An Introduction to Solution-NMR Structure Determination

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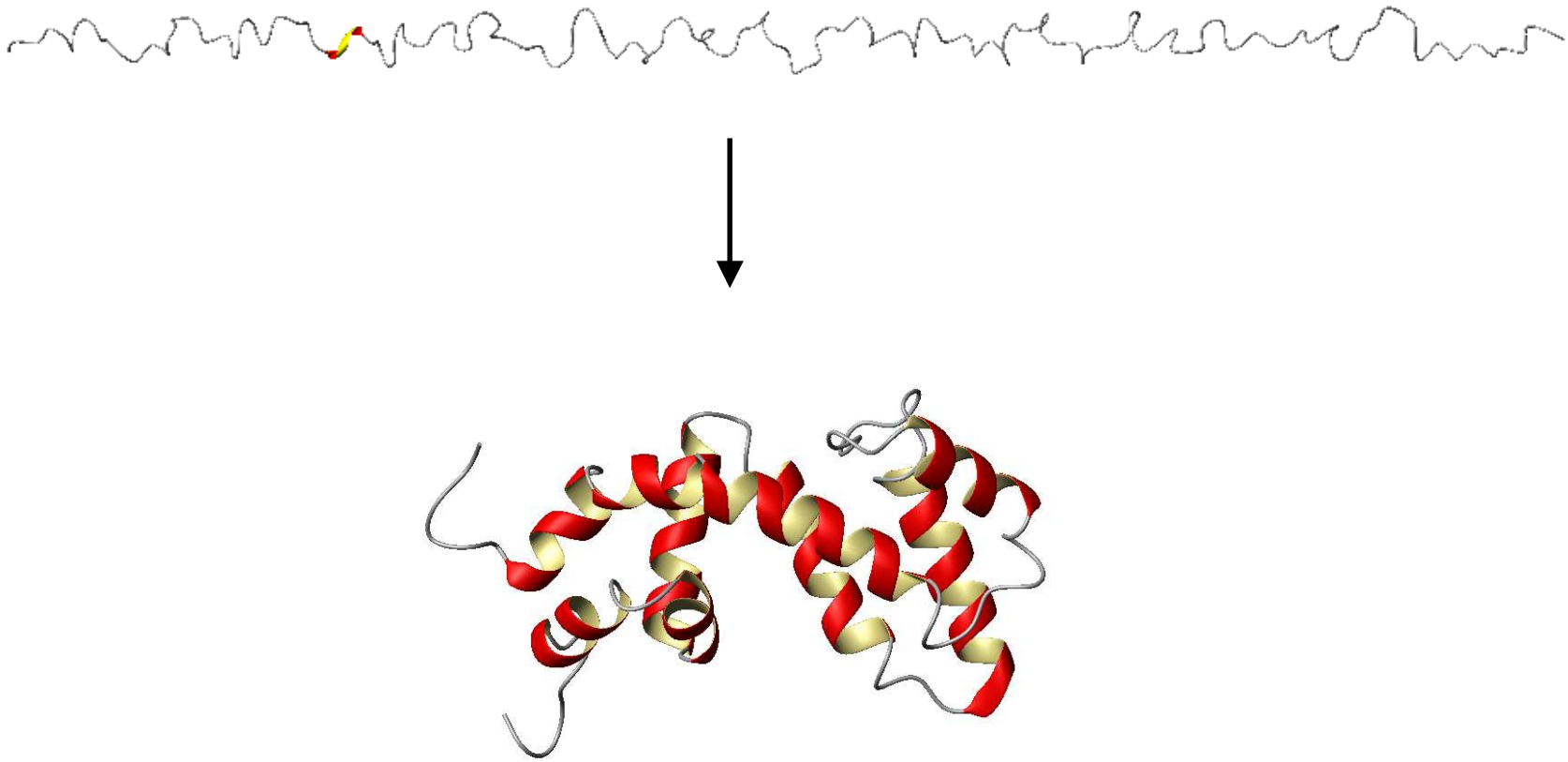
Basic Principle

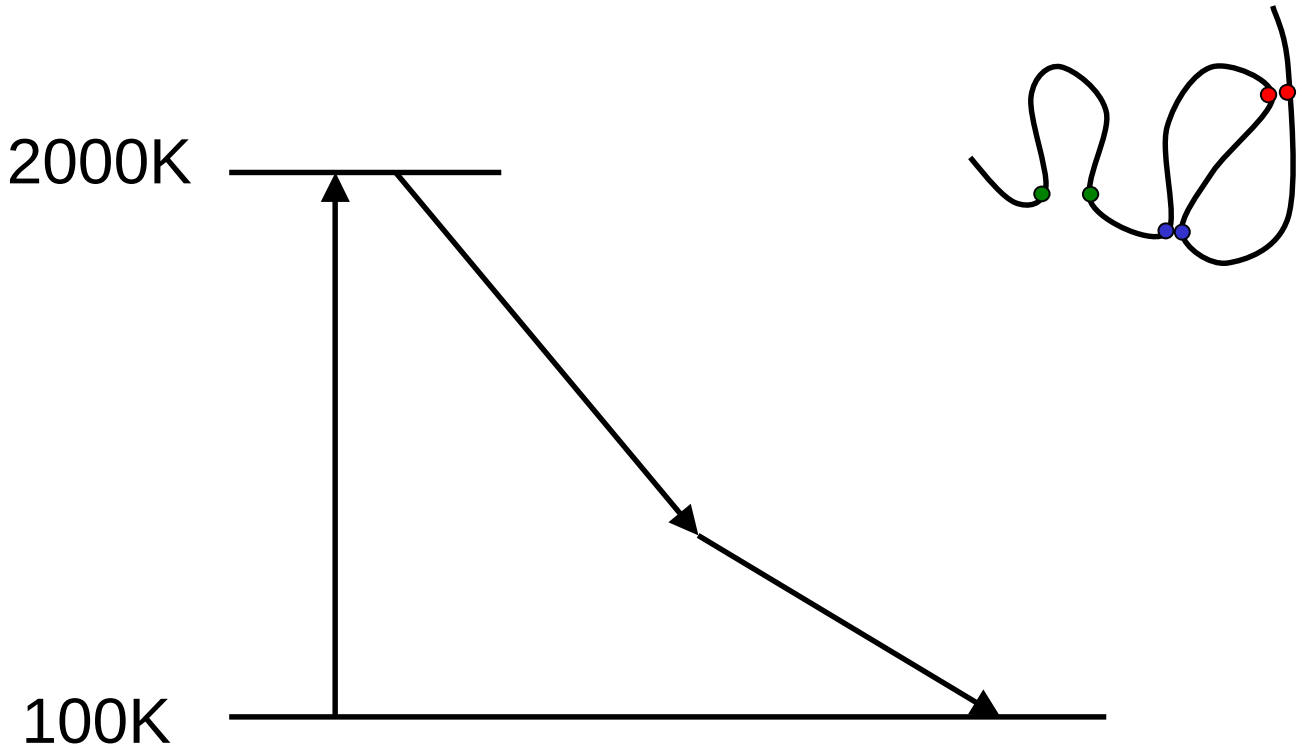
- Experimental restraints – measured
  - Distance between two atoms
  - Angle between two bonds
- Force field - known information
  - Primary sequence of the protein
  - Atoms cannot overlap one another (!)
  - Bond angles
  - Stereochemistry

$$E = k\,(D_{calc} - D_{obs})^2$$

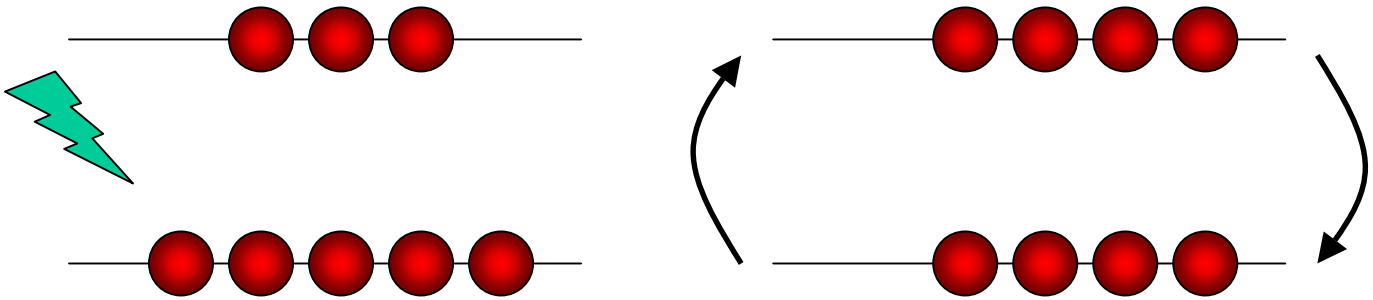
Basic Principle



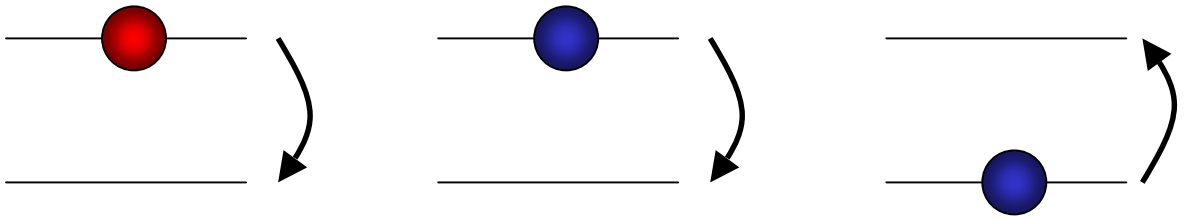
Simulated Annealing



NOE (Nucelar Overhauser Effect)



NOE (Nucelar Overhauser Effect)



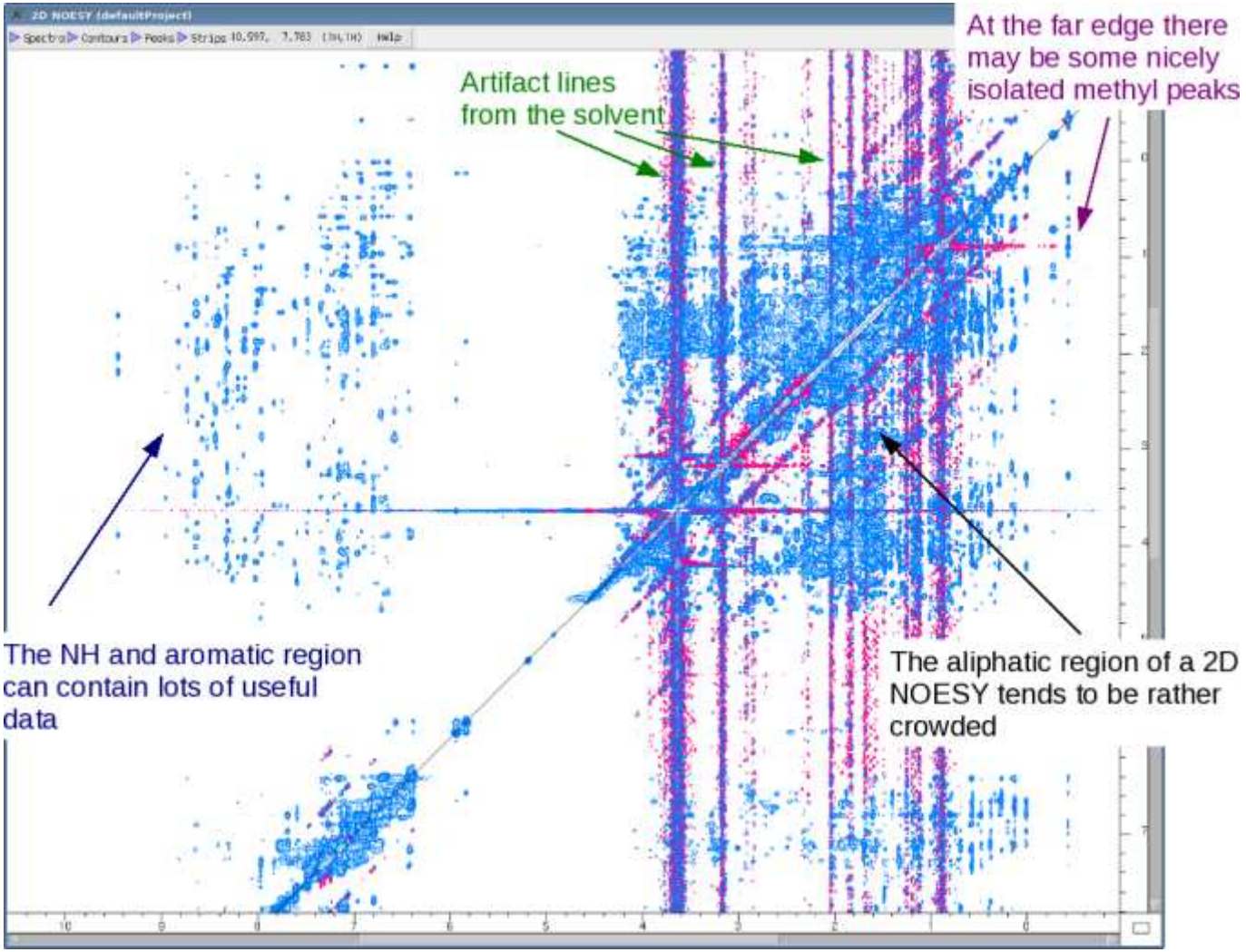
Cross correlation

$I \propto 1/d^6$

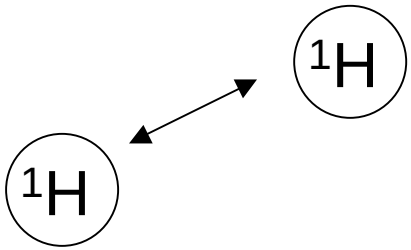
$I = k / d^6$

$\Rightarrow$ active up to about 6-7 Å

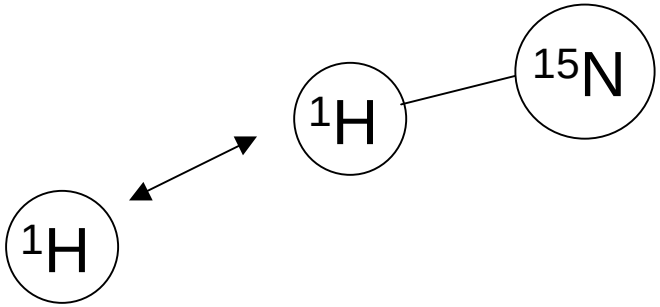
2D NOESY


Artifact lines
from the solvent
At the far edge there
may be some nicely
isolated methyl peaks
The NH and aromatic region
can contain lots of useful
data
The aliphatic region of a 2D
NOESY tends to be rather
crowded

