**Institution: University of Oxford**

**Unit of Assessment: 8 Chemistry**

**Title of case study: UOA08-05: Oxford Nanopore Technologies: a successful company built on innovative DNA sequencing**

## 1. Summary of the impact

Hagan Bayley's research on nanopore sensing for DNA sequencing at the University of Oxford led to the formation of the spin-out company Oxford Nanopore Technologies Ltd (ONT) in 2005. Since 2008, ONT has raised £ 97.8M to support research and product development. This level of investment arises as a direct result of the pioneering technology ONT has developed, based on research in the UOA, which has the potential to revolutionise DNA sequencing and other single molecule analyses. ONT currently employs 145 people, nearly six times as many as in 2008, and was recently valued at $ 2 billion. Evidence from ONT was used in a 2009 House of Lords report on genomic medicine, demonstrating ONT's position at the forefront of this new technology.

## 2. Underpinning research

Nucleic acid sequencing is likely to become a core part of personalised medicine and has many additional applications including in agriculture and crop science, food safety and security, and defence. However, it is currently an expensive and time-consuming enterprise. In 2004, the US National Human Genome Research Institute (NHGRI) announced a series of grants to support research with the aim of sequencing a human genome for the cost of $ 1000 or less. At this cost, the technology should become affordable enough to enable routine use in personalised medicine. The speed and reliability of sequencing is also important for use in the clinic. Conventional high-throughput sequencing technologies are relatively slow; most require DNA samples to be amplified, cut to an appropriate length, attached to a bead or surface and labelled with a fluorescent tag which is read with expensive optical imaging equipment. There is thus a need for 'new generation sequencing' technologies which use different approaches to sequencing and can therefore transform the speed and reliability of genome sequencing, and hence the utility of 'personal' genome sequencing.

Hagan Bayley joined the Department of Chemistry in 2003 to carry out fundamental research on membrane proteins. His research led to breakthrough developments in the area of 'new generation DNA sequencing'; specifically, his group worked on perfecting techniques to permit the sensing of individual molecules using engineered membrane protein pores. Bayley established that staphylococcal alpha-haemolysin, a bacterial pore-forming toxin, could be modified by protein engineering to bind a wide variety of partial blockers and that these pores might be used to detect many different analytes at the single-molecule level. For example, a paper published in 2005 (with researchers at Texas A&M University) demonstrated how an engineered pore could be used to detect binding events to monitor the concentration of a critical cell signalling enzyme, cAMP-dependent protein kinase [1]. This research built on previous work by Bayley at Texas A&M on the assembly, structure and functional properties of the alpha-haemolysin protein pore, and made use of the technique of 'stochastic sensing', in which the modulation by an analyte of the ionic current flowing through a single protein pore produces a signal that reveals both the concentration of the analyte and its identity. Bayley has also used stochastic sensing to distinguish between enantiomeric drug molecules. In 2005, Oxford Nanopore Technologies Ltd (ONT) was formed to support and further translate his research to applications in sequencing and sensing.

The work on engineered pores suggested that DNA and RNA might be sequenced by pulling single DNA strands through a nanopore and detecting nucleobases (including non-canonical bases) through their different effects on the ion current passing through the channel pore. In 2006, a breakthrough paper from the Bayley group demonstrated for the first time that the four bases of DNA could be distinguished using an alpha-haemolysin pore equipped with a cyclodextrin adapter [2]. Following this result, in 2009 -10 his group addressed one of the key issues in strand sequencing: the need to be able to sense several different DNA bases within the nanopore at once. The group showed for the first time that an alpha-haemolysin pore could be engineered to contain more than one recognition site, and thus identify groups of different nucleobases

simultaneously in an immobilised DNA strand [3, 4]. This marked a critically important step towards continuous strand sequencing.

In 2010, the Bayley group demonstrated that it was possible to create solid-state nanopores suitable for integration into wafer-scale devices, by attaching a double strand of DNA to a single alpha-haemolysin pore and threading it into a solid-state nanopore by electrophoretic translocation. This research, in collaboration with Delft University of Technology, showed that the hybrid nanopore remained functional in terms of ability to sense individual DNA molecules [5]. It formed the foundation for the development of ONT's GridION and MinION devices for electronic single molecule sensing and DNA and RNA sequencing. In parallel with the DNA-focused research, the Bayley group has worked on similar methods to enable nanopore sensing of protein molecules [1].

In 2005, the Bayley laboratory was awarded an NHGRI '$ 1,000 Genome' grant, which was renewed in 2010 – the only such grant outside the USA. The total award has been in the region of US$ 10M. In 2009, Bayley was awarded the Royal Society of Chemistry World Entrepreneur of the Year Award in recognition of his contribution to the commercialisation of nanopore research.

