BIOC530 Protein NMR Sessions
Outline

Wednesday, Nov. 19, 2014	Friday, Nov. 21, 2014
Introduction to Protein NMR (RK)	Case Study (VV)
Practical Considerations (SD)	How to study binding (MS)
How to look at an NMR spectrum: 2D &3D (KD)	Strategies for large proteins (MS)
How to assign a spectrum (LT)	New frontiers: solid-state NMR (MS)
	Q & A (all)

BIOC530: NMR Unit, Part 2
Protein NMR: What can it do?

Wednesday	Friday
Intro: Protein NMR… definitely NOT your one-trick pony (RK)	Case Study (VV)
Practical Considerations (SD)	How to study binding (MS)
How to look at an NMR spectrum (KD)	Strategies for large proteins (MS)
How to assign a spectrum (LT)	New frontiers: solid-state NMR (MS)
	Q & A

Protein NMR…
definitely NOT your one-trick pony

1. CHEMICAL SHIFTS (i.e., frequency at which a given nucleus resonates)

Benefits: easy to measure (2D spectra), with good S/N, high accuracy, and high precision

Information content:

- Protein backbone shifts ($^{1}H^{15}N$, $^{13}C_{\alpha}$, $^{13}C'$) ⟶ secondary structure info
- $^{13}CH_3$ (Ile) ⟶ distinguish α-helix and β-strand in very large proteins/complex
- As *f*(pH), ⟶ pK_R values of individual sidechains in protein
  pH-dependent conformational change
  tautomeric states of histidines
- As *f*([ligand]), ⟶ binding site identification (at residue-level resolution)
  estimate exchange rate/lifetime of bound species
  estimate K_D (but not very well!)

Protein NMR…
definitely NOT your one-trick pony

2. Nuclear Overhauser Effects (NOE), interaction bet. Nuclei w/ r^{-6} dependence

Benefits:

- under optimal conditions, can measure hundreds – thousands of pairwise distances that can define a 3D structure *de novo*.
- Even limited NOE information can guide structure determination using sparse constraints.
- Heteronuclear NOEs (hNOEs) such as ^{1}H, ^{15}N provide information about dynamics in the ps - ns timescale on a residue level. (great for segmental motions)

Limitations:

- Low sensitivity experiment (not hNOE).
- Difficult/tedious to assign unambiguously to specific pairs
- Not strictly r^{-6}, so have to use distance bins rather than explicit distances
- Practically speaking, limited to protein systems with fewer than 200 residues.

Protein NMR…
definitely NOT your one-trick pony

3. Paramagnetic Relaxation Enhancement (PRE), effect of "spin label" on NMR resonances.

Benefits:

- Measurements are made using robust 2D spectra (^{15}N or ^{13}C)
- Can be either intramolecular (especially useful for IDPs) or intermolecular
- Can be used in highly flexible systems (IDPs, fuzzy complexes, etc.)
- Effects go out to longer distances than NOEs

Limitations:

- Need to attach a paramagnetic label via a single Cys residue.
- Label is long and flexible, so data is often used qualitatively.

Protein NMR…
definitely NOT your one-trick pony

4. Residual Dipolar Couplings (RDCs), information on bond vector orientation relative to external frame (external magnetic field)

Benefits:

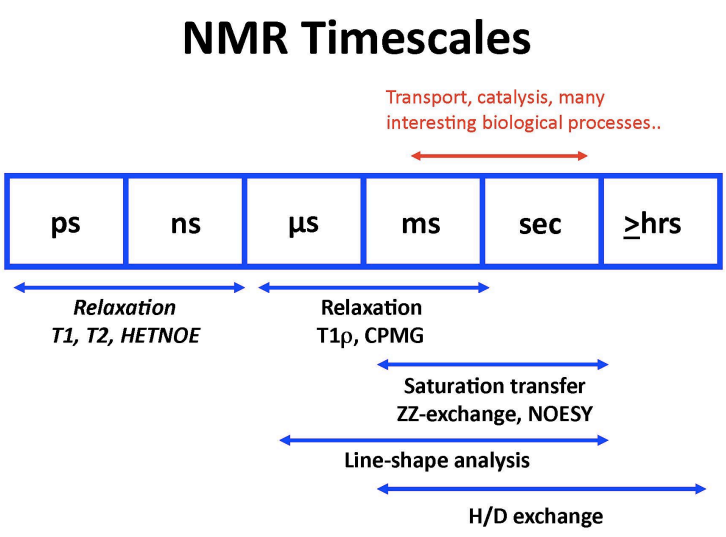
- Measurements are made using robust 2D spectra (^{15}N or ^{13}C)
- Very useful for structure determination using sparse constraints.
- Can provide long-range information, so good for defining domain-domain orientations, subunit-subunit orientation.

Limitations:

- Requires partial alignment of sample in the magnetic field. Alignment media include polyacrylamide gels, phage, organic solvents.
- Spectrum can degrade due to peak broadening.

Protein NMR…
definitely NOT your one-trick pony

5. Dynamics! A whole lecture of its own…



SCOTT'S SLIDES

Practical Considerations for Protein NMR

NMR SAMPLES

- Proteins < 25kDa
  - reduced signal from relaxation
  - spectral crowding
  - methods to overcome this limitation (later!)
- 100-500µM
  - >90% pure, structurally homogenous
  - concentrated samples required for low sensitivity experiments
  - some data can be collected at lower concentrations (20µM)
- 300-500µL

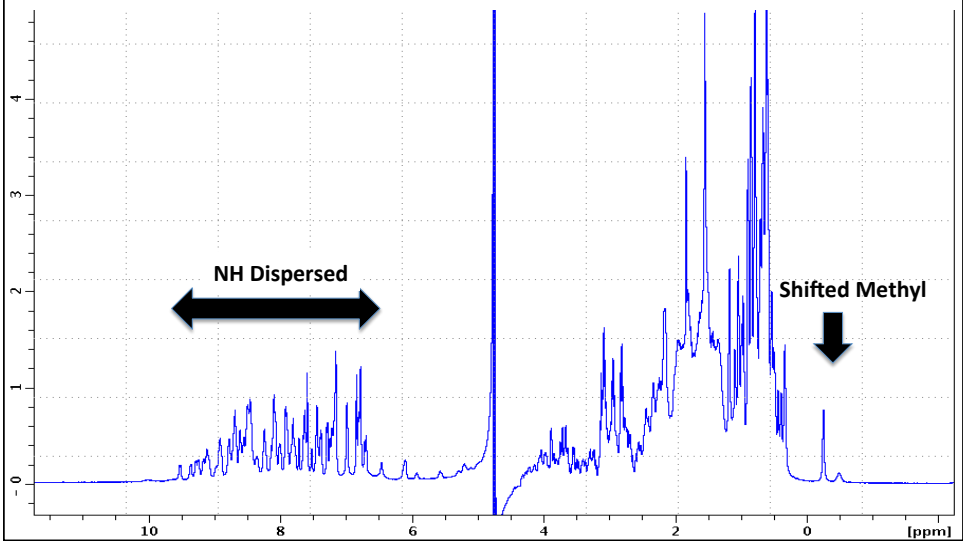
Solution Conditions

- Buffers "invisible" in experiments
  Phosphate is preferred, Tris is not
- Optimize conditions for data collection
  seeking the strongest signal
  Vary pH, ionic strength, temperature, and [protein]
  mutagenesis
- For binding experiments, material must be in matching solutions

1D ^{1}H Spectra

- "Quick" experiments ~15 minutes
- Natural abundance
- Is your protein folded?
- Mutagenesis
- New Constructs
- Sample quality
- Degradation can often be observed

