Exploring the diffusion and interaction dynamics of the cytosolic peroxisomal import receptor PEX5 via FCS and STED-FCS

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Molecular interactions play a crucial role in cell signaling, thereby measuring diffusion dynamics in living cells is an essential step for a deep understanding of such interactions. While multiple approaches have been developed to study diffusion modalities in the cellular plasma membrane, this is less explored for measurements inside the cytosol. Here we present a detailed study of diffusion and interaction dynamics of the import receptor PEX5 responsible for the recognition of peroxisomal matrix proteins in the cytosol. Peroxisomes are small organelles that fulfil many anabolic and catabolic functions in mammalian cells by importing all required proteins post-translationally. Dysfunction of the peroxisomal import process leads to severe diseases making the molecules involved in this process a study of utmost importance. Here, we combine advanced live-cell microscopy and spectroscopy techniques such as fluorescence correlation spectroscopy (FCS) and super-resolution STED microscopy to present a detailed characterization of the diffusion and thus interaction dynamics of PEX5 in the cytosol. In this study we disclose an unexpectedly slow diffusion of PEX5, independent of many factors such as aggregation, target binding or cytoskeleton but associated with larger cytosolic interaction partners via the protein N-terminal half. In addition to these specific insights, our study highlights the potential of using complementary microscopy tools to reveal molecular interactions in the cytosol via studying their diffusion dynamics.