Reactions on Monolayers: Organic Synthesis in Two Dimensions

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Self-assembled monolayers (SAMs) provide ideal molecularly defined platforms to study reactions in two dimensions. The surface chemistry of SAMs can easily be controlled by the head group of the surfactant molecules, which self-assemble into perfectly ordered, crystalline monolayers. A wide range of organic transformations can then be carried out to change the surface chemistry. These reactions have important applications in biological microarray fabrication, such as DNA and peptide chips. In this Microreview we will give an overview of all the classes of reactions that have been performed on SAMs. We will discuss the fundamental difficulties related to synthesis on monolayers, focussing on issues like steric hindrance, characterization, determination of yields and possibilities for multistep syntheses.

Introduction

Self-assembled monolayers (SAMs) have aroused widespread interest because of their potential applications related to the control of wettability, biocompatibility, and corrosion resistance of the surfaces of a wide range of materials.[1,2] Monolayers provide an opportunity to define the chemical functionality with molecular precision. Chemical transformations on SAMs can be studied in detail and may provide new mechanistic insights as well as routes to tailored surface properties. Such transformations allow, for example, the tethering of biologically important molecules to surfaces at precisely controlled positions, which can be of significant importance in a wide range of studies in chemical biology and microarray technology.[3]

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Solid-phase organic synthesis (developed by R. B. Merrifield in the early 1960s[4] and now widely used in peptide and DNA synthesis) and the use of solid-supported reagents are increasingly important tools in the design of clean, efficient, multistep routes to complex molecules or libraries of molecules.[5] The chemical transformations and reactions in solid-phase synthesis take place at the solid/liquid interface, and although this interface is not very well defined, synthetic organic chemists have achieved impressive control over these reactions. A tremendous amount of effort has gone into optimizing reaction conditions to overcome diffusion problems, swelling of beads and steric hindrance, and to minimize the influence of the solid support itself on the reaction.

This review deals with organic reactions at the solid-liquid interface, but instead of 3D solid-supported reagents, we will focus on 2D model surfaces formed by self-assembled monolayers. Central to this review are issues that

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MICROREVIEWS: This feature introduces the readers to the authors' research through a concise overview of the selected topic. Reference to important work from others in the field is included.

deal with the relationship between 2D chemistry and solution phase synthesis, and the potential to harness and control the reactions on monolayers to such an extent that the synthesis of complex molecules becomes possible.

It should be noted that reactions on monolayers have already been commercially exploited. The most widely used way to synthesize so-called DNA chips, which have dramatically revolutionized genotyping over the last decade, is by multistep reactions on self-assembled monolayers.[3] This approach is exemplified by work from Fodor et al.[6] who have developed photolithographic techniques to pattern and activate monolayer surfaces and synthesize libraries of DNA oligonucleotides.[7] The spectacular progress in the fabrication of 2D DNA chips does not mean that there is no room for improvement in the design of optimum surfaces for organic synthesis. We think that a review of reactions on monolayers could therefore benefit a wide audience of researchers working in many different areas of organic chemistry and chemical biology.

We will first consider the structural features of monolayers of thiols on gold and of alkylsilanes on Si/SiO_2. The analytical tools used in the characterization of monolayers and to follow their chemical transformations are quite different from those found in the standard organic chemistry toolbox, and we will give a brief introduction to some of the most important techniques. The ability to pattern monolayers laterally at the (sub)micrometer level is of great importance when the surfaces are to be used in (optoelectronic) devices, sensors, or arrays of biomolecules. It also aids the characterization of reactions on the surface, as it introduces a potential internal background that remains unchanged and can be used to check products and yields. The main technique for patterning SAMs is microcontact printing (μCP),[8] which utilizes an elastomeric stamp to deliver molecules to a surface.

The following sections include an overview of the different reactions that have been studied, followed by a more detailed look at those reactions of specific importance in the attachment of biomolecules to surfaces.

Self-Assembled Monolayers

Self-assembled monolayers are highly ordered molecular assemblies, which form spontaneously by chemisorption of functionalized, surfactant-like molecules.[9]

Once adsorbed to the surfaces, these molecules organize themselves laterally through van der Waals interactions between long aliphatic chains. In this review, we will focus our attention on SAMs of alkanethiols on gold and alkylsilanes on silicon, as these represent the bulk of the SAMs described in the literature (Figure 1). The preparation of SAMs on gold is simple. The clean substrate is immersed in a 1−10 mM solution of the desired alkanethiol at room temperature and after approximately 1 h, the surface is covered with a near perfect monolayer. It is generally believed that the thiol group binds to the gold as a thiolate (RS^-), located at threefold hollow sites of the Au(111) surface.[10] The overall result is an extremely densely packed, crystalline monolayer, where each thiolate occupies 21.4 $Å^2$ (which corresponds to ca. 2×10^{14} molecules/cm^2). For more detailed information regarding the formation of thiol monolayers on gold we refer the reader to the book by Ulman.[16] The choice of the head group thus determines the "chemical flavor" of the surface, as the underlying substrate becomes completely "invisible" to molecules in solution. To illustrate this point, clean gold is naturally hydrophilic, but the formation of SAMs makes it possible to control the contact angle of water on the surface to any value between 0° (−OH and $-CO_2H$ groups) and 118° ($-CF_3$ groups) depending on the functional group on the surface.[11]

The surface energy of a given SAM can also be altered by making a "mixed" SAM with two (or more) components. The problem of bulky head groups is avoided by mixing with less bulky thiols in the feed solution. When the alkanethiols are of equal chain length, the ratio of thiols in the SAM will resemble the ratio in solution.[12] The advantage of this method is that it can overcome problems of steric hindrance between bulky head groups or between nearby strands of molecules on the surface.[13]

The formation of SAMs of alkylsilanes on silicon or glass is more complex. These monolayers are covalently bound to surface hydroxy groups through Si−O bonds. The molecules used in the formation of such monolayers are either chlorodimethyl long chain alkylsilanes, alkyltrichlorosilanes, or trialkoxy(alkyl)silanes.[14] The alkylchlorosilane derivatives react spontaneously with clean Si/SiO_2 or glass, whereas the alkoxysilanes need to be heated, in order to convert the alcohols into leaving groups. The more commonly used alkylchlorosilanes are either deposited from the vapor phase or from solution. These molecules partially hydrolyze in solution, forming oligomers before settling down on the surface into a polymeric network. The hydrolysis of trichlorosilane derivatives can, however, also result in polymer networks "dangling off" the surface.[15] The experi-

