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Performance testing and commercialization of regenerable biotinylated sensor slide

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Tiivistelmä

Kinetiikka ja sitoutumismittaukset ovat usein keskeisen tärkeitä biolääketieteeseen liittyvässä tutkimuksessa ja teollisuudessa. Erittäin herkkää ja ilman leima-aineita tehtävää pintaplasmoniresonanssiin (SPR) perustuvaa biosensoritekniikkaa on käytetty vakiintuneena menetelmänä biomolekulaariseen analyysiin. Multiparametrinen plasmoniresonanssi (MP-SPR) laajentaa sitoutumisanalytiikan soveltamismahdollisuuksia ja tarjoaa uusia liiketoimintamahdollisuuksia. Kehittyneiden regenerointiominaisuuksien soveltamismahdollisuudet luovat merkittävää kilpailu-etua. Lisäksi mahdollisuudet kasvattaa liikevoittoja biosensorimarkkinoilla ovat lisääntyneet. Bioteknologian ja terveysalojen kannattavuus paranee yhdistämällä dataa ja analytiikkaa yhdessä teknologian ja liiketoiminnan kanssa.

Tässä työssä käytetyt kultapäällystetyt sensoripinnat olivat biotinyloituja ja muunneltu tiolijohdannaisilla. Sensoreiden optimaalista toimintaa tutkittiin regeneroinnin, säilytysolosuhteiden ja ikääntymisen suhteen. Muunneltujen pintojen ominaisuuksia ja laatua tutkittiin BioNavis –yhtiön MP-SPR laitteella. Switchavidiini valittiin tutkittavaksi molekyyliksi sen suotuisien ominaisuuksien vuoksi. Switchavidiinilla on esimerkiksi kyky sitoa reversiibelisti biotiinia ja se soveltuu hyvin biosensorisovelluksiin. Immunoglobuliini G (IgG) valittiin tähän tutkimukseen, sillä vasta-aine on tyypillinen tutkimuksessa käytetty väline. Biosensorimarkkinoita tutkittiin markkinoiden tarpeiden ymmärryksen kehittämiseksi, BioNavis -yrityksen kilpailukyvyn vahvistamiseksi sekä liiketoimintamahdollisuuksien kasvattamiseksi. Tässä tutkielmassa pyrittiin lisäämään ymmärrystä tieteen ja kaupallisuuden välisestä yhteydestä sekä hyödyntämään tutkimustuloksia liiketoiminnan edistämisessä.

Sensoreiden regeneroituvuus säilyi hyvin ja BioNavis voi käyttää tutkimuksen tuloksia myynnissä ja markkinoinnissa. Tulosten avulla voidaan vahvistaa regenerointiprosessin toimivuus ja sensoreille optimaaliset olosuhteet. Sensoreiden käyttöajan ja säilyvyyden arviointi edellyttää

lisätutkimusta. Lisäksi erillinen sensorit ja reagenssit sisältävä pakkaus voisi tuoda lisäarvoa yritykselle. Kokeiden tulokset ja havainnot antavat hyödyllistä tietoa jatkotutkimukselle. Tulosten avulla voidaan lisä-optimoida ja tehdä jatkotutkimuksia, jotka mahdollistavat innovatiivisia liiketoiminta strategioita ja kehittää olemassa olevien asiakkuuksien arvoa. Tämä Pro Gradu työ korostaa biolääketieteen, teknologian ja liiketoiminnan yhteistyön tärkeyttä yritysten ja akateemisen sektorin välillä.

Avainsanat: pintaplasmoinresonanssi, biosensori, multiparametrinen pintaplasmoniresonanssi, diagnostiikka, switchavidin, biotinyloidut sensoripinnat, liiketoimintamalli, strategia, innovaatio, kaupallistaminen, regeneraatio, asiakaslähtöisyys, regeneroitavuus, kultapäällysteiset sensoripinnat

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Abstract

Kinetics and binding measurements are often inevitable in biomedicine related research and industry. The highly sensitive and label free surface plasmon resonance (SPR) biosensor method has been used as an established method for biomolecular analysis. Multi-Parametric Surface Plasmon Resonance (MP-SPR) broadens the application range for interaction analyses and offers new insights for growing business markets. Enhanced regeneration capabilities would create significant competitive advantage possibilities for various applications. In addition, possibilities to gain revenues in the biosensor related markets have been growing. At the forefront of biotech and health related sectors, profitability is often made by combining data, analytics with technology and business.

In this thesis gold coated sensor slide surfaces were biotinylated and modified with thiol derivatives to examine the optimal functionality of sensors in terms of regeneration, storage circumstances and aging. The characteristics and quality of the modified surfaces were investigated with BioNavis multi parametric surface plasmon resonance (MP-SPR) device. Switchavidin was selected for injections because of its remarkable properties and ability to bind reversible to biotin. Immunoglobulin G (IgG) was chosen because the antibody is a relevant and widely used molecular tool. Biosensor related bio business aspects were studied in order to characterize market economy and understand universal needs to create business opportunities in order to grow revenues. Features related for attaining and maintaining competitiveness in biosensor related markets were studied. In addition, the results accomplished in this Master's Thesis work, was aimed to gather additional information for BioNavis in order to enhance further business strategies. Biobusiness orientation was highlighted through the Thesis in order to advance translation of research results to industry and provide understanding about the relationships between science, technology, development and business.

Sensor slides regenerated surprisingly good and BioNavis can use the results as a part of the material in selling and marketing. The functional regeneration process and features for sensor slide's optimal environment can be confirmed with the results. Aging and shelf life estimation needs further research in

order to gain more specific results but provides an agenda for further research. Additionally, the kit could bring further value for the company. The results and observations made from the experiments provide useful data for the further research. Additionally, further optimization and studies can be made with results in order to innovate new business strategy interventions and build cross-sell services. This Master's Thesis advocates further cooperation on biomedical science, technology and business among enterprises and academic sector.

Keywords: surface plasmon resonance, biosensor, multi parametric surface plasmon resonance, diagnostics, switchavidin, biotinylated sensor slides, business model, strategy, innovation, commercialization, customer focus, regeneration, gold coated sensor slides

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Contents

ABBREVIATIONS

CAGR	Compound annual growth rate
CE	Conformité Européene
d_n/d_c	Specific refractive index increment
EC	ELISA coating
ELISA	Enzyme-linked immunosorbent assay
FC	Flow channel
FDA	Food and Drug Administration
GDPR	Greater Data Protection Regulation
IgG	Immunoglobulin subclass G molecule
ISO	International Organization of Standardization
K_a	Affinity constant. Unit M^{-1} is the affinity measure of the binding between two molecules. Higher number indicates tighter binding.
K_d	Dissociation constant. Unit M^{-1}. The smaller indicated tighter binding and thus lower dissociation.
KOL	Key opinion leader
LOC	Lab on a chip
mDeg	Millidegree unit of angle
M_w	Molecular weight
MP-SPR	Multi parametric surface plasmon resonance
NASDAQ	National Association of Securities Dealers Automated Quotations
NYSE	New York Stock Exchange
PBS	Phosphate buffered saline
POC	Point of care
QMS	Quality Management System
R&D	Research and Development
RI	Refractive index

SAM	Self-assembled monolayer
SDS	Sodium dodecyl sulphate
SERS	Surface enhanced Raman scattering
SOP	Standard Operation Procedure
SPR	Surface plasmon resonance
TIR	Total internal reflection

1 INTRODUCTION

Biosensors provide sophisticated technology for quantitative and qualitative analysis of interaction. Biosensor related markets have been growing rapidly (Patching 2014; Vigneshvar et al. 2016). Surface plasmon resonance (SPR) makes real-time analysis possible and sensitivity is high in optical based SPR devices. New ways have been discovered to immobilize analytes with polymers or nanomaterials in order to improve both detection limit and sensitivity of SPR (Patching 2014; Vigneshvar et al. 2016). Yet, sensor slides with both desired surface properties and appropriate bulk properties are challenging to manufacture (Holzinger et al. 2014; Paladiya and Kiani 2018). Most of the challenges are related with the long-term usage of sensor slides and discoveries for better sensor regeneration technologies are needed. Especially from the biosensor markets point of view, competitive advantage can be created by enhancing these properties (Vigneshvar et al. 2016). Additionally, in medical and biotechnological related research and industry, reduction or prevention of undesired biofouling is necessary. Sensor slides may go through diverse of circumstances and environments that are affecting to the aging process and shelf life. The aim of this Master's Thesis was to study the functionality of biotin-coated sensor slides in combination with switchavidin (Taskinen et al. 2014) and their commercial potential. This thesis work represents co-operative work between the Faculty of Medicine and Life Sciences and BioNavis Ltd. in order to advance science and translation of research results to industry and business. Important part of the study was to research and evaluate profoundly what are the optimal circumstances to achieve optimal regeneration cycles in order to advance competitiveness of BioNavis products in the biosensor related markets. The surface chemistry of all the gold coated and biotinylated sensor slides was the same but the storage conditions were different from each other. Altogether four different environments were studied. Each environment had two adjacent sensor slides, respectively. Additionally, one 100 cycle measurement was made to study the regeneration capacity over extended measurement. Measurements were made by using MP-SPR Navi™ 420A ILVES instrument. Altogether nine sensor slides were included in the final analysis. The results of the analysis supported the MP-SPR instrument

combined with the optimal sensor slides as a reliable and valid measurement technology.

Improved regeneration process attributes can provide significant value for business. The present market research studies have been suggested that in the near future surface plasmon resonance related innovations will be even more significant in the field of medicine and pharma. Furthermore, biosensors can be used widely e.g. in the environment, security, defense and food safety areas (Deng 2017; Turner 2013; Vigneshvar et al. 2016). In particular, even though biomolecular interactions have been studied decades based on surface plasmon resonance, developments in the field of biosensor technology may provide e.g. significant enhancement for portable technologies in the near future. This includes the important themes about personalized medicine, a lab on a chip (LOC) and point of care (POC) devices (Nguyen et al. 2015; Zanchetta et al. 2017). Scientific substance knowledge is supporting the Thesis's business orientation and customer-focused approach. Furthermore, post-marketing studies can be made based on the results obtained here.

Regeneration was a specific objective in this research because the challenges related to this process have been recognized universally. However, the events and properties that are determining the regeneration process details, are deeply complex. Aging process and shelf life were additional objectives. Surface related characteristics e.g. chemical and physical properties as well as the topographies are defining surface mechanics and functionalization that are affecting to aging processes and shelf life (Guegan et al. 2014; Tallawi et al. 2015; Yang et al. 2014). Biosensors are broadly utilized and the markets are globally promising. For instance, Grand View Research (2017) have demonstrated that especially optical based biosensors have one of the most fastest growing future perspectives. Chapters addresses the multidisciplinary entities and focuses on business policy and other measures necessary to enhance competitiveness. The cumulative technical and business based continuous learning was achieved during the work.

In this thesis gold coated sensor slide surfaces were modified with coatings consisting of biotin-thiol derivatives in order to achieve optimal functionality and fouling properties. Functionality assessment included regeneration process studies and aging process studies. Switchavidin and biotinylated immunoglobulin G were selected to be

immobilized on the biotin surface. The role of switchavidin is established as one of the most optimal tool for biosensor applications and immunoglobulin G is relevant model molecule to use in the second attached layer. At first, in the review of the literature section, the SPR and MP-SPR are represented with emphasis on physical aspects and assay design insights. Sensor slides and factors affecting the surface fouling are discussed, with a focus on the main characteristics of the research. The review of the literature section is also representing the diversity of the factors affecting bio business with emphasis on biosensor related markets. Potential inventions may not be succeeded without a solid business plan and this topic is discussed. Specifically, the aspects of commercial marketing benefits are regarded. Later, the experimental setup and results are represented. Finally, comprehensive discussion and conclusion are combining the most significant scientific and business-related findings made in this thesis work.

2 LITERATURE REVIEW

2.1 Biosensors and surface plasmon resonance

According to International Union of Pure and Applied Chemistry (IUPAC), biosensor can be defined as a device that can detect chemical compounds. Turner et al. (1987) defined biosensor as an analytical device with sensing element that incorporates with physiochemical transduces (Turner et al. 1987; Thevenot et al. 1999; Wang W. et al. 2017). Clark and Lyons (1962) described biosensors at a New York Academy of Sciences symposium as sensors incorporating with biological material. One of the first enzyme related transducers were then introduced by Clark and Lyons (Clark and Lyons 1962). Surface plasmon resonance biosensing is highly sensitive and label-free technique. Transduction mechanism of biosensor devices are based e.g. on optical, thermal or electrical signals. Surface plasmon resonance is based on optical detection (Homola et al. 1999; Ozkan-Ariksoyal 2013; Thevenot et al. 1999). The measurement technology has been developed based on the biotechnological multidisciplinary work that combines life sciences and engineering. For example, Carrara (2010) describes about how interactions of a laser beam with metallic surface have been studied already in 1960s. The quantum phenomenon of surface plasmon resonance was discovered already then and many applications have been made in the rapidly growing area of research after the discovery. Sensing technique development have been rapid. Systems that are based on a real-time measurement techniques have specific detection methods for analyte detection. Biotechnological advances have been providing wide range of novel biochemical materials with advanced properties (Bosch et al. 2007; Plaxco and Soh 2011, Wilson and Hu 2000).

Besides the real-time monitoring, one of the advantages is the sensitivity of the surface plasmon resonance measurement systems. Nanoparticles and nanostructure usage and development can provide even more sensitive measurement technology methods (Mayer and Hafner 2011). New techniques provide possibilities to make long-term experiments with cells in order to permit cell survival. Cellular responses of the living cells can be monitored in real-time (Vigneshvar et al. 2016; Viitala et al. 2013). Simultaneous multiple analyte detection is relevant in order to make better applications e.g. to measure protein interactions (Heeres and Hergenrother 2011) or screen and detect toxins (Brennan et al. 2012). While proteins can be immobilized to the sensor

slide surface via adsorption to study protein-protein interactions, it is also possible to use biosensors to get dynamic response information about living cell behavior and investigate long-term changes. The surface of the sensor slide must be chemically functionalized in order to detect only the target-analytes that bind to the surface. Modern SPR instruments enable multiple detection of the analytes simultaneously when usually challenges may emerge with only one output channel (Cetin et al. 2014; Dong et al. 2008). In general, both industry and academia can use biosensors in a wide scale. Interest towards biosensors has grown in many different areas such as environment, medical sciences and military field. Biosensors can be used e.g. in drug discovery for validation, concentration management or target identification (Grand View Research 2017, Ronkainen et al. 2010).

