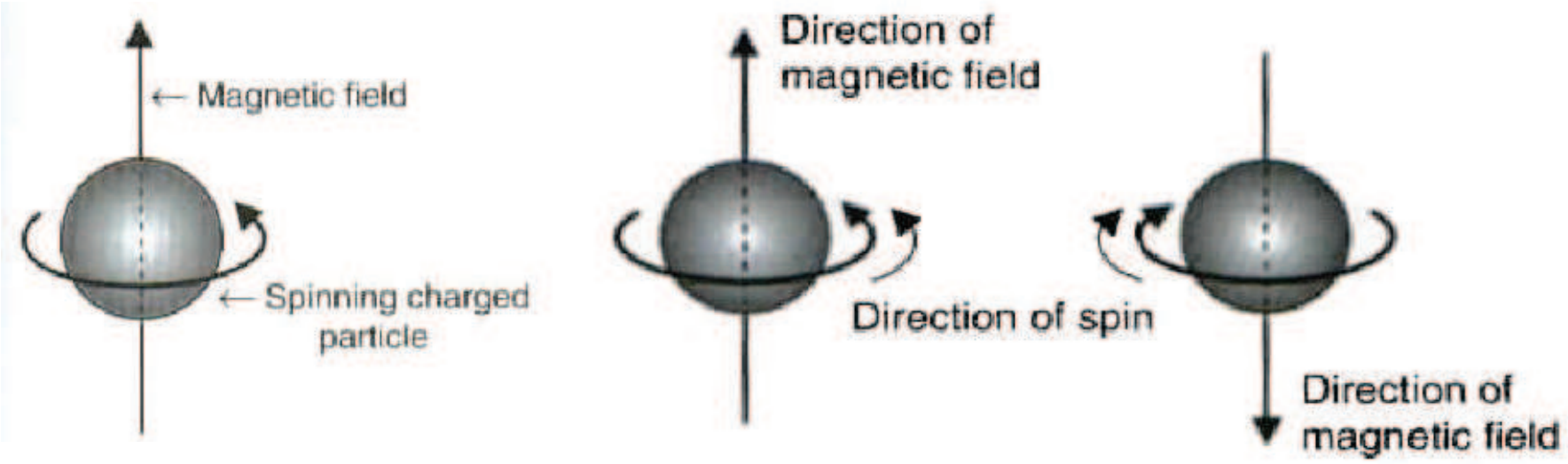
Lecture 3

Tutorial on semi-automatic assignment of ubiquitin via NMRView J

Adelajda Zorba

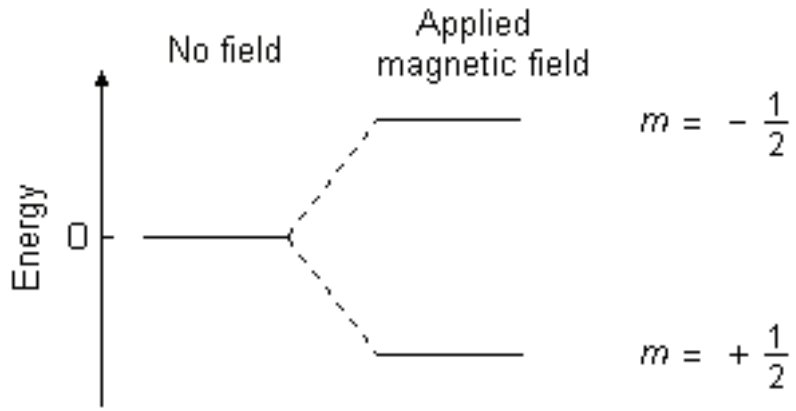
NMR exploits the properties of nuclear spins



http://www.umkcradres.org/education/neuro/Spec/RADPAGE/Magnetized%20nuclear%20spin%20systems.htm

An external magnetic field is required to generate the ground and excited spin states

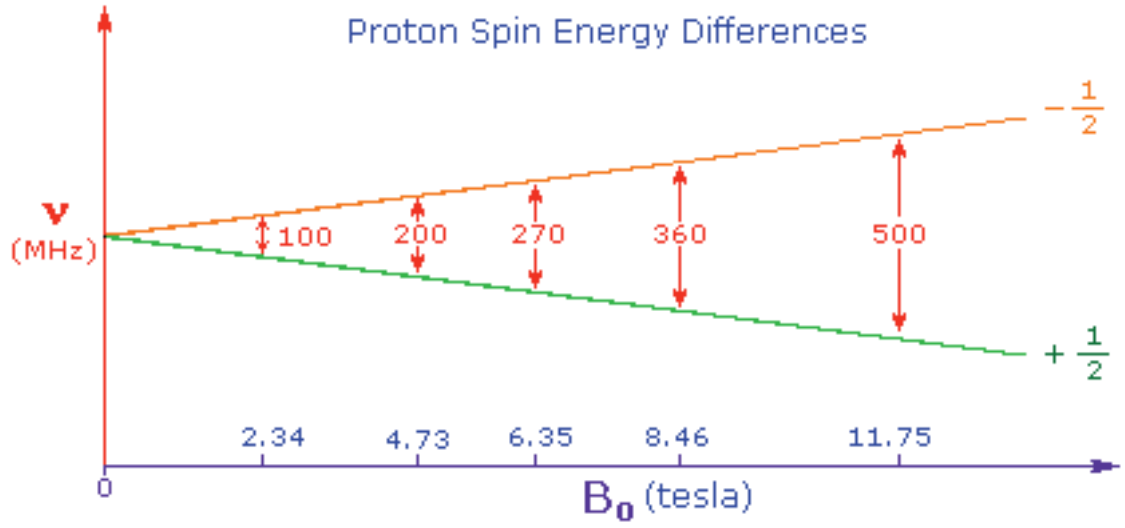
Energy levels for a nucleus with spin quantum number 1/2



http://www.umkcradres.org/education/neuro/Spec/RADPAGE/Magnetized%20nuclear%20spin%20systems.htm

Three important observations:

1. The greater the magnetic field strength, the greater the energy difference between the states

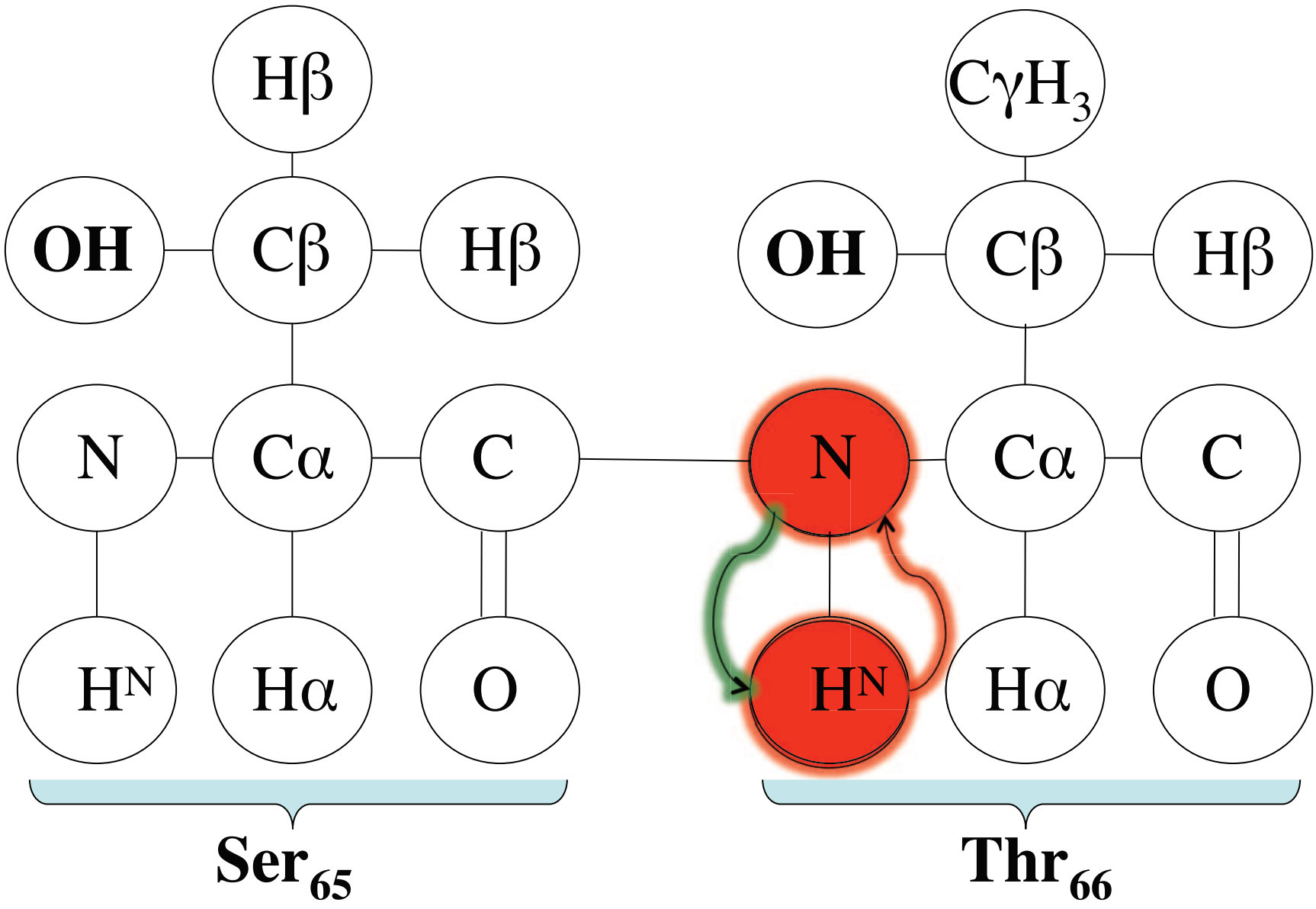


2. ^{1}H, ^{13}C, ^{15}N, ^{19}F, ^{31}P are biologically-relevant isotopes that have a spin state.

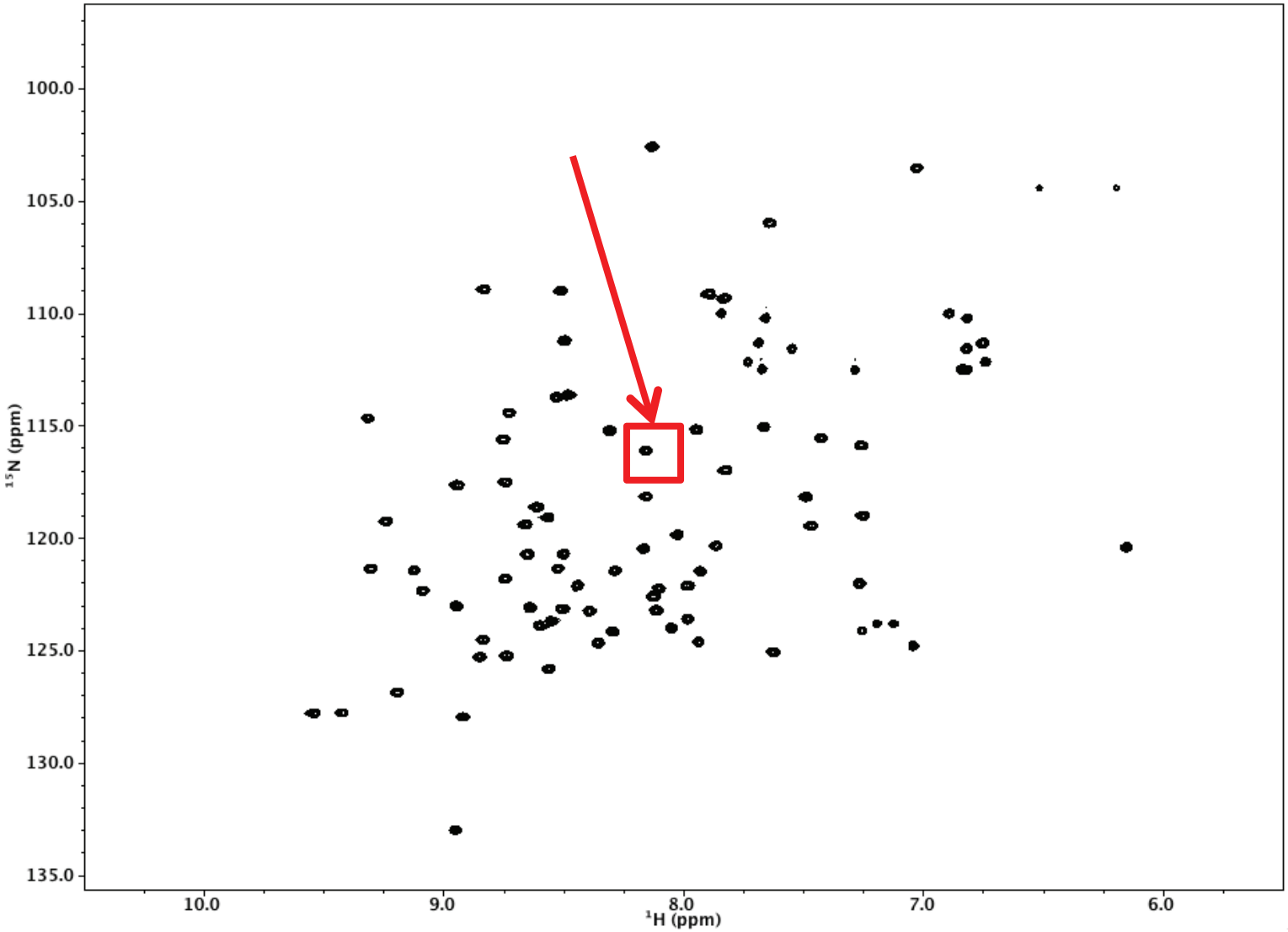
3. The NMR excited states has lifetimes that are 10^9 longer than excited electronic states: τ prop. $(1/\omega^3)$

