says, "It was actually my student Ed Schwartz who bravely took that big leap and moved it into the fly system. We were very happily surprised how straightforward it was." By feeding the fly food laced with rapamycin they could switch luciferase activity on within minutes.

It turns out that the rate-determining step in this technology is getting it to work initially in culture by finding the right points in the target protein to split the molecule. Muir suggests that going forward it may be possible to develop some empirical rules to simplify the process, but there will always be an element of trial and error requiring careful testing and validation. To justify this effort you have to have a problem that can only be addressed by this type of method.

What kinds of applications are these new methods appropriate for? Most likely it will be ones that require titration of protein levels, something that is very difficult to achieve with transcriptional regulation. Muir believes this is going to be the 'killer app' for this kind of technology. "Any system where the protein levels oscillate as a function of some sort of intrinsic or extrinsic control [is] worth exploring with this kind of small molecule–regulated system," says Muir.

"What we'd really like to do with this technology is adapt it such that we get away from small molecules altogether," says Muir. "One thing we are looking at carefully in the lab is trying to control this with light. That would ultimately provide the best level of control." Muir is unwilling to make any bets on which of the recent methods developed to regulate proteins at the post-transcriptional level will ultimately prove the most useful but you can bet his intein-based methods will be contenders.

Daniel Evanko

RESEARCH PAPERS

Mootz, H.D. & Muir, T.W. Protein splicing triggered by a small molecule. *J. Am. Chem. Soc.* **124**, 9044–9045 (2002).

Schwartz, E.C. *et al.* Post-translational enzyme activation in an animal via optimized conditional protein splicing. *Nat. Chem. Biol.* **3**, 50–54 (2007).

the cell nucleus and in different cell types as well as the ability to quantify binding affinities, it has inherent limitations, being a FRET-based method. The fluorescent proteins must be brought to within about 6 nm of each other for FRET to occur, and thus the system is limited to studying smaller proteins or parts of proteins.

Daugherty points out that an exciting application of this method would be domain-level protein interaction screening, for example, to build a higher-resolution interaction map after a Y2H screen. The information obtained in a FRET assay—such as which domains interact and their binding affinities—could be used to assemble more detailed protein interaction maps with enough information build models about the system. But to build such a map, a huge effort would be required to examine each of the domains of a given system: first determining which proteins are potentially involved in the system using expression data, then cloning individual domains of the identified proteins and finally screening individual domains against a cDNA fragment library. "That would yield interesting data that are not showing up in the current Y2H screens," adds Daugherty. "We are contemplating whether to jump in or not."

Irene Kaganman

RESEARCH PAPERS

Nguyen, A.W. & Daugherty, P.S. Evolutionary optimization of fluorescent proteins for intracellular FRET. *Nat. Biotechnol.* **23**, 355–360 (2005).

You, X. *et al.* Intracellular protein interaction mapping with FRET hybrids. *Proc. Natl. Acad. Sci. USA* **103**, 18458–18463 (2006).

NEWS IN BRIEF

IMMUNOCHEMISTRY

Using plants to make a better antibody

It is well known that the efficacy of an antibody can be influenced by modifying the glycosylation pattern. Cox *et al.* describe a new approach to optimize antibody glycan configuration by using a genetically modified aquatic plant, *Lemna minor*, in which several components of the glycosylation machinery have been silenced. This robust system led to the generation of antibodies with optimized activity, containing only a single major *N*-glycan species.

Cox, K.M. *et al. Nat. Biotechnol.* **24**, 1591–1597 (2006).

PROTEIN BIOCHEMISTRY

Experimental validation of computational design

In contrast to error-prone PCR, a more 'intelligent' way to introduce new function onto protein scaffolds is to use computational tools to design and optimize a library of alternative protein sequences. Few of these libraries, however, have been experimentally characterized. Treynor *et al.* report the systematic evaluation of seven library-design algorithms with the goal of introducing shifted fluorescence emission to GFP variants.

Treynor, T.P. *et al. Proc. Natl. Acad. Sci. USA* **104**, 48–53 (2007).

GENOMICS

Sorting out *Mos1* insertion mutants in worms

Mutants exist for about a quarter of *Caenorhabditis elegans* genes. As a complement to other large-scale mutant screens, Duverger *et al.* present a high-throughput approach to generating *Mos1* transposon insertion mutants. Using a combination of fluorescence sorting, a liquid-handling robot and parallel cultivation of several thousand strains in liquid culture, they generated more than 17,500 homozygous insertional mutants.

Duverger, Y. *et al. Nucleic Acids Res.*; published online 12 December 2006.

IMAGING AND VISUALIZATION

Imaging bacterial infections *in vivo*

Using near-infrared fluorescent zinc(II) dipicolyamine–based dyes that specifically target Gram-positive bacterial cell surfaces, Leevy *et al.* show that bacterial infections can be imaged in living mice at a tissue depth of more than two centimeters. They introduced the dye into the bloodstream of mice and found that it accumulated at a site of local *Staphylococcus aureus* infection.

Leevy, W.M. *et al. J. Am. Chem. Soc.* **128**, 16476–16477 (2006).

MICROFLUIDICS

Optimizing experiments the microfluidic way

Using crystallization trials of membrane proteins as a proof-of-principle system, Li *et al.* demonstrate a simple microfluidics approach for simultaneous screening and optimization of experimental parameters in nanoliter volumes. They combined reagents in a controlled manner in microfluidic channels to generate hundreds of 10-nl droplets, each representing an individual experiment. They obtained protein crystals diffracting at 1.9–2.5 Å, validating the approach.

Li, L. *et al. Proc. Natl. Acad. Sci. USA* **103**, 19243–19248 (2006).

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