

Solution NMR: small molecules and HSQC/Titration

AUTUMN SCHOOL
FMP 2010

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AG Solution NMR

www.fmp-berlin.de/schmieder/teaching/autumn_school.htm

The program

2/22

practical demonstration:

how to measure a sample

perform a titration of a protein with a ligand

measure spectra for the assignment of a small molecule

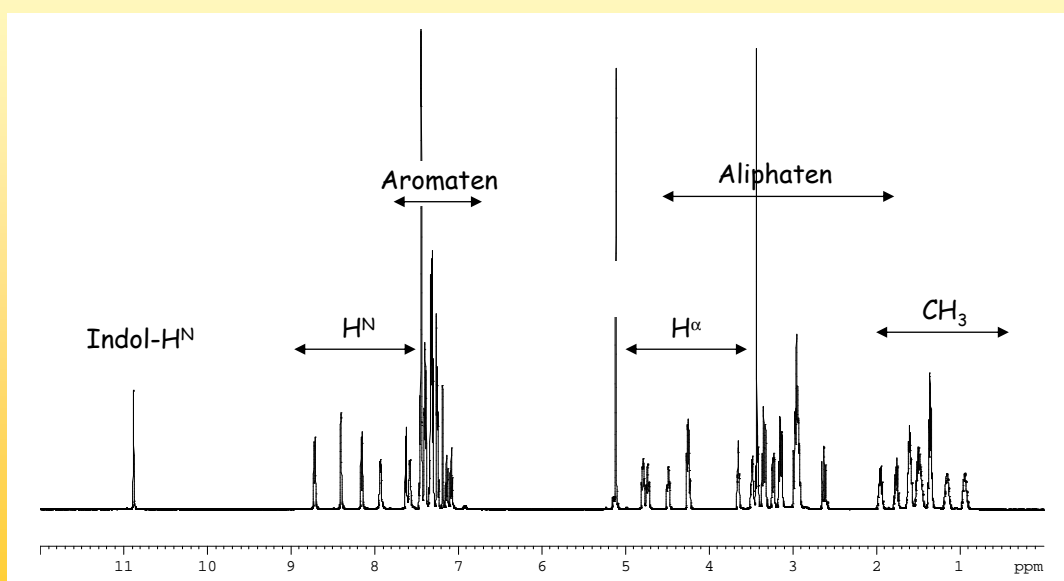
NMR exercise:

assignment of a small molecule

important NMR experiments

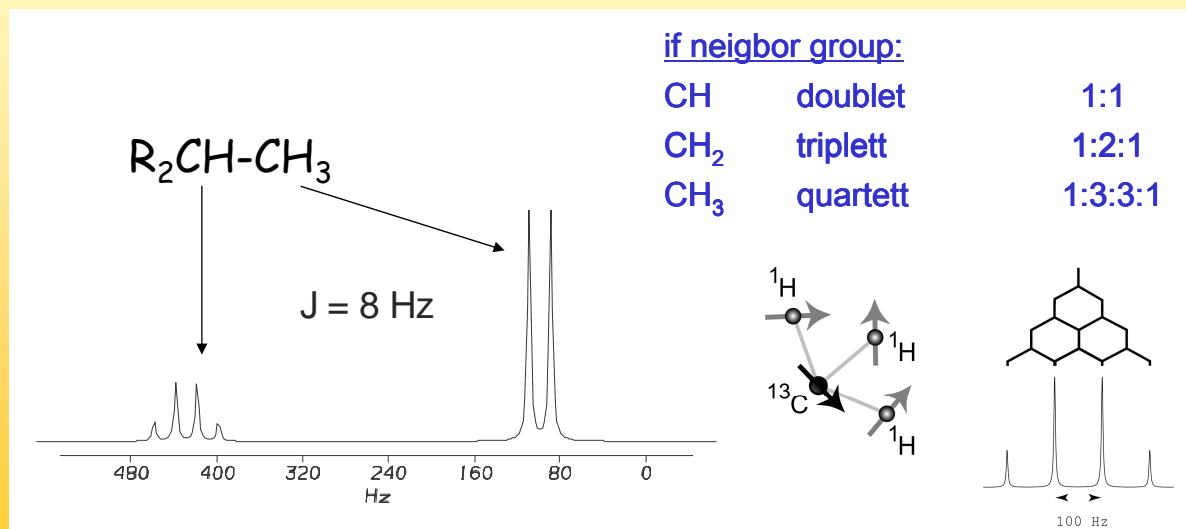
important NMR experiments

In a 1D protons appear in different chemical shift regions depending on their chemical environment.