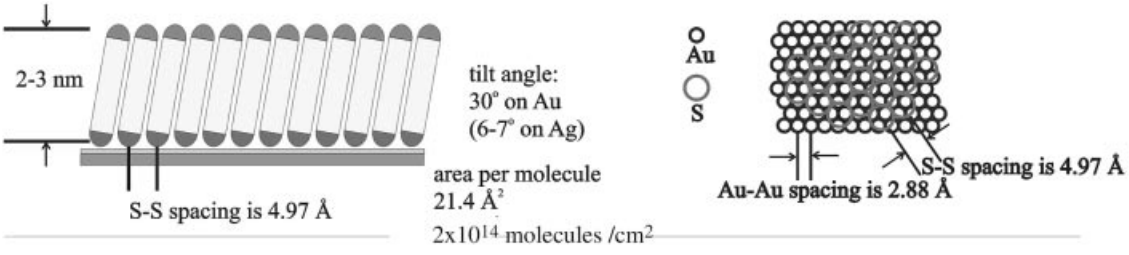


Figure 1. Schematic drawing of self-assembled monolayers of thiols on gold

mental conditions therefore need to be controlled to ensure clean, complete monolayer formation. Often, a "baking" step is necessary to ensure covalent bond formation between the SAM and the surface hydroxy groups. However, experimental evidence suggests that long alkylsilanes do form very tightly packed monolayers that are only slightly less dense than alkanethiols on gold.[16]

Patterning Self-Assembled Monolayers

Microcontact Printing (μCP) is a "soft-lithographic" method, which has now become a routine method to form patterned SAMs containing (sub)micron regions terminated by different chemical functionalities.[8,17] The procedure is illustrated in Figure 2. The first step in μCP involves "inking" the stamp with a reagent (e.g. alkanethiols or silanes); subsequently, the stamp is brought into contact with the surface. Where the stamp is in contact with the surface, a monolayer is formed. Typically, SAM formation is complete within 30 s. After the initial printing, a second, different SAM can be formed on the underivatized regions by exposing the patterned substrate to a dilute solution containing a second reagent.

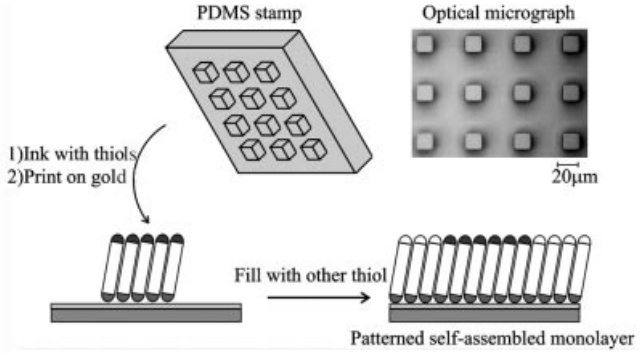


Figure 2. Outline of the μCP procedure to produce patterned SAMs

The resolution of contact printing is not diffraction-limited, which means that it is possible to print smaller than the wavelength of light. However, it is difficult to produce very small features (< 100 nm) due to the instability of the stamp, and problems related to diffusion of the inks during printing.[18–20] We have recently used high molecular weight dendrimers to overcome these diffusion problems and produce structures in the 100 nm range.[21]

Larger amounts of materials (e.g. reagents for synthesis, deposition of proteins) can be delivered with micron-sized resolution using a variety of techniques, most notably "spotting",[75] ink jet printing,[22,23] and microfluidic networks.[24] Another possible way of exploiting SAMs in order to direct molecules to certain sites on a surface is by taking advantage of hydrophilic and hydrophobic properties. Oligonucleotide arrays have recently been made using differences in surface tension (surface tension arrays) to define each synthesis site.[25,26]

Surface Characterization

The products of organic synthesis in solution can easily be purified and subsequently analyzed with rapid, sensitive techniques such as NMR spectroscopy, mass spectrometry, elemental analysis and X-ray spectroscopy. Solid-phase synthesis greatly facilitates the purification of products and has become the backbone of modern combinatorial chemistry, but the characterization of products bound to the solid supports is more difficult. Solid-state MAS NMR spectroscopy is one possible way to monitor a reaction, but in general, the products can only be fully analyzed after cleavage from the support. When working with reactions on monolayers, the problems of monitoring the reaction, determining the products and estimating the yield become quite significant. The extremely small quantities involved render most analytical tools useless, and very often, a combination of techniques is necessary to prove the structure on the surface. An exception is the use of monolayers on gold nanoparticles.[27] Monolayers on gold colloids (20 nm size range) have been used as models for 2D SAMs.[28] The advantage of colloids is that their reactivity can easily be studied by NMR spectroscopy in solution. As the small particles are highly curved, it is not always straightforward to extrapolate yields from nanoparticles to planar surfaces.

One of the advantages of SAMs on smooth, reflective surfaces, is that reactions on these monolayers can be studied by a wide range of techniques including infrared spectroscopy,[29–31] scanning electron microscopy,[32,33] contact angle measurements,[34,35] atomic force microscopy (AFM),[36,37] surface plasmon resonance,[38,39] ellipsometry,[40] low-angle X-ray reflectometry,[41,42] surface acoustic wave and acoustic plate mode devices,[43,44] X-ray photoelectron spectroscopy,[45] sum frequency spectroscopy,[46] quartz crystal microbalance,[47] electrochemical methods,[48] confocal and optical microscopes,[49] secondary ion mass spectrometry (SIMS)[50,103] and near-edge X-ray absorption fine structure (NEXAFS).[51,66] We will not discuss these techniques in detail, but refer the readers to the references given and the classic book by Ulman.[16] In practice, IR spectroscopy, ellipsometry and XPS are the techniques most widely used to study chemical transformations, whereas AFM is particularly useful to image-patterned surfaces. Here a change inside the pattern can be easily compared with the nonchanging background that acts as an internal reference.

The introduction of fluorescent tags and their detection using (confocal scanning) fluorescence microscopy is widely used to study the attachment of labelled biomolecules to a substrate (e.g. the hybridization of DNA in solution with DNA arrays). The quantitative analysis can be quite difficult, because a significant amount of quenching can occur due to the close proximity of fluorescent probes on the surface.[52] To overcome this problem the fluorescent reagent has been diluted with an equally reactive but nonfluorescent reagent.[53] It was found that the optimum surface coverage for the fluorescent probe is around 60%.

Instead of determining the yield while the molecules are in the monolayer, it is also possible to cleave the products from the solid support and analyze the molecules "off-line". Using very sensitive analytical tools, even the tiny amounts of material cleaved from substrates can be characterized. Butler et al.[25] measured the efficiency of phosphoramidite-based oligonucleotide synthesis on surface tension arrays using capillary electrophoresis of cleaved products.

