Spin filter–based sample preparation for shotgun proteomics

To the Editor: Wiśniewski *et al.* recently reported a sample preparation method for proteome analysis using spin filter microcentrifugation devices[1]. The procedure described is almost identical to a method we reported in 2005 (ref. 2). In our paper, we described the use of spin filters to remove sodium dodecyl sulfate (SDS) and other contaminants, followed by the reduction, alkylation and tryptic digestion of proteins on the filter and finally the isolation of peptides by centrifugation. We described the application of the spin filter preparation method to purified proteins, protein mixtures, cell lysates and subcellular fractions, which are the major elements of the method described by Wiśniewski *et al.*[1]. Our spin filter method already has seen considerable use: we are aware of at least 18 publications in which it was applied (**Supplementary Note**).

These publications show that this approach is useful in some applications, but is not necessarily "universal" as Wiśniewski *et al.*[1] suggest. We and others have found that the use of spin filters has considerable limitations because of poor peptide recoveries when relatively small (<50 µg) protein samples are analyzed. Even at higher sample loads, digestion efficiencies and peptide recoveries are variable[3]. In our previous work with detergent-solubilized membrane vesicles, the spin filter preparation did not yield protein identifications, apparently owing to the difficulty of removing detergent (1% CHAPS) that interfered with protein digestion. In that work, we used a 'short' SDS–polyacrylamide gel electrophoresis (SDS-PAGE) separation ~1–2 cm into the gel, followed by in-gel tryptic digestion and multidimensional liquid chromatography–tandem mass spectrometry (LC-MS/MS) to identify several dozen vesicle-associated proteins[4]. To analyze cell and tissue proteomes, we also have used the in-solution digestion method of Wang *et al.*[5], which uses trifluoroethanol (TFE) instead of detergent to solubilize hydrophobic and membrane proteins.

We compared the performance of the spin filter method (performed as described by Wiśniewski *et al.*[1]) with that of the short SDS-PAGE and TFE methods for analysis of proteins from RKO colon carcinoma cells (**Table 1** and **Supplementary Data**). We analyzed samples corresponding to a high protein load (50 µg) and a low protein load (150 ng). Then we analyzed all digests under identical conditions by reverse phase LC-MS/MS (**Supplementary Methods**). At the high protein load, the spin filter preparation yielded 8% more protein identifications than the gel and TFE methods. However, at the low protein load, the spin filter method yielded just 44% of the protein identifications found with the gel method and only 31% of the identifications found with the TFE method.

Table 1 | Comparison of spin filter, short SDS-PAGE and TFE methods

Method	Protein load	Peptide identifications	Protein identifications
Spin filter		5,369	642
Short SDS-PAGE	50 µg	4,176	593
TFE		4,663	593
Spin filter		86	46
Short SDS-PAGE	150 ng	298	106
TFE		626	150

Samples of human RKO colon carcinoma cells containing the indicated amounts of protein were prepared in triplicate by the indicated methods and analyzed by reverse phase LC-MS/MS. Peptide identifications are total MS/MS spectrum-to-sequence database matches at 5% false discovery rate; protein identifications are nonredundant identifications with at least two identified peptides and parsimonious protein assembly. Reported values are the means of three technical replicate analyses.

Thus we conclude that spin filter-based approaches are subject to substantial losses of identifications at low sample loads, probably owing to binding of proteins and peptides to the spin filters. We note that Wiśniewski *et al.*[1] only analyzed complex cell proteomes with their spin filter method. However, nonspecific binding and protein or peptide losses would make this method a poor choice for the analysis of less complex samples (for example, multiprotein complex pull-downs), which often represent very small protein loads. The short SDS-PAGE approach is much better suited to such samples.

Note: Supplementary information is available on the Nature Methods website.

ACKNOWLEDGMENTS

This work was supported in part by the National Cancer Institute Clinical Proteomic Technologies Assessment for Cancer program grant 1U24CA126479. We thank K. Cheek for technical assistance and R. Slebos for valuable comments.

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Wiśniewski & Mann reply: We welcome the correspondence by Liebler and Ham[1] because it gives us the opportunity to correct an embarrassing oversight in our Brief Communication describing the filter-aided sample preparation (FASP) method published earlier this year[2]. The method by Manza *et al.*[3] indeed has similarities to our protocol and had we been aware of it, we would certainly have cited it. Unfortunately, neither we nor the reviewers, nor the many people that have already used our protocol for a year were aware of the paper. More importantly, there are fundamental differences between the methods. Both perform digestion in a 'chemical reactor' (in this case a spin column) as do many other protocols in proteomics (for example, ref. 4). However, we completely eliminated sodium dodecyl sulfate (SDS) and other detergents by urea exchange, which we had previously introduced for complete sample solubilization[5]. This was the main advance of our protocol, which