Introduction to Protein Structure
Bioinformatics 2004
NMR Spectroscopy

CH.EMBnet course 28.9.2004

Biozentrum, Basel

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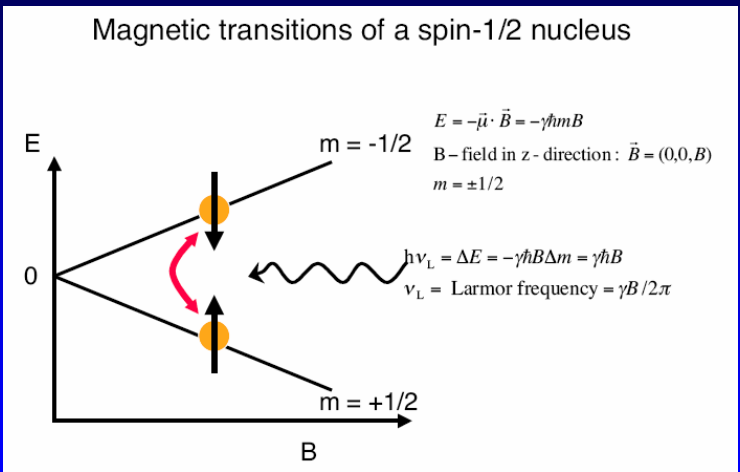
Overview

1. Basic principles of NMR
2. Structure Determination by Solution NMR
3. Beyond Structure
4. Questions

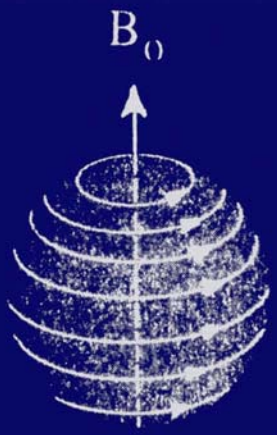
The principle of magnetic resonance

- When molecules are placed in a strong magnetic field, the magnetic moments of the nuclei align with the field
- This equilibrium alignment can be changed to an excited state by applying radio frequency (RF) pulses
- When the nuclei revert to the equilibrium they emit RF radiation that can be detected

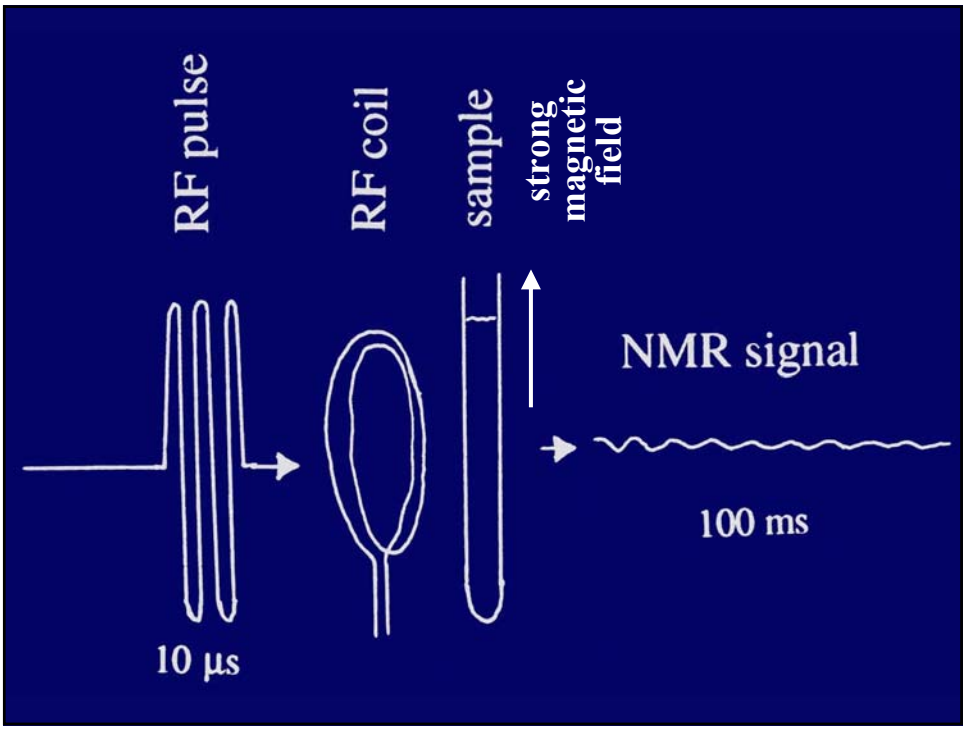
Basic principle of NMR



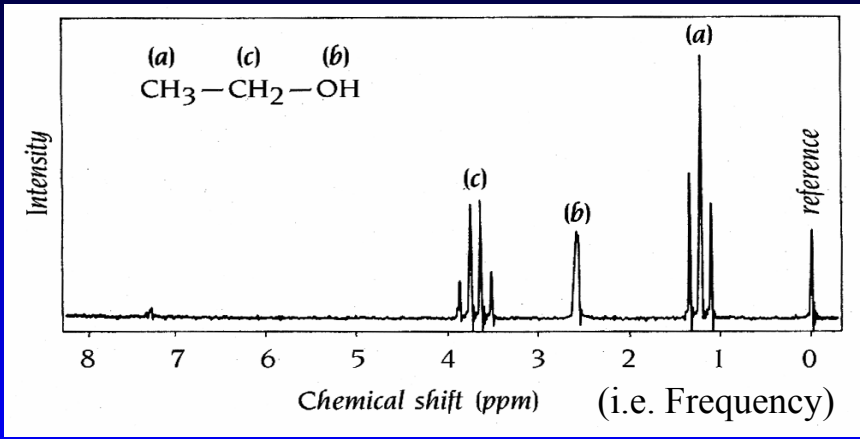
Magnetic shielding of nucleus by surrounding electron cloud



$$\nu = \gamma B_0 (1-\sigma)$$



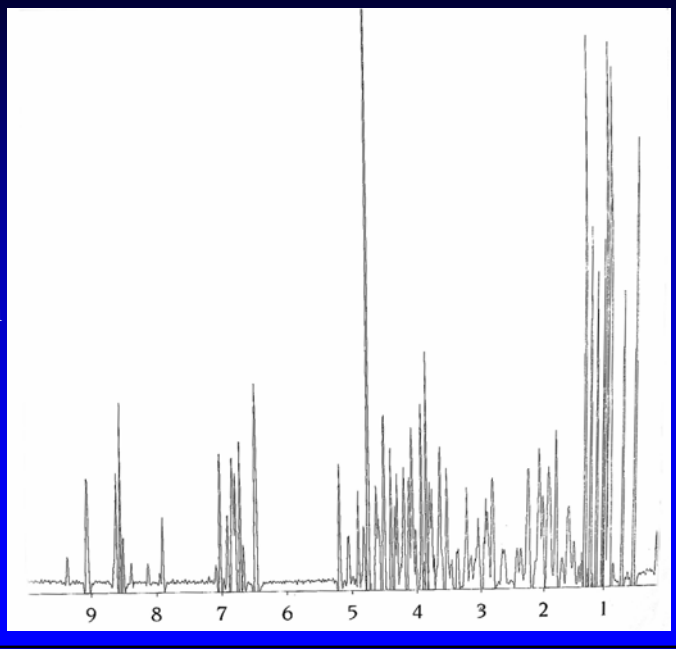
The frequency spectrum of the emitted NMR RF signal is obtained by a mathematical analysis that is called Fourier transform

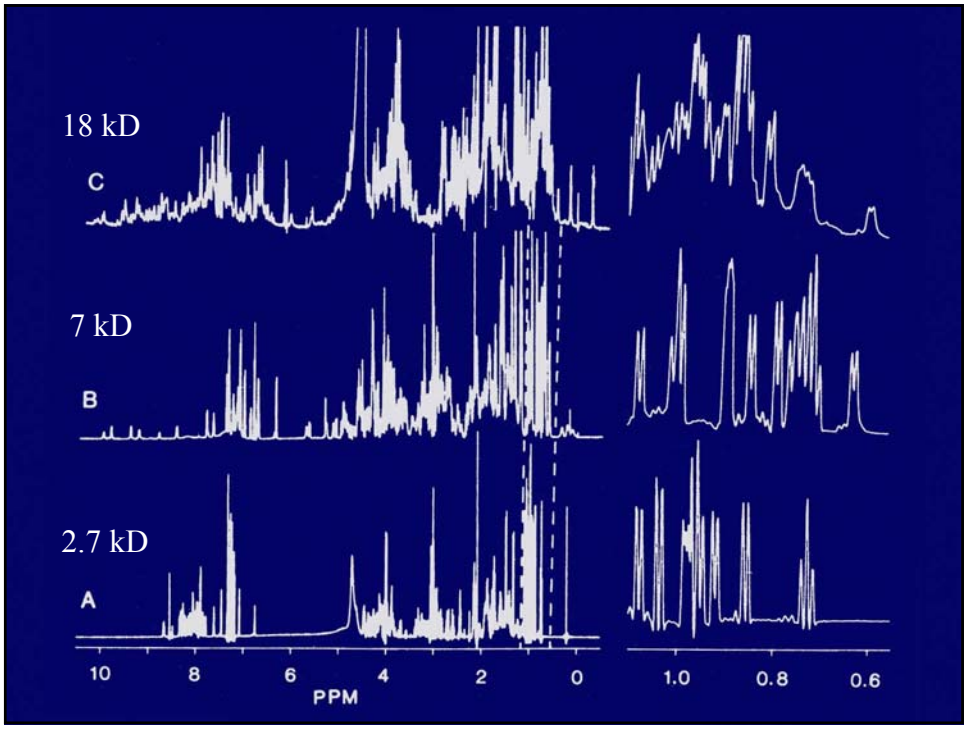


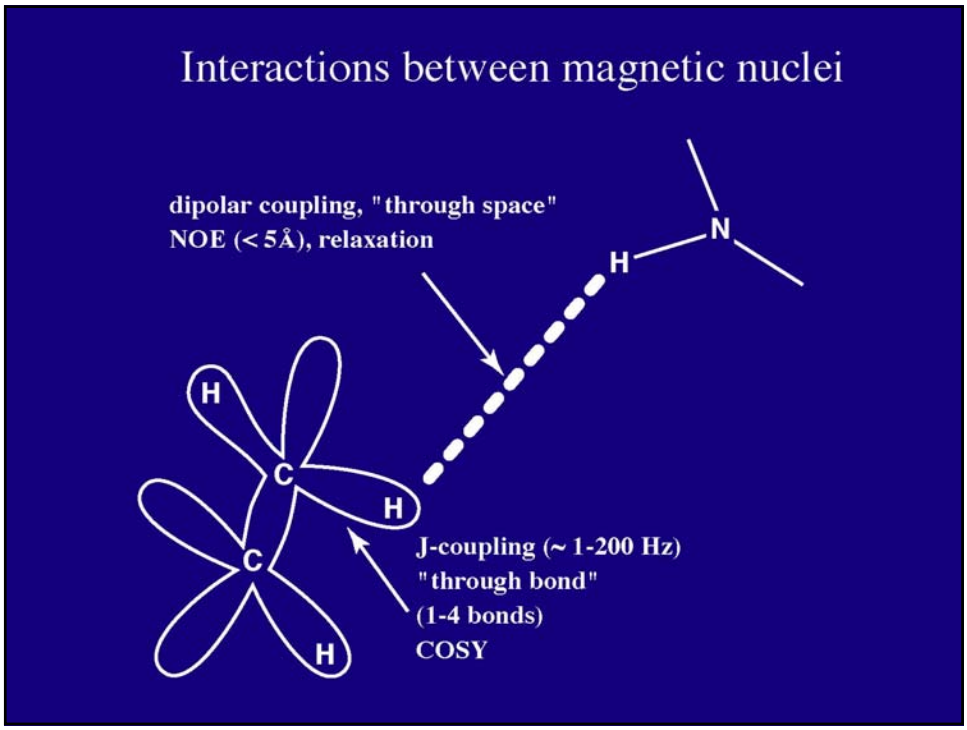
The exact frequency of the emitted radiation depends on the chemical environment. The frequency is determined relative to a reference signal. As this relative frequency it is called ***chemical shift***.