THIS PROTEIN IS FOLDED



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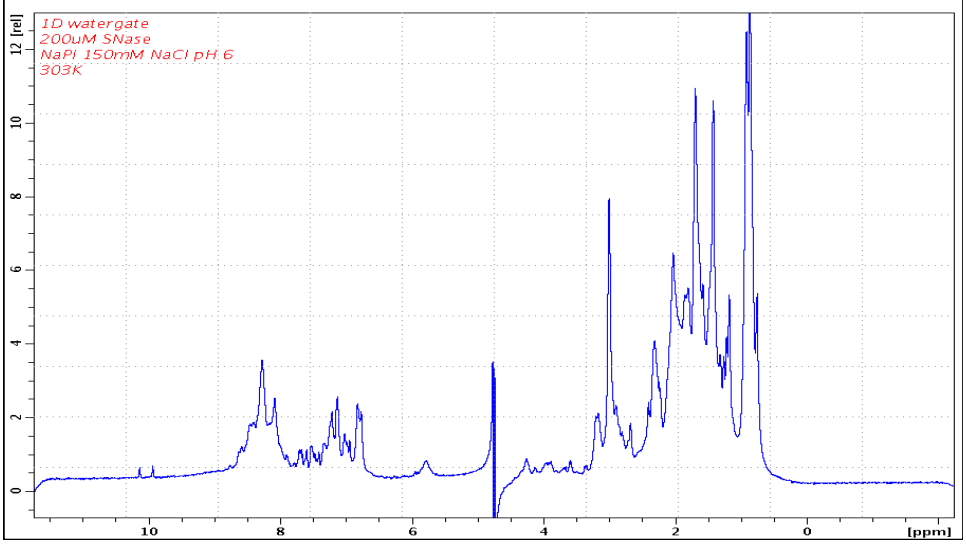


Isotopic Labeling

- Multi-dimensional NMR (2D, 3D) has many advantages over 1D NMR
- ^{15}N, ^{13}C, proteins

  Purified from bacteria grown in minimal media with ^{15}N-NH_4Cl (20$/L) and/or ^{13}C-glucose (100-200$/L)

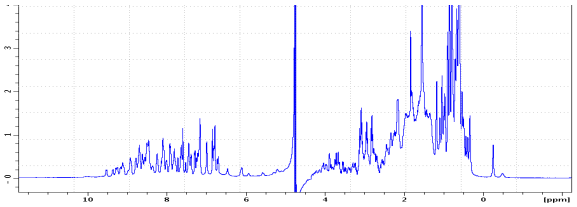
  Other metabolic precursors may be used
- Deuteration

  Reduced relaxation (more robust signal)

  grown in D_2O media (up to1000$/L)

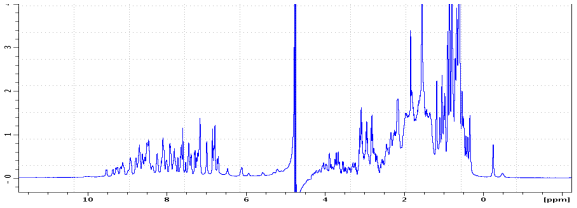
Add one dimension for better resolution and more information

1D



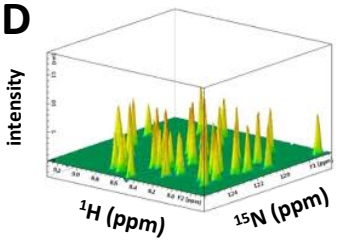
Add one dimension for better resolution and more information

1D



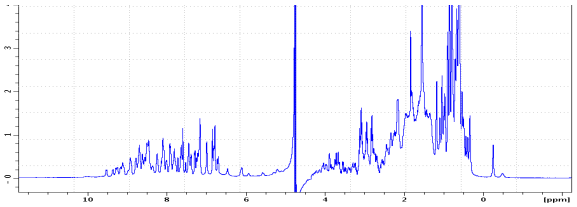
Stacked Plot

2D



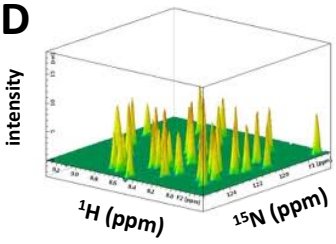
Add one dimension for better resolution and more information

1D

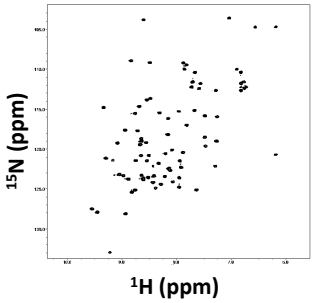


2D

Stacked Plot

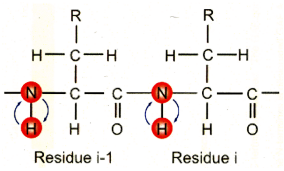


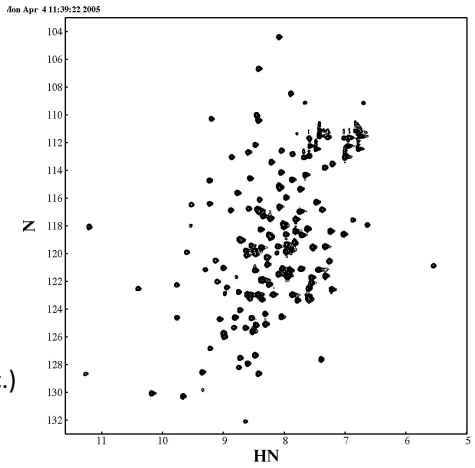
Contour Plot



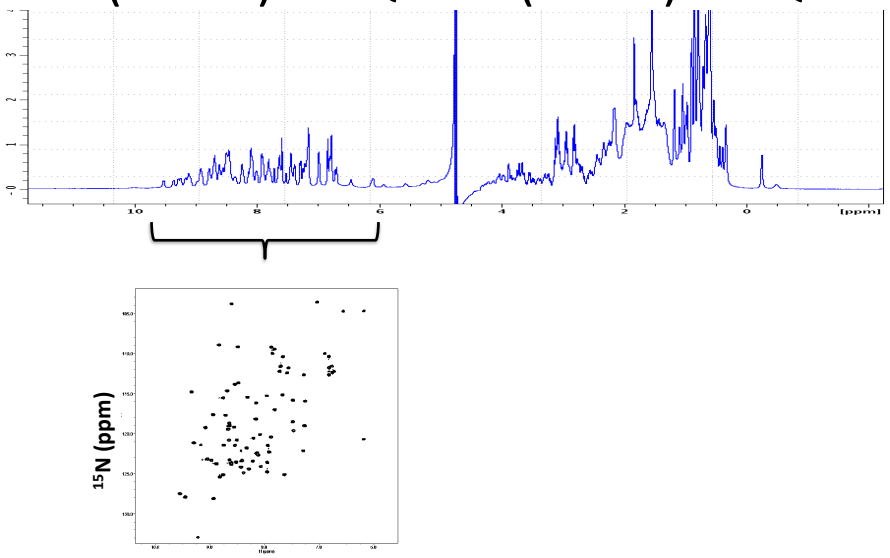
(^{1}H, ^{15}N)-HSQC: the simplest heteronuclear 2D spectrum

- Essentially, 1 peak/ residue:
  1. amide NH
  2. Asn & Gln NH_2
  3. Trp indole NH
  4. His imidazole NH
  5. (Arg NH, Lys NH_3)
- "fingerprint" of protein
- BUT…chemical shifts of NH resonances are sensitive to:
  1. pH
  2. temperature
  3. (ionic strength)
  4. other (ligand binding, etc.)



