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Commentary

Multidimensional separation prior to mass spectrometry: Getting closer to the bottom of the iceberg

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While prefractionation has previously been shown to improve results in MS analysis, a novel combination provides an additional dimension of separation: protein fractionation by SDS-PAGE followed by IEF of tryptic peptides before separation by RP-LC [Atanassov and Urlaub, *Proteomics* 2013, *13*, 2947–2955]. This three-step separation procedure prior to MS/MS substantially increases proteome coverage and represents a further step toward a more comprehensive analysis of complex proteomes.

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Undersampling of the proteome remains one of the major issues in proteomics. It is a main limitation of 2D gel-based proteomics but also of shotgun proteomics. Even though the latest mass spectrometers perform thousands of MS/MS events during a standard RP-LC run, only a relatively small percentage of the eluting peptides (usually in the order of 15–20%) are targeted for fragmentation [1]. Incomplete databases, low abundance of the precursor, PTM, nontryptic cleavages, and interference by co-eluting peptides or contaminants further hamper peptide identifications. To overcome undersampling, various prefractionation strategies have been proposed prior to the final separation of peptides by RP-LC. Increasing the number of separation steps, however, has two major drawbacks. First, it increases the MS time required to analyze the proteome of interest. Second, fractionation introduces variation in the proteomics workflow, as documented recently for several fractionation schemes [2]. Whereas reduced throughput is commonly regarded as a reasonable trade-off to increase proteome coverage, additional variability is problematic. Inevitably, quantitative accuracy is compromised and information gets lost once the technical variability exceeds the biological differences in the samples. Thus, it remains a challenge to balance sensitivity and reproducibility.

Two-dimensional fractionations (e.g. gel-LC-MS/MS: SDS-PAGE of proteins followed by RP-LC of tryptic peptides) are widely used but not satisfactory with regards to sensitivity and proteome coverage. It is thus tempting to introduce a third separation step, as recently proposed for protein-based separations [3]. In this issue, Atanassov and Urlaub [4] report that adding a third dimension, namely, IEF of tryptic peptides after SDS-PAGE separation and in-gel digestion of proteins offers improved resolution by RP-LC resulting in a substantial increase in proteome coverage with comparably little technical variability. Increasing the LC gradient time in the gel-LC-MS/MS experiment

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resulted in fewer new protein identifications than adding an extra dimension by IEF. It appears that with the method proposed by Atanassov and Urlaub [4], the separation of peptides by IEF has improved peptide resolution to such an extent that the mass spectrometer samples has more low abundant peptides compared to the conventional gel-LC-MS/MS approach. Peptides belonging to the same protein are not just contained within neighboring gel slices but are spread out across the entire experiment according to their p*I*s. This could also be advantageous for PTMs by allowing a better separation of modified peptides from their nonmodified counterparts.

In summary, the 3D workflow for shotgun proteomics established by Atanassov and Urlaub may take proteomics a step closer to the "bottom of the iceberg" of the cellular proteome.

The authors have declared no conflict of interest.

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