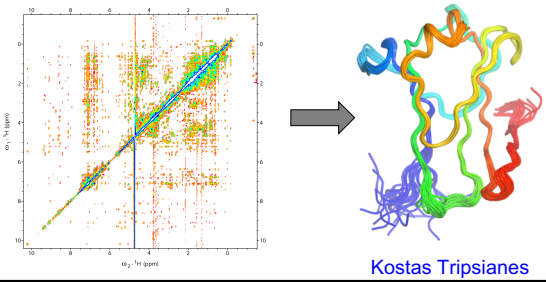
NMR structure calculation

What Data
How to get Structures
Examples

What Information
What Software
Precautions



Kostas Tripsianes

from data to structures

- NMR experiments
- Spectra analysis
  - Sparky, NMRview, CARA, CcpNmr, Olivia
- Resonance assignments
  - Backbone
  - Sidechain
  - Software: AUTOASSIGN, MARS, MBA, GARANT, PISTACCHIO
- Structural restraints
  - NOE assignment / distances
  - torsion angles → TALOS+!!!
  - RDCs / orientation restraints
- Structure calculations
  - Software: ARIA/CNS, CANDID/CYANA, XPLOR-NIH, UNIO
- Structure validation
  - Software: iCing

Why use NMR ?

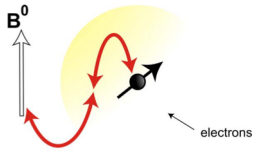
- some proteins do not crystallize
- crystals do not diffract well, or at all
- can not solve the phase problem
- functional differences in crystal vs in solution
- can get information about dynamics

NMR active nuclei

Nucleus	Nuclear spin	Natural abundance	Relative NMR sensitivity
^{1}H	½	99.98%	100
^{2}H	1	0.02%	0.96
^{13}C	½	1.1%	1.6
^{15}N	½	0.366%	0.1
^{19}F	½	100%	83.3
^{31}P	½	100%	6.6

Chemical shift

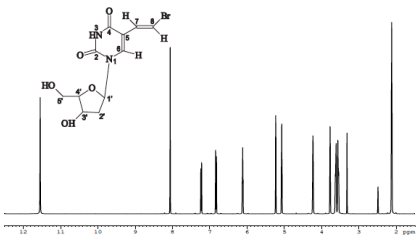
Electrons around the nucleus shield it from the external magnetic field, the more electrons the weaker the field



$B_{eff} = (1 - \sigma) B_0$
$\omega = \gamma (1 - \sigma) B_0$
$\delta = (\omega - \omega_{ref}) / \omega_0 \times 10^6$

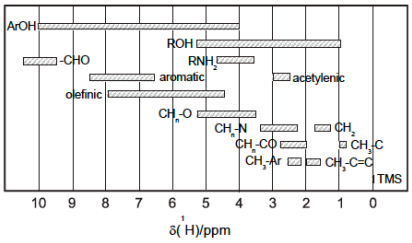
Proton resonance spectrum

Each proton atom in the molecule gives rise to a resonance line

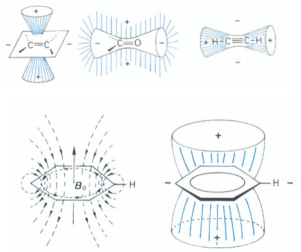


Chemical environment

The chemical shift depends on the chemical environment



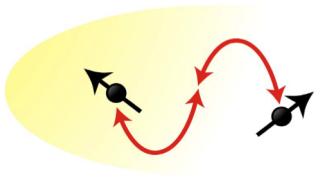
Chemical Shift Anisotropy



An important factor influencing the chemical shift are anisotropy effects, that are created by small additional fields

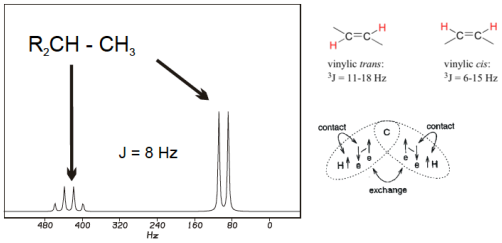
Scalar or J-coupling

Electrons in the bonds between the nuclei mediate an interaction, the scalar coupling

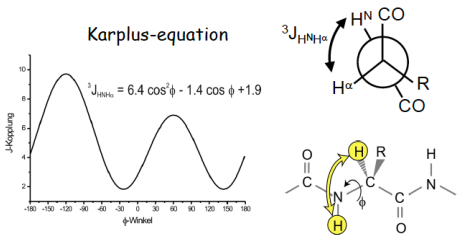


Scalar or J-coupling

Scalar coupling splits the signals according to the number of neighboring nuclei

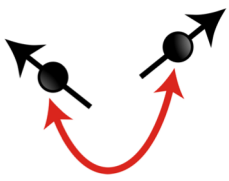


Scalar coupling contains structural information



Dipolar coupling

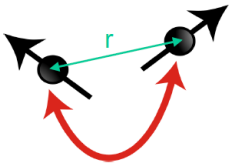
The nuclei interact directly through space via a dipole-dipole interaction



In solution NMR this interaction is averaged to zero due to the fast isotropic movement of the molecules but it is still a source of relaxation

NOE effect

$I_{NOE} \sim 1/r^6$

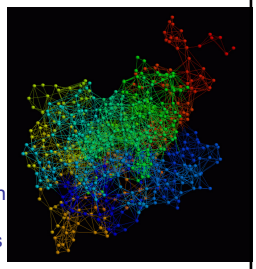


The intensity drops quickly with increasing distance. The effect can only be observed up to 6 Angtrong

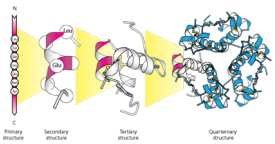
Source of structural information!!!

