NMR Spectral Assignment and Structural Calculations

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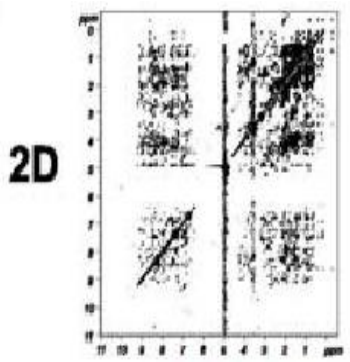
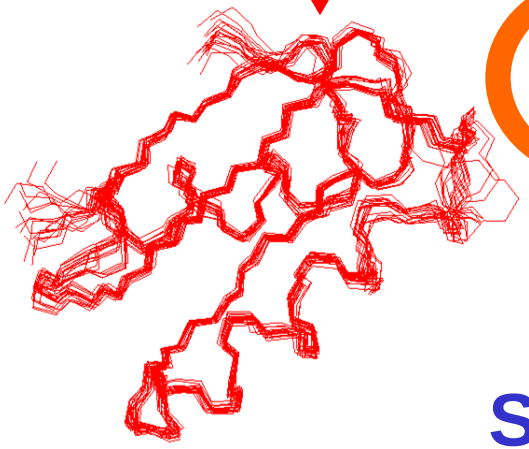
instruct
Integrating
Biology

Structure determination through NMR





Protein Sample

↓

NMR spectroscopy

↓

Sequential resonance assignment

↓

Collection of conformational constraints

↓

3D structure calculations

↓

Structure refinement and Analysis

The protein in the NMR tube!

- Protein overexpression
- Purification
- $^{15}N/^{13}C$ labelling

< 25 KDa
about 240 AA → **^{13}C, ^{15}N labeling**

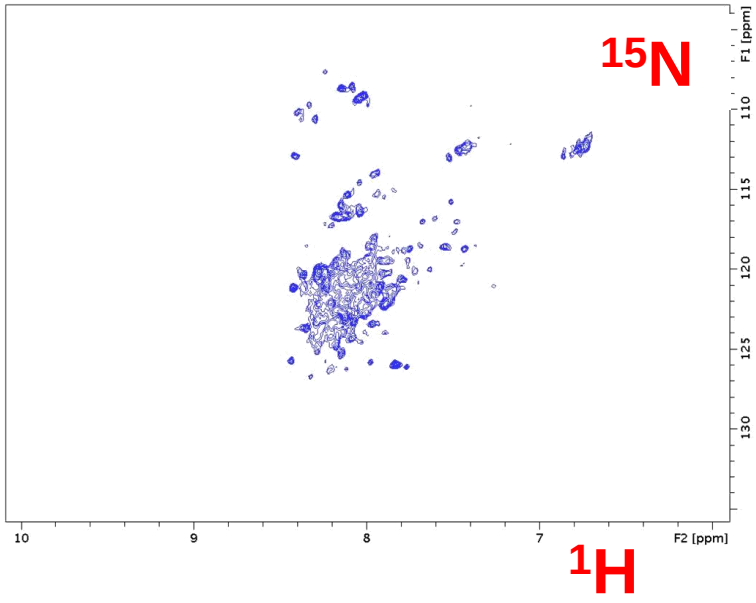
> 25 kDa
about 240 AA → **^{13}C, ^{15}N labeling**
+ ^{2}H labeling necessary!!

Which experiments should I run?

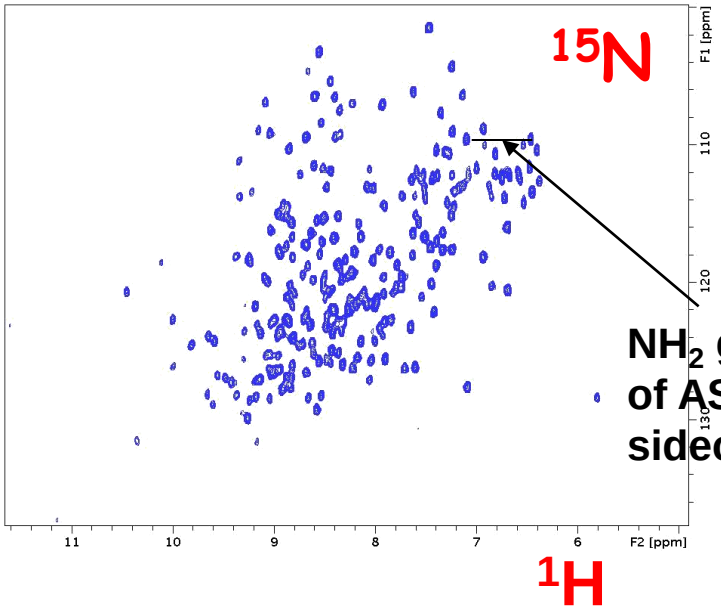
Is my sample OK for NMR?

^{1}H-^{15}N HSQC gives the protein fingerprint

unfolded



folded



NH_2 groups of ASN, GLN sidechains

Signals of unfolded proteins have little ^{1}H dispersion, that means the ^{1}H frequencies of all residues are very similar.

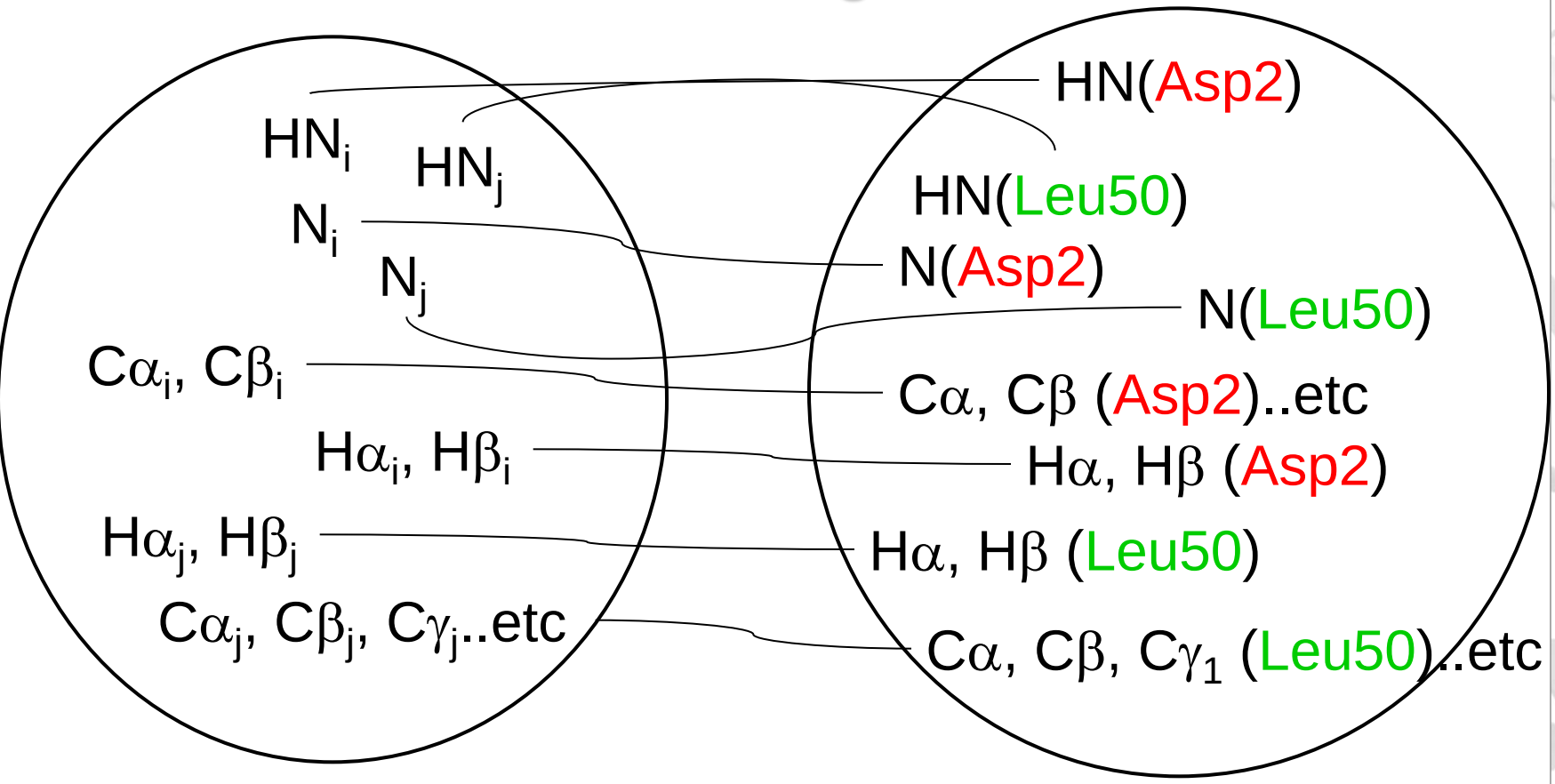
Folded proteins have larger dispersion

Can I see all the peaks I expect?

Count the peaks! → Backbone NH (excluding prolines!)

Making resonance assignment

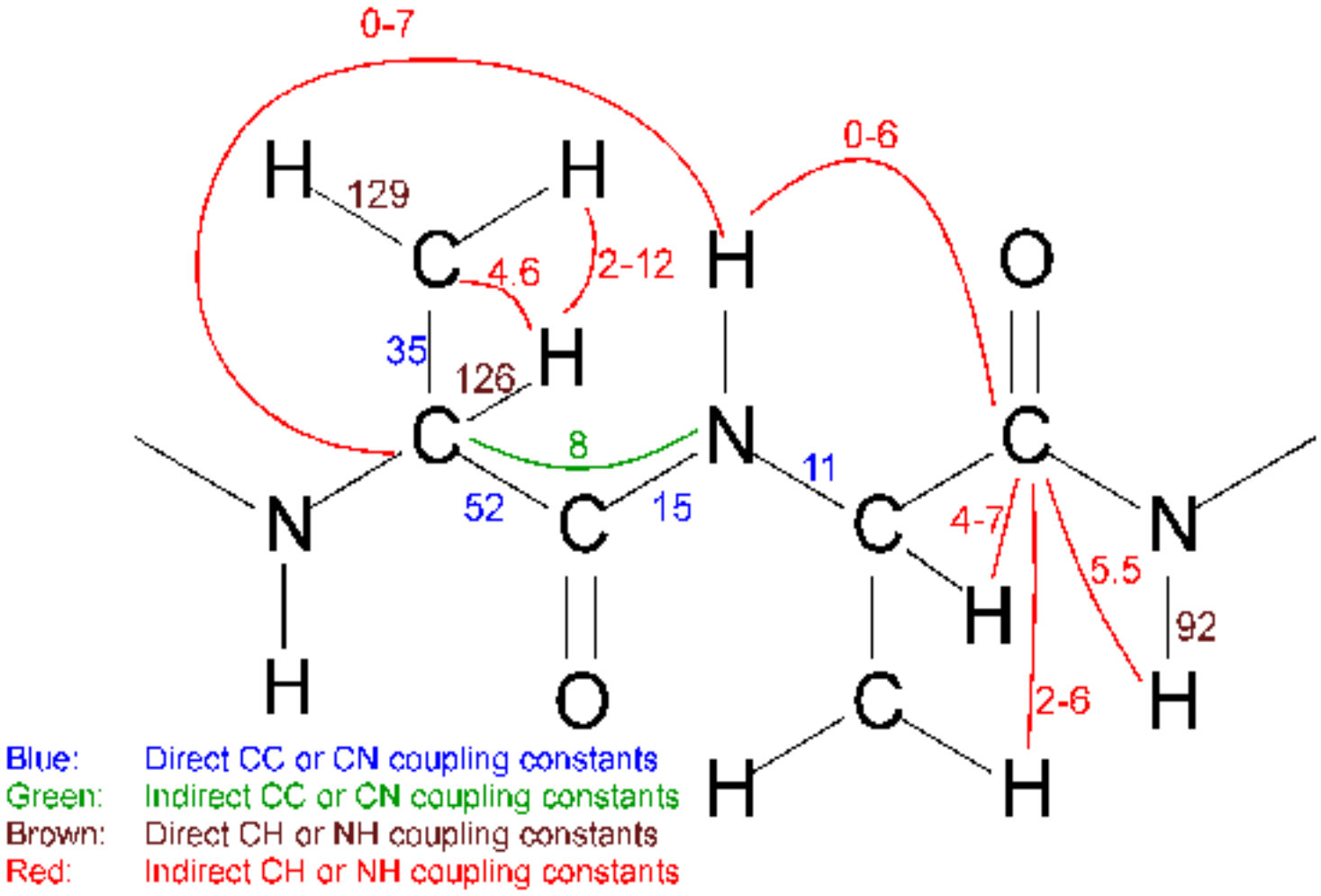
What does it mean to make sequence specific resonance assignment ?



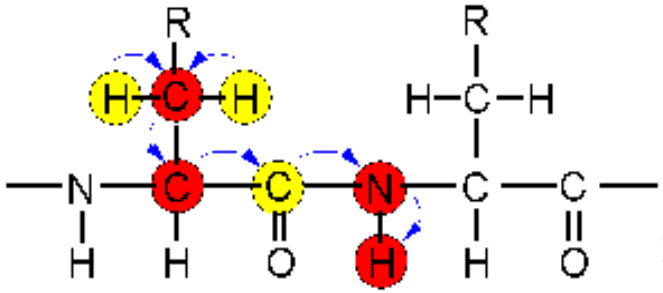
To associate each resonance frequency to each atom of the individual residues of the protein

Assignment Strategy

The strategy for assignment is based on scalar couplings



Experiments for backbone assignment



CBCA(CO)NH

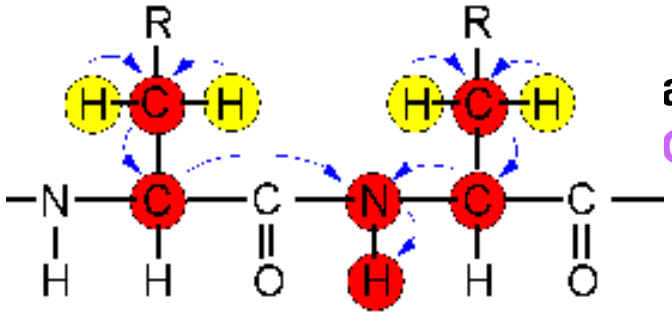
$^{1}H_{i}$-$^{15}N_{i}$-$^{13}C\alpha_{i-1}$ $^{13}C\beta_{i-1}$

Res_{i-1} Res_{i}

CBCANH

$^{1}H_{i}$-$^{15}N_{i}$-$^{13}C\alpha_{i}$ $^{13}C\beta_{i}$

$^{1}H_{i}$-$^{15}N_{i}$-$^{13}C\alpha_{i-1}$ $^{13}C\beta_{i-1}$



Res_{i-1} Res_{i}

CBCA(CO)NH and CBCANH correlate amide groups (H and ^{15}N) with Cα and Cβ resonances.

