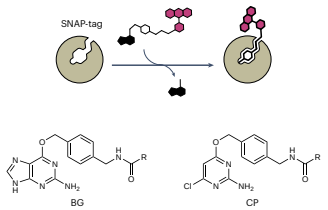
Chemical biology

SNAP-tag2 improves live-cell imaging



SNAP-tag2 labeling scheme with two example substrates, O^6-benzylguanine (BG) and chloropyrimidine (CP). Reproduced from S. Kühn et al. *Nat. Chem. Biol.* https://doi.org/10.1038/s41589-025-01942-z (2025), CCBY 4.0.

Self-labeling protein tags (SLPs) are genetically encodable proteins that can form a covalent bond with synthetic dyes or fluorogenic probes. SLPs, including HaloTag, SNAP-tag and CLIP-tag, are commonly used for real-time bioimaging for the visualization of protein dynamics and other cellular events, with HaloTag7 being the most popular SLP for live cell imaging. SNAP-tag has limited cell permeability and reduced labeling kinetics and fluorogenicity, making is less suitable for live-cell imaging applications.

A group of researchers led by Julien Hiblot and Kai Johnsson from the Max Planck Institute for Medical Research, Heidelberg, Germany, have been working on expanding the toolbox for live cell imaging. Their recent publication presents SNAP-tag2, an advanced version of SNAP-tag with improved cell permeability, increased labeling kinetics and greater brightness. "SNAP-tag2 allows labeling at much lower concentrations and much shorter timescales, which directly translates into a much powerful system for fluorescence labeling," explains Johnsson.

The authors first developed different substrate scaffolds that had better reactivity in vitro for improved labeling efficiency and higher live-cell compatibility. Then they used computational modeling, directed evolution and deep mutational scanning to improve the reactivity and stability of SNAP-tag. The new system, SNAP-tag2, carries 11 substitutions and replacement of an 18-amino acid segment with one 8 amino acids long.

Using three different cells lines, various substrates and direct comparisons to SNAP-tag, the researchers show that SNAP-tag2 is 100-fold faster and maintains high specificity and minimal cellular disruption. They also showed that SNAP-tag2 performs well for super-resolution microscopy in yeast cells.

"Conceptually, SNAP-tag2 is not different from HaloTag or CLIP-tag; however, what is important that the community now has two covalent labeling systems, i.e., SNAP-tag2 and HaloTag7, that enable very efficient/clean labeling with (far-red) fluorophores. The two systems can also be used simultaneously for multi-color experiments," explains Johnsson. However, when asked about the current limitations, Johnsson adds, "Applications in vivo are still challenging for all current labeling systems and will require the further engineering of the pharmacokinetic properties of the substrates used for labeling in vivo."

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Microbiology

Optical pooled screens shed light on Ebola infection

Filoviruses, a group of negative-sense single-stranded RNA viruses that encompasses Ebola, Marburg and Sudan viruses, among others, cause frequent deadly outbreaks. While some therapies and vaccines have been developed, treatment options are still limited, and the need to better understand the interplay between host factors and viral infection throughout the viral life cycle is urgent.

To tackle this challenge, researchers led by Paul Blainey at the Broad Institute and Robert Davey at Boston University used optical pooled screening (OPS) technology to assay the effects of genetic perturbations of host factors on different stages of the life cycle of Ebola, uncovering previously unknown positive and negative modulators of infection.

OPS, originally developed for mammalian cells in the Blainey lab, is a high-throughput genetic screening technology that couples CRISPR-based genetic perturbation with microscopic observation of markers of interest in fixed or live cells and in situ sequencing to link each cell's perturbation with its phenotype. While genetic screens have been used to study Ebola infection, these have been biased toward strong early inhibitors selected by preventing virus-induced cytotoxicity. "We saw the potential of high-content screening with OPS to go beyond previous screening approaches to identify host factors relevant to multiple different stages of the filovirus lifecycle," notes Davey. To this end, team members Rebecca Carlson and Justin Patten generated OPS data to identify 998 host regulators of Ebola virus infection in 39,085,093 human cells. The team evaluated viral protein and RNA synthesis as markers of infection and applied a deep learning model to associate each host factor with a particular viral replication step.

Working with highly pathogenic viruses is bound to face challenges. "The assay development and first steps of the screening had to be carried out using complex high-containment procedures to ensure this work was done safely," says Blainey. The big data aspect of the work presented different hurdles, he notes. "The scale of the data from this genome-wide screen, approximately 40 million high-quality cells with identified perturbations, is by itself similar to the largest cell atlas projects completed, so handling the data and the machine-learning aspects of our project were also challenging."

Building on their intriguing results, Blainey and coauthors would like to "leverage OPS further to go deeper into filoviral biology and identify new opportunities for therapies that can help protect people everywhere from these devastating infections." They also hope that protocol, instrumentation and software development will broaden access to allow even larger-scale genetic screens to be completed routinely and uncover yet more viral regulators. "Of course, we look forward to many virologists interacting with the data to see what more they can discover from it and how the results might accelerate their own independent work," says Blainey. "We also hope we inspire more labs to take advantage of this powerful functional genomic technology."

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