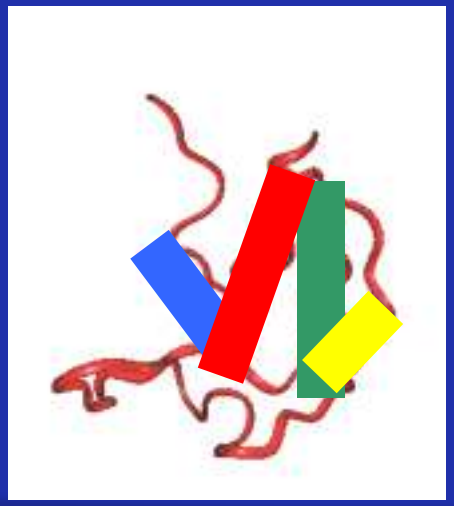
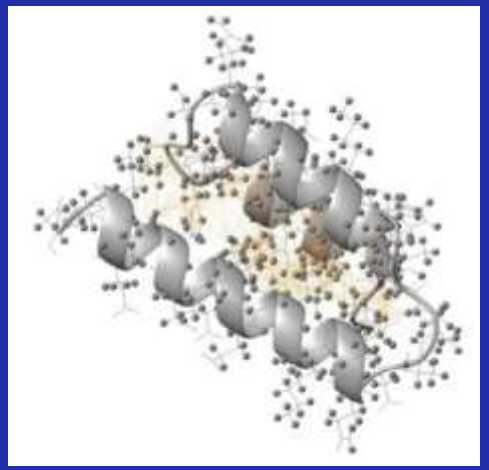
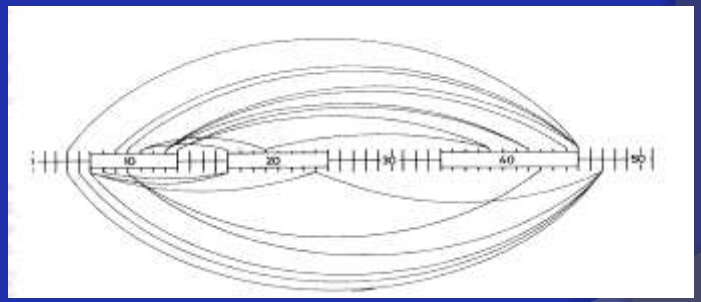
蛋白质核磁共振结构解析示意图

Unfolded



Correctly folded

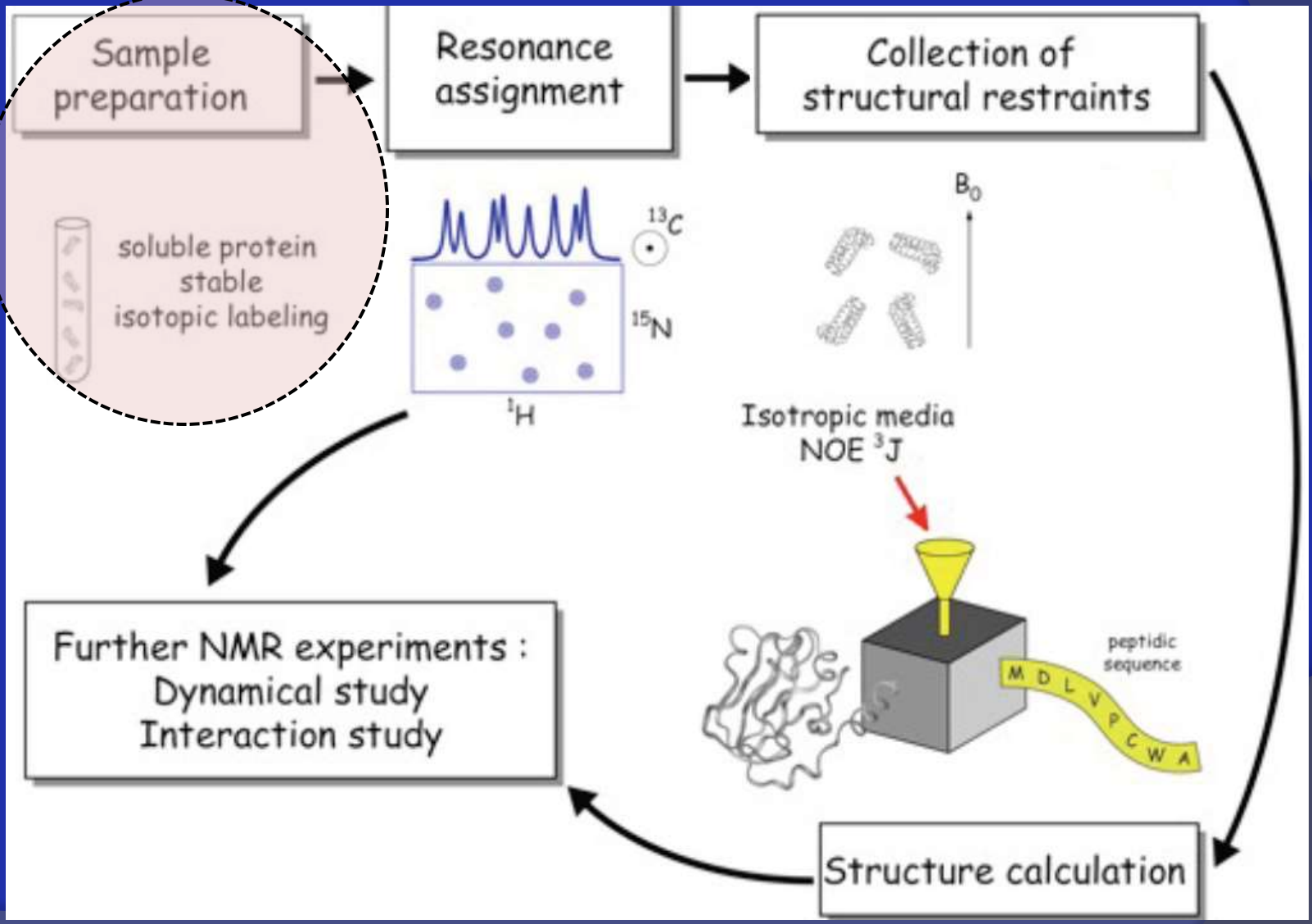




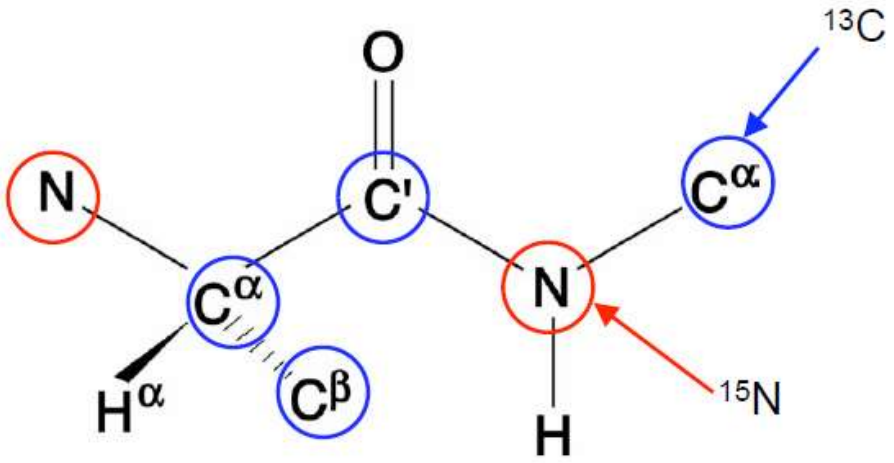
Schematic showing long range nOes in the *lac* headpiece protein

蛋白质核磁共振流程

同位素标记



How to label proteins with stable isotopes?



^{15}N labeling: use ^{15}N-labeled ammonium chloride as a source for nitrogen.

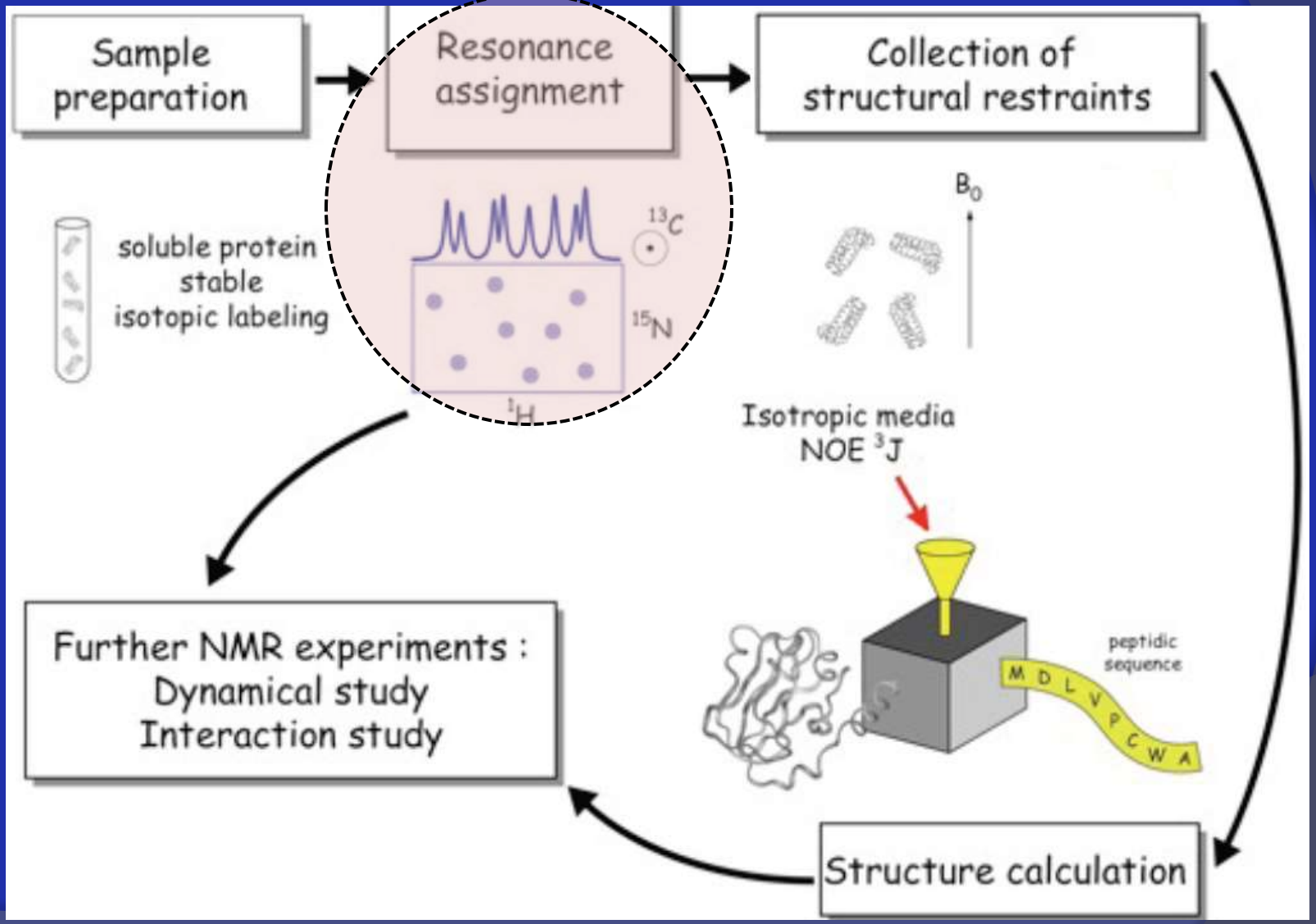
^{13}C labeling: use ^{13}C-labeled glucose as a source for carbon.