When a larger number of different atoms is present, more lines are observed

Proton NMR spectrum of 36 amino acid protein (C-terminal domain of cellulase)

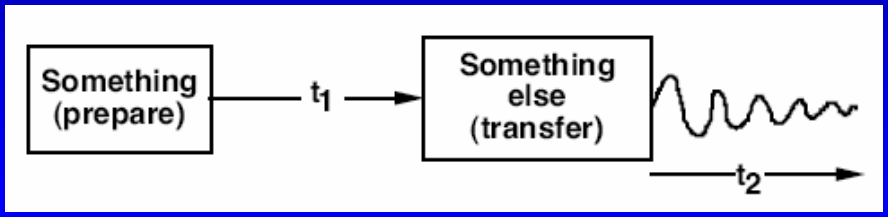



18 kD
C
7 kD
B
2.7 kD
A
10
8
6
4
2
0
PPM
1.0
0.8
0.6


Interactions between magnetic nuclei
dipolar coupling, "through space"
NOE (< 5Å), relaxation
H
N
H
C
H
C
H
J-coupling (~ 1-200 Hz)
"through bond"
(1-4 bonds)
COSY

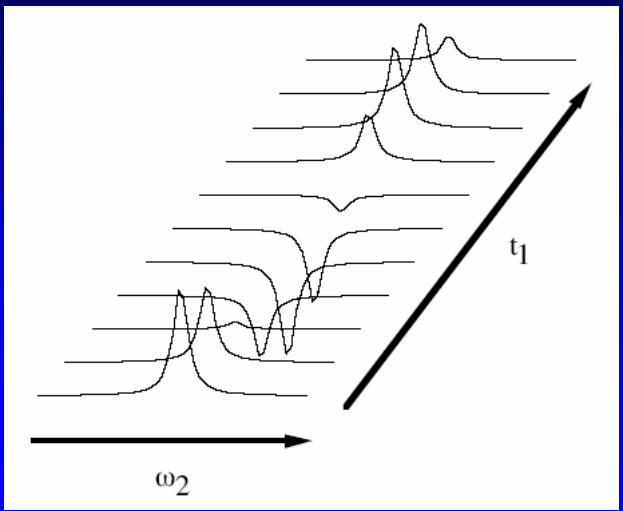
From one-dimensional to two-dimensional NMR spectroscopy

A two-dimensional NMR experiment consists of a large number (e.g. 512) of one-dimensional experiments. Between each experiment a time t1 delay is incremented

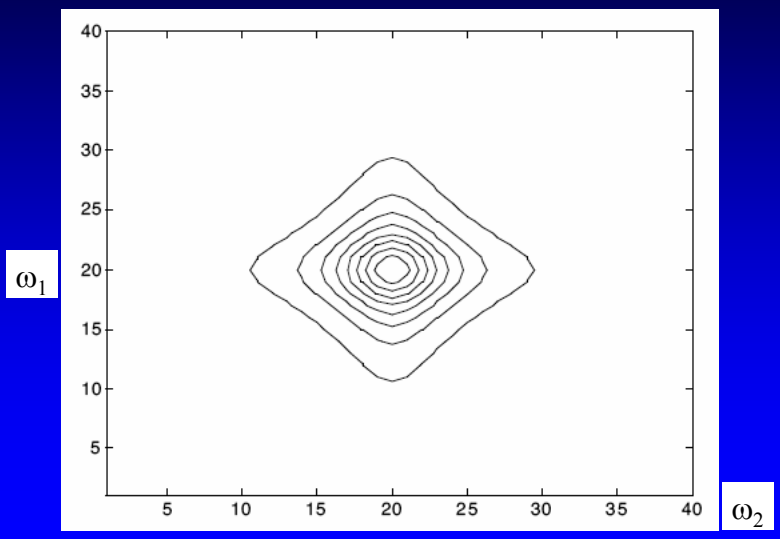


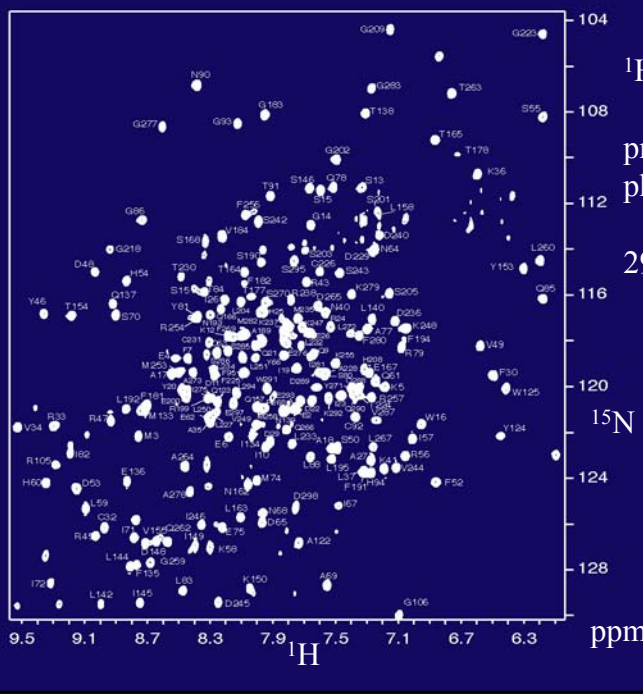
Interferogram of a two-dimensional spectrum

time domain in the first dimension
frequency domain in the second dimension



Second Fourier transformation ->
two-dimensional spectrum (contour lines)

