Structural investigations of the heterotetrameric E5/PDGF-receptor β complex by oriented circular dichroism and solid state NMR

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Poster #

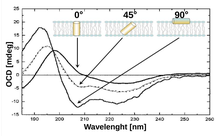
Tools

- (SR-) CD
- high-resolution NMR

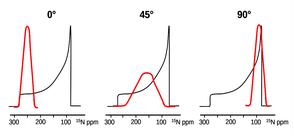
} **secondary structure**

- Oriented CD (OCD)
- solid-state NMR

} **orientation in membranes**



Oriented CD basis spectra: the 208 nm „fingerprint" absorption band depends on helix orientation.

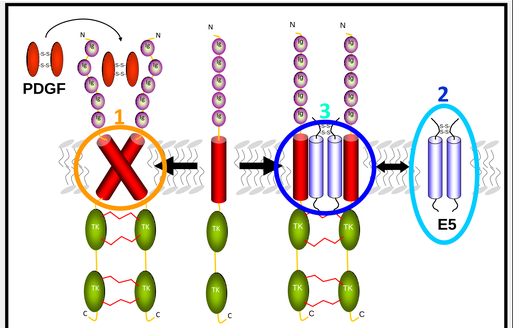


theoretical solid-state 1D ^{15}N-NMR spectra: the shape and position of the signal (red) depends on helix orientation. ^{15}N powder tensor (black) for comparison.

Activation of the PDGF-receptor β

The Platelet-derived growth factor receptor β is a member of the receptor-tyrosine-kinase family involved in development. The receptor can be activated by two different ways: 1) The receptor is activated when the natural ligand PDGF binds simultaneously to the ectodomains of two receptors, resulting in spatial proximity and subsequent cross-activation of the cytosolic kinase domains.[1] 2) The receptor can also be activated independently of the natural ligand by binding of the small E5 oncoprotein from bovine papillomavirus.[2] E5 is a small membrane protein of only 44 amino acids and it is thought to manipulate the function of the receptor by specific helix-helix-contacts to the transmembrane of the receptor which then result in sustained receptor activation and cell transformation.

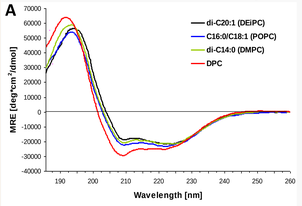
To elucidate the helix-helix-interactions in receptor complex, we investigate the structure and membrane alignment of both proteins, first for each protein and later in the heterotetrameric four-helix-bundle. For the structural analysis we combined circular dichroism (CD) spectroscopy and liquid-state NMR to determine the secondary structure. Oriented CD and solid-state NMR were used to resolve the orientation of both proteins in their native environment. Therefore the E5 protein and the transmembrane domain of the receptor (PDGFR-TMD: aa 494-531) were ^{15}N-isotope labelled by bacterial protein expression and reconstituted in detergent micelles and in oriented lipid bilayers. By CD and liquid-state NMR we found that both proteins are predominantly α-helical in detergent micelles and in lipid bilayers.[3,4] Furthermore, high resolution NMR measurements showed that PDGFR-TMD forms a left-handed coiled-coil structure. A complementary OCD and solid-state NMR analysis of E5 and PDGFR-TMD reconstituted in different lipid bilayers showed that the orientation of both proteins depends on the bilayer thickness, where both proteins were more tilted in thin membranes and less tilted in thick membranes.



step-by-step strategy:
1) PDGFR-TMD dimer
2) E5 dimer
3) heterotetrameric E5/PDGFR-TMD complex

1) structural investigations of PDGFR-TMD

1) Secondary structure of the TMD in micelles and liposomes



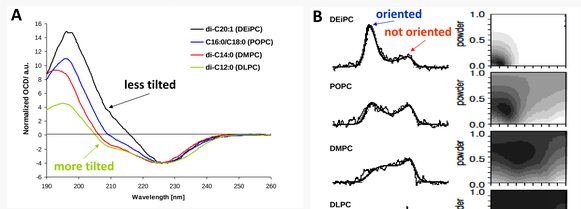
B

CONTIN	α-Helix			
DEiPC	65,2	6,7	9,9	18,2	0,045
POPC	63,9	4,4	10,9	20,8	0,048
DMPC	64,9	5,1	11	18,9	0,049
DPC	74,4	0,1	10,6	14,6	0,031

A: CD analysis of the TMD reconstituted in micelles and liposomes. The CD spectra indicate an α-helical folding. **B:** Secondary structure contents calculated using CONTIN. The TMD has α-helical content of ~65% in liposomes and of ~75% in micelles.

