

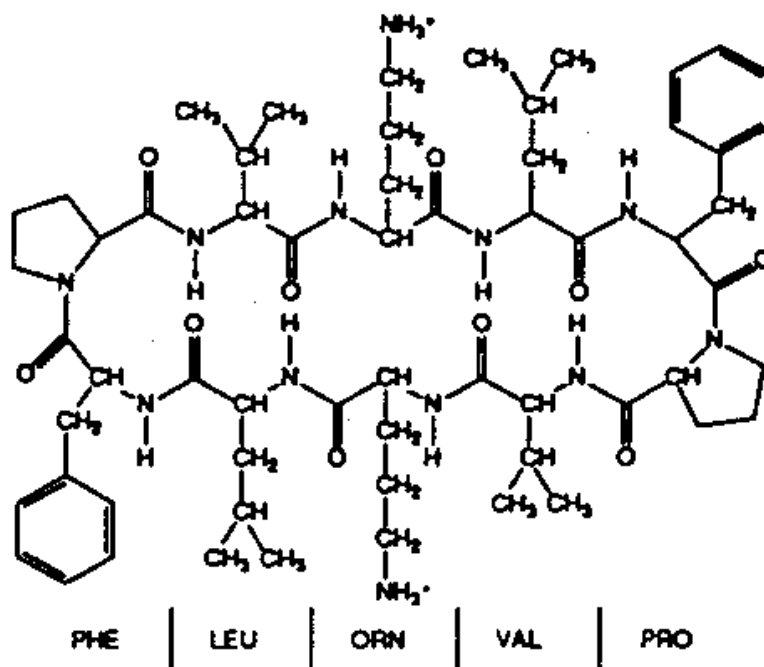
Laboratory for CHEM 781

Experiment 7: Conformational analysis using NOESY and P.E. COSY

20 points due date: Dec. 10 2008

Besides information about the connectivity of the atoms through bonds (experiment 5), NMR can provide information about the relative spatial orientation of the atoms with respect to each other, i. e. configuration and conformation. While a full three dimensional determination of the three dimensional structure can be obtained, this is beyond the scope of this lab. In this experiment, distance information derived from a NOESY spectrum and coupling constants derived from a P.E. COSY spectrum will be used to obtain stereo chemical assignments on methylene protons and determine elements of secondary structure. Also, a ROESY spectrum will be used to identify exchange peaks.

Sample: Gramicidin-S in DMSO-d₆



Gramicidin-S, 16

Assignment of proton and carbon resonances:

The assignment of the protons and carbons is shown below:

¹H NMR:

PRO_1:HA	4.311
PRO_1:HB	1.471
PRO_1:HB'	1.960
PRO_1:HG*	1.510
PRO_1:HD	3.591
PRO:HD'	2.500
VAL_2:HN	7.221
VAL_2:HA	4.412
VAL_2:HB	2.080
VAL_2:HG1*	0.768
VAL_2:HG2*	0.811
ORN+_3:HN	8.650
ORN+_3:HA	4.767
ORN+_3:HD	2.818
ORN+_3:HB	1.744
ORN+_3:HB'	1.615
ORN+_3:HG*	1.645
ORN+_3:HD'	2.818
LEU_4:HN	8.321
LEU_4:HA	4.577
LEU_4:HB*	1.331
LEU_4:HG	1.400
LEU_4:HD1*	0.790
LEU_4:HD2*	0.804
PHE_5:HN	9.114
PHE_5:HA	4.361
PHE_5:HB	2.967
PHE_5:HB'	2.880

¹³C NMR:

PRO_1:CA	60.286
PRO_1:CB	29.388
PRO_1:CG	23.580
PRO_1:CD	46.428
VAL_2:CA	57.142
VAL_2:CB	31.520
VAL_2:CG2	19.398
VAL_2:CG1	18.445
ORN+_3:CA	51.378
ORN+_3:CB	30.021
ORN+_3:CG	23.531
ORN+_3:CD	38.80
LEU_4:CA	50.003
LEU_4:CB	41.400
LEU_4:CG	24.423
LEU_4:CD*	23.158
PHE_5:CA	54.309
PHE_5:CB	36.075

Spectra on workstation:

<i>disk</i>	<i>user</i>	<i>Name</i>	<i>expno</i>	<i>Experiment</i>
u	labx	expt7	1	1D proton spectrum (500 MHz)
u	labx	expt7	2	1D carbon spectrum (125 MHz)
u	labx	expt7	3	2D P.E. COSY spectrum (500 MHz)
u	labx	expt7	4	2D NOESY spectrum ($\tau = 100$ ms) (300 MHz)
u	labx	expt7	5	2D ROESY spectrum ($\tau = 400$ ms) (300 MHz)
u	labx	expt7	6	2D HMBC spectrum (500 MHz)

Tasks:

- 1) **Processing and phasing of phase sensitive spectra:** Both the P.E. COSY, NOESY and ROESY spectra are taken in the phase sensitive mode and thus need manual phasing. Process and phase the two dimensional spectra such that the cross peaks appear as anti phase multiplets in the P.E. COSY, and in phase in the NOESY and ROESY. Spectra. For the COSY experiments, select H^N - H^α crosspeaks on both sides of the diagonal for the manual phase correction as they give a simple pattern. Phase them as anti-phase doublets. For the NOESY and ROESY spectra, select diagonal peaks and phase them positive.
- 2) **H^N - H^α coupling constants** Use the 1D and P.E. COSY spectra to measure the ${}^3J_{HNH^\alpha}$ coupling constants. Where do the measured values differ and why (and which ones are more reliable). Does the P.E. COSY offer any advantage over a DQF COSY in this case ?
- 3) **Phe- H^α - H^β and Phe- H^β -CO coupling constants:** From the $H^\alpha H^\beta$ cross peaks in the P.E. COSY spectrum determine the $J_{H^\alpha H^\beta}$ coupling constants for the Phenylalanine residue. What is the relative sign of ${}^2J_{H^\beta 1H^\beta 2}$ and ${}^3J_{H^\alpha H^\beta}$ and how can it be obtained from the spectrum ?
From the HMBC, compare the relative intensities of the $H^{\beta 2}$ -CO and $H^{\beta 1}$ -CO cross peaks. How is that intensity related to the respective coupling constant ?
- 4) **Interresidual NOE's:** NOE's and From the NOESY spectrum, identify $H^N H^\alpha$ and $H^N H^N$

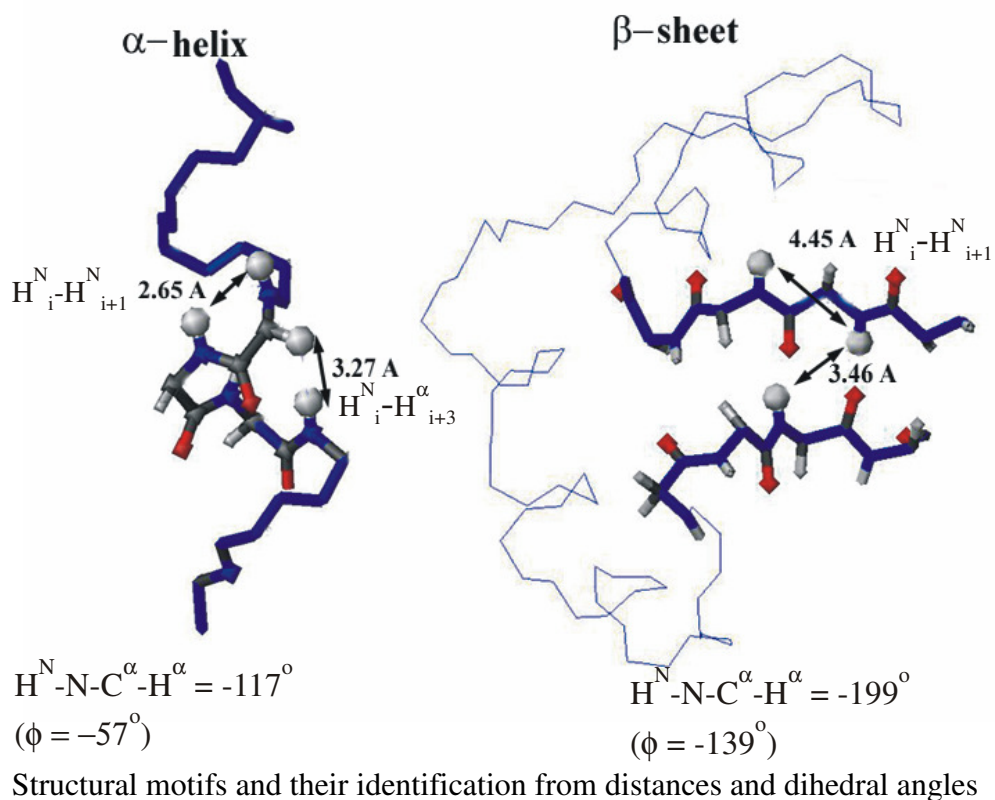
cross peaks between *different* amino acids. Which ones are between neighboring amino acids and which are most likely across the peptide ring ?

