

# RMN multidimensional em biologia estrutural

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IQUSP

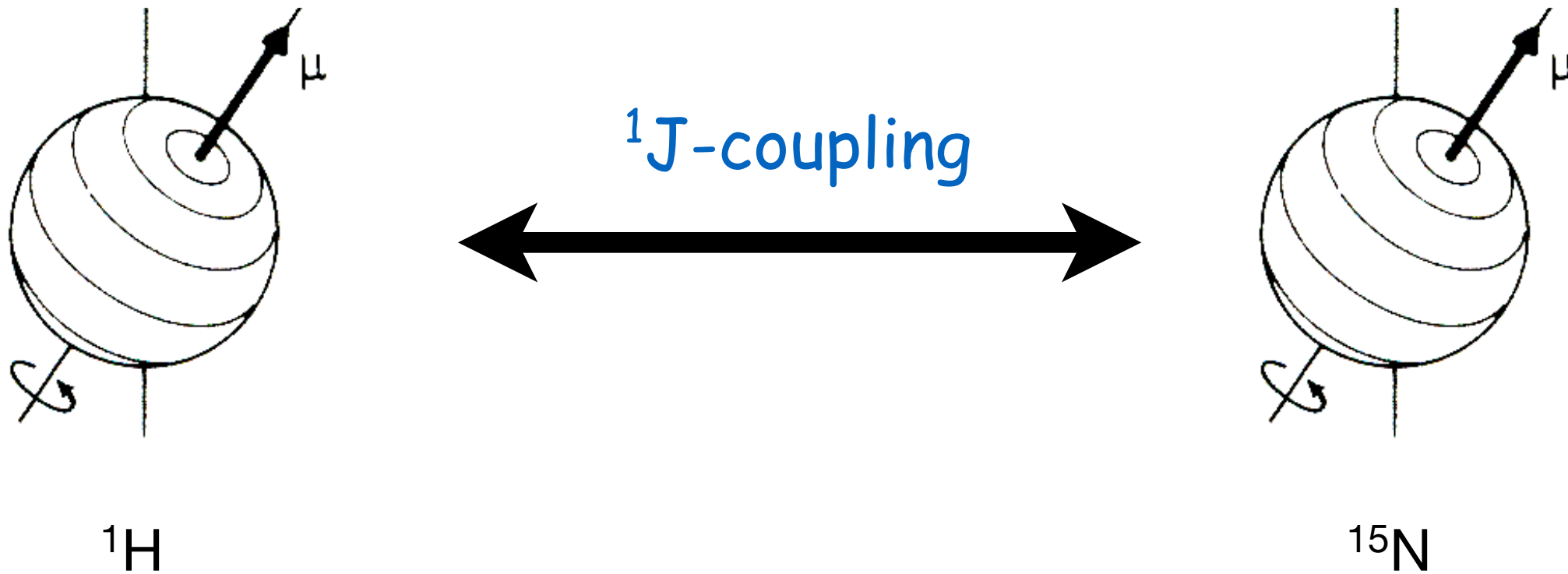
# The assignment problem

## Strategy 2: Multidimensional approach

- (Additional) Heteronuclear 2D & 3D experiments
  - ✓ Triple resonance - assign "spin systems" (residue-type)
  - ✓ Requires isotope labelling with  $^{15}\text{N}$  and  $^{13}\text{C}$  (< 20 kDa) or  $^2\text{H}$ ,  $^{15}\text{N}$  and  $^{13}\text{C}$  (20 - 50 kDa)
  - ✓ Use pairs of experiments to sequentially assign all resonances

