Automated protein in-gel digestion for MALDI-TOF-MS

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In-gel digestion is a major bottleneck for large scale proteome analyses and is therefore a prime candidate for automation. The Proteomics Group at the Max Planck Institute (MPI), Göttingen, in collaboration with Tecan, has developed an innovative automated solution based on Tecan liquid handling workstations.

Two-dimensional gel electrophoresis with subsequent in-gel digestion of the separated proteins followed by the analysis of those peptides using matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS) is one of the fundamental techniques of modern proteome research. A major restriction, however, is the bottleneck created by in-gel digestion and, for this reason, the Proteomics Group at MPI Göttingen, in association with Tecan, has developed a system that automates in-gel digestion, extraction of the proteolytic peptides and sample preparation for MALDI-TOF-MS, all on a single pipetting platform. Liquid handling is a major technical challenge during in-gel digestion, because very small volumes of liquid are repeatedly added and removed, readily leading to loss of sample and blockage of the pipetting needles, but the objective was to achieve the same quality of results as with the manual method while reducing hands-on time to a minimum.

The system the team has developed is based on a Genesis Freedom® liquid handling workstation equipped with a liquid handling arm with eight pipetting needles, a robotic manipulator arm and a Te-VacS™ vacuum separation module (Fig. 1). Two cooling racks, with lids to prevent evaporation, ensure that the consumable reagents are kept cool, while a microplate incubator allows incubation steps at 45 °C or 50 °C.

A specially designed in-gel digestion stack is made up of four components (Fig. 2a), including a 96-well protein digestion plate developed for this purpose by ABgene®/ Thermo Fisher Scientific. Each well of the digestion plate has two 250 µm holes to allow the aspiration of reagents using the Te-VacS. The protein digestion plate fits

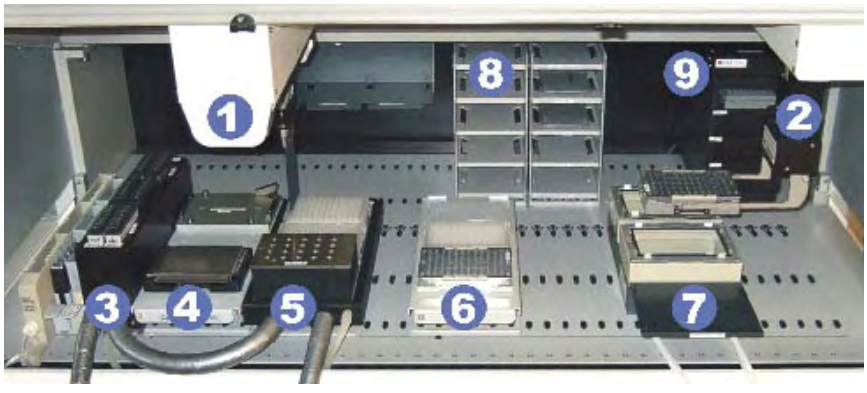


Figure 1: Tecan Genesis Freedom liquid handling workstation, set up for automated in-gel digestion and sample spotting on MALDI target plates. 1, liquid handling arm with eight pipetting needles; 2, robotic manipulator arm with in-gel digestion stack (see Fig. 2a for detail); 3, cooling rack with cover for consumable reagents; 4, carrier for MALDI target (front) and downholder position for separating the in-gel digestion stack (rear); 5, cooling rack for microplates (rear) and test tubes (front); 6, carrier with supporting areas for metal lid; 7, Tecan Te-VacS vacuum separation module; 8, microplate hotel; 9, microplate incubator with temperature control.

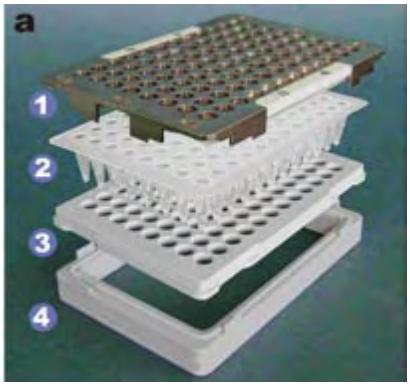


Figure 2: In-gel digestion stack for automated in-gel digestions. a, sandwich assembly; b, stack configuration on the vacuum module for pipetting and aspiration of reagents; c, stack configuration on the vacuum module for the elution of tryptic peptides into the collecting plate. 1, microplate cover plate with silicone mat;

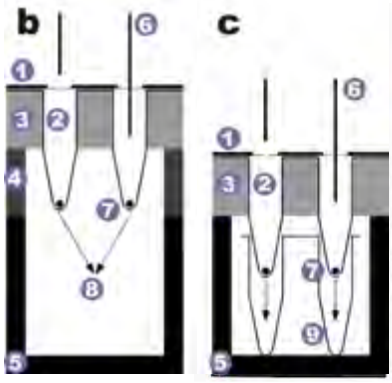
into a holder on a frame and is covered with a heavy metal plate perforated with 96 holes. A pre-perforated silicone mat on the underside of the metal plate protects the samples from evaporation and contamination and can be penetrated by the pipetting needles, while the intrinsic weight of the metal lid prevents the stack from lifting during withdrawal of the pipetting needles.