- 5) **Backbone structure:** From the NOE patterns and H^N-H^α coupling constants, which of the two structural motifs listed in the appendix is consistent with the data ?
- 6) **Phenylalanine side chain conformation:** For the Phenylalanine residue, use coupling, NOE information and H^β/CO HMBC crosspeak intensities determine the $N-C_\alpha-C_\beta-C_\gamma$ dihedral angle (χ^1) according to the possible conformations listed in the appendix. Obtain stereospecific assignment of diastereotopic Phe- H^β protons (HB2 vs. HB3).
- 7) **NOE vs. exchange:** The ROESY experiment will always show crosspeaks due to NOE (more accurate: ROE) with the sign opposite to diagonal, whereas crosspeaks due to exchange will always have the same sign as the diagonal. Comparing the NOESY and ROESY spectra, which cross peak(s) is/are clearly arising from exchange and not NOE (ROE)? Is the tumbling rate of gramicidin in DMSO larger or smaller than the Larmor frequency of the protons ?

APPENDIX:

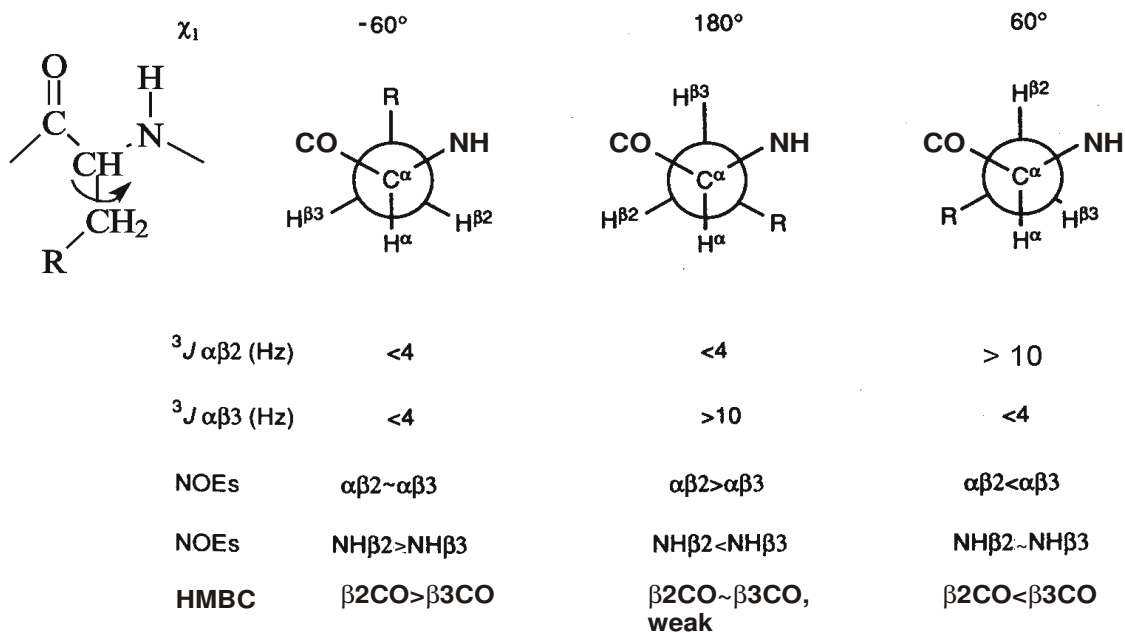
Determination of backbone conformation from NMR data

Two common structural motifs in proteins are α -helices and β -sheets. They both show very distinct NOE patterns and coupling constants. In an α -helix, usually strong sequential $H^N_i-H^N_{i+1}$ and $H^N_i-H^\alpha_{i+3}$ NOE's across a helix turn are observed. In β -sheets N-H protons are arranged in an *anti*-fashion and sequential NOE's are weak, but H^N-H^N and H^N-H^α NOE's across the β -sheet are observed. Coupling constants can give information about dihedral angles according to the Karplus equation, with the coupling exhibiting a maximum value for dihedral angles of 0° and 180° , and a minimum at 90° . Thus the H^N-H^α coupling constant is also a measure of backbone conformation given by the ${}_{C_{i-1}}N_i-C^\alpha_i-C_{i+1}$ dihedral angle (ϕ).



