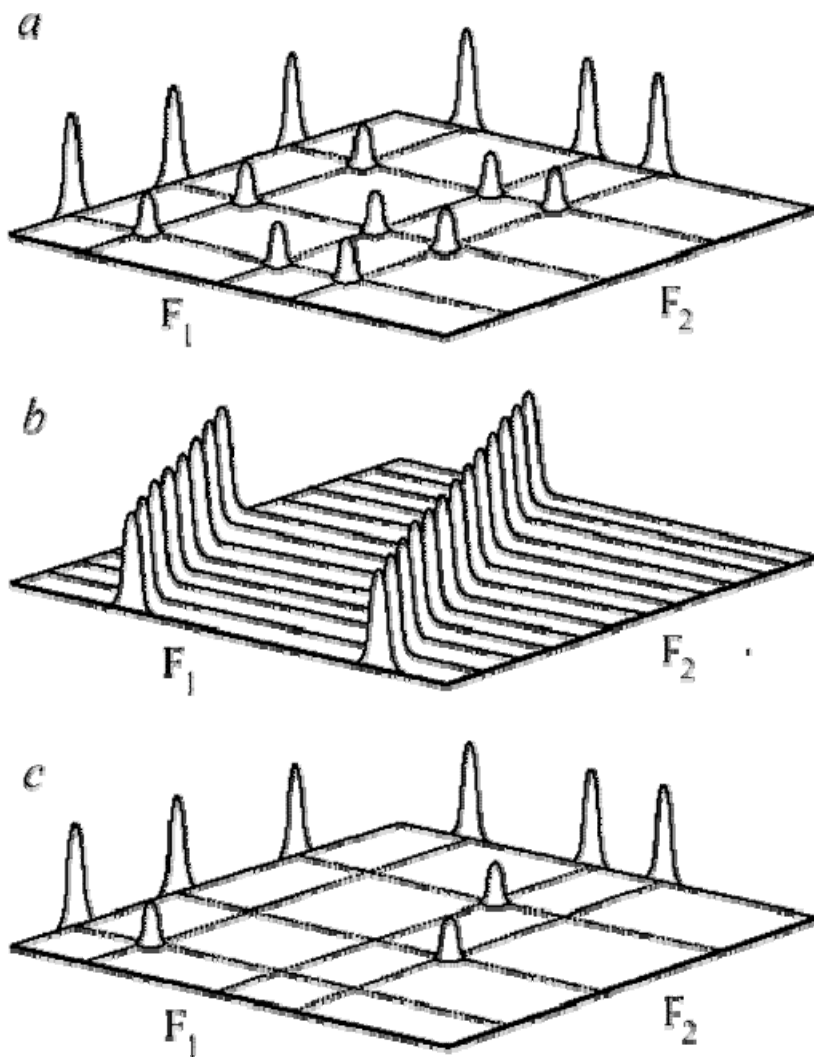
1H-15N/13C plane
of HNCO



tilted H-CN plane



Reconstruction Procedure



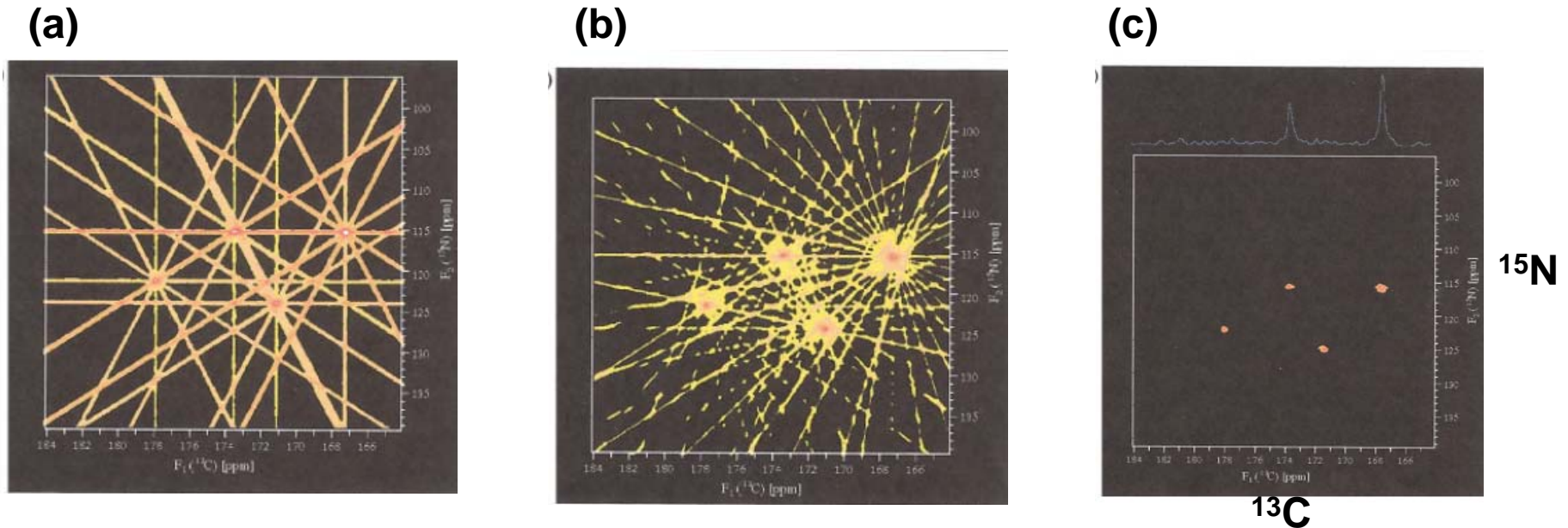
One F1F2 (C-N) plane at a time:

- (a).** A provisional (F1F2) spectrum is created by convolution of the projections on the F1 and F2 axes. "True + false" peaks are all present.
- (b)** A "mask" is created by back-projection of the tilted projection, generating parallel ridges, in this case two in number. This mask is superimposed on spectrum **(a)** and intensities at corresponding locations compared; retaining the lower intensity at each point.
- (c)** This eliminates peaks not overlapped by the ridges, leaving only three genuine correlation peaks.

Methods of reconstruction

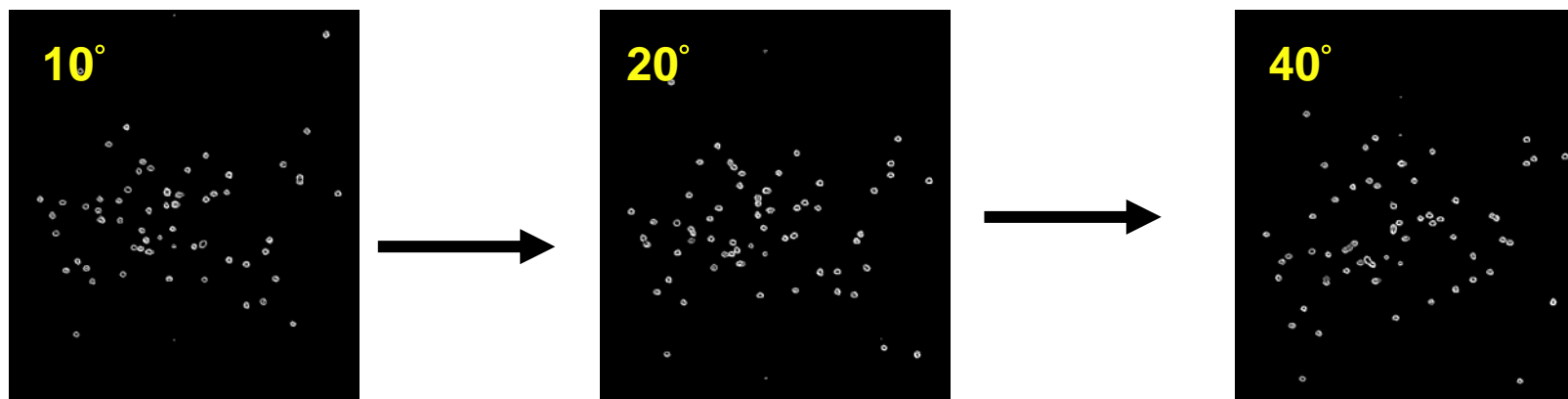
- **Good sensitivity:** (1). “Lower value” method for “all positive” cross peaks spectrum.
- (2). Use the “multiplication” method for spectrum containing “positive + negative” cross peaks (i.e. HNCACB).
- **Very poor S/N:** “addition” method (many more tilted angles acquired).

For a very low S/N case, use the “addition” reconstruction routine



The inverse Radon transform. Reconstruction of the cross-peaks in a typical plane of the 600-MHz three dimensional HNCO spectrum of ubiquitin, based on the addition of back-projections. **(a)**. Starting with only six projections at 0, 30, 60, and 90°. **(b)**. With 18 projections at 0, 10, 20, 30, 40, 50, 60, 70, 80, and 90° **(c)**. The same spectrum as panel (b) but with contours set above the level of the artifacts.

By changing the ratio between the two simultaneously evolving dimensions (^{13}C & ^{15}N), we can change the angle of the tilted plane. In Bruker's implementation, the tilt angle is specified directly with the parameter "cnst31".



Pulse programming:

```
"PI=3.141592653589793"
```

```
"in0=in1*cos(2*PI*cnst31/360)/2"
```

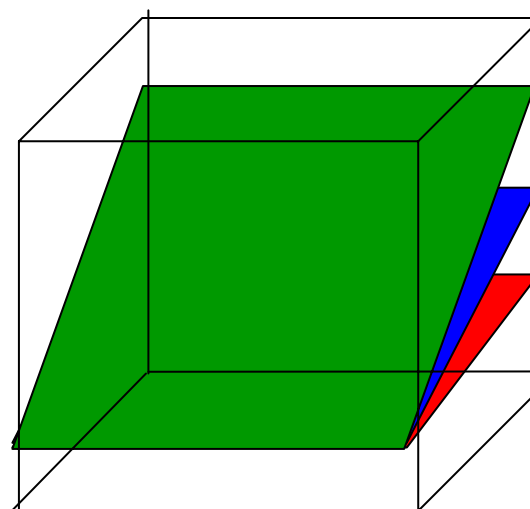
```
"in10=in2*sin(2*PI*cnst31/360)/4"
```

```
;nd0: 2
```

```
;in1: 1/sw (F1)
```

```
;in2: 1/sw (F2)
```

```
;nd10: 4
```

