Elucidating Receptor Cluster Formation in CD95 Signaling via DNA Origami and Multiparametric Image Spectroscopy

Nina Bartels[1], Nicolaas van der Voort[2], Ricarda Berger[3], Johann Weck[4], Simon Kempe[3], Tim Liedl[3], Joachim Rädler[3], Claus Seidel[2], Amelie Heuer-Jungemann[4] and Cornelia Monzel[1]

[1] *Experimental Medical Physics, Heinrich-Heine University Düsseldorf*
[2]*Molecular Physical Chemistry, Heinrich-Heine University Düsseldorf*
[3]*Faculty of Physics and Center for NanoScience (CeNS), Ludwig-Maximilians-University, Munich*
[4]*Max Planck Institute of Biochemistry and Center for Nanoscience (CeNS), Ludwig-Maximilians-University, Munich*

Cell fate is decided by the CD95 oligomerization state. Cluster of Differentiation 95 (CD95) is a membrane receptor protein that signals for controlled cell death (apoptosis) after ligand-induced activation. Some types of cancer show a deregulation of this CD95 apoptosis mechanism leading to proliferation rather than cell death[1]. As the molecular conditions leading to either proliferation or apoptosis are fundamentally not understood, we investigate the structural preconditions on the membrane as decisive mechanism to trigger a particular cell fate decision. However, measuring the oligomerization state of proteins on a cell membrane remains challenging. Here, we present two strategies to uncover the receptor activity states: on the one hand, we use DNA origami sheets exhibiting nanoscale tunable CD95 ligand arrangements. By mimicking characteristic receptor geometries, the sheets act as signaling platforms and enable to derive molecular benchmarks in apoptosis signal initiation[2]. On the other hand, we use calibrated time-resolved FRET experiments to quantitatively measure the supramolecular multimeric state of the CD95 receptor on live membranes directly. We further develop novel confocal methodologies in EGFP-bleaching experiments supported by super-resolved STED images to obtain an unambiguous interpretation of the CD95 oligomerization state.

[1] G. Gülcüler Balta, C. Monzel, S. Kleber, J. Beaudouin, T. Kaindl, M. Thiemann, C.R. Wirtz, M. Tanaka and A. Martin-Villalba, "3D cellular architecture modulates tyrosine activity thereby switching CD95 mediated apoptosis to survival". Cell Reports, 29, 2295-2306 (2019)

[2] Ricarda M. L. Berger, Johann M. Weck, Simon M. Kempe, Oliver Hill, Tim Liedl, Joachim O. Raedler, Cornelia Monzel*, and Amelie Heuer-Jungemann*, "Nanoscale FasL Organization on DNA Origami to Decipher Apoptosis Signal Activation in Cells". Small, 2101678 (2021) *corresponding authors