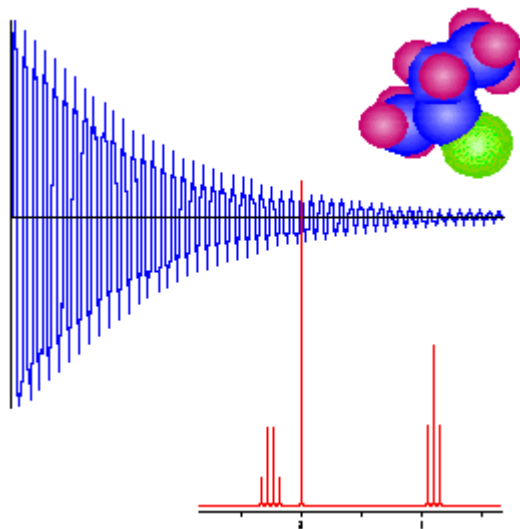


COURSE#1022: Biochemical Applications of NMR Spectroscopy

<http://www.bioc.aecom.yu.edu/labs/girvlab/nmr/course/>

**Lab 7: 2D & 3D ^1H - ^{15}N NMR of Labeled
Proteins and Data Analysis Using
NMRView**



LAST UPDATE: 3/26/2007

Objective:

- To understand how to acquire and analyze 3D ^{15}N NOESY/TOCSY-HSQC spectra. You will obtain a 2D ^1H - ^{15}N HSQC spectrum of a labeled peptide and make sequential assignments using 3D ^{15}N NOESY-HSQC and 3D ^{15}N TOCSY-HSQC spectra and NMRView analysis software.

Reading:

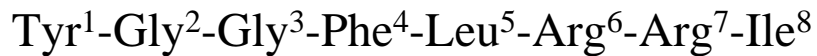
- refer to lecture on “3D NMR” and Labs #4 and #5

Outline:

- the sample
- the 2D ^1H - ^{15}N HSQC NMR experiment
- Strategy for Assigning Spin Systems of Peptides/Proteins using 3D ^{15}N TOCSY-HSQC and 3D ^{15}N NOESY-HSQC experiments
- the 3D ^{15}N TOCSY-HSQC experiment
- the 3D ^{15}N NOESY-HSQC experiment
- Problem Set

The Sample:

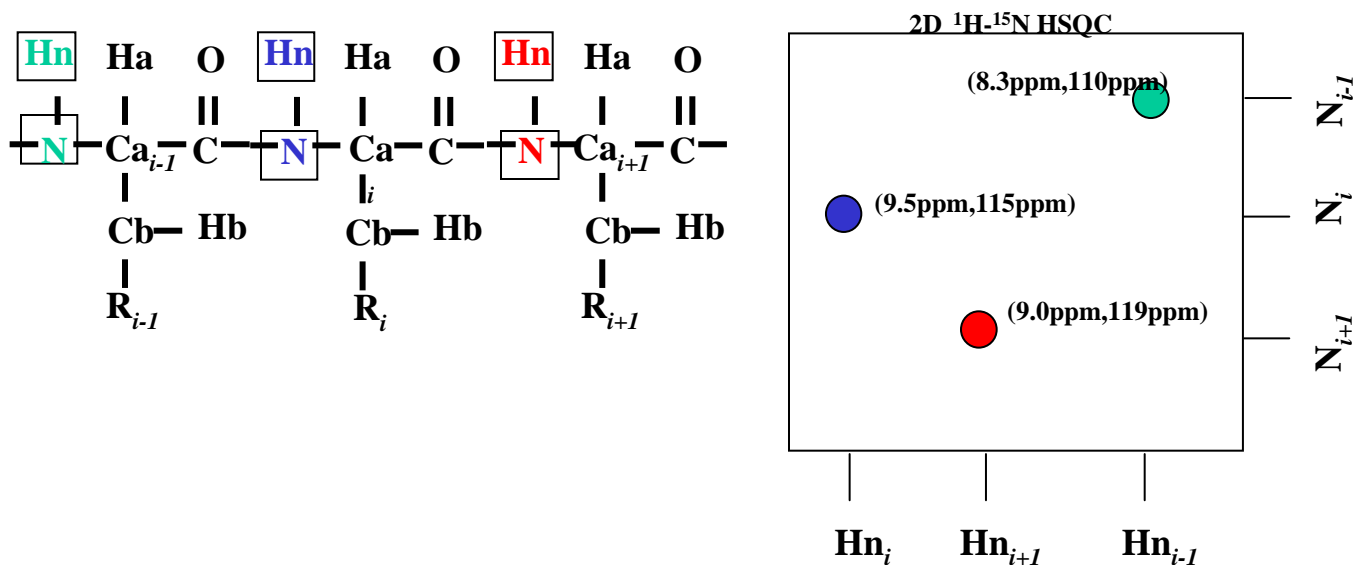
The “GFL” peptide used for this lab is an octapeptide and has the following sequence:



