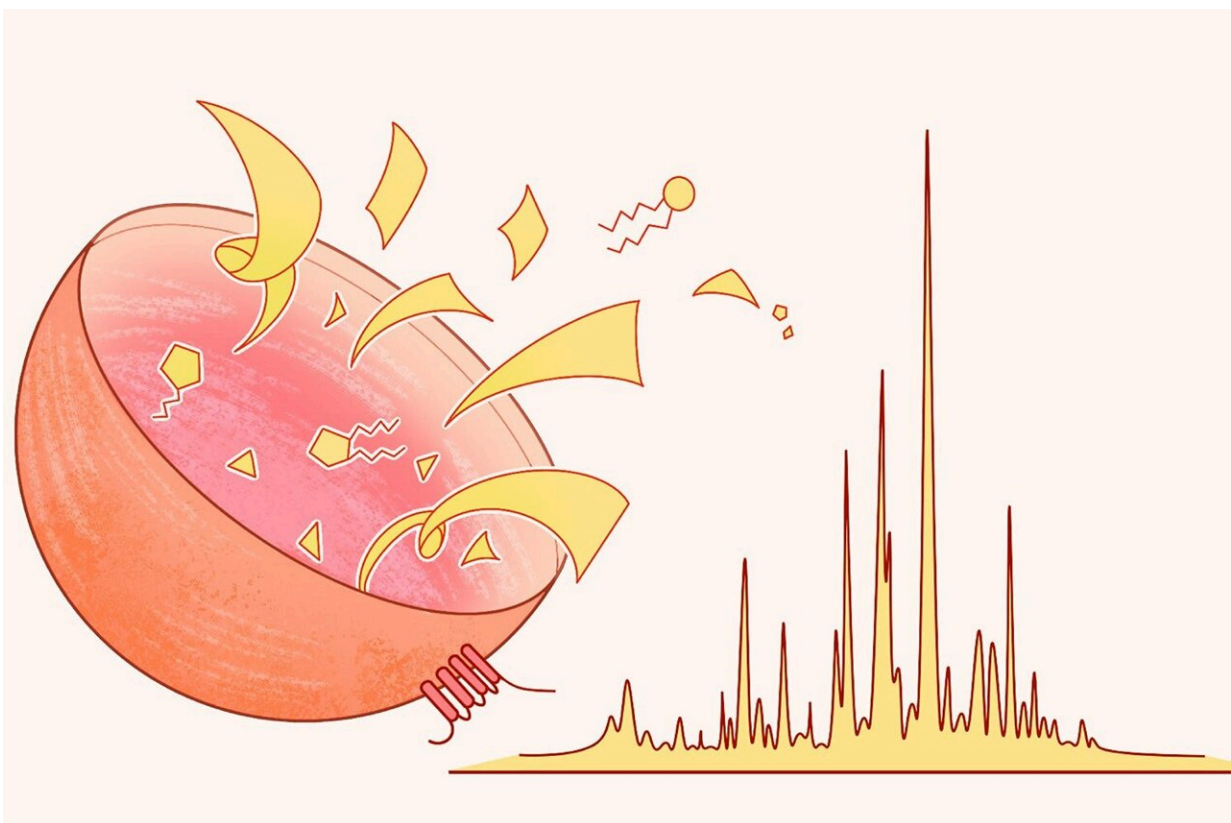


# New method allows scientists to determine all the molecules present in the lysosomes of mice

September 21 2022, by Rebecca McClellan

---



A cartoon representation of the new method, which allows scientists to isolate the lysosomes (left) of any cell in a mouse to analyze and identify using mass spectrometry (right) all the molecules inside them. Credit: Cindy Lin

Small but mighty, lysosomes play a surprisingly important role in cells despite their diminutive size. Making up only 1-3% of the cell by volume, these small sacs are the cell's recycling centers, home to enzymes that break down unneeded molecules into small pieces that can then be reassembled to form new ones. Lysosomal dysfunction can lead to a variety of neurodegenerative or other diseases, but without ways to better study the inner contents of lysosomes, the exact molecules involved in diseases—and therefore new drugs to target them—remain elusive.

A new method, reported in *Nature* on Sept. 21, allows scientists to determine all the molecules present in the lysosomes of any cell in mice. Studying the contents of these molecular recycling centers could help researchers learn how the improper degradation of cellular materials leads to certain diseases. Led by Stanford University's Monther Abu-Remaileh, institute scholar at Sarafan ChEM-H, the study's team also learned more about the cause for a currently untreatable neurodegenerative [disease](#) known as Batten disease, information that could lead to new therapies.

"Lysosomes are fascinating both fundamentally and clinically: they supply the rest of the cell with nutrients, but we don't always know how and when they supply them, and they are the places where many diseases, especially those that affect the brain, start," said Abu-Remaileh, who is an assistant professor of chemical engineering and of genetics.

