Selection of the fluorophore panel for a given experiment, however, remains a trial-and-error process. Based on their extensive experience working with the fluorochromes, Roederer and colleagues match the brightest reagents with less-abundant markers, keeping in mind that spectral overlap reduces sensitivity. Yet to develop a large panel of fluorophores, they test several different colors of each of the antibodies they want to use. "We start by mixing several different combinations of six or eight antibodies and see how that performs, and then we start layering in additional reagents and continually revising the panel," explains Roederer.

In new work reported in *Nature Medicine*, they used quantum dot–conjugated antibodies in combination with organic fluorochrome–conjugated antibodies to assay the phenotype of antigen-specific T cells. In this 17-color analysis, they were able to identify the expected markers, but also found new phenotypic differences between the populations.

As in imaging, the addition of quantum dots to the palette of fluorescent tags affords greater sensitivity. As Roederer puts it, "Historically, we've been limited to three or four different tags, but now we are working our way to where we have several dozen, and that gives us just much more flexibility in designing and implementing experiments where we want to detect fluorescent tags."

Irene Kaganman

RESEARCH PAPERS

Chattopadhyay, P.K. *et al.* Quantum dot semiconductor nanocrystals for immunophenotyping by polychromatic flow cytometry. *Nat. Med.* **12**, 972–977 (2006).

This low-tech method allowed Fraser and colleagues to identify 349 interactions out of ~65,000 tested, which means that pairwise combinations of mutations are many times more likely to result in nonviable phenotypes than single mutations. And this is only looking at the highest-confidence hits.

To try to identify weaker interactions, Fraser is now building an automated image analysis system. He explains, "Now at least we have test sets so we know how well the screen works, what it gives us, and that it is worth doing." Fraser is quick to add, however, that even if they get the automated method working well they will likely do a very rapid manual screen as a first pass and then do all the repeats in a quantitative way using the automated screening.

So before you embark on your next high-throughput screening experiment, it might be worth considering whether it might be good to do an old-fashioned manual screen before investing time and effort in something that does not work out quite as planned.

Daniel Evanko

RESEARCH PAPERS

Lehner, B. *et al.* Systematic mapping of genetic interactions in *Caenorhabditis elegans* identifies common modifiers of diverse signaling pathways. *Nat. Genetics* **38**, 896–903 (2006).

NEWS IN BRIEF

PROTEIN BIOCHEMISTRY

Experimental generation of electrostatic potential maps

Red, white and blue electrostatic potential maps are often computationally generated for protein structures to assess the roles of electrostatics in various protein functions. Suydam *et al.* now demonstrate that these maps can be experimentally generated for the active site of human aldose reductase, by mapping the effects of the local electrical field on a nitrile-based inhibitor using vibrational spectroscopy.

Suydam, I.T. *et al. Science* **313**, 200–204 (2006).

MOLECULAR LIBRARIES

Improving the display of proteins on phage

Phage display is a powerful tool for the high-throughput screening of proteins for desired properties. Unfortunately, many proteins display poorly on phage despite attempts to address the problem. Steiner *et al.* have vastly improved the range of proteins amenable to phage display by using signal sequences that harness the signal-recognition particle translocation pathway rather than the traditional Sec-dependent translocation pathway.

Steiner, D. *et al. Nat. Biotech.* **24**, 823–831 (2006).

GENE TRANSFER

On-target gene delivery with lentiviral vectors

Lentiviral vectors have shown great potential as *in vivo* gene delivery vehicles, but assuring on-target delivery is very difficult. Yang *et al.* now show that efficient delivery of the vector to specific cell types is possible by separating the functions of target recognition and membrane fusion into two unique moieties on the lentiviral surface—an antibody and a pH-responsive fusogenic envelope glycoprotein.

Yang, L. *et al. Proc. Natl. Acad. Sci. USA* **103**, 11479–11484 (2006).

CHEMICAL TOOLS

Fluorescent dyes for live-cell imaging of RNA

By using combinatorial organic synthesis to generate a large fluorescent styryl dye library, Li *et al.* discovered three RNA-selective probes that can be used for the live-cell imaging of RNA distribution within the nucleus. These three dyes exhibited higher selectivity, photostability and lower cytotoxicity compared with a commercially available alternative.

Li, Q. *et al. Chem. Biol.* **13**, 615–623 (2006).

SPECTROSCOPY

Probing membrane protein orientation with EPR spectroscopy

Inbaraj *et al.* report a new technique for determining integral membrane protein topology in bicelles by electron paramagnetic resonance (EPR) spectroscopy. Using the nicotinic acetylcholine receptor as an example, they show that the incorporation of a nitroxide spin probe facilitates measurement of hyperfine splittings, which are used to accurately calculate the helical tilt of the transmembrane domain.

Inbaraj, J.J. *et al. J. Am. Chem. Soc.* **128**, 9549–9554 (2006).