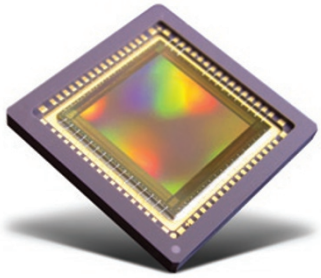
SEQUENCING

Speed-reading DNA in the dark

A new chip with a capability to read genomes uses arrays of tiny pH sensors on an integrated circuit to sequence DNA without the need for optics.

It has been six years since massively parallel sequencing was introduced, and a recent report in *Nature* by Jonathan Rothberg and colleagues of Ion Torrent Systems proves that our appetite for low-cost, large-scale technologies is not on the wane. Rothberg, a pioneer of next-generation sequencing, believed that "we needed a more direct way to convert biological information into digital information." The historical limitation, in his view, has been the need for sequencing platforms to image at the detection step.

To sidestep the imaging requirement, Rothberg and his colleagues focused on a byproduct of DNA polymerization, a single hydrogen ion. Their key insight was in using an ion-sensitive field-effect transistor, which translates changes in pH from proton release to changes in current, to underlie each microwell in a high-density array. The group fabricated the integrated circuit using the complementary metal-oxide semiconductor (CMOS) process, the same technology used to construct microprocessors and digital camera sensors.



Ion Torrent Systems, Inc.

Each chip packs millions of ion-sensitive transistors under wells in which individual sequencing reactions take place.

As in other next-generation sequencing methods, the 'ion chip' needs to start with millions of short, isolated DNA sequences, each at high-enough copy numbers for detection. The system uses emulsion PCR, whereby fragmented DNA is ligated to adapters, fixed at one end to 2-micrometer acrylamide beads and clonally amplified in individual droplets of an oil emulsion. Sequencing reactions are kept isolated by the 1.2 million wells etched on the chip's surface, each measuring 3.5 micrometers and only accepting a single bead.

To sequence, each of the four DNA bases is successively flooded across the chip and then washed away. The process takes 4–5 seconds and is repeated over 100 times. Protons are only detected when a base is added, with repeated bases yielding a current proportional to the number of added bases. In this way, the ion sensors take a chemical picture of incorporation events for every nucleotide. Rothberg likens it to "the CMOS imager, which converts photons to electrons and allows us to see light—only we created a chip that 'sees' ions."

The advantages of ion sequencing are speed, cost and scalability. Its simplicity keeps the footprint small and the costs low because imaging and special reagents such as fluorescently labeled nucleotides are avoided. An average run of 100-base-pair (bp) reads takes only 2 hours and produces 25 Mbp of sequence. Rothberg says that his group is currently sequencing 200 bp routinely and has broken 300 bp. To demonstrate scalability, they produced 6.1-million-sensor and 11-million-sensor

CELL BIOLOGY

WATCHING NEUROTRANSMISSION *IN VITRO*

An ***in vitro*** system for neurotransmitter release is reported.

A well-established approach to study a cellular process is to reconstitute the system *in vitro*. Researchers in several laboratories, including that of Axel Brunger at Stanford University, have been using this strategy to understand neurotransmitter release. In a recently published paper, Brunger and colleagues describe an *in vitro* system that qualitatively recapitulates several of the fundamental characteristics of the process as it occurs in neurons.

The researchers had to begin with very stable preparations of proteoliposomes, incorporating full-length, functional components of the known neuronal fusion machinery, the SNAREs, at physiological protein levels. For most experiments, this minimal reconstituted system included the calcium-sensing protein synaptotagmin and the synaptic protein complexin.

Critically, they included dyes in the donor vesicle preparations that would report on both lipid mixing (DiD) and content mixing (sulforhodamine B) during the vesicle fusion process, a unique feature of their system. The dyes are quenched at concentrations at which they are incorporated into the proteoliposomes and become dequenched upon fusion. But some of the dyes themselves destabilize the liposomes, explains Brunger, so optimizing the system to minimize leakage was very important early on.

Equally importantly, the researchers set up their system so that they could begin with a very well-defined set of conditions. They immobilized acceptor vesicles onto a surface and then allowed labeled donor vesicles to bind in the absence of calcium, a known trigger for neuronal fusion. They monitored the system with very sensitive cameras, using total internal reflection microscopy to detect interacting vesicles via the weak signal from the quenched lipid dye in the donor. After extensive washing and a sufficiently long incubation to allow calcium-independent processes to reach a plateau, Brunger and colleagues had in hand a meta-stable preparation of interacting vesicles mimicking the ready-releasable pool of synaptic vesicles in the neuron. "When they are docked," explains Minjoung Kyoung, first author on the paper describing this work, "the vesicles don't burst; they don't leak; they stay very well together, just interacting; and then when we add calcium, they fuse."

As the system includes both lipid- and content-mixing reporters, and because the researchers monitor single vesicles, different events have characteristic fluorescent signals and can be distinguished on this basis. "We can distinguish interacting vesicles from just

chips by increasing surface area and dropping the number of transistors.

