Institution: EaStCHEM School of Chemistry

Unit of Assessment: UoA 8: Chemistry

Title of case study: Direct detection of microRNA enables business growth through commercialisation of fast, accurate and quantitative detection of disease biomarkers

Period when the underpinning research was undertaken: 2007 – 2012

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:
Mark Bradley	Professor of Chemical Biology	March 2005 – present
Juan J. Díaz-Mochón	PDRA	March 2005 – December 2010

Period when the claimed impact occurred: 1 August 2013 – 31 December 2020

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

1. Summary of the impact

EaStCHEM Professor Mark Bradley and his group have developed a novel method able to detect small microRNAs (miRNAs) directly from patient plasma samples. miRNAs are key biomarkers of disease states, and this technology provides a breakthrough solution for their detection that is fast, accurate and quantitative.

This research has underpinned the sustained growth of the spin-out company, *DESTINA Genomics* (*DESTINA*), and the creation of a new company, *Vetsina Animal Diagnostics* (*Vetsina*). In this REF period, investment of EUR1,200,000 and GBP250,000 has been raised to support the activity of *DESTINA* and *Vetsina* respectively, and employment has been created for 12 staff (headcount: 12; FTEs: 12) The technology has generated over EUR1,000,000 in commercial revenue, with *DESTINA* assays the only method available commercially to analyse miRNA directly from plasma. A further EUR1,470,000 in development grant income has supported *DESTINA*'s work to develop the technology towards new applications. *DESTINA*'s lead assay kit *LiverAce*, based on their proprietary *ChemiRNA™ Tech,* was commercialised for R&D use in the pharmaceutical development sector in 2019 and provides rapid absolute quantification of liver disease biomarkers. *LiverAce* is being assessed by two major pharmaceutical companies for improved screening of liver toxicity of new drug candidates. *DESTINA*'s pipeline of assays currently under development targets lung cancer screening, acute kidney failure and heart failure. Economic benefit extends to industry partners *Optoi Microelectronics* and *Advanced Wave Sensors* through collaborative projects developing new diagnostic platforms and associated grant funding of over EUR800,000.

2. Underpinning research

The challenge: unleashing the potential of miRNA biomarkers

Micro-RNAs are powerful biomarkers whose levels in biological fluids are indicative of multiple disease states that include cancer, liver failure, and heart disease. The development of a rapid and accurate method for their detection and quantification will provide a crucial tool in clinical research and drug development and deliver improved diagnosis and treatment of disease. However, miRNAs have been exceptionally problematic to analyse due to their small size, poor stability and their low levels found in plasma, resulting in significant difficulties in amplification to allow accurate detection. Detection methods to date have required a complex and expensive process of sample preparation, conversion and amplification, with large variations found between samples/labs and kits. As a result, the utilisation of miRNAs as valuable biomarkers has historically not been possible.

Dynamic covalent chemistry for error-free analysis of nucleic acids

Research in the group of EaStCHEM Professor Mark Bradley between 2007 and 2012 led to the development and validation of a novel method using dynamic covalent chemistry (i.e. reversible

bond-formation under thermodynamic control) to enable error-free genetic analysis **[R1]**. Key to the method is the generation of peptide nucleic acid (PNA) oligomers ("probes") designed to hybridise a target nucleic acid, with a "blank position" where one of the bases (in the middle of the strand) is deliberately omitted. When the PNA probe hybridises with the target nucleic acid, this blank position creates a "pocket" containing the unknown nucleotide under interrogation (Figure 1). Subsequently, four bespoke artificial aldehyde-containing nucleobases are added, which insert into the pocket, via reversible, dynamic, imine chemistry, to generate a duplex structure with the "best fitting" nucleobase incorporated. The aldehyde-bases can be functionalised with fluorophores or biotin to permit their easy detection.

