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Research Article

# High MS-compatibility of silver nitrate-stained protein spots from 2-DE gels using ZipPlates and AnchorChips for successful protein identification

The availability of easy-to-handle, sensitive, and cost-effective protein staining protocols for 2-DE, in conjunction with a high compatibility for subsequent MS analysis, is still a prerequisite for successful proteome research. In this article we describe a quick and easy-to-use methodological protocol based on sensitive, homogeneous, and MS-compatible silver nitrate protein staining, in combination with an in-gel digestion, employing the Millipore 96-well ZipPlate system for peptide preparation. The improved quality and MS compatibility of the generated protein digests, as compared to the otherwise weakly MS-compatible silver nitrate staining, were evaluated on real tissue samples by analyzing 192 Coomassie-stained protein spots against their counterparts from a silver-stained 2-DE gel. Furthermore, the applicability of the experimental setup was evaluated and demonstrated by the analysis of a large-scale MALDI-TOF MS experiment, in which we analyzed an additional ~1000 protein spots from 2-DE gels from mouse liver and mouse brain tissue.

**Keywords:**
2-DE / In-gel digestion / MALDI-TOF MS / Protein staining / Proteomics
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## 1 Introduction

2-DE, in combination with sensitive silver nitrate-based protein staining protocols, has the capacity to separate and visualize complex protein populations of up to 10 000 protein spots in a single experiment [1]. MALDI-TOF MS allows the fast, reliable, and sensitive identification of large numbers of isolated proteins [2]. As a consequence, the combination of 2-DE and MALDI-TOF MS has proven to be a powerful combination for the analysis of complex protein samples from different organisms in recent years (610 publications in the NCBI PubMed literature database for the combination of the keywords 2-DE and MALDI until April 2007).

As a prerequisite to efficiently perform 2-DE-based proteome analysis, the employed protein stain has to yield high detection sensitivity, possess a large dynamic range, and have a good reproducibility between independently processed gels [3]. Hence, the choice of the appropriate protein stain has a major influence not only on the image analysis of the 2-DE gels, but also on the outcome of the subsequent MS analysis. Unfortunately, the requirements for successful mass spectrometric protein identification often conflict with the requirements for 2-DE image analysis and spot detection. For example, many sensitive protein staining protocols, such as silver nitrate stains [4], show low MS compatibility [5, 6], while more MS-suited protein staining protocols, like the Coomassie protein dyes [7], often lack detection sensitivity [3]. To overcome some of these problems, improved staining protocols have been developed, such as MS-compatible silver nitrate stains [8, 9] or colloidal Coomassie stains with enhanced sensitivity [10]. However, these improvements still suffer from most of the previously described problems. Recently, other highly MS-compatible protein stains like zinc-imidazole stains [11] and also new fluorescent stains [3] were introduced into proteome analysis. Both techniques show good detection sensitivity, but the post-staining handling of protein spots from these 2-DE gels is much more difficult because it requires sophisticated, and in the case of the fluorescent dyes, expensive scanners and robotics for the processing of the protein samples. These tools are often not readily available in standard laboratories [12]. Additionally, the basic costs for the fluorescent dyes are still several folds higher than the ones for the classical light absorption-based dyes [13].

In the present publication we assembled methods and protocols that allow circumventing many of the above-mentioned sensitivity and MS-compatibility related problems for

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**Abbreviations: MTP,** microtiter plate; **RT,** room temperature

2-DE-based proteome analysis. For this purpose we made use of a reproducible and sensitive MS-compatible silver nitrate-based protein staining protocol for the staining of 2-DE gels. The stained protein spots were then excised from the gels and digested, without any destaining steps, by a simple tryptic in-gel digestion protocol, employing 96-well $C_{18}$ ZipPlates. The digested peptides were then collected in chemically inert, low affinity binding Mulitichem microtiter plates (MTPs, Whatman, Brentford, UK), before they were finally analyzed in a Bruker Reflex IV MALDI-TOF MS (Bruker Daltonics, Bremen, Germany) on AnchorChip targets employing 2,5-dihydroxy benzoic acid (DHB) as a MALDI matrix. The combination of these robust and easy to handle methods was used, in an initial proof of concept experiment, to identify a set of about 1000 protein spots from 2-DE gels obtained from mouse brain and liver samples.

## 2 Materials and methods

### 2.1 Tissue protein extraction, 2-DE gel separation, and image analysis

Cytosolic protein extracts from mouse brain and mouse liver tissue (*Mus musculus*, strain C57BL/6 (B6)) were prepared and extracted as described previously [14, 15]. The proteins were separated by the large-gel 2-DE system, employing the carrier ampholyte technique for the IEF (first dimension) [1, 16]. IEF of extracts for analytical gels was performed loading 8 μL of protein extract (~8 μg/μL protein concentration), on $0.9 \times 400$ mm IEF-gels, while preparative IEF-gels ($1.5 \times 400$ mm), were loaded with 40 μL of the same protein extract. IEF-gels were cut by half after the separation. Each half gel was loaded onto a $233 \times 0.75 \times 300$ mm (analytical) or a $233 \times 1 \times 300$ mm (preparative) SDS PAGE gel, respectively.