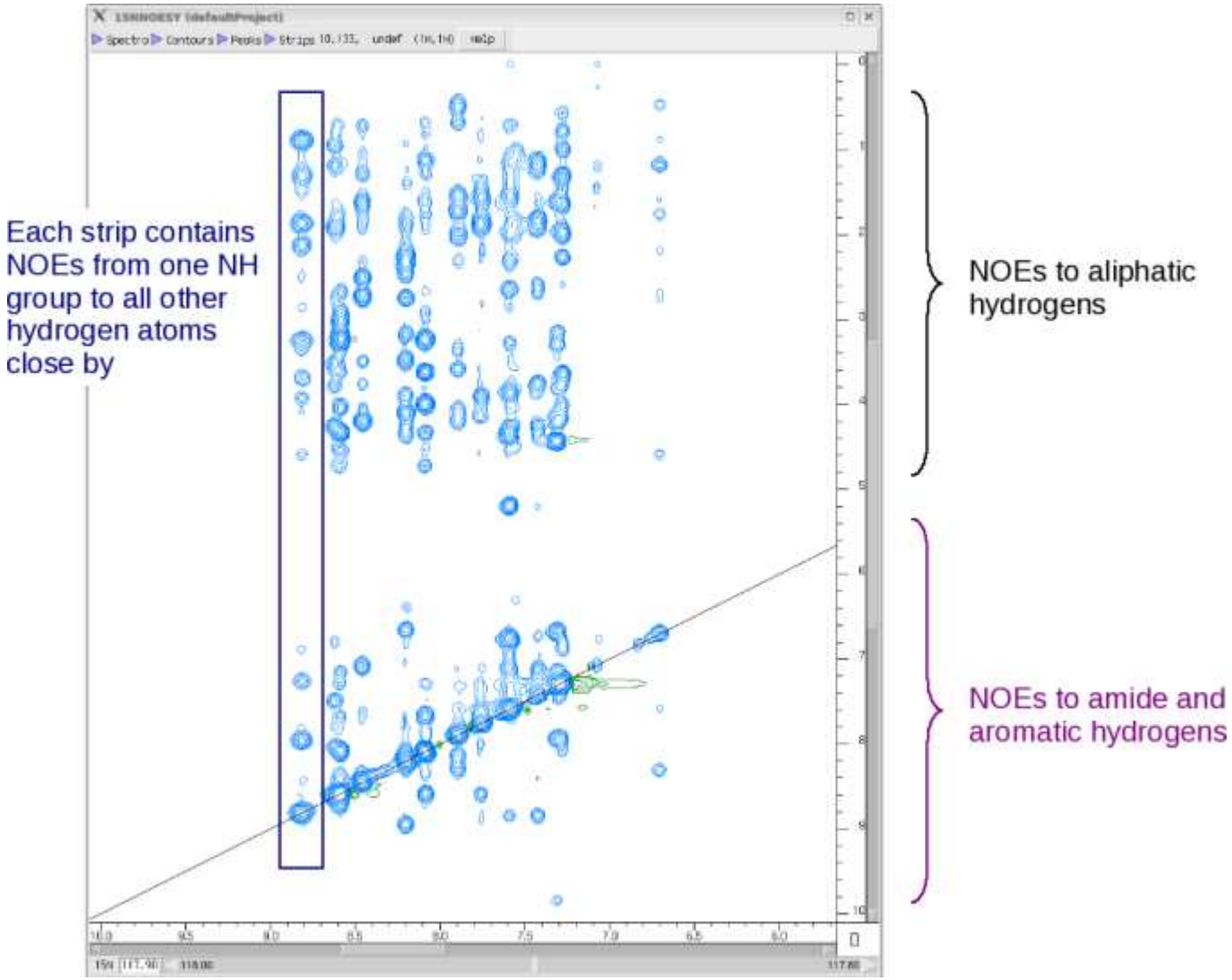
2D NOESY



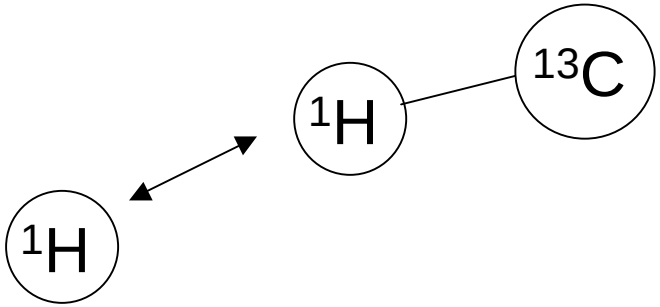
3D NOESY



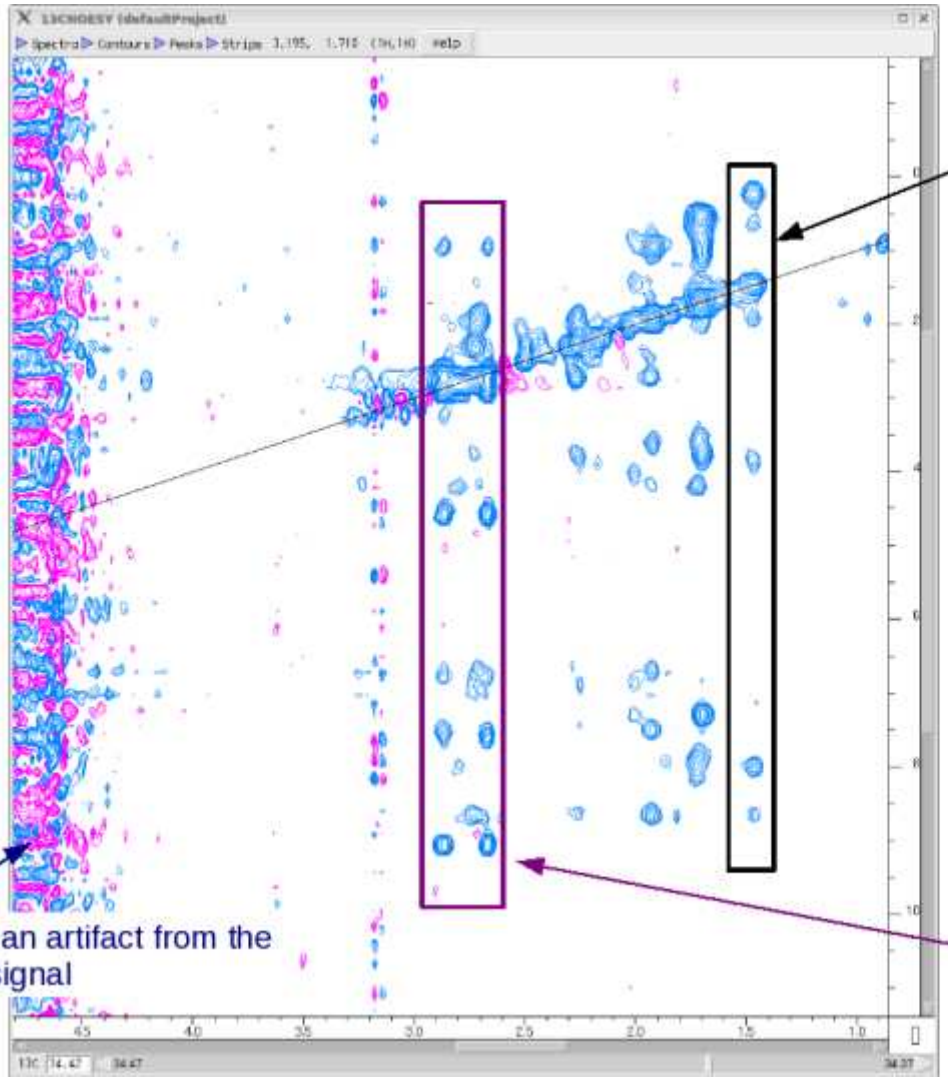
3D ^{15}N-NOESY-HSQC



3D NOESY



3D ^{13}C-NOESY-HSQC

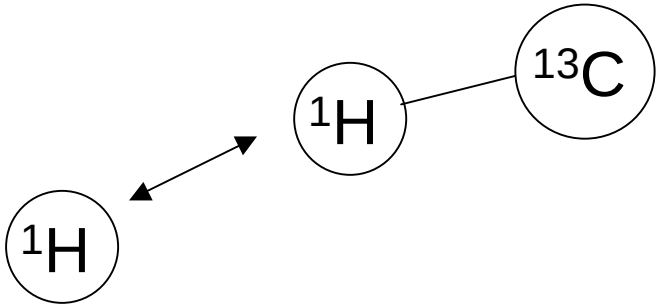


Each strip contains NOEs from one CH group to all other hydrogens nearby

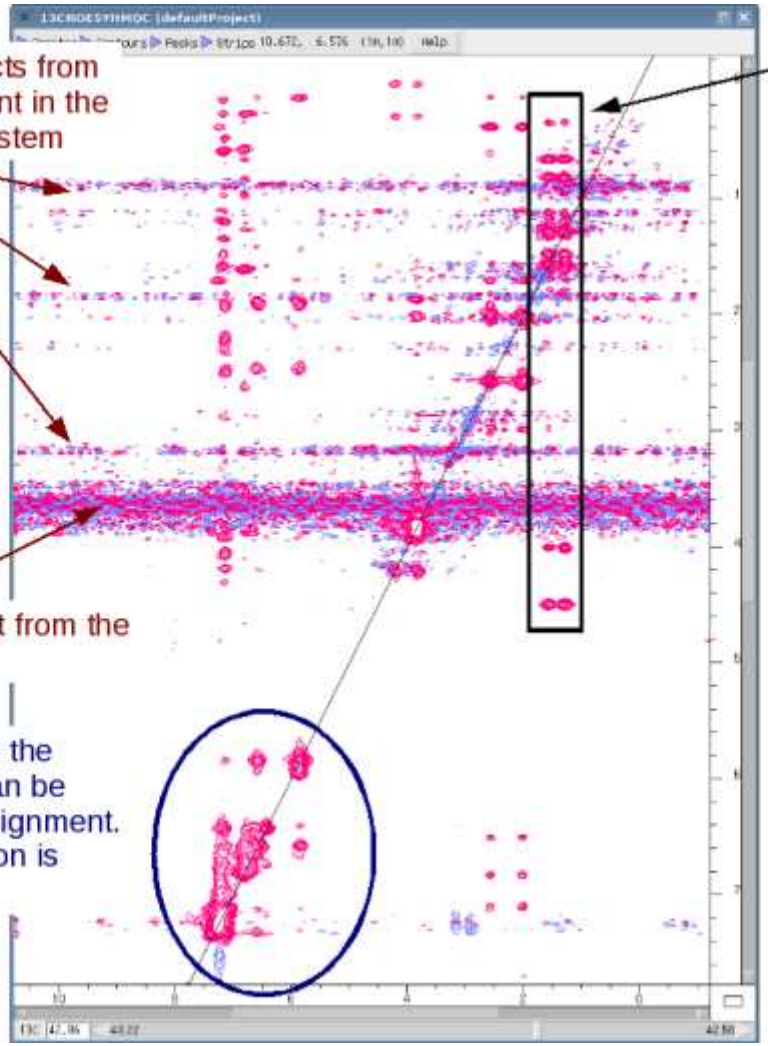
A double set of peaks usually arises from a CH_2 group in which the two hydrogen atoms have different chemical shifts.

