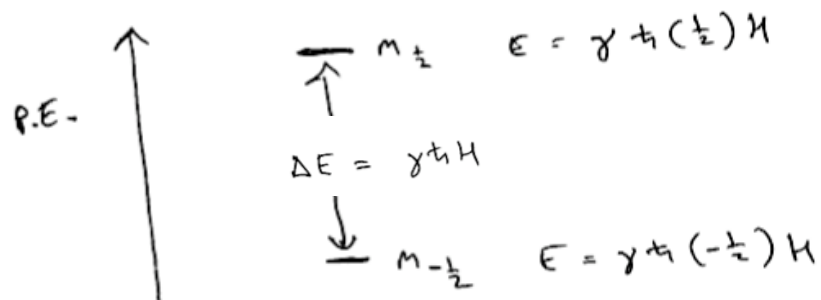


Today's planned topics:

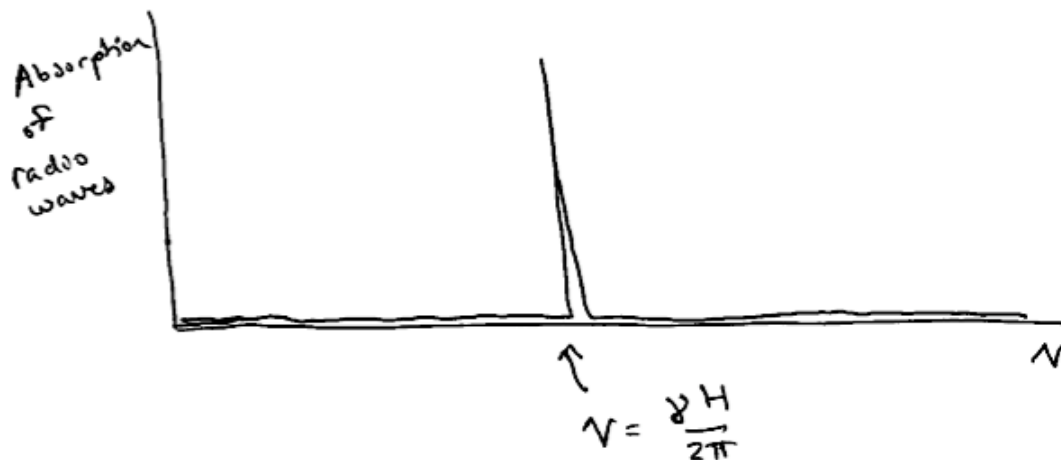
- 1) Acquiring an NMR spectrum: Fourier transforms & FT-NMR.
- 2) Solving the "spectrum assignment problem".

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:

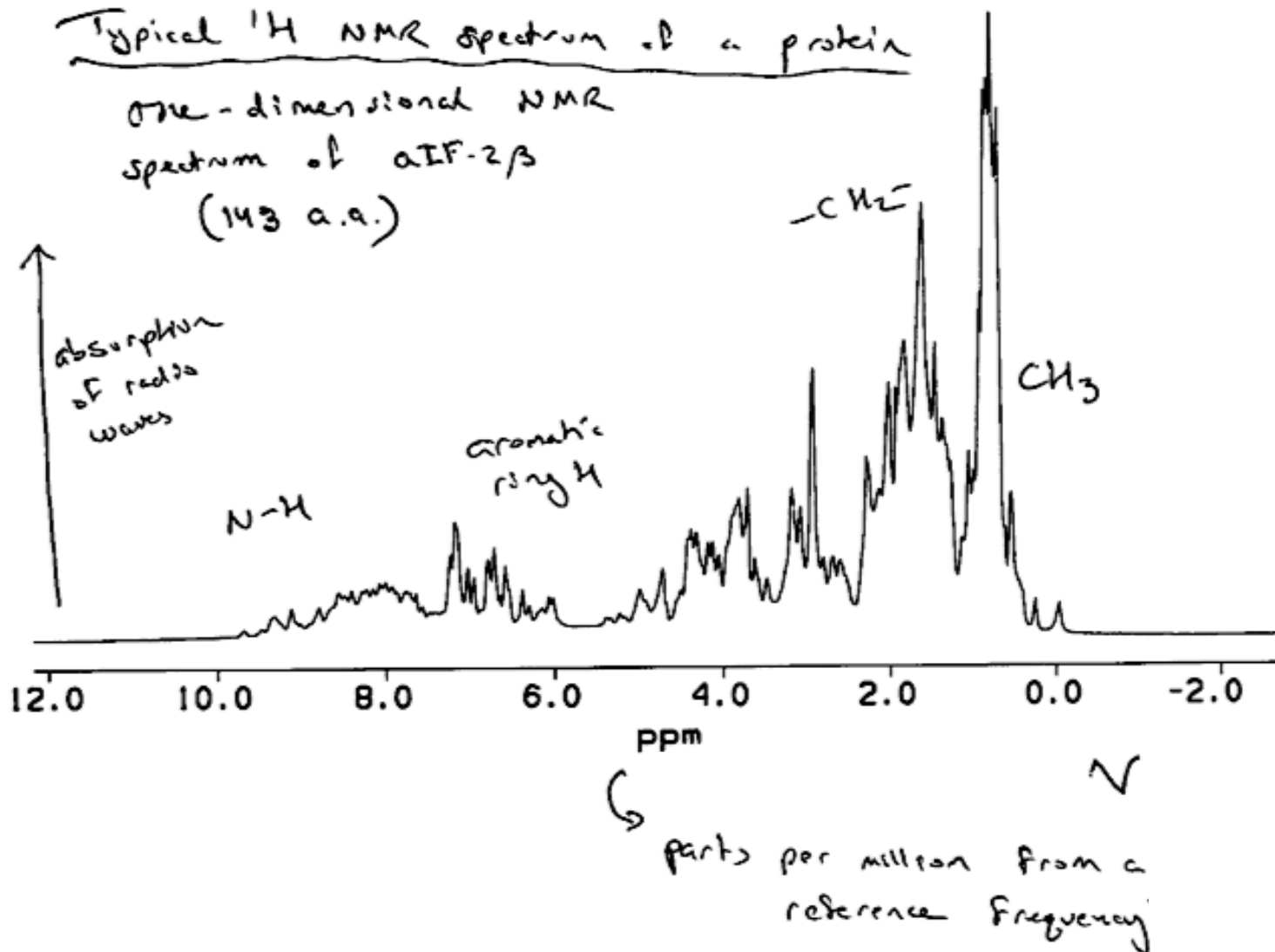


You could irradiate sample with radio waves, and there will be absorption when the energy of the radio wave matches the energy difference between the two allowed energy states. You would need to scan the frequency spectrum, one frequency at a time:



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

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

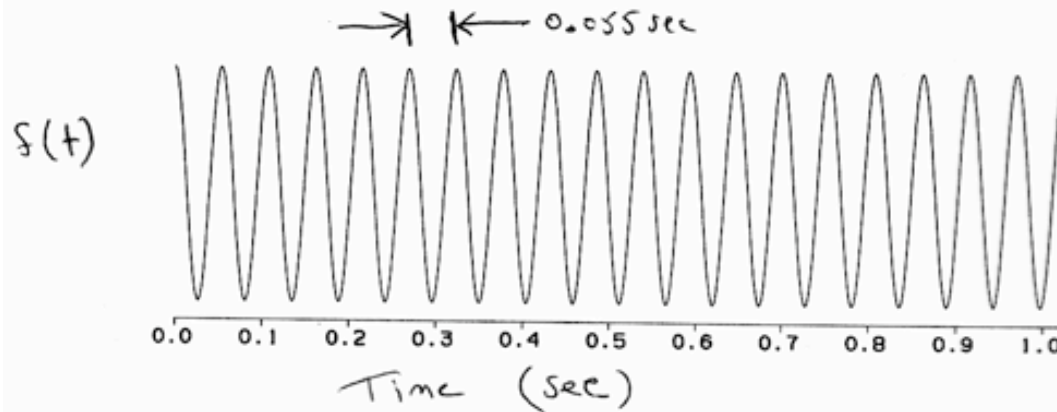
$f(\nu)$ = frequency spectrum.

$f(t)$ = time varying signal.

Examples of Fourier Transforms:

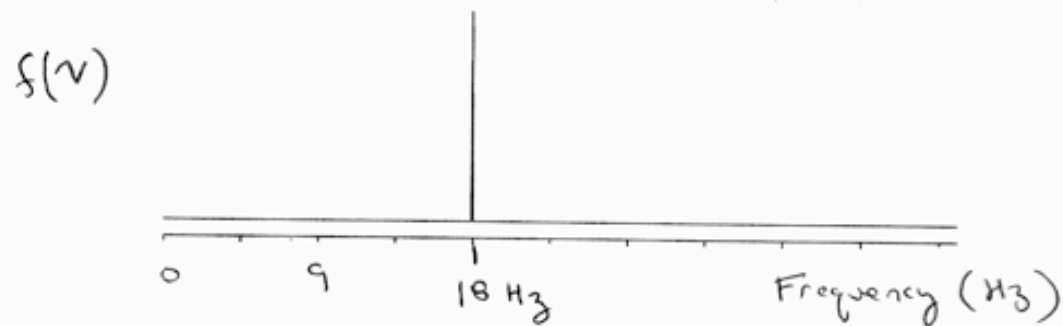
Fourier Transform of time-varying signal:

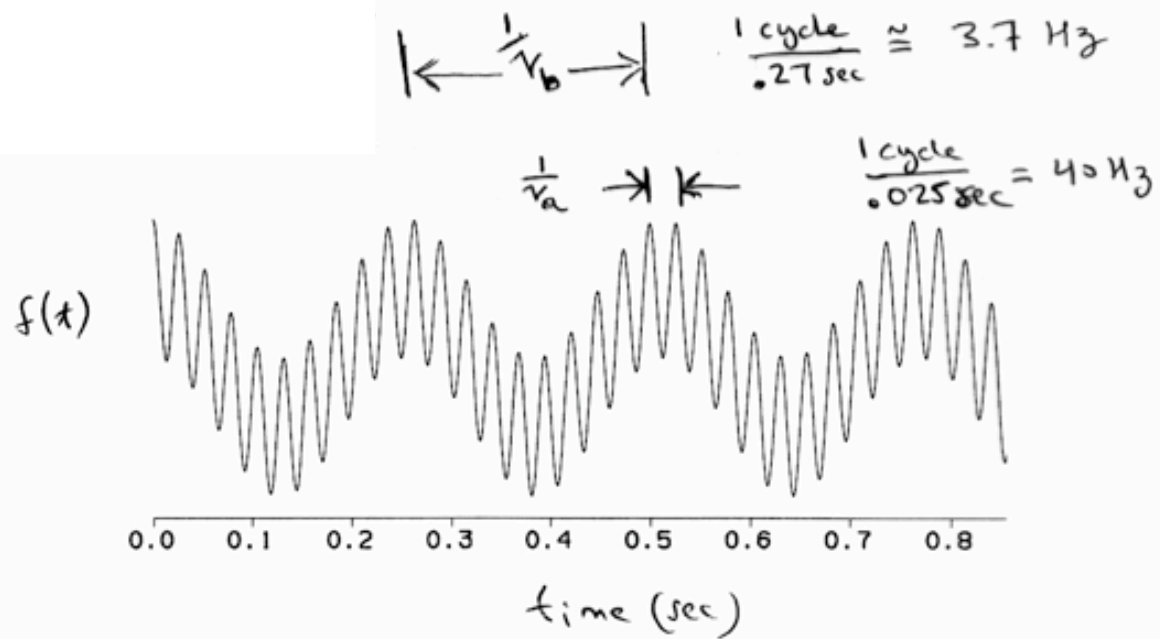
$$\text{frequency} = \frac{1 \text{ cycle}}{0.055 \text{ sec}} \approx 18 \text{ Hz}$$



↓ F.T.

