Institution: University of Oxford

Unit of Assessment: 8 - Chemistry

Title of case study: Oxford Nanopore: Nucleic acid sequencing and diagnostics on any scale from laboratory to point of care

Period when the underpinning research was undertaken: 2004 - 2020

Details of staff conducting the underpinning research from the submitting unit:

Name(s):	Role(s) (e.g. job title):	Period(s) employed by submitting HEI:
Professor Hagan Bayley	Chair of Chemical Biology	2003 – present
Evan Spruijt	Marie Curie Fellow	01/07/2015 – 31/08/2016
Yujia Qing	Postdoctoral Fellow	25/03/2019 – present
Ellina Mikhailova	Technician	01/12/2003 – present
Mariam Ayub	Postdoctoral Fellow	26/07/2010 – 15/08/2016
Andrew Heron	Postdoctoral Fellow	03/01/2006 – 03/12/2010
Giovanni Maglia	Postdoctoral Fellow	14/02/2006 – 31/10/2010
Lakmal Jayasinghe	Postdoctoral Fellow	06/03/2006 – 30/09/2008
Matt Holden	Postdoctoral Fellow	30/06/2006 – 30/06/2008
Yann Astier	Postdoctoral Fellow	09/10/2004 – 09/10/2006

Period when the claimed impact occurred: 01/08/2013 to 31/12/2020

Is this case study continued from a case study submitted in 2014? N

1. Summary of the impact (indicative maximum 100 words)

Professor Hagan Bayley, along with external partners Dr Gordon Sanghera and Dr Spike Willcocks, founded Oxford Nanopore Technologies (ONT) in 2005, based on Bayley's research on single-molecule nanopore sensing in Oxford's Department of Chemistry. ONT's inexpensive, fast, portable, nanopore-based direct DNA and RNA sequencing has generated widespread impact, as evidenced by the sequencing of viruses in public health emergencies including Ebola and Nigerian Lassa fever in Africa and Zika in Brazil, and more recently the global coronavirus pandemic, where it identified the strain of the coronavirus when it first appeared in China. ONT also applied their technology to develop LamPORE, a novel diagnostic platform for detecting SARS-CoV-2 RNA in use by the UK government. ONT has raised GBP507,800,000 since 1 August 2013 to support research and development, as well as commercialisation of its products worldwide. In December 2020 ONT employed 600 people, 4 times as many as in July 2013, and the most recent transactions valued the company at approximately GBP1,700,000,000.

2. Underpinning research (indicative maximum 500 words)

DNA and RNA sequencing are changing the world. Nucleic acid sequencing impacts all aspects of fundamental genetics and genomics, including human ancestry, biology, zoology, botany and microbiology, and has numerous applications in areas including medicine, forensics and agriculture.

As recently as the early 2000s, nucleic acid sequencing was expensive, cumbersome and time-consuming. In 2004, the US National Human Genome Research Institute (NHGRI) announced support for research to sequence a human genome for just USD1,000. At this cost, the technology would be affordable for routine use in medicine, including point of care diagnostics. Cheap, high-speed, direct nucleic acid sequencing would be of immense value in personal genome sequencing, and portability would be an added advantage. In 2005 Hagan Bayley was awarded an NHGRI '$1,000 Genome' grant, the only such grant outside the USA. The research was very successful and the grant was renewed in 2009 and 2013, and ran until 2018, the end of the scheme. The total award to Bayley since 2013 was around USD1,500,000.