This is an artifact from the water signal

3D NOESY



3D ^{13}C-HMQC-NOESY



These are artifacts from molecules present in the solvent/buffer system

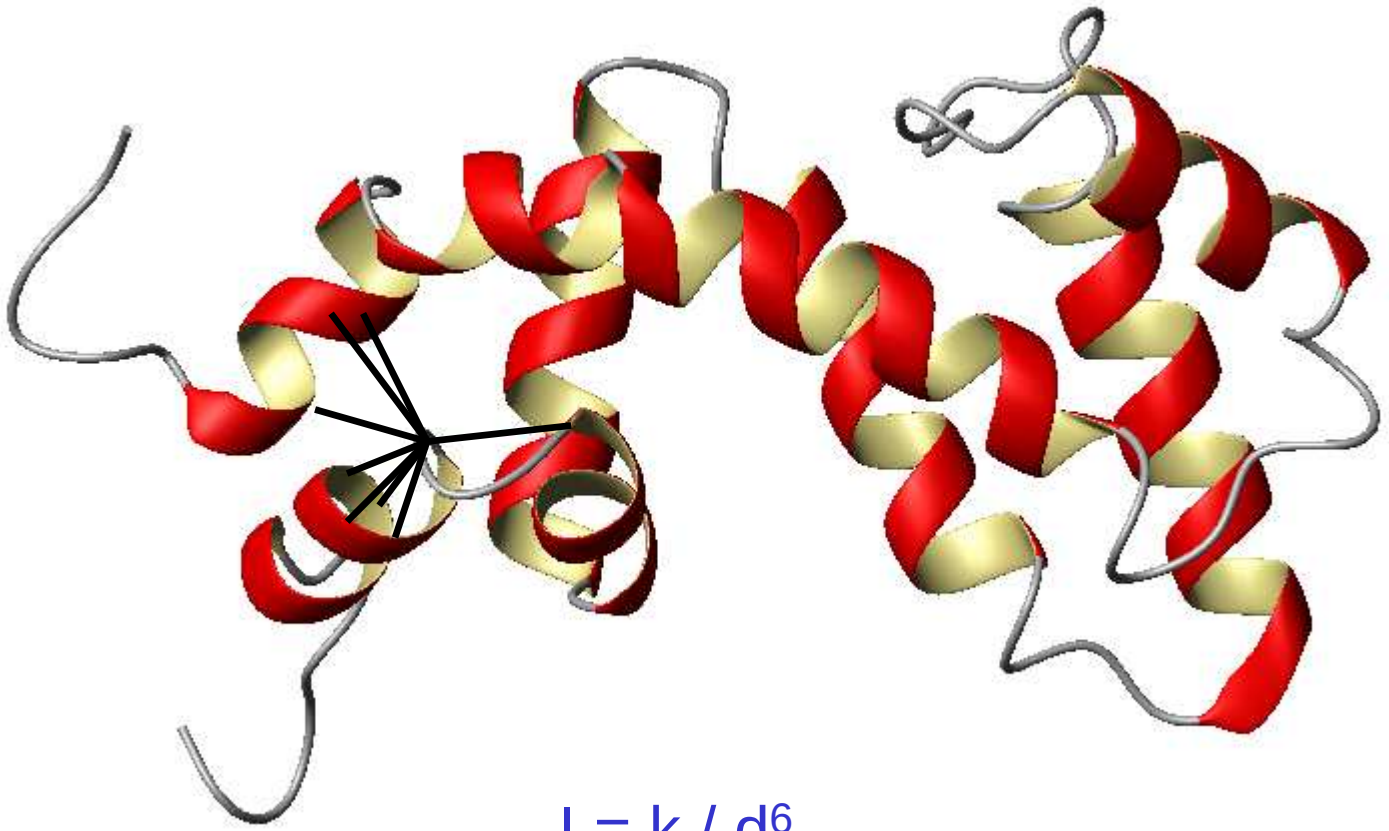
This is an artifact from the water signal

Crosspeaks within the aromatic region can be very useful for assignment. Note that this region is aliased.

Each strip contains NOEs from one CH group to all other hydrogens nearby. A double set of peaks usually arises from a CH_2 group in which the two hydrogen atoms have different chemical shifts.

The resolution in the NOE dimension isvery high, as this is the detected dimension

NOEs in Structure Calculations



$$I = k / d^6$$

NOEs in Structure Calculations

Parallel β-sheet

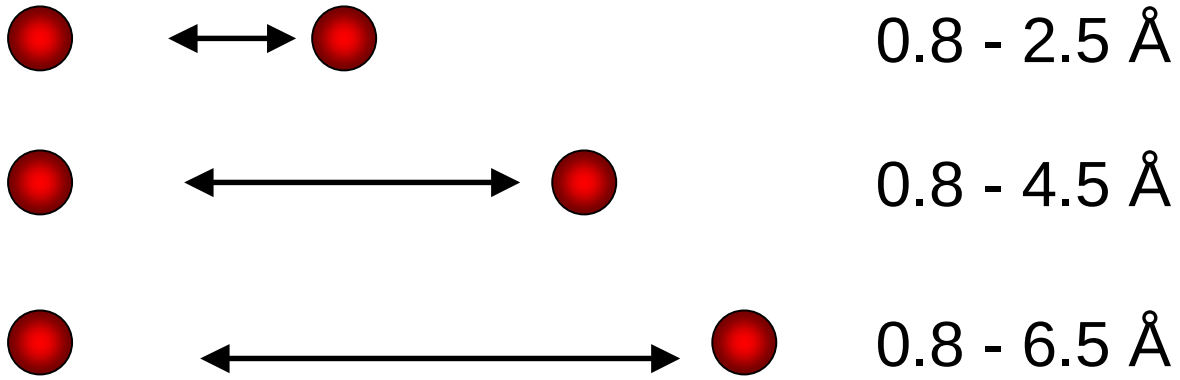
H^{α}_{i}- H^{α}_{j}	= 2.3 Å
H^{α}_{j}- H^{N}_{j}	= 3.2 Å
H^{N}_{i}- H^{N}_{j}	= 3.3 Å

α-helix

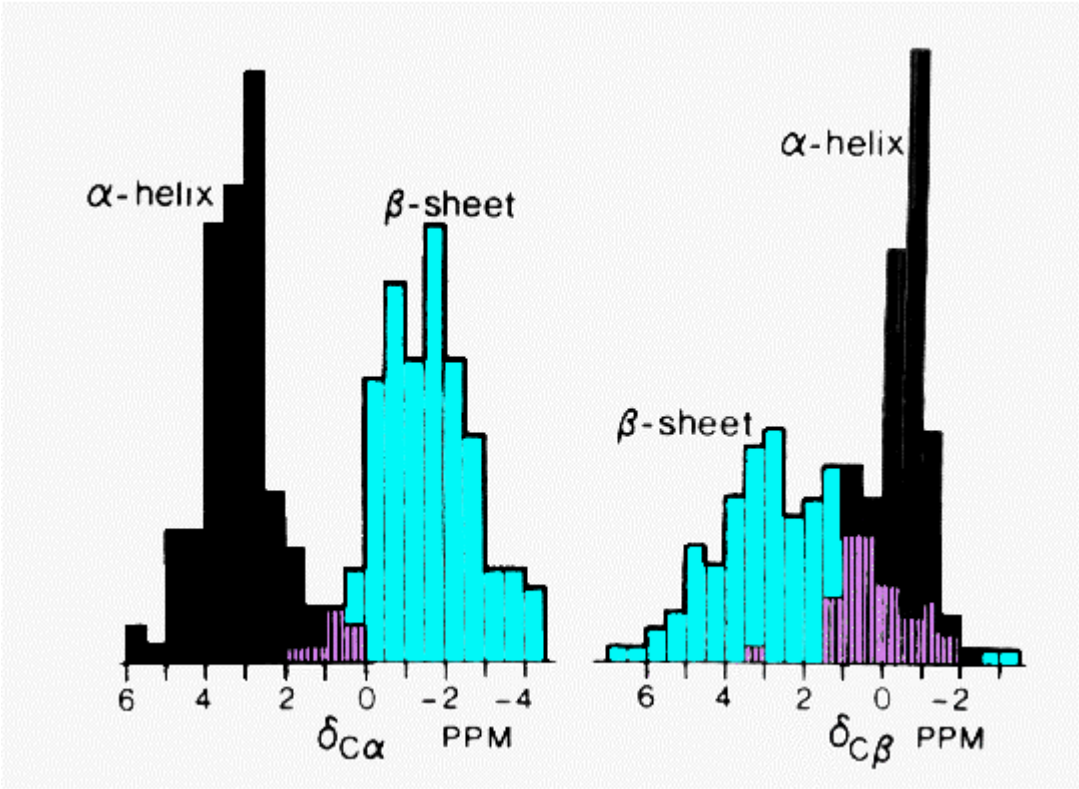
H^{N}_{i}-H^{N}_{i+1}	= 2.8 Å
H^{α}_{i}-H^{N}_{i+3}	= 3.4 Å
H^{α}_{i} - H^{N}_{i+4}	= 4.2 Å
H^{N}_{i} - H^{N}_{i+2}	= 4.2 Å
H^{α}_{i} -H^{β}_{i+3}	= 2.5-4.4 Å

$$I = k / d^6$$

NOEs in Structure Calculations



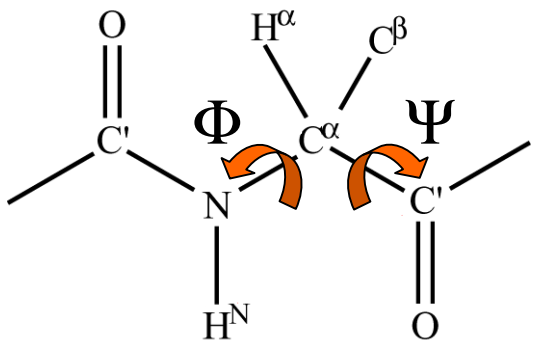
Chemical Shifts

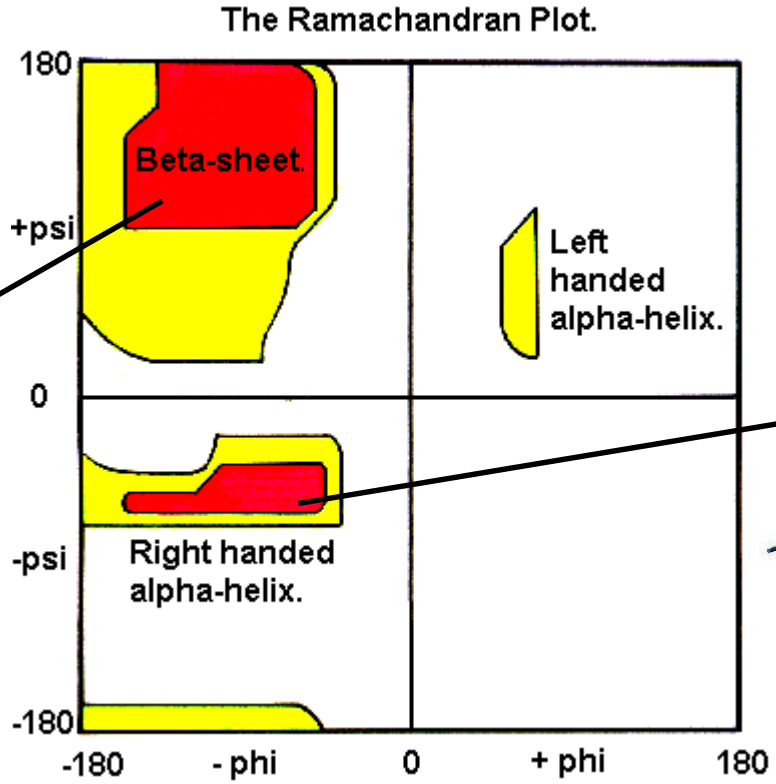


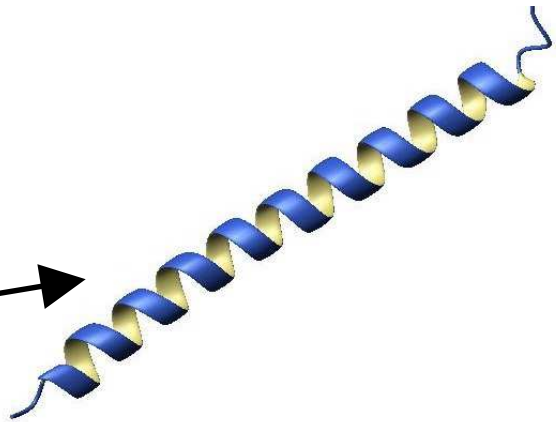
$\delta C\alpha = C\alpha_{\text{random coil}} - C\alpha$