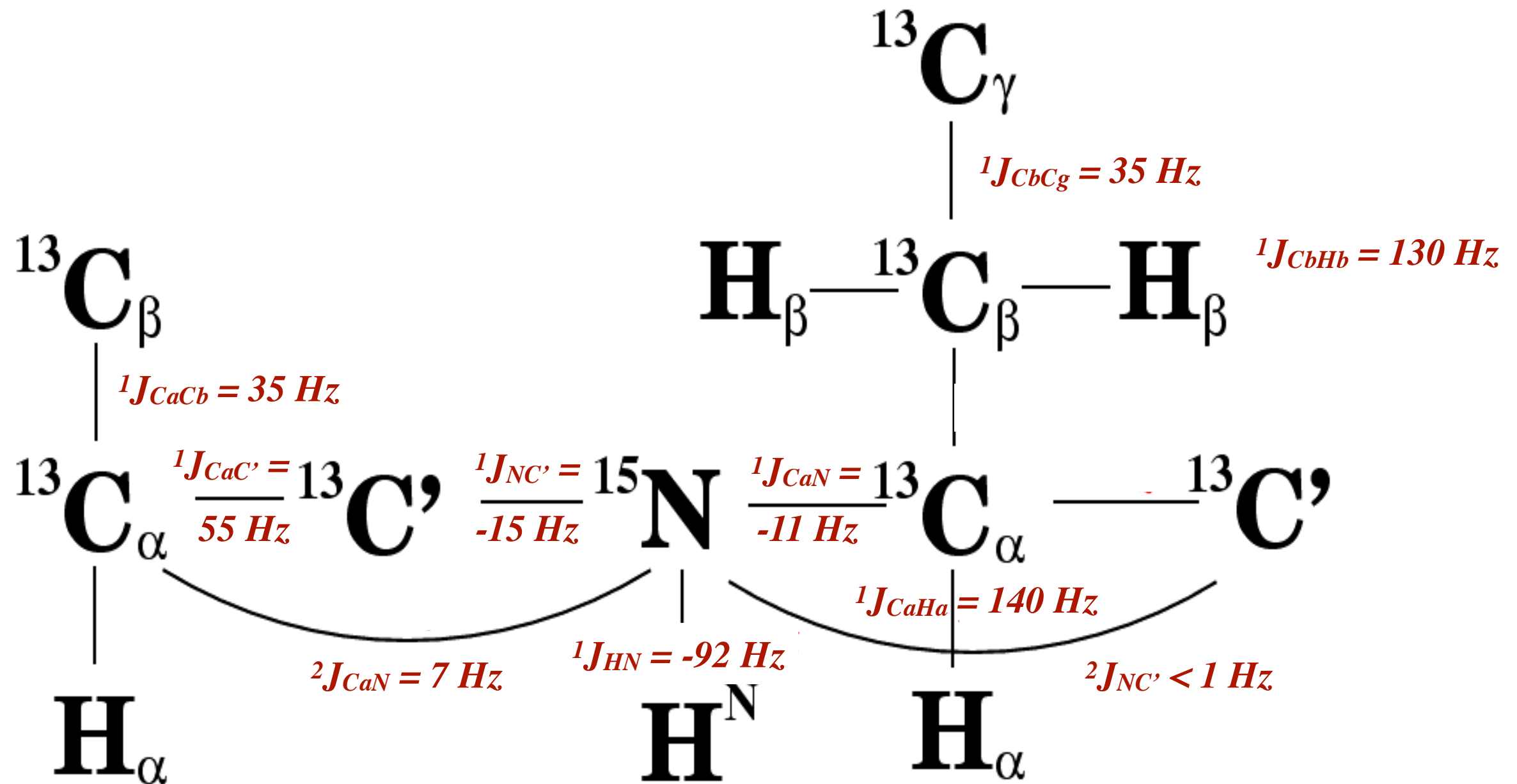
RMN multidimensional  
heteronuclear

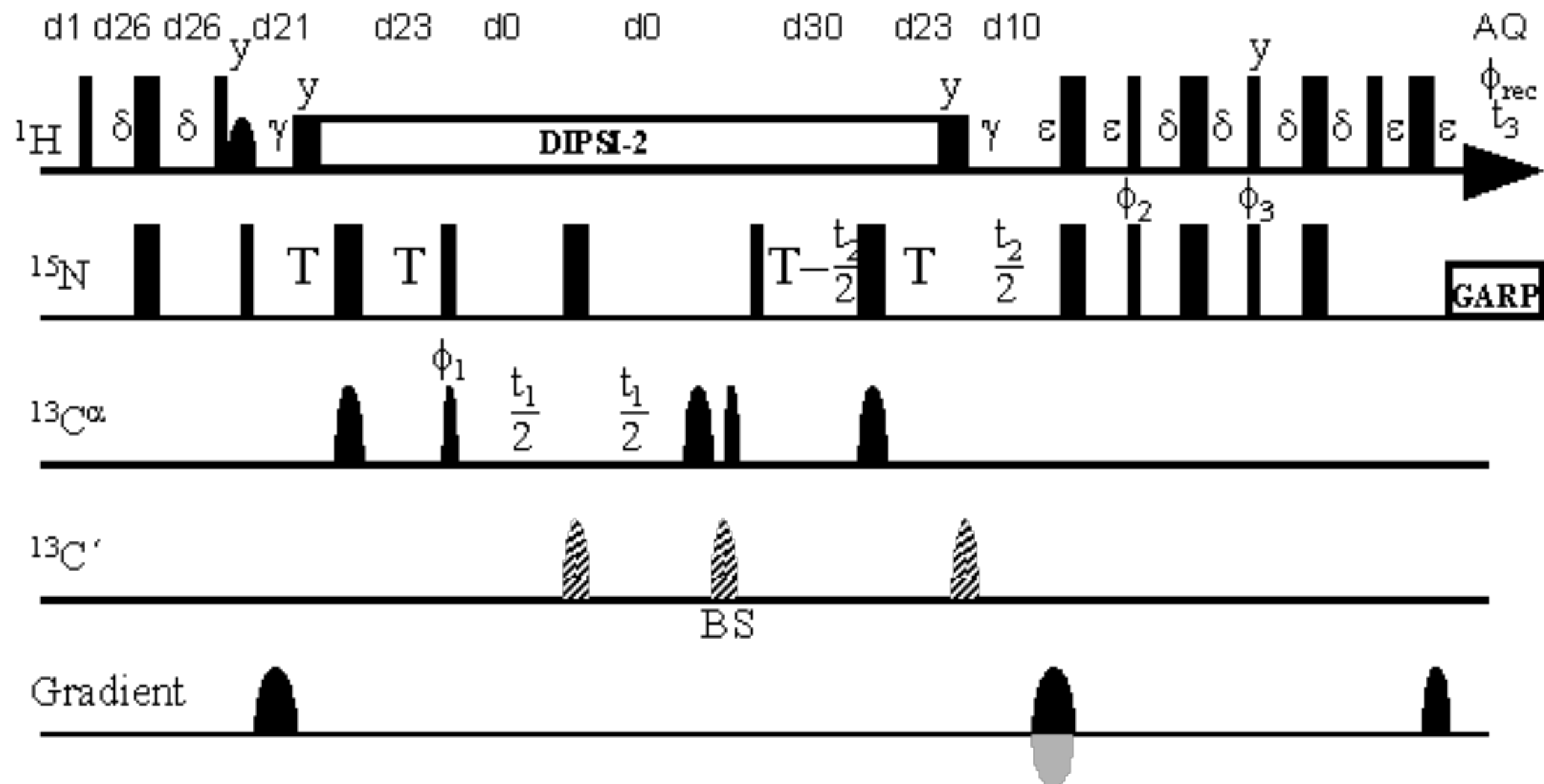
# heteronuclear NMR



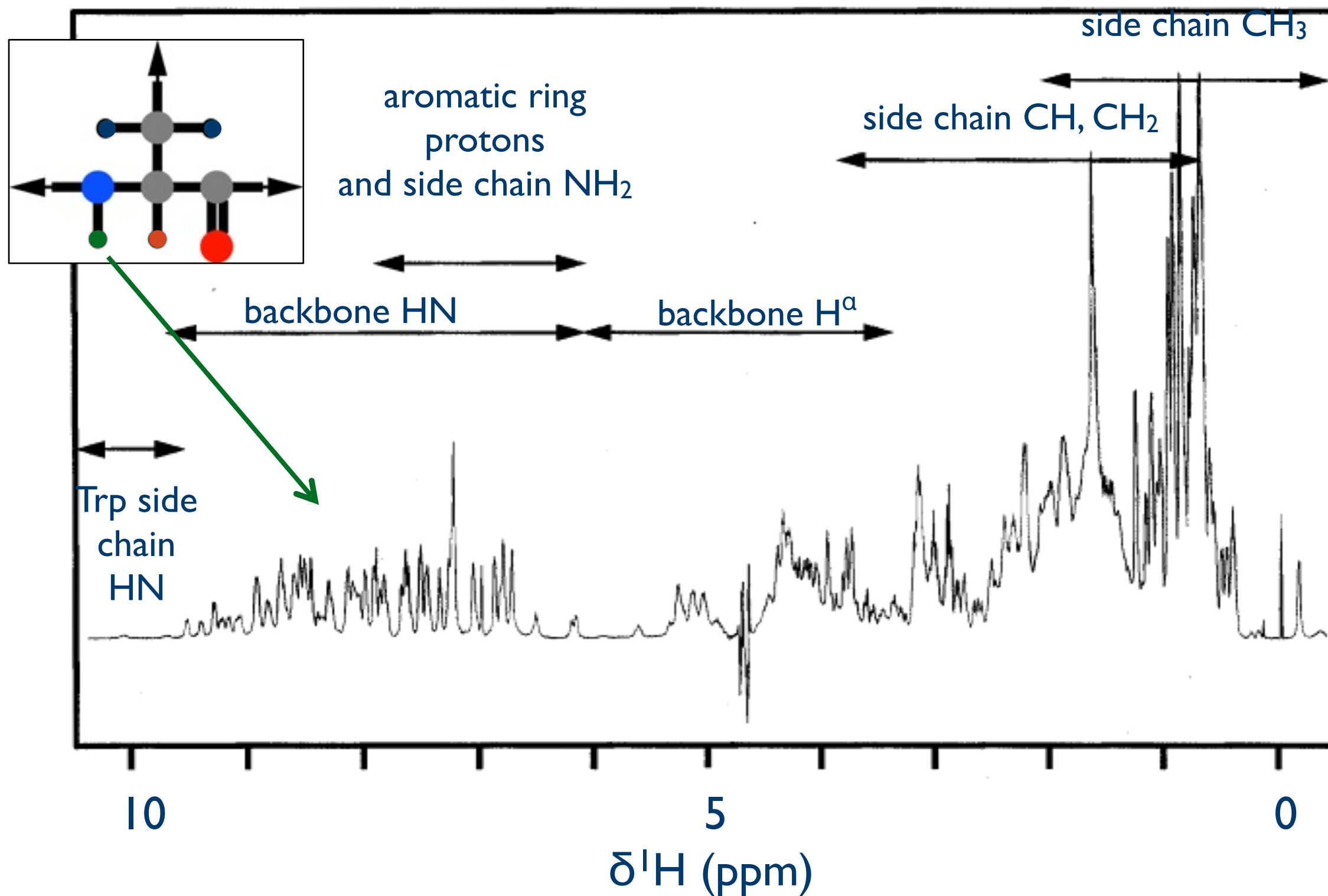
- measure frequencies of different nuclei; e.g.  $^1\text{H}$ ,  $^{15}\text{N}$ ,  $^{13}\text{C}$
- no diagonal peaks
- mixing not possible using NOE, only via J

# J coupling constants





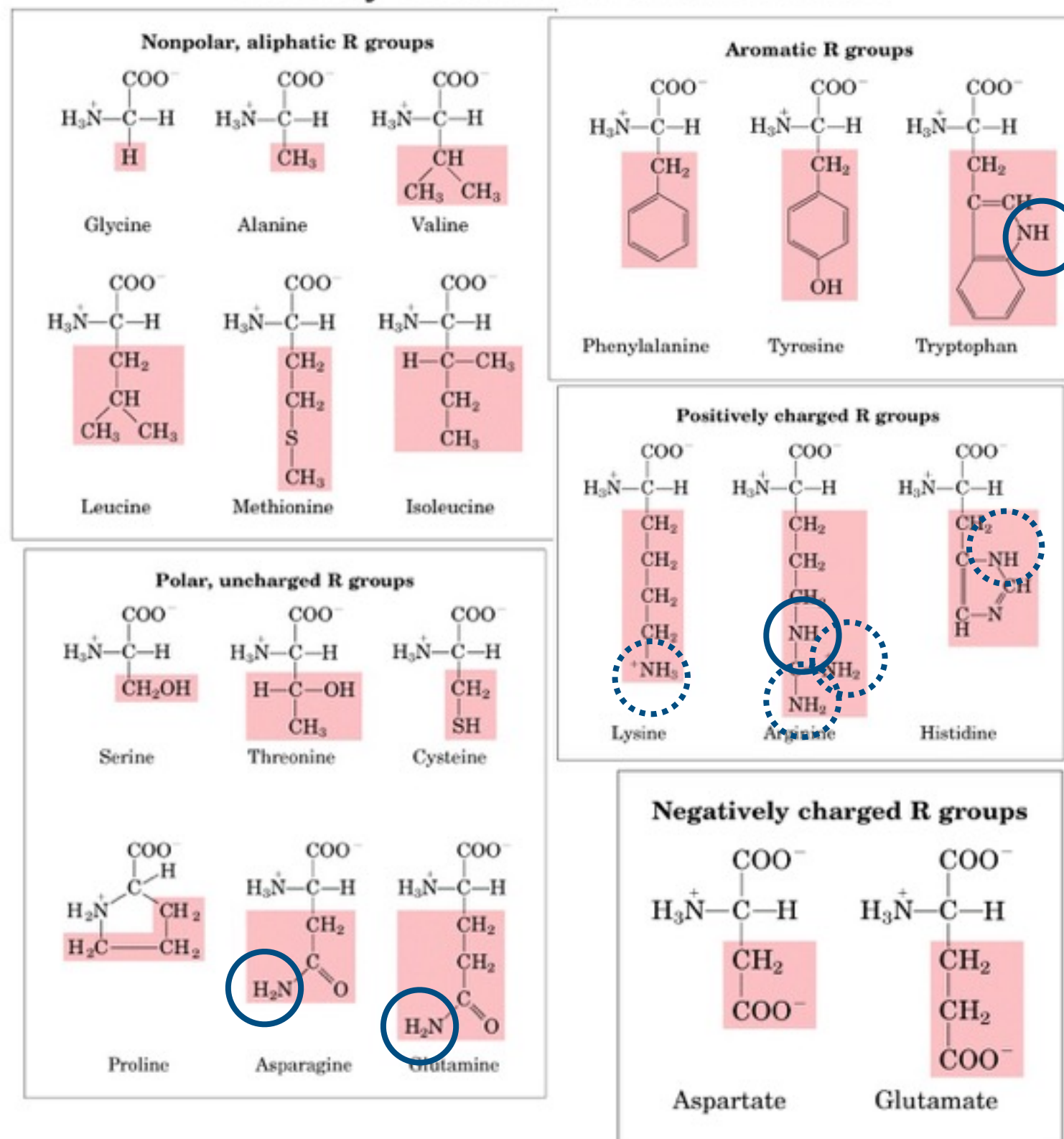
# <sup>15</sup>N-filter - selecting a subspectrum



