DISSERTATIONES CHIMICAE UNIVERSITATIS TARTUENSIS
58

SURFACE SILANIZATION AND ITS APPLICATION IN BIOMOLECULE COUPLING

KRISTJAN SAAL

TARTU UNIVERSITY
PRESS

Department of Chemistry, Institute of Organic and Bioorganic Chemistry, University of Tartu, Estonia

Department of Physics, Institute of Physics, University of Tartu, Estonia.

Dissertation is accepted for the commencement of the degree of Doctor of Philosophy in Chemistry on April 4, 2006, by the Doctoral Committee of the Department of Chemistry, University of Tartu.

Doctoral advisor:	Prof. Ago Rinken, University of Tartu Dr. Ants Lõhmus, University of Tartu
Opponents:	Dr. Donats Erts, University of Latvia
Commencement:	June 21, 2006, 2 Jakobi St., room 430

ISSN 1406–0299
ISBN 9949–11–372–5 (trükis)
ISBN 9949–11–373–3 (PDF)

Tartu Ülikooli Kirjastus
www.tyk.ee
Tellimus nr. 299

CONTENTS

LIST OF ORIGINAL PUBLICATIONS

Paper I K. Saal, V. Sammelselg, A. Lõhmus, E. Kuusk, G. Raidaru, T. Rinken and A. Rinken, Characterization of glucose oxidase immobilization onto mica carrier by atomic force microscopy and kinetic studies, *Biomolecular Engineering* **19** (2002) 195–199.

Paper II T. Tätte, K. Saal, I. Kink, A. Kurg, R. Lõhmus, U. Mäeorg, M. Rahi, A. Rinken and A. Lõhmus, Preparation of smooth siloxane surfaces for AFM visualization of immobilized biomolecules, *Surface Science*, **532–535** (2003) 1085–1091.

Paper III K. Saal, T. Tätte, I. Tulp, I. Kink, A. Kurg, U. Mäeorg, A. Rinken and A. Lõhmus, Sol-gel films for DNA microarray applications, *Materials Letters* **60** (2006) 1833–1838.

Paper IV K. Saal, M. Plaado, I. Kink, A. Kurg, V. Kiisk, J. Koževnikova, U. Mäeorg, A. Rinken, I. Sildos, T. Tätte and A. Lõhmus, Aminopropyl Embedded Silica Films as Potent Substrates in DNA Microarray Applications, *Biological and Bio-Inspired Materials and Devices* (Mater. Res. Soc. Symp. Proc. **873E**, Warrendale, PA, 2005) K9.3.

Author's contribution

As seen from the list of authors of included papers there were many people involved in the research, which was because of the interdisciplinary nature of the work. With minor exceptions, the author of this thesis performed all sol-gel syntheses and silanizations and AFM (atomic force microscopy) measurements of prepared substrates, as well as substrate preparations for different analyses. The spectroscopic measurements of prepared samples and biochemical experiments (enzyme kinetics and DNA assays) were performed and interpreted by coauthors. The author of this thesis was responsible for assembling and processing different experimental data and their further analysis for publications. The author also compiled the manuscripts of papers [II–IV]. However, the intellectual merit of outcome produced in the frame of this work is a group effort and the contribution of every author cannot be overestimated.

Paper I: responsible for substrate pretreatment and immobilization of glucose oxidase. Performed most of the AFM imaging. Actively participated in preparation of the manuscript.

Paper II: responsible for sol-gel synthesis, film-making, AFM imaging and sample preparation for spectroscopic analyses. Responsible for composing template and final (revised) versions of the manuscript.

Paper III: responsible for sol-gel synthesis, film-making, AFM imaging and sample preparation for spectroscopic analyses. Responsible for composing template and final (revised) versions of the manuscript.
Paper IV: responsible for sol-gel synthesis, film-making and sample preparation for spectroscopic analyses. Responsible for composing final (revised) versions of the manuscript.

ABBREVIATIONS

AFM	atomic force microscope/microscopy
APTES	3-aminopropyltriethoxysilane/γ-aminopropyltriethoxysilane/$NH_2CH_2CH_2CH_2Si(OC_2H_5)_3$
APTMS	3-aminopropyltrimethoxysilane/γ-aminopropyltrimethoxysilane/$NH_2CH_2CH_2CH_2Si(OCH_3)_3$
CGH	comparative genomic hybridization
GPS	3-glycidoxypropyltrimethoxysilane /γ-glycidoxypropyltrimethoxysilane
OTS	n-octadecyltrichlorosilane/$CH_3(CH_2)_{17}SiCl_3$
PTMS	n-propyltrimethoxysilane/$CH_3CH_2CH_2Si(OCH_3)_3$)
SAM	self-assembled monolayer
SNP	single nucleotide polymorphism
TEOS	tetraethoxysilane/$Si(OC_2H_5)_4$
TMOS	tetramethoxysilane/$Si(OCH_3)_4$

PREFACE

The world is constantly searching for new technological advances in perspective of possible synergy to the present ones. Undoubtedly, materials science and biotechnology provide a significant part of progress in our everyday lives. This work is an attempt to highlight important aspects of three different disciplines like science of self-assembled monolayers (SAMs), sol-gel chemistry and DNA microarray technology. Often the interdisciplinary aspects of these disciplines have led to contradicting conclusions or have been misinterpreted. E.g., in DNA microarray analyses SAMs or sol-gel substrates are used as platforms where the biochemical reactions are performed, but often their influence on the analyses and results is neglected. From the other side, despite being very same by the chemical origin and applications, the relevant aspects of SAMs and sol-gel films are rarely confronted in frame of a single research work. In this dissertation several aspects of SAMs, sol-gel chemistry and DNA microarray technology are discussed with the objective to achieve a three-in-one compendium that connects the main principles of its components.

The current work was mainly carried out at the Institute of Physics and Institute of Organic and Bioorganic Chemistry, University of Tartu. A significant contribution was added by the Institute of Molecular and Cell Biology, Estonian Biocentre. Also, the practical assistance by the Institute of Biochemistry, University of Tartu and Asper Biotech Ltd was of considerable importance.

The thesis is divided into four chapters. The first chapter gives short overview about the silanization chemistry. The second chapter takes a brief look at DNA microarray technology, giving a wider explanation to the motivations behind the work presented in [III] and [IV]. The third chapter defines the aims of this study and in the fourth chapter a summary of the results is given. The summary has been provided with the comments on the evolutionary stages of the current research, followed by a deeper look at the original papers and related problems individually.

1. SILANIZATION: PROCESSES AND FILM FORMATION

1.1. Historical background

Silanization is a method of organic thin film preparation, used in a multitude of applications like coatings for corrosion inhibition, adhesion promotion, anti-reflection, anti-statics, biomolecule immobilization etc. Nowadays, silanization classifies as an independent scientific orientation, which synergies the competence of organic chemistry and materials science. However, the complete history of silanization remains obscure, because systematic studies on the topic began only two decades ago. In general, the problems most often encountered in silanization/related research notably intrigued scientists already more than 200 years ago. Back then Franklin observed the calming influence of oil on water surfaces [1]. In the 19th century, Pockels prepared monolayers at the air-water interface [2–5], followed by the works of Rayleigh [6], Hardy [7], Devaux [8], and others. Later, monolayers of amphiphilic molecules on the water surface were named after Langmuir [9,10]. On solid substrates, Blodgett did the first study on the deposition of long-chain carboxylic acids [11,12]. Around that time, amphiphilic monolayers were already used to control the wetting behavior of metal condenser plates in steam engines [13–15].

At present times silanization is a major part of discipline of SAMs. Systematic research on SAMs was first performed by Zisman in 1946, who studied preparation of a monomolecular layer by adsorption (self-assembly) of a surfactant onto a clean metal surface [16]. At that time, the potential of self-assembly was not recognized, and this publication initiated only limited interest. The work by Blackman and Dewar more than a decade later can be spotlighted as a methodical approach to SAMs formation [17]. The early works on SAMs focused on thiol assemblies on metal surfaces. Until now these systems remain as the most studied SAMs. One of the first papers on silane based SAMs was published by Sagiv in 1980 [18]. Since then interest in silane SAMs grew rapidly focusing primarily on octadecyltrichlorosilane (OTS) monolayers, which by now have become the most studied silane based SAM systems. Surprisingly, self-assembling of short-chain silanes has received only moderate attention. OTS films are true monolayers and therefore other silane films are often erroneously considered to be monolayers as well. However, most of them display fairly more complex structures. Recently, Schreiber have published an exhaustive review about various SAM-systems, covering very different aspects of the subject [19].

Silanization processes are certainly also a part of sol-gel chemistry. The first studies on alkoxide hydrolysis, polymerization and effects of pH and solvent on these processes appeared in the early 1980s by Yoldas [20,21], Uhlmann [22] and Sakka [23], after which the sol-gel chemistry quickly became an important field of chemistry where it remains also today.

1.2. Silanization processes

1.2.1. General aspects

In most cases silanization is used in order to give specific properties to a substrate of interest. Consequently, silanizing agent should bear a functional group of interest and also relevant groups needed for coupling to substrate surface. Most often alkoxysilanes and chlorosilanes are used for latter purpose (Figure 1). These compounds attach to substrate surface via alkoxy or chloro endings, and for silane coupling the substrate should bear available hydroxyl groups on its surface.

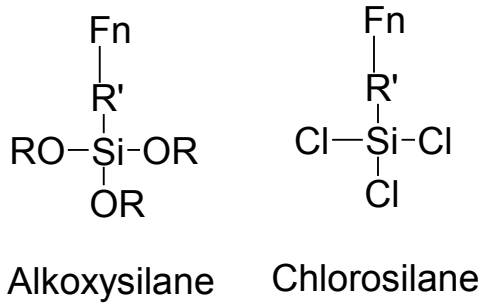

Figure 1. Schematic representation of functional alkoxy and chlorosilanes; Fn – a functional group.