Reactions on SAMs

Variation of the head group of the monolayer makes it possible to control wettability etc., and also allows the introduction of chemically more interesting properties such as nonspecific binding of proteins to surfaces. The introduction of oligoethylene glycol functionality to the end of the alkyl chain results in protein-resistant properties.[54] Further research in this area has led to a number of different functional groups that are all capable, to some extent, of resisting the adsorption of proteins to the surface.[55] Instead of synthesizing different thiols/silanes with different head groups, it is more convenient to use a number of "standard" SAMs and subsequently perform reactions on SAMs to modify the surface chemistry. Performing reactions on SAMs allows us to tune the properties of surfaces at the molecular level, but due to the nature of SAMs (tightly packed, movements of molecules within monolayers restricted) the choice of reaction is important. One must consider that steric effects are likely to be exacerbated for certain surface reactions, leading to an energy barrier higher than would be expected in solution chemistry. To successfully functionalize a SAM, reaction conditions must not cause destruction of the monolayer or damage the underlying substrate. This is a special consideration with monolayers such as alkanethiols on gold, where desorption of the monolayer can occur more readily than in systems such as the covalent attachment of trichlorosilanes on silicon ox-

Table 1. Reactions which have been carried out on SAMs.

SAM	Reaction type	Reagents	Towards	Refs.
Alkenes	Oxidation	Potassium permanganate	Ketol/diol	[59,60]
Amine	Nucleophilic substitution	*N*-hydoxysuccinimide (NHS) ester of (9-fluorenyl)methoxycarbonyl (Fmoc)	Fmoc-protected amine	[78]
		4-Nitrobenzaldehyde (chromophore)		
		Carboxylic acids	Imine (reversible reaction)	[63]
		Chlorodimethylsilane (S_N2)	Amides	[62]
			Aminosilane	[61,62]
	Nucleophilic addition	Isothiocyanates	Thioureas	[57,58]
		Diisocyanates	Carbamate (urethane)	[7967]
		Polymer-bound isocyanates	Urethane (substituted ureas)	[80]
	Acylation	Acid chlorides	Amides	[93]
		Active esters		[94,95]
		Quinones		[96]
Alcohol	Acylation	Acid chlorides	Esters	[81]
		Anhydrides	Esters	[81]
	Alcoholysis of anhydrides	Fluoroacetic anhydrides	Fluorinated Ester	[99,65]
	Nucleophilic substitution	Alkyltrichlorosilanes	Double SAM layer	[69]
	Nucleophilic addition	Phenyl isocyanate	Urethane	[66,67]
	S_N1	Dimethoxytrityl chlorides	DMT-protected, followed by acid deprotection	[78]
	Nucleophilic substitution	*N*-Hydroxysuccinimide (NHS) ester of (9-fluorenyl)methoxycarbonyl (Fmoc)	Fmoc-protected followed by deprotection with base	[78]
Carboxylic acid	Nucleophilic substitution (esterification)	Trifluoroethanol/di-*tert*-butylcarbodiimide	Ester	[65,72]
	Aliphatic nucleophilic substitution/dehydration	Intramolecular carboxylic acid + Trifluoroacetic anhydride	Interchain anhydrides	[102]
	Nucleophilic addition	Phenyl isocyanate	Mixed anhydride	[66,67]
	Nucleophilic substitution/ acylation of amines	Amines	Amides	[82]
Aldehyde	Nucleophilic substitution	Amines	Imines	[74]
Epoxide	Nucleophilic substitution	Primary Amines	Secondary amine	[75]
	Alcoholysis of epoxides	Hydroxy (Glycols)	β-Hydroxy ether	[76]
	Nucleophilic substitution	Carboxyl-terminated polymers	β-Hydroxyalkyl carboxylates	[77]
Thiol	Oxidation	Proteins	Chemisorption of proteins via a disulfide link	[83]
Sulfide	Oxidation	Hydrogen peroxide	Sulfoxides	[84]
Alkyl halides	Nucleophilic substitution/ halide exchange	Sodium iodide	Alkyl iodide (very slow)	[85]
	S_N2	Strong anionic nucleophiles (SCN^-, N_3^-)	Substituted product	[86,87]
Hydroquinone	Oxidation followed by Diels–Alder	cyclopentadiene linked to peptide	Peptide for attachment of cells	[88,89]

ides. Over the last decade, a considerable number of reactions has been studied:[56] (i) olefins − oxidation,[57−60] hydroboration, and halogenation;[57,58] (ii) amines − silylation,[61,62] amidation,[62] and imine formation;[63] (iii) hydroxy groups − reaction with anhydrides,[64,65] isocyanates,[66,67] epichlorohydrin[68] and chlorosilanes;[69] (iv) carboxylic acids − formation of acid chlorides,[70] mixed anhydrides[71] and activated esters;[64,72] (v) carboxylic esters − reduction and hydrolysis;[73] (vi) aldehydes − imine formation;[74] (vii) epoxides − reactions with amines,[75] glycols[76] and carboxyl-terminated polymers.[77] A list of all the major classes of reactions on SAMs plus relevant examples is given in Table 1.

The following sections will provide a more detailed look at some of the reactions listed above, especially those that are of relevance for the tethering of biomolecules to surfaces and for the combinatorial build-up of bio-assays.

Reactions of Amine-Terminated SAMs

The formation of aminopropylsilane SAMs on glass or Si/SiO_2 is a standard surface modification that allows the covalent coupling of DNA fragments to arrays,[90−92] or the base-by-base synthesis of oligonucleotides (Figure 3).[6,7,53]

The synthesis of the DNA strands is based on a series of phosphoramidite coupling steps, alternating with primary alcohol-DMT-deprotection reactions. The deprotection/coupling cycle is reported to have a ca. 95% yield. The attachment to the surface is via a linker, coupled to an aminopropyl-terminated silane monolayer. The advantage of this method is that it allows the principles of combinatorial chemistry to be applied, so that the number of oligonucleotide sequences far exceeds the required number of chemical steps. Another very important advantage of this method is that no purification steps are required.

