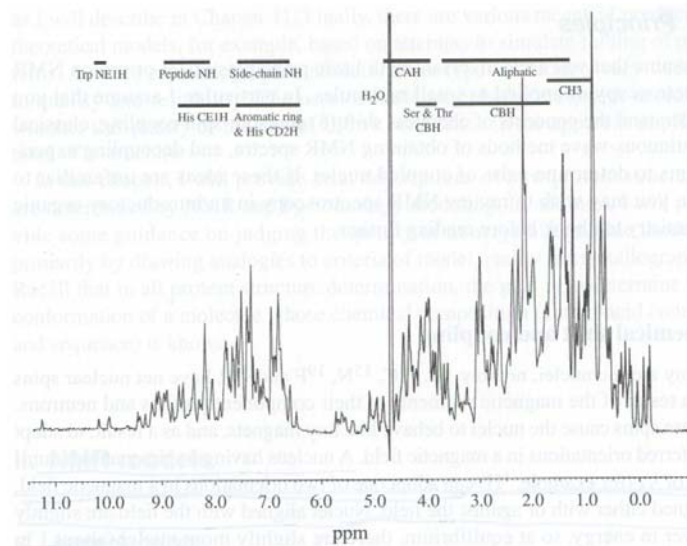


## *2D and 3D NMR for CHEM 645*

**Brian Bahnson**  
Department of Chemistry & Biochemistry  
University of Delaware

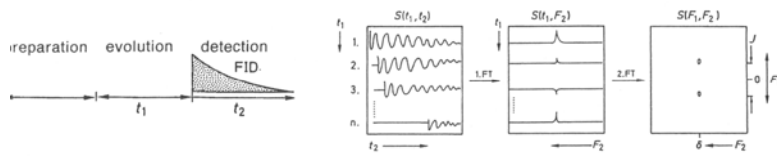
### A Protein's $^1\text{H-NMR}$ Spectra is Complex



**Figure 10.1**  $^1\text{H-NMR}$  spectrum of thioredoxin, reduced form.

To assign peaks of proteins, this needs to be spread into a 2<sup>nd</sup> or 3<sup>rd</sup> dimension.

## Two Dimensional NMR



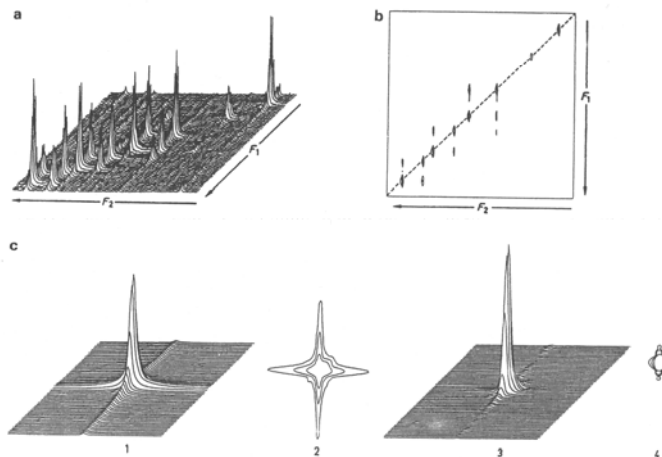
Preparation: Prepare the spin system for the experiment, generation of transverse magnetization.

Evolution: Prepared spin system develops during  $t_1$  under certain interactions, such as scalar spin-spin coupling, exchange, etc.