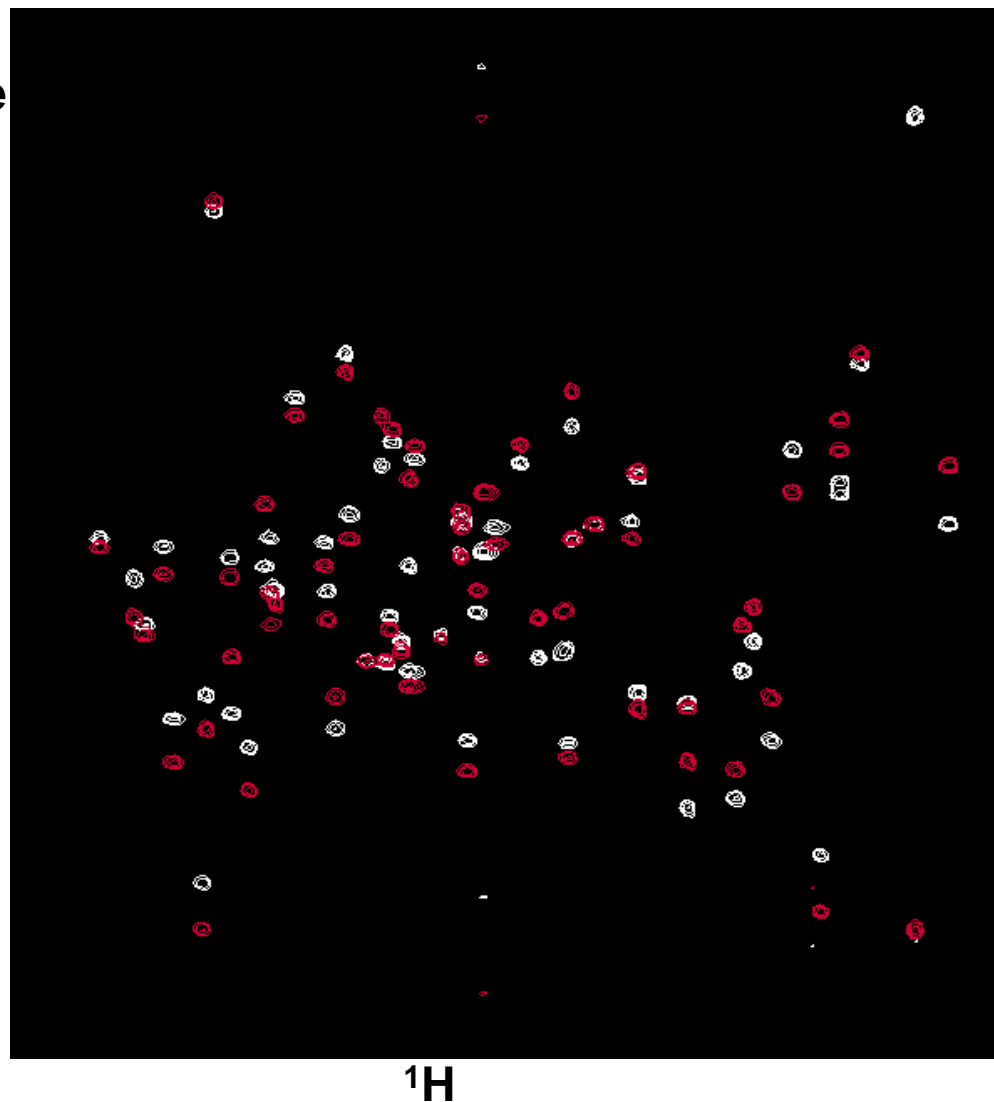


θ

Changing cnst31

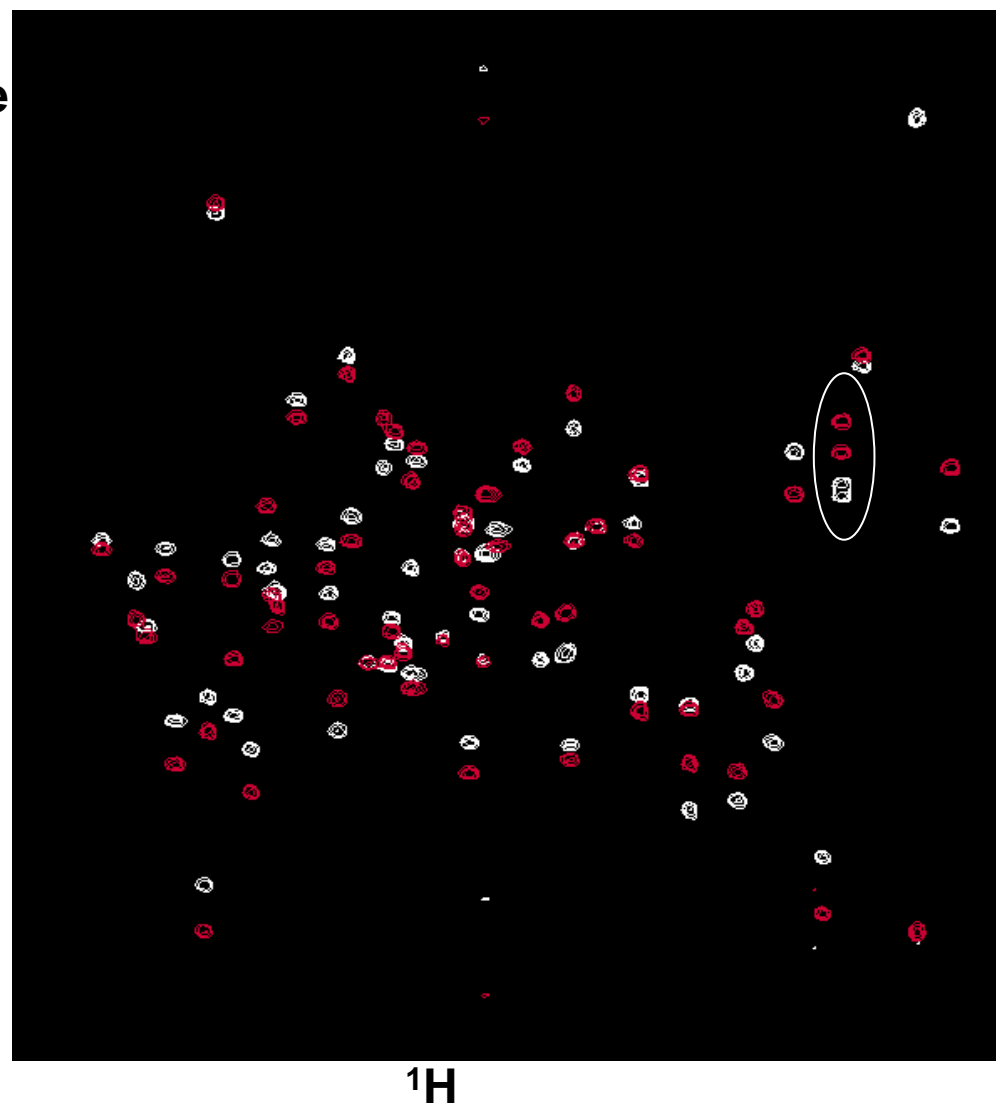
Overlapped peaks may be resolved by viewing from a different angle

10° tilted plane: white
20° tilted plane: red



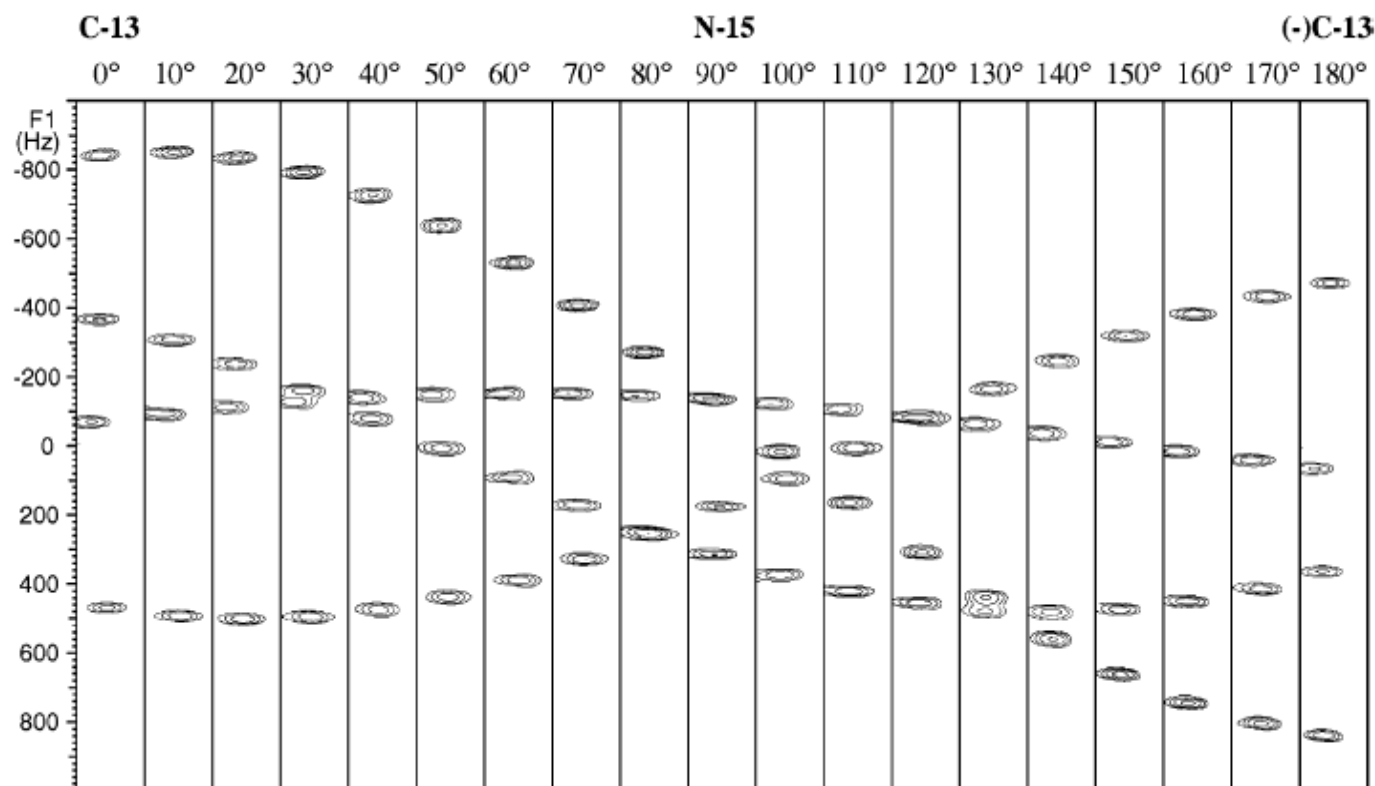
Overlapped peaks may be resolved by data from a different tilted angle

10° tilted plane: white
20° tilted plane: red

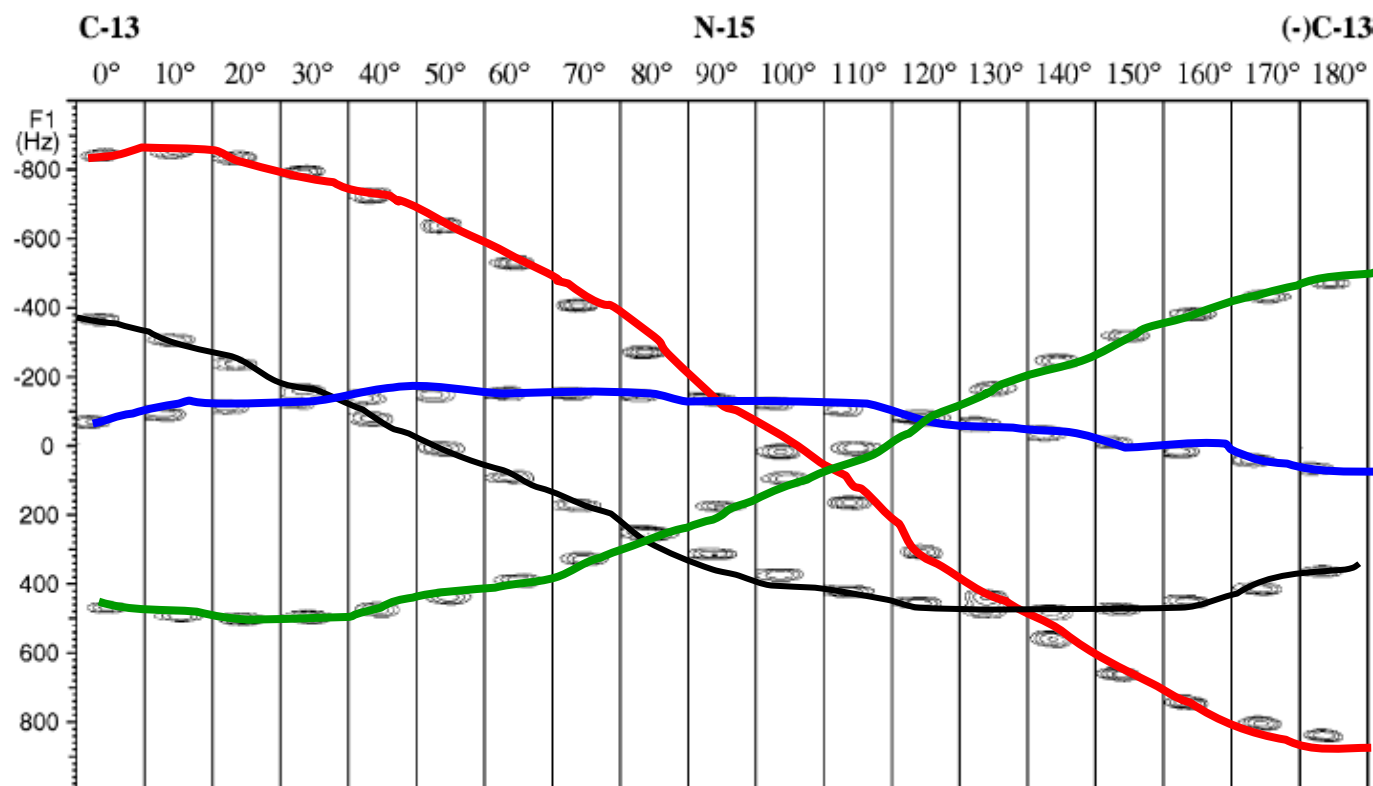


$^{13}\text{C}/^{15}\text{N}$

^1H



Projections of a selected section of the three-dimensional HNCO spectrum of ubiquitin onto planes that are progressively tilted in 10 increments starting from the C-H plane, running through the N-H plane (center) and back to the C-H plane.



Projections of a selected section of the three-dimensional HNCO spectrum of ubiquitin onto planes that are progressively tilted in 10° increments starting from the C-H plane, running through the N-H plane (center) and back to the C-H plane.

