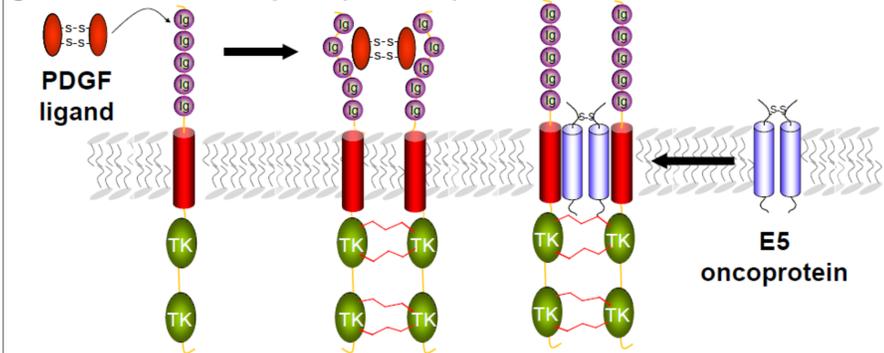


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How does the viral oncoprotein E5 activate the platelet-derived growth factor receptor (PDGFR)?



PDGFR

- cell surface receptor
- involved in development and angiogenesis
- platelet-derived growth factor (PDGF) is the normal ligand
- binding of PDGF to the extracellular domain leads to dimerization of two receptor monomers

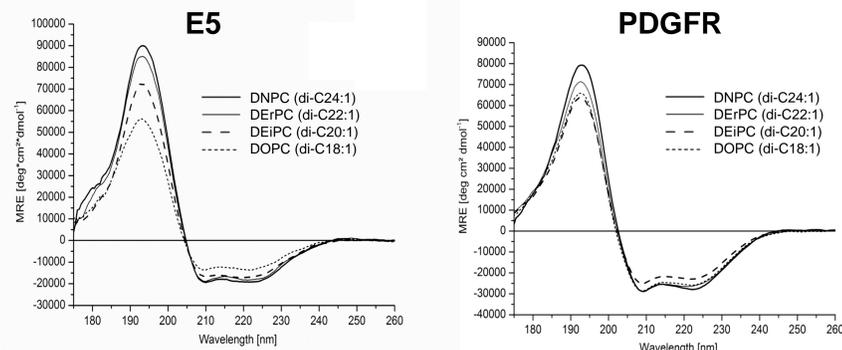
E5

- short transmembrane protein (44 amino acids)
- activates the PDGFR in ligand-independent manner through specific helix-helix interactions [1]
- sustained activation can cause cancer
- dimerization of two receptor monomers via transmembrane segment of E5
- E5 is dimeric per se

Aim: study the helix-helix interactions between E5 and PDGFR

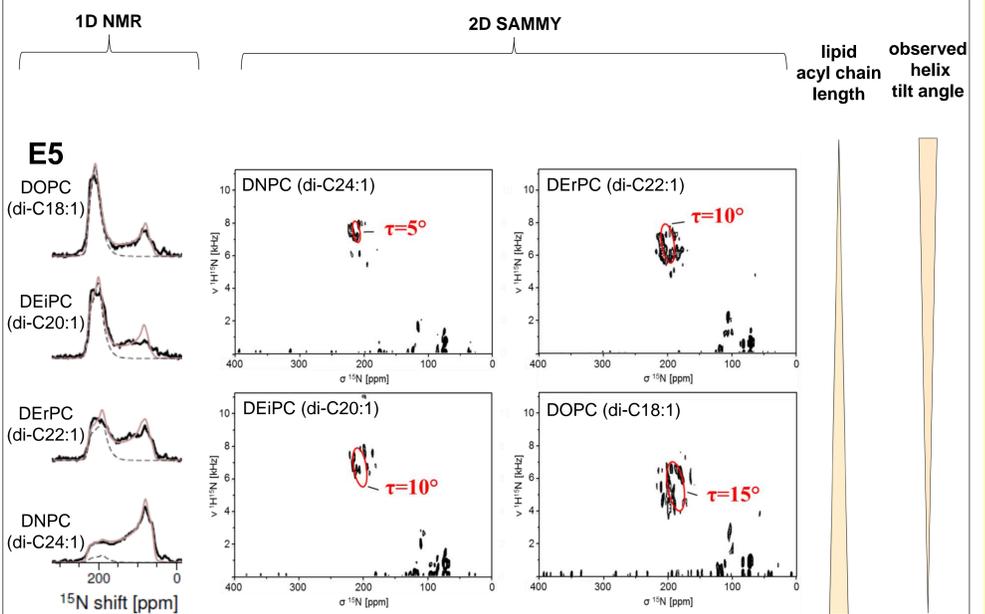
Strategy: 1) study E5 alone
2) study PDGFR alone as monomer and dimer
3) observe changes in **hetero-oligomeric complex**