HNCO $^{1}H_{(i)}$-$^{15}N_{(i)}$-$^{13}CO_{(i-1)}$

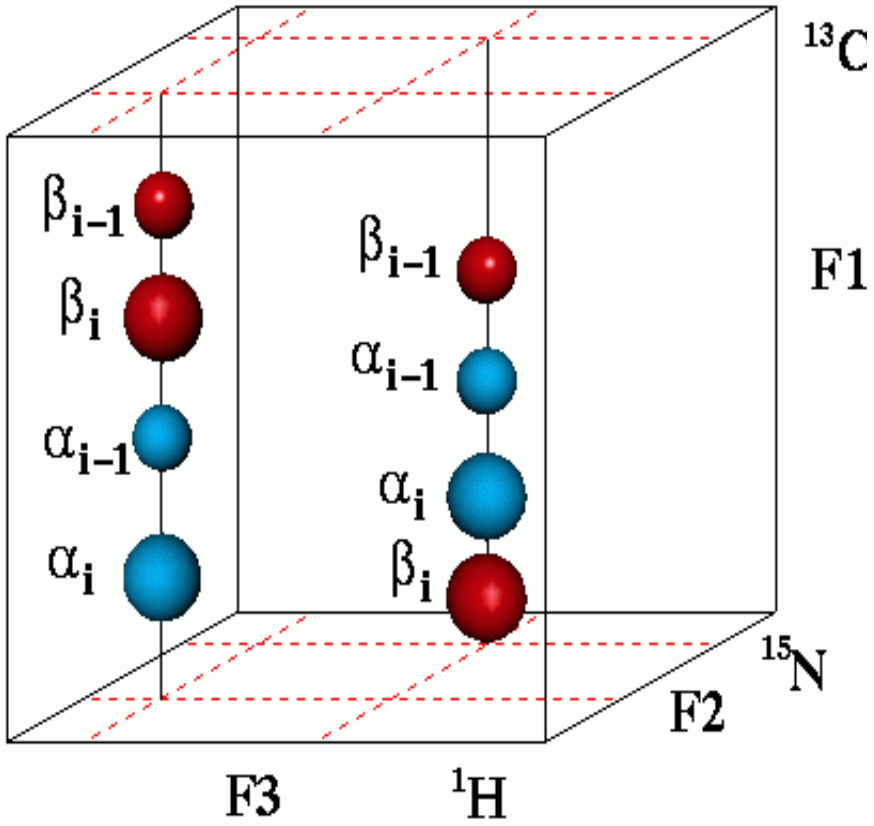
HN(CA)CO { $^{1}H_{(i)}$-$^{15}N_{(i)}$-$^{13}CO_{(i-1)}$, $^{1}H_{(i)}$-$^{15}N_{(i)}$-$^{13}CO_{(i)}$

HNCA { $^{1}H_{(i)}$-$^{15}N_{(i)}$-$^{13}C_{\alpha(i)}$, $^{1}H_{(i)}$-$^{15}N_{(i)}$-$^{13}C_{\alpha(i-1)}$

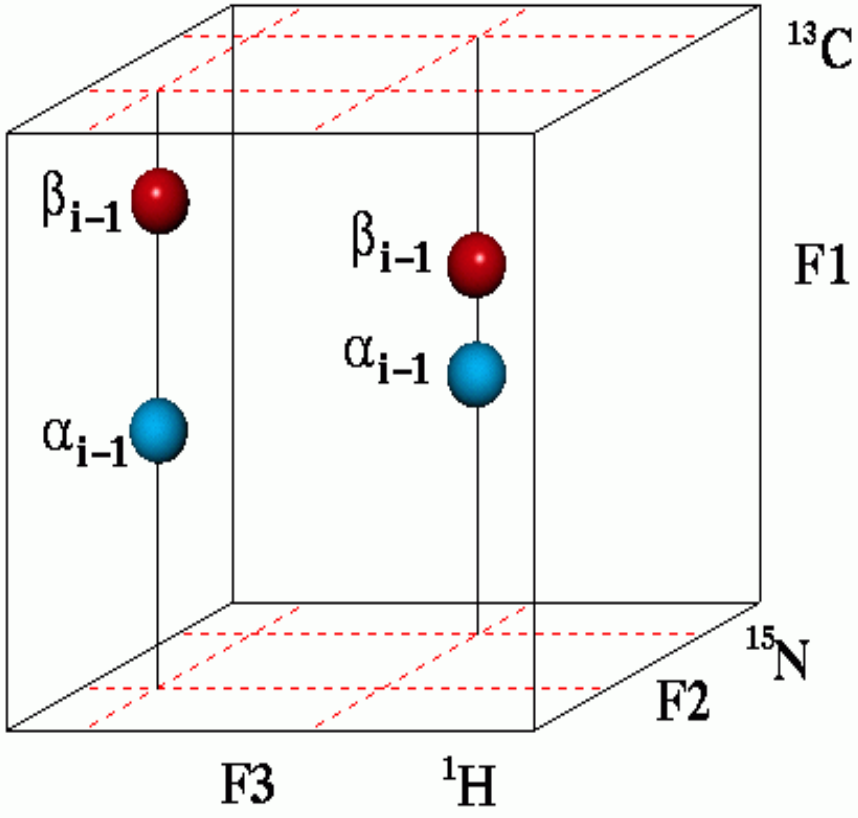
HN(CO)CA $^{1}H_{(i)}$-$^{15}N_{(i)}$-$^{13}C_{\alpha(i-1)}$

Experiments for backbone assignment

CBCANH



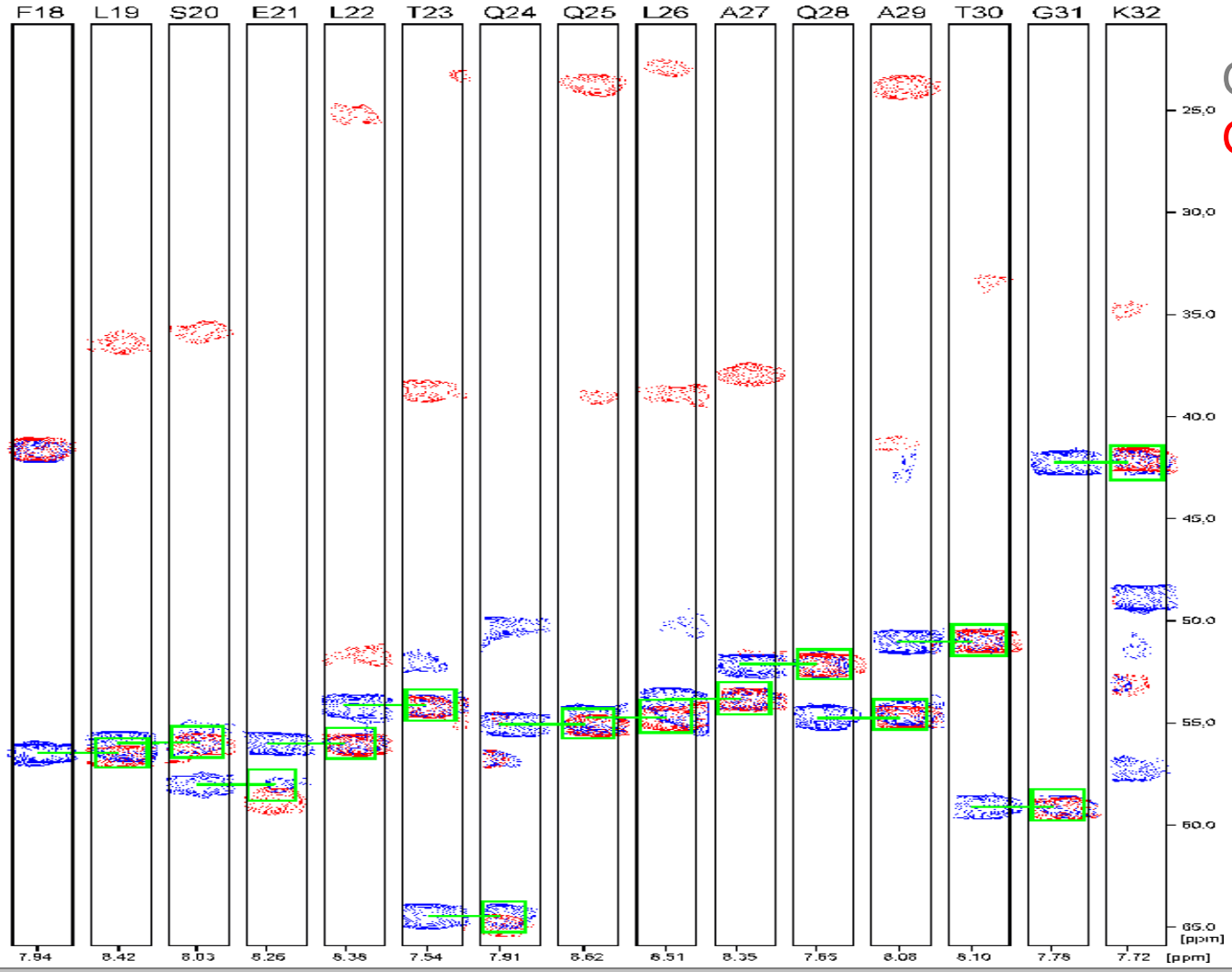
CBCA(CO)NH



The chemical shifts of Cα and Cβ atoms can be used for a preliminary identification of the amino acid type.

Sequential Assignment

The 'domino pattern' is used for the sequential assignment with triple resonance spectra



CB CANH
CBCA(CO)NH

Green boxes indicate sequential connectivities from each amino acid to the preceeding one

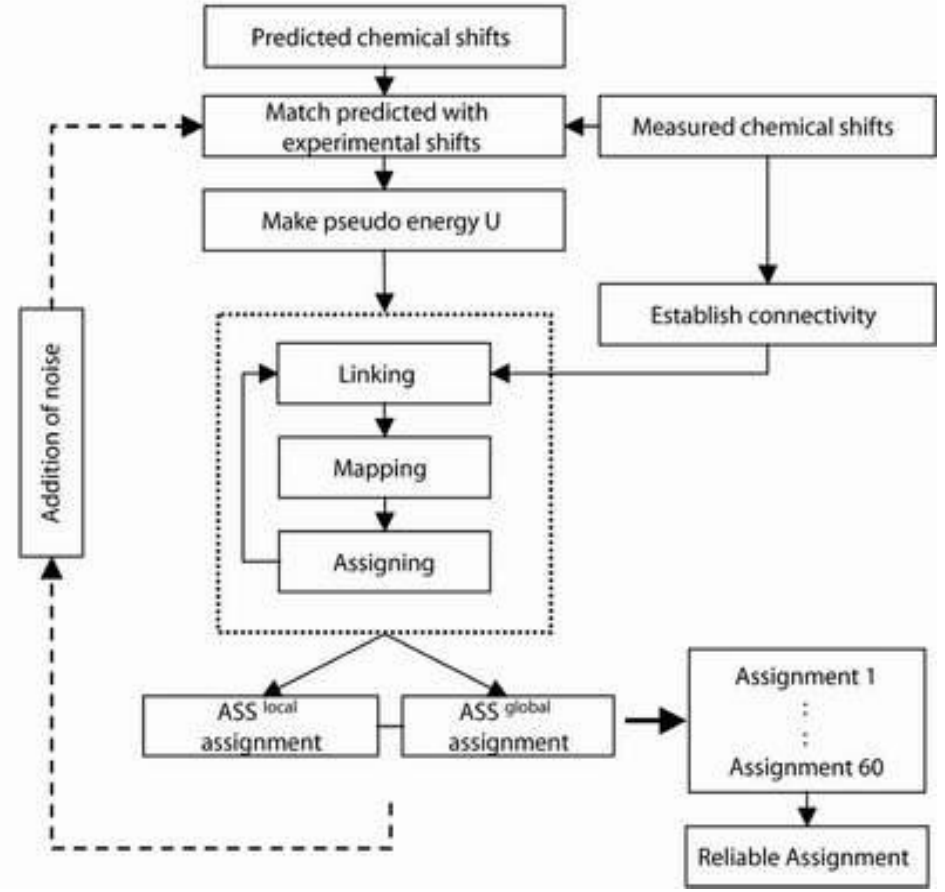
Automated assignment programs

MARS

Used for automated backbone assignment (NH, CO, Cα, Cβ) .
It requires manually pick-peaking of 3D spectra for backbone assignment, such as CBCANH, CBCACONH etc

Input:

- Primary sequence
- Spectral data, i.e chemical shifts of resonances grouped per residue and those of its preceding residue.
- Chemical shift tolerances
- Secondary structure prediction data (PSI-PRED)



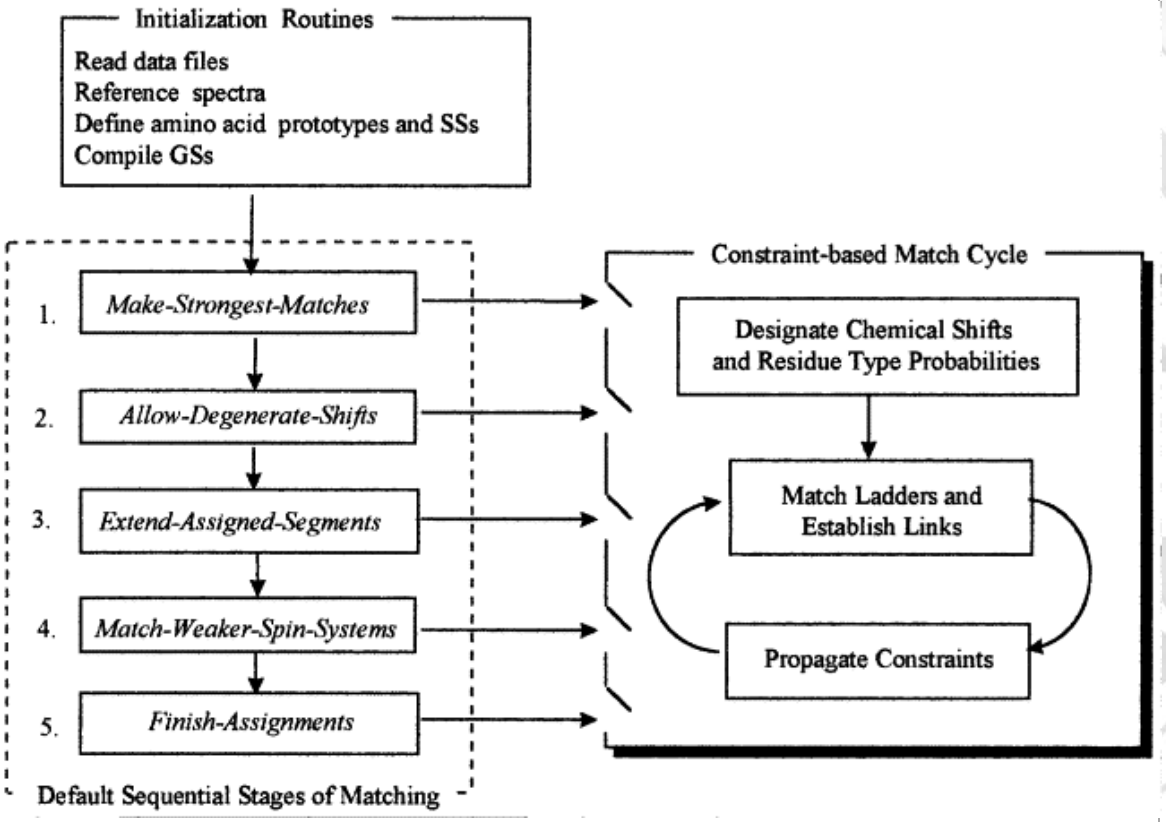
Automated assignment programs

AutoAssign

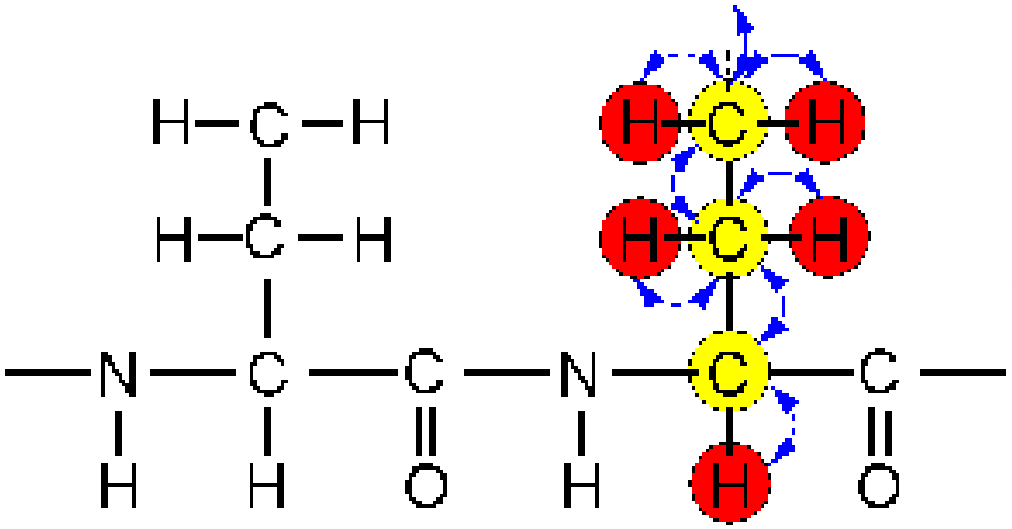
For automated backbone assignment (NH, CO, Cα, Cβ, Hβ and Hα). It requires manually pick-peaking of 3D spectra for backbone assignment, such as CBCANH, CBCACONH etc.

