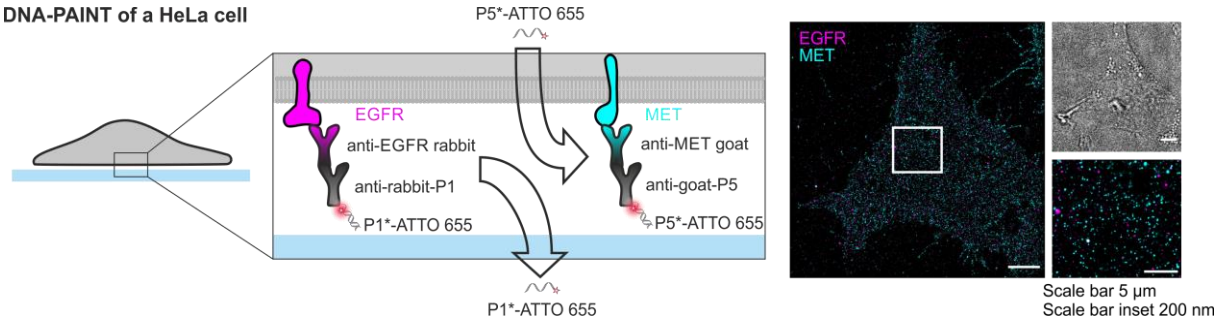
Multicolor super-resolution microscopy to quantify cellular expression and localization of EGFR/MET family receptor tyrosine kinases

K. R. Mertinkus*, M.-L. I. E. Harwardt, M. S. Dietz, M. Heilemann
Institute of Physical and Theoretical Chemistry
Johann Wolfgang Goethe-University, Max-von-Laue-Str. 7, 60438 Frankfurt, Germany
Email: mertinkus@chemie.uni-frankfurt.de

Keywords: Receptor tyrosine kinases, DNA-PAINT, Exchange-PAINT

Receptor tyrosine kinases (RTKs) respond to growth factors and regulate the development of vertebrates by triggering various signaling pathways for cell proliferation and migration. [1] Malfunction or mutation of RTKs are involved in several human diseases, such as cancer. Known oncogenes are amongst others the members of the epidermal growth factor receptor (EGFR) family [2] and hepatocyte growth factor receptor (HGFR or c-Met) [3]. Activation upon ligand binding leads to the formation of either RTK homo- or heterodimers. [4] However, the interplay of different RTKs is poorly understood until now.
Expression, distribution, activation and interaction of EGFR/ErbB2/ErbB3 and MET were studied in different cell lines using the single-molecule localization microscopy technique DNA-point accumulation for imaging in nanoscale topography (DNA-PAINT) [5]. For this, we used short, complementary DNA oligonucleotides that transiently bind and generate single-molecule blinking events. A DNA docking strand (PX) tagged to an antibody is used as a recognition label binding solely to the corresponding fluorophore-bound imager strand (PX*). Advantage of this method is the possibility to exchange imager strands using different, orthogonal DNA pairs. This multiplexing experiment, called Exchange-PAINT [6,7], allows to study the complex network of multiple RTKs at the cell membrane, and to record "molecular fingerprints" for different cell lines to understand the interplay of EGFR/ErbB2/3 and MET.



[1] M. A. Lemmon, J. Schlessinger, *Cell* **2010**, *141*, 1117.
[2] Blume-Jensen, P., Hunter, T. *Nature* **2001**, 411, 355.
[3] Birchmeier, C.; Birchmeier, W.; Gherardi, E.; Vande Woude, G. F., *Nature reviews. Molecular cell biology* **2003**, 4, 915.
[4] Hubbard, Stevan R.; Miller, W. Todd, *Current opinion in cell biology* **2007**, 19, 117.
[5] R. Jungmann, C. Steinhauer, M. Scheible, A. Kuzyk, P. Tinnefeld, F. C. Simmel, *Nano letters* **2010**, *10*, 4756.
[6] R. Jungmann, M. S. Avendaño, J. B. Woehrstein, M. Dai, W. M. Shih, P. Yin, *Nature methods* **2014**, *11*, 313.
[7] J. L. Werbin, M. S. Avendaño, V. Becker, R. Jungmann, P. Yin, G. Danuser, P. K. Sorger, *Scientific reports* **2017**, *7*, 12150.