Summary of the main research achievement

Development and practical application of fluorescent probes for reliable detection and visualisation of bioinorganic substances in biological systems

The research achievement presented in this application aimed towards the improvement of the detection of challenging, biologically relevant and poorly understood bioinorganic analytes, such as metal ions and reactive oxygen and nitrogen species (RONS), in biological contexts with fluorescent tools. Responsive fluorescent probes are among the most intensely studied and promising tools for investigating biological processes and have enjoyed extensive interest from the research community. However, most of such tools have never been successfully applied in biological systems beyond proof of concept largely due to insufficient characterisation and limited reliability. To address this problem, I focused on three dimensions: design, probe development, and methodology / practical application protocols. By introducing novel design strategies and validated recommendations, I have been able to develop improved probes based on improved specificity, ratiometricity, and fluorescence lifetime, and to create reliable methodologies and workflows for data extraction and analysis in biological models.

Developing new criteria and approaches for the design and validation of fluorescent probes for bioinorganic analytes in biological models. I established strict criteria for designing highly specific probes for RONS by improving the understanding of the relationship between structure, reactivity, and fluorescence response. Furthermore, I introduced comprehensive prerequisites for designing reversible probes and protocols to maximise their reliability. These theoretical advancements and meta-analyses yielded ground-breaking conclusions that contribute to the emergence and improve the use of more reliable tools for studying of challenging analytes in cells.

Harnessing structure-reactivity-responsiveness relationship to develop specific probes for bioinorganic analytes. On the examples of nicotinamide-coumarin conjugates and platinum chelators, I demonstrated the use of in-depth interrogation of the relationship between structure, reactivity, and specificity of fluorescence response to improve reliability of detection of bioinorganic analytes. This led also to the development of highly specific RPt1 probe and microwell plate assay to quickly and accurately assess the stability of aryl-transplatin complexes with potential therapeutic or biological applications.

Design and use of reliable probes for bioinorganic analytes in biological context. In order to overcome a major limitation of most fluorescent tools, I have developed and applied probes and protocols that generate a signal independent of probe's concentration. To that end, I co-developed two ratiometric probes for labile metal ion pools (Fe(II), Cu(I)) with improved reliability of detection in complex biological models, including 3D tumour spheroids and subtle changes in mitochondria, and applied them to study mechanisms and efficacy of anticancer therapeutic candidates. In addition, I eliminated the formation of the artifacts in RONS detection by selection of appropriate probes as well as protocols for data acquisition and analysis. This allowed us to monitor oxidative stress in mitochondria of RSV virus-infected human cells, independently on mitochondrial membrane potential and to confirm its role in viral infection. I also demonstrated a possibility of improving reliability of measuring oxidative stress with intensity-based responsive probes by instead measuring the changes in their fluorescence lifetime in cells.

Introduction and advancement of dual-analyte probe design for biological applications. I systematised theoretical foundation for a new field of fluorescent probe design aimed at dual-analyte sensing in biology. These probes have the potential to deepen our understanding of interdependencies between bioanalytes by providing a new, previously inaccessible dimension of data. Through this work I showed that dual-analyte probes must exhibit an AND-type specific response simultaneously to two analytes and identified parameters necessary for compatibility with biological environments. This work has been highly cited, and since its publication, tens of new dual-analyte probes based on our guidelines have been reported for use in biology.

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