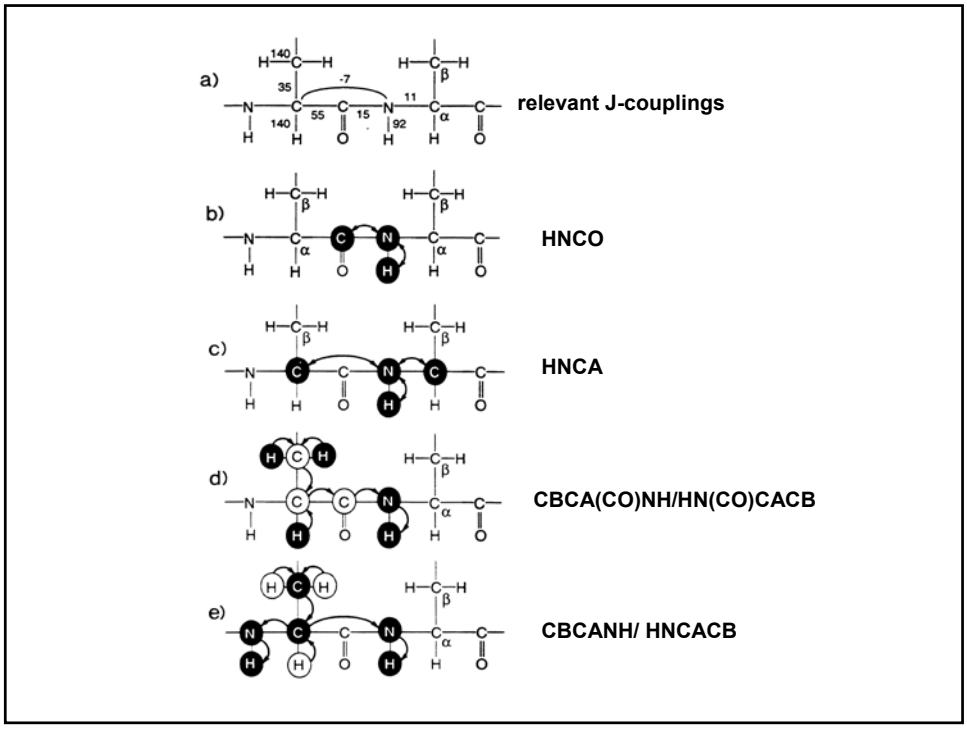


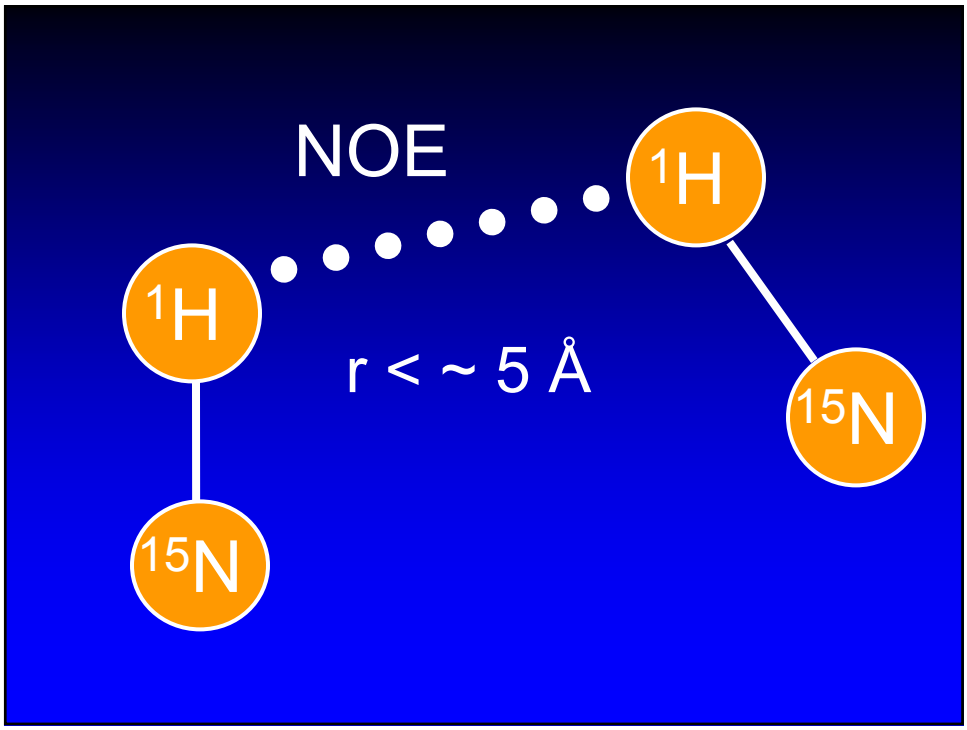


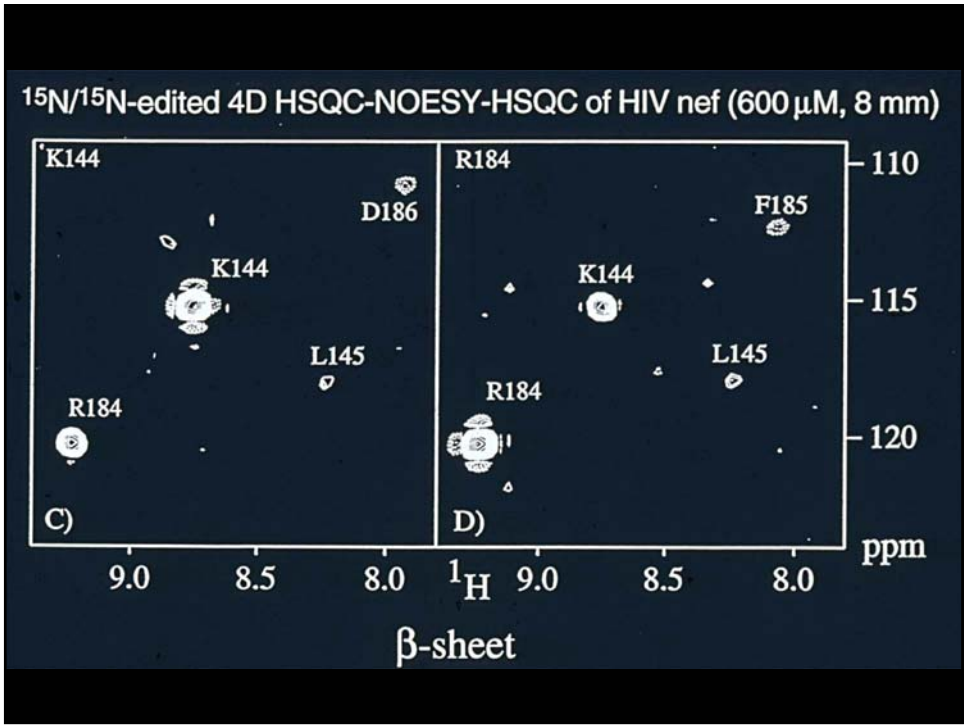
^{1}H-^{15}N HSQC COSY

protein tyrosine phosphatase 1B

298 aa ~ 35 kDa


a)
relevant J-couplings
b)
HNCO
c)
HNCA
d)
CBCA(CO)NH/HN(CO)CACB
e)
CBCANH/ HNCACB


NOE
1H
1H
15N
r < ~ 5 Å
15N



Sample requirements

- ~ 0.25 ml 0.5 mM protein
  (= 2.5 mg for 20 kDa protein)
- ^{15}N, ^{13}C, (^{2}H) labelled (*E. coli*)
- MWT < ~ 60 kDa for 3D structure
- MWT < ~100 (800) kDa for secondary structure, functional tests, etc.

Summary Part 1

- NMR uses nuclear magnetic moments of atoms
- 1D-spectra:
  chemical shifts, line widths, coupling constants
- 2D (3D,4D,etc.)-spectra:
  connectivities (COSY)
  proximity in space (NOESY)

Part 2: Structure Determination of Proteins in Solution

- Resonance assignment (COSY)
- Distance assignment (NOESY)
- Structure calculation

Resonance assignment

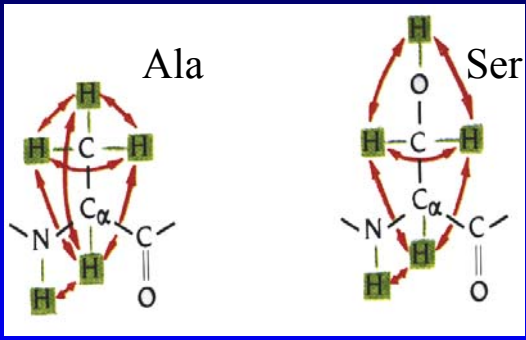
- The crosspeaks in NOESY spectra cannot be interpreted without knowledge of the frequencies of the different nuclei
- These frequencies are not known in the beginning
- The frequencies can be obtained from information contained in COSY (correlation spectroscopy) spectra
- The process of determining the frequencies of the nuclei in a molecule is called resonance assignment

  (and can be lengthy…)

COSY (Correlation Spectroscopy)

Two-dimensional COSY NMR experiments give correlation signals that correspond to pairs of hydrogen atoms which are connected through chemical bonds.

Typical COSY correlations are observable for "distances" of up to three chemical bonds.

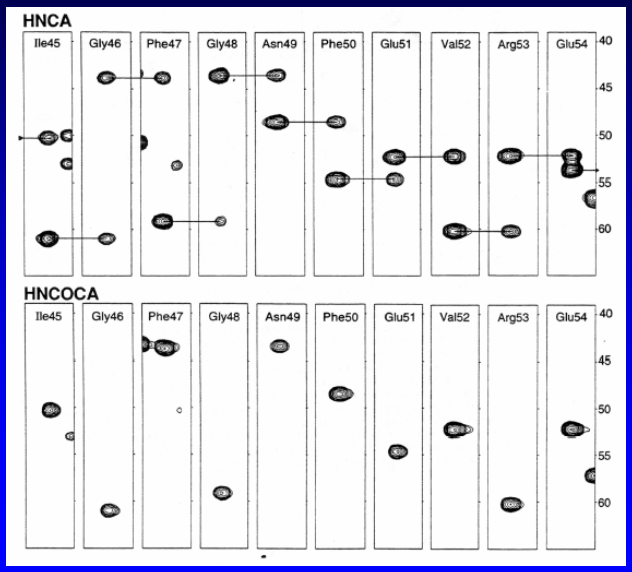


COSY correlations between covalently bonded hydrogen atoms

Resonance assignment by COSY

- COSY spectra show frequency correlations between nuclei that are connected by chemical bonds
- Since the different amino acids have a different chemical structure they give rise to different patterns in COSY spectra
- This information can be used to determine the frequencies of all nuclei in the molecule. This process is called resonance assignment
- Modern assignment techniques also use information from COSY experiments with ^{13}C and ^{15}N nuclei

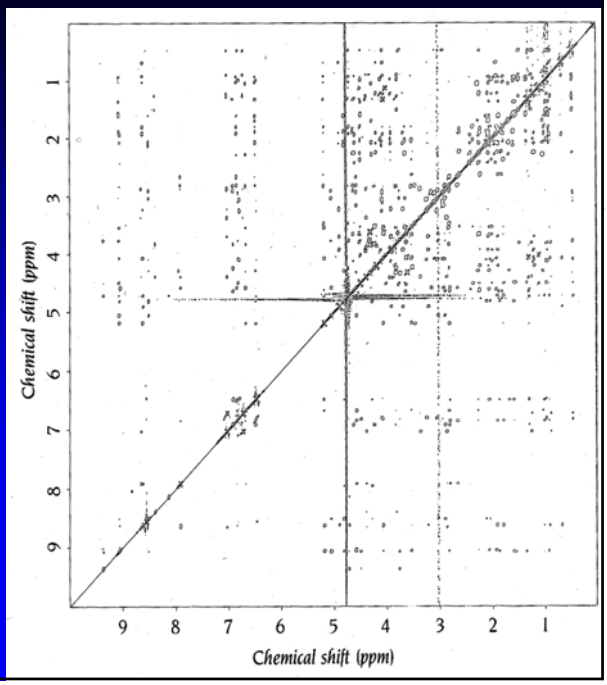
Example of an assigned HNCA/HNCOCA



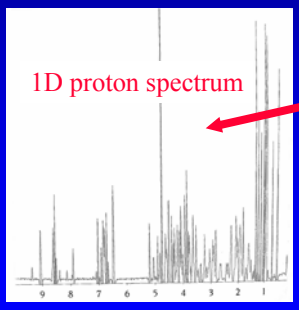
Distances from NOESY spectra:

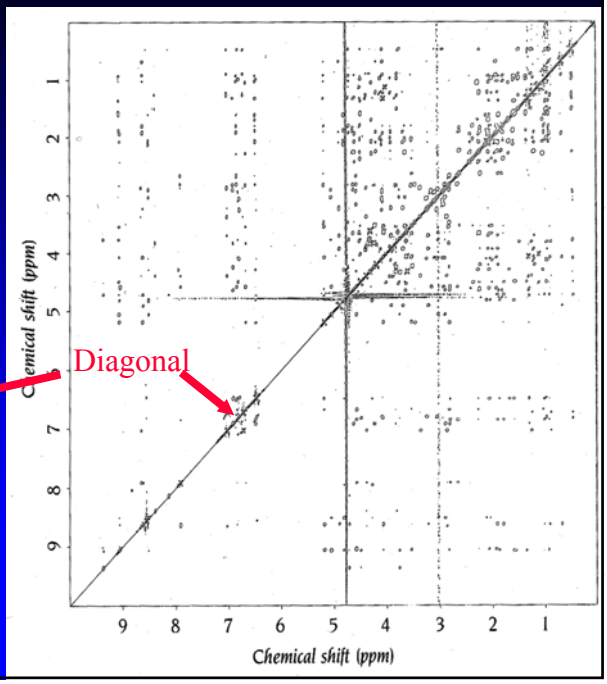
- secondary structure elements
- calculation of three-dimensional structure

