A polymeric solution for enriching the phosphoproteome

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Solution polymers such as dendrimers promise to provide valuable new tools for proteomics researchers using mass spectrometry (MS) to probe protein modifications.

Over the past decade, MS has rapidly emerged as an incredibly powerful tool for proteomics studies. Yet despite their utility, MS-based methodologies do not obviate the need for traditional biochemical techniques. Instead, the sheer complexity and dynamic nature of living organisms requires the continued development of new approaches to fractionate various subsets of the overall protein complement to allow more targeted mass spectrometric analyses. In this issue of *Nature Methods*, Aebersold and coworkers describe a new enrichment strategy in which synthetic soluble polymers are used to selectively isolate their phosphorylated biological counterparts[1].

Protein phosphorylation is a reversible post-translational modification that has been implicated in such diverse processes as subcellular localization, complex formation, regulation of transcription factors and the modulation of protein lifetime[2]. As such, understanding the highly interconnected regulatory networks modulated by this post-translational modification requires nothing less than the identification and quantitation of changes in multiple sites of phosphorylation on many proteins over time. Although MS is having an ever-increasing role in meeting this challenge, many factors complicate the analysis of phosphorylation in complex mixtures by MS-based methods[3]. Thus, various sample fractionation methods are used to focus the analysis on the phosphoproteome.

Detection with antibodies remains the most widely used phosphorylation-specific enrichment technique, although it is typically limited to specific amino-acid sequences or phosphotyrosine-containing species[4]. Alternatively, immobilized metal affinity chromatography has been utilized for the large-scale enrichment of many native phosphorylated species[5]. Although this approach has yielded considerable results, its selectivity appears to be dependent on several operational parameters including the metal ion, the ligand and the stationary phase used. Finally, various chemical transformations have also been described. By far the most prevalent of these involves removal of the phosphate moiety under basic conditions followed by reaction with an appropriate affinity handle. This reaction scheme, however, is only applicable to phosphoserine- or phosphothreonine-containing species and is known to suffer from numerous side reactions[6].

Here Aebersold and coworkers describe an alternative general chemical strategy for the enrichment and subsequent mass spectrometric analysis of phosphopeptides, which represents a major advance compared to their previously reported methodology[7]. This improvement arises primarily from directly capturing phosphopeptides in a single-step reaction with a primary amine–containing solution polymer (in this case, a Generation-5 polyamidoamine dendrimer) rather than the complicated

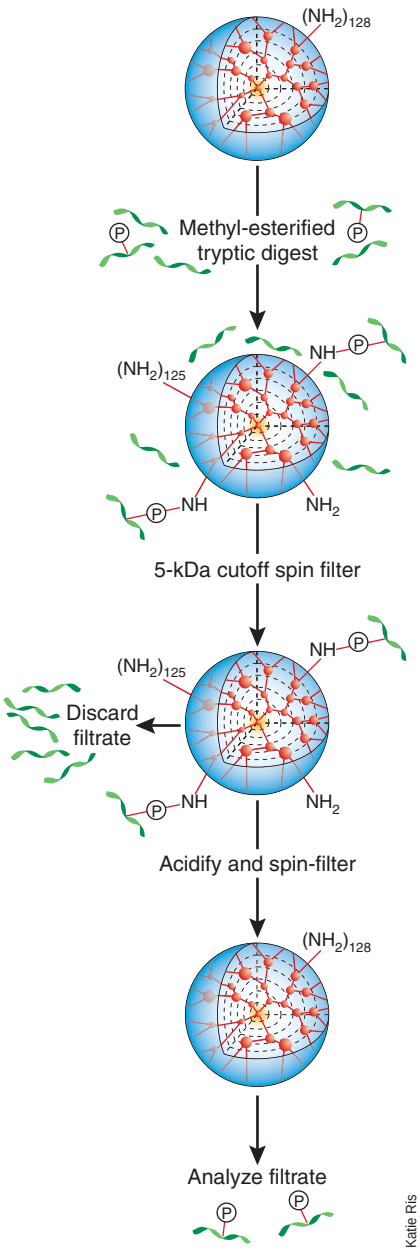

Figure 1 | Schematic of the solution polymer–based phosphopeptide enrichment procedure. Dendrimer polymers containing 128 free amino groups bind any phosphopeptides. The resulting complexes are separated from unbound peptide using a molecular-weight cutoff filter. Finally, the phosphopeptides are separated from the polymers by acidification and filtration, and the sequences and phosphorylation sites of the phosphopeptides are determined by MS.

