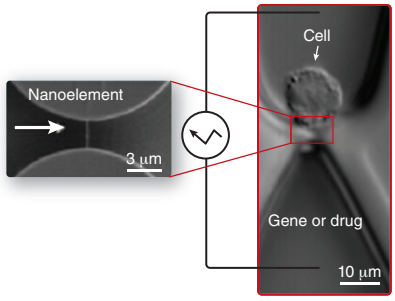
CELL BIOLOGY

Nanoelectroporation

Precise amounts of DNA and quantum dots can be moved into cells through tiny channels.

Cells elegantly and precisely control what substances pass in and out of their membranes. In contrast, human researchers hoping to transport materials into cells are stuck with clumsier techniques, which often damage cells and which provide limited scope over how much, if any, material is delivered. A tiny device designed by L. James Lee and colleagues at Ohio State University might help solve this problem.

Nucleic acids are typically introduced into cells by bulk electroporation. In this procedure, suspended cells and reagents are placed together in a vessel, and an electric field is applied to increase the permeability of the cell membrane. The technique is simple, but it also kills many cells and leaves more untransfected. Another technique, microfluidics-based electroporation, positions cells next to small openings that focus electric fields on only a small section of the cell membrane. This results in lower rates of cell death and higher rates of transfection, but it does not allow control over how much material is delivered. The device invented by Lee and colleagues allows electroporation on the nanometer scale. Not only is the electric field applied to an area one-hundredth the size of those used in microfluidic-based methods, the volume of material delivered to cells can be precisely controlled through the duration and number of electric pulses.



Nanochannel electroporation. Paired microchannels are connected by a nanochannel through which materials can move into cells. Figure courtesy of the Lee laboratory.

Although stochastic techniques such as bulk electroporation can be very useful, says Lee, they do not allow researchers to investigate effects of dose levels. Microinjection can deliver precise dose levels, but this method works best with large cells, which are less easily damaged by the injection needle. "A method allowing precisely controlled transfection for small cells—most primary cells are small—would remove this limitation," he says.

The device Lee and colleagues describe is made of a series of paired microchannels, each connected by an even tinier nanochannel with a diameter of about 90 nanometers. This nanochannel is made by laying gold-coated DNA strands into a low-viscosity resin into which microchannels have been stamped, and then etching out the strands' impression. Cells are placed in one microchannel and the transfection material in the other. A voltage pulse creates a tiny pore in the cell membrane through which a precise amount of material can be driven.

Lee and colleagues tested their device by transfecting cells with 18-mer oligonucleotides attached to a fluorescent marker as well as with an RNA-based molecular beacon

BIOCHEMISTRY

SEQUENCING FOR SUGARS

The hypothesis that many glycans may have a regular sequence gains support, with new evidence of a sequence for the simplest proteoglycan, bikunin.

The glycome—the dizzying array of carbohydrate structures found in the cell—is still very poorly understood. One of the fundamental questions still to be addressed in the glycomics field is whether the glycan sugar chains that are post-translationally attached to proteins, called proteoglycans, have a regular, deterministic sequence. This question has interested Robert Linhardt of Rensselaer Polytechnic Institute for three decades.

"Our working hypothesis has always been that nature invests so much energy in biosynthesizing these very complex molecules, that they must have some defined structure," says Linhardt. However, experimentally testing this hypothesis has proved extremely difficult. Unlike nucleic-acid or protein synthesis, glycan synthesis is not template-driven; despite much work put into understanding how glycans are biosynthesized in the Golgi, a template-driven process has never been found. Moreover, some glycans, such as starch, serve mainly as energy storage molecules and may not require a specific sequence to carry out their biological function. Proteoglycans, however, are mainly found on the cell surface and are thought to be responsible for helping to assemble signaling complexes; such an important function could suggest the need for proteoglycan sequence, Linhardt notes.

The glycomics field has also been challenged by the lack of available methods for sequencing glycans. The first hurdle is just obtaining enough material for mass spectrometry–based sequencing. Linhardt's collaborator, Toshihiko Toida of Chiba University in Japan, was instrumental in helping the team to obtain sufficient quantities of the simplest known proteoglycan, bikunin, which is used as a drug for treating acute pancreatitis in Japan.

Second, proteoglycans consist of one or more linear, sulfated glycosaminoglycan (GAG) chains linked to a core protein, and most core proteins contain multiple different GAG chains of different lengths, presenting a potentially major separation headache. Whereas bikunin contains only a single GAG chain, still, this GAG chain is heterogeneous and was challenging to purify. Linhardt's team first used proteolysis to generate a simple mixture of peptidoglycosaminoglycan chains and then applied a very efficient separation method, continuous elution preparative polyacrylamide gel electrophoresis, to fractionate the chains into mixtures of similar sizes and charges to simplify the analysis.

The third component to analyzing whether bikunin contained sequence required the expertise of collaborator Jonathan Amster of the University of Georgia. At Amster's mass spectrometry facility, the team analyzed the peptidoglycosaminoglycan fractions using two different instruments: the extremely sophisticated Fourier transform ion cyclotron resonance instrument and the more common Orbitrap

(a probe designed to fluoresce upon hybridization to a target RNA or DNA molecule). They demonstrated dose control by transfecting cancer cells with varying levels of a small interfering RNA targeting a protein that inhibits apoptosis, demonstrating that varying dose levels affected cell viability. The technique also allowed controlled delivery of specified numbers of quantum dots and large DNA molecules into cells. With other forms of electroporation, nanoparticles tend to get stuck in the cell membrane, but nanochannel electroporation allowed the particle to reach cells' interiors. The fact that the device works with larger molecules is encouraging, says Lee, because nanoparticles and large nucleic acids (larger than 4,000,000 Daltons, or about 6.6 kilobases) are difficult to transfect using existing methods.

