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Hydrogen Peroxide Sensing for Reproductive Health

Hydrogen Peroxide Sensing for Reproductive Health

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For Ainsley: my patient, beautiful, longsuffering wife.

*If we knew what it was we were doing, it would not be called research,
would it?*

Albert Einstein

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PUBLICATIONS

Journal Papers

These papers comprise full chapters in this thesis:

Purdey, M. S.; Connaughton, H. S.; Whiting, S.; Schartner, E. P.; Monroe, T. M.; Thompson, J. G.; Aitken, R. J.; Abell, A. D., Boronate probes for the detection of hydrogen peroxide release from human spermatozoa. *Free Radical Biology and Medicine* **2015**, *81* (0), 69-76.

Purdey, M. S.; Thompson, J. G.; Monroe, T. M.; Abell, A. D.; Schartner, E. P., A dual sensor for pH and hydrogen peroxide using polymer-coated optical fibre tips. *Sensors* **2015**, *15* (12), 31904-31913.

Purdey, M. S.; Abell, A. D., New BODIPY-based probes for the detection of hydrogen peroxide. **2015**, *In Preparation*.

These publications represent other work by the author described in this thesis:

Sutton-McDowall, M. L.; Purdey, M.; Brown, H. M.; Abell, A. D.; Mottershead, D. G.; Cetica, P. D.; Dalvit, G. C.; Goldys, E. M.; Gilchrist, R. B.; Gardner, D. K.; Thompson, J. G., Redox and anti-oxidant state within cattle oocytes following in vitro maturation with bone morphogenetic protein 15 and follicle stimulating hormone. *Molecular Reproduction and Development* **2015**, *82* (4), 281-294.

Sutton-McDowall, M. L.; Wu, L.; Purdey, M.; Brown, H. M.; Abell, A. D.; Goldys, E. M.; MacMillan, K. L.; Robker, R. L.; Thompson, J. G., Non-Esterified Fatty Acid-Induced Endoplasmic Reticulum Stress in Cattle Cumulus Oocyte Complexes Alters Cell Metabolism and Developmental Competence. *Biology of Reproduction* **2015**, Available online, doi:10.1095/biolreprod.115.131862

Zuber, A.; Purdey, M.; Schartner, E.; Forbes, C.; van der Hoek, B.; Giles, D.; Abell, A.; Ebdorff-Heidepriem, H., Detection of gold nanoparticles with different sizes using absorption and fluorescence based method. *Sensors and Actuators B: Chemical* **2016**, *227*, 117-127.

Schartner, E. P.; Henderson, M. R.; Purdey, M. S., Dhattrak, D.; Monroe, T. M.; Gill, P. G.; Callen, D. F., Tumour detection in human tissue samples using a fibre tip pH probe. **2016**, *In preparation*.

Patent

Australian patent application 2015902890 – “*Detection of Gold Nanoparticles*” (Deep Exploration Technologies CRC Limited) – 21 July **2015**

Conference presentations

Conference paper:

Purdey, M. S.; Schartner, E. P.; Sutton-McDowall, M. L.; Ritter, L. J.; Thompson, J. G.; Monroe, T. M.; Abell, A. D., Localised hydrogen peroxide sensing for reproductive health. *Proceedings of SPIE* **2015**, 9506, 950614.

Invited talks:

Purdey, M. S.; Schartner, E. P.; Heng, S.; Zhang, X. Z.; Stubing, D. B.; Monroe, T. M.; Abell, A. D.; Functionalisation of Optical Fibres for Biosensing Applications. *ANFF Research Showcase*, Canberra, ACT, Australia, November **2014**.

Purdey, M. S.; Schartner, E. P.; Sutton-McDowall, M. L.; Ritter, L. J.; Monroe, T. M.; Thompson, J. G.; Abell, A. D.; A Non-invasive Sensor for Hydrogen Peroxide and pH. *Society for Reproductive Biology*, Adelaide, SA, Australia, August **2015**.

