(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).