2.1.1 MP-SPR

Multi-parametric surface plasmon resonance (MP-SPR) is based on real-time and label free measurement technique like the normal SPR but MP-SPR provides a different optical setup. Both can be used to detect and analyze biomolecular interactions (Lakayan et al. 2018; Sonny et al. 2010). MP-SPR system enables i.e. real-time angular scanning with variable wavelength. Additionally, biosensing can be optimized with the selection of the sensor surface material. Surface plasmon resonance is highly sensitive and fast technique (Lakayan et al. 2018; Sonny et al. 2010). Multiparametric surface plasmon resonance (MP-SPR) devices offer modern technology for molecular interaction studies. Multi fluidic channel systems for high throughput need measurements with modern software offers platform to make advanced assay design. MP-SPR instruments are good options e.g. for surface interaction studies and nanolayer characterization (Kari et al. 2017; Korhonen et al. 2015; Viitala et al. 2013). Additionally, MP-SPR is offering insights for controlled drug release and cell and tissue engineering. MP-SPR can be combined with electrochemistry and catalytic interactions can be measured (BioNavis 2017; Kari et al. 2017; Korhonen et al. 2015; Grieshaber et al. 2008). Table 1 is describing more specifically the comprehensive possibilities that MP-SPR have in life sciences, material sciences and biosensor related assay development and point of care (POC) biosensor development. Table 2 is highlighting the parameters the can be measured only with MP-SPR (BioNavis 2017;

Grieshaber et al. 2008; Kari et al. 2017; Korhonen et al. 2015; Lakayan et al. 2018; Viitala et al. 2013).

	Biosensors		Life Sciences		Material Sciences	
	Assay development	***Development of point of care biosensors***	***Drug development***	***Biophysics***	***New materials and coatings***	***Process optimization of thin film manufacturing***
	Agriculture Food and feed safety Environmental Clinical Border and process control	EC SERS ELISA Fluorescence printed biosensors Nanoparticles	Drug formulation Controlled drug release Drug-cell interaction Nanoparticle targeting Uptake mechanisms	Lipid interactions	Antimicrobial Antireflective surfaces Filter membranes Contact lenses Implants Carbon nanotubes Plasmonic materials Graphene Green materials	Display technologies Solar cells Batteries
Features	Different possibilities of sensing substrate materials (→ variety of markets). In-line bulk effect measurement Simultaneously possibility to determine thickness and refractive index.		Possibility to measure interaction kinetics and layer structural changes. In real-time and label-free possibility to distinguish trans- and para-cellular drug/nanoparticle transport on cell monolayer. In-line bulk effect measurement.		Commercial SPR for material sciences. Biochemical properties of functional coatings can be studied with good sensitivity. Alternating nanolaminate thickness is possible to measure from 5Å, with optical density and its barrier functionality. Simultaneously possibility to determine thickness and refractive index.	

Table 1. Application possibilities of the MP-SPR portfolio products. Table is showing some of the specific features of the MP-SPR technology. The most important features are introduced and highlighted (Modified from BioNavis 2017. Based on Grieshaber et al. 2008; Kari et al. 2017; Korhonen et al. 2015; Lakayan et al. 2018).

Parameters measured only with MP-SPR	**Characteristics**
Intensity of SPR peak min	• in liquid • in gas • on metals and metal oxides • hydrogels - Sensor material and media are depending factors
(Another wavelength pair) *not in device used in these measurements	• Resolving the layer thickness and RI

	• Possible to use for all measurements
SPR-width at 3 different levels	• Defines the shape of the SPR peak • Enables absorbing coating and sample characterization because the absorption of light by the binding molecules is informed
Steepest falling and raising slope	• The most sensitive measurement area is shown • For the quick fixed angle measurements
Total Internal Reflection (TIR) value	• Utilized for dn/dc calculations • Bulk properties around the sensor are depending factors

Table 2. Characteristics of the SPR/MP-SPR technology. Angular position of SPR peak min can be measured with both traditional SPR and MP-SPR but the parameters listed here can be measured only with MP-SPR (Modified from BioNavis 2017. Based on Grieshaber et al. 2008; Kari et al. 2017; Korhonen et al. 2015; Lakayan et al. 2018; Viitala et al. 2013).

2.1.2 Physical aspects

Plasmons are solutions to Maxwell's equations that is valid for certain metal-dielectric geometries (Maier 2007). The polarization for a metal nanoparticle is enhanced at certain frequencies of light. Phenomena is occurring for visible light in noble metals (Maier 2007). By the use of subwaveleght structures, the propagation of light can be controlled. In science and technology, plasmonics is an emerging area and the applications of this phenomena includes SPR sensors (Anwar et al 2017). Common plasmonic biosensors are utilizing refractometric detection (Anwar et al 2017; Quinn et al. 2000; Špačková et al. 2016; Wang S. et al. 2017). By optical spectroscopy, changes in intensity of light for different wavelengths can then be detected.

Surface plasmon resonance (SPR)-based transducers measures plasmon resonance angle change. Angle change can be measured using light which is reflected from the sensor slide's surface. Surface with the immobilized receptors is enabling the detection of the binding and release events (Anwar et al 2017; Quinn et al. 2000; Špačková et al. 2016; Wang W. et al. 2017). The surface properties of the sensor slides can vary a lot. For example, sensor slide can be gold coated with the specific surface

chemistry. Wide range of sensor surface materials from inorganic layers (SiO2, TiO2) to functionalized sensor surfaces such as carboxymethyldextran (CDM) are available (BioNavis 2017; Patching 2014). SPR angle change is growing when the mass that bound onto the surface increases (Cooper 2002; Homola 2008). SPR based devices can be based on a technology that have an influence on a refractive index change that can be monitored (Sonny 2010).

This thesis introduces shortly the basis on the physical phenomena behind SPR biosensing because it is relevant to understand the core science behind the biosensor functions. The profound explanation of the physics is left out because the most specific focus in this thesis is in the biomedical research related to the bio business aspects.

2.2 MP-SPR Assay Design and Reagents

There are many available methods to immobilize bait molecules on biosensor surface. In label-free biosensors the detection is based on the reaction where the binding of the analyte molecules occurs on the sensing surface (Pollheimer et al. 2013). Sophisticated MP-SPR devices includes run table to help to design the assay. Flow setting can be adjusted optimally in order to prevent diffusion effects and to reach Langmuir behavior. Higher diffusive flux can be reached with small sensors because of the edge effects and on the other hand multiple molecules are lost due the flow that reduces capture efficiency (Dahlin 2017).

2.2.1 Switchable mutant of avidin

Switchavidin is a chicken avidin mutant suitable for manufacturing of regenerable functional layers on biosensor surface (Pollheimer et al. 2013; Tallawi et al. 2105 Taskinen et al. 2014). Avidins are homotetrameric glycoproteins (MW 68 kDa) found in egg white with four identical subunits. Avidins are able to bind biotin with high affinity ($K_d \sim 10^{-15}$ M) and specificity. Switchavidin can be used for the reversible immobilization of biotinylated sensor surfaces because switchavidin is displaying a reversible binding to biotin. Reversibility possibilities are better when attaching biotinylated biomolecules to biotinylated substrates via switchavidin bridges (Holzinger et al. 2014; Pollheimer et al. 2013). Due the reduced surface charge, switchavidin has a low nonspecific binding capacity but an excellent binding affinity toward conjugated biotin. Vitamin H is also known as biotin and its MW is 244.3 kDa.

The four biotin binding sites of (switch)avidin enable the stable complex formation between switchavidin and biotin. (Pollheimer et al. 2013; Tallawi et al. 2105 Taskinen et al. 2014).

Furthermore, this kind of immobilization method enables the reproducibility of the biosensoring. The ideal situation is to have a rapid method for the reversible immobilization of biotinylated bait molecules on biotinylated sensor slides and switchavidin has been used in this thesis work because of these characteristic properties (Pollheimer et al. 2013; Tallawi et al. 2105 Taskinen et al. 2014).

2.2.2 Immunoglobulin G

The immunoglobulin G (IgG) molecules are produced by the adaptive immune system in humans and other vertebrates. These molecules are also called antibodies. Antibodies are glycoproteins (Grieshaber et al. 2008; Panda and Ding 2015). In this research, selected biotinylated antibody IgG is used as for a verification of the biosensor functionalization after every switchavidin injection. Antibodies can bind to particular target molecules with excellent specificity and affinity. These properties make antibodies suitable for MP-SPR experiments and verification. Antibody fragments are also used as a reliable alternative to antibodies (Grieshaber et al. 2008). Usually antibody consists of two Fab' binding sites and one Fc. Antibody fragments are smaller and suitable for biosensor applications. Binding is highly specific, even though part of the heavy chains is missing in Fab fragments (Grieshaber et al. 2008). Antibodies can be easily engineered and they can recognize peptides, proteins and almost any other structures.

2.2.3 Sodium dodecyl sulfate

The optimal situation is when biotinylated bait molecules can be immobilized and removed as often as needed. There are naturally occurring limit for the time span for sensor slides and furthermore for their optimal functionality (Pollheimer et al. 2013; Taskinen et al. 2014; Zauner et al. 2016). According to Zauner and co-workers (2016) the combination of 0.25% sodium dodecyl sulfate (SDS) and 2.5% citric acid (pH 2) can cause the instant cleavage for the bridges that switchavidin and biotin form i.e. biotin-avidin-biotin bridges and the same strategy was utilized by Pollheimer et al. (2013).

2.3 Sensor slides, surface related factors and materials involved in the SPR experiments and technology

2.3.1 Sensor slides and surface architecture - Surface materials and modifications.

SAMs are self-assembled monolayers that present the variety of the polymers that can be used on the surfaces of the sensor slides (Chapman et al. 2000; Grieshaber et al. 2008). Metallic surfaces such as gold can be coated with self-assembled monolayers (SAMs) of sulfides (thiols). Highly organized functionalized structures can be formed with thiol-containing molecules (Chapman et al. 2000; Grieshaber et al. 2008). Chemical and physical modifications e.g. grafting and cross-linking have been enabling new ways to customize material properties (Benhabbour et al. 2008; Müller et al. 2017). The assay design defines what polymers and substrates to use. For example, the sensor slide's surface can be biotinylated using biotin molecules conjugated into thiol groups like in this thesis work. Alternatively, direct immobilization by covalent derivatization can be made e.g. with aldehyde modification or amine coupling (Vachali et al. 2015). Furthermore, because of the biotinylated surfaces, the switchavidin was used in this thesis work to form biotin-avidin bridges, followed by biotinylated IgG for the verification of the avidin attachment.

Reagents and polymers must be carefully chosen. There are multiple ways to immobilize ligand on the sensor surface such as using thiol modification. Both adsorption and binding events are detected due the changes in the surface plasmon waves and in the local index of refraction (Lakayan et al. 2018; Pollheimer et al. 2013; Taskinen et al. 2014).

2.3.2 Surface materials and modifications - Adhesion to gold-coated surfaces

Gold is the metal that is often used in biosensor technology. Sensor surface needs to be conductive and compared to other metals, gold is an inert metal and it does not cause toxic side effects (Long et al. 2013; Spivak et al. 2013; Zhang 2010). Nanogold and colloidal gold have been considered as one of the most promising biocompatible materials for many healthcare applications because of the electronic and tunable optical properties. (Long et al. 2013; Spivak et al. 2013; Zhang 2010). Thin layer of

gold on the surfaces, approximately 50 nm, is used for supporting the electron plasmon. The downside of this metal layer is where the incident laser beams and reflected laser beams are controlled. The metal layer`s topside is used to immobilize probe antibodies. Furthermore, the topside is utilized for protein and antigen interaction studies by using a microfluidic system (Carrara 2010; Ray et al. 2010). This thesis focuses on gold plated sensor slides. Besides the research, the specificity of the sensor slide's surface chemistry is related to the commercialization and marketing entirety.

2.3.3 Functionalized sensor slide and the biochemistry

The functionalized sensor slide is the bio recognition element in surface plasmon resonance (Liu et al. 2015). The biotinylated gold surface makes possible that the switchavidin can attach to it. Running buffer in flowing through the sensor surface. Ligand gets immobilized on the sensor chip and the analyte is flowing through the sensor slide surface (Patching 2014; Vachali et al. 2015).

2.3.4 Biosensors and transducers

SPR method is providing information about the changes on the sensor surface in real-time and the signal is transduced into optical response. Different types of biosensors have different types of characteristics and application possibilities. There are variety of transducers to use in the detection, depending on the application (Grieshaber et al. 2008; Soper et al. 2006; Tothill 2009). Ideal biosensor can meet many requirements like specificity, sensitivity, real-time monitoring possibility with high-throughput detection system for multiple analytes and the recording possibilities for reversible responses. Biosensors and specifically the transducers can achieve more possibilities in one application with the new methods of fabrication techniques. Interdisciplinary knowledge from areas such as electrical engineering and bioengineering, medicine, quantum, bioelectronics surface physics and biochemistry are required (Grieshaber et al. 2008; Soper et al. 2006; Tothill 2009).

2.4 Data analysis

Reporting and processing biosensor related data requires a lot of manual performing but developed software platforms and tools are helping to interpret big entities of data.

Surface plasmon resonance data analysis tools may vary a lot depending on the manufacturer. Data analysis is time consuming and high requirements are set. Multiparametric data analysis requires robust and accurate responses from surface plasmon resonance systems (Dahl et al. 2017).

2.4.1 Association and dissociation

Association phase is the first phase when the target analyte is binding (Homola 1999). In the next phase molecules are constantly binding to the surface and dissociating from it even though binding seems to reach equilibrium and a plateau. Both affinity constants and external factors i.e. temperature and buffer composition are affecting to binding and dissociating events. The accessible binding sites on the sensor slide surface are never fully occupied. This is the stage when the affinity of the analyte-ligand interaction can be seen (Homola 1999, Homola 2008). Final phase of biosensor measurement cycle is typically the dissociation phase (Cooper 2002, Homola 2008). In reality, the surface of the sensor is a dynamic system as some binding and dissociation is constantly occurring. This needs to be considered already in the assay design phase. Injection times, temperature, concentration, buffer composition and all the washing steps are meaningful in the assay design. First, when equilibrium is reached, the total number of bound molecules can be calculated. Second, the washing steps are important for the dissociation phase to become visible (Cooper 2002, Homola 2008).

2.4.2 Regeneration process

Total dissociation of the bound molecules is occurring in the regeneration process as described in Picture 1. Regeneration condition selection is delicate as descripted in studies made by Pollheimer et al. (2013), Taskinen et al. (2014) and Zauner et al. (2016). Regeneration conditions in this thesis were selected based on these studies and because of that, the literature of review is underlining these conditions. The phases from association to regeneration phase are repeated after the regeneration pulse. After every regeneration pulse, the aim is to retain the target molecules (biofunctionalization layer) on the surface. In the ideal environment, the original and functionally optimal conditions are retained. Some of the biosensor related challenges are associated with the regeneration process (Vigneshvar et al. 2016).