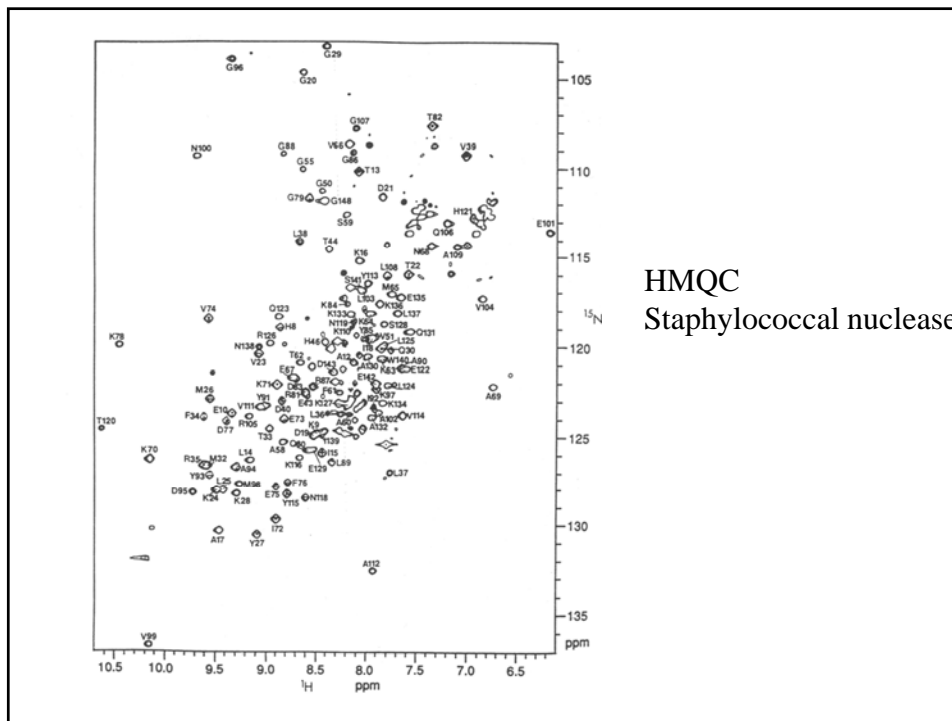
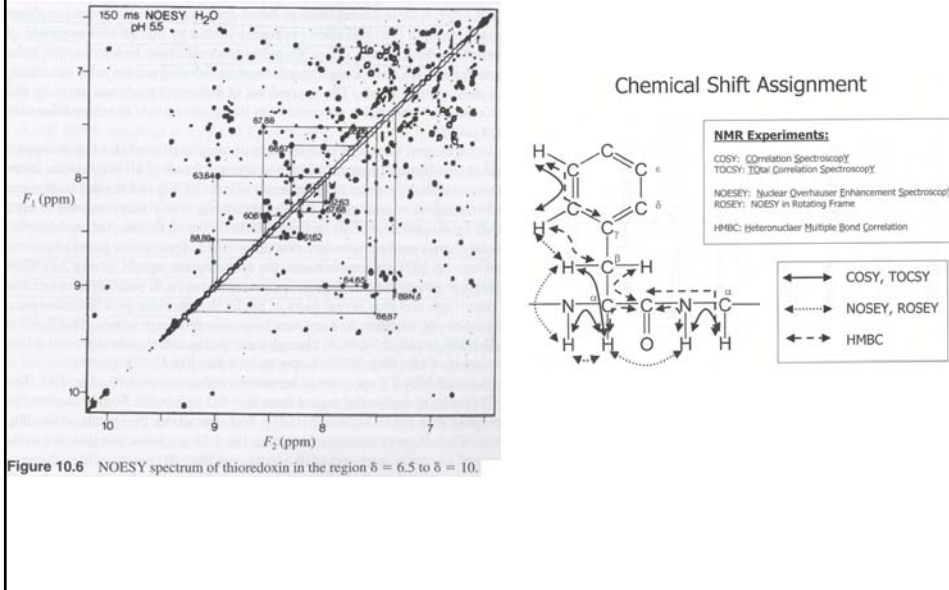
Detection: Usually detecting Larmor precession ( $\delta$ ).

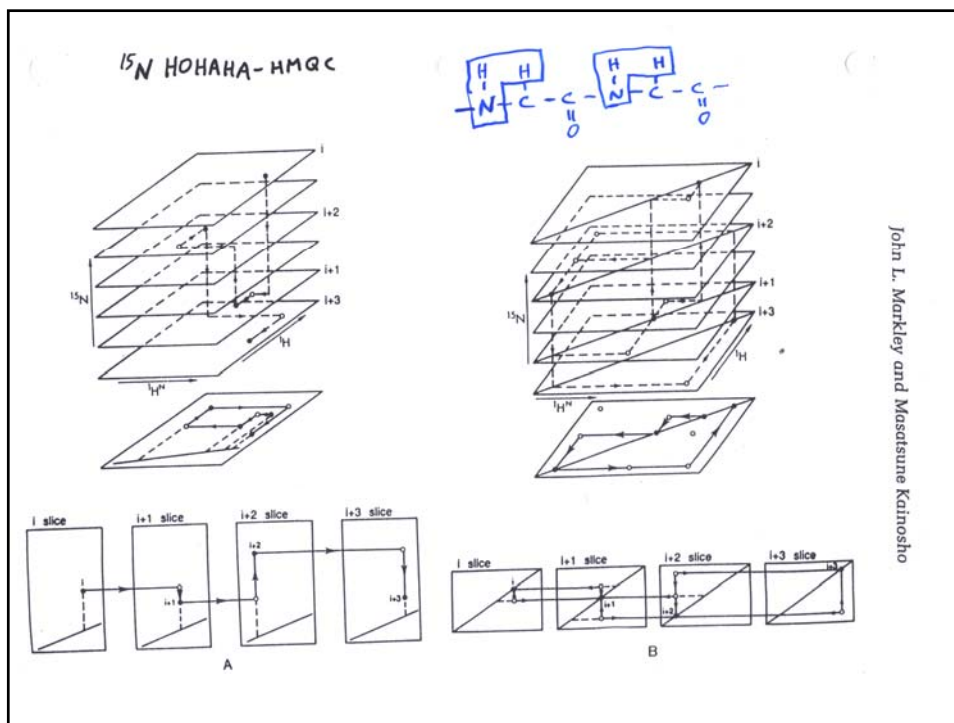
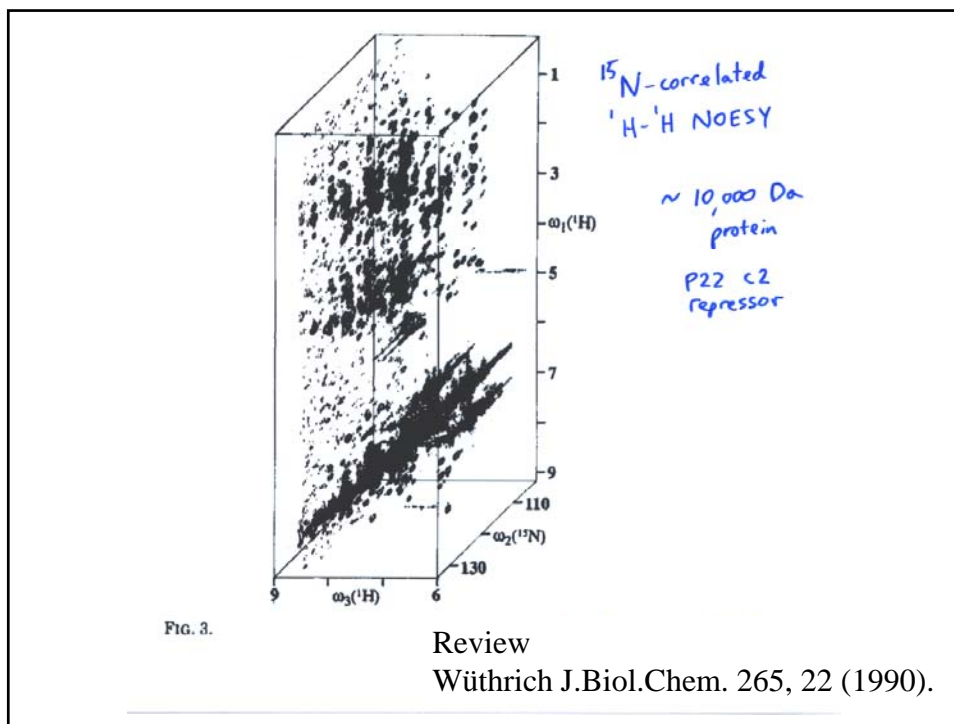
Data processing: A series of 1D experimental data are stored as data matrix as a function of time  $t_1$  and  $t_2$ . Fourier transform is then separately performed with respect of  $t_1$  and  $t_2$ .

