

ABSTRACT

Disclosed in the present invention is a method for detecting a protein having changes in an energy state, and affinity of a ligand to a protein. Specifically, after the energy state of a protein changes, its tolerance to proteolytic cleavage destruction changes. The structure of the protein in a low-energy state is also destroyed under a non-denaturation condition by using a large amount of enzymes, and small peptide fragments, which have molecular weight of less than 5 KDa and can be directly used for bottom-top mass spectrometry analysis, are directly generated. The method has extremely high sensitivity. Quantitative proteomics is used to find enzyme cleavage differential peptide fragments, and proteins to which the differential peptide fragments belong and the positions in the proteins are analyzed, so that a protein having changes in an energy state, and a change region can be determined in the whole proteome range. If the energy state of the protein changes due to addition of a ligand, the method can determine a binding protein and a binding region of the ligand; and the output of a quantitative result on the peptide fragment level further enables the method to determine the local affinity of binding of the ligand to the protein.