DEVELOPMENT AND APPLICATION OF SELECTIVE SYNTHETIC RECEPITORS FOR PROTEIN DETECTION

Summary of the PhD thesis

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1 Introduction

The analytical use of highly selective biological receptors and biochemical reactions, such as antibody-antigen, enzyme-substrate or the hybridization of complementary nucleic acids, has revolutionized the field of bioanalysis. In this thesis we use the term “receptor” to refer to molecular recognition agents able to recognize their complementary target compound through non-covalent interactions. The exquisite selectivity of biological origin receptors led on one hand to the development of biosensors that are able to measure directly the target compound in complex samples and on the other hand to biochips and high-throughput bioanalyzers to detect multiple components and interactions in a highly parallel manner. Despite of all their advantages, biological receptors have also some limitations. Thus, in many cases their application is restricted to experimental conditions closely resembling their natural environment and they are very susceptible to various chemicals and temperature changes. In many cases further difficulties are encountered in terms of cost-effective, large scale and reproducible production as well as long term storage. Therefore, the development of synthetic receptors having appropriate custom tailored physical-chemical properties would represent a significant progress.

With this motivation in mind my doctoral research focused on the development and application of robust synthetic receptors designed explicitly for analytical purposes. Two, conceptually different kind of receptors were developed and evaluated for protein targets: DNA aptamers and molecularly imprinted polymers.

Aptamers are 40-80 mer oligonucleotides able to bind selectively a given target molecule. Although nucleic acid – protein interactions are well known in nature, the sequences of nucleic acid aptamers for a given target compound are determined by a synthetic, in-vitro procedure referred to as SELEX (Systematic Evolution of Ligands by Exponential Enrichment). This involves screening an oligonucleotide library consisting of a large number of random sequences against a target compound and ultimately identifying through a series of washing, separation and amplification steps those sequences, aptamers, exhibiting the highest affinity for the target. Consequently, their applicability is wider compared to antibodies, since aptamers can be selected also for small or toxic molecules, and the establishment of physiological conditions is not mandatory during their application.

Since the first publication of the SELEX procedure in 1990\(^1\)\(^2\) a large number of aptamers have been selected for an impressive range of target molecules.\(^3\) However,


still most of analytically aimed studies are performed in “ideal conditions” on a relatively limited number of well characterized model aptamers, which were not intended originally for analytical application. This means that in the majority of the reports the analytical method development uses aptamers that have not been optimized for the analytical task and consequently, analytical applications that start from the selection of aptamers custom tailored for the intended analytical task are scarce. The importance of such an integrated approaches reside in the possibility of including counter selection steps during the SELEX process to remove from the selection process oligonucleotides binding to critical interfering species present in the sample. Such a procedure has the potential to dramatically enhance the selectivity of the selected aptamers.

Therefore, a major goal of my research was to implement an integrated approach to develop an aptamer-based label-free sensor. As the fast generation of highly discriminating receptors makes aptamers one of the most prospective candidates for the detection of various virus strains and their mutants, we attempted for the first time, the development of an aptamer-based assay against the coat proteins of a plant virus, Apple Stem Pitting Virus (ASPV). To evaluate the effectiveness of the counter-selection process we have developed aptamers to discriminate between two closely homologous virus coat proteins, PSA-H and MT32, with amino acid sequences identical in 81%. The label-free detection of ASPV was ensured by developing surface plasmon resonance sensor chips based on original aptamer sequences, which also allowed the kinetic analysis of aptamer-target interactions.

The second part of my thesis focused on the development of **molecularly imprinted polymers** for protein recognition. Molecularly imprinted polymers (MIPs) are selective, synthetic sorbents prepared from a mixture of functional monomers in the presence of a target molecule. During polymerization, the target molecule acts as a template inducing binding sites in the polymer matrix, which are capable of selectively recognizing (rebinding) the target molecule. Although the universal concept of molecular imprinting proved to be very successful for small molecule imprinting, its application for biomacromolecules is still a major challenge. The bulk synthesis method, with excellent results in generating MIPs for recognition of low-molecular-weight compounds, is hardly applicable to macromolecules due to their hindered mobility in the highly reticulated polymeric networks. Thus, in the worst case the macromolecules become entrapped in the polymeric material with both their removal and rebinding prohibited. Therefore, the essential prerequisite of generating macromolecular imprints should be clearly to create accessible binding sites amenable

