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Exploring cellular isotopic fractionation of essential elements for biomedical purposes *in vitro*: challenges and limitations

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High-precision isotopic analysis has been demonstrated to be an outstanding tool for identifying changes in metabolic processes. Research on high-precision isotopic analysis of essential elements, such as Cu, Fe and Zn, via multi-collector (MC)-ICP-MS in (human) cell cultures is still limited although the study of cellular isotope fractionation *in vitro* has been shown to provide further insights in the pathways of these essential elements under different experimental conditions.[1-5] Hypoxia,[1] oxidative stress[2] and cell differentiation[3] have been shown to induce Cu isotope fractionation and pro-inflammatory conditions Zn isotopic fractionation.[4] Cell experiments must be carefully optimized from both a biological and an analytical point of view to ensure the quality of the isotope ratio data. Moreover, the major contribution to the uncertainty on the isotope ratios relies on the cell experiments itself due to the heterogeneity of the cells in a single culture, within experiments and/or between cell batches; all affecting the reproducibly of the experiments. Slight modifications of the cell culture protocol can hinder data interpretation and comparison, as cells can show variations in transcriptome, genome and metabolome based on the culture conditions and/or treatment applied, in turn affecting the isotopic signatures. Results of various experiments using different human cell types and analytical approaches will be presented.

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