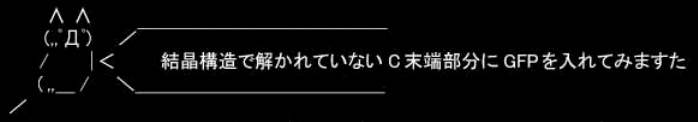
Protein NMR

Part V



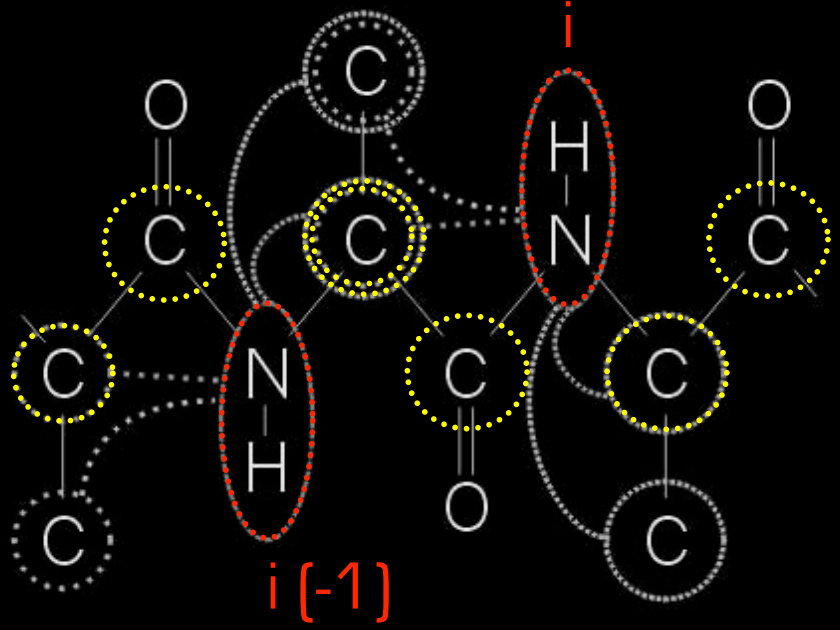
GroEL/GroES (Xu et al. *Nature* **388**: 720)
GFP (Ormo et al. *Science* **273**: 1392)

This is really going to be for the last time .....

1. Backbone NMR experiments

Magnetization transfer through space > NOE

Magnetization transfer through bonds > J-coupling



$^{1}H/^{15}N$ Correlation (2D)

HSQC or HMQC-type

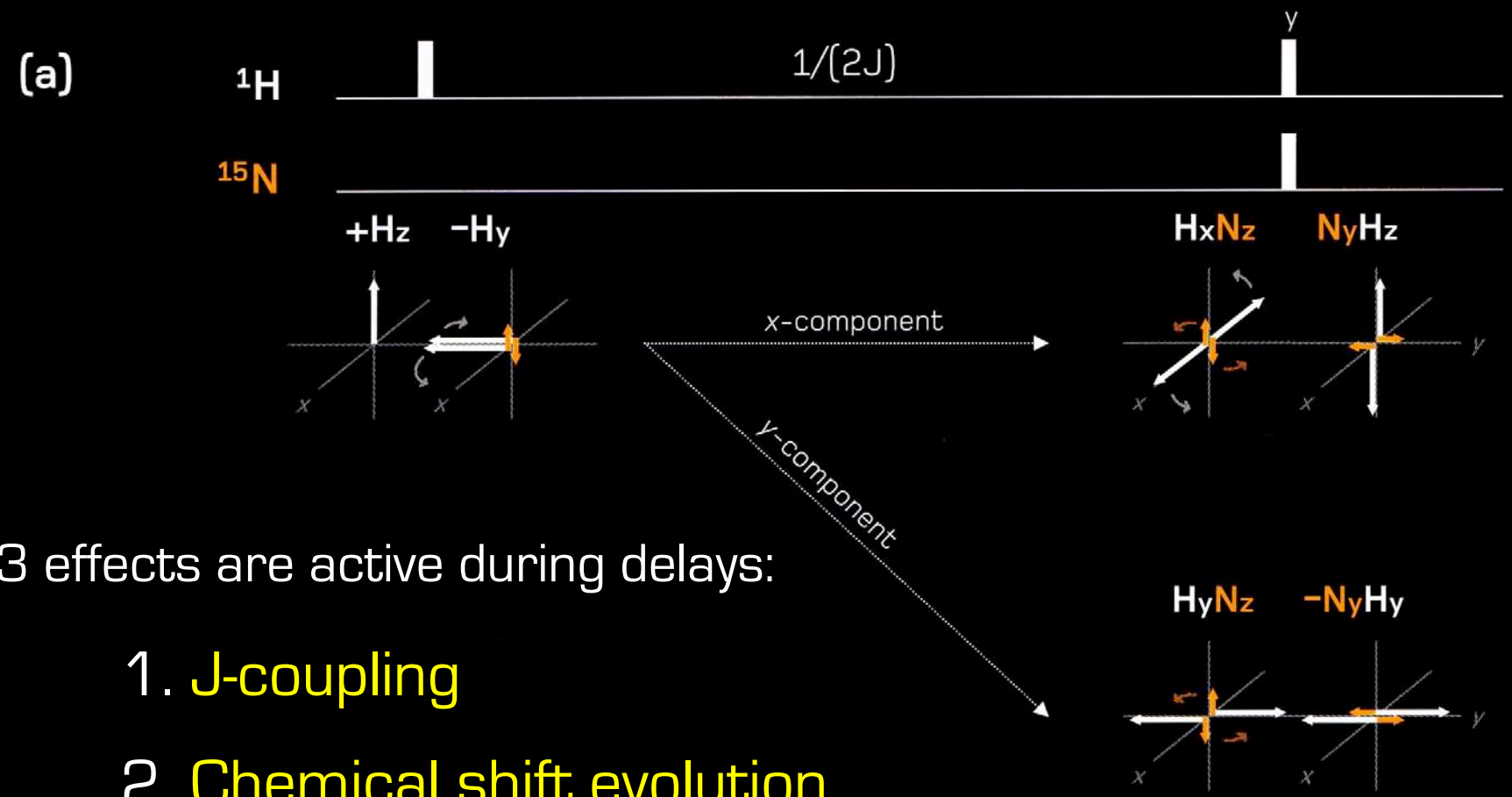
HNCO Experiment (3D)

HNCA Experiment (3D)

HN(CO)CA Experiment (3D)

2. Chemical shift evolution

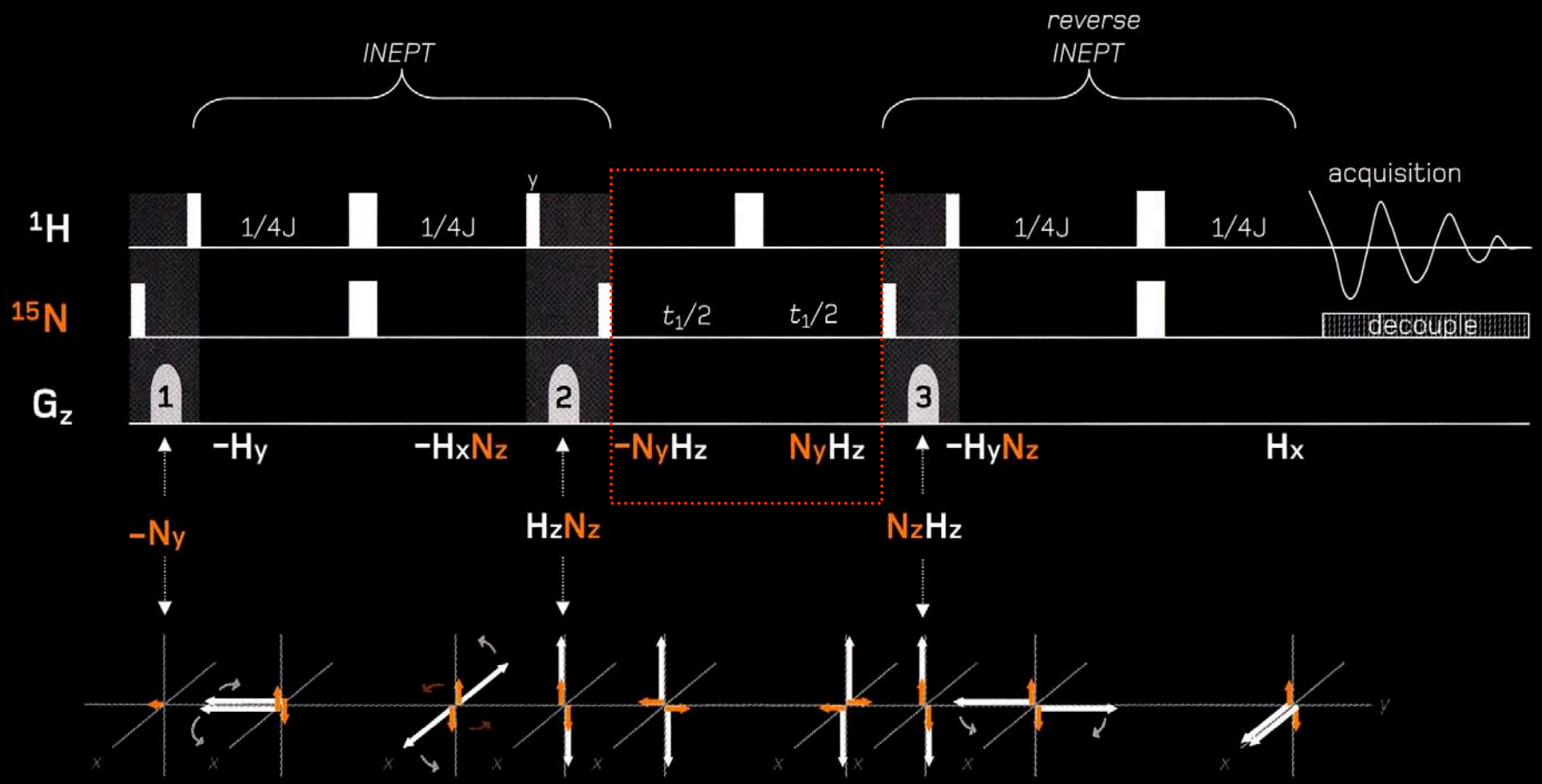
J-coupling and chemical shift evolve SIMULTANEOUSLY during 'every' delay period (for all nuclei with transverse components)!



