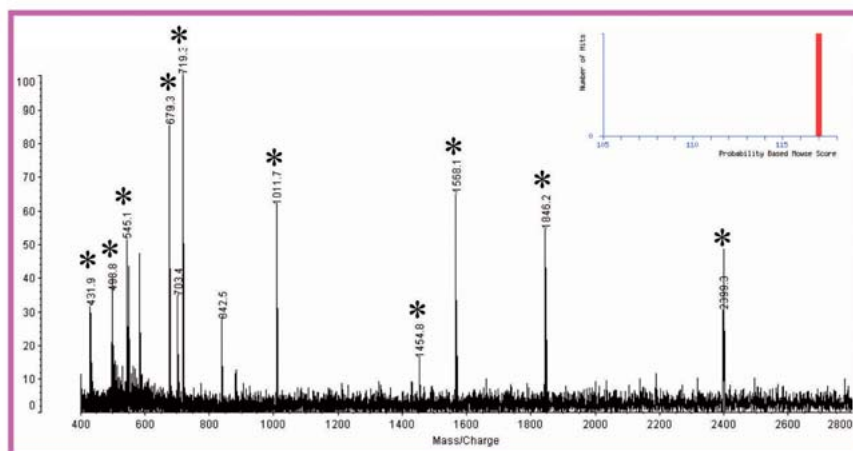
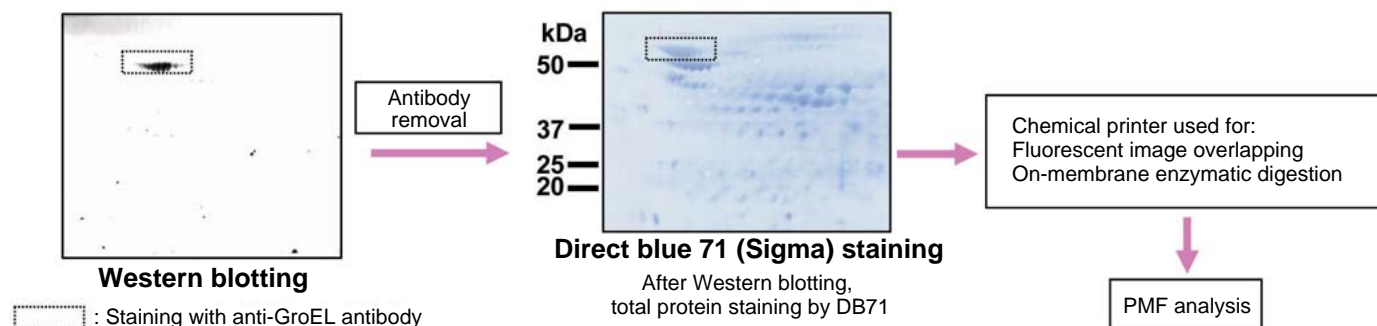


# Western Blotting Method: Direct On-Membrane Identification of Target Molecules Detected by Western Blotting

Until now, "detection" of target proteins via Western blotting and "identification" by PMF analysis required electrophoresis of the same sample on multiple gels. In this application, we developed a new, groundbreaking Western MS method which enables "detection" and "identification" on the same membrane.

After transferring a 2-dimensional electrophoresis gel of *E. coli* extract to a PVDF membrane, GroEL was detected by Western blotting (fluorescent antibody method) using Alexa488-labeled secondary antibody against anti-GroEL (primary antibody). The antibodies were then removed, and microscale, on-membrane enzymatic digestion (tryptic digestion) of the detected GroEL was conducted using a chemical printer (ref. Application 14). On-membrane Direct PMF analysis of the obtained peptide fragments using the AXIMA-CFRplus confirmed the GroEL identification (Fig. 1).



MS Spectrum and Database Search (Mascot)

\*: GroEL-derived proteins

Fig. 1: Western MS System Flow

Using the Western MS system in this way allows identification of antigens by Western blotting analysis and mass spectrometric analysis on the same membrane without cumbersome procedures such as preparing multiple gels or extracting digested peptides.

Literatures cited: I. Ohtsu *et al.*, *J. Proteome Res.*, 2005, 4, 1391-1396



JQA-0376