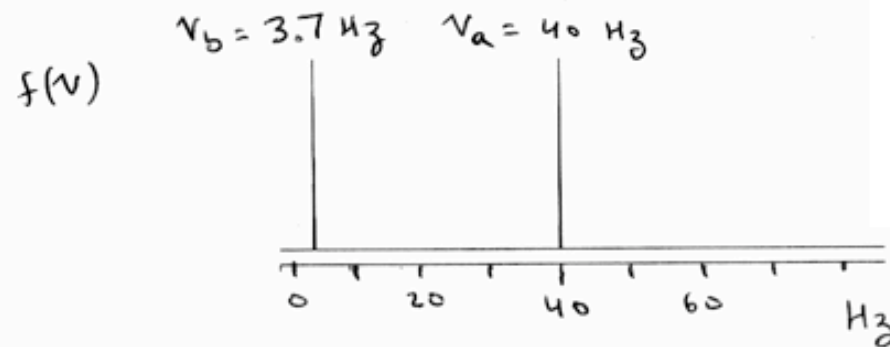
Converts the time-varying signal into its frequency spectrum.





\Downarrow F.T.

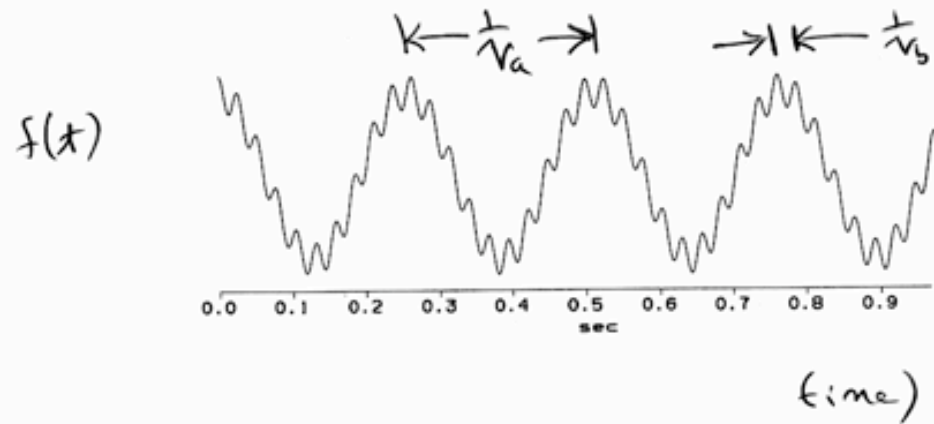
Converts the time-varying signal into its frequency spectrum.



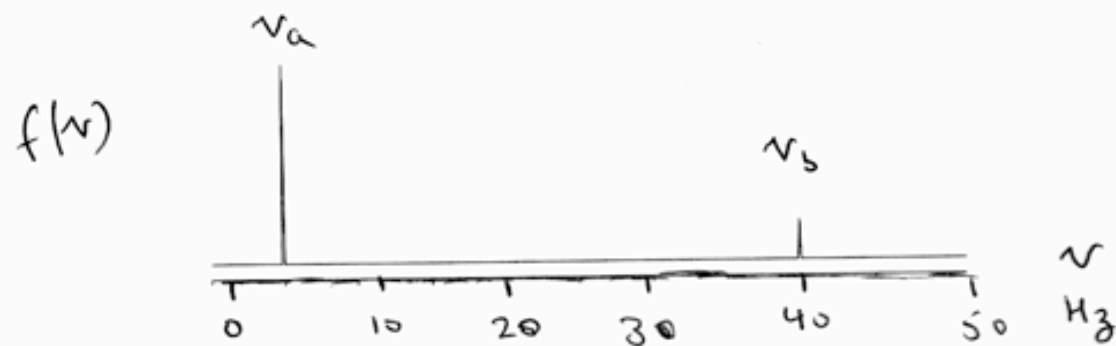
Two frequencies with unequal amplitudes:

small amplitude frequency $\approx 40 \text{ Hz} = \nu_b$

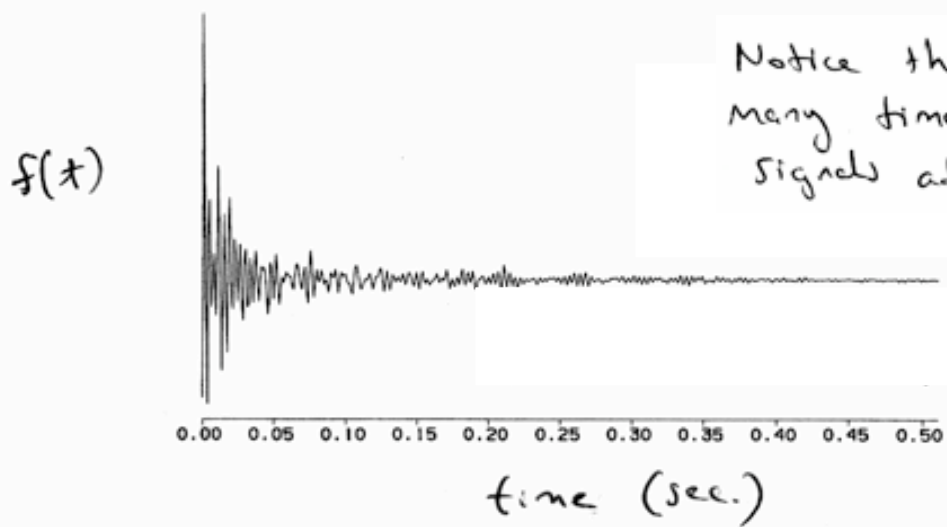
large amplitude frequency $= \nu_a \approx 3.9 \text{ Hz}$



\Downarrow F.O.T.



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

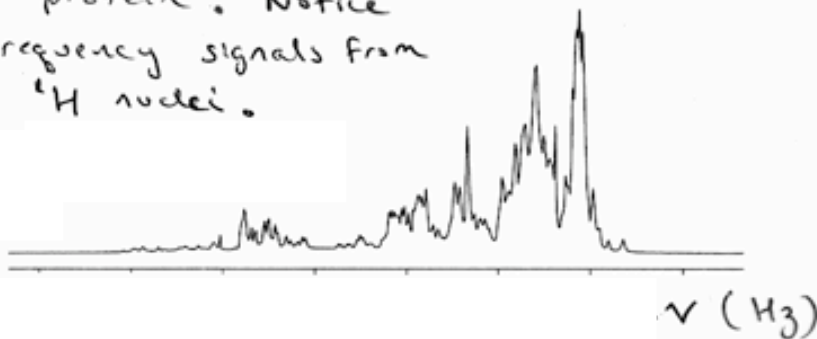


Notice there are many time-varying signals added together.

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.

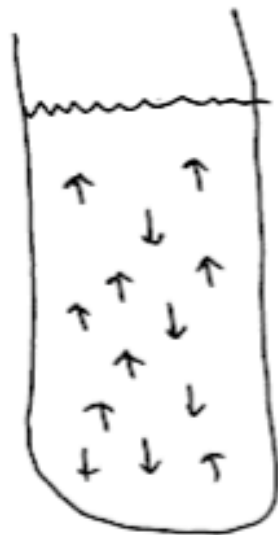


For FT-NMR, it is necessary to identify a time-varying signal that can be measured and Fourier transformed to yield the NMR frequency spectrum.

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 \uparrow

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

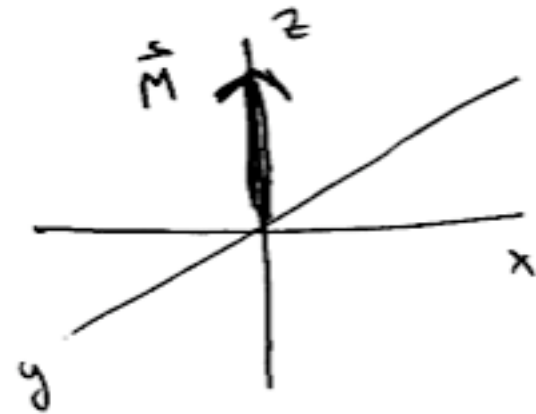
Slight excess of nuclei in
the lower energy state
(by Boltzmann distribution)

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

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

↑ bulk magnetization of sample ↑ sum ↑ all individual magnetic dipoles.

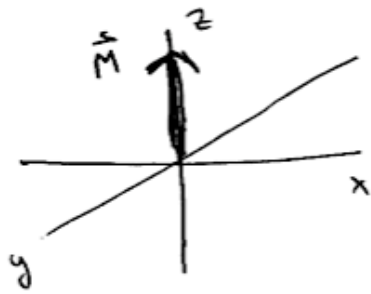
At equilibrium, \vec{M} is parallel to the magnetic field \vec{H} (\vec{H} is always considered to define the z-direction).



at equilibrium

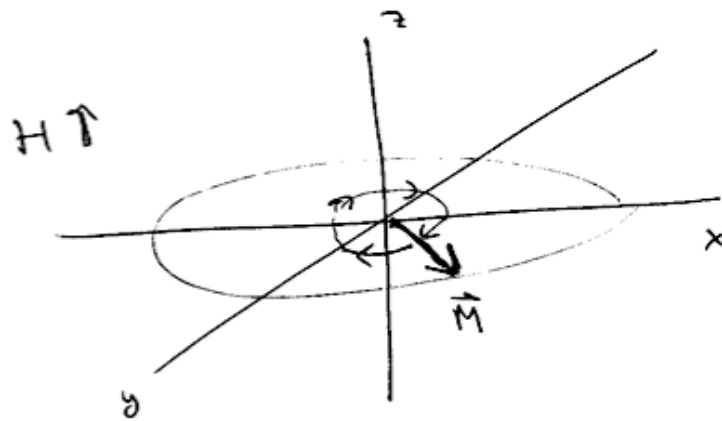
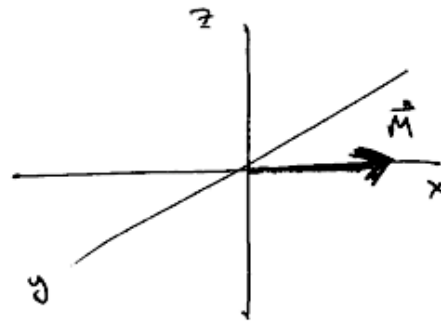
If \vec{M} is not at equilibrium (not parallel to \vec{H}) it will experience a force that causes \vec{M} to precess around \vec{H} with a frequency ν

$$\text{where } \nu = \gamma H / 2\pi$$



at equilibrium

pulsed magnetic field
"90" pulse

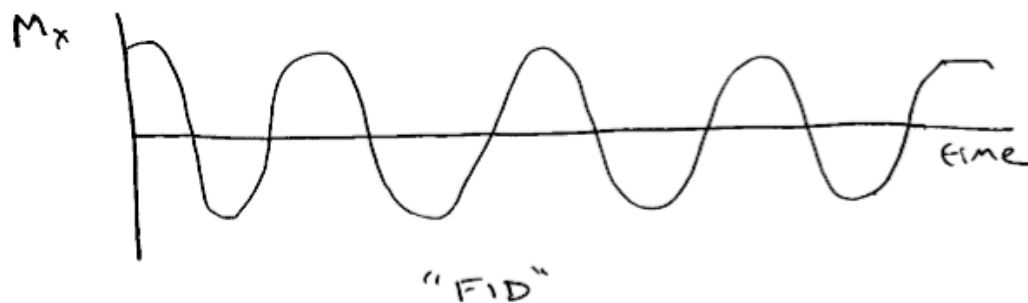
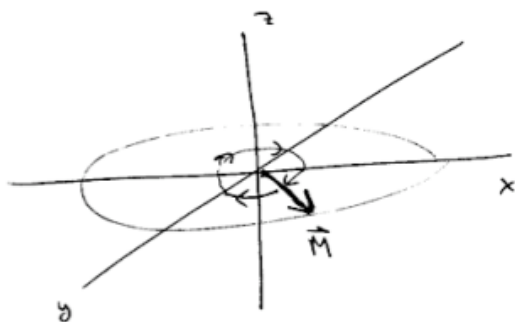