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for free exchange of the target between the MIP and the sample phase, i.e. to have them confined to the surface of the MIPs. To address this fundamental limitation we have developed a novel method for the synthesis of surface imprinted polymers compatible with conventional photolithographic technology directly onto the surface of surface plasmon resonance (SPR) sensor chips. Apart from the overwhelming majority of the MIPs prepared by chemical synthesis, we used electrochemical oxidation to form surface-imprinted poly(3,4-ethylenedioxythiophene) (PEDOT) doped with poly(styrenesulfonate) (PSS).

To characterize the synthetic receptors prepared, i.e., DNA aptamers and surface imprinted polymers, in terms of their interaction strength with target proteins I have mainly used imaging surface plasmon resonance (iSPR). This technique enabled besides real-time and label-free monitoring of the relevant interactions also to perform multiplexed measurements aiding the effective optimization of the receptor-modified surfaces. Given the advantages of methods providing high-throughputs but involving simple assay methodology such homogeneous assays I have also developed an Amplified Luminescent Proximity Homogeneous Assay (ALPHA) based method for characterizing aptamer-protein interactions. This method has the prospect to significantly simplify the selection of the best aptamer candidates during the SELEX process.

2 Experimental

Aptamer selection

Plant virus protein specific aptamers were selected using modified SELEX process from a DNA library containing 40-mer random oligonucleotides flanked by two 18-mer primer binding regions. For the selection we used bacterial overexpressed 6×His tagged MT32 and PSA-H ASPV coat proteins immobilized to affinity beads through Ni²⁺-His interaction. The SELEX process consisted of 15 selection cycles, with the amount of target protein decreased after each cycle. Counter-selection was performed after the 3rd, 6th and 9th rounds. Following the selection, to obtain the sequence of the aptamers the PCR product was ligated into the pGEM-T Easy (Promega) vector system and Escherichia coli (DH5a) competent cells were transformed with the ligation mixture. Twenty colonies were picked and sequenced from both selections.

Preparation of aptamer modified SPR sensors

Thiol modified aptamers were immobilized onto gold surface plasmon resonance sensors. For this purpose virus coat protein aptamers were synthetized with “TTTT-(CH₂)₆-SH” modification at their 3’terminus. Mixtures with various molar ratios of the thiolated aptamer probe and thiolated oligo- and polyethylene glycol derivatives were spotted by using solid contact pins (350 mm) and a microarray spotter (Calligrapher Microarrayer, Bio-Rad, Hercules, CA). The immobilization parameters of the aptamers were determined and optimized in terms of sensitivity and selectivity by iSPR
measurements. The surface density of different length of oligonucleotides was determined by voltammetry based on the measurement of the Ru(NH$_3$)$_6^{3+}$ amount electrostatically adsorbed to compensate the negative surface charge of the immobilized DNA sequences.

**Preparation of surface imprinted polymer based SPR sensors**

The protein imprinted PEDOT/PSS micropatterns, i.e., bands of 5 µm width and ca. 200 nm height, were fabricated directly on the surface of SPR gold sensor chips. First a photoresist (AZ 1518) micropattern was generated on the gold coated glass support using photolithography, then a polycarbonate (PC) sacrificial layer was spin coated to fill the gap between the microbands. After removing the photoresist the template protein, avidin (Av, 66 kDa, pI = 10.5), was adsorbed onto the PC micropattern left behind and followed by the electrosynthesis of PEDOT/PSS conducting polymer. The surface imprinted sites on the lateral walls of the PEDOT/PSS microbands were liberated by removing the PC. For characterizing the selectivity of Av surface imprinted polymers ExtrAvidin (EA, pI = 6.5), streptavidin (ST, 60 kDa, pI = 5.5), NeutrAvidin (NA, 60 kDa, pI = 6.3), lysozyme (Lys, 14.3 kDa, pI = 11.35) and bovin serum albumin (BSA, 66.4 kDa, pI = 4.7) were used.

