APPLICATION NOTE

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Online SPR- Mass Spectrometry Epitope & Interaction Analyzer

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Bioaffinity analysis using biosensors such as surface pasmon resonance (SPR) has become an established technique for detection and quantification of biomolecular interactions. However, a principal limitation of biosensors is their lack of providing chemical structure information of affinity-bound ligands. Proteolytic excision/extraction (Protex-MS), hydrogen-deuterium exchange (HDX-MS) of peptide backbone hydrogens, and Fast-Photochemical Oxidation (FPOP) are major techniques for mass spectrometry based elucidation of protein- ligand interactions, but none of these tools alone provide quantitative affinity data.

Using a surface plasmon resonance (SPR) biosensor, we have developed a continuous online biosensor-MS combination with electrospray ionization mass spectrometry that enables the simultaneous affinity isolation, structure identification and affinity quantification of biopolymer ligands from a protein- ligand complex immobilized on a gold chip. Key tool of the online biosensor-MS epitope analyzer is a integrated, automated interface that provides sample concentration and in-situ desalting for the direct MS analysis of the ligand eluate [1]. ESI-MS systems from all major manufacturers can be coupled, using a newly developed software. The broad application potential of the online-SPR-MS epitope analyzer has been shown by recent studies of an unusual mixed-disulfide antibody epitope of the rheumatoic target protein, HLA-B27; and the interaction site identification of chaperone complexes of lysosomal enzymes [2, 3]. Interaction epitopes as diverse as antigen-antibody and lectin- carbohydrate complexes [4], and affinity binding constants (K_D) from milli- to nanomolar ranges can be analysed. Applications amenable with the online-SPR-MS epitope analyzer include affinity-based biomarker identification, identification of protein and peptide epitopes, precise antibody affinity determinations, and direct label-free antigen quantification.

To establish and validate the analytical feasibility and accuracy of the online SPRMS interface, direct analysis of horse heart myoglobin (HHM) by ESI-MS and SPR-biosensor was assessed to analysis through the microfluidic interface. Anti-myoglobin antibody was immobilized on a dextran-SPR affinity chip, as well as on a Sepharose™ micro-column. K_D determination of myoglobin and the corresponding anti-myoglobin antibody was performed by investigation of the protein-antibody interaction by SPR biosensor followed by ESI-MS analysis after sample processing through the interface. Subsequent online desalting of analyte prior to MS detection was performed after elution of affinity captured HHM from the affinity chip. Comparative data was recorded by straight analysis of the protein in each separate instrument. Following the analysis of HHM and anti-myoglobin antibody by both mass spectrometry and SPR biosensor analysis, the protein interaction and molecular identification was carried out by using the microfluidic interface.

Myoglobin is a well characterized protein, with molecular masses of 17558,132 Da or 16941,955 Da for holo- and apoprotein, respectively. Native HHM spectra were recorded under physiological pH in order to compare them to biosensor analysis conditions. Subsequent MS revealed denaturing sample processing conditions: HHM samples were eluted from the affinity column and desalted with 0.1 % TFA for ESI-MS analysis. The recorded spectra presented the apo-myoglobin with charge distributions of $[M+14H]^{14+}$ to $[M+24H]^{24+}$ showing the unfolded, denatured protein (without heme). Kinetic studies of the affinity interaction from the biosensor with and without interface coupling were performed. After antibody immobilization, the K_D values obtained were in comparable ranges both obtained through direct analysis and on-line SPR-MS, proving the validity of the procedure: Formation of myoglobin anti-myoglobin antibody complexes exhibited high affinity with a KD of 8.99*E-8 M. The lowest detection limit found during on-line analysis was determined to be in the low micro-molar range.

The new online SRP-MS system is capable to detect and identify the affinity interaction of the myoglobin anti-myoglobin antibody pair in real time and the recorded spectra showed recognition of the apo-myoglobin. The reliability of the online interface was established by repeatable and comparable K_D determinations and precise mass spectrometric identification of protein. Fast on-line sample processing allows fast throughput of different analytes for biomolecular interaction studies.

