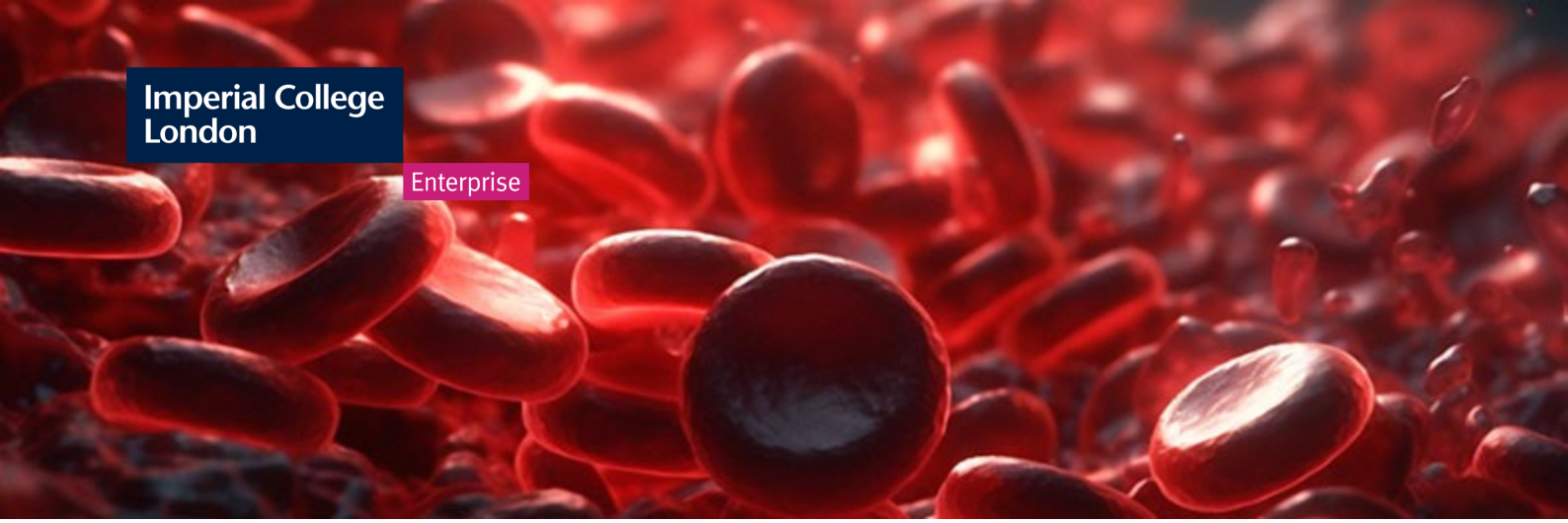

Imperial College London

Enterprise

# A real-time fluorescence probe for Heme Oxygenase activity

**Novel heme oxygenase 1 (HO-1) probe compositions, and easy-to-use methods covering rapid readouts of cell protection in cardiovascular disease, haemorrhage and red cell disorders.**

## Proposed uses

- Fluorescent probe compositions for heme oxygenase 1 detection can be used as research reagents, form kits or other commercial products and services such as diagnostics assessing HO-1 activity and cell self-protection.
- Real-time point of care assessment of red cell disorders or hemorrhage.
- Probe compatibility with applications in medical imaging of coronary and cerebrovascular disease.

## Problem addressed

Heme oxygenase 1 is an enzyme that catalyses the degradation of heme into biliverdin, carbon monoxide and iron. HO-1 is a vital homeostatic enzyme exerting protective effects, and it impacts the defence against oxidative stress, inflammation and tissue injury. Additionally, Heme Oxygenase, particularly the inducible gene, Heme Oxygenase 1, is critical for vessel health and for regulation of levels of blood pigment molecules. Heme oxygenase 1 has an implicated role in vascular diseases. For example, HO-1 is elevated in the most impacted vessel tissues in coronary artery disease patients vulnerable to heart attacks and is an elevated blood marker succeeding hemorrhagic stroke. Additionally, HO-1 shows elevated expression in sickle cell disease (putative protective role) and correlates with red cell damage.

Accurate measurement of HO-1 activity could provide valuable insights into the pathophysiology and prognosis of various disease. However, HO-1 measurement is a difficult and slow process. Current commercial solutions (namely assays, kits based on monoclonal antibodies or spectrophotometric probes) are insufficient, due to low HO-1 sensitivity and specificity and a lack of standardisation across sample types, which can lead to high readout variability of limited reproducibility. The HO-1 fluorescent probes developed by Imperial researchers have the potential to significantly improve upon existing approaches in terms of sensitivity, specificity, speed of results and probe cost. Addressing these bottlenecks will catalyse research on this promising enzymatic disease marker and accelerate innovative HO-1-associated diagnostic technologies for patients.

## Benefits

- The first fluorescent imaging reagent to report on Heme Oxygenase activity in real-time, dramatically improving current methods of detection.
- Low-cost probes which can facilitate basic and translational research into an important protective enzyme and cell signaling system.
- Real-time and potential point-of-care assessment of hemorrhagic, hemolytic and cardiovascular disorders.
- Potential imaging of symptomatic or immediately pre-symptomatic cardiovascular disease in patients.