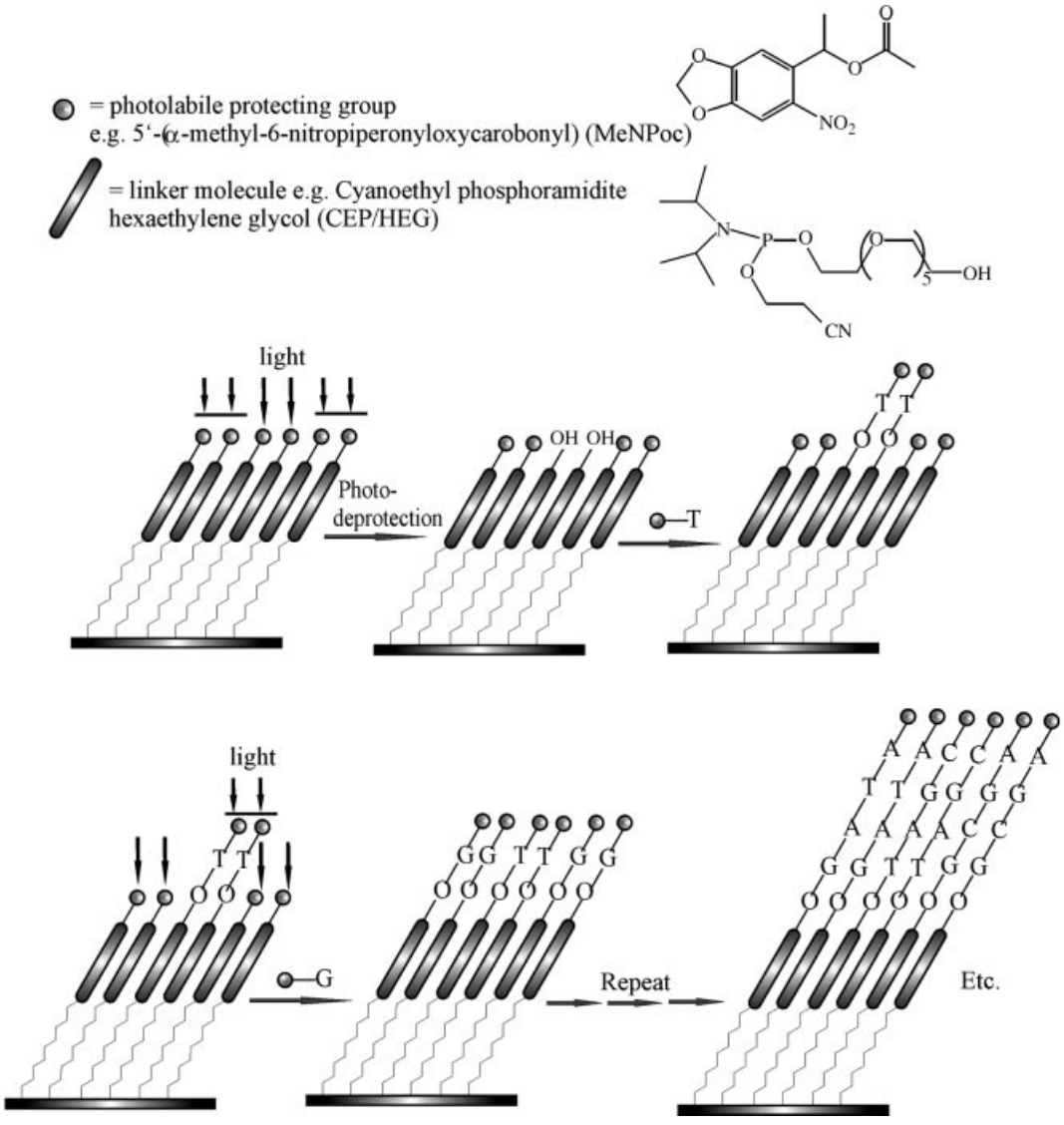


Figure 3. Schematic illustration of DNA chip synthesis

Because of their use in DNA chips, reactions on amine-terminated monolayers have received considerable attention and are well understood. Amine SAMs react readily with acylating reagents such as acid chlorides,[93] active esters[94,95] and quinones.[96] The initially hydrophilic amine surface was found to become hydrophobic with the coupling of a bulky protecting group to the amine. The bulky group was shown to be easily cleaved under acidic or basic conditions depending on the protecting group to yield the original amine surface.[78] We recently probed amino SAMs using a carboxylic acid terminated fluorophore, rhodamine B. The SAM was allowed to react with the dye both in solution and by microcontact printing. Fluorescence microscopy clearly showed the presence of the dye in the printed regions. (Figure 4).[98]

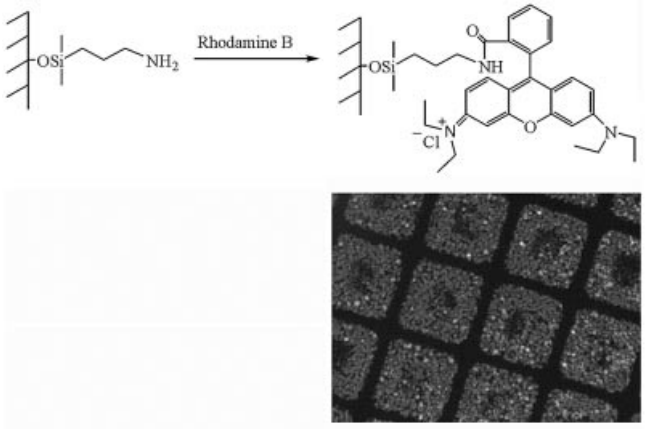
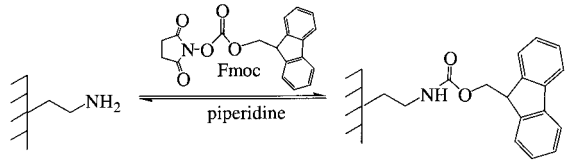
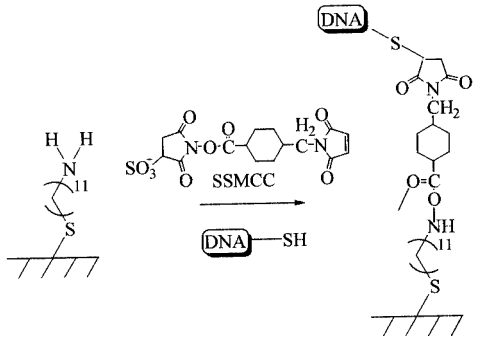


Figure 4. Reaction of rhodamine B with an amino SAM and fluorescence microscope image

To demonstrate their use in "classical" organic synthesis, amine groups on SAMs were capped with Fmoc protecting groups and subsequently treated with base to regenerate the original surface (Scheme 1).[78]



Scheme 1

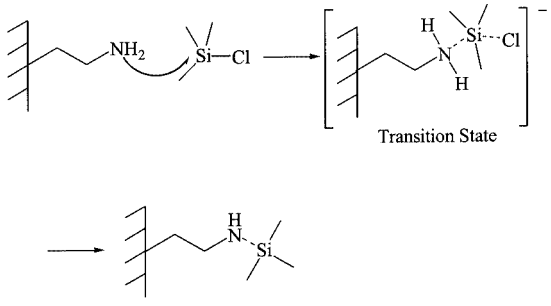


Scheme 2

Corn and co-workers also described the use of a heterobifunctional linker, which contained an NHS ester functionality (reactive toward amines) and a maleimide functionality (reactive toward thiols), Scheme 2.[92]

