Transmembrane helix-helix interactions between a receptor tyrosine kinase and a viral oncoprotein

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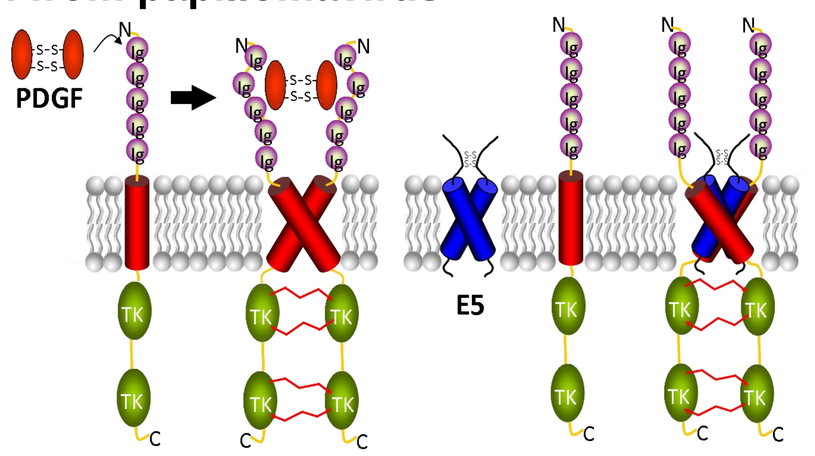
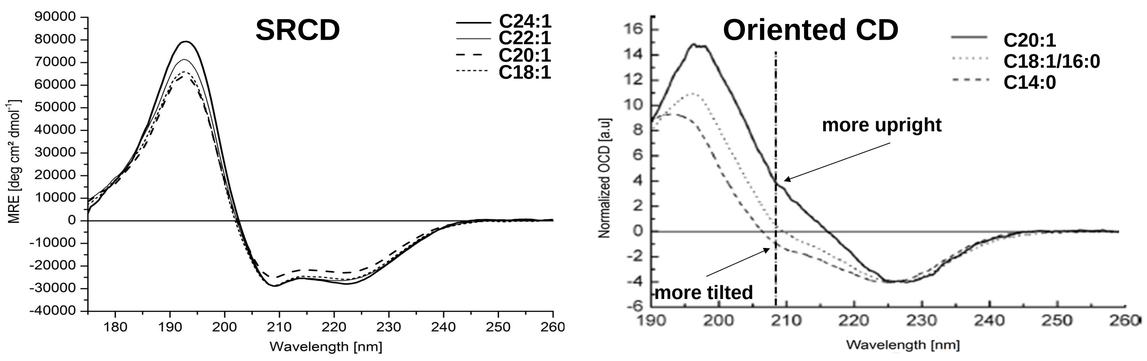
Activation of the PDGF-receptor by the viral E5 oncoprotein from papillomavirus

The platelet-derived growth factor receptor (PDGFR) is a receptor tyrosine kinase that gets constitutively activated by the oncogenic E5 protein from papillomavirus, leading to uncontrolled proliferation and cancer. Bovine E5 with a length of only 44 amino acids consists largely of a transmembrane helix that can engage in specific helix-helix interactions with the transmembrane segment of PDGFR [1,2]. Our aim is to elucidate the structural criteria by which these transmembrane segments recognize each other, and to describe the oligomeric bundle formed in the lipid bilayer. We reconstituted each of the two recombinantly expressed polypeptides in lipid bilayers with different acyl chain lengths, in order to (i) confirm their intact helical conformation, to (ii) determine their molecular alignment in the lipid membrane, to (iii) monitor any changes in response to bilayer thickness, and to (iv) observe the structural effect of one partner on the other. Complementary spectroscopic measurements were carried out using solid-state NMR and synchrotron-radiation circular dichroism (SRCD) on macroscopically oriented membrane samples [3,4,5,6].