The concept and detection method evolved from the Bradley group's expertise in PNA chemistry and was developed through their work on PNA synthesis and the encoding of peptide libraries **[R2, R3]**. A key insight leading to the development of the method was an understanding of the effect that the presence of a pocket has on the stability of the DNA/PNA duplex and duplex formation. This led to the insight that a PNA strand with a missing base will only hybridise to a fully complementary DNA sequence, giving rise to the exquisite selectivity of the technology. The team also recognised that the pocket left by the use of the blank PNA strand would only allow dynamic selection and incorporation of the correct nucleobase.

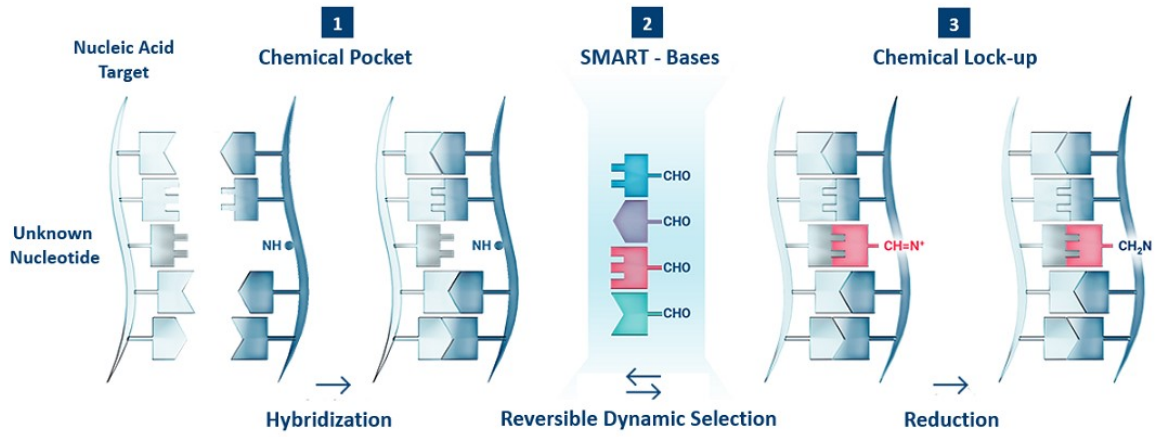

Figure 1. *Bradley's research: nucleic acid analysis based on functionalised "aldehyde nucleobases" and unique peptide nucleic acid (PNA) capture probes with a blank position. Image credit: DESTINA.*

The method guarantees specificity by dynamic covalent chemistry, with signal detection only achievable if the target nucleic acid sequence binds in perfect alignment on the PNA probe and the correct aldehyde-base fits into the chemical pocket. As a consequence, no false positives (or negatives) can be generated. Clinically, the method was validated by its ability to distinguish genetic variations in cystic fibrosis patients with 100% read accuracy **[R4]**.

Importantly, the method is unique in providing direct detection and quantification of miRNAs from body fluids (e.g. serum), without the need for PCR amplification processes, which introduce errors and increase time and costs.

The granted patented technology for the method **[R5]** provides one of the first examples of dynamic covalent chemistry applied for a practical real-world use.

3. References to the research

The underpinning research was supported by peer-reviewed grants (EP/E027660/1, E18330/2), published as peer-reviewed outputs in well-regarded academic journals **[R1-R4]**, and has resulted in a granted patent **[R5]**.

R1. F.R. Bowler, **J.J. Diaz-Mochon**, M.D. Swift and **M. Bradley**, "DNA Analysis by Dynamic Chemistry", *Angew. Chem., Int. Ed.*, **2010**, *49*, 1809-1812. DOI: 10.1002/anie.200905699.

R2. N. Svensen, **J.J. Diaz-Mochon** and **M. Bradley**, "Microwave-assisted orthogonal synthesis of PNA–peptide conjugates", *Tetrahedron Lett.*, **2008**, *46*, 6498-6500. DOI: 10.1016/j.tetlet.2008.08.104.