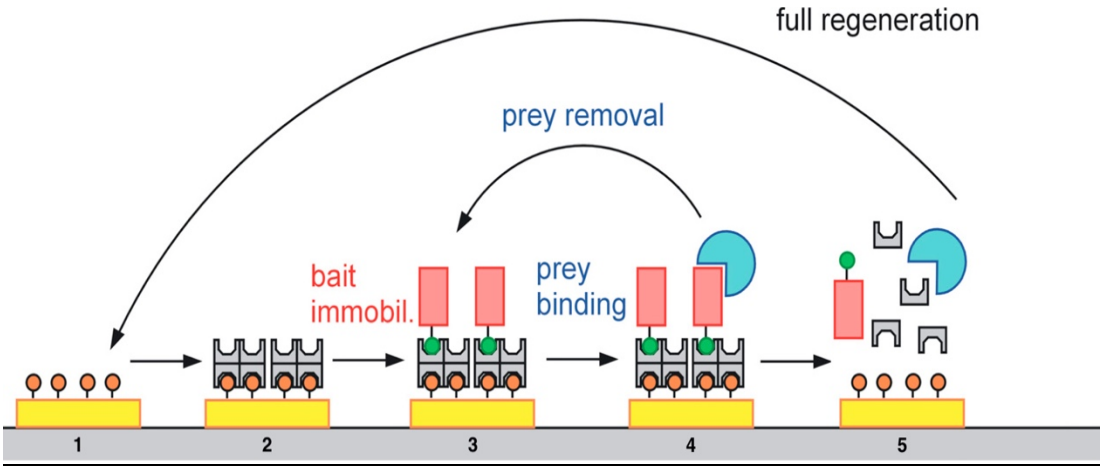

Figure 1: Full regeneration cycle of biotinylated biosensor surface. Removal of switchavidin (5) is a key feature as gold coated biotinylated sensor surfaces can tolerate rigorous regeneration cycles. Four binding sites of switchavidin are illustrated (2). The switchavidin interaction mechanism towards biotinylated sensor surface (1) and binding of biotinylated IgG (3) on it are described in the picture. Modified from Knoglinger et al. (2018).

2.4.3 Sensor performance and aging process

Product performance knowledge is useful and important for end users. For example, depending on the product, physical stability and kinetic stability can be estimated. The shelf life of a sensor slide is depending on many things. In this thesis estimations about sensor slides' shelf life and aging process are made because this information is supporting the experimental part's data analysis. Accelerated aging test can be used in order to determine lifespan of product and the tests are made especially for novel products to become introduced into market. Accelerated aging test can include e.g. heat, oxygen to speed up the normal aging process of sensor slides. Different temperatures and humidity conditions can support accelerated aging tests (Maxwell et al. 2005). In many cases, packaging information of the product must include and provide an assessment of the shelf life.

2.5 The variety of biosensor applications possibilities

Specificity in a real-time analysis and low detection limit for analyte detection is

important e.g. in a disease diagnosis (Kim et al. 2015). Biosensor applications can utilize e.g. a combination of electrochemical sensing and high-throughput biosensors. Drug-cell interactions can be monitored in real time with SPR that is a huge advantage in the field of drug discovery and development. Furthermore, the measurements can be done under constant flow e.g. living cells can be monitored under dynamic conditions (Knoglinger et al. 2018; Viitala et al. 2013). Lateral-flow technology is used in MP-SPR devices to induce the formation of specific interactions (Kari et al. 2017; Liu et al 2015; Viitala et al. 2013). SPR technology provides excellent time resolution and it makes possible to report volume changes in living cells attached on the biosensor. Cell volume regulation and the changes in it are often very important indicators for many cell pathologies.

MP-SPR is also suitable method for material sciences. It can provide optimization for solid films in a wide range from ångströms to 100 nanometers. Kinetic parameters can be solved with the SPR technology and its applications (Kari et al. 2017; Liu et al 2015; Viitala et al. 2013). This means that e.g. K_a and K_d can be estimated with the SPR instrument. Different biosensor applications can be used for many purposes in the field of medicine. Besides medicine, the markets are wide in the field of environment, food safety, defense, agriculture, industry and security.

2.6 Biosensors and the markets

There have been continuous advances in biosensor applications and future estimations for the markets are great. Picture 2 describes how in April 24, 2018, the S&P Biotechnology Select Industry index turned in a 16 percent return from over in a 10-year period. Total price returns for S&P biotechnology selected industry index as an index level from a same period was almost 6.9 units (S&P Down Jones Indices, 2018) The index knowledge is relevant for investors. Biosensor markets are valued to be 27.06 billion dollars in 2022 (Markets and Markets 2017) and according to Report Buyer's market research (Report Buyer 2017) the biosensor markets were estimated to be 16.9 billion dollars in 2017. Both international research and industry requires reliable products that can offer automatization and cost-effectiveness. Medical and health related areas have tremendous markets. There is estimated to be significant growth in the non-medical markets as well. Companies are about to make significant growth also in such areas as environment, agriculture and food production. According

the governmental initiatives global biosensor markets are expected to accelerate also by the development of genomics and proteomics (Grand View Research 2017).

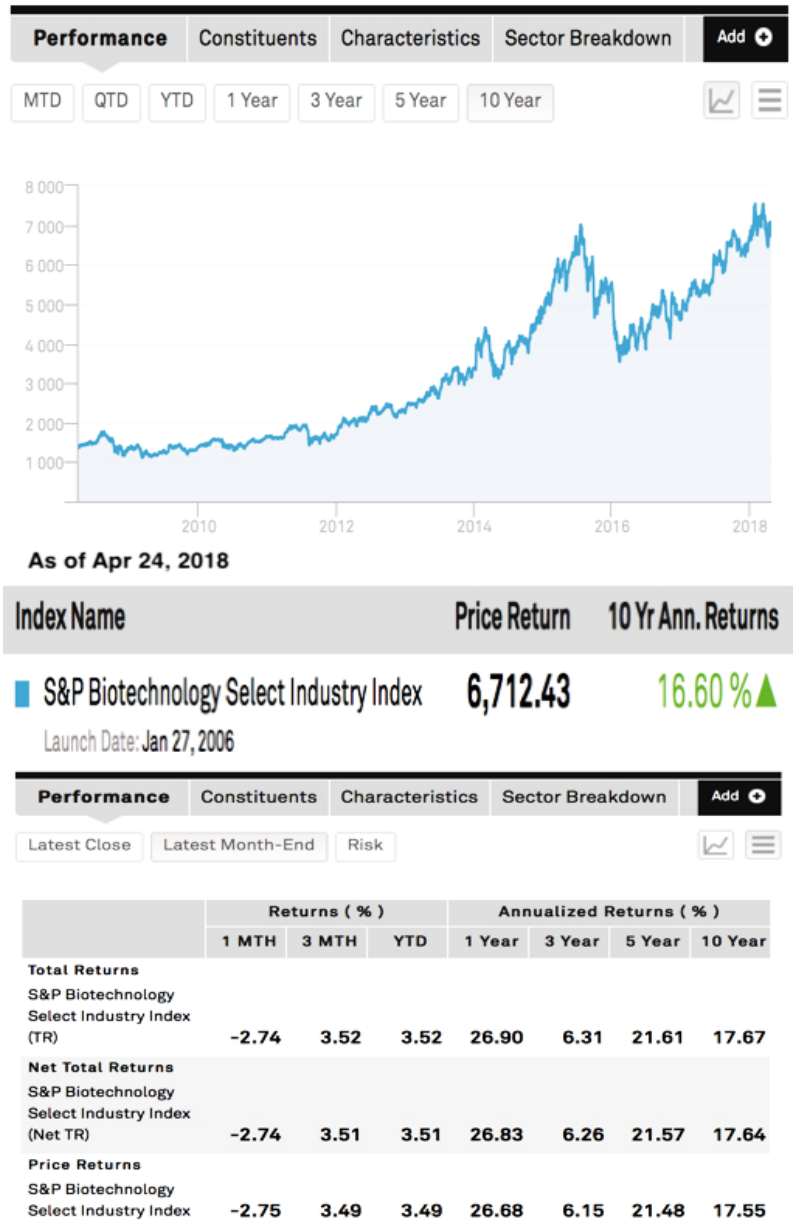

Figure 2. S&P Biotechnology Select Industry Index. S&P Dow Jones Indices. From Jan 27, 2006 to Apr 24, 2018. S&P is an American stock market index. It is based on having common stock listed on NASDAQ and NYSE by the market capitalizations of 500 large companies (Modified from S&P 24.4.2018). The most noticeable is that S&P Biotechnology Select Industry index turned in a 16 percent return from over in a 10-year period.

Biosensor markets can be categorized based on the technology; optical, electrochemical, piezoelectric and thermal (Grand View Research 2017; Ozkan-Ariksoysal 2013). Especially electrochemical biosensor segment markets have been

very successful. Many significant applications for quantification and analysis have been invented and sold related to the biological and biochemical processes (Grand View Research 2017; Ozkan-Ariksoysal 2013) The fastest growing segment is excepted to be the optical segment of biosensors. In this light, SPR and MP-SPR related applications have very potential future perspectives. The optical sensors have multiple application possibilities e.g. related to structural studies, receptor-cell interactions studies, kinetic studies, equilibrium studies and concentration studies (Grand View Research 2017).

2.6.1 Commercializing successful biomedical technologies

Business today is quite turbulent. Standards in health technology and industry have raised as well as the standards in the environment and food safety areas. Real impact is delivered by having sufficient internal recourses and by creating fast solutions while improving the productivity of assets constantly (Turner 2013; Wirtz et al. 2016). Success in the bio business field requires interdisciplinary work. A great innovation is only rarely enough to gain success in the markets (Dollar and Kraay 2003). Label-free biosensors can be developed for various detection methods and wide application possibilities can be provided for markets (Citartan et al. 2013; Turner 2013). Furthermore, various commercializing possibilities can be created via this basis. Commercializing process has its unique power to elevate the innovation and the final product to the next level. People with negotiation skills are needed to close the deals and help to gain repeated further purchases. Substance knowledge is important but social and emotional intelligence is needed when negotiating deals with the customers. Companies have to individualize customers and context. They also need to prescribe and deliver the easiest and fastest solutions for the customer needs (Grand View Research 2017; Neethirajan et al. 2018). Companies have to be able to improve self-service tools, troubleshooting, present solutions, resolve issues and customize the interaction. Successful commercializing process is big entirety (Nevens et al. 1990; Ozkan-Ariksoysal 2013; Sveiby 1997). Navigating company to more profitable future is often reflected by the diversity of interdisciplinary team work. Profit oriented commercialization processes can provide opportunity for further expansion and revenue generation. For example, in the pharma industry aligned medical and

commercial actions can be used. Early iterative engagement with payers, providers and key opinion leader (KOL) can support market shaping (Sax 2016).

2.6.2 Translating the technology into success; from lab to business

Building and sustaining competitive advantage from lab to business can bring unexpected challenges. Scale of challenges exists in the strategy formulation, planning and business development, also distribution and logistics have their own challenges. Furthermore, successful marketing, selling, customer service, sales and account management have significant positive impact on earnings (Neethirajan et al. 2018; Ozkan-Ariksoysal 2013). Creating a successful route from lab to business means revenues that are increasing faster than expenses. And it requires understanding about how to mobilize the right resources and not to focus only on short-time results. It is an asset to have investors who can recognize if company's strategy is not working and identify investment risks. Value-conscious segmented market thinking is needed in business. Costly but ineffective technologies are the worst scenario and it is essential to define the end user, customers and understand the customer needs (Nevens et al. 1990; Pellikka et al. 2012; Thevenot 1999). Laboratory circumstances and easy data capture may not always be possible and biosensor applications can offer solutions for these issues. It requires a lot to translate the technology into a very successful business; from lab to business Nevens et al. 1990; Pellikka et al. 2012; Thevenot 1999) Great business intelligence creates more value to increase the cash flow in the range that desired and have more revenues.

2.6.3 Business model – innovation

A great business model can make a significant difference when multiple innovations and products are competing against each other in the markets (Stähler 2002). Real advantage possibilities are laying in the marketplace and the business model have to offer extensive support for the company (Wintjes 2006; Wirtz et al. 2016). SPR and MP-SPR are technology driven-innovations that can offer optimal solutions for the customer needs in many ways as demonstrated in Table 1 and Table 2. Yet, markets are indicating that even better solutions and business models can be created. Current business world is dynamic, demanding a lot multidisciplinary knowledge and skills. In the field of biotech products, lots of research and development (R&D) have been done

before finalizing the product. Constant resilience is needed all the time. Creating new value is important because competitive advantage is not sustainable. Furthermore, this indicates towards ideology where strategies and business models needs to be smartly updated. (Teece DJ 2010; Wirtz et al. 2016). The heart of the innovations and business models are the customers and their needs. Building sustainable long-term client relationships in the dynamic business world can be challenging. Balancing between risks and opportunities may even lead to difficult strategic decisions (Amit and Zott 2012; Osterwalder and Pigneur 2005). Finally, a successful biotech business cannot be built without great management skills that are the essential core of the business. Great management skills are often combination of business intelligence and comprehensive substance knowledge (Branstetter et al 2014; Gibbons et al. 2010) e.g. from the field of biomedicine, biotechnology and business.

2.6.4 Product and services

Different instruments, devices and products are constantly heading to be far more sophisticated than before. Lots of R&D is required before finalizing the product and developing a knowledge based economy requires adequate levels of investment besides R&D (Wintjes 2006). Product improvements and even some new product opportunities can be generated while resolving user and client experiences in later phases. The most important is to be able to clarify the value of the new product or design for the intended user. The biosensor related technology area is really interesting regarding the markets. The technology in itself can be really specialized. Yet, there are laying great possibilities to attract really wide customer bases from different fields of technology and industry. Novel, useful and liable applications in the forefront of health industry have really wide customer bases (Grand View Research 2017; Neethirajan et al. 2018; Ozkan-Ariksoysal 2013). Biosensor related portable devices, kits and services have remarkable potential to create business value. Multiple possibilities in more than one technology and industry area will help to increase the connections and odds to build long-term success (Wintjes 2006). Besides the product, brilliant software can bring significant value for the company. Continuous development is necessary because markets are evolving rapidly, especially with data-enhanced products (Delgado et al. 2012; Schwab 2017).

2.6.5 Regulatory constraints and quality controls in the cycle of development

The awareness of different intellectual property types is needed in the field of bio business and it is relevant to make sure to understand what is needed for patent procedure. Also in biotech -industry some trade secret may be difficult or even challenging to maintain. When it comes to business models and business thinking, it is essential to be aware of intellectual property rights and also have knowledge about options e.g. licensing agreements and royalties. Manufacturing takes regulatory compliance, validation and standards to fulfill (Brunet 2012; Van Norman 2016). Device development also takes its own time and needs optimization and design. The Food and Drug Administration (FDA) has a significant role i.e. in the drug and device approvals as in Europe the marketed medical device must have a Conformité Européenne (CE) mark. Tens of notified bodies are operating in EU are there are differences in safety, performance and clinical efficiency aspects depending on the authorization (Brunet 2012; Van Norman 2016). Additionally, new greater data protection regulation (GDPR) is setting some new standards and practices for the industry. The scope of GDPR is extensive and compliance policy is strict (European Parliament 2016).

