IMAGING AND VISUALIZATION

Bioluminescent quantum dots

Bioluminescence is widely used for *in vivo* imaging of nude mice. By conjugating luciferase protein to quantum dots, bioluminescence resonance energy transfer (BRET) turns these useful fluorophores into a new class of bioluminescent probe.

Quantum dots have several advantageous characteristics that have made them the fluorophore of choice in many applications. Unfortunately they are not particularly suited to whole-animal *in vivo* imaging, an application that is becoming increasingly popular. Now Jianghong Rao, Sanjiv Gambhir and colleagues from the Molecular Imaging and Bio-X Program for interdisciplinary research at Stanford describe the synthesis and the use of bioluminescent quantum dots for *in vivo* imaging.

Although quantum dots can emit light in the red and infrared regions of the spectrum that have good tissue penetration, they all require blue light for efficient excitation. Blue light, however, does not penetrate tissue well and also produces high background owing to excitation of endogenous fluorophores. During *in vivo* imaging, this results in low excitation efficiency and high background. Rao and colleagues thought there must be a way to solve this problem.

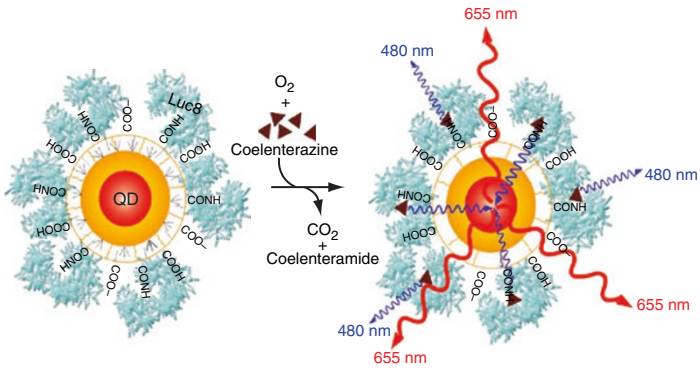

Figure 1 | A schematic showing the design and function of the bioluminescent quantum dot. Oxidation of coelenterazine by Luc8 conjugated to a quantum dot releases bioluminescence energy. Some of this energy excites the quantum dot, resulting in the release of red light. Reprinted with permission from *Nature Biotechnology*.

Rao says they decided to try using luminescent light to excite quantum dots. This light can come from a bioluminescent protein that produces light via a chemical reaction. "We tried firefly luciferase first, and it was very hard to do the conjugation," says Rao. "The reason was probably that the firefly luciferase is very fragile and sensitive to any chemical modification. This caused it to dramatically loose its luciferase activity."

The Gambhir laboratory has been developing methods to use BRET imaging in living mice and has also developed a variant of *Renilla reniformis* luciferase (Luc8) that showed greater stability and improved

GENE REGULATION

HARNESSING A TINY HAIRY BEAST

With the goal of using the ciliate *Tetrahymena thermophila* for biotechnology applications—as a heterologous protein expression system—researchers have identified a copper-inducible and repressible promoter.

Although it is not a widely known model system, research on Tetrahymena has contributed to many fundamental discoveries, including identification and purification of dynein, discovery of telomere structure and telomerase enzyme, the Nobel prize–winning discovery of ribozymes, and elucidation of the function of histone acetylation, among others. A single-celled organism covered with hair-like cilia, Tetrahymena is not the most glamorous eukaryote, but in addition to being a powerful model organism, it has promise in biotechnology for overexpression of eukaryotic proteins that cannot be expressed in the current workhorse systems such as *Escherichia coli*.

The biggest advantage of Tetrahymena, as Theodore Clark of Cornell University points out, is that "it's a very simple-to-grow microorganism that has a lot of structural and behavioral complexity. So it's much easier to grow than mammalian tissue culture; it's also much cheaper. But at the same time it can handle proteins that *E. coli* can't handle in terms of overexpression." For example, eukaryotic membrane proteins do not traffic properly in *E. coli*, but Tetrahymena have a vast membrane system, which may be an advantage for expressing these proteins.

To create such an expression system, researchers tried to identify robust inducible promoters in Tetrahymena. In a recent article in *Eukaryotic Cell*, Francesco Boldrin, a postdoc in Ester Piccinni's laboratory at the University of Padua, now working in the Clark lab at Cornell University, and colleagues describe a *T. thermophila* metallothionein gene (*MTT2*) that can be activated rapidly by adding copper and subsequently turned off by removing the inducer from the growth medium. To determine whether the 5′ region of this gene could drive expression of other genes, they cloned the potential promoter in front of a gene encoding an NRK2 kinase–GFP fusion and introduced the entire construct at an ectopic site in the Tetrahymena genome. In cells carrying this construct, the researchers could observe fluorescence 2 hours after exposure to copper.

efficiency compared to other luciferases. Furthermore, Luc8 emits shorter-wavelength light than firefly luciferase, which overlaps better with the absorption spectrum of quantum dots and its activity is not dependent on the use of ATP. Rao tried using this new Luc8 variant, and it worked. He says, "The beauty of this Luc8 is that it is very tolerant of chemical modifications, and this makes it very easy to do the conjugation and retain the luciferase activity."