## 3. References to the research

Asterisked outputs denote best indicators of quality; University of Oxford authors are underlined.


1. * Xie, H., Braha, O., Gu, L.-Q., Cheley, S., Bayley, H. Single-molecule observation of the catalytic subunit of cAMP-dependent protein kinase binding to an inhibitor peptide. Chemistry and Biology 12, 109-210 (2005). DOI: 10.1016/j.chembiol.2004.11.013
   *Demonstrates how stochastic sensing can be used in an engineered pore to detect binding events at the single-molecule level.*
2. Astier, Y., Braha, O. and Bayley, H. Toward single molecule DNA sequencing: direct identification of ribonucleoside and deoxyribonucleoside 5′-monophosphates by using an engineered protein nanopore equipped with a molecular adapter. J. Am. Chem. Soc. 128, 1705-1710 (2006). DOI: 10.1021/ja057123+.
3. * Stoddart, D., Heron, A., Mikhailova, E., Maglia, G. and Bayley, H. Single nucleotide discrimination in immobilized DNA oligonucleotides with a biological nanopore. Proc. Natl. Acad. Sci. USA 106, 7702-7707 (2009). DOI: 10.1073/pnas.0901054106
   *Paper describing how an engineered αHL pore containing 3 recognition sites can be used to identify all 4 DNA bases in an immobilized single-stranded DNA molecule.*
4. Stoddart, D., Maglia, G., Mikhailova, E., Heron, A. and Bayley, H. Multiple base-recognition sites in a biological nanopore: two heads are better than one. Angew. Chem. Int. Ed. 49, 556-559 (2010). DOI: 10.1002/anie.200905483.
5. * Hall, A.R., Scott, A., Rotem, D., Mehta, K.K., Bayley, H. and Dekker, C. Hybrid pore formation by directed insertion of alpha hemolysin into solid-state nanopores. Nature Nanotechnology 5, 874-877 (2010). DOI: 10.1038/nnano.2010.237 *Paper describing the creation of hybrid nanopores suitable for integration into wafer-scale devices.*


## 4. Details of the impact

The research described above has underpinned the formation of a very successful company, Oxford Nanopore Technology (ONT), which has attracted more than £ 105M investment, £ 97.8M of this since 1st January 2008. This very high level of investment arises as a direct result of ONT's pioneering technology, which has the potential to revolutionise the fields of DNA sequencing and single-molecule analysis.

ONT was spun-out of the Department of Chemistry in 2005, founded on intellectual property from the University of Oxford, to exploit the technology developed by the Bayley laboratory and develop it into a robust, commercial product. From the outset the company aimed to create 'disruptive technology' (one that establishes an entirely new market or new behaviours in an existing market,

thus disrupting the old one). From 2008 onwards, ONT forged collaborations with leading nanopore researchers at other institutions including Harvard University, the University of California Santa Cruz and Boston University, in addition to the company's existing relationship with the University of Oxford. This work has generated an IP portfolio for the company comprising >300 issued patents and patent applications in >80 patent families, including 12 key patents arising from Bayley's work at Oxford [7]. A new agreement in 2010, renewed in 2012, strengthened ONT's collaboration with the University of Oxford through funding (>£ 2M) for the Bayley laboratory. The deliberate decision to engage multiple institutions with relevant IP has given ONT a world-leading position in nanopore sensing and ensured that a UK-based company stands to profit from the enormous potential of this technology, with associated benefits to the UK economy.

In February 2012, ONT revealed the GridION system at the Advances in Genome Biology and Technology conference in Florida. GridION is a nanopore-based platform that utilises key elements of Bayley's research, in addition to research from other ONT partners. The primary emphasis of GridION is efficient DNA/RNA strand sequencing (efficient both in time and cost), but it can also be adapted for the direct electronic analysis of target proteins, with applications in the discovery and validation of disease biomarkers and the development of subsequent diagnostics (e.g. in relation to drug response or status of a disease). ONT has revealed that the technology within the GridION platform can be miniaturised into MinION, a portable device for electronic single-molecule sensing that plugs into a PC and is the size of a USB memory stick. These devices measure single molecules directly, without the need for amplification of the target molecule, fluorescent or chemical labelling, or optical instrumentation; in addition analysis of data can be performed in real time, and multiple nanopore measurements can be made in parallel, making it both cheaper and faster than current sequencing technologies [8]. MinION will be made available to selected researchers to use, assess and evaluate from late November 2013 [9].

Currently, ONT's nanopore-sensing technology is the only near-market method which looks set to break the $ 1,000 barrier (it is expected that MinION will be marketed at around $ 900), and importantly it will provide extremely fast and robust sequencing, differentiating it from other sequencing technologies [9]. ONT calculates that if 20 of its second-generation GridION nodes were used together, a human genome could be sequenced in 15 minutes at a highly competitive cost (since the single-molecule techniques developed at ONT do not require the cyclic addition of reagents) [9]. Application of this technology will not just be in human health and personalised medicine but also in agriculture and crop science, environmental science (e.g. detection of toxins in water), energy and defence (e.g. detection of explosives). The potential global market for 'next generation sequencing' is significant: in 2012 it stood at $ 232M and is expected by market researchers such as BCC Research to be approximately $ 7.6bn by 2018. The announcement of GridION and MinION attracted considerable worldwide media attention from national and international newspapers including the New York Times, Guardian, Sunday Times and Financial Times, as well as from scientific journals including Science. These reports highlighted the low cost and high speed of ONT's devices, and the two sequencers were described as 'impressive, credible, possibly amazing' and as a potential 'game-changer' [10, 11].

The enormous potential of ONT's new technology, coupled with its wide intellectual property portfolio and product pipeline, has generated a very high level of investment during the impact period. Seed funding that enabled ONT to be established was obtained in two rounds from IP Group in 2005, and in June 2006 the company raised £ 7.75M. Since 1st January 2008, ONT has raised a further £ 97.8M (representing 92% of total investment since company start-up) from a range of sources in the UK and the US. Over a third of this (£ 34.1M) was raised in May 2012 almost entirely from existing investors in the company – a clear indication of the extent to which investors see the potential for GridION and MinION to become highly successful. ONT is currently well funded for the next phase of corporate development [12].

The success of ONT is also reflected in its workforce. During the impact period the number of people ONT employed increased nearly 6-fold, from 25 in 2008 to 145 in July 2013. The vast majority of current staff are employed in research and development; they have backgrounds in multiple disciplines including nanopore science, molecular biology and applications, informatics,

engineering, electronics, manufacturing and commercialisation. ONT has UK offices in Oxford and Cambridge and US offices in Boston and New York, and is building a commercial team in advance of the launch of the products. Since 2008, ONT has also benefited from the transfer of 3 highly-skilled staff from the Bayley laboratory. Two of these bring academic research expertise to an industrial R&D setting and work in application development and advanced research, and the third works for ONT's IP team [9]. The company was valued at $ 2bn by a Numis Securities analyst in November 2012 [13].

ONT made an important contribution to the 2008 - 09 House of Lords Science and Technology Committee session on Genomic Medicine. ONT provided evidence to the effect that the new technologies were advancing so rapidly that the government needed to take action. This evidence helped to shape the recommendations in the 2009 final report [14], which are now being taken forward by the Government (for example, via the Human Genomics Strategy Group, established by the government to address questions raised by the report [15]). This influence further reflects ONT as a pioneering leader in the field of new generation sequencing.

**5. Sources to corroborate the impact**

7. https://www.google.com/patents/US7939270
   The first Bayley patent to be licensed to ONT, US 7939270 B2, granted May 2011: 'Delivery of molecules to a lipid bilayer', part of the technology underpinning GridION and MinION.
8. https://www.nanoporetech.com/technology/introduction-to-nanopore-sensing/introduction-to-nanopore-sensing
   ONT webpage confirming details of the GridION and MinION technology and what it can do.
9. The Corporate and Communications Director at ONT can corroborate details of the '15 minute genome', the projected cost of MinION, its availability to researchers from late 2013, details of company employees, and the role of staff who have joined ONT from the Bayley laboratory.
10. http://www.nytimes.com/2012/02/18/health/oxford-nanopore-unveils-tiny-dna-sequencing-device.html?_r=0
    February 2012 article in the New York Times, announcing the release of MinION and corroborating the descriptions of the device quoted here.
11. http://www.sciencemag.org/content/336/6081/534.full?sid=9d6cc8c3-4c55-443d-9818-3cebd1228d27
    May 2012 article in Science News, announcing the release of MinION and corroborating the descriptions of the device quoted here.
12. https://www.nanoporetech.com/about-us/for-investors
    ONT webpage, confirming investments in the company.
13. http://www.bio-itworld.com/2012/11/29/numis-reiterates-2billion-valuation-oxford-nanopore.html
    BioIT World report corroborating the $ 2 billion valuation of ONT.
14. http://www.publications.parliament.uk/pa/ld200809/ldselect/ldsctech/ldsctech.htm
    Reports from the House of Lords Science and Technology Committee session on Genomic Medicine, confirming the evidence provided by ONT to the committee.
15. https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/213705/dh_132382.pdf
    Human Genomics Strategy Group report, January 2012, confirming actions as a result of the report cited at [14].