

Differentially Expressed Proteins Detected on Chromatographic Retentive Surface Capture by Mass Spectrometry

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Introduction

The effects of chromatographic enrichment on both sensitivity and selectivity of protein detection have been long established for linear TOF MS top-down biomarker profiling. In this study we utilize various chromatographic surface functionalities to demonstrate differential expression of native peptides and proteins in beer yeast lysates, prepared after incubation with and without three different sweeteners. Profiling utilized the Lucid Proteomics System™, a combination of ProteinChip arrays with high-resolution MALDI-TOF/TOF mass spectrometry. Direct MS/MS sequence analysis of peptides from chromatographic arrays, with and without small scale chromatographic enrichment, was performed to identify peptides of interest.

Methods

• Sample preparation:

Safbrew S-33 beer yeast and sweeteners were purchased from commercial suppliers. Yeast (0.5 g) was dissolved in 10 mL milliQ water with and without the following additives: 0.1 g priming sugar (dextrose), 0.1 g table sugar (sucrose), or 0.033 g Splenda® (sucralose). Yeast samples were passively lysed by gentle agitation at room temperature for ten minutes. Samples were centrifuged at 5000 x g for five minutes at 4°C. The resulting supernatants were transferred to a new tube prior to analysis.

• ProteinChip array preparation:

Cation exchange (CM10), metal affinity (IMAC30), and anion exchange (Q10) ProteinChip arrays were pre-equilibrated with their corresponding binding and washing buffers: 0.1 M NaPhosphate + 0.5 M NaCl, pH 7.0 for IMAC-Cu arrays; 100 mM NaAcetate, pH 4.0 for CM10 arrays; and 50 mM Tris-HCl, pH 9.0 for Q10 arrays. Crude supernatants were diluted 1:10 in binding buffer using a bioprocessor and allowed to bind for 30 minutes with shaking at room temperature. Each spot was then washed three times with binding buffer and rinsed three times with water within the bioprocessor. Sinapinic acid (50% saturated) and α -cyano-4-hydroxycinnamic acid (CHCA, 25% saturated) were used as matrices. Details on samples applied to each array spot were annotated within a virtual notebook in Lucid™ Proteomics Software version 2.0 (Bio-Rad).

• Data collection:

Arrays were analyzed using an ultrafleXtreme MALDI-TOF/TOF mass spectrometer (Bruker Daltonics) in linear mode. After laser optimization, AutoExecute runs were generated within Lucid Proteomics Software and exported for use with Bruker flexControl software. System set-up and optimization and testing of MS parameters for reproducible protein profiling was performed prior to analysis using the Lucid System Qualification Kit (Bio-Rad). External calibration was performed with Bio-Rad's QC Peptide Array using calibrants from 1 to 12 kDa.