= secondary chemical shift

Chemical Shifts





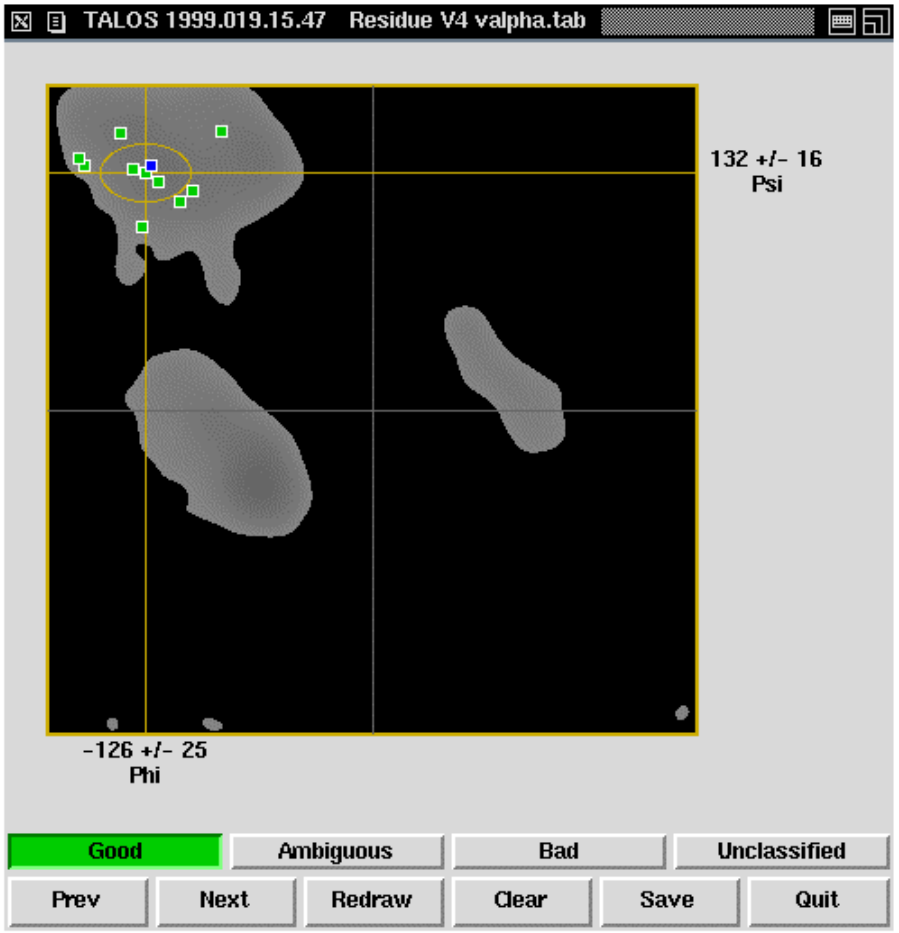


Chemical Shifts

- TALOS – Torsion Angle Likelihood Obtained form Shift and sequence similarity

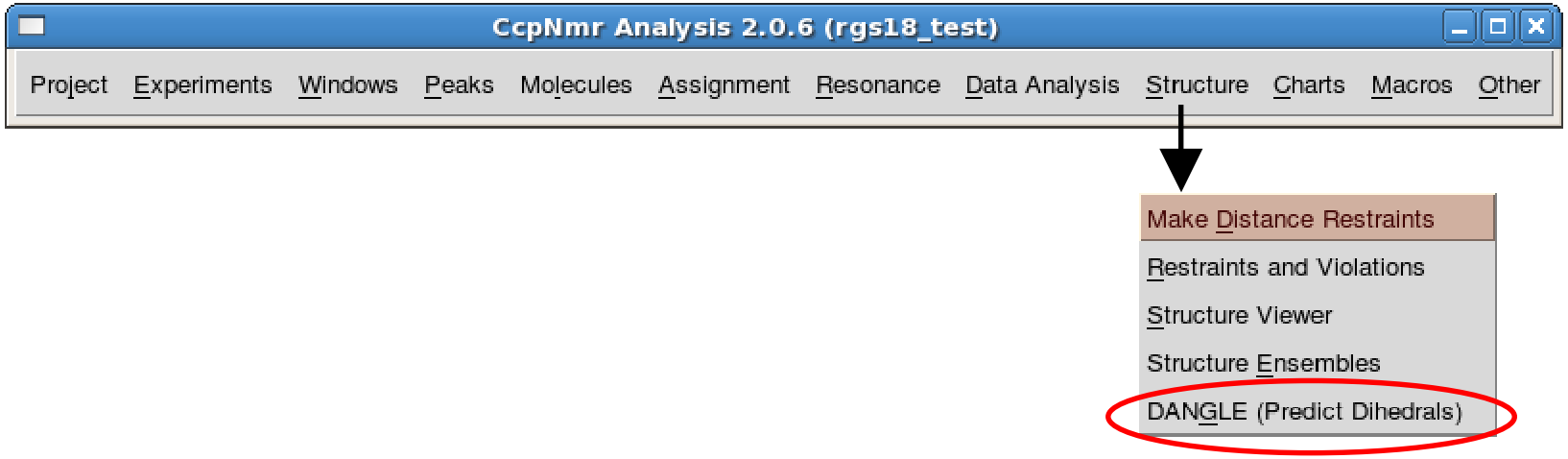
Residue V4, Triplet Q3 V4 R5

-119	127	21.46	S65 T66 L67		ubiquitin
-160	136	22.21	V40 K41 M42		dehydrase
-163	140	22.59	K131 R132 I133		dehydrase
-126	132	22.61	E110 V111 K112		dehydrase
-100	122	24.28	R57 Q58 Y59		HIVprotease
-133	134	24.38	D33 V34 I35		cutinase
-128	102	25.50	R177 S178 S179		alpha_LP
-84	155	25.54	D86 S87 Y88		hca_l
-107	116	27.55	H68 L69 V70		ubiquitin
-140	154	27.77	S84 F85 V86		alpha_LP
-126	132	24.39			Average



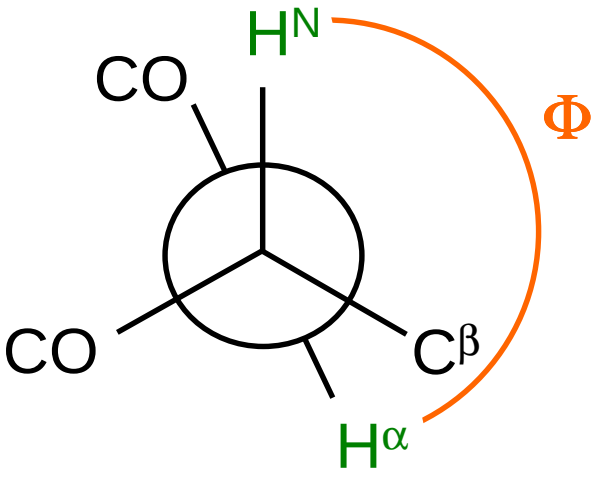
Chemical Shifts

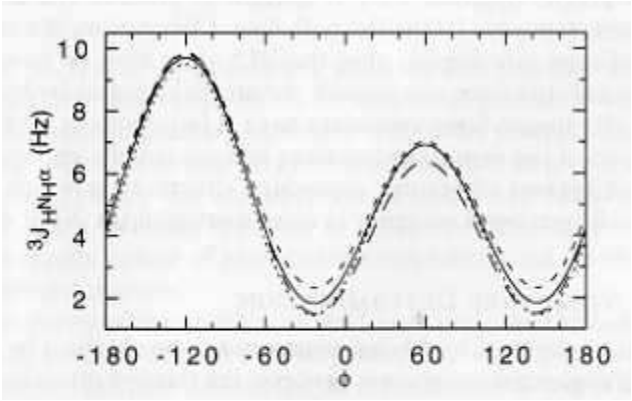
- DANGLE – Dihedral ANGLE



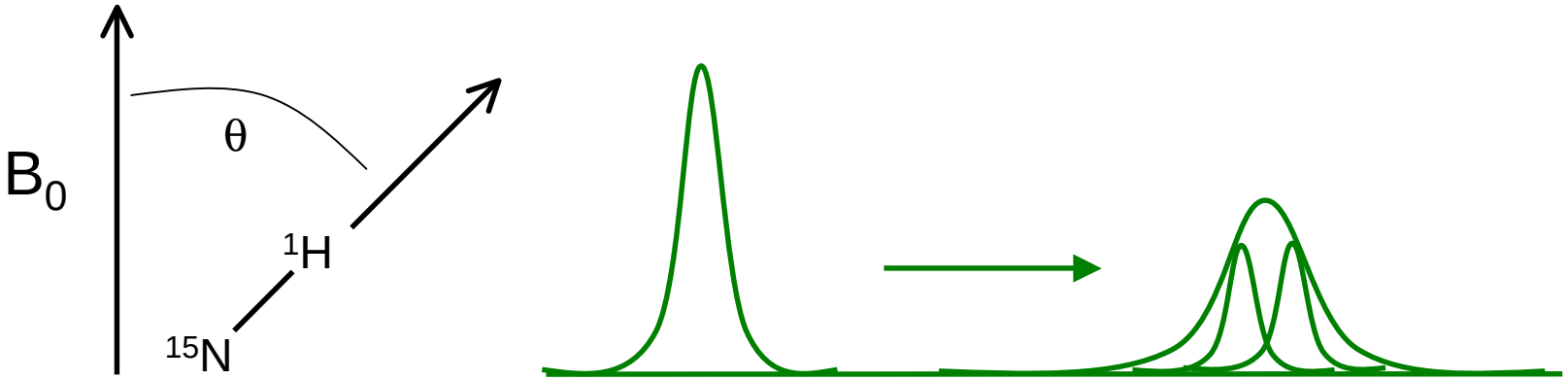
J-Couplings

- Karplus Kurve



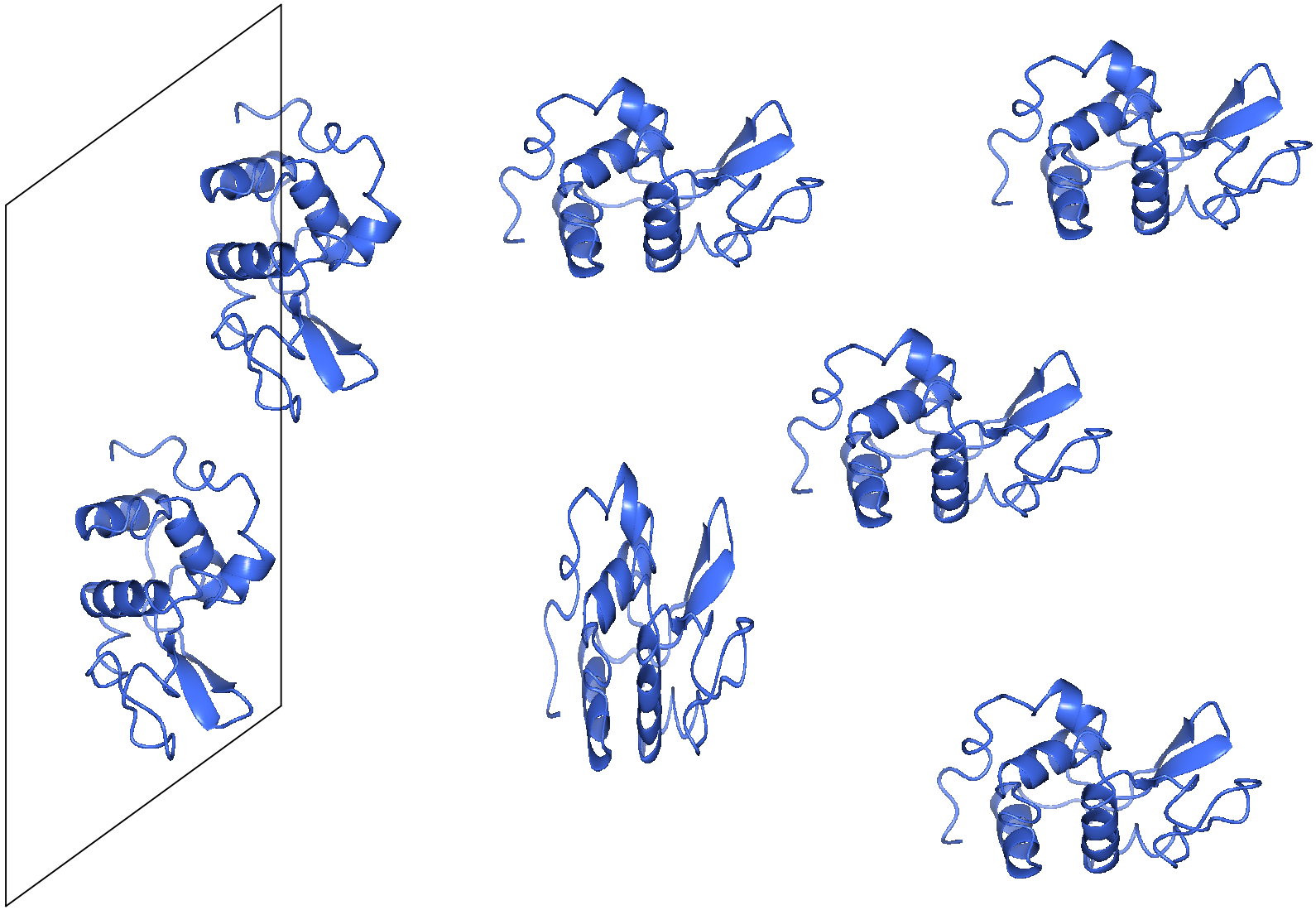


Residual Dipolar Couplings



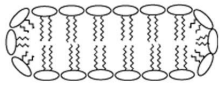
$$D = C\, r_{XY}^{-3} (3\cos^2\theta - 1)$$

