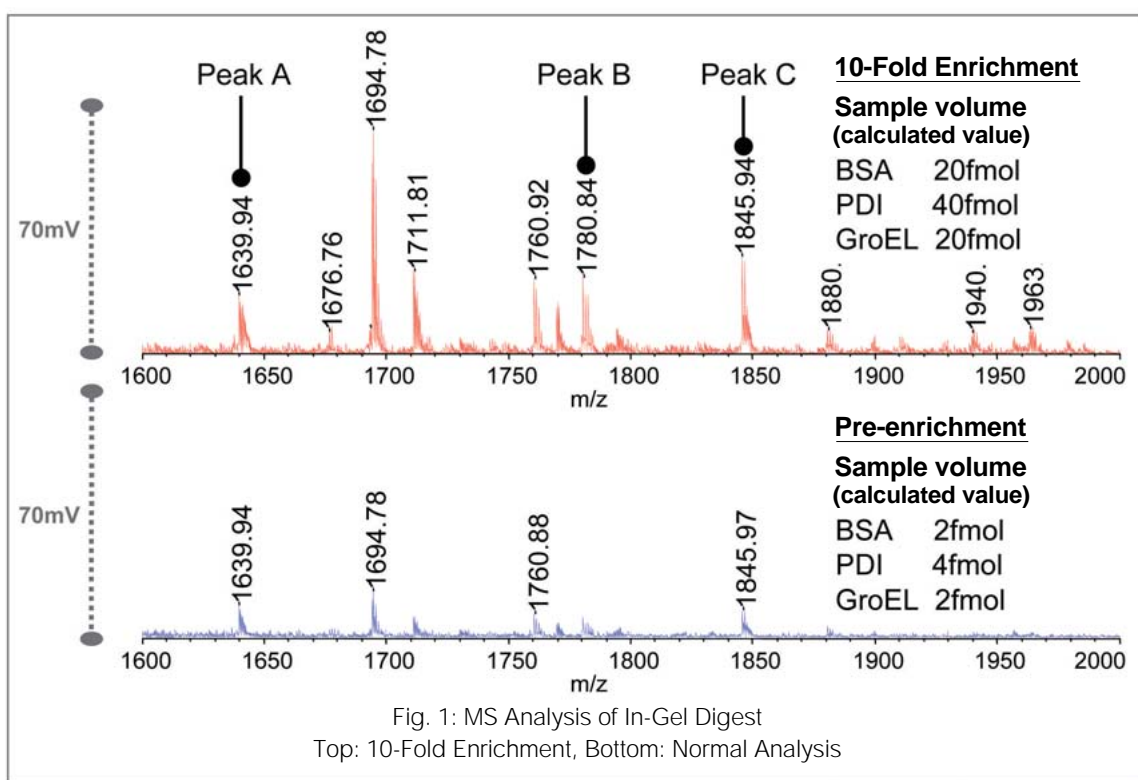


AXIMA-QIT Analysis using FocusMass-T3 Enrichment Effect (1) Measurement of Mixture using High-Sensitivity MS/MS

The FocusMass-T3 MALDI plate can enrich up to 20 μL of sample at one time. A key feature of the AXIMA-QIT is its ability to perform high-sensitivity MS^n measurements; however, since the volume of sample that can be deposited is limited, it is sometimes difficult to analyze low concentration samples. Using the FocusMass-T3 plate further utilizes the high-sensitivity MS^n measurement capability of the AXIMA-QIT.



Sample Preparation

After SDS-PAGE analysis of 1 pmol each of bovine serum albumin (BSA), protein disulfide isomerase (PDI), and E. coli-derived GroEL, we performed gel digestion. Following desalting by ZipTip C₁₈, the digest solutions were mixed together and diluted to suitable concentrations.