The resulting 2-DE gels were stained according to the corresponding staining protocols (see Section 2.2 and 2.3), before digitizing them with a TMA 1600 Scanner (Mictrotek, Umax Systems, Willich, Germany). The scanner was set to 300 dpi and 16 bit color scale. Image analysis was performed using the ProteomWeaver software, version 3.2.0.5 Beta (BioRad, Hercules, CA, USA), as described previously [17].

### 2.2 MS-compatible silver nitrate staining

Proteins were detected by silver nitrate staining using a modified protocol of Heukeshoven *et al.* [4]. The gels were incubated for 2 h in a fixing solution containing 50% v/v ethanol and 10% v/v acetic acid (Merck, Darmstadt, Germany). Afterwards they were rinsed for 10 min in 20% v/v ethanol, before they were incubated for 1 min in a 0.02% w/v sodium thiosulfate solution (Sigma, St. Louis, MO, USA). This step was followed by a $2 \times 1$ min rinsing of the gels in water. The gels were then incubated in 0.1% w/v silver nitrate (Merck) for 30 min. After this step the gels were rinsed once in water for 30 s and once with 2.5% w/v sodium carbonate (Merck) for 1 min. The stain was finally developed in a 2.5% w/v sodium carbonate, 0.02% v/v formaldehyde solution (Merck), 0.025% w/v thimerosal (Sigma), for 2–10 min. Stain development was stopped with an aqueous solution containing 18.5 g/L Titriplex (Merck) for 20 min. Afterwards, the gels can be stored in water until spot excision. For longer storage periods, the gels can be sealed between polyethylenglycol sheets (VWR International, Darmstadt, Germany) and stored at 4°C.

### 2.3 Colloidal Coomassie G250 staining (G250)

The Coomassie G250 staining was performed according to a modified protocol by Doherty *et al.* [18]. Protein fixation was performed by incubating the gels in a 50% v/v methanol and 2% v/v phosphoric acid solution for a minimum of 2 h. The gels were then rinsed for 20 min in water, before they were equilibrated for 1 h in a solution containing 30% v/v methanol, 2% v/v phosphoric acid, and 17% w/v ammonium sulfate (Merck). 660 mg/L Coomassie G250 (BioRad) will then be added and the staining proceeded for 1–5 days under constant shaking. Finally the gels can be stored in water, or for longer storage periods the gels can be sealed between polyethylenglycol sheets and stored at 4°C before spot excision.

### 2.4 Protein spot excision and tryptic in-gel digest using ZipPlates

Gel pieces of 2 mm diameter were excised from MS-compatible silver nitrate- and Coomassie-stained 2-DE gels by using the GelPal manual spot excision unit (Genetix, New Milton, UK). The tryptic in-gel digestion and desalting steps were performed using 96-well ZipPlates (Millipore, Bedford, MA, USA) according to the instructions of the manufacturer, except that destaining and reduction/alkylation steps were omitted.

In brief, gel pieces were transferred to 96-well ZipPlates and washed by adding 100 μL of 5% v/v ACN (Merck) in 25 mM $NH_4CO_3$ (pH 8) (Sigma). After a 15 min incubation at room temperature (RT) the supernatant was removed using a vacuum manifold system (Millipore). Gel pieces were further washed by adding 100 μL of 50% v/v AcN in 25 mM $NH_4CO_3$ (pH 8). The incubation lasted for 15 min at RT. The supernatant was removed and this washing step was repeated once. Gel pieces were then dehydrated by a 10 min RT incubation of the gel pieces in 200 μL of 100% AcN. After removing the supernatant, the gel pieces were rehydrated in 10 μL 50 mM $NH_4CO_3$ (pH 8), containing 10 ng/μL sequencing-grade modified porcine trypsin (Promega WI, USA). The digestion was performed for 3 h at 37°C. After 30 min incubation another 5 μL, preheated (37°C), 50 mM $NH_4CO_3$ (pH 8) was added to each digest. Peptides were extracted by adding 8 μL 100% AcN. These gel pieces were incubated for 15 min at 37°C, before 130 μL of 0.2% TFA

 

(Merck) was added. The gel pieces were incubated for additional 30 min at RT, before they were concentrated onto the $C_{18}$ material at the bottom of the 96-well plates. Subsequently, the enriched peptides, bound to the $C_{18}$ material, were washed and desalted twice with 100 μL of 0.2% TFA. In a final step the peptides were eluted with 15.5 μL of 50% v/v AcN in 0.1% v/v TFA and collected in chemically inert 96-well Multichem MTPs. These peptide extracts could be stored at −20°C, after they have been sealed with cap mats (Whatman).

