NEW FLUORESCENT LYSOSOMAL MARKER DYE WITH SUPERIOR STABILTY FOR LONG TERM LIFE CELL IMAGING

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Live cell imaging using fluorescence microscopy is widely used to follow dynamics in cells. Its application ranges from assessing alterations in ion concentrations, distribution of larger molecules as second messengers, proteins and lipids to analyse the uptake of larger assemblies like pathogens or drug formulations. To assess spatial distribution of different molecules or assemblies, fluorescent biomarkers for the different subcellular compartments are required. Ideally these labels should specifically enrich in one compartment, they should be brightly fluorescent, photostable and not be (photo)toxic. During the time course of measurement, the biomarkers should remain attached to the compartment and not be degraded or eliminated by the cells. Here, we present a new rylene-based dye which specifically labels lysosomes. These small acidic compartments are involved in the digestions of extracellular and intracellular content in eukaryotic cells. Using a HeLa cell model we investigated the cellular distribution and stability of the new dye over a time period of 48 h. The new biomarker is readily taken up by cells and colocalises with commercial lysosomal biomarkers. Even after 48 h of incubation, lysosomes appeared brightly fluorescent using the new label, whereas the commercial lysosomal marker had faded considerably after this time period. We characterised the spectroscopic properties of the dye by measuring absorption, excitation and emission spectra as well as fluorescence life times at different pH values. Remarkably, the dye exhibits spectral and life time changes upon changing the acidity of the solvent which are most pronounced in the pH range of 4.5-5 which represents the physiologic pH value of lysosomes. The new lysosomal biomarker might, thus, be utilized to define the physiological status of the lysosomes and their cargo over time. Fluorescence life time images and spectral scans in HeLa cells suggest different pH values between individual lysosomes.

References

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