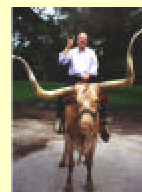
Structure Determination by Multidimensional NMR

Questions:

1. What are the requirements and limitations of multidimensional NMR methods?
2. NMR spectra - many types of experiments.
3. What is the "Assignment Problem"?
5. How are "Assignments" made?
6. From peaks to secondary structure.
7. How is the protein "model" obtained?
8. Comparison of structure determination by X-ray vs. NMR.

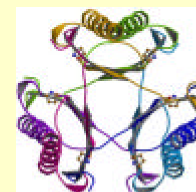
CH370 – Hackert (with examples from Dr. David Hoffman)

Image Formation



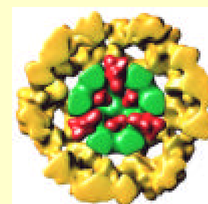
• Light Photography

l ~ 400 - 700 nm



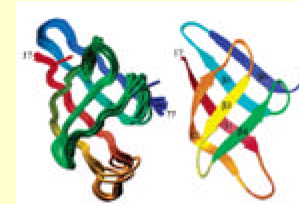
• X-Ray

l ~ 0.1 nm



• Electron Microscopy

l ~ 0.001 - 0.1 nm



• NMR

NMR Methods

Nuclear spin (nuclear spin Quantum Number *I*)

No spin: #neutrons and #protons both even - 12C, 16O

Half-integer spin (1/2, 3/2, 5/2): #neutrons + #protons odd - 1H, 13C, 15N

Integer spin (1, 2, 3): #neutrons and #protons both odd - 2H, 14N

Spin Quantum Numbers of Common Nuclei

Element	^{1}H	^{2}H	^{12}C	^{13}C	^{14}N	^{16}O	^{17}O	^{19}F
Nuclear Spin Quantum No (I)	1/2	1	0	1/2	1	0	5/2	1/2
No. of Spin States	2	3	0	2	3	0	6	2

Elements with odd mass or odd atomic number have nuclear "spin".

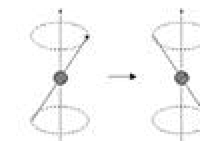
http://www.chem.umd.edu/courses/chem243davis/

NMR Methods

Nuclear spin and the splitting of energy levels in a magnetic field

Nucleus of spin *I* ; 2*I* + 1 orientations:

(m = -*I* to +*I*) e.g. *I* = ½ ; *m* = -1/2 , +1/2



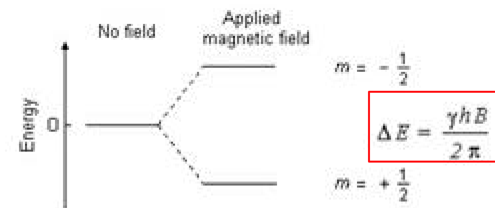
Energy levels for a nucleus with spin quantum number 1/2

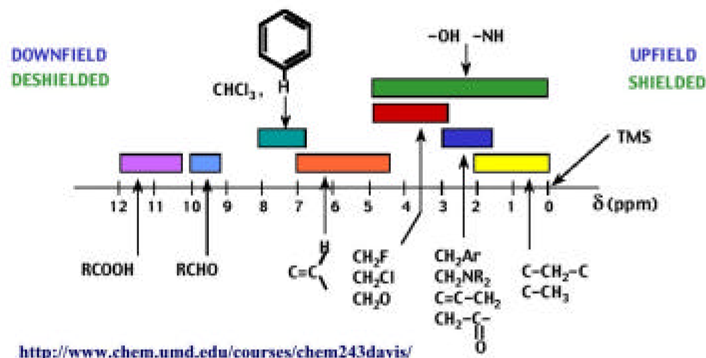
Nuclear magnetic moment

$$\mu = \frac{\gamma I h}{2\pi}$$

Energy of level "*m*"

