Communications

Molecular Recognition by Self-Assembled Monolayers Detected with Surface Plasmon Resonance**

By Bart-Hendrik Huisman, Rob P. H. Kooyman,*
Frank C. J. M. van Veggel, and David N. Reinhoudt*

During the past decades, the modification of surfaces by means of self-assembly has been investigated extensively, partly in view of the potential applications.^[1] In particular, organosulfur-modified gold surfaces have been studied,^[2] because of their high stability and high degree of order. Monolayers have been reported with a variety of functionalities. Applications have been mentioned such as in piezoelectric devices, nonlinear optics, and microsensors.^[3]

Self-assembled monolayers are appealing as a chemical interface in sensors as they are thermodynamically stable, and well defined on a molecular level. Fast response times are obtained because of the short diffusion lengths. Furthermore, a flexible design of the chemical interface is possible. Sensitivity and selectivity can be obtained by molecular recognition at the monolayer interface.^[4] Good examples are found in the field of immunosensors, where the superior selectivity of a biological molecule, the antibody, is exploited.^[5] In the field of supramolecular chemistry efforts are made to mimic this selectivity with synthetic receptors. Demonstrations of the successful use of these receptors can be found in fields such as membrane transport,^[6] and the development of CHEMFETs.^[7]

Only a few functionalized self-assembled monolayers produced with the objective of improving selectivity have been reported. In the presence of copper ions simple monolayers containing carboxylic acid end groups showed selectivity for organophosphonates.^[8] Mixed monolayers of isophthalamide adsorbates and thiols were shown to bind barbiturate.^[9] Very recently, a resorcin-based assembly was described by Stirling in which the binding of vitamin C to the monolayer was observed.^[10] Kaifer reported mixed monolayers of β -cyclodextrin and pentanethiol, and showed with cyclic voltammetry that ferrocene was complexed in the cavity.^[11]

Previously, we have described highly-ordered self-assembled monolayers of resorcin[4]arene^[12] and calix[4]arene^[13] based tetrasulfides. These adsorbates were shown to form well-ordered assemblies if the alkyl chains can cover an area that is comparable to the "arene" head group.^[14] In this system, the head group is readily available for complexation as it is directed to the outer interface, in contrast to a resorcin[4]arene monolayer reported by Moore et al.^[15] In our case, the alkyl chains form a densely packed monolayer providing order to the monolayer, and efficiently block the heterogeneous electron transfer during electrochemical experiments.^[14] In sensor experiments this is advantageous because unspecific interactions are thus prevented. These unspecific interactions have been observed in Langmuir-Blodgett films of calix[n]arenes during selective permeation experiments, i.e. permeation across these films is governed by diffusion between neighboring molecules and not through the molecular pores of the calix[n]arenes.^[16]

With a quartz crystal microbalance (QCM), modified with the resorcin[4]arene cavitand adsorbate **1** (Scheme 1), we have observed high sensitivity for tetrachloroethylene.^[17] In order to better understand the adsorption behavior we have now investigated this monolayer system with a surface

Scheme 1.

plasmon resonance set-up (SPR). We have used a home-built vibrating mirror SPR set-up^[18] that, owing to its high stability, is particularly suited for further investigations of the interaction of vapors with monolayers of cavitand **1**. The adsorption behavior to monolayers of **1** and octadecanethiol has been studied, and was compared with theoretical adsorption isotherms. To the best of our knowledge, this is the first example where self-assembled monolayers are used as an interface for vapor detection with SPR as a transducer.

Surface plasmon resonance is a technique where changes in the refractive index near an interface can be readily

[*] Prof. D. N. Reinhoudt, Dr. B.-H. Huisman, Dr. F. C. J. M. van Veggel
Laboratory of Organic Chemistry, MESA Research Institute
University of Twente
P.O. Box 217, NL-7500 AE Enschede (The Netherlands)
Dr. R. P. H. Kooyman
Department of Applied Physics, MESA Research Institute
University of Twente
P.O. Box 217, NL-7500 AE Enschede (The Netherlands)

[**] We thank A. T. M. Lenferink (UT) for assistance with the SPR measurements.

detected. Several optical configurations have been reported for sensor applications over the past decade.^[19] One of the first examples where SPR was used as a transducer in sensing devices is reported by Nylander. He applied a film of silicone oil as a chemical interface to detect the anaesthetic gas halothane.^[20] Shortly afterwards the application of SPR as an immunosensor was reported.^[21] Whitesides very recently illustrated the use of SPR combined with self-assembled monolayers by the detection of carbonic anhydrase and non-specific binding of protein.^[22] To date, SPR is a technique that is frequently applied in immunosensing, but still less common in other fields.