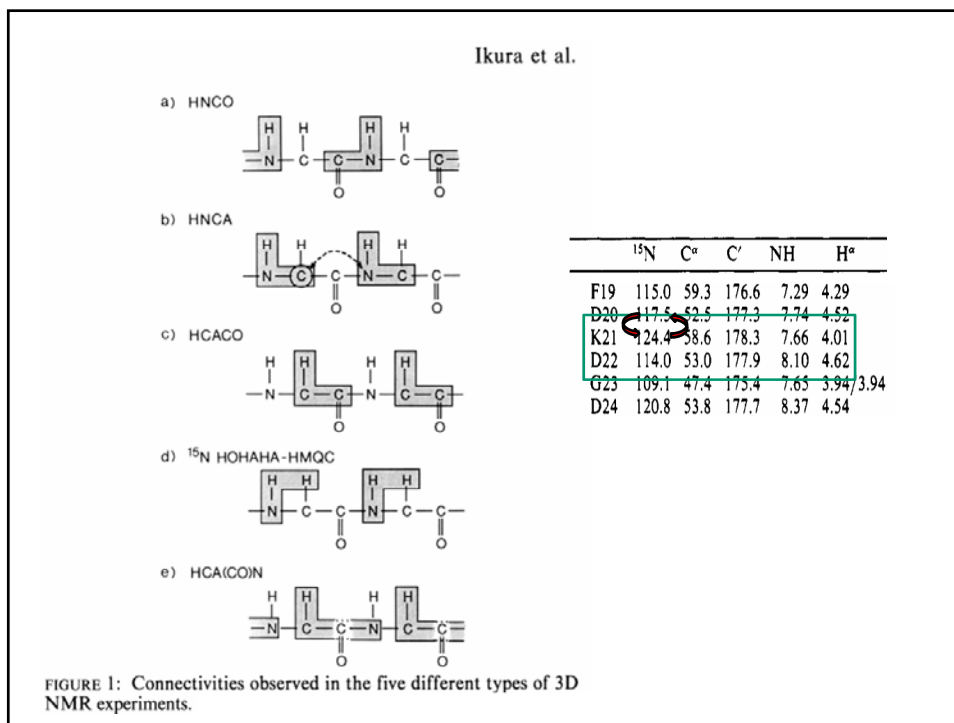
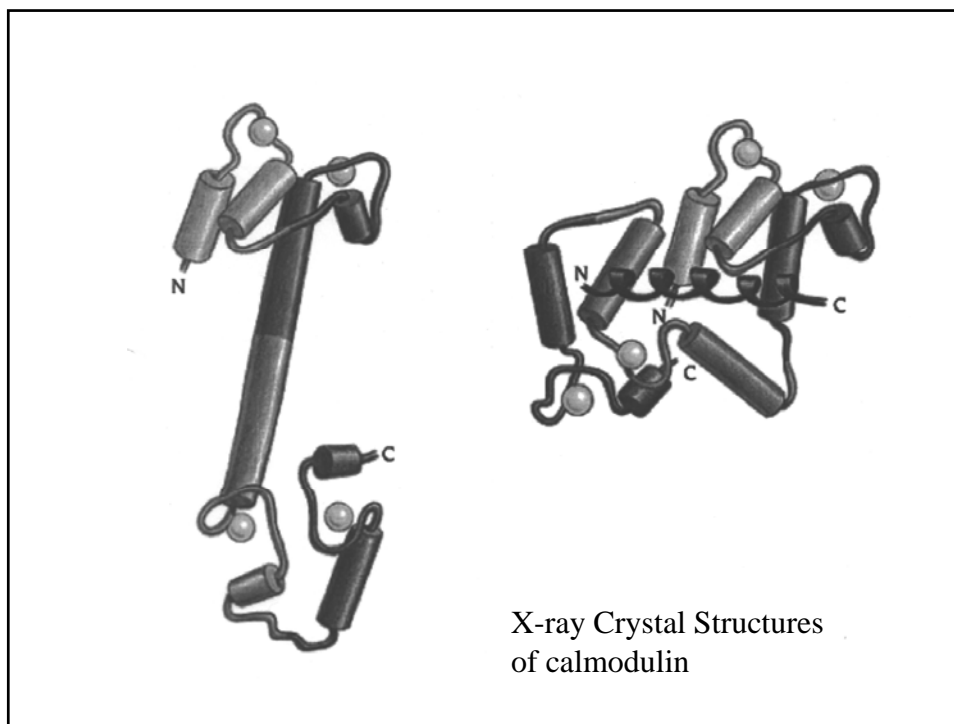
## Presentation of Two Dimensional NMR