The most common substrates subjected to silanization are glass, quartz, silicon and aluminum, and all these materials provide sufficient amount of hydroxyl groups needed for silane coupling. In a clean, zero-aqueous environment these silanes remain stable for long time periods (for years). But wherever there are even traces of water present in the medium, the silanization processes immediately start to progress. Water initiates hydrolysis of initial compounds, which subsequently condensate (polymerize) (Figure 2) [24]. Rates of these processes are different for different silanes.

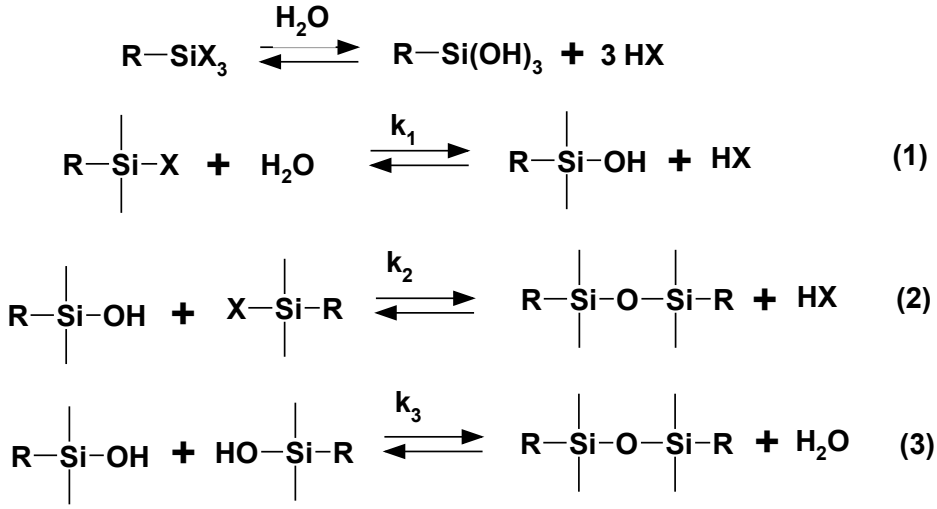

Figure 2. Hydrolysis and condensation of trifunctional silanes [24].

These rates determine the ability of silanes to form preferably either monolayers or three-dimensional clusters. If rate constants k_2 and k_3 are relatively small compared to k_1, formation of SAMs is favored. Alkoxysilanes (X=OR) are more stable to hydrolysis than chlorosilanes [24–26]. A major reason for this is that both steps 1 and 2 in Figure 2 result in the production of acid when X=Cl, as in the hydrolysis of chlorosilanes. This acid catalyses further reactions and quickly leads to gel formation. Nevertheless, hydrolysis takes place in both cases. For alkoxysilanes it has been shown that each one of the three hydrolysis steps leaves next one to occur more rapidly [27,28]. It has been shown empirically that moisture must be rigorously excluded from trichlorosilanes, whereas alkoxysilanes can be stored and used for extended periods with minimal protection from hydrolysis [24]. However, this empirical fact only applies to long-chain silanes, but clearly shows up controversial in the case of short-chain ones. For example, the hydrolysis kinetics of short silanes γ-glycidoxypropyltrimethoxysilane (GPS) and γ-aminopropyltriethoxysilane (APTES) differ a lot (APTES is known to hydrolyze rapidly) because of an autocatalytic reaction of APTES [27].

1.2.2. Catalysis

Both silanization processes, hydrolysis and condensation, are regulated by acid-base catalyses. Although hydrolysis can occur without addition of an external catalyst, it is more rapid and complete when the catalyst is employed. Mineral

acids (e.g. HCl) and ammonia are most widely used, however, other catalysts like acetic acid, KOH, amines, KF, and HF are also effective [29]. The rate and extent of the hydrolysis reaction is often determined by the strength and concentration of the acid- or base catalysts [30]. As with hydrolysis, condensations can proceed without catalyst, however, these reactions are acid and base specific as well [31]. Iler has shown that under more basic conditions times of gel formation have tendency to increase [32]. However, the most important issue raising from the catalysis is the fact that it determines the structure of the formed polymer. For example, sol-gel derived silicon oxide molecular networks under acid-catalyzed conditions yield primarily linear or randomly branched polymers, which entangle and form additional branches resulting in gelation. On the other hand, silicon oxide networks derived under base-catalyzed conditions yield more highly branched clusters, which do not interpenetrate prior to gelation and thus behave as discrete clusters [23].

1.2.3. Water/silane molar ratio

Water/silane molar ratio, also known as term R in sol-gel chemistry is another parameter strongly affecting the kinetics of hydrolysis and condensation processes. The most straightforward effect of water (increased value of R) is the acceleration of the hydrolysis. At higher R values more complete hydrolysis of monomers occurs before significant condensation takes place. But since water is one of the products of the reversible condensation reaction (Figure 2 (3)), large excess of water may promote hydrolysis of siloxane bonds (reverse of Figure 2 (3)) [33].

Similarly to the catalysts, R value is also important factor in determination of the structure of forming gels [34].

1.2.4. Organically modified silanes

The kinetics of sol-gel processes has been thoroughly studied mainly on the basis of tetraethoxysilane (TEOS) [35]. Organically modified silanes (alkyl-substituted trialkoxysilanes) have the same hydrolysis–polycondensation mechanism as TEOS [36]. However, the reaction rates are different. For example, vinyl-TEOS and especially methyl-TEOS hydrolyze-polycondensate faster than TEOS. Methyl group is a weak electron donor compared to ethoxy groups, which are moderate electron acceptors. The substitution of ethoxy group with methyl group decreases the positive charge on silicon, increasing the negative charge on oxygen. The latter increases the degree of protonation of oxygen atoms around silicon atom leading to a higher hydrolysis rate in the case of methyl-TEOS comparatively with TEOS [36].

1.3. Formation of silane films

1.3.1. General aspects, adsorption of long-chain silanes

The typical scheme for a silane film formation is depicted in Figure 3. Similar representation can be met in recent papers [37] and monographs [38]. However, this scheme is simplified, as the structure and uniformity of the film depend on the type of silane, its concentration, reaction medium and substrate.

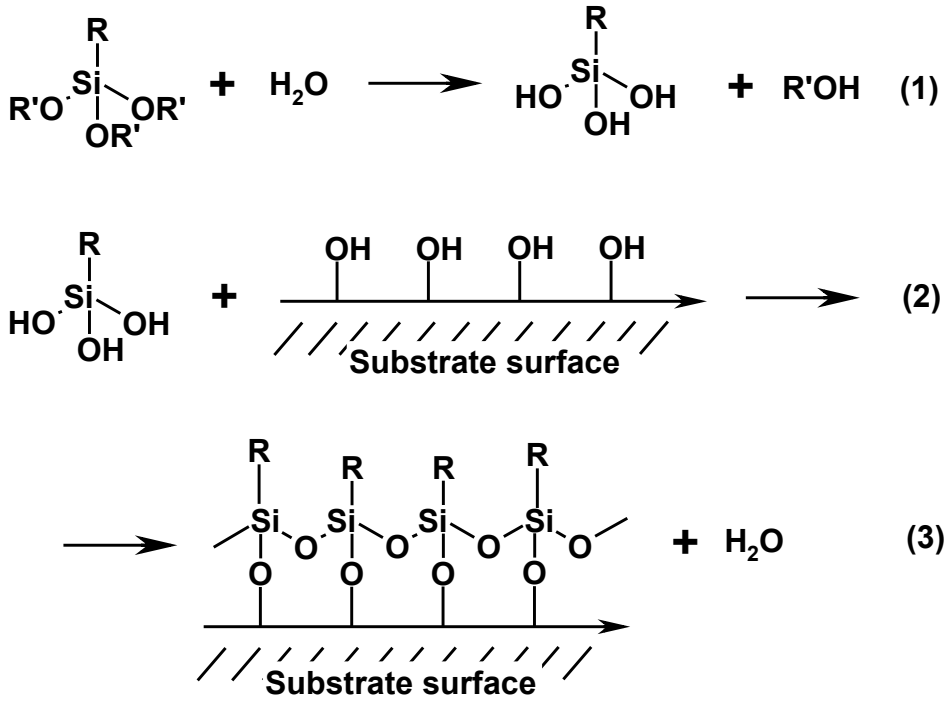

Figure 3. Schematic representation of the silane film formation.

Prior to film formation silanization processes occur (see 1.2.). As a result, silane molecules polycondensate and form three-dimensional clusters, which compete with hydrolyzed monomers in filling the vacant sites on the substrate surface. Polycondensation is highly sensitive to even trace quantities of water present in reaction medium and on the substrate. McGovern *et al* suggested that water concentration of 1.5 ppm is optimal for forming closely packed OTS monolayers [39]. Nevertheless, there are a large number of reports on formation of true OTS monolayers on different substrates [18,24,40–56]. Biernbaum *et al* claimed that n-alkyltrichlorosilanes with longer as well as with shorter chain-lengths than OTS form more disordered films in terms of the orientation of the

chains [56]. They also showed that OTS SAMs exhibit so-called island growth [55]. Longer-chain molecules showed similar growth mechanisms, whereas short-chain molecules did not show island growth behavior [55]. However, on flat substrates like silicon OTS forms uniform monomolecular layers (e.g. see Figure 5 in [42]). On the contrary, other silanes, especially short-chain ones, do not form uniform monolayers, but monolayers mixed with polycondensates originating from silanization processes [57].