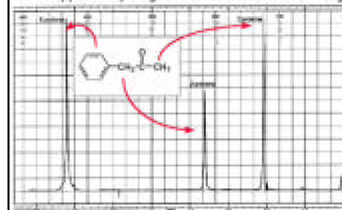
$$E = -\frac{\gamma h}{2\pi} m B$$



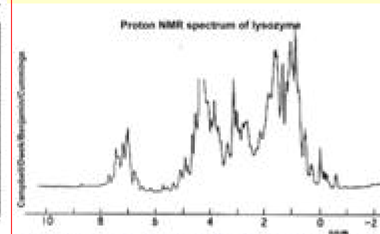


NMR Methods

Small molecule NMR



"Big" molecule NMR

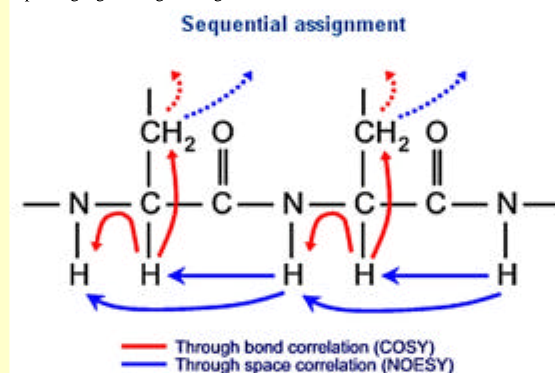


Limitations for Structure Determination by Multidimensional NMR Methods

1. Protein must be "smallish" (< 300 amino acid residues)
2. Protein must be soluble and well behaved in solution. (1-2 mM or 30 mg/mL for a 20kDa protein)
3. Must be able to solve the "Assignment" Problem
4. Must have sufficient number of distance restraints

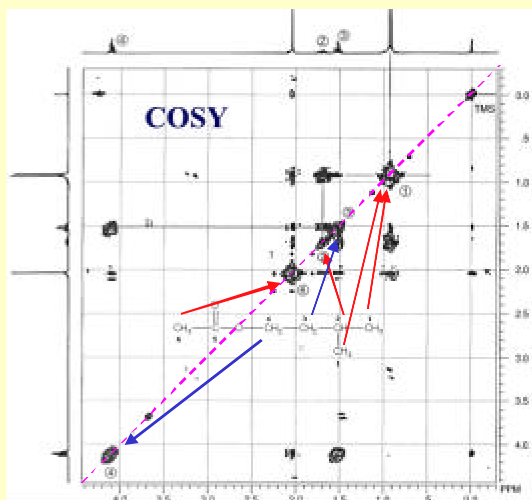
NMR Methods – COSY

Two-dimensional COSY (COrrelation SpectroscopY) experiments allow you to determine the connectivity of a molecule by determining which protons are spin-spin coupled. One could accomplish the same task by a detailed analysis of spin-spin splittings, given high enough resolution.