## 2-D and 3-D Separates Out Spectra







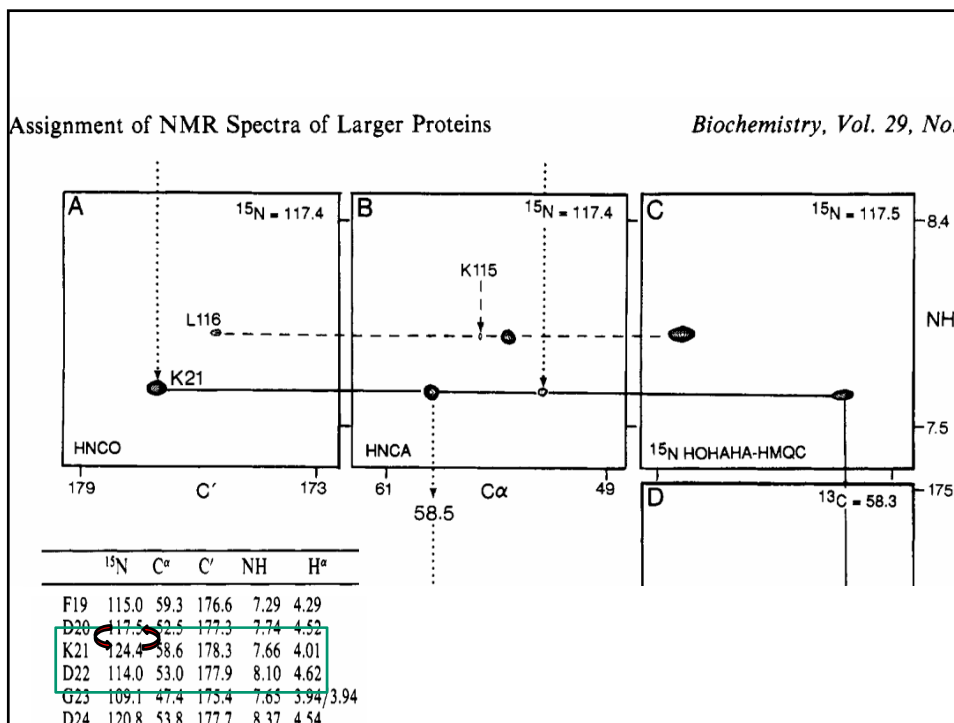
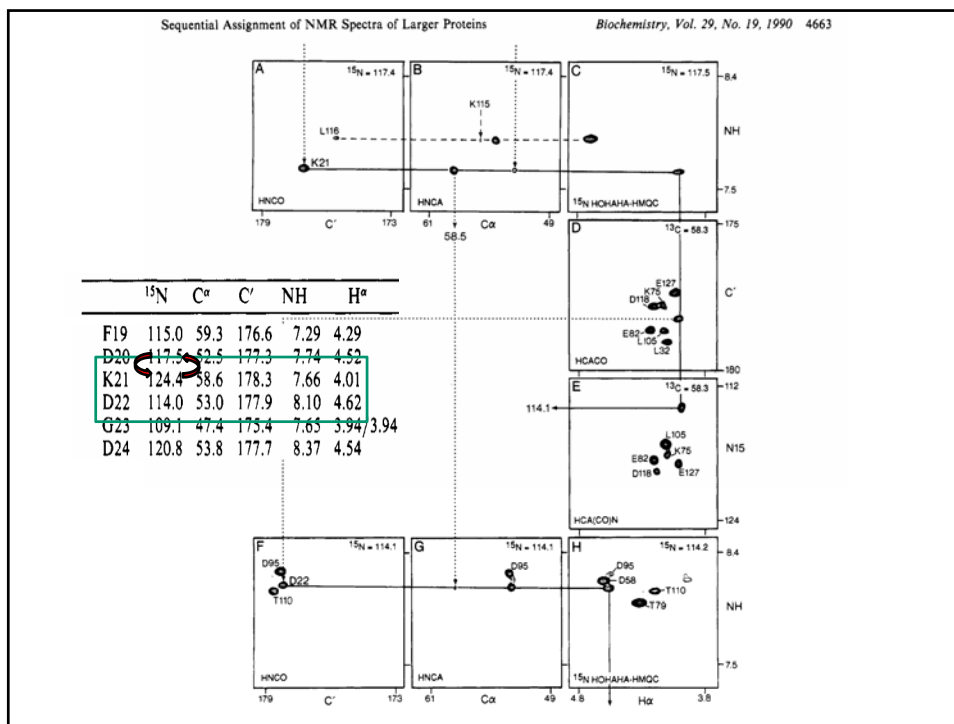
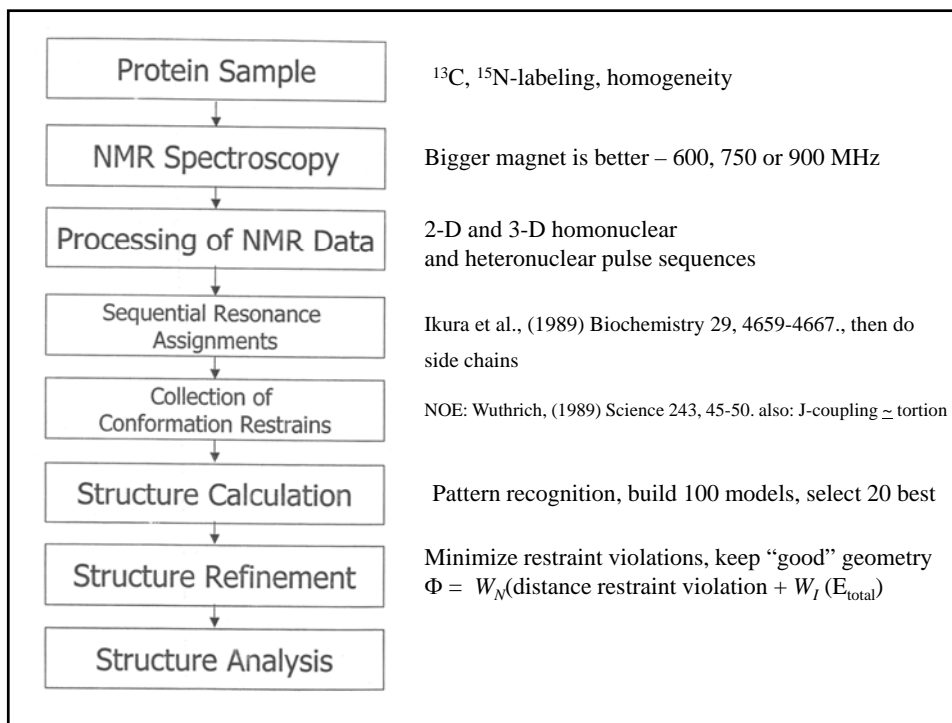