Input:

- peak list table of triple resonance spectra
- primary sequence



Experiment for side-chain assignment

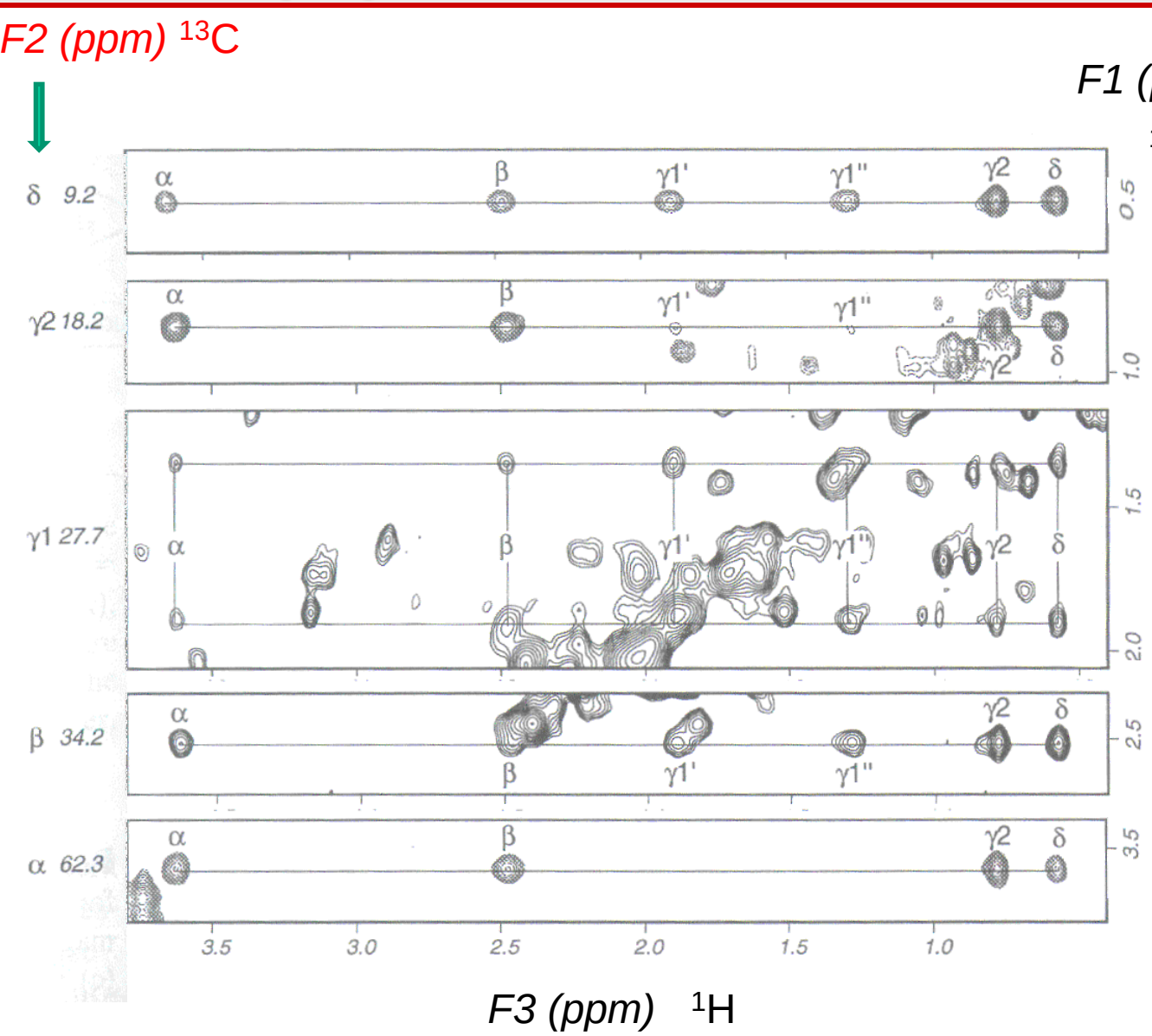


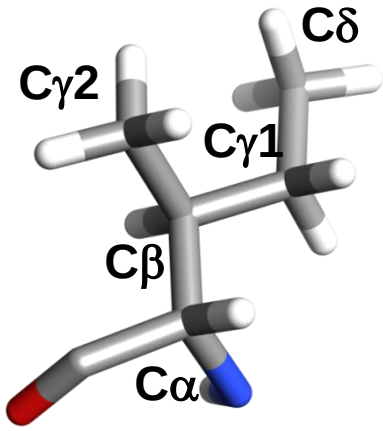
$^1H_i\alpha$, $^1H_i\beta$, $^1H_i\gamma1$.....

Res_{i-1} Res_i

In H(C)CH-TOCSY, magnetization coherence is transferred, through ^1J couplings, from a proton to its carbon atom, to the neighboring carbon atoms and finally to their protons.

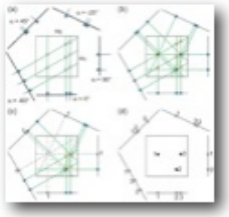
H(C)CH-TOCSY experiment

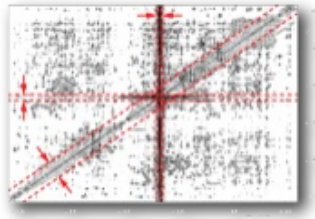


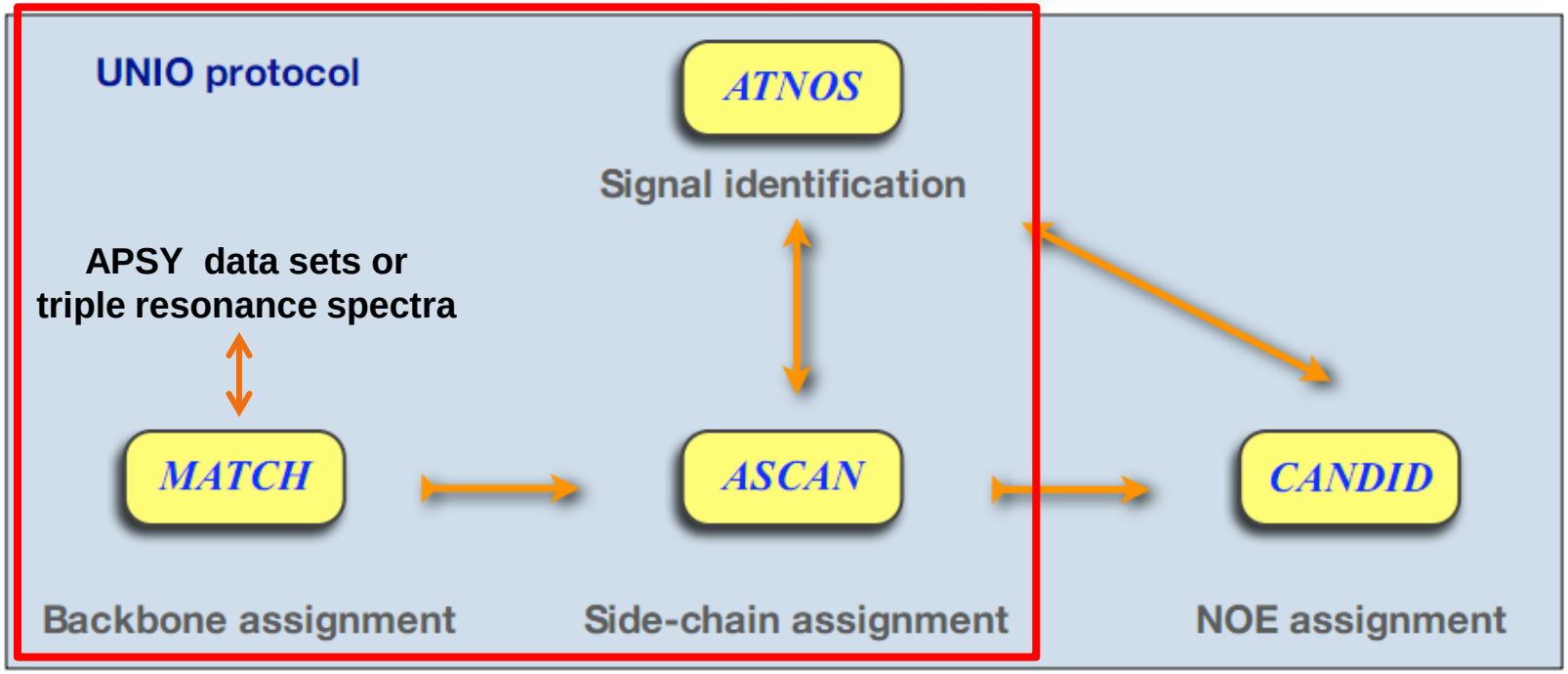


Isoleucine

UNIO for protein structure determination







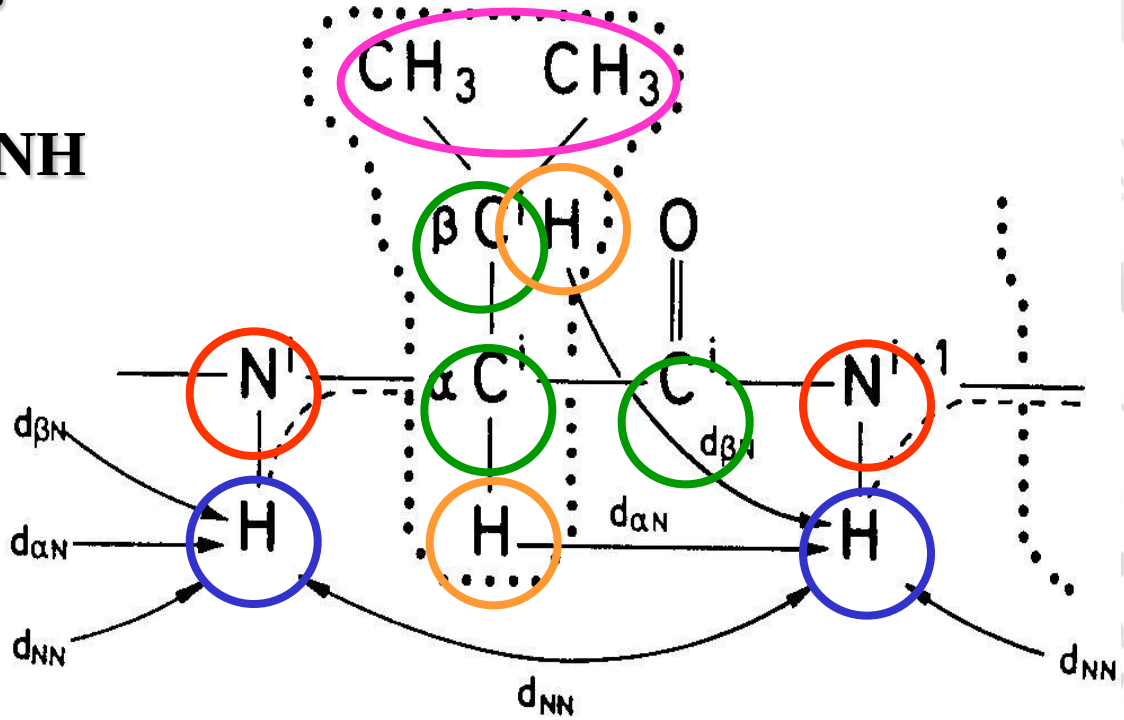
UNIO protocol operates directly on the NMR spectra.

http://perso.ens-lyon.fr/torsten.herrmann/Herrmann/Software.html

(1) Volk, J.; Herrmann, T.; Wüthrich, K. J. Biomol.NMR. 2008, 41, 127-138.
(2) Fiorito, F.; Damberger, F.F.; Herrmann, T.; Wüthrich, K. J. Biomol. NMR 2008, 42, 23-33.
(3) Herrmann, T.; Güntert, P.; Wüthrich, K. J. Mol. Biol. 2002, 319, 209-227.

Resonance assignment

- Triple resonance exps and NOESY/TOCSY ^{15}N-HSQC
- Triple resonance exps
- HNHA and HNHB or HBHA(CBCACO)NH
- (H)CCH-TOCSY/ H(C)CH-TOCSY

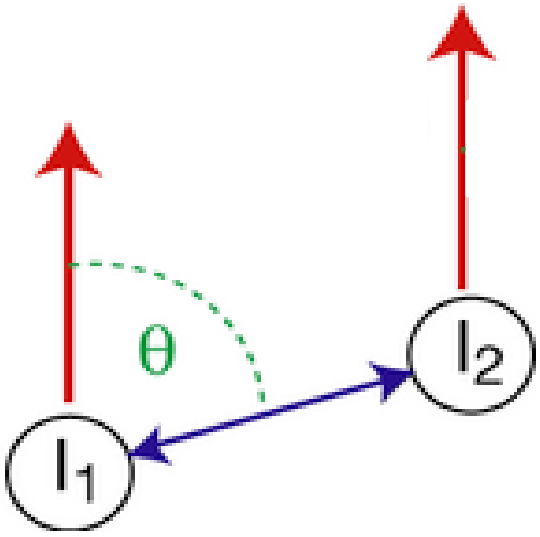


Conformational restraints

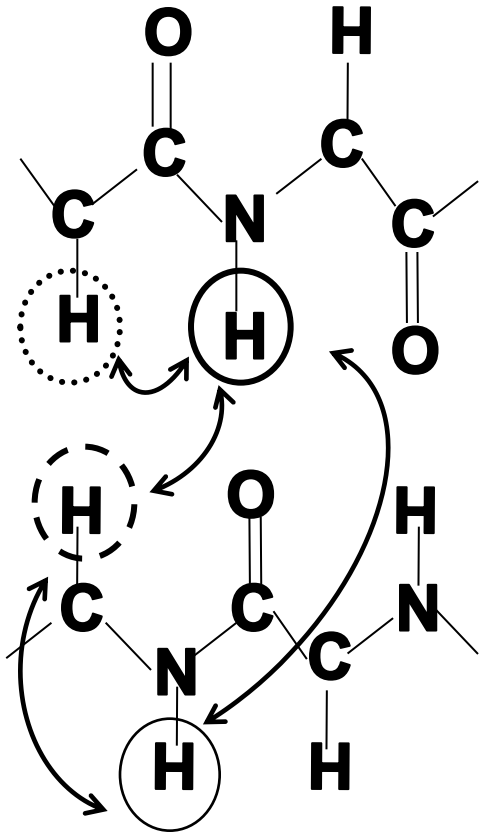
NMR experimental data		Structural restraints
NOEs	→	Proton-proton distances
Coupling constants	→	Torsion angles
Chemical shifts	→	Torsion angles
H -bonds	→	Proton-proton distances
RDCs	→	Bond orientations
Relaxation times	→	Metal-nucleus distances
PCSs	→	{ Metal-nucleus distances Orientation in the metal χ frame
Contact shifts	→	Torsion angles

Distance constraints

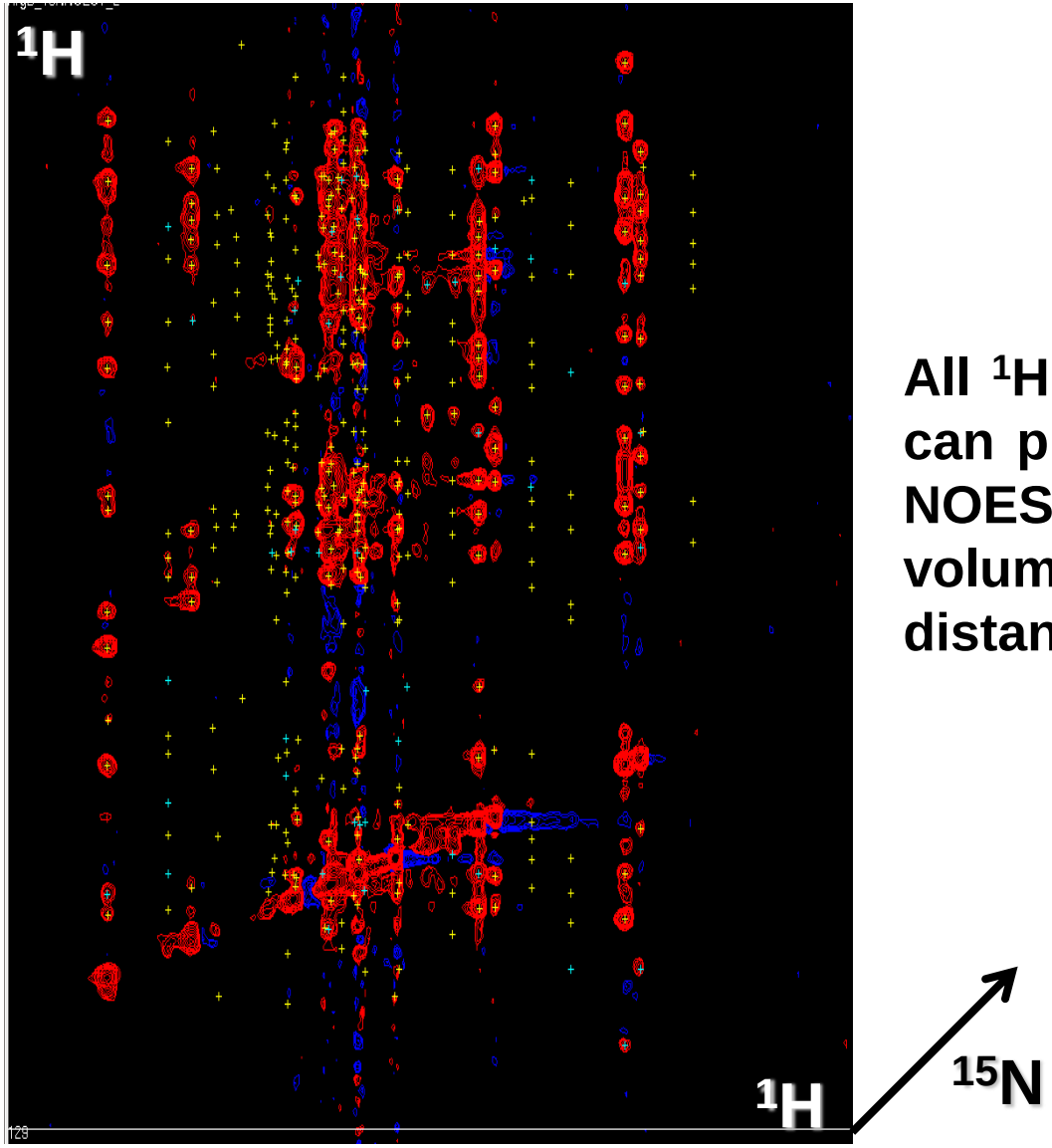
NOESY volumes are proportional to the inverse of the sixth power of the interproton distance (upon vector reorientational averaging)



$$\eta_{IJ} \propto \frac{\tau_c}{r_{IJ}^6}$$



The NOESY experiment:



All ^{1}H within 5-6 Å from a ^{1}H can produce a cross-peak in NOESY spectra whose volume provides ^{1}H-^{1}H distance restraints

How are the distance constraints obtained from NOEs intensities?