http://www2.chemistry.msu.edu/faculty/reusch/VirtTxtJml/Spectrpy/nmr/nmr1.htm

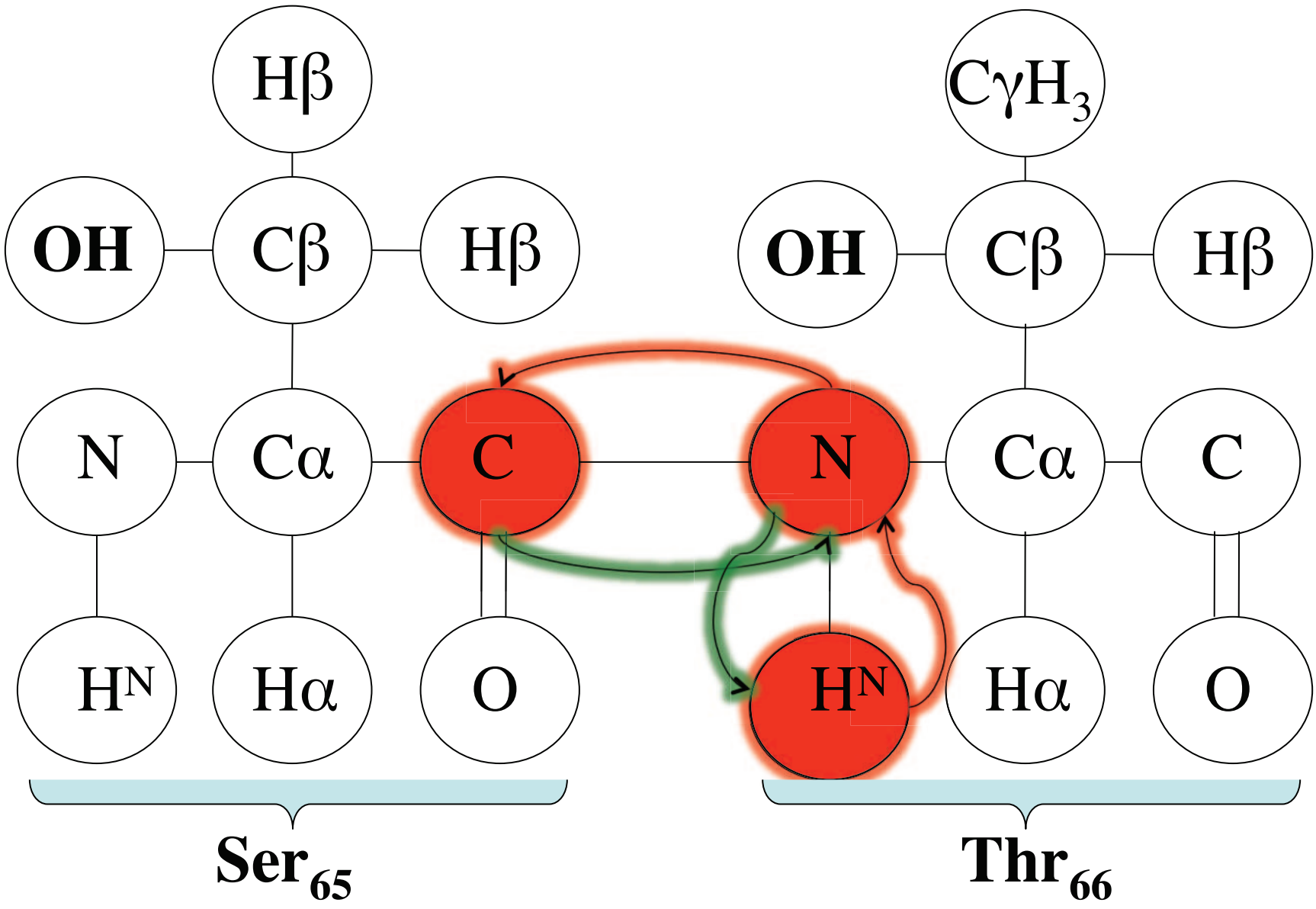
Heteronuclear Single Quantum Coherence (**HSQC**)