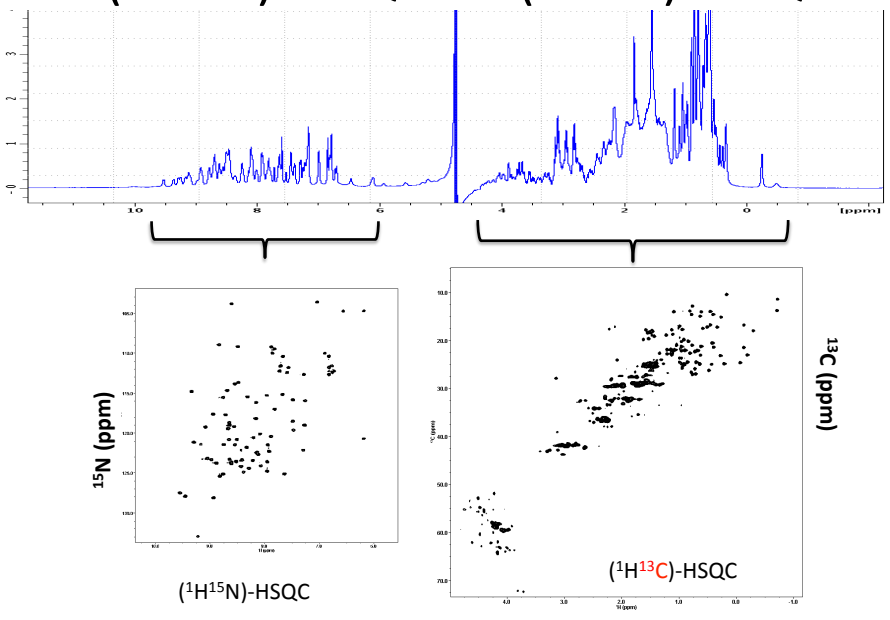


($^1H^{15}N$)-HSQC vs. ($^1H^{13}C$)-HSQC



($^1H^{15}N$)-HSQC

($^1H^{15}N$)-HSQC vs. ($^1H^{13}C$)-HSQC



($^1H^{15}N$)-HSQC

3D spectra: increased dispersion and information

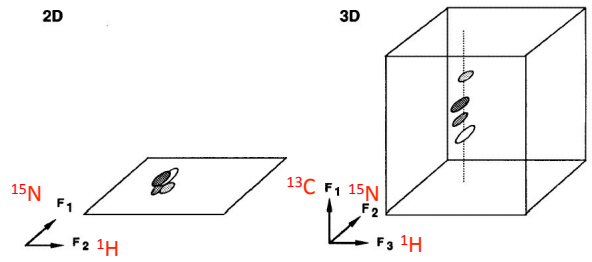
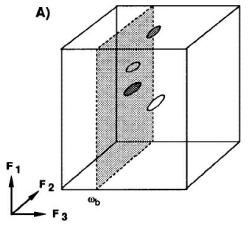
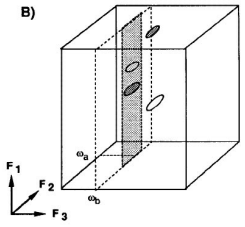


Figure 1.2.4

"triple resonance"

Planes

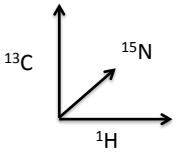


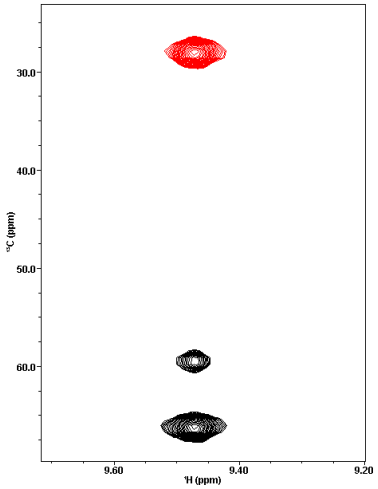


Strips

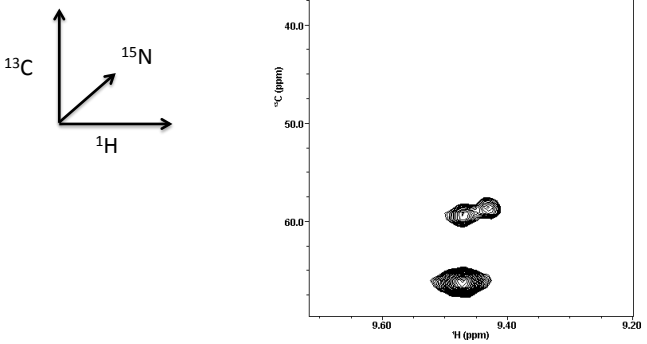
Figure 1.2.6. Cross sections (A) and strips (B).

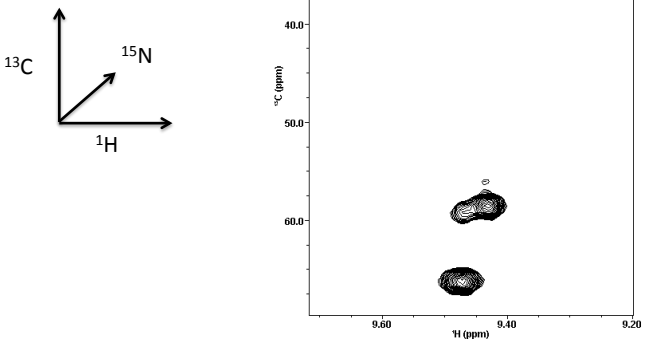
3D NMR data set

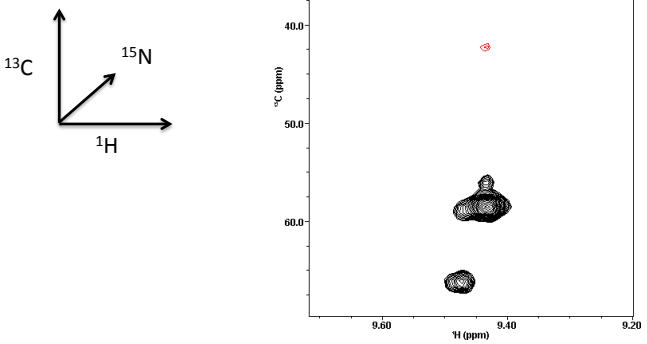


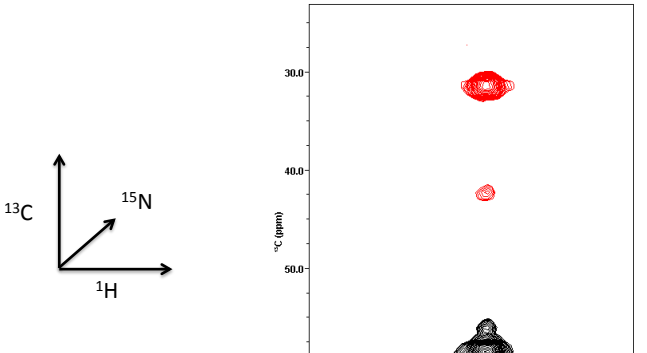


"walk" through ^{15}N dimension

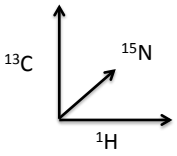

^{13}C
^{15}N
^{1}H
30.0
40.0
50.0
60.0
^{13}C (ppm)
9.60
9.40
9.20
^{1}H (ppm)

