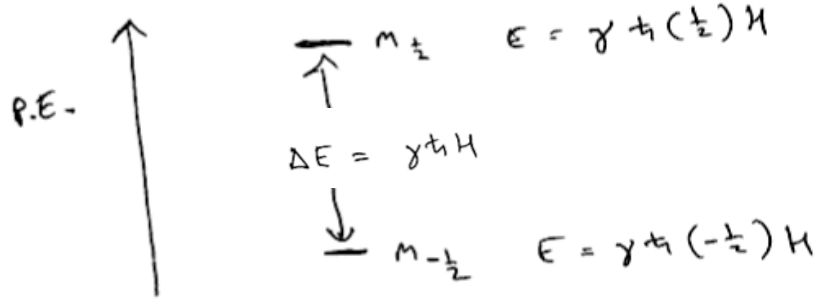
Today's planned topics:

1) Acquiring an NMR spectrum: Fourier transforms & FT-NMR.

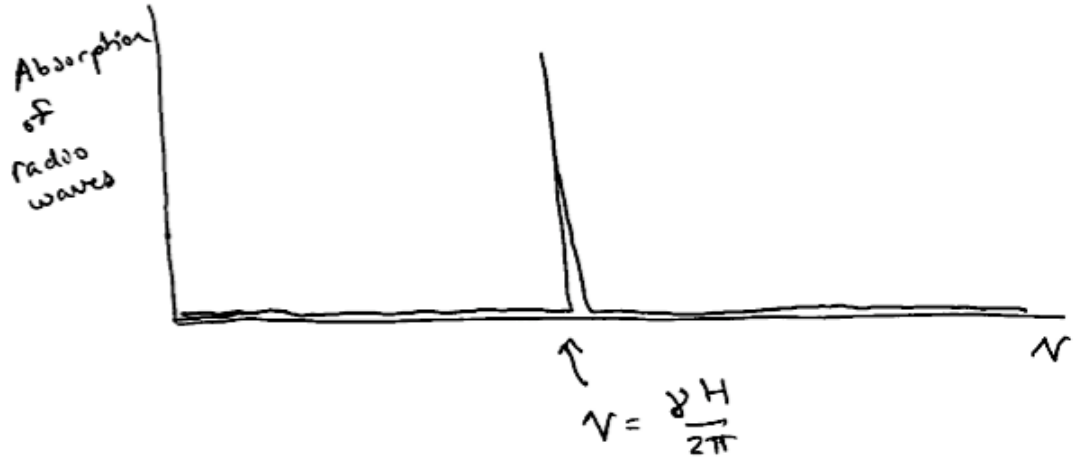
2) Solving the "spectrum assignment problem".

Obtaining an NMR spectrum: a simple (but inefficient) way.

Put nuclei in magnetic field. For ^1H (with I=1/2) there are two allowed energy states:

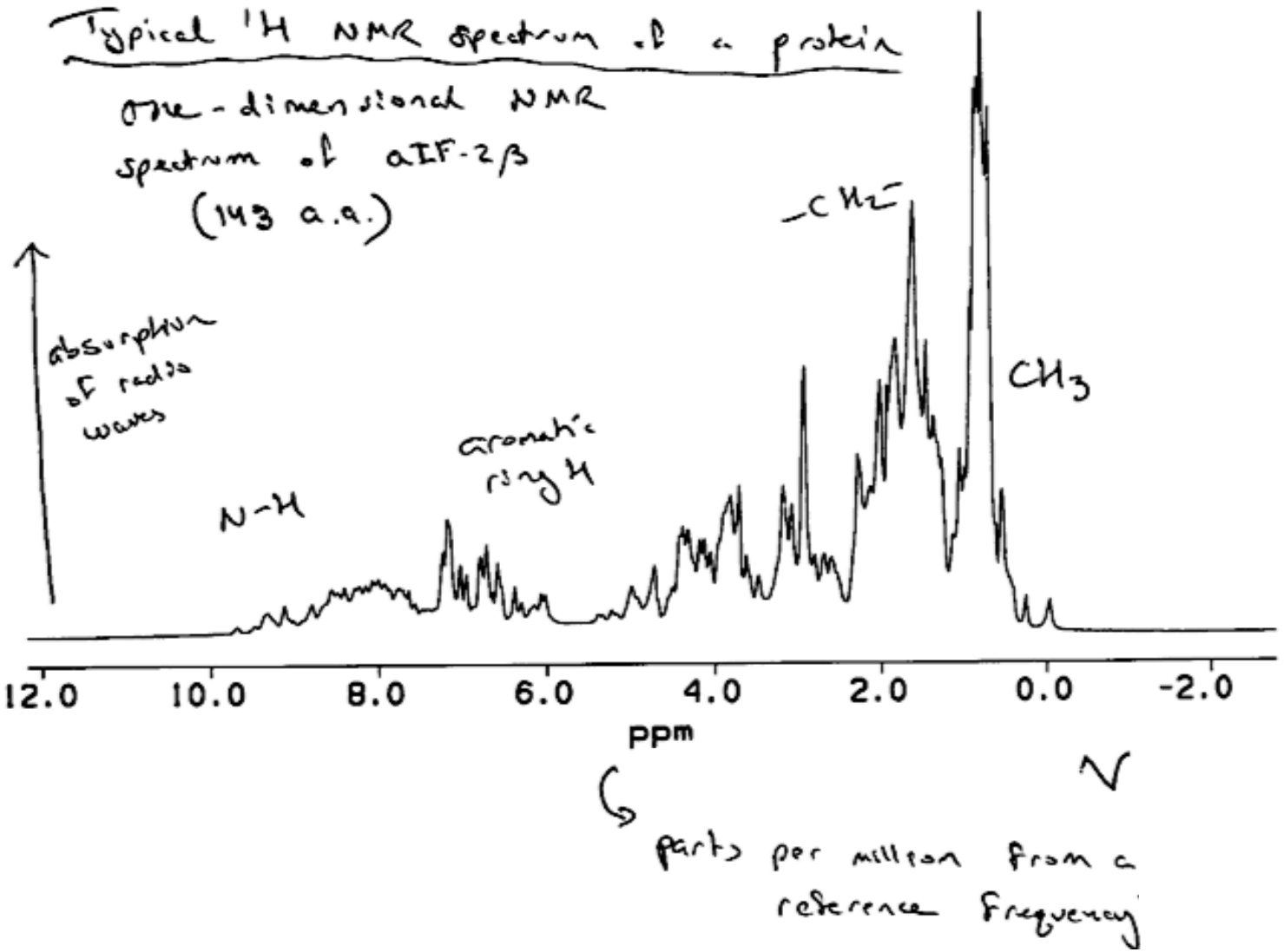


You could irradiate sample with radio waves, and there will be absorption when the energy of the radio wave matches the energy difference between the two allowed energy states. You would need to scan the frequency spectrum, one frequency at a time:



This is one way to get an NMR spectrum. It is not very efficient, but it would work!

To obtain an NMR spectrum of a protein, you could measure the absorption of radio waves, one frequency at a time:



A much more efficient way to get an NMR spectrum uses a "Fourier transform" method.

What is a Fourier transform?

Fourier transform is a mathematical operation that converts a time-varying signal to its frequency spectrum:

time varying signal $\xrightarrow{\text{F.T.}}$ frequency spectrum

$$f(\nu) = \int_{-\infty}^{\infty} f(t)\, e^{i\nu t}\, dt$$

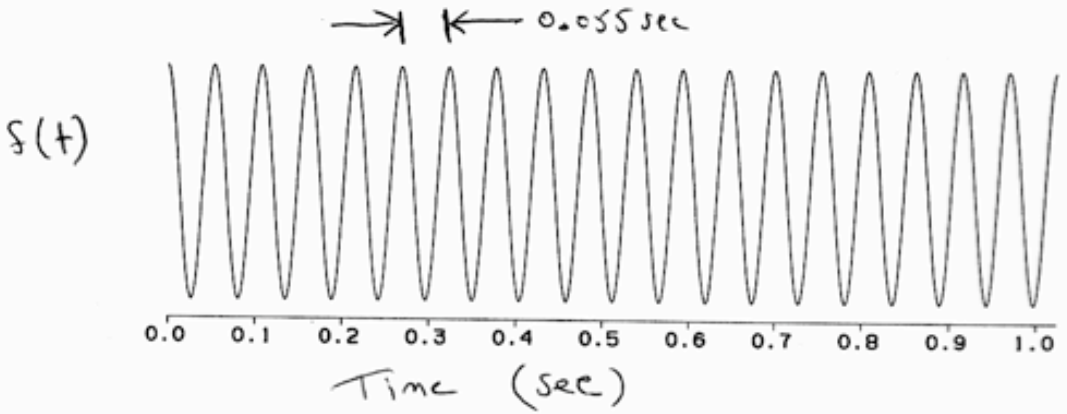
$f(\nu)$ = frequency spectrum.

$f(t)$ = time varying signal.

Examples of Fourier Transforms:

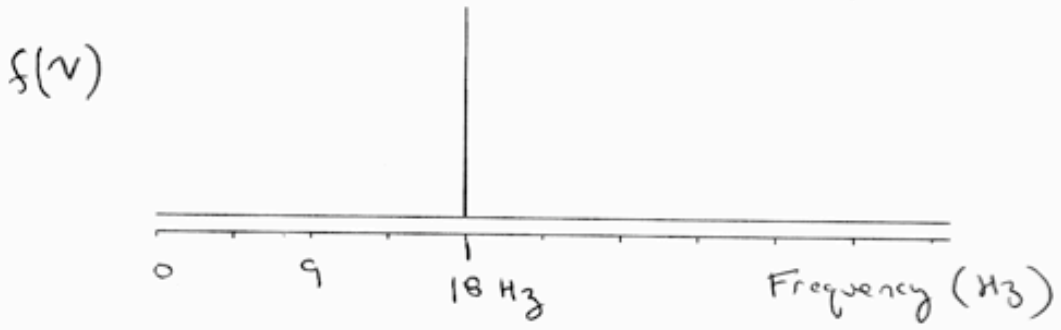
Fourier Transform of time-varying signal:

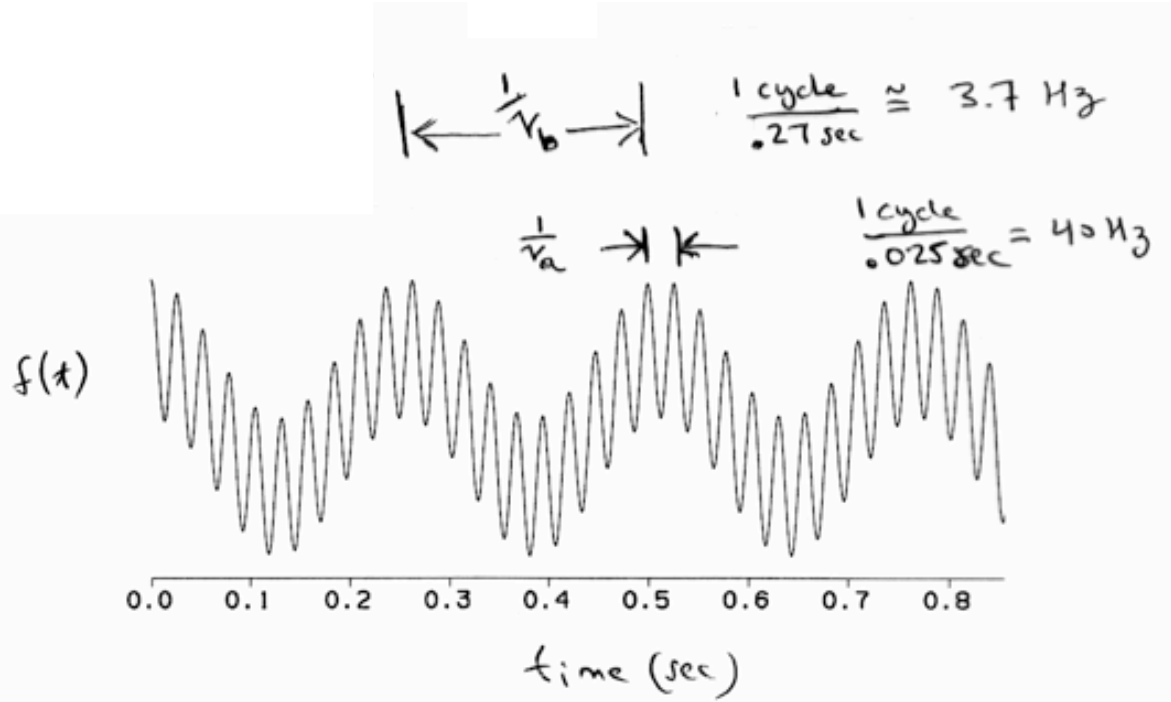
$$\text{Frequency} = \frac{1 \text{ cycle}}{0.055 \text{ sec}} \cong 18 \text{ Hz}$$



F.T.

