DNA SEQUENCING

Read with quantum mechanics

Electron tunnelling can be used to selectively identify the basic constituents of DNA, indicating that the approach could be used to efficiently read a DNA sequence.

Thomas Thundat

A wealth of genetic information, ranging from hereditary traits to predisposition to diseases, is coded in our DNA as a sequence of four bases — adenine, thymine, cytosine and guanine. However, it took the Human Genome Project 13 years and $3 billion to sequence the three billion bases of the human genome[1]. Moreover, DNA sequencing remains challenging and is typically accomplished using techniques that are inherently inefficient, naturally slow and exorbitantly expensive. Although the introduction of new techniques has reduced the cost of DNA sequencing to less than $100,000 per human genome, it still remains very expensive.

The National Human Genome Research Institute has been supporting the development of new sequencing technologies under the '$1,000 Genome' initiative, which aims to make DNA sequencing routine and affordable, thus making 'personalized medicine' possible[2]. The key to popularizing this concept is to develop a new generation of technologies that are faster and cheaper. Over the years it has been established that techniques for sequencing single molecules of DNA are a more attractive approach than those that use multiple molecules, because they are potentially faster and avoid expensive and time-consuming sample preparation[3]. Furthermore, the short read lengths of current techniques make discovery of repeated sequences and genes difficult. An electronic method that can directly read bases of large DNA molecules without any chemical manipulation or labelling of DNA would be particularly attractive, because such a device could be mass-produced[3].

The quantum-mechanical phenomenon of electron tunnelling has been proposed as a readout mechanism for such a DNA sequencing method[4–6]. The tunnelling current between two electrically biased electrodes, placed a few nanometres apart, is exponentially sensitive to electrode separation. The tunnel current is also exponentially sensitive to the position of atoms within the tunnel gap between the electrodes. This extremely high sensitivity can be exploited to discriminate chemical species present in the gap based on their size, shape and orientation[5]. However, to use the enormous potential of this approach, two challenges must be addressed. First, the tunnelling amplitude is extremely sensitive to changes in the atomic and molecular positions caused by thermal fluctuation, and also to thermal drift of the electrodes. Second, as the tunnelling does not have high intrinsic chemical selectivity, the presence of contaminant molecules can be a source of potential interference.

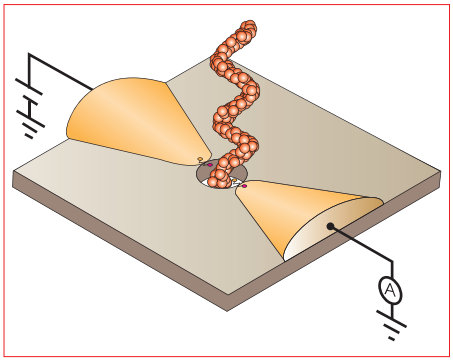

Figure 1 | DNA sequencing by electron tunnelling. A single-stranded DNA molecule (shown in red) passes through the nanogap between two functionalized nanoelectrodes (shown in orange). The interaction between the DNA molecule and the functional groups of the electrodes orient the DNA bases to create a reproducible tunnelling current between the electrodes.

Two independent groups have now overcome these challenges indicating that the use of electron tunnelling to sequence DNA may be an achievable goal. Writing in *Nano Letters*, Stuart Lindsay and colleagues[7] at Arizona State University report the selective identification by tunnelling current of all four DNA nucleosides — a base joined to a sugar — using functionalized electrodes and a nanoscale gap. And writing in *Nature Nanotechnology*, Masateru Taniguchi, Tomoji Kawai and colleagues[8] at Osaka University report the selective identification by electron tunnelling of three of the four DNA nucleotides — a base joined to a sugar and one or more phosphate groups — using nanofabricated, reconfigurable electrodes.

Electron tunnelling through a nanoscale gap is affected by the presence of DNA nucleosides in the gap, and because the nucleosides can have a wide range of molecular orientations in the gap, this can lead to large variations in the contact resistance and, therefore, the tunnel current. However, reproducible tunnelling current can be obtained if the nucleosides in the gap are oriented in the same direction every time. The Arizona State group use gold electrodes functionalized with a mercapto benzoic acid — a benzene ring with a COOH group at one end and a sulphur atom (that can bind to a gold electrode) at the other end — to control the orientation of the nucleosides in the tunnelling gap. At the right gap distances, the protruding benzoic acid group (COOH) can form hydrogen bonds with passing nucleosides. The hydrogen bonds are highly position sensitive and orient the nucleosides such that only one nucleoside configuration is allowed in the gap. This basically overcomes the issue of nucleoside rotation that can result in poor selectivity. The hydrogen bonding provides higher coupling and thus higher tunnel current, which increases the signal-to-noise ratio[9]. This coupling is, however, still weak enough to allow DNA translocation through the gap. As the number of hydrogen bonds varies depending on the nucleosides, the tunnelling current intensity shows a change depending on the identity of the base traversing through the tunnelling gap, which allows all four nucleosides to be discerned.

In the approach reported by the Osaka group, the challenge associated with maintaining a precise and reliable control of the nanogap separation is overcome by using nanofabricated, mechanically controllable, break junctions to measure how the tunnel current changes as a molecule passes between the electrodes. This technique allows control of the nanogap dimension to match the size of a DNA nucleotide with picometre precision. The presence of nucleotides is determined by changes in tunnel current. These experiments, carried out in water,

show discernable and characteristic tunnel-current distributions for three of the four nucleotides, though the current distributions are much wider than those reported when functionalized electrodes are used. The Arizona State group also obtained broad distributions when bases were read with bare electrodes, but showed, through an analysis of the bound-state lifetimes, that the source of these distributions was most probably the well-known promiscuous binding of the imines and amines in bases to gold. The signal appears to be broadened by the large number of configurations that can bind a bare metal electrode, and not the free diffusion of bases, as was previously suggested[6].

Both these approaches are the first strides towards sequencing DNA using a physics-based technique that is label-free and capable of potentially obtaining sequence information at a very high rate. There are, however, many challenges to be overcome before these proof-of-principle experiments can be considered as a basis for a routine DNA sequencer. Even with functionalized electrodes, where distributions are quite narrow[7], there is some overlap of the signatures of the different nucleotides. Statistical techniques can resolve these signatures, but this costs time. Another challenge will be developing simple techniques for creating reliable and precise nanogaps. Other issues include localizing the nucleotide in the gap, controlling its speed, and thermal noise suppression. Furthermore, a practical challenge will be forcing the DNA to pass one base at a time through the tunnelling gap in a reproducible fashion, though the recent introduction of metallic carbon nanotubes as conducting nanopores for DNA control represents an important development in this direction[10].

Eventually, tunnelling in combination with nanopores could permit read lengths that approach a significant fraction of a whole genome. For practical applications this method should be adapted for DNA in buffered aqueous solutions. The methods reported by the Arizona State and Osaka groups partially answer many of these issues, and combining these approaches (as shown in Fig. 1) can potentially overcome some of the variations in the tunnel current signatures of the bases. Moreover, an improved understanding of the variables that contribute to the statistical spread of the currents will allow spatial and temporal control of bases in the gap, leading to rapid, single-shot experiments that can read DNA sequences for routine use. Just as similar challenges have been met before, these issues can be surmounted through the continuing development of a range of creative approaches. ❐

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SELF-HEALING MATERIALS

Get ready for repair-and-go

Computer simulations have shown that hydrophobic nanoparticles encapsulated in a deformable shell can repair surfaces in a manner that is similar to the way white blood cells work in the body.

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We are fortunate that our bodies are able to heal themselves with little or no intervention after a minor injury such as a small cut. This self-healing ability has inspired scientists to invent a range of different methods for restoring function to damaged materials[1]. However, there are many challenges to be overcome before self-healing materials are available for practical applications such as circuit boards and airplane wings[2]. Now, writing in *ACS Nano*, Anna Balazs and co-workers[3] at the University of Pittsburgh and the University of Massachusetts introduce a new concept in self-healing that is inspired by the ability of white blood cells to heal wounds in the body.

When the body is injured, certain molecules called mediators are released to help white blood cells find the site of the injury and activate a healing process[4]. Thus, white blood cells are recruited to the site of damage where they recognize the injured

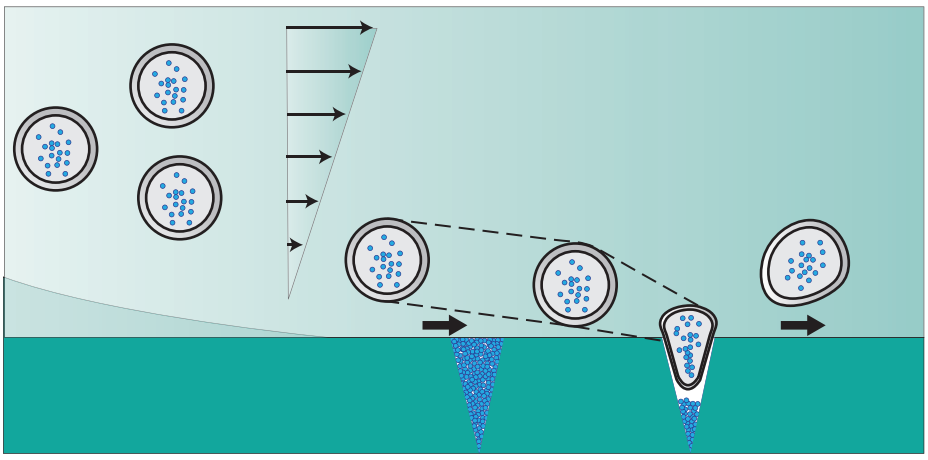

Figure 1 | The 'repair-and-go' approach to self-healing systems. Amphiphilic capsules (grey spheres) that contain hydrophobic nanoparticles (blue spheres) are released in a flow field. The capsules can recognize cracks, which have hydrophobic interiors, in the surface of the material (dark green), which is hydrophilic. Entropic forces cause the nanoparticles to be released into the crack. Once the crack has been repaired, or the flow conditions change, the capsules are released and go with the flow again.