IMAGING AND VISUALIZATION

Double delivery

With tunable properties, quantum dot–lipid hybrid vesicles can be constructed for use as intracellular delivery vehicles as well as for the fluorescent staining of plasma membranes.

Quantum dots are highly regarded for their superior photostability, narrow emission spectra and multitude of colors as compared to organic dyes; hence, they are increasingly being used for biological imaging applications. Lipid vesicles are commonly used for delivering molecules—from DNA to drugs—into cells. Their properties can be easily tuned to facilitate different uptake mechanisms, from adsorption to fusion to endocytosis.

So why not put these two powerful technologies together? That is just what Horst Vogel and his colleagues at the Swiss Federal Institute of Technology in Lausanne decided to do. They discovered that hydrophobic quantum dots could be stably incorporated into a lipid bilayer, offering a new way to image lipid vesicles. "High-resolution confocal imaging can now be performed for hours on model membrane systems," explains Vogel, owing to the photostability of quantum dots. These lipid–quantum dot hybrid vesicles could be readily formed from any class of lipids and in a variety of sizes.

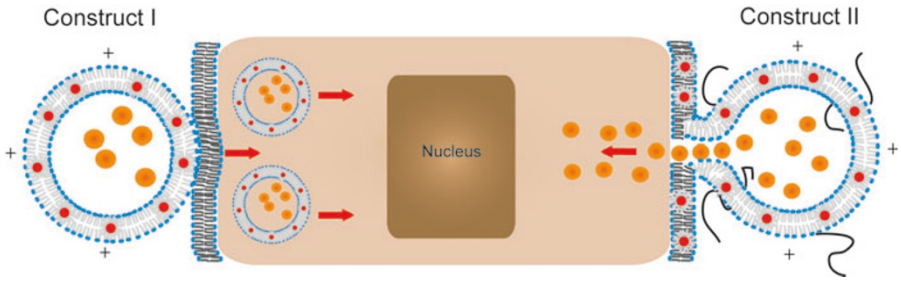

Figure 1 | The interaction of Constructs I and II with living cells. Quantum dots, red; nanocontainer cargo, orange. Reprinted with permission from *Angewandte Chemie International Edition*.

They did not stop there, however. Curious as to whether the hybrid vesicles could be used for nanobiotechnology applications, they made two hybrid vesicle constructs: one designed for transfer into cells (Construct I), and one designed to fuse with the plasma membrane (Construct II; **Fig. 1**). Their lipid compositions were almost identical, except for the addition of a very small percentage of PEG-lipid molecules, which appears to prevent the internalization of Construct II vesicles.

The Construct I hybrid vesicles are internalized by a fairly well-established transfection process; the fluorescent vesicles were

GENE REGULATION

AN EASIER WAY TO TAKE CONTROL

By targeting regulation at the translational rather than the transcriptional level, researchers have uncovered a potentially simpler way to effectively control transgene expression.

Contemporary strategies for the external control of gene expression typically involve modified promoters and engineered transcription factors, and a number of such options—including various systems based on tetracycline-induction—are now available. These systems have the benefit of being well-characterized and offering strong induction, but also suffer from the need to introduce multiple engineered sequences into a target cell and the inability to regulate expression from an unmodified endogenous promoter.

In 2004, Richard Mulligan and his colleagues at Harvard Medical School described an alternative to conventional transcriptional control schemes, in which target transcripts were linked to a ribozyme motif whose cleavage activity is regulated by a small-molecule ligand (Yen *et al.*, 2004). More recently, Mulligan's group has developed an even simpler solution for exogenous gene regulation, a strategy requiring only the introduction of a single trinucleotide sequence to exercise strong regulation of protein production.

Several studies have shown that aminoglycoside antibiotics such as G418 (Geneticin) are capable of suppressing nonsense mutations in mammalian cells. Based on these findings, Mulligan's group tested the ability of G418 to regulate expression of a human apolipoprotein CII-luciferase fusion gene with a nonsense mutation a few codons downstream of the initiator ATG (Murphy *et al.*, 2006). They observed only low levels of luciferase activity in human cells lentivirally transduced with this transgene; however, administration of G418 led to a concentration-dependent increase in luciferase activity, up to a maximum of 72.8% of wild-type activity. They detected restoration of translation as early as an hour after induction, with maximal effect observed by 48 hours. This approach worked well with several different human cell lines, and other aminoglycosides also proved suitable for nonsense suppression, although G418 was the most effective. G418 also proved capable of strongly and specifically inducing luciferase activity *in vivo*, both in mice intratracheally infected with the lentiviral construct, and in lethally irradiated mice whose marrow had been reconstituted with lentivirally transduced hematopoietic stem cells.

The researchers did not observe toxic effects at the doses used in these studies, but there is nonetheless considerable evidence for G418 toxicity, and Mulligan's group also tested the effectiveness of two nonaminoglycoside compounds with nonsense suppression capabilities. Neither was as effective as

observed in cells only seconds after the start of incubation. "We believe, if one would endow the Construct I hybrid vesicles with specific receptor molecules, it should be possible to target them to specific cellular organelles," says Vogel. Notably, the lipid-coated quantum dots did not appear to have substantial cytotoxic effects.