Some proteins that are usually located in lysosomes are linked to a number of diseases. Mutations in the genetic instructions for making those proteins lead to these "lysosomal storage disorders," as they are collectively called, but the functions of some of these proteins have long puzzled scientists. Information about how these proteins work could help scientists develop better ways to diagnose, monitor, or treat these

diseases.

If scientists want to study the role a particular protein plays in the cell, they might either block or stimulate its function and see if certain molecules appear or disappear in response. But studying the contents of lysosomes is a problem of scale. "If something happens and a molecule grows in abundance 200-fold in the [lysosome](#), you would see only a two-fold increase if you look at the whole cell," said Nouf Laqtom, first author on the study. The revealing results get buried in the noise.

To quiet the noise, the researchers would have to separate lysosomes from everything else in the cell. They had previously developed a method to do just that in cells grown in labs, but they wanted to develop a way to do the same thing in mice.

## **Fishing for magnets**

The first step in their quest to isolate lysosomes was making a small change in the genes of the mice to install a little molecular tag on the surface of every lysosome in the entire animal. At any point when they want to stop and check on the molecules in the mouse lysosomes, like after fasting or feeding them a specific food, they turn on the tag in the cells they want to examine, and then remove the tissue and carefully grind it up to break open the cells without disrupting the lysosomes inside.

To fish lysosomes from the cellular sludge, the team relies on magnets. To their slurry they add tiny magnetic beads that are each decorated with molecular clamps that grab ahold of the lysosomal tag they had previously installed. They can selectively collect all the lysosomes using a second magnet, and then break apart the lysosomes to reveal the molecules that had been safely tucked inside. Mass spectrometry, a set of tools that determines the weights of different molecules in a mixture,

then helps the researchers identify the individuals in their lysosomal molecular potpourri. Those that grow or decrease would point scientists to certain pathways or functions.

Except for the little extra tag on each lysosome, these "LysoTag" mice are otherwise normal laboratory mice. Now, almost any researcher can use these mice to study the role of lysosomes in different diseases.

"These mice are freely available for anyone in the research community to use, and people are already starting to use them," said Abu-Remaileh. "We hope that this will become the gold standard."

## **Knowing where to look**

The team was eager to apply their method to study the lysosomes found in brain cells to better understand the neurodegenerative lysosomal storage diseases, starting with CLN3 disease or juvenile Batten disease. "We really see this as one of the most urgent problems we can help solve," said Abu-Remaileh.

Caused by a mutation in the gene that codes for a protein called CLN3, juvenile Batten disease is fatal and leads to vision loss, seizures, and progressive motor and mental deterioration in children and young adults. The CLN3 protein is found on the membrane of the lysosome, but no one has ever determined its function in the cell or how its dysfunction leads to the observed symptoms.

Using their LysoTag mice, the researchers collaborated with experts in both the Sarafan ChEM-H Metabolomics Knowledge Center and the Whitehead Institute Metabolomics Core Facility and found a dramatic increase in the amount of a kind of molecule called a glycerophosphodiester, or GPD for short, in [mice](#) with the CLN3 disease mutation. These GPDs are temporarily formed during the degradation of

the fatty molecules that make up the membranes of every cell in our body.

In healthy [cells](#), the GPDs do not accumulate in the lysosome; they get exported to a different part of the cell, where they are then degraded into smaller pieces. The researchers now believe that the CLN3 protein plays an important role in that export, either by directly shuttling out the molecules or by helping another [protein](#) do that job. They found GPD molecules in the cerebral spinal fluid of patients with CLN3 disease, which suggests that clinicians could potentially monitor GPD levels to measure the success of future treatments. The team is now determining which of the GPD [molecules](#) might be toxic and how the proteins involved in making and exporting GPDs could be targeted with [new drugs](#). They also are applying their method to look at other diseases that involve mutations in lysosomal genes, like Parkinson's disease.

"You can't develop new ways to diagnose or treat diseases if you don't know what is changing in the [lysosomes](#)," said Laqtom, a former postdoctoral scholar in the Abu-Remaileh lab. "This method helps you make sure you're looking in the right direction. It points you down the right path and keeps you from getting lost."

**More information:** Nouf N. Laqtom et al, CLN3 is required for the clearance of glycerophosphodiesterases from lysosomes, *Nature* (2022). [DOI: 10.1038/s41586-022-05221-y](https://doi.org/10.1038/s41586-022-05221-y)

Provided by Stanford University

Citation: New method allows scientists to determine all the molecules present in the lysosomes of mice (2022, September 21) retrieved 25 April 2024 from <https://phys.org/news/2022-09-method-scientists-molecules-lysosomes-mice.html>

This document is subject to copyright. Apart from any fair dealing for the purpose of private study or research, no part may be reproduced without the written permission. The content is provided for information purposes only.