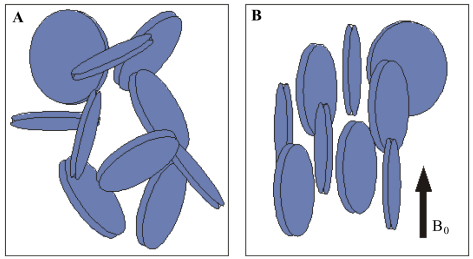
Residual Dipolar Couplings

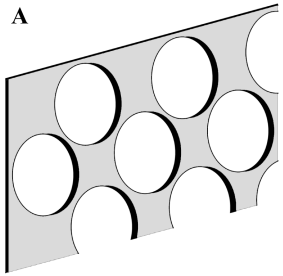


RDCs – Alignment Media

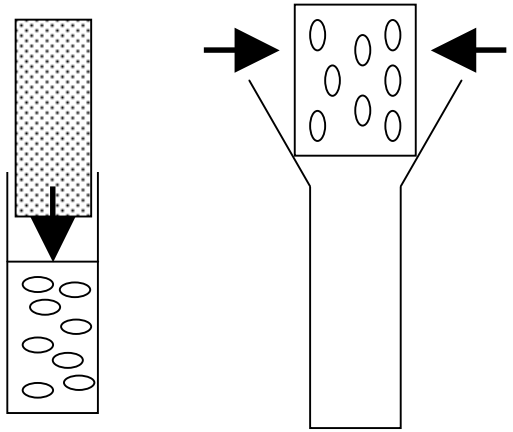
Bicelles



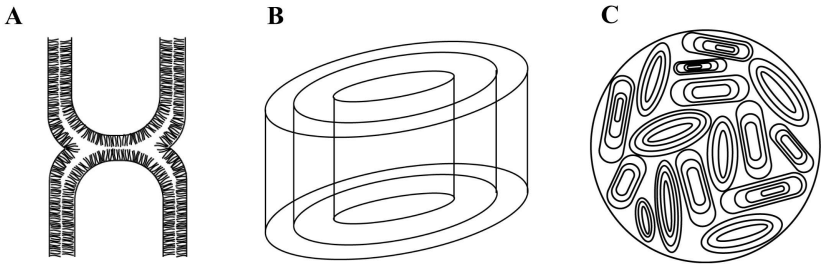




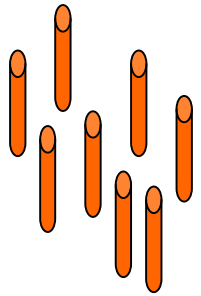
Strained gels



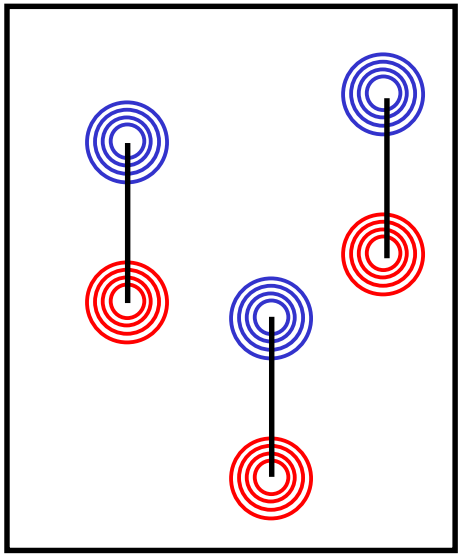
n-alkyl-PEG / alcohol



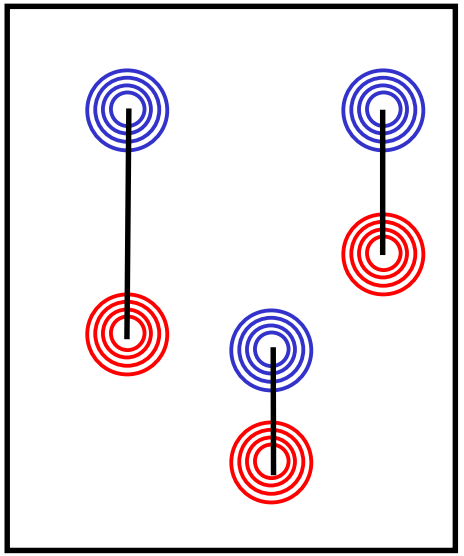
Phage



RDCs – Measurement



isotropic



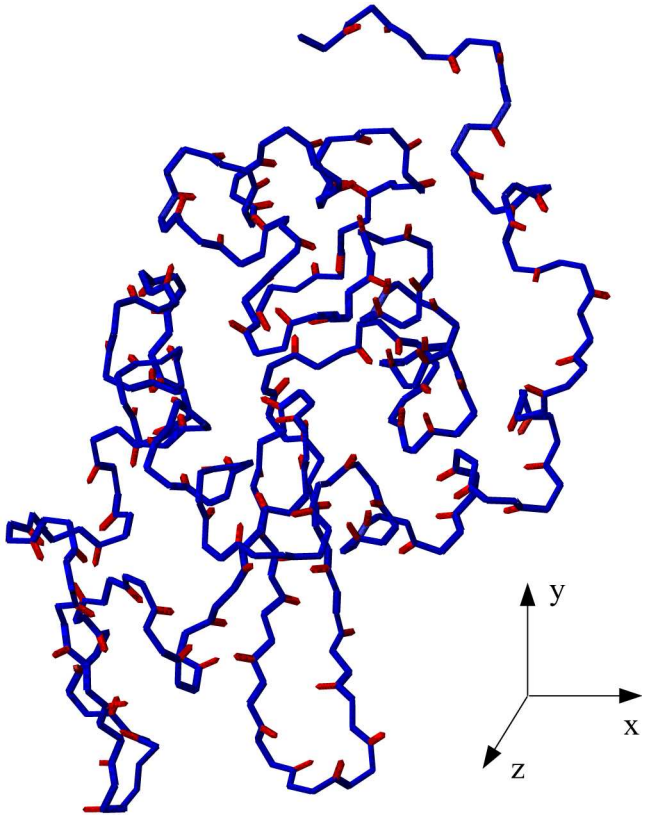
anisotropic

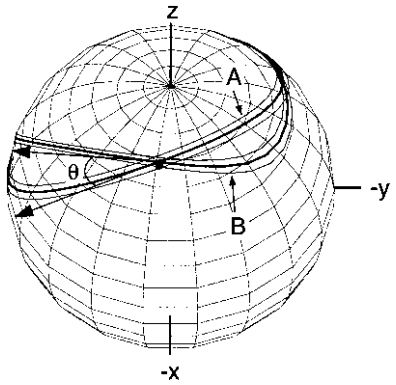
$J_{iso,obs} = J$

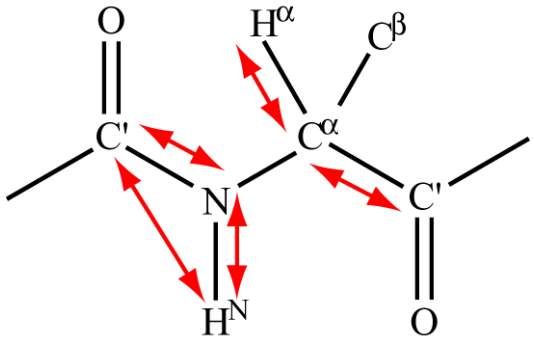
$J_{aniso,obs} = J + D$

$\therefore D = J_{aniso,obs} - J_{iso,obs}$

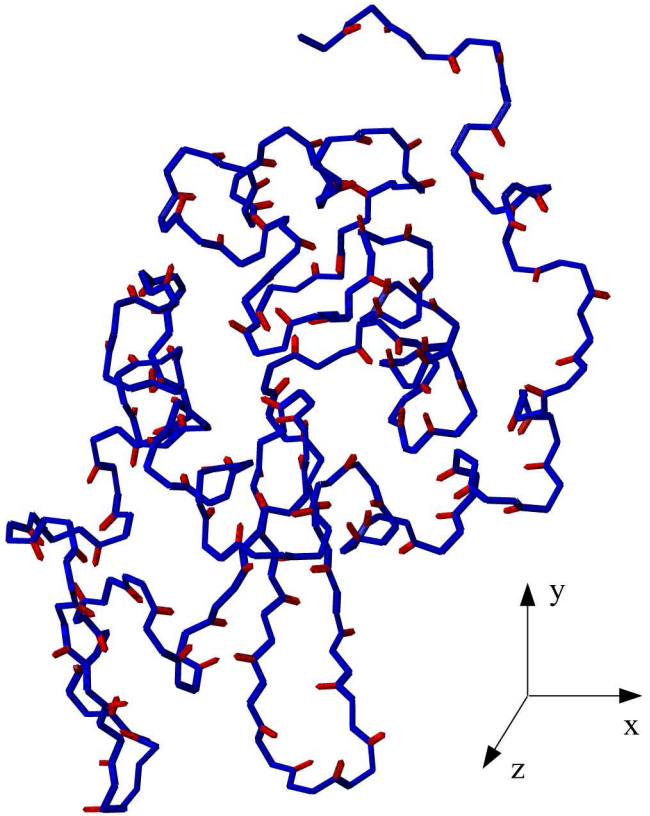
RDCs in Structure Calculations

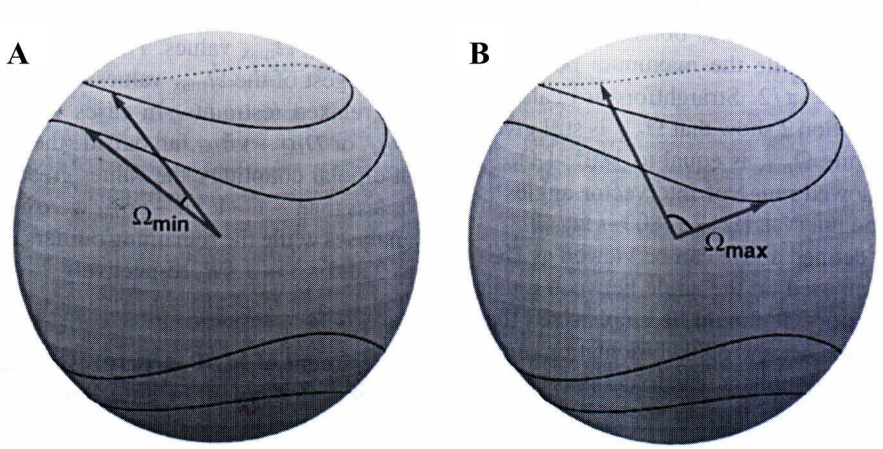