### 2.5 MALDI-TOF MS analysis and database searches

1.5 μL of peptide extract was mixed with 1.5 μL of matrix solution (3.3 g/L 2,5-dihydroxybenzoic acid in one part of AcN and two parts of 0.1% TFA) directly on the 800 μm AnchorChip MALDI target. Mass spectra from peptide mixtures were generated using a Bruker Reflex IV MALDI-TOF mass spectrometer operated in reflector mode. Signals corresponding to mass-to-charge ($m/z$) ranging from 0 to 3500 were monitored. The XMASS/NT 5.1.16 software package (Bruker Daltonics, Bremen, Germany) was used for data processing. Internal calibration was performed with mass peaks 842,509 $[M + H]^+$ and 2211, 104 $[M + H]^+$, which were derived from autoproteolytic trypsin digestion. Peak picking was performed automatically, using the following settings: Mass range 800 to 3500 Da, maximal 200 peaks *per* sample, SNAP algorithm and Peak sensitivity at least 3. Masses from an exclusion list, containing known background peaks and trypsin specific autoproteolytic peptide masses, were deleted from the generated mass lists automatically. For database searches these mass lists were searched against the NCBInr protein databases (National Center for Biotechnology Information, Bethesda, USA) using Mascot Daemon 2.1.0 (Matrix Science, London, UK). Search parameters allowed for one missed cleavage site. Peptide mass tolerance was set to 100 ppm and Methionine oxidation and acrylamide derived Cysteine alkylation (cysteinyl-*S*-propionamide) were considered as possible modifications. Searches were restricted taxonomically to *Mus musculus*. Proteins were evaluated by considering the Mascot MOWSE Score ($p$-value $> 0.05$), the number of matched peptides, and the percentage coverage of protein sequence.

## 3 Results and discussion

### 3.1 Comparison of silver nitrate- and Coomassie-stained 2-DE gels

We performed a direct comparison of mouse brain tissue derived 2-DE gels under three staining conditions: (i) stained with a sensitive, glutardialdehyde-based, non-MS-compatible silver nitrate stain (analytical silver), (ii) a modified silver nitrate staining protocol, omitting the cross-linking glutardialdehyde sensitizing step to make this stain MS-compatible (preparative silver), and (iii) an MS-compatible colloidal Coomassie G250 stain (preparative Coomassie). Since the analytical silver stain is known to be more sensitive than the employed preparative stains, we tried to adjust for this difference by increasing the amount of protein loaded on the preparative 2-DE gels. For this purpose the analytical gel (0.9 mm diameter for the 1-D gel and 0.75 mm diameter for the 2-D gel) was loaded with ~64 μg total protein (8 μL of protein extract), while the two preparative 2-DE gels (1.5 mm diameter for the 1-D gel and 1 mm diameter for the 2-D gel) were loaded with ~320 μg total protein (40 μL of protein extract).

Two common problems, often discussed for preparative silver nitrate-stained 2-DE gels, namely the appearance of a strong background in the low-molecular-weight area of the gels, which often results from insufficient removal of comigrating carrier ampholytes, and the appearance of inhomogeneously stained, chromatic protein spots [12], were barely observable in our 2-DE gels (Fig. 1B). Both forementioned problems, which would disturb the efficient and accurate detection and quantification of protein spots by image analysis software, were therefore foreclosed from our preparative silver gels, allowing us to perform automated, software-driven spot detection and analysis.

As expected, the analytical silver stain provided the highest detection sensitivity, with ~2321 detected protein spots on the acidic half of the 2-DE gels. Interestingly, the preparative silver nitrate stain showed a quite comparable spot detection sensitivity, with a total of ~2059 detected protein spots (88%). This demonstrated that the loading difference of 5 times more protein on the preparative gel could almost compensate for the differences in the detection sensitivity between these two silver staining protocols. Nevertheless, based on the identical staining mechanism of these two stains, a very good comparability of the detected spot patterns was achieved. This is visualized in the magnified region from the 2-DE gels, where most of the 121 detected protein spots from the analytical gel can also be detected among the 110 detected protein spots in the preparative silver gel (Figs. 1A, B). In contrast to this, the Coomassie-stained gels provided much lower detection sensitivity. For this stain a total number of ~1545 protein spots were detected on the acidic half of the gel (66% of the spots detected on the analytical gel; 75% of spots detected on the preparative silver gel), even though an amount of 5 times more protein, compared to the analytical 2-DE gel, had been loaded. The magnified areas from the 2-DE gels underline the insufficient sensitivity of Coomassie staining, with the silver-stained gels (Figs. 1A–C). Only 63 of the 121 detected protein spots (52%) from this region of the analytical silver gel could be found in the coomassie gel.

This quantitative reduction in protein spot detection provides an essential problem for successful 2-DE-based proteome projects, not only if the total number of displayed protein spots determines the efficiency of the experiment, but also for the more often used differential display-based 2-DE analysis, where comparisons of, *i.e.*, different environmental conditions or various developmental stages are dis-

 