3 effects are active during delays:

1. J-coupling
2. Chemical shift evolution
3. Relaxation (!)

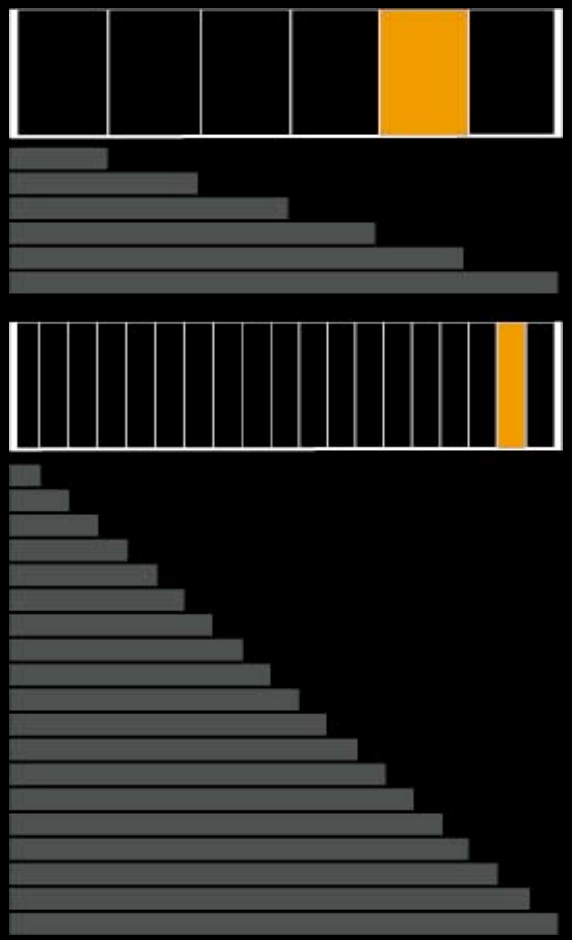
3. PFG-HSQC (decoupled)



^{15}N chemical shift evolves during the t_1 period (which is incremented in every scan). ^{1}H chemical shift does NOT evolve because magnetization is safely 'stored' along z. J-coupling however IS refocused.

4. Chemical shift evolution (cont.)

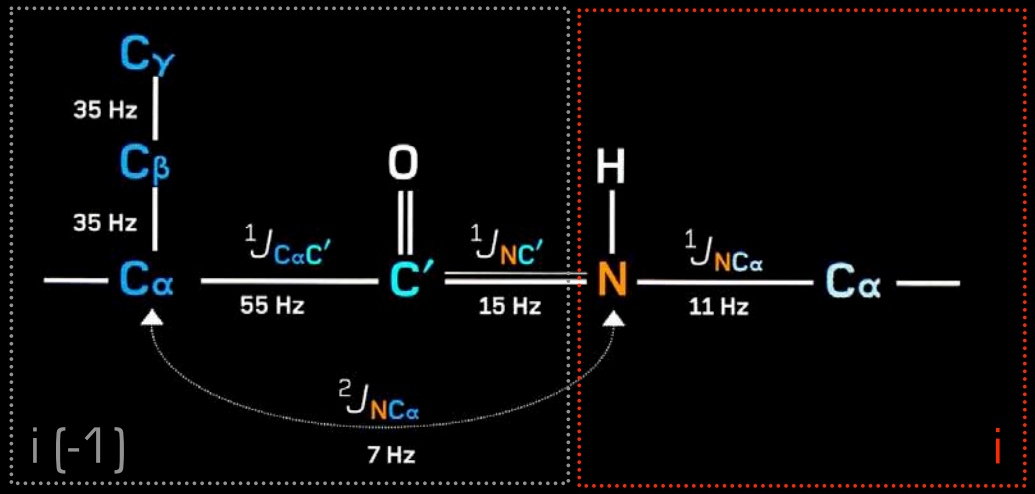
The resolution of your 2D (3D) NMR spectrum in the indirect dimension(s) (^{15}N or ^{13}C chemical shift evolution) will depend on the size of the chosen increments (NUMBER OF POINTS i.e. TD (128, 256 etc.))



Setting the SWEEP-WIDTH (SWH) to a value that is appropriate for the expected chemical shift range AND choosing the right NUMBER OF POINTS (TD) can greatly reduce the time requirements of your NMR measurements!

The NUMBER OF POINTS (on Bruker machines) is a complex number (i.e. real+imaginary). Meaning that the actual number of increments that you record is only HALF the number of points that you define (real data points).

4. J-coupling in proteins

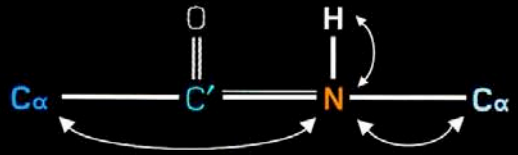


(b)

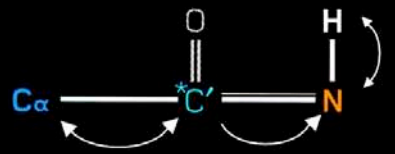
HNCO

(1) Strong J-coupling > Short delays
(2) Weak J-coupling > Long delays
(remember the INVERSE relationship
i.e. 1/2J, 1/4J etc.)

HNCA (+HNCA)

