Development of methodologies for the retention and contextual imaging of endogenous metals in fixed biological samples

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Endogenous metals play a key role in several essential biological processes, including immunity,[1] tumor development,[2] and degenerative diseases.[3] To better understand how metal ions contribute to these processes, it would be highly informative to simultaneously access their distribution alongside that of key biological components (such as organelles, proteins, or nucleic acids). However, this remains challenging, as imaging techniques for biological structures and those for metal ions are often incompatible.[4,5]

Indeed, classical methods used in bioimaging, and particularly antibody-based staining procedures generally require chemical fixation with an aldehyde or alcohol, followed by cell permeabilization before antibody exposure. Aldehyde-based fixation crosslinks cellular components, while alcohol provokes the precipitation of proteins. These processes can rigidify membrane transporters and pores, or deteriorate the membrane integrity, increasing membrane permeability and causing leakage of part of the cellular metal content.[6,7] Additionally, permeabilization with detergents, which is necessary for antibody penetration, further exacerbates this leakage. Metal imaging is thus generally performed using cryofixation processes instead of chemical fixation, or using live imaging with metal-sensing probes, which strongly limit the staining strategies for proteins. Overall, despite the wealth of information they could provide, antibody-based staining strategies are currently incompatible with metal imaging, and thus inaccessible today for biological mechanism studies.

The objective of the present project is to establish a procedure that would enable endogenous transition metal elemental imaging using chemical fixation. For this purpose, the PhD candidates will implement the synthesis of chemically fixable chelators that would enable metal retention within the cells during cell fixation and further steps, in order to preserve the native metal distribution. These chelators will be characterized to determine the kinetics and thermodynamics of their association with relevant metal ions (Fe(II/III), Cu(I/II), Mn(II), Zn(II)). They will then be implemented in cellular studies to optimize the fixation procedure, and the compatibility with antibody staining. Finally, to avoid the pitfalls of multimodal imaging approaches, a protocol to functionalize antibodies with rare earth elements, that can be imaged using elemental approaches such as X-fluorescence or mass spectrometry, will be developed. The candidate will contribute to designing and implementing procedures that will pave the way for an easier and more comprehensive study of the role of metal ions in both healthy or pathological biological processes, with the potential for future valorization.

The CPCV laboratory (ENS-PSL, CNRS, SU) will provide to the PhD candidate a multidisciplinary environment, with chemists and biologists working together in the host team Methrox (metal in biology and redox homeostasis). The PIs and team experience cover all the aspects of the project, including

organic synthesis, coordination complexes characterization, elemental analytical analysis and imaging, and cellular studies.

References

1. Murdoch, C. C. & Skaar, E. P. Nutritional immunity: the battle for nutrient metals at the host–pathogen interface. *Nat Rev Microbiol* (2022) doi:10.1038/s41579-022-00745-6.
2. Pfeifhofer-Obermair, C., Tymoszuk, P., Petzer, V., Weiss, G. & Nairz, M. Iron in the Tumor Microenvironment—Connecting the Dots. *Front. Oncol.* **8**, 549 (2018).
3. Wang, L., Yin, Y. L., Liu, X. Z., Shen, P., Zheng, Y. G., Lan, X. R., ... & Wang, J. Z. (2020). Current understanding of metal ions in the pathogenesis of Alzheimer's disease. *Translational neurodegeneration*, *9*, 1-13.
4. New, E. J., Wimmer, V. C. & Hare, D. J. Promises and Pitfalls of Metal Imaging in Biology. *Cell Chemical Biology* **25**, 7–18 (2018).
5. Perry, W. J. *et al.* Integrated molecular imaging technologies for investigation of metals in biological systems: A brief review. *Current Opinion in Chemical Biology* **55**, 127–135 (2020).
6. Jin, Q. *et al.* Preserving elemental content in adherent mammalian cells for analysis by synchrotron-based x-ray fluorescence microscopy. *Journal of Microscopy* **265**, 81–93 (2017).
7. Schaier, M. *et al.* Multiparametric Tissue Characterization Utilizing the Cellular Metallome and Immuno-Mass Spectrometry Imaging. *JACS Au* **3**, 419–428 (2023).