^{2}H labeling: replace H_2O with D_2O.

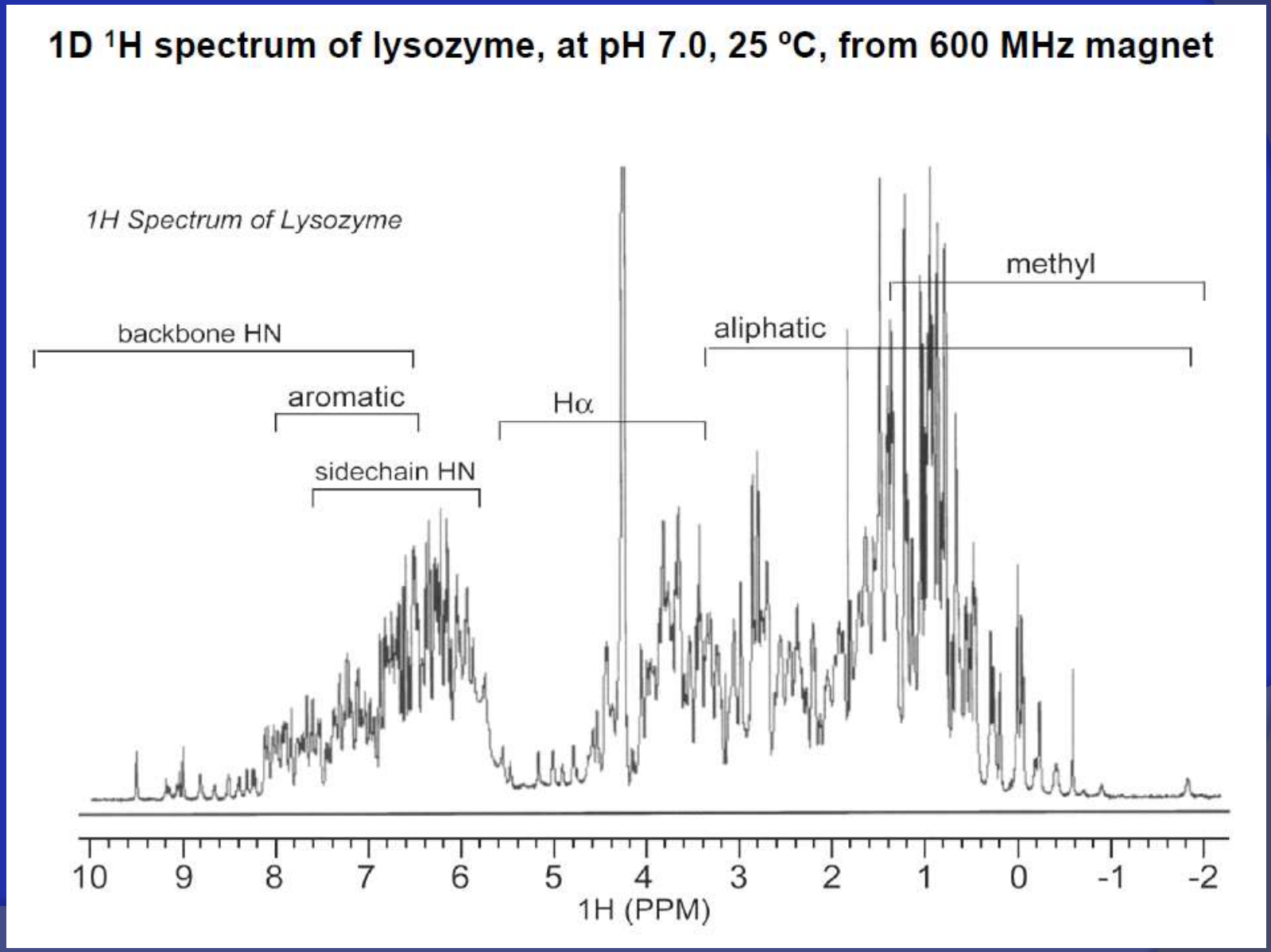
Selective amino acid labeling: use cells that cannot make the amino acids you want to label and feed the cells with ^{15}N / ^{13}C-labeled amino acids.

