The Analyst

Tutorial Review

Self-assembled Monolayers for Biosensors

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The use of self-assembled monolayers (SAMs) in various fields of research is rapidly growing. In particular, many biomedical fields apply SAMs as an interface-layer between a metal surface and a solution or vapour. This review summarises methods for the formation of SAMs upon the most commonly used materials and techniques used for monolayer characterisation. Emphasis will lie on uniform, mixed and functionalised monolayers applied for immobilisation of biological components including (oligo-)nucleotides, proteins, antibodies and receptors as well as polymers. The application of SAMs in today's research, together with some applications will be discussed.

Keywords: *Self-assembled monolayer; immunoassay; electrochemistry; surface plasmon resonance; (electrochemical) quartz crystal microbalance; biosensor*

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High selectivity provided by biomolecules (antibodies, enzymes, nucleic acids) or biological systems (receptors, whole cells) is exploited in biosensors, in which a biological sensing element is integrated with an electrochemical, optical or piezoelectric transducer.[1,2] Most commonly, the biological component (capture molecule) is immobilised on, or in close proximity to, the surface of the transducer. As a consequence, immobilisation strategies for biomolecules are of paramount importance in order to preserve their biological activity. At biological recognition, *i.e.*, when the immobilised molecule and its ligand (the analyte) have interaction, a signal is generated which is (proportionally) related to the concentration or amount of the analyte in the sample.

Direct immobilisation of biomolecules involves physisorption and is applied, *e.g.*, in microtiterplate immunoassays, in which usually no control over the orientation of the molecule is achieved.[3] Interaction sensors based upon surface plasmon resonance (SPR) or monomode dielectric waveguides,[4] as well as microtiterplate immunoassays,[3] require more sophisticated approaches to avoid random orientation. Protein A or G[5] as well as biotin-avidin chemistry[6–11] can improve control over the immobilisation process. Other strategies exploit hydrogels[12] or poly-l-lysine[5] with specific linkage reagents (*e.g.*, carbodiimide,[13,14] or periodate[15]). Photochemical[16] activation and, most of all, functionalised self-assembled monolayers (SAMs)[17–20] offer promising possibilities.

Almost any surface can be equipped with functionalised monolayers which possesses a required specific electrical, optical or chemical property. An ideal monolayer is depicted as perfectly aligned, closely packed alkane chains, attached to a smooth surface (Fig. 1). There are two methods to deposit molecular layers on solid substrates (*e.g.*, glass or metal): Langmuir–Blodgett transfer[21] and self assembly. The Langmuir–Blodgett technology[21] will not be discussed in this review. The formation of stable monolayers on glass or aluminium oxide by alkanesilanes[22] is also beyond the scope of this review. Emphasis, however, lies on another class of monolayers, based on the strong adsorption of disulfides (R–S–S–R), sulfides (R–S–R) and thiols (R–SH) on a metal (particularly gold) surface. Nuzzo and Allara, pioneers in the assembly of sulfur-containing molecules,[23] noticed that dialkane sulfides form highly ordered monolayers on metal surfaces. Sulfur donor atoms coordinate strongly on a gold substrate. Van der Waals forces between methylene groups orient and stabilise the monolayer. Porter *et al.*[24] showed that long-chain (number of methylene groups $n > 10$) alkanethiols assemble in a crystalline-like way. A reduction in chain length leads to less ordered structures.

Adsorption studies of unsymmetrical dialkane sulfides[25] revealed that these monolayers are significantly less densely

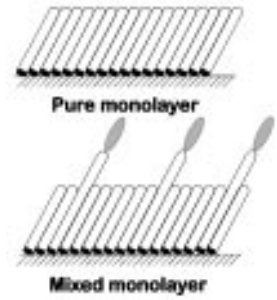

Fig. 1 Schematic drawing of a pure and a mixed monolayer. Sulfur (black) is attached to a gold surface. Functional groups (shaded) are elevated into the solution or gaseous phase.

packed and less ordered, compared to alkanethiol monolayers. A series of ω-substituted long-chain alkanethiols[26] also form both highly oriented and ordered monolayers. Almost simultaneously a mixed monolayer, composed of different thiols, was introduced which offers a very promising basis for immobilising biomolecules *via* a functional group.[27]

The formation and characterisation of organic SAMs and their applications in sensor technology will be discussed in this review.

Formation of Monolayers

Disulfides, sulfides or thiols coordinate very strongly onto a variety of metals, *e.g.*, gold, silver, platinum or copper. The structure of a self-assembled monolayer depends on the morphology of the metal. Au(111) is mostly applied for the formation of monolayers. Gold films adopt this crystallographic orientation predominantly when deposited upon polished glass, silicon or freshly cleaved mica. Gold is favoured, because it is reasonably inert.

