APPLICATION INFORMATION

ProteomeLab™ Automation

USING THE BIOMEK® 3000 LABORATORY AUTOMATION WORKSTATION TO INTERFACE THE PROTEOMELAB PF 2D SYSTEM WITH THE MALDI-TOF MS FOR MULTIDIMENSIONAL PROTEOME PROFILING

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Introduction

The discovery stage of proteome profiling typically involves the comparison of different states of a cell or tissue. One approach utilizes fractionation of the proteome into intact proteins followed by mass spectrometry (MS). A two-dimensional, liquid chromatographic fractionation system, the ProteomeLab™ PF 2D, followed by a 3rd-dimension with MALDI-TOF MS has been used for this approach. The 1st-dimension separation is chromatofocusing where proteins are separated by pI and collected in fractions based on pH intervals. Upwards of 20 pI fractions are then separated in a 2nd-dimension by reversed-phase chromatography.

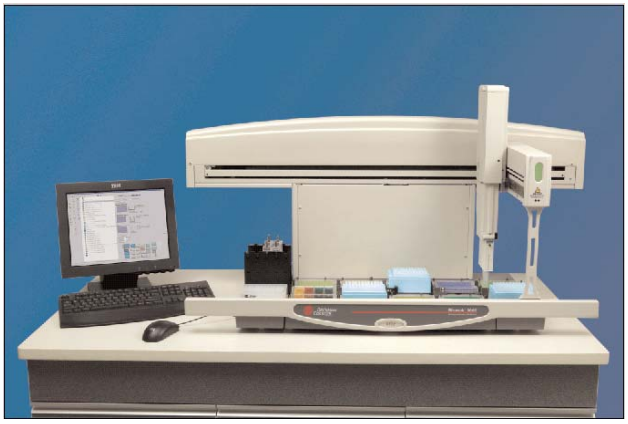

Figure 1. *An automated method for spotting samples onto the MALDI plate was developed on the Biomek 3000 Laboratory Automation Workstation.*

From each 2nd-dimension run, 80 to 90 fractions can be collected. To accommodate the large number of fractions generated by the first two dimensions for subsequent MS analysis, the Biomek® 3000 Laboratory Automation Workstation (Figure 1) was used. The Biomek 3000 Laboratory Automation Workstation prepared and spotted the fractions from the 2nd-dimension runs along with the appropriate matrix on a 384-well format, MALDI target. The target plate was analyzed directly by MALDI-TOF MS. This paper illustrates a "proof of concept" by performing automatic two-dimensional separation of human plasma with the ProteomeLab PF 2D followed by analysis of intact proteins by MALDI-TOF MS as the 3rd dimension.

Materials and Method

Plasma Sample Preparation

20 mL of blood was drawn from a healthy adult male. The blood sample was collected into tubes containing K_3-EDTA and centrifuged at 1000 rpm for 45 minutes at 8°C. The plasma was carefully removed, aliquoted and frozen at -80°C. The protein concentration, as determined by biuret assay, was 82.51 mg/mL.

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ProteomeLab™ PF 2D Fractionation

The components, principle of operation, materials and method of separation of the ProteomeLab PF 2D system have been previously described.[1] The plasma sample was injected at 2.5 mg of protein onto the 1st dimension of the ProteomeLab PF 2D. To equilibrate the plasma sample to the starting pH, 30 µL of plasma were mixed with Start Buffer to a final volume of 400 µL and the entire volume of the sample was injected.

2nd-Dimension Fraction Collection

Fifteen-second fractions were collected from the 2nd-dimension run of the pH fractions 5.33-5.63 and 6.21-6.48. Fractions were collected from 6 to 24 minutes of the 2nd dimension into a Greiner V-bottom polypropylene plate using the Gilson FC 204 Fraction Collector.