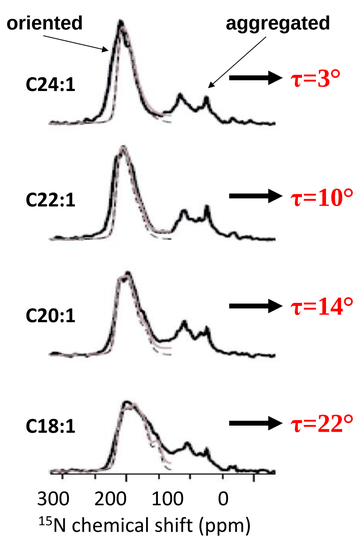
PDGFR dimer crosslinked by the natural ligand PDGF | *PDGFR dimer crosslinked by the viral E5 oncoprotein*

Structure and alignment of the PDGFR-TMD in lipid bilayers

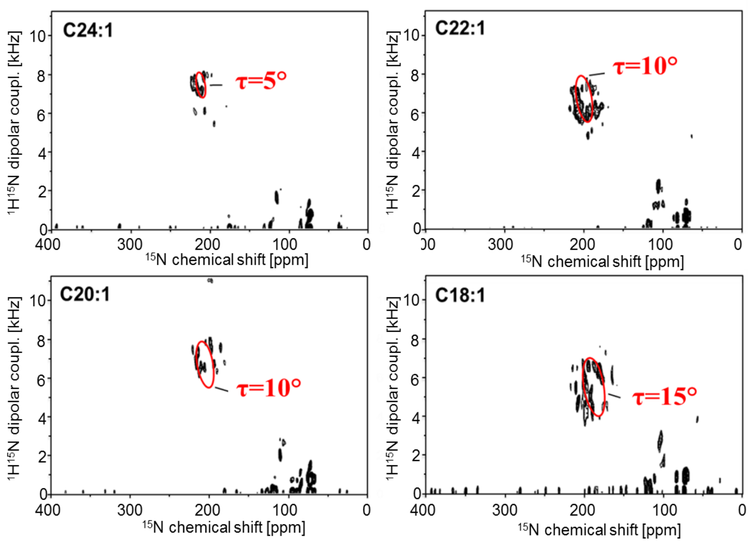


Helical conformation confirmed | *Transmembrane orientation found*

Solid-state 1D ^{15}N-NMR

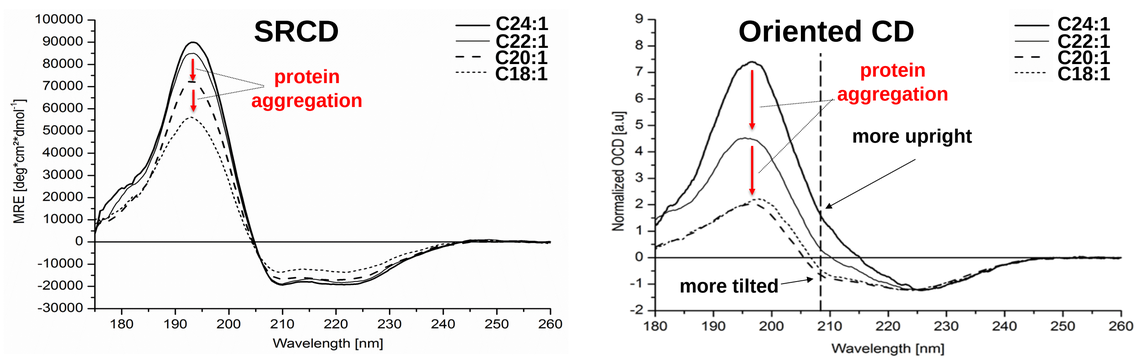


Solid-state 2D PISEMA ^{15}N-NMR



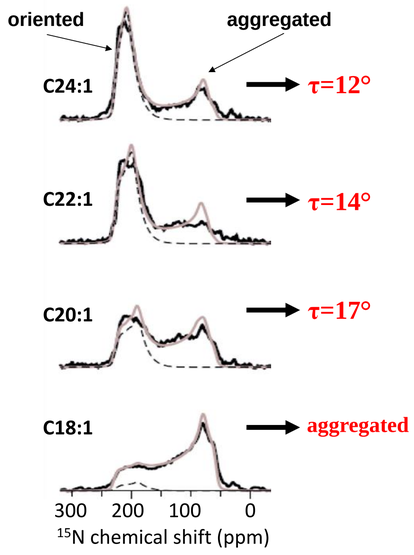
Increase of the helix tilt angle (C24:1 -> C18:1) as a consequence of increased hydrophobic mismatch in thin lipid bilayers observed.