Conference posters:

Purdey, M. S.; Connaughton, H. S.; Whiting, S.; Thompson, J. G.; Aitken, R. J.; Abell, A. D.; Reactive Oxygen Species Detection in Human Spermatozoa with Aryl Boronates. *RACI National Congress*, Adelaide, SA, Australia, December **2014**.

Purdey, M. S.; Schartner, E. P.; Monroe, T. M.; Aitken, R. J.; Thompson, J. G.; Abell, A. D.; Hydrogen Peroxide Sensing for Reproductive Health. *249th ACS Nation Congress*, Denver, CO, USA, March **2015**.

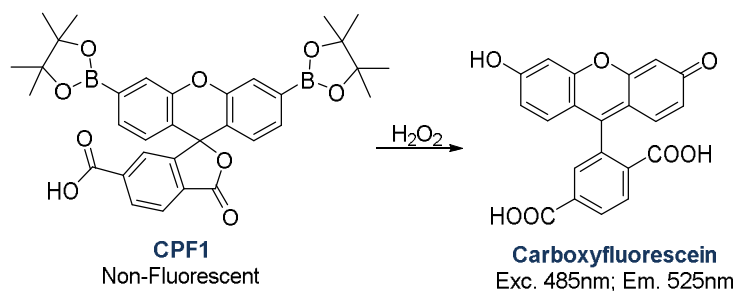
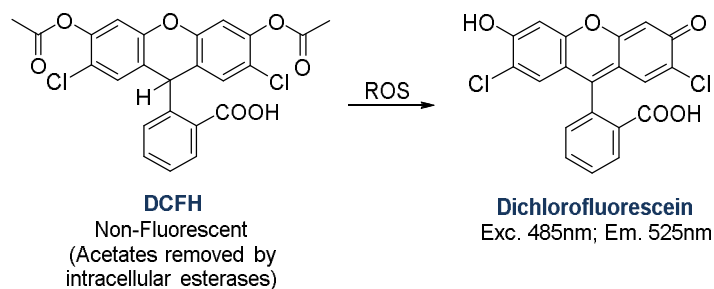
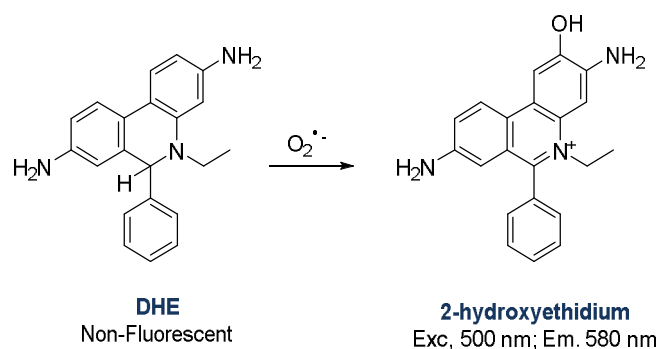
ABBREVIATIONS