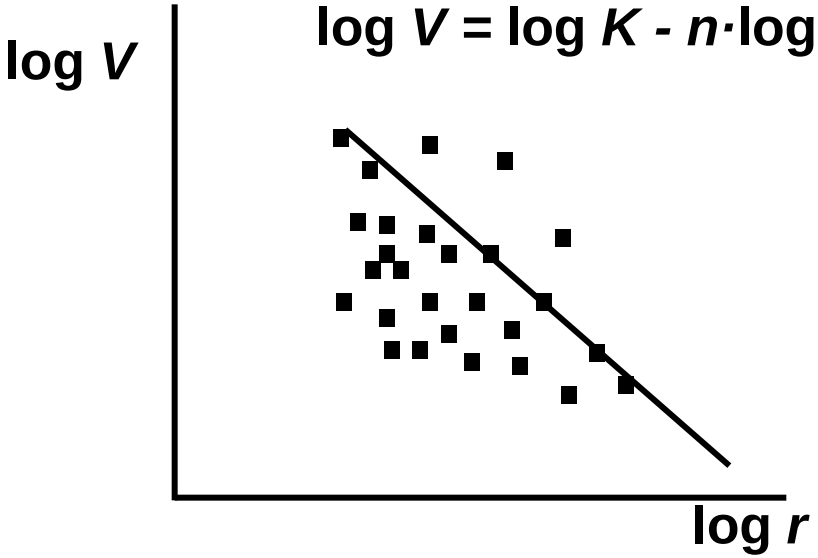
CYANA NOEs calibration

The NOESY cross-peak intensities (V) are converted into upper distance limits (r) through the relation:

$$V = \frac{K}{r^n}$$

where K is a constant and *n* can vary from 4 to 6.

***K* constant is initially determined from NOE's between protons at fixed distance**



Classes of constraints

1. Backbone	**$V = A/d^6$**
2. Sidechain	**$V = B/d^4$**
3. Methyl	**$V = C/d^4$**

Distances are given as value range

Wuthrich, K. (1986) "NMR of Proteins and Nucleic Acids"

How are the distance constraints obtained from NOEs intensities?

Xplor-NIH Calibration of NOEs

The NOESY cross-peak intensities are converted into upper distance limits

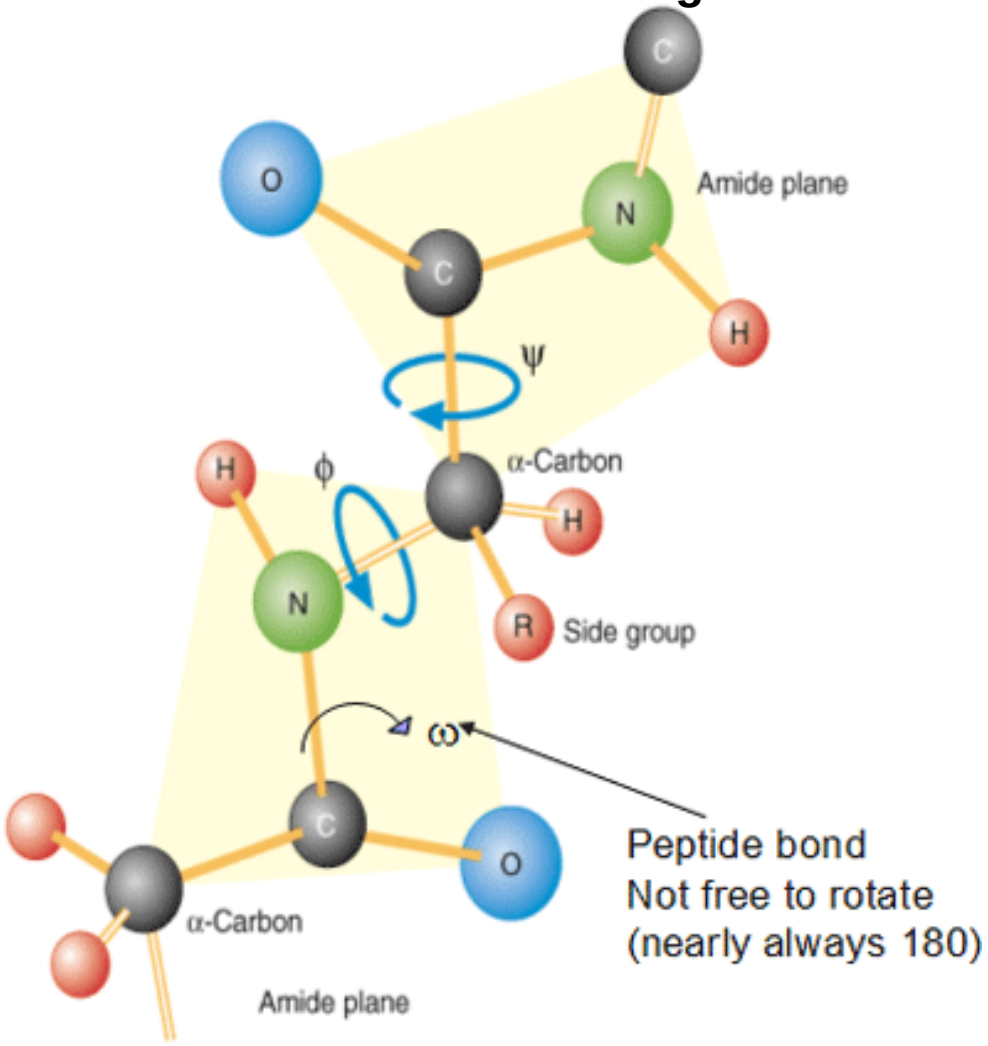
Classes of restraints		Distance ranges
1. Very Weak	0 – 20%	1.8–6.0 Å
2. Weak	20 – 50%	1.8–5.0 Å
3. Medium	50 – 80%	1.8–3.3 Å
4. Strong	80 –100%	1.8–2.7 Å

0.5 Å are added to the upper bound of distances involving methyl groups in order to correct for the larger than expected intensity of methyl crosspeaks

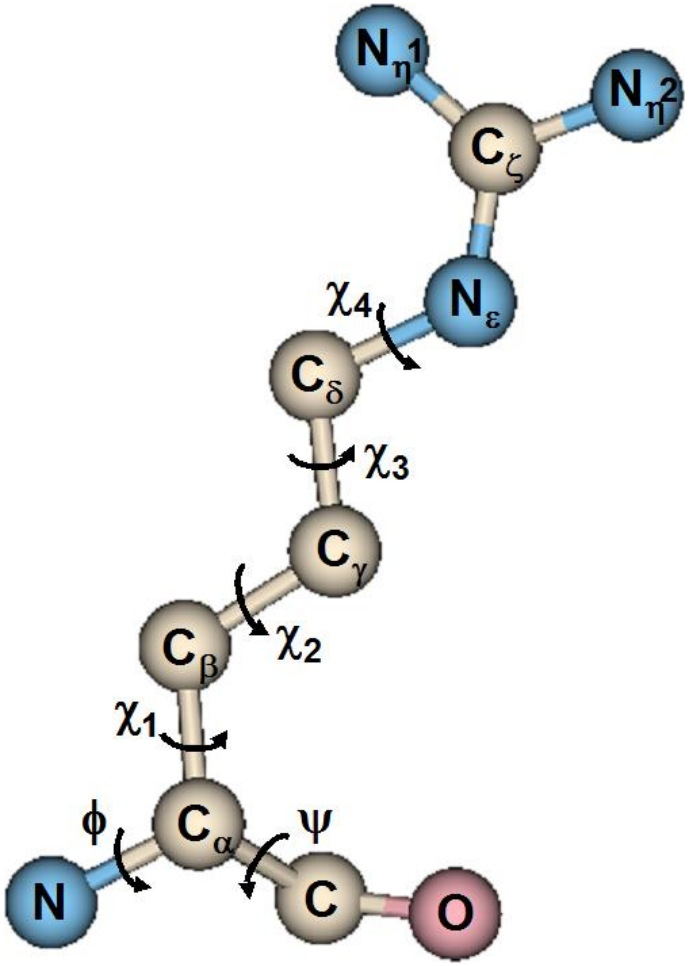
J. J. Kuszewski, R. A. Thottungal, G. M. Clore, Charles D. Schwieters *J Biol NMR* 2008

Dihedral angles

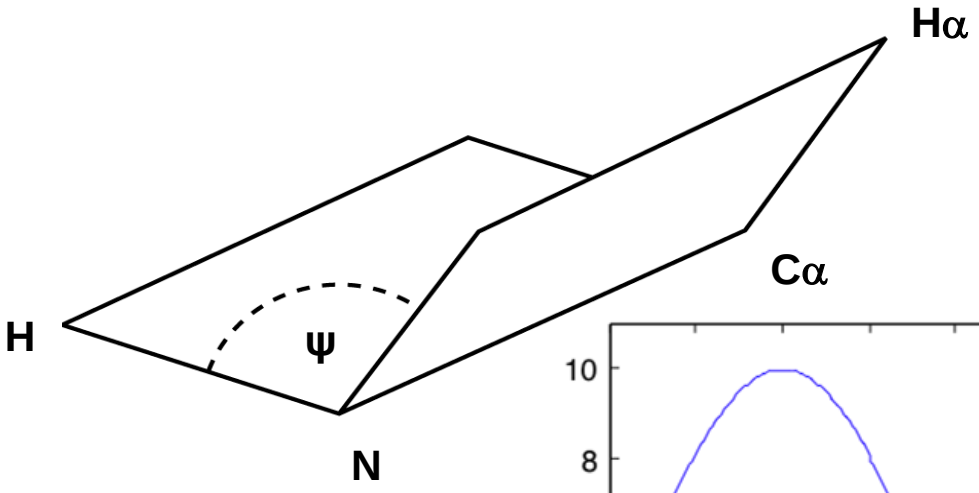
Backbone dihedral angles



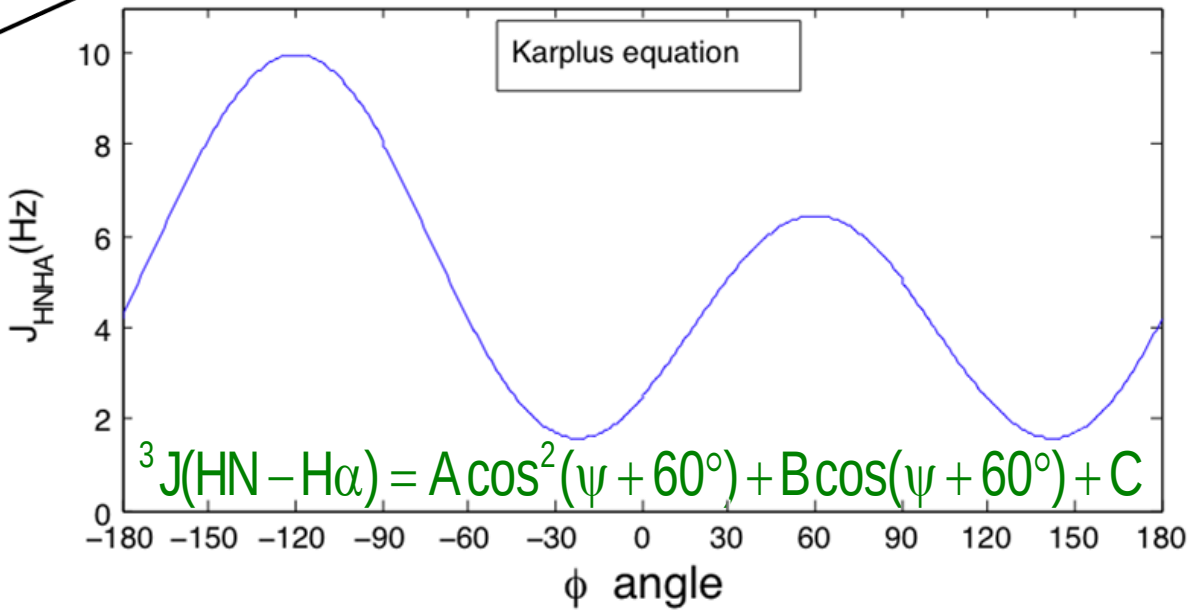
Sidechains dihedral angles



Dihedral angle restraints



^{3}J coupling constants are related to dihedral angles through the Karplus equation



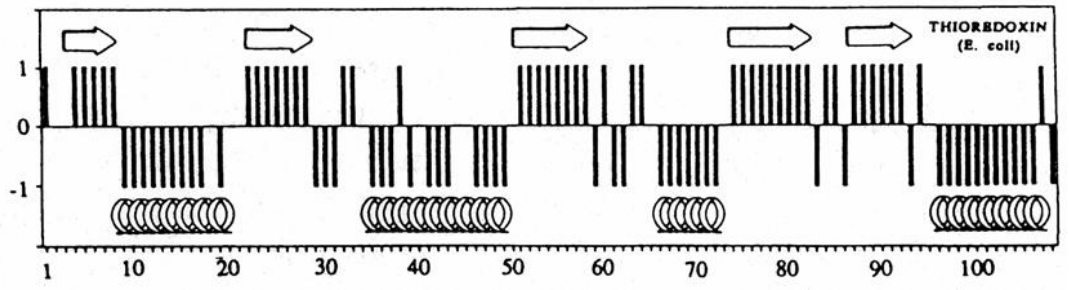
$J_{HNH\alpha}$ > 8Hz
$J_{HNH\alpha}$ < 4.5Hz
4.5Hz < $J_{HNH\alpha}$ < 8Hz

– 155° < ϕ=ψ+120° < – 85° β strand conformation
– 70° < ϕ=ψ+120° < – 30° α helix
ϕ,ψ values depend on $J_{HNC'}$

Chemical Shift Index

As chemical shifts depend on the nucleus environment, they contain structural information. Correlations between chemical shifts of Cα, Cβ,CO, Hα and secondary structures have been identified.

Chemical Shift Index:



CSI's are assigned as:
Cα and carbonil atoms chemical shift difference with respect to reference random coil values:

-0.7 ppm < Δδ < 0.7 ppm	0
Δδ < - 0.7 ppm	-1
Δδ > +0.7 ppm	+1

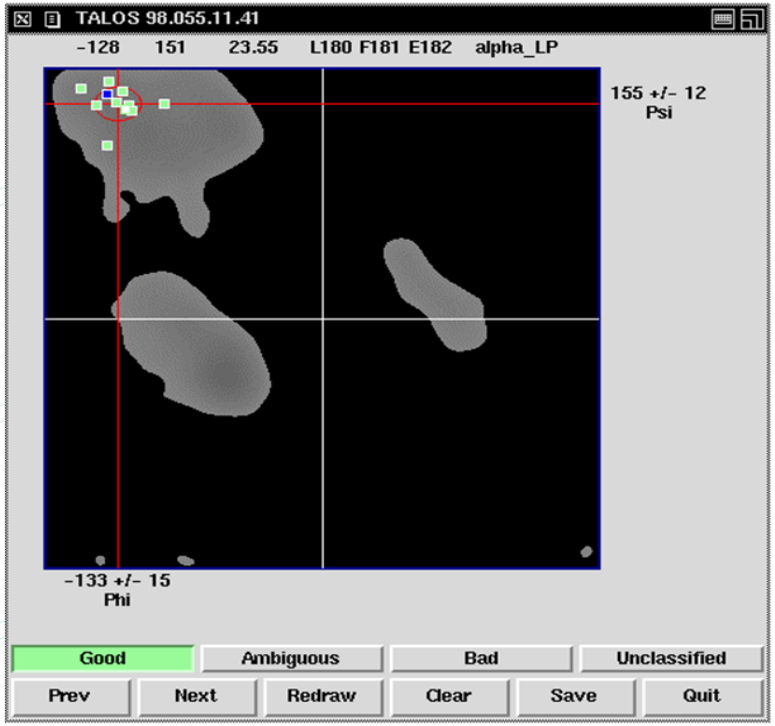
For Cβ the protocol is the same but with opposite sign than Cα

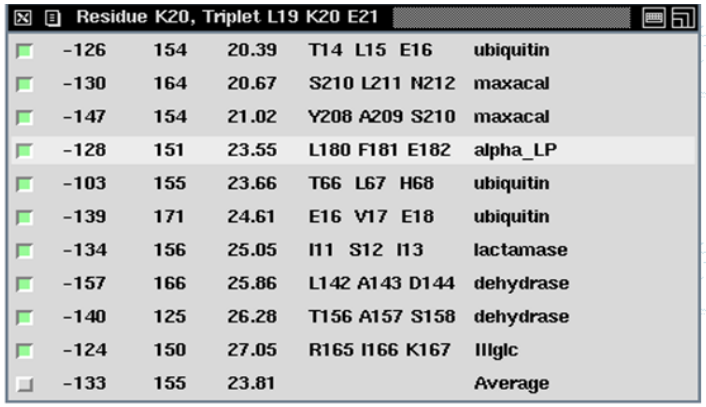
Any "dense" grouping of four or more "-1's", uninterrupted by "1's" is assigned as a helix, while any "dense" grouping of three or more "1's", uninterrupted by "-1's", is assigned as a β-strand. Other regions are assigned as "coil".

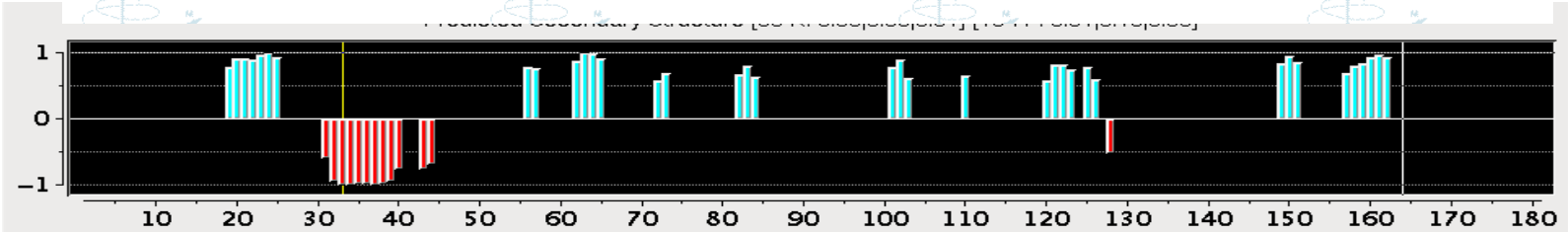
A "dense" grouping means at least 70% nonzero CSI's.