Table II: Polypeptide Backbone  $^1\text{H}$ ,  $^{13}\text{C}$ , and  $^{15}\text{N}$  Chemical Shifts for *Drosophila* Calmodulin (1.5 mM) at pH 6.3 and 47 °C, 6.1 mM  $\text{CaCl}_2$  and 0.1 M  $\text{KCl}^a$ 

$^{15}\text{N}$					$^{13}\text{C}$					$^1\text{H}$							
	$\text{C}^\alpha$	$\text{C}^\beta$	NH	$\text{H}^\alpha$		$\text{C}^\alpha$	$\text{C}^\beta$	NH	$\text{H}^\alpha$		$\text{C}^\alpha$	$\text{C}^\beta$	NH	$\text{H}^\alpha$			
A1	51.9	174.0	4.15		M51	119.3	59.5	178.8	7.84	4.09	I100	127.3	60.5	175.8	10.15	4.87	
D2	120.5	54.7	175.8	4.67	I52	118.0	65.0	177.9	7.74	3.57	S101	123.8	56.0	175.5	9.01	4.90	
Q3	119.6	55.7	175.8	8.24	4.42	N53	117.5	56.0	177.5	8.47	4.42	A102	123.0	56.1	179.4	9.16	3.97
L4	122.8	54.6	177.7	8.16	4.70	E54	116.4	59.0	177.8	7.58	4.09	A103	118.1	55.3	181.3	8.15	4.08
T5	112.9	60.7	175.8	8.56	4.52	V55	108.9	61.1	176.0	7.22	4.47	E104	119.4	59.4	179.9	7.79	4.09
E6	120.5	60.2	179.4	8.93	4.03	D56	121.6	54.0	176.4	7.72	4.67	L105	120.8	58.5	178.7	8.53	4.16
E7	119.3	60.0	179.1	8.63	4.12	A57	131.7	54.3	178.8	8.47	4.25	R106	117.3	60.0	178.8	8.51	3.85
Q8	119.8	58.7	178.3	7.67	3.93	D58	113.9	52.8	178.1	8.18	4.67	H107	118.6	59.5	177.4	7.91	4.38
D9	119.4	66.1	177.9	8.31	3.77	G59	108.2	47.4	175.2	7.55	3.95/3.85	V108	118.7	66.2	178.1	7.93	3.56
A10	121.3	55.6	180.9	7.97	4.15	N60	118.4	52.7	177.0	8.07	4.67	M109	116.5	57.8	178.7	8.18	4.37
E11	119.1	55.4	180.2	7.78	4.17	G61	113.3	45.8	173.6	10.51	4.22/3.55	T110	114.4	65.8	177.6	8.10	4.22
F12	119.6	59.5	178.8	8.52	5.01	T62	109.0	59.7	173.5	7.68	4.79	N111	121.9	65.7	176.5	7.85	4.54
K13	123.1	60.3	179.4	9.09	4.06	I63	124.0	60.0	175.6	8.90	5.19	L112	118.7	55.6	177.5	7.78	4.37
E14	120.2	59.5	179.5	7.82	4.20	D64	128.3	52.4	176.7	8.87	5.39	G113	106.3	45.6	174.6	7.79	4.27/3.78
A15	122.2	55.4	179.0	8.01	4.28	F65	119.0	63.7	173.8	8.90	4.04	E114	120.3	55.5	175.8	7.90	4.46
F16	119.0	62.1	177.4	8.73	3.31	P66	117.2	66.7	180.1	3.95		K115	123.5	55.8	175.7	8.41	4.41
S17	112.4	61.6	175.2	7.90	4.18	E67	117.4	58.8	178.8	7.80	4.15	L116	124.2	54.4	178.0	7.92	4.78
L18	120.8	57.3	178.0	7.45	4.04	F68	123.3	61.3	177.1	8.76	4.00	T117	114.0	60.8	175.6	8.91	4.52
F19	115.0	59.3	176.6	7.29	4.29	L69	118.8	58.1	179.1	8.31	3.39	D118	122.5	58.5	179.9	8.44	4.22
D20	124.4	58.6	178.3	7.54	4.53	T70	114.9	66.5	176.2	7.47	3.83	E119	119.8	59.5	178.6	8.27	4.11
K21	124.4	58.6	178.3	7.66	4.01	M71	121.4	59.0	178.1	7.75	3.84	E120	118.7	59.5	179.9	8.15	4.19
D22	114.0	53.0	177.9	8.10	4.62	M72	116.4	56.0	178.4	7.98	4.06	V121	120.8	67.0	177.4	8.02	3.68
D23	109.1	59.4	175.4	7.85	3.94	A73	121.8	54.5	179.6	8.08	4.13	D122	119.6	57.8	179.2	8.02	4.38