Two-dimensional NOESY spectrum of C-terminal domain of cellulase

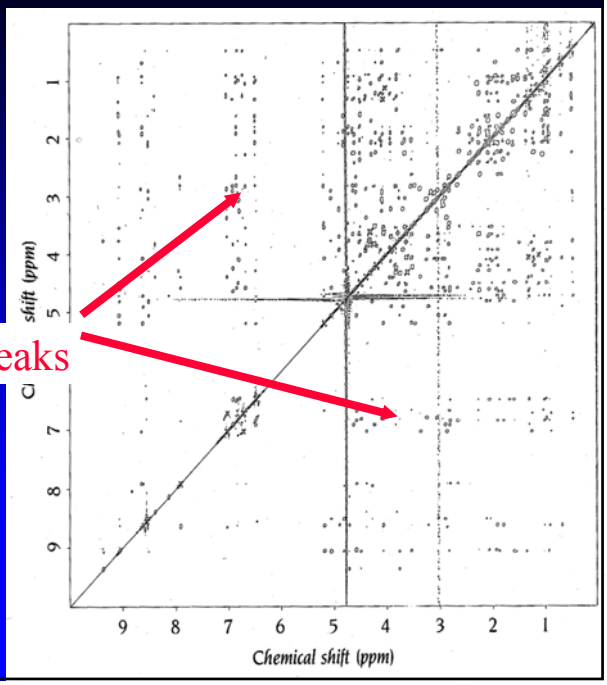


The diagonal in the NOESY contains the one-dimensional spectrum





The off-diagonal peaks in the NOESY represent interactions between hydrogen nuclei that are closer than 5Å to each other in space

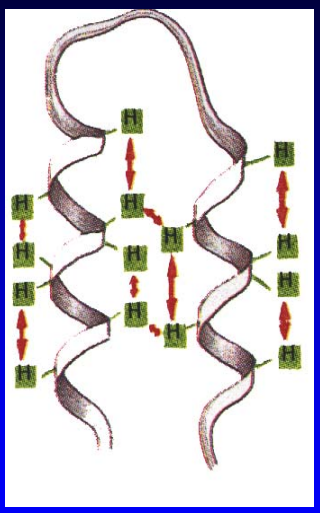


E.g:. a crosspeak at position (7 ppm, 3 ppm) in the NOESY means that there are two protons with frequencies 7 and 3 ppm and these two protons are closer than 5 Å to each other in the molecule.

Structure information from NOEs

NOESY experiments give signals that correspond to hydrogen atoms which are close together in space (< 5Å), even though they may be far apart in the amino acid sequence.

Structures can be derived from a collection of such signals which define distance constraints between a number of hydrogen atoms along the polypeptide chain.

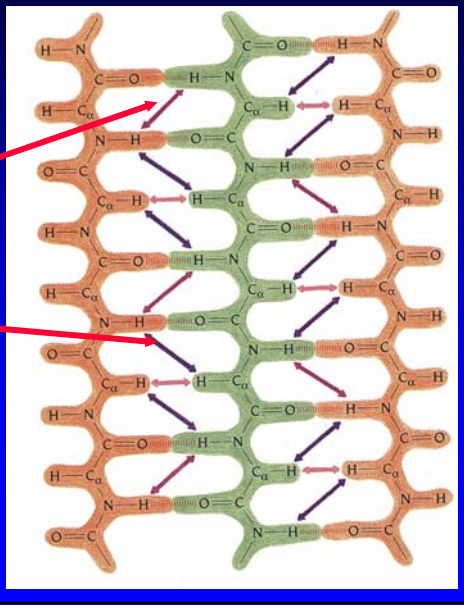


Example: short distance (< 5 Å, NOE) correlations between hydrogen atoms in a helix

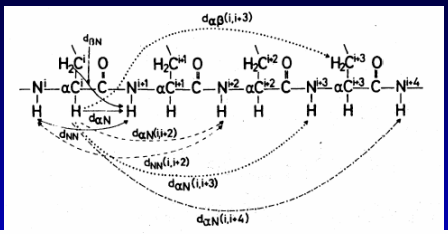
Example of NOE-observable hydrogen-hydrogen distances (< 5 Å) in an antiparallel beta sheet

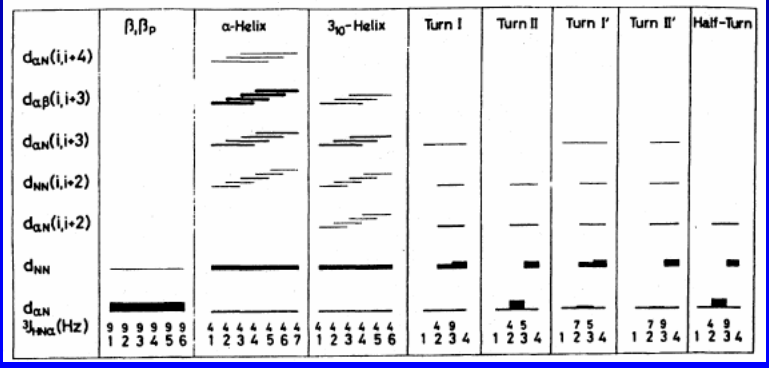
Cross-strand H^N- H^N

Cross-strand H^N-H^α



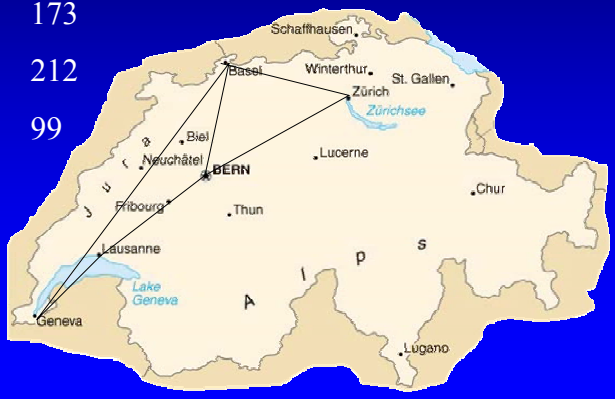
NOE pattern observed for different types of secondary structure elements



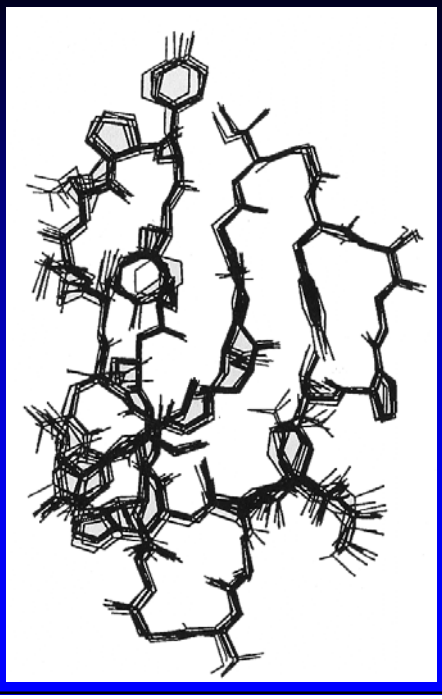


Two-dimensional structure from distance information

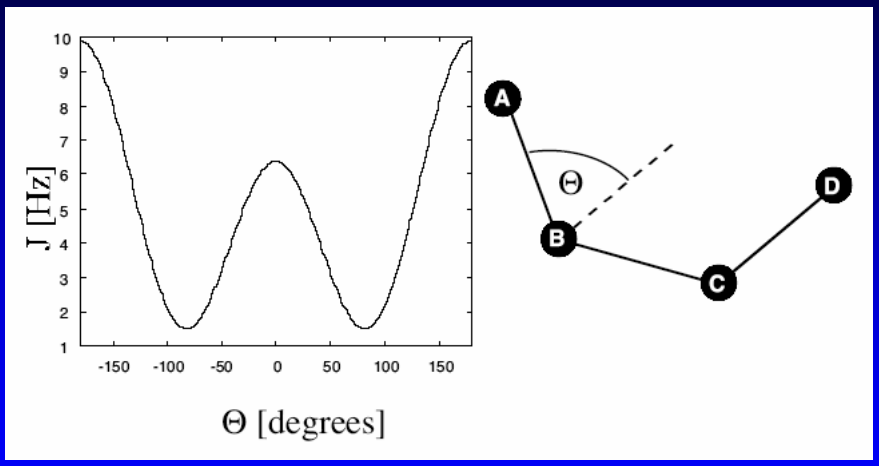
Basel - Bern	93
Basel - Zürich	98
Zürich - Bern	102
Genf - Bern	173
Genf - Basel	212
Genf - Lausanne	99
…	



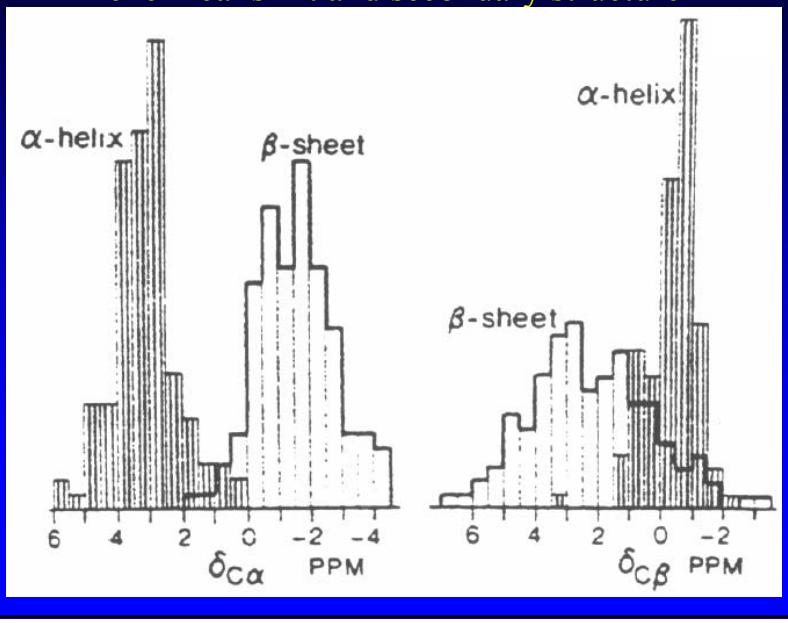
Example of a set of 10 calculated structures based on NOESY data.
All 10 structures are compatible with the determined distances constraints.