It is far beyond the scope of the present paper to give even a superficial discussion of SPR. Experimentally, the main quantity is the 'plasmon angle', which is the angle under which light, reflected at a prism/metal interface in the so-called Kretschmann configuration, undergoes a minimum in the reflectance. The change of the plasmon angle during a surface binding experiment can be shown to be proportional to the amount of material bound to the interface.^[23]

A two-channel vibrating-mirror angle scan set-up based on the Kretschmann configuration was used for SPR experiments, as was described in detail before.^[24] With this equipment (operating wavelength 632 nm) changes in the plasmon angle can be determined with high accuracy ($< 0.001^\circ$). One channel is used to measure monolayers of **1**, while simultaneously at the other channel an octadecanethiol monolayer is measured as a reference. The octadecanethiol monolayer does not contain any recognition sites, and can thus be used as a reference layer in a study of the specific interaction between the guest and the cavity monolayer. The layers were in contact with different vapors via a gas-flow system in which saturated vapors were mixed with nitrogen to obtain the required concentration.

The change in plasmon angle ($\Delta\alpha$) was determined as a function of the concentration of toluene, tetrachloroethylene, tetrachloromethane, chloroform, dichloromethane, and 1,2-dichloroethane. Throughout the measurements the baseline was stable within $\Delta\alpha = 0.01^\circ$. These were gradual changes, most likely caused by small changes in temperature under influence of the laser beam. Since the response to changes in vapor concentrations is fast, these variations in the baseline did not affect the angular resolution. Consequently, the error in the change of the plasmon angle is about 0.001° .

Changes in the concentration of different vapors resulted in a change of plasmon angle, both for the cavity monolayer and to a much lesser extent octadecanethiol. A typical experimental response curve is shown in Figure 1, where different concentrations were subsequently applied to the monolayers. The response time is fast (typically $t_{90\%} < 1$ s). The initial response to changes in concentrations was faster than the time between two data points (1 s). In general a stable signal was obtained after a few seconds. After an initial stabilization, the interaction of the guest with the host was fully reversible. Furthermore, the response was

Fig. 1. Typical SPR time response curve for tetrachloroethylene in contact with monolayers of cavitand **1** (top) and octadecanethiol (bottom) as a function of the applied concentration; angle change on relative scale.

independent of the concentration measured immediately prior to the new concentration, i.e. no memory effect was observed for any of the layers or vapors.

Table 1. SPR differential sensitivity S ($^\circ/\text{ppm}$) at 50 % vapor saturation.

Vapor	Formula	S (10^{-7} degree per ppm)	
		cavitand	octadecanethiol
tetrachloroethylene	$\text{Cl}_2\text{C}=\text{CCl}_2$	60	5
1,2-dichloroethane	$\text{CH}_2\text{Cl}-\text{CH}_2\text{Cl}$	6.0	3
dichloromethane	CH_2Cl_2	1.5	1
chloroform	CHCl_3	4.5	2
tetra	CCl_4	12	6
toluene	$\text{C}_6\text{H}_5-\text{CH}_3$	20	11

In Table 1 the SPR differential sensitivities S are presented for the various vapors in contact with both the cavity and the octadecanethiol monolayer. A large difference in sensitivity to different vapors is observed. In all cases S for the monolayer containing cavitand **1** was larger than that for the octadecanethiol monolayer. This shift was about a factor two larger for the cavitand monolayer compared to the octadecane monolayer for all vapors, except for tetrachloroethylene. In the latter case, a considerably larger shift was observed in contact with the cavitand monolayer. However, the response of tetrachloroethylene in contact with the octadecanethiol monolayer was comparable to that of the other vapors. As in general the enhancement is about a factor two, this effect may be partly attributed to enlargement of the surface area as a consequence of the cavities. For tetrachloroethylene, however, this effect is too large (twelve times enhancement compared to octadecanethiol). This strongly indicates molecular recognition of tetrachloroethylene by the cavitand monolayer.^[25]

The change in plasmon angle as a function of the concentration of tetrachloroethylene and 1,2-dichloroethane in

