Development of dual-analyte fluorescent probes for the detection of hypoxia in cellular models

Francesca Canyelles i Font, MSc

Abstract

This work concerns the development and characterisation of fluorescent probes that respond to two chemical species concurrently by turning from a quenched to a fluorescent state (off-on mode response).

The aim of this thesis was to develop stable dual-analyte fluorescent probe for visualising hypoxia in cellular environments. This goal was achieved through a comprehensive approach from initial probes' design through synthesis and characterisation, culminating in the validation of these probes in biological systems. The conceptual design of probes capable of multiparametric sensing of hypoxic state involved careful selection of the fluorophore core (hemicyanine dye) and the responsive moieties for the corresponding target analytes – pH and reducing environment (nitroreductase activity), which would serve as proxies for hypoxia.

Following the design phase, a new hemicyanine-based fluorescent scaffold, **HCy-Jul**, that is more robust and resistant to biological interferents than other commonly used hemicyanines was synthesized, characterised and successfully validated. Subsequently, using this scaffold, two new dual-analyte responsive probes, **NHCy** and **NHCy-C** were synthesized, characterised to confirm their structure and purity, and tested for photophysical response to target analytes. Finally, the developed probes were validated *in vitro* and *in cellulo* to determine their performance, sensitivity, reliability and effectiveness in biological environments.

Critically, **NHCy** successfully reported on hypoxia cells through a fluorescence turn-on response to bio-reduction and low pH, two hallmarks of hypoxia, but its residual fluorescence in the off state (common for most turn-on probes) limited the reliability of detection. **NHCy-C probe,** on the other hand, demonstrated a ratiometric response to the presence of both hypoxia surrogate analytes that allowed for reliable hypoxia detection in cells via fluorescence microscopy and flow cytometry.

Designs and methodologies (synthesis, spectroscopic characterisation, live-cell imaging) described herein enabled the development and validation of new fluorescent probes for reliable multiparametric sensing of hypoxia in cancer cell models. This constitutes a major advancement in the field of cellular imaging since a dual-analyte detection with a single probe allows for a more extensive and comprehensive monitoring of biological processes occurring in cells. This knowledge key in the better understanding of diseases, discovery of new functional biomarkers and drug targets and validation of new therapies.

Keywords: responsive fluorescent probes · dual-analyte · hemicyanine dye · hypoxia · pH · nitroreductase activity · cancer