Hagan Bayley's research established "stochastic sensing" for the detection of a wide range of analytes at the single-molecule level by monitoring currents flowing through engineered membrane protein nanopores with exquisite sensitive. Analytes have included metal ions, small biological molecules and pharmaceuticals, peptides, oligonucleotides (DNA) and chemically reactive substances. The approach is selective enough to distinguish optical

isomers. Bayley, along with Sanghera, and Willcocks, formed Oxford Nanopore Technologies (ONT) in 2005 to commercialise this research. The company set its sights on the daunting challenge of DNA sequencing, which was facilitated by a series of papers from the Bayley group from 2004 to 2010, including the critical work on DNA nucleobase identification [**Figure 1**, **R1**]. Work from the Bayley laboratory remains cutting-edge. For example, research underpinning the potential utility of more than one constriction in the nanopore was published in 2010 [**R2**] and was subsequently leveraged by ONT in their development of a proprietary class of nanopore structure. Other important work introduced into the commercial world by ONT includes RNA base identification [R3] and the direct reading of epigenetic base modifications [**R4**]. These are both highly significant because they are relevant to many medically important processes including gene expression, and such additional high-level information is not obtainable from standard DNA sequencing that relies on the use of polymerase activity in a 'sequencing by synthesis' design.

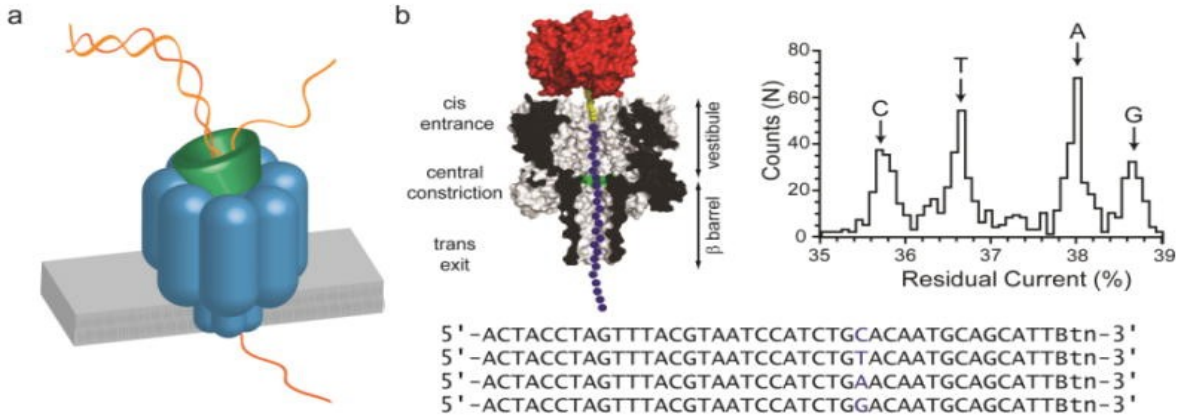

Figure 1: a), Nanopore sequencing. ssDNA is fed through an individual protein pore by an enzyme that handles dsDNA. Sequence is determined by analysis of fluctuations in the ion current. b), Early base identification experiments. ssDNAs suspended in an αHL pore by attachment to streptavidin mimic the ratcheting motion of the enzyme. The DNA bases each gave a different residual ionic current even in a complex background.

Work in the Bayley lab from August 2013 has continued to push forward the frontiers of nanopore technology. Improved throughput is crucial in the DNA sequencing field, and in 2015 optical detection was shown to be potentially advantageous in this context for highly parallel nucleobase detection [**R5**]. In 2018 Bayley showed that a molecule can be moved in an electric field along a track in a nanopore by a non-enzymatic chemical ratcheting mechanism. The molecular motion has defined start and end points, has no chemical fuel requirement, is externally controlled with precision and the motion is directional. A DNA molecule can be ratcheted along the nanopore track in either direction to repeatedly read the sequence, an ideal scenario for sequencing [**R6**].

In 2019, Bayley won the prestigious Royal Society Mullard Award for the invention of stochastic sensing. The Mullard Award is given to 'those who have an outstanding academic record in any area of natural science, engineering or technology and whose work is currently making or has the prospect to make a contribution to national prosperity in the United Kingdom.'