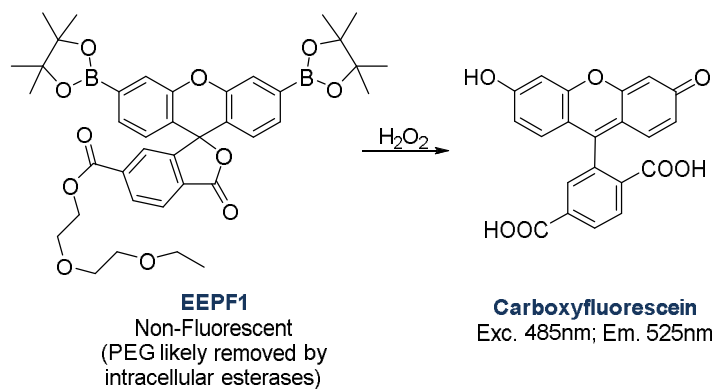
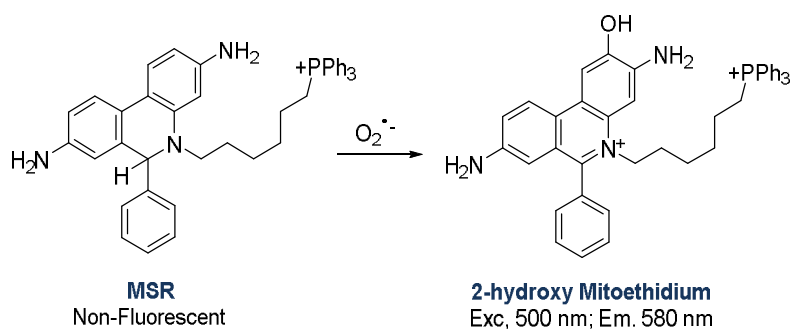
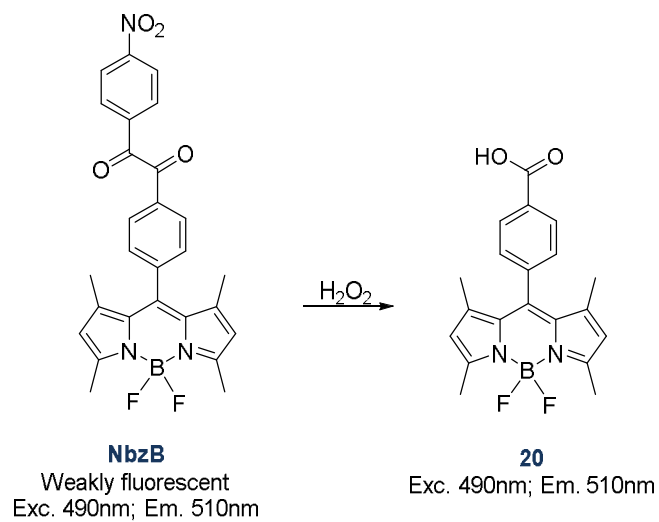
4HNE	4-Hydroxynonenal
AA	Arachidonic Acid
ACN	Acetonitrile

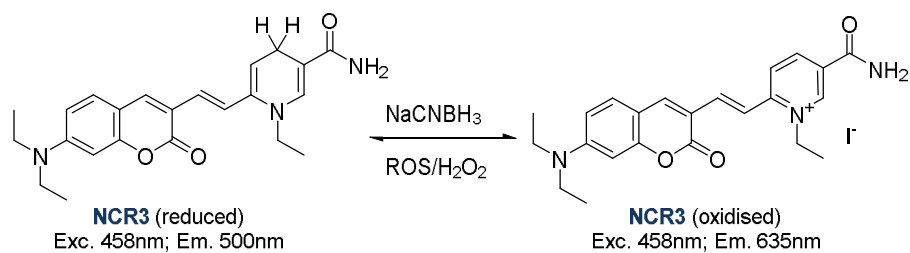
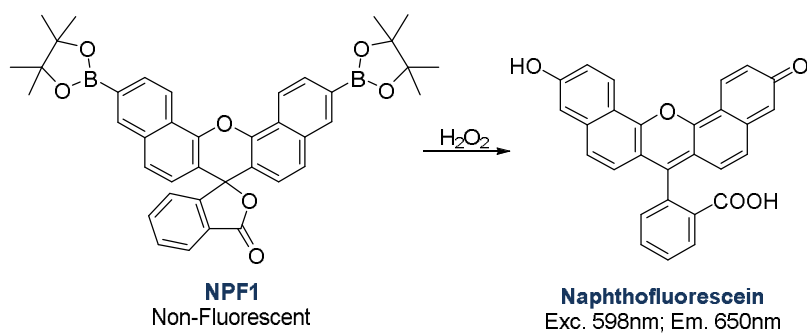
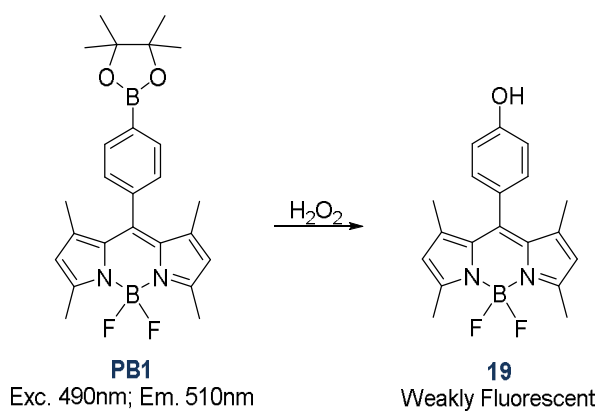
APTES	3-(Aminopropyl)triethoxysilane
BODIPY	Boron Dipyrromethene
CDCl ₃	Deuterated chloroform
CHCl ₃	Chloroform
CPF1	Carboxy Peroxyfluor-1
CPF1-NHS	N-Succinimide ester of Carboxy Peroxyfluor-1
DCFH	2',7'-Dichlorohydrofluorescein diacetate
DCM	Dichloromethane
DHE	Dihydroethidium
DMF	Dimethylformamide
DMSO	Dimethylsulfoxide
DPI	Diphenyl Iodonium
EEPF1	2-Ethoxy(2-Ethoxyethoxy) Peroxyfluor-1
ETC	Electron Transport Chain
FCR2	Flavin Coumarin Redox sensor 2
FRET	Förster Resonance Energy Transfer
H ₂ O ₂	Hydrogen Peroxide
HEPES	4-(2-Hydroxyethyl)piperazine-1-ethanesulfonic acid
HNO	Nitroxyl
HPLC	High Performance Liquid Chromatography
HRMS	High Resolution Mass Spectrometry
IVF	<i>In Vitro</i> Fertilisation
MeOH	Methanol
MitoPY1	Mitochondrial PeroxyYellow-1