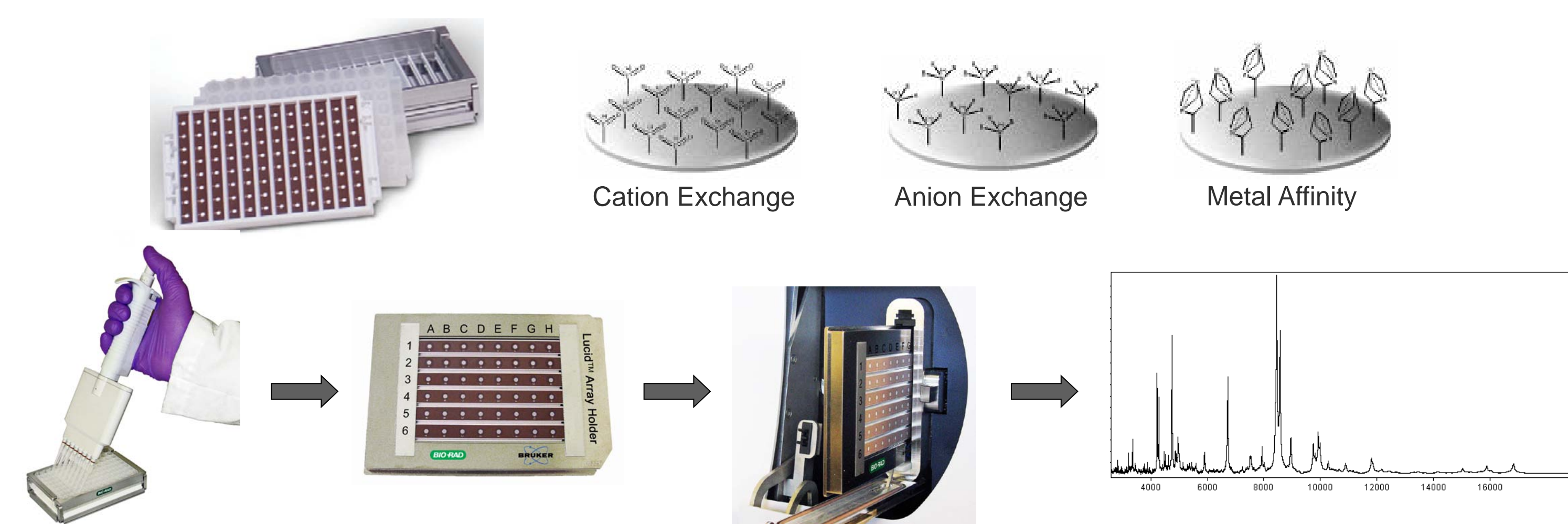


Figure 1. ProteinChip arrays, array preparation, and TOF data collection.

• Data analysis:

Data was imported into Lucid Proteomics Software for determination of differentially expressed peptides and proteins. Spectra were sorted into individual folders based on profiling condition (array type and matrix), and each set was normalized by TIC (total ion current). Baseline subtraction, filtering, and noise settings were unaltered from defaults. Automatic labeling of peaks (from 2 to 25 kDa) with a (minimum) S/N ratio of 5.0 and valley depth of 3.0 was executed using the Lucid software Cluster Wizard. Clusters were completed (across all spectra for a given profiling condition) when a given peak was detected in at least 20% of spectra.

• Enrichment for identification:

Reverse phase beads (200 μ l, Varian Inc.) in 10% acetonitrile, 0.1% TFA were added to 3 mL of each of the beer yeast lysates (control, +dextrose, +sucrose, +sucralose). Lysates and beads were incubated overnight. Fractions of increasing acetonitrile (10%, 20%, 30%, 40%, 50%, in 0.1% TFA) were collected and applied to CM10 arrays by diluting 1:10 in binding buffer. CHCA was used as the matrix. Note that while many biomarker candidates were present on both CM10 and Q10 arrays, CM10 arrays were selected for profiling of reverse phase fractions for increased signal and buffer compatibility.

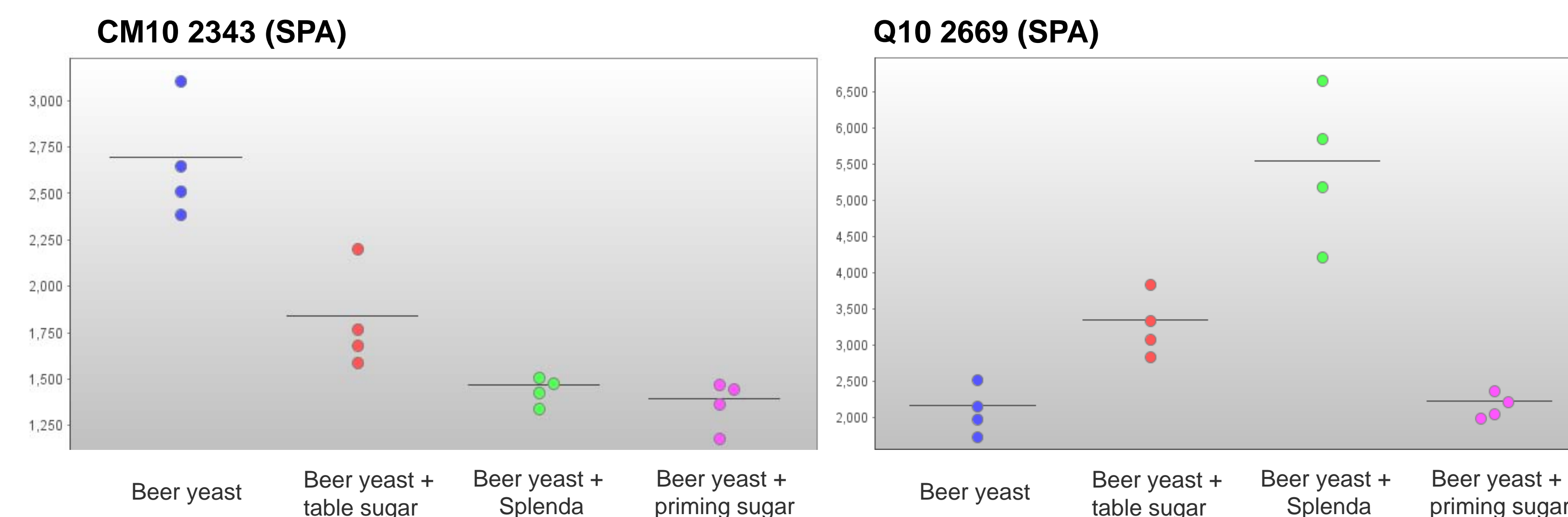


Figure 2. Differentially expressed proteins. Scatter plots for two differentially expressed peptides selected for identification.