http://www.bch.bris.ac.uk/staff/pfdg/teaching/nmr.htm

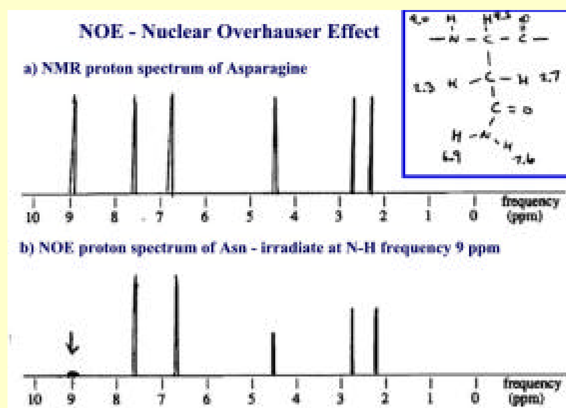
NMR Methods – COSY



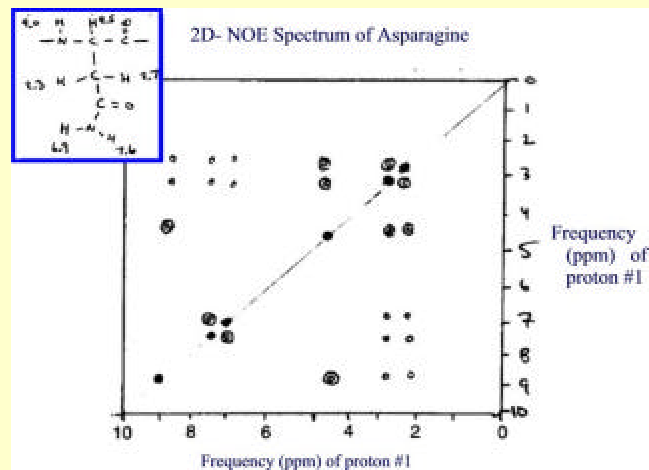
Structure determination is performed using **Nuclear Overhauser Effect (NOE)** spectra, to find protons that are near each other in the structure.

NOESY is a acronym for **Nuclear Overhauser Effect Spectroscopy**. The nuclear Overhauser effect is the perturbation of the magnetization of one spin due to **dipolar coupling** with another spin. Since this interaction is detected through **space** the NOESY experiment provides important information on **inter-nuclear distances.**

Nuclear Overhauser Effect (NOE) - 1 Dimension



Nuclear Overhauser Effect (NOE) – 2 Dimensions



Protein Structure Determination

The determination of protein structure by NMR methods is largely based on the ability to detect and quantify **inter-proton distances** by **measurement of the dipolar coupling between protons.**

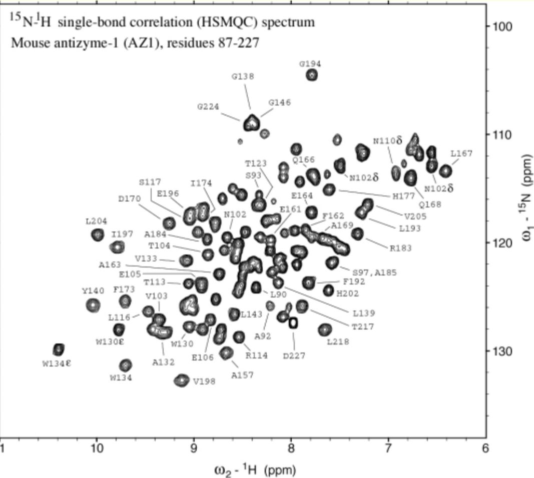
However - In order to obtain these distances it is necessary to **assign resonance frequencies to the protons with the protein**. The latter is referred to as "assigning the spectrum", or the ***assignment problem***.

The **assignments** are based largely on the detection of **scalar coupling through chemical bonds**. Analysis of spectra will give assignments of proton type (e.g. amide, alpha, beta etc.) and carbon type.

First step of NMR analysis is spectrum assignment:
(Identifying the NMR frequency of as many nuclei as possible).

Proton Chemical Shifts

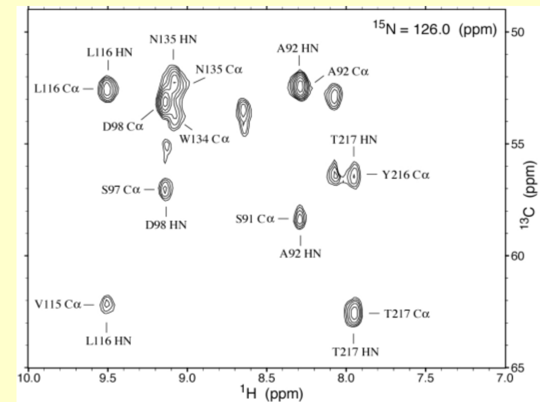
Carbon Chemical Shifts



The ^{15}N-^{1}H correlated HSQC spectrum of AZ-1D86. The relatively disperse resonances are typical of a protein that is folded and suitable for structural study by NMR methods. Some of the assigned resonances are labeled.