2.6.6 Quality Management System

A quality management system (QMS) is a collection of business processes. ISO (International Organization of Standardization) 9001:2008 was updated to ISO9001:2015 because there have been significant changes in business needs and expectations (Croasdell 2001; International Organization of Standardization 2015; Thevenot 1999). Standards are implementing and maintaining e.g. organizational processes, documenting, polices and goals. QMS focuses to improve customer satisfaction and align the organization's strategic direction. Different management system standards are harmonized by ISO. QMS is applicable to any size or type of organizations. ISO9001:2015 sets out criteria for QMS and it is certifiable. The new version of ISO9001 includes separately e.g. product and services section, instead of product in order to make services more applicability. Risk-based thinking is introduced more profoundly in the new standards in order to adapt it depending on the organization or business situation. ISO9001:2015 leadership requirements have

increased and integrated management system has opportunity to address elements such as environment (Croasdell 2001; International Organization of Standardization 2015; Thevenot 1999) QMS principles always includes strong customer focus and according to Nevans (2001) “manufacturing quality is marked as one of the key elements that would give firms an edge competitively in the market”. Additionally, organizations usually have their own standard operation procedures (SOPs).

2.7 Marketing and sales

Harvard Business review (2017) highlights the strategic and staggering business implications in the year 2017 latest issue. Digitalization and new tools are changing the way how enterprises serve customers, train employees, design and create products. The ultimately important is how the enterprise can compete in the markets (Porter and Heppelmann 2017; West et al. 2010). Innovation and the product are rarely enough, if the marketing and sales are lacking the competence. Products are transferred into very real business benefits with marketing and sales (West et al. 2010). Right marketing strategy is helping to eliminate the geographic distance and the navigation through the global challenges in business (Wintjes 2006). It is easier to make substantial improvements in quality, productivity, and other measures of performance when there is knowledge about the markets, selling and customer needs. Most of the companies are struggling at some point in the business with challenges and own specific business model is needed to gain the real business benefits (Ozkan-Ariksoysal 2013; Schwab 2017; Sosna et al. 2010).

2.7.1 Market analysis

Market analysis can be used for different purposes like developing the business strategy or doing evaluation about the size of markets in overall. Biotech products and services can be developed further with the information of the analysis. Up-to-date information eases to create more assets to the company in the complex markets (Grand View Research 2017; Sveiby 1997). Specialized market research can even reduce the financial risks. Sales and trends may change quickly. Comprehensive market condition check is also an efficient way to know how to improve customer satisfaction. It can even assess new viewpoints to the future markets potential (Markets and Markets 2017; Sveiby 1997). For example, biosensors can be used in the different fields of

technology. Specific customer groups can be approached with the unique way because of the market research knowledge. Furthermore, different marketing message can be used in sales. Even verbatim comments from the clients can directly have influence on the company's services, sales, product development and customer support activities in overall. Specialized market research can be used in overall for many purposes in the field of biotech business to reduce the financial risks that are always involved in the processes of developing, selling and serving (Markets and Markets 2017).

2.7.2 Strategy and making the successful market plan

Strategy delivery is just as important as the design of the product. Commercialization of advanced detection methodologies in biosensor area has not been as advanced as research output (Ozkan-Ariksoysal 2013; West et al. 2010). Medical science related innovations are disciplining the engineering principles to biomedical technologies and defined strategy can deliver benefits. Business strategies can be often multiplied in the field of technology and services.

Important is to create functional strategies that really are working. Fast and cost-effective solutions are needed all the time to the problems that biosensors can solve. According to the latest market research results (Grand View Research 2017; Markets and Markets 2017; Report Buyer 2017), biosensor related markets are accelerating rapidly. According to these studies the compound annual growth rate (CAGR) between 2017 and 2022 is estimated to reach 8.1%. Significant growth is about to be in the Asia Pacific markets with a CAGR of 11.4%. The most significant end-user markets for biosensors are estimated to be in the North America. The fastest growing segment is excepted to be the optical segment of biosensors (Grand View Research 2017; Markets and Markets 2017; Report Buyer 2017). This implicates the importance of making finalized strategical decisions in the global markets.

2.8 Insights about customers and competitors

The concept that product can be created in a collaboration with the customers is relaying on the ideology that customers can give significant insights to help to build strong and sustainable relationships. Recent advances in the biosensor technology are providing multiple options from electrochemical biosensors to the optic based biosensor technology that includes the SPR based instruments (Kim et al. 2015; Wang

et al. 2013). For now, glucose monitoring sensors and the pregnancy tests have been among the most successful biosensor devices in the markets. There is often correlation between some practical ideas that customers have and the ones that will lead to the most successful products. Quantitative glucose sensors have been changing the lives of diabetes patients (Yao et al. 2011). Qualitative pregnancy tests are based on a lateral flow assay in order to detect human chorionic gonadotropin hormone. Achieving desired processes have been resulting to improve customer satisfaction further. Both of these biosensors have significantly large markets and there has been fundamental competition between different company's products. Neither of product is competing straight with e.g. MP-SPR but both are great examples of biosensor related business development. The products are representing intelligent strategical commercialization process development in the competitive markets (Delgado et al. 2012; Nevens et al. 1990; Wintjes 2006).

In the literature, many competitive theories are still based on Porter´s (1990) Diamond Model. In the examples above, customer base has not been automatically lost in any cases even if competitor may have lower-cost alternatives. This fundamental rule is still valid in the business world. Many aspects are affecting to the final decision about closing the deal (Eisenhardt 1989). For example, easier data handling with specific software can be a deal breaker, rather than the device's price. Each company has the ability to compete while meeting quality goals. Identifying segments of customers with similar needs can help to avoid losing any market share. Some governments may also have their own role when it comes to protecting and promoting competition (Dosi et al. 2015; Wintjes 2006). All stakeholders have important roles when creating real competition and changes in the health technology related industry.

2.8.1 Customer focus

The field of biosensor technology is dependent on the most fundamental force in economics that is the principle of supply and demand. Tailored solutions are good to provide to any user problems. Workforce needs to understand and address the needs that customer have. Relationships with vendors are also important and the principle to bring benefit for all the investors. It is not just about closing the deals but also reframing the customer relationships and keep actively extending customer base in order to have revenues. It is essential not to depend just on the former sales

intermediates which requires the capability to respond changes and not to hold too long to any strategy that has been once successful (West et al. 2010). One part of the customer focus can be to create anonymous feedback channels and incorporate novel information. Explicitly consider some alternative perspectives and strategies. When there is a deep market understanding, it is easier to identify and analyze the problems and make the most powerful solutions. Organizational capabilities can be always developed which increases the level of competence in overall (Salazar et al. 2012; Sveiby 1997). Changes are inevitable but they need to be accepted and develop organizational capabilities in order to become more profitable. Having the right and relevant regulatory and financial expertise can make a tremendous difference in the outcomes of the decisions. Most of all market understanding and result orientation is needed in the context of the customer focus.

2.8.2 Competitive advantage

Competitive advantage is the attribute that is outperforming the company from its competitors. Michael Porter defined different forms of generic competitive advantage decades ago (Porter 1990). His book is one of the most influential management book still on the 20th century. Competitive advantage can be achieved by developing robust plans, products, new strategies, promoting, improving and serving the customers better that competitors do. Differential strategy and focus strategy may require significantly new and creative ways to provide excellent products for different customer segments. Cost leadership is one option to gain competitive advantage but it often requires lower-cost base for materials and facilities (Porter 1985; Porter 1996).

Besides high-quality products, a company can achieve competitive advantage in e.g. with customary post-sales support, kits and other value-adds that can facilitate the use and purchases of instruments, devices and supplies. Competitive advantage can be achieved specially over those companies who are not making that much effort to clarify their customer needs (Liu 2017; Porter 1985). Despite developing and implementing the strategies, unexpected challenges may emerge. Achieved competitive advance can help to outline the strengths and weaknesses of the current situation and ease to make better decisions in the markets (Salazar et al. 2012; West et al. 2010). Vice versa; competitive advance is hard to achieve without outlining the strengths and weaknesses. Organizations might pursue changes in order to remain

competitive and productive while trying to drive down the costs (Salazar et al. 2012; West et al. 2010). According to Schwab (2017) there are about to emerge even more new strategic ways to create competitive advantage. In this light, more deal-driven customers and investors can be attracted in the biotech business field by making new and better strategic planning.

2.9 Business development - Measuring success

Based on the previous chapters, economic growth is a significant success indicator. Customer satisfaction or profitability per district can be success measures (Almquist et al. 2018; Bocken et al. 2014; Freeman and Beale 1992). The secondary sector business opportunities are really important regarding the business development. New business opportunities can be also maximized by developing the long-term customer relationships (Almquist et al. 2018, Bocken et al. 2014; Freeman and Beale 1992). Some companies are more market-driven than others and number of ways such as increased awareness or strategic partnerships can be also used as success indicators. Ultimately, company should be worthy by all stakeholders.

2.10 State of art and future perspectives

In the forefront of science there is always a need for the new analysis methods that are delivering more cost-effective solutions for the problems related to human health end environment. Future perspectives are covering the application possibilities from medical research and clinical use to the wide market areas of industry. Biosensor technology is expected to achieve even more sophisticated possibilities in one application due the new methods of fabrication techniques (Tothill 2009; Soper et al. 2006). Advances in microbead and layer-by-layer techniques are expected to develop innovations more towards 3D biosensors (Dias 2014; Vigneshvar et al. 2016). Patient-specific treatments are expected to develop even further when disease screening and clinical diagnosis methods are improved based on the multiple analyte detection (Tothill 2009; Vigneshvar et al. 2016). Major rapid advances have been already made in recent years. For example, significant detection limit and sensitivity improvements have been made by using right polymers or nanomaterials in the analyte immobilizing process (Wang et al. 2013). Point of care technology is the state of the art in many innovations. According to the Report Buyer´s Market research (2017), the largest

application area for the biosensors is excepted to be the POC testing. The markets are great for the portable devices. MP-SPR is a suitable method for point of care applications in assay development. The label-free platform is providing robust and quick tool for preclinical use (Kari et al. 2017). Especially there has been development in the surface plasmon resonance imaging (SPRi) detection. This powerful technology may be the most promising technique that will ultimately improve point of care testing and lab on a chip (LOC) technology (Puiu and Bala 2016). Furthermore, future applications can offer more sophisticated biosensors e.g. for almost every stage, that are included in the drug discovery processes. Portability and novel technologies are making more and more possibilities to achieve competitive advantage in the biosensor markets (Grand View Research, 2017). Cell survival is possible under some measurements techniques and new possibilities for *in vivo* measurements are expected (Dias 2014; Vigneshvar et al. 2016). Markets are wide for robust regenerative biosensors. Customers have need for long-term use in the field of diagnostic, drug discovery, environment, agriculture, food industry, security and defense (Citartan et al. 2013; Fracchiolla et al. 2013). Semiconductor materials and quantum dot technology have also significant potential for applications (Peng et al. 2014; Shen et al. 2014). Optic-based biosensors are expected to offer significant technology application solutions in biosensing. Fiber-optic techniques have possibilities to enable even more variety to transducers (Kwon and Bard, 2012). Advances in biosensing and biofabrication are together allowing powerful application possibilities for fast growing markets (Dias 2014; Vigneshvar et al. 2016).

3 AIMS OF STUDY

The aim of this thesis was to design SPR assay for MP-SPR Navi™ 420A ILVES instrument. In the assay the regeneration processes of biotin-coated sensor slides were studied. The specific focus was to characterize different sensor slides in different storage conditions. This process also included more specific aims that are listed below:

I Research biotinylated sensor slides' ability to bind switchavidin after different storage conditions and capability of the bound switchavidin to bind IgG.

II Research and interpret aging and shelf life of biotin-coated sensor slides.

III Research regeneration abilities of gold coated sensor slides in order to give insights for the preparation of business and marketing material.

IV Translate scientific results into business opportunities

4 MATERIALS AND METHODS

4.1 Materials

Switchavidin protein was dissolved in water and 2.5ml aliquots were made in 50 µg/ml concentration and stored in in -20 °C. Switchavidin was originally produced, purified, characterized and lyophilized by the Protein Dynamics research group in the Faculty of Medicine and Life Sciences in the University of Tampere. For every run a new aliquot was taken from the freezer (-20 °C) approximately 20 minutes before the injections. Biotinylated immunoglobulin G was ordered from Jackson Immuno Research Laboratories, INC. Baltimore, USA. This protein was Biotin-SP-conjugated AffiniPure Bovine Anti-Goat IgG (H+L) with minimal cross-reaction to bovine, chicken, guinea pig, syrian hamster, horse, human, mouse, rabbit and rat serum proteins. IgG was delivered in volume of 0.5 ml and antibody concentration was 1.6 mg/ml. IgG was dissolved in the filtrated buffer that was 50 mM sodium phosphate, 150 mM NaCl, pH 7.2. The final concentration for IgG was 1µg/ml. Aliquots were made for extended storage after rehydration and stored in -20 °C. The number of aliquots in the whole research was estimated and calculated by the need of the injections. New IgG aliquot was taken from the fridge (-20 °C) approximately 20 minutes before the injections. The running buffer in the measurements was phosphate buffered saline (PBS) and its pH was adjusted in 7.3 with concentrated HCl. Regeneration solution was citric acid 2.5% + SDS 0.25% (pH 2.1). Wash solution contained SDS 0.5 %. 3 minutes hellmanex (Sigma-Aldrich Z805939 – 1EA, Hellmanex™ III, Lot 2728318) 2% injections were used between sensor slide changes. Used storage bottles were H_2O_2 treated in the fume hood before making the solutions. Device was connected beforehand so that the temperature between the device and all reagents would be equal when the measurement were about to begin. The device temperature was set in 22 °C. 96-well plate format was used for the switchavidin, antibody IgG and hellmanex injections. SDS 0.5% and citric acid 2.5% with SDS 0.25% was injected from the 50ml polypropylene tubes that were placed in the sidewall of the device. Needle height was set in 1 mm and run table was filled before every measurement. All measurements were made with the BioNavis MP-SPR Navis™ 420A ILVES instrument. Gold plated sensor slides were made by Martin Albers from

BioNavis (Tampere, Finland). Sensor slides were biotinylated using a company´s own protocol.

4.1.1 Sensor slides and assay design

Thin glass slides were coated with a 50-nm thin gold layer. Sensor slides used in this Masters' Thesis were manufactured together with Dr. Albers (Bionavis Ltd.) because the gold coated sensor slides and their biotin coating surface chemistry is a business secret of the company. Sensor slides were stored in four different conditions that were +4 ° C, RT (room temperature), +30 °C and humid conditions in RT. In every environment two different sensor slides were used for parallel measurements respectively (sensor slide 1 and sensor slide 2). Measurements in the first part of the research were repeated every week in scheduled timescale precisely in all environments. Measurements in the long measurement entirety were made with one sensor slide (sensor slide 1) and repeated in different timescale than in the first part of the research. The overall assay design is demonstrated in the Table 3 and in the Table 4 comprehensively. After finishing the measurements with a sensor slide, it was placed back to its storage environment in a shell. Measurement days are not equal in all environments because the timing of measurement days had to exact and some preference had to be made because there were four different environments with two sensors in each to measure. The amount of antibody IgG was highly limited which is why it was not injected after every switchavidin injection.

Circumstance	+4 °C	RT	RT Humid	+30 °C
Sensor slides	Sensor Slide 1, Sensor Slide 2	Sensor Slide 1, Sensor Slide 2	Sensor Slide 1, Sensor Slide 2	Sensor Slide 1, Sensor Slide 2
Days/Switchavidin	14	14	10	12
Days/IgG	12	12	10	12
Measurement cycles	4	4	4	4
Total measurement cycles	28	28	20	24
Total IgG injections	18	18	14	18
Total Switchavidin injections	14	14	10	12

Table 3. Measurements entity 1. Measurements in different storage conditions. Sensor slides and their storage conditions. The timing and the total number of switchavidin and IgG injections are demonstrated.

