Solving structures by NMR

Qinghua Wang
1232B LGRT
545-3473; qwang@biochem.umass.edu

1946 Bloch, Purcell first nuclear magnetic resonance

1955 Solomon NOE (nuclear Overhauser effect)

1966 Ernst, Anderson Fourier transform NMR

1975 Jeener, Ernst 2D NMR

1985 Wüthrich first solution structure of a small protein (BPTI) from NOE derived distance restraints

1987 3D NMR + 13C, 15N isotope labeling of recombinant proteins

1990 pulsed field gradients (artifact suppression)

1996/7 new long range structural parameters: projection angle restraints from residual dipolar couplings (RDCs) or cross-correlated relaxation
TROSY (molecular weight > 100 kDa)

Nobel prizes

1944 Physics Rabi (Columbia)

1952 Physics Bloch (Stanford), Purcell (Harvard)

1991 Chemistry Ernst (ETH)

2002 Chemistry Wüthrich (ETH)

2003 Medicine Lauterbur (Urbana), Mansfield (Nottingham)

Lecture overview

1. A few reminders from last lecture
2. The problem of sequential assignment, and how it is solved
3. Calculating an NMR structure from inter-nuclear distances
4. How to assess the quality of an NMR structure

The energy state of one nucleus can affect other nuclei:

Scalar couplings between nuclei connected by three or fewer bonds

Dipolar couplings between nuclei that are close together in space (<~5Å)

For example: a two-dimensional NOESY correlation spectrum

- Off-diagonal peaks correspond to NOEs between two protons that are close to each other in the 3-dimensional structure of the protein
- The intensity of the peak is proportional to r^{-6} (r = distance between protons)
- Limited to protons within about 5 Å of each other

23

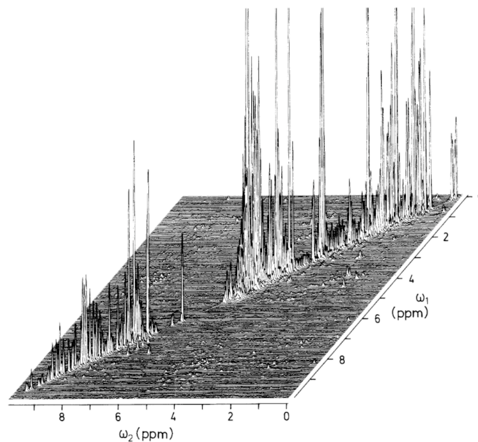


Figure 18 Two-dimensional (2D) $[^1H,^1H]$-NOE spectroscopy ($[^1H,^1H]$-NOESY). A stacked plot representation of a spectrum of the small protein bull seminal proteinase inhibitor IIA (BUSI IIA, M ≈ 6000) is shown (500 MHz, 45 °C, H_2O-solution).

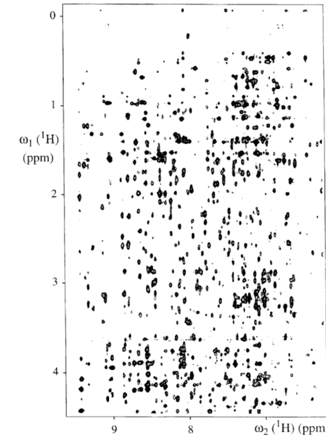


Figure 19 2D $[^1H,^1H]$-NOESY spectrum of the plant pathogenesis-related protein P14A (M ≈ 15000). A contour plot of the spectral region $[\omega_1(^1H) = 0–4.3$ ppm, $\omega_2(^1H) = 6.3–9.5$ ppm$]$ is shown (750 MHz, 30 °C, H_2O-solution).

Wüthrich, *J. Biomol. NMR,* **27:** 13-39, 2003

NMR structure calculation relies primarily on NOEs

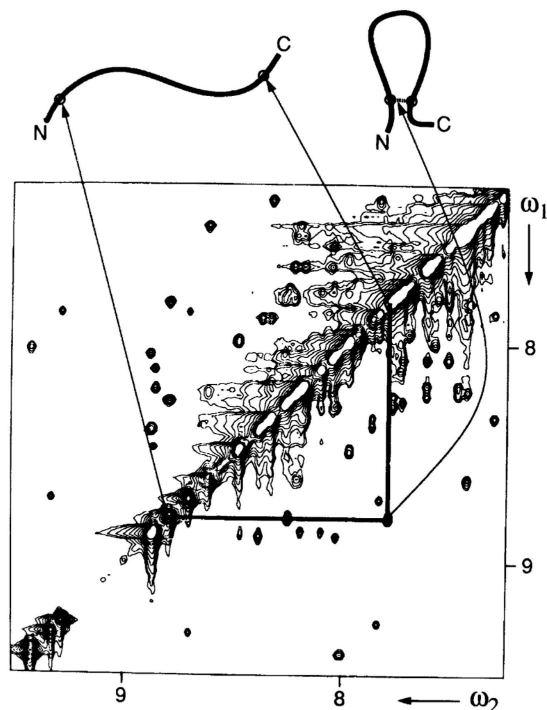


Figure 24 Scheme indicating the relations between an experimental 2D [^{1}H,^{1}H]-NOESY spectrum, a polypeptide with the chain ends indicated by N and C, sequence-specific assignments for two hydrogen atoms in the polypeptide chain indicated by circles, and the NOE upper distance constraint derived from the NOESY cross-peak connecting the chemical shift positions of the two assigned hydrogen atoms (see text).

Wüthrich, *J. Biomol. NMR,* **27:** 13-39, 2003

The Sequential Assignment Problem!

- <u>If the chemical shift of each proton is known</u>, every off-diagonal peak can be assigned to a short-distance interaction between two specific protons within the protein sequence.

- Peak volume or intensity relates to the interproton distance.

- Hundreds or thousands of inter-proton distance restraints are used to calculate three-dimensional structures that are consistent with the NOE data.

The Problem of Sequential Assignment

Solution: Use “through-bond” scalar couplings (as opposed to the “through-space” correlations that underlie the NOE) to trace from one nucleus to another.

Different strategies are utilized for small proteins versus larger proteins, where peak overlap (or chemical shift degeneracy) becomes more of a problem.

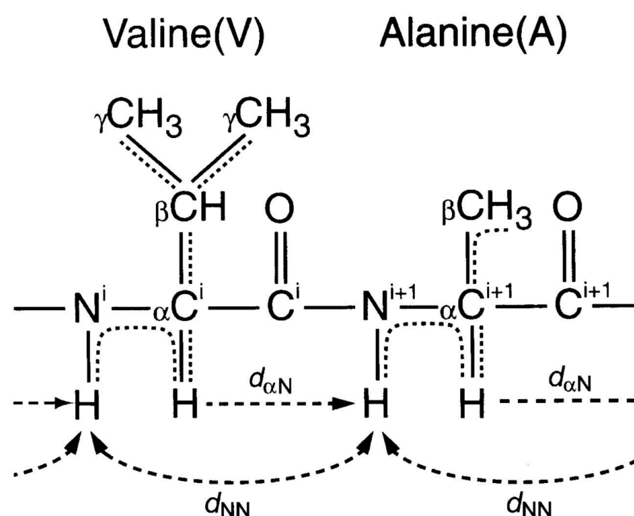


Figure 16 Sequential ^{1}H NMR assignment of proteins. The drawing shows the chemical structure of a – valine – alanine – dipeptide segment in a polypeptide chain. The dotted lines connect groups of hydrogen atoms that are separated by at most three chemical bonds and can therefore be connected using scalar spin–spin couplings. The broken arrows link pairs of hydrogen atoms in neighbouring amino acid residues that are separated by short through-space distances, $d_{\alpha N}$ and d_{NN}, and can therefore be connected by ‘sequential NOEs’.

Wüthrich, *J. Biomol. NMR,* **27:** 13-39, 2003

Sequential Assignment for proteins <15 kDa

▪Two-dimensional $^1H,^1H$-COSY spectrum shows correlations between protons connected through three or fewer bonds (indicated by ······, below left).

▪Each residue is a closed system in this experiment, called a “spin system”, isolated by the carbonyl.

Can usually identify a spin system as a particular amino acid type based on the number of resonances and their chemical shifts.

▪Spin systems are connected sequentially using short-range NOE correlations from a 2D NOESY spectrum, usually $d_{\alpha N}$ and d_{NN} (indicated by ------, below left).

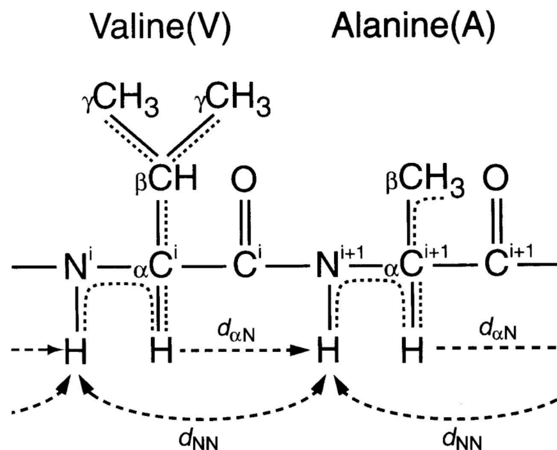


Figure 16 Sequential ^{1}H NMR assignment of proteins. The drawing shows the chemical structure of a – valine – alanine – dipeptide segment in a polypeptide chain. The dotted lines connect groups of hydrogen atoms that are separated by at most three chemical bonds and can therefore be connected using scalar spin–spin couplings. The broken arrows link pairs of hydrogen atoms in neighbouring amino acid residues that are separated by short through-space distances, $d_{\alpha N}$ and d_{NN}, and can therefore be connected by ‘sequential NOEs’.