蛋白质核磁共振流程

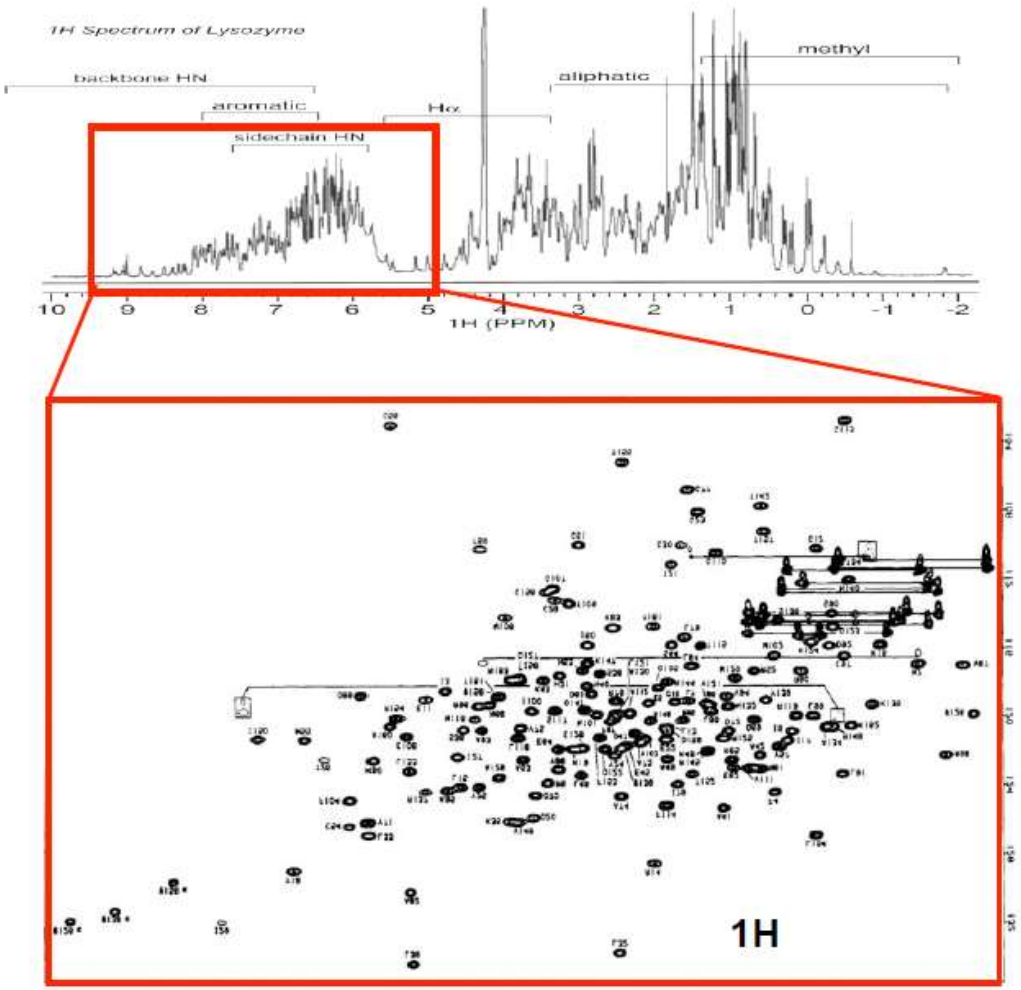
骨架信号归属
支链信号归属



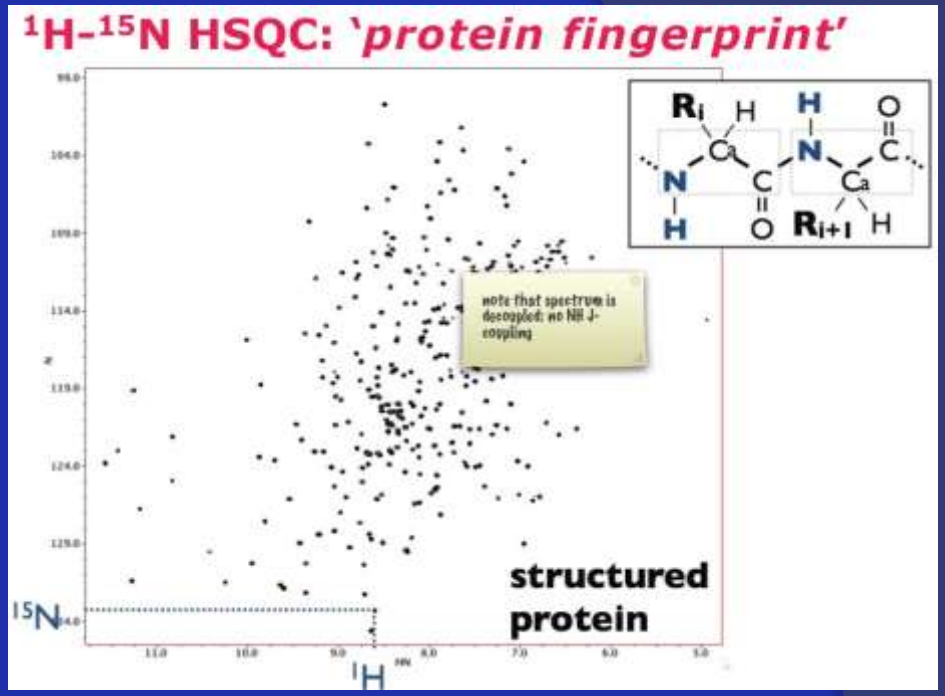
蛋白质核磁共振信号归属的基本步骤

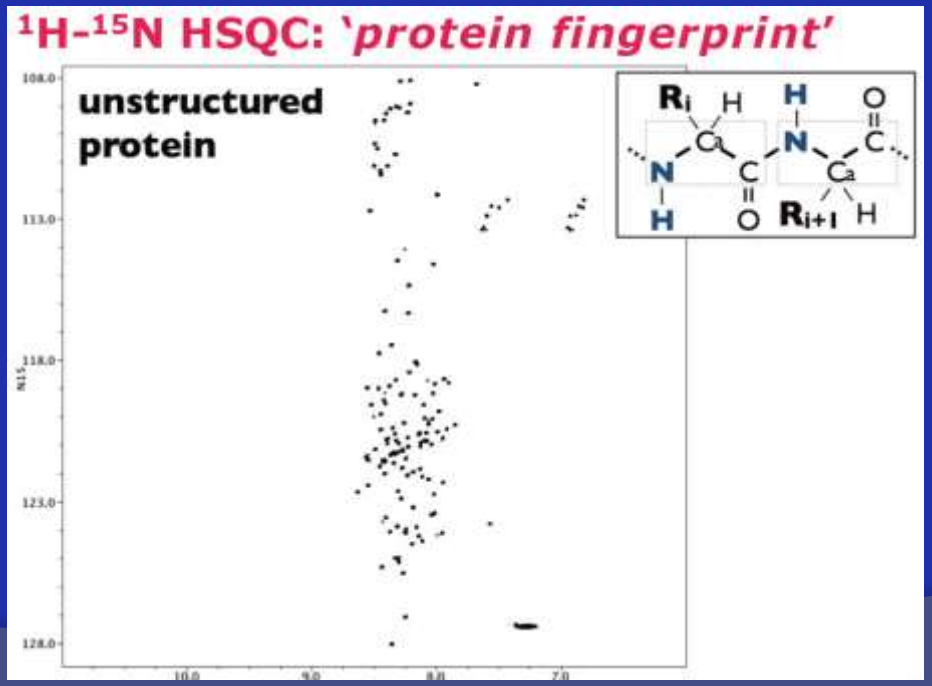


2D ^{1}H - ^{15}N correlation spectrum of lysozyme (HSQC)



蛋白指纹区

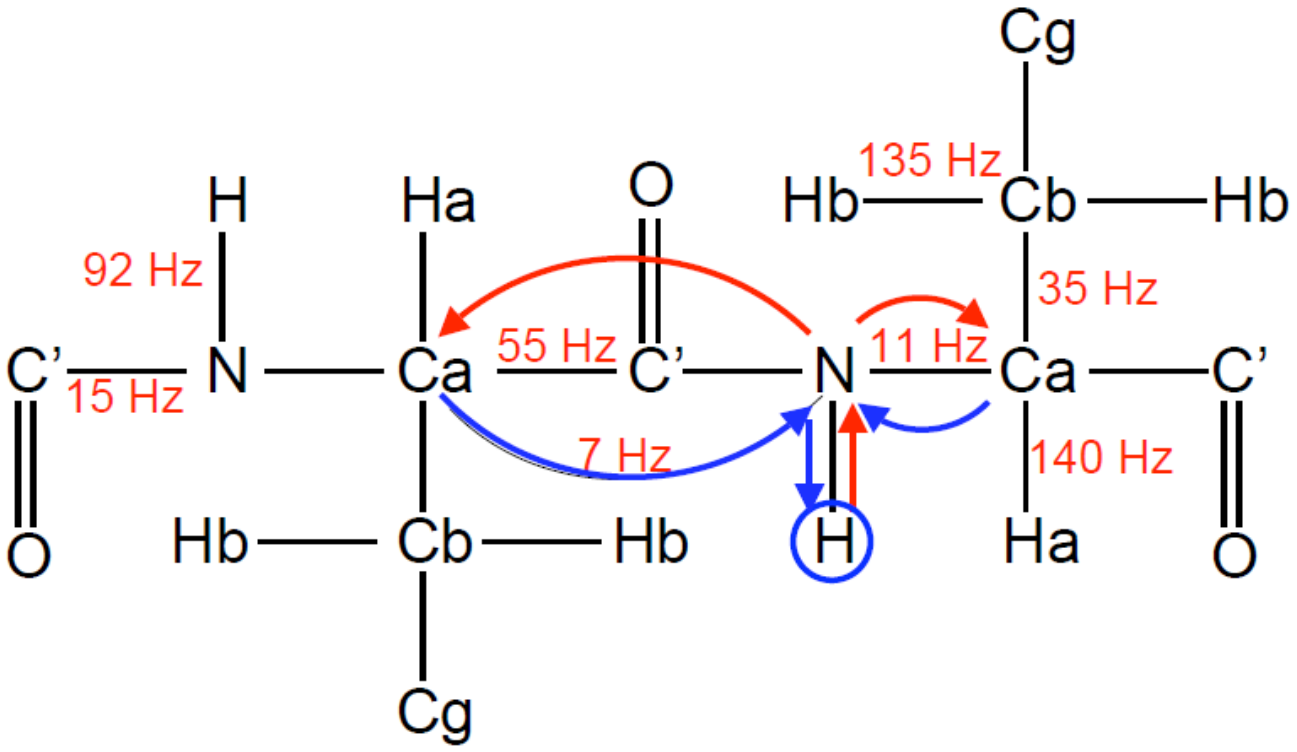




有结构蛋白和无结构蛋白的差异

蛋白质骨架核磁共振信号归属示例

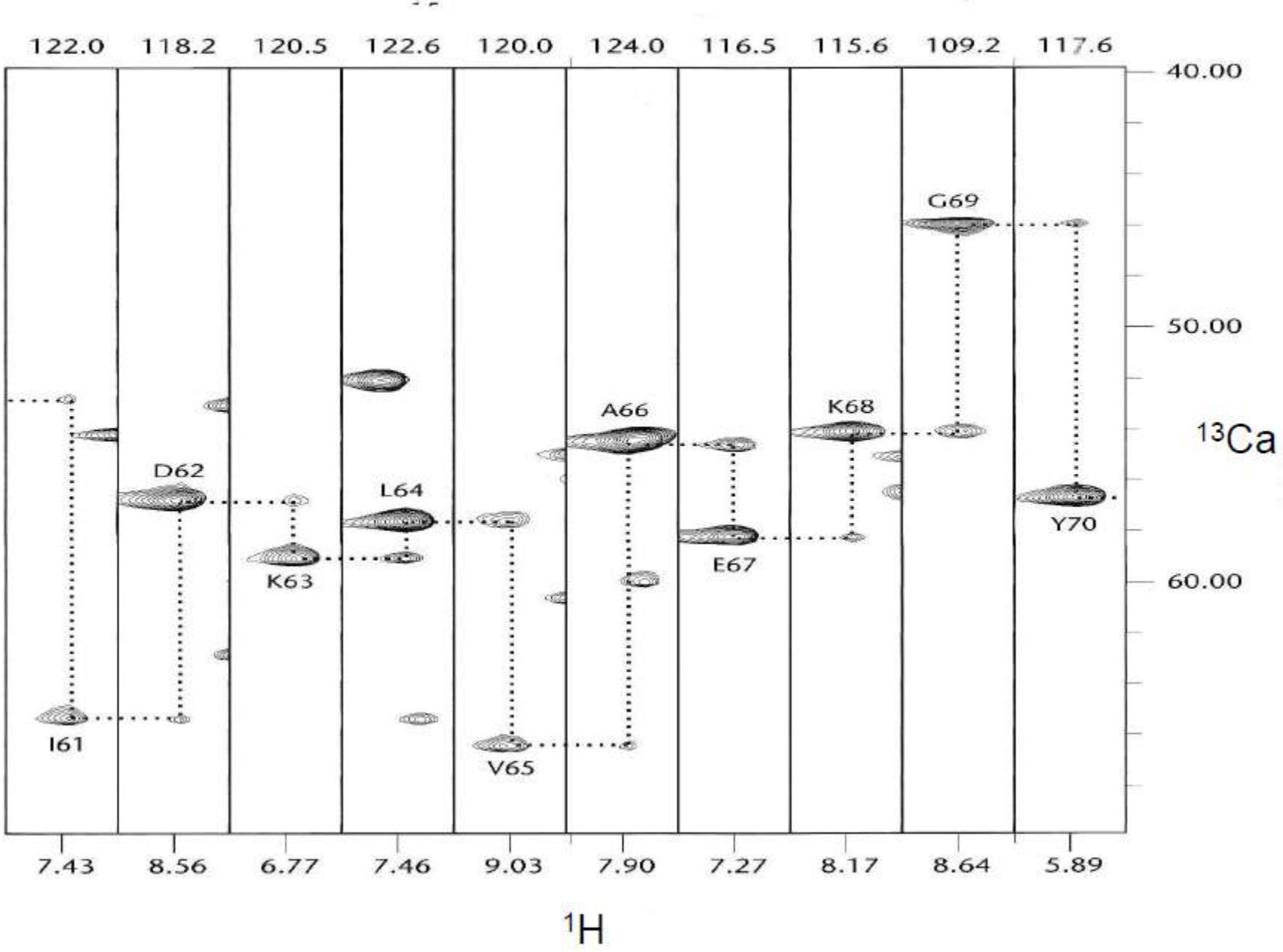
HNCA - correlate Ca resonances of *i* and *i* -1 residues



$$\mathrm{H} \rightarrow \mathrm{N} \rightarrow \mathrm{C}_i^{\alpha} / \mathrm{C}_{i-1}^{\alpha} \rightarrow \left(\mathrm{C}_i^{\alpha} / \mathrm{C}_{i-1}^{\alpha} \text{ evolution t1}\right) \rightarrow \mathrm{N}+\left(\mathrm{N} \text{ evolution t2}\right)$$