Online SPR-Mass Spectrometry Epitope Analyzer

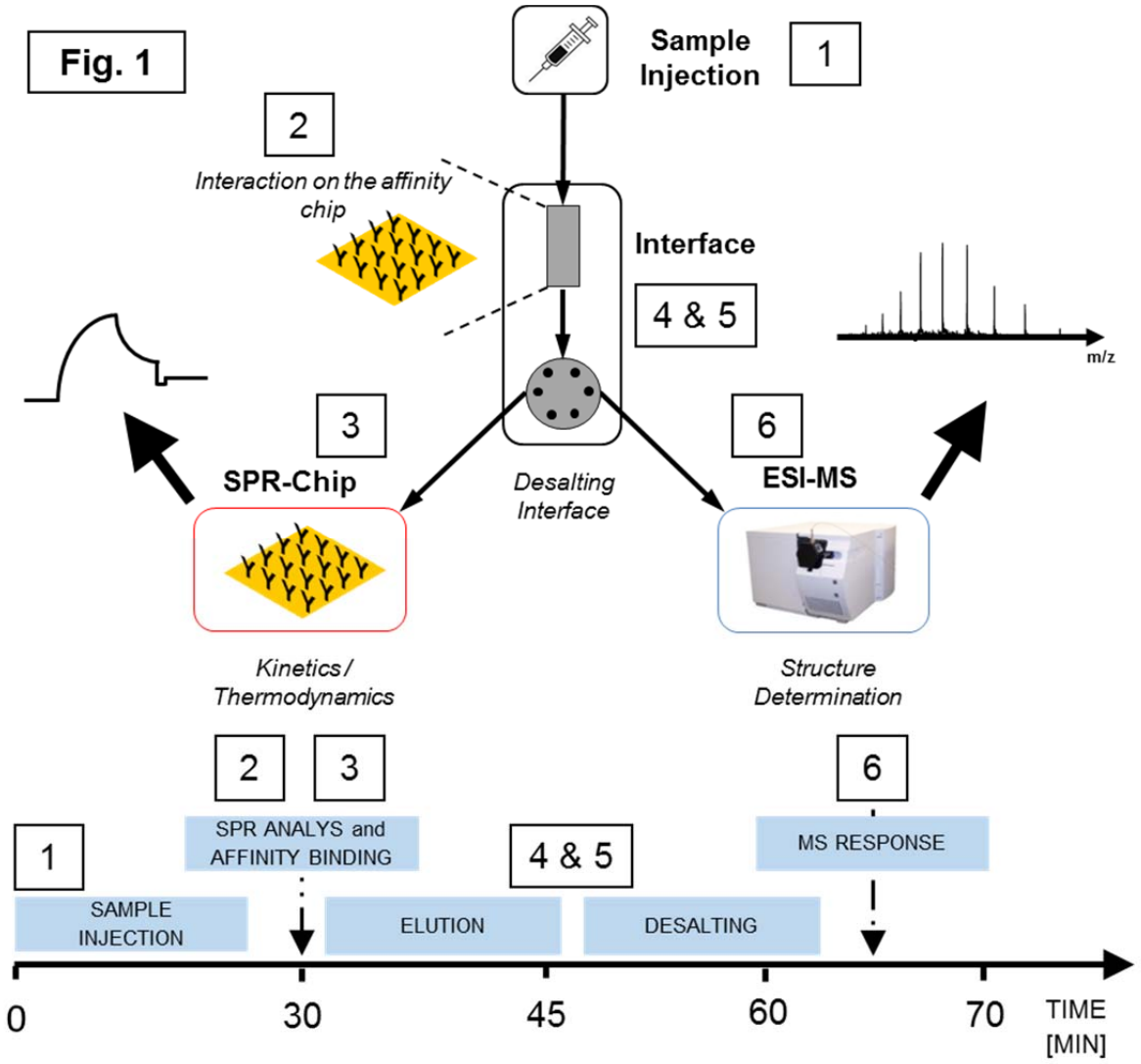

Fig. 1: Schematic workflow of epitope & interaction analysis using the online SPR-MS Epitope Analyzer. After sample injection (1), the analyte is captured on the affinity chip (2) followed by the SPR-chip (3) for kinetic analysis of the affinity interaction. After sample processing through the desalting interface (4 and (5), structural analysis is performed by ESI-MS. Time scale is represented on the time axis below.