Currently, only a handful of cells can be transfected at a time with the nanochannel electroporation device because cells are loaded into the microchannel using optical tweezers. However, the team is currently at work on a second-generation device that would allow parallel transfection of 100,000 cells.

Lee anticipates that the device can be used for studying fundamental biological questions. "The greater control over dose delivered to individual cells can provide information not achievable by existing methods," he says. However, he predicts that the most important applications will be for modifying cells in gene therapy and reprogramming. Current techniques can result in overdosing and other transfection-caused toxicity, he says: "We believe that a precisely controlled high dose delivered to the precursor cells can achieve high successful rates with low chance to form cancerous cells."

Monya Baker

RESEARCH PAPERS

Boukany, P.E. *et al.* Nanochannel electroporation delivers precise amounts of biomolecules into living cells. *Nat. Nanotechnol.* **6**, 747–754 (2011).

instrument. Still, the analysis was not straightforward; one challenge the team had to overcome is that not all glycan bonds are equally sensitive to fragmentation by collision-induced dissociation. The sulfate bonds tend to break first, resulting in the loss of information before the glycan backbone can be sequenced. The team came up with a breakthrough trick to keep the charge state of the glycans higher than the number of sulfate groups, which prevented loss of the sulfate group and allowed them to obtain sequence information, using both mass spectrometry platforms.

In the end, this overall strategy led to the finding that bikunin indeed contains a regular sequence. In particular, the researchers identified two well-ordered domains in the GAG sequence: a 12-residue sulfated domain near the reducing end and a 6–22-residue nonsulfated domain at the nonreducing end.

Still, before a sweeping conclusion of whether all proteoglycans have sequence can be made, much more work remains to be done. "I would say that when we started this work, 20 years ago, our hypothesis that GAG chains had sequence was deeply contested," says Linhardt. "The hypothesis is now stronger, but to generalize it to other proteoglycans would help prove it." But as all other proteoglycans have more complex structures than bikunin, additional advances both in separation and in mass spectrometry technologies will likely be necessary to make glycan sequencing routine.

Allison Doerr

RESEARCH PAPERS

Ly, M. *et al.* The proteoglycan bikunin has a defined sequence. *Nat. Chem. Biol.* **7**, 827–833 (2011).

NEWS IN BRIEF

BIOPHYSICS

Dark-state exchange saturation transfer

Biology is full of interactions between free molecules in solution and a large supramolecular structure or surface. Fawzi *et al.* present a way to study such interactions at the atomic level with solution nuclear magnetic resonance (NMR) spectroscopy, with a method called dark-state exchange saturation transfer (DEST). They applied DEST to study the exchange reaction between amyloid-β monomers and very large protofibrils (too large to be observed with NMR spectroscopy)—a process implicated in Alzheimer's disease.

Fawzi, N.L. *et al. Nature* advance online publication (30 October 2011).

MICROSCOPY

Inverted selective plane illumination microscopy

Caenorhabditis elegans is an ideal model organism for studying neural development, but current imaging methods are not well suited for such studies, owing to the rapid movements of worm embryos. Wu *et al.* describe inverted selective plane illumination microscopy (iSPIM), which allowed them to perform high-speed, noninvasive, volumetric imaging of *C. elegans* neural development. iSPIM can be performed by simply adding a selective plane illumination module to a conventional inverted microscope.

Wu, Y. *et al. Proc. Natl. Acad. Sci. USA* **108**, 17708–17713 (2011).

SEQUENCING

Oligonucleotide-selective sequencing

Targeted resequencing can require lengthy enrichment protocols prone to sample loss. Myllykangas *et al.* provide an efficient alternative in which targets are captured directly on an Illumina flow cell by fixed probes that act as primers. Subsequent amplification creates substrates for conventional bridge amplification and sequencing. The method, called oligonucleotide-selective sequencing (OS-seq), has comparable sensitivity and uniformity to other enrichment methods and can be automated.

Myllykangas, S. *et al. Nat. Biotechnol.* **29**, 1024–1027 (2011).

CHEMISTRY

Simple xanthene dye synthesis

Photoactivatable xanthene fluorophores are very useful for photoactivated localization microscopy imaging but are challenging to synthesize because of the unique properties of such dyes to exist in both 'open' fluorescent and 'closed' colorless conformations. Wysocki *et al.* now describe a simple synthetic scheme for making caged xanthene fluorophores, including caged Q-rhodamine, rhodamine 110 and Oregon Green. With these dyes, they demonstrated super-resolution imaging of labeled cellular DNA.

Wysocki, L.M. *et al. Angew. Chem. Int. Ed.* advance online publication (26 September 2011).

STRUCTURAL BIOLOGY

'Mutate-and-map' RNA structure characterization

Kladwang *et al.* describe a 'mutate-and-map' strategy for inferring RNA structures, involving systematic mutagenesis of one nucleotide in a base pair, combined with high-throughput, single-nucleotide-resolution chemical accessibility mapping. This approach allowed them to model secondary structures of domains from diverse noncoding RNAs, including ribosomal RNA, ribozymes and riboswitches.

Kladwang, W. *et al. Nat. Chem.* advance online publication (30 October 2011).