R3. D. Pouchain, **J.J. Diaz-Mochon**, L. Bialy and **M. Bradley**, "A 10.000 Member PNA-Encoded Peptide Library for Profiling Tyrosine Kinases", *ACS Chem. Biol.*, **2007**, *2*, 810-818. DOI: 10.1021/cb700199k.

R4. F.R. Bowler, P.A. Reid, A.C. Boyd, **J.J. Diaz-Mochon** and **M. Bradley**, "Dynamic chemistry for enzyme-free allele discrimination in genotyping by MALDI-TOF mass spectrometry", *Anal. Methods*, **2011**, *3*, 1656-1663. DOI: 10.1039/C1AY05176H.

R5. **M. Bradley** and **J.J. Diaz-Mochon**, "Nucleobase characterisation", *Granted patent* WO 2009/037473, **2009**.

4. Details of the impact

The dynamic covalent chemistry method for fast, error-free and quantitative detection of nucleic acids directly from patient plasma, pioneered by Professor Mark Bradley and his group, underpins the growth of two spin-out companies, *DESTINA Genomics* and *Vetsina Animal Diagnostics*. In this REF period, they have raised investment of EUR1,200,000 and GBP250,000 respectively, commercial revenue of over EUR1,000,000, and development grant income of EUR1,470,000. *DESTINA* provides the only method available commercially to analyse miRNA direct from patient plasma, including lead assay *LiverAce*, which is being assessed by two major pharmaceutical companies for improved screening of liver toxicity of new drug candidates. Economic benefit extends to industry partners through collaborative projects to develop new diagnostic platforms.

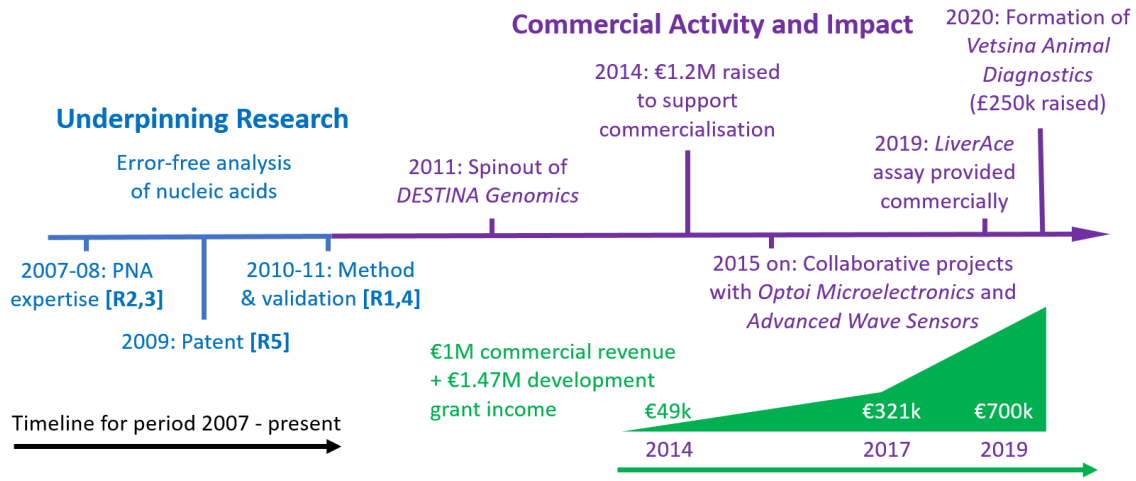

***Figure 2**. Timeline showing the development of impact from the primary underpinning research described in Section 2 (blue line/text refers to outputs referenced in Section 3) **[S1]**.*

Growth of *DESTINA Genomics Ltd* and formation of *Vetsina Animal Diagnostics Ltd*

DESTINA spun out of the University of Edinburgh in 01-2011 with a worldwide, exclusive license to develop the Bradley group technology **[R5] [S1]**. A second spin-out company, *Vetsina*, was incorporated in 02-2020 to apply the technology for applications in animal health **[S1, S2]**.