Preparation of Matrix Solutions

The HCCA matrix solution was prepared by dissolving 9 mg of HCCA in 3 mL of acetone, which was then diluted with 6 mL of ethanol to prepare the working solution. To prepare the SA matrix solution, acetonitrile was mixed with 0.1% TFA in water (9:1) and 10 mL of this mixture was used to dissolve 20 mg of SA which was the working solution. Aliquots of each matrix working solution were then distributed into each well of a 96 U-bottom polypropylene plate. The best results were obtained when the matrix stock solution was prepared immediately before use.

Preparation of Recrystallization Solutions

Two different solutions were used for recrystallization depending on the matrix selected. For HCCA matrix, the recrystallization solution was prepared by adding ethanol, acetone, and 0.1% TFA in water at a ratio of 6:3:1 to a total volume of 10 mL. For SA matrix, the recrystallization solution was made by mixing acetonitrile with 0.1% TFA in water (9:1) to a total volume of 10 mL. 100 µL aliquots of the appropriate recrystallization solution was then distributed into each well of a 96 U-bottom polypropylene plate. The best results were obtained when the recrystallization solutions were prepared immediately before use.

Spotting the MALDI AnchorChip with the Biomek® 3000

Table 1 shows the MALDI plate spotting method summary. The method utilizes four 96-well sample plates to spot one AnchorChip* MALDI plate (600 µm anchor diameter / 384 anchors). A list of chemicals used for spotting on the AnchorChip target plate is also shown.

MALDI Plate Spotting Method Summary		
Method Name	MALDISpottingB3K.bmt	
Labware		Quantity
	AP20 Tip Racks	5
	Greiner 96 Round PPN Plates	4
	AnchorChip MALDI Plate (600/384)	1
	Reservoir Holder	1
	Quarter Reservoirs	2
Hardware	Tool Rack	1
	MP20 Tool	1
	Labware Holders	11
Software	Biomek® Software (Version 3.0)	
Samples	96-Well Sample Plates	4
Throughput	384 Targets on AnchorChip Plate	
Run Time	~22 minutes (excluding dry time)	
Chemicals Used for Spotting MALDI Plate		
MALDI Matrices	α-Cyano-4-Hydroxycinnamic Acid (α-HCCA) Sinapinic Acid (SA)	
Solvents	Acetone Acetonitrile Ethyl Alcohol Trifluoroacetic acid HPLC-grade Water	

Table 1. *MALDI spotting on the Biomek 3000.*

Figure 2 shows the setup for the Biomek 3000 to perform the MALDI spotting.

The following method was developed to spot matrix and sample together onto the MALDI plate. Biomek Software is used to control the Biomek 3000. It is a common software architecture among Beckman Coulter's Laboratory Automation Workstations (Biomek FX, Biomek 3000 and Biomek NX). Software features include variables and version control. A view of the MALDI spotting method is shown in Figure 3.

MALDI-TOF MS Analysis

The m/z values were determined for the intact proteins from spotted 2nd-dimension fractions with a Reflex III* MALDI-TOF MS. The MS analysis was performed by the Consortium for Life Science at the University of Minnesota (St. Paul, MN).