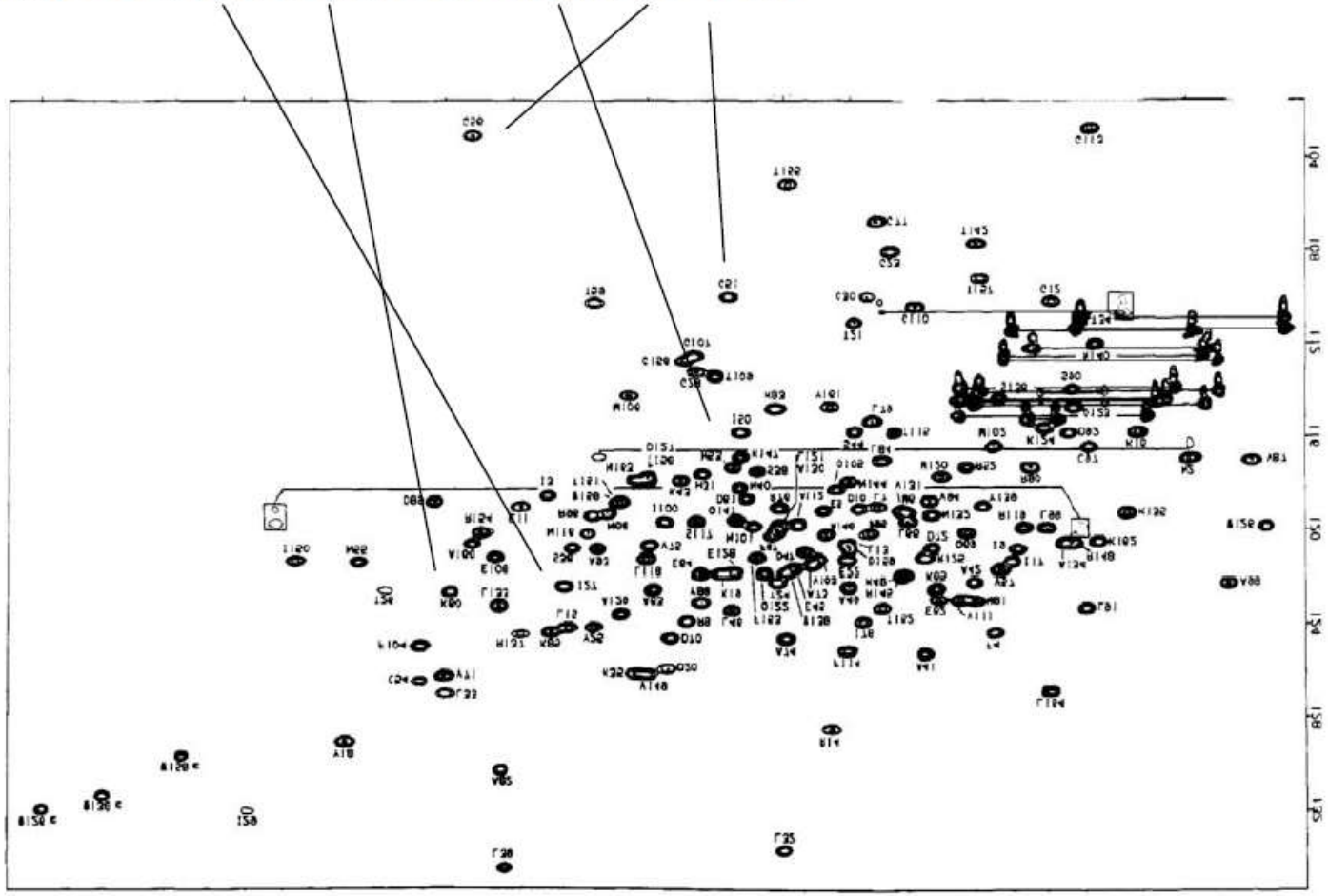
$$\rightarrow \mathrm{H} \rightarrow \left(\mathrm{H} \text{ evolution t3}\right)$$

HN strips from a 3D HNCA Spectrum



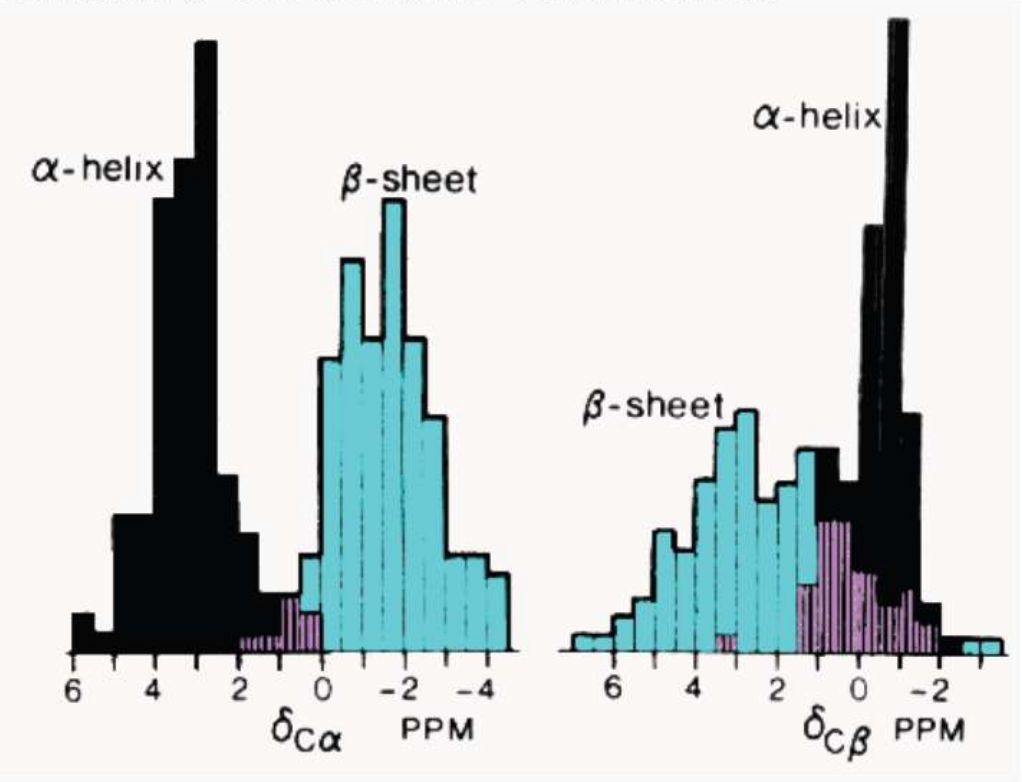
Sequence specific assignments of NMR resonances

