1. FINAL PUBLISHABLE SUMMARY REPORT

This section normally should not exceed 2 pages.

This is a comprehensive summary overview of results, conclusions and the socio-economic impacts of the project. The publishable report shall be formatted to be printed as a stand alone paper document. This report should address a wide audience, including the general public.

Please ensure that it:

- *Is of suitable quality to enable direct publication by the REA.*

- *Is comprehensive, and describes the work carried out to achieve the project's objectives; the main results, conclusions and their potential impact and use and any socio-economic impact of the project. Please mention any target groups such as policy makers or civil society for whom the research could be relevant.*

- *Includes where appropriate, diagrams or photographs and the project logo, illustrating and promoting the work of the project.*

- *Provides the address of the project Website (if applicable) as well as relevant contact details.*

Publishable Summary

The first aim of the project was to produce a new generation of optical molecular imaging probes that will permit the immediate detection of critical inflammatory/infective events within the lung thereby allowing the real time optical imaging to be used in lung inflammation and infection. The longer-term vision is that these optical imaging probes will enable clinicians in intensive care units to be able to apply this method to rapidly and accurately stratify patients for appropriate therapy.

Pulmonary infiltrates, often encountered in mechanically ventilated patients, represent a major diagnostic challenge in the intensive care unit (ICU). Acute Lung Injury (ALI), which is a neutrophil-predominant clinical syndrome characterised by pulmonary infiltrates, results in a heavy burden of morbidity and mortality in ICU. Critically ill patients are at risk of also developing ventilator-associated pneumonia (VAP). Both ALI and VAP increase hospital mortality and result in poor functional long-term patient outcomes. Defining and tracking ALI and diagnosing VAP are key challenges to critical care clinicians. Current therapies are generally poor, because the diagnostic tools available to define the inflammatory/infective state *in situ* are very limited and crude. As a result clinicians utilise broad-spectrum empiric antibiotic strategies and often lack the confidence to de-escalate or stop antimicrobial treatment, even though this could reduce emergence of antibiotic resistance, such as Methicillin-Resistant *Staphylococcus aureus* (MRSA). Since VAP is frequently associated with ALI, it would be extremely advantageous to be able to simultaneously detect and monitor both of these processes.

In this project, activity-based smart probes, that can discriminate between sterile and bacterial inflammation in the lung, *i.e.*, confirm/exclude ALI and VAP, have been developed. These imaging probes target neutrophils (PMNs), which characterise ALI. The PMN probes have been designed to be activated by certain biomarkers in lung injury or inflammation. Other imaging probes are designed to target and label gram-negative and gram-positive bacteria (Figure 1).

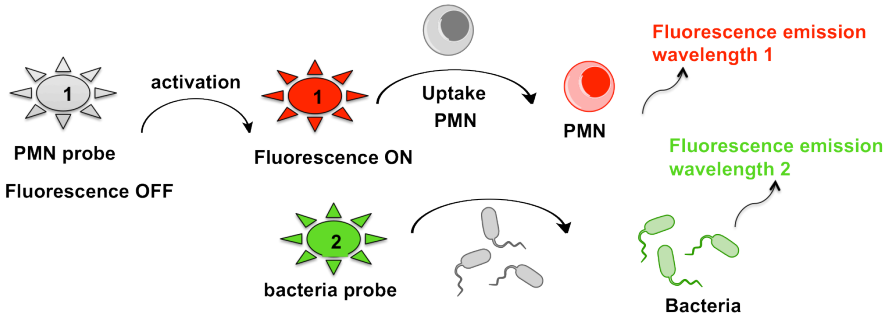

Figure 1. *The basic concept of imaging probes for neutrophils and bacteria.*

During this project several probe constructs were designed and synthesised. As a part of the project, a selective bacteria labelling moiety was identified. A selective PMN labelling probe has also been identified. As a "proof-of-concept", we have successfully shown that our probes, selectively label bacteria and/or PMNs in bacteria–PMN coculture with no bystander labelling of lung epithelial cells. We are currently moving ahead with the probes and tuning them to be applicable with our *in vivo* lung imaging platforms.

