

# Abstract

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CD4 co-receptor of main T cell receptor (TCR) is essential for proper development of T lymphocytes and their function in adaptive immune responses. It is believed that CD4 stabilizes the interaction of TCR with antigenic ligand, peptide-MHC, and thereby improves T cell-dependent responses during immune reaction. CD4 is transmembrane glycoprotein with a number of structural motifs in its intracellular domain which do not dramatically affect its sorting to the plasma membrane but can influence its local organization at nanoscale. CD4 was shown to transiently accumulate in the immunological synapse formed between T cell and antigen-presenting cell. Such accumulation is rapidly followed by its internalization and/or delocalization outside the synapse. This is in contrast with TCR which accumulates strongly in the immunological synapse and is later found enriched in the central area of this structure. It is therefore unclear how TCR and its CD4 co-receptor function together when binding to their common ligand during the initiation of signaling in T cells.

We aim to study localization of CD4 at nanoscale using advanced fluorescence microscopy techniques achieving significant improvements in resolution. In this work, CD4 and its mutant variants, potentially causing its different localization at the membrane, were fused with fluorescent proteins and characterized. All constructs were confirmed to yield in expression of expected recombinant proteins which were localized to the plasma membrane and intracellular vesicles in human T cells. The constructs with photoconvertible fluorescent protein were further tested in localization microscopy.

This work succeeded in generation and thorough characterization of plasmids encoding CD4 and its mutants required for further work focused on precise localization of these molecules at the surface of activated T cells. Such knowledge can help to understand relation between TCR and CD4 during the early phase of T cell activation, yet further investigation will be required to clarify dynamics of immune synapse formation, and thereby the T cell activation.