Institution: University of Glasgow (UofG)		
Unit of Assessment: UoA8 Chemistry		
Title of case study: Commercial exploitation of novel mitochondrial probes		
Period when the underpinning research was undertaken: 2007−ongoing		
Details of staff conducting the underpinning research from the submitting unit:		
Name(s): Prof. Richard Hartley	**Role(s) (e.g. job title):** Professor	**Period(s) employed by submitting HEI:** 1995−present
Period when the claimed impact occurred: 2011-present		
Is this case study continued from a case study submitted in 2014? N		

1. Summary of the impact

Mitochondria are powerhouses of the cell, sources of oxidative stress, and are key to healthy cell functioning. They play a critical role in metabolism and signalling. Over a decade of chemistry research at UofG has resulted in the design and synthesis of a suite of molecular probes targeted to mitochondria, including the first and currently best probe for quantifying hydrogen peroxide in the mitochondria of living organisms. These probes are marketed by specialist chemical suppliers including Cayman Chemical and Abcam across Europe, Asia and North America. They have reduced animal use in numerous laboratories world-wide, by an average of 25%. [Text removed for publication.]

2. Underpinning research

Mitochondria are vital to the healthy functioning of the cell. Mitochondria are powerhouses of the cell, sources of oxidative stress, and are central to metabolism and signalling. A symbiotic research programme between Prof. Hartley's Chemical Biology laboratory and Prof. Michael Murphy (MRC Mitochondrial Biology Unit) over the past decade has developed a portfolio of molecular probes which enable researchers across the globe to gain knowledge on the function of mitochondria in health and disease both at the cellular and whole animal level.

Functional Molecular Probes

Hartley's UofG laboratory designed and synthesised a suite of mitochondria-targeted molecular probes. These have been used by Prof. Murphy *et al.* to elucidate the mitochondrial metabolic and redox signalling processes involved in oxidative stress and a wide range of diseases including reperfusion injury in the heart, stroke, neurodegeneration, cancer, ageing and inflammation. Recently this has been expanded to include cell permeable forms of mitochondrial metabolites.

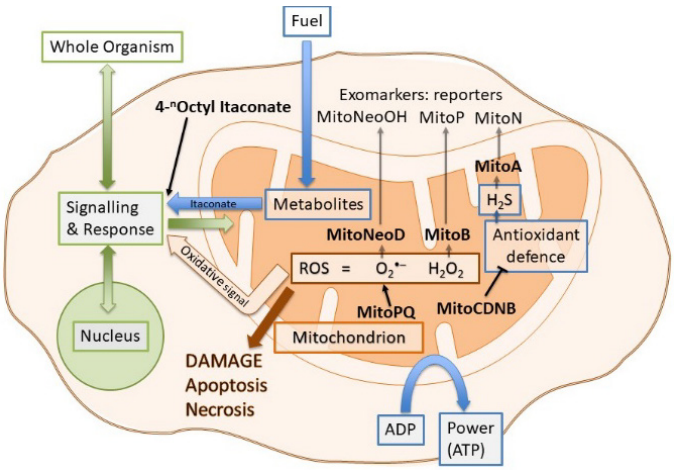

Figure1: The UofG Mito series of molecular probes accumulate several-hundred-fold in the mitochondrial matrix. MitoNeoD, MitoB and MitoA detect specific reactive species and report through their exomarkers MitoNeoOH, MitoP and MitoN. MitoPQ generates superoxide, while MitoCDNB removes the thiol antioxidant defence.
4-ⁿOctyl itaconate is a cell permeable version of the mitochondrial metabolite, itaconate, which is produced as an antioxidant negative feedback signal in inflammatory macrophages.

MitoB/MitoP: Hydrogen peroxide is a key mediator in redox signalling, and is central to mitochondrial oxidative damage. The probe MitoB (CAS Registry No. 1247025-84-8) was developed by Hartley *et. al* in UofG from 2007 to 2011. This is comprised of a tetraphenylphosphonium (TPP) cation attached to an arylboronic acid. The TPP cation results in several-hundred-fold accumulation of the Mito molecular probes within the mitochondrial matrix, where the arylboronic acid moiety reacts with hydrogen peroxide to form a phenol, MitoP (CAS 74597-01-6). Quantifying the MitoP/MitoB ratio by liquid chromatography-tandem mass spectrometry (LC-MS/MS), against known amounts of deuterated standards added at extraction,

enabled measurement of a weighted average of mitochondrial H_2O_2 within mitochondria of whole living organisms for the first time and this was reported by Hartley and Murphy in 2011 [3.1]. MitoB/P and the d15-deuterated standards developed by Hartley *et. al.* provided the paradigm for the exomarker approach for quantification of reactive species in cells, tissues, organs and whole organisms.

Building on this successful collaboration, Prof Hartley has designed and synthesised a suite of other molecular probes to specifically target key areas of mitochondrial biochemistry. Evidence of their efficacy and their applicability to a diverse range of applications was demonstrated by Prof. Murphy and a wider network of collaborators which includes medics and biomedical scientists.

