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

Katharina Becker, Colin Ziegler, Dirk Windisch, Anne S. Ulrich

Karlsruhe Institute of Technology (KIT), Institute for Biological Interfaces (IBG-2), POB 3640, 76021 Karlsruhe, Germany
contact: Katharina.Becker@kit.edu

The 44-amino acid oncogenic E5 protein from bovine papillomavirus is a short integral membrane protein. It activates the platelet-derived growth factor receptor β (PDGFR) in a ligand-independent manner through highly specific helix-helix interactions of the transmembrane segments.[1] This leads to receptor-dimerization followed by trans-autophosphorylation of the cytosolic kinase domains, and thus to sustained mitogenic signaling, which results in tumorigenic cell transformation.[2] 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. For this, complementary solid-state NMR and oriented CD measurements on macroscopically aligned samples are used to determine the conformation, alignment and dynamics of the two membrane-embedded proteins, first of each protein alone and then together in the hetero-oligomeric complex.
Our structural investigations showed that the transmembrane segment of PDGFR forms a left-handed helical coiled-coil dimer, whose orientation in the membrane is sensitive to the thickness of the lipid bilayer.[3] The dimer is stably inserted in membranes of proper thickness, but it becomes destabilzed when the membrane gets too thin. Notably, a similiar orientational behavior was also found for the E5 protein, which means that E5 can interact with the PDGFR through equally aligned transmembrane helices.[4]
In the next step we intend to investigate the molecular details of the E5/PDGFR-complex. The analysis of the molecular structure of the complex can give new insights in viral oncogenesis and in the activation of transmembrane proteins in general. For this, we want to measure distance constraints within the complex using NHHC spin diffusion NMR experiments.[5]

References

[1] Surti, T. et al., 1998, Structural models of the bovine papillomavirus E5 protein, *Proteins: Structure, Function, and Bioinformatics, Wiley Subscription Services, Inc., A Wiley Company,* 33, 601-612
[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] 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, ASBMB,* 287, 26178-26186
[4] Windisch, D. et al, in preparation vor publication.
[5] Lange, A. et al., 2002, Structural Constraints from Proton-Mediated Rare-Spin Correlation Spectroscopy in Rotating Solids, *Journal of the American Chemical Society,* 124, 9704-9705