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chemical transformations described previously. A dendrimer is a perfectly branched, unimolecular solution polymer that has functional groups only at its surface[8]. Such soluble reagents permit faster reactions than the chemically functionalized controlled pore glass beads used previously, owing to their higher capacity and the homogeneous nature of the reaction medium. Selective reaction of phosphorylated peptides with the amine groups of the dendrimer produces phosphopeptide-polymer conjugates that are physically larger than unmodified peptides. Thus, these conjugates can readily be separated from other peptides and excess reagents using a simple 5-kDa size filtration spin column. Afterward, the phosphopeptides are released from the dendrimer under acidic conditions, and isolated from the dendrimer using the same membrane-based filtration device. Notably, this chemistry is equally applicable to all classes of phosphopeptides, although in practice the relatively low abundance of phosphotyrosine-containing species still necessitates the use of phosphotyrosine antibodies.

Using this approach, the authors identified and quantitated the relative changes in over 150 sites of phosphorylation (including 75 sites of tyrosine phosphorylation) from Jurkat T cells treated with the phosphatase inhibitor pervandate for either two or ten minutes.

Given the massive complexity of the proteome, the development of new efficient fractionation techniques is always welcome, and the authors certainly present very high-quality data demonstrating the validity of their approach. The real importance of this work, however, may not necessarily be in the future application of this exact methodology to the phosphorylation profiling of other biological systems, as results of similar scope have been reported using classical phosphorylation-specific enrichment techniques.

Instead, the true value of this work is in the authors' effective adaptation of techniques from other nonbiologically oriented fields to solve a biochemical problem. Specifically, the use of solution polymers, such as dendrimers, as auxiliaries in organic reactions has a long-established history[9], but this is the first report of their effective use in a proteomics application. More importantly, the control that polymer chemists can now exert over the physical and chemical properties of these synthetic macromolecules suggests that similar enrichment schemes could readily be developed for other naturally occurring or artificially introduced protein functionalities for which no tailored affinity schemes such as immobilized metal affinity chromatography exist. By looking to the fields of polymer and organic chemistry, proteomic researchers may find new solutions for the complex biochemical problems they face[10].

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Insect transgenesis by site-specific recombination

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Targeted genomic insertion will improve the qualitative and quantitative functional comparison of similar transgenes and provide suitable integration points for transgenes of applied interest.

Transposon-based vectors have been the most suitable gene transfer systems for insect transgenesis so far. Both for basic laboratory research as well as for future applications, such as insect bioreactors to produce heterologous proteins or biotechnologically engineered insect pest control, transposable elements have been the prime choice to introduce transgenes into the genome[1]. In this issue, Small and colleagues demonstrate the efficient use of a non–transposition-based strategy to generate transgenic insects[2]. Several such site-specific recombination systems have recently been investigated to serve for transgene integration into insect genomes, which indicates the importance of this approach[3].

Transposon insertion requires usually only few specific base pairs at the target loci, which are spread randomly within the genome. Therefore transposition is undirected and the transgenes that are usually imbedded into nonautonomous transposons can end up in quite diverse chromosomal environments. As neighboring *cis*-regulatory DNA elements or the local chromatin configuration will influence the pattern or level of expression, almost each transgene insertion will experience a distinct position effect (Fig. 1a). Thus, the same transgene can be quite differently expressed, depending on its genomic landing site. For clean qualitative and quantitative comparisons between similar but distinct transgenes, however, the generation of true allelic situations is needed. This requires that the diverse transgenes be placed at the very same position in the insect genome. Homologous recombination[4], which could in principle serve for directed transgene integration, is a much too rare event to target several different genes to the same genomic position in an efficient way.

Another way to effectively direct recombination in insect genomes is by using site-specific recombinases, such as the bacteriophage P1–derived Cre ('causes recombination'), the yeast-derived Flp ('flipase') or the phage ϕC31–derived Int ('integrase')[5]. Cre- or Flp-mediated site-specific recombination at their respective target sites, *loxP* and *FRT*, are reversible processes. The recombinases should thus

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