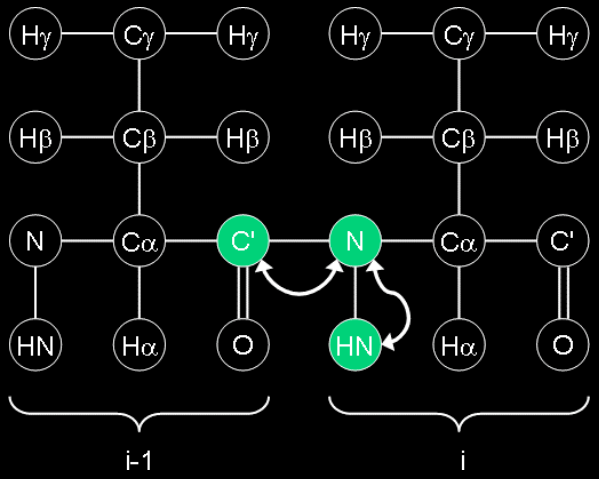


HN(CO)CA



(1) Short delays > Little relaxation
(2) Long delays > More relaxation

5. 3D HNCO



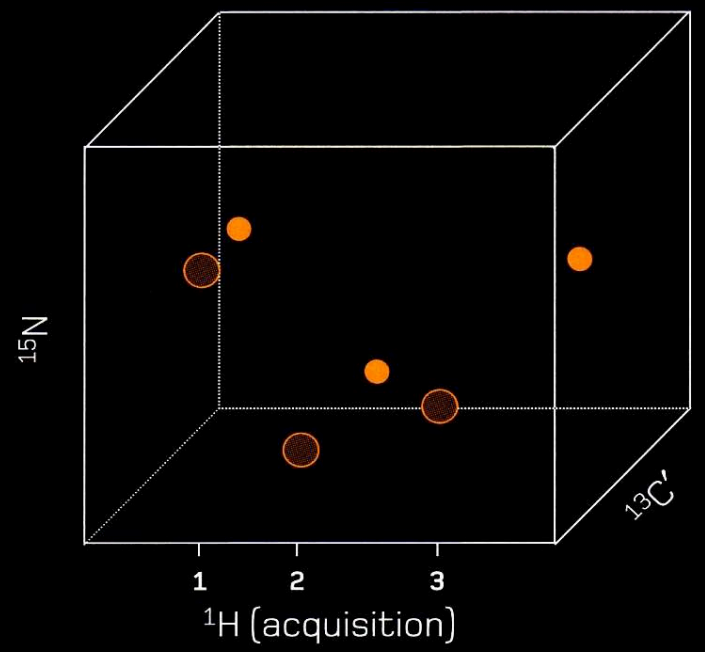
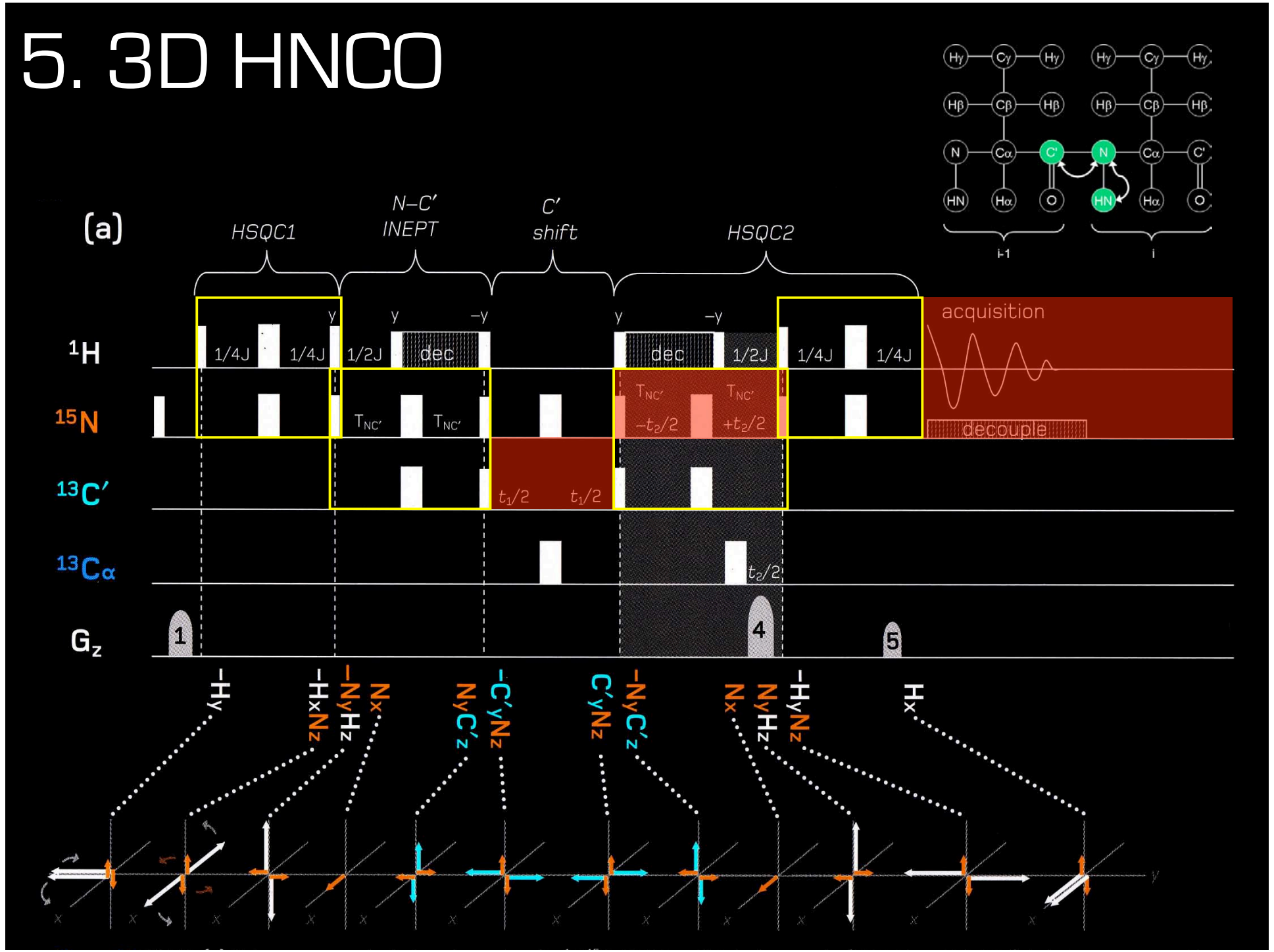


Figure 6.2. HNCO. (b) Schematic spectrum corresponding to the three amino acid region shown in Figure 4.2b: each amino acid produces a single peak (dark blue) along the $^{13}C'$ dimension (into the page), directly behind the corresponding HSQC peak (light blue) of Figure 4.2b (the light blue peaks do not appear in this spectrum and are only included as a guide). The position of the peaks along the $^{13}C'$ dimension specifies the chemical shift of $^{13}C'_{(i-1)}$ that was encoded during t_1.

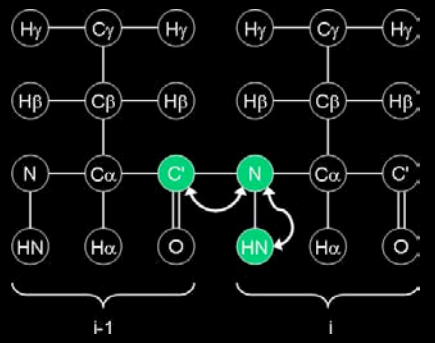
Starting from a 2D ^{1}H ^{15}N correlation (HSQC) C' Chemical Shift Evolution yields the 3rd. Dimension.

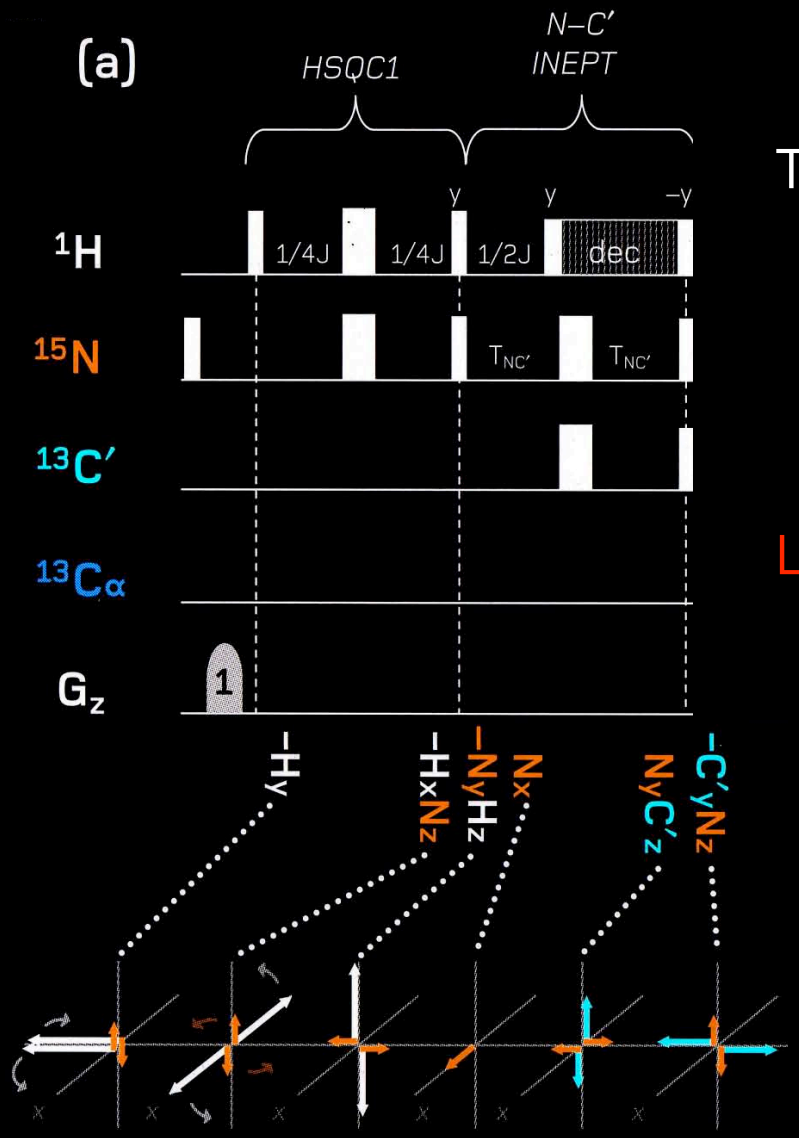
The 3D HNCO is the MOST SENSITIVE of all the Triple Resonance Experiments for protein bb-assignment.

