

Interaction MS Method: Direct On-Membrane Identification of Target Molecules Detected by Lectin Staining on Membrane

Previously, we developed a Western MS method (AXIMA Application 20) for detection of target proteins via Western blotting and identification by PMF analysis on the same membrane. In this application, we developed a new Interaction MS Method that allows PMF or MS/MS analysis of glycoproteins detected by lectin staining on the same membrane. After running different concentrations of ovalbumin by SDS-PAGE and transferring the separated proteins to a PVDF membrane, ovalbumin detection was conducted by lectin blotting (fluorescence method) using Alexa488-labeled concanavalin A. The lectin was then removed, and microscale, on-membrane enzymatic digestion (trypsin digestion) of the detected ovalbumin was conducted using a chemical printer (ref. Application 14). On-membrane Direct MS/MS analysis of the obtained peptide fragments using the AXIMA-QIT confirmed the ovalbumin identification (Fig. 1).

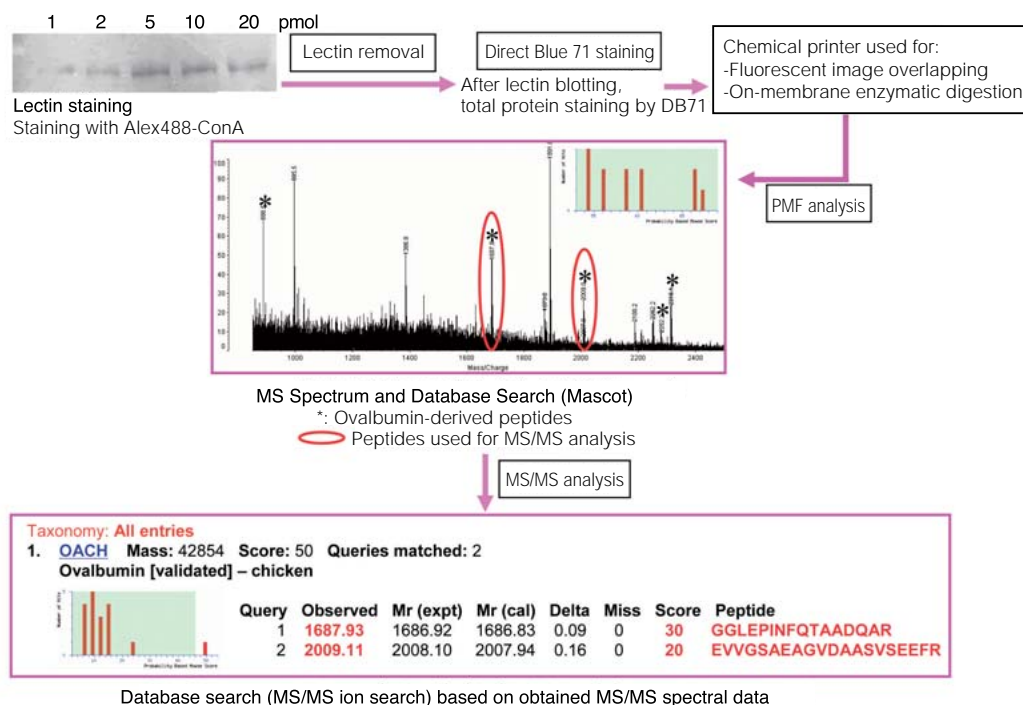


Fig. 1: Interaction MS System Flow

As shown above, Interaction MS allows detection of a glycoprotein (ovalbumin), which interacts with a sugar chain-recognizing protein (lectin), and subsequent identification of the detected glycoprotein by mass spectrometric analysis on the same membrane.

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



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