Wüthrich, *J. Biomol. NMR,* **27:** 13-39, 2003

2D COSY

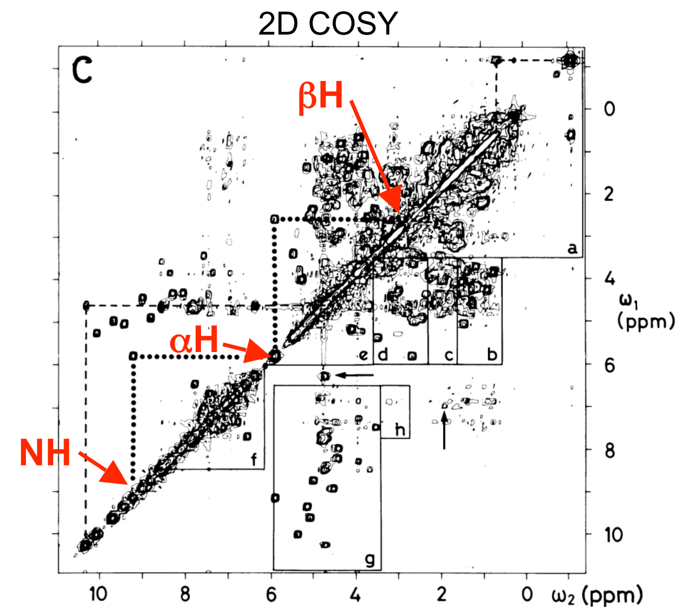


Figure 5.8. (*Continued*)

From “NMR of Proteins & Nucleic Acids, by K. Wüthrich, pp. 54-55

Sequential assignment for larger proteins (>15 kDa)

Two problems with larger proteins:

1. Many more protons lie in same spectral range, and peaks overlap.
2. Molecule tumbles more slowly as a whole, leading to broad peaks.

- Problem #1 can be overcome by labeling protein with other NMR-sensitive nuclei, such as ^{13}C and ^{15}N.
- Overcrowded spectra can then be spread out in additional dimensions.
- Accomplished by growing cells in a minimal growth medium with single carbon/nitrogen sources (*e.g.* ^{13}C-glucose and $^{15}NH_4Cl$ for *E. coli*).
- Disadvantage is the substantial cost of isotopic labeling.

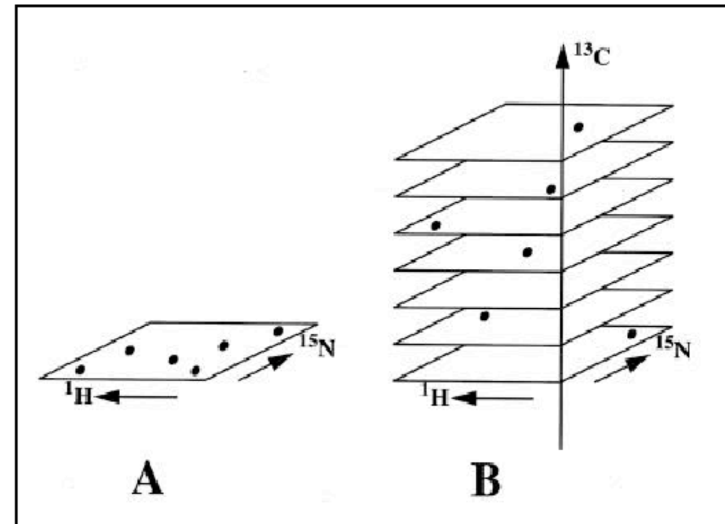


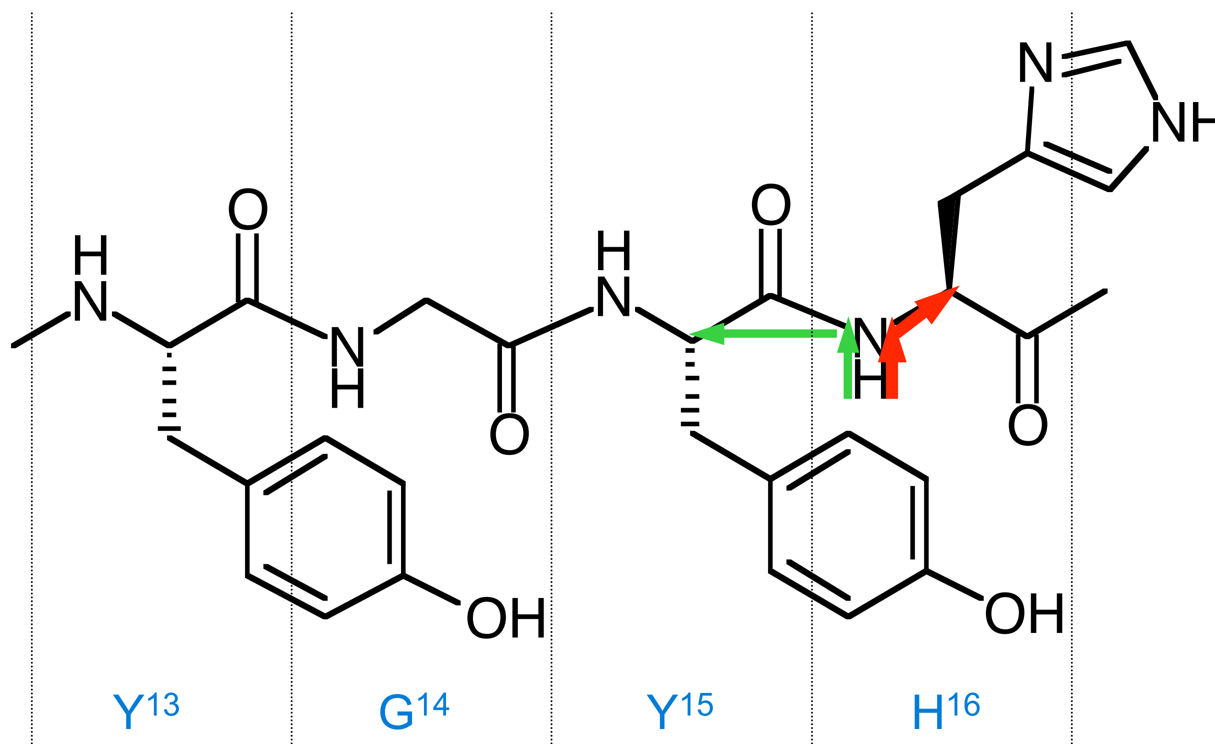
Figure 3. Schematic 2-D and 3-D NMR spectrum. The drawing illustrates the representation of a 3-D spectrum and the increased resolution obtained when going from 2-D to 3-D NMR spectra. The six peaks in the 2-D [^{1}H,^{15}N]-correlation spectrum (A) are separated in different planes of a 3-D spectrum (B) by an additional correlation with the α-carbon nuclei (^{13}C) attached to the nitrogen nuclei (^{15}N) in the same amino acid residue. The chemical shifts of the carbon nuclei are used to spread the resonances from the 2-D plane into a third dimension.

Wider, *Biotechniques,* **29:** 1278-1294, 2000

Sequential assignment for larger proteins (>10-15 kDa)

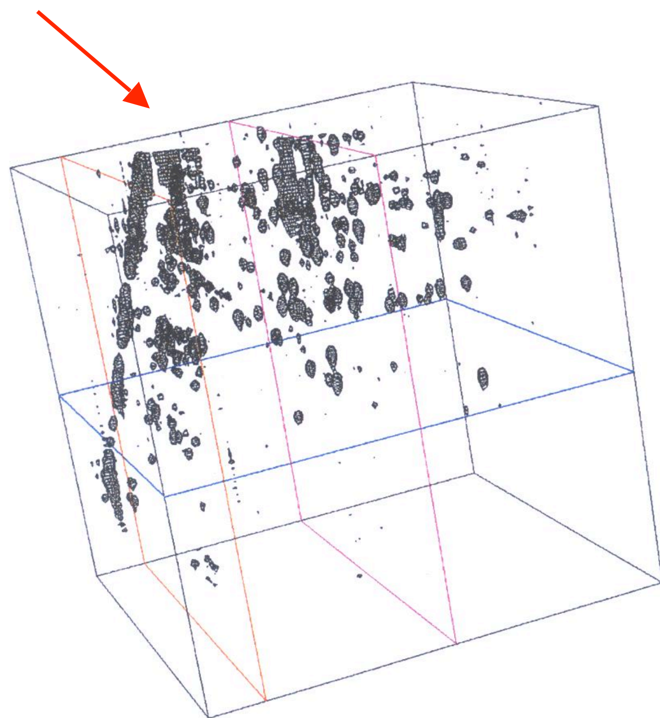
Carbon and nitrogen labeling enable tracing directly along backbone from one amino acid to the next via scalar “through-bond” couplings.