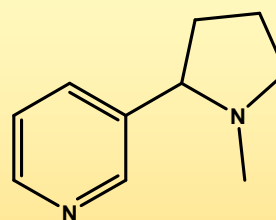
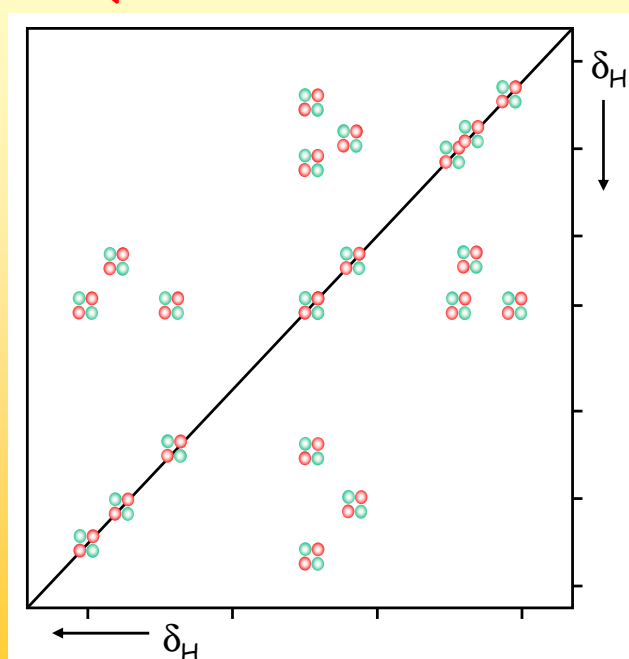
important NMR experiments

The peak integrals of a 1D correspond to the number of protons that cause the signal. The splitting pattern contains information about the number of proton neighbors.



important NMR experiments

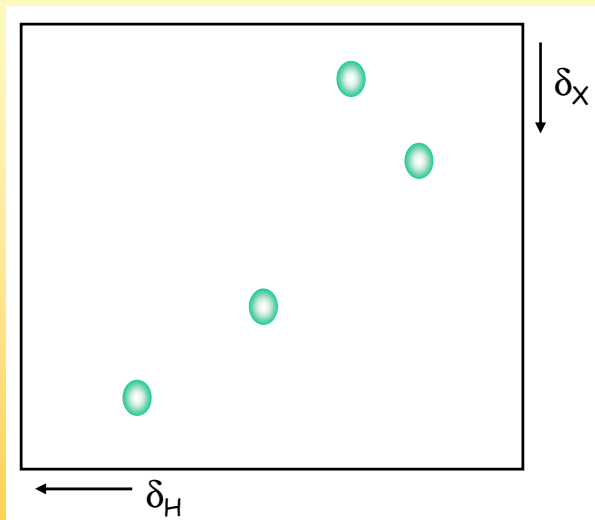
DQF-COSY



DQF-COSY accomplish a proton-proton transfer via not more than three bonds.

important NMR experiments

HMQC = Heteronuclear Multiple Quantum Correlation
or **HSQC** = Heteronuclear Single Quantum Correlation



A signal indicates a direct bond between the proton and the heteronucleus. Every proton gives rise to only one signal to the hetero nucleus to which it is bonded.

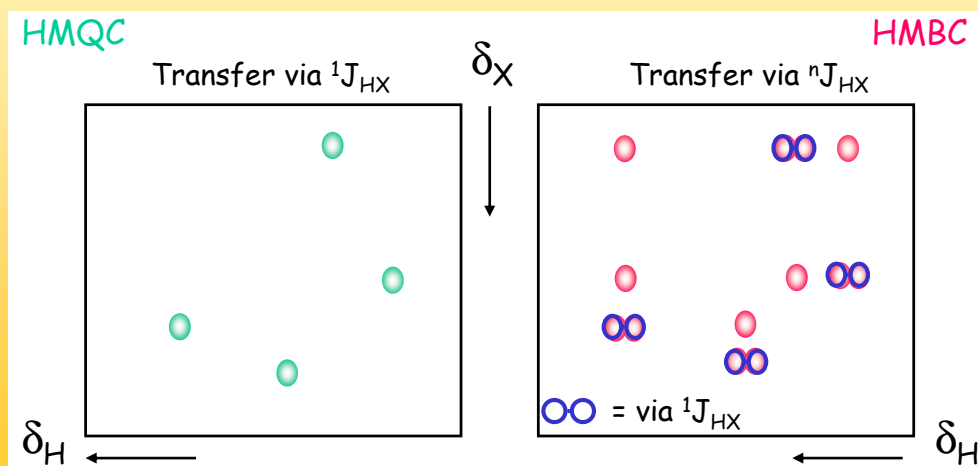
Can be measured as ^{13}C spectrum for carbon bound protons or as ^{15}N spectrum for nitrogen bound protons.