in which 3 sequential residues, Gly³-Phe⁴-Leu⁵, are ¹⁵N/¹³C-labeled. The peptide is dissolved in DMSO-d₆ and has a concentration of ~1 mM.

This sample costs > \$1000 because of the high price of ¹⁵N/¹³C-labeled amino acids used in its chemical synthesis so ... please be careful!

The 2D ^1H - ^{15}N HSQC Experiment



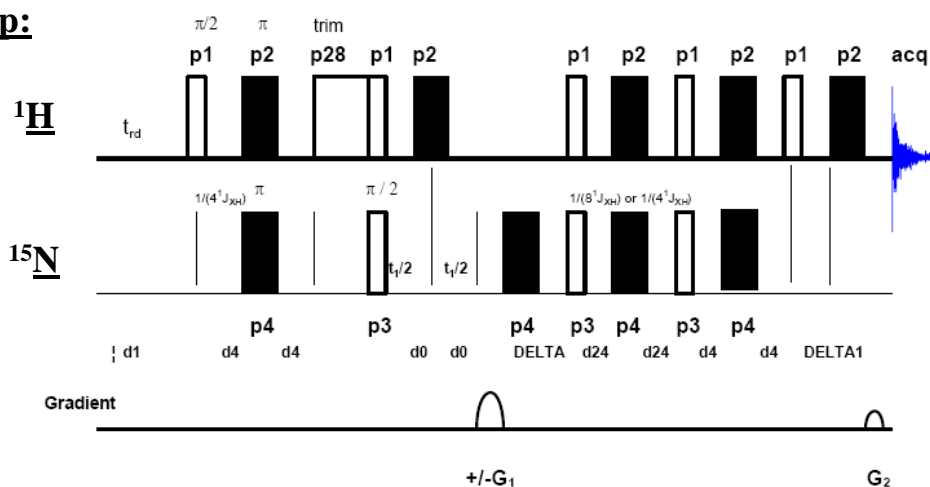
Set-up:

1. make sure that instrument is set-up properly (probe is tuned for ^{15}N and ^1H using the command `n15tune` and sample is locked and shimmed).
2. create a new dataset.
3. run macro by typing “2DhnHSQC” – this macro will set up your dataset with the appropriate parameters for running a 2D ^1H - ^{15}N HSQC experiment.
4. change any acquisition parameters to suit your experimental needs. Use command “`expt`” to calculate total experiment time.
5. run the experiment using the command “`zg`” (starts the experiment) or “`azg`” (starts the experiment and puts you into acqu window to monitor the data collection).
6. after the experiment ends, you can process the data using the command “`xfb`”.
7. since this is a phase-sensitive experiment, you may have to adjust the phasing if cross-peaks do not appear “in-phase”

The 2D ^1H - ^{15}N HSQC Experiment

macro = 2DhnHSQC

spdisp:



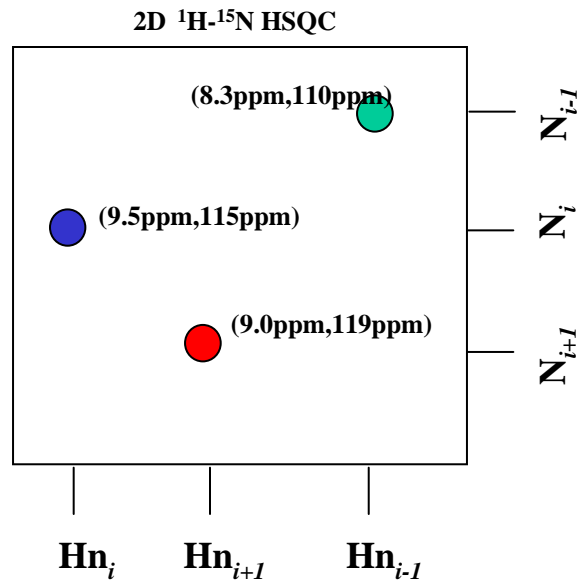
acquisition parameters in F2 (^1H):

pulprog	invietgpsi	Sensitivity-enhanced pulse sequence w/ gradient select
td	1k	number of data points (time domain size)
ns	1*n	minimum number of scans
sw	14 ppm	sweep width for ^1H
aq	122 ms	acquisition time – determined by sweep width and number of data points
o1p	4.7 ppm	center of spectrum for ^1H
d1	1.3 s	delay parameter in seconds
d4,d24	2.8 ms	$1/4J$ for inept transfer where $^1J_{\text{NH}} \sim 90\text{hz}$
expt	~15 min	experiment time using default param. (8 scans)
gp1,gp2	80:8.1	gradient ratio to select ^1H - ^{15}N

acquisition parameters in F1 (^{15}N):

td	64	number of data points (time domain size)
sw	40 ppm	^{15}N sweep width (opt. for backbone amides)
o2p	120 ppm	center of spectrum for ^{15}N (opt. for backbone amides)
in0	$1/2 * \text{sw}$	increment delay
MC2	echo-antiecho	phase sensitive

The 2D ^1H - ^{15}N HSQC Experiment, cont.



processing parameters in F2 (^1H):

si	1k	real spectrum size, si = td (zero fill 1x)
wdw	QSINE	sinebell squared window function
ssb	2	shift of sinebell window
PH_mod	pk	phase sensitive

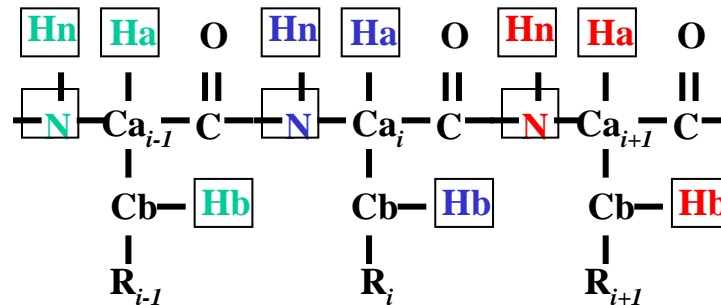
processing parameters in F1 (^{15}N):

si	64	real spectrum size
wdw	QSINE	sinebell window function
ssb	2	no shift of sinebell window
PH_mod	pk	phase sensitive