# $^{15}\text{N}$ HSQC

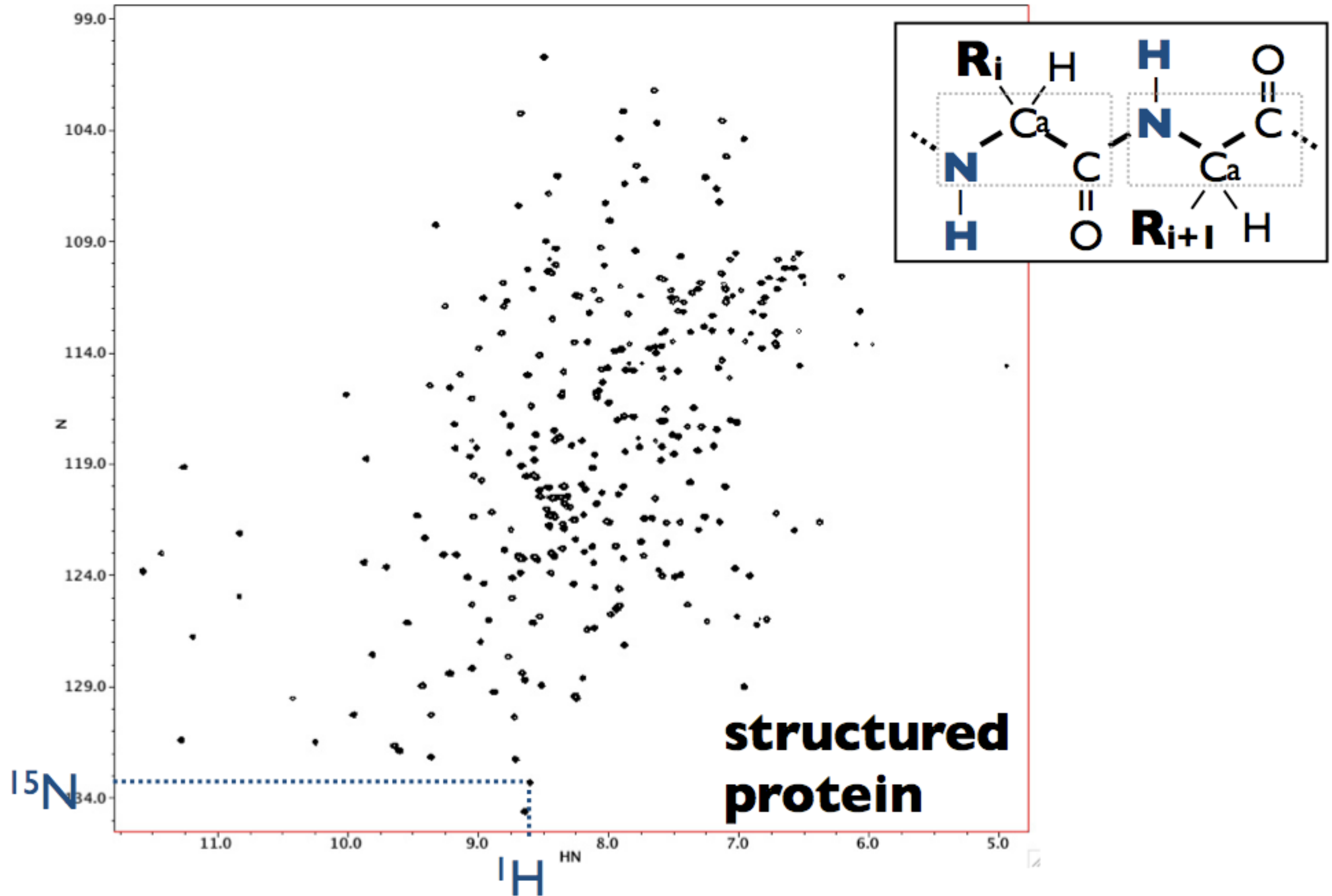
- Backbone HN
- Side-chain NH and  $\text{NH}_2$