Circumstance	+4 °C
Sensor Slides	Sensor Slide 1
Days	5
Measurement cycles/day	20
Total measurement cycles	100
IgG injections	Day 1: Injections 1, 7,14, 20 Day 2: Injections 7,14, 20 Day 3: Injections 7,14, 20 Day 4: Injections 7,14, 20 Day 5: Injections 1, 7,14, 20
Switchavidin injections	Day 1: Injections 1-20 Day 2: Injections 1-20 Day 3: Injections 1-20 Day 4: Injections 1-20 Day 5: Injections 1-20
Total IgG injections	17
Total Switchavidin injections	100

Table 4. Measurement entity 2. 100 cycles measurement with one sensor slide. Storage condition +4 °C. The timing and total number of switchavidin and IgG injections are demonstrated.

4.1.2 Injection protocol

For each measurement injection parameters and running actions were set in the MP-SPR Navi Control – Autosampler Run table. Injection flow rate was 30 µl/min for each sample. The average of the flow channels was calculated after exporting the data from Trace Drawer (BioNavis) to Excel. Figure 3 and Figure 4 are demonstrating the injections and injection protocol. The specific regeneration evaluation was based on the baseline change analyzation (R=IV-I) that is demonstrated in the Figure 4. Injection volume for switchavidin and IgG were 300 µl. Injection volume for SDS 0.5% and citric acid 2.5% with SDS 0.25% were 462 µl. Running buffer (PBS) flow was in pre-injection delays for all samples 2 minutes and post injection delays 5 minutes. Running buffer was automatically flowing through between every injection. Injection length for switchavidin and IgG were 6 minutes. Injection length for SDS

0.5% and citric acid 2.5% with SDS 0.25% were 3 minutes. Every measurement cycle started with SDS 0.5%. Measurement cycles consisting of (1) citric acid 2.5% with SDS 0.25% (2) switchavidin and (3) biotinylated antibody injection are demonstrated in the Figure 3 and Figure 4. The cycle was repeated four times for each sensor slide in different environments. Furthermore, one specific assay was made with one sensor slide that was stored in +4 ℃ if the measurement was not active (during nights) for five days. Sensor slide were left in +4 ℃ for further studies. Same cycle for this sensor slide was repeated 100 times.

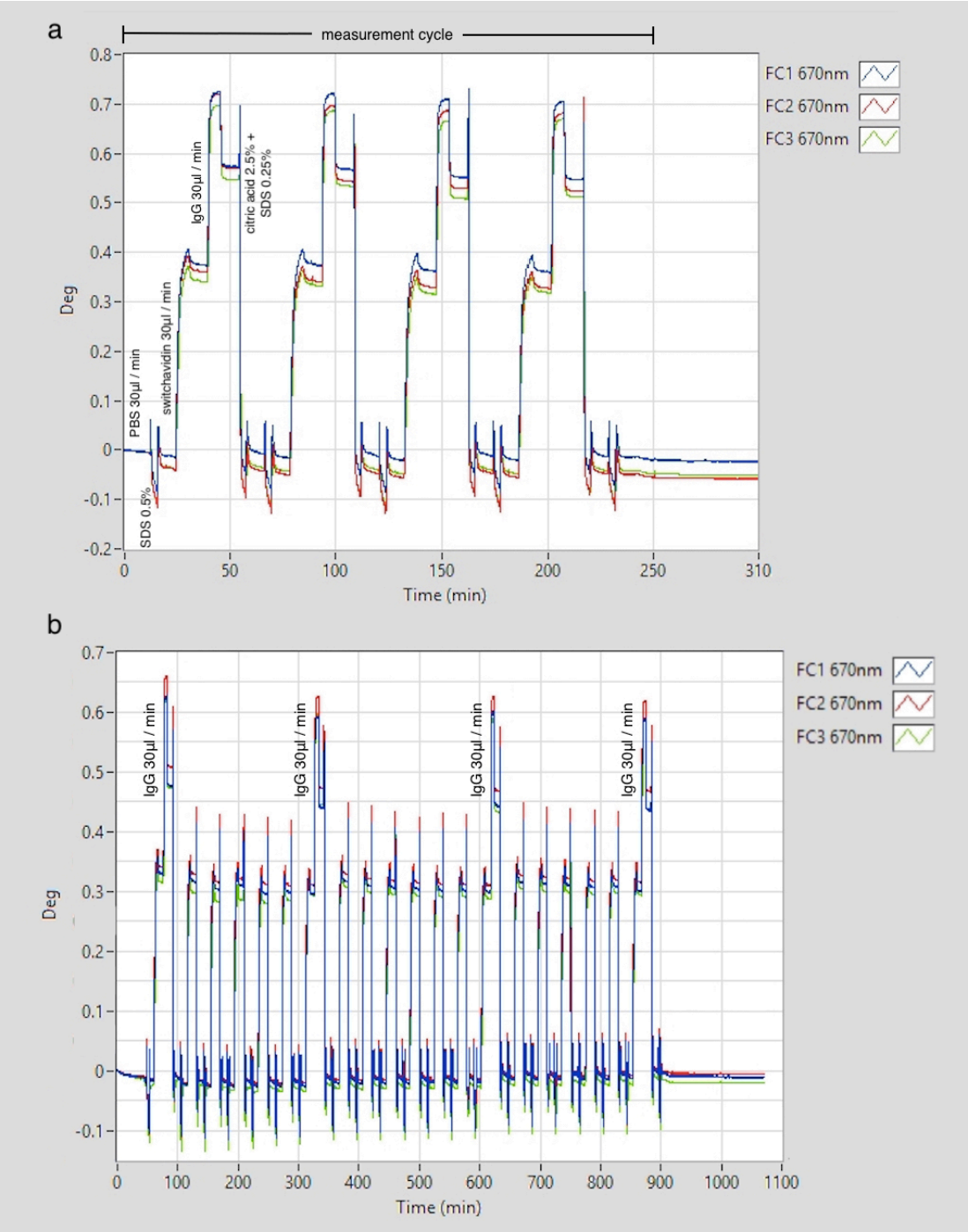

Figure 3. *Biosensor measurement protocol. 3a is demonstrating the injection protocol. The cycle entirety was repeated four times for each sensor slide stored in different environments. One measurement cycle is consisting of (1) citric acid 2.5% with SDS 0.25% (2) switchavidin, (3) biotinylated antibody injection and running buffer PBS. 3b is showing a part of the 100 cycle measurement injections that were made during one day. 100 cycle measurement was made with one sensor slide. Same cycle entirety was repeated 100 times for this sensor slide as described in 1a. Data is illustrated by MP-SPR Navi™ Control software.*

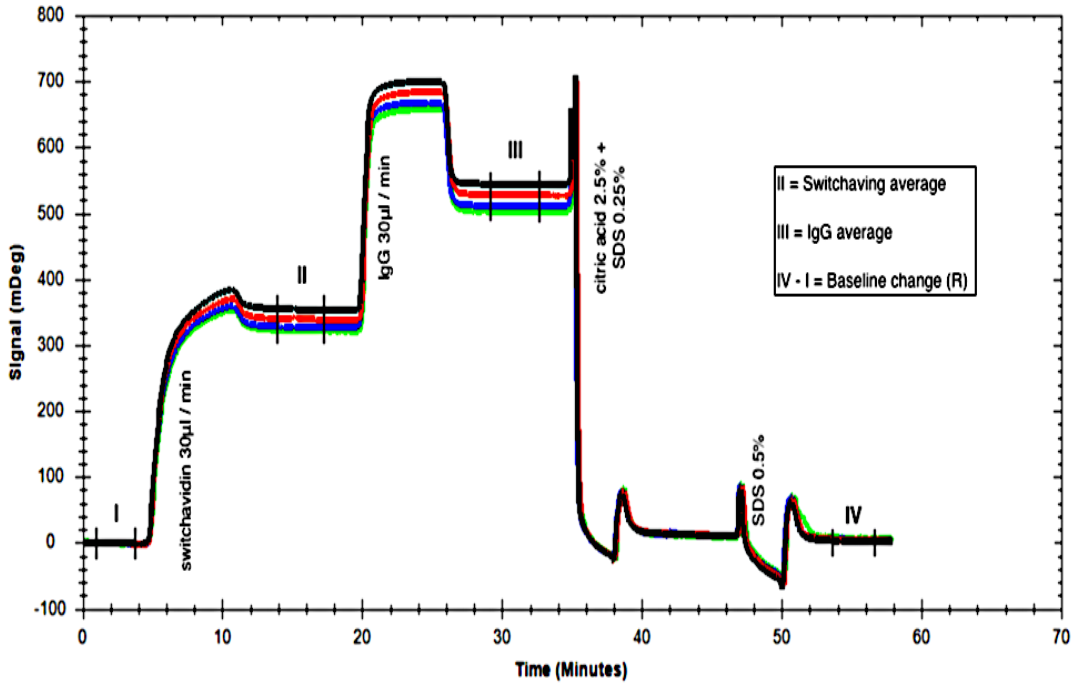

Figure 4: The injections from one measurement cycle. Intervals I-IV are demonstrating the time points of flow channel averages. I = baseline, II = determined total switchavidin binding after wash, III = determined total IgG binding after wash, IV = baseline. R (IV-I) corresponds to the baseline change. It characterizes the regeneration performance. Data is viewed by Trace Drawer™ after processing and exporting it from MP-SPR Navi™ Data Viewer.

4.1.3 SPR experiments and device control

Flow cell was opened and sensor slide was inserted in its place between prism and elastomer. Flow cell was closed and "Initializing Scan" (Init. scan) was selected and executed. Different procedures e.g. "wash all and kick the bubble" were needed if the SPR peak in the init. scan wasn`t good enough. After respectable results, angular scan was made. Liquid range was selected for both measurements respectively. Measurements were made in high refractive index media so that the angle of incidence was between 50-78 degrees. Experiment was started only after all the parameters were detailed. Each experiment started with baseline measurement that took approximately 8 to 15 minutes for each run. Hellmanex (2%) injection (300 µl/min, 5 minutes) from the well plate was done between experiments with different sensor slides.

4.2 Data analysis

Measured data was first analyzed in the MP-SPR Navi™ Control and exported into MP-SPR Navi™ Data Viewer. Degrees were changed in mDeg after exporting the measured from the MP-SPR Navi™ Control and MP-SPR Navi™ Data Viewer into Trace Drawer™ and every flow channel was layered according the channel, injection and day. For advanced analysis data was exported to Trace Drawer™ where the data was layered by different flow channels, injections and days. After Trace Drawer analysis, the data was exported to the Excel for the final calculations and analysis. In Excel, an average from every injections' different flow channels were calculated.

5 RESULTS

5.1 Performance of the biosensor surface stored in different environments

5.1.1 Biotinylated sensor's ability to bind switchavidin and sensor's performance after storage

Biotinylated sensor slides were found to bind switchavidin differently depending on the storage conditions. Switchavidin is attaching onto sensor slide's surface like Pollheimer et al. (2013) and Zauner et al. (2016) have published in their work (Figure 5 and Figure 8).

As can be seen from Figure 5, almost all sensor slides are showing clear decrease in switchavidin binding when the measurement days are about to reach 8 to 12 days. However, in humid conditions, there are no significant drop observed after such storage time. Both of the biotinylated sensors stored in humid conditions have switchavidin binding capacity values above 300 mDeg during the whole measurement protocol. Additionally, both sensor slide 1 and sensor slide 2 are performing the most equal fashion as compared to the sensors stored in other conditions.

Room temperature conditions have the most descending linear change in switchavidin binding values and days 8-12 are indicating slightly weakening capacity to bind switchavidin (Figure 5). The breakdown of the sensor slides in +30 °C can be seen already in day 8 and the measured response is only slightly above 100 mDeg (Figure 5). For the sensors stored at +4 °C, there aren`t significant decrease observed in the measured response in day 8 or after two weeks (Figure 5). The switchavidin binding values in +4 °C are varying most if compared to other environments. Sensor 2 have slightly higher values than sensor slide 1 expect in day 10. Overall, +4 °C and RT humid show the best performance over time but the binding signal measured using slides stored at +4 °C are much lower than those observed for slides stored at RT in humid conditions. The values in RT humid environments are constantly above 300 mDeg and even close to 350 mDeg during the whole measurements.

Different biotin functionalization trials were made for the gold surfaces using different reagents and the fresh reagents seemed to yield the most optimal functionalization. This might be one interesting point of view to research more in order to manufacture even better sensor slides. Overall there appeared to be no significant differences between the qualities of the parallel sensor slides in different environments. Only a few significant deviations can be seen such as temperature + 30 °C in day 8 (injection 1) is statistically deviant. In environment RT, the sensor slide 1 has the most deviant values from the sensor slide 2. Yet, differences are rather small regarding the scaling is in mDeg.

