Basic principles of multidimensional NMR in solution

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Peter Schmieder

AG Solution NMR
General aspects
Basic principles
Parameters in NMR spectroscopy
Multidimensional NMR-spectroscopy
Protein structures
NMR-spectra of proteins
Sequence specific assignment
Protein structure determination
Ligand-screening
General aspects of NMR-spectroscopy
Nuclear Magnetic Resonance

NMR-spectroscopy observes the resonance interaction of atomic nuclei with electromagnetic waves. The effect is only detectable in a strong magnetic field. Every atomic nucleus is observed separately and in addition interactions between nuclei can be visualized. NMR therefore corresponds well to the chemists view of a molecule as atoms connected by bonds.
General aspects of NMR spectroscopy

Analytical method accompanying synthetic work

[Chemical structure image]

Basic principles of multidimensional NMR spectroscopy

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Structure elucidation of natural compounds

NMR is very powerful in the determination of the constitution of natural products.
General aspects of NMR spectroscopy

Determination of the three-dimensional structure of proteins

NMR can help to determine the 3D structure of proteins at atomic resolution, in solution as well as in the solid state.
Determining of molecular interactions

NMR can be used to detect the interaction between proteins and ligands.
Basic principles of NMR-spectroscopy
Basic principles of NMR-spectroscopy

Basis of the effect of nuclear magnetic resonance is the nuclear spin, that can be imagined as a mixture of gyroscope and magnetic needle.
A gyroscope has an angular momentum whose axis is stable in three-dimensional space.
An alignment of the "magnetic needle" with an external magnetic field is prevented by the properties of a gyroscope, a precession begins.
The resonance frequency of the spins is determined by the magnetic field, as is the sensitivity and the resolution of the spectra.

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### Magnetic properties of relevant nuclei

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Parameters in NMR-spectroscopy
Chemical shift

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

$B_{\text{eff}} = (1 - \sigma) B_0$

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

$\delta = (\omega - \omega_{\text{ref}}) / \omega_0 \times 10^6$

$= (\sigma_{\text{ref}} - \sigma) \times 10^6$
Each atom in the molecule gives rise to a resonance line
The chemical shift depends on the chemical environment.
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 coupling splits the signals according to the number of neighboring nuclei.
Scalar coupling contains structural information

Karplus-equation

\[ ^3J_{HN\alpha} = 6.4 \cos^2 \phi - 1.4 \cos \phi + 1.9 \]
Dipolar coupling

The nuclei interact directly through space via a dipol-dipol 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
One aspect of relaxation is the NOE-Effect, that depends on the distance between two nuclei.

\[ I_{NOE} \sim \frac{1}{r^6} \]

Since the intensity drops quickly with increasing distance the effect can only be observed up to 500 pm.
Multidimensional NMR-spectroscopy
Basic principles of multidimensional NMR spectroscopy

1D-NMR:
- 2 axis
- intensity vs. frequency

2D-NMR:
- 3 axis
- intensity vs. frequency (1)
  vs. frequency (2)
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.
Basic principles of multidimensional NMR spectroscopy

Transfer of magnetization takes place between like nuclei. Both axis 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

Transfer of magnetization takes place between nuclei of different types. The two axis 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.
Basic principles of multidimensional NMR spectroscopy

3D-NMR
Protein structures
Protein structures

Primary structure
20 proteinogenic amino acids
Protein structures

secondary structure

α-helix

β-sheet
Levels of structural organization

Protein structures
NMR-spectroscopy of proteins
The major problem of protein NMR results from the fact that proteins are polymers, i.e. the repetition of almost identical subunits.
NMR-spectroscopy of proteins

(-)-Menthol
cyclic hexapeptide

NMR-spectroscopy of proteins

Basic principles of multidimensional NMR spectroscopy

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$^1\text{H}$ NMR-spectrum of a protein
Differences in chemical shifts can be produced by structure and the accompanying anisotropy effect.

NMR-spectroscopy of proteins
NMR-spectroscopy of proteins

$^1$H NMR-spectrum of an unfolded protein
Sequence specific assignment
The solution of the assignment problem is the sequence-specific assignment.

Two strategies exist:
In case of small proteins or peptides where usually only unlabeled material is available the strategy is based on homonuclear spectra (COSY, TOCSY, NOESY).
In case of larger proteins labeling with $^{13}C$ and $^{15}N$ is necessary and heteronuclear triple resonance experiments (CBCA(CO)NNH, CBCANNH) are recorded.
Sequence-specific assignment

1. Which amino acid type is present (which color)
2. Which amino acid is next to which (neighborhood)
3. Comparison of subsequences with that of the protein
Assignment using homonuclear spectra:
Each amino acid represents a separate set of signals, a spin system, since amino acids are separated by the carbonyl carbon that does not have a proton attached. Homonuclear spectra that utilize scalar couplings (COSY, TOCSY) are used to establish the amino acid type.
The neighborhood of the amino acids are then detected by through space interactions, i.e. in NOESY spectra.