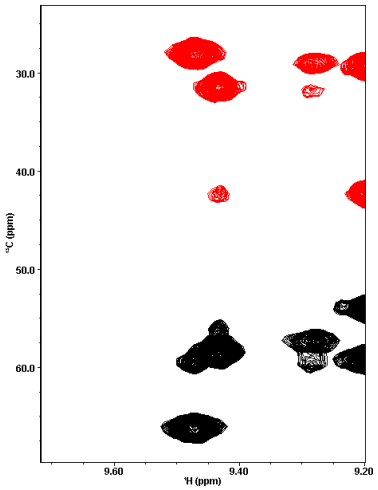

^{13}C
^{15}N
^{1}H
30.0
40.0
50.0
60.0
^{13}C (ppm)
9.60
9.40
9.20
^{1}H (ppm)


^{13}C
^{15}N
^{1}H
30.0
40.0
50.0
60.0
^{13}C (ppm)
9.60
9.40
9.20
^{1}H (ppm)

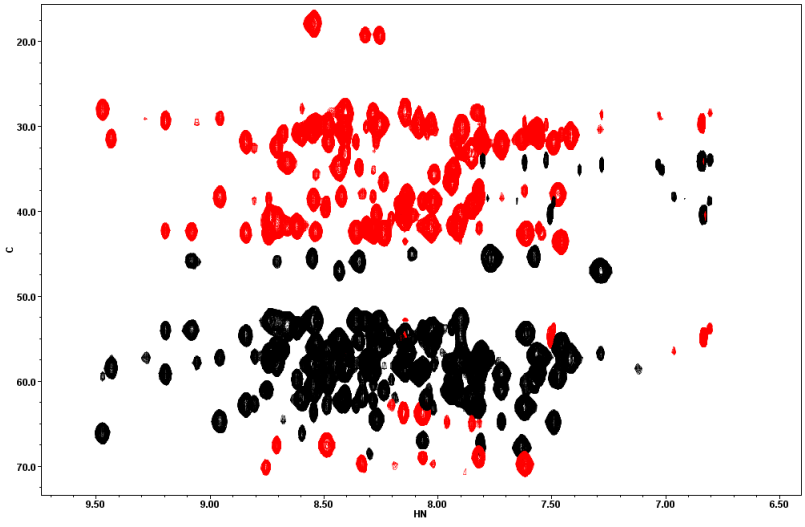

^{13}C
^{15}N
^{1}H
30.0
40.0
50.0
60.0
^{13}C (ppm)
9.60
9.40
9.20
^{1}H (ppm)

Strip of Collapsed ^{15}N dimension

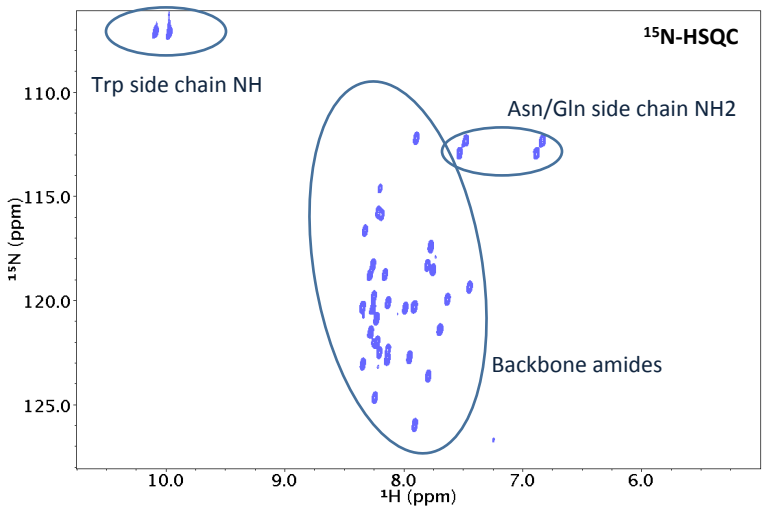




^{13}C-^{1}H Plane of Collapsed ^{15}N dimension



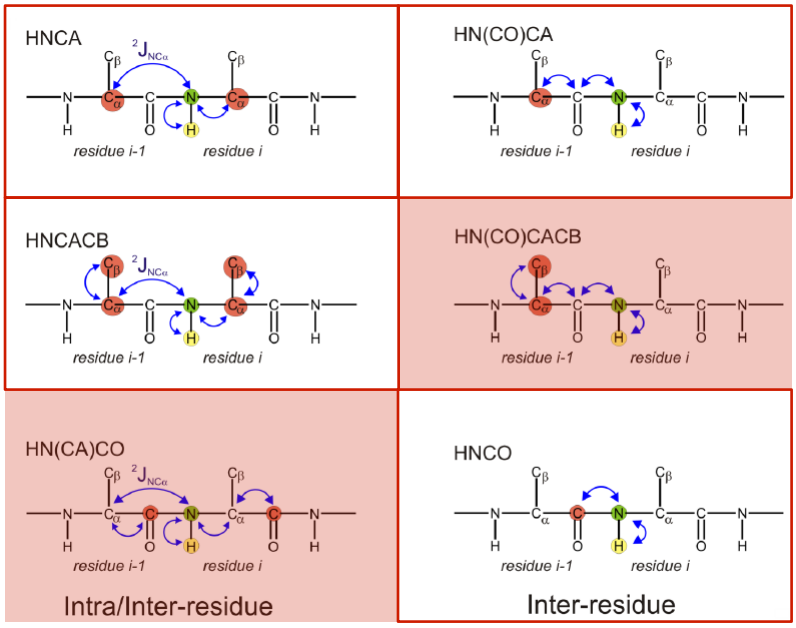
NMR Assignments – A simple example



110 120 130

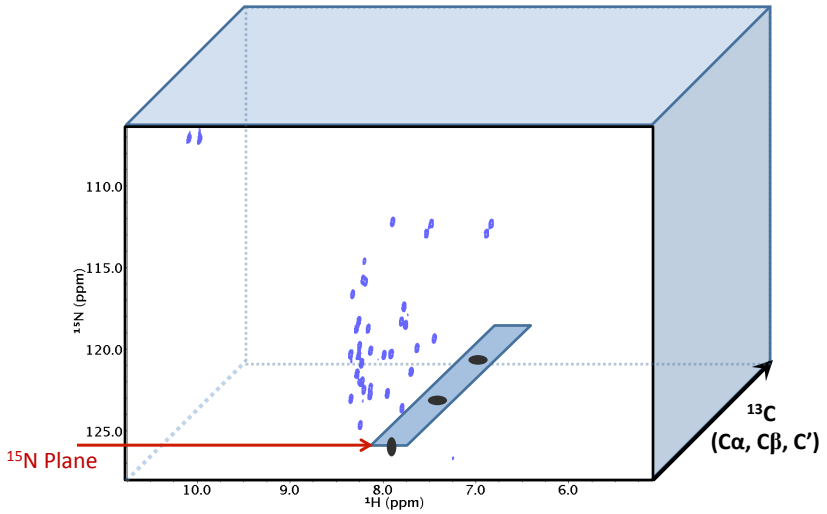
34 Residue peptide: STDST PMFEY ENLED NSAFW MWLFA TDIPV TTDD

Backbone triple resonance experiments (need ^{1}H, ^{13}C, ^{15}N sample)