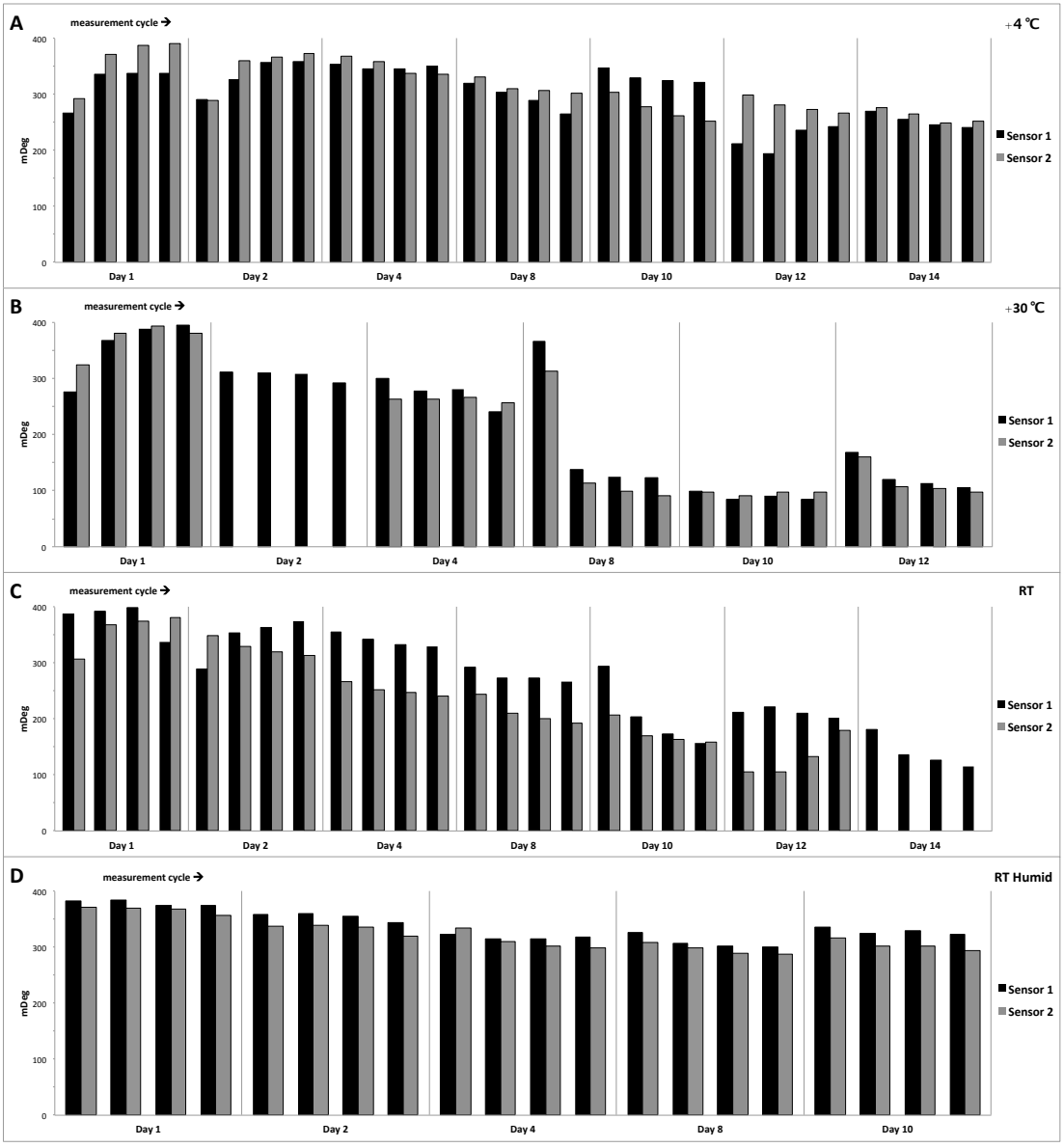

Figure 5. Switchavidin binding on biotinylated sensor stored in different conditions. Binding of switchavidin has been measured using two parallel sensors, and four measurement cycles were carried out. (a) sensor stored at +4°C (b) sensor stored at +30°C RT, (c) sensor stored

at RT, (d) sensor stored in humid conditions at RT. Please note that only one sensor was analyzed for the day 2 in the case of sensor stored at +30°C (b) and in the case of the day 14 for sensor stored at RT (d).

5.1.2 Capability of switchavidin to bind IgG after different storage conditions

The cycles shown in Figure 6 are demonstrating the verification that was made by using the antibody IgG. Until day 4, all the sensor slides and environments are showing a ratio of IgG/Switchavidin close to 1.5 (Figure 7). The ratio is prominently good in all environments during the whole measurements even in the highest temperature but a little unexpected variation can be seen in the RT environment. The results are indicating that after two weeks the binding capacity of the bound switchavidin layer is not collapsed. It is challenging to extrapolate how different environments would performance after 3 weeks. Nonetheless, RT humid environment is showing the most promising results as can be seen in every D graphs, respectively.

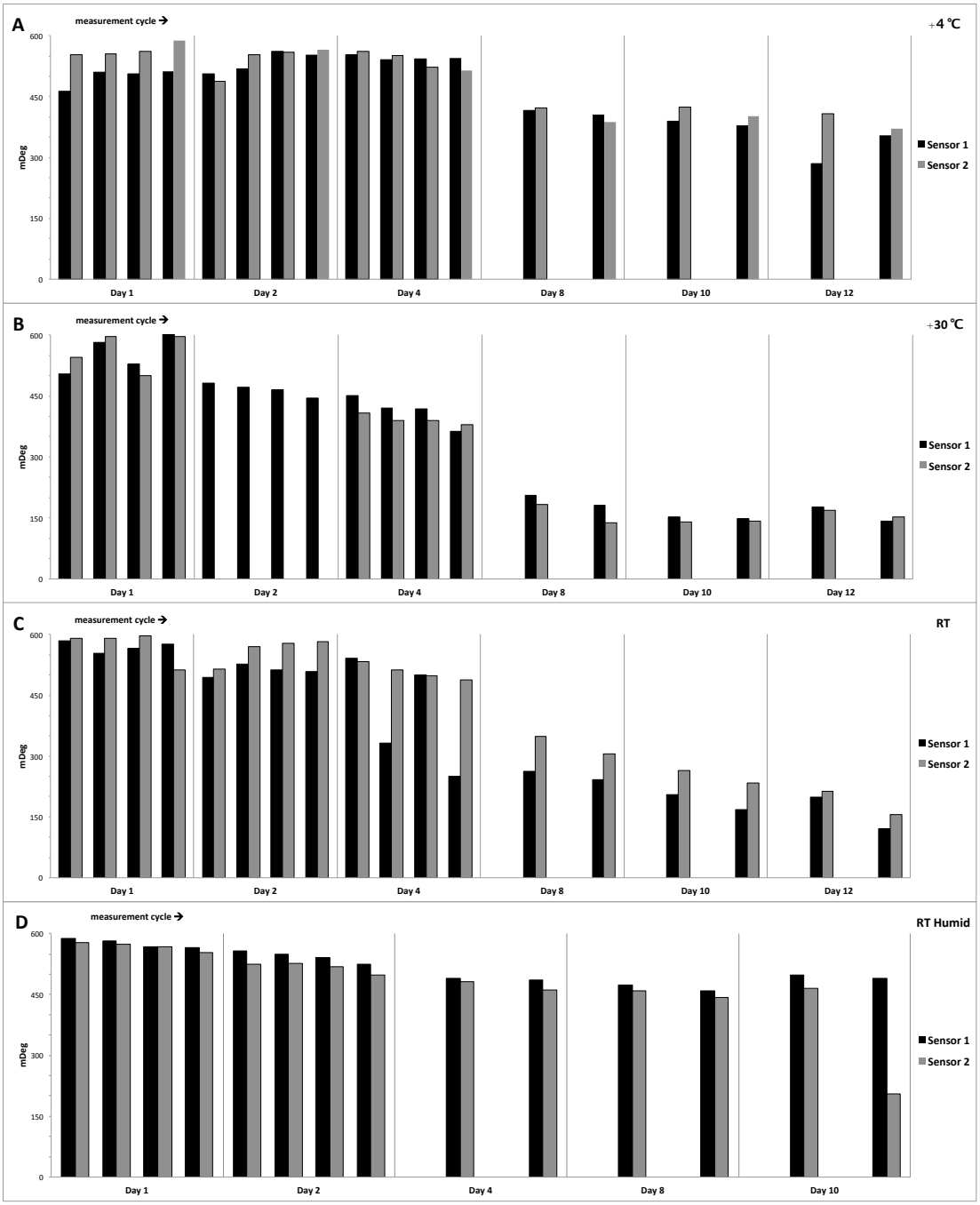

Figure 6. Switchavidin performance to bind IgG after different environmental storage conditions. Effects of binding are measured and demonstrated in environments 4°C, 30°C, RT, and RT Humid.

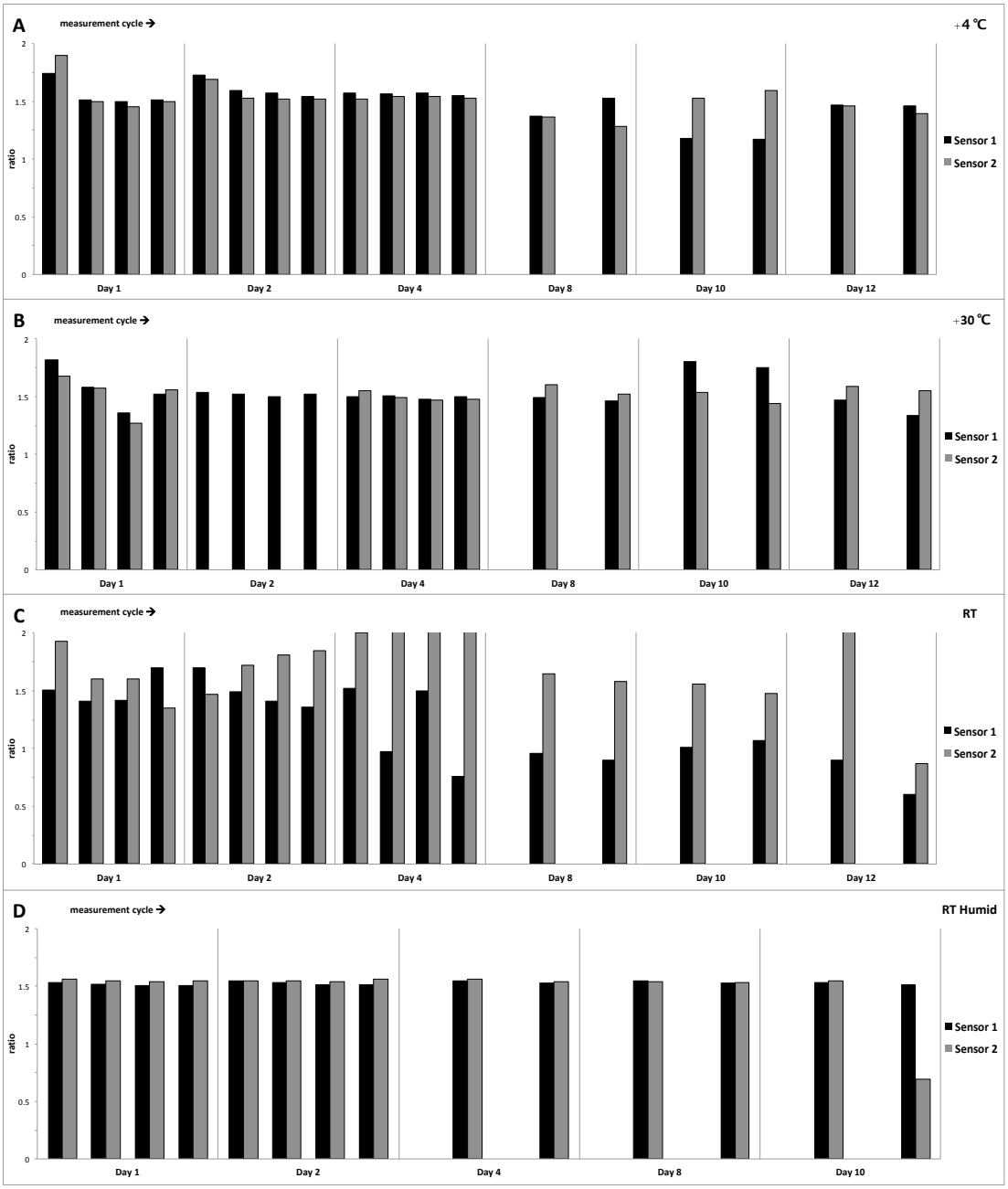

Figure 7. Ratio of IgG/Switchavidin binding for sensors with different storage conditions. Demonstration of IgG/Switchavidin ratio are measured in environments 4°C, 30°C, RT, and RT Humid.

Interestingly mere RT environment or +4°C environment isn't indicating as good results as expected as seen in graphs A and C, if compared to D graphs in Figure 6 and Figure 7. It was expected that +30 °C environment is about to set too harsh conditions for biotin-functionalized gold sensor slides but it was interesting to observe the time point when the sensor slides were about to diminish their optimal performance. As

seen in every B graphs, the sensor slides in the highest temperature are about to break between day 4 and day 8.

Nonetheless, the collected data show that switchavidin binding was occurring and verification with IgG seemed to function rather well. Next step could be to continue measurements until 3-4 weeks to see how the binding capacity is about to start to decrease.

Knoglinger and co-workers (2018) reported about transferring biotinylated sensor slide into argon filled Falcon™ tube that was stored at +4 °C. This might be a good method to use in the future experiments. Even though the data is in mDeg, the deviations in +4 °C environment are the quite significant as results in the 5.1 - 5.3 are demonstrating. According to Knoglinger et al. (2018), the +4°C temperature should be relatively good choice for storage conditions.

Additionally, the repeated removal of IgG and switchavidin after each IgG injection, that was performed with mixture of citric acid 2.5% and SDS 0.25%, seemed to remove proteins rather good. Typical another choice for e.g. antigen removal can be performed with 100 mM glycine buffer (pH 2.7, GE Healthcare Data File 18-1012-91 AC, as Pollheimer et al. (2013) was performed in their research. Nonetheless, Pollheimer et al. also reported in their article in 2013, about how the instantaneous cleavage of the biotin-avidin-biotin bridges can be caused by citric acid 2.5% and SDS 0.25% combination.

5.2 100 cycles measurement

Binding and desorption of switchavidin was repeated with good reproducibility in 100 cycles measurement, as illustrated in the Figure 8 and Figure 9. As the results shows, the study design was successful and reliable. Results are indicating that the gold coated biotinylated sensor slide can have great ability for complete regeneration cycles. Yet, further measurements are needed to give more information about the specific storage conditions but the sensor slide accomplished the goal to regenerate. Results are representing comprehensive and steady average of the flow cells as mentioned in the materials and methods. Significant deviations are indicating also about possible disturbances as air bubbles in one flow cell channel. This is why the average of three flow channels have been calculated instead of one flow cell channel. The average was

calculated in order to minimize bias that could have occurred if only one flow channel was observed for the analyzes.

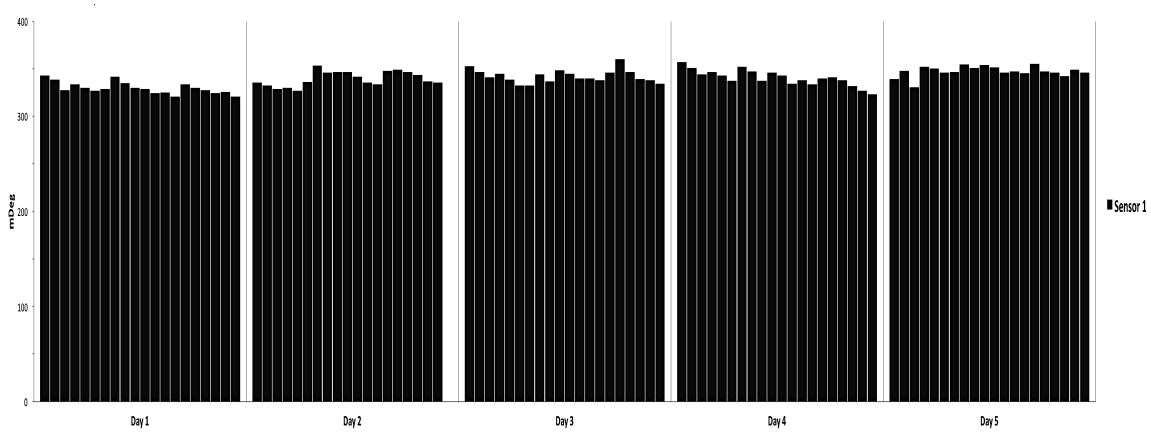

Figure 8. Biotinylated sensor slide's ability to bind switchavidin. 100 measurement cycles are shown. This 100 cycles measurement study was conducted in 5 days. Measurement was active 24/7. Only reagents (switchavidin and IgG) were changed between the days.