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Technology reference: **10512**

Imperial College London Enterprise

## Technology overview

We have patented a family of turn-on breakapart fluorescence resonance energy transfer (FRET)-based probes for Heme Oxygenase. With these, fluorescence emission relates to actual activity of Heme Oxygenase, and not simply its protein levels, and thus can detect Heme Oxygenase within minutes after application of probe and ready-mix support medium. Other methods require days and are destructive procedures. This is the first known small molecule probe to report on HO-1 activity or utilise the FRET-breakapart approach in any analogue of a metabolite. Embodiments of the core technology principally include:

- Probes designed for Heme Oxygenase 1 detection, in particularly claimed are porphyrin, chlorin, bacteriochlorin, and isobacteriochlorin compounds which have desired tetrapyrrole backbones and fluorophores incorporated.
- Probe applications cover *in-vitro/in-vivo* diagnostic methods, and *ex vivo* imaging methods and/or methods to use in treatment for hemorrhage, intraplaque hemorrhage, acute coronary syndrome, stroke and/or atherosclerosis among other cardiovascular diseases.

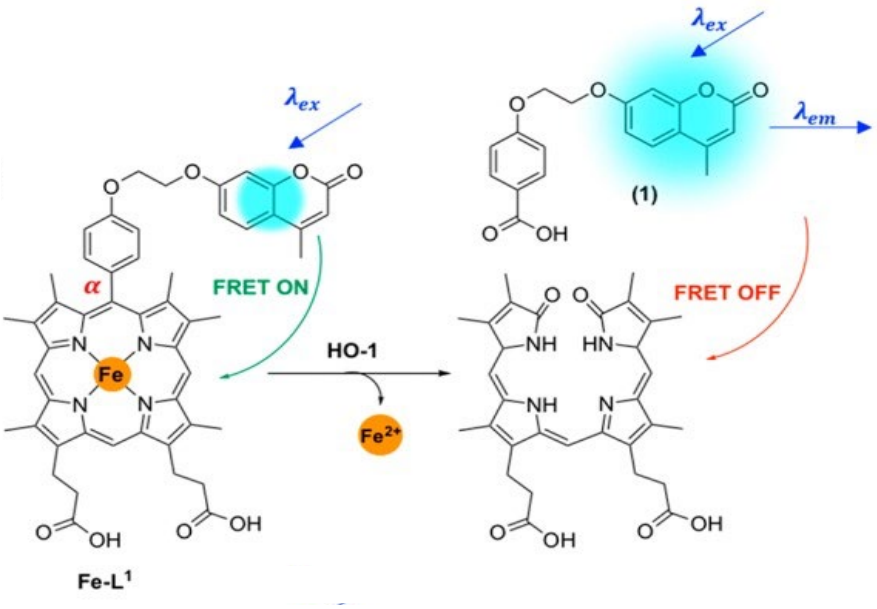


**Figure 1.** An entirely novel small molecule construct was synthesised. This links together a coumarin fluorophore that is ultraviolet-excited and blue-emissive with a porphyrin analogue that is blue-excited and red-emissive. In this molecule, the light-derived energy passes directly from the coumarin to the porphyrin. Crucially, they are linked via the part that is lost during cleavage of endogenous analogue hemin by Heme Oxygenase. Consequently, the molecule is cleaved into two separate fluorescence molecules by catalytic action of Heme Oxygenase, freeing the coumarin molecule to emit blue light. This is conceptually similar to a number of fluorescence probes for nucleases and peptidases and the first time it has been applied to a small molecule metabolite. IP covers a family of related constructs.

Please see patent, and inventors listed manuscripts or contact Imperial Enterprise for further technical details.

## Intellectual property information

- The IP portfolio owned by Imperial College London and managed by Imperial Enterprise includes:

### Patent asset:

**COMPOUNDS FOR THE DETECTION OF HEME OXYGENASE 1 (HO-1) AND METHODS AND USES INVOLVING THE SAME**

- GB priority patent filing completed on 11/12/2020, entering PCT Stage 12/11/2021 (PCT/GB2021/052929 and published: WO2022101635-A1). National entry has been prioritised in E.U, U.S., Japan, China and India. Substantive examination to be pursued in all jurisdictions; EPO (EP4243929A1), CHIPO (CN116745297A), JPO, USPTO and IPO.

### Know-how and expertise

- Regarding applied synthetic inorganic and organometallic chemistry, improved 2-3rd generation fluorescent HO-1 probes, and methods of use for probes in diagnostic applications. Consultancy projects can be arranged, via ICON, to facilitate pilot testing of the technology prior to licencing, customise probes and methods towards sample and indication application specific requirements.

## Publications


Walter ERH, Ge Y, Mason JC, Boyle JJ, Long NJ. A Coumarin-Porphyrin FRET Break-Apart Probe for Heme Oxygenase-1. J Am Chem Soc. 2021 May 5;143(17):6460-6469. doi: 10.1021/jacs.0c12864. Epub 2021 Apr 12. PMID: 33845576; PMCID: PMC8154531. https://pubmed.ncbi.nlm.nih.gov/33845576/

Walter ERH, Ge Y, Mason JC, Boyle JJ, Long NJ. A Coumarin-Porphyrin FRET Break-Apart Probe for Heme Oxygenase-1. J Am Chem Soc. 2021 May 5;143(17):6460-6469. doi: 10.1021/jacs.0c12864. Epub 2021 Apr 12. PMID: 33845576; PMCID: PMC8154531. https://pubmed.ncbi.nlm.nih.gov/34605500/

Boyle JJ. Heme and haemoglobin direct macrophage Mhem phenotype and counter foam cell formation in areas of intraplaque haemorrhage. Curr Opin Lipidol. 2012 Oct;23(5):453-61. doi: 10.1097/MOL.0b013e328356b145. PMID: 22777293. https://pubmed.ncbi.nlm.nih.gov/22777293/


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