CHEMICAL TOOLS

A synthetic solution to gene delivery

A self-assembling polymer-DNA nanoparticle serves as a vehicle for highly efficient gene delivery to cells.

There is great medical interest in developing safe and effective delivery systems for gene therapy. Although modified viruses have shown promise as delivery vehicles, they are not an ideal solution as their specificity can be difficult to manipulate and they can cause severe immune reactions. Synthetic polymers have also been test driven, but it has proven a challenge to adjust their chemical properties to achieve efficient gene delivery while avoiding cellular toxicity.

Toward an innovative synthetic solution, Paula Hammond, in collaboration with Robert Langer (both of MIT), recently reported a self-assembling hybrid block copolymer system that targets plasmid DNA to cells with high efficiency, serum stability, low toxicity and, perhaps most importantly, chemical tunability. The block copolymer (so named because chemicaly distinct repeating units, or 'blocks', are linked together) consists of a cationic branched polymer to bind DNA, a biocompatible hydrophilic linear polymer to prevent nonspecific uptake, and a sugar ligand for cell-surface receptor targeting. Each of these blocks is synthetically addressable, as Hammond explains: "We can take elements that we know work extremely effectively for a delivery system and isolate them using the block copolymer format."

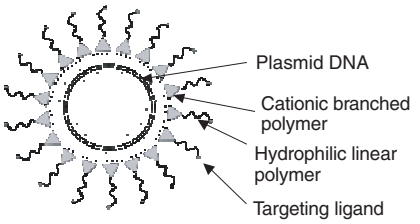

Figure 1 | Self-assembling polyplex of DNA and hybrid polymer.

The hybrid polymer and plasmid DNA self-assemble into a 'polyplex' (**Fig. 1**), as lead author Kris Wood explains: "The cationic hybrid polymer condenses negatively charged DNA to form nanoparticles with a series of concentric, functional 'shells' possessing independently tunable properties." As proof that the polyplex could be efficiently endocytosed, they introduced the gene encoding firefly luciferase into two cell lines, and 72 hours later, the cells were glowing.

Wood, Langer and Hammond are not quite finished yet, however. They plan to test their gene delivery system *in vivo*, which may require tuning of the targeting ligand to recognize surface receptors on specific cell types. They also envision that the block copolymer format could be modified to deliver other molecules into cells, such as hydrophobic drugs. "We have a lot of chemical space to work in," says Hammond.

Allison Doerr

RESEARCH PAPERS

Wood, K.C. *et al.* A family of hierarchically self-assembling linear-dendritic hybrid polymers for highly efficient targeted gene delivery. *Angew. Chem. Int. Ed.*; published online 20 September 2005.

IMAGING AND VISUALIZATION

Labeling on the surface

A strategy for labeling cell-surface proteins in living cells with small-molecule fluorophores allowed scientists to study receptor trafficking by single-cell FRET imaging in real time.

When imaging living cells, it is still a challenge to specifically and efficiently label the target protein, and to do so without disrupting protein function. Fluorescent protein fusions have been a popular means to achieve labeling, but they have their drawbacks. Although it's easy to create these fusions, according to David Golan of Harvard University, "The problem from an imaging point of view is that when you synthesize fluorescent protein inside the cell, the whole biosynthetic pathway becomes fluorescently labeled." Small synthetic fluorescent probes, which have higher sensitivity and resolution for imaging studies, have been developed as alternatives, but their use is limited by a lack of methods to quickly and covalently label proteins with these probes.

Recently, Christopher Walsh's group developed a method for site-specific post-translational labeling of proteins in cell lysates with small molecules (Yin *et al.*, 2004). They fused an 80-residue peptide carrier protein (PCP) to the target protein and then used the enzyme Sfp phosphopantetheinyl transferase to catalyze the covalent modification of a specific serine in PCP.

Now, in an article in *Chemistry & Biology*, Christopher Walsh, David Golan, Marianne Wessling-Resnick and colleagues have collaborated to extend this method to specifically label a cell-surface protein in living cells (Yin *et al.*, 2005). They labeled a transferrin receptor–PCP fusion and its ligand, transferrin, with Alexa fluorophores, which allowed them to use fluorescence resonance energy transfer (FRET) imaging to follow trafficking of this receptor-ligand pair through the cell in real time. Using this method, they showed that transferrin is bound to the receptor throughout the endocytic and exocytic cycle, corroborating prior biochemical studies.

According to Golan, this work shows "the power of a great method," and the authors plan to use the same approach to study other trafficking pathways. There are also efforts underway to use this method to label intracellular proteins, potentially extending its applicability to many more fields.

Irene Kaganman

RESEARCH PAPERS

Yin, J. *et al.* Labeling proteins with small molecules by site-specific posttranslational modification. *J. Am. Chem. Soc.* **126**, 7754–7755 (2004).

Yin, J. *et al.* Single-cell FRET imaging of transferrin receptor trafficking dynamics by Sfp-catalyzed, site-specific protein labeling. *Chem. Biol.* **12**, 999–1006 (2005)