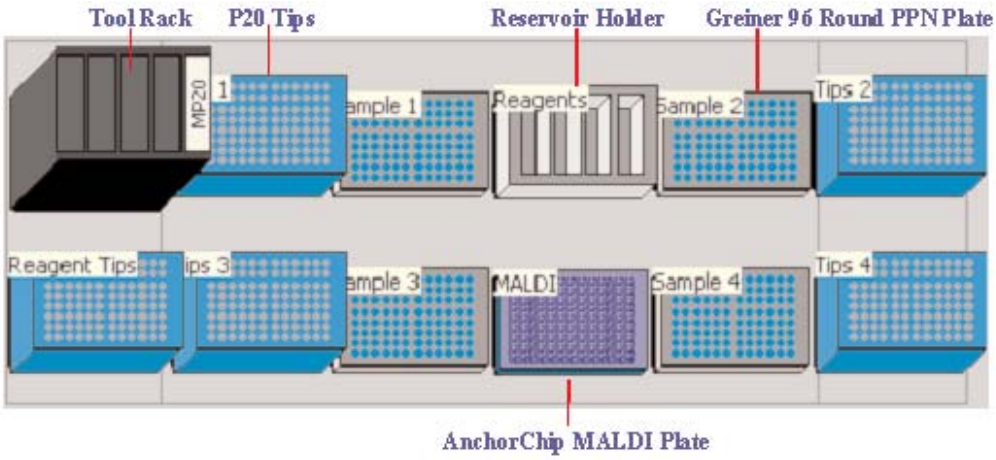

Figure 2. *Instrument Setup for the MALDI plate spotting method.*

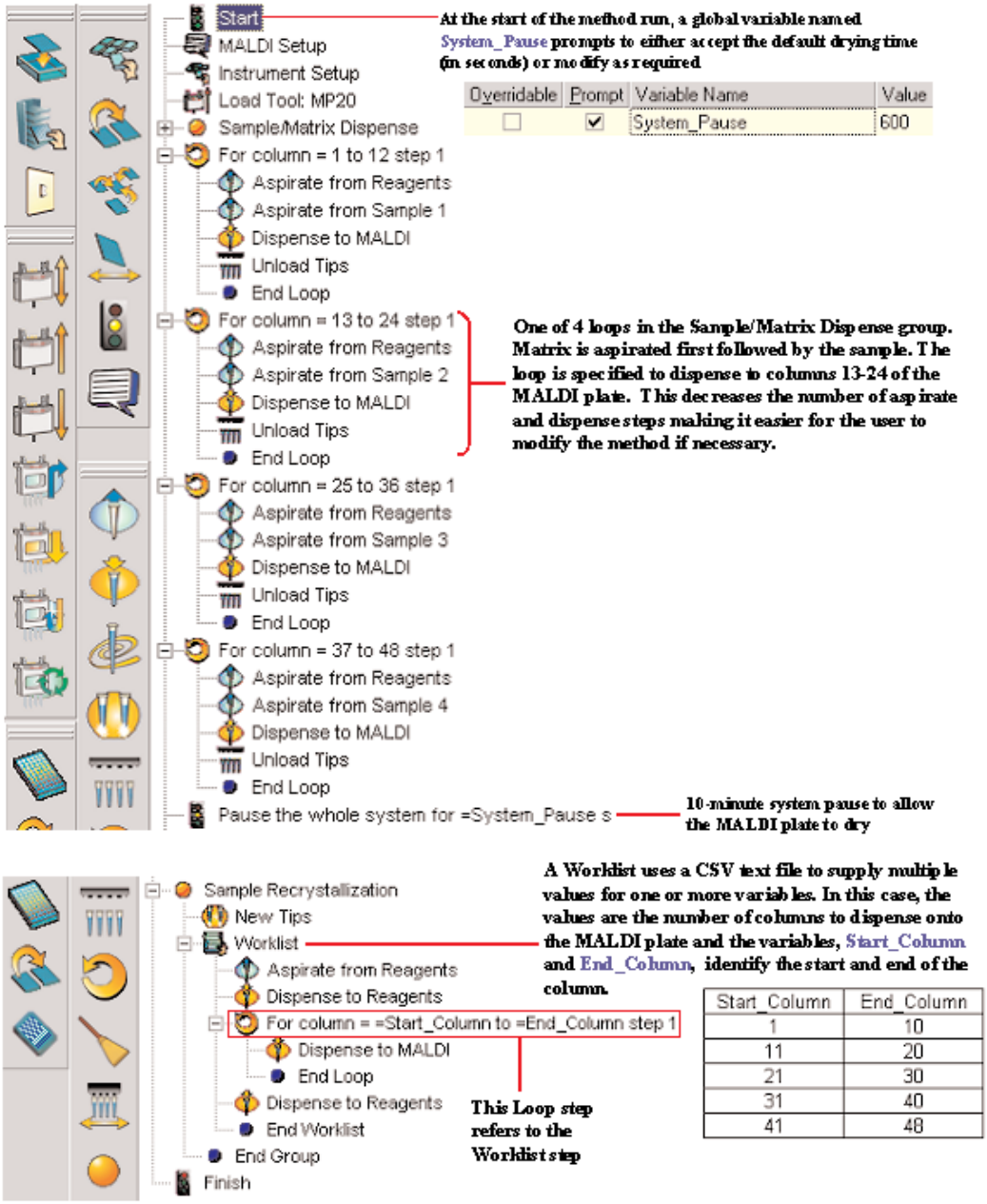

Figure 3. *MALDI spotting method details.*

Results and Discussion

The ProteomeLab™ PF 2D effectively fractionates proteomes for subsequent MS analysis. Because the fractions are liquid, no sample solubilization is required prior to MS. Two of the 1st-dimension fractions from human plasma were run on the 2nd dimension and 15-second fractions were collected from this dimension. One 1st-dimension fraction had a pH range of 5.33-5.63 and the second had a pH range of 6.21-6.48. An aliquot of each 2nd-dimension fraction was spotted with the Biomek® 3000 onto the MALDI target plate for analysis of the intact proteins MS. The mass spectra are shown in Figures 4-6.

With the SA matrix, two prominent peaks were observed in the MS spectra from the 2nd-dimension run of the pH fraction 5.33-5.63. One peak, which had a 2nd-dimension retention time of 16.75 to 17.0 minutes, had an m/z value 66,844 (Figure 4). This retention time corresponds to that for human serum albumin standard on the 2nd dimension and this m/z