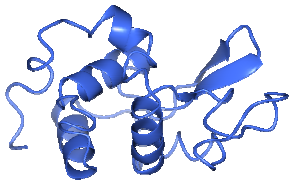


RDCs in Structure Calculations





Hydrogen Bonds



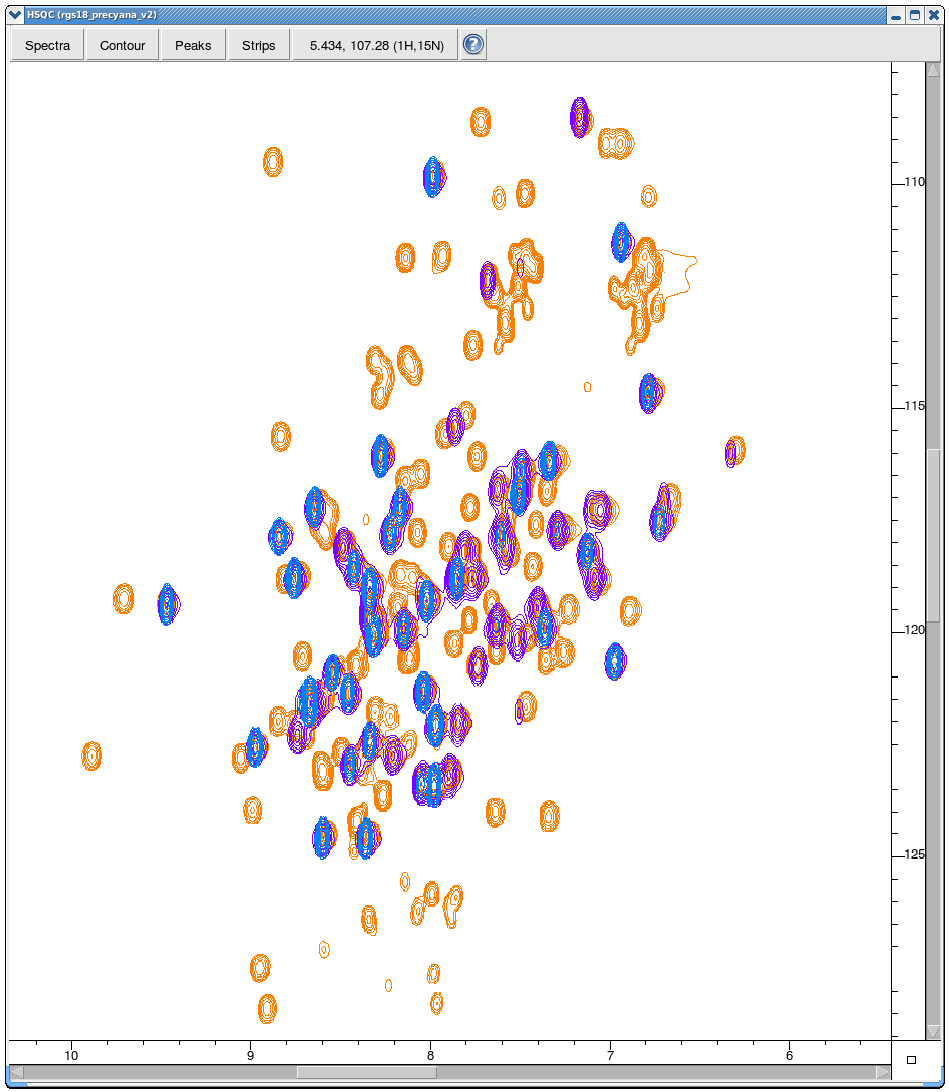
+ H_2O

↓

Freeze dry

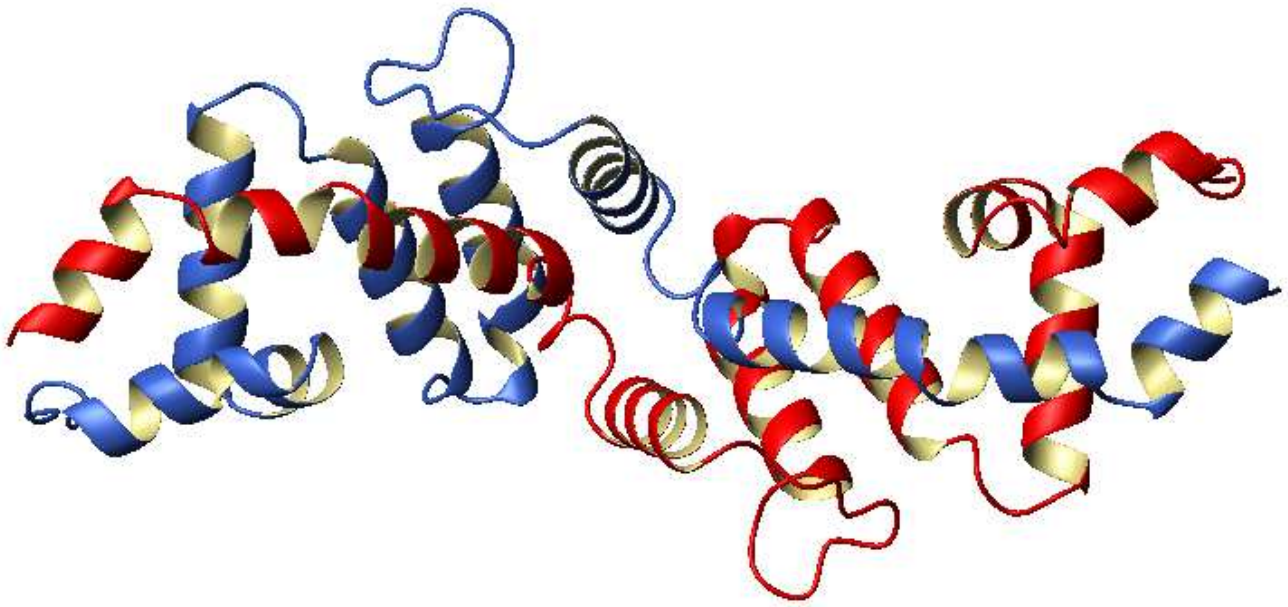
↓

D_2O



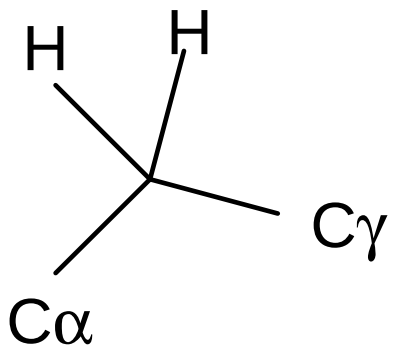
Other Restraint Types

- Symmetry



Other Restraint Types

- Symmetry
- Prochiral atoms



Other Restraint Types

- Symmetry
- Prochiral atoms
- Conformational Database Potentials

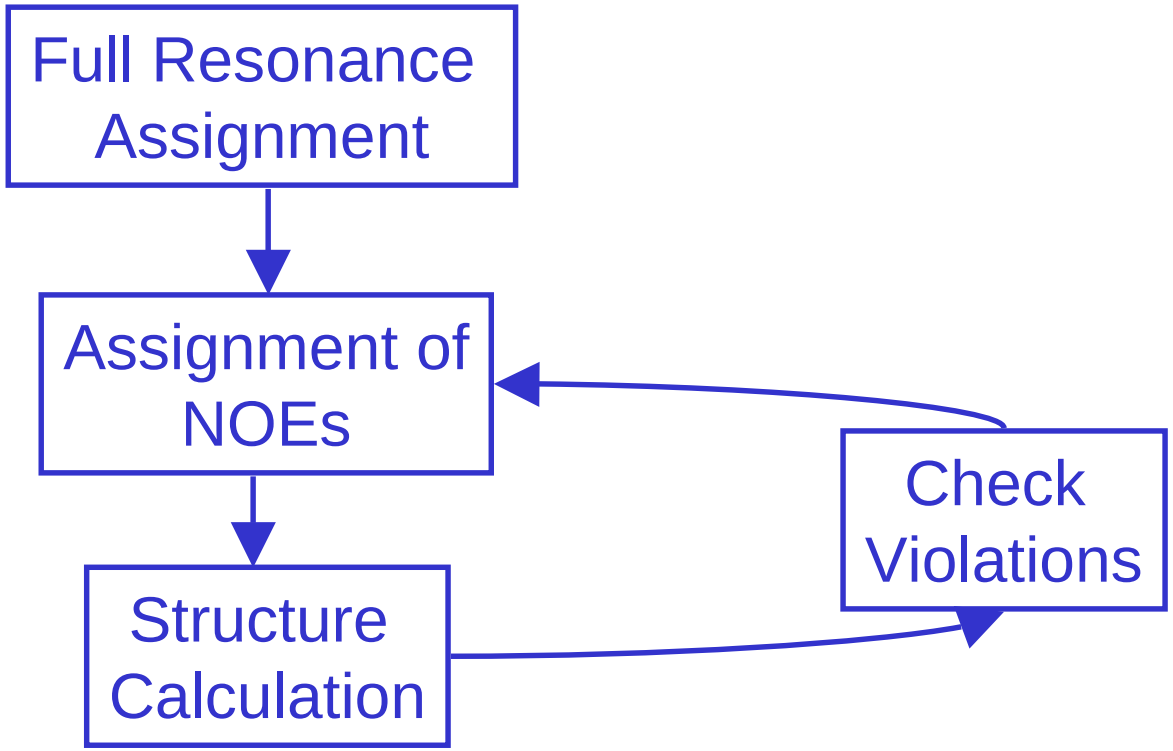
Other Restraint Types

- Symmetry
- Prochiral atoms
- Conformational Database Potentials
- Radius of Gyration

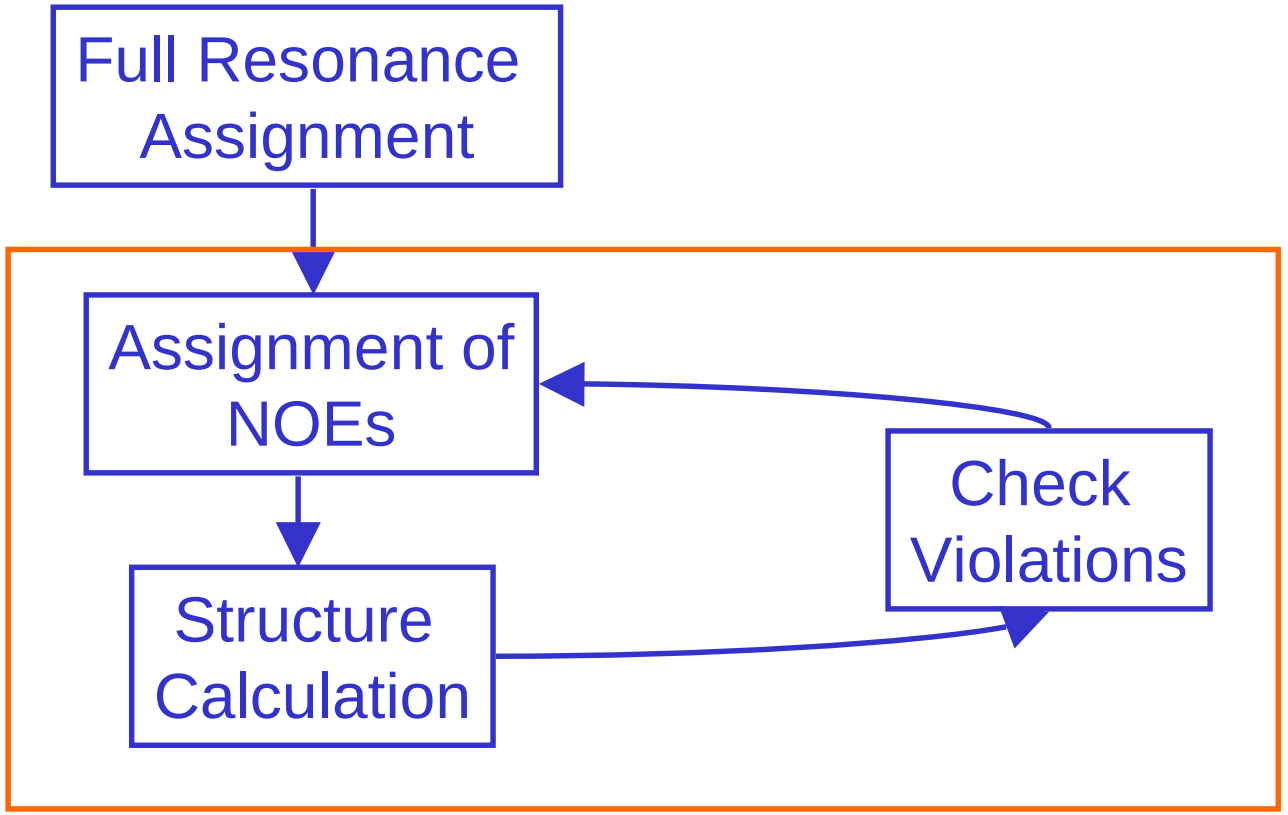
Other Restraint Types

- Symmetry
- Prochiral atoms
- Conformational Database Potentials
- Radius of Gyration
- Pseudoatoms