Myoglobin – Holoprotein complex Analysis

Fig. 2

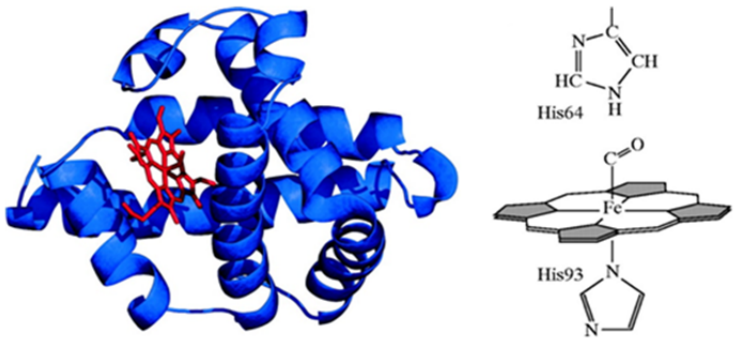

Fig. 2: Ribbon model of the holoprotein complex consisting of the myoglobin protein together with the heme prostetic group, stabilized by histidine residues inside the globular protein.

Fig. 3

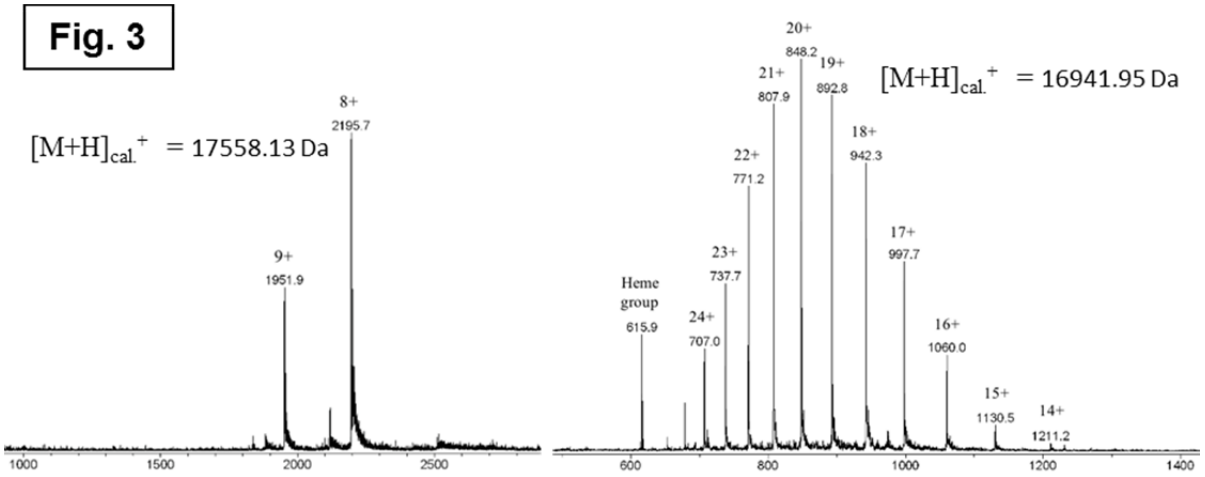

Fig. 3: Direct ESI-MS analysis of myoglobin. Both the holoprotein under native conditions (pH 7) as well as the denatured apoprotein (pH 2) are shown.