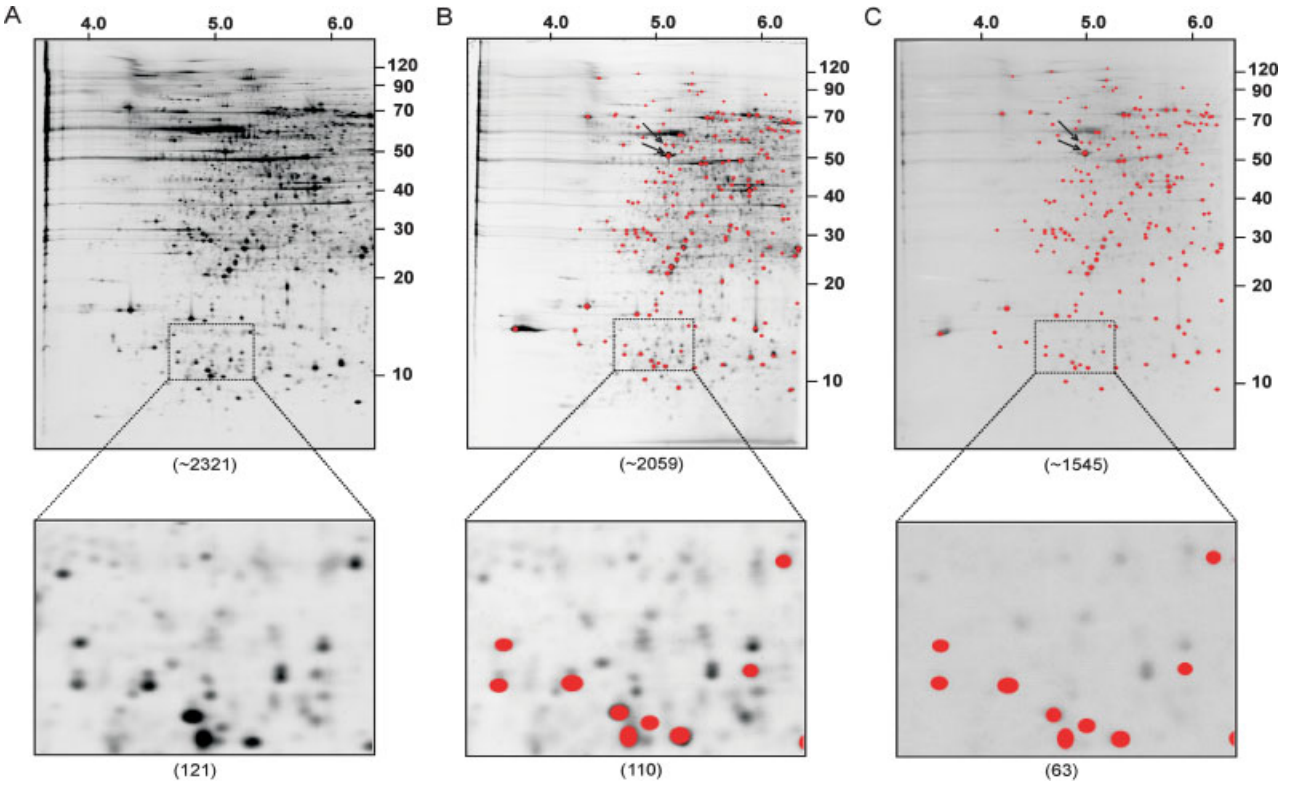


**Figure 1.** Comparative analysis of differentially stained 2-DE gels from cytosolic protein extracts of mouse brain tissue. Red marked protein spots were analyzed by MALDI MS. (A) Acid side of an analytical silver nitrate-stained 2-DE gel, loaded with ~64 μg total protein. (B) Acid side of a preparative, MS-compatible silver nitrate-stained 2-DE gel loaded with ~320 μg of total protein. (C) Acid side of a preparative, Coomassie-stained 2-DE gel, loaded as (B). The isoelectric point is indicated above the gels horizontally, while the molecular mass is indicated left to each gel in kDa. Numbers in brackets below the gels indicate the number of detected protein spots. The arrows in (B) and (C) indicate the selected protein spots for which the MALDI MS spectra are shown in Fig. 3.

played and analyzed. In such an experiment the image analysis of the gels is performed on multiple analytical 2-DE gels, which are usually prepared from a low amount of protein sample (often a limiting factor in proteomic research). In a second step one or few preparative gels, which require higher protein loads, are prepared for the final MS analysis. If the preparative stain would not display the differential protein spots, as it would be the case for ~40–50% of the spots in the comparison between the analytical silver and the preparative Coomassie gels, the whole experiment would fail. This leads to the conclusion that the preparative silver stain offers a large advantage over the classical coomassie stain.

### 3.2 Evaluation of the MS compatibility of silver nitrate-stained protein

Because we have shown that the preparative silver stain, at least based on its visual properties, was superior to the coomassie stain, we evaluated the MS compatibility of this staining protocol. Since we already know from previously performed experiments that the MS spectra, derived from preparative silver-stained protein spots were of lower quality as compared to MS spectra derived from coomassie-stained protein spots [19, 20], we decided to developed an improved strategy for the analysis of these silver-stained samples. Supported by observations of Mortz *et al.* [21], who showed that the desalting and concentration of tryptic digests derived from silver-stained BSA improved the quality of the MS spectra, we made use of a similar approach. Our approach was based on the use of Millipore $C_{18}$ ZipPlates for the tryptic protein digest. These 96-well ZipPlates could be used for all the steps performed in the sample digestion, starting from the spot picking and ending with the sample desalting and concentration. No sample transfer was necessary, since all the needed buffers and chemicals could be simply added by pipetting, while the waste was removed by evacuation or simple centrifugation.