**Characterization of aptamer-protein interaction by iSPR**

The SPR method is suitable for real-time, label-free monitoring of the interactions between surface immobilized receptors and their ligands in solution. Therefore, SPR chips modified with the relevant receptors were first synthesized and the relevant ligands were injected at different concentration levels into the SPR flow cell. Both the association and the dissociation phases were monitored in real time and between two consecutive measurements the surface was regenerated with a suitable chaotropic agent. The resonance angle transients obtained for different ligand concentrations were fitted with theoretical expressions for biomolecular interaction kinetics either by using Origin (OriginLabs Co., Northampton, MA USA) or Scrubber2 (BiaLogic Ltd, Campbell, Australia) software to determine the association and dissociation rate constants and consequently the equilibrium dissociation constants.

**Characterization of aptamer-protein interactions using ALPHA**

ALPHA (Amplified Luminescent Proximity Homogenous Assay) technology is a sensitive method for measuring biological interactions using a microplate reader equipped with AlphaScreen optical module. This technique is based on donor and acceptor nanobead pairs that are modified with the two reaction partners, i.e., aptamer and target protein, respectively. The aptamer-ligand binding brings the two beads in close proximity that triggers by a succession of photoluminescent reactions an energy transfer from the donor beads to acceptor beads producing a luminescent signal. Both saturation and competitive assays were used to determine the $K_D$ values of aptamer-protein interactions. Accordingly, the experimental calibration curves were fitted with dose response curves for 1:1 stoichiometry that takes into account the deviation of the
free target concentration from the total target concentration as well as with the one-site homologous competition model, respectively.

3 Results

Aptamer-based SPR biochip development for label-free detecting plant virus coat proteins

Receptors responsible for recognition should be immobilized on the surface of the SPR sensor chip. Aptamers have large conformational flexibility and bind to their ligands by adaptive recognition mechanism. Thus, their application to SPR requires a more thorough optimization of the surface density and properties of the sensor chips than in the case of antibodies. We have explored the direct attachment of aptamers to bare gold chips by using thiolated probes. We have investigated the effect of the aptamer surface density and the length of coimmobilized spacer thiols and the presence of primer regions (flank) on the specific and non-specific interactions.

For the evaluation of binding affinity and binding kinetics it is important that the measurements reflect exclusively the interaction of the binding partners, thus the non-specific interaction should be negligible. Earlier studies have shown that longer ethylene glycol chains better resist non-specific adsorption of proteins, therefore, we have investigated the applicability of commercially available thiolated poly(ethylene glycol)s (PEG-SH) of MW 5 and 20 kDa as well as thiolated tetra- (TEG-SH) and hexaethylene glycol (HEG-SH). The non-specific adsorption was extremely low for all ethyleneglycol derivatives as tested for with different plant extracts and protein solutions. A slight superiority of PEG-SH was identified, however these molecules in case of mixed aptamer/EG monolayers also reduced the level of the specific interaction (Figure 1). Thus a properly optimized surface layer apparently should involve only spacer molecules that do not exceed the length of the linker used to immobilize the aptamer sequence, as they can otherwise sterically hinder the binding of target molecules. As the aptamers were synthesized with HS-(CH$_2$)$_6$-TTTT linkers at the 3’ end to attain proper spacing between the surface and the binding sequence obviously PEG-SH compounds do not comply with this requirement.

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The effect of the length of ethylene glycol molecules coimmobilized in a mixed monolayer with (a) a reference DNA strand (HS-T44), and (b) HS-MT32 aptamer onto the surface of SPR chips on the recognition of 500 nM MT32 protein.

The largest difference between the specific and non-specific binding was obtained for TEG-SH that was found also suitable for PSA-H detection coimmobilized with PSA-H aptamer as shown in Figure 1. Therefore TEG-SH was used as blocking agent in all subsequent experiments.

![Figure 1](image1.png)

Typical sensorgram showing a succession of experiments starting with the immobilization of thiol modified aptamer (1 µM PSA-H aptamer in 1 M KH₂PO₄), blocking the unmodified surface with TEG-SH (0.1 mM in PBS-T), and finally the binding curves of PSA-H protein injected in increasing concentrations. The upward pointing arrows mark the time instances of the regeneration steps.