If we ignore the polycondensation processes, the product presented in Figure 3 (3) cannot be even theoretically possible. Such full cross-linking cannot be achieved. With a typical Si-O bond length of 1.6 Å, the O-O distance has to be 3.2 Å or less (for a O-Si-O bond angle of less than 180°), which would not leave enough space for parallel, upright-standing hydrocarbon chains. These chains would have to be splayed apart, implying that the cross-linked head-groups would prevent formation of a full-covered layer or, reverse, that the full-covered layer cannot be fully cross-linked [19,24,58]. The exact mechanism of the anchorage of SAMs to substrate surface remains to be resolved.

1.3.2. Adsorption of functional silanes

Even though the film formation of non-functional short-chain silanes has not been extensively studied, it has been shown that their films are less ordered than OTS films [56]. These films are mainly made of short silanes bearing an active functional group like NH_2 and SH. These functional groups seriously affect the film formation, but there is very little systematic information available about this influence. Lee and Wool investigated the co-silanization of aluminum substrate using 3-aminopropyltrimethoxysilane (APTMS, $H_2NCH_2CH_2CH_2Si(OMe)_3$) and n-propyltrimethoxysilane (PTMS, $CH_3CH_2CH_2Si(OMe)_3$), which only differ by the presence/non-presence of terminal NH_2 group [37]. The formation of mixed silane coatings on the substrate was proposed. However, there was no clear evidence that these films were monolayers, so they were called as 'monolayer-like silane coatings'. In addition, APTMS adsorbed twice as fast as PTMS. A similar study was performed by Wu et al, who investigated the co-adsorption of OTS and 4-aminobutyldimethylmethoxysilane (ABS, $NH_2C_4H_9Si(CH_3)_2OCH_3$) to silicon substrate [59]. They obtained microscopically heterogeneous surfaces instead of a uniformly mixed monolayer. It was proposed that OTS formed a smooth monolayer, which was mixed with three-dimensional multilayer domains of ABS up to 7 nm in thickness. The reason why aminosilanes do not form regular monolayers may be connected with their tendency to form hydrogen bonds between the functional groups [60]. Wei *et al* measured the force of interactions between one pair of amino groups in water to be 200.0±43.6 pN [61], while this parameter for hydroxyl groups was 181±35 pN [62]. The ordered monolayers were not obtained also with long aminosilanes, such as (17-aminohepta-decyl)trimethoxysilane, probably due to hydrogen bond formation between

amine groups and also with the surface silanols [56]. Thus, literature data show that in liquid environment aminosilanes form only disordered layers [38], whereas ordered aminosilane monolayers can be obtained by utilization of vapor phase silanization techniques [38,63,64], in which case polycondensation can still occur on substrate surface when exposed to water molecules [27]. It is also important to note that polymeric silane structures adsorb onto active centers of the surface faster than monomers [65], and in the case of aminosilanes these structures are mainly kept together by non-covalent forces [27].

The uniformity of silane films heavily depends on the concentration of silane in reaction medium, either in liquid [57] or in gas phase [66], but also on the reaction times [67]. Hu *et al* investigated film formation of 3-mercaptopropyltrimethoxysilane (MPTMS, $(CH_3O)_3SiCH_2CH_2CH_2SH$) in benzene [57]. At low silane concentrations and water content below 30 ppm monolayer-like films were formed, while the increase in silane concentration led to appearance of polymeric particles into the monolayer (see Figure 7 in [57]). They proposed a nice scheme that in a compact way summarizes the formation of a silane film in dilute solutions (Figure 4).

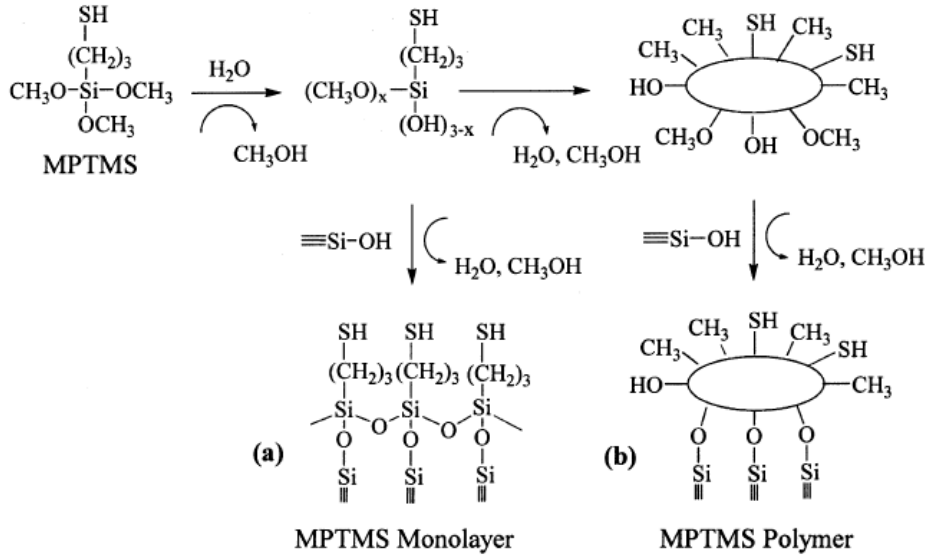

Figure 4. Competitive reactions in the formation of self-assembled MPTMS layers on the SiO_2 surface: (a) surface dehydration on the SiO_2 surface; (b) self-polymerization in the solvent [57].

1.3.3. Formation of sol-gel silane films

Sol-gel films are alternatives to SAMs and SAM-like systems described above. These materials have been known for their very high level of chemical homogeneity [68]. In preparing the sol-gel films the condensation processes (see Figure 2) are even favorable. In a typical sol-gel coating process, the monomer silane is first polymerized and the product (polymer silane solution or "sol") is transferred to a substrate by dip- or spin-coating (Figure 5). Then the solvent is evaporated and a glassy "gel" is formed onto the substrate. Thereafter the surface is thermally treated in order to remove remains of a solvent and water from the system. During the "baking" the sol-gel processes depicted in Figure 2 will be completed, the film constringes and becomes harder [69]. The sol-gel processing enables also to produce other glass-like materials in various forms like fibers, monolithic optical lenses and composite glass [70]. TMOS and TEOS are the most widely used starting compounds for the fabrication of silica-based sol-gels. The introduction of various functional groups into alkoxide has led to organically modified sol-gel glasses, known as ormosils (Figure 6).

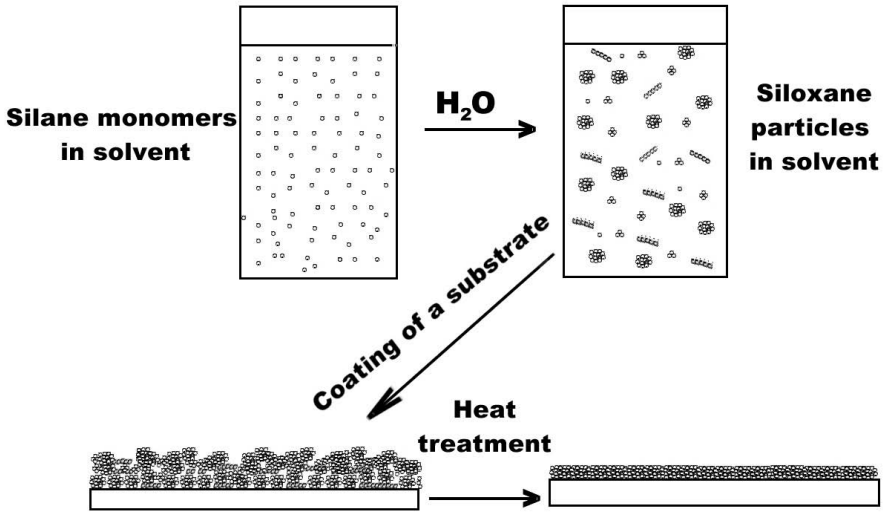

Figure 5. Formation of sol-gel films.

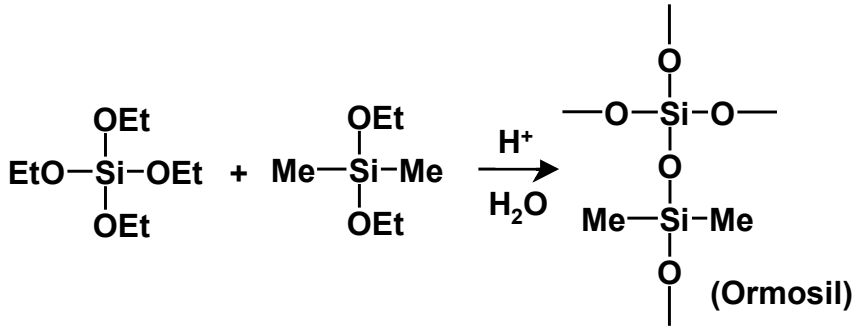

Figure 6. Reaction scheme for ormosil formation [71].

The covalently bound functional groups decrease mechanical tension during the drying process [72]. In order to avoid phase separation, functionalized alkoxides F-R′-Si(OEt)$_3$, where F is a functional group such as amino or isocyanate and R′ is an alkyl spacer, are usually used to graft covalently dopants onto the matrix. The structure and topography of ormosils depend on the size of the organic subsistent F-R' [71]. In frame of current study, the preparation and properties of APTMS and APTMS-TMOS ormosils was investigated. The obtained films had very high homogeneity and nanoscale smoothness, which made them suitable substrates for immobilization of biomolecules [II,III].