The Construct II hybrid vesicles, serving as 'nanocontainers,' can be filled with water-soluble cargo for delivery inside the cells. Even more interestingly, when the Construct II vesicles fuse with the plasma membrane of a live cell, the quantum dots integrate into the membrane. "Combining the delivery followed by membrane staining is, for instance, helpful for monitoring the events happening at the cell membrane while the cargo is inside," explains Vogel. The researchers filled the nanocontainers with calcium chloride and incubated them with cells loaded with a fluorescent calcium indicator, to demonstrate that both an intracellular increase in calcium and quantum dot membrane staining could be imaged.

Vogel and his colleagues are quite excited about the nanobiotechnology applications that may become possible by using this intriguing method. For example, magnetic nanoparticles carried in the lipid bilayer of the hybrid vesicles could be delivered to cell membranes. Vogel explains that then by applying an external magnetic field, this could allow "cell sorting, selective isolation of plasma membranes, selective and local manipulation of certain microdomains within the plasma membrane where quantum dots would preferentially be inserted and thereby influence cellular signaling reactions, and the manipulation and isolation of cellular organelles."

Allison Doerr

RESEARCH PAPERS

Gopalakrishnan, G. *et al.* Multifuctional lipid/quantum dot hybrid nanocontainers for controlled targeting of live cells. *Angew. Chem. Int. Ed.* **45**, 5478-5483 (2006).

G418, but both proved capable of partially restoring luciferase activity in transduced cell lines without apparent toxic effects. These compounds are still relatively new and poorly characterized from a toxicity perspective, but demonstrate the potential for identifying other effective small-molecule activators—although the authors note that the lack of G418 toxicity observed here should still encourage use of this approach in experimental settings.

This system appears to offer a surprisingly simple alternative for effective genetic control, minimally requiring only the insertion of a single terminator codon. Many elements still remain to be clarified for this system—for example, the effect of different terminator codons appears to be variable and context-dependent, and the potential effects of terminator codon insertion on target proteins are unclear. Nonetheless, Mulligan and colleagues believe that with further refinement, this could be a powerful tool for both research and gene-therapy applications. "It should be possible to achieve regulation in the context of virtually any expression vector," they conclude, "and to provide for the regulation of expression of protein-coding sequences within the context of their normal, endogenous control elements."

Michael Eisenstein

RESEARCH PAPERS

Murphy, G.J. *et al.* Exogenous control of mammalian gene expression via modulation of translational termination. *Nat. Med.* **12**, 1093–1099 (2006).

Yen, L. *et al.* Exogenous control of mammalian gene expression through modulation of RNA self-cleavage. *Nature* **431**, 471–476 (2004).

NEWS IN BRIEF

DRUG DISCOVERY

Three-hybrid for mammalian cells

As an extension of the yeast two-hybrid systems for detecting protein-protein interactions, yeast three-hybrid systems are used to screen for proteins that interact with a small molecule or vice versa. For drug discovery, however, it would be advantageous to perform these types of screens in mammalian cells. Caligiuri *et al.* now describe MASPIT, or mammalian small molecule–protein interaction trap, a mammalian three-hybrid system.

Caligiuri, M. *et al. Chem. Biol.* **13**, 711–722 (2006).

CHEMICAL BIOLOGY

A new tag-probe system for labeling proteins

Short peptide tag–fluorescent small molecule pairs such as the tetracysteine motif–FlAsH dye are indispensable for cell-biology applications. Ojida *et al.* report that an oligo-aspartate tag and a fluorescently labeled multinuclear zinc(II) complex can serve as a new system for labeling cell-surface proteins. Notably, the properties of the probe can be easily tuned to suit the application.

Ojida, A. *et al. J. Am. Chem. Soc.* **128**, 10452–10459 (2006).

CELL BIOLOGY

Multiplex measurements of neuronal signaling

Signal propagation through individual neurons is often studied using electrophysiology techniques. Patolsky *et al.* have now designed a cell culture–compatible field-effect transistor array of silicon nanowire for the multiplex arraying of neurons. This array facilitates highly sensitive, spatially resolved detection of neuronal signaling, with potential applications in drug discovery and testing.

Patolsky, F. *et al. Science* **313**, 1100–1104 (2006).

PROTEOMICS

Measuring the half-lives of proteins

Using a TAP-tagged yeast library and western blotting after inhibition of protein synthesis, Belle *et al.* have measured the half-lives of more than 3750 proteins in the yeast proteome. This was the final piece of the puzzle that has now allowed them to construct a quantitative model of protein metabolism, using previous large-scale measurements of mRNA levels, translation rates and protein abundances.

Belle, A. *et al. Proc. Natl. Acad. Sci. USA* **103**, 13004–13009 (2006).

IMAGING AND VISUALIZATION

Quadruplex molecular beacons

The hairpin-shaped molecular beacons are becoming increasingly important tools for highly sensitive and specific DNA and RNA detection. When closed, the fluorophore on one end of the molecular beacon is quenched by the quencher at the other end. When bound to a target sequence, however, the hairpin opens and fluorescence is restored. Bourdoncle *et al.* now demonstrate that molecular beacons employing a G-quadruplex motif can be constructed, which may give the user more control over their thermodynamic and kinetic properties.

Bourdoncle, A. *et al. J. Am. Chem. Soc.* **128**, 11094–11105 (2006).