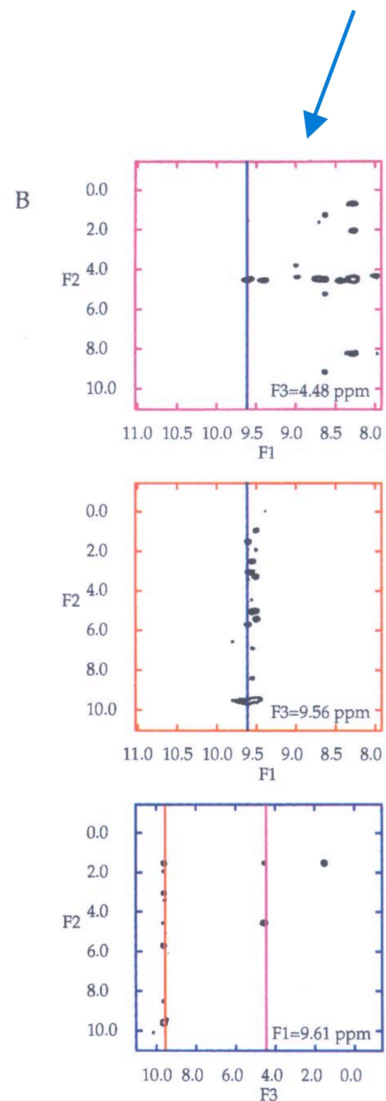
Example: An HNCA experiment yields a strong intra-residue correlation between the amide proton, nitrogen and alpha carbon, plus a weak correlation from the amide proton and nitrogen to the alpha carbon of the i-1 (preceding) residue.



Remove slices to analyze in 2D contour plots

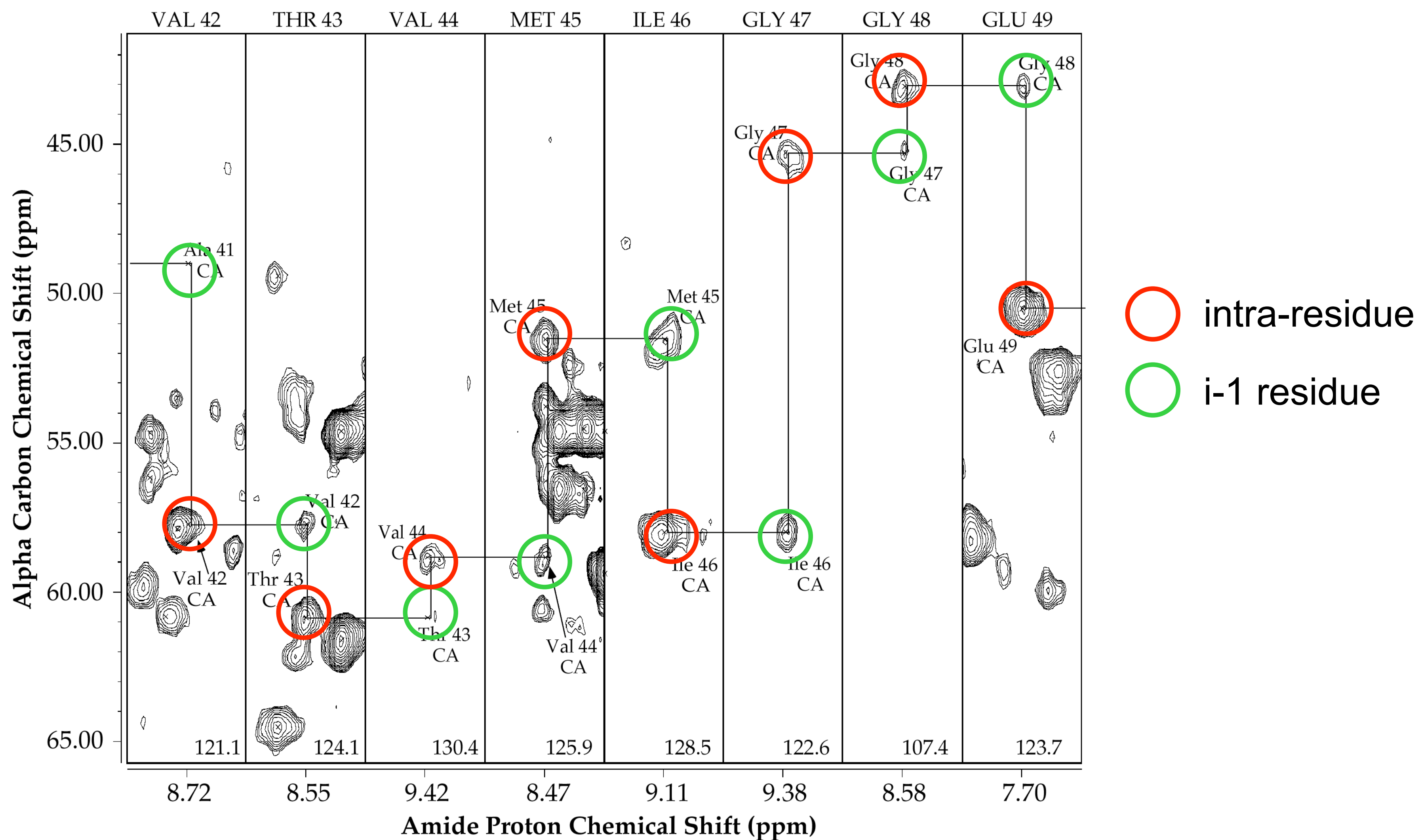
3D spectra are difficult to look at in 3D mode!





From “NMR Data Processing” by Hoch and Stern

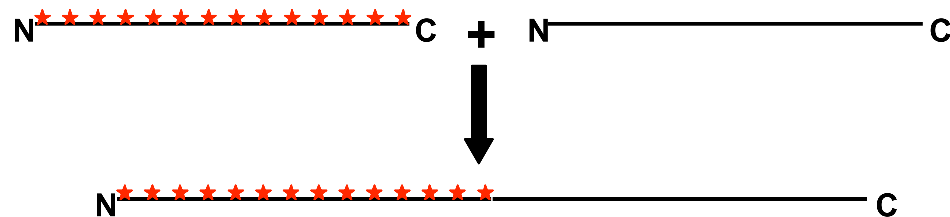
Slices from a 3D HNCA experiment



More tricks for even larger proteins (>25 kDa)

▪Segmental isotopic labeling can solve problems with peak overlap:

- •Two portions of protein are expressed separately, with only one isotopically labeled.
- •Two segments are then ligated *in vitro* to re-create the full-length protein.



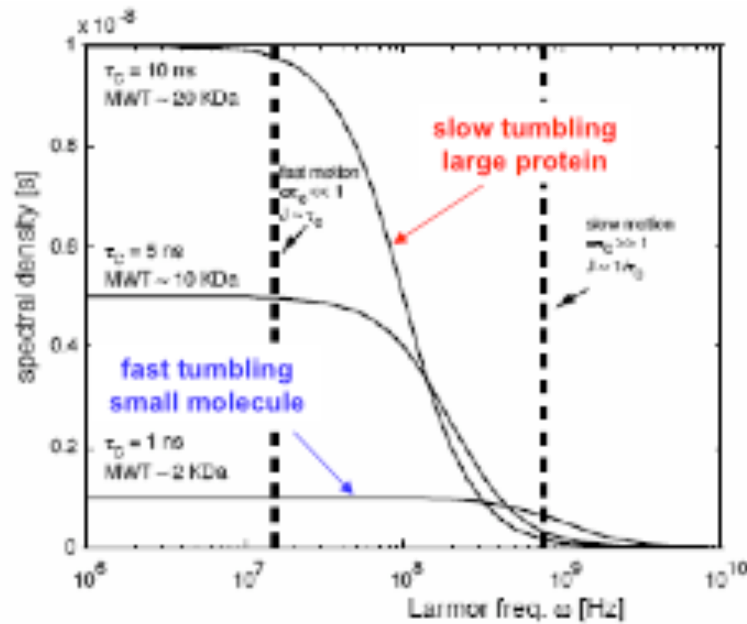
▪Partial labeling with deuterium slows relaxation of NMR signals, and can narrow peaks that are broad due to slow molecular tumbling.

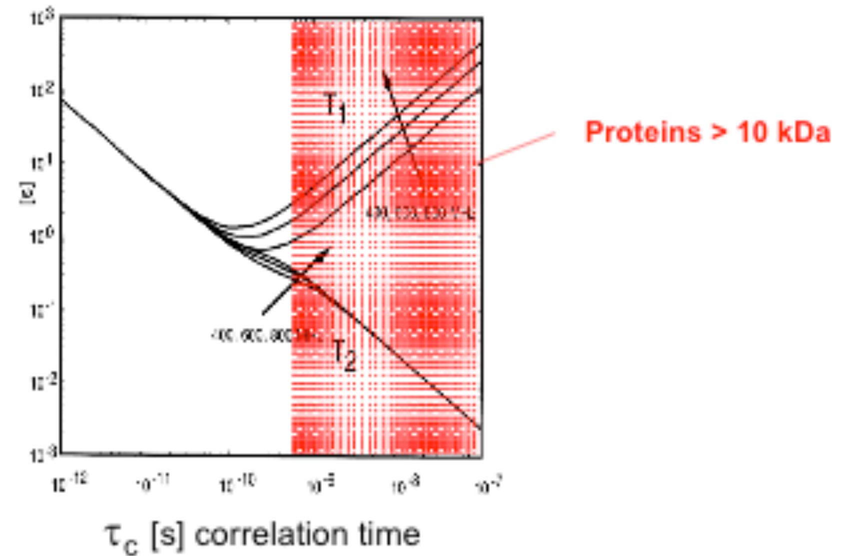
▪New TROSY and CRINEPT experiments give sharper peaks for very large proteins, especially with high-field spectrometers (900 MHz).