- **Each of the four cross-peaks follows a sinusoidal trajectory with relatively few exact frequency degeneracies with other cross-peaks, suggesting that the selection of the tilt angle is not particularly critical.**

E. Kupče and R. Freeman J. Am. Chem. Soc., 126, 6429 -6440, 2004.

Some math in the processing of Projection Reconstruction NMR

$t_1 = t (\cos\Phi)$, $t_2 = t (\sin\Phi)$
 Φ : tilted angle

δ_A : ^{13}C , δ_B : ^{15}N

$$M_1 = \cos(2\pi\delta_A t \cos \phi) \cos(2\pi\delta_B t \sin \phi). \quad [3]$$

$$M_1 = \cos(2\pi\delta_A t \cos \phi) \cos(2\pi\delta_B t \sin \phi). \quad [3]$$

$$M_2 = \sin(2\pi\delta_A t \cos \phi) \cos(2\pi\delta_B t \sin \phi) \quad [4]$$

$$M_3 = \cos(2\pi\delta_A t \cos \phi) \sin(2\pi\delta_B t \sin \phi) \quad [5]$$

$$M_4 = \sin(2\pi\delta_A t \cos \phi) \sin(2\pi\delta_B t \sin \phi) \quad [6]$$

$$\text{Re} = \cos(2\pi\delta_A \cos \phi - 2\pi\delta_B \sin \phi) \quad [7]$$

$$\text{Im} = \sin(2\pi\delta_A \cos \phi - 2\pi\delta_B \sin \phi), \quad [8]$$

which gives the difference frequency $2\pi\delta_A \cos \phi - 2\pi\delta_B \sin \phi$. The same data set gives the complex conjugate by negating the $\sin \phi$ terms:

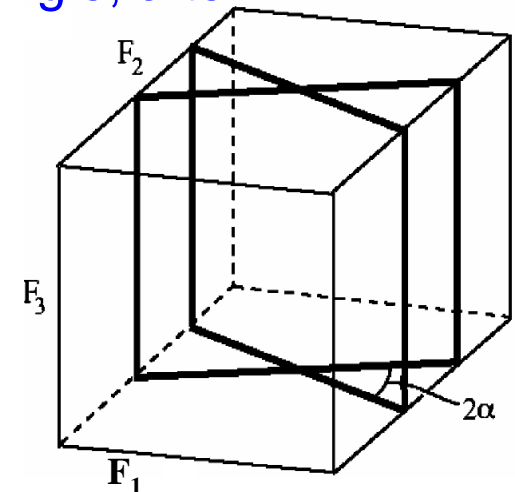
$$\text{Re} = \cos(2\pi\delta_A \cos \phi + 2\pi\delta_B \sin \phi) \quad [9]$$

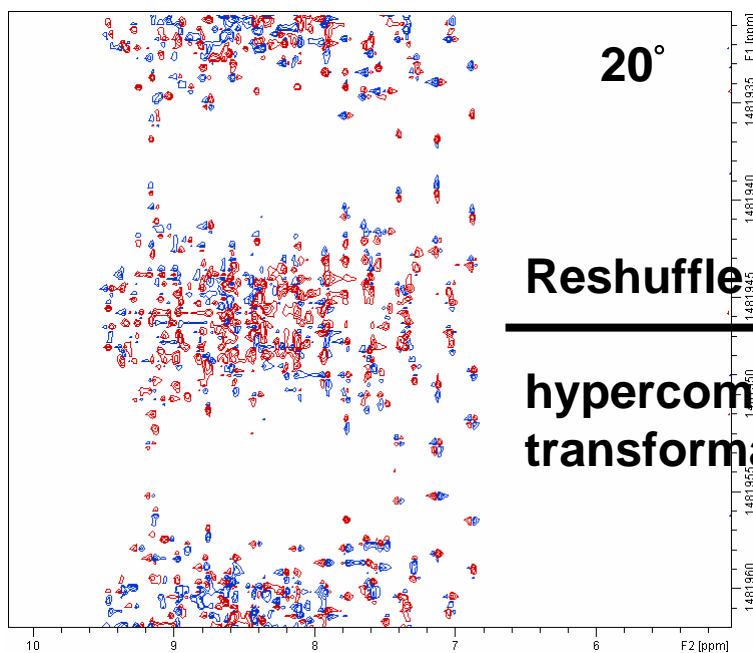
$$\text{Im} = \sin(2\pi\delta_A \cos \phi + 2\pi\delta_B \sin \phi), \quad [10]$$

which gives the sum frequency $2\pi\delta_A \cos \phi + 2\pi\delta_B \sin \phi$. This explains why projections always occur in pairs tilted at $\pm\phi$. The factors $\cos \phi$ and $\sin \phi$ simply reflect the tilting effect.

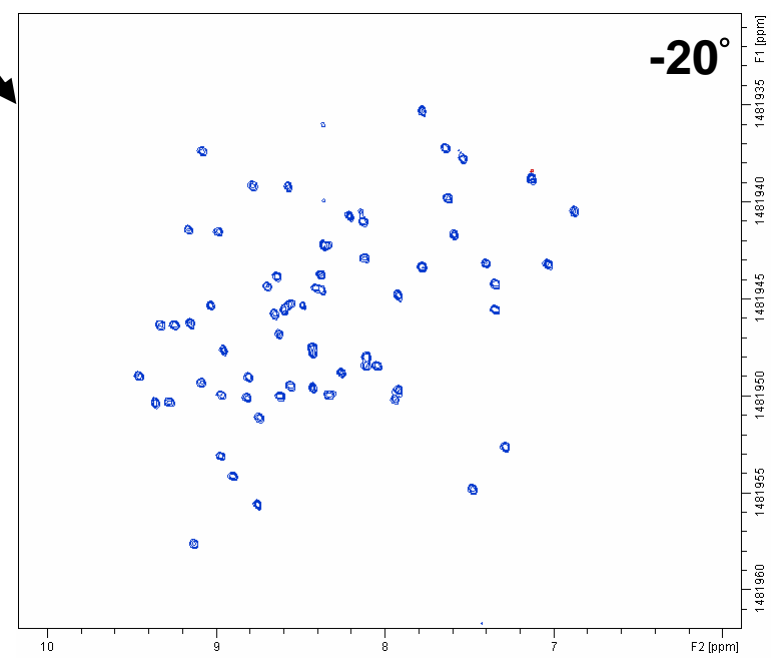
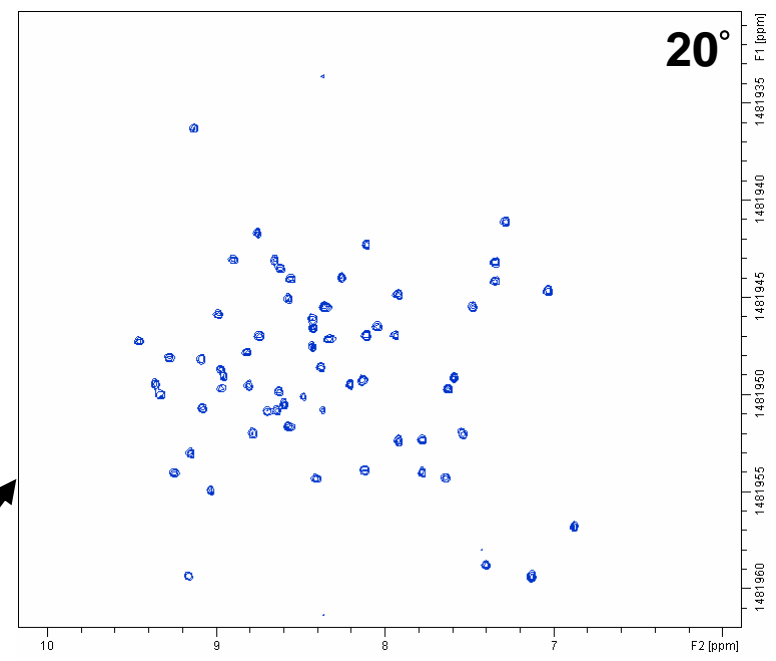
The projections always appear in pair tilted at α and $-\alpha$

- With the quadrature (complex) detection in both of the ^{13}C and ^{15}N dimension, and after the standard hypercomplex Fourier transformation:
- Sum frequency: $2\pi\delta_{\text{C}}\cos\alpha + 2\pi\delta_{\text{N}}\sin\alpha \dots\dots(1)$
- Difference frequency: $2\pi\delta_{\text{C}}\cos\alpha - 2\pi\delta_{\text{N}}\sin\alpha \dots\dots(2)$,
Since the tilted angle α is known, δ_{C} and δ_{N} can be obtained individually now.
- Two projections tilted through angle $\pm\alpha$ about the ^1H axis are generated. Therefore, **when we acquire a $+40^\circ$ tilted angle, after processing, we also obtain a -40° tilted angle data.**





**Reshuffle &
hypercomplex
transformation**



Sensitivity

- The sensitivity per unit time remains the same, but the overall sensitivity is reduced in proportion to the square root of the acquisition time. Fortunately modern high-field spectrometer, particularly those with a cryoprobe, often have a high enough intrinsic sensitivity that the final S/N is still viable.
- Increase sensitivity: higher sample concentration; use a cryoprobe (R.T. probe feasible); more scans in each of the tilted 2D experiments. (this may be still much faster than the conventional mode).

Tilted angle

- Choose a proper tilted angle to avoid overlap.
- More tilted angles are needed for a crowded spectrum.
- Higher field is preferred for PR-NMR (better dispersion).

Availability of Projection Reconstruction NMR

AU programs (-> /TOPSPIN/exp/stan/nmr/au/src)

- pr_setup.be (set up)
- pr_proc.be (rearrange data)
- pr_recos.be (3d reconstruction)
- pr_alpha.be (tilt angle prediction)

Pulse programs (-> /TOPSPIN/exp/stan/nmr/lists/pp)

- pr_hncogp3d.t1.be
- **pr_hncogp3dsc.ww (¹⁵N-semi constant time)**
- pr_hncagp3d.t1.be
- pr_hncagp3dsc.ww (¹⁵N-semi constant time)
- pr_hncocagp3d.ww
- **pr_hncocagp3dsc.ww (¹⁵N-semi constant time)**
- pr_hncacogp3d.ww
- pr_hncacogp3dsc.ww (¹⁵N-semi constant time)
- pr_hncacbgp3d_dp9.ww
- **pr_hncacbgp3dsc_dp9.ww (¹⁵N-semi constant time)**
- pr_hncbcp3d_dp9.ww
- pr_hncbcp3dsc_dp9.ww (¹⁵N-semi constant time)
- **pr_cbcaconhgp3dsc.ww (¹⁵N-semi constant time)**

Software Requirement for PR-NMR

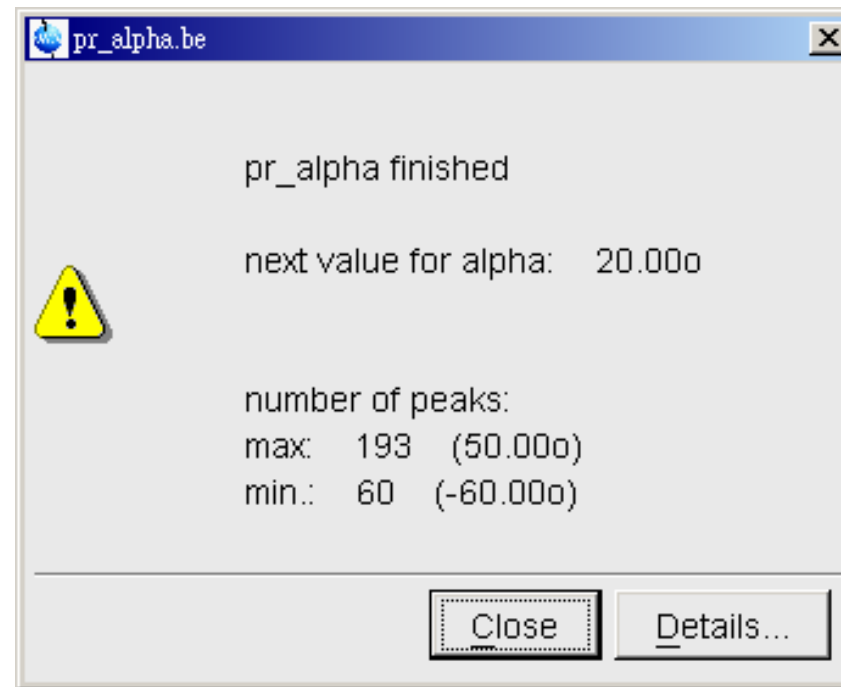
Requirement:

Any NMR spectrometers capable of performing conventional H-C-N triple resonance experiment and have **TopSpin 1.3 and above**. And the AU program and pulse sequences in the previous slides.

Prediction of the next tilted angle for data collection

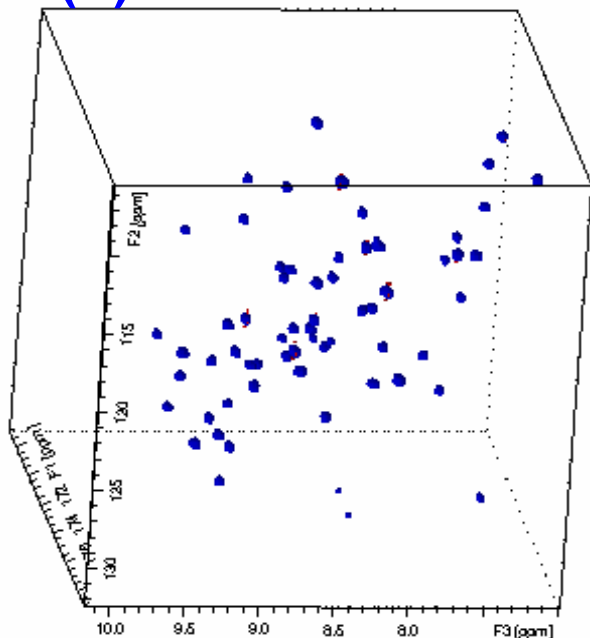
- **The next tilted angle:** The program “pr_alpha.be” calculates an F1F3 projection from the reconstructed cube and counts the peaks therein. It will do this for angles between -80° and $+80^\circ$ and will search for a maximum. The idea is that the best suited projection angle is the one with the largest number of peaks. So the program will suggest the next projection angle to be recorded.

next:	20.0	107
max:	50.0	193
min: -60.0	60	
proj. mode:	2	
-80.0	67	
-70.0	73	
-60.0	60	
-50.0	107	
-40.0	155	
-30.0	125	
-20.0	102	
-10.0	66	
0.0	127	
10.0	77	
20.0	107	
30.0	121	
40.0	177	
50.0	193	
60.0	111	
70.0	114	
80.0	81	



3D HNCO

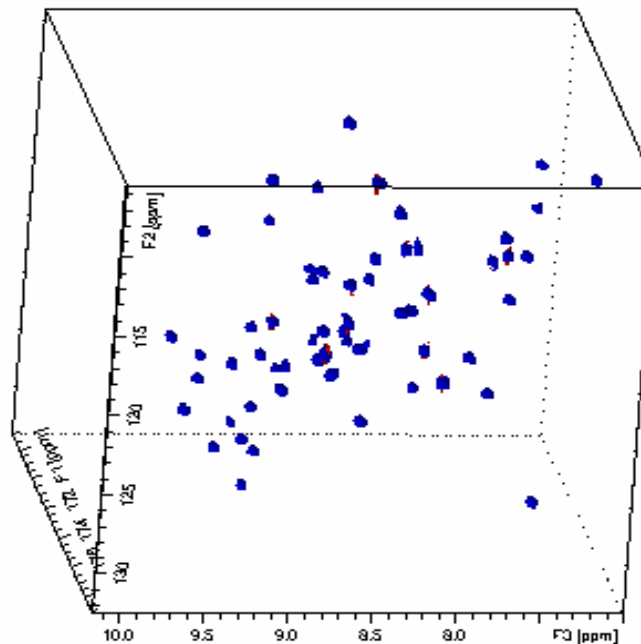
(A). Conventional



Acquisition time: 12 hr

Ni=32 (^{15}N), 32 (^{13}C),

(B). Projection reconstruction



Acquisition time: 51 min

(reconstructed from 0° , 90° , $\pm 40^\circ$)

Only three 2D spectra acquired.

