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Functionalization of Oxide-Free Silicon Surfaces for Biosensing Applications

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As an alternative to standard silicon biofunctionalization protocol based on alkoxysilanes that bind to SiO_2 on oxidized silicon substrates, several protocols to form bifunctional interlayers that directly bind to the silicon surface via a strong Si—C bond have been developed during the last decades. These interlayers are more stable, and the lack of an insulating layer between the substrate and the interlayer reduces electric noise in biosensor devices. Si—C monolayers are not yet regularly applied as the protocols are more complex and generally require an inert gas atmosphere. However, a variety of applications and new methods to form bifunctional Si—C interlayers are recently developed. Here, the role these innovative protocols and interlayers play in biofunctionalization of silicon surfaces is reviewed and their applications in electrochemical, microelectromechanical, and optical biosensing are reported. From the analysis of the current situation, it can be concluded that the advantageous properties offered with this approach in many cases more than outweigh the additional processes to form Si—C bonded interlayers and the approach is predicted to expand the applications of future silicon-based biosensors.

1. Introduction

The rapid development of complementary metal-oxide-semiconductor (CMOS) technology was made possible by the engineering of the Si—SiO_2 interface. With the reduction of the interface state density below a value of 10^{11} cm^{-2}, the charge carrier channel between source and drain regions in MOS field effect transistors can be converted to a state of inversion as required for the amplification and switching functions of MOSFETs. It was therefore interface engineering on which CMOS technology, including scaling, was built and which made the enormous advances in information and communications technology in recent decades possible. We are probably facing a similar situation today, where microelectronics is on the verge of being used in biological systems, but the signal exchange between semiconductors and the biomilieu is still dominated by the influence of defect-rich interfaces.

The rapid development in semiconductor technology has also been captured by new miniaturized biosensors[1–3] whose sensitivity and performance have been significantly improved by micro- and nanotechnologies. Nano-biosensors benefit from an efficient transduction mechanism due to a high surface to volume ratio[4] and theoretical faster analyte diffusion attributed to a lower fractional dimension.[5] Furthermore, biocompatibility, standardized fabrication processes and a wide scope of available biofunctionalization protocols makes nano-sized silicon in many ways an ideal substrate for biochemical sensing.

Due to the miniaturization of silicon devices, surface properties and surface functionalization have gained in importance through which the properties of semiconductor devices can be adjusted. Chemical functionalization of various silicon substrates, such as crystalline, or porous silicon, or nanowires with well-defined organic films may change its surface wetting properties dramatically,[6] may induce doping effects,[7] and allows the integration of molecular wires into conventional semiconductor technology.[8] While functionalization of miniaturized silicon substrate offers many opportunities to tune its proprieties to your demands, immobilizing biomolecules onto nanosized structures may sometimes be challenging. This may be due to limited diffusion into the nano holes of porous substrates or a less propensity to form well defined molecular layers on surfaces with nanosized curvatures.[9]

Efficiently grafting of bioreceptors onto surfaces is however essential for reliable biosensing that allows selective and sensitive detection of target analytes.

The most frequently applied approaches to functionalize silicon is to take advantage of the thin native oxide that forms above silicon surfaces in ambient environment.

Silanization with bifunctional alkoxy silanes indeed represents an attractive approach for this strategy, as it is relatively simple, reliable, cheap, and generate high quality monolayers.[10–12]

Consequently, this approach has been applied for the great majority of reported biomolecules covalently attached to

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Table 1. Characterization of silicon before and after oxide etching.

Method	Before etching	After etching Si(111)	After etching SI(100)
Ellipsometric[28] Measurement	1–2 nm	N/A	3 Å
Atomic force microscopy (RMS)[29,30]		<1 Å (atomic flat)	0.1–0.3 nm
FTIR[9]	1242, 1052 $\nu(SiO_2)$	ν(2085 cm^{-1}) monohydride	ν(2085 cm^{-1}) mono-, ν(2115 cm^{-1}) di- and ν(2142 cm^{-1}) tri-hydride stretching
XPS[31]	Si 2p, 104 eV (oxide peak)	Absence of oxide peak	Absence of oxide peak
Water contact angle[32]	< 10°	≈84°	≈84°

silicon. Among the many suitable bifunctional silanes, APTES [(3-aminopropyl)triethoxysilane] has become the most frequently applied linker. The terminal amine group on APTES functionalized surfaces may either couple biomolecules with an activated carboxyl group (aspartate, glutamate) or after treatment with glutaraldehyde bind to Lysine side chains via imine formation.[13,14] While the use of alkoxy silanes is a very attractive approach for surface functionalization it is also associated with several issues such as multilayer formation and hydrolysis in aqueous media.[15,16]

These issues might hump the formation of a dense layer of immobilized bioreceptor at the transducer surface, consequently preventing sensitive and reliable biosensing. Furthermore, the oxide layer between the receptor and the silicon surface are unfavorable for various transducer mechanisms. An isolating layer between the receptor and molecular binding event may hamper the electron transfer in electrochemical based sensors as well as increasing the noise level.[17]

An alternative to the silane chemistry is to graft the organic molecules direct to the silicon surface via Si—C bond. The Si—C bond is stronger attached to the surface and the lack of the silicon oxide makes the electric properties of the surface more predictable. These features make the interlayer very attractive for biosensing applications, but its implication has been hampered by complex functionalization protocols.

Recent reviews are discussing the applications of such monolayer for general applications,[18] redox active surfaces[17] or for tuning photoluminescence of porous substrates.[19] In this paper we are focusing on opportunities and challenges for the biofunctionalization of hydrogen-terminated silicon and its application in biosensors.

2. The Silicon Surface

Several approaches have been described for the functionalization of hydrogen terminated silicon. They all have their advantages and disadvantages but independent on what strategy being used the first crucial step is to remove the native oxide, covering the surface.

The most common applied etching agents are based on aqueous fluoride solutions. After the oxide etch the silicon surface is passivated with hydrogen atoms. The preference of hydrogen over fluoride might seem slightly surprising, as the Si—F bond is very strong with a bonding energy of 5.7 eV, almost twice compared to Si—H (3.1 eV).[20] While the etching mechanism seems to be complex,[21,22] one reason for the hydrogen passivation would be a less bipolar bond that are more similar to the bulk state of the silicon, thus making the Si—H bond more energetically favorable and the passivation more stable.[23]

The exact composition of the aqueous F^- containing etch solution may vary depending on the silicon substrate. Silicon (111) surfaces are for example generally etched in a 40 vol% aqueous ammonium fluoride solution. This generates an atomically flat surface with the silicon surface atoms bonded to one hydrogen atom, monohydride-surface.[24] Oxidized Si(100) -surfaces are etched in 1–5 vol% aqueous hydrogen fluoride (HF) that generates a slightly rougher surface of about a few Ångström.[25] This is due to strengths in the silicon back-bond and a more complex hydrogen termination, containing a mixture of mono-, di-, and tri-hydrides. To generates atomically flat surfaces ultra-high vacuum techniques are generally required.[26,27] The removal of the oxide may be monitored by standard surface analytical methods (**Table 1**).

Oxidized porous and nanostructured surfaces are also generally etched in 1–5 vol% HF solutions. The hydrogen termination is similar to flat Si(100) surfaces mixed hydrides however the distribution of mono-, di-, and tri-hydrides are strongly correlated to the porosity of the substrate.[33] The different hydrogen termination and roughness at the different substrates do influences their ability to graft organic molecules. In that sense, Si(111) surfaces are more prone to form self-assembled monolayer (SAM) than Si(100) substrates. Furthermore, monolayer formation on porous substrate may be hampered by steric effects.

While the HF solutions selectively etch SiO_2 over the silicon substrate it is not selective over metal structures that may be crucial to the function of biosensor devices. For example, Ni silicide applied to connect nanowires to Ni contact by the fabrication of Silicon nanowire field effect transistors (SiNW-FETs) may be etched in dilute HF within a minute (**Figure 1**). Etch solutions that are less aggressive to such metal structures may be prepared by adding glycol to the HF solution. Mixture of HF, ammonium fluoride and ethylene glycol in suitable ratios will maintain a pH of ≈7 that is less corrosive to metals and those enable selective etching of the SiO_2 over for example aluminum, aluminum/copper, titanium, tungsten, titanium nitride, and common metal alloys.[34,35]

3. General about Bioconjugation

Among the many strategies developed for biofunctionalization of surfaces, the least complex procedures are based on

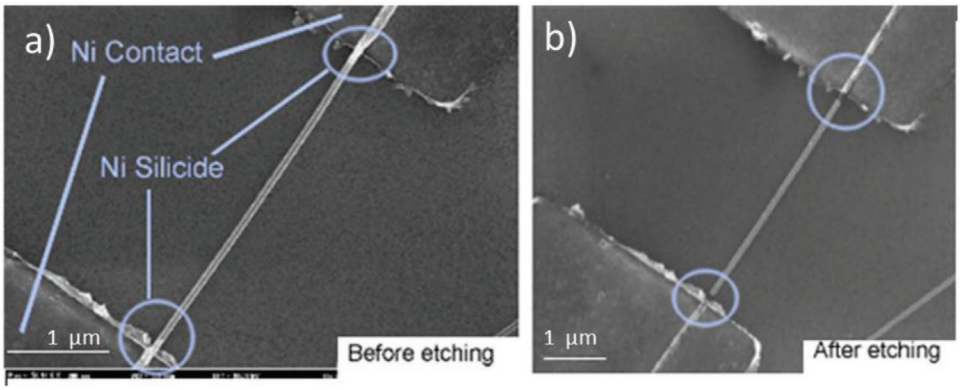

Figure 1. Silicon nanowire transistors before and after treatment with 5% HF: a) Two nickel contacts connected to a silicon nanowire by a Ni-silicide layer. b) The same transistors after being treated 30 s with 5% HF. The Ni-Silicide contacts are clearly etched away. Reproduced with permission.[175] Copyright 2012, The Author, published by TU Wien Bibliothek.

non-covalent physical adsorption such as Van der Waals, electrostatic, and hydrophobic forces. While these strategies are convenient, demand minimal preparations and may allow for the regeneration of the sensor surface, as a drawback they offer very little control over the orientation of the immobilized biomolecules. Consequently, the active site may be inaccessible to molecular interaction, "soft" proteins such as Bovine serum albumin (BSA), immunoglobulin G (IgG), fibrinogen, and α-lactoglobulin may lose their activity due to surface induced irreversible conformational changes, and weak interaction may induce surface detachment due to weak interaction.[13]

For a strong attachment to the surface, strategies based on covalently bonded receptor molecules are preferable. This is usually achieved in a two-step procedure, where a α,ω-disubstituted bifunctional linker (**Figure 2**) is first grafted to the surface via the α-group, forming a so called SAM with the terminal ω-functionality accessible for further reactions.