## Twenty standard Amino Acids

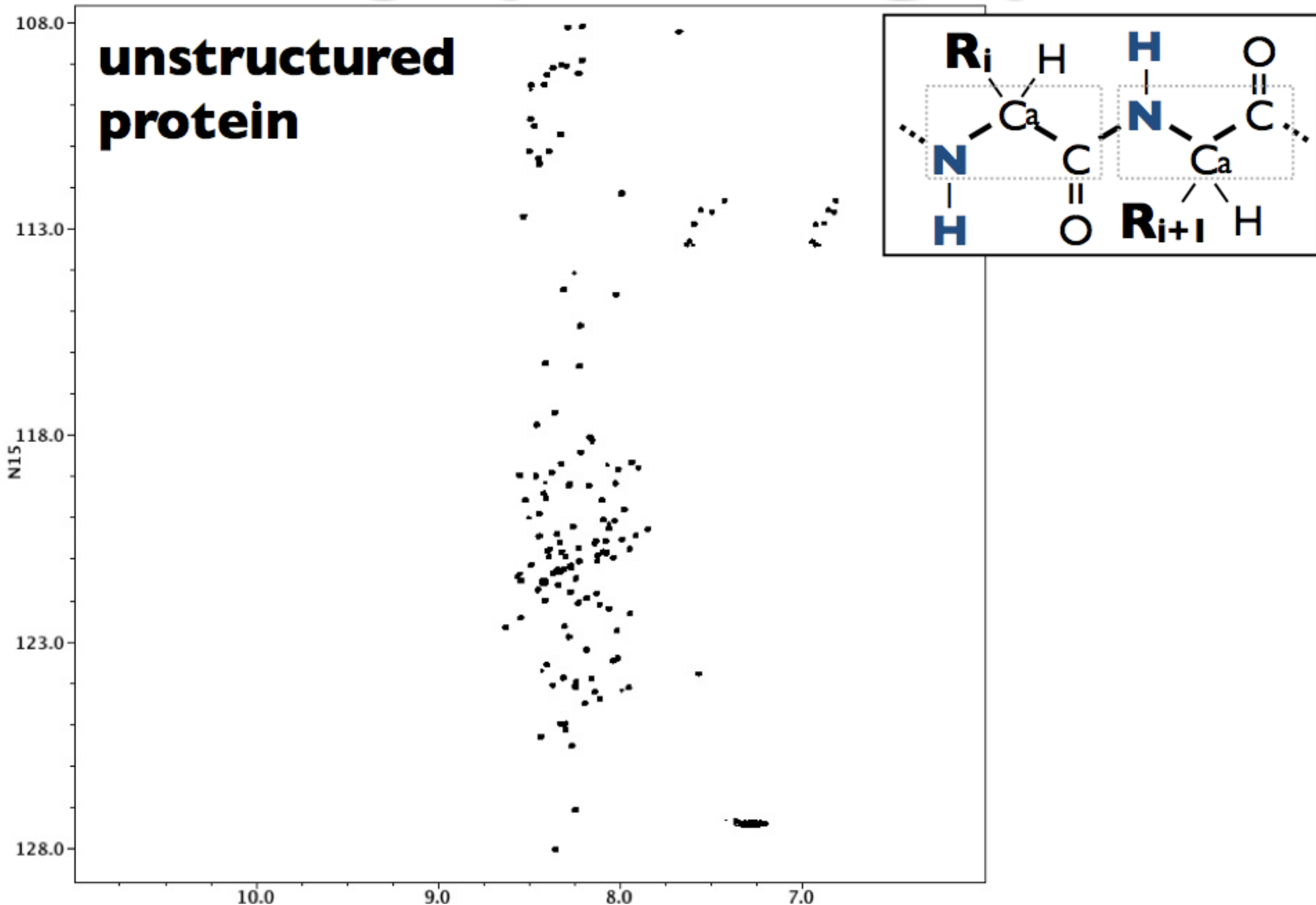


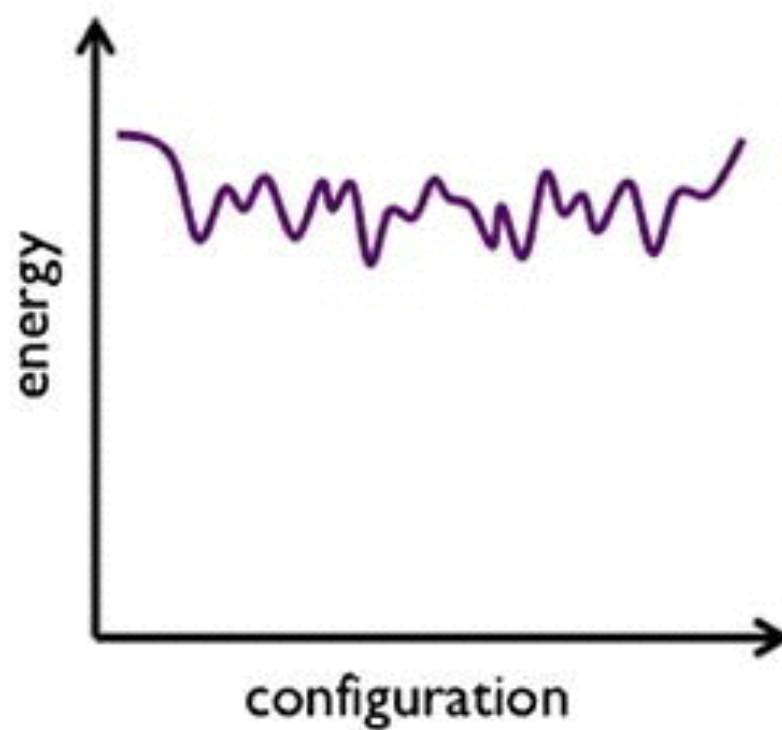
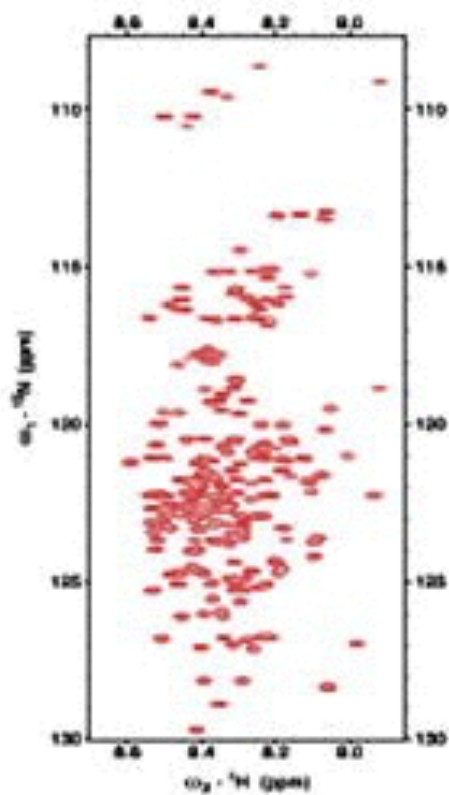
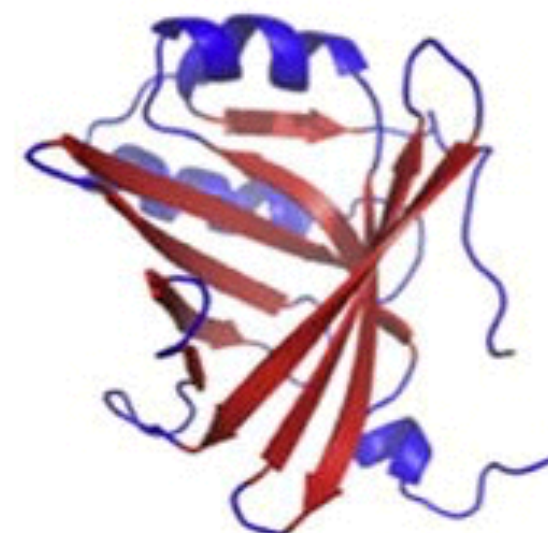
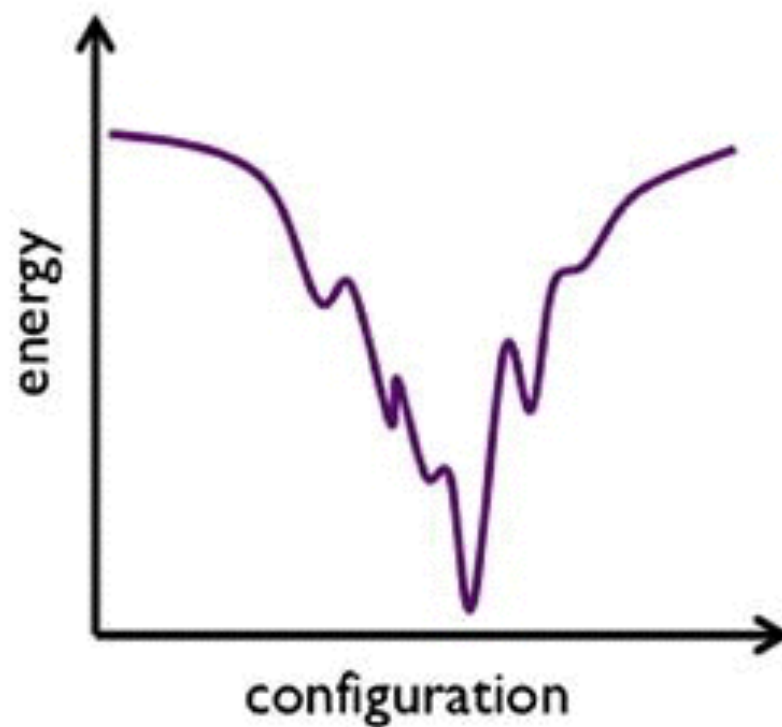
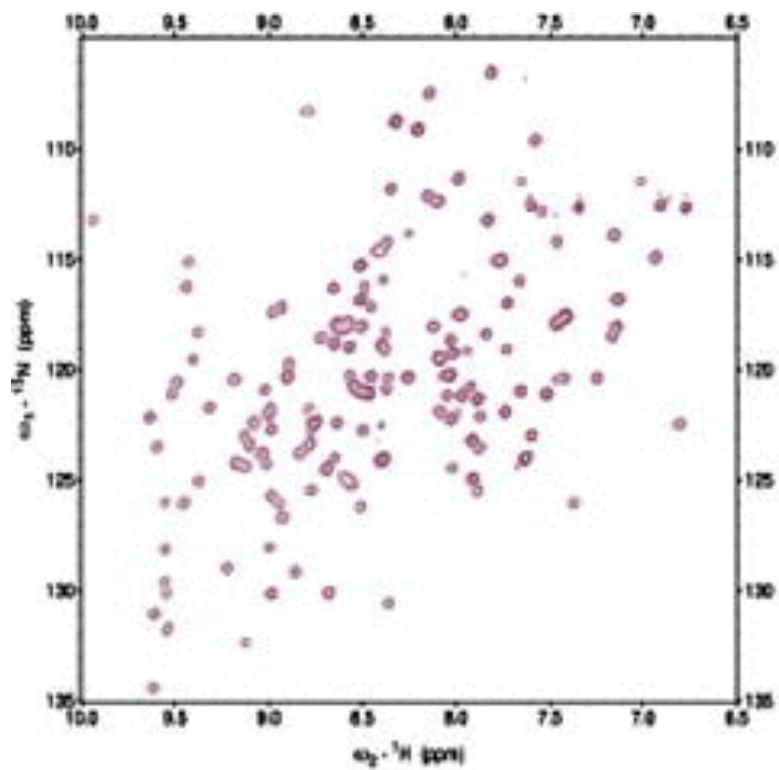


# $^{15}\text{N}$ -HSQC: fingerprint



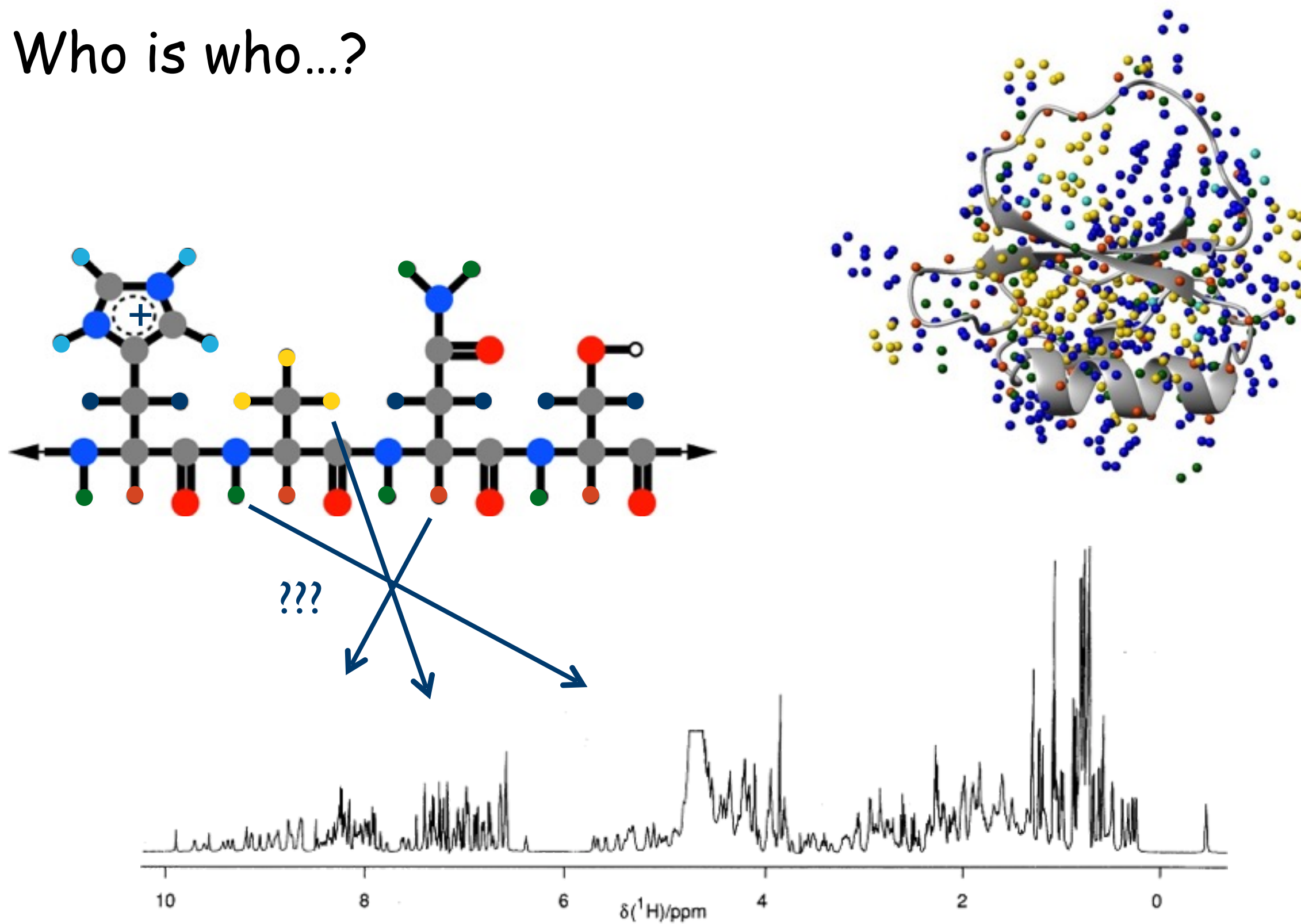
# $^1\text{H}$ - $^{15}\text{N}$ HSQC: '*protein fingerprint*'