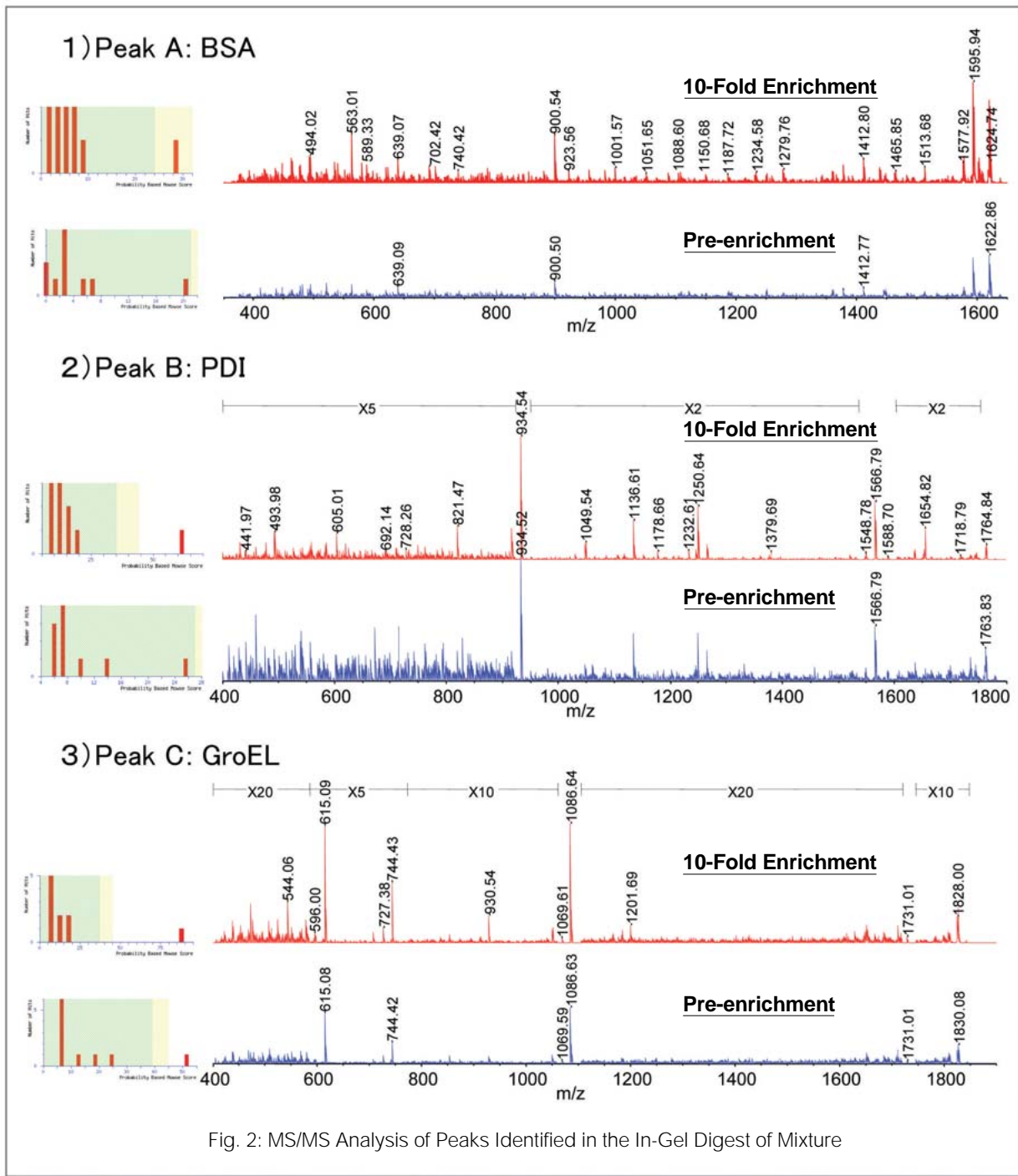
MS Analysis

Instrument: AXIMA-QIT
Matrix: Reagent kit (DHB) for FocusMass-T3

Fig. 1 shows the effect of enrichment on MS measurement. Compared to the lower, blue spectrum, the red spectrum at top was generated following enrichment of a 10-fold sample volume on the plate. The increased peptide signal intensity due to enrichment is clearly evident.

The protein quantities shown in the figure are calculated values. As up to 30-50% of sample is lost in desalting and other processes, measurement is actually conducted on quantities lower than the values shown.

Fig. 2 shows the results of MS/MS measurement of Peaks A, B and C in Fig. 1.



The data in Fig. 2 shows comparisons of the MS/MS spectra and MS/MS ion search results between spectra obtained using un-enriched samples and spectra obtained via 10-fold volume-enriched samples. Peaks A, B and C are peptides originating from the BSA, PDI and GroEL digests, respectively. Although they differ in degree, the fragment ions of the spectra obtained from FocusMass-enriched samples show markedly increased intensity, as well as higher search scores.

In this way, the inherently high-sensitivity AXIMA-QIT MSⁿ measurement function is even further improved and strengthened with the use of the FocusMass-T3 MALDI plate.



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