Thermal evaporation and deposition upon silicon wafers is the most convenient way to prepare gold substrates. To render a hydrophilic wafer surface, it is pretreated with 'piranha' solution[27] (hot H_2O_2–H_2SO_4) and successively rinsed with deionised water and absolute alcohol. Another method is treatment with hot basic and acidic oxidising mixtures.[28,29] An adlayer of chromium (50–150 Å)[30] or titanium (~40 Å)[31] promotes the adhesion of gold. Deposition with a rate of 2 Å s^{-1} at a pressure of ~10^{-8} Torr (1 Torr = 133.322 Pa) produces a polycrystalline surface, but extended Au(111) terraces are present.[30] The thiols form the highest ordered and oriented monolayers upon these terraces. An uncontaminated gold surface is important but not essential; the high affinity of the thiol moiety for gold even displaces contaminants.[25,32] Most commonly, as a precaution, possible contaminants are removed by 'piranha' solution and a hydrophilic gold surface will be obtained. Residual contaminants on bare gold can be removed by scanning the potential between +0.5 and +1.4 V *versus* SCE in dilute (0.5 m) sulfuric acid. Clean gold shows a characteristic anodic peak current near +1.1 V and a single cathodic peak near +0.9 V.[33]

Thiols, sulfides or disulfides can be directly adsorbed from appropriate high purity solvents (commonly ethanol for non-polar or water for polar ω-substituted alkanethiols).

Alkanethiols from dilute solution form a densely packed monolayer in less than 1 h. The adsorption time seems to be independent of the chain length, but high concentrations lead to shorter adsorption times. A general protocol for self-assembly of monolayers is hard to give, because the preparation depends on the desired properties.

The mole ratio of a mixture of thiols in solution results in the same ratio in the mixed SAM. The two components do not phase segregate into islands.[34] This interesting feature can be exploited to immobilise biomolecules in such a manner that steric hindrance between these molecules and their binding partners is avoided.

Although dense monolayers assemble quickly, well ordered monolayers can take days to form.[35] For alkanethiols ($n > 5$) adsorption stops at the monolayer level, a stable multilayer is not formed. However, multilayer formation of an alkanethiol is observed after 6 d of immersion.[36] The assembling kinetics of a monolayer is biphasic: the diffusion-controlled adsorption is followed by a slow (re-)crystallisation process.

Characterisation of Monolayers

Monolayers can be characterised by a wide variety of methods, of which the non-electrochemical methods are not extensively reviewed. Several chapters[35,37] and a book[38] discuss this topic. Of interest are the (*a*) pinholes or defect structures, (*b*) gold–sulfur bonding, (*c*) molecular orientation of the polymethylene chains and (*d*) order and orientation of the tail groups.

(*a*) *Pinholes or Defect Structures*

Monolayers may completely cover the metal surface. Microscopy techniques (scanning tunnelling[39–41] and atomic force[42]) have revealed that normally 'a very low concentration' of pinholes is present. Electrochemistry can easily detect pinholes in a monolayer covering gold.[33] A bare gold electrode in an acidic aqueous solution yields a well defined set of peaks, due to surface oxidation. Suppression of these faradaic currents implies that even water is excluded from a SAM-covered electrode. Residual oxidation can be caused by pinholes in the SAM or defect structures in the gold surface, so care should be taken when interpreting experimental results. Porter *et al.*[24] have estimated the maximum pinhole radius as 8 μm and a fractional uncovered area of one per cent. or less.[37]

An extreme decrease in the faradaic current of a redox couple [$Ru(NH_3)_6^{3+}$ or $Fe(CN)_6^{3-}$] is indicative of a (hydrophobic) SAM. A small residual current is a strong indication of the presence of pinholes. A pinhole-free monolayer is impermeable to aqueous ions, and will act as an ideal capacitor.[43] A SAM-covered electrode, compared to a bare one, demonstrates a strong decrease in capacity. Charging currents will be relatively larger if the monolayer is not impermeable. Differential capacitance measurements are less sensitive than voltammetry, but they additionally provide a manner in which to measure the film thickness and the permeability for simple ions.

(*b*) *Gold-Sulfur Bonding*

Voltammetric studies indicate that thiol groups are deprotonated upon adsorption.[44] The assumed formation of a gold–thiolate bond is: $RSH + Au \rightleftharpoons RS\text{–}Au + e^- + H^+$.

