

Insight into signaling: combining genetics, nanotechnology and microscopy to reveal molecular details and new mechanisms of tyrosine receptor kinases

**Donna J. Arndt-Jovin, Cornelia Fritsch^a, Guy M. Hagen,
Diane S. Lidke^b, and Thomas M. Jovin**

Max Planck Institute for Biophysical Chemistry, 37070 Göttingen, Germany

emails: djovin@gwdg.de, ghagen@gwdg.de, cfritsch@sussex.ac.uk,

dlikde@salud.unm.edu, tjovin@gwdg.de

The erbB family of receptor tyrosine kinases includes erbB1 (the classical EGF receptor, EGFR), erbB2, erbB3 and erbB4. Activation induced by the extracellular binding of EGF or other peptide ligands triggers signaling cascades responsible for cellular motility, cell division, and differentiation. A number of genetically constructed expression tags can be fused to the RTKs so as to specifically label them with intrinsic and extrinsic fluorescent probes for localization by microscopy. We have extensively used the visible fluorescent proteins and, more recently, the acyl carrier protein (ACP) sequence to which a fluorescent moiety of a coenzyme A (CoA) derivative can be enzymatically transferred (to a specific serine residue). We have targeted the ectodomains of the RTKs, such that only the transmembrane cohort is labeled on the external cell surface. Advances in nanotechnology have led to the emergence of photo- and chemically-stable semiconductor “quantum dots” (QDs), which can be excited by a single wavelength but emit according to their size and composition in discrete and separated spectral bands. By combining ligands attached to QDs and high-resolution microscopy techniques, we have gained new insights into the molecular interactions and the downstream signaling pathways of the erbB family of receptors [1-4]. We will report the newest findings of these investigations.

(1) *Activated ErbB1 mobility and retrograde transport.* QD-coupled EGF allows the visualization in living cells of individual EGFR receptors, the diffusion of which has been determined with emCCD cameras [4,5]. Utilizing these probes we discovered a systematic retrograde transport on filopodia of EGFR following EGF binding and activation. The process is linked to treadmilling of actin filaments. We propose that this phenomenon acts as a biosensor, in that receptors are transferred from remote sites of detection/activation to the transduction mechanisms in the cell body[2]. We have mutated specific tyrosine residues in the cytoplasmic tail of the EGFR so as to identify the adaptor molecules mediating the transport.

(2) *Association state of the erbB family.* The behavior of various hetero- and homodimers of the erbB family upon ligand activation determines both the direction and extent of the response. The overexpression and/or unrestrained activation of the RTKs, particularly of erbB2, are implicated in many types of cancer. Cluster size and location are being probed by hetero- and homo-FRET and STED microscopy.

(3) *Partition of activated receptor complexes.* The fate of activated receptors determines the extent and magnitude of signaling, and may provide insights as to how to inhibit oncogenic growth. Pulse chase experiments [3] conducted with CLSMs and high-speed widefield sectioning microscopy (“PAM” system) shed new light on the effect of various ligands in cells expressing different combinations of the RTKs.

[1] D.S. Lidke, et al. “Quantum Dot ligands provide new insights into receptor-mediated signal transduction” *Nat. Biotech.* 22, 198-203 (2004)

[2] D.S. Lidke, et al. “Reaching out for signals: filopodia sense EGF and respond by directed retrograde transport of activated receptors” *J. Cell Biol.* 170, 619-626 (2005)

[3] D.J. Arndt-Jovin, et al. “In vivo cell imaging with semiconductor quantum dots and noble-metal nanodots” *Proc. SPIE* 6096, 60960P1-11 (2006).

[4] G. Hagen, et al. In *Single Molecule Dynamics*, Ishii and Yanagida, eds., Wiley, (in press).