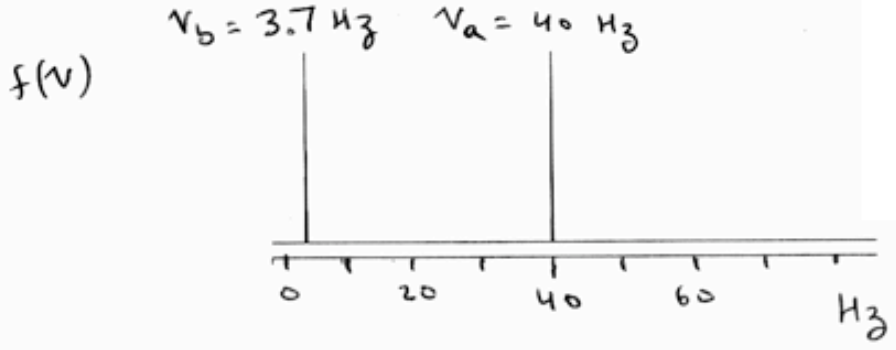
Converts the time-varying signal into its frequency spectrum.





F.T.

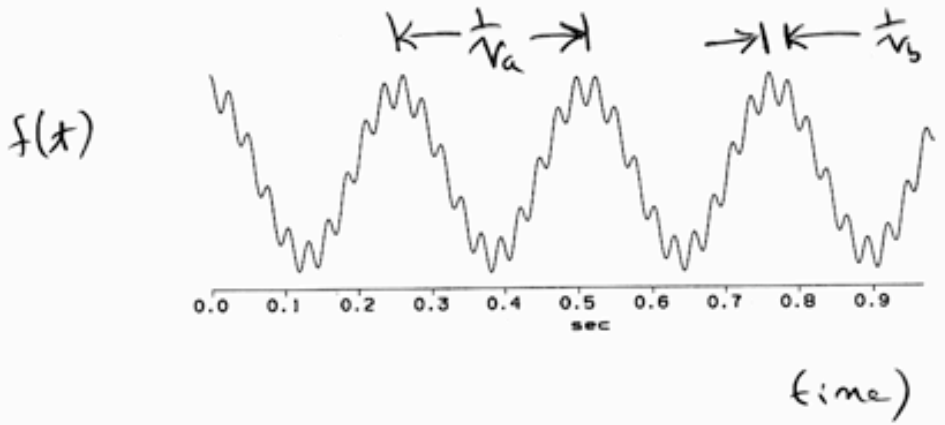
Converts the time-varying signal into its frequency spectrum.



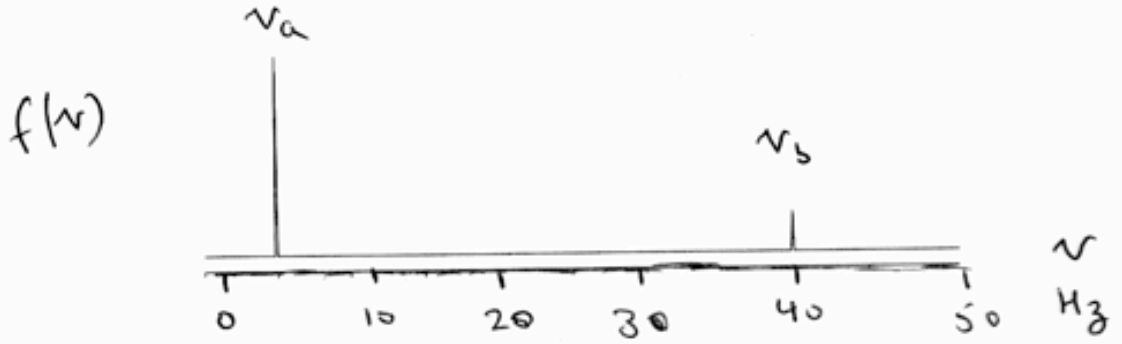
Two frequencies with unequal amplitudes:

small amplitude frequency $\cong 40\ Hz = \nu_b$

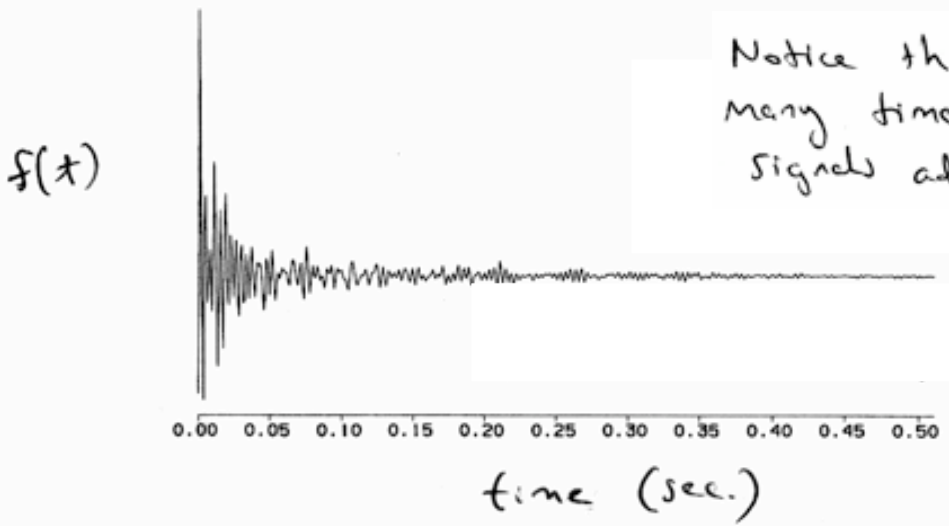
large amplitude frequency $= \nu_a \cong 3.9\ Hz$



F.T.



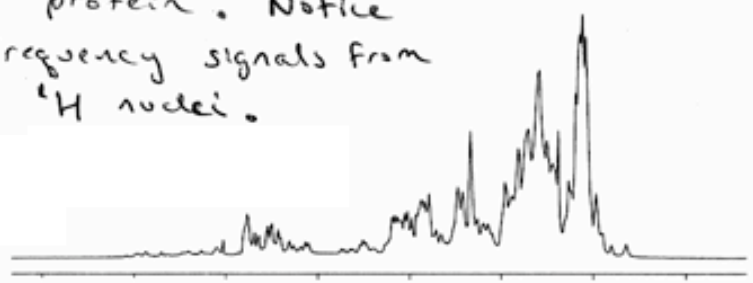
Fourier Transform of a time-varying NMR signal from a protein



Notice that the entire NMR frequency spectrum is obtained by recording the time-varying signal for 0.5 seconds!

F.T.

NMR frequency spectrum of a protein. Notice many frequency signals from distinct ^1H nuclei.

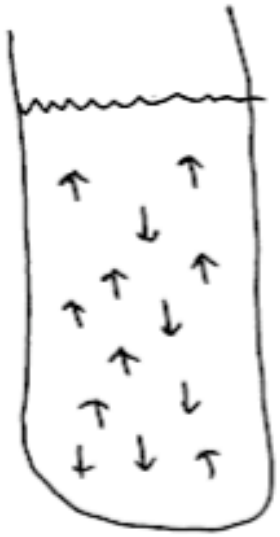


For FT-NMR, it is necessary to identify a time-varying signal that can be measured and Fourier transformed to yield the NMR frequency spectrum.

Obtaining an NMR spectrum by F.T. NMR:

Start w/ sample of nuclei:

Sample of nuclei, place in magnetic field



Some nuclei will be in the $m = +\frac{1}{2}$ state ↑

Some will be in the $m = -\frac{1}{2}$ state ↓

Slight excess of nuclei in the lower energy state (by Boltzmann distribution)

Each individual nucleus is a small magnetic dipole (has an energy of interaction with magnetic field)

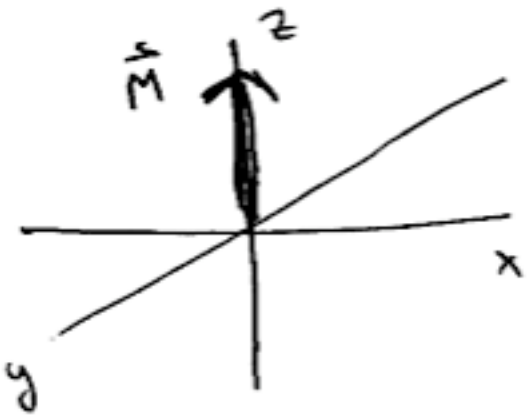
$$\vec{M} = \sum \vec{\mu}$$

$\vec{M}$: bulk magnetization of sample

$\sum$: sum

$\vec{\mu}$: of individual magnetic dipoles.

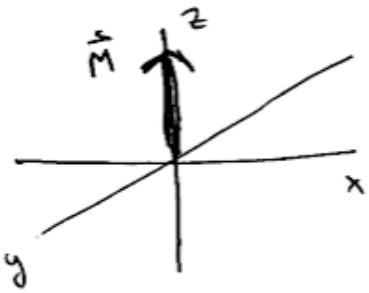
At equilibrium, $\vec{M}$ is parallel to the magnetic field $\vec{H}$ ($\vec{H}$ is always considered to define the z-direction).



at equilibrium

If $\vec{M}$ is not at equilibrium (not parallel to $\vec{H}$) it will experience a force that causes $\vec{M}$ to precess around $\vec{H}$ with a frequency ν

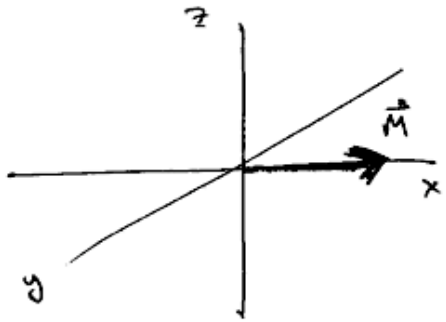
where $\nu = \gamma H / 2\pi$

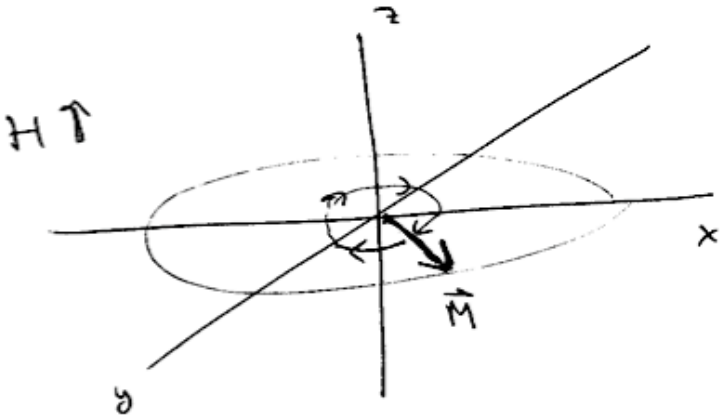


at equilibrium

pulsed magnetic field

"90° pulse"

