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A simple strategy for preparation of sensor arrays: molecularly structured monolayers as recognition elements†

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The spreader-bar approach is a simple method for producing a huge variety of receptors with different selectivities. A sensor-array consisting of five such receptors is presented. A pattern recognition provides selective detection of different purines and pyrimidines.

An application of pattern recognition techniques, based on principal component analysis or neuronal networks, allows one to reach a high selectivity of chemical analysis but requires a pre-formation of an array of chemical sensors with essentially distinguished properties of single sensors. Instead of the traditional way of receptor preparation by means of a time-consuming chemical synthesis, we suggest use of the spreader-bar approach. Hence, thiol modified purines and pyrimidines (spreader-bar molecules) have been co-adsorbed together with dodecanethiol (matrix molecules) onto a gold surface, thus forming self-assembled nanostructured monolayers with recognition properties towards different purines and pyrimidines. This technology provides a simple way to form a huge variety of chemoreceptors with different selectivity properties and can be used for fabrication of large sensor arrays.

A co-adsorption of linear (matrix) and planar (template) molecules on a solid surface followed by a desorption of the template leads to the formation of artificial receptors to these molecules.[1] This principle has been used for creation of photochemical imprints.[2,3] We have reproduced and confirmed the principle[1] on the gold–alkanethiol system, but discovered the limited stability of the system — most probably because of the lateral diffusion, the affinity properties were lost in about one hour.[4] It can be overcome if the template remains on the surface thus forming binding cavities (Fig. 1). This 'spreader-bar' approach is based on creation of mixed monolayers of two different compounds, none of them exhibiting recognition properties alone. One component, called the matrix, is an alkanethiol, the second component, called the spreader-bar molecule, has a rigid planar shape. The matrix molecules must be able to form a monolayer thicker than a monolayer of the spreader-bar molecules.[4] Both molecules can be co-adsorbed on the gold surface.[5] The structures formed were found to be able to interact selectively with certain molecules in a solution.[4,6,7] In this paper, the spreader-bar approach is applied for preparation of sensor arrays based on thiolated bases of nucleic acids as spreader-bars; adenine, cytosine, thymine, uracil, caffeine and uric acid were used as analytes. Analyte binding was detected as changes of peak amplitude in cyclic voltammetry or as modification of electrochemical impedance: the binding modifies reaction resistance and electrode capacitance while the Warburg impedance does not change. Monitoring of the capacitive current[4,8] was used as the main detection method.

For gold electrodes covered by a monolayer of a single component of either matrix- or spreader-bar molecules, no recognition abilities were found. For example, the changes of capacitive current at 80 Hz due to adsorption of purines and pyrimidines from the solution of 300 μmol L^{-1} on dodecanethiol coated electrodes were 0.7% for adenine or even less for every other substance.

The behaviour of mixed monolayers consisting of dodecanethiol and the one of thiolated purines or pyrimidines was quite different: an adsorption of adenine, cytosine, thymine, uracil, caffeine or uric acid resulted in over 25% change of the capacitive current (Fig. 2). To obtain systems displaying such properties, the mixed monolayers have to be formed at a definite concentration range of spreader-bar and matrix molecules in the coating solutions. An investigation of the obtained monolayers by IR reflection absorption spectroscopy and contact angle measurements has shown that this range of coating conditions corresponds to the formation of mixed monolayers with comparable surface concentrations of both components. A

