



levels, this would yield 10,000 codes. Additional sizes could be added with stricter filtering requirements, perhaps at the cost of dynamic range of intensity levels.

The synthetic methodology for CdSe–ZnS quantum dots is now well established. The dots are robust and do not photodegrade. One major hurdle in commercialization is the difficulty of ensuring reproducible batch processing of the QD-tagged microbeads. Nevertheless, it appears that the availability of QD bar-coded beads is on the horizon³. The next hurdle is to modify existing microbead formats to take advantage of this increase in multiplexing capability.

This significant advance in optical bar-coding technology has potential to impact genomics, proteomics, and other high-throughput screening assays. In comparison to planar DNA chip technology, microbeads offer potentially improved hybridization, improved statistics, and increased flexibility. For example, typically 10–20 µl of unknown, labeled target DNA is deposited on a chip. Hybridization is limited by diffusional transport. The microbeads described by Han *et al.* could accelerate hybridization by allowing more uniform mixing.

Second, DNA chip technology is typically performed in duplicate or triplicate. As the QD microbeads can be assayed at 20,000 per second, it would be reasonable to perform 50 or 100 replicates in a single experiment.

Finally, the DNA chips are a fixed assay (what's on the chip is on the chip) whereas the microbeads are a flexible assay, different beads can be "dialed in" to screen for different genes based on the results of a previous assay. In the realm of medical diagnoses, microbeads could be used to analyze HIV titers, screen for drugs of abuse, and test for allergies.

One significant obstacle remains before QDs have a wide impact in medical diagnostics. CdSe–ZnS nanocrystals do not emit in the near infrared, so they cannot be used for analyses in blood. Nevertheless, new, robust nanocrystal methodologies are emerging rapidly and will make the concepts described by Han *et al.* applicable to even a wider range of biological problems.

1. Bruchez, M. Jr., Moronne, M., Gin, P., Wiess, S. & Alivisatos, A.P. *Science* **281**, 2031–2016 (1998).
2. Chan, W.C.W. & Nie, S., *Science* **281**, 2016–2018 (1998).
3. Han, M., Gao, X., Su, J.Z. & Nie, S. *Nat. Biotechnol.* **19**, 631–635 (2001).

bias, the oligonucleotides partially obstruct the nanopore and reduce its ion conductivity (Fig. 1B, C). The reduction of ionic current is particularly striking when an oligonucleotide extends into, and blocks, a constriction whose diameter is barely large enough to allow translocation of the oligonucleotide's narrowest dimension (Fig. 1D).

Both of the papers discussed here use α -hemolysin, a protein toxin produced by the bacterium *Staphylococcus aureus*, to form a nanopore. The toxin self-assembles into a lipid bilayer, as shown in Figure 1. By covalently tethering a DNA oligonucleotide near the entrance to the pore's lumen, Howorka and Bayley implement this molecular amplifier as a biosensor that can distinguish between polynucleotides whose sequence perfectly complements that of the tethered oligomer and those whose sequence imperfectly complements, or does not complement, the tethered oligomer. When a short complementary polynucleotide is drawn into the lumen of the nanopore by a voltage bias, it is likely to form a DNA duplex with the tethered oligomer, producing a characteristic current reduction that may last tens of milliseconds. On the other hand, a polynucleotide that does not complement the tethered oligomer is rapidly drawn by the voltage bias into the constriction of the transmembrane region, producing a fleeting but marked current reduction that signals molecular translocation without duplex formation.

A series of experiments with polynucleotides each containing a different sequence showed that, as expected, even a single-base mismatch can influence the distribution of duration times for duplex formation. When an individual oligonucleotide with an unknown codon sequence is tethered to the nanopore and analyzed by the application of a series of different untethered polynucleotides of known sequence, the unknown codon sequence of the single tethered oligomer molecule can be determined. Alternatively, if an array of electrically addressable nanopores with different known tethered oligonucleotides could be implemented, such an array could be used in place of today's surface-mounted microarrays to identify unknown sequence variations in a solution of untethered polynucleotides.