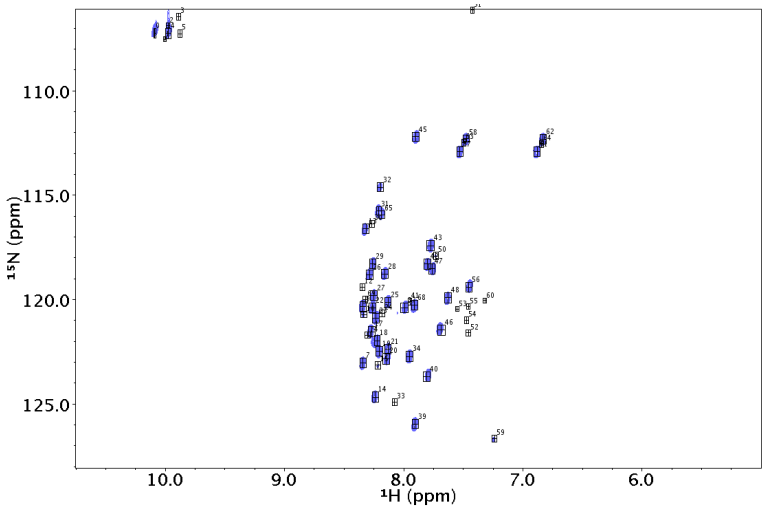


***i* and *i-1* peaks** ***i-1* peaks**

3D spectra for backbone assignments

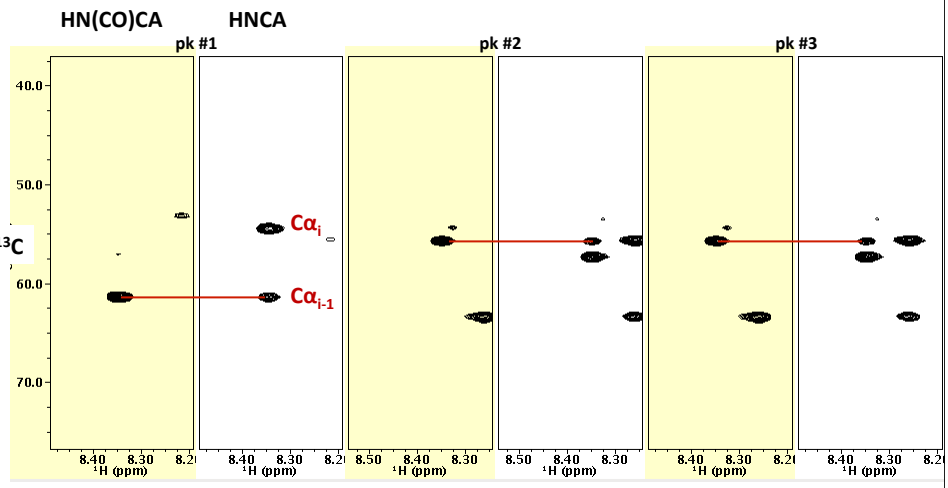


Backbone Assignments – Step 1: Pick the peaks



34 Residue peptide: STDST PMFEY ENLED NSAFW MWLFA TDIPV TTDD

Backbone Assignments



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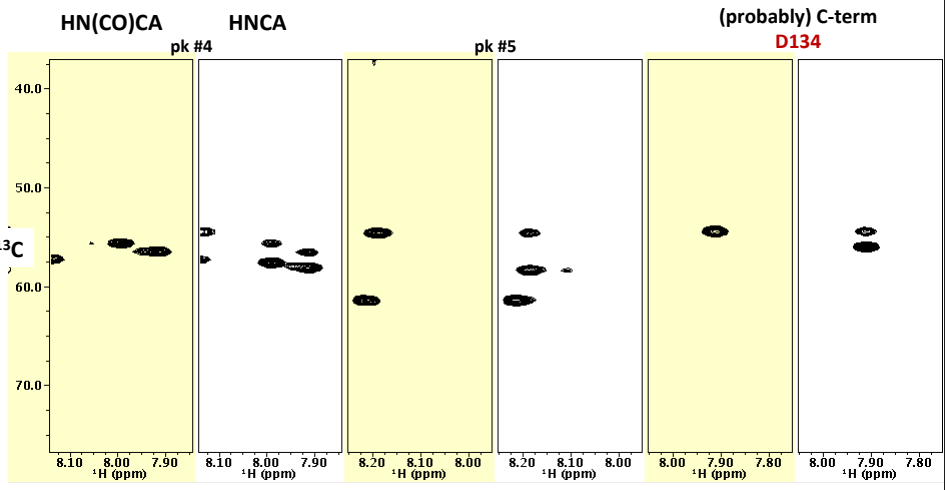
Backbone Assignments



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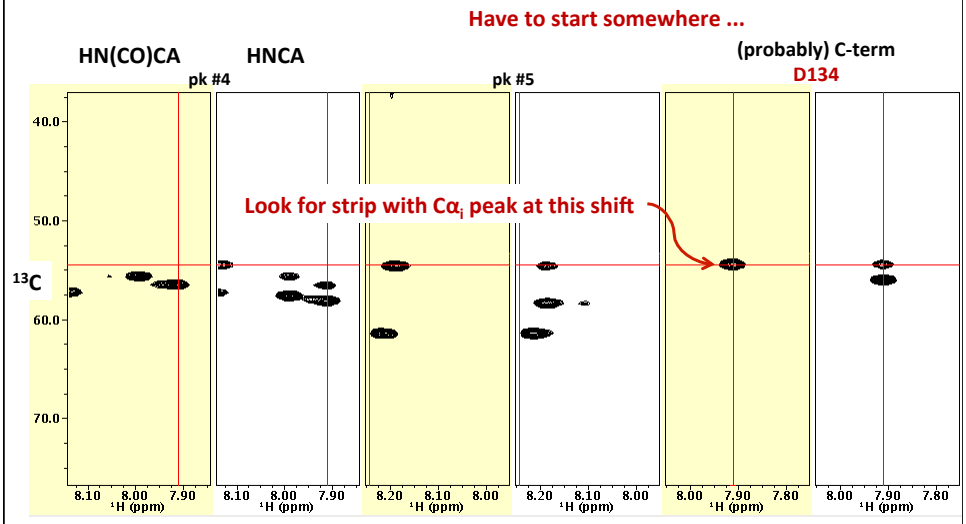
Backbone Assignments