Fig. 2. Shift of plasmon angle $\Delta\alpha$ as a function of the concentration tetrachloroethylene (top), and 1,2-dichloroethane (bottom). Cavitand monolayer: $\square$; octadecanethiol monolayer: $\circ$. Note the difference of the scale in horizontal axis.

contact with both the cavitand and the octadecanethiol monolayer is plotted in Figure 2. Note the difference in concentration needed for a similar response for these two vapors. It was determined that at a concentration of 1600 ppm tetrachloroethylene still results in a response of 0.005° being measured. With the current equipment this implies that the detection limit is about 300 ppm tetrachloroethylene, which may be improved by a stronger binding of the guest via molecular engineering of the monolayer. The interaction between the cavity monolayer and tetrachloroethylene showed a progressively increasing response at higher vapor concentrations. In contact with octadecanethiol this trend was absent. For the other vapors, the concave concentration dependency was less pronounced. A shift in plasmon angle of 0.01° corresponds to about 1–2 monolayers with an assumed refractive index of 1.5, indicating that the solvent does not condense during the experiments. Also previous experiments showed that no condensation at the interface occurs.^[17]

To obtain more insight in the mechanism of the adsorption of tetrachloroethylene to the monolayer, the experimental response was fitted with various standard

isotherms, each of them characteristic for a certain adsorption process. For our experiments it is reasonable to assume that the plasmon angle is linearly dependent on the refractive index (n) of the film in contact with the metal, and that n changes linearly with the amount of substance adsorbed to the surface.^[23] Then, the shift of the plasmon angle is a linear measure for the surface coverage (θ), as shown in Equation 1, where θ is the surface coverage which depends on the pressure p , and K is an instrument-dependent constant.

$$\Delta\alpha(p) = K\theta(p) \quad (1)$$

This formula was used to adapt the standard isotherms to the shift in plasmon angle. Three standard isotherms that describe the shape of the curve (BET,^[26] Freundlich,^[27] and Sips^[28]) were fitted with the experimental data, as represented by the lines in Figure 2a. The best correlations are obtained with the BET and the Freundlich isotherm, for which the fitted curves remain within the experimental error. These isotherms are based on systems allowing two, and multiple adsorption enthalpies, respectively. The adsorption enthalpies can be determined experimentally if temperature-dependent measurements are performed. The good agreement with the BET and the Freundlich isotherm for adsorption of perchloroethylene to cavitand monolayer **1** indicates that after monolayer adsorption to one type of sites, adsorption occurs to sites with a different heat of adsorption. In other words, adsorption sites with different adsorption energies are present in the cavity monolayer.

The results of the surface plasmon experiments are in agreement with the qualitative experiments performed with the QCM, where also a high response for tetrachloroethylene was observed.^[17] The molecular recognition sites induce an increased affinity for the guests and thus a higher sensitivity. The use of SPR allowed us a more detailed study of the adsorption behavior to monolayers, compared to the QCM experiments. The sensitivity of both techniques is comparable, but SPR was found to be a very stable technique, i.e. virtually no base-line shift was observed. High-frequency SAW devices allow an even higher sensitivity,^[15,29] but like QCM show a considerable amount of base-line drift.

In conclusion, the vibrating mirror SPR set-up was shown to be a suitable transducer with high stability and sensitivity. Due to the use of self-assembled monolayers as the chemical interface, fast response times are possible. Molecular recognition sites in the monolayer show a high affinity for tetrachloroethylene, resulting in a high sensitivity. Adsorption of tetrachloroethylene to the cavitand monolayer can be described by a BET or a Freundlich isotherm, which implies an interface with more than one adsorption enthalpy.

Experimental

All chemicals (highest purity) were purchased from Janssen Chimica or Aldrich, and were used as received. Cavitand **1** was synthesized as described

before.^[30] Monolayers were prepared on substrates of 47.5 nm gold evaporated onto glass of 25 mm diameter covered with 2 nm chromium for adhesion purposes. These substrates were cleaned by oxygen-plasma for 10 min. immediately prior to adsorption. Monolayers were formed by immersion of a gold substrate into a 1 mM solution of the adsorbate in a mixture of ethanol:chloroform (7:3, v/v). Monolayers of octadecanethiol were left in solution for 6 h, while monolayers of **1** were left for 13 h at 60 °C.^[12] Subsequently the layers were rinsed extensively with chloroform, ethanol, and ultra pure water, respectively. The layers were characterized by FT-IR spectroscopy and contact-angle measurements prior to use in the SPR set-up. The monolayer characterization was in full agreement with the previously reported results.^[14]

