

A novel photoconvertible protein for accurate single molecule counting

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Keywords: quantitative SMLM, Phocl

The analysis of molecular clustering in membranes by SMLM is hampered by artefacts resulting from multiple localisations of the same molecule, leading to apparent clusters originating from a single molecule. Unfortunately, even single photoconvertible molecules such as mEOS2 exhibit significant blinking over timescales that make them incompatible with clustering SMLM experiments(1). Here we show, that Phocl(2), a green-to-red photoconvertible protein that disintegrates shortly after photoconversion, exhibits single molecule fluorescence compatible with SMLM. Furthermore, we perform quantitative SMLM on Phocl oligomers and verify if it can be used to represent a single molecule in quantitative SMLM.

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