2. DNA MICROARRAYS

2.1. Definition and working principle

DNA microarray technology is among the most widely used advanced applications of silane coatings. The pioneering work on the microarray technology was published in 1991 by Fodor et al, where a light-directed, spatially addressable parallel chemical synthesis method for generation of peptide microarrays was described [73]. The technology era began in mid-1990s with the famous work by Schena et al [74].

The DNA microarray consists of a flat, solid substrate (typically glass) with a smooth coating, typically functionalized alkoxysilane. The surface is then grafted (by printing or *in situ* synthesis) with various known DNA probes at predefined locations (see Figure 3 in [75] for illustration).

DNA probes on a chip can be oligonucleotides or longer DNA sequences and the chips can have several applications. To determine the expression level or location of a gene of interest, "expression chips" are used. Probes are generated to be complementary to the cDNA synthesized from mRNA sequences of known genes and spotted onto chips. cDNAs from mRNA, using reverse transcription, of normal and diseased tissues are labeled with dissimilar fluorescent dyes (i.e. the healthy targets can be labeled with a green dye, while the cancerous targets are labeled with red) and hybridized on the same chip. Hybridization occurs when the sequences of the target and probe are complementary, resulting in strong hydrogen bonding between the target and probe. Noncomplementary targets and probes do not form such strong hybrids and are subsequently removed from the array surface using stringent washing. A scanning process is conducted after hybridization and washing to image the fluorescent dyes simultaneously, which represent labeled targets that have hybridized with their probe complements on the microarray surface. Spots fluorescing strongly in the green or red channels are indicative of strong gene activity in the healthy or cancerous tissues respectively, while spots exhibiting a yellow appearance indicate minimal difference in gene activity between the healthy and cancerous tissues.

It has been hypothesized that certain chromosomal gains and losses are related to cancer progression and that the patterns of these changes are relevant to clinical prognosis. Genomic gains and losses can also be detected by DNA chips using Comparative Genomic Hybridization (CGH). CGH allows to look for gains and losses or for a change in the number of copies of a particular gene involved in a disease state. In CGH each spot of the target DNA in the array has a known chromosomal location. DNA from tumor tissue and from normal control tissue (reference) is labeled with a different fluorescent dye and hybridized together on the same chip. If there is a difference in copy number it will be revealed by different intensities of the fluorescent signal.

DNA chips can also be used in a mutation microarray analysis for detection of small genetic variations called Single Nucleotide Polymorphism or SNPs. SNPs may fall within coding sequences of genes and therefore might cause changes in amino acid sequence of proteins which in turn might result in a disease.

Thus, the DNA microarrays have become glass-based, biological sensors that can contain over 30 000 distinct, known probes at specific locations. This powerful biological sensor provides researchers a tool to characterize a human genomic state with a single, miniature experiment.

2.2. Importance of the glass substrate

The DNA microarray contains three key components:

- A solid, inert, impermeable support (usually inorganic glass);
- A coating that smoothes the surface and generates functional groups for biomolecule binding; and
- A biomolecules' array for hybridization probes.

Standard 25 mm × 75 mm glass microscope slides were the first supports commonly used for microarray assays, mainly because of their availability in most molecular biology laboratories where DNA microarray technologies were invented. However, the widespread adoption of glass as the substrate of choice for microarray applications is also attributable to its intrinsic properties, including low fluorescence, excellent flatness, chemical inertness, and low cost. Low intrinsic fluorescence is especially important property for microarray supports, since most assays are characterized using fluorescent imaging equipment with excitation wavelengths in the visible spectrum (most commonly 523 nm and 635 nm), and high background would considerably decrease microarray sensitivity [75].

The flatness of the glass is also important parameter for microarray applications. A total flatness of <50 μm (peak-to-valley over a 25 mm × 75 mm area) is required during a microarray assay to ensure precise biomolecule immobilization (by spotting or photosynthesis) and accurate scanning, since some laser scanners have a depth of focus of only ±30 μm [75].

Another important property of glass for microarray applications is its chemical stability. The glass must provide effective biomolecule immobilization, but should not dissolve or leach alkali ions or other components, which may change composition of the buffer solutions used during the microarray printing and hybridization processes [75].

Other solid-supports applied besides the glass include cellulose sheets [76], polymer-based membranes, or other materials. Glass coated with polymers [77] or dendrimers [78] have also been used.

2.3. Importance of the coatings

The second important component of a microarray is its coating, which provides a uniform surface containing essential functional groups for biomolecule immobilization. Organo-functional alkoxysilane coatings are widely used and accepted within the microarray industry as they are well suited to coat glass surfaces and can provide a variety of possible chemical functionalities. γ-aminopropyltriethoxysilane (APTES) and γ-glycidoxypropyltrimethoxysilane (GPS) are most commonly used as the formation of almost perfect monolayers for biomolecule immobilization is expected with these reagents. However, as already discussed in section 1., formation of homogeneous surfaces using short functionalized silanes is highly complicated. Therefore, in current commercial practice, silane coatings are generally disordered and display a distribution of bonding configurations (including self-condensation/cross-linking), although skewed in such way that the majority of the organic endgroups extend out from the coating and are free to interact with the DNA [75]. The DNA can be coupled to silanized surface by either non-covalent (ionic) [75,79,80] or covalent means [75,80–84].

3. AIMS OF THE STUDY

The first and general aim of the study was to find new and optimal methods of substrate pretreatment for biomolecule coupling onto solid surfaces. During the studies the particular aims have changed but this general line has been followed in this work. The particular objectives of the study were:

- Finding optimal surface pretreatment conditions of biomolecule immobilization onto solid carrier for AFM visualization and biosensor and DNA microarray applications.
- Comparison of different silanization strategies (silanization in dilute solutions and sol-gel silanization) in order to obtain homogeneous, smooth and stable films.
- Comparative characterization of obtained sol-gel silane films by different experimental means (AFM and different spectroscopies).
- Characterization of the coupling of aminated oligonucleotide DNA to silanized substrates and estimation of the applicability of sol-gel silane films in DNA microarray analyses.

4. RESULTS AND DISCUSSION

4.1. Approaches on the studies of surface silanization

The main objective of the study was fabrication of homogeneous and smooth coatings for biomolecule coupling. In original papers silanization and biomolecule immobilization to silanized substrates have been discussed, based on existing understanding and knowledge about the problems. When we started our research on glucose oxidase immobilization to mica carrier [I], we expected to achieve the conditions corresponding to maximal coupling and clear visualization of the immobilized enzyme with AFM. First we searched literature for the reliable chemical routes for covalent coupling of biomolecules to a solid substrate. We found that silanization was the most widely spread technique for that purpose. Furthermore, silanization was the cheapest and at the first glance it seemed relatively simple by its nature. However, in [I] we have found that fulfilling of the two settled objectives simultaneously was impossible. The problems occurred with the "invisible vertical silane structures", which did not allow to visualize particular molecules, but determined the ultimate enzyme load to the surface. In our subsequent study we focused on silanization processes rather than biomolecule immobilization [II]. We opposed the substrate silanization in dilute solutions (i.e. preparation of SAMs or SAM-like films) to the sol-gel silanization, and found some answers to the problems we met in [I]. The main claim in [II] was that short-chain aminosilane APTMS could not be deposited uniformly in liquid medium, which gave the base for the discussion in section 1.3.2. of this thesis. However, in [II] the explanation to this fact remained insufficient as we focused on the description of the alternative, sol-gel silane system. This system was uniform and smooth, an ideal for biomolecule immobilization, and we proposed that it was because of the "linear polymerization" of monomer silane. In the next paper we explicated the idea further more to sol-gel discipline, by incorporating a cross-linker silane TMOS in the system [III]. We showed that this made possible to control the density of amino groups on the film surface. From the practical point of view, the obtained films could be applied as substrates for DNA microarray analyses. As the logical continuation of that work, we carried on with the insight to the application itself by running arrayed primer extension analysis (APEX) on different sol-gel films [IV]. The analyses indicated to the role of complex factors on the intensity and selectivity of APEX signals. Here our studies have reached on the next level, where sol-gel films not only enable to perform microarray analysis, but also allow to generate specific substrates for particular analyses. We hope that we are able to describe these processes in the future.

4.2. Silanization of a surface and the enzyme coupling to silanized substrate [I]

The main objective in [I] was to characterize the localization and packing of glucose oxidase on a solid carrier. The mica surface was treated in 0.0001–1% w/v APTMS solution in 95% w/w acetone/water, activated with 1,4-phenylenediisothiocyanate (PDC) and eventually the enzyme was immobilized by incubation in glucose oxidase solution (50 ng/ml–50 mg/ml) (Figure 7). The active enzyme load onto mica surface depended on the concentration of APTMS used in silanization step (Figure 4 in [I]). However, the AFM imaging of these samples did not reveal significant differences in the texture of the enzyme layer (Figure 3 in [I]). The silanized substrates had "homogeneously wavy surface with the maximal height difference of 4–6 nm", which could not have been the reason of the variations in enzyme loading. Comparison of the enzyme activity data with the loading capacity data indicated that there had to be additional vertical structures in the enzyme layer, mounting all the activity on the surface. However, these APTMS-generated vertical structures for enzyme immobilization could not be visualized by AFM. In frame of [I] these intriguing questions largely remained veiled and our further studies focused primarily on silanization, neglecting the structuring of the enzyme, as that required more complex approach than we could achieve. However, the intriguing problems proposed in [I] have received quite an intensive feedback especially by biologists. Recently, Blasi et al published their investigations on enzyme–solid substrate systems with references to our papers [I] and [II], which can be considered as the attempts to unravel the loose ends that we had left in terms of these systems [85,86].

