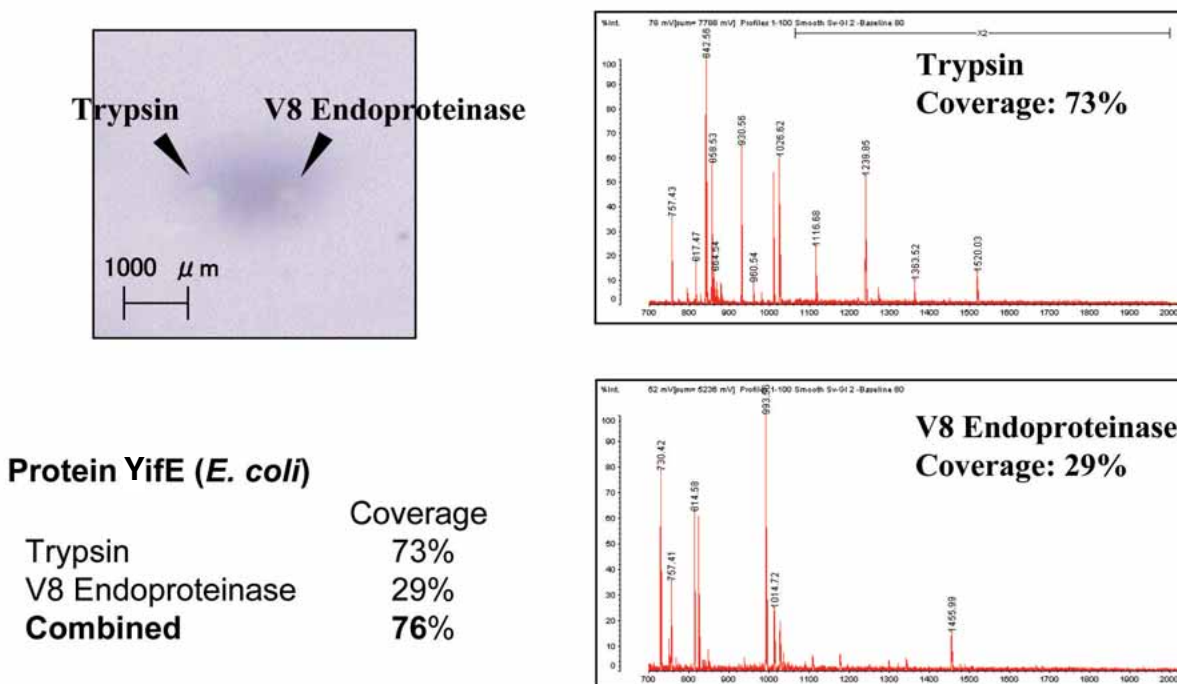


PMF Analysis with Multiple Enzymes of Proteins Blotted on PVDF Membrane using Chemical Inkjet Printer (CHIP-1000)

The chemical inkjet printer can microdispense picoliter volume of reagents and allow the on-membrane PMF analysis using the microscale area of the protein spot. With this technology, multiple analyses with different enzymes can be performed on a single spot, generating detailed information about the target protein.

The results of PMF analysis with multiple enzymes of *Escherichia coli* extract are shown below. Proteins separated with 2-dimensional electrophoresis and blotted onto PVDF membrane were used. PMF analyses with multiple enzymes (Trypsin and V8 proteinase: Fig.1, Trypsin and Chymotrypsin: Fig.2) on the single protein spots were carried out using AXIMA-CFR, and the database search was performed on the basis of the obtained MS spectrum.

Figure 1 shows the result of multi-enzyme analysis on a single protein spot with the two enzymes, trypsin and V8 proteinase.