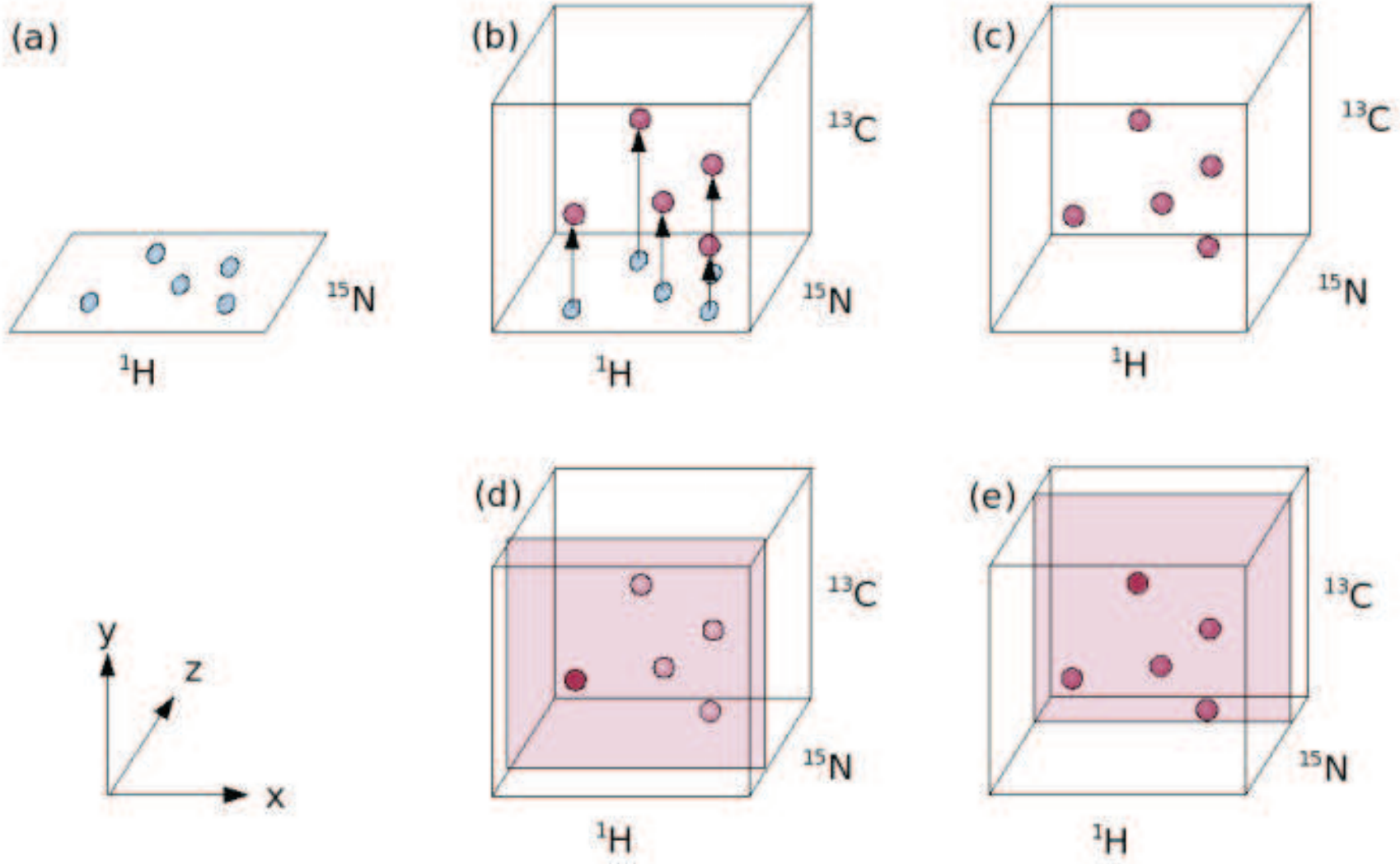
HSQC of ubiquitin



HNCO

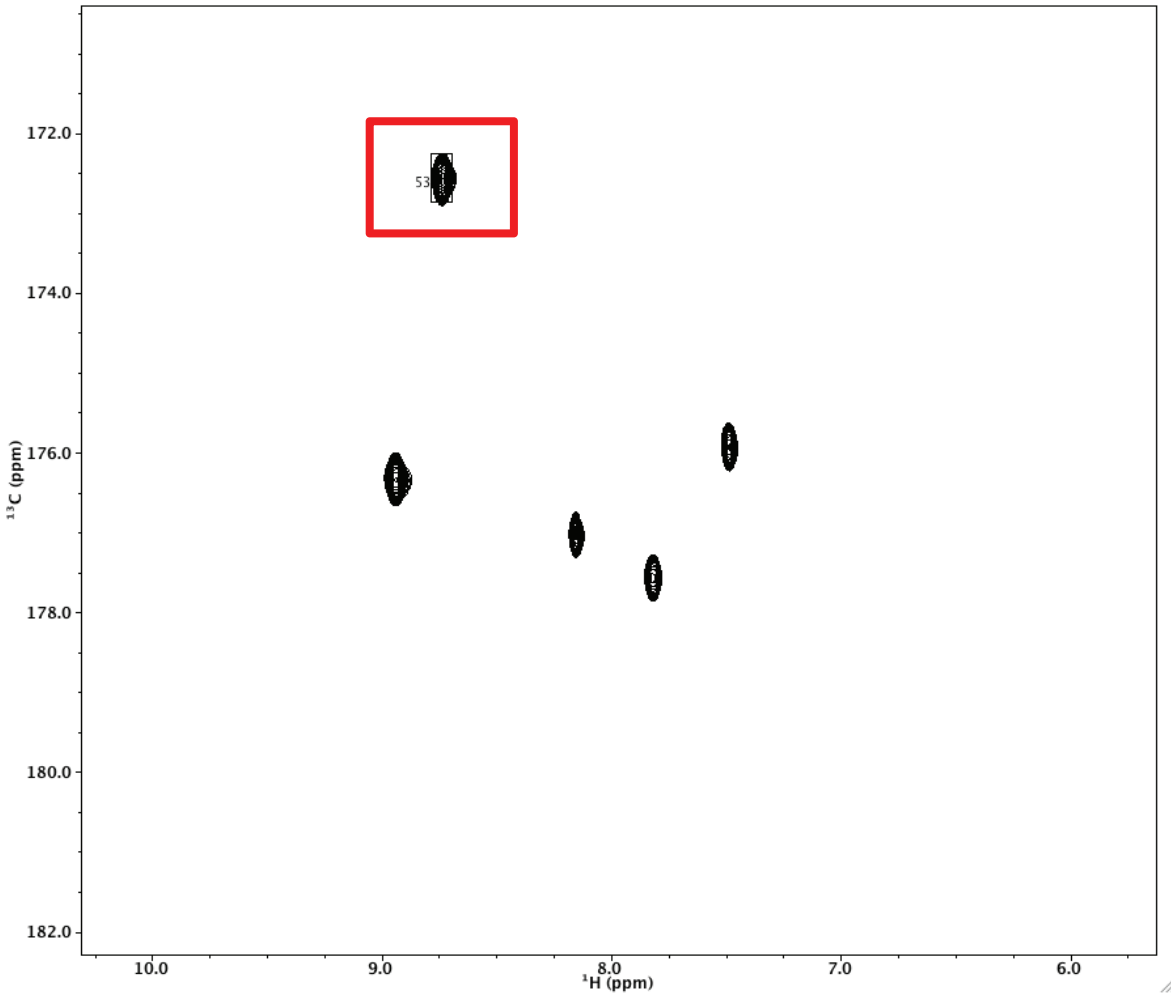


HNCO – in theory

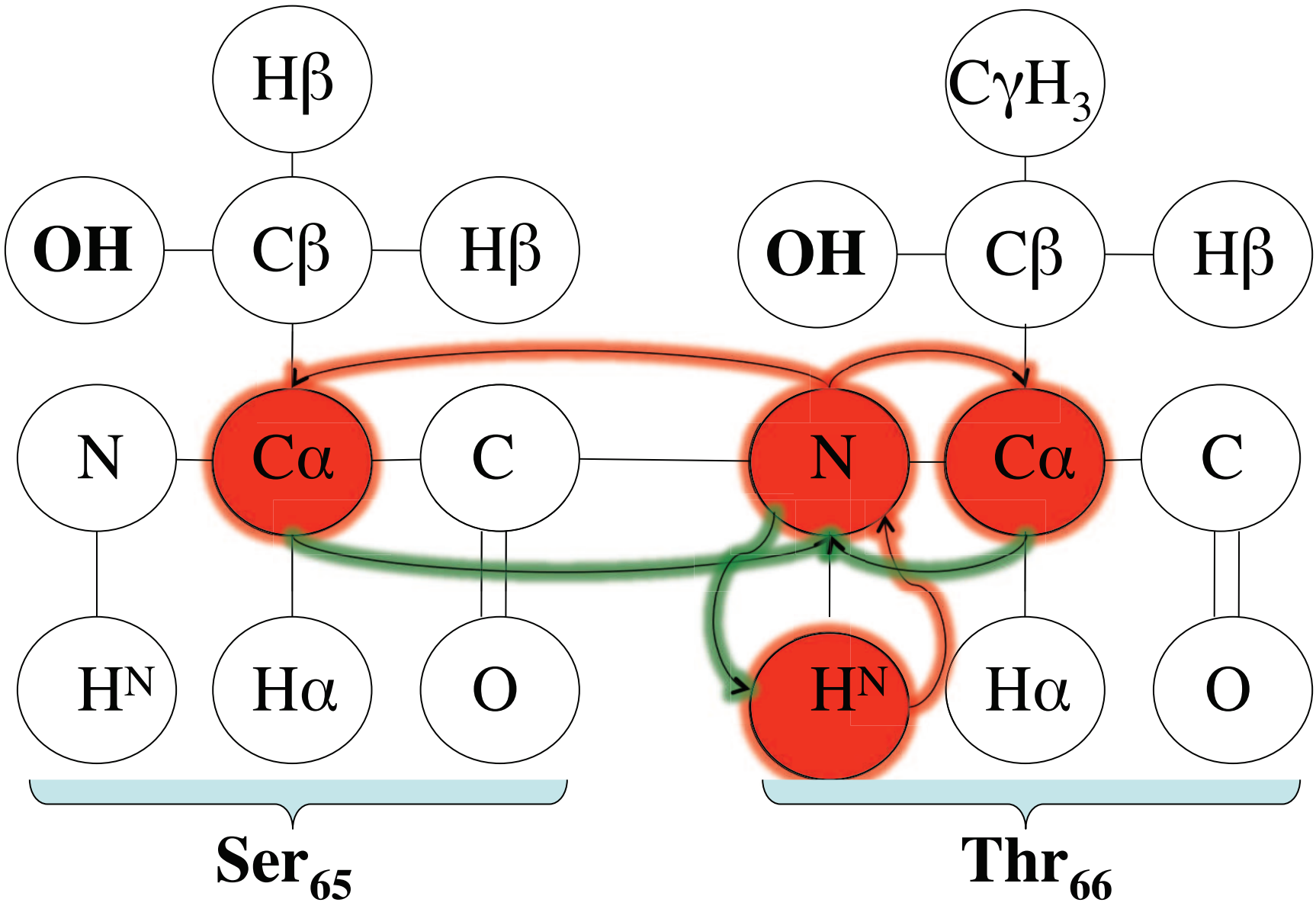


http://www.protein-nmr.org.uk/assignment_theory.html

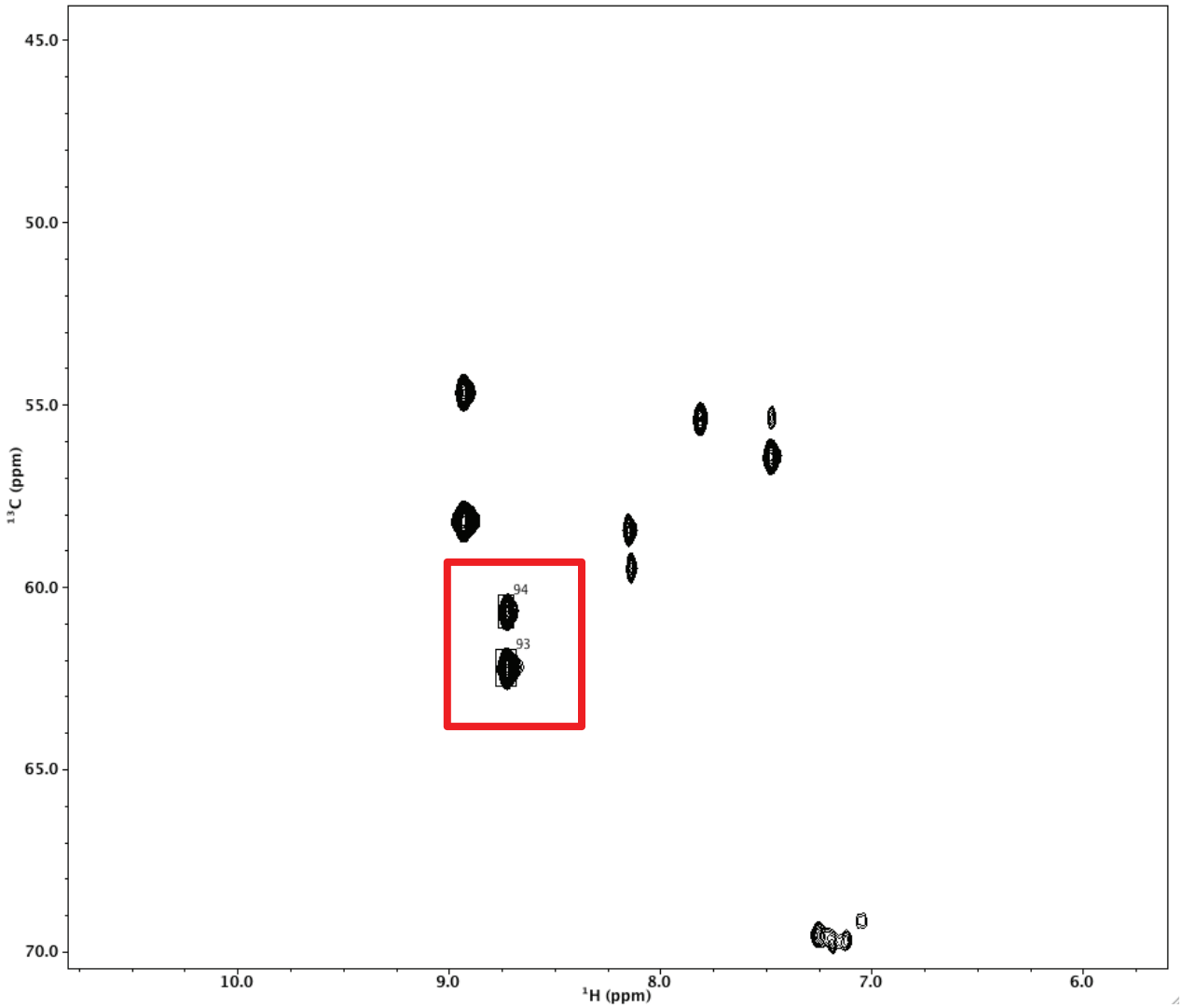
HNCO of ubiquitin



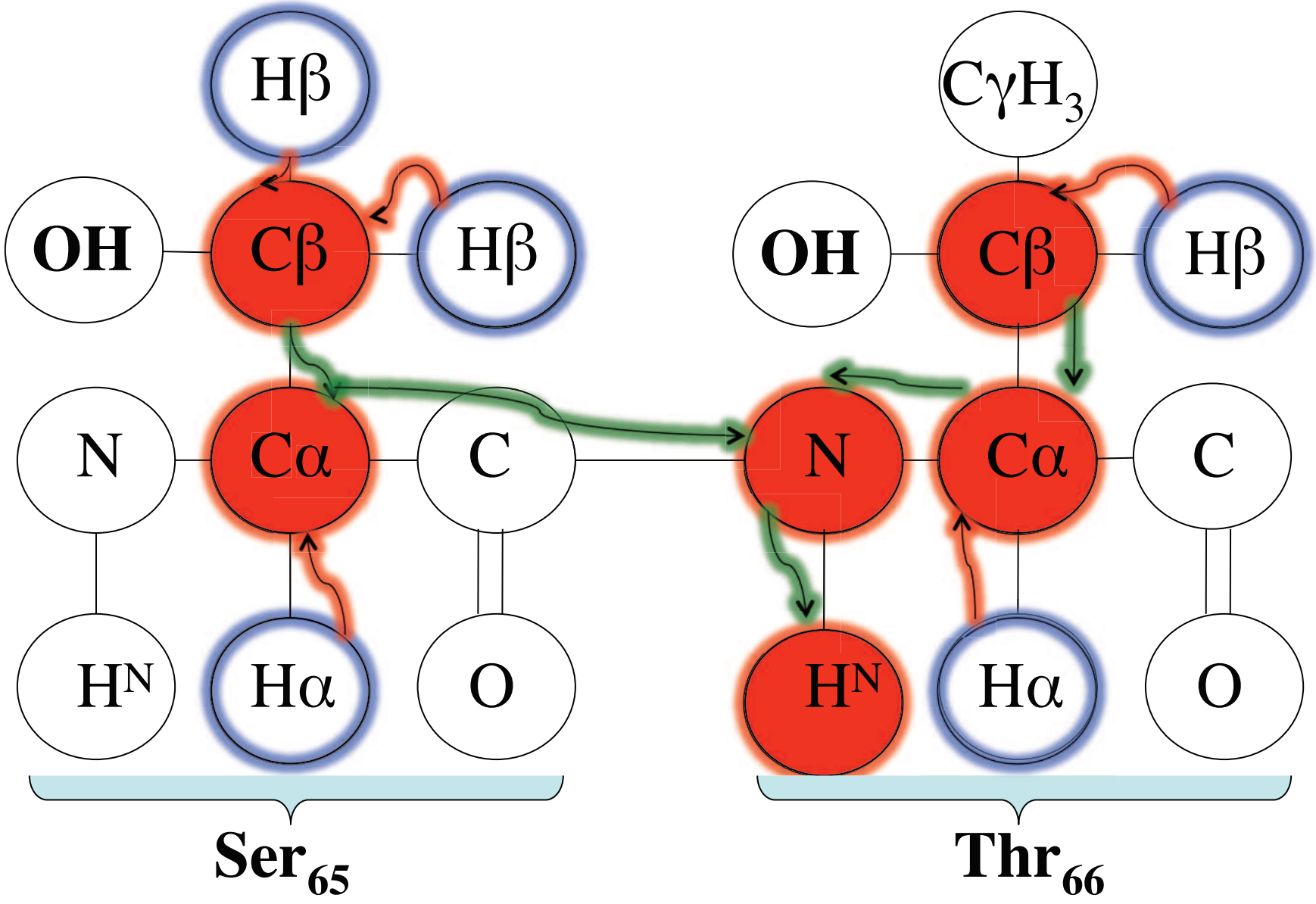
HNCA



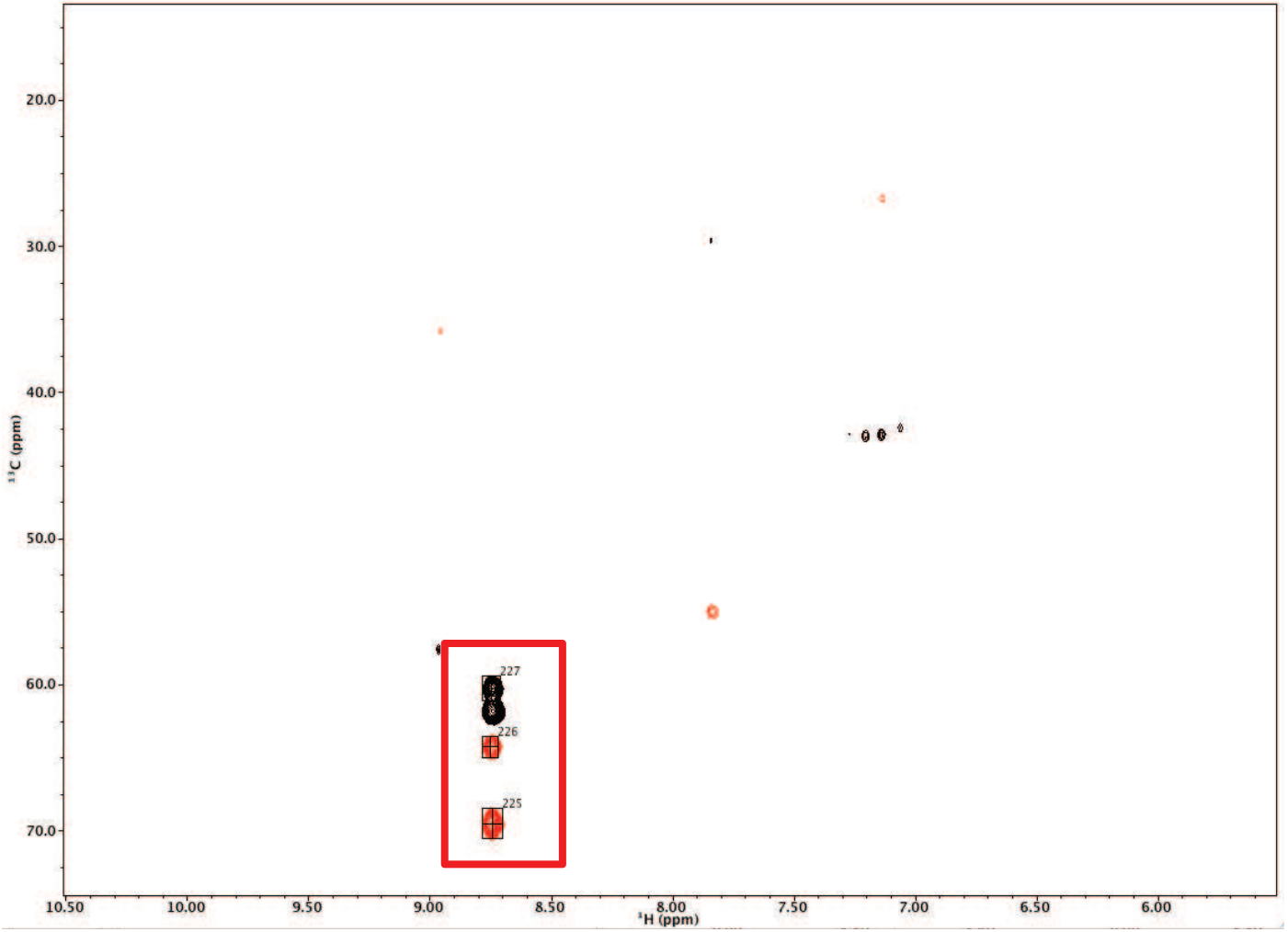
HNCA of ubiquitin

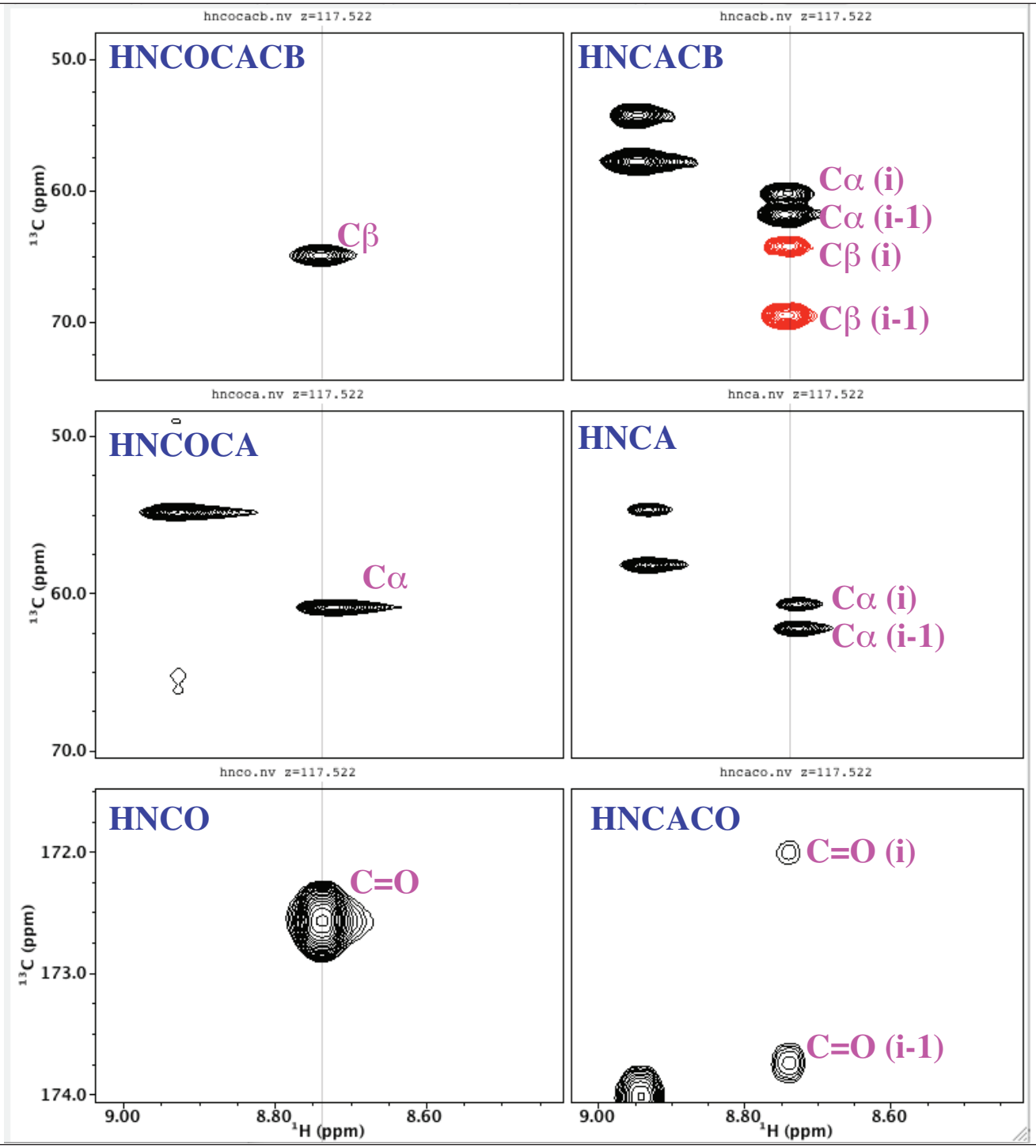


HNCACB

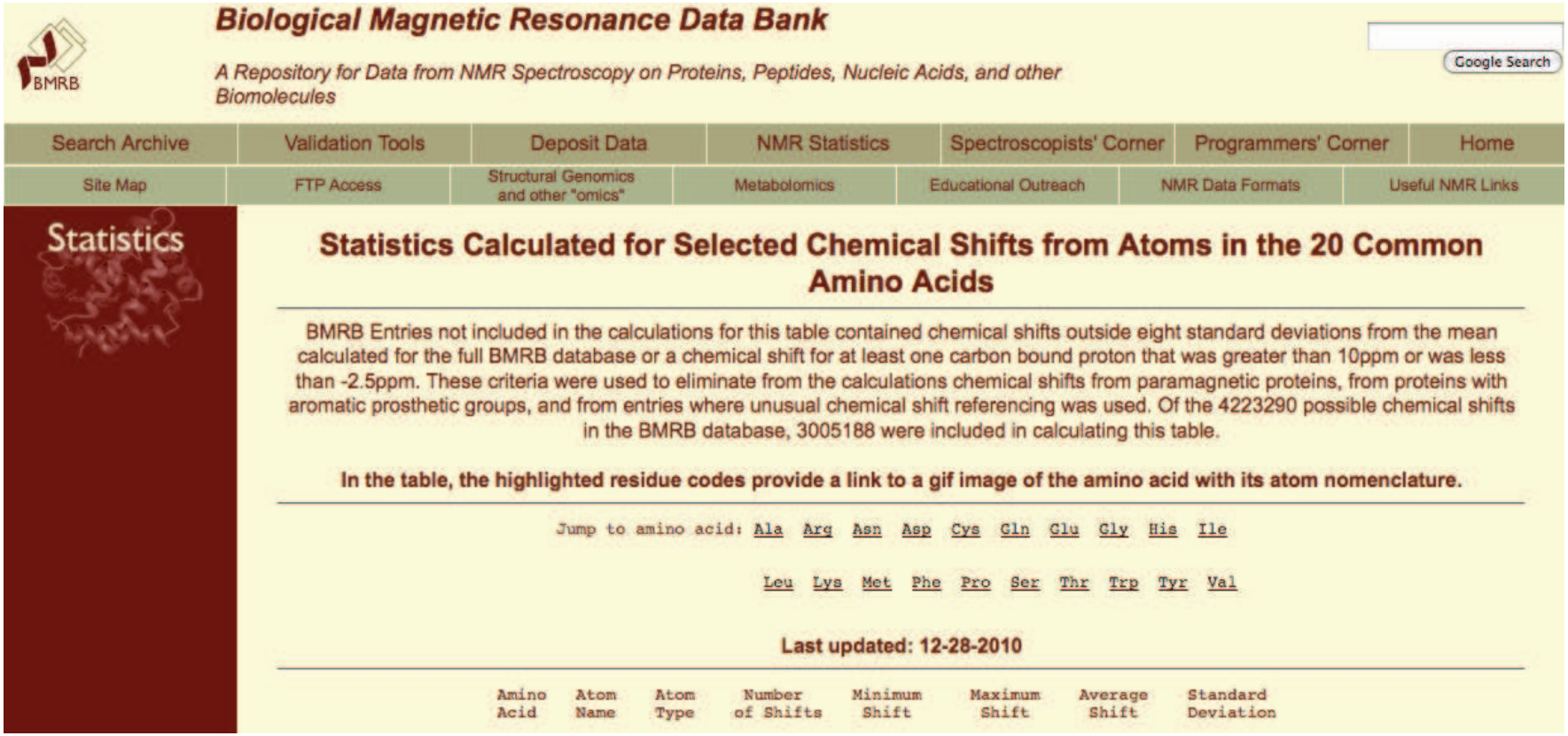


HNCACB of ubiquitin




hncocacb.nv z=117.522
HNCOCACB
Cβ
hncacb.nv z=117.522
HNCACB
Cα (i)
Cα (i-1)
Cβ (i)
Cβ (i-1)