MitoPhotoDNP: In 2012, Hartley designed and synthesised the photo-activated protonophore, MitoPhotoDNP, which was demonstrated to depolarize mitochondria with complete spatio- and temporal control [3.2].

MitoParaquat (MitoPQ) was developed in 2015 and generates superoxide, specifically in the mitochondrial matrix without interfering with the biological machinery and so can be used in any cell, tissue or organism to study the effect of elevating mitochondrial ROS. [3.3].

MitoA/MitoN: In 2017, UofG research developed MitoA and its accompanying deuterated internal standards for use with LC-MS/MS in an exomarker approach [3.1]. These compounds address the lack of suitable probes to examine the role of hydrogen sulfide (H_2S) on signalling pathways and cell function *in vivo* [3.4].

4-^{n}Octyl itaconate: In 2018, Hartley designed 4-^{n}octyl itaconate (CAS 3133-16-2), a cell permeable version of the anti-inflammatory mitochondrial metabolite, itaconic acid [3.5]. 4-^{n}Octyl itaconate (marketed commercially as 4-Octyl itaconate) has been key to understanding the role of itaconate, the endogenous activator of the transcription factor Nuclear factor erythroid-2-related factor 2 (Nrf2). Itaconate is produced as an antioxidant negative feedback signal in inflammatory macrophages.

MitoCDNB: The maintenance of mitochondrial thiol redox state is thought vital for cell survival. In 2019, MitoCDNB was developed to specifically remove the thiol antioxidant defence in mitochondria [3.6], enabling researchers, for the first time, to manipulate the organelle's thiol systems independently of those in other cell compartments.

More recent additions to the UofG suite of mitochondrial probes are **MitoNeoD**, a versatile and robust probe to assess changes in mitochondrial superoxide from isolated mitochondria to animal models, and **MitoGamide**, a potential therapeutic for oxidative damage during diabetes.

Together, this suite of mitochondrial molecular probes has provided a step-change in understanding the role of mitochondrial metabolites in a variety of preparations from isolated mitochondria and cell culture, through to whole organs and organisms, and are used by laboratories across the globe.

3. References to the research

3.1 Cocheme, *et. al.* Measurement of H_2O_2 within Living Drosophila during Aging Using a Ratiometric Mass Spectrometry Probe Targeted to the Mitochondrial Matrix. Cell Metabolism 2011, 13 (3), 340−350. doi: 10.1016/j.cmet.2011.02.003

3.2 Chalmers, S.; Caldwell, S. T.; Quin, C.; Prime, T. A.; James, A. M.; Cairns, A. G.; Murphy, M. P.; McCarron, J. G.; **Hartley, R.C.**, Selective Uncoupling of Individual Mitochondria within a Cell Using a Mitochondria-Targeted Photoactivated Protonophore. Journal of the American Chemical Society 2012, 134 (2), 758−761. doi:10.1021/ja2077922

3.3 Robb, E. L.; Gawel, J. M.; Aksentijevic, D.; Cocheme, H. M.; Stewart, T. S.; Shchepinova, M. M.; Qiang, H.; Prime, T. A.; Bright, T. P.; James, A. M.; Shattock, M. J.; Senn, H. M.; **Hartley, R.C.;** Murphy, M. P., Selective superoxide generation within mitochondria by the

targeted redox cycler MitoParaquat. Free Radical Biology and Medicine 2015, 89, 883−894. doi:10.1016/j.freeradbiomed.2015.08.021

3.4 Arndt, S.; Baeza-Garza, C. D.; Logan, A.; Rosa, T.; Wedmann, R.; Prime, T. A.; Martin, J. L.; Saeb-Parsy, K.; Krieg, T.; Filipovic, M. R.; **Hartley, R.C.;** Murphy, M. P., Assessment of H2S in vivo using the newly developed mitochondria-targeted mass spectrometry probe MitoA. Journal of Biological Chemistry 2017, 292 (19), 7761−7773. doi:10.1074/jbc.M117.784678

3.5 Mills *et. al.* Itaconate is an anti-inflammatory metabolite that activates Nrf2 via alkylation of Keap1. Nature 2018, 556, 113−117. doi: 10.1038/nature25986

3.6 Booty *et. al.* Selective Disruption of Mitochondrial Thiol Redox State in Cells and In Vivo. Cell Chemical Biology 2019, 26 (3), 449−461 doi:10.1016/j.chembiol.2018.12.002

4. Details of the impact

Mitochondria are present in almost all animal, plant and fungal cell types, generating adenosine triphosphate (ATP), the energy currency of cells. Their role in cellular signalling is increasingly being understood and they are also key to cell death. Mutations in nuclear or mitochondrial DNA can cause life-threatening and currently incurable mitochondrial diseases which affect 1 in 4,300 adults. Mitochondrial dysfunction is a much wider phenomenon, and contributes to many common disorders in the general population, including heart disease, neurodegeneration and inflammation. Therefore, understanding the role of mitochondria in health and disease is critical to the treatment of any dysfunction but requires reliable and high fidelity tools such as the suite of molecular probes developed by Prof. Hartley. This has delivered commercial impacts in a specialised market, and contributed to a reduction in the number of animals required in research to develop therapies for common diseases.