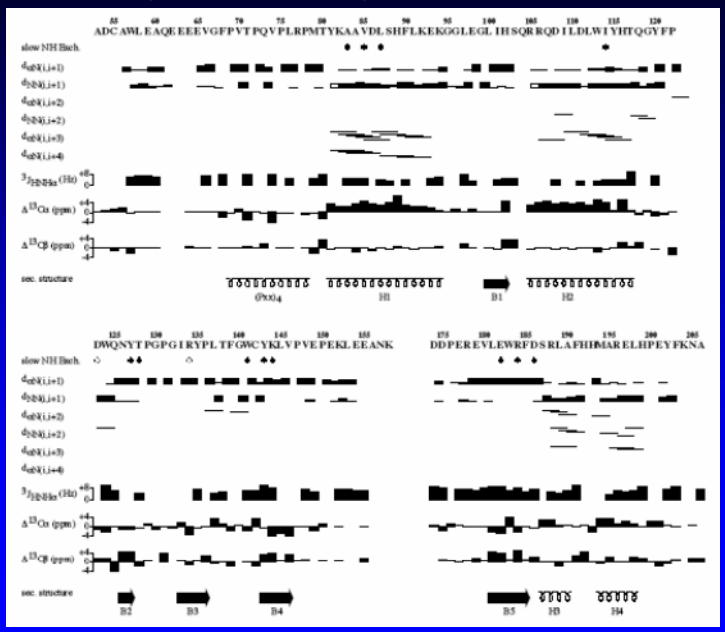
Karplus relationship between $^3J_{HN\alpha}$ and Θ



Relation between deviation from random coil chemical shift and secondary structure

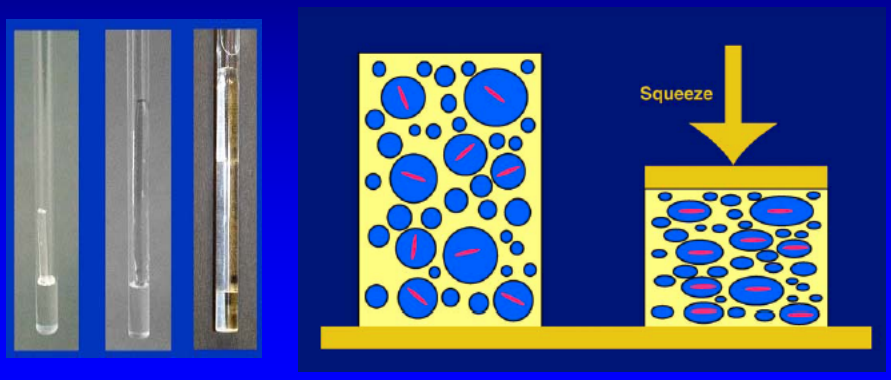


Summary on Secondary Structure Information



Residual Dipolar Couplings

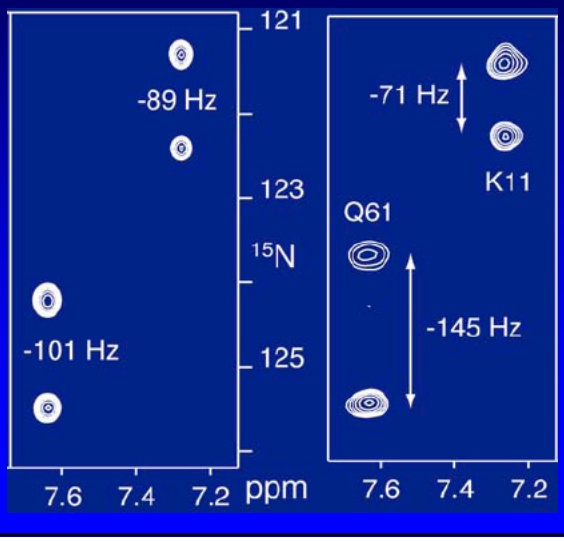
Partial orientation in a gel



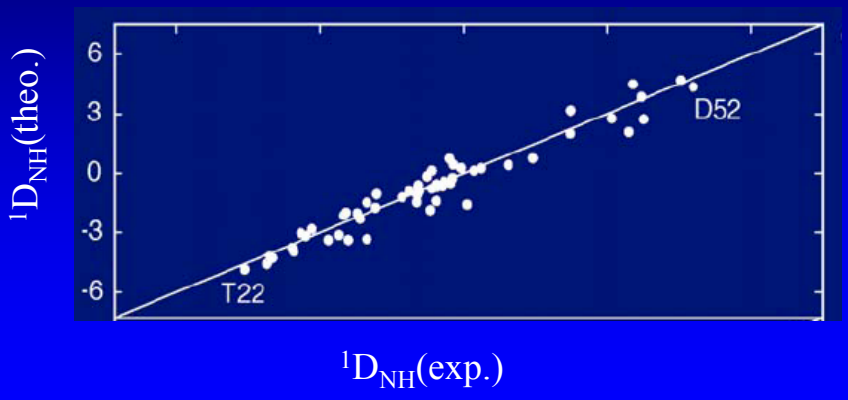
Residual Dipolar Couplings

Ubiquitin in acrylate/acrylamide gel

Coupled ^{1}H-^{15}N COSY-spectra



Residual Dipolar Couplings
Ubiquitin in acrylamide gel
comparison experimental/calculated $^1D_{NH}$
(improving an existing structure)



Information used for structure calculation:

- Distance restraints (NOESY)
- Torsion angles ($^3J_{HN\alpha}$ from HNHA)
- Chemical shifts (COSY-type experiments)
- Hydrogen bonds
- RDCs

Two different approaches:

(which can be combined)

- Distance geometry (DG)

  converts a set of distances constraints into cartesian coordinates which are optimized using trial values

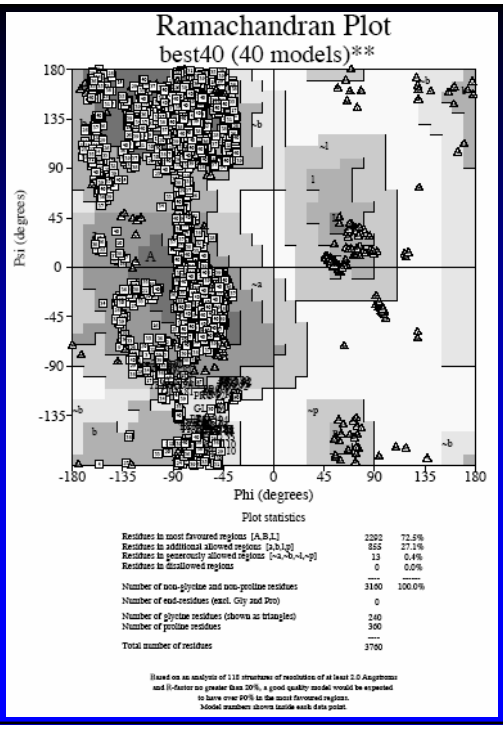
- Simulated annealing (SA)

  protein is “heated” to 2000 K to sample the entire conformational space; then T is lowered, while NOE energy terms are increased

HIV-1 Nef

Quality control for NMR structures:

- number of restraints per residue
  < 7 low resolution
  > 16 high resolution
- Ramachandran plot analysis
- rmsd between individual structures of a bundle
- Q-factor for RDCs



Problems with NOE accuracy

- Spin diffusion

  larger mixing times can not be described as a two spin problem -> simulation

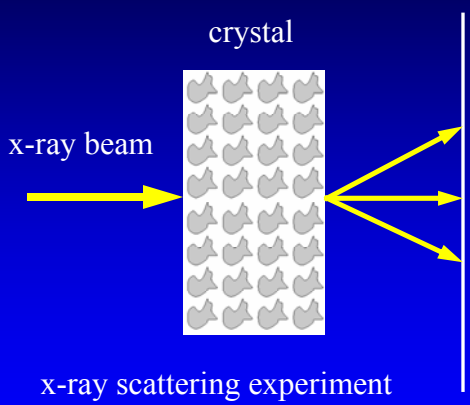
- Local motion (methyl rotation, ring flips etc.

  -> “model-free” S^2-parameter

Part 3: Beyond structure

Example: multidrug resistance:
thiostrepton induced protein A

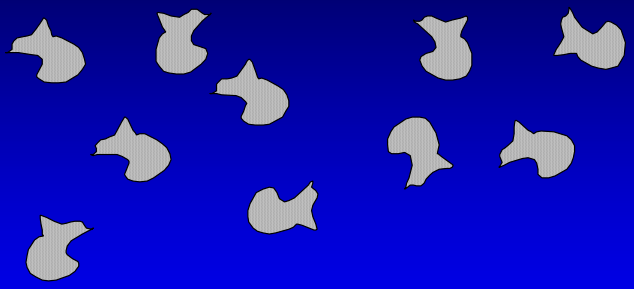
X-ray crystallography of biomacromolecules needs crystals



protein crystal

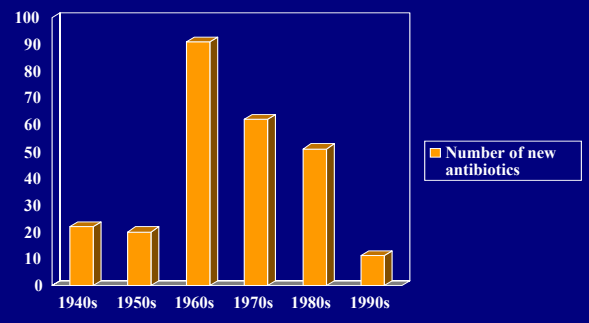
Photograph by P. Storici

Structure determination by high resolution NMR works in solution



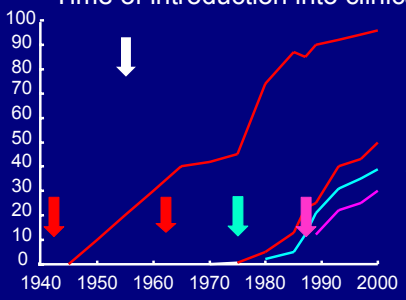
molecules in solution: ligand binding, dynamics etc.

