

# Investigations of membrane polypeptides by solid-state NMR spectroscopy: Structure, dynamics, aggregation and topology

**Burkhard Bechinger**

Université Louis Pasteur / CNRS FRE2446, Faculté de Chimie, Institut le Bel, 4, rue Blaise Pascal, 67070 Strasbourg, France, [bechinger@chimie.u-strasbg.fr](mailto:bechinger@chimie.u-strasbg.fr), [www.chimie.u-strasbg.fr/~rmmmc](http://www.chimie.u-strasbg.fr/~rmmmc)

Solid-state NMR spectroscopy has a proven record during the investigation of the structure and dynamics of membrane-associated polypeptides. In particular from oriented samples considerable details on the structure and topology of the protein is obtained. The investigation of single-site or selectively labeled polypeptides has distinct advantages as it avoids problems with the assignment of resonances, allows the resolution and analysis also of broadened resonances, as well as investigations of dynamic features from non-oriented samples. The preparation of polypeptides labeled at single sites is most easily achieved by solid-phase chemical peptide synthesis.

A solid-state NMR approach which allows for the accurate determination of the tilt and rotational pitch angles of peptides reconstituted into uniaxially oriented membranes will be presented. The method works with transmembrane or in-plane oriented peptides that have been labelled with 3,3,3-<sup>2</sup>H<sub>3</sub>-alanine and <sup>15</sup>N-leucine at two selected sites. Proton-decoupled <sup>15</sup>N and <sup>2</sup>H solid-state NMR spectroscopy at sample orientations of the membrane normal parallel to the magnetic field direction have been used to characterize the tilt and rotational pitch angle of several peptides in considerable detail. Relative changes in tilt angle of less than 2° can be monitored using the deuterium NMR method. When many sample conditions have to be tested, e.g. in order to monitor the pH-dependent realignment of membrane-bound DNA transfectants, other physical methods such as oriented ATR-FTIR measurements can provide very valuable complementary information.

When the same samples are inserted into the magnetic field at 90 degrees tilted alignments provide valuable information on the rotational diffusion constants in membranes and thereby of the association and size of peptide complexes within the membrane environments. Whereas monomeric transmembrane peptides exhibit spectral averaging and well-defined resonances, larger complexes are characterized by broad spectral line shapes. In particular the deuterium line shape is sensitive to association of a few transmembrane helices. In contrast, the formation of much larger complexes affects the <sup>15</sup>N chemical shift spectrum.

The biological systems investigated by us using solid-state NMR spectroscopy include antibiotic peptides, viral or fungal channels, signal sequences, DNA transfectants, viral inhibitors of the intracellular TAP transporter, Alzheimer fibrils, colicins and proteins of the Bcl-family.

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