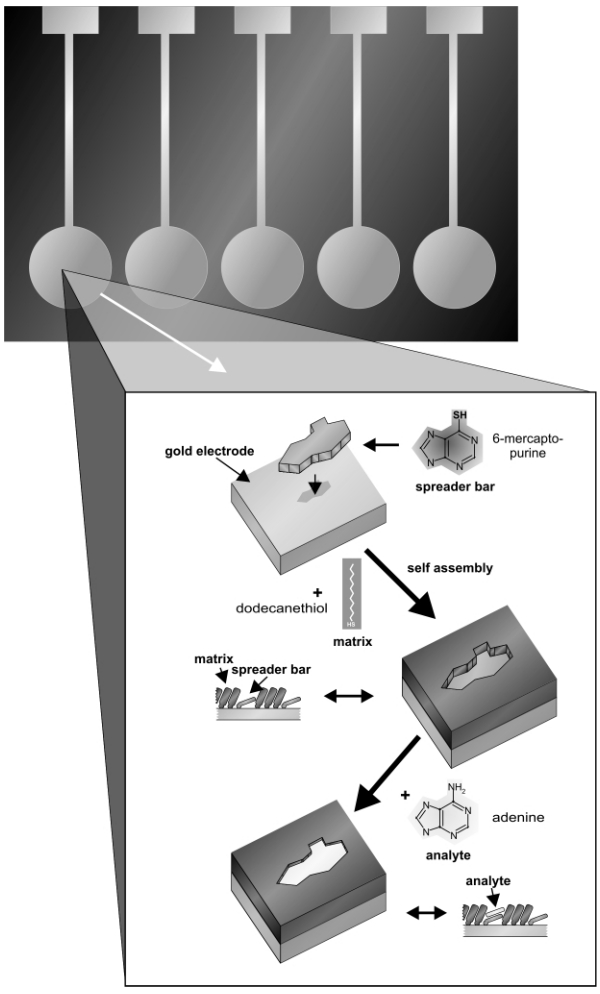

Fig. 1 Formation of a sensor array by spreader-bar technology. The co-adsorption of a thiol modified analyte and alkanethiol leads to the formation of a mixed monolayer exhibiting recognition abilities to particular analytes.

† Electronic supplementary information (ESI) available: experimental details and electrochemical measuring conditions. See http://www.rsc.org/suppdata/cc/b2/b210554c/

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decreased spreader-bar : matrix ratio (less than 10 : 1 for 6-mercaptopurine as spreader-bar) in the coating solutions or lower deposition time (less than 1.5 days at 22 °C) results in formation of monolayers displaying only weak adsorption of analytes which does not differ from the properties of pure monolayers.

Concentration dependences of an electrode array consisting of dodecanethiol and one of 6-mercaptopurine (ASH), 2-amino-6-purinethiol (GSH), 4-amino-2-mercaptopyrimidine (CSH), 4-hydroxy-5-methyl-2-mercaptopyrimidine (TSH) or 4-hydroxy-2-mercaptopyrimidine (USH) on addition of adenine are is shown in Fig. 2. The response depends on a specific combination of spreader-bar and adsorbate. The electrode coated by a mixture of TSH and dodecanethiol exhibit the highest change of the signal on adenine addition; for electrodes with other spreader-bars, the signal decreased according to the order: USH > GSH > ASH > CSH. The observed interaction of adenine with mixed monolayers consisting of ASH was a reason for testing this system as an artificial receptor for ATP. The experiment confirmed this suggestion: an ATP addition resulted in a concentration dependent decrease of the capacitive current through the mixed monolayer with saturation at 2.2% and the concentration, corresponding to 50% of the maximal response, at about 50 μmol L^{-1}.

Variations of spreader-bars lead to essential modifications in sensor behaviour. A study of relative signal changes on adsorption of the same concentrations of different purines and pyrimidines onto mixed monolayers formed with either ASH, GSH, TSH, USH or CSH, results in signal patterns which are typical for every specific analyte used (Table 1). The sequences are valid for the whole concentration range (20 to 470 μmol L^{-1}) studied. This set of five artificial receptors based on these mixed monolayers allows one to identify each of the six