SAM is a one molecule thick layer of material that bonds to the surface in an ordered anisotropic way as a result of physical or chemical forces during a deposition process and are now established as crucial interlayers for the biofunctionalization of sensors.

The desired biomolecules are subsequently attached to the surface via a suitable coupling reaction. Commonly attached functional ω-groups on the linker include sulfhydryls, carbonyls, carbohydrates, or carboxylic acids that may interact with functionalities (e.g., primary amines, sulfhydryls) available on proteins or other biomolecules. Additionally chemical agents are used to modify the biomolecules such as the amino acid side chains on proteins and peptides to further expand the available bioconjugation strategies.[36] This enables strategies based on the streptavidin–biotin interaction, C- or N-terminal histidine, and FLAG tags, silicon bonded protein and click chemistry.

4. Grafting Organic Molecules to H Passivated Silicon

The ability to graft organic molecules onto H-passivated silicon is of greatest importance for biosensor applications. The formed monolayers serve as an interlayer between the surface and the biomolecule aiming both to prevent oxidation of the silicon substrates as well as to enable the coupling of the biomolecules and preserve the electric properties.[37,38]

It has been shown that the interlayer quality in terms of a well-ordered and densely packed structure of the film molecules is of utmost importance for this purpose. Slightly defect interlayers may preclude reproducible electric properties which is in accordance with theoretical models. These films tends to be dominated by defect properties and no longer reflect molecule properties such as chain lengths or the presence of specific functional groups.[39] Disordered isotropic films (**Figure 3**) can also make the ω-functionality sterically inaccessible to further coupling reactions and in this way block biofunctionalization. For this reason, time and efforts should be invested in developing robust and reliable protocols to form a highly ordered monolayer that may serve as an interlayer between the bifunctionality and the silicon surface.

The hydrosilylation reaction is the most common approach to form such interlayers on oxide free silicon surface. In this approach a hydrogen terminated silicon substrate, formed after HF etching may react with terminal alkenes or alkynes after activation. A number of different activation methods have been reported to induce this reaction including thermal,[40] or UV-light induced cleavage of the Si—H bond,[41] sonochemical activation,[42]white light irradiation,[43] or chemical activation.[44]

The thermally activated hydrosilylation reaction is probably the most well-studied hydrosilylation reaction for the formation of SAMs on silicon. The mechanism is the least debated and is generally accepted as a radical chain propagation.[45] The initial step is a thermal cleavage of a Si—H bond, forming a silyl radical at the hydrogen-terminated silicon surface, known as a dangling bond. The silyl radical then attacks the alkene or alkyne resulting in a new covalent Si—C bond and a migration of the radical to the β-carbon atom. Subsequently one hydrogen atom is abstracted from a neighboring Si—H bond at the hydrogen terminated silicon surface resulting in a Si—C bonded alkyl or alkenyl group and a new dangling bond at the silicon surface. This dangling bond can then react with another hydrocarbon chain and in this way the monolayer formation propagates across the surface. Using deuterium passivated silicon surfaces, this mechanism has been experimentally confirmed on different silicon substrates such as flat silicon,[45] porous silicon,[46]

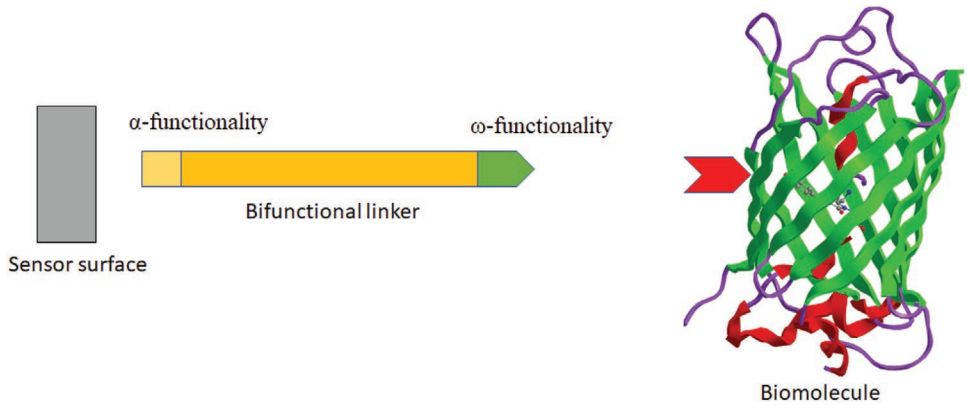

Figure 2. Schematic figure of the strategy for biofunctionalization of oxide free silicon.

and SiNPs[47] and was theoretical studied by density functional theory (DFT) calculations.[48] The DFT study indicated that due to a lower H-abstraction energy barrier and a larger overall reaction enthalpy the reaction with terminal alkynes should be faster than with corresponding alkenes and should also lead to a more stable organic overlayer. This was later experimentally confirmed by two independent studies by Ciampi et al.[49] and Scheres et al[50] Typical reaction conditions for thermally induced hydrosilylation reactions are the neat alkyne/alkene at temperatures above 160 °C, a reaction time of at least 3 h and rigorous exclusion of oxygen. Hydrosilylation on porous silicon seems to demand less harsh reaction condition compared to flat silicon and has been successfully carried out in refluxing toluene at 110 °C.

For photo-initiated hydrosilylation reactions, four mechanisms have been proposed:[41] i) Si H bond homolysis followed by a radical reaction as described above,[51] ii) photoemission: photo induced electron emission followed by a nucleophilic attack on the positively charged silicon and hydrogen migration,[52] iii) plasmon-mediated,[53] and iv) exciton-mediated initiation:[45] a valence band electron is excited to the conduction band and is stabilized through coulomb interaction with the formed electron hole in the valence band.

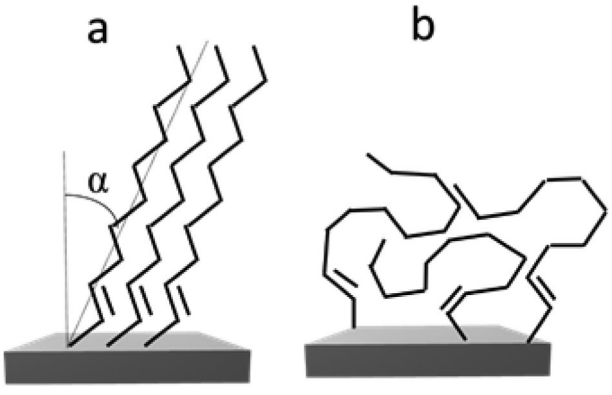

Figure 3. Proposed structural models of monolayers of decyne adsorbed on silicon surfaces. a) Anisotropic model with all-trans hydrocarbon chains tilted with angle α to the substrate normal. b) Isotropic model with randomly oriented molecules.[28]

The mechanisms proposed for UV-initiated hydrosilylation are Si-bond homolysis and photoemission. Visible-light induced hydrosilylation is in addition to photoemission proposed to involve the formation of plasmons or quantum-confined exciton (**Figure 4**). The radical chain reaction is not likely to happen, since visible light has insufficient energy to break the Si—H bond. The mechanism of white-light initiated hydrosilylation reactions seems to follow a different route compared to UV or thermal initiation and proposals involving both plasmons and excitons have been presented. A result strongly in favor of the exciton mechanism is that efficient white-light adsorption has only been observed on photoluminescent, porous silicon. As photoluminescence originates in the recombination of excitons, this is a strong indication supporting the exciton hypothesis.[41]

Si(111) surfaces tend to be easier to functionalize than Si(100), independent of the functionalization strategy. The reason for this is the higher surface roughness of Si(100) disturbing the monolayer formation. Although Si(100) is the most common crystal geometry in electronic device, it is easily oxidized and thus is more sensitive to oxygen in the reaction vessel.[27]

For biosensors applications it is worth paying extra attention to the functionalization of porous silicon substrates due to its slightly different surface chemistry and its versatile sensing applications ascribed to simple fabrication processes, interesting optical and morphological properties of the material, and large internal surface area.

While functionalization of silicon based nano materials may be achieved by applying similar approaches as to flat substrates, quantum mechanical effects open up new reaction mechanisms, and new activation opportunities.

On porous silicon substrates variations in the reaction mechanism of light induced hydrosilylation as well as Si—C formation on porous silicon at low temperatures have been reported (Figure 4). Furthermore, room temperature and light induced hydrosilylation of alkene esters onto silicon nanocrystals was described and evidenced a strong correlation between the dimension of the nanocrystal and successful grafting.[54–56] Another possible factor that may have an influence is variations in the hydrogen termination that is dependent on the porosity of the substrate.[55]

An alternative to hydrosilylation, which is used in many applications to form Si—C bonds, especially on porous