Acquisition time: (B)/(A)= 1/14

Sample: chicken SH3 (62 A.A.), 1.5 mM, pH 3.6

Predicted tilted angle

¹⁵N-semi constant time PR-HNCO of SH3 (62 a.a.)

40,-40

next: 50
 max: 80
 min: -60
 proj. mode: 2

-80	61
-70	61
-60	58
-50	62
-40	63
-30	60
-20	62
-10	60
0	61
10	61
20	61
30	59
40	61
50	62
60	60
70	61
80	63

50,-50

next: -20
 max: -80
 min: 50
 proj. mode: 2

-80	65
-70	61
-60	61
-50	61
-40	61
-30	62
-20	63
-10	62
0	60
10	59
20	60
30	59
40	60
50	59
60	61
70	62
80	62

20,-20

next: 70
 max: -80
 min: 50
 proj. mode: 2

-80	65
-70	61
-60	61
-50	61
-40	61
-30	62
-20	63
-10	62
0	60
10	59
20	60
30	59
40	60
50	59
60	62
70	62
80	62

70,-70

next: 60
 max: -80
 min: 50
 proj. mode: 2

-80	65
-70	61
-60	61
-50	60
-40	61
-30	62
-20	63
-10	62
0	60
10	59
20	60
30	59
40	60
50	59
60	62
70	62
80	62

60,-60

next: 10
 max: 80
 min: -10
 proj. mode: 2

-80	61
-70	61
-60	58
-50	59
-40	61
-30	61
-20	61
-10	57
0	60
10	60
20	61
30	59
40	62
50	62
60	60
70	61
80	62

10,-10

next:
 max:
 min:
 proj.

Predicted tilted angle

¹⁵N-semi constant time PR-HNCO of SH3 (62 a.a.)

40,-40

next:	50
max:	80
min:	-60
proj. mode:	2
-80	61
-70	61
-60	58
-50	62
-40	63
-30	60
-20	62
-10	60
0	61
10	61
20	61
30	59
40	61
50	62
60	60
70	61
80	63

50,-50

next:	-20
max:	-80
min:	50
proj. mode:	2
-80	65
-70	61
-60	61
-50	61
-40	61
-30	61
-20	61
-10	61
0	61
10	61
20	61
30	59
40	60
50	59
60	61
70	62
80	62

20,-20

next:	70
max:	-80
min:	50
proj. mode:	2
-80	65
-70	61
-60	61
-50	61
-40	61
-30	62
-20	63
-10	62
0	60
10	59
20	60
30	59
40	60
50	59
60	62
70	62
80	62

70,-70

next:	60
max:	-80
min:	50
proj. mode:	2
-80	65
-70	61
-60	61
-50	60
-40	61
-30	62
-20	63
-10	62
0	60
10	59
20	60
30	59
40	60
50	59
60	62
70	62
80	62

60,-60

next:	10
max:	80
min:	-10
proj. mode:	2
-80	61
-70	61
-60	58
-50	59
-40	61
-30	61
-20	61
-10	57
0	60
10	60
20	61
30	59
40	62
50	62
60	60
70	61
80	62

10,-10

next:	
max:	
min:	
proj. mode:	

For a well-dispersed spectrum, as few as one tilted angle is sufficient and the exact tilted angle is not so important!

pr_recos

- **Reconstruction of a 3D cube from 2D tilted planes:** The program “**pr_recos.be**” reconstructs the 3D cube; it also calculates an F1F2 projection and counts the peaks therein. The number can be used to check how the reconstruction improves as the number of peaks should converge towards the end.

Status of reconstruction

¹⁵N-semi constant time PR-HNCO
of SH3 (62 a.a.)

18 (total # angles) Expt#	Angle	P.R.Mode	#p_peak	#n_peak
1200	0.0	1		
1201	90.0	1		
1206	30.0	1		
1207	-30.0	1	64	600
1216	80.0	1		
1217	-80.0	1	61	517
1208	40.0	1		
1209	-40.0	1	61	541
1210	50.0	1		
1211	-50.0	1	60	594
1204	20.0	1		
1205	-20.0	1	60	586
1214	70.0	1		
1215	-70.0	1	60	547
1212	60.0	1		
1213	-60.0	1	60	
1202	10.0	1		
1203	-10.0	1	60	

Status of reconstruction

¹⁵N-semi constant time PR-HNCO
of SH3 (62 a.a.)

18 (total # angles) Expt#	Angle	P.R.Mode	#p_peak	#n_peak
1200	0.0	1		
1201	90.0	1		
1206	30.0	1		
1207	-30.0	1	64	600
1216	80.0	1		
1217	-80.0	1	61	517
1208	40.0	1		
1209	-40.0	1	61	541
1210	50.0	1		
1211	-50.0	1	60	594
1204	20.0	1		
1205	-20.0	1	60	586
1214	70.0	1		
1215	-70.0	1	60	547
1212	60.0	1		
1213	-60.0	1	60	
1202	10.0	1		
1203	-10.0	1	60	

Status of reconstruction

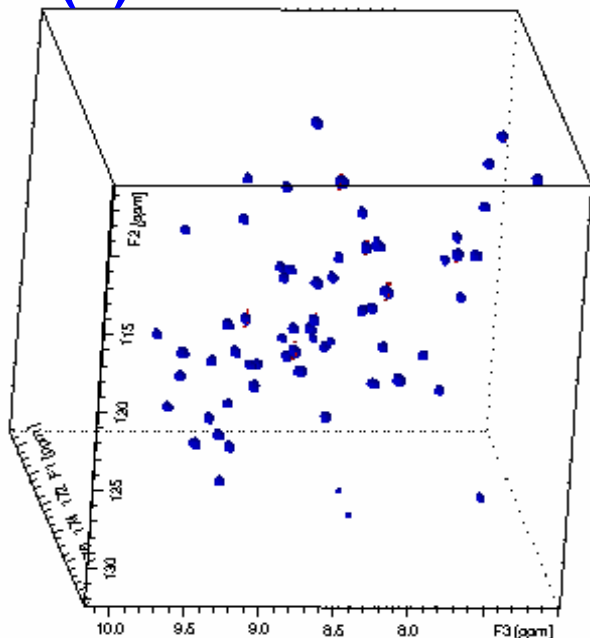
¹⁵N-semi constant time PR-HNCO
of SH3 (62 a.a.)

18 (total # angles) Expt#	Angle	P.R.Mode	#p_peak	#n_peak
1200	0.0	1		
1201	90.0	1		
1206	30.0	1		
1207	-30.0	1	64	
1216	80.0	1		
1217	-80.0	1	61	
1208	40.0	1		
1209	-40.0	1	61	541
1210	50.0	1		
1211	-50.0	1	60	594
1204	20.0	1		
1205	-20.0	1	60	586
1214	70.0	1		
1215	-70.0	1	60	547
1212	60.0	1		
1213	-60.0	1	60	
1202	10.0	1		
1203	-10.0	1	60	

One additional
tilted angle is
sufficient to
reconstruct a 3D
HNCO for SH3

3D HNCO

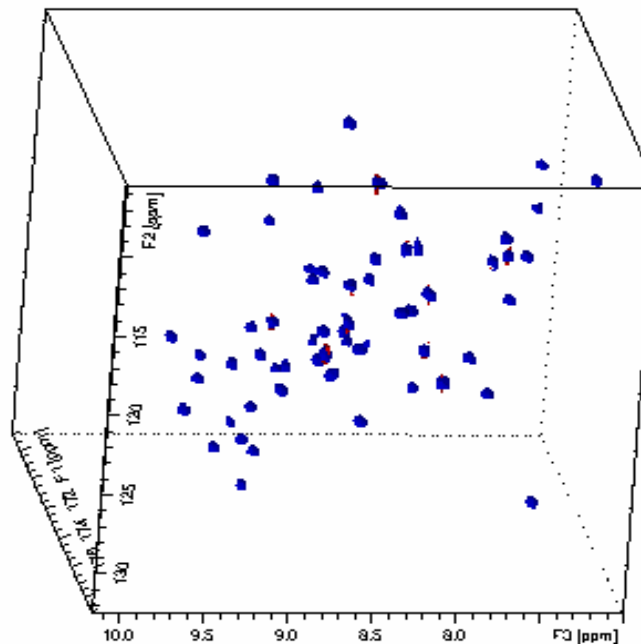
(A). Conventional



Acquisition time: 12 hr

Ni=32 (^{15}N), 32 (^{13}C),

(B). Projection reconstruction



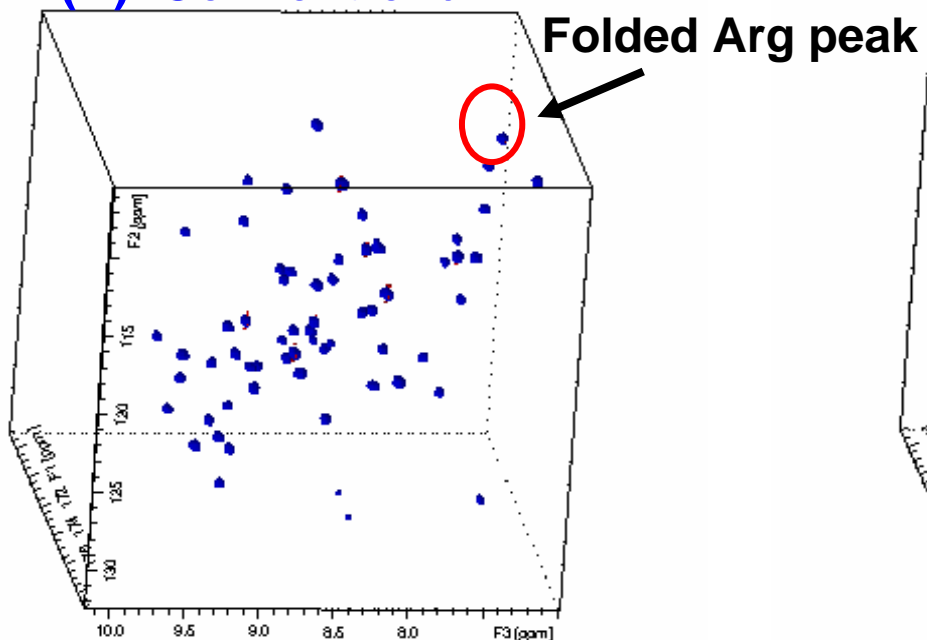
Acquisition time: 51 min
(reconstructed from 0° , 90° , $\pm 40^\circ$)
Only three 2D spectra acquired.

Acquisition time: (B)/(A)= 1/14

Sample: chicken SH3 (62 A.A.), 1.5 mM, pH 3.6

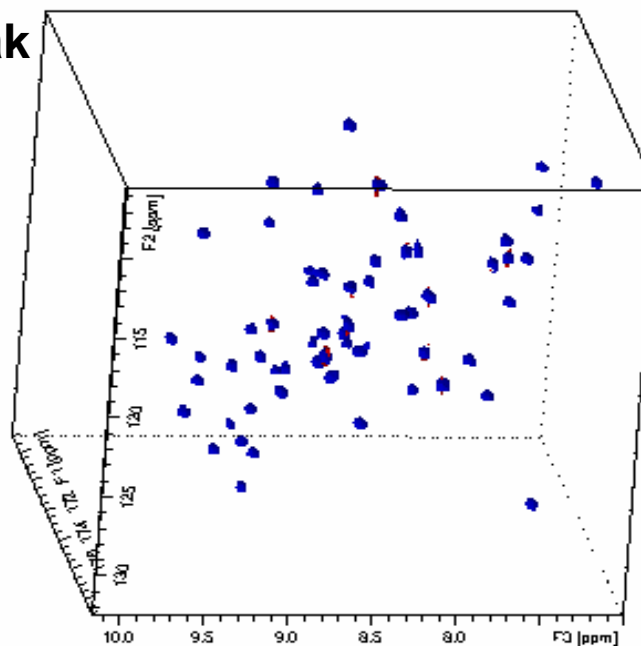
3D HNCO

(A). Conventional



Acquisition time: 12 hr

(B). Projection reconstruction



Acquisition time: 51 min
(reconstructed from 0° , 90° , $\pm 40^\circ$)

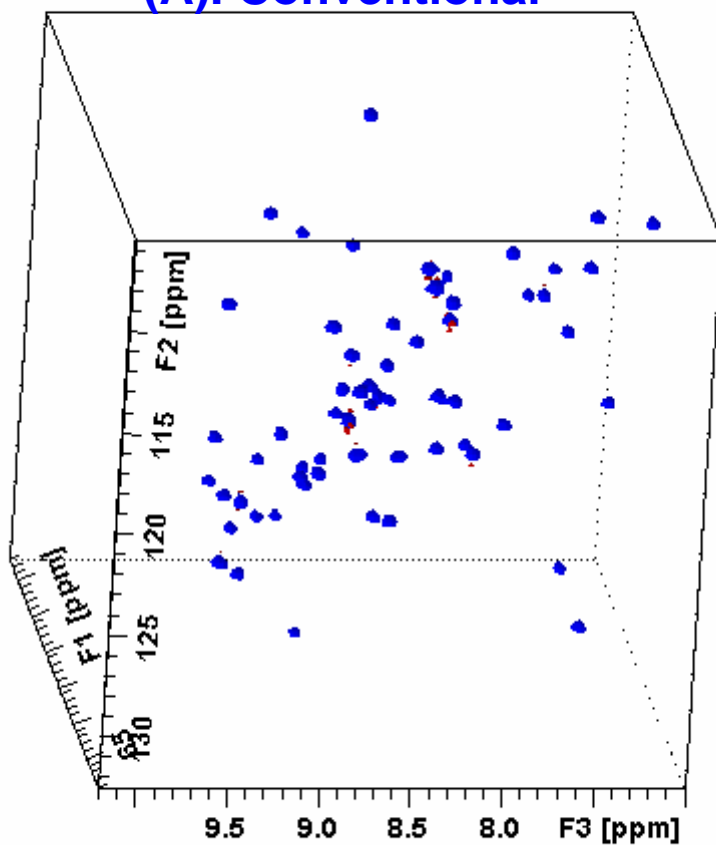
Acquisition time: (B)/(A)= 1/14

Note: Folded peaks can not be observed in projection-reconstruction NMR !