3-dimensional "triple-resonance" NMR

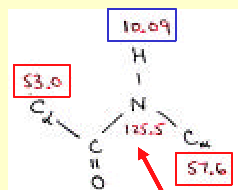
is used for solving the **assignment problem**.

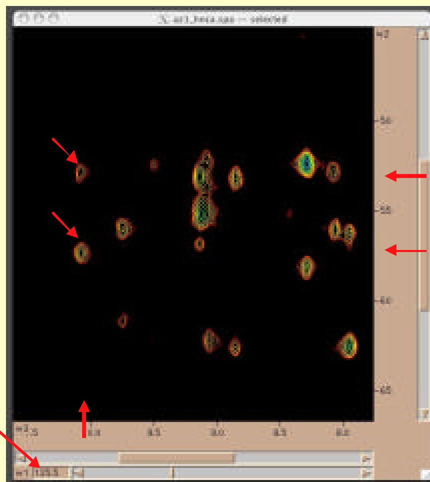


3-D HNCA spectrum of the mouse antizyme (AZ-1D86). A plane corresponding to a single 15N resonance frequency is shown, obtained using our 500 MHz cryo-probe equipped instrument. Resonance peaks correlate the backbone amide ^{1}H and ^{15}N nuclei with the alpha 13C of within the same and preceeding amino acid.

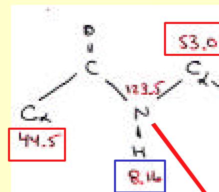
Assignments: AZ-1

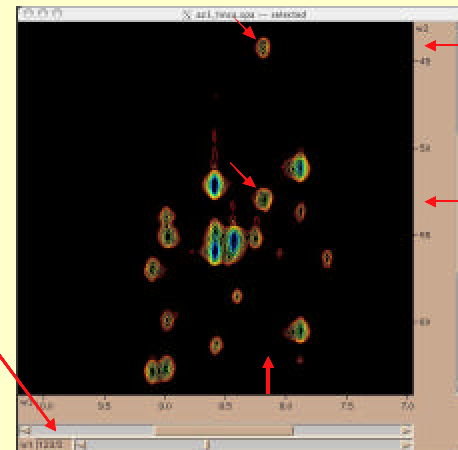
"HNCA" spectrum.