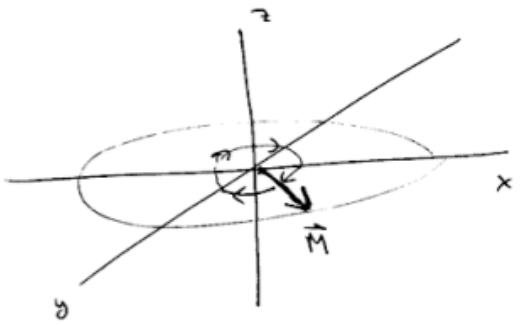




$\vec{M}$ precesses about $\vec{H}$ at frequency ν

$$\nu = \frac{\gamma H}{2\pi}$$

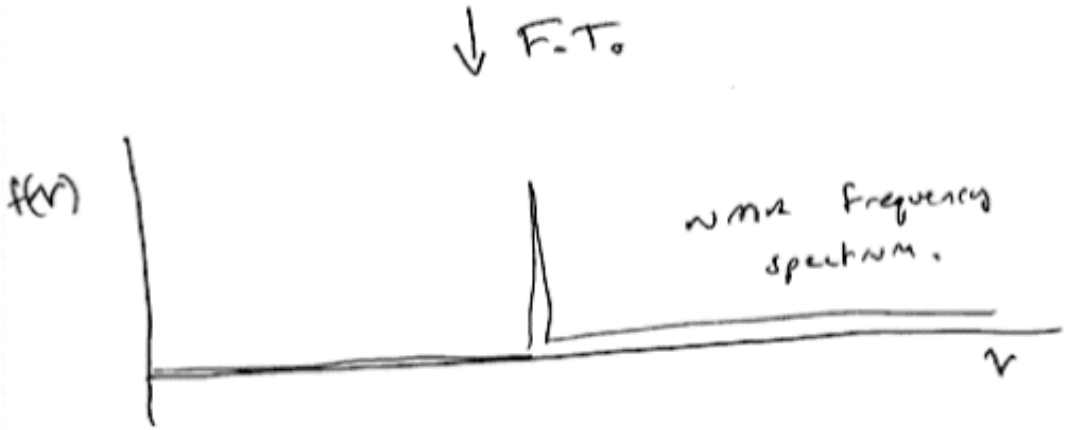
What is recorded in an FT-NMR experiment:



The x-component of $\vec{M}$ is recorded as a function of time as $\vec{M}$ precesses:

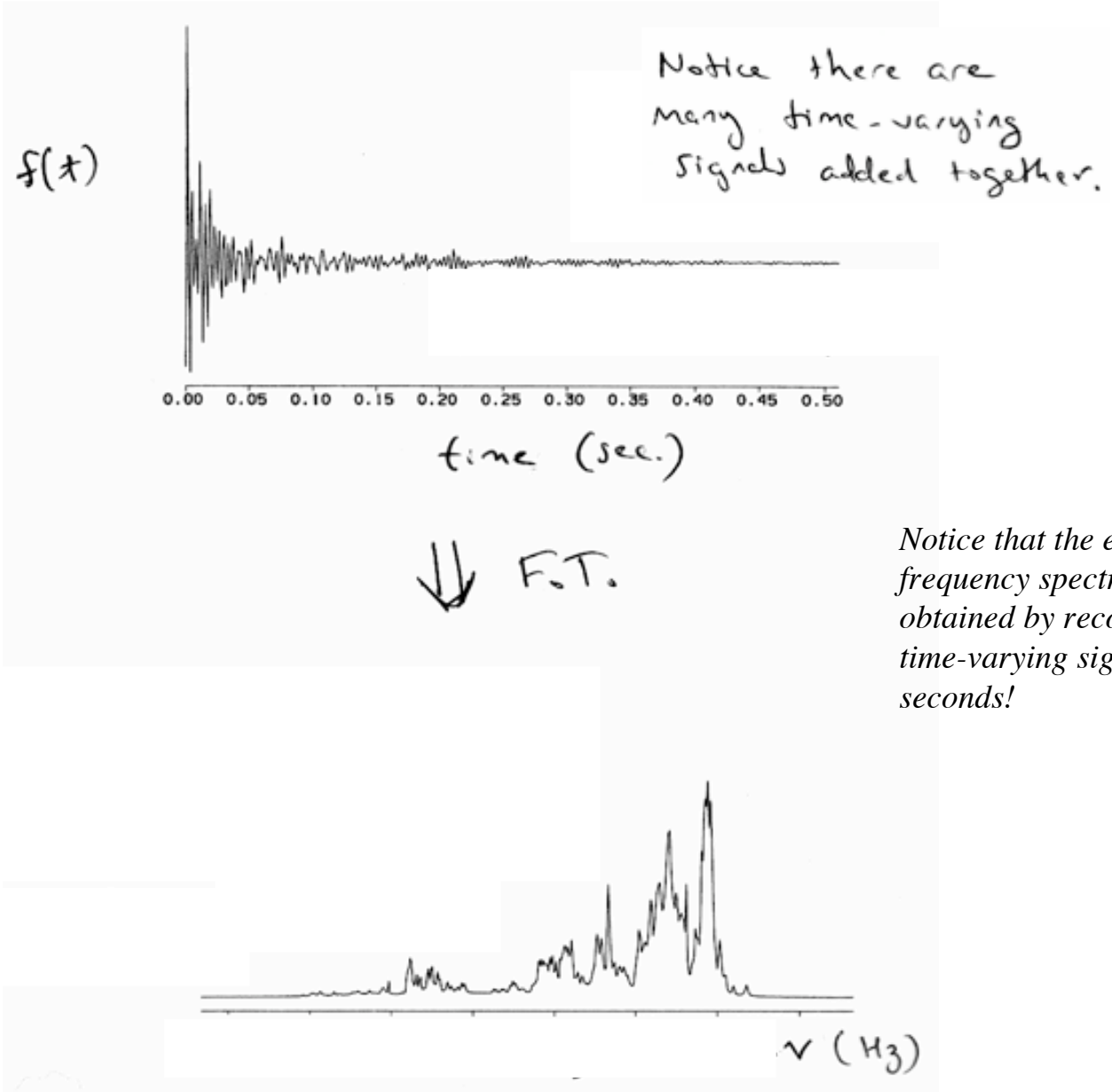
This is called the Free Induction Decay (or "FID").

(actually, what is recorded is the voltage in a RF receiver coil placed around the sample. This voltage is proportional to M_x).



Typical time-varying signal from a protein, where the different nuclei have many different NMR frequencies:

90 degree pulse, then record f(t):



Notice that the entire NMR frequency spectrum is obtained by recording the time-varying signal for 0.5 seconds!

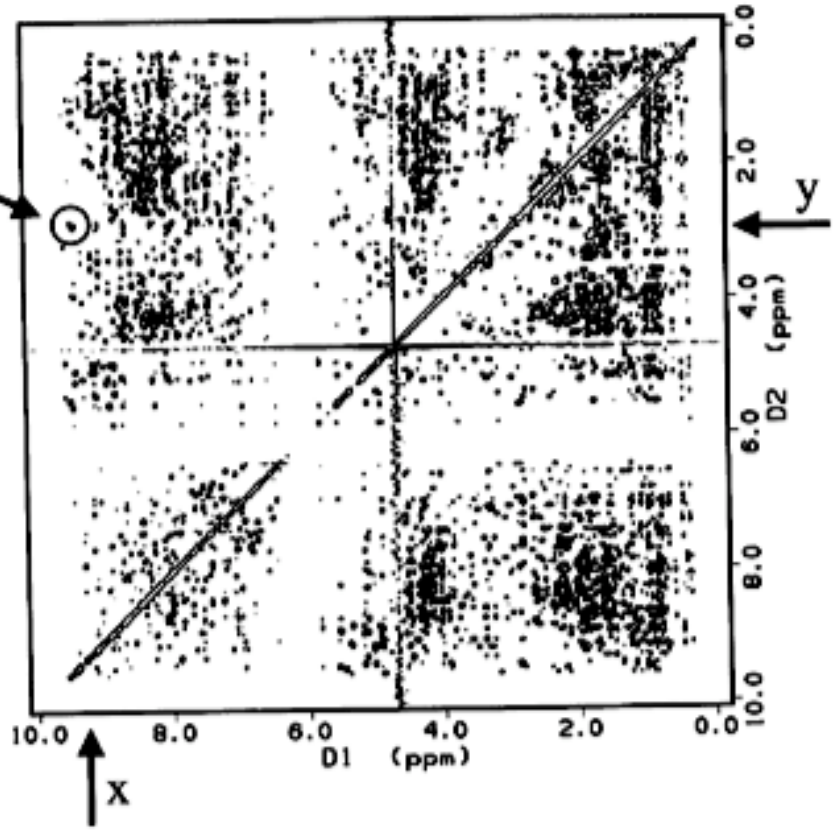
For multi-dimensional NMR, 2 time varying signals are recorded, and Fourier transformed is used two times to generate the 2-D NMR spectrum.

2-D nuclear Overhauser effect (NOE) spectrum

Peak has 2 coordinates (9.5, 3.0) ppm

The proton with NMR frequency of 9.5 ppm is near the proton with NMR frequency 3.0 ppm.

"Near" means < 6 Å



Today's topics:

1) Acquiring an NMR spectrum: Fourier transforms and FT-NMR.

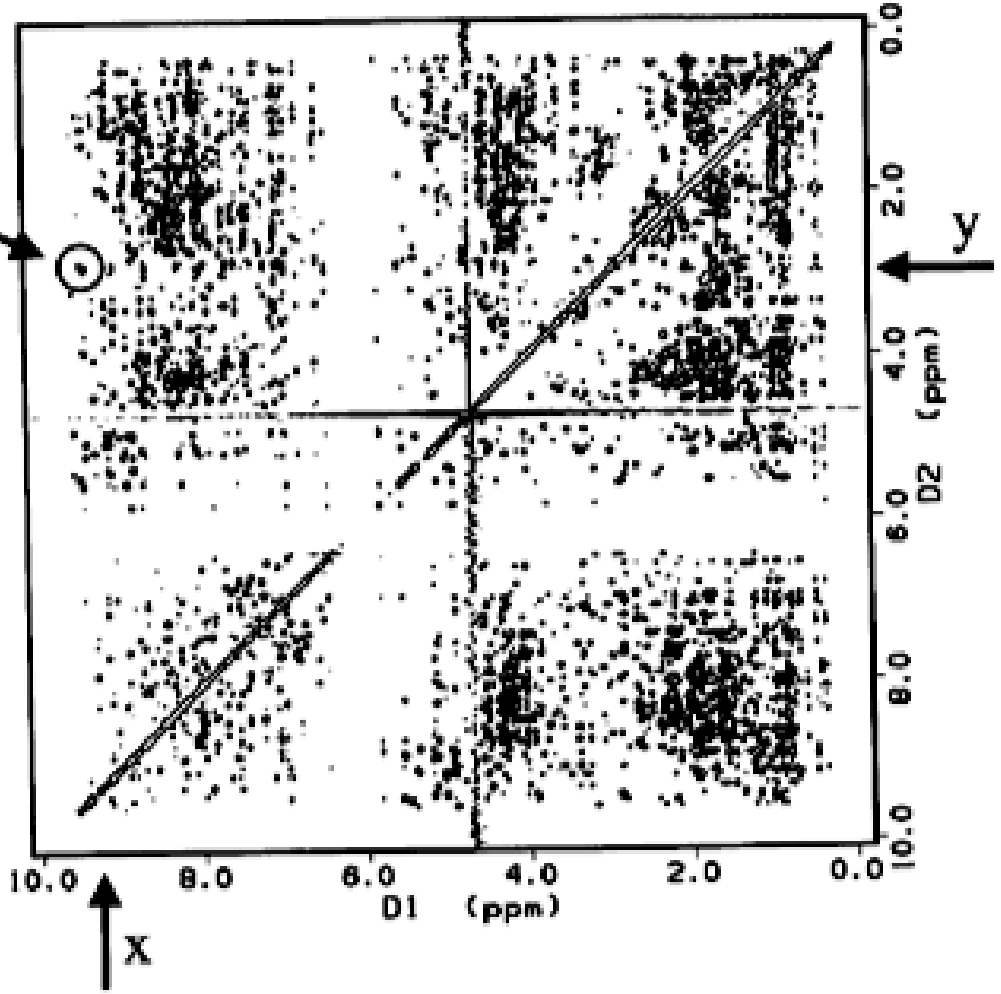
2) Solving the NMR spectrum assignment problem.