Surface plasmon resonance: The substrates with monolayers were optically connected to the prism with a matching oil. Vapor concentrations were varied between 7 and 100 % of saturation at standard pressure at 22 °C. The total flow in the sample compartments was constant during each measurement. The vapor pressures at saturation were calculated using Antoine constants.^[31]

Received: November 30, 1995
Final version: March 13, 1996

- [1] J. D. Swalen, D. L. Allara, J. D. Andrade, E. A. Chandross, S. Garoff, J. Israelachvili, T. J. McCarthy, R. Murray, R. F. Pease, J. F. Rabolt, K. J. Wynne, H. Yu, *Langmuir* **1987**, 3, 932.
- [2] For a recent review see: L. H. Dubois, R. G. Nuzzo, *Annu. Rev. Phys. Chem.* **1992**, 43, 437.
- [3] A. Ulman, *An Introduction to Ultrathin Organic Films*, Academic Press, San Diego, CA **1991**.
- [4] a) R. C. Hughes, A. J. Ricco, M. A. Butler, S. J. Martin, *Science* **1991**, 254, 74. b) F. L. Dickert, A. Haunschild, *Adv. Mater.* **1993**, 5, 887.
- [5] W. Müller, H. Ringsdorf, E. Rump, G. Wildburg, X. Zhang, L. Angermaier, W. Knoll, M. Liley, J. Spinke, *Science* **1993**, 262, 1706.
- [6] For example: D. M. Rudkevich, J. D. Mercer-Chalmers, W. Verboom, R. Ungaro, F. de Jong, D. N. Reinhoudt, *J. Am. Chem. Soc.* **1995**, 117, 6124.
- [7] P. L. H. M. Cobben, R. J. M. Egberink, J. G. Bomer, P. Bergveld, D. N. Reinhoudt, *J. Electroanal. Chem.* **1994**, 368, 193.
- [8] L. J. Kepley, R. M. Crooks, A. J. Ricco, *Anal. Chem.* **1992**, 64, 3191.
- [9] K. Motesharei, D. C. Myles, *J. Am. Chem. Soc.* **1995**, 117, 6124.
- [10] H. Adams, F. Davis, C. J. M. Stirling, *J. Chem. Soc., Chem. Commun.* **1994**, 2527.
- [11] M. T. Rojas, R. Königer, J. F. Stoddart, A. E. Kaifer, *J. Am. Chem. Soc.* **1995**, 117, 336.
- [12] E. U. Thoden van Velzen, J. F. J. Engbersen, D. N. Reinhoudt, *J. Am. Chem. Soc.* **1994**, 116, 3597.
- [13] B.-H. Huisman, E. U. Thoden van Velzen, F. C. J. M. van Veggel, J. F. J. Engbersen, D. N. Reinhoudt, *Tetrahedron Lett.* **1995**, 36, 3273.
- [14] E. U. Thoden van Velzen, J. F. J. Engbersen, P. J. de Lange, J. W. G. Mahy, D. N. Reinhoudt, *J. Am. Chem. Soc.* **1995**, 117, 6853.
- [15] L. W. Moore, K. N. Springer, K.-X. Shi, X. Yang, B. I. Swanson, D. Li, *Adv. Mater.* **1995**, 7, 729.
- [16] P. Dekek, A. S. Webber, V. Janout, R. A. Hendel, S. L. Regen, *Langmuir* **1994**, 10, 3943.
- [17] K.-D. Schierbaum, T. Weiß, E. U. Thoden van Velzen, J. F. J. Engbersen, D. N. Reinhoudt, W. Göpel, *Science* **1994**, 265, 1413.
- [18] A. T. M. Lenferink, R. P. H. Kooyman, J. Greve, *Sens. Actuators B* **1991**, 3, 261.
- [19] K. Matsubara, S. Kawata, S. Minami, *Appl. Opt.* **1988**, 27, 1160.
- [20] C. Nylander, B. Liedberg, T. Lind, *Sens. Actuators* **1982/83**, 3, 79.
- [21] B. Liedberg, C. Nylander, I. Lundström, *Sens. Actuators* **1983**, 4, 299.
- [22] a) M. Mrksich, J. R. Grunwell, G. M. Whitesides, *J. Am. Chem. Soc.* **1995**, 117, 12009. b) M. Mrksich, G. B. Sigal, G. M. Whitesides, *Langmuir* **1995**, 11, 4383.
- [23] H. Raether, in *Physics of Thin Films* (Eds: G. Has, M. Francombe, R. Hoffman), Academic Press, New York **1977**, Vol. 9.
- [24] R. P. H. Kooyman, A. T. M. Lenferink, R. G. Eenink, J. Greve, *Anal. Chem.* **1991**, 63, 83.
- [25] Specific adsorption is further evidenced by the desorption enthalpies reported before (see ref. 17).
- [26] T. L. Hill, *Adv. Catal.* **1952**, 4, 225.
- [27] G. D. Halsey, *Adv. Catal.* **1952**, 4, 259.
- [28] R. Sips, *J. Chem. Phys.* **1948**, 16, 490; R. Sips, *J. Chem. Phys.* **1950**, 18, 1024.
- [29] F. L. Dickert, T. Bruckdorfer, H. Feigl, A. Haunschild, V. Kuschow, E. Obermeier, W.-E. Bulst, U. Knauer, G. Mages, *Sens. Actuators B* **1993**, 13-14, 297.
- [30] E. U. Thoden van Velzen, J. F. J. Engbersen, D. N. Reinhoudt, *Synthesis* **1995**, 8, 989.
- [31] *Vapor-Liquid Equilibrium Data* (Ed: S. Ohe), Elsevier, Amsterdam **1989**.