A new route for fast electrodeposition of monolayers has been proposed by Weisshaar *et al.*[45] In this study it is proven that a monolayer can be reversibly adsorbed and desorbed by electrochemical means. This process can serve as a basis for the determination, by capacitance measurements, of the monolayer surface coverage Γ,[46] by measuring the charge, *Q*, needed to desorb an ω-mercaptoalkane ferrocenecarboxylate monolayer. From $\Gamma = Q/nFA$, where *n* is the number of electrons involved in the electron-transfer process, *F* the Faraday constant and *A* the geometric electrode surface area, the value of Γ can be calculated. The values of *Q* are determined by integration of the area under the *i*–*E* curves (obtained in 1.0 m $HClO_4$) after compensating for charging current and compared to those obtained from infrared reflection absorption spectroscopy.[46]

Monolayers are stable in the potential range from −400 to +1400 mV *versus* SCE in dilute sulfuric acid solutions.[33]

Surface methods based on electron or photon irradiation should not destroy the gold–sulfur bond.[47] Techniques used for the determination of the chemical composition of the monolayers, including infrared,[23,48] X-ray photoelectron spectroscopy,[49] and near edge X-ray absorption fine structure measurements[50] are reviewed by Finklea.[37]

(*c*) *Molecular Orientation of the Polymethylene Chains*

Infrared data show that monolayers of ω-substituted alkanethiols ($n > 10$) are densely packed crystalline-like structures, exhibiting a typical tilt angle in the range of 28–40° from the surface normal and a twist of chain axes of approximately 55°.[31] The methylene groups exhibit strong Van der Waals inter-

actions, stabilising the monolayer. Helium diffraction and transmission electron microscopy studies[51] reveal that methylene groups are ordered (crystalline like) at low temperatures and less ordered (semi-crystalline) at room temperature.

(*d*) *Order and Orientation of the Tail Groups*

These groups are important for the interaction of a monolayer with a biomolecule.

Grazing incidence infrared spectroscopy[31] shows that ω-substituted alkanethiols also are densely packed, highly oriented and ordered. As long as the end group (NH_2, OH) is relatively small (< 5Å),[43] the orientation of the monolayer is not influenced. More bulky groups (COOH, ferrocene) decrease the density of packing and ordering.[43,46] A mixed monolayer, consisting of a ω-substituted and a (shorter) alkanethiol is adsorbed in the same mole fraction as in the solution. A phase separation is not observed, indicating random ordering of the two constituents.[34] This feature offers the possibility to 'dilute' ω-substituted alkanethiols with shorter non-substituted thiols in order to have anchor groups available for immobilisation procedures in which steric hindrance is possibly reduced (Fig. 2).

Wetting studies by contact angle measurements between the monolayer surface and a liquid provide structural information.[27] Comparing contact angles of polar and non-polar liquids can provide insight into the three dimensional structure of the monolayer. Contact angle measurements probe the outermost few angstroms of the surface.[26] High contact angles of water and hexadecane on methyl-terminated thiol monolayers and the low contact angles on carboxylic acid- and alcohol-terminated monolayers indicate that the surface of the monolayers consist of densely packed arrays of the tail groups of the thiols.

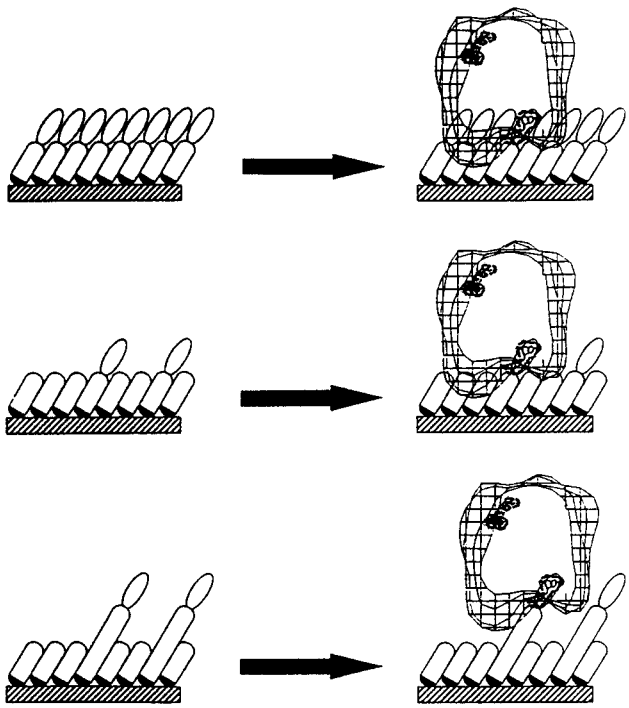

Fig. 2 Schematic representation of a SAM of thiols and the binding of streptavidin to them. (Top) A pure monolayer. Binding of streptavidin is severely sterically hindered. (Center) A monolayer of a mixture of thiols with the same length of the alkane moiety. There still is steric hindrance. (Bottom) Addition of a spacer allows binding without steric hindrance.[83] (With kind permission from Elsevier Science Ltd.)

