Commentary

Bromodomains shake the hegemony of pan-acetyl antibodies

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Acetylation signaling pathways are involved in numerous cellular processes and are used as therapeutic targets in several disease contexts. However, acetylated proteins only represent a minor fraction of the full proteome, and the identification and quantification of acetylated sites remain a technological challenge. Currently, pan-acetyl antibodies are used to increase the abundance of acetylated peptides through affinity purification before MS analysis. These antibodies are powerful reagents, but they are hampered by a lack of specificity, affinity, and batch-to-batch reproducibility. In this issue, Bryson et al. (*Proteomics* 2015 *15*, 1470–1475) present an interesting alternative to these antibodies, in the form of bromodomains. These domains specifically recognize acetylated lysines, and were successfully used in this study to enrich for acetylated peptides before MS analysis. Future development of this pioneering approach could help overcome this limiting step in the characterization of acetylproteomes.

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In this issue, Bryson et al. [1] suggest the use of bromodomains, specifically binding acetylated lysines, to enrich for acetylated peptides when performing acetylproteome studies. The efficiency of bromodomain-based enrichment is similar to that afforded by pan-acetyl antibodies, opening up possibilities of a new generation of biological molecules for use in acetylproteome studies.

Proteomics now allows for the systematic detection and quantification of protein PTMs in various contexts with increasing accuracy and completeness. However, gaining access to the subproteome defined by a specific PTM remains challenging because it is severely underrepresented within the cell's protein mixture. For this reason, modified peptides are usually enriched before their analysis by MS. This enrichment step is crucial when seeking to detect a maximum number of modified peptides, but at the same time, it can be prone to biases. Indeed, there is a high risk that the reagent used for this enrichment will not cover the entire diversity of the subproteome, thus resulting in better enrichment of some modified peptides compared to others.

Different classes of reagents have been developed for the enrichment step. For example, a range of methods exists for the purification of phosphopeptides, such as chemical derivatization, chromatography, and affinity purification using inorganic species [2]. Antibodies are also widely used to study the landscape of phosphorylation, methylation, and acetylation in different cellular and disease contexts [3–5]. However, their use for the enrichment of modified peptides relies on paradoxical traits. The modification has to be highly specifically recognized, excluding any bias due to the surrounding amino acids. This requirement can be met by using polyclonal antibodies as the combination of numerous clonal antibodies dilutes any bias toward neighboring

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residues. However, polyclonal antibodies notoriously suffer from batch-to-batch variability. This important limitation has several consequences, the most important of which is the limited availability of any validated batch, obliging labs to constantly test new batches. Monoclonal antibodies could overcome the challenge of batch-to-batch variability, but are more sensitive to the sequence context surrounding any modified residue. It is tempting to speculate that a mixture of monoclonal antibodies produced in highly standardized conditions could help to optimize enrichment procedures.

A recent comment signed by 112 authors urges the standardization of antibodies used in research to save millions of dollars [6]. This comment suggests that polyconal antibodies should be avoided, while monoclonal antibodies should be defined based on their sequence and produced as recombinant proteins. This initiative echoes another source of inspiration in specific protein enrichment, based on protein modules identified within cellular machineries. Indeed, eukaryotic cells use dedicated protein domains to sense and interpret PTMs. When these domains are produced as recombinant proteins, they can be used to detect and enrich for specific modifications. For example, SH2 domains bind phosphorylated tyrosine and have been used to profile phosphospecific signaling pathways [7]. Two groups have also engineered methyl-binding domains and used them successfully for methylproteome studies [8, 9].

Similarly, bromodomains can be used to enrich for acetylated peptides [1]. This domain was first discovered in the 1990s in the *brahma* gene from *Drosophila melanogaster* [10]. Bromodomains are present in a variety of proteins, most of which have functions related to the biology of chromatin and transcription regulation [11]. Although their primary sequence is not particularly conserved, they possess a characteristic fold, made up of four alpha helices linked by loop regions, which creates a central acetyl-lysine binding pocket. In their study, Bryson et al. analyzed the specificity of all 14 *Saccharomyces cerevisiae* bromodomains. They assessed their binding properties using an array of degenerate peptides anchored on a centered acetylated lysine residue. They found that the different bromodomains presented variable binding specificity, revealing their potential to capture the diversity of acetylated peptides. The yeast protein Bdf1 has two bromodomains, and Bryson et al. used the second of these bromodomains to capture acetylated peptides for MS analysis. The efficiency of Bdf1 bromodomain-based enrichment was similar to that afforded by pan-acetyl antibodies. Interestingly, bromodomains are present in pairs in many proteins (five out of ten in yeast, and 10 out of 46 in human). Bryson et al. further demonstrate in their article that, when the two Bdf1 bromodomains are used together, their specificity for acetylated histones increases tenfold [1].

In conclusion, bromodomains constitute a new class of bioreagent which can be used to enrich for acetylated peptides. Future studies should explore whether new combinations of bromodomain pairs could optimize their properties for the enrichment of acetylated peptides. In addition, many research facilities are currently investigating the potential of bromodomains as therapeutic targets in various diseases [11]. This quest for specific bromodomain inhibitors has led to a detailed understanding of how their binding pocket functions. Ultimately, this knowledge might be useful in engineering synthetic bromodomains, optimized for the capture of acetylated peptides for use in acetylproteome studies.

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