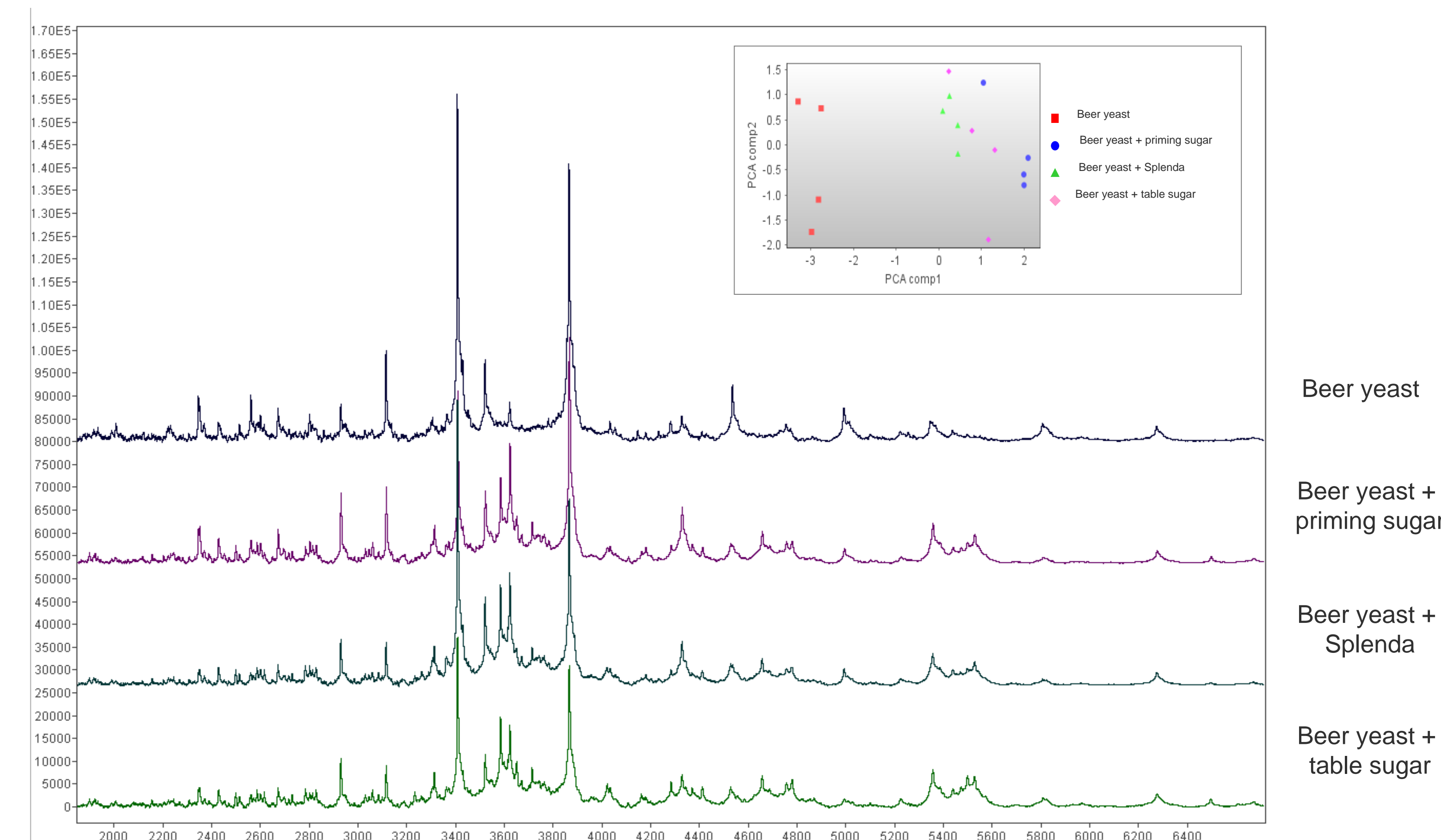


Figure 2. Differential expression overlay. Representative spectral profiles comparing the four sample treatments. Data from CM10 arrays, CHCA matrix is shown. The PCA plot (inset) demonstrates clear separation between the control and +sugar groups, with some segregation between sugar types.

• Peptide Identification:

Arrays were analyzed using an ultrafleXtreme MALDI-TOF/TOF mass spectrometer in reflector mode followed by LIFT mode. Fragmentation data was analyzed using flexAnalysis software followed by BioTools software searching the Saccharomyces Cerevisiae SwissProt protein database.