Applications

SAM technology is most advantageous for electrochemical, SPR and (electrochemical) quartz crystal microbalance [(E)QCM] sensors. Modifying a sensor surface with SAMs generates model systems with a specific property or function.

Some considerations to use SAMs in analytical applications include: (*a*) the (alkane)thiols form easy-to-manufacture, pinhole-free, stable monolayers from dilute solutions, ensuring a uniform immobilisation surface; (*b*) SAMs shield biological substances from the sensor surface, preventing possible denaturation;[52–56] (*c*) contamination of metal surfaces (non-specific adsorption altering the hydrophobic c.q. hydrophilic properties) impairs analysis and has to be avoided; and (*d*) the monolayer can be tailored with functional terminal groups for immobilisation purposes.

This last feature offers numerous challenges, *e.g.*, improvement of detection limits, the ability to regenerate the sensor, prevention of aspecific adsorption, as well as development of generic assays.

In the following sections a number of applications of SAMs in electrochemical, optical (SPR) and (E)QCM sensors is presented. A representative selection of articles has been made in this very rapidly expanding research field.

Electrochemical Sensors

Within the potential limits as stated before, SAMs based on thiols or related compounds are not desorbed from electrode surfaces in aqueous solutions. SAMs can either be used for studying (non-electroactive) blocking properties, or for obtaining a selective electroactive surface. The blocking properties of a SAM have already been presented in the section dealing with the detection of pinholes. SAMs with long alkanethiols ($n > 10$) cause a dramatic decrease in electrode/electrolyte capacitance compared with bare gold.[33] This involves a reduction in noise and a relative enhancement of the faradaic current (provided that this current is not significantly attenuated by the monolayer). On the other hand SAMs can decrease electron-transfer rates, which can be used for kinetic studies. Detailed theoretical aspects are presented by Finklea.[37]

An 'electroactive electrode' consists of a SAM with both blocking behaviour and selective electron tunnelling or 'gates' for the analyte. In the following section, several approaches for using SAMs in electrochemical sensors are presented.

In an amperometric sensor a carboxylic acid-terminated SAM [$HS(CH_2)_nCOOH$ with $n = 15$ or 11] is used to immobilise cytochrome c (a mediator in cell redox reactions) *via* carbodiimide activation.[57] In contrast to electrostatically adsorbed cytochrome c, carbodiimide-mediated attached molecules could not be desorbed by a solution of saturated potassium nitrate. Also, carboxylic acid-terminated SAMs ($n = 2$, 5 or 10) are applied in detecting the neurotransmitter dopamine in the presence of ascorbic acid.[58] At neutral pH, the negatively charged SAM repels ascorbic acid, while positively charged dopamine can be detected at sub-millimolar levels. An optimum in electrochemical discrimination between ascorbic acid and dopamine is achieved using the medium length thiol ($n = 5$). Electrochemically polymerised pyrrole (polypyrrole), a conducting polymer, is widely used for incorporating biomolecules.[59,60] Polypyrrole initially deposits as oligomers onto the electrode surface. Growth upon this nucleation sites continues in a fairly uncontrolled manner. Scanning tunnelling micrographs reveal a 'cauliflower'-like structure. A novel approach is controlled growth on a SAM from a pyrrole-derivatised thiol.[61,62] Pyrrole is electrochemically polymerised to form an adlayer. Control over the final deposition of the polymer film is provided by the number and location of the ordered nucleation sites. Adhesion of multilayers of polypyrrole on this adlayer is enhanced. Growth of the film proceeds layer by layer, as

indicated by a less porous surface. The resulting film, in contrast to deposition of polypyrrole on a bare surface, is not easily removed. The thickness-corrected conductivity is enhanced by a factor of 3.

Redox enzymes linked to SAMs have been studied by Creager and Olsen.[63] A glucose sensor is prepared by cross-linking glucose oxidase (GOx) to a SAM from ω-hydroxy alkanethiol by glutaric dialdehyde. Electrical communication between electrode and GOx is achieved *via* freely diffusing hydroxymethylferrocene. Normally, redox currents are diminished by a SAM, but the response of this redox mediator was less affected. Background currents of interferents (uric acid, ascorbic acid and 4-acetamidophenol) were dramatically reduced relative to untreated electrodes. Rikling and Willner[64] coupled GOx enzyme, derivatised with ferrocene, to a monolayer of cystamine: $[NH_2(CH_2)_2S]_2$. Since one adlayer of GOx did not yield a detectable signal, seven consecutive adlayers were covalently attached, enhancing the signal substantially.

A highly selective and thermodynamically favourable electron transfer pathway consists of a SAM from 3,3′-dithiobissulfosuccinimidylpropionate on which the enzyme GOx is immobilised. The active site of the enzyme is assumed to be in direct contact with the electrode.[65]

Another approach for a glucose sensor is presented by Rubin *et al.*[66] They used a mixed monolayer of ferrocenylhexadecanethiol, as mediator for electron transfer, and aminoethanethiol, for immobilising GOx.