Secondary structure and reconstitution of E5 and PDGFR

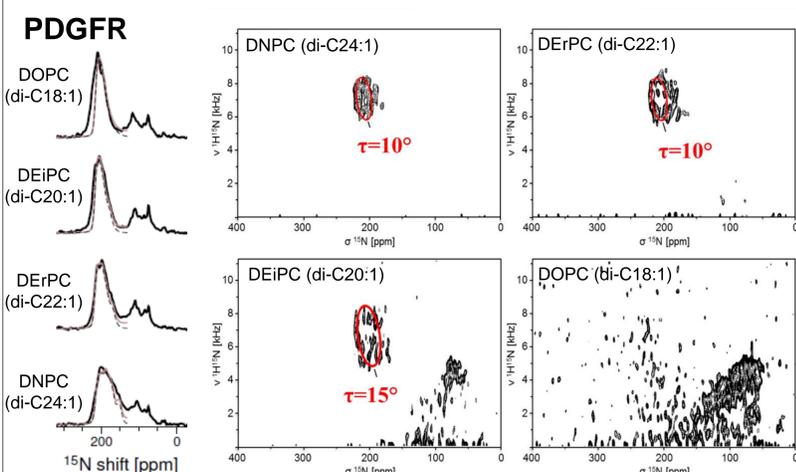


Synchrotron circular dichroism analysis of E5 and PDGFR in membranes of varying thickness: both proteins are predominantly α -helical in lipid bilayers [2][3].

Influence of membrane thickness on the helix tilt angle (τ)

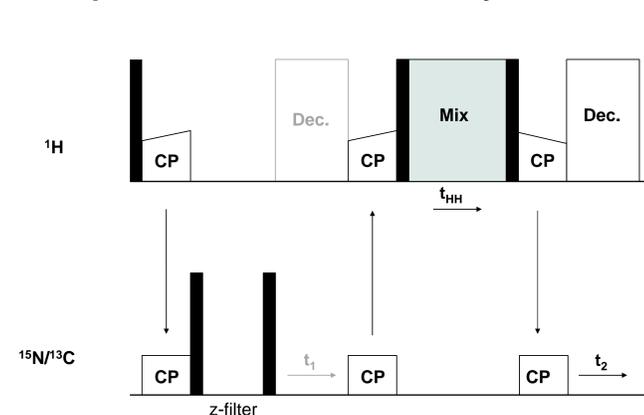


Solid-state ^{15}N -NMR 1D and 2D PISEMA spectra of E5 reconstituted in lipid bilayers of different acyl chain length. PISA wheels reflect the helix tilt angle (τ).

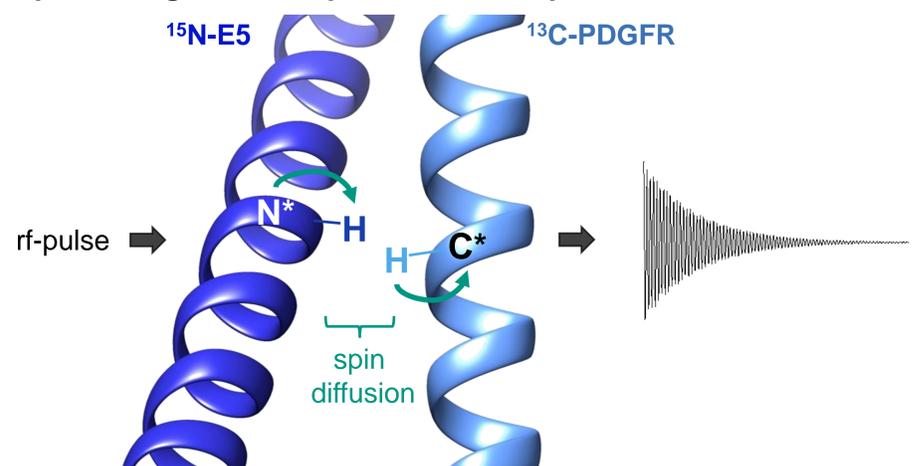


The PDGFR helix is stably inserted in membranes of proper thickness, but becomes destabilized and more tilted when the membrane gets too thin. Both proteins show similar behavior, which means that E5 can interact with PDGFR through equally aligned transmembrane helices [2][4].

Future plans: solid-state NMR analysis of the hetero-oligomeric complex using a NHC spin diffusion experiment



Pulse sequence of the NHC spin diffusion experiment. With this method, heterogeneous mixtures of uniformly ^{13}C - and ^{15}N -labeled proteins can be examined under MAS conditions when reconstituted in liquid crystalline membranes [5].



Magnetization transfer-chain: magnetization is transferred from a ^{15}N - to a ^{13}C -labeled protein via protons and makes helix-helix interactions within the complex traceable.

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