Abstract Dissertation Marco Lindner, 1456131

Membrane proteins are the interface of cell communication and the target of more than 60 % of all medical drugs. However, their quantitative analysis remains challenging, as the highly complex local environment, including other proteins, is critical to their functionality. In 2008, our group presented a method that allows to track the interaction of membrane proteins in space and time. For this purpose, the cells are placed on micropatterned substrates, which fix the membrane proteins in vivo on periodic areas. The lateral distribution of a fluorescently labeled target protein is monitored in the living cell by total internal reflection fluorescence microscopy (TIRFM). In case of interaction, the spatial distribution of the target protein will be that of the fixed membrane protein, while otherwise a more even distribution across the available space will be present.

The basis of this method is the substrate, which structures the arrangement of the membrane proteins. For this purpose, a two-dimensional pattern of anchor and block proteins is applied to a TIRFM-suitable substrate. The lateral resolution of the method for interaction detection is primarily defined by the periodicity of this protein pattern. Analysis of spatial heterogeneities in the protein-protein interaction requires the interrogation of contrast values at a spatial frequency which is higher than the desired resolution of the pattern. To improve the lateral resolution down to length-scales of one micrometer, a period of less than 500nm is necessary due to the Nyquist theorem. In this work a simple method to produce such substrates with protein patterns that can reach a period well below the optical resolution limit of 200nm is presented. For this purpose, nanocontact printing (nCP) is performed with a silicone that was originally developed in 2009 for a variation of nanoimprint lithography (NIL) and, to our knowledge, has not yet been used for contact printing.

First, masters were produced by phase transition mastering (PTM) and silicone stamps were molded from them. Two types of silicones with high Young’s modulus were used and the structural shape, diameter and period were varied to the technical limits of PTM. From this it could be deduced which degree of hardness of the silicone is required for which geometry. An equally important part of nCP is substrate preparation and coating, and several variants have been reviewed. A coating material that has not yet been used for µCP or nCP has been shown to give the best results. The functionality of the produced protein patterns was checked by TIRFM and super-resolution microscopy. Subsequently, a master was acquired that had a period of 140nm and nanopatterns were successfully created. At these length scales, the choice of the protein turned out to be crucial for success. These protein patterns were examined with atomic force microscopy (AFM) and stimulated emission depletion (STED) microscopy. With these nanopatterns, it will be possible to decrease the resolution of the protein interaction assay to below 300nm.