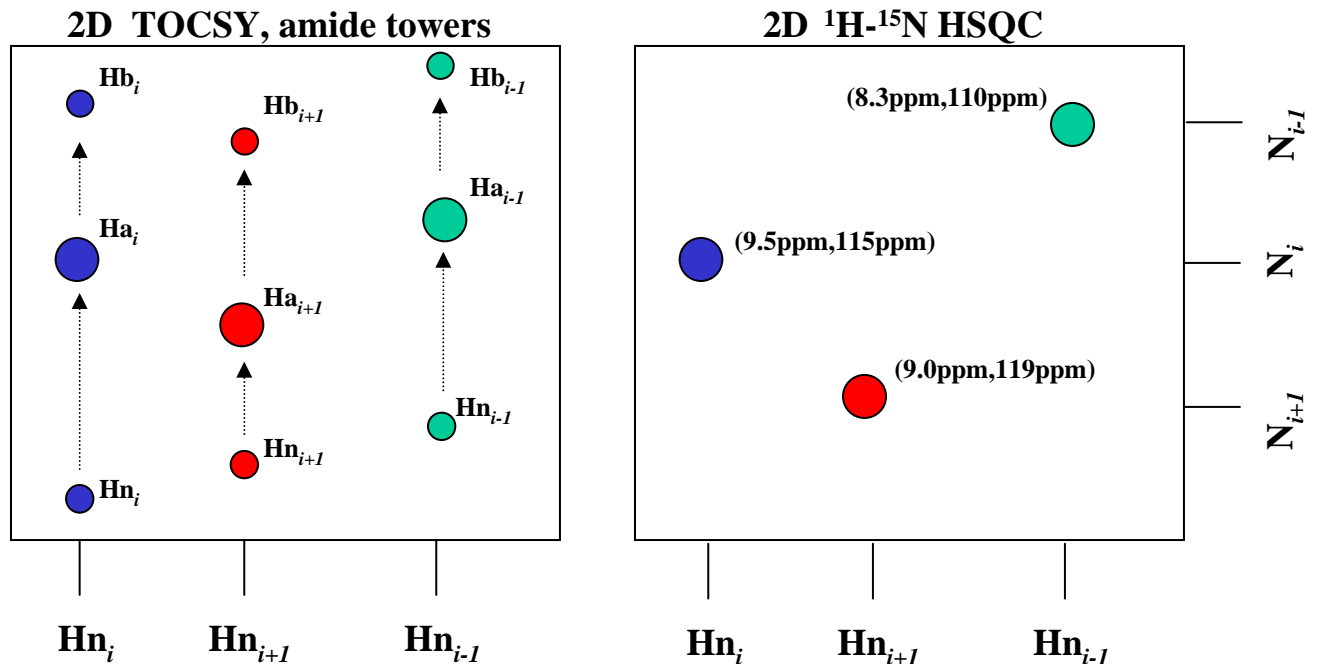
Strategy for Assigning Spin Systems of Peptides/Proteins using 3D ¹⁵N TOCSY-HSQC and 3D ¹⁵N NOESY-HSQC experiments

3D ¹⁵N TOCSY-HSQC Example:

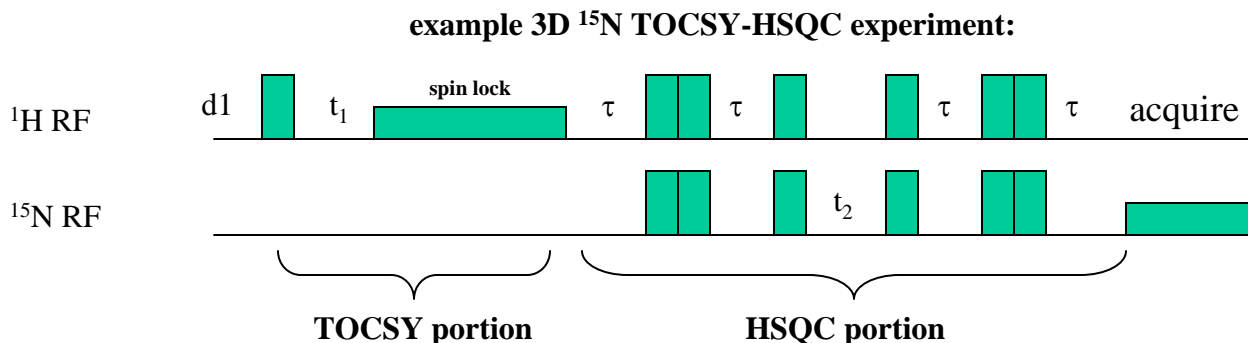
- consider the following peptide fragment:



- assume this peptide fragment has the following signals in a 2D TOCSY and a 2D ¹H-¹⁵N HSQC experiment:

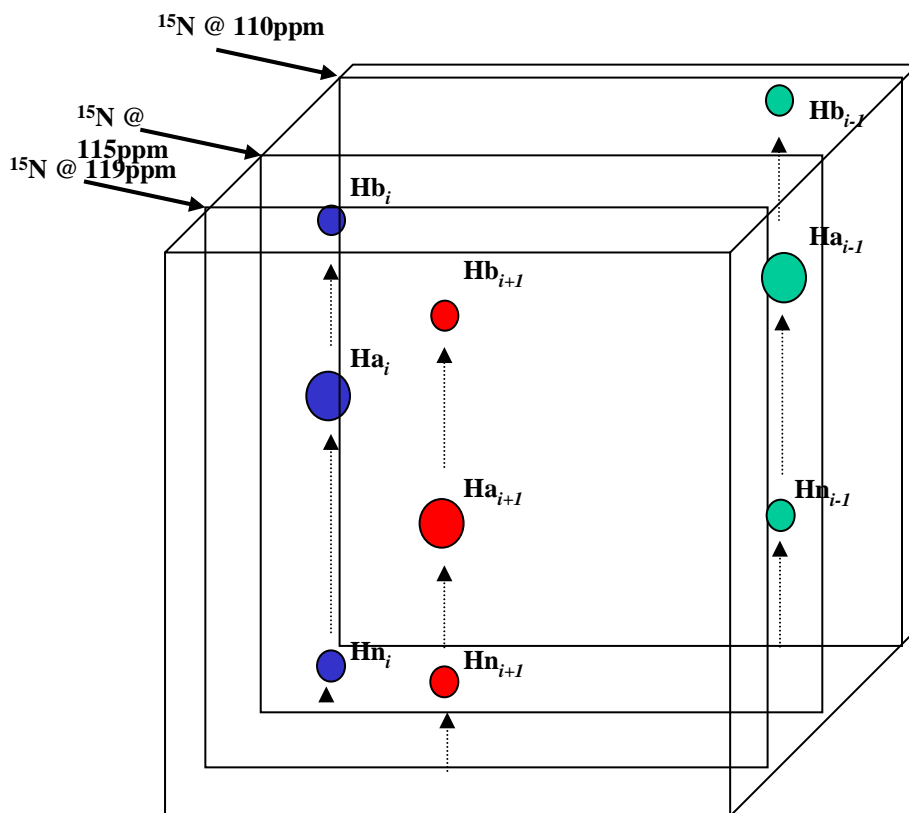


- The information contained in the 2D TOCSY and 2D ^1H - ^{15}N HSQC experiments can be obtained in a single 3D ^{15}N TOCSY-HSQC experiment enabling resolution of the amide strips in the TOCSY experiment according to the amide ^{15}N chemical shift:



the 3D experiment is typically set up with the following parameters:

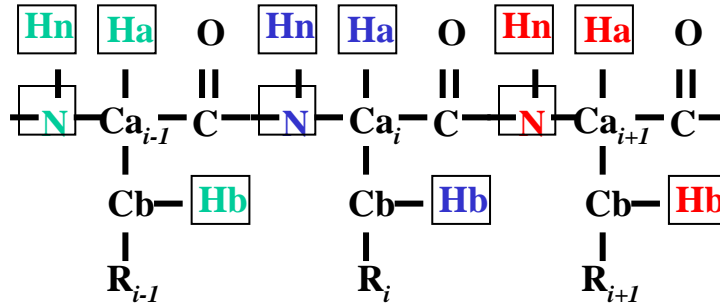
- ^1H acquisition period: $\text{TD}=1\text{k}$, $\text{sw}=14\text{p}$, $\text{aq}=122\text{ms}$, $d1=1.3\text{s}$
- ^{15}N evolution period (t_2): $\text{TD}=64$, $\text{sw}=40\text{p}$ @ 120p
- ^1H evolution period (t_1): $\text{TD}=400$, $\text{sw}=14\text{p}$
- tocsy spin lock is set-up the same way as 2D ^1H - ^1H TOCSY (see Lab #4)
- the total experiment time is determined by the number of scans (NS) which depends on concentration of sample - under above conditions, $\text{NS}=8$ will give expt~3.5 days



- Using the 2D ^1H - ^{15}N HSQC spectrum as a guide, ^1H - ^1H TOCSY planes can be extracted from the 3D dataset at the appropriate ^{15}N chemical shift in order to assign the residue type for each spin system (see Lab #4).