D24	120.8	53.8	177.7	8.37	4.54	R74	116.5	58.1	177.8	7.52	4.15	E123	119.2	59.2	178.1	7.89	4.05
G25	113.1	45.6	174.0	10.57	4.28/3.74	K75	118.7	57.1	177.6	7.71	4.28	M124	119.4	59.5	179.1	7.80	4.06
T26	112.9	60.1	173.2	8.14	5.31	M76	118.4	56.5	176.5	7.85	4.42	I125	118.2	64.2	177.3	7.92	3.55
I27	127.1	60.5	176.3	9.71	5.00	K77	120.3	60.2	176.5	7.81	4.37	R126	118.4	59.9	179.3	8.13	4.05
T28	116.6	59.8	176.8	8.53	4.86	D78	121.4	54.6	176.7	8.21	4.74	E127	115.8	58.5	177.4	7.87	4.05
T29	112.8	66.8	177.2	8.99	3.83	T79	114.0	62.3	174.7	8.01	4.37	A128	119.1	52.3	177.8	7.37	4.45
K30	120.4	59.3	179.9	7.65	4.22	D80	122.8	54.8	176.9	8.35	4.75	N129	117.3	54.2	176.4	7.83	4.57
E31	121.3	59.4	179.2	7.60	4.10	S81	116.7	59.9	175.7	8.30	4.50	I130	127.4	63.2	178.0	8.50	4.03
L32	120.4	58.2	179.1	8.64	4.13	E82	120.9	58.2	178.7	8.76	4.26	D131	117.1	53.8	178.5	8.27	4.61
G33	105.3	48.4	175.5	8.59	4.00/3.62	E83	118.8	60.0	179.2	8.63	4.13	G132	108.3	47.6	175.5	7.57	4.01/3.85
T34	117.8	67.0	177.2	7.85	3.99	E84	120.2	59.3	179.4	7.68	4.06	D133	120.6	53.8	177.8	8.34	4.51
V35	122.2	66.5	179.1	7.64	3.50	I85	121.3	64.5	178.0	7.93	4.07	G134	112.8	46.0	173.2	10.27	4.07/3.47
M36	117.9	59.1	179.2	8.38	4.16	R86	121.6	60.2	179.3	8.32	4.20	Q135	115.4	53.3	175.0	7.96	4.88
R37	118.9	59.2	181.1	8.38	4.81	E87	118.4	59.1	178.7	8.07	4.18	V136	125.2	61.8	176.1	9.12	5.24
S38	118.4	61.8	174.9	7.93	4.42	A88	121.8	55.2	179.2	7.93	4.23	N137	129.0	51.4	175.2	9.62	5.28
L39	120.1	54.7	177.6	7.38	4.52	F89	118.5	62.2	176.7	8.47	3.23	V138	118.2	62.9	176.3	8.36	3.50
G40	106.4	45.7	174.7	7.82	4.24/3.81	R90	115.4	58.9	178.3	7.64	3.94	E139	118.2	60.5	180.5	8.00	3.72
Q41	118.4	54.6	174.4	7.82	4.53	V91	118.1	65.7	177.4	7.52	3.56	E140	120.1	58.7	179.2	8.73	4.08
N42	116.5	51.4	172.3	8.59	5.20	F92	116.4	60.0	177.0	7.47	4.25	F141	124.0	61.6	177.0	8.86	4.05
P43	115.6	62.7	177.9	4.80		D93	116.9	52.4	177.6	7.74	4.59	V142	119.4	67.2	179.6	8.48	3.21
T44	112.6	60.7	175.4	8.60	4.50	K94	125.8	59.0	178.4	7.66	3.97	T143	116.5	66.7	176.1	7.67	3.81
E45	120.7	60.3	179.0	8.74	4.02	D95	114.2	53.2	177.9	8.24	4.62	M144	121.8	58.7	178.0	7.93	4.13
A46	120.5	55.2	180.2	8.16	4.14	G96	109.1	47.3	175.4	7.76	3.85/3.82	M145	114.8	55.7	177.8	7.89	4.34
E47	118.6	59.2	180.1	7.63	4.08	N97	119.4	52.8	176.2	8.32	4.68	T146	110.0	62.5	175.1	7.64	4.45
L48	120.2	58.0	178.6	8.11	4.12	G98	112.8	45.3	172.8	10.59	4.04/3.44	S147	117.8	59.1	173.7	7.74	4.52
Q49	118.1	58.8	178.6	8.13	3.89	F99	115.6	56.0	174.9	7.64	5.19	K148	127.7	57.7	181.2	7.65	4.21
D50	120.0	57.7	178.8	8.01	4.46												