\vec{M} precesses about
 \vec{H} at frequency ν

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

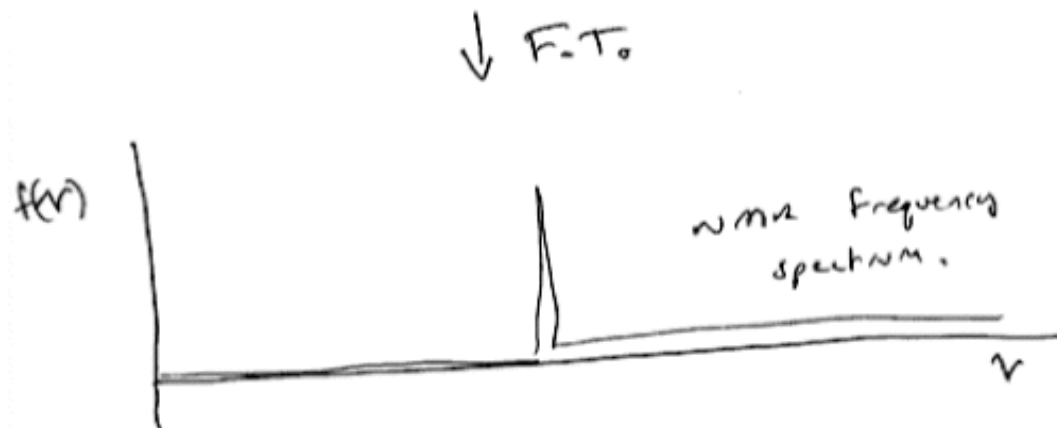
What is recorded in an FT-NMR experiment:

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



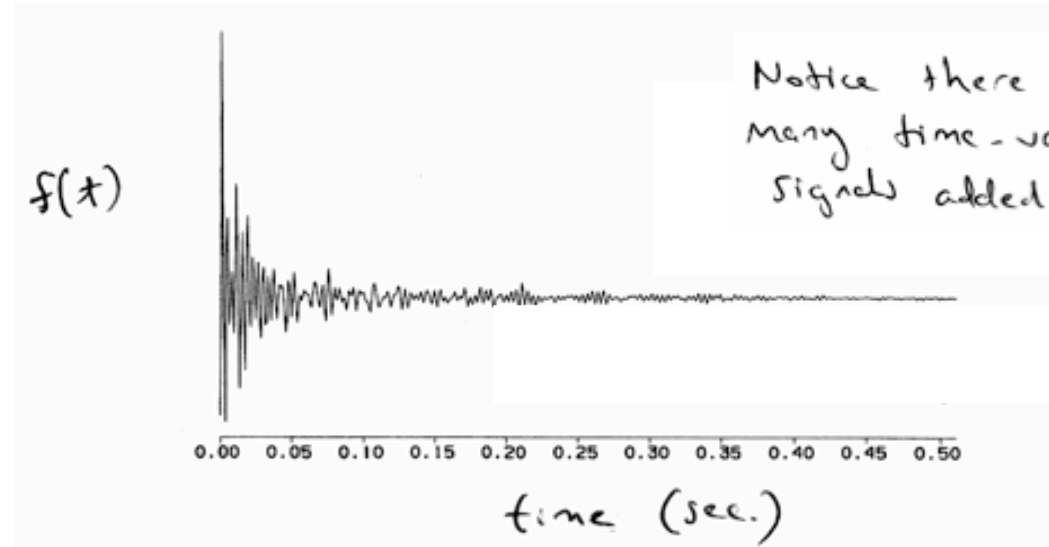
This is called the Free Induction Decay (or "FID").

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



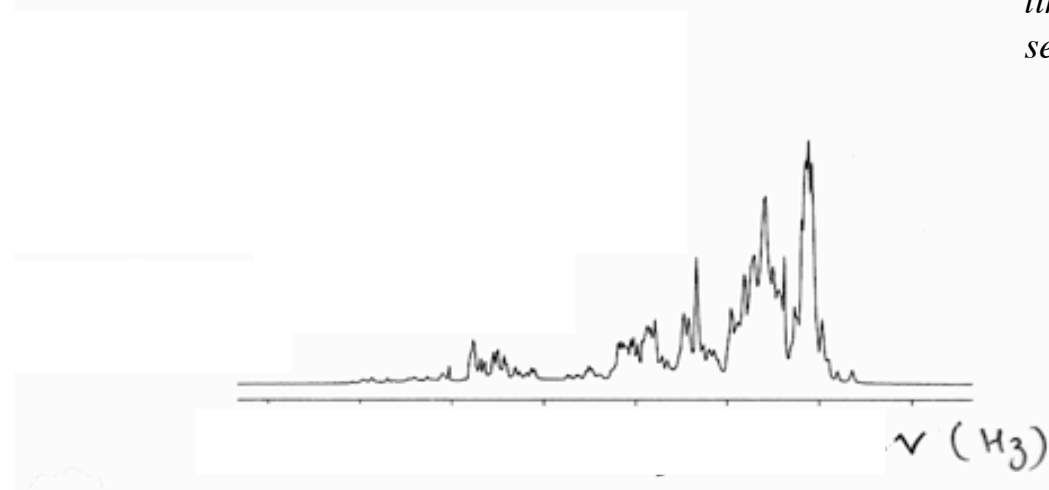
Typical time-varying signal from a protein, where the different nuclei have many different NMR frequencies:

90 degree pulse, then
record $f(t)$:



Notice there are
many time-varying
signals added together.

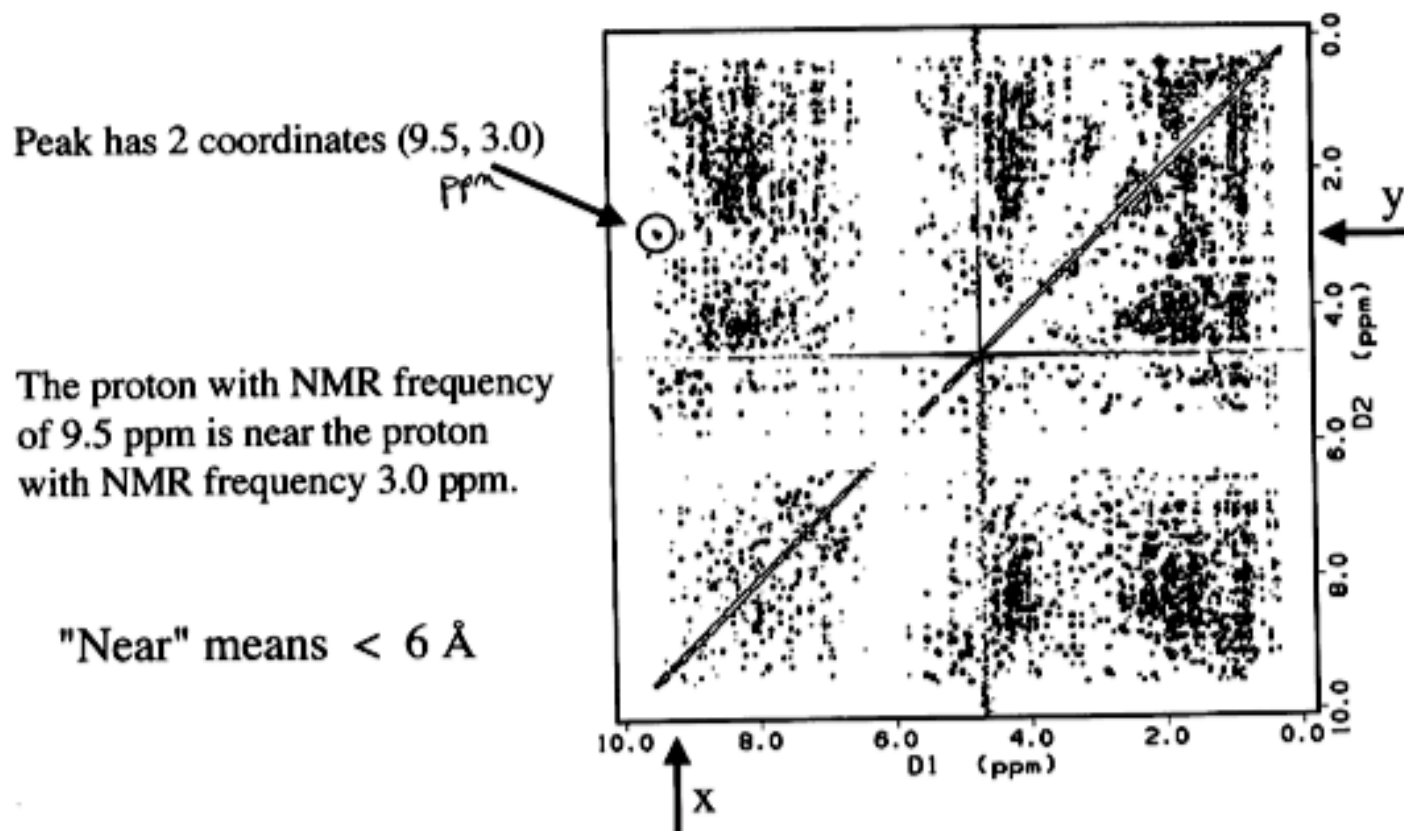
⇓ F.T.