hncoca.nv z=117.522
HNCOCA
Cα
hnca.nv z=117.522
HNCA
Cα (i)
Cα (i-1)
hnco.nv z=117.522
HNCO
C=O
hncaco.nv z=117.522
HNCACO
C=O (i)
C=O (i-1)
50.0
60.0
70.0
172.0
173.0
174.0
13C (ppm)
9.00
8.80
8.60
1H (ppm)

Compare chemical shift data with BMRDB



Biological Magnetic Resonance Data Bank

A Repository for Data from NMR Spectroscopy on Proteins, Peptides, Nucleic Acids, and other Biomolecules

Google Search

Search Archive | Validation Tools | Deposit Data | NMR Statistics | Spectroscopists' Corner | Programmers' Corner | Home

Site Map | FTP Access | Structural Genomics and other "omics" | Metabolomics | Educational Outreach | NMR Data Formats | Useful NMR Links

Statistics

Statistics Calculated for Selected Chemical Shifts from Atoms in the 20 Common Amino Acids

BMRB Entries not included in the calculations for this table contained chemical shifts outside eight standard deviations from the mean calculated for the full BMRB database or a chemical shift for at least one carbon bound proton that was greater than 10ppm or was less than -2.5ppm. These criteria were used to eliminate from the calculations chemical shifts from paramagnetic proteins, from proteins with aromatic prosthetic groups, and from