Point of VIEW

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Climbing to the Next Step: Photosensitive Protein Labels

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ABSTRACT The tool set for intracellular labeling has received its newest addition. It is now possible to attach a fluorescent molecule to proteins inside living cells and to change the fluorescent properties at some point in time by a flash of light. Hence the labeled protein is easily and unambiguously trackable against a background of nonilluminated proteins from different locations.

The fate of molecules in cells is frequently determined by attached fluorophores. This technique is not always reliable, as the fluorophore itself may interfere with the metabolism or localization of the molecule of interest. For proteins, however, the fusion to a fluorescent protein or the introduction of a small molecule fluorophore seems only in some cases to have undesired effects on protein behavior. In particular, the addition of β-barrel-shaped fluorescent proteins is often without effect on protein performance other than decreased proteolytic cleavage. Despite easy-to-apply labeling strategies, fluorescent proteins have their limitations. Especially, sharp long wavelength excitation fluorescent proteins are hard to come by. For these and other cases, alternative labeling methods that permit the introduction of small fluorophores are very helpful. The most common technique in this respect to date is the tagging by the self-alkylating SNAP-tag fusion proteins, pioneered by the group of Kai Johnsson (*1*). In an article in this issue of *ACS Chemical Biology*, this group now introduced a photoswitchable variety of the SNAP technique (*2*). For this purpose, a fluorophore and a quencher molecule are introduced that exhibit almost complete fluorescence resonance energy transfer (FRET). In the linker region between the two fluorophores, a photocleavable nitroveratryl-type unit permits the separation of the two dyes and hence the complete loss of FRET. This leads to large unquenching of the donor fluorescence and to a substantial increase in fluorescence. In cases where the quencher is also a fluorophore as described for the FRET pair Cy3-Cy5, photocleavage literately results in a change from dark red to orange fluorescence (Figure 1). Thus the new probes complement the now common photosensitive fluorescent proteins such as photoactivatable mCherry (*3*), photoconvertible Dendra (*4*), or photoswitchable Dronpa (*5*), to name just a few examples.

The preparation of these tools required significant efforts in preparative organic chemistry. The SNAP tag design was based on the substrate properties for the alkyl guanine transferase (AGT) fusion protein. Therefore, an O^6-alkylated guanine was linked to a fluorophore-taggable lysine unit via a standard linker. The side chain amino group of the lysine was used to attach the photoactivatable nitroveratryl-like group by a carbamate group. The quencher was attached in the last step by employing an azido alkyl group on the exocyclic benzyl carbon and click chemistry. As donor fluorophores, the green fluorescent dye fluorescein and the orange dye Cy3 were used, and as quenchers the diaminoxanthene dye QSY7 or the red Cy5 was used. All dyes are charged and hence impermeant to cell membranes. Therefore, the constructs were most useful for applications on cell surfaces, for instance, to label GPI-anchored proteins. However, the authors used an interesting strategy to mechanically treat cell membranes in a way that permitted construct entry. The method is called bead-loading and was originally developed by McNeil and Warder (*6*).

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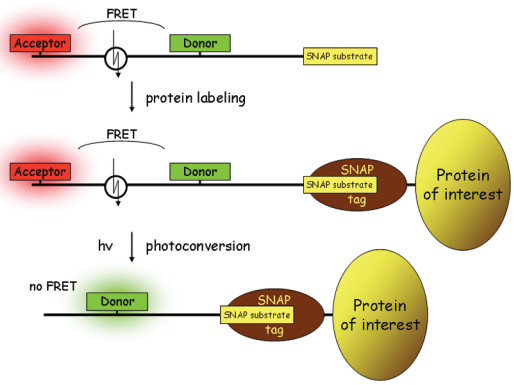

Figure 1. Mode of action of photoconvertible protein tags such as Cy3-Cy5. After selective protein labeling via SNAP tag, the cleavage of a photosensitive nitroveratryl unit by UV light releases the FRET acceptor from the donor leading to a strong increase in donor fluorescence and a decrease in acceptor fluorescence. In the case of Cy3-Cy5, the emission of both dyes can be recorded ratiometrically before and after photocleavage. Cy3 is an orange and Cy5 a red fluorescent cyanine dye. Alternatively, nonfluorescent dyes may be used as FRET acceptors leading to photoactivatable probes that increase fluorescence after acceptor release.