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



Today's topics:

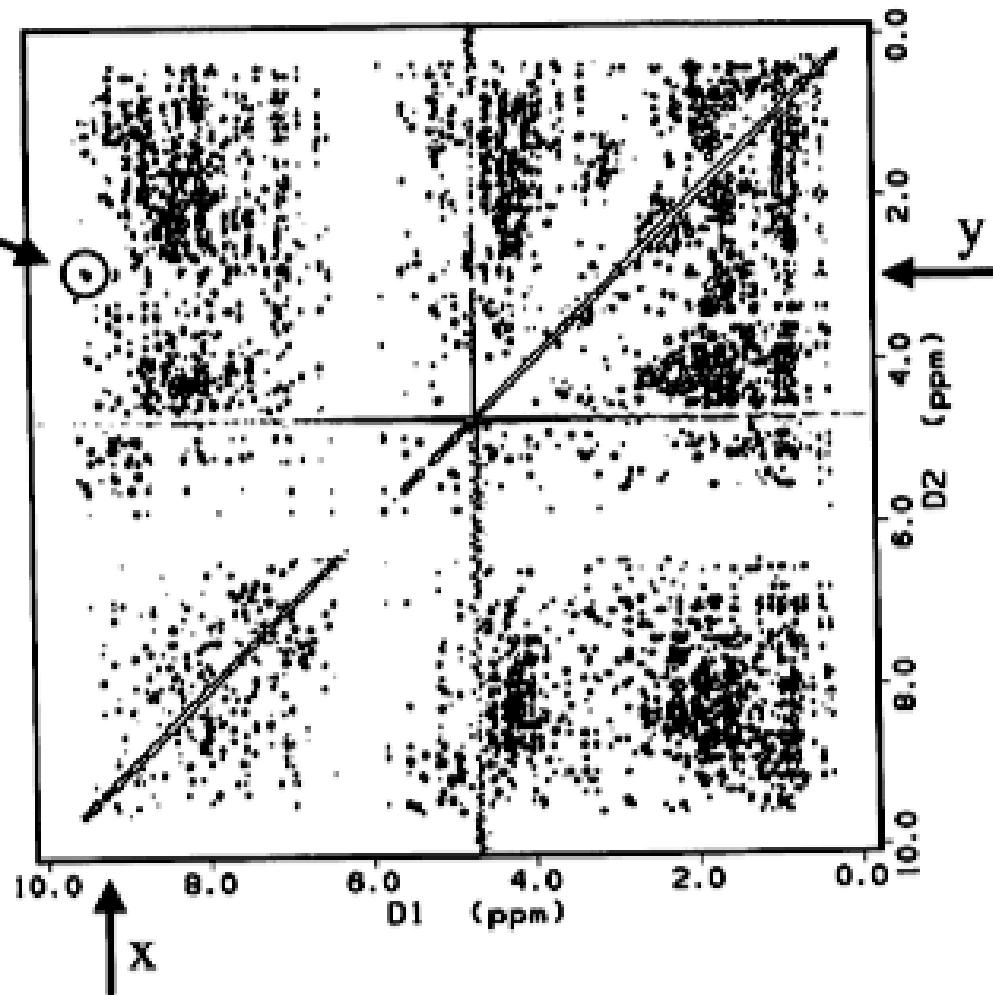
- 1) Acquiring an NMR spectrum: Fourier transforms and FT-NMR.
- 2) Solving the NMR spectrum assignment problem.

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)
ppm

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

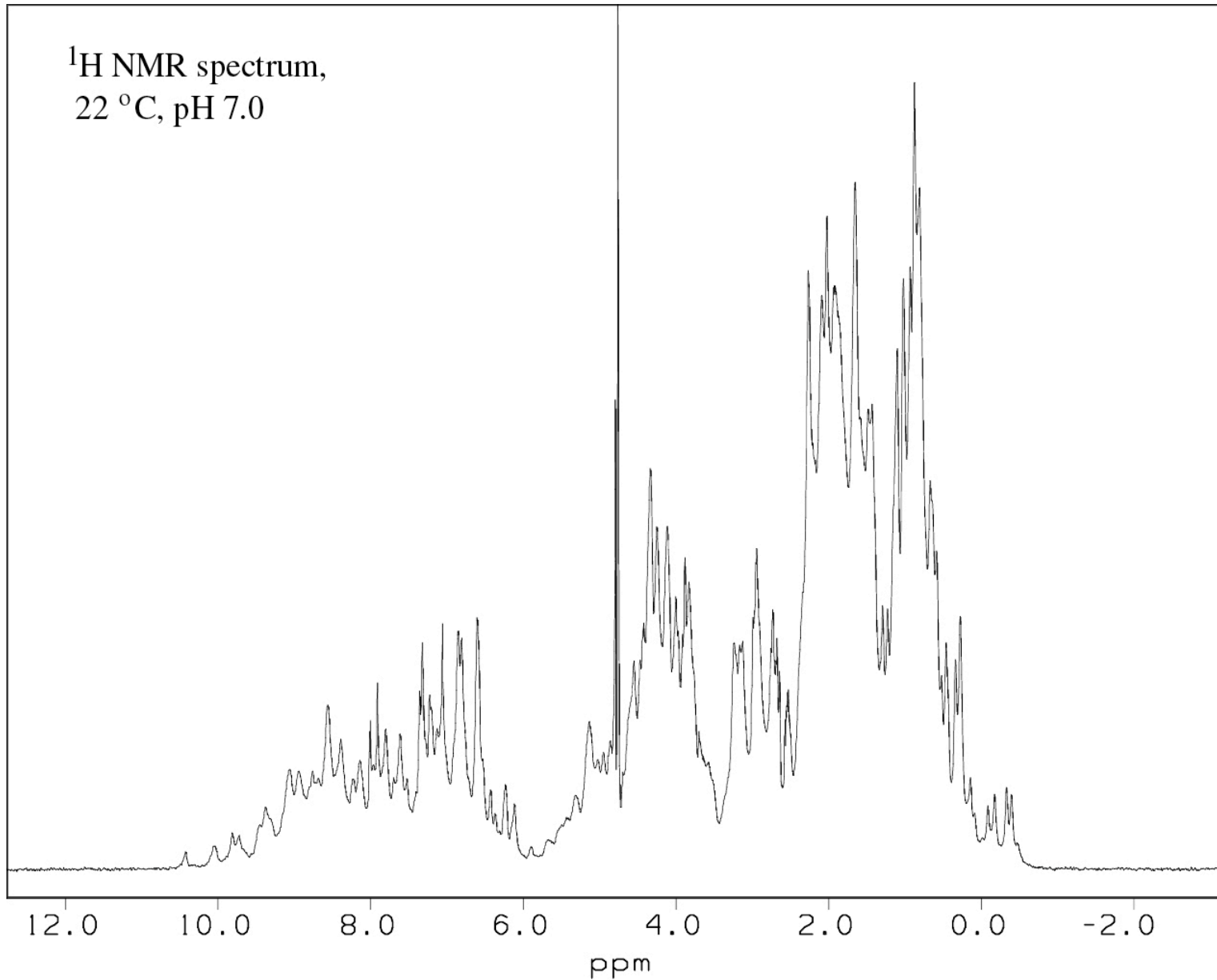
"Near" means $< 6 \text{ \AA}$



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

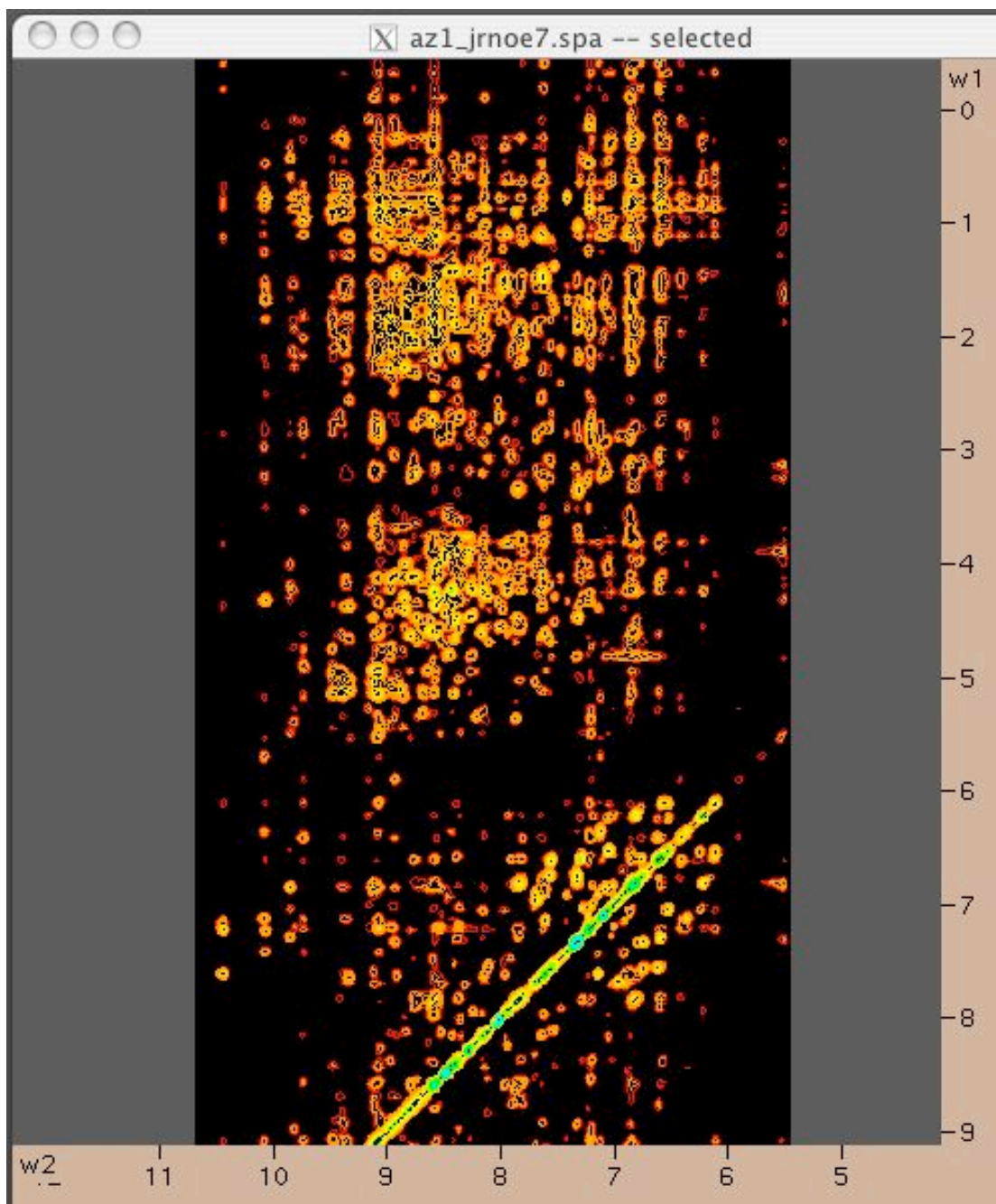
NMR analysis of a protein called "Antizyme",
an inhibitor of the enzyme ornithine decarboxylase (ODC).
Antizyme binds and inhibits ODC, and targets ODC for
degradation.

