A FRET-FLIM STUDY REVEALS THE INTERACTION BETWEEN ALCAM AND ACTIN AS A POTENTIAL REGULATOR OF ALCAM BINDING ACTIVITY

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Interactions between T cells and antigen-presenting cells represent the first step in the induction of an adaptive immune response. CD6 is a cell surface receptor expressed on mature T cells that specifically binds to activated leukocyte cell adhesion molecule (ALCAM). It has been shown that CD6 and its ligand ALCAM are actively recruited to the antigen-induced DC-T cell contact zone and that CD6-ALCAM interactions are also required during the proliferative phase of the T cell response. The molecular mechanism controlling ALCAM mediated interactions still remains unclear. Specifically, how the cytoskeleton dynamically regulates ALCAM binding activity at the cell-cell contact remains unknown. Transient cotransfection with Actin-RFP of a K562 cell line stably transfected with ALCAM-GFP was performed in order to investigate by FRET-FLIM the interaction between ALCAM and Actin. By measuring the donor fluorescence lifetime (GFP) in the absence and the presence of acceptor (RFP) the FRET efficiency and the distance between donor- and acceptor- labeled proteins were estimated. This FRET-FLIM study demonstrates the interaction between ALCAM and Actin (Figure 1) and opens the door for further investigation of the role of ALCAM-Actin interactions in the formation and stabilization of the immunological synapse.

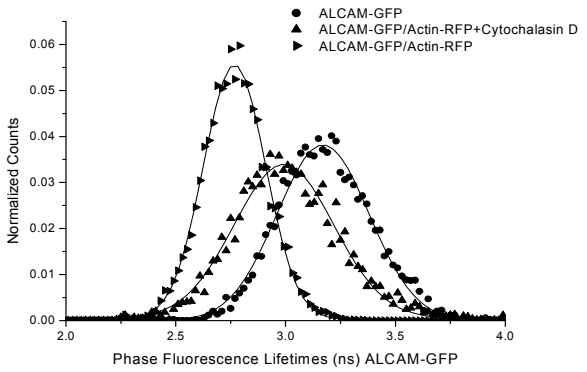

***Figure 1**. FRET-FLIM results – Histogram of averaged fluorescence lifetimes of ALCAM-GFP in K562 cells in selected regions of interest (ROI). A fluorescence lifetime decrease was detected for ALCAM-GFP in the presence of Actin-RFP. An average FRET efficiency of ~14% and a distance between donor and acceptor of ~ 7.7 nm were calculated for this experiment.In the presence of Cytochalasin D the FRET efficiency is reduced to ~10%. The solid lines represent the Gaussian fits.*

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