![Figure 2](image2.png)

The optimization of the mixed aptamer–TEG monolayers for optimal specific binding was made by changing the three essential parameters that were previously identified as determinant on the surface coverage of self-assembled nucleic acids, i.e., the concentration of the two thiolated compounds (HS-aptamer and HS-TEG) and the ionic strength of the surface-modifying solution. The results show that optimal aptamer–ligand binding, i.e., exhibiting the lowest non-specific and highest specific response, requires aptamer concentrations of at least 5 µM, while the HS-TEG concentration should be kept at its highest value in the studied concentration range, 100 mM (Figure 3.).
Figure 3. Two-dimensional representation of the resonant angle shifts for (A) specific aptamer–ASPV-infected plant protein extract (dilution, 10×) interactions and (B) 1% BSA non-specific adsorption onto the surface as a function of the thiolated aptamer and TEG-SH concentration. The values associated with the individual contour lines are the resonant angle shifts corresponding to a given mixing ratio of the two thiols.

The electrochemical assessment of the optimal aptamer surface coverage gave a value of 1.9×10^{12} molecules/cm^2 for the PSA-H aptamer, which corresponds to an average of 2 molecules on a 100 nm^2 surface. This is, indeed, very reasonable if little steric hindrance between the surface-bound proteins is expected. We also determined the surface densities of 81 (PSA-H-Flank) and 20 (thrombin) base length aptamers immobilized using the same optimal procedure. The results show that with the longer aptamer, the surface density decreases only by 25% (1.3×10^{12} molecules/cm^2), while the use of shorter aptamers results in a more than 3 times larger aptamer surface coverage (6.2×10^{12} molecules/cm^2).

The concentration dependence of the SPR signal was investigated, first for MT32 and PSA-H viral proteins (purified from bacterial lysates) dissolved in PBS (Figure 4.).

Figure 4. SPR calibration curves of (A) MT32 and (B) PSA-H proteins by using MT32 (a), PSA-H (b), and PSA-H-Flank (c) aptamer-modified biochips. The experimental curves are fitted with a hyperbolic equation for specific binding.

Specificity was obtained only in the case of the PSA-H-Flank aptamer, which gave no response at all to MT32 protein. However, the PSA-H-Flank aptamer also
showed a lower sensitivity to PSA-H than the shorter PSA-H aptamer. The sensitivity decrease for PSA-H can be explained by the ca. 25% smaller surface coverage (binding capacity) of the longer aptamer strand as compared with its shorter counterpart. The selectivity order correlates with the dissociation constants ($K_D$) of the aptamer–protein complexes derived from the kinetic analysis of SPR transients. Thus, in the case of MT32 protein, the HS-MT32 aptamer was characterized by a $K_D$ that was lower with ca. 1 order of magnitude than that of the HS-PSA-H aptamer, i.e., $5 \times 10^{-8}$ M as opposed to $2.9 \times 10^{-7}$ M. The PSA-H protein–aptamer dissociation constants also increased in the order PSA-H ($8.0 \times 10^{-9}$ M), PSA-H-Flank ($2.6 \times 10^{-8}$ M), and MT32 ($8.3 \times 10^{-8}$ M), in agreement with the observed selectivity behavior.

For detection of virus coat proteins in real plant samples the mixed aptamer-SH/TEG-SH layers were evaluated in terms of non-specific adsorption. While the TEG-SH surfaces have shown very low non-specific adsorption the mixed surfaces exhibited significant levels of non-specific adsorption (10-35 m°). Therefore, we considered the possibility of having the non-specific adsorption caused by the high negative surface charge densities of aptamer-modified surfaces that by electrostatic interaction can led to the adsorption of proteins with high isoelectric points (pI). The non-specific interaction was tested using 250 nM of avidin (pI=10.5) and NeutrAvidin (pI=6.3). Avidin, which is positively charged at pH 7.4, gave a resonant angle shift of 220.5 (±3.0) m°, while NeutrAvidin, slightly negatively charged at this pH, only gave 8.5 (±0.5) m°. Due to the similarity in size (~60 kDa), the large resonant angle shift obtained for avidin is difficult to be explained by anything else other than electrostatic interaction.