^1H NMR spectrum,
22 °C, pH 7.0



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

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

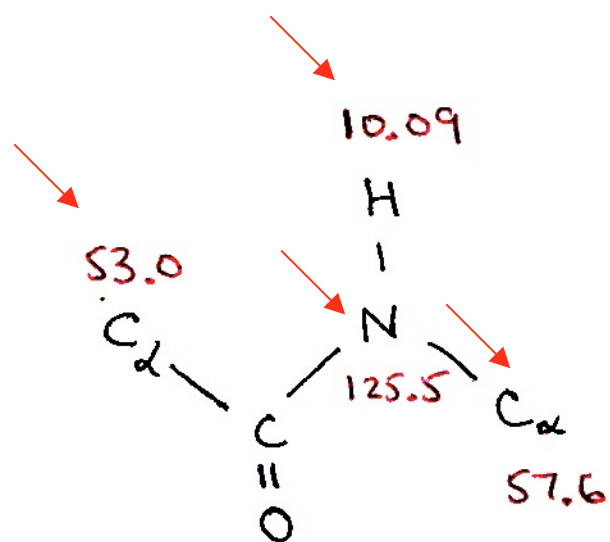


First step of NMR analysis of Antizyme is spectrum assignment:

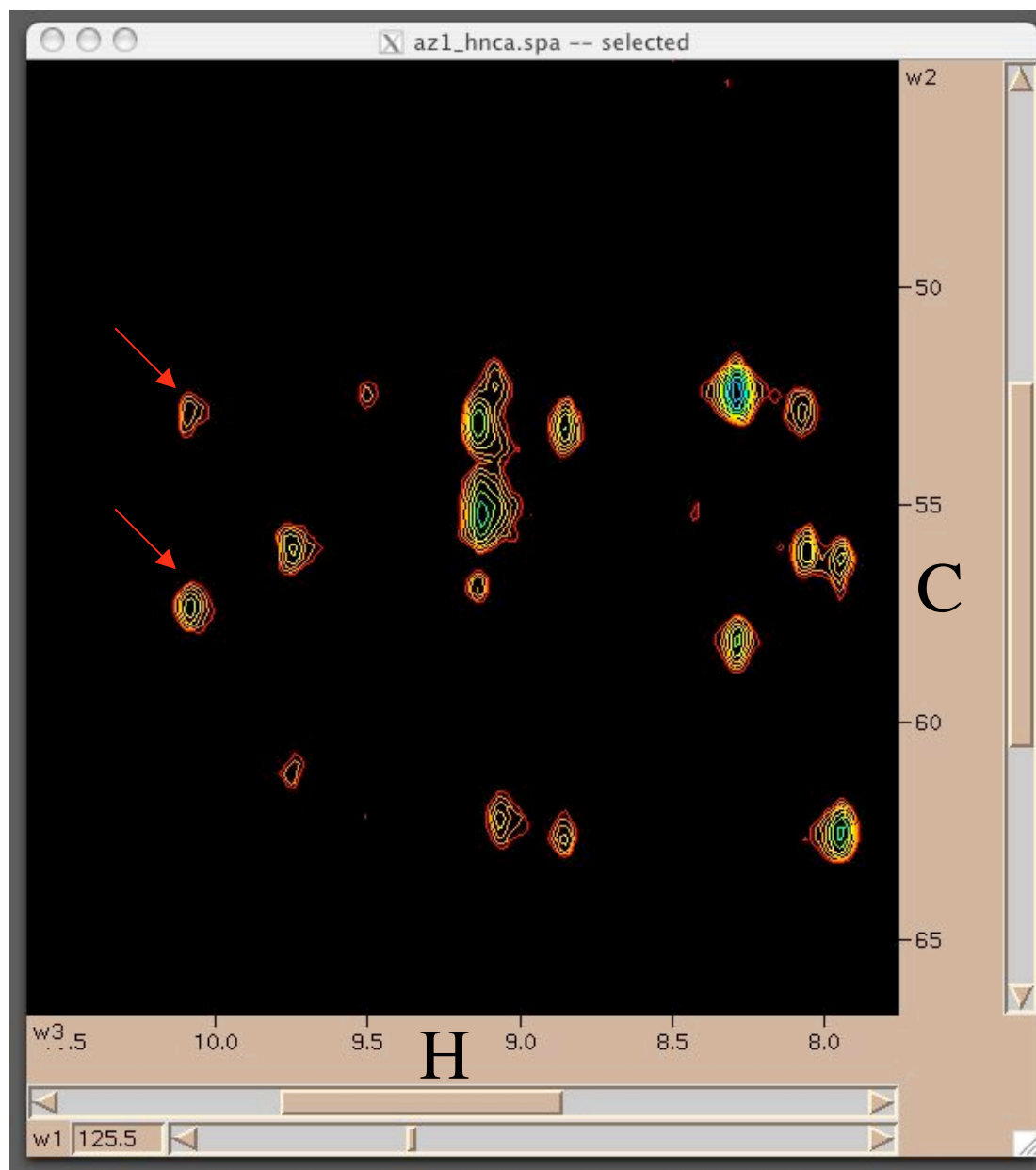
(Identifying the NMR frequencies of as many specific nuclei in the protein as possible).

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

3-D “HNCA” spectrum.

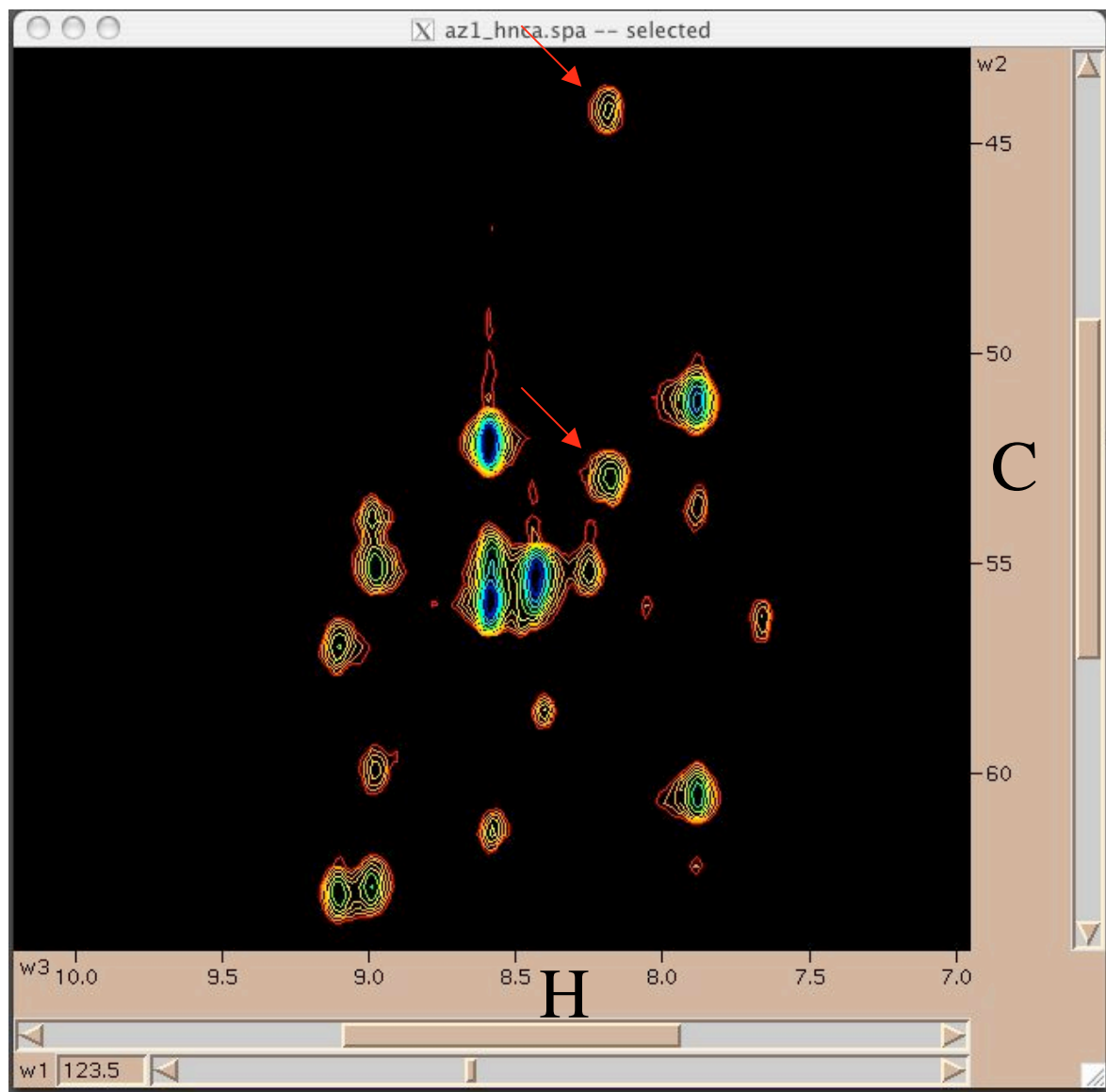
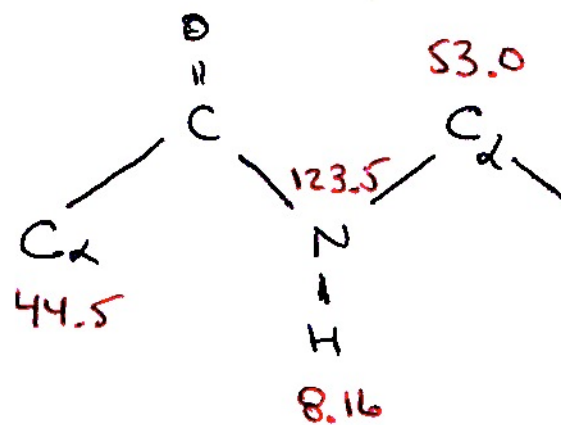


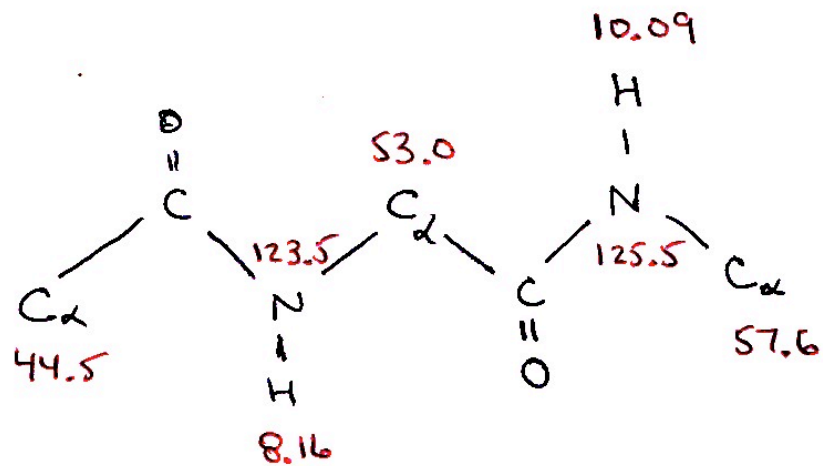
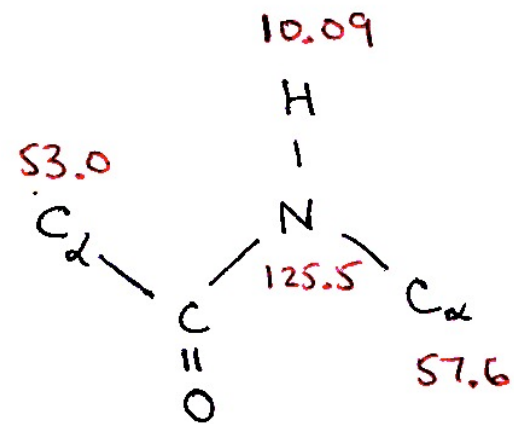
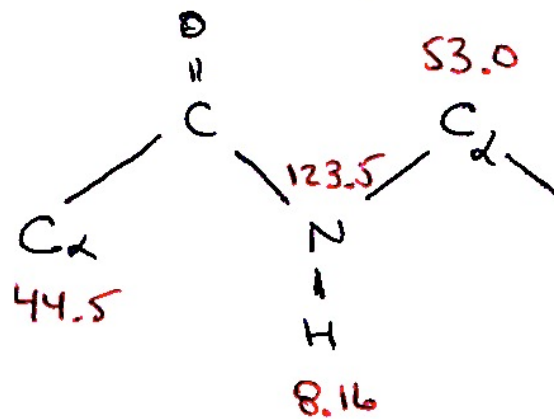
3-D spectrum, with H on first axis, $^{13}\text{C}_\alpha$ on 2nd axis, and ^{15}N on 3rd axis.



