6112 *J. Am. Chem. Soc.* **2000,** *122,* 6112−6113

Monolayer of a Na^+-Selective Fluoroionophore on Glass: Connecting the Fields of Monolayers and Optical Detection of Metal Ions

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Received February 28, 2000

We[1] and others[2] have recently reported, that monolayers of selective receptors on gold can be used to measure metal ion concentrations in solution with cyclic voltammetry and impedance spectroscopy. However, as a transduction principle for molecular recognition, fluorescence has advantages over electrochemical methods, for example, there is no need for reference electrodes or additional electrolytes. Nevertheless, examples of fluorescent monolayers on gold used for sensing are rare,[3] which may be related to the inherent disadvantage of fluorescence quenching by metal surfaces. Glass would be the best substrate for fluorescent monolayers, and we have found that the host−guest interaction between a monolayer of dansyl on glass and β-cyclodextrin can be detected by fluorescence spectroscopy.[4] In this contribution, we describe a monolayer of Na^+-selective fluorescent receptor **1** on glass, prepared by covalent coupling of the bis-isocyanate derivative of the receptor to a self-assembled monolayer (SAM) of 3-aminopropyltriethoxysilane (APTES). This is the first example of a monolayer of a selective receptor for detection of metal ions by fluorescence. The optical response of the monolayer to Na^+ ions is compared to that of fluoroionophore **2** in solution. Fluoroionophore **1** was prepared in four steps from calix[4]arene **3** as depicted in Scheme 1.[5] The ion receptor site is formed by a calix[4]arene[6] substituted with four amide moieties and has the size and polarity for Na^+ selectivity over other alkali metal ions.[7] Such ion receptors based on calix[4]arenes have already been applied in ion transport,[8] optical[9] and potentiometric sensors.[10] Two pyrene fluorophores transduce the complexation of a metal ion into an optical signal.[11] The tethered p-nitrophenylcarbamate groups allow the covalent attachment of **1** to the APTES SAM. The strategy of making monolayers of complex molecules on glass by coupling them to a preformed SAM is necessary, because the trichloro- or trialkoxysilane groups that form SAMs on glass[12] are not compatible with many functional groups that molecular receptors may contain. Following another two-step procedure, Hieftje et al.[13] have immobilized nucleic acid aptamers with fluorescent labels on a glass surface modified with activated carbamides.

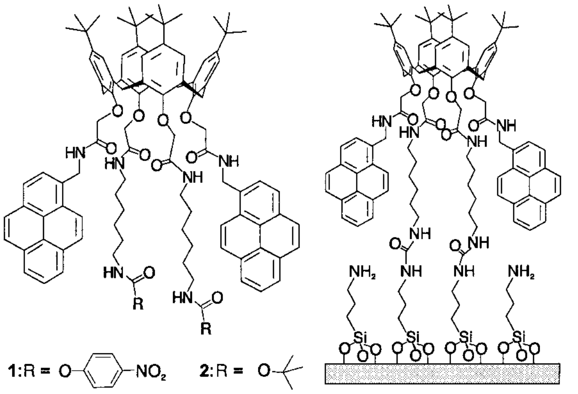
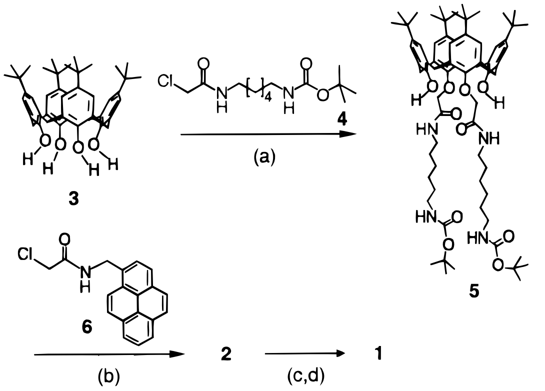


Figure 1. Structures of fluoroionophores **1** and **2** and schematic representation of the monolayer.

Scheme 1. Synthesis of Fluoroionophores **1** and **2**[a]



[a] (a) **4**, K_2CO_3, KI, acetonitrile, reflux, 16 h, 65%; (b) **6**, Na_2CO_3, NaI, acetonitrile, reflux, 4 days, 21%; (c) 1:1 TFA and dichloromethane, 3 h; (d) p-nitrophenylchloroformate, N,N-diisopropylethylamine, dichloromethane, 10%.

The monolayers were prepared by immersing the substrates, functionalized with an APTES SAM,[14] in a solution of compound **1** and N,N-diisopropylethylamine in dichloromethane overnight, after which the layers were rinsed with acetonitrile, ethanol, and dichloromethane and dried in a stream of nitrogen. In the coupling reaction, the p-nitrophenylcarbamate groups are converted with

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(12) The monolayers formed by trichloro- and trialkoxysilanes on glass are not strictly "self-assembled", because the coupling to the surface is covalent and not reversible, but it is common to refer to them as SAMs, as they share many characteristics with "true" self-assembled monolayers. See also: (a) Sagiv, J. *J. Am. Chem. Soc.* **1980**, *102*, 92−98. (b) Ulman, A. *Chem. Rev.* **1996**, *96*, 1533−1554.