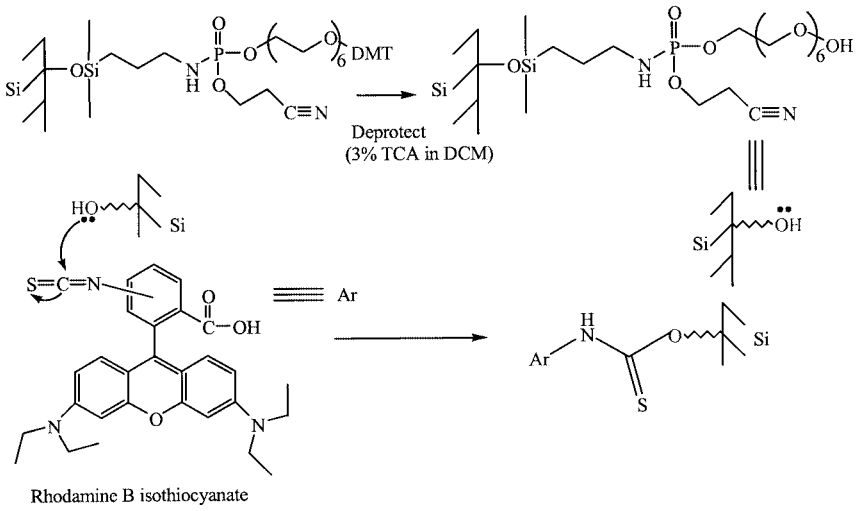
Park and co-workers[63] chemisorbed various alkoxy(amino)silanes onto a silicon surface and treated them with an aldehyde-functionalized chromophore (4-nitrobenzaldehyde) to probe the relative surface density of the underlying monolayer. The condensation was driven to completion by the removal of water and it was found that the hydrolysis of the imine restored the primary amine. This reversible process could be repeated a number of times on the same substrate.[63] Park also showed that the density of amines and the nature of the spacer molecule are important factors in determining the amount of chromophore that can be attached to the surface.[97] As a general point of caution, the precise determination of the number of amine molecules per unit area, as well as the percentage of these molecules that are reactive, is very difficult. The number of reactive groups is sometimes called the "loading capacity", which is likely to depend on the monolayer quality and the preparation method. This is especially the case for the aminopropylsilane SAMs that are very prone to multilayer formation.

The amine group in (3-aminopropyl)triethoxysilane SAMs on silicon has been quantitatively treated with chlorotrimethylsilane (Scheme 3). It was found that in this S_N2-type reaction, where the incoming nucleophile was a surface-bound amine, steric hindrance was not significant.[61,62] The reaction was studied using reflection absorbance IR spectroscopy (RAIRS), quartz crystal microbalance, ellipsometry and contact angle measurements and was found to give high conversions.



Scheme 3

The reactions of surface-attached amines with isothiocyanates,[58] diisocyanates[79] and polymer-bound isocyanates[80] to form urea linkages have all been reported. An isocyanate-functionalized polymer, poly(1-methylvinyl isocyanate)-alt-(maleic anhydride), was chemisorbed from solution. Evidence from FTIR spectroscopy suggests that the anhydride groups bound to the polymer also reacted with the amine giving amides and carboxylic acids. Unchanged anhydride and isocyanate groups were observed, leading to the possibility of further functionalization.[80] The number of free isocyanate groups attached to the adsorbed surface was determined by the number of groups initially present



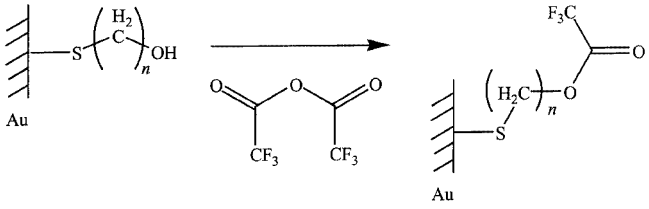
Scheme 4

on the unbound polymer. Furthermore, all organization gained on the surface during the process of self-assembly was lost due to the random coil nature of the polymer.

Reactions of Hydroxy-Terminated SAMs

Hydroxy-terminated linker molecules on a silicon surface react with isothiocyanate groups of a rhodamine B isothiocyanate dye at elevated temperatures (Scheme 4).[98]

The reaction between substrate-bound alcohols and fluoroacetic anhydrides has been estimated to give a 80−90% yield. The reaction was thought not to go to completion because the larger fluorine atoms lead to a sterically hindered environment, although no unchanged hydroxy groups could be detected by infrared reflection-absorption spectroscopy.[99]



Scheme 5

The reaction was studied in more detail by Leggett et al.[65] XPS experiments showed that trifluoroacetic anhydride completely reacted with the hydroxy monolayer, but longer chain anhydrides were found to give only an 80% conversion. As this reaction was performed in the gas phase, the lower conversion for the longer chain molecules might be explained by their higher boiling points. The possibility that CF_3 groups pack less densely due to their larger size than the corresponding alkyl chain was disputed in a more detailed study of the reaction.[64] The authors concluded that steric considerations were not significant, as the difference between the cross sectional areas of CH_3 and CF_3 is not very large. The variation in intensity of OH and CF bands in the IR spectrum, as plotted against time, suggested a complete reaction within 15 min. This evidence was supported by XPS data and negative ion TOF-SIMS spectra. This latter technique is highly sensitive, and showed the complete disappearance of any signals attributable to the alcohol and the appearance of signals assigned to the derivatized species.

Hydroxy-terminated SAMs on gold react with alkyltrichlorosilanes in an analogous manner to the chemisorption of trichlorosilane on hydroxysilicon substrates. This reaction represents the first example of the preparation of double layers using both the trichlorosilane and thiol methods of monolayer formation.[69]

The reaction between phenyl isocyanate and hydroxy-bearing SAMs has been described by Himmel et al.[66] The resulting urethane linkage was obtained in an 87% yield in the condensed phase reaction. The resulting monolayer-bound phenyl groups were found to be ordered with respect to the original monolayer. The urethane linkage was thermally unstable at temperatures above room temp., as shown by the strong decrease of the nitrogen signal in XPS. The use of protecting groups, widely used in the solid-phase synthesis of peptides and DNA, has been extended to monolayer chemistry by Frutos et al.[78] who selectively protected and deprotected hydroxy groups bound to a surface using base-labile (9-fluorenyl)methoxycarbonyl (Fmoc) (Scheme 1) and acid-labile di-*p*-methoxytrityl (DMT). A chlorine derivative of Fmoc was treated with a mercaptoundecanol monolayer to form a carbonate linkage in 1 h and the cleavage was demonstrated to be complete within 15 min. Similar reactivities were found for the DMT protection of the hydroxy groups.