Bifunctional Homopolymeric Hydrogen-Bonded Tapes**

By Peter R. Ashton, George R. Brown, Wayne Hayes,
Stephan Menzer, Douglas Philp, J. Fraser Stoddart,* and
David J. Williams

The ability to predict both molecular and supramolecular structures in the solution and solid states is one of the contemporary goals^[1] of those searching for functioning molecular devices.^[2] Extensive studies of hydrogen bonding motifs^[3,4] in supramolecular systems have resulted in the construction, via self-assembly^[2,5] processes, of linear arrays—such as ribbons, sheets and tapes—possessing an interesting range of physical properties.^[6] The use of ‘natural’ hydrogen bonding motifs, involving purine and pyrimidine bases, to facilitate assembly of molecular subunits features strongly in the majority of the supramolecular systems studied to date.

Hydrogen bonding between the hydrogen atoms of carboxylic acids and the nitrogen atoms of pyridine derivatives has been observed and investigated thoroughly in a variety of systems incorporating these two functionalities.^[7,8] Although these studies have revealed that this linear recognition motif predominates^[7b] for systems incorporating these two functionalities, there are several exceptions wherein carboxylic acid dimers^[9] or internal hydrogen bonding networks between the carboxylic acid hydrogen atom and alternative acceptor functionalities^[10] present in the molecule have been observed.

Recently, the linear recognition motif has been examined by Fréchet and co-workers^[11] as a potential vehicle for liquid crystal formation between a range of pyridine and carboxylic acid derivatives. In addition, several linear covalently-linked polybipyridinium systems have revealed novel liquid crystalline and conductive properties.^[12] Concurrently, we have designed a simple linear cationic pyridylpyridinium system **1** · PF₆ (Fig. 1), possessing a terminal carboxylic acid group in order to investigate its potential to form the hydrogen bonded polymeric tape arrays and exhibit liquid crystalline behavior. Here, we

[*] Prof. J. F. Stoddart, P. R. Ashton, Dr. W. Hayes, Dr. D. Philp
School of Chemistry, University of Birmingham
Edgbaston, Birmingham, B15 2TT (UK)

Dr. G. R. Brown
ZENECA Pharmaceuticals
Mereside, Alderley Park
Macclesfield, Cheshire SK10 4TG (UK)

Prof. D. J. Williams, Dr. S. Menzer
Chemical Crystallography Laboratory
Department of Chemistry, Imperial College
South Kensington, London SW7 2AY (UK)

[**] This research was supported by the Engineering and Physical Sciences Research Council, the Shell International Petroleum Company Limited, the Wolfson Foundation and ZENECA Pharmaceuticals. We acknowledge gratefully the discussions and helpful suggestions of Prof. H. Ringsdorf and Dr. J. A. Preece from the Institut für Organische Chemie, Johannes Gutenberg-Universität in Mainz, Germany.