GLKVTSEVALLKREDNNAAGPT …



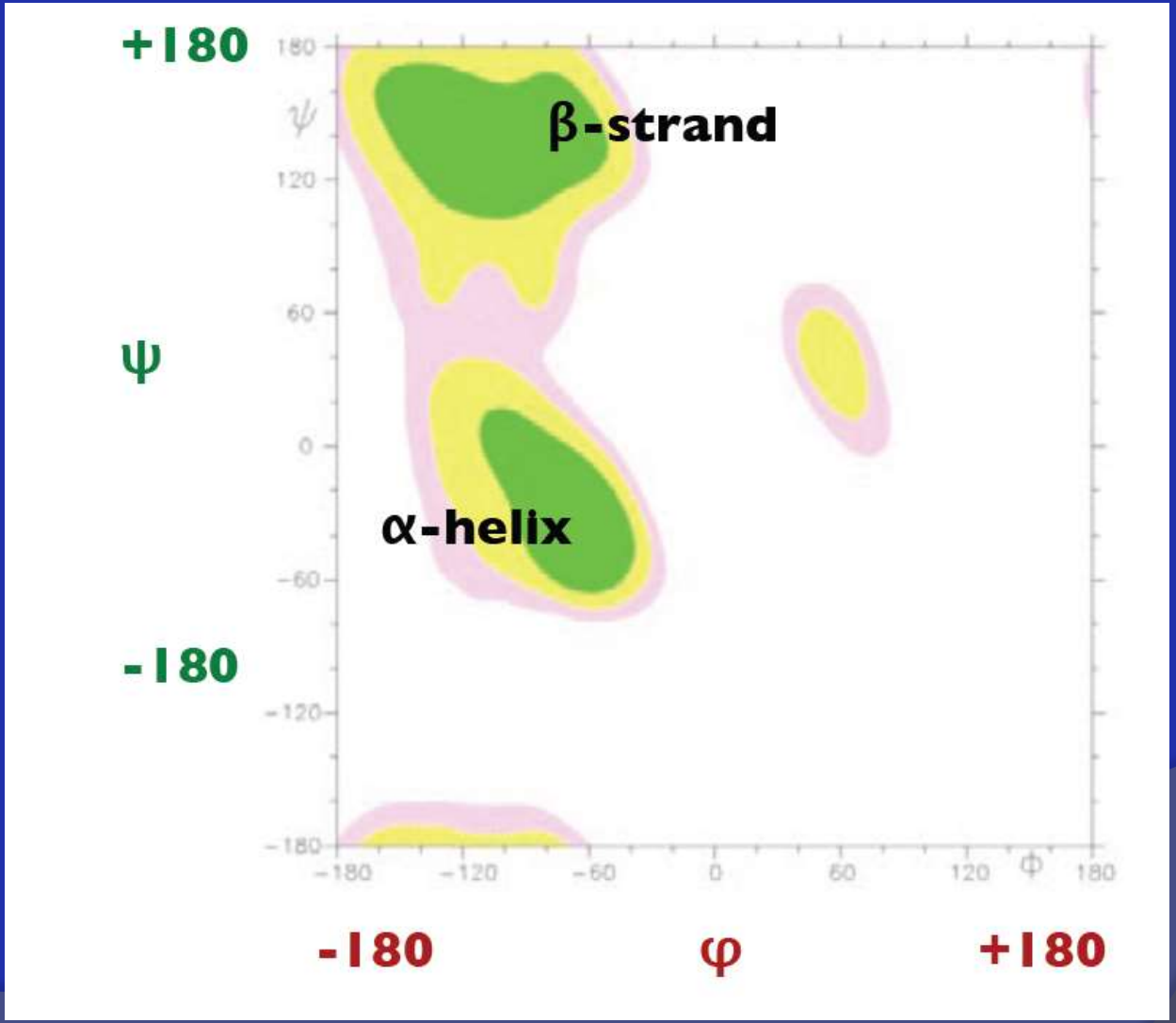
化学位移与二面角的关系

- $^{13}C_\alpha$ and $^{13}C_\beta$ chemical shifts
  - sensitive to dihedral angles
  - report on secondary structure elements

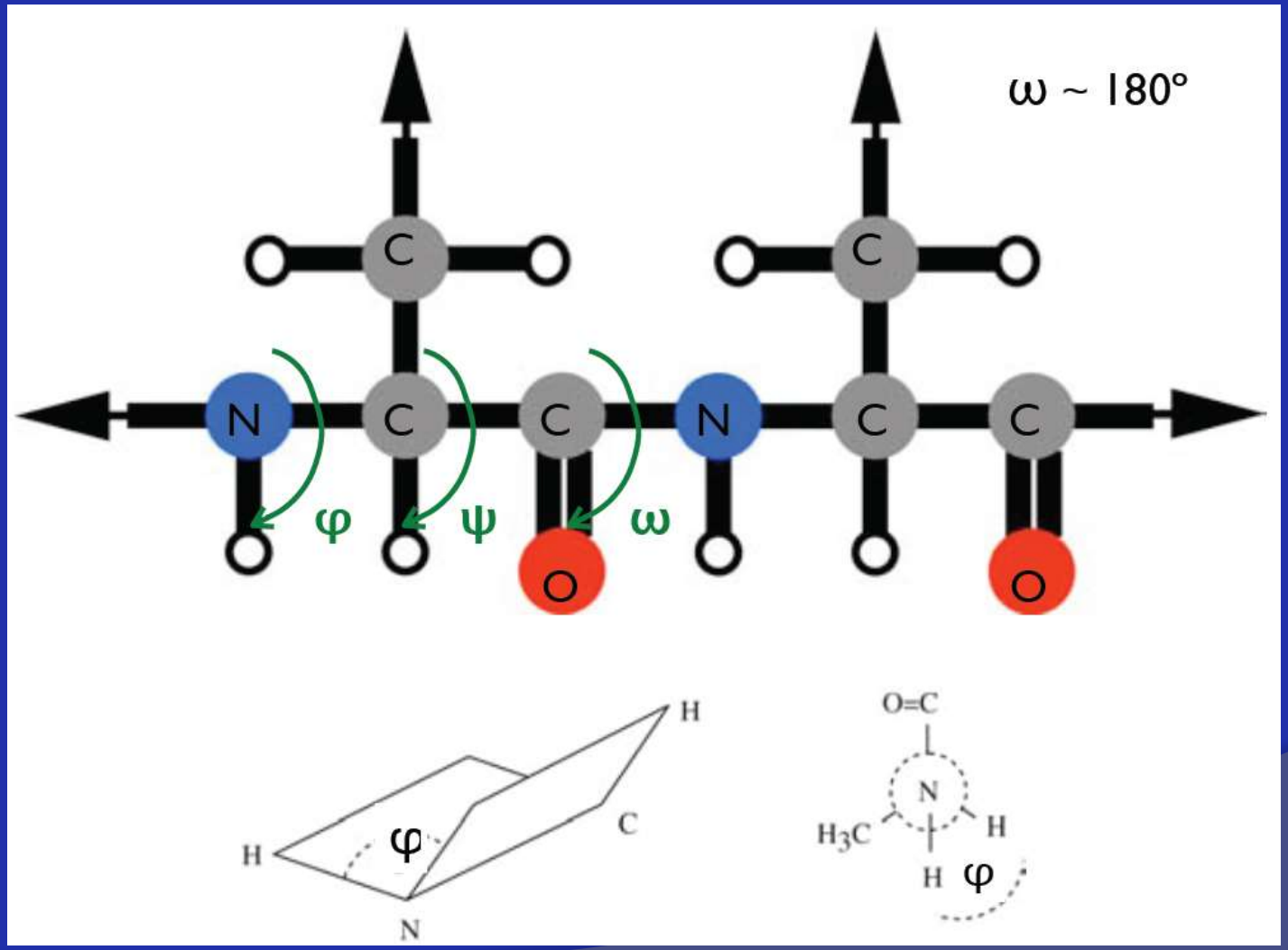


Dihedral angle restraint

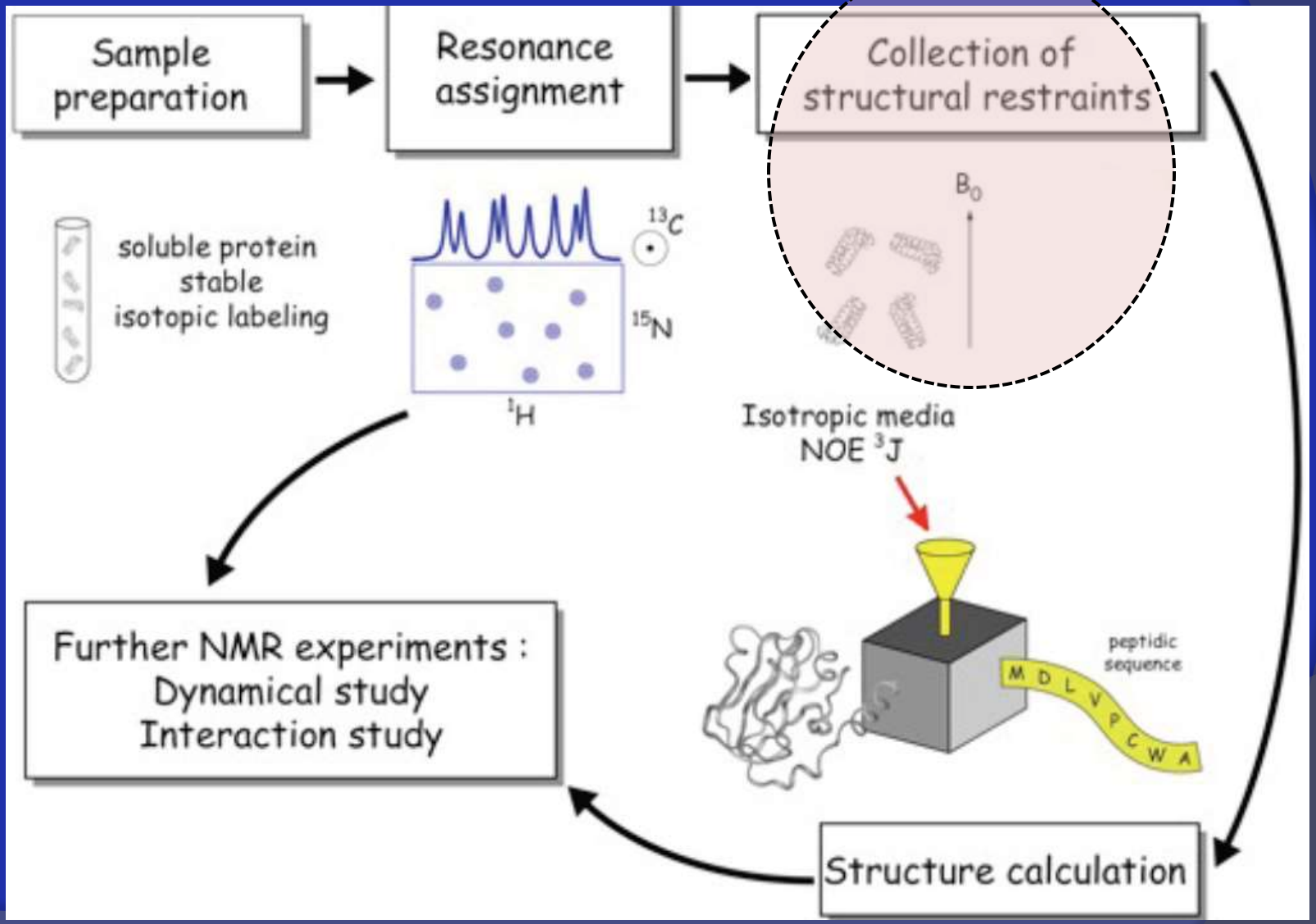
Ramachandran plot



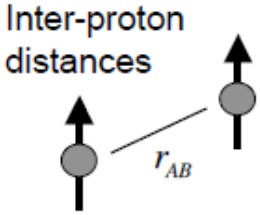
二面角定义

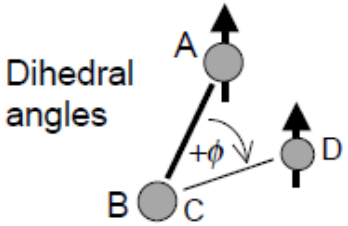


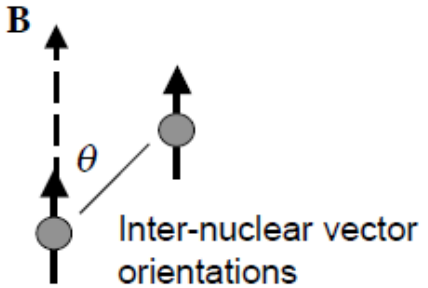
蛋白质核磁共振流程


NOEs和J耦合常数
Sample preparation
Resonance assignment
Collection of structural restraints
soluble protein
stable
isotopic labeling
^{13}C
^{15}N
^{1}H
B_0
Isotropic media
NOE ^{3}J
peptidic sequence
M D L V P C W A
Further NMR experiments :
Dynamical study
Interaction study
Structure calculation