Reactions of Carboxylic Acid Terminated Monolayers

Carboxylic acid terminated monolayers self-assembled onto Au substrates have been studied by Leggett et al.[65] The reaction between a carboxylic acid functionalized SAM and trifluoroethanol in the presence of di-*tert*-butylcarbodiimide, an activator added to make the carboxylic acid monolayer more susceptible towards nucleophilic attack,[72] was found to proceed slowly with only a 60% rate of conversion after several days. Subsequent reduction of the esters led to the formation of hydroxy groups, which can be exploited in the preparation of multilayers with trichlorosilane derivatives. These sluggish reaction rates are in agreement with comparably slow reactions on poly(methacrylic acid) where steric interactions are believed to be responsible for the long reaction times.[100] The authors concluded that the slow reaction on the monolayer was due to a combination of a) bulky *tert*-butyl groups on the diimide combined with the lack of space within the carboxylic acid SAM preventing attack; b) the sterically hindered nature of backside attack from the approaching alcohol directed towards the carbonyl group, and c) the adsorption of alcohol contaminants due to hydrogen bonding between the carboxylic acid and the ethanol used in preparation of the monolayer. It may be possible to refine Leggett's hypothesis with experiments on mixed monolayers where the surfaces can be engineered to provide a less hindered environment for the approaching diimide.

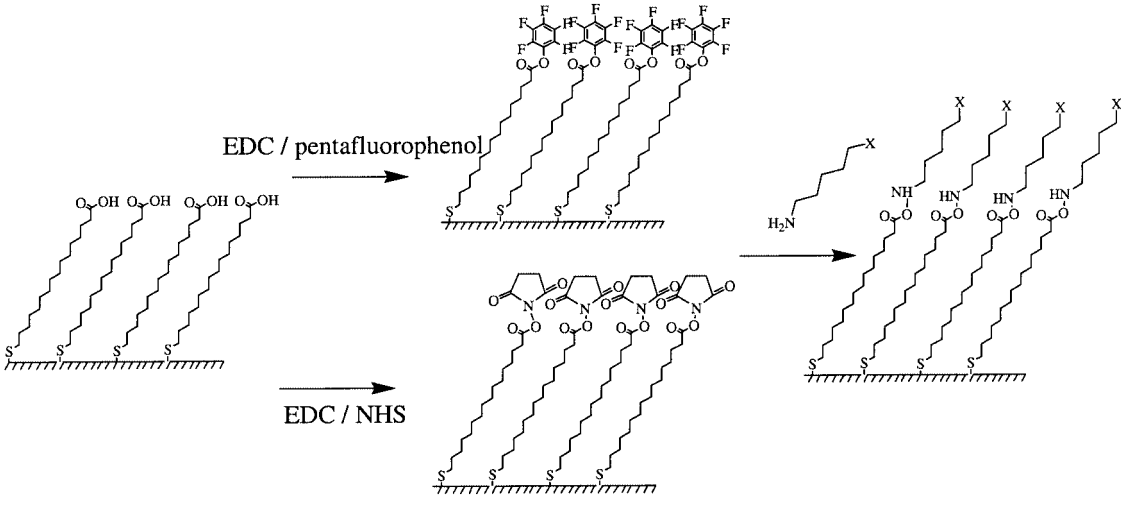
Carboxylic acid functionalized SAMs have been exposed to phenyl isocyanate vapors.[66] Vapor-phase reactions have the advantage that solvent influences can be excluded, and additionally, the effect of contaminants such as water and alcohols adsorbing to polar surfaces will be reduced under high vacuum conditions. Surprisingly, upon exposure of the carboxylic acid monolayer to the phenyl isocyanate at room temperature, the reaction did not proceed. When phenyl isocyanate was condensed onto the monolayer and subsequently warmed to room temp., the reaction was found to proceed with an 83% conversion, similar to the solution phase reaction. Near-edge X-ray absorption fine structure (NEXAFS) measurements showed that many of the carboxyl head groups were buried within the unchanged monolayer which was evident from the high proportion of gauche defects characteristic of a disordered system. However, the same results were found for the more ordered hydroxy-bearing monolayers, suggesting that the reaction rate is probably dependent on concentration rather than steric hindrance.

The reaction of carboxylic acid terminated SAMs with amines by the formation of amide bonds has been demonstrated by the reaction of bases of various strengths. A good correlation was found between stronger bases and faster reactions with the monolayer.[82] This example of acid/base chemistry on monolayers has been exploited by grafting an amine-terminated poly(amidoamine) dendrimer G0−G4 to a mercaptohexadecanoic acid monolayer. IR spectroscopy confirmed the absence of carboxylic acid carbonyl groups after the reaction.[71]

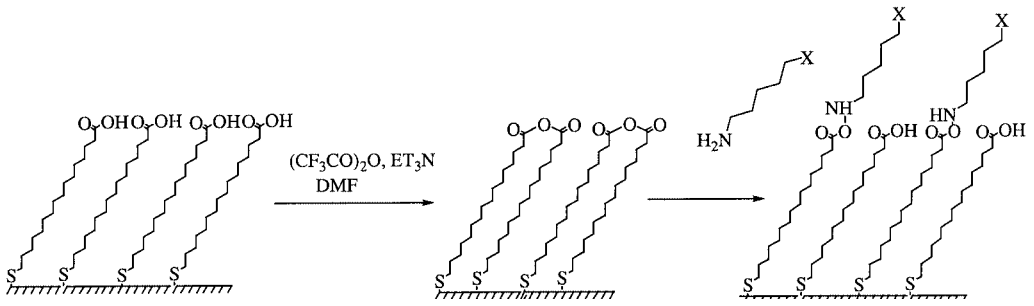
A so-called "activated ester" method is widely used for the introduction into SAMs of ligands that can be used for the binding of biological molecules to the surface.[13] The NHS functionality is often used to introduce a variety of amine-terminated molecules to SAMs under mild conditions. The reaction proceeds quickly, giving high yields and is compatible with a wide range of functional groups.[92,101]

Alternatively, carboxylic acid terminated SAMs were converted into reactive esters by immersing the surface into a solution of 3-(dimethylamino)-1-ethylpropylcarbodiimide (EDC) and pentafluorophenol in DMF (Scheme 6). The reaction proceeded quickly (ca. 20 min) under mild conditions. The carboxylic acid groups were activated to pentafluorophenyl groups because these esters are ca. 10 times more active than the corresponding NHS esters.

The close proximity of bound chains on the surface permits "intraSAM" reactions between these chains. Yan et al. showed that the dehydration of carboxylic acid monolayers with trifluoroacetic anhydride in the presence of triethylamine formed interchain anhydride SAMs.[102] It is thought



Scheme 6



Scheme 7

that the intermediate product is the mixed trifluoroanhydride, and that this subsequently reacts with another carboxylic acid functionality on the surface. These intra-SAM anhydrides were formed in quantitative yields and provided a reactive model surface to which a range of amines can conveniently be coupled (Scheme 7). For example, after reaction with *n*-undecylamine the characteristic signals for the C=O stretches of the anhydride disappeared completely, and two new absorption bands at 1742 and 1563 cm^{-1} appeared. These bands were assigned to the reformed carboxylate and the amide II band, respectively. XPS data showed the complete disappearance of the carbonyl group attributed to the carboxylic acid and appearance of the carboxylic anhydride. The reaction obviates the need for complicated synthesis of large molecules containing thiol end groups and has been used to introduce *n*-alkyl groups, perfluorinated *n*-alkyl chains,[103] peptides,[104] charged groups (sulfonate and guanidine groups)[103] and polymers containing amines (e.g. polyethylene imine).[105]