Sample: chicken SH3 (62 A.A.), 1.5 mM, pH 3.6

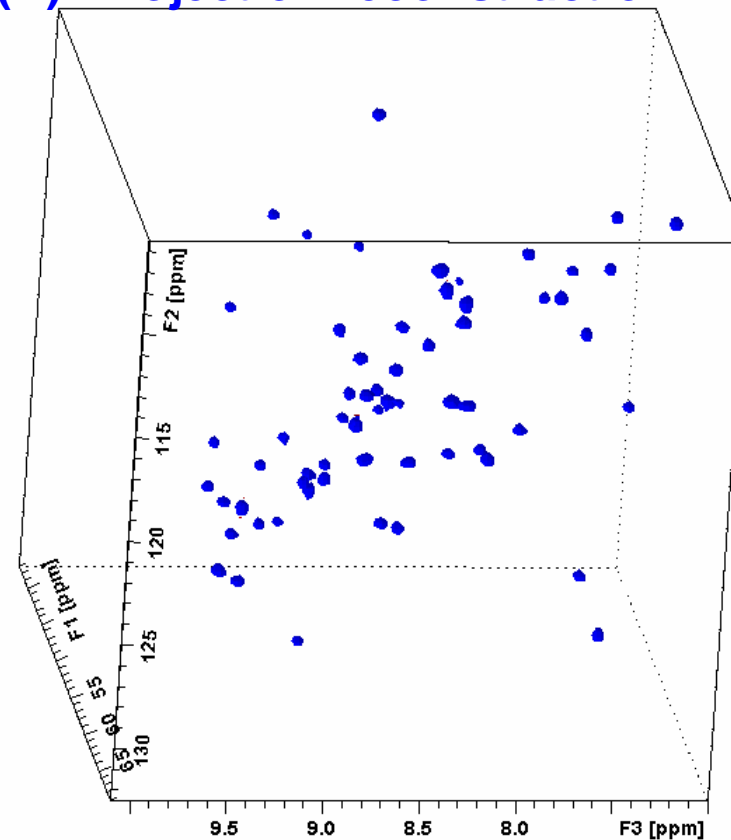
HN(CO)CA

(A). Conventional



Acquisition time: 35 hr

(B). Projection reconstruction



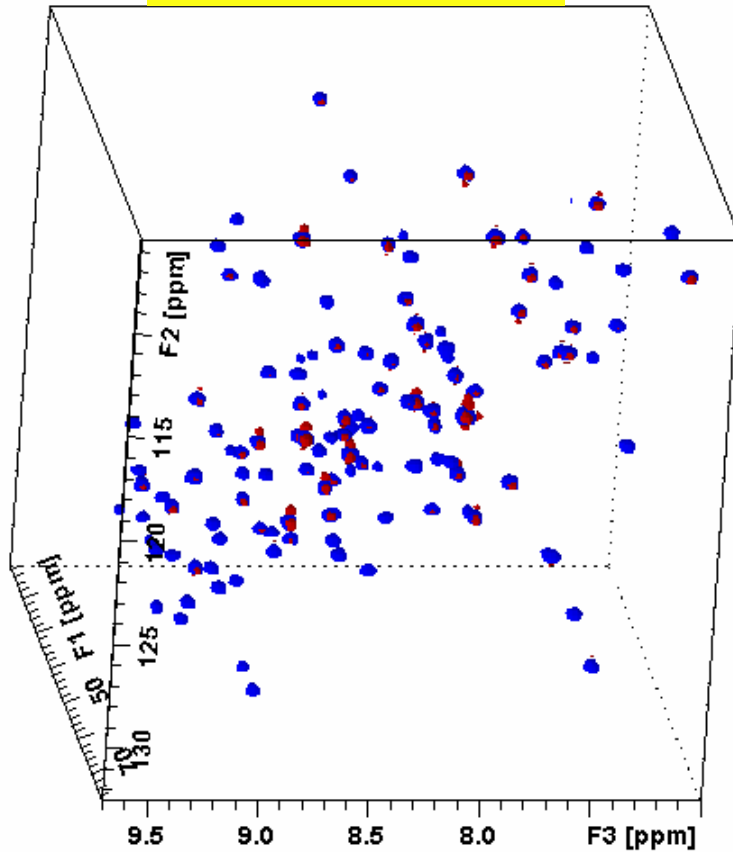
Acquisition time: 2 hr 15 min

(reconstructed from 0° , 90° , $\pm 40^\circ$)

Acquisition time: (B)/(A)= 1/15

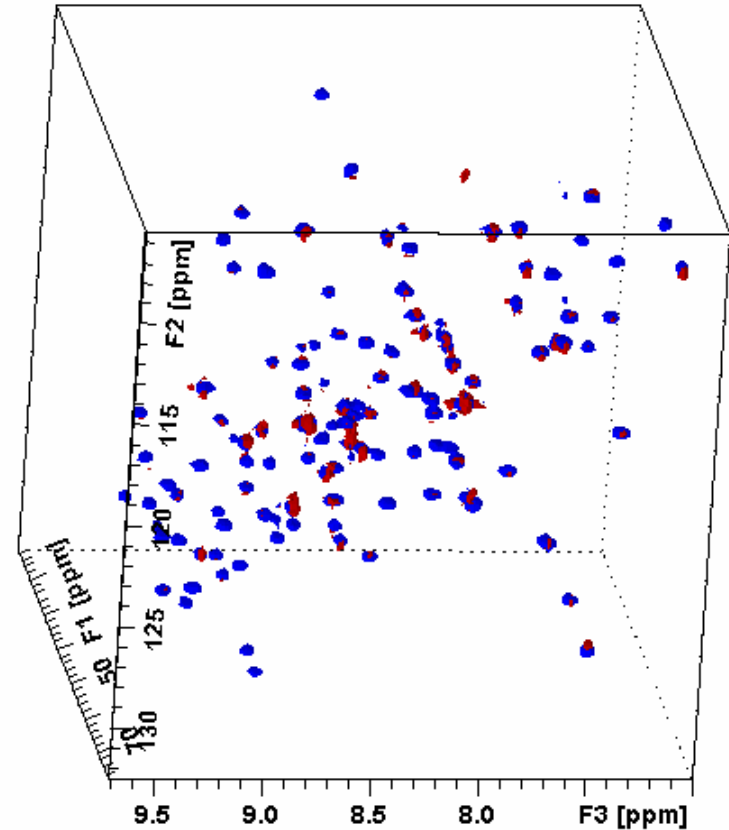
CBCA(CO)NH

Conventional 3D



Data collection time :40 hr

Projection reconstruction

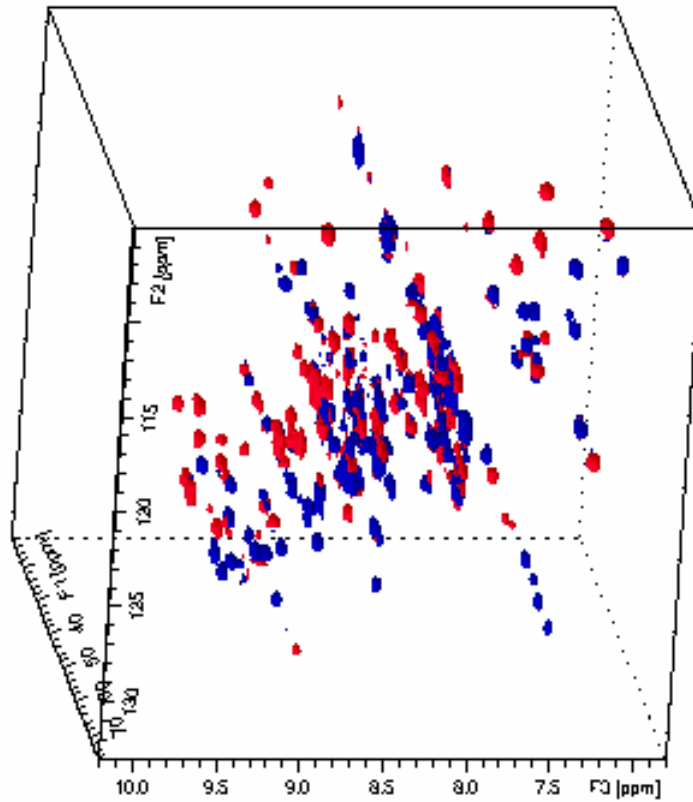


Data collection time: 6 hr

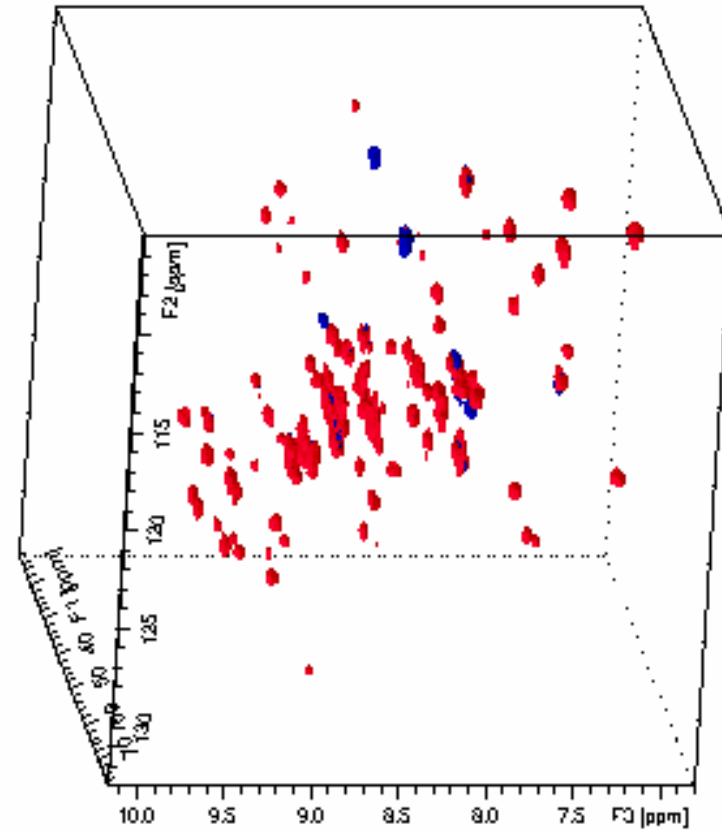
Reconstructed from $0^\circ, 90^\circ, \pm 20^\circ, \pm 40^\circ, \pm 60^\circ, \pm 80^\circ$

Acquired on a 600 MHz room temperature probe, $ns=32$

PR-HN(CA)CB



PR-HNCACB

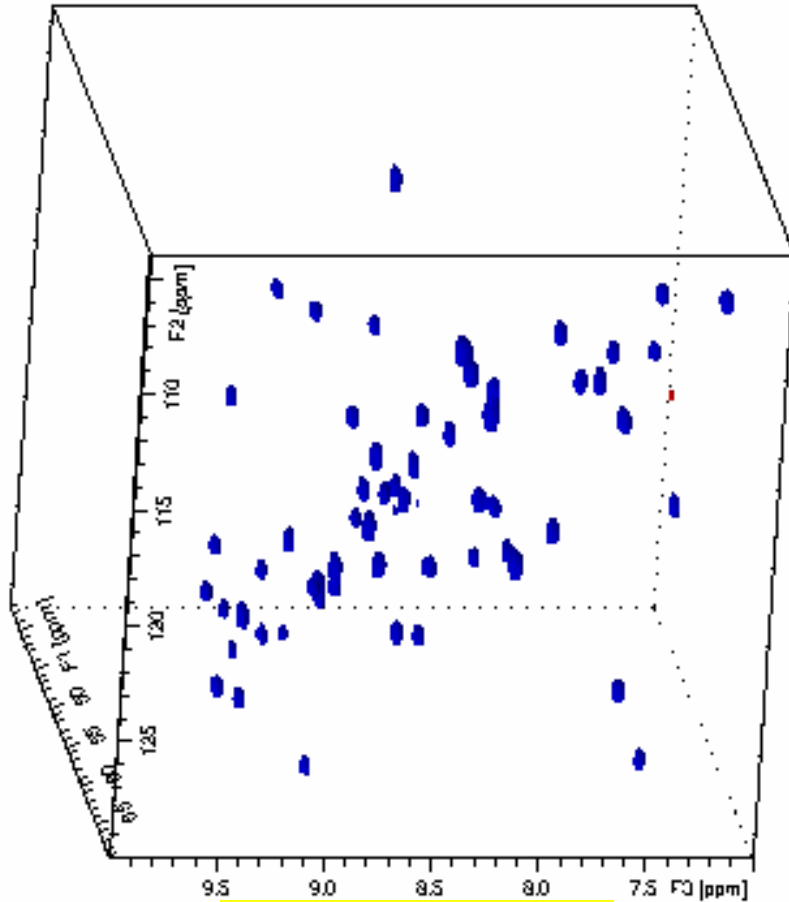


PR-HN(CA)CB

**Resolution Enhancement
via
 ^{15}N -semi constant time
chemical shift evolution**

PR-HN(CO)CA

Sample: SH3, 62 a.a.