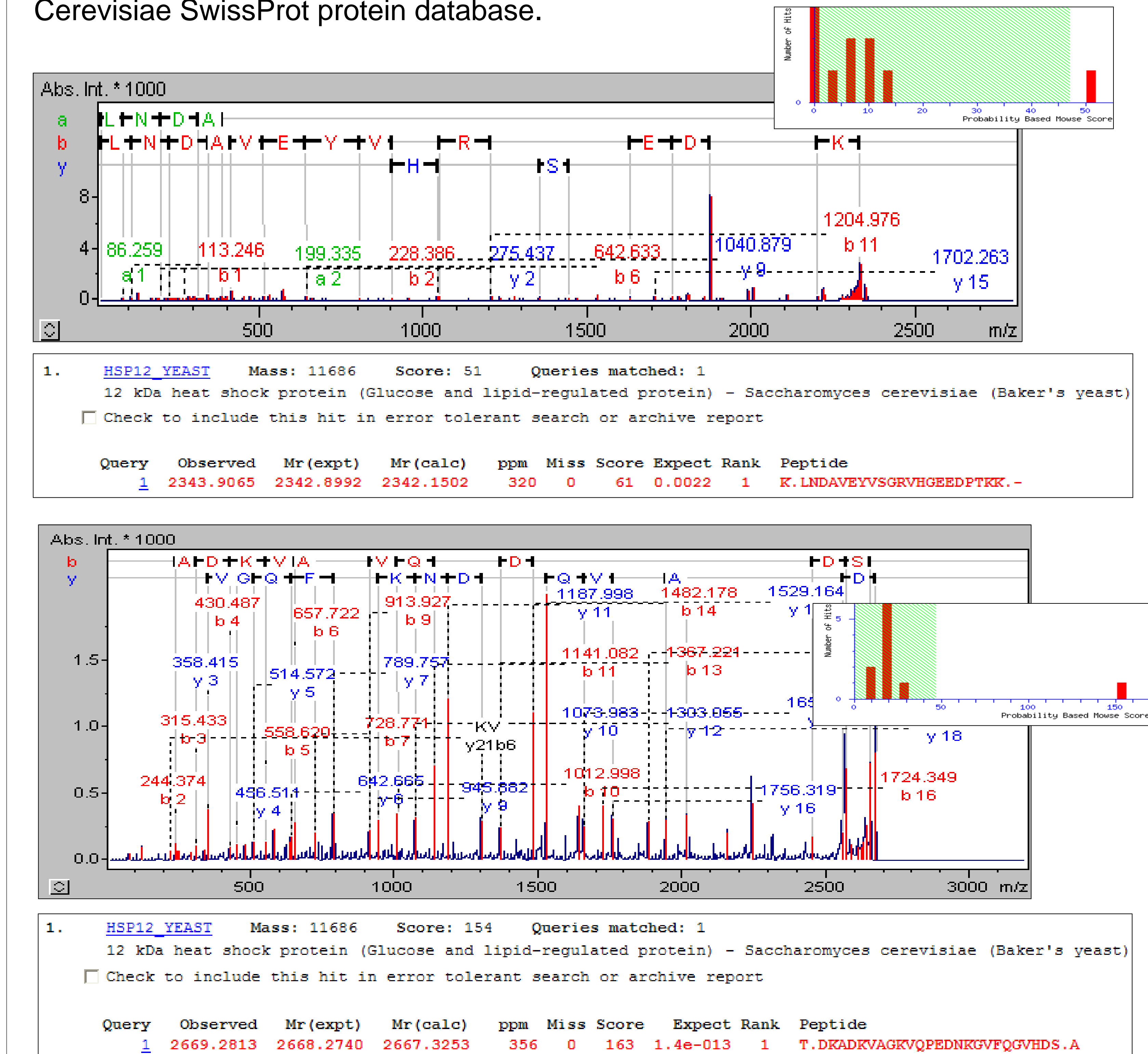


Figure 4. Identification of HSP12 peptides. Identification of differentially expressed peptides from enriched yeast+sugar preparations at 2343 Da (top) and 2669 (bottom) as two different peptides (amino acids 89–109 and 35–59, respectively) from the 12 kDa yeast heat shock protein (HSP12).

Conclusions

Top-Down proteomic profiling using the Lucid Proteomics System was shown to generate reproducible profiles and detect statistically significant differentially expressed protein fragments from beer yeast after incubation with and without three different sweeteners prior to lysis. The strength of this top-down approach was clearly illustrated in the discovery – for the same parent protein, one fragment was upregulated and one was downregulated with exposure to sugar, a distinction that may have been missed by other approaches. It was noted that the profiles of the yeast +sugar(s) were generally similar to each other, including the artificial sweetener Splenda. Selected differentially expressed peptides representing different regions of the 12 kDa yeast heart shock protein, also known as the glucose and lipid-regulated protein, were directly identified following a single step enrichment from ProteinChip arrays via TOF/TOF analysis.

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