

Cellular recycling caught in the act

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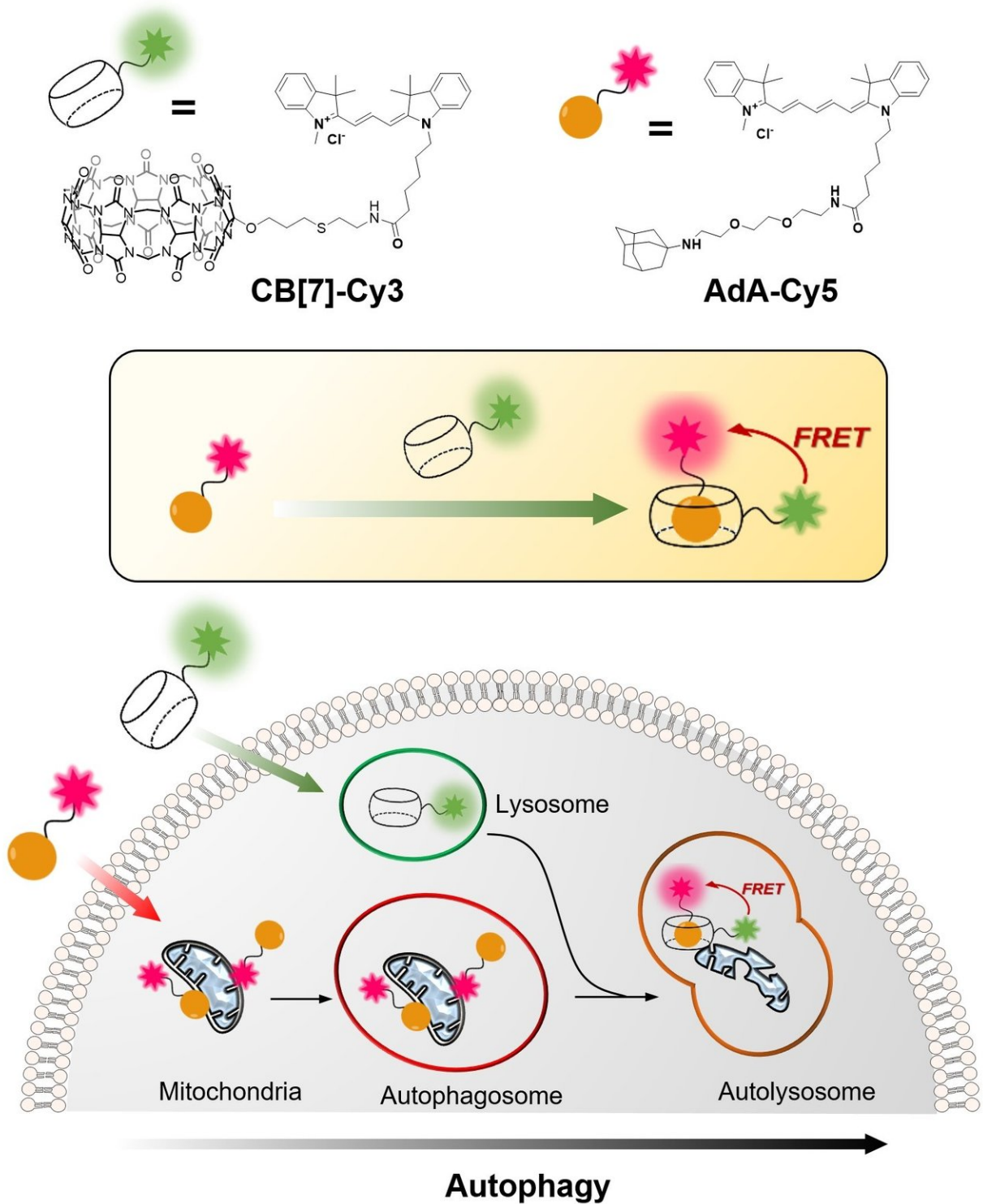


Figure 1: Relatively simple and inexpensive technique to visualize the cellular recycling mechanism (autophagy) in real time. The process is based on the binding between CB[7] and AdA. CB[7] is bound to the fluorescent dye Cy3