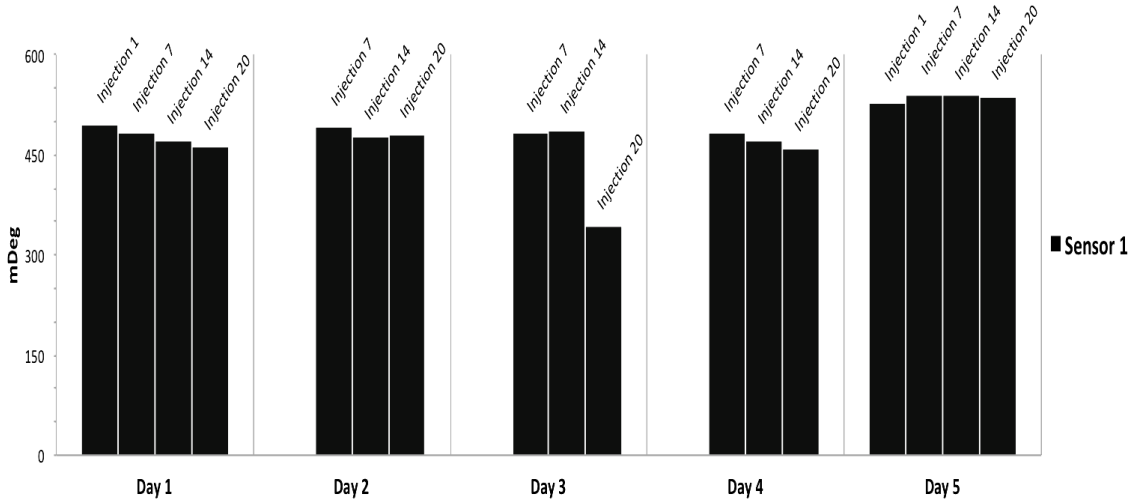

Figure 9. Capability of immobilized switchavidin to bind IgG along the 100-measurement experiment. This 100 cycles measurement study was conducted in 5 days. Measurement was active 24/7. Only reagents (switchavidin and IgG) were changed between the days.

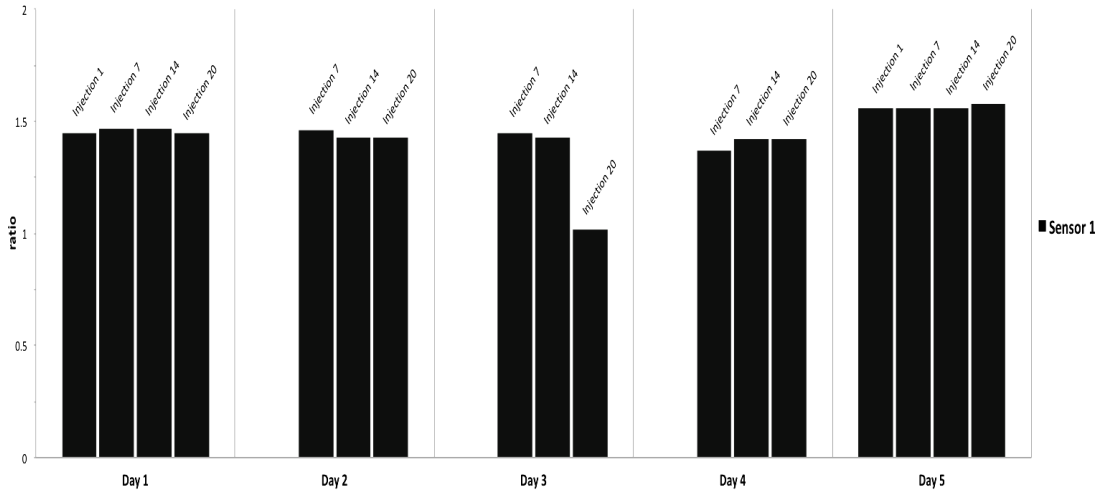

Figure 10. Ratio of IgG/switchavidin binding capacity along the 100-measurement experiment. The reason for the decrease in the injection 20 shall be related to a contemporary deviation in one flow channel. Result is still valid and it is included in the flow channel measurement average as well as in all the other measurements.

The results indicate that stable binding of switchavidin was occurring and verification with IgG seemed to function rather well. Like shown in the results section 5.1-5.3, the assay design strategy enabled to accomplish information about different environmental factors and constant strain caused by continuous measurements and about how the aging process evolves as a function of time. Stable binding of switchavidin was proven to function with these biotinylated sensor slides and verification with IgG seemed to function well. Additionally, aliquots were successfully made and stored.

5.3 Regeneration and baseline change

The regeneration evaluation was supported by the baseline change analysis as demonstrated in the Figure 4. The data obtained indicate that the layer formed on the biotinylated biosensor consisting of biotinylated IgG and switchavidin is removed successfully after each measurement cycle (Figure 12). Fortunately, it seems that almost complete repeated removal of all the protein molecules was achieved with some of the biotinylated sensor slides during the measurement. Figure 12 is demonstrating the sensor slide that is performing multiple cycles of binding and removal events. Regeneration was found to be complete. As a consequence, it would be interesting to make even longer measurements i.e. to include more days to the measurement protocol to evaluate the regeneration ability over extended time period.

Alternatively, studies in more comprehensive set of storage conditions with different humid conditions would be beneficial. Such research would require lots of reagents, time and possible additional measurement devices.

The results provide basement for further research in the sensor manufacturing and storage. One part of this research was focused mostly on how storage conditions effect the sensor performance, while 100 cycles measurements were testing sensor slide's performance during the constant rigorous regeneration cycles. As rationalized in the literature of review, Figure 1 and Figure 3 – Figure 4, the quantitative dissociation of the biotin–switchavidin–biotin bridges from the surface of the sensor is occurred in the regeneration. In this Master's Thesis, reproducibility of chip regeneration was studied. The recovery of these biotinylated sensor slide after fresh switchavidin and IgG injections can be successful if the storage environment have been optimal as results in the 5.1 - 5.3 are demonstrating.

Like mentioned in the introduction, one of the main goals was to research the regeneration ability. As Knoglinger et al. (2018) have written in their article, regeneration protocols for sensor slides are useful only if the complete regeneration is occurring fast enough. Likewise, in this research, the regeneration phase for sensor slides was executed only for a few minutes. For this reason, the good regeneration performance obtained in such short time scale is indicating good regeneration performance. Results are indicating important further aspects where to concentrate in the follow-up studies. Neither citric acid 2.5% + SDS 0.25% nor SDS 0.5% alone are remarkably harming the surface of the sensor slide during the two weeks' measurement cycles, though switchavidin and IgG are removed from the biotinylated surface. Indeed, the Figure 12 and Figure 3b are illustrating almost complete removal of switchavidin, without significant changes in baseline. Considering the strain caused to the sensor slides under measurements, results are indicating very good performance ability rather than collapse attributes.

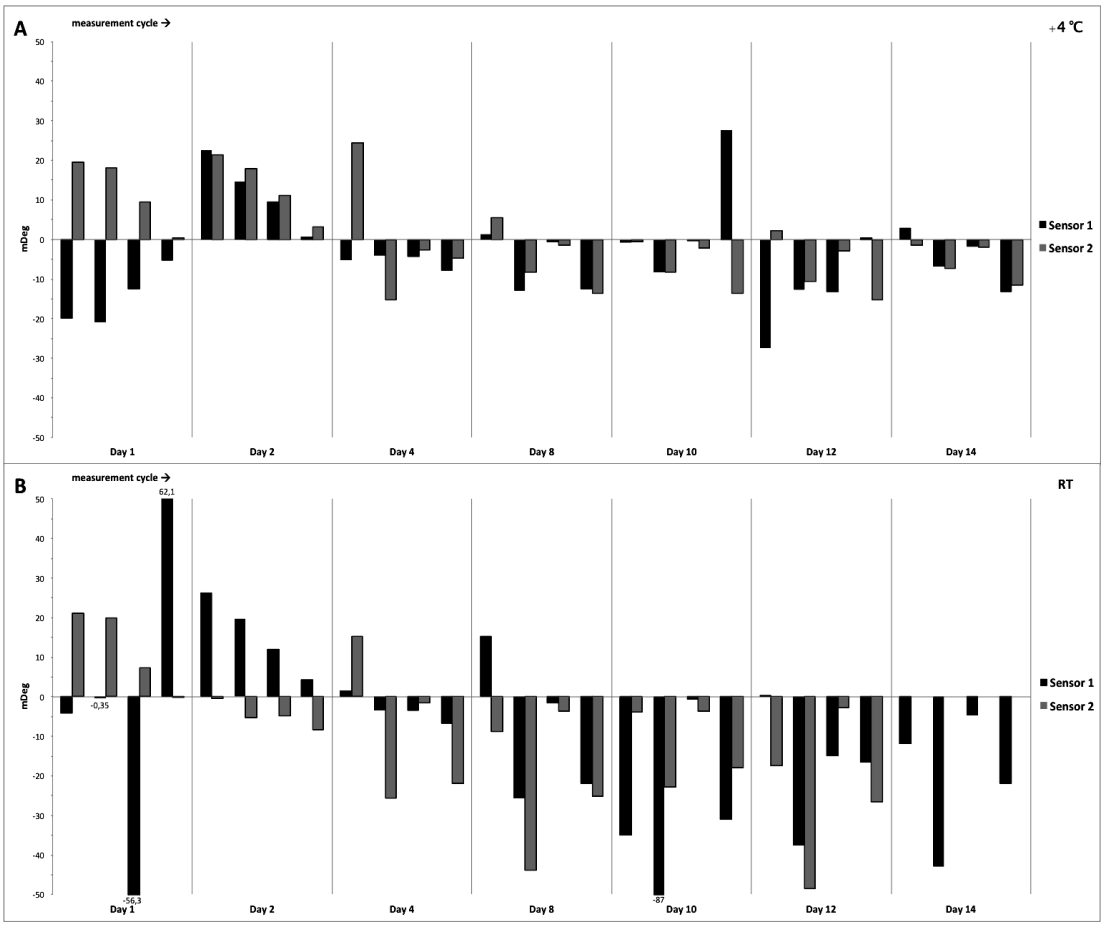

Figure 11. Regeneration, +4°C and RT. Regeneration has been measured using two parallel sensors, and four measurement cycles were carried out during each day. (A) sensor stored at +4°C (B) sensor stored at RT. Please note that only one sensor was analyzed for the day 14 for sensor stored at RT. Also, please note that the graphs are scaled differently as compared to those showing the measured binding data.

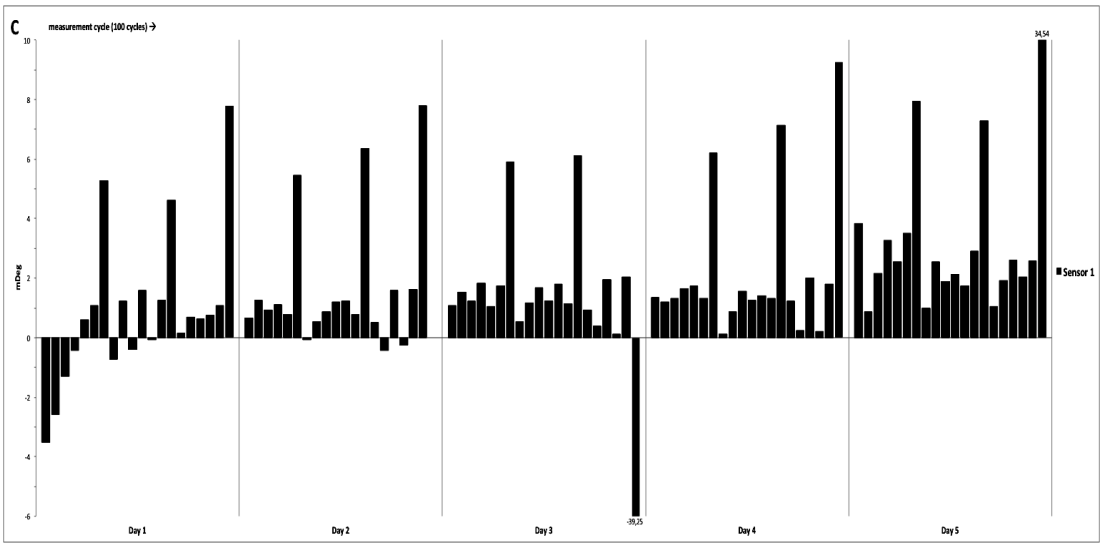

Figure 12. Regeneration, 100 cycles measurement. This 100 cycles measurement study was conducted in 5 days and measurement was active 24/7. Twenty measurement cycles were carried out during each day. Only reagents (switchavidin and IgG) were changed between the days.

As illustrated in Figure 11 and Figure 12, the baseline is often starting below zero and stabilizes only after few measurement cycles. This may demonstrate the recovery of the biotinylated sensor slide after storage. However, such a significant baseline drift was not seen in the subsequent cycles with sensor slide that was used in 100 cycles measurement. As the Figure 12 demonstrates, the baseline growth is relatively small (mDeg) and the change is beginning to increase slightly only after day 4 (i.e. after >50 measurement cycles).

The term sensor slide regeneration is illustrating that all bound injected molecules are removed from the sensor slide. This procedure is making biotinylated surface ready for the next injection of sample. This is why stability of sensor slide is important during removal. It was good choice to use regeneration buffer that have physiological ionic strength as Zauner et al. discussed in their work in 2016. Their regeneration experiments were made with sensor slides that were functionalized with avidin mutants (Zauner et al 2016). Results in the section 5.1-5.3 are indicating that regeneration can be done in reproducible manner if the sensor surface manufacturing is done with the right protocol and if the storage environment is optimal. SDS 0.5% is washing away the weakly bound materials from the sensor surface before the switchavidin injection. The phenomena can be seen also clearly before the first switchavidin injection in the Figure 1 (raw data), where the baseline in slightly dropped

after the SDS injection (1min – 10 min). Baseline have been changing relatively minimally between the injections in the 100 cycles measurement, even though the sensor slide has mots probably experienced lots of physical and chemical strain during the measurement cycles. However, the first injection with SDS is causing change of the baseline which is indicating that there have been proteins or other impurities adsorbed on the sensor slides' surface.

Based on the information provided in the literature review, biotin and switchavidin are binding each other non-covalently on the sensor surface (Knoglinger et al. 2018; Taskinen et al. 2014; Zauner et al. 2016). Figure 1 is demonstrating the binding of switchavidin in different storage conditions. Yet, some deviation in the regeneration graphs can be seen. It is possible that all the non-covalently binding molecules have not been washed away after the SDS 0.5% and citric acid 2.5% + SDS 0.25% wash. There may also be a possibility that the biotin coating of the sensor slides is not homogenous enough.

5.4 Sensor slide's performance and aging process for shelf life estimations

Performance and durability under specified storage conditions was demonstrated in 5.1 - 5.3 and results show that if biotinylated sensors are stored in right environment, they can be used repeatedly. 100 cycles measurements and different environmental measurements are indicating lots of information about the rates when sensors are about to lose their performance. In 30 °C this occurs already between day 4 and day 8. Comparison of RT and RT humid is clearly indicating that humid conditions can lengthen the shelf life and to slow down the aging. In the future, further studies could be potentially improved by identifying more facts associated with shelf life. In addition, shelf life could be followed for additional years. For example, follow up can be executed with the sensor slides used in this study.

