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Conditional control of fluorescent protein degradation by an auxin-dependent nanobody

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Most biological processes involve spatial-temporal changes in the concentration of proteins that ensure that the right protein acts at the right place at the right time. Due to its high temporal resolution and minimal photo bleaching light sheet microscopy is ideally suited to visualize such protein dynamics given that the protein of interest is labelled with a fluorescent probe. Indeed, GFP-traps and increasingly CRISPR/Cas9-mediated fluorescent knock in's exist in several experimental systems ranging from tissue cell culture to model organisms and thus are great resources for light sheet microscopy experiments. To understand complex biological systems, however, we do not only need to visualize the emergent behaviour of protein, cells or organisms, but also to have to ability to interrogate the system. Here, we present an auxin-dependent GFP-nanobody to regulate the levels of overexpressed and endogenous GFP-tagged proteins in a conditional and reversible manner. We demonstrate efficient and reversible inactivation of the anaphase promoting complex/cyclosome (APC/C) in human tissue cell culture and thus provide new means to study the functions of this essential ubiquitin E3 ligase. Further, utilizing light sheet imaging, we show that the auxin-dependent GFP-nanobody can be applied to zebrafish. Hence, in principle the auxin-dependent GFP-nanobody has the potential to make any existing GFP-line in this and other model organisms compatible with auxin-mediated protein degradation thus enabling advanced functional studies.

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