important NMR experiments

HMBC = Heteronuclear Multiple Bond Correlation

A signal indicates a correlation via two, three or four bonds between the proton and the heteronucleus.

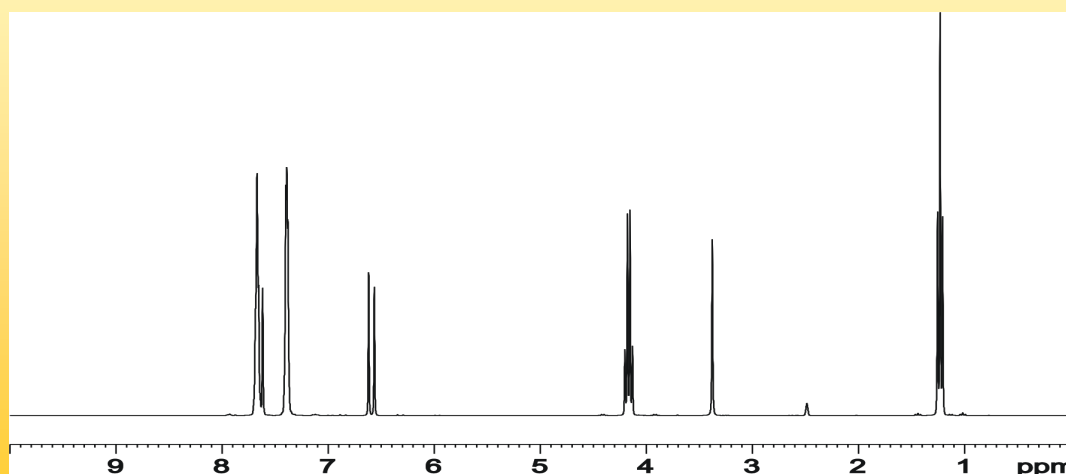
In contrast to the HMQC quaternary carbons are detected.



assignment procedure for small molecules

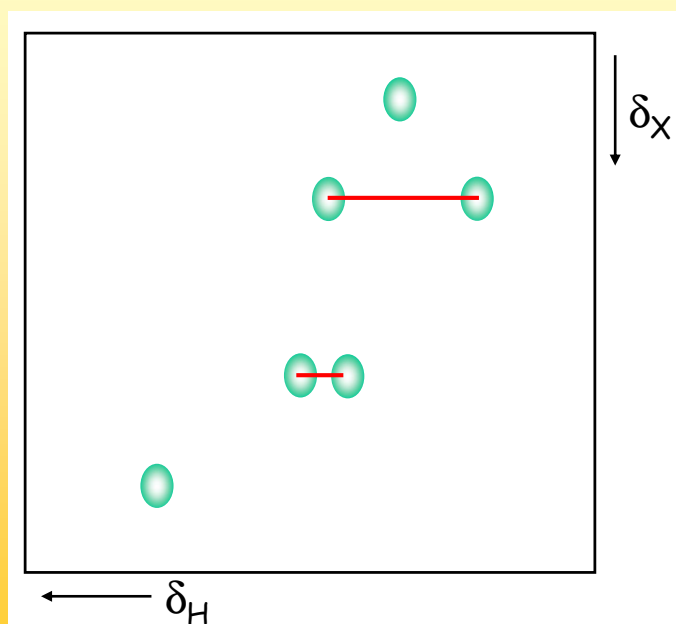
assignment procedure

First have a look at your 1D proton spectrum and your structure. Is the substance clean? Think about how many signals you should expect in the aliphatic and in the aromatic region and count your signals.