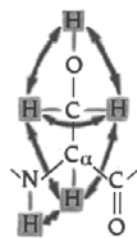
<sup>1</sup>H Chemical shifts are expressed relative to (trimethylsilyl)propionic-d<sub>4</sub> acid, <sup>15</sup>N shifts relative to liquid NH<sub>3</sub> (Live et al., 1984), and <sup>13</sup>C shifts relative to hypothetical internal (trimethylsilyl)propionic acid (Bax & Subramanian, 1986).



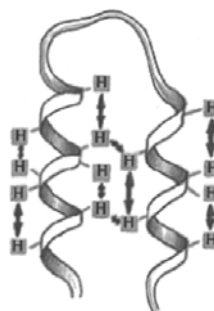
## Distance Restraints



Ala



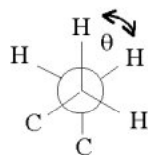
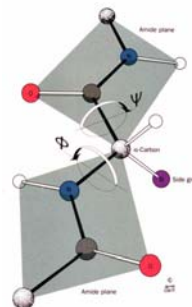
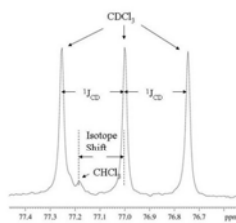
Ser



Through space - NOE

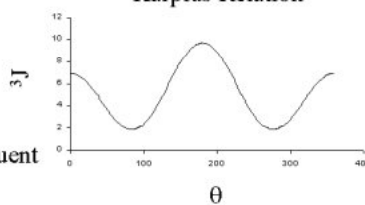
$$\text{NOE} \propto 1/r^6 \cdot f(\tau_c)$$

## Torsional Restraints through bond J-coupling



A, B and C on the substituent  
electronegativity.