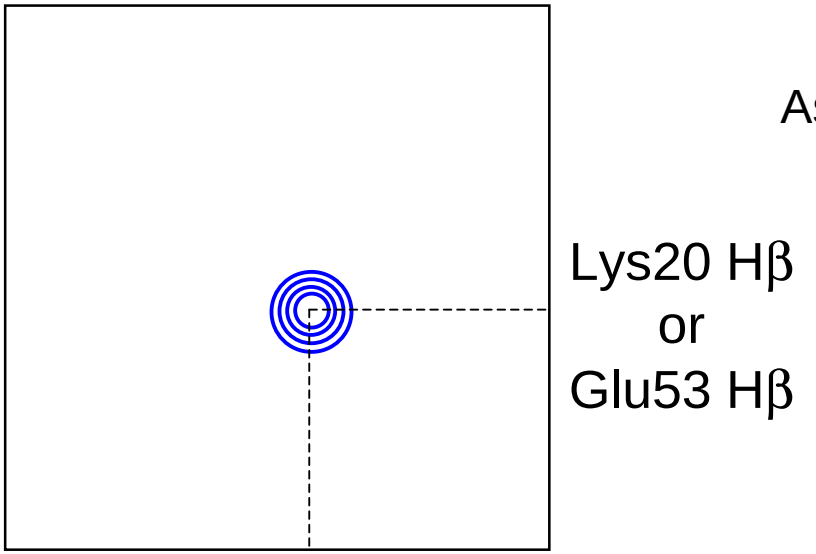
Traditional Structure Determination

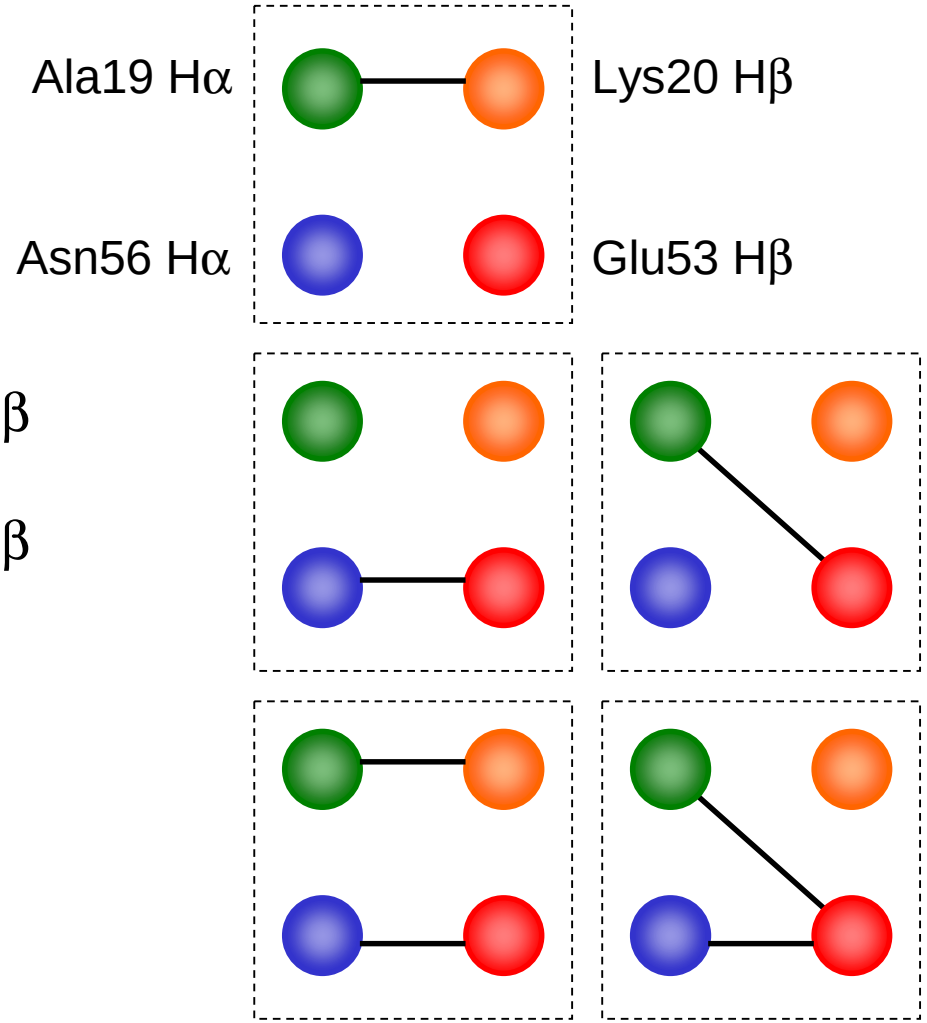


Automatic NOE Assignment

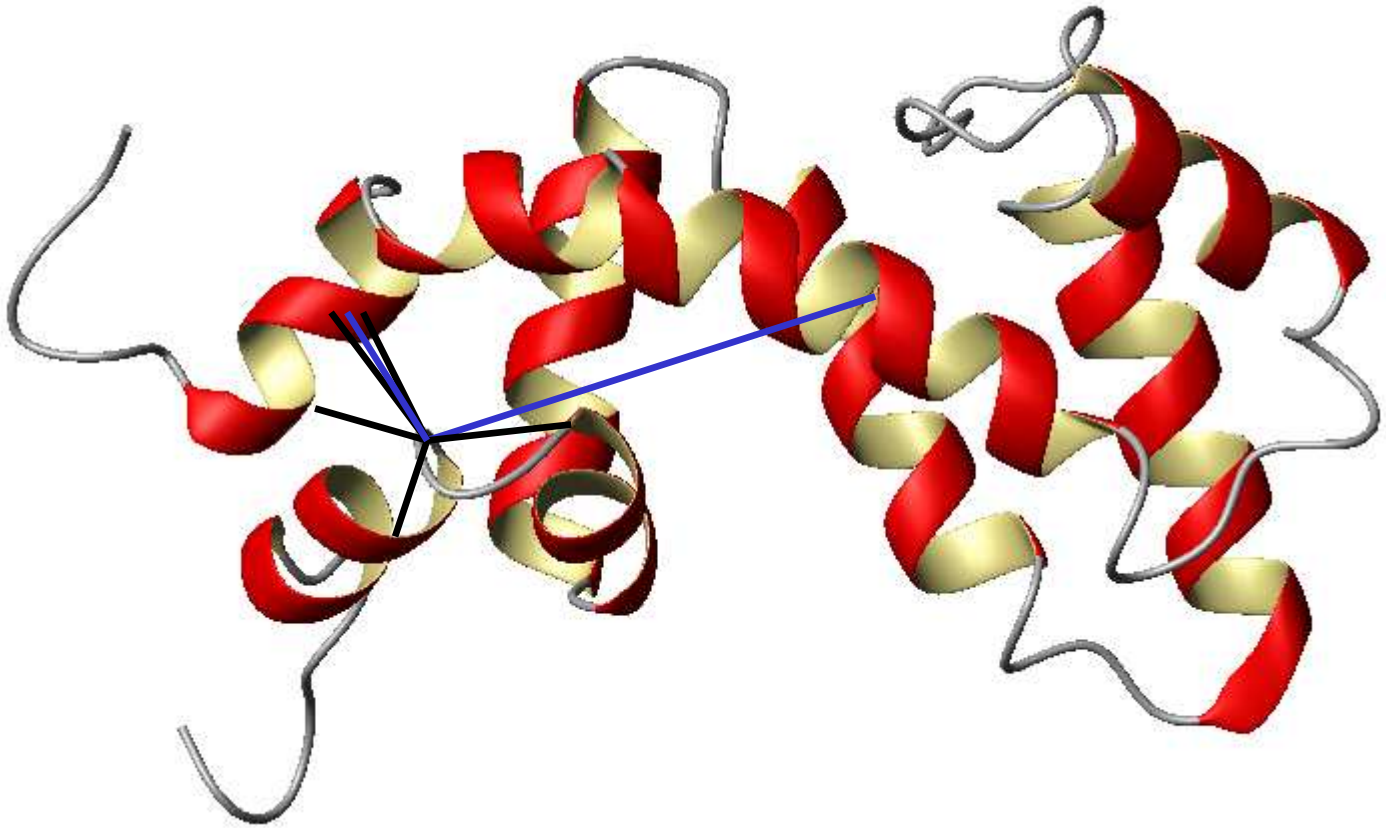


Ambiguous NOE Restraints

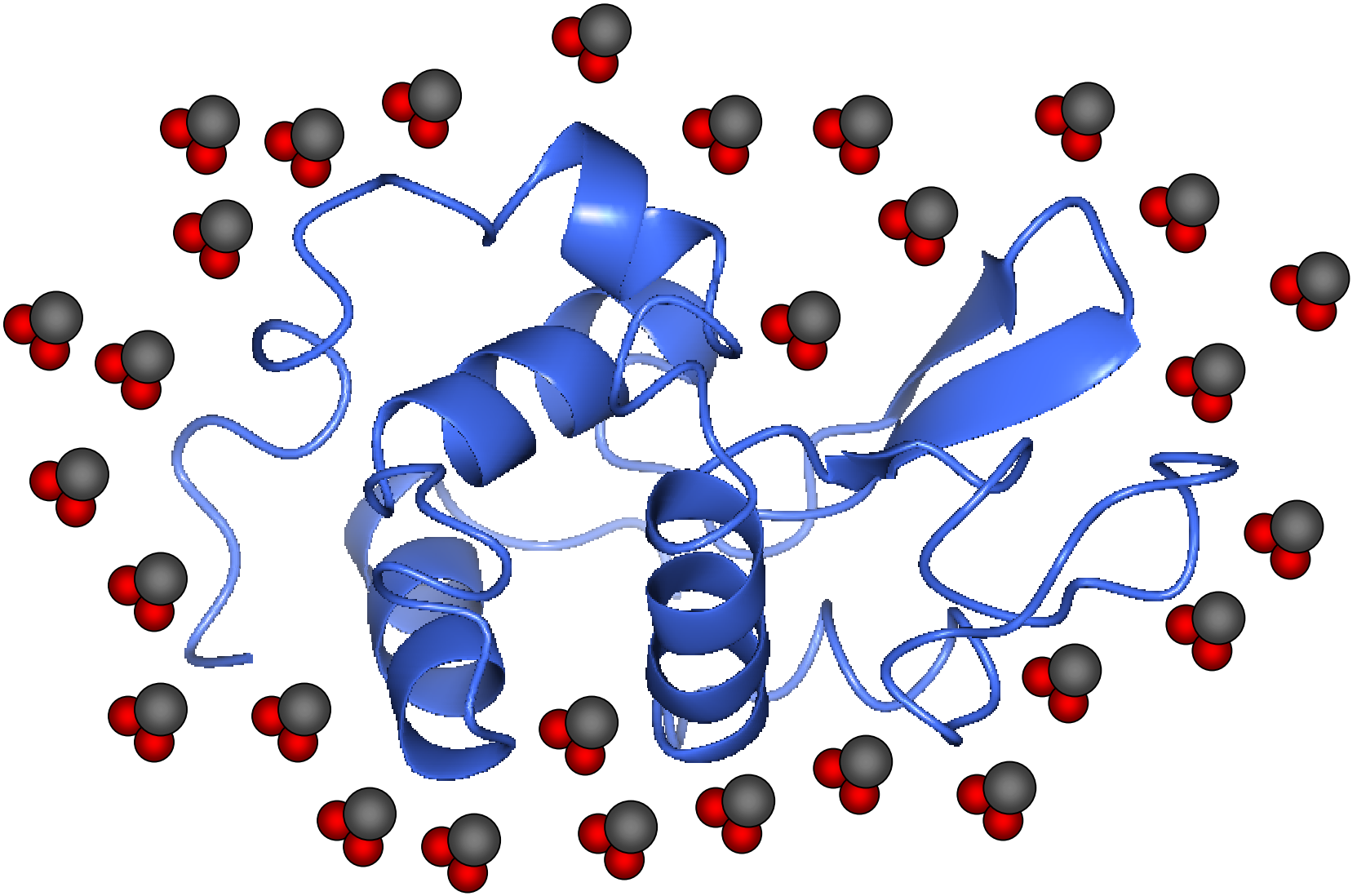




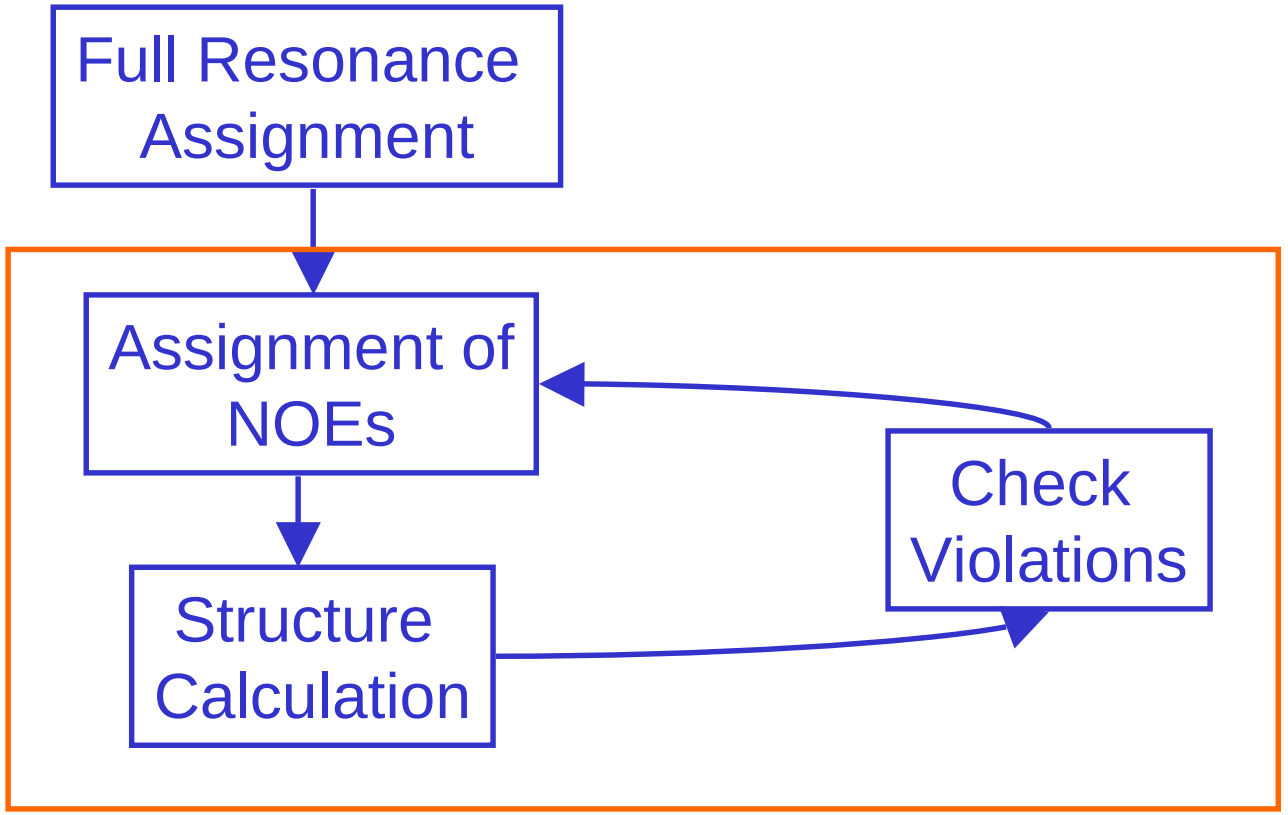
Network Anchoring



Water Refinement



Validation


Full Resonance Assignment
Assignment of NOEs
Structure Calculation
Check Violations

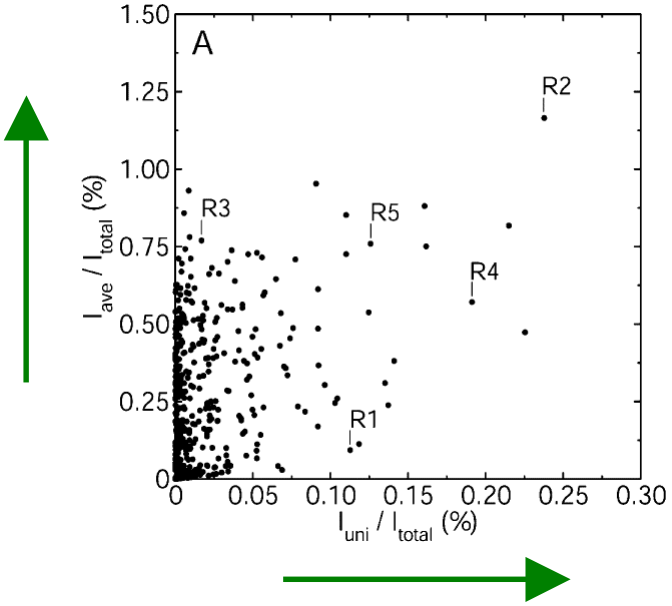
Validation

- Violations to experimental restraints (CCPNmr Analysis, PSVS)
- Dihedral Angles (Procheck, PSVS)
- Inter-atomic Clashes (PSVS)
- RMSD (PSVS)
- Monomer or Multimer? (Relaxation measurements or AUC)
- Information Content of Restraints (QUEEN)