Another conclusion of the experiments aimed to evaluate the extent of non-specific adsorption form various plant extract was that while the non-specific adsorption from a single batch of plant extract was practically the same, the non-specific adsorption varied to a much greater extent from plant to plant and even from batch to batch of the same plant species. Thus the conventional approach to eliminate non-specific effects by subtracting the values obtained for a blank sample is not feasible.

On the other hand it was striking that similar length DNA strands exhibited very similar levels of non-specific adsorption. Therefore, for measuring MT32 spiked apple leaf extract we implemented a reference surface modified with a random sequence DNA strand of similar length as the aptamer probe and immobilized at the same surface density. The obtained data are comparable to those measured in ideal solutions, the longer PSA-H-Flank and shorter PSA-H aptamers showing a very low response, while the MT32 aptamer produced almost linear resonant angle shift increments with increasing MT32 protein concentrations (Figure 5.).
Figure 5. Calibration curves of MT32 in apple leaf extract with the three different aptamer probes. The curves were corrected for non-specific interaction by using the average SPR angle shifts recorded on reference spots consisting of a mixed TEG-SH and a random sequence thiolated single stranded DNA. The experimental data are fitted with a hyperbolic equation for specific binding.

The self-referencing method used for detecting virus coat proteins in plant extracts was tested with the ASPV-positive plant extracts obtained from Bioreba AG (Reinach, Switzerland) (Figure 6.).

Figure 6. Responses to ASPV-infected apple leaf extracts at a total protein concentration of 500 mg mL\(^{-1}\): (a) MT32, (b) HS-PSA-H, (c) HS-PSA-H-Flank aptamers.

The results clearly show that a proper discrimination of the positive and negative controls can be made by label-free detection based on HS-PSA-H and HS-MT32 aptamers. The lack of response of the highly specific HS-PSA-H-Flank aptamer and the highest response obtained from MT32 aptamer suggest that the plant extract contains only the MT32 protein, indicating the presence of the ASPV in agreement with the specifications of the manufacturer.
Figure 7. (A) Background-corrected SPR response curves of HS-MT32-based aptamer chips to ASPV-contaminated apple leaf extracts at different dilutions of the total protein content. (B) Concentration dependence determined for the three different aptamers: (a) HS-MT32, (b) HS-PSA-H, (c) HS-PSA-H-Flank

Figure 7 shows typical response curves for ASPV-infected samples and of the negative control at different dilutions. As expected, the SPR signals decrease upon dilution, and an excellent discrimination of the negative control was obtained. While the lack of response of the PSA-H-Flank aptamer was preserved throughout the investigated concentration range, HS-MT32 and HS-PSA-H exhibited a well distinguishable response in the studied concentration range, which again demonstrates the feasibility of detecting ASPV infections even in considerably diluted plant extracts.

As a control Figure 8 shows the SEM images of an aptamer-modified surface and a HS-TEG protected surface. Filamentous virus fragments (2.2×10^7 virus fragments/cm^2) are clearly visible on the aptamer-modified surfaces, while they are missing on all images recorded on the aptamer-free surfaces.

Figure 8. Typical scanning electron micrographs: (A) of the reference surface without immobilized aptamer and (B) the HS-MT32-modified surface after incubation in ASPV-contaminated plant extract. Fixation with glutaraldehyde and negative staining was used to image the surface bound virus segments. The scale bars are 500 nm.

The selectivity of MT32 aptamer was tested by using plant protein extracts contaminated with ASPV, apple mosaic virus (APMV), or apple chlorotic leaf spot virus (ACLSV). The imaging SPR measurements and subsequent two-sample t test revealed no significant resonant angle shift difference at 0.05 level between the surface
bound reference oligoT and MT32 upon challenging them with APMV and ACLSV-infected plant samples (Figure 9.). These results indicated that the MT32 aptamer is able to discriminate between the different virus species.