Chemical Shift Restraints

TALOS+ uses $^{13}C\alpha$, $^{13}C\beta$, $^{13}C'$, $^{1}H\alpha$ and ^{15}N chemical shifts together with sequence information/chemical shift databases to predict values for backbone dihedral angles φ and ψ







Shen, Delaglio, Cornilescu, Bax *J. Biomol NMR*, 2009

H-bonds as Structural restraints

Experimental Determination of H-Bonds:

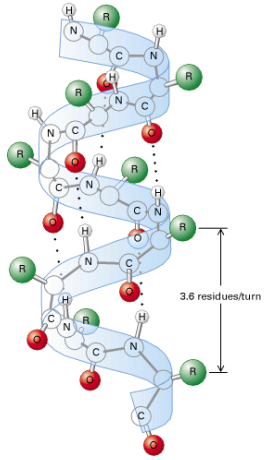
- **HNCO** — direct method
- **H/D exchange** — indirect method

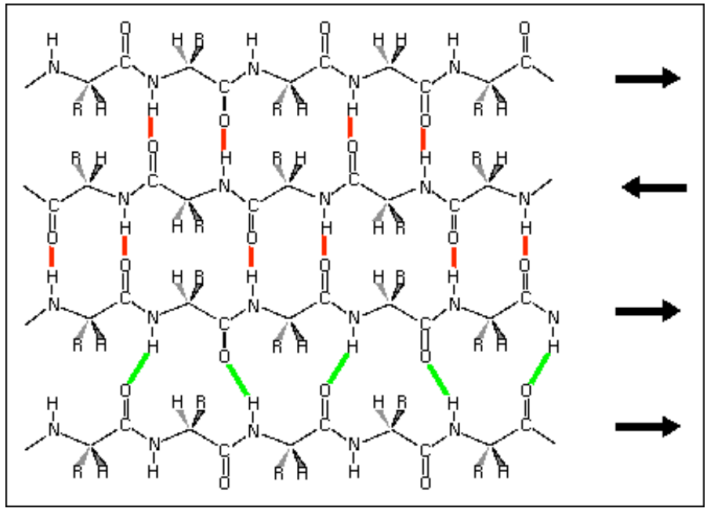
Distance and angle restraints

- **Upper distance limit**
- **Lower distance limit**

α-Helix **β–Sheet**

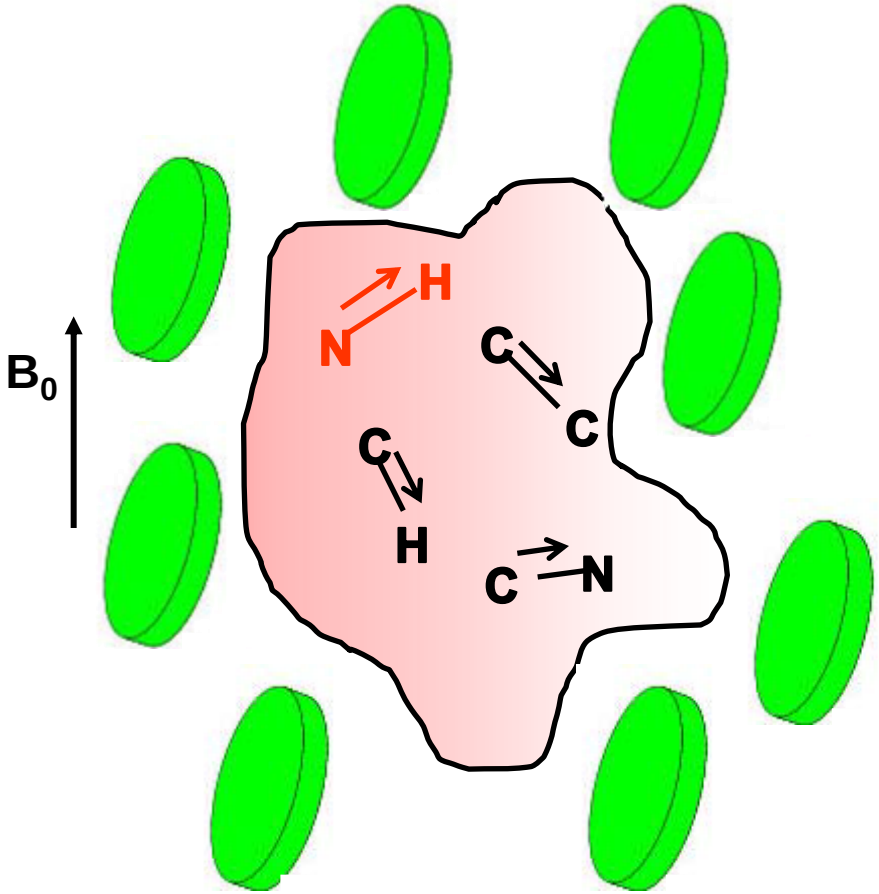
Distance between the donor and the acceptor atoms is in the range **2.7- 3.2 Å**

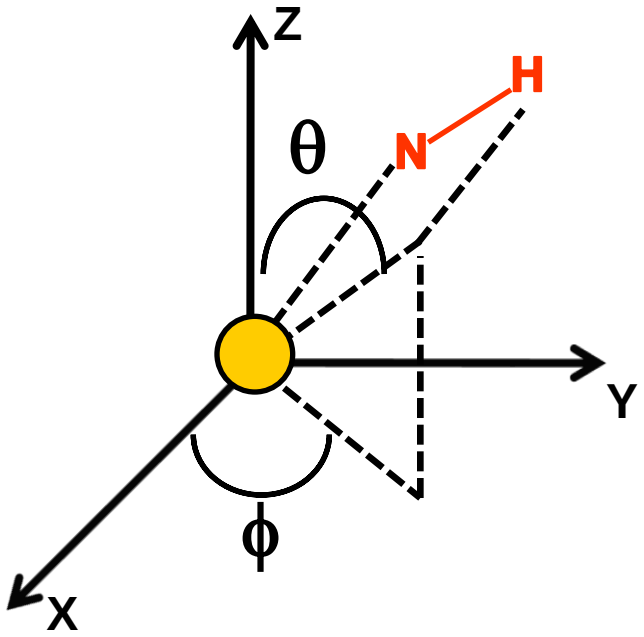




140° < N-H···O < 180°

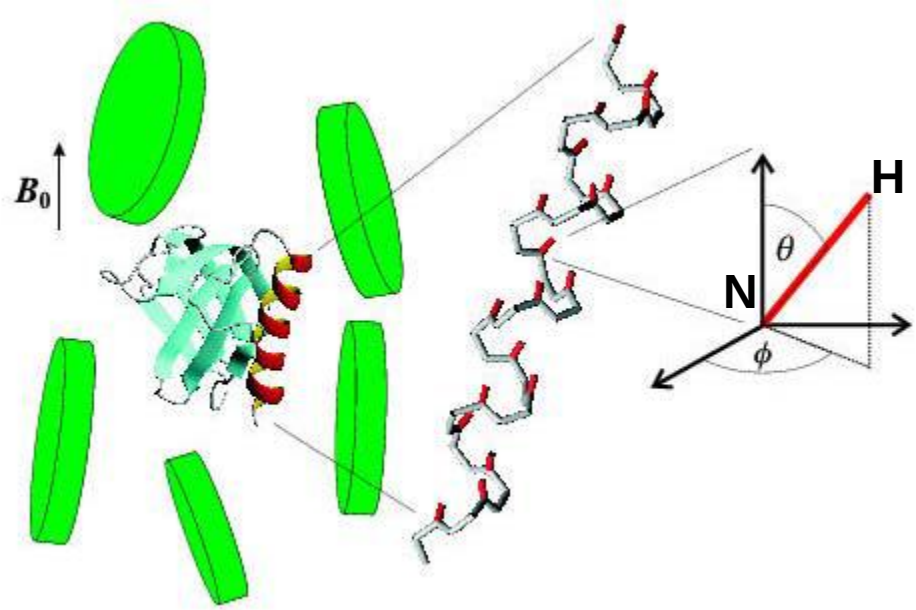
Residual dipolar couplings





RDCs provide information on the orientation of (in principle each) bond-vector with respect to the molecular frame and its alignment in the magnetic field

Residual dipolar couplings



$$RDC_{(IS)_i} \propto \Delta\chi \; f(\theta_i, \varphi_i)$$

where χ is the molecular alignment tensor with respect to the magnetic field and θ_i, φ_i are the angles between the bond vector and the tensor axes

Proteins dissolved in liquid, orienting medium

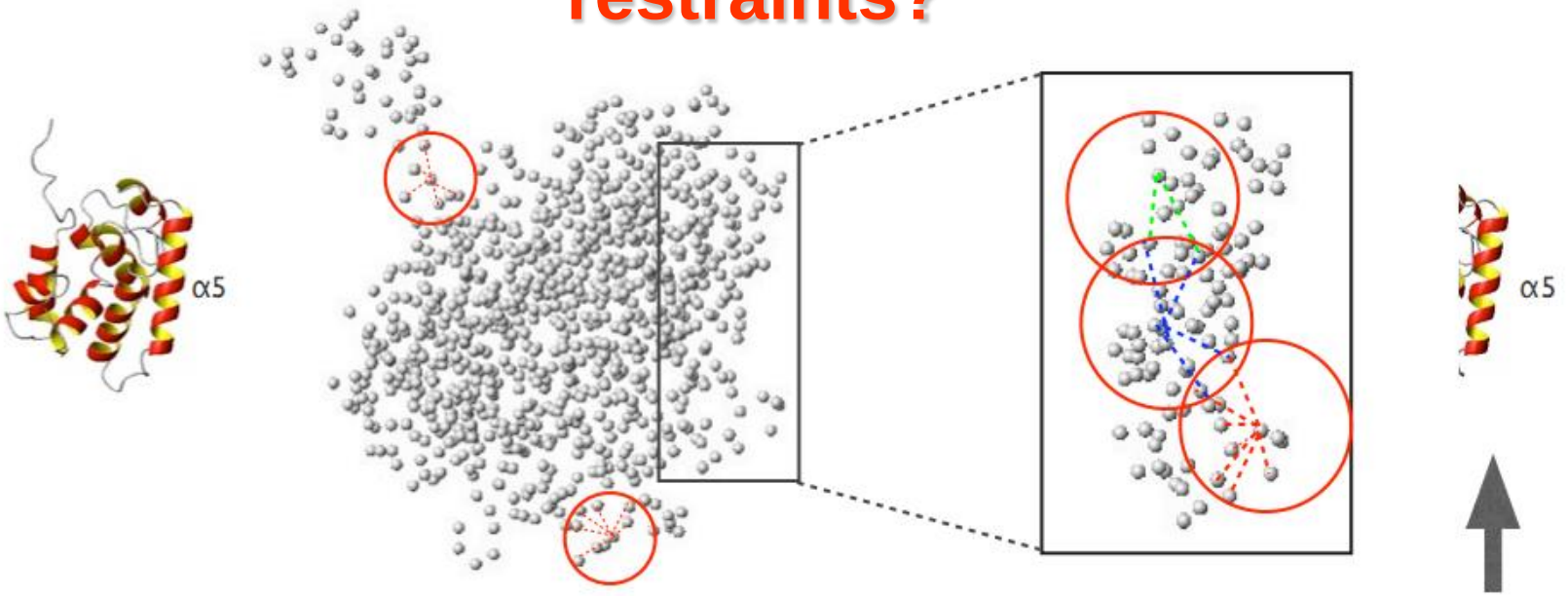
Some media (e.g. bicelles, filamentous phage, cellulose crystallites) induce to the solute some orientational order in a magnetic field

A small "residual dipolar coupling" results

Relative orientation of secondary structural elements can also be determined

General Consideration

How complete are the NMR structural restraints?



Hydrogen cloud of the protein

Successive distance measurements in an alpha helix.

100 of distance measurements with NMR.

NMR mainly determines short range structural restraints but provides a complete network over the entire molecule

3D structure calculations

Most Common Algorithms

- MD in cartesian coordinates/Simulated annealing

  XPLOR-NIH

- MD in torsion angle space/Simulated annealing

  XPLOR-NIH and CYANA

A random coil polypeptide chain is generated, which is folded through MD/SA calculations and applying experimental constraints

Molecular Dynamics (MD)

How the algorithms work:

- MD calculations numerically solve the equation of motion to obtain trajectories for the molecular system
- In Cartesian coordinates, the Newton's equation of motion is:

$$m_a \frac{\mathrm{d}^2 \mathbf{r}_a}{\mathrm{d}t^2} = \frac{\partial}{\partial \mathbf{r}_a} U\left[t \middle| \sigma\left(\mathbf{r}_1, \ldots, \mathbf{r}_N\right)\right],$$

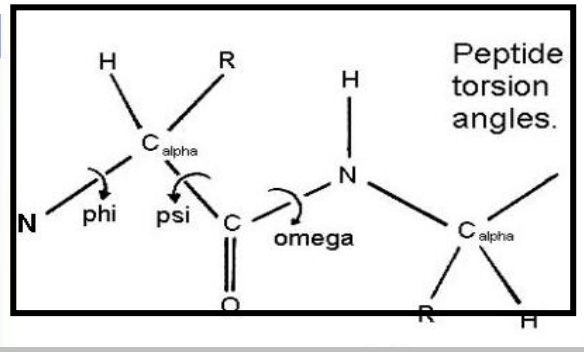
- In torsion angle space the equations of motion (Lagrange equations) are solved in a system with N torsion angles as the only degrees of freedom. Conformation of the molecule is uniquely specified by the values of all torsion angles.

About 10 times less degrees of freedom than in Cartesian space

$$\frac{\mathbf{d}}{\mathbf{dt}}\left(\frac{\partial \mathbf{L}}{\partial \dot{\mathbf{q}}_\mathbf{k}}\right) - \frac{\partial \mathbf{L}}{\partial \mathbf{q}_\mathbf{k}} = \mathbf{0}$$

$\mathbf{L = E_{kin} - E_{pot}}$

q = generalized coordinate



How MD is used to find the lowest energy conformation?

- The potential energy landscape of a protein is very complex and studded with many local minima where a conformation can become "trapped" during MD calculations

- A distinctive feature of MD simulations, when compared to the straightforward minimization of an energy function, is the presence of kinetic energy that allows the protein conformations to cross barriers of the potential surface

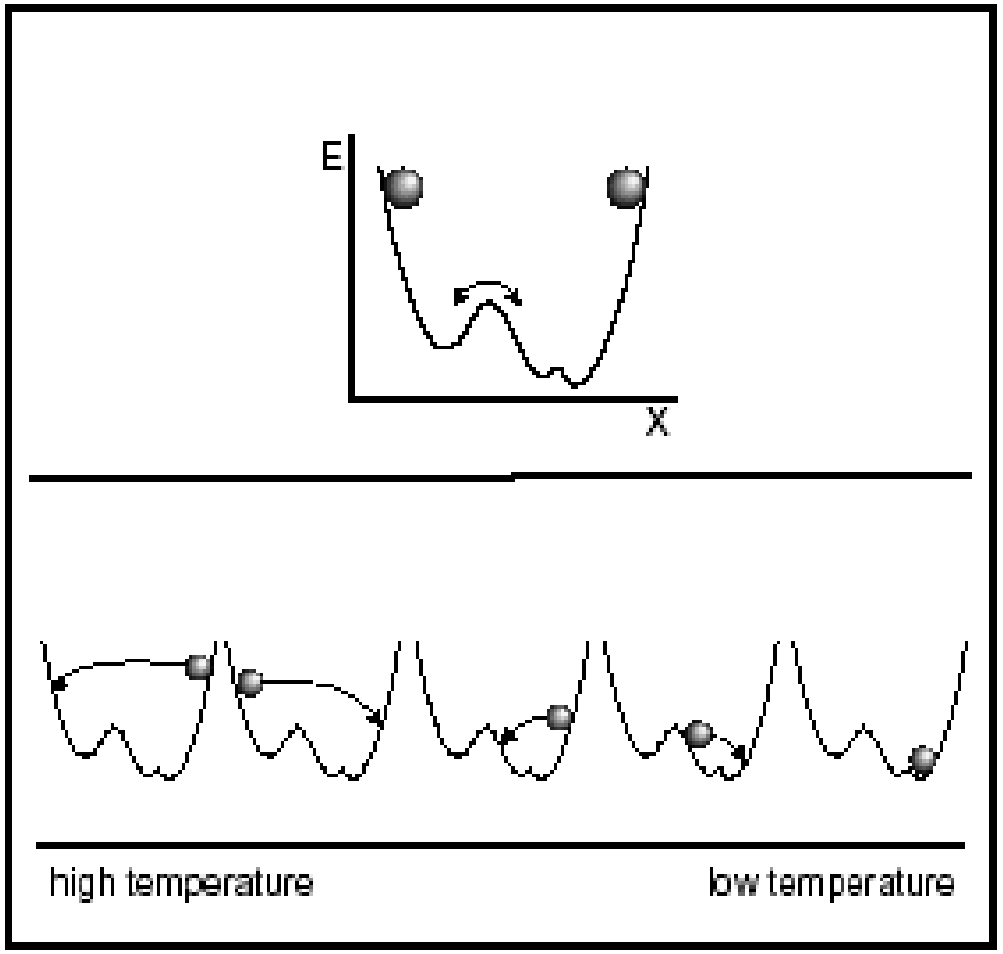
Simulated annealing (SA)

- MD is combined with simulated annealing protocols
- The kinetic energy (provided in terms of temperature) defines the maximal height of energy barrier that can be overcome in MD simulations
- In protein structure calculations, temperature is varied along the MD simulation so as to sample a broad conformational space of the protein and to facilitate the search of the minimum of the hybrid energy function
-

How the algorithms work:

A sketch of what SA does

- A starting random structure is heated to very high temperature
- During many cooling steps the starting structure evolves towards (i.e., folds into) the energetically favorable final structure under the influence of the force field derived from the restraints



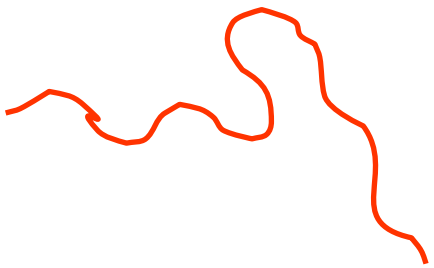
Simulated annealing (SA)

- Through SA, a molecule reaches its minimum energy configuration by slow cooling it after having sampled a broad conformation range at high temperatures
- It is a general optimization method used to search for the minimum of very complex functions
- Elaborated SA protocols have been developed to optimize the exploration of protein conformational space (e.g., several stages of heating and cooling, switching on/off atom-atom repulsion, etc.)

