DNA NANOTECHNOLOGY

Lysosomes uncovered

Two-ion measurements can potentially be used to screen therapeutics for lysosomal disorders in an unbiased way and to better identify patient cohorts for clinical trials

Lysosomes are not only the waste disposal system of cells, but they also regulate key cellular functions, including autophagy and innate immunity. Assigning specific functions to populations of lysosomes has been challenging thus far owing to difficulties in resolving lysosome chemotypes. Now, writing in *Nature Nanotechnology*, Yamuna Krishnan and colleagues demonstrate how a DNA-based reporter can be used to simultaneously measure lysosomal pH and chloride concentration in live cells, revealing chemically distinct sub-populations that play a role in lysosomal diseases.

Ionic levels, in particular proton and chloride concentration, are

critical for lysosome function, ensuring organelle maturation, cargo degradation and recycling. “Changes in pH and chloride concentration can be correlated to lysosome dysfunction, which is central to the pathology of Alzheimer’s disease, Parkinson’s disease as well as lysosomal storage disorders,” says Krishnan. Therefore, the researchers developed a DNA-based nanodevice that integrates a chloride-sensitive fluorophore and a pH reporter.

The fluorescent combination reporter — named ChloropHore — is made of a 61-base pair DNA duplex. The duplex comprises a chloride reporter domain, which contains a peptide nucleic acid sequence conjugated to a fluorescent, chloride-sensitive molecule, and a DNA switch that is triggered by protons and functions as a pH sensor based on fluorescence resonance energy transfer. The anionic DNA interacts with scavenger receptors on cells, which causes cellular uptake of the DNA and entry in the endolysosomal pathway. Thus, the DNA nanodevice can be targeted to individual lysosomes in cells, where it has a half-life of 20 hours and is stable for at least 10 hours.

In the lysosomal lumen, the DNA nanodevice can measure pH between 5.5 and 6.5 (and between 4.5 and 6.5 if a variant with modified 5’-bromocytosines is used). Moreover, emission intensities of the chloride-sensitive fluorophore show a linear response to chloride concentrations between 5 mM and 120 mM. Importantly, pH sensing is independent of chloride concentration and vice versa.

Therefore, the DNA reporter can simultaneously measure chloride concentration and pH, allowing the chemical profiling of individual lysosomes in a concentration range relevant to this acidic organelle.

“We have applied the two-ion measurement approach to lysosomes in fibroblasts derived from healthy humans and from patients suffering from lysosomal storage disorders,” explains Krishnan. “The chemical lysosome profiles reveal a sub-population of acidic, chloride-rich lysosomes in healthy humans that is lost in patients with lysosomal disorders.”

Krishnan and colleagues tested the effect of a therapeutic, which is used for the treatment of lysosomal storage disorders, on the chemical profile of lysosomes. Interestingly, when treated with the drug, the chloride-rich highly acidic lysosome population, characteristic of healthy cells, can be recovered in cell culture models of lysosomal storage disorders.

By directly visualizing relative abundances of lysosome chemotypes, and correlating these with known functions, the researchers hope to deconvolute chemotype–function relationships of lysosomes and to correlate these with disease. “Two-ion measurements can potentially be used to screen therapeutics for lysosomal disorders in an unbiased way and to better identify patient cohorts for clinical trials,” comments Krishnan.

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Credit: Adapted from Leung et al. (2018), Springer Nature Limited.

ORIGINAL ARTICLE Leung, K. et al. A DNA nanomachine chemically resolves lysosomes in live cells. *Nat. Nanotechnol.* <https://doi.org/10.1038/s41565-018-0318-5> (2018)