Challenges for determining protein structures using NMR

- Proteins have thousands of signals
- Assign the specific signal for each atom
- Thousands of interactions between protons also need to be assigned
- Need to transform the representation from spectra through interactions between atoms to spatial coordinates



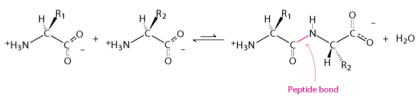
Hierarchical structure of proteins

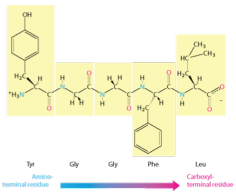


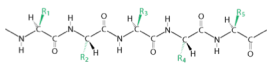
TAKE-HOME MESSAGE: *function is derived from three-dimensional structure, and three-dimensional structure is specified by amino acid sequence*

- All natural proteins are constructed from the same set of 20 amino acids
- Amino acids are classified based on the properties of R groups or sidechains
  hydrophobic, polar, charged
- The primary structure (sequence) of a protein is the linear arrangement of the amino acids that compose it
- The amino acids in a polypeptide are linked by peptide bonds
  planarity, hydrogen bonding potential, uncharged
- The secondary structure refers to periodic structures stabilized by backbone hydrogen bonds
  α helix, β sheets
- The tertiary structure refers to the overall conformation of a polypeptide (three-dimensional structure)
  hydrophobic interior, hydrophilic surface
- Proteins consisting of more than one polypeptide chain display quaternary structure
- The highest level of protein structure is the association of proteins into macromolecular assemblies

Primary structure of proteins



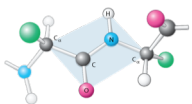


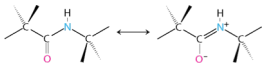


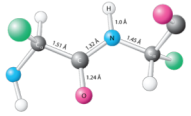
Repeating part: backbone
Variable part: sidechain (R group)

Most proteins contain 100 to 1000 residues
Muscle protein titin consists of more than 27000 residues

The peptide bond

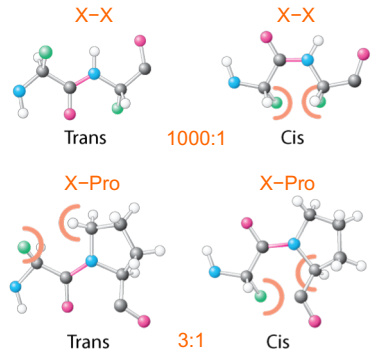




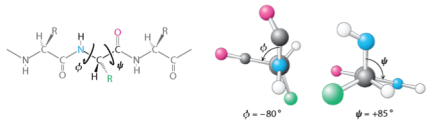


C-N single bond (1.49 Å)
C=N double bond (1.27 Å)

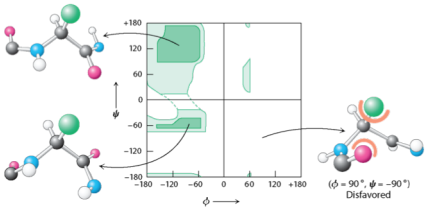
Peptide bond configurations



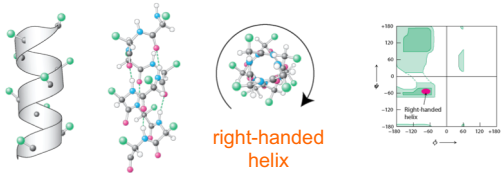
Backbone torsion angles



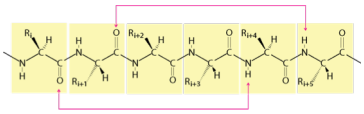
Ramachandran plot

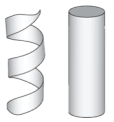


Secondary structure *α helix*

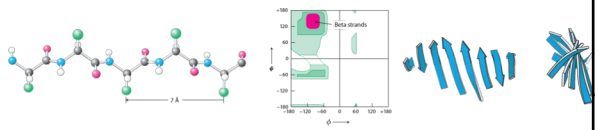


right-handed helix

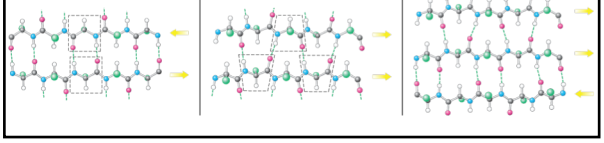




Secondary structure *β sheets*



antiparallel parallel mixed



Protein Structures from an NMR Perspective

Overview of Some Basic Structural Principals:

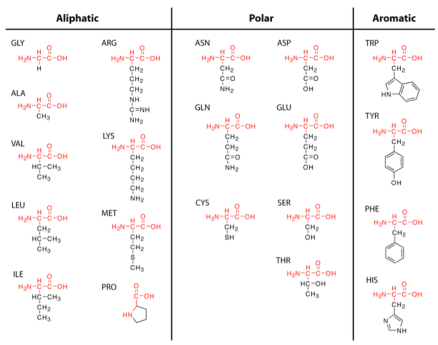
a) Primary Structure: the amino acid sequence arranged from the amino (N) terminus to the carboxyl (C) terminus → *polypeptide chain*

b) Secondary Structure: regular arrangements of the backbone of the polypeptide chain without reference to the side chain types or conformation

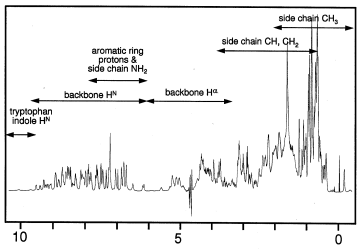
c) Tertiary Structure: the three-dimensional folding of the polypeptide chain to assemble the different secondary structure elements in a particular arrangement in space.

d) Quaternary Structure: Complexes of ***2*** or more polypeptide chains held together by noncovalent forces but in precise ratios and with a precise three-dimensional configuration.

The twenty amino acids



Proton NMR
A simple yet valuable NMR experiment

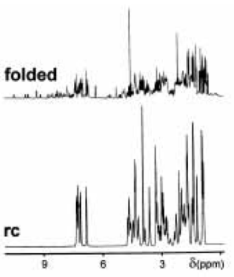


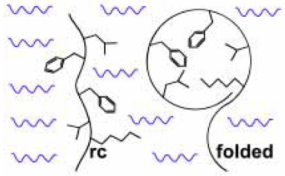
Protons: natural abundance NMR active nuclei
10-50 μM protein concentration
Acquisition time seconds
What is the value of this simple experiment?

Is my protein folded?