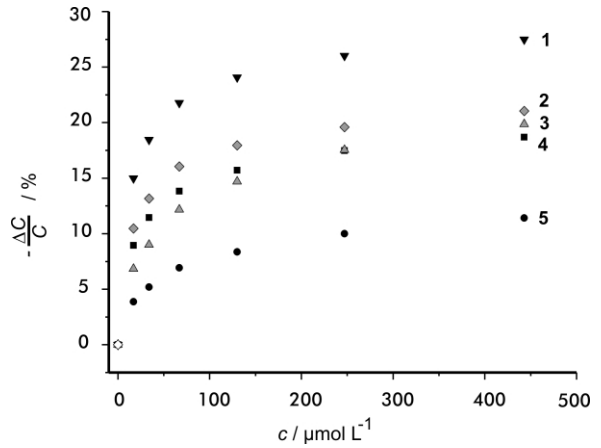

Fig. 2 Concentration dependence of the relative changes in capacitive current on addition of adenine for an array of five different mixed monolayers with the spreader-bar molecules 1: TSH, 2: USH, 3: GSH, 4: ASH, and 5: CSH.

Table 1 Pattern of the effectiveness of spreader-bars for the recognition of different analytes

Analyte	Signal pattern[a]
Adenine	T > U > A > G > C
Cytosine	U > G > A > t > c
Thymine	g > a > t > u > C
Uracil	T > U > C > A > G
Caffeine	A > C ≅ T > U > g
Uric acid	U > G > A > T > C

[a] The spreader-bar molecules are named by the first letter (A represents ASH) and ordered by decreasing response. A lower case letter indicates signal changes lower then 0.3%.

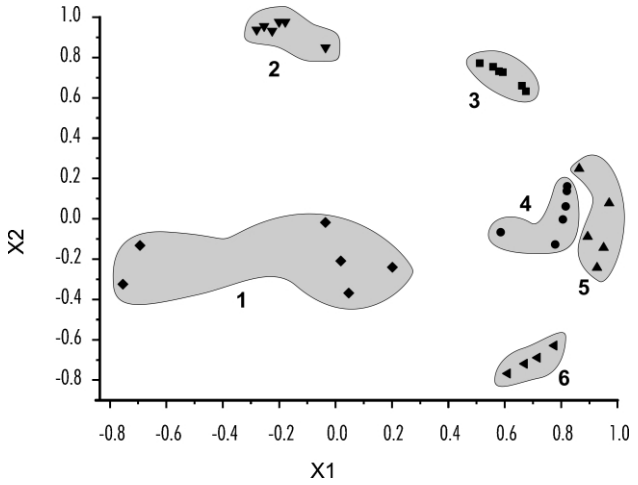

Fig. 3 Patterns of different concentrations of caffeine **1**, uracil **2**, adenine **3**, cytosine **4**, thymine **5** and uric acid **6** on an array of artificial receptors formed by thiolated derivatives of purines (ASH, GSH) and pyrimidines (CSH, TSH, USH) presented in the plot of principal components. X1 and X2 signify the first and the second principal components. Capacitive transducing was used.

different analytes tested. For cytosine and uric acid the patterns are the same, however the magnitude of the signal changes for uric acid was 5 to 17 times higher.

An analysis of principal components of the data array has shown that the first two components contain about 75% of the data variation. The data obtained at different concentrations for different analytes, being plotted in the virtual plane of the first and second principal components, form an arrangement in groups corresponding to individual substances (Fig. 3).

The results show, that in spite of limited selectivity of every single sensor element, the sensor array can be used for recognition of bases of nucleic acids as well as caffeine and uric acid. This first application of spreader-bar technology in sensor arrays illustrates its high potential in creation of large variety of chemoreceptors with different selectivity, thus fitting the main requirement in the development of modern analytical systems based on pattern recognition.[9] The spreader-bar technique provides a simple way to manufacture an almost unlimited number of such receptors: practically every thiol derivative can be used. Here we have demonstrated an application of this approach to form an array of only five sensors, but there is no technical limit to preparing such an array with hundreds of sensing elements.

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