entries where unusual chemical shift referencing was used. Of the 4223290 possible chemical shifts in the BMRB database, 3005188 were included in calculating this table.

In the table, the highlighted residue codes provide a link to a gif image of the amino acid with its atom nomenclature.

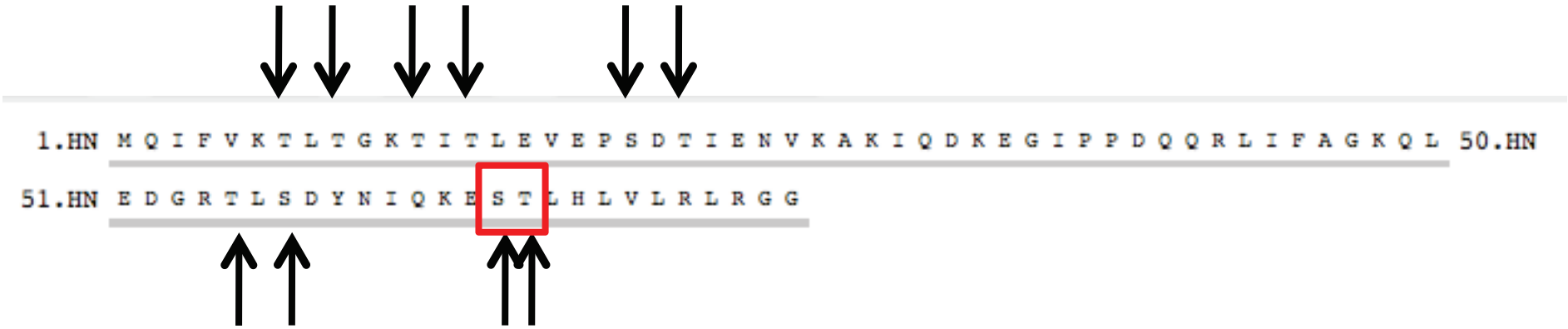
Jump to amino acid: Ala Arg Asn Asp Cys Gln Glu Gly His Ile

Leu Lys Met Phe Pro Ser Thr Trp Tyr Val

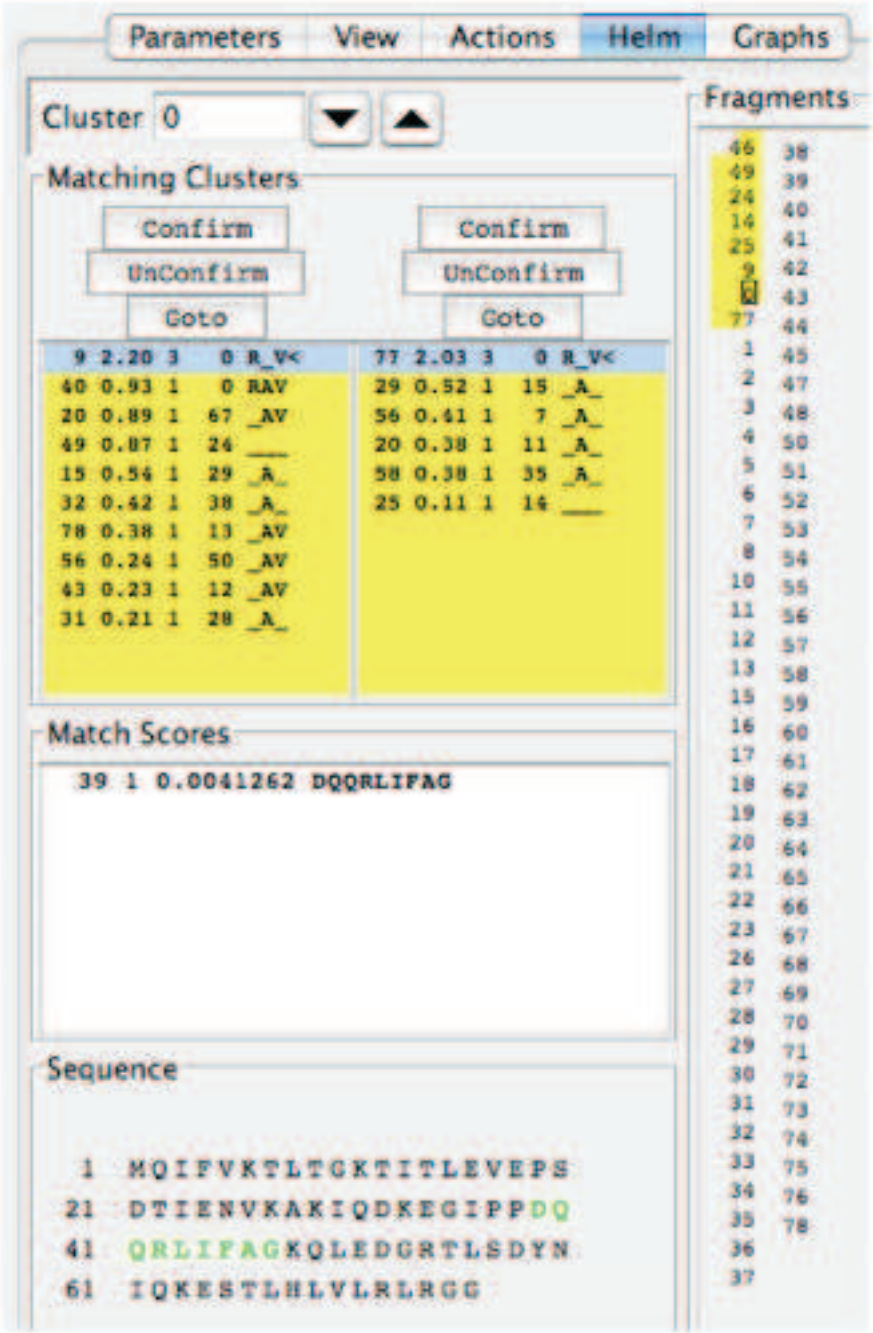
Last updated: 12-28-2010

Amino Acid	Atom Name	Atom Type	Number of Shifts	Minimum Shift	Maximum Shift	Average Shift	Standard Deviation