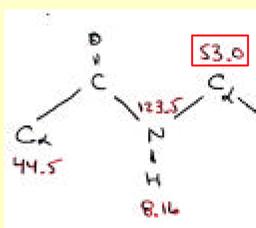


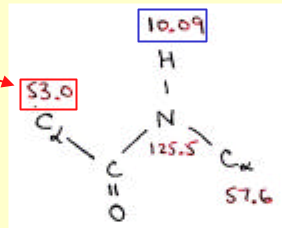
Assignments: AZ-1

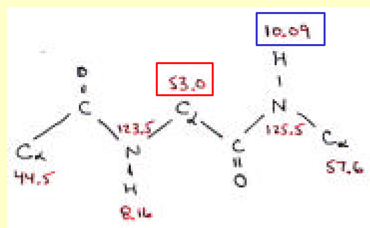




Assignments: AZ-1



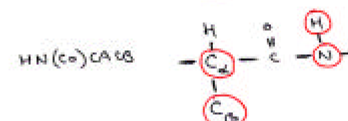




Triple Resonance NMR of Antizyme

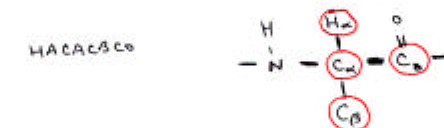
HNCA

HN(co)CACB



HNCO

HACACBCO



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	E											
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	1.62,1.57
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	131.2	55.9	61.1	43.9	37.6	175.9	175.0	4.35	1.62	
11	96	V	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	53.2	29.0	40.8	176.2	174.8	4.14	2.22,2.06	
15	100	K	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	S	8.96	122.7	55.0	60.0	21.9	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	na		56.9		32.2						
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	8.89	123.8	58.0	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	E			56.9		28.6		172.5		5.19		
28	113	T	9.89	123.6	62.9	56.9	69.1	28.6	172.2	173.5	4.35	4.14	1.16
29	114	K	8.58	128.8	55.1	62.5		69.1		173.3	4.36	1.68, 1.80	
30	115	V	9.85	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	9.88	117.8	57.1		63.1		173.6	175.4	5.87	3.85, 3.72	
33	118	T	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	

Distance Restraints:

AZ-1

2-D NOE spectrum.

Distance Restraints:

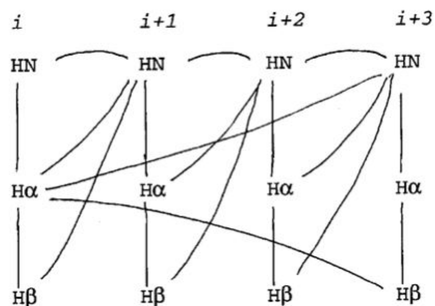
AZ-1

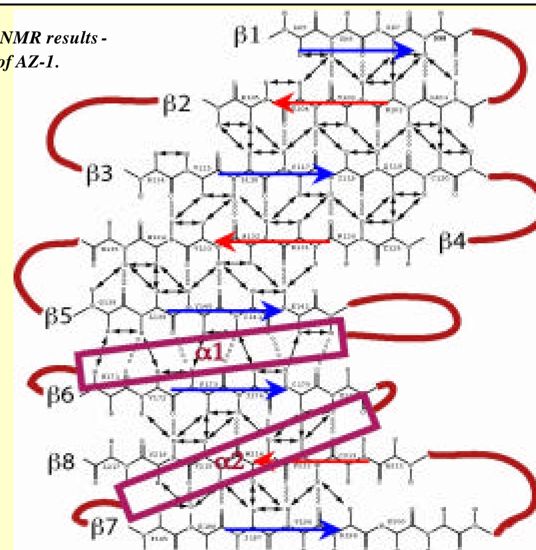
10.09
5.71
9.12
6.12

Distance Restraints:

AZ-1

L139
Y140
10.09
5.71
9.12
6.12
W134
V133





Energy Refinement

$$E_{TOTAL} = E_{EMPIRICAL} + E_{EFFECTIVE}$$

$$E_{EFFECTIVE} = E_{XREF} + E_{NOE} + E_{HARM} + E_{CDIH} + E_{NCS} + E_{DG} + E_{RELA} + E_{PLAN}$$

$$E_{EMPIRICAL} = S^{N}_{p=l}\,[w^{p}_{BOND}E_{BOND} + w^{p}_{ANGL}E_{ANGL} + w^{p}_{DIHE}E_{DIHE} + w^{p}_{IMPR}E_{IMPR} + w^{p}_{VDW}E_{VDW} + w^{p}_{ELEC}E_{ELEC} + w^{p}_{PVDW}E_{PVDW} + w^{p}_{PELE}E_{PELE} + w^{p}_{HBON}E_{HBON}].$$

