COMBINING SURFACE PLASMON RESONANCE WITH MASS SPECTROMETRY: A POWERFUL TOOK IN FUNCTIONAL PROTEOMICS

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The rapid growth in activities around functional proteomics will lead to a comprehensive understanding of biological pathways and the rapid development of new pharmaceuticals. Many of these advances have been possible because of the development of and effective interplay between complementary technologies. We will present a tool in functional proteomics that is generated by the integration of two well-established techniques, mass spectrometry (MS) and surface plasmon resonance (SPR). SPR-MS allows the selective binding, recovery, identification, and characterization of specific proteins from complex biological mixtures and can be used to tackle functional proteomics applications, such as, ligand fishing, epitope mapping, and the study of macromolecular complexes and novel molecular interactions.

SPR technology provides a sensitive real time detection of the binding and dissociation of biomolecules on a sensor chip. Sensor chips are available for use with various immobilization chemistries and here we used chips where biomolecules can bind specifically to ligands that are covalently attached to a carboxymethyl dextran layer, or chips that are covered with a nickel/nitriloacetic acid layer to capture his-tagged proteins. After injection of complex biological mixtures, the biomolecules of interest are captured on the sensor chip, eluted, and further processed for structural analysis with MS. The binding and recovery of the biomolecule of interest are fully automated with a microfluidic system suitable for handling microliter volumes and typically nanomolar ligand concentrations.

To demonstrate the general applicability of the combination of SPR with MS, examples will be given in which a protein of interest are fished out from mixtures. In one of these examples, calmodulin is selectively captured on the sensor chip after injecting bovine brain extract. Calmodulin fishing is based on the specific interaction of calmodulin with a peptide that is part of the myosin light chain kinase. In a typical experiment between 200 and 500 femtomoles are captured on the sensor chip and eluted in four microliter. After elution, the samples were prepared in various ways for MS analysis, including direct application on MALDI targets, enzymatic digestion, and reverse phase purification prior to MS sample preparation. The samples were structurally characterized by their intact molecular weight and identified, including post-translational modifications, based on peptide mapping in combination with database searches.

In another example, recombinant histidine-tagged cytochrome P450 is selectively captured on the NTA sensor chip from the membrane extract of *E.coli*. The functionality of cytP450 was then tested by studying its interaction with the ligand 4-phenylimidazole. After recovery, cytP450 was enzymatically digested and its structure was confirmed by MALDI MS.

Furthermore, new software, recently developed within Biacore's R&D lab will also be described. This software allows the sample to be recovered in two microliter that can be automatically deposited on a MALDI target.