Institution: University of Nottingham

Unit of Assessment: 5 - School of Life Sciences

Title of case study:

Development and Commercialisation of Fluorescent Ligand Technologies for Advancing Receptor Pharmacology and Drug Screening

1. Summary of the impact

Fluorescent ligand technologies developed by Professor Hill and Dr Briddon in the Pharmacology research group, in collaboration with Professor Kellam in the School of Pharmacy, permitted biophysical analysis of G-protein coupled receptors (GPCRs) at the individual cell and molecule level for the first time. The technologies have been commercialised through the spin-out business, CellAura Technologies (and their distributors Abcam, Sigma-Aldrich and others), generating revenues and making the products available to researchers and drug discovery communities worldwide. Custom product developments with global pharmaceutical companies and drug screening reagent providers have generated further partnership revenues and technology benefits. Nottingham-trained researchers are now employed worldwide, broadening the technology's impacts.

2. Underpinning research

Fluorescent ligand technologies encapsulate the synthesis of fluorescently-tagged small molecule compounds that bind specifically and with high affinity to G-protein coupled receptors (GPCRs) and other membrane receptor classes, to allow biophysical properties of the ligand-receptor interaction to be determined at the single cell and single molecule level.

The underpinning research by Professor Stephen Hill and Dr Stephen Briddon in the Pharmacology research group was undertaken between 2003 and 2008, to investigate single ligand-receptor interactions in order to understand how different signalling pathways can be activated by the same GPCR in different cellular locations[1,2,3,]. This could not be achieved using conventional reagents, such as radiolabelled ligands, available at that time, and required the synthesis of novel, fluorescently-labelled small molecule ligands. Professor Hill's expertise in GPCR signalling pathways directed the research to appropriate receptors and ligands; Dr Briddon (Principal Research Fellow) provided fluorescence detection techniques to analyse single ligand-receptor interactions; collaborator Professor Barrie Kellam (School of Pharmacy) provided chemistry expertise to synthesize the necessary fluorescent ligands. Funding was provided by the Wellcome Trust[13], BBSRC[14], University of Nottingham (UoN), MRC[15] and EU IMI[16]. The initial publication in PNAS[1] was the first describing the application of fluorescence correlation spectroscopy (FCS) to study non-peptide GPCRs in membrane micro-domains of living cells. This study also provided the first description of a pharmacologically-validated fluorescent ligand for a biogenic amine GPCR. This work, and the associated patents[9,10], formed the basis for the establishment of the spin-out company, CellAura Technologies.

FCS) studies with fluorescent agonists allowed the group to measure ligand binding to active conformations of GPCRs in single living cells for the first time[4]. In-house development of a patented live cell perfusion system for fluorescence imaging[12] also allowed the group to measure ligand-receptor binding kinetics, and the effects of allosteric modulators on orthosteric ligand binding in single live cells in real-time[5,6,7]. This expertise has made the group a centre for training key international scientists in these techniques (L May and R Bathgate, Australia; R Corriden, USA; V Segura, Spain; M Arruda, Brazil). Furthermore, the development, both at the University of Nottingham and at CellAura, of high throughput fluorescence binding assays has facilitated GPCR drug discovery screens[8]. Complementary ligands with alternative red and green fluorophores, have also advanced the understanding of receptor dimerization / oligomerization, and its impact on receptor pharmacology (Comps-Agrar et al., 2011; *Methods in Molecular Biology* **756**, 201-214. doi: 10.1007/978-1-61779-160-4_10).

Dr Briddon was awarded the BPS Bill Bowman Travelling Lectureship in 2009 to present this work in academic and industrial centres in the UK. Dr Briddon has also worked closely with Zeiss in

developing the appropriate FCS methodology and with Hill and Kellam in use of custom-designed fluorescent ligands. Co-authored reviews have been produced with authors from Pfizer (Williams and Hill (2009) *Methods Mol. Biol.* **552**, 39-50. doi: 10.1007/978-1-60327-317-6_3) and Zeiss (Weisshart, Jungel and Briddon (2004) *Curr. Pharm. Biotechnol*. **5**, 135-154. doi: 10.2174/1389201043376913).

3. References to the research

Key Publications (UoN authors in bold, key author(s) underlined)

