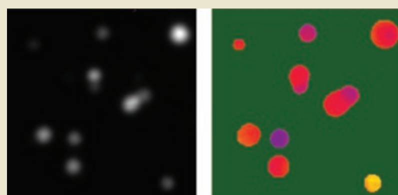
Fluorescent proteins in a lifetime



Traditionally, the search for ever brighter fluorescent proteins has been conducted by determining the brightness of individual cells or bacterial colonies expressing mutated versions of a parent protein. Although this approach has created many improved variants, properties other than the molecular brightness—such as expression level, maturation efficiency and colony morphology—often influence the results. Goedhart *et al.* screen mutant libraries for intrinsically brighter cyan fluorescent proteins by measuring the fluorescence lifetime of bacterial colonies. Fluorescence lifetime is correlated with the quantum yield of the fluorophore and can be evaluated independent of confounding factors. Starting with the brightest available cyan fluorescent protein, SCFP3A, the authors find a variant, mTurquoise, which is ~50% brighter than the commonly used mCerulean and ~30% brighter than the parent protein. The high quantum yield also makes this protein an excellent donor for Förster resonance energy transfer (FRET) experiments. The comparatively long lifetime extends the range of cyan fluorescent proteins that can be imaged simultaneously by lifetime unmixing, and the monoexponential decay of the fluorescence simplifies the quantitative analysis of FRET by lifetime imaging. (*Nat. Methods* **7**, 137–139, 2010) *ME*

Avoiding vaccine-vector antibodies

Despite the potential of adenovirus-5 and vaccinia virus for use as vaccine vectors, the utility of these platforms can be limited by the magnitude of the CD8$^+$ T-cell responses elicited (vaccinia) and preexisting antibody immunity (adenovirus). Furthermore, both platforms elicit vector-specific antibody immunity, which interferes with booster vaccinations. Flatz *et al.* show that replication-defective lymphocytic choriomeningitis virus (LCMV) may be an attractive alternative vaccine delivery platform. They generate replication-defective LCMV by deleting the envelope glycoprotein gene and replacing it with diverse antigens, including a foreign viral envelope protein and tumor antigens. LCMV is known to stimulate a weak neutralizing antibody response, and in this study even multiple doses of LCMV vector did not elicit inhibitory antibody levels. In mice as well as in cultured human blood cells, LCMV vectors selectively target and activate dendritic cells, thereby eliciting strong CD8$^+$ T-cell responses. In mice, LCMV vector-induced CD8+ T cells are more effective at destroying solid tumors than those induced by recombinant adenovirus-5 or vaccinia virus, and they confer long-lived antibacterial protection as well as antiviral immunity. What's more, LCMV vectors elicit protective antibody immunity against vaccine antigens. (*Nat. Med.* advance online publication, doi:10.1038/nm.2104, 7 February 2010) *CM*

Synthetic enhancer diversity explored

Natural transcriptional enhancers discovered in organisms such as cytomegalovirus are workhorses of biological research. Elledge and colleagues synthesize ~50,000 artificial enhancer sequences, screen them for activity in six mammalian cell lines and discover cell type–specific enhancers. They started by synthesizing a pool of synthetic enhancers comprising all possible 10-nt motifs repeated ten times in 100-nt sequences. They then cloned each enhancer upstream of a green fluorescent protein reporter on a retroviral vector, transfected cells with the virus, recovered cells with the strongest signal by fluorescence-activated cell sorting and subsequently sequenced the inserts. In many cases, the recovered enhancers contain motifs that match known transcription factor binding sites. Notably, the enhancers recovered span a wide range of strengths up to twice as strong as the wild-type cytomegalovirus enhancer. Other enhancers are active only in specific cell lines. These results suggest that the method may be useful for generating highly optimized experimental reagents. (*Proc. Nat. Acad. Sci. USA* **107**, 2538–2543, 2010) *CM*

Nanofactories disrupt bacterial communication

Interfering with quorum sensing—a process involved with biofilm formation, bioluminescence and virulence—could control bacterial growth without eliciting drug resistance. Bentley and colleagues design a multifunctional protein assembly (a 'nanofactory') that can trigger quorum-sensing behavior in bacteria. The self-assembling nanofactory comprises four modules: a targeting module (bacteria-specific antibody), a sensing module, a synthesis module (a fusion protein of the enzymes that synthesize the quorum sensing molecule AI-2) and an assembly module (protein G, which attaches the assembly module to the targeting module). The researchers show that the nanofactory can both pick out specific bacteria from a mixture and induce quorum-sensing behavior. Finally, they show that after functionalizing *Escherichia coli* with a specific nanofactory, unrelated species can sense their presence owing to the production of the quorum-sensing molecule by the functionalized *E. coli*. Bacteriostatic nanofactories could combat specific pathogens by inducing biofilm formation before the concentration of bacteria is high enough to damage the host, potentially avoiding the emergence of drug resistance. (*Nat. Nanotechnol.* advance online publication, doi:10.1038/nnano.2009.457, 17 January 2010) *LD*

Biofuel-producing bacterial factories

Complex chemical processes are now required to convert expensive plant and animal oils into fuels and chemicals. But in a *tour de force* of microbial engineering, Keasling and colleagues, along with scientists at LS9 (San Carlos, CA), demonstrate that it is feasible to consolidate many key reactions into a single strain of *Escherichia coli* that can then convert inexpensive plant biomass into fatty acid–based fuels and chemicals. They introduce or delete ~10 microbial enzymes so that a single bacterial strain liberates simple sugars from plant biomass and, when grown on glucose alone, overproduces free fatty acids and synthesizes the fatty acids into fatty acid ethyl esters (FAEE; a component of biodiesel) or medium chain fatty alcohols (which are commercially important). Although the function of each enzyme had been characterized previously and each of these steps can be accomplished by adding purified enzymes to a reaction vessel, performing the steps in a single organism has the potential to simplify processing and to reduce costs. An optimized strain produces 647 mg l^{-1} of FAEE when grown on 2% glucose, which is 9.4% of the theoretical yield and is within an order of magnitude of that required for commercial production. The remaining necessary improvements are conceivably achievable with existing strain and process engineering strategies. (*Nature* **463**, 559–562, 2010) *CM*

Written by Markus Elsner, Laura DeFrancesco & Craig Mak

© 2010 Nature America, Inc. All rights reserved.