Solid-state NMR and oriented CD of a receptor tyrosine kinase transmembrane segment and its interactions with a viral oncoprotein

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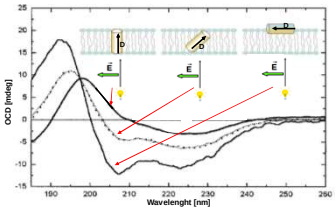
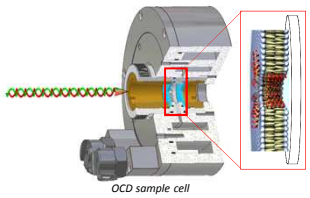
Membrane proteins and polypeptides are key players in many biological processes, as they control the flow of information and material between cells and their environment, and they are prime drug targets. The focus of our group lies on the structure-function analysis of membrane-active peptides and transmembrane protein segments of cell surface receptors. We are especially interested to find out how they insert into the membrane, how they fold and align within the hydrophobic core of the bilayer, and how they find and bind to their corresponding interaction partners. Complementary solid-state NMR and oriented CD measurements on macroscopically aligned samples are used to determine the conformation, alignment and dynamics of membrane-bound peptides and proteins in the quasi-native environment of a lipid bilayer. Here, we will present two interacting proteins: the PDGF-receptor β (PDGFR), a receptor tyrosine kinase, that gets activated by the oncogenic E5 protein via transmembrane helix-helix interactions [1,2]. A complementary PISEMA-NMR and OCD analysis of the PDGFR transmembrane domain and the E5 protein was used to resolve the structures and orientations of both proteins in their native environment [3,4,5].

Oriented circular dichroism

- ideal to study the membrane alignment of membrane proteins
- usage of membrane-like lipid bilayers
- identification of conformational changes possible
- lipid screens to optimize reconstitution
- fast and sensitive



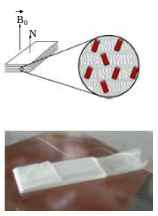
OCD spectra of helical membrane proteins at different orientations (transmembrane, tilted or surface aligned) in lipid bilayers. The 208 nm fingerprint band is sensitive to changes in the helix alignment and can be used to monitor the protein orientation. [6,7]



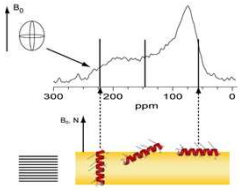
OCD sample cell (built in-house). Membrane proteins are reconstituted lipid vesicles which were spotted on a glass plate. The sample cell provide defined conditions (temperature and humidity) and can be rotated to minimize LD artifacts. [8]

Solid-state NMR

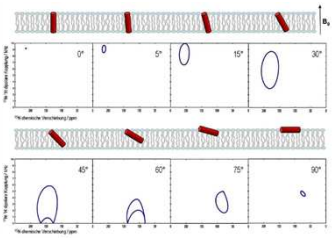
- Chemical shifts and heteronuclear dipolar couplings are sensitive to the secondary structure and topology
- structural investigations of tilt angle, rotation angle and mobility
- characteristic 1D and 2D spectra



Solid-state NMR sample of mechanically aligned lipids and reconstituted proteins on glass plates (wrapped in foil to prevent drying)



Solid-state ^{15}N-NMR 1D spectrum of a powder of a labeled peptide in combination with the peak positions corresponding to different orientations of a peptide in a lipid bilayer.

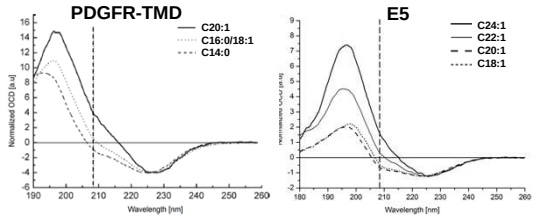


Solid-state ^{15}N-NMR 2D PISEMA spectra of a helix at different orientations in a lipid bilayer. PISA wheels display distinctive resonance patterns which provide a direct measure of the helix tilt and rotation angle, and are highly sensitive for any deviations of the helix structure, like helix curvature of kinks. [9,10]