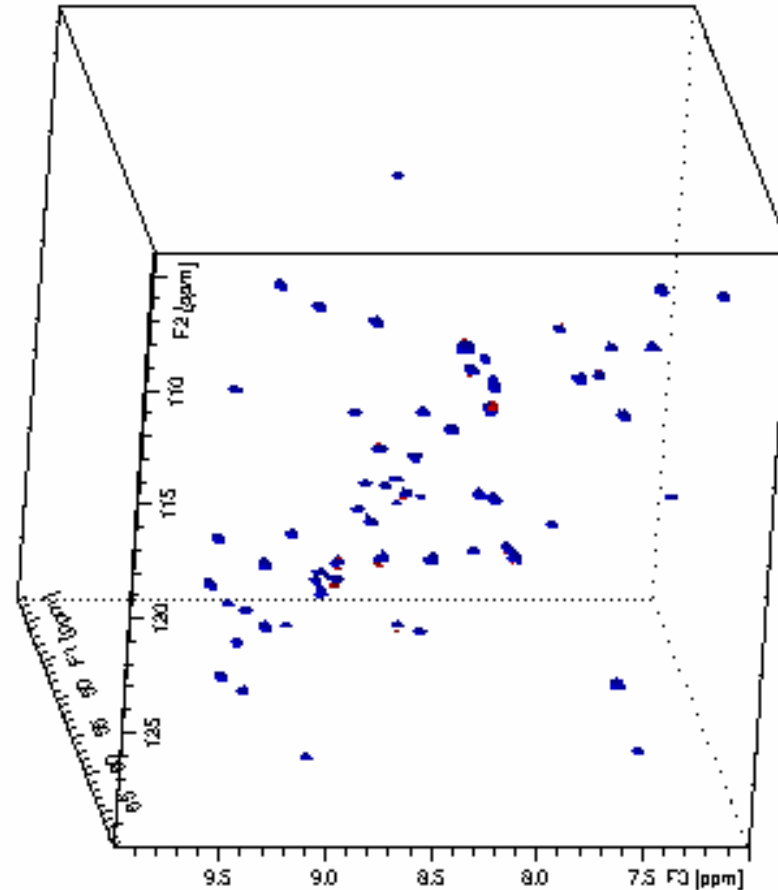


Conventional 3D

^{15}N -constant time

Ni=24 (^{15}N) * 32 (^{13}C)

ns=2; acq. 31 min.



PR-NMR ($0^\circ, 90^\circ, \pm 30^\circ$)

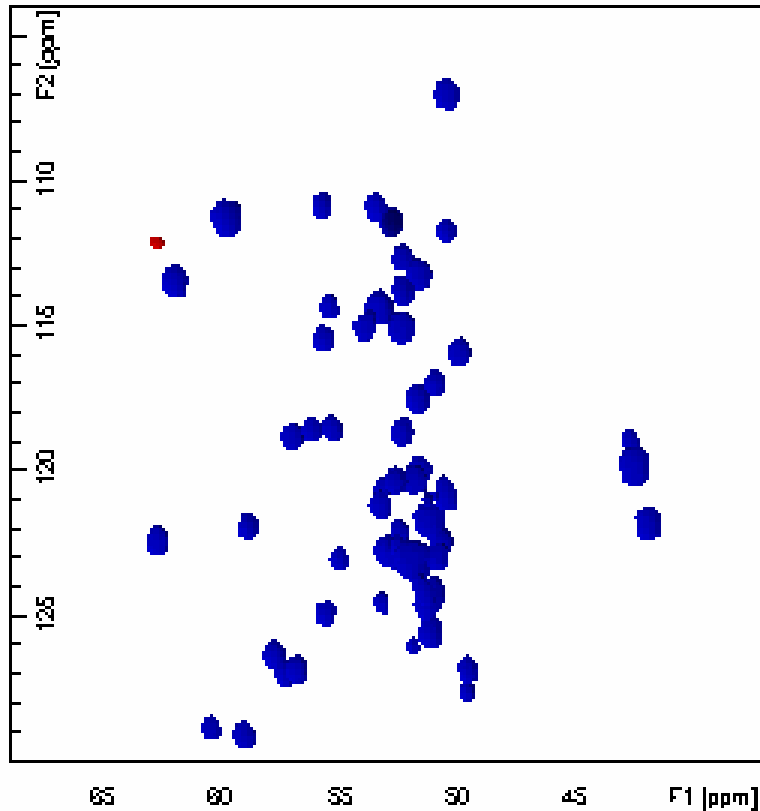
^{15}N - semi-constant time

Ni=128 (^{15}N & ^{13}C)

ns=2; acq. 22 min.

PR-HN(CO)CA, ^{15}N -semi constant time

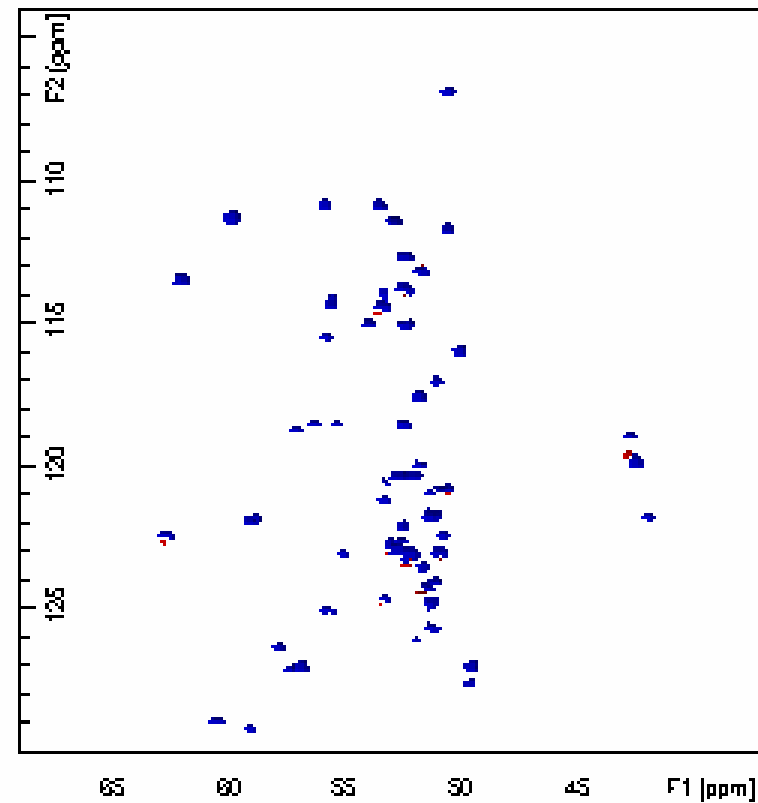
Sample: SH3, 62 a.a.



Conventional 3D

Ni=24 (^{15}N) *32 (^{13}C)

ns=2; acq. 31 min.



PR-NMR ($0^\circ, 90^\circ, \pm 30^\circ$)
 ^{15}N - semi-constant time

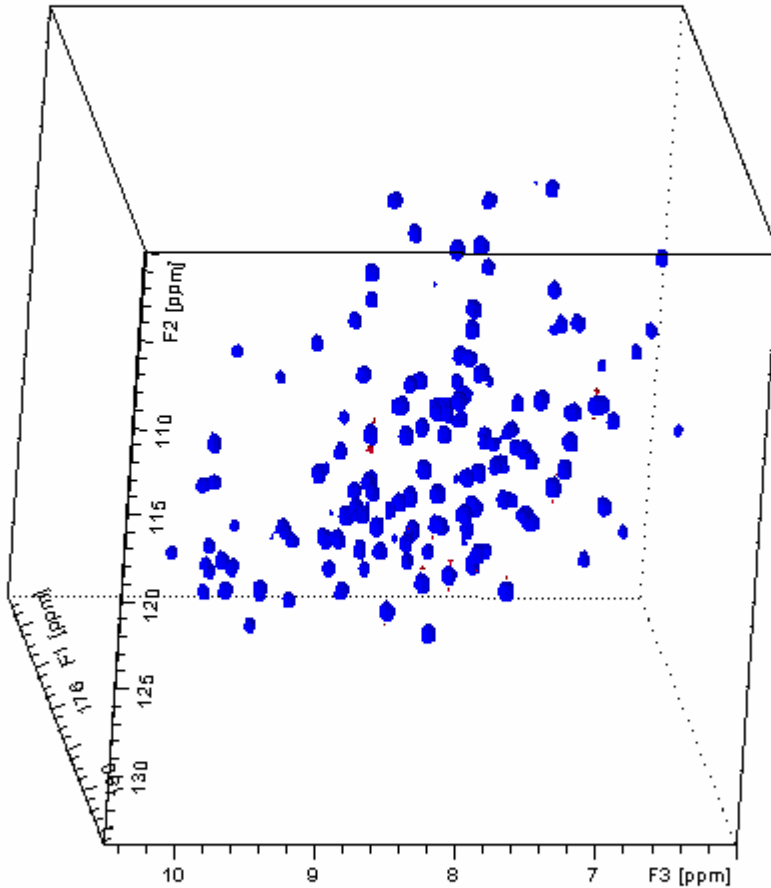
Ni=128 (^{15}N & ^{13}C)

ns=2; acq. 22 min.

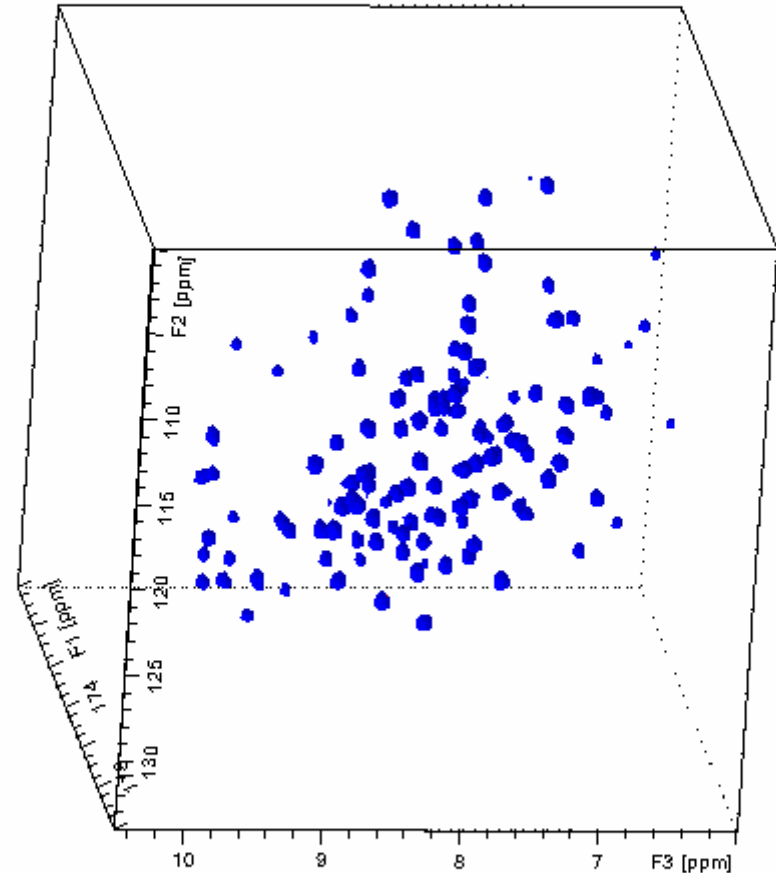
**Testing
for
larger proteins**

HNCO of KP1966v0_CoA, 138 a.a.