Measuring structural restraints

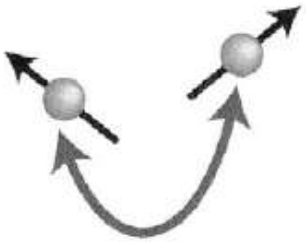


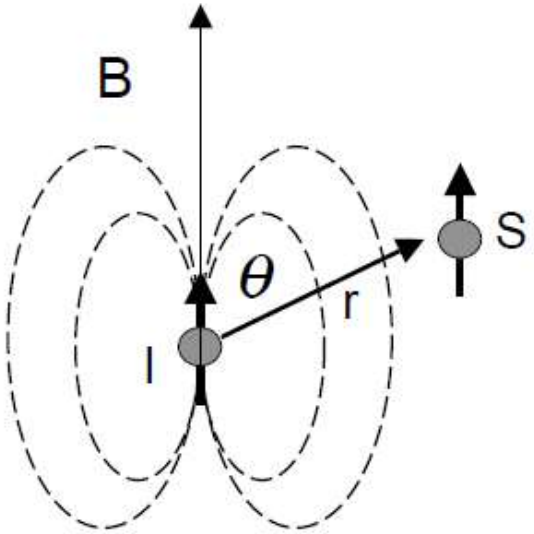




Interaction between two magnetic dipole moments

dipolar interaction,
through-space

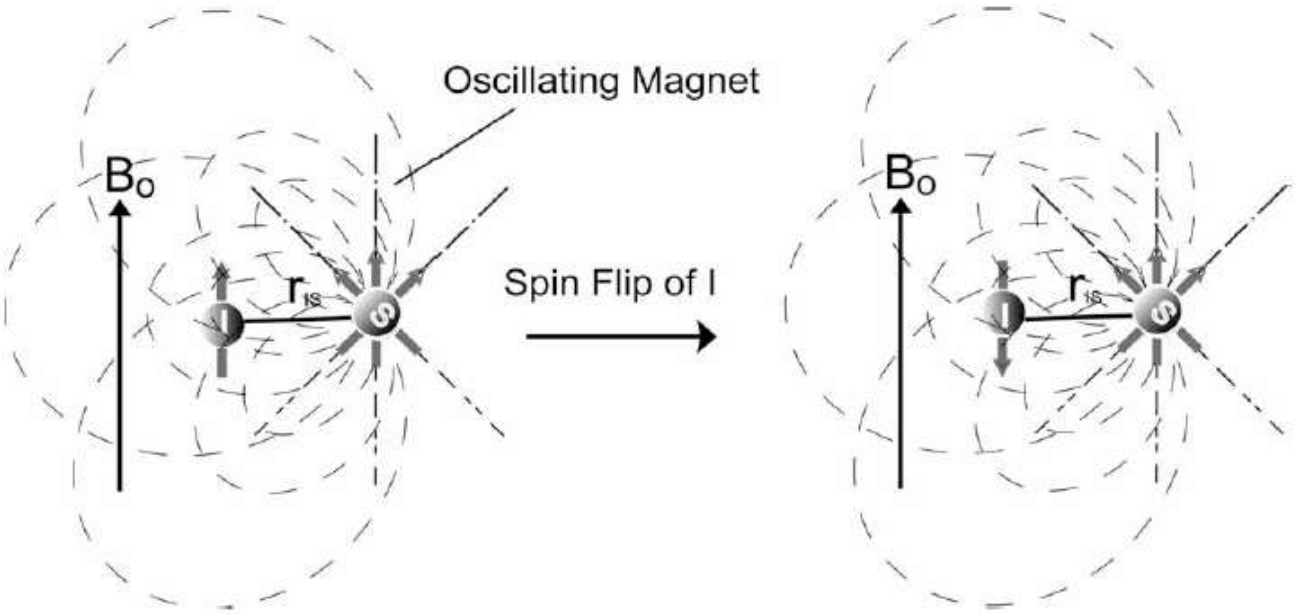




$$DC = -\frac{\mu_0 \gamma_I \gamma_S h}{16\pi^3 r^3}\left(3\cos^2\theta - 1\right)$$

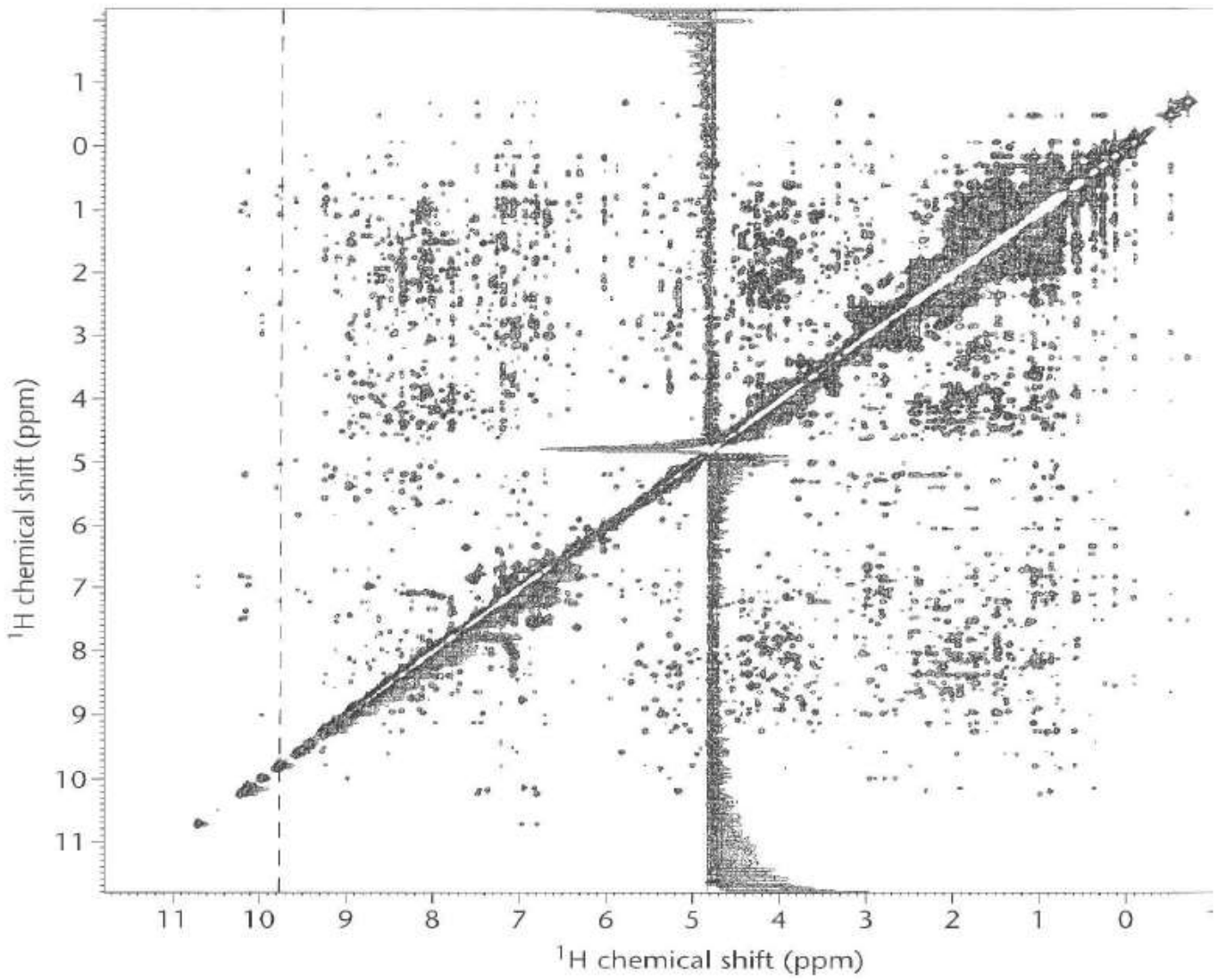
Nuclear Overhauser Enhancement (NOE) - distance restraints

Because of the dipole-dipole interaction and molecular tumbling, two dipolar-coupled spins in a molecule do not relax independently. The cross-relaxation between them lead to observation of NOE, or transfer of magnetization from one spin to another.



NOE measures ^{1}H-^{1}H distances and is proportional to $1/r^6$. Hence NOE is usually observed when $r < 5$ Å. NOE-derived distance restraints are essential for protein structure determination.

