Abstract

Plasma membrane of T cells is abundant in diverse receptors and other molecules orchestrating immune responses. Numerous studies demonstrate that the localisation of proteins in the cell is non-random and that mislocalisation either in the context of plasma membrane at nanoscale or with respect to the cell interior can lead to the protein malfunction and subsequent aberrant T-cell response. In my first Ph.D. project we focused mainly on the role of the transmembrane domain length and amino acid composition, proximal sequences and the presence or absence of palmitoylation on the localisation of transmembrane adaptor proteins LAT, PAG and NTAL in T cells. We showed that plasma membrane localisation of PAG and NTAL is controlled by the amino acid composition of their TMD and is palmitoylation independent. We propose that NTAL localisation to the plasma membrane is, despite its suboptimal length, facilitated by the electrochemical asymmetry of its TMD. Among transmembrane adaptor proteins, LAT was the most interesting one. Dependency of LAT plasma membrane localisation on palmitoylation in combination with unusual amino acid composition of its TMD led us to investigate it in a separate project. My first author Ph.D. project was thus to elucidate the role of highly conserved helix-breaking amino acids, proline and glycine in the dynamics of LAT TMD and sorting to the plasma membrane. We found that the presence of central proline disrupts the α-helical structure of LAT TMD by inducing a kink and that the other proline and glycine amino acid residues have a role in overall helix properties. Exchange of helix-breaking amino acids for alanine or leucine led to the presence of these non-palmitoylatable LAT variants on the plasma membrane but surprisingly without capacity to support TCR-induced T-cell responses. Based on our data we hypothesised that palmitoylation of LAT is important for its proper nanoscopic localisation on the plasma membrane of T cells. Unfortunately, due to the changes in cellular morphology, we were unable to analyse the data from superresolution microscopy to further address this theory. The goal of my second co-author project was to investigate the importance of structural motifs for the proper membrane nanoscopic organisation of CD4 coreceptor. For the analysis of our superresolution data, we developed a model-free quantitative approach based on SOFI algorithm and discovered that plasma membrane organisation of CD4 relies on the presence of palmitoylation and its extracellular domain. This interested finding motivated us to further investigate the origin of those clusters. Using advanced algorithm which enabled us to map protein distribution in 3D nanometer resolution, we discovered, that CD4 coreceptor localizes to the tips of microvilli cells and that this localisation is driven by palmitoylation.