Online SPR-ESI MS of native Holo-myoglobin and unfolded Apo-myoglobin

Fig. 4

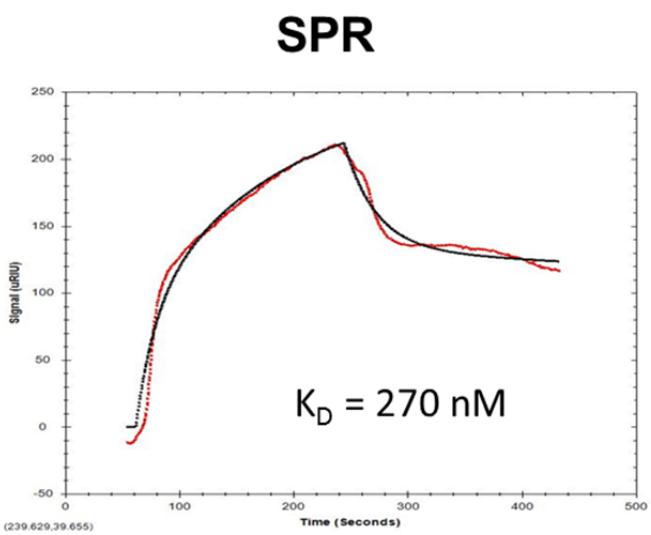

Fig. 4: SPR kinetic evaluation of holo-myoglobin during online processing rendered the calculated K_D of 270 nM.

Fig. 5

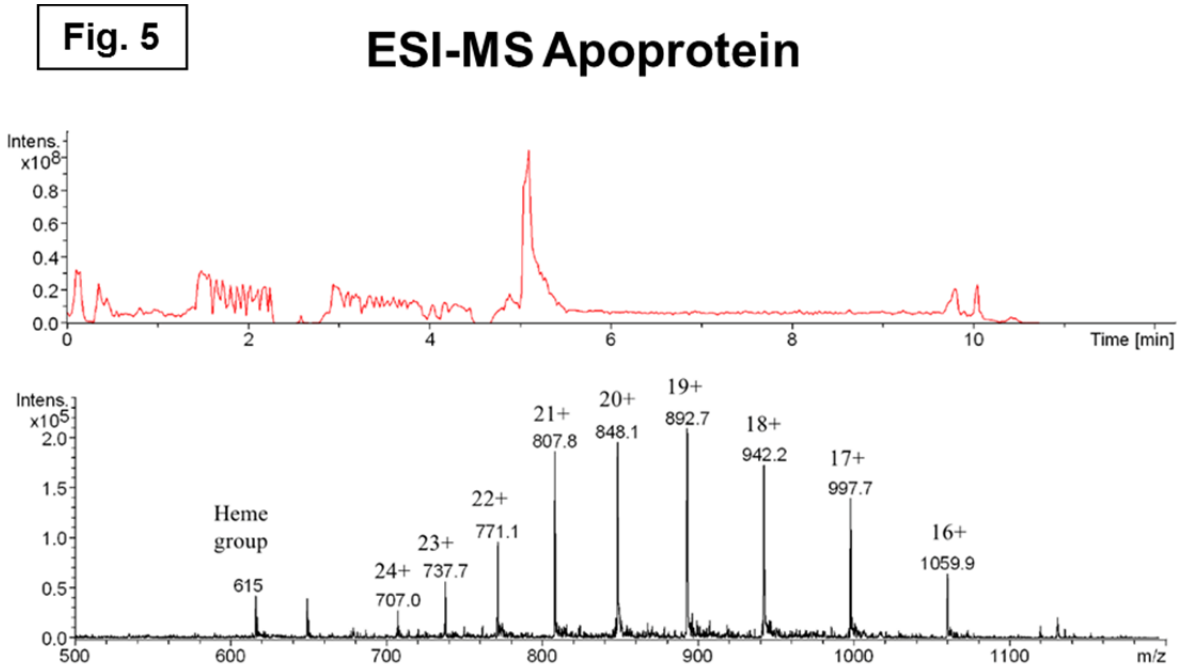

Fig. 5: ESI-MS spectra of apo-myoglobin after desalting via the interface. Total ion chromatogram showes a sharp elution signal around 5.3 min. The multiply charged protonated molecular ions identify the unfolded apoprotein recognized by the antibody (Bruker Esquire 3000+ ESI-MS).