The 2-D NOE spectrum of a 140 a.a. protein is used in obtaining the protein structure:

Peak has 2 coordinates (9.5, 3.0)

The proton with NMR frequency of 9.5 ppm is near the proton with NMR frequency 3.0 ppm.

"Near" means < 6 Å

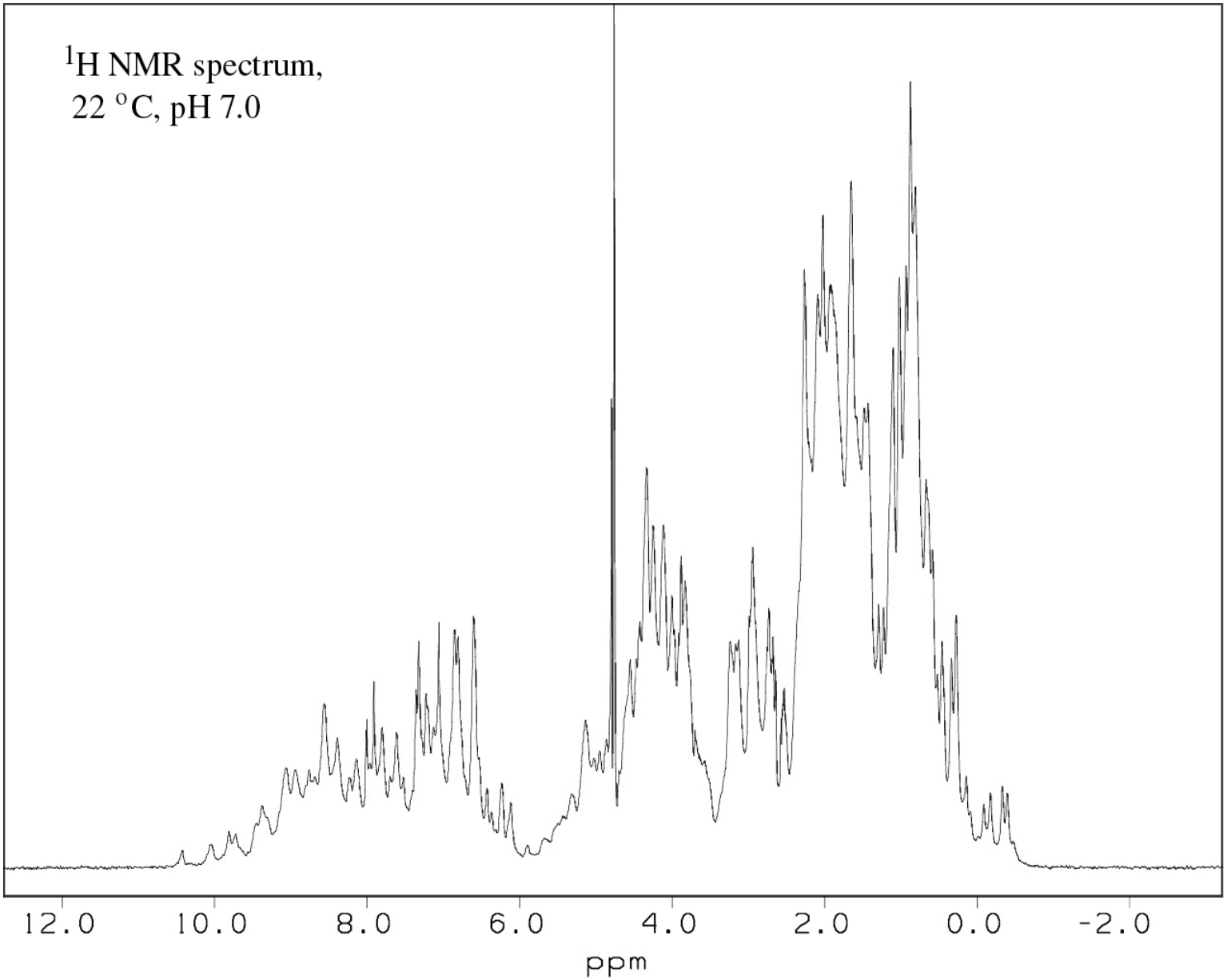


An example of a protein structure determination by NMR (including solving the spectrum assignment problem).

NMR analysis of a protein called "Antizyme",

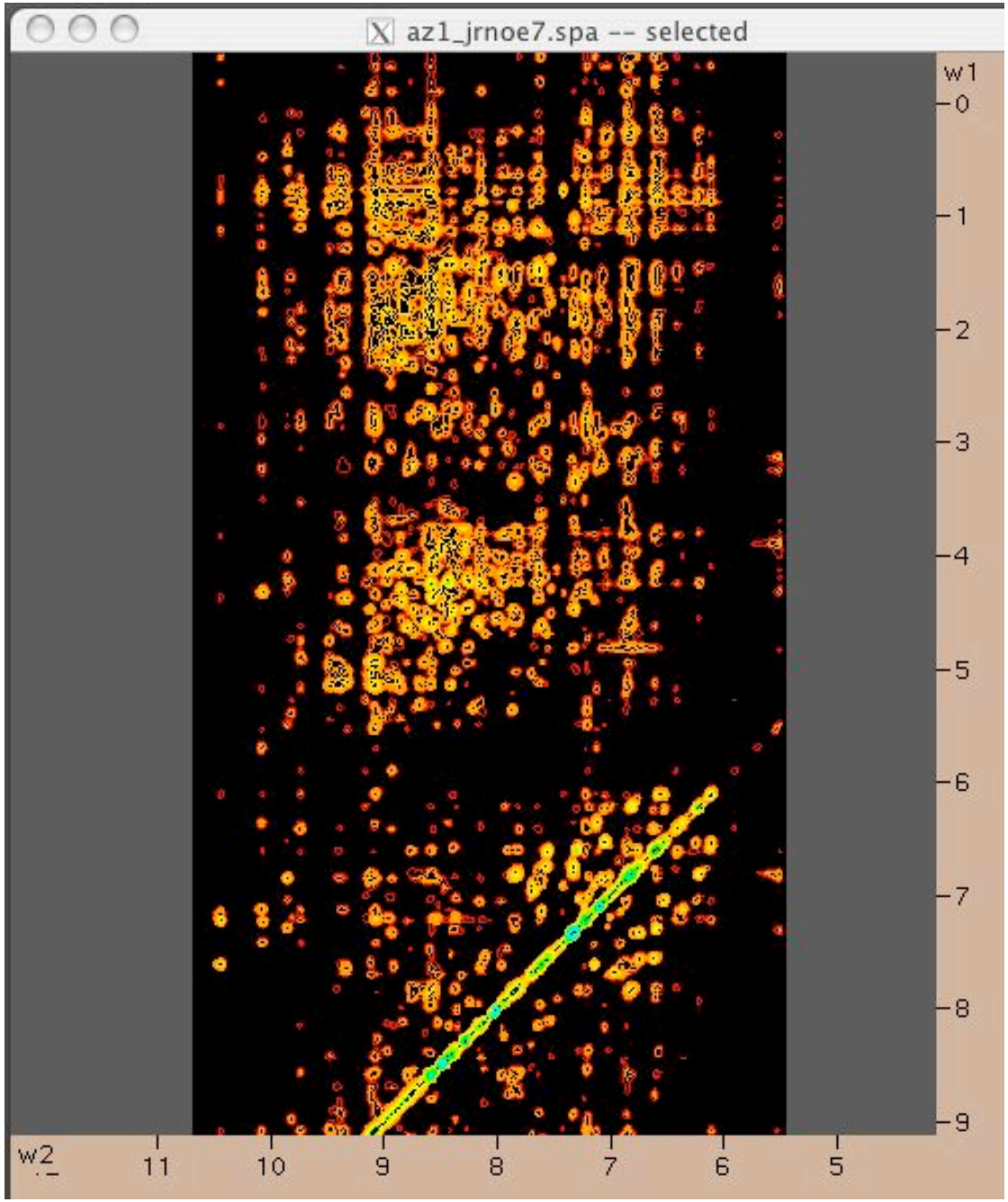
an inhibitor of the enzyme ornithine decarboxylase (ODC).

Antizyme binds and inhibits ODC, and targets ODC for degradation.



Frequency of radio waves absorbed by ^{1}H nuclei in sample.

A section of the 2-D NOE spectrum of Antizyme.

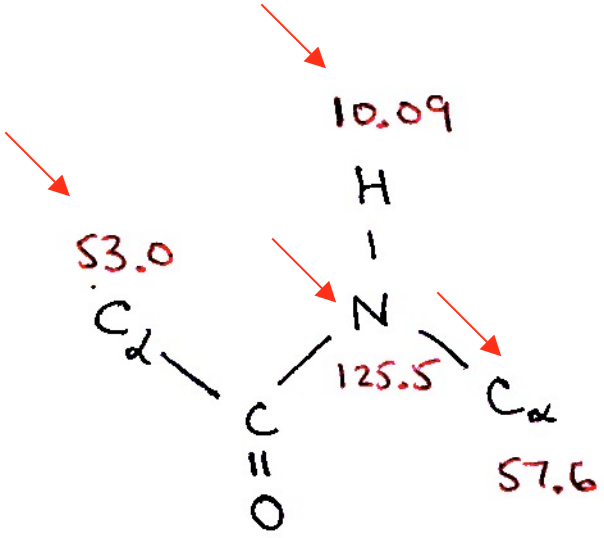


First step of NMR analysis of Antizyme is spectrum assignment:

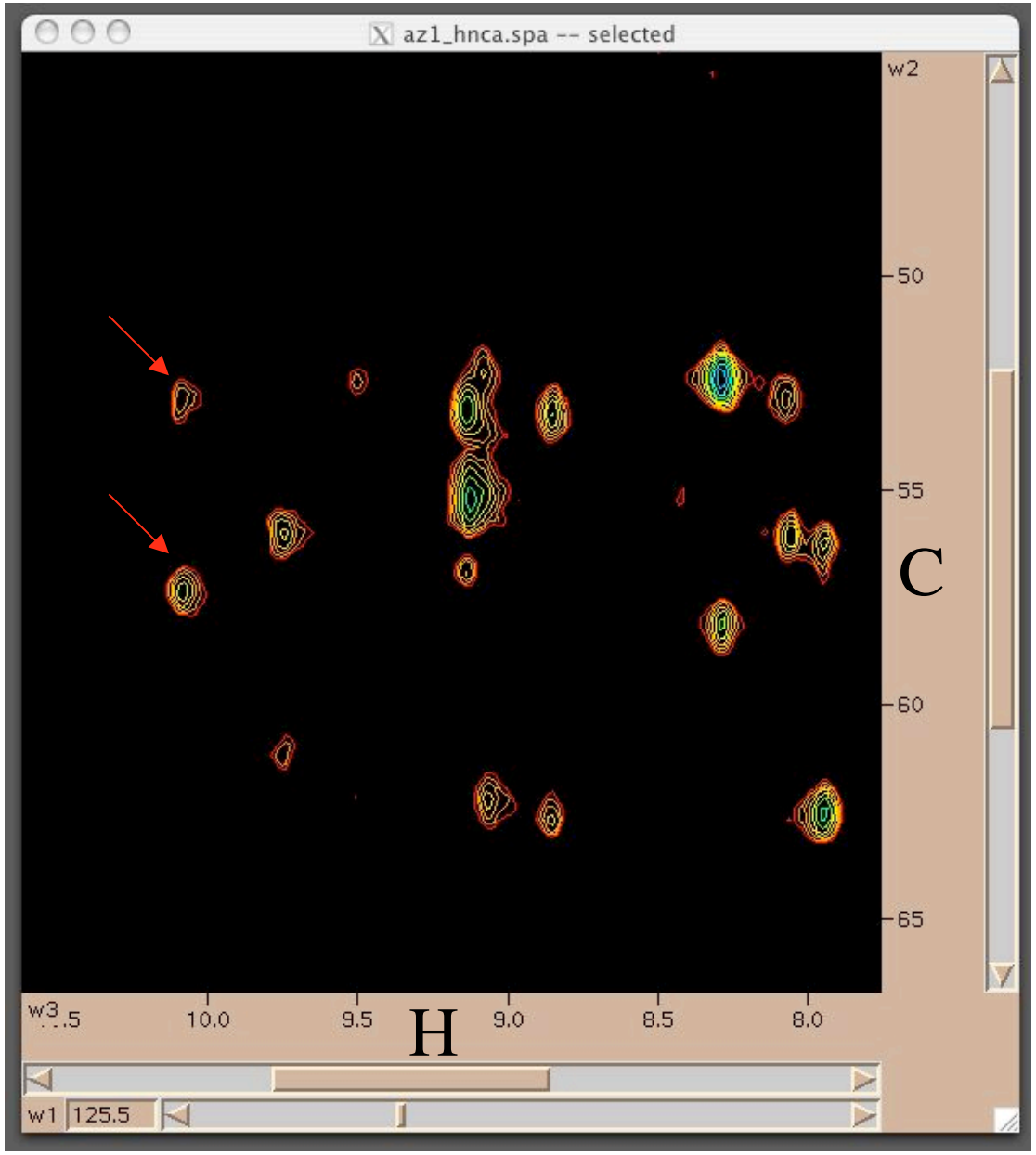
(Identifying the NMR frequencies of as many specific nuclei in the protein as possible).

3-dimensional "triple-resonance" FT-NMR is used for solving the assignment problem.

3-D “HNCA” spectrum.

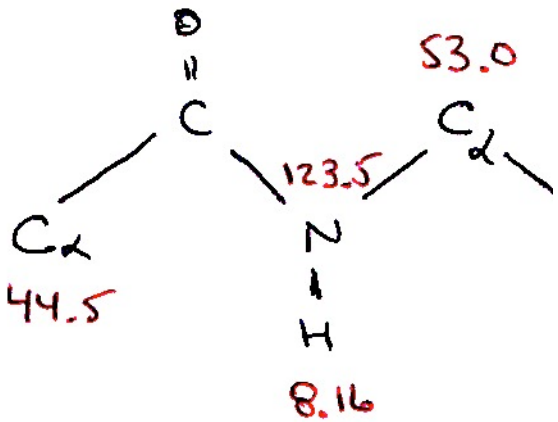


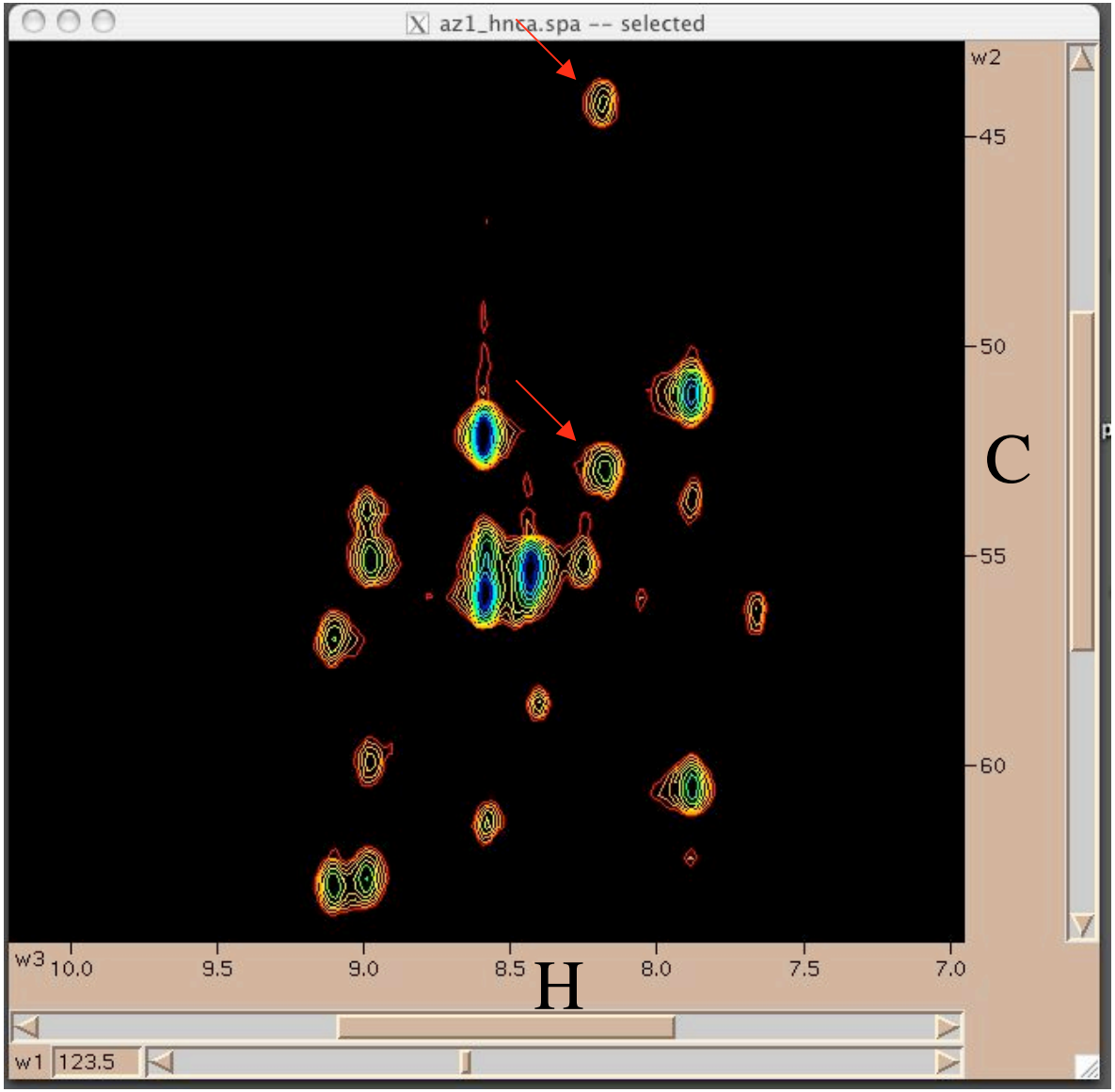
3-D spectrum, with H on first axis, $^{13}C\alpha$ on 2nd axis, and ^{15}N on 3rd axis.