Vercoutere *et al.* take a somewhat different approach and examine the duplex formed by the stems of molecules that take on a hairpin conformation. They use hairpins because the hairpin loops of these molecules adopt conformations that prevent entry into the nanopore lumen, thus keeping the duplex stem portion of a hair-

Nanopores with a spark for single-molecule detection

Progress continues in exploiting ionic conductance of a membrane channel for single-molecule DNA sequence detection.

Hui Wang and Daniel Branton

Elegant and powerful technologies are usually based on a simple concept. And it is hard to imagine a simpler concept than measuring changes of ionic conductivity caused by threading an RNA or DNA molecule through a membrane channel or nanopore¹. Such measurements enable direct, microsecond-time scale nucleic acid characterization without the need for amplification, chemical modification, surface adsorption, or the binding of probes

or intercalators^{2,3}. Now two reports, one in this issue by Howorka *et al.*⁴ and another published previously by Vercoutere *et al.*⁵, demonstrate the use of a nanopore to sequence a codon in a single molecule of DNA or detect both single-base pair and single-nucleotide differences between molecules.

In both papers, the molecular amplifier⁶ used to detect single molecules is simply the ionic current-carrying capacity of an electrolyte-filled nanopore that spans an insulating membrane (see Fig. 1). When the nanopore is filled only with electrolyte (Fig. 1A), a voltage bias induces ions to flow through the nanopore. The current flow is tiny (at the picoampere level), but readily measured and recorded. When single- or double-stranded oligonucleotides are drawn into the nanopore by the voltage

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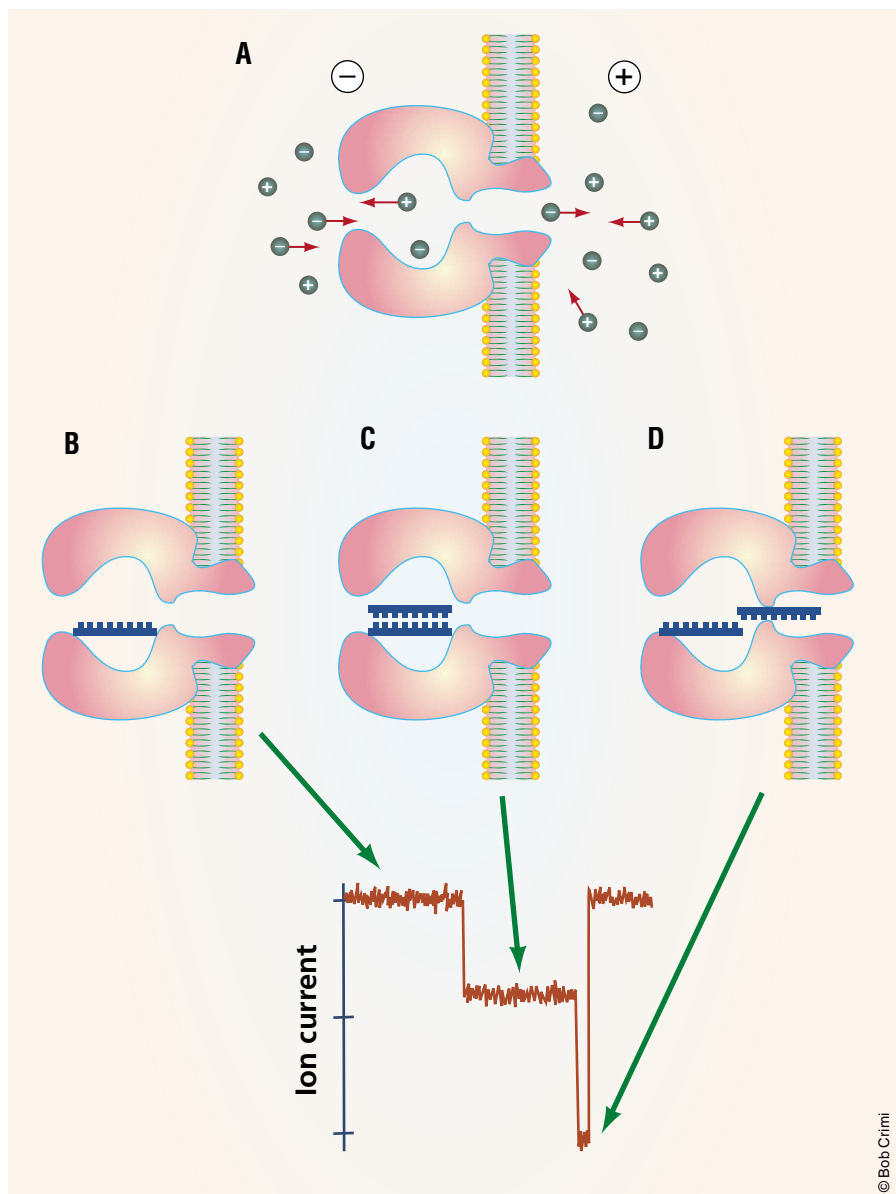