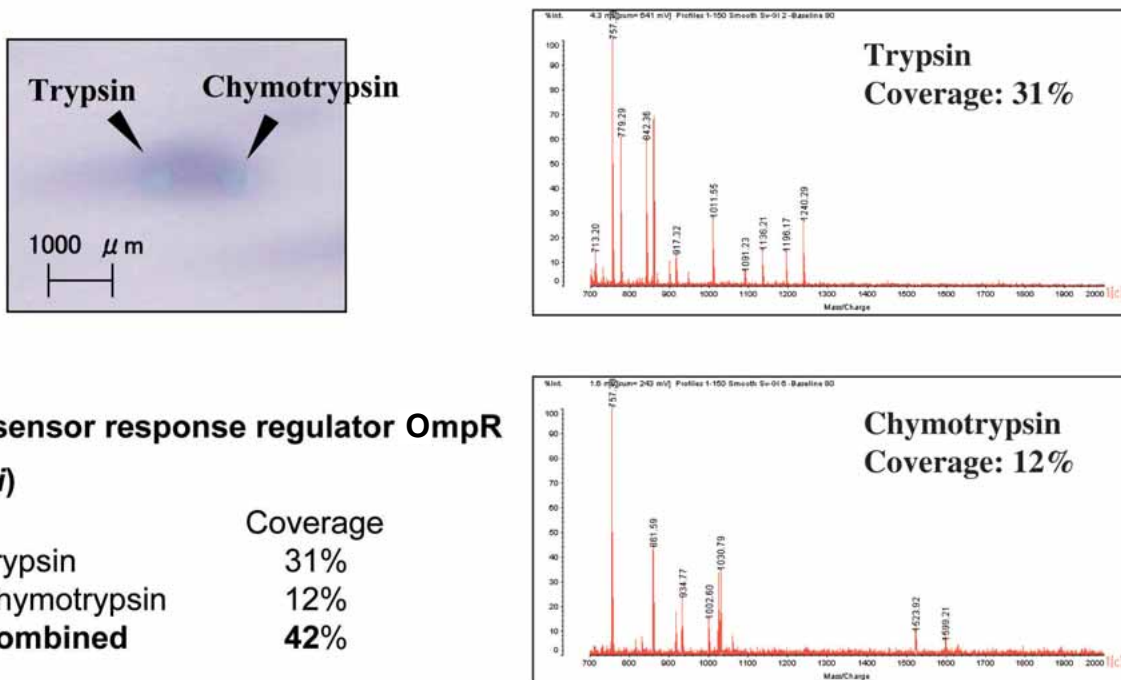


1 **AESFTTTNR** YFDNKHYPRG **FSRHGDFTIK** **EAQLLERHGY** AFNELDLGKRE
 51 PVTEEEKLF VAVCRGEREP VTEAERVWSK YMTRIKRPKR **FHTLSGGKPQV**
 101 **EGAEDYTDS** **DD**

red letter: trypsin-digested fragments, underline: V8 endoproteinase-digested fragments

Figure 1 MS spectra obtained with multi-enzyme PMF analysis on a single protein spot with trypsin and V8 endoproteinase, the sequence coverage and the covered amino acid sequence resulting from the database search.

Subsequently, the result of multi-enzyme analysis on a single protein spot with the two enzymes, trypsin and chymotrypsin is shown.



1 MQENYKILVV DDDMRLRALL ERYLTEQGFQ VRSVANAEQM DRLLTRESFH
 51 LMVLDLMLPG EDGLSICRRL RSQSNPMP II MVTAKGEEVD RIVGLEIGAD
 101 DYIPKPFNPR ELLARIRAVL RRQANELPGA PSQEEAVIAF GKFKLNLGTR
 151 EMFREDEPMP LTSGEFAVLK ALVSHPREPL SRDKLMNLAR GREYSAMERS
 201 IDVQISRLRR MVEEDPAHPR YIQTVWGLGY VFVPDGSKA

red letter: trypsin-digested fragments, underline: chymotrypsin-digested fragments

Figure 2 MS spectra obtained with multi-enzyme PMF analysis on a single protein spot with trypsin and chymotrypsin, the sequence coverage and the covered amino acid sequence resulting from the database search.

The chemical inkjet printer allows independent PMF analyses using multiple enzymes in a single protein spot. The combination of the results from those database searches can generate the highly reliable result with increased sequence coverage.