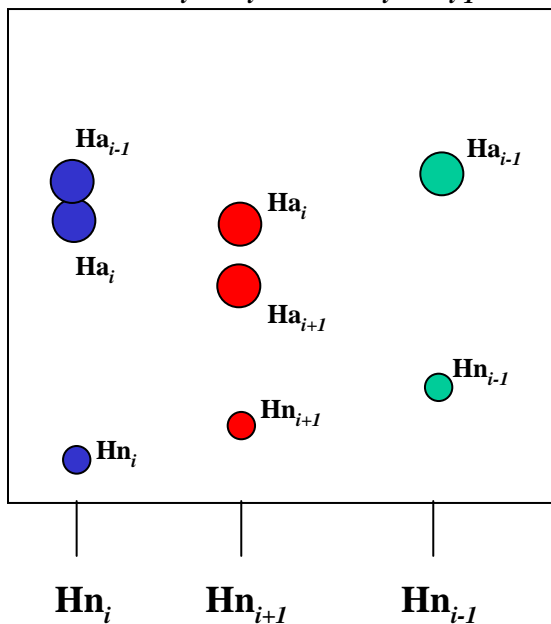
3D ^{15}N NOESY-HSQC Example:

- consider the following peptide fragment:

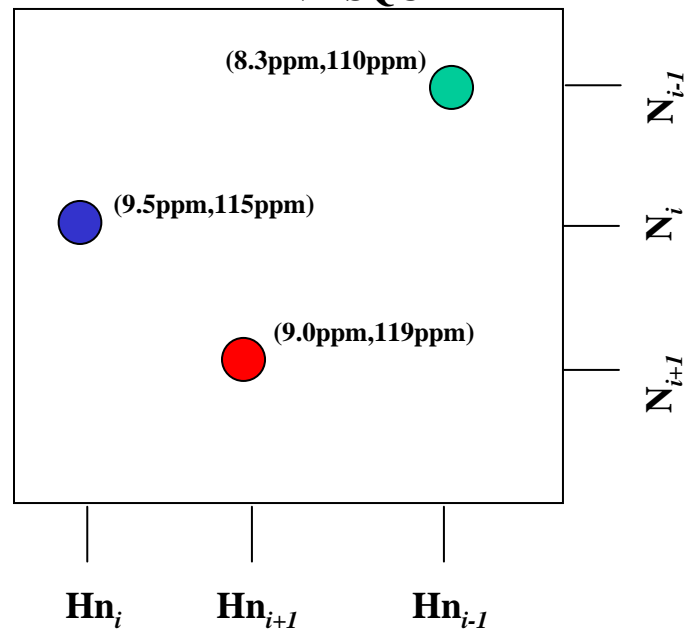


- assume this peptide fragment has the following signals in a 2D NOESY and a 2D ^1H - ^{15}N - HSQC experiment:

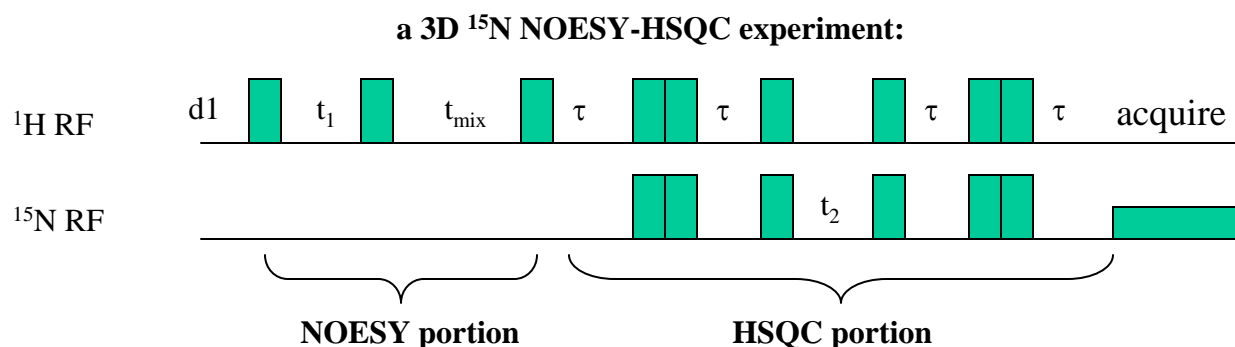
2D NOESY, amide towers
showing Hn_i - Ha_i and Hn_i - Ha_{i-1} NOEs