Figure 1. Amplifying nanoscale events. (A) A lipid bilayer forms a nearly perfect insulating membrane between two electrolyte-filled chambers, labeled “-” and “+”. Ions flow through the open pore when a voltage bias is applied across the membrane. (B–D) Depending on the physical state, position, and other properties of oligonucleotides that are drawn into the nanopore lumen, the path of ion flow in the nanopore will be altered. For example, the characteristic electrical signal whose pattern is shown here would be produced if an oligonucleotide base-pairs with a second oligonucleotide that is tethered in the pore’s lumen, then dissociates from the tethered oligo, and is rapidly drawn by the voltage bias through the pore’s narrow transmembrane region.

pin entrapped in the nanopore lumen during tens of milliseconds. By briefly reversing the voltage bias, a molecule can be ejected from the nanopore, and a new molecule entrapped. Thus, Vercoetere *et al.* can evaluate thousands of perfectly base-paired, or imperfectly base-paired, stems in a relatively brief period of time.

This paper is significant because it advances beyond the detection of duplex formation *per se* to ask whether single-base pair and single-nucleotide differences in

stem duplexes modify nanopore conductivity to produce a discernibly different electrical signal. The results show that single-base pair differences between individual molecules are easily resolved and visually discriminated. By automating signal analysis and classification using a support vector machine (SVM) with a training phase, even single-nucleotide differences between molecules are clearly resolved. The single-nucleotide discrimination observed suggests that if a DNA molecule could be

induced to slowly translocate through a suitable nanopore, the atomic-scale changes in the chemical and electrostatic environment within the nanopore might be sufficient to directly reveal the sequence of the translocating DNA as a series of ionic conductivity “signatures”.

What was demonstrated by both papers is proof-of-principle for the extraordinary sensitivity of a nanopore detector and the wealth of information stored in the electrical signals. The papers’ significance lies in recognizing pattern features in individual electrical signals. More than simply detecting the presence of nucleic acids in the nanopore, information regarding the dynamic state of the captured DNA molecule is obtained. Because the authors can relate this change of state to a clear model—base pairing—they can derive sequence information.

What prospects do these systems hold for routine DNA analysis and sequencing? The authors are clearly aware that routine DNA analysis and sequencing will require a robust nanopore. Solid-state nanopores could be ideal, but today’s fabrication methods will have to be substantially improved to develop an electrically addressable array of pores with reproducible diameters in the required 10^{-9} m range. Resolving the different current signatures of individual nucleotides in the nanopore requires that the time a nucleotide spends in the pore be long enough to allow accurate ionic current measurements. A simple method that enables efficient, not too hasty, electrophoretic translocation of a DNA strand through the nanopore remains to be devised. This will require a better understanding of the factors that regulate polymer translocation through a nanopore^{7–9}.

With better physical models of the molecular interactions and ion current flow within the nanopore, it is likely that much more information could be harvested from the electrical signals. In the meantime, the results reported here demonstrate that much remains to be explored, learned, and potentially realized from the lipid bilayer–protein nanopore system.

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