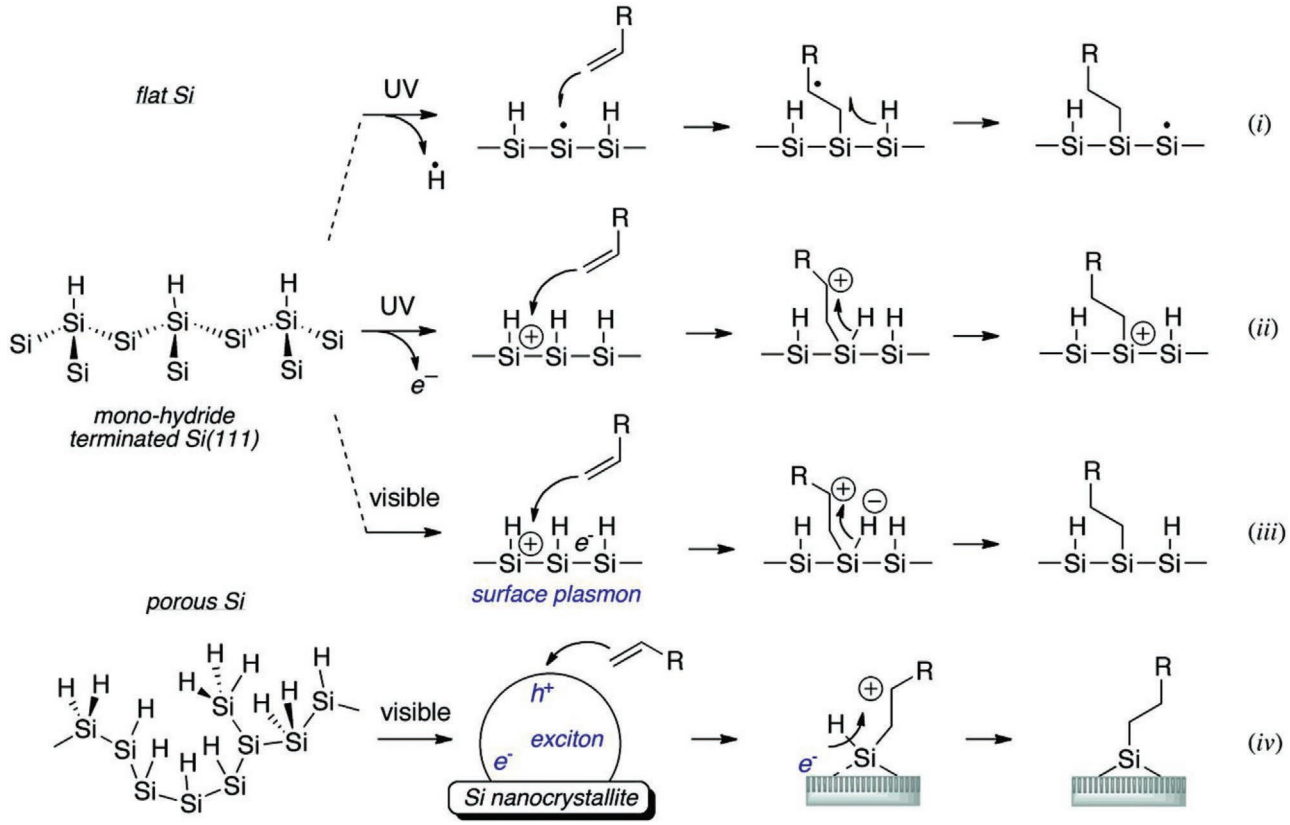

Figure 4. Four mechanisms previously proposed for the photoinitiated hydrosilylation on hydrogen-terminated silicon surfaces. i) Si—H bond homolysis, ii) photoemission, iii) plasmon-mediated, and iv) exciton-mediated. Reproduced with permission.[45] Copyright 2012, American Chemical Society.

substrates is the thermal carbonization reaction, which uses high temperatures and gaseous carbon sources such as acetylene and ethylene. This functionalization strategy is especially used for porous substrates, as the gases diffuse well into the pores, there is no steric hindrance, and the stability in aqueous environment is higher compared to the hydrosilylation reaction.[57–59] Otherwise, the thermal carbonization reaction is very similar to the hydrosilylation reaction, as for both a hydrogen terminated silicon surface is crucial and it generally reacts with alkenes at high temperatures.

In a recent review by Salonen,[58] a few loose criteria were set to differentiate between the two reactions. For Salonen, temperatures are the main criterion, as hydrosilylation generally proceeds below 200 °C in liquid reagents, while carbonization with gaseous substrates requires temperatures above 400 °C.

In addition to the hydrosilylation and thermal carbonization reaction a third established way to functionalize oxide-free silicon surfaces with an organic layer is through electrochemical deposition of diazonium salts that carry the desired functionality.[60–62] Thin films with a broad variety of functionalities have been prepared and the grafting can be carried out in aqueous as well as non-aqueous environments. The diazonium salts are relatively easy to prepare and the functionalized aromatic groups could be further modified by classical chemical reactions. Furthermore, the strategy can be applied to prepare patterned surfaces[63] by selectively applying the electrochemical activation on the desired areas to be functionalized. The grafted aromatic compounds are also beneficial for efficient electron transfer in electrochemical applications.[62,64]

Difficulties in producing monolayers in a reproducible and controllable manner was commonly associated with the strategy and especially the formation of multilayers has been an issue.[65] However the multilayer formation may be avoided by radical scavengers[66] or by a controlled steric hindrance approach[65] and it has been shown that the silicon surface has a low defect concentration after the functionalization.[67]

Silicon surfaces can also be functionalized by reaction of a Grignard reagent (R-MgX) carrying the desired functionality with a halogenated silicon surface,[68] An almost 100% surface coverage was obtained when methyl groups were introduced using this approach, resulting in an excellent protection against oxidation.[17,69] however, few options for further functionalization, limit its practical applicability. An interesting alternative is the electrochemical functionalization by ethynyl groups via electrochemical oxidation of ethynyl-MgBr, forming a thin alkyne terminated polymer that may be further functionalized via a click a coupling reaction.[70,71]

While the above-mentioned four grafting strategies are the most frequently applied to form Si—C bonded monolayer, several new approaches have been reported the last decade.

A palladium-catalyzed immobilization strategy for the grafting of aromatic compounds onto a hydrogen-terminated Si surfaces without the formation of multilayers was reported by Yamanoi et al.[29] The strategy has been successful on Si(111),

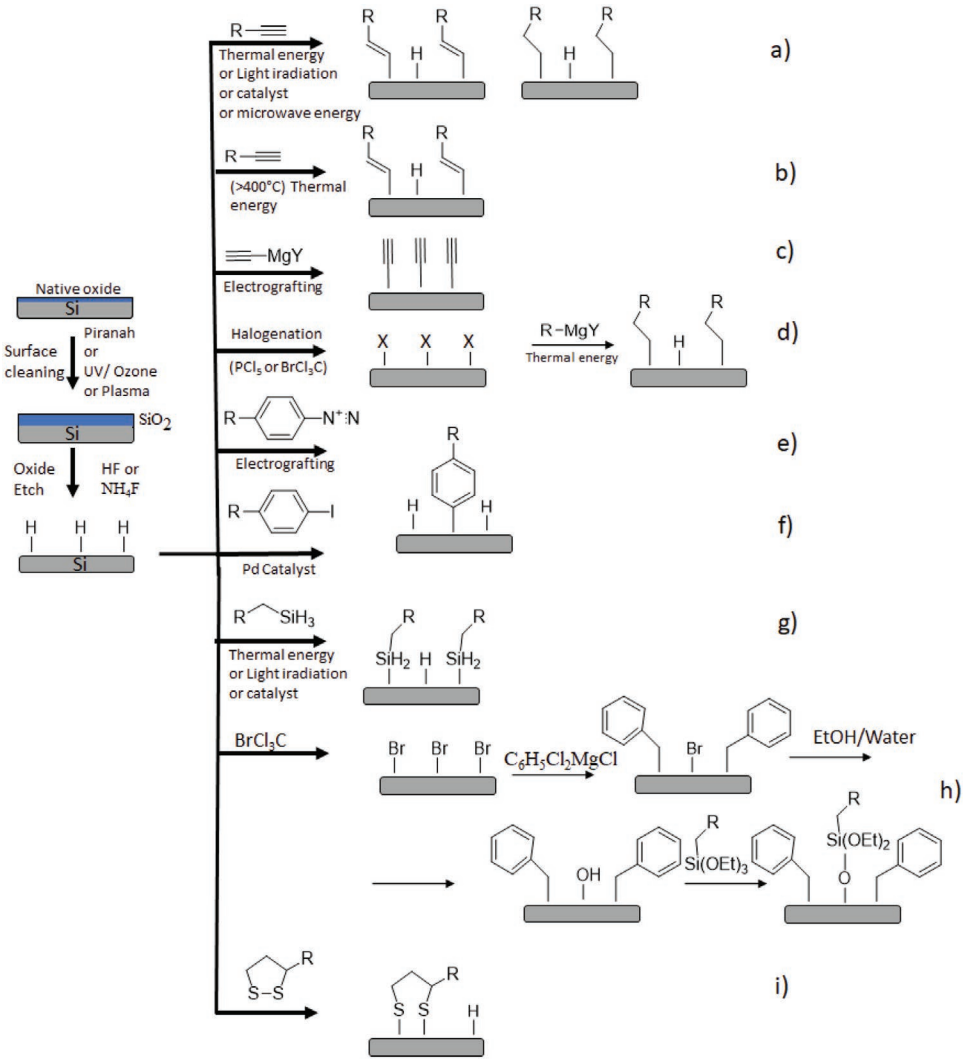

Figure 5. Different approaches to graft organic molecules with functionality R to oxide free silicon surfaces. a) Hydrosilylation b) Therman carbonization c) Electrografting of ethynyl Grignard reagent, Y = Cl, Br. d) Grignard reaction, x = Br or Cl, and Y = Cl, Br, or I. e) Electrografting with diazonium salt. f) Palladium catalyzed arylation. g) Dehydrocoupling h) Formation of air-stable hydroxyl groups followed by silane chemistry i) disulfide reduction.

Si(100) as well as on porous substrates[31] and is an additional tool that may expand the number of aromatic molecules that can be attached to the surface, thus improving for example the electron transfer in electrochemical sensor devices.

It has also been shown that hydrosilanes may be attached to silicon via thermal activation. The first grafting to silicon substrates as reported by Li et al.[72] suffered from several side reactions. The coupling strategy has since been improved by Veinot et al.[73] via a Wilkinsson catalyst as well as by Arkles et al.[74] and Kim et al.[75] that both reported catalyst-free grafting to hydrogen terminated silicon surfaces under mild thermal condition. Kim et al. further confirmed stable robust films as well as retention of electronic quality, thus the feasibility of the strategy to functionalize electric devices.

Another interesting method was recently published by Hänisch et al.,[76] who presented a chemical reaction protocol to prepare air-stable hydroxyl groups on the Si surface that can be further functionalized with well-established silane chemistry without the need of a SiO_2 layer. The hydroxyl functions were embedded in a benzyl matrix that prevented further oxidation of the substrates. While the electric properties of the monolayers yet are to be characterized it may open up a large variety of silane-based strategies to functionalize silicon-based sensors with better device performance.