A monolayer from 2-mercaptobenzothiazole can electrically be opened and closed for underpotential deposition of copper,[67] without affecting the electron-transfer rate of an Fe^{2+}/Fe^{3+} redox reaction. Cysteamine $[NH_2(CH_2)_2SH]$ was assembled and a quinone mediator (pyrroloquinolinquinone: a catalyst for electro-oxidation of NADPH and NADH) covalently linked.[68] This yielded a pH-sensitive detector for nicotinamide cofactors. When to this quinone ($NADP^+$-dependent) malic enzyme is covalently bound, maleic acid can be measured amperometrically in the range from 10^{-7} to 10^{-3} m. Porphyrins (catalysts for oxygen reduction) linked with thiols on the 'edges' of the molecule are assembled on an optical transparent electrode.[69] The effect of the electrode surface on the mechanism of oxygen reduction by metalloporphyrin films could be studied (Fig. 3). Using a transparent electrode, UV/VIS absorption spectra can be obtained simultaneously for the adsorption process. Surprisingly, long adsorption times led to the formation of multilayers, but these could be removed by extensive rinsing.

Thioctic acid, a cyclic disulfide containing a carboxylic acid, is used as a pH-dependent SAM[70,71] for the redox response of

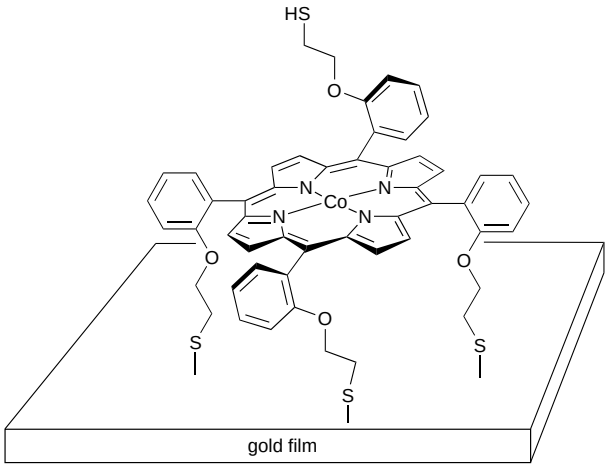

Fig. 3 Schematic illustration of the most probable average surface structure of tetra-thiolated cobalt porphyrin.[69] (With kind permission from the American Chemical Society.)

$Ru(NH_3)_6^{3+}$ and $Fe(CN)_6^{3-}$. At increasingly higher pH values, the SAM becomes more negatively charged and the faradaic current for $Fe(CN)_6^{3-}$ is reduced. In this way it acts as a cationic sensor. At low pH values when carboxylic acid is neutral, both $Fe(CN)_6^{3-}$ and $Ru(NH_3)_6^{3+}$ show a fast response.

Thioctic acid serves as a basis for a 'novel separation-free sandwich-type enzyme immunoassay' for proteins.[72] On a gold-coated microporous nylon membrane a capture antibody against human chorionic gonadotrophin (hCG) is covalently immobilised. Simultaneously, hCG and an alkaline phosphatase labelled detecting antibody are incubated. Aminophenyl phosphate is enzymatically converted into aminophenol, which is detected electrochemically.[73] The detection limit for hCG was determined to be 0.8 U l^{-1} ($\approx$ 160 ng l^{-1}).

There is a growing demand for detection systems of specific DNA sequences.[74] An electrochemical method for the determination of the amount of DNA adsorbed onto an electrode surface is presented by Pang *et al.*[75] Single-stranded DNA is covalently attached to a monolayer of thioctic acid, activated by carbodiimide and *N*-hydroxysuccinimide. The amount of immobilised DNA is determined indirectly through the redox reaction of tris(2,2′-bipyridyl)cobalt(iii), which complexes strongly with DNA.[76]

Surface Plasmon Resonance

When *p*-polarised laser light reflects internally at the interface of an optically dense and less dense medium, an evanescent field is generated. This field will, at a specific angle, be enhanced by collective motions (resonance) of conducting electrons (plasmons) in a thin ($\approx$50 nm) metal layer.[77] Silver and gold films are commonly used, because of a low imaginary dielectric constant. Changes in refractive index at the gold/air or gold/solution interface shifts the resonance angle. Interactions can be followed in real time, without any labelling of the reaction partners. Again, SAM technology has already proven its merits in this sensor type.