Interestingly, during the evaluation of different digestion protocols, it was possible to optimize and shorten the whole sample preparation, omitting even the silver destaining step. This step, which is frequently employed to improve the MS compatibility for silver-stained proteins, is based on the reoxidation of the silver with either hydrogen peroxide [22] or farmers reagent [23] and is followed by extensive washing steps. Furthermore, we could remove the time consuming reduction-alkylation step from the digestion protocol, since

 

this treatment did not significantly increase the quality of the obtained MS spectra (data not shown). Nevertheless, all these steps could optionally be reintroduced into the digestion scheme, if needed. The whole procedure, which is described in Section 2, ends with the sample collection in specific, low affinity binding, chemically inert, 96-well Multichem MTPs. The MALDI sample preparation was then performed employing AnchorChip MALDI targets with DHB as the MALDI matrix, which showed the best performance in our hands. We also tested α-cyano-(3,4-dihydroxy) cinnamic acid (data not shown), which might allow easier automation of the matrix and sample application to the target [24].

After having set up these protocols, we performed a MALDI-MS-based qualitative comparison of preparative silver- and Coomassie-stained protein spots from mouse brain 2-DE gels. For this purpose 192 identical protein spots from the acidic sides of a Coomassie and a preparative silver-stained 2-DE gel were excised, digested, PMF maps were recorded, and database searches were performed. As can be seen from the marked dots in Figs. 1B and C, the excised spots were distributed over the entire area of these 2-DE gels, providing a representative mixture of different protein samples. We reasoned that this kind of analysis, also limited by the detection sensitivity of the Coomassie stain, would provide a significant advantage over staining comparisons of a limited number of protein standards separated by 1-D SDS-PAGE.

The number of proteins identified from the preparative silver-stained gels exceeded the number of spots identified from the Coomassie-stained gel. We identified 170 protein spots in the silver-stained gel (success rate 88.5%), and 153 identified spots (success rate 79.7%) in the Coomassie-stained gel. This was quite unexpected, since Coomassie is known to show an excellent MS compatibility. An explanation for this observation might be related to the fact that the silver-stained proteins benefit more from the ZipPlate cleanup than the Coomassie proteins. A similar phenomenon was observed for SYPRO Ruby-stained protein spots, which showed greater improvement upon the use of ZipTip cleanup than comparable colloidal Coomassie blue-stained proteins [25].

Further analysis of the generated data showed that both stains resulted in an overlap of 149 protein spots identically identified from the analyzed 2-DE gels. This result demonstrated the reliability of the applied protein identification methods, and further confirmed the accurate picking of the selected protein spots. A detailed comparison of the MS spectra from the preparative silver-stained gels not only confirmed the excellent protein identification rates, but additionally it also showed that high quality mass spectra, similar to the quality of the Coomassie-derived spectra, were obtained from all the silver-stained protein spots. Figure 2 gives a summary of the parameters underlying the protein identifications: the number of peptides *per* identified protein, the sequence coverage, and the MASCOT MOWSE score which were sorted according to the molecular weight of the identified proteins. As can be seen, the silver stain matches or even exceeds all of the benchmarks in the three categories set by the Coomassie stain.

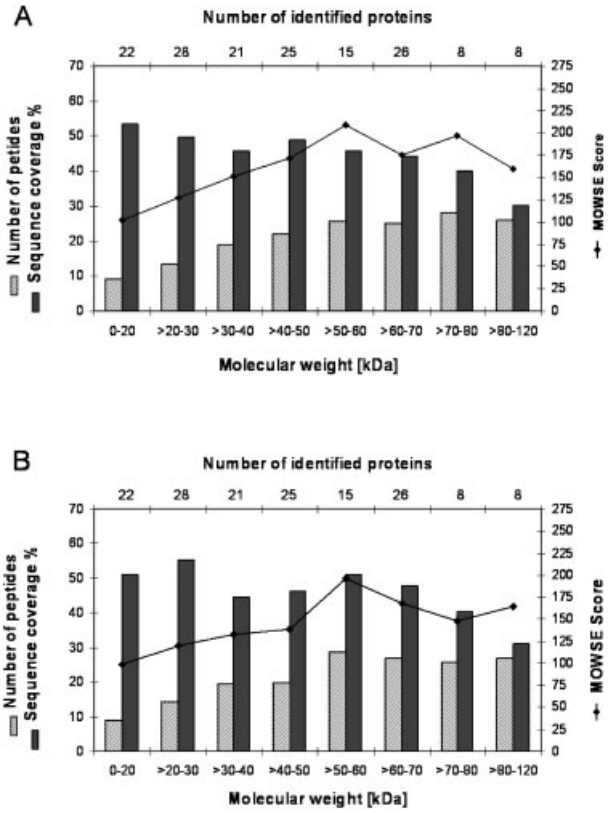


**Figure 2.** Comparative representation of different quality indicators (number of matched peptides, sequence coverage and MOWSE score) for 149 identical identified tryptic digests of protein spots from MS-compatible silver nitrate-stained (A) and Coomassie-stained (B) 2-DE gels. The parameters are grouped according to the different mass ranges indicated below each bar duplet.