Grafting of sulfhydryls on to Si—H surfaces have been investigated with some success by UV illumination,[77] heat,[78] or electrochemical grafting.[79] The later recently published electrochemical grafting protocol interestingly showed monolayer formation under ambient condition that may even proceed spontaneously without applied voltage or catalyst. They suggested that the formation of the monolayer, formed within an hour is guided by water generating local nanoscale oxidation of the Si surface. By applying the electric potential of 0.8 V a full monolayer was formed within 15 min. Furthermore, the electron transfer through the monolayer to the substrate was reported to be similar to monolayers on gold surfaces. While these Si—S

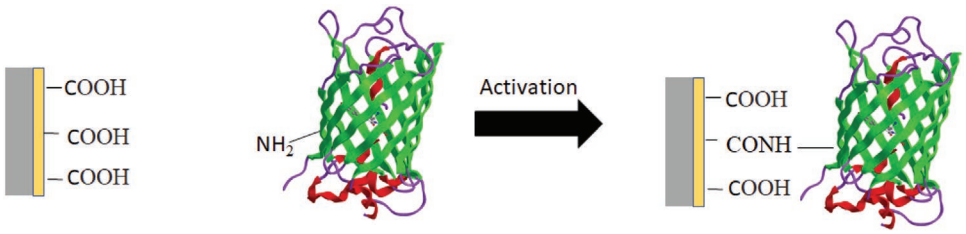

Figure 6. Bioconjugation with carboxylic acid terminated interlayer.

monolayers are not as stable as the Si—C monolayers, the mild conditions without the need of an inert atmosphere seems very promising and may be accessible for research groups without much chemistry experience. Furthermore, as interlayers from sulfhydryls are frequently applied for biosensing purposes on gold surfaces, those systems may potentially be transferred to silicon-based devices.

The various functionalization protocols summarized in **Figure 5** all have their advantages and disadvantages, and the application will decide which one is the most suitable. For the purpose of device functionalization, however, metal catalysts and high temperatures are often unfavorable as it may alter the performance of microelectronic devices. Thus, light or electrochemical activation is a less harsh alternative that are more suitable for sensitive applications. Light and electrochemical activation may also allow a more selective chemical functionalization in which you only activate the desired spot on the surface. On the other hand, thermal activated hydrosilylation is well studied and known to generate high quality monolayers while more new immobilization protocols such as the one presented by Dief et al. and Hänisch et al. may expand the available biofunctionalization strategies.

5. Functional Surfaces for Efficient Bioconjugation

5.1. Amine and Carboxylic Acid Terminated Surfaces

One of the most widely applied strategies to attach biomolecules to surfaces is to graft a COOH terminated linker followed by amide-coupling between an amine and a carboxylic acid (**Figure 6**).

A common reported procedure applied on oxide free silicon surfaces involves hydrosilylation with undecylenic acid to introduce the carboxylic acid functionality onto the silicon surface[80] followed by coupling to the amine group of a biomolecule via NHS/EDC chemistry. The method is one of the more reliable coupling strategies and is frequently applied for efficient bioconjugation on various substrates. Furthermore, the acid functionality has been shown to assist in the hydrosilylation reaction, and thus allowing faster grafting at lower temperature.[54,56] On nanosized silicon structures such as silicon nanocrystals, grafting may occur even at room temperature. Successful immobilization of both unprotected and protected undecylenic acid has been reported as well as its subsequent biofunctionalization. One of the earlier biofunctionalizations of hydrogen terminated silicon was the attachment of oligonucleotides reported in 2000 by Strother et al.[81] by grafting ω-undecylenic acid methyl or trifluoroethyl ester by UV irradiation. The group subsequently added poly-L-lysine and reaction of the lysine-amin groups with the heterobifunctional crosslinker SSMCC. This results in a maleimide-activated surface that could be coupled in aqueous solution with a thiol modified oligodeoxynucleotide to yield the DNA-modified surface. Oligonucleotides have since been grafted to carboxylic acid terminated oxide free silicon for various applications, by applying the same SSMCC linker[82,83] but more frequently by applying NHS/EDC chemistry.

A mixed interlayer by grafting 1-decene and undecylenic acid followed by NHS coupling at the carboxylic acid functionality may allow a less dense layer of ssDNA on the surface thus preventing steric hindrance and ease the hybridization.[84] To further optimize the hybridization the cation to the DNA string may be varied. A relation between the degree of conformational variation of the DNA SAM as well as the efficiency regarding hybridization depending on metal cations present could be confirmed by Asanuma et al.[84]

Oligodeoxyribonucleotide monolayers was also prepared by Cataruzza et al.[85] as probe systems for DNA recognition by first attaching 10-undecynoic acid on the silicon surface, via cathodic electrografting, attaining a carboxylic acid terminated interlayer followed by coupling of oligonucleotides via NHS/EDAC.

In similar manners, proteins and peptides have been coupled to non-oxidized Si.[71,86,87]

The reversed coupling between an amine terminated surface and the carboxylic acid functionality of biomolecules have also been reported by applying a variety of approaches such as light activated grafting of ethylenediamine[88] and hydrosilylation of protected amines[89]

The amine functionality may also be useful in order to attach bifunctional polyethylene glycol (PEG) linkers that prevents biofouling or as a way of attaching other functionalities such as thiol, biotin,[82] or maleimide.[90]

5.2. Azide, Alkene, and Alkyne Functionalized Surfaces for Biofunctionalization via Click Chemistry

Click chemistry as described by Sharples et al.[91] is a collection of reactions that follow certain criteria such as high yielding, wide in scope and absence of by-products. The concept has been widely spread the last two decades as it allow the reliable coupling of molecules in a straight forward manner.[92–94]

The workhorse of click chemistry is the copper catalyzed Huisgen cycloaddition in there an azide building block is coupled to an alkyne building block (**Figure 7**). Lummerstorfer et al.

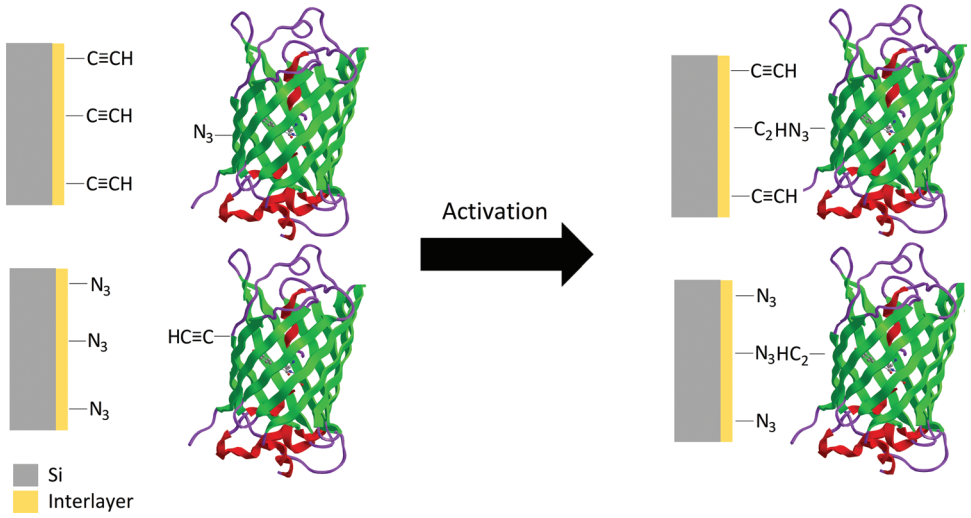

Figure 7. Bioconjugation via click chemistry.

first applied this reaction to functionalize silicon surfaces, using a silane chemistry approach.[95] Since then this coupling strategy became a common approach for the biofunctionalization of a large variety of substrates, including hydrogen passivated silicon.[38]

The grafting of a $\alpha\omega$-diyne followed by a copper catalyzed cycloaddition was first reported in 2008 by Ciampi et al.[96] Since then it is a frequently applied strategy to functionalize oxide free silicon electrodes and has been employed to immobilize redox active groups, for the functionalization of nano particles,[97,98] solar energy devices,[99] for monolayer induced doping,[7] and patterning of SAM.[100]

The bifunctional interlayer has shown a remarkable ability to protect the surface from oxidation and allow efficient electron transfer.[101,102] Electron transfer between the silicon electrode and a ferrocene functionalized monolayer could proceed over several hundred redox cycles without any apparent oxidation of the underlying silicon with close to ideal surface electrochemistry.

Click chemistry have also been frequently applied to immobilize biomolecules onto the surfaces, with some examples on oxide free silicon. Ciampi et al. functionalized stepwise a Si(100) surface with cytochrome c via isonicotinic acid derivates that was clicked to an alkyne terminated silicon and coordinated to the cyt c hemgroup via the pyridine moieties.[103]

α,ω-alkenynes, with the alkynyl terminalis protected with a trimethylgermanyl (TMG) group, were grafted on hydrogen-terminated silicon substrates by Qin et al.[104] The TMG groups that prevents back bonding on the film could be removed in aqueous solutions in the presence of Cu(I), thus allowing simultaneous alkyne deprotection and azide alkyne coupling. Biotin and mannose oligo(ethylene glycol) (OEG) with an azido-tag was attached to the TMG-alkyne surfaces, leading to biofunctional OEG-terminated surfaces that prevents non-specific adsorption of protein.[105] It was also demonstrated that the monolayer platform could be functionalized with mannose for highly specific capturing of living target.

Molina et al.[106] studied streptavidin immobilization by clicking a tripod-shaped penta(p-phenylene) on an acetylene terminated surface followed by the coupling of an active theophylline derivative with high affinity to streptavidin. The tripod shaped linker assures a spatial distance between the immobilized proteins.

One major drawback with the Huisgen cycloaddition, however, is the use of copper, which may get incorporated in the modified electrode and change its electronic properties drastically.[107,108]

For this reason copper free strain promoted click chemistry approaches[109] are widely applied for biosensing application.[110] This functionality requires however an extra functionalization step to attach it to oxide-free silicon.

Another approach for copper free click chemistry was reported by Henriksson et al. An electron withdrawing carbonyl group next to the alkyne functionality allowed successful coupling onto Si(100) and silicon nanowires without a metal catalyst.[9,111]

It is also possible to prepare Azide-functionalized surfaces that may be employed to couple alkyne labeled biomolecules. While hydrosilylation in one step is generally not possible these surfaces may be prepared via hydrosilylation of ω-halide followed by a substitution reaction with NaN_3,[95] via electrografting of diazonium salts[112] or via Pd catalyzed attachment of aromatic compounds.[29]

In most published work, the azide-alkyne coupling is used to couple a linker with a terminal functionality that may conjugate to a functionality on the biomolecule. However, an ever-increasing demand for site specific protein fluorescent labeling, has driven the evolvement of new protocols for preparing biological active azide functionalized clickable proteins that can be directly clicked to the surface. Azides may be fairly site specific incorporated into protein by post-functionalization of Cysteine side chains. However, also by applying recently developed protocols of metabolic pathway engineering in bacteria, unnatural azide-functionalized amino acids can be inserted at specific positions with high precision.[113,114] This scalable method is a convenient way to functionalize proteins and allows covalent immobilization of biomolecules at a specific position without side reactions. This technology was further developed so that

L-azidohomoalanine can be directly produced from sodium azide and is efficiently and specifically incorporated into a recombinant model protein. This method is efficient and scalable, so that larger amount of azide-modified proteins can be produced.

