IMAGING AND VISUALIZATION

Highlighting protein movement in living cells

A new photoactivatable fluorescent protein combines some of the best features of existing proteins used for *in vivo* labeling.

The development of photoactivatable fluorescent proteins began in the mid 1990s and finally yielded truly useful tools in 2002. Exposure of these proteins to light of a certain wavelength and intensity changes their fluorescence, allowing researchers to highlight proteins in discrete regions of cells and track their movement directly rather than watching the movement of proteins into a bleached space as was previously done. A report by Lukyanov and colleagues in the November issue of Nature Biotechnology describes the creation of a new dual-color photoactivatable monomeric fluorescent protein they call photoswitchable cyan fluorescent protein, or PS-CFP, that promises to further simplify protein tracking experiments (Chudakov *et al.*, 2004).

PS-CFP represents the next generation of optical highlighter molecules and has some distinct advantages over the currently published proteins. It offers a higher contrast between pre- and postactivation fluorescence than either PA-GFP (Patterson and Lippincott-Schwartz, 2002) or KFP1 (Chudakov *et al.*, 2003). This contrast is on the same scale as that for Kaede (Ando *et al.*, 2002), but the protein is a monomer instead of an obligate tetramer. Following exposure to relatively intense 405-nm light, this cyan fluorescent protein undergoes a 5-fold decrease in cyan fluorescence and a 300-fold increase in green fluorescence, resulting in an optical contrast of 1,500-fold. Because the resolution that can be obtained using photoactivated tags is largely dependent on the optical contrast triggered by photoactivation, PS-CFP should allow investigators to resolve finer changes in protein trafficking without complications caused by self-association of the tag.

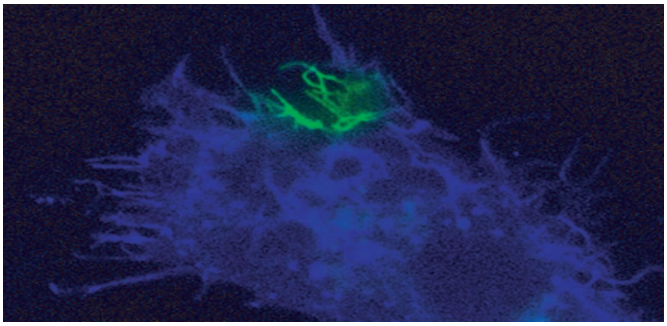

Figure 1 | A living endothelial cell transiently expressing the human dopamine transporter (hDAT) fused with the new photoswitchable cyan fluorescent protein (PS-CFP). The transporter is localized to the integral membrane, cellular processes and constitutive endosomes. A subpopulation of PS-CFP was selectively photoswitched at the cell edge.

Another advantage of PS-CFP over photoactivatable proteins such as PA-GFP is that it possesses significant fluorescence before photoactivation. This can be particularly helpful in cases where the protein is localized to discrete cellular regions. Lukyanov and col-

DRUG DISCOVERY

DON'T MIND THE GAP

A new study may have surprising implications for drug-design research, suggesting that multivalent ligands targeting multiple binding sites need not fully span the distance between sites to be highly effective.

Erkang Fan's team at the University of Washington (Seattle, WA) studies compounds designed to inhibit cholera toxin (CT), the protein responsible for cholera disease, which interacts with the cell-surface ganglioside GM1 in the small intestine via five identical B subunits. The Fan lab works with ligands consisting of a designed multivalent core linked with multiple ligand units, considerably improving binding affinity relative to monomeric antagonists. This in itself is not unexpected, but a recent article by Fan's group in *Chemistry & Biology* demonstrates that the drug design process can still hold some twists.

"Basically, the paper describes kind of a surprise in our ongoing research," says Fan. His early efforts at ligand synthesis had focused on structure-based design, tailoring molecules that fit the surface of the targeted site on their cognate protein, and pentavalent ligands designed to match the five binding sites on the B pentamer of a CT-like toxin yielded promising results (Fan *et al.*, 2000). To further enhance ligand affinity, the group developed variants with branched ends, turning the pentamer into a decamer, with each tip presenting two branches bearing galactose analogues.

"Since we didn't know precisely how best the two [molecules] should be connected on the tip, we wanted to make a range of linkers," Fan continues. "We realized that maybe the longer linkers will give... higher potency because they may actually occupy two binding sites simultaneously. But the surprise to us was actually that something in the middle is the best." Indeed, linkers incapable of spanning the distance between binding sites proved no impediment to significant improvement of CT binding by the decavalent molecule (Zhang *et al.*, 2002).

Their new article (Pickens *et al.*, 2004) confirms the efficiency of bivalent inhibitors of CT, even when the effective linker length is insufficient to span the 35 Å separating adjacent binding sites. Fan's group connected pairs of galactose-derived ligand molecules with linkers of varying lengths, measuring each molecule's capacity to block CT-ganglioside interaction *in vitro*. Four different ligands, each incapable of effectively bridging the gap between

leagues took advantage of PS-CFP's preactivation fluorescence and stability at low pH to track the movement of tagged human dopamine transporter (hDAT) within filopodia (**Fig. 1**) and endosomes. They were also able to successfully photoactivate selected endosomes and track their movement in the cytoplasm. When two activated and nonactivated endosomes made contact, the authors observed, for the first time ever, the direct mutual exchange of cargo proteins between these cellular compartments, thus highlighting the value of PS-CFP for such studies.