Determination of side chain angle from NMR data

A combination of NMR information is needed to determine the conformation of the $\text{HN-C}^\alpha\text{-C}^\beta\text{-C}$ dihedral angle χ_1 . The $\text{H}^\alpha\text{-H}^\beta$ NOE's are correlated with the coupling constant and do not give additional information. Thus $\text{H}^\text{N}\text{-H}^\beta$ NOE's and $\text{H}^\beta\text{-CO}$ coupling information has to be used in addition:



Determination of side chain N-C-C-C dihedral angle χ_1 and stereo-specific assignment of H^β protons from NOE and coupling information.

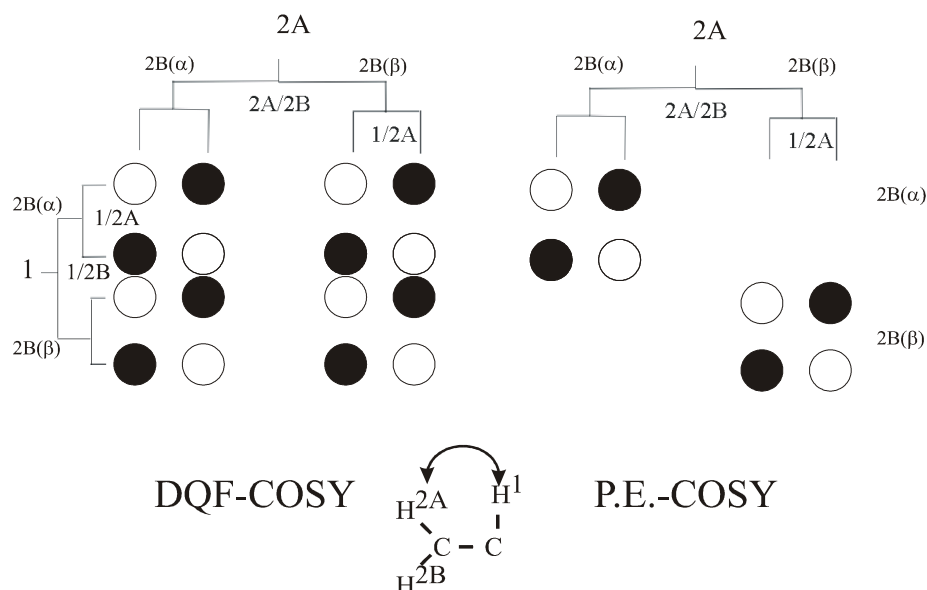
DQF-COSY versus P.E. COSY

For more complex molecules, the extraction of coupling constants from one dimensional spectra becomes very tedious or impossible. The regular COSY experiment, on the other hand, has the diagonal peaks 90° out of phase with respect to the cross peaks.

In the DQF-COSY the dispersive diagonal is suppressed by applying a double quantum filter, retaining only cross peaks and in-phase components of the diagonal. All singlet signals will be suppressed. The cross peaks will be split into multiplets in both dimensions, with the active coupling giving rise to antiphase (+ -) splittings and passive couplings resulting causing in phase splittings (+ + or + -).

As shown in the figure below, even for just three spins one can observe up to 16 peaks, and four spins would result in 64 signals. If some coupling constants become similar in magnitude, overlap of positive and negative signals can result in partial cancellation of multiplet components.

The P.E. COSY (Primitive Enhanced COSY) allows to simplify the multiplet pattern by replacing the second 90° pulse in a regular COSY by a small pulse angle, typically a 36° pulse. This retains only signals in the cross peaks between lines with the passive spin in the same spin state. In the example below lines of spin 2A coupled to 2B in the β state correlate only with lines of 1 coupled to 2B in the β state, and 2A coupled to 2B in the α state correlates only with 1 coupled to 2B in α . This allows often to measure even small coupling constants without overlap.



Schematic DQF-COSY and P.E. COSY cross peaks for a three spin system with $|^2J_{2A2B}| > ^3J_{1,2B} > ^3J_{1,2A}$ and $^2J_{2A2B} < 0$.