Bonded Energy Terms

$$E_{BOND} = \underset{bonds}{S}\, k_b(t - t_o)^2$$

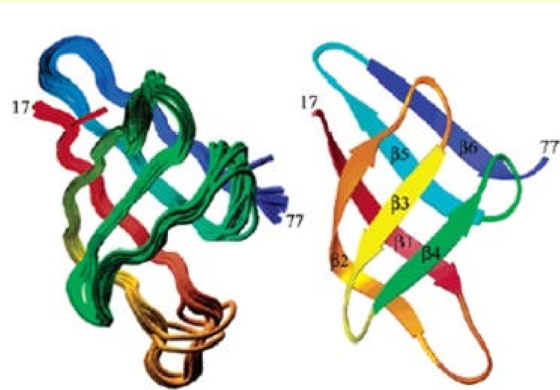
$$E_{ANGL} = \underset{angles}{S}\, (k_?(? - ?_0)^2 + k_{ub}(r_1 3 - r_{ub})^2)$$

$$E_{DIHE} = \underset{dihedrals}{S}\ \underset{i=1,m}{S}\, k_{f\,i}(1 + COS(nf_i + d_i)) \text{ if } n_i > 0$$

$$\underset{dihedrals}{S}\ \underset{i=1,m}{S}\, k_{f\,i}(f_i - d_i)^2 \text{ if } n_i = 0$$

$$E_{IMPR} = \underset{impropers}{S}\ \underset{i=1,m}{S}\, k_{f\,i}(1 + COS(nf_i + d_i)) \text{ if } n_i > 0$$

$$\underset{impropers}{S}\ \underset{i=1,m}{S}\, k_{f\,i}(f_i - d_i)^2 \text{ if } n_i = 0$$



Biochemistry 2003, 42, 13541−13550

NMR Structure of an Archaeal Homologue of Ribonuclease P Protein Rpp29

David J. Sidote and David W. Hoffman*

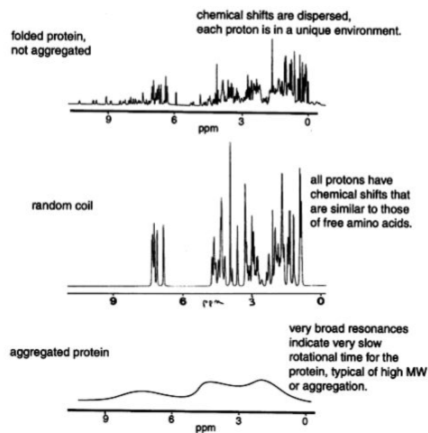
Department of Chemistry and Biochemistry, Institute for Cellular and Molecular Biology, University of Texas, Austin, Texas 78712

Table 1: Summary of Refinement and Structural Statistics for the *A. fulgidus* aRpp29 Protein (Residues 17−77)ª

intraresidue NOEs	215
sequential NOEs (residue *i* to *i* + 1)	178
medium-range NOEs (residue *i* to *i* + 2, 3, 4)	18
long-range NOEs	143
dihedral angle restraints	70
hydrogen bond restraints	27
total structural restraints	651
no. of unique starting structures for simulated annealing	10
no. of simulated annealing runs, differing in initial trajectories	200
rmsd for backbone atoms (residues 17−77)	0.87 Å
rmsd for side chain atoms (residues 17−77)	1.78 Å
av no. of NOE violations > 0.2 Å (per structure)	3.2 ± 1.0
av no. of NOE violations > 0.5 Å (per structure)	0
residues in most favored regions of the Ramachandran plot	71.2 ± 2.6%
residues in additionally allowed regions of the Ramachandran plot	21.2 ± 4.0%
residues in generously allowed regions of the Ramachandran plot	5.8 ± 2.7%
residues in disallowed regions of the Ramachandran plot	1.9 ± 0.9%
rmsd for covalent bonds	0.0034 ± 0.0001
rmsd for covalent angles	0.511 ± 0.015
rmsd for improper angles	0.581 ± 0.016

Synergy between

NMR and crystallography.



Comparison of X-ray vs. NMR Structure Determination

1.	Large protein, crystals	X-ray
2.	Small protein, no crystals	NMR
3.	Small protein, crystals	X-ray + NMR
4.	High resolution	X-ray
5.	Surface features of side chains	X-ray
6.	Flexibility / Motions	NMR
7.	Interactions	NMR