Concerns have been voiced about the ability of quantum dots to function as fluorescence resonance energy transfer acceptors for organic fluorophores. After testing both noncovalent and covalent methods to conjugate Luc8 to quantum dots, however, Rao found that covalent conjugation allowed very efficient bioluminescence energy transfer from Luc8 to the quantum dot (**Fig. 1**).

When injected into mice, these bioluminescent quantum dot conjugates produced good signals with no background after injection of the luciferase substrate coelenterazine into the bloodstream. Furthermore, Rao and colleagues were able to exploit the fact that quantum dots with distinct emission spectra all have similar absorption profiles allowing all of them to function as luciferase energy acceptors. Quantum dots with emission peaks from 605 nm to 800 nm all performed well and permitted multiplex detection of signals *in vivo*.

The properties of these conjugates should make them suitable for use with animals larger than mice, and Rao says they are testing these conjugates for use for functional imaging *in vivo*. Gambhir says that these probes will allow multiplexing so that multiple events can be monitored in the same living subject. They are also exploring ways to exploit modulation of the BRET signal to detect enzyme or protein function.

Daniel Evanko

RESEARCH PAPERS

So, M.K. *et al.* Self-illuminating quantum dot conjugates for *in vivo* imaging. *Nat. Biotechnol.* **24**, 339–343 (2006).

This work is an important advance for the Tetrahymena system because previously researchers used the related *MTT1* promoter, which is sensitive to cadmium—a toxic metal that must be removed during protein purification. In preliminary studies, the upstream region of *MTT2* could drive expression of a heterologous gene, and as Boldrin notes, "This promoter can be improved once we find out what its regulatory elements are."

In the research lab as well as at their company, Tetragenetics, Inc., Clark and colleagues intend to use this promoter in some of the ongoing projects, including expression of potential vaccine antigens for two human pathogens—SARS and avian flu. The use of this promoter should also benefit basic biology studies in the Tetrahymena system. As Clark points out, "There is a crying need for alternative protein expression systems, and we think Tetrahymena is going to be one of the good ones."

Irene Kaganman

RESEARCH PAPERS

Boldrin, F. *et al.* Metallothionein gene from *Tetrahymena thermophila* with a copper-inducible-repressible promoter. *Eukaryot. Cell* **5**, 422–425 (2006).

NEWS IN BRIEF

MICROBIOLOGY

Quantitative imaging of Plasmodium transmission from mosquito to mammal

Using epifluorescence microscopy, Amino *et al.* track the *in vivo* behavior of GFP-expressing *Plasmodium berghei* after its transmission into mice via mosquito bite. By observing the initial entry of sporozoites into the dermis, followed by invasion of blood and lymphatic vessels, they obtain insights into malaria pathology that may assist vaccine design.

Amino, R. *et al. Nat. Med.* **12**, 220–224 (2006).

BIOINFORMATICS

GeneDesign: rapid, automated design of multikilobase synthetic genes

Many research projects require the construction or modification of synthetic genes. Design can be tricky, however, and careful attention must be given to such factors as restriction-site usage and codon selection. Richardson *et al.* now describe GeneDesign, a publicly accessible, modular online system that can assist users with every stage of the design and assembly process.

Richardson, S.M. *et al. Genome Res.*; published online 15 February 2006.

PROTEIN BIOCHEMISTRY

Self-assembly of synthetic collagen triple helices

Collagen is a useful substrate for several cell biology and biomaterials applications, but the use of natural collagen preparations has sometimes proven problematic. Kotch and Raines have designed two short 'sticky' polypeptide fragments capable of self-assembly into long, triple-helical collagen-like fibers, with the potential for chemical modification for the design of tailored collagen variants.

Kotch, F.W. & Raines, R.T. *Proc. Natl. Acad. Sci. USA* **103**, 3028–3033 (2006).

BIOINFORMATICS

Structure modeling of all identified G protein–coupled receptors in the human genome

G protein–coupled receptors (GPCRs) are considered important drug targets for numerous diseases, but detailed structural data are lacking for the majority of these proteins. To address this, Zhang *et al.* conducted a genome-wide computational analysis of putative GPCRs, and estimate that their TASSER algorithm has predicted accurate folds for nearly 820 human GPCRs.

Zhang, Y. *et al. PLoS Comp. Biol.* **2**, e13 (2006).

IMAGING AND VISUALIZATION

Automated cell lineage tracing in *Caenorhabditis elegans*

To date, automated methods for tracking cell lineage in *C. elegans* development in live embryos have only succeeded as far as the eight-cell stage. Bao *et al.* now describe an improved method that combines effective fluorescence labeling of cell nuclei with highly sensitive image analysis algorithms, allowing accurate *in vivo* tracking of cell lineage up to the 350-cell stage.

Bao, Z. *et al. Proc. Natl. Acad. Sci. USA* **103**, 2707–2712 (2006).