Orientation of E5 and the transmembrane domain of PDGFR

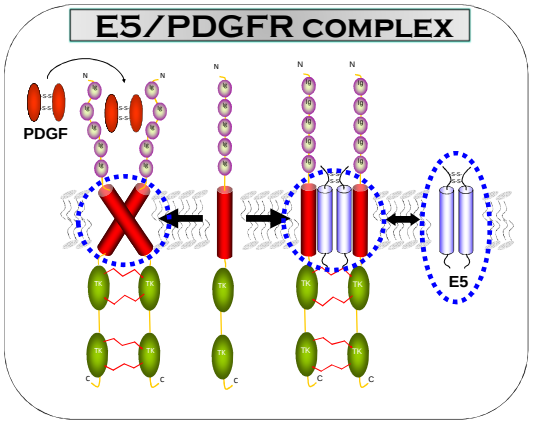
Analysis of the membrane alignment of E5 and the TMD of PDGFR. The proteins were reconstituted in lipid bilayers of different thickness and the influence on reconstitution, tilt angle (with respect to the bilayer normal), rotation angle (with respect to the helix axis) and mobility was investigated by oriented CD, solid-state 1D and 2D NMR.

Oriented CD analysis

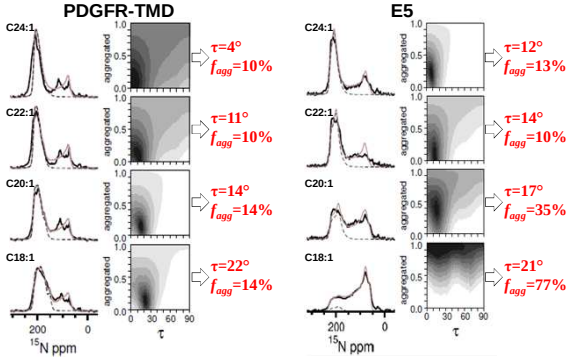


Oriented CD analysis of the membrane alignment of E5 and PDGFR-TMD in different membranes. In thick membranes (C20:1) PDGFR-TMD was upright inserted (positive 208 nm band), while with decreasing thickness (C16:0/18:1 and C14:0) the protein was more tilted (negative 208 nm band). E5 was upright inserted in thick C24:1 membranes and more tilted in thinner lipid bilayers.

E5/PDGFR complex

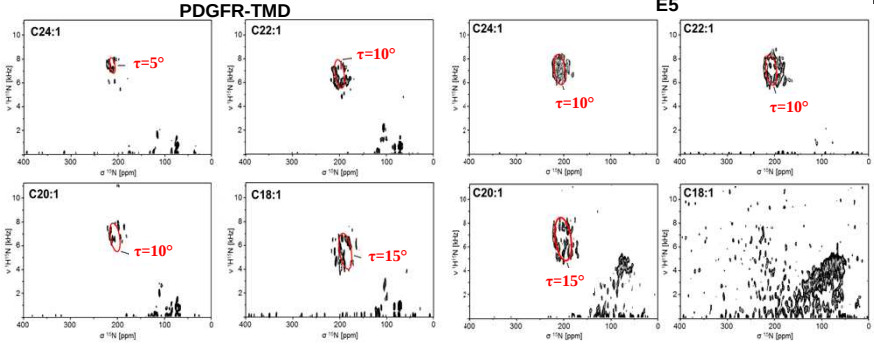


Solid-state 1D NMR analysis



Solid-state 1D NMR analysis of the membrane alignment of E5 and PDGFR-TMD to quantify the tilt angle (τ) and the content of protein aggregation (f_{agg}). In thick lipid bilayers both proteins were upright inserted (signals at ~200 ppm), while with decreasing membrane thickness the tilt angles increased (signals moved to lower ppm) as well as the contents of protein aggregation (signals ~60 ppm evolved).

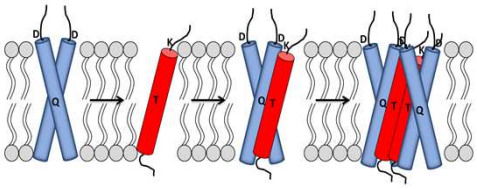
Solid-state 2D NMR PISEMA analysis



Solid-state 2D NMR PISEMA analysis of the membrane alignment of E5 and PDGFR-TMD. The tilt angles of both proteins in the different lipid bilayers systems can be obtained by fitting simulated PISA wheels (with defined tilt angles) to the measured spectra. For both proteins an increase of the tilt angle was observed from thick bilayers (C24:1) to thin bilayers (C18:1). At the same time the content of aggregated protein increased (signals 60 - 200 ppm). For E5 in C18:1 no PISA wheel was observed due to severe protein aggregation.

Conclusion

When present in the same lipid bilayer, E5 and PDGFR share a similiar orientation This should allow a close packing of the helices in the heterotetrameric bundle, which is stabilized by interhelical hydrogen bonds (Q-T) and salt bridges (D-K). E5 clusters two receptor subunits to form a functional receptor dimer.



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