3. References to the research (All journal articles)

R1. Clarke J, Wu H, Jayasinghe L, Patel A, Reid S, Bayley H: Continuous base identification for single-molecule nanopore DNA sequencing. Nature Nanotechnology 2009, 4:265-270. DOI: 10.1038/NNANO.2009.12. Citations: 1,023 (Web of Science, 10/12/2020)
R2. Stoddart D, Maglia G, Mikhailova E, Heron A, Bayley H: Multiple base-recognition sites in a biological nanopore– two heads are better than one. Angew Chem Int Ed 2010, 49:556-559. DOI: 10.1002/anie.200905483
R3. Ayub M, Bayley H: Individual RNA base recognition in immobilized oligonucleotides using a protein nanopore. Nano Lett 2012, 12:5637-5643. DOI: 10.1021/nl3027873

R4. Wallace EVB, Stoddart D, Heron AJ, Mikhailova E, Maglia G, Donohoe TJ, Bayley H: Identification of epigenetic DNA modifications with a protein nanopore. ChemComm 2010, 46:8195-8197. DOI: 10.1039/C0CC02864A
R5. Huang S, Romero-Ruiz M, Castell OK, Bayley H, Wallace MI: High-throughput optical sensing of nucleic acids in a nanopore array. Nature Nanotechnology 2015, 10:986-991. DOI: 10.1038/nnano.2015.189
R6. Qing Y, Ionescu SA, Pulcu G and Bayley H: Directional control of a processive molecular hopper. Science 2018, 361, 6405, 908-912. DOI: 10.1126/science.aat3872

Evidence of the quality of the research:

Title	Start date	End date	Funder	Amount
Hybrid nanopores	2015	2018	BBSRC	GBP726,201
$1,000 Genome Project	2005	2018	NHGRI	USD1,500,000

4. Details of the impact (indicative maximum 750 words)

Pathway to impact: from research to commercialisation

Hagan Bayley's research in Oxford Chemistry gave rise to a key patent on the crucial original lipid bilayer (WO2008012552A1, *Formation of bilayers of amphipathic molecules)* that enabled the formation and furtherance of a very successful company, Oxford Nanopore Technologies (ONT), which he spun out from the University in 2005 with support from IP Group (intellectual property commercialisation company).

ONT has attracted GBP507,800,000 in investments during the REF period (GBP613,550,000 since formation) [**E1**] and in December 2020 employed 600 people (headcount: 600), 4 times as many as in 1 August 2013 [**E11**]. In January 2020 ONT was valued at around GBP1,700,000,000 [**E2**]. It is a truly international company with headquarters at the Oxford Science Park and offices or facilities in Cambridge (UK), Shanghai, Tokyo, Singapore, San Francisco, Boston and New York. Revenues are growing rapidly (2015: GBP700,000; 2016: GBP4,500,000; 2017: GBP13,800,000; 2018: GBP32,500,00; 2019: GBP52,100,000) [**E5**].

Pioneering DNA, RNA and epigenetic sequencing technology

ONT's pioneering technology is revolutionising DNA sequencing and the emerging fields of RNA sequencing and epigenetic sequencing. The latter two in particular are crucial for our understanding of gene expression and its relationship to development, ageing and disease. The global market for next generation sequencing was valued at USD7,800,000,000 in 2019 (source: Research and Markets) and is expanding rapidly. Oxford Nanopore's sequencing technology is opening up a range of new opportunities owing to the novel and unique properties of this wide-ranging single-molecule detection platform (rapid, low cost sequencing; direct read-out of sequence information; sequencing of ultra-long DNA strands; portable, diagnostic applications at point of care; applicability to other biomolecules).

ONT has developed a range of DNA sequencing devices for various throughput levels in different environments (e.g. core laboratories, individual research laboratories, in the field) [**E3**]. The MinION sequencer, available for free alongside a USD1,000 purchase of consumables, typifies ONT's innovative power. This portable device is the size of a mobile phone and can sequence 500 individual DNA strands asynchronously in parallel (Figure 2) with a best-in-field yield of 42Gb. It is capable of sequencing fragments of DNA of over 1 Megabase in length and tricky repetitive DNA elements and RNA molecules. The GridION can run five MinION flow cells (chips), and PromethION enables entire human genome sequencing.

Figure 2: Portable MinION DNA sequencer

A 3,200m^2 UK manufacturing facility at Harwell, the MinION Building, produces flow cells for ONT's sequencing devices. The facility, opened in 2019, houses supersize clean rooms and its capacity exceeds 1,000,000 flow cells per year [**E4**].