YES: structure possible

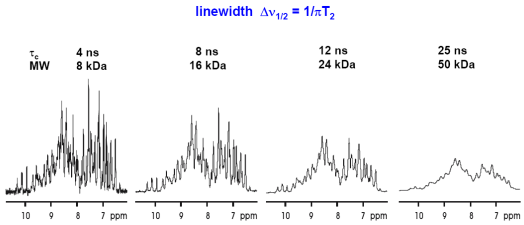
NO: structure impossible➔magnificent disorder



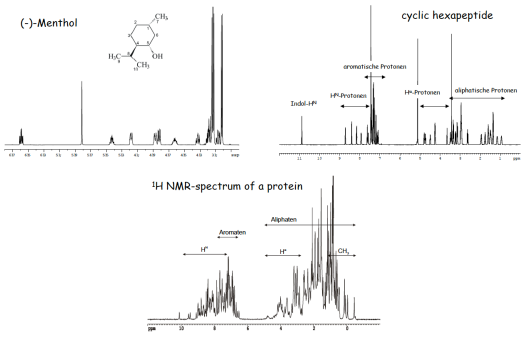


Each proton in a protein resonates at a characteristic frequency on the NMR chemical shift scale, defined by its local structural environment.

Illustrations of the Relationship Between MW, τ_c and T_2



Why multidimensional NMR?



Advances of multidimensional NMR?

The two major advantages of multidimensional NMR are:

Improved resolution: Signals are spread over a surface (2D) or in a three-dimensional space (3D, 4D)

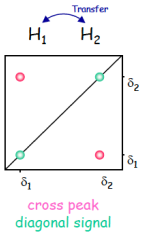
Magnetization transfer: Signals result from the interaction between nuclei. That can be interactions through bond (via J-coupling) or through space (via NOE)

Taken together this eases the interpretation and the assignment of the spectra considerably

Homonuclear spectra (2D)

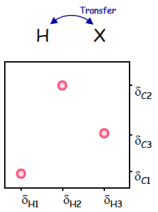
Magnetization transfer between same nuclei. Both axes exhibit the chemical shift of the same type of nucleus. If a transfer has taken place, the signal has different frequencies in the two dimensions:cross peak

If no transfer has taken place, the shifts are the same in both dimensions:diagonal signal

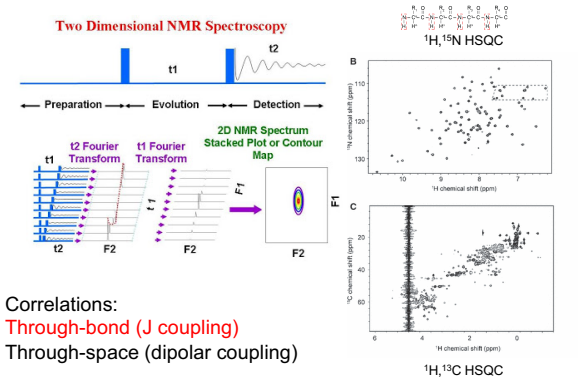


Heteronuclear spectra (2D)

Magnetization transfer different nuclei. The two axes show the chemical shift of the respective type of nucleus. If a transfer has taken place, a signal appears at the intersection of the two frequencies, without a transfer there is no signal.



Resolving protons ($^{13}C/^{15}N$ labeling)



$^1H,^{15}N$ HSQC

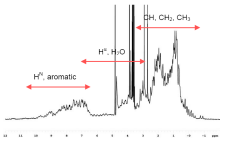
Correlations:
Through-bond (J coupling)
Through-space (dipolar coupling)

$^1H,^{13}C$ HSQC

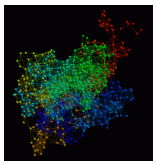
NMR assignment problem

Each observable NMR resonance needs to be assigned or associated with the atom in the protein structure.

- NMR spectra of proteins are complex, where the complexity increases with the size or number of residues of the protein
- Use ^{13}C & ^{15}N isotope enrichment to simplify the NMR spectra need to assign these NMR resonances
- a typical protein will have hundreds of ^1H, ^{13}C and ^{15}N NMR resonances to assign

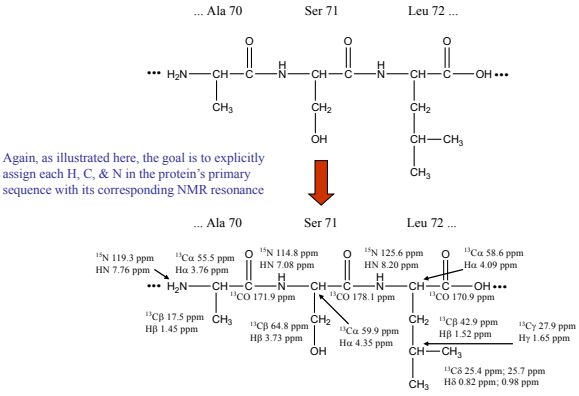


^1H NMR Spectra



Protein PDB File

NMR Assignments



Assignment strategy

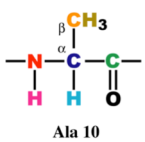
1. Identify resonances for each amino acid
2. Put amino acids in order
   - Sequential assignment (R-G-S,T-L-G-S)
3. Place fragments in aa sequence
   - Sequence-specific assignment

What do we mean by resonance assignment?

First we make a list of the NMR active nuclei in the protein

Second, we identify their chemical shifts from NMR spectra

Third, we associate the chemical shifts with amino acids in the protein sequence



Ala 10

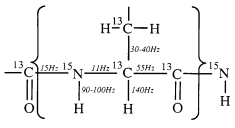
HN	8.613 ppm
N	125.932 ppm
Cα	51.900 ppm
Hα	4.371 ppm
Cβ	19.219 ppm
Hβ	1.285 ppm
C	174.531 ppm

Note: In this example the protein should be isotopically labeled with ^{15}N and ^{13}C.

NMR Assignments

3D NMR Experiments

- Takes advantage of ^{13}C and ^{15}N labeling
- Extends assignments to proteins in the 20-25 kDa range
- Extends Connectivity by Scalar Coupling (J) into 3D dimensions
  - Primarily uses one-bond heteronuclear coupling (^1H-^{13}C, ^1H-^{15}N)
    - ^1J generally stronger than ^3J

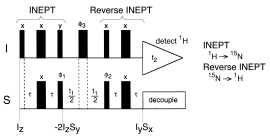


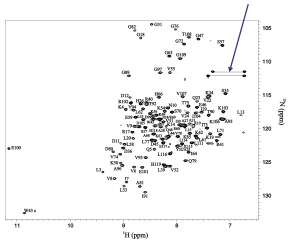
  - 2D 1H-15N HSQC is the root experiment of most of the standard *triple-resonance* (^1H, ^{13}C, ^{15}N) NMR experiments
- 3D NMR simplifies data and removes overlap by spreading information into third dimension
- Requires multiple experiments (≥ 6) to "walk through" the backbone assignments
- Requires a similar number of additional experiments to obtain the side-chain assignments