value corresponds to that of the single charged species of this protein. MALDI-TOF MS analysis of tryptic fragments of this fraction identified this protein as human serum albumin (data not shown).

The second peak, which had a 2nd-dimension retention time of 19.00 to 19.25 minutes, had a m/z value of 28,273 (Figure 5). MALDI-TOF MS analysis of tryptic fragments of this fraction identified this protein as human apolipoprotein A1 (data not shown).

With the SA matrix, one peak was observed in the MS spectra from the 2nd-dimension run of the pH fraction 6.21-6.48 (Figure 6). The fraction corresponds to the peak with retention time of 16.25-16.50 minutes in the 2nd dimension. It has an m/z value of 82,289. This is comparable to the mass of native human transferrin, which is a glycoprotein. Human transferrin standard has the same retention time in the 2nd dimension and a theoretical pI of 6.70,[2] which is similar to the pH range of the 1st-dimension fraction.

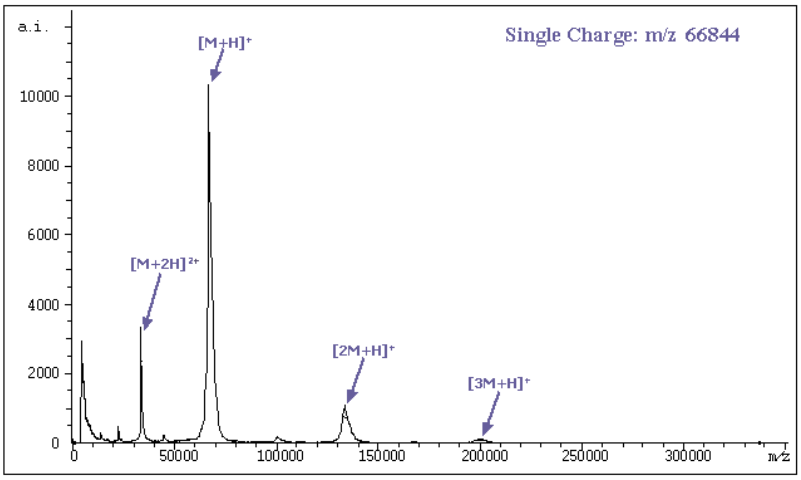

Figure 4. *Mass spectrum of intact protein of the peak with a retention time of 16.75-17.0 minutes in the 2nd dimension from the pH fraction 5.33-5.63.*

