ProDOL: a general method to determine the degree of labeling for staining optimization and molecular counting

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Abstract

Determining the label to target ratio, also known as the degree of labeling (DOL), is crucial for quantitative fluorescence microscopy and a high DOL with minimal unspecific labeling is beneficial for fluorescence microscopy in general. Yet robust, versatile and easy-to-use tools for measuring cell-specific labeling efficiencies are not available. Here we present a DOL determination technique named protein-tag DOL (ProDOL), which enables fast quantification and optimization of protein-tag labeling. With ProDOL various factors affecting labeling efficiency, including substrate type, incubation time and concentration, as well as sample fixation and cell type can be easily assessed. We applied ProDOL to investigate how human immunodeficiency virus-1 pathogenesis factor Nef modulates CD4 T cell activation measuring total and activated copy numbers of the adapter protein SLP-76 in signaling microclusters. ProDOL proved to be a versatile and robust tool for labeling calibration, enabling determination of labeling efficiencies, optimization of strategies and quantification of protein stoichiometry.