The root spectrum

2D ^{1}H-^{15}N HSQC experiment

- correlates backbone amide ^{15}N through one-bond coupling to amide ^{1}H
- in principal, each amino acid in the protein sequence will exhibit one peak in the ^{1}H-^{15}N HSQC spectra
  - also contains side-chain NH_2s (ASN,GLN) and NεH (Trp)
  - position in HSQC depends on local structure and sequence
  - no peaks for proline (no NH)

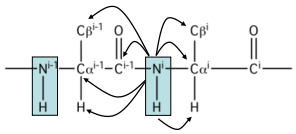


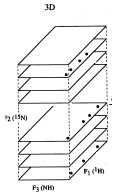


NMR Assignments

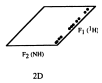
3D NMR Experiments

- Consider a 3D experiment as a collection of 2D experiments
  - z-dimension is the ^{15}N chemical shift
- ^{1}H-^{15}N HSQC spectra is modulated to include correlation through coupling to another backbone atom





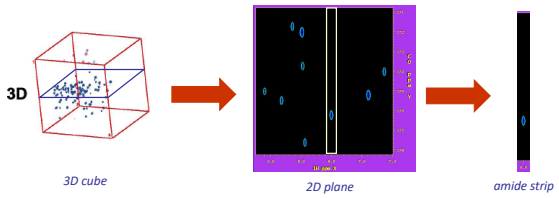
- All the 3D triple resonance experiments are then related by the common ^{1}H,^{15}N chemical shifts of the HSQC spectra
- The backbone assignments are then obtained by piecing together all the "jigsaw" puzzles pieces from the various NMR experiments to reassemble the backbone



NMR Assignments

3D NMR Experiments

- Amide Strip

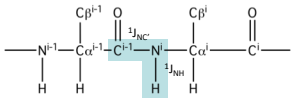


Strips can then be arranged in backbone sequential order to visual confirm assignments

NMR Assignments

3D NMR Experiments

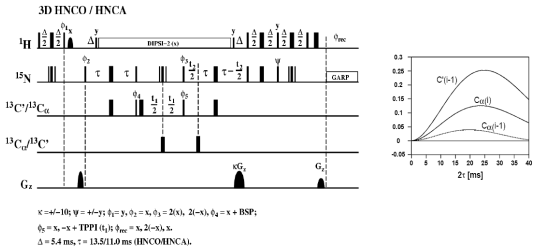
- 3D HNCO Experiment
  - common nomenclature → letters indicate the coupled backbone atoms
  - correlates NH^i to C^{i-1} (carbonyl carbon, CO or C')
  - no peaks for proline (no NH)
- Like the 2D ^{1}H-^{15}N HSQC spectra, each amino acid should display a single peak in the 3D HNCO experiment
  - identifies potential overlap in 2D ^{1}H-^{15}N HSQC spectra, especially for larger MW proteins
  - most sensitive 3D triple resonance experiment



NMR Assignments

3D NMR Experiments

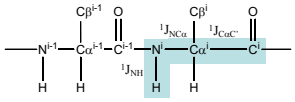
- 3D HNCO Experiment



NMR Assignments

3D NMR Experiments

- 3D HN(CA)CO Experiment
  - correlates NH^i to Co^i and Co^{i-1}
  - relays the transfer through $C\alpha^i$ without chemical shift evolution
  - contains both intra and sequential correlation
  - provides a means to sequential connect NH and CO chemical shifts
    - match NH^i-CO^i (HN(CA)CO with NH^i-CO^{i-1} (HNCO)
    - not sufficient to complete backbone assignments because of overlap and missing information
    - every possible correlation is not observed
    - need 2-3 connecting inter and intra correlations for unambiguous assignments
  - no peaks for proline (no NH) breaks assignment chain
    - but can identify residues i-1to prolines

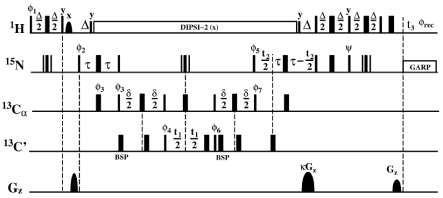


NMR Assignments

3D NMR Experiments

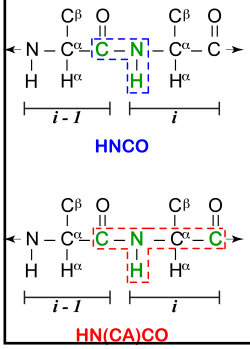
- 3D HN(CA)CO Experiment

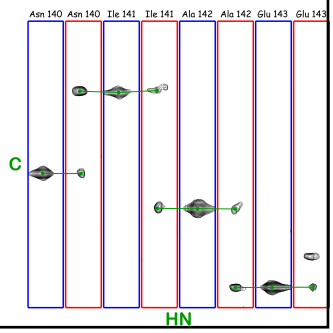
3D HN(CA)CO (HSQC)



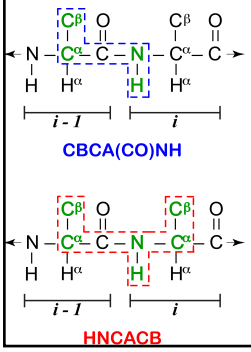
κ = +/−10; ψ = +/−y; ϕ_1 = x; ϕ_2 = x, −x; ϕ_3 = x ; ϕ_4 = x + BSP; ϕ_5 = x;
ϕ_6 = 4(x), 4(−x) + TPPI(t_1); ϕ_7 = 2(x), 2(−x), ϕ_{rec} = x, 2(−x), x, −x, 2(x), −x.
Δ = 5.4 ms, τ = 11 ms, δ = 6.8 ms, η = 5.5 ms.

