Institution: The University of Nottingham promoting

Unit of Assessment: UoA5

Title of case study: The development of a real-time NanoBRET ligand-binding assay resulted in its successful commercialisation and implementation by global pharma.

Period when the underpinning research was undertaken: 2013-present

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:
Stephen Hill	Professor of Molecular Pharmacology	1981-present
Jeanette Woolard	Professor of Cardiovascular Physiology & Pharmacology	2010-present

Period when the claimed impact occurred: 2015-present

Is this case study continued from a case study submitted in 2014? No

1. Summary of the impact

Research led by Professors Hill and Woolard at the University of Nottingham, as part of a long-term and ongoing collaboration with the multi-national biotechnology company Promega Corporation, has developed a technology to enable the high-throughput study of ligand-binding to cell surface receptors. The NanoBRET (Nanoluciferase Bioluminescence Resonance Energy Transfer) technology is highly sensitive and specific for a given target receptor. This innovation has resulted in the introduction of new commercial products with positive impacts on commerce. Major beneficiaries are Promega, who are successfully commercialising the technology and as a result have increased employment to support product development, as well as SMEs and major pharma including GSK, who have optimised their drug discovery processes by adopting the Promega NanoBRET technology.

2. Underpinning research

G-Protein Coupled Receptors (GPCRs) mediate a variety of fundamental physiological processes and represent the largest group of proteins targeted by existing therapeutics. The development of new effective therapies that target GPCRs is only possible with the comprehensive understanding of their pharmacology and therefore new approaches that facilitate this understanding will enable the discovery of novel therapeutics.

In 2015 Professor Hill and colleagues at the University of Nottingham (UoN), the University of Western Australia and Promega developed an innovative method (NanoBRET) to monitor ligand binding to GPCRs on the surface of living cells. NanoBRET combines the use of fluorescently-labelled ligands, previously developed and presented as an Impact Case Study in REF2014, with Promega's NanoLuc protein (a small luciferase with superior luminescence) tagged to receptors. NanoBRET technology enables the measurement of ligand binding to GPCRs using Bioluminescence Resonance Energy Transfer (BRET) and standard multi-well bioluminescence/fluorescence plate readers. Using this technology for the first time, Professor Hill and colleagues' research described the real-time binding of a red fluorescent analogue of the antagonist propranolol to the human β_2-adrenoreceptor, and of fluorescent agonists and antagonists to the adenosine A_1 and A_3 receptors in living cells **[1]**. This technology is ground-breaking in comparison to traditional and established radioligand assays because it is safer (avoiding the used of radioisotopes), high-throughput, cost-effective, highly sensitive. It is also physiologically relevant, as it allows ligand binding kinetics to be measured in live cells at 37°C in real time **[1,2,4].**

Professors Hill and Woolard went a step further in 2018 and using the NanoBRET assay measured ligand binding *in vivo* in a mouse model of triple-negative human breast cancer

cells expressing nanoluciferase-tagged β_2-adrenoreceptor **[3]**. This was the first *in vivo* study of real-time ligand binding using NanoBRET and it demonstrated the visualisation and measurement of the extent to which target engagement is achieved in an animal cancer model, where the vascular environment is typically compromised and often impairs access and binding of the drug to the malignant cells.

Professors Hill and Woolard further applied NanoBRET, underpinned by a BBSRC LINK Award with Promega **[11],** to fluorescently label Vascular Endothelial Growth Factor (VEGF) isoforms and monitor their binding to a VEGF receptor (VEGFR2); a Receptor Tyrosine Kinase (RTK), and co-receptor neuropilin-1 **[5, 6]**. RTKs are a different type of cell surface receptor to GPCRs. They initiate signalling pathways that regulate cell proliferation, migration, differentiation and cell survival, and therefore are also popular targets for cancer drug development. With these two publications, Professors Hill and Woolard validated a novel method for monitoring ligand binding to RTKs making it an attractive method for new therapeutics development that target RTKs.

More recently, Professor Hill and colleagues have used CRISPR/Cas9 genome editing technique to incorporate a NanoBiT fragment (HiBiT) of nanoluciferase into the N-terminus of the chemokine receptor CXCR4 under the control of its endogenous promoter. The full nanoluciferase activity can be reconstituted by addition of purified LgBiT and this has allowed ligand binding using NanoBRET to be monitored in cells that endogenously express the CXCR4 receptor at native and therefore physiological levels **[7]**.