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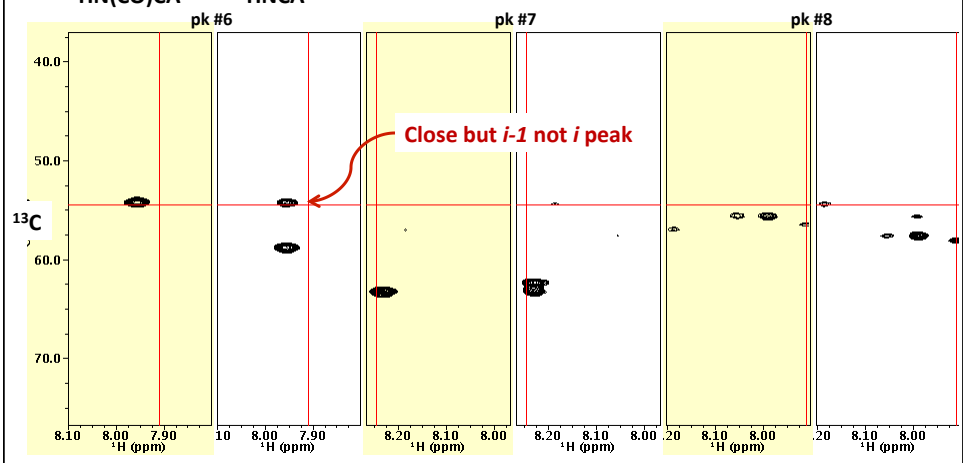
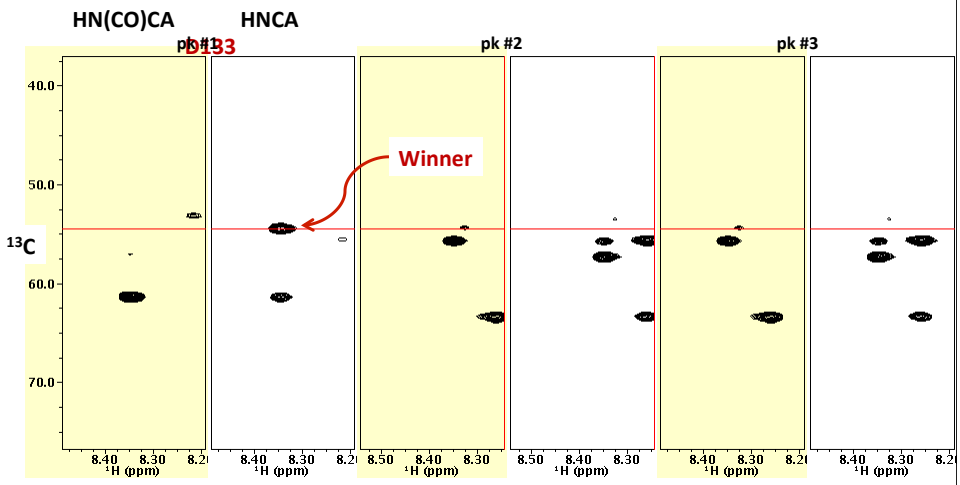
Backbone Assignments



34 Residue peptide: STDST PMFEY ENLED NSAFW MWLFA TDIPV TTDD

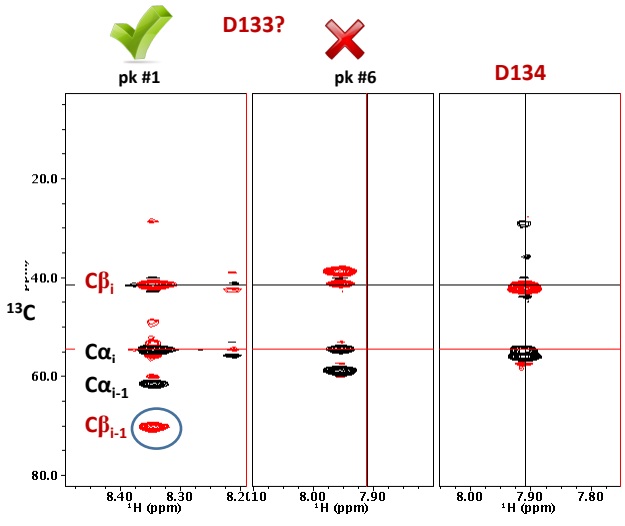
Backbone Assignments



34 Residue peptide: STDST PMFEY ENLED NSAFW MWLFA TDIPV TTDD

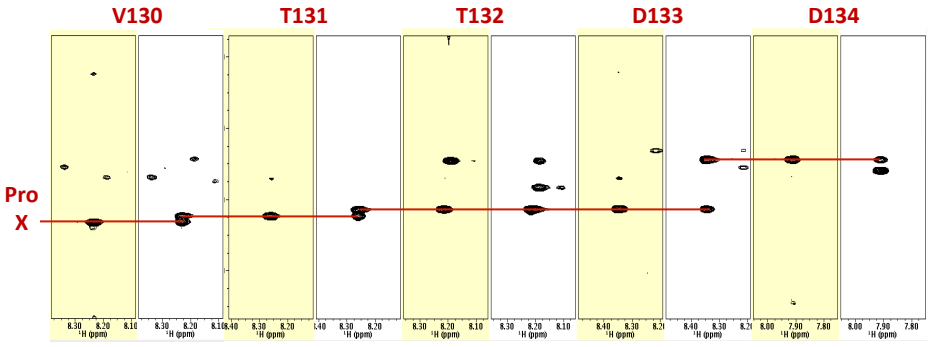
Backbone Assignments

Can confirm with HNCACB



34 Residue peptide: STDST PMFEY ENLED NSAFW MWLFA TDIPV TTDD

Backbone Assignments

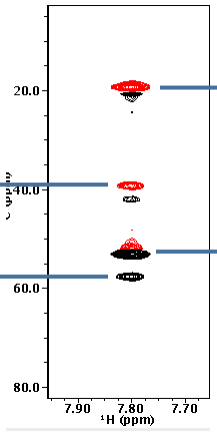


Chain stops here

34 Residue peptide: STDST PMFEY ENLED NSAFW MWLFA TDIPV TTDD

Backbone Assignments

Alanine
118 or 125?



Alanines have distinctive Cβ shifts

So do Thr & Ser

Look for *i-1* peaks

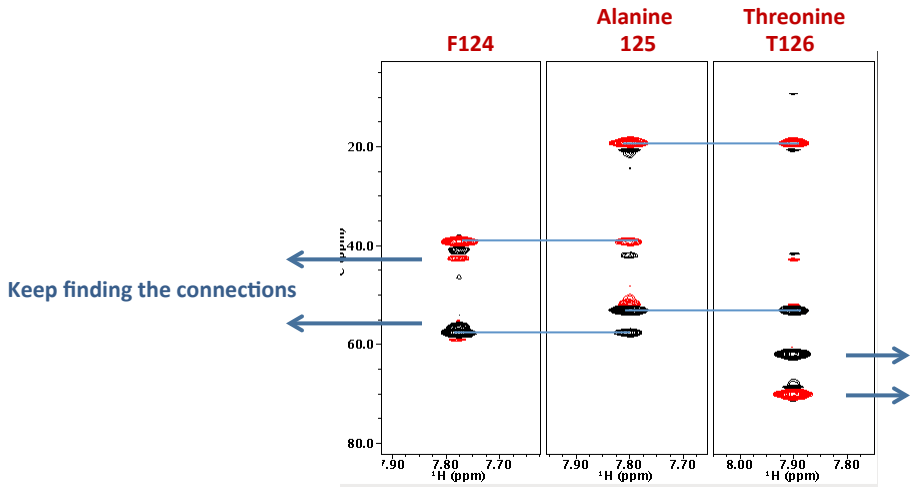
Look for *i* peaks

Peak is **A118** if the previous strip looks like a **Ser**

Peak is **A125** if the next strip looks like a **Thr**

34 Residue peptide: STDST PMFEY ENLED NSAFW MWLFA TDIPV TTDD

Backbone Assignments

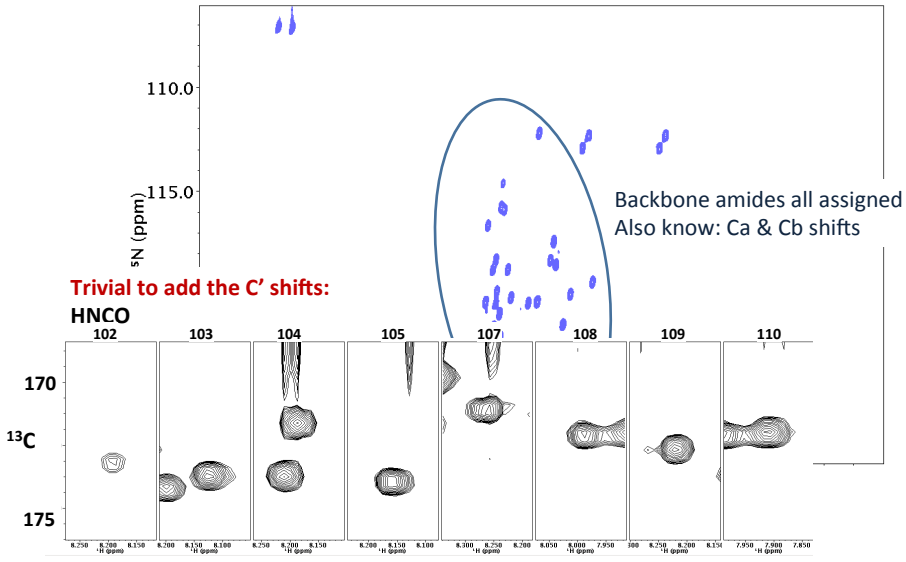


Repeat for remaining sections ...