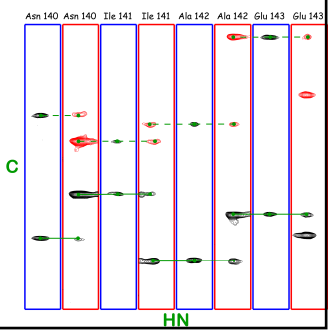
CO connectivities



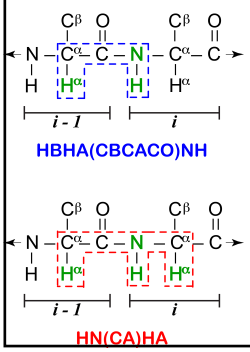


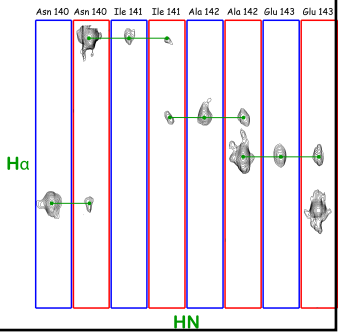
CA-CB connectivities





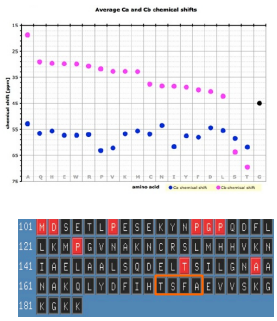
HA connectivities





Sequential assignments

Starting points: Thr, Ser, Ala, Gly



NMR Assignments

3D NMR Experiments

- Backbone Assignments
  - The process is a multi-step approach:

(1) correlate all the experimental data with each NH root observed in the 2D ^{1}H-^{15}N HSQC spectra

Pk-ID	NH	N15	Cα	Cβ	Cαi-1	Cβi-1
2.00	8.58	129.50	60.65	38.63	64.84	69.56
3.00	8.68	128.63	53.65	18.58	53.27	43.21
4.00	8.98	128.56	53.07	45.72	60.66	32.82
5.00	8.93	127.98	61.03	40.67	60.58	34.68
6.00	9.15	127.45	60.20	32.32	61.13	40.71
7.00	9.38	126.47	53.76	44.74	61.70	69.26
8.00	9.38	126.46	54.26	44.74	61.70	69.26
9.00	8.63	125.79	60.91	29.76	57.23	30.09
10.00	8.79	125.73	60.61	34.73	54.47	35.21
11.00	8.19	125.61	58.67	42.86	61.38	62.40
12.00	8.21	125.51	57.15	****	61.31	62.40
13.00	8.11	125.59	60.76	32.89	61.17	36.07
14.00	9.01	125.50	59.76	41.21	57.95	35.22
15.00	8.22	125.40	57.22	****	55.69	29.56
16.00	8.22	125.40	55.83	****	55.69	29.56
17.00	9.04	125.12	54.75	39.51	58.80	33.07
18.00	7.82	124.78	54.62	32.46	62.56	33.07
19.00	8.57	124.32	57.99	35.22	59.26	36.57
20.00	9.05	123.83	64.05	31.96	53.90	42.80
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NMR Assignments

3D NMR Experiments

- Backbone Assignments
  - The process is a multi-step approach:

(2) Match pairs of NH roots based on i and i-1 correlations

Pk-ID	NH	N15	Cα	Cβ	Cα(i-1)	Cβ(i-1)
2.00	8.58	129.49	60.61	38.63	64.82	69.56
202.00	8.55	116.39	62.55	69.49	60.62	38.62
3.00	8.68	128.63	53.65	18.58	53.27	43.21
230.00	8.78	105.35	45.64	****	53.72	18.60
4.00	8.98	128.57	52.96	45.72	60.64	32.82
193.00	8.22	117.39	54.54	36.27	52.95	45.73
5.00	8.93	127.98	60.90	40.67	60.57	34.68
6.00	9.16	127.45	60.14	32.32	61.10	40.71
6.00	9.16	127.45	60.14	32.32	61.10	40.71
108.00	8.78	119.65	58.97	34.36	60.16	32.27
7.00	9.38	126.46	54.17	44.74	61.65	69.26
197.00	8.95	117.12	55.46	37.23	54.14	44.78
8.00	8.64	125.80	60.88	29.76	57.16	30.09
206.00	8.85	116.15	58.95	****	60.86	29.65
9.00	8.79	125.73	60.59	34.73	54.37	35.21
5.00	8.93	127.98	60.90	40.67	60.57	34.68
10.00	8.19	125.62	58.60	42.86	61.31	62.40
203.00	8.55	116.32	62.15	69.49	58.61	42.85

NMR Assignments

3D NMR Experiments

- Backbone Assignments
  - The process is a multi-step approach:

(3) Extend pairs of NH roots and match to protein primary sequence

Identify overlapping spin-system pairs

5.00	8.93	127.98	60.90	40.67	60.57	34.68
6.00	9.16	127.45	60.14	32.32	61.10	40.71
6.00	9.16	127.45	60.14	32.32	61.10	40.71
108.00	8.78	119.65	58.97	34.36	60.16	32.27

connect spin-system pairs

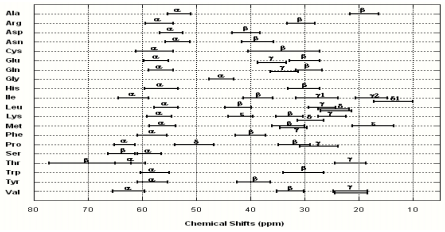
5.00	8.93	127.98	60.90	40.67	60.57	34.68
6.00	9.16	127.45	60.14	32.32	61.10	40.71
108.00	8.78	119.65	58.97	34.36	60.16	32.27

NMR Assignments

3D NMR Experiments

- Backbone Assignments
  - The process is a multi-step approach:

(3) Extend pairs of NH roots and match to protein primary sequence



Identify possible residue types by chemical shift ranges

NMR Assignments

3D NMR Experiments

- Backbone Assignments
  - The process is a multi-step approach:

(3) Extend pairs of NH roots and match to protein primary sequence

F,F,I,C	5.00	8.93	127.98	60.90	40.67	60.57	34.68
F, W, C	6.00	9.16	127.45	60.14	32.32	61.10	40.71
F, W, C	108.00	8.78	119.65	58.97	34.36	60.16	32.27

Find potential match in sequence

MTLKQVIVVRDDLKLSRGKLAVQVAHAAIIGYLKSDSSLRRKWLDEGQKKVVLKVKS
LEELLGIKHKAESLGLVTGLVQDAGLTEVPPGTITAVVIGPDEERKIDKVTGNLPLLKLE
HHHHHH