5. 3D HNCO


(a)
HSQC1
N–C′ INEPT
C′ shift
HSQC2
1H
15N
13C′
13Cα
Gz
1/4J
1/2J
dec
TNC′
t1/2
t2/2
−t2/2
+t2/2
acquisition
decouple
1
4
5
Hγ
Cγ
Hβ
Cβ
N
Cα
C′
HN
Hα
O
i-1
i

5. 3D HNCO





The $-N_yH_z$ term is subject to 4 J-couplings:

(1) $^1J_{HN}$

(2) $^1J_{NC'}$

(3) $^1J_{NC\alpha(i)}$ and $^2J_{NC\alpha(i-1)}$

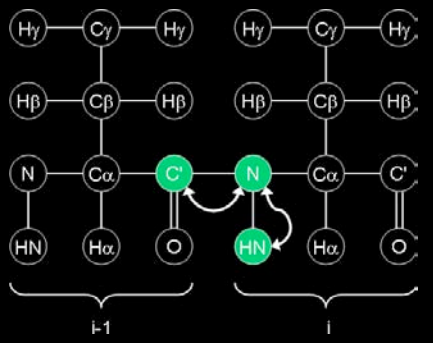
Let's analyze them one by one:

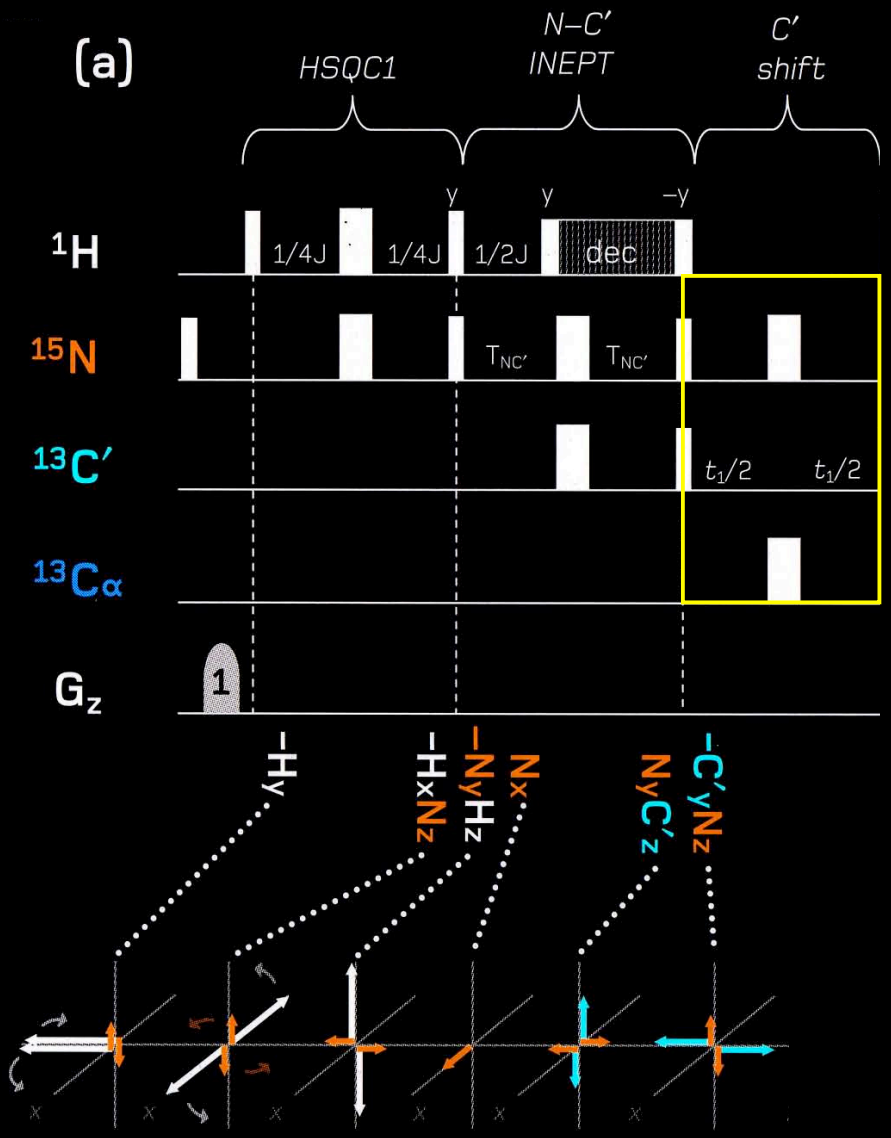
(1) During $1/(2\ ^1J_{HN})$ $-N_yH_z > N_x$

(2) Simultaneous 180deg. pulses on N and C' keep $^1J_{NC'}$ active during the entire $2T_{NC'}$ period > setting $2T_{NC'}$ to $1/(2\ ^1J_{NC'})$ $N_x > N_yC'_z$

(3) No 180deg. pulse on Cα > no $J_{NC\alpha}$

5. 3D HNCO





Evolve CHEMICAL SHIFT of C' > decoupling pulses on N and Cα

5. 3D HNCO

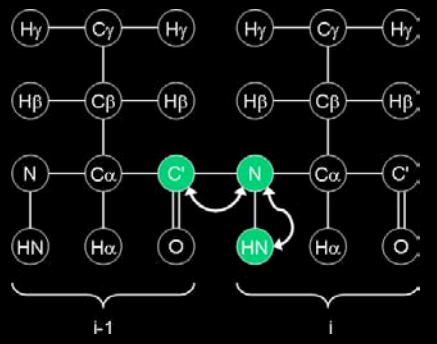
4 things must be achieved during the 2nd. $T_{NC'}$ period:

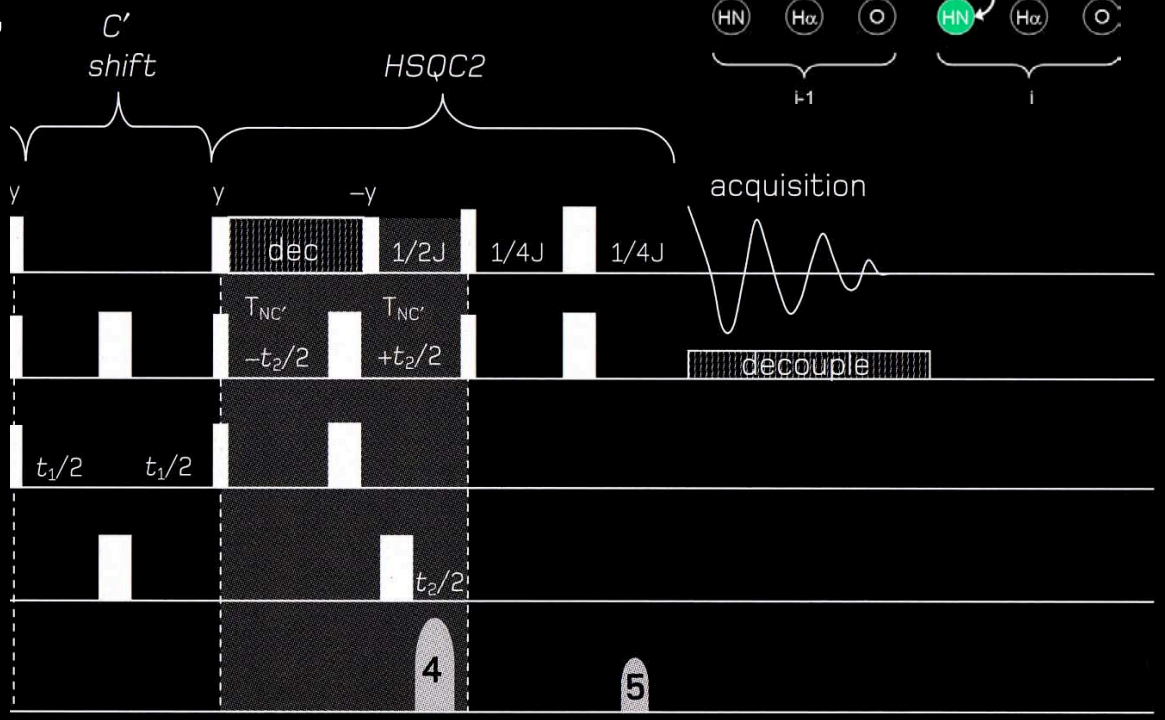
(1) $-N_yC'_z$ > N_x in-phase to C'

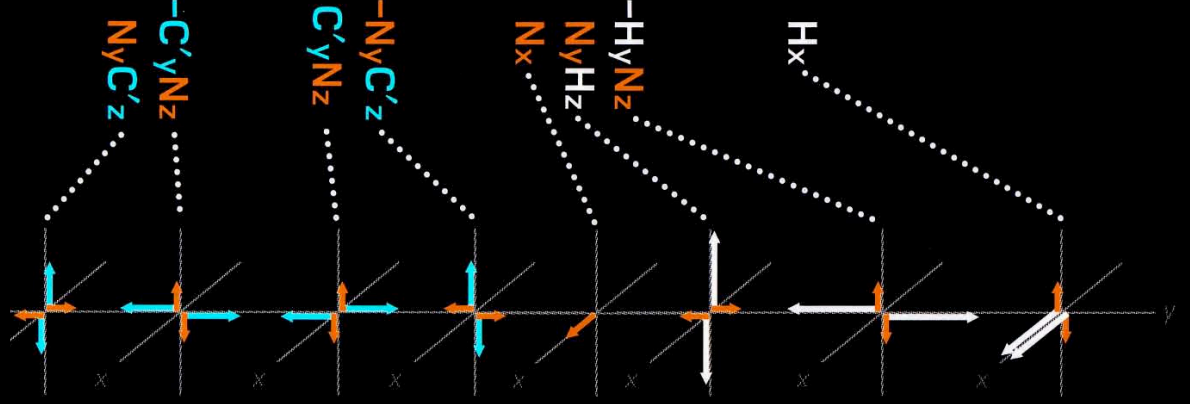
(2) Anti-phase term to ^1H must be created i.e. N_yH_z

(3) $^1J_{NC\alpha(i)}$ and $^2J_{NC\alpha(i-1)}$ must be suppressed

(4) Nitrogen chemical shift must evolve!