To illustrate the high quality of the silver stain-derived spectra and to show the comparability of the spectra to the Coomassie-derived spectra in further detail, we displayed the spectra from two representative protein spots in Figs. 3a–d and summarized the obtained criteria in Table 1. Again, the achieved sequence coverage, the number of matching peptides, and the resulting MOWSE score were at least as good as the values observed for the Coomassie-stained spots. In this comparison we also introduced another measure, namely the intensity coverage, which is a measure for the percentage of matched peaks and peak intensities from the whole set of detected peaks in a mass spectrum. This value therefore not only provides an indicator for the amount of nonmatching peaks, but also for the number of "big" peaks assigned to the peak list of the identified protein. As can be seen from the asterisks in the spectra (Figs. 3a–d) and the values provided in Table 1, the preparative silver stain in combination with our digestion and sample preparation protocol showed very good results with up to 90% of the intensity coverage. Interestingly, the results of the intensity coverage uncovered another phenomenon that could be

 

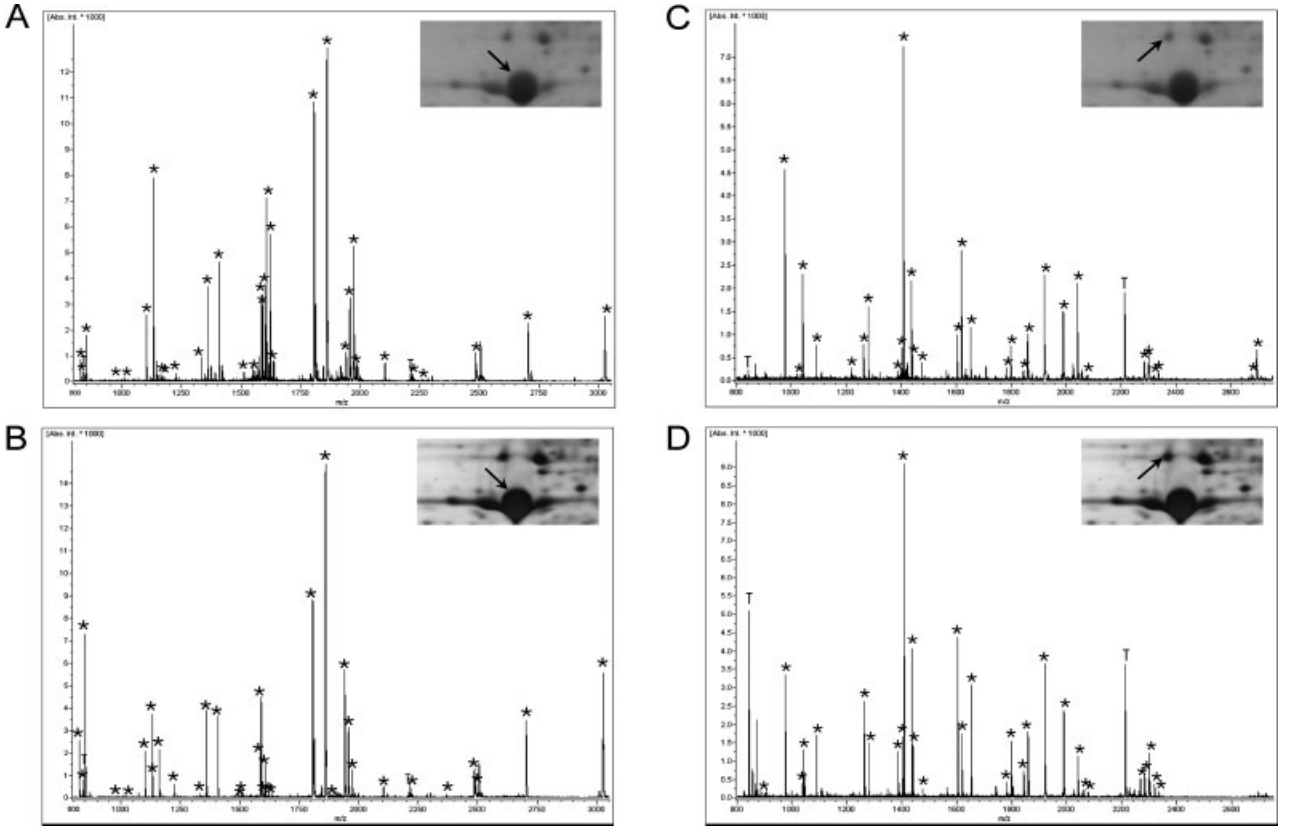


**Figure 3.** Representative MALDI mass spectra of the tryptic digested protein spots indicated by an arrow in Fig. 1 and shown in the upper right corner of each spectrum. (B and D) MS-compatible silver nitrate-stained protein spots. (A and C) Coomassie-stained protein spots. (A and B) Protein spots identified as enolase 2, gamma. (C and D) Protein spots identified as ATP synthase, $H^+$ transporting mitochondrial F1 complex, beta subunit. Asterisk-labeled peaks match to the protein sequences. Peaks derived from autoproteolysis of trypsin are labeled with (T).