Validation – Information Content

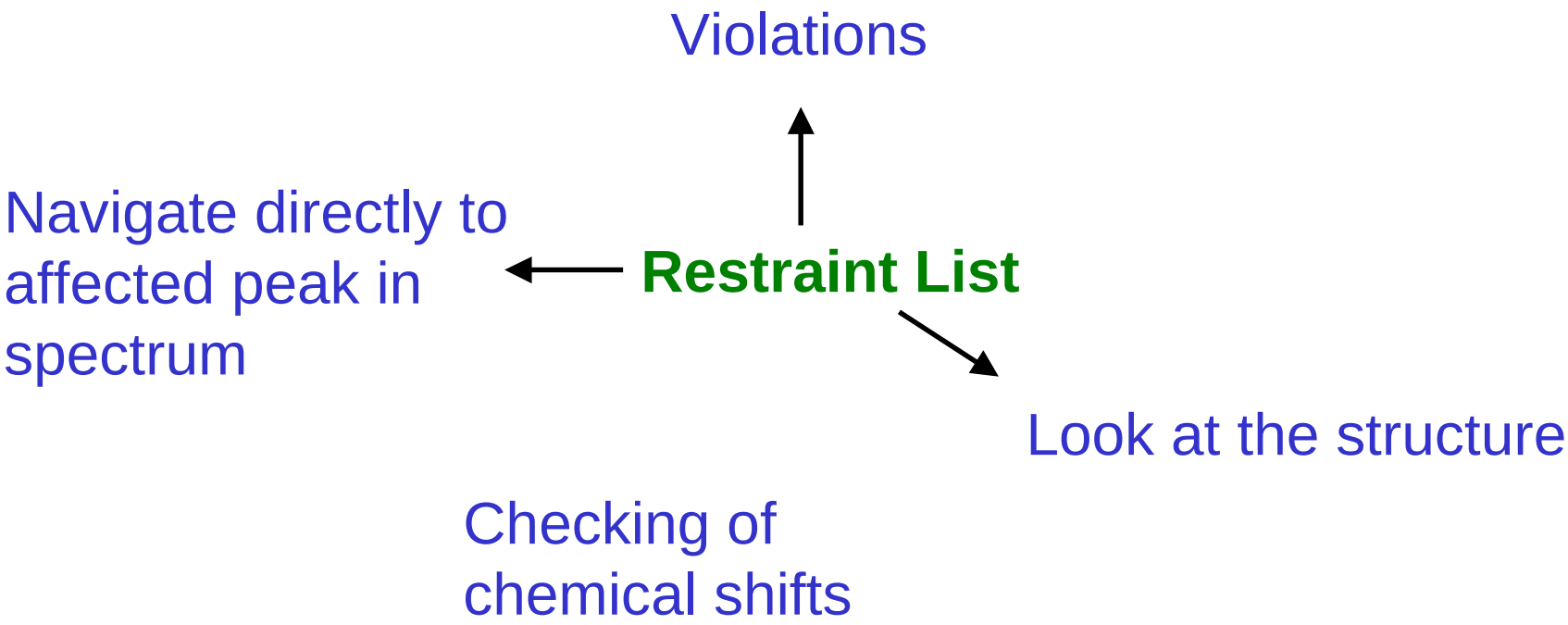
- QUEEN – QUantitative Evaluation of Experimental Nmr restraints

How much does this restraint influence the structure?

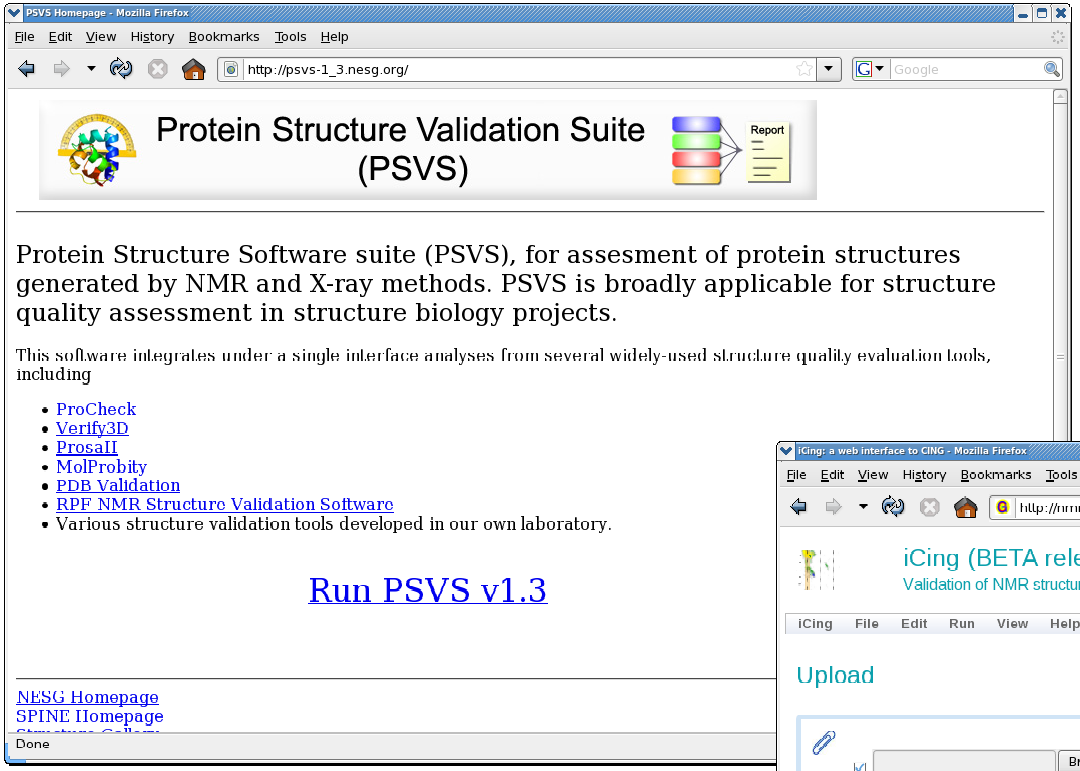


How unique is the information provided by this restraint?

Validation - CCPN

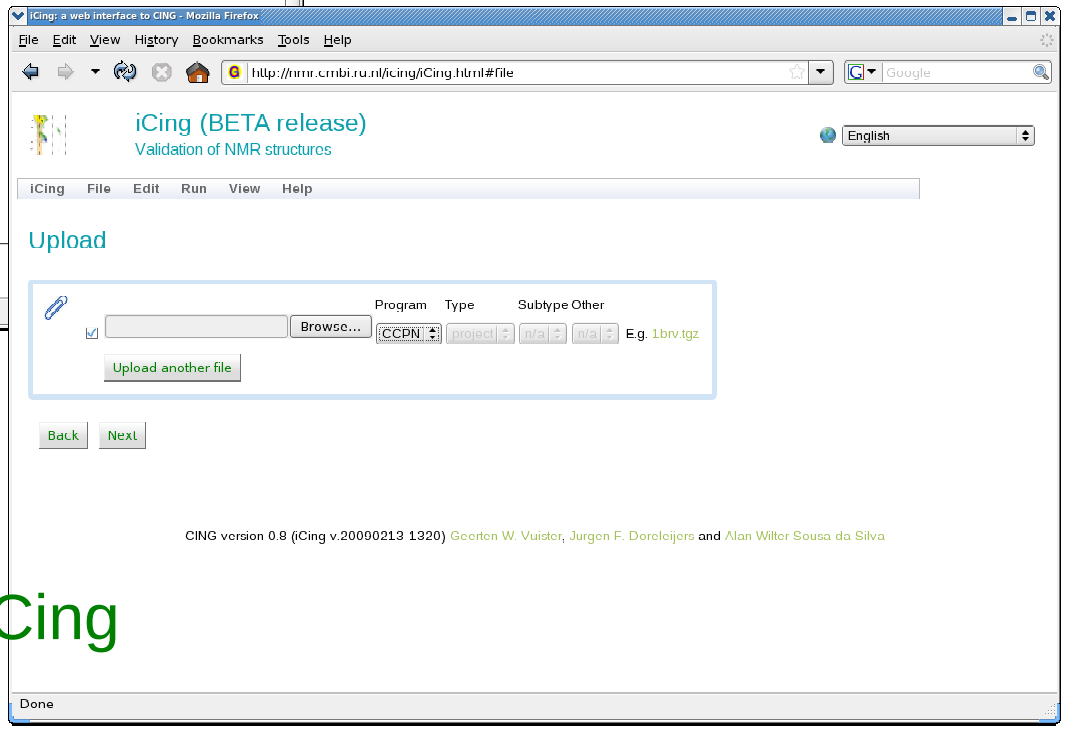


Validation - Other



PSVS

http://psvs-1_3.nesg.org/



iCing

http://nmr.cmbi.ru.nl/icing/iCing

Structure Determination from Chemical Shifts

- CS-ROSETTA
- CHESHIRE
- CS23D (www.cs23d.ca)

Inferential Structure Determination

- www.isd.bio.cam.ac.uk/isd

@FMP

- CYANA
- ARIA
- XPLOR-NIH
- CNS

- QUEEN

- Cluster [48 processors]: matrix
  - http://matrix.fmp-berlin.de

Good Luck!