Probing protein-protein interactions on the nm scale using TIRF/PALM

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Superresolution beyond the diffraction limit by e.g. photoactivation localization microscopy (PALM) has become indispensable for studying cellular processes and structures. PALM offers the powerful possibility to study the distribution of single labeled proteins in the nm range. The output of this technique is a spatial point pattern of molecular positions of a given labeled protein ensemble. These point patterns, however, should contain information about spatial correlations like clustering. Clustering is characterised by the deviation of the pattern from the arrangement of independent random events. The random distribution is given by a Poisson process. Thus, deviations, i.e. the degree and spatial scale of clustering or repulsion in such patterns provide information about the underlying molecular interactions. Here we explored whether spatial statistics based on k-Nearest Neighbour Analysis, Ripley's K- or L-functions or pair correlation analysis can be utilized to determine the stoichiometry of macromolecular assemblies in cell membranes. As simple models we expressed fusion constructs of the Shaker-IR potassium channel or the vesicle membrane protein Synaptobrevin 2, which is known to dimerize with its transmembrane domain, in the plasma membrane of HEK293 and HeLa cells. Using quantitative analysis of TIRF-PALM image stacks we succeeded in counting the exact number of monomers in these membrane protein complexes. Comparison with simulations of point patterns of different clustering degrees and of differing localization precision corroborates our finding that TIRF-PALM data can be used to faithfully extract information about protein-protein interactions on the nm scale.