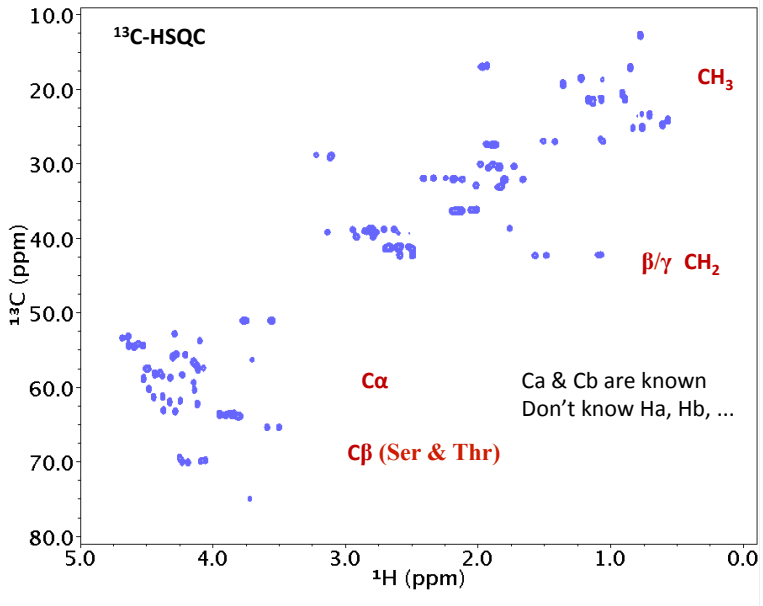
34 Residue peptide: STDST PMFEY ENLED NSAFW MWLFA TDIPV TTDD

Backbone Assignments: HN, N, Ca, Cb, C'

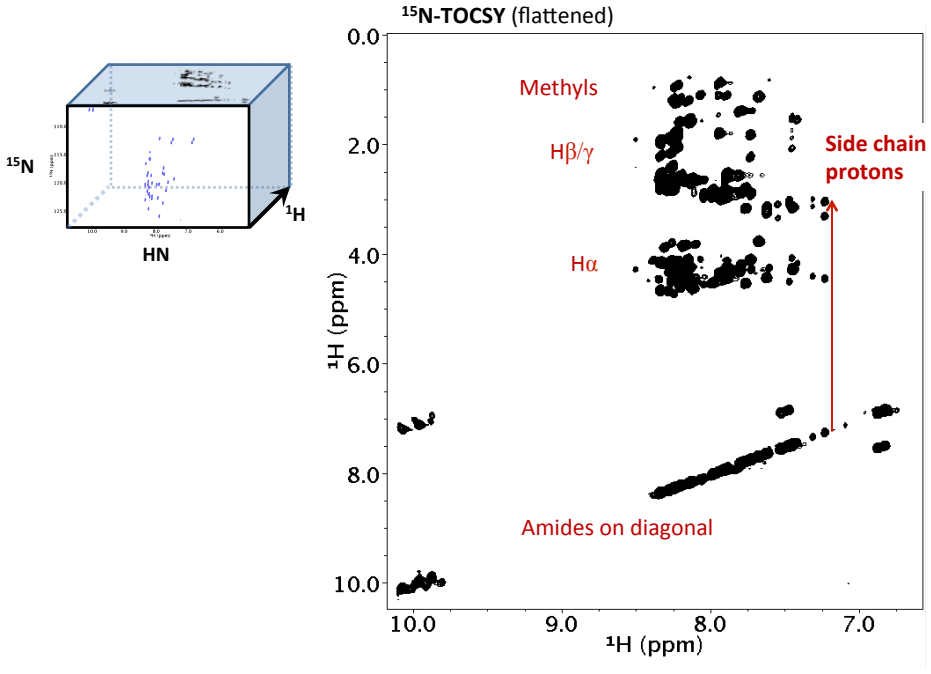
34 Residue peptide: STDST PMFEY ENLED NSAFW MWLFA TDIPV TTDD