N15 = 125.5 ppm plane

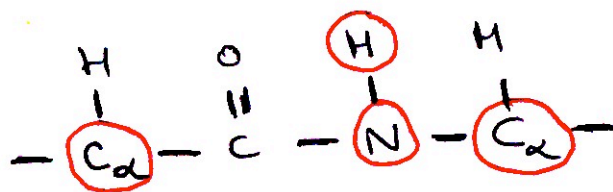
N15 = 125.5 ppm plane



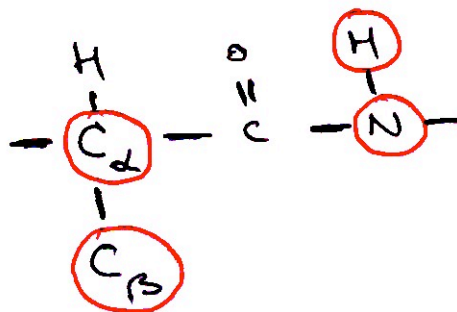


Triple Resonance NMR of Antizyme

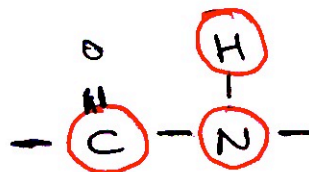
HNCA



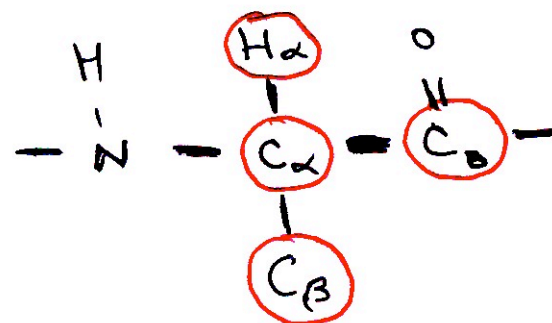
HN(CO)CACB



HNCO



HACACBCO

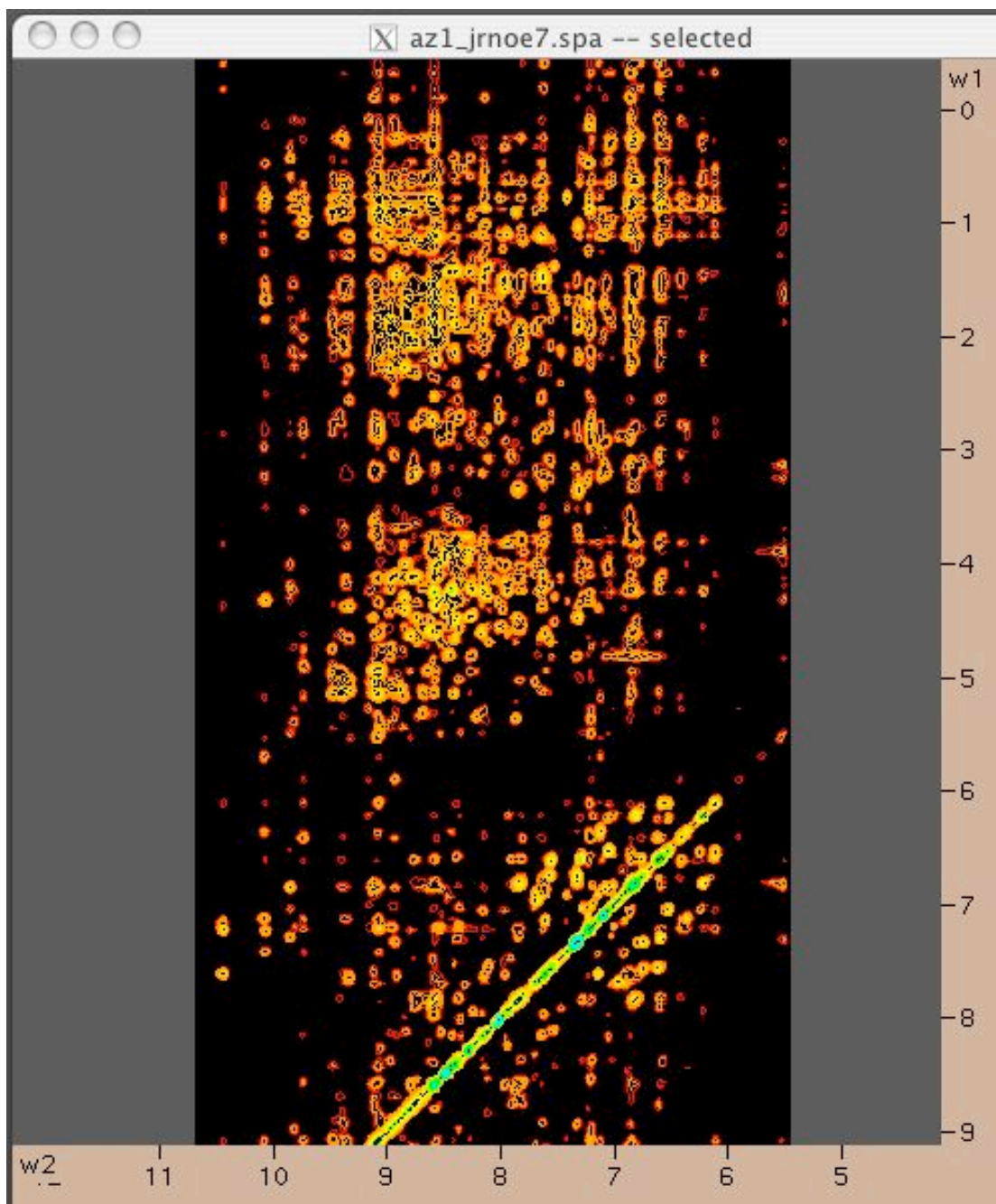


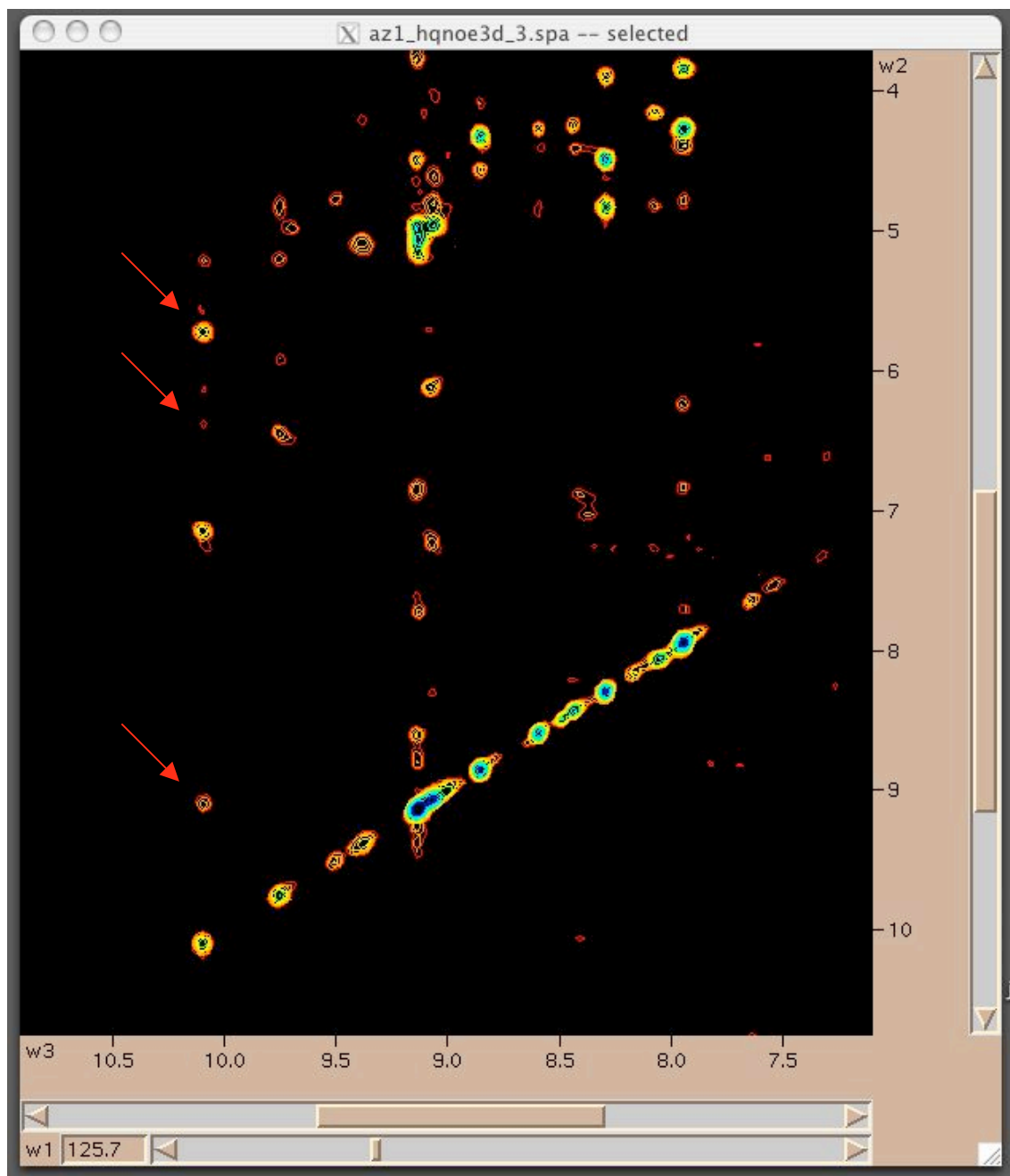
Eventually generate a table of the NMR frequencies of (almost) all the nuclei in the protein:

	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.

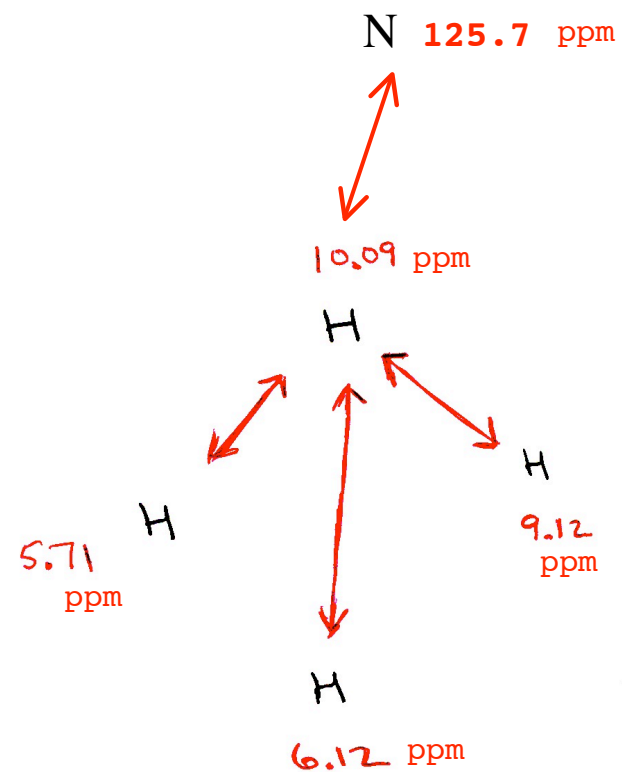
2-D NOE spectrum.