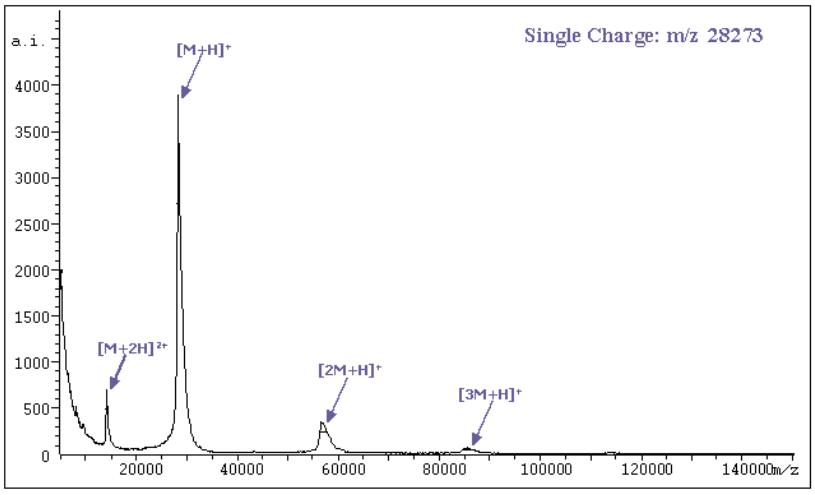

Figure 5. *Mass spectrum of intact protein of the peak with a retention time of 19.00-19.25 minutes in the 2nd dimension from the pH fraction 5.33-5.63.*

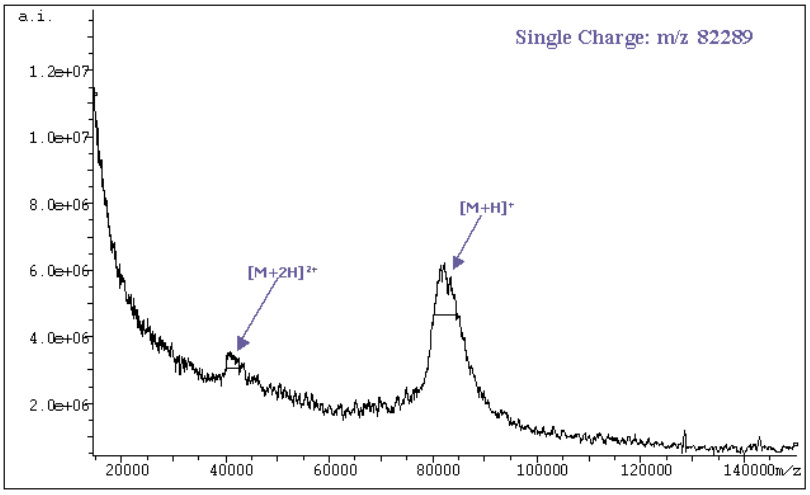

Figure 6. *Mass spectrum of intact protein of the peak with a retention time of 16.25-16.50 minutes in the 2nd dimension from the pH fraction 6.21-6.48.*

Summary

The combination of ProteomeLab™ PF2D separation of complex protein mixtures and MALDI-TOF MS is an advanced analytical tool for proteomics. MALDI-TOF MS analysis of 2nd-dimension fractions gives information on exact masses of intact proteins. To interface this combination, the Biomek® 3000 Laboratory Automation Workstation was used. Even with the volatile working matrix and recrystalization solutions, the pipetting of 1 µL volumes of these solutions was very accurate with the Biomek 3000 Laboratory Automation Workstation. The Biomek 3000 Laboratory Automation Workstation facilitated the analysis of a fractionated proteome by removing the potential bottleneck resulting from the large number of samples collected from the ProteomeLab PF2D.

References

1. Simonian, M. H, Betgovargez, E. "Proteome Analysis of Human Plasma with the ProteomeLab PF 2D System." Beckman Coulter, Inc. *Application Information Bulletin A-1963A* (2003).
2. SWISS-2DPAGE database, *http://www.expasy.ch/ch2d/*

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