Inter- and intra-residue signals are separated by comparison between the scalar-coupling spectra that can only show intra-residual peaks and the NOESY
The distance from the $H^N$ to the $H^\alpha$ of the same amino acids, $d_{N\alpha}(i,i)$, is always short enough to yield an NOE. The same is true for the distance from the $H^N$ to the $H^\alpha$ of the amino acid (i-1), $d_{\alpha N}$.
A neighborhood of amino acids is thus established.
Triple resonance experiments use the couplings between $^1H$, $^{13}C$ und $^{15}N$. 
Sequence specific assignment

Mainchain assignment using tripel resonance experiments

CBCA(CO)NNH

CBCANNH
A structure determination using NMR-spectroscopy
A protein structure determination

Bioinformatics

Protein expression

Data acquisition

Structure calculation

Structurally relevant information

Resonance assignment

Basic principles of multidimensional NMR spectroscopy

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Bioinformatics (1)

A protein structure determination
A protein structure determination

Bioinformatics (2)

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| 1cku | 1cku | ASSOC_SITE | |
|------|------|-------------||
| 1bpd | 1bpd | ASSOC_SITE | |
| 2abk | 2abk | ASSOC_SITE | |

**Basic principles of multidimensional NMR spectroscopy**

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A protein structure determination

Protein expression and purification

(1) Database analysis

(2) Plasmid

(3) Escherichia coli

(4) Cell harvest

(5) Column

(6) SDS gel
A protein structure determination

600 MHz $^1$H 1D-Spektrum of the EphB2 SAM Domain

ppm  10  8  6  4  2  0
A protein structure determination

2D-NOESY (100 msec) of the EphB2 SAM Domain
With increasing size of the protein the interpretation of homonuclear spectra alone becomes increasingly difficult.

With the introduction of nitrogen and carbon labels this problem can be ameliorated because of the better resolution in the heteronuclear spectra and the option to record well resolved 3D spectra.
A protein structure determination

$^{15}$N-HSQC of the EphB2 SAM Domain
A protein structure determination

2D NOESY

3D NOESY

$^{15}$N=120.68 ppm

Basic principles of multidimensional NMR spectroscopy

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Mainchain assignment using triple resonance experiments

CBCA(CO)NNH

CBCANNH

F2 (ppm)
A protein structure determination

List of relevant experiments

$^{15}$N-HSQC, $^{13}$C-HSQC

$^{15}$N-NOESY-HSQC, $^{13}$C-NOESY-HSQC

CBCA(CO)NNH, CBCANH

HNCO, HN(CA)CO

HNCA, HN(CO)CA

(H)C(CO)NNH, H(CCO)NNH

$^{15}$N-relaxation time experiments
A protein structure determination

Structurally relevant information

distances

angles

Basic principles of multidimensional NMR spectroscopy

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Distances give information on elements of secondary structure
A protein structure determination

Structurally relevant information

![Graph showing structural information](image-url)
Distances determine the overall structure of the protein.

Few distances are enough to "fold up" the protein.
A protein structure determination

As a result a 3D structure can be calculated
Ligand-screening using NMR-spectroscopy
An increasingly important application of NMR-spectroscopy is the screening of compound libraries to identify new interaction partners for a given protein and subsequently lead structures.

There are two major types of approach, the "ligand-detecting techniques" and the "protein detecting techniques".
A technique of major importance from the class of protein-detecting techniques is called „SAR-by-NMR“

Starting point is a completely assigned two-dimensional HSQC spectra of the protein of interest
HSQC-spectra with and without the addition of a potential ligand are compared. A shift in the spectrum with ligand relative to the one without indicates an interaction.

The method can be used in a „high-throughput“ manner.
There are numerous techniques in the class of the "ligand detecting techniques". Two techniques of particular importance are WATERlogsy and STD-NMR.

The major principle is the alteration of certain properties of the ligand by the protein when bound.
Screening can be commercially interesting....
...but it can also be used to detect specific interactions

\[ ^{15} \text{N-HSQC of the synthrophin PDZ domain} \]
Summary

using protein-NMR-spectroscopy it is possible

to determine the structure of small to medium sized proteins

to study protein-protein or protein-ligand interactions
The NMR facility
That's it