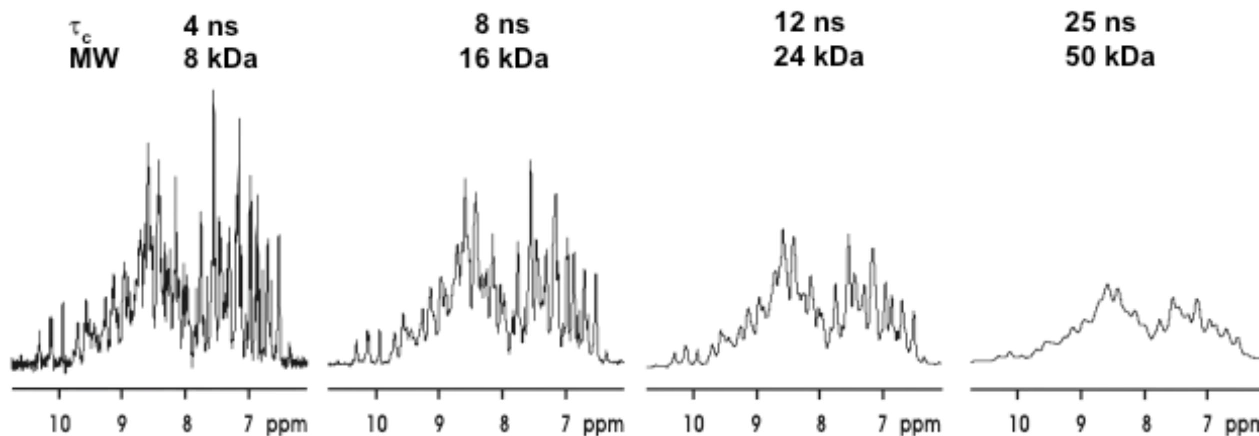
Some of the biggest proteins studied so far:

▪40 kDa hHR23a protein structure (Walters et al., *PNAS* 100:12694-12699, 2003)
▪42 kDa maltodextrin-binding protein global fold determined (Müller et al., JMB 300:197-212, 2000)
▪110 kDa aldolase octamer assigned (Salzmann et al., JACS 122:7543-7548, 2000)
▪81 kDa Malate Synthase G assigned (Tugarinov et al., JACS 124:10025-10035, 2002)
▪900 kDa GroEL/ES tetradecamer partially assigned (Fiaux et al., Nature 418:207-211, 2002)

Spin-spin relaxation becomes very efficient when tumbling is slow, leading to short T_2





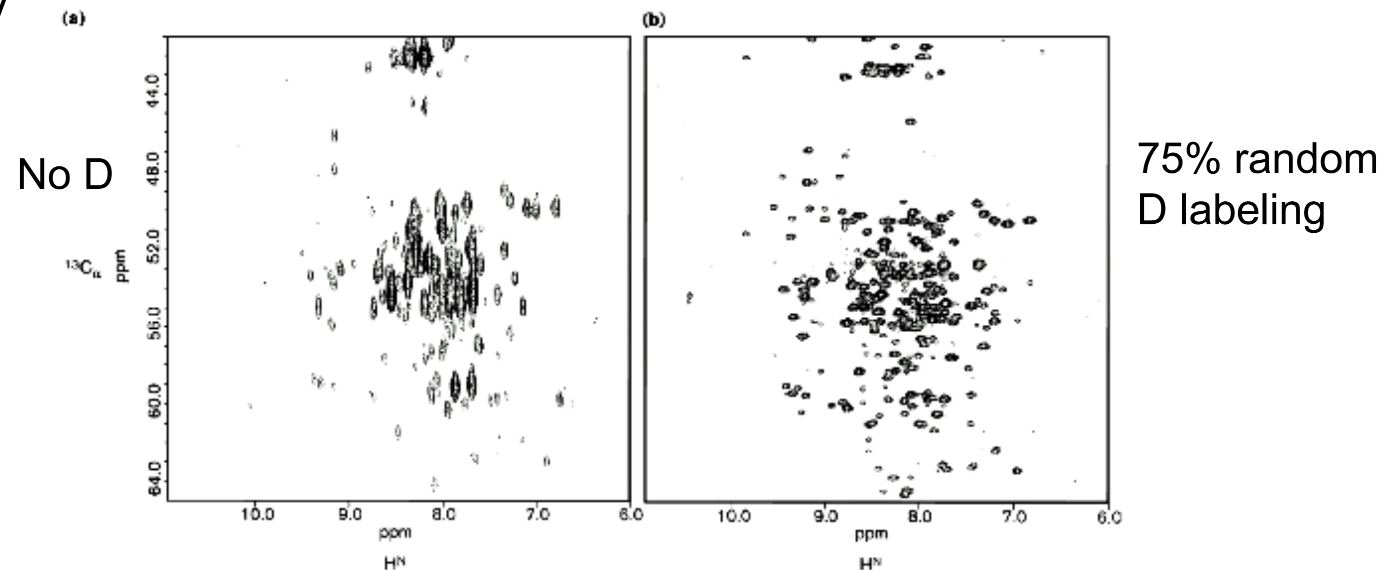


$$\Delta\upsilon_{1/2} = 1/\pi T_2$$

www.embl-heidelberg.de/**nmr**/sattler/ teaching/teaching_pdf/bio**nmr**_theo+appl.pdf

How to overcome broad peaks:

- Replacing most (or all non-labile) protons with deuterons reduces the primary contribution to relaxation: dipolar interactions with protons nearby

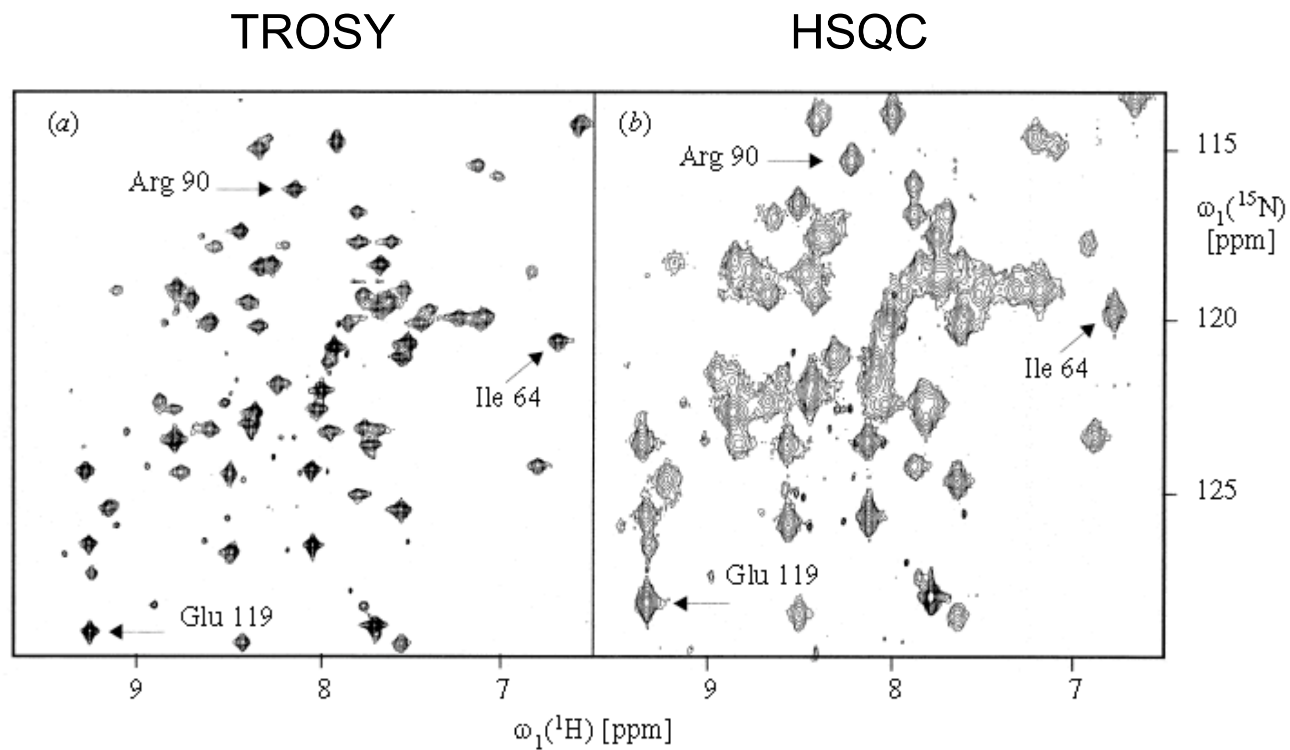


- Transverse relaxation-optimized spectroscopy (TROSY)

  Takes advantage of relaxation interference between chemical shift anisotropy and dipolar interactions to select for the narrowest component of a multiplet

Example: a 110 kDa protein complex at 750 MHz

TROSY HSQC



Pervushin, K., Q. Rev. Biophys., 33:161-197, 2000

Structure Calculation

Once assignments are complete (chemical shifts of most protons are known), NOESY peaks are interpreted as distance restraints between pairs of protons, starting with peaks that can be unambiguously assigned.

Assemble a list of distances between pairs of protons, called structural restraints.

