Solid-state NMR analysis of the membrane alignment of the PDGF-receptor β and the E5 oncoprotein

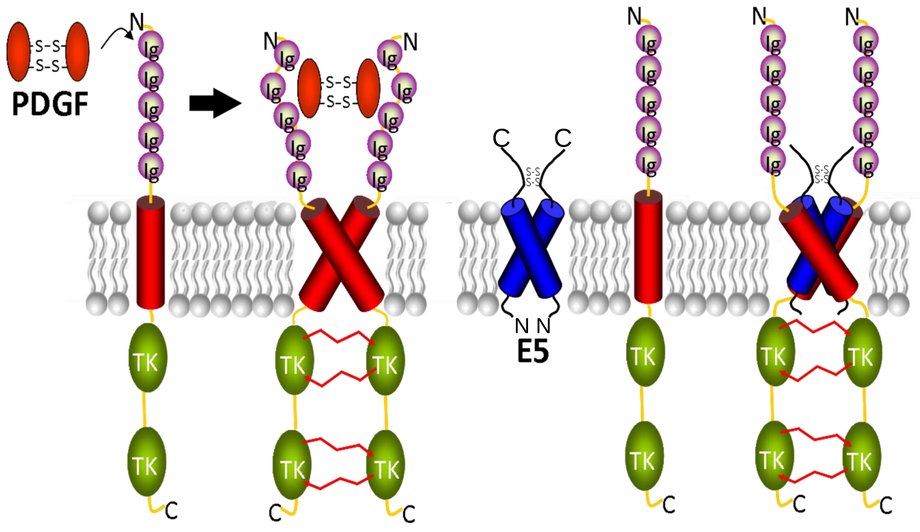
Dirk Windisch[1], Colin Ziegler[2], Stephan L. Grage[1], Parvesh Wadhwani, Anne S. Ulrich[1,2]

[1] KIT, Campus North, Institute for Biological Interfaces (IBG-2), POB 3640, 76021 Karlsruhe, Germany;

[2] KIT, Campus South, Institute of Organic Chemistry (IOC), Fritz-Haber-Weg 6, 76131 Karlsruhe, Germany.

Contact: Dirk.Windisch@kit.edu

The E5 oncoprotein from papillomavirus is a short membrane protein that manipulates the platelet-derived growth factor receptor β (PDGFRβ) in a ligand-independent manner by specific helix-helix interactions [1,2]. The conformation of this heterooligomeric signal transduction complex remains vague, as no structure of the E5 protein and the E5/PDGFRβ-complex is available. However, recent progress in the biophysical characterization of E5 and the transmembrane domain (TMD) of PDGFRβ unravelled insights in how these two proteins are aligned in the membrane [3,4]. For both proteins slightly tilted orientations in the membrane and a bilayer thickness dependent behaviour was observed. Here, we now have investigated the azimuthal helix rotation angle of E5 and the PDGFRβ-TMD to determine into which direction the helices incline. Using two-dimensional (2D) solid-state ^{15}N-NMR measurements of selectively amino acid labelled analogues we were able to characterize the alignment of both proteins in the membrane. For E5 we found an orientation in which the helical TMD tilts into the direction of Ala14. In this alignment, homodimerization is possible via the dimer-interface described by molecular dynamics studies earlier [5]. Moreover, Gln17, which is important for interaction with PDGFRβ, is located in a position accessible for Thr513 of the receptor. For the TMD of PDGFRβ we observed an orientation in which the proteins tilt into the direction of Lys499/Leu517. This alignment matches with the structures found for the isolated TMD and for the full-length receptor [4,6].

