SFB 35 Colloquia in Membrane Transport

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Gerhard Schütz

(Johannes-Kepler-Universität Linz, Austria)

"Single Molecule Biology - Studying Movements and Meetings within the Plasma Membrane"

Current scientific research throughout the natural sciences aims at the exploration of the Nanocosm, the collectivity of structures with dimensions between 1 and 100nm. In the life sciences, the diversity of this Nanocosm attracts more and more researchers to the emerging field of Nanobiotechnology. In my lecture, I will show examples how to obtain insights into the organization of the cellular Nanocosm by single molecule experiments. Our primary goal is to understand the molecular organization of the plasma membrane and its impact on signaling processes. For this, we apply single molecule tracking to resolve the plasma membrane structure at sub-diffraction-limited length-scales by employing the high precision for localizing biomolecules of ~15nm. Brightness and single molecule colocalization analysis allows for studying stable or transient molecular associations in vivo. In particular, we developed a technique to detect molecular cluster formation directly in the live cell plasma membrane. With this methodology, individual aggregates can be selectively imaged, and the load of each cluster can be determined. We applied this technique to investigate the association of a fluorescent lipid analogues and lipid anchored proteins (1). Aggregates containing up to 4 probe lipids were observed to diffuse freely as stable platforms, shedding new light on the current debate concerning the existence of “lipid rafts”. Using the same technique we could further quantify the subunit stoichiometry of ion channels (2). Next, single molecule tools enable the direct observation of the random transitions a biomolecule experiences during its movements through the plasma membrane. For example, transient proximity due to interactions with other proteins can be directly monitored and quantified. I will present results on the interaction between antigen-loaded MHC and the T cell receptor directly in the interface region of a T cell with a mimicry of an antigen-presenting cell, using single molecule FRET (3). Finally, we developed a method for in vivo micropatterning of plasma membrane proteins to measure molecular interactions (4). This technology brings together

our interest in immune signaling, and the capability for ultra-sensitive readout of large biochip surfaces. Cells transfected with a fluorescent fusion protein ("prey") are grown on micropatterned surfaces functionalized with specific antibodies to the extracellular domain of a membrane protein ("bait"); the fluorescence copatterning is used as readout for the baitprey interaction. We applied this technology for the study of the interaction between CD4 – the major coreceptor for T cell activation – and Lck, an important tyrosine kinase in early T cell signaling. In addition to the well-known zinc-clasp structure, we found strong contributions of Lck membrane anchorage to the binding of the two proteins.

1. M. Brameshuber et al., J Biol Chem 285, 41765 (2010).
2. J. Madl et al., J Biol Chem 285, 41135 (2010).
3. J. B. Huppa et al., Nature 463, 963 (2010).
4. M. Schwarzenbacher et al., Nat Methods 5, 1053 (2008)