Fluorescent probes that enable cell-based imaging of lysosomal enzyme activities and protein markers

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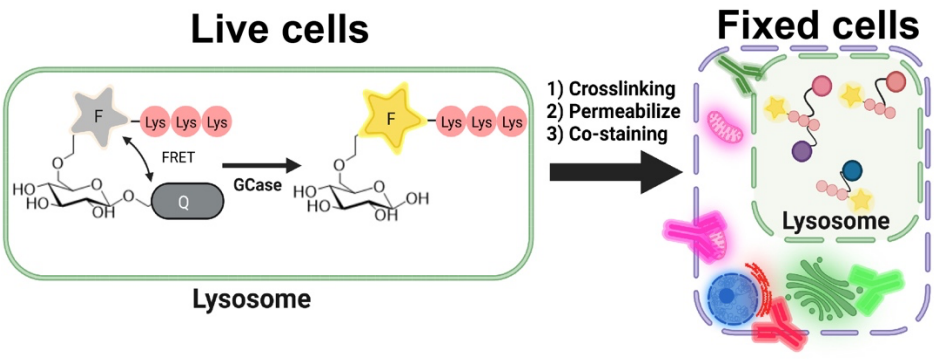
The ability to accurately quantify enzyme activity in live cells is of high interest both from an academic perspective as well as for translational research to enable new medicines, including gene therapies and next-generation enzyme replacement therapy[1,2]. The use of chemical tools such as fluorescence-quenched substrates is a promising approach to studying enzyme activity within live cells[3,4]. However, substrates for quantifying enzyme activity within fixed cells are lacking. Yet, such fixable substrates would permit the preservation of tissue samples for long-term storage as well as enable the concomitant use of other experimental methods such as immunofluorescence. Ideally, the fixing conditions used for such substrates would be the spatial distribution of protein factors of interest.

Here we present the first fixable substrate that enables quantitative analysis of enzyme activity within lysosomes of both live and fixed cells. We incorporated a small, fixable motif that enables the efficient chemical fixation of a fluorescence-quenched substrate for the Parkinson's Disease (PD) associated enzyme glucocerebrosidase (GCase) while preserving its fluorescence after fixation. Using fluorescence microscopy allows one to quantify the turnover of this probe, LysoFix-GBA, within both live and fixed cells. We demonstrate that LysoFix-GBA operates as a robust and selective tool to quantify chemical and genetic perturbations of lysosomal GCase activity. Furthermore, we apply LysoFix-GBA to multiplexed co-localization studies of GCase activity with various subcellular protein markers using immunocytochemistry. We expect that LysoFix-GBA will be broadly useful for compound screening and studying the role of GCase activity in PD, as well as for the development of new approaches targeting the GCase pathway for therapeutic benefits.



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