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Data Article

Phospho-iTRAQ data article: Assessing isobaric labels for the large-scale study of phosphopeptide stoichiometry

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ABSTRACT

The ability to distinguish between phosphopeptides of high and low stoichiometry is essential to discover the true extent of protein phosphorylation. We here extend the strategy whereby a peptide sample is briefly split in two identical parts and differentially labeled preceding the phosphatase treatment of one part (Pflieger et al., 2008. *Mol. Cell. Proteomics*, 7: 326–46 [1]; Wu et al., 2011. *Nat. Methods*, 8: 677–83 [2]). Our Phospho-iTRAQ method focuses on the unmodified counterparts of phosphorylated peptides, which thus circumvents the ionization, fragmentation, and phospho-enrichment difficulties that hamper quantitation of stoichiometry in most common phosphoproteomics methods. Since iTRAQ enables multiplexing, simultaneous (phospho)proteome comparison between internal replicates and multiple samples is possible. The technique was validated on multiple instrument platforms by adding internal standards of high stoichiometry to a complex lysate of control and EGF-stimulated HeLa cells. To demonstrate the flexibility of PhosphoiTRAQ with regards to the experimental setup and data mining, the proteome coverage was extended through gel fractionation, while an internal replicate measurement creates more stringent data analysis opportunities. The latter allows other researchers to set their own threshold for selecting potential phosphorylation events in the dataset presented here, depending on the biological question or corroboration under investigation. The latest developments in MS

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instrumentation promise to further increase the resolution of the stoichiometric measurement of Phospho-iTRAQ in the future. The data accompanying the manuscript on this approach (Glibert et al., 2015, *J. Proteome Res.* **14**: 2015, 839–49 [5]) have been deposited to the ProteomeXchange with identifier PXD001574.

Specifications Table

Subject area	*Biology, Chemistry*
More specific subject area	*Phosphoproteomics and mass spectrometry* (*MS*)
Type of data	*MS data and annotations*
How data was acquired	*MS: Data-dependent acquisition acquired on three different MS instruments: SynaptG2Si* (*Waters*), *TripeTOF 5600* (*Sciex*) *and QExactive* (*Thermo*)
Data format	*Raw* (**.raw, *.wiff and *.raw, respectively*), *mgf peak lists, *.dat and *.XML identified files from Mascot* (*Matrix Science*)
Experimental factors	*HepG2 cells were treated or not with EGF and after tryptic digest spiked with an internal standard of isotopically heavy AQUA phosphopeptides.*
Experimental features	*The respective proteomes were fractionated using 1DPAGE, followed by tryptic digest. Peptides were briefly split and each half labeled with a different iTRAQ label before enzymatic dephosphorylation of one half and subsequent LC–MS analysis of the merged peptide mixture.*
Data source location	*Gent, Belgium*
Data accessibility	*Deposited to the ProteomeXchange with identifier PXD001574* (*http://proteomecentral.proteomexchange.org/cgi/GetDataset?ID=PXD001574*)

Value of the data

- As a new approach to quantify the stoichiometry of phosphorylation events, “Phospho-iTRAQ” is applicable to a wide range of biological models.
- This first “Phospho-iTRAQ” dataset is generated on MS instruments from three different vendors, illustrating that the approach can be applied platform-independent.
- As described in detail in the accompanying manuscript (*DOI: 10.1021/pr500889v*), a new data analysis approach is presented that uses internal duplicate measurements unique to this experimental workflow. It allows mining data at different thresholds. Depending on the biological question under investigation, other investigators can choose to mine the presented dataset at the threshold of their picking.

1. Experimental design, materials and methods

Fig. 1 shows the general workflow of the Phospho-iTRAQ approach. (A) Experimental workflow: in the “in-solution” approach (left panel), the soluble protein extracts (R1) of control and EGF-stimulated HeLa cells were spiked with an internal peptide standard (IS2) of heavy phosphopeptides and compared by Phospho-iTRAQ. When only one sample is mined (EGF), the flexibility of the protocol allows for gel purification and fractionation and thus for complementing the soluble protein extract (R1) with the hydrophobic fraction diluted in strongly denaturing buffer (R3) to increase the number of annotated phosphopeptides (“in-gel” approach, right panel). The EGF-stimulated cells were spiked with a phosphoprotein internal standard (IS1) before fractionation on a 1D PAGE into four molecular weight