In 10-2014, *DESTINA* secured investment of EUR1,200,000 to support the commercialisation of their technology **[S1, S3]**. Licensing and service contracts from 2014 onwards, and roll out of *DESTINA*'s first assay kit (*LiverAce*) in 2019, generated total commercial revenue of over EUR1,000,000 **[S1]**. In addition, development grant income of EUR1,470,000 to *DESTINA* has supported work to develop their technology towards new applications **[S1]**. Investment of GBP250,000 has been raised to date to support the set-up of *Vetsina* **[S1]**. *DESTINA* has created 11 jobs (headcount: 11; FTEs: 11) **[S1]**; a further initial position has been created at *Vetsina* **[S1, S2]**.

First commercially-available direct analysis of miRNA and the *LiverAce* assay for detecting the key biomarker of liver injury

DESTINA's *DGL-Tech*™, based on the research and IP of Professor Bradley **[R5, S1]**, forms the basis of their *ChemiRNA*™ *Tech* for the direct detection and quantification of miRNAs in body fluids **[S4]**. *ChemiRNA*™ *Tech* uses a single biotinylated "*SMART-Base*" to label the duplex formed with a miRNA sequence. The duplex is read out using a reporter molecule that recognises the biotin tag (Figure 3).

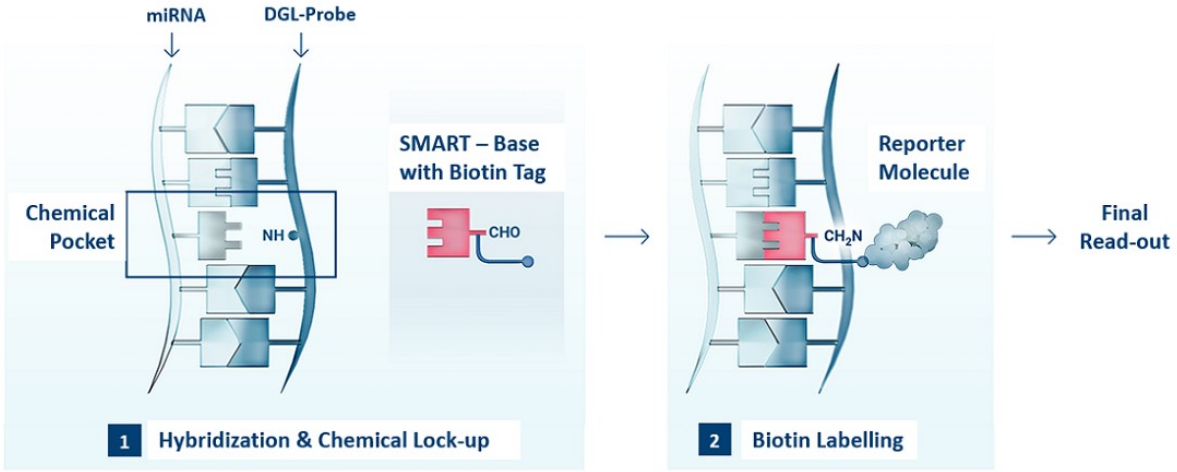

Figure 3. *ChemiRNA™ Tech based on DESTINA DGL-Tech™ for the direct detection and quantification of miRNAs in body fluids. Image credit: DESTINA* ***[S4]****.*

DESTINA's *ChemiRNA*™ *Tech* is the only method currently available commercially to analyse miRNA directly from patient plasma samples **[S1]**, avoiding the complex, expensive and time-consuming processes of preparation, conversion and amplification required by previous methods, and the risk of errors those bring. Studies have shown that it provides a 1,000-fold increase in miRNA detection limits compared to current methods **[S5]**.