A hydrogel[78] can be linked with a SAM onto a gold surface. Electrostatic interactions preconcentrate positively charged proteins (at low pH, below their isoelectric point) at residual negative charges in the hydrogel. Activation with carbodiimide and *N*-hydroxysuccinimide covalently binds the proteins to the matrix.[12] Other methods for (covalent) coupling chemistries for this carboxymethylated dextran hydrogel matrix are presented by Löfås *et al.*[79]

A versatile and useful biotin-functionalised SAM has been presented by Knoll and coworkers.[80,81] Biotin (vitamin H) has an extremely high binding constant (K_{diss} = 10^{-15} m) for tetravalent (strept)avidin. When (strept)avidin is bound to this monolayer, a biotinylated antibody (anti-hCG) is readily immobilised. The accessibility for (strept)avidin can be optimised by preparing a monolayer of biotinylated long-chain alkanethiols, diluted with shorter hydroxythiols.[82] The long-chain alkanethiol acts as a spacer molecule, elevating the biotin group from the monolayer surface.

Cyclodextrins are cyclic oligosaccharides that chelate with small molecules. Derivatised with multiple long-chain thiol spacers, these cyclodextrin molecules assemble parallel to the surface. Oriented in this way they are freely accessible for dye molecules.[83]

Specific recognition with high sensitivity for cholera toxin is achieved by creating an artificial membrane with entrapped ganglioside.[84] A lipid layer (membrane) can be immobilised through hydrophobic interaction upon a SAM of an alkanethiol. To form a lipid membrane upon this SAM, various deposition methods were compared. A simple and fast way to deposit an artificial membrane has been achieved by a lipid/detergent dilution technique that finally yields a micellar solution. These

micelles spontaneously assemble onto the hydrophobic SAM as an artificial membrane. The detection limit for cholera toxin (M ≈ 50 kDa for the B_5 subunit) can be estimated as about 10^{-9} m with SPR.

Thiol-derivatised synthetic peptides (M_r ≈ 2 kDa) form a sensing layer for specific protein molecules.[85,86] An optimum response for an antibody (M_r ≈ 150 kDa) is found when the SAM consisted of only 3 mol % thiolated peptide.

To study the effects of surface properties on protein adsorption,[87] SAMs are excellent model systems. Hydrophilic hexa(ethylene glycol)-terminated SAMs effectively reduce non-specific adsorption for a wide range of proteins found in biological fluids.

A similar type of SAM is used by Duschl *et al.*[88] 11-Mercaptoundecanol-linked antigenic peptide (mimicking the surface of a malaria parasite) is diluted with underivatised 11-mercaptoundecanol. Non-specific antibodies did not adsorb, but the specific antibody (10^{-9} m) gave a small, but noticeable shift when incubated.

A series of six histidine moieties (a His tag) is incorporated in the primary sequence of recombinant proteins, in order to simplify purification. Thiols, terminated with a nitrilotriacetic acid (NTA) group and a tri(ethylene glycol) group (mentioned before) in a molar ratio 1 : 10 form a mixed SAM.[89] NTA coordinates with Ni^{2+}, leaving two vacant binding sites, which selectively chelates with the His tag. Detection of DNA hybridisation with SPR is demonstrated by Piscevic *et al.*[90] A ten-mer oligonucleotide, with the 5′-phosphate group attached to mercaptopropyl has been immobilised on gold. Surprisingly, formation of an 'incomplete' SAM is preferred over dilution. The self-assembly process was stopped in an early stage of the formation. In real-time, the hybridisation process is followed,