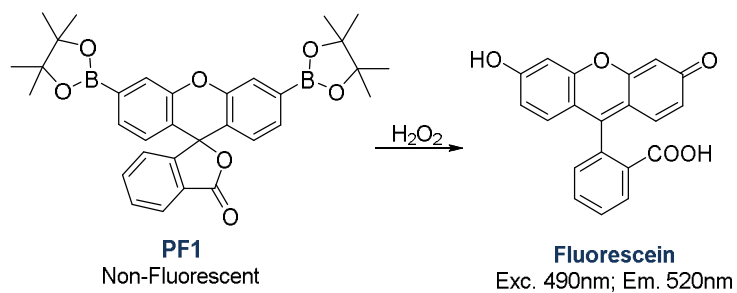
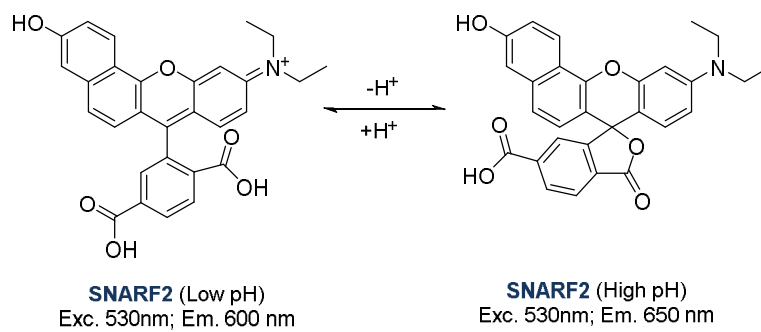
MRI	Magnetic Resonance Imaging
MSR	MitoSOX Red
NaCNBH ₃	Sodium Cyanoborohydride
NbzB	NitrobenzoylBODIPY
NbzF	NitrobenzoylFluorescein
NCR3	NicotinamideCoumarin Redox sensor 3
NEFA	Non-Esterified Fatty Acid
NMR	Nuclear Magnetic Resonance
NO	Nitric Oxide
NPF1	Naphthoperoxyfluor-1
NpFR1	Naphthalimide Flavin Redox sensor 1
O ₂ ^{•-}	Superoxide
⁻ OCl	Hypochlorite
[•] OH	Hydroxyl radical
ONOO ⁻	Peroxynitrite
PB1	PeroxyBODIPY-1
PBS	Phosphate Buffer Solution
PEG	Poly Ethylene Glycol
PET	Photoinduced Electron Transfer
PF1	Peroxyfluor-1
ROS	Reactive Oxygen Species
SE	Standard Error of the mean
SNARF2	Seminaphthorhodofluor-2
TBHP	Tert-Butyl Hydroperoxide

