

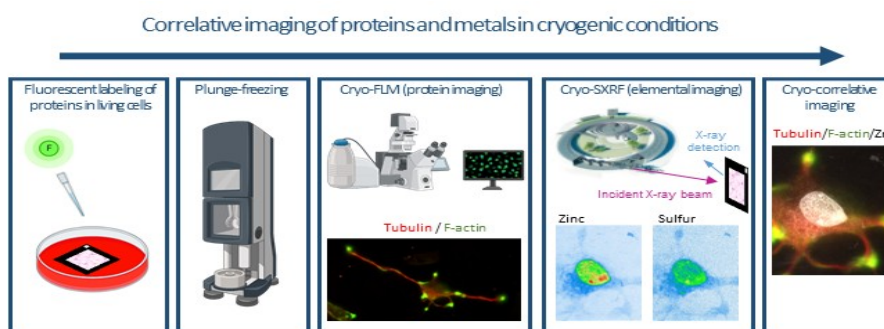
Cryogenic correlative imaging of proteins and metals in primary neurons

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Essential metals such as iron, copper and zinc are required for a wide variety of biological processes. For example, they act as cofactors in many proteins, conferring enzymatic activity or structural stability. Interactions between metals and proteins are often difficult to characterize due to the low concentration of metals in biological tissues [1]. To better understand the cellular functions of essential metals, we correlate protein localization, using fluorescence light microscopy (FLM), and metal distribution with synchrotron X-ray fluorescence (SXRF), a high-sensitivity and high-spatial-resolution technique for metal imaging [2]. Both chemical imaging modalities are implemented under cryogenic conditions to preserve native cell structure and chemical element distribution. As a proof of concept, we applied cryo-FLM and cryo-SXRF correlative imaging to cultured primary hippocampal neurons. Neurons were labeled under live conditions with fluorescent F-actin and tubulin dyes, then samples were flash-frozen and observed in a frozen hydrated state. This methodology, cryo-FLM combined to cryo-SXRF, revealed the distribution of iron, copper and zinc relative to F-actin and tubulin in the growth cones, dendrites, axons, and axonal *en passant* boutons of developing neurons.



[1] I. Kelkoul, V. Puente Munoz, R. Ortega, A. Carmona, *Metalomics*, **2025**, 17(2), mfaf003.

[2] R. Ortega, M. Fernández-Monreal, N. Pied, S. Roudeau, P. Cloetens, A. Carmona, *Chemical & Biomedical Imaging*, **2024**, 2, 744-754.