PROTEIN BIOCHEMISTRY

Sorting out the best targets

A new fluorescence-based bacterial display assay could greatly accelerate the identification of target sequences for an increasing number of uncharacterized proteases.

As the number of identified proteases continues to grow—to date, nearly 700 have been found in the human proteome—the need for a reliable and high-throughput method for characterizing target-sequence specificity has become more and more apparent. A variety of screens have been tested in recent years, including methods based on viral display, pooled substrate libraries and peptide arrays, and these have yielded some valuable information regarding the target specificity and kinetics of previously uncharacterized proteases.

University of California at Santa Barbara researcher Patrick Daugherty and graduate student Kevin Boulware have now added another promising method to the proteomics toolbox, based on the fluorescence-assisted cell sorting (FACS) of a bacterial display library. Their library is composed of *Escherichia coli* cells expressing chimeric cell-surface proteins, which consist of a streptavidin-binding peptide linked to a variable six-residue 'substrate' region. The library is labeled with a fluorescent streptavidin-conjugated phycoerythrin probe, which binds to the chimeric display protein; then fluorescently tagged cells are treated with a protease of interest. Cells expressing suitable target sequences lose their fluorescence as a result of protease cleavage, and can be sorted via FACS for expansion and characterization. Boulware and Daugherty have termed this method of screening with cellular libraries of peptide substrates 'CLiPS'.

They tested CLiPS with two proteases for which canonical substrates have been identified, caspase-3 and enteropeptidase. After multiple rounds of sorting, they identified several individual clones as containing appropriate substrate sequences for each protease. DNA sequencing of clones with the most efficient cleavage kinetics revealed a caspase-3 consensus target sequence, which closely matched the previously identified canonical target, DxVDG; in contrast, the team was surprised to identify several enteropeptidase substrates that differed substantially from but were cleaved more efficiently than the established DDDDK target sequence. They subsequently confirmed the relative kinetics of the different target sequences in additional assays with synthetic fluorogenic peptide substrates.

The authors tout their method as a rapid means for examining the specificity and cleavage kinetics of proteases, and suggest that it should be likewise extensible for use with mammalian or yeast cells, which could potentially allow investigators to examine the impact of post-translational modifications on cleavage.

Michael Eisenstein

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Boulware, K.T. & Daugherty, P.S. Protease specificity determination by using cellular libraries of peptide substrates. *Proc. Natl. Acad. Sci. USA* **103**, 7583–7588 (2006).

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CHEMICAL TOOLS

SHEDDING LIGHT ON THE BARRIER PROBLEM

Researchers have harnessed the luciferin system to monitor passage of releasable luciferin–transporter conjugates into the cell.

An understanding of how molecules cross biological barriers is important both for pharmacological applications and basic research. Present methods, however, allow only a qualitative assessment of passage across membranes. Paul Wender and his colleagues at Stanford University hoped to go from this qualitative understanding to a more quantitative tool.

They designed a releasable luciferin–transporter system comprising an octaarginine transporter conjugated to a luciferin molecule via a disulfide linker. Upon entry into the cell, this linker is cleaved, releasing free luciferin. The uptake of this construct into cells can be measured in cells expressing luciferase, which catalyzes the oxidation of luciferin, resulting in the release of a photon. "The beauty of the system is that unlike fluorescence, we get a photon for every turnover event, so it's pristinely quantifiable," says Wender.

In the work described in the *Journal of the American Chemical Society*, Wender and colleagues report the synthesis of releasable luciferin–transporter conjugates and validation of this system in prostate cancer cells stably transfected with a luciferase-encoding gene. They were able to quantify, in real time, the uptake and release of the conjugates by measuring the luminescence.

Armed with this assay, researchers now can study transport and delivery of cargo in different cell types and tissues, as well as design conjugates that transport molecules effectively and release them at the desired rates. Further, this assay can be used to quantify the expression levels of bioactivatable targets, such as proteases, in cells and tissues: researchers could design a transporter with a linker containing a specific protease cleavage site and then use the luciferase assay to determine the activity of this enzyme. Using this information, they can design conjugates that release their cargo only in specific tissues.

One important advantage of this luciferin-based system is that researchers do not have to fix cells or section tissues or even kill animals. As Wender summarizes, "It gets about as close to where you'd like to be with research. And that is, you are just observing something, you are not perturbing it. You are sending something in and something is coming out, and that is the photon that allows you to count the effect."

Irene Kaganman

RESEARCH PAPERS

Jones, L.R. *et al.* Releasable luciferin–transporter conjugates: tools for the real-time analysis of cellular uptake and release. *J. Am. Chem. Soc.* **128**, 6526–6527 (2006).