Ion Torrent Systems, the company founded by Rothberg and now part of Life Technologies, made this technology commercially available in the form of their Personal Genome Machine late last year. In the current work, they show that it is capable of whole-genome sequencing. They first produced robust sequence data for three bacterial genomes at five- to tenfold coverage. Accuracy lagged somewhat behind that of other methods for the first 50 bp, but was greater for sequences over 100 bp. One limitation was single-base repeats, for which data accuracy drops the longer they are. Using one thousand 1.2-million-sensor chips, the researchers produced a low-coverage personal genome sequence of Gordon Moore, author of the eponymous law, which states that the number of transistors on an integrated circuit will double every two years. Although the results were roughly comparable to those for the same genome sequenced at low coverage on a different platform, ion sequencing is currently best suited to small genomes and diagnostic detection of variants in targeted stretches of DNA.

As a young technology, accuracy and sequence yield are expected to improve. In addition to their own tweaks, Rothberg is banking that innovations in CMOS chip fabrication will also drive improvements. As he puts it, "the publication used a factory built using technology from 1995, but we can use accumulated Moore's law and time travel by making our next chips in a 2005 factory." He expects it will be possible to make a 1-billion-sensor chip capable of rapidly sequencing personal genomes in the near future.

Tal Nawy

RESEARCH PAPERS

Rothberg, J.M. *et al.* An integrated semiconductor device enabling non-optical genome sequencing. *Nature* **475**, 348–352 (2011).

lipid mixing and from full fusion, so we know the nature of the events," says Brunger. Also, in contrast to ensemble measurements, in which single vesicles are not observed and which is how reconstituted systems have been monitored in the past, this allows one to effectively distinguish between fusing vesicles and those that may burst or leak accidentally.

Notably, the reconstituted system showed rapid full vesicle fusion upon injection of calcium and could recapitulate known *in vivo* effects of mutant synaptotagmin and complexin. As *in vivo*, the system behaves cooperatively as calcium concentration is increased, though the levels of calcium that trigger fusion are one to two orders of magnitude higher than those *in vivo*. The minimal system is most probably lacking components needed to perfectly mimic the situation in the cell. It is, however, undoubtedly an excellent starting point to quantitatively study the functions of additional players in neurotransmitter release.

Brunger and colleagues hope to reach a complete mechanistic understanding of synaptic vesicle fusion at the single-molecule level. "We want," says Brunger, "to make movies of this process, and this system is a major stepping stone to this goal."

Natalie de Souza

RESEARCH PAPERS

Kyoung M. *et al. In vitro* system capable of differentiating fast Ca^{2+}—triggered content mixing from lipid exchange for mechanistic studies of neurotransmitter release. *Proc. Natl. Acad. Sci. USA* **108**, 304–313 (2011).

NEWS IN BRIEF

GENETICS

Large-scale genome editing in *Escherichia coli*

Isaacs *et al.* achieve large-scale genome editing in *E. coli* by stitching together smaller-scale viable intermediates. They started by using multiplex automated engineering to produce strains with mutations in different sections of the chromosome. Through a clever combination of integrated resistance markers and a conjugation-initiating gene, their hierarchical conjugative assembly genome engineering approach allowed them to seamlessly convert all stop codons from TAG to TAA.

Isaacs, F.J. *et al. Science* **333**, 348–353 (2011).

CHEMISTRY

Clickable, stable fluorophores

Perylene tetracarboxylic acid diimides (perylenediimides, PDIs) are stable fluorophores with high quantum yields, making them promising for single-molecule studies. To make these compounds water-soluble for use in biological experiments, Yang *et al.* report encapsulating PDIs in polyglycerol dendrons. They also introduced a single azide group as a 'click' chemistry handle. The resulting PDI-cored dendrimers were used in labeling proteins on the surface of live bacterial and mammalian cells.

Yang, S.K. *et al. J. Am. Chem. Soc.* **133**, 9964–9967 (2011).

NEUROSCIENCE

Optogenetic tools for social disorders

Behavioral problems in psychiatric disorders such as autism and schizophrenia have been hypothesized to arise from an elevated cellular excitation to inhibition balance in neural microcircuitry. Yizhar *et al.* now test this E-I by using optogenetic tools to elevate the cellular E-I balance in the medial prefrontal cortex of freely moving mice. This resulted in social and cognition impairments, and could be ameliorated by elevating the inhibitory cell activity, lending support to the hypothesis.

Yizhar, O. *et al. Nature* advance online publication 27 July 2011.

STRUCTURAL BIOLOGY

High-resolution structures from low-resolution data

Electron crystallography is a powerful method for obtaining structural information about membrane proteins from two-dimensional crystals. A challenge, however, is collecting image data that can provide experimental phase information to solve structures at high resolution. Wisedchaisri and Gonen describe a way around this by obtaining phase information from low-resolution images using a fragment-based phase-extension method. This allowed them to solve the high-resolution structures of aquaporin-4, bacteriorhodopsin and aquaporin-0.

Wisedchaisri, G. & Gonen, T. *Structure* **19**, 976–987 (2011).

SYNTHETIC BIOLOGY

RNA scaffolds for biosynthesis in bugs

There is great interest in engineering synthetic pathways in bacteria to produce useful compounds, drugs and biofuels. Delebecque *et al.* describe the engineering of RNA modules into scaffolds to spatially organize bacterial proteins involved in the hydrogen production pathway. They captured the proteins [FeFe]-hydrogenase and ferredoxin onto a scaffold with RNA aptamer binding domains. As a result of scaffolding, they observed a 48-fold increase in hydrogen production.

Delebecque, C.J. *et al.* Science **333**, 470–474 (2011).