Both spectrum acquired on AV500_CRP



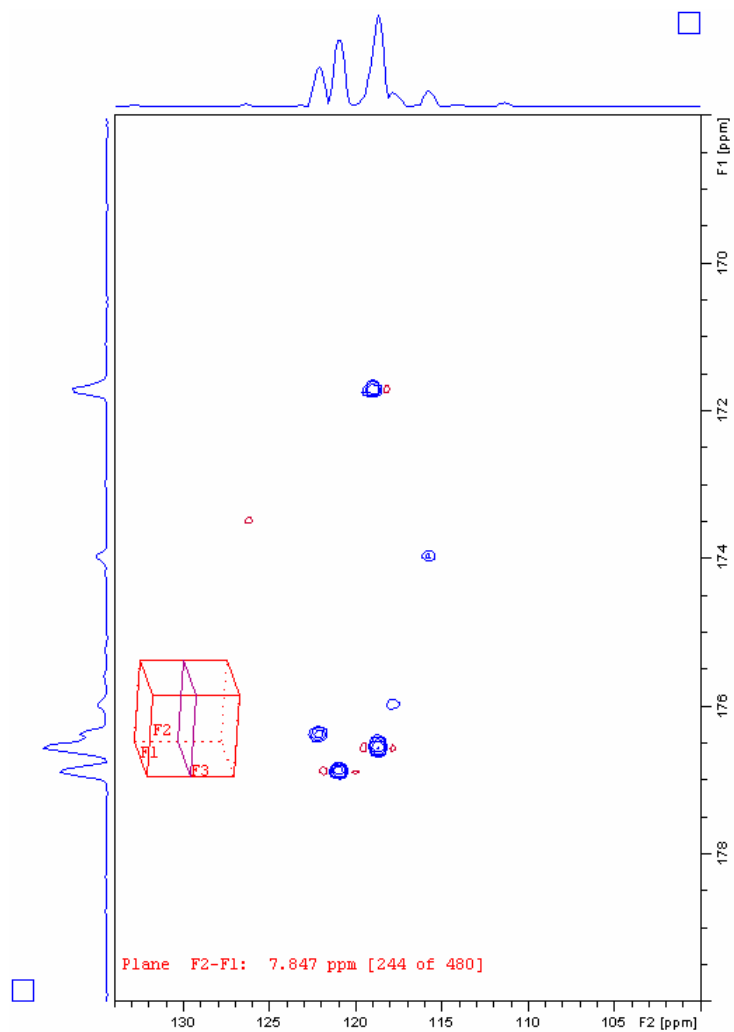
Conventional 3D HNCO
Acquisition time: 793 min



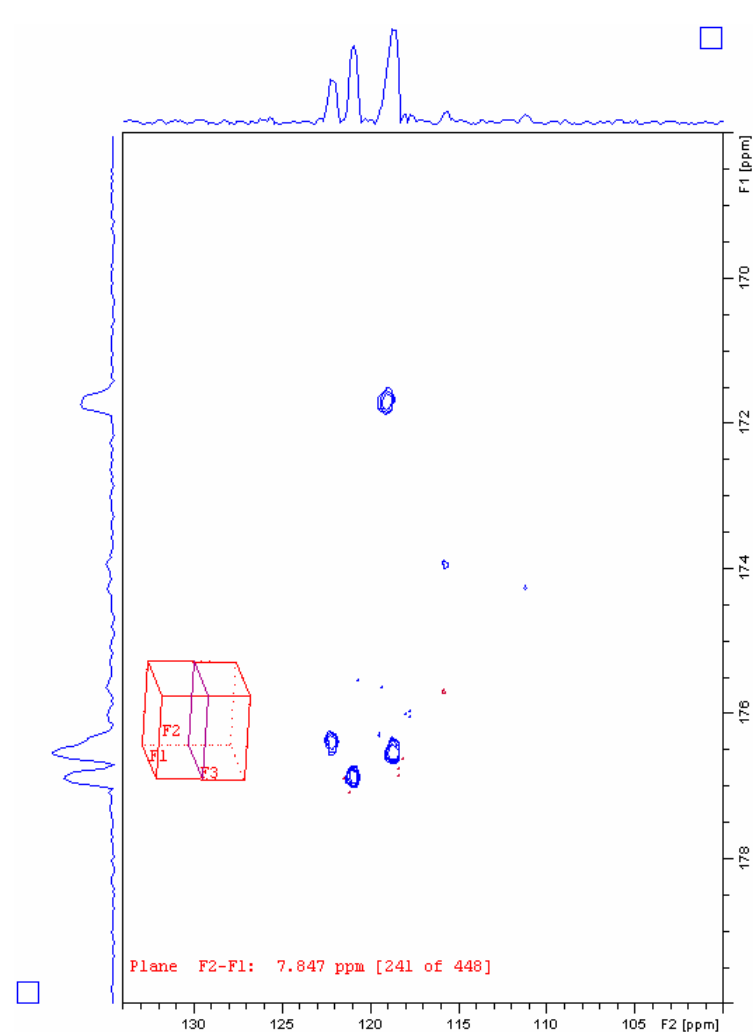
PR-HNCO, (0°, 90°, ±30°, ±40°, ±50°, ±60°)
Acquisition time=225 min
(Semi-constant time in ¹⁵N)

HNCO of KP1966v0_CoA

AV500_CRP

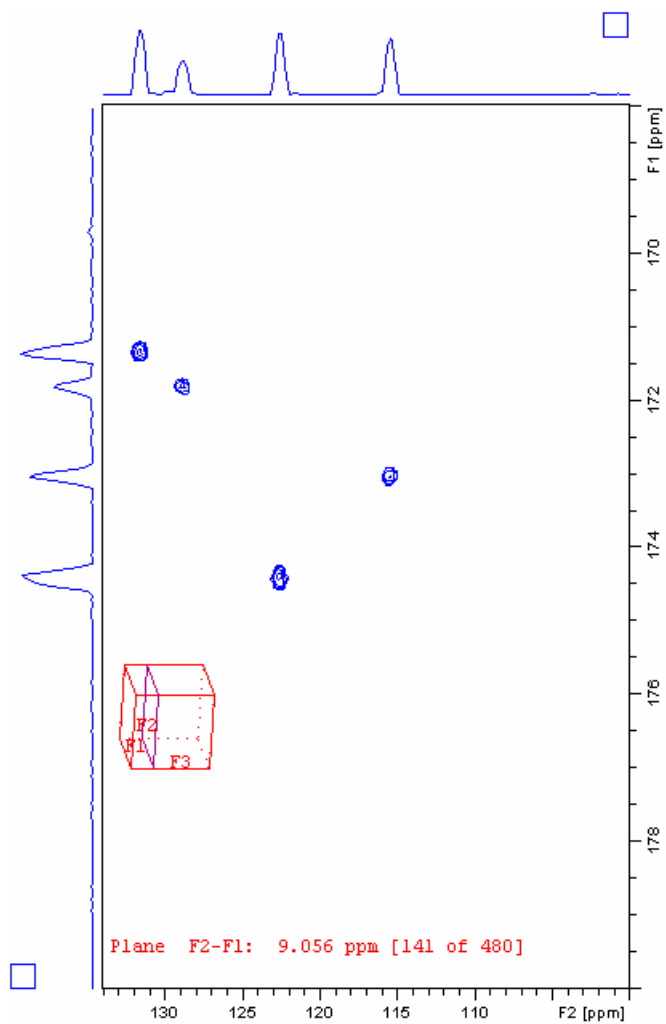


Conventional 3D HNCO

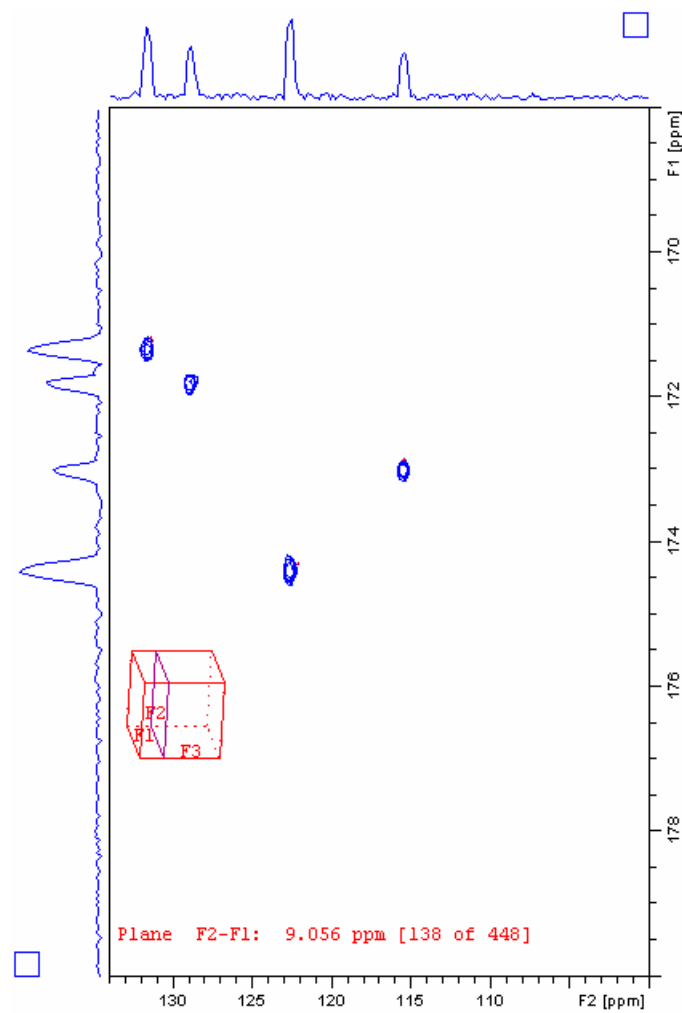


PR-HNCO, 0° , 90° , 30° , 40° , 50° , 60°
(Semi-constant time in ^{15}N)

HNCO of KP1966v0_CoA



Conventional 3D HNCO



PR-HNCO, (0°, 90°, ±30°, ±40°, ±50°, ±60°)

(Semi-constant time in ¹⁵N)

Nc-kp1966v0-coa/507/pr_recos HNCO_semi-constant time

18

1100	0.0	1		
1101	90.0	1		
1106	30.0	1		
1107	-30.0	1	309	4091
1108	40.0	1		
1109	-40.0	1	154	4206
1110	50.0	1		
1111	-50.0	1	132	4305
1112	60.0	1		
1113	-60.0	1	126	4504
1114	70.0	1		
1115	-70.0	1	126	4723;
1102	10.0	1		
1103	-10.0	1	125	
1104	20.0	1		
1105	-20.0	1	123	
1116	80.0	1		
1117	-80.0	1	127	4976

4 angles are sufficient on 500 MHz

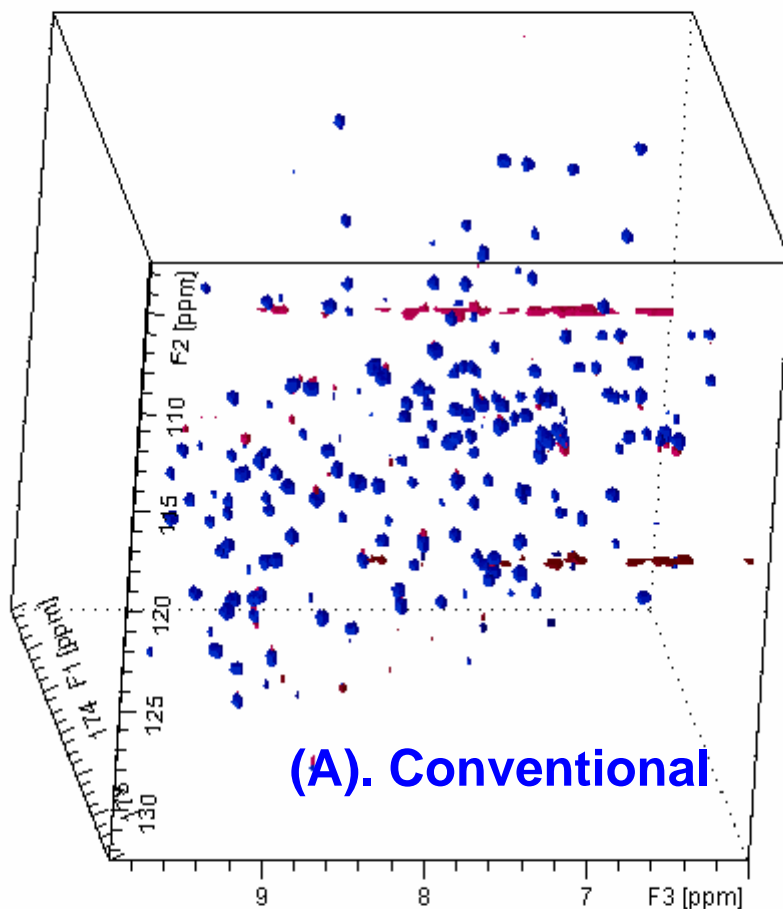
Fact:

In PR-NMR, S/N reduces with the square root of time saving factor.