GLOSSARY OF FLUORESCENT PROBES

Carboxyfluor-1 (**CPF1**)See **Chapters 2-4, 6 and 7**2',7'-Dichlorohydrofluorescein Diacetate (**DCFH**)See **Chapters 1 and 2**Dihydroethidium (**DHE**)See **Chapters 1 and 2**

2(2-Ethoxyethoxy)ethoxy Peroxyfluor-1 (**EFPF1**)See **Chapters 2, 6 and 7**MitoSOX red (**MSR**)See **Chapters 1 and 2**NitrobenzoylBODIPY (**NbzB**)See **Chapters 5-7**

Nicotinamide Coumarin Redox Sensor 3 (**NCR3**)See **Chapters 6** and **7**Naphthoperoxyfluor-1 (**NPF1**)See **Chapters 6** and **7**PeroxyBODIPY-1 (**PB1**)See **Chapters 5-7**

Peroxyfluor-1 (PF1)See **Chapters 1-7****Seminaphtharhodafleur-2 (SNARF2)**See **Chapters 4 and 7**

ABSTRACT

The research presented in this thesis details the synthesis, surface functionalisation and photochemical studies of fluorescent probes for the detection of hydrogen peroxide (H_2O_2) in reproductive health.

Chapter 1

H_2O_2 is an important reactive oxygen species (ROS) that is detrimental to the health of spermatozoa and embryos. Fluorescent probes are commonly used for the detection of ROS and here examples with different mechanisms of detection are examined; such as turn-on probes, turn-off probes, Förster resonance energy transfer (FRET)-based and photoinduced electron transfer (PET)-based probes. Specific reference is given to the aryl boronate and benzil classes of probe, which show good selectivity for H_2O_2 over other ROS. The attachment of the fluorescent probe to an optical fibre as a non-invasive sensing platform is discussed. This then allows sensing in a sensitive biological environment, such as an embryo, without exposure to the probe in solution. Fibre tip sensors and microstructured optical fibre-based sensors are discussed for use in such biological environments. Finally, a summary is provided detailing the objectives of this thesis and the chapters in which these are addressed.

Chapter 2

Three aryl boronate probes [peroxyfluor-1 (**PF1**), carboxy peroxyfluor-1 (**CPF1**) and a novel probe 2(2-ethoxyethoxy)ethoxy peroxyfluor-1 (**EEPF1**)] were synthesised for use in the detection of H_2O_2 in human spermatozoa. The activity and selectivity of these probes was then compared to three commonly used commercial probes, 2',7'-dichloro-4,6-diamino-2-methylrhodine diacetate (**DCFH**), dihydroethidium (**DHE**) and MitoSOX red (**MSR**). **PF1** and **EEPF1** were found to be effective at detecting H_2O_2 and peroxynitrite (ONOO^-) produced by spermatozoa when stimulated with menadione or 4-hydroxynonenal. Flow cytometry was used to demonstrate that **EEPF1** is more effective at detecting ROS in spermatozoa compared to **DCFH**, **DHE** and **MSR**. Furthermore, **EEPF1** distinguished poorly motile sperm from motile sperm as revealed by an enhanced production of ROS.