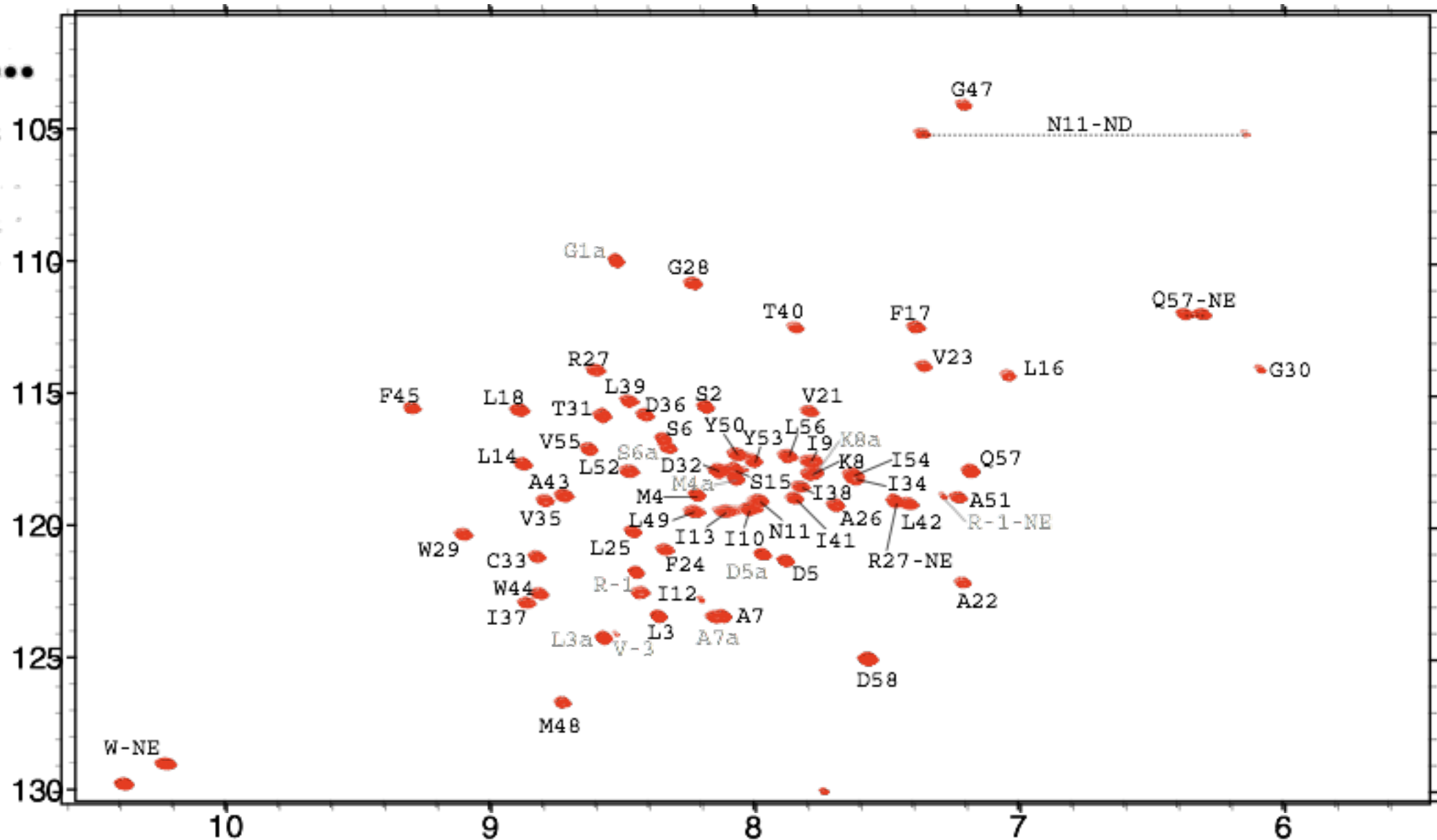
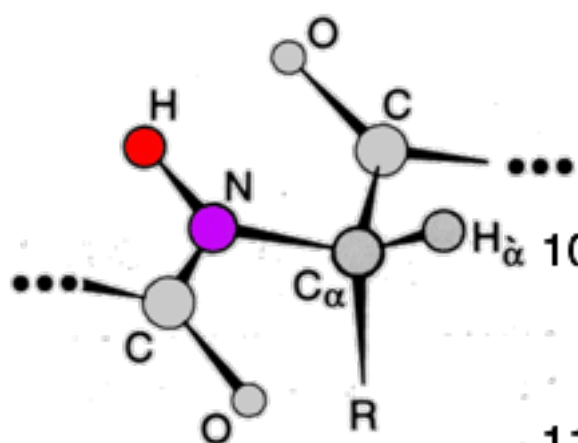


# The assignment problem

Who is who...?



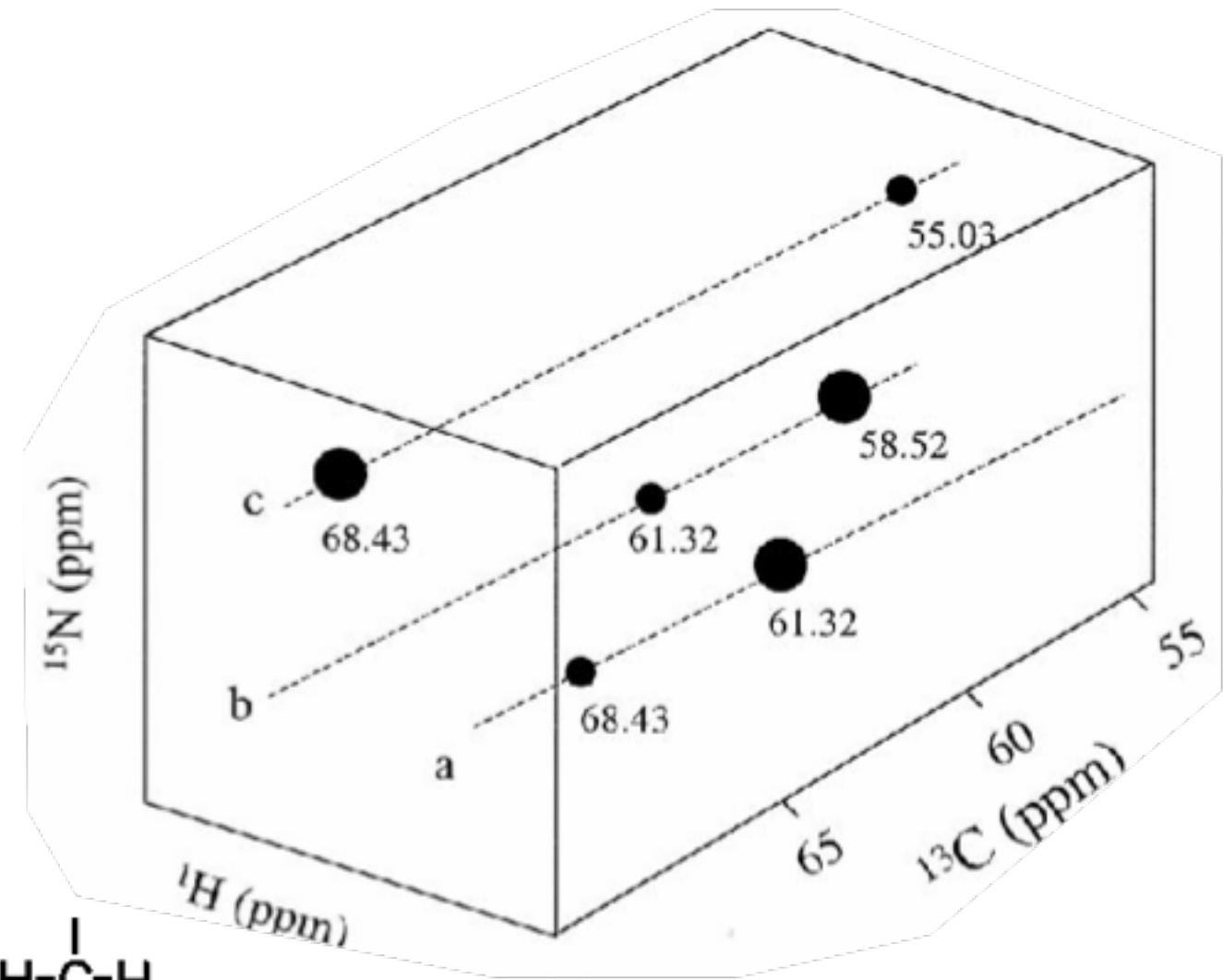
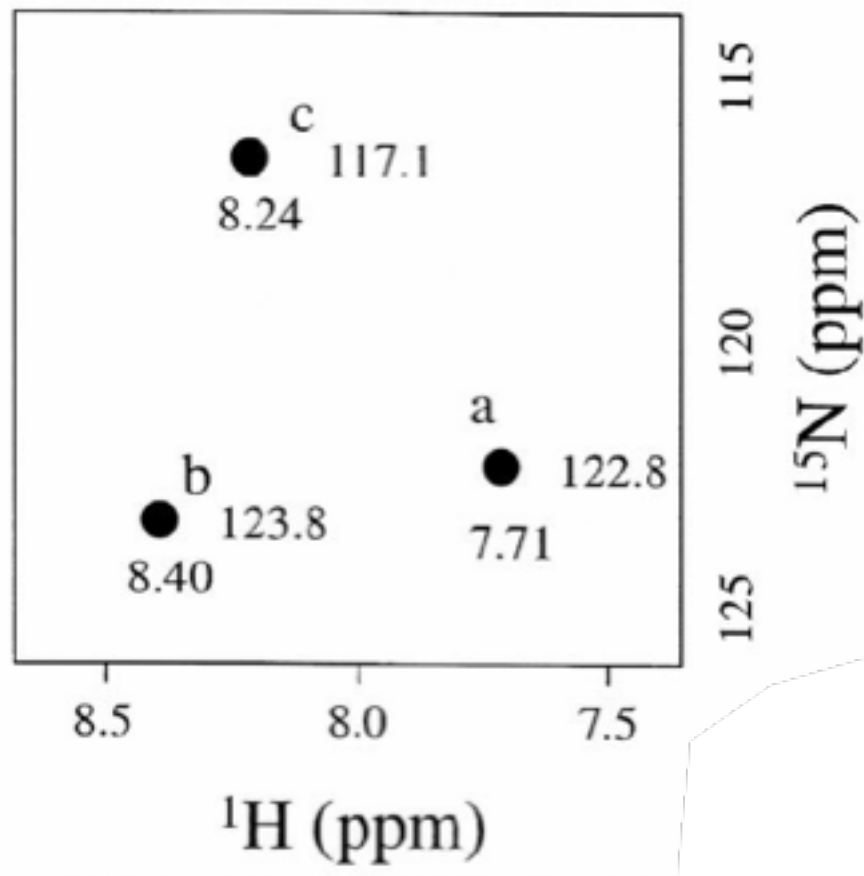
# Assigned $^1\text{H}$ - $^{15}\text{N}$ HSQC



