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

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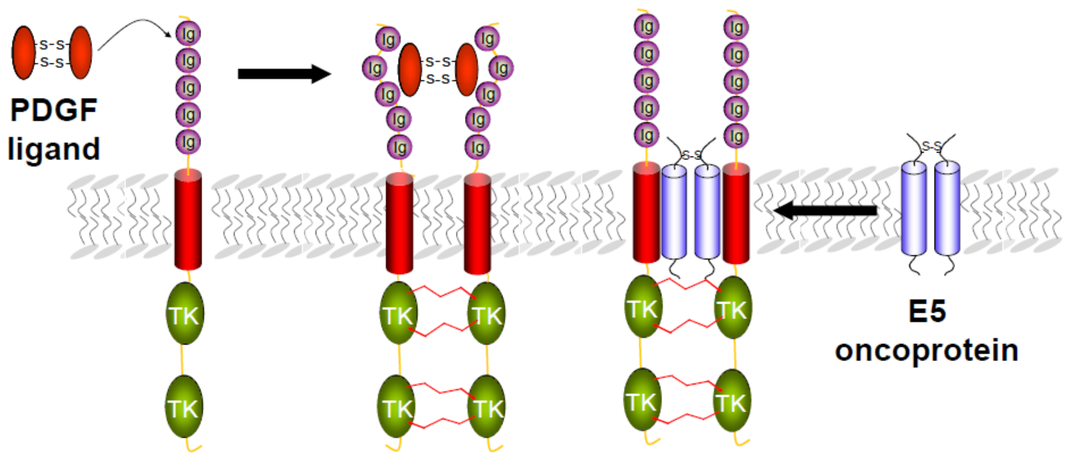
How does the viral oncoprotein E5 manipulate the PDGFR receptor β ?

Plateled derived growth factor receptor β (PDGFR)

- cell surface receptor
- involved in development and angiogenesis
- activation by its natural ligand PDGF via extracellular domain leads to dimerization of two receptor monomers



E5 oncoprotein of the bovine papillomavirus

- short 44 amino acids transmembrane protein, dimeric per se
- ligand-independend dimerization of two receptor monomers via the transmembrane segment of E5 through specific helix-helix interactions
- sustained activation can cause cancer

Aim

The focus of our group lies on the structure-function analysis of the E5/PDGFR-complex under quasi-native conditions in liquid crystalline lipid bilayers.

Strategy

1) study the structure of each protein in the membrane
2) compare E5 and PDGFR
3) study the helix-helix interactions between E5 and PDGFR