Amino Acid	Atom Name	Atom Type	Number of Shifts	Minimum Shift	Maximum Shift	Average Shift	Standard Deviation
SER	H	H	23411	2.96	13.13	8.28	0.59
SER	HA	H	18361	1.58	6.85	4.48	0.41
SER	HB2	H	16748	1.74	5.41	3.88	0.25
SER	HB3	H	15243	1.54	5.01	3.85	0.28
SER	HG	H	266	0.00	8.97	5.35	1.00
SER	C	C	14212	164.47	184.88	174.68	1.74
SER	CA	C	20036	45.13	68.40	58.76	2.10
SER	CB	C	18372	46.69	76.39	63.80	1.49
SER	N	N	21101	99.62	133.68	116.27	3.56
THR	H	H	20776	5.54	11.73	8.25	0.62
THR	HA	H	15844	0.87	6.36	4.46	0.48
THR	HB	H	14472	0.92	8.35	4.17	0.33
THR	HG1	H	488	0.32	8.95	4.96	1.57
THR	HG2	H	14332	-1.19	3.54	1.14	0.23
THR	C	C	12262	165.50	184.43	174.58	1.76
THR	CA	C	17226	51.61	72.80	62.24	2.60
THR	CB	C	15798	33.00	80.22	69.73	1.67
THR	CG2	C	10742	11.70	36.73	21.57	1.10
THR	N	N	18781	95.77	138.27	115.44	4.74


1.HN M Q I F V K T L T G K T I T L E V E P S D T I E N V K A K I Q D K E G I P P D Q Q R L I F A G K Q L 50.HN
51.HN E D G R T L S D Y N I Q K E S T L H L V L R L R G G

NMRView J
RunAbout



Output:
List of
Chemical
shifts for
each residue

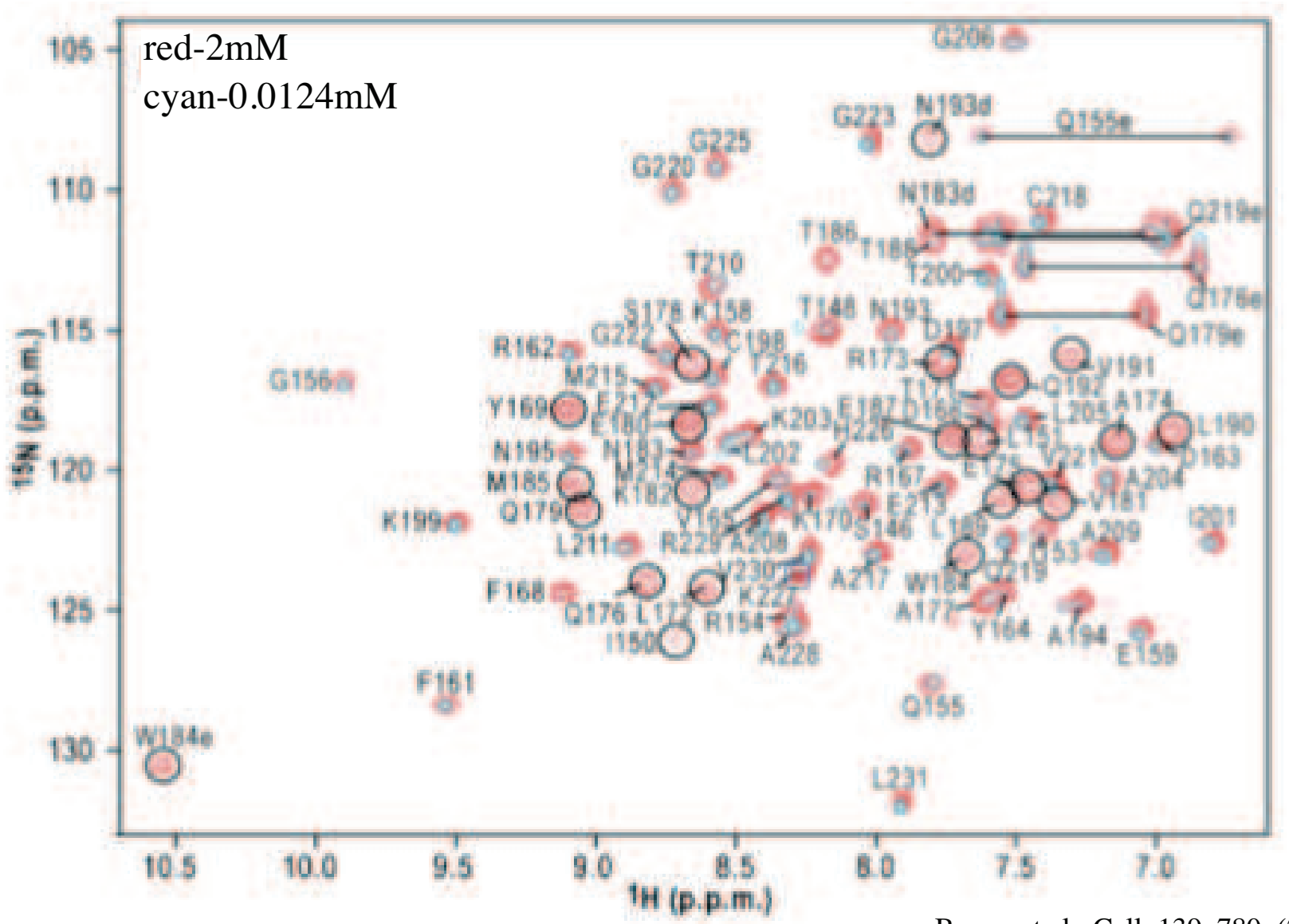
```
RES_ID  65
RES_TYPE       SER
SPIN_SYSTEM_ID  65
   N 114.73
   HN 7.67
   HA 4.64
   C 172.16
   CA 60.89
   CB 64.91
   HB1 3.89
   HB2 3.65
END_RES_DEF

RES_ID  66
RES_TYPE       THR
SPIN_SYSTEM_ID  66
   N 117.11
   HN 8.73
   HA 5.27
   C 173.95
   CA 62.34
   CB 70.08
   HB 4.08
   CG2 21.57
   HG2# 0.94
END_RES_DEF
```

Still need:
- Distance constraint data (NOESY/RDCs)
- Side chains data (TOCSY, COSY)

-Use structure calculation software
- Ramachandran plots

BMRB entry 5387

Some beautiful work on CA CTD dimer



Byeon et al., Cell, 139, 780, (2009)

For the sake of full disclosure…

Vol 460 | 23 July 2009 | doi:10.1038/nature08200 nature

LETTERS

Increased mortality and AIDS-like immunopathology in wild chimpanzees infected with SIVcpz

Brandon F. Keele[1]†, James Holland Jones[4], Karen A. Terio[5], Jacob D. Estes[6], Rebecca S. Rudicell[2], Michael L. Wilson[7,8], Yingying Li[1], Gerald H. Learn[1], T. Mark Beasley[3], Joann Schumacher-Stankey[8], Emily Wroblewski[8], Anna Mosser[9], Jane Raphael[9], Shadrack Kamenya[9], Elizabeth V. Lonsdorf[10], Dominic A. Travis[11], Titus Mlengeya[12], Michael J. Kinsel[5], James G. Else[13], Guido Silvestri[14], Jane Goodall[15], Paul M. Sharp[16], George M. Shaw[1], Anne E. Pusey[8] & Beatrice H. Hahn[1,2]

These results indicate that SIVcpz, like HIV-1, is associated with progressive $CD4^+$ T-cell loss, lymphatic tissue destruction and premature death. These findings challenge the prevailing view that all natural SIV infections are non-pathogenic and suggest that SIVcpz has a substantial negative impact on the health, reproduction and lifespan of chimpanzees in the wild.