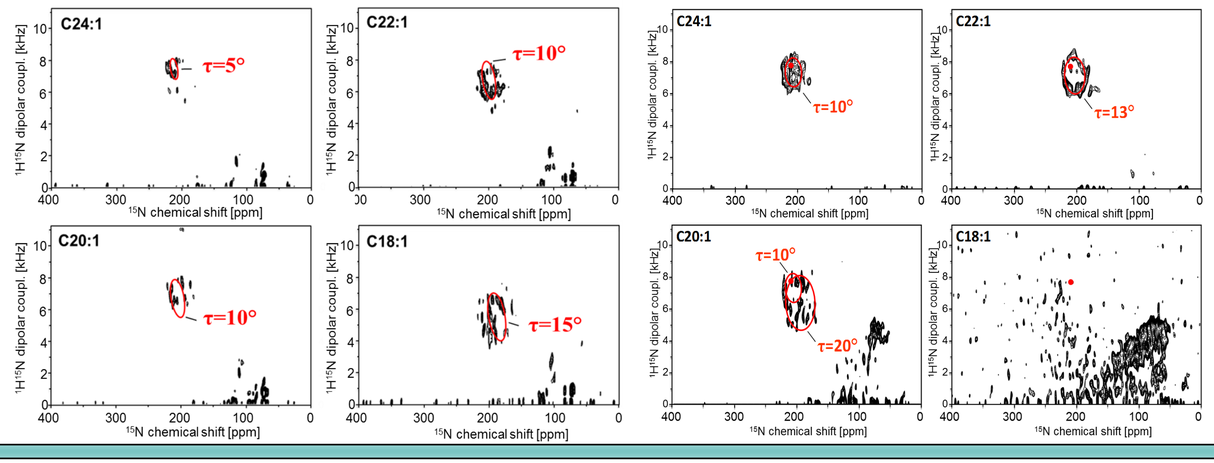


PDGFRβ dimer (red) crosslinked by the natural ligand PDGF

PDGFRβ dimer crosslinked by the viral E5 oncoprotein (blue)

Helix tilt angles of the TMD of PDGFRβ and E5 in membranes

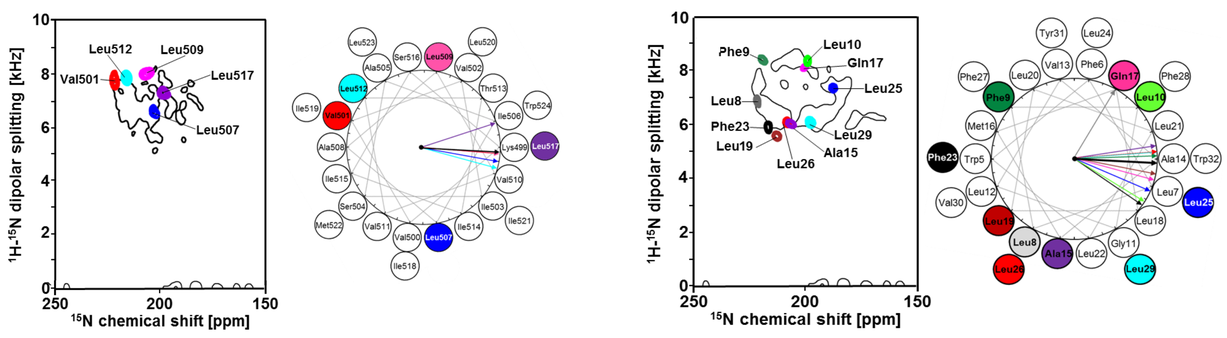
PDGFRβ-TMD **E5**



- ***Definition***: *the helix tilt angle τ is the angle between the membrane normal and the helical axis*
- ***Method***: *solid-state 2D ^{15}N-NMR measurements of fully labeled analogues of PDGFRβ-TMD and E5*
- ***Results***: *- Decrease bilayer thickness -> increase helix tilt angles*
  - in C22 bilayers: τ=10° for PDGFRβ-TMD and τ=13° for E5
  - E5 is aggregated in very thin membranes

