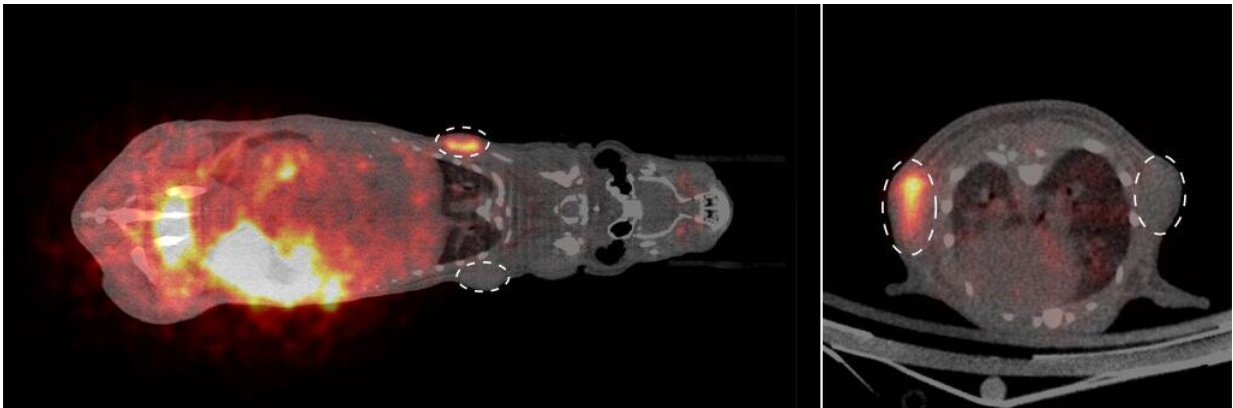


Cell labeling method from microscopy adapted for use in whole-body imaging

October 1 2021, by Doris Niederhoff



PET imaging of tumours (dashed circles) in the body of a mouse (right in cross-section). Researchers used a newly developed radioactive substrate to label tumour cells in a living organism. Cells genetically modified to produce a so-called SNAP-tag enzyme took up the radioactive marker (orange), while cells without this enzyme did not. Credit: Depke et al.

Processes and structures within the body that are normally hidden from the eye can be made visible through medical imaging. Scientists use imaging to investigate the complex functions of cells and organs and search for ways to better detect and treat diseases. In everyday medical practice, images from the body help physicians diagnose diseases and monitor whether therapies are working. To be able to depict specific processes in the body, researchers are developing new techniques for

labeling cells or molecules so that they emit signals that can be detected outside the body and converted into meaningful images. A research team at the University of Münster has now adapted a cell labeling strategy currently used in microscopy—the so-called SNAP-tag technology—for use in whole-body imaging with positron emission tomography (PET) for the first time.

This method labels cells in two steps that work for completely different cell types such as tumor and [inflammatory cells](#). First, the cells are genetically modified to produce a so-called SNAP-tag enzyme on their surface that is unique to the targeted cells. The enzyme is then brought into contact with a suitable SNAP-tag [substrate](#). The substrate is labeled with a signal emitter and chemically structured so that it is recognized and split by the enzyme allowing the signal emitter to be transferred to the enzyme. In the process, the enzyme is modified so that it is no longer active and, as a result, the signal emitter remains tightly coupled to it. "Through its [biological activity](#), the SNAP-tag enzyme labels itself, so to speak—this happens very quickly and without disturbing the natural processes in the organism," explains Dominic Depke, a biology doctoral student and one of the lead authors of the new study.

In microscopy, fluorescent dyes are used to label cells, but they are mostly not suitable for whole-body imaging because their signals are scattered by thicker tissue layers with the result that they can no longer be measured. To solve this problem, the scientists synthesized a new SNAP-tag substrate using the radioactive signal emitter fluorine-18. The team have successfully labeled tumor cells in mice by injecting this substrate into the organism via the bloodstream and were then able to visualize the tumors using PET imaging. "The exciting thing for us about SNAP-tag technology is that it opens up the prospect of visualizing genetically encoded cells in the body with different imaging modalities and at different temporal stages—we call it multiscale imaging," explains nuclear medicine specialist Prof Michael Schäfers. "Radioactive signals

from fluorine-18 remain stable for only a short time," adds radiochemist Dr. Christian Paul Konken, "but as we can repeat the second labeling step, we can potentially visualize the same cells again and again over days and weeks." The high level of detail provided by microscopy makes it possible to study how individual cells communicate with each other. The big picture view provided by whole-body imaging enables scientists to assess how these cells function as part of whole organ systems. Time may reveal what role individual cell types play in inflammation, for example, as it begins, continues and resolves. "Only by combining all this information can we understand how everything is connected in the body," says Michael Schäfers.