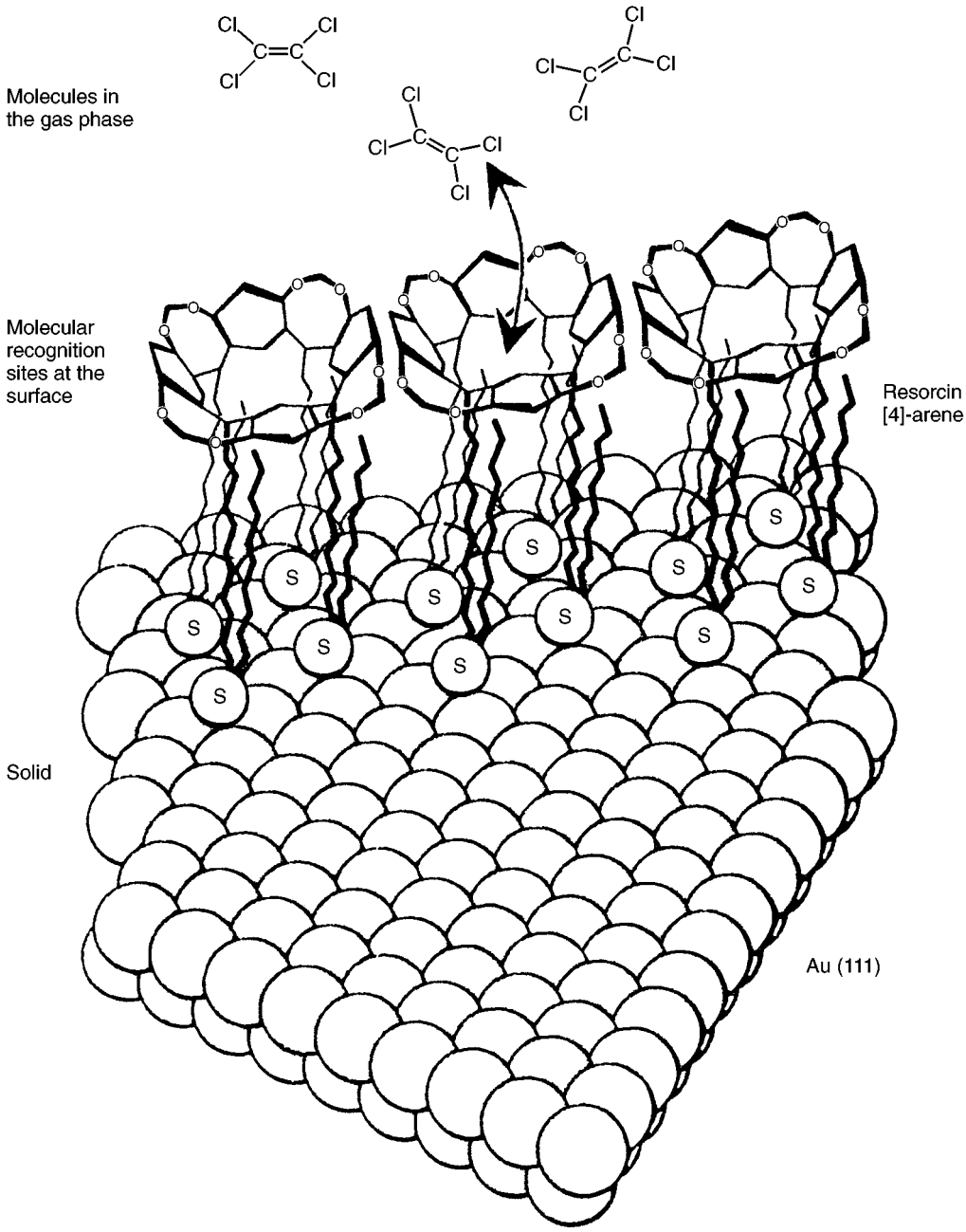

Fig. 4 Schematic representation of the molecular structure and interaction of a self-assembled monolayer of resorcin[4]arenes on an Au(111) surface with perchloroethylene molecules from the gas phase.[96] (With kind permission from the American Association for the Advancement of Science).

providing a promising basis for detection of DNA hybridisation reactions with SPR.

Quartz Crystal Microbalance

A QCM senses mass changes that result in a shift in resonance frequency. A piezoelectric crystal oscillates at a very sharp frequency. Small mass changes on the crystal surface shifts this frequency. In liquids, the performance of a QCM is in many cases inferior to that in air.[91] Sensitivity and selectivity are comparable with SPR.[92] If both sides of the crystal are coated with gold, these layers can be used as electrodes. Simultaneous measurements of electrochemical parameters and mass changes are an interesting feature of the EQCM.[93]

With a mass-sensitive instrument, organophosphonates (nerve agents) can be selectively and reversibly detected, as shown by Kepley *et al.*[94] Copper(ii)-ions, bound to a SAM of carboxylate-terminated alkanethiols act as the sensing layer.

EQCM has been used to study the kinetics of the formation, and the oxidative or reductive desorption of alkanethiol SAMs.[94] It was concluded that better SAMs are formed from poorer solvents for the thiol. Choosing the appropriate conditions (solvent, formation time, thiol concentration) is of importance when well defined monolayers are required.

