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# Combination of a SAW-biosensor with MALDI mass spectrometric analysis

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**Abstract**

A S-sens® K5 surface acoustic wave biosensor was coupled with mass spectrometry (SAW-MS) for the analysis of a protein complex consisting of human blood clotting cascade factor α-thrombin and human antithrombin III, a specific blood plasma inhibitor of thrombin. Specific binding of antithrombin III to thrombin was recorded as a function of time with a S-sens® K5 biosensor. Two out of five elements of the sensor chip were used as references. To the remaining three elements coated with RNA *anti*-thrombin aptamers, thrombin and antithrombin III were bound consecutively. The biosensor measures mass changes on the chip surface showing that 20% of about 400 fmol/cm$^2$ thrombin formed a complex with the 1.7-times larger antithrombin III. Mass spectrometry (MS) was applied to identify the bound proteins. Sensor chips with aptamer-captured (1) thrombin and (2) thrombin–antithrombin III complex (TAT-complex) were digested with proteases on the sensor element and subsequently identified by peptide mass fingerprint (PMF) with matrix assisted laser desorption/ionization time-of-flight (MALDI-ToF) mass spectrometry. A significant identification of thrombin was achieved by measuring the entire digest with MALDI-ToF MS directly from the sensor chip surface. For the significant identification of both proteins in the TAT-complex, the proteolytic peptides had to be separated by nano-capillary-HPLC prior to MALDI-ToF MS. SAW-MS is applicable to protein interaction analysis as in functional proteomics and to miniaturized diagnostics.


*Keywords:* MALDI-ToF MS; Surface acoustic wave sensor; Thrombin; Aptamer; Protein-complexes

## 1. Introduction

Biosensor research and development has grown rapidly over the years. In parallel, technological advances were achieved in protein analytical and biochemical methods, in structural and molecular biology, electronics, optics, micro fluidics and chemical approaches to surface modification. Biosensors have potential applications not only in basic research, but also in medical diagnostics, explosives detection, food quality determination, genetic screening and environmental monitoring. Mass spectrometry, especially matrix assisted laser desorption/ionization time-of-flight (MALDI-ToF), has become a common laboratory technique in proteomics, protein characterization, protein visualization in cells and even in clinical research. By the combination of both techniques, it is feasible to gain more information on proteins and protein–protein interactions even for clinical applications, useful for detecting biomarker proteins (Nedelkov and Nelson, 2001a; Nedelkov et al., 2006) in diagnostics (Nedelkov and Nelson, 2003).

The S-sens® K5 surface acoustic wave sensors in Love-geometry are highly sensitive towards surface effects and are used to detect and quantify mass and viscosity changes due to biomolecular interactions. Selectivity of the sensor surface for defined analyte molecules can be achieved by adding specific ligands, e.g. antibodies or aptamers. Previously, a S-sens® K5 biosensor with a detection limit of <1 pg/mm$^2$ (Perpeet et al., 2006; Schlensog et al., 2004) was used to characterize human thrombin, a serine protease acting as a component of the blood-coagulation cascade and by use of a sensor-immobilized *anti*-thrombin RNA-aptamer (White et al., 2001). The main role of thrombin as the last protease in the clotting cascade (Holland et al., 2000) is to process numerous enzymatic actions on other blood proteins and on receptors (Coughlin, 2005). Aptamers are nucleic acid-based receptor molecules that are isolated from combinatorial libraries of synthetic oligonucleotides by *in vitro*

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selection on nearly every class of ligand molecules. The resulting oligonucleotides rival antibodies in selectivity and affinity (Ellington and Szostak, 1990; Robertson and Joyce, 1990; Tuerk and Gold, 1990). Aptamers have been used in basic research or drug development (Proske et al., 2005) for aptamer-based biosensor arrays for detection and quantification of different biological macromolecules (McCauley et al., 2003), especially for aptamer-based quartz crystal protein biosensors (Liss et al., 2002) and for many other analytical applications (Tombelli et al., 2005). We have evaluated und quantified the complex formation of aptamer-immobilized thrombin with thrombin-specific inhibitors and natural anticoagulants (Olson and Chuang, 2002) antithrombin III (ATIII) and heparin with a S-sens® K5 biosensor (Gronewold et al., 2005). Analysis of the generated data have shown that heparin activated the formation of tighter ternary inhibitory complexes of antithrombin III with thrombin 2.7-fold of up to 74% of bound thrombin. The immobilization of the RNA aptamer did not inhibit its binding ability to their cognate analytes (Gronewold et al., 2005). Furthermore, affinity captured thrombin has been detected by its molecular mass with MALDI-ToF MS, even from biological fluids (Dick and McGown, 2004).