(colored in green) and enters the lysosomes, while AdA is bound to the fluorescent Cy5 (colored in red) which accumulates in mitochondria. In the autophagy process, the two cellular organelles fuse together, leading to the encounter of CB[7] and AdA. The scientists can detect the fusion, because when the two fluorescent dyes move closer, they interact with each other via the fluorescence resonance energy transfer (FRET) mechanism. In this way, the research team can analyze the autophagy process step by step. Credit: Institute for Basic Science

A team of researchers at the Center for Self-assembly and Complexity, within the Institute for Basic Science (IBS) have observed a normal physiological process, called "self-eating", that cells use to recycle their components. They developed an accurate technique that visualizes how mitochondria, cells' energy factories, are fused with lysosomes, cells' recycling machines, in order to get material destroyed and recycled. Since irregularities in this mechanism can lead to Alzheimer's, Parkinson's, and other complications, this research could help future advances in degenerative brain disease diagnosis and drug development. The study was published in *Angewandte Chemie International Edition*.

The name "autophagy" comes from the Greek language and means "self-eating". As weird as it sounds, it is an indispensable process used to clean [cells](#) from damaged organelles and proteins. Inside lysosomes, old cellular parts are broken down to their building blocks, which are employed to build new ones. Recently, autophagy became a very active field of study and was at the center of Yoshinori Ohsumi's Nobel Prize in Physiology or Medicine in 2016.

One example of cellular organelle that must be periodically recycled is the mitochondrion. The careful regulation of this process is essential, and cells that do not divide regularly, like nerve cells, are particularly vulnerable. If cells accumulate [defective mitochondria](#), they can damage

themselves. This occurs, for example, in Alzheimer's and Parkinson's diseases, where build-up of damaged mitochondria and aggregated proteins leads to neuronal death.

It is known that autophagy (or mitophagy, in the specific case of mitochondria) occurs by fusion of two different organelles, in this case: mitochondria and lysosomes. However, observing the behavior and fusion of mitochondria and lysosomes inside the cells has been challenging. To date, most of these studies rely on [fluorescent proteins](#) attached to one organelle, which allows the scientists to observe only one organelle at a time. Moreover, fluorescent proteins themselves are degraded during autophagy, making it difficult to accurately study the mechanism.