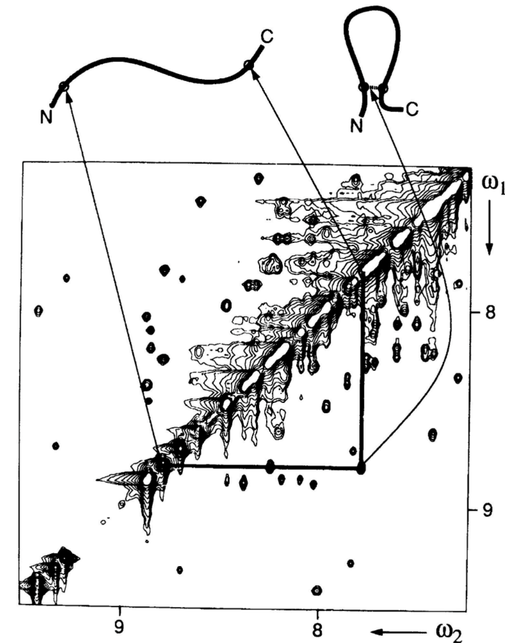
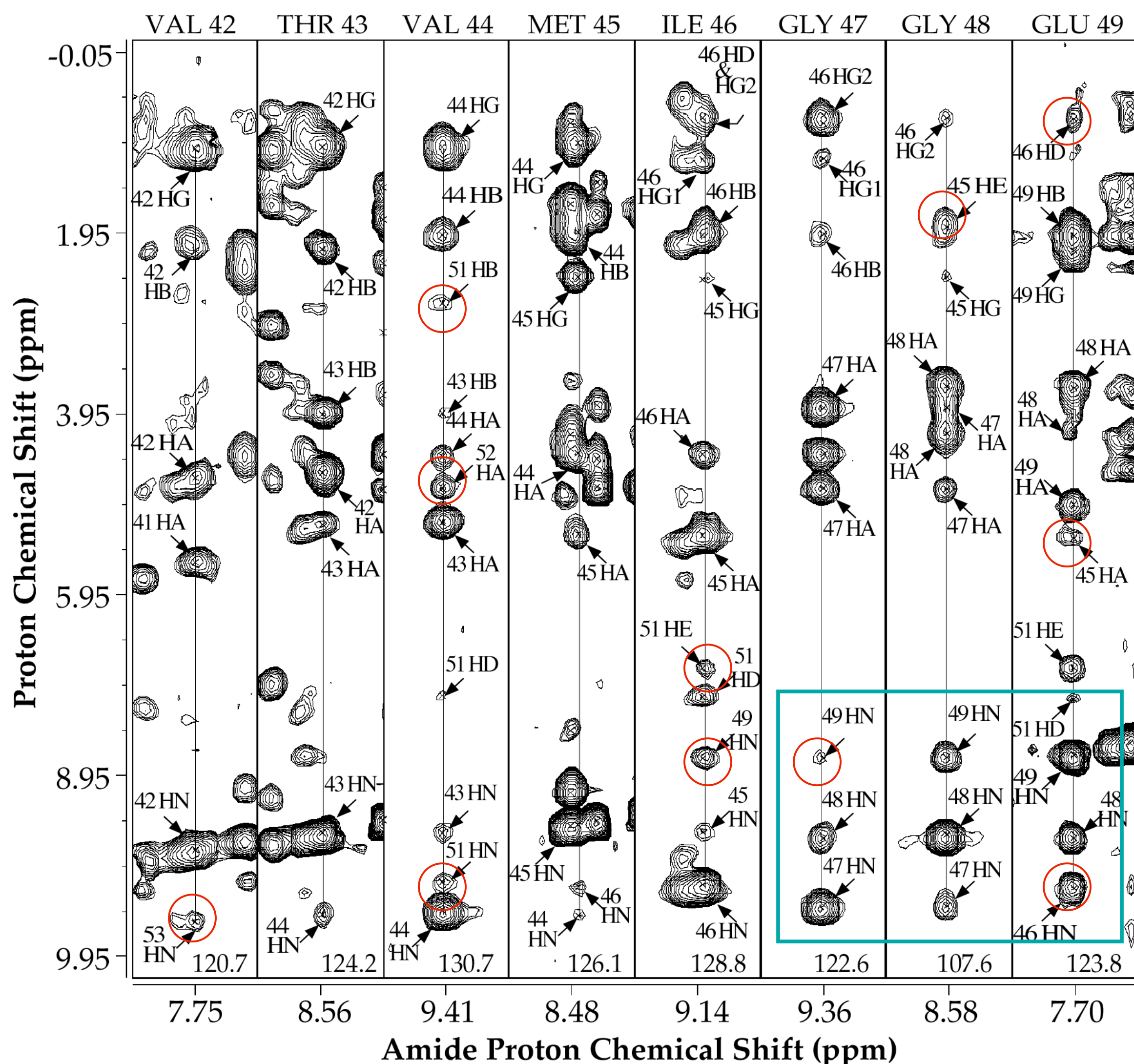


Figure 24 Scheme indicating the relations between an experimental 2D [^{1}H,^{1}H]-NOESY spectrum, a polypeptide with the chain ends indicated by N and C, sequence-specific assignments for two hydrogen atoms in the polypeptide chain indicated by circles, and the NOE upper distance constraint derived from the NOESY cross-peak connecting the chemical shift positions of the two assigned hydrogen atoms (see text).

Sample slices from a 3D 15N-edited NOESY experiment

- Initially, not all peaks can be unambigously assigned
- Peak volumes are related to the inverse sixth power of the distance between the two protons
- Volumes are hard to accurately measure in crowded regions of spectrum



NOEs between residues that are distant in protein sequence are extremely important restraints for structure calculation

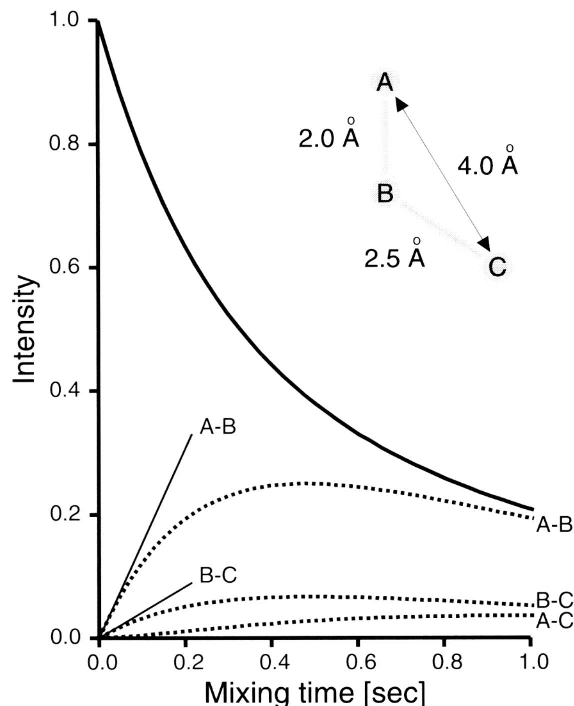
Sequential HN-HN NOEs indicate turn or helix

Sources of error in NOE restraints

•Incorrect volume of peak due to overlap
•Mixing time in pulse sequence too long - spin diffusion occurs

(Thus NOE restraints are given wide distance ranges, or are merely classified as strong, medium and weak)

•Incorrect assignment (will hopefully become clear later)



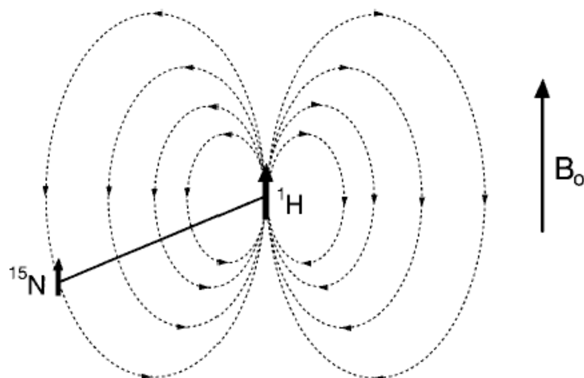
Spin diffusion

The longer the mixing time, the more likely that magnetization mixes from A to B and then from B to C, ultimately resulting in a A-C peak that is larger than it ought to be and an A-B peak that is smaller than it ought to be.

Fundamentals of Protein NMR Spectroscopy, by Rule and Hitchens, Springer 2006

Residual dipolar couplings (RDCs): a new structural restraint that rivals the NOE

A nearby dipole affects the local net magnetic field, and depends on whether the dipole is oriented with or against the external magnetic field.



The degree of coupling depends on the orientation of the internuclear vector - maximum when parallel to B_o

$$D^{PQ} = D^{PQ}{}_{max} \langle (3\cos^2\theta - 1)/2 \rangle,$$

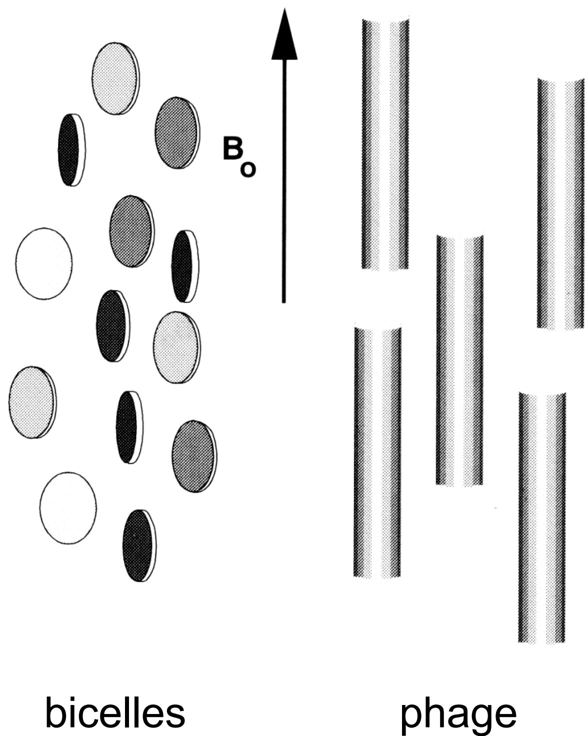
In solution, protein tumbling averages the dipolar coupling to zero, since all molecular orientations with respect to the external magnetic field are possible.