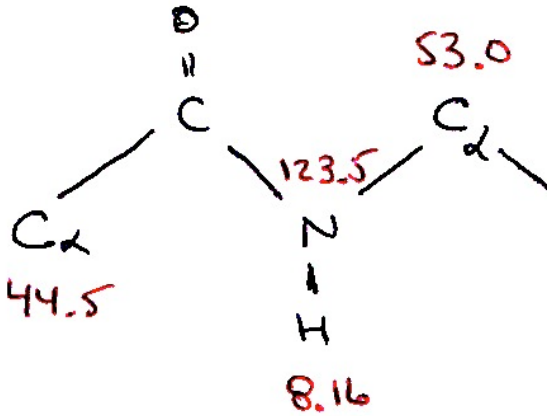


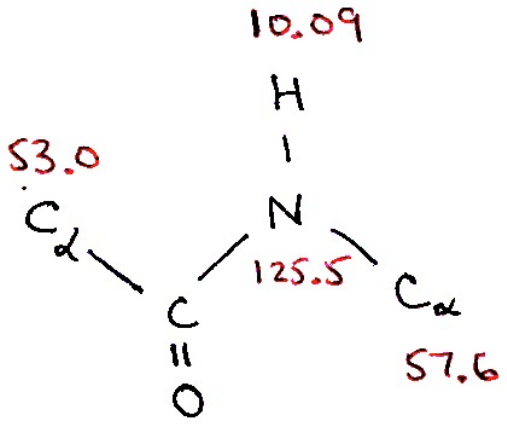
N15 = 125.5 ppm plane

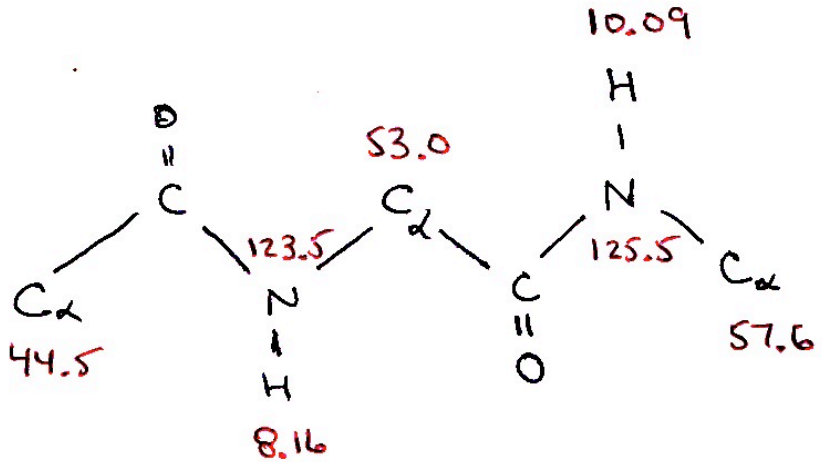
N15 = 125.5 ppm plane





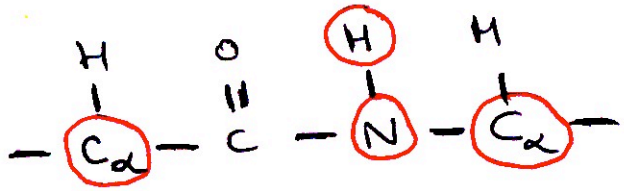

O
C
53.0
Cα
123.5
Cα
44.5
N
H
8.16


10.09
H
N
53.0
Cα
125.5
Cα
C
O
57.6

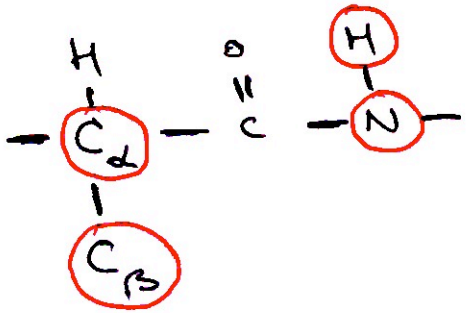

10.09
H
O
N
C
53.0
Cα
123.5
Cα
125.5
C
O
57.6
Cα
44.5
N
H
8.16

Triple Resonance NMR of Antizyme

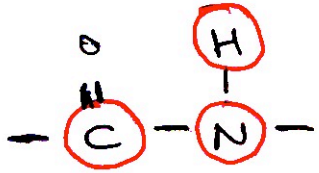
HNCA



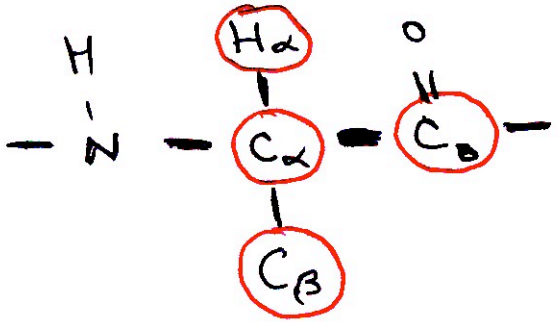
HN(CO)CACB



HNCO



HACACBCO



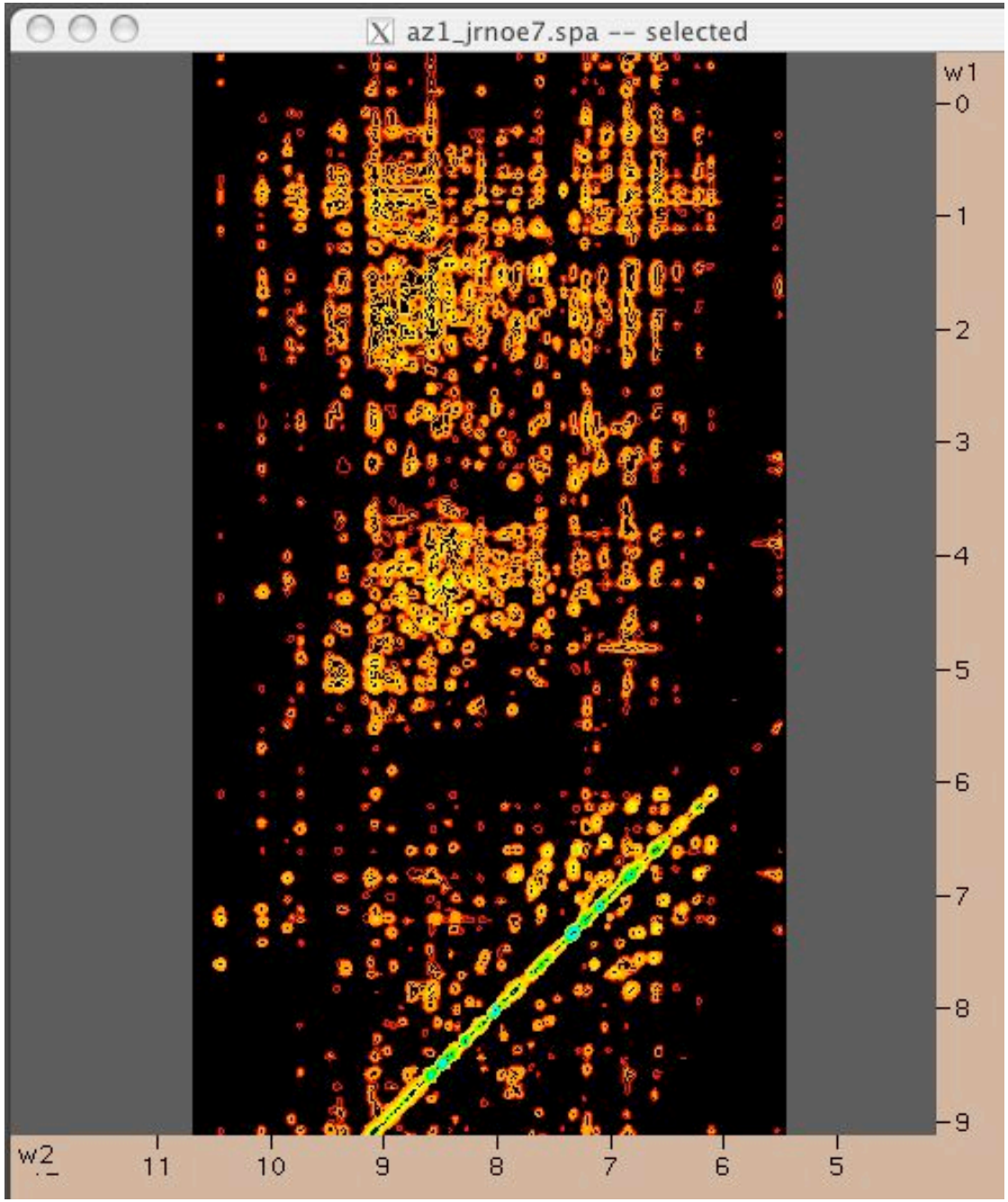
Eventually generate a table of the NMR frequencies of (almost) all the nuclei in the protein:

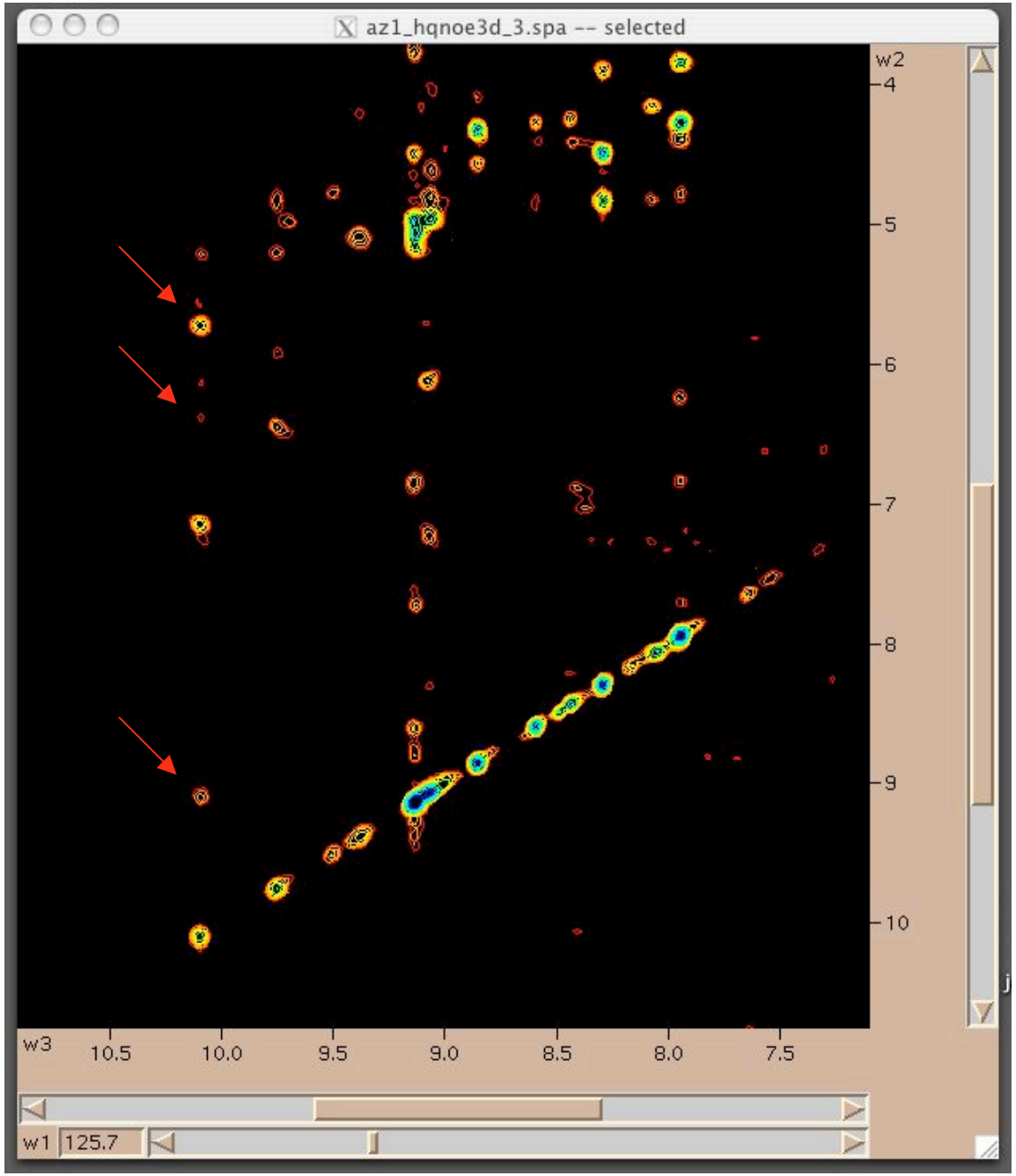
az1_80_136.xls

	A	B	C	D	E	F	G	H	I	J	K	L	M
1	no	type	HN	N	CA	CA-1	CB	CB-1	CO	CO-1	HA	HB	HG
2	87	D											
3	88	H											
4	89	S			58.6		63.7		173.9		4.42		
5	90	L	8.42	124.2	55.2	58.8	42.3	63.6	176.7	174.1	4.44	1.67	1.82,1.57
6	91	S	8.24	116.1	58.1	55.1	63.8	42.0	173.3	176.7	4.48	3.92	
7	92	A	8.27	125.6	52.4	58.2	19.4	63.8	176.6	173.3	4.48	1.45	
8	93	S	8.39	115.2	57.8	52.3	64.2	19.4	172.8	176.7	4.60	3.94	
9	94	I	8.62	124.3	61.1	57.8	37.6	64.1	175.0	173.0	4.22		
10	95	L	8.70	130.2	55.9	61.1	43.9	37.6	175.9	175.0	4.55	1.62	
11	96	Y	7.82	119.1	58.1	55.9	42.5	43.9	171.9	175.9	4.62		
12	97	S	7.62	121.6	57.0	58.0	65.3	42.4	171.4	171.9	4.99	3.77,3.66	
13	98	D	9.14	126.0	53.1	57.0	40.8	65.2	174.8	171.3	4.48		
14	99	E	8.76	115.8	59.1	53.3	29.0	40.8	176.3	174.8	4.14	2.33,2.06	
15	100	R	8.82	118.1	56.8	59.0	32.4	29.0	174.8	176.3	4.66		
16	101	L	8.60	121.5	53.9	56.4	46.7	32.6	174.3	174.8	5.32	1.66,1.60	1.26
17	102	N	8.70	119.6	53.7	53.9	42.0	46.5	173.5	174.2	5.20	2.75	
18	103	V	9.41	127.0	61.3	53.8	33.4	42.0	174.2	173.5	5.11	2.05	
19	104	T	8.89	121.1	60.0	61.3	71.4	33.4	172.0	174.2	5.13		1.17
20	105	E	8.96	123.7	55.0	60.0	31.9	71.6	175.2	172.2	5.21		
21	106	E	8.87	127.1	54.0	55.0	29.7	32.0		175.4	4.78	1.85	
22	107	P	no		56.8		32.3						
23	108	T	7.94	114.4	61.4	56.8	71.0	32.5	172.3	176.0	4.66	4.04	1.12
24	109	S	8.59	123.8	55.0	61.6		70.9		172.5	4.31		
25	110	N			53.1		39.3						
26	111	D	8.47	119.8	55.1	53.0		39.3		173.9	4.51	2.76	
27	112	K			56.9		28.6		173.5		5.19		
28	113	T	9.09	123.6	62.9	56.9	69.1	28.6	173.3	173.5	4.35	4.14	1.16
29	114	R	8.58	128.8	55.1	62.9		69.1		173.3	4.56	1.68, 1.80	
30	115	V	9.05	125.2	62.3	54.8	33.2	32.6	174.0	174.0	4.79	2.05	1.03,0.98
31	116	L	9.50	126.4	52.7	62.3	44.9	33.2	175.3	173.9	5.45	1.8	
32	117	S	9.08	117.8	57.1		63.1		173.6	175.4	5.07	3.85, 3.72	
33	118	I	9.38	128.0	61.0	57.1	39.3	63.1		173.7	4.41	2.12	
34	119	Q	8.95	127.9	54.8	61.1	30.9	39.3		172.7	5.01	1.95, 2.12	

Structure determination is performed using Nuclear Overhauser Effect (NOE) spectra, to find protons that are near each other in the structure.

