

## **Kinetics and identification of non-covalently interacting partners in a multiplex way with SPRI-MALDI MS**

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By coupling SPRI and MALDI MS investigations of non-covalently interacting biomolecules in a multiplexed and high-throughput fashion with high sensitivity (low fmol range) become feasible. It provides information on binding kinetics, binding affinity in real time and identifies the interaction partners and without any need for purification or labelling.

SPR imaging measurements were performed on the SPR Plex II (Horiba, France) working with polyoxyethylene functionalized gold slides that are carrying NHS-ester groups for immobilizing ligands. Mass spectrometric detection was done with a commercial MALDI TOF mass spectrometer (Ultraflex II TOF, Bruker Daltonics, Germany). The gold slide was mounted in an adapter. MS measurements were performed in the positive ion reflectron mode with standard settings and a 532 nm pulsed laser. Each mass spectrum was the average of 2500 laser shots acquired at random sample positions. One of the problems when solutions (e.g. trypsin or matrix) are spotted by pipets is the low reproducibility due to spreading and therefore the dilution of the sample. To overcome this, we developed a protocol to spray trypsin or matrix solution. This works in a short time and preserves the multiplexing SPRI measurements.

As a proof of concept we will show an investigation of specific binding of various designed ankyrin repeat proteins (DARPin)s against different proteins, like maltose-binding protein (MBP), ribosomal protein S6 kinase 2 (RPS6KA2), Kirsten rat sarcoma protein (K-Ras), mitogen-activated protein kinase 8 (MAPK8) and green fluorescent protein (GFP). Most of these proteins fulfill important functions within cellular processes. RPS6KA2 was recently identified as a potential drug target and seems to be suitable for the development of novel inhibitors for pancreatic cancer therapy. K-Ras works as a regulating molecular switch. Mutations can lead to the production of permanently activated K-Ras (GTP bound) followed by a permanent growth stimulating signal in the cell. MAPK8 is involved in processes such as proliferation, differentiation and programmed cell death. Stress stimulates the MAP kinase signaling pathway where MAPK8 is activated and in turn phosphorylates a number of transcription factors. The two other proteins are commonly applied as a protein expression tag (MBP) or fluorescent label (GFP). DARPin)s are a very promising class of non-immunoglobulin binders that rival antibodies for target recognition. Importantly and in contrast to antibody-derived binders, DARPin)s predominantly bind to structural rather than linear epitopes and thus allow recognition of their respective targets with very high specificities and sensitivities.

In this study, DARPin)s were immobilized, dissolved pure proteins were injected, and the  $K_D$ s were determined based on the measured SPR data ( $K_D$ s between 0.4 and 27.7 nM were found). When injecting a mixture of all proteins, a MALDI MS measurement on the SPRI-chip directly after the SPRI experiment analyzed peptides resulting from an on-chip tryptic digest. This verifies the specific non-covalent interactions even in the low fmol range.