It involves a temporary rupture of the plasma membrane by mechanical impact and is therefore an invasive method that requires a recovery phase for the cells under investigation. During this time the authors speculate that the excess of free dye is released from the cell. Indeed, little background was detectable, demonstrating that the method worked reliably in and on cells. Double labeling of two cell compartments with a SNAP-tag and a CLIP-tag, the latter using an O^2-benzylcytosine derivative (*7*), was successfully shown, as was the application in fluorescence recovery after photobleaching (FRAP) experiments to determine plasma membrane protein diffusion coefficients.

In conclusion, the use of photocleavable tools to switch on fluorescence or change emission wavelengths is a wonderful addition for looking into cell activity in real time and with spatial resolution. Compared to simple SNAP-tag labeling, probe preparation is much more extensive. Therefore, while the strategy provided by Johnsson and co-workers has promise to be generally applicable, the required tailoring of each new tool requires a lot of chemistry. The availability of the reagents will be crucial for the method to reach wide distribution. Furthermore, it will be interesting to have membrane-permeant constructs, for instance, with uncharged dyes or with dye derivatives equipped with bioactivatable protecting groups. In the future, photocleavable tools for cell biology will become more common as these methods permit the local manipulation of molecules including their localization and activity. The field would generally benefit from new photoactivatable groups, especially with longer excitation wavelengths or those that can be cleaved by two-photon excitation (*8, 9*). Apart from improved biocompatibility, these would potentially permit the intracellular manipulation of two orthogonal events, an important prerequisite for interfering with complex structures such as signaling networks.

REFERENCES

1. Keppler, A., Gendreizig, S., Gronemeyer, T., Pick, H., Vogel, H., and Johnsson, K. (2003) A general method for the covalent labeling of fusion proteins with small molecules *in vivo*, *Nat. Biotechnol. 21*, 86–89.
2. Maurel, D., Banala, S., Laroche, T., and Johnsson, K. (2010) Photoactivatable and photoconvertible fluorescent probes for protein labeling, *ACS Chem. Biol.* Epub March 10, 2010; DOI: 10.1021/cb1000229.
3. Subach, F. V., Patterson, G. H., Manley, S., Gillette, J. M., Lippincott-Schwartz, J., and Verkhusha, V. V. (2009) Photoactivatable mCherry for high-resolution two-color fluorescence microscopy, *Nat. Methods 6*, 153–159.
4. Gurskaya, N. G., Verkhusha, V. V., Shcheglov, A. S., Staroverov, D. B., Chepurnykh, T. V., Fradkov, A. F., Lukyanov, S., and Lukyanov, K. A. (2006) Engineering of a monomeric green-to-red photoactivatable fluorescent protein induced by blue light, *Nat. Biotechnol. 24*, 461–465.
5. Ando, R., Mizuno, H., and Miyawaki, A. (2004) Regulated fast nucleocytoplasmic shuttling observed by reversible protein highlighting, *Science 306*, 1370–1373.
6. McNeil, P. L., and Warder, E. (1987) Glass-beads load macromolecules into living cells, *J. Cell Sci. 88*, 669–678.
7. Gautier, A., Juillerat, A., Heinis, C., Correa, I. R., Kindermann, M., Beaufils, F., and Johnsson, K. (2008) An engineered protein tag for multiprotein labeling in living cells, *Chem. Biol. 15*, 128–136.
8. Warther, D., Bolze, F., Leonard, J., Gug, S., Specht, A., Puliti, D., Sun, X. H., Kessler, P., Lutz, Y., Vonesch, J. L., Winsor, B., Nicoud, J. F., and Goeldner, M. (2010) Live-cell one- and two-photon uncaging of a far-red emitting acridinone fluorophore, *J. Am. Chem. Soc. 132*, 2585–2590.
9. Gug, S., Bolze, F., Specht, A., Bourgogne, C., Goeldner, M., and Nicoud, J. F. (2008) Molecular engineering of photoremovable protecting groups for two-photon uncaging, *Angew. Chem., Int. Ed. 47*, 9525–9529.