5. 3D HNCO

How we achieve this:

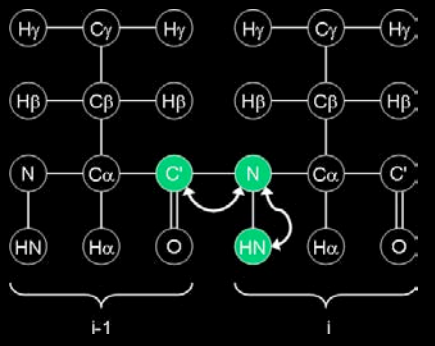
(1) Simultaneous 180deg. pulses on N and C'

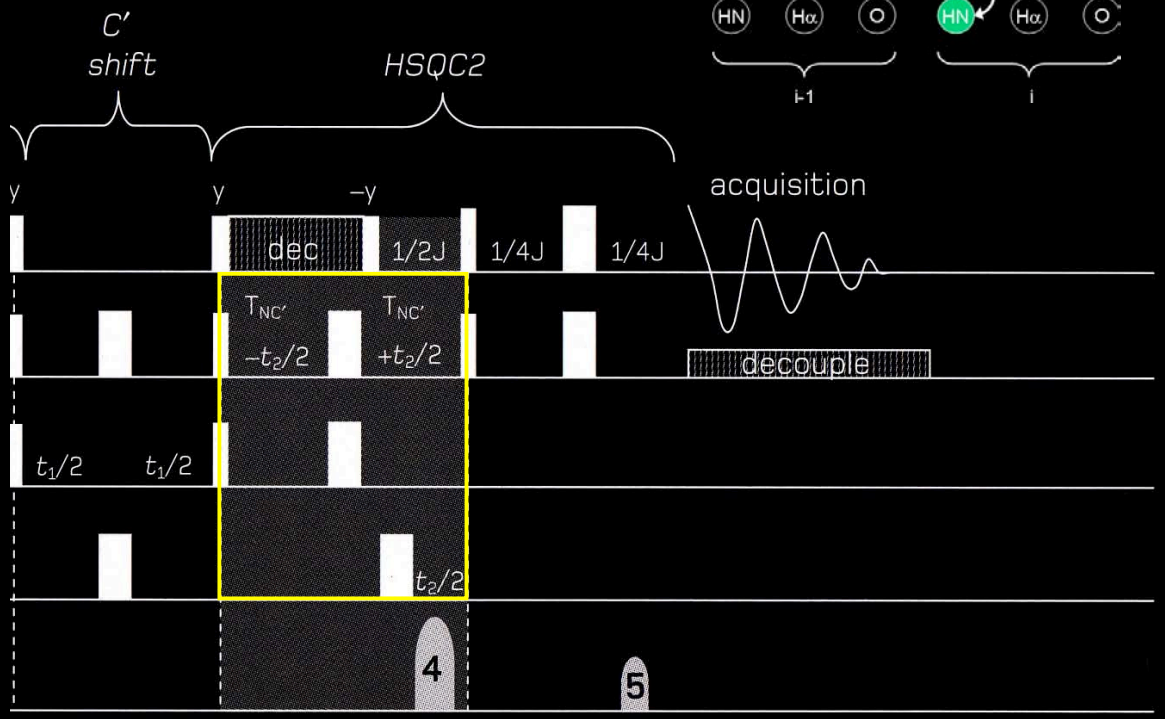
(2) Decoupling ^{1}H, only allow 1/2 $^{1}J_{HN}$ period

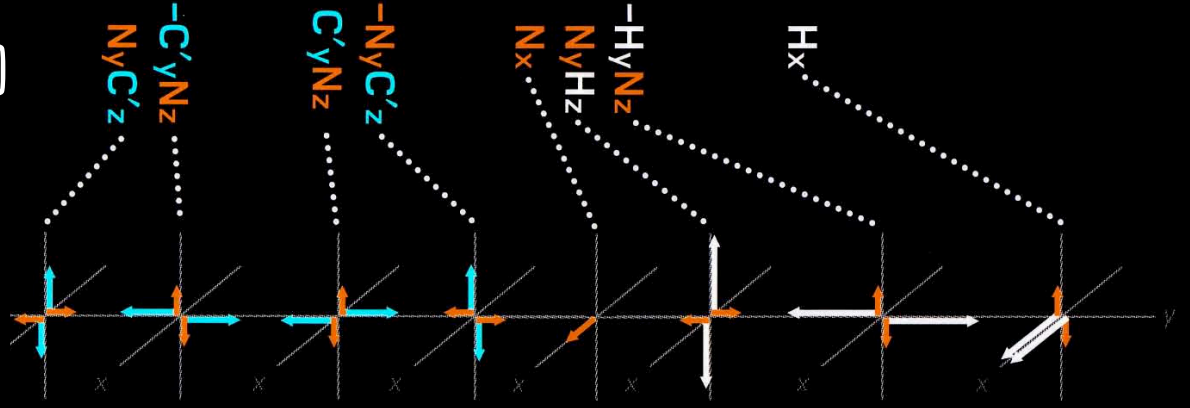
(3) $^{1}J_{NC\alpha(i)}$ and $^{2}J_{NC\alpha(i-1)}$ suppressed by 180deg.

(4) ^{15}N chemical shift evolution via Constant Time (CT) procedure!

$T_{NC'}$-$t_2/2$ - ($T_{NC'}$+$t_2/2$) = -t_2

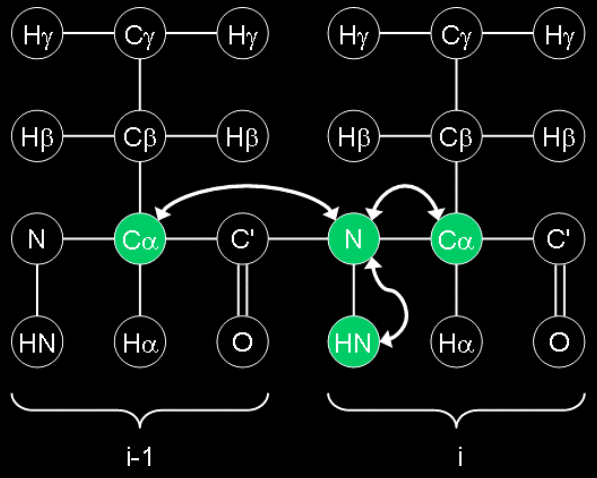






Animation 1

6. 3D HNCA



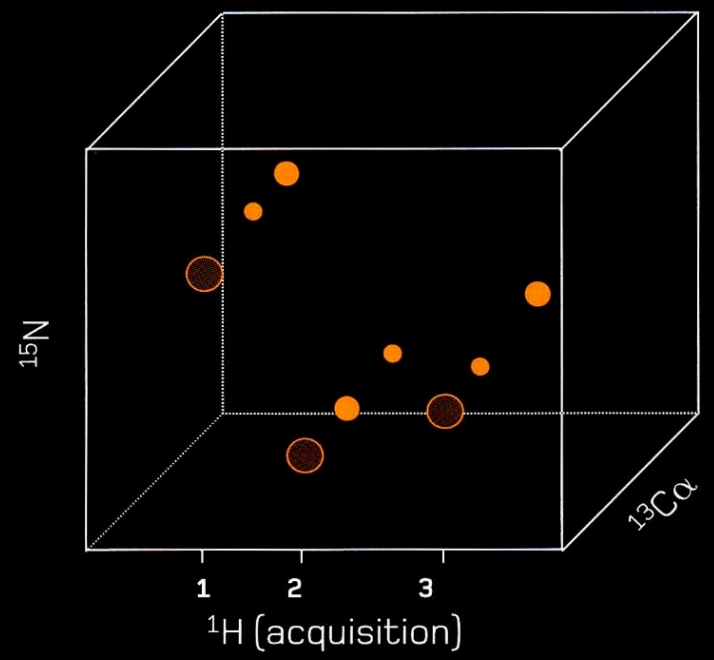
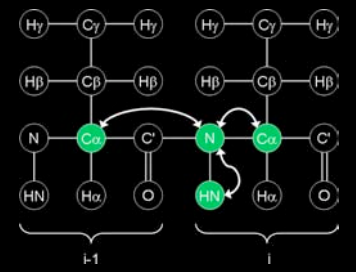


