# INVESTIGATION OF MOLECULES INVOLVED IN CHRONIC INFLAMMATORY PROCESSES USING SINGLE MOLECULE LOCALIZATION MICROSCOPY IN CELLS

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Chronic inflammatory processes are associated with many major diseases like asthma, multiple sclerosis, or Alzheimer's disease. A key role in chronic inflammation is played by different pattern recognition receptors (PRRs), such as the Toll-like receptor 4 (TLR4) [1]. Binding of lipopolysaccharides (LPS) from gram-negative bacteria to TLR4 leads to the activation of different downstream signalling pathways. Eventually NF-κB translocates into the nucleus, where it initiates the transcription of pro-inflammatory cytokines. Interestingly, not only LPS can activate TLR4, but also a class of nutritional proteins, the wheat amylase-trypsin inhibitors (ATIs), which are resistant to intestinal proteolysis, can activate TLR4 as well [2,3].

Up to now the influence of LPS and ATIs on the spatial distribution of TLR4 are unknown, despite that effects in receptor downstream signalling are observed. The emergence of super resolution microscopy enabled the investigation of receptor arrangement at the nanoscale. It was reported that several membrane receptors are recruited in nanoclusters on the cell membrane, indicating a functional mechanism in receptor downstream signalling [4].

In order to investigate the spatial organization of TLR4 upon treatment with LPS and ATIs we used single molecule localization microscopy (SMLM) [5] and single molecule photobleaching. The distribution of TLR4 was accessed by using a density based clustering algorithm as well as pair-correlation analysis. Our measurements indicate a pre-clustered and non-random distribution of TLR4. Cluster analysis yielded a mean cluster diameter of 60 nm to 80 nm. The cluster size was independent of the cell line used, the stimulation time and the type of stimulation. To investigate the effect of different anti-inflammatory drugs on TLR4 signalling, we started to treat cells with pharmaceutical herbal extracts that inhibit the TLR4 activation pathway before adding LPS and ATIs, looking for changes in cluster size and density.

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