Make assignment

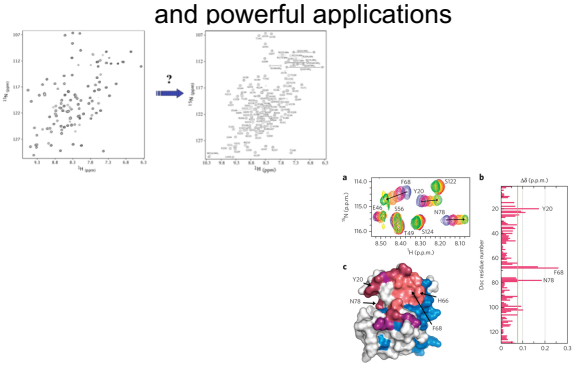
I 7	5.00	8.93	127.98	60.90	40.67	60.57	34.68
V 8	6.00	9.16	127.45	60.14	32.32	61.10	40.71
V 9	108.00	8.78	119.65	58.97	34.36	60.16	32.27

NMR Assignments

Relative sensitivity of triple resonance experiments

Experiment	Assignment	Comment	Relative S/N [%]
HNCO	H(i), N(i), C'(i-1)	<20 kD, above use ^{2}H labeling	100
HNCA	H(i), N(i), C_α(i).C_α(i-1)	<20 kD, above use ^{2}H labeling	50/15
HN(CO)CA	H(i), N(i), C_α(i-1)	<20 kD, above use ^{2}H labeling	71
HN(CA)CO	H(i), N(i), C'(i)	<20 kD, above use ^{2}H labeling	13/4
CBCA(CO)NH	H(i), N(i), C_α(i-1), C_β(i-1)	<20 kD, above use ^{2}H labeling	13/9 α/β
HBHA(CO)NH	H(i), N(i), H_α(i-1), H_β(i-1)	<20 kD, above use ^{2}H labeling	13/9 α/β
CBCANH, HNCACB	H(i), N(i), C_α(i), C_β(i), C_α(i-1).C_β(i-1)	<15 kD, above use ^{2}H labeling	4/1.7 α/β(i) 1.3/0.5 α/β(i-1)
(H)CC(CO)NH-TOCSY	H(i), N(i), $C^{aliph.}$(i-1)	<15-20 kD, above use ^{2}H labeling	
H(CC)(CO)NH-TOCSY	H(i), N(i), $H^{aliph.}$(i-1)	<15-20 kD, above use ^{2}H labeling	
HCCH-TOCSY	$H^{aliph.}$, $C^{aliph.}$	<25 kD, - sensitive, but tedious to analyze, combine with HCCONH type experiments	

Sequence-Specific Assignment and powerful applications



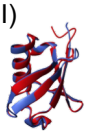
The Chemical Shift Index (CSI)

With the exception of Glycines

Helix		Strand	
Hα	↕	Hα	↓
Cα	↓	Cα	↕
Cβ	↕	Cβ	↓
C′	↑	C′	↕

Backbone chemical shifts report protein secondary structure

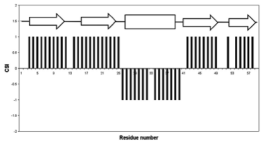
★ Structure from chemical shifts ★
CS-ROSETTA



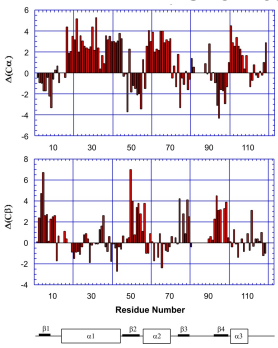
D.S. Wishart / Progress in Nuclear Magnetic Resonance Spectroscopy 58 (2011) 62–87

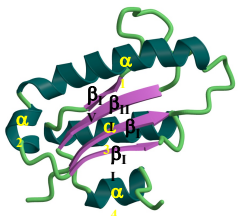
Table 7
Statistically derived reference chemical shifts (in ppm) for backbone shifts in helix, β-sheet and random coil secondary structures.

Residue type	$^{13}C\alpha$			$^{13}C\beta$			$^{13}C'$			^{15}N			^{1}HN			$^{1}H\alpha$		
	Helix	Sheet	Coil	Helix	Sheet	Coil	Helix	Sheet	Coil	Helix	Sheet	Coil	Helix	Sheet	Coil	Helix	Sheet	Coil
Ala	54.8	51.5	52.8	18.3	21.1	19.1	179.4	176.1	177.7	121.4	124.5	123.6	8.08	8.44	8.15	4.03	4.77	4.26
Cys (ox)	58.0	55.0	55.6	39.4	43.9	41.0	176.2	173.6	174.9	117.7	121.0	118.0	8.20	8.80	8.25	4.15	5.15	4.65
Cys (red)	61.3	56.9	57.5	27.8	30.2	29.4	176.2	173.6	174.9	117.7	121.0	118.0	8.20	8.80	8.25	4.15	5.15	4.65
Asp	56.7	53.9	54.2	40.5	42.3	40.9	178.0	175.5	176.3	119.2	122.2	120.0	8.18	8.51	8.36	4.43	4.94	4.60
Glu	59.1	55.5	56.9	29.4	32.0	30.2	178.6	175.4	176.4	119.0	122.1	120.4	8.22	8.53	8.37	4.01	4.78	4.28
Phe	60.8	56.7	58.0	38.8	41.5	39.5												
Gly	46.9	45.2	45.5	N/A	N/A	N/A												
His	59.0	55.1	55.9	29.5	31.9	30.0												
Ile	64.6	60.1	61.0	37.6	39.9	38.7												
Lys	58.9	55.4	56.6	32.3	34.6	32.8												
Leu	57.5	54.1	54.9	41.6	43.8	42.4												
Met	58.1	54.6	55.7	32.3	35.1	33.4												
Asn	55.5	52.7	53.2	38.6	40.1	38.6												
Pro	65.5	62.6	63.5	31.5	32.3	31.9												
Gln	58.5	54.8	56.1	28.5	31.3	29.1												
Arg	58.9	55.1	56.4	30.1	32.2	30.7												
Ser	60.9	57.5	58.4	63.1	65.2	64.0												
Thr	65.6	61.1	61.6	68.9	70.8	70.1												
Val	66.2	60.8	62.1	31.5	33.9	32.7												
Trp	60.0	56.4	57.8	29.3	31.5	29.7												
Tyr	61.0	56.8	58.0	38.3	41.0	39.0												



Protein Secondary Structure and Carbon Chemical Shifts

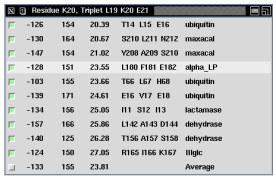




NMR Structure Determination

Protein Secondary Structure and Carbon Chemical Shifts

- TALOS+
  - Given the backbone chemical shifts and primary sequence
  - Compares the secondary chemical shifts against database of chemical shifts and associated high-resolution structure
    - comparison based on "triplet" of amino acid sequences present in database structures with similar chemical shifts and secondary structure
  - Provides potential ϕ , ψ backbone torsion constraints