Figure 9. Reference-corrected surface plasmon resonance responses to ASPV-, APMV- and ACLSV-infected plant extracts at 100 μg/mL total protein concentration.

Amplified luminescent proximity homogenous assay (ALPHA) for evaluation of aptamer-protein interactions

An Amplified Luminescent Proximity Homogenous Assay (ALPHA) was developed to assess the $K_D$ value of aptamer–protein complexes. The obtained $K_D$ values for PSA-H and MT32 aptamers were $5.3\pm 3.9$ nM and $70.5\pm 28.0$ nM, respectively, in very close agreement with the previously published values determined by SPR: $8$ nM (PSA-H aptamer) and $83$ nM (MT32 aptamer) (Figure 10.).

Figure 10. Saturation curves for PSA-H protein interaction with MT32 (•) and PSA-H (▪) aptamers.

We also explored the feasibility of using a competitive assay format for the determination of $K_D$ (Figure 11.). The $K_D$ of $28.4\pm 16.6$ nM obtained by the competitive assay slightly overestimates the value determined by SPR; however, based on an independent two-sample t-test the results of the competitive and saturation assays were found not to be significantly different at 95% confidence level.
Figure 11. Competitive assay for PSA-H aptamer. The experimental data were fitted with the one-site homologous competition model resulting in $K_D = 28.4 \pm 16.6$ nM

Selective label-free detection of proteins using micropatterned surface imprinted artificial receptors

The schematic of the novel concept to generate micropatterned surface-imprinted polymers (SIPs) for protein recognition using a fabrication methodology that is compatible with standard photolithographic techniques is shown in Figure 12. The synthesis of surface imprinted sites is restricted to the lateral walls of the PEDOT/PSS micropatterns.

![Schematics of the surface imprinting procedure for fabricating molecularly imprinted polymer microbands on bare Au SPR chips (a–f, left side) and the optical (a–c,e,f) microscopy images of the relevant microstructures (right side). In case of (d) the protein adsorption on PC microbands was visualized by using fluorescently labeled protein (avidin-fluorescein isothiocyanate (Av-FITC)) and epifluorescence imaging.](image)

On the surface of the SPR sensor chips both SIP and non-imprinted polymer (NIP) structures were formed to allow the simultaneous measurement of the binding events on both types of surfaces. The additive calibration curves shown in Figure 13. clearly demonstrate the effect of surface imprinting by enhanced Av binding of the Av–SIP micropatterns as compared to the NIP. At the highest Av concentration tested, the response of NIP microstructures was lower than that of SIPs by ca. one order of magnitude, which means that the imprinting factor is among the highest reported for protein MIPs.
Calculated based on fitting the experimental saturation assay data with the hyperbolic equation for specific binding gave a $K_D$ of 125 nM, which is somewhat better than it was reported earlier for surface-imprinted PEDOT/PSS microrods and also more than tenfold lower than that obtained for NIP (1.4 µM).

The selectivity of SIPs was further investigated with respect to Av analogues, i.e., streptavidin (ST), neutravidin (NA), and extravidin (EA) as well as proteins with similarly high isoelectric points (pIs) at the same concentration, such as lysozyme (Lys, pI 11.35) (Figure 14.). The study also included bovine serum albumin (BSA), a protein of significantly lower pI (4.7) but similar molecular weight. In general, the NIPs gave a lower response to the tested proteins than Av-SIPs, which is due to the low protein adsorption on native PEDOT/PSS surfaces. As expected, the highest response on SIP surfaces was recorded for Av followed by EA, NA, and ST (Figure 14.) in close agreement with the structural homology.

Investigating various chaotropic agents for surface regeneration we found 1 M NaCl solution to provide the best results. It is likely that imprinting with a positively

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Figure 13. A) SPR sensorgrams recorded on micropatterned Av-SIP (a) and NIP (b) surfaces upon consecutive addition of solutions with increasing Av concentration; B) the respective cumulative calibration curves fitted with a 4-parameter logistic function

Figure 14. Response of Av-SIP (a) and NIP (b) surfaces for various proteins at a concentration of $10^{-2}$ mg/mL.