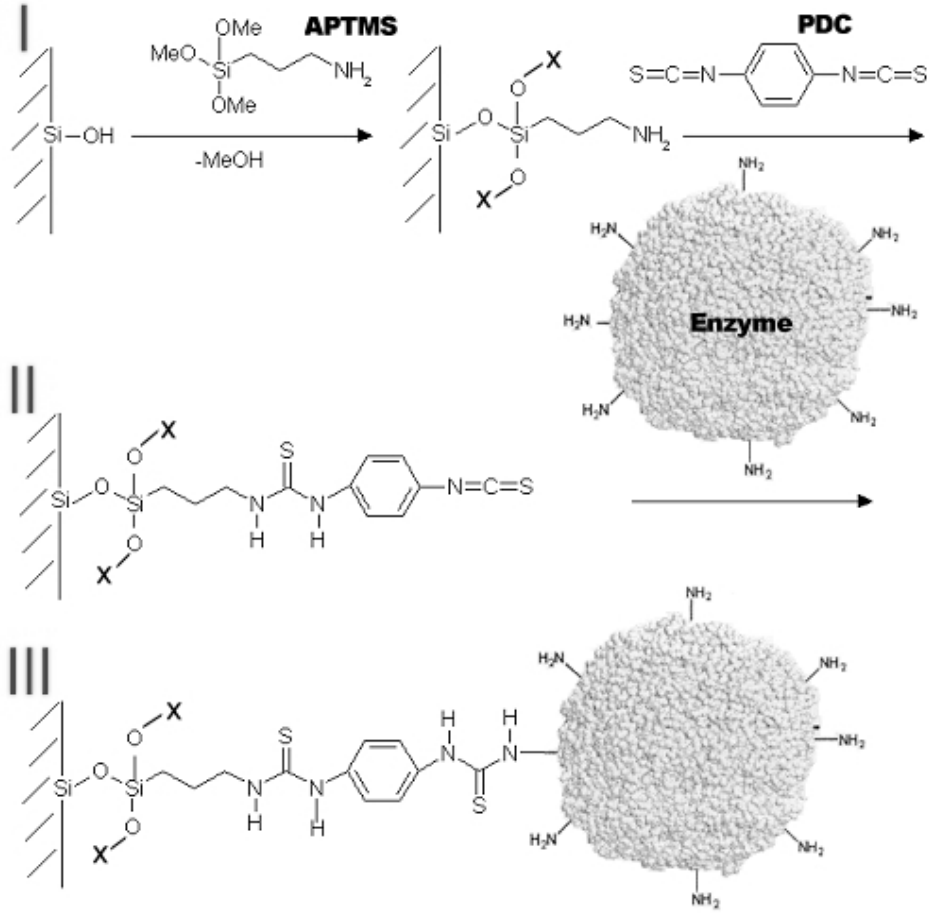

Figure 7. Immobilization of enzyme onto surface of mica using silanization with APTMS and coupling with PDC.

4.3. Comparison of silanization methods for making smooth silane films [II]

During the next step we have paid the main attention on the silanization processes in order to find out the factors that play an important role in biomolecule visualization with AFM. We compared the siloxane layers obtained by silanization in dilute solutions of APTMS with the layers prepared with methods of sol-gel chemistry. The mechanisms proposed (Scheme 1 in [II]) were supported by AFM data (Figures 1 and 2 in [II]). Of course, it has to be recognized that presented schemes may be oversimplified and reflect only a part of the real situation, as they do not take into account for example the

interactions and bonds between individual siloxane particles. The film formation of linearly polymerized APTMS might be more complex, but revealed considerably better surfaces, which dominate over other surfaces for biomolecule immobilization and especially for visualization with AFM.

In addition to the data published in [II] there was an intriguing series of experiments performed after their publication. The surfaces were formed by dipping of the substrates into prepolymerized silane solution for 2 minutes. Using 2% w/w solution of APTMS in 95% v/v acetone/water a clear correlation between preincubation times and sizes of siloxane particles was observed (Figure 8). These results are also in good agreement with model presented as Scheme 1 A in [II].

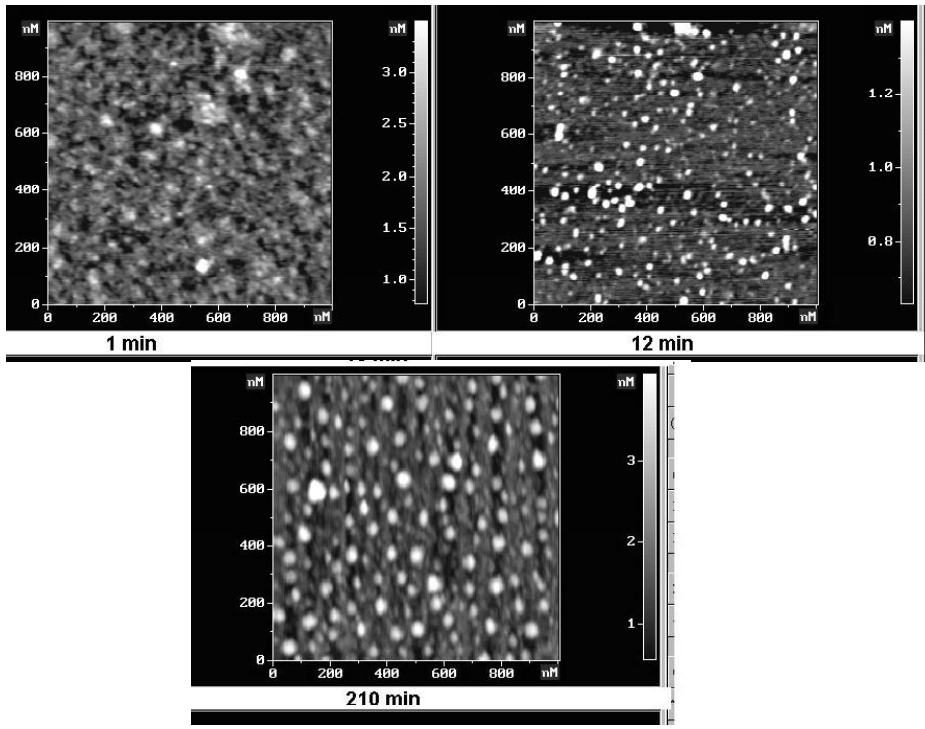

Figure 8. AFM images of APTMS nanoparticles deposited onto mica surfaces. Time values correspond to the preincubation times of silane.

4.4. Hybrid sol-gel films for coupling of DNA [III]

Proceeding from the data obtained in [II], our next efforts were focused on sol-gel processing in order to find the optimal conditions for the preparation of slides for binding of DNA. We used different APTMS-TMOS ratios for the

preparation of hybrid films and compared their chemical stability and properties in binding of DNA oligomers [III]. The aminated DNA was bound onto surface via PDC linker, similarly as depicted in Figure 7. Higher APTMS excess over TMOS concentration revealed higher biomolecule binding, but TMOS was required for cross-linking of the molecules to achieve stability of the film in aqueous medium at higher temperatures. Thus, the optimal APTMS/TMOS ratio was found to be 1:1 to 3:1, which formed also smooth surfaces with average height difference less than 2 nm (Fig. 5 in [III]). Due to the chemical flexibility of the sol-gel methods, we expected that these films extenuate the reproducibility problems that still hinder the microarray industry.

4.5. Hybrid sol-gel films for DNA microarray analyses [IV]

During the next step [IV] the suitability of APTMS-TMOS films for APEX analysis was studied. We used spin coating for the preparation of slides, but this gave considerably lower DNA binding rate to APTMS-TMOS films in comparison with slides prepared by dip coating (Figure 1 in [IV] and [III], respectively). The difference between spin- and dip-coated films can be remarkable, as was recently shown by Deepa et al [87]. They found that the grain size of sol-gel films was strongly dependent on the deposition technique as dip coated films consisted of larger grains. This observation correlates to our results, because dip-coated films bound more DNA probably because of the larger surface area (see discussions in [III] and [IV]). From other side, we have found that the optimum APEX signal does not correspond to maximum amino-density on the surface (Figure 2 in [IV]). Here, most likely the steric hindrances of the APEX reactions play crucial role and sol-gel chemistry allows to optimize reaction conditions for efficient study of the specific aspects of biochemical surface processes.

Last but not least we can conclude that our slides obtained by sol-gel methods have several preferences in APEX analysis. In comparison with commercial analogues the sol-gel films showed relatively low fluorescence background and more clearly formed spots (Figure 10), which is very important in APEX analyses.