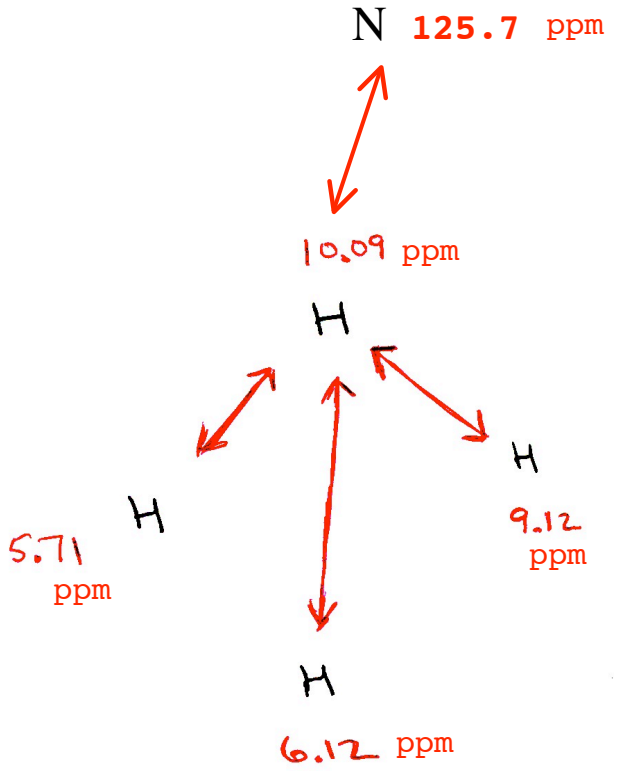
2-D NOE spectrum.

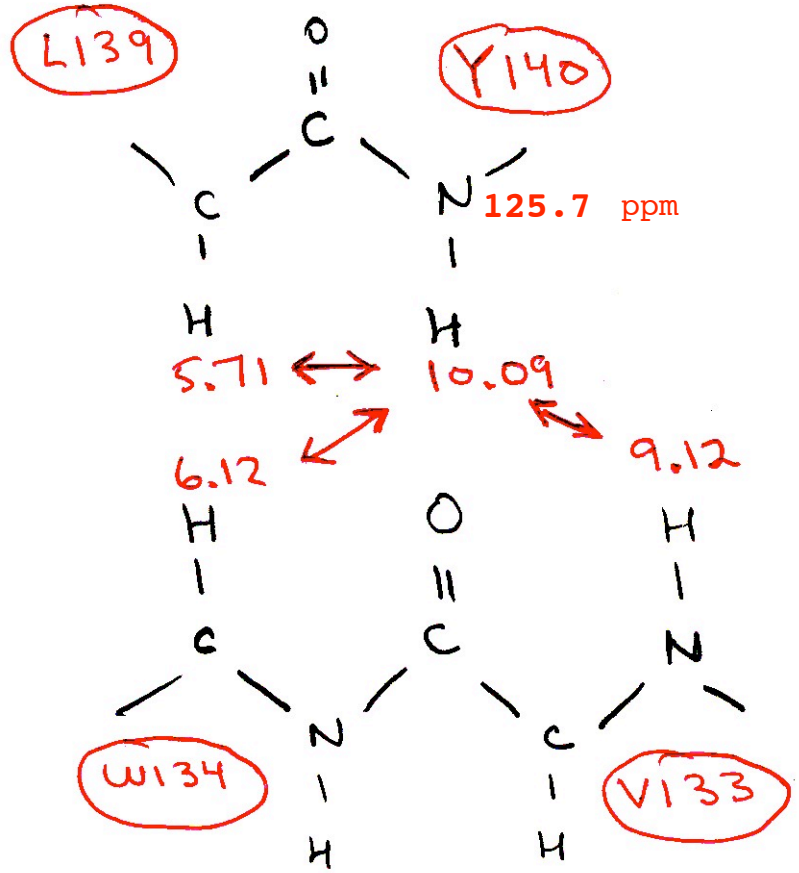


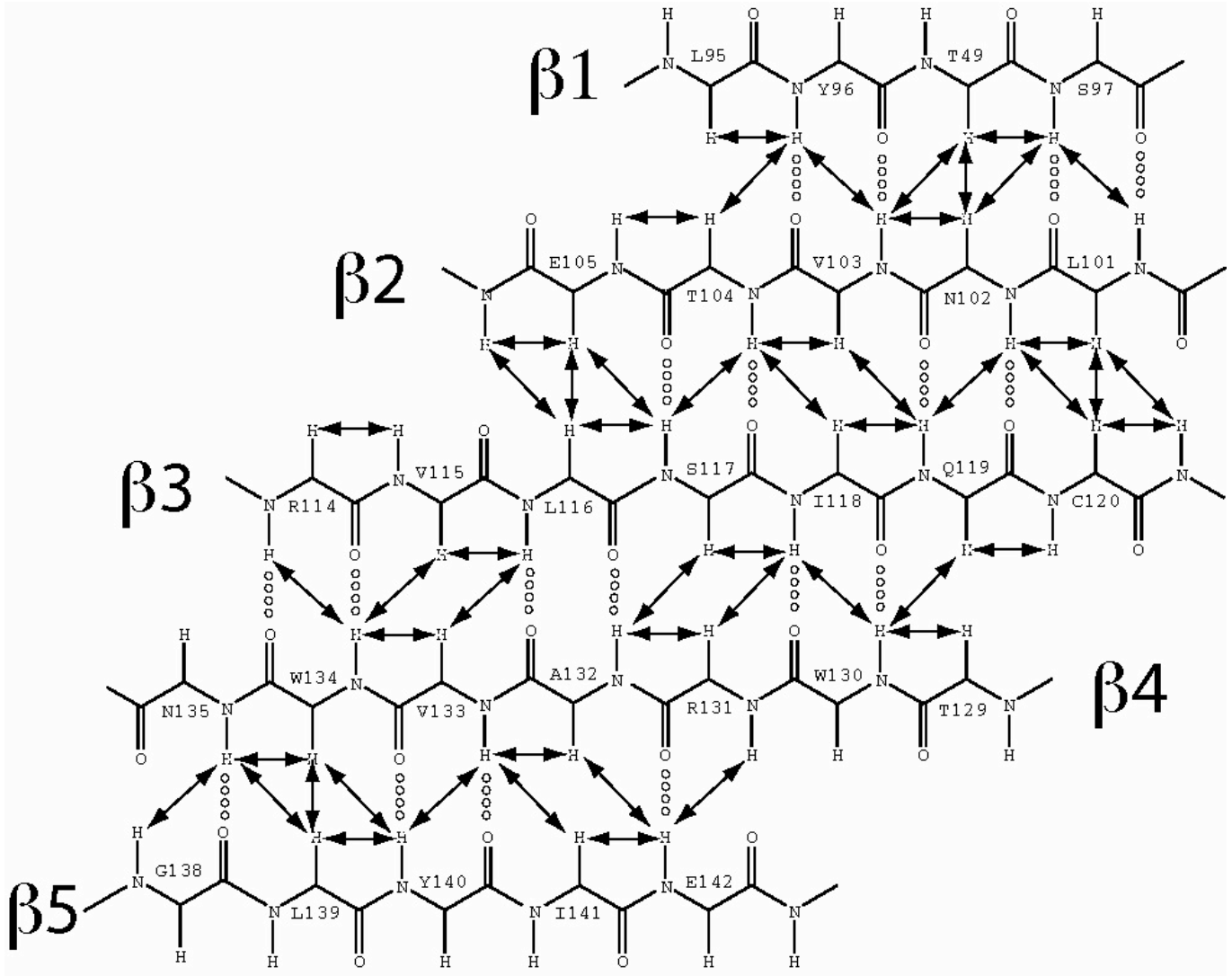


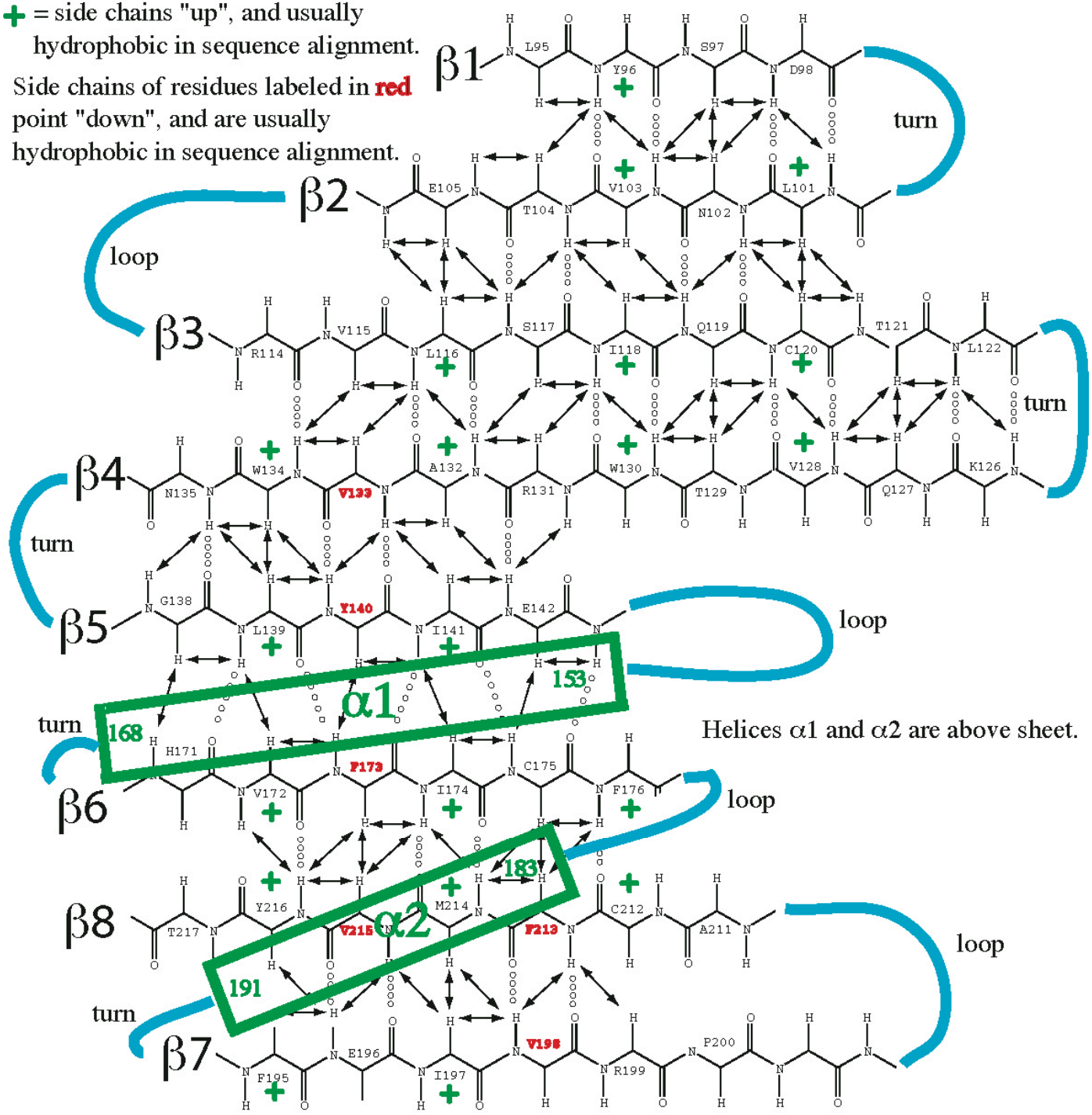
N15 = 125.7 ppm plane

3-D NOE spectrum.



