SUMMARY

Proteins represent one of the basic building blocks of all organisms. To understand their function at the molecular level is one the critical goals of current biological, biochemical and biophysical research. It is important to characterise all aspects that affect the localisation of proteins into different compartments with specific functions, the dynamic structure of proteins and their role in multiprotein assemblies, because altering these properties can lead to various diseases.

Most of the proteomic studies are nowadays performed using biochemical approaches that allow us to study multicellular organism or tissue at once. The disadvantage of these methods is complex preparation of sample and the need for a large number of cells, which leads to the loss of information at the molecular level and in individual cells.

On the contrary, microscopy can provide rather detailed information about proteins of interest and at the level of a single cell. A variety of fluorescence microscopy methods in combination with recombinant DNA techniques were applied to elucidate subcellular localisation of transmembrane adaptor proteins (TRAPs) in human lymphocytes and their nanoscopic organisation at the plasma membrane. Linker of activation of T lymphocytes (LAT), phosphoprotein associated with glycosphingolipid-enriched membrane microdomains (PAG) and non-T-cell activation linker (NTAL) were selected for our studies. These are single spanning proteins that lack significant extracellular domain, are palmitoylated and share comparable hydrophobicity and length of their transmembrane domain (TMD).

The necessity of palmitoylation for plasma membrane sorting of LAT, but not for other studied TRAPs, was demonstrated using transient expression of fluorescent fusion proteins. This different behaviour could be caused by the presence of helix-breaking residues introducing a dynamic kink in the centre of the structure of LAT TMD. The positive charge inside-rule was confirmed using model TRAP-like proteins carrying artificial TMD with different symmetry at the ends of the transmembrane segments. Also the presence of the DxE/ YxxØ amino acid sequence motifs responsible for release of proteins from endoplasmic reticulum was confirmed in the C-terminal part of LAT and PAG.

New sample preparation procedure for super-resolution microscopy was developed. This procedure radically improved the quality of acquired single molecule fluorescence data and, in addition, preserved the surface morphology of lymphocytes immobilised on the optical surface. The origin of previously described microclusters of CD4 was discovered by combination of our new protocol and improved and simplified version of the quantitative 3D analysis of single molecule localisation microscopy data. These accumulation patterns of CD4 at the tips of microvilli are palmitoylation dependent. In comparison, CD45 molecule was shown to be randomly distributed over the shaft and the base of microvilli. Indeed, its segregation from the tips of microvilli suggests the mechanism how surface morphology can regulate signalling in T cells.

In summary, the presented data indicate the importance of quantitative fluorescence microscopy for characterisation of proteins in their physiological environment, in individual cells and, in some cases, at the single molecule level.

Key words: LAT, PAG, NTAL, TRAP, CD4, CD45, fluorescence microscopy, super-resolution microscopy, T cell, transmembrane protein sorting, cell surface morphology