How the algorithms work:

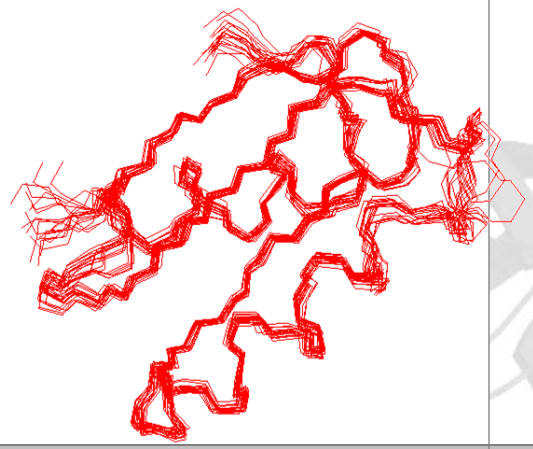
Molecular Dynamics (MD)

- Steps:
  - a random coil conformation is generated
  - an MD trajectory is calculated using the hybrid energy function as the potential energy
  - During MD the temperate is gradually decreased to zero
  - the end point of the trajectory is (close to) the minimum of the hybrid energy function

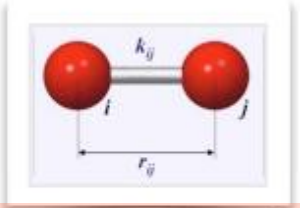


MD calculation with restraints

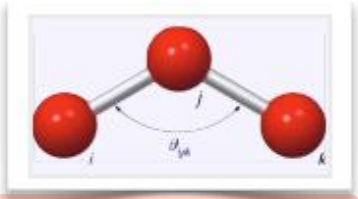
Lower hybrid energy



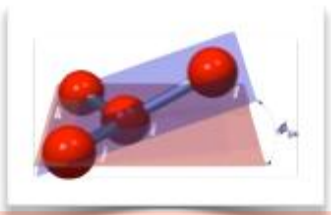
Hybrid energy function



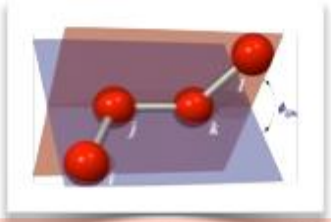
$$V_B = k_{ij}\left(r_{ij} - r_0\right)^2$$



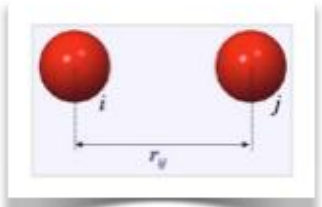
$$V_A = k^H_{ijk}\left(\theta_{ijk} - \theta_0\right)^2$$



$$V_I = k_{ijkl}\left(\phi_{ijkl} - \phi_0\right)^2$$



$$V_D = k^H_{ijkl}\left(\phi_{ijkl} - \phi_0\right)^2$$



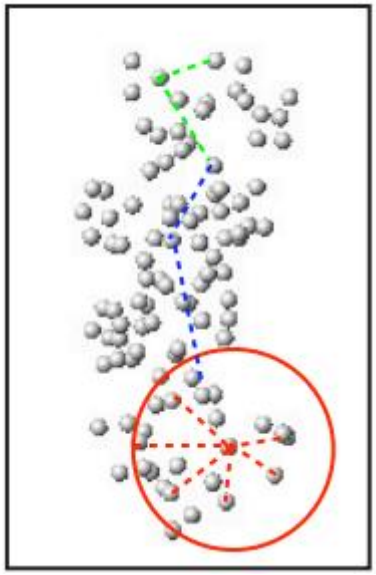
$$V_{vdW} = 4\,\epsilon_{ij}\left(\left(\frac{\sigma_{ij}}{r_{ij}}\right)^{12} - \left(\frac{\sigma_{ij}}{r_{ij}}\right)^{6}\right)$$

+

NMR experimental conformational restraints

$$\sum_{\text{distance restraints}} k_d\,(d - d_0)^2 +$$

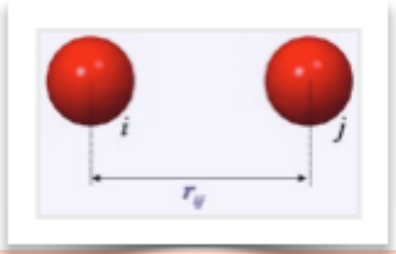
$$\sum_{\text{torsional restraints}} k_\psi\,(\psi - \psi_0)^2 + \ldots$$



A hybrid energy function is defined, that incorporates *a priori* information and NMR structural restraints as potential and pseudopotential energy terms, respectively

CYANA TARGET FUNCTION (hybrid energy function)

The CYANA target function is built up from van der Waals terms as well as upper limit, lower limit and torsion angle potential energy components for the input restraints.



$$V_{vdW} = 4\,\epsilon_{ij}\left(\left(\frac{\sigma_{ij}}{r_{ij}}\right)^{12} - \left(\frac{\sigma_{ij}}{r_{ij}}\right)^{6}\right)$$

Bond lengths and angle values are kept fixed

+

$$\sum_{\text{distance restraints}} k_d (d - d_0)^2 +$$

$$\sum_{\text{torsional restraints}} k_\psi (\psi - \psi_0)^2 + \ldots$$

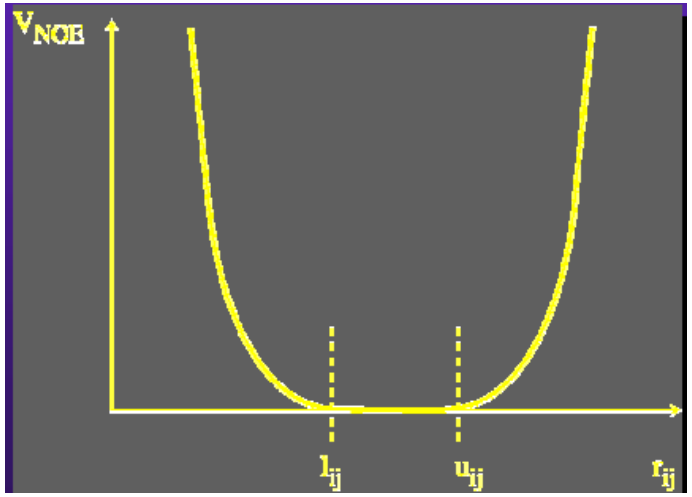
- The CYANA target function is defined such that it is zero if and only if all experimental distance constraints and torsion angle constraints are fulfilled in the calculated structure and all nonbonded atom pairs satisfy a check for the absence of steric overlap. A conformation that satisfies the constraints more closely than another one will lead to a lower target function value.
- In CYANA the final energy of each calculated structure is reflected by the target function which increases when the distance and dihedral restraints do not agree with the calculated structure.

Pseudopotential energy terms: the NOEs

- The atom pair distance r_{ij} (derived from NOE) is restrained between an upper (u_{ij}) and a lower (l_{ij}) limit as:

$$
\begin{aligned}
V_{NOE} &= k(r_{ij} - u_{ij})^2 && \text{if} \quad r_{ij} > u_{ij} \\
&= 0 && \text{if} \quad l_{ij} \le r_{ij} < u_{ij} \\
&= k(l_{ij} - r_{ij})^2 && \text{if} \quad r_{ij} < l_{ij}
\end{aligned}
$$

- The shape of the energy term looks like (if l_{ij} is not available, the sum of the atomic radii is used):



Knowledge about the topology of the system is needed:

- Experimental data are supplemented with information on the covalent structure of the protein (bond lengths, bond angles, planar groups...) and the atomic radii (i.e. each atom pair cannot be closer than the sum of their atomic radii)

CYANA and Xplor-NIH

	Cyana	Xplor-NIH
Covalent structure	Fixed	Restrained by potential energy terms
MD in Cartesian coordinates	No	Yes
MD in Torsion Angle Space (TAD)	Yes	Yes
SA protocol	Yes	Yes
Structure refinement (in explicit water)	No	Yes

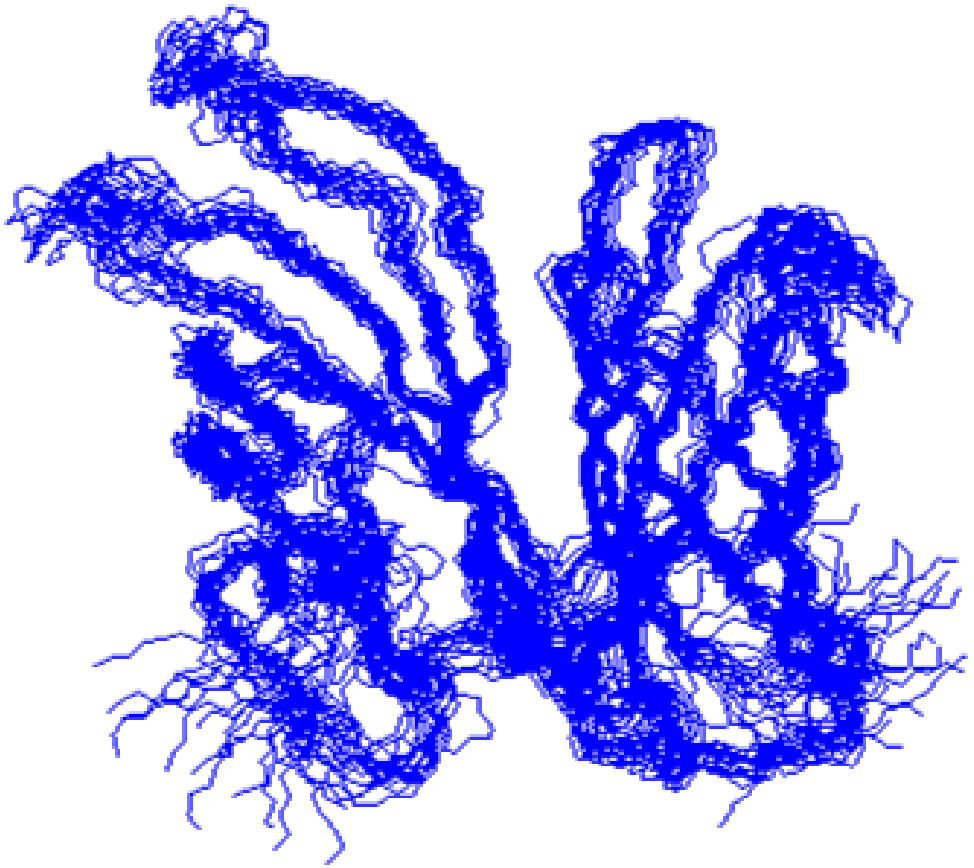
NMR structure determination & GRID

http://wenmr.eu/wenmr/nmr-services

Not just one time

- NMR structure calculations are always performed by computing, using the same restraints and algorithm, several different conformers, each starting from different initial random coil conformations
- In general, some of the conformers will be good solutions (i.e. exhibit small restraint violations) whereas others might be trapped in local minima
- The usual representation of an NMR structure is thus a bundle of conformers, each of which being an equally good fit to the data
- Conformational uncertainty may originate from true flexibility of the molecule

Bundles of conformers



- ***2987 meaningful NOE***
- ***158*** *dihedral* ψ *and* ***158*** *dihedral* ϕ *angle constraints*
- ***RMSD*** *to the mean structure is* ***1.25 ± 0.23 Å*** *for the backbone and* ***1.75 ± 0.14 Å*** *for all heavy atoms*

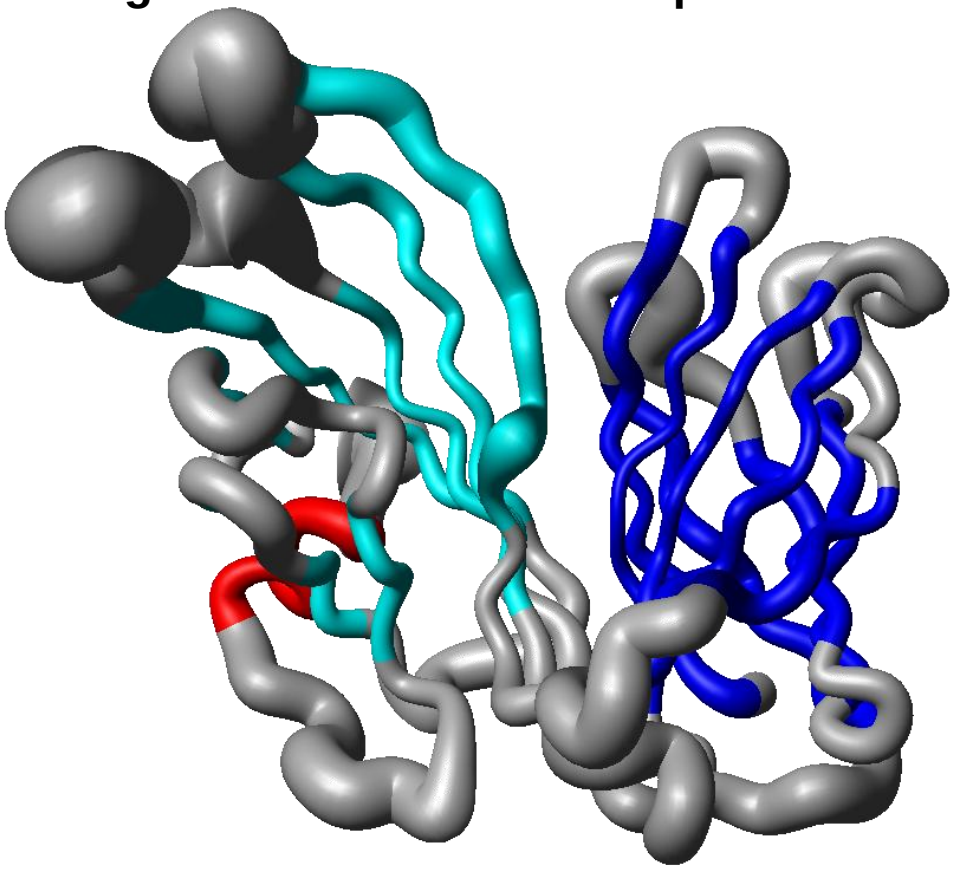
NMR structure must simultaneously fulfill all distance measurements.

The NMR solution structure of a protein is hence represented by a bundle of equivalent conformers.

Cantini, F., Veggi, D., Dragonetti, S., Savino, S., Scarselli, M., Romagnoli, G., Pizza, M., Banci, L., and Rappuoli, R. (2009) *J. Biol. Chem. 284*, 9022-9026.

Bundles of conformers

The backbone of a protein structure can be displayed as a cylindrical "sausage" of variable radius, which represents the global displacements among the conformers of the protein family:



- ***2987 meaningful NOE***
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Structure refinement

(Restrained) Energy Minimization (EM) and MD on the bundle of conformers

The calculated conformes are then refined applying the complete force field

- EM: the conformation with the local energy minimum is obtained. It will only locate the closest minimum. Cannot cross energy barriers
- MD: the conformational space is sampled through internal motions which depend on the potential generated by the atoms in the molecule and the kinetic energy, defined by the temperature.
- (R)EM/(R)MD: in addition to the classical force field, the structural restraints are also applied
- Performed in vacuum and in explicit solvent (water)

Structure refinement

- With CYANA an external MD program is needed (e.g., AMBER). Xplor-NIH itself can perform refinement

$$E = \sum K_r (r - r_0)^2 + \sum K_\theta (\theta - \theta_0)^2 + \sum_n \sum \frac{V_n}{2} [\cos(\eta_n \phi - \gamma_n)] +$$

$$\sum_{i<j} \varepsilon_{ij} \left[\left(\frac{R_{ij}}{r_{ij}} \right)^{12} - \left(\frac{R_{ij}}{r_{ij}} \right)^{6} \right] + \sum_{i<j} \frac{q_i q_j}{r_{ij}}$$

Analysis of the results

- *How many conformers should be used to represent the solution structure?*

Around 10% of calculated structures. It should be a number that is a reasonable compromise between statistics significance and data size with respect to their manageability in graphics and analysis programs.