### Karplus Relation

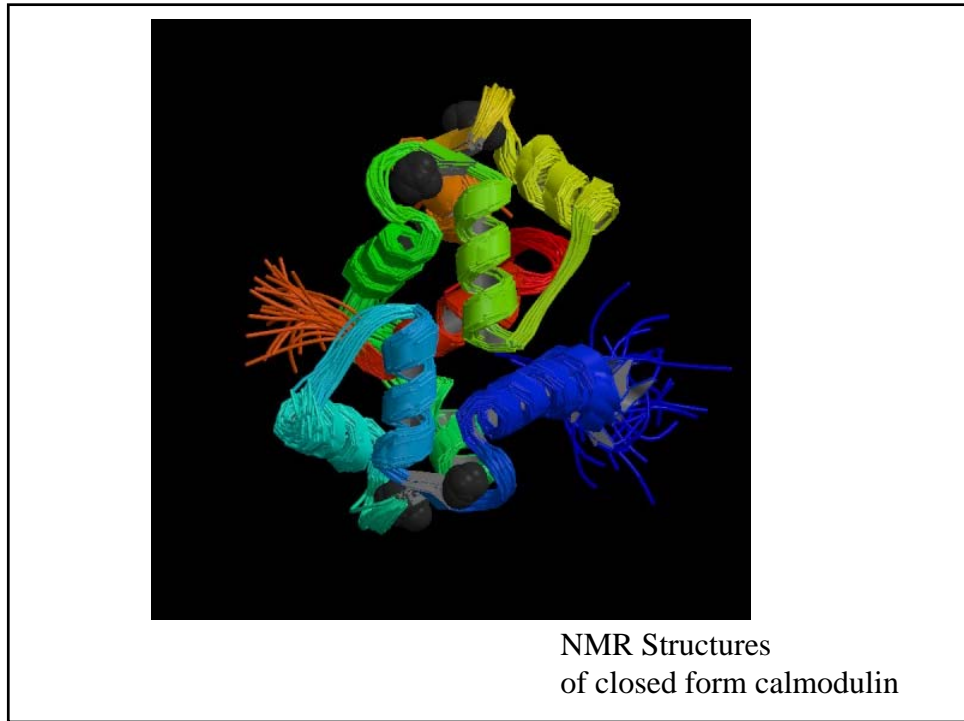


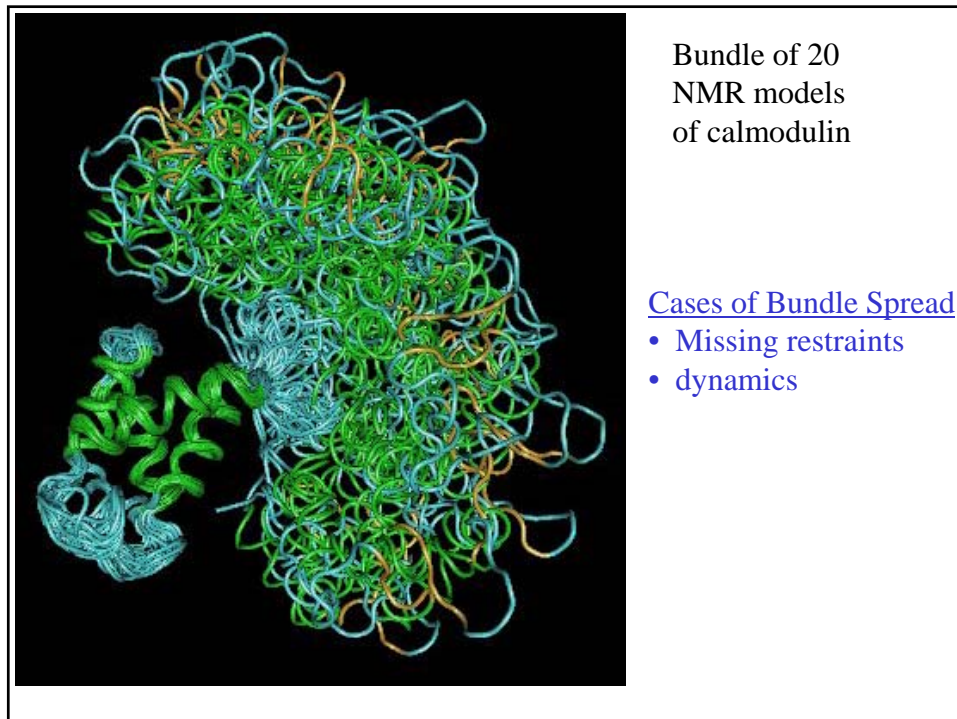
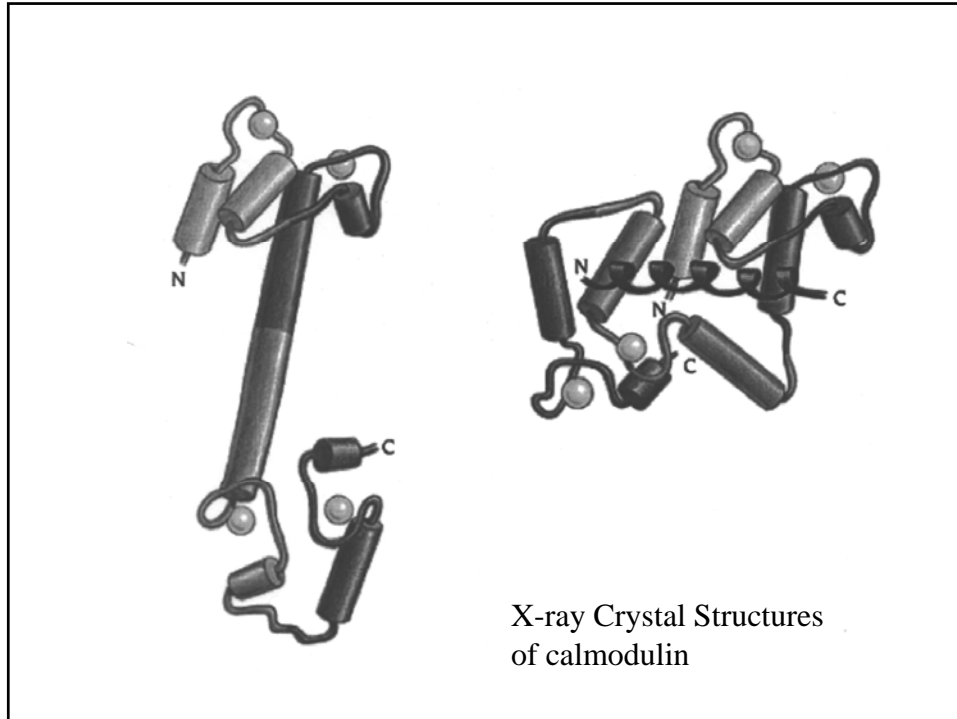
**NMR Refinement**

$$\Phi = w_{\text{NMR}} \left( \underbrace{\sum \text{distance restraint violations}}_{\text{NMR term}} + \underbrace{\sum \text{torsional restraint violations}}_{\text{NMR term}} \right) + w_{\text{ideal}} \underbrace{E_{\text{total}}}_{\text{ideal geometry}}$$
  

**X-ray Refinement**

$$\Phi = w_{\text{F}} \sum_{hkl} w_{hkl} \left( \underbrace{|\text{Fo}|}_{\text{observed}} - \underbrace{|\text{Fc}|}_{\text{calculated}} \right)_{hkl}^2 + w_{\text{ideal}} \underbrace{E_{\text{total}}}_{\text{ideal geometry}}$$





### Crystallography vs. NMR – advantage/disadvantages

- 1) Experimental difficulties  
need for homogeneity in common  
need good crystals for crystallography  
need  $^{13}\text{C}$  and  $^{15}\text{N}$  label for NMR  
size limits of NMR technique  
solubility an issue for each technique
- 2) Reported structure(s) look different  
crystal structure vs. solution structure bundle
- 3) Complementary information  
high resolution vs. dynamics  
positional                      amplitude,  
certainty                        time domains