1. **Briddon SJ, Middleton RJ, Cordeaux Y, Flavin FM, Weinstein JA, George MW, Kellam B, Hill SJ** (2004) Quantitative analysis of the formation and diffusion of A1-adenosine receptor-antagonist complexes in living cells. *Proc. Natl. Acad. Sci. (USA)* **101**: 4673-4678. doi: 10.1073/pnas.0400420101
2. **Briddon SJ**, **Middleton RJ**, **Yates, AS**, **George, MW**, **Kellam B**, **Hill SJ** (2004) Application of fluorescence correlation spectroscopy to the measurement of agonist binding to a G-protein coupled receptor at the single cell level. *Faraday Discussions* **126**: 197-207. Doi: 10.1039/B307407B
3. **Middleton RJ, Briddon SJ, Dale C, George MW, Baker JG, Hill SJ, Kellam B**. (2007). New fluorescent adenosine A1-receptor agonists which allow quantification of ligand-receptor interactions in microdomains of single living cells *J. Med. Chem.* **50**: 782-793. doi: 10.1021/jm061279i
4. **Cordeaux Y, Briddon SJ, Alexander SPH, Kellam B, Hill SJ** (2008) Agonist-occupied A3-adenosine receptors exist within heterogeneous complexes in membrane microdomains of individual living cells. *FASEB J.* **22**: 850-860. doi: 10.1096/fj.07-8180com
5. **May LT**, **Briddon SJ**, **Hill SJ** (2010). Antagonist selective modulation of adenosine A1 and A3 receptor pharmacology by the food dye Brilliant Black BN: evidence for allosteric interactions. *Mol. Pharmacol.* **77**: 678-86. doi: 10.1124/mol.109.063065
6. **May LT, Self TJ, Briddon SJ, Hill SJ**. (2010). The effect of allosteric modulators on the kinetics of agonist-G protein-coupled receptor interactions in single living cells. *Mol. Pharmacol.* **78**: 511-23. doi: 10.1124/mol.110.064493
7. **May LT, Bridge LJ, Stoddart LA, Briddon SJ and Hill SJ** (2011). Allosteric interactions across native adenosine -A_3 receptor homodimers: Quantification using single cell ligand binding kinetics. *FASEB J.* **25**: 3465-76. doi: 10.1096/fj.11-186296
8. **Stoddart LA, Vernall AJ, Denman JL, Briddon SJ, Kellam B** and **Hill SJ.** (2012). Fragment screening at adenosine A3-receptors in living cells using a fluorescence-based binding assay. *Chemistry & Biology* **19**: 1105-1115. doi: 10.1016/j.chembiol.2012.07.014

Key Patents

9. **Kellam B**, **Middleton RJ**, **George MW**, **Hill SJ**. (2003) Library having several tagged non-peptide ligands or their salts, useful for assessing pharmacological properties of ligand, comprising ligand moieties linked to tag moieties through linker moieties. WO2004088312-A2; EP1623223-A2; AU2004225696-A1; US2006211045-A1; JP2006523203-W; IN200501873-P2; CN1860364-A; IN231463-B
10. **Hill SJ**; **Briddon SJ**, **Kellam B**. (2004) Improvements in High Content Screening. WO2006032926-A2; EP1792182-A2; US2009093001-A1
11. **Hill SJ**, **Kellam B**, Middleton RJ. (2007) Method for generating a recombinant clonal cell line and novel reagents for use in the method. US201130116; WO2009040555; GB2468447; EP2238248; CN101896604
12. **Morris B**, **Self T** and **Hill SJ**. Observation cell arrangement. (2011) EP2486386; WO2011042755; CN102639987

Key Research Grants

13. **Wellcome Trust**: 1999-2002, **£881,988**; 2002-2005, **£226,310**; 2011-2014; **£374,000**.
14. **BBSRC**: 2005-2008, **£295,119**; 2006-2008, **£95,223**.
15. **MRC**: 2009-2014; **£1,313,190**.
16. **EU Innovative Medicines Initiative**: 2012-2017, **€16M** (~ €500K to Nottingham).

4. Details of the impact

Impact 1: Technology Transfer, Exploitation, and the Principal Beneficiary

The development of fluorescent ligand technologies by Professors Hill and Kellam led to creation of the University of Nottingham spin-out company, CellAura Technologies, in 2004 for the commercialisation of a wide range of novel fluorescent ligands. Professors Hill and Kellam, as founding directors, secured initial funding from Lachesis that allowed CellAura's successful establishment and start of trading at BioCity in Nottingham in 2006. CellAura received further investments in 2008 and 2010 from several regional investors totalling approximately £1.8 million[A].

Four University patents were licensed exclusively to CellAura in exchange for ~12% equity[A]. The original two patents[9,10], derived from UoN research, underpin CellAura's core fluorescent ligand business. Subsequent development by CellAura of a further 12 ligands added to the catalogue from 2008 onwards and an additional 34 development ligands are founded on the original patents. The third patent[11] describes the novel use of fluorescent ligands as alternatives to antibodies for fluorescence activated cell sorting (FACS) that has been adopted by the biotech community[B,C], in part due to significant assay-time reductions[D]. The fourth patent[12] describes a perfusion instrument that allows measurement of ligand binding kinetics in live cells[5,6,7]. The value of these patents to the University is equivalent to its equity share in the company valuation (approximately £215,000). Their value to CellAura is crucial to permit commercialisation and further development of the catalogue. A pipeline agreement between the University and CellAura (dated 2008)[A] feeds technology refinements into the business, creating continued benefits for both parties. Annual turnover figures for CellAura since 2008 show a steady increase to 2012 (2009: £33,572; 2010: £92,821; 2011: £174,782; 2012: £179,978; 2013: £96,950)[A]. CellAura is therefore the principal beneficiary of the technology transfer and commercialisation.