**Table 1.** Comparison of in-gel digestion efficiency of proteins spots from MS-compatible silver nitrate- and Coomassie-stained 2-DE gels

| | MOWSE score | Peptides match/total | Sequence coverage (%) | Intensity coverage (%) |
|---|---|---|---|---|
| A[a)] | 189 | 33/108 | 70 | 73 |
| B[b)] | 277 | 36/83 | 78 | 87 |
| C[a)] | 187 | 30/63 | 53 | 85 |
| D[b)] | 181 | 30/61 | 48 | 91 |

(A, B) enolase 2, gamma.
(C, D) ATP synthase $H^+$ transporting mitochondrial F1 complex, beta subunit.
a) Silver nitrate-stained protein spot.
b) Coomassie-stained protein spot.

observed within the comparison of the spectra from both stains. In our analysis, Coomassie mass spectra generally contained a higher number of mass peaks, without increasing the number of matching peptides or increasing the sequence coverage. This effect might be related to the fact that our digestion protocol avoids long destaining of the silver-stained gel slices and hence the Coomassie spots were still slightly blue during the digestion process, which could be the cause for the increased number of dye-related background peaks and therefore for the lower values for the intensity coverage.

### 3.3 Large-scale analysis of silver nitrate-stained protein spots from mouse brain and mouse liver tissue

As a proof of concept experiment, to validate the results obtained from the staining comparison of the Coomassie and the preparative silver staining, we performed a proteome analysis using the developed methods and protocols. For this purpose, we excised 384 protein spots from a mouse brain 2-DE gel (loaded with ~320 μg total protein) and 602 protein spots from a mouse liver 2-DE gel (loaded with ~870 μg total protein). These ~1000 silver-stained protein spots, which were distributed over the whole 2-DE gels, not only contained proteins of different molecular weights and isoelectric points, but they also represented proteins of highly diverse

 

abundance. The latter point was of some importance, since the silver staining, with its superior detection sensitivity, enabled the MS analysis of protein spots, previously not analyzed by our Coomassie-stained 2-DE gels. Therefore it was interesting to see if either the identification success rate or the quality of the spectra would drop for these protein spots of lower abundance.

The processing of this large number of protein spots was performed in a total of 11 96-well ZipPlates. One person, using a multichannel pipette, could comfortably handle up to four ZipPlates *per* analysis. The resulting peptide mixtures resulting from these four plates were then spotted on one 384-anchor-containing MALDI AnchorChip targets and analyzed. The MS data derived from the analyzed spots are summarized in Figs. 4A and B. As can be seen from these figures, the data confirmed the previously generated results from the Coomassie-silver comparison with no decrease in quality or success rate. We could identify a total of 851 protein spots from the two 2-DE gels (358 protein spots from the brain 2-DE gel, success rate 93%; and 493 from the liver 2-DE gels, 82% success rate). Not only was the absolute success rate good, but also the MOWSE score, the number of matching peptides, and the reached sequence coverage values were also high, exceeding even results from previously performed proteome analyses, using Coomassie-stained 2-DE gels [19, 20]. We observed, as summarized in Table 2, that three-fourth of all the spectra that led to protein identification had a MOWSE score above 100, and one-fourth of all the identified MS spectra even showed a MOWSE score above 200. Further, we found that approximately 50% of all the spectra had 20 or more matching peptides, providing a sequence coverage of 50% or higher for more than half of the identified protein spots. Detection of such a large number of matching peptides in combination with the broad sequence coverage, may enable us, using a further analytical step, to determine some of the post-translational protein modifications of the analyzed protein spots, adding another layer of information to the provided proteome data.

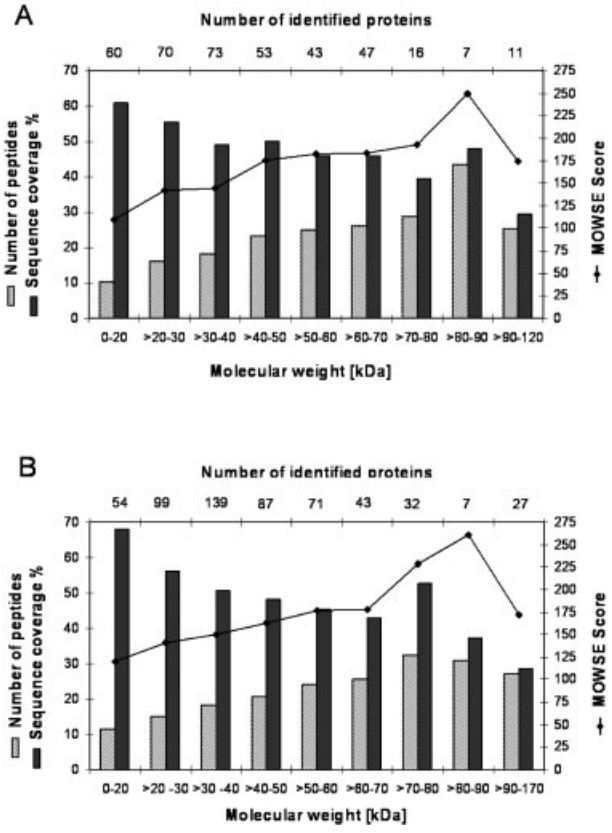


**Figure 4.** Statistical representation of quality indicators of MALDI MS identified proteins from silver nitrate-stained mouse brain and mouse liver tissue 2-DE gels. The figures summarize the number of matching peptides, the sequence coverage and the MOWSE score of the identified proteins. (A) Summary of data derived from 358 identified mouse brain protein spots. (B) Summary of data derived from 493 identified mouse liver protein spots. The parameters are grouped according to the different mass ranges indicated below each bar duplet.