2D ^1H - ^{15}N HSQC

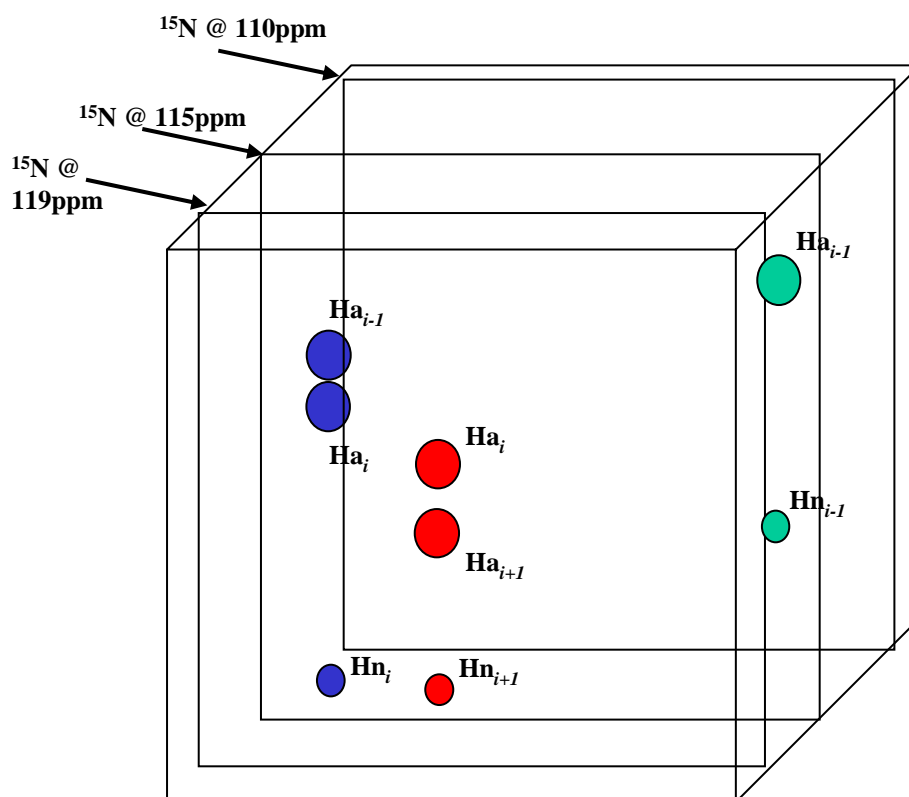


- The information contained in the 2D NOESY and 2D ^1H - ^{15}N HSQC experiments can be obtained in a single 3D ^{15}N NOESY-HSQC experiment enabling resolution of the amide strips in the NOESY experiment according to the amide ^{15}N chemical shift:



the 3D experiment is typically set up with the following parameters:

- ^1H acquisition period: TD=1k, sw=14p, aq=122ms, d1=1.3s
- ^{15}N evolution period (t_2): TD=64, sw=40p @ 120p
- ^1H evolution period (t_1): TD=400, sw=14p
- noesy mixing time is set-up the same way as 2D ^1H - ^1H NOESY (see Lab #5)
- the total experiment time is determined by the number of scans (NS) which depends on concentration of sample - under above conditions, NS=8 will give expt~3.5 days



- Using the 2D ^1H - ^{15}N HSQC spectrum as a guide, ^1H - ^1H NOESY planes can be extracted from the 3D dataset at the appropriate ^{15}N chemical shift in order to sequentially assign each spin system (see Lab #5).