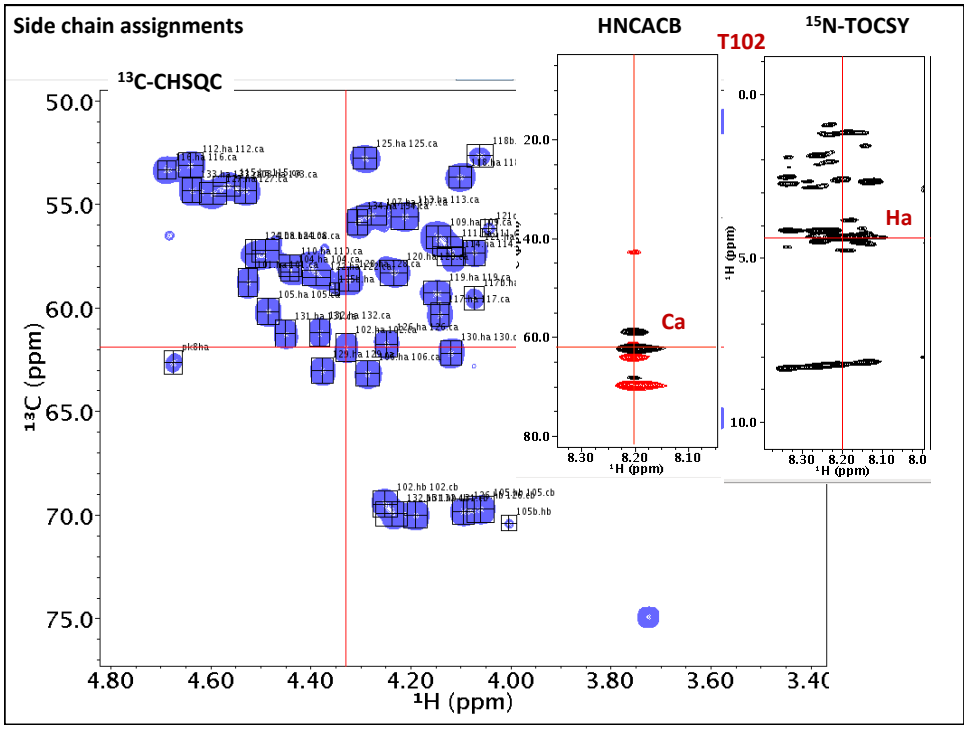


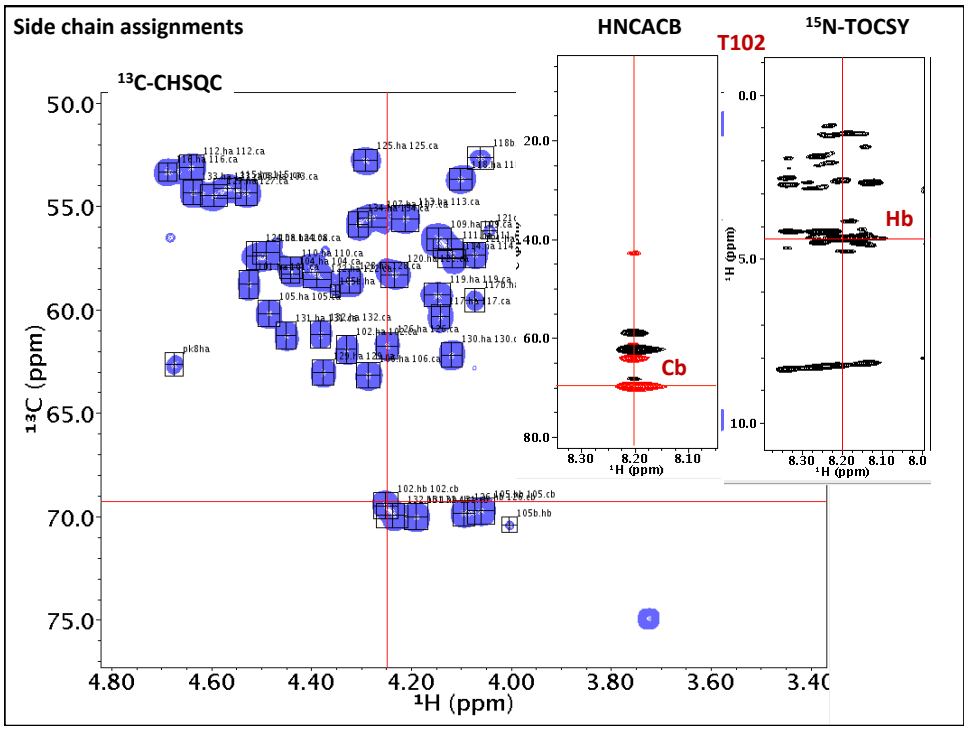
Side chain assignments

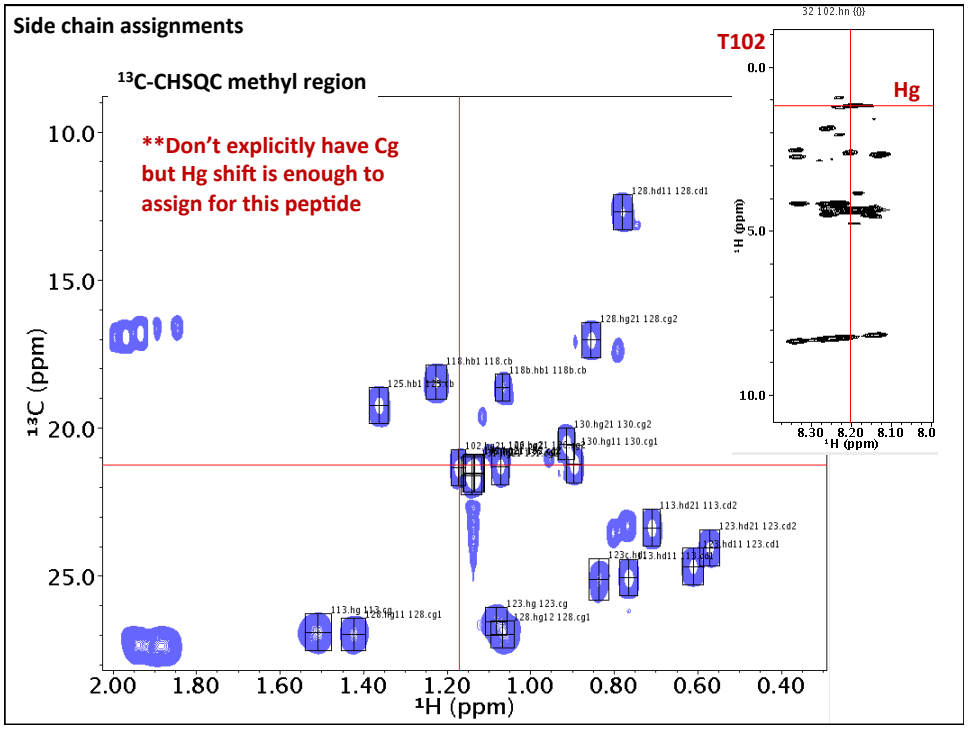


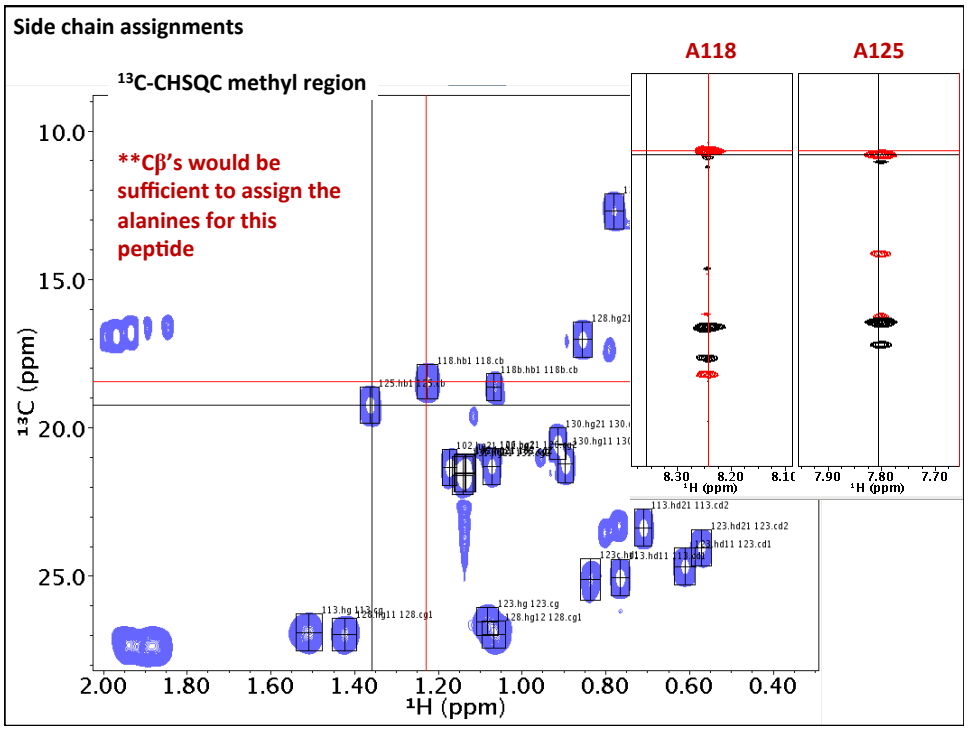
Side chain assignments




Side chain assignments
HNCACB
T102
15N-TOCSY
13C-CHSQC
Ca
Ha
13C (ppm)
1H (ppm)


Side chain assignments
HNCACB
T102
15N-TOCSY
13C-CHSQC
Cb
Hb
13C (ppm)
1H (ppm)


Side chain assignments
^{13}C-CHSQC methyl region
**Don't explicitly have Cg but Hg shift is enough to assign for this peptide
T102
Hg
^{13}C (ppm)
^{1}H (ppm)


Side chain assignments
^{13}C-CHSQC methyl region
**Cβ's would be sufficient to assign the alanines for this peptide
A118
A125
^{13}C (ppm)
^{1}H (ppm)

Side chain assignments: Ha, Ca, Hb, Cb, Hg, Hd … Cg, Cd inferred