The dipolar couplings can be reintroduced by partial alignment of protein molecules in solution. In a slightly anisotropic environment, the orientational distribution of the proteins is no longer random. In such an environment, the large one-bond inter-nuclear dipolar interactions no longer average to zero and report on the average orientation of the corresponding vectors relative to the magnetic field.

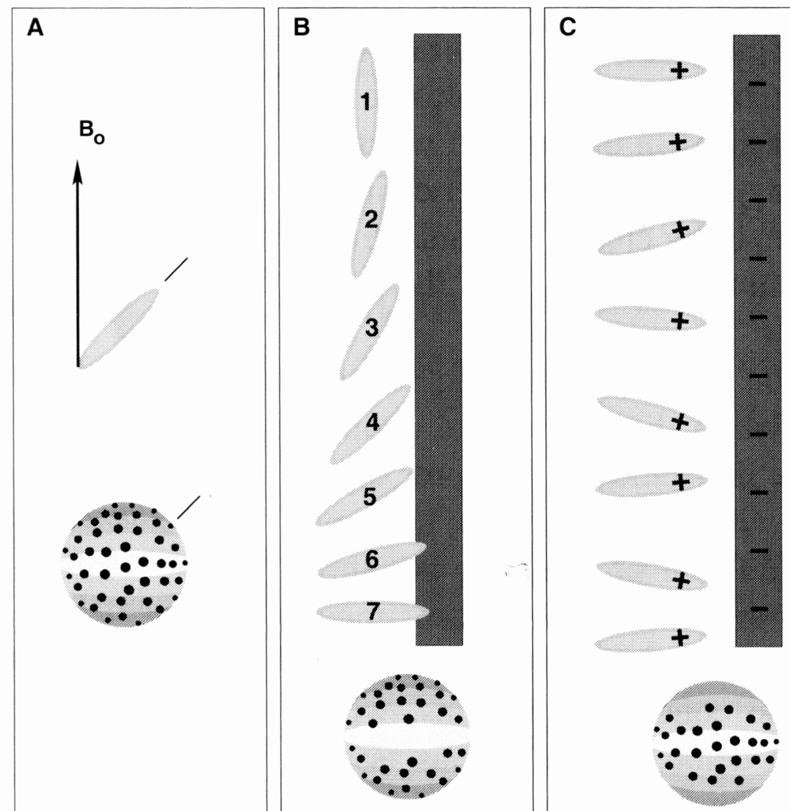
Bax, A., Protein Sci 12:1-16, 2003

Partial alignment is accomplished by adding bicelles, filamentous phage, or aqueous nematic liquid crystalline suspensions into protein solution, or by incorporating protein into anisotropically compressed hydrogels

Weak interaction of protein with alignment media causes some molecular orientations to be disfavored, others favored.



Fundamentals of Protein NMR Spectroscopy
by Rule and Hitchens, Springer 2006



no alignment | Bicelles (mechanical) | Phage (electrostatic)

Bax, A., Protein Sci 12:1-16, 2003

Simple data collection: HSQC without proton decoupling during ^{15}N chemical shift evolution, collected with and without alignment media

When aligned, splitting of peaks corresponds to the scalar coupling constant plus the dipolar coupling: $J_{NH} + D_{NH}$

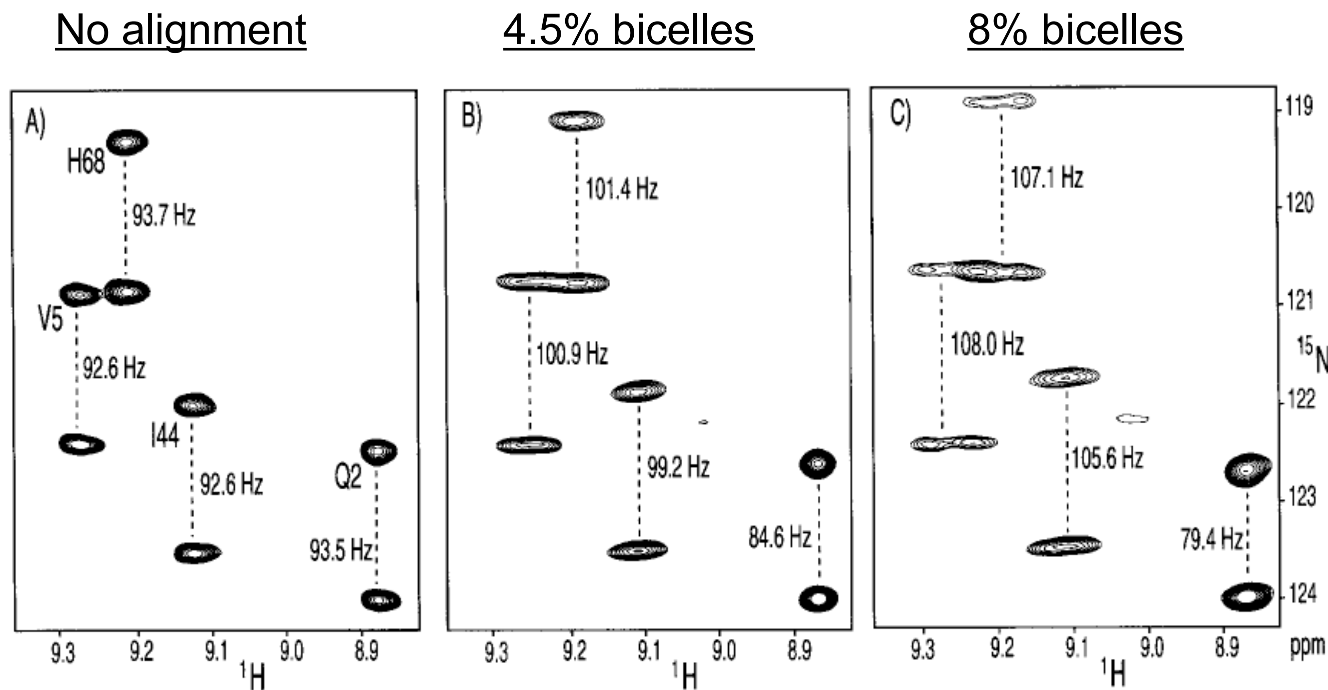
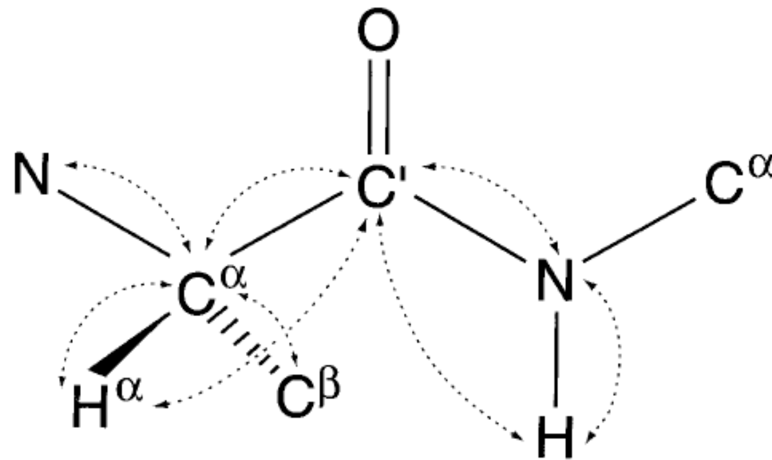


Figure 4. Small regions of the 600 MHz ^{15}N-^{1}H correlation spectra of ubiquitin, recorded in the absence of ^{1}H decoupling in the ^{15}N dimension, at three different levels of molecular alignment. (*A*) Isotropic spectrum, with the marked splitting corresponding to $^{1}J_{NH}$. (*B*) Spectrum recorded in 4.5% (w/v) bicelles, consisting of a 30:10:1 molar ratio of DMPC, DHPC, and cetyl-trimethyl ammonium bromide (CTAB). (*C*) Spectrum recorded in 8% (w/v) bicelles. Marked splittings in panels *B* and *C* correspond to the sum of the $^{1}J_{NH}$ and dipolar coupling. The broadening in the ^{1}H dimension, observed in panels *B* and *C* relative to *A* is caused by ^{1}H-^{1}H dipolar couplings.

Bax, A., Protein Sci 12:1-16, 2003

Many internuclear vectors can be measured using partial alignment



Residual dipolar couplings are especially useful for orienting domains of known structure in a multidomain protein, or for orienting proteins that interact.

Bax, A., Protein Sci 12:1-16, 2003

Other restraints are sometimes incorporated into structure calculation:

▪Residual dipolar couplings (RDCs) measured in weakly-aligned samples give the angle of bond vectors with respect to the magnetic field (and therefore to each other).

increasingly used, some recent structures rely more on RDCs than NOEs.