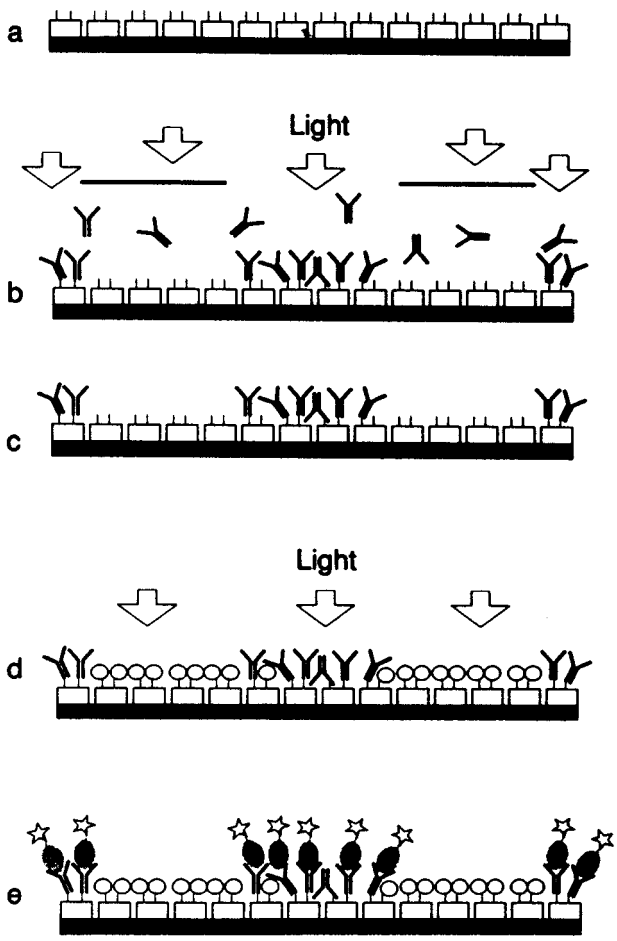

Fig. 5 The immobilisation process used for patterning of proteins. a, Avidin with photobiotin immobilised onto the surface. b, Exposure of selected areas to light through a mask results in activation of the photobiotin molecule, specifically immobilising the antibody in the solution. c, Unbound material is removed by washing. d, The entire surface is exposed to light, and a blocking molecule bound to all unreacted photobiotin groups. e, Following washing the surface is exposed to fluorescently labeled antigen, which is bound by the patterned antibody.[100] (With kind permission from Elsevier Science Ltd.)

Fundamental knowledge about the adsorption kinetics of alkanethiols can be obtained both from SPR and QCM measurements, because the process is monitored in real-time.[95]

Very small mass changes of small gaseous molecules were measured with QCM using a resorcin[4]arene monolayer (Fig. 4) for detection.[96] Perchloroethylene molecules are, according to the authors, recognised by these hydrophobic cavitants and could be measured in the nanogram range. However, other authors doubt their conclusions.[97]

A synthetic antigenic peptide [of the foot-and-mouth disease virus (FMDV)] has been derivatised with ω-hydroxy-undecanethiol (HUT) and was adsorbed onto gold.[91] Various procedures to prepare sensing layers from (mixed) SAMs are compared and (contrarily) the best layer is the undiluted modified-peptide SAM. The common strategy to avoid steric hindrance, diluting the HUT-modified peptide in a mixed monolayer with pure HUT, yielded a lower response to anti-FMDV-antibody.

Uniform Immobilisation Strategies

In developing sensitive sensors, the self-assembly technique offers interesting perspectives. By generating functionalised surfaces through modification of thiols, it is possible to selectively attach the biomolecules of interest. Another option is to derivatise a biomolecule with a thiol functionality. For repetitive measurements (cost savings), the sensor surface ought to be regenerable. A sensor surface for phosphate biomolecules can be regenerated using a pH-dependent, electrostatically attached pentamidine layer upon a monolayer of mercapto-alkanoic acid.[98]

An *N*-hydroxysuccinimide ester functionalised SAM can be applied for a uniform covalent immobilisation of amino group-containing biomolecules.[99]

Photo-immobilisation for proteins *via* benzophenon derivatization can be another strategy (Fig. 5). Upon UV irradiation, a 10,10′-dithiobis(decanoic acid *N*-hydroxysuccinimide ester) derivatised with benzophenon crosslinks with an antibody. A homogeneous single layer of antibodies results, with retention (at least in part) of the activity.[100]

Polymer layers are widely applied in sensors and reviewed by Harsányi.[101] The films can be deposited upon a substrate by (*a*) spinning or casting, (*b*) electrochemical polymerisation or (*c*) vacuum deposition. Polymerisation to a thin film, after being adsorbed as thiol monomers, was first described by Ford *et al.*[102] An SAM of 4-(mercaptomethyl)styrene was polymerised in an aqueous solution of azo-initiator by irradiation with a laser, yielding a hydrophobic surface.

General Conclusions

Monolayers from thiol-containing molecules are easy to prepare, quickly assembled and well ordered. A general protocol for self-assembly conditions is hard to give; the preparation route depends on the desired properties. Electrochemistry, especially cyclic voltammetry, can detect pinholes or defect structures in the monolayers. SAMs are stable in a potential range from −400 to +1400 mV *versus* SCE; beyond these potentials the layer desorbs, yielding a clean gold surface.

Small ω-functional groups exhibit no influence on the formation of the monolayer. Preparing a mixed SAM from a long-chain ω-functionalised thiol ‘diluted’ with a shorter-chain alkanethiol offers great challenges for analytical purposes. In this way steric hindrance of the functionality can largely be reduced. The SAM technology is widely applied in electrochemical, SPR-based and (E)QCM-based sensors.

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