ONT's nanopore sequencing technology has impacted in many fields from zoology and microbiology, to human genetics and cancer research. The portability of the MinION has enabled it to be used for real-time sequencing of polar ocean microbes in the Arctic, and the first ever DNA sequencing in microgravity on board the International Space Station. One of the areas where it has had the most impact is in sequencing viruses to aid global public health emergencies.

Sequencing of viruses in public health emergencies

ONT's technology has been used for the sequencing of viruses including yellow fever and swine flu. The portability of the MinION enables surveillance in the field, for example:

- **Ebola virus in West Africa (2015-16):** a team led by Public Health England used the MinION to establish a field laboratory in Guinea for real-time genomic surveillance of the ongoing epidemic. The data, reported to the World Health Organization (WHO), identified two lineages and showed transmission between Sierra Leonne and Guinea. After a resurgence in 2016, MinION sequencing showed that survivors could reactivate the outbreak, which led to a WHO programme of vaccination of contacts of survivors in Guinea. [**E8**]
- **Zika virus in Brazil (2017):** the ZIBRA project, a UK-Brazil collaboration, used MinION to sequence Zika virus genomes in north-east Brazil. Results demonstrated the region was a focal point of the epidemic and played a key role in its spread within Brazil, before spreading across the Americas. [**E9**]
- **Nigerian Lassa fever (2018):** an upsurge in cases led the Nigeria Centre for Disease Control and WHO to urgently request sequencing information. A team led by the German Center for Infection Research used MinIONs to conduct in-country, mid-outbreak viral genome sequencing. Results showed zoonotic transmission, rather than an emergent strain or extensive human-to-human transmission, was the main source. [**E10**]

Sequencing of SARS-CoV-2 and development of LamPORE testing

At the onset of the coronavirus pandemic ONT sent hundreds of MinION devices to China's Centre of Disease Control and Prevention, which allowed the first RNA sequences of SARS-CoV-2 to be recorded. This was key to identifying the strain of the coronavirus when it first appeared and important for vaccine development and understanding viral transmission and evolution [**E6**]. The MinION device was used in work published as early as January 2020 which indicated person-to-person transmission of the virus through air travel [**E6**]. The conclusions of the study – including to isolate patients, and trace and quarantine contacts as early as possible – helped to inform the global response to the pandemic. Surveillance of the mutating SARS-CoV-2 RNA sequence has helped to provide information about its mode and speed of evolution, geographical spread and adaptation to human hosts. Recent examples include how COVID-19 can transmit from mink to humans and back again, and how the 439K variant evades antibody-mediated immunity [**E13**].

ONT acted with remarkable speed to develop COVID-19 diagnostics including LamPORE, a parallel viral RNA amplification and barcoding diagnostic protocol, which utilises rapid MinION or GridION sequencing. This 90-minute test can be carried out on-site (e.g. in hospitals). The UK government ordered 450,000 LAMP swab tests from ONT in August 2020 [**E7**]. The Department of Health and Social Care (DHSC) conducted a validation study of LamPORE on 1,200 asymptomatic NHS staff and symptomatic patients who had undergone previous respiratory pathogen testing. A total of 3,966 swab and 18,435 saliva samples were tested across 4 NHS hospital sites. Results published in December 2020 show the technical performance of the LamPORE assay demonstrated a sensitivity of 99.57% and specificity of 99.40%. When the technology was evaluated in the setting of saliva samples using RNA extraction, the sensitivity is 98.94% and specificity 99.39% across all samples tested [**E14**].

The DHSC report concludes that *'the key advantage of LamPORE, as part of a testing regime for both saliva and swabs, is its capability to offer high throughput testing of SARS-CoV-2 for both asymptomatic or symptomatic testing... The LamPORE assay in the RNA mode has been successfully applied in this use case to populations of healthcare workers with the aim of curtailing transmission between staff and to patients within healthcare settings. The NHS*

asymptomatic staff saliva testing pilot successfully facilitated daily on-site testing as an additional test to support SARS-CoV-2 detection' [**E14**].