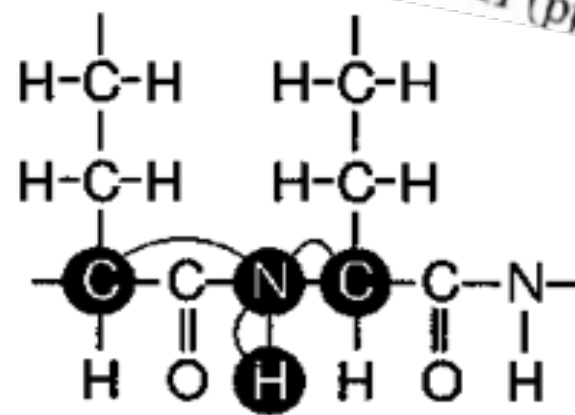


# Example: 3D HNCA

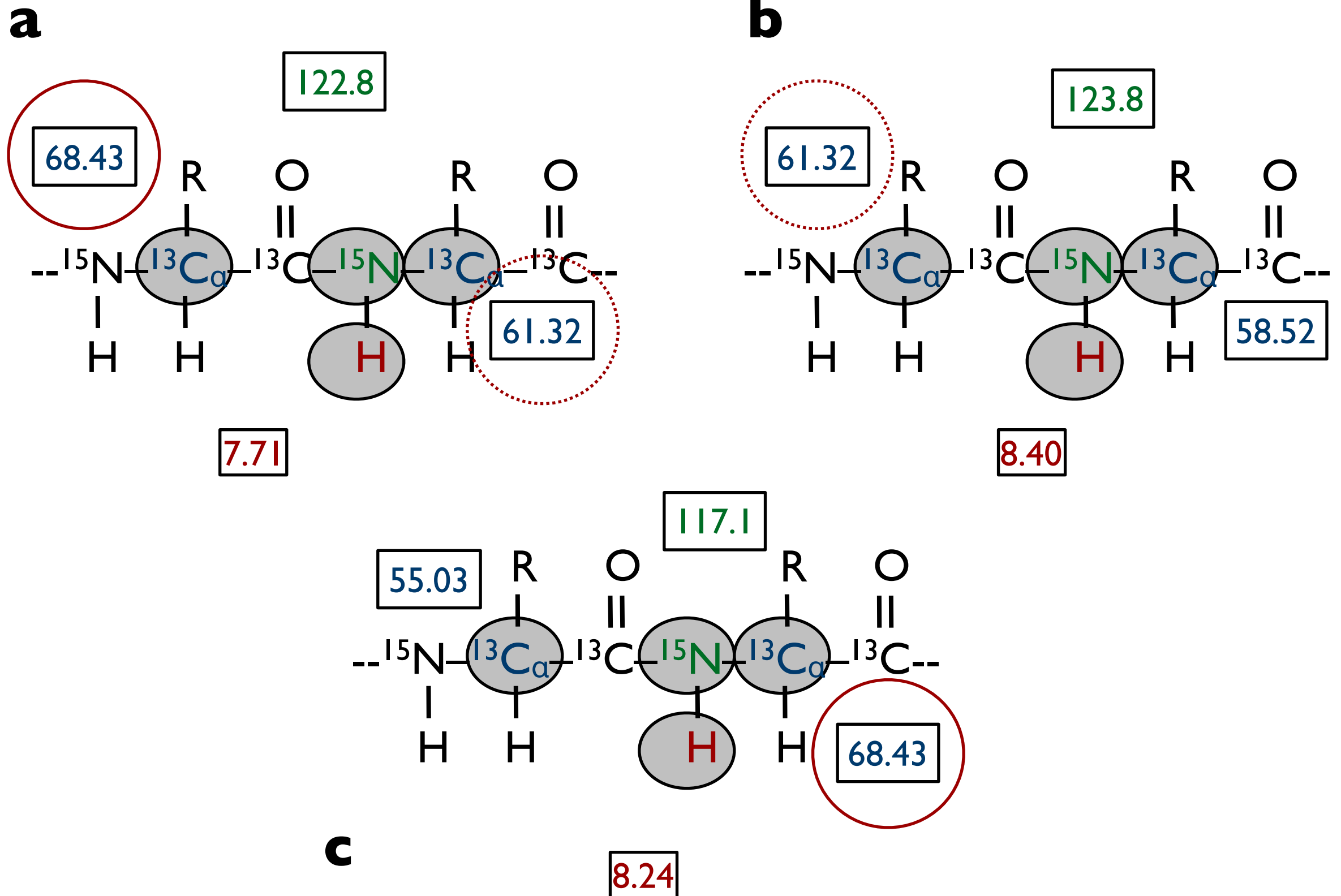
**[<sup>1</sup>H, <sup>15</sup>N]-HSQC → HNCA**



	c	a	b
<sup>1</sup> H <sub>N<sub>i</sub></sub>	8.24	7.71	8.40
<sup>15</sup> N <sub>i</sub>	117.1	122.8	123.8
<sup>13</sup> C <sup>α</sup> <sub>i</sub>	68.43	61.32	58.52
<sup>13</sup> C <sup>α</sup> <sub>i-1</sub>	55.03	68.43	61.32



# Example: analyze frequencies



# Example: link the spin-systems

## Numerically...

- c:  $C_{\alpha}(i) = a: C_{\alpha}(i-1)$
- a:  $C_{\alpha}(i) = b: C_{\alpha}(i-1)$

**Sequence: c – a – b**

	c	a	b
${}^1\text{H}_i$	8.24	7.71	8.40
${}^{15}\text{N}_i$	117.1	122.8	123.8
${}^{13}\text{C}_{\alpha_i}^{\alpha}$	68.43	61.32	58.52
${}^{13}\text{C}_{\alpha_{i-1}}^{\alpha}$	55.03	68.43	61.32



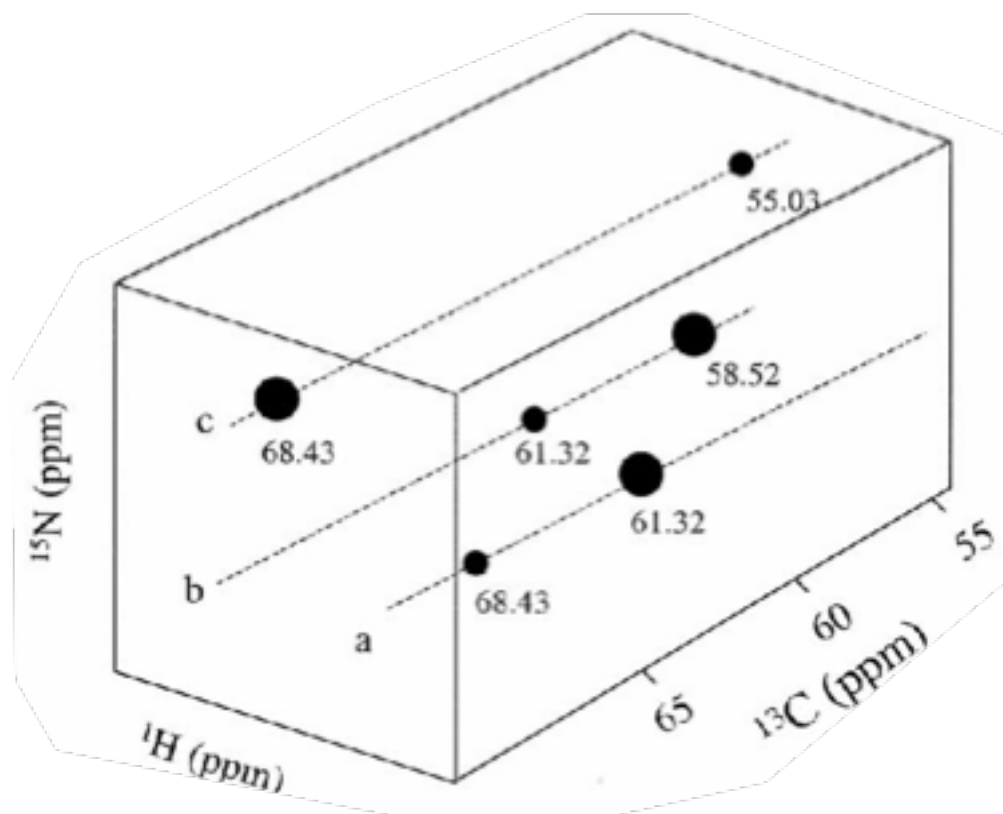
# Example: link the spin-systems

## Numerically...

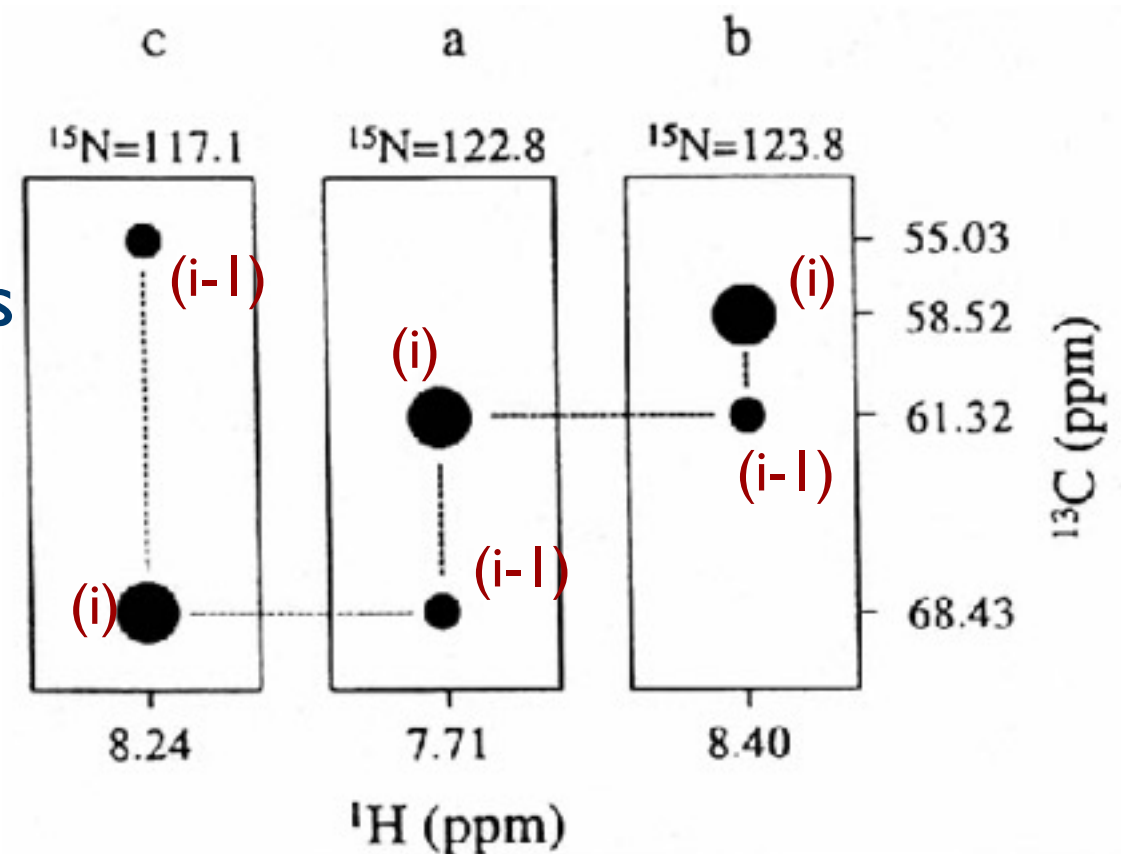
- $c: C_{\alpha}(i) = a: C_{\alpha}(i-1)$
- $a: C_{\alpha}(i) = b: C_{\alpha}(i-1)$