As a further aid in localization and tracking, PS-CFP can be visualized with standard ECFP and FITC filters, making it straightforward to use in multilabel experiments with red fluorophores. A potential problem with PS-CFP is that unlike Kaede, which uses a different wavelength for photoactivation and visualization of the nonactivated form, PS-CFP uses the same wavelength at different intensities for both processes. PS-CFP is also slightly more sensitive to bleaching than EGFP, which could further complicate some experiments. However, the authors clearly demonstrate that as long as researchers are careful in their experiments, these drawbacks do not obviate the significant advantages afforded by this new addition to the photoactivatable fluorescent protein family.

Daniel Evanko

RESEARCH PAPERS

Chudakov, D.M. *et al.* Photoswitchable fluorescent label for protein tracking. *Nat. Biotechnol.* published online 17 October 2004 (doi:10.1038/nbt1025).

Patterson, G.H. & Lippincott-Schwartz, J. A photoactivatable GFP for selective photolabeling of proteins and cells. *Science* **297**, 1873–1877 (2002).

Chudakov, D.M. *et al.* Kindling fluorescent proteins for precise *in vivo* photolabeling. *Nat. Biotechnol.* **21**, 191–194 (2003).

Ando, R. *et al.* An optical marker based on the UV-induced green-to-red photoconversion of a fluorescent protein. *Proc. Natl. Acad. Sci. USA* **99**, 12651–12656.

binding sites, all showed strong inhibition; the strongest, BV3, with an IC_{50} 200-fold better than that of the monomeric ligand, contained a linker with an effective length of only 22 Å.

Fan's group believe that these ligands inhibit CT-ganglioside interaction partly through steric blocking, occupying a binding site while the unassociated ligand moiety physically interferes with further protein surface interactions. They are currently developing new assays, including cell-based systems, to further characterize this inhibition. Fan believes these findings could ultimately provide new directions for future drug design projects, as bivalent ligands appear to offer a simplified strategy for improving inhibition and ultimately "it's probably much easier to make large quantities of bivalent compound rather than the heavily designed, generally hard-to-make multivalent ligand."

Michael Eisenstein

RESEARCH PAPERS

Pickens, J.C. *et al.* Nonspanning bivalent ligands as improved surface receptor binding inhibitors of the cholera toxin B pentamer. *Chem. Biol.* **11**, 1205–1215 (2004).

Fan, E. *et al.* High-affinity pentavalent ligands of *Escherichia coli* heat-labile enterotoxin by modular structure-based design. *J. Am. Chem. Soc.* **122**, 2663–2664 (2000).

Zhang, Z. *et al.* Solution and crystallographic studies of branched multivalent ligands that inhibit the receptor-binding of cholera toxin. *J. Am. Chem. Soc.* **124**, 12991–12998 (2002).

NEWS IN BRIEF

CHEMICAL BIOLOGY

LEAPT: Lectin-directed enzyme-activated prodrug therapy

Robinson *et al*. describe a glycosylation-based system for cell-specific activation of a prodrug compound. A nonmammalian enzyme, α-rhamnosidase, is artificially tagged with a carbohydrate that enables targeting to and internalization by cells of interest; following introduction of a similarly glycosylated, rhamnoside-capped prodrug, strong, tissue-specific enzymatic drug activation is observed.

Robinson, M.A. *et al. Proc. Natl. Acad. Sci. USA* **101**, 14527–14532 (2004).

BIOINFORMATICS

Textpresso: an ontology-based information retrieval and extraction system for biological literature

Müller *et al.* have devised a system for the analysis of text from the abstracts, titles and body of published articles, after which the data is organized ontologically according to established categories. This system, which the authors term Textpresso, makes it possible to conduct more productive and efficient literature searches.

Müller, H.-M. *et al. PLoS Biol.*, published online 21 September 2004.

GENE TRANSFER

Self-inactivating retroviral vectors with improved RNA processing

Self-inactivating (SIN) vectors offer a safer alternative to conventional retroviruses for gene delivery, but typically suffer from reduced titer and inefficiency of RNA processing. Kraunus *et al.* have developed a modified SIN vector incorporating a favorable intron that considerably improves transgene expression from the construct, and a viral regulatory element that strongly elevates the titer.

Kraunus, J., *et al. Gene Therapy*, published online 16 September 2004.

PROTEIN BIOCHEMISTRY

The site-specific incorporation of *p*-iodo-L-phenylalanine into proteins for structure determination

Using a directed-evolution strategy, Xie *et al.* generated an orthogonal, variant tyrosyl-tRNA synthetase capable of specifically directing the incorporation of the unnatural amino acid *p*-iodo-L-phenylalanine at the amber stop codon in *Escherichia coli*. Introduction of this residue into proteins is shown to improve the quality of structure determination via single wavelength anomalous dispersion (SAD) phasing.

Xie, J. *et al. Nat. Biotechnol.*, **22**, 1297–1301 (2004).

BIOINFORMATICS

Comparative homology agreement search: an effective combination of homology-search methods

Alam *et al.* introduce a tool called comparative homology agreement search (CHASE), which integrates five different sequence homology search methods to obtain a combined 'E value' confidence estimate for homology that surpasses the analytical performance of any of the individual algorythms.

Alam, I. *et al. Proc. Natl. Acad. Sci. USA* **101**, 13814–13819 (2004).