Registrations of New Antibiotics



Emergence of Resistance

Time of introduction into clinical use



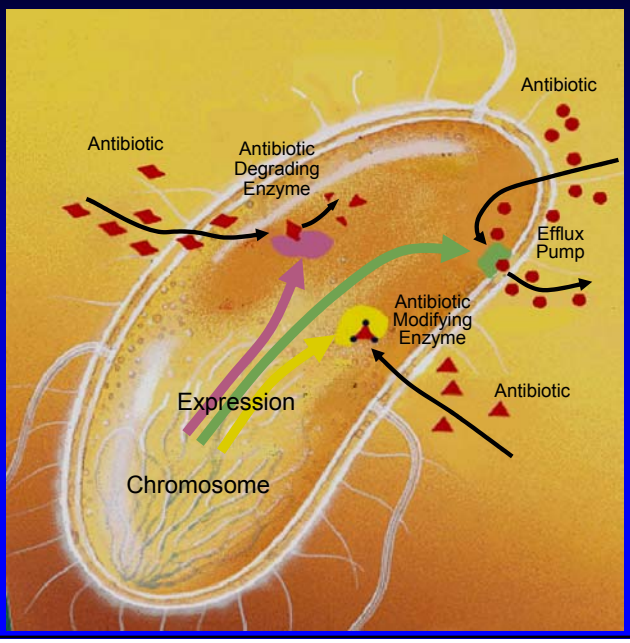
Penicillinase-producing staphylococci

Methicillin-resistant *S. aureus*

3rd gen. Cephalosporin resistant *E. cloacae*

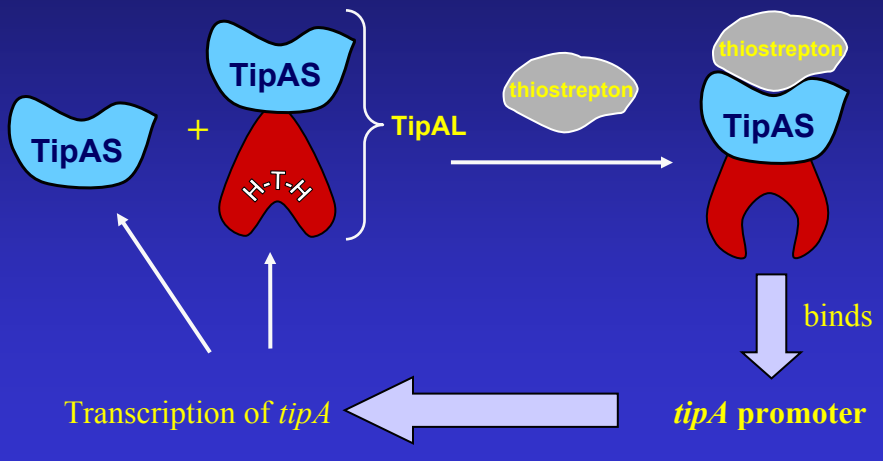
Ciprofloxacin-resistant *P. aeruginosa*

Mechanisms of bacterial antibiotic resistance



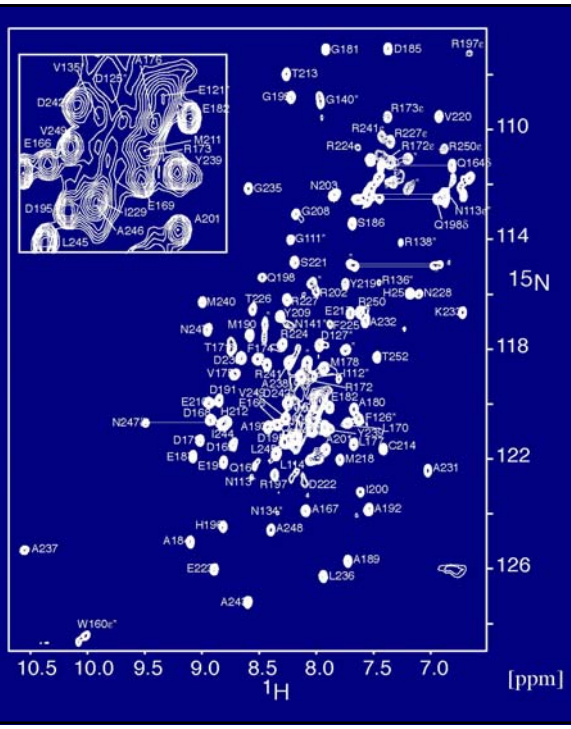
How do multidrug resistance proteins bind to different molecular shapes?

The TipA Multidrug Resistance Protein from *S. lividans* (C. Thompson)

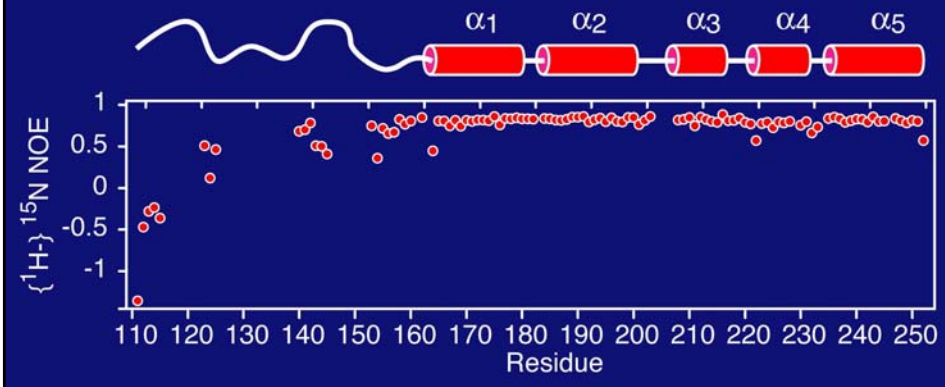


^{1}H-^{15}N-HSQC spectrum of free TipAS

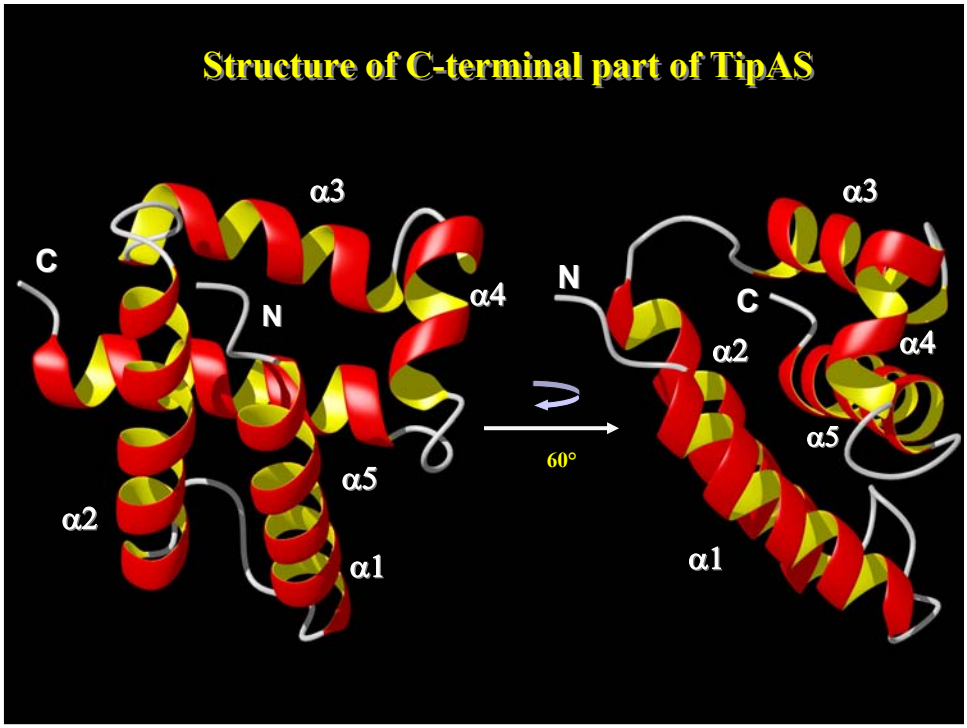
- NH resonances:
  - Expected 137
  - Observed 102
- smear in random coil region
- protein has unstructured parts



