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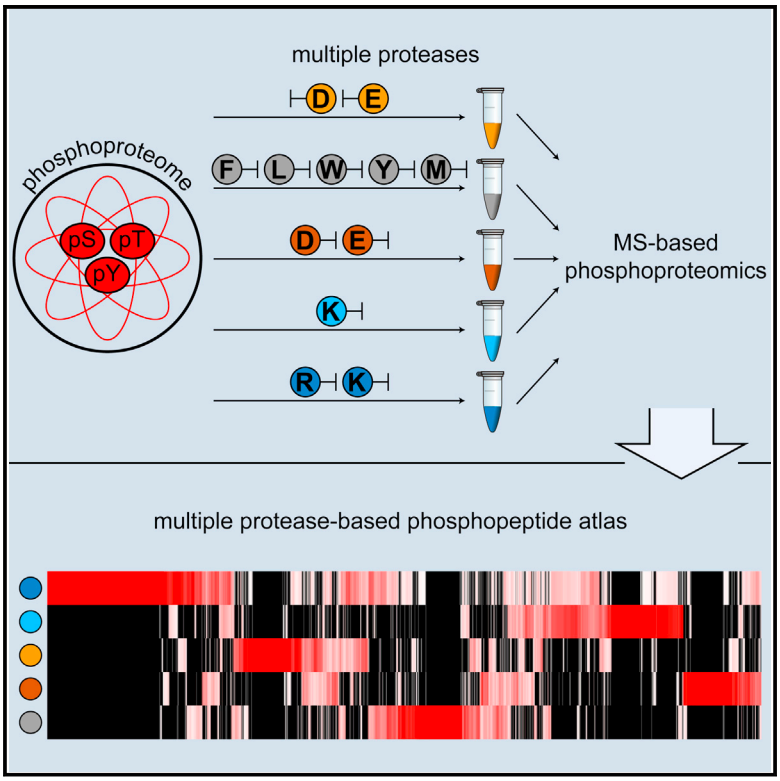
An Augmented Multiple-Protease-Based Human Phosphopeptide Atlas

Graphical Abstract



Highlights

- There is a considerable bias in the current deposited phosphoproteome
- Key phosphosites can be occluded by using trypsin alone
- Optimal MS detection of a phosphorylation site is linked to a favorable protease
- An augmented human phosphopeptide atlas is presented

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In Brief

Giansanti et al. present an augmented human phosphopeptide atlas of 37,771 unique phosphopeptides. Using a multiple protease workflow in combination with highly selective Ti^{4+}-IMAC phosphopeptide enrichment, the authors show that optimal detection of each protein individual phosphorylation event is linked to a preferred protease.

Giansanti et al., 2015, Cell Reports *11*, 1834–1843
June 23, 2015 ©2015 The Authors
http://dx.doi.org/10.1016/j.celrep.2015.05.029

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An Augmented Multiple-Protease-Based Human Phosphopeptide Atlas

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http://dx.doi.org/10.1016/j.celrep.2015.05.029

SUMMARY

Although mass-spectrometry-based screens enable thousands of protein phosphorylation sites to be monitored simultaneously, they often do not cover important regulatory sites. Here, we hypothesized that this is due to the fact that nearly all large-scale phosphoproteome studies are initiated by trypsin digestion. We tested this hypothesis using multiple proteases for protein digestion prior to Ti^{4+}-IMAC-based enrichment. This approach increases the size of the detectable phosphoproteome substantially and confirms the considerable tryptic bias in public repositories. We define and make available a less biased human phosphopeptide atlas of 37,771 unique phosphopeptides, correlating to 18,430 unique phosphosites, of which fewer than 1/3 were identified in more than one protease data set. We demonstrate that each protein phosphorylation site can be linked to a preferred protease, enhancing its detection by mass spectrometry (MS). For specific sites, this approach increases their detectability by more than 1,000-fold.

INTRODUCTION

Cellular signaling proceeds largely via cascades of post-translational modifications, in which reversible protein phosphorylation provides a key mechanism (Huang and White, 2008; Rigbolt and Blagoev, 2012). Site-specific protein phosphorylation can be monitored by site-specific phospho-antibodies, such as those raised against, for instance, pERK T202/Y204 and pSRC Y419. Although powerful, there are only a limited number of these antibodies available, hardly sufficient to monitor the more than 100,000 unique phosphosites present in a human cell. Other caveats in using these antibodies are their limited specificity, recognizing multiple sites in a single protein, or similar phospho-sequences in other proteins. Moreover, these approaches are difficult to multiplex for use in high-throughput assays.

Mass spectrometry (MS)-based phosphoproteomics has recently surfaced as the method of choice for global and high-throughput protein analysis. Immense progress in both mass spectrometric instrumentation (Hebert et al., 2014; Michalski et al., 2011, 2012) and sample preparation and analysis (Di Palma et al., 2012; Ruprecht and Lemeer, 2014; Wiśniewski et al., 2009; Yates et al., 2014) have allowed this technology to confidently identify as many as thousands of proteins and phosphorylation sites in a single experiment and to accurately quantify changes in protein expression or post-translational modifications (PTMs). Recently, we demonstrated the high potential of a new material for phosphopeptide enrichment (Zhou et al., 2013). This affinity matrix termed titanium (IV)-IMAC (Ti^{4+}-IMAC) was shown to possess high selectivity, sensitivity, and quantification reproducibility, allowing in-depth monitoring of more than 10,000 phosphorylation events by a single-step phosphopeptide enrichment (de Graaf et al., 2014).

Predominantly, large-scale phosphoproteomics analyses are based on peptides derived from the tryptic digestion of proteins in a lysate (Mallick et al., 2007; Sharma et al., 2014). Trypsin represents a valid choice because it is highly specific, very effective and, compared to the other available proteases, generates a higher number of peptides in the preferred mass range suitable for identification by MS (Guo et al., 2014; Swaney et al., 2010). As a result, the vast majority of the reported workflows in proteomics are dominated by using exclusively trypsin (Tsiatsiani and Heck, 2015; Wilhelm et al., 2014). However, potentially interesting sequences, and thus particular relevant phosphosites, will remain occluded by this approach, as the tryptic peptides generated do not always possess appropriate physicochemical properties that make them suitable for detection by LC-MS/MS.

Although it has been shown that proteome coverage can be increased by using multiple alternative proteases (Bian et al., 2012; Gauci et al., 2009; Guo et al., 2014; Peng et al., 2012; Swaney et al., 2010), this approach has not systematically been investigated for the analysis of PTMs. Here, we report a systematic study using five commercially available proteases to extend the coverage of the phosphoproteome. We targeted the phosphoproteome of human Jurkat T cells stimulated by PGE_2 and used Ti^{4+}-IMAC for the enrichment of the phosphopeptides from lysates digested in parallel by AspN, chymotrypsin, GluC,