After electrophoretic separation by SDS-PAGE and staining with Coomassie® Brilliant Blue G-250, 1.5 mm gel plugs, equivalent to 29 fmol to 34 pmol protein per gel plug, are punched out of the protein bands, inserted into the protein digestion plate and placed in the stack (Fig. 2b). The plugs are destained with ammonium bicarbonate, dehydrated with acetonitrile, dried at 50 °C and reduced with dithiothreitol (DTT). The plugs are then alkylated with iodoacetamide in the dark, washed, dehydrated and dried again, in order to ensure optimal uptake of the trypsin solution. Trypsin digestion is carried out for two hours at 45 °C and stopped by the addition of trifluoroacetic acid. Tryptic peptides are extracted by passive elution and recovered on the Te-VacS (Fig. 1, no. 7, Fig. 2c, no. 5) into a 96-well collecting plate (Fig 2c, no. 9) by contactless aspiration, which practically eliminates loss of sample and prevents blockage of the pipetting needles.
The peptide eluates collected are then spotted onto a MALDI target plate (Bruker Daltonics AnchorChip™ 600/384). The entire sequence of steps is controlled by flexible and fully programmable Tecan software, and the whole technical set-up can also be installed on the Tecan Freedom EVO® pipetting platform.

At present, the new automated method can process 192 in-gel digestion samples automatically in eight hours, and validation experiments have confirmed that its efficiency is at least equivalent to the manual method (Fig. 3). The automated procedure takes an hour longer than the manual method because of the pipetting needle washing steps but, after the preparation time of about an hour, which is the same for both methods, there is no further hands-on time required. In comparison, the manual method for in-gel digestion ties up one person, almost full time, for about seven hours (Fig. 4) so the labor saving is considerable, and, by using a scheduling software, the automated throughput could be increased further without changing the technical set-up. In addition, the liquid handling arm ensures precise positioning of the samples on the MALDI target plate, improving the reproducibility of results[1].

Overall, the method of automated sample preparation in conjunction with the MALDI-TOF-MS analysis has made routine protein identification very reliable and easy. The system described provides automated and verified high throughput proteome analyses with a high standard of significance and reproducibility, and can be implemented in principle in any laboratory. MPI is now using this method to conduct large scale proteome analyses of various biological samples, including myelin from mouse brain, frog oocytes and peroxysomes from plant leaves.

Reference
1 Olaf Jahn, Dörte Hesse, Marina Reinelt, Hartmut D. Kratzin: Technical innovations for the automated identification of gel-separated proteins by MALDI-TOF mass spectrometry. Anal. Bioanal. Chem. (2006) 386: 92-103



2, 96-well protein digestion plate with 250 µm holes (ABgene/Thermo Fisher Scientific); 3, holder for protein digestion plate; 4, bottom frame; 5, Tecan Te-VacS vacuum separation module; 6, pipetting needles; 7, gel plug; 8, waste; 9, 96-well collecting plate for peptide eluates.

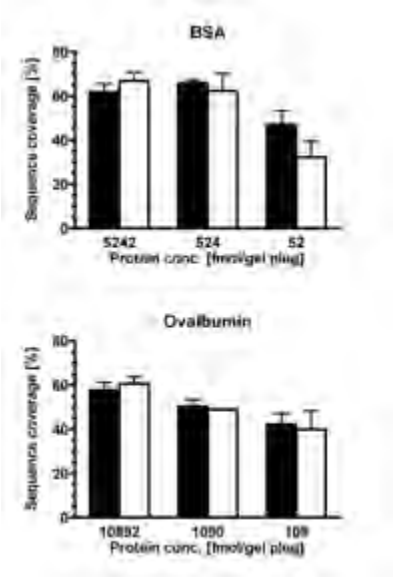


Figure 3: Typical results of automated in-gel digestion, comparing the automated method (black bars) with the manual method (white bars). The charts show the data for two of the six standard proteins used, bovine serum albumin (66 kDa) and ovalbumin (54 kDa), analyzed at three different protein concentrations. The bars represent the mean percentage sequence coverage from two independent in-gel digestions and sample preparations with twin samples (n = 4). The error bars show the standard deviation.

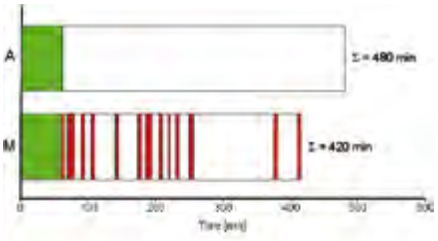


Figure 4: Time required for 192 in-gel digestion samples. Both the automated (A) and the manual (M) process need approximately one hour of preparation time (green). In contrast to the automated solution, this is followed by further hands-on times (red) in the case of the manual method.