**Sequence: c - a - b**

## Or visually

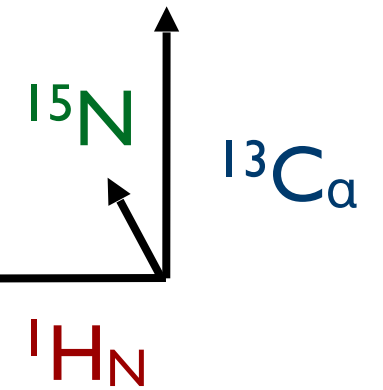
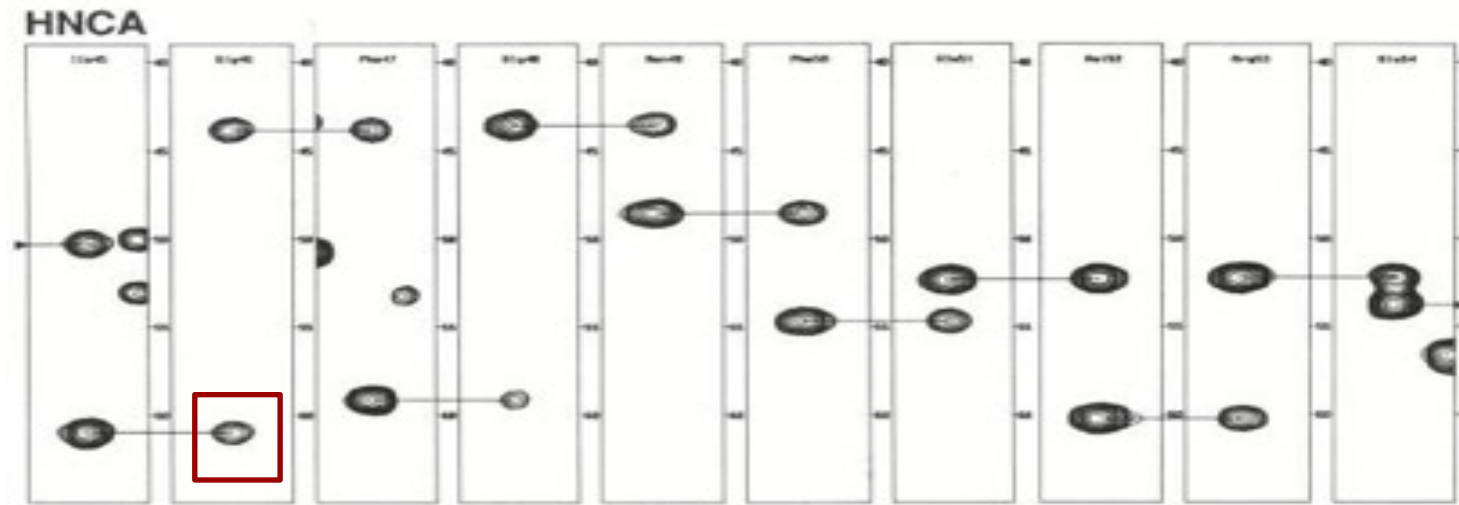
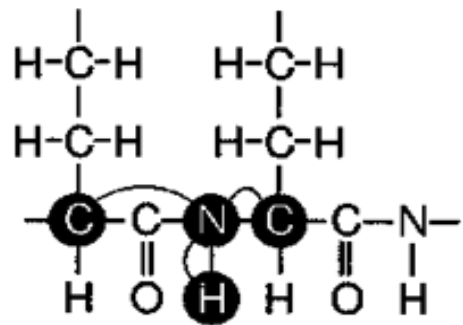


make strips

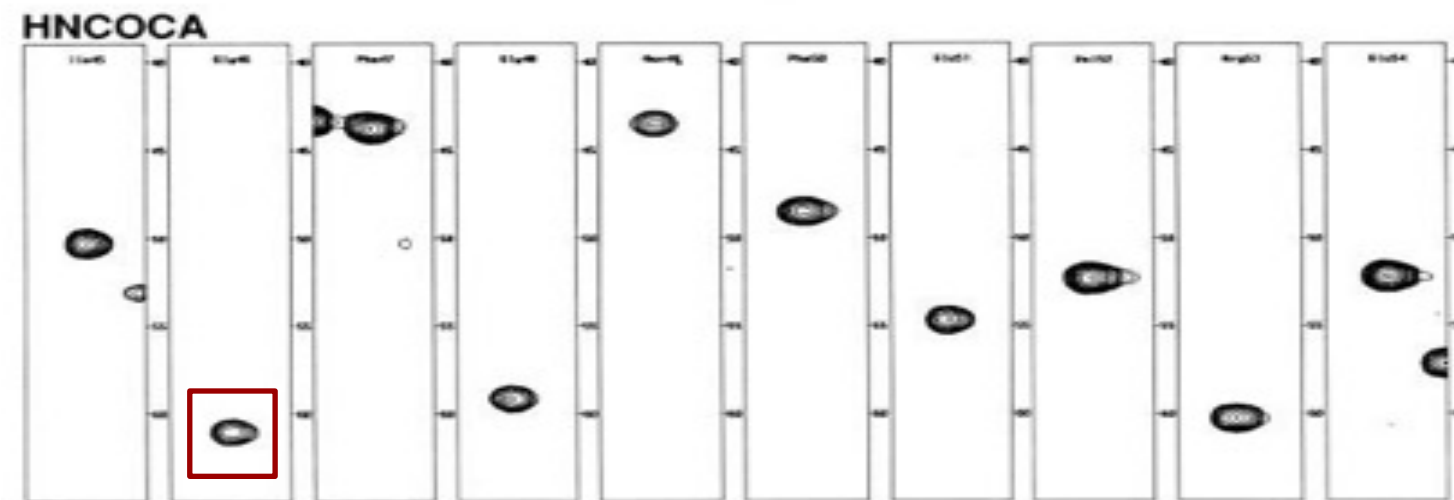
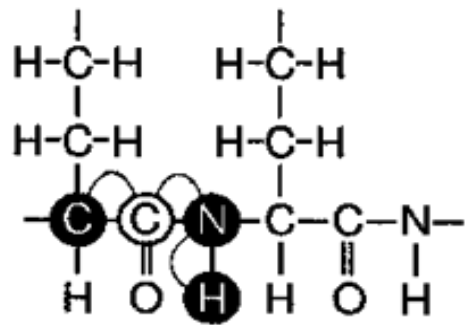