Chapter 3

A fibre-tip based probe constructed by encapsulating **CPF1-NHS** in a polyacrylamide matrix is reported for the detection of H_2O_2 . This non-invasive platform avoids the need to introduce an organic fluorophore into a sensitive cell such as an embryo as discussed above. A number of derivatives of **PF1** were investigated, with carboxylated fluorophore **CPF1** proving to be the easiest to synthesise and characterise. **CPF1** was functionalised to glass slides

using layer-by-layer deposition of polyelectrolytes. This functionalised surface showed a fluorescent response to H_2O_2 comparable to solution-based measurements. Three surface functionalisation methods were then investigated for attachment to an optical fibre tip, specifically polyelectrolyte deposition, silane monolayer formation, and light-catalysed polymerisation of acrylamide. The most effective method of functionalisation was found to be light-catalysed formation of a polyacrylamide matrix with the **CPF1** embedded. These polyacrylamide fibre tip probes were then guided into microdroplets of bovine *in vitro* fertilisation (IVF) media using a micromanipulator. This was visualised under an optical microscope to detect the controlled release of H_2O_2 . This fibre probe is thus compatible with imaging techniques used in IVF research laboratories.

Chapter 4

This chapter presents the development of a single optical fibre tip probe capable of detecting both the concentration of H_2O_2 and the pH of the associated solution. The sensor was constructed by embedding two fluorophores [**CPF1** and seminaphtharhodafuor-2 (**SNARF2**) for H_2O_2 and pH detection respectively] on the tip of an optical fibre using the previous developed polyacrylamide matrix methodology. The functionalised fibre probes reproducibly sensed pH with a resolution of 0.1 pH units. The probe also accurately detected H_2O_2 over a biologically significant concentration range, of 50-100 μM . This study revealed the importance of simultaneous detection of H_2O_2 and pH, where changes in pH were shown to affect the fluorescent response of **CPF1**. This new fibre probe offers potential for non-invasive detection of pH and H_2O_2 in biological environments using a single optical fibre.

Chapter 5

Two new cell-permeable boron-dipyrromethene (**BODIPY**) based fluorescent probes for the detection of H_2O_2 were designed and synthesised. The aryl boronate peroxyBODIPY-1 (**PB1**) gave rise to a decrease in fluorescence on reaction with H_2O_2 , while the fluorescence of the benzil-based nitrobenzoylBODIPY (**NbzB**) probe increased on reaction with H_2O_2 . The benzil probe **NbzB** exhibited a high degree of selectivity for H_2O_2 over other ROS. The aryl boronate **PB1** showed a greater change in fluorescence on reaction with H_2O_2 compared to **NbzB**, and **PB1** also detected H_2O_2 in bovine oocytes under oxidative stress. These results suggest that aryl boronates (i.e. **PB1**) and benzils (i.e. **NbzB**) have use in biological environments requiring higher sensitivity or selectivity to H_2O_2 .

Chapter 6

The research discussed here extends the solution-based and fibre tip experiments to the detection of H_2O_2 in biological environments. Detection of H_2O_2 within cells is often frustrated

by autofluorescence in the green emission region. Contrastingly, the red emission region in biological systems shows a lower autofluorescence background signal. Therefore a red-emitting fluorescent probe for H_2O_2 , naphthoperoxyfluor-1 (**NPF1**), was synthesised. However, when incubated with H_2O_2 in cuvette, **NPF1** showed a greater than 20-fold reduced fluorescent response to H_2O_2 compared with **CPF1**. This poor sensitivity suggests that **NPF1** should not be used for the detection of H_2O_2 , but rather fluorophores with a greater fluorescent response should be utilised (e.g. **CPF1**). A reversible optical fibre-based sensor for H_2O_2 was then explored by attaching a reversible fluorescent probe for ROS (nicotinamide coumarin redox sensor 3, **NCR3**) to an optical fibre tip. The sensor was constructed using light-catalysed polymerisation to give a polymer matrix on the tip containing **NCR3**. This allowed the fibre tip to be reversibly oxidised by H_2O_2 and reduced by NaCNBH_3 . The sensor exhibited good reversibility over at least seven cycles of oxidation and reduction, with consistent fluorescent ratios of its maxima at 500 and 635 nm. However, its fluorescence intensity decreased over time, suggesting that **NCR3** leached from the polymer into the buffer solution. This nevertheless represents the first example of a reversible fibre sensor for ROS and is as such an important first step towards a reusable optical fibre probe for H_2O_2 .

DECLARATION

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Tuesday, 8 December 2015

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