2D ^{1}H-^{1}H NOESY of dioxygenase (179 AA) at pH 7.0, 25 °C



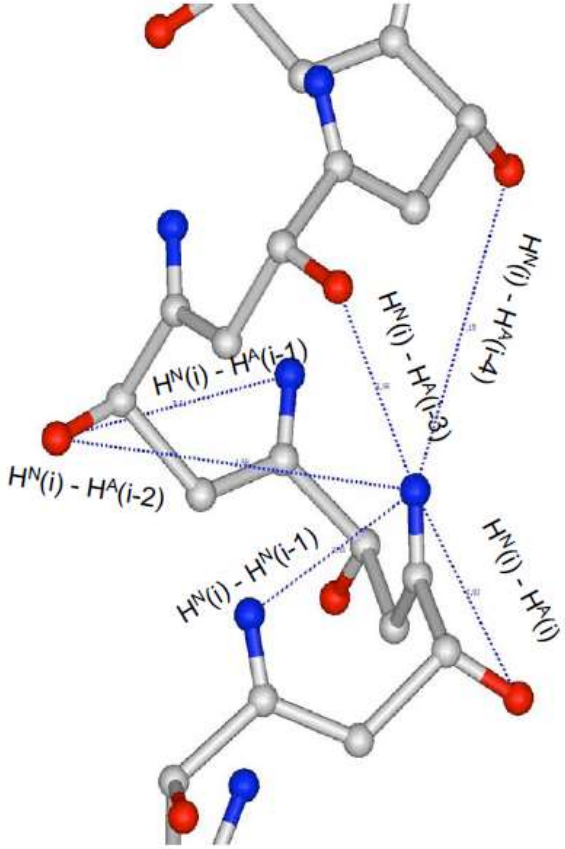
Characteristic NOEs for helical structures

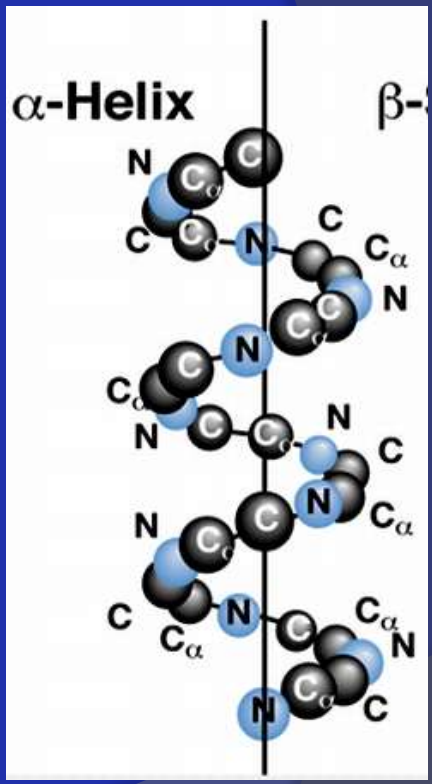
α-helix

HN(i) – HN(i+1)	2.8	Å
Ha(i) – HN(i+1)	3.5	Å
Ha(i) – HN(i+2)	4.4	Å
Ha(i) – HN(i+3)	3.4	Å

3_{10}-helix

HN(i) – HN(i+1)	2.6	Å
Ha(i) – HN(i+1)	3.4	Å
Ha(i) – HN(i+2)	3.8	Å
Ha(i) – HN(i+3)	3.3	Å





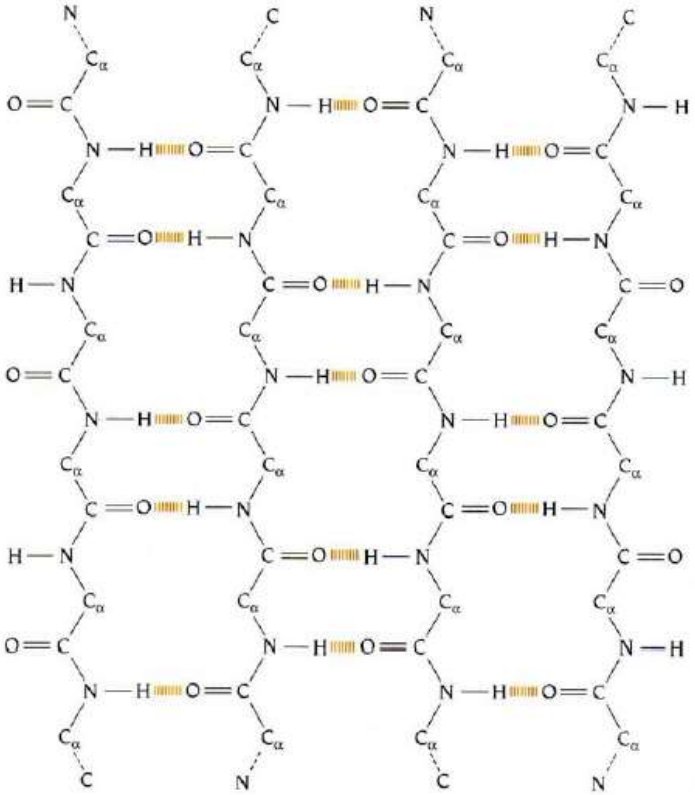
Characteristic NOEs for β structures

antiparallel β-sheet

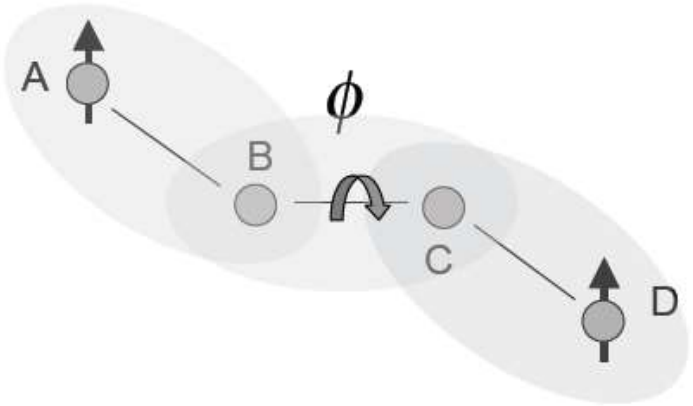
Ha(i) – HN(i+1)	2.2	Å
Ha(i) – Ha(j)	2.3	Å
Ha(i) – HN(j)	3.2	Å
HN(i) – HN(j)	3.3	Å

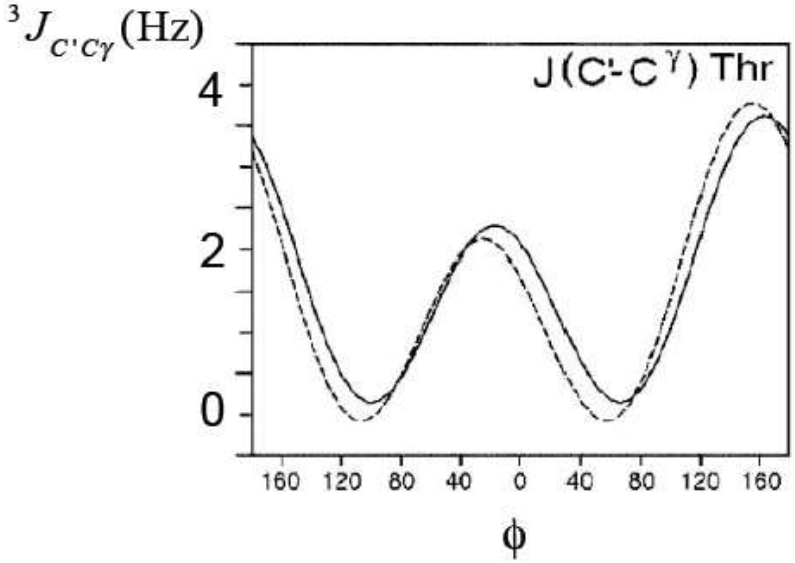
parallel β-sheet

Ha(i) – HN(i+1)	2.2	Å
Ha(i) – Ha(j)	4.8	Å
Ha(i) – HN(j)	3.0	Å
HN(i) – HN(j)	4.0	Å



3-bond *J* couplings - dihedral angle restraints

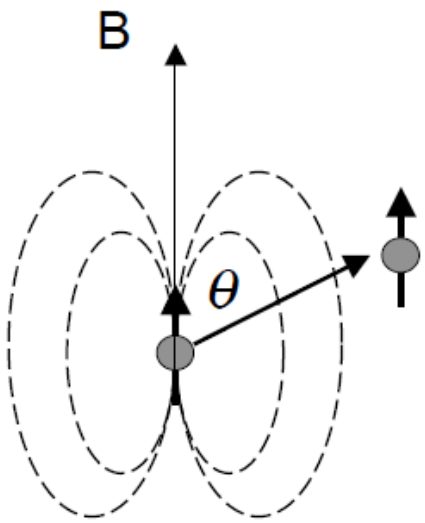




$$^3J = \mathrm{A}\cos^2(\phi) + \mathrm{B}\cos(\phi) + \mathrm{C}$$

Karplus equation

Dipolar couplings - orientation restraints



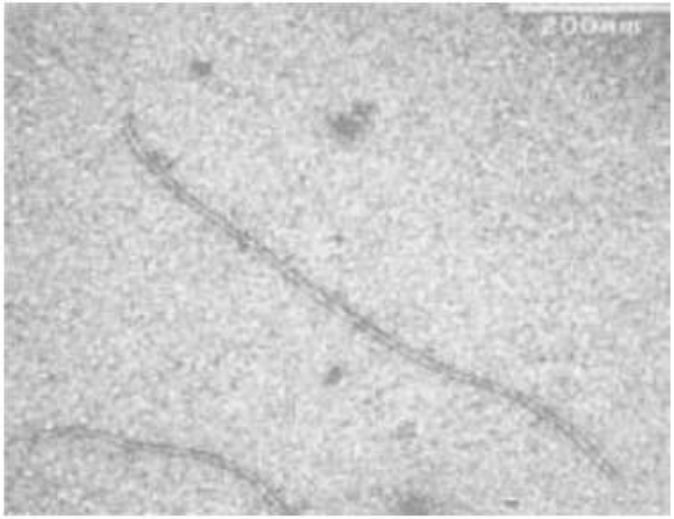
$$DC \propto 3\cos^2\theta - 1$$

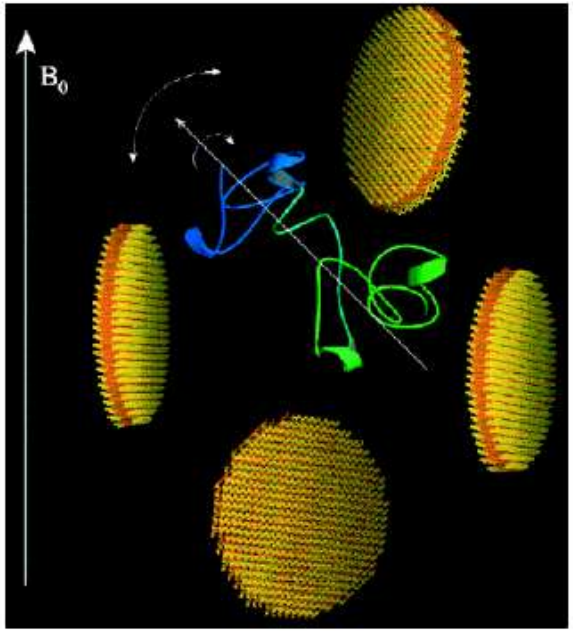
In solution, proteins tumble isotropically. DC from all orientations sum up to zero in the timescale NMR signals are observed.