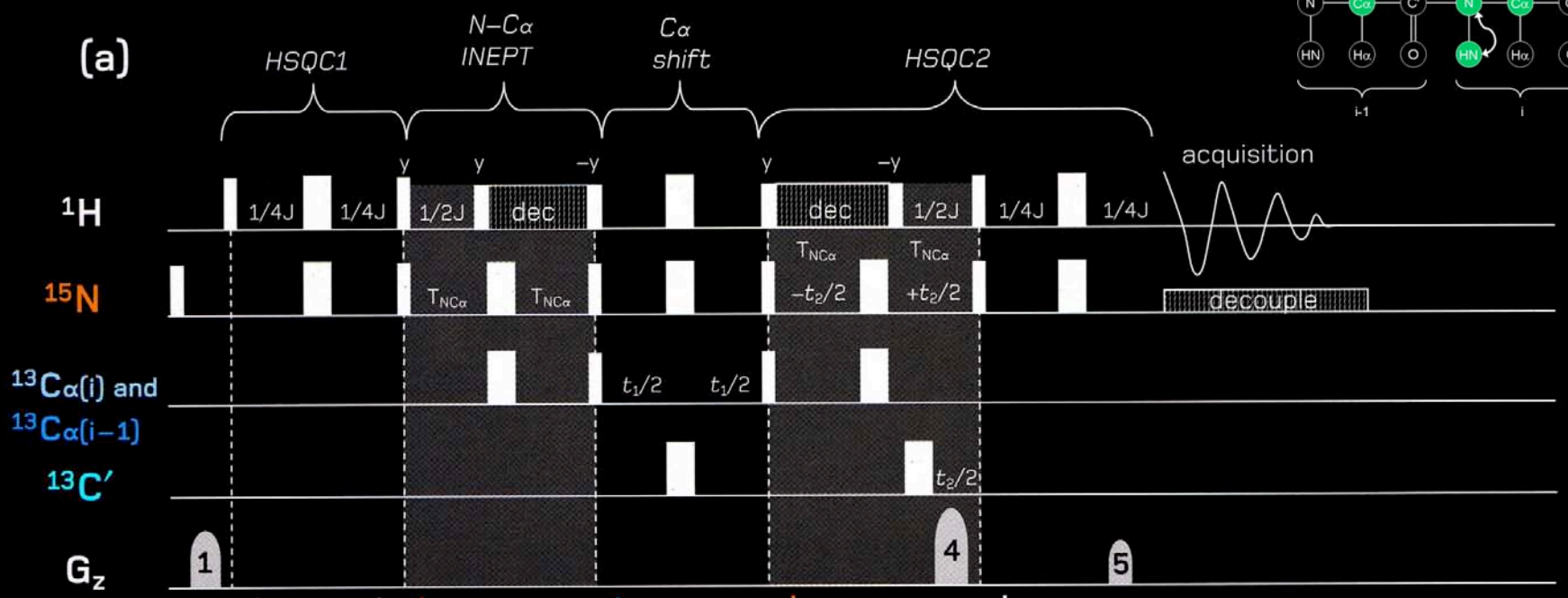
Figure 6.3. HNCA. (b) Schematic spectrum corresponding to the three amino acid region shown in Figure 4.2b: each amino acid produces two peaks (dark blue) along the $^{13}C\alpha$ dimension (into the page), directly behind the corresponding HSQC peak (light blue) of Figure 4.2b (the light blue peaks do not appear in this spectrum and are only included as a guide]. The position of the peaks along the $^{13}C\alpha$ dimension specify the chemical shifts of the $C\alpha_{(i)}$ and $C\alpha_{(i-1)}$ spins encoded during t_1. The $C\alpha_{(i)}$ peak is generally the more intense of the two.

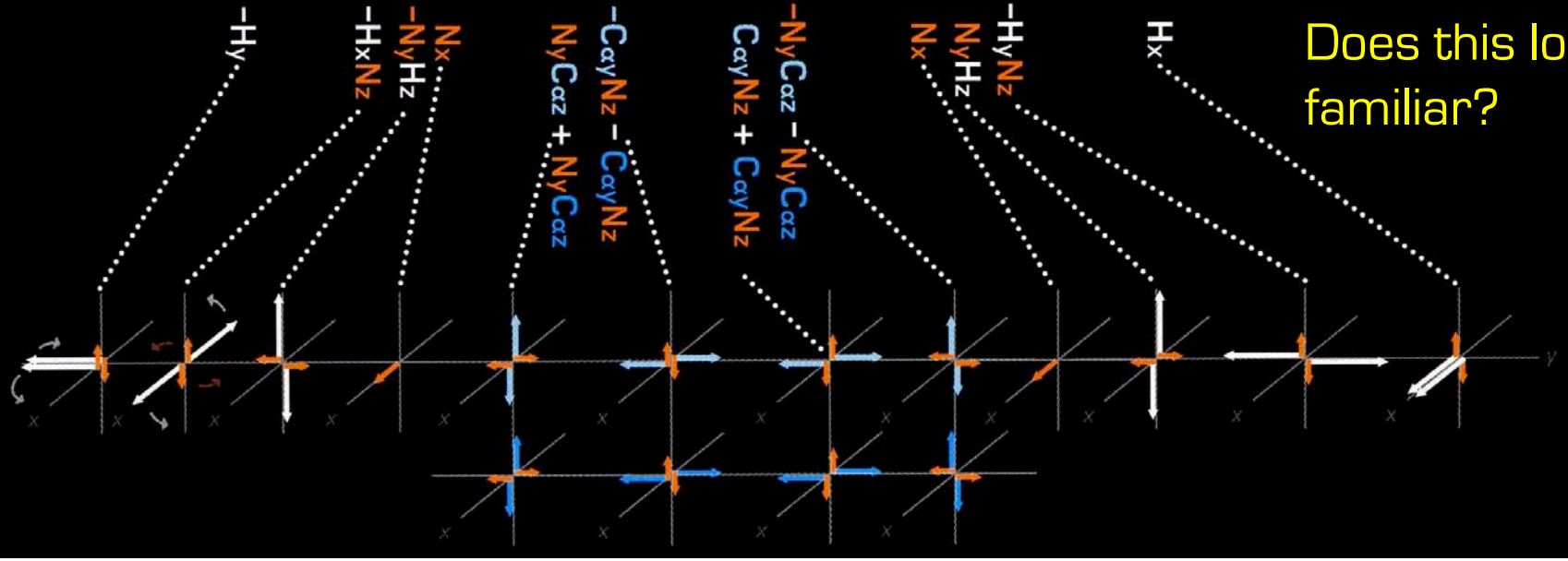
The 3D HNCA yields chemical shift information about $C\alpha_{(i)}$ and $C\alpha_{(i-1)}$

> Provides the basis for CONNECTIVITIES between individual protein residues.

6. 3D HNCA

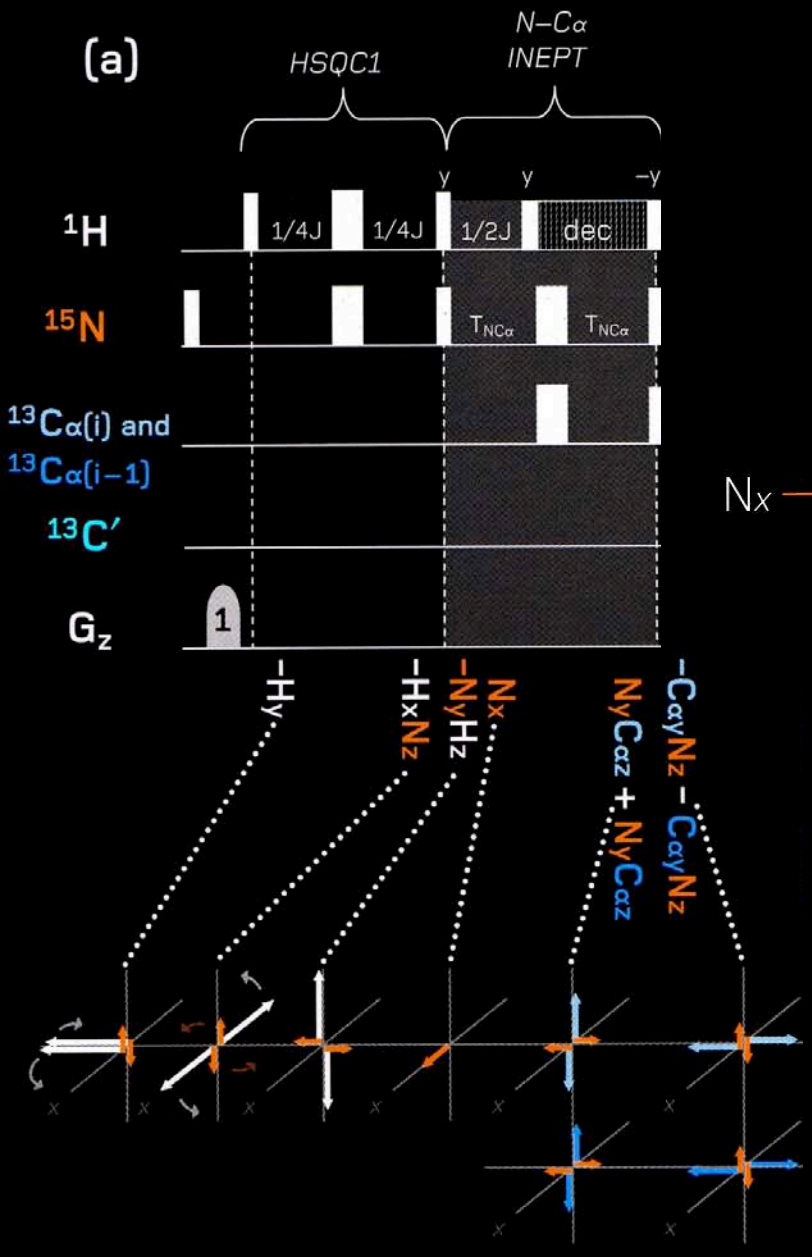


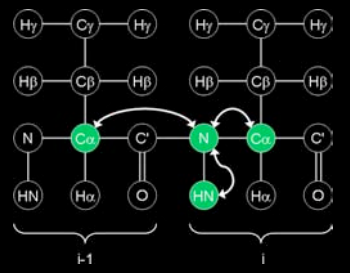




Does this look familiar?