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charged protein induces an increase in the concentration of PSS on the surface of PEDOT/PSS films. This phenomenon, would explain the higher affinity of the SIP for proteins of high pI and also the higher adsorption of Lys on SIP than on NIP surfaces. Still, the interaction between Av and SIP cannot be of purely electrostatic nature, as suggested by the higher response to Av and the neutrally charged EA as compared with that to Lys.

The response of regenerated surfaces shown in Figure 15. (A) indicates an insignificant baseline drift but somewhat lower imprinting factors. This suggests that the highly concentrated NaCl solutions might affect the binding sites of the SIP.

Figure 15. SPR response of a micropatterned Av-SIP surface corrected for nonspecific interaction to various Av concentrations with subsequent regeneration between the different samples (A) and the relevant calibration curve (B) fitted with a 4-parameter logistic function.

4 Novel scientific results

1. I have developed surface plasmon resonance (SPR) sensor chips based on original DNA aptamers for selective detection of apple stem pitting virus (ASPV) coat proteins. Using surface plasmon resonance imaging (SPRi) technique I have optimized the immobilization parameters of aptamers on planar gold sensor chips. I have identified and determined the effect of several parameters influencing the selectivity of aptamer based SPR sensors: (a) the presence of primer regions, (b) the aptamer surface density, (c) the spacer molecules, (d) the electrostatic interaction of aptamers with the sample matrix.\textsuperscript{P1}

2. I have shown that by using original counter-selected aptamers the two forms of apple stem pitting virus (ASPV) coat protein can be selectively detected. Using an appropriate reference surface I have developed a label-free method to detect the ASPV virus in infected plant extracts. This method proved to be selective against other plant viruses (apple mosaic virus - APMV, apple chlorotic leafspot virus - ACLSV).\textsuperscript{P1,P2}
3. I have reported the first application of ALPHA (Amplified Luminescent Proximity Homogeneous Assay) for the characterization of DNA aptamer-protein interactions and demonstrated its suitability for determining the equilibrium dissociation constants of the interactions. P4

4. I have developed a novel method based on standard photolithographic technology for in-situ preparation of micropatterned surface imprinted polymer based SPR sensors. Using avidin as model protein target I have shown that this method provides excellent means for the preparation of selective surface imprinted polymers (SIPs) for proteins and that the respective SIP-based SPR chips are suitable for label-free real time monitoring of protein binding interactions. P3

5 List of scientific publications

Publications directly related to the PhD thesis


Presentations in Hungarian

Lautner, G., Növényi vírus burokfehérjék jelölésmentes detektálása aptameren alapuló iSPR biochip kezeléssel, Oláh György Doktori Iskola Doktoráns konferencia, 2010, Budapest

Lautner, G., Syrytski, V., Rappich, J. Gyurcsányi, R., Felületi lenyomatú, mikromintázott polímeren alapuló érzékelés, Kémiai Szenzorok Workshop, 2010, Pécs

Other coauthored presentations and posters


Lautner, G., Szűcs, J., Gyurcsányi, R.E., Bardóczy, V., Mészáros, T., Aptamereken alapuló SPR biochipek fejlesztése növényi vírusok meghatározására, Oláh György Doktori Iskola Doktoráns konferencia, 2008, Budapest


Lautner, G. Rappich, J., Syritsky, V., Gyurcsányi, R.E., Selective Artificial Receptors Based on Micropatterned Surface-Imprinted Polymers for Label-free Detection of Proteins by SPR Imaging, Oláh György Doktori Iskola Doktoráns konferencia, 2009, Budapest

Balogh, Zs., Bardóczy, V., Lautner, G., Komorowska, B., Gyurcsányi, R.E., Mészáros, T., Aptamers as Virus Detecting Molecules, Pittcon, 2010, Orlando FL, USA

Gyurcsányi, R.E., Lautner, G., Mészáros, T., Szintetikus receptorokon alapuló chipek vírusok és proteinek jelölés nélküli meghatározására felületi plazmon rezonanciás képkockatással, Francelab Tudományos Napok 2011, Budapest

Lautner, G., Képkockó felületi plazmon rezonancia bioanalitikai alkalmazása, Francelab Tudományos Napok 2012, Budapest