

Product Specifications Pf1 for NMR (RDC)

RDC stands for “**residual dipolar coupling**”. Residual dipole coupling (RDC) measurements give information about the topology of molecules. The method is used to complement or even substitute classic NOEs (Nuclear Overhauser Effect), which are applied for NMR-structure definition of macromolecules. Residual dipolar coupling arises from the partial alignment of molecules in orienting media or via an intrinsic anisotropic magnetic susceptibility of the molecule itself (predominantly the magnetic susceptibility of aromatic groups in nucleic acids and protein side chains, paramagnetic ligands, or to a weaker extent of the peptide group (helices!)) when samples are placed in a magnetic field. Practically useful alignment leaves residual (to a large extent averaged) dipolar couplings of up to ca. 30 Hz from the several-kHz-couplings observed in solids where no averaging occurs.

Molecules of the solution in an anisotropic medium are oriented by steric clashing, electrostatic interactions and/or weak transient binding. Dipolar couplings are mostly determined for C-H and N-H-groups in non-decoupled, spin-state separated HSQC-like experiments (IPAP, DSSE) and for H-H-couplings in COSY-type experiments. The value is determined by comparison of the splitting in an aligned state with a reference spectrum in isotropic phase where only the J-splitting is detected. Dipolar couplings serve as angular restraints in the structure determination process.

A great advantage of RDC-Measurements is to get geometric information about the arrangement of protein domains or ligand-receptors without doing time consuming NMR-measurements in a first step. Measuring is carried out in nematic, fluid-crystalline phases, so that the action of the molecules is only partly but not completely inhibited. The characteristic features of the molecules are maintained.

Basis of residual dipole coupling is the magnetic interaction of cores over their space:

The interactions of the cores of different molecules are induced by the magnetic dipole moment of every single atom. A fast rotation movement of the molecules in fluids with low viscosity leads to average forces, means they annul. The magnetic forces reappear by suppressing the motion of the cores. This results in the coupling between the cores and can be visualized as a splitting of the NMR-resonance spectral lines. The structure of the splitting gives information about the geometry of the molecule.

There are liquid crystals (metamorphe) phases, like bicelles or filamentous phages to reduce the motion of the molecules. But the bicelle liquid crystalline phase is unstable in the presence of certain proteins and offers only a limited temperature range.

The alignment media for RDC are:

- bicelles consisting of various charged or uncharged lipids
- filamentous phage Pf1
- mechanically stressed polyacrylamide gels
- purple membrane of *Halobacterium salinarum* with bacteriorhodopsin in two-dimensional crystalline arrangement