6. 3D HNCA





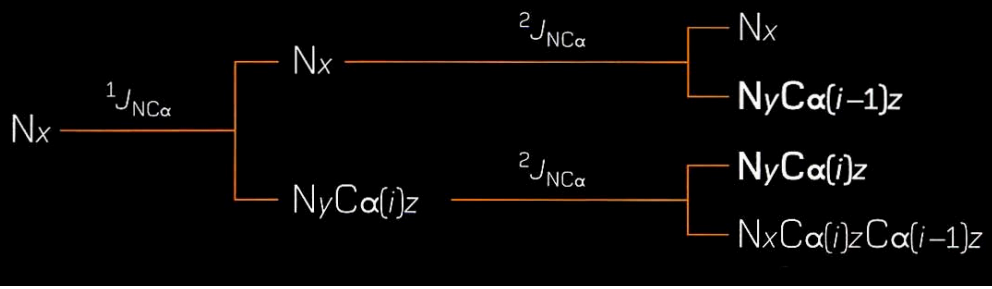
However, ONE PROBLEM arises:

Let's assume that $^{1}J_{NC\alpha(i)} = {}^{2}J_{NC\alpha(i-1)}$ and that $T_{NC\alpha}$ is set to $1/(4\ {}^{1}J_{NC\alpha})$.

$$N_x \xrightarrow{{}^{1}J_{NC\alpha}} N_yC\alpha(i)_z \xrightarrow{{}^{2}J_{NC\alpha}} N_xC\alpha(i)_zC\alpha(i-1)_z$$

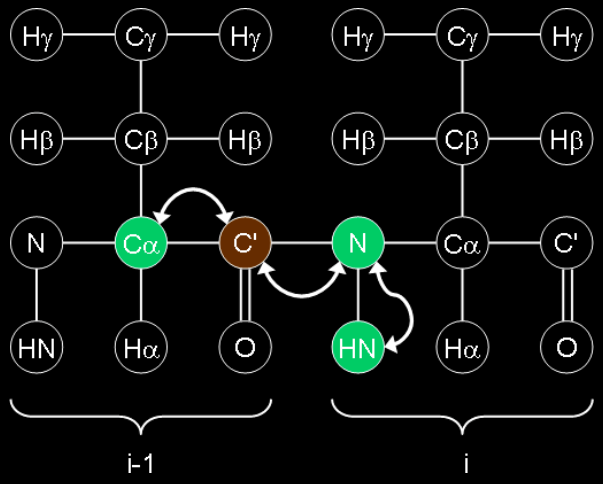
SOLUTION (as always a compromise):

Let's set $T_{NC\alpha}$ to $1/(8\ {}^{1}J_{NC\alpha})$ > development of anti-phase magnetization is incomplete (i.e. some in-phase term N_x remains)



Animation 2

7. 3D HN(CO)CA (b)



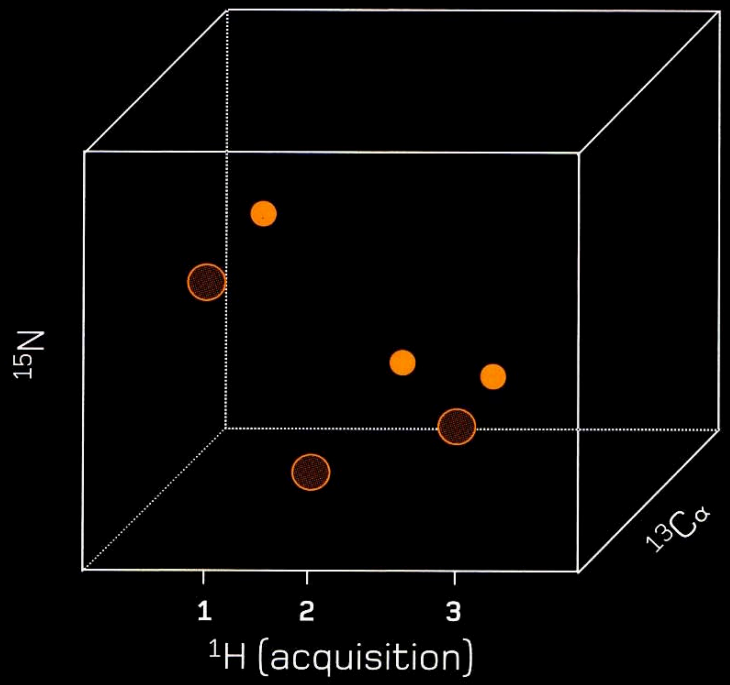
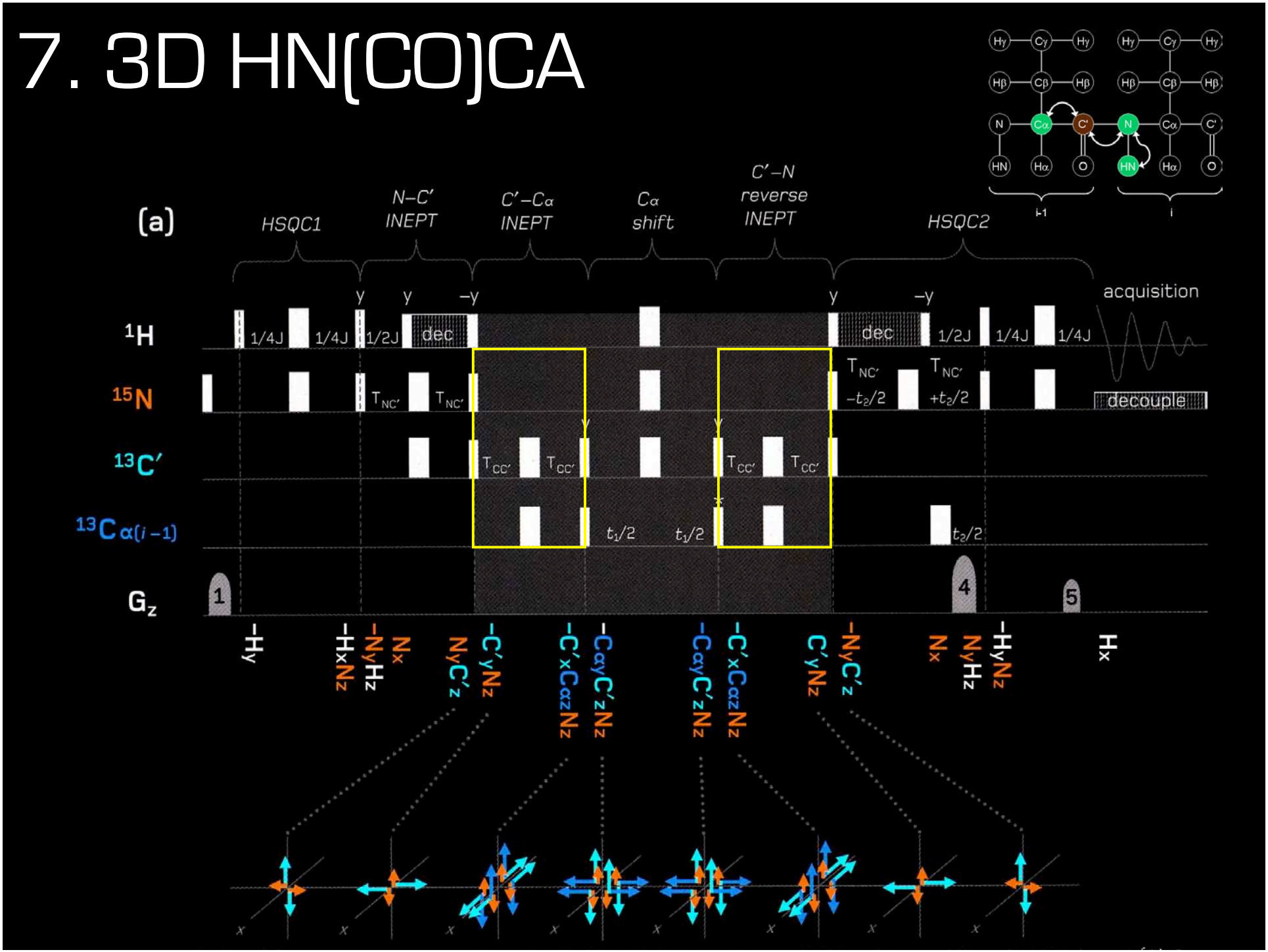