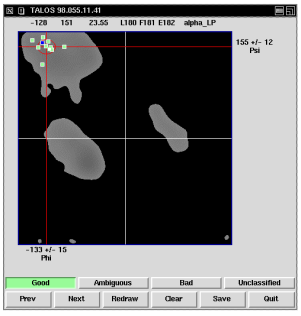


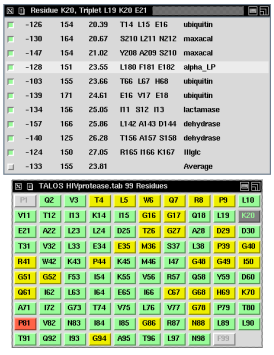
-126	154	20.39	T14 L15 E16	ubiquitin	
-130	164	20.67	S210 L211 N212	maxacal	
-147	154	21.02	Y208 A209 S210	maxacal	
-128	151	23.55	L180 F181 E182	alpha_LP	
-103	155	23.66	T66 L67 H68	ubiquitin	
-139	171	24.61	E16 V17 E18	ubiquitin	
-134	156	25.05	I11 S12 I13	lactamase	
-157	166	25.86	L142 A143 D144	dehydrase	
-140	125	26.28	T156 A157 S158	dehydrase	
-124	150	27.05	R165 I166 K167	IIIgic	
-133	155	23.81		Average	

NMR Structure Determination

Protein Secondary Structure and Carbon Chemical Shifts

- TALOS +





Shen *et al.* (2009) *J. Biomol NMR* 44:213

NMR Structure Determination

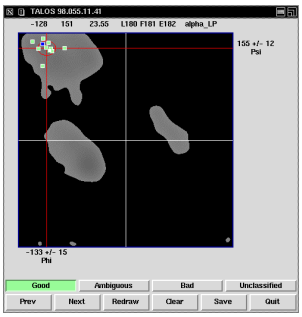
Protein Secondary Structure and Carbon Chemical Shifts

- TALOS+

TALOS may provide relatively tight error bounds associated with the predicted ϕ,ψ.

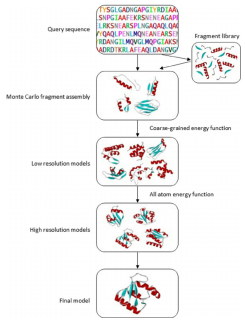
It is better being more conservative by using minimal errors of:

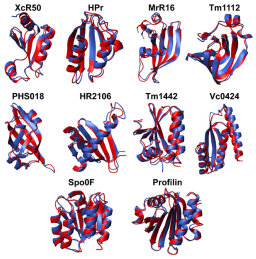
ϕ ± 30
ψ ± 50
χ ± 20



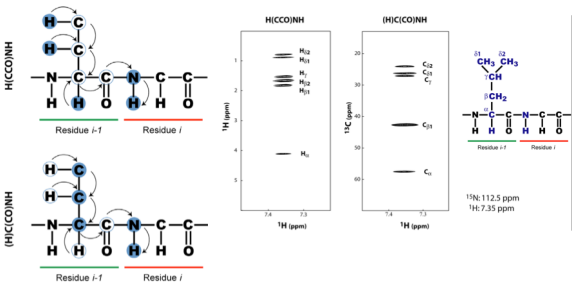
CS-Rosetta

$^{13}C^{\alpha}$, $^{13}C^{\beta}$, $^{13}C'$, ^{15}N, $^{1}H^{\alpha}$ and $^{1}H^{N}$ NMR chemical shifts





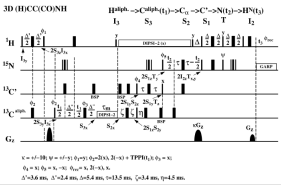
Sidechain assignments (1)



NMR Assignments

3D NMR Experiments

- Side-chain Assignments
  - Help confirm the backbone assignment
  - Similar in principal to 2D assignment approach
    - Correlate entire spin-system with NH backbone
    - Use TOCSY to observe entire spin-system
    - CC(CO)NH & HCC(CO)NH
      - Relay magnetization from NH through side-chain carbon or hydrogen chemical shifts
      - Start simultaneously on all side-chain hydrogens
      - Also, overlap with Cα and Cβ chemical shifts from other triple-resonance experiments to confirm side-chain assignments



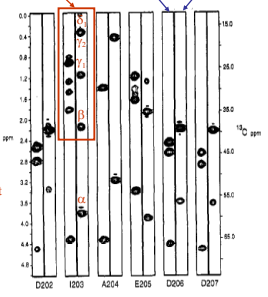
NMR Assignments

3D NMR Experiments

- Side-chain Assignments
  - CC(CO)NH & HCC(CO)NH
  - Can assign residue type by the number of observed resonances and the chemical shift ranges
    - may be able to assign Cγ, Cδ, Cε from chemical shift values and from previously assigned Cα and Cβ
    - less likely to assign Hγ, Hδ and Hε, unless unique chemical shift
    - need companion experiments to connect carbon and hydrogen chemical shifts.

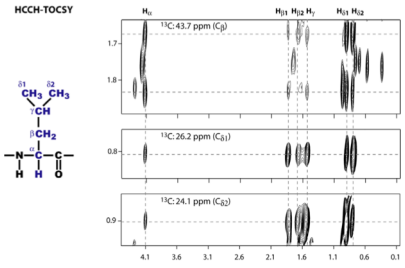
Which H's match the C's?

HCC(CO)NH CC(CO)NH



Biochemistry, Vol. 34, No. 42, 1995

Sidechain assignments (2)



This experiment correlates a ^{1}H/^{13}C pair to all other protons in the same aminoacid sidechain.

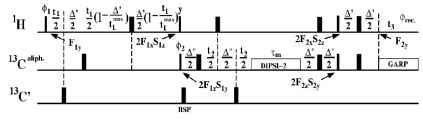
Also, very useful for determining which ^{1}H is directly attached to which ^{13}C

NMR Assignments

3D NMR Experiments

- Side-chain Assignments
  - HCCH-TOCSY & HCCH-COSY
    - relays magnetization from side-chain and backbone ^{1}H & ^{13}C via coupling constants
    - Experiments have symmetry
      - ^{1}Hα-^{13}Cα diagonal shows cross peak to ^{1}Hβ
      - *AND*
      - ^{1}Hβ-^{13}Cβ diagonal shows cross peak to ^{1}Hα
    - does not correlate to backbone NH→ no direct connection with other triple-resonance experiments
      - sample can be collected in D2O

a) **3D HC(C)H–TOCSY**



ϕ_1= x, –x + TPPI(t_1); ϕ_2 = 2(x), 2(–x)+ TPPI(t_2); ϕ_{rec}= x, 2(–x), x, –x, 2(x), –x.

