# Solid-state $^{15}$N- and $^{19}$F-NMR analysis of the interaction of the viral E5 oncoprotein with the PDGF receptor in membranes

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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 was to elucidate the structural criteria by which these transmembrane segments recognize each other and to describe the oligomeric bundle formed in the membrane. We used solid-state $^{15}$N-NMR to characterize the structure and alignment of E5 and PDGFR in lipid bilayers, each one alone and together in the hetero-oligomeric complex.[3,4] When reconstituted alone in lipid bilayers, we observed that the E5 helix is inserted almost upright in thick membranes, but it starts to tilt and gets slightly deformed in moderately thinner bilayers, and it becomes aggregated in very thin membranes due to hydrophobic mismatch. On the other hand, when reconstituted together with the receptor, E5 can compensate for the hydrophobic mismatch by binding to the transmembrane segment of the receptor. This hydrophobic mismatch behaviour may be responsible for driving the two interacting partners together within the thin membrane of the Golgi compartment (before reaching the thicker membrane environments of the plasma membrane). They can recognize each other by forming a closely packed bundle of transmembrane aligned helices. As E5 is supposed to be present as a dimer to bind to the receptor, we performed solid-state $^{19}$F-NMR CODEX and CPMG experiments to characterize the homo-oligomerization interface of this protein. We were able to detect inter-molecular distance-dependent dipolar couplings between certain pairs of $^{19}$F-labels, namely for positions 6, 17, 28 in E5. These data providing direct evidence for E5 dimerization and allowed to construct a viable model for the E5 dimer in lipid membranes.

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