5.3. Other Applied Functionalities for Bioconjugation

Other functionalities grafted to the surface for biofunctionalization purposes include sulfhydryl, hydroxide, or epoxides groups. Sulfhydryl may bind biomolecules via disulfide bridges to cys sidechains or post functionalized biomolecules or by thiolene click chemistry.[115]

Sulfhydryl terminated monolayer have been prepared on oxide free silicon surfaces in various ways.

Hart et al.[90] attached the sulfhydryl functionality in several steps, starting with hydrosilylation of an alkyne with a ω-CN group which were reduced to NH_2 followed by coupling of *N*-succinimidyl-(2-pyridyldithio)propionate and subsequent treatment with ditiotreitol.

Böcking et al.[116] attached trifluoroacetyl (TFA)-protected thiol groups by thermal hydrosilylation of *S*-undec-10-enyl-2,2,2-trifluoroethanethioate (C11-S-TFA). The protecting group did assist to avoid side reactions of the sulfhydryl group with the hydrogen terminated silicon surface as well as to avoid degradation and contamination of the monolayer surface during storage. They chose the relatively small TFA group over bulkier protecting groups such as the dimethoxytrityl (DMT) group to avoid steric interference with the packing of the alkyl chains in the monolayer and because it could be removed rapidly at room temperature.

A bifunctional PEG with alkene and thiol ester at opposite sites was attached to porous silicon. Reduction with $LiAlH_3$ gave the thiol followed by reaction with β-maleimido propionic acid NHS crosslinker that could further couple to Albumin.[117]

Hydroxyl functionalities may be applied for bioconjugation often via coupling to carbonyl diimidazole that can further react with hydroxyl groups, creating an active imidazolyl carbamate intermediate that is capable of binding amine-containing biomolecules.[36]

Pike et al.[118] prepared hydroxy functionalized oxide free silicon surfaces via hydrosilylation of undencenol in toluene. The alcohol functionality was protected with 4,4_-dimethoxytrityl-protected that could be removed in anhydrous CH_3NH_2.

McInnes[119] approach involves the fabrication of porous silicon (pSi) microparticles and their functionalization via hydrosilylation reactions to generate a DMT-protected alcohol on the pSi surface as an initiation point for the solid synthesis of short oligonucleotides. The alcohol terminated surfaces were prepared in several steps starting with hydrosilylated with fmoc-protected aminoundecene. Subsequently, the fmoc-protecting group was removed followed by a reaction with succinic anhydride. The carboxy group was further reduced with $LiAlH_3$ to yield OH groups on the surface.

Epoxy functionalities are frequently applied for bioconjugation of variety of molecules, often via interaction of the amine groups on the biomolecule and have been useful for the immobilization of oligonucleotides and proteins.

One example was reported by Böcking et al.[120] as they immobilized the functionality onto hydrogen terminated silicon via hydrosilylation of alkenes with epoxide-terminated tri(ethylene oxide) moieties. This allowed biofunctionalization by spotting a solution of oligo nucleotides onto the surface. Further the ethylene oxide prevented unspecific bonding.

6. Recent Application of Oxide Free Silicon-Based Biosensors

6.1. Electrochemical Sensors

Transducers based on electrical and electrochemical principles are frequently applied to sensors for a large variety of bio detection events.[121,122] Examples include field effect transistors, various amperometric and potentiometric approaches and impedance spectroscopy on a large variety of substrates including silicon.

The state of the art biofunctionalization strategy of silicon substrates via silane chemistry is however for these applications unfavorably as the oxide layer renders silicon an insulator and decelerates interfacial electron transfer.[123] Electrically active surface defects of about an order of magnitude higher than Si—C monolayers are generated thereby.[124,125]

For this reason, Si—C bonded interlayers are a highly attractive and a promising strategy that may expand the applications of electrochemical sensors to also include silicon substrates.

As interlayer defects strongly influences the electric properties of the system and thus the applicability of the sensing platform[39,103] it is worth investing time in the formation of an ordered high quality monolayer to succeed in this endeavor (**Table 2**).

The silicon based electrochemical biosensors that have gained most attention are biosensors based on the principle of SiNW-FETs.[132]

They offer label-free real time sensing with very high sensitivity and fabrication methods compatible with state-of-the-art semiconductor technology. A field-effect transistor-based biosensor, (Bio-FET), is a field-effect transistor (based on the MOSFET structure) that is gated by changes in the surface potential induced by the binding of molecules. When charged molecules, such as biomolecules, bind to the FET they can change the charge distribution of the underlying semiconductor material resulting in a change in conductance of the FET channel. In SiNW FETs the transconductance between a source and the drain connected by silicon nanowires are measured.

Immobilization of proteins to an oxide-free SiNW-based FET was first reported by Stern et al.[89] via a biotin-avidin/streptavidin or immunosensing systems. A functional device was obtained via photo activated hydrosilylation followed by bioconjugation via ethylene dicarbodiimide coupling chemistry.

The resulting devices showed a correlation between the source–drain conductance versus streptavidin concentration in the range between 10 fM and 1 nM with a noise level indicating a detection limit of 70 aM. Furthermore mouse-IgG or mouse-IgA at 100 fM for goat anti-mouse IgG-functionalized sensor were demonstrated.

Another example was investigated by Zhang et al.[126] They prepared PNA-functionalized SiNWFET devices for the detection of ss-DNA via UV-initiated hydrosilylation of 10-*N*-boc-amino-dec-1-ene followed by removal of the Boc group to

Table 2. Electrochemical sensors on oxide free silicon surfaces.

Sensor type	Receptor/Analyte	Surface coverage	LOD	Ref.
SiNWFET	Antimouse IgG/mouse IgG	N/A	100 fM	Stern et al.[89]
SiNWFET	PNA/DNA	N/A	10 fM	Zhang et al.[126]
SiNW	DNA/DNA	2.5×10^{13} molecules cm^{-2}	10 pM	Bunimovich.[127]
Impedimetric	DNA/DNA	N/A	500 pM	Wei et al.[128]
Amperometric	—COOH/LPS	N/A	1.8 ng mL^{-1}	Reta et al.[129]
LAE	DNA/DNA	N/A	500 pM	Zarei et al.[130]
LAPS	N/A	N/A	N/A	Zhou et al.[131]

generate an amine terminated surface. The surface was subsequently chemically modified with glutaraldehyde, allowing bioconjugation with amines. The resistance changes of the SiNWs increased linearly with the logarithm of DNA concentration between 1 nM to the detection limit of 10 fM.

PNA was considered a more suitable receptor than DNA as it is not only neutral but is also independent of the salt concentration that is an important parameter in FET sensors.

That an increased performance of the FET sensors may be attributed to the lack of oxide was further confirmed by Bunimovich et al.[127]

They functionalized identical nanowires by either UV-initiated hydrosilylation chemistry or silanization for detection of DNA hybridization. By comparing the different sensing interfaces oxide free surfaces was shown to increase the limit of detection by 1 order of magnitude, with an accompanying increase in the dynamic range. The results highlight the importance of controlling surface chemistry of SiNWs for their optimization as biological sensors and the advantage of Si—C bonded interlayer.

Beyond the favorable electronic properties of the oxide free surfaces Si—C bonded monolayer also offers potentials to selectively functionalize the active silicon surface over enclosed SiO_2 that often function as an insulting layer in silicon-based sensor devices.

One approach was explored by Veeerbeck et al.,[133] who coupled PNA onto Si nanowires on SiO_2 substrates by applying a thermal hydrosilylation reaction to graft 1,8-nonadiyne followed by copper catalyzed click chemistry (**Figure 8**). The functionalization strategy failed to selectively functionalize the silicon nanowire but led to a covalently bonded monolayer at both the Si—H and the SiO_2 regions, as could be followed by fluorescence microscopy and X-ray photoelectron spectroscopy (XPS). The selectivity could however be increased by an extra HF treatment after the initial monolayer formation to partly remove the monolayer from the oxidized regions. While still requiring optimization, this strategy seemed to be successful. Further the authors suggested to replace the thermal hydrosilylation with photo activate-hydrosilylation to increase selectivity.[133,134] This was also previously shown to be successful.[135] Furthermore, Veerbeck et al. suggest a mixture of silane-based and alkyne-based molecules to improve the selectivity further.

Amperometric detection is another category of electrochemical sensors that is frequently applied on various substrates to monitor a large variety of biointeraction events coupled to a redox reaction such as enzymatic reactions and hybridization of labeled oligonucleotide strains.[136] Furthermore, CMOS based technologies enable the realization of user-friendly and relatively small size diagnostic detection systems[137] The implication of silicon as a working electrode has been hampered because of the oxide layer that is generally separating the electrolyte and the silicon surface, thus precluding efficient electron transfer. CMOS based amperometric

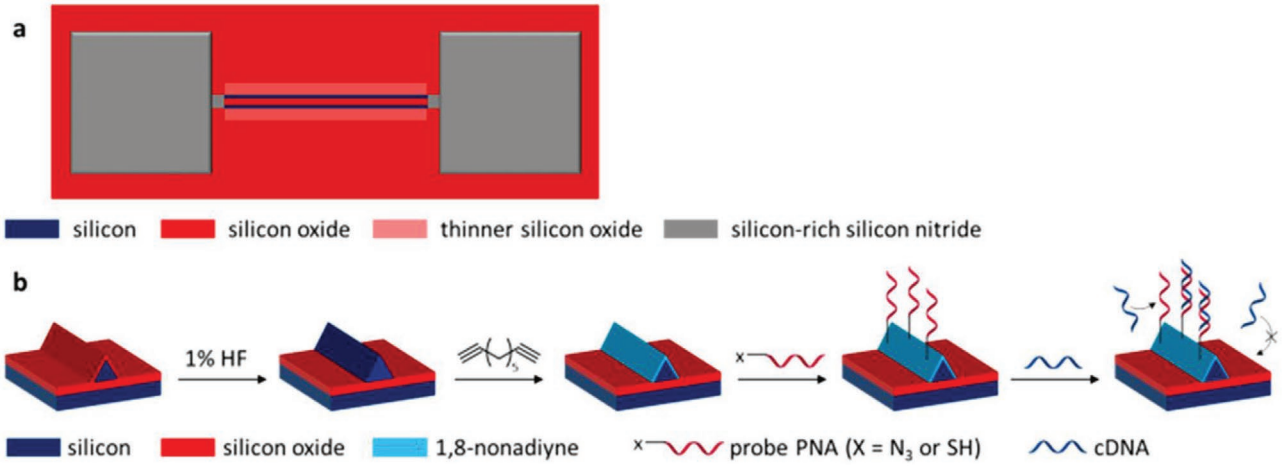

Figure 8. a) Illustration of a Si Nanowires on SiO_2 chip and b) Schematic Illustration of PNA functionalization that selectively bind to Si over enclosed SiO_2. a,b) Reproduced with permission.[133] Copyright 2018, American Chemical Society.

detection may thus often require backend processes to passivate the working electrodes with a conductive layer. Surface functionalization based on SAM on oxide free silicon surfaces offers efficient and predictable electron transfer[102,138] and could serve as an alternative and may simplify the production of the biosensors.

