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HIGH RESOLUTION DYNAMIC STUDIES OF PEPTIDE PHOSPHORYLATION USING ELEMENT MASS SPECTROMETRY

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Cells continuously receive stimulus from outside to which they have to respond. It is well recognized today that phosphorylation plays a pivotal role in such responses so it is strictly necessary to quantify the phosphorylation dynamics of each protein in order to clarify the relationship between signalling reactions and eventually observed biological responses. Molecular mass spectrometry has currently established as the most powerful tool for the study of phosphorylation temporal changes because of its versatility and sensitivity. MS-based approaches to study temporal dynamics of cellular signalling are mainly based on the use of stable isotope labelling strategies. All these label-based approaches mostly provides the relative quantification of protein phosphorylation and the time resolution achievable is always limited by the number of different isotopic tags used and the relatively high variability of quantitative results (10-20 % RSD). Only recently, absolute quantification methods have been reported (AQUA, QCAT) but requiring the synthesis of heavy isotope labelled counterparts to be used as internal standards for each peptide/protein sought.

The response by elemental mass spectrometry (ICPMS) is species and matrix independent allowing the absolute and site specific quantification of every phosphorylated protein (unknown or not) after its enzymatic digestion. Quantitative results obtained by capillary HPLC-ICPMS also show exceptional accuracy and precision (<5 % RSD). Using this approach, we herein report the absolute quantification of phosphopeptides and we show its potential to discriminate between very small temporal changes in protein phosphorylation levels. The strong point of the approach proposed is that the high precision achieved in the quantification can be applied to discriminate between close phosphorylation states, therefore leading to very high time resolution in protein phosphorylation dynamics investigations.