- *How should they be selected from the ensemble of conformers?*

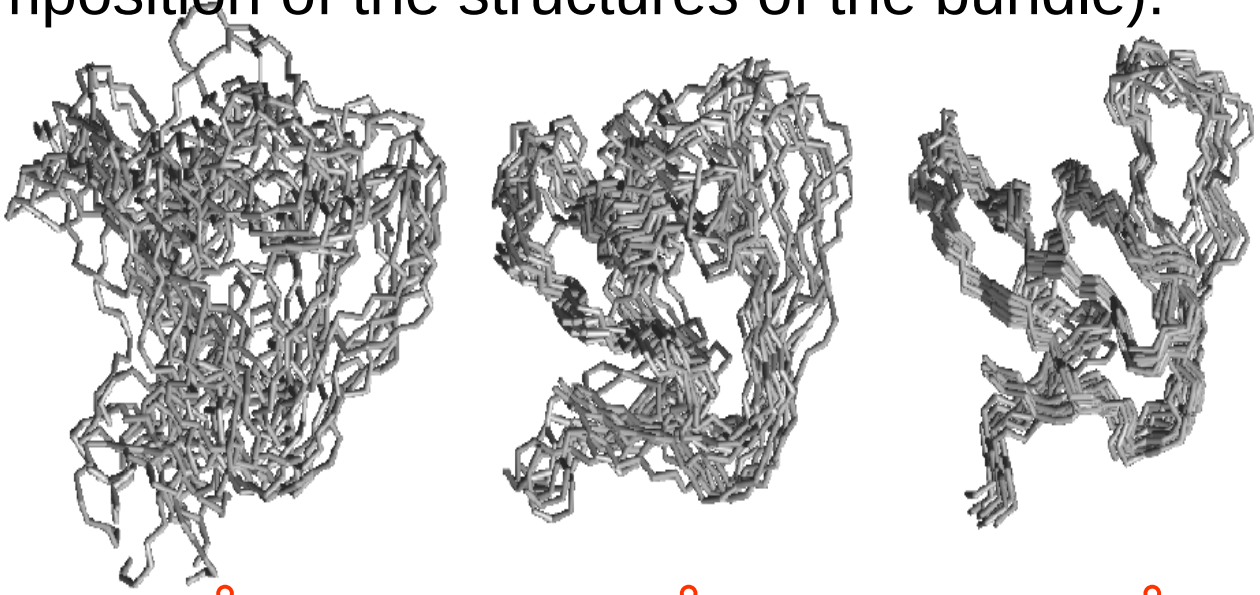
The conformers with the lowest target/penalty function, i.e. with the best agreement with the experimental structural restraints are selected

Accuracy of the Structure

RMSD

For two sets of n atoms, RMSD is defined as the normalized sum of the root mean square deviations of the position of a given atom with that of the same atom in the second set (after superimposition of the structures of the bundle):

Precision of the structure



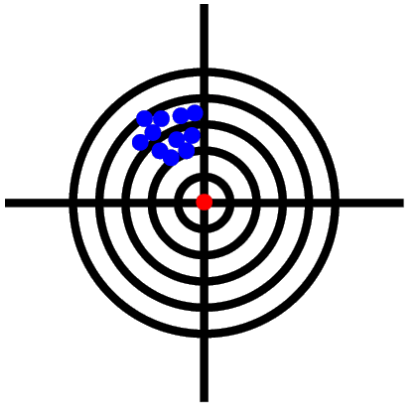
RMSD: 4.2 Å 1.9 Å 1.1 Å

$$RMSD = \sqrt{\frac{\sum (r_{ai} - r_{bi})^2}{n}}$$

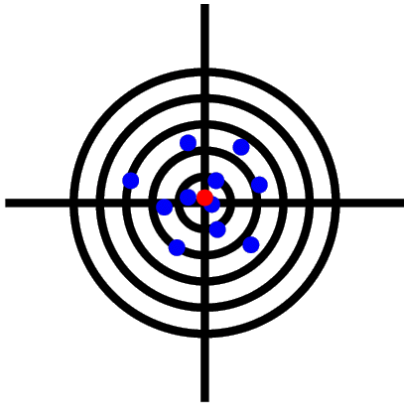
- two identical structures will have an rmsd of 0Å
- larger is the rmsd and more dissimilar are the structures

Precision versus Accuracy

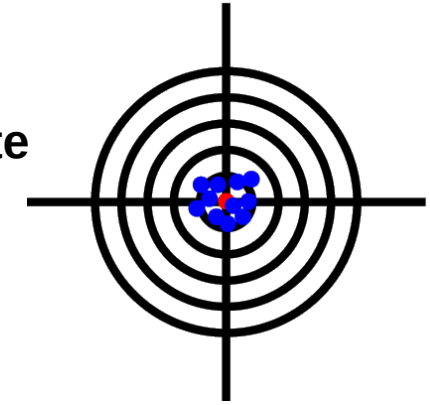
Precise,
not accurate



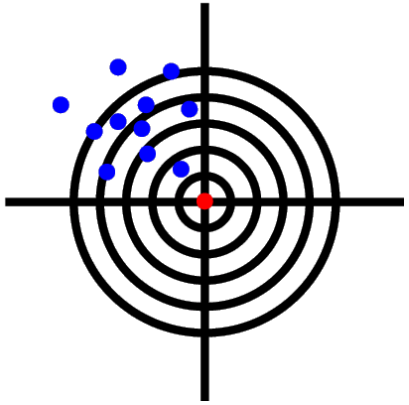
Accurate,
not precise



Precise
and accurate



Not accurate
and not precise



Validation criteria

Protein Structures are assessed with respect to:

- **Back-calculation of the experimental restraints**
- **Local geometry:**
  - **Bond lengths, bond angles, chirality, omega angles, side chain planarity**
- **Overall quality:**
  - **Ramachandran plot, rotameric states, packing quality, backbone conformation, side-chain planarity**
- **Others:**
  - **Inter-atomic bumps, buried hydrogen-bonds, electrostatics, packing quality**

Validation of the NMR Structures

The most common programs used to evaluate the quality of the structures are:

- **WHATIF (swift.cmbi.ru.nl)**
- **QUEEN**
- **CiNG (http://nmr.cmbi.ru.nl/icing) (WHATIF and PROCHECK-NMR)**
- **PSVS (http://psvs-1_4-dev.nesg.org/) (PROCHECK-NMR, MolProbity, Verify3D, Prosa II)**

Kay, L. E., Xu, G. Y., Singer, A. U., Muhandiram, D. R., and Forman-Kay, J. D. (1993) *J.Magn.Reson.Ser.B* 101, 333-337

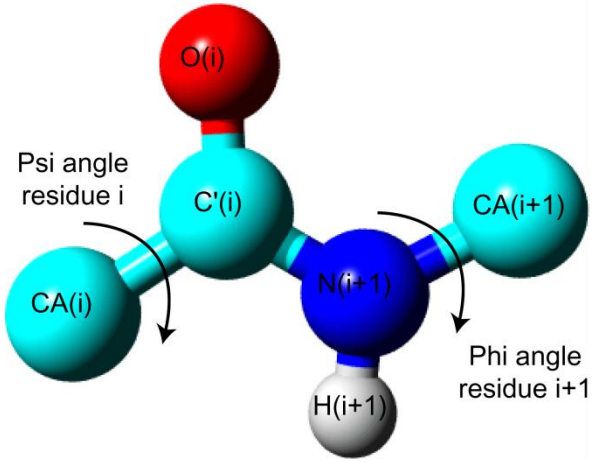
Zhang, O., Kay, L. E., Olivier, J. P., and Forman-Kay, J. D. (1994) *J.Biomol.NMR* 4, 845-858

Farrow, N. A., Muhandiram, R., Singer, A. U., Pascal, S. M., Kay, C. M., Gish, G., Shoelson, S. E., Pawson, T., Forman-Kay, J. D., and Kay, L. E. (1994) *Biochemistry* 33, 5984

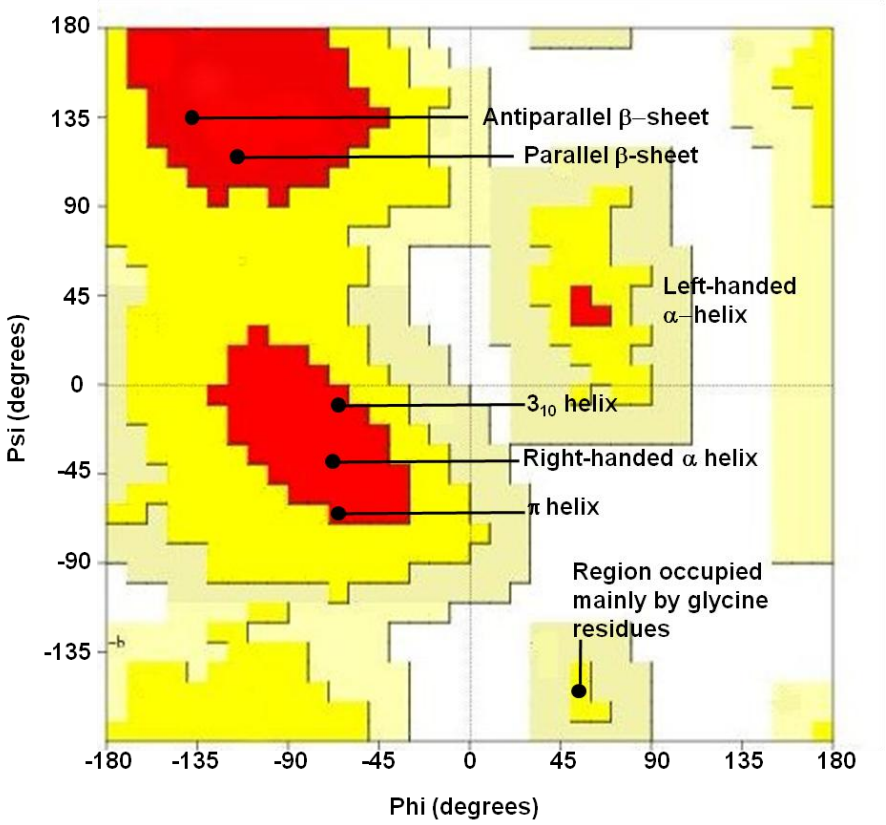
Battacharya, A., Tejero, R., and Montelione, G. T. (2007) *Proteins* 66, 778-795

Structural Parameters

Ramachandran Plot



Phi and Psi angles



Ramachandran plot

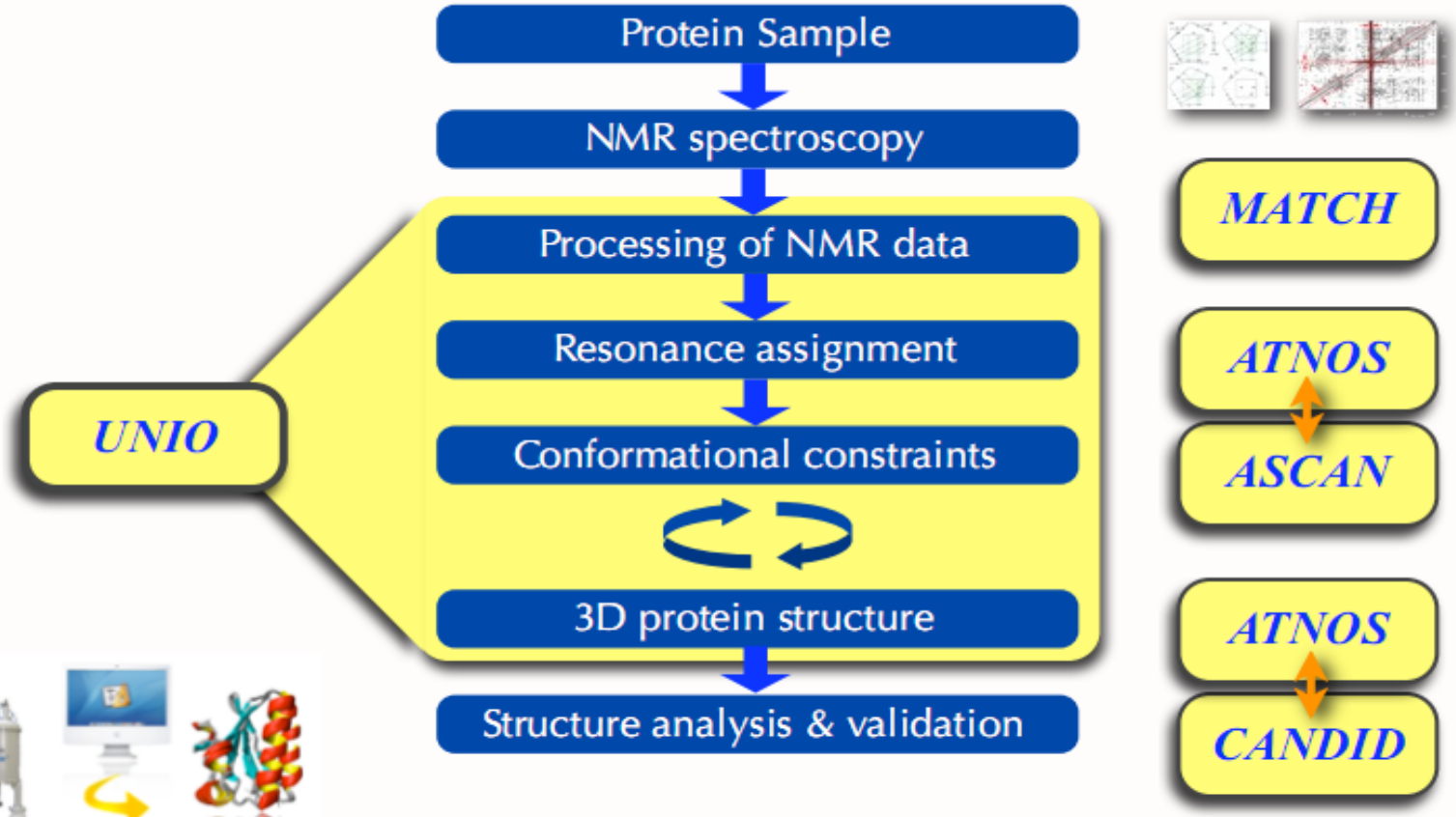
Generously allowed

Disallowed

Ideally, over 90% of the residues should be in the "core" regions

Automated Structure determination

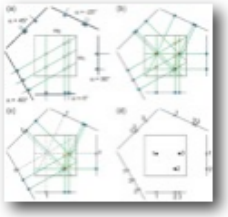
UNIO – Computational suite for fully/highly Automated NMR protein structure determination

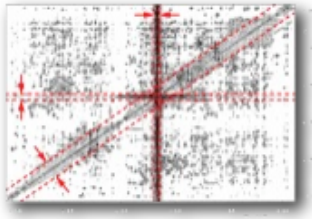


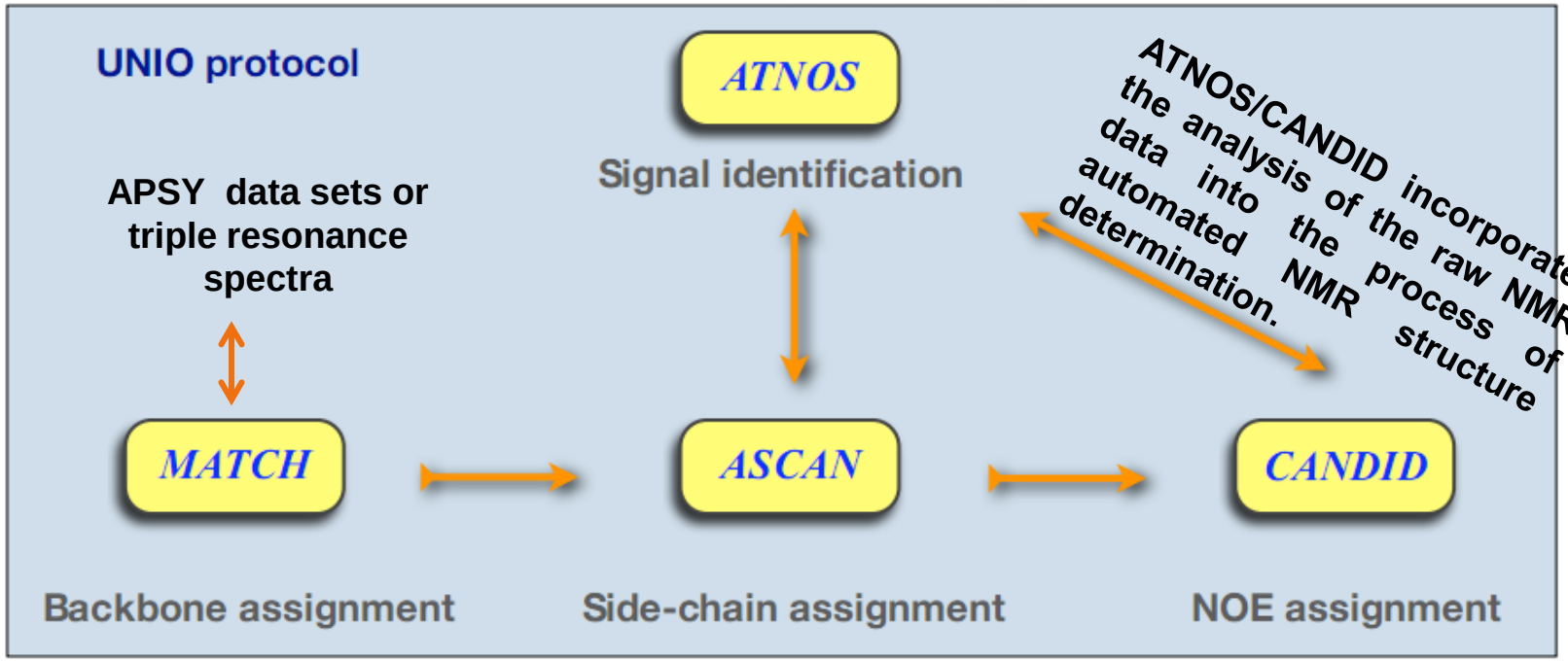
- UNIO provides accurate and automated 3D protein structure determination.
- UNIO enables protein NMR structure determination within one week including the collection of NMR experiments.