The examples of such approaches are sparse and require high quality interlayers. One of the few reported work was published by Reta et al.[129] They developed a porous silicon membrane-based amperometric biosensor that allowed sensitive detection and differentiation of various bacterial lipopolysaccharides (LPS). The sensing platform is a promising alternative to current tests based on colorimetric measurements, such as limunus amebocyte lysate assay and various enzyme-linked immunosorbent assays.

A particular promising application that indeed highlights the opportunities that Si—C functionalization strategies offer is the report of silicon based light addressable electrochemical sensors, realized either in a potentiometric setup or an amperometric setup.

The light addressable potentiometric sensors (LAPS) measures the localized non-faradaic AC current across an electrolyte–insulator–semiconductor structure.[140]

In the amperometric mode, light-addressable electrochemistry (LAE) allows faradaic electrochemistry to occur in a specific region and thus enables electrochemical sensing. Illumination of the silicon electrode results in a locally increase in charge carrier that allows electron transfer through the interlayer to the silicon substrate in the illuminated region. In this way any location on an electrode surface can be interrogated electrochemically with microscale resolution allowing the realization of electrochemical microarrays that doesn't have to be individually wired (**Figure 9**). The small bandgap of silicon (1.1 eV) is such that visible light can be used, which becomes important for biological applications. This new approach was successfully implemented by Choudhury et al.[130,139,141] and allowed amperometric validation of DNA hybridization.

Crucial for the functionality of LAPS and LAE is a surface passivation with few electric active defects with low surface recombination velocity that allow enough surface carrier for the

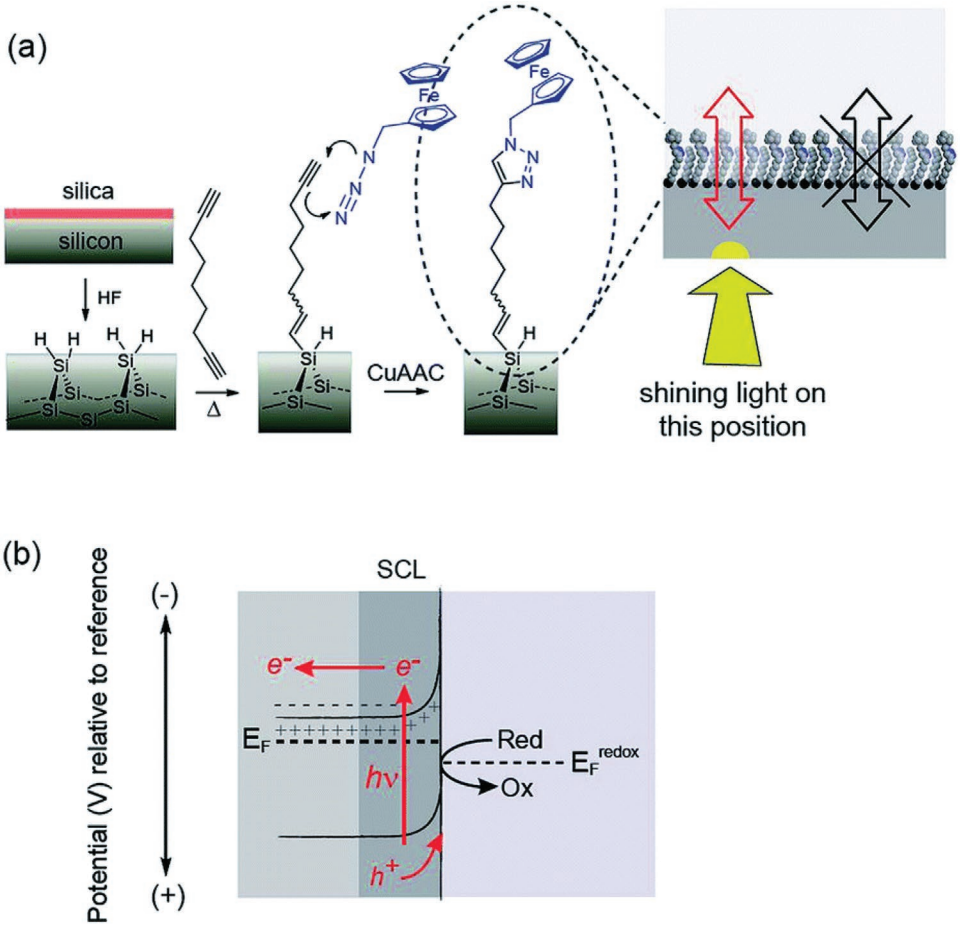

Figure 9. Schematic figure illustrating the realization of LAE on silicon substrates: a) Oxide free silicon substrate is passivated by an organic monolayer via hydrosilylation with 1.8-nonadiyne. The terminal alkyne is subsequently used to couple a redox active ferrocene functionality. The electrochemistry is locally activated by illumination. b) Diagram showing the working principles of how electrochemistry can be modulated on and off at a monolayer modified silicon surface using light. Illumination of the silicon electrode results in a locally increase in charge carrier that allows electron transfer through the interlayer to the silicon substrate in the illuminated region. a,b) Reproduced with permission.[139] Copyright 2015, The Royal Society of Chemistry.

photocurrent as well as efficient electron transfer through the interlayer.

Hydrogen terminated silicon surfaces exhibit unusually few defects and low recombination velocity (0.25 cm s^{-1}) that is an order of magnitude lower than even the most carefully prepared oxide interfaces.[142] As the native oxide grow on the hydrogen terminated silicon, defects are introduced that dramatically increases the velocity with several orders of magnitude up to 10^6 cm s^{-1}.[143]

Organic monolayer on oxide free silicon surfaces have appeared to generate few defects with low recombination velocities in the order of 10^2 cm s^{-1}, enough to generate an efficient photocurrent.[143] Alkyl monolayers generally yield a maximum coverage of ≈50% of the reactive Si—H sites, bulkier molecules even lower.[17,69] Thus a dense interlayer that prevents penetration of water molecules that may cause oxidation and introduce surface defects is crucial for the device performance.

The authors realized this by thermally activated grafting of 1.8-nonadiyne that enables conjugation of the DNA strain via click chemistry.

Zou et al.[131] further showed that functionalized oxide free silicon substrates may be a suitable for LAPS imaging. The authors presented an innovative photo-electrochemical imaging sensing platform that combines the ability of LAPS to analyze dynamic ion fluxes with LAE that may give information about dynamic changes in cell attachment and permeability of the cell membrane. While LAPS on oxide free silicon substrate were demonstrated, the authors choose a hematite substrate instead of the silicon substrate for the combined imaging, which illustrates that silicon based amperometric measurements are still on an early stage.

6.2. Microelectromechanical Biosensors

Mechanical transducers respond to changes in a physical quantity or condition with a mechanical and subsequent electrical output. Example of frequently applied strategies in biosensing includes to measure the quasi-static deflection caused by biomolecules binding to the surface of a cantilever, or a mass induced change in resonance frequency of a piezoelectric material such as in surface acoustic wave sensors or quartz crystal microbalance sensors.[144–147]

Kanyong et al.[148] studied the electrografting of a diazonium salt bearing a maleimide functionality carried out on both Au-coated quartz crystals as well as Au-quartz crystals that had been additionally coated with 110 nm of microcrystalline Si by plasma enhanced chemical vapor deposition. Combining electrochemical quartz crystal microbalance with an IR ellipsometric method allowed in situ monitoring of the adsorption process and confirmed a dense monolayer. The method is promising for the situ monitoring adsorption and desorption processes of biomolecules.

Bivardi et al. functionalized a silicon micro cantilever (Si-MC) via photochemical hydrosilylation.[149,150] The hydrosilylation reaction was chosen to achieve a robust and stable receptor immobilization that was not prone to hydrolyses in water as would be the case with silane monolayers. A "tetraphosphonate cavitand" receptor (synthetic receptors that are capable of binding inorganic and organic cations[149]) bearing ω-decylenic functionality was covalently grafted on the H-terminated Si(100) face of Si-MC. The developed sensor was found to efficiently detect whole class of mehamphetamine in water with detection limit in tenths of ppm.

6.3. Optical Sensors

Optical biosensors include for example surface plasmon resonance sensors,[151] ellipsometric sensors[152] waveguide and interferometric sensors,[153,154] and reflectometric interference spectroscopy biosensors.[155] They represent the most common type of bioanalytical sensors and are often silicon-based.

There are several examples of optical biodetection on oxide-free silicon surfaces. Cattaruza et al.[85] presented fluorescence based detection of the hybridization of complementary oligonucleotides. Liao, et al.[156] developed a Fourier transform infrared spectroscopy (FTIR)-ATR based biosensing method for the interaction of organic molecules with DNA double helix with sequence specific photo cleavage (**Table 3**).

Colas et al.[112] immobilized DNA onto a hydrogen passivated ring-resonator by electrografting of an azide functionalized

Table 3. Optical sensors with oxide free silicon substrates.