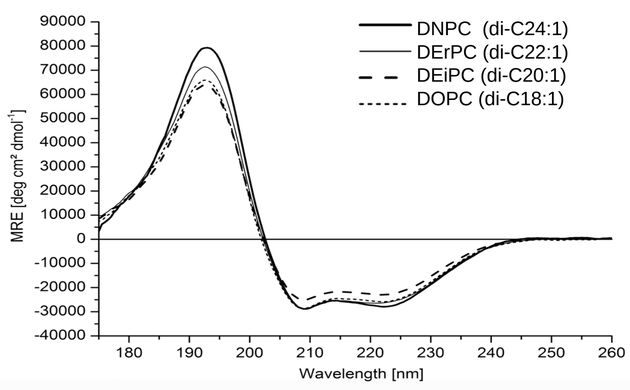
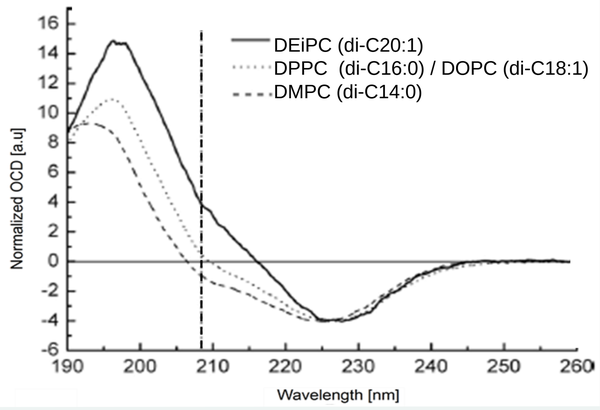
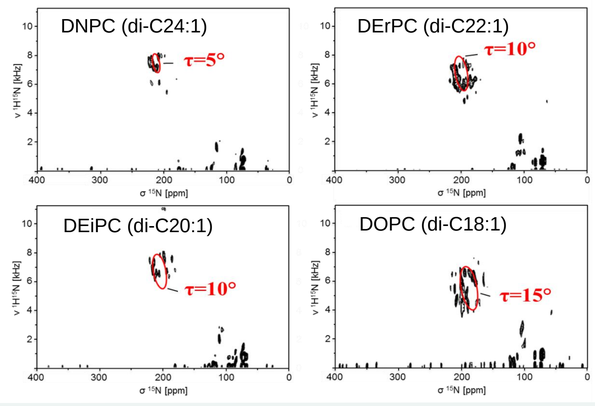
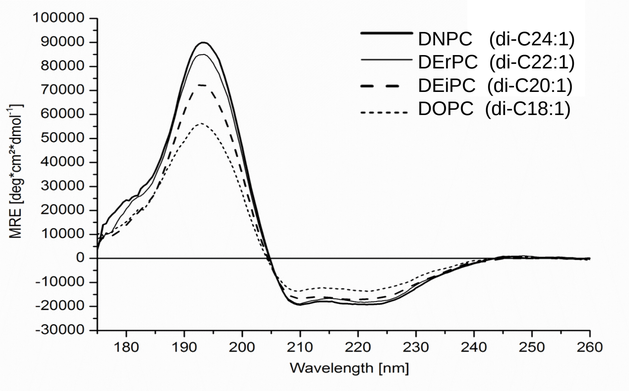
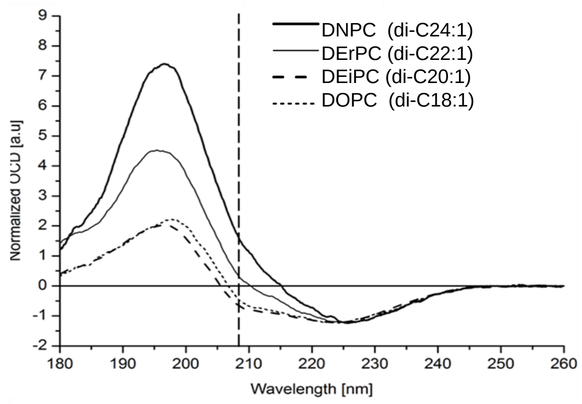
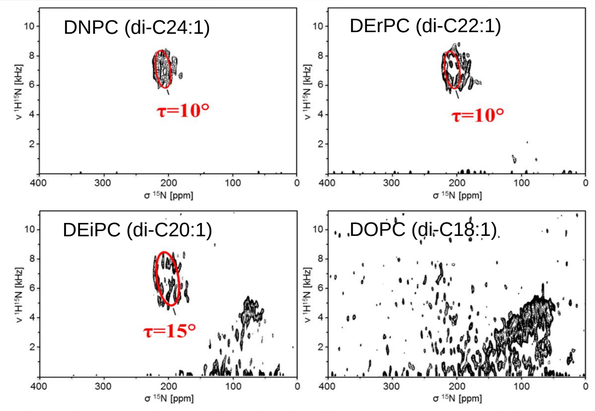
Methods