A small beginning with great potential

"Our investigations are a very first step, in which we have shown that labeling cells with SNAP-tags works, in principle, in living organisms," says biochemist Prof Andrea Rentmeister. "What matters here is that the substrate is distributed rapidly in the organism and that it binds exclusively to the cells to be studied." The next crucial steps will be to test how many cells are needed to obtain a sufficiently strong signal and whether the method can also be used to visualize cells that move within the organism—in particular immune system cells. If the approach continues to prove successful, the technique may become important for future research into immunotherapies in which the body's own immune cells are genetically modified in the laboratory so that they can combat a specific disease. Such therapies are already being used for cancer treatment and have the potential to help treat inflammatory diseases as well. Imaging could help develop and improve such treatments.

When the scientists presented their results for the first time at a scientific symposium, they were in for a surprise—colleagues from Tübingen presented a similar study there at the same time. Independently of each other, both research teams had the same fundamental idea, a

SNAP-tag substrate labeled with fluorine-18. Chemically speaking, they implemented the idea differently but they tested the resulting substrates using the same biological model system and arrived at similar findings. "This shows how topical our question is and that our results are reproducible and really promising," says Michael Schäfers. He adds that the Tübingen team is developing new labeling methods to study immune cells in cancer, while the team in Münster is focusing on inflammatory diseases, so the research complements each other very well. The research team from Münster published their study in the scientific journal *Chemical Communications*; only a few days later, the publication from Tübingen was released in *Pharmaceuticals*.

Creating a new substrate for the SNAP-tag

Like all SNAP-tag substrates, the newly developed molecule is based on benzylguanine to which the scientists attached the radioactive isotope fluorine-18, which is, in turn, ideally suited for PET imaging. "Our goal was to design the synthesis in a few quick steps so that we get as strong a signal as possible—because fluorine-18 has a short half-life, its radioactivity is reduced by half after every 110 minutes," explains Christian Paul Konken. Initially, the scientists found that the fluorine-18 did not attach to the desired position on the molecule. "The benzylguanine was apparently too sensitive to be labeled directly with fluorine-18," says Lukas Rösner a biochemistry doctoral student, "so we first labeled a small molecule that is insensitive to the necessary chemical reactions—the fluoroethylazide—and then attached it to the benzylguanine using a click reaction, which is very fast and selective."

Tests in test tube, cell cultures and the organism

The scientists first checked whether the synthesized substrate remained stable when in contact with blood in the test tube and then examined how

the cells interacted with the substrate in the first practical tests in cell cultures. In doing so, they compared human tumor cells into which they had genetically incorporated the SNAP-tag enzyme with those that did not produce the enzyme.

"We could see very clearly that the radioactivity was only taken up by the cells that produced the SNAP-tag enzyme," says Dominic Depke. Finally, the team conducted targeted studies on individual mice. "This step was decisive once again," says Michael Schäfers, "because how a molecule behaves in the complex biological environment in a living organism cannot be fully simulated in cell culture or with artificially produced organs."

The scientists were able to show that once the substrate is injected into the bloodstream it is distributed through the body very quickly. Additionally, they identified the pathways through which it is excreted. They then compared how tumor cells with and without the SNAP-tag enzyme reacted to the substrate in living organisms. For this purpose, the tumor [cells](#) were injected under the skin of mice and removed again after the examination in order to confirm the results with autoradiography.

More information: Dominic Alexej Depke et al, A novel ¹⁸F-labeled clickable substrate for targeted imaging of SNAP-tag expressing cells by PET in vivo, *Chemical Communications* (2021). [DOI: 10.1039/D1CC03871K](https://doi.org/10.1039/D1CC03871K)

Provided by University of Münster

Citation: Cell labeling method from microscopy adapted for use in whole-body imaging (2021, October 1) retrieved 25 April 2024 from <https://medicalxpress.com/news/2021-10-cell-method->

[microscopy-whole-body-imaging.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.