Direction towards which the TMD of PDGFRβ and E5 are inclined in C22 membranes

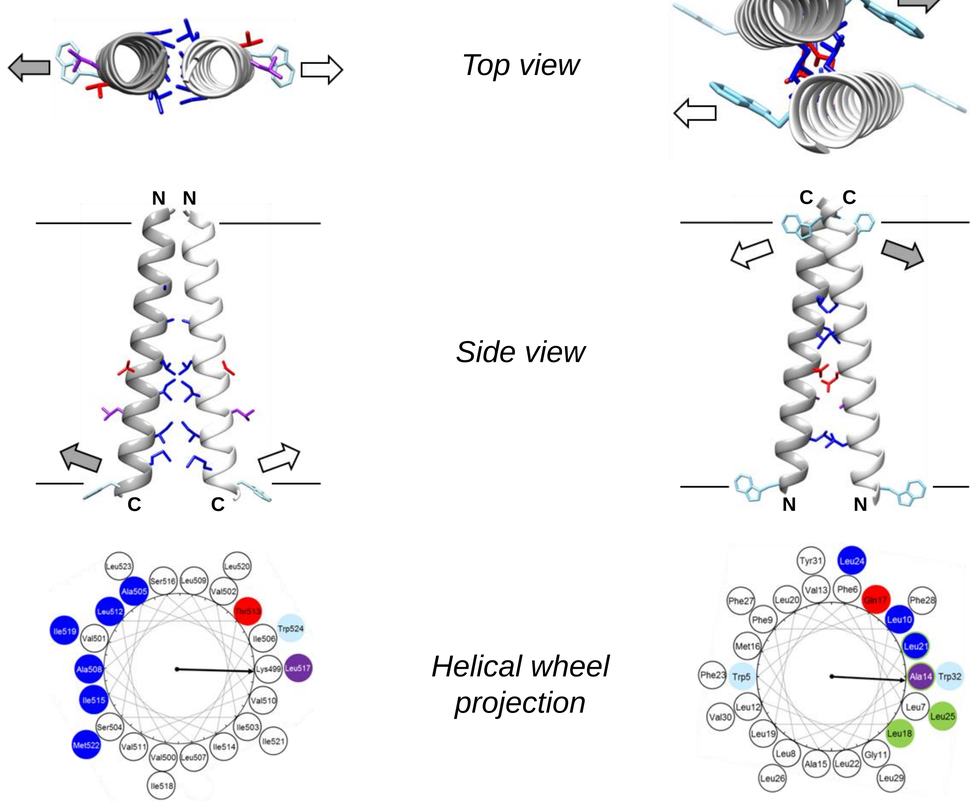
PDGFRβ-TMD **E5**



- ***Definition***: *The azimuthal rotation angle ρ is the angle of a residue around the helix axis with respect to the tilt direction (ρ=0°)*
- ***Method***: *solid-state 2D ^{15}N-NMR measurements of five specific ^{15}N-amino acid labeled analogues of PDGFRβ-TMD and ten analogues of E5*
- ***Results***: *- PDGFRβ-TMD inclines towards Lys499/Leu517*
  - E5 inclines towards Ala14/Trp32

Orientation of the TMD of PDGFRβ and E5 in the membrane

PDGFRβ-TMD **E5**



- ***PDGFRβ-TMD***:

The two subunits tilt toward Leu517 (purple). In this orientation the two subunits are assembled in a back-to-back conformation using the dimer-interface [Ala505, Ala508, Leu512, Ile515, Ile519, Met522 (dark blue)] found by solution NMR [4]. Thr513 (red) is rotated ~-40° away from the direction of the tilt and is accessible for interaction with Gln17 of E5.

- ***E5***:

The two subunits tilt towards Ala14 (purple). In this alignment the subunits can form a face-to-face dimer via the dimer-interface [Leu10, Ala14, Leu21, Leu24 (blue) and Gln17 (red)] predicted by molecular dynamics simulations [5]. Without PDGFRβ, Gln17 participates in E5 homodimerization mediated by this dimer-interface. In the presence of PDGFRβ the subunits then are slightly rotated in a clockwise manner to form a second dimer-interface (green), resulting in a breakage of the Gln17-Gln17 interaction, allowing the Gln17 side chain to form a hydrogen bond with Thr513 of the receptor TMD.

References:
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