the TMD is α-helical

2) orientation of the TMD in lipid bilayers



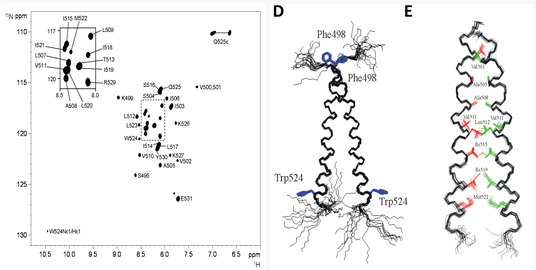
C

	thickness	tilt	powder	S	rmsd
DEiPC	31,5 Å	10°	0,04	0,98	0,0002
POPC	27,1 Å	17°	0,26	1	0,00024
DMPC	25,4 Å	31°	0,54	0,94	0,0003
DLPC	20,9 Å	n.d.	>0,70	0,98	0,00031

A: Oriented CD analysis of PDGFR-TMD in macroscopically aligned lipid bilayers of different thickness. The intesity increase (DLPC->DMPC->POPC->DEiPC) of the 208 nm band indicates a more upright orientation of the TMD in thick membranes.

B: Complementary solid-state NMR analysis in oriented bilayers. The TMD is much better reconstituted in thick membranes. **C:** Summary solid state NMR analysis: The TMD is well reconstituted and less tilted in thick membranes.

the TMD adjusts its tilt in response to lipid bilayer thickness (to compensate hydrophobic mismatch)

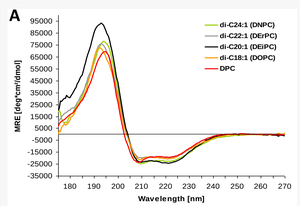


C: ^{1}H^{15}N 2D-HSQC spectrum of the TMD in DPC micelles with amino acid assignment. **D:** Superposition of the 10 best NMR structures of the PDGFR-TMD dimer in DPC. The TMD forms a left-handed coiled-coil with a dimer crossing angle of ~20°. The side chains of Phe498 and Trp524 are shown in blue for visual orientation. **E:** Details of the interface after a rotation of 30° around the z-axis. The contributing residues are depicted in red and green, and the disordered termini are removed for clarity.

the TMD forms a left-handed coiled coil dimer

2) structural investigations of E5

1) Secondary structure E5



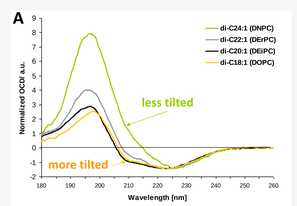
B

CONTIN	Helix	β-Strand		
DNPC	80%	5%	5%	10%
DErPC	78%	8%	6%	8%
DEiPC	85%	9%	3%	2%
DOPC	60%	5%	11%	24%
POPC	69%	10%	7%	15%
DPC	85%	3%	5%	9%

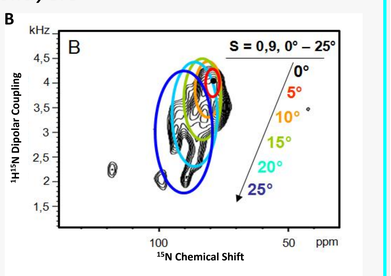
A: SR-CD analysis of E5 in micelles and liposomes. The CD spectra indicate an α-helical folding. **B:** Secondary structure contents calculated using CONTIN. The E5 protein has a higher α-helical content in thick lipid bilayers and micelles (80-85%).

the E5 protein is α-helical

2) orientation of E5 in lipid bilayers



A: Oriented CD spectra of the E5 dimer in macroscopically aligned lipid bilayers of different thickness. The intesity increase (DOPC -> DEiPC -> DErPC -> DNPC) of the 208 nm fingerprint band indicates a more upright orientation of the E5 dimer in thick membranes (DNPC and DErPC) and a more tilted alignment in thin membranes (DOPC and DEiPC).



B: 2D solid-state NMR PISEMA analysis of the orientation of E5 in magnetically aligned bicelles (DMPC/DMPG[80:20]/6OPC; q=5). The weakly resolved PISA-Wheel of E5 (black) can be superimposed with theoretical PISA-Wheels of 0°-25° (with an order parameter S_{mol} = 0,9).

E5 adjusts its tilt in response to lipid bilayer thickness (to compensate hydrophobic mismatch)

next steps: investigations of the heterotetrameric E5/PDGFR-TMD complex

References