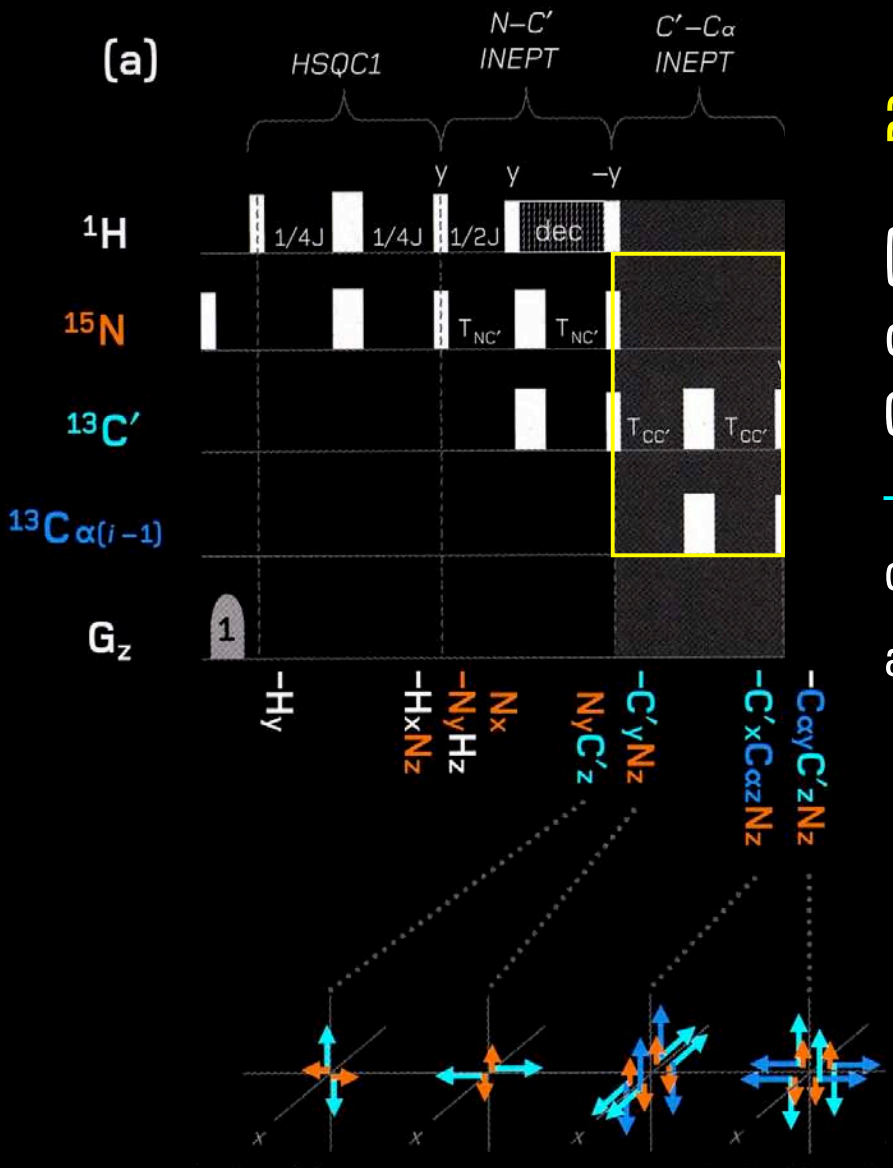
Figure 6.4. HN(CO)CA. (b) Schematic spectrum corresponding to the three amino acid region shown in Figure 4.2b: each amino acid produces one peak (dark blue) along the $^{13}C\alpha$ dimension (into the page), corresponding to the generally weaker $C\alpha_{(i-1)}$ peaks from Figure 6.3b. (The light blue peaks do not appear in this spectrum and are only included as a guide.)

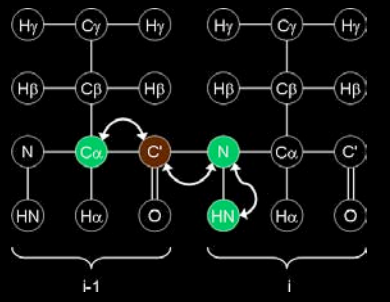
Starting from a 2D ^{1}H ^{15}N correlation (HSQC) $C\alpha_{(i-1)}$ Chemical Shift Evolution yields the 3rd. Dimension.

7. 3D HN(CO)CA


(a)
HSQC1
N–C′ INEPT
C′–Cα INEPT
Cα shift
C′–N reverse INEPT
HSQC2
acquisition
decouple
dec
1/4J
1/2J
$T_{NC'}$
$T_{CC'}$
$t_1/2$
$t_2/2$
$-t_2/2$
$+t_2/2$
^1H
^{15}N
$^{13}C'$
$^{13}C\alpha(i-1)$
G_z
i-1
i

7. 3D HN(CO)CA





$2T_{NC'}$ is different to $2T_{CC'}$

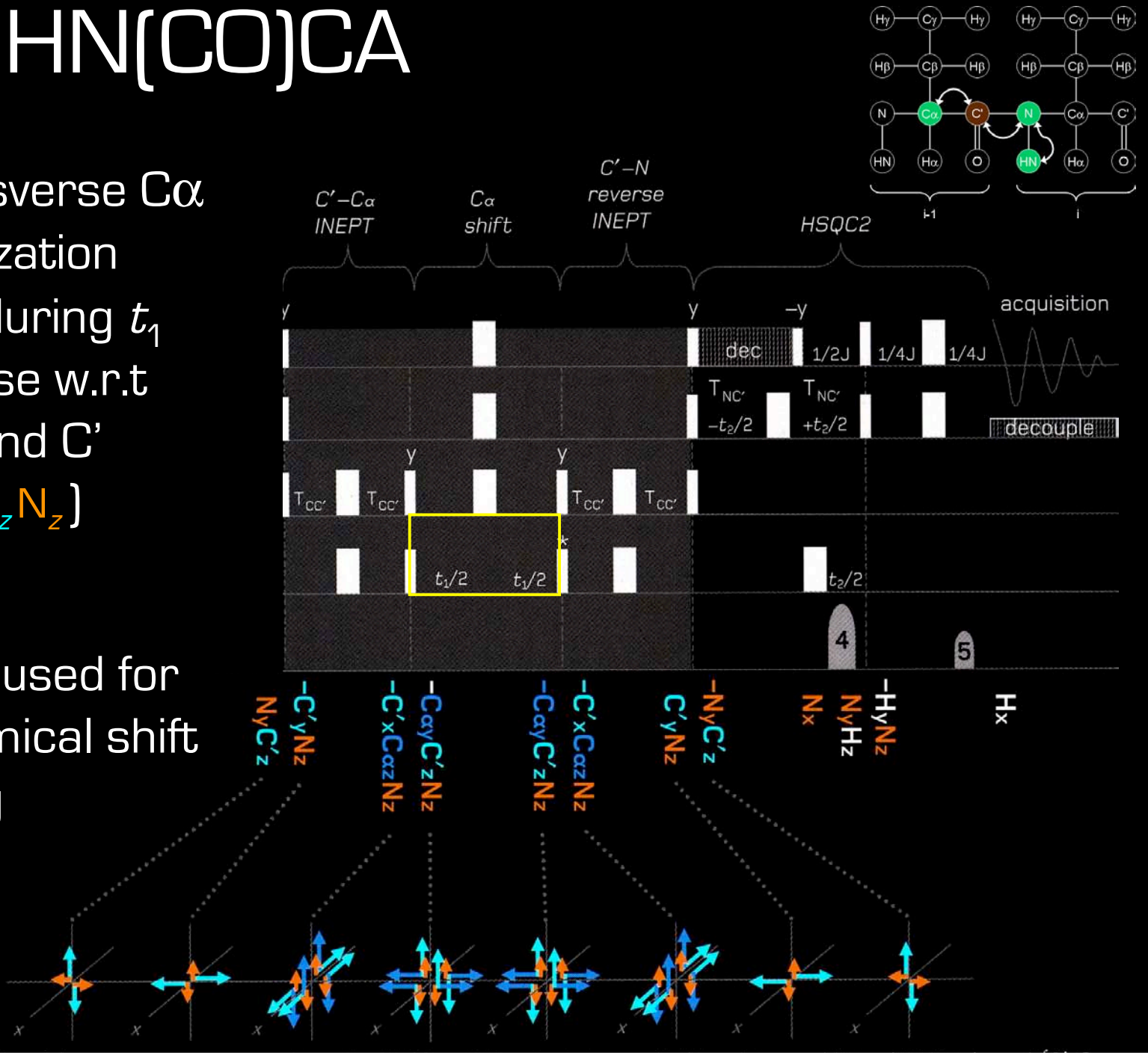
(1) No ^{15}N 180deg. pulse during magnet. transfer C' to $C\alpha_{(i-1)}$ > $^{1}J_{NC'}$ evolution ignored

$-C'_y N_z$ term remains anti-phase > double anti-phase term ($-C'_x C\alpha_z N_z$) at the end of $2T_{CC'}$.

7. 3D HN(CO)CA

(2) Transverse Cα magnetization evolves during t_1
-anti-phase w.r.t both N and C'
> ($-C\alpha_y\ C'_z N_z$)

(3) CT is used for ^{15}N chemical shift encoding



Animation 3

8. .... Eh, voila!

A back-bone assigned protein allows you to do a great deal of simple, but highly useful NMR experiments!