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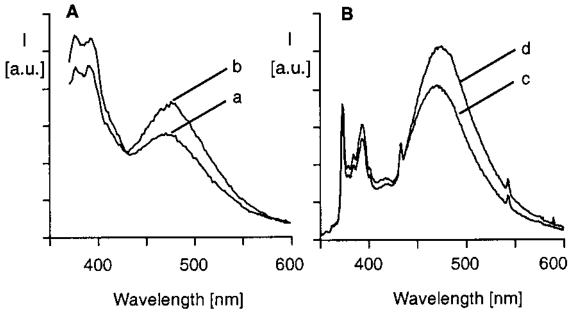

Figure 2. Emission spectra of monolayer and fluoroionophore in solution. (A) Monolayer of **1** in methanol, [NaOAc] = 0 (a) and 17 (b) mM. (B) Fluoroionophore **2** in methanol (3.6 μM), [NaOAc] = 0 (c) and 50 mM (d).

base into isocyanates (releasing *p*-nitrophenolate),[15] which subsequently react with the amino-terminated surface under formation of urea bonds. From ellipsometry data[16] and UV−vis absorption spectroscopy[17] a surface coverage of 80% was calculated. This can be considered high, when it is taken into account that the fluoroionophores are large molecules and that the attachment to the surface is irreversible. In wettability studies, advancing and receding contact angles of 90 ± 2° and 35 ± 2°, respectively, were found. This means that the monolayers are less hydrophobic than a calix[4]arene tetrasulfide monolayer on gold (advancing contact angle = 100−105°),[18] indicating that the polar functionalities are somewhat exposed to the outer interface. The large hysteresis indicates that the monolayers are very disordered.

The response of the monolayers to sodium acetate in methanol was monitored using the emission of the fluoroionophores (see Figure 2). Upon complexation of Na^+, the pyrene monomer fluorescence (maxima at 385 and 395 nm) decreased in intensity, while the eximer fluorescence (broad band at 480 nm) increased. In the Na^+ complex, the pyrene units are apparently in a better relative position to form eximers than in the free ligand. The behavior of compound **2** in solution (3.6 μM in methanol) is very similar to that of the monolayer. The response is opposite to that found by Shinkai et al. and Koyama et al., who studied calix[4]arene tetraesters with two pendant pyrene units in solution and found a decrease of the excimer fluorescence upon complexation of alkali metal ions.[11] It is likely that this is caused by the fact that the secondary amides in our system affect the conformation of the calix[4]arene receptors differently than esters.

Fitting[19] of the monomer fluorescence response (see Figure 3) gave a binding constant of $K_{395\ \mathrm{nm}} = 1540 \pm 700\ \mathrm{M}^{-1}$, and fitting

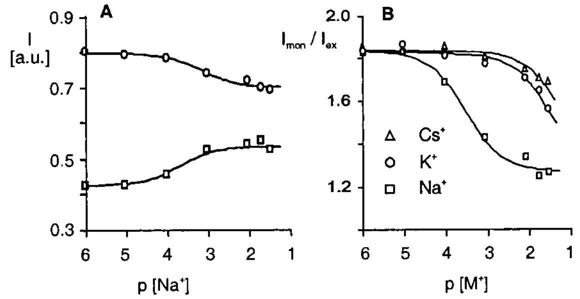

Figure 3. Response of the monolayer of **1** to alkali metal ions. (A) Fluorescence intensity at 395 nm (○) and 475 nm (☐) in dependence of the NaOAc concentration. The solid lines are calculated using association constants of $K = 1540\ \mathrm{M}^{-1}$. (B) Fluorescence response to the acetate salts of Na^+ (☐), K^+ (○), and Cs^+ (△). The response is expressed as the ratio of the monomer (395 nm) and excimer (475 nm) emission intensity. Excitation at 325 nm. The solid lines are drawn to guide the eye.

of the excimer fluorescence gave $K_{475\ \mathrm{nm}} = 5000 \pm 3000\ \mathrm{M}^{-1}$. Although the error is relatively large, these numbers correlate with the binding constant of $1275 \pm 191\ \mathrm{M}^{-1}$ for **2**, calculated from the excimer fluorescence response measured in solution. These binding constants are in the range that can be expected for calix[4]arene-based ionophores with four secondary amide ligating groups.[20] In titration experiments with both the monolayer and compound **2**, potassium and cesium acetate failed to produce a significant response up to concentrations of 5 mM. Exact binding constants could not be obtained, but it can be estimated that the selectivity constants for Na^+ over K^+ and Cs^+ are larger than 100.

This work presents a new approach to the immobilization of chromoionophores, which remains a major problem in the development of optical sensors for metal ions. It offers an alternative to physical immobilization in membranes, in which the partition of ions into the membrane both affects the selectivity and limits the response rates.[21] Since the receptor molecules in SAMs are directly exposed to analytes in solution, their response is fast. Most importantly, the similarities between the optical response as well as the binding constants of the monolayer and free ligand show that the fluoroionophores on the surface function independently and that the confinement in a monolayer does not affect the complexation behavior. We are currently expanding the method toward other fluoro- and chromoionophores.

Acknowledgment. We thank The Technology Foundation for financial support and Stephen I. Klink for his kind assistance with the fluorescence measurements.

Supporting Information Available: Preparative procedures and analytical data for compounds **1**−**6**; detailed procedures for monolayer preparation and surface reactions (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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[16] Measured monolayer thickness: 23 ± 2 Å. Estimated from CPK models for full surface coverage: 25.5 Å.

[17] Using the Lambert−Beer law with an estimated extinction coefficient for the fluoroionophores on the surface (using the solution value of **2**, 60 000 $\mathrm{M}^{-1}\ \mathrm{cm}^{-1}$) a density of one fluoroionophore per 200 Å^2 was found. The area of the fluoroionophore as estimated from a CPK model is 160 Å^2.

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