DESTINA's lead assay kit, *LiverAce*, was commercialised for R&D use in the pharmaceutical development sector in 2019 and is the first assay in the world to offer reliable screening for liver injury based on miRNA **[S1, S4]**. *LiverAce* uses *ChemiRNA*™ *Tech* to provide direct detection of miRNA-122, a key biomarker for hepatic conditions including drug-induced liver injury as well as hepatitis, non-alcoholic liver disease and liver cancer **[S1, S4]**. It provides rapid, absolute quantification of miRNA-122 from body fluids, with high sensitivity and specificity to single-base resolution on a platform that is compatible with widely used instruments **[S1, S4]**. In clinical studies, the assay has been shown to accurately identify patients at risk of drug induced liver injury after acetaminophen overdose **[S5]** within 4 hours, rather than the 24 hours required previously **[S1]**.

LiverAce is being assessed by two major pharmaceutical companies for more effective evaluation of liver toxicity of new drugs, aiming for earlier and better screening of drug candidates **[S1]** (details confidential). In addition to *LiverAce*, *DESTINA's* pipeline of assays under development targets lung cancer screening (in clinical validation), acute kidney failure (in analytical validation), and heart failure (biomarker discovery phase) **[S1]**. Collaborations with industry and research partners have developed the *DESTINA* technology towards new applications and generated economic benefit for SME partners, including **[S6]**:

- With *Advanced Wave Sensors S.L.* (Spain), a novel diagnostic platform for the early and rapid detection of mutations associated with colorectal cancer from blood samples (grant funding of EUR415,668 between 2016 and 2018);
- With *Optoi Microelectronics*, an Italian company specialising in advanced sensors and measuring systems, a novel bead-based detection platform for miRNAs as biomarkers for detection of lung cancer (grant funding of EUR126,000 between 2015 and 2019); also

participation in a project to provide low-cost testing for the presence, and drug-resistance profile, of TB (grant funding of EUR271,250 between 01-2019 and 06-2021).

5. Sources to corroborate the impact

S1. Letter from *DESTINA Genomics* Chief Scientific Officer. Confirms underpinning role of EaStCHEM research in *DESTINA* technology and resulting economic benefit and success.

S2. "*Vetsina Animal Diagnostics launched to improve the speed of diagnosis and facilitate the development of point-of-care products*." News article from *Vetsina* website confirming company launch and technology. https://vetsina.com/news/vetsina-animal-diagnostics-launched-to-improve-the-speed-of-diagnosis-and-facilitate-the-development-of-point-of-care-products

S3. "*DESTINA Genomics* raises €1,200,000 to develop novel tests for cancer and infectious diseases." Scottish Enterprise news article, 03-10-2014, confirming funding raised. https://www.scottish-enterprise-mediacentre.com/news/DESTINA-genomics-raises-1200000-to-develop-novel-tests-for-cancer-and-infectious-diseases

S4. *DESTINA* web pages confirming technology, products and services provided by the company.
- **a)** Technology https://destinagenomics.com/technology
- **b)** Products https://destinagenomics.com/products-services/products
- **c)** Services https://destinagenomics.com/products-services/services

S5. Direct Detection of miR-122 in Hepatotoxicity Using Dynamic Chemical Labeling Overcomes Stability and isomiR Challenges, López-Longarela, B, et al., Analytical Chemistry, 2020, 92, 4, 3388–3395 10.1021/acs.analchem.9b05449. Confirms ability of *DESTINA*'s *LiverAce* assay to diagnose liver injury in humans and dramatic improvement in levels of detection.

S6. EU CORDIS web pages for collaborative projects using the *DESTINA* technology. Confirms wider economic benefit to partner companies.
- **a)** Liqbiopsens (with *Advanced Wave Sensors*) https://cordis.europa.eu/project/id/687785
- **b)** miRNA-DisEASY (with *Optoi*) https://cordis.europa.eu/project/id/690866
- **c)** ARREST-TB (with *Optoi*) https://cordis.europa.eu/project/id/825931