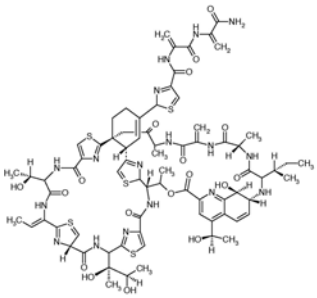
Flexibility of apo TipAS



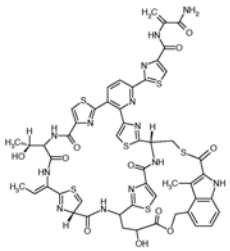
Structure of C-terminal part of TipAS



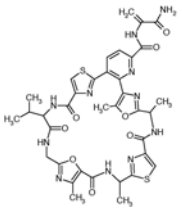
tipA inducing thiopeptide antibiotics



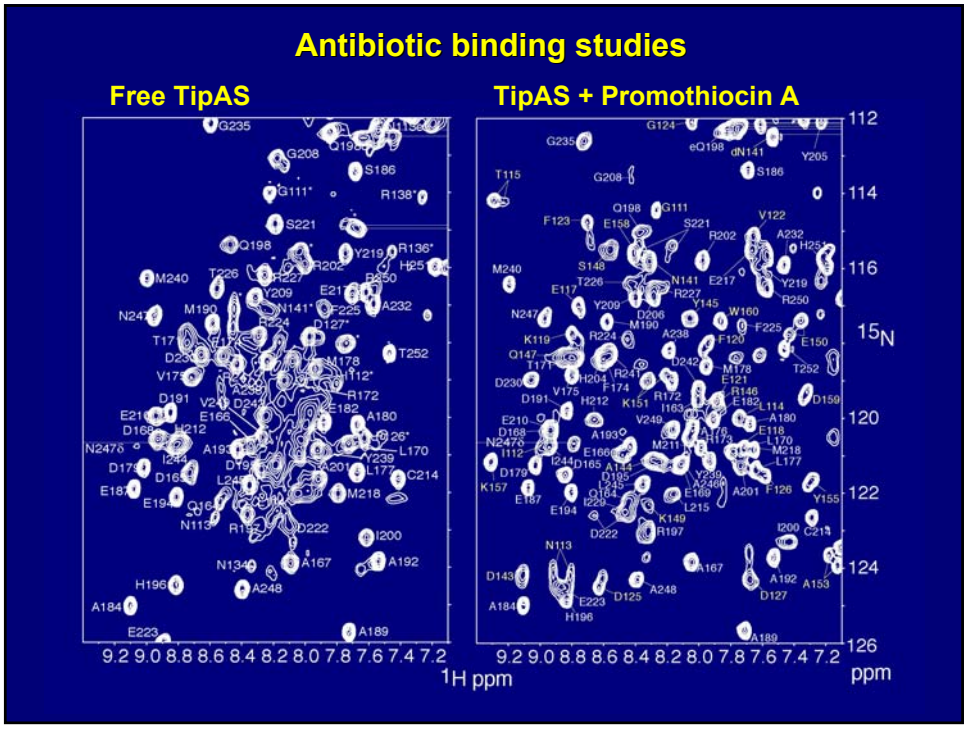
Thiostrepton

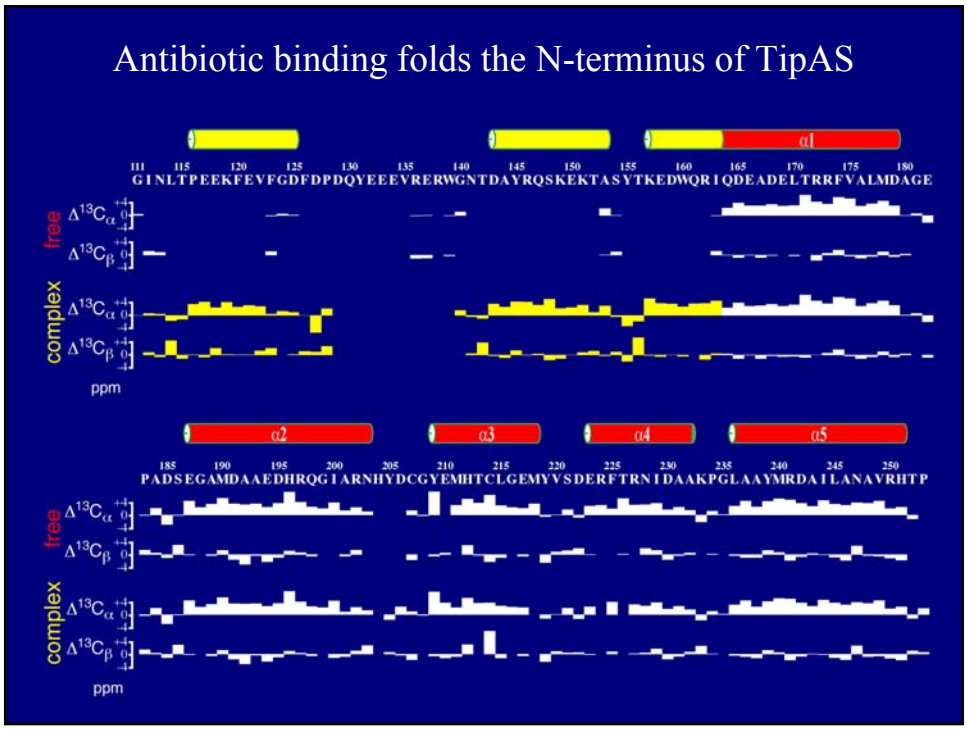


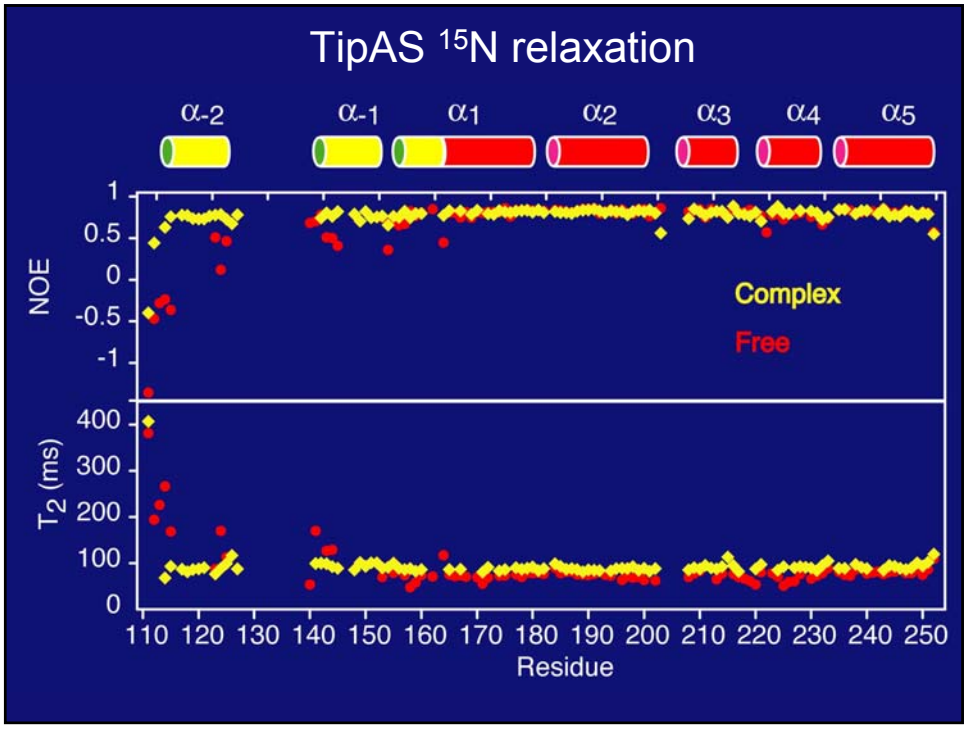
Nosiheptide

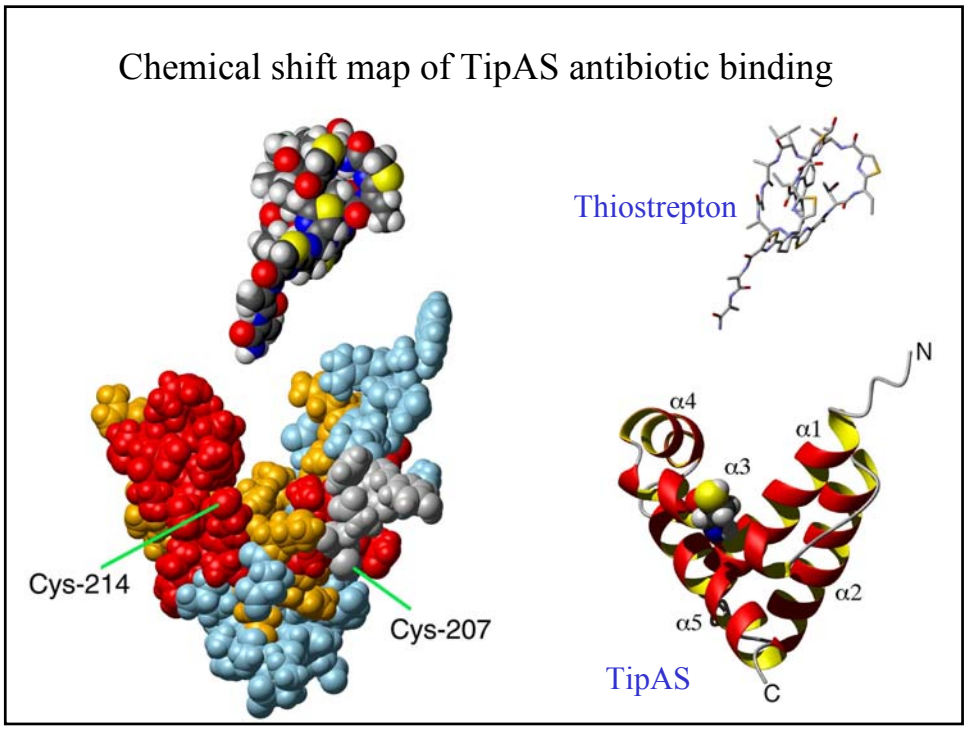


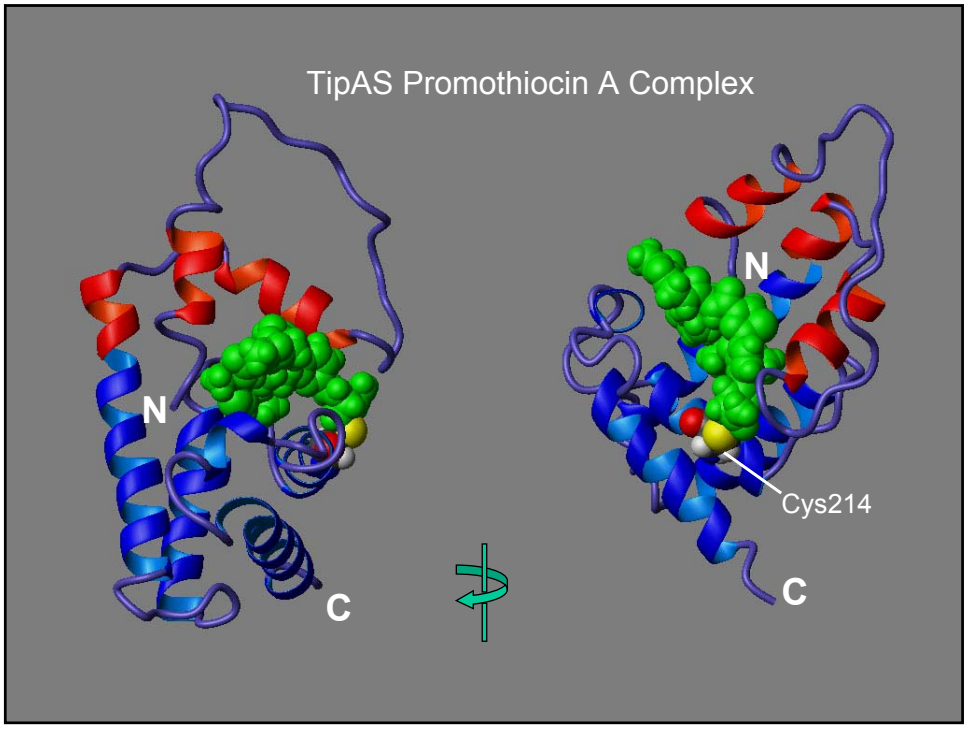
Promothiocin A


Antibiotic binding studies
Free TipAS
TipAS + Promothiocin A
15N
1H ppm
ppm

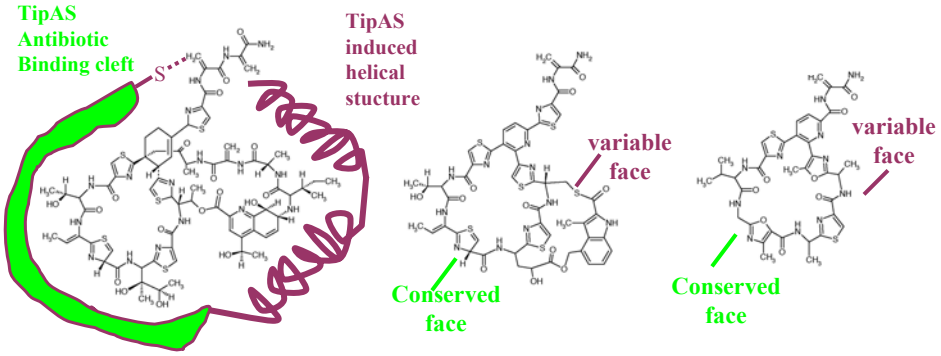

Antibiotic binding folds the N-terminus of TipAS
α1
α2
α3
α4
α5
free
complex
ppm


TipAS 15N relaxation
α-2
α-1
α1
α2
α3
α4
α5
NOE
1
0.5
0
-0.5
-1
Complex
Free
T2 (ms)
400
300
200
100
0
110 120 130 140 150 160 170 180 190 200 210 220 230 240 250
Residue


Chemical shift map of TipAS antibiotic binding
Cys-214
Cys-207
Thiostrepton
N
α4
α1
α3
α2
α5
C
TipAS



