HIDE for a closer look

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Preview for "Gupta, A., Rivera-Molina, F., Xi, Z. et al. Endosome motility defects revealed at super-resolution in live cells using HIDE probes. Nat Chem Biol 16, 408–414 (2020). https://doi.org/10.1038/s41589-020-0479-z".

Endosome is a membrane-bound organelle that plays a major role in material transportation in eukaryotic cells. Along the maturation process from early endosomes to late endosomes, the lumen pH lowers, the membrane proteins destined for recycling usage are concentrated to return to the cytoplasm membrane via budding, and degradation materials gathered from endocytosis remain in the late endosomal lumen to fuse with lysosomes. The disruption of endosomal motility can lead to atherosclerosis, Alzheimer's disease, and lysosomal storage diseases[1]. Because overexpressing organelle specific marker proteins fused with fluorescent proteins is not feasible in primary or patient-derived cells, organelle specific targeting probes would provide a valuable chemical too to visualize the morphology, distribution, and motility of these subcellular compartments.

Three major types of fluorescent probes for labeling late endosomes and lysosomes (LE/LY) for living cells are currently available, each with a different targeting mechanism. The first type of the probes contains mild amine moieties and accumulates to LE/LY by targeting their acidic environment[2-5]. The second type has an epoxysuccinyl scaffold[6, 7] that targets cysteine cathepsins which resides in LE/LY. The third type contains nanoparticles[8], epidermal growth factor[9] or dextrans moiety which will undergo endocytosis to reach LE/LY. Despite the LE/LY targeting mechanisms, current LE/LY fluorescent probes have their second part of the molecule as classical fluorophores, which are suitable for confocal or two photon microscopies.

The resolution of confocal, around 0.8 um, is limited by the detraction of light. It is not suitable to visualize the interaction between endosomes with other vesicles, which can be smaller than 200 nm. The super resolution stimulated emission depletion (STED) microscopy[10] can be employed for this purpose. However, the high intensity laser required in STED can easily photobleach the fluorophores in the current LE/LY probes when doing long time lapse for monitoring vesicle motility.

A Silicon Rhodamine (SiR) dye[11, 12] was previously developed to overcome the high laser intensity for STED. High photostability of organelle specific fluorescent probes can be achieved by linking SiR with distinct organelle targeting molecules. However, the conjugated molecules were shown failing to target the correct subcellular compartment. And thus, a two-step labeling was developed to target certain subcellular compartments, including Golgi[13, 14], ER and mitochondria[15]. First, the lipid-based organelle targeting molecule was delivered to cells and accumulated at the destination compartments. Then, a modified SiR is provided and will covalently bind to the targeting molecules through a "tetrazine-click" reaction *in cellulo*. A similar strategy was successfully applied to label late endosomes in this recent publication.

In this paper, Gupta *et al.* developed novel high-density environmentally sensitive (HIDE) probes that selectively label the endo-lysosome compartments with a super resolution STED microscopy for long time-lapse acquisition. This new chemical tool can evaluate endosome motility defects in Nieman-Pick C disease (NPD) patients.

The probe design consists of cell diffusible dialkylindocarbocyanine dye $DiIC_{16}$ analogues marking late endosomes and lysosomes, conjugated to a TCO moiety either at the aromatic core ($DiIC_{16}$,TCO) or at the terminus of a single lipid tail ($DiIC_{16'}$,TCO). The addition of silicon-rhodamine-tetrazine (SiR-Tz) upon tetrazine ligation generates a high-density environmentally sensitive (HIDE) probe. $DiIC_{16}$,-SiR and $DiIC_{16'}$,-SiR were verified to colocalize with the late endosome marker Rab7 and lysosome marker lamp1, and with a low colocalization coefficient with the early endosome marker Rab5, which indicates that these HIDE probes can be used to selectively visualize LE/LY *in cellulo*.

Using the galectin-recruitment assay and the epidermal growth factor receptor (EGFR) trafficking as read-outs, the authors demonstrated that neither endosome integrity nor its function is interfered. upon the endo-lysosomal HIDE probes treatment With STED microscopy, a resolution of 80nm was achieved for late endosome imaging, compared to the previously reported 400nm. Moreover, the endo-lysosomal HIDE probes survived up to 7 min exposure with STED without losing the fluorescent signal, which enabled the visualization of “kiss-and-run” events over a longer time-lapse.

The authors applied these novel endo-lysosomal HIDE probes to evaluate the endosome motility in wild-type fibroblasts and fibroblasts from NPD patients, which carry lysosomal transmembrane proteins NPC1 mutations, including I1061T, the heterozygous P237S/I1061T and R404Q. All three mutations were believed to have defects in endosome motility in correlation with cholesterol accumulation in endo-lysosomes, where the first two mutations have misfolded NPC1 in the endoplasmic reticulum and R404Q only reduces the binding of NPC1 with NPC2 on lysosomal membranes. Gupta *et al.* quantified the maximum velocity of the endosomes by STED microscopy over 7 minutes and found that the maximum velocity was significantly reduced in cells carrying I1061T and P237S/I1061T mutations, but not R404Q. Together, these results indicated that NPC1 correct folding is crucial for endosome motility, while cholesterol accumulation is not correlated to those motility defects.

These novel endo-lysosomal HIDE probes contributed to the study of endosome trafficking in cells expressing endogenously misfolded NPC proteins, and revealed that endosome motility is decreased only when the protein activity is impaired. A variety of lysosomal storage disorders are characterized by a dysfunctional enzyme or transporter that result in the accumulation of a specific substrate. NPD patients show cholesterol accumulation in the endosome lumen and endo-lysosome motility aberrations within their cells, but the details linking distinct NPD-mutations to endosome motility are still elusive.

Applications of HIDE probes with STED microscopy achieved higher resolution and enabled the discovery of the mechanistic endocytic pathway regulation. Future directions augmenting imaging capabilities include modifications at the dye design to increase fluorogenicity, development of membrane specific lipids that can discriminate endosomes from lysosomes, and conjugating SiR with distinct endo-lysosomal targeting molecules that employ a different mechanism of action that will not affect endo-lysosomal pH and therefore their functions. Those advanced chemical probes combined with powerful imaging technologies will provide a new toolkit for exploring distinct intriguing questions that remain unsolved in cell biology. Among those examples, endocytic

dynamics have emerged as crucial intracellular platforms for cargo delivering, neuropeptide release as well as virus or toxin intake. Screening for genes that modulate those processes will highlight unknown molecular key players that can be used to dissect “kiss-and-run” fission and fusion events, independent and clathrin-mediated endocytosis that can potentially provide therapeutic targets to improve metabolic diseases associated with endocytic dysfunctions.

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