The interchain anhydride reaction has been exploited in the rapid and efficient introduction of a large variety of functional groups, which were screened for protein-resistant properties.[106] This work has also been extended to study the resistance of SAM-grafted polymeric thin films towards the adsorption of proteins and bacteria.[107]

Instead of treating the anhydrides with molecules in solution, it is also possible to print amines down using μCP. Instead of inking the PDMS stamps with thiols and placing the stamps on a clean gold substrate, the stamps were inked with *n*-undecylamine and the stamp was subsequently placed on the reactive anhydride SAM. Yan et al.[103] have shown that the yields obtained by μCP and by immersion were consistent with each other. Secondary ion mass spectrometry (SIMS) and scanning electron microscopy (SEM) were used to define the edge resolution (which was < 100 nm) and the composition of the patterned SAM. Microcontact printing to pattern reactive SAMs has also been used in the pentafluorophenol-derivatized SAMs discussed above.[13]

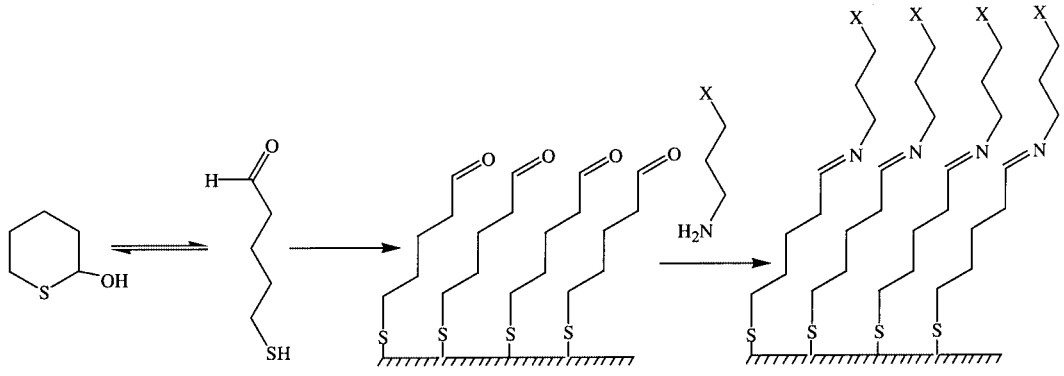
Aldehyde-Terminated SAMs

The ring-opening equilibrium of tetrahydro-2*H*-thiopyran-2-ol has been used to generate aldehyde-terminated surfaces, Scheme 8. The adsorption of the thiol pushes the equilibrium towards the linear aldehyde, which resulted in a densely packed monolayer within 3 h.

Aldehydes are a potentially useful functional group to immobilize onto a surface as they easily react with a variety of primary amines, forming imines.[74]

Oxidation Reactions on SAMs

Silane SAMs on Si/SiO_2 or glass with hydroxy or carboxylate head groups cannot be prepared directly from monomeric precursors due to hydrolysis. The oxidation of surface-bound alkene-terminated SAMs with potassium permanganate has been exploited as a useful alternative.



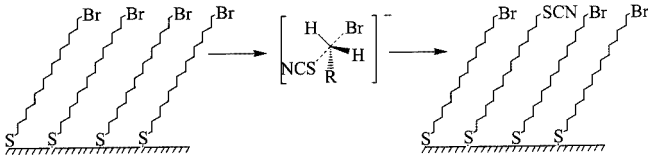
Scheme 8

The disappearance of the characteristic ethylenic bending at 964 cm^{-1} and the appearance of carbonylic absorbances in the 1500−1700 cm^{-1} region demonstrated the oxidation reaction.[59,60] These observations were consistent with changes in wettability as the hydrophobic alkene becomes oxidized. The close-packed nature of the SAMs allowed only the surface-exposed species to be oxidized, as any functionality within the monolayer is impenetrable to MnO_4^- ions.

The oxidation of thiol-terminated SAMs formed from α,ω-difunctionalized dithiols has allowed the chemisorption of proteins via a disulfide bond[83] and the formation of covalently bonded multilayers from the oxidation of dithiols on planar gold substrates. Ellipsometry measurements showed that the disulfide multilayers were preferentially formed over intralayer disulfides. This observation may be a result of higher ring strain in the intralayer product, leading to multilayer formation. Further oxidation revealed the presence of SO_x moieties.[79] These observations are consistent with an earlier study showing the oxidation of surface-exposed sulfides by H_2O_2 to form sulfoxides.[84]