Herrmann, T., Güntert, P., Wüthrich, K. (2002). J. Biomol. NMR 24
Herrmann, T., Güntert, P., Wüthrich, K. (2002). J. Mol. Biol. 319
Volk, J., Herrmann, T., Wüthrich, K. (2008). J. Biomol. NMR 41.
Fiorito, F., Damberger, F.F., Herrmann, T., Wüthrich, K. (2008). J. Biomol. NMR 42.

UNIO for protein structure determination







UNIO protocol operates directly on the NMR spectra.

Herrmann, T., Güntert, P., Wüthrich, K. (2002). J. Biomol. NMR 24
Herrmann, T., Güntert, P., Wüthrich, K. (2002). J. Mol. Biol. 319
Volk, J., Herrmann, T., Wüthrich, K. (2008). J. Biomol. NMR 41.
Fiorito, F., Damberger, F.F., Herrmann, T., Wüthrich, K. (2008). J. Biomol. NMR 42.

UNIO standard protocol

Amino acid sequence of the protein

MATCH backbone assignment

Input : 4D and 5D APSY spectra or triple resonance spectra

Output :backbone chemical shifts

ATNOS/ASCAN side chain assignment

Input : 3D NOESY spectra

Output :side-chain chemical shifts

ATNOS/CANDID NOE assignment

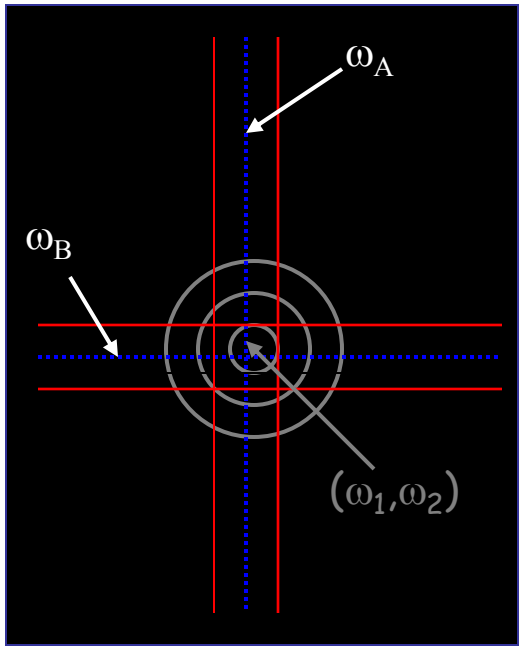
Input : 3D NOESY spectra

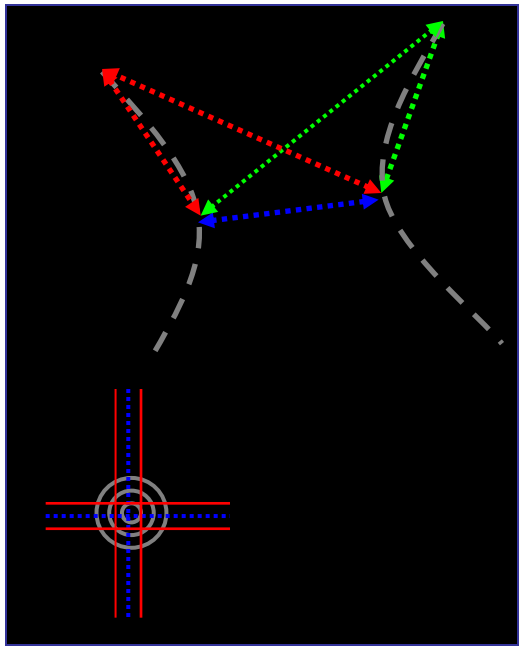
Output :assigned 3D NOESY peak lists and 3D protein structure with external program (XPLOR, CYANA, CNS etc)

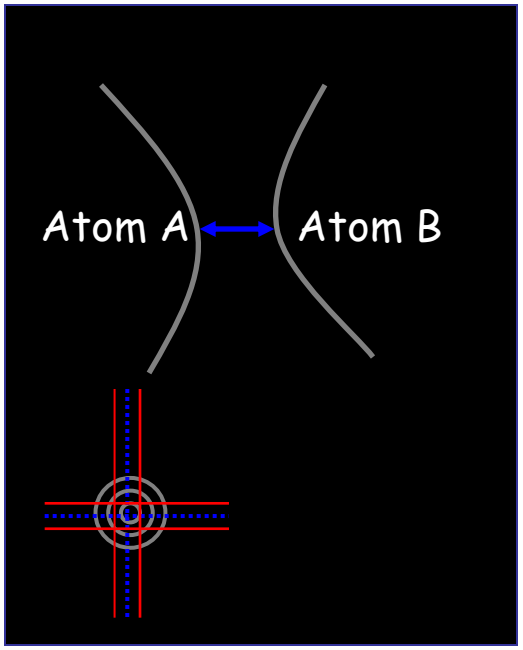
Criteria for NOE assignment

for each cross-peak the initial possible assignments are weighted with respect to several criteria , and initial assignments with low overall score are then discarded.

- ✓ **Chemical shift agreement**
- ✓ **NOEs network-anchoring**
- ✓ **Compatibility with intermediate structure**







Herrmann, T., Güntert, P., Wüthrich, K. (2002). J. Biomol. NMR
Herrmann, T., Güntert, P., Wüthrich, K. (2002). J. Mol. Biol.

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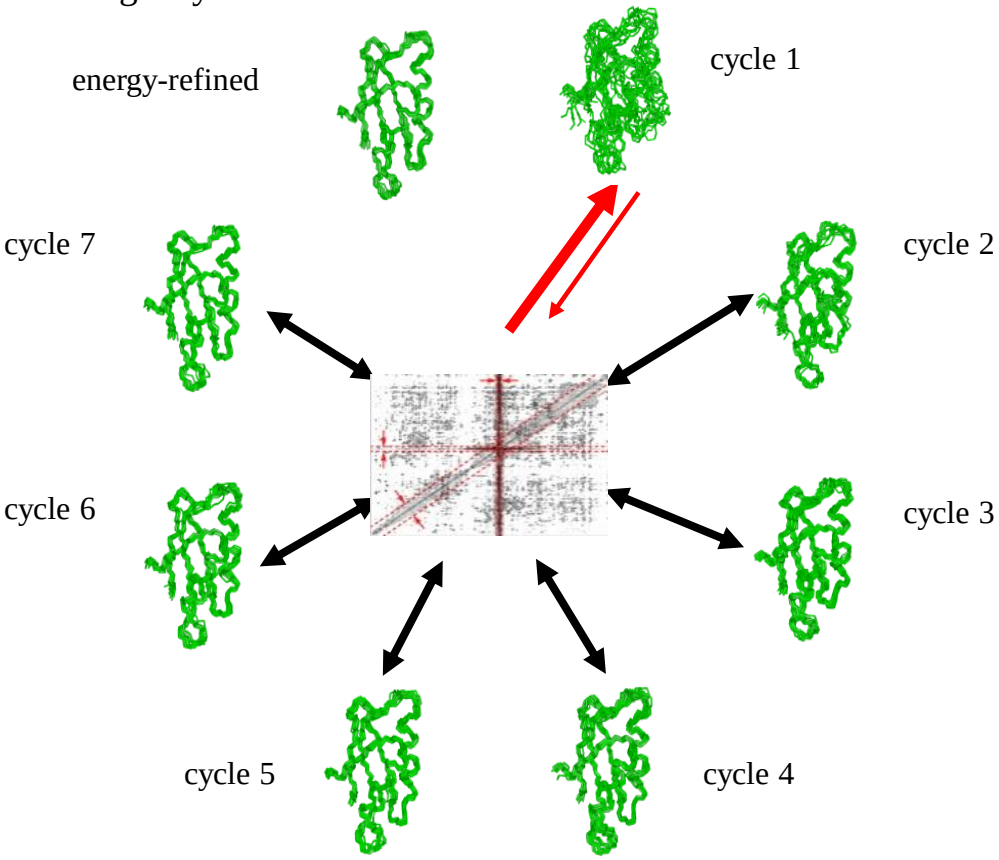
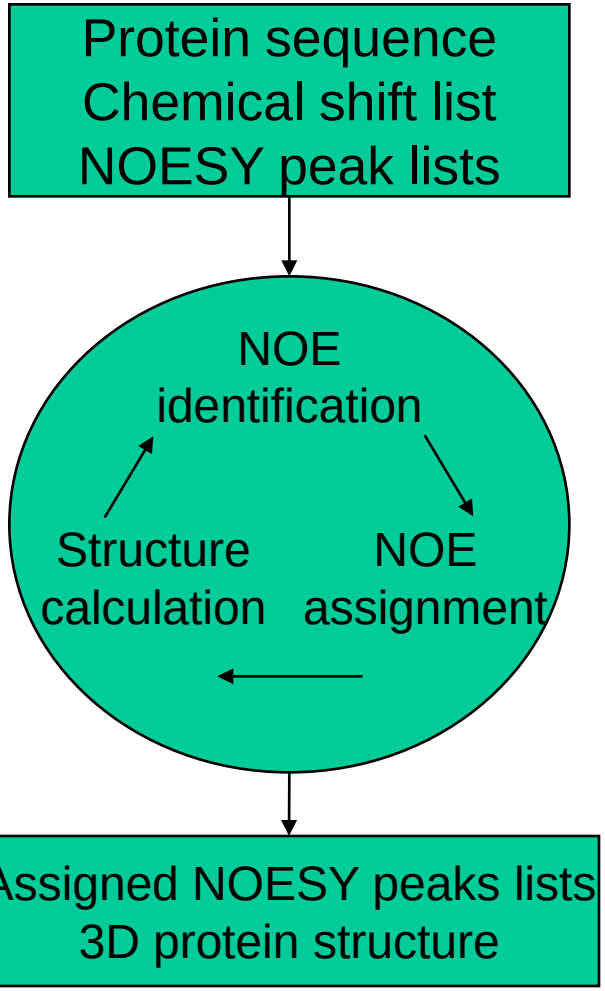
Automated NMR structure determination

•Automated NOESY spectral analysis using ATNOS-CANDID/CYANA

- the automated ATNOSCANDID algorithm assembled in UNIO ***proceeds in iterative cycles*** of ambiguous NOE assignment followed by structure calculation using torsion angle dynamics



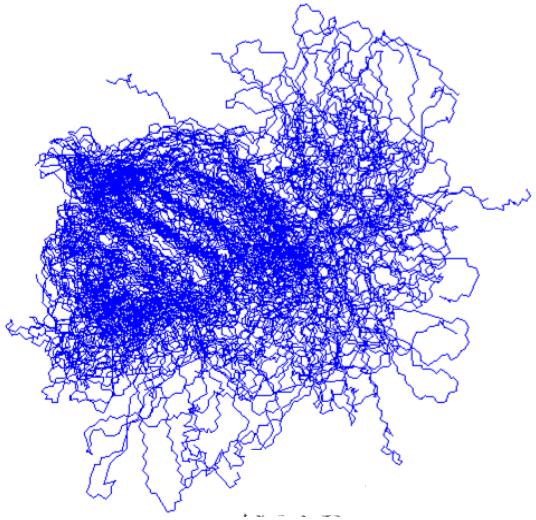


T. Herrmann K. Wüthrich and F. fiorito

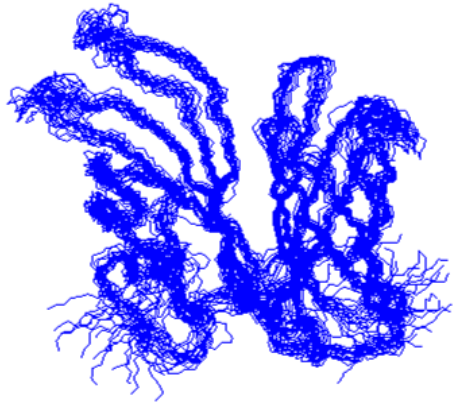
Does it always work ??

Automatic **Manual**

atx- like domain of hCCS protein (70 aa)



fHbp (274 aa)

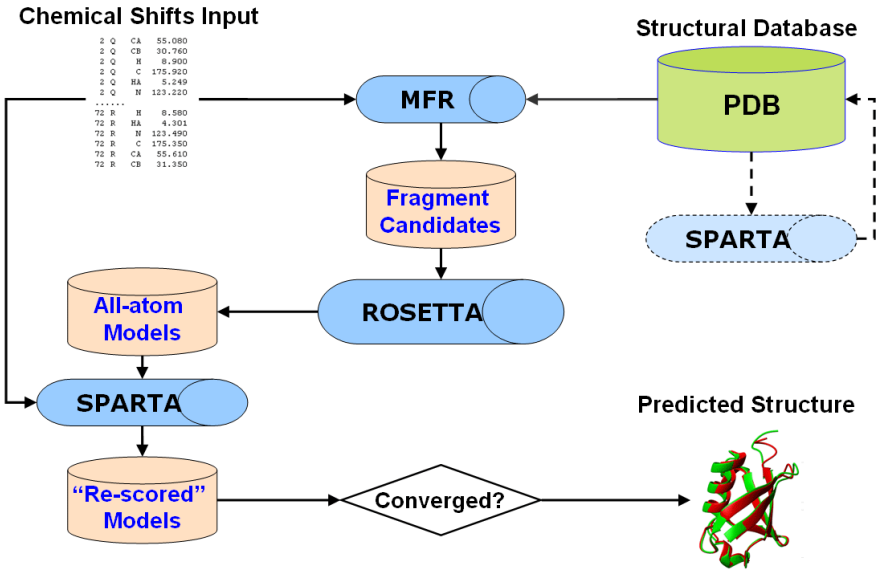


Chemical Shift-based structure calculations

CS ROSETTA generates 3D models of proteins, using only the $^{13}C\alpha$, $^{13}C\beta$, $^{13}C'$, ^{15}N, $^{1}H\alpha$ and ^{1}HN NMR chemical shifts as input

CS-ROSETTA involves two separate stages:

1. Polypeptide fragments are selected from a protein structural database, based on the combined use of $^{13}C\alpha$, $^{13}C\beta$, $^{13}C'$, ^{15}N, $^{1}H\alpha$, and ^{1}HN chemical shifts and the amino acid sequence pattern.
2. These fragments are used for generate a structural model, using the standard ROSETTA Monte Carlo assembly and relaxation methods



Thank you

Automated NMR structure determination

- **Automated NOESY spectral analysis using ATNOS-CANDID/CYANA**

➢ In the first cycle, network-anchoring has a dominant impact, since structure-based criteria cannot be applied yet. All cross-peaks with a poor score are temporarily discarded.

➢Correctness of cycle 1 is crucial for reliablity of automated approach as all the following cycles use the intermediate structures from the preceding cycle.

➢The input for the second and subsequent CANDID cycles is indeed derived from the three-dimensional protein structure of the previous cycle, in addition to the complete input used for the first cycle (amino acid sequence, the chemical shift and NOESY spectra).

Ambiguous distance constraints

- A NOESY cross peak with a single initial assignment (n=1) gives rise to a conventional upper distance constraint.
- A NOESY cross peak with initial multiple possible assignments (n>1) gives rise to an ambiguous distance constraint.

$$d_{eff} \equiv (\sum d_k^{-6})^{-1/6} \leq b$$

Sums run over all assignment possibilities

b : upper distance bound

d_k: distance for assignment possibility *k*

Each of the distances d_k in the sum corresponds to one assignment possibility to a pair of hydrogen atoms, αk and βk. In this way, information from cross-peaks with an arbitrary number of initial assignment possibilities can be used for the structure calculation, and although inclusion of erroneous assignments for a given cross-peak can result in wrong information, it will not lead to inconsistencies as long as the correct assignment is among the initial assignments.

Nilges et al., 1997, J. Mol. Biol. **269**, 408-422

Output criteria

The correctness of resulting 3D protein structure

- **Residual CYANA target function value:**

  $TF^{cycle1} < 200Å^2$, $TF^{cycle7} < 2Å^2$

- **Root mean square deviation (RMSD) value:**

  $RMSD^{cycle1} < 3Å$

- **Evolution of $RMSD^{drift}$ value:**

The RMSD value between the mean coordinates of the k-th and the subsequent cycle should be in the order of the RMSD value of the k-th cycle.