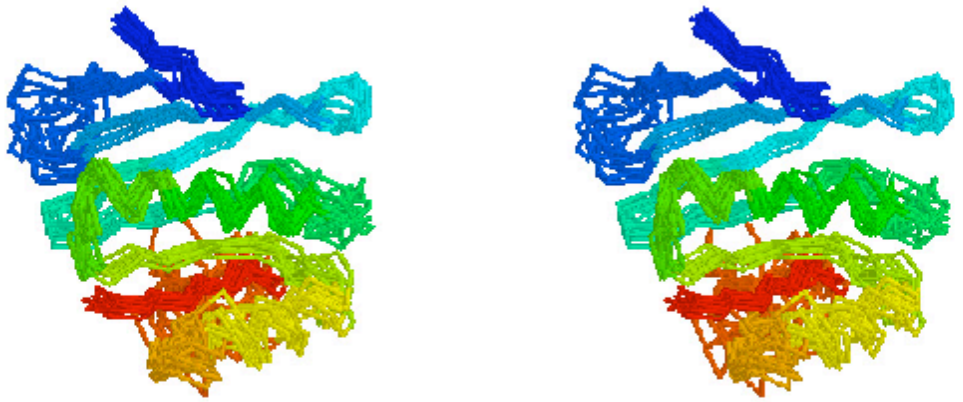

L139
Y140
125.7 ppm
5.71
10.09
6.12
9.12
W134
V133


β1
β2
β3
β4
β5
L95
T49
Y96
S97
E105
V103
L101
T104
N102
V115
S117
Q119
R114
L116
I118
C120
W134
A132
W130
N135
V133
R131
T129
G138
Y140
E142
L139
I141

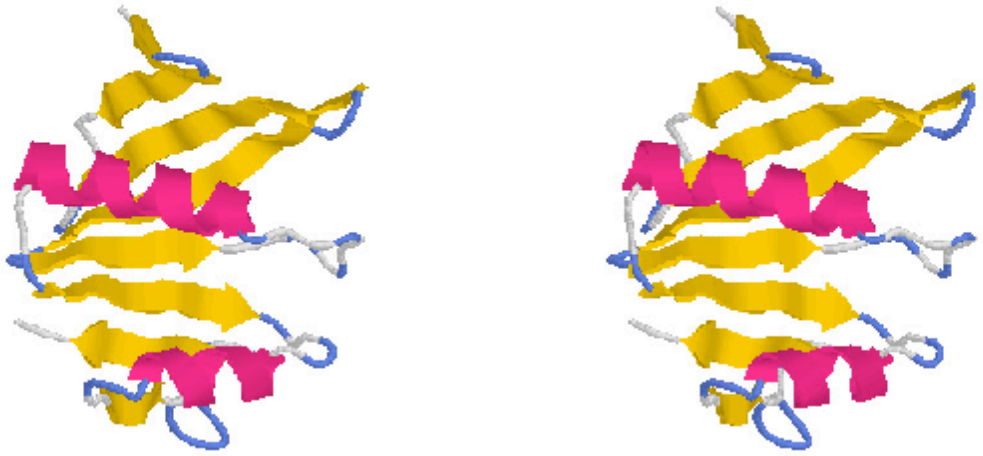

+ = side chains "up", and usually hydrophobic in sequence alignment.
Side chains of residues labeled in red point "down", and are usually hydrophobic in sequence alignment.
β1
β2
β3
β4
β5
β6
β7
β8
α1
α2
loop
turn
Helices α1 and α2 are above sheet.

Results after simulated annealing, using 1400 NMR-derived restraints:

Overlay 12 structures,
residues 94 - 218.



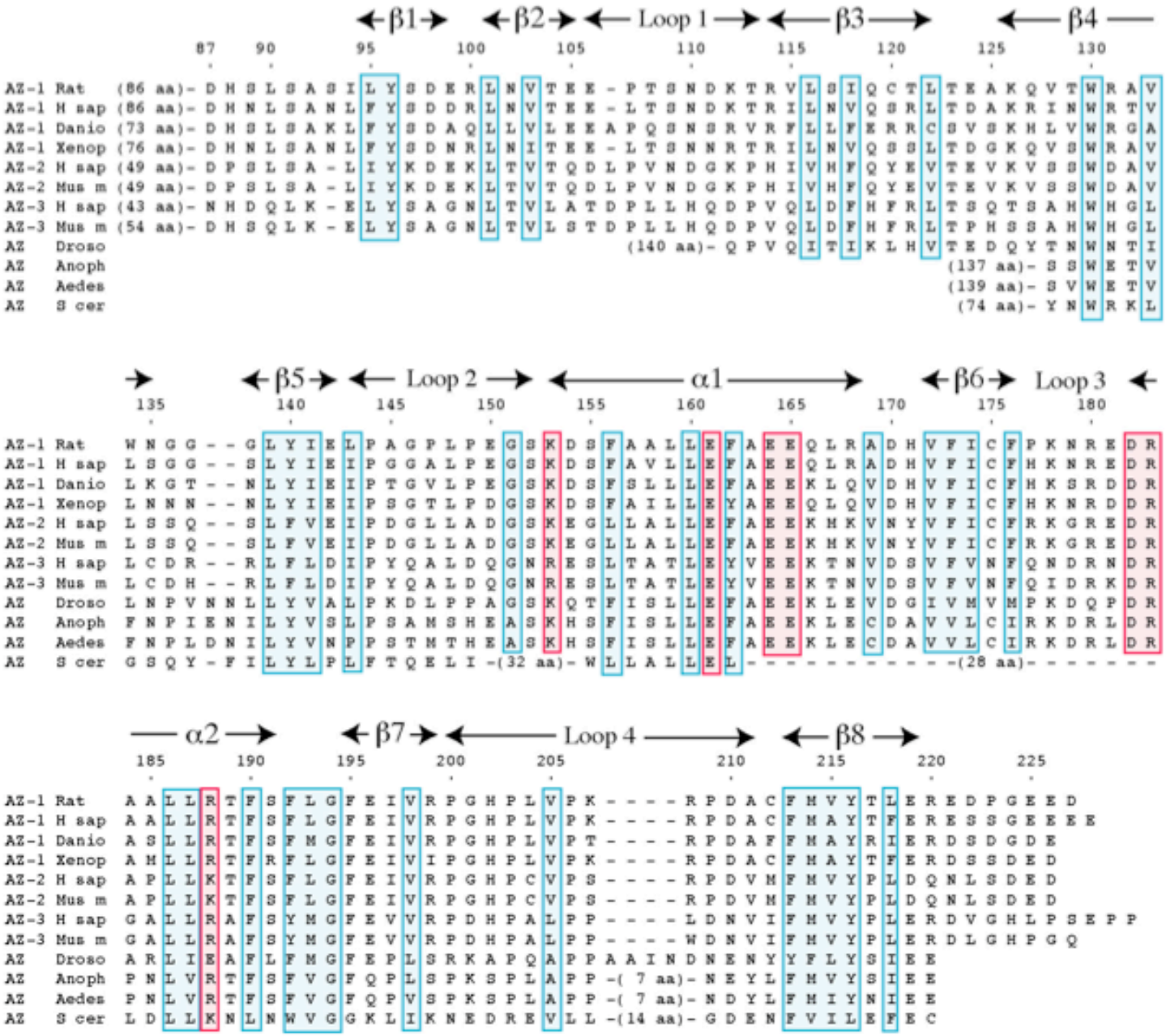
One structure,
residues 94 - 218.

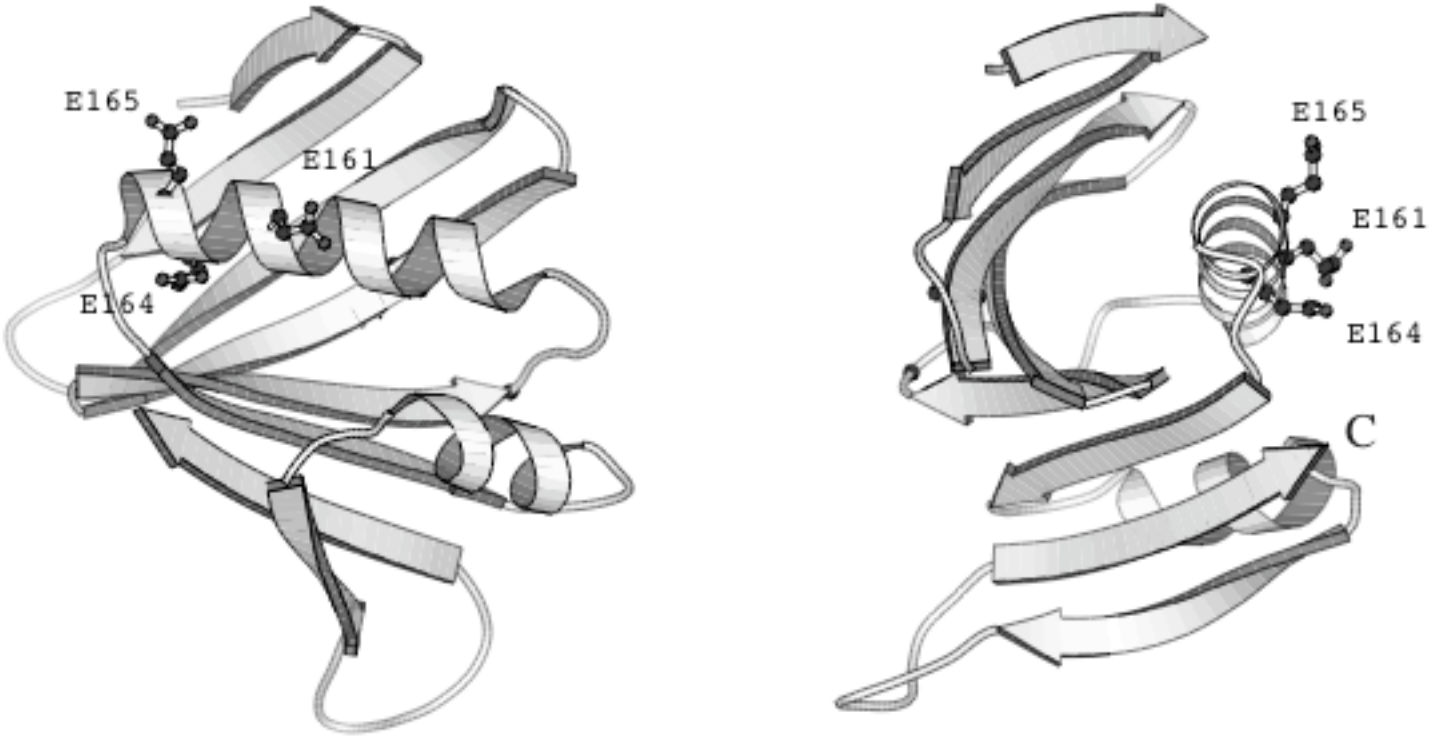


Once the structure of Antizyme is known, how do you identify candidates for which parts of the protein are functionally important?

(In this case, "functionally important" may mean binding and inhibiting the enzyme Ornithine Decarboxylase).

Blue = conserved, inside protein ; Red = conserved, on surface of protein.





Structure, plus locations of conserved amino acids, leads to hypotheses regarding which a.a. may be directly involved in binding to ornithine decarboxylase.