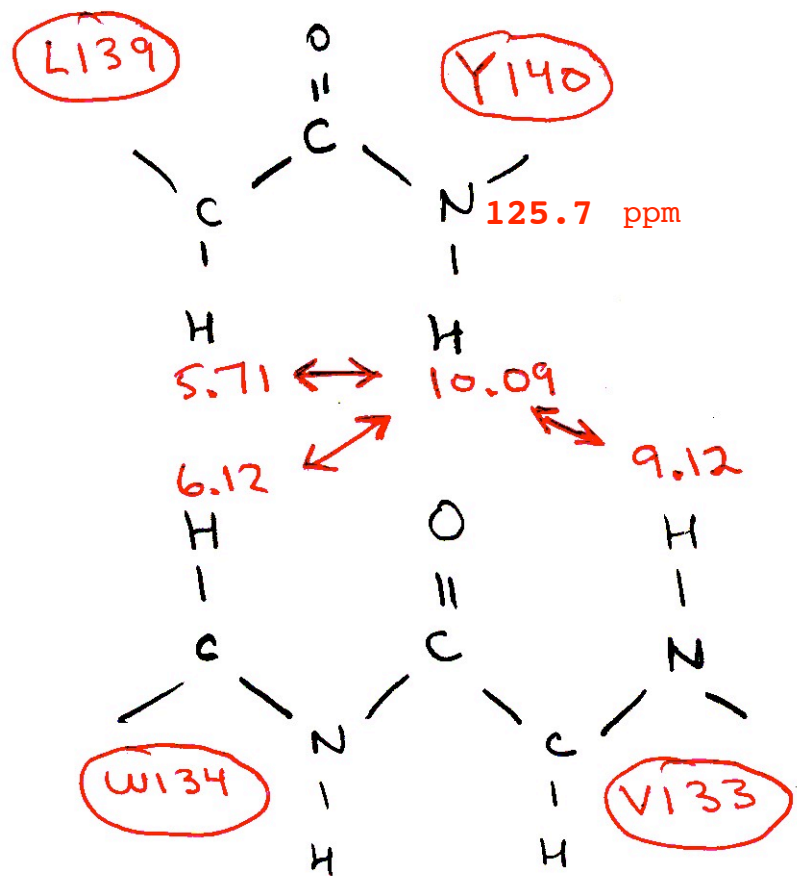


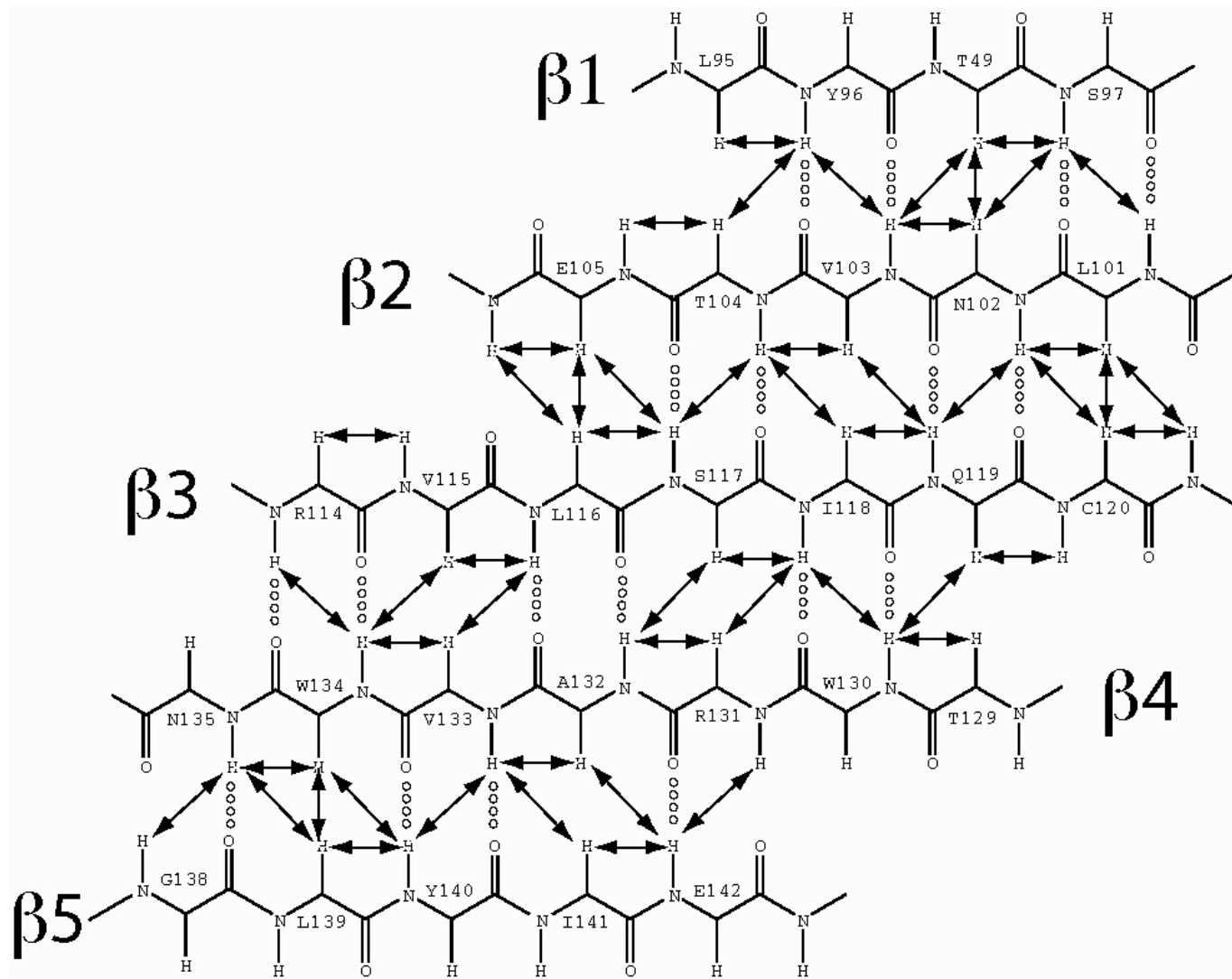


N15 = 125.7 ppm plane

3-D NOE spectrum.

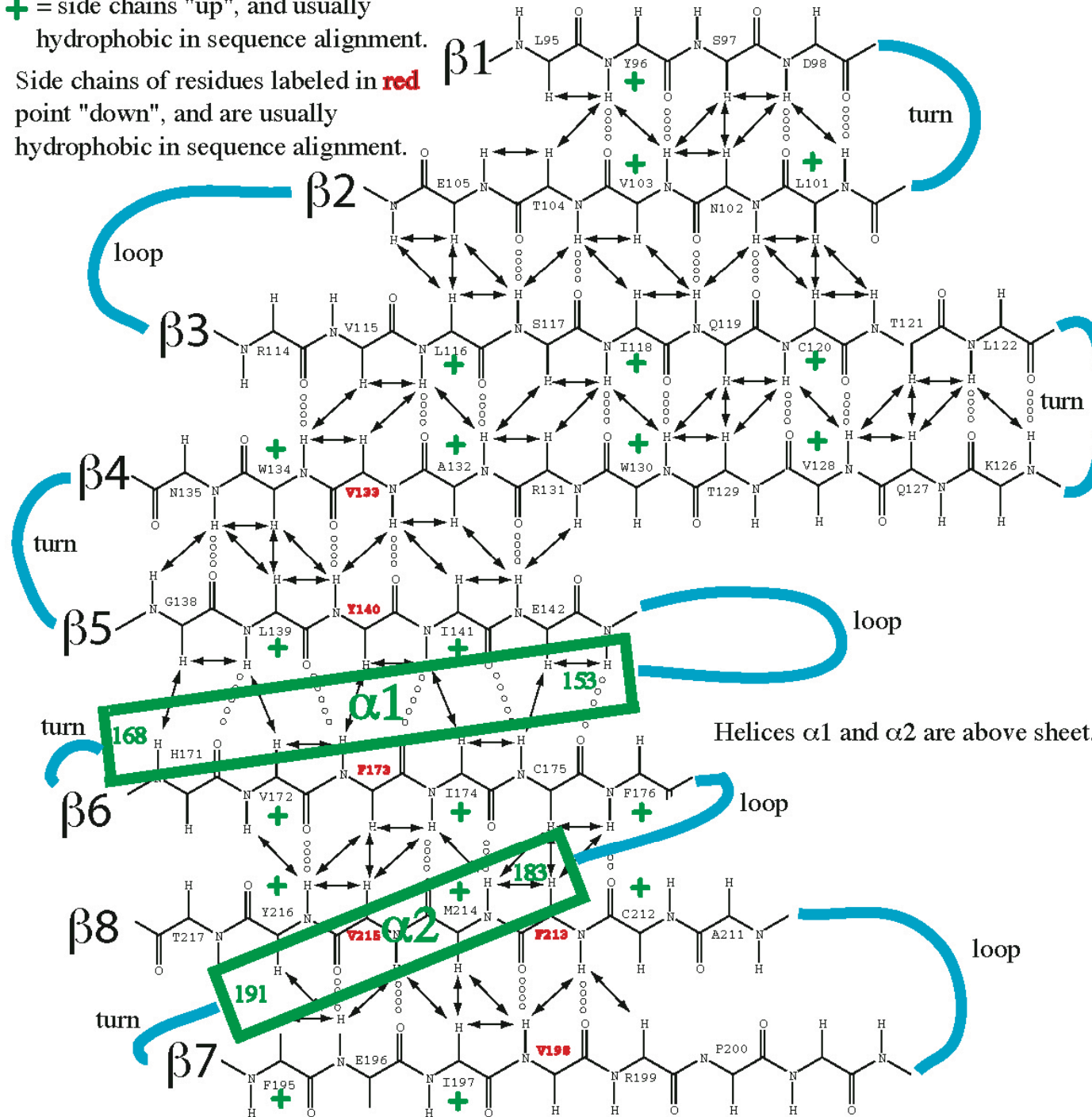






+ = side chains "up", and usually hydrophobic in sequence alignment.

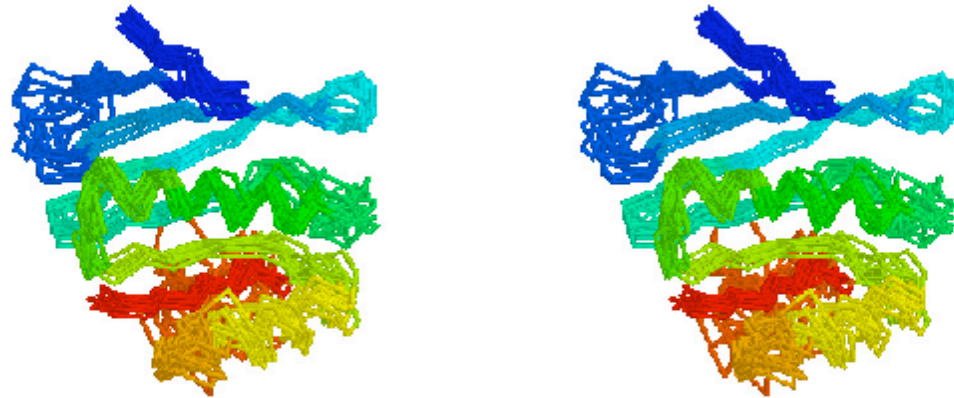
Side chains of residues labeled in red point "down", and are usually hydrophobic in sequence alignment.