Synchrotron CD: secondary structure and reconstitution in model membranes

Oriented CD: orientation within model membranes

Solid-state NMR: PISEMA: helix tilt angle

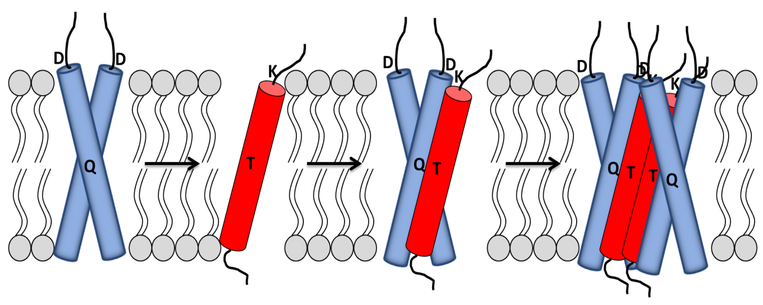
Structure of the PDGF receptor and the E5 protein in the membrane

	Synchrotron CD	Oriented CD	PISEMA NMR
PDGFR			
E5			

Synchrotron CD-measurements show that PDGFR and E5 both have predominantly an α-helical secondary structure in lipid bilayers

Oriented CD measurements and PISEMA NMR analysis show that both helices are stably inserted in membranes of proper thickness, but become destabilized and more tilted when the membrane gets too thin. E5 is aggregated in thin membranes. Notably, both proteins have the same orientation in the membrane.

Results: similar behaviour of both proteins

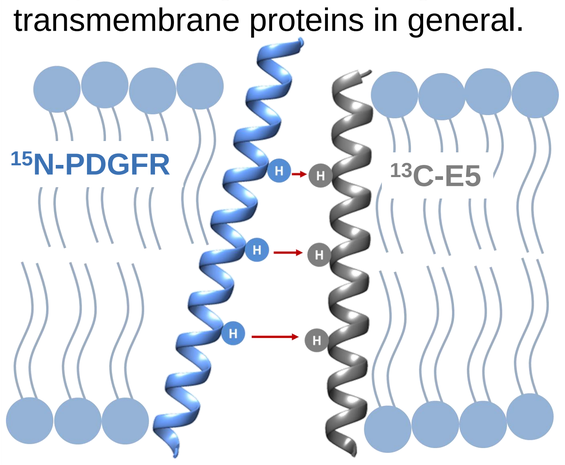


Our experiments showed that E5 and PDGFR have the same tilt angle in the membrane. Both peptides can therefore interact through a perfectly parallel alignment in the membrane.

Future plans

Solid-state NMR analysis of the hetero-oligomeric complex

The analysis of the molecular structure of the E5/PDGFR hetero-oligomeric complex can give new insights in viral oncogenesis and in the activation of transmembrane proteins in general.



For this aim, we want to measure inter-molecular distance constraints within the E5/PDGFR-complex using ^{1}H-^{1}H spin diffusion techniques that allow the investigation of heterogeneous mixtures of uniformly labeled proteins when reconstituted in liquid crystalline model membranes to make helix-helix interactions traceable.

References

[1] Muhle-Goll, C. et al., 2012, Hydrophobic matching controls the tilt and stability of the dimeric platelet-derived growth factor receptor PDGFR-β transmembrane segment, *Journal of Biological Chemistry, SBMB,* 287, 26178-26186
[2] Talbert-Slagle, K. et al., 2009, The bovine papillomavirus E5 protein and the PDGF receptor β: It takes two to tango, *Virolog,* 384, 345 – 351
[3] Windisch, D. et al, 2010, Structurall Role of the Conserved Cysteines in the Dimerization of the Viral Transmembrane Oncoprotein E5, *Biophysical Journal, 99*, 1764 - 1772
[4] Dirk Windisch, Colin Ziegler, Jochen Bürck and Anne S. Ulrich, Structural verification of a truncated E5 oncoprotein from papillomavirus in lipid bilayers, *Biological Chemsitry*, in revision
[5] Dirk Windisch, Colin Ziegler, Stephan L. Grage, Jochen Bürck, Marcel Zeitler, Peter Gor´kov, Timothy A. Cross and Anne S. Ulrich, Preference of the oncogenic E5 transmembrane protein for thick membranes drives its interaction with the PDGF receptor β, *Biophysical Journal*, in preparation for publication