▪Dihedral angles calculated from scalar coupling constants (ϕ, φ, χ_1)

▪Hydrogen bond restraints

1. From hydrogen exchange measurements: Simplest method is to dissolve lyophilized protein into D2O-containing buffer, and monitor loss of amide protons as they exchange for deuterons by collecting successive experiments. Hydrogen-bonded amides will exchange very slowly.
2. Measured directly *via* very weak scalar coupling across hydrogen bond

▪Chemical shift data: alpha proton and alpha, beta and carbonyl carbon chemical shifts have been empirically related to ϕ/φ dihedral angles

Structure Calculation

- Full list of unambiguous structural restraints are input into distance geometry or simulated annealing protocol
  - a set of 30-100 structures are calculated that are consistent with restraints
  - structures are refined by restrained molecular dynamics or energy minimization
- Initial structures are usually of poor quality due to inadequate numbers of NOEs (or incorrectly assigned NOEs).
  - initial structures help to assign NOEs that were previously ambiguous, and to fix incorrect ones.
- Repeat this process iteratively. 15-25 “best” structures are selected for NMR model.

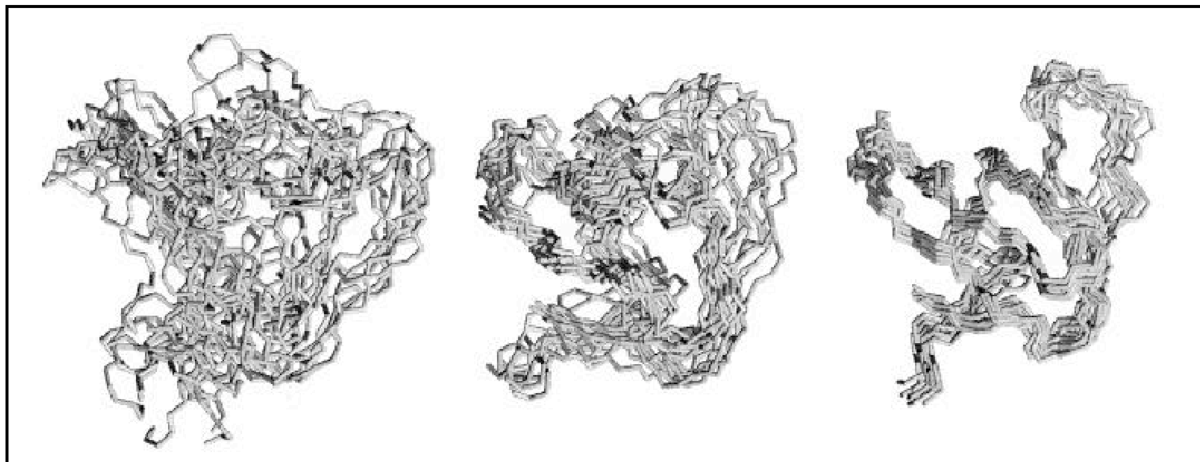


Figure 6. Protein backbone structures calculated with different numbers of NMR constraints. The structures show the SH_3 domain of human p56 Lck tyrosine kinase (26) at various stages of the assignment of additional distance constraints on the basis of preliminary structures (courtesy M. Salzmann). Backbone superpositions of ten conformers are shown with 1113 constraints (left), 1336 (middle) and 1687 constraints (right). (The RMSD between the positions of the polypeptide backbone atoms in the different conformers of the three bundles of structures are 4.2, 1.9 and 1.1 Å, respectively).

Wider, *Biotechniques,* **29:** 1278-1294, 2000

Assessing Structural Quality

1998 IUPAC Task Force recommended the following structural statistics be reported:

1. Number and type of NOEs used {intraresidue, sequential, medium range (≤5 residues apart), long range (>5 residues apart), intermolecular}
2. Number of torsion angle restraints
3. Number of hydrogen bond restraints
4. Maximum restraint violation and the average violation per constraint
5. Deviations from idealized geometry (*i.e.,* unusual bond lengths or bond angles)
6. Precision of structures: RMSD with respect to the mean structure (backbone versus all heavy atoms)
7. Percentage of residues falling into allowed regions of $\phi\varphi$ space

RMSD: root mean square deviation (in Å)

1. Calculate a mean structure from the ensemble of *n* structures by averaging the position of each atom in all the structures. The average structure is then energy-minimized to fix all the problems with bond angle/length, etc.

2. Calculate rmsd relative to this mean structure:

   For each atom, measure the distance, *r*, between its position in structure *i* and the mean structure.

$$rmsd = \left(\sum_{i=1}^{n} r_i^2 \Big/ n \right)^{1/2}$$

   This gives an rmsd for each atom in the protein.

3. For “heavy atom rmsd”, average the rmsds for all the non-hydrogen atoms.

   For global rmsd, average all atomic rmsds.

Assessing Structural Quality

1998 IUPAC Task Force recommended the following structural statistics be reported:

1. Number and type of NOEs used {intraresidue, sequential, medium range (≤5 residues apart), long range (>5 residues apart), intermolecular}
2. Number of torsion angle restraints
3. Number of hydrogen bond restraints
4. Maximum restraint violation and the average violation per constraint
5. Deviations from idealized geometry (*i.e.,* unusual bond lengths or bond angles)
6. Precision of structures: RMSD with respect to the mean structure (backbone versus all heavy atoms)
7. Percentage of residues falling into allowed regions of $\phi\varphi$ space

Ramachandran Plot showing allowed regions of ϕφ space

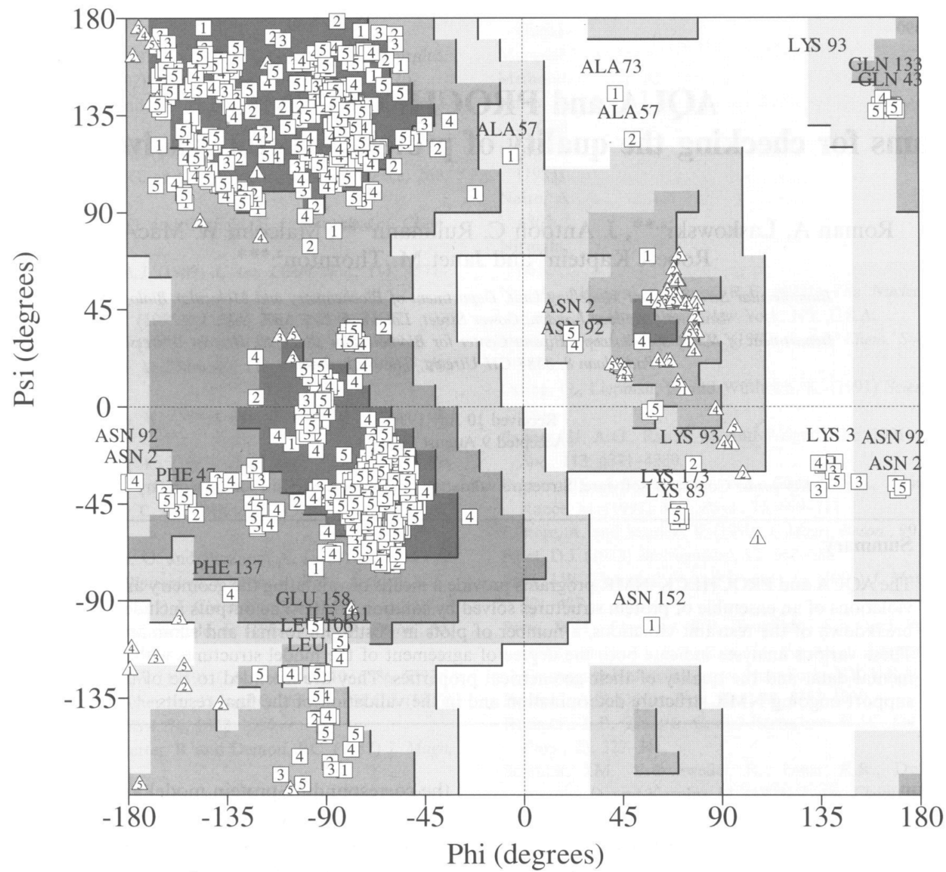


Fig. 1. The Ramachandran plot shows the distribution of ϕ–ψ values for all the residues in the structure. Here, only models 1 to 5 have been selected from the entire ensemble of 25 models. Each data point is labelled with its model number, while the names of any residues in disallowed regions of the Ramachandran plot are printed above their respective points. The shading indicates the favourable and unfavourable regions of the plot, the darker the shading the more favourable the region. A separate plot can be generated for each model in the ensemble, and even for each residue (see Fig. 2).

Black: favored regions
Dark Grey: additionally-allowed regions
Light Grey: generously-allowed regions
White: disallowed regions

Laskowski *et al.*, *J. Biomol. NMR,* **8:** 477-486, 1996

Assessing Structural Quality

1998 IUPAC Task Force recommended the following structural statistics be reported:

1. Number and type of NOEs used {intraresidue, sequential, medium range (≤5 residues apart), long range (>5 residues apart), intermolecular}
2. Number of torsion angle restraints
3. Number of hydrogen bond restraints
4. Maximum restraint violation and the average violation per constraint
5. Deviations from idealized geometry (*I.e.,* unusual bond lengths or bond angles)
6. Precision of structures: RMSD with respect to the mean structure (backbone versus all heavy atoms)
7. Percentage of residues falling into allowed regions of $\phi\varphi$ space

1 and 6 are the best indicators of structural quality.