NanoBRET has become the ligand-binding assay of choice for many researchers, both in academic and pharma/biotech environments, due to its enhanced sensitivity, ease of use and capacity for real time monitoring in live cells. NanoBRET is now a well-established method for investigating ligand binding to cell surface receptors and intracellular proteins.

3. References to the research

Key publications (University of Nottingham UoA5 researchers, at the time of publication, are highlighted in bold; researchers of Promega are underlined)

1 **Stoddart LA**, Johnstone EKM, **Wheal AJ**, **Goulding J,** Robers MB, Machleidt T, Wood KV, **Hill SJ** & Pfleger KDG (2015). Application of BRET to monitor ligand binding to GPCRs. *Nature Methods*. 12: 661-663. doi: 10.1038/nmeth.3398

2 **Stoddart LA**, Vernall AJ, **Bouzo-Lorenzo M**, Bosma R, Kooistra AJ, Vischer HF, Leurs R, **Briddon SJ**, Kellam B, **Hill SJ** (2018) Development of novel fluorescent H_1-receptor antagonists to study ligand-binding kinetics in living cells. *Sci. Rep*. 8:1572. doi: 10.1038/s41598-018-19714-2

3 **Alcobia DC**, Ziegler, AI, Kondrashov A, Comeo E, Mistry S, Kellam B, Chang E, **Woolard J, Hill SJ**, Sloan EK (2018). Visualising ligand-binding to a GPCR *in vivo* using nanoBRET. *iScience* 6: 280-88. doi: 10.1016/j.isci.2018.08.006.

4 **Bouzo-Lorenzo M**, **Stoddart LA**, Xia, L, Ijzerman AP, Heitman LH**, Briddon SJ**, **Hill SJ** (2019) A live cell NanoBRET binding assay allows the study of ligand-binding kinetics to the adenosine A_3 receptor. *Purinergic Signalling*. 15: 139–153. doi: 10.1007/s11302-019-09650-9.

5 **Kilpatrick LE**, Friedman-Ohana R, **Alcobia DC**, Riching K, **Peach CJ**, **Wheal AJ**, **Briddon SJ**, Robers MB, Zimmerman Machleidt T, Wood KV, **Woolard J, Hill SJ** (2017) Real-time analysis of the binding of fluorescent $VEGF_{165}$a to VEGFR2 in living cells: Effect of receptor tyrosine kinase inhibitors and fate of internalized agonist-receptor complexes. *Biochem. Pharmacol*. 136:62-75. doi: 10.1016/j.bcp.2017.04.006

6 **Peach CJ**, **Kilpatrick LE**, Friedman-Ohana R, Zimmerman K, Robers MB, Wood KV, **Woolard J, Hill SJ** (2018). Real-time ligand binding of fluorescent VEGF-A isoforms that discriminate between VEGFR2 and NRP1 in living cells. *Cell Chem Biol.* 25: 1208-1218. doi: 10.1016/j.chembiol.2018.06.012.

7 **White CW, Caspar B**, Vanyai, HK, Pfleger KDG, **Hill SJ** (2020) CRISPR-mediated protein tagging with Nanoluciferase to investigate chemokine receptor function and

conformational changes at native expression levels. *Cell Chem Biol* 27: 499-510. doi: 10.1016/j.chembiol.2020.01.010.

Key Grants:

8 2012-2017, European Commission FP7-JTI, Innovative Medicines Initiative (IMI), "Kinetics for Drug Discovery (K4DD)", **Hill Nottingham PI**, Total EUR20,860,250, UoN share EUR409,999.

9 2016-2021, MRC Research Grant, "Use of fluorescence correlation to study GPCR oligomerisation and allosterism in membrane microdomains of single living cells", **Hill** PI, **Woolard** CoI, GBP1,917,102

10 2013-2015, Australian Research Council Linkage Projects, "Development of class-leading bioluminescence resonance energy transfer technologies for real-time monitoring of molecular interactions", **Hill** PI, AUD201,412

11 2014-2017, BBSRC Industrial Link Grant (with Promega) for "Novel BRET approaches to unravel the molecular pharmacology of VEGFR2 receptors", **Hill** PI, **Woolard** CoI, GBP406,282 from BBSRC plus GBP578,413 in-kind support from Promega Corporation.