**Table 2.** MS results of the identified 358 mouse liver and 493 mouse brain tissue protein spots

| | MOWSE Score (%) | | Peptides matched (%) | Sequence coverage (%) |
|---|---|---|---|---|
| | 100–200 | >200 | ≥20 | ≥50 |
| Liver | 45 | 27 | 45 | 54 |
| Brain | 54 | 23 | 53 | 53 |

## 4 Concluding remarks

In this article, we provided data that demonstrate that the combination of our preparative silver nitrate staining protocol, in combination with a ZipPlate- and AnchorChip-based sample preparation, provides an easy to handle set of methods enabling high-throughput analysis of 2-DE-derived protein spots. These protocols combine the advantages of improved protein detection sensitivity, provided by silver nitrate staining, with high-quality mass spectra. These two qualities enable not only efficient differential display proteome analysis, but also high-throughput protein identification with excellent identification success rates. Due to the fact that the whole procedure is based on silver nitrate, a light absorption-based detection method, no sophisticated or expensive protein detection and handling devices are required and all the analytical steps can therefore be performed in standard laboratories.

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## 5 References

[1] Klose, J., Kobalz, U., *Electrophoresis* 1995, *16*, 1034–1059.

[2] Aebersold, R., Mann, M., *Nature* 2003, *422*, 198–207.

[3] Patton, W. F., *J. Chromatogr. B* 2002, *771*, 3–31.

[4] Heukeshoven, J., Dernick, R., *Electrophoresis* 1988, *9*, 28–32.

[5] Scheler, C., Lamer, S., Pan, Z., Li, X. P., Salnikow, J., Jungblut, *Electrophoresis* 1998, *19*, 918–927.

[6] Gevaert, K., Vandekerckhove, J., *Electrophoresis* 2000, *21*, 1145–1154.

[7] Neuhoff, V., Arold, N., Taube, D., Ehrhardt, W., *Electrophoresis* 1988, *9*, 255–262.

[8] Blum, H., Beier, H., Gross, H. J., *Electrophoresis* 1987, *8*, 93–99.

[9] Shevchenko, A., Wilm, M., Vorm, O., Mann, M., *Anal. Chem.* 1996, *68*, 850–858.

[10] Candiano, G., Bruschi, M., Musante, L., Santucci, L. *et al.*, *Electrophoresis* 2004, *25*, 1327–33.

[11] Hardy, E., Castellanos-Serra, L. R., *Anal. Biochem.* 2004, *328*, 1–13.

[12] Chevallet, M., Diemer, H., Luche, S., van Dorsselaer, A. *et al.*, *Proteomics* 2006, *6*, 2350–4.

[13] Rabilloud, T., Strub, J. M., Luche, S., van Dorsselaer, A., Lunardi, J., *Proteomics* 2001, *1*, 699–704.

[14] Klose, J., in: Link, A. J. (Ed.), *2-D Proteome Analysis Protocols*, Humana Press, Totowa, NJ 1999, pp. 67–85.

[15] Klose, J., Nock, C., Herrmann, M., Stuhler, K. *et al.*, *Nat. Genet.* 2002, *30*, 385–393.

[16] Klose, J., in: Link, A. J. (Ed.), *2-D Proteome Analysis Protocols*, Humana Press, Totowa, NJ 1999, pp. 147–172.

[17] Weingarten, P., Lutter, P., Wattenberg, A., Blueggel, M. *et al.*, *Methods Mol. Med.* 2005, *109*, 155–74.

[18] Doherty, N. S., Littman, B. H., Reilly, K., Swindell, A. C. *et al.*, *Electrophoresis* 1998, *19*, 355–363.

[19] Giavalisco, P., Nordhoff, E., Kreitler, T., Kloppel, K. D. *et al.*, *Proteomics* 2005, *5*, 1902–1913.

[20] Giavalisco, P., Kapitza, K., Kolasa, A., Buhtz, A., Kehr, J., *Proteomics* 2006, *6*, 896–909.

[21] Mortz, E., Krogh, T. N., Vorum, H., Görg, A., *Proteomics* 2001, *1*, 1359–1363.

[22] Sumner, L. W., Wolf-Sumner, B., White, S. P., Asirvatham, V. S., *Rapid Commun. Mass Spectrom.* 2002, *16*, 160–8.

[23] Gharahdaghi, F., Weinberg, C. R., Meagher, D. A., Imai, B. S., Mische, S. M., *Electrophoresis* 1999, *20*, 601–5.

[24] Schuerenberg, M., Luebbert, C., Eickhoff, H., Kalkum, M. *et al.*, *Anal. Chem.* 2000, *72*, 3436–42.

[25] Finehout, E. J., Lee, K. H., *Electrophoresis* 2003, *24*, 3508–3516.

 