assignment procedure

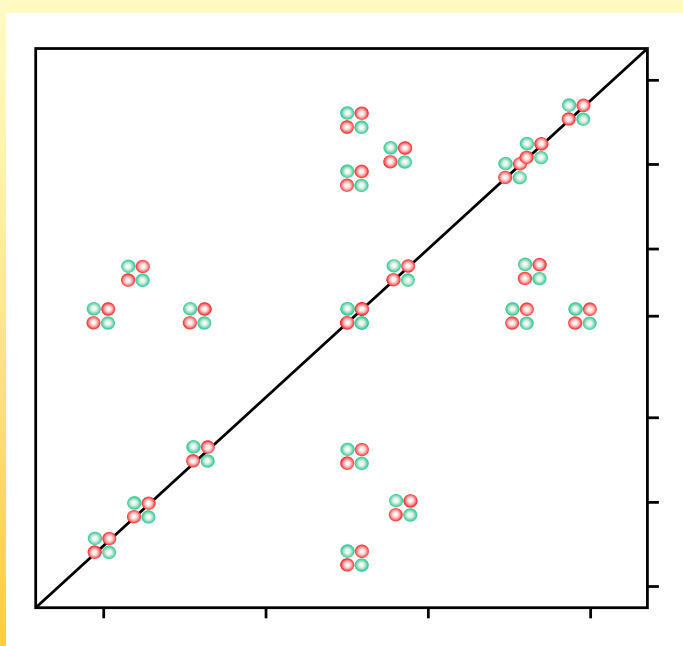
HMQC



If the DQF-COSY is relatively confusing, first have a look at the HMQC to establish which protons are attached to the same carbon atom. Carry this information to the 1D and the DQF-COSY.

assignment procedure

DQF-COSY



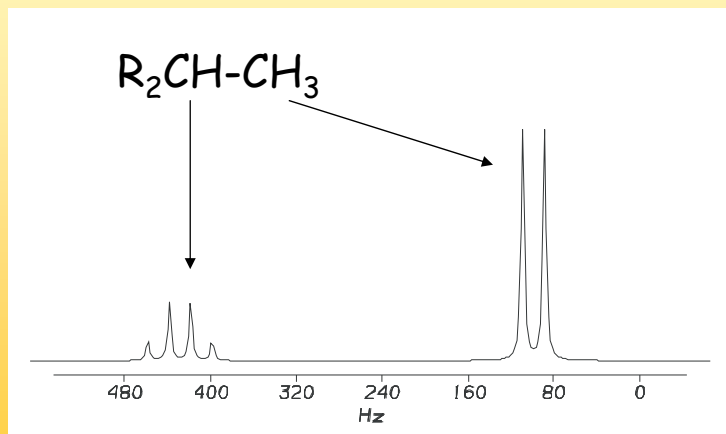
Then look at the DQF-COSY to find out which protons are neighbors (not more than 3 bounds between them).

The protons from your 1D can be found on the diagonal, neighbors are linked by cross peaks.

Try to assign protons.

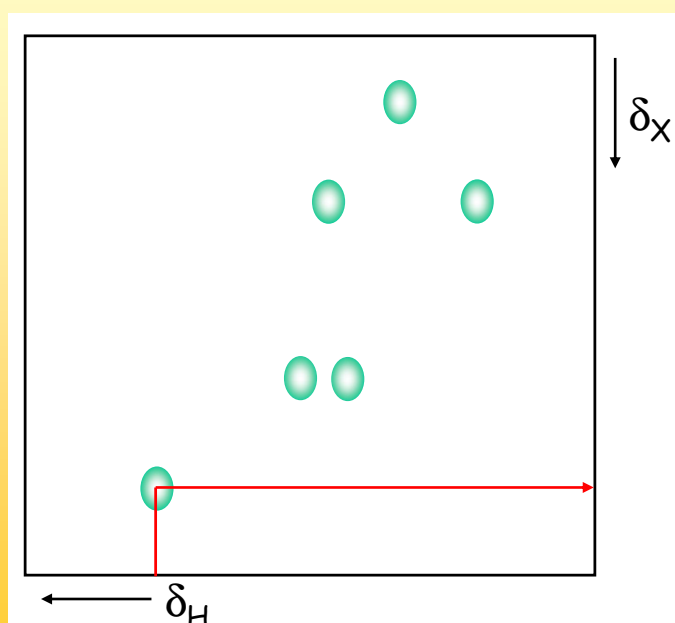
assignment procedure

The 1D can give you an additional idea of to the number of neighboring nuclei.