To measure DC, proteins need to have, on average, a preferred orientation. When proteins are weakly aligned relative to the B field, residual dipolar couplings (RDCs) can be measured.

Various liquid crystals for marginally orientating proteins

filamentous phage (Pf1)



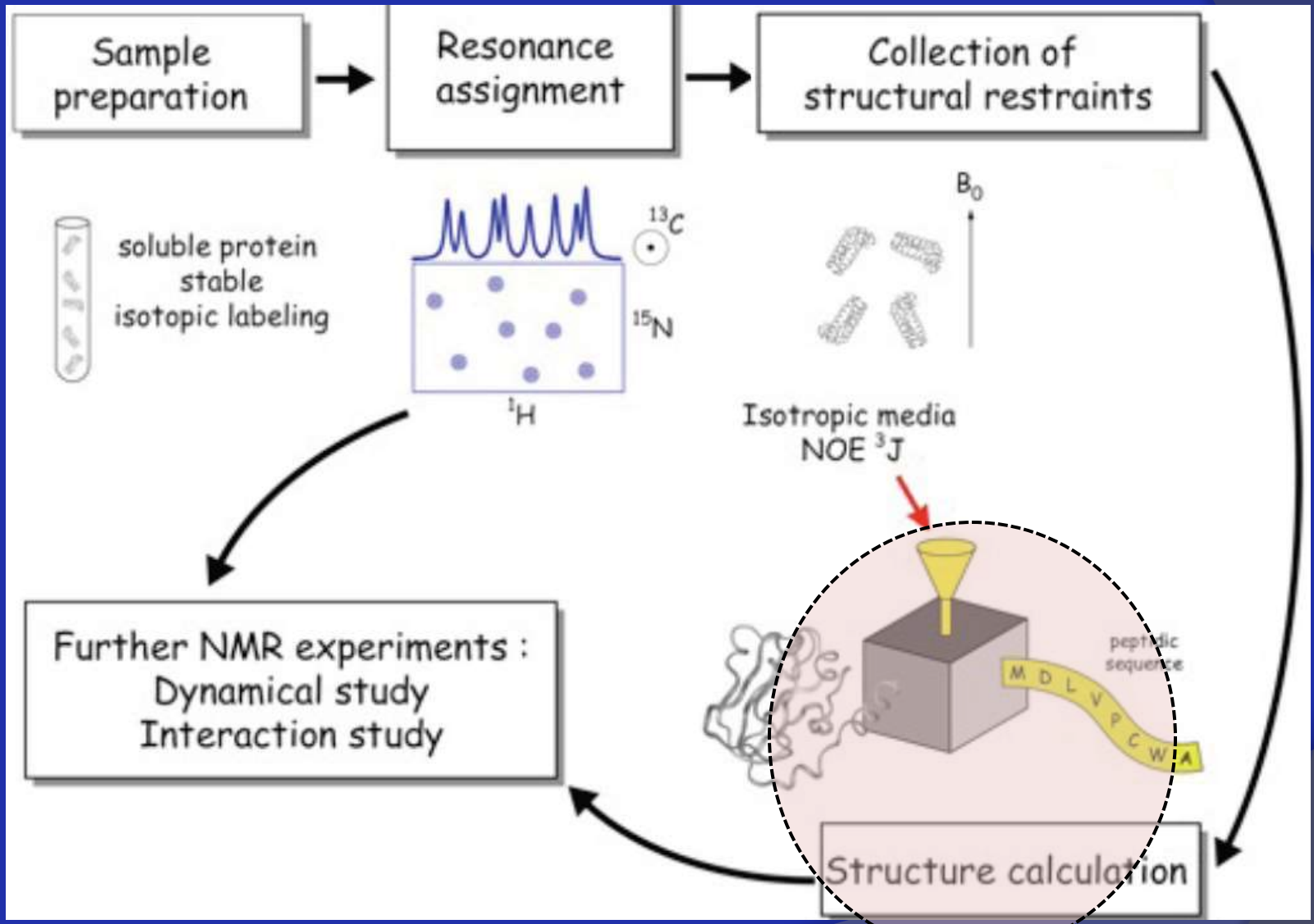


DMPC:DHPC Bicelles

Polyethylene Glycol, hexanol

Cellulose Crystallites, DNA nanotubes

蛋白质核磁共振流程



结构计算

Calculate structures using NMR-derived restraints

Goal: find a structural solution which satisfies all experimental restraints

Restrained Molecular Dynamics

Basic idea is to solve Newton's equations of motion

$$-\nabla_{qi} \mathbf{V} = m_i \frac{d^2 r_i}{dt^2},$$

r_i – position of the ith atom
m_i – mass of the ith atom
V – total potential energy of the system

What is V?

Defining the NMR refinement potential

Total potential V includes both physical and pseudo potential energies

Physical potentials:
- chemical bonds
- angles
- VDW

Pseudo potentials from experimental restraints:

NOE distance restraints (including HB restraints)

Example:

$$U_{NOE} = c(r_{ij} - r_{ij}^{0})^2$$

c is force constant (kcal mol^{-1} A^{-2})

Dihedral restraints: phi, psi, chi1, and chi2

Dipolar coupling restraints – vector orientation

The simulated annealing structure calculation protocol

Why use simulated annealing?

Due to the complexity of the potential and thus the energy landscape, MD often gets stuck in false local minima.

Protocol

increase the momentum of all particles in a MD run by raising the temperature or increasing the mass of particles

lower all other potentials except for the NMR potentials; this is to get around the local minima by satisfying the NMR restraints

annealing: slowly reduce temperature (mass) and increase the VDW force constants to reach the global minimum

An example of SA/MD calculation protocol in XPLOR-NIH

T (initial) = 2000 K T (final) = 200 K

Compute energy

While T > T (final)

T = T - dT

Compute energy

Increase the force constant of NMR restraint potential

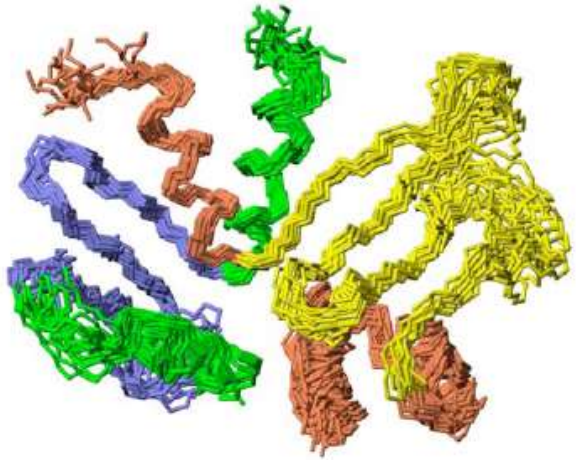
Increase VDW

Verlet dynamics at T for ~ 10 ps

End Cooling Loop

Final Powell energy minimization

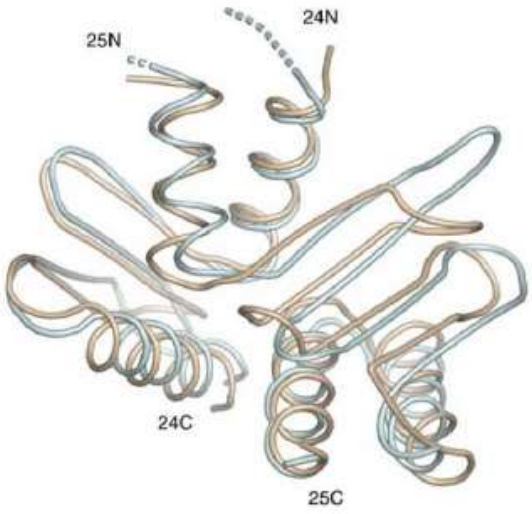
Calculate many structures starting from the random templates each time



Select an ensemble of 20 structures with lowest energy

Coordinate precision

All heavy atoms	1.55 A
Backbone heavy atoms	1.05 A



Comparison between the NMR and X-ray structures

Table 1 NMR structural statistics and atomic r.m.s. differences[1]

Quantity	Number of restraints	Vio. per struct.
NOEs	681	0.25 +/- 0.55 (> 0.2 Å)
Intramolecular	165 / 467	
Intermolecular	49	
Dihedral angle restraints	34	0
χ^1	10 / 13	0
χ^2	11 / 0	0
Dipolar coupling restraints (Hz) [2]	173	2.55 +/- 0.12
NH	50 / 62	2.05 +/- 0.11
$C'C^{\alpha}$	0 / 61	3.30 +/- 0.19
Other restraints		
H-bond	26 / 38	0.45 +/- 0.69 (> 0.1 Å)
ϕ/ψ	58 / 115	0.2 +/- 0.41 (> 2.5°)

[1] Statistics are calculated and averaged over the 20 structures with the lowest overall energy. Numbers are reported for the combined dimer or the individual monomers (spc24 / spc25).

[2] Violations are given as the r.m.s. difference (in Hz) between individual sets of experimental dipolar couplings and those predicted by the 20 final structures by means of SVD fit. The $^1D_{C'C_\alpha}$ couplings are normalized to $^1D_{NH}$.

[3] The precision of the atomic coordinates is defined as the average r.m.s. difference between the 20 final structures and their mean coordinates.