The second aim of this project was to develop an efficient, selective and generic labelling method for proteins both *in vitro* and *in vivo*. The approach included the development of a new protein tag that can be employed in selective protein labelling with a reactive probe developed in conjunction. The protein tag sequence incorporates a well-established hexa-histidine (His-Tag) in addition to a strategically placed tyrosine, a residue for selective covalent labelling. Tyrosines can selectively undergo irreversible reaction with a cyclic diazodicarboximide derivative (PTAD) under mild biocompatible conditions. In addition to the PTAD moiety for tyrosine labelling, the new probe developed bears a NTA ligand that, by binding to the hexa-histidine sequence in the protein tag, gains selectivity over other proteins present. The new His_6-Tyr-Tag is being identified by screening on peptide microarrays in order to identify the ideal tag sequence with a NTA-PTAD-probe containing Cy5 fluorophore (Figure 2). For the probes, a flexible synthesis route is being developed employing solid phase and solution phase synthesis and enabling efficient optimisation of, for example, spacer lengths and fluorophores.

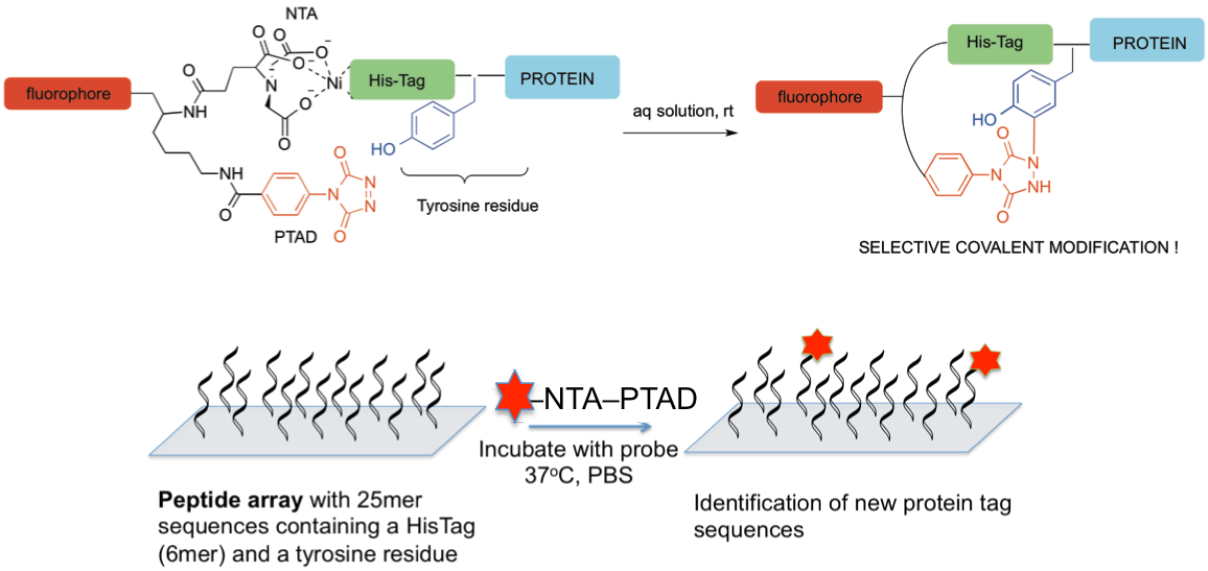

Figure 2. *The platform for identifying a new* His_6-Tyr-Tag *for selective protein labelling with an NTA-PTAD probe.*

USE AND DISSEMINATION OF FOREGROUND

Section A (public) – DISSEMINATION MEASURES

This section should describe the dissemination measures, including any scientific publications relating to foreground and specify any applications for patents etc in accordance with article II.11. Its content will be made available in the public domain thus demonstrating the added-value and positive impact of the project on the European Community.

- **Dissemination activities**

Patenting
The Bradley and Haslett research groups are currently constructing patents concerning the work related to the development of novel imaging probes with a pulmonary focus. An invention disclosure form was submitted to the University of Edinburgh in November 2013.

Planned Publications
The project has produced publishable results. The publication of the research is pending patent filing

Presentations
Dr Lilienkampf has presented part of the work in an invited talk at the National Hellenic Research Foundation (NHRF), Athens, Greece, November 2012. Title of the talk: Development of Optical Imaging Probe with Pulmonary Focus.
In addition, Dr Lilienkampf is scheduled to present her work in an invited talk at the University of Helsinki in December 2013. Upon patenting, the complete work will be presented in scientific conferences related to the field.