assignment procedure

HMQC

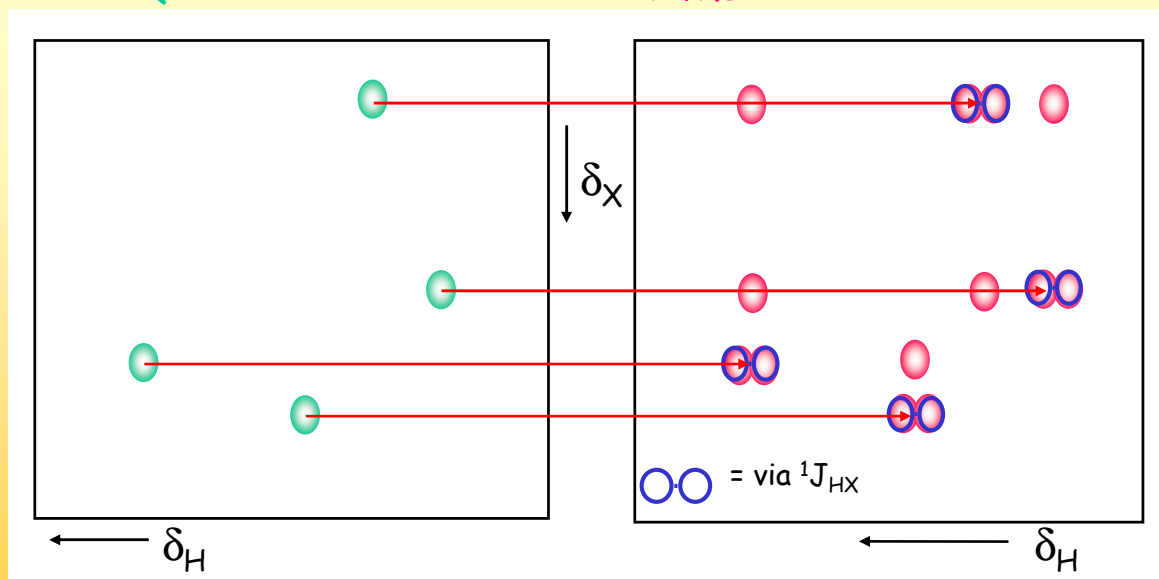


Go back to the HMQC.
Derive from your
proton chemical shifts
what the corresponding
 ^{13}C shifts are.

assignment procedure

HMQC

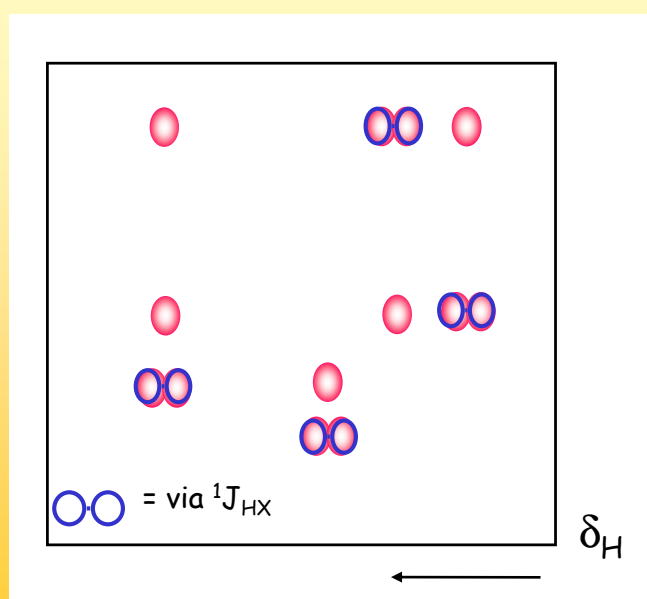
HMBC



Carry your proton and carbon chemical shifts from the HMQC to the HMBC. Maybe use an overlay of both.

assignment procedure

HMBC



With the HMBC you can confirm and complete your assignment. You can find correlations via more than 3 bonds and assign carbons with no protons attached.

Strongest peaks are normally caused by 3J couplings (3 bonds between carbon and proton).