Structure and alignment of the E5 oncoprotein in lipid bilayers

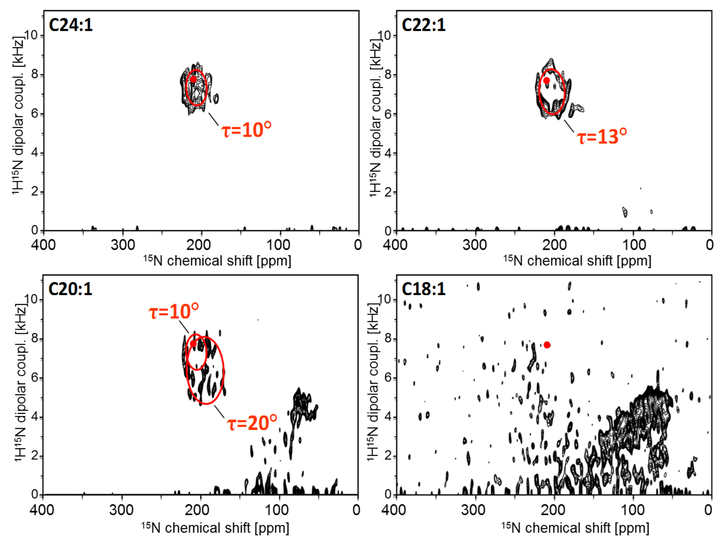


Helical conformation in thick bilayers, but aggregation in thin bilayers. | *Transmembrane orientation in thick bilayers, aggregation in thin membranes.*

Solid-state 1D ^{15}N-NMR

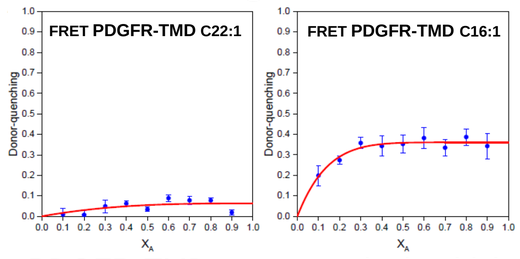


Solid-state 2D PISEMA ^{15}N-NMR

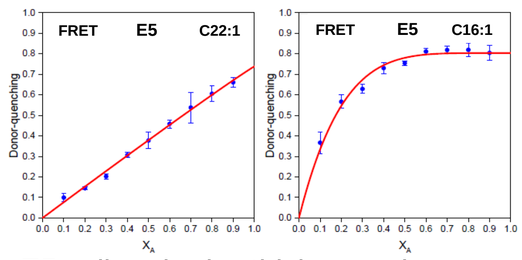


E5 tilted only slightly (C24:1 -> C22:1 -> C20:1) to compensate hydrophobic mismatch, but then it aggregated in thin lipid bilayers (C18:1).

Oligomeric state of PDGFR-TMD and E5



PDGFR-TMD: monomeric in thick and thin membranes.

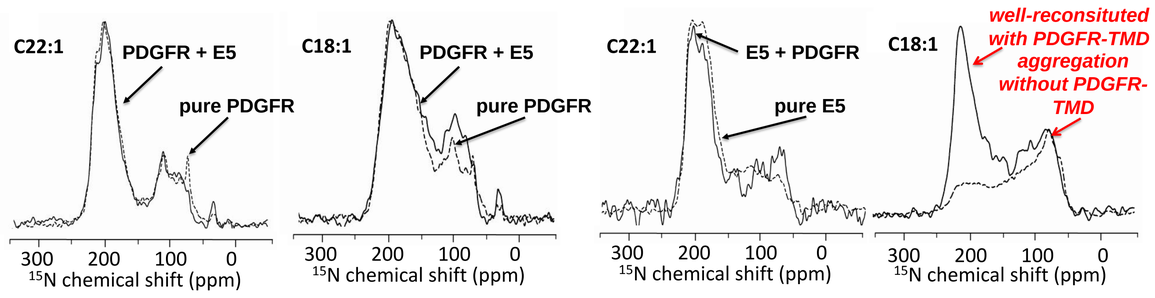


E5: dimeric in thick membranes, aggregated in thin membranes.