Δ'=3.6 ms, Δ''=2.4 ms.

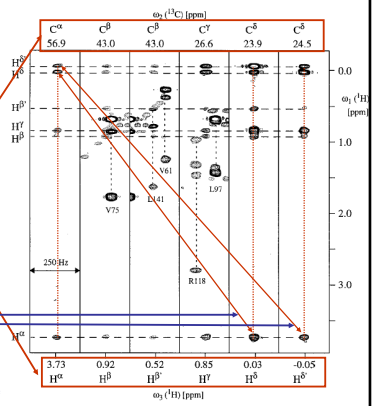
NMR Assignments

3D NMR Experiments

- Side-chain Assignments
  - HCCH-TOCSY

Slices taken from different ^{13}C chemical shift planes at different ^{1}H chemical shifts illustrates the entire spin system for a single side-chain

Symmetry – each HCδ shows a cross peak to Hα and the HCα shows a crosspeak to both HCδ



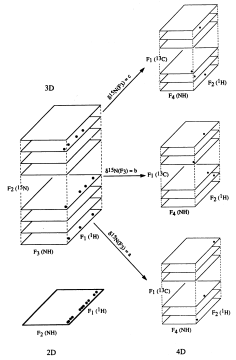
Note: Symmetry peaks may not always be present (separate pathways, separate relative sensitivity). Presence of a symmetry peak increase the likelihood of correct assignment

Journal of Biomolecular NMR, 9 (1997) 445–446

NMR Assignments

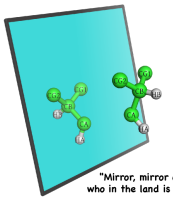
4D NMR Experiments

- Consider a 4D NMR experiment as a collection of 3D NMR experiments
  - still some ambiguities present when correlating multiple 3D triple-resonance experiments
  - 4D NMR experiments make definitive sequential correlations
    - increase in spectral resolution
      - Overlap is unlikely
    - loss of sensitivity
      - an additional transfer step is required
      - relaxation takes place during each transfer



rotameric states (VAL / LEU)

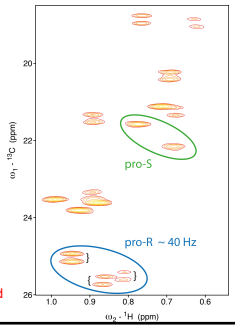
Two chemically identical substituents on a tetrahedral carbon may be geometrically distinct.
Two such atoms are referred to as being prochiral.
Designated as pro-R or pro-S based on same criteria as R and S.



"Mirror, mirror on the wall,
who in the land is fairest of all?"

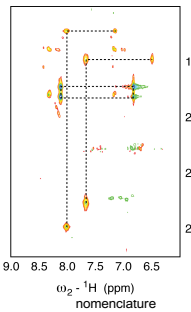
System in use at BioMagResBank
(IUPAC/IUB Biochemistry 9, 3471-3479 [1970])
for VAL CG1=pro-R / CG2=pro-S
for LEU CD1=pro-R / CD2=pro-S

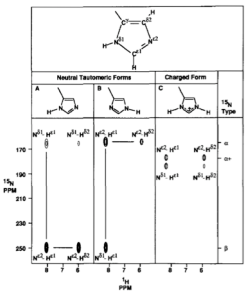
Switch off swapping when rotameric states are obtained
(e.g. using 10% ^{13}C enriched medium)



tautomeric state HIS

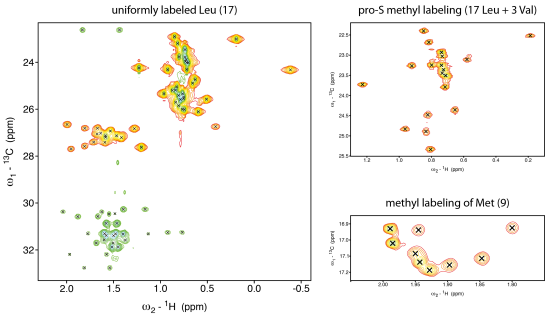
^1H-^{15}N long-range HSQC





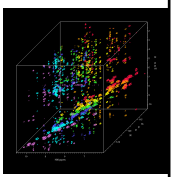
nomenclature	CYANA			
CNS	HIS	HIS+	HIST	
	HISD	HISE	HIS	

labeling schemes

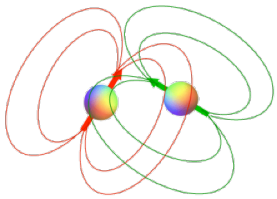


NMR experiments

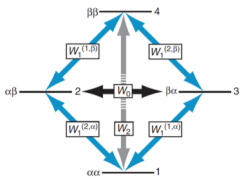
- Backbone experiments
  CBCA(CO)NH & HNCACB
  HNCO & HN(CA)CO
  HAHB(CBCACO)NH & HN(CA)HA
  3D spectra (4D, 5D …)
  C(CCO)NH & HNHtocsy identify the spin-system
- Sidechain experiments
  hCCH-TOCSY & HcCH-TOCSY (fold coupled carbon in both)
- Aromatic rings
  CBHD CBHE but better rely on the noesy
- Stereospecific assignments for VAL/LEU methyl groups
  ^1H-^{13}C HSQC on 10% labeled sample / pro-S labeling using an acetolactate precursor
- Tautomeric states of histidines
  ^1H-^{15}N long-range HSQC
- For larger proteins or complicated systems
  selective isotopic labeling of amino acids and stereospecific isotopic labeling of methyl groups
- NOESY experiments
  HNH_noesy
  HCH_noesy obtain fold
  HCHaro_noesy
  CNH_noesy
  CCH_noesy optimize fold
  2d_noesy homonuclear



Nuclear Overhauser Effect (NOE)



Distance: The strength of the dipolar interaction depends on the distance between the spins. The effect is inversely proportional to the sixth power of distance ($1/r^6$)



two spins, four energy levels
dipolar interaction causes relaxation-induced transitions
between any two levels