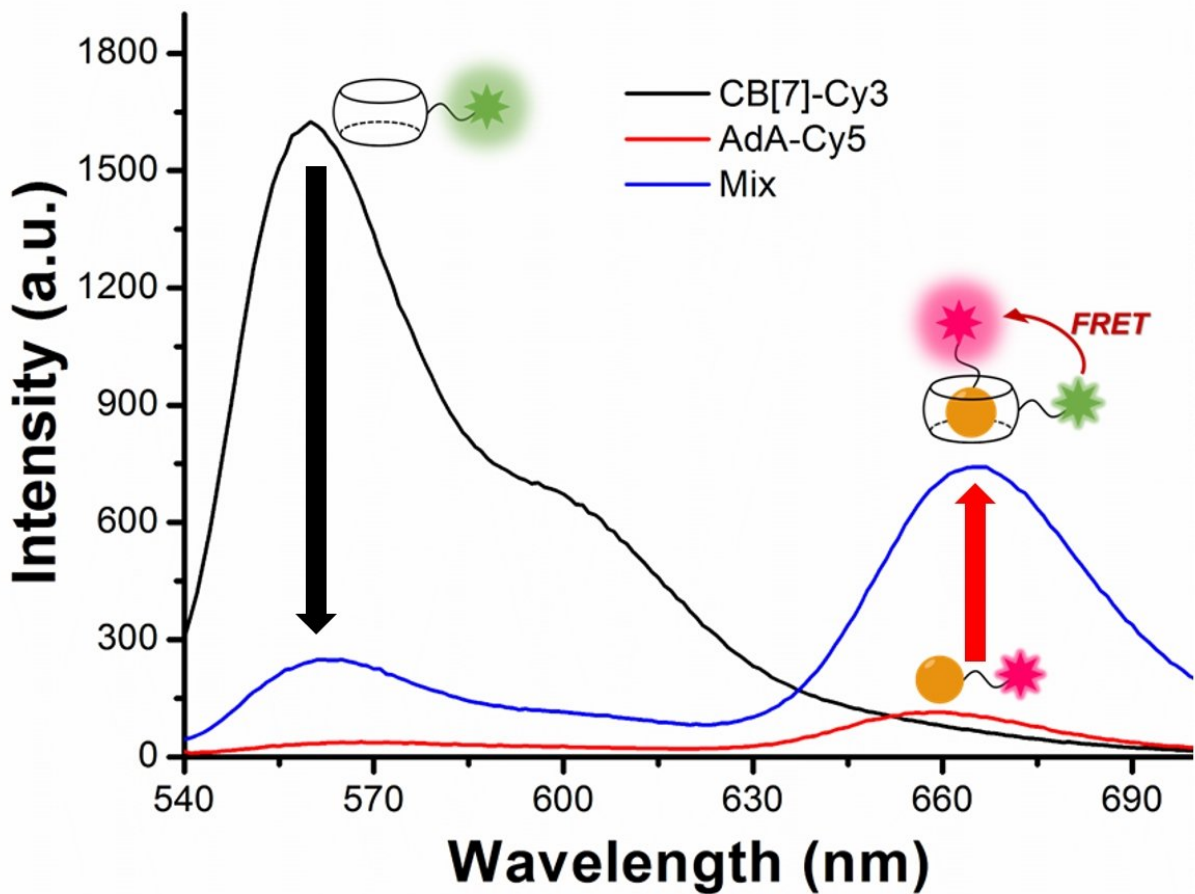


Figure 2: Studying autophagy with fluorescence resonance energy transfer (FRET) between the greenish fluorescent dye Cy3 and the red fluorescent dye Cy5. Cy3 emits fluorescence with a peak wavelength at 563 nanometers (black line), while Cy5 at 670 nanometers (red line). In the experiment, Cy3 is attached to CB[7] and Cy5 to AdA. During autophagy, the two dyes come closer and Cy3 transfers some energy to its nearby partner dye Cy5, following the FRET phenomenon. The scientists capture the meeting of the two dyes as an increase in fluorescence with wavelength 644-800 nanometers (blue line). Credit: Institute for Basic Science

IBS scientists at POSTECH devised a quantitative procedure to visualize

both mitochondria and lysosomes over time. The relatively simple and inexpensive technique is more accurate than the current ones. It uses hollow barrel-shaped synthetic molecules, known as cucurbituril (CB[7]), that binds with exceptionally high binding strength to a molecule called adamantylamine (AdA) and cannot be degraded by the lysosomes. CB[7] was decorated with a fluorescent dye (Cy3), while AdA with another dye (Cy5). Initially, CB[7]-Cy3 enters the lysosomes and AdA-Cy5 the mitochondria, and then, when the two organelles fuse in the recycling process, CB[7]-Cy3 and AdA-Cy5 bind together. In this way, the research team followed the behavior of different organelles and observed the mitophagy process occurring in live cells. Moreover, the experiments showed that the two compounds are not toxic for the cells below a 800 nanomolar dose.

"The most challenging part of the experiment was the choice of the chemicals. We chose chemicals with the right hydrophilicity, charge, and molecular size to be selectively incorporated into [mitochondria](#) and lysosomes," explains PARK Kyeng Min, one of the corresponding authors of the study.

In more technical terms, the novelty of this paper relies on the application of fluorescence resonance energy transfer (FRET) to the study of autophagy. FRET is a distance-dependent transfer of energy between two different fluorescent dyes; in this case Cy3 and Cy5. When the two are close enough, Cy3 donates energy to Cy5. The scientists capture the meeting of the two dyes as the transfer of energy from Cy3 to Cy5 leads to a reduction in Cy3 fluorescence intensity in favor of an increase in Cy5 emission intensity.

In the future, a similar technique could be used to study [autophagy](#) processes that involve other cellular organelles, like the protein factories Golgi apparatus and endoplasmic reticulum.

More information: Meng Li et al. Autophagy Caught in the Act: A Supramolecular FRET Pair Based on an Ultrastable Synthetic Host-Guest Complex Visualizes Autophagosome-Lysosome Fusion, *Angewandte Chemie* (2017). [DOI: 10.1002/ange.201711629](https://doi.org/10.1002/ange.201711629)

Provided by Institute for Basic Science

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