Structural characterization of the PDGFR-TMD/E5-complex

^{14}N-^{15}N-hetero-labeling scheme used for ^{15}N-NMR measurements of the complex:

^{15}N-PDGFR-TMD + ^{14}N-E5 ⟷ *^{15}N-E5 + ^{14}N-PDGFR-TMD*



Alignment of the PDGFR-TMD not affected by the presence of the E5 protein.

Reconstitution and orientation of E5 strongly influenced by the presence of the PDGFR-TMD in thin membranes.

References:

[1] L. Petti, D. DiMaio, ***EMBO J.*** 10, 845-855 (1991)

[2] L. Petti, D. DiMaio, ***Proc. Natl. Acad. Sci USA*** 89, 6736-6740 (1992)

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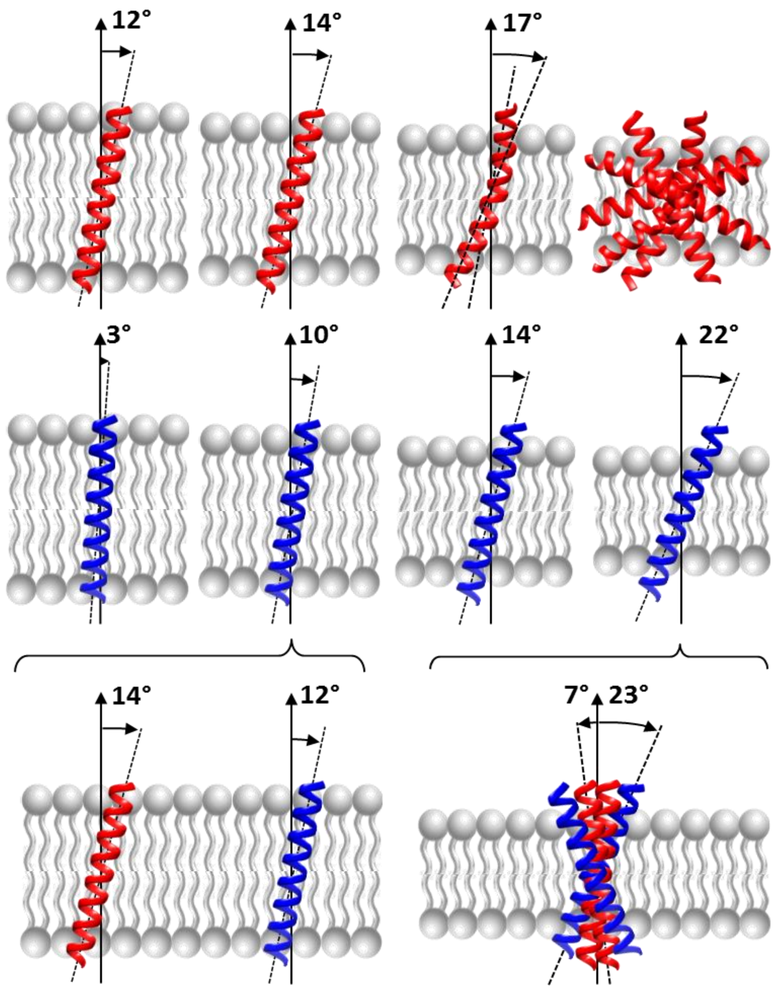
Conclusion



The E5 protein can slightly tilt to compensate hydrophobic mismatch, but then the protein aggregates if the membrane is too thin.

The TMD of the PDGFR can respond to hydrophobic mismatch by tilting.

In thin bilayers E5 can compensate the hydrophobic mismatch by binding to the TMD of PDGFR resulting in a stable heterotetrameric complex. In thick membranes hydrophobic mismatch is less critical, hence E5 does not bind to PDGFR.

In conclusion, the hydrophobic mismatch in thin lipid membranes might be the underlying driving force which induces the binding of the E5 oncoprotein to the TMD of the receptor. This could explain why E5 binds to the receptor already within the thin membranes of the ER and Golgi [7].