In summary, as the sensor ages the decrease in sensor binding capacity appears to accelerate (Figure 12). In a normal use, sensor slides can maintain their performance at least over two weeks in right conditions (Figure 5 – Figure 7). Specific shelf life of the biotinylated sensor slides' can be studied further with these results and the sensor slides that were put in specific storage for further shelf life studies. This will give more information about specifications about how long sensor slide can performance well

after the immediate packaging is opened for the first time. Results are indicating that each sensor slide may have a different stability and shelf life due the different surface ratios which is why the manufacturing part of the sensor slides is really delicate and important. This may have to be pointed out in the future research.

5.5 Value adds - Kit design

One strategic decision is to create kits for specific customer needs as discussed in the review of literature. There are different features that can be highlighted already in the design phase. The kit is creating value for the customer needs. Basically, the kit saves customers time because it includes materials and reagents with instructions. Customer who uses the instruments from specific company could also buy custom made kits and repeat the purchases from this specific company. This requires high quality condition supply chain and logistics from the company. For example, customer from the Switzerland's pharmaceutical industry can buy the customized kits for their device and final costs for the customer are defined based on the reagents and the materials needed for the kit. Multiple purchases can reduce the total costs. Marketing and strategy planning could be created based on the different segments in the customer base. For example, pharmaceutical industry, and food safety industry. Under these main categories there could be target segments for new customers and the key customers. The core of the kit is the specific amount of the sensor slides with the specific surface chemistry. It includes specifications also about different environments as results 5.1 – 5.3 are demonstrating.

The company's product portfolio combined to the customized kits can bring relevant competitive advantage and more revenues for the company. Furthermore, it creates more assets to maintain and create stronger customer relationships. Additionally, the customer definition needs to be specific. Is customer the one who is buying the product or using the product? In some cases, customer can be the end-user or the supplier, depending on the context. For example; if a diagnostic biosensor has been developed for therapeutics, it can help both clinicians and patient. Revenues for the company are coming from the specific cluster, who eventually are buying the product. Nevertheless, the company needs the feedback from both clinicians and patients in order to gain comprehensive information to improve the products and the marketing strategies.

6 DISCUSSION

6.1 Sensor slides

In this study, switchavidin association on the biotinylated gold coated sensor slides exposed to variable environments was studied. Additionally, evidence emerged of sensor performance in relation with measurement days (storage time). Examination with the biotinylated gold coated sensor slides built the knowledge about the regeneration possibilities and understanding for the further research was gained. As the 100 cycles measurement shows (Figure 8 and Figure 12), the dissociation of switchavidin from the biotinylated surfaces as a function of time was remaining significantly robust during the whole measurement. Multiple analyses with switchavidin yielded similar results to Knoglinger et al. (2018), Taskinen et al. and (2014) Zauner et al. (2016) as avidin and it mutants being one of the optimal tools for biosensor related studies. As Knoglinger et al. (2018) are pointing out in their research, the major difficulties have been finding a method for switching off the robust bond between streptavidin and the biotin chip. Both Knoglinger et al. (2018) and Zauner et al. (2016) have been describing of wild-type streptavidin characteristics to remaining tetrameric in the presence of acid and SDS as in contrast to switchavidin mutants. In this study only switchavidin was used. Conversely avidin and streptavidin were mostly used in these above-mentioned studies. However, additional knowledge about biosensor related studies with switchavidin and biotinylated sensor slide's performance was built in this thesis.

The findings agree with several the other studies as sensor slide functionalization being one of the most important part of the biosensor related studies (Taskinen et al. 2014; Zauner et al. 2016). For example, some minor differences between sensor 1 and sensor 2 are indicating that the functionalization might have been not as even i.e. homogenous as possible. In environment +4 °C, sensor 2 have slightly higher values than sensor slide 1 expect in day 10 (Figure 3 – Figure 5). The average for the flow channels was calculated in order to minimize the bias that could have occurred if only one flow channel was observed for the analyzes. However, additional error data analysis with different channels could give more specific information about the data. Average calculation was a good choice for data analyzation, rather than choosing just a one

flow channel. However, further information about channel differences might have been interesting to research and analyze. Yet, mDeg changes are easy to interpret as in a function of days and different environmental effects are readable.

Assay design was succeeded even though some preference had to be made because there was only a limited timescale for the measurements due the parallel multiple measurements with exact timing. With two devices, the execution might have been more fluent. Multitude of ovens for multiple environments of stability and performance research could have been tested but the disadvantage of this approach was that one oven is dedicated to a single experimental set. Next step could be to have multiple ovens that in turn requires a more facilities and time frame to execute experiments because of the exact measurement timing of sensor slides as a function of time.

One of the most interesting findings is that results in the RT Humid environment are indicating that optimal storage conditions of the gold coated and biotinylated sensor slides needs humidity (Figure 3 - Figure 5). One of the strength of the study included the 100 cycles measurement that especially indicates promising results for the regeneration performance. As Vigneshvar et al. (2016) mentioned, regeneration can cause challenges in the field of biosensor related technology. Some unnecessary drifting in the early phase of the measurement can be caused by the lack of a dummy injection that Zauner et al. (2016) descried to be performed with regeneration buffer before starting repeated binding measurements. Even though reagents were placed early in the right temperature with the device before starting the measurement, discontinuity in the temperature dependence was observed at some time points. Biosensor are highly sensitive devices and as Bates (1962) already observed decades ago, temperature changes can change the pH of a solution even with buffers present (Bates 1962). Even though hellmanex washes were made, it is not excluded that cross contaminations incipient impurities could have occurred during the measurements because many people were using the device with different reagents.

According the relative humidity changes as a function of temperature, it would be interesting to see whether variance in humidity correlates with different temperatures and affects to the sensor slide's properties and performance. Additionally, according to the results, humidity shall have some positive impact on the storage conditions. The

phenomena could be further studied and discussed in the future studies. Further analysis about prediction of stability in packaged product is still needed but the results of are indicating the main points worth of focusing on. In the future shelf life and aging tests could be executed by controlled standard test methods. One additional measure of sensor slide's product stability could be the color and surface change analyze. With atomic force microscopy (AFM) some differences between the sensors could be taken into account and analyzed during the measurements. Additional shelf life estimations based on the observed rate of the changes could be made as a function of time.

6.2 Biosensors and market perspectives

Besides the scientific biotechnological studies with MP-SPR, business aspects were highlighted through the thesis because biosensor related technology is very wide and has promising future perspectives. Global market research results are indicating significant areas where to concentrate and how to build strategic planning intelligently further (Grand View Research 2017). Strategic competitive related planning is needed to strengthen the equity, also in the biosensor related markets in order to gain more profits. New competitive marketplaces are emerging in the biotech field. Company can create significant acts of implicating in order to shape the customer's brand choices. Biosensor markets are accelerating rapidly and this Master's Thesis is suggesting that also smaller companies invest in consulting firms in order to make profound market research and in order to make business more profitable. Profound consulting assessment from different sectors of medicine, industry and technology can reduce business risks significantly. It is important to have knowledge to identify risks and key unknowns from different parts of the distinct market places (Dias 2014; Salazar et al. 2012). As discovered in the introduction, key account managers and engineers might have significantly different perspectives about product specifications and market research results can help to create more optimal solutions for complex problems. Unique features can have role in product life cycle evaluation and planning.

Interest toward the new products and services is clearly existing and new revenues can be achieved also by developing services among other strategic planning as described in the review of literature (Vigneshvar et al. 2016; West et al. 2010; Wintjes 2006). The constant quality of sensor slides is essential. These results indicate for significantly better regeneration achieving methods in the future markets. Results did show

differences between sensor slides in the functional recovery levels. Via regeneration process improvements, significant competitive advantage can be achieved. This Master´s Thesis work is indicating that even more competitive advantage could be achieved by developing e.g. kit products further while improving some of the features in the existing devices. Besides product development, segmented strategies, marketing and selling messages should be created to the different customer sectors.

On the basis of the market research, marketing should place strong input on the product. Especially in the new product development, pricing should be strongly influenced by marketing that has to play a strategic role. Green values, ethical and moral issues are namely important factors now days also in the field of business. Many companies have make a difference by owning green values to be an important part of a business plan e.g. in packaging (D'angelico et al. 2010). Strong service fundamentals and quick responds to challenges is needed to create value in the dynamic business world. Substantial growth and profit are created by attracting new customers and offering value propositions to their needs while responding to valid requirements of the key customers. Most successful solutions are often reflected of a diversity of multidisciplinary work than can lead to another benefit; opportunity for competitive differentiation (Dosi et al. 2015). By generating new ways to analyze data fast, biosensor companies can create a great cumulative advantage. Company can make a significant difference to other companies that are trying to appeal to the same customers by creating a value proposition and familiarity that is unique. Services have become significant value adds for the business, besides the actual product. More companies should offer profound scientific support to ensure compliance and assist with installation and software upgrades. Business transitions may take unexpected turns and key strategic questions needs great management skills. In the field of biosensor markets both substance knowledge and business intelligence are needed. Many companies are now investing to the digital supply chains and via that even new business models can be made.

6.3 Sensor slide properties and business perspectives

Many areas like drug research, clinical analysis, defense and environment research need the cost-effective solutions. Surface plasmon resonance biosensors are providing excellent analytical performance specs for precise use and various biosensors with the

diverse potential of usage creates very wide application possibilities. Furthermore, this creates more profit possibilities because the biosensors can be used in the field of drug discovery, biomedicine, security and defense. Food safety standards and environmental monitoring are also important examples about the imperative utilization of the biosensors (Dias 2014; Vigneshvar et al. 2016). Hence, diverse of various biosensors and customers makes the strategy planning challenging but it can bring new and fresh multidisciplinary benefits considering the future product development.

Sensor slides were classified into four different groups based on the environments. This was a good indicator to evaluate the possible harms that are caused to the sensor slides by different storage conditions. Besides the storage conditions, the end user sites can vary a lot in terms of transport distance. Therefore, the results obtained here can be used to evaluate the optimal packaging, further shelf life research and storage environments from the very first time point when the sensor slides are manufactured. Information is necessary for the company but also for the customers. There are multiple ways to make business analyses to help to value the market size and growth prospects. Success requires quick capability to respond to customer needs and requests regarding all the products and services (Almquist et al. 2018, Bocken et al. 2014; Freeman and Beale 1992). Customers are willing to know how to use the sensor slides and instruments correctly in order to achieve the optimal sensor slide performance. Additionally, a holistic approach to a temperature controlled shipping can support to overcome the transportation obstacles and meet the regulatory requirements. Sensor slides that are reproducible and constant quality with the valid regeneration features would offer significant competitive advantage in the markets.

Differences in functionality observed in between the sensor slides were slightly diverse, indicating that the surface chemistry properties could have been even more equal. Yet, range between parallel sensor slides analyzed in the same environment were varying expectedly less than that observed between different storage conditions. Nevertheless, there were surprisingly wide differences between some parallel measurements. This can be seen from the results 5.1 – 5.3 where the parallel sensor slides may differ from each other at some time points quite markedly (Figure 3 - Figure 5). Yet, this is a great finding for the manufacturing department in order to make some adjustments. Additionally, it was noticed that the functionalization of the sensor slide

is better as the slides are made using fresh reagents. Both systematic and random errors may have occurred during and between the measurements. Many people were working in the same laboratory and the device was also used by other people. There are likely some biases that have been affecting to the results, even though work was really accurately and carefully executed. However, biases have been occurred mostly from the functionalization of the sensor slides, rather than working conditions. These findings can offer several practical implications for further studies. As described in the review of literature, in order to have meaningful market value, the satisfactory results are not good enough (Schwab 2017; Teece DJ 2010; Wirtz et al. 2016). First, company needs to have tools to identify profitability improvements to make better results. Second, recognizing the product problems in the early phase and diagnosing what changes are needed to achieve the right solution are important to consider. Third, it shows business intelligent to coordinate innovations strategically in order to bring more assets and value for the company.

Sensor slides that are reproducible and constant quality with valid regeneration features would offer significant competitive advantage in the markets. Surface chemistry of the sensor slides needs to be in excellent condition in order to gain relevant revenues from the markets. Customers are willing to know the facts about the products in order to make repeated purchases. Besides the substance knowledge, the business intelligence about sales and marketing are very essential assets. Business model should include flexibility for new enhancements and innovations (Massa and Tucci 2013). This leads to the core question about what kind of risks the company can or have to take in order to be agile enough to outcome competitors? The right decision making requires powerful team with great management skills (Sveiby 1997; Wintjes 2006).

7 CONCLUSIONS

Possibility for repetitive measurement cycles with multiparametric surface plasmon resonance (MP-SPR) device can lower the research costs. Supporting the Knoglinger et al. (2018) and Zauner et al. (2016), it is necessary to eliminate sensor slide-to-sensor slide variation, which is a problem with some product lines. In this research, biotinylated sensor slides were found to be suitable for functionalization with switchavidin and they showed good performance in regeneration. When concerning all the results together at the end of the study period, sensor slide performance is generally starting to decrease after 12 days (Figure 3 – Figure 5). Further studies are needed to underline the specific days for different conditions.

It was also found that the storage specifications of the sensor slides are important. The portfolio of the products and services defines the strategic lines besides the company's awareness about the facts how the customer needs are met. Besides other aspects, the research is valid as Zauner et al. 2016 discussed about the importance of reagents and the sensor slide's surface that must be stable under ambient conditions.

The results are highlighting the fundamental importance of reagent choice and storage environment of the gold coated and biotinylated sensor slides that must be made with care, in order to exploit the full potential for regenerative biosensing. Results are supporting the findings Knoglinger et al. (2018) and Zauner et al. (2016) who showed that switchavidin is as an optimal tool for biosensor related applications. The accelerated and predictive performance and stability measurements can give insights for the future advances, needed in the field of accelerated aging methods for packaging, storage and sensor slide improvements. In addition, the main findings can aid to prevent unexpected instabilities in future researches and studies. Possible factors for instability were identified. Further research could identify if bigger number of sensors lose their performance ability at the same point of time as they are exposed to the exactly same environment for a prolonged time period. In conclusion, the experiments made in this research demonstrate that the regenerative gold coated biotinylated sensor slide is well suited for the reconstruction of the interaction mechanism between switchavidin and IgG. This study provides potential and cost-

efficient approach to evaluate effects and costs of various biosensor related application possibilities on markets. Additionally, approach to improve sensor slide properties and functionalization further.

Biosensors are associated with significant adverse applications and increased market potential. Value in the business markets is more than entity of the both technical, economical, service and social related benefits. As Schwab (2017) describes the complexity of the theory about fourth industrial revolution, biotech-related markets are growing and new solutions are constantly needed to meet the requirements of different customer segments. At the moment, in order to achieve and maintain competitive advantage, multiple companies still need employees and management with both business and technological substance intelligence combined to a capability for emotional intelligence. Managing flexible market offerings successfully is a part of company's ability gain more revenues. Because of these extensive causal-connections, this Master's Thesis successfully underlined the value of multidisciplinary skills in order to push understanding and innovations further.

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