# HNCA versus HN(CO)CA

**i & i-1**



**i-1**



# Specific $^{13}\text{C}_\alpha$ & $^{13}\text{C}_\beta$ chemical shifts

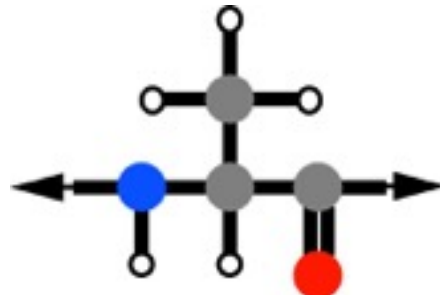
## Gly

- $\text{C}_\alpha$  43-47 ppm
- no  $\text{C}_\beta$



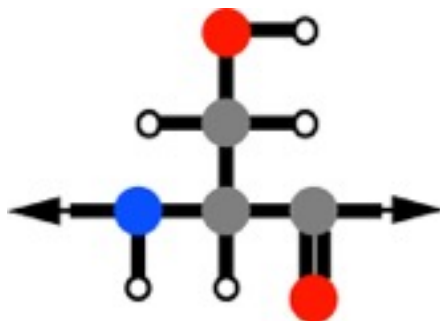
## Ala

- $\text{C}_\alpha$  53-55 ppm
- $\text{C}_\beta$  18-23 ppm



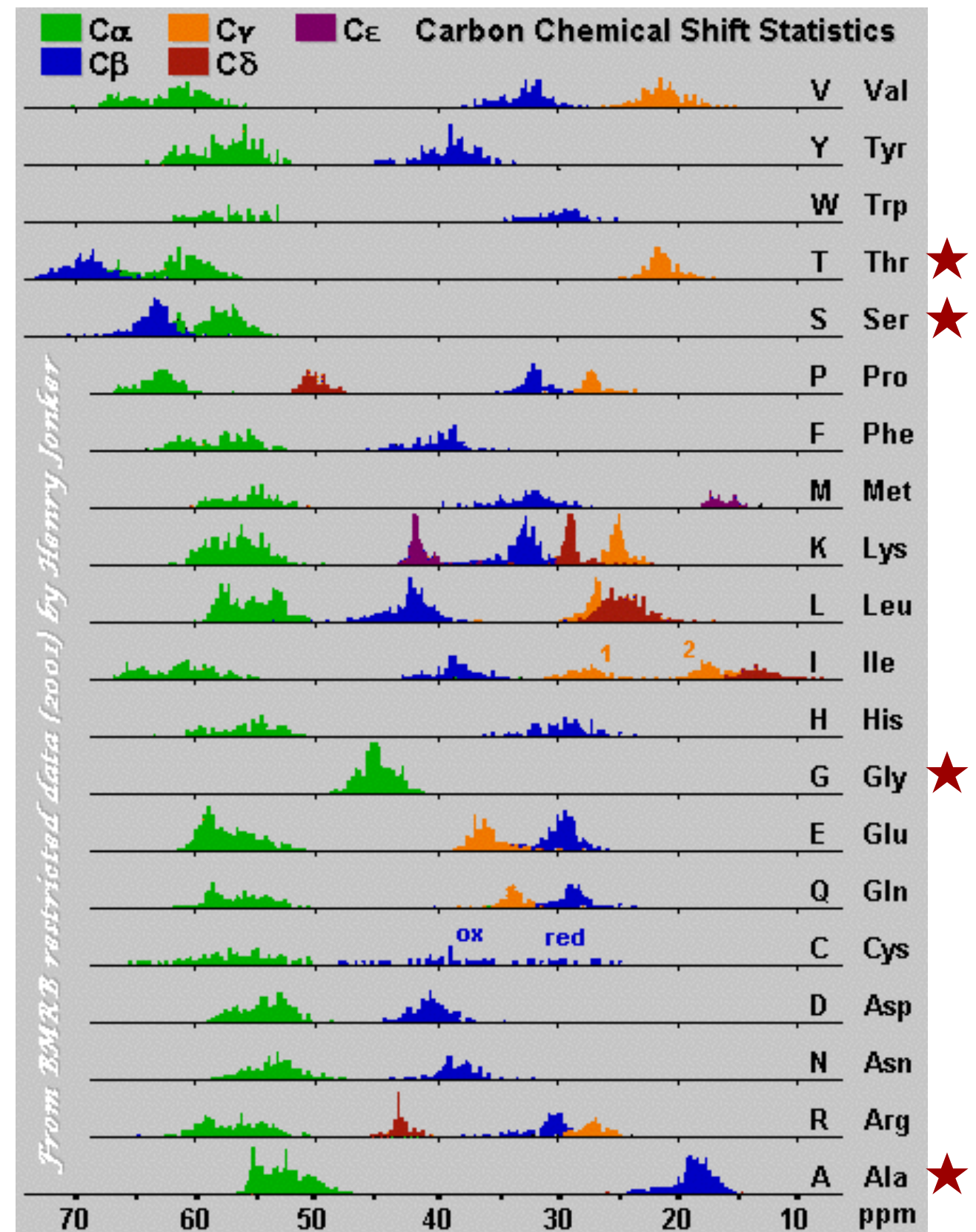
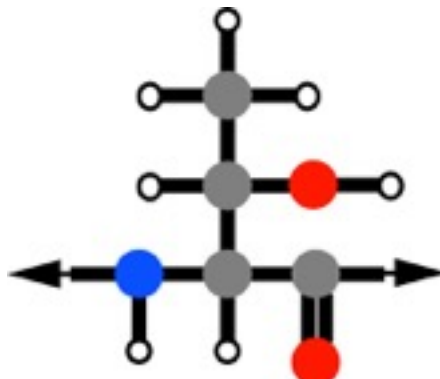
## Ser

- $\text{C}_\alpha$  57-61 ppm
- $\text{C}_\beta$  62-67 ppm



## Thr

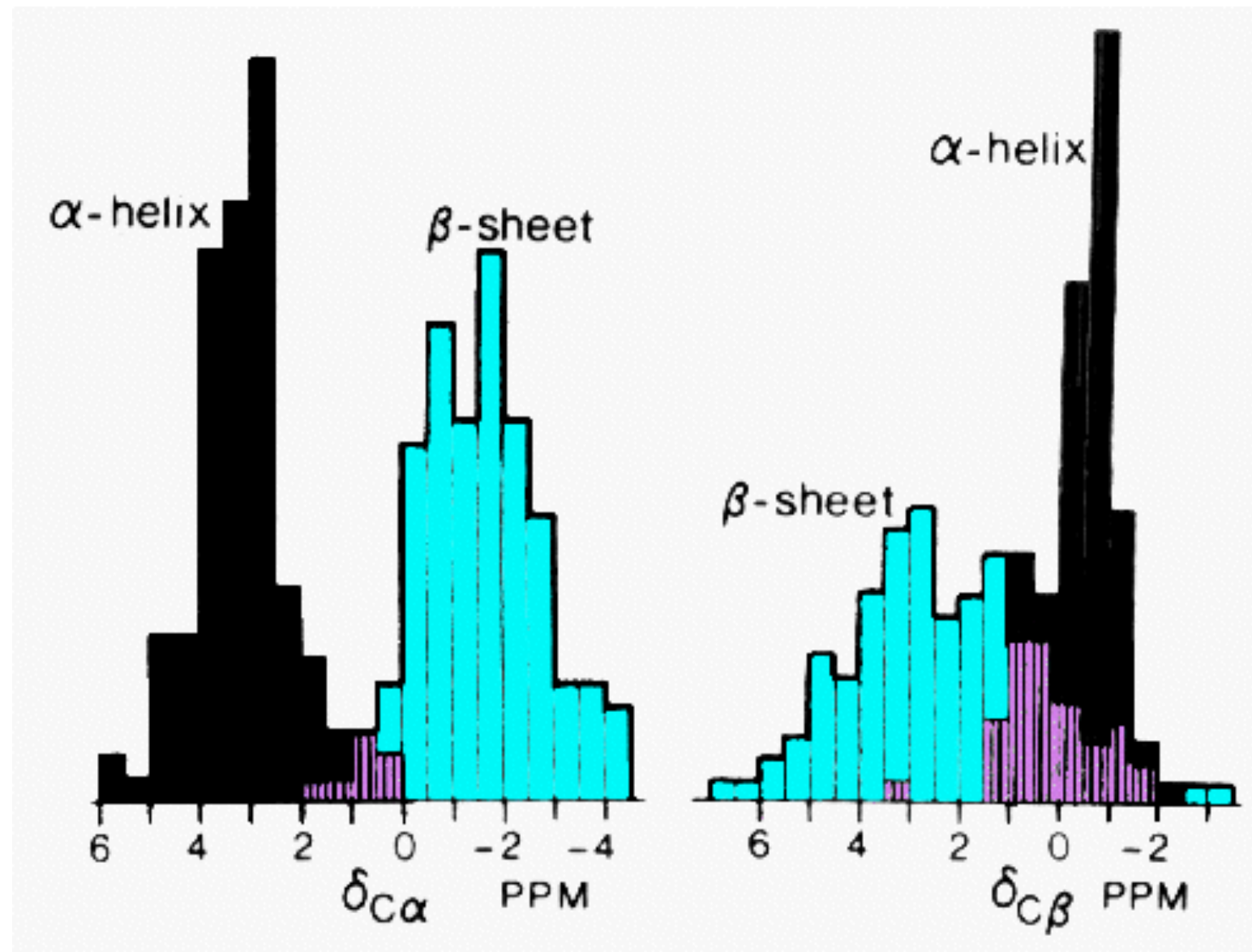
- $\text{C}_\alpha$  58-68 ppm
- $\text{C}_\beta$  68-72 ppm



# OBSERVABLE: chemical shifts

## $^{13}\text{C}_\alpha$ and $^{13}\text{C}_\beta$ chemical shifts

- Sensitive to dihedral angles
- Report on secondary structure elements



# Larger proteins: What are the problems?

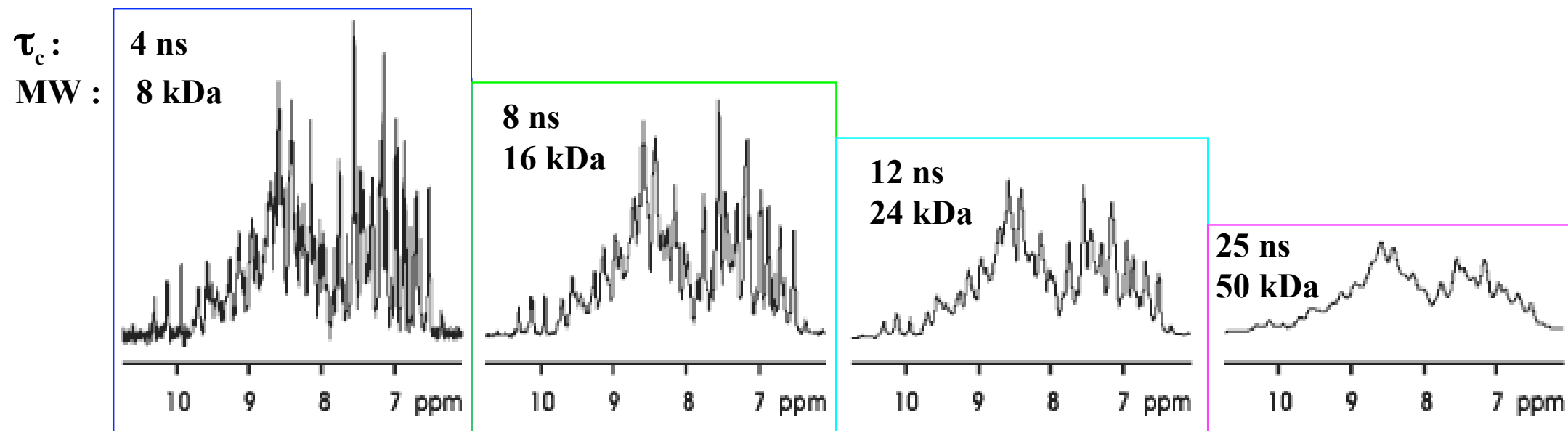
Nuclei relax faster due to slower tumbling:

-broader lines

-lower sensitivity of NMR experiments

Number of signals increase with higher MW:

-increased signal overlap



The larger the molecular weight...



...the lower the resolution and the sensitivity

**Teste: considere as três fatias dos espectros 3D HNCA e 3D HN(CO)CA abaixo. Indique qual é a sequência dos resíduos 1, 2 e 3. Qual deles é uma glicina?**