Sensor type	Receptor/Analyte	Surface coverage	LOD	Ref.
Fluorescence	DNA/DNA	6×10^{-11} mol cm^{-2}	0.30×10^{-9} M	Cattaruza et al.[85]
FTIR-ATR	Aptamer/thrombin	N/A	10 nM	Liao et al.[128]
Microring resonator	DNA/DNA	N/A	N/A	Colas et al.[112]
Reflectance spectroscopy	DNA/DNA	N/A	N/A	Layouni et al.[57]
Reflectance spectroscopy (Optical rugate filter)	N/A/fibroblast	N/A	N/A	Tong et al.[157]
RIS	Antibody/Insulin	N/A	1.9 μg mL^{-1}	Chhasatia et al.[158]
photoluminescence	Protein A/N/A	N/A	N/A	Moretta et al.[159]
UV/Vis absorbance	tyrosinase enzyme/pyrocatheol	1.135×10^{15} molecules	0.43 μM	Lasmi et al.[160]
Fluorescence	DNA/DNA	N/A	0.2 μM	Böcking et al.[120]
Reflectance (smartphone)	Biotin/Streptavidin	N/A	500 nM	Cao et al.[161]

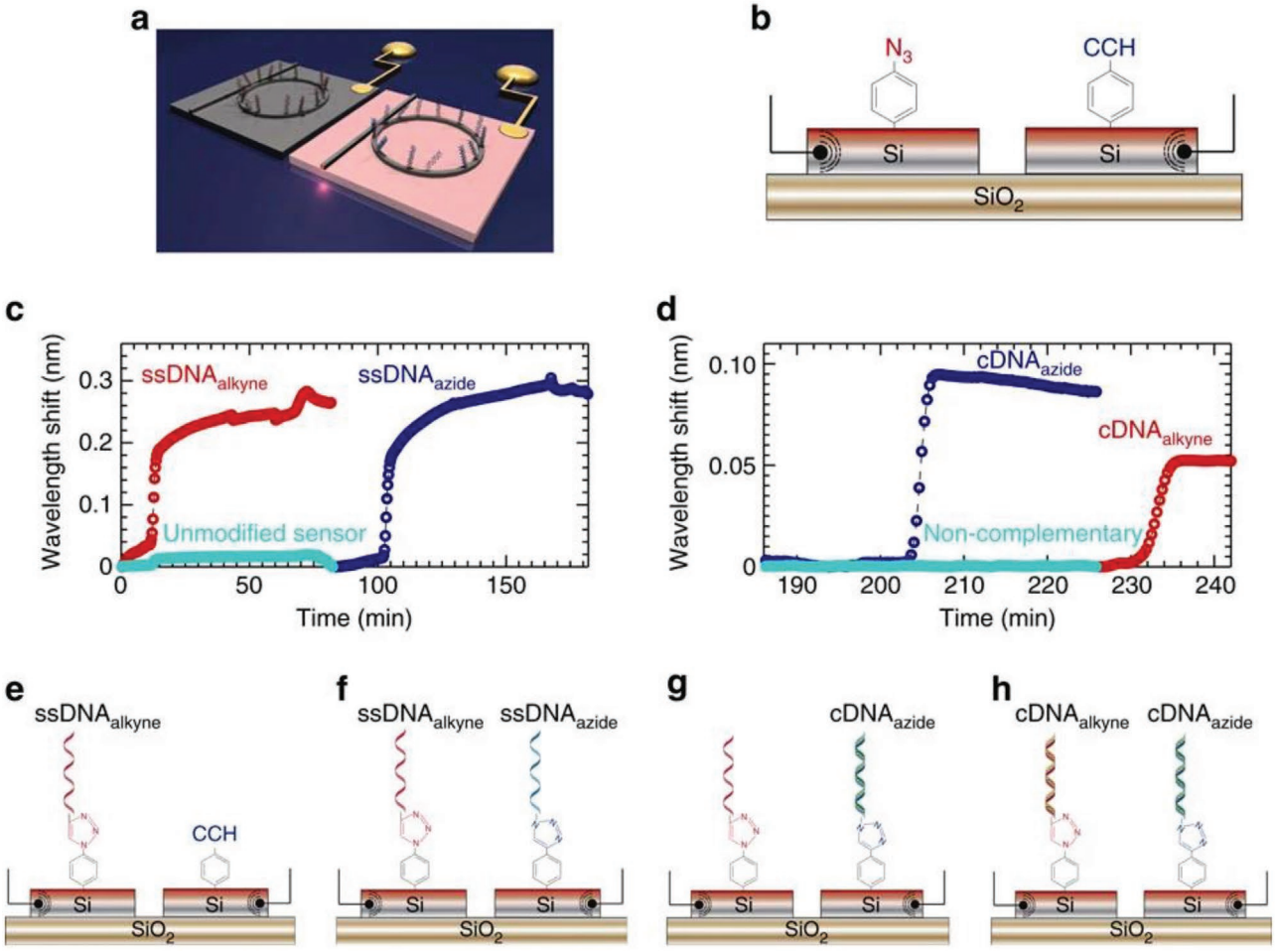

Figure 10. Schematic illustration of selectively functionalized optical ring resonator DNA array. a) Schematic illustration of the sensor. b) Different diazonium salt can be electrografted on each sensor to provide an orthogonal chemical functionality. c) The individual resonators are functionalized with modified DNA sequences using copper-catalyzed azide–alkyne click reaction, showing selective binding to the microrings functionalized with the pertinent chemical moiety. d) The selectivity of the functionalization process was confirmed by exposing the sensor array to DNA of the reverse complement of $ssDNA_{azide}$ and $ssDNA_{alkyne}$. e–h) Schematic illustrations of the sensor functionalities. a–h) Reproduced under the terms of the CC-BY 4.0 license.[112] Copyright 2016, The Authors, published by Springer Nature.

aromatic compound followed by copper catalyzed coupling with an alkyne labeled oligo nucleotide.

The microring resonators were optimally n-doped to support high Q resonances alongside electrochemical processes in situ.

They were able to selectively functionalize desired rings on the chip, thus allowing the formation of an array and multiple analytes detection (**Figure 10**). The combination of photonic and electrochemical characterization also provides additional quantitative information and unique insight into chemical reactivity that is unavailable with photonic detection alone. Furthermore, as electrografting only proceed in the area in which a voltage is applied, the approach allows the silicon sensor area to be selectively functionalized over the enclosed buried oxide on the silicon on insulator (SOI) substrate, thus preventing waste of receptor molecules.

A great number of recent published studies of optical biosensors are based on porous silicon substrates[162] and porous silicon nanoparticles.[163–166] The transducing mechanism is often based on changes in photoluminescence or a spectral shift in the optical properties due to changes in the mean refractive index of the porous layer. The effect of a change in refractive index can be enhanced greatly by creating optical elements such as Bragg mirrors or rugate filters.[162,167] In Bragg mirrors two dielectric media with different refractive indices are stacked alternately, whereas by rugate filters a smooth index profile is fabricated. The variation in refractive index can be achieved by changing the porosity of the substrate.[168]

The performance of photoluminescence sensors may be tuned by optimizing nano dots and pore dimensions in porous silicon. Furthermore, chemical passivation of the oxide-free substrates is known to stabilize the photoluminescence[75,169] and prevent recombination. Therefore, organic interlayer prepared via hydrosilylation or electrografting that both conserve the photoluminescent properties as well as allow further biofunctionalization may play a great role in these devices. Furthermore, due to differences in surface chemistry[41,58] compared to bulk substrates, porous silicon opens up different

routes for surface functionalization such as room temperatures hydrosilylation reaction and thermal carbonization.

Thermal carbonization is an interesting alternative as a strategy for the functionalization of porous silicon.[170–172] While the strategy generally fails to maintain the photoluminescence properties of the substrate,[173] thermal carbonization has other advantages such as efficient penetration of grafting molecules into the porous structures, less steric hindrance, and a surface that better prevents against oxidation and corrosion.[57,58] The stability of the interlayer is extra interesting for biosensor applications and this was nicely illustrated by Layouni et al.[57] who compared the stability of porous silicon passivated with a) SiO_2 followed by DNA immobilization via an APTES protocol, 2) hydrosilylation of undecylenic acid followed by DNA coupling via NHS chemistry and 3) thermal carbonization of acetylene followed DNA coupling. They found the thermal carbonization superior in preventing corrosion of the pSi substrate. Corrosion may be a problem, in particular when monitoring hybridization of DNA due to its negative charge. The group further demonstrated successful monitoring of DNA hybridization using a reflectance measurement set-up. The stability of the interlayer was also demonstrated by Tong et al.[157] who proposed the interlayer would be suitable for implantable devices.

Another recent example is a biosensor based on reflectance interferometric spectroscopy (RIS) using both an antibody and aptamer bioreceptor motif for the detection of insulin.[158] The bioreceptors were covalently attached to a thermally hydrosilylated *p*-doped Si surface through amide coupling, while unreacted surface area rendered stable and low fouling by incorporation of PEG moieties. The insulin detection ability of each biosensor was determined, using a range of different media both with and without serum.

This was the first time a pSi based IRS sensing platform has been applied for the detection of insulin from a human islets sample.

A different surface functionalization approach was carried out by Moretta et al.[159] who took advantage of the 2D photoluminescent material graphene oxide (GO) to prepare a device for label-free sensing with a transducing mechanism based on both changes in reflectivity and photoluminescence. Porous silicon was functionalized in four steps starting with a thermal induced hydrosilylation reaction using undecylenic acid, followed by a PEGylation process of by EDC/NHS, immobilization of GO by EDC/NHS on the PEGylated pSi and immobilization of PrA.

Finally Cao et al.[161] demonstrated a smartphone biosensor based on analyzing structural color of porous silicon. Captured analytes attached to the pore walls, generated an increase in the effective refractive index of the porous silicon increases and results in a redshift. Instead of measuring this shift as a reflectance spectrum with a spectrometer they analyzed color change of the porous silicon that may be detected by the smartphone camera. The Specific detection of streptavidin molecules was demonstrated with an estimated detection limit of 500 nM. The sensing approach is a promising contribution as a low-cost platform for the detection of chemical and biomolecular species. In this example the pSi were modified by thermal hydrosilylation of undecenoic acid biotinylation via NHS/EDC chemistry (**Table 4**).

7. Conclusion and Future Prospects

Since the pioneering work in the 90s, a large variety of protocols that allow surface functionalization of oxide free silicon surfaces has been developed.