**To overcome the sensitivity
loss in PR-NMR,
apply TROSY and deuteration
for larger proteins**

PR-HNCO on an effective 36 kDa protein

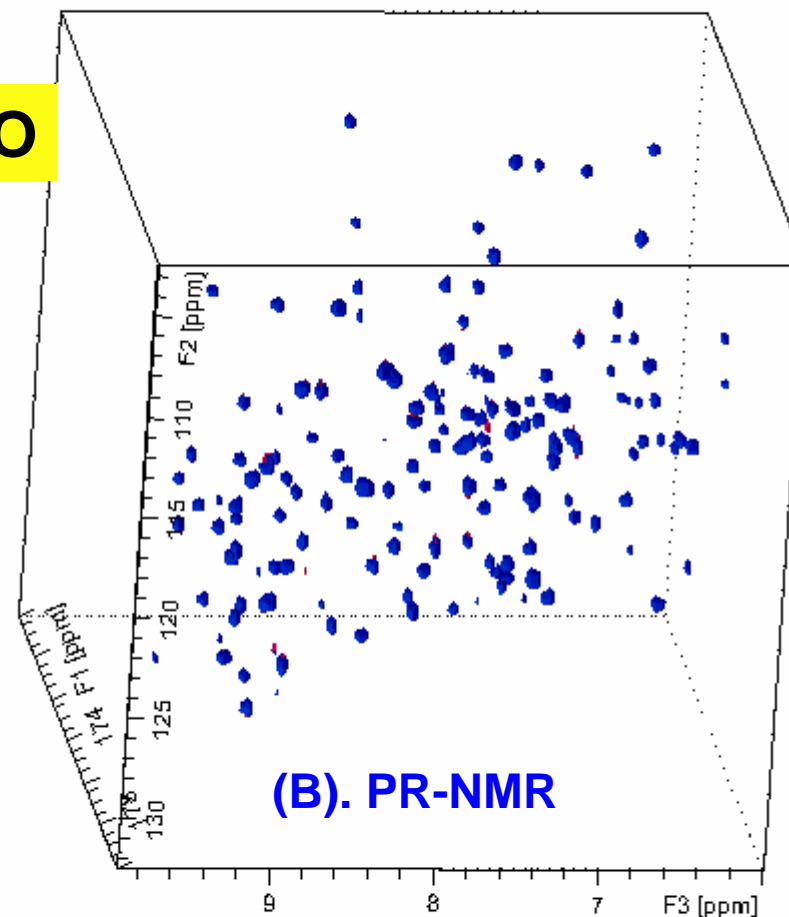
60% ^2H , ^{13}C , ^{15}N -Pol X, 174 A.A. (20 kDa, at 4 °C ~36 kDa), 0.7 mM



Acquisition time: 11 hr 37 min

ns=4, AV800-Cryoprobe

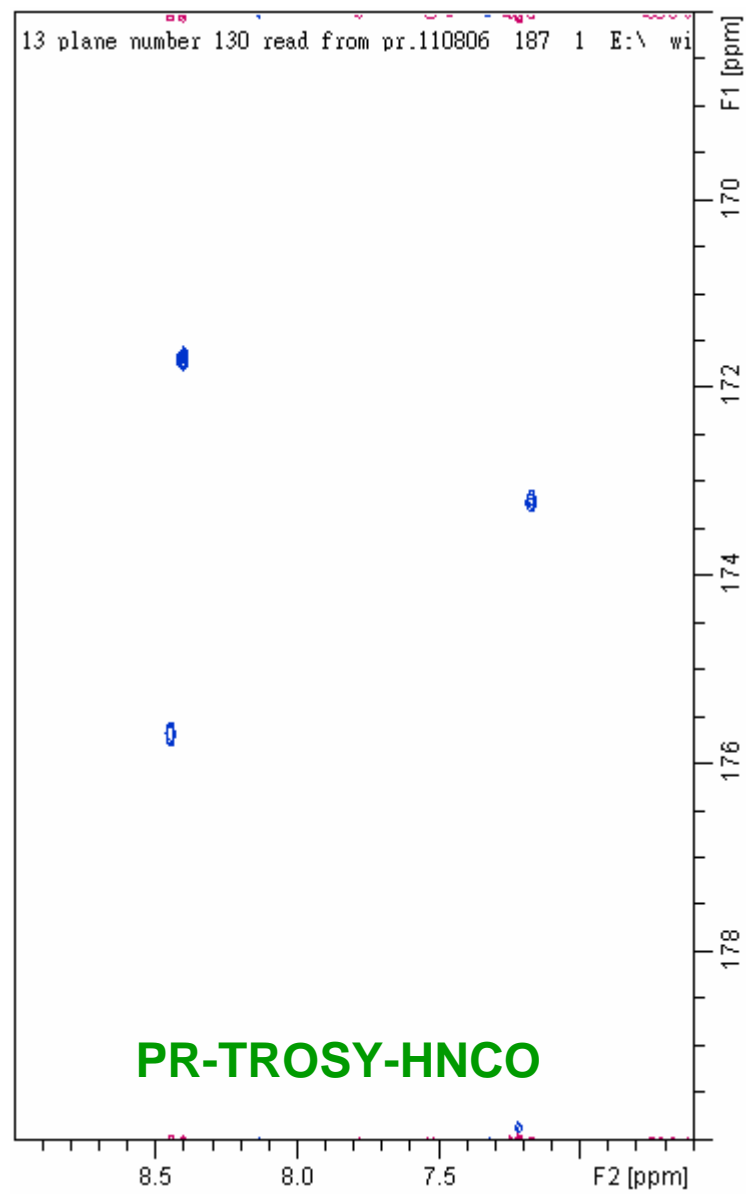
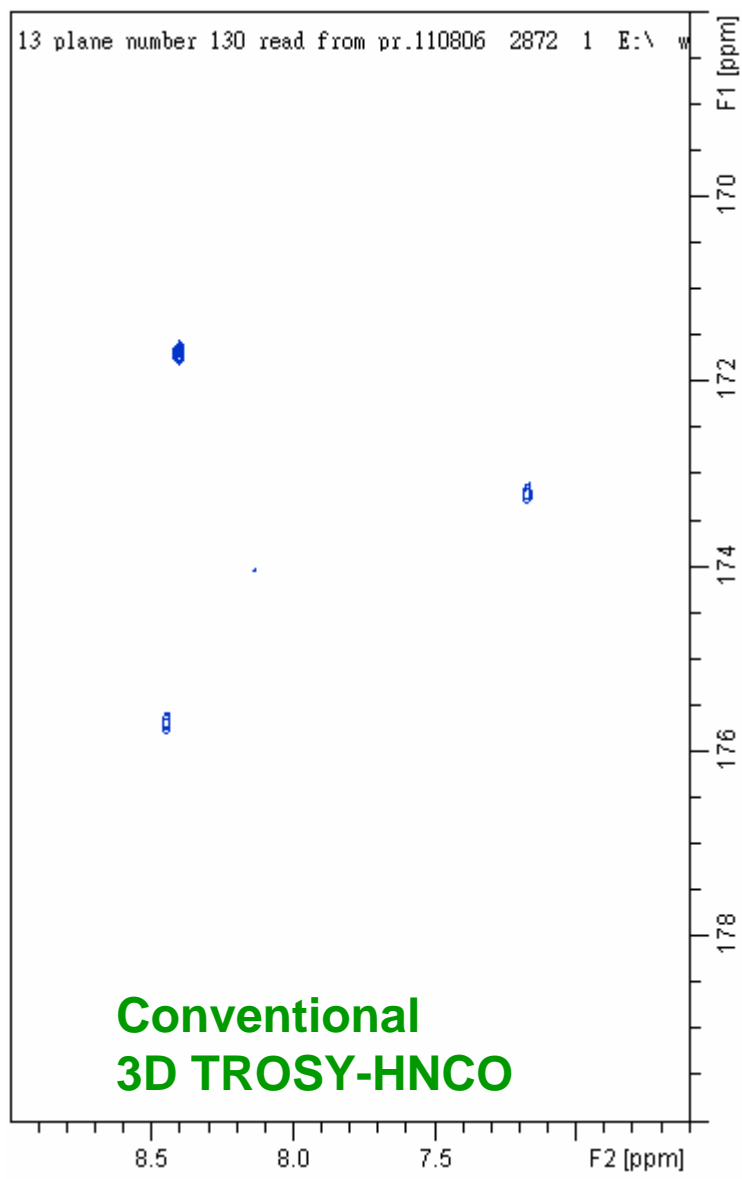
HNCO

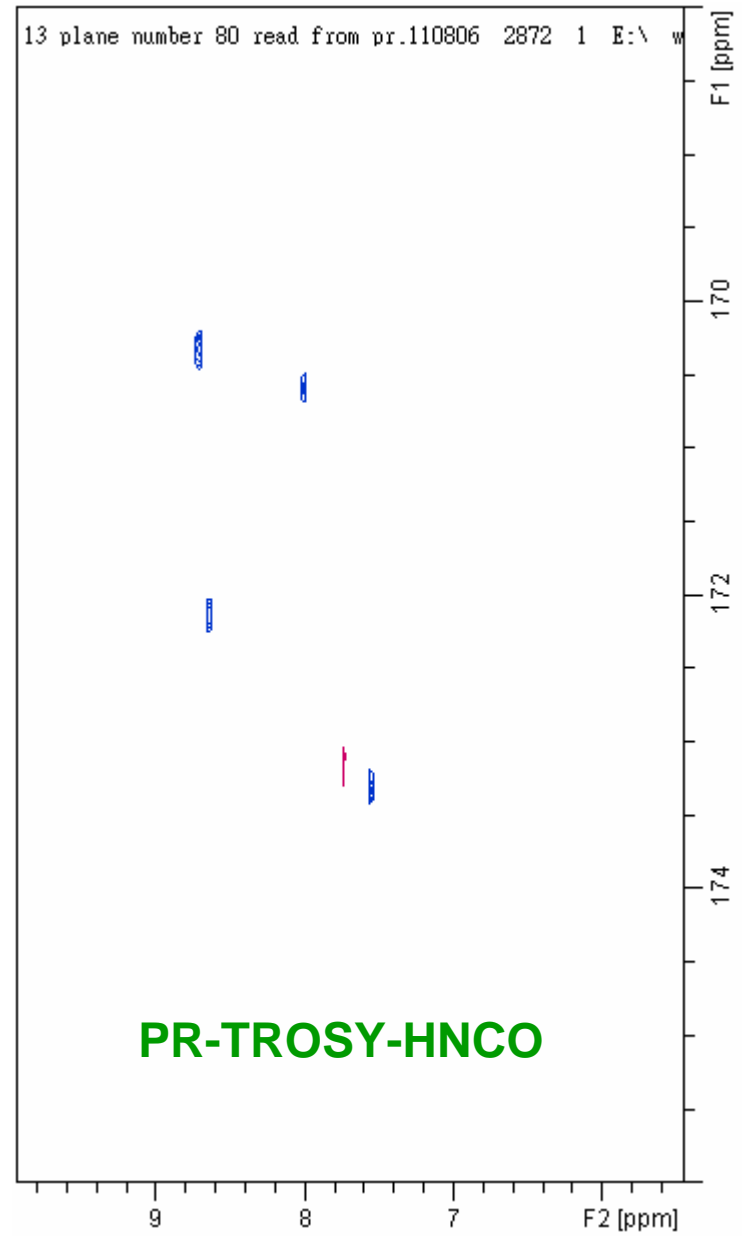
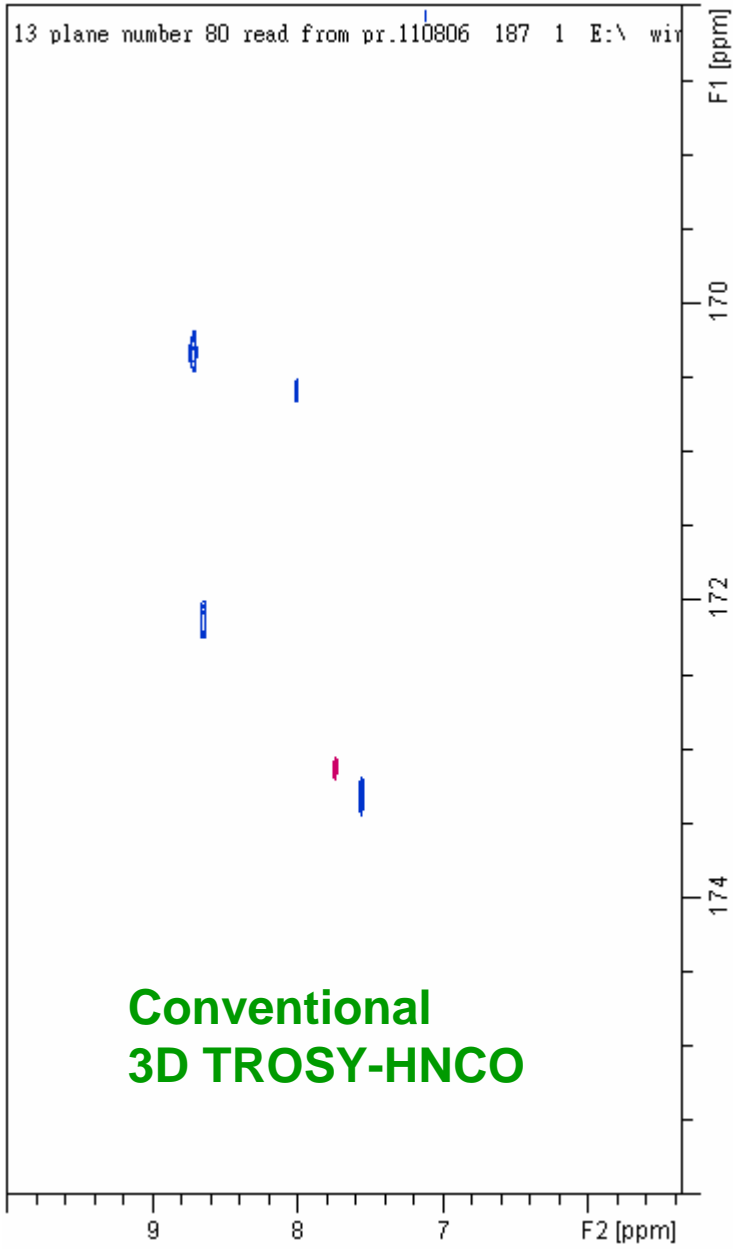


Acquisition time: 51 min

(reconstructed from 0°, 90°, ±10°, ±40°)

Note: The Pol X protein sample was kindly provided by Dr. Mei-I Su of Dr. Ming-Daw Tsai's group of the Genomic Research Center of Academia Sinica.





References

- Kupce, E. & Freeman, R. *J. Biomol. NMR* **2003**, 25, 349-354.
- Freeman, R.; Kupce, E. *J. Biomol. NMR* **2003**, 27, 101-113.
- Kupce, E. & Freeman, R. *J. Am. Chem. Soc.* **2003**, 125, 13958-13959.
- Kupce, E. & Freeman, R. *J. Am. Chem. Soc.* **2004**, 126, 6429-6440.