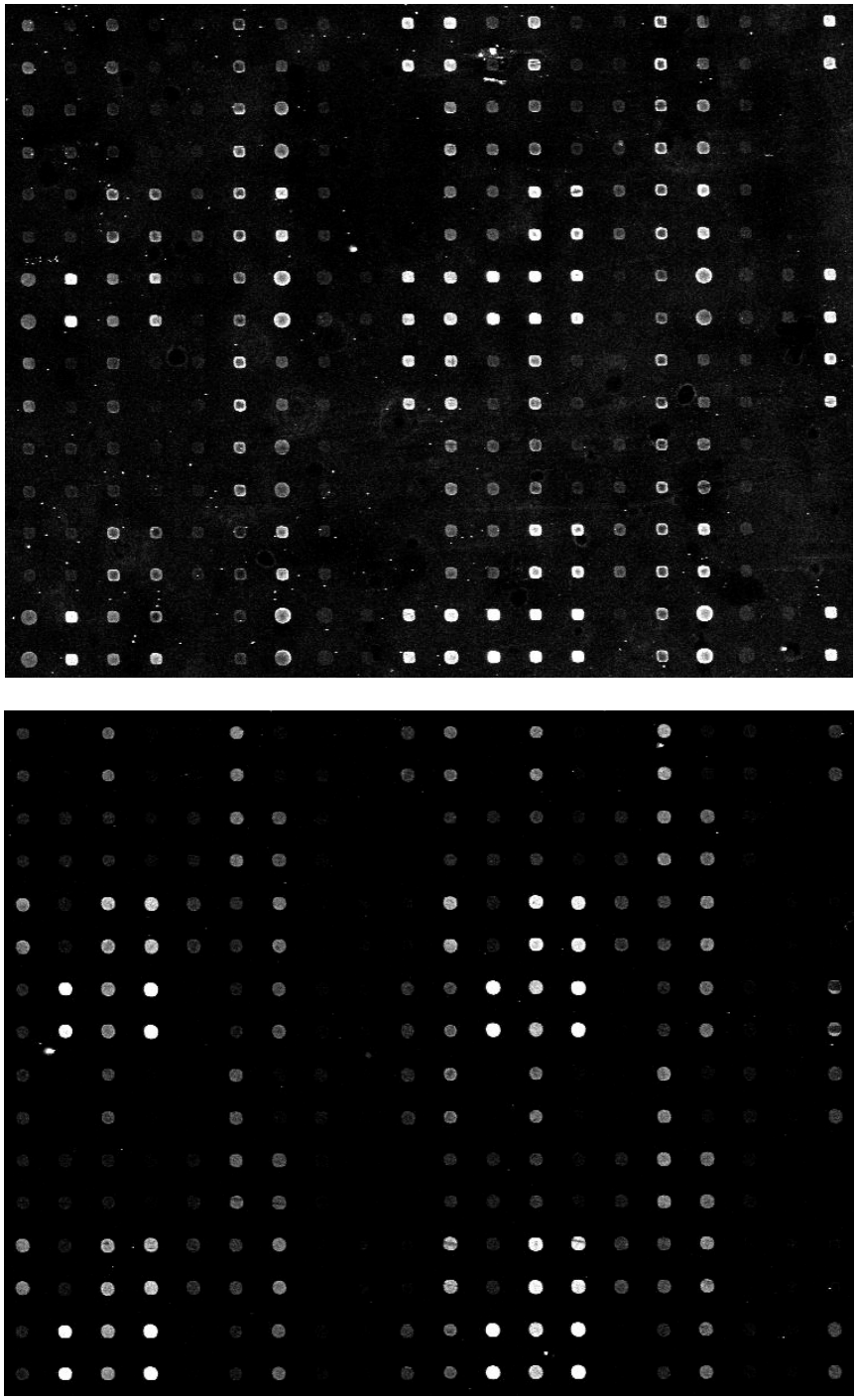

Figure 10. Fluorescence images of APEX results obtained on SAL-slide (a commercial reference slide) (upper) and on APTMS-TMOS 1:7 film (lower) (see [IV] for details).

CONCLUSIONS

Following conclusions can be drawn from the study:

- The dilute aqueous solutions of APTMS form three-dimensional surfaces, which have high capacity for biomolecule binding, but have very irregular surface for APEX analysis and AFM visualization of bound biomolecules.
- Sol-gel processing of the mixture of APTMS/TMOS enables to prepare homogeneous siloxane films. APTMS-TMOS hybrid films consist of densely packed three-dimensional nanometric particles. The surface density of amino groups is determined by the initial ratio of APTMS/TMOS.
- The amount of DNA immobilized onto APTMS-TMOS films and the structure of their spots depend on the molar ratio of APTMS to TMOS, and coating method.
- The optimum of the APEX signal does not correspond to maximal amino-density on the surface of APTMS-TMOS, probably due to the steric hindrances of the APEX reactions in the case of tightly packed molecules. Therefore, the optimization of the amino-density on hybrid films allows to optimize the efficiencies of the APEX signals as well.

SUMMARY IN ESTONIAN

Pindade silüülimine biomolekulide immobiliseerimiseks

Käesoleva doktoritöö eesmärgiks oli iseloomustada tahkete kandjate silüülimisprotsesse ning nende rakendatavust biomolekulide immobiliseerimiseks. Töö teoreetilises osas on käsitletud silaanikilede valmistamist lahjadest lahustest ja sool-geel meetodil. Lisaks on toodud ülevaade DNA mikrojada-analüüsist kui ühest levinumast silüülitud pindade rakendusalast. Töö uurimuslikus osas on vaadeldud vilkaluse silüülimist 3-aminopropüültrimetoksüsilaani (APTMS) lahjades (kuni 2%) lahustes ning ensüümi (glükoosi oksüdaas) immobiliseerimist valmistatud pindadele. Lisaks on uuritud APTMS ja tetrametoksüsilaani (TMOS) segakilede valmistamist sool-geel meetodil, amineeritud oligomeerse DNA sidumist nendele kiledele ning saadud pindade kasutatavust DNA mikrojadaeksperimentide (APEX ehk *arrayed primer extension*) läbiviimisel. Teostatud töö põhjal võib teha alljärgnevad järeldused:

- Lahjades lahustes APTMS polümeriseerub, moodustades kolmedimensionaalseid nanomõõtmetes osakesi, mis seovad hästi biomolekule, kuid moodustavad ebaregulaarseid pindu, mis ei ole kasutatavad biomolekulide visualiseerimiseks aatomjõumikroskoopia abil ega analüüsimiseks DNA mikrojadaeksperimentides.
- APTMS-TMOS segu kasutamine võimaldab sool-geel meetoditega valmistada homogeenseid kilesid, mis koosnevad tihedalt pakitud siloksaaniosakestest, kusjuures aminorühmade tihedus kile pinnal sõltub APTMS/ TMOS lähtekontsentratsioonist.
- Seostuva DNA hulk ja moodustuvate seostumispiirkondade (ing. k. *spots*) mõõtmed ja struktuur APTMS-TMOS hübriidkiledel sõltub kasutatavast APTMS ja TMOS vahelisest moolsuhtest, eelinkubatsiooni ajast ja kile pealekandmise tehnikast.
- APEX-analüüsi optimaalne efektiivsus ei lange kokku kile pinnal moodustunud maksimaalse aminorühmade pindkontsentratsiooniga, viidates, et APEX reaktsioonide läbiviimisel ilmnevad steerilised takistused tihedalt pakitud molekulide vahel. Seetõttu omab olulist väärtust metoodika, mis võimaldab valmistada APEX reaktsioonide jaoks optimaalse pindtihedusega substraate.

REFERENCES

1. B. Franklin, Philos. *Trans. R. Soc. London* **64** (1774) 445.
2. A. Pockels, *Nature* **43** (1891) 437.
3. A. Pockels, *Nature* **46** (1892) 418.
4. A. Pockels, *Nature* **48** (1893) 152.
5. A. Pockels, *Nature* **50** (1894) 223.
6. L. Rayleigh, *Philos. Mag.* **48** (1899) 321.
7. W.B. Hardy, *Proc. R. Soc. London A* **86** (1912) 610.
8. H. Devaux, *Smithson. Inst. Ann. Rep.* 261 (1913).
9. I. Langmuir, *J. Am. Chem. Soc.* **39** (1917) 1848.
10. I. Langmuir, *Trans. Faraday Soc.* **15** (1920) 62.
11. K. Blodgett, *J. Am. Chem. Soc.* **57** (1935) 1007.
12. K. Blodgett, *Phys. Rev.* **51** (1937) 964.
13. E. Schmidt, W. Schurig, W. Sellschopp, *Tech. Mech. Thermodyn.* **1** (1930) 53.
14. W.M. Nagle, T.B. Drew, *Trans. Am. Inst. Chem. Eng.* **30** (1933) 217.
15. H. Emmons, *Trans. Am. Inst. Chem. Eng.* **35** (1939) 109.
16. W.C. Bigelow, D.L. Pickett, W.A. Zisman, *J. Colloid Interface Sci.* **1** (1946) 513.
17. L.C.F. Blackman, M.J.S. Dewar, *J. Chem. Soc.* **162** (1957), Part I–IV.
18. J. Sagiv, *J. Am. Chem. Soc.* **102** (1980) 92.
19. F. Schreiber, *Prog. Surf. Sci.* **65** (2000) 151.
20. B.E. Yoldas, *J. Non-Cryst. Solids* **51** (1982) 105.
21. B.E. Yoldas, *J. Non-Cryst. Solids* **63** (1984) 145.
22. B.J.J. Zelinski and D.R. Uhlmann. *J. Phys. Chem. Solids* **45** (1984) 1069.
23. S. Sakka, H. Kozuka, *J. Non-Cryst. Solids* 100 (1988) 142.
24. C.R. Kessel, S. Granick, *Langmuir* **7** (1991) 532.
25. W. Noll, Chemistry and Technology of Silicones; Academic: New York 1968.
26. R.J.H. Voorhoeve, Organohalosilanes; Elsevier: Amsterdam, 1967.
27. M.-L. Abel, J.F. Watts, R.P. Digby, *Int. J. Adhes.&Adhes.* **18** (1998) 179.
28. J. Chojnowski. In: Jones RG, editor. Silicon containing polymers, pp. 59–72. Proceedings of RSC Macro Group Symposium, 6–8 July 1994, Canterbury Kent.
29. C.J. Brinker and G.W. Scherer, Sol-Gel Science: The Physics and Chemistry of Sol-Gel Processing (Academic Press, Inc.: New York, 1990).
30. R. Aelion, A. Loebel, F. Eirich, *J. Am. Chem. Soc.* **72** (1950) 5705.
31. E.R Pohl and F.D. Osterholtz in: Molecular Characterization of Composite Interfaces; eds. H. Ishida and G. Kumar, (Plenum: New York, 1985) p. 157.
32. R.K. Iler, The Chemistry of Silica (Wiley: New York, 1979).
33. L.C. Klein, *Ann. Rev. Mater. Sci.* **15** (1985) 227.
34. V.N. Balbyshev, K.L. Anderson, A. Sinsawat, B.L. Farmer, M.S. Donley, *Progr. Org. Coat.* **47** (2003) 337.
35. J. Šefčík, A.V. McCormick, *Catal. Today* **35** (1997) 205.
36. A. Jitianu, A. Britchi, C. Deleanu, V. Badescu, M. Zaharescu, *J. Non-Cryst. Solids* **319** (2003) 263.
37. I. Lee, R.P. Wool, *Thin Solid Films* **379** (2000) 94.
38. R.H. Wieringa, Surface-Grafted Polyglutamate Films with Reaction-Induced Polar Order (Ph.D. Thesis), University of Groningen, The Netherlands (ISBN 90–367–1306–4) 2000.