We describe the combination of the biosensor with MALDI-ToF MS (Schmid et al., 2002). An RNA thrombin-binding aptamer (Jeter et al., 2004) was covalently coupled to an activated self-assembled monolayer (SAM) on a gold surface. Binding of molecules was quantified from the sensor signal. Proteins bound to the sensor surface were digested on the chip and either measured directly with MALDI-ToF MS on-chip, thus avoiding further regeneration procedures. Alternatively, the bound proteins were eluted from the sensor surface, digested the resulting peptides separated by HPLC and further analyzed with MALDI. We here demonstrate the first identification of a thrombin–antithrombin III complex from an aptamer-based SAW-MS biosensor.

## 2. Materials and methods

### 2.1. Reagents

α-Cyano-4-hydroxycinnamic acid was from Bruker Daltonik (Bremen, Germany). Bombesin, human adrenocorticotropic hormone fragments 18–39 (ACTH 18–39), human angiotensin II, Tris(2-carboxyethyl)phosphine (TCEP) and iodoacetamide (IAA) were obtained from Sigma–Aldrich (Taufkirchen, Germany). Human α-thrombin and human antithrombin III were purchased from Haematologic Technologies (Essex Junction, VT, USA). Endoproteinase LysC (sequencing grade) was bought from Roche Diagnostics (Mannheim, Germany). Somatostatin-28 was purchased from Bachem (Basel, Switzerland). Disodium-hydrogenphosphate was bought from J.T. Baker (Deventer, Netherlands). *N*-Octyl-b-D-glucopyranoside (nOGP), trifluoroacetic acid (TFA), $NH_4HCO_3$ and acetonitrile (HPLC gradient grade) were delivered from Merck (Darmstadt, Germany). Ethylenediamine-tetraaceticacid-disodium-dihydrate (EDTA) was purchased from Roth (Karlsruhe, Germany).

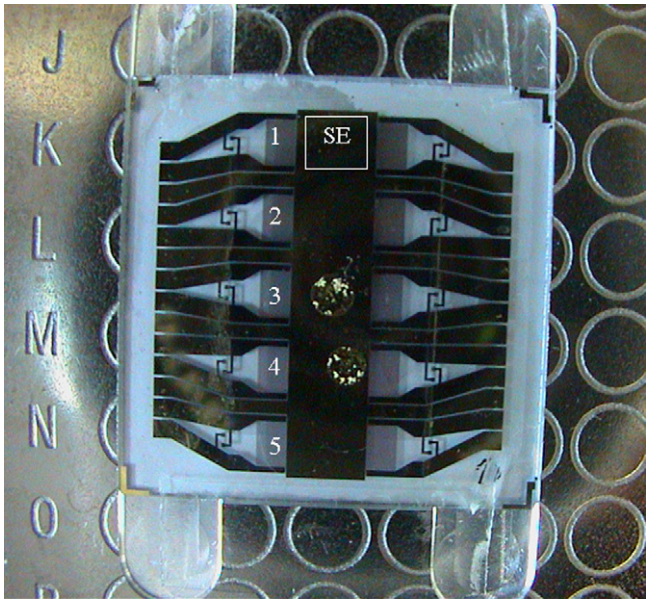


Fig. 1. S-sens(R) K5 chip with five sensor elements (SE) mounted onto a MALDI target plate. Each element is flanked by transducer elements and connectors for electric contact. On the third and fourth sensor element HCCA matrix was added to the dried protein digest mixture for MALDI measurements. On the top sensor element matrix with calibration peptides were applied for MS calibration.

### 2.2. Love-wave sensor set-up and immobilization of aptamers

For preparation of the sensor, an AT cut quartz crystal was processed by photolithography as described previously (Perpeet et al., 2006). The obtained sensor chips of Love-wave sensor type with five sensor elements (Fig. 1) were placed into an S-sens® K5 read out system (S-sens analytics, Nanofilm, Germany), and the detected signals were recorded in real-time using a standard PC. A self-assembled monolayer of 11-mercaptoundecanoic acid (Sigma) was formed on the gold shielding. Its carboxyl groups were activated using 50 mM *N*-hydroxysuccinimide (NHS) and 200 mM *N*-(3-dimethylaminopropyl)-*N*-ethylcarbodiimide (EDC). RNA aptamers with the sequence 5′-$NH_2$–$(CH_2)_3$–GGG AAC AAA GCU GAA GUA CUU ACC C-3′ (Jeter et al., 2004), synthesized by Dharmacon Inc. (Lafayette, CO, USA), were coupled to the activated SAM via a 5′-amino linker (Gronewold et al., 2005).

### 2.3. Aptamer-analyte binding experiments

Binding experiments with human thrombin (33,600 Da) and antithrombin III (58,000 Da) were performed at 23 °C in binding buffer (20 mM Tris–HCl, pH 7.4, 140 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$). Displayed are the phase shifts measured with the Love-wave sensors. First, the difference between sensor and reference signal was evaluated. Bound masses were calculated using the sensitivity of the Love-wave sensor of 515° ($cm^2/\mu g$), under the assumption that the value is generally applicable. Additional sensitivity values have been determined for proteins in the range from 2000 to 200,000 g/mol, showing variations of