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ABSOLUTE PEPTIDE/PROTEIN QUANTIFICATION USING ELEMENTAL MASS SPECTROMETRY (ICPMS)

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Changes resulting from alterations of the biological systems can only be detected if quantitative information is obtained. Unfortunately, reliability and accuracy (trueness and precision) of the results published on quantitative proteomics so far is a central point of concern. Further research and standards are required to address the quality assurance requirements urgently demanded. Unfortunately, ionization process in electrospray and MALDI mass spectrometry (MS) is extremely depending on the physic-chemical properties of the peptide, which obliges to synthesise standards for every individual species when you look for its absolute quantification in a particular sample.

One possible way out to this problem is to resort to MS with an elemental ion source (inductively coupled plasma, ICPMS). Interestingly, the elemental response by ICPMS could be directly proportional to the absolute amount of the element introduced (any different from C, H, N and O). Therefore, in contrast to molecular MS techniques, this signal is independent of the species and sample matrix. In this way, every individual species (peptide) containing the heteroatom could be easily quantified using a simply heteroatom-containing species as a generic standard. Such high-quality absolute quantitative data becomes then directly traceable to a certified standard providing constancy of the results across time and space and a known level of accuracy.

At best, these heteroatoms detectable by ICPMS are naturally present in the protein/peptide (i.e. P, S, Se). In our approach we propose to introduce an ICP-detectable element into the amino acid sequence to make every protein detectable by ICPMS. To do so, we have bioconjugated 2 iodine atoms specifically to the tyrosine residues. Then, capillary HPLC-ICPMS provided absolute quantification of every tyrosine-containing peptide present with extremely high sensitivity (below the nM level) and robustness by simply spiking the sample with a generic iodine-containing standard (iodo-benzoic acid). Quantitative results have been validated using a Reference Material (NIST 8327).