39. M.E. McGovern, K.M.R. Kallury and M. Thompson, *Langmuir* **10** (1994) 3607.
40. D.A. Styrkas, J.L. Keddie, J.R. Lu, T.J. Su and P.A. Zhdan, *J. Appl. Phys.* **85** (1999) 868.
41. I. Markovich, D. Mandler, *J. Electroanal. Chem.* **500** (2001) 453.
42. K. Kojio, A. Takahara, T. Kajiyama, *Colloid. Surface. A* **169** (2000) 295.
43. A. Ulman, *Chem. Rev.* 96 (1996) 1533.
44. R. Maoz, J.J. Sagiv, *J. Colloid Interf. Sci.* **100** (1984) 465.
45. L. Netzer, J.J. Sagiv, *J. Am. Chem. Soc.* **105** (1983) 674.
46. T. Vallant, J. Kattner, H. Brunner, U. Mayer, H. Hoffnamm, *Langmuir* **15** (1999) 5339.
47. N. Tillman, A. Ulman, J.F. Elman, *Langmuir* **5** (1989) 1020.
48. S.R. Wasserman, Y.Y. Tao, G.M. Whitesides, *Langmuir* **5** (1989) 1074.
49. S.R. Wasserman, G.M. Whitesides, I.M. Tidswell, B.M. Ocko, P.S. Pershan, J.D. Axe, *J. Am. Chem. Soc.* **111** (1989) 5852.
50. A. Ulman, Characterization of Organic Thin Films, Academic Press, San Diego, 1995.
51. C.P. Tripp, M.L. Hair, *Langmuir* **11** (1995) 1215.
52. J.B. Brzoska, I. BenAzouz, F. Rondelez, *Langmuir* **10** (1994) 4367.
53. A.N. Parikh, D.L. Allara, I. BenAzouz, F. Rondelez, *J. Phys. Chem.* **98** (1994) 7577.
54. D.K. Schwartz, S. Steinberg, J. Israelachvili, J.A.N. Zasadzinski, *Phys. Rev. Lett.* **69** (1992) 3354.
55. K. Bierbaum, M. Grunze, A.A. Baski, L.F. Chi, W. Schrepp, H. Fuchs, *Langmuir* **11** (1995) 2143.
56. K. Bierbaum, M. Kinzler, C. Wöll, M. Grunze, G. Hähner, S. Heid, F. Effenberger, *Langmuir* **11** (1995) 512.
57. M. Hu, S. Noda, T. Okubo, Y. Yamaguchi and H. Komiyama, *Appl. Surf. Sci.* **181** (2001), 307.
58. M.J. Stevens, *Langmuir* **15** (1999) 2773.
59. B. Wu, G. Mao, K.Y. Simon Ng, *Colloids Surf. A* **162** (1999) 203.
60. T.J. Horr, P.S. Arora, *Colloids Surf. A* **126** (1997) 113.
61. Z.Q.Wei, C. Wang, C.F. Zhu, C.Q. Zhou, B. Xu, C.L. Bai, *Surf. Sci.* **459** (2000) 401.
62. J.M. Williams, T. Han, T.P. Beebe Jr., *Langmuir* **12** (1996) 1291.
63. H.G. Hong, M. Jiang, S.G. Sligar, P. W. Bohn, *Langmuir* **10** (1994) 153.
64. D.G. Kurth, T. Bein, *Langmuir* **11** (1995) 3061.
65. R. Resch, M. Grasserbauer, G. Friedbacher, T. Vallant, H. Brunner, U. Mayer, H. Hoffmann, *Appl.*
66. *Surf. Sci.* **140** (1999) 168.
67. K.C. Popat, R.W. Johnson, T. A. Desai, *Surf. Coat. Techn.* **154** (2002) 253.
68. Y.-Z. Du, L.L. Wood, S. Scott Saavedra, *Mat. Sci. Eng. C* **7** (2000) 161.
69. G.J. McCarthy, R. Roy, R.M. McKay, *J. Am. Ceram. Soc.* **54** (1971) 637.
70. D.R. Uhlmann, T. Suratwala, K. Davidson, J.M. Boulton, G. Teowee, *J. Non-Cryst. Solids* **218** (1997) 113.
71. L.L. Hench, J.K. West, *Chem. Rev.* **90** (1990) 33.
72. V.S. Tripathi, V.B. Kandimalla, H. Ju, *Sensor. Actuat. B* **114** (2006) 1071.
73. G. Philipp and H.J. Schmidt, *J. Non-Cryst. Solids* **63** (1984) 283.
74. S.P. Fodor, J.L. Read, M.C. Pirrung, L. Stryer, A.T. Lu and D. Solas, *Science* **251** (1991) 767.
75. M. Schena, D. Shalon, R.W. Davis, P.O. Brown, *Science* **270** (1995) 467.

76. S.D. Conzone, C.G. Pantano, *Mater. Today* **7** (2004) 20.
77. R. Frank, *Tetraheddron* **48** (1992) 9217.
78. B.A. Stillman, J.L. Tonkinson, *BioTechniques* **29** (2000) 630.
79. R. Benters, C.M. Niemeyer and D. Wohrle, *ChemBioChem* **2** (2001) 686.
80. B.D. Martin, B.P. Gabaer, C.H. Patterson, D.C. Turner, *Langmuir* **14** (1998) 3971.
81. http://www.asperbio.com/coated_glass.htm (by Asper Biotech Ltd).
82. G. MacBeath, A.N. Koehler, S.L. Schreiber, *J. Am. Chem. Soc.* **121** (1999) 7967.
83. P.J. Hergenrother, K.M. Depew, S.L. Schreiber, *J. Am. Chem. Soc.* **122** (2000) 7849.
84. J.R. Falsey, M. Renil, S. Park, S. Li, K.S. Lam, *Bioconjug. Chem.* **12** (2001) 346.
85. G. MacBeath, S.L. Schreiber, *Science* **289** (2000) 1760.
86. L. Blasi, L. Longo, G. Vasapollo, R. Cingolani, R. Rinaldi, T. Rizzello, R. Acierno, M. Maffia, *Enzyme Microb. Tech.* **36** (2005) 818.
87. L. Blasi, L. Longo, P.P. Pompa, L. Manna, G. Ciccarella, G. Vasapollo, R. Cingolani, R. Rinaldi, A. Rizzello, R. Acierno, C. Storelli, M. Maffia, *Biosens. Bioelectron.* **21** (2005) 30.
88. M. Deepa, T.K. Saxena, D.P. Singh, K.N. Sood, S.A. Agnihotry, *Electrochim. Acta* **51** (2006) 1974.

ACKNOWLEDGEMENTS

Many people have contributed to my thesis and I would like to express my sincere gratitude to all of them. I would like to thank:

- My supervisor Prof. Ago Rinken for his knowledge, patience and that unique mild strictness that has guided me through all these years.
- My supervisor Dr. Ants Lõhmus for his commitment in sharing his erudition with young researchers, and also for his contagious urge on all kind of innovations.
- Dr. Uno Mäeorg for his immense knowledge and energy, and for showing how to make more time out of 24 hours.
- Tanel Tätte for bold ideas and interesting discussions on sol-gel chemistry, and for his bohemian ways that have brought him many fans, including myself.
- Dr. Ants Kurg for sharing his expertise on DNA microarray technology, for fruitful collaboration and always accompanying great sense of humor.
- Dr. Ilmar Kink for his erudition and interesting discussions on science, and for showing how to be and stay young.
- Dr. Rünno Lõhmus for his practical support in the field of science and beyond.
- Prof. Väino Sammelselg for sharing his solid expertise in SPM and materials science.
- Silvar Kallip and Jaanus Eskusson for their help in specific AFM nuances.
- Madis Lobjakas and Dr. Andrus Kilk for technical assistance.
- Dr. Toonika Rinken, Dr. Gerda Raidaru, Edith Kuusk, Jevgenia Koževnikova, Indrek Tulp, Dr. Ilmo Sildos, Valter Kiisk and Dr. Märt Rahi for their collaboration.
- Sven Parkel for assistance in specific chapters of this thesis.
- Margo Plaado and Madis Paalo for interesting discussions on sol-gel chemistry and for making everyday life more diverse.
- My parents and sister for their support and being with me.
- Finally: Piret for showing me the true meaning of life.

The support from Estonian Science Foundation grants no 5105 and 6492 and from European Science Foundation Nanotribology Program is also greatly acknowledged.