The greatest advantages with the mentioned functionalization strategies compared to silanization are improved electrical properties of the surface and more stable interlayers. The lifetime of surface minority charge carriers has been reported to be an order of magnitude higher on hydrogen terminated surfaces compared to the most carefully prepared oxides.

It has been shown that a grafted organic monolayer on silicon surfaces can conserve these electronic properties and at the same time protect the surface from oxidation. Furthermore, there is no insulating layer separating the desired organic functionality from the surface which allow direct electron transfer, the monolayers are highly stable also in alkaline solutions and there are few interface traps that may take out the photoluminescence of the substrate. Crucial to form such high quality interlayer however, is optimized functionalization protocols. Just as the control of the density of states at the Si/SiO_2 interface was a milestone for the establishment of the MOSFET technology preventing electrically active surface defects at the Si/interlayer interface will be crucial to develop silicon based electrochemical devices.

A variety of approaches including thermal, ultraviolet, and visible light hydrosilylation, electrografting of diazonium salts and Pd catalyzed reactions leaves many options to adapt the adsorption conditions to the specific, required surface functionalities. Many reports in the literature have proven successful covalent grafting of a large variety of functionalities either through pre-syntheses adsorption or through multi step attachment procedures. H-terminated silicon surfaces can be prepared through etching of the native silicon oxide layer in HF. Due to steric reasons, monolayers of alkyl chains yield a maximum coverage of ≈50% of the reactive Si—H sites. In spite of that, excellent oxidation protection and electrical insulating properties have been reported.

Despite these advantageous, biofunctionalization of silicon is for the most part carried out via silanization chemistry due to more manageable functionalization protocols that can be carried out by researchers with limited knowledge about chemistry laboratory work. To graft organic molecules to oxide free silicon surfaces, expert training is usually required to handle poisonous liquids such HF and the grafting is generally carried out under inert atmosphere, often with custom-made flasks.

Therefore, biofunctionalized oxide-free silicon surfaces are probably best suited for applications where silane chemistry is not an option, as well as for applications that aim at detecting analytes at very low concentrations or where hydrolysis must be prevented such as long term continues measurements in aqueous conditions.

Silicon based sensors are often fabricated from a SOI wafer where the sensing elements and possible integrated circuit devices are built on the device layer, with the buried oxide enclosing the device, functioning as an effective etch-stop during wafer processing. As a consequence, by applying silane based biofunctionalization approaches the receptor molecules will be immobilized all over the chip and not specifically at

Table 4. Strategies toward biofunctionalization of oxide free silicon surfaces.

Surface/Functionality	Precursor molecule/Reaction conditions	Biomolecule/micro-organism	Bioconjugation strategy	Ref.
Si(100) / −C≡CH	1,8-nonadiyne Thermal hydrosilylation Neat, 220 °C, 3 h	Cytochrome C	Azide-alkyne coupling (Cu(I) + ascorbic acid)	[103]
Si(111)/ −C≡C−TMG	$HC{=}CH(CH_2)_8O(CH_2CH_2O)_{10}{-}CH_2CH_2C{\equiv}CH$ /hydrosilylation UV light 2H	Biotin followed by avidin. Or mannose followed by *E. coli*	Azide-alkyne coupling (Cu(I) + ascorbic acid)	[104]
Si (111) −C≡CH	1,8-nonadiyne Hydrosilylation, UV light, 2 h	Biotine followed by Streptavidine	Azide-alkyne coupling (Cu(I))	[106]
Si(100) −OOC≡CH	$HC{\equiv}CCOOCH_2CH_2OOCHC{\equiv}CH$ 120 °C 12 h	N/A	Copper free Azide-alkyne coupling	[111]
Si(100) −COOH	10-undecanoic acid Hydrosilylation 200 °C 2 h	Thiol modified oligodeoxynucleotide	Amine coupling of maleimide derivative followed by Michael addition.	[81]
Si(111) COOH	1-decene and undecylenic acid Hydrosilylation 160 °C 3 h	DNA	NHS coupling	[84]
Si(100)	10-undecynoic acid Cathodic electrografting	Oligodeoxyribonucleotide	NHS coupling	[85]
SiNW COOH	5-hexynoic acid Electrografting	BSA	NHS coupling	[86]
pSi COOH	4-(2-aminoethyl)benzoic acid Anodic electrografting Grignard	Tyrokinase	NHS coupling	[71]
pSi COOH	NHS-Undecyl-1-en Hydrosilylation UV	Biotin	NHS coupling	[87]
pSi −SH	$HC{=}CH(CH_2)_8O(CH_2CH_2O)_3{-}SCOCH_2$ Hydrosilylation	Albumin	1) $LiAlH_4$ 2) NHS maleimido crosslinker 3) Michael addition	[117]
Si(111) −OH	Protected undencenol Hydrosilylation 110 °C	N/A	N/A	[118]
Si(111) Epoxide	$HC{=}CH(CH_2)_8O(CH_2CH_2O)_3{-}CHCH_2O$, hydrosilylation	Thiolated DNA	Thiolysis	[120]
Si(100) / −C≡CH	1,8-nonadiyne Thermal hydrosilylation Neat, 165 °C, 3 h	Mouse IgG	1) Light activated Cu catalyzed azide-alkyne coupling of a carboxylic acid linker 2) NHS chemistry.	[119]
Si(111) Si−H	N/A	Azurin	Disulfide reduction, Spontaneous or electrografting (−0.8 V vs Ag/AgCl)	[79]
Si (111) −COOH	1) Methyl undecylenate Hydrosilylation UV 1 h 2) Acidic hydrolysis	DNA	NHS chemistry	[128]
pSi −COOH	Undeylenic acid Hydrosilylation 150 °C 12 h	LPS	NHS Chemistry	[129]
Si(100) −C≡CH	1,8-nonadiyne Thermal hydrosilylation Neat, 165 °C, 3 h	ssDNA	Cu catalyzed azide alkyne coupling.	[130,139,141]
SiNW $-NH_2$	Boc-10-aminodec-1-ene Hydrosilylation, UV, 1 h	ssDNA	Electrostatic interactions	[127]
SiNW $-NH_2$	1.2-tert-butyl allylcarbamate Hydrosilylation UV 3 h (t-Boc deprotection TFA/MeOH) Hydrosilylation, UV 21 h	avidin/streptavidin and Antibody	ethylene dicarbodiimide coupling chemistry.	[89]
SiNW $-NH_2$	10-*N*-boc-amino-dec-1-ene Hydrosilylation, UV 3 h	PNA	Glutaraldehyde strategy	[126]
SiNW −C≡CH	1,8-nonadiyne Thermal hydrosilylation	PNA	Azide-alkyne coupling. Cu catalyst	[133]
Si(100) S−H	N/A	ω-decylenic functionalized tetraphosphonate cavitand receptor	Hydrosilylation, UV 2 h	[149]
Si(111) −COOH	Undecylenic acid Hydrosilylation, UV	Thrombin, Oligonucleotides	NHS Chemistry	[156]

Table 4. Continued.

Surface/Functionality	Precursor molecule/Reaction conditions	Biomolecule/micro-organism	Bioconjugation strategy	Ref.
SiNW C≡CH or $-N_3$	$HC{\equiv}C-Bn-N^+_2X^-$ or $N_3-Bn-N^+_2X^-$ Electrografting	ssDNA	Azide/alkyne coupling	[112]
pSi −COOH	undecylenic acid Thermal hydrosilylation 150 °C 24 h	Insulin antibody or aptamer	NHS Chemistry	[158]
pSi −COOH	1) Undecylenic acid Thermal hydrosilylation 110 °C 18 h 2) BOC-NH-PEG-NH_2 NHS chemistry 3) GO NHS chemistry	Protein A	NHS Chemistry	[159]
pSi −COOH	Undecenoic acid Thermal hydrosilylation, 120 °C 5 h	Streptavidin	1) Biotin (NHS chemistry) 2) Biotin-streptavidin interaction.	[161]
pSi −COOH	undecylenic acid thermal hydrosilylation 150 °C 15 h	Tyrosinase	NHS Chemistry	[160]
Si(111) $-NH_2$	Boc-1-Amino-10-undecene hydrosilylation	1) SSMC linker 2) 5′-biotin-DNA-3′thiol 3) Streptavidin coated AU-NP	1) Amide coupling 2) Substitution reaction 3) Affinity interaction	[82]
Si(111) −maleimide	para-maleimidophenyl diazonium tetrafluoroborate (p-MPDT) Electrodeposition	Cys-Peptide	Michael addition	[174]

the sensing region. Biointeraction events that take place at the enclosed oxide region will not be detected thus potentially reducing the LOD. More work should therefore be devoted to the functionalization SOI devices focusing on protocols enabling selective functionalization of the silicon surface over the SiO_2 to enhance the performance of the sensors.

Another application that will benefit from these biofunctionalization approaches are light-addressable biosensors. Oxide-free silicon surfaces stands out over metals or oxidized substrates in their ability to generate a photocurrent. A favorable bandgap allows visible light to electrochemically activate any location on an electrode surface with microscale resolution allowing the realization of electrochemical microarrays that doesn't have to be individually wired. The approach may simplify both the production and read out of electrochemical sensor arrays and be an interesting alternative in modern automatized laboratories, by combining high specificity and high throughput.

In order to expand the applications even further and be a more competitive alternative to silane chemistry, time should be invested in the development of simpler grafting protocols that ideally are also compatible with materials implicated in standard microfluidic devices such as polydimethylsiloxane. This may require a switch away from common applied organic solvent such as toluene and mesitylene as well as refrainment from thermal activation. Approaches to form interlayer like the very recent reported grafting of a sulfhydryl that can be carried out in ambient atmosphere at low temperatures would be a step in this direction. Another approach to make the functionalization strategy more widely accessible could be to develop strategies and infrastructure, where the initial hydrogen passivation is carried out by experts in a centralized lab and the user may simple couple the desired biomolecules using simpler protocols. Such strategies should ideally be reversible, allowing the sensor to be used multiple times with different receptor molecules.

As the functionalization protocols get more accessible to a broader research community, we expect that silicon-based biosensors will find more applications, taking advantage of the improved stability, selectivity, and electric properties generated by these interlayers.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords

biofunctionalization, biosensors, hydrosilylation, oxide-free silicon surface, self-assembled monolayers, silicon passivation, silicon surfaces

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