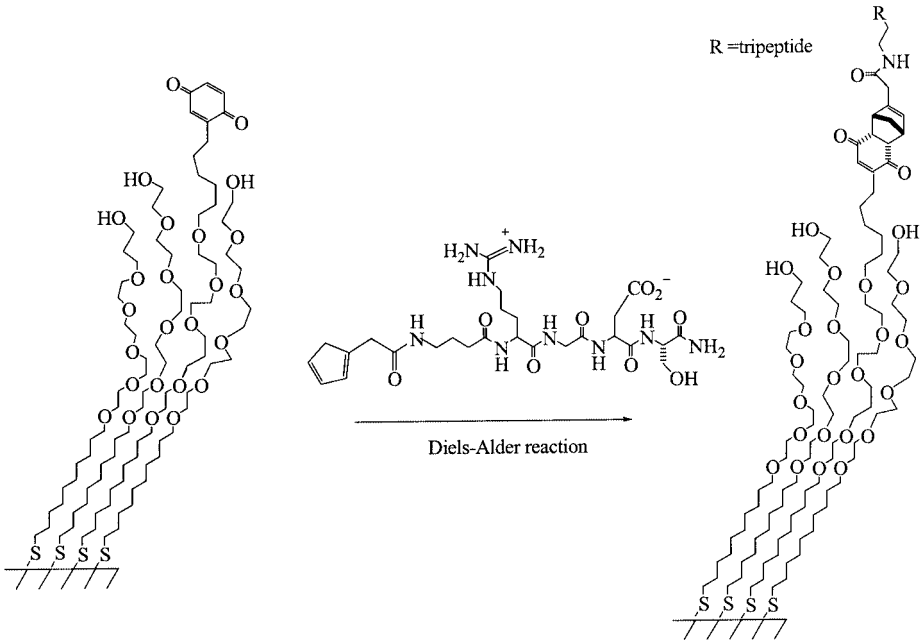
Reactions of Halogen-Bearing SAM Films

Exchange reactions of alkyl chloride monolayers with sodium iodide have shown that the reaction does not go to completion, and that the rate is approximately ten times slower on a monolayer than in solution. It is thought that the relatively large size of the incoming iodine atoms simply prevents them from fitting into the monolayer.[85] The S_N2 reaction of alkyl bromide SAMs has been studied, and the reaction was found to go to completion with strong anionic nucleophiles (SCN^-, N_3^-). The alkyl bromide is not sufficiently electrophilic to react with weaker nucleophiles such as amines, alcohols and thiols.[86] In a similar study using mixed monolayers, the effect of anionic nucleophiles on alkyl bromides was tested. The small, very nucleophilic azide was found to displace 100% of all available bromides, but the reaction took 48−60 h. Thiocyanate anions gave only a 75% yield after 48 h.[87] This observation was explained with reference to the "backside" or "*anti*" approach of the incoming nucleophile. The incoming nucleophile cannot attack from the front, due to electrostatic repulsions encountered from the halogen atom. The σ* antibonding orbital of the C−Br bond is orientated towards the surface and any approaching nucleophile would have to move into the densely packed monolayer to make a nucleophilic substitution possible. Only a small, reactive nucleophile such as azide could be envisaged to be able to overcome this increased energy barrier. This mechanistic description also accounts for the general observation that a nucleophile bound to a surface does not experience such an increased energy barrier and the reaction proceeds smoothly.



Scheme 9

In order to test this hypothesis, Fryxell et al.[87] employed radical tin chemistry unreliant on the backside attack trajectory to the halogen atom. The reaction was shown to go to completion much faster than the analogous S_N2 reaction, thus giving further support for this model.



Scheme 10

To test the effect of increasing electrophilicity on surface-exposed monolayers a variety of iodide-bearing monolayers were synthesized. The classical leaving group tendency I > Br > Cl was observed in solution and on the surface. Monolayers of an iodoacetyl moiety were found to react with decanethiol, *p*-nitrothiophenol and primary amines; in competition experiments, preferential substitution of the *p*-nitrothiophenol was observed, due to its superior nucleophilicity.[108] The reactivity of benzyl halides has been studied[85,109] although they have been found to be less effective than acetyl halides described above.

Dynamic Surfaces

For a number of applications (e.g. cell-binding studies or self-cleaning surfaces) it would be ideal if the surface chemistry could be modulated in situ and relatively fast. A number of ingeneous systems have been developed, using electrochemical or photochemical reactions and isomerizations. Mrksich reported the covalent attachment of cell-binding ligands by a Diels−Alder reaction of a substituted cyclopentadiene with benzoquinone attached to the SAM. The benzoquinone derivative could be activated or deactivated by a reversible oxidation/reduction step into the hydroquinone form, Scheme 10.[2,89,110] Mrksich described the use of a cyclopentadiene−peptide conjugate as an immobilization route for peptides used in the attachment of cells such as 3T3 fibroblasts.[88]

A similar strategy was employed to develop SAMs that released groups rather than immobilized ligands. A catechol orthoformate group was used as the electroactive linker that tethered the ligand to the SAM.[111] Upon electrochemical oxidation, the catechol orthoformate was converted into the corresponding orthoquinone, along with the hydrolyzed formate product. The reaction was complete within a single cyclic voltammetric scan, demonstrating the efficiency and speed of electrochemical reactions on monolayers.

Willner and co-workers exploited the photoisomerization of (dinitrophenyl)spiropyrans to tune the binding of antibodies to surfaces.[112] Illumination of the spiropyran with 370-nm UV light resulted in the isomerization of the double bond and ring-opening of the pyran moiety. Subsequent exposure to 500-nm light reversed this isomerization back to the starting (dinitrophenyl)spiropyran.

Polymer Brushes

We have discussed a great number of organic reactions on planar surfaces with very high densities of functional groups. However, these functional groups are not always available for further functionalization, which leads to incomplete reactions, especially when attempting to link large molecules (e.g. DNA strands) to these surface groups. One possible solution is to construct a "pseudo-3D" surface where the number of available reactive sites is increased by allowing the monolayers to extend into solution. This can be achieved by growing polymer brushes from the surface that contain side groups for further linkage. The polymers are grown from monolayers that contain an initiator head group, from which polymer chains grow, monomer by monomer, from the surface.

In addition, such ultrathin films of polymers have been shown to introduce control over surface properties such as wettability, adhesion and corrosion resistance.[113] There are a number of ways in which polymers can be grown in a controlled way from surfaces including the use of "living" cationic,[114] anionic,[115] nitroxide-mediated,[116a−116c] ring-opening[117] and Atom Transfer Radical Polymerization (ATRP).[118] The disadvantages of some of these methods are that even though controlled brush growth can be obtained, the process often requires high temperatures, the addition of a sacrificial initiator, and long reaction times resulting in polymerization of the monomer in solution. More recently, an alternative method of surface-initiated polymerization was developed in aqueous media, offering controlled growth of brushes with a predetermined length and the possibility of block copolymers.[119] The density of these brushes can be controlled by "diluting" the initiator monolayer by mixing with unreactive CH_3-terminated thiols.[120]

Polymer brushes can also be used to modulate the surface properties in real time, which can be another route towards dynamic surfaces. We have recently grown poly(*N*-isopropylacrylamide) brushes from patterned self-assembled monolayers exploiting the same chemistry as outlined above.[121] Below the so-called Lower Critical Solution Temperature (LCST) the brushes were hydrophilic and fully hydrated, but by increasing the temperature to 30 °C (above the LCST), the brushes collapsed and became hydrophobic, due to their insolubility in water. By lowering the temperature below the LCST, the brushes expanded again into their hydrophilic state. This reversible switching could be used in self-cleaning, anti-fouling surfaces, where bacteria adsorbed to the hydrophilic polymers detached upon raising the temperature.

Summary and Outlook

The use of self-assembled monolayers allows the study of a vast number of reactions on surfaces. A better understanding of these reactions is important in the design of better solid supports for solid-phase synthesis, but also in the field of biological microarrays. We believe that most types of reactions can be performed, though steric hindrance and diffusion barriers can hamper the yield or rate of reactions at the surface. Although impressive results have been achieved in DNA-array synthesis, multistep synthesis of complex molecules on SAMs has not been widely explored. Better techniques for the characterization of complex molecules on surfaces and the determination of the yield of reactions on SAMs are required. As the purification of products on SAMs is impossible, reactions that go to completion are a prerequisite, unless sufficiently efficient "masking" reagents can be found that react with all unchanged head groups in SAMs, thus eliminating them from

further reactions. Tuning the chemical character of the SAM by introducing certain molecules has proved to be a very powerful tool for biological applications. Such applications have been briefly exploited but we believe there are many more possible applications, especially concerning the attachment to substrates of more and more complex biomolecules. The ability to chemically pattern the monolayer surfaces using soft-lithographic techniques is another attractive aspect of the planar surface. As many of these techniques are becoming more available to a wide range of research groups in organic chemistry, we fully expect self-assembled monolayers to become a standard synthetic platform in the (near) future.

Acknowledgments

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