Commercial adoption of UofG probes:
Cayman Chemical is a specialist manufacturer and supplier of biochemical reagents used primarily by pharmaceutical companies and universities life sciences research. Cayman Chemical has grown its customer base through the provision of a comprehensive portfolios of related products; they currently hold the largest portfolio of UofG mitochondria-targeted molecular probes.

> "*Starting in 2015, Cayman Chemical added MitoP and MitoB to our catalogue and quickly invested resources by building application notes and other web content to help promote these unique reagents. In addition, reagents were packaged both as a standalone reagent as well as value-added kit formats*." [5.1]

Cayman Chemical also provides mitochondrial hydrogen peroxide measurements as a contracted service using MitoP/MitoB. Since the company's introduction of MitoB in March 2015, the Hartley portfolio of products has had sales in over 15 countries [text removed for publication.] Within the United States alone, customers include biopharmaceutical companies, governmental laboratories, hospitals, private institutions, clinical research organisations, research laboratories and universities.

> *"The portfolio of Hartley compounds available from Cayman recently expanded in 2018 with the inclusion of MitoPQ and octyl itaconate, further expanding the catalog of these reagents to researchers worldwide."* [5.1]

AbCam, a leading international supplier of life science research tools, reports that, since its inclusion in the Abcam catalogue in 2016, MitoParaquat sales have grown year-on-year, [text removed for publication] with sales across Europe, Asia and North America enabling investigations into the role of mitochondria in areas including cancer biology, parasitology and inflammation.

Non-commercial distribution:
UofG has also supplied its molecular probes to non-direct collaborators, consistent with its commitment to promote widespread use of the compound. Since August 2013, they have been supplied to 43 institutions in 17 countries through Material Transfer Agreements.

The open-access publication of synthesis protocols has enabled high-volume users to synthesise probes themselves. The Director of the USA's National Heart, Lung, and Blood Institute noted that

the ability to manufacture MitoPhotoDNP in-house has saved them USD50,000-100,000 in synthesis and development costs since 2013:

> "*This probe opened up a whole new area of research into the structure of the mitochondria in health and disease. Frankly, without the demonstrations that this probe provided this area of research would still be struggling to provide direct evidence of a mitochondria conductive network in striated muscle. This is likely one of the major insights into the metabolic design of muscle in the last 50 years"* [5.5a].

Reducing the use of animals in research

The breeding of transgenic animals for research purposes is highly cost- and labour-intensive, requiring the breeding of many animals over and beyond those used in the experiments. The Hartley suite of mitochondrial molecular probes reduces the need for animals in research [5.5b] by avoiding the need for knock-out/knock-in of key enzymes and in the case of MitoPhotoDNP allowing for the use of autologous controls (where animals serve as their own control).

We identified 31 institutions internationally which have published research articles using Hartley's molecular probes since 2013, including in the study of disease aetiologies such as cardiovascular disease, atherosclerosis, diabetes, cancer, neurology, metabolic disorders and aging. Of these, 19 laboratories were contacted whose research outputs used either wild-type or transgenic mammals *in vivo/ex vivo;* 14 responded to a survey request (74% return rate). All revealed that the UofG molecular probes had reduced the number of animals used for research by ~25% on average by changing their experimental design. This was accomplished by reducing the requirement for breeding of transgenic animals, reducing the crossbreeding of transgenic animals and facilitating the use of more robust wild-type animals.

> *"The use of these compounds allowed us to decipher the mechanisms in vitro and carefully plan focused in vivo studies with limited mice*." Feinberg School of Medicine, Northwestern University [5.5c]
>
> *"The use of these compounds has… offered a more streamlined approach towards the effective assessment of the oxidative state of a cell in vivo. The ability to do this without a need for the crossing to complex reporter mice has been a major step forward".* The University of Texas Southwestern Medical Center [5.5d].

[Text removed for publication.]

5. Sources to corroborate the impact

5.1. Evidence from Cayman Chemical
a) Testimonial; b) sales figures/metrics from Cayman; c) documentation including product info sheets and the promotional material for the kit
https://www.caymanchem.com/news/measure-mitochondrial-h2o2-directly-in-vivo-or-in-cells

5.2. Evidence from Abcam
a) Statement from Business Development Manager, Abcam; b) Datasheet for MitoPQ (ab146819); c) ab146819 Citations

5.3. Material Transfer Agreements for non-commercial work

5.4. Institutional use of compounds

5.5. Collated survey responses and statements

[Text removed for publication.]