12 2016-2019, Australian Research Council Linkage Projects, "Development of technologies to monitor multimolecular complexes", **Hill** PI, AUD520,170

13 2018-2022, BBSRC and GSK Training Grant, "Application of novel cellular target engagement technologies to interrogate the IL23R-JAK TyK2-STAT signalling pathway", **Hill** Training Grant Holder**,** GBP99,034 from BBSRC and GBP32,000 from GSK

Awards

14 2018-2019, President of the British Pharmacological Society, **Hill,** web link

15 2018, awarded the "Select Science Life Sciences Video of the Year 2018" for the use of NanoBRET to study ligand-binding to GPCRs and RTKs, **Hill,** web link

16 2019, Ariens Award and Lecture from the Dutch Pharmacological Society for outstanding scientific achievements in pharmacology, **Hill**, web link

17 2020, Honorary Fellowship of the British Pharmacological Society in recognition of sustained excellence and leadership in science and service to the Society, **Hill,** web link

18 2019, Bill Bowman Award from the British Pharmacological Society, **Woolard**

19 2020, Fellowship from the British Pharmacological Society, **Woolard**, web link

4. Details of the impact

The extensive collaboration between the UoN research team and Promega has contributed to innovation within an existing business through the introduction of new products, positively impacting commerce and the economy, with two main beneficiaries:

i. Promega, through the commercialisation of NanoBRET technology and the provision of contract research using NanoBRET binding assays.
ii. Biotech, Pharma companies and CROs using NanoBRET binding assays for optimised drug discovery pipelines.

Promega Corporation is a US company with branches in 16 countries including the UK. Professors Hill and Woolard have maintained a long-standing and active relationship with Promega over the last seven years that was reinforced by a BBSRC Link grant (2014-2017) allowing the NanoBRET technology to be applied to RTKs **[11]**. The BBSRC LINK grant formalised the partnership, through the implementation of a collaboration agreement, and included a substantial GBP578,413 in kind contribution from Promega that covered reagents, cell lines, the appointment of a postdoctoral scientist and substantial intellectual input from key members of the Promega Advanced Technology Group (Madison, USA). Promega has further developed the NanoBRET approach described in this Impact Case Study for GPCR and RTK target engagement and expanded it successfully to monitor target engagement at intracellular kinases and a variety of other proteins. Professor Hill and GSK also have an ongoing collaboration to develop NanoBRET ligand-binding approaches for the IL-23

cytokine receptor. Kits and reagents exploiting the technology are now sold by Promega **[S1]**. Additionally, Promega has established a range of commercial collaborative projects with Pharma and Contract Research Organisations to further exploit the technology.

Economic impact for Promega and its Pharma/Biotech customers.

The collaboration between the UoN research group and Promega established a proof of concept of a commercial kit to query GPCR target engagement. Since then, this technology has been successfully applied to hundreds of targets, spanning a sizeable fraction of the druggable proteome. To date, Promega offers over 300 live cell kinase target engagement assays based on NanoBRET technology and has recently expanded that commercial offering to non-kinase proteins including HDACs, demethylases, bromodomains, heat-shock proteins, poly-ADP ribosylases and E3 ligases. Promega has further built on the technology precedent established under the UoN collaboration by developing a panel of reagents, including fluorescent ligands, cell lines and DNA plasmids, which enable pharma/biotech scientists to develop novel target engagement tracers using in-house synthetic chemistry resources. Head of Biology at Promega Corporation said *"…Promega has increased its R&D headcount to support this platform… Together, these assay kits and reagents have resulted in a major platform for Promega that has aggressive growth expectations for years to come"* **[S2].** Consistent with this aggressive growth expectation, Promega are expanding their branch facilities in the UK and Germany. Promega have also recently opened a new R&D building in Madison **[S3].**

Evidence of the commercial value for Promega of the NanoBRET Target Engagement technology is its highlight in the company's corporate reports (2017-2020): *"Over the years, we have developed a broad portfolio of assay reagents that meet the needs of these pharmaceutical researchers and have been used widely during various phases of drug discovery and development. For example, our NanoBRET™ Target Engagement Assays allow researchers to quantitatively measure the interaction between a molecule and a protein in live cells reliably in a high-throughput manner."* **[S3]**.

