

## Matrix to Analyte Concentration Ratio: A Critical Factor in the Successful Identification of Low-Abundance Proteins by AP-MALDI

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### Introduction

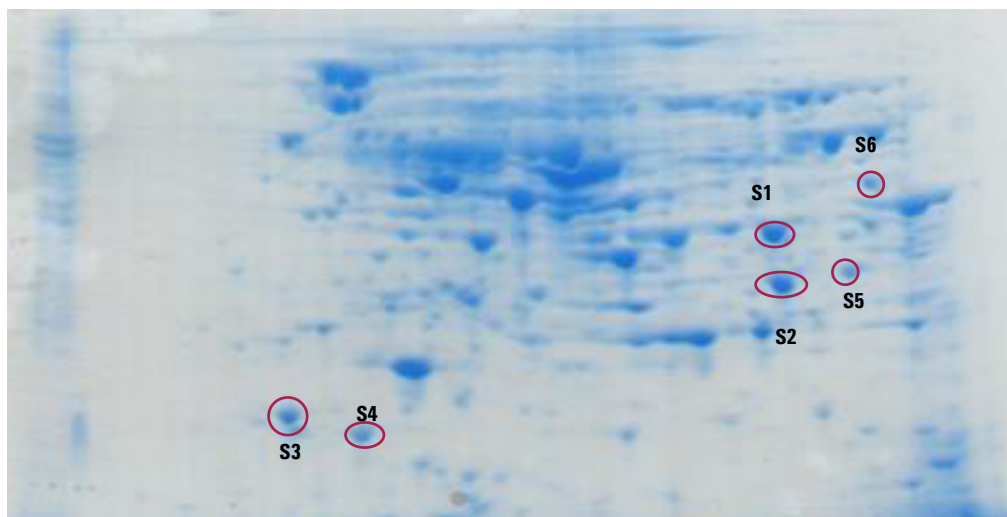
A major challenge in the field of proteomics is the identification of low-abundance proteins from complex protein mixtures. A common approach used in addressing the issue has been to run the protein mixture on 2-D gels. The resolving power of the gel and subsequent in-gel digestions followed by mass spectrometry analysis has facilitated the identification of these low-abundance proteins from their more abundant counterparts. Atmospheric pressure- or AP-MALDI is among the most sensitive techniques for the identification of proteins and is well-suited for the identification of proteins from in-gel digests. Our results show that the technique is particularly successful at identifying low levels of proteins from digests when an optimal matrix to analyte concentration ratio is used.

### Methods and Instrumentation

Standard protein digests and in-gel digestions of proteins were analyzed using an Agilent LC/MSD Trap XCT plus with an AP-MALDI source. Various matrix (CHCA) to protein digest concentration ratios were spotted on target plates to determine the ratio yielding the optimal sensitivity.

### Results and Discussion

We found that the matrix to analyte concentration ratio was particularly important when examining low levels of sample (sub-femtomole amounts). A two-fold difference in matrix concentration (0.25 mg/ml to 0.5 mg/ml of CHCA) had a five-fold effect on the absolute signal intensity of peptides from standard digests. On the other hand, the relative effect of varying the matrix concentration was less pronounced on samples at the fmol level or higher. While traditional vacuum MALDI sample preparations have used a matrix concentration of 5-10 mg/ml for the analysis of low pmol quantities of digests, we find that diluting the matrix ~20-fold (0.75 mg/ml) is ideal for the analysis of gel spots of varying intensities.



**Figure 1:** Coomassie stained 2D-gel of an *E. coli* lysate. Two darkly-stained (S1 and S2), medium-stained (S3 and S4) and lightly-stained (S5 and S6) gels spots were excised, digested and extracted for subsequent AP-MALDI MS/MS analysis. A final matrix concentration of 0.75 mg/ml was used in the analysis.

| Agilent Spectrum Mill - Protein/Peptide Summary                                    |                  |                |             |                       |                                    |               |                                 |                 |            |                                 |                      |  |
|--|------------------|----------------|-------------|-----------------------|------------------------------------|---------------|---------------------------------|-----------------|------------|---------------------------------|----------------------|--|
| Spectrum Mill  | Summary Settings | Autovalidation | Build TIC   | MS/MS Search          | Spectrum Summary                   | Tool Belt     | Help                            |                 |            |                                 |                      |  |
| Results Shown Filtered by Validation Category: all                                 |                  |                |             |                       |                                    |               |                                 |                 |            |                                 |                      |  |
| Data Directory: msdataSM/JEM/ASMS2004/S1-S6 - SpecFeatures read Files: 99 Hits: 86 |                  |                |             |                       |                                    |               |                                 |                 |            |                                 |                      |  |
| Run #  | Run Name         | Group (#)      | Spectra (#) | Distinct Peptides (#) | Distinct Summed MS/MS Search Score | % AA Coverage | Mean Peptide Spectral Intensity | Protein MW (Da) | Protein pI | Species                         | Database Accession # | Protein Name   |
| 1  | In-gel01         | S1             | 6           | 6                     | 46.39                              | 22            | 2.11e+005                       | 34489.8         | 5.83       | Escherichia coli O157:H7 EDL933 | 15802947             | cysteine synthase A, O-acetylserine sulphydolase A         |
| 2  | In-gel03         | S2             | 12          | 10                    | 85.67                              | 36            | 3.23e+006                       | 28556.5         | 5.85       | Escherichia coli O157:H7 EDL933 | 15800464             | phosphoglyceromutase 1                                     |
| 3  | In-gel04         | S3             | 1           | 1                     | 13.76                              | 8             | 5.48e+006                       | 18266.1         | 4.73       | Escherichia coli O157:H7 EDL933 | 15802950             | PTS system, glucose-specific IIA component                 |
| 4  | In-gel07         | S4             | 4           | 4                     | 47.70                              | 42            | 1.24e+006                       | 17704.2         | 4.75       | Escherichia coli                | 1742169              | Thiol peroxidase (EC.1.11.1.-) (P20)                       |
| 5  | In-gel08         | S5             | 11          | 11                    | 72.05                              | 36            | 1.66e+005                       | 28556.5         | 5.85       | Escherichia coli O157:H7 EDL933 | 15800464             | phosphoglyceromutase 1                                     |
| 6  | In-gel11         | S6             | 4           | 4                     | 37.27                              | 13            | 1.71e+005                       | 38009.7         | 6.14       | Escherichia coli O157:H7 EDL933 | 15800463             | Phospho-2-dehydro-3-deoxyheptonate aldolase, Phe-sensitive |

**Figure 2:** Spectrum Mill summary report of the six 2D-gel spots analyzed. A positive identification was made for each of the spots analyzed with multiple distinct peptides identified for five of the six proteins. Spot S3 yielded only one peptide, but of very good MS/MS quality (see Fig. 8). Additionally, the protein molecular weight and PI are consistent with what is observed on the 2D-gel. Furthermore, the protein ID was confirmed by LC-ESI.

## Conclusions

- Matrix concentration is more critical when analyzing low attomole levels of protein digests.
- Matrix concentration is less critical when analyzing samples at femtomole levels or higher, which are the expected quantity yields for faintly-stained coomassie gel bands/spots.
- AP-MALDI is a robust, sensitive technique that is well-suited for the identification of proteins from in-gel digests.