Goal:
1. 15-20 restraints per residue
2. 0.6Å rmsd for backbone atoms, 1.0Å rmsd for heavy atoms

Comparing NMR structures and crystal structures

Very rough rule of thumb (with many many exceptions): an NMR structure calculated with ≥20 restraints per residue is equivalent to a 2-2.5Å crystal structure

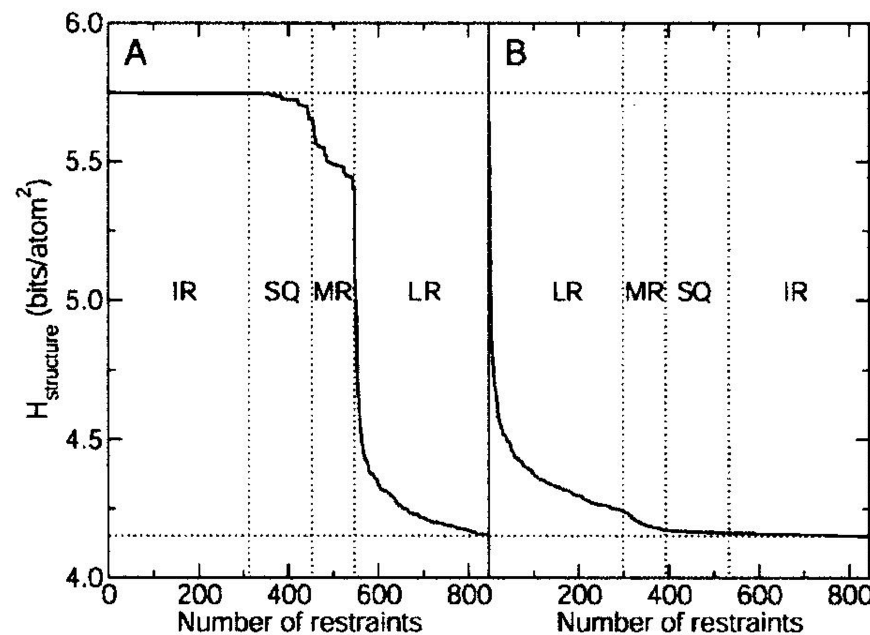


Figure 1. The structural uncertainty, $H_{structure}$, of the IgG-binding domain of protein G as a function of the number of distance restraints incorporated. The interproton distance restraints are grouped into four sets: intraresidual restraints (IR), sequential restraints (SQ), medium-range restraints (MR), and long-range restraints (LR). Two different orders of addition of the experimental data are shown: (A) IR-SQ-MR-LR and (B) LR-MR-SQ-IR.

Nabuurs *et al.*, *J. Am. Chem. Soc.*, **125:** 12026-12034, 2003

But… long range restraints are much more important than medium range, sequential or intraresidue ones for making a high quality NMR structure

Comparing two NMR structures

19.5 restraints/residue

30% medium-long restraints

Table 1 Summary of restraints and structural statistics

Restraints		
NOEs[1]		
Intraresidue	1,474	
Sequential	429	
Medium range (i < 5)	341	
Long range	460	
Unambiguous	2,699	
Ambiguous	5	
Total NOEs	2,704	
Others		
ϕ,ψ	150	
Hydrogen bonds[2]	48 (× 2)	
Total number of restraints	2,948	
Deviations from experimental	<SA>[3]	Lowest Energy
R.m.s. deviation of NOE	0.020 (0.001)	0.018
NOE violations > 0.3 Å	0.4 (0.6)	0
ϕ,ψ violations > 5°	0.3 (0.5)	0
Deviations from ideal geometry		
Bonds (Å)	0.0018 (0.0001)	0.0016
Angles (°)	0.360 (0.006)	0.351
Impropers (°)	0.277 (0.011)	0.270
Precision		
Backbone helices[4]	0.43 (0.07)	
Heavy atoms helices[5]	0.91 (0.10)	
Heavy atoms 10–34, 48–136	1.09 (0.17)	
Structure quality		
Procheck (%; mf / aa / ga / da)[6]	74 / 18 / 6 / 2	77 / 17 / 4 / 2
WhatIf[7]	-1.80 (0.07)	-1.79
X-PLOR energy[8]	163.1 (6.5)	149

[1]NOEs were counted with explicit inclusion of all H atoms of methyl and methylene groups (that is, no pseudoatoms). Trivial distances were not included.
[2]Hydrogen bonds were included as a restraint of 1.5 (0.8) Å between HN_i and O_{i-3} atoms and a restraint of 2.5 (0.8) Å between N_i and O_{i-3} for those residues whose amides where determined to be in slow to intermediate exchange within helices. The value in parentheses is the upper bound on the restraint.
[3]Values are reported as the average values over 15 of the lowest energy structures with standard deviations in parentheses.
[4]The average r.m.s. deviation for the coordinate set was calculated by superimposing each of the 15 structures onto the mean coordinate set. This superposition was over backbone N, C, O and Cα atoms of residues 13–20, 27–30, 48–66, 79–92, 100–114, 120–133.
[5]This superposition was over nonhydrogen atoms of residues 13–20, 27–30, 48–66, 79–92, 100–114, 120–133.
[6]Procheck analysis[39]: mf, most favored; aa, additionally allowed; ga, generously allowed; da, disallowed.
[7]WhatIf score (QUACHK)[15].
[8]Energy calculated from X-PLOR 3.851[38] with force constants of 50 kcal mol^{-1} for the NOE restraints and 200 kcal mol^{-1} rad^{-2} for the torsion angle restraints. All other force constants used were the default values.

Altieri *et al.*, *Nat. Struct. Biol.*, **7:** 470-474, 2000

~ same precision

11.7 restraints/residue

45% medium-long restraints

Table 1 Structural statistics for LC1

Data set	<SA>[1]	$(SA)_r$[2]
R.m.s. deviations with respect to mean for residues 3–197		
Heavy backbone atoms (Å)	0.61 ± 0.11	
All heavy atoms (Å)	1.10 ± 0.10	
Number of experimental restraints		
Interresidue sequential (|i - j| = 1)	764	
Interresidue medium range (1 < |i - j| ≤ 5)	491	
Interresidue long range (|i - j| > 5)	551	
Meaningful intraresidue	513	
Hydrogen bonds[3]	103	
Dihedral angles	165 ϕ, 85 ψ, 16 χ_1	
Restraint violations[4]		
NOE distances with violations >0.3 Å	0.4 ± 0.7	0
Dihedrals with violations >3°	0.4 ± 0.6	0
R.m.s. deviations for experimental restraints[5]		
All distance restraints (2319) (Å)	0.022 ± 0.001	0.025
Torsion angles (266) (°)	0.331 ± 0.059	0.278
X-PLOR energies from simulated annealing[6]		
F_{noe} (kcal mol^{-1})	63.9 ± 7.9	48.2
F_{tor} (kcal mol^{-1})	1.8 ± 0.6	1.2
F_{repel} (kcal mol^{-1})	159.1 ± 9.8	142.5
E_{L-J} (kcal mol^{-1})[7]	-251.6 ± 30.0	-263.8
R.m.s. deviations from idealized covalent geometry		
Bonds (Å)	0.004 ± 0.000	0.004
Angles (°)	0.489 ± 0.012	0.457
Impropers (°)	0.390 ± 0.016	0.390
Ramachandran analysis (residues 1–198)		
Residues in favored regions (%)	67.1 ± 1.6	68.9
Residues in additional allowed regions (%)	30.0 ± 1.8	29.0
Residues in generously allowed regions (%)	2.7 ± 1.0	2.1
Residues in disallowed regions (%)	0.2 ± 0.3	0

[1]<SA> represents the 17 structures calculated in DYANA and refined by simulated annealing in X-PLOR using energy terms for NOE distance restraints, dihedral angle restraints, bonds, angles, impropers and hard sphere van der Waals contacts.
[2]$(SA)_r$ represents the structure calculated by restrained minimization of the mean of the <SA> structures.
[3]Two distance restraints per hydrogen bond were used, providing a total of 206 restraints.
[4]No distance restraints were violated by >0.5 Å, and no dihedral angles were violated by >5°.
[5]The number of restraints for each experimental class is shown in parentheses.
[6]The force constants used for the potential energy terms were 50 kcal mol^{-1} $Å^{-2}$ for F_{noe} and 200 kcal mol^{-1} rad^{-2} for F_{tor}. The hard sphere van der Waal repulsion term F_{repel} was calculated with a force constant of 4 kcal mol^{-1} $Å^{-2}$ with the van der Waals radii set to 0.8 times the value in the CHARMM-19 parameter set.
[7]The Lennard-Jones 6-12 energy was calculated within QUANTA (Molecular Simulations, Inc.) using the CHARMM-22 parameters.

Wu *et al.*, *Nat. Struct. Biol.*, **7:** 575-579, 2001

Take-home points:

1. NMR structures are built up from many short discrete distance restraints, primarily utilizing NOE data.

2. NOEs result from dipole-dipole interactions between protons close in space, and the closer the protons, the more intense the NOE peak.

3. Medium-to-long range restraints (those between non-adjacent residues) are crucial for calculating a high-quality structure.

3. Deposited structures are typically the result of 20-30 structure calculations, and the better they overlay (low RMSD), the higher the structural quality.