The NanoBRET Target Engagement Intracellular Kinase Assay was nominated for Scientist's Choice Award by SelectScience as one of the *Best New Drug Discovery & Development Products of 2017* **[S4]** and awarded the Reviewer's Choice Award for *Drug Discovery & Development Product of 2018* by SelectScience **[S5]**. A review of the product by a customer and user published in 2018 on SelectScience website said *"Essential and field-changing technology…Easy to use, high quality and reproducible results. Highly recommended to others!"* **[S6].**

Economic impact for biotech and pharma companies using NanoBRET technology.

The NanoBRET technology addresses a critical need in pharma and biotech industries to be able to assess ligand-binding kinetics with high throughput early in a drug discovery project. The rates of successful drug discovery could be improved by optimising the drug's binding kinetics to clinical need, thereby reducing the time and cost associated with delivering new medicines to patients. This need was identified by major pharma companies who, together with the European Commission under the Innovative Medicines Initiative, funded research to deliver robust new tools to assess ligand-binding kinetics, including NanoBRET in a high throughput plate-reader based format. The Kinetics for Drug Discovery (K4DD) consortium (k4dd.eu) included (GSK, AstraZeneca, Bayer, Heptares, Janssen, Merck, Roche and Sanofi) alongside academic collaborators including the UoN. K4DD funding **[8]** resulted in key publications including [**2** and **4**] which, due to the regular interactions between consortium partners, have been shared widely among all pharma partners during and after the project.

The global health-care company GSK has changed its processes as a result of adopting the new NanoBRET ligand-binding technology. In their letter of support for the UoN researchers, Director of Chemical Biology at GSK and Director of the Crick-GSK Biomedical LinkLabs said *"NanoBRET is now regularly used [at GSK] to both identify small molecules and*

characterize cellular ligand-binding kinetics early in a drug discovery project. This has enabled us to make informed, quicker project progression decisions, which has had a direct impact on the GSK drug discovery pipeline. To date, GSK have commissioned Promega to develop six novel NanoBRET reagents, in addition to the purchase of five established assays." **[S7].**

There is further evidence that international pharma companies are adopting NanoBRET technology at different stages of early hit prioritization steps of compound screening. Recent studies by Amgen Inc and Bristol-Myers Squibb have reported the development and implementation of a high-throughput NanoBRET Target Engagement assay to assess compound engagement to two kinases associated with human disease **[S8, S9].** Additional evidence supporting the wide-spread adoption of this technology is that major manufacturers of microplate reader instruments have also adapted their devices to be able to read the signal from NanoBRET assays. These include global companies BMG LABTECH, Thermo Fisher Scientific, PerkinElmer and Molecular Devices **[S10]**.

5. Sources to corroborate the impact (websites were last accessed on 18/01/2021)

S1 Promega website sells kits and reagents for NanoBRET Technology, web link

S2 Letter of support from Head of Biology of the Advanced Technologies Group at Promega Corporation.

S3 Combined PDF including: Promega corporate report 2017, p18; Promega corporate report 2018, p22; Promega corporate report 2019, p23; Promega corporate report 2020, p24

S4 Nomination for Scientist's Choice Award by SelectScience as one of the *Best New Drug Discovery & Development Products of 2017,* web link

S5 Reviewer's Choice Award for *Drug Discovery & Development Product of 2018* by SelectScience, web link

S6 Review of the product by a customer and user published in 2018 on SelectScience website, web link

S7 Letter of Support from Director of Chemical Biology at GSK and Director of the Crick-GSK Biomedical LinkLabs.

S8 High-Throughput Implementation of the NanoBRET Target Engagement Intracellular Kinase Assay to Reveal Differential Compound Engagement by SIK2/3 Isoforms, doi: 10.1177/2472555219893277

S9 A High-Throughput BRET Cellular Target Engagement Assay Links Biochemical to Cellular Activity for Bruton's Tyrosine Kinase, doi: 10.1177/2472555219884881

S10 Combined PDF including BMG Labtech, Thermofisher, PerkinElmer and Molecular Devices plate readers.