PUBLICATIONS

CURRICULUM VITAE

KRISTJAN SAAL

General dada

Born	10. Dec. 1977 Tartu
Citizenship	Estonian
Marital status	Single
Contact	Institute of Physics University of Tartu (IPUT) 142 Riia St, 51014 Tartu Tel.: (+372) 738 3037 Fax.: (+372) 738 3033 E-mail: saal@fi.tartu.ee
Current position	Scientist (IPUT)

Education

2002–2006	Ph.D. Student (University of Tartu)
2000–2002	M.Sc. Student (University of Tartu)
1996–2000	Graduate Student (University of Tartu)

Work experience

2003–	Scientist (IPUT)
2002–2003	Engineer (IPUT)

Professional training

2002	Scanning Probe Microscopy Factory Training by NT-MDT (IPUT)

Publications

1. K. Saal, T. Tätte, I. Tulp, I. Kink, A. Kurg, U. Mäeorg, A. Rinken, A. Lõhmus, Sol-gel films for DNA microarray applications, *Materials Letters* **60** (2006) 1833–1838.
2. M. Aunapuu, K. Kurrikoff, H. Tamm, A. Adojaan, T. Järveots, T. Suuroja, S. Kõks, E. Vasar, K. Saal, A. Arend, Morphological Investigation

of Transgenic with CCK2 Receptor Over-expression Mice Brain. A Pilot Study, *Eesti Arst* Lisa 6 (Proc. of the Conference Tissue Biology VI) (2005), 30–33.

3. K. Saal, M. Plaado, I. Kink, A. Kurg, V. Kiisk, J. Koževnikova, U. Mäeorg, A. Rinken, I. Sildos, T. Tätte, and A. Lõhmus, Aminopropyl Embedded Silica Films as Potent Substrates in DNA Microarray Applications, *Biological and Bio-Inspired Materials and Devices* (Mater. Res. Soc. Symp. Proc. **873E**, Warrendale, PA, 2005) K9.3.
4. V. Jacobsen, T. Tätte, R. Branscheid, U. Mäeorg, K. Saal, I. Kink, A. Lõhmus, M. Kreiter, Electrically conductive and optically transparent Sb-doped SnO_2 STM-probe for local excitation of electroluminescence, *Ultramicroscopy* **104** (2005) 39–45.
5. T. Tatte, V. Jacobsen, M. Paalo, R. Branscheid, M. Kreiter, U. Maeorg, K. Saal, A. Lohmus, I. Kink, Preparation of Sb doped SnO2 SPM tips and their use as transparent probes in STM induced light hybrid microscopy, *NSTI Nanotech 2005*, NSTI Nanotechnology Conference and Trade Show, Anaheim, CA, United States, May 8–12, 2005 (2005), **3** 305–308.
6. K. Saal, T. Tatte, M. Plaado, I Kink, A. Kurg, R. Lohmus, U. Maeorg, A. Lohmus, Sol-gel silane films for DNA microarray experiments, *NSTI Nanotech 2004*, NSTI Nanotechnology Conference and Trade Show, Boston, MA, United States, Mar. 7–11, 2004 (2004), **3** 358–361.
7. I. Kink, V. Kisand, K. Saal, T. Tatte, M. Lobjakas and A. Lohmus, Laser ablation for 3D nanometric imaging of solids, *Proceedings of the Estonian Academy of Sciences, Engineering* **10(1)** (2004) 30–38.
8. I. Kink, R. Lõhmus, M. Adamovich, R. Jaaniso, K. Saal, M. Lobjakas and A. Lõhmus, Three-dimensional subsurface imaging with laser ablation/AFM, *Proceedings of SPIE (International Society for optical Engineering)* **5123** (2003) 266–269.
9. T. Tätte, K. Saal, I. Kink, A. Kurg, R. Lõhmus, U. Mäeorg, M. Rahi, A. Rinken and A. Lõhmus, Preparation of smooth siloxane surfaces for AFM visualization of immobilized biomolecules, *Surface Science* **532–535** (2003) 1085–1091.
10. K. Saal, V. Sammelselg, A. Lõhmus, E. Kuusk, G. Raidaru, T. Rinken and A. Rinken, Characterization of glucose oxidase immobilization onto mica carrier by atomic force microscopy and kinetic studies, *Biomolecular Engineering* **19** (2002) 195–199.
11. K. Saal, V. Sammelselg, A. Lõhmus, R. Lõhmus, E. Kuusk, G. Raidaru, T. Rinken and A. Rinken, AFM characterization of enzyme immobilisation onto glass and mica — Problems and perspectives, *Physics of Low-Dimensional Sstructures*, **3–4** (2001) 151–157.
12. K. Saal, V. Sammelselg, A. Lõhmus, E. Kuusk, S. Kopantshuk, G. Raidaru, T. Rinken and A. Rinken, Characterization of glucose oxidase immobilization on surface of mica by atomic force microscopy and kinetic studies, *Mikrosistemnaya Tekhnika* **12** (2001) 30–33 (in russian).

ELULOOKIRJELDUS

KRISTJAN SAAL

Üldandmed

Sündinud	10.12.1977 Tartu
Kodakondsus	Eesti
Perekonnaseis	Vallaline
Kontaktandmed	Tartu Ülikooli Füüsika Instituut (TÜFI) Riia 142, 51014 Tartu Tel.: (+372) 738 3037 Fax.: (+372) 738 3033 E-mail: saal@fi.tartu.ee
Töökoht	Teadur (TÜFI)

Haridus

2002–2006	Doktorant (Tartu Ülikool)
2000–2002	Magistrant (Tartu Ülikool)
1996–2000	Üliõpilane (Tartu Ülikool)

Teenistuskäik

2003–	Teadur (TÜFI)
2002–2003	Insener (TÜFI)

Erialane enesetäiendus

2002	Firma NT-MDT teravikmikroskoopia-alane koolitus (TÜFI)

Publikatsioonid

1. K. Saal, T. Tätte, I. Tulp, I. Kink, A. Kurg, U. Mäeorg, A. Rinken, A. Lõhmus, Sol-gel films for DNA microarray applications, *Materials Letters* **60** (2006) 1833–1838.
2. M. Aunapuu, K. Kurrikoff, H. Tamm, A. Adojaan, T. Järveots, T. Suuroja, S. Kõks, E. Vasar, K. Saal, A. Arend, Morphological Investigation of Transgenic with CCK2 Receptor Over-expression Mice Brain. A Pilot

Study, *Eesti Arst* Lisa 6 (Proc. of the Conference Tissue Biology VI) (2005), 30–33.

3. K. Saal, M. Plaado, I. Kink, A. Kurg, V. Kiisk, J. Koževnikova, U. Mäeorg, A. Rinken, I. Sildos, T. Tätte, and A. Lõhmus, Aminopropyl Embedded Silica Films as Potent Substrates in DNA Microarray Applications, *Biological and Bio-Inspired Materials and Devices* (Mater. Res. Soc. Symp. Proc. **873E**, Warrendale, PA, 2005) K9.3.
4. V. Jacobsen, T. Tätte, R. Branscheid, U. Mäeorg, K. Saal, I. Kink, A. Lõhmus, M. Kreiter, Electrically conductive and optically transparent Sb-doped SnO_2 STM-probe for local excitation of electroluminescence, *Ultramicroscopy* **104** (2005) 39–45.
5. T. Tatte, V. Jacobsen, M. Paalo, R. Branscheid, M. Kreiter, U. Maeorg, K. Saal, A. Lohmus, I. Kink, Preparation of Sb doped SnO2 SPM tips and their use as transparent probes in STM induced light hybrid microscopy, *NSTI Nanotech 2005*, NSTI Nanotechnology Conference and Trade Show, Anaheim, CA, United States, May 8–12, 2005 (2005), **3** 305–308.
6. K. Saal, T. Tatte, M. Plaado, I. Kink, A. Kurg, R. Lohmus, U. Maeorg, A. Lohmus, Sol-gel silane films for DNA microarray experiments, *NSTI Nanotech 2004*, NSTI Nanotechnology Conference and Trade Show, Boston, MA, United States, Mar. 7–11, 2004 (2004), **3** 358–361.
7. I. Kink, V. Kisand, K. Saal, T. Tatte, M. Lobjakas and A. Lohmus, Laser ablation for 3D nanometric imaging of solids, *Proceedings of the Estonian Academy of Sciences, Engineering* **10(1)** (2004) 30–38.
8. I. Kink, R. Lõhmus, M. Adamovich, R. Jaaniso, K. Saal, M. Lobjakas and A. Lõhmus, Three-dimensional subsurface imaging with laser ablation/AFM, *Proceedings of SPIE (International Society for optical Engineering)* **5123** (2003) 266–269.
9. T. Tätte, K. Saal, I. Kink, A. Kurg, R. Lõhmus, U. Mäeorg, M. Rahi, A. Rinken and A. Lõhmus, Preparation of smooth siloxane surfaces for AFM visualization of immobilized biomolecules, *Surface Science* **532–535** (2003) 1085–1091.
10. K. Saal, V. Sammelselg, A. Lõhmus, E. Kuusk, G. Raidaru, T. Rinken and A. Rinken, Characterization of glucose oxidase immobilization onto mica carrier by atomic force microscopy and kinetic studies, *Biomolecular Engineering* **19** (2002) 195–199.
11. K. Saal, V. Sammelselg, A. Lõhmus, R. Lõhmus, E. Kuusk, G. Raidaru, T. Rinken and A. Rinken, AFM characterization of enzyme immobilisation onto glass and mica — Problems and perspectives, *Physics of Low-Dimensional Sstructures*, **3–4** (2001) 151–157.
12. K. Saal, V. Sammelselg, A. Lõhmus, E. Kuusk, S. Kopantshuk, G. Raidaru, T. Rinken and A. Rinken, Characterization of glucose oxidase immobilization on surface of mica by atomic force microscopy and kinetic studies, *Mikrosistemnaya Tekhnika* **12** (2001) 30–33 (in russian).