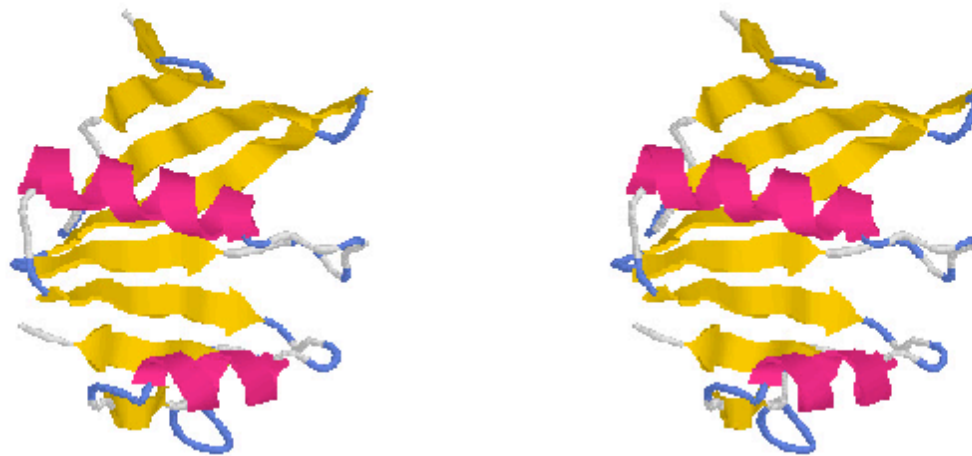
Helices α1 and α2 are above sheet.

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?

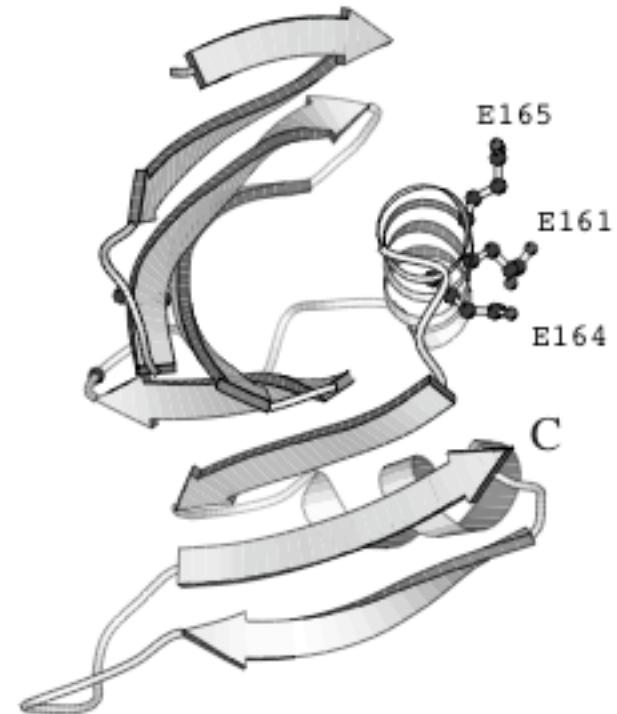
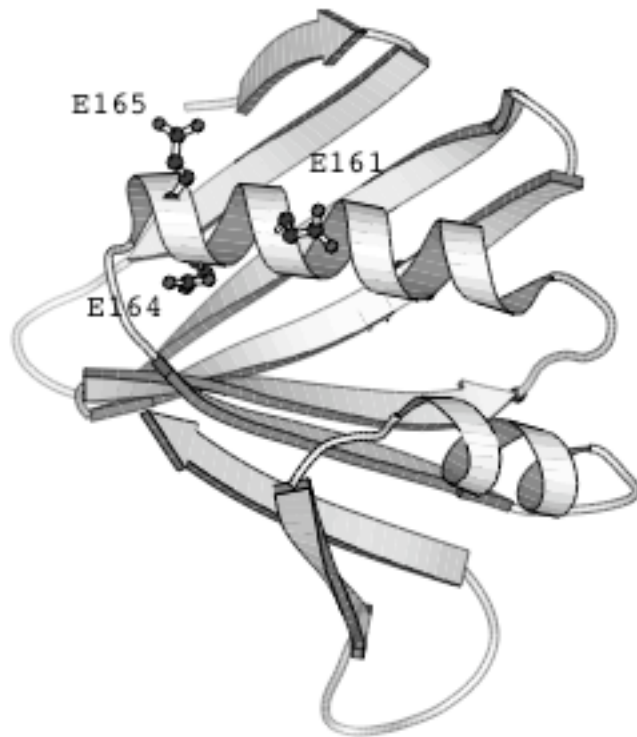
(In this case, “functionally important” may mean binding and inhibiting the enzyme Ornithine Decarboxylase).

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

			←β1→	←β2→	← Loop 1 →	←β3→	←β4→				
	87	90	95	100	105	110	115	120	125	130	
AZ-1 Rat (86 aa)	-	D H S L S A S I	L Y	S D E R	L N V	T E E	- P T S N D K T R V	L S I	Q C T	L T E A K Q V T	W R A V
AZ-1 H sap (86 aa)	-	D H N L S A N L	F Y	S D D R	L N V	T E E	- L T S N D K T R I	L N V	Q S R L	T D A K R I N W R T V	
AZ-1 Danio (73 aa)	-	D H S L S A K L	F Y	S D A Q	L L V	L E E	A P Q S N S R V R F L	L F E	R R C	S V S K H L V W R G A	
AZ-1 Xenop (76 aa)	-	D H N L S A N L	F Y	S D N R	L N I	T E E	- L T S N N R T R I	L N V	Q S S L	T D G K Q V S W R A V	
AZ-2 H sap (49 aa)	-	D P S L S A -	L I	Y K D E K L	T V	T Q D L	P V N D G K P H I	V H F	Q Y E	V T E V K V S S W D A V	
AZ-2 Mus m (49 aa)	-	D P S L S A -	L I	Y K D E K L	T V	T Q D L	P V N D G K P H I	V H F	Q Y E	V T E V K V S S W D A V	
AZ-3 H sap (43 aa)	-	N H D Q L K -	E L	Y S A G N L	T V	L A T D	P L L H Q D P V Q	L D F	H F R L	T S Q T S A H W H G L	
AZ-3 Mus m (54 aa)	-	D H S Q L K -	E L	Y S A G N L	T V	L S T D	P L L H Q D P V Q	L D F	H F R L	T P H S S A H W H G L	
AZ Droso						(140 aa)	- Q P V Q	I T	I K L H	V T E D Q Y T N W N T I	
AZ Anoph										(137 aa)	- S S W E T V
AZ Aedes										(139 aa)	- S V W E T V
AZ S cer										(74 aa)	- Y N W R K L

	→	←β5→	← Loop 2 →	←α1→	←β6→	← Loop 3 →					
	135	140	145	150	155	160	165	170	175	180	
AZ-1 Rat	W N G G - - G	L Y I E	L P A G P L P E	G S K	D S F	A A L L E F	A E E	Q L R A	D H V F I C	F P K N R E	D R
AZ-1 H sap	L S G G - - S	L Y I E	I P G G A L P E	G S K	D S F	A V L L E F	A E E	Q L R A	D H V F I C	F H K N R E	D R
AZ-1 Danio	L K G T - - N	L Y I E	I P T G V L P E	G S K	D S F	S L L L E F	A E E	K L Q V	D H V F I C	F H K S R D	D R
AZ-1 Xenop	L N N N - - N	L Y I E	I P S G T L P D	G S K	D S F	A I L L E F	A E E	Q L Q V	D H V F I C	F H K N R D	D R
AZ-2 H sap	L S S Q - - S	L F V E	I P D G L L A D	G S K	E G L	L A L L E F	A E E	K H K V	N Y V F I C	F R K G R E	D R
AZ-2 Mus m	L S S Q - - S	L F V E	I P D G L L A D	G S K	E G L	L A L L E F	A E E	K H K V	N Y V F I C	F R K G R E	D R
AZ-3 H sap	L C D R - - R	L F L D	I P Y Q A L D Q	G N R	E S L	T A T L E Y	V E E	K T N V	D S V F V N	F Q N D R N	D R
AZ-3 Mus m	L C D H - - R	L F L D	I P Y Q A L D Q	G N R	E S L	T A T L E Y	V E E	K T N V	D S V F V N	F Q I D R K	D R
AZ Droso	L N P V N N L	L Y V A	L P K D L P A G	S K Q T	F I S L	L E F A	E E K	L E V	D G I V M V	M P K D Q P	D R
AZ Anoph	F N P I E N I	L Y V S	L P S A M S H E	A S K H S F	I S L L E F	A E E	K L E C	D A V V L C	I R K D R L	D R	
AZ Aedes	F N P L D N I	L Y V N	P P S T M T H E	A S K H S F	I S L L E F	A E E	K L E C	D A V V L C	I R K D R L	D R	
AZ S cer	G S Q Y - F I	L Y L F	L F T Q E L I	- (32 aa)	- W L	L A L L E L	- - - - -	- - - - -	- - - - -	- (28 aa)	- - - - -

	←α2→	←β7→	← Loop 4 →	←β8→					
	185	190	195	200	205	210	215	220	225
AZ-1 Rat	A A L L R T	F S F L G F	F E I V	R P G H P L	V P K - - - -	R P D A C	F H V Y T	L E R E D P	G E E D
AZ-1 H sap	A A L L R T	F S F L G F	F E I V	R P G H P L	V P K - - - -	R P D A C	F H A Y T	F E R E S S	G E E E E
AZ-1 Danio	A S L L R T	F S F M G F	F E I V	R P G H P L	V P T - - - -	R P D A F	F H A Y R	I E R D S D	G D E
AZ-1 Xenop	A H L L R T	F R F L G F	F E I V	I P G H P L	V P K - - - -	R P D A C	F H A Y T	F E R D S S	D E D
AZ-2 H sap	A P L L K T	F S F L G F	F E I V	R P G H P C	V P S - - - -	R P D V M	F H V Y P	L D Q N L S	D E D
AZ-2 Mus m	A P L L K T	F S F L G F	F E I V	R P G H P C	V P S - - - -	R P D V M	F H V Y P	L D Q N L S	D E D
AZ-3 H sap	G A L L R A	F S Y M G F	F E V V	R P D H P A	L P P - - - -	L D N V I	F H V Y P	L E R D V G	H L P S E P P
AZ-3 Mus m	G A L L R A	F S Y M G F	F E V V	R P D H P A	L P P - - - -	W D N V I	F H V Y P	L E R D L G	H P G Q
AZ Droso	A R L I E A	F L F M G F	F E P L	S R K A P Q	A P P A A I	N D N E N Y	Y F L Y S I	E E	
AZ Anoph	P N L V R T	F S F V G F	Q P L S	P K S P L A	P P - (7 aa)	- N E Y L	F H V Y S I	E E	
AZ Aedes	P N L V R T	F S F V G F	Q P V S	P K S P L A	P P - (7 aa)	- N D Y L	F H I Y N I	E E	
AZ S cer	L D L L K N	L N W V G	G K L I	K N E D R E	V L L - (14 aa)	- G D E N	F V I L E F	E C	



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