Impact 2: Commercialisation and Subsidiary Beneficiaries

CellAura makes direct-to-customers sales in Europe, the US, and Australasia, to major pharma companies (e.g. Pfizer, Sanofi-Aventis, Amgen, Takeda), to GPCR drug discovery biotechs (e.g Addex, Heptares)[A] and to academia. To facilitate access to CellAura's products, regional distributor agreements were arranged with Funakoshi (Japan) and Fischer Scientific (UK and Scandinavia) in 2009, and worldwide agreements with Abcam and Sigma-Aldrich in 2011[A]. Between 2009 and 2012, CellAura developed custom fluorescent ligands for CisBio Bioassays for use in their Tag-lite™ GPCR high throughput screening (HTS) assays. CisBio accepted 18 'active' ligands from CellAura for inclusion in its assay kits[E]. Promega has also demonstrated that CellAura's fluorescent ligands can be used very successfully in their NanoLuc-GPCR ligand binding technology, stating that: "*The suite of fluorescent ligands offered by CellAura have helped validate a novel, enabling assay principle for studying receptor pharmacology in a simplified, live cell format. The combined technologies strongly position Promega as an innovative leader in the drug discovery community*"[F]. These companies are therefore subsidiary beneficiaries of the technology.

Since 2008, CellAura has successfully completed custom ligand development projects for AstraZeneca (2008: 5 GPCR targets) and Novartis (2009: a GPCR ligand that met multiple physicochemical and biological parameters, allowing Novartis to "*better understand the pharmacological mechanism of drug compounds and to direct new research on longer acting drugs targeting GPCRs"*)[G]. A number of smaller custom synthesis projects for industrial and academic researchers in Europe, US and the Far East were also completed by CellAura from 2008 onwards. AstraZeneca also established a CASE studentship with the University in 2009, supported by CellAura, to develop a fluorescent agonist for an orphan GPCR (completed in 2011)[H]. The custom fluorescent ligands all resulted in technology benefits for project partners, such as real-time imaging of fluorescent ligand binding kinetics and internalisation in live cells to optimise a drug candidate's pharmacokinetics. For Novartis fluorescent ligands are *"an enabling technology that brings benefits to the pharmaceutical industry by allowing pursuit of approaches that were previously unavailable"*[G] and for AstraZeneca, the benefits fitted well with the company's "*principles of lean and responsible procurement to improve the company's efficiency, sustainability and environmental footprint*."[H]

CellAura has validated the performance of its products on the Applied Biosystems FMAT reader (2008), and the high content analysis (HCA) imaging platforms of GE Healthcare (InCell Analyser; 2008), PerkinElmer (Opera; 2008), Molecular Devices (ImageXpress Ultra; 2009) and

ThermoFisher/Cellomics (Arrayscan; 2010), resulting in scientific posters and marketing materials[I]. CellAura earned a 'best new technology' award at the SBS /ELRIG Drug Discovery meeting in 2008. In 2012, CellAura established a successful collaboration with BMG Labtech that showed binding of CellAura's ligands could be read on a conventional (non-imaging) PheraStar fluorescence reader in whole-cell receptor binding assays for HTS[J]. Together, the reagent + instrument combinations provide improved methods for investigating ligand interactions with GPCRs for drug discovery (Comley J, (2009); *Drug Discovery World* **Spring 2009**, 32-50), bringing additional benefits to instruments manufacturers and their customers.

Fluorescent ligands also have advantages over radioligands (a £60-65 million annual global market) by avoiding radioisotope safety and waste disposal issues, reducing their detrimental health and environmental impacts.

Impact 3: Employment and Wealth Creation

CellAura has created employment for 15 individuals in up to 7 full-time and 4 part-time posts since 2008. Three current or past members of the CellAura scientific staff were trained in the laboratories of Hill and Briddon (Carter, Rose, Spencer), and a further four (Middleton, Adams, Beardsell, McCarroll) in other schools at the UoN. Professors Hill and Kellam continue as non-executive directors and shareholders, with Dr Briddon serving as a consultant to CellAura.

5. Sources to corroborate the impact

A. Statement from the CEO, CellAura Technologies, on file and available on request
B. Zwier JM et al (2010) J Biomol Screen. 15(10):1248-59. doi: 10.1177/1087057110384611.
C. Kamiya K et al (2010) Biotechnol Bioeng. 2010 107(5):836-43. doi: 10.1002/bit.22845.
D. Statement from Managing Director, InScreenEx (Germany) on file; see also: Schucht R et al, 2011 J Biomol Screen 16(3): 323-331; doi: 10.1177/1087057110396371
E. Contract Termination Statement from CisBio Bioassays (France), on file and available on request
F. Statement from a Senior Research Scientist, Promega Corporation (USA), on file and available on request
G. Statement on behalf of Novartis Horsham Research Centre, Novartis (UK), on file and available on request
H. Statement from an Associate Principal Scientist, AstraZeneca-Alderley Park (UK); on file and available on request; see also: http://www.pa2online.org/abstracts/vol10issue1abst031p.pdf
I. http://www.cellaura.com/resources/index.html#posters
J. http://www.bmglabtech.com/application-notes/fluorescence-intensity/gpcr-cellaura-pherastar-fs-227.cfm

Corroborative documents and copies of webpages are held on file and are available on request.