The flexible N-terminus of TipAS can recognize a variety of antibiotics

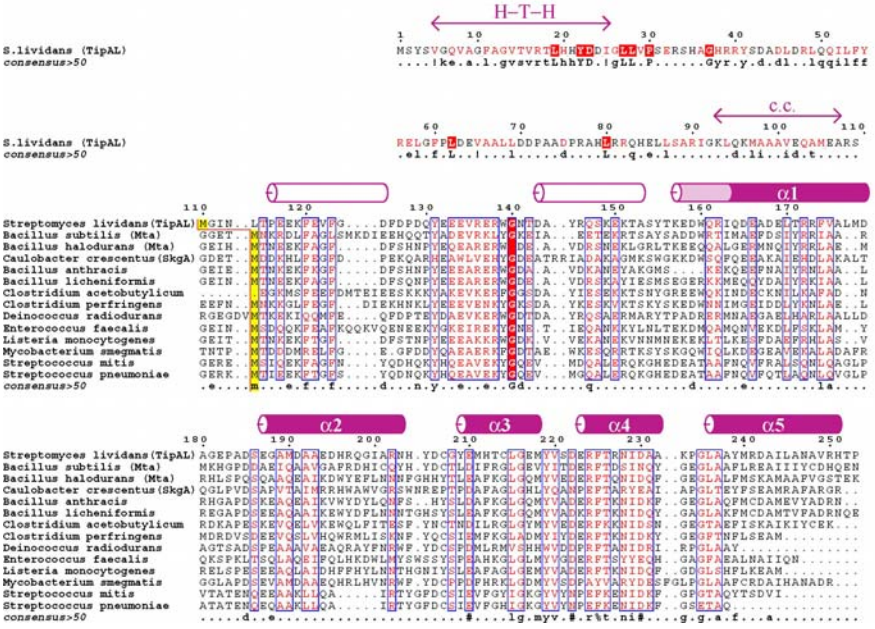


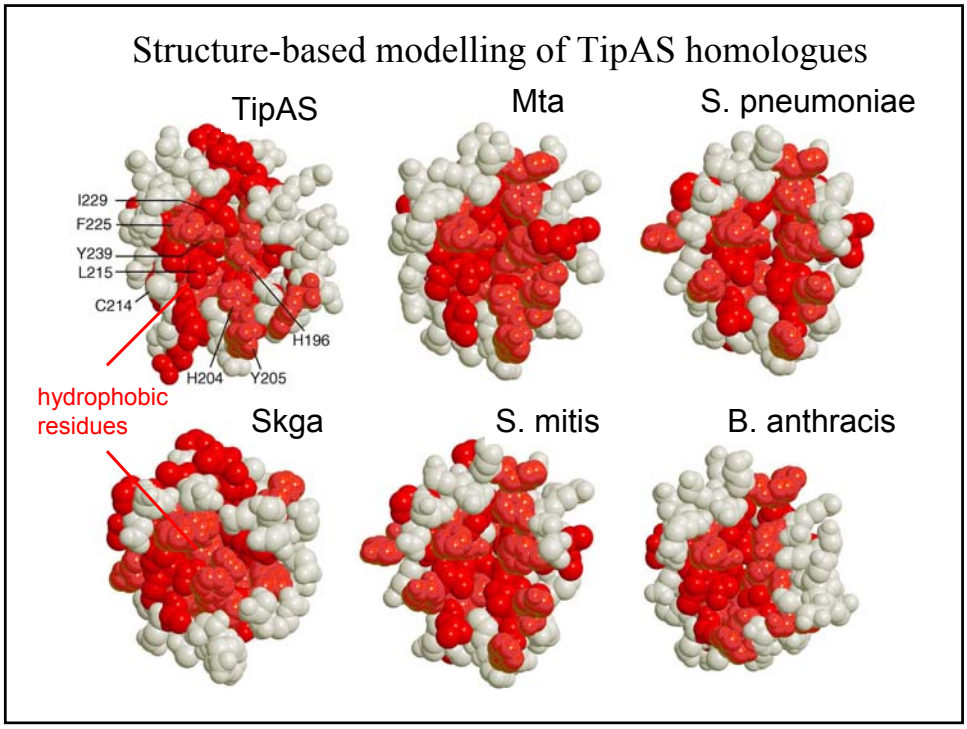
Thiostrepton **Nosiheptide** **Promothiocin A**

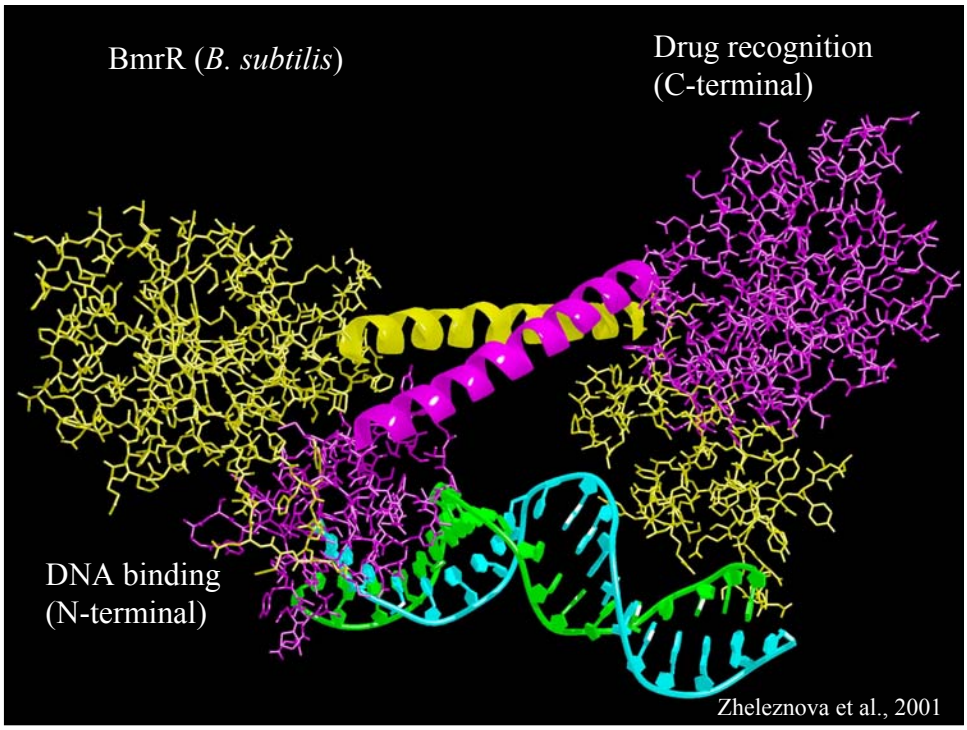
Dyson, H.J. and Wright, P.E. (2002) Coupling of folding and binding for unstructured proteins. *Curr Opin Struct Biol*, **12**, 54-60.

Increasing the capture radius by unfolding

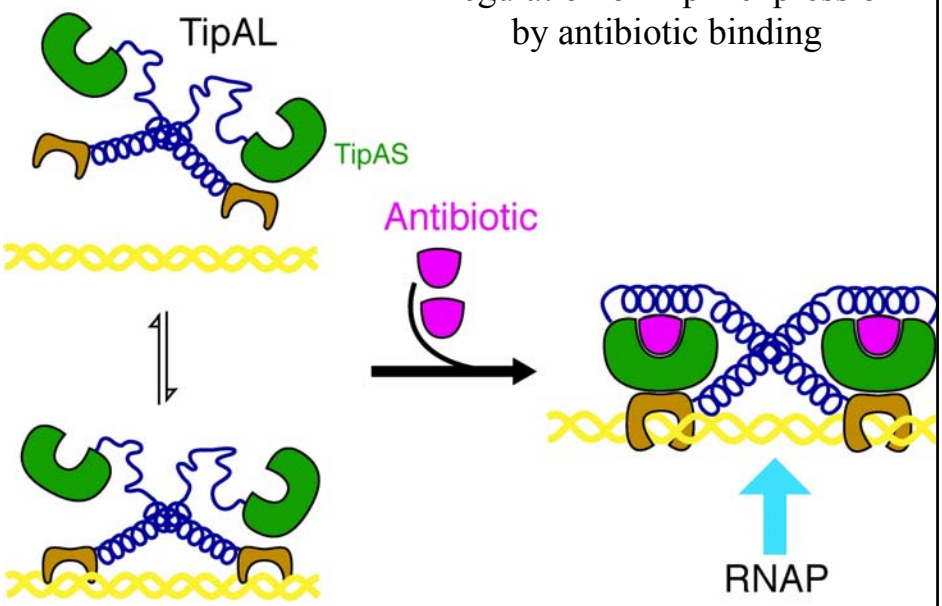
Sequence alignment of TipA




Structure-based modelling of TipAS homologues
TipAS
Mta
S. pneumoniae
I229
F225
Y239
L215
C214
H196
H204
Y205
hydrophobic residues
Skga
S. mitis
B. anthracis


BmrR (*B. subtilis*)
Drug recognition (C-terminal)
DNA binding (N-terminal)
Zheleznova et al., 2001

Regulation of TipA expression by antibiotic binding



1.4 Suggested Reading

1.4.1 Introductions:

1. Henry Rattle, An NMR Primer for Life Scientists, Partnership Press, Fareham Hants, 1995. Good introductory book with special focus on applications in life sciences.
2. Andrew E. Derome, Modern NMR Techniques for Chemistry research, Pergamon Press, Oxford, 1987. Good introductory book describing all the details for 1- and 2-dimensional, homonuclear NMR + general ideas. No mathematical effort.
3. Robin K. Harris, Nuclear magnetic resonance spectroscopy, Longman, Essex, 1983, reprinted 1997. Very thorough and very detailed introductory and (!) reference book.
4. Kessler, H., Gehrke, M., Griesinger, C. 1988. Angewandte Chemie. Int. Ed. Engl. 27, 490-536. Journal article describing the product operator formalism
5. Cavanagh, W. J. Fairbrother, A.G. Palmer, N.J. Skelton, Protein NMR Spectroscopy, Academic Press, San Diego, 1996. Good introductory book for the modern, homonuclear and heteronuclear protein NMR techniques. Some mathematical effort.
6. Clore and A.M. Gronenborn (Eds.), "NMR of Proteins", CRC Press, Boca Raton, 1993. Collection of scientific texts from different laboratories doing modern NMR. Good overview of modern protein applications.

1.4.2 Further Reading:

1. Wuthrich, NMR of Proteins and Nucleic Acids, Wiley, New York, 1986. Classical text on the homonuclear method of structure determination. Many specific details for proteins and nucleic acids.
2. Ernst, G. Bodenhausen, A. Wokaun, "Principles of Nuclear Magnetic Resonance in One and Two Dimensions", Clarendon Press, Oxford, 1987. Classical book on product oparators and Fourier transform spectroscopy. Very mathematical.
3. Abragam, A., "Principles of Nuclear Magnetism", Clarendon Press, Oxford, 1961. Classical book on NMR, if you really want a deep understanding. Very mathematical.

Acknowledgements:

S. Grzesiek (NMR-teaching and slides)
J. Kahmann and M. Allan (TipA)