The DHSC Minister for Innovation said of the report: *'With one in three people not displaying symptoms of Covid-19, broadening asymptomatic testing is critical to protect those at highest risk. Oxford Nanopore's LamPORE test is another example of British innovation leading the way, and is an incredibly useful addition to our Covid-19 testing toolkit – delivering accurate results to people with and without symptoms'* [**E12**]. The LamPORE assay is being expanded to encompass other respiratory infections such as Influenza and Respiratory Syncytial Virus (RSV), crucial in the winter months.

Nanopore sequencing has been of paramount importance in the global response to the COVID-19 pandemic and ONT continues to develop yet more technological advances backed by the company's intense R&D programme and large IP portfolio, including 7 patents arising from Bayley's work at Oxford during the REF period. Developments include the SmidgION (a smartphone attachment), the smallest sequencing device ever developed, and the Plongle, which offers high-throughput, real-time sequencing in a 96-well plate format, compatible with automation.

5. Sources to corroborate the impact (indicative maximum of 10 references)

E1. ONT website showing details of investments in ONT over REF period
E2. The Times newspaper article reporting on 2020 valuation of ONT
E3. ONT website showcasing the current ONT nanopore sequencing technology
E4. ONT press release on new UK manufacturing centre at Harwell near Oxford (05/07/2019)
E5. ONT Ltd Annual Report & Financial Statements for year ended 31/12/2019, corroborating sales revenues from 2015-19 (page 6)
E6. Journal articles: Sequencing SARS-CoV-2 in China: N. Zhu et al, *A Novel Coronavirus from Patients with Pneumonia in China, 2019*, N Engl J Med 2020; 382:727-733; the viral transmission of COVID-19: J.F Chan et al, *A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster*, The Lancet, 2020, 395, 514-523
E7. UK Government press release: government orders 450,000 LamPORE tests (03/08/20)
E8. Journal articles on MinION sequencing of Ebola: B. Diallo et al, *Resurgence of Ebola Virus Disease in Guinea Linked to a Survivor With Virus Persistence in Seminal Fluid for More Than 500 Days*, Clinical Infectious Diseases, 2016, 63:10, 1353–1356; J. Quick et al, *Real-time, portable genome sequencing for Ebola in Africa*, Nature, 2016, 530, 228–232; M. Carroll, *Retrospective versus real-time Ebola virus sequencing*, The Lancet Infectious Diseases, 2019, 19:6, 567-568
E9. ONT website: ZiBRA project: real-time sequencing of Zika virus in Brazil (30/03/20); Journal article: J. de Jesus et al, *Acute Vector-Borne Viral Infection: Zika and MinION Surveillance*, Microbiology Spectrum, 2019, 7:4; both detailing MinION sequencing of Zika
E10. Journal article on MinION sequencing of Nigerian Lassa Fever: L.E. Kafetzopoulou et al, *Metagenomic sequencing at the epicenter of the Nigeria 2018 Lassa fever outbreak*, Science, 2019, 363:6422, 74-77
E11. Letter of support from Chief Executive Officer of Oxford Nanopore Technologies (Jan 2021) corroborating Bayley's research and company details of ONT
E12. Mirror Online news article reporting on effectiveness of LamPORE, including corroborating quote from DHSC Parliamentary Under Secretary of State (Minister for Innovation), 28/01/2021
E13. Journal articles on surveillance of the mutating SARS-CoV-2 RNA sequence: E. Thompson et al, *The circulating SARS-CoV-2 spike variant N439K maintains fitness while evading antibody-mediated immunity*, bioRxiv, Nov 2020; B. Oude Munick et al, *Jumping back and forth: anthropozoonotic and zoonotic transmission of SARS